Application of Nitrile Oxide-Isoxazoline Chemistry for the Synthesis of 2-Ulosonic Acid Analogues

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What does man gain from all his labour
at which he toils under the sun?
Generations come and generations go,
but the earth remains for ever.
The sun rises and the sun sets,
and hurries back to where it rises.
The wind blows to the south
and turns to the north;
round and round it goes,
ever returning on its course.
All streams flow into the sea,
yet the sea is never full.
To the place the streams come from,
there they return again.
All things are wearisome,
more than one can say.
The eye never has enough of seeing,
nor the ear its fill of hearing.
What has been will be again,
what has been done will be done again;
there is nothing new under the sun.
Is there anything of which one can say,
"Look! This is something new"?
It was here already, long ago;
it was here before our time.
There is no remembrance of men of old,
and even those who are yet to come
will not be remembered
by those who follow.

Ecclesiastes **1** Vs 3-11

Declaration

I declare that this thesis was composed my myself and that it describes my own work except where specifically stated in the text. The work was carried out from October 1991 to October 1994 in the department of chemistry at The University of Edinburgh under the supervision of Dr. R.M. Paton.

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Glossary of Terms, Symbols and Abbreviations

AIBN	azoisobutyronitrile
aq.	aqueous
[α]	specific optical rotation
b.p.	boiling point
Bz	benzoyl
Bzl	benzyloxy
δ	chemical shift
m-CPBA	*meta*-chloroperbenzoic acid
CMP-KDO	cytidine 5′-monophospho-3-deoxy-D-*manno*-2-octulosonic acid
CTP	cytidine 5′-triphosphate
d	doublet
DAH	3-deoxy-D-*arabino*-2-heptulosonic acid
DAHP	3-deoxy-D-*arabino*-heptulosonate-7-phosphate
DEAE	diethylaminoethyl
DHQ	dehydroquinate
DIBAL	diisobutylaluminium hydride
DMAP	dimethylaminopyridine
DMF	*N*, *N*-dimethylformamide
DMP	dimethoxypropane
DMSO	dimethysulphoxide
E.coli	Escherichia coli
EPSP	5-enolpyruvyl-shikimate-4-phosphate

ether	diethyl ether
FAB	fast atom bombardment
FMO	frontier molecular orbital
g	gram
HOMO	highest occupied molecular orbital
HPLC	high performance liquid chromatography
hr	hour
Hz	hertz
IC_{50}	inhibition constant
IR	infra-red
J	coupling constant
KDN	3-deoxy-D-*glycero*-D-*galacto*-2-nonulosonic acid
KDO	3-deoxy-D-*manno*-2-octulosonic acid
K_i	inhibition constant
K_m/K_i	ratio of inhibition constant for substrate DAHP to inhibitor
LAH	lithium aluminium hydride
LDA	lithium diisopropylamide
Lipid-A	(*R*)-3-hydroxytetradecanoic acid
lit.	literature
LPS	lipopolysaccharide
L-Phe	L-phenylalanine
L-Trp	L-tryptophan
L-Tyr	L-tyrosine

LUMO	lowest unoccupied molecular orbital
m	multiplet
M	moles per litre
M^+	molecular ion
M eq	molar equivalent
mg	milligram
min	minute
mm Hg	pressure in millimetres of mercury
mmol	millimole
mol	mole
m.p.	melting point
MPLC	medium performance liquid chromatography
ms	mass spectrometry
Ms	methanesulphonyl
m/z	mass to charge ratio
NAD^+	nicotinamide adenine dinucleotide
NBS	*N*-bromosuccinamide
nd	not determined
Neu5Ac	*N*-acetyl-5-amino-3,5-dideoxy-D-*glycero*-D-*galacto*-2-nonulosonic acid
NMO	*N*-methylmorpholine *N*-oxide
NMR	nuclear magnetic resonance
PCC	pyridinium chlorochromate

PDC	pyridinium dichromate
PEP	phosphoenol pyruvate
PP_i	pyrophosphate
ppm	parts per million
Py	pyridine
q	quartet
r.t.	room temperature
s	singlet
t	triplet
TBAF	tetrabutylammonium fluoride
TBDMS	*tert*-butyldimethylsilyl
TBDPS	*tert*-butyldiphenylsilyl
TDI	tolylene 2,4-diisocyanate
TEA	triethylammonium
Tf	trifluoromethanesulphonyl
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TLC	thin layer chromatography
TMS	trimethylsilyl
TPSCl	2,4,6-triisopropylbenzenesulphonyl chloride
Ts	*p*-toluenesulphonyl
TsOH	*p*-toluenesulphonic acid

Abstract

Nitrile oxide-isoxazoline methodology has been employed in a novel convergent approach towards 2-ulosonic acid analogues, in particular those of 3-deoxy-D-*arabino*-2-heptulosonic acid (DAH) and 3-deoxy-D-*manno*-octulosonic acid (KDO). It involves regio- and diastereoselective [3+2] cycloaddition of a nitrile oxide to carbohydrate alk-1-enes containing four, five or six carbons, yielding 2-isoxazolines. Subsequent deprotection followed by reductive hydrolytic cleavage affords the 2-ulosonic acid analogues.

The four carbon alkene 1,2-dideoxy-3,4-*O*-cyclohexylidene-D-*glycero*-1-enitol was chosen as a model alkene on which to establish the methodology which could then be applied directly to the two target classes of compounds. Cycloadditions were performed using four nitrile oxides: ethoxycarbonylformonitrile oxide, benzonitrile oxide, acetonitrile oxide and (diethoxyphosphoryl)acetonitrile oxide. These proceeded in 40-88% yield with moderate π-facial selectivity (54-64% d.e.) in favour of *erythro* adducts, with the major product in each case possessing *S*-configuration at the new asymmetric centre, C-5. This selectivity can be rationalised in terms of the 'inside alkoxy effect' proposed by Houk *et al*, and the 'homoallylic' modification offered by De Micheli *et al*.

In an attempt to control selectivity in the nitrile oxide cycloaddition reactions, a dispiroketal protecting group was utilised. However, the increase in steric bulk and presence of a six-membered ring as the protecting group incorporating the diol, rather than the five-membered ring, does not appear to influence π-facial selectivity.

Cycloadditions were also performed using the five carbon alkenes 1,2-dideoxy-3,5-*O*-ethylidene-D-*erythro*-pent-1-enitol and 1,2-dideoxy-3,5-*O*-benzyl-

idene-L-*threo*-pent-1-enitol. With the former alkene these proceeded in 40-80% yield but with minimal π-facial selectivity (4-6% d.e.). However, changing the homoallylic substituent from equatorial to axial in the latter alkene significantly increased the selectivity (to 62-64% d.e.). In both instances the major product was shown by X-ray crystallography to possess *S*-configuration at the new chiral centre (C-5) corresponding to an *erythro* relationship about the C-5/C-6 bond. The analogous acetate derivatives also yielded *erythro* adducts preferentially.

Using the six carbon alkene 1,2-dideoxy-3,4:5,6-di-*O*-isopropylidene-D-*arabino*-hex-1-enitol the cycloadditions (30-88% yield) showed poor π-facial selectivity (25-34% d.e.). Although the isoxazolines were chromatographically inseparable, X-ray crystal analysis of the major isomer established that the new chiral centre at C-5 had *R*-configuration, corresponding to an *erythro* relationship about the C-5/C-6 bond. An alternative approach to the same target compound involved addition to methyl 5,6-dideoxy-2,3-*O*-isopropylidene-α-D-*lyxo*-hex-5-enofuranoside. This proved to be more selective (62-76% d.e.) and yielded separable products. The selectivities in both cases were rationalised in terms of the 'inside alkoxy effect'.

Deprotection of the 2-isoxazolines to provide the precursors for the final reductive hydrolytic cleavage occurred with good yields. For the hexulose series a 2-ulosonic acid analogue has been isolated, and its structure established by mass spectrometry and NMR spectroscopy which shows that it exists predominantly in its keto form. A KDO analogue has also been prepared and is mainly in the ring closed hemiketal form. Reductive hydrolytic cleavage of the 2-isoxazolines using Raney-Nickel catalysed hydrogenolysis also provided the desired β-hydroxyketones, along with small quantities of diastereoisomeric mixtures of γ-amino alcohols.

CONTENTS

1 INTRODUCTION

1.1 Foreword 1

1.2 3-Deoxy-2-ulosonic Acids 1

1.3 Nitrile Oxide/Isoxazoline Methodology 4

1.3.1 Nitrile Oxide Cycloaddition Chemistry 4

1.3.1.1 Introduction 4

1.3.1.2 Generation of Nitrile Oxides 6

1.3.1.3 Dimerisation and Rearrangement Reactions 8

1.3.1.4 Cycloaddition to Alkenes and Alkynes 9

1.3.2 Synthetic Utility of Nitrile Oxide-Isoxazoline Chemistry 10

1.4 DAH/DAHP Inhibitors 13

1.4.1 Shikimate Pathway 13

1.4.2 Mechanism for the Conversion of DAHP to DHQ 15

1.4.3 Inhibitors of Dehydroquinate (DHQ) Synthase 17

1.4.5 Syntheses of DAH and Analogues 20

1.4.5.1 Syntheses from Carbohydrates 20

1.4.5.2 Syntheses from Non-Carbohydrates 23

1.4.5.3 Chemoenzymatic Approaches 24

1.4.5.4 Whole Cell Syntheses 27

1.4.5.5 Miscellaneous Appproaches 28

1.5 KDO Inhibitors 30

1.5.1 Introduction 30

1.5.2 Structure of KDO 31

1.5.3 Structure of LPS 32

1.5.4 Biosynthesis of KDO and Incorporation into Lipid A 34

1.5.5 Inhibitors of CMP-KDO Synthetase 37

1.5.5 Syntheses of KDO and Analogues 41

1.5.5.1 Syntheses from Carbohydrates 43

1.5.5.2 Syntheses from Non-Carbohydrates 48

1.5.5.3 Enzymatic Approaches 52

2 RESULTS AND DISCUSSION

2.1 Introduction 54

2.2 Synthesis of Nitrile Oxide Precursors 56

2.3 3-Deoxy-hexulosonic Acid Analogues 58

2.3.1 Synthesis of 1,2-Dideoxy-3,4-*O*-cyclohexylidene-D-*glycero*-but-1-enitol (**195**) 58

2.3.2 Investigation of π-Facial Selectivity in Nitrile Oxide Cycloadditions to Alkene(**195**) 60

2.3.2.1 Nitrile Oxide Cycloadditions to Alkene (**195**) 60

2.3.2.2 Characterisation of Isoxazoline Cycloadducts 63

2.3.2.3 Rationale for Stereoselectivity of Cycloadditions 68

2.3.3 Synthetic Utility of (Diethoxyphosphoryl)acetonitrile 74

Oxide (**216**)

2.3.4 Stereoselective Cycloadditions of Nitrile Oxides to a Dispiroketal-Protected *S*-but-3-ene-1,2-diol (**226**) 76

2.3.4.1 Synthesis of (6*R*,7*R*,14*S*)-14-Ethenyl-1,8,13,16-tetraoxadispiro[5.0.5.4]hexadecane (**226**) 76

2.3.4.2 Nitrile Oxide Cycloadditions to Alkene (**226**) 77

2.3.4.3 X-Ray Crystal Structure of Adduct (**229**) 79

2.3.4.4 Characterisation of Isoxazoline Cycloadducts 81

2.3.5 Selectivity of Nitrile Oxide Cycloadditions to Alkene (**226**) 84

2.3.6 Attempted Cycloadditions of Nitrile Oxides to Trans-3,4-*didehydro*-3,4-*dideoxy*-1,2:5,6-*di*-O-*cyclohexylidene*-D-threo-*hexitol* (**232**) 87

2.3.6.1 Synthesis of Alkene (**232**) 87

2.3.6.2 Attempted Cycloadditions to Alkene (**232**) 88

2.3.7 Reduction of 3-Ethoxycarbonyl Substituent to Hydroxymethyl 89

2.3.8 Conversion of 2-Isoxazolines to 2-Hexulosonic Acid Analogues 90

2.3.8.1 Reductive Hydrolytic Cleavage of 2-Isoxazolines to β-Hydroxyketones 91

2.3.8.2 Reductive Hydrolytic Cleavage of Isoxazolines Followed by Deprotection (Route A) 95

2.3.8.3 Characteristics of β-Hydroxyketones 98

2.3.8.4 Attempted Removal of Cyclohexylidene Protection from β-Hydroxyketone (**244**) 102

2.3.8.5 Deprotection of Isoxazolines Followed by Reductive Hydrolytic Cleavage (Route B) 103

2.3.8.6 Characteristics of *S*-But-3-ene-1,2-diols 104

2.3.8.7 Deduction of Stereochemistry at C-5 in Dispiroketal-protected Isoxazoline (**227**) 108

2.3.8.8 Reductive Hydrolytic Cleavage of 2-Isoxazoline (**249**) 109

2.3.9 Mesylation and Tosylation of 3-Hydroxymethyl-Substituted Isoxazoline (**204**) 114

2.4.10 Conclusions 116

2.4 3-Deoxy-D-*arabino*-heptulosonic Acid (DAH) Analogues 117

2.4.1 Synthesis of 1,2-*Dideoxy*-3,5-O-*ethylidene*-D-erythro-*pent*-1-*enitol* (**260**) 118

2.4.2 Investigation of π-Facial Selectivity in Nitrile Oxide Cycloadditions to Alkene (**260**) 119

2.4.2.1 Nitrile Oxide Cycloadditions to Alkene (**260**) 119

2.4.2.2 Characterisation of Isoxazoline Cycloadducts 121

2.4.3 Conclusion 125

2.4.4 Synthesis of 1,2-*Dideoxy*-4-O-*acetyl*-3,5-O-*ethylidene*-D-erythro-*pent*-1-*enitol* (**271**) 126

2.4.5 Investigation of π-Facial Selectivity in Nitrile Oxide Cycloadditions to Alkene (**271**) 126

2.4.5.1 Nitrile Oxide Cycloadditions to Alkene (**271**) 126

2.4.5.2 Characterisation of Isoxazoline Cycloadducts 129

2.4.6 X-Ray Crystallography to Determine Configuration at New Asymmetric Centre C-5 for Compounds (**263**), (**272**) and (**273**) 133

2.4.7 Synthesis of 1,2-*Dideoxy*-D-erythro-*pent*-1-*enitol* (**276**) 138

2.4.8 Reduction of 3-Ethoxycarbonyl Substituent to Hydroxymethyl 140

2.4.9 Deprotection of 2-Isoxazolines 141

2.4.9.1 Characteristics of Triols 142

2.4.9.2 Deprotection of 2-Isoxazolines Containing an Acetoxy Homoallylic Substituent 145

2.4.9.3 Characteristics of Triols 146

2.4.10 Conclusion 149

2.4.11 Synthesis of 1,2-*Dideoxy*-3,5-O-*benzylidene*-L-threo-*pent*-1-*enitol* (**286**) 150

2.4.12 Investigation of π-Facial Selectivity in Nitrile Oxide Cycloadditions to Alkene (**286**) 151

2.4.12.1 Nitrile Oxide Cycloadditions to Alkene (**286**) 151

2.4.12.2 Characterisation of Isoxazoline Cycloadducts 153

2.4.13 X-Ray Crystal Structure for Adduct (**289**) 155

2.4.14 Conclusions 157

2.5 3-Deoxy-D-*manno*-octulosonic Acid (KDO) Analogues 160

2.5.1 Synthesis of 1,2-Dideoxy-3,4:5,6-di-*O*-isopropylidene-D-*arabino*-hex-1-enitol (**293**) 161

2.5.2 Investigation of π-Facial Selectivity in Nitrile Oxide 163

Cycloadditions to Alkene (**293**)

2.5.2.1 Nitrile Oxide Cycloadditions to Alkene (**293**) 163

2.5.2.2 Characterisation of Isoxazoline Cycloadducts 166

2.5.3 X-Ray Crystal Structure of (**299**) 169

2.5.4 Reduction of 3-Ethoxycarbonyl Substituent to Hydroxymethyl 171

2.5.5 Deprotection of 2-Isoxazolines (**305**)/(**306**) 172

2.5.5.1 Characteristics of Deprotected Adducts (**307**)/(**308**) and (**309**)/(**310**) 174

2.5.6 Reductive Hydrolytic Cleavage of 2-Isoxazolines (**305**)/(**306**) 177

2.5.6.1 Characteristics of β-Hydroxyketones 178

2.5.7 Mesylation of 3-Hydroxymethyl-Substituted Isoxazoline (**305**)/(**306**) 180

2.5.8 Conclusion 181

2.5.9 Alternative Approach to KDO Analogues 182

2.5.10 Synthesis of Methyl 5,6-Dideoxy-2,3-*O*-isopropylidene-α-D-*lyxo*-hex-5-enofuranoside (**317**) 183

2.5.10.1 Cycloaddition to Methyl 5,6-Dideoxy-2,3-*O*-isopropylidene-α-D-*lyxo*-hex-5-enofuranoside (**317**) 184

2.5.10.2 Characterisation of Isoxazoline Cycloadducts 185

2.5.11 Reduction of 3-Ethoxycarbonyl Substituent to Hydroxymethyl 188

2.5.12 Deprotection of 2-Isoxazolines 189

2.5.12.1 Characterisation of Deprotected Compounds 191

2.5.13 Ring Opening of Furanose 194

2.5.14 Reductive Hydrolytic Cleavage of 2-Isoxazolines 196

2.5.14.1 Characteristics of β-Hydroxyketones 197

2.5.15 Reductive Hydrolytic Cleavage of 2-Isoxazoline (**307**) 200

2.5.16 Mesylation of 3-Hydroxymethyl-Substituted Isoxazoline (**327**) 204

2.5.17 Modification of Side Chain at C-3 of 2-Isoxazoline 205

2.5.17.1 Reduction of 3-Ethoxycarbonyl Substituent to Aldehyde 205

2.5.17.2 Oxidation of Alcohols (**327**) and (**328**) to Aldehydes (**344**) and (**345**) 206

2.5.18 Synthesis of Aldehyde Derivatives 207

2.5.19 Conclusions 209

2.6 Conclusions and Future Work 211

3.0 EXPERIMENTAL

3.1 General 214

3.1.1 Instrumentation 214

3.1.2 Chromatography 215

3.1.3 Solvents and Reagents 215

3.2 Synthesis of Nitrile Oxide Precursors 216

3.2.1 Ethyl Chloro-oximinoacetate (**184**) 216

3.2.2 Benzohydroximoyl Chloride (**185**) 216

3.2.3 (Diethoxyphosphoryl)acetohydroximoyl Chloride (**189**) 217

3.3 Synthesis of Sugar Alkenes 220

3.3.1 1,2-Dideoxy-3,4-*O*-cyclohexylidene-D-*glycero*-but-1-enitol (**195**) 220

3.3.2 1,2-*Dideoxy*-3,5-O-*ethylidene*-D-erythro-*pent*-1-*enitol* (**260**) 223

3.3.3 1,2-*Dideoxy*-3,5-O-*benzylidene*-L-threo-*pent*-1-*enitol* (**286**) 227

3.3.4 1,2-Dideoxy-3,4:5,6-di-*O*-isopropylidene-D-*arabino*-hex-1-enitol (**293**) 230

3.3.5 Methyl 5,6-dideoxy-2,3-*O*-isopropylidene-α-D-*lyxo*-hex-5-enofuranoside (**317**) 235

3.3.6 (6*R*,7*R*,14*S*)-14-Ethenyl-1,8,13,16-tetraoxadispiro[5.0.5.4]-hexadecane (**226**) 238

3.3.7 Trans-3,4-*didehydro*-3,4-*dideoxy*-1,2:5,6-*di*-O-*cyclohexylidene*-D-threo-*hexitol* (**232**) 239

3.4 Nitrile Oxide Cycloadditions 242

3.4.1 Cycloadditions to 1,2-Dideoxy-3,4-*O*-cyclohexylidene-D-*glycero*-but-1-enitol (**195**) 243

3.4.2 Cycloadditions to 1,2-*Dideoxy*-3,5-O-*ethylidene*-D-erythro-*pent*-1-*enitol* (**260**) 251

3.4.3 Cycloadditions to 1,2-*Dideoxy*-3,5-O-*benzylidene*-L-threo-*pent*-1-*enitol* (**286**) 257

3.4.4 Cycloadditions to 1,2-*Dideoxy*-4-O-*acetal*-3,5-O-*ethylidene*-D-erythro-*pent*-1-*enitol* (**271**) 261

3.4.5 Cycloadditions to 1,2-*Dideoxy*-D-erythro-*pent*-1-*enitol* (**276**) 265

3.4.6 Cycloadditions to 1,2-Dideoxy-3,4:5,6-di-*O*-isopropylidene-D- 266

arabino-hex-1-enitol (**298**)

3.4.7 Cycloadditions to Methyl 5,6-dideoxy-2,3-*O*-isopropylidene-α-D-*lyxo*-hex-5-enofuranoside (**317**) 271

3.4.8 Cycloadditions to (6*R*,7*R*,14*S*)-14-Ethenyl-1,8,13,16-tetra-oxadispiro-[5.0.5.4]hexadecane (**226**) 274

3.4.9 Cycloadditions to trans-3,4-*Didehydro*-3,4-*Dideoxy*-1,2:5,6-*di*-O-*cyclohexylidene*-D-threo-*hexitol* (**232**) 278

3.5 Synthesis of Acetate Derivatives 279

3.5.1 1,2-*Dideoxy*-4-O-*acetyl*-3,5-O-*ethylidene*-D-erythro-*pent*-1-*enitol* (**271**) 279

3.5.2 5S-5-(2-O-*Acetyl*-1,3-O-*ethylidene*-D-erythro-*triitol*-1-*yl*)-3-*ethoxycarbonyl*-2-*isoxazoline* (**272**) 279

3.5.3 5R-5-(2-O-*Acetyl*-1,3-O-*ethylidene*-D-erythro-*triitol*-1-*yl*)-3-*ethoxycarbonyl*-2-*isoxazoline* (**273**) 280

3.5.4 5S-5-(2-O-*Acetyl*-1,3-O-*ethylidene*-D-erythro-*triitol*-1-*yl*)-3-*phenyl*-2-*isoxazoline* (**274**) and 5R-5-(2-O-*acetyl*-1,3-O-*ethylidene*-D-erythro-*triitol*-1-*yl*)-3-*phenyl*-2-*isoxazoline* (**275**) 281

3.6 Reduction of Ethyl Ester to Alcohol 282

3.6.1 5S-5-(1,2-O-*Cyclohexylidene*-D-glycero-*diitol*-1-*yl*)-3-*hydroxy-methyl*-2-*isoxazoline* (**204**) 282

3.6.2 5R-5-(1,2-O-*Cyclohexylidene*-D-glycero-*diitol*-1-*yl*)-3-*hydroxy-methyl*-2-*isoxazoline* (**205**) 282

3.6.3 5S-5-(1,3-O-*Ethylidene*-D-erythro-*triitol*-1-*yl*)-3-*hydroxy*- 284

methyl-2-*isoxazoline* (**269**)

3.6.4 5R-5-(1,3-O-*Ethylidene*-D-erythro-*triitol*-1-*yl*)-3-*hydroxy*- 284
methyl-2-*isoxazoline* (**270**)

3.6.5 5R-5-(2,3-O-*Isopropylidene*-O-*methyl* α-D-lyxo-*tetro*- 286
furanos-4-*yl*)-3-*hydroxymethyl*-2-*isoxazoline* (**327**)

3.6.6 5S-5-(2,3-O-*Isopropylidene*-O-*methyl*-α-D-lyxo-*tetro*- 286
furanos-4-*yl*)-3-*hydroxymethyl*-2-*isoxazoline* (**328**)

3.6.7 5R-5-(1,2:3,4-*di*-O-*Isopropylidene*-D-arabino-*tetritol*-1-*yl*)- 288
3-*hydroxymethyl*-2-*isoxazoline* (**305**) and 5S-5-(1,2:3,4-*di*-O-
isopropylidene-D-arabino-*tetritol*-1-*yl*)-3-*hydroxymethyl*-
2-*isoxazoline* (**306**)

3.7 Deprotection of Isoxazolines 290

3.7.1 - 3.7.6 (1,2-O-*Cyclohexylidene*-D-glycero-*diitol*-1-*yl*)- 290
protected-isoxazolines (**196**) - (**199**), (**204**) and (**205**)

3.7.7 1,2-*Dideoxy*-3,5-O-*ethylidene*-D-erythro-*pent*-1-*enitol* (**260**) 295

3.7.8 - 3.7.12 (1,3-*O-Ethylidene*-D-erythro-*triitol*-1-*yl*)-protected- 296
isoxazolines (**263**) - (**265**), (**269**) and (**270**)

3.7.13 - 3.7.14 (2-O-*Acetyl*-1,3-O-*ethylidene*-D-erythro-*triitol*-1-*yl*)- 300
protected isoxazolines (**272**) and (**273**)

3.7.15 5S/R-5-(1,3-O-*ethylidene*-D-erythro-*triitol*-1-*yl*)-3-(*dideoxy*- 302
phosphorylmethyl)-2-*isoxazolines* (**267**)/(**268**)

3.7.16 5R-5-(1,2:3,4-*Di*-O-*isopropylidene*-D-arabino-*tetritol*-1-*yl*)-3- 303
hydroxymethyl-2-*isoxazoline* (**305**) and 5S-5-(1,2:3,4-*di*-O-

isopropylidene-D-arabino-*tetritol*-1-*yl*)-3-*hydroxymethyl*-2-*isoxazoline* (**306**)

3.7.17 5R-5-(1,2:3,4-Di-O-*isopropylidene*-D-arabino-*tetritol*-1-*yl*)-3-*hydroxymethyl*-2-*isoxazoline* (**305**) and 5S-5-(1,2:3,4-*di*-O-*isopropylidene*-D-arabino-*tetritol*-1-*yl*)-3-*hydroxymethyl*-2-*isoxazoline* (**306**) 306

3.7.18 5S-3-*Ethoxycarbonyl*-5-(6R,7R,14S-1,8,13,16-*tetraoxadispiro*-[5.0.5.4]*hexadecan*-14-*yl*)-4,5-*dihydroisoxazole* (**227**) 306

3.8 Acid Hydrolysis followed by Reduction 308

3.8.1 5R-5-(1-O-*Methyl*-α-D-lyxo-*tetrofuranoside*-4-*yl*)-3-*hydroxy*-*methyl*-2-*isoxazoline* (**329**) 308

3.8.2 5R-5-(α-D-lyxo-*tetrofuranoside*-4-*yl*)-3-*hydroxymethyl*-2-*isoxazoline* (**331**) 308

3.8.3 5R-5-(D-arabino-*tetritol*-1-*yl*)-3-*hydroxymethyl*-2-*isoxazoline* (**307**) 309

3.8.4 5S-5-(D-arabino-*tetritol*-1-*yl*)-3-*hydroxymethyl*-2-*isoxazoline* (**308**) 310

3.8.5 5R-5-(1-O-*methyl*-α–D-lyxo-*tetrofuranoside*-4-*yl*)-3-*phenyl*-2-*isoxazoline* (**330**) 310

3.9 Mesylation 312

3.9.1 5S-5-(1,2-O-*Cyclohexylidene*-D-glycero-*diitol*-1-*yl*)-3-*methanesulphonyloxymethyl*-2-*isoxazoline* (**258**) 312

3.9.2 5R-5-(1,2:3,4-*Di*-O-*isopropylidene*-D-arabino-*tetritol*-1-*yl*)- 313

3-*methanesulphonyloxymethyl*-2-*isoxazoline* (**315**) and
5R-5-(1,2:3,4-*di*-O-*isopropylidene*-D-arabino-*tetritol*-1-*yl*)-3-*methanesulphonyloxymethyl*-2-isoxazoline (**316**)

3.9.3 5R-5-(2,3-O-*Isopropylidene*-1-O-*methyl*-α-D-lyxo-*tetrofuranoside*-4-*yl*)-3-*methanesulphonyloxymethyl*-2-*isoxazoline* (**315**) 314

3.10 Tosylation 314

3.10.1 5S-5-(1,2-O-*Cyclohexylidene*-D-glycero-*diitol*-1-*yl*)-3-p-*toluenesulphonyloxymethyl*-2-*isoxazoline* (**259**) 314

3.11 Reduction of Ester to Aldehyde 315

3.11.1 5R-5-(2,3-O-*Isopropylidene*-1-O-*methyl*-α-D-lyxo-*tetrofuranoside*-4-*yl*)-3-*formyl*-2-*isoxazoline* (**344**) 315

3.12 Oxidation of Alcohol to Aldehyde 316

3.12.1 5R-5-(2,3-O-*Isopropylidene*-1-O-*methyl*-α-D-lyxo-*tetro-furanoside*-4-*yl*)-3-*formyl*-2-*isoxazoline* (**344**) 316

3.12.2 5S-5-(2,3-O-*Isopropylidene*-1-O-*methyl*-α-D-lyxo-*tetro-furanoside*-4-*yl*)-3-*formyl*-2-*isoxazoline* (**345**) 317

3.13 Synthesis of Aldehyde Derivatives 318

3.13.1 5R-5-(2,3-O-*Isopropylidene*-1-O-*methyl*-α-D-lyxo-*tetro-furanos*-4-*yl*)-*eth*-3-*enyl*-2-*isoxazoline* (**346**) 318

3.13.2 5R-5-(2,3-O-*Isopropylidene*-1-O-*methyl*-α-D-lyxo-*tetro-furanos*-4-*yl*)-*eth*-3-*enyl*-(2-*ethoxycarbonyl*)-2-*isoxazoline* (**347**) 318

3.13.3 5R-5-(2,3-O-*Isopropylidene*-1-O-*methyl*-α-D-lyxo-*tetro-furanos*-4-*yl*)-3-*aldoximino*-2-*isoxazoline* (**348**) 319

3.14 Reductive Hydrolytic Cleavage of 2-Isoxazolines to β-Hydroxyketones 322

3.14.1 Raney-Nickel Induced Hydrogenolysis of 2-Isoxazolines 322

3.14.2 Palladium-on-Charcoal Catalysed Hydrogenolysis of 2-Isoxazolines 327

3.15 Reductive Hydrolytic Cleavage of 2-Isoxazolines to 2-Uloses 329

3.15.1 To 1-*Phenyl*-2-*deoxy*-D-*xylose* (**255**) 329

3.15.2 To 3-*deoxy*-D-manno-2-*octulose* (**339**) 330

Appendix 1 : DHQ Synthase Inhibitors 332

Appendix 2 : CMP-KDO Inhibitors 336

Appendix 3 : π-Facial Selectivities for Nitrile Oxide Cycloadditions 345

References 346

1 INTRODUCTION

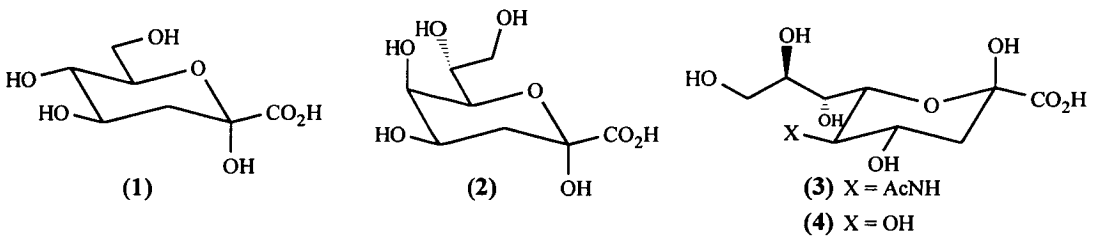
1.1 Foreword

The subject of this thesis is the application of nitrile oxide-isoxazoline methodology to accomplish the key synthetic steps in the synthesis of 2-ulosonic acid analogues. In the introduction three topics are discussed. First a section describing the importance of 3-deoxy-2-ulosonic acids; then an outline of nitrile oxide-isoxazoline chemistry and rationalising its choice for the present work; and finally reviews of 3-deoxy-D-*arabino*-2-heptulosonic acid (DAH) (**1**) and 3-deoxy-D-*manno*-2-octulosonic acid (KDO) (**2**), including their mode of action, analogues previously synthesised, and recent approaches to their construction.

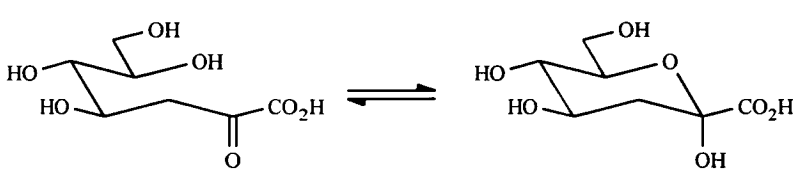
1.2 3-Deoxy-2-ulosonic Acids

Higher 3-deoxy-2-ulosonic acids containing seven, eight and nine carbons are widely diffuse natural carbohydrates which participate in various important biological processes. For example the 7-phosphate of the seven-carbon compound 3-deoxy-D-*arabino*-2-heptulosonic acid (DAH) (**1**) is a key intermediate in the biosynthesis *via* the shikimate pathway of aromatic amino acids from glucose in plants and bacteria but not in mammals;[1] the eight-carbon analogue 3-deoxy-D-*manno*-2-octulosonic acid (2-keto-3-deoxy-D-*manno*-octonic acid) (KDO) (**2**) occurs in the lipopolysaccharide region of the cell surface of all Gram-negative bacteria and is an essential component for their replication;[2,3] and the nine-carbon compound *N*-acetyl-5-amino-3,5-dideoxy-D-*glycero*-D-*galacto*-2-nonulosonic acid (*N*-acetyl-neuraminic acid, Neu5Ac or NANA) (**3**) is a sialic acid (aminononulosonic acid)

which is found at the terminal positions of glycolipids, glycoproteins, and oligosaccharides, often in nerve tissues and cellular membranes of various living organisms.[4] These sialyl conjugates play an important role in biological molecular recognition processes such as differentiation phenomena and cell adhesion.[5] The 5-deamino compound 3-deoxy-D-*glycero*-D-*galacto*-2-nonulosonic acid (KDN) (**4**) also occurs naturally, but is less common; it was isolated in 1986 from rainbow trout egg polysialoglycoprotein and is believed to play a role in activation by protecting against the action of sialidase.[6]



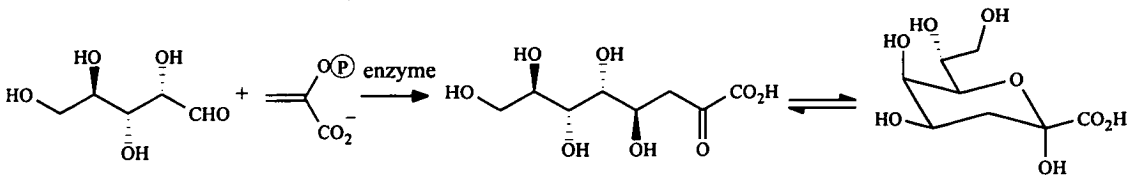
3-Deoxy-2-ulosonic acids exist in both open chain and cyclic hemiketal forms in the same way as the simple sugar D-fructose. The pyranose form results from intramolecular nucleophilic addition of the hydroxyl group at C-6 to the C-2 ketone group (Scheme 1). The CO_2H group occupies the equatorial position.



Scheme 1

The biosynthesis of sialic and ulosonic acids and particularly DAH, KDO, Neu5Ac and KDN, is thought to involve the stereoselective aldol condensation of

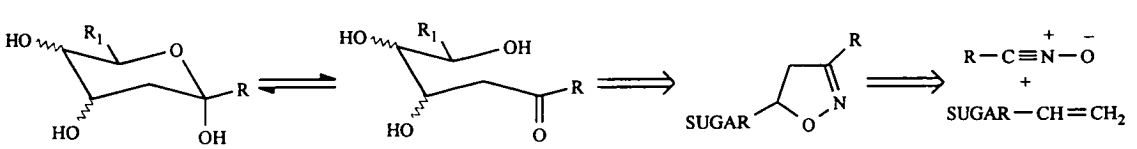
aldoses with phosphoenol pyruvate catalysed by the appropriate aldolase enzyme.[2,4] For example, Scheme 2 shows the biosynthesis of KDO from D-arabinose.



(2)

Scheme 2

Much effort has been devoted to the isolation, characterisation and preparation of these unusual carbohydrates and to investigating their biological functions. As these compounds are potential herbicidal and antibacterial agents, many synthetic studies are being carried out throughout the world, with particular interest directed towards the development of short and efficient syntheses of DAH and KDO analogues as specific enzyme inhibitors. Most approaches to 3-deoxy-2-ulosonic acids have made use of readily accessible carbohydrate precursors[7] such as D-glucose (DAH),[8] D-arabinose (KDO),[9] D-glyceraldehyde (KDO),[10] D-mannose (KDO)[11] and D- or L-mannose (KDN).[7] Our retrosynthetic strategy to 3-deoxy-2-ulosonic acids is shown in Scheme 3.



Scheme 3

1.3 Nitrile Oxide/Isoxazoline Methodology

The principal aim of the work is to develop a general route from readily available precursors which is appropriate for the whole series of 2-ulosonic acids and which is sufficiently flexible to provide access to a wide range of analogues with control of stereochemistry. Nitrile oxide cycloaddition methodology is considered to be well suited for the construction of 2-ulosonic acids, in particular DAH and KDO.

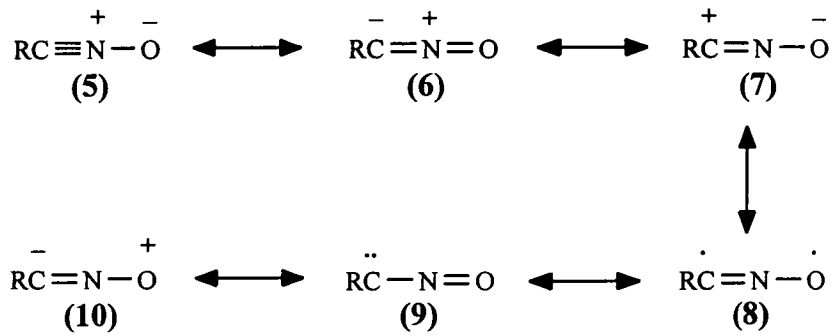
1.3.1 Nitrile Oxide Cycloaddition Chemistry

1.3.1.1 Introduction

The concept of 1,3-dipolar cycloadditions for the construction of five-membered ring heterocycles was developed by Huisgen[12,13] in the 1960's, and since then much work has been performed in this area. Due to the immensity of this field, 1,3-dipolar cycloaddition chemistry will not be reviewed extensively. Instead a brief overview of the properties of nitrile oxides and their reactions will be presented. Further details can be obtained from Padwa's comprehensive monograph '1,3-Dipolar Cycloaddition Chemistry',[13] Grundmann and Grünanger's text 'The Nitrile Oxides',[14] Torssell's more recent book 'Nitrile Oxides, Nitrones and Nitronates in Organic Synthesis'[15] and a recent review article entitled 'Recent Advances in Synthetic Applications on Nitrile Oxide Cycloadditions'[16] by Kanemasa and Tsuge.

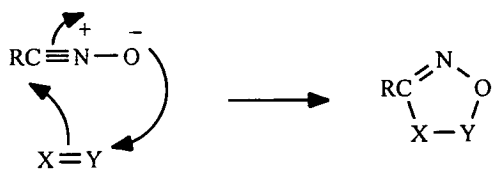
Nitrile oxides are members of the nitrilium betaine family of 1,3-dipoles and exist as a three atom four π-electron system. The most common representation of a nitrile oxide is that of a zwitterionic octet structure of the linear propargyl-allenyl type (**5**) which contains an orthogonal π-bond. They can be represented as a resonance

hybrid of the structures (**5**)-(**10**) shown in Scheme 4. The main contributions in the ground state are the octet structures (**5**) and (**6**).



Scheme 4

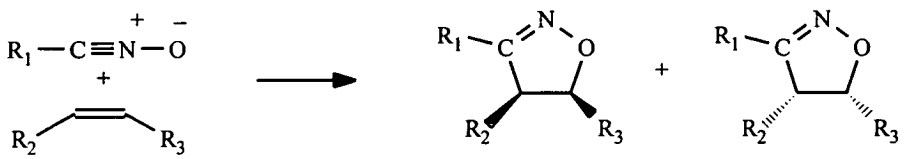
The most significant synthetic reaction of nitrile oxides is their [3+2] cycloaddition to dipolarophiles to form five-membered heterocyclic rings (Scheme 5). A range of multiple bonds (X=Y, X≡Y) have been utilised including C=C, C≡C, C=N, C≡N and C=O.



Scheme 5

There has been much controversy regarding the mechanism of nitrile oxide cycloadditions. The most widely accepted mechanism involves a concerted,[19,20] but not necessarily synchronous, one-step process proposed by Huisgen.[12,17,18] 1,3-Dipolar cycloaddition reactions to asymmetric dipolarophiles are stereospecific with formation of four possible stereoisomers, two stereoisomers for each regioisomer (Scheme 6). Stereoelectronic factors normally control the regioselectivity with the

least hindered side of the dipolarophile being favoured by the approaching dipole. Hence, often only two stereoisomers are observed. Retention of dipolarophile stereochemistry is maintained in the hererocyclic adduct.

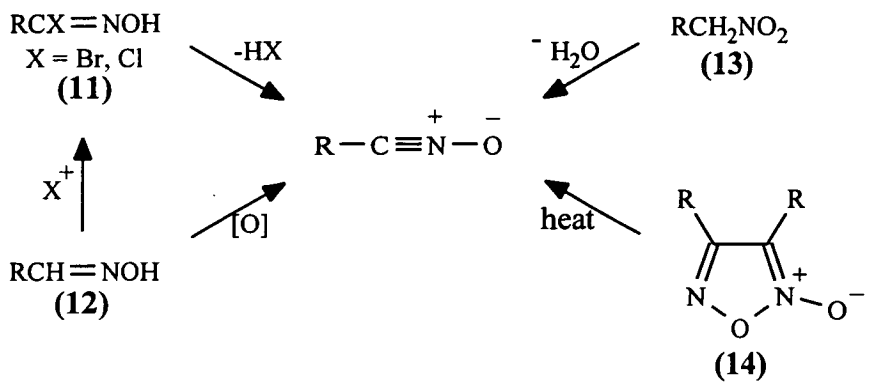


Scheme 6

The observed reactivities and regioselectivities have been rationalised by Sustmann[21,22] using Frontier Molecular Orbital (FMO) theory, in which the interactions of the dipole and dipolarophile are classified into three cases depending on whether the reaction is under dipole HOMO or dipole LUMO control (Sustmann Type I or III respectively) or a combination of both (Type II). Nitrile oxide FMO interactions are normally dipole LUMO controlled (Type III). The regioselectivity of nitrile oxide cycloadditions can be predicted by FMO theory with the major regioisomer being formed from maximum overlap of like orbitals.[23] Steric factors can also influence the relative abundance of each stereoisomer.

1.3.1.2 Generation of Nitrile Oxides

Nitrile oxides are reactive species and have a tendency to dimerise [Section 1.3.1.3], and so they are usually generated slowly *in situ* at low concentration in the presence of excess dipolarophile, thus minimising the competing formation of furoxans (furazan *N*-oxides). The routes normally employed for their preparation are summarised in Scheme 7.



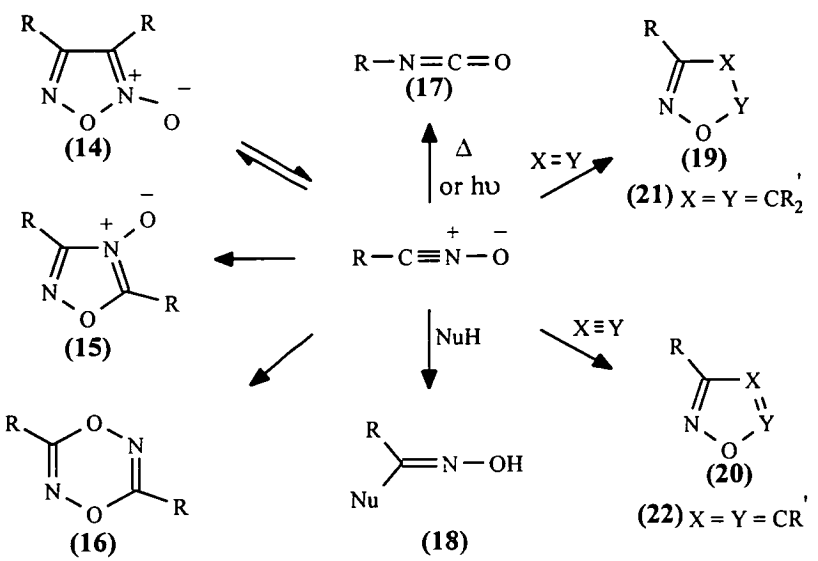
Scheme 7

One of the most common methods is that developed by Huisgen *et al*[24] which involves triethylamine-mediated, and recently potassium fluoride[25] or thermally-induced,[26,27] dehydrohalogenation of hydroximoyl halides (**11**). These are readily accessible from the reaction of aldoximes (**12**) with halogens[28] or nitrosyl chloride,[29] or by milder *in situ* treatment with *N*-bromosuccinimide (NBS)[30] or *N*-chlorosuccinimide (NCS).[31] One-pot generation of nitrile oxides *via* their hydroximoyl halides has also been accomplished using alkaline sodium hypochlorite,[32] hypobromite[33] and chloramine-T.[34] Lead tetraacetate has been used for direct dehydrogenation of *syn* aldoximes[35] (**12**).

An equally important and useful alternative for the generation of nitrile oxides is the Mukaiyama[36] method involving dehydration of a primary nitro compound (**13**) using a catalytic amount of triethylamine and an isocyanate as a dehydrating agent. Access to nitrile oxides is also provided by the thermal cycloreversion[37-39] of furoxan dimers (1,2,5-oxadiazole-2-oxides) (**14**).

1.3.1.3 Dimerisation and Rearrangement Reactions (Scheme 8)

In the absence of a dipolarophile, nitrile oxides usually dimerise to yield furazan *N*-oxides (**14**).[40,41] These dimers are often also formed as by-products in cycloaddition reactions. The rate of dimerisation is variable and depends on the electronic nature and steric bulk of the R substituent. Electron deficient analogues are prone to rapid dimerisation; eg. at room temperature the half-life varies from seconds to minutes for a neat sample of an aliphatic nitrile oxide, whereas many sterically hindered nitrile oxides can be isolated and stored indefinitely.[14] Dimers such as 1,2,4-oxadiazole-4-oxide (**15**) and 1,4,2,5-dioxadiazine (**16**) are also sometimes formed.



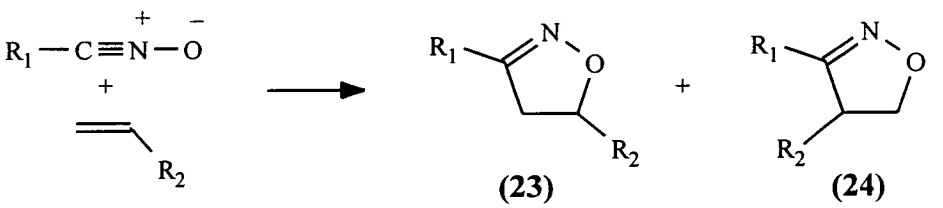
Scheme 8

Other reactions competing with cycloaddition include thermal and photochemical rearrangements to isocyanates[14] (**17**) and 1,3-addition using nucleophiles (**18**).

1.3.1.4 Cycloaddition to Alkenes and Alkynes (Scheme 8)

Nitrile oxides are sufficiently reactive to undergo cycloaddition to a wide range of dipolarophiles affording five membered heterocycles containing the C=N-O unit, (**19**) and (**20**). Of direct relevance to the work being discussed is the cycloaddition to an alkene yielding 2-isoxazolines (4,5-dihydroisoxazoles) (**21**). The corresponding reaction with alkynes affords isoxazoles (**22**).

Cycloaddition to most monosubstituted and 1,1-disubstituted alkenes occurs regiospecifically yielding the 5-substituted- (**23**) (Scheme 9) and 5,5-disubstituted-2-isoxazolines respectively. The reaction is regiospecific when the dipolarophile contains electron donating or conjugating substituents, eg. hex-1-ene or styrene. However, the presence of a strong electron-withdrawing group results in a mixture of regioisomers (**23**) and (**24**).[42]



(**23**) (**24**)

R_1	R_2	Ratio (23) : 24)
Ph	Bu	100 : 0
Ph	Ph	100 : 0
Ph	CO_2CH_3	95 : 5

Scheme 9

1,2-disubstituted alkenes undergo cycloaddition with complete retention of dipolarophile stereochemistry to produce a mixture of regioisomers. More highly substituted alkenes show decreased reactivity. Sustmann Type III [LUMO (dipole)]

controlled reactions become more Sustmann Type II [HOMO + LUMO (dipole)] for dipolarophiles containing electron withdrawing substituents or dipoles with electron donating groups and this explains the differences in reactivity observed.

1.3.2 Synthetic Utility of Nitrile Oxide-Isoxazoline Chemistry

For many years the 1,3-dipolar cycloaddition reactions of nitrile oxides have been used for the synthesis of 5-membered heterocycles incorporating the C=N-O unit, but it is only in the last decade that their potential for natural product synthesis has been realised.[15,16,43-45] Their utility stems from their ease of generation under mild conditions from readily accessible precursors and the regio- and stereo-chemical control of their cycloaddition reactions with alkenes. The well established chemistry of the resulting 2-isoxazoline cycloadducts[43-45] permits rapid accumulation of polyfunctionality in a small molecular framework, and this methodology has been used successfully for the preparation of a variety of natural products including prostaglandins,[46] terpenoids,[47] and various alkaloids[43] and carbohydrates.[45] The use of nitrile oxides in synthesis has been reviewed by Jäger,[44] Kozikowski,[43] Curran[45] and Torssell.[15]

Three basic steps are involved in the nitrile-oxide/isoxazoline route to natural products and analogues. The first is the 1,3-dipolar cycloaddition with predictable regio- and stereochemistry of a nitrile oxide to an alkene. The resulting 2-isoxazoline can be modified if required. Isoxazolines are stable ring structures, and this allows a range of transformations to be performed on the ring and/or side chains by appropriate reagents without disruption of the heterocycle. The final stage involves ring cleavage of the isoxazoline at the N-O bond to reveal the target functionalities.[43]

Modification of a 3,5-substituted isoxazoline ring at the 4-position can be effected by a method developed by Jäger[48,49] which involves deprotonation using lithium di-isopropylamide (LDA) or butyllithium at -78°C, and reaction of the resulting carbanion with an electrophile to produce stereoselectively the 4-substituted isoxazoline.

Cleavage of the isoxazoline N-O bond has been reported by Curran,[45] Jäger[44] and Kozikowski[43] and can lead to a number of acyclic products, depending on the reaction conditions (Scheme 10). Reductive hydrolytic cleavage[45] releases the latent β-hydroxyketone functionality and is an alternative to the classical aldol condensation. It also avoids the problems normally encountered with aldol reactions : reversibility, cross and self-aldol side reactions, lack of stereocontrol and selective enolate formation. The two approaches produce different, but complementary C-C bonds (Figure 1).

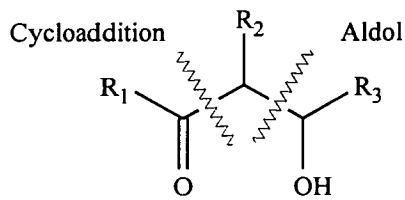
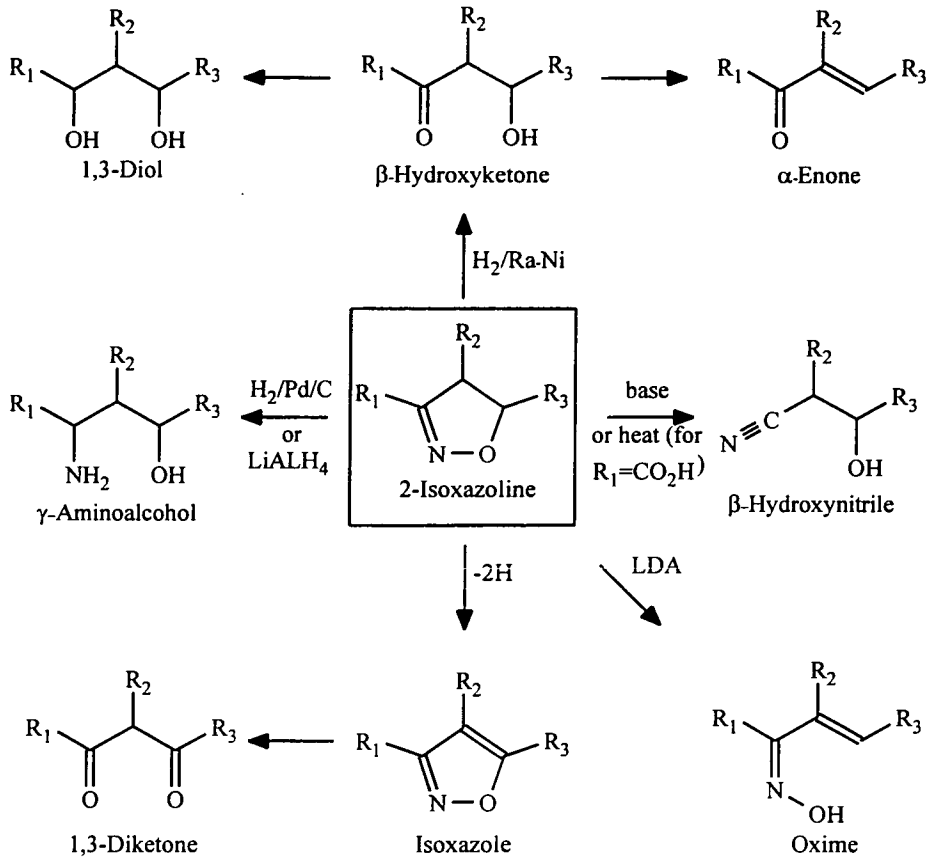


Figure 1

The most widely used procedure for ring cleavage of isoxazolines is catalytic hydrogenation[45] with a palladium-on-charcoal or Raney-nickel catalyst under aqueous methanolic conditions. Alternative reagents include titanium trichloride,[50] molybdenum hexacarbonyl[51] and ozone.[52] The β-hydroxyketone can then provide access to 1,3-diols by reduction, or α-enones by dehydration.



Scheme 10

A γ-aminoalcohol is another functionality that can be unmasked from isoxazolines. This is accomplished using lithium aluminium hydride (LAH)[43] reduction or non-aqueous catalytic hydrogenation.[53] The reaction is stereoselective, although the extent of this diastereoselectivivty is dependent on the substituents on the isoxazoline ring. LAH reduction is more stereoselective due to complexation with the nitrogen of the isoxazoline ring.

Other functionalities can also be unmasked. Oxidation of 2-isoxazolines to isoxazoles,[54] and subsequent ring opening provides access to 1,3-diketones and β-keto nitriles. The presence of base releases β-hydroxynitriles from 3-unsubstituted isoxazolines,[55] as does thermal decarboxylation[56] of isoxazoline 3-carboxylic acids.

1.4 DAH/DAHP Inhibitors

1.4.1 Shikimate Pathway

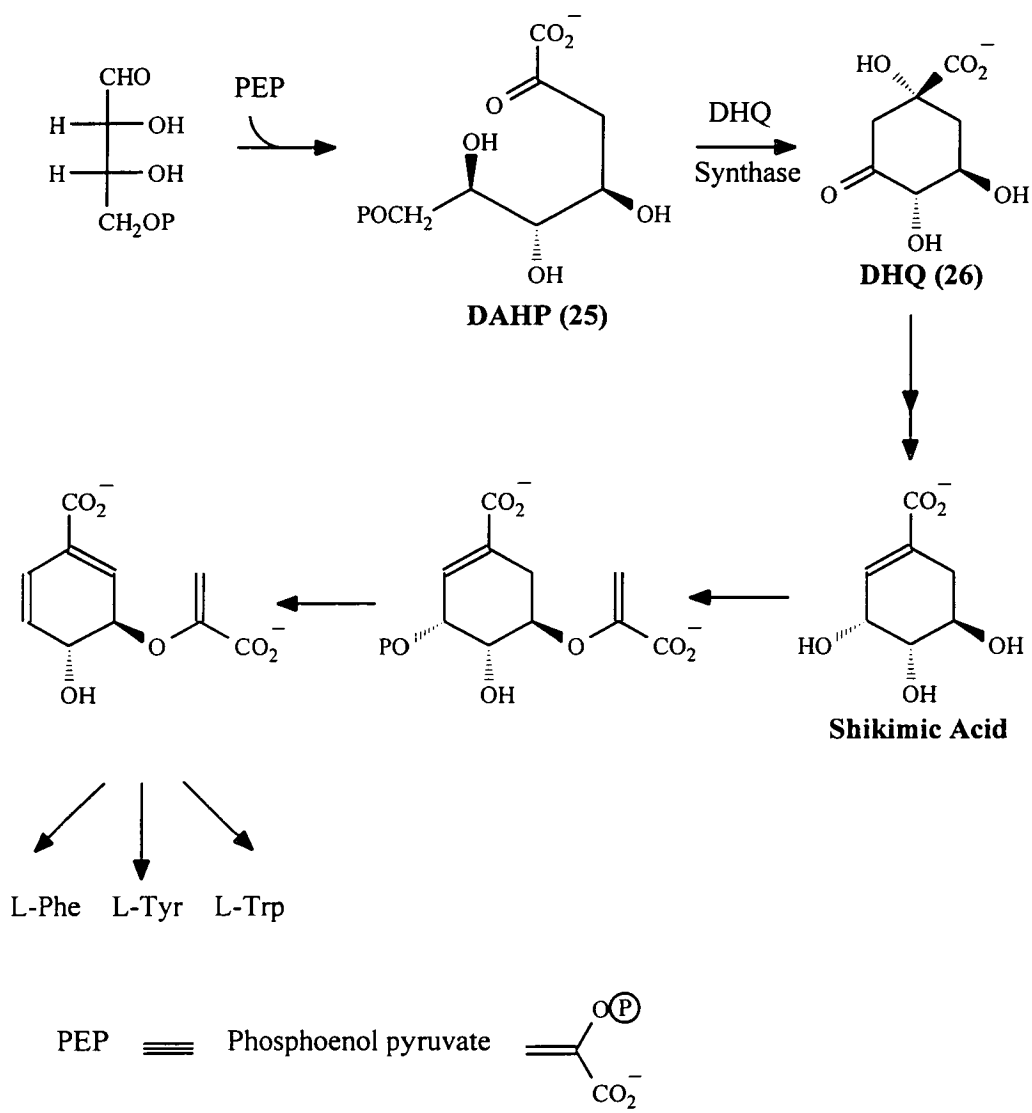
The shikimate pathway (Scheme 11) is a major biosynthetic pathway in plants and microorganisms[1,57,58] and is responsible for the production of a range of important primary and secondary metabolites, including the three aromatic amino acids L-phenylalanine (L-Phe), L-tyrosine (L-Tyr) and L-tryptophan (L-Trp). These aromatic compounds are essential for the growth of these hosts, but cannot be synthesised *de novo* by animals.

In prokaryotes and plants, 3-deoxy-D-*arabino*-heptulosonate 7-phosphate (DAHP), a seven-carbon acid, is produced from phosphoenol pyruvate (PEP) and erythrose 4-phosphate, the condensation of which is mediated by DAHP synthase.[59] DAHP is present at the beginning of the enzyme-catalysed cascade that starts with this seven-carbon carbohydrate and ends with the amino acids. It is an important intermediate, standing at the crossover point from the sugar phosphates to the cyclitols that eventually are transformed into the aromatic amino acids.

The conversion of the keto-acid DAHP to the six-membered cyclic intermediate (DHQ), the first carbocyclic metabolite in the pathway is catalysed by DHQ synthase. The chemical mechanism for this process is one of the more fascinating steps in the shikimate pathway,[60-62] and will be discussed briefly later. Subsequently shikimic acid is obtained in two steps from DHQ. This then reacts further with PEP to eventually produce the desired aromatic acids.

The rational design of herbicidal chemicals has been influenced by recognition that plant death can result from chemical disruption of the aromatic amino acid biosynthesis. For years, the synthesis of large numbers of chemical

compounds followed by screening led the way for the discovery of new herbicides. Thus the development of specific inhibitors for the shikimate pathway (not found in animals) offers a non-toxic approach to potential herbicides as well as antimicrobial agents. A convenient synthetic route to DAHP could provide access to structural analogues of DAHP and strategies for achieving potent inhibition of DHQ synthase might also serve as examples for inhibition of other closely related and medicinally important enzymes.



Scheme 11 : Shikimate Pathway

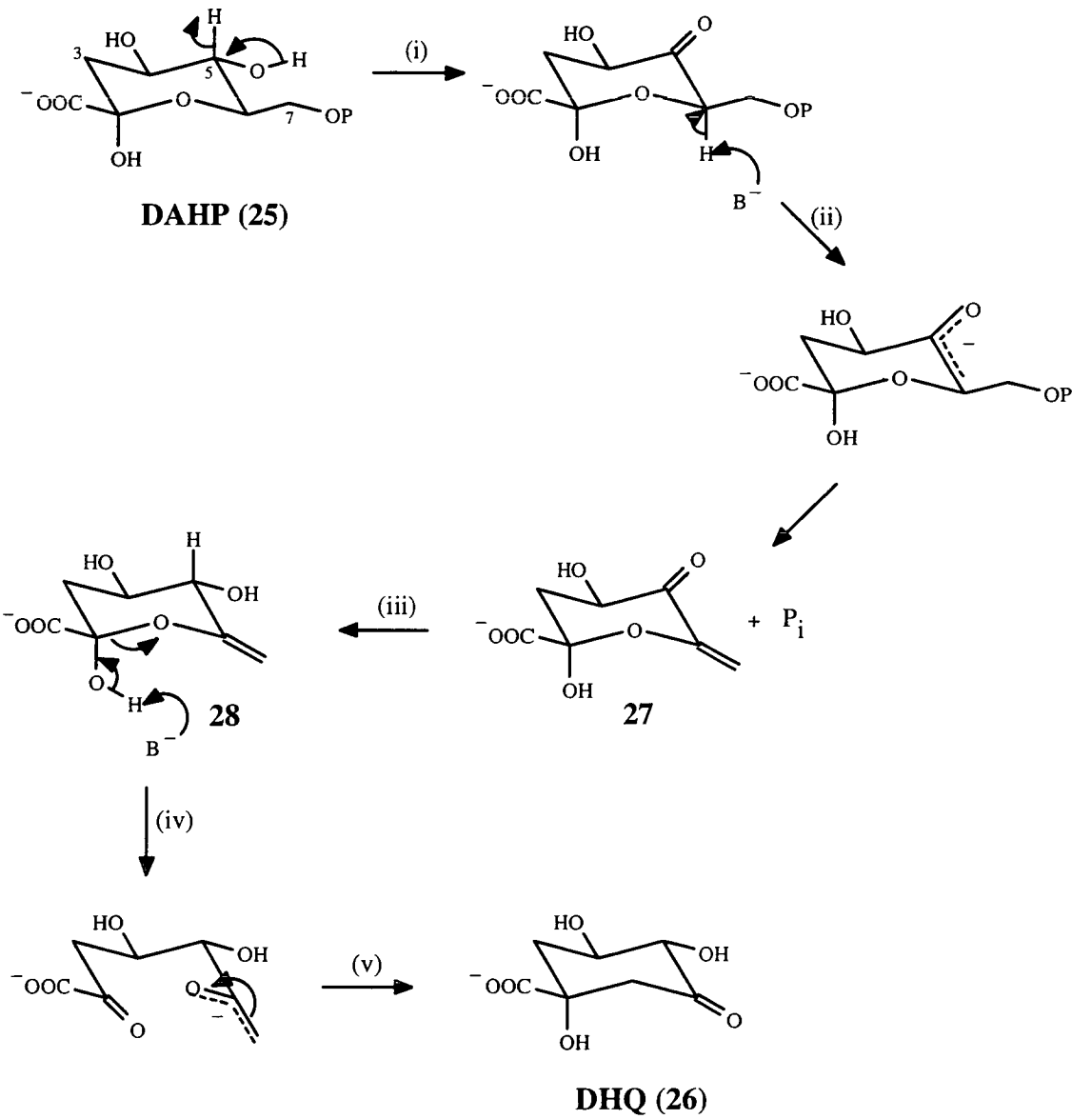
1.4.2 Mechanism for the Conversion of DAHP to DHQ

The proposed pathway for catalytic conversion of DAHP (**25**) to DHQ (**26**) is shown in Scheme 12. In this sequence, which is mechanistically diverse for a single enzyme, the DHQ synthase[60] is involved in five transformations: (i) NAD^+ mediated oxidation of the secondary alcohol at C-5; (ii) β-elimination of inorganic phosphate across C-6 and C-7; (iii) reduction of the resulting enone at C-5; (iv) ring opening of the enol pyranose; (v) and the final intramolecular aldol-like reaction which proceeds *via* a chairlike transition state to produce DHQ. Recently it has also been proposed[63] that kinetic parameters influence these transformations. The key features of DHQ synthase and its role in this catalytic conversion are summarised below.

1. DHQ synthase is a homogeneous monomeric metalloenzyme (MW = 39000) that contains a precisely stoichiomeric amount of tightly bound Co(II) (1 mol) and also 1 mol of NAD^+, the presence of which are essential for both catalytic activity and stability of the enzyme.[60-62]
2. The metal ion has a central role in the catalytic mechanism, since it not only influences the catalytic activity of the enzyme but also affects the rate of NAD^+ dissociation from the active site during turnover.
3. NAD^+ is employed catalytically to effect a transient oxidation at C-5 of the substrate, and the redox cofactor need never dissociate from the active site.
4. It is suggested by Bartlett[64] that DHQ synthase catalyses the reaction up to the reduction of ketone **27** and that **28** is the actual product of the enzymatic reaction.
5. β-Elimination of inorganic phosphate is *syn*.
6. The aldol transition state is chair-like.

7. The C-7 side chain has to have a gauche conformation, to allow proton abstraction from C-6. This positions the C-6 proton close to one of the peripheral oxygens of the phosphate group.

In summary the enzyme DHQ synthase possesses remarkable properties for the catalytic conversion of DAHP to DHQ.



Scheme 12

1.4.3 Inhibitors of Dehydroquinate (DHQ) Synthase

There have been numerous strategies directed towards the synthesis of substrate analogues of DAHP as potential inhibitors of DHQ synthase,[58-71] and *in vitro* inhibition studies on these compounds have been performed. Representative examples of such analogues are shown in Appendix 1. From the results the following conclusions can be drawn about the structural features known to be essential for inhibition of this enzyme.

Micromolar competitive inhibition of the enzyme was first achieved with a organophosphonate analogue (**29**) of substrate DAHP.[65] The discovery of the reversible slow-binding, carbocyclic analogue (**30**)[61,66] resulted in inhibition being lowered to nanomolar levels. Ketocarbaphosphonate (**31**)[67] constituted the next step in inhibitory potency; it was the first reported irreversible inhibitor of the enzyme, and is ideal for maintaining *in vivo* enzyme inhibition. It is a lead compound for a large class of reaction-intermediate analogues of considerable agricultural and pharmacological importance.

Enhanced binding to DHQ synthase has been achieved by incorporation of features into an inhibitor that favour oxidation at C-5 eg. analogue **32**. Also the importance of the phosphate monoester in substrate binding to DHQ synthase has been demonstrated by the inability of DAH itself to serve as a substrate of DHQ synthase.[58]

Numerous phosphonate and homophosphonate analogues have been synthesised, with the phosphonomethyl moiety becoming an influential feature of many inhibitors of DHQ synthase.[68] A major advance was the discovery of a new class of inhibitory substrate analogues where the pyranosyl ring oxygen was replaced

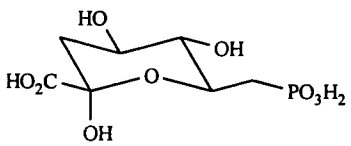
with a methylene group.[61] These carbocyclic analogues bind 25-100 times more tightly than the corresponding oxacyclic compounds (**33** *cf* **34**).[61] This has been attributed to a change in conformation of the enzyme. Other carbocyclic analogues eg. **35** have been prepared in an attempt to generate a Michael acceptor at the enzyme active site which is capable of suicide inactivation of DHQ synthase.[69]

The conformation of the alkenyl side chain also appears to be important for inhibitory activity,[61] and the preferred binding mode has the side chain bound in a gauche conformation for increased activity (**36**). Analogues that are more nearly isosteric with the substrate bind less tightly to the enzyme than those containing a shortened side chain; for example (**29**) is a competitive inhibitor of the enzyme whereas (**33**) is inactive.[58]

Substrate analogues (**30**) and (**37**) have been synthesised[63,66] where one or both of the secondary hydroxyl groups have been removed from a carbaphosphonate inhibitor. Removal of a single hydroxyl group from the carbocyclic backbone of carbaphosphonate (eg. **30** *cf* **34**) appears to decrease the inhibitor potency by a factor of 100. Tight interactions between active site residues and the C-4 carbanophosphate (**34**) are responsible for its nanomolar-level inhibition.[66] However, it does not seem to matter which hydroxyl group, (at C-4 or C-5) is removed for the observed decrease in inhibition. Carbaphosphonate (**34**) is thus a lead compound for the design and synthesis of future *in vivo* inhibitors of DHQ synthase.

The enzyme does not tolerate the acyclic substrate (**38**)[62] which does not bind to the active site. Also replacement of the carboxyl group by an isopolar and isosteric tetrazole unit[70] (eg. **39**) results in no significant inhibition even at sub millimolar concentrations. Finally *in vitro* inhibition[68] of DHQ synthase has been

previously shown not to be very selective in the nature of the products afforded,[71] whereas *in vivo* inhibition has been found to be highly diastereoselective.



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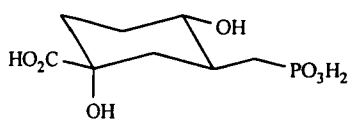

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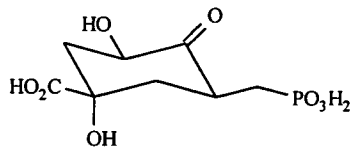

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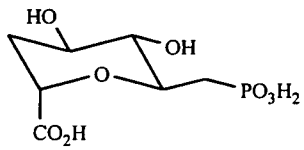

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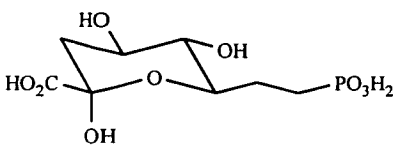

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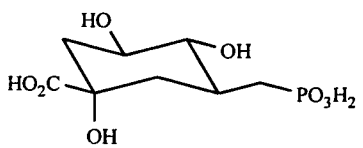

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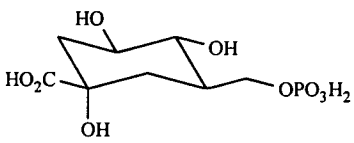

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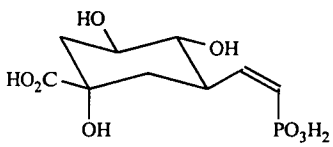

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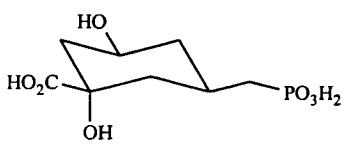

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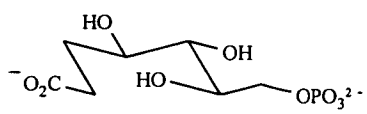

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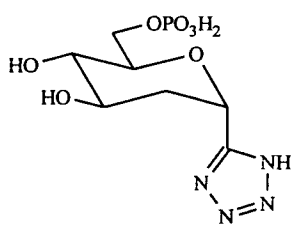


(39)

1.4.5 Syntheses of DAH and Analogues

The potential herbicidal and antimicrobial benefits of DAH/DAHP inhibitors of the shikimate pathway have been the focus of much interest in recent years, as has the development of short, efficient and stereoselective routes to such compounds and their analogues. Indeed, more potent and specific inhibitors are still sought.

There have been many reported routes for the chemical syntheses of DAH/DAHP and their analogues.[59,74-82] However, to date no reference has used nitrile oxide/isoxazoline chemistry. Most approaches have employed 2-deoxy-D-glucose (a relatively costly carbohydrate) as the starting material, and require a one-carbon chain extension of the aldose followed by specific phosphorylation at the primary hydroxyl group at C-7, and oxidation at C-2. The most efficient[59] gives 6% overall yield after eight steps [Section 1.4.5.1.1]. Since the first preparation of DAH in 1963,[77] there have been numerous successful syntheses. Several of these methods are detailed in Haslam's comprehensive monograph 'Shikimic Acids'.[1]

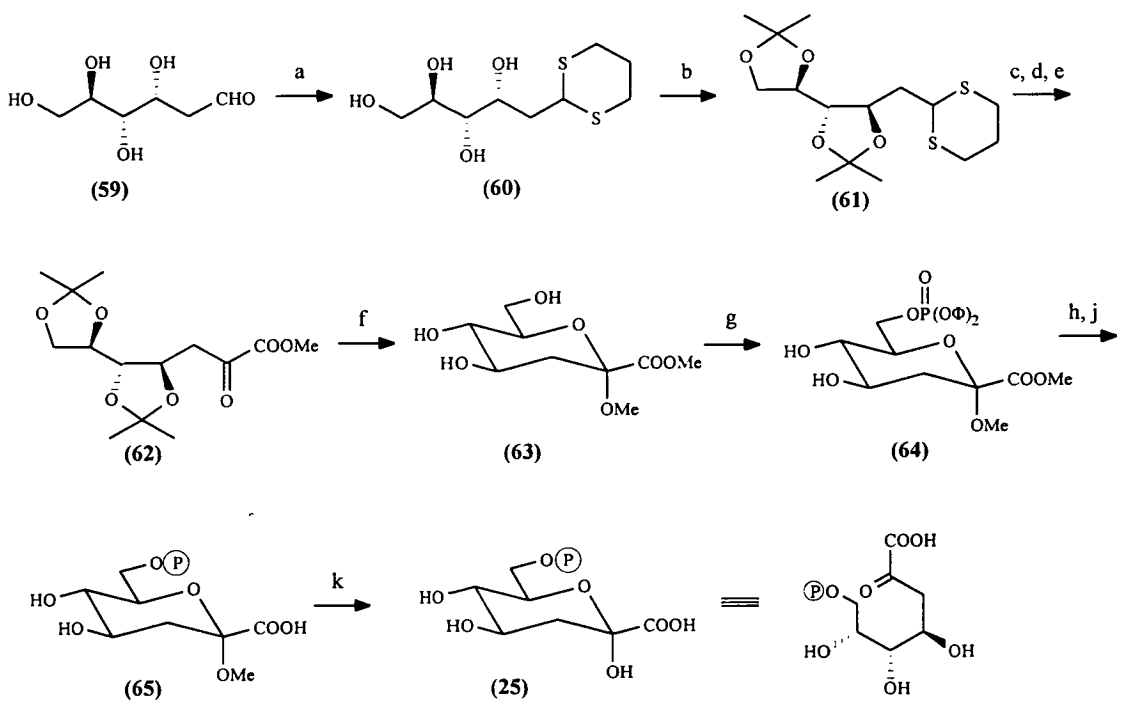
The following account presents some of the recent procedures used for the formation of DAH/DAHP and analogues. The first section deals with their preparation *via* the modification of carbohydrates, while the second covers non-carbohydrate based approaches. Chemoenzymatic and whole cell syntheses follow and finally a miscellaneous section covering work not categorised elsewhere is presented.

1.4.5.1 Syntheses from Carbohydrates

1.4.5.1.1 From 2-Deoxy-D-glucose

This route[59] enables the production of substrate analogues of both DAH and DAHP (Scheme 13). Conversion of 2-deoxy-D-glucose (**59**) to the propylene

dithioacetal (**60**) is achieved using acid catalysis and subsequent reaction with acetone containing a trace of sulphuric acid leads to the 3,4:5,6-di-*O*-isopropylidene derivative (**61**). Metallation of (**61**) with butyllithium was followed by methoxycarbonylation with methyl chloroformate. NBS in aqueous acetone was then used to remove the dithioacetal protection. The resulting methyl 3-deoxy-heptulosonate (**62**) was converted to methyl glycoside (**63**) by heating in refluxing acidic methanol. Phosphorylation of this cyclic heptulosonate (**63**) with diphenyl phosphochloridate gave (**64**). Exhaustive hydrogenation removed the phenyl ester groups and was followed by basic hydrolysis of the methyl ester. Finally, heating with Dowex 50 (H^+ form) hydrolysed the methyl pyranoside (**65**) to yield DAHP (**25**).

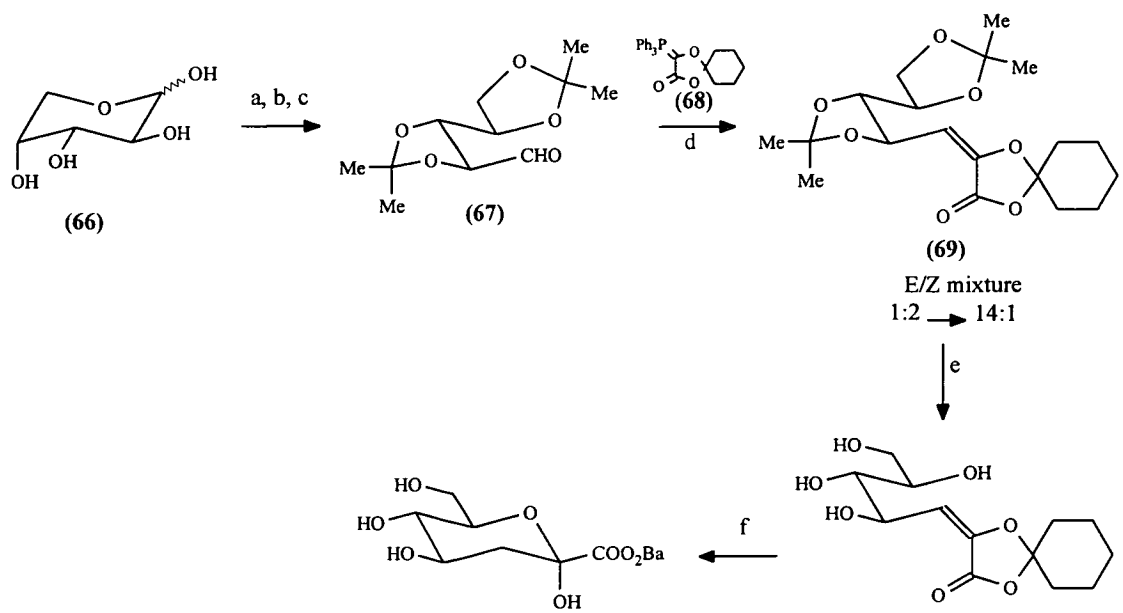


(a) HCl, $HS(CH_2)_3SH$, EtOH; (b) $(CH_3)_2CO$, H_2SO_4; (c) n-BuLi; (d) $ClCOOCH_3$; (e) NBS, acetone; (f) HBr, MeOH; (g) $(PhO)_2POCl$, pyridine; (h) PtO_2/ H_2; (j) KOH; (k) Dowex 50 (H^+)

Scheme 13

1.4.5.1.2 *Via* 5-Ylidene-1,3-dioxalan-4-one Intermediates[83,84]

The advantage of this approach results from the nature of the dioxalanone ring system which is relatively stable to acidic cleavage, but which can produce salts of the α-keto carboxylic acid when hydrolysed by alkali under very mild conditions. The synthesis of DAH has two key stages; the first involves the preparation in three steps from D-arabinose (**66**) of protected aldehyde (**67**) containing the desired stereochemistry at each carbon atom. In the second, crucial step the protected aldehyde (**67**) is reacted with the Wittig reagent (**68**) thus introducing the masked α–keto acid function. The ulosonic acid is then released by hydrolysis as shown in Scheme 14.



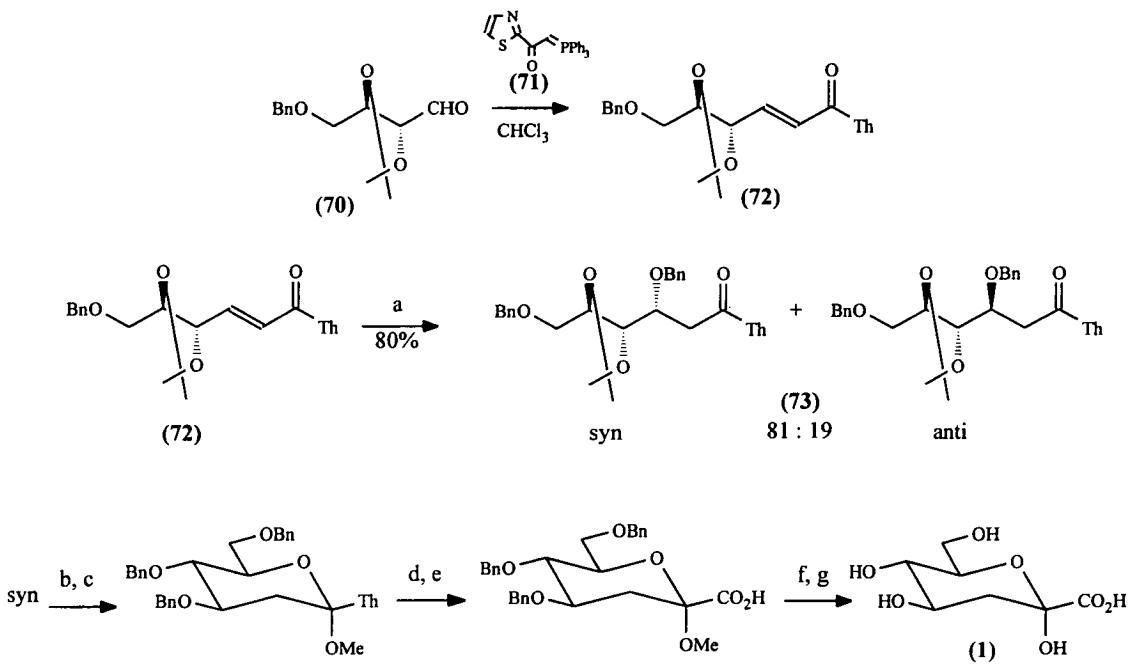
(a) C_2H_5SH, conc. HCl, O°C; (b) acetone, conc. H_2SO_4; (c) $HgO/HgCl_2$, 2 hr, 30°C, 1 hr 50°C, 2 hr reflux; (d) 60°C under N_2, 10 min, then r.t. 30 min; (e) CF_3COOH/H_2O(3:2), 80°C, 14 hr; (f) 0.5 M eq $Ba(OH)_2$ in aq. MeOH

Scheme 14

1.4.5.2 Synthesis from Non-Carbohydrates

1.4.5.2.1 Wittig Olefination-Michael Addition Route

Dondoni *et al*[85] have developed a strategy which involves the stereoselective carbon-carbon bond formation *via* Wittig olefination of an aldose (**70**) with a thiazole-armed acyl ylide (**71**), followed by carbon-oxygen bond formation by conjugate Michael-type addition of a benzyloxy anion to the resultant trans-α-enone (**72**) (Scheme 15).



(a) BnONa, 80%; (b) HCl/MeOH, 90%; (c) BnBr-NaH, 100%; (d) TfOMe, then $NaBH_4$, then $CuCl_2$-CuO-H_2O, 76%; (e) Ag_2O, 96%; (f) H_2-Pd/C, 100%; (g) AcOH-H_2O, 70%

Scheme 15

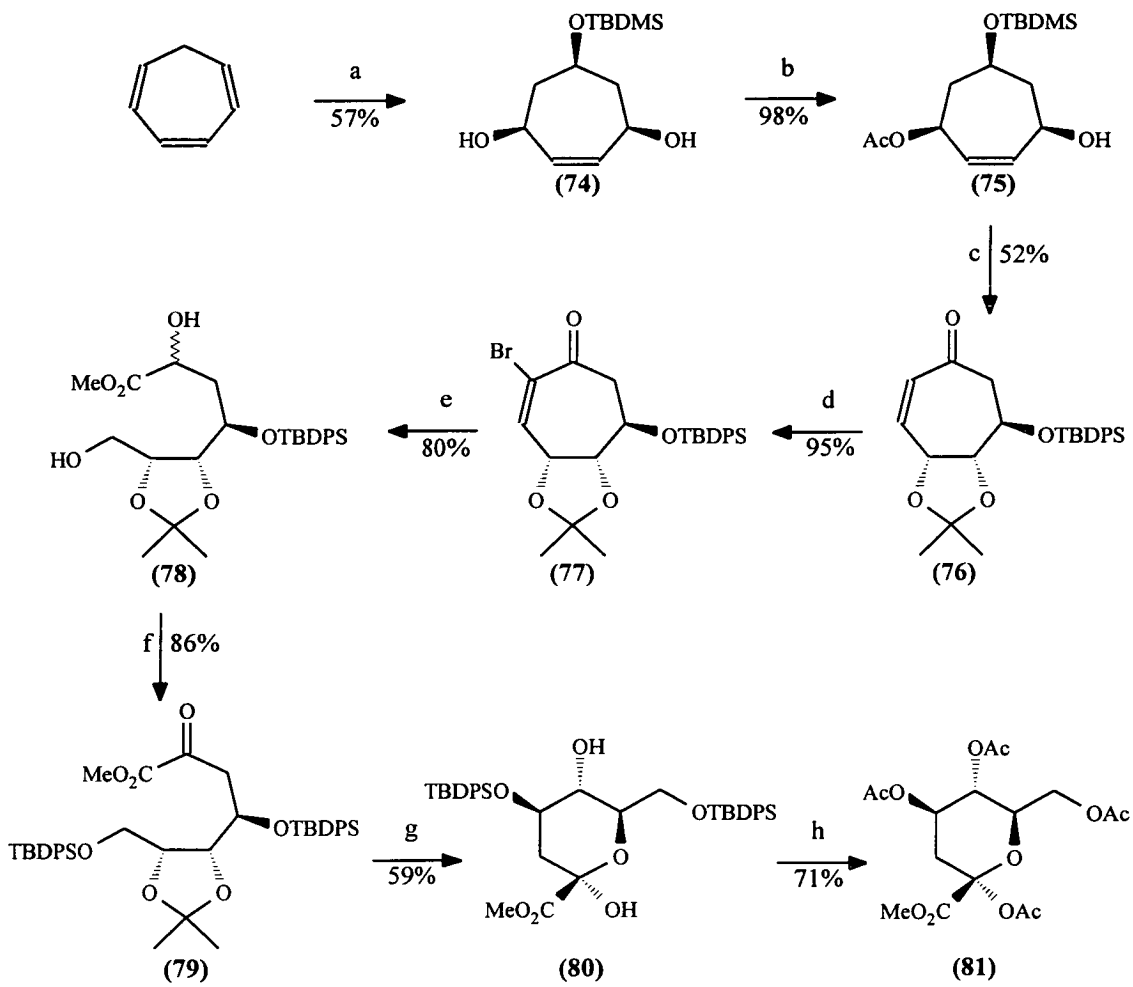
A Felkin-Anh transition-state model,[85] predicts the stereochemical outcome for the carbonyl and alkene addition, with the large α-substituent being orientated antiperiplanar to the forming new bond and the medium-size substituent in the inside

position. The thiazole ring serves as a masked formyl group and is important in the success of this approach, being stable to a wide variety of conditions and yet undergoing efficient conversion to the aldehyde under neutral conditions. This formyl unmasking involves a one-pot sequence using methyl triflate for *N*-methylation to a thiazolium salt, sodium borohydride reduction, and $CuCl_2$ and CuO for the metal assisted hydrolysis of the resulting thiazolidine. In this way aldehyde (**70**), formed by one-carbon homologation of D-glyceraldehyde acetonide, is converted into DAH.

1.4.5.3 Chemoenzymatic Approaches

In this section two different routes will be described. The first very recent approach involves a fully stereocontrolled total synthesis of the parent DAH by a strategy which uses a simple, cyclic hydrocarbon cycloheptatriene as a non-carbohydrate, achiral starting material.[86] Monoacetate (**75**) (and its derivatives) are readily prepared form cycloheptatriene *via* an enzymatic asymmetrisation[87,88] of the meso-diol (**74**) with *Pseudomonas cepacia* lipase in isopropenyl acetate (Scheme 16). The monoacetate was then converted into the α,β-unsaturated ketone (**76**)[89] which yielded the α-bromo enone (**77**) on treatment with bromine and triethylamine. A mixture of epimeric alcohols was afforded by Luche reduction[90] of (**77**) and ozonolysis of this mixture followed by chemoselective reduction of the intermediate aldehyde with sodium borohydride gave ester (**78**) as a mixture of alcohols epimeric at C-2. Conversion of the terminal hydroxy group of (**78**) to the *tert*-butyldiphenylsilyl ether followed by PDC oxidation[91] of the secondary alcohol yielded protected heptulosonic acid (**79**) possessing the required *arabino* stereochemistry. Cyclised

DAH derivative (**80**) was obtained by treatment of (**79**) with methanol containing an acid catalyst. DAH was formed by cleavage of the silyl ethers and characterised as its tetraacetate derivative (**81**).

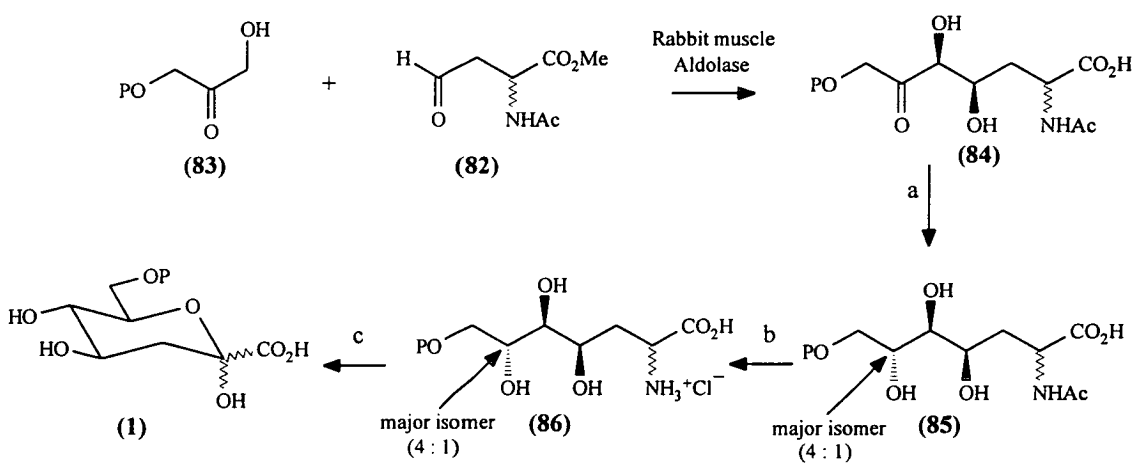


(a)i HBF_4, Ph_3COH; ii Na_2CO_3, CH_3CN; iii $NaBH_4$, MeOH; iv TBDMSCl, imidazole, DMF; v $^1O_2$, CH_2Cl_2/MeOH; vi Zn/HOAC, CH_2Cl_2; (b) *Pseudomonas cepacia* lipase (Amino PS-30), isopropenyl acetate; (c)i TBDPSCl, imidazole, DMF; ii OsO_4, NMO, acetone, H_2O iii dimethoxypropane, *p*-TsOH; iv KOH, MeOH; v MsCl, Et_3N, CH_2Cl_2; vi *p*-TsOH, MeOH; vii $(COCl)_2$, DMSO, Et_3N; (d) Br_2, Et_3N, CH_2Cl_2; (e)i $NaBH_4$, $CeCl_3$, MeOH, -78°C; ii O_3, DMS, MeOH; iii $NaBH_4$; (f)i TPSCl, Et_3N, $CHCl_2$; ii PDC/4Å sieves, CH_2Cl_2; (g) TsOH, MeOH, 59%; (h)i TBAF, THF ii Ac_2O/Py/ DMAP.

Scheme 16

An earlier report by Whitesides *et al*[92] described an alternative approach to the synthesis of DAHP using a rabbit muscle aldolase to catalyse the formation of the C-

4/C-5 bond. (Scheme 17). Two chiral centres are generated diastereospecifically in this aldol condensation. Quantitative production of methyl ester was obtained by treatment of *N*-acetyl-D/L-allylglycine with diazomethane. Ozonolysis, followed by reductive workup with dimethyl sulphide afforded the aldehyde (**82**). In the presence of aldolase reaction of aldehyde (**82**) with dihydroxyacetone phosphate (**83**) gave 40% maximum conversion, and the product (**84**) as a mixture of diastereomers epimeric at C-2 (2*R*:2*S* = 1.5:1.0) was obtained in 37% yield after ion-exchange chromatography. Hydrolysis of the methyl ester also occurs. The sodium salt of (**84**) was reduced using tetramethylammonium triacetoxyborohydride to give the desired *anti* configuration as the dominant isomer. Hydrolysis of (**85**) gave a diastereomeric mixture of amino acids (**86**). The final transformation of (**86**) to ketoacid (**1**) was accomplished by a transamination reaction with sodium glyoxylate.



(a) $Me_4N^+BH(OAc)_3^-$, AcOH; (b) 6 N HCl, 100°C, 90 min; (c)i Sodium glyoxylate (5 M eq), Al^{3+}; ii Ion-exchange chromatography

Scheme 17

This route to DAHP has four attractive features. First, there are few protection and deprotection steps, and the reactions are all carried out in aqueous media. Second,

formation of the carbon-carbon bond is accomplished simultaneously with the introduction of the phosphate group. In other syntheses this step often occurs with low yields. Third, use of an appropriately labelled aldehyde (**82**) or dihydroxyacetone phosphate (**83**) can allow for the preparation of isotopically labelled DAHP. Finally, this route provides a potentially useful method for synthesising analogues of DAHP due to the high tolerance of rabbit muscle aldolase for the aldehyde component, and also the possibility of structural modification at centres other than C-4 and C-5 (those formed in the aldolase-catalysed reaction).

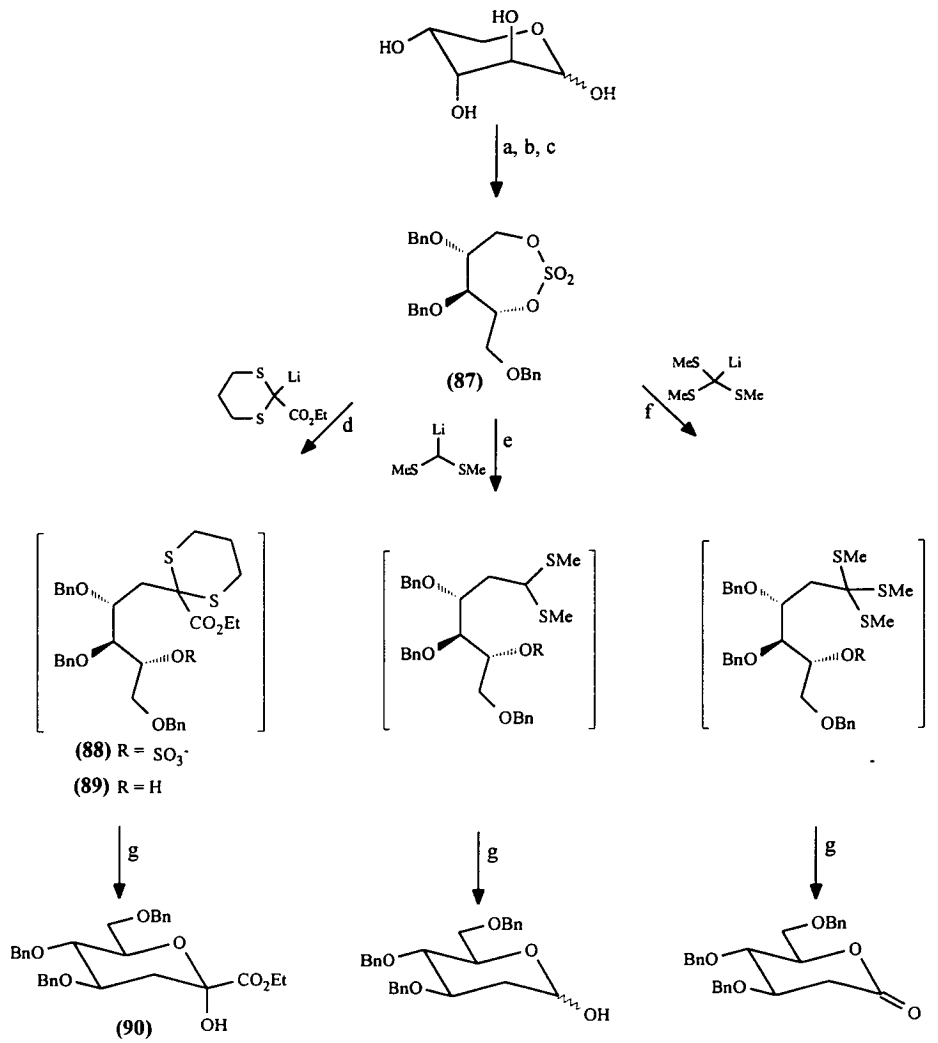
1.4.5.4 Whole Cell Syntheses

One of the difficulties of exploiting whole cells for the production of intermediary metabolites results from the accumulation in the growth medium of contaminants produced in normal cellular processes. Manipulation of large volumes of crude product is required during purification of such metabolites as well as isolation of the target molecules free from contaminants of closely similar structure and charge type. Frost and Knowles[59] have successfully synthesised DAHP using mutants of *E.coli* that contain no dehydroquinate synthase. Two different *E.coli* were used and shown to secrete considerable quantities of the dephospho material DAH (in addition to DAHP). The DAHP was purified by anion-exchange chromatography on DEAE-cellulose. These refinements allowed the ready preparation in a few days of 100 mg quantities of pure DAHP. The advantage of the microbial route is that the process is shorter and less skill is required. However, the concomitant production of substrate analogues is prohibited.

1.4.5.5 Miscellaneous Approaches

1.4.5.5.1 1,4-Cyclic Sulphate Approach

Nucleophilic opening of a sugar cyclic sulphate[93] gives easy access to interesting precursors of DAHP analogues. The key intermediate required for the preparation of such analogues is 2,3,5-tri-*O*-benzyl-D-arabinitol 1,4-cyclic sulphate (**87**) (Scheme 18).



(a)i MeOH/HCl; ii BnBr/NaH; (b)i AcOH/H_2O (4:1); ii $NaBH_4$; (c)i $SOCl_2/Et_3N$; ii $NaIO_4/RuCl_3$ (d) THF, 20°C, 16 hr; (e) THF, 40°C, 1 hr; (f) THF, -40°C, 0.5 hr; (g) NBS/MeCN, ET_3NHCO_3/H_2O, O°C, 5 min

Scheme 18

The cyclic sulphate (**87**) was prepared from 2,3,5-tri-*O*-benzyl-D-arabinitol using thionyl chloride, followed by ruthenium chloride oxidation. Removal of the sulphate group in for example intermediate (**88**) by mild acidic hydrolysis was effected *in situ* to give (**89**), and final unmasking with NBS of the dithioketal function in (**89**) yielded anomerically pure (α form) (**90**).

This methodology has also been applied to the successful synthesis of KDO and its corresponding derivatives,[94] and is also applicable to a 1,4-diol system present in an open-chain sugar configuration. It allows for exclusive formation of the required D-*arabino* or D-*manno* configuration, respectively, and not the concomitant formation of the unwanted D-*gluco* configuration as observed in most routes to KDO *via* an aldol or Wittig-type elongation.

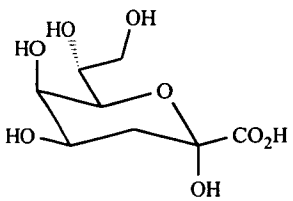
1.4.5.5.2 Plasmid-Based Biocatalysis

Plasmid-based biocatalysis[95] is useful for the synthesis of small molecules which are normally difficult to obtain, and for their production in quantities suitable for use in chemical synthesis. The synthesis is accomplished without the need for adenosine triphosphate regeneration, co-factor, co-substrate, enzyme immobilisation and enzyme purification required by enzymatic synthesis.

1.5 KDO Inhibitors

1.5.1 Introduction

The eight carbon carbohydrate 3-deoxy-D-*manno*-octulosonic acid (KDO) (**2**) is an essential and unique constituent of the outer membrane lipopolysaccharide (LPS) of all Gram-negative bacteria,[2,96] and has been identified in several acidic capsular exopolysaccharides (K-antigens).[2,97,98] It has also been found in the primary cell wall of higher plants,[99,100] the protozoan *Trypanosona cruzi*[101,102] and green alga *Tetraselmis striata,*[103] but has never been detected in Gram-positive bacteria.[104] Since its discovery in 1959,[3] KDO has steadily grown in importance as a natural product of bacterial origin. While Gram-positive bacteria are susceptible to many antibacterial agents, Gram-negative bacteria are more difficult to target effectively.[105]



(**2**)

A continuing problem in chemotherapy is the development of resistance by bacteria to existing antibacterial agents. There is thus an increasing need to discover novel classes of compounds[106-111] which could be used in conjunction with those presently employed in the treatment of bacterial infections. Recent efforts have therefore been directed toward identifying new biochemical pathways that are essential to bacterial survival, but are unique to prokaryotes. Specific compounds are

then designed with the hope that they will possess antibacterial activity without being toxic to eukaryotic cells.

The vital rate-limiting step in LPS biosynthesis involves incorporation of KDO which is required for the growth and proliferation of Gram-negative bacteria. Therefore, since mutants unable to produce KDO are non-viable,[103,112] KDO is an attractive target in the rational design of novel antibacterial agents and chemotherapy.[113] In addition, as KDO is not present in mammalian cells,[99] agents directed against KDO metabolism ought to have a high degree of selective toxicity. There is thus a great interest in the immunochemistry, biochemistry and synthetic carbohydrate chemistry involving KDO and in particular the synthesis of KDO analogues as specific enzyme inhibitors of the KDO pathway.[2,7,100,106-108,113,114]

1.5.2 Structure of KDO

The initial structural determination on the KDO molecule was carried out by Levin and Racker,[3] who observed the enzyme-catalysed formation of KDO 8-phosphate from enolpyruvate phosphate and D-arabinose-5-phosphate. They assigned the configuration of C-5 to C-7 as *arabino* and this was verified by Heath and Ghalambor[115,116] who also tentatively assigned the D-*manno* configuration to KDO derived from LPS. This was subsequently confirmed by a Wittig synthesis of KDO,[117] NMR analysis[118,119] and X-ray crystallography[120] of the α-D-ketosidic KDO derivative (**91**).

1.5.3 Structure of LPS

The outer membrane of Gram-negative bacteria is composed of a bilayer with endotoxic LPS outside and an inner phospholipid region. The most important component is LPS which serves as an effective barrier towards the penetration of a wide variety of lipophilic compounds including xenobiotics of molecular weight >600 which would be harmful to the organism.

LPS[121] consists of three regions. The hydrophobic portion, a 2-amino-2-deoxy-D-glucosyl disaccharide substituted with the unique fatty acid Lipid A [*O*-(β-D-glucosaminyl)-(1,6)-D-glucosamine], possesses the lipid anchor required for membrane binding, and determines the endotoxic, antigenic specificity, adhesiveness, invasiveness, penetrability and immunostimulatory properties of LPS. The hydrophilic polysaccharide domain is composed of two regions. The first is a complex oligosaccharide that projects from the cell surface into solution. The other is an inner carbohydrate core region comprising a non-repetitive sequence of residues. The last constituent of the core, always KDO,[2] is situated at the reducing ends of the polysaccharide domains linking them through a α-(2-6′) ketosidic bond to the O-6′ of the D-glucosamine disaccharide backbone, Lipid A. This structural unit appears to be a prerequisite for the growth and survival of the bacterial cell. One or more KDO units may be present (normally three) and very often one of these is substituted by L-*glycero*-D-*manno*-heptopyranose to form the sequence : heptose-(1,5)-KDO-(KDO)-(2,6)-glucosamine. Figure 2 shows the structure of the lipid A-KDO region of LPS.[96]

Evidence suggests that one of the final stages involved in constructing LPS[96] is the incorporation of KDO, allowing nascent LPS molecules to move from the inner

surface of the plasma membrane to the exterior of the cell, where final assembly takes place.

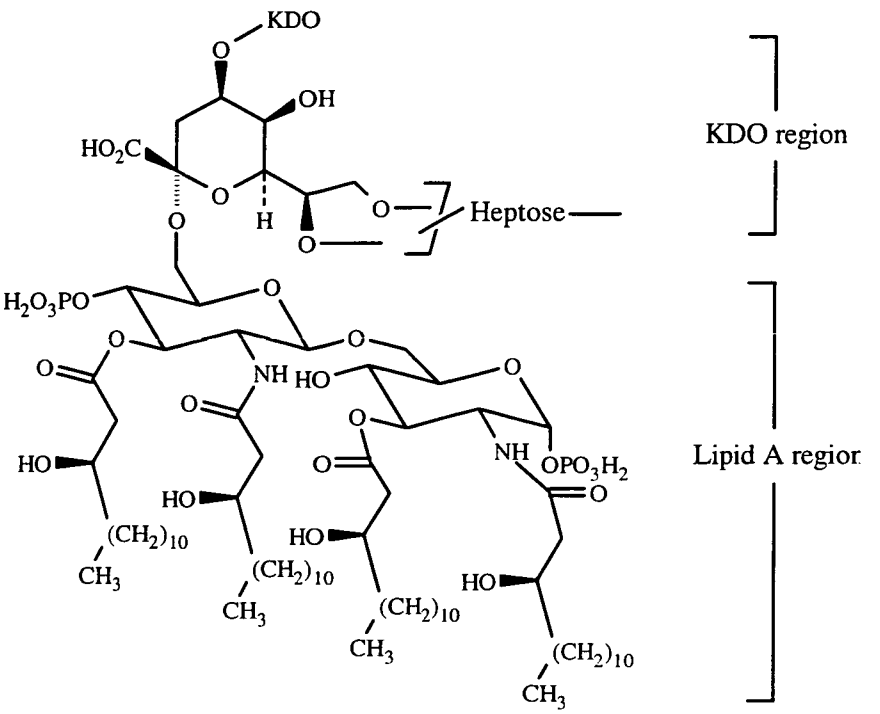
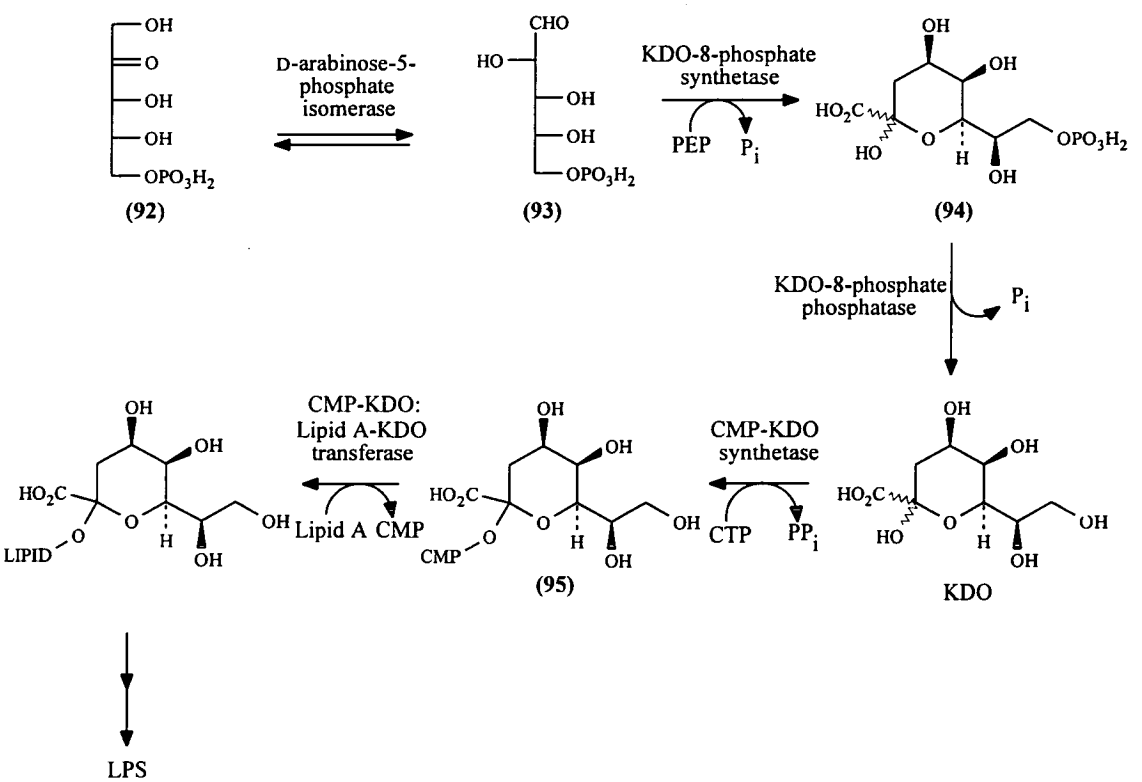


Figure 2

1.5.4 Biosynthesis of KDO and Incorporation into Lipid A

In lipopolysaccharide biosynthesis the incorporation of KDO[96] consists of two steps: the formation of cytidine 5′-monophospho-3-deoxy-D-*manno*-2-octulosonic acid (CMP-KDO) by CMP-KDO synthetase[122] and the subsequent coupling with Lipid A precursor. Since the rate limiting step is the activation of the KDO moiety,[122] inhibitors of CMP-KDO synthetase are potentially useful as bacterial agents. Kohlbrenner and Fesik[123] elucidated the stereochemistry of this reaction which provides the basis for a rational design of enzyme inhibitors.

The biosynthetic pathway of KDO is well understood[96] and is shown in Scheme 19.



Scheme 19

Five enzymes are required for the biosynthesis and incorporation of KDO into LPS. Each has a specific role. D-Arabinose-5-phosphate isomerase is involved in isomerisation of D-ribulose-5-phosphate (**92**) to D-arabinose-5-phosphate (**93**). KDO-8-phosphate synthetase catalyses the condensation of (**93**) with phosphoenol pyruvate (PEP) to afford KDO-8-phosphate (**94**). KDO-8-phosphate phosphatase hydrolyses the phosphate ester to provide KDO. The fourth enzyme, CMP-KDO synthetase, is involved in activation of the anomeric hydroxyl group; KDO reacts with CTP to produce labile CMP-KDO (**95**). The final stage requires glycosylation for incorporation of KDO into LPS.

The key enzyme is CMP-KDO synthetase, which determines the rate of reaction, and hence this enzyme has attracted most interest as a target for inhibition of the biosynthetic pathway. The design of inhibitors for this enzyme is the focus of part of this thesis.

A number of conclusions about the enzyme CMP-KDO synthetase can be made.[123,124]

1. It is specific for the β-pyranose form of KDO (a minor form in solution) and this anomeric configuration is retained in the CMP-KDO product. KDO is assumed to be in the $^5C_2$ ring conformation.
2. CMP-KDO formation is *via* a nucleophilic displacement mechanism[124] (Figure 4) and is in agreement with the transition-state model[125] shown in Figure 3.
3. The biosynthesis of CMP-KDO appears to occur in a manner completely analogous to the biosynthesis of CMP-*N*-acylneuraminic acids.

4. Transfer of KDO in the β-configuration from CMP-KDO to LPS is thought[2] to occur with inversion of configuration at the anomeric centre, C-2, resulting in KDO α-linked in LPS.

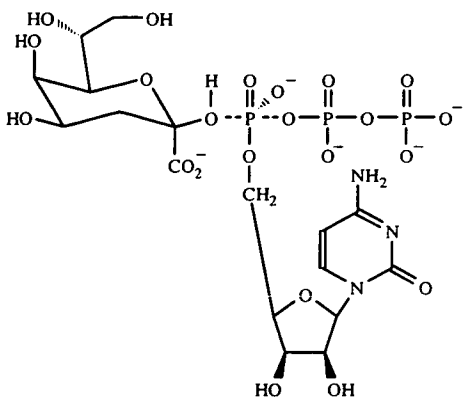


Figure 3 : Transition-state Model

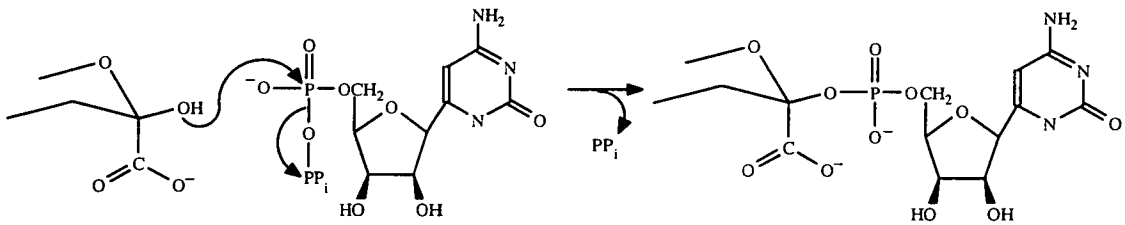


Figure 4 : Nucleophilic Displacement Mechanism for CMP-KDO Formation

The metabolism of KDO in Gram-negative bacteria still has many interesting questions which need to be answered, not only in the role KDO plays in the functioning of the outer membrane macromolecular structures, but also in the reaction mechanisms involved. Of particular interest are the mechanism for transfer of KDO into LPS, the rates of interconversion of KDO forms in the cell, and the role KDO performs in maintaining the outer membrane barrier of Gram-negative bacteria.

1.5.5 Inhibitors of CMP-KDO Synthetase

A large number of analogues of KDO have been synthesised *via* different strategies[2,102,106-111,113,125-138] as potential inhibitors of the enzyme CMP-KDO synthetase, and *in vitro* inhibition studies performed. Representative examples of such analogues are presented in Appendix 2. The following conclusions can be drawn about the requirements for an enzyme inhibitor.

Inhibitors possessing high affinity are likely to be those which are able to form many strong interactions with the enzymes active site. When the active site is fully occupied these requirements can be satisfied. A ternary complex between enzyme, inhibitor and CTP is the probable mode of binding of an inhibitor, eg. (**96**),[113] in the active site of CMP-KDO synthetase.

2-Deoxy-β-KDO (**96**)[111] was found to be a potent inhibitor of CMP-KDO synthetase, whereas 2-deoxy-α-KDO (**97**) was inactive.[111,132] This finding led to the synthesis of many analogues of 2-deoxy-β-KDO in an attempt at retaining, or even enhancing, the inhibitory activity.[131] Modification of the anomeric centre is desirable since this is the reacting centre during incorporation of KDO into the developing LPS of the outer membrane. Carbocyclic analogues of β-KDO,[130,132] eg. (**98**) the cyclic analogue of 2-deoxy-β-KDO (**99**),[109] analogues of 2-deoxy-β-KDO modified at the carboxylic group[102] such as (**100**), and an isosteric phosphonate analogue[107] of CMP-KDO (**101**) have all been shown to be inferior to 2-deoxy-β-KDO as inhibitors of CMP-KDO synthetase.

A potential advantage of carbocyclic analogues[134] of 2-deoxy-β-KDO is that they are likely to be more stable than 2-deoxy-KDO as well as being more lipophilic,

thus allowing easier access across the bacterial cell membrane. However, this was not found to be the case, and analogues possessing the required α-orientation for the carboxyl group (**98**) lack substrate or inhibitory activity.[130,132] Retention of the ring oxygen of KDO appears to be required for activity,[129] with this oxygen being involved in a critical hydrogen bond to the enzyme.[136] The mandatory α-stereochemistry of the anomeric centre is maintained in the azacyclic analogue of 2-deoxy-β-KDO[109] (**102**) and can donate a strong hydrogen bond[21] to the enzyme. Despite this, the azacyclic derivative (**102**) has poor binding capability compared to the oxacyclic analogue (**96**) which is an excellent inhibitor. This can be attributed to replacement of the ring oxygen with nitrogen rather than to removal of the C-2 hydroxyl group.

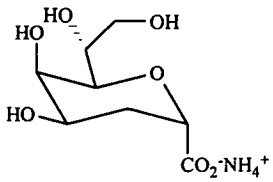
Compounds such as (**103**) resulting from replacement of H-2 with small substituents of varying polarity were found to display low activity (<15% inhibition). The potent enzyme inhibitor 2-deoxy-β-KDO (**96**) has a close fit in the enzyme active site, whereas there is almost complete loss of inhibitory activity[131] for the corresponding methyl compound (**104**). This great difference in inhibitory activity between the 2-methyl-2-deoxy-KDO (**104**) and 2-deoxy-β-KDO (**96**) has been explained through a hypothesis proposed by Claesson *et al,*[113] which suggests that KDO binds to the active site together with CTP. However, when other substituents are introduced an unfavourable thermodynamic equilibrium develops thus resulting in loss of activity.

Conformational factors appear to be irrelevant,[131] since alteration of the 2-substituent does not affect the conformation of the pyranose ring. It is observed that the compound with CH_2OH replacing the anomeric H (**103**), which is closely related

the compound with CH_2OH replacing the anomeric H (**103**), which is closely related to KDO, exhibits low activity. The 8-substituted derivatives[111] of 2-deoxy-β-KDO eg. (**105**) are all potent inhibitors of CMP-KDO synthetase with the exception of the azido derivative (**99**). Compounds (**105**), (**106**) and (**107**) containing the substituents F, CH_3 and NH_2 respectively at the 8-position are all inhibitors, indicating that the electronic properties of the substituent do not influence activity. However, steric factors are suggested to be important from the observation that the derivative (**108**) containing the larger thiol group is noticeably less active than 2-deoxy-β-KDO and that with the linear azido group (**99**) is even less active.

In general, the enzyme binds isosteric phosphonate analogues several times more weakly than the naturally occurring phosphate[107] [(**101**) *cf* (**109**)]. This effect has been attributed to small differences in bonding geometries, deletion of a hydrogen bond to the phosphonate oxygen and steric interactions with the methylene hydrogen.

One of the main difficulties encountered with analogues synthesised to date is that, although they may exhibit potent *in vitro* inhibition of the isolated enzyme eg. (**96**), their inability to penetrate the bacterial cell membrane results in limited, if any, antibacterial activity. However, by linking the 8-amino group or carboxyl substituent at C-2 to a dipeptide, eg. (**110**) and (**111**), it is possible to obtain derivatives with good antibacterial activity.[96,111,133] This involves the transport of inhibitor across the cytoplasmic membrane by the ubiquitous bacterial peptide permeases, with subsequent hydrolysis to give the active inhibitor.



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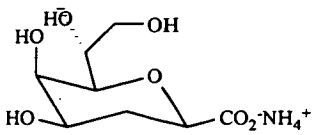

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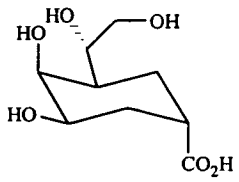

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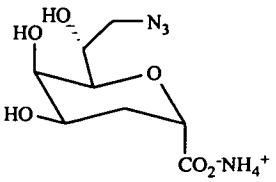

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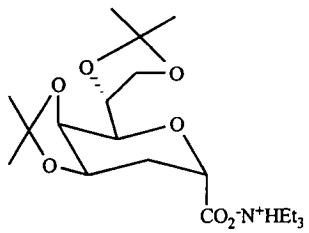

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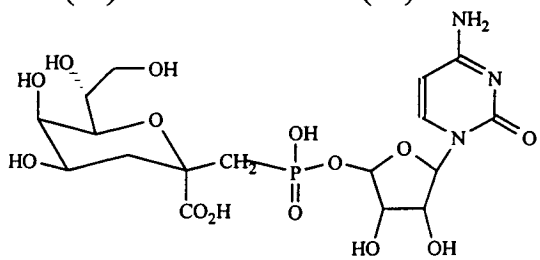

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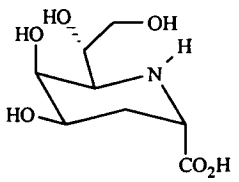

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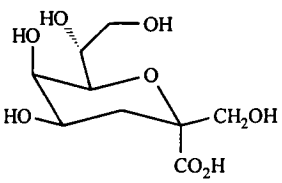

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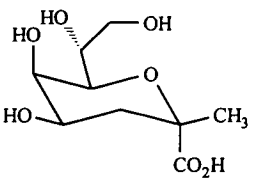

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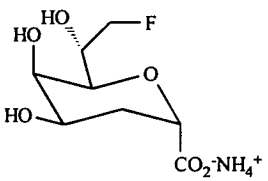

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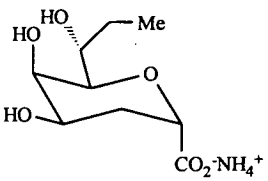

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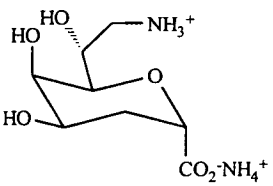

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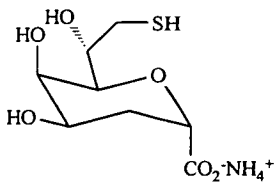

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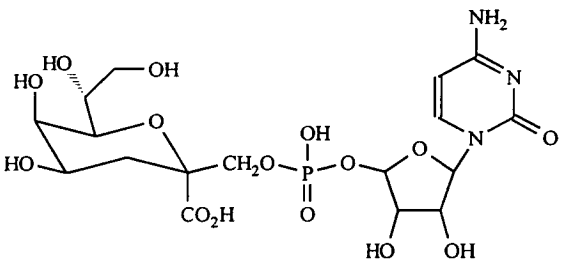

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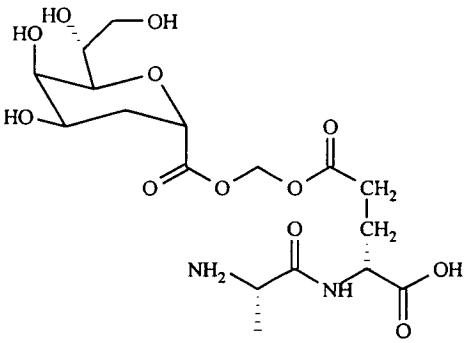

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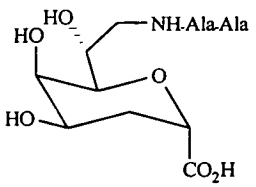

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1.5.6 Syntheses of KDO and Analogues

There is an increasing interest[139] in short and efficient syntheses of KDO due to the continuing difficulty of its isolation from biological material as well as the requirement for the biological function of such complex glycoconjugates to be studied. The synthesis of KDO analogues is also important for the development of an entirely new class of Gram-negative antibacterials targeting the KDO biosynthetic pathway.[132]

Most of the approaches in the literature assemble the eight carbon skeleton of KDO by extending a five or six carbon chiral synthon, either D-arabinose or D-mannose, with a two[83,140,141] or three[142,143] carbon unit respectively *via* aldol or Wittig reactions. The methods required for the construction of the D-*manno* structure involve many steps for the completion of the structure of KDO and also separation of KDO and its isomers is often required. To date no reference has been found which makes use of nitrile oxide/isoxazolines for the formation of 2-ulosonic acids.

Many enzymatic (based on sialic acid aldolase[144] or KDO-8 phosphate synthase[145]) and chemical[2,140-169] syntheses have been developed, however the Cornforth method[170,171] is considered to be the most practical involving chemical aldol condensation of D-arabinose with the four-carbon unit oxalacetic acid. Despite this, although several improved procedures have been reported,[159,164] eg. one pot reaction using Ni^{2+} catalysed decarboxylation of the aldol adduct, the yield, stereoselectivity, and reproducibility of the reaction are not satisfactory. KDO and epi-KDO (epimeric at C-4) are both produced in a ratio >10:1 respectively. Oxalacetic acid, the starting material, is also rather expensive. The enzymatic aldol reaction[165] of D-arabinose and

pyruvate and reactions under nearly neutral and mild conditions catalysed by KDO aldolase may thus be useful for the synthesis of KDO and analogues.

Kuhn[2,118] applied a different approach utilising the formation of lactone esters which could be hydrolysed to the corresponding enol lactone, then to the KDO salt.

There have been many syntheses using the Wittig reaction, in particular those by Kochetkov,[142] Hellwig[117] and Collins.[140] Kochetkov uses protected D-arabinose and condenses this with a phosphonium salt (ylide) to yield a betaine, which is unstable and decomposes to form an alkene. In most of these procedures several additional steps are required to liberate the latent 2-keto group.

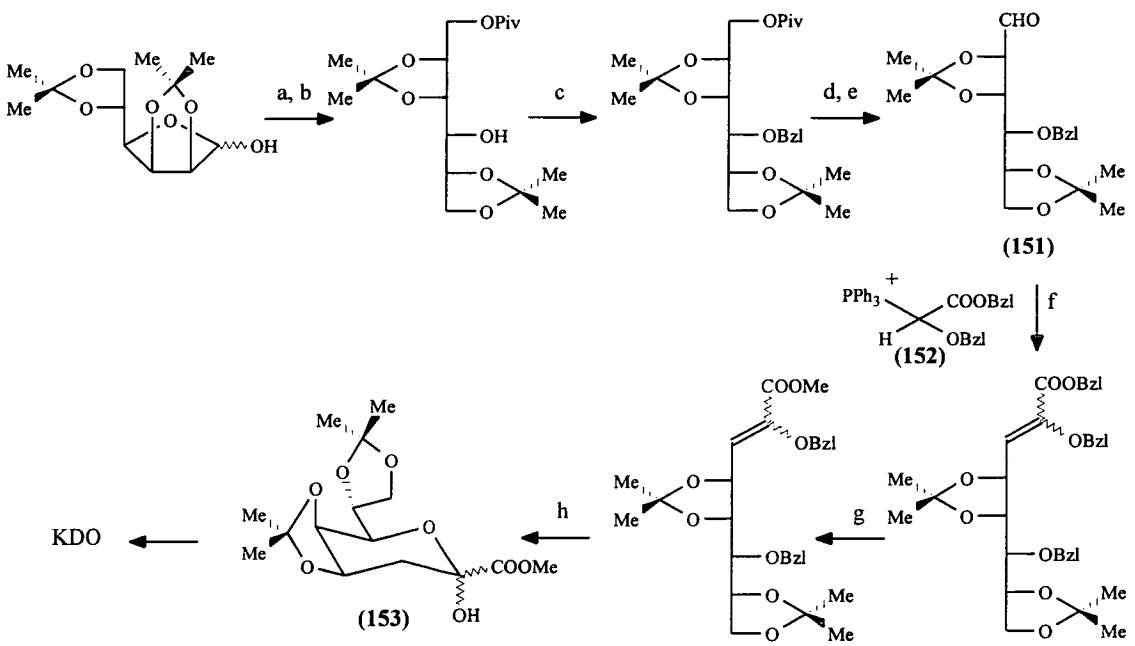
Paquet and Sinay[166] suggested the use of a phosphonate of a functionalised acceptor sugar, followed by mercuration/demercuration, thus providing directly a KDO-containing disaccharide in good yield but with low α/β selectivity. Shiba *et al*[155] reported the preparation of KDO by nucleophilic substitution of a triflate group at C-1 of a D-mannitol derivative with the anion of methyl glyoxylate dithioacetal. An interesting variation of this approach, using the 1,4-cyclic sulphate of D-mannitol, was reported by van Boom *et al.*[94] Branchaud *et al*[152] have described the use of cobaloxime-mediated radical alkyl-alkenyl cross coupling to construct the C-C bond.

The total synthesis of KDO has many advantages, allowing analogues to be produced as potential inhibitors of the key rate determining enzyme. The following account will present some of the recent procedures used for the formation of KDO and its analogues. The first section deals with their preparation *via* the modification of carbohydrates, the second covers non-carbohydrate based approaches, and finally the enzymatic syntheses will be presented.

1.5.6.1 Syntheses from Carbohydrates

1.5.6.1.1 Wittig Approach

Schmidt *et al*[139] have reported the chain elongation of 4-*O*-benzyl-2,3:5,6-di-*O*-isopropylidene-D-mannose (**151**) by a Wittig reaction with [(benzyloxy)(benzyl-oxycarbonyl)methyl]triphenylphosphonium bromide (**152**) (Scheme 20). This Wittig salt is obtained *via* a one pot reaction from glyoxylic acid. Hydrogenolytic removal of the benzyl groups yielded the 4,5:7,8-di-*O*-isopropylidene protected KDO (**153**) and subsequent deprotection afforded KDO.

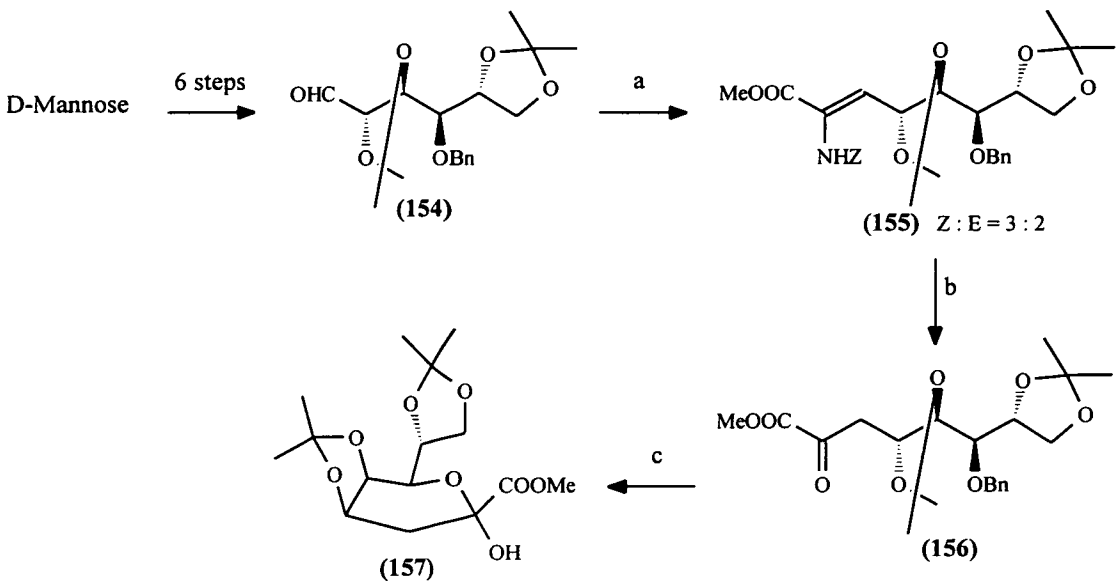


(a) $NaBH_4$; (b) PivCl, Py; (c) NaH, BzlBr; (d) NaOMe, 60°C; (e) DMSO, Ac_2O; (f) NaOBzl; (g) NaOMe, MeOH; (h) Pd/C, H_2

Scheme 20

KDO has also been synthesised *via* the Horner-Wittig adduct[172] (Scheme 21). The synthesis starts with protected D-mannose (**154**) which then reacts with methyl 2-benzyloxycarbonylamino-2-(diethoxyphosphoryl)acetate in the presence of

sodium hydride to give the Horner-Wittig adduct (**155**). The methyl α-oxoalkanoate derivative (**156**) was obtained by selective debenzyloxycarbonylation of (**155**). Subsequent debenzylation yielded the required derivative (**157**).

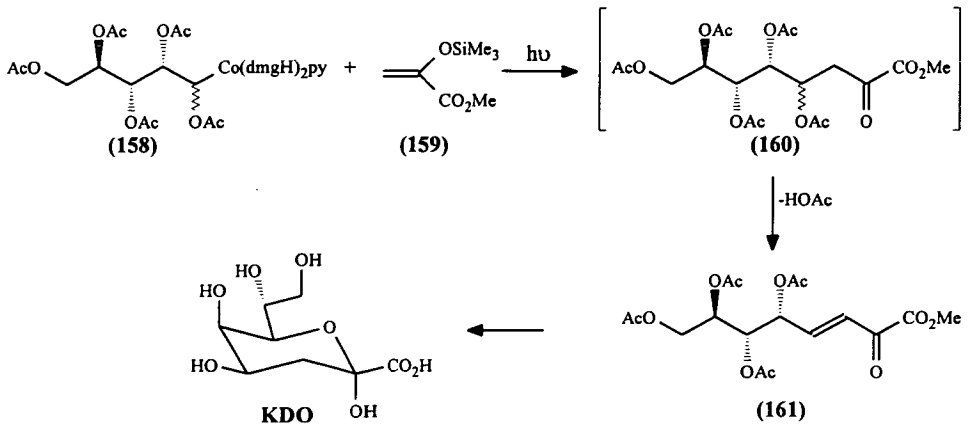


(a) $(EtO)_2P(O)CH(NHZ)COOMe$, NaH/CH_2Cl_2, 80%; (b) 5% Pd/C, H_2, benzene; (c) 10% $Pd(OH)_2$, H_2, EtOH

Scheme 21

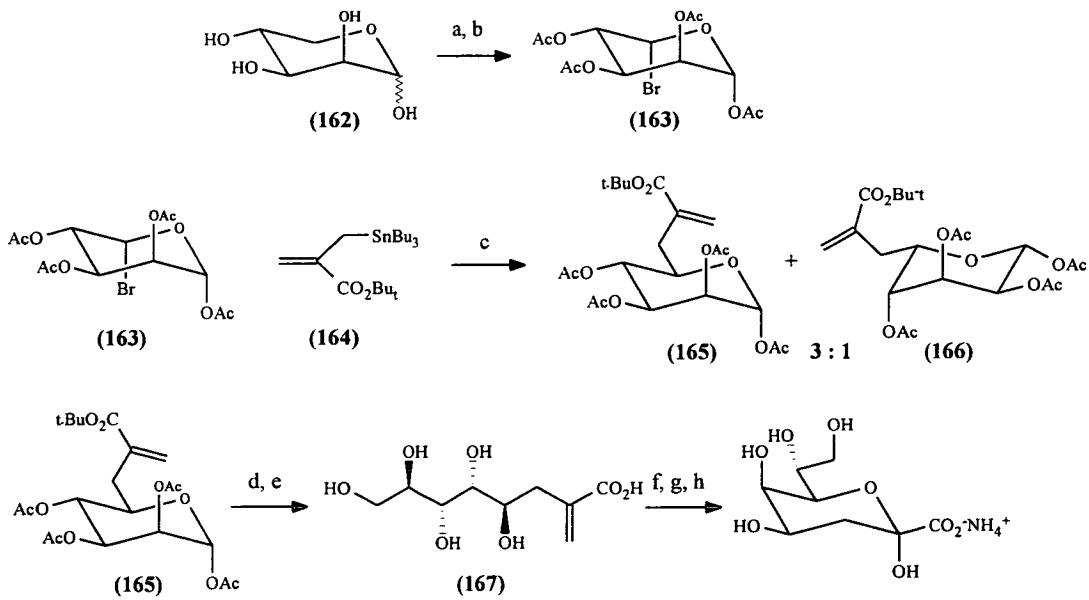
1.5.6.1.2 Radical Carbon-Carbon Bond Formation

Giese *et al*[162] have reported the formation of a chain elongated product (**161**)[173] *via* the reaction of arabinosyl cobaloxime (**158**) with the pyruvate ester (**159**) in a radical C-C bond forming reaction. An intermediate in this reaction is assumed to be the acyclic derivative (**160**) of KDO, which eliminates acetic acid. Subsequent hydrolysis of the double bond of (**161**) affords KDO[142] (Scheme 22).



Scheme 22

Another approach *via* radical C-C bond formation was reported by Giese[161] in the same year (Scheme 23). The ammonium salt of KDO is synthesised from D-lyxose (**162**). 5-Bromo compound (**163**) reacts with alkene (**164**) *via* radical C-C bond formation and results in the production of a diastereoisomeric mixture of (**165**) and (**166**). After deprotection and reduction of (**165**), ozonolysis of 2,3-dideoxy-2-methylene-D-*manno*-octonic acid (**167**) affords the target molecule.

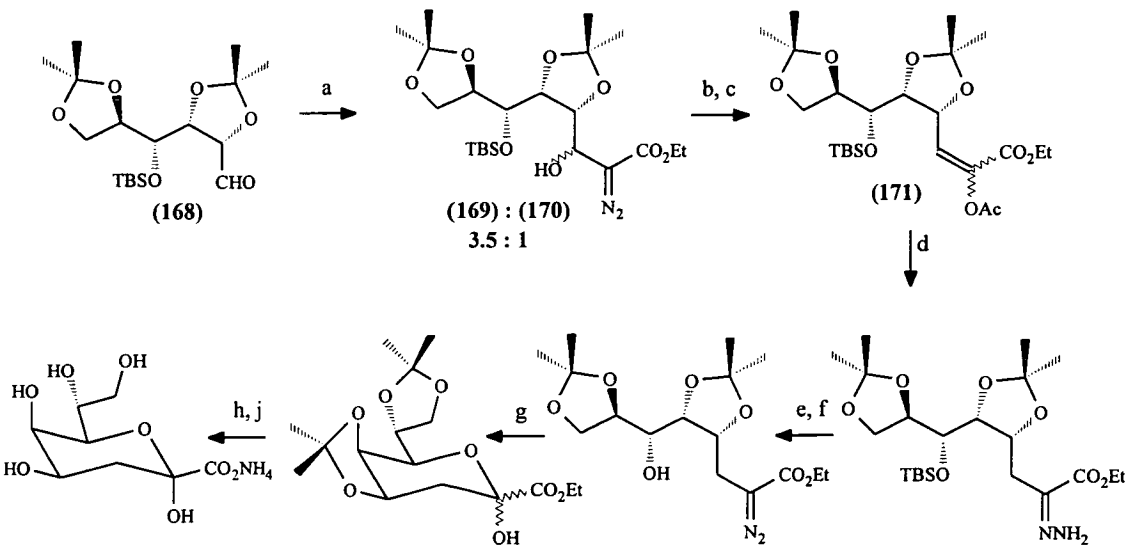


(a) Ac_2O/Py, 68%; (b) NBS/CCl_4, reflux, 1 hr, hυ, 35%; (c) AIBN/benzene, 80°C, 1.5 hr; (d) NaOH/H_2O, r.t., 30 min; (e) $NaBH_4$/H_2O, r.t., 30 min; (f) O_3/THF-H_2O (9:1), 0°C, 10 min; (g) Me_2S, r.t., 10 hr; (h) NH_4OH

Scheme 23

1.5.6.1.3 β-Oxy-α-diazo Carbonyl Compounds

Both KDO and DAH are readily prepared by this method.[163] Reasonably high yields of both 3-deoxy-2-keto aldonic acids are produced under very mild conditions. For synthesising KDO, the β-hydroxy-α-diazo carbonyl derivatives (**169**) and (**170**) are formed by aldol-like condensation of aldehyde (**168**)[174] with ethyl diazoacetate. The Z:E enol acetates (**171**) are afforded after acetylation and rhodium catalysed decomposition. These acetates are then converted through to the ammonium salt of KDO (Scheme 24).



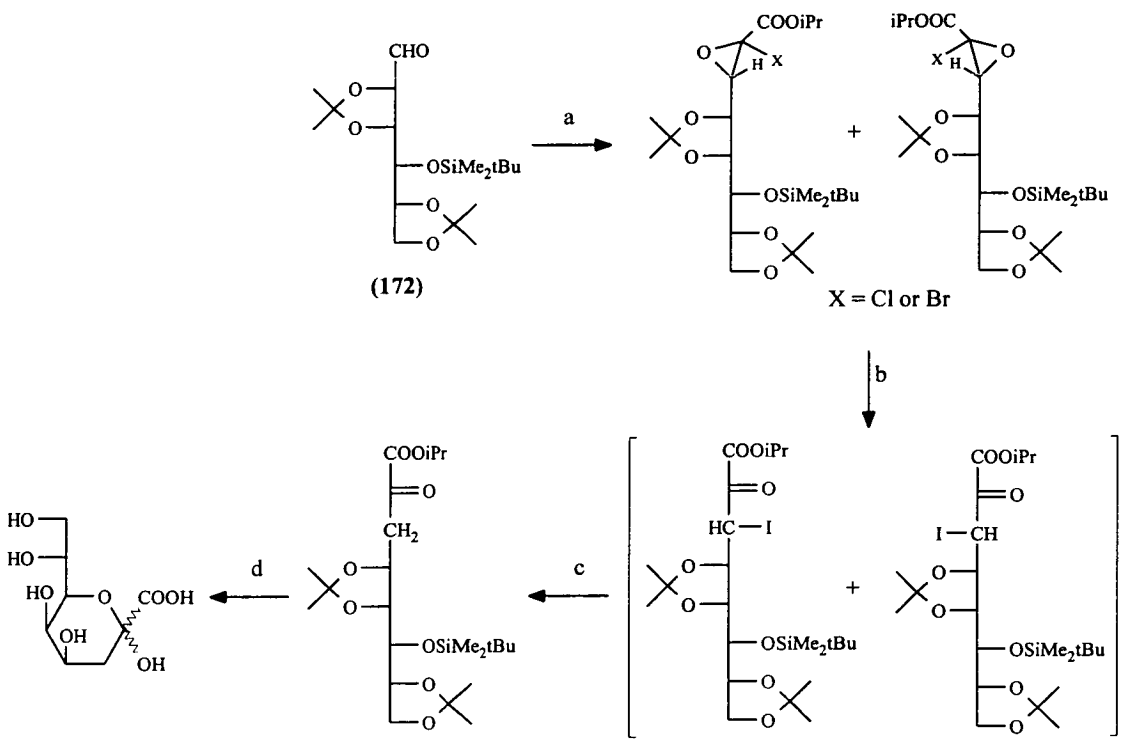
(a) HCN_2CO_2Et, 74%; (b) Ac_2O/Py; (c) $Rh_2(AcO)_4$; (d) NH_2NH_2/MeOH, 88%;
(e) $MnO_2/HCCl_3$; (f) TBAF/THF; (g) m-CPBA/$HCCl_3$; (h) AcOH, 90°C, 15 min; (j) NH_4OH

Scheme 24

1.5.6.1.4 Dihalogeno Ester Carbanions

This approach[11] involves the direct introduction of an α-keto ester moiety onto a carbonyl containing substrate *via* an alkyldichloro or dibromoacetate anion (Scheme 25). Starting from the protected D-mannose (**172**), the methodology allows

for the installation of a pyruvic acid unit onto a glucidic aldehyde. This has two advantages; firstly the commercial availability of the alkyl dihalogenacetate, and secondly the desired ketosidic carboxylic acid is also readily accessible. Since the overall yield of the (+)-KDO ammonium salt from protected D-mannitol (**172**) is 63%, this is far better than any previously reported chemical syntheses of KDO, the best ones being those of Dondoni[9] (6.8%) and Shing[175] (37%).

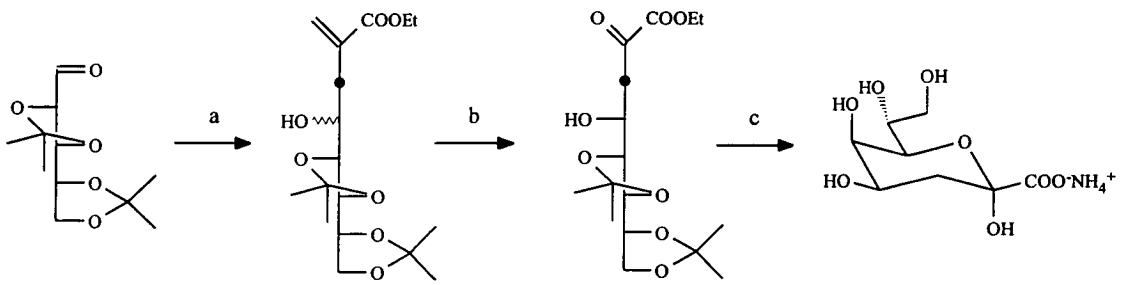


(a) $CHX_2COOiPr$, iPrOK/iPrOH; (b) MgI_2, ether; (c) $NaHSO_3$, H_2O; (d) 90% AcOH, 90°C, 15 min, then K_2CO_3/H_2O, 60°C, 15 min

Scheme 25

1.5.6.1.5 Indium Mediated Allylation in Aqueous Media

Whitesides *et al* have demonstrated[176] the use of metal mediated allylation for the construction of KDO. Addition of indium metal to 2,3:4,5-di-*O*-isopropylidene-D-arabinose (**173**) and ethyl 2-(bromoethyl)acrylate afforded a mixture of diastereomers (**174**) (Scheme 26). The stereochemistry of this reaction is based on similar work performed by Schmidt[177] using acetonide-protected aldoses. After separation of the isomers, ozonolysis produces the α-keto ester (**175**), which is converted through to the ammonium salt of KDO.



(173) (174) erythro : threo 2 : 1 (175)

(a) In ethyl α-(bromomethyl)acrylate, 10% formic acid, aq. CH_3CN; (b)i O_3, MeOH, -78°C; ii Me_2S, MeOH, 78°C to r.t.; (c) aq. TFA; NH_4OH

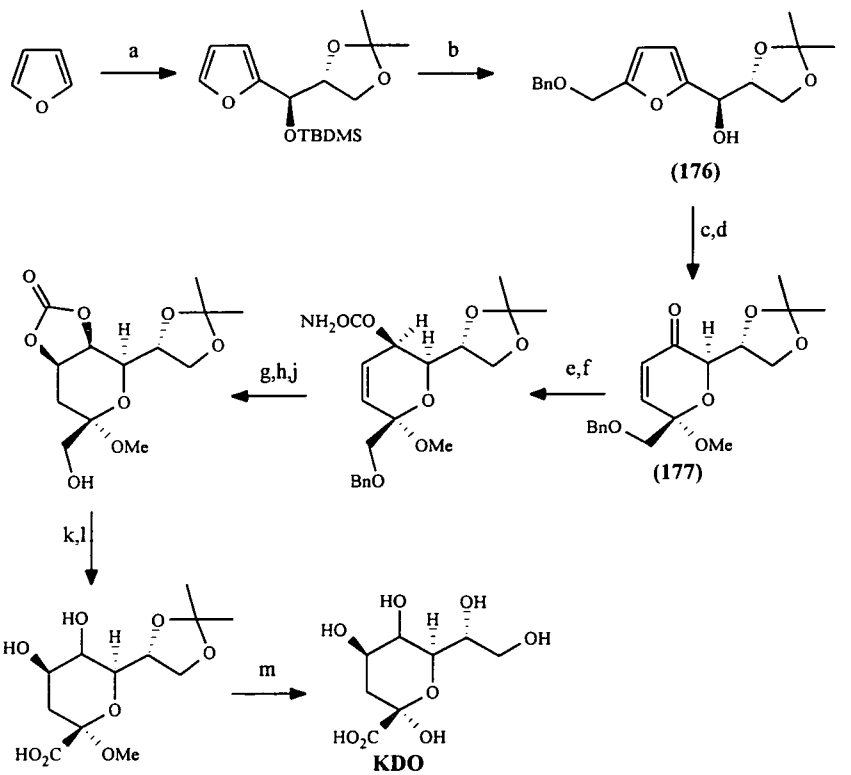
Scheme 26

1.5.6.2 Syntheses from Non-Carbohydrates

A number of syntheses using non-carbohydrate based starting materials have been utilised for the synthesis of KDO. The use of thiazoles as the synthetic equivalent of a formyl group has also been demonstrated in the synthesis of carbohydrates,[178] as already mentioned for the synthesis of DAH. The octulosonic acid KDO has been produced in 6.8% by chain elongation of D-arabinose diacetonide.[178]

1.5.6.2.1 Furan Route

This route[10,179] provides a concise and efficient strategy for the asymmetric synthesis of (+)-KDO in twelve steps from furan and isopropylidene-D-glyceraldehyde (Scheme 27). The methodology can be extended to the *de novo* syntheses of other higher monosaccharides. The approach to KDO involves the oxidative conversion of a suitably functionalised furfuryl carbinol (**176**) into the desired dihydro-3-pyranone (**177**). The dihydropyranone then provides an excellent template for efficient, stereoselective introduction of new functional groups and substituents onto the hydropyran ring.

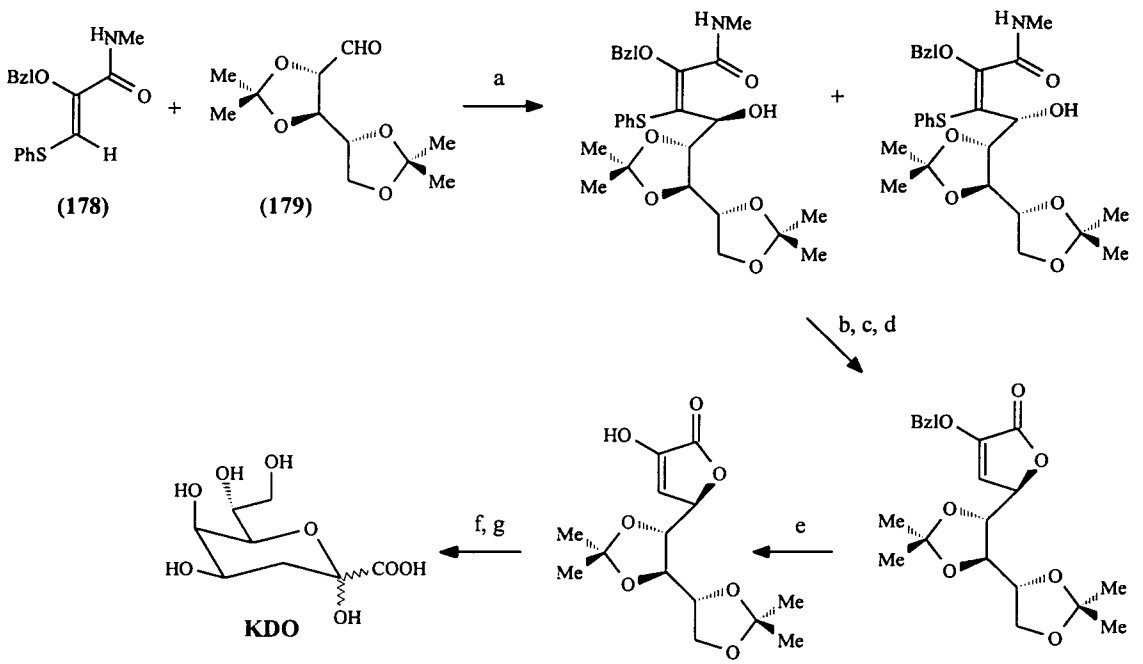


(a)i *n*-BuLi, THF, -78°C; ii $ZnBr_2$, isopropylidene-D-glyceraldehyde, THF, 0°C: iii *t*-TBDMS, THF, r.t.; (b)i *t*-BuLi, THF, -78°C; ii $BnOCH_2Cl$, *n*-Bu_4NF, r.t., 10 hr; (c) $VO(acac)_2$, *t*-BuOOH, CH_2Cl_2, r.t., 3 hr; (d) MeI, Ag_2O, r.t., 12 hr; (e) K-Selectride, THF, -78°C; (f) trichloroacetyl isocyanate, CH_2Cl_2; (g) $I(Collodone)_2ClO_4$, CH_3CN, 24 hr; (h) $HSn(n\text{-}Bu)_3$, AIBN, toluene, reflux, 3 hr; (j) Ra-Ni/H_2, 48 hr; (k) Oxalyl chloride; (l) Ag_2O, aq. NaOH, aq. EtOH, r.t., 24 hr; (m)i DOWEX 50W(H^+), H_2O, 80°C, 1 hr (pH<2); ii 5% NH_4OH, 0°C, 24 hr (pH 10); iii DOWEX 50W(H^+) (pH 7); iv sequential chromatography on cellulose and Sephadex G-10.

Scheme 27

1.5.6.2.2 Using Functionally Substituted Vinyl Carbanions

This synthesis[151] uses a *C*-lithiated, functionally substituted acrylic acid (2-benzyloxy-3-(phenylthio)acrylic acid N-methylamide) (**178**) as a pyruvic acid-equivalent three carbon building block and 2,3:4,5-di-*O*-isopropylidene-D-arabinose (**179**) as a five carbon unit (Scheme 28). Steric and stereoelectronic influences favour *re*-attack to give the D-*manno* product, rather than *si* attack to give the D-*gluco* product.



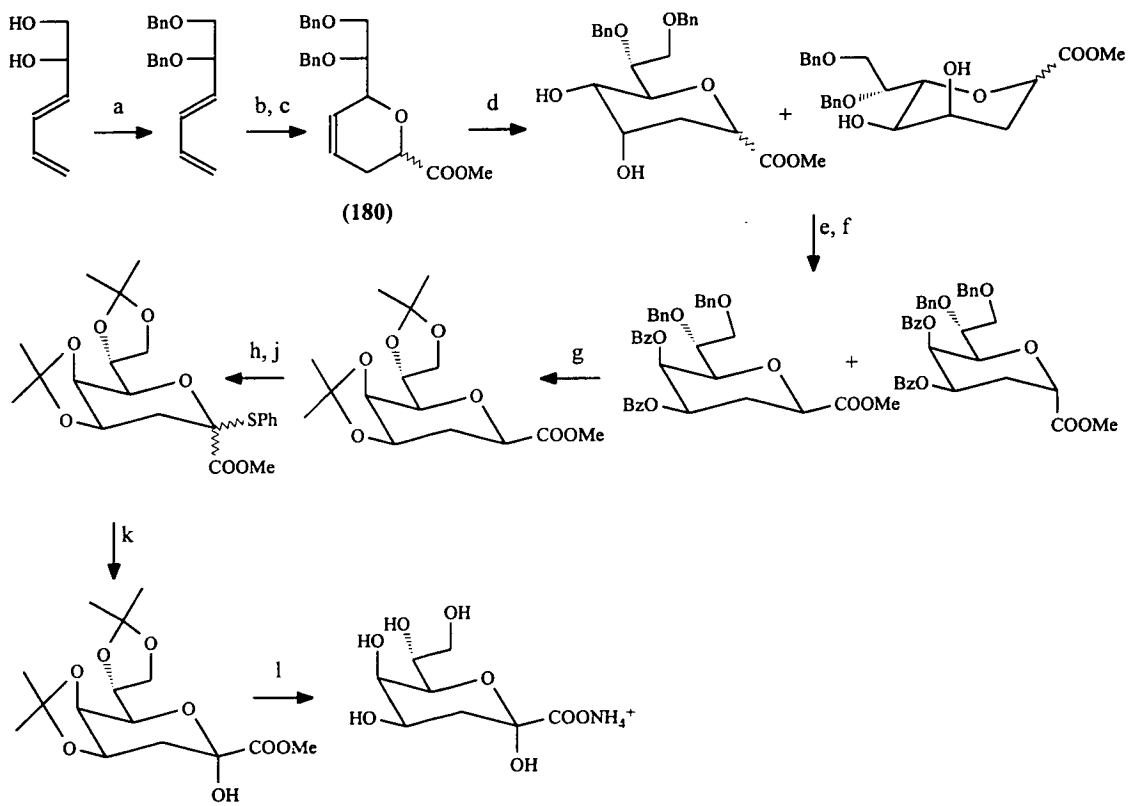
(a) LDA, THF, HMPT; (b) AcOEt/ petroleum ether, reflux, 6 hr; (c) Bu_3SnH, benzene, AIBN, reflux, 8 hr; (d) Py, HBr, reflux, 24 hr; (e) Pd/C, H_2; (f) CF_3COOH; (g) NH_3

Scheme 28

1.5.6.2.3 Aqueous Hetero Diels-Alder Reaction

This route[105] involves synthesis of 2-deoxy-KDO as a synthetic intermediate, which can then be used as a precursor of glycosyl donors of KDO, leading to a large range of compounds (Scheme 29).

The strategy is based upon three key steps leading to an activated form of KDO. The first step which has never been used before for the construction of carbohydrates is *via* a hetero-Diels Alder reaction involving the formation of the C-2/C-3 and C-6-O bonds in (**180**). Cis dihydroxylation *via* osmylation of the newly created double bond, followed by oxidation (bis inversion) of a 2-deoxy-ulosonic acid provided access to KDO. The heterocycloaddition, both in water or in neat conditions, exhibits only modest facial stereoselectivity (74:26), however the next two steps are totally stereospecific. The advantage of this method is that it uses the cheap commercial aqueous solution of glyoxylic acid.



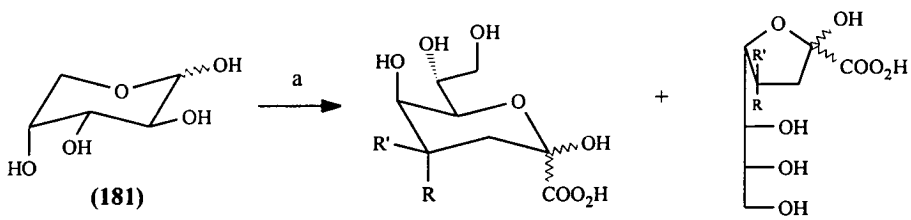
(a) NaH, $PhCH_2Br$; (b) HCOCOOBu, 18 hr, 130°C; (c) MeOH, TsOH; (d) OsO_4 cat., NMO, acetone-water; (e) Tf_2O, -10°C, CH_2Cl_2, Py; (f) $PhCOO^-NBu_4^+$, toluene; (g) CH_3ONa, MeOH, 20 min, then H_2, Pd/C, then dimethoxypropane, acetone, *p*-TsOH; (h) LDA, TFA; (j) PhSSPh; (k) NBS-H_2O; (l) Dowex 50 (H^+), NaOH, Dowex 50 (NH_4^+)

Scheme 29

1.5.6.3 Enzymatic Approaches

Enzyme catalysis has a major advantage synthetically in that the stereoselectivity of the resulting products can be predicted.[180] However, different solvents,[181] substrate structures,[182] or temperatures[183] can occasionally change the stereoselectivity. The diastereofacial selectivity of the aldehyde component in enzymatic aldol reactions is often consistent and completely controlled by the enzyme.[165]

Sialic acid aldolase-catalysed condensation of pyruvate with D-arabinose (**181**) (Scheme 30) results in a lack of stereoselectivity of the aldolase with equimolar amounts of KDO and epi-KDO being observed both in their pyranose and furanose forms.[144,168,184,185]



R = H, R' = OH (KDO)
R = OH, R' = H (epi-KDO)

R = H, R' = OH
R = OH, R' = H

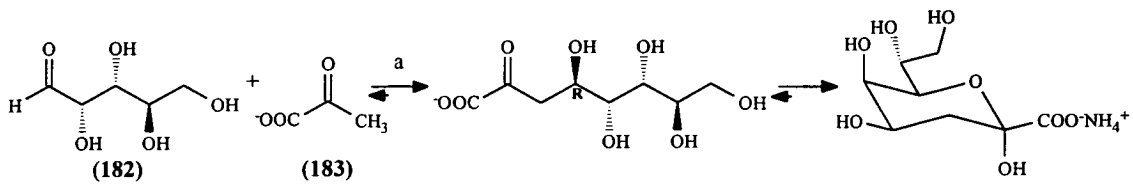
(a) sialyl aldolase, pyruvate

Scheme 30

However, a practical preparation of KDO can be obtained by this method using a high concentration of the acceptor D-arabinose, and this leads to an increase in percentage of KDO up to 83%. The inverted stereoselectivity originates from the $^1C_4$ major D-arabinose conformer. Complete inversion of stereoselectivity has been noticed recently[168,169] for this aldolase with various sugars such as 6-deoxy-L-mannose and L-mannose which are known to adopt the preferred $^1C_4$ conformation. In each of these cases facial attack was exclusively *re* resulting in generation of a single product

in 80% yield with *R* configuration at the new stereogenic centre. Synthesis of KDO was achieved with a 25-fold excess of D-arabinose in an enzyme membrane reactor allowing a continuously operating process. Anion-exchange chromatography[185] was used to successfully separate the diastereoisomers and isolate pure KDO.

Enzymic synthesis of KDO has been reported using KDO-8-phosphate synthetase to catalyse the reaction of D-arabinose-5-phosphate with phosphoenolpyruvate.[145] The enzymatic synthesis using KDO aldolase[7] on a multi-mmol scale can also be achieved using D-arabinose (**182**) and a 10 molar excess of pyruvate (**183**) to give 67% yield of ammonium KDO (Scheme 31).



(a) KDO aldolase

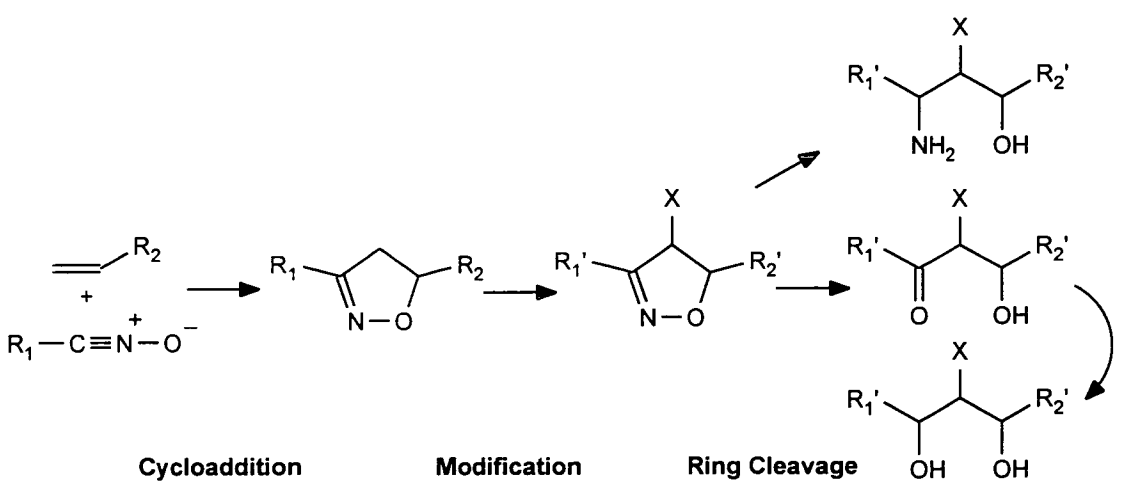
Scheme 31

The *re* face of the carbonyl group of the acceptor substrate is attacked by the pyruvate. This is complementary to the majority of sialic acid aldolase reactions which are under *si* face attack. The yield of enzymatic reaction is comparable to the highest one obtained by the modified Cornforth synthesis (66%).[164] So KDO aldolase-catalysed condensation is effective for the synthesis of KDO and analogues. The reactions are stereospecific with formation of a new *R* stereocentre at C-3 from D-arabinose and related substructures.

2 RESULTS AND DISCUSSION

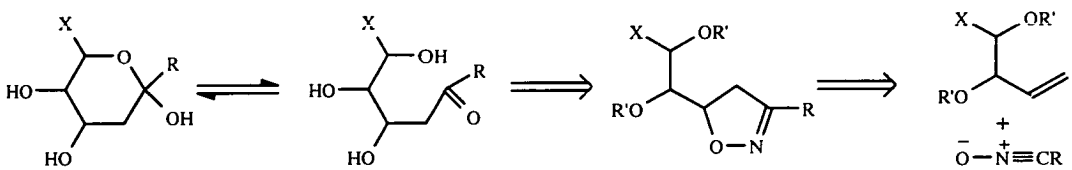
2.1 Introduction

There is considerable interest in the role of ulosonic acids in various biological systems and in their potential pharmaceutical and agrochemical applications. Although much effort[2,4,68,96,144] has been devoted to the isolation, characterisation and preparation of the ulosonic acids themselves, and to investigating their biological functions, there is a need for novel synthetic routes designed to produce a variety of analogues. A number of methods, including iterative procedures, convergent and non-convergent syntheses have been used (see Sections 1.4.5 and 1.5.7 for surveys). The approach adopted in the present work has been to construct ulosonic acid analogues *via* an intermediate isoxazoline formed by the cycloaddition of a nitrile oxide to a sugar alkene. In comparison to the complexity of the routes outlined in the above mentioned surveys, our approach is short and offers access to a wide range of analogues. Nitrile oxide/isoxazoline methodology, which is widely used in natural product synthesis,[186] involves three basic steps (Scheme 32).



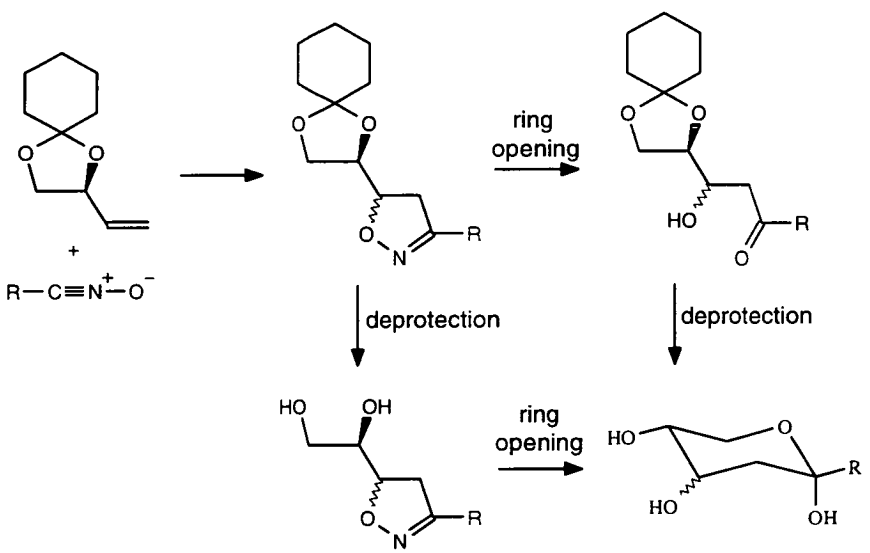
Scheme 32

This chemistry is considered to be particularly well suited for the construction of the ulosonic acid framework. The overall synthetic strategy (Scheme 33) involves regiospecific and diastereoselective cycloaddition of the nitrile oxide to the appropriate sugar alk-1-ene. Deprotection and reductive hydrolytic ring cleavage of the resulting isoxazoline should then afford the target compound, initially in its open chain form. Analogues can be produced by varying the alkene and nitrile oxide components and by modification of substituents at the isoxazoline stage (Scheme 32).



Scheme 33

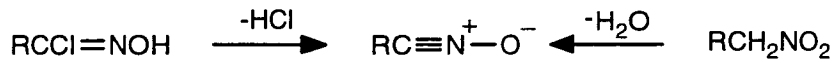
A model system (Scheme 34) involving hexulosonic acid analogues was first investigated in order to establish the methodology which could then be applied to the synthesis of higher 2-ulosonic acid derivatives of biological significance.



Scheme 34

2.2 Synthesis of Nitrile Oxide Precursors

Two methods were used for the generation of the nitrile oxides: dehydrochlorination of the parent hydroximoyl chloride with triethylamine and dehydration of nitroethane with isocyanate and triethylamine (Scheme 35). In each case the nitrile oxide was formed *in situ* to minimise the formation of unwanted side products.



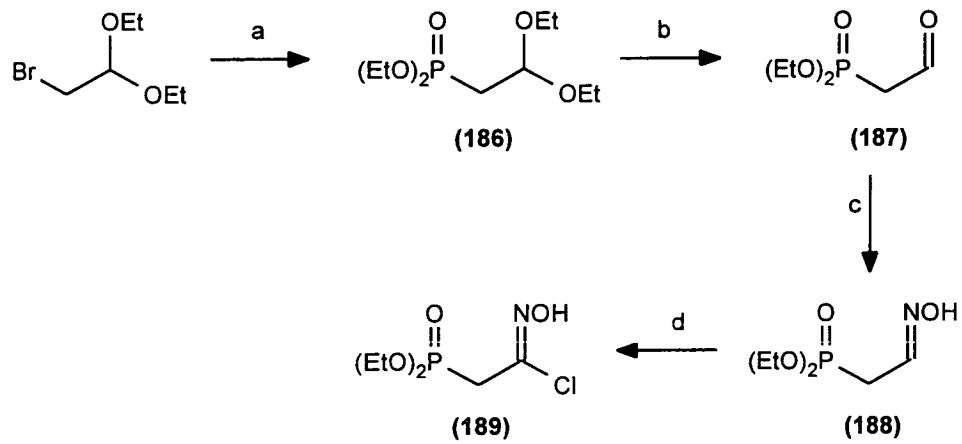
(**184**) R = CO_2Et
(**185**) R = Ph

Scheme 35

Ethyl chloro-oximinoacetate (EtO_2CCCl=NOH) (**184**) was prepared[187] in 48% yield by nitrosation of glycine ester hydrochloride using hydrochloric acid and sodium nitrite. Synthesis of benzohydroximoyl chloride (PhCCl=NOH) (**185**)[188] involved conversion of benzaldehyde to the corresponding oxime with subsequent chlorination using chlorine gas to the desired compound in 80% yield.

(Diethoxyphosphoryl)acetohydroximoyl chloride (**189**) was prepared by the method of Tsuge[189] from bromoacetaldehyde diethyl acetal (Scheme 36). After heating with triethyl phosphite fractional distillation yielded the phosphonate derivative (**186**) which was converted to aldehyde (**187**) by treatment with aqueous hydrochloric acid. Reaction of the aldehyde (**187**) with hydroxylamine hydrochloride provided the oxime (**188**) in 61% yield which was shown to be present as an approximately 7:4 mixture of the anti/syn isomers. The hydroximoyl chloride (**189**)

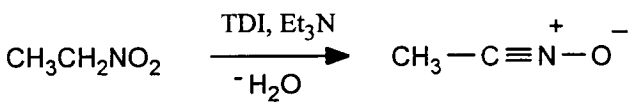
was produced by addition of chlorine gas to oxime (**188**) and then *in situ* dehydrochlorination afforded the desired nitrile oxide.



(a) $(EtO)_3P$, reflux, 5 hr; (b) aq. HCl (2% w/v), reflux, 20 min; (c) $NH_2OH.HCl$, r.t., 17 hr; (d) Cl_2, -10°C

Scheme 36

Acetonitrile oxide (**191**) was generated by a modification of the Mukaiyama method.[36] This involved *in situ* dehydration of nitroethane (**190**) using tolylene-2,4-diisocyanate (TDI) in conjunction with a catalytic amount of triethylamine (Scheme 37).



(**190**) (**191**)

Scheme 37

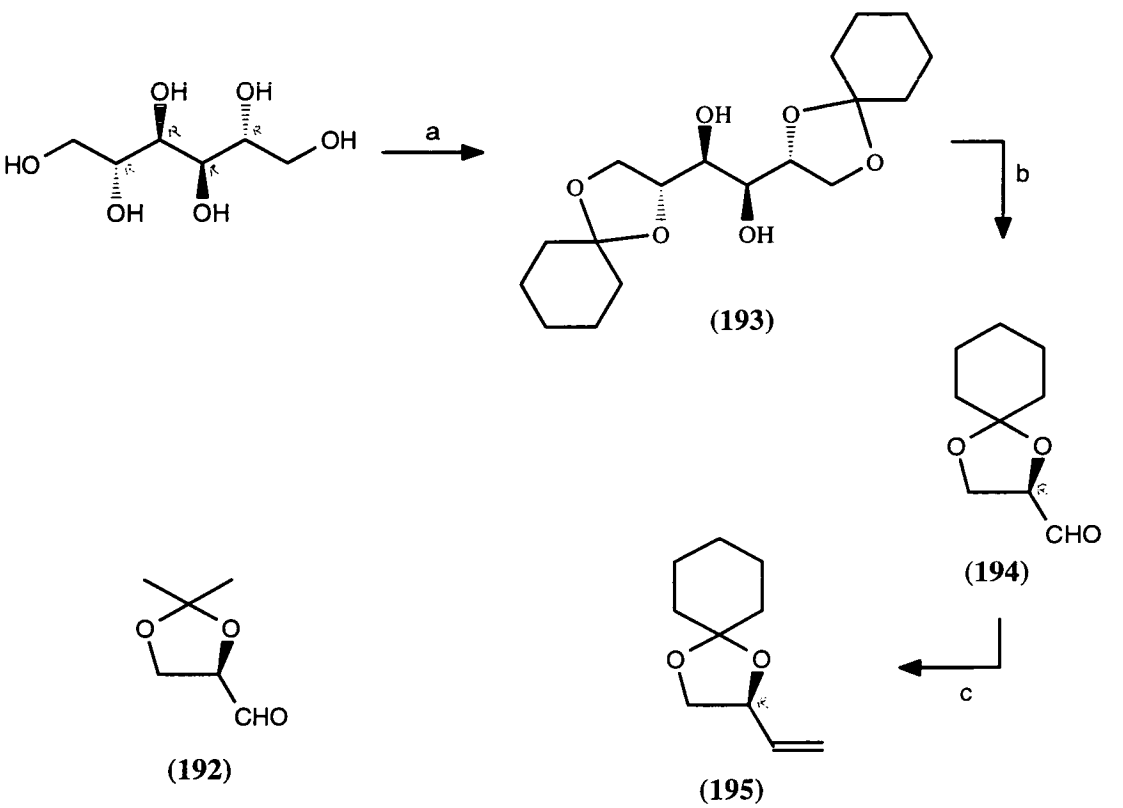
2.3 3-Deoxy-hexulosonic Acid Analogues

The approach selected for the synthesis of hexulosonic acid analogues is *via* the four carbon alkene (**195**) and involves three stages; cycloaddition of the alkene to an appropriate nitrile oxide followed by, either deprotection and subsequent reductive hydrolytic ring opening of the isoxazoline or *vice versa* (Scheme 34). Previous observations[190] have shown that isopropylidene-protected D-glyceraldehyde (**192**) is difficult to handle with a tendency to polymerise, as well as being unstable in the presence of acids. To minimise these difficulties cyclohexylidene protection[190] which affords a more stable aldehyde was chosen as the source of our model alkene (**195**).

2.3.1 Synthesis of 1,2-Dideoxy-3,4-*O*-cyclohexylidene-D-*glycero*-but-1-enitol (**195**)

1,2-Dideoxy-3,4-*O*-cyclohexylidene-D-*glycero*-but-1-enitol (**195**) has previously been prepared from but-3-ene-1,2-diol and cyclohexanone/2,2-dimethoxypropane/*p*-TsOH(catalyst) in benzene by transketalisation.[191,192] However the starting material is not readily accessible. In the present work, the route selected involved Wittig olefination of the easily prepared D-glyceraldehyde derivative (**194**) (Scheme 38),[190] and the title compound was thus available in three steps from D-mannitol with an overall yield of 45%. Selective protection[190] of the free C-1, C-2 and C-5, C-6 *vicinal* hydroxyls using cyclohexanone, triethyl orthoformate and boron trifluoride etherate as a catalyst yielded the dicyclohexylidene adduct (**193**). The reaction is suggested[190] to proceed in two steps: formation initially of cyclohexanone

diethyl acetal, followed by acetal exchange with mannitol. Subsequent treatment with sodium periodate and tetrabutylammonium bromide as phase transfer catalyst afforded the aldehyde (**194**). Since (**194**) polymerises on standing, its purification by distillation/sublimation using a cold-finger distillation apparatus was performed immediately prior to the Wittig reaction using methyl triphenylphosphorane for the conversion to alkene (**195**).



(a) Cyclohexanone, $HC(OEt)_3$, $BF_3.Et_2O$, DMSO; (b) $NaIO_4$, Bu_4NBr;
(c) $Ph_3P^+CH_3I^-$, $(CH_3)_3COK$, THF

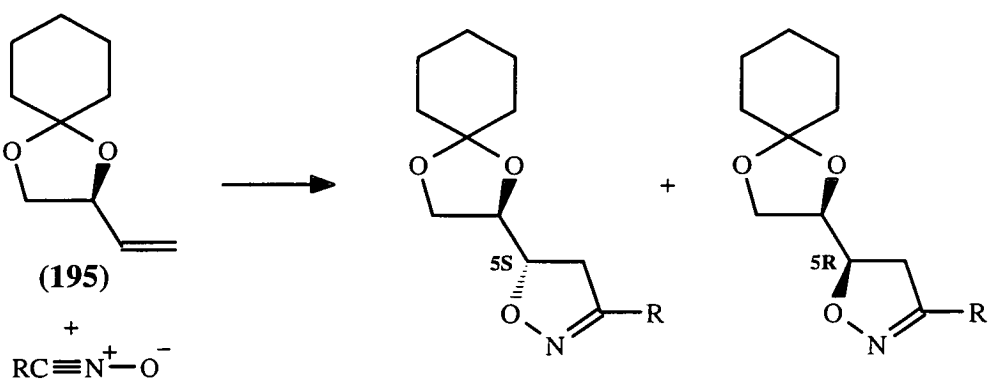
Scheme 38

2.3.2 Investigation of π-Facial Selectivity in Nitrile Oxide Cycloadditions to 1,2-Dideoxy-3,4-*O*-cyclohexylidene-D-*glycero*-but-1-enitol (195)

2.3.2.1 Nitrile Oxide Cycloadditions to Alkene (195)

Four nitrile oxides were examined: ethoxycarbonylformonitrile oxide [$EtO_2CC{\equiv}N^+$-O^-], benzonitrile oxide [$PhC{\equiv}N^+$-O^-], (diethoxyphosphoryl)acetonitrile oxide [$(EtO)_2P(O)CH_2C{\equiv}N^+$-$O^-$] and acetonitrile oxide [$CH_3C{\equiv}N^+$-O^-]. The first three were generated *in situ* by slow addition, *via* a motorised syringe pump, of triethylamine (1.1 M eq) in diethyl ether to a chilled solution of the hydroximoyl chloride and a slight excess of the appropriate alkene (1:1.5) in diethyl ether at 0°C. These conditions were chosen to generate a low concentration of the nitrile oxide in the presence of excess dipolarophile in order to minimise the competing dimerisation of the nitrile oxide to unwanted furazan *N*-oxide (furoxan) by-products. The diastereoisomeric cycloadducts were separated from the excess alkene and dimers by chromatography. A small quantity of the adduct mixture was retained for isomer ratio determination, while the remainder was separated by flash chromatography to afford the pure isoxazolines. The diastereoisomeric ratios were determined by comparison of corresponding integrals in the ^1H NMR spectra of the cycloadduct mixtures. The recovered alkene was sufficiently pure for reuse. The individual adducts were characterised by ^1H and ^{13}C NMR spectroscopy, FAB mass spectroscopy and optical rotation. Chemical formulae were verified by high resolution FAB mass spectroscopy and/or combustion analysis. The assignment of configuration for the newly created chiral centre at C-5 is based on correlation of NMR and optical rotation data with a previously reported X-ray analysis[191] for benzonitrile oxide adduct (**196**).

Cycloaddition of alkene (**195**) to benzonitrile oxide (Scheme 39) was carried out as outlined above. A mixture of diastereoisomeric isoxazolines (**196**) and (**197**) in combined yield of 88% and ratio of 79:21 was obtained after chromatography to remove 3,4-diphenylfurazan *N*-oxide (the nitrile oxide dimer) (5%). The isomer ratio was determined by comparison of the integrals for the epimeric 5-H signals in the ^{1}H NMR spectrum of the mixture. The assignment of 5*S*-configuration for the major isomer (**196**) corresponding to an *erythro* relationship about the C-5/C-6 bond was made by correlation of ^{13}C NMR shift differences with those reported by Jäger[191] for addition of benzonitrile oxide to the same alkene (**195**), and also on optical rotation data and TLC properties.



(**196**) R = Ph
(**198**) R = CO_2Et
(**200**) R = $CH_2P(O)(OEt)_2$
(**202**) R = Me
(**204**) R = CH_2OH

(**197**) R = Ph
(**199**) R = CO_2Et
(**201**) R = $CH_2P(O)(OEt)_2$
(**203**) R = Me
(**205**) R = CH_2OH

Scheme 39

Similarly, the corresponding cycloaddition of ethoxycarbonylformonitrile oxide to the same alkene (**195**) afforded the adducts (**198**) and (**199**) in combined yield of 68% and ratio 77:23 together with the nitrile oxide dimer 3,4-

diethoxycarbonylfurazan *N*-oxide (17%). The ratio was again determined by comparison of the integrals of the 5-H signals in the ^{1}H NMR of the mixture. Although crystalline solids were obtained for both adducts, X-ray analysis was not necessary since the configuration at C-5 for the main product could be confidently assigned by correlation of ^{13}C NMR shift differences,[191] optical rotation data and TLC properties. The major adduct (**198**) was thereby assigned *S*-configuration at C-5, corresponding to an *erythro* relationship about the C-5/C-6 bond.

Cycloaddition of (diethoxyphosphoryl)acetonitrile oxide to the title alkene (**195**) produced a 40% combined yield of the diastereoisomeric 2-isoxazolines (**200**) and (**201**) in a ratio of 81:19 (Scheme 39). Again the ratio was determined by comparison of the integrals for the 5-H signals in the ^{1}H NMR. Although the isoxazolines were chromatographically inseparable the isoxazoline ring protons for each isomer could be assigned in the ^{1}H NMR of the mixture due to differences in signal intensities. The major isomer was identified as the *erythro* adduct (**200**) based on comparison of its spectral parameters with those of similar adducts, as above.

Finally, the cycloaddition of acetonitrile oxide to alkene (**195**) was accomplished using the Mukaiyama procedure. *In situ* dehydration of nitroethane (**190**) using tolylene-2,4-diisocyanate (TDI) in conjunction with a catalytic amount of triethylamine generated the desired acetonitrile oxide and the diastereoisomeric 2-isoxazolines (**202**) and (**203**) were formed in a ratio 82:18 and a combined yield of 65% (Scheme 39). The ratio was determined from the epimeric 5-H proton signals which were well resolved. Again the major isomer was assigned as having *erythro* stereochemistry based on comparison of spectral and TLC properties.

The nitrile oxide cycloadditions to alkene (**195**) all proceed with moderate π-facial selectivity in favour of *erythro* adducts with d.e. values ranging from 54% for ethoxycarbonylformonitrile oxide to 64% for acetonitrile oxide. The *erythro:threo* ratios for the four case are presented in Table 1.

Nitrile Oxide	***erythro:threo***
EtO_2CCNO	77:23
PhCNO	79:21 81:19[191]
$(EtO)_2P(O)CH_2CNO$	81:19
MeCNO	82:18

Table 1 : Isomer Ratios for Cycloaddition of Nitrile Oxides to Alkene (**195**)

2.3.2.2 Characterisation of Isoxazoline Cycloadducts

It has been reported by Jäger *et al*[191] that correlation of ^{1}H and ^{13}C NMR data and optical rotation is a reliable method for predicting the stereochemistry at the new chiral centre at C-5 of the isoxazoline. A similar approach has been adopted for assigning structures to the individual major and minor adducts in the present work. These characteristic properties are therefore summarised below, along with those for (**204**) and (**205**) obtained after borohydride reduction (Section 2.3.7).

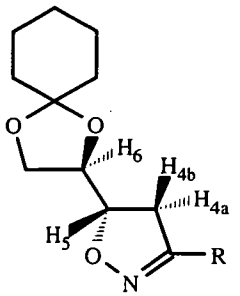
TLC

The major *erythro* adduct in each case has a larger R_f value on silica than the corresponding minor *threo* adduct [0.23 *cf* 0.14 for (**196**)/(**197**), 0.15 *cf* 0.09 for

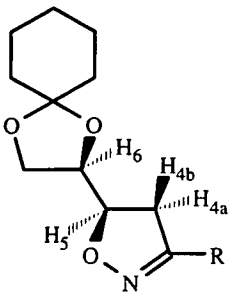
(**198**)/(**199**) (30% ether/hexane); 0.28 *cf* 0.21 for (**202**)/(**203**), 0.57 *cf* 0.52 for (**204**)/(**205**) (100% ether)].

^{1}H NMR

The ^{1}H NMR spectra show major differences between the isomers for protons 4a-H, 4b-H, 5-H and 6-H. The isoxazoline ring protons 4a-H and 4b-H give rise to a characteristic ABX system with 5-H, which is adjacent to the ring oxygen at highest chemical shift. The ^3J values of 6-11 Hz for 4ab-H/5-H and the geminal coupling of *ca* 18 Hz for 4a-H/4b-H are also typical of 3,5-disubstituted isoxazolines.[193-195] It is observed that for the major *erythro* adduct the 5-H signal absorbs at significantly lower frequency than for the corresponding minor *threo* product [$\Delta\delta_H$ -0.17 ppm for (**196**)/(**197**), -0.13 ppm for (**198**)/(**199**), -0.08 ppm for (**200**)/(**201**), -0.18 ppm for (**202**)/(**203**), -0.14 ppm for (**204**)/(**205**)]. The signals for the methylene protons of the isoxazoline ring, 4a-H and 4b-H, are closer together in the major *erythro* adducts. This is particularly noticeable when comparing isomers (**198**) and (**199**). In the case of major *erythro* adduct (**198**) they are coincident, while in the minor *threo* diastereoisomer (**199**) they are separated by 0.10 ppm. It is noteworthy that the coupling between 5-H and 6-H in the *erythro* isomer is significantly greater than that in the minor *threo* product [7.6 *cf* 4.4 Hz for (**196**)/(**197**), 7.0 *cf* 4.2 Hz for (**198**)/(**199**), 7.8 *cf* 4.6 Hz for (**202**)/(**203**), 7.1 *cf* 4.4 Hz for (**204**)/(**205**)]. This suggests that the two compounds adopt different conformations in solution. The chemical shifts and coupling constants are summarised in Tables 2 and 3.



Erythro



Threo

R	Isoxazoline	δ_H/ppm			
		4a-H	4b-H	5-H	6-H
Ph	*Erythro* (**196**)	3.37	3.47	4.64	4.05
	Threo (**197**)	3.88	3.26	4.81	4.33
CO_2Et	*Erythro* (**198**)	3.26	3.26	4.70	4.08
	Threo (**199**)	3.14	3.24	4.83	4.25
$CH_2P(O)(OEt)_2$	*Erythro* (**200**)	3.12	3.20	4.54	nd
	Threo (**201**)	3.25	3.17	4.62	nd
Me	*Erythro* (**202**)	2.91	3.01	4.41	3.95
	Threo (**203**)	2.94	2.81	4.59	4.20
CH_2OH	*Erythro* (**204**)	3.06	3.14	4.53	4.02
	Threo (**205**)	2.97	3.10	4.67	4.23

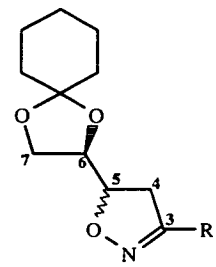
Table 2 : Selected ^{1}H NMR data for cycloadducts

R	Coupling	*J*/Hz			
		4a,4b	**4a,5**	**4b,5**	**5,6**
Ph	*Erythro* (**196**)	17.0	7.3	9.3	7.6
	Threo (**197**)	17.1	10.7	8.3	4.4
CO_2Et	*Erythro* (**198**)	-	8.9	8.9	7.0
	Threo (**199**)	17.8	11.4	8.5	4.2
$CH_2P(O)(OEt)_2$	*Erythro* (**200**)	nd	6.5	10.6	6.5
	Threo (**201**)	nd	11.1	8.0	5.2
Me	*Erythro* (**202**)	17.3	6.4	10.1	7.8
	Threo (**203**)	17.2	10.9	7.9	4.6
CH_2OH	*Erythro* (**204**)	17.5	6.9	10.1	7.1
	Threo (**205**)	17.5	11.0	7.9	4.4

Table 3 : Selected ^{1}H NMR data for cycloadducts

^{13}C NMR

The ^{13}C NMR spectra for the pairs of isomers show significant differences for C-4 to C-7. In each case the major *erythro* adduct has a higher δ-value for C-4 [$\Delta\delta_C$ +1.2 ppm for (**196**)/(**197**), +0.7 ppm for (**198**)/(**199**), +0.7 ppm for (**202**)/(**203**), +1.0 ppm for (**204**)/(**205**)] as does C-5 ($\Delta\delta_C$ +1.0 ppm for each of the pairs of isomers). There is also a marked difference shown for C-7 [$\Delta\delta_C$ +2.0 ppm for (**196**)/(**197**), +1.8 ppm for (**198**)/(**199**), +2.1 ppm for (**202**)/(**203**), +1.9 ppm for (**204**)/(**205**)]. These findings are shown in Table 4.



R		δ_C/ppm			
		C-4	C-5	C-6	C-7
Ph	*Erythro* (**196**)	37.7	81.3	75.5	66.8
	Threo (**197**)	36.5	80.3	75.8	64.8
CO_2Et	*Erythro* (**198**)	36.3	83.5	75.1	66.4
	Threo (**199**)	35.6	82.5	75.6	64.6
Me	*Erythro* (**202**)	36.4	80.3	75.6	66.8
	Threo (**203**)	35.7	79.3	75.8	64.7
CH_2OH	*Erythro* (**204**)	37.6	81.0	75.5	66.7
	Threo (**205**)	36.6	80.0	75.5	64.8

Table 4 : Selected ^{13}C NMR data for cycloadducts

Optical Rotation

The major *erythro* isomer in each case was found to have a more positive $[\alpha]_D$ value. These values were measured at similar concentrations in chloroform. This result is important for correlation with known adducts[191] and for thus assigning configuration at the new chiral centre, C-5. Table 5 summarises this information.

Erythro (**196**)	*Threo* (**197**)	*Erythro* (**198**)	*Threo* (**199**)	*Erythro* (**204**)	*Threo* (**205**)
+23.4	-39.1	+58.1	-153.8	+59.5	-68.3

Table 5

Melting Points

It is observed that the minor *threo* isomer always has a slightly higher m.p. than the corresponding major *erythro* adduct (Table 6).

R	m.p.°C	
	Erythro	***Threo***
Ph	61-63[191]	96-98
CO_2Et	40.8-41.9	47-49
CH_2OH	80-82	83.5-85.5

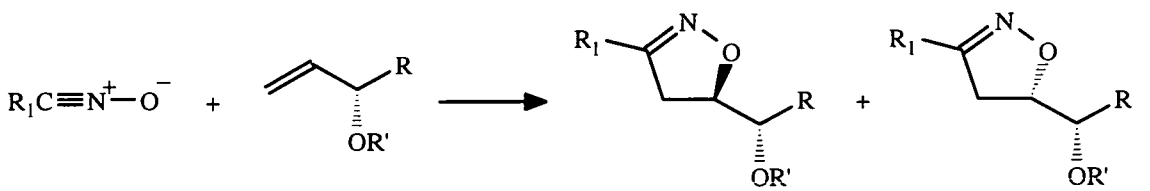
Table 6

2.3.2.3 Rationale for Stereoselectivity of Nitrile Oxide Cycloadditions

The degree of π-facial selectivity observed for cycloadditions to alkene (**195**) in favour of *erythro* adducts can be rationalised in terms of the so-called "inside alkoxy effect" hypothesis proposed by Houk *et al*[196] to account for nitrile oxide cycloadditions to chiral allyl ethers.

Control of selectivity in nitrile oxide cycloaddition reactions is a key target in the development of the nitrile oxide-isoxazoline synthetic route to natural products and analogues, and has been the subject of intensive investigation.[15,16,43-45,193] Whereas the reaction with monosubstituted alkenes is either regiospecific or highly regioselective (>90:10) in favour of 5-substituted 2-isoxazoline cycloadducts,[13] the extent of π-facial discrimination in additions to alkenes bearing an allylic stereocentre is more variable.[196-199] The ratio of products is dependent on the steric and electronic nature of both the allylic and homoallylic substituents, and appears to be subject to

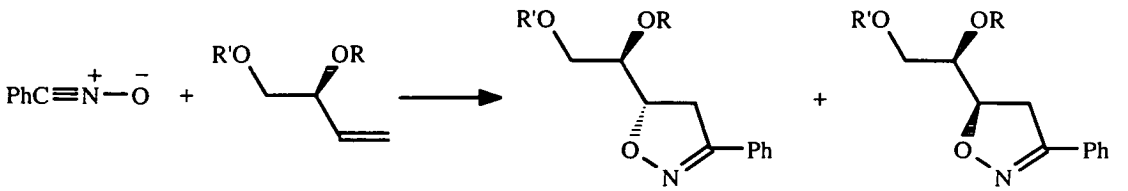
subtle variations in geometry. Chiral allyl ethers (**206**) yield predominantly *erythro* adducts[197] resulting from *anti* addition (Scheme 40, R′ ≠ H), this preference being attributed to the so-called "inside alkoxy effect" proposed by Houk *et al.*[196-198] There is negligible effect on the selectivity by varying the alkoxy substituent R′.



(**206**) *Erythro* *Threo*

Scheme 40

However, for the special case of allyl alcohols (R′ = H), the selectivity can be markedly different, with a slight preference for the *threo* product sometimes being observed.[197] Changing the bulkiness of the R group results in an increase in *erythro* selectivity. In contrast, for *S*-but-3-ene-1,2-diol (**207**) and its diacetate derivative (**208**) the reported[196] diastereoselectivity is low with d.e. values of 22% and 6% respectively for the formation of isoxazolines (**210**)/(**211**) and (**212**)/(**213**) on reaction with benzonitrile oxide (Scheme 41). Somewhat higher levels of selectivity (50-60% d.e.) were found for the corresponding addition to 2-vinyl-1,3-dioxolane (**209**), the cyclic analogue in which the 1,2-diol is protected as its isopropylidene derivative.[196,198] This level of selectivity is similar to that observed in our present work involving cyclohexylidene protection.



Erythro | *Threo*

(**207**) R = R′ = H
(**208**) R = R′ = Ac
(**209**) RR′ = CMe_2

(**210**) R = R′ = H
(**212**) R = R′ = Ac

(**211**) R = R′ = H
(**213**) R = R′ = Ac

Scheme 41

As already mentioned Houk's 'inside alkoxy effect' hypothesis can account for the observed π-facial selectivity. Six possible staggered transition state structures can be drawn for the cycloaddition of a nitrile oxide to a chiral allyl ether (Figure 5). Theoretical calculations by Houk *et al*[196] of the relative energies of these transition states places them in ascending order of energy : A<A′<B<B′<C′<C. The major *erythro* adduct results from A, B and C, and the *threo* adduct from A′, B′ and C′.

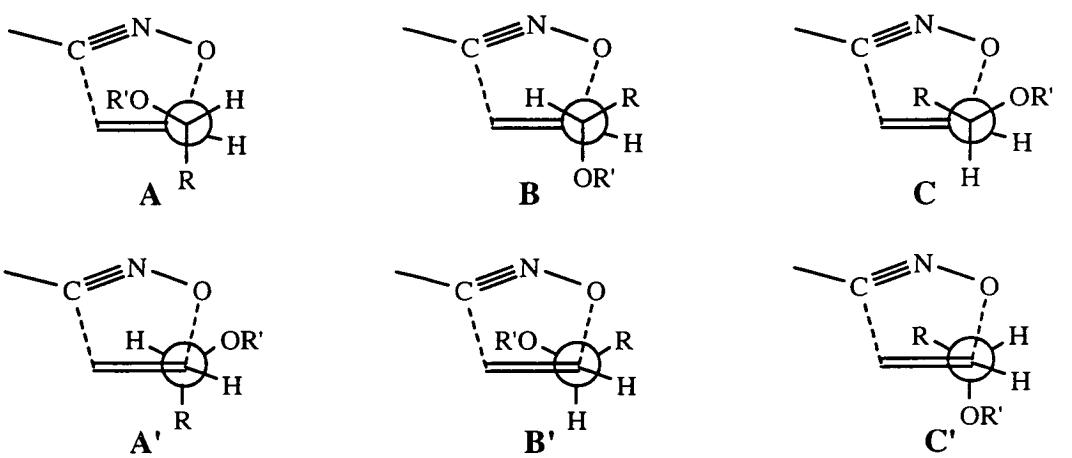


Figure 5

The preferred lowest energy transition state based on a Felkin[200]-Ahn[201] model has the largest substituent (R) *anti*, the smallest (H) "outside", and the alkoxy (OR′) in the "inside" position (Figure 6).

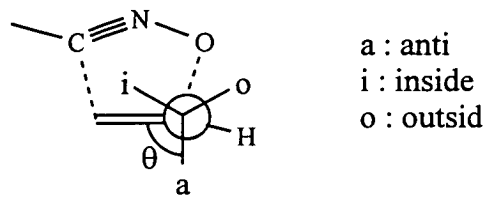


Figure 6

This results in formation of the major isomer with *erythro* stereochemistry. The minor *threo* isomer is produced from the second lowest energy transition state A′. Increase in size of the R group causes an increase in the C=CCR dihedral angle θ, and hence increased π-facial selectivity. The reverse is true for A′. A is stabilised relative to A′, since the unfavourable lone pair interactions between the allylic oxygen and nitrile oxide oxygens are reduced due to the two oxygens being forced further apart.

Secondary orbital interactions were proposed by Houk[196] to explain the preference of the *inside* position for the alkoxy group over the *anti* position. Unfavourable oxygen lone pair interactions between the oxygen of the nitrile oxide and the allylic alkoxide make the outside position less favourable. Nitrile oxide cycloadditions are mildly electrophilic; hence electron donating substituents on the dipolarophile will stabilise the resulting transition state. With the alkoxy (OR′) group *anti* there is maximum overlap of the σ_{CO}* and π-orbitals, resulting in destabilisation of the transition state. In comparison, with the OR′ group inside this unfavourable

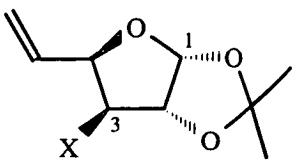
overlap is minimised. Electron donating orbitals σ_{CH} and σ_{CR} further stabilise this transition state by overlap with the π-orbital.

The low selectivities observed for addition to chiral allyl alcohols may be explained in terms of a hydrogen bond interaction between the oxygen of the nitrile oxide and the allyl hydroxyl, resulting in A′ being the slightly preferred transition state; hence a reduction in *erythro* selectivity. The isomer ratios predicted correlate well with experimentally determined values.

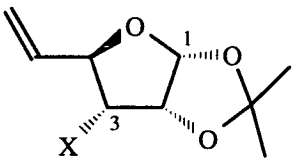
An alternative, non-theoretically based model has been proposed by Kozikowski.[199] It predicts that the major isomer is formed by antiperiplanar addition of the incoming nitrile oxide to the alkoxy group (equivalent to transition state B in Houk's analysis, Figure 5). He argues that the transition state minimises secondary antibonding orbital interactions, a direct contradiction of Houk's proposal. The more widely accepted explanation is that proposed by Houk.

The hypothesis that the stereochemistry of the cycloadditions is determined by electrostatic interactions between the nitrile oxide oxygen and allylic oxygen has been reinforced recently by improved calculations.[202] These have also shown that the exact diastereoselectivity is dependent on the charge distribution in the nitrile oxide.[203]

As will become apparent in later discussions (Section 2.4.2) the presence of homoallylic substituents also has a marked influence on selectivity. This effect has been studied by De Micheli *et al*[204] and Paton *et al*[205] for nitrile oxide cycloadditions to the D-*xylo* and D-*ribo* alkenes (**214**) and (**215**) (Table 7).



(214)



(215)

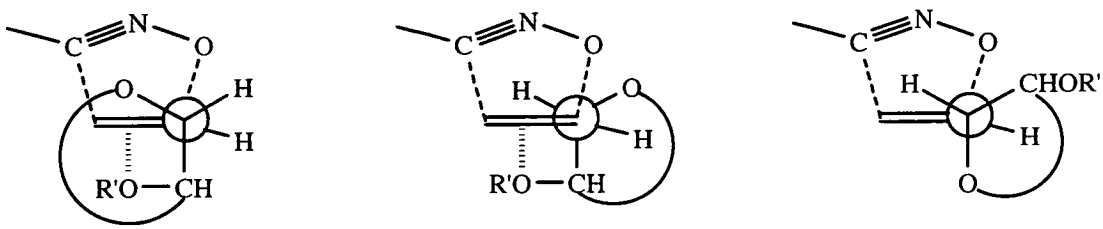
X	Alkene	Nitrile Oxide	*erythro* : *threo*	
			Expt.	Calc.
H	(**214**)	HCNO	73.5 : 26.5[b]	65 : 35
OMe	(**214**)	HCNO	96.5 : 3.5[b]	67 : 37
OBn	(**214**)	EtO_2CCNO	86 : 14[c] 84 : 16[b]	-
	(**214**)	PhCNO	90 : 10[c] 94 : 6[b]	-
	(**214**)	MesCNO[a]	74 : 26[b]	-
OBn	(**215**)	EtO_2CCNO	51 : 49[c]	-
	(**215**)	PhCNO	42 : 58[c]	-

[a] 2,4,6-Trimethylbenzonitrile oxide [b] Ref. 204 [c] Ref. 205

Table 7 : Isomer ratios for addition of nitrile oxides to alkenes (**214**) and (**215**)

It was observed that for the *xylo*-alkenes (**214**) homoallylic alkoxy substituents at C-3 resulted in significantly higher *erythro* selectivity than would be predicted by Houk's 'inside alkoxy effect' model. For the deoxy analogue (X = H), the experimental and calculated values are much more comparable. From these results De Micheli *et al*[204]concluded that the lone pair on the homoallylic oxygen (3-OR) must be exerting a stereoelectronic effect. They proposed that in the lowest transition states A and A′ there is an unfavourable and destabilising interaction of the homoallylic oxygen

lone pair with the double bond. However, in B the next lowest transition state, this interaction (lone pair/π-bond) cannot occur and hence the relative importance of B increases and there is an increase in *erythro* selectivity (Figure 7). In marked contrast the D-*ribo* alkene (**215**) exhibits low selectivities[205] indicating the the 'inside alkoxy effect' exerted by the furanose ring oxygen (O-4) is not the only factor governing selectivity, and that the effect of the allylic group may be either reinforced or counteracted by homoallylic groups.

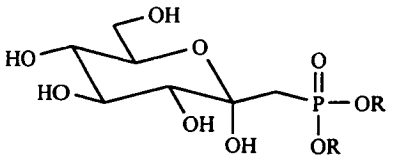


A **A'** **B**

Figure 7

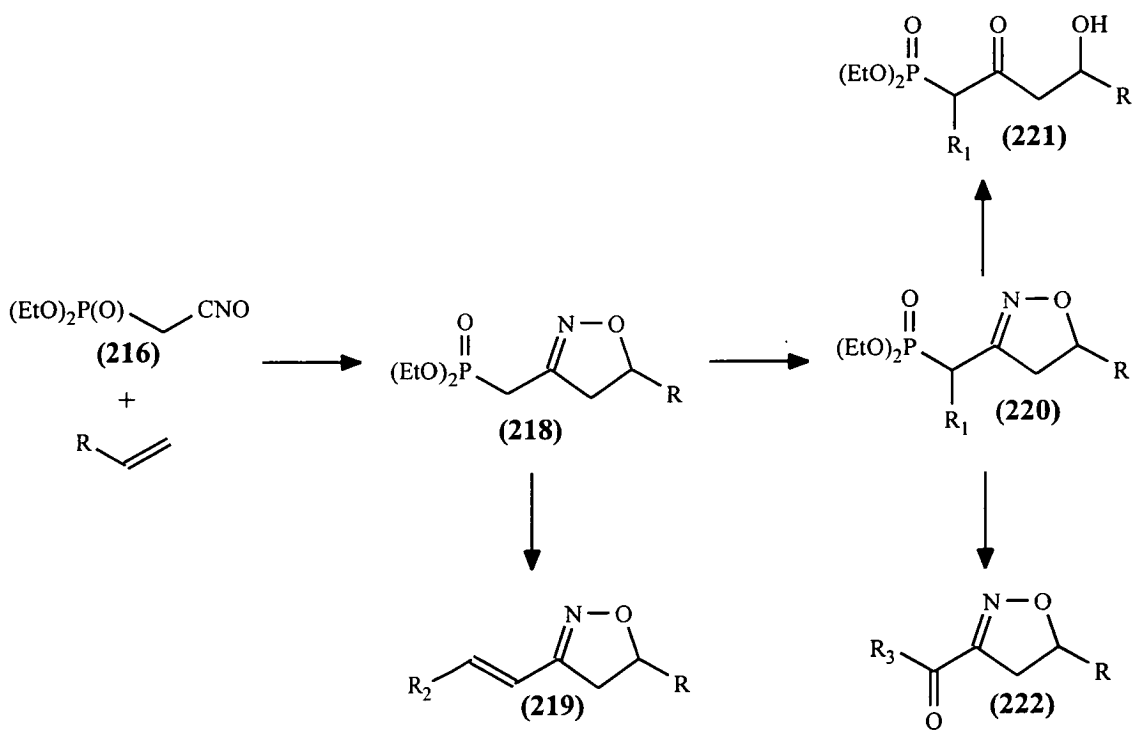
2.3.3 Synthetic Utility of (Diethoxyphosphoryl)acetonitrile Oxide (216)

There are many advantages of using (diethoxyphosphoryl)acetonitrile oxide (**216**) as an α-functionalised dipole, not least its synthetic versatility[16,189] (Scheme 42). It should afford *C*-glycosides eg.(**217**) which might prove to be inhibitors for the glycosyl transferases.[206] As already mentioned in Section 1.4.3, the presence of a phosphorus containing substituent (as a phosphonate) in DAH analogues enhances the inhibitory activity. This therefore makes the introduction of phosphorus substituents an important area of study.



(217)

It may also allow *in vivo* inhibition by facilitating transport of these phosphonate compounds across the protein cell membrane. Often analogues are able to act *in vitro*, but penetration of the membrane is the stumbling block preventing antibacterial action. Furthermore, the resulting 2-isoxazolines (**218**) could be altered[16] by replacement of the phosphorus moiety with an alkene substituent to give (**219**). Other possible modifications include reductive ring cleavage of the isoxazoline (**220**) using Raney-Ni affording phosphorus-functionalised β-hydroxyketones (**221**), and displacement of the phosphorus group by aldehydes or alkyl halides to yield (**222**).



Scheme 42

Hence, the use of (diethoxyphosphoryl)acetonitrile oxide (**216**) has great potential for the synthesis of inhibitors of DAH and KDO. However, its application still needs to be investigated in more detail, since the 2-isoxazoline mixtures have so far proved to be inseparable.

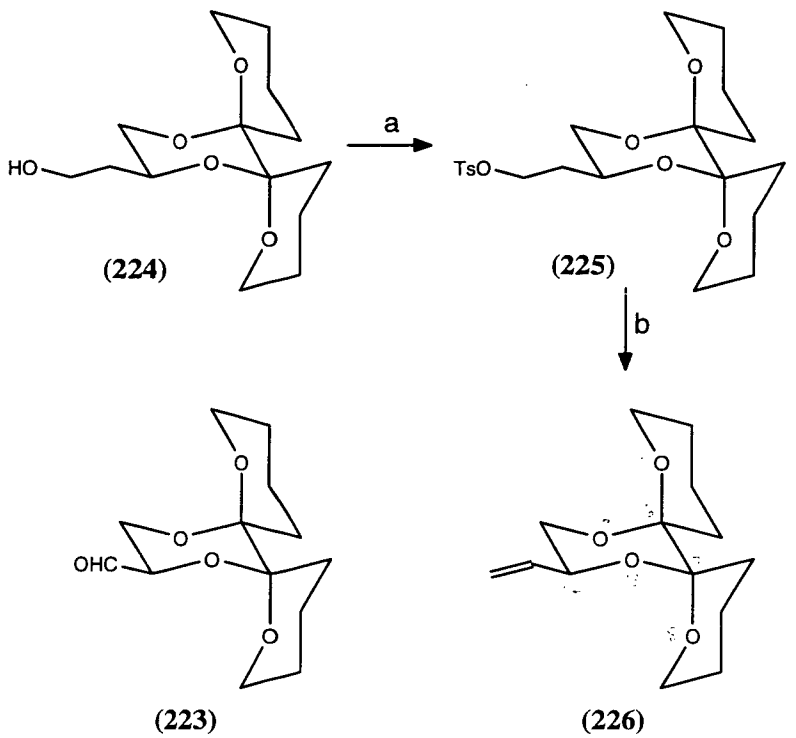
2.3.4 Stereoselective Cycloadditions of Nitrile Oxides to a Dispiroketal-Protected *S*-But-3-ene-1,2-diol (226)

In an attempt to control selectivity in nitrile oxide cycloaddition reactions to but-3-ene-1,2-diols, a dispiroketal protecting group has been utilised. This is a new protecting group developed by Ley *et al*[207] for vicinal diols and incorporates the diol into a six membered 1,4-dioxane ring as a spiroketal. Prompted by a report[208] that the addition of various organometallic reagents to the carbonyl group of a dispiroketal-protected D-glyceraldehyde (**223**) is highly selective, an effect attributed to the rigidly-defined geometry of the dioxane ring and the large steric bulk of the dispiroketal moiety, the corresponding 2-vinyl-1,4-dioxane spiroketal alkene (**226**) has been prepared, and its cycloaddition reactions with nitrile oxides examined.

2.3.4.1 Synthesis of (6*R*,7*R*,14*S*)-14-Ethenyl-1,8,13,16-tetraoxadispiro[5.0.5.4] hexadecane (226)

The required 2-vinyl-1,4-dioxane spiroketal dipolarophile (**226**) was prepared in two steps from β-hydroxyethyl compound (**224**) according to Scheme 43, with an overall yield of 35%. (**224**) was converted to the tosyl derivative (**225**), as previously

described,[208] followed by elimination of toluenesulphonic acid using potassium *tert*-butoxide to give the desired vinyl compound (**226**) which was purified by dry flash chromatography.

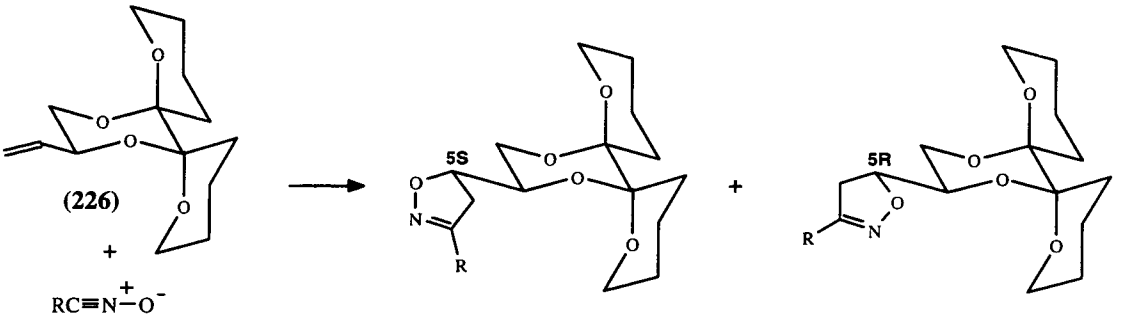


(a) TsCl, Py, r.t., 3 hr; (b) *t*-BuOK, DMSO, r.t., 2 hr

Scheme 43

2.3.4.2 Nitrile Oxide Cycloadditions to Alkene (226)

The cycloaddition of ethoxycarbonylformonitrile oxide to alkene (**226**) (Scheme 44) was carried out as outlined in Section 2.3.1. After chromatography to remove 3,4-diethoxycarbonylfurazan *N*-oxide (6%), a mixture of diastereoisomeric isoxazolines (**227**) and (**228**) in a ratio 72:28 and combined yield of 53% was obtained.



(227) R = CO_2Et
(229) R = Ph

(228) R = CO_2Et
(230) R = Ph

Scheme 44

The isomer ratio was measured from the ^1H NMR spectrum of the product mixture by comparison of the 5-H signals, which were well separated. HPLC analysis gave a similar value (71:29). In order to identify the individual isomers, and assign the relationship between the newly created chiral centre (C-5) and the adjacent asymmetric centre (C-14′) of the 1,3-dioxane unit, the major isomer was deprotected and the product compared with authentic samples generated from cyclohexylidene-protected adducts. These correlation experiments will be discussed in Section 2.3.8.6. The major isomer (**227**) was therefore assigned *S*-configuration at C-5, corresponding to an *erythro* relationship between C-5 and C-14′. This predominance of *erythro* adduct can be rationalised in terms of the 'inside alkoxy effect' proposed by Houk *et al*[196] to account for nitrile oxide cycloadditions to chiral allyl ethers.

Similarly the corresponding cycloaddition of benzonitrile oxide to the same alkene (Scheme 44) afforded the adducts (**229**) and (**230**) in a ratio 75:25 and a combined yield of 40%, together with 3,4-diphenylfurazan *N*-oxide (8%). The ratio was again determined by comparison of the 5-H signals in the ^1H NMR spectrum of the mixture. The structures of the individual isomers were assigned by comparison of

their physical and spectroscopic properties with those of the ethoxycarbonyl analogues (**227**) and (**228**). Further confirmation of the *erythro* configuration for the major isomer was obtained from X-ray crystallography of isomer (**229**).

The nitrile oxide cycloadditions to alkene (**226**) both proceeded with moderate π-facial selectivity in favour of the *erythro* adducts with d.e. values of 44% for ethoxycarbonylformonitrile oxide and 50% for benzonitrile oxide.

2.3.4.3 X-Ray Crystal Structure of Adduct (229)

The major adduct (**229**) obtained from addition of benzonitrile oxide to alkene (**226**) was isolated as a crystalline solid suitable for X-ray analysis. The crystal structure (Figure 8) provided further confirmation that the new asymmetric centre at C-5 possessed *S*-configuration, thus establishing the *erythro* relationship between C-5 and C-14′. This assignment is consistent with that predicted by the 'inside alkoxy effect.'

Using the Haasnoot parameterisation[209] of the Karplus equation the proton-proton coupling constants were calculated from the H-C-C-H torsion angles obtained, and compared to those observed in solution (Table 8). Consideration of the data indicates satisfactory correlation between the observed and calculated *J*-values for the isoxazoline ring protons, as well as between $J_{4,5a}$ and $J_{4,5e}$. However, there is a significant deviation associated with the coupling between 3-H/4-H at the ring junction, indicating a difference in preferred conformation in solution and in the crystal.

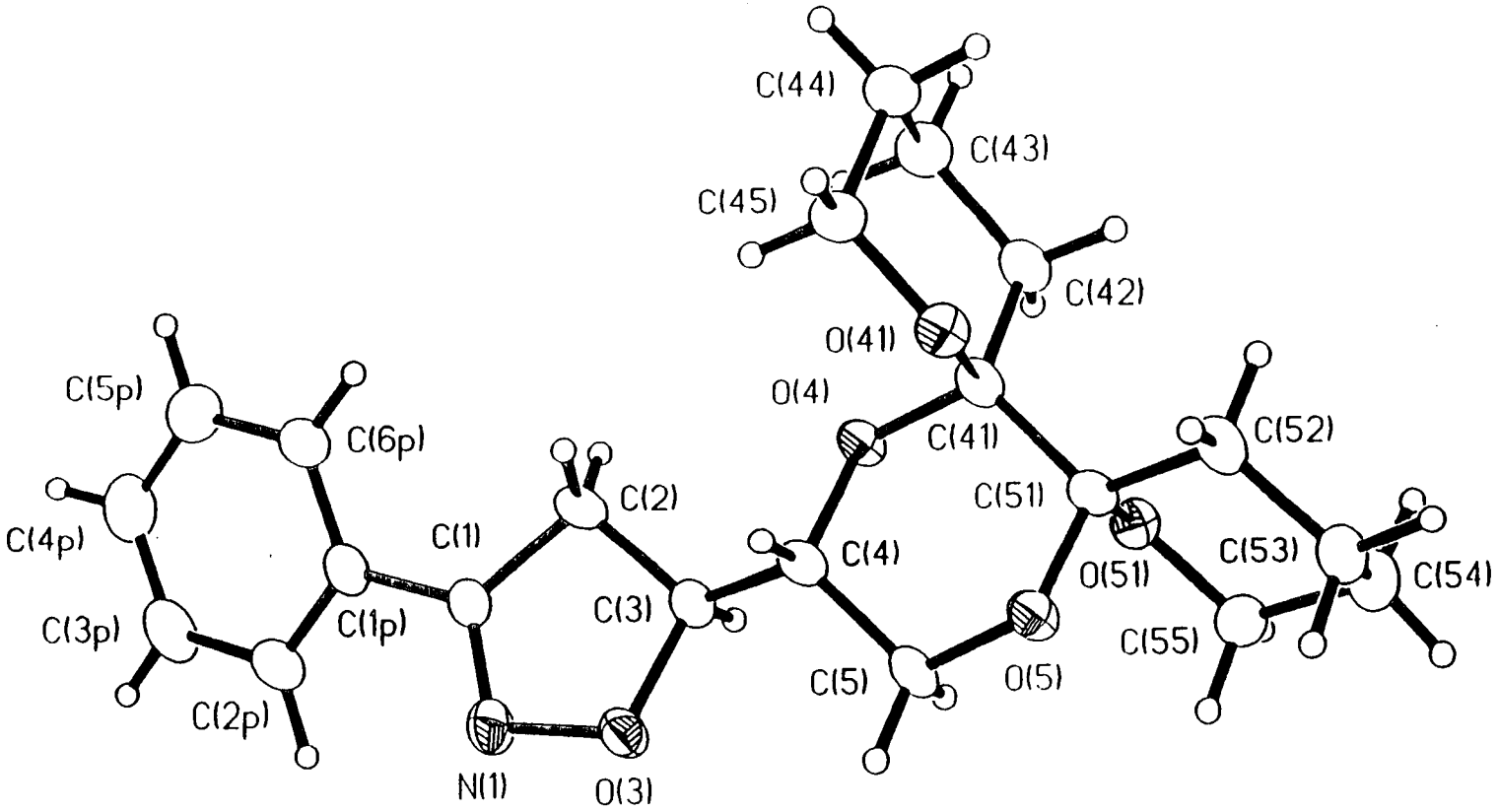
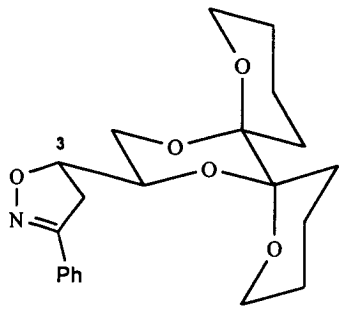

C(44)
C(43)
C(45)
C(42)
O(41)
O(4)
C(41)
C(52)
C(5p)
C(6p)
C(2)
C(51)
C(4p)
C(1)
C(4)
O(51)
C(53)
C(54)
C(3p)
C(1p)
C(3)
C(55)
C(5)
O(5)
C(2p)
N(1)
O(3)

Figure 8



	H_x,H_y[1]				
	2a,3	**3b,3**	**3,4**	**4,5a**	**4,5e**
θ_{Obs}	-150.5	-27.0	178.2	174.7	56.0
J_{Obs}	8.8	8.8	7.7	9.3	4.7
J_{Calc}	8.2	6.6	10.3	10.2	3.2

$^3J_{calc} = 7.76Cos^2\theta - 1.1Cos\theta + 1.4$

Table 8 : Calculated and observed coupling constants for (**229**)

2.3.4.4 Characteristics of Isoxazoline Cycloadducts

Comparison of the physical and spectroscopic properties of the major and minor cycloadducts obtained, together with those previously reported for cycloadditions to the cyclohexylidene-protected alkene (**195**), indicated a number of distinctive features upon which the prediction of the stereochemistry at the new chiral centre could be made. These characteristic properties are summarised below.

TLC

The major *erythro* adduct in each case has a larger R_f value on silica (60% ether/hexane) than the corresponding minor adduct [0.33 *cf* 0.23 for (**227**)/(**228**), 0.37 *cf* 0.26 for (**229**)/(**230**)].

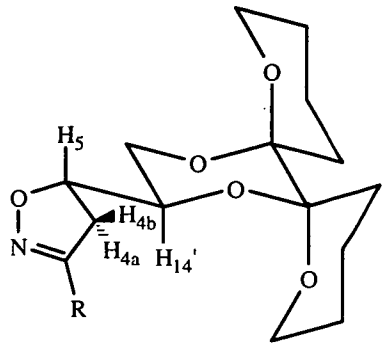
[1] Labelling of protons in X-ray picture is different from that used in characterisation of products

^{1}H NMR

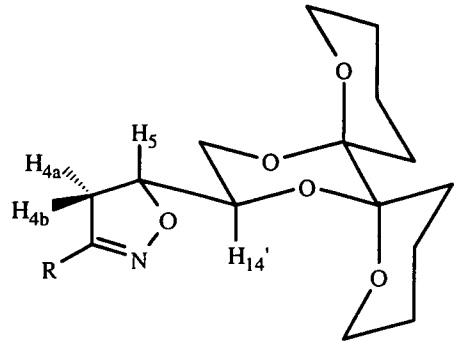
The ^{1}H NMR spectra show major differences between the isomers for protons 4a-H, 4b-H and 5-H. The isoxazoline ring protons 4a-H and 4b-H give rise to a characteristic ABX system with 5-H, which is adjacent to the ring oxygen, at highest chemical shift. It is observed that for the major *erythro* adducts the 5-H signal absorbs at significantly lower frequency [$\Delta\delta_H$ -0.12 ppm for (**227**)/(**228**), -0.10 ppm for (**229**)/(**230**)] and 4b-H at higher frequency [$\Delta\delta_H$ +0.07 ppm for (**227**)/(**228**), +0.09 ppm for (**229**)/(**230**)] than the corresponding peaks for the minor *threo* product. Again the ^3J values of 8-11 Hz for 4ab-H/5-H and the geminal coupling of *ca* 18 Hz for 4a-H/4b-H are observed for the isoxazolines.[193-195] It is noteworthy that the coupling between protons 5-H and 14′-H in the major *erythro* isomer is significantly greater than that for the minor *threo* product [7.0 *cf* 4.8 Hz for (**227**)/(**228**), 7.7 *cf* 5.3 Hz for (**229**)/(**230**)]. This suggests that in each case the two compounds adopt different conformations in solution. Tables 9 and 10 give selected chemical shifts and coupling constants for the isomers.

^{13}C NMR

The ^{13}C NMR for the pairs of isomers are very similar. The major difference is associated with the signal for C-4. In each case the major *erythro* adduct has a higher δ-value than the minor [$\Delta\delta_C$ +0.9 ppm for (**227**)/(**228**), +1.3 ppm for (**229**)/(**230**)]. These findings are shown in Table 11.



Erythro



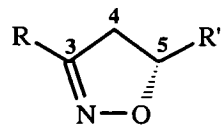
Threo

R	Isoxazoline	δ_H/ppm		
		4a	4b	5
CO_2Et	*Erythro* (**227**)	3.18	3.25	4.64
	Threo (**228**)	3.18	3.18	4.76
Ph	*Erythro* (**229**)	3.40	3.40	4.63
	Threo (**230**)	3.31	3.31	4.73

Table 9 : Selected ^{1}H NMR data for cycloadducts

R	Coupling	*J*/Hz		
		4a,5	4b,5	5,14′
CO_2Et	*Erythro* (**227**)	10.9	8.1	7.0
	Threo (**228**)	10.2	10.2	4.8
Ph	*Erythro* (**229**)	8.8	8.8	7.7
	Threo (**230**)	9.8	9.8	5.3

Table 10 : Selected ^{1}H NMR data for cycloadducts



	Isoxazoline			
	R = CO_2Et		R = Ph	
δ_C/ppm	*Erythro* (**227**)	*Threo* (**228**)	*Erythro* (**229**)	*Threo* (**230**)
C-4	35.4	34.5	37.2	35.9

Table 11 : Selected ^{13}C NMR data for cycloadducts

Optical Rotation

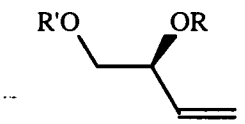
The major *erythro* isomer in each case was found to have a more positive/less negative $[\alpha]_D$ value (Table 12).

Erythro (**227**)	*Threo* (**228**)	*Erythro* (**229**)	*Threo* (**230**)
+14.9	-200.5	-3.5	-175.7

Table 12

2.3.5 Selectivity of Nitrile Oxide Cycloadditions to Alkene (226)

The π-facial selectivities resulting from cycloadditions of ethoxycarbonyl-formonitrile oxide and benzonitrile oxide to dispiroketal-protected but-3-ene-1,2-diol (**226**) are compared in Table 13 with those found for cycloadditions to 2-vinyl-1,3-dioxolanes and acyclic analogues.[191,197,199]



R	R′	Nitrile Oxide	*Erythro*	*Threo*	Reference
dispiroketal		EtO_2CCNO	75	25	210
		PhCNO	72	28	210
H	H	PhCNO	61	39	197
H	CPh_3	PhCNO	54	46	197
Ac	Ac	PhCNO	53	47	197
$SiMe_3$	$SiMe_3$	PhCNO	75	25	197
$-CMe_2-$		EtO_2CCNO	80	20	199
		PhCNO	85 83	15 17	191, 197 199
$-C(CH_2)_5-$		EtO_2CCNO	77	23	210
		PhCNO	81 79	19 21	191, 197 210
-CO-		PhCNO	82	18	197
-COCO-		PhCNO	66	34	197
PhCO	PhCO	PhCNO	54	46	197
Me	H	PhCNO	40	69	197

Table 13

The predominance of *erythro* adducts in each case can be rationalised in terms of the "inside alkoxy effect " proposed by Houk *et al.*[196-198] For 2-vinyl-1,3-dioxanes and 2-vinyl-1,3-dioxolanes the *anti* substituent is linked *via* the five membered ring to the inside alkoxy as illustrated in Figure 9. The increased selectivity observed in these systems may be associated with a through-space interaction of the alkene π bond and a lone pair of the homoallylic oxygen.[204]

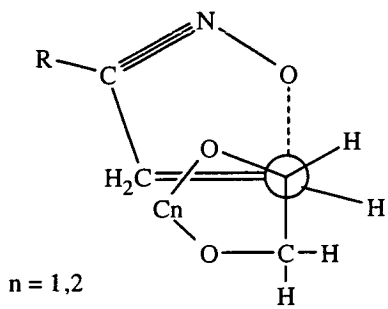


Figure 9

In contrast to nucleophilic additions to the carbonyl group of the dispiroketal-protected D-glyceraldehyde (**223**), which are reported[208] to be highly selective, the preference for *erythro* adducts for nitrile oxide cycloadditions to alkene (**236**) is only moderate and similar to that found for the same nitrile oxides with cyclohexylidene (Section 2.3.2) and isopropylidene protected alkenes. It is therefore concluded that the large steric bulk of the dispiroketal moiety and changing from a five to six-membered ring system appears to have negligible effect on diastereoselectivity.

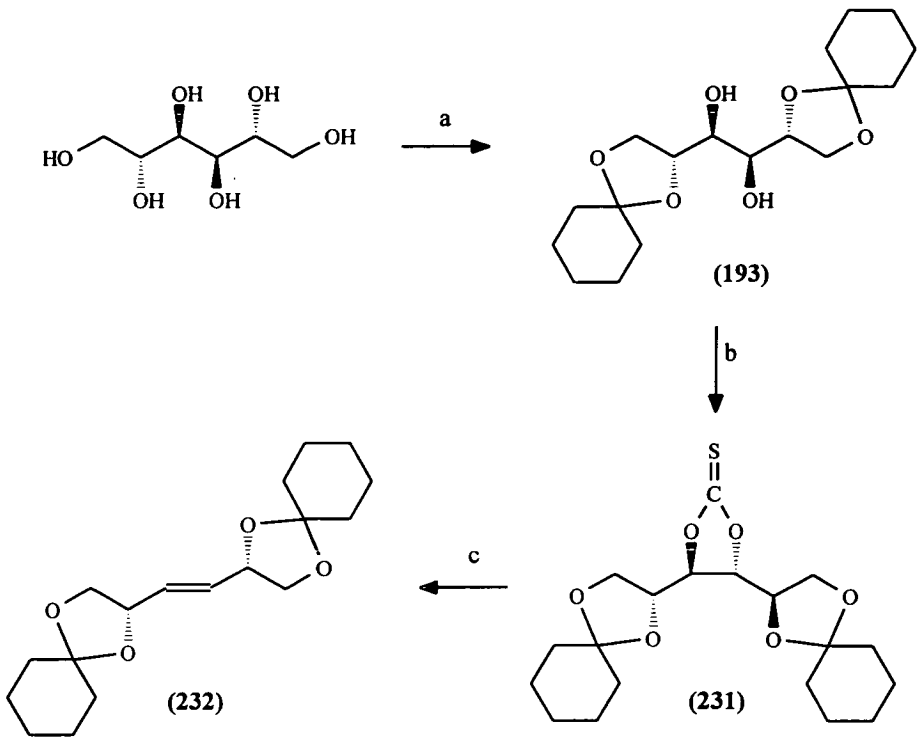
2.3.6 Attempted Cycloadditions of Nitrile Oxides to trans-3,4-*Didehydro*-3,4-*di*deoxy-1,2:5,6-*di*-O-*cyclohexylidene*-D-threo-*hexitol* (232)

In order to investigate the influence of bulky substituents on reactivity and hence selectivity, alkene (**232**) was synthesised and its reaction with nitrile oxides examined. As both faces of the alkene are sterically hindered by the cyclohexylidene groups it was anticipated that this would have a significant effect on the reaction.

2.3.6.1 Synthesis of trans-3,4-*Didehydro*-3,4-*dideoxy*-1,2:5,6-*di*-O-*cyclohexylidene*-D-threo-*hexitol* (232)

Alkene (**232**) was prepared in three steps from D-mannitol according to Scheme 45, with an overall yield of 26%. Selective protection of D-mannitol as referred to in Section 2.3.1 yielded the dicyclohexylidene adduct (**193**). This 3,4-diol was then refluxed with 1,1′-thiocarbonyldiimidazole in toluene to afford the cyclic thionocarbonate derivative (**231**) in good yield. Conversion to the symmetrically substituted alkene (**232**) was *via* an elimination reaction (desulphurisation-decarboxylation) using triethyl phosphite, which is acting as both solvent and reactant. Addition of alkali to the reaction mixture hydrolysed excess phosphoesters, and the alkene was purified by dry flash chromatography.

Specific *cis*-elimination[211] is observed for conversion of (**231**) to the symmetrically substituted *trans*-alkene (**232**).



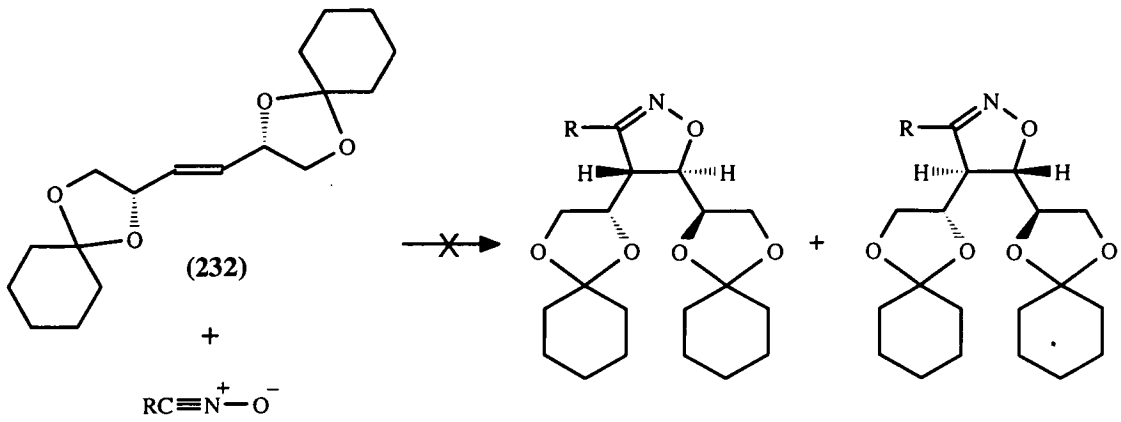
(a) Cyclohexanone, $HC(OEt)_3$, $BF_3.Et_2O$, DMSO; (b) 1,1'-thiocarbonyldiimidazole, toluene, reflux; (c) $(EtO)_3P$, reflux

Scheme 45

2.3.6.2 Attempted Cycloadditions to trans-3,4-*Didehydro*-3,4-*dideoxy*-1,2:5,6-*di*-O-*cyclohexylidene*-D-threo-*hexitol* (232)

Ethoxycarbonylformonitrile oxide was generated in the presence of alkene (**232**) (Scheme 46). However, there was no evidence by TLC or ^1H NMR analysis of the reaction mixture that cycloadducts (**233**) or (**234**) had been produced. Similarly attempted cycloaddition of benzonitrile oxide to alkene (**232**) did not yield adducts (**235**) and (**236**).

The failure to form cycloadducts suggests that the presence of the bulky substituents is preventing the incoming nitrile oxide from getting close enough to the dipolarophile for reaction to occur.



(233) R = CO_2Et
(235) R = Ph

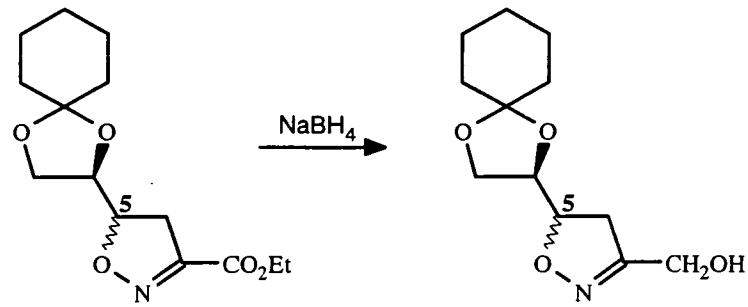
(234) R = CO_2Et
(236) R = Ph

Scheme 46

2.3.7 Reduction of 3-Ethoxycarbonyl Substituent to Hydroxymethyl

In order to convert the 3-ethoxycarbonyl isoxazolines (**198**) and (**199**) to hexulosonic acid analogues, reduction of the ester group to provide a hydroxymethyl substituted isoxazoline is required. This modification is a prerequisite for the reductive hydrolytic cleavage of the 2-isoxazoline to the β-hydroxyketone, since isoxazolines containing an ester substituent at the 3-position are known to be resistant to ring opening.[53]

Adducts (**198**) and (**199**) were therefore reduced with sodium borohydride in ethanol at room temperature to provide the corresponding alcohols (**204**) and (**205**) in excellent yields, 94% and 96% respectively (Scheme 47).



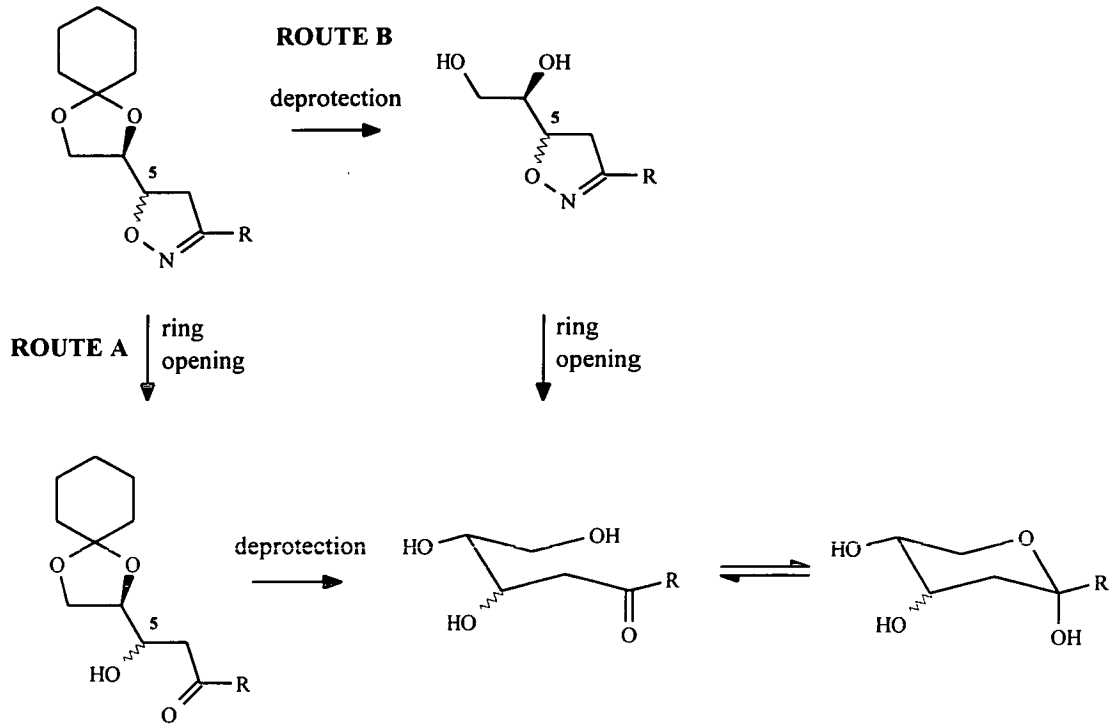
(**198**) 5*S* (**204**) 5*S*
(**199**) 5*R* (**205**) 5*R*

Scheme 47

The presence of the hydroxymethyl group should also allow access to a wide range of derivatives including mesylate and tosylate. These will be discussed in Section 2.3.9. The characteristic properties of the isomers (**204**) and (**205**) are compared with those of the parent major *erythro* and minor *threo* cycloadducts (**198**) and (**199**) in Section 2.3.2.2.

2.3.8 Conversion of 2-Isoxazolines to 2-Hexulosonic Acid Analogues

Two approaches for the conversion of the 2-isoxazolines to the desired 2-ulosonic acid analogues have been investigated: either initial reductive hydrolytic cleavage of the N-O bond of the isoxazoline to the β-hydroxyketone, followed by removal of the cyclohexylidene protection (Route A), or *vice versa* (Route B) (Scheme 48). In the final step it was envisaged that pyranose ring structures would be formed by ring closure of the resulting δ-hydroxyketone.



Scheme 48

Each of these approaches will be considered in turn, starting with ring opening followed by deprotection ie. Route A, and their relative merits discussed. However, before either route is considered a brief overview of the conditions required to bring about ring cleavage will be presented.

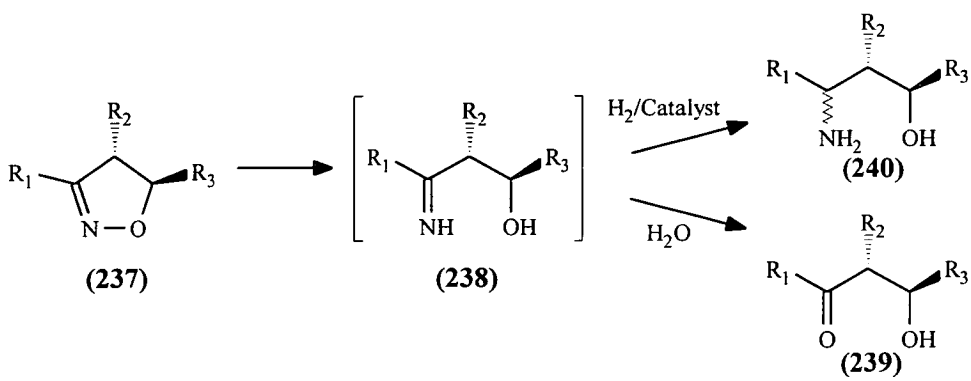
2.3.8.1 Reductive Hydrolytic Cleavage of 2-Isoxazolines to β-Hydroxyketones

While a number of reductive procedures have been devised to release the β-hydroxyketone from the isoxazoline, the choice of precise reaction conditions does depend on the type of functionality present within the substrate and on the necessity of preserving chirality at C-5. The most widely used method[212] involves catalytic hydrogenation in the presence of palladium-on-charcoal or Raney-nickel and boric acid, using aqueous methanol as the solvent. These optimised conditions specifically

incorporating boric acid have been shown by Curran[45] to be particularly effective at minimising epimerisation, and are critical to the overall success of the process.

In this ring opening, for which the mechanism is now well understood, the N-O bond of the isoxazoline (**237**) is cleaved by addition of hydrogen to form initially a β-hydroxyimine intermediate (**238**). Subsequent hydrolysis of (**238**) produces a β-hydroxyketone (**239**) (Scheme 49). This hydrolysis competes with further reduction to the γ-amino alcohols (**240**).

Curran[45] has rationalised the key role of the acid as follows: Firstly, it promotes imine hydrolysis by protonation of the imine nitrogen; it can also neutralise the ammonia produced during the hydrolysis and finally, when Raney-nickel is used, it neutralises excess hydroxide present on the catalyst.

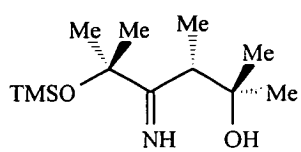


Scheme 49

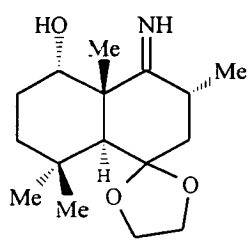
A number of other acidic additives have previously been employed including acetic acid,[213] aluminium[52]and boron trichlorides,[214] concentrated HCl,[52] trimethyl borate[212] and acetate or phosphate buffers.[212] However, boric acid has proved to be the best available, and remains the most commonly used reagent.

The reaction uses mildly acidic conditions (pH 5.5-6.0) resulting in the β-hydroxyimine undergoing rapid hydrolysis[45] and hence it is not normally detectable. However, some β-hydroxyimines, eg. (**241**)[215] and (**242**),[47] have been isolated on hydrolysis of the corresponding isoxazolines; their increased stability is attributed to steric hindrance by the bulky neighbouring groups slowing down the rate of hydrolysis.

Strong evidence for the proposed mechanism is given by subsequent conversion of these imines to β-hydroxyketones when resubjected to hydrolysis conditions.

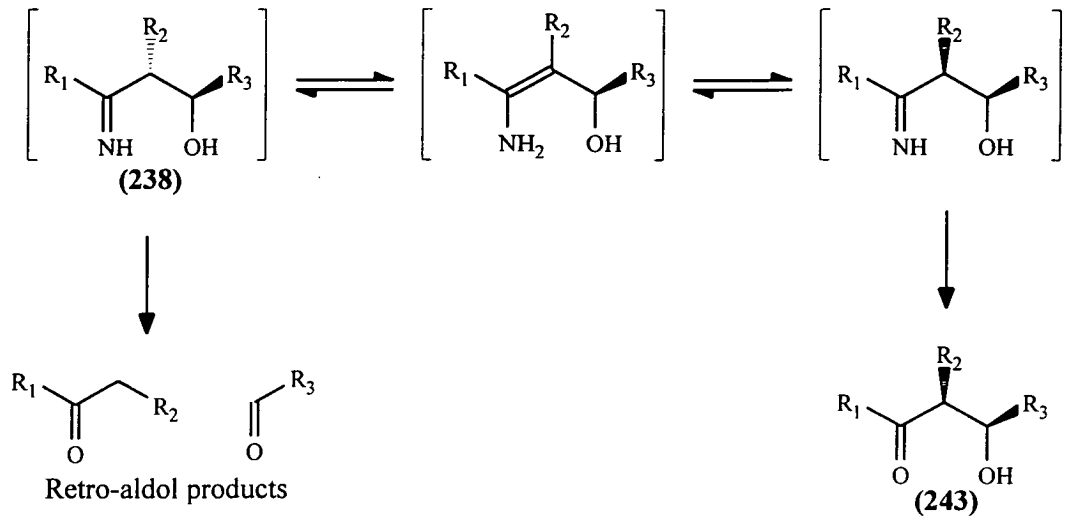


(**241**)



(**242**)

The use of acetate or phosphate buffers minimises the formation of γ-amino alcohols but other unwanted products, sometimes as the major constituent, are often obtained. These include products resulting from epimerisation at the α-position which leads to diastereoisomeric mixtures of ketones (**239**) and (**243**), and also from retro-aldol reactions. Curran[45] has proposed that the epimerisation is due to imine-enamine tautomerisation of the intermediate β-hydroxyimine (Scheme 50).



Scheme 50

Using boric acid as the acid additive minimises the formation of the above unwanted products (retro-aldol compounds and diastereoisomeric ketones), and in most cases eliminates their formation altogether. This additive is believed[43] to form a cyclic borate ester (Figure 10), that is capable of increasing the rate of imine hydrolysis and prevent the undesired tautomerisation.

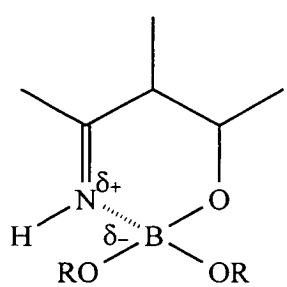


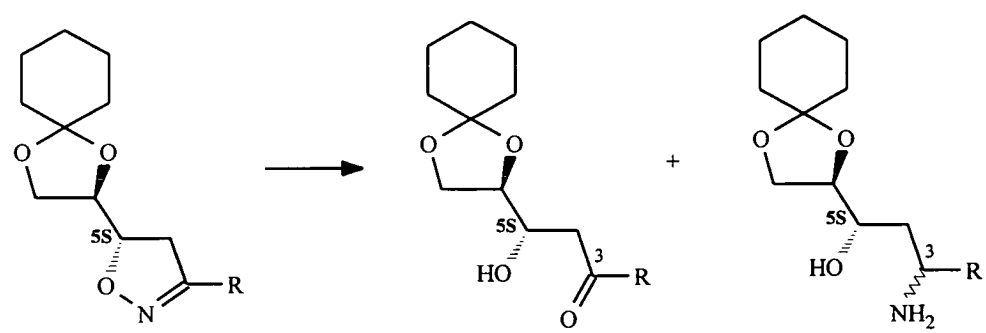
Figure 10

2.3.8.2 Reductive Hydrolytic Cleavage of Isoxazolines Followed by Deprotection (Route A)

The first stage in this route to the desired 3-deoxy-hexulosonic acid analogues involved reductive hydrolytic cleavage of the isoxazoline. It was therefore important to establish optimum conditions for this ring opening process. Hydrogenolysis of isoxazolines (**196**) and (**204**) using both palladium-on-charcoal (Pd/C) and Raney-nickel (Ra-Ni) catalysts has been investigated in order to determine whether changing the reaction conditions alters the yields of β-hydroxyketone produced. Pd/C was initially employed for the proposed N-O bond cleavage of the isoxazolines.

Palladium-on-Charcoal Catalysed Hydrogenolysis of 2-Isoxazolines

The procedure selected for reductive hydrolytic cleavage of 2-isoxazolines uses 10% (Pd/C) as the catalyst using conditions optimised by Curran.[45] The reaction involved vigorous stirring of the isoxazoline (1 M eq), boric acid (6 M eq) and catalyst under an atmosphere of hydrogen for 18 hours in a 5:1 mixture of methanol/water (Scheme 51).



(**196**) R = Ph
(**204**) R = CH_2OH

(**244**) R = Ph
(**246**) R = CH_2OH

(**245**) R = Ph
(**247**) R = CH_2OH

Scheme 51

Initial experiments were performed using 3-phenyl-isoxazoline (**196**). TLC analysis of the reaction mixture indicated when all the starting material had been consumed. Two products were identified and separated by preparative TLC; the less polar component gave a positive stain with Brady's reagent and was assumed therefore to be the desired β-hydroxyketone (**244**) (64%). The more polar (baseline) component was proven to be an inseparable mixture of diastereoisomeric γ-amino alcohols (**245**) (28%). Similarly, ring cleavage of hydroxymethyl-isoxazoline (**204**) resulted in the formation of β-hydroxyketone (**246**) (60%) and a mixture of γ-aminoalcohols (**247**) (25%).

The β-hydroxyketones were identified by ^{1}H and ^{13}C NMR spectroscopy and FAB mass spectrometry and verification of the chemical formulae made by high resolution FAB mass spectrometry. Complicated ^{1}H NMR spectra were produced for the mixtures of γ-amino alcohols. However, characteristic signals[216] within the region $\delta \sim 53\text{-}57$ ppm were observed in the ^{13}C NMR spectra for the $CHNH_2$ (C-3) resonance. Further evidence for these compounds is given by a positive ninhydrin stain on TLC, together with high resolution FAB mass spectrometry analysis. Due to their instability the structures were not verified further. These γ-amino alcohols are formed by reduction of the intermediate β-hydroxyimine implying that this reduction competes with the hydrolysis to the β-hydroxyketone.

Raney-Nickel Induced Hydrogenolysis of 2-Isoxazolines

In an attempt to increase the yields of β-hydroxyketones (**244**) and (**246**) and minimise the competing formation of γ-amino alcohols (**245**) and (**247**), the alternative Ra-Ni catalysed process was pursued. Standard conditions as detailed above were employed, with Ra-Ni substituted for the Pd/C catalyst. The starting material in each case was consumed in 4 hours. Isoxazoline (**196**) was initially employed and after preparative TLC, the reaction provided the desired β-hydroxyketone (**244**) (80%), identical to that obtained from the previous Pd/C process, together with an inseparable mixture of γ-amino alcohols (**245**) (10%).

Isoxazoline (**204**) was treated in the same manner. Again, after preparative TLC the more polar and unwanted inseparable mixture of γ-amino alcohols (**247**) (8%) was obtained along with β-hydroxyketone (**246**) (78%).

The consistency of the yields obtained is influenced by the quality of the Ra-Ni used. Curran[45] has reported that best results are obtained using Ra-Ni washed with water (~ ×20) and stored in a freezer under methanol for 3-4 weeks prior to use, and which was used within 6 weeks of initial storage. Our findings are in agreement with this.

Comparison of the yields obtained from the two methods is shown in Table 14. These results indicate that yields of the desired β-hydroxyketones relative to the γ-amino alcohols are improved by using Ra-Ni rather than Pd/C as the catalyst for reductive hydrolytic cleavage of the isoxazoline N-O bond. All subsequent hydrogenations were therefore performed using these optimised conditions.

Isoxazoline	Catalyst	% Yield	
		β-hydroxyketone	γ-amino alcohol
(196)	Pd/C	64	28
	Ra-Ni	80	10
(204)	Pd/C	60	25
	Ra-Ni	78	8

Table 14 : Palladium-on-charcoal *versus* Raney-Nickel Induced Hydrogenolysis

2.3.8.3 Characteristics of β-Hydroxyketones

TLC

The parent 2-isoxazoline has a larger R_f value on silica (100% ether) than the corresponding β-hydroxyketone [eg. 0.58 *cf* 0.23 for (**196**)/(**244**)]. Brady's Reagent also stains the β-hydroxyketone thus aiding in its identification.

^{1}H NMR

The ^{1}H NMR data for protons 4-H to 6-H reflect major differences between the 2-isoxazolines and β-hydroxyketones. Tables 15 and 16 compare the chemical shifts and coupling constants for the D-mannitol derived isoxazolines (**196**) and (**204**) with those of the β-hydroxyketones (**244**) and (**246**). It is observed that the 5-H signal absorbs at significantly higher frequency for the isoxazoline than for the corresponding β-hydroxyketone [$\Delta\delta_H$ +0.61 ppm for (**196**)/(**244**), +0.50 ppm for (**204**)/(**246**)]. Comparison of the large coupling constant for $J_{4a,5}$ (8.7 Hz) and the smaller value for $J_{4b,5}$ (2.5 Hz and 3.1 Hz respectively) for (**244**) and (**246**), is

consistent with a hydrogen-bonded half-chair conformation for the β-hydroxyketone (Figure 11) with 5-H and 4a-H occupying *pseudo* axial positions and 4b-H an equatorial position. The bulky cyclohexylidene substituent R_1 occupies the sterically least demanding quasi-equatorial position in this conformation. Similar observations have been reported for 6-deoxy-7-undecosuloses.[217]

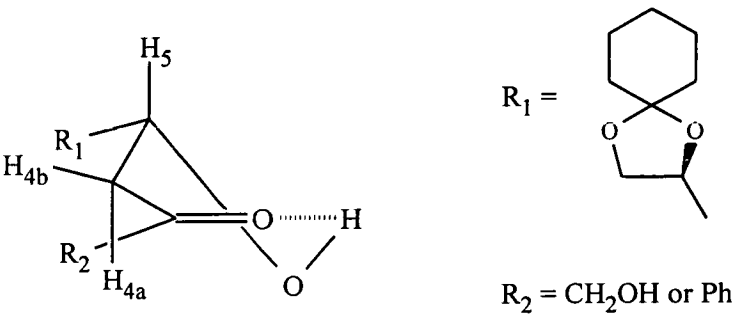
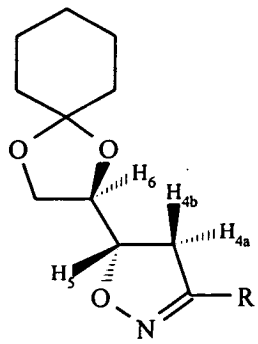
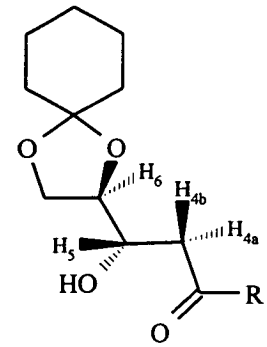


Figure 11

^{13}C NMR

Major differences for C-4 to C-6 are observed in the spectra for the β-hydroxyketones compared to those of the respective isoxazolines. These differences are shown in Table 17 . In both cases, C-4 resonates at a higher frequency for the β-hydroxyketone [$\Delta\delta_C$ +3.3 ppm for (**196**)/(**244**), +4.1 ppm for (**204**)/(**246**)]. In comparison C-5 resonates at a higher frequency for the isoxazolines [$\Delta\delta_C$ +4.6 ppm for (**196**)/(**244**), +3.7 ppm for (**204**)/(**246**)], as does C-6 [$\Delta\delta_C$ +6.7 ppm for (**196**)/(**244**), +6.6 ppm for (**204**)/(**246**)].



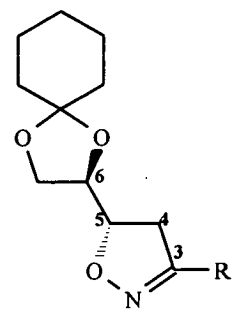


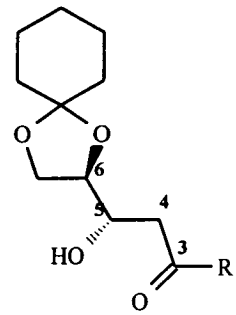
R	Proton	δ_H/ppm			
		4a-H	4b-H	5-H	6-H
Ph	Isoxazoline (**196**)	3.37	3.47	4.64	4.05
	β-Hydroxyketone (**244**)	3.12	3.42	4.03	4.12
CH_2OH	Isoxazoline (**204**)	3.06	3.14	4.53	4.02
	β-Hydroxyketone (**246**)	2.58	2.74	4.03	4.05

Table 15 : Selected ^{1}H NMR data

R	Coupling	*J*/Hz			
		4a,5	4b,5	5,6	7a,7b
Ph	Isoxazoline (**196**)	7.3	9.3	7.6	7.7
	β-Hydroxyketone (**244**)	8.7	2.5	4.9	14.1
CH_2OH	Isoxazoline (**204**)	6.9	10.1	7.1	8.4
	β-Hydroxyketone (**246**)	8.7	3.1	5.7	12.0

Table 16 : Selected ^{1}H NMR data





R	Carbon	δ_C/ppm			
		C-3	C-4	C-5	C-6
Ph	Isoxazoline (**196**)	156.5	37.7	81.3	75.5
	β-Hydroxyketone (**244**)	200.0	41.0	76.7	68.8
CH_2OH	Isoxazoline (**204**)	158.5	37.6	81.0	75.5
	β-Hydroxyketone (**246**)	209.6	41.7	77.3	68.9

Table 17 : Selected ^{13}C NMR data

Optical Rotation

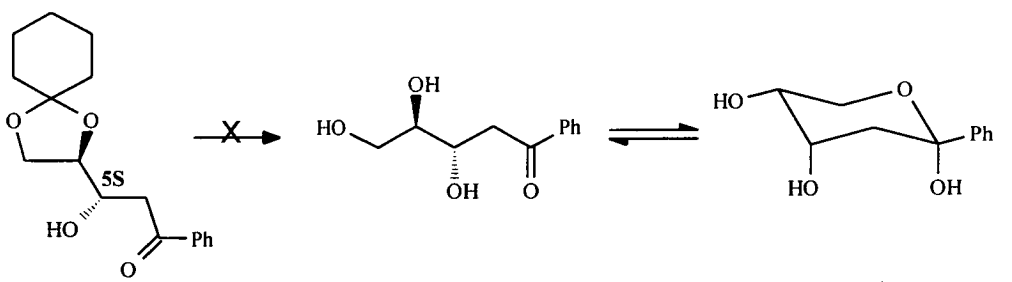
The β-hydroxyketone in each case was found to have a more negative $[\alpha]_D$ value (Table 18).

Isoxazoline (196)	β-Hydroxyketone (244)	Isoxazoline (204)	β-Hydroxyketone (246)
+23.4	-22.6	+59.5	-11.5

Table 18

2.3.8.4 Attempted Removal of Cyclohexylidene Protection from β-Hydroxyketone (244)

Deprotection of β-hydroxyketone (**244**) was attempted by stirring with a mixture of water/glacial acetic acid (1:4) at 80°C for 23 hr (Scheme 52). Although TLC analysis indicated that reaction had occurred (all starting material being consumed) analysis by ^{1}H NMR and FAB MS showed no evidence for the desired product (**248**). This may be due to the β-hydroxyketone being unstable under the conditions required to remove the cyclohexylidene protecting group, and also to the possibility of side reactions producing for example α-enones.



(**244**) (**248**)

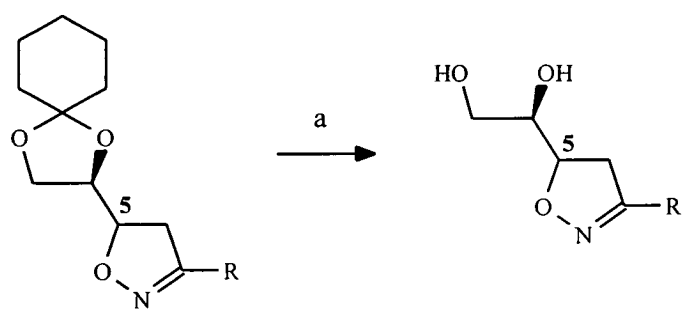
Scheme 52

It was therefore decided that access to the desired analogues would be more likely to be achieved *via* deprotection followed by ring opening, ie. Route B. This approach was thus adopted and is considered next.

2.3.8.5 Deprotection of Isoxazolines Followed by Reductive Hydrolytic Cleavage (Route B)

The alternative approach to the desired hexulose analogues involves removal of the cyclohexylidene protection of the 2-isoxazoline prior to reductive ring opening of the isoxazoline.

The procedure for the first stage involved stirring the isoxazoline with a mixture of water/glacial acetic acid (1:4) at 80°C (Scheme 53). TLC of the mixture indicated when reaction was complete (2-10 hours). Deprotection of each pair of cycloadducts resulted in yields ranging from 70% for (**249**) to 80% for (**253**).



5*S*	(**196**) R = Ph	5*S*	(**249**) R = Ph
	(**198**) R = CO_2Et		(**250**) R = CO_2Et
	(**204**) R = CH_2OH		(**251**) R = CH_2OH
5*R*	(**197**) R = Ph	5*R*	(**252**) R = Ph
	(**199**) R = CO_2Et		(**253**) R = CO_2Et
	(**205**) R = CH_2OH		(**254**) R = CH_2OH

(a) Water/glacial acetic acid (1:4), 80°C

Scheme 53

2.3.8.6 Characteristics of Isoxazolines

Removal of the cyclohexylidene or dispiroketal protecting groups provided the corresponding diols in moderate yields (70-80%). The characteristic properties of these adducts compared to the starting isoxazolines are summarised below.

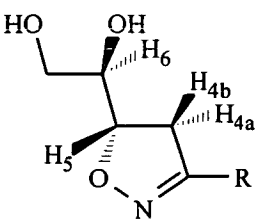
TLC

The parent 2-isoxazoline has a larger R_f value on silica (100% ethyl acetate) than the corresponding diol. It is also observed that the major diol occurs at larger R_f value than the minor isomer.

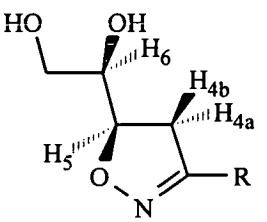
^{1}H NMR

Considering initially the deprotected diastereoisomeric adducts. The ^{1}H NMR spectra reflect a major difference between the 5-H signal for the major *erythro* and minor *threo* isomers. As before the isoxazoline ring protons 4a-H and 4b-H give rise to a characteristic ABX system with 5-H at highest chemical shift. It is observed that for the minor *threo* adduct the 5-H signal absorbs at significantly higher frequency than for the corresponding major *erythro* product [$\Delta\delta_H$ +0.07 ppm for **(249)/(252)**, +0.05 ppm for **(250)/(253)**, +0.08 ppm for **(251)/(254)**]. Compared to the protected adducts (see Section 2.3.2.2) 5-H has shifted to slightly higher frequency. Available data also indicates that 6-H, 7a-H and 7b-H shift to lower frequency in the deprotected adducts. The coupling between 5-H and 6-H is smaller for both the major *erythro* and minor *threo* isomers compared to the protected products [eg. 7.0 *cf* 4.5 Hz for **(198)/(250)**, and 4.2 *cf* 3.1 Hz for **(199)/(253)**]. It is also noticeable that $\Delta\delta_H$

between the major *erythro* and minor *threo* isomers is significantly less when they are deprotected. $J_{5,6}$ changes much more markedly for the major *erythro* isomers than the minor *threo* isomers ($\Delta\delta_H$ 1.5-2.5 Hz *cf* 0.5-1.1 Hz). There is a marked increase in the coupling between 7a-H and 7b-H on going to the deprotected adducts [7.7 *cf* 11.1 Hz for (**196**)/(**249**), 8.4 *cf* 10.8 Hz for (**204**)/(**251**). The chemical shifts and coupling constants for the deprotected adducts are summarised in Tables 19 and 20.



Erythro



Threo

R	Isoxazoline	δ_H/ppm		
		4a-H	4b-H	5-H
Ph	*Erythro* (**249**)	3.48	3.37	4.75
	Threo (**252**)	3.47	3.40	4.82
CO_2Et	*Erythro* (**250**)[a]	3.30	3.18	4.88
	Erythro (**250**)[b]	3.29	3.17	4.86
	Threo (**253**)	3.28	3.20	4.91
CH_2OH	*Erythro* (**251**)	3.14	3.04	4.53
	Threo (**254**)	3.17	3.05	4.61

Table 19 : Selected ^{1}H NMR data

[a] Product from dispiroketal-protected isomer (**227**)
[b] Product from cyclohexylidene-protected isomer (**198**)

R	Coupling	*J*/Hz			
		4a,4b	**4a,5**	**4b,5**	**5,6**
Ph	*Erythro* (**249**)	16.9	8.1	10.8	5.4
	Threo (**252**)	16.8	10.7	8.9	3.5
CO_2Et	*Erythro* (**250**)[a]	17.6	8.2	11.3	nd
	Erythro (**250**)[b]	17.6	8.5	11.4	4.5
	Threo (**253**)	17.4	11.3	9.0	3.1
CH_2OH	*Erythro* (**251**)	17.3	8.0	10.7	5.6
	Threo (**254**)	17.2	10.5	8.8	3.9

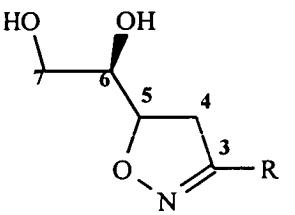
Table 20 : Selected ^{1}H NMR data

[a] Product from dispiroketal-protected isomer (**227**)
[b] Product from cyclohexylidene-protected isomer (**198**)

^{13}C NMR

The ^{13}C NMR spectra for the deprotected isomers show significant differences for C-4 and C-6. In each case the minor *threo* adduct has a higher δ-value for C-4 [$\Delta\delta_C$ 0.9 ppm for (**249**)/(**252**), 1.5 ppm for (**250**)/(**253**), 0.7 ppm for (**251**)/(**254**)]. This is the reverse of that seen for the protected isomers where C-4 for the major *erythro* isomer appears at higher chemical shift (see Section 2.3.2.2). The major *erythro* deprotected isomers have shifted to lower frequency by ~2 ppm compared to the protected adducts, whereas the minor *threo* isomers have remained at the same chemical shift in the protected and deprotected isomers. There is also a marked difference shown for C-6 in the deprotected adducts [$\Delta\delta_C$ +1.0 ppm for (**249**)/(**252**), +1.3 ppm for (**250**)/(**253**), +0.9 ppm for (**251**)/(**254**)]. C-6 appears at a lower chemical shift relative to the protected adducts. It is noteworthy that in the protected isomers

there is no difference in C-6. Considering C-7 for the deprotected isomers one can see no difference in the values, whereas the corresponding protected isomers show a noticeable difference [eg. $\Delta\delta_C$ 1.8 ppm for (**198**)/(**199**)]. However, the δ-values for the deprotected adducts do occur at lower frequency than for the corresponding isomers. The differences between the deprotected adducts are shown in Table 21.



R		δ_C/ppm			
		C-4	**C-5**	**C-6**	**C-7**
Ph	*Erythro* (**249**)	35.5	81.8	72.3	63.5
	Threo (**252**)	36.4	81.6	73.3	63.3
CO_2Et	*Erythro* (**250**)[a]	34.1	84.1	71.8	63.0
	Erythro (**250**)[b]	33.8	84.2	71.8	63.1
	Threo (**253**)	35.3	83.8	73.1	63.0
CH_2OH	*Erythro* (**251**)	36.0	80.8	72.3	63.5
	Threo (**254**)	36.7	80.8	73.2	63.3

Table 21 : Selected ^{13}C NMR data

[a] Product from dispiroketal-protected isomer (**227**)
[b] Product from cyclohexylidene-protected isomer (**198**)

Optical Rotation

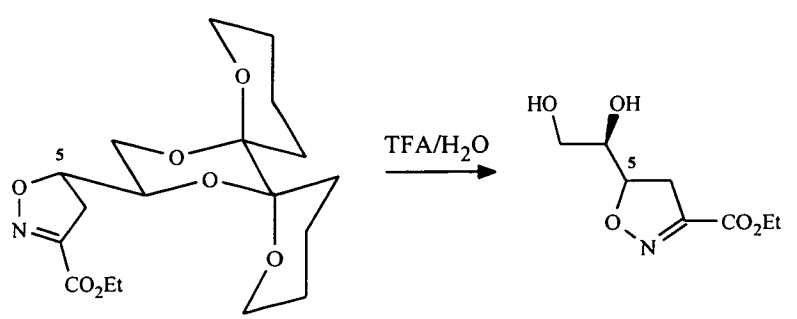
The major isomer was found to have a more positive $[\alpha]_D$ value (Table 22).

Erythro **(249)**	*Threo* **(252)**	*Erythro* **(250)**	*Threo* **(253)**
+83.1	-158.9	+110.0	-124.9

Table 22

2.3.8.7 Deduction of Stereochemistry at C-5 in Dispiroketal-protected Isoxazoline (227)

Access to the deprotected isomers (**250**) and (**253**) allowed the identity of the adducts from the dispiroketal-protected *S*-but-3-ene-1,2-diol (**226**) experiments (discussed earlier in Section 2.3.4) to be identified by correlation. The major adduct (**227**) was deprotected by treatment with aqueous trifluoroacetic acid (TFA) at room temperature, to afford the corresponding 1,2-dihydroxyethyl-isoxazoline (Scheme 54) and the product compared with authentic samples of compounds (**250**) and (**253**). The structures of the adducts from which these were derived are firmly established.[191]



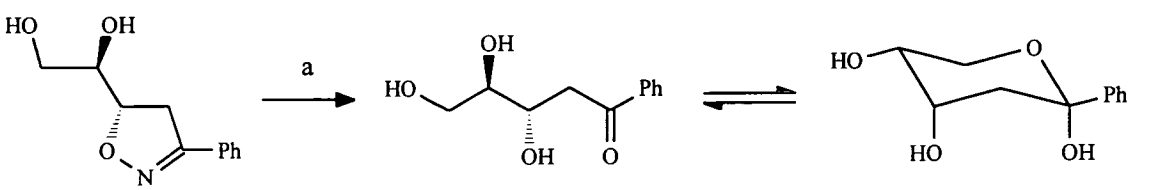
(227) **(250)**

Scheme 54

The product resulting from deprotection of the major dispiroketal adduct (**227**) proved to be identical by ^{1}H and especially ^{13}C NMR, and also R_f value to product (**250**) obtained from deprotection of isomer (**196**). Therefore, the adduct (**227**) is assigned as having *S*-configuration at the new chiral centre C-5 corresponding to an *erythro* relationship between this carbon and the adjacent asymmetric centre (C-14'). The minor isomer (**228**) therefore has a *threo* structure.

2.3.8.8 Reductive Hydrolytic Cleavage of 2-Isoxazoline (249)

The reductive hydrolytic cleavage of deprotected isoxazoline (**249**) constitutes the final stage in our approach to 2-ulosonic acid analogues. The procedure selected was based on the model studies discussed earlier (Section 2.3.8.3) and involves vigorous stirring of the isoxazoline (1 M eq), boric acid (6 M eq) and Raney-Nickel catalyst in a 5:1 mixture of methanol/water under an atmosphere of hydrogen (Scheme 55). TLC analysis of the reaction mixture indicated when reaction was complete (4 hours).



(249) **(255)** **(256)**

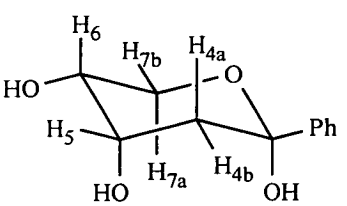
(a) Raney-Ni, MeOH/H_2O (5:1), H_3BO_3 (6 M eq)

Scheme 55

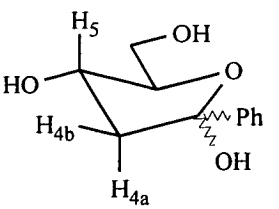
The product, which has a smaller TLC R_f value on silica (100% ethyl acetate) than the parent 2-isoxazoline (0.27 *cf* 0.10), was isolated by removal of solvent *in vacuo* with subsequent addition and evaporation of methanol to remove boric acid. The resulting pale yellow oil (37%) was not purified further. It was characterised by mass spectrometry and from its NMR spectra.

The FAB mass spectrum showed a characteristic peak at 212 for M^++2H. Accurate mass analysis (212.10483) of this peak gave the elemental composition as $C_{11}H_{14}O_4$, consistent with compounds (**255**) or (**256**).

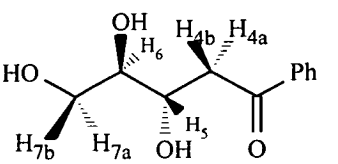
It was anticipated that the product could exist in open-chain and cyclic hemiacetal forms. Three structures were thus considered: a pyranose-like chair conformation (**256**), a furanose envelope conformation (**257**) with the 5-hydroxyl and hydroxymethyl groups occupying equatorial positions, and the open-chain structure (**255**).



(**256**)



(**257**)



(**255**)

To identify which of the above structures was most likely for the product, ^1H NMR data was initially considered. The product was tentatively assigned as the pyranose-chair with $^1C_4$ conformation (**256**). Examination of the ^1H NMR spectrum (Figure 12) allowed a number of deductions to be made. The two protons at lowest chemical shift are assigned to 4a-H and 4b-H, which have no oxygen attached. The

pattern shown is a key feature of an ABX system. There is a large geminal coupling of 16.5 Hz between 4a-H and 4b-H, and both are coupled to 5-H which appears as a multiplet at 4.22 ppm. 5-H has a large coupling of 8.7 Hz to 4a-H (larger than would normally be expected for axial-equatorial coupling) and a smaller coupling of 3.1 Hz to 4b-H. The smaller geminal coupling for 7a-H/7b-H of 10.6 Hz is consistent with this methylene group being next to an oxygen. Protons 7a-H and 7b-H also appear at higher chemical shift than those at C-4 as expected. However, the couplings for 5-H/6-H (6.7 Hz) and 6-H to both 7a-H and 7b-H (5.5, 4.1 Hz) are not consistent with this structure. Therefore an alternative furanose envelope conformation (**257**) was considered. The large geminal coupling between 4a-H/4b-H is still appropriate, as are the couplings 4a-H/5-H and 4b-H, but now the couplings between 5-H/6-H, 6-H/7a-H and 6-H/7b-H are more consistent with this structure.

However, when the ^{13}C NMR was examined a peak was detected at δ_C 200.7 ppm attributed to a carbonyl group. This observation is consistent only with the open chain form (**255**). All the ^{1}H-^{1}H couplings observed also fit with this structure.

The data obtained for the open chain product (**255**) are compared with those for the isoxazoline precursor (**249**) in Tables 23 and 24.

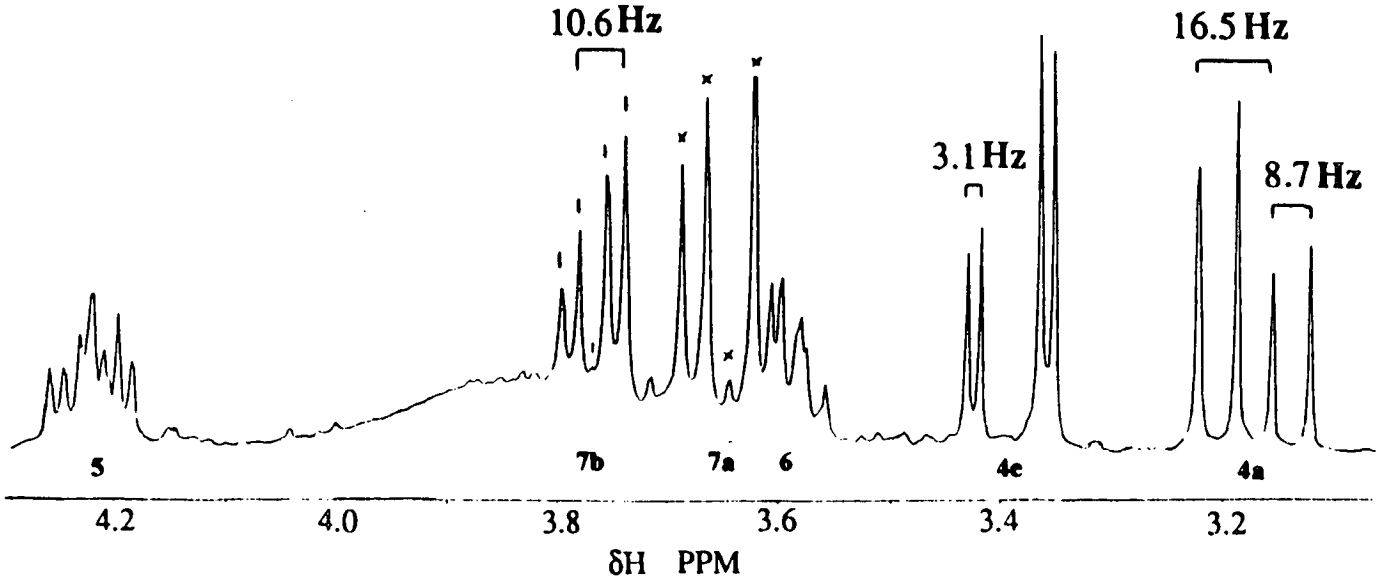
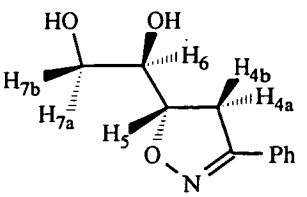
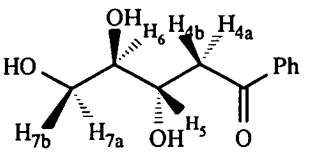


Figure 12 : NMR Spectrum for (255)



(249)



(255)

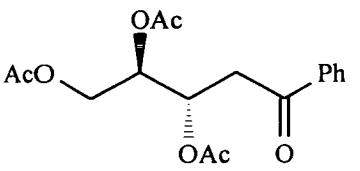
Proton	δ_H/ppm		Coupling	*J*/Hz	
	(249)	(255)		(249)	(255)
4a-H	3.48	3.17	4a,4b	16.9	16.5
4b-H	3.37	3.39	4a,5	8.1	8.7
5-H	4.75	4.22	4b,5	10.8	3.1
6-H	3.90	3.61	5,6	5.4	6.7
7a-H	3.61	3.77	6,7a	6.0	5.5
7b-H	3.68	3.68	6,7b	4.5	4.1
			7a,7b	11.1	10.6

Table 23 : ^{1}H NMR data

Compound	δ_C/ppm				
	C-3	C-4	C-5	C-6	C-7
(249)	156.6	35.5	81.8	72.3	63.5
(255)	200.7	41.9	74.4	68.7	62.9

Table 24 : ^{13}C NMR data

To provide further evidence for the proposed structure, compound (**255**) was converted to its triacetate derivative (**257**) using acetic anhydride and pyridine. Accurate mass measurement of 337.12873 (M^++H) gave an elemental formulae of $C_{17}H_{20}O_7$ in accord with compound (**255**).



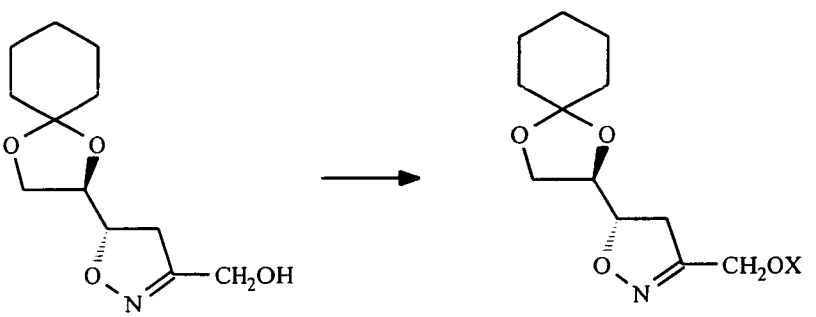
(**257**)

It is therefore proposed that the product formed from reductive hydrolytic cleavage of isoxazoline (**249**) exists predominantly in its open-chain form, rather than the pyranose ring structure. This may be a consequence of conjugation of the C=O to the phenyl substituent. It is predicted that replacement of Ph with non-conjugating groups such as CH_2OH would be more likely to allow ring closure to occur, although this experiment has not yet been performed.

2.3.9 Mesylation and Tosylation of 3-Hydroxymethyl-Substituted Isoxazoline (204)

One of the objectives of our work is to synthesise a range of 3-deoxy-hexulosonic acid analogues. Hence, conversion of the 3-hydroxymethyl group of (**204**) to the corresponding mesylate (**258**) or tosylate (**259**) should allow for alternative functionalisation of the isoxazoline by providing a suitably positioned leaving group for manipulation at later stages if required.

Alcohol (**204**) was treated with methanesulphonyl chloride in dry dichloromethane using triethylamine as base, affording the desired mesylate (**258**) in 96% yield (Scheme 56). The corresponding tosyl derivative (**259**) (40%) was prepared in a similar way. Both derivatives were yellow oils which were purified by dry flash column chromatography.



(**204**)

(**258**) X = SO_2Me
(**259**) X = $SO_2C_6H_4Me$-*p*

Scheme 56

Comparison of the ^1H NMR data of the mesylate and tosylate with those of the parent alcohol indicates that changing from OH to OMs or OTs has little effect on the chemical shift values apart from that due to CH_2OR, which moves to much higher frequency for OMs as expected ($\Delta\delta_H$ +0.53 ppm). There is a smaller change for OTs (Δ_H -0.12 ppm). 5-H also shifts to slightly lower frequency [$\Delta\delta_H$ -0.07 ppm for (**258**), -0.09 for (**259**)]. However, there is a marked change in coupling between 5-H and 6-H [7.1 *cf* 3.8 Hz for (**258**)] indicating that the presence of OMs influences the conformation adopted in solution. This difference in coupling is not observed in the tosylate (**259**). In the ^{13}C NMR for (**258**) there is a noticeable increase in the chemical

shift for CH_2OR on changing from OH to OMs ($\Delta\delta_C$ +5.0 ppm) and also in the adjacent quaternary carbon ($\Delta\delta_C$ -5.3 ppm).

2.3.10 Conclusions

A synthetic route to hexulosonic acid analogues using nitrile oxide-isoxazoline chemistry has been established using alkene (**195**) as the starting material. Cycloaddition of nitrile oxides to cyclohexylidene-protected alkene (**195**) and also the dispiroketal-protected alkene (**226**) have afforded adducts in good yields (40-88%) and moderate π-facial selectivity (72:28 to 79:21) with preference for *erythro* adducts in each case. The large steric bulk of the dispiroketal moiety and changing from a five to six membered ring system appears to have negligible effect on diastereoselectivity.

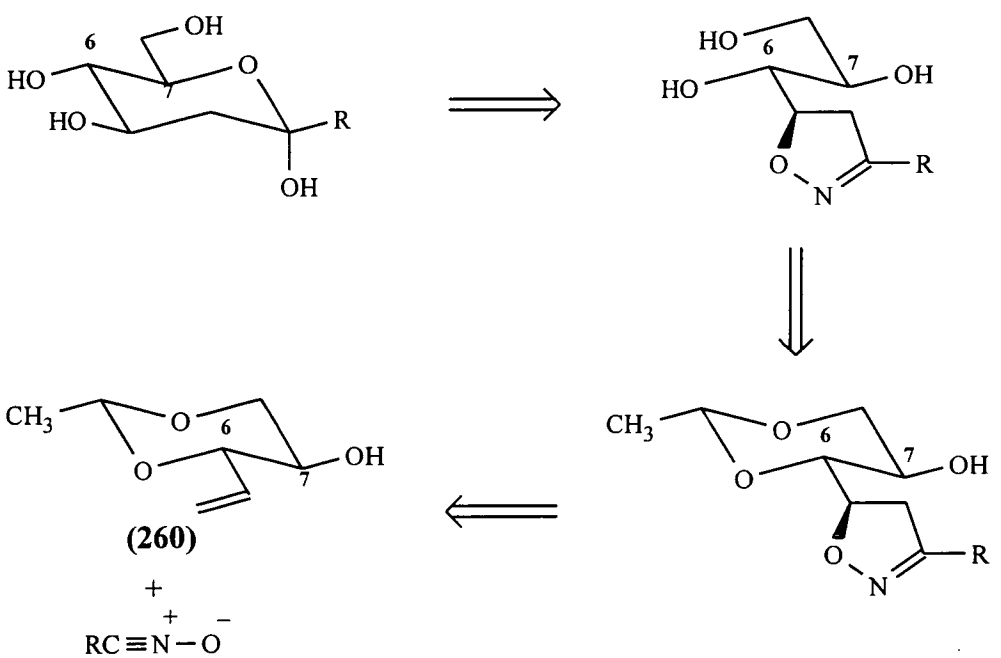
Two approaches for the conversion of the 2-isoxazoline to the desired 2-ulosonic acid analogues were investigated; either initial reductive hydrolytic cleavage of the N-O bond of the isoxazoline to the β-hydroxyketone followed by removal of the cyclohexylidene protection or *vice versa*. It was found that the desired analogues could best be formed *via* deprotection followed by Raney-nickel catalysed hydrogenolysis. One such analogue has been synthesised and proposed to exist predominantly in its open-chain form (**255**).

In summary, this work may provide access to a wide range of hexulosonic acid analogues *via* nitrile oxide-isoxazoline chemistry. Future work could involve the synthesis of the CH_2OH analogue to determine whether this also exists in an open-chain form or as predicted in the pyranose-ring structure.

2.4 3-Deoxy-D-*arabino*-heptulosonic Acid (DAH) Analogues

The approach selected for the synthesis of heptulosonic acid (DAH) analogues is *via* the five carbon sugar alkene (**260**) which incorporates the correct stereochemistry for C-6 and C-7 of the DAH framework. The choice of ethylidene protection for D-glucose was made based on literature precedent[218] which suggests that synthesis *via* the alternative 4,6-*O*-benzylidene-D-glucose would be unreliable and difficult.

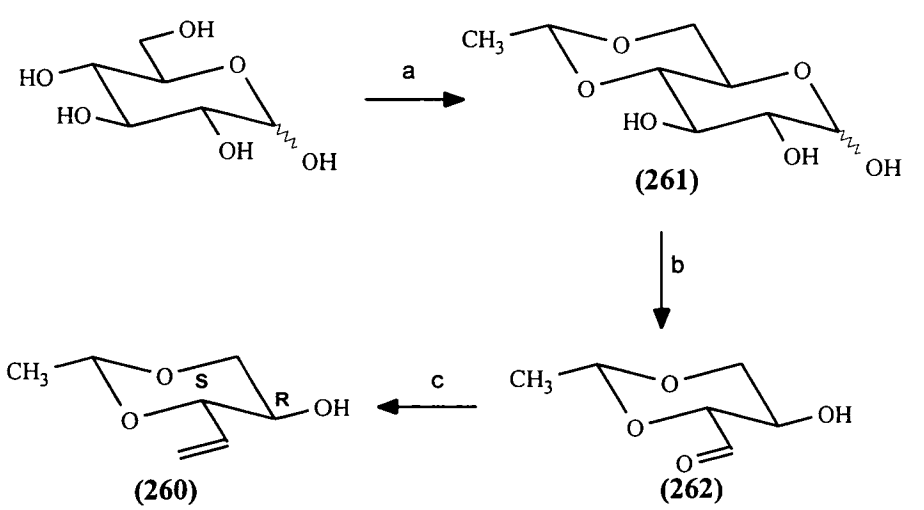
By analogy with the route described earlier for the six-carbon analogues (Section 2.3.2) cycloaddition of the alkene to an appropriate nitrile oxide, followed by deprotection and subsequent reductive hydrolytic ring opening of the isoxazoline, should provide access to heptulosonic acid analogues (Scheme 57).



Scheme 57

2.4.1 Synthesis of 1,2-*Dideoxy*-3,5-O-*ethylidene*-D-erythro-*pent*-1-*enitol* (260)

Alkene (**260**) was prepared in three steps from D-glucose as outlined in Scheme 58, with an overall yield of 32%. Treatment of D-glucose with freshly distilled paraldehyde (1.01 M eq) containing concentrated sulphuric acid afforded the desired 4,6-*O*-ethylidene-D-glucose (**261**)[219] as a mixture of α and β anomers (α:β 79:21 shown by ^{1}H NMR). It is interesting to note that on addition of d_6-acetic acid, the ratio of α to β changes to 37:63, with the β form becoming predominant. This mixture of anomers was used for later stages without any attempt at separation, since the anomeric carbon is lost in the following steps. Subsequent metaperiodate cleavage afforded aldehyde (**262**) as a white glass. The aldehyde is reported[220] to exist in more than one form; as a dimer m.p. 149-150°C, and also in a different crystalline form with m.p. 110-111°C indicating the presence of water of hydration. The conversion of aldehyde (**262**) to alkene (**260**) using a Wittig reaction was then performed and the product purified by dry flash chromatography to afford a pale yellow oil in 70% yield.



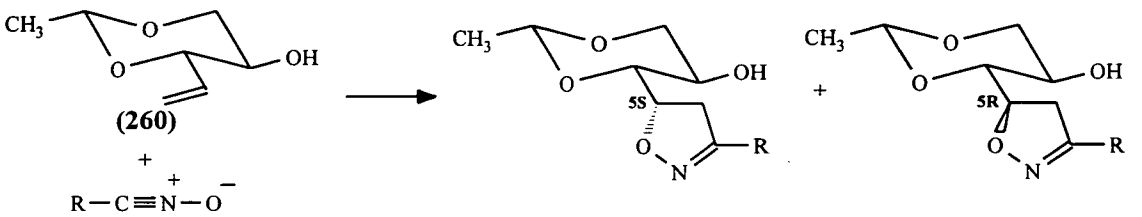
(a) $(CH_3CHO)_3$, conc. H_2SO_4; (b) $NaIO_4$; (c) $Ph_3P^+CH_3I^-$, $(CH_3)_3COK$, THF

Scheme 58

2.4.2 Investigation of π-Facial Selectivity in Nitrile Oxide Cycloadditions to 1,2-Dideoxy-3,5-*O*-ethylidene-D-*erythro*-pent-1-enitol (260)

2.4.2.1 Nitrile Oxide Cycloadditions to Alkene (260)

Three nitrile oxides were examined: ethoxycarbonylformonitrile oxide, benzonitrile oxide and (diethoxyphosphoryl)acetonitrile oxide. Cycloaddition of alkene (**260**) to ethoxycarbonylformonitrile oxide (Scheme 59) was carried out as outlined in Section 2.3.2. A mixture of diastereoisomeric isoxazolines (**263**) and (**264**) in combined yield of 77% and ratio 53:47 was obtained after chromatography to remove 3,4-diethoxycarbonylfurazan *N*-oxide (10%).



(**263**) R = CO_2Et (**264**) R = CO_2Et
(**265**) R = Ph (**266**) R = Ph
(**267**) R = $CH_2P(O)(OEt)_2$ (**268**) R = $CH_2P(O)(OEt)_2$
(**269**) R = CH_2OH (**270**) R = CH_2OH

Scheme 59

The isomer ratio was determined by comparison of the integrals of the epimeric 5-H signals in the ^1H NMR spectrum of the mixture. The assignment of 5*S*-configuration for the major isomer (**263**) corresponding to a *erythro* relationship about the C-5/C-6 bond was deduced unambiguously by X-ray analysis (Section 2.4.6).

The overall stereochemistry for the isomers can be assigned as D-*ribo* for the 5*S* isomer and D-*arabino* for the 5*R* isomer. However, in the following discussion the

terms *erythro* and *threo* will be used, referring to the relationship about the newly created chiral centre at C-5 and the adjacent centre C-6.

Similarly the corresponding cycloaddition of benzonitrile oxide to the same alkene (**260**) afforded the adducts (**265**) and (**266**) in a combined yield of 75% and ratio of 52:48, together with the nitrile oxide dimer 3,4-diphenylfurazan *N*-oxide (1%). The ratio was again determined by comparison of the integrals of the 5-H signals in the ^{1}H NMR of the mixture. The assignment of configuration for the major isomer as *erythro* was made by correlation of NMR shift differences, optical rotation data and TLC properties with those of the ethoxycarbonylformonitrile adducts.

Cycloaddition of (diethoxyphosphoryl)acetonitrile oxide to the title alkene (**260**) produced a 38% combined yield of the inseparable diastereoisomeric 2-isoxazolines (**267**) and (**268**) in a ratio of 50:50 in favour of the *erythro* adduct. Again the ratio was determined by comparison of the integrals for the 5-H signals in the ^{1}H NMR. Although the isoxazolines were chromatographically inseparable, the isoxazoline ring protons for each isomer could be assigned in the ^{1}H NMR of the mixture due to differences in signal intensities. The major isomer was identified as the *erythro* adduct (**267**) based on comparison of its spectral parameters with those of similar adducts, as above.

The nitrile oxide cycloadditions to alkene (**260**) all proceed with little π-facial selectivity with d.e. values ranging from 0% for (diethoxyphosphoryl)acetonitrile oxide to 6% for ethoxycarbonylformonitrile oxide. The *erythro:threo* ratios for the three cases are presented in Table 25.

Nitrile Oxide	*erythro* : *threo*
EtO_2CCNO	53 : 47
PhCNO	52 : 48
$(EtO)_2P(O)CH_2CNO$	50 : 50

Table 25 : Isomer Ratios for Cycloaddition of Nitrile Oxides to Alkene (**260**)

2.4.2.2 Characterisation of Isoxazoline Cycloadducts

The stereochemistry at the new chiral centre C-5 of the isoxazoline can be predicted by correlation of ^1H and ^{13}C NMR and optical rotation data. These characteristic properties of the major and minor isomers are therefore summarised below, along with those for (**269**) and (**270**) obtained after borohydride reduction of the ethoxycarbonyl compounds (**263**) and (**264**) (Section 2.4.8).

TLC

The *erythro* adduct has a larger R_f value on silica (100% ether) than the corresponding *threo* isomer [0.25 *cf* 0.21 for (**263**)/(**264**), 0.28 *cf* 0.24 for (**265**)/(**266**)].

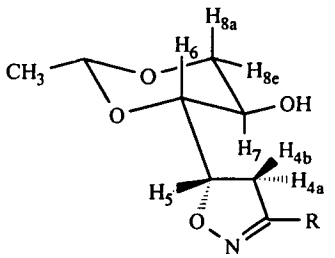
^1H NMR

The ^1H NMR spectra reflect major differences between the isomers for protons 4-H to 7-H. The isoxazoline ring protons 4a-H and 4b-H give rise to a characteristic ABX system with 5-H, which is adjacent to the ring oxygen, at highest chemical shift, and the ^3J values observed are typical of 3,5-disubstituted isoxazolines.[193-195] It is observed that for the *threo* adduct the 5-H signal absorbs at

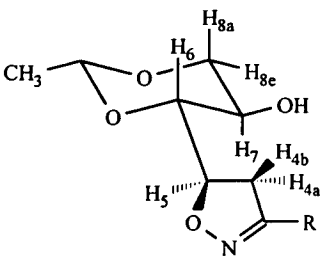
significantly higher frequency than for the corresponding *erythro* product [$\Delta\delta_H$ +0.14 ppm for (**263**)/(**264**), +0.18 ppm for (**265**)/(**266**), +0.12 ppm for (**267**)/(**268**)]. The exception is that for the pair of isomers (**269**) and (**270**) where 5-H resonates at the same frequency for each isomer. The signals for the methylene protons of the isoxazoline ring, 4a-H and 4b-H, are closer together in the *erythro* adducts. It is noteworthy that the coupling between 5-H and 6-H in the *erythro* isomer is significantly greater than in the *threo* product [4.8 *cf* 2.9 Hz for (**263**)/(**264**), 5.3 *cf* 3.2 for (**265**)/(**266**), 6.2 *cf* 3.4 for (**267**)/(**268**)]. This may be due to the two compounds adopting different conformations in solution. There is also an appreciable difference in the proton 7-H signal, which resonates at a higher frequency for the *threo* isomer compared to the *erythro* isomer [$\Delta\delta_H$ +0.35 ppm for (**263**)/(**264**), +0.17 ppm for (**265**)/(**266**), +0.28 ppm for (**269**)/(**270**)]. Evidence for the chair conformation of the 1,3-dioxane ring is shown by the large diaxial coupling between 7-H and 8a-H (*ca* 10 Hz) and the smaller axial-equatorial coupling between 7-H and 8e-H. Selected data for the chemical shifts and coupling constants are summarised in Tables 26 and 27.

^{13}C NMR

The ^{13}C NMR spectra for the pairs of isomers show significant differences for C-4 to C-7. In the adducts (**263**) to (**266**) the *erythro* adduct has a higher δ-value for C-4 [$\Delta\delta_C$ +1.1 ppm for (**263**)/(**264**), +1.9 ppm for (**265**)/(**266**)] as does C-5 [$\Delta\delta_C$ +2.9 ppm for (**263**)/(**264**), +1.7 ppm for (**265**)/(**266**)]. There is also a marked difference shown for C-7 [$\Delta\delta_C$ -2.9 ppm for (**263**)/(**264**), -3.2 ppm for (**265**)/(**266**)]. These findings are shown in Table 28.



Erythro



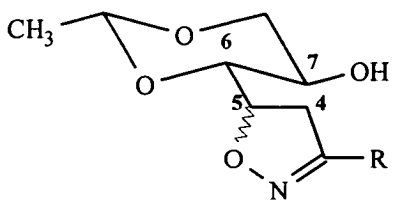
Threo

R	Isoxazoline	δ_H/ppm					
		4a-H	4b-H	5-H	6-H	7-H	8a-H
CO_2Et	*Erythro* (**263**)	3.34	3.23	5.01	3.53	3.63	3.36
	Threo (**264**)	3.21	3.38	5.15	3.45	3.98	3.38
Ph	*Erythro* (**265**)	3.44	3.45	4.89	3.45	3.77	3.38
	Threo (**266**)	3.34	3.45	5.07	3.53	3.94	3.46
$CH_2P(O)(OEt)_2$	*Erythro* (**267**)	3.20-2.85		4.83	3.42	3.86	nd
	Threo (**268**)	3.20-2.85		4.95	3.48	3.73	nd
CH_2OH	*Erythro* (**269**)	3.19	3.07	4.85	3.58	3.48	3.35
	Threo (**270**)	3.10	3.13	4.85	3.36	3.76	3.33

Table 26 : Selected ^{1}H NMR data for cycloadducts

R	Coupling	*J*/Hz				
		4a,4b	4a,5	4b,5	5,6	7,8a
CO_2Et	*Erythro* (**263**)	17.8	7.7	11.4	4.8	10.0
	Threo (**264**)	17.6	11.7	8.8	2.9	10.6
Ph	*Erythro* (**265**)	17.5	7.1	11.5	5.3	10.2
	Threo (**266**)	16.7	11.5	8.6	3.2	9.9
$CH_2P(O)(OEt)_2$	*Erythro* (**267**)	nd	6.2	11.4	6.2	9.3
	Threo (**268**)	nd	11.5	8.2	3.4	9.8
CH_2OH	*Erythro* (**269**)	16.9	8.3	11.2	2.6	10.0
	Threo (**270**)	-	10.7	9.3	2.8	10.4

Table 27 : Selected ^{1}H NMR data for cycloadducts



R		δ_C/ppm			
		C-4	C-5	C-6	C-7
CO_2Et	*Erythro* (**263**)	35.1	83.8	79.8	63.6
	Threo (**264**)	34.0	80.9	80.5	60.7
Ph	*Erythro* (**265**)	37.5	82.1	79.8	64.6
	Threo (**266**)	35.6	80.4	78.8	61.4
CH_2OH	*Erythro* (**269**)	35.1	81.1	80.0	62.8
	Threo (**270**)	35.6	81.7	77.7	61.5

Table 28 : Selected ^{13}C NMR data for cycloadducts

Optical Rotation

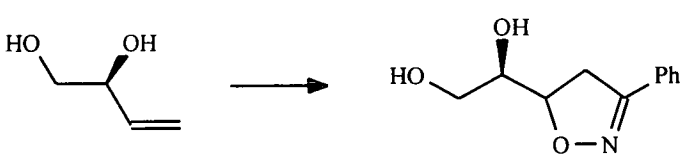
For the pair of ethoxycarbonyl adducts (**263**, **264**) and their reduced analogues (**269**, **270**) the *erythro* isomer was found to have a more positive $[\alpha]_D$ value. This result is important for correlation and for thus assigning the configuration at the new chiral centre C-5. Table 29 summarises this information.

Erythro (**263**)	*Threo* (**264**)	*Erythro* (**269**)	*Threo* (**270**)
+105.6	-213.5	+115.9	-225.8

Table 29

2.4.3 Conclusion

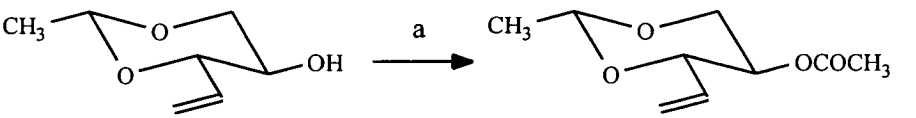
These results indicate that there is negligible π-facial selectivity for nitrile oxide cycloaddition reactions to alkene (**260**). This lack of selectivity is possibly due to the presence of the homoallylic hydroxyl at C-7. It has previously been observed[204,205] (Section 2.3.2.3) that homoallylic alkoxy substituents have a significant effect on selectivities. For example, with the *ribo*-alkene (**215**) the selectivity is 42:58 (*erythro*:*threo*) compared to 90:10 (*erythro*:*threo*) for the *lyxo*-alkene (**214**). Hydrogen-bonding interactions between an alcohol group on the dipolarophile and the oxygen of the nitrile oxide may also influence the isomer ratio.[196] In order to investigate the influence of the homoallylic substituent on π-facial selectivity the alkene (**260**) was therefore acetylated and subsequent cycloaddition reactions performed. Removal of the ethylidene protecting group of alkene (**260**) followed by cycloaddition reactions to this deprotected alkene have also been carried out in order to investigate the effect on selectivity. It has been reported[196] that cycloaddition to (**207**) gives an *erythro*:*threo* ratio of 61:39. This represents a marked decrease in selectivity compared to cycloadditions to alkenes containing cyclohexylidene or isopropylidene protecting groups (see Table 13, Section 2.3.5).



(**207**)

2.4.4 Synthesis of 1,2-*Dideoxy*-4-O-*acetal*-3,5-O-*ethylidene*-D-erythro-*pent*-1-*enitol* (271)

Alkene (**271**) was prepared as a pale yellow oil in 62% yield by acetylation of (**260**) using dry acetic anhydride in dry pyridine (Scheme 60).



(**260**) (**271**)

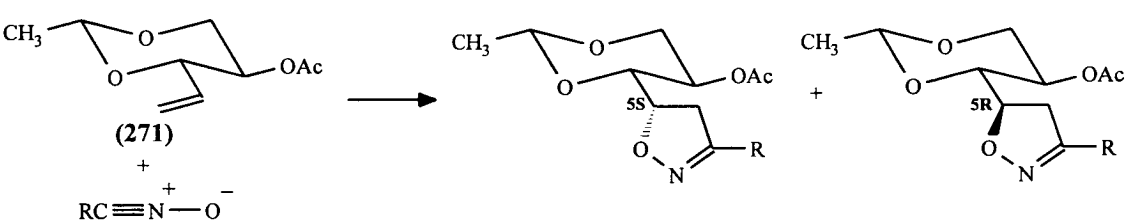
(a) $(CH_3CO)_2O$, Pyridine

Scheme 60

2.4.5 Investigation of π-Facial Selectivity in Nitrile Oxide Cycloadditions to 1,2-*Dideoxy*-4-O-*acetal*-3,5-O-*ethylidene*-D-erythro-*pent*-1-*enitol* (271)

2.4.5.1 Nitrile Oxide Cycloadditions to Alkene (271)

Two nitrile oxides were examined: ethoxycarbonylformonitrile oxide and benzonitrile oxide. Cycloaddition of alkene (**271**) to benzonitrile oxide (Scheme 61) was carried out as outlined in Section 2.3.2 and a mixture of diastereoisomeric isoxazolines (**272**) and (**273**) in combined yield of 74% and ratio 63:37 was obtained after chromatography to remove 3,4-diphenylfurazan *N*-oxide (4%).



(**272**) R = Ph
(**274**) R = CO_2Et

(**273**) R = Ph
(**275**) R = CO_2Et

Scheme 61

The isomer ratio was determined by comparison of the integrals of the 6-H signals in the ^{1}H NMR spectrum of the mixture. X-ray crystal structures for both the phenyl adducts (**272**) and (**273**) were obtained to assign unambiguously the configuration of the new asymmetric centre C-5. These are discussed in Section 2.5.6. The results show that the major isomers (**272**) and (**274**) have *S*-configuration at C-5, corresponding to an *erythro* relationship about the C-5/C-6 bond.

Similarly the corresponding cycloaddition of ethoxycarbonylformonitrile oxide to alkene (**271**) afforded the adducts (**274**) and (**275**) in a combined yield of 46% and ratio of 59:41, together with the nitrile oxide dimer 3,4-diethoxycarbonyl furazan *N*-oxide (12%). The ratio was again determined by comparison of the integrals for the 6-H signals in the ^{1}H NMR of the mixture. The assignment of configuration for the major isomer as *erythro* was made by correlation of NMR shift differences, optical rotation data and TLC properties with the benzonitrile adducts (**272**) and (**273**).

The stereoselectivity of the nitrile oxide cycloadditions to alkene (**271**) both proceed with moderate π-facial selectivity with d.e. values ranging from 18% for ethoxycarbonylformonitrile oxide to 26% for benzonitrile oxide. Table 30 gives a comparison of the selectivities observed using alkenes (**260**) and (**271**).

Alkene	**Nitrile Oxide**	***erythro* : *threo***
(**260**)	EtO_2CCNO	52 : 48
	PhCNO	53 : 47
(**271**)	EtO_2CCNO	59 : 41
	PhCNO	63 : 37

Table 30 : Isomer Ratios for Cycloaddition of Nitrile Oxides to Alkenes (**260**) and (**271**)

The results obtained indicate that, although there is enhanced selectivity in favour of the *erythro* isomer, the extent of this increase is small.

The small increase in selectivity (d.e. 4% *cf* 26%) may be attributed to the presence of the acetyl group which may be preventing the hydrogen bonding interactions which are possible for the free hydroxy compound. Six possible transition states (Figure 13) can be drawn for the cycloaddition of alkene (**260**) to a nitrile oxide. The *erythro* adduct results from A, B and C, and the *threo* adduct from A′, B′, and C′. The influence of an equatorial hydroxy at the homoallylic position effects the transition states B and C′ (Figure 14), with these two states being favoured by hydrogen bonding. Figure 14 represents the hydrogen bonding interactions that exist in these two states.

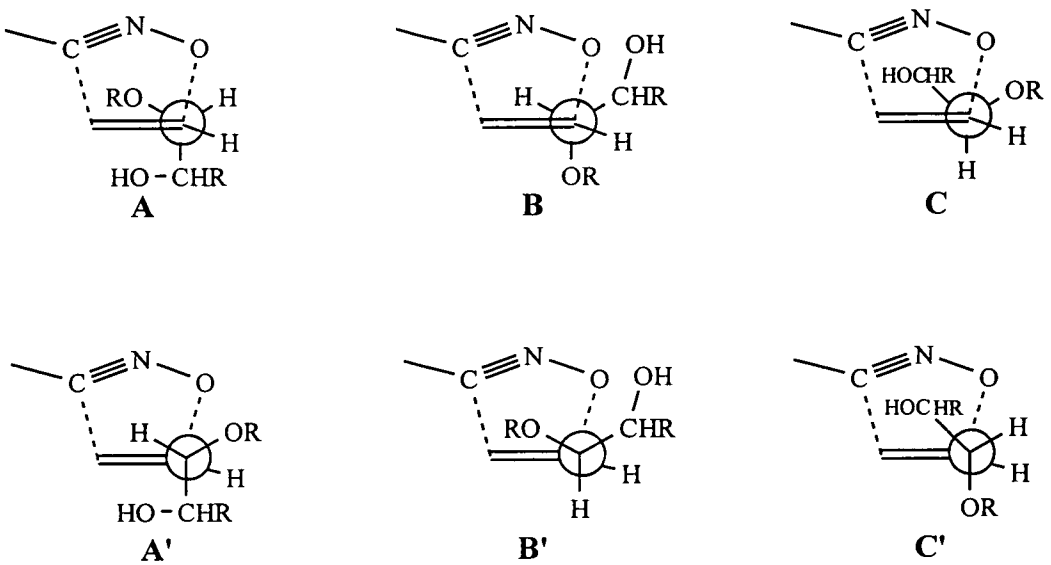


Figure 13[2]

[2] Configuration of allylic centre in this case is of opposite stereochemistry to that in Houk model

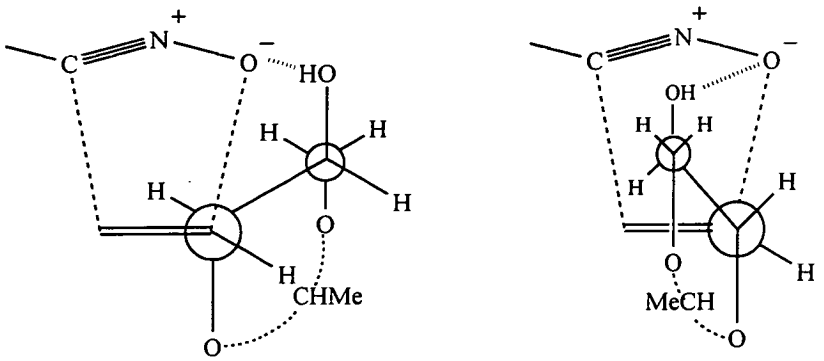


Figure 14

The selectivity for the 1,3-dioxan is also low compared to 1,3-dioxolanes (~60:40 *cf* ~80:20). Replacing acetate with a more bulky substituent eg. benzoate might be expected to increase the selectivity further. However, additional investigations need to be performed to determine the precise influence of this equatorial homoallylic substituent.

2.4.5.2 Characterisation of Isoxazoline Cycloadducts

Correlation of ^{1}H and ^{13}C NMR and optical rotation data allow prediction of the stereochemistry at the new chiral centre C-5. Comparison of results with those from cycloadditions to alkene (**260**), which contains a hydroxyl substituent at the homoallylic position, can also be made. These characteristic properties of the major and minor isomers are therefore summarised below.

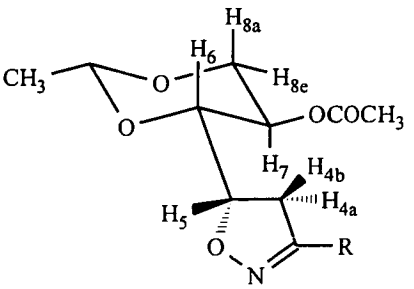
TLC

The *erythro* adduct has a larger R_f value on silica (100% ether) than the corresponding *threo* isomer [0.50 *cf* 0.41 for (**272**)/(**273**), 0.47 *cf* 0.39 for (**274**)/(**275**)]. This is in agreement with results obtained from cycloadditions to alkene (**260**) (Section 2.4.2.2).

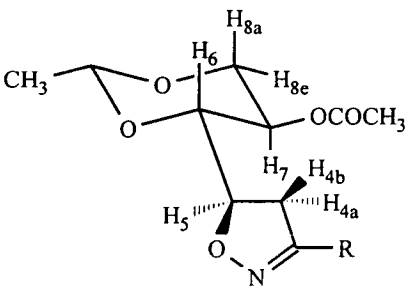
^{1}H NMR

The ^{1}H NMR spectra reflect major differences between the isomers for protons 4-H to 7-H. As observed in previous systems, the isoxazoline ring protons 4a-H and 4b-H give rise to a characteristic ABX system. It is observed that for the *threo* adduct the proton 5-H signal absorbs at significantly higher frequency than for the corresponding *erythro* product ($\Delta\delta_H$ +0.11 ppm for each of the pairs of isomers). This difference is essentially the same as for the previously mentioned cycloadditions to alkene (**260**). However, changing from 7-OH to 7-$OCOCH_3$, has resulted in proton 5-H shifting to lower frequency [eg. 4.79 *cf* 5.01 ppm for (**274**)/(**263**)]. The signals for the methylene protons of the isoxazoline ring, 4a-H and 4b-H, are closer together in the *threo* adducts, which is a reversal to that obtained in the hydroxyl compounds, and may be a consequence of conformational change. Again it is noteworthy that proton 7-H resonates at a higher frequency for the *threo* isomer compared to the *erythro* isomer [$\Delta\delta_H$ +0.22 ppm for (**272**)/(**273**), +0.27 ppm for (**274**)/(**275**)]. This proton also occurs at ~1.0 ppm higher frequency in these acetylated adducts than the corresponding hydroxyl compounds [4.69 *cf* 3.63 ppm for (**274**)/(**263**), 4.96 *cf* 3.98 ppm for (**275**)/(**264**), 4.82 *cf* 3.77 ppm for (**272**)/(**265**), 5.04 *cf* 3.94 ppm for (**273**)/(**266**)]. There is also a noticeable difference for 6-H. In this case it absorbs at a significantly higher frequency than for the corresponding 7-OH compounds [$\Delta\delta_H$ +0.27 ppm for (**274**)/(**263**), +0.16 ppm for (**275**)/(**264**), +0.39 ppm for (**272**)/(**265**), +0.18 ppm for (**273**)/(**266**)]. The difference is much more pronounced for the *erythro* isomers. The coupling between 5-H and 6-H for both isomers is essentially the same. This is in comparison to the values obtained for the 7-OH compounds, and may be

due to an altered conformation of the *erythro* isomer in solution on changing the homoallylic substituent. The chemical shifts and coupling constants are summarised in Tables 31 and 32.



Erythro



Threo

R	Isoxazoline	δ_H/ppm					
		4a-H	4b-H	5-H	6-H	7-H	8a-H
Ph	*Erythro* (**272**)	3.51	3.32	4.77	3.84	4.82	3.46
	Threo (**273**)	3.32	3.41	4.88	3.71	5.04	3.37
CO_2Et	*Erythro* (**274**)	3.38	3.15	4.79	3.80	4.69	3.44
	Threo (**275**)	3.16	3.24	4.90	3.61	4.96	3.32

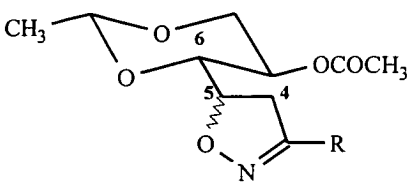
Table 31 : Selected ^{1}H NMR data for cycloadducts

R	Coupling	*J*/Hz				
		4a,4b	4a,5	4b,5	5,6	7,8a
Ph	*Erythro* (**272**)	16.6	7.9	11.2	3.2	10.2
	Threo (**273**)	16.4	11.2	8.4	2.9	10.0
CO_2Et	*Erythro* (**274**)	17.7	8.3	11.7	3.2	10.1
	Threo (**275**)	17.5	11.4	8.6	2.6	9.9

Table 32 : Selected ^{1}H NMR data for cycloadducts

^{13}C NMR

The ^{13}C NMR spectra for the pairs of isomers show significant differences for C-4 to C-6. In each case the *threo* adduct has a higher δ-value for C-4 ($\Delta\delta_C$ +0.5 ppm for each of the pairs of isomers) as does C-6 [$\Delta\delta_C$ +0.3 ppm for (**272**)/(**273**), +0.6 ppm for (**274**)/(**275**)]. However, the *erythro* isomer has a higher frequency for C-5 than the corresponding *threo* isomer [$\Delta\delta_C$ +2.0 ppm for (**272**)/(**273**), +2.4 ppm for (**274**)/(**275**)]. These findings are shown in Table 33.



R		δ_C/ppm		
		C-4	**C-5**	**C-6**
Ph	*Erythro* (**272**)	35.1	80.2	77.9
	Threo (**273**)	35.6	78.2	78.2
CO_2Et	*Erythro* (**274**)	33.6	82.5	77.5
	Threo (**275**)	34.1	80.1	78.1

Table 33 : Selected ^{13}C NMR data for cycloadducts

Optical Rotation

The *erythro* isomer was found in each case to have a more positive $[\alpha]_D$ value (Table 34). This result is again important for correlation and assignment of configuration at C-5.

Erythro (**272**)	*Threo* (**273**)	*Erythro* (**274**)	*Threo* (**275**)
+41.8	-200.0	+68.6	-224.0

Table 34

2.4.6 X-Ray Crystallography to Determine Configuration at New Asymmetric Centre C-5 for Compounds (263), (272) and (273)

The major adduct (**263**) (first isomer recovered by chromatography) obtained from addition of ethoxycarbonylformonitrile oxide to alkene (**260**), was isolated as a crystalline solid suitable for X-ray analysis. Examination of the crystal structure (Figure 15) established *S*-configuration at the new asymmetric centre C-5 representing an *erythro* relationship between C-5 and C-6. The assignment of *S*-configuration for the acetate analogue (**272**) confirmed this *erythro* relationship. Each asymmetric unit of (**263**) was shown by the X-ray data to be composed of two molecular shapes within the crystal. The other two X-ray structures for (**272**) and (**273**) were single shapes (Figures 16 and 17).

The H-C-C-H torsion angles obtained for each X-ray structure were used to calculate the proton-proton coupling constants by applying the Haasnoot parameterisation[209] of the Karplus equation. These values are compared to those observed in solution in Tables 35, 36 and 37. Consideration of the data indicates satisfactory correlation for some of the data, eg. $J_{4,5}$, $J_{5,6a}$ and $J_{5,6e}$; however significant deviations are noticed elsewhere. The relationship between the calculated and observed *J*-values for the isoxazoline ring protons is poor; this has previously been observed for other isoxazolines.[221,222] A noteworthy feature of the crystal structure for (**263**) is the -72.0/-75.5 torsion angle for H(3)-C(3)-C(4)-H(4) involving the protons attached to the carbon atoms linking the six membered 1,3-dioxane ring and newly formed isoxazoline. In solution the corresponding ^{1}H-^{1}H coupling is 4.8 Hz, which is

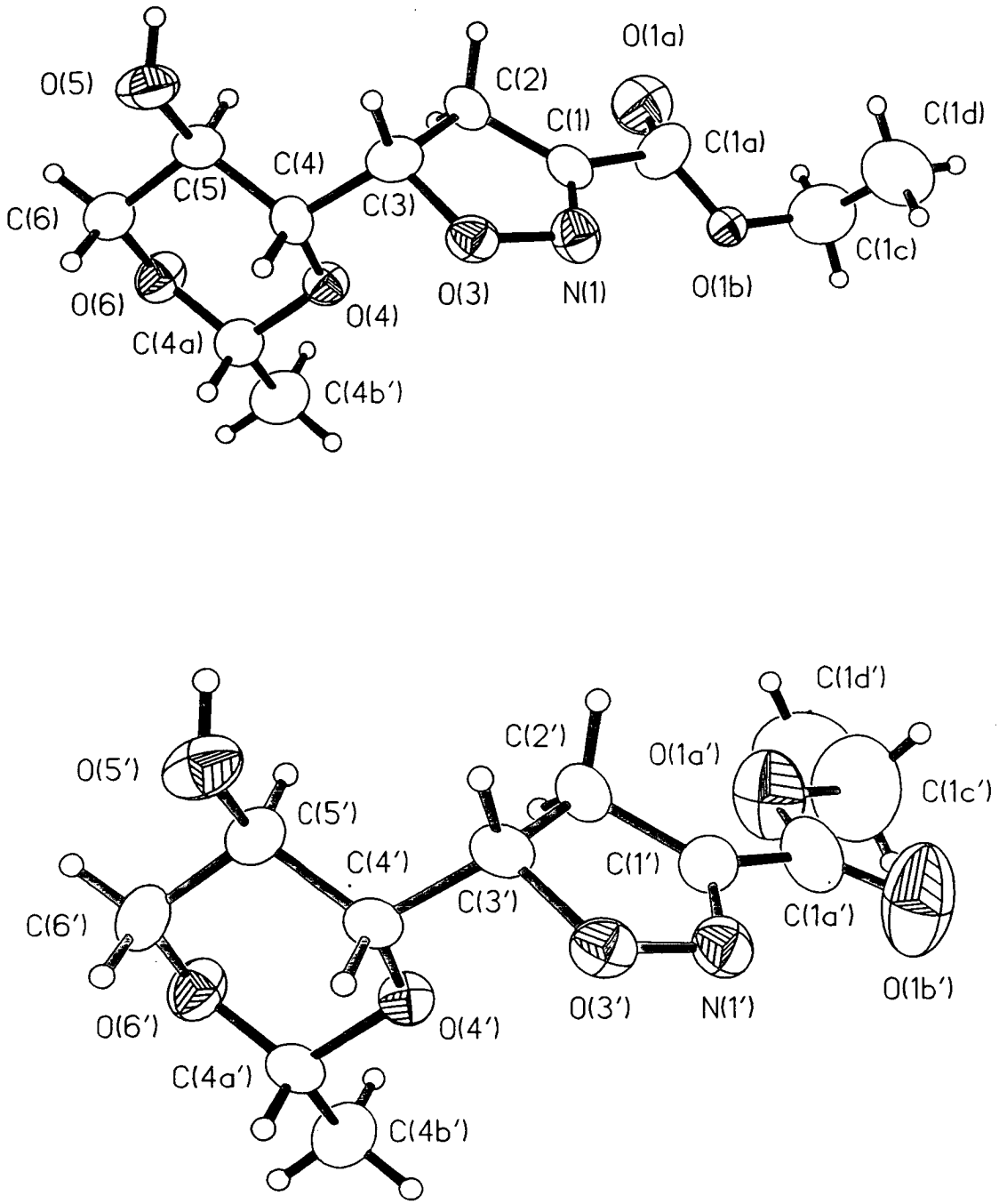
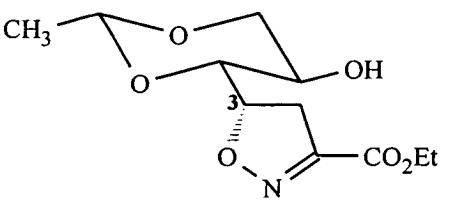

O(5)
C(2)
O(1a)
C(1)
C(1d)
C(1a)
C(4)
C(5)
C(3)
C(6)
C(1c)
O(6)
O(3)
N(1)
O(1b)
O(4)
C(4a)
C(4b')
C(1d')
O(5')
C(2')
O(1a')
C(1c')
C(5')
C(4')
C(1')
C(3')
C(6')
C(1a')
O(1b')
O(3')
N(1')
O(6')
O(4')
C(4a')
C(4b')

Figure 15

much greater than the calculated value (1.8/1.6 Hz). This suggests that the preferred conformation in solution differs markedly from that found in the crystal. In contrast, the data for (**272**) and (**273**) shows good correlation between the two values, which suggests that the presence of the equatorial 7-OAc locks the molecule in a conformation which is the same in solution or crystal.



	H_x,H_y[1]					
	2a,3	**2b,3**	**3,4**	**4,5**	**5,6e**	**5,6a**
θ_{Obs}[a]	-123.3	-1.2	-72.0	175.4	-55.6	-174.2
J_{Obs}	7.7	11.4	4.8	9.3	5.3	10.0
J_{Calc}[a]	5.3	8.1	1.8	10.2	3.3	10.2
θ'_{Obs}[a]	-122.5	-0.4	-75.5	174.4	-54.9	-173.4
J_{Obs}	7.7	11.4	4.8	9.3	5.3	10.0
J'_{Calc}[a]	4.2	8.1	1.6	10.2	3.3	10.2

a : θ and J_{Calc} represent the torsion angles and coupling constants respectively for one crystal type , θ' and J'_{Calc} are the corresponding values for the alternative.
$^3J_{calc} = 7.76\ Cos^2\theta - 1.1\ Cos\theta + 1.4$

Table 35 : Calculated and observed coupling constants for the two crystal types of adduct (**263**)

[1] Labelling of protons in X-ray pictures is different from that used in characterisation of products

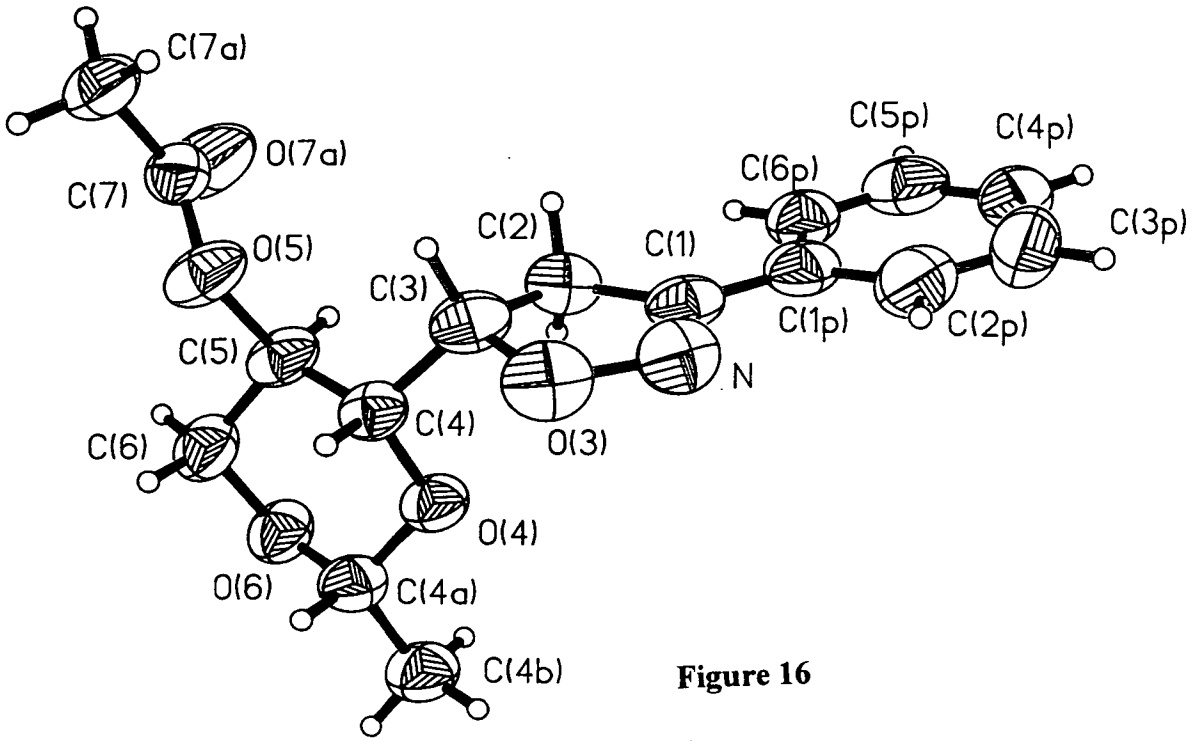


Figure 16

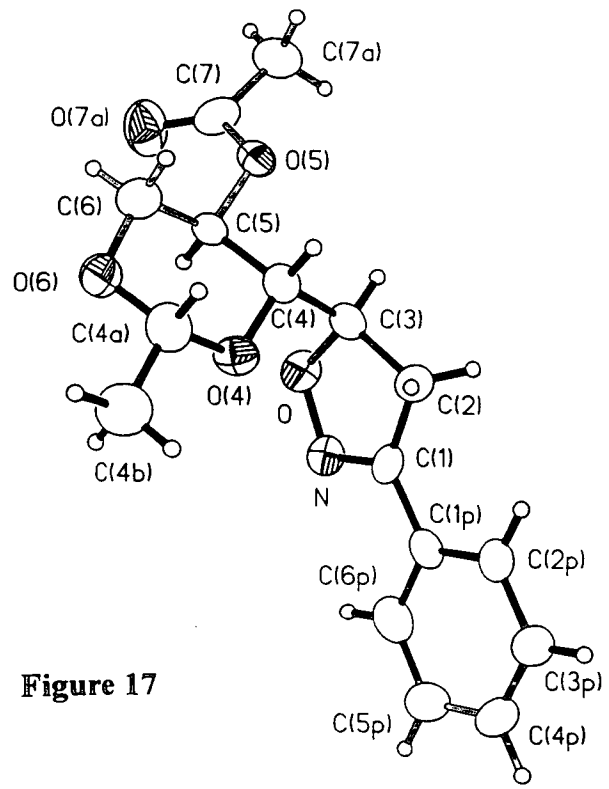
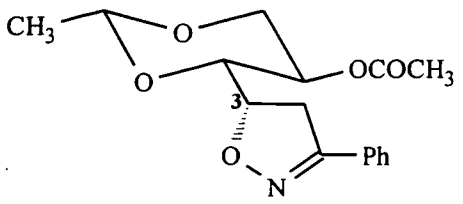
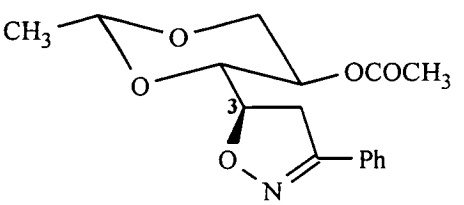


Figure 17



	H_x,H_y					
	2a,3	**2b,3**	**3,4**	**4,5**	**5,6e**	**5,6a**
θ_{Obs}	-134.5	-12.0	-68.3	172.0	-51.5	-170.3
J_{Obs}	7.9	11.2	3.2	9.8	5.4	10.2
J_{Calc}	6.0	7.7	2.1	10.1	3.7	10.0

Table 36 : Calculated and observed coupling constants for adduct (**272**)

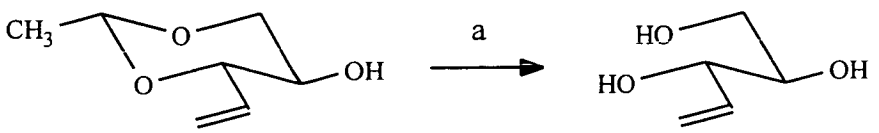


	H_x,H_y					
	2a,3	**2b,3**	**3,4**	**4,5**	**5,6e**	**5,6a**
θ_{Obs}	-4.3	118.5	-59.6	175.5	-54.3	-174.1
J_{Obs}	11.2	8.4	2.9	9.7	5.4	10.0
J_{Calc}	8.0	3.7	2.8	10.2	3.4	10.2

Table 37 : Calculated and observed coupling constants for adduct (**273**)

2.4.7 Synthesis of 1,2-*Dideoxy*-D-erythro-*pent*-1-*enitol* (276)

The deprotected alkene (**276**) was afforded as a colourless oil (41%) by stirring of alkene (**260**) with a mixture of water/glacial acetic acid (1:4) at 80°C for 7 hours (Scheme 62) and the product purified by preparative TLC. The parent alkene has a larger R_f value on silica (50% ether/ethyl acetate) than the corresponding triol as expected (0.59 *cf* 0.09).



(**260**) (**276**)

(a) Glacial acetic acid/water (4:1), 80°C

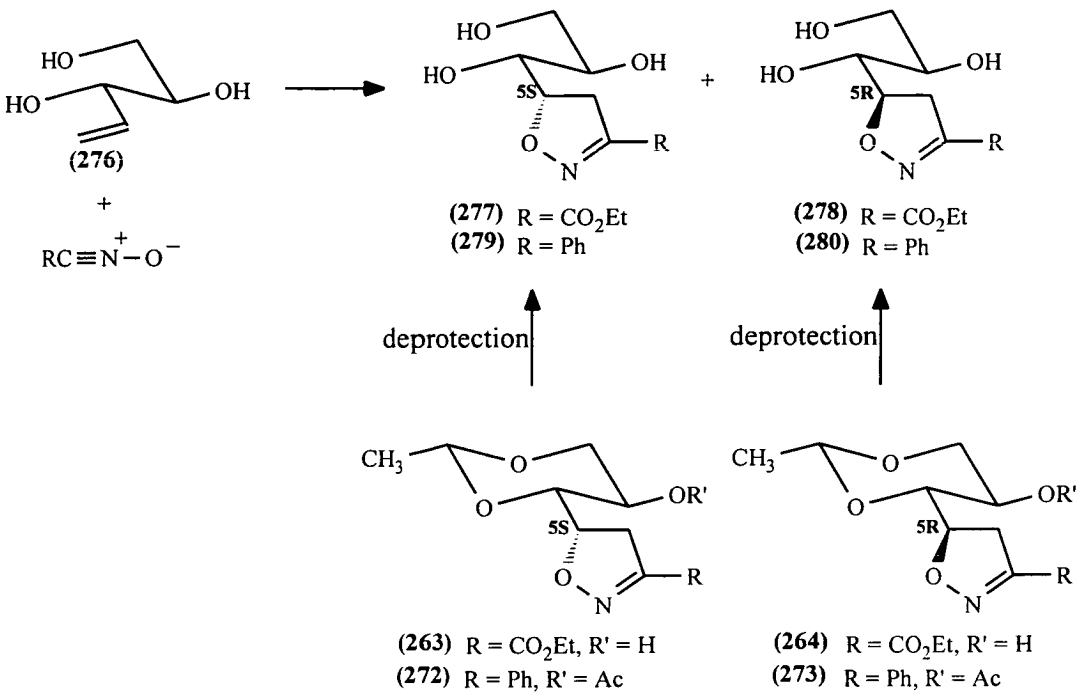
Scheme 62

2.4.7.1 Nitrile Oxide Cycloadditions to Alkene (276)

Cycloaddition of ethoxycarbonylformonitrile oxide to alkene (**276**) (see Scheme 63) was carried out as outlined in Section 2.3.2. The reaction mixture was analysed by ^{1}H NMR and from this a ratio of 55:45 for the isoxazolines (**277**) and (**278**) obtained. The ratio of products was determined from the crude reaction mixture. Unreacted alkene:furoxan:isoxazolines was estimated to be in a ratio of 1.0:0.7:1.0.

Similarly the corresponding cycloaddition of benzonitrile oxide to the same alkene (**276**) afforded the adducts (**279**) and (**280**) in a combined yield of 25% and ratio of 62:38. The ratio was again determined by comparison of the integrals in the crude reaction mixture.

The products were identified by comparison with authentic samples prepared by removal of the ethylidene group (Scheme 63) from (**263**) and (**264**) or (**272**) and (**273**). The major adducts (**277**) and (**279**) were thereby assigned *S*-configuration at C-5, corresponding to an *erythro* relationship about the C-5/C-6 bond.



Scheme 63

The nitrile oxide cycloadditions to alkene (**276**) both proceed with low π-facial selectivity with d.e. values of 10% for ethoxycarbonylformonitrile oxide and 24% for benzonitrile oxide. The ratios obtained (Table 38) indicate there is little change in π-facial selectivity for cycloadditions to the triitol alkene (**276**) compared with those to the ethylidene protected alkene (**260**).

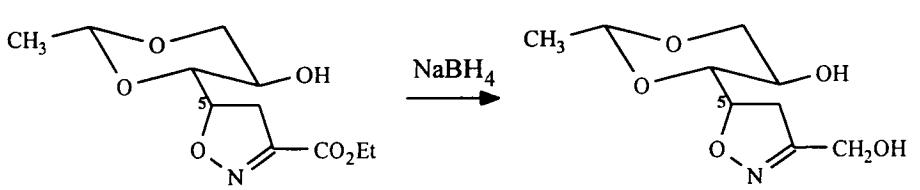
Alkene	Nitrile Oxide	*erythro* : *threo*
(**260**)	EtO_2CCNO	53 : 47
	PhCNO	52 : 48
(**276**)	EtO_2CCNO	55 : 45
	PhCNO	62 : 38

Table 38 : Isomer Ratios for Cycloaddition of Nitrile Oxides to Alkenes (**260**) and (**276**)

2.4.8 Reduction of 3-Ethoxycarbonyl Substituent to Hydroxymethyl

Conversion of the 3-ethoxycarbonyl isoxazolines (**263**) and (**264**) to heptulosonic acid analogues requires reduction of the ester group to provide the hydroxymethyl substituted isoxazoline. This transformation should also provide access to a range of analogues in addition to facilitating the hydrolytic ring cleavage of the 2-isoxazoline to the β-hydroxyketone.

Isoxazolines (**263**) and (**264**) were therefore reduced with sodium borohydride in ethanol at room temperature to afford alcohols (**269**) and (**270**) respectively (Scheme 64). Each adduct was obtained in good yield (77% and 84% respectively). The characteristic properties of the isomers (**269**) and (**270**) are compared with those of the parent major and minor cycloadducts (**263**) and (**264**) in Section 2.4.2.2.



(**263**) 5*S*
(**264**) 5*R*

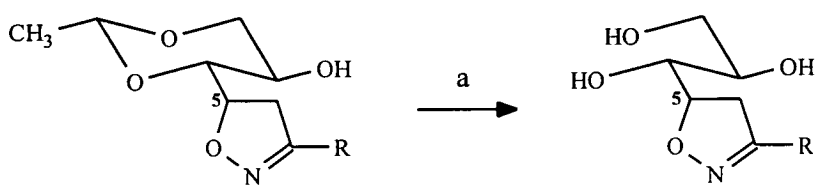
(**269**) 5*S*
(**270**) 5*R*

Scheme 64

2.4.9 Deprotection of 2-Isoxazolines

It has been established from model studies carried out for the hexulosonic acid series that initial deprotection followed by reductive hydrolytic ring cleavage (Section 2.3.8.5) is the better approach to the required analogues. Thus the route selected to DAH analogues first involved removal of the ethylidene protecting group from the 3-substituent of the 2-isoxazoline. Subsequent ring opening of the isoxazoline would then be expected to afford the product in hemiketal form.

The procedure involved stirring of the isoxazoline with a mixture of water/glacial acetic acid (1:4) at 80°C (Scheme 65) and TLC of the mixture indicated when reaction was complete (4-10 hours). Deprotection of each pair of cycloadducts resulted in yields ranging from 48% for (**278**) to 87% for (**277**)



5*S* (**263**) R = CO_2Et	5*S* (**277**) R = CO_2Et
(**265**) R = Ph	(**279**) R = Ph
(**269**) R = CH_2OH	(**281**) R = CH_2OH
5*R* (**264**) R = CO_2Et	5*R* (**278**) R = CO_2Et
(**266**) R = Ph	(**280**) R = Ph
(**270**) R = CH_2OH	(**282**) R = CH_2OH
5*R/S* (**267**)/(**268**) R = $CH_2P(O)(OEt)_2$	5*R/S* (**283**) R = $CH_2P(O)(OEt)_2$

(a) Glacial acetic acid/water (4:1), 80°C

Scheme 65

2.4.9.1 Characteristics of Triols

The characteristic properties of the triols resulting from removal of the ethylidene protecting group are compared with those for the starting isoxazolines below.

TLC

The parent 2-isoxazoline has a larger R_f value on silica (100% ethyl acetate) than the corresponding triol [eg. 0.49 *cf* 0.09 for (**265**)/(**279**)]. The *erythro* triol also occurs at larger R_f value than the *threo* isomer.

^{1}H NMR

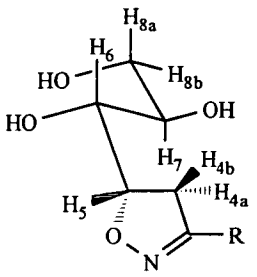
The deprotected diastereoisomeric adducts will be considered initially, and then these results compared with the parent isoxazolines. The ^{1}H NMR spectra reflect a major difference between the 5-H signal for the *erythro* and *threo* isomers. It is observed that for the *threo* adduct the 5-H signal absorbs at significantly higher frequency than for the corresponding *erythro* product [$\Delta\delta_H$ +0.04 ppm for (**277**)/(**278**), +0.05 ppm for (**279**)/(**280**), +0.04 ppm for (**282**)/(**283**)]. Compared to the protected adducts (see Section 2.4.2.2) 5-H has also shifted to higher frequency for the *erythro* adduct whereas the value for the *threo* isomer remains unchanged. It is noteworthy that the coupling between 5-H and 6-H is smaller for both the *erythro* and *threo* isomers compared to the protected products [eg. 4.8 *cf* 2.6 Hz for (**263**)/(**277**), 2.9 *cf* 2.4 Hz for (**264**)/(**278**)]. The $\Delta\delta_H$ for $J_{5,6}$ between the *erythro* and *threo* isomers is also significantly less when they are deprotected with $J_{5,6}$ changing much more markedly for the *erythro* isomers than the *threo* isomers ($\Delta\delta_H$ ~2 Hz *cf*

0.5 Hz). The coupling $J_{6,7}$ (*ca* 7 Hz) and $J_{7,8a}$ (*ca* 5.5 Hz) indicates that once ethylidene protection has been removed the isomers no longer have diaxial couplings between these protons and adopt a different conformation in solution, perhaps due to loss of rigidity imposed by the 1,3-dioxane ring. A torsion angle of 131° is predicted from the 5.5 Hz coupling. The chemical shifts and coupling constants for the deprotected adducts are summarised in Tables 39 and 40.

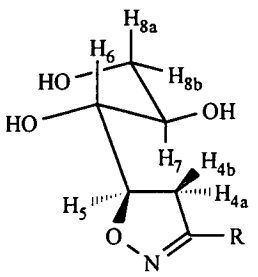
^{13}C NMR

The ^{13}C NMR spectra for the deprotected isomers show significant differences for C-4 to C-6. In each case the *threo* adduct has a higher δ-value for C-4 [$\Delta\delta_C$ +2.7 ppm for (**277**)/(**278**), +1.4 ppm for (**279**)/(**280**)]. This the reverse of that seen for the protected isomers where C-4 for the *erythro* isomer appears at higher chemical shift (see Section 2.4.2.2). The peaks for the *erythro* deprotected isomers have shifted to lower frequency by ~3 ppm compared to the protected adducts. There is also a marked difference shown for C-5 in the deprotected adducts [$\Delta\delta_C$ +1.7 ppm for (**277**)/(**278**), +2.7 ppm for (**279**)/(**280**)]. This was also observed in the protected adducts. It is noteworthy that C-6 in the deprotected adducts occur at much lower frequency than in the corresponding protected isomers [$\Delta\delta_C$ -7.4 ppm for (**277**)/(**263**), -7.3 ppm for (**264**)/(**278**)]. This is a consequence of C-6 now being adjacent to a hydroxyl group. There is no difference between the isomers for C-7 in the deprotected compounds whereas the corresponding protected isomers show a noticeable variance [eg. $\Delta\delta_C$ 2.9 ppm for (**263**)/(**264**)]. However, the signals for the deprotected adducts occur at much lower frequency than for the corresponding

protected isomers (*ca* 8 Hz for the *erythro* adduct and 10 Hz for the *threo* isomer). The data for the deprotected adducts are shown in Table 41.



Erythro



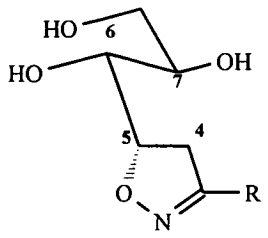
Threo

R	Isoxazoline	δ_H/ppm				
		4a-H	**4b-H**	**5-H**	**6-H**	**8a-H**
CO_2Et	*Erythro* (**277**)	3.30	3.14	5.10	3.92	3.65
	Threo (**278**)	3.29	3.20	5.14	3.73	3.67
Ph	*Erythro* (**279**)	3.51	3.34	5.01	3.98	3.69
	Threo (**280**)	3.47	3.44	5.06	3.58	nd
CH_2OH	*Erythro* (**281**)	3.16	3.00	4.79	nd	nd
	Threo (**282**)	3.11	3.10	4.83	nd	nd

Table 39 : Selected ^{1}H NMR data

R	Coupling	*J*/Hz					
		4a,4b	**4a,5**	**4b,5**	**5,6**	**6,7**	**7,8a**
CO_2Et	*Erythro* (**277**)	17.4	9.1	11.6	2.6	7.9	5.6
	Threo (**278**)	17.5	11.5	9.0	2.4	nd	5.1
Ph	*Erythro* (**279**)	16.7	8.9	11.1	3.3	7.0	5.8
	Threo (**280**)	-	10.7	9.1	2.6	4.7	5.4
CH_2OH	*Erythro* (**281**)	17.1	9.0	10.9	3.6	nd	nd
	Threo (**282**)	-	10.5	9.4	3.0	nd	nd

Table 40 : Selected ^{1}H NMR data



R		δ_C/ppm			
		C-4	C-5	C-6	C-7
CO_2Et	*Erythro* (**277**)	32.6	85.1	72.4	71.4
	Threo (**278**)	35.3	83.4	73.2	71.5
Ph	*Erythro* (**279**)	34.1	82.6	72.8	71.9
	Threo (**280**)	35.5	79.9	72.5	70.8

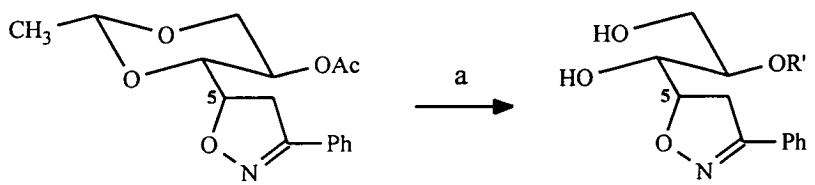
Table 41 : Selected ^{13}C NMR data

2.4.9.2 Deprotection of 2-Isoxazolines Containing an Acetoxy Homoallylic Substituent

The ethylidene protecting group for each of the isoxazolines (**272**) and (**273**) was removed by stirring with a mixture of water/glacial acetic acid (1:4) at 80°C for 5 hours (Scheme 66) and the products purified by preparative TLC.

The reaction was initially performed using isoxazoline (**272**). TLC analysis of the reaction mixture indicated the presence of two products which could be separated by preparative TLC. By comparison of R_f values with an authentic sample of (**279**), the deprotected isomer obtained from (**265**), the more polar component was predicted to be the fully deprotected compound in which the acetate ester has also been

hydrolysed. This was confirmed by its ^{1}H and ^{13}C NMR spectra which were identical to those of (**279**). The conditions used have also been successful at partial removal of the acetyl group to provide (**284**). The analogous reaction was performed using isoxazoline (**273**). However, in terms of ease of reaction, although the acetylated cycloadducts (**272**) and (**273**) are easier to separate by nature of their R_f values, the subsequent deprotection is achieved much more readily for the 7-OH adduct (**265**) and much higher yields are obtained (82% *cf* 12%). The less polar components were characterised by ^{1}H and ^{13}C NMR data and identified as the deprotected isomers (**284**) and (**285**) containing an intact homoallylic acetyl group at C-7.



(**272**) 5*S*

(**273**) 5*R*

(**279**) 5*S* R'=H
(**284**) 5*S* R'=Ac
(**280**) 5*R* R'=H
(**285**) 5*R* R'=Ac

(a) Glacial acetic acid/water (4:1), 80°C

Scheme 66

2.4.9.3 Characteristics of Triols (279), (280), (284) and (285)

^{1}H NMR

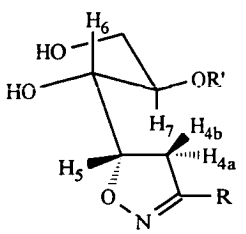
The deprotected diastereoisomeric adducts will first be compared. The ^{1}H NMR spectra reflect a major difference between proton 5-H for the *erythro* and *threo* isomers. It is observed that for the *threo* adduct the 5-H signal absorbs at significantly higher frequency than for the corresponding *erythro* product ($\Delta\delta_H$ +0.05

ppm for each of the pairs of isomers). Compared to the protected adducts (see Section 2.4.5.2) 5-H has also shifted to significantly higher frequency [$\Delta\delta_H$ +0.24 ppm for (**272**)/(**279**), +0.18 ppm for (**273**)/(**280**)]. It is worth noting that changing from 7-OH to 7-$OCOCH_3$ has a marked influence on the chemical shift. This has previously been remarked upon for the protected isomers (**272**) to (**275**) in Section 2.4.5.2. Proton 7-H resonates at higher frequency for the *threo* isomer compared to the *erythro* isomer [$\Delta\delta_H$ +0.02 ppm for (**279**)/(**280**), +0.61 ppm for (**284**)/(**285**)]. This difference is much more pronounced for the acetylated adducts (**284**) and (**285**) and may be due to the conformation adopted in solution. Again the coupling constant $J_{7,8a}$ is very much smaller in the deprotected adducts compared to the parent isoxazolines [eg. 5.8 Hz in (**279**) *cf* 10.2 Hz in (**272**), 6.5 Hz in (**284**) *cf* 10.2 Hz in (**272**)] suggesting that there is no longer diaxial coupling between 7-H and 8a-H. The $J_{7,8e}$ value is also smaller for the deprotected adducts [eg. 3.7 Hz in (**279**) *cf* 5.4 Hz in (**272**), 3.0 Hz in (**284**) *cf* 5.4 Hz in (**273**)]. The chemical shifts and coupling constants for the deprotected adducts are summarised in Tables 42 and 43.

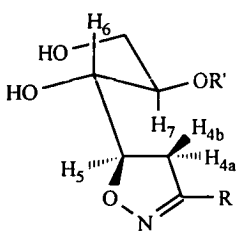
^{13}C NMR

The ^{13}C NMR spectra for the deprotected isomers show significant differences for C-4 to C-7. The *threo* adduct has a higher δ-value for C-4 [$\Delta\delta_C$ +1.4 ppm for (**279**)/(**280**)]. There is a marked difference shown for C-5 in the deprotected adducts [$\Delta\delta_C$ 2.7 ppm for (**279**)/(**280**)]. This was also observed for the protected adducts. It is noteworthy that the signals for C-6 in the deprotected adducts occur at much lower frequency than in the corresponding protected compounds [$\Delta\delta_C$ -5.1 ppm

for (**272**)/(**279**), -5.7 ppm for (**273**)/(**280**)]. This is a consequence of C-6 now being adjacent to a hydroxyl group. Once again the C-7 δ-values for the protected adducts occur at much higher frequency than for the corresponding protected isomers (8 Hz for both the *erythro* and *threo* isomers). The differences for the deprotected adducts are shown in Table 44.



Erythro



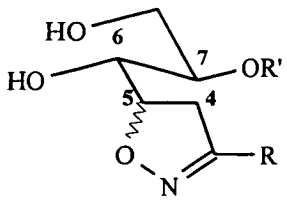
Threo

R	R′		δ_H/ppm				
			4a-H	**4b-H**	**5-H**	**6-H**	**7-H**
Ph	H	*Erythro* (**279**)	3.51	3.34	5.01	3.98	3.93
	H	*Threo* (**280**)	3.47	3.44	5.06	3.58	3.95
	Ac	*Erythro* (**284**)	3.51	3.36	5.03	3.91	3.37
	Ac	*Threo* (**285**)	3.51	3.43	5.08	3.58	3.98

Table 42 : Selected ^{1}H NMR data

R	R′		*J*/Hz			
			5,6	**6,7**	**7,8a**	**7,8e**
Ph	H	*Erythro* (**279**)	3.3	7.0	5.8	3.7
	H	*Threo* (**280**)	2.6	7.7	5.4	3.7
	Ac	*Erythro* (**284**)	3.4	4.7	6.5	3.0
	Ac	*Threo* (**285**)	2.5	8.9	6.3	2.6

Table 43 : Selected ^{1}H NMR data



R	R'		δ_C/ppm				
			C-4	C-5	C-6	C-7	C-8
Ph	H	*Erythro* (**279**)	34.1	82.6	72.8	71.9	64.0
	H	*Threo* (**280**)	35.5	79.9	72.5	70.8	63.2
	Ac	*Threo* (**285**)	36.6	80.6	73.0	70.0	66.7

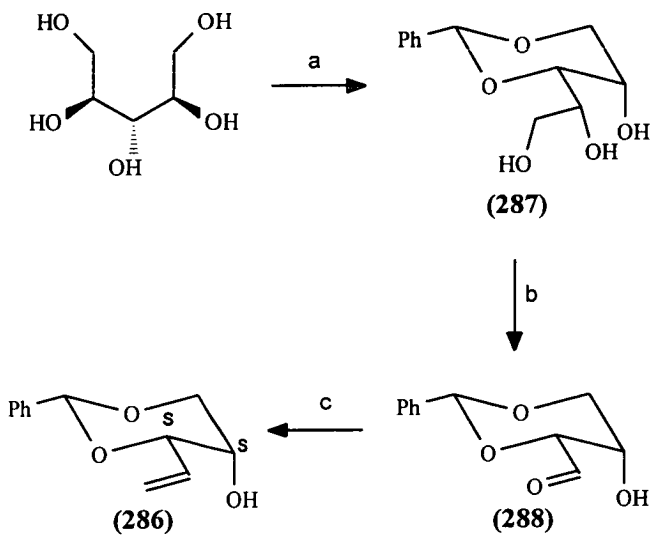
Table 44 : Selected ^{13}C NMR data

2.4.10 Conclusion

Cycloaddition of nitrile oxides to D-*erythro*-alkenes (**260**), (**271**) and (**276**) afforded adducts in variable yields (38-77%) and with poor π-facial selectivity (50% *erythro* : 50% *threo* to 63% *erythro* : 37% *threo*) with slight preference for *erythro* adducts. In order to investigate further the influence of homoallylic substituents at C-7 on selectivity an alternative five-carbon alkene containing an axial hydroxyl at C-7 was synthesised.

2.4.11 Synthesis of 1,2-*Dideoxy*-3,5-O-*Benzylidene*-L-threo-*pent*-1-*enitol* (286)

The five-carbon sugar alkene (**286**) which contains an axial hydroxyl at C-7 was prepared in three steps from D-arabinitol (equivalent to L-arabinitol) according to Scheme 67, with an overall yield of 22%. The route involves protection of the 1- and 3- positions as the benzylidene derivative, periodate cleavage of the *vicinal* 5,6-diol, and finally Wittig olefination of the resulting aldehyde.



(a) PhCHO, dry HCl(g); (b) $NaIO_4$; (c) $Ph_3P^+CH_3I^-$, $(CH_3)_3COK$, THF

Scheme 67

A series of experiments was performed using various solvents, catalysts and temperature conditions to try and optimise conditions for the first step. The technique selected involved passage of a rapid stream of dry hydrochloric acid gas into a suspension of D-arabinitol in benzaldehyde to afford the 1,3-*O*-benzylidene-L-arabinitol (**287**).[223,224] This reaction, however, proved to be very unpredictable yielding mixtures of isomers. In order to assign the structure of (**287**) unambiguously NOE experiments were carried out. Irradiation of 6-H resulted in the expected

enhancement of the 3-H and 5a-H signals (Figure 18) thus confirming that the phenyl substituent occupies the equatorial position. There was no observed enhancement of 5e-H or 4-H. These results are consistent with the structure shown since, if the phenyl were axial and 6-H equatorial there would be no enhancement of 5a-H or 3-H on irradiation of 6-H. It also confirms that 3-H is axial rather than equatorial.

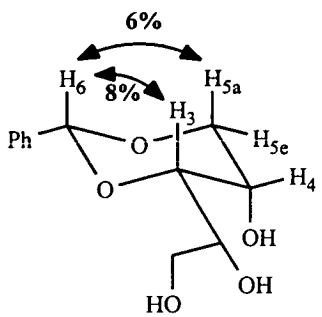


Figure 18

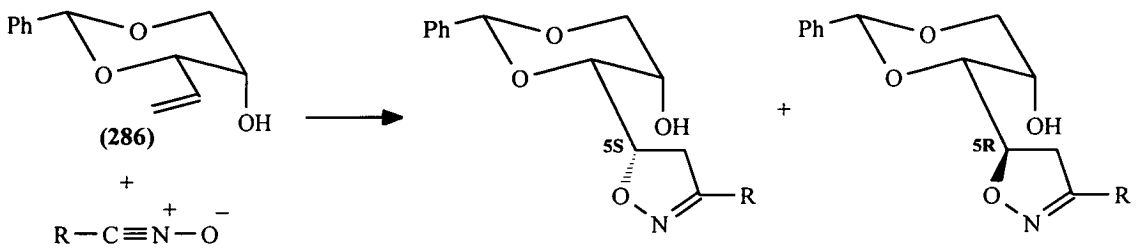
Subsequent metaperiodate cleavage of (**287**) at pH 4 followed by rapid hydrolysis at pH 6.5 afforded aldehyde (**288**) as white crystals (70%). This was converted *via* a Wittig reaction with methyltriphenylphosphorane to the desired alkene (**286**) in 49% yield.

2.4.12 Investigation of π-Facial Selectivity in Nitrile Oxide Cycloadditions to 1,2-*Dideoxy*-3,5-O-*benzylidene*-L-threo-*pent*-1-*enitol* (286)

2.4.12.1 Nitrile Oxide Cycloadditions to Alkene (286)

Two nitrile oxides were examined: ethoxycarbonylformonitrile oxide and benzonitrile oxide. Cycloaddition of benzonitrile oxide to alkene (**286**) (Scheme 68) was carried out as outlined in Section 2.3.2 and a mixture of diastereoisomeric

isoxazolines (**289**) and (**290**) in combined yield of 64% and ratio 81:19 obtained after chromatography to remove 3,4-diphenylfurazan *N*-oxide.



(**289**) R = Ph
(**291**) R = CO_2Et

(**290**) R = Ph
(**292**) R = CO_2Et

Scheme 68

The product ratio could not be determined from the ^1H NMR spectrum of the mixture. Instead it was measured by reverse phase HPLC. An X-ray structure of (**289**) was obtained to assign unambiguously the configuration at the new asymmetric centre C-5 and this will be discussed in Section 2.4.13. The major isomer (**289**) was thus assigned *S*-configuration at C-5, corresponding to an *erythro* relationship about the C-5/C-6 bond. The overall stereochemistry for the isomers can be assigned as L-*lyxo* for the 5*S* isomer and L-*xylo* for the 5*R* isomer.

The corresponding cycloaddition of ethoxycarbonylformonitrile oxide to alkene (**286**) afforded the adducts (**291**) and (**292**) in a combined yield of 69% and ratio 83:17, together with the nitrile oxide dimer 3,4-diethoxycarbonylfurazan *N*-oxide. The ratio was determined by comparison of the integrals for the 6-H signals in the ^1H NMR, as well as by reverse phase HPLC (ratio 82:18). The assignment of configuration for the major isomer as *erythro* was made by comparison of its spectral parameters with those of the benzonitrile oxide adducts (**289**) and (**290**).

The nitrile oxide cycloadditions to alkene (**286**) proceed selectively with d.e. values of ~64%. The *erythro:threo* ratios for the reactions studied are presented in Table 45.

Alkene	Nitrile Oxide	*erythro:threo*
(**286**)	EtO_2CCNO	83 : 17
	PhCNO	81 : 19

Table 45 : Isomer Ratios for Cycloaddition of Nitrile Oxides to Alkene (**286**)

2.4.12.2 Characterisation of Isoxazoline Cycloadducts

The characteristic properties of the cycloadducts obtained from cycloadditions to alkene (**286**) are summarised below.

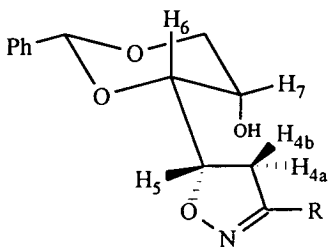
TLC

The *erythro* adduct has a larger R_f value on silica (100% ether) than the corresponding *threo* isomer [eg. 0.23 *cf* 0.14 for (**289**)/(**290**)].

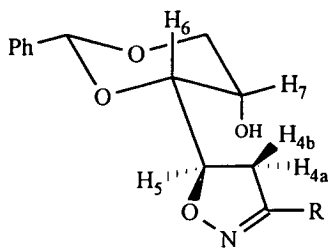
^{1}H NMR

The ^{1}H NMR reflect major differences between the isomers for protons 4-H to 7-H. It is observed that for the *erythro* adduct the 5-H signal absorbs at a slightly higher frequency than for the corresponding *threo* product [$\Delta\delta_H$ +0.03 ppm for (**289**)/(**290**), +0.01 ppm for (**291**)/(**292**)]. The signals for the methylene protons of the isoxazoline ring, 4a-H and 4b-H, are closer together in the *erythro* adducts. It is also

worth noting that proton 7-H resonates at a higher frequency for the *erythro* isomer compared to the *threo* isomer [$\Delta\delta_H$ +0.19 ppm for (**289**)/(**290**), +0.15 ppm for (**291**)/(**292**)]. There is also a noticeable difference for proton 6-H which absorbs at a higher chemical shift for the *threo* isomer than the *erythro* isomer [$\Delta\delta_H$ +0.17 ppm for (**289**)/(**290**), +0.10 ppm for (**291**)/(**292**)]. In comparison to the results obtained for cycloadditions to alkenes (**260**) and (**271**) (Sections 2.4.2 and 2.4.5 respectively), opposing trends are observed here for 6-H and 7-H. This can be rationalised in terms of changing from an equatorial homoallylic substituent OR′ in alkenes (**260**) and (**271**) to an axial substituent in alkene (**286**). In this system containing the axial 7-OH there is no observable difference in the coupling between 5-H and 6-H for either of the pairs of isomers. Tables 46 and 47 show selected ^{1}H NMR data.



Erythro



Threo

R		δ_H/ppm				
		4a-H	**4b-H**	**5-H**	**6-H**	**7-H**
Ph	*Erythro* (**289**)	3.46	3.45	5.06	3.86	3.90
	Threo (**290**)	3.49	3.26	5.03	4.03	3.71
CO_2Et	*Erythro* (**291**)	3.35	3.26	5.07	3.88	3.78
	Threo (**292**)	3.33	3.13	5.06	3.98	3.63

Table 46 : Selected ^{1}H NMR data for cycloadducts

R		*J*/Hz			
		4a,4b	**4a,5**	**4b,5**	**5,6**
Ph	*Erythro* (**289**)	17.1	7.7	9.2	8.4
	Threo (**290**)	16.7	10.8	8.4	7.4
CO_2Et	*Erythro* (**291**)	18.2	7.3	10.9	7.5
	Threo (**292**)	17.7	11.4	8.9	7.5

Table 47 : Selected ^1H NMR data for cycloadducts

2.4.13 X-Ray Crystal Structure of Adduct (289)

The major adduct (**289**) obtained from cycloaddition of benzonitrile oxide to alkene (**286**) was isolated as a crystalline solid suitable for X-ray analysis. Examination of the crystal structure (Figure 19) established the *S*-configuration at the new asymmetric centre at C-5 representing an *erythro* relationship between C-6 and C-5. This assignment is consistent with those obtained for the adducts (**263**), (**272**) and (**273**), which were also determined by X-ray crystallography (Section 2.4.6).

The torsion angles obtained for the X-ray crystal structure were used to calculate the proton-proton coupling constants by applying the Haasnoot parameterisation[209] of the Karplus equation and these then compared to those observed in solution (Table 48). In this case the relationship between the observed and calculated *J*-values for the isoxazoline ring protons is satisfactory. There is also reasonable correlation for $J_{4,5}$, $J_{5,6a}$ and $J_{5,6e}$. However, there is a significant deviation associated with the coupling between the hydrogens at the ring junction 3-H/4-H, indicating a difference of preferred conformation in solution and in the crystal.[4]

[4] Labelling of protons in X-ray picture is different from that used in characterisation of products

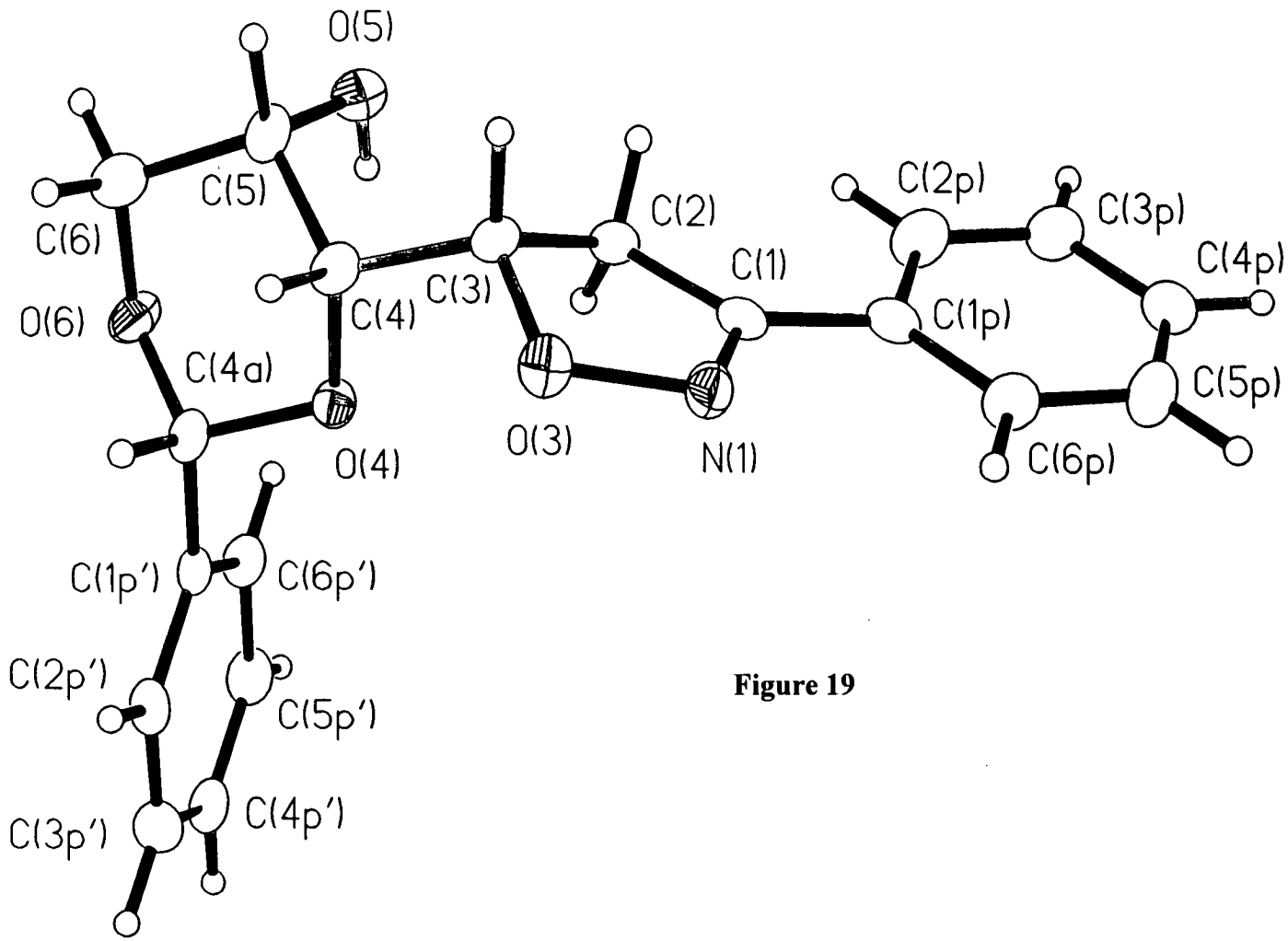
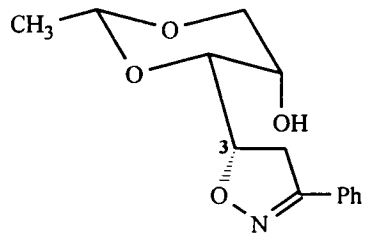

O(5)
C(5)
C(6)
O(6)
C(4a)
O(4)
C(4)
C(3)
C(2)
C(1)
O(3)
N(1)
C(2p)
C(3p)
C(4p)
C(1p)
C(5p)
C(6p)
C(1p')
C(6p')
C(2p')
C(5p')
C(3p')
C(4p')

Figure 19



	H_x,H_y					
	2a,3	**2b,3**	**3,4**	**4,5**	**5,6a**	**5,6e**
θ	-147.9	-25.1	-68.7	54.2	67.0	-51.7
J_{Obs}	9.2	7.7	8.4	1.1	1.2	1.9
J_{Calc}	7.9	6.8	2.0	3.4	2.2	3.7

$$^3J_{Calc} = 7.76\ Cos^2\theta - 1.1\ Cos\theta + 1.4$$

Table 48 : Calculated and observed coupling constants for adduct (**289**)

2.4.14 Conclusions

The approach selected for the synthesis of heptulosonic acid (DAH) analogues is *via* nitrile oxide-isoxazoline chemistry using the five-carbon alkene (**260**) as the starting material. Cycloaddition of nitrile oxides to D-*erythro*-alkene (**260**) afforded adducts in variable yields (38-77%) and with negligible π-facial selectivity (0-6% d.e.). The low selectivity is possibly due to the presence of the homoallylic hydroxyl at C-7.

In order to investigate the influence of the homoallylic substituent on π-facial selectivity cycloadditions to alkene (**271**) containing an equatorial acetoxy homoallylic substituent at C-7 were performed. These afforded adducts in variable yields (46-74%) with moderate π-facial selectivity (18-26% d.e.) and again with

preference for *erythro* products. Cycloadditions to alkene (**276**), resulting from removal of the ethylidene protection of (**260**), also proceed with low selectivities (10-24% d.e.).

In contrast, the corresponding cycloaddition to the isomeric L-*threo*-alkene (**286**), which has an axial hydroxyl at C-7, proceeded with high *erythro* selectivity (60-62% d.e.)

These results illustrate the influence that can be imparted on selectivity merely by changing from an equatorial to axial homoallylic substituent at C-7 (Table 49). This change may be attributed to subtle changes in the relative energies of the transition states proposed by Houk *et al*[196] (Figure 13, Section 2.4.5.1).

	Alkene	**Nitrile Oxide**	***erythro*:*threo***
Equatorial Homoallylic Substituent	(**260**)	EtO_2CCNO	53:47
		PhCNO	52:48
	(**271**)	EtO_2CCNO	59:41
		PhCNO	63:37
	(**276**)	EtO_2CCNO	55:45
		PhCNO	62:38
Axial Homoallylic Substituent	(**286**)	EtO_2CCNO	83:17
		PhCNO	81:19

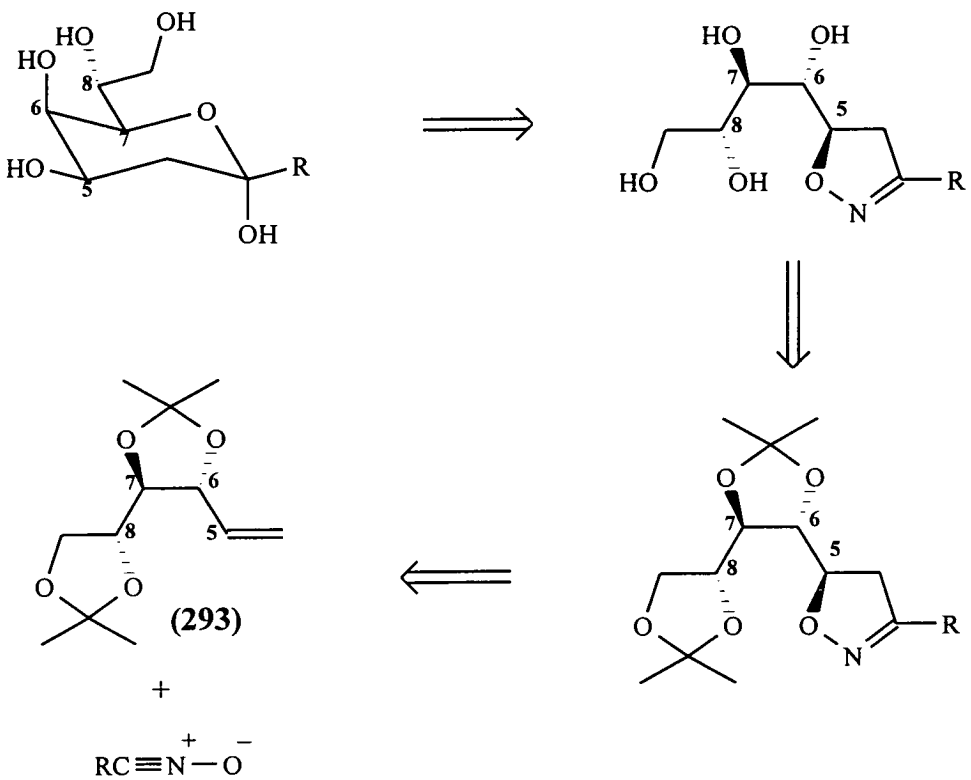
Table 49 : Isomer Ratios for Cycloaddition of Nitrile Oxides to Alkenes (**260**), (**271**), (**276**) and (**286**)

Removal of the ethylidene protecting group from the 5-substituent of the 2-isoxazolines derived from alkene (**260**) has also been accomplished to yield precursors required for the final ring opening of the 2-isoxazoline.

This final stage has yet to be investigated. Reductive hydrolytic cleavage of the isoxazoline would be expected to afford the hemiketal form for the hydroxymethyl analogues. In contrast the phenyl compounds may be expected to exist in the open-chain keto form as previously observed in the six-carbon model system (Section 2.3.8.8).

2.5 3-Deoxy-D-*manno*-octulosonic Acid (KDO) Analogues

The approach selected for the synthesis of octulosonic acid (KDO) analogues initially focussed on preparation of the D-arabinose-derived six-carbon alkene (**293**), which has the required configuration for C-6, C-7 and C-8 of the KDO framework. Cycloaddition of this alkene to an appropriate nitrile oxide, followed by deprotection and subsequent reductive hydrolytic cleavage of the isoxazoline, should therefore provide access to octulosonic acid analogues (Scheme 69).



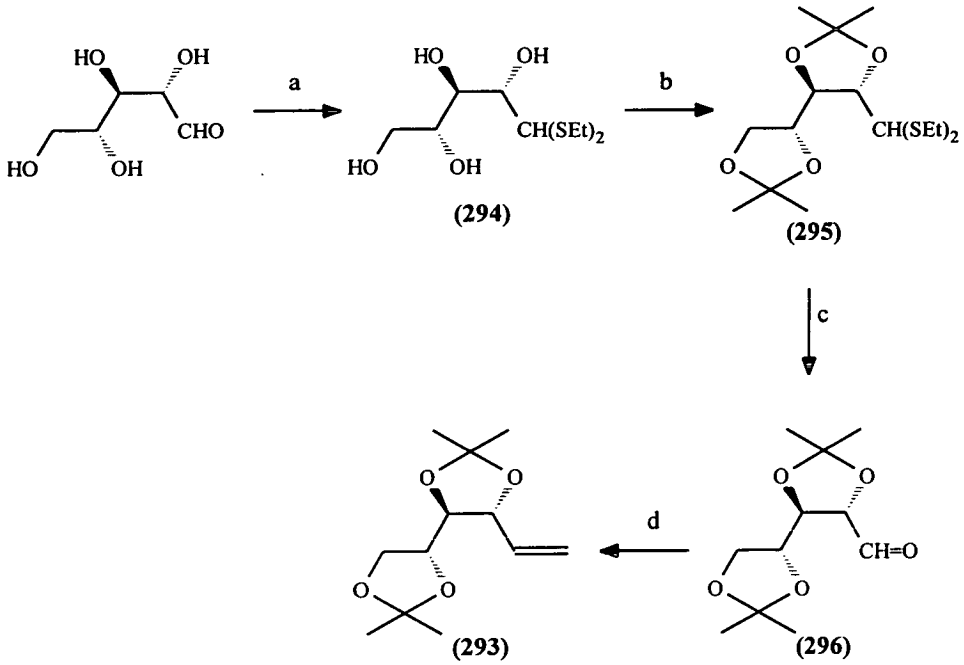
Scheme 69

2.5.1 Synthesis of 1,2-Dideoxy-3,4:5,6-di-*O*-isopropylidene-D-*arabino*-hex-1-enitol (293)

The title compound was prepared in four steps using two different synthetic pathways.

1. From D-arabinose (Scheme 70) by the method of Zinner[225] in 29% overall yield.
2. From D-mannitol (Scheme 71) by the method of Wiggins[226] in 14% overall yield.

The method of Zinner was first considered. The aldehyde function of D-arabinose was initially protected as a thioacetal (**294**) by reaction with ethanethiol.[225] This thioacetal (**294**) was then treated with acetone containing 1.5% sulphuric acid, followed by anhydrous copper sulphate in acetone, to afford the 2,3:4,5-di-*O*-isopropylidene derivative (**295**).[225] Treatment with mercury(II)oxide and mercury(II)chloride unmasked[225] the aldehyde function (Scheme 70). Since (**296**) was found to degrade on standing, purification by distillation/sublimation using a cold-finger distillation apparatus was performed immediately prior to the Wittig olefination for the conversion to alkene (**293**). This Wittig reaction was performed in the same way using methyltriphenyl-phosphonium iodide as for the lower homologues already examined.



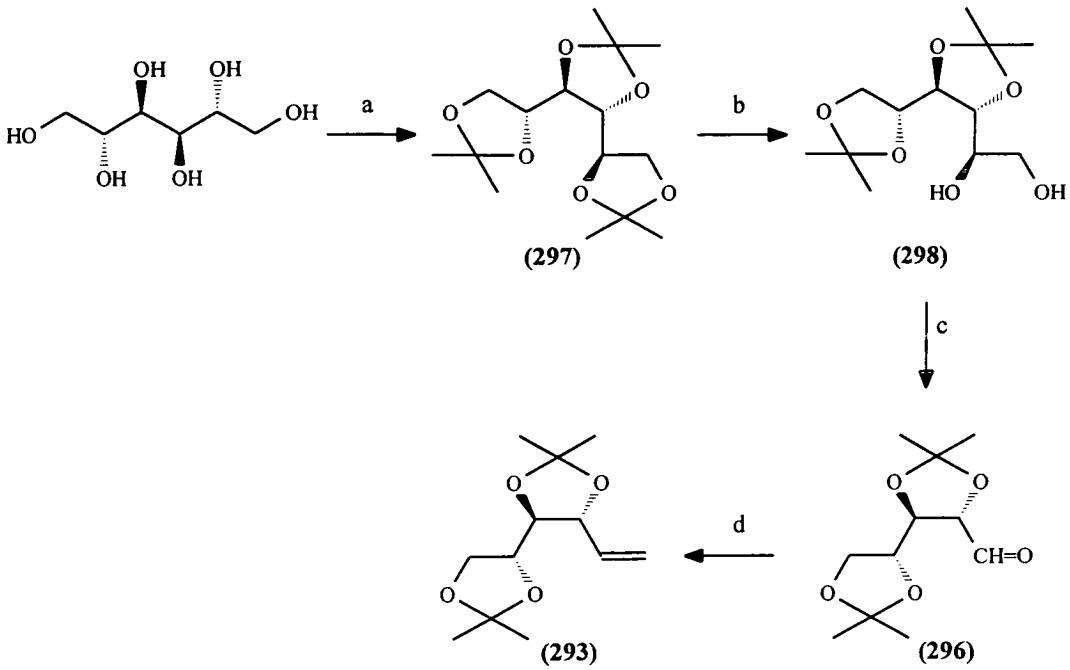
(a) C_2H_5SH, HCl; (b) Acetone, H_2SO_4; (c) HgO, $HgCl_2$; (d) $PhP^+CH_3I^-$, $(CH_3)_3COK$, THF

Scheme 70

The alternative Wiggins preparation of aldehyde (**296**) (Scheme 71) starts from D-mannitol and avoids the use of ethanethiol. 1,2:3,4:5,6-Tri-*O*-isopropylidene-D-mannitol (**297**), prepared by reaction of D-mannitol with acetone using a catalytic amount of concentrated sulphuric acid,[226] was hydrolysed selectively at the more reactive terminal ketal using 70% ethanol containing concentrated hydrochloric acid. The desired 1,2:3,4-di-*O*-isopropylidene-D-mannitol (**298**) (35%) was separated from the mono- and tri-ketal derivatives by column chromatography. Subsequent treatment with sodium periodate afforded aldehyde (**296**) which was purified by distillation, and then converted *via* a Wittig reaction to alkene (**293**).

The overall yield (14%) of alkene (**293**) prepared by this method is slightly less than that for the preceding method (29%), mainly due to losses during

chromatography. However, this is a more environmentally friendly route to the desired aldehyde (**296**) and thence alkene (**293**).



(a) Acetone, H_2SO_4; (b) 70% EtOH, HCl, 40-45°C; (c) $NaIO_4$, 0°C;
(d) $Ph_3P^+CH_3I^-$, $(CH_3)_3COK$, THF

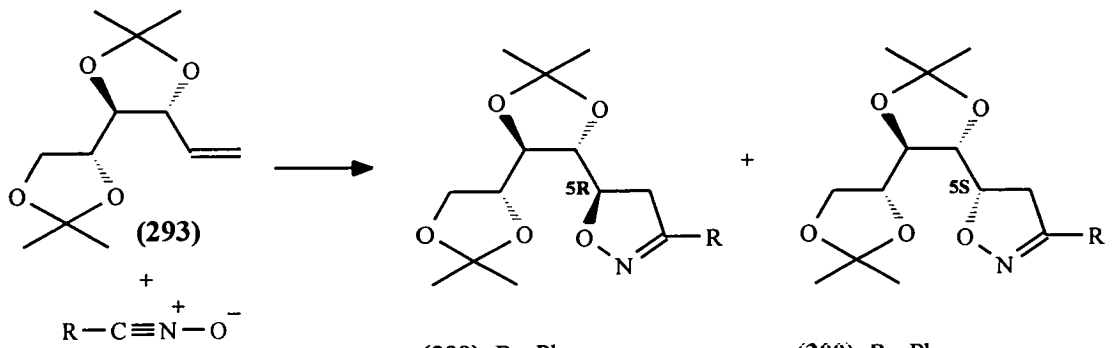
Scheme 71

2.5.2 Investigation of π-Facial Selectivity in Nitrile Oxide Cycloadditions to 1,2-Dideoxy-3,4:5,6-di-*O*-isopropylidene-D-*arabino*-hex-1-enitol (293)

2.5.2.1 Nitrile Oxide Cycloadditions to Alkene (293)

Three nitrile oxides were examined: ethoxycarbonylformonitrile oxide, benzonitrile oxide and (diethoxyphosphoryl)acetonitrile oxide. Cycloaddition of benzonitrile oxide to the title alkene (**293**) (Scheme 72) was carried out as outlined in Section 2.3.2. After chromatography to remove 3,4-diphenylfurazan *N*-oxide (26%),

a mixture of isoxazolines (**299**) and (**300**) in a ratio of 67:33 and a combined yield of 60% was obtained.



(**299**) R =Ph
(**301**) R = CO_2Et
(**303**) R = $CH_2P(O)(OEt)_2$
(**305**) R = CH_2OH

(**300**) R =Ph
(**302**) R = CO_2Et
(**304**) R = $CH_2P(O)(OEt)_2$
(**306**) R = CH_2OH

Scheme 72

The isomer ratio was determined by comparison of the integral of the epimeric 5-H signals at 4.98 and 4.87 ppm in the ^1H NMR spectrum of the mixture. Although the isoxazolines were chromatographically inseparable, the ^1H NMR of the mixture was readily interpreted on the basis of the differing signal intensities. Repeated recrystallisation from ether provided a small quantity of the pure major isomer. Subsequent X-ray analysis of this sample showed that the new chiral centre at C-5 possessed *R*-configuration, corresponding to an *erythro* relationship about the C-5/C-6 bond. Thus, the major isomer was identified as (**299**) and the minor isomer as the *threo* adduct (**300**).

The overall stereochemistry for the isomers can be assigned as D-*manno* for the major isomer and D-*gluco* for the minor isomer. However in the subsequent discussion the terms *erythro* and *threo* will be used, referring to the relationship about the newly created chiral centre at C-5 and the adjacent centre C-6.

The corresponding cycloaddition of alkene (**293**) to ethoxycarbonylformonitrile oxide afforded an inseparable mixture of diastereoisomeric isoxazolines (**301**) and (**302**) in combined yield of 88% together with the nitrile oxide dimer 3,4-diethoxycarbonylfurazan *N*-oxide (24%). The isomer ratio was determined as 62.5:37.5 in favour of *erythro* adduct (**301**) by comparison of the integrals of the 5-H signals at 5.06 and 4.92 ppm in the ^{1}H NMR of the mixture. Again, although the isoxazolines were chromatographically inseparable the ^{1}H NMR of the mixture was readily interpreted on the basis of the differing signal intensities. The assignment of configuration for the major isomer (**301**) was made by correlation of its NMR shift differences with those of the benzonitrile oxide adducts.

Cycloaddition of (diethoxyphosphoryl)acetonitrile oxide to alkene (**293**) (Scheme 72) furnished a 30% combined yield of the inseparable diastereoisomeric 2-isoxazolines (**303**) and (**304**) in a ratio of 63:37 in favour of the *erythro* adduct. Again the ratio was determined by comparison of the integrals for the 5-H signals in the ^{1}H NMR. Although the isoxazolines were chromatographically inseparable, the ^{1}H NMR of the mixture was readily interpreted for the isoxazoline ring protons. The major isomer was assigned as the *erythro* adduct (**303**) based on comparison of its spectral parameters with those of similar adducts, as above.

The nitrile oxide cycloadditions to alkene (**293**) all proceed with low π-facial selectivity with d.e. values ranging from 25% for ethoxycarbonylformonitrile oxide to 34% for benzonitrile oxide. The *erythro*:*threo* ratios for the three cases are presented in Table 50.

Nitrile Oxide	*erythro:threo*
EtO_2CCNO	62.5:37.5
PhCNO	67:33
$(EtO)_2P(O)CH_2CNO$	63:37

Table 50 : Isomer Ratios for Cycloaddition of Nitrile Oxides to Alkene (**293**)

2.5.2.2 Characterisation of Isoxazoline Cycloadducts

The stereochemistry at the new chiral centre C-5 of the isoxazoline can be predicted by correlation of ^1H and ^{13}C NMR and optical rotation data. The characteristic properties of the major and minor isomers are summarised below, along with those for (**305**) and (**306**) obtained after borohydride reduction (Section 2.5.4).

TLC

The major *erythro* adduct has a slightly larger R_f value on silica (50% ether/hexane) than the corresponding minor *threo* adduct [0.26 *cf* 0.24 for (**299**)/(**300**), 0.26 *cf* 0.22 for (**301**)/(**302**)]. However, this difference was not sufficient to be able to resolve the isomers by dry flash chromatography.

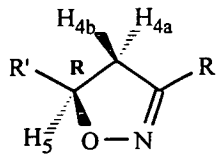
^1H NMR

Since the cycloadducts were unable to be separated, the ^1H NMR spectra obtained show the signals for both isomers. However, major differences between the isoxazoline ring protons 4a-H, 4b-H and 5-H could still be identified. As seen in

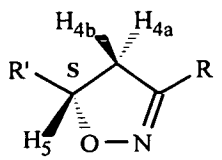
previous systems the isoxazoline ring protons 4a-H and 4b-H give rise to a characteristic ABX system. It is observed that for the major *erythro* adducts the proton 5-H signal absorbs at significantly higher frequency than for the corresponding minor *threo* product [$\Delta\delta_H$ +0.11 ppm for (**299**)/(**300**), +0.14 ppm for (**301**)/(**302**), +0.10 ppm for (**303**)/(**304**), +0.11 ppm for (**305**)/(**306**)]. The isoxazoline methylene proton signals (4a-H and 4b-H) are coincident in the minor *threo* adducts (**302**) and (**306**), while in the major *erythro* adducts (**301**) and (**305**) they are separated by 0.17 ppm and 0.10 ppm respectively. This pattern has previously been observed for other cycloadducts, involving different alkenes.[217] The couplings between 5-H and 6-H [eg. 3.9 *cf* 3.8 Hz for (**299**)/(**300**), 3.0 *cf* 2.2 Hz for (**301**)/(**302**)] are similar for the two isomers, indicating that they adopt similar conformations in solution. The chemical shifts and coupling constants are summarised in Tables 51 and 52.

^{13}C NMR

The ^{13}C NMR spectra were assigned for each isomer in the mixture. The major differences between the adducts are associated with the signals for the epimeric center at C-5 and also that for C-4. In each case the major *erythro* adduct has a lower δ-value for C-4 [$\Delta\delta_C$ -1.5 ppm for (**299**)/(**300**), -0.8 ppm for (**301**)/(**302**), -2.1 ppm for (**305**)/(**306**)], and for C-5 [$\Delta\delta_C$ -1.2 ppm for (**299**)/(**300**), -1.8 ppm for (**305**)/(**306**)]. These differences are shown in Table 53.



Erythro



Threo

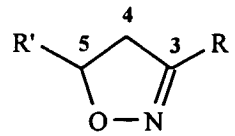
R	Isoxazoline	δ_H/ppm		
		4a-H	4b-H	5-H
Ph	*Erythro* (**299**)	3.34	3.45	4.98
	Threo (**300**)	3.47	3.38	4.87
CO_2Et	*Erythro* (**301**)	3.15	3.32	5.06
	Threo (**302**)	3.27	3.27	4.92
$CH_2P(O)(OEt)_2$	*Erythro* (**303**)	3.03*	2.94^{+}	4.85
	Threo (**304**)	2.88^{+}	2.99*	4.75
CH_2OH	*Erythro* (**305**)	3.02	3.12	4.87
	Threo (**306**)	3.11	3.11	4.76

Table 51 : Selected ^{1}H NMR data for cycloadducts

*, $^{+}$ Alternative assignments

R	Coupling	*J*/Hz			
		4a,4b	4a,5	4b,5	5,6
Ph	*Erythro* (**299**)	16.5	11.2	7.8	3.9
	Threo (**300**)	16.5	7.9	10.8	3.8
CO_2Et	*Erythro* (**301**)	17.5	11.7	8.4	3.0
	Threo (**302**)	-	8.6	11.0	2.2
$CH_2P(O)(OEt)_2$	*Erythro* (**303**)	15.0	10.6	8.4	3.8
	Threo (**304**)	15.0	8.4	10.8	2.4
CH_2OH	*Erythro* (**305**)	17.0	11.1	7.8	3.5
	Threo (**306**)	-	8.8	10.0	2.5

Table 52 : Selected ^{1}H NMR data for cycloadducts



R	Carbon	δ_C /ppm	
		C-4	C-5
Ph	*Erythro* (**299**)	35.6	80.7
	Threo (**300**)	37.1	81.9
CO_2Et	*Erythro* (**301**)	35.1	nd
	Threo (**302**)	35.9	nd
CH_2OH	*Erythro* (**303**)	35.1	80.2
	Threo (**304**)	37.2	82.0

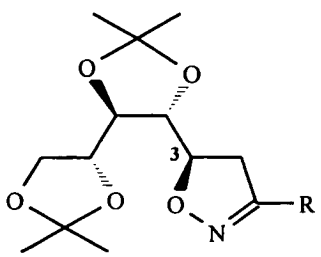
Table 53 : Selected ^{13}C NMR data for cycloadducts

2.5.3 X-Ray Crystal Structure of Adduct (299)

A small amount of major isomer (**299**) obtained from addition of benzonitrile oxide to alkene (**293**) was isolated as a crystalline solid suitable for X-ray analysis. The crystal structure (Figure 19) showed that the new asymmetric centre at C-5 possessed *R*-configuration, thus indicating an *erythro* relationship between C-6 and C-5. This assignment is consistent with that predicted by the 'inside alkoxy effect'.

Table 54 shows the proton-proton coupling constants calculated for the crystal from the torsion angles using the Haasnoot parameterisation[209] of the Karplus equation, compared to those observed in solution. From the data available, there is satisfactory correlation for $J_{4,5}$. However, the relationship between the observed and calculated *J*-values for the isoxazoline ring protons is poor. Also there is a significant

deviation associated with the coupling between the hydrogens at the ring junction 3-H/4-H, indicating a difference of preferred conformation in solution and in the crystal.[5]



	H_x,H_y						
	2a,3	**2b,3**	**3,4**	**4,5**	**5,6**	**6,7a**	**6,7b**
θ	8.7	131.8	79.3	-142.7	175.1	91.4	-30.2
J_{Obs}	11.2	7.8	3.9	8.2	nd	nd	nd
J_{Calc}	6.5	5.6	1.5	7.2	10.2	1.4	6.2

$$^3J_{Calc} = 7.76\ Cos^2\theta - 1.1\ Cos\theta + 1.4$$

Table 54 : Calculated and observed coupling constants for adduct (**299**)

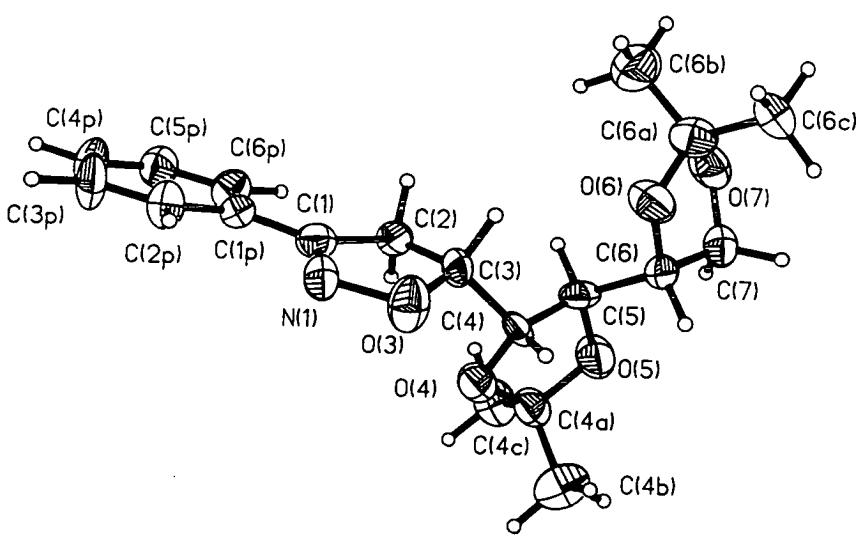


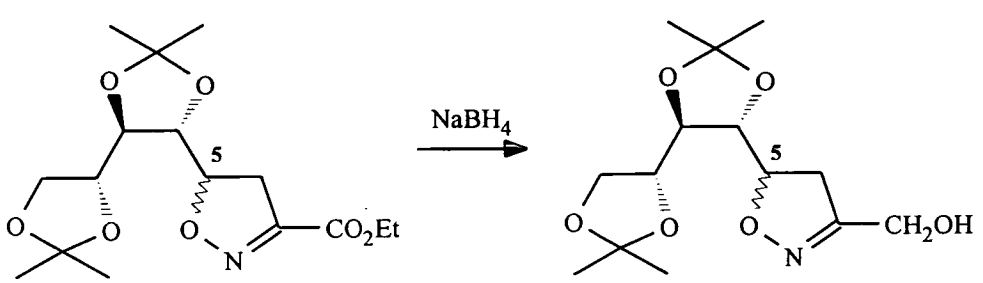
Figure 20

[5] Labelling of protons in X-ray picture is different from that used in characterisation of products

2.5.4 Reduction of 3-Ethoxycarbonyl Substituent to Hydroxymethyl

In order to convert the 3-ethoxycarbonyl isoxazolines (**301**) and (**302**) to octulosonic acid analogues, reduction of the ester group to provide a hydroxymethyl substituted isoxazoline is required. A wide range of derivatives should also become available by this modification including, for example, the mesylate.

The mixture of adducts (**301**) and (**302**) was therefore reduced by sodium borohydride in ethanol to provide the corresponding diastereoisomeric mixture of alcohols (**305**) and (**306**) in excellent yield (88%) (Scheme 73). As for the parent compounds these were found to be chromatographically inseparable. The isomer ratio (60 : 40), which was measured by integration of the 5-H signals at 4.87 and 4.76 ppm in the ^{1}H NMR, is similar to the value obtained for the ester mixture from which the alcohols were derived. The characteristic properties of the isomers (**305**) and (**306**) are compared with those of the parent major *erythro* and minor *threo* cycloadducts (**301**) and (**302**) in Section 2.5.2.2.



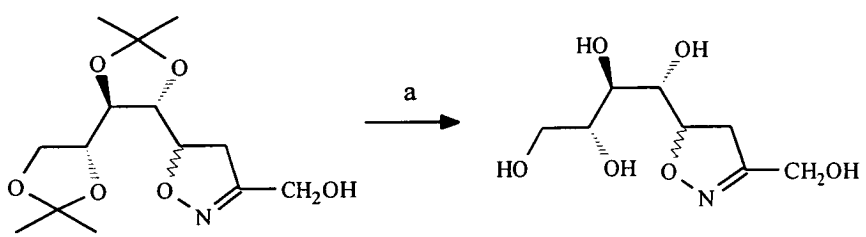
(**301**)/(**302**) (**305**)/(**306**)

Scheme 73

2.5.5 Deprotection of 2-Isoxazolines (305)/(306)

Based on the model studies carried out for the hexulosonic acid series where initial deprotection was followed by reductive hydrolytic ring opening (Section 2.3.8.5) the route selected to the KDO analogues therefore first involved removal of the isopropylidene protection of the 2-isoxazolines (Scheme 74). Subsequent ring opening of the isoxazoline would then be envisaged to provide the desired pyranose ring structure.

A series of pilot experiments were carried out in order to establish conditions for deprotection. The results are summarised in Table 55.



(305)/(306) **(307)/(308)**

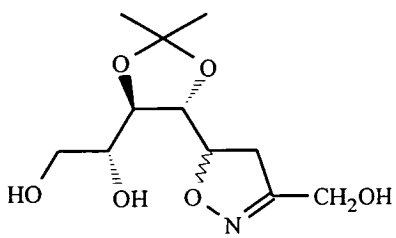
(a) Glacial acetic acid/water (3:2), 80°C, 2.5 hr

Scheme 74

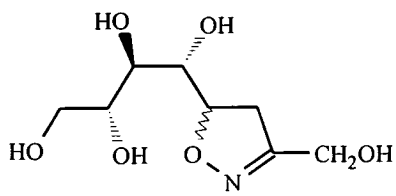
Reagent	Temperature, Time	Deprotection
MeOH containing 4% HBr	Reflux, 24 hr	No
CH_3CO_2H/H_2O (3:2)	40°C, 16 hr	Yes
90% CH_3CO_2H	90°C, 2 hr r.t, 18 hr	No
TFA containing 5% H_2O	r.t, 1 hr	No

Table 55

Examination of the ^{1}H NMR spectra gave no evidence of deprotection having occurred using any of these methods apart from under conditions 2, where partial deprotection had occurred. The product obtained was analysed by ^{1}H NMR and proved to be a mixture of the inseparable isomers (**309**)/(**310**) in a ratio *erythro*:*threo* 5:3, and combined yield of 66%. This product results from hydrolysis at the more reactive terminal positions. The chemical formulae were verified by high resolution FAB mass spectrometry.



(**309**)/(**310**)



(**307**)/(**308**)

Since conditions had been established for partial deprotection, it was envisaged that increasing the temperature of the reaction, without altering any other factors, would facilitate the complete removal of the isopropylidene protecting groups. Two different experiments were performed, the first at 60°C and the second at 80°C. At 60°C, monitoring the reaction by TLC, it was evident that deprotection was occurring, however there were still two products present: that due to the partially deprotected product (**309**)/(**310**), and also a more polar component assumed to be the desired product (**307**)/(**308**). Increasing the temperature to 80°C, resulted in only one detectable product being observed by TLC after 2.5 hours. This product (**307**)/(**308**) was recrystallised from ethanol in 66% yield. The identity of both the partially and

fully deprotected products is discussed below. Each of these products is present as a mixture of inseparable isomers.

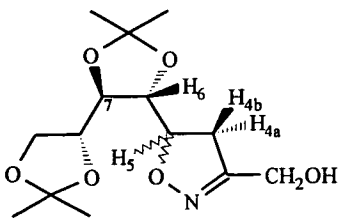
2.5.5.1 Characteristics of Deprotected Adducts (307)/(308) and (309)/(310)

Removal of either one or both of the isopropylidene protecting groups resulted in the formation of the partially and fully deprotected products respectively. The characteristic properties of these adducts compared to the starting isoxazoline are summarised below.

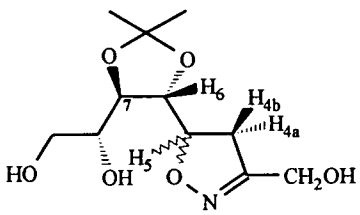
^{1}H NMR

When the partially and fully deprotected isoxazolines are compared with the parent isoxazolines major differences are observed. The ^{1}H NMR spectra reflect a major variation between the 5-H signal for the major *erythro* and minor *threo* isomers in all cases. It is observed that for the minor *threo* isomer this proton absorbs at significantly lower frequency than the corresponding major *erythro* adduct [$\Delta\delta_H$ - 0.11 ppm for (**305**)/(**306**), -0.11 ppm for (**309**)/(**310**)]. For the deprotected compounds the methylene protons of the isoxazoline ring, 4a-H and 4b-H are closer together in the major *erythro* adducts and in the fully deprotected major *erythro* adduct they are coincident. It is worth noting that the coupling between 5-H and 6-H changes dramatically on going from (**305**)/(**306**) to (**307**)/(**308**) [3.5 *cf* 6.8 Hz for (**305**)/(**307**), 2.5 *cf* 6.9 Hz for (**306**)/(**308**)]. A significant change is also observed for the coupling between 6-H and 7-H, but in the opposite direction, ie. a decrease in

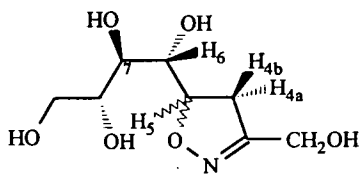
coupling [$\Delta\delta_H$ -5.8 ppm for (**305**)/(**307**), -6.5 ppm for (**306**)/(**308**). Selected chemical shifts and coupling constants for the isomers are shown in Table 56 and 57.



(**305**)/(**306**)



(**309**)/(**310**)



(**307**)/(**308**)

Isoxazolines	δ_H/ppm			
	4a-H	4b-H	5-H	6-H
(**305**)	3.02	3.12	4.87	4.16
(**306**)	3.11	3.11	4.76	4.12
(**309**)	3.29	3.22	4.97	3.95
(**310**)	3.05	3.36	4.86	3.90
(**307**)	3.21	3.21	4.80*	3.91
(**308**)	2.96	3.24	4.86*	3.86

Table 56 : Selected ^{1}H NMR data for cycloadducts

* Data obtained from deprotection of (**327**) and (**328**) (Section 2.5.13).

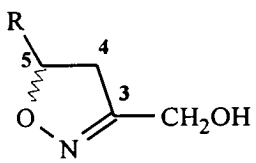
Isoxazolines	*J*/Hz				
	4a,4b	4a,5	4b,5	5,6	6,7
(**305**)	17.0	11.1	7.8	3.5	7.3
(**306**)	-	8.8	10.0	2.5	8.5
(**309**)	17.1	10.9	7.4	3.6	6.9
(**310**)	17.4	8.3	11.0	3.3	6.7
(**307**)	-	9.9*	7.0*	6.8	1.5
(**308**)	17.6	8.3	10.7	6.9	2.0

Table 57 : Selected ^{1}H NMR data for cycloadducts

* Approximate values.

^{13}C NMR

The ^{13}C NMR spectra for the partially and fully deprotected isomers show significant differences for C-4 and C-5. In each case the major *erythro* adduct has a lower chemical shift for C-4 [$\Delta\delta_C$ -2.1 ppm for (**305**)/(**306**), -1.5 ppm for (**309**)/(**310**), -0.3 ppm for (**307**)/(**308**)]. It is also interesting to note that as the isopropylidene groups are removed there is a marked decrease in $\Delta\delta_C$ for C-4. There is also an observable increase for C-5 [$\Delta\delta_C$ +1.8 ppm for (**305**)/(**306**), +1.5 ppm for (**307**)/(**308**)]. These differences are shown in Table 58.



Isoxazoline	δ_C/ppm		
	C-3	C-4	C-5
(**305**)	158.3	35.1	80.2
(**306**)	158.1	37.2	82.0
(**309**)	160.8	35.2	-
(**310**)	160.6	36.7	-
(**307**)	161.2	36.6	80.3
(**308**)	160.9	36.9	81.8

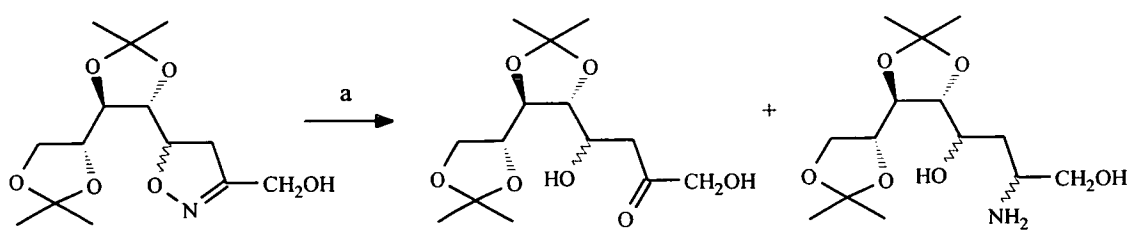
Table 58 : Selected ^{13}C NMR data for cycloadducts

2.5.6 Reductive Hydrolytic Cleavage of 2-Isoxazolines (305)/(306)

In order to synthesise the desired 3-deoxy-octulosonic acid analogues, reductive hydrolytic cleavage of the isoxazoline has to be accomplished. Although it has previously been shown that it is best to remove the protecting group first, followed by Raney-nickel catalysed hydrogenolysis (Sections 2.3.8.7 and 2.3.8.8), it was decided to perform the ring opening to allow characterisation of the resulting β-hydroxyketones in the hope that the products would be separable.

The reductive hydrolytic cleavage of isoxazolines (**305**)/(**306**) was carried out using Raney-nickel in the same manner as for (**196**) and (**204**) (Section 2.3.8.2). After preparative TLC the desired β-hydroxyketones (**311**)/(**312**) (65%) (a mixture of inseparable isomers) together with a complex mixture of diastereoisomeric γ-amino alcohols (**313**)/(**314**) (10%) were isolated (Scheme 75).

The β-hydroxyketones were characterised by ^{1}H and ^{13}C NMR spectroscopy and FAB mass spectrometry, and the chemical formulae verified by high resolution FAB mass spectometry. Brady's reagent also stains the β-hydroxyketone aiding in its identification. The γ-amino alcohols were identified by a positive ninhydrin stain on TLC, together with high resolution FAB mass spectrometry analysis.



(**305**)/(**306**) (**311**)/(**312**) (**313**)/(**314**)

(a) Ra-Ni, MeOH/H_2O (5:1)

Scheme 75

2.5.6.1 Characteristics of β-Hydroxyketones

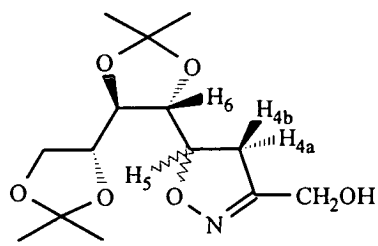
^{1}H NMR

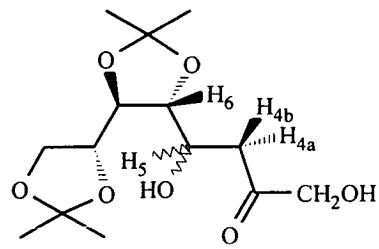
The ^{1}H NMR data for protons 4-H to 6-H reflect major differences between the 2-isoxazolines and β-hydroxyketones. Tables 59 and 60 compare the chemical shifts and coupling constants for the isoxazolines (**305**)/(**306**) with those of the β-hydroxyketones (**311**)/(**312**). It is observed that the 5-H signal absorbs at significantly higher frequency for the isoxazoline than for the corresponding β-hydroxyketone [$\Delta\delta_H$ +0.91ppm for (**305**)/(**311**), +0.72ppm for (**306**)/(**312**)]. This trend has previously been observed in the six-carbon series (Section 2.3.8.3). Although there is a change in conformation of the β-hydroxyketone in solution relative to the isoxazoline, as seen by the marked reduction in $J_{6,7}$ [$\Delta\delta_H$ -4.7 ppm for (**305**)/(**311**), -5.9 ppm for (**306**)/(**312**)], the β-hydroxyketone does not appear to be adopting a hydrogen bonded half-chair conformation as in previous examples. This may be due to steric interactions of the bulky isopropylidene side chain preventing such a structure being assumed. It is also noticeable that the methylene protons (4a-H and 4b-H) occur at higher δ-value (~0.4 ppm) in the isoxazoline than in the corresonding β-hydroxyketone. Proton 6-H has also moved to much lower frequency in the β-hydroxyketones ($\Delta\delta_H \sim 0.45$ ppm).

^{13}C NMR

Major differences for C-3 and C-4 are observed in the spectra for the β-hydroxyketones compared to those of the respective isoxazolines (Table 61). As seen

in previous examples (Section 2.3.8.3) C-4 resonates at a higher frequency for the β-hydroxyketone [$\Delta\delta_C$ + 7.5 ppm for (**305**)/(**311**), + 5.7 ppm for (**306**)/(**312**)].



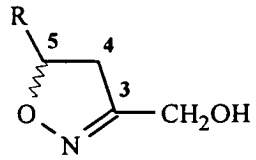


Proton	δ_H/ppm			
	4a-H	**4b-H**	**5-H**	**6-H**
Isoxazoline (**305**)	3.02	3.12	4.87	4.16
Isoxazoline (**306**)	3.11	3.11	4.76	4.12
β-Hydroxyketone (**311**)	2.63	2.76	3.96	3.70
β-Hydroxyketone (**312**)	2.76	2.76	4.04	3.70

Table 59 : Selected ^{1}H NMR data

Coupling	*J*/Hz			
	4a,5	**4b,5**	**5,6**	**6,7**
Isoxazoline (**305**)	11.1	7.8	3.5	7.3
Isoxazoline (**306**)	8.8	10.0	2.5	8.5
β-Hydroxyketone (**311**)	11.5	8.3	3.8	2.6
β-Hydroxyketone (**312**)	8.9	11.1	4.0	2.6

Table 60 : Selected ^{1}H NMR data



Carbon	δ_C/ppm	
	C-3	C-4
Isoxazoline (**305**)	158.3	35.1
Isoxazoline (**306**)	158.1	37.2
β-Hydroxyketone (**311**)	209.0	42.6
β-Hydroxyketone (**312**)	209.0	42.9

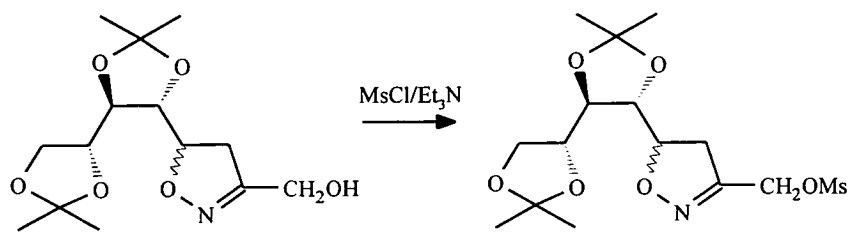
Table 61 : Selected ^{13}C NMR data

2.5.7 Mesylation of 3-Hydroxymethyl-Substituted Isoxazoline (305)/(306)

One of the objectives of our work is to synthesise a range of 3-deoxy-octulosonic acid analogues. Hence, conversion of the 3-hydroxymethyl group of (**305**)/(**306**) to the corresponding mesylates (**315**)/(**316**) should allow for the alternative functionalisation of the isoxazoline, and provide a suitably positioned leaving group for manipulation at later stages if required.

The mixture of alcohols (**305**) and (**306**) was treated with methanesulphonyl chloride in dry dichloromethane using triethlyamine as base, to afford a mixture of mesylates (**315**) and (**316**) in 80% yield (Scheme 76). As for the parent compounds these mesylates were found to be chromatographically inseparable. The isomer ratio, measured by integration of the 5-H signals at 4.17 ppm [6-H (*erythro*)] and 4.82 ppm

[5-H (*threo*)] in the ^{1}H NMR was found to be 59:41, consistent with the value obtained for the alcohol mixture. The chemical formula was verified by high resolution FAB mass spectrometry. Comparison of ^{1}H NMR of the mesylates with the parent alcohols indicates that changing from OH to OMs has little effect on the chemical shift values apart from that due to the CH_2OR, which moves to much higher frequency as expected ($\Delta\delta_H$ -0.55 ppm for both isomers). In the ^{13}C NMR there is a noticeable increase in the chemical shift for CH_2OR on changing from OH to OMs ($\Delta\delta_H$ ~5.2 ppm) and also in the adjacent quaternary carbon ($\Delta\delta_C$ -5.4 ppm). These trends for $\Delta\delta_H$ and $\Delta\delta_C$ were also observed in the hexulose series (Section 2.3.9).



(305)/(306) **(315)/(316)**

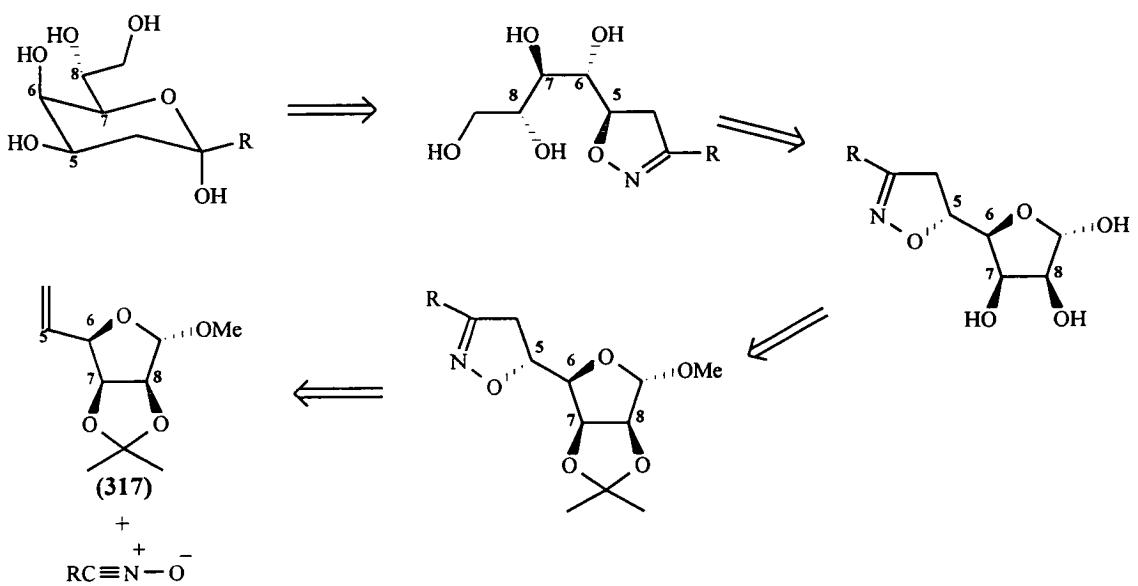
Scheme 76

2.5.8 Conclusion

Cycloaddition of nitrile oxides to the D-*arabino*-alkene (**293**) afforded adducts in variable yields (30-88%) and moderate π-facial selectivity (63:37 to 67:33) with preference for *erythro* adducts in each case. Unfortunately, it has proved impossible to separate these isomers by dry flash chromatography to afford sufficient quantities of pure isomer for subsequent reactions. Therefore an alternative strategy was required for the synthesis of KDO analogues.

2.5.9 Alternative Approach to KDO Analogues

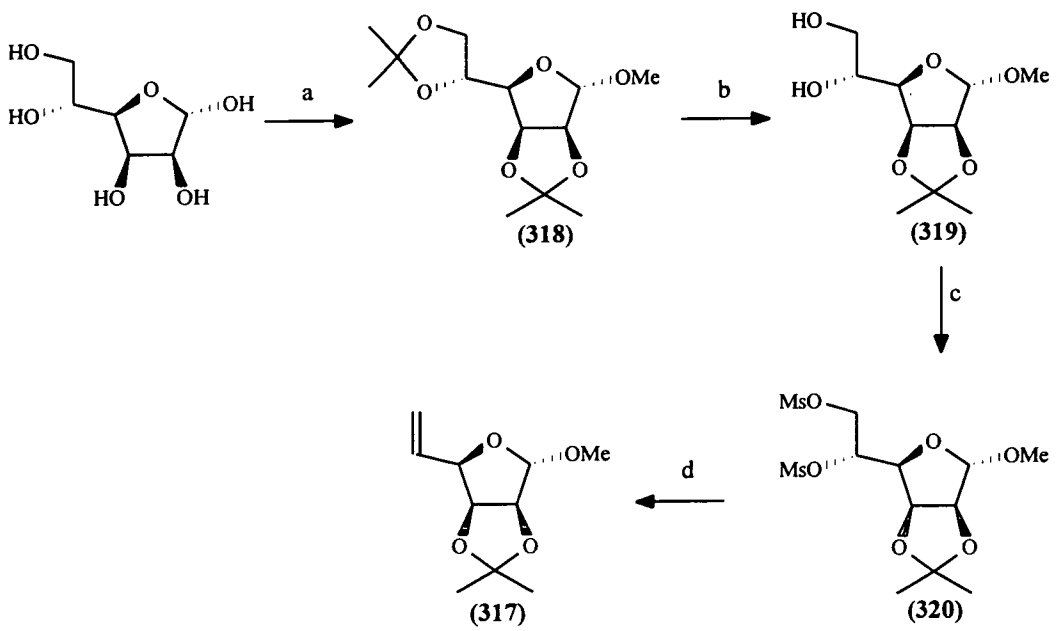
This approach involves cycloaddition of nitrile oxides to the D-mannose derived alkene (**317**) in which the alkene moiety is at the non-reducing end of the sugar dipolarophile. A known literature procedure[227] is used to synthesise alkene (**317**) which incorporates the correct configuration for C-6, C-7 and C-8 of the KDO framework. This alkene has been shown previously[217,221] to cycloadd to nitrile oxides with a high degree of selectivity, and to also yield isomers separable by chromatography. Subsequent deprotection, reduction of the aldehyde, followed by reductive hydrolytic ring opening of the isoxazoline should provide access to octulosonic acid analogues (Scheme 77).



Scheme 77

2.5.10 Synthesis of Methyl 5,6-Dideoxy-2,3-*O*-isopropylidene-α-D-*lyxo*-hex-5-enofuranoside (317)

Alkene (**317**) was prepared from D-mannose in four steps (Scheme 78) using the literature procedure,[227] in an overall yield of 49%.



(a) Acetone, MeOH, conc HCl, reflux; (b) conc HCl, H_2O, 23°C; (c) MsCl, pyridine; (d) Zn/Cu couple, NaI, 133°C

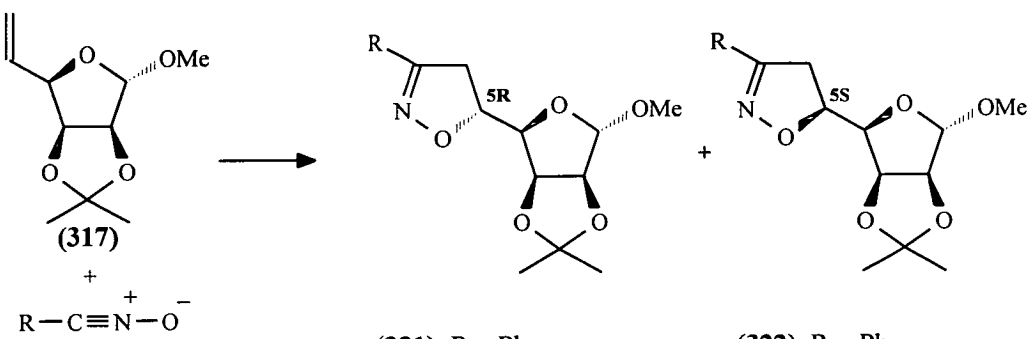
Scheme 78

Acid catalysed reaction of D-mannose with acetone and methanol[227] yielded the fully protected glycoside (**318**). Selective hydrolysis of the 5,6-isopropylidene group and subsequent treatment of the diol (**319**) with methanesulphonyl chloride and pyridine afforded the dimesylate (**320**)[227] as a white crystalline solid, without the need for purification of the intermediate compounds (**318**) and (**319**). The dimesylate is a stable compound with a good shelf-life, whereas the resulting alkene (**317**) is found to decompose on prolonged storage. Therefore, conversion to *lyxo*-alkene

(**317**) by the zinc/copper couple mediated reduction of the dimesylate, in a modification[228] of the Tipson-Cohen[229] reaction, was carried out as required.

2.5.10.1 Cycloaddition of Nitrile Oxides to Methyl 5,6-dideoxy-2,3-*O*-isopropylidene-α-D-*lyxo*-hex-5-enofuranoside (317)

Cycloaddition of benzonitrile oxide to the title alkene (**317**) (Scheme 79) was carried out as outlined in Section 2.3.2. Removal of excess alkene and 3,4-diphenylfurazan *N*-oxide (14%) afforded a mixture of known[217] cycloadducts (**321**) and (**322**) in a ratio of 81:19 and a combined yield of 83%.



(**321**) R = Ph
(**323**) R = CO_2Et
(**325**) R = $CH_2P(O)(OEt)_2$
(**327**) R = CH_2OH

(**322**) R = Ph
(**324**) R = CO_2Et
(**326**) R = $CH_2P(O)(OEt)_2$
(**328**) R = CH_2OH

Scheme 79

The isomer ratio was determined by comparison of the integrals for the glycosidic methyl proton signals which are well separated. In this case the individual adducts were readily separated by chromatography. This cycloaddition had previously been performed by K. McGhie,[217] so there was no need to characterise the adducts.

The major isomer was assigned by comparison of its TLC data and melting point to an authentic sample. It had already been established unambiguously by X-

ray analysis[195] that the new chiral centre C-5 had *R*-configuration, indicating the preferential formation of the *erythro* adduct. The overall stereochemistry for the isomers can be assigned as D-*manno* for the major isomer and D-*gluco* for the minor isomer.

The corresponding cycloaddition of ethoxycarbonylformonitrile oxide to alkene (**317**) (Scheme 79) afforded the adducts (**323**) and (**324**) in ratio 82:18 and a combined yield of 80%, together with the nitrile oxide dimer 3,4-diphenylfurazan *N*-oxide (20%). This cycloaddition had previously been carried out by K. Penman[195,221] and G. Kirkpatrick,[195,230] and the major isomer was assigned as being the *erythro* adduct by comparison of its TLC data and melting point with an authentic sample.

Cycloaddition of (diethoxyphosphoryl)acetonitrile oxide to alkene (**317**) (Scheme 79) afforded a 30% combined yield of the inseparable diastereoisomeric 2-isoxazolines (**325**) and (**326**) in a ratio of 88:12. The ratio was determined by comparison of the integrals for the methoxy signals in the ^{1}H NMR and the major isomer was assigned as the *erythro* adduct (**325**) based on comparison of its spectral parameters with those of similar adducts.

2.5.10.2 Characterisation of Isoxazoline Cycloadducts

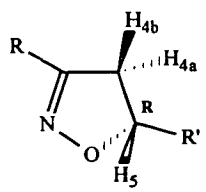
The new chiral centre C-5 of the isoxazolines (**325**) to (**328**) was assigned by correlation of ^{1}H and ^{13}C NMR and optical rotation data, to cycloadducts previously characterised by K. McGhie[217] and K. Penman.[221] The stereochemistry for the major isomer (**321**) had also previously been determined[195] by X-ray crystallography. These characteristic properties of the major and minor isomers are summarised below along with those for (**327**) and (**328**) obtained after borohydride reduction (Section 2.5.11).

^{1}H NMR

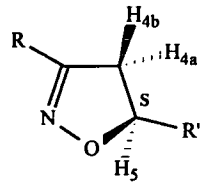
The ^{1}H NMR spectra reflect major differences between the isomers for the isoxazoline ring protons 4a-H, 4b-H and 5-H. It is observed that for the major *erythro* adduct the 5-H signal absorbs at significantly higher frequency than for the corresponding minor *threo* product [$\Delta\delta_H$ +0.11 ppm for (**321**)/(**322**), +0.06 ppm for (**323**)/(**324**), +0.10 ppm for (**327**)/(**328**)]. The signals for the methylene protons of the isoxazoline ring, 4a-H and 4b-H, are closer together in the major *erythro* adducts. Indeed, in the case of the major *erythro* adduct (**321**) they are coincident, while in the minor *threo* diastereoisomer (**322**) they are separated by 0.45 ppm. It is worth mentioning that the coupling between 5-H and 6-H in the minor *threo* isomer, is significantly greater than that in the major *erythro* product [8.6 *cf* 6.3 Hz for (**322**)/(**321**), 8.5 *cf* 5.3 Hz for (**324**)/(**323**) and 8.5 *cf* 6.0 Hz for (**328**)/(**327**)]. This suggests that the two compounds adopt different conformations in solution. Also observed are larger chemical shift values for the OMe signal of the minor *threo* isomers. The chemical shifts and coupling constants are summarised in Tables 62 and 63.

^{13}C NMR

The ^{13}C NMR spectra for the pairs of isomers are very similar. The major difference is associated with the signal for the epimeric center at C-5. In each case the major *erythro* adduct has a lower δ-value than the minor *threo* adduct [$\Delta\delta_C$ -1.5 ppm for (**321**)/(**322**), -0.6 ppm for (**323**)/(**324**), -1.8 ppm for (**327**)/(**328**)]. These findings are shown in Table 64.



Erythro



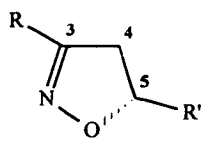
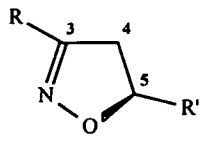
Threo

R	Isoxazoline	δ_H/ppm			
		4a-H	4b-H	5-H	OMe
Ph (Ref. 217)	*Erythro* (**321**)	3.45	3.45	5.05	3.30
	Threo (**322**)	3.57	3.12	4.94	3.34
CO_2Et (Ref. 195)	*Erythro* (**323**)	3.32	3.20	5.03	3.26
	Threo (**324**)	3.40	2.99	4.97	3.32
$CH_2P(O)(OEt)_2$	*Erythro* (**325**)	3.19	3.18	4.91	3.98
	Threo (**326**)	3.20	3.18	4.91	3.98
CH_2OH	*Erythro* (**327**)	3.13	3.12	4.90	3.29
	Threo (**328**)	3.24	2.85	4.80	3.32

Table 62 : Selected ^{1}H NMR data for cycloadducts

R	Coupling	*J*/Hz			
		4a,4b	4a,5	4b,5	5,6
Ph (Ref. 217)	*Erythro* (**321**)	-	8.6	9.7	6.3
	Threo (**322**)	16.9	10.7	8.7	8.6
CO_2Et (Ref. 195)	*Erythro* (**323**)	18.1	8.3	11.2	5.3
	Threo (**324**)	18.0	11.2	9.1	8.5
$CH_2P(O)(OEt)_2$	*Erythro* (**325**)	-	8.5	9.6	6.4
	Threo (**326**)	-	10.5	8.8	6.4
CH_2OH	*Erythro* (**327**)	-	8.5	10.0	6.0
	Threo (**328**)	17.5	10.6	8.8	8.5

Table 63 : Selected ^{1}H NMR data for cycloadducts

δ_C/ppm	Isoxazoline					
	R = Ph		R = CO_2Et		R = CH_2OH	
C-5	*Erythro* (**321**)	78.1	*Erythro* (**323**)	78.8	*Erythro* (**327**)	80.2
	Threo (**322**)	79.6	*Threo* (**324**)	79.4	*Threo* (**328**)	82.0

Table 64 : Selected ^{13}C NMR data for cycloadducts

Optical Rotation

The major isomer was found to have a more negative $[\alpha]_D$ value. This information is summarised in Table 65.

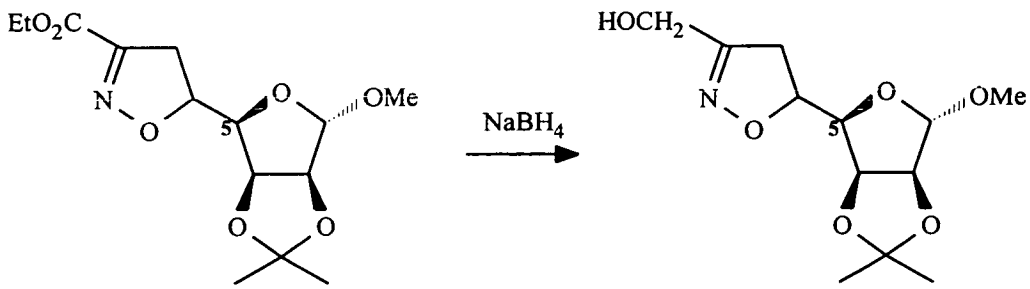
Erythro (**321**)	*Threo* (**322**)	*Erythro* (**323**)	*Threo* (**324**)	*Erythro* (**327**)	*Threo* (**328**)
-35.2	+84.8	-43.8	+90.1	-5.6	+77.9

Table 65

2.5.11 Reduction of 3-Ethoxycarbonyl Substituent to Hydroxymethyl

In order to convert the 3-ethoxycarbonyl isoxazolines (**323**) and (**324**) to octulosonic acid analogues, reduction of the ester group to provide a hydroxymethyl substituted isoxazoline is required. This should also allow access to a wide range of derivatives as well as facilitating the hydrolytic ring cleavage of the 2-isoxazoline to the β-hydroxyketone.

Isoxazolines (**323**) and (**324**) were therefore reduced by sodium borohydride in ethanol at room temperature to provide the corresponding alcohols (**327**) and (**328**) in excellent yields, 99% and 98% respectively (Scheme 80). The characteristic properties of the isomers (**327**) and (**328**) are compared with those of the parent major *erythro* and minor *threo* cycloadducts (**323**) and (**324**) in Section 2.5.10.2.



(**323**) 5*R*
(**324**) 5*S*

(**327**) 5*R*
(**328**) 5*S*

Scheme 80

2.5.12 Deprotection of 2-Isoxazolines

The next step *en route* to KDO analogues involves the removal of the isopropylidene protecting group[231,232] and hydrolysis of the methyl glycoside of the 2-isoxazolines (Scheme 81). Subsequent ring opening of the furanose followed by reduction and hydrogenolysis of the isoxazoline would be envisaged to provide the desired pyranose ring structure.

Removal of the isopropylidene protecting group and hydrolysis of the methyl glycoside was accomplished for hydroxymethyl compounds (**327**) and (**328**) after a number of pilot experiments had been performed using (**327**) (Table 66). Monitoring

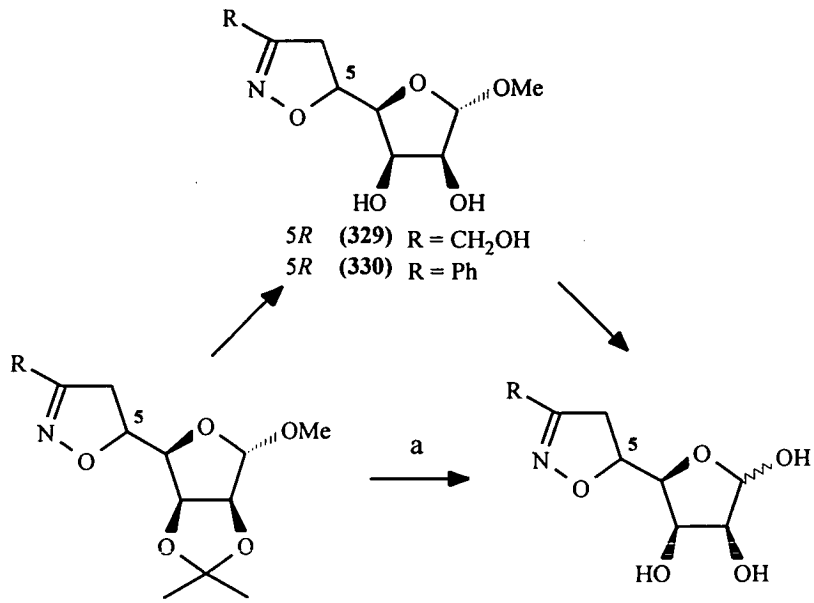
the reactions by TLC, it was evident that much more polar components were being produced, indicative of the formation of partially and fully deprotected adducts.

Reagent	Temperature, Time	Isopropylidene Deprotection	Hydrolysis of Methyl glycoside
CF_3CO_2H/H_2O (9:1)	r.t., 20 min	Yes	No
	40°C, 3 hr	Yes	Yes
	60°C, 2 hr	Yes	Yes
H_2SO_4 (1 M)	100°C, 5 hr	No evidence of required product	No evidence of required product

Table 66

Analysis of these experiments indicated that total deprotection could be acccomplished at 40°C or 60°C. From these results it was decided to perform all subsequent deprotections at a temperature intermediate to these, i.e. at ~50°C to ensure total deprotection occurred. The partially and fully deprotected products (**329**) and (**331**) were obtained as oils in 70% and 60% yields respectively. Each was identified and characterised by ^{1}H and ^{13}C NMR and FAB mass spectrometry. (**331**) was shown to be present as a mixture of anomers. The chemical formulae were verified by high resolution FAB mass spectrometry.

Pilot experiments for the phenyl analogue (**321**) under similar conditions show partial deprotection to intermediate (**330**). To take this compound through to the fully deprotected isoxazoline derivative (**333**) will require more forcing conditions.



5*R* (**329**) R = CH_2OH
5*R* (**330**) R = Ph

5*R* (**327**) R = CH_2OH
5*S* (**328**) R = CH_2OH
5*R* (**321**) R = Ph

5*R* (**331**) R = CH_2OH
5*S* (**332**) R = CH_2OH
5*R* (**333**) R = Ph

(a) CF_3CO_2H/H_2O (9:1), 50°C, 3 hr

Scheme 81

2.5.12.1 Characterisation of Deprotected Compounds (329) to (331)

The characteristic properties of the partially and fully deprotected isoxazoline derivatives resulting from removal of the isopropylidene protecting group and hydrolysis of the methyl glycoside are compared below with those of the starting isoxazolines.

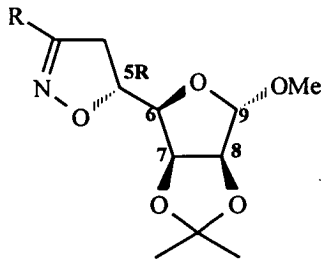
^{1}H NMR

When the partially and fully deprotected isoxazolines are compared with the parent isoxazolines major differences are observed. On removal of the isopropylidene protecting group the signal due to 6-H absorbs at significantly higher frequency [$\Delta\delta_H$ +0.27 ppm for (**327**)/(**329**), +0.36 ppm for (**321**)/(**330**)]. In comparison protons 7-H

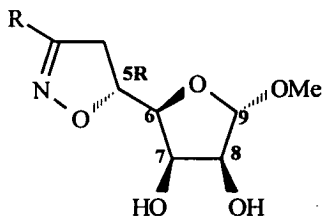
and 8-H both shift to lower frequency [eg. 4.74 *cf* 4.34 for (**327**)/(**329**)]. However, there is no observable change in δ-value for 7-H and 8-H on removal of the methoxy substituent. It is noticeable that as deprotection is accomplished 9-H gives rise to higher chemical shifts [4.88 *cf* 4.99 *cf* 5.33 for (**327**)/(**329**)/(**331**)]. The main difference in coupling values is that due to $J_{8,9}$ which increases dramatically on deprotection [eg. 0.0 *cf* 4.1 *cf* 5.1 Hz for (**327**)/(**329**)/(**331**)]. These differences are shown in Tables 67 and 68.

^{13}C NMR

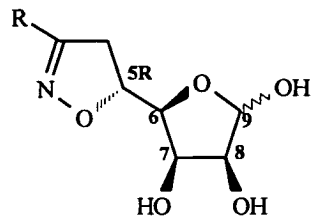
The ^{13}C NMR spectra for the deprotected isomers show significant differences for C-4 and C-9 (Table 69). On removal of the isopropylidene group C-4 shifts to a much lower frequency [$\Delta\delta_C$ -2.7 ppm for (**327**)/(**329**), -2.6 ppm for (**321**)/(**330**)]. However, once the methoxy substituent has been removed to yield the free OH C-4 moves to a higher chemical shift than in the original protected isomers [$\Delta\delta_C$ +4.6 ppm for (**329**)/(**331**)]. C-9 moves to lower frequency on deprotection.



5*R* (**327**) R = CH_2OH
5*R* (**321**) R = Ph



5*R* (**329**) R = CH_2OH
5*R* (**330**) R = Ph



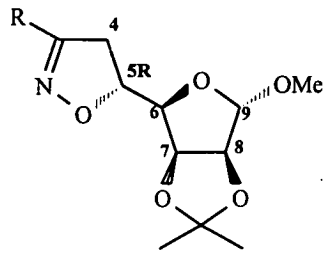
5*R* (**331**) R = CH_2OH

Isoxazoline	δ_H/ppm				
	6-H	**7-H**	**8-H**	**9-H**	**OMe**
(**327**)	4.01	4.74	4.54	4.88	3.29
(**328**)	4.28	4.34	4.14	4.99	3.44
(**331**)	4.23	4.40	4.35 4.14	5.33	-
(**321**)	4.08	4.81	4.58	4.91	3.30
(**330**)	4.44	4.39	4.21	5.04	3.49

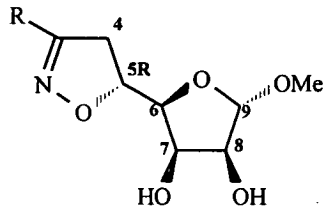
Table 67 : Selected ^{1}H NMR data

Isoxazoline	*J*/Hz			
	5,6	**6,7**	**7,8**	**8,9**
(**327**)	6.0	3.7	5.9	0.0
(**328**)	5.9	3.8	4.7	4.1
(**331**)	5.4	3.7	5.9 6.3	5.1
(**321**)	6.3	3.6	5.9	<1
(**330**)	5.7	3.9	4.6	4.0

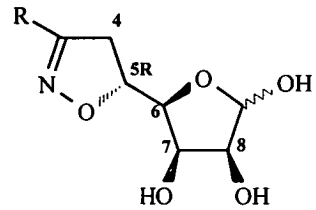
Table 68 : Selected ^{1}H NMR data



(**327**) R = CH_2OH
(**321**) R = Ph



(**329**) R = CH_2OH
(**330**) R = Ph



(**331**) R = CH_2OH

Isoxazoline	δ_C/ppm						
	C-4	**C-5**	**C-6**	**C-7**	**C-8**	**C-9**	**OCH_3**
(**327**)	37.4	78.0	84.7	79.3*	79.1*	107.1	54.6
(**329**)	34.7	77.6	76.1	73.6	68.4	106.2	53.9
(**331**)	39.3	81.9, 81.0, 79.2, 73.3				103.6	-
(**321**)	37.3	78.1	84.8, 79.4, 79.1			107.1	54.6
(**330**)	34.7	77.8	76.7	73.6	68.5	106.2	53.9

Table 69 : ^{13}C NMR data

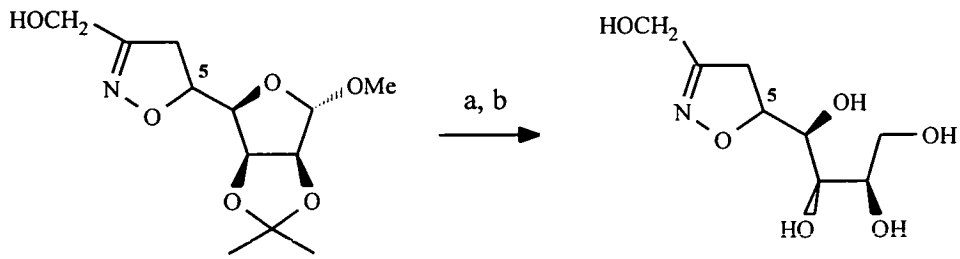
* Alternative assignments

2.5.13 Ring Opening of Furanose

The route to KDO analogues requires reduction of the aldehyde afforded on the removal of the isopropylidene protecting group and hydrolysis of the methyl glycoside. Therefore sequential hydrolysis and reduction was examined. In practice the fully deprotected adduct (**331**) was not isolated and reduction was performed on this product as soon as deprotection had occurred.

Reduction at 0°C by sodium borohydride[231] in water (after successful deprotection) was initially accomplished for hydroxymethyl-isoxazoline (**327**) (Scheme 82). Removal of sodium ions with Amberlite IR-120 (H^+) followed by

addition and evaporation of methanol to remove boric acid afforded alcohol (**307**) as a white solid (74%). Isoxazoline (**328**) was treated in the same manner yielding (**308**) as a white solid (80%).



5*R* (**327**)
5*S* (**328**)

5*R* (**307**)
5*S* (**308**)

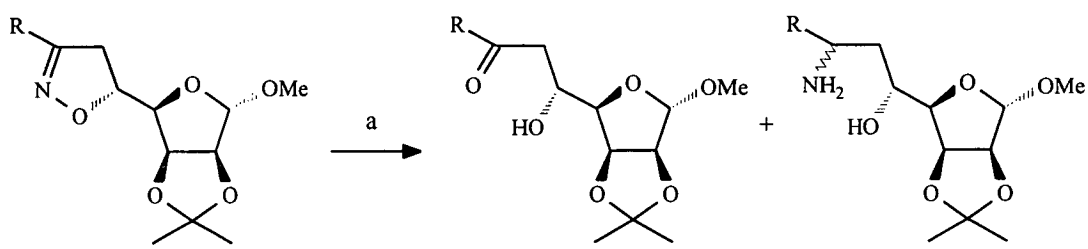
(a) CF_3CO_2H/H_2O (9:1), 50°C, 3 hr; (b) $NaBH_4$, 0°C, 3 hr

Scheme 82

The products isolated [(**307**) and (**308**)] are identical to those obtained by deprotection of the mixture of hydroxymethyl isoxazolines (**305**)/(**306**) (Section 2.5.5). However this route is much better since it provides access to each compound as a pure isomer. This is a significant advantage for the subsequent reductive hydrolytic ring cleavage of the isoxazoline. The NMR data for (**307**) and (**308**) are shown in Tables 56 and 57 (Section 2.5.5.1). As deprotection and ring cleavage of the furanose occur it is observed that the coupling between 8-H and 9-H increases dramatically [0.0 *cf* 6.2/2.7 for (**327**)/(**307**), 0.0 *cf* 6.0/2.8 for (**328**)/(**308**)] suggesting that the products (**307**) and (**308**) adopt different conformations in solution to the starting isoxazolines (**327**) and (**328**).

2.5.14 Reductive Hydrolytic Cleavage of 2-Isoxazolines

It has been established in the six-carbon series (Section 2.3.8) that deprotection of the isoxazoline adducts followed by ring opening of the isoxazoline is the better route to adopt for conversion to ulosonic acid analogues. However, in order to characterise β-hydroxyketones (6-deoxy-7-octosuloses) the ring opening of the protected compounds (**327**) and (**321**) was first examined. This was performed by catalytic hydrogenolysis using Raney-nickel as outlined in Section 2.3.8.2. After preparative TLC the desired β-hydroxyketones (**334**) (51%) and (**336**) (47%) were afforded, together with inseparable mixtures of the diastereoisomeric γ-amino alcohols (**335**) (9%) and (**337**) (16%) (Scheme 83).



(**327**) R = CH_2OH
(**321**) R = Ph

(**334**) R = CH_2OH
(**336**) R = Ph

(**335**) R = CH_2OH
(**337**) R = Ph

(a) Ra-Ni, MeOH/H_2O (5:1)

Scheme 83

The β-hydroxyketones were characterised by ^1H and ^{13}C NMR spectroscopy and FAB mass spectrometry, and their chemical formulae verified by high resolution FAB mass spectrometry. The γ-amino alcohols were identified by a positive ninhydrin stain on TLC, together with high resolution FAB mass spectrometry.

2.5.14.1 Characteristics of β-Hydroxyketones

^{1}H NMR

The ^{1}H NMR data for protons 4-H to 6-H reflect major differences between the parent 2-isoxazolines and β-hydroxyketones. Tables 70 and 71 compare the chemical shifts and coupling constants for the D-*manno* isoxazolines (**327**) and (**321**) with those of the β-hydroxyketones (**334**) and (**336**). It is observed that in both series the 5-H signal absorbs at significantly higher frequency for the isoxazoline than for the corresponding β-hydroxyketone [$\Delta\delta_H$ +0.52 ppm for (**327**)/(**334**), + 0.47 ppm for (**321**)/(**336**)]. The large coupling constant for $J_{4,5a}$ (8.9 Hz and 8.6 Hz respectively) and the smaller value for $J_{4b,5}$ (3.3 Hz and 2.9 Hz respectively) for (**334**) and (**336**) are consistent with the β-hydroxyketone adopting a hydrogen-bonded half-chair conformation (Figure 21). A similar obervation was noted in Section 2.3.8.3 for the six-carbon analogues.

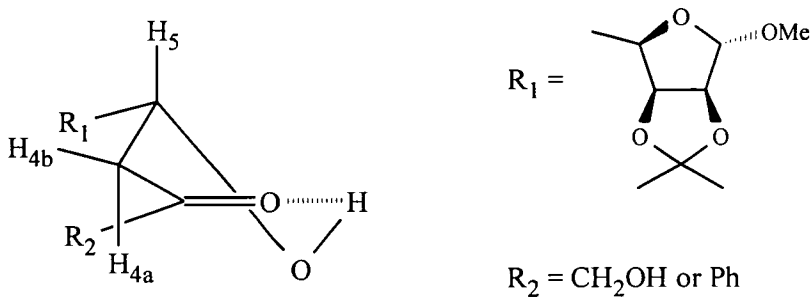
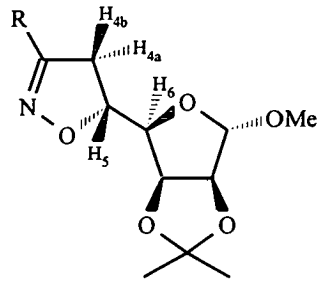
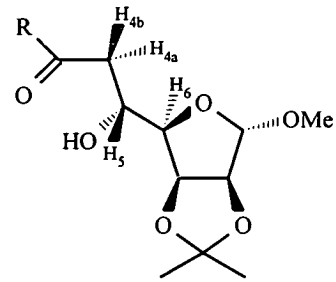


Figure 21

R	Proton	δ_H/ppm			
		4a-H	4b-H	5-H	6-H
CH_2OH	Isoxazoline (**327**)	3.13	3.12	4.90	4.01
	β-Hydroxyketone (**334**)	2.67	2.80	4.38	3.77
Ph	Isoxazoline (**321**)	3.32	3.20	5.03	4.06
	β-Hydroxyketone (**336**)	3.22	3.43	4.56	3.91

Table 70 : Selected ^{1}H NMR data

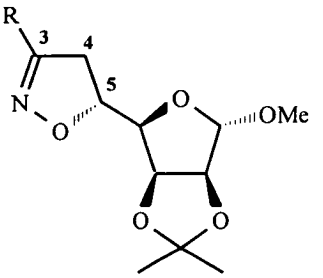
R	Coupling	*J*/Hz			
		4a,4b	4a,5	4b,5	5,6
CH_2OH	Isoxazoline (**327**)	17.5	8.5	10.0	6.0
	β-Hydroxyketone (**334**)	16.1	8.9	3.3	8.3
Ph	Isoxazoline (**321**)	18.1	8.3	11.2	5.3
	β-Hydroxyketone (**336**)	17.3	8.6	2.9	8.3

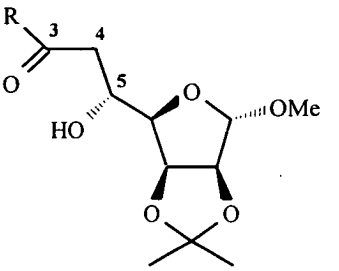
Table 71 : Selected ^{1}H NMR data

^{13}C NMR

Major differences for C-3 to C-5 are observed in the spectra for the β-hydroxyketones compared to those of the respective isoxazolines (Table 72). As for the six-carbon analogues (Section 2.3.8.3), C-4 resonates at a higher frequency for

the β-hydroxyketone [$\Delta\delta_C$ +5.3 ppm for (**327**)/(**334**), + 6.0 ppm for (**321**)/(**336**)] and C-5 at lower frequency [$\Delta\delta_C$ -11.9 ppm for (**327**)/(**334**), - 12.7 ppm for (**321**)/(**336**)].





R	Carbon	δ_C/ppm		
		C-3	C-4	C-5
CH_2OH	Isoxazoline (**327**)	159.0	37.4	78.0
	β-Hydroxyketone (**334**)	209.0	42.7	66.1
Ph	Isoxazoline (**321**)	159.4	36.0	78.8
	β-Hydroxyketone (**336**)	200.5	42.0	66.1

Table 72 : Selected ^{13}C NMR data

Optical Rotation

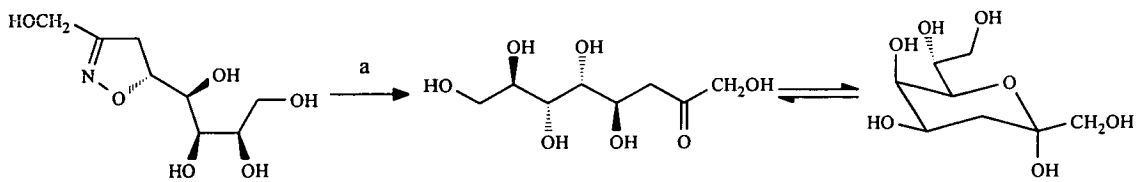
The β-hydroxyketone in each case was found to have a more positive $[\alpha]_D$ value than the isoxazoline (Table 73).

Isoxazoline (**327**)	β-Hydroxyketone (**334**)	Isoxazoline (**321**)	β-Hydroxyketone (**336**)
-5.6	+37.5	-43.8	+1.3

Table 73

2.5.15 Reductive Hydrolytic Cleavage of 2-Isoxazoline (307)

The final stage in the route to 3-deoxy-D-*manno*-octulosonic acid analogues involved the reductive hydrolytic cleavage of deprotected isoxazoline (**307**). This was performed by stirring the isoxazoline (1 M eq), boric acid (6 M eq) and Raney-nickel catalyst in a 5:1 mixture of methanol/water under an atmosphere of hydrogen (Scheme 84). The reaction was judged to be complete (2 hours) by TLC analysis.



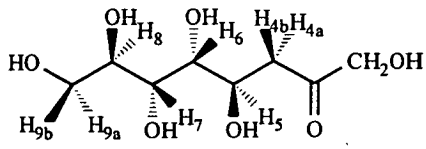
(307) **(338)** **(339)**

(a) Raney-Ni, MeOH/H_2O (5:1), H_3BO_4 (6 M eq)

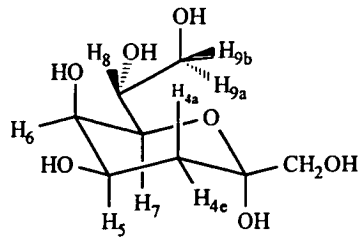
Scheme 84

The parent 2-isoxazoline has a greater TLC R_f value on silica (50% methanol/ethyl acetate) than the product (0.20 *cf* 0.05). The product which was not purified further was isolated as a pale yellow oil (43 %) by removal of solvent *in vacuo* with subsequent addition and evaporation of methanol to remove boric acid. It was characterised by mass spectrometry and from its NMR spectra. There was a characteristic peak at 222 for M^+-2H in the FAB mass spectrum, and accurate mass analysis (222.07424) of this peak gave the elemental composition as $C_8H_{16}O_7$ consistent with compounds (**338**) or (**339**).

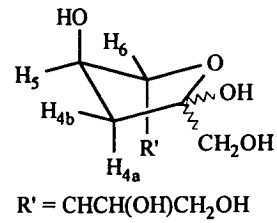
Three structures were considered for the product: a pyranose-like chair conformation (**339**), a furanose envelope conformation (**340**) with the 5-hydroxyl occupying an axial position, and the open-chain structure (**338**).



(338)

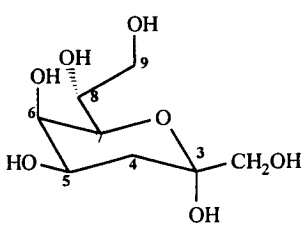


(339)

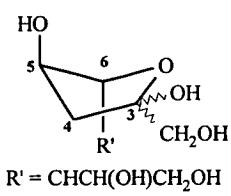


(340)

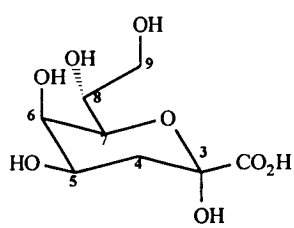
^{13}C NMR data were initially considered to identify which of the above structures was most likely. When the spectrum was examined three distinct CH_2 peaks and four CH peaks were observed, but no peak was detected at δ_C ~200 ppm, indicating the absence of a carbonyl group and thus eliminating structure (**338**). In order to distinguish between the hemiacetal forms (**339**) and (**340**) the δ_C values were compared to literature data[185] for 3-deoxy-D-*manno*-octulosonic acid in its pyranose (**341**) and furanose (**342**) forms (Table 74). The corresponding data for the parent isoxazoline (**307**) are also included for reference.



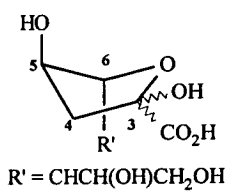
(339)



(340)



(341)



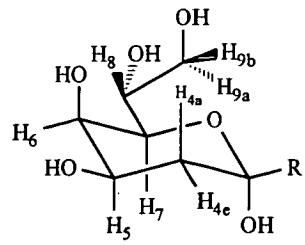
(342)

Compound	δ_C/ppm						
	C-3	C-4	C-5	C-6	C-7	C-8	C-9
(**307**)	159.1	34.6	78.3	68.6	67.8	76.2	60.9
(**339**) or (**340**)	80.4	36.7	73.3, 70.8, 70.0, 69.4				63.0
(**341**)	96.5	33.7	66.7	66.2	71.2	69.5	62.9
(**342**)	103.1	44.7	72.4	85.2	70.6	71.7	60.5

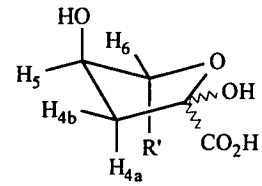
Table 74 : ^{13}C NMR data

The δ_C values for C-4 to C-9 are much closer to those for the pyranose structure (**341**) than the furanose structure (**342**), and the product is therefore tentatively assigned the $^1C_4$ structure (**339**). As expected the hemiketal carbon C-3 resonates at lower chemical shift (80.4 ppm *cf* 96.5 ppm) in ketose (**339**) due to the adjacent carboxyl group in KDO itself.

A number of deductions can be made by examination of the ^1H NMR spectrum. The two protons at lowest chemical shift are assigned to 4a-H and 4b-H which have no oxygen attached. These protons occur at low frequency (1.78 and 1.83 ppm respectively) in accord with the hemiketal form. If they had been next to a carbonyl group as in (**338**) they would have been expected to occur at a higher δ-value of ~3.00 ppm. There is a large geminal coupling of 13.0 Hz between 4a-H and 4b-H (smaller than would normally be expected), and both are coupled to 5-H which appears as a multiplet at 4.09 ppm. 5-H has a large coupling of 9.0 Hz to 4a-H and smaller couplings of 6.0 Hz to 4b-H and 3.0 Hz to 6-H. These couplings indicate that the pyranose-ring may be slightly distorted. Comparison of this data to literature values for KDO in its pyranose and furanose forms[185] further confirms that the product is existing predominantly in the pyranose form (Table 75). The methylene group at C-9 has a geminal coupling of 11.8 Hz consistent with being adjacent to an oxygen. Each of these protons 9a-H and 9b-H also appear at higher chemical shift than 4-H as expected. The couplings between 8-H/9a-H and 8-H/9b-H (2.6, 5.5 Hz) are also consistent with the proposed structure. The data obtained for product (**339**) are compared for reference with the isoxazoline precursor (**307**) in Table 76.



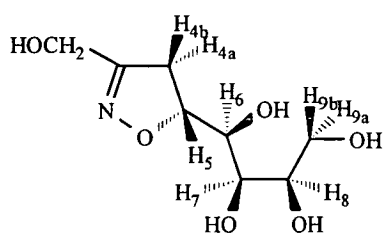
(339) R = CH_2OH
(341) R = CO_2H



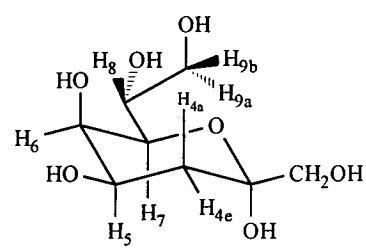
(342)
R′ = $CHCH(OH)CH_2OH$

Proton	δ_H/ppm[6]			Coupling	*J*/Hz	
	(339)	(341)	(342)		(339)	(341)
4a-H	1.78	1.92	2.53	4a,4b	13.0	12.5
4b-H	1.83	1.83	2.02	4a,5	9.0	12.5
				4b,5	6.0	5.5

Table 75 : Selected ^{1}H NMR data



(307)



(339)

Proton	δ_H/ppm		Coupling	*J*/Hz	
	(307)	(339)		(307)	(339)
4a-H	3.25	1.78	4a,4b	-	13.0
4b-H	3.25	1.83	4a,5	8.4	9.0
5-H	4.80	4.09	4b,5	10.6	6.0
6-H	3.95	3.87-3.80	5,6	6.7	3.0
7-H	3.69	3.87-3.60	6,7	1.4	nd
8-H	3.78	3.87-3.80	7,8	8.7	nd
9a-H	3.88	4.02	8,9a	2.7	2.6
9b-H	3.69	3.63	8,9b	6.2	5.5
CH_2OH	4.43	3.87-3.60	9a,9b	11.7	11.8

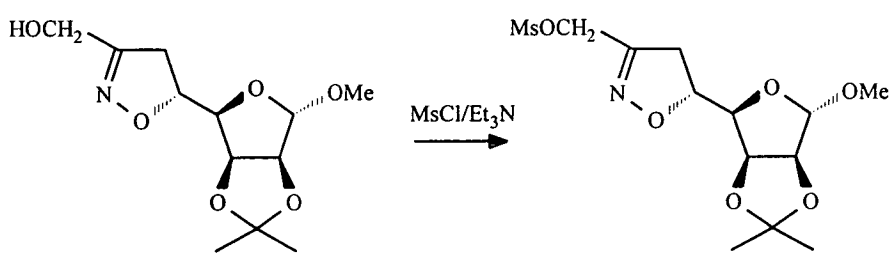
Table 76

[6] All NMR data measured in D_2O.

It is therefore proposed that the product resulting from reductive hydrolytic cleavage of isoxazoline (**307**) exists predominantly in the pyranose-ring form (**339**) although the presence of small amounts of furanose cannot be discounted.[233] This is as predicted with the non-conjugating CH_2OH group facilitating ring closure. Confirmation for this structure could be obtained by more detailed NMR experiments and by the preparation of derivatives.

2.5.16 Mesylation of 3-Hydroxymethyl-Substituted Isoxazoline (327)

One of the aims of our current work is to be able to synthesise a range of 3-deoxy-octulosonic acid analogues. Thus conversion of the 3-hydroxymethyl group of (**327**) to the corresponding mesylate (**343**) allows for alternative functionalisation of the isoxazoline. Alcohol (**327**) was reacted with methanesulphonyl chloride in dry dichloromethane using triethylamine as base, affording the desired mesylate (**343**) in 81% yield (Scheme 85). This mesylate was purified by preparative thin layer chromatography.



(**327**) (**343**)

Scheme 85

Comparison of ^1H NMR of the mesylate with the parent alcohol indicates that changing from OH to OMs has little effect on the chemical shift values apart from

that due to CH_2OR, which moves to much higher frequency as expected ($\Delta\delta_H$ +0.59 ppm). 5-H shifts to slightly lower frequency ($\Delta\delta_H$ - 0.07 ppm). There is no significant change in coupling between 5-H and 6-H (6.0 *cf* 5.3 Hz) indicating that the presence of OMs is not influencing the conformation adopted in solution. In the ^{13}C NMR there is a noticeable increase in the chemical shift for CH_2OR on changing from OH to OMs ($\Delta\delta_C$ +5.4 ppm) and a decrease in the adjacent quaternary carbon ($\Delta\delta_C$ -5.4 ppm).

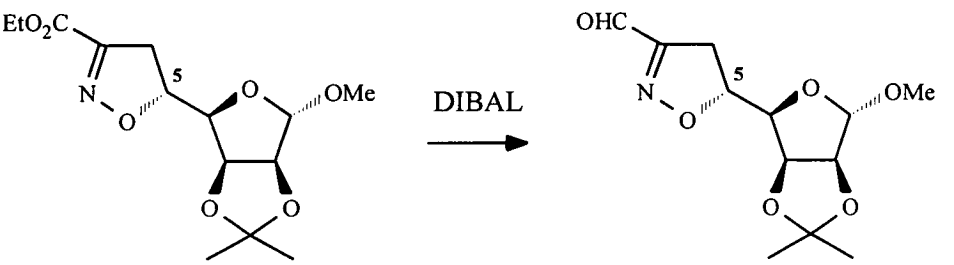
2.5.17 Modification of Side Chain at C-3 of 2-Isoxazoline

2.5.17.1 Reduction of 3-Ethoxycarbonyl Substituent to Aldehyde

Conversion of the ester substituent at C-3 to an aldehyde was carried out to allow further manipulations of the side chain and to provide easy access to a wide variety of isoxazoline derivatives containing different functional groups. These aldehydes are potential precursors for KDO analogues differing only in the substituent at the anomeric centre, thus providing a whole series of derivatives for screening for antibacterial action. The presence of an aldehyde at C-3 would permit the introduction of side chains containing $-(CH_2)_nR$ *via* Wittig reactions and subsequent hydrogenolysis/reduction, and allow the effect of separation of the CO_2H from the pyranose ring on activity to be examined. The procedure for the direct conversion of ester (**323**) to aldehyde (**344**) involves DIBAL reduction (Scheme 86).

This approach was developed for 3-formylisoxazolines by Wade and coworkers.[234] The aldehyde (**344**) was purified by preparative TLC and isolated as a colourless oil in 68% yield. It was difficult to get the reaction to go to completion,

and increasing the amount of DIBAL caused overreduction of the aldehyde to alcohol. It has been reported[234] that at -78°C a small amount (<5% by TLC and NMR) of alcohol is produced, probably due to the high reactivity of the carbonyl group attached to a carbon-nitrogen double bond. Also in this case, aldehyde and ester run at the same R_f value on TLC, independent of the solvent conditions tried, making it difficult to monitor completion of the reaction. Although pure aldehyde was obtained, an alternative approach was investigated.

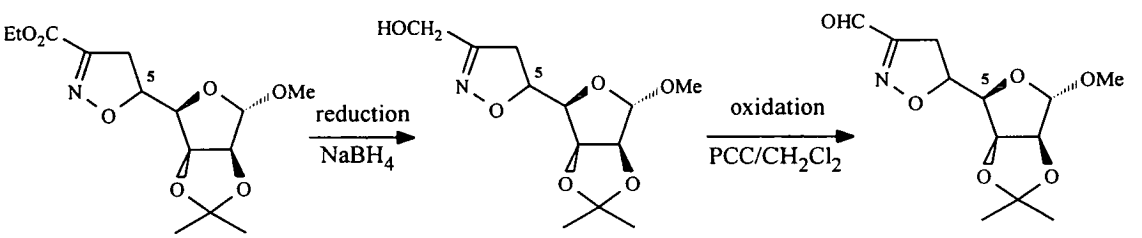


(323) (344)

Scheme 86

2.5.17.2 Oxidation of Alcohols (327) and (328) to Aldehydes (344) and (345)

This second and less direct approach to aldehyde (**344**) involved reduction of ester to alcohol followed by partial reoxidation using pyridinium chlorochromate (PCC)[235] (Scheme 87).



5R (**323**) *5R* (**327**) *5R* (**344**)
5S (**324**) *5S* (**328**) *5S* (**345**)

Scheme 87

The oxidation proceeded to completion with no TLC evidence of any unconsumed starting material. There was no need for purification of the aldehyde which was obtained in 70% yield. Spectral comparisons indicated that it was identical to that obtained by reduction of ester (**323**).

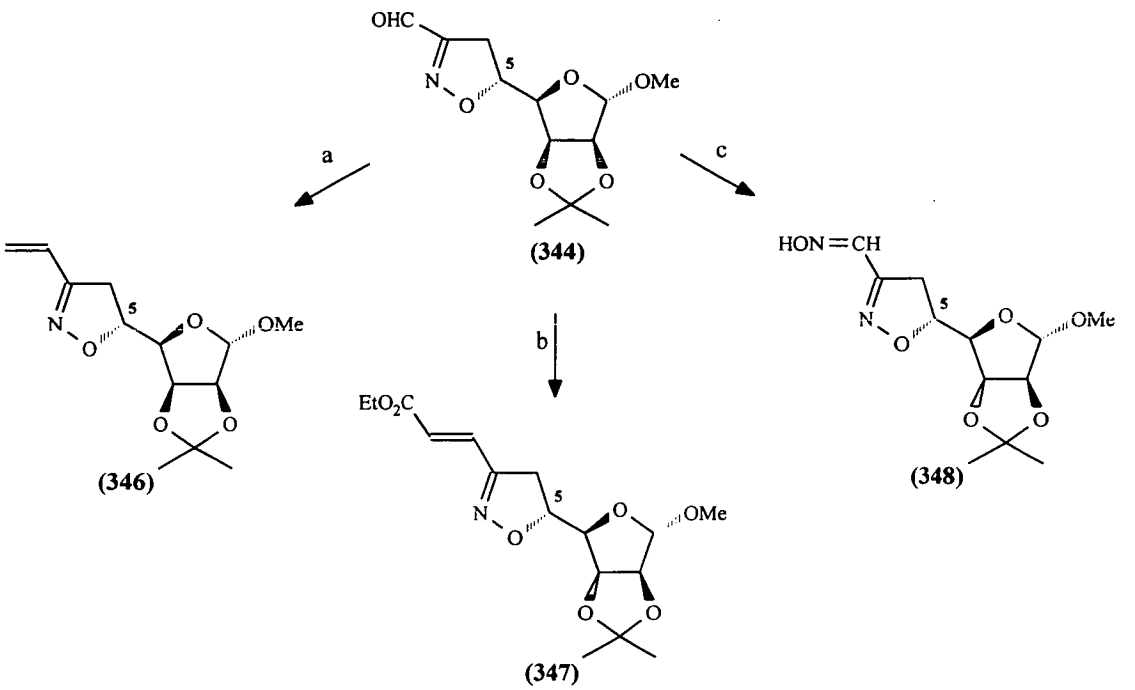
Having used both methods, and compared the ease of isolation of the resulting aldehydes, it was decided to adopt the latter approach (ie. PCC oxidation of the alcohol) for the preparation of aldehyde (**345**) (Scheme 87). This was prepared as a white solid in 54% yield. The direct reduction ($-CO_2Et \rightarrow -CHO$) method is quicker, but the two step approach ($-CO_2Et \rightarrow -CH_2OH \rightarrow -CHO$) requires less stringent control of conditions, and isolation of pure aldehyde is easier.

2.5.18 Synthesis of Aldehyde Derivatives

Once a method had been established for the successful synthesis of pure aldehyde (**344**), a number of manipulations of this group were performed. The aldehyde was converted *via* Wittig reactions to two different alkenes (**346**) and (**347**) and also to the corresponding oxime (**348**).

Conversion of aldehyde (**344**) to alkene (**347**) was achieved using the Wadsworth-Emmons modification of the Wittig reaction which utilises a resonance-stabilised phosphonate anion[336] (Scheme 88). Alkene (**347**) was purified by preparative thin layer chromatography and afforded as a colourless oil in 75% yield. As expected, in this case the *E*-alkene was formed exclusively. This was deduced from the characteristic ^1H NMR spectrum which had well separated doublets for the olefinic protons ($\Delta\delta_H$ = 1.47 ppm and J = 16.1 Hz) which indicates *trans* geometry.

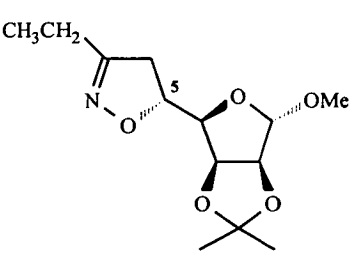
The 3-vinyl analogue (**346**) was prepared as a colourless oil in 80% from aldehyde (**344**) *via* Wittig olefination using methyltriphenylphosphorane (Scheme 88).



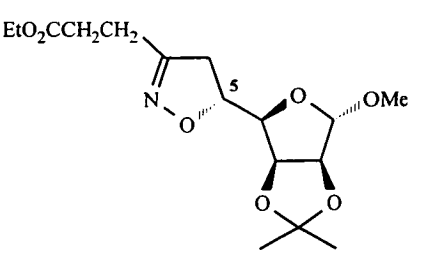
(a) $Ph_3P^+CH_3I^-$, $(CH_3)_3COK$, THF; (b) $(CH_3CH_2O)_2P(O)CH_2CO_2C_2H_5$, $(CH_3)_3COK$, ethylene glycol dimethyl ether; (c) $NH_2OH.HCl$, NaOH

Scheme 88

Hydrogenation of the unsaturated alkene double bonds of (**346**) and (**347**) may provide access to active analogues of KDO with an alkane side chain, and ester substituent separated by two methylenes removed from the isoxazoline ring [eg. (**349**) and (**350**) respectively].



(**349**)



(**350**)

Preparation of oxime (**348**) involved use of a two phase system and addition of hydroxylamine hydrochloride to an aqueous solution of sodium hydroxide, followed by addition of an ethereal solution of the aldehyde (**344**) (Scheme 88). The oxime (**348**), a colourless oil (75%), was present as a 2 : 3 mixture of *syn* and *anti* isomers as determined by the ^{1}H NMR spectrum. The methylene protons (4a-H and 4b-H) are well separated making identification and ratio determination straightforward.

2.5.19 Conclusions

Two approaches using nitrile oxide-isoxazoline chemistry have been employed for the synthesis of octulosonic acid (KDO) analogues. In the first route cycloaddition of nitrile oxides to D-*arabino*-alkene (**293**) occurred in variable yields (30-88%) and with moderate π-facial selectivity (63:37 to 67:33) and afforded adducts which proved to be chromatographically inseparable. Preference for *erythro* adducts was observed in each case. All subsequent manipulations to substituents at C-3 on the isoxazoline ring also gave inseparable isomers, so an alternative strategy was sought.

Using the D-mannose derived alkene (**317**), a synthetic route to KDO analogues *via* nitrile oxide-isoxazoline chemistry has been established yielding separable cycloadducts. Cycloadditions of nitrile oxides to alkene (**317**) yielded adducts in variable yields (30-83%) and good π-facial selectivity (81:19 to 88:12), again with preference for *erythro* adducts. Sequential hydrolysis of the

isopropylidene protecting group and methyl glycoside and reduction of the aldehyde resulting from deprotection has been successfully accomplished for the hydroxymethyl compounds (**327**) and (**328**). Subsequent conversion of the resulting 2-isoxazoline (**307**) to the KDO analogue has been performed in one case by Raney-nickel catalysed hydrogenolysis, and the product tentatively assigned the pyranose ring structure (**339**) on the basis of its NMR data.

Modification of the side chain at C-3 of the isoxazoline has also been investigated. The hydroxymethyl and 3-ethoxycarbonyl substituents have been converted to an aldehyde function suitable for subsequent manipulation. In the present work, Wittig olefinations have resulted in the synthesis of isoxazolines containing alkene side chains at C-3.

In summary, this approach may provide access *via* nitrile oxide-isoxazoline chemistry to a wide range of octulosonic acid analogues.

2.6 Conclusions and Future Work

The general approach adopted for the construction of ulosonic acid analogues which is suitable for all members of the homologous series has been *via* an intermediate isoxazoline formed by the cycloaddition of a nitrile oxide to a sugar alkene.

The strategy proposed for assembling the hexulosonic acid analogues in our model system was *via* the four-carbon D-mannitol-derived alkene (**195**) which gave moderate π-facial selectivity in the nitrile oxide cycloadditons. In an attempt to control selectivity to but-3-ene-1,2-diols, a dispiroketal protecting group was utilised [alkene (**226**)]. However the large steric bulk of the dispiroketal moiety and changing from a five to six-membered ring system appears to have negligible effect on diastereoselectivity.

The seven-carbon DAH skeleton has been created by combination of a five-carbon alkene dipolarophile such as the D-glucose-derived alkene (**260**) with the appropriate nitrile oxide, and the eight-carbon (KDO) in a similar way from the six-carbon alkene (**317**) prepared from D-mannitol.

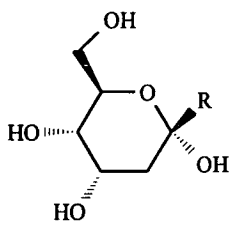
The π-facial selectivity for the key cycloaddition step in which the carbon frameworks are assembled was found to vary, with d.e. values ranging from 0% to 76% (Appendix 3).

Deprotection of the 2-isoxazolines to provide the precursors for the final reductive cleavage of the N-O bond has occurred with variable yields (48% to 87%). It has been established in the six-carbon series that deprotection of the isoxazoline adducts followed by ring opening of the isoxazoline is the better route to adopt for

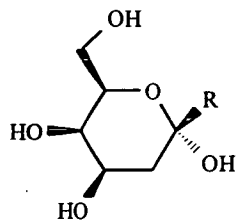
the conversion to ulosonic acid analogues. For the hexulose series a 2-ulosonic acid analogue containing a 3-phenyl substituent has been isolated, and its structure established by mass spectrometry and NMR spectroscopy which shows that it exists predominantly in its open-chain keto form. Preliminary experiments for the eight-carbon KDO system indicate that an analogue containing a non-conjugating CH_2OH attached to the anomeric centre exists mainly in the pyranose-ring form. Further work is required to optimise the ring cleavage conditions and establish techniques for purifying the products in order to prepare a whole series of seven and eight carbon analogues of DAH and KDO. The final reductive cleavage of the isoxazoline has still to be performed for the DAH analogues; however it is anticipated to proceed in the same way as for those already mentioned.

Having established the methodology for the key synthetic steps, a series of analogues could be prepared to demonstrate the scope of this route. Either the nitrile oxide component can be altered, or modifications performed at the isoxazoline stage. In the KDO series, the 3-ethoxycarbonyl substituent has been converted to an aldehyde, which provides access to a wide range of analogues.

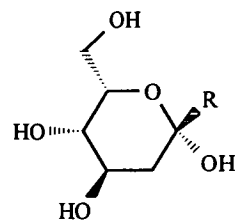
The route is ideally suited, not only for replacement of the carboxylate function at the anomeric centre, but also for the synthesis of analogues with different configurations at the various ring positions. For DAH analogues for example, the 6-epimer (**351**), and (**352**) and (**353**) could be formed by a different choice of starting alkene component or by manipulation of the isoxazoline. It should also be possible to introduce functionality at the 4-position of the isoxazoline[44] by treatment with base followed by addition of an electrophile (E), to give for example (**354**).



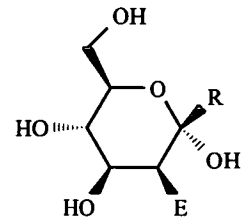
(351)



(352)

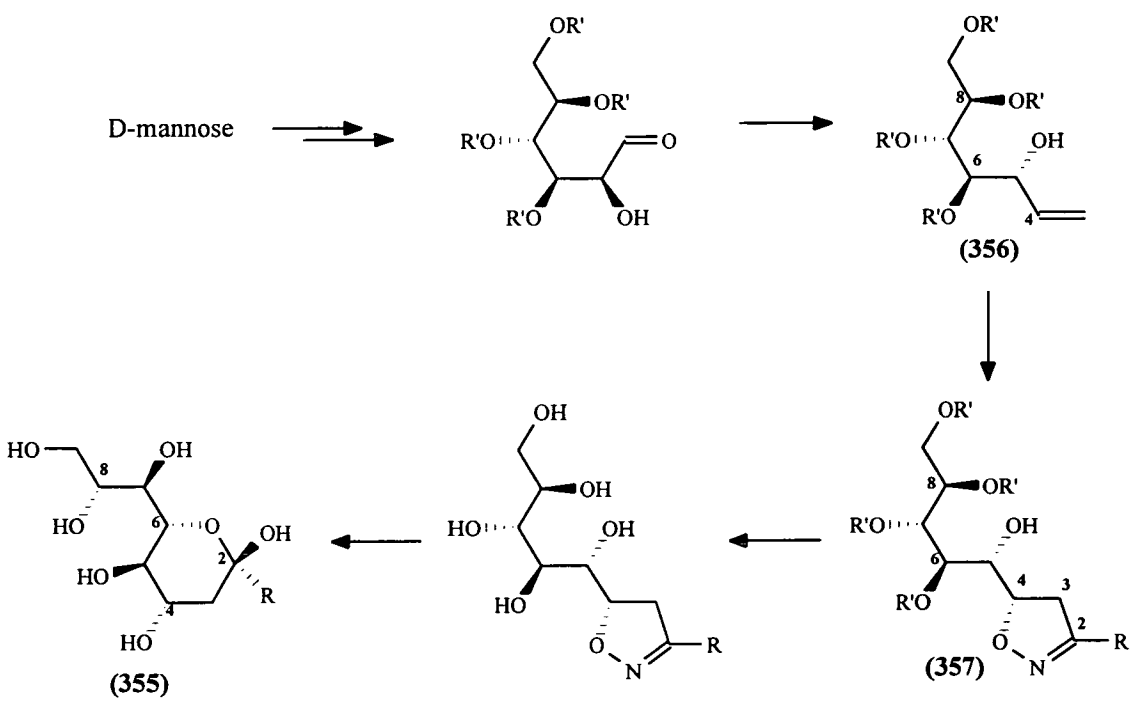


(353)



(354)

The methodology described above for DAH and KDO compounds can be applied equally well to the synthesis of higher homologues, eg. the nine carbon KDN analogues (**355**). The approach envisaged for the assembly of nonulosonic acid analogues is shown in Scheme 89. The dipolarophile component (**356**) has D-*manno* stereochemistry and it is proposed that it could be prepared by chain elongation at the reducing end of D-mannose. Reductive hydrolytic ring opening of isoxazoline (**357**), followed by deprotection and ring closure should afford KDN compounds.



Scheme 89

3 EXPERIMENTAL

3.1 General

3.1.1 Instrumentation

Elemental analyses were performed by Mrs. L. Eades using a Perkin Elmer 2400 elemental analyser.

Infrared spectra were recorded as films or nujol mulls on a Perkin Elmer 781 spectrometer and on a Bio-Rad FTS-7 spectrometer.

FAB mass spectra and exact mass measurements were recorded by Mr. A. Taylor and Miss. E. Stevenson on a Kratos MS50TC instrument using either glycerol or thioglycerol as a matrix.

Melting points were measured on a Gallenkamp capillary tube apparatus and are uncorrected.

^{1}H NMR spectra were recorded on Bruker WP200SY, AX250 and WH360 instruments by Miss H. Grant, Mr. J. R. A. Millar and Dr. D. Reed. ^{13}C NMR spectra were recorded on the WP200SY, AX250 and WH360 instruments. Chemical shifts (δ) in all spectra are measured in parts per million using tetramethylsilane ($\delta = 0.0$) as the reference signal.

Optical rotations were measured on a Perkin Elmer 141 polarimeter using 1.8 ml of filtered solution.

X-ray diffraction analyses were performed on a Stoë STADI-4 four circle diffractometer by Dr. A. J. Blake and Dr. R. O. Gould.

3.1.2 Chromatography

Preparative thin layer chromatography was carried out on glass plates (20 × 20 cm) coated with a layer of Kieselgel GF_{254} (0.5 mm) which contains 13% calcium sulphate and a fluorescent indicator. Analytical thin layer chromatography was carried out on Merck aluminium-backed plates coated with Kieselgel GF_{254} (0.2 mm).

Dry flash chromatography was carried out with a variety of sintered funnels filled with Kieselgel GF_{254} and eluted under water pump vacuum.

3.1.3 Solvents and Reagents

All reagents and solvents were standard laboratory grade and were used as supplied unless otherwise stated.

Dry ether and toluene were Analar grade dried over sodium wire.

Dry acetone was Analar grade stored over 4Å molecular sieve.

Pyridine was dried by distillation from and stored over KOH.

Dry THF was freshly distilled from sodium and benzophenone.

Dry methanol was obtained by distillation from calcium hydride.

Dry chloroform was obtained by distillation from phosphorous pentoxide and stored over molecular sieve.

Dry DMSO was obtained by allowing the solvent to stand over activated calcium sulphate for 24 hr, filtering and distilling from calcium hydride under water pump vacuum. The distilled solvent was stored over molecular sieve.

Acetic anhydride was purified by fractional distillation and stored over 4Å molecular sieve.

3.2 Synthesis of Nitrile Oxide Precursors

The nitrile oxides were generated *in situ* by dehydrochlorination of the parent hydroximoyl chloride with triethylamine.

3.2.1 Ethyl Chloro-oximinoacetate (184)

This was prepared utilising the method of Skinner.[187] Glycine ethyl ester hydrochloride (29.30 g, 0.21 mol) in water (90 ml) was cooled to -35°C and hydrochloric acid (36% w/w, 18 ml, 0.21 mol) added followed by dropwise addition of sodium nitrite (14.45 g, 0.21 mol) in water (25 ml). The additions were then repeated, and the cooled solution left stirring for 1 hr. The white precipitate which formed was filtered, washed with petroleum ether (b.p. 40-60°C, 5 ml) and dried to afford a white crystalline solid (15.69 g, 48%), m.p. 76-78°C (lit.[187] 79-80°C).

3.2.2 Benzohydroximoyl Chloride (185)

This was prepared according to the method of Chiang.[188]

To a stirred solution of sodium hydroxide (23.10 g, 0.58 mol) in water (66 ml) and freshly distilled benzaldehyde (33 ml, 0.33 mol) was added hydroxylamine hydrochloride (24.75 g, 0.36 mol) in small portions. The benzaldehyde eventually disappeared, and upon cooling at -5°C, a crystalline mass of the sodium derivative of the oxime formed. Sufficient water was added to form a clear solution, and carbon dioxide added until the solution was saturated. A colourless emulsion of the α-aldoxime separated out, which was extracted with ether, dried ($MgSO_4$), and evaporated *in vacuo*. Distillation under reduced pressure yielded the pure α-

benzaldoxime as a liquid which solidified on cooling to a white solid (31.94g, 80%). Chlorine gas was passed through a cooled solution (-20°C) of the α-benzaldoxime (10.27 g, 85.00 mmol) in chloroform (300 ml) until the solution had gone yellow/green (*via* an intermediate blue colour), when chlorination was judged to be complete. Nitrogen was then bubbled through the solution for 40 min to remove dissolved chlorine gas, the solution going very pale yellow. The solvent was evaporated *in vacuo*, and afforded a colourless oil. Trituration with petroleum ether (b.p. 80-100°C) (5 ml) and chloroform (4 ml) followed by cooling to 0°C, afforded white crystals (5.56 g, 42%), m.p. 48-50°C (lit.[188] 50-51°C).

3.2.3 (Diethoxyphosphoryl)acetohydroximoyl Chloride (189)

This was prepared from bromoacetaldehyde diethyl acetal in four steps according to Scheme 36.

3.2.3.1 Diethyl 2,2-diethoxyethylphosphonate (186)[189]

Bromoacetaldehyde diethyl acetal (21.8 ml, 0.15 mol) was heated with triethylphosphite (26.47 g, 0.16 mol) at reflux for 5 hr. After this period the low boiling material (below 100°C) was distilled under reduced pressure (~20 mmHg) and the residual oil fractionated under reduced pressure to yield diethyl 2,2-diethoxyethyl phosphonate (**186**) (16.57 g, 45%) as a colourless oil, b.p. 52°C, 2 mmHg (lit.[189] 146-149°C, 14 mmHg; 101-103°C, 8 mmHg); δ_H (360 MHz, $CDCl_3$) 4.75 (1H, dt, J_{CH2CH} = 5.6 Hz, $J_{CH,P}$ = 5.5 Hz, CH_2CH), 3.96 [2H, qd, J_{CH3CH2} = 7.1 Hz, $J_{CH2,P}$ = 7.5 Hz, $(CH_3CH_2O)_2PO$], 3.46 [2H, 2 × qd, J_{CH3CH2} = 7.1 Hz, $J_{CH2,P}$ =

8.2 Hz, $CH(OCH_2CH_3)_2$], 2.05 (2H, dd, J_{CH2CH} = 5.6 Hz, $J_{CH2,P}$ = 18.8 Hz, CH_2CH), 1.18, 1.06 [12H, 2 × t, J_{CH3CH2} = 7.1 Hz, $(CH_3CH_2O)_2CH$, $(CH_3CH_2O)_2PO$); δ_C (90 MHz, $CDCl_3$) 98.1 (CH_2CH), 61.4 (OCH_2CH_3, d, J_{CP} = 8.7 Hz), 31.5 (CH_2CH, d, J_{CP} = 139.9 Hz), 16.1 (OCH_2CH_3), 14.9 [$P(O)(OCH_2CH_3)$ d, J_{CP} = 6.3 Hz]; δ_P (90 MHz, $CDCl_3$) 26.65 (P=O); m/z (FAB) 255.13610 (M^++H), $C_{10}H_{24}O_5P$ requires 255.13612; υ_{max}/cm^{-1} (film) 2976.9, 2930.9, 2904.9 (CH), 1609.9, 1479.6 (P=O), 1391.9, 1254.8.

3.2.3.2 (Diethoxyphosphoryl)acetaldehyde (187)[189]

Diethyl 2,2-diethoxyethylphosphonate (**186**) (5.28g, 20.80 mmol) was heated at reflux with aqueous hydrochloric acid (2% w/w, 33.8 ml) for 20 min. To the cooled mixture was added sodium chloride to saturation and the mixture extracted with CH_2Cl_2. The combined organic extracts were dried ($MgSO_4$), filtered and evacuated *in vacuo* to yield (diethoxyphosphoryl)acetaldehyde (**187**) (3.67 g, 98%) as a colourless oil; δ_H (360 MHz, $CDCl_3$) 9.56 (1H, m, $J_{CHO,CH2}$ = 3.2 Hz, CHO), 4.13-4.00 (4H, 2 × q, J_{CH3CH2} = 7.1 Hz, CH_3CH_2O), 3.01 (2H, dd, $J_{CHO,CH2}$ = 3.2 Hz, $J_{CH2,P}$ = 22.0 Hz, CH_2); δ_C (90 MHz, $CDCl_3$) 192.6 (CHO, d, J_{CP} = 5.6 Hz), 62.4 (C_2, d, J_{CP} = 5.7 Hz), 42.6 (OCH_2CH_3, d, J_{CP} = 128.4 Hz), 15.9 (OCH_2CH_3, d, J_{CP} = 5.3 Hz); m/z (FAB) 181.06297 (M^++H), $C_6H_{14}O_4P$ requires 181.06296; υ_{max}/cm^{-1} (film) 2980.8, 2907.4 (CH), 1723.8 (C=O), 1393.2, 1254.9, 1029.3, 966.0.

3.2.3.3 (Diethoxyphosphoryl)acetaldehyde oxime (188)[189]

To a solution of (diethoxyphosphoryl)acetaldehyde (**187**) (4.30 g, 24.00 mmol) in water (10 ml) was added aqueous hydroxylamine hydrochloride (2.78 g,

40.00 mmol) in water (20 ml) and the mixture stirred for 17.5 hr at room temperature. After this period the mixture was poured into water (40 ml) and extracted with CH_2Cl_2 (3 × 50 ml). NaCl was added to aid extraction into the organic layer. The combined organic extracts were dried ($MgSO_4$), filtered and evaporated *in vacuo* to yield (diethoxyphosphoryl)acetaldehyde oxime (**188**) (3.02 g, 65%) as a very pale yellow oil; δ_H (360 MHz, $CDCl_3$) 9.06 (1H, b, OH), 7.30 [0.36 × 1H, dt, $J_{1,2b}$ (*syn*) = 6.5 Hz, $J_{1,P}$ = 5.2 Hz, CH=N (*syn*)], 6.70 [0.64 × 1H, dt, $J_{1,2a}$ (*anti*) = 6.0 Hz, $J_{1,P}$ = 5.0 Hz, CH=N (*anti*)], 4.05 (4H, dq, J_{CH3CH2} = 7.1 Hz, $J_{CH2,P}$ = 8.1 Hz, CH_3CH_2), 2.98 (0.64 × 1H, dd, $J_{2a,2b}$ = 6.0, $J_{2a,P}$ = 21.9 Hz, H_{2a}), 2.70 (0.36 × 1H, dd, $J_{2a,2b}$ = 6.5 Hz, $J_{2b,P}$ = 21.8 Hz, H_{2b}), 1.24 (6H, t, J_{CH3CH2} = 7.1 Hz, CH_3CH_2); δ_C (90 MHz, $CDCl_3$) 141.7 [CH=N (*syn*), d, J_{CP} = 9.5 Hz], 140.2 [CH=N (*anti*), d, J_{CP} = 7.8 Hz], 62.3 (C_2, d, J_{CP} = 5.7 Hz), 27.8 [OCH_2CH_3 (*syn*), d, J_{CP} = 141.0 Hz], 23.2 [OCH_2CH_3 (*anti*), d, J_{CP} = 139.9 Hz], 16.0 (OCH_2CH_3, d, J_{CP} = 5.5 Hz); m/z (FAB) 196.07387 (M^++H), $C_6H_{15}NO_4P$ requires 196.07386; υ_{max}/cm^{-1} (film) 3240.3, 2984.1, 2908.4 (CH), 1647.9 (P=O), 1246.9, 1023.4, 971.3, 813.0, 807.0.

3.2.3.4 (Diethoxyphosphoryl)acetohydroximoyl chloride (189)

Chlorine gas was passed through a cooled solution (-20°C) of (diethoxyphosphoryl)acetaldehyde oxime (**188**) (7.00 g, 35.90 mmol) in chloroform (140 ml) until the solution had gone yellow/green (*via* an intermediate blue colour), when chlorination was judged to be complete. Nitrogen was then bubbled through the solution for 40 min to remove dissolved chlorine gas, the solution going very pale yellow. The solvent was evaporated *in vacuo*, and afforded a very pale blue oil (5.00 g, 61%), m/z (FAB) 229 (M^++H).

3.3 Synthesis of Sugar Alkenes

3.3.1 1,2-Dideoxy-3,4-*O*-cyclohexylidene-D-*glycero*-but-1-enitol (195)

This alkene was prepared in three steps from D-mannitol according to Scheme 38.

3.3.1.1 1,2:5,6-Di-*O*-cyclohexylidene-D-mannitol (193)[190]

A mixture of D-mannitol (30.00 g, 0.16 mol), cyclohexanone (60 ml), triethyl orthoformate (20 ml) and boron trifluoride etherate (2 ml) in dry DMSO (80 ml) was stirred overnight at room temperature. The mixture was poured into ice/sodium hydrogen carbonate (200 ml) and extracted with ether (6 × 100 ml). The extract was washed with water (200 ml), brine (200 ml), dried ($MgSO_4$) and evaporated *in vacuo* to yield crude product containing excess cyclohexanone. The residual syrup was crystallised from hexane and further recrystallised from hexane-ether (2 : 1) (3 × 40 ml) to yield (**193**) as a white powder (38.20 g, 70%); m.p. 98-101°C (lit. 105-105.5°C,[190] 101-102°C[190]); δ_H (60 MHz, $CDCl_3$) 4.50-3.67 (4H, m, H_1, H_2, H_5, H_6), 2.67-2.23 (2H, d, $J_{3,OH}$ = 6 Hz, 2 × OH), 3.03-2.67 (2H, br, H_3, H_4), 2.23-1.30 (20H, m, CH_2); m/z (FAB) 343.21205 (M^++H), $C_{18}H_{31}O_6$ requires 343.21205.

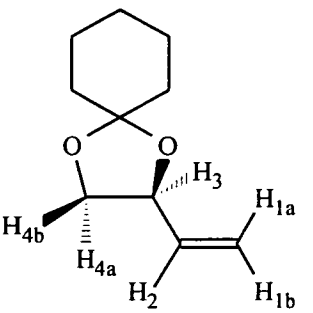
3.3.1.2 2,3-*O*-Cyclohexylideno-D-glyceraldehye (194)[190]

To a solution of 1,2:5,6-di-*O*-cyclohexylidene-D-mannitol (**193**) (30.22 g, 88.40 mmol) in ether (300 ml) was added a solution of sodium metaperiodate (22.72 g, 88.40 mmol) and tetrabutylammonium bromide (603 mg, 1.88 mmol) in water

(270 ml), and the mixture stirred for 3 hr at room temperature. The organic layer was separated, and the aqueous layer extracted with ether (3 × 200 ml). The combined organic extracts were washed with water (180 ml), dried ($MgSO_4$) and evaporated *in vacuo*. The crude product was purified by distillation/sublimation to yield aldehyde (**194**) (21.94 g, 73%) as a colourless syrup, (b.p. 90-92°C, 2 mmHg), (lit.[190] 102°C, 1 mmHg); δ_H (200 MHz, $CDCl_3$) 9.65 (1H, d, $J_{1,2}$ = 1.9 Hz, H_1), 4.32 (1H, ddd, $J_{1,2}$ = 1.9 Hz, $J_{2,3a}$ = 7.1 Hz, $J_{2,3b}$ = 5.0 Hz, H_2), 4.10 (1H, dd, $J_{2,3a}$ = 7.1 Hz, $J_{3a,3b}$ = 8.7 Hz, H_{3a}), 4.02 (1H, dd, $J_{2,3b}$ = 5.0 Hz, $J_{3a,3b}$ = 8.7 Hz, H_{3b}), 1.61-1.57 (10H, m, CH_2); m/z (FAB) 171.10097 (M^++H), $C_9H_{15}O_3$ requires 171.10212.

3.3.1.3 1,2-Dideoxy-3,4-*O*-cyclohexylidene-D-*glycero*-but-1-enitol (195)

Potassium *tert*-butoxide (11.38 g, 100.00 mmol) in dry THF (80 ml) was added to methyltriphenyl-phosphonium iodide (25.76 g, 64.00 mmol) under nitrogen, and the solution stirred for 1.5 hr at room temperature. Freshly distilled aldehyde (**194**) (6.60 g, 38.80 mmol) in dry THF (50 ml) was added dropwise over 10 min, and the solution stirred for 3 hr. Water (80 ml) was added and the mixture extracted with hexane (4 × 150 ml). The combined organic extracts were dried ($MgSO_4$), and the solvent removed *in vacuo* to afford a syrup which was purified by dry flash chromatography on silica using 30% ether/hexane as eluent, to afford the product as a colourless oil (5.80 g, 89%); δ_H (200 MHz, $CDCl_3$) see Table 77; m/z (FAB) 167.10870 (M^+-H), $C_{10}H_{15}O_2$ requires 167.10870.



(195)

Resonance	δ_H/ppm	Coupling	*J*/Hz
1a	5.33	1a,1b	1.6
1b	5.19	1a,2	17.2
2	5.81	1b,2	10.2
3	4.49	2,3	7.1
4a	3.58	3,4a	7.6
4b	4.08	3,4b	6.2
cyclohexyl.CH_2	1.63-1.60	4a,4b	8.1
		1a,3	0.9

Table 77

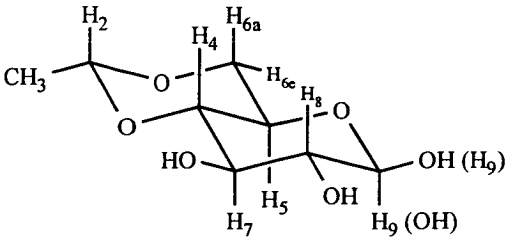
3.3.2 1,2-*Dideoxy*-3,5-O-*ethylidene*-D-erythro-*pent*-1-*enitol* (260)

This alkene was prepared in three steps from D-glucose (Scheme 58).

3.3.2.1 4,6-*O*-Ethylidene-D-glucose (261)[219]

This was prepared utilising the method of Hockett *et al*[219] modified in the following fashion.

D-Glucose (30.00 g, 0.17 mol) was treated with paraldehyde (22.5 ml, 0.17 mol, 1.01 M eq) containing concentrated sulphuric acid (0.17 ml). The reaction was stirred vigorously until it became semi-solid (30 min) and then set at room temperature for 3 days. The mixture was slurried in ethanol (100 ml) and the pH adjusted to 6.5-7.0 with ethanolic KOH (1 M). The solids were brought into solution by heating, and the pH kept at 6.5 by addition of alkali. Decolourising charcoal (1 g) was added, the solution filtered through a pad of celite and washed with hot ethanol. On standing the filtrate deposited crystalline material, which was recrystallised from ethanol. The filtrate was concentrated to dryness to remove excess paraldehyde and from the crystalline residue more material of the same m.p. obtained by recrystallisation from ethanol. 4,6-*O*-Ethylidene-D-glucose (**261**) was afforded as white crystals (16.13 g, 47%), m.p. 178-180°C (lit.[219]179-181°C); [Found : C, 46.5; H, 6.9. $C_8H_{14}O_6$ requires C, 46.6; H, 6.8]; δ_H (400 MHz, CD_3OD) see Table 78; δ_C (90 MHz, CD_3OD) 98.9, 98.8 (C_2), 96.8, 92.6 (C_9), 80.4, 79.8 (C_4), 75.1, 72.6, 72.3, 69.7 (C_7, C_8), 67.7, 67.2 (C_6), 65.7, 61.5 (C_5), 18.7 (CCH_3); m/z (FAB) 205.07073 (M^+-H), $C_8H_{13}O_6$ requires 205.07121.



(261)

Anomer	Resonance	δ_H/ppm	Coupling	*J*/Hz
α	CH_3	1.21	CH_3CH	5.1
	2	4.70	6a,6e	10.1
	4	-	6a,5	10.1
	5	3.63	6e,5	4.8
	6a	3.42*	4,5	9.9
	6e	3.90	4,7	9.3
	7	3.53	7,7OH	5.0
	8	3.25	7,8	9.3
	9	4.93	8,8OH	6.8
	7OH	4.99	8,9	3.7
	8OH	4.72*	9,9OH	4.6
	9OH	6.46		
β	CH_3	1.21	CH_3CH	5.1
	2	4.70	6a,6e	10.1
	4	-	6a,5	9.9
	5	3.20*	6e,5	4.8
	6a	3.44*	4,5	9.7
	6e	3.97	4,7	nd
	7	-	7,7OH	nd
	8	2.96	7,8	8.7
	9	4.39	8,8OH	nd
	7OH	5.11	8,9	7.7
	8OH	5.11	9,9OH	6.5
	9OH	6.76		

Table 78

* Alternative assignments

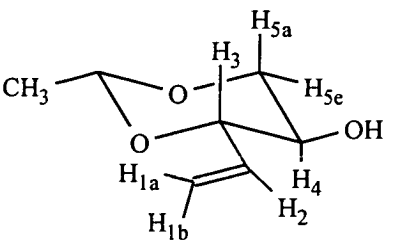
3.3.2.2 2,4-*O*-Ethylidene-D-erythrose (262)[220]

4,6-*O*-Ethylidene-D-glucose (**261**) (12.00 g, 58.30 mmol) was dissolved in water (30 ml) and added dropwise over a period of 30 min to a well stirred solution of sodium periodate (25.17 g, 117.70 mmol) in water (233 ml). The temperature of the reaction was kept below 10°C with an ice bath and the pH maintained at 4, by dropwise addition of 8 N NaOH. The pH was then adjusted to 6.5 by addition of 8 N NaOH, causing rapid hydrolysis of the formyl group. The reaction mixture was concentrated at 40-45°C, and the resulting solids dried overnight at room temperature and 0.1 mmHg pressure, and then thoroughly extracted with hot ethyl acetate (600 ml). The combined organic extracts were dried (Na_2SO_4), and concentrated *in vacuo* to afford 2,4-*O*-ethylidene-D-glucose (**262**) (8.23 g, 97%), white crystalline glass, m.p. 108-110°C (lit.[220] 110-111°C, 149-150°C); δ_H (250 MHz, $CDCl_3$) 9.70 (1H, s, C*H*O); m/z (FAB) 145.050081 (M^+-H), $C_6H_9O_4$ requires 145.05008.

3.3.2.3 1,2-*Dideoxy*-3,5-O-*ethylidene*-D-erythro-*pent*-1-*enitol* (260)

Potassium *tert*-butoxide (15.99 g, 142.50 mmol) in dry THF was added to methyltriphenyl-phosphonium bromide (31.32 g, 87.70 mmol) in dry THF, under argon, and the solution stirred for 1.5 hr at room temperature. 2,4-*O*-Ethylidene-D-erythrose (**262**) (8.00 g, 54.8 mmol) dissolved in dry THF was added dropwise over 10 min, and the solution stirred overnight. Water (150 ml) was added and the mixture extracted with hexane (6 × 150 ml). The combined organic extracts were dried ($MgSO_4$), and the solvent removed *in vacuo* to afford a syrup which was purified by MPLC (silica; hexane/ether gradient elution 10 : 0 → 6 : 4), and afforded alkene

(**260**) as a pale yellow oil (5.52 g, 70%); $[\alpha]_D^{25}$ -22.7° (c = 1.29, $CHCl_3$); [Found : C, 57.6; H, 8.2. $C_7H_{12}O_3$ requires C, 58.3; H, 8.3]; δ_H (360 MHz, $CDCl_3$) see Table 79; δ_C (90 MHz, $CDCl_3$) 134.5 (C_2), 118.9 (C_1), 98.6 (*C*Me), 82.5 (C_3), 70.2 (C_5), 64.8 (C_4), 20.3 (CH_3); m/z (FAB) 145.08564 (M^++H), $C_7H_{13}O_3$ requires 145.08647.



(**260**)

Resonance	δ_H/ppm	Coupling	*J*/Hz
1a	5.37	1a,1b	1.3
1b	5.27	1a,2	17.3
2	5.84	1b,2	10.4
3	3.72	2,3	6.7
4	3.41	3,4	8.7
5a	3.36	4,5a	10.1
5e	4.05	4,5e	4.6
CH_3CH	4.66	5a,5e	10.3
CH_3CH	1.24	CH_3CH	5.0
4OH	2.65	4,4OH	nd

Table 79

3.3.3 1,2-*Dideoxy*-3,5-O-*benzylidene*-L-threo-*pent*-1-*enitol* (286)

This alkene was prepared in three steps from D-arabinitol according to Scheme 67.

3.3.3.1 1,3-*O*-Benzylidene-L-arabinitol (287)[223]

A rapid stream of dry hydrochloric acid gas was passed into a suspension of D-arabinitol (1.50 g, 9.90 mmol) in benzaldehyde (0.95 ml) at room temperature; the D-arabinitol dissolved completely in 10 min. The reaction mixture was allowed to stand for 18 hr, during which period it became a magma of white crystals. The mass was broken up and hydrogen chloride removed as far as possible at room temperature and 0.1 mmHg. Final traces were removed by storage for 24 hr in an evacuated desiccator containing KOH and H_2SO_4. The residue was dissolved in aqueous ammonia (20 ml) containing Na_2CO_3 (0.5 g), and extracted with light petroleum (b.p. 60-80°C) to remove benzaldehyde. Concentration of the aqueous solution yielded a white solid which was recrystallised from ethanol. 1,3-*O*-Benzylidene-L-arabinitol (**287**) was afforded as white needles (1.50 g, 63%), m.p. 142-143°C (lit.[223] 151-152°C); $[\alpha]_D^{23}$ +7.7° (c = 0.89, $CHCl_3$); [Found : C, 59.7; H, 6.7. $C_{12}H_{16}O_5$ requires C, 60.0; H, 6.7]; δ_H (400 MHz, DMSO-d_6) 5.52 (1H, s, PhC*H*), 4.69 (1H, d, $J_{CHOH,CHOH}$ = 6.4 Hz, 2-OH), 4.68 (1H, d, $J_{4OH,4}$ = 6.3 Hz, 4-OH), 4.39 (1H, t, $J_{CH2OH,CH2OH}$ = 5.7 Hz, CH_2OH), 4.03 (1H, dd, $J_{4,5a}$ = 1.7 Hz, $J_{5a,5b}$ = 11.8 Hz, H_{5a}), 3.71 (1H, dd, $J_{3,4}$ = 1.3 Hz, J_{CHOH} = 8.6 Hz, H_3), 3.64 (1H, ddd, $J_{3,4}$ = 1.3 Hz, $J_{4,5a}$ = 1.7 Hz, $J_{4,5e}$ = 1.5 Hz, H_4), 3.99 (1H, dd, $J_{4,5e}$ = 1.5 Hz, $J_{5a,5e}$ = 11.8 Hz, H_{5e}), 3.68 (1H, ddd, $J_{1,2}$ = 2.8 Hz, $J_{3,CHOH}$ = 8.6 Hz, H_2), 3.57-3.40 (2H, m, $J_{CHOH,CH2OH}$ = 2.8

Hz, $J_{CH2OH,CH2OH}$ = 5.7 Hz, H_1); δ_C (90 MHz, CD_3OD) 127.8, 127.1, 125.5 (5 × PhCH), 100.6 (*C*Ph), 78.1 (C_3), 71.9 (C_5), 69.0 (C_4), 62.2 (C_1), 62.0 (C_2); m/z (FAB) 241.10760 (M^++H), $C_{12}H_{17}O_5$ requires 241.10759.

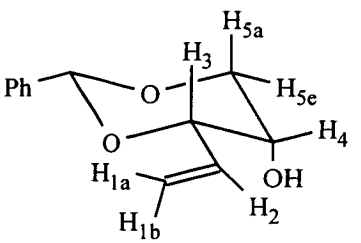
3.3.3.2 2,4-*O*-Benzylidene-L-threose (288)[224]

A solution of 1,3-*O*-benzylidene-L-arabinitol (**287**) (1.50 g, 6.30 mmol) and sodium periodate (2.14 g, 0.01 mol, 1.6 M eq) in water (100 ml) was stirred for 2 hr at room temperature, and the pH of the solution maintained at 4. The reaction mixture was then adjusted to pH 6.5 by the addition of saturated $NaHCO_3$, and the solution [containing a small amount (0.1 g) of solid Na_2CO_3] concentrated at 40°C. The residue was dehydrated using ethanol (3 × 10 ml), more ethanol added and the suspension filtered. The filtrate was concentrated *in vacuo* to yield 2,4-*O*-benzylidene-L-threose (**288**) as white crystals (0.91 g, 70%), m.p. 161-163°C (lit.[224] 165°C); δ_H (250 MHz, $CDCl_3$) 9.65 (1H, s, CHO), 7.60-7.30 (5 × PhCH); m/z (FAB) 209.08137 (M^++H), $C_{11}H_{13}O_4$ requires 209.08138.

3.3.3.3 1,2-*Dideoxy*-3,5-O-*benzylidene*-L-threo-*pent*-1-*enitol* (286)

Potassium *tert*-butoxide (0.98 g, 8.75 mmol) in dry THF was added to methyltriphenyl-phosphonium bromide (2.18 g, 5.38 mmol) in dry THF, under nitrogen and the solution stirred for 1.5 hr at room temperature. 2,4-*O*-Benzylidene-L-threose (**288**) (0.70 g, 3.37 mmol) dissolved in dry THF was added dropwise over 10 min, and the solution stirred overnight. Water (30 ml) was added and the mixture extracted with hexane (3 × 50 ml). The combined organic extracts were dried

($MgSO_4$), and the solvent removed *in vacuo* to afford a syrup which was purified by dry flash chromatography (silica; hexane/ether gradient elution 10 : 0 → 4 : 6), and afforded alkene (**286**) as a yellow oil (0.34 g, 49%); $[\alpha]_D^{26}$ -3.3° (c = 0.23, $CHCl_3$); δ_H (360 MHz, $CDCl_3$) see Table 80; δ_C (90 MHz, $CDCl_3$) 134.6 (C_2), 128.8, 128.0, 125.8 (5 × PhCH), 117.3 (C_1), 101.0 (*C*Ph), 80.1 (C_3), 72.1 (C_5), 65.6 (C_4); m/z (FAB) 207.102112 (M^++H), $C_{12}H_{15}O_3$ requires 207.10211.



(**286**)

Resonance	δ_H/ppm	Coupling	*J*/Hz
1a	5.45	1a,1b	1.6
1b	5.33	1a,2	17.4
2	5.98	1b,2	10.7
3	4.46	2,3	5.2
4	3.57	3,4	1.5
5a	4.24	4,5a	1.9
5e	4.07	4,5e	1.3
PhC*H*	5.63	5a,5e	11.9
Ph	7.55-7.35	4,4OH	nd
4OH	2.65		

Table 80

3.3.4 1,2-Dideoxy-3,4:5,6-di-*O*-isopropylidene-D-*arabino*-hex-1-enitol (293)

This alkene was prepared in four steps utilising two different synthetic pathways.

1. From D-arabinose, (Scheme 70) by the method of Zinner[225] in 29% overall yield.
2. From D-mannitol, (Scheme 71) by the method of Wiggins[226] in 14% overall yield.

3.3.4.1 D-Arabinose diethyldithioacetal (294)

To a stirred solution of D-arabinose (10.03 g, 66.80 mmol) in concentrated hydrochloric acid (10 ml) was added ethanethiol (10 ml) dropwise over 20 min. On cooling the solution to 0°C a precipitate formed. After 5 min, water (40 ml) was added to form a slurry, which on filtering yielded an off-white precipitate, which was washed with cold water (2 × 25 ml). Recrystallisation from boiling water (50 ml) with hot filtration yielded D-arabinose diethyldithioacetal (**294**) as fine white needles (8.40 g, 49%), m.p. 124-126°C (lit.[225] 126°C from isobutanol); m/z (FAB) 256.08228 (M^+), $C_9H_{20}O_4S_2$ requires 256.08030; υ_{max}/cm^{-1} (nujol) 3260 (OH).

3.3.4.2 2,3:4,5-Di-*O*-isopropylidene-D-arabinose diethyldithioacetal (295)

D-Arabinose diethyldithioacetal (**294**) (4.02 g, 15.70 mmol) was added to dry acetone (40 ml) containing concentrated sulphuric acid (0.6 ml), and the mixture shaken overnight at room temperature. The reaction mixture was neutralised with anhydrous ammonia gas, filtered over a pad of celite and the filtrate stirred for 58 hr at room temperature with anhydrous copper sulphate (1.50 g). The filtrate was concentrated under reduced pressure and the resulting oil chromatographed on silica

with ethyl acetate-hexane (1 : 9) as eluent to produce 2,3:4,5-di-*O*-isopropylidene-D-arabinose diethyldithioacetal (**295**) (3.97 g, 75%) as a yellow syrup.

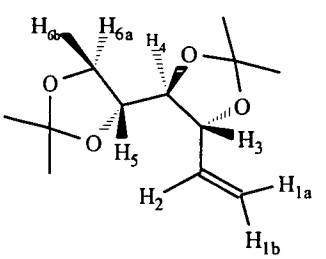
3.3.4.3 2,3:4,5-Di-*O*-isopropylidene-*aldehydo*-D-arabinose (296)

Mercuric(II)oxide (6.03 g, 27.00 mmol) and mercuric(II)chloride (6.03 g, 22.20 mol) were added to a magnetically stirred solution of the bisacetonide (**295**) (4.04 g, 15.8 mmol) in acetone (37 ml) and water (3 ml) and then the mixture heated for 2 hr at 30°C, 1 hr at 50°C, and 2 hr at reflux. After cooling, the reaction mixture was filtered over a pad of celite and the filtrate concentrated *in vacuo* to give a yellow oily residue. This was dissolved in chloroform (125 ml), shaken with saturated potassium iodide (2 × 125 ml), washed with water (90 ml), and dried ($MgSO_4$). The solvent was evaporated *in vacuo* to afford the crude aldehyde as a yellow oil, which was purified by distillation/sublimation to yield aldehyde (**296**) (2.91 g, 80%) as a colourless oil, (b.p. 60-65°C, 0.08 mmHg); δ_H (200 MHz, $CDCl_3$) 9.72 (1H, d, $J_{1,2}$ = 1.1 Hz, H_1), 4.38 (1H, dd, $J_{1,2}$ = 1.1 Hz, $J_{2,3}$ = 5.9 Hz, H_2), 4.12 (1H, dd, $J_{4,5b}$ = 6.7 Hz, $J_{5a,5b}$ = 5.9 Hz, H_{5b}), 4.09 (1H, dd, $J_{4,5a}$ = 6.6 Hz, $J_{5a,5b}$= 5.9 Hz, H_{5a}), 4.02 (1H, dd, $J_{2,3}$ = 5.9 Hz, $J_{3,4}$ = 3.7 Hz, H_3), 3.94 (1H, ddd, $J_{3,4}$ = 3.7 Hz, $J_{4,5a}$ = 6.6 Hz, $J_{4,5b}$ = 6.7 Hz, H_4), 1.44, 1.39, 1.34, 1.31 (12H, 4 × s, 4 × CH_3).

3.3.4.4 1,2-Dideoxy-3,4:5,6-di-*O*-isopropylidene-D-*arabino*-hex-1-enitol (293)

Potassium *tert*-butoxide (0.82 g, 7.28 mmol) in dry THF (6 ml) was added to methyltriphenyl-phosphonium iodide (1.96 g, 4.85 mmol) in dry THF (13 ml) under nitrogen, and the solution stirred for 1.5 hr at room temperature. Freshly

distilled aldehyde (**296**) (0.52 g, 2.26 mmol) in dry THF (6 ml) was added dropwise over 10 min, and the solution stirred for 3 hr. Water (12 ml) was added and the mixture extracted with hexane (3 × 40 ml). The combined organic extracts were dried ($MgSO_4$) and the solvent removed *in vacuo* to afford a syrup which was purified by dry flash chromatography on silica using 10% ether/hexane as eluent, to afford the product (**293**) as a yellow oil (0.29 g, 56%); [Found : C, 62.8; H, 9.0. $C_{12}H_{20}O_4$ requires C, 63.2; H, 8.8]; δ_H (360 MHz, $CDCl_3$) see Table 81; δ_C (90 MHz, $CDCl_3$) 135.7 (C_2), 117.1 (C_1), 109.5, 109.2 [2 × $C(Me)_2$], 81.0 C_3), 80.3 (C_4), 76.5 (C_5), 66.8 (C_6), 26.8, 26.5, 25.1 (4 × CH_3); m/z (FAB) 229 (M^++H).



(**293**)

Resonance	δ_H/ppm	Coupling	*J*/Hz
1a	5.38	1a,1b	1.5
1b	5.18	1a,2	17.2
2	5.88	1b,2	10.5
3	4.33	1b,3	1.2
4	3.67	2,3	6.1
5	3.92	3,4	7.6
6a	4.10	4,5	7.1
6b	4.06	5,6a	4.6
isopropyl.CH_3	1.38-1.31	5,6b	6.7
		6a,6b	6.3

Table 81

3.3.4.5 1,2:3,4:5,6-Triisopropylidene-D-mannitol (297)

A mixture of D-mannitol (10.00 g, 54.90 mmol), anhydrous acetone (125 ml, 1.71 mol) and concentrated sulphuric acid (1 ml) was shaken for 18 hr at room temperature, neutralised with concentrated aqueous ammonia solution (4 ml), and sodium carbonate (6.25 g, 59.00 mmol), and filtered. The filtrate and acetone washings were combined, and concentrated at water pressure (12 mmHg), in the presence of a small amount of sodium carbonate, to *ca* 30 ml amd poured into ice-water (300 ml). Recrystallisation of the white precipitate from aqueous acetone containing a trace of ammonia, yielded white needles of the required product (**297**) (10.01 g, 60%), m.p. 64-66°C (lit.[226] 69-70°C); δ_H (200 MHz, $CDCl_3$) 4.22-3.90 (8H, m, 2 × H_1, H_2, H_3, H_4, H_5, 2 × H_6), 1.39-1.32 (18H, m, 6 × CH_3); δ_C (50 MHz, $CDCl_3$) 110.0, 109.4 (3 × $C(Me)_2$), 80.5, 79.2, 76.2 (C_2, C_3, C_4, C_5), 66.1 (C_1, C_6), 27.3, 26.6, 25.3, 26.2, 25.1, 24.9 (6 × CH_3); m/z (FAB) 303.18073 (M^++H), $C_{15}H_{27}O_6$ requires 303.18075.

3.3.4.6 1,2:3,4-Diisopropylidene-D-mannitol (298)

1,2:3,4:5,6-Triisopropylidene-D-mannitol (**297**) (5.92 g, 19.60 mmol) was dissolved in 70% ethanol (118 ml) containing concentrated hydrochloric acid (0.4 ml) and the solution warmed at 40-45°C for 60 min. The acid was neutralised with barium carbonate, the filtrate solution evaporated to dryness, and the residue extracted with acetone. The acetone extract, on evaporation, gave a residue which was dissolved in a little ethanol and poured into water, when unchanged 1,2:3,4:5,6-triisopropylidene-D-mannitiol (**297**) was precipitated (0.59 g, 10%) as white needles.

Evaporation of the filtrate *in vacuo* followed by column chromatography yielded, in order of elution, further 1,2:3,4:5,6-triisopropylidene-D-mannitol (**297**) (0.53 g, 9%), the desired 1,2:3,4-diisopropylidene-D-mannitol (**298**) (1.77 g, 35%), and 3,4-mono-isopropylidene-D-mannitol (**298**) (1.39 g, 32%). 1,2:3,4-Diisopropylidene-D-mannitol (**298**), white needles, m.p. 33-35°C; δ_H (200 MHz, $CDCl_3$) 4.19-3.72 (8H, m, 2 × H_1, H_2, H_3, H_4, H_5, 2 × H_6), 2.52 (2H, b, 2 × OH), 1.43, 1.35 (12H, d, 4 × CH_3); δ_C (50MHz, $CDCl_3$) 110.0, 109.5 [2 × $C(Me)_2$], 80.6 (C_3, C_4), 76.3 (C_5), 72.1 (C_2), 67.8 (C_6), 63.8 (C_1), 26.6, 26.2, 24.9 (4 × CH_3); m/z (FAB) 263.14840 (M^++H), $C_{12}H_{23}O_6$ requires 263.14946.

3.3.4.7 2,3:4,5-Di-*O*-isopropylidene-*aldehydo*-D-arabinose (296)

Using this synthetic route the above compound (**296**) was prepared by oxidation of 1,2:3,4-diisopropylidene-D-mannitiol (**298**). In a well stirred and cooled flask was placed a solution of 1,2:3,4-diisopropylidene-D-mannitiol (**298**) (1.41 g, 5.37 mmol) dissolved in water (4.5 ml). A solution of sodium periodate (1.29 g, 6.03 mmol) in water (20 ml) was added, maintaining the mixture at 0-5°C. The reaction mixture was allowed to remain at this temperature for 1 hr, then saturated with sodium chloride, and extracted with chloroform (10 × 10 ml). After drying over $NaSO_4$, removing the solvent at room temperature and distillation, (**296**) was afforded as a colourless oil (0.60 g, 49%) (b.p. 80°C, 0.01 mmHg); δ_H (200 MHz, $CDCl_3$) see Section 3.3.1.3.

3.3.4.8 1,2-Dideoxy-3,4:5,6-di-*O*-isopropylidene-D-*arabino*-hex-1-enitol (293)

This was prepared in the same way as previously described in Section 3.3.4.4.

3.3.5 Methyl 5,6-dideoxy-2,3-*O*-isopropylidene-α-D-*lyxo*-hex-5-eno-furanoside (317)[227]

This alkene was prepared in four steps from D-mannose (Scheme 78).

3.3.5.1 Methyl 2,3:5,6-di-*O*-isopropylidene-α-D-*manno*-furanoside (318)

The title compound was prepared by a literature method.[227] A solution of D-mannose (25.00 g, 0.14 mol), 2,2-dimethoxypropane (85 ml), acetone (82.5 ml), methanol (82.5 ml) and concentrated hydrochloric acid (2.5 ml) was refluxed for 2 hr. The solution was cooled, water added (250 ml) and concentrated to ~ 250 ml at <30°C. The product was not isolated, but instead carried directly onto the next stage.

3.3.5.2 Methyl 2,3-*O*-isopropylidene-α-D-*manno*-furanoside (319)[227]

To the stirred solution of (**318**) prepared in Section 3.3.5.1, methanol (250 ml) and concentrated hydrochloric acid (6.25 ml) were added and the resulting solution stirred at 23°C for 200 min. The solution was neutralised by addition of sodium hydrogen carbonate solution (1 M, 160 ml) and then concentrated to remove the methanol. The resulting aqueous solution was continuously extracted with chloroform for 3 hr in a liquid-liquid extractor, the extract dried ($MgSO_4$) and then

concentrated to a syrup. This syrup was not purified, but taken directly onto the next stage.

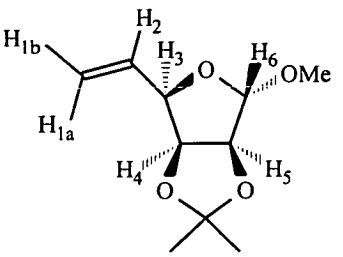
3.3.5.3 Methyl 2,3-*O*-isopropylidene-5,6-di-*O*-methylsulphonyl-α-D-*manno*-furanoside (320)[227]

Syrup (**319**) was dissolved in pyridine (125 ml) and methanesulphonyl chloride (37.5 ml) added while keeping the stirred solution below 35°C. The solution was stirred at 20°C for 2 hr and then excess methanesulphonyl chloride decomposed by slow addition of water keeping the temperature below 50°C. More water (300 ml) was added and the product filtered off, washed with water and dried. The crude product was recrystallised from ethanol to yield white needles (29.79 g, 55% from D-mannose); m.p. 145-146°C (lit.[227] 144.5-146.0°C); m/z (FAB) 391.07392 (M^++H). $C_{12}H_{23}O_{10}S_2$ requires 391.07327.

3.3.5.4 Methyl 5,6-Dideoxy-2,3-*O*-isopropylidene-α-D-*lyxo*-hex-5-eno-furanoside (317)

This alkene was prepared by reduction[228] of dimesylate (**320**). (**320**) (19.54 g, 50.10 mmol), sodium iodide (38.50 g, 0.26 mol, dried over P_2O_5), Zn/Cu couple (prepared[228] from 17.60 g of zinc powder), DMF (229 ml) and dimethoxyethane (48 ml) were stirred together at reflux for 70 min. After cooling, the mixture was poured into water (500 ml) with rapid stirring. Toluene (300 ml) was added and the mixture filtered through celite. The filter pad was washed with toluene (2 × 300 ml) and the washes used to extract the aqueous layer. The combined organics were washed with

water (2 × 200 ml), dried ($MgSO_4$) and the solvent removed *in vacuo* to produce a syrup which was purified by dry flash chromatography (silica gel, hexane/ether 80 : 20) to yield alkene (**317**) as a colourless oil (9.54 g, 89 %); δ_H (360 MHz, $CDCl_3$) see Table 82; δ_C (90 MHz, $CDCl_3$) 132.2 (C_2), 118.9 (C_1), 107.0 (C_6), 85.1 (C_3), 81.3, 81.0 (C_4, C_5), 54.5 (OMe), 25.9, 24.8 (2 × CH_3).



(**317**)

Resonance	δ_H/ppm	Coupling	*J*/Hz
1a	5.37	1a,1b	1.3
1b	5.30	1a,2	17.6
2	5.95	1b,2	10.4
3	4.35	2,3	7.4
4	4.64	3,4	3.7
5	4.54	4,5	5.9
6	4.87	5,6	-
OMe	3.31		
isopropyl.CH_3	1.43, 1.27		

Table 82

3.3.6 (6*R*,7*R*,14*S*)-14-Ethenyl-1,8,13,16-tetraoxadispiro[5.0.5.4]-hexadecane (226)

This alkene was prepared in two steps from (6*R*,7*R*,14*S*)-14-(2-hydroxyethyl)-1,8,13,16-tetraoxadispiro[5.0.5.4]hexadecane (**224**).

3.3.6.1 (6*R*,7*R*,14*S*)-14-[2-(4-Methylphenylsulphonyl)ethyl]-1,8,13,16-tetra-oxadispiro[5.0.5.4]hexadecane (225)[208]

To a stirred solution of (**224**) (300 mg, 1.10 mmol) in pyridine (0.8 ml) under argon was added at 0°C a solution of TsCl (315 mg, 1.65 mmol) in pyridine (0.19 ml). On completion of the addition, the reaction mixture was allowed to warm up to room temperature. After 3 hr the reaction was quenched by addition of water (6 ml) and then extracted with CH_2Cl_2 (3 × 50 ml). The organic extracts were washed with 10% HCl (3 × 10 ml) and water (3 × 10 ml), dried ($MgSO_4$), filtered and concentrated *in vacuo* to give (**225**) after purification by column chromatography, as an off-white crystalline solid (375 mg, 80%), m.p. 97.8-100.0°C [lit.[208] 104-105°C (Et_2O)]; δ_H (360 MHz, $CDCl_3$) 7.77 (2H, d, J = 8.3 Hz, *o*-tolyl), 7.32 (2H, d, J = 8.3 Hz, *m*-tolyl), 4.32-4.25 (1H, m, H_{18}), 4.18-4.13 (1H, m, H_{18}), 4.06-3.99 (1H, m, H_{14}), 3.65-3.43 (5H, m), 3.38 (1H, dd, $J_{15a,15e}$ = 11.2 Hz, $J_{14,15e}$ = 3.1 Hz, H_{15e}), 2.43 (3H, s, CH_3), 1.77-1.37 (14H, m); δ_C (90 MHz, $CDCl_3$) 144.7, 133.0 (*C*Ph), 129.7, 127.8 (PhCH), 96.2, 95.2 (C_6, C_7), 62.2 (C_{14}), 66.0, 62.4 (C_{17}, C_{18}), 60.7, 30.4, 28.4, 28.2, 24.8, 17.9, 17.8 (C_2, C_3, C_4, C_5, C_9, C_{10}, C_{11}, C_{12}, C_{15}), 21.5 [CH_3(Tos)]; m/z (FAB) 426.17121 (M^+), $C_{21}H_{30}O_7S$ requires 426.1708.

3.3.6.2 (6*R*,7*R*,14*S*)-14-Ethenyl-1,8,13,16-tetraoxadispiro[5.0.5.4]hexadecane (226)

To (**225**) (375 mg, 0.88 mmol) at room temperature were added potassium *tert*-butoxide (1.19 g, 10.56 mmol) and DMSO (20 ml) and the solution stirred for 2 hr. The resultant red-brown solution was added to saturated aqueous $NaHCO_3$ (35 ml) and extracted with ether (35 ml). The ethereal extract was washed with water (2 × 35 ml) and dried ($MgSO_4$), filtered, concentrated *in vacuo* and the residue purified by dry flash chromatography on silica gel, eluting with ether/ hexane (1 : 1) to give (**226**) as a colourless oil (0.63 g, 71%); δ_H (360 MHz, $CDCl_3$) see Table 83; δ_C (90 MHz, $CDCl_3$) 134.1 (C_{17}), 117.3, (C_{18}), 96.3, 95.0 (C_6, C_7), 67.8 (C_{14}), 62.0, 61.0, 60.6 (C_2, C_9, C_{15}), 29.6, 28.5, 28.3, 24.9, 24.86, 18.0, 17.8 (C_3, C_4, C_5, C_{10}, C_{11}, C_{12}); m/z (FAB) 255.15759 (M^++H), $C_{14}H_{23}O_4$ requires 255.15963.

3.3.7 trans-3,4-*Didehydro*-3,4-*Dideoxy*-1,2:5,6-*di*-O-*cyclohexylidene*-D-threo-*hex*itol (232)

This alkene was prepared in three steps from D-mannitol according to Scheme 45.

3.3.7.1 1,2:5,6-Di-*O*-cyclohexylidene-D-mannitol (193)[190]

This was prepared according to the method previously outlined in Section 3.3.2.1.

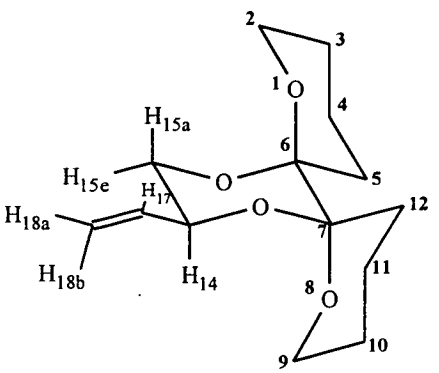
3.3.7.2 1,2:5,6-*Di*-O-*isopropylidene*-D-*mannitol*-3,4-*thionocarbonate* (231)

1,2:5,6-Di-*O*-cyclohexylidene-D-mannitol (**193**) (5.76 g, 16.90 mmol) was refluxed in toluene (80 ml) with 1,1'-thiocarbonyldiimidazole (3.00 g, 16.90 mmol) for 16 hr. After cooling the mixture, water (70 ml) was added, and the mixture extracted with dichloromethane (3 × 70 ml). The combined organic extracts were dried ($MgSO_4$) and evaporated *in vacuo* to yield a yellow oil, which was purified by dry flash chromatography (silica; hexane/ether gradient elution 10 : 0 → 1 : 4). (**231**) was afforded as white platelets (4.62 g, 71%), m.p. 130-131°C; $[\alpha]_D^{25}$ +9.1° (c = 2.04, $CHCl_3$); [Found : C, 59.3; H, 7.3. $C_{19}H_{28}O_6S$ requires C, 59.4; H, 7.3]; δ_H (360 MHz, $CDCl_3$) 4.68 (2H, dd, $J_{2,3}$ = 6.4 Hz, H_3), 4.25 (2H, ddd, $J_{1a,2}$ = 3.8 Hz, $J_{1b,2}$ = 6.7 Hz, H_2), 4.12 (1H, dd, $J_{1b,2}$ = 6.7 Hz, $J_{1a,1b}$ = 9.3 Hz, H_{1b}), 3.94 (1H, dd, $J_{1a,2}$ = 3.8 Hz, $J_{1a,1b}$ = 9.3 Hz, H_{1a}), 1.61-1.52 (20H, m, cyclohexyl. CH_2); δ_C (50 MHz, $CDCl_3$) 190.5 (C=S), 111.3 (cyclohexyl. quat.), 82.8 (C_3), 73.2 (C_2), 65.1 (C_1), 33.9, 33.7, 24.8, 23.8, 23.4 (cyclohexyl. CH_2); m/z (FAB) 385.16845 (M^++H), $C_{19}H_{29}O_6S$ requires 385.16847.

3.3.7.3 trans-3,4-*Didehydro*-3,4-*dideoxy*-1,2:5,6-*di*-O-*cyclohexylidene*-D-threo-*hexitol* (232)

(**231**) (3.60 g, 9.38 mmol) was refluxed in triethyl phosphite (14.4 ml, 84.1 mmol, 9 M eq), under nitrogen for 12 hr. After cooling, saturated sodium hydroxide solution (300 ml) was added, and the mixture extracted with ether (3 × 300 ml). The combined organic extracts were dried ($MgSO_4$) and evaporated *in vacuo* to afford a syrup which was purified by dry flash chromatography (silica, hexane/ether gradient

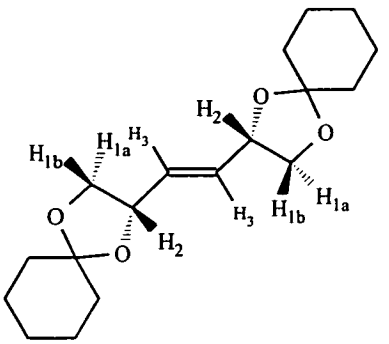
elution 10 : 0 → 6 : 4), and yielded alkene (**232**) as a colourless oil (2.75 g, 95%); $[\alpha]_D^{25}$ +24.4° (c = 2.69, $CHCl_3$); δ_H (200 MHz, $CDCl_3$) see Table 84; δ_C (50 MHz, $CDCl_3$) 131.0 (C_3), 109.9 (cyclohexyl. quat.), 75.6 (C_2), 68.8 (C_1), 36.1, 35.3, 25.0, 23.8, 23.7 (cyclohexyl. CH_2); m/z (FAB) 308.19876 (M^+), $C_{18}H_{28}O_4$ requires 308.19875.



(226)

Resonance	δ_H/ppm	Coupling	*J*/Hz
18a	5.19	18a,18b	1.5
18b	5.34	17,18a	10.6
17	5.76	17,18b	17.3
14	4.42	14,17	6.0
15a	3.70-3.62	14,15a	9.4
15e	3.45	14,15e	3.2
2a, 2e, 9a, 9e	3.70-3.62	15a,15e	11.2
3, 4, 5, 10, 11, 12	1.87-1.45		

Table 83



(232)

Resonance	δ_H/ppm	Coupling	*J*/Hz
1a	3.56	1a,1b	8.1
1b	4.05	1a,2	6.2
2	4.50	1b,2	7.6
3	5.76	2,3	3.9
cyclohexyl. CH_2	1.61-1.53		

Table 84

3.4 Nitrile Oxide Cycloaddition Reactions

General Procedure

A solution of triethylamine (1.1 M eq) in dry ether was added over 32-45 hr using a motorised syringe pump, to an ice-cooled stirred solution of alkene (1.5 M eq) and hydroximoyl chloride (1.0 M eq) in dry ether. The solution was left stirring for a further 8 hr. After filtration of the precipitated triethylamine hydrochloride, and removal *in vacuo* of the solvent, TLC of the crude reaction mixture showed evidence of either three or four spots. 10% of the crude mixture was retained for isomer ratio

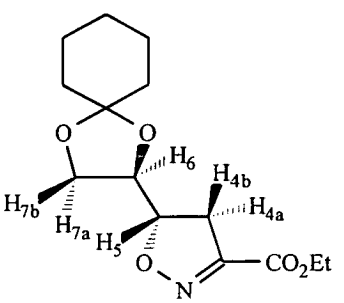
determination by ^{1}H-NMR and/or reverse phase HPLC. From the reaction mixture was isolated by chromatography on silica (gradient elution) : unreacted alkene, furazan *N*-oxide [identified by comparison (TLC) with authentic sample] and a pair of diastereomeric isoxazoline cycloadducts. In some cases these cycloadducts could not be separated by chromatography.

3.4.1 Cycloadditions of Nitrile Oxides to 1,2-Dideoxy-3,4-*O*-cyclohexylidene-D-*glycero*-but-1-enitol (195)

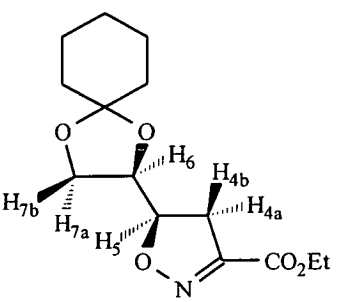
3.4.1.1 Ethoxycarbonylformonitrile Oxide

Addition of ethyl chloro-oximinoacetate (**186**) (433 mg, 2.86 mmol) to alkene (**195**) (720 mg, 4.29 mmol) was carried out utilising the general procedure outlined above. The syrup was purified by dry flash chromatography (silica; hexane/ether gradient elution 10 : 1 → 1 : 1), and afforded in order of elution, unreacted alkene (104.5 mg, 22%), 3,4-diethoxycarbonylfurazan *N*-oxide (57.3 mg, 17%) and a pair of diastereomeric isoxazoline cycloadducts (**198**) and (**199**) in a ratio 77 : 23 (*erythro* : *threo*) as determined by the ratio of the integral of the H_5 protons in the ^{1}H-NMR (360 MHz, $CDCl_3$). The major isomer was identified as 5S-5-(1,2-O-*cyclohexylidene*-D-glycero-*diitol*-1-*yl*)-3-*ethoxycarbonyl*-2-*isoxazoline* (**198**), (421 mg, 52%), white needles, m.p. 40.8-41.9°C; $[\alpha]_D^{24}$ +58.1° (c = 0.31, $CHCl_3$); [Found : C, 59.7; H, 7.7; N, 5.0. $C_{14}H_{21}NO_5$ requires C, 59.4; H, 7.4; N, 5.0]; δ_H (360 MHz, $CDCl_3$) see Table 85; δ_C (90 MHz, $CDCl_3$) 160.3 (C_3), 151.6 (C=O), 110.5 (cyclohexyl. quat.), 83.5 (C_5), 75.1 (C_6), 66.4 (C_7), 62.0 (CH_3CH_2O), 36.3 (C_4), 36.0, 34.4, 24.9, 23.8, 23.6 (cyclohexyl. CH_2), 14.0 (CH_3CH_2O); m/z (FAB) 284.14980

(M^++H), $C_{14}H_{22}NO_5$ requires 284.14979. The minor isomer was identified as 5R-5-(1,2-O-*cyclohexylidene*-D-glycero-*diitol*-1-*yl*)-3-*ethoxycarbonyl*-2-*isoxazoline* (**199**), (132 mg, 16%), cream needles, m.p. 47-49°C; $[\alpha]_D^{24}$ -153.8° (c = 0.10, $CHCl_3$); [Found : C, 59.4; H, 7.7; N, 4.6. $C_{14}H_{21}NO_5$ requires C, 59.4; H, 7.4; N,5.0]; δ_H (360 MHz, $CDCl_3$) see Table 85; δ_C (90 MHz, $CDCl_3$) 160.4 (C_3), 151.4 (C=O), 110.7 (cyclohexyl. quat.), 82.5 (C_5), 75.6 (C_6), 64.6 (C_7), 62.0 (CH_3CH_2O), 35.6 (C_4), 35.3, 34.6, 24.9, 23.7, 23.6 (cyclohexyl. CH_2), 14.0 (CH_3CH_2O); m/z (FAB) 284.14980 (M^++H), $C_{14}H_{22}NO_5$ requires 284.14979.



***Erythro* isomer (198)**



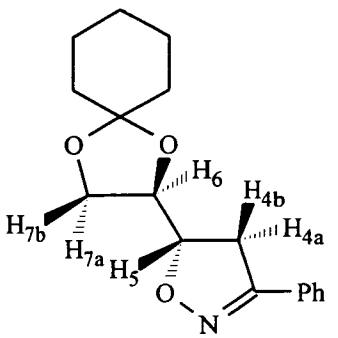
***Threo* isomer (199)**

Resonance	δ_H/ppm		Coupling	*J*/Hz	
	***Erythro* (198)**	***Threo* (199)**		***Erythro* (198)**	***Threo* (199)**
4a	3.26	3.24	4a,4b	-	17.8
4b	3.26	3.14	4a,5	8.9	11.4
5	4.70	4.83	4b,5	8.9	8.5
6	3.85	4.25	5,6	7.0	4.2
7a	4.10	4.05	6,7a	6.3	6.8
7b	4.06	3.84	6,7b	4.0	6.3
CH_3CH_2	4.33	4.33	7a,7b	7.7	8.7
CH_3CH_2	1.35	1.35	CH_3CH_2	7.1	7.1
cyclohexyl. CH_2	1.61-1.50	1.61-1.50			

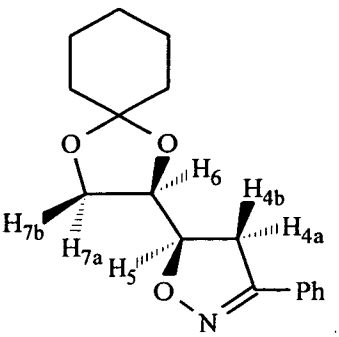
Table 85

3.4.1.2 Benzonitrile Oxide

Addition of benzohydroximoyl chloride (**185**) (2.16 g, 13.87 mmol) to alkene (**195**) (3.50 g, 20.80 mmol) was carried out utilising the general procedure outlined above. The syrup was purified by dry flash chromatography (silica; hexane/ether gradient elution 10 : 1 → 1 : 1), and afforded in order of elution, unreacted alkene (171 mg, 7%), 3,4-diphenylfurazan *N*-oxide (84 mg, 5%) and a pair of diastereomeric isoxazoline cycloadducts (**196**) and (**197**) in a ratio 79 : 21 (*erythro* : *threo*) as determined by the ratio of the integral of the H_5 protons in the ^{1}H-NMR (200 MHz, $CDCl_3$). The major isomer was identified as 5S-5-(1,2-O-*cyclohexylidene*-D-glycero-*diitol*-1-*yl*)-3-*phenyl*-2-*isoxazoline* (**196**), (2.78 g, 70%), cream needles, m.p. 61-63°C, (lit.[191] 73.5°C (after 5 recrystallisations)); $[\alpha]_D^{24}$ +23.4° (c = 0.14, $CHCl_3$); [Found : C, 71.1; H, 7.6; N, 4.7. $C_{17}H_{21}NO_3$ requires C, 71.1, H, 7.3; N, 4.9]; δ_H (200 MHz, $CDCl_3$) see Table 86; δ_C (50 MHz, $CDCl_3$) 156.5 (C_3), 129.2 (*C*Ph), 130.1, 128.6, 126.6 (5 × PhCH), 110.2 (cyclohexyl. quat.), 81.3 (C_5), 75.5 (C_6), 66.8 (C_7), 37.7 (C_4), 36.4, 34.5, 25.0, 23.9, 23.6 (cyclohexyl. CH_2); m/z (FAB) 288.15997 (M^++H), $C_{17}H_{22}NO_3$ requires 288.15996. The minor isomer was identified as 5R-5-(1,2-O-*cyclohexylidene*-D-glycero-*diitol*-1-*yl*)-3-*phenyl*-2-*isoxazoline* (**197**), (0.72 g, 18.3%), white needles, m.p. 96-98°C; $[\alpha]_D^{24}$ -39.1° (c = 0.23, $CHCl_3$); [Found : C, 70.7; H, 7.7; N, 4.7. $C_{17}H_{21}NO_3$ requires C, 71.1; H, 7.3; N, 4.9]; δ_H (200 MHz, $CDCl_3$) see Table 86; δ_C (50 MHz, $CDCl_3$) 156.3 (C_3), 129.2 (*C*Ph), 130.0, 128.5, 126.6 (5 × PhCH), 110.4 (cyclohexyl. quat.), 80.3 (C_5), 75.8 (C_6), 64.8 (C_7), 36.5 (C_4), 35.7, 34.6, 25.0, 23.8, 23.6 (cyclohexyl. CH_2); m/z (FAB) 288.15997 (M^++H), $C_{17}H_{22}NO_3$ requires 288.15996.



***Erythro* isomer (196)**



***Threo* isomer (197)**

Resonance	δ_H/ppm		Coupling	*J*/Hz	
	***Erythro* (196)**	***Threo* (197)**		***Erythro* (196)**	***Threo* (197)**
4a	3.37	3.88	4a,4b	17.0	17.1
4b	3.47	3.26	4a,5	7.3	10.7
5	4.64	4.81	4b,5	9.3	8.3
6	4.05	4.33	5,6	7.6	4.4
7a	4.14	4.08	6,7a	5.7	6.7
7b	3.95	3.88	6,7b	4.1	6.3
Ph	7.64, 7.39	7.65, 7.38	7a,7b	7.7	8.6
cyclohexyl.	1.68-1.52	1.68-1.52			

Table 86

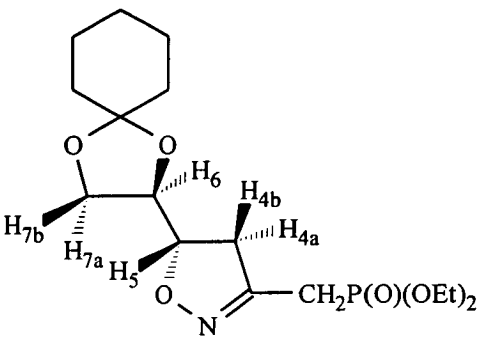
3.4.1.3 (Diethoxyphosphoryl)acetonitrile Oxide

Addition of (diethoxyphosphoryl)acetohydroximoyl chloride (**189**) (453 mg, 1.99 mmol) to alkene (**195**) (500 mg, 2.98 mmol) was carried out utilising the general procedure outlined above. The syrup was purified by dry flash chromatography (silica; hexane/ether/ethyl acetate gradient elution 10 : 0 → 1 : 1), and afforded in order of elution, unreacted alkene (100 mg, 30%), 3,4-di-(diethoxyphosphorylmethyl)-furazan-*N*-oxide (15 mg, 15%) and a pair of diastereomeric cycloadducts, 5S-5-(1,2-O-*cyclohexylidene*-D-glycero-*diitol*-1-*yl*)-3-(*diethoxyphosphorylmethyl*)-2-*isoxazoline* (**200**) and 5R-5-(1,2-O-*cyclohexylidene*-D-glycero-*diitol*-1-*yl*)-3-(*diethoxyphosphorylmethyl*)-2-*isoxazoline* (**201**), (287 mg, 40%) in a ratio 79 : 21 (*erythro* : *threo*) as determined by the ratio of the integral of the H_5 protons in the ^{1}H-NMR. The isomers were unable to be separated. δ_H (360 MHz, $CDCl_3$) see Table 87; m/z (FAB) 362.17285 (M^++H), $C_{16}H_{29}NO_6P$ requires 362.17325.

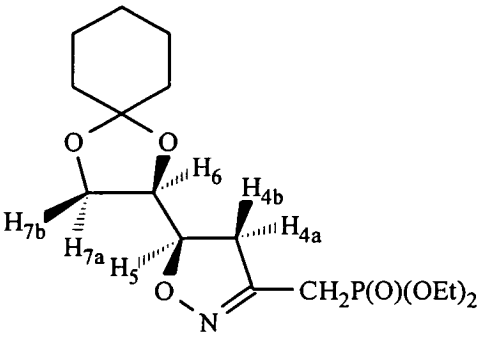
3.4.1.4 Acetonitrile Oxide

A solution of nitroethane (89 mg, 1.19 mmol) in 1,1,1-trichloroethane (44 ml) was added at room temperature to a stirred solution of alkene (**195**) (200 mg, 1.19 mmol), TDI (620 mg, 3.56 mmol) and triethylamine (60 mg, 0.59 mmol) in 1,1,1-trichloroethane (20 ml) *via* a motorised syringe pump over a period of 40 hr. The solution was cooled to 0°C, and then 1,2-diaminoethane (0.32 ml) in ether (5 ml) added over 30 min to the ice cooled solution which was then stirred for a further 30 min. The precipitated polymeric urea was filtered off through celite and the filter pad

washed with ether (5 ml) and chloroform (5 ml). The filtrate and washings were combined and reduced *in vacuo*. The residue was dissolved in ethyl acetate (10 ml) and filtered through a silica pad which was then washed with ethyl acetate (2 × 10 ml). The combined filtrate and washings were reduced *in vacuo* to afford an oil. This was purified by dry flash chromatography (silica; hexane/ether gradient elution 10 : 0 → 1 : 9), and afforded in order of elution, unreacted alkene (50 mg, 22%) and a pair of diastereomeric isoxazoline cycloadducts (**202**) and (**203**) in a ratio 82 : 18 (*erythro* : *threo*) as determined by the ratio of the integral of the H_5 protons in the ^{1}H-NMR. The major isomer was identified as 5S-5-(1,2-O-*cyclohexylidene*-D-glycero-*diitol*-1-*yl*)-3-*methyl*-2-*isoxazoline* (**202**), (87 mg, 33%), pale yellow oil; δ_H (200 MHz, $CDCl_3$) see Table 88; δ_C (90 MHz, $CDCl_3$) 155.3 (C_3), 110.0 (cyclohexyl. quat.), 80.3 (C_5), 75.6 (C_6), 66.8 (C_7), 36.4 (C_4), 34.5, 24.9, 23.8, 23.6 (cyclohexyl. CH_2), 12.9 (CH_3); m/z (FAB) 226.14432 (M^++H), $C_{12}H_{20}NO_3$ requires 226.14431. The minor isomer was identified as 5R-5-(1,2-O-*cyclohexylidene*-D-glycero-*diitol*-1-*yl*)-3-*methyl*-2-*isoxazoline* (**203**), pale yellow oil, (85 mg, 32%); δ_H (200 MHz, $CDCl_3$) see Table 88; δ_C (90 MHz, $CDCl_3$) 155.3 (C_3), 110.2 (cyclohexyl. quat.), 79.3 (C_5), 75.8 (C_6), 64.7 (C_7), 35.7 (C_4), 34.5, 24.9, 23.8, 23.6 (cyclohexyl. CH_2), 12.8 (CH_3); m/z (FAB) 225.13647 (M^+), $C_{12}H_{19}NO_3$ requires 225.13649.



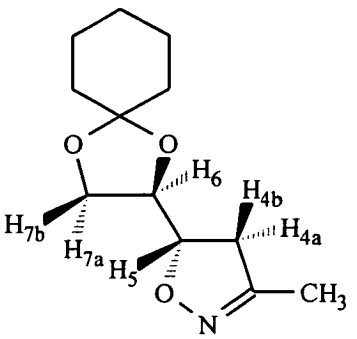
***Erythro* isomer (200)**



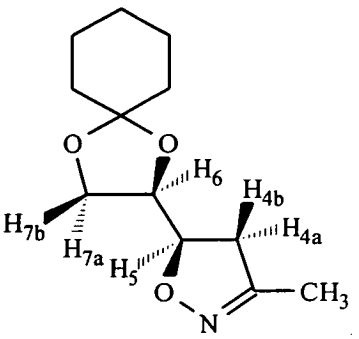
***Threo* isomer (201)**

Resonance	δ_H/ppm		Coupling	*J*/Hz	
	***Erythro* (200)**	***Threo* (201)**		***Erythro* (200)**	***Threo* (201)**
4a	3.12	3.25	4a,4b	-	-
4b	3.20	3.17	4a,5	6.5	11.1
5	4.54	4.62	4b,5	10.6	8.0
6	4.20-4.00	4.20-4.00	5,6	6.5	5.2
7a	4.20-4.00	4.20-4.00	6,7a	nd	nd
7b	4.20-4.00	4.20-4.00	6,7b	nd	nd
$CH_2P(O)$	3.04	3.04	7a,7b	nd	nd
OCH_2CH_3	nd	nd	4a, P	3.9	4.4
OCH_2CH_3	1.57	1.57	4b, P	4.4	4.3
cyclohexyl.CH_2	1.32, 1.30, 1.28	1.32, 1.30, 1.28			

Table 87



***Erythro* isomer (202)**



***Threo* isomer (203)**

Resonance	δ_H/ppm		Coupling	*J*/Hz	
	***Erythro* (202)**	***Threo* (203)**		***Erythro* (202)**	***Threo* (203)**
4a	2.91	2.94	4a,4b	17.3	17.2
4b	3.01	2.81	4a,5	6.4	10.9
5	4.41	4.59	4b,5	10.1	7.9
6	3.95	4.20	5,6	7.8	4.6
7a	4.05	4.00	6,7a	6.1	6.7
7b	3.83	3.76	6,7b	4.8	6.2
CH_3	1.32-1.30	1.32-1.30	7a,7b	8.5	8.6
cyclohexyl. CH_2	1.60-1.50	1.60-1.50			

Table 88

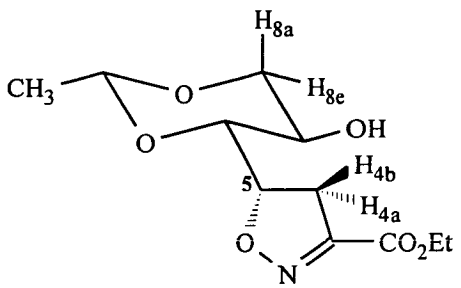
3.4.2 Cycloadditions of Nitrile Oxides to 1,2-*Dideoxy*-3,5-O-*ethylidene*-D-erythro-*pent*-1-*enitol* (260)

3.4.2.1 Ethoxycarbonylformonitrile Oxide

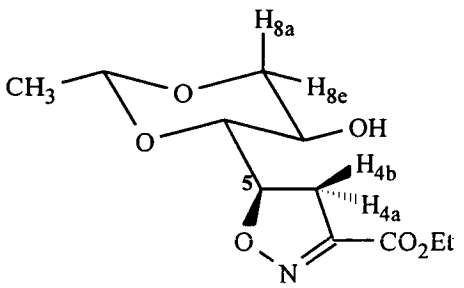
Addition of ethyl chloro-oximinoacetate (**284**) (3.25 g, 21.47 mmol) to alkene (**260**) (4.64 g, 32.20 mmol) was carried out utilising the general procedure outlined above. The syrup was purified by MPLC (silica; hexane/ether gradient elution 10 : 0 → 3.5 : 6.5 and hexane/ethyl acetate 10 : 0 → 6.5 : 3.5) and afforded in order of elution, unreacted alkene (1.67 g, 54%), 3,4-diethoxycarbonylfurazan *N*-oxide (247 mg, 10%) and a pair of diastereomeric isoxazoline cycloadducts (**263**) and (**264**) in a ratio 53 : 47 (*erythro* : *threo*) as determined by the ratio of the integral of the H_5 protons in the ^{1}H-NMR (360 MHz, $CDCl_3$). The major isomer was identified as 5S-5-(1,3-O-*ethylidene*-D-erythro-*triitol-1-yl*)-*3-ethoxycarbonyl-2-isoxazoline* (**263**), (2.30 g, 41%), white needles, m.p. 82.8-85.4°C; $[\alpha]_D^{25}$ +105.6° (c = 0.80, $CHCl_3$); [Found : C, 51.2; H, 6.2; N, 5.3. $C_{11}H_{17}NO_6$ requires C, 51.0; H, 6.6; N, 5.4]; δ_H (400 MHz, $CDCl_3$) see Table 89; δ_C (90 MHz, $CDCl_3$) 160.3 (C=O), 151.9 (C_3), 98.7 (*C*Me), 83.8 (C_5), 79.8 (C_6), 70.1 (C_8), 63.6 (C_7), 62.1 (CH_3CH_2O), 35.1 (C_4), 20.1 (CCH_3), 13.9 (CH_3CH_2O); m/z (FAB) 260.11343 (M^++H), $C_{11}H_{18}NO_6$ requires 260.11340. The minor isomer was identified as 5R-5-(1,3-O-*ethylidene*-D-erythro-*triitol*-1-*yl*)-3-*ethoxycarbonyl*-2-*isoxazoline* (**264**), (1.99 g, 36%), white needles, m.p. 99-100°C; $[\alpha]_D^{25}$ -213.5° (c = 0.56, $CHCl_3$); [Found : C, 51.0; H, 6.7; N, 5.2. $C_{11}H_{17}NO_6$ requires C, 51.0; H, 6.6; N, 5.4]; δ_H (400 MHz, $CDCl_3$) see Table 89; δ_C* (90 MHz, $CDCl_3$) 160.1 (C=O), 151.7 (C_3), 98.3 (*C*Me), 80.9 (C_5), 80.5 (C_6),

* Unambiguous assignments made by ^{1}H-^{13}C NMR correlation experiments

70.3 (C_8), 61.3 (CH_3CH_2O), 60.7 (C_7), 34.0 (C_4), 20.3 (CCH_3), 13.9 (CH_3CH_2O); m/z (FAB) 260.11343 (M^++H), $C_{11}H_{18}NO_6$ requires 260.11340. Unseparated cycloadducts (1.30 g, 23%) were also obtained.



***Erythro* isomer (263)**



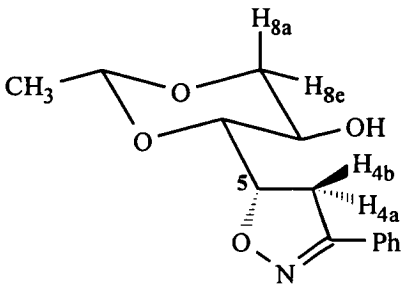
***Threo* isomer (264)**

Resonance	δ_H/ppm		Coupling	*J*/Hz	
	***Erythro* (263)**	***Threo* (264)**		***Erythro* (263)**	***Threo* (264)**
4a	3.34	3.21	4a,4b	17.8	17.6
4b	3.23	3.38	4a,5	7.7	11.7
5	5.01	5.15	4b,5	11.4	8.8
6	3.53	3.45	5,6	4.8	2.9
7	3.63	3.98	6,7	9.3	9.3
8a	3.36	3.38	7,8a	10.0	10.6
8e	4.10	4.15	7,8e	5.3	5.4
CH_3CH	4.65	4.66	8a,8e	10.9	10.7
CH_3CH	1.27	1.38	CH_3CH_2	7.1	7.2
CH_3CH_2	4.32	4.35	CH_3CH	5.0	5.0
CH_3CH_2	1.34	1.32	7,7OH	4.1	-
7OH	2.65	1.95			

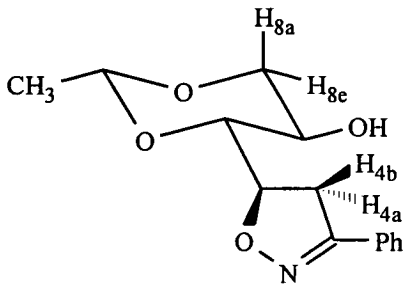
Table 89

3.4.2.2 Benzonitrile Oxide

Addition of benzohydroximoyl chloride (**185**) (3.30 g, 21.20 mmol) to alkene (**260**) (4.58 g, 31.80 mmol) was carried out utilising the general procedure outlined above. The syrup was purified by dry flash chromatography (silica; hexane/ether gradient elution 10 : 0 → 0 : 10) and afforded in order of elution, unreacted alkene (1.79 g, 59%), 3,4-diphenylfurazan *N*-oxide (30 mg, 1%) and a pair of diastereomeric isoxazoline cycloadducts (**265**) and (**266**) in a ratio 52 : 48 (*erythro* : *threo*) as determined by the ratio of the integral of the H_5 protons in the ^{1}H- NMR (360 MHz, $CDCl_3$). The combined yield of isoxazolines was 75%. The major isomer was identified as 5S-5-(1,3-O-*ethylidene*-D-erythro-*triitol*-1-*yl*)-3-*phenyl*-2-*isoxazoline* (**265**), (2.50 g, 45%), white needles, m.p. 164.8-166.2°C; $[\alpha]_D^{25}$ +47.2° (c = 0.17, $CHCl_3$); [Found : C, 63.8; H, 6.7; N, 5.1. $C_{14}H_{17}NO_4$ requires C, 63.9; H, 6.5; N, 5.3]; δ_H (360 MHz, $CDCl_3$) see Table 90; δ_C (90 MHz, $CDCl_3$) 157.2 (C_3), 130.4, 128.7, 128.66, 126.8 (5 × PhCH), 98.8 (*C*Me), 82.4 (C_5), 77.3 (C_6), 69.9 (C_8), 65.0 (C_7), 38.1 (C_4), 20.2 (CH_3); m/z (FAB) 264.12358 (M^++H), $C_{14}H_{18}NO_4$ requires 264.12357. The minor isomer was identified as 5R-5-(1,3-O-*ethylidene*-D-erythro-*triitol*-1-*yl*)-3-*phenyl*-2-*isoxazoline* (**266**). Pure minor isomer was not isolated. (NMR data are obtained from spectra containing both isomers). δ_H (360 MHz, $CDCl_3$) see Table 90; δ_C (90 MHz, $CDCl_3$) 157.2 (C_3), 130.3, 129.1, 128.8, 126.7 (5 × PhCH), 99.1 (*C*Me), 80.4 (C_5), 78.8 (C_6), 70.4 (C_8), 61.4 (C_7), 35.6 (C_4), 20.2 (CH_3); m/z (FAB) 264.12306 (M^++H), $C_{14}H_{18}NO_4$ requires 264.12358.



***Erythro* isomer (265)**



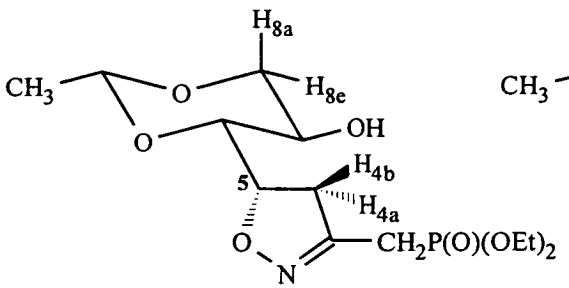
***Threo* isomer (266)**

Resonance	δ_H/ppm		Coupling	*J*/Hz	
	***Erythro* (265)**	***Threo* (266)**		***Erythro* (265)**	***Threo* (266)**
4a	3.44	3.34	4a,4b	17.5	16.7
4b	3.45	3.45	4a,5	7.1	11.5
5	4.89	5.07	4b,5	11.5	8.6
6	3.45	3.53	5,6	5.3	3.2
7	3.77	3.94	6,7	8.7	9.9
8a	3.38	3.46	7,8a	10.2	9.9
8e	4.15	4.14	7,8e	5.5	5.4
CH_3CH	4.66	4.66	8a,8e	11.0	10.8
CH_3CH	1.30	1.27	CH_3CH	5.0	5.0
Ph	7.68-7.37	7.67-7.36	7,7OH	nd	nd
7OH	3.10	3.10			

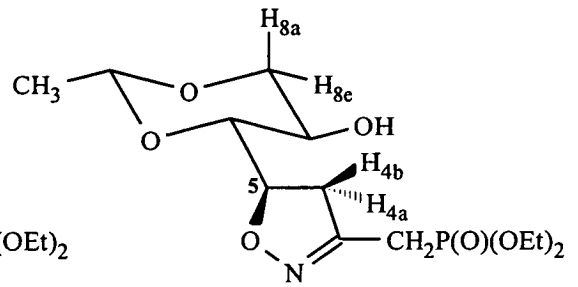
Table 90

3.4.2.3 (Diethoxyphosphoryl)acetonitrile Oxide

Addition of (diethoxyphosphoryl)acetohydroximoyl chloride (**189**) (4.22 g, 18.53 mmol) to alkene (**195**) (4.00 g, 27.78 mmol) was carried out utilising the general procedure outlined above. The syrup was purified by MPLC (silica; hexane/ether/methanol gradient elution 10 : 0 → 0 : 10) and afforded in order of elution, unreacted alkene (1.40 g, 53%), 3,4-di-(diethoxyphosphorylmethyl)-furazan *N*-oxide (150 mg, 5%) and a pair of diastereomeric cycloadducts, 5S-5-(1,3-O-*ethylidene*-D-erythro-*triitol*-1-*yl*)-3-(*diethoxyphosphorylmethyl*)-2-*isoxazoline* (**267**) and 5R-5-(1,3-O-*ethylidene*-D-erythro-*triitol*-1-*yl*)-3-(*diethoxyphosphorylmethyl*)-2-*isoxazoline* (**268**), in a ratio 50 : 50 (*erythro* : *threo*) as determined by the ratio of the integral of the H_5 protons in the ^{1}H-NMR (360 MHz, $CDCl_3$). The combined yield of isomers was 38% and they were unable to be separated. δ_H (360 MHz, $CDCl_3$) see Table 91; m/z (FAB) 338.13686 (M^++H), $C_{13}H_{25}NO_7P$ requires 338.13685.



***Erythro* isomer (276)**



***Threo* isomer (268)**

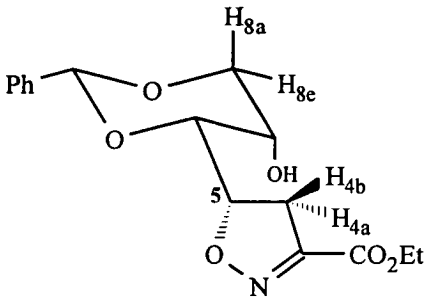
Resonance	δ_H/ppm		Coupling	*J*/Hz	
	***Erythro* (267)**	***Threo* (268)**		***Erythro* (267)**	***Threo* (268)**
4a	3.20-2.85	3.20-2.85	4a,4b	nd	nd
4b	3.20-2.85	3.20-2.85	4a,5	6.2	11.4
5	4.83	4.95	4b,5	11.4	8.2
6	3.42	3.48	5,6	6.2	3.4
7	3.86	3.73	6,7	9.6	9.4
8a	nd	nd	7,8a	9.3	9.8
8e	3.32	3.15	7,8e	4.2	4.2
CH_3CH	4.67	4.67	8a,8e	11.0	11.2
CH_3CH	1.37-1.25	1.37-1.25	CH_3CH	5.0	5.0
CH_3CH_2O	4.19-4.10	4.19-4.10	CH_2P	21.8	21.8
CH_3CH_2O	1.37-1.25	1.37-1.25			
$CH_2P(O)$	2.96	2.96			

Table 91

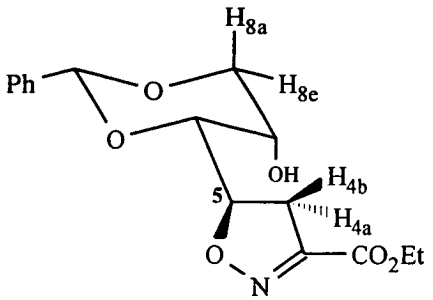
3.4.3 Cycloadditions of Nitrile Oxides to 1,2-*Dideoxy*-3,5-O-*benzylidene*-L-threo-*pent*-1-*enitol* (286)

3.4.3.1 Ethoxycarbonylformonitrile Oxide

Addition of ethyl chloro-oximinoacetate (**184**) (74 mg, 0.49 mmol) to alkene (**286**) (150 mg, 0.73 mmol) was carried out utilising the general procedure outlined above. The syrup was purified by dry flash chromatography (silica; hexane/ether gradient elution 10 : 0 → 0 : 10) and afforded in order of elution, unreacted alkene (30 mg, 30%) and a pair of diastereomeric isoxazoline cycloadducts (**291**) and (**292**) in a ratio 83 : 17 (*erythro* : *threo*) as determined by the ratio of the integral of the H_6 protons in the ^{1}H-NMR (360 MHz, $CDCl_3$). [82 : 18 ratio measured by reverse phase HPLC (55% H_2O/MeOH)]. The major isomer was identified as 5S-5-(1,3-O-*benzylidene*-L-threo-*triitol*-1-*yl*)-3-*ethoxycarbonyl*-2-*isoxazoline* (**191**), (89 mg, 57%), white needles; δ_H (360 MHz, $CDCl_3$) see Table 92; m/z (FAB) 322.12908 (M^++H), $C_{16}H_{20}NO_6$ requires 322.12905. The minor isomer was identified as 5R-5-(1,3-O-*benzylidene*-L-threo-*triitol*-1-*yl*)-3-*ethoxycarbonyl*-2-*isoxazoline* (**292**), (19 mg, 12%), pale yellow oil; δ_H (360 MHz, $CDCl_3$) see Table 92; m/z (FAB) 322.12908 (M^++H), $C_{16}H_{20}NO_6$ requires 322.12905.



***Erythro* isomer (291)**



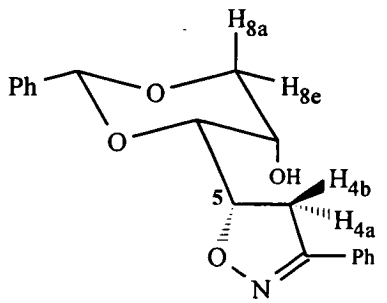
***Threo* isomer (292)**

Resonance	δ_H/ppm		Coupling	*J*/Hz	
	***Erythro* (291)**	***Threo* (292)**		***Erythro* (291)**	***Threo* (292)**
4a	3.35	3.33	4a,4b	18.2	17.7
4b	3.26	3.13	4a,5	7.3	11.4
5	5.07	5.06	4b,5	10.9	8.9
6	3.88	3.98	5,6	7.5	7.5
7	3.78	3.63	6,7	1.3	1.2
8a	4.24	4.23	7,8a	1.9	2.0
8e	4.04	4.06	7,8e	1.3	1.4
PhC*H*	5.57	5.59	8a,8e	12.1	12.1
Ph	7.49-7.34	7.99-7.31	CH_3CH_2	7.1	7.2
CH_3CH_2	4.33	4.34			
CH_3CH_2	1.35	1.35			
7OH	2.76	~2.9			

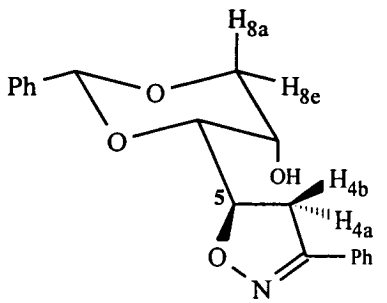
Table 92

3.4.3.2 Benzonitrile Oxide

Addition of benzohydroximoyl chloride (**185**) (37 mg, 0.24 mmol) to alkene (**286**) (75 mg, 0.36 mmol) was carried out utilising the general procedure outlined above. The syrup was purified by dry flash chromatography (silica; hexane/ether gradient elution 10 : 0 → 0 : 10) and afforded in order of elution, unreacted alkene (13 mg, 26%) and a pair of diastereomeric isoxazoline cycloadducts (**289**) and (**290**) in a ratio 81 : 19 (*erythro* : *threo*) as determined by reverse phase HPLC (45% H_2O/MeOH). The major isomer was identified as 5S-5-(1,3-O-*benzylidene*-L-threo-*triitol*-1-*yl*)-3-*phenyl*-2-*isoxazoline* (**289**), (39 mg, 50%), white needles, m.p. 152.6-153.9°C; [Found : C, 69.7; H, 5.6; N, 4.3. $C_{19}H_{19}NO_4$ requires C, 70.2; H, 5.9; N, 4.3]; δ_H (360 MHz, $CDCl_3$) see Table 93; δ_C (90 MHz, $CDCl_3$) 156.9 (C_3), 130.3-125.8 (5 × PhCH), 101.4 (*C*Me), 79.2 (C_5), 78.0 (C_6), 72.3 (C_8), 63.2 (C_7), 37.1 (C_4); m/z (EI) 325.13140 (M^+), $C_{19}H_{19}NO_4$ requires 325.1314. The minor isomer was identified as 5R-5-(1,3-O-*benzylidene*-L-threo-*triitol*-1-*yl*)-3-*phenyl*-2-*isoxazoline* (**290**), (9 mg, 12%), pale yellow oil; δ_H (360 MHz, $CDCl_3$) see Table 93; δ_C (90 MHz, $CDCl_3$) 134.3 (C_3), 130.3-125.9 (5 × PhCH), 101.3 (*C*Me), 80.9 (C_5), 80.5 (C_6), 72.5 (C_8), 62.0 (C_7), 37.1 (C_4); m/z (FAB) 326.13926 (M^++H), $C_{19}H_{20}NO_4$ requires 326.13922.



***Erythro* isomer (289)**



***Threo* isomer (290)**

Resonance	δ_H/ppm		Coupling	*J*/Hz	
	Erythro (289)	*Threo* (290)		*Erythro* (289)	*Threo* (290)
4a	3.46	3.49	4a,4b	17.1	16.7
4b	3.45	3.26	4a,5	7.7	10.8
5	5.06	5.03	4b,5	9.2	8.4
6	3.86	4.03	5,6	8.4	7.4
7	3.90	3.91	6,7	1.1	1.2
8a	4.28	4.25	7,8a	1.9	2.0
8e	4.07	4.08	7,8e	1.2	1.3
PhC*H*	5.58	5.62	8a,8e	12.0	12.0
Ph	7.71-7.36	7.70-7.36	7,7OH	11.2	10.8
7OH	2.72	2.96			

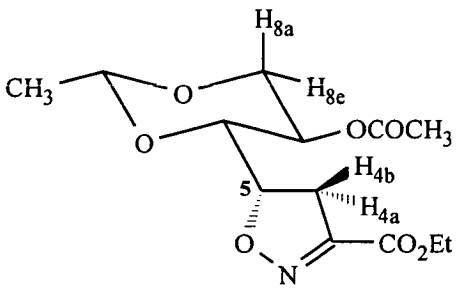
Table 93

3.4.4 Cycloadditions of Nitrile Oxides to 1,2-*Dideoxy*-4-O-*acetal*-3,5-O-*ethylidene*-D-erythro-*pent*-1-*enitol* (271)

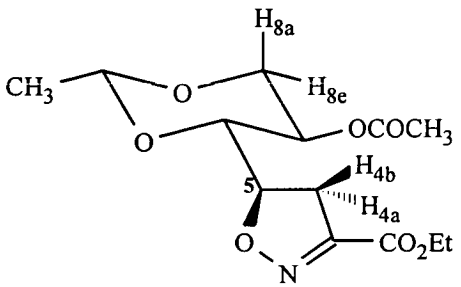
3.4.4.1 Ethoxycarbonylformonitrile Oxide

Addition of ethyl chloro-oximinoacetate (**184**) (163 mg, 1.07 mmol) to alkene (**271**) (300 mg, 1.61 mmol) was carried out utilising the general procedure outlined above. The syrup was purified by dry flash chromatography (silica; hexane/ether gradient elution 10 : 0 → 0 : 10) and afforded in order of elution, unreacted alkene (120 mg, 60%), 3,4-diethoxycarbonylfurazan *N*-oxide (17 mg, 12%) and a pair of diastereomeric isoxazoline cycloadducts (**274**) and (**275**) in a ratio 59 : 41 (*erythro* : *threo*) as determined by the ratio of the integral of the H_6 protons in the ^{1}H-NMR (360 MHz, $CDCl_3$). The major isomer was identified as 5S-5-(2-O-*acetyl*-1,3-O-*ethylidene*-D-erythro-*triitol*-1-*yl*)-3-*ethoxycarbonyl*-2-*isoxazoline* (**274**), (90 mg, 28%), white needles, as prepared by acetylation of 5S-5-(1,3-*ethylidene*-D-erythro-*triitol*-1-*yl*)-3-*ethoxycarbonyl*-2-*isoxazoline* (**263**) (57 mg, 99%), m.p. 72-7°C; $[\alpha]_D^{25}$ + 68.6° (c = 0.68, $CHCl_3$); [Found : C, 51.4; H, 6.5; N, 4.6. $C_{13}H_{19}NO_7$ requires C, 51.8; H, 6.3; N, 4.7]; δ_H (360 MHz, $CDCl_3$) see Table 94; δ_C (90 MHz, $CDCl_3$) 169.8 (CH_3C=O), 160.3 (C=O), 151.4 (C_3), 98.9 (*C*Me), 82.5 (C_5), 77.5 (C_6), 67.3 (C_8), 63.4 (CH_3CH_2O), 61.9 (C_7), 33.6 (C_4), 20.6 (CH_3C=O), 20.1 (CCH_3), 14.0 (CH_3CH_2O); m/z (FAB) 302.12395 (M^++H), $C_{13}H_{20}NO_7$ requires 302.12396. The minor isomer was identified as 5R-5-(2-O-*acetyl*-1,3-O-*ethylidene*-D-erythro-*triitol*-1-*yl*)-3-*ethoxycarbonyl*-2-*isoxazoline* (**275**), (58 mg, 18%), white needles, as prepared by acetylation of 5R-5-(1,3-*ethylidene*-D-erythro-*triitol*-1-*yl*)-3-*ethoxycarbonyl*-2-*isoxazoline* (**264**) (57 mg, 98%), m.p. 68.5-70.5°C; $[\alpha]_D^{25}$ -224.0°

(c = 1.19, $CHCl_3$); [Found : C, 51.8; H, 6.7; N, 4.5. $C_{13}H_{19}NO_7$ requires C, 51.8; H, 6.3; N, 4.7]; δ_H (360 MHz, $CDCl_3$) see Table 94; δ_C (90 MHz, $CDCl_3$) 169.3 (CH_3C=O), 160.4 (C=O), 151.3 (C_3), 99.4 (*C*Me), 80.1 (C_5), 78.1 (C_6), 67.2 (C_8), 63.0 (CH_3CH_2O), 61.9 (C_7), 34.1 (C_4), 20.6 (CH_3C=O), 20.1 (CCH_3), 13.9 (CH_3CH_2O); m/z (FAB) 302.12395 (M^++H), $C_{13}H_{20}NO_7$ requires 302.12396.



***Erythro* isomer (274)**



***Threo* isomer (275)**

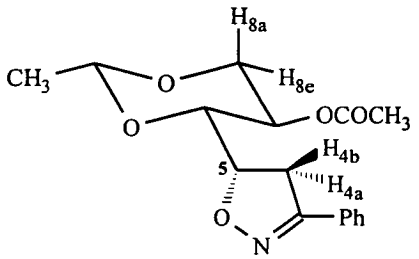
Resonance	δ_H/ppm		Coupling	*J*/Hz	
	Erythro (274)	*Threo* (275)		*Erythro* (274)	*Threo* (275)
4a	3.38	3.16	4a,4b	17.7	17.5
4b	3.15	3.24	4a,5	8.3	11.4
5	4.79	4.90	4b,5	11.7	8.6
6	3.80	3.61	5,6	3.2	2.6
7	4.69	4.96	6,7	9.9	9.8
8a	3.44	3.32	7,8a	10.1	9.9
8e	4.13	4.28	7,8e	5.4	5.3
CH_3CH	4.69	4.66	8a,8e	10.8	10.7
CH_3CH	1.28	1.28	CH_3CH_2	7.1	7.1
CH_3CH_2	4.31	4.30	CH_3CH	5.0	5.0
CH_3CH_2	1.33	1.32			
CH_3CO	2.02	2.02			

Table 94

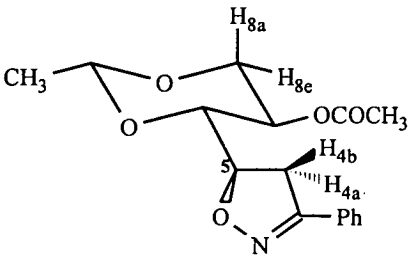
3.4.4.2 Benzonitrile Oxide

Addition of benzohydroximoyl chloride (**185**) (178 mg, 1.15 mmol) to alkene (**271**) (319 mg, 1.72 mmol) was carried out utilising the general procedure outlined above. The syrup was purified by dry flash chromatography (silica; hexane/ether gradient elution 10 : 0 → 0 : 10) and afforded in order of elution, unreacted alkene (93 mg, 44%), 3,4-diphenylfurazan *N*-oxide (5 mg, 4%) and a pair of diastereomeric isoxazoline cycloadducts (**272**) and (**273**) in a ratio 63 : 37 (*erythro* : *threo*) as determined by the ratio of the integral of the H_6 protons in the ^{1}H-NMR (360 MHz, $CDCl_3$). [65 : 35 ratio measured by reverse phase HPLC (60% H_2O/MeOH)]. The major isomer was identified as 5S-5-(2-O-*acetyl*-1,3-O-*ethylidene*-D-erythro-*triitol*-1-*yl*)-3-*phenyl*-2-*isoxazoline* (**272**), (157 mg, 45%), white needles, m.p. 92-92.6°C; $[\alpha]_D^{25}$ + 41.5° (c = 0.65, $CHCl_3$); [Found : C, 62.9; H, 6.4; N, 4.4. $C_{16}H_{19}NO_5$ requires C, 63.0; H, 6.2; N, 4.6]; δ_H (360 MHz, $CDCl_3$) see Table 95 ; δ_C (90 MHz, $CDCl_3$) 169.8 (CH_3C=O), 156.3 (C_3), 129.9, 129.2, 128.5, 126.6 (5 × PhCH), 98.9 (*C*Me), 80.2 (C_5), 77.9 (C_6), 67.3 (C_8), 63.8 (C_7), 35.1 (C_4), 20.6 (CH_3C=O), 20.1 (CCH_3); m/z (FAB) 306.13415 (M^++H), $C_{16}H_{20}NO_5$ requires 306.13414. The minor isomer was identified as 5R-5-(2-O-*acetyl*-1,3-O-*ethylidene*-D-erythro-*triitol*-1-*yl*)-3-*phenyl*-2-*isoxazoline* (**273**), (93 mg, 27%), white needles, m.p. 130.1-130.9°C; $[\alpha]_D^{25}$ -200.0° (c = 0.34, $CHCl_3$); [Found : C, 63.0; H, 6.4; N, 4.3. $C_{16}H_{19}NO_5$ requires C, 63.0; H, 6.2; N, 4.6]; δ_H (360 MHz, $CDCl_3$) see Table 95 ; δ_C (90 MHz, $CDCl_3$) 169.3 (CH_3C=O), 156.3 (C_3), 129.9, 129.3, 128.5, 126.5 (5 × PhCH), 99.4 (*C*Me), 78.2 (C_5), 78.2 (C_6), 67.3 (C_8), 63.3 (C_7), 35.5 (C_4), 20.6

(CH_3C=O), 20.1 (CCH_3); m/z (FAB) 306.13415 (M^++H), $C_{16}H_{20}NO_5$ requires 306.13414.



***Erythro* isomer (272)**



***Threo* isomer (273)**

Resonance	δ_H/ppm		Coupling	*J*/Hz	
	***Erythro* (272)**	***Threo* (273)**		***Erythro* (272)**	***Threo* (273)**
4a	3.51	3.32	4a,4b	16.6	16.4
4b	3.32	3.41	4a,5	7.9	11.2
5	4.77	4.88	4b,5	11.2	8.4
6	3.84	3.71	5,6	3.2	2.9
7	4.82	5.04	6,7	9.8	9.7
8a	3.46	3.37	7,8a	10.2	10.0
8e	4.16	4.31	7,8e	5.4	5.4
CH_3CH	4.72	4.69	8a,8e	10.7	10.6
CH_3CH	1.28	1.29	CH_3CH	5.0	5.0
Ph	7.67-7.36	7.65-7.36			
CH_3CO	2.00	2.04			

Table 95

3.4.5 Cycloadditions of Nitrile Oxides to 1,2-*Dideoxy*-D-erythro-*pent*-1-*enitol* (276)

3.4.5.1 Ethoxycarbonylformonitrile Oxide

Addition of ethyl chloro-oximinoacetate (**184**) (53 mg, 0.35 mmol) to alkene (**276**) (61 mg, 0.52 mmol) was carried out utilising the general procedure outlined above. The syrup was purified by dry flash chromatography (silica; ethyl acetate/ethanol gradient elution 10 : 0 → 9 : 1) and afforded in order of elution, unreacted alkene (18 mg, 45%), 3,4-diethoxycarbonylfurazan *N*-oxide (3 mg, 7%) and a pair of diastereomeric isoxazoline cycloadducts 5S-5-(D-erythro-*triitol*-1-*yl*)-3-*ethoxycarbonyl*-2-*isoxazoline* (**277**) and 5R-5-(D-erythro-*triitol*-1-*yl*)-3-*ethoxy-carbonyl*-2-*isoxazoline* (**278**) (24 mg, 30%) in a ratio 55 : 45 (*erythro* : *threo*) as determined by ^{1}H-NMR [360 MHz, $(CD_3)_2CO$]. These adducts are identical to those obtained by deprotection of 5S-5-(1,3-O-*ethylidene*-D-erythro-*triitol*-1-*yl*)-3-*ethoxycarbonyl*-2-*isoxazoline* (**263**) (Section 3.7.8) and 5R-5-(1,3-O-*ethylidene*-D-erythro-*triitol*-1-*yl*)-3-*ethoxycarbonyl*-2-*isoxazoline* (**264**) (Section 3.7.9).

3.4.5.2 Benzonitrile Oxide

Addition of benzohydroximoyl chloride (**185**) (132 mg, 0.85 mmol) to alkene (**276**) (150 mg, 1.27 mmol) was carried out utilising the general procedure outlined above. The syrup was purified by dry flash chromatography (silica; ethyl acetate/ethanol gradient elution 10 : 0 → 9 : 1) and afforded in order of elution, unreacted alkene (50 mg, 50%), 3,4-diphenylfurazan *N*-oxide (10 mg, 10%) and a pair of diastereomeric isoxazoline cycloadducts 5S-5-(D-erythro-*triitol*-1-*yl*)-3-

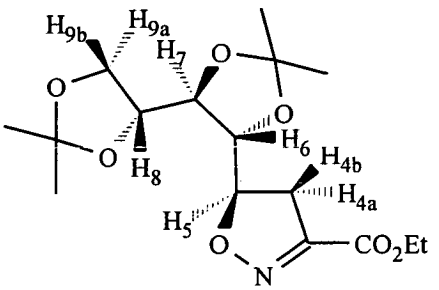
phenyl-2-*isoxazoline* (**279**) and 5R-5-(D-erythro-*triitol*-1-*yl*)-3-*phenyl*-2-*isoxazoline* (**280**) (50 mg, 25%) in a ratio 62 : 38 (*erythro* : *threo*) as determined ^{1}H-NMR [360 MHz, $(CD_3)_2CO$] The major adduct is identical to that obtained by deprotection of 5R-5-(1,3-O-*ethylidene*-D-erythro-*triitol*-1-*yl*)-3-*phenyl*-2-*isoxazoline* (**265**) (Section 3.7.12).

3.4.6 Cycloadditions of Nitrile Oxides to 1,2-Dideoxy-3,4:5,6-di-*O*-isopropylidene-D-*arabino*-hex-1-enitol (293)

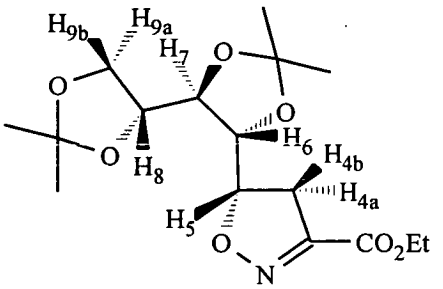
3.4.6.1 Ethoxycarbonylformonitrile Oxide

Addition of ethyl chloro-oximinoacetate (**184**) (209 mg, 1.38 mmol) to alkene (**293**) (472 mg, 2.07 mmol) was carried out utilising the general procedure outlined above. The syrup was purified by dry flash chromatography (silica; hexane/ether gradient elution 10 : 0 → 1 : 1), and afforded in order of elution, unreacted alkene (138 mg, 44%), 3,4-diethoxycarbonylfurazan *N*-oxide (38 mg, 24%) and a pair of diastereomeric isoxazoline cycloadducts 5R-5-(1,2:3,4-*di*-O-*isopropylidene*-D-arabino-*tetritol*-1-*yl*)-3-*ethoxycarbonyl*-2-*isoxazoline* (**301**) and 5S-5-(1,2:3,4-*di*-O-*isopropylidene*-D-arabino-*tetritol*-1-*yl*)-3-*ethoxycarbonyl*-2-*isoxazoline* (**302**) [418 mg (combined yield), 88%] which could not be separated. The ratio of (**301**) to (**302**) was 62.5 : 37.5 (*erythro* : *threo*) as determined by ^{1}H-NMR. NMR data have been obtained from the mixed isomer spectrum. δ_H (360 MHz, $CDCl_3$) see Table 96; δ_C (62 MHz, $CDCl_3$) 160.4 (C_3, *threo*), 160.2 (C_3, *erythro*), 152.0 (*C*=O, *erythro*), 151.5 (*C*=O, *threo*), 110.1 [*C*$(Me)_2$, *erythro*], 109.8 [*C*$(Me)_2$, *threo*], 83.5, 79.2, 78.9, 72.4 (C_5, C_6, C_7, C_8, *erythro*), 83.0, 81.7, 80.7, 72.7 (C_5, C_6, C_7, C_8, *threo*), 63.7 (C_9,

erythro), 63.5 (C_9, *threo*), 62.1 (CH_3CH_2O, *erythro*), 62.0 (CH_3CH_2O, *threo*), 35.9 (C_4, *threo*), 35.1 (C_4, *erythro*), 29.6, 26.9 [$C(CH_3)_2$, *erythro*], 27.2, 26.3 [$C(CH_3)_2$, *threo*], 14.0 (CH_3CH_2O); m/z (FAB) 344.17255 (M^++H), $C_{16}H_{26}NO_7$ requires 344.17093.



***Erythro* isomer (301)**



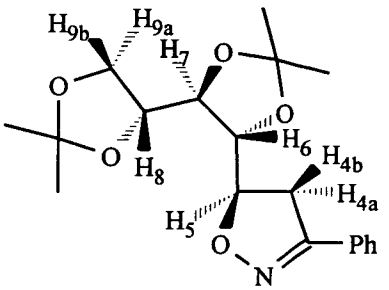
***Threo* isomer (302)**

Resonance	δ_H/ppm		Coupling	*J*/Hz	
	***Erythro* (301)**	***Threo* (302)**		***Erythro* (301)**	***Threo* (302)**
4a	3.15	3.28	4a,4b	17.5	-
4b	3.32	3.26	4a,5	11.7	8.6
5	5.06	4.92	4b,5	8.4	11.0
6	4.20	4.40-3.96	5,6	3.0	2.2
7	4.40-3.96	4.40-3.96	6,7	7.4	nd
8	4.40-3.96	4.40-3.96	7,8	nd	nd
9a	3.91	4.06	8,9a	4.9	4.7
9b	4.12	4.14	8,9b	6.2	6.0
CH_3CH_2	4.34	4.34	9a,9b	8.4	8.4
CH_3CH_2	1.42-1.32	1.42-1.32	CH_3CH_2	7.1	7.1
isopropyl.CH_3	1.37-1.35	1.37-1.35			

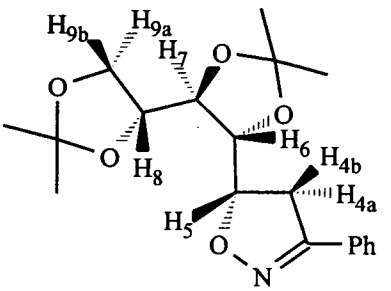
Table 96

3.4.6.2 Benzonitrile Oxide

Addition of benzohydroximoyl chloride (**185**) (595 mg, 3.83 mmol) to alkene (**293**) (1.31 g, 5.74 mmol) was carried out utilising the general procedure outlined above. The syrup was purified by dry flash chromatography (silica; hexane/ether gradient elution 10 : 0 → 1 : 1), and afforded in order of elution, unreacted alkene (380 mg, 44%), 3,4-diphenylfurazan *N*-oxide (12 mg, 26%) and a pair of diastereomeric isoxazoline cycloadducts 5R-5-(1,2:3,4-*di*-O-*isopropylidene*-D-arabino-*tetritol*-1-*yl*)-3-*phenyl*-2-*isoxazoline* (**299**) and 5S-5-(1,2:3,4-*di*-O-*isopropylidene*-D-arabino-*tetritol*-1-*yl*)-3-*phenyl*-2-*isoxazoline* (**300**) [791 mg (combined yield), 60%] which could not be separated. The ratio of (**299**) to (**300**) was 67 : 33 (*erythro* : *threo*) as determined by ^{1}H-NMR. A small sample of pure major isomer (5*R*) was obtained after repeated recrystallisation from ether, m.p. 131.6-133.3°C; [Found : C, 65.4; H, 7.2; N, 4.1. $C_{19}H_{25}NO_5$ requires C, 65.7; H, 7.2; N, 4.0]. NMR and mass spectrometry data have been obtained from the mixed isomer spectrum. δ_H (360 MHz, $CDCl_3$) see Table 97; δ_C (50 MHz, $CDCl_3$) 156.4 (C_3, *erythro*), 156.2 (C_3, *threo*), 110.1, 109.9, 109.6 [*C*(Me)$_2$], 81.9 (C_5, *threo*), 80.7 (C_5, *erythro*), 80.5, 79.4, 78.9, 77.5, 77.4, 76.9, 76.6, 76.2 (C_6, C_7, C_8), 67.8 (C_9, *threo*), 67.2 (C_9, *erythro*), 37.1 (C_4, *threo*), 35.6 (C_4, *erythro*), 29.6, 29.2, 29.0, 27.1, 26.7, 26.3, 25.1 [C(*C*H$_3$)$_2$]; m/z (FAB) 348.18107 (M^++H), $C_{19}H_{26}NO_5$ requires 348.19108.



***Erythro* isomer (299)**



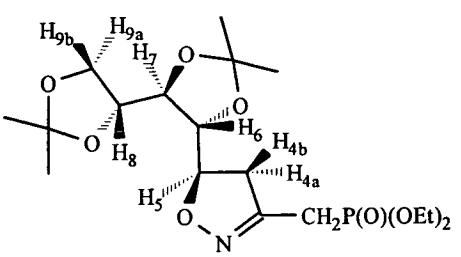
***Threo* isomer (300)**

Resonance	δ_H/ppm		Coupling	*J*/Hz	
	***Erythro* (299)**	***Threo* (300)**		***Erythro* (299)**	***Threo* (300)**
4a	3.34	3.47	4a,4b	16.5	16.5
4b	3.45	3.38	4a,5	11.2	7.9
5	4.98	4.87	4b,5	7.8	10.8
6	4.01	4.19	5,6	3.9	3.8
7	4.16-4.04	4.16-4.04	6,7	8.2	7.1
8	4.16-4.04	4.16-4.04	7,8	nd	nd
9a	4.16-3.78	4.16-3.78	8,9a	nd	nd
nd	4.16-3.78	4.16-3.78	8,9b	nd	nd
Ph	7.66, 7.39	7.66, 7.39	9a,9b	nd	nd
isopropyl. CH_3	1.46-1.28	1.46-1.28			

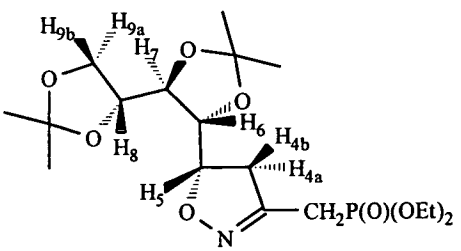
Table 97

3.4.6.3 (Diethoxyphosphoryl)acetonitrile Oxide

Addition of (diethoxyphosphoryl)acetohydroximoyl chloride (**189**) (1.00 g, 4.39 mmol) to alkene (**293**) (1.50 g, 6.58 mmol) was carried out utilising the general procedure outlined above. The syrup was purified by MPLC (silica; hexane/ether/ethyl acetate gradient elution 10 : 0 → 0 : 10), and afforded in order of elution, unreacted alkene (300 mg, 30%), 3,4-di-(diethoxyphosphorylmethyl)-furazan *N*-oxide (106 mg, 15%) and a pair of diastereomeric isoxazoline cycloadducts (**303**) and (**304**) (554 mg, 30%) which could not be separated. The ratio of (**303**) to (**304**) was 63 : 37 (*erythro* : *threo*) as determined by ^{1}H-NMR. NMR data have been obtained from the mixed isomer spectrum. δ_H (360 MHz, $CDCl_3$) see Table 98; m/z (FAB) 422.19437 (M^++H), $C_{18}H_{33}NO_8P$ requires 422.19436.



***Erythro* isomer (303)**



***Threo* isomer (304)**

Resonance	δ_H/ppm		Coupling	*J*/Hz	
	Erythro (303)	*Threo* (304)		*Erythro* (303)	*Threo* (304)
4a	3.03*	2.88+	4a,4b	15.0	15.0
4b	2.94+	2.99*	4a,5	10.6	8.4
5	4.85	4.75	4b,5	8.4	10.8
CH_2CH_3	4.31-4.10	4.31-4.10	5,6	3.8	2.4
CH_2CH_3	1.43-1.29	1.43-1.29			
isopropyl. CH_3	1.43-1.29	1.43-1.29			

Table 98

*, + Alternative assignments

3.4.7 Cycloadditions of Nitrile Oxides to Methyl 5,6-dideoxy-2,3-*O*-isopropylidene-α-D-*lyxo*-hex-5-enofuranoside (317)

3.4.7.1 Ethoxycarbonylformonitrile Oxide

Addition of ethyl chloro-oximinoacetate (**184**) (2.27 g, 15.00 mmol) to alkene (**317**) (4.50 g, 22.50 mmol) was carried out utilising the general procedure outlined above. The syrup was purified by dry flash chromatography (silica; hexane/ether gradient elution 10 : 0 → 4 : 6) and afforded in order of elution, unreacted alkene (0.60 g, 20%), 3,4-diethoxycarbonylfurazan *N*-oxide (0.47 g, 20%) and a pair of diastereomeric isoxazoline cycloadducts (**323**) and (**324**) in a ratio 81 : 19 (*erythro* : *threo*) as determined by ^{1}H-NMR. The major isomer was identified as 5R-5-(2,3-O-*isopropylidene*-1-O-*methyl*-α-D-lyxo-*tetrofuranos*-4-*yl*)-3-*ethoxy-carbonyl*-2-*isoxazoline* (**323**), (3.07 g, 65%), white needles, m.p. 72-73°C (from ethanol) and by TLC comparison to authentic sample; m/z (FAB) 316.14038 (M^++H), $C_{14}H_{22}NO_7$ requires 316.13963. The minor isomer was identified as 5S-5-(2,3-O-*isopropylidene*-1-O-*methyl*-α-D-lyxo-*tetrofuranos*-4-*yl*)-3-*ethoxycarbonyl*-2-*isoxazoline* (**324**), (0.71 g, 15%), white needles, m.p. 85.1-86.0°C (from ethanol); m/z (FAB) 316.14130 (M^++H), $C_{14}H_{22}NO_7$ requires 316.13963.

3.4.7.2 Benzonitrile Oxide

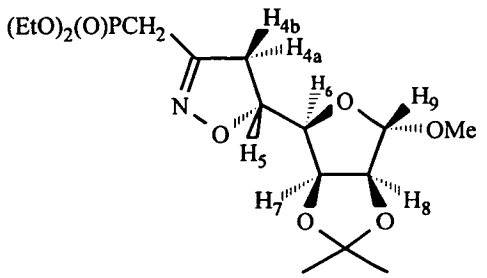
Addition of benzohydroximoyl chloride (**185**) (1.94 g, 12.50 mmol) to alkene (**317**) (3.75 g, 18.75 mmol) was carried out utilising the general procedure outlined above. The syrup was purified by dry flash chromatography (silica; hexane/ether gradient elution 10 : 0 → 4 : 6) and afforded in order of elution,

unreacted alkene (1.04 g, 26%), 3,4-diphenylfurazan *N*-oxide (208 mg, 14%) and a pair of diastereomeric isoxazoline cycloadducts (**321**) and (**322**) in a ratio 81 : 19 (*erythro* : *threo*) as determined by ^{1}H-NMR. The major isomer was identified as 5R-5-(2,3-O-*isopropylidene*-1-O-*methyl*-α-D-lyxo-*tetrofuranos*-4-*yl*)-3-*phenyl*-2-*isoxazoline* (**321**), (2.67 g, 67%), white needles, m.p. 123-125°C (from methanol); m/z (FAB) 320.14830 (M^++H), $C_{17}H_{22}NO_5$ requires 320.14980. The minor isomer was identified as 5S-5-(2,3-O-*isopropylidene*-α-D-lyxo-*tetrofuranos*-4-*yl*)-3-*phenyl*-2-*isoxazoline* (**322**), (0.64g, 16%), white needles, m.p. 130-131°C (from methanol); m/z (FAB) 320.14806 (M^++H), $C_{17}H_{22}NO_5$ requires 320.14980.

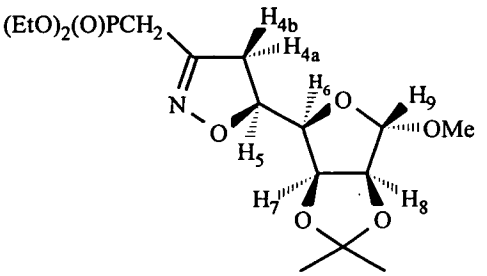
3.4.7.3 (Diethoxyphosphoryl)acetonitrile Oxide

Addition of (diethoxyphosphoryl)acetohydroximoyl chloride (**189**) (304 mg, 1.33 mmol) to alkene (**317**) (400 mg, 2.00 mmol) was carried out utilising the general procedure outlined above. The syrup was purified by dry flash chromatography (silica; hexane/ether/ethyl acetate gradient elution 10 : 0 → 3 : 7) and afforded in order of elution, unreacted alkene (67 mg, 25%), 3,4-di-(diethoxyphosphorylmethyl)-furazan *N*-oxide (39 mg, 18%) and a pair of diastereomeric isoxazoline cycloadducts 5R-5-(2,3-O-*isopropylidene*-1-O-*methyl*-α-D-lyxo-*tetrofuranos*-4-*yl*)-3-(*diethoxyphosphorylmethyl*)-2-*isoxazoline* (**325**) and 5S-5-(2,3-O-*isopropylidene*-1-O-*methyl*-α-D-lyxo-*tetrofuranos*-4-*yl*)-3-(*diethoxy-phosphorylmethyl*)-2-*isoxazoline* (**326**) (67 mg, 30%) in a ratio 88 : 12 (*erythro* : *threo*) as determined by ^{1}H-NMR. The isomers were unable to be separated. δ_H (360 MHz, $CDCl_3$) see Table 99; δ_C (90 MHz, $CDCl_3$) 147.6 (C_3), 112.5 [$C(Me)_2$], 107.1 (C_9), 84.7 (C_6), 79.3, 79.1 (C_7, C_8), 78.0 (C_5), 62.4 (CH_3CH_2O), 54.5 (OMe), 39.7

(C_4), 26.9, 25.4 [$CH_2P(O)$, $J_{CH2,P}$ = 141.0 Hz], 25.7, 24.2 [$C(CH_3)_2$], 16.2 (CH_3CH_2O); m/z (FAB) 394.16308 (M^++H), $C_{16}H_{29}NO_8P$ requires 394.16306.



***Erythro* isomer (325)**



***Threo* isomer (326)**

Resonance	δ_H/ppm		Coupling	*J*/Hz	
	***Erythro* (325)**	***Threo* (326)**		***Erythro* (325)**	***Threo* (326)**
4a	3.19	3.20	4a,4b	-	-
4b	3.18	3.18	4a,5	8.5	10.5
5	4.91	4.91	4b,5	9.6	8.8
6	3.98	3.98	5,6	6.4	6.4
7	4.74	4.74	6,7	3.7	3.7
8	4.54	4.54	7,8	5.9	5.9
9	4.87	4.87	8,9	-	-
OMe	3.29	3.32	CH_2,P	21.7	21.7
isopropyl.CH	1.44, 1.34-1.29		CH_2CH_3	7.1	7.1
$CH_2P(O)$	2.95	2.95			
OCH_2CH_3	4.18-4.08	4.18-4.08			
OCH_2CH_3	1.34-1.29	1.34-1.29			

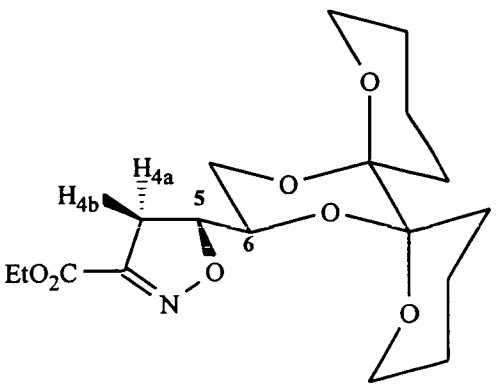
Table 99

3.4.8 Cycloadditions of Nitrile Oxides to (6*R*,7*R*,14*S*)-14-Ethenyl-1,8,13,16-tetraoxadispiro[5.0.5.4[hexadecane (226)

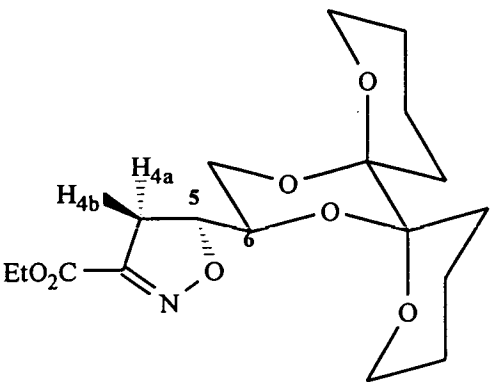
3.4.8.1 Ethoxycarbonylformonitrile Oxide

Addition of ethyl chloro-oximinoacetate (**184**) (90 mg, 0.59 mmol) to alkene (**226**) (225 mg, 0.89 mmol) was carried out utilising the general procedure outlined above. The syrup was purified by dry flash chromatography (silica ; hexane/ether gradient elution 10 : 0 → 6 : 4), and afforded in order of elution, unreacted alkene (79 mg, 53%), 3,4-diethoxycarbonylfurazan *N*-oxide (4 mg, 6%) and a pair of diastereomeric isoxazoline cycloadducts (**227**) and (**228**) in a ratio 72 : 28 (*erythro* : *threo*) as determined by the ratio of the integral of the H_5 protons in the ^{1}H-NMR (360 MHz, $CDCl_3$). [71 : 29 ratio measured by reverse phase HPLC (45% H_2O/MeOH)]. The major isomer was identified as 5S-3-*ethoxycarbonyl*-5-(6R,7R,14S-1,8,13,16-*tetraoxadispiro*[5.0.5.4]*hexadecan*-14-*yl*)-4,5-*dihydro*-2-*isoxazoline* (**227**), (87 mg, 42%), white needles (from hexane/diethyl ether), m.p. 110-111°C; $[\alpha]_D^{24}$ +14.9° (c = 0.33, $CHCl_3$); [Found : C, 58.7; H, 7.2; N, 3.3. $C_{18}H_{27}NO_7$ requires C, 58.5; H, 7.3; N, 3.8]; δ_H (360 MHz, $CDCl_3$) see Table 100; δ_C (90 MHz, $CDCl_3$) 160.5 (*C*=O), 151.4 (C_3), 96.3, 95.2 (C_8, C_9), 82.4 (C_5), 66.7 (C_6), 62.0, 60.9, 60.7, 59.7 (C_{11}, C_{13}, C_{18}, CH_3CH_2O), 38.4 (C_4), 28.2, 28.1, 24.7, 17.8, 17.7 (C_{14}, C_{15}, C_{16}, C_{19}, C_{20}, C_{21}), 13.9 (CH_3CH_2O); m/z (FAB) 370.18657 (M^++H), $C_{18}H_{28}NO_7$ requires 370.18656. The minor isomer was identified as 5R-3-*ethoxycarbonyl*-5-(6R,7R,14S-1,8,13,16-*tetraoxadispiro*[5.0.5.4]*hexadecan*-14-yl)-4,5-*dihydro*-2-*isoxazoline* (**228**), (41 mg, 17%), pale yellow oil; $[\alpha]_D^{24}$ -206.5° (c = 0.68, $CHCl_3$); δ_H (360 MHz, $CDCl_3$) see Table 100; δ_C (90 MHz, $CDCl_3$) 151.1 (C_3),

96.5, 95.2 (C_8, C_9), 82.1 (C_5), 67.1 (C_6), 61.9, 60.9, 60.6, 58.4 (C_{11}, C_{13}, C_{18}, CH_3CH_2O), 34.5 (C_4), 28.5, 28.2, 24.9, 24.7, 17.9, 17.8 (C_{14}, C_{15}, C_{16}, C_{19}, C_{20}, C_{21}), 13.9 (CH_3CH_2O); m/z (FAB) 370.18657 (M^++H), $C_{18}H_{28}NO_7$ requires 370.18656.



***Erythro* isomer (227)**



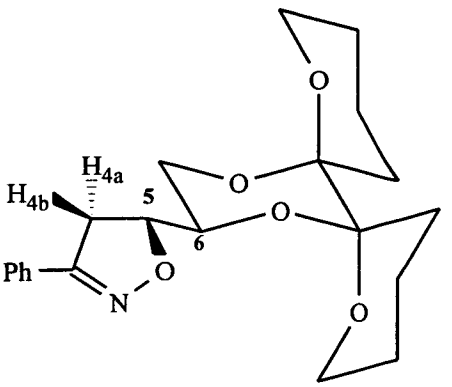
***Threo* isomer (228)**

Resonance	δ_H/ppm		Coupling	*J*/Hz	
	Erythro (227)	*Threo* (228)		*Erythro* (227)	*Threo* (228)
4a	3.18	3.18	4a,4b	17.8	-
4b	3.25	3.18	4a,5	10.9	10.2
5	4.64	4.76	4b,5	8.1	10.2
6	nd	4.01	5,6	7.0	4.8
7e	nd	3.45	6,7a	nd	7.5
CH_3CH_2	4.29	4.32	6,7e	nd	2.8
CH_3CH_2	1.31	1.33	7a,7e	nd	10.9
			CH_3CH_2	7.1	7.1

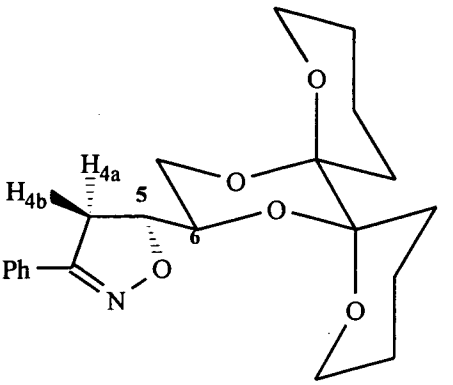
Table 100

3.4.8.2 Benzonitrile Oxide

Addition of benzohydroximoyl chloride (**185**) (92 mg, 0.59 mmol) to alkene (**226**) (225 mg, 0.89 mmol) was carried out utilising the general procedure outlined above. The syrup was purified by dry flash chromatography (silica ; hexane/ether gradient elution 10 : 0 → 0 : 10), and afforded in order of elution, unreacted alkene (81 mg, 54%), 3,4-diphenylfurazan *N*-oxide (6 mg, 8%) and a pair of diastereomeric isoxazoline cycloadducts (**229**) and (**230**) in a ratio 75 : 25 (*erythro* : *threo*) as determined by the ratio of the integral of the H_5 protons in the ^{1}H-NMR (360 MHz, $CDCl_3$). The major isomer was identified as 5S-3-*phenyl*-5-(6R,7R,14S-1,8,13,16-*tetraoxadispiro*[5.0.5.4]*hexadecan*-14-*yl*)-4,5-*dihydro*-2-*isoxazoline* (**229**), (66 mg, 30%), white needles (from hexane/ether), m.p. 189.2-190.3°C $[\alpha]_D^{24}$ -3.5° (c = 0.56, $CHCl_3$); [Found : C, 67.8; H, 7.4; N, 3.7. $C_{21}H_{28}NO_5$ requires C, 67.6; H, 7.2; N, 3.8]; δ_H (360 MHz, $CDCl_3$) see Table 101; δ_C (90 Hz, $CDCl_3$) 156.2 (C_3), 96.3, 95.1 (C_8, C_9), 80.2 (C_5), 67.0 (C_6), 60.9, 60.7, 60.3 (C_{11}, C_{13}, C_{18}), 37.2 (C_4), 28.24, 28.17, 24.7, 17.88, 17.86 (C_{14}, C_{15}, C_{16}, C_{19}, C_{20}, C_{21}); m/z (FAB) 374.19674 (M^++H), $C_{21}H_{28}NO_5$ requires 374.19673. The minor isomer was identified as 5R-3-*phenyl*-5-(6R,7R,14S-1,8,13,16-*tetraoxadispiro*[5.0.5.4]*hexadecan*-14-*yl*)-4,5-*dihydro*-2-*isoxazoline* (**230**), (33 mg, 10%), pale yellow oil, $[\alpha]_D^{24}$ -175.7° (c = 0.33, $CHCl_3$); δ_H (360 MHz, $CDCl_3$) see Table 101; δ_C (90 MHz, $CDCl_3$) 156.1 (C_3), 96.5, 95.3 (C_8, C_9), 79.9 (C_5), 69.2 (C_6), 60.9, 60.6, 58.6 (C_{11}, C_{13}, C_{18}), 35.9 (C_4), 28.6, 28.3, 24.82, 24.77, 18.0, 17.8 (C_{14}, C_{15}, C_{16}, C_{19}, C_{20}, C_{21}); m/z (FAB) 374.19674 (M^++H), $C_{21}H_{28}NO_5$ requires 374.19673.



***Erythro* isomer (229)**



***Threo* isomer (230)**

Resonance	δ_H/ppm		Coupling	*J*/Hz	
	***Erythro* (229)**	***Threo* (230)**		***Erythro* (229)**	***Threo* (230)**
4a	3.40	3.31	4a,4b	-	-
4b	3.40	3.31	4a,5	8.8	9.8
5	4.63	4.73	4b,5	8.8	9.8
6	3.95	4.12	5,6	7.7	5.3
7e	3.60	3.52	6,7a	9.3	8.2
Ph	7.68-7.37	7.67-7.36	6,7e	4.0	2.9
			7a,7e	10.9	11.0

Table 101

3.4.9 Cycloadditions of Nitrile Oxides to Trans-3,4-*didehydro*-3,4-*dideoxy*-1,2:5,6-*di*-O-*cyclohexylidene*-D-threo-*hex*itol (232)

3.4.9.1 Ethoxycarbonylformonitrile Oxide

Addition of ethyl chloro-oximinoacetate (**184**) (328 mg, 2.17 mmol) to alkene (**232**) (1.00 g, 3.25 mmol) was carried out utilising the general procedure outlined above. The syrup was purified by dry flash chromatography (silica; hexane/ether gradient elution 10 : 0 → 0 : 10), and afforded in order of elution, unreacted alkene (500 mg, 75%) and 3,4-diethoxycarbonylfurazan *N*-oxide (20 mg, 8%). There was no evidence by TLC or ^{1}H-NMR of any cycloaddition reaction occurring.

3.4.9.2 Benzonitrile Oxide

Addition of benzohydroximoyl chloride (**185**) (95 mg, 0.61 mmol) to alkene (**232**) (284 mg, 0.92 mmol) was carried out utilising the general procedure outlined above. The syrup was purified by dry flash chromatography (silica ; hexane/ether gradient elution 10 : 0 → 0 : 10), and afforded in order of elution, unreacted alkene (135 mg, 71%) and 3,4-diphenylfurazan *N*-oxide (7 mg, 10%). There was no evidence by TLC or ^{1}H-NMR of any cycloaddition reaction occurring.

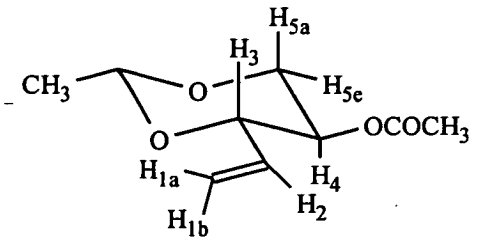
3.5 Synthesis of Acetate Derivatives

3.5.1 1,2-*Dideoxy*-4-O-*acetyl*-3,5-O-*ethylidene*-D-erythro-*pent*-1-*enitol* (271)

Dry acetic anhydride (1.31 ml, 13.89 mmol, 5 M eq) was added to a stirred solution of 1,2-*dideoxy*-3,5-O-*ethylidene*-D-erythro-*pent*-1-*enitol* (**260**) (400 mg, 2.77 mmol) in dry pyridine (24 ml). After stirring at room temperature for 16 hr, the mixture was concentrated *in vacuo*, and purified by preparatory TLC on silica using 100% ether as solvent, to afford 1,2-*dideoxy*-4-O-*acetyl*-3,5-O-*ethylidene*-D-erythro-*pent*-1-*enitol* (**271**) as a very pale yellow oil (319 mg, 62%); δ_H (360 MHz, $CDCl_3$) see Table 102; δ_C (90 MHz, $CDCl_3$) 169.5 (CH_3CO), 134.0 (C_2), 118.7 (C_1), 98.8 (CCH_3), 79.7 (C_3), 67.5 (C_5), 65.9 (C_4), 20.6 (CH_3CO), 20.3 (CCH_3); m/z (FAB) 187 (M^++H).

3.5.2 5S-5-(2-O-*Acetyl*-1,3-O-*ethylidene*-D-erythro-*triitol*-1-*yl*)-3-*ethoxy*-*carbonyl*-2-*isoxazoline* (274)

Using the same procedure as for (**271**), isoxazoline (**263**) (50 mg, 0.19 mmol) afforded 5S-5-(2-O-*acetyl*-1,3-O-*ethylidene*-D-erythro-*triitol*-1-*yl*)-3-*ethoxy*-*carbonyl*-2-*isoxazoline* (**274**) as white needles (57 mg, 99%), m.p. 72-74°C; δ_H (360 MHz, $CDCl_3$) see Table 94; δ_C (90 MHz, $CDCl_3$) see Section 3.4.5.1; m/z (FAB) 302.12395 (M^++H), $C_{13}H_{20}NO_7$ requires 302.12396. This product (**274**) is identical to that obtained from the cycloaddition of ethoxycarbonylformonitrile oxide to 1,2-*dideoxy*-4-O-*acetyl*-3,5-O-*ethylidene*-D-erythro-*pent*-1-*enitol* (**271**). (By comparison of m.p., TLC and FAB MS data).



(271)

Resonance	δ_H/ppm	Coupling	*J*/Hz
1a	5.42	1a,1b	1.0
1b	5.22	1a,2	17.2
2	5.79	1b,2	10.5
3	3.94	2,3	6.7
4	4.67	3,4	9.6
5a	3.41	4,5a	10.2
5e	4.16	4,5e	5.3
CH_3CH	4.71	5a,5e	10.6
CH_3CH	1.33	CH_3CH	5.1
CH_3CO	1.98		

Table 102

3.5.3 5R-5-(2-O-*Acetyl*-1,3-O-*ethylidene*-D-erythro-*triitol*-1-*yl*)-3-*ethoxy-carbonyl*-2-*isoxazoline* (275)

Using the same procedure as for (**271**), isoxazoline (**264**) (50 mg, 0.19 mmol) afforded 5R-5-(2-O-*acetyl*-1,3-O-*ethylidene*-D-erythro-*triitol*-1-*yl*)-3-*ethoxy-carbonyl*-2-*isoxazoline* (**275**) as white needles (57 mg, 98%), m.p. 68.5-70.5°C; δ_H (360 MHz, $CDCl_3$) see Table 94; δ_C (90 MHz, $CDCl_3$) see Section 3.4.5.1; m/z (FAB) 302.12395 (M^++H), $C_{13}H_{20}NO_7$ requires 302.12396. This product (**275**) is

identical to that obtained from the cycloaddition of ethoxycarbonylformonitrile oxide to 1,2-*dideoxy*-4-O-*acetyl*-3,5-O-*ethylidene*-D-erythro-*pent*-1-*enitol* (**264**). (By comparison of m.p., TLC and FAB MS data).

3.5.4 5S-5-(2-O-*Acetyl*-1,3-O-*ethylidene*-D-erythro-*triitol*-1-*yl*)-3-*phenyl*-2-*isoxazoline* (272) and 5R-5-(2-O-*acetyl*-1,3-O-*ethylidene*-D-erythro-*triitol*-1-*yl*)-3-*phenyl*-2-*isoxazoline* (273)

Using the same procedure as for (**271**), mixed isomers (**265**) and (**266**) (400 mg, 1.52 mmol) were acetylated and then separated by dry flash chromatography (silica; hexane/ether gradient elution 10 : 0 → 0 : 10). 5S-5-(2-O-*Acetyl*-1,3-O-*ethylidene*-D-erythro-*triitol*-1-*yl*)-3-*phenyl*-2-*isoxazoline* (**272**) was isolated as white needles (218 mg, 47%); m/z (FAB) 306.13414 (M^++H), $C_{16}H_{20}NO_5$ requires 306.13415 and 5R-5-(2-O-*acetyl*-1,3-O-*ethylidene*-D-erythro-*triitol*-1-*yl*)-3-*phenyl*-2-*isoxazoline* (**273**) (209 mg, 45%); m/z (FAB) 306.13415 (M^++H), $C_{16}H_{20}NO_5$ requires 306.13414. These products (**272**) and (**273**) are identical to those obtained by cycloaddition of benzonitrile oxide to 1,2-*dideoxy*-4-O-*acetyl*-3,5-O-*ethylidene*-D-erythro-*pent*-1-*enitol* (**271**). (By comparison of TLC and FAB MS data). Table 95 shows the data obtained (Section 3.4.5.2).

3.6 Reduction of Ethyl Ester to Alcohol

General Procedure

The isoxazoline (1 M eq) was stirred in ethanol and THF added dropwise until it had completely dissolved. Sodium borohydride (6 M eq) was added in portions and the resulting mixture stirred at room temperature overnight. After pouring into water and extracting with chloroform, the organic phase was dried ($MgSO_4$) and the solvent removed *in vacuo* to afford the products, which were purified by recrystallisation.

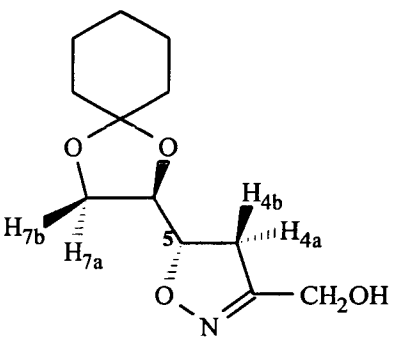
3.6.1 5S-5-(1,2-O-*Cyclohexylidene*-D-glycero-*diitol*-1-*yl*)-3-*hydroxymethyl*-2-*isoxazoline* (204)

Obtained from (**198**) (423 mg, 1.49 mmol) using the general procedure above, (**204**) was isolated as white needles (338 mg, 94%), m.p. 80-82°C (from 1 : 1 ether : hexane); $[\alpha]_D^{24}$ +59.5° (c = 0.10, $CHCl_3$); [Found : C, 59.5; H, 8.2; N, 5.6. $C_{12}H_{19}NO_4$ requires C, 59.8; H, 7.9; N, 5.8]; δ_H (360 MHz, $CDCl_3$) see Table 103; δ_C (90 MHz, $CDCl_3$) 158.5 (C_3), 110.2 (cyclohexyl. quat.), 81.0 (C_5), 75.5 (C_6), 66.7 (C_7), 58.0 (CH_2OH), 37.6 (C_4), 36.4, 34.4, 24.9, 23.9, 23.6 (cyclohexyl. CH_2); m/z (FAB) 242.139221 (M^++H), $C_{12}H_{20}NO_4$ requires 242.13922.

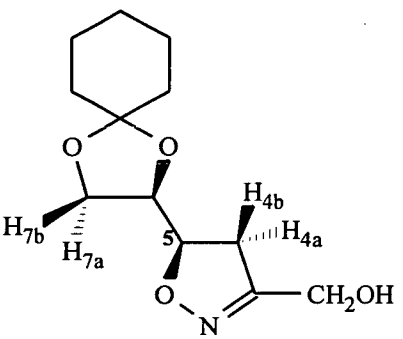
3.6.2 5R-5-(1,2-O-*Cyclohexylidene*-D-glycero-*diitol*-1-*yl*)-3-*hydroxymethyl*-2-*isoxazoline* (205)

Obtained from (**199**) (300 mg, 1.06 mmol) using the general procedure above, (**205**) was isolated as white needles (244 mg, 96%), m.p. 83.5-85.5°C (from 1

: 1 ether : hexane); $[\alpha]_D^{24}$ -68.3° (c = 0.50, $CHCl_3$); δ_H (360 MHz, $CDCl_3$) see Table 103; δ_C (90 MHz, $CDCl_3$) 158.2 (C_3), 110.5 (cyclohexyl. quat.), 80.0 (C_5), 75.5 (C_6), 64.8 (C_7), 58.0 (CH_2OH), 36.6 (C_4), 35.7, 34.6, 25.0, 24.9, 23.8, 23.7 (cyclohexyl. CH_2); m/z (FAB) 242.139221 (M^++H), $C_{12}H_{20}NO_4$ requires 242.13922.



(204)



(205)

Resonance	δ_H/ppm		Coupling	*J*/Hz	
	(204)	(205)		(204)	(205)
4a	3.06	3.10	4a,4b	17.5	17.5
4b	3.14	2.97	4a,5	6.9	11.0
5	4.53	4.67	4b,5	10.1	7.9
6	4.02	4.23	5,6	7.1	4.4
7a	4.08	4.04	6,7a	6.2	6.8
7b	3.85	3.82	6,7b	4.5	6.3
CH_2OH	4.40	4.40	7a,7b	8.4	8.6
CH_2OH	2.40	nd			
cyclohexyl. CH_2	1.62-1.50	1.64-1.50			

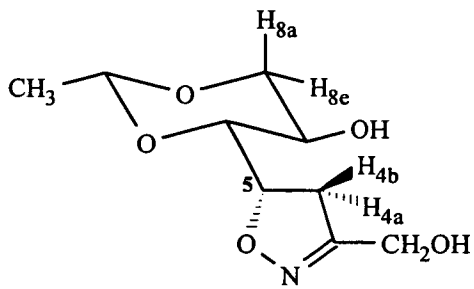
Table 103

3.6.3 5S-5-(1,3-O-*Ethylidene*-D-erythro-*triitol*-1-*yl*)-3-*hydroxymethyl*-2-*isoxazoline* (269)

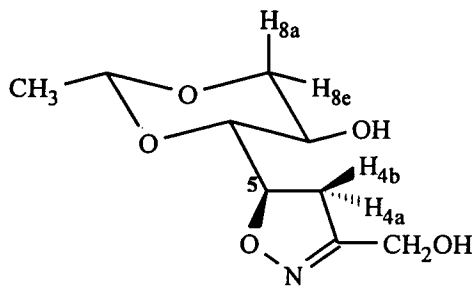
Obtained from (**263**) (300 mg, 1.16 mmol) using the general procedure above including continuous extraction ($CHCl_3$), (**269**) was isolated as a very pale yellow oil (193 mg, 77%), $[\alpha]_D^{26}$ +115.9° (c = 1.01, MeOH); δ_H [360 MHz, $(CD_3)_2CO$] see Table 104; δ_C [90 MHz, $(CD_3)_2CO$] 158.9 (C_3), 98.4 (CCH_3), 81.1 (C_5), 80.0 (C_6), 70.6 (C_8), 62.8 (C_7), 57.1 (CH_2OH), 35.1 (C_4), 20.1 (CCH_3); m/z (FAB) 218.10285 (M^++H), $C_9H_{16}NO_5$ requires 218.10285.

3.6.4 5R-5-(1,3-O-*Ethylidene*-D-erythro-*triitol*-1-*yl*)-3-*hydroxymethyl*-2-*isoxazoline* (270)

Obtained from (**264**) (200 mg, 0.77 mmol) using the general procedure above including continuous extraction ($CHCl_3$), (**270**) was isolated as white needles (141 mg, 84%), m.p. 147-148.5°C; $[\alpha]_D^{24}$ -225.8° (c = 0.96, MeOH); [Found : C, 50.3; H, 7.3; N, 6.1. $C_9H_{15}NO_5$ requires C, 49.8; H, 6.9; N, 6.5]; δ_H [360 MHz, $(CD_3)_2CO$] see Table 104; δ_C [90 MHz, $(CD_3)_2CO$] 158.8 (C_3), 98.0 (CCH_3), 81.7 (C_5), 77.7 (C_6), 70.8 (C_8), 61.5 (C_7), 57.1 (CH_2OH), 35.6 (C_4), 20.1 (CCH_3); m/z (FAB) 218.10284 (M^++H), $C_9H_{16}NO_5$ requires 218.10285.



(269)



(270)

Resonance	δ_H/ppm		Coupling	*J*/Hz	
	(269)	(270)		(269)	(270)
4a	3.19	3.10	4a,4b	16.9	-
4b	3.07	3.13	4a,5	8.3	10.7
5	4.85	4.85	4b,5	11.2	9.3
6	3.58	3.36	5,6	2.6	2.8
7	3.48	3.76	6,7	9.4	9.4
8a	3.35	3.33	7,8a	10.0	10.4
8e	4.00	4.02	7,8e	5.1	5.3
CH_3CH	4.69	4.66	8a,8e	10.5	10.6
CH_3CH	1.20	1.20	CH_3CH	5.0	5.0
CH_2OH	4.28	4.28	7,7OH	-	5.8
CH_2OH	2.14	3.01			
7OH	2.61	4.38			

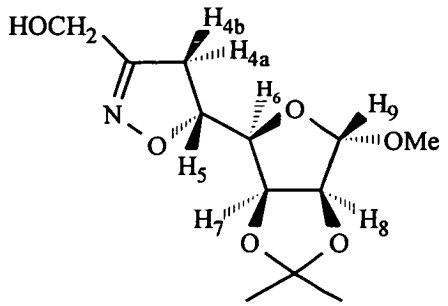
Table 104

3.6.5 5R-5-(2,3-O-*Isopropylidene*-O-*methyl*-α-D-lyxo-*tetrofuranos*-4-*yl*)-3-*hydroxymethyl*-2-*isoxazoline* (327)

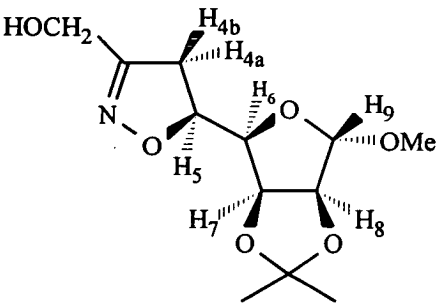
Obtained from (**323**) (1.52 g, 4.83 mmol) using the general procedure above, (**327**) was isolated as a colourless oil (1.30 g, 99%); $[\alpha]_D^{24}$ -5.6° (c = 1.12, $CHCl_3$); δ_H (360 MHz, $CDCl_3$) see Table 105; δ_C (90 MHz, $CDCl_3$) 159.0 (C_3), 112.6 [*C*$(Me)_2$], 107.1 (C_9), 84.7 (C_6), 79.3 (C_7), 79.1 (C_8), 78.0 (C_5), 57.9 (*C*H_2OH), 54.6 (OMe), 37.4 (C_4), 25.6, 24.2 (2 × Me); m/z (FAB) 274.12906 (M^++H), $C_{12}H_{20}NO_6$ requires 274.12905.

3.6.6 5S-5-(2,3-O-*Isopropylidene*-1-O-*methyl*-α-D-lyxo-*tetrofuranos*-4-*yl*)-3-*hydroxymethyl*-2-*isoxazoline* (328)

Obtained from (**324**) (350 mg, 1.11 mmol) using the general procedure above, (**328**) was isolated as white needles (298 mg, 98%), m.p. 95-97°C; $[\alpha]_D^{24}$ +77.9° (c = 0.83, $CHCl_3$); δ_H (360 MHz, $CDCl_3$) see Table 105; δ_C (90 MHz, $CDCl_3$) 158.6 (C_3), 112.8 [*C*$(Me)_2$], 107.4 (C_9), 84.6 (C_6), 79.9 (C_7), 79.5 (C_5, C_8), 57.8 (*C*H_2OH), 54.7 (OMe), 37.6 (C_4), 25.8, 24.6 (2 × Me); m/z (FAB) 274.12906 (M^++H), $C_{12}H_{20}NO_6$ requires 274.12905.



(327)



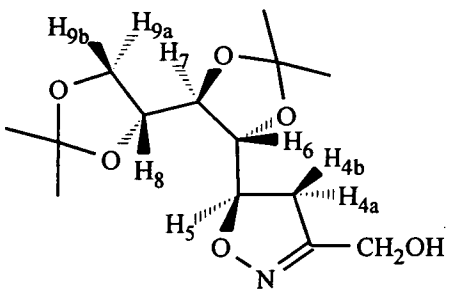
(328)

Resonance	δ_H/ppm		Coupling	*J*/Hz	
	(327)	(328)		(327)	(328)
4a	3.13	3.24	4a,4b	-	17.5
4b	3.12	2.85	4a,5	8.5	10.6
5	4.90	4.80	4b,5	10.0	8.8
6	4.01	3.98	5,6	6.0	8.5
7	4.74	4.68	6,7	3.7	3.8
8	4.54	4.54	7,8	5.9	5.9
9	4.88	4.94	8,9	-	-
OMe	3.29	3.32			
C*H*$_2$OH	4.38	4.38			
CH$_2$O*H*	2.69	nd			
isopropyl.	1.43, 1.28	1.42, 1.25			

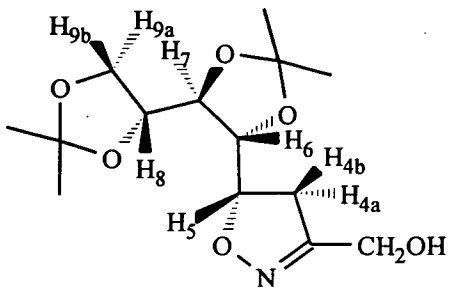
Table 105

3.6.7 5R-5-(1,2:3,4-*Di*-O-*isopropylidene*-D-arabino-*tetritol*-1-*yl*)-3-*hydroxymethyl*-2-*isoxazoline* (305) and 5S-5-(1,2:3,4-*di*-O-*isopropylidene*-D-arabino-*tetritol*-1-*yl*)-3-*hydroxymethyl*-2-*isoxazoline* (306)

Obtained from mixed isomers (**301**) and (**302**) (137 mg, 0.40 mmol) using the general procedure above, (**305**) and (**306**) were isolated as a white solid (106 mg, 88%). The isomers were unable to be separated. [Found : C, 55.4; H, 7.9; N, 4.5. $C_{14}H_{23}NO_6$ requires C, 55.8; H, 7.6; N, 4.7]; m/z (FAB) 302.16036 (M^++H), $C_{14}H_{24}NO_6$ requires 302.16035. The major isomer was identified as 5R-5-(1,2:3,4-*di*-O-*isopropylidene*-D-arabino-*tetritol*-1-*yl*)-3-*hydroxymethyl*-2-*isoxazoline* (**305**), δ_H (360 MHz, $CDCl_3$) see Table 106; δ_C (90 MHz, $CDCl_3$) 158.3 (C_3), 110.2, 109.2 [*C*$(Me)_2$], 80.2 (C_5), 80.3, 78.7, 76.6 (C_6, C_7, C_8), 67.2 (C_9), 58.0 (*C*H_2OH), 35.1 (C_4), 27.2, 26,6, 25.0 (4 × CH_3). The minor isomer was identified as 5S-5-(1,2:3,4-*di*-O-*isopropylidene*-D-arabino-*tetritol*-1-*yl*)-3-*hydroxymethyl*-2-*isoxazoline* (**306**), δ_H (360 MHz, $CDCl_3$) see Table 106; δ_C (90 MHz, $CDCl_3$) 158.1 (C_3), 109.9, 109.7 [*C*$(Me)_2$], 82.0 (C_5), 79.0, 77.3, 77.0 (C_6, C_7, C_8), 67.8 (C_9), 58.0 (*C*H_2OH), 37.2 (C_4), 29.6, 27.0, 26.3, 25.2 (4 × CH_3).



(305)



(306)

Resonance	δ_H/ppm		Coupling	*J*/Hz	
	(305)	(306)		(305)	(306)
4a	3.02	3.11	4a,4b	17.0	-
4b	3.12	3.11	4a,5	11.1	8.8
5	4.87	4.46	4b,5	7.8	10.0
6	4.16	4.12	5,6	3.5	2.5
7	nd	nd	6,7	7.3	8.5
8	nd	nd	7,8	nd	nd
9a	nd	nd	8,9a	nd	nd
9b	nd	nd	8,9b	nd	nd
C*H*$_2$OH	4.39	4.39	9a,9b	nd	nd
CH$_2$O*H*	2.30	2.30			
isopropyl.	1.42-1.32	1.42-1.32			

Table 106

3.7 Deprotection of Isoxazolines

General Procedure

The isoxazoline in a mixture of water/glacial acetic acid (1 : 4) was stirred at 80°C for 2-10 hr. The solution was concentrated *in vacuo*, and after repeated addition (4 × 10 ml) and removal of a 1 : 1 mixture of toluene and heptane, the residue was triturated with isopropanol to afford the product, which was purified by preparative TLC on silica gel (100 % ethyl acetate or 50/50 ethyl acetate/diethyl ether).

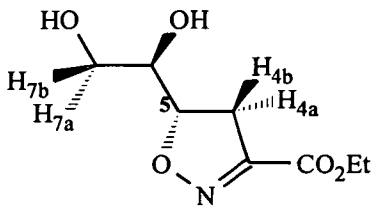
3.7.1 5S-5-(1,2-O-*Cyclohexylidene*-D-glycero-*diitol*-1-*yl*)-3-*ethoxycarbonyl*-2-*isoxazoline* (198)

Using the general procedure above, isoxazoline (**198**) (80 mg, 0.28 mmol) afforded 5S-5-(D-glycero-*diitol*-1-*yl*)-3-*ethoxycarbonyl*-2-*isoxazoline* (**250**) as a yellow oil (44 mg, 77%); $[\alpha]_D^{26}$ +109.9° (c = 0.78, CH_3OH); δ_H [360 MHz, $(CD_3)_2CO$] see Table 107; δ_C [90 MHz, $(CD_3)_2CO$] 160.7 (*C*=O), 151.9 (C_3), 84.2 (C_5), 71.8 (C_6), 63.1 (C_7), 61.4 (CH_3CH_2O), 33.8 (C_4), 13.7 (CH_3CH_2O); m/z (FAB) 204.08720 (M^++H), $C_8H_{14}NO_5$ requires 204.08719.

3.7.2 5R-5-(1,2-O-*Cyclohexylidene*-D-glycero-*diitol*-1-*yl*)-3-*ethoxycarbonyl*-2-*isoxazoline* (199)

Using the general procedure above, isoxazoline (**199**) (80 mg, 0.28 mmol) afforded 5R-5-(D-glycero-*diitol*-1-*yl*)-3-*ethoxycarbonyl*-2-*isoxazoline* (**253**) as a yellow oil (46 mg, 80%); $[\alpha]_D^{26}$ -124.9° (c = 0.56, CH_3OH); δ_H [360 MHz, $(CD_3)_2CO$] see Table 107 ; δ_C [90 MHz, $(CD_3)_2$ CO] 160.8 (*C*=O), 151.9 (C_3), 83.8

(C_5), 73.1 (C_6), 63.0 (C_7), 61.4 (CH_3CH_2O), 35.3 (C_4), 13.7 (CH_3CH_2O); m/z (FAB) 204.08720 (M^++H), $C_8H_{14}NO_5$ requires 204.08719.



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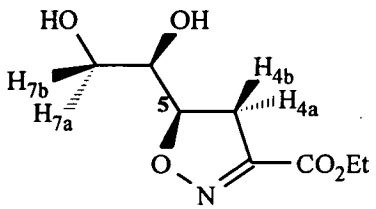


(253)

Resonance	δ_H/ppm			Coupling	*J*/Hz		
	(250)[a]	(250)[b]	(253)		(250)[a]	(250)[b]	(253)
4a	3.29	3.30	3.28	4a,4b	17.6	17.6	17.4
4b	3.17	3.18	3.20	4a,5	8.5	8.2	11.3
5	4.86	4.88	4.91	4b,5	11.4	11.3	9.0
6	nd	nd	nd	5,6	4.5	nd	3.1
7a	nd	nd	nd	6,7a	nd	nd	nd
7b	nd	nd	nd	6,7b	nd	nd	nd
CH_3CH_2	4.28	4.28	4.38	7a,7b	nd	nd	nd
CH_3CH_2	1.30	1.30	1.30	CH_3CH_2	7.1	7.1	7.1

Table 107

[a] Product from cyclohexylidene-protected isomer (**198**)
[b] Product from dispiroketal-protected isomer (**227**)

3.7.3 5S-5-(1,2-O-*Cyclohexylidene*-D-glycero-*diitol*-1-*yl*)-3-*hydroxymethyl*-2-*isoxazoline* (204)

Using the general procedure above, isoxazoline (**204**) (300 mg, 1.24 mmol) afforded 5S-5-(D-glycero-*diitol*-1-*yl*)-3-*hydroxymethyl*-2-*isoxazoline* (**251**) as a pale yellow oil (142 mg, 71%); $[\alpha]_D^{24}$ +97.3° (c = 0.72, CH_3OH); δ_H [360 MHz, $(CD_3)_2CO$] see Table 108; δ_C [90 MHz, $(CD_3)_2CO$] 159.2 (C_3), 80.8 (C_5), 72.3 (C_6), 63.5 (C_7), 57.1 (CH_2OH), 36.0 (C_4); m/z (FAB) 162.07664 (M^++H), $C_6H_{12}NO_4$ requires 162.07663.

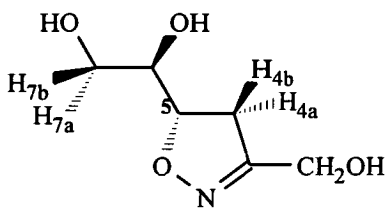
3.7.4 5R-5-(1,2-O-*Cyclohexylidene*-D-glycero-*diitol*-1-*yl*)-3-*hydroxymethyl*-2-*isoxazoline* (205)

Using the general procedure above, isoxazoline (**205**) (150 mg, 0.62 mmol) afforded 5R-5-(D-glycero-*diitol*-1-*yl*)-3-*hydroxymethyl*-2-*isoxazoline* (**249**) as a colourless oil (75 mg, 75%); δ_H [360 MHz, $(CD_3)_2CO$] see Table 108; δ_C [90 MHz, $(CD_3)_2CO$] 159.2 (C_3), 80.8 (C_5), 73.2 (C_6), 63.3 (C_7), 57.1 (CH_2OH), 36.7 (C_4); m/z (FAB) 162.07664 (M^++H), $C_6H_{12}NO_4$ requires 162.07663.

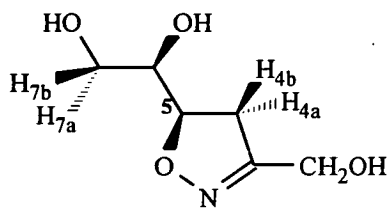
3.7.5 5S-5-(1,2-O-*Cyclohexylidene*-D-glycero-*diitol*-1-*yl*)-3-*phenyl*-2-*isoxazoline* (196)

Using the general procedure above, isoxazoline (**196**) (500 mg, 1.74 mmol) afforded 5S-5-(D-glycero-*diitol*-1-*yl*)-3-*phenyl*-2-*isoxazoline* (**249**) as white needles (253 mg, 70%), m.p. 104-106°C (from acetone : hexane 1 : 4); $[\alpha]_D^{24}$ +83.1° (c = 0.10, CH_3OH); [Found : C, 63.5; H, 6.5; N, 6.6. $C_{11}H_{13}NO_3$ requires C, 63.8; H, 6.3;

N, 6.8]; δ_H [360 MHz, $(CD_3)_2CO$] see Table 109; δ_C [90 MHz, $(CD_3)_2CO$] 156.6 (C_3), 129.9, 128.9, 126.7 (5 × PhCH), 81.8 (C_5), 72.3 (C_6), 63.5 (C_7), 35.5 (C_4); m/z (FAB) 208.09736 (M^++H), $C_{11}H_{14}NO_3$ requires 208.09737.



(251)



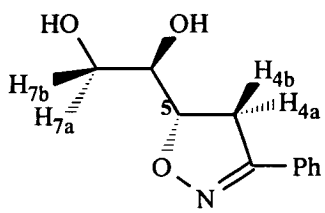
(254)

Resonance	δ_H/ppm		Coupling	*J*/Hz	
	(251)	(254)		(251)	(254)
4a	3.14	3.17	4a,4b	17.3	17.2
4b	3.04	3.05	4a,5	8.0	10.5
5	4.53	4.61	4b,5	10.7	8.8
6	3.64	nd	5,6	5.6	3.9
7a	3.61	nd	6,7a	5.8	nd
7b	3.52	nd	6,7b	4.2	nd
C*H*$_2$OH	4.28	4.28	7a,7b	10.8	nd

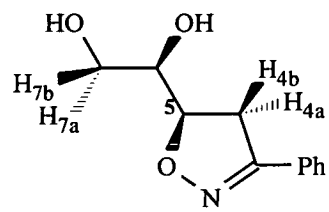
Table 108

3.7.6 5R-5-(1,2-O-*Cyclohexylidene*-D-glycero-*diitol*-1-*yl*)-3-*phenyl*-2-*isoxazoline* (197)

Using the general procedure above, isoxazoline (**197**) (300 mg, 1.05 mmol) afforded 5R-5-(D-glycero-*diitol*-1-*yl*)-3-*phenyl*-2-*isoxazoline* (**252**) as white needles (160 mg, 74%), m.p. 128-130°C; $[\alpha]_D^{25}$ -158.9° (c = 0.30, CH_3OH); δ_H [360 MHz, $(CD_3)_2CO$] see Table 109; δ_C [90 MHz, $(CD_3)_2CO$] 156.6 (C_3), 129.9, 128.8, 126.7 (5 × PhCH), 81.6 (C_5), 73.3 (C_6), 63.3 (C_7), 36.4 (C_4); m/z (EI) 207.08954 (M^+), $C_{11}H_{13}NO_3$ requires 207.08900.



(249)



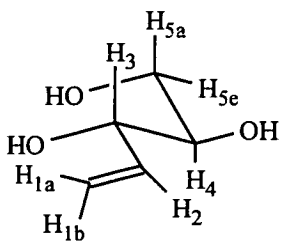
(252)

Resonance	δ_H/ppm		Coupling	*J*/Hz	
	(249)	(252)		(249)	(252)
4a	3.48	3.47	4a,4b	16.9	16.8
4b	3.37	3.40	4a,5	8.1	10.7
5	4.75	4.82	4b,5	10.8	8.9
6	3.90	3.94-3.67	5,6	5.4	3.5
7a	3.61	3.94-3.67	6,7a	6.0	nd
7b	3.68	3.94-3.67	6,7b	4.5	nd
Ph	7.72-7.43	7.72-7.41	7a,7b	11.1	nd
6OH, 7OH	3.20-3.00	nd			

Table 109

3.7.7 1,2-*Dideoxy*-3,5-O-*ethylidene*-D-erythro-*pent*-1-*enitol* (260)

Using the general procedure above, alkene (**260**) (300 mg, 2.08 mmol) afforded 1,2-*dideoxy*-D-erythro-*pent*-1-*enitol* (**276**) as a colourless oil (101 mg, 41%), $[\alpha]_D^{24}$ -30.6° (c = 0.51, CH_3OH); δ_H [360 MHz, $(CD_3)_2CO$] see Table 110; δ_C [90 MHz, $(CD_3)_2CO$] 138.9 (C_2), 114.6 (C_1), 74.8 (C_3)*, 73.8 (C_4)*, 63.5 (C_5); m/z (FAB) 119.07080 (M^++H), $C_5H_{11}O_3$ requires 119.07081.



(**276**)

Resonance	δ_H/ppm	Coupling	*J*/Hz
1a	5.27	1a,1b	1.9
1b	5.10	1a,2	17.2
2	6.00	1b,2	10.6
3	3.54	1a,3	1.7
4	4.10	1b,3	1.5
5a	nd	2,3	5.7
5e	3.65	3,4	4.3
3OH, 4OH, 5OH	4.20 (2OH)	4,5a	nd
		4,5e	3.9
		5a,5e	10.5

Table 110

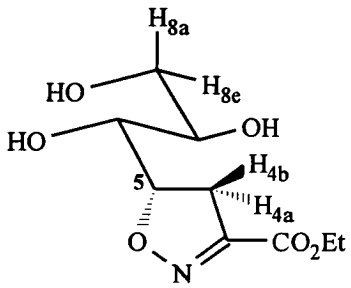
* Alternative assignments in ^{13}C-NMR

3.7.8 5S-5-(1,3-O-*Ethylidene*-D-erythro-*triitol*-1-*yl*)-3-*ethoxycarbonyl*-2-*isoxazoline* (263)

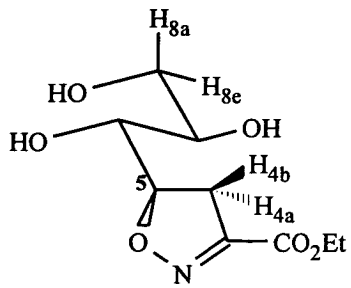
Using the general procedure above, isoxazoline (**263**) (200 mg, 0.77 mmol) afforded 5S-5-(D-erythro-*triitol*-1-*yl*)-3-*ethoxycarbonyl*-2-*isoxazoline* (**277**) as a white solid (157 mg, 87%), m.p. 123.6-125.2°C; $[\alpha]_D^{25}$ +145.9° (c = 0.70, CH_3OH); δ_H [360 MHz, $(CD_3)_2CO$] see Table 111; δ_C [90 MHz, $(CD_3)_2CO$] 160.8 (*C*=O), 151.9 (C_3), 85.1 (C_5), 72.4 (C_6), 71.4 (C_7), 63.8 (C_8), 61.3 (CH_3CH_2O), 32.6 (C_4), 13.7 (CH_3CH_2O); m/z (FAB) 234.09776 (M^++H), $C_9H_{16}NO_6$ requires 234.09775.

3.7.9 5R-5-(1,3-O-*Ethylidene*-D-erythro-*triitol*-1-*yl*)-3-*ethoxycarbonyl*-2-*isoxazoline* (264)

Using the general procedure above, isoxazoline (**264**) (150 mg, 0.58 mmol) afforded 5R-5-(D-erythro-*triitol*-1-*yl*)-3-*ethoxycarbonyl*-2-*isoxazoline* (**278**) as white needles (65 mg, 48%), m.p. 100.2-102.5°C; $[\alpha]_D^{25}$ -175.7° (c = 0.82, CH_3OH); δ_H [360 MHz, $(CD_3)_2CO$] see Table 111; δ_C [90 MHz, $(CD_3)_2CO$] 160.8 (*C*=O), 152.0 (C_3), 83.3 (C_5), 73.2 (C_6), 71.5 (C_7), 64.1 (C_8), 61.3 (CH_3CH_2O), 35.3 (C_2), 13.7 (CH_3CH_2O); m/z (FAB) 234.09776 (M^++H), $C_9H_{16}NO_6$ requires 234.09775.



(277)



(278)

Resonance	δ_H/ppm		Coupling	*J*/Hz	
	(277)	(278)		(277)	(278)
4a	3.30	3.29	4a,4b	17.4	17.5
4b	3.14	3.20	4a,5	9.1	11.5
5	5.10	5.14	4b,5	11.6	9.0
6	3.92	3.73	5,6	2.6	2.4
7	3.52	3.55	6,7	7.9	nd
8a	3.65	3.67	7,8a	5.6	5.1
8e	3.76	3.80	7,8e	3.9	3.6
CH_3CH_2	4.27	4.27	8a,8e	11.1	10.4
CH_3CH_2	1.30	1.29	CH_3CH_2	7.1	7.1
5OH	nd	3.57			

Table 111

3.7.10 5S-5-(1,3-O-*Ethylidene*-D-erythro-*triitol*-1-*yl*)-3-*hydroxymethyl*-2-*isoxazoline* (269)

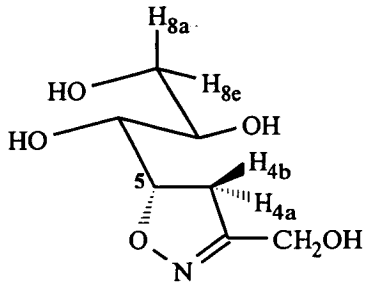
Using the general procedure above, isoxazoline (**269**) (63 mg, 0.75 mmol) afforded 5S-5-(D-erythro-*triitol*-1-*yl*)-3-*hydroxymethyl*-2-*isoxazoline* (**281**) as a pale yellow oil (89 mg, 62%); δ_H [360 MHz, $(CD_3)_2CO$] see Table 112; δ_C [90 MHz, $(CD_3)_2CO$] 158.1 (C_3), 80.6 (C_5), 71.6 (C_6), 70.8 (C_7), 62.9 (C_8), 56.1 (CH_2OH), 33.5 (C_4); m/z (FAB) 192.08760 (M^++H), $C_7H_{14}NO_5$ requires 192.08720.

3.7.11 5R-5-(1,3-O-*Ethylidene*-D-erythro-*triitol*-1-*yl*)-3-*hydroxymethyl*-2-*isoxazoline* (270)

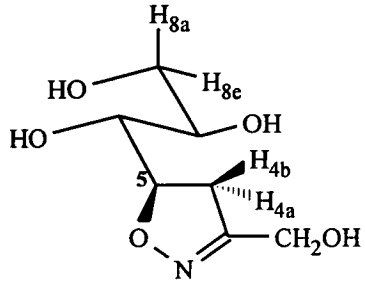
Using the general procedure above, isoxazoline (**270**) (70 mg, 0.32 mmol) afforded 5R-5-(D-erythro-*triitol*-1-*yl*)-3-*hydroxymethyl*-2-*isoxazoline* (**282**) as a pale yellow oil (31 mg, 50%); δ_H [360 MHz, $(CD_3)_2CO$] see Table 112; m/z (FAB) 192.08720 (M^++H), $C_7H_{14}NO_5$ requires 192.08719.

3.7.12 5S-5-(1,3-O-*Ethylidene*-D-erythro-*triitol*-1-*yl*)-3-*phenyl*-2-*isoxazoline* (265)

Using the general procedure above, isoxazoline (**265**) (97 mg, 0.37 mmol) afforded 5S-5-(D-erythro-*triitol*-1-*yl*)-3-*phenyl*-2-*isoxazoline* (**279**) as white needles (72 mg, 82%), m.p. 115.5-119.5°C; $[\alpha]_D^{24}$ +99.6° (c = 0.55, CH_3OH); [Found : C, 61.1; H, 6.6; N, 5.4. $C_{12}H_{15}NO_4$ requires C, 60.8; H, 6.3; N, 5.9]; δ_H [360 MHz, $(CD_3)_2CO$] see Table 113; δ_C [90 MHz, $(CD_3)_2CO$] 156.5 (C_3), 129.8, 128.8, 126.7 (5 × PhCH), 82.6 (C_5), 72.8 (C_6), 71.9 (C_7), 64.0 (C_8), 34.1 (C_2); m/z (EI) 237.1001 (M^+), $C_{12}H_{15}NO_4$ requires 237.1002.



(281)



(282)

Resonance	δ_H/ppm		Coupling	*J*/Hz	
	(281)	(282)		(281)	(282)
4a	3.16	3.11	4a,4b	17.1	-
4b	3.00	3.10	4a,5	9.0	10.5
5	4.79	4.83	4b,5	10.9	9.4
6	3.81-3.50	3.89-3.44	5,6	3.6	3.0
7	3.81-3.50	3.89-3.44	6,7	nd	nd
8a	3.81-3.50	3.89-3.44	7,8a	nd	nd
8e	3.81-3.50	3.89-3.44	7,8e	nd	nd
C*H*$_2$OH	4.29	4.28	8a,8e	nd	nd
CH$_2$O*H*	3.91	nd	7,7OH	4.7	nd
7-OH	4.12	nd	C*H*$_2$OH,O*H*	5.0	nd

Table 112

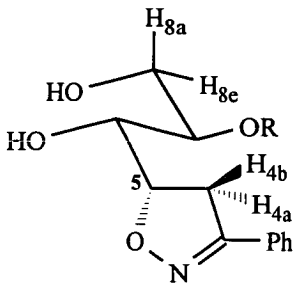
3.7.13 5S-5-(2-O-*Acetyl*-1,3-O-*ethylidene*-D-erythro-*triitol*-1-*yl*)-3-*phenyl* 2-*isoxazoline* (272)

Using the general procedure above, isoxazoline (**272**) (150 mg, 0.49 mmol) afforded two products after preparative TLC. 5S-5-(D-erythro-*triitol*-1-*yl*)-3-*phenyl*-2-*isoxazoline* (**279**) was isolated as white needles (14 mg, 12%), m.p. 115-119°C; δ_H [360 MHz, $(CD_3)_2CO$] see Tables 113 and 114; δ_C [62 MHz, $(CD_3)_2CO$] 156.5 (C_3), 129.8, 128.8, 126.7 (5 × PhCH), 82.6 (C_5), 72.8 (C_6), 71.9 (C_7), 64.0 (C_8), 34.0 (C_4); m/z (FAB) 238.10794 (M^++H), $C_{12}H_{16}NO_4$ requires 238.10792. This product (**279**) is identical to that obtained from deprotection of 5S-5-(1,3-O-*ethylidene*-D-erythro-*triitol*-1-*yl*)-3-*phenyl*-2-*isoxazoline* (**265**). (See Section 3.7.12). The desired product 5S-5-(2-O-*acetyl*-D-erythro-*triitol*-1-*yl*)-3-*phenyl*-2-*isoxazoline* (**284**) was also afforded as a white solid (32 mg, 24%); δ_H [360 MHz, $(CD_3)_2CO$] see Tables 113 and 114; m/z (FAB) 280.11850 (M^++H). $C_{14}H_{18}NO_5$ requires 280.11849.

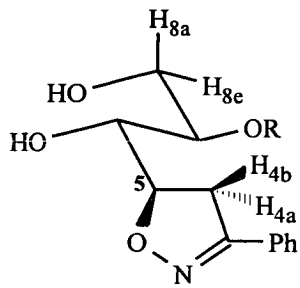
3.7.14 5R-5-(2-O-*Acetyl*-1,3-O-*ethylidene*-D-erythro-*triitol*-1-*yl*)-3-*phenyl*-2-*isoxazoline* (273)

Using the general procedure above, isoxazoline (**273**) (100 mg, 0.33 mmol) afforded two products after preparative TLC. 5R-5-(D-erythro-*triitol*-1-*yl*)-3-*phenyl*-2-*isoxazoline* (**280**) was isolated as a pale yellow oil (8 mg, 10 %); δ_H [250 MHz, $(CD_3)_2CO$] see Tables 113 and 114; δ_C [62 MHz, $(CD_3)_2CO$] 155.6 (C_3), 130.3, 129.5, 127.9, 127.7, 125.6 (5 × PhCH), 79.5 (C_5), 72.5 (C_6), 70.8 (C_7), 63.2 (C_8), 35.5 (C_4); m/z (FAB) 238.10814 (M^++H), $C_{12}H_{16}NO_4$ requires 238.10794. The desired product 5R-5-(2-O-*acetyl*-D-erythro-*triitol*-1-*yl*)-3-*phenyl*-2-*isoxazoline* (**285**)

was also afforded as a pale yellow oil (23 mg, 25%); δ_H [360 MHz, $(CD_3)_2CO$] see Tables 113 and 114; δ_C [90 MHz, $(CD_3)_2CO$] 156.7 (C_3), 129.9, 128.9, 126.7 (5 × PhCH), 80.6 (C_5), 73.0 (C_6), 70.0 (C_7), 66.7 (C_8), 36.6 (C_4), 20.2 (CH_3CO); m/z (FAB) 280.11850 (M^++H), $C_{14}H_{18}NO_5$ requires 280.11849.



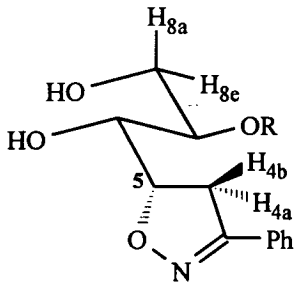
(**279**) R = H
(**284**) R= Ac



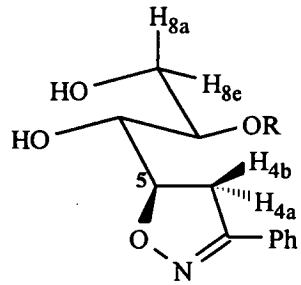
(**280**) R= H
(**285**) R = Ac

R	H		$COCH_3$	
Resonance	δ_H/ppm		δ_H/ppm	
	(279)	(280)	(284)	(285)
4a	3.51	3.47	3.51	3.51
4b	3.34	3.44	3.36	3.43
5	5.01	5.06	5.03	5.08
6	3.98	3.58	3.91	3.58
7	3.93	3.95	3.37	3.98
8a	3.69	3.78-3.75	4.18	4.18
8e	3.81	3.78-3.75	3.36	4.41
Ph	7.74-7.37	7.74-7.42	7.73-7.41	7.72-7.41
7OH	4.27		-	-
$COCH_3$	-	-	2.07-2.05	2.10-2.00

Table 113



(**279**) R = H
(**284**) R = Ac



(**280**) R = H
(**285**) R =Ac

R	H		$COCH_3$	
Coupling	*J*/Hz		*J*/Hz	
	(279)	(280)	(284)	(285)
4a,4b	16.7	16.3	16.7	16.8
4a,5	8.9	10.7	8.7	11.1
4b,5	11.1	9.1	11.0	8.9
5,6	3.3	2.6	3.4	2.5
6,7	7.0	7.7	4.7	8.9
7,8a	5.8	5.4	6.5	6.3
7,8e	3.7	3.7	3.0	2.6
8a,8e	10.9	nd	11.4	11.4

Table 114

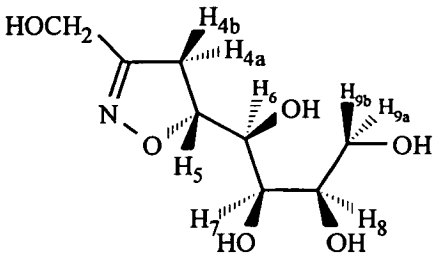
3.7.15 5S/R-5-(1,3-O-*ethylidene*-D-erythro-*triitol*-1-*yl*)-3-(*dideoxyphosphoryl-methyl*)-2-*isoxazolines* (267)/(268)

Using the general procedure above, isoxazoline (**267**)(**268**) (150 mg, 0.63 mmol) afforded after preparative TLC (5 : 0.5 ethyl acetate/ethanol) 5S/R-5-(D-erythro-*triitol*-1-*yl*)-3-(*dideoxyphosphorymethyl*)-2-*isoxazolines* (**283**) as a colourless oil (50 mg, 36%), a mixture of both isomers, which crystallised from acetone to give

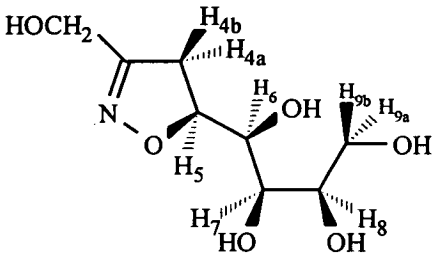
fine needles; δ_H [360 MHz, $(CD_3)_2CO$] 4.86 (1H, m, H_5), 4.13 (2H, t, J_{CH3CH2} = 7.0 Hz, CH_3CH_2), 3.79 (1H, b, H_{8e}), 3.67 (1H, b, H_{8a}), 3.18-3.05 (2H, 2 × dd, H_{4a} and H_{4b}), 2.99 [2H, d, $J_{CH2,P}$ = 21.6 Hz, $CH_2P(O)$], 1.30 (3H, t, J_{CH3CH2} = 7.0 Hz, CH_3CH_2); m/z (FAB) 312.12123 (M^++H), $C_{11}H_{23}NO_7P$ requires 312.12120.

3.7.16 5R-5-(1,2:3,4-*Di*-O-*isopropylidene*-D-arabino-*tetritol*-1-*yl*)-3-*hydroxymethyl*-2-*isoxazoline* (305) and 5S-5-(1,2:3,4-*di*-O-*isopropylidene*-D-arabino-*tetritol*-1-*yl*)-3-*hydroxymethyl*-2-*isoxazoline* (306)

Using the general procedure above, isoxazolines (**305**) and (**306**) (50 mg, 0.17 mmol) afforded a mixture of 5R-5-(D-arabino-*tetritol*-1-*yl*)-3-*hydroxymethyl*-2-*isoxazoline* (**307**) and 5S-5-(D-arabino-*tetritol*-1-*yl*)-3-*hydroxymethyl*-2-*isoxazoline* (**308**) as a white solid recrystallised from isopropanol (36 mg, 98%); δ_H (360 MHz, D_2O) see Tables 115 and 116; δ_C (90 MHz, D_2O) 161.2 (C_3, *erythro*), 160.9 (C_3, *threo*), 81.8 (C_5, *threo*), 80.3 (C_5, *erythro*), 70.9 (C_6, *threo*), 70.7 (C_6, *erythro*), 69.9 (C_7, *erythro*, C_7, *threo*), 69.3 (C_8, *erythro*, C_8, *threo*), 62.9 (C_9, *erythro)*, 62.7 (C_9, *threo*), 56.50 (CH_2OH, *erythro*), 56.47 (CH_2OH, *threo*), 36.9 (C_4, *threo*), 36.6 (C_4, *erythro*); m/z (EI) 222.09774 (M^++H), $C_8H_{16}NO_6$ requires 222.09775.



(307)

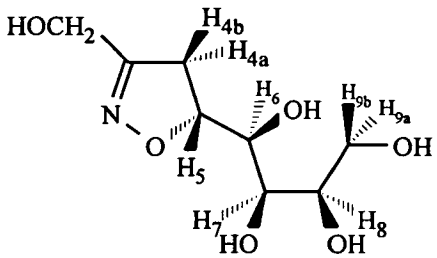


(308)

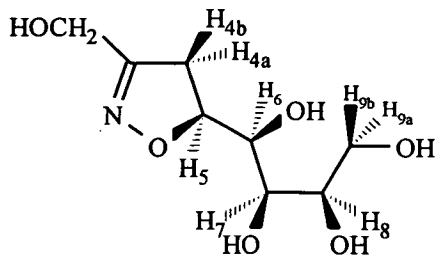
Resonance	δ_H/ppm			
	(307)[a]	(308)[a]	(307)[b]	(308)[c]
4a	3.21	3.24	3.25	3.28
4b	3.21	2.96	3.25	3.00
5	4.82	4.82	4.80	4.86
6	3.91	3.86	3.95	3.90
7	3.64	3.59	3.69	3.63
8	3.73	3.76	3.78	3.80
9a	3.65	3.65	3.69	3.69
9b	3.84	3.83	3.88	3.87
CH_2OH	4.39	4.48	4.43	4.42

Table 115

[a] Product from deprotection of **(305)**/**(306)**
[b] Product from deprotection of **(327)**
[c] Product from deprotection of **(328)**



(307)



(308)

Coupling	*J*/Hz			
	(307)[a]	(308)[a]	(307)[b]	(308)[b]
4a,4b	-	17.6	-	17.6
4a,5	7.0	10.7	8.4	10.6
4b,5	9.9	8.3	10.6	8.3
5,6	6.8	6.9	6.7	7.1
6,7	1.5	2.2	1.4	2.0
7,8	8.8	8.4	8.7	8.3
8,9a	6.2	6.2	6.2	6.0
8,9b	2.8	2.8	2.7	2.8
9a,9b	11.8	11.5	11.7	11.6

Table 116

[a] Product from deprotection of **(305)**/**(306)**
[b] Product from deprotection of **(327)**
[c] Product from deprotection of **(328)**

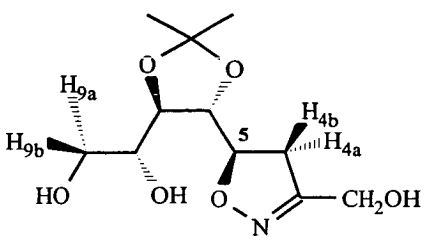
3.7.17 5R-5-(1,2:3,4-*Di*-O-*isopropylidene*-D-arabino-*tetritol*-1-*yl*)-3-*hydroxymethyl*-2-*isoxazoline* (305) and 5S-5-(1,2:3,4-*di*-O-*isopropylidene*-D-arabino-*tetritol*-1-*yl*)-3-*hydroxymethyl*-2-*isoxazoline* (306)

Isoxazolines (**305**) and (**306**) (50 mg, 0.17 mmol) in a mixture of water/glacial acetic acid (2 : 3) were stirred at 40°C for 16 hr. Solvent was removed *in vacuo*, and after repeated addititon (4 × 10 ml) and removal of a 1 : 1 mixture of toluene and heptane, the residue was triturated with cold petroleum ether/ether (1 : 1) to afford a mixture of 5R-5-(3,4-O-*isopropylidene*-D-arabino-*tetritol*-1-*yl*)-3-*hydroxymethyl*-2-*isoxazoline* (**309**) and 5S-5-(3,4-O-*isopropylidene*-D-arabino-*tetritol*-1-*yl*)-3-*hydroxymethyl*-2-*isoxazoline* (**310**) as a white solid (25 mg, 66%) after recrystallisation from hot ethanol, which were unable to be separated, δ_H (360 MHz, D_2O) see Table 117; δ_C (90 MHz, D_2O) 160.8 (C_3, *erythro*), 160.6 (C_3, *threo*), 110.7 [*C*$(Me)_2$], 81.7, 80.7, 80.4, 80.3, 79.3, 79.0, 77.5, 76.5, 72.2, 70.8, 70.6, 69.8, 69.2 (C_5, C_6, C_7, C_8), 63.0 (C_9, *threo*), 62.9 (C_9, *erythro*), 56.5 (*C*H_2OH, *threo*), 56.4 (*C*H_2OH, *erythro*), 36.7 (C_4, *threo*), 35.2 (C_4, *erythro*), 26.3, 26.0, 25.9, 25.8 (*C*H_3); m/z (FAB) 262.12871 (M^++H), $C_{11}H_{20}NO_6$ requires 262.12906.

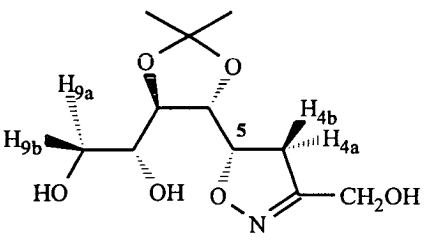
3.7.18 5S-3-*Ethoxycarbonyl*-5-(6R,7R,14S-1,8,13,16-*tetraoxadispiro*[5.0.5.4]-*hexadecan*-14-*yl*)-4,5-*dihydroisoxazole* (227)

Isoxazoline (**227**) (30 mg, 81.30 mmol) in a mixture of TFA (1 ml) and water (0.1 ml) was stirred at room temperature for 1 hr. Solvent was removed *in vacuo* and after repeated addition (4 × 10 ml) and removal of a 1 : 1 mixture of toluene and heptane, the residue was purified by preparative TLC on silica gel using

100 % ether as eluent, to afford 5S-5-(D-glycero-*diitol*-1-*yl*)-3-*ethoxycarbonyl*-2-*isoxazoline* (**250**) as a yellow oil (15 mg, 91%); δ_H [360 MHz, $(CD_3)_2CO$] see Table 107; m/z (FAB) 204.08720 (M^++H), $C_8H_{13}NO_5$ requires 204.08719. This product is identical to that formed by deprotection of isoxazoline (**198**) (Section 3.7.1).



(309)



(310)

Resonanc	δ_H/ppm		Coupling	*J*/Hz	
	(309)	(310)		(309)	(310)
4a	3.29	3.05	4a,4b	17.1	17.4
4b	3.22	3.36	4a,5	10.9	8.3
5	4.97	4.86	4b,5	7.4	11.0
6	3.95	3.90	5,6	3.6	3.3
7	3.69	3.63	6,7	6.9	6.7
8	3.86	3.86	7,8	8.4	8.4
9a	3.78	3.77	8,9a	2.6	2.6
9b	3.69	3.63	8,9b	6.0	6.0
CH_2OH	4.40	4.40	9a,9b	11.5	11.4

Table 117

3.8 Acid Hydrolysis Followed by Reduction

3.8.1 5R-5-(1-O-*Methyl*-α-D-lyxo-*tetrofuranoside*-4-*yl*)-3-*hydroxymethyl*-2-*isoxazoline* (329)

A solution of octofuranose derivative 5R-5-(2,3-O-*isopropylidene*-1-O-*methyl*-α-D-lyxo-*tetrofuranoside*-4-*yl*)-3-*hydroxymethyl*-2-*isoxazoline* (**327**) (100 mg, 0.37 mmol) in TFA/water (9 : 1) (3 ml) was kept at room temperature for 20 min and then evaporated under diminished pressure with occassional additions of water. The residue was purified by dry flash chromatography on silica using ethyl acetate as eluent, to afford the partially deprotected product 5R-5-(1-O-*methyl*-α-D-lyxo-*tetrofuranoside*-4-*yl*)-3-*hydroxymethyl*-2-*isoxazoline* (**329**) as a colourless oil (60 mg, 70%); δ_H (360 MHz, D_2O) see Tables 118 and 119; δ_C (90 MHz, D_2O) 159.2 (C_3), 106.2 (C_9), 77.6 (C_5), 76.1 (C_6), 73.6 (C_7), 68.4 (C_8), 54.3 (CH_2OH), 53.9 (OMe), 34.7 (C_4); m/z (FAB) 234.09776 (M^++H), $C_9H_{16}NO_6$ requires 234.09775.

3.8.2 5R-5-(α-D-lyxo-*tetrofuranoside*-4-*yl*)-3-*hydroxymethyl*-2 *isoxazoline* (331)

A solution of octofuranose derivative 5R-5-(2,3-O-*isopropylidene*-1-O-*methyl*-α-D-lyxo-*tetrofuranoside*-4-*yl*)-3-*hydroxymethyl*-2-*isoxazoline* (**327**) (50 mg, 0.18 mmol) in TFA/water (9 : 1) (2 ml) was kept at 40°C for 2 hr. Evaporation under vacuum with occassional additions of water afforded 5R-5-(α-D-lyxo-*tetrofuranoside*-4-*yl*)-3-*hydroxymethyl*-2-*isoxazoline* (**331**) as a colourless oil (24 mg, 60%), a mixture of isomers (50 : 50) at the anomeric centre C_9; δ_H (360 MHz, D_2O) see Tables 118 and 119; δ_C (90 MHz, D_2O) 163.9 (C_3), 103.4, 98.0 (C_9), 81.9, 81.0,

79.2, 73.3 (C_5, C_6, C_7, C_8), 59.0 (CH_2OH), 39.3 (C_4); m/z (FAB) 220.08213 (M^++H), $C_8H_{14}NO_6$ requires 220.08210.

3.8.3 5R-5-(D-arabino-*tetritol*-1-*yl*)-3-*hydroxymethyl*-2-*isoxazoline* (307)

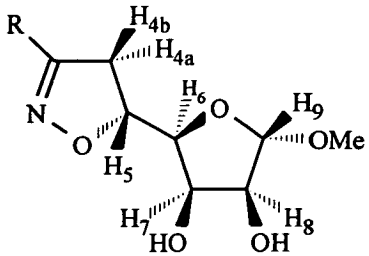
A solution of octofuranose derivative 5R-5-(2,3-O-*isopropylidene*-1-O-*methyl*-α-D-lyxo-*tetrofuranoside*-4-*yl*)-3-*hydroxymethyl*-2-*isoxazoline* (**327**) (100 mg, 0.37 mmol) in TFA/water (9 : 1) (3 ml) was kept at 50°C for 3 hr and then evaporated under diminished pressure with occassional additions of water. To a cooled (0°C) and stirred solution of the residue in water (6 ml) was gradually added sodium borohydride (0.1 g, 2.60 mmol), and the reaction mixture stirred for 3 hr at 0°C, and then overnight at room temperature. Sodium ions were removed from the reaction mixture with Amberlite IR-200 (H^+) resin (3.5 g), and the resin filtered off and washed thoroughly with water. The filtrate and washings were combined and evaporated *in vacuo*, and methanol was added to, and evaporated from, the residue until no boric acid remained. The residue was triturated with methanol and yielded 5R-5-(D-arabino-*tetritol*-1-*yl*)-3-*hydroxymethyl*-2-*isoxazoline* (**307**) as a white solid (60 mg, 74%), m.p. 139-142°C (from methanol); $[\alpha]_D^{25}$ -76.2° (c = 0.55, $CHCl_3$); δ_H (360 MHz, D_2O) see Tables 115 and 116; δ_C (62 MHz, D_2O) 159.1 (C_3), 78.3 (C_5), 68.6 (C_6), 67.8 (C_7), 67.2 (C_8), 60.9 (C_9), 54.4 (CH_2OH), 34.6 (C_4); m/z (FAB) 222.09984 (M^++H), $C_8H_{16}NO_6$ requires 222.09776.

3.8.4 5S-5-(D-arabino-*tetritol*-1-*yl*)-3-*hydroxymethyl*-2-*isoxazoline* (308)

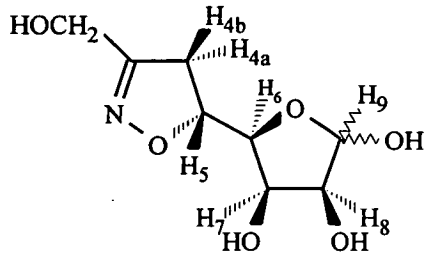
A solution of octofuranose derivative 5S-5-(2,3-O-*isopropylidene*-1-O-*methyl*-α-D-lyxo-*tetrofuranoside*-4-*yl*)-3-*hydroxymethyl*-2-*isoxazoline* (**328**) (100 mg, 0.37 mmol) in TFA/water (9 : 1) (3 ml) was kept at 50°C for 3 hr. The reaction mixture was worked up in the same way as described in the preparation of 5R-5-(D-arabino-*tetritol*-1-*yl*)-3-*hydroxymethyl*-2-*isoxazoline* (**307**). 5S-5-(D-arabino-*tetritol*-1-*yl*)-3-*hydroxymethyl*-2-*isoxazoline* (**308**) was afforded as a white solid (65 mg, 80%); $[\alpha]_D^{25}$ +100.1° (c = 1.02, CH_3OH); δ_H (360 MHz, D_2O) see Tables 115 and 116; δ_C (90 MHz, D_2O) 160.9 (C_3), 81.7 (C_5), 70.9 (C_6), 70.6 (C_7), 70.5 (C_8), 62.7 (C_9), 56.5 (CH_2OH), 36.9 (C_4); m/z (FAB) 222.09624 (M^++H), $C_8H_{16}NO_6$ requires 222.09776.

3.8.5 5R-5-(1-O-*Methyl*-α-D-lyxo-*tetrofuranoside*-4-*yl*)-3-*phenyl*-2-*isoxazoline* (330)

Using the same procedure as for 5R-5-(1-O-*methyl*-α-D-lyxo-*tetrofuranoside*-4-*yl*)-3-*hydroxymethyl*-2-*isoxazoline* (**329**) (Section 3.8.1), octofuranose derivative 5R-5-(2,3-O-*isopropylidene*-1-O-*methyl*-α-D-lyxo-*tetrofuranoside*-4-*yl*)-3-*phenyl*-2-*isoxazoline* (**321**) (100 mg, 0.31 mmol) afforded the partially deprotected product 5R-5-(1-O-*methyl*-α-D-lyxo-*tetrofuranoside*-4-*yl*)-3-*phenyl*-2-*isoxazoline* (**330**) as a colourless oil (51 mg, 61%); δ_H (360 MHz, D_2O) see Tables 118 and 119; δ_C (62 MHz, D_2O) 157.5 (C_3), 128.8, 126.8, 126.0, 124.7 (5 × PhCH), 106.2 (C_9), 77.8 (C_5), 76.7 (C_6), 73.6 (C_7), 68.5 (C_8), 53.9 (OMe), 34.7 (C_4); m/z (FAB) 280.111850 (M^++H), $C_{14}H_{18}NO_5$ requires 280.11849.



(329) R = CH_2OH
(330) R = Ph



(331)

Resonance	δ_H/ppm		
	(329)	**(330)**	**(331)**
4a	3.19	3.63	3.20, 3.33
4b	3.22	3.64	3.27, 3.22
5	4.88	5.06	5.01, 4.93
6	4.28	4.39	4.23
7	4.34	4.44	4.40
8	4.14	4.21	4.35, 4.14
9	4.99	5.04	5.33
OMe	4.44	3.49	-
CH_2OH	4.38	-	4.43
Ph	-	7.76-7.53	-

Table 118

Coupling	*J*/Hz		
	(329)	**(330)**	**(331)**
4a,4b	-	-	17.6
4a,5	8.6	8.4	8.4, 8.0
4b,5	10.1	10.0	10.0, 10.2
5,6	5.9	5.7	5.3, 5.4
6,7	3.8	4.0	3.7
7,8	4.8	4.6	5.9, 6.3
8,9	4.1	4.0	5.1

Table 119

3.9 Mesylation

3.9.1 5S-5-(1,2-O-*Cyclohexylidene*-D-glycero-*diitol*-1-*yl*)-3-*methanesulphonyl-oxymethyl*-2-*isoxazoline* (258)

To an ice-chilled solution of the alcohol (**204**) (50 mg, 0.21 mmol) in dichloromethane (1 ml) was added successively triethylamine (42 mg, 0.42 mmol) and methanesulphonyl chloride (47 mg, 0.41 mmol) under argon. After stirring for 4 hr the mixture was concentrated *in vacuo*, dichloromethane (10 ml) was added and then washed with water (2 × 4 ml). The organic fraction was dried ($MgSO_4$), evaporated *in vacuo* and the crude product purified by preparative TLC on silica gel (50/50 ethyl acetate/hexane) to afford 5S-5-(1,2-O-*cyclohexylidene*-D-glycero-*diitol*-1-*yl*)-3-*methanesulphonyloxymethyl*-2-*isoxazoline* (**258**) (64 mg, 96%), very pale yellow oil; $[\alpha]_D^{26}$ +33.8° (c = 1.58, $CHCl_3$); δ_H (360 MHz, $CDCl_3$) 4.93 (2H, s, CH_2OMs), 4.60 (1H, ddd, $J_{4a,5}$ = 7.1 Hz, $J_{4b,5}$ = 10.1 Hz, $J_{5,6}$ = 3.8 Hz, H_5), 4.07 (1H, dd, $J_{6,7a}$ = 6.3 Hz, $J_{7a,7b}$ = 7.8 Hz, H_{7a}), 4.03 (1H, ddd, $J_{5,6}$ = 3.8 Hz, $J_{6,7a}$ = 6.3 Hz, $J_{6,7b}$ = 4.0 Hz, H_6), 3.80 (1H, dd, $J_{6,7b}$ = 4.0 Hz, $J_{7a,7b}$ = 7.8 Hz, H_{7b}), 3.15 (1H, dd, $J_{4b,5}$ = 10.1 Hz, $J_{4a,4b}$ = 17.5 Hz, H_{4b}), 3.08 (1H, dd, $J_{4a,5}$ = 7.1 Hz, $J_{4a,4b}$ = 17.5 Hz, H_{4a}), 3.06 (3H, s, $MeSO_2$), 1.60-1.51 (10H, m, cyclohexyl.CH_2); δ_C (90 MHz, $CDCl_3$) 153.2 (C_3), 110.4 (cyclohexyl. quat.), 81.9 (C_6), 75.3 (C_5), 66.4 (C_7), 63.0 (CH_2OMs), 37.8 (Me_{Ms}), 36.9 (C_4), 36.3, 34.4, 31.4, 24.9, 23.8, 23.6 (cyclohexyl. CH_2); m/z (FAB) 319.10780 (M^++H), $C_{13}H_{21}NO_4S$ requires 319.10896.

3.9.2 5R-5-(1,2:3,4-*Di*-O-*isopropylidene*-D-arabino-*tetritol*-1-*yl*)-3-*methane-sulphonyloxymethyl*-2-*isoxazoline* (315) and 5S-5-(1,2:3,4-*di*-O-*isopropylidene*-D-arabino-*tetritol*-1-*yl*)-3-*methanesulphonyloxymethyl*-2-*isoxazoline* (316)

Obtained from (**305**)/(**306**) (50 mg, 0.17 mmol) using the same procedure as for (**258**), (**315**)/(**316**) was isolated as a colourless oil (50 mg, 80%); δ_H (360 MHz, $CDCl_3$) 4.94 (CH_2OMs), 4.93 [0.59H, ddd, $J_{4a,5}$ = 11.5 Hz, $J_{4b,5}$ = 7.4 Hz, $J_{5,6}$ = 3.3 Hz, H_5, (*erythro*)], 4.82 [0.41H, ddd, $J_{4a,5}$ = 7.0 Hz, $J_{4b,5}$ = 9.5 Hz, $J_{5,6}$ = 2.2 Hz, H_5, (*threo*)], 4.17 [0.59H, dd, $J_{5,6}$ = 3.3 Hz, $J_{6,7}$ = 7.5 Hz, H_6, (*erythro*)], 4.11 [0.59H, dd, $J_{8,9b}$ = 6.1 Hz, $J_{9a,9b}$ = 8.2 Hz, H_{9b} (*erythro*)], 4.10 [0.41H, dd, $J_{8,9b}$ = 6.3 Hz, $J_{9a,9b}$ = 7.9 Hz, H_{9b} (*threo*)], 4.13-3.87 [m, H_7, H_8 (*erythro*), H_6, H_7, H_8 (*threo*)], 4.06 [0.41H, dd, $J_{8,9a}$ = 4.5 Hz, $J_{9a,9b}$ = 8.2 Hz, H_{9a} (*threo*)], 3.91 [0.59H, dd, $J_{8,9a}$ = 3.5 Hz, $J_{9a,9b}$ = 7.9 Hz, H_{9a} (*erythro*)], 3.14 [0.59H, dd, $J_{4b,5}$ = 7.4 Hz, $J_{4a,4b}$ = 17.0 Hz, H_{4b}, (*erythro*)], 3.08 [1H, dd, $J_{4a,5}$ = 11.5 Hz (*erythro*), $J_{4a,4b}$ =17.0 Hz (*erythro*), $J_{4b,5}$ = 9.5 Hz (*threo*), $J_{4a,4b}$ = 17.0 Hz (*threo*)], 3.06 [0.41H, dd, $J_{4a,5}$ = 7.0 Hz, $J_{4a,4b}$ = 17.0 Hz, H_{4a} (*threo*)], 1.40-1.30 (15H, m, isopropyl. CH_3 and SO_2CH_3); δ_C (90 MHz, $CDCl_3$) 152.9 (C_3, *erythro*), 152.7 (C_3, *threo*), 110.3, 109.9 [$C(Me)_2$, *erythro*], 109.74, 109.68 [$C(Me)_2$, *threo*], 81.9, 81.3, 80.1, 79.9, 78.6, 77.2, 76.9, 76.6 (C_5, C_6, C_7, C_8), 67.8 (C_9, *threo*), 67.3 (C_9, *erythro*), 63.4 (CH_2OMs, *threo*), 63.2 (CH_2OMs), *erythro*), 37.9 (Me_{Ms}, *threo*), 37.8 (Me_{Ms}, *erythro*), 36.9 (C_4, *threo*), 34.6 (C_4, *erythro*), 27.1, 27.0, 26.6, 26.3, 25.1, 25.0 [$C(Me)_2$]; m/z (FAB) 380.13627 (M^++H), $C_{15}H_{25}NO_8S$ requires 380.13791.

3.9.3 5R-5-(2,3-O-*Isopropylidene*-1-O-*methyl*-α-D-lyxo-*tetrofuranoside*-4-*yl*)-3-*methanesulphonyloxymethyl*-2-*isoxazoline* (343)

Obtained from (**327**) (39 mg, 0.14 mmol) using the same procedure as for (**258**), (**343**) was isolated as a pale yellow oil (40 mg, 81%); δ_H (360 MHz, $CDCl_3$) 4.97 (2H, s, CH_2OMs), 4.97 (1H, ddd, $J_{4a,5}$ = 8.3 Hz, $J_{4b,5}$ = 10.8 Hz, $J_{5,6}$ = 5.3 Hz, H_5), 4.89 (1H, s, H_9), 4.74 (1H, dd, $J_{6,7}$ = 3.8 Hz, $J_{7,8}$ = 5.9 Hz, H_7), 4.55 (1H, d, $J_{7,8}$ = 5.9 Hz, H_8), 4.07 (1H, dd, $J_{5,6}$ = 5.3 Hz, $J_{6,7}$ = 3.8 Hz, H_6), 3.29 (3H, s, OMe), 3.22 (1H, dd, $J_{4a,5}$ = 8.3 Hz, $J_{4a,4b}$ = 17.7 Hz, H_{4a}), 3.13 (1H, dd, $J_{4b,5}$ = 10.8 Hz, $J_{4a,4b}$ = 17.7 Hz, H_{4b}), 3.06 (3H, s, $MeSO_2$), 1.44, 1.28 [6H, m, $C(Me)_2$]; δ_C (90 MHz, $CDCl_3$) 153.6 (C_3), 112.7 [$C(Me)_2$], 107.1 (C_9), 84.7 (C_6), 79.2, 79.14, 79.07 (C_5, C_7, C_8), 63.3 (CH_2OMs), 54.7 (OMe), 37.9 (Me_{Ms}), 36.7 (C_4), 25.6, 24.2, [$C(CH_3)_2$]; m/z (FAB) 352.10982 (M^++H), $C_{13}H_{22}NO_8S$ requires 352.10661.

3.10 Tosylation

3.10.1 5S-5-(1,2-O-*Cyclohexylidene*-D-glycero-*diitol*-1-*yl*)-3-p-*toluenesulphonyl-oxymethyl*-2-*isoxazoline* (259)

To an ice-chilled solution of the alcohol (**204**) (52 mg, 0.22 mmol) in dichloromethane (1 ml) was added successively triethylamine (44 mg, 0.43 mmol) and *p*-toluenesulphonyl chloride (82 mg, 0.43 mmol) under argon. After stirring for 2.5 hr the mixture was concentrated *in vacuo*, dichloromethane (10 ml) was added and then washed with water (2 × 4 ml). The organic fraction was dried ($MgSO_4$), evaporated *in vacuo* and the crude product purified by preparative TLC on silica gel

(50/50 ethyl acetate/hexane) to afford 5S-5-(1,2-O-*cyclohexylidene*-D-glycero-*diitol*-1-*yl*)-3-p-*toluenesulphonyloxymethyl*-2-*isoxazoline* (**259**) (34 mg, 40%), pale yellow oil; δ_H (360 MHz, $CDCl_3$) 7.90 (2H, d, $J_{o,m}$ = 8.3 Hz, 2 × PhCH), 7.39 (2H, d, $J_{o,m}$ = 8.3 Hz, 2 × PhCH), 4.58 (1H, ddd, $J_{4a,5}$ = 7.0 Hz, $J_{4b,5}$ = 10.4 Hz, $J_{5,6}$ = 7.0 Hz, H_5), 4.28 (2H, s, CH_2OTs), 4.08 (1H, dd, $J_{6,7a}$ = 6.3 Hz, $J_{7a,7b}$ = 8.3 Hz, H_{7a}), 4.04 (1H, ddd, $J_{5,6}$ = 6.3 Hz, $J_{6,7a}$ = 6.3 Hz, $J_{6,7b}$ = 4.3 Hz, H_6), 3.84 (1H, dd, $J_{6,7b}$ = 4.3 Hz, $J_{7a,7b}$ = 8.3 Hz, H_{7b}), 3.18 (1H, dd, $J_{4b,5}$ = 10.4 Hz, $J_{4a,4b}$ = 17.5 Hz, H_{4b}), 3.10 (1H, dd, $J_{4a,5}$ = 7.0 Hz, $J_{4a,4b}$ = 17.5 Hz, H_{4a}), 2.47 (3H, s, Me_{Tos}), 1.59-1.54 (10H, m, CH_2).

3.11 Reduction of Ester to Aldehyde

3.11.1 5R-5-(2,3-O-*Isopropylidene*-1-O-*methyl*-α-D-lyxo-*tetrofuranoside*-4-*yl*)-3-*formyl*-2-*isoxazoline* (344)

DIBAL (1 M in hexanes, 24 ml, 24 mmol) was added slowly to a -78°C solution of ester (**323**) (200 mg, 0.63 mmol) in dry toluene (8 ml) (temperature maintained below -70°C throughout DIBAL addition). After stirring the mixture for a further 2 hr at -78°C, the reaction was quenched by slow addition of cold (-78°C) methanol at such a rate that the internal temperature did not exceed -65°C. The mixture was allowed to warm to room temperature, poured onto ice cold 1 M HCl (100 ml) and extracted with ethyl acetate (3 × 100 ml). The combined organics were washed with brine (2 × 60 ml), dried ($MgSO_4$) and evaporated *in vacuo* to an oil which was purified by preparative TLC on silica gel (100% ethyl acetate) yielding

aldehyde (**344**) as a colourless oil (135 mg, 68%); $[\alpha]_D^{25}$ -39.4° (c = 1.37, $CHCl_3$); δ_H (360 MHz, $CDCl_3$) see Table 120; δ_C (90 MHz, $CDCl_3$) 185.6 (*C*HO), 159.8 (C_3), 112.8 [*C*$(Me)_2$], 107.1 (C_9), 84.7, 82.1, 79.0, 78.9 (C_5, C_6, C_7, C_8), 54.7 (OMe), 32.2 (C_4), 25.6, 24.1 (2 × Me); m/z (FAB) 272.11372 (M^++H), $C_{12}H_{18}NO_6$ requires 272.11341.

3.12 Oxidation of Alcohol to Aldehyde

General Procedure

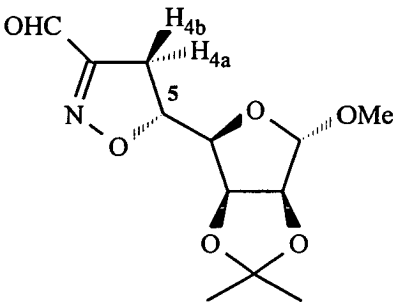
Pyridinium chlorochromate (316 mg, 1.47 mmol) was suspended in dry dichloromethane (13 ml) and a solution of the alcohol (150 mg, 0.55 mmol) in dichloromethane (6 ml) added. After stirring for 3 hr, TLC (100% ether) indicated all starting material had been consumed. The reaction mixture was diluted with dry ether (70 ml), and the supernatant liquid decanted off. The residual brown sludge was washed with ether (3 × 20 ml), and the combined organic extract filtered through a silica pad and then concentrated *in vacuo*.

3.12.1 5R-5-(2,3-O-*Isopropylidene*-1-O-*methyl*-α-D-lyxo-*tetrofuranoside*-4-*yl*)-3-*formyl*-2-*isoxazoline* (344)

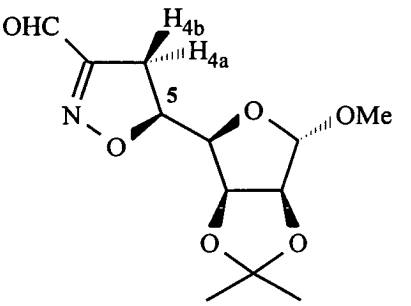
Obtained from (**327**) (150 mg, 0.55 mmol) as a colourless oil (104 mg, 70%) identical to that formed by reduction of ester (**328**). For characterisation see Section 3.11.1.

3.12.2 5S-5-(2,3-O-*Isopropylidene*-1-O-*methyl*-α-D-lyxo-*tetrofuranoside*-4-*yl*)-3-*formyl*-2-*isoxazoline* (345)

Obtained from (**328**) (50 mg, 0.18 mmol) as a white solid (27 mg, 54%), m.p. 91.9-92.2°C; $[\alpha]_D^{25}$ +5.9° (c = 0.89, $CHCl_3$); δ_H (360 MHz, $CDCl_3$) see Table 120; δ_C (90 MHz, $CDCl_3$) 185.3 (*C*HO), 159.1 (C_3), 113.0 [*C*(Me)$_2$], 107.5 (C_9), 84.6, 83.8, 80.8, 79.4 (C_5, C_6, C_7, C_8), 54.8 (OMe), 33.0 (C_4), 25.7, 24.5 (2 × Me); m/z (FAB) 272.11159 (M^++H), $C_{12}H_{18}NO_6$ requires 272.11341.



(344)



(345)

Resonance	δ_H/ppm		Coupling	*J*/Hz	
	(344)	(345)		(344)	(345)
4a	3.26	3.30	4a,4b	17.9	17.8
4b	3.12	2.90	4a,5	8.4	11.3
5	5.10	5.02	4b,5	11.4	9.1
6	4.12	4.00	5,6	4.9	8.4
7	4.76	4.69	6,7	3.8	3.8
8	4.56	4.55	7,8	5.9	5.9
9	4.90	4.97	8,9	-	-
OMe	3.29	3.33			
C*H*O	9.90	9.91			
isopropyl.CH_3	1.45, 1.29	1.42, 1.26			

Table 120

3.13 Synthesis of Aldehyde Derivatives

3.13.1 5R-5-(2,3-O-*Isopropylidene*-1-O-*methyl*-α-D-lyxo-*tetrofuranos*-4-*yl*)-*eth*-3-*enyl*-2-*isoxazoline* (346)

Potassium *tert*-butoxide (0.23 g, 2.05 mmol) in dry THF was added to methyltriphenyl-phosphonium bromide (0.54 g, 1.34 mmol) in dry THF, under nitrogen and the solution stirred for 1.5 hr at room temperature. Aldehyde (**344**) (75 mg, 0.28 mmol) dissolved in dry THF was added dropwise over 10 min, and the solution stirred overnight. Water (20 ml) was added and the mixture extracted with hexane (3 × 50 ml). The combined organic extracts were dried ($MgSO_4$), and evaporated *in vacuo* to an oil which was purified by preparative thin layer chromatography yielding alkene (**346**) as a colourless oil (60 mg, 80%); δ_H (360 MHz, $CDCl_3$) see Tables 121 and 122; δ_C (90 MHz, $CDCl_3$) 157.8 (C_3), 126.8 (C_2), 122.0 (C_1), 112.6 [*C*$(Me)_2$], 107.1 (C_9), 84.8 (C_6), 79.4, 79.1 (C_7, C_8), 78.2 (C_5), 54.6 (OMe), 35.6 (C_4), 25.7, 24.3 (2 × Me); m/z (FAB) 270.13673. $C_{13}H_{20}NO_5$ requires 270.13415.

3.13.2 5R-5-(2,3-O-*Isopropylidene*-1-O-*methyl*-α-D-lyxo-*tetrofuranos*-4-*yl*)-*eth*-3-*enyl*-(2-*ethoxycarbonyl*)-2-*isoxazoline* (347)

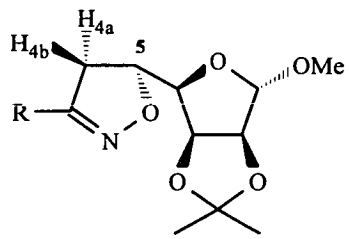
Potassium *tert*-butoxide (88 mg, 0.78 mmol) in dry ethylene glycol dimethyl ether (50 ml) was added to triethyl phosphonoacetate (117 mg, 0.52 mmol) in dry ethylene glycol dimethyl ether, under nitrogen and the solution stirred for 1.5 hr at room temperature. Aldehyde (**344**) (66 mg, 0.24 mmol) dissolved in dry ethylene glycol dimethyl ether was added dropwise over 10 min, and the solution stirred

overnight. Water (50 ml) was added and the mixture extracted with hexane (3 × 75 ml). The combined organic extracts were dried ($MgSO_4$), and evaporated *in vacuo* to an oil which was purified by preparative thin layer chromatography yielding alkene (**347**) as a colourless oil (62 mg, 75%); δ_H (360 MHz, $CDCl_3$) see Tables 121 and 122; δ_C (90 MHz, $CDCl_3$) 156.5 (C_3), 133.0 (C_1), 125.6 (C_2), 112.6 [$C(Me)_2$], 84.7 (C_6), 60.9 (CH_3CH_2O), 54.7 (OCH_3), 34.8 (C_4), 25.7, 24.2 [$C(CH_3)_2$], 14.1 (CH_3CH_2O); m/z (FAB) 342.15480 (M^++H), $C_{16}H_{24}NO_7$ requires 342.15528.

3.13.3 5R-5-(2,3-O-*Isopropylidene*-1-O-*methyl*-α-D-lyxo-*tetrofuranos*-4-*yl*)-3-*aldoximino*-2-*isoxazoline* (348)

To an aqueous solution of sodium hydroxide (2.3 g, 57.5 mmol) in water (10 ml) was added hydroxylamine hydrochloride (2.4 g, 34.5 mol), followed by a solution of aldehyde (**344**) (50 mg, 0.18 mmol) in ether (10 ml) and the solution stirred overnight at room temperature. The organic layer was separated and the aqueous portion extracted with ethyl acetate (2 × 50 ml). The combined organic extract was washed with water (2 × 50 ml), brine (50 ml), dried ($MgSO_4$) and concentrated *in vacuo* to yield the title compound, a colourless oil as a ~ 2 : 3 mixture of *cis* and *trans* isomers (**348**) (40 mg, 75%); δ_H (360 MHz, $CDCl_3$) 5.10-5.00 (1H, b, OH), 4.88 (1H, s, H_9), 4.83 [1H, ddd, $J_{4a,5}$ = 3.8 Hz (cis), $J_{4a,5}$ = 2.8 Hz (trans), $J_{4b,5}$ = 6.9 Hz (cis), $J_{4b,5}$ = 8.4 Hz (trans), $J_{5,6}$ = 8.5 Hz (cis), $J_{5,6}$ = 8.1 Hz (trans), H_5 (cis + trans)], 4.58 [0.40H, d, $J_{7,8}$ = 5.9 Hz, H_8 (cis)], 4.55 [0.60H, d, $J_{7,8}$ = 5.9 Hz, H_8 (trans)], 4.17 [1H, dd, $J_{6,7}$ = 3.8 Hz (cis), $J_{6,7}$ = 3.7 Hz (trans), $J_{7,8}$ = 5.9 Hz, H_7 (cis + trans)], 3.88 [0.40H, dd, $J_{5,6}$ = 8.5 Hz, $J_{6,7}$ = 3.8 Hz, H_6 (cis)], 3.79

[0.60H, dd, $J_{5,6}$ = 8.1 Hz, $J_{6,7}$ = 3.7 Hz, H_6 (trans)], 3.30 [0.60H, s, OMe(trans)], 3.29 [0.40H, s, OMe (cis)], 2.78 [0.40H, dd, $J_{4a,5}$ = 3.8 Hz, $J_{4a,4b}$ = 16.8 Hz, H_{4a} (cis)], 2.64 [0.40H, dd, $J_{4b,5}$ = 6.9 Hz, $J_{4a,4b}$ = 16.8 Hz, H_{4b} (cis)], 2.56 [0.60H, dd, $J_{4a,5}$ = 2.8 Hz, $J_{4a,4b}$ = 14.9 Hz, H_{4a} (trans)], 2.23 [0.60H, dd, $J_{4b,5}$ = 8.4 Hz, $J_{4a,4b}$ = 14.9 Hz, H_{4b} (trans)], 1.87 (0.40H, s, C*H*=NOH), 1.86 (0.6H, s, C*H*=NOH).



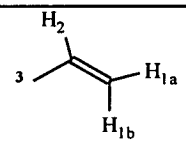
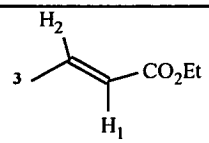
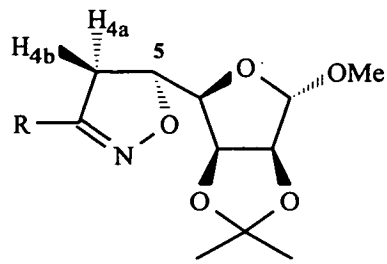
R		
Resonance	**δ_H/ppm**	
1a	5.56	/
1b	5.48	6.07
2	6.68	7.54
4a	3.18	3.21
4b	3.15	3.12
5	4.94	5.02
6	4.00	4.06
7	4.77	4.76
8	4.56	4.56
9	4.89	4.89
OMe	3.30	3.29
isopropyl. CH_3	1.46, 1.30	1.46, 1.30
CH_3CH_2	/	4.24
CH_3CH_2	/	1.30

Table 121



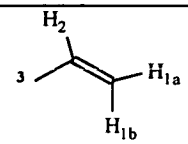
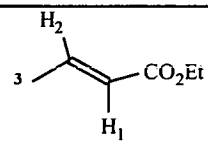
R		
Resonance	***J*/Hz**	
1a,1b	0.6	/
1a,2	10.8	/
1b,2	17.7	/
1,2	/	16.1
4a,4b	-	16.9
4a,5	9.2	8.3
4b,5	9.2	10.9
5,6	6.5	5.7
6,7	3.7	3.8
7,8	5.9	5.9
8,9	-	-
CH_3CH_2	/	7.1

Table 122

3.14 Reductive Hydrolytic Cleavage of 2-Isoxazolines to β-Hydroxyketones

3.14.1 Raney-Nickel Induced Hydrogenolysis of 2-Isoxazolines

General Procedure

The isoxazoline (1 M eq) and boric acid (6 M eq) were dissolved in a mixture of methanol and water (5 : 1) (8-12 ml per 100 mg of isoxazoline) and Raney-Nickel[a] (3 spatula tips per 100 mg of isoxazoline) added. The mixture was degassed and hydrogen filled several times, then left to stir vigorously under a hydrogen atmosphere. Upon reaction completion (monitored by TLC) the mixture was filtered through a celite pad and concentrated *in vacuo* (bath temperature <25°C). The excess boric acid was removed by repeated methanol addition and evaporation. The resulting oil was purified by preparative TLC on silica gel.

3.14.1.1 Hydrogenolysis of 5S-5-(1,2-O-*Cyclohexylidene*-D-glycero-*diitol*-1-*yl*)-3-*phenyl*-2-*isoxazoline* (196)

According to the general procedure given above, isoxazoline (**196**) (63 mg, 0.22 mmol) yielded after 4 hr, a mixture of crude products. Separation by preparative TLC (100% ether) afforded pure 4,5-O-*cyclohexylidene*-2-*deoxy*-1-*phenyl*-D-erythro-5-*pentose* (**244**) as a very pale yellow oil (51 mg, 80%); $[\alpha]_D^{25}$ -22.6° (c = 1.01, $CHCl_3$); δ_H (360 MHz, $CDCl_3$) 7.99-7.44 (5H, 5 × PhCH), 4.12 (1H, ddd, $J_{5,6}$ = 4.9 Hz, $J_{6,7a}$ = 7.1 Hz, $J_{6,7b}$ = 2.8 Hz, H_6), 4.03 (1H, ddd, $J_{4a,5}$ = 2.5 Hz, $J_{4b,5}$ = 8.7 Hz, $J_{5,6}$ = 4.9 Hz, H_5), 4.02 (1H, dd, $J_{6,7b}$ = 2.8 Hz, $J_{7a,7b}$ = 14.1 Hz, H_{7b}), 3.47 (1H, dd, $J_{6,7a}$ =

[a] Best results were obtained when Raney-nickel was washed with water (stir, decant × 20) then stored under methanol in a freezer for 3-4 weeks prior to use

7.1 Hz, $J_{7a,7b}$ = 14.1 Hz, H_{7a}), 3.42 (1H, dd, $J_{4b,5}$ = 2.5 Hz, $J_{4a,4b}$ = 17.8 Hz, H_{4b}), 3.12 (1H, dd, $J_{4a,5}$ = 8.7 Hz, $J_{4a,4b}$ = 17.8 Hz, H_{4a}), 1.59-1.56 (10H, m, cyclohexyl. CH_2); δ_C (90 MHz, $CDCl_3$) 200.0 (C_3), 135.9 (*C*Ph), 132.9, 128.0, 127.5 (5 × PhCH), 109.4 (cyclohexyl. quat.), 76.7 (C_5), 68.9 (C_6), 66.0 (C_7), 41.0 (C_4), 35.7, 34.0, 24.4, 23.3, 23.0 (cyclohexyl. CH_2); m/z (FAB) 290.15181 (M^+), $C_{17}H_{23}O_4$ requires 290.15180, and an oil which gave a positive ninhydrin stain on TLC, believed to contain an inseparable mixture of γ-aminoalcohols 3-*amino*-4,5-O-*cyclohexylidene*-3,4-*dideoxy*-D-erythro-*pentose* and 3-*amino*-4,5-O-*cyclohexylidene*-3,4-*dideoxy*-D-threo-*pentose* (**245**) (6 mg, 10%); δ_H (360 MHz, $CDCl_3$) 128.8, 126.2 (5 × PhCH), 4.54-3.70 (8H, m, H_3, H_{4a}, H_{4b}, H_5, H_6, H_{7a}, H_{7b}, 5OH), 2.15-1.90 (2H, b, NH_2); δ_C (90 MHz, $CDCl_3$) 129.2, 128.8, 128.4, 127.8, 126.7, 126.2 (5 × PhCH), 110.0 [*C*$(Me)_2$], 78.1 and 77.5 (C_5), 72.8 and 69.0 (C_6), 65.3 and 65.0 (C_7), 56.3 and 53.0 (C_3), 38.3 and 38.0 (C_4), 38.3, 38.0, 36.1, 34.2, 34.0, 25.0, 24.2, 23.9 (cyclohexyl. CH_2); m/z (FAB) 292.19057 (M^++H), $C_{17}H_{26}NO_3$ requires 292.19127.

3.14.1.2 Hydrogenolysis of 5S-5-(1,2-O-*Cyclohexylidene*-D-glycero-*diitol*-1-*yl*)-3-*hydroxymethyl*-2-*isoxazoline* (204)

According to the general procedure given above, isoxazoline (**204**) (80 mg, 0.33 mmol) yielded, after 4 hr, a mixture of crude products. Separation by preparative TLC (100% ether) afforded pure 5,6-O-*cyclohexylidene*-3-*deoxy*-D-erythro-2-*hexulose* (**246**) as a colourless oil (63 mg, 78%); $[\alpha]_D^{25}$ -11.5° (c = 0.54, $CHCl_3$); δ_H (360 MHz, $CDCl_3$) 4.29 (2H, s, C*H*$_2$OH), 4.05 (1H, ddd, $J_{5,6}$ = 5.7 Hz, $J_{6,7a}$ = 7.8 Hz, $J_{6,7b}$ = 5.2 Hz, H_6), 4.03 (1H, ddd, $J_{4a,5}$ = 8.7 Hz, $J_{4b,5}$ = 3.1 Hz, $J_{5,6}$ =

5.7 Hz, H_5), 3.95 (1H, dd, $J_{6,7b}$ = 5.2 Hz, $J_{7a,7b}$ = 12.0 Hz, H_{7b}), 3.91 (1H, dd, $J_{6,7a}$ = 7.8 Hz, $J_{7a,7b}$ = 12.0 Hz, H_{7a}), 3.10, 2.94 (2H, 2 × s, CH_2OH, 5-OH), 2.74 (1H, dd, $J_{4b,5}$ = 3.1 Hz, $J_{4a,4b}$ = 16.7 Hz, H_{4b}), 2.58 (1H, dd, $J_{4a,5}$ = 8.7 Hz, $J_{4a,4b}$ = 16.7 Hz, H_{4a}), 1.57-1.54 (10H, m, cyclohexyl. CH_2); δ_C (90 MHz, $CDCl_3$) 209.6 (C_3), 110.1 (cyclohexyl. quat.), 77.3 (C_5), 68.8 (C_6), 68.7 (CH_2OH), 66.0 (C_7), 41.7 (C_4), 36.2, 34.4, 24.9, 23.8, 23.6 (cyclohexyl. CH_2); m/z (FAB) 246.14608 (M^++H), $C_{12}H_{22}O_5$ requires 246.14672, and an oil which gave a positive ninhydrin stain on TLC, believed to contain an inseparable mixture of γ-aminoalcohols, 3-*amino*-5,6-O-*cyclohexylidene*-3,4-di*deoxy*-D-erythro-2-*hexulose* and 3-*amino*-5,6-O-*cyclohexylidene*-3,4-di*deoxy*-D-threo-2-*hexulose* (**247**) (7 mg, 8%); m/z (FAB) 246.17185 (M^++H), $C_{12}H_{24}NO_4$ requires 246.17053.

3.14.1.3 Hydrogenolysis of 5R-5-(2,3-O-*Isopropylidene*-1-O-*methyl*-α-D-lyxo-*tetrofuranos*-4-*yl*)-3-*hydroxymethyl*-2-*isoxazoline* (327)

According to the general procedure given above, isoxazoline (**327**) (80 mg, 0.29 mmol) yielded, after 3 hr, a mixture of crude products. Separation by preparative TLC (100% ether) afforded *methyl* 6-*deoxy*-2,3-O-*isopropylidene*-α-D-manno-*octulose*-(1,4) (**334**) as a colourless oil (41 mg, 51%); $[\alpha]_D^{24}$ +37.5° (c = 1.45, $CHCl_3$); δ_H (360 MHz, $CDCl_3$) 4.85 (1H, s, H_9), 4.78 (1H, dd, $J_{6,7}$ = 3.8 Hz, $J_{7,8}$ = 5.9 Hz, H_7), 4.54 (1H, d, $J_{7,8}$ = 5.9 Hz, H_8), 4.38 (1H, ddd, $J_{4a,5}$ = 8.9 Hz, $J_{4b,5}$ = 3.3 Hz, $J_{5,6}$ = 8.3 Hz, H_5), 4.29 (1H, s, CH_2OH), 3.77 (1H, dd, $J_{5,6}$ = 8.3 Hz, $J_{6,7}$ = 3.8 Hz, H_6), 3.27 (3H, s, OMe), 3.30-3.10 (1H, b, CH_2OH), 2.80 (1H, dd, $J_{4b,5}$ = 3.3 Hz, $J_{4a,4b}$ = 16.1 Hz, H_{4b}), 2.67 (1H, dd, $J_{4a,5}$ = 8.9 Hz, $J_{4a,4b}$ = 16.1 Hz, H_{4a}), 1.44 (3H, s, Me),

1.33 (3H, s, Me); δ_C (90 MHz, $CDCl_3$) 209.0 (C_3), 112.7 [*C*(Me)$_2$], 106.9 (C_9), 84.7 (C_6), 81.3 (C_7), 79.3 (C_8), 68.9 (*C*H$_2$OH), 66.1 (C_5), 54.5 (OMe), 42.7 (C_4), 25.8, 24.5 [C(*Me*)$_2$]; m/z (FAB) 277.12774 (M^++H), $C_{12}H_{21}O_7$ requires 277.12873, and an oil which gave a positive ninhydrin stain on TLC, believed to contain an inseparable mixture of γ-amnioalcohols *methyl* 7-*amino*-6,7-di*deoxy*-2,3-O-*isopropylidene*-D-gluco-α-D-manno-2-*octulose*-(1,4) (**335**) (7 mg, 9%); m/z (FAB) 278.15992 (M^++H), $C_{12}H_{24}NO_6$ requires 278.16036.

3.14.1.4 Hydrogenolysis of 5R-5-(2,3-O-*Isopropylidene*-1-O-*methyl*-α-D-lyxo-*tetrofuranos*-4-*yl*)-3-*phenyl*-2-*isoxazoline* (321)

According to the general procedure given above, isoxazoline (**321**) (120 mg, 0.38 mmol) yielded, after 3 hr, a mixture of crude products. Separation by preparative TLC (100% ether) afforded pure *methyl* 6-*deoxy*-2,3-O-*isopropylidene*-7-*phenyl*-α-D-manno-7-*heptulose*-(1,4) (**336**) as a colourless oil (57 mg, 47%); $[\alpha]_D^{25}$+ 1.3° (c = 1.83, $CHCl_3$); δ_H (360 MHz, $CDCl_3$) 7.58-7.43 (5H, m, 5 × PhCH), 4.87 (1H, s, H_9), 4.85 (1H, dd, $J_{6,7}$ = 3.7 Hz, $J_{7,8}$ = 5.9 Hz, H_7), 4.56 (1H, d, $J_{7,8}$ = 5.9 Hz, H_8), 4.56 (1H, ddd, $J_{4a,5}$ = 8.6 Hz, $J_{4b,5}$ = 2.9 Hz, $J_{5,6}$ = 8.3 Hz, H_5), 3.91 (1H, dd, $J_{5,6}$ = 8.3 Hz, $J_{6,7}$ = 3.7 Hz, H_6), 3.50 (1H, d, $J_{5,5OH}$ = 4.7 Hz, 5-OH), 3.27 (3H, s, OMe), 3.43 (1H, dd, $J_{4b,5}$ = 2.9 Hz, $J_{4a,4b}$ = 17.3 Hz, H_{4b}), 3.22 (1H, dd, $J_{4a,5}$ = 8.6 Hz, $J_{4a,4b}$ = 17.3 Hz, H_{4a}), 1.45 (3H, s, Me), 1.32 (3H, s, Me); δ_C (90 MHz, $CDCl_3$) 200.5 (C_3), 136.7, 133.3, 128.5, 128.0 (5 × PhCH), 112.5 [*C*(Me)$_2$], 107.0 (C_9), 84.7 (C_6), 81.1 (C_7), 79.5 (C_8), 66.1 (C_5), 54.4 (OMe), 42.0 (C_4), 25.9, 24.5 [C(*Me*)$_2$]; m/z (FAB) 323.14932 (M^++H), $C_{17}H_{23}O_6$ requires 323.14946, and an oil which gave a positive

ninhydrin stain on TLC, believed to contain an inseparable mixture of γ-aminoalcohols *methyl* 7-*amino*-6,7-*dideoxy*-2,3-O-*isopropylidene*-7-*phenyl*-D-gluco-α-D-manno-*heptulose*-(1,4) and *methyl* 7-*amino*-6,7-*dideoxy*-2,3-O-*isopropylidene*-7-*phenyl*-D-manno-α-D-manno-*heptulose*-(1,4) (**337**) (20 mg, 16%); m/z (FAB) 324.18312 (M^++H), $C_{17}H_{26}NO_5$ requires 324.18110.

3.14.1.5 Hydrogenolysis of 5R-5-(1,2:3,4-*di*-O-*isopropylidene*-D-arabino-*tetritol*-1-*yl*)-3-*hydroxymethyl*-2-*isoxazoline* (305) and 5S-5-(1,2:3,4-*di*-O-*isopropylidene*-D-arabino-*tetritol*-1-*yl*)-3-*hydroxymethyl*-2-*isoxazoline* (306)

According to the general procedure given above, isoxazolines (**305**) and (**306**) (58 mg, 0.19 mmol) yielded, after 4 hr, a mixture of crude products. Separation by preparative TLC (100% ether) yielded a mixture of 3-*deoxy*-5,6:7,8-*di*-O-*isopropylidene*-D-gluco/manno-2-*octuloses* (**311**) and (**312**), which were unable to be separated, as a colourless oil (38 mg, 65%); δ_H (360 MHz, $CDCl_3$) 4.28 (2H, s, CH_2OH), 4.18 (1H, dd, $J_{8,9a}$ = 5.7 Hz, $J_{9a,9b}$ = 8.3 Hz, H_{9a}), 4.13 (1H, dd, $J_{8,9b}$ = 5.9 Hz, $J_{9a,9b}$ = 8.3 Hz, H_{9b}), 4.04 [1H, ddd, $J_{4a,5}$ = 11.5 Hz, $J_{4b,5}$ = 8.9 Hz, $J_{5,6}$ = 4.0 Hz, H_5 (*threo*)], 3.96 [1H, ddd, $J_{4a,5}$ = 8.3 Hz, $J_{4b,5}$ = 11.1 Hz, $J_{5,6}$ = 3.8 Hz, H_5 (*erythro*)], 3.89 (1H, dd, $J_{6,7}$ = 2.6 Hz, $J_{7,8}$ = 5.3 Hz, H_7), 3.70 [1H, dd, $J_{5,6}$ = 3.8 Hz (*erythro*), $J_{5,6}$ = 4.0 Hz (*threo*), $J_{6,7}$ = 2.6 Hz, H_6 (*erythro*) and H_6 (*threo*)], 2.75 [2H, 2 × dd, $J_{4b,5}$ = 11.1 Hz (*erythro*), $J_{4b,5}$ = 8.9 Hz (*threo*), $J_{4a,4b}$ = 15.3 Hz (*erythro*), $J_{4a,4b}$ = 15.1 Hz (*threo*), H_{4b} (*erythro*) and H_{4b} (*threo*)], 2.63 [2H, 2 × dd, $J_{4a,5}$ = 8.3 Hz (*erythro*), $J_{4a,5}$ = 11.5 Hz (*threo*), $J_{4a,4b}$ = 15.3 Hz (*erythro*), $J_{4a,4b}$ = 15.1 Hz (*threo*),

H_{4a} (*erythro*) and H_{4a} (*threo*)], 1.43-1.33 (12H, m, 4 × CH_3); δ_C (90 MHz, $CDCl_3$) 209.0 [C_3 (*erythro*) and C_3 (*threo*)], 110.2, 109.2 [$C(Me)_2$ (*erythro*)], 109.8, 109.7 [$C(Me)_2$ (*threo*)], 82.7, 81.0, 76.1, 69.1 [C_5, C_6, C_7, C_8 (*erythro*)], 82.4, 77.1, 77.0, 66.8 [C_5, C_6, C_7, C_8 (*threo*)], 69.1 [CH_2OH (*erythro*) and CH_2OH (*threo*)], 67.8 [C_9 (*erythro*) and C_9 (*threo*)], 42.9 [C_4 (*threo*)], 42.6 [C_4 (*erythro*)], 27.0, 26.7, 26.5, 25.1 [$C(CH_3)_2$ (*threo*)], 26.6, 26.58, 26.3, 24.9 [$C(CH_3)_2$ (*erythro*)]; m/z (FAB) 305.15940 (M^++H), $C_{14}H_{25}O_7$ requires 305.16003, and an oil which gave a positive ninhydrin stain on TLC, believed to contain an inseparable mixture of four γ-aminoalcohols 4-*amino*-3,4-*dideoxy*-5,6:7,8-*di*-O-*isopropylidene*-D-gluco/manno-2-*octuloses* (**313**)/ (**314**) (8 mg, 14%); m/z (FAB) 306.19300 (M^++H), $C_{14}H_{28}NO_6$ requires 306.19166.

3.14.2 Palladium-on-Charcoal Catalysed Hydrogenolysis

3.14.2.1 Hydrogenolysis of 5S-5-(1,2-O-*Cyclohexylidene*-D-glycero-*diitol*-1-*yl*)-3-*phenyl*-2-*isoxazoline* (196)

Isoxazoline (**196**) (50 mg, 0.17 mmol) and boric acid (65 mg, 1.05 mmol) were dissolved in methanol and water (5 : 1) (9 ml). 10% Palladium-on-charcoal (17 mg) was added and the mixture degassed using a water pump and hydrogen flushed five times, then left to stir vigorously under a hydrogen atmosphere for 18 hr. The mixture was filtered through celite and evaporated several times *in vacuo* (no heating). Methanol was added and evaporated several times to remove the excess boric acid as the volatile trimethylborate. TLC analysis indicated the presence of unreacted isoxazoline and two more polar components. The reaction was set up again

with the same quantities of reagents and left to stir vigorously for a further 20 hr. Work up as above afforded, after preparative TLC (100% ether), unreacted isoxazoline (**196**) (4 mg, 7%), 4,5–O–*cyclohexylidene*-2-*deoxy*-1-*phenyl*-D-erythro-5-*pentose* (**244**) (32mg, 64%), very pale yellow oil, and γ-amino alcohols, 3-*amino*-4,5–O–*cyclohexylidene*-2,3-*dideoxy*-1-*phenyl*-D-erythro-5-*pentose* and 3-*amino*-4,5–O–*cyclohexylidene*-2,3-*dideoxy*-1-*phenyl*-D-threo-5-*pentose* (**245**) as an oil, (14 mg, 28%). See Section 3.14.1.1 for ^{1}H and ^{13}C NMR and m/z (FAB) data. These products were identified by comparison of TLC data.

3.14.2.2 Hydrogenolysis of 5S-5-(1,2-O-*Cyclohexylidene*-D-glycero-*diitol*-1-*yl*)-3-*hydroxymethyl*-2-*isoxazoline* (204)

Using the same procedure as in Section 3.14.2.1, isoxazoline (**204**) (156 mg, 0.65 mmol) yielded, after 24 hr, a mixture of crude products. Separation by preparative TLC (100% ether) afforded 5,6–O–*cyclohexylidene*-3-*deoxy*-D-erythro-*hexulose* (**246**) (95 mg, 60 %), colourless oil, and γ-aminoalcohols 4-*amino*-5,6–O–*cyclohexylidene*-3,4-*dideoxy*-D-erythro-5-*hexulose* and 4-*amino*-5,6–O–*cyclohexylidene*-3,4-*dideoxy*-D-threo-5-*hexulose* (**247**) as an oil (40 mg, 25%). See Section 3.14.1.2 for ^{1}H and ^{13}C NMR and m/z (FAB) data. These products were identified by comparison of TLC data.

3.15 Reductive Hydrolytic Cleavage of 2-Isoxazolines to 2–Uloses

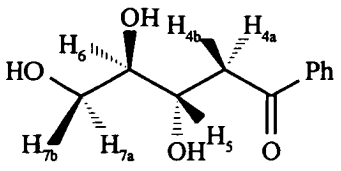
General Procedure

Isoxazoline (1 M eq) and boric acid (6 M eq) were dissolved in a mixture of methanol and water (5 : 1) (8-12 ml per 100 mg of isoxazoline) and Raney-Nickel[a] (3 spatula tips per 100 mg of isoxazoline) added. The mixture was degassed and hydrogen filled several times, then left to stir vigorously under a hydrogen atmosphere. Upon reaction completion (monitored by TLC) the mixture was filtered through a celite pad and concentrated *in vacuo* (bath temperature <25°C). The excess boric acid was removed by repeated methanol addition and evaporation. The resulting oil was not purified further.

3.15.1 Hydrogenolysis of 5S-5-(D-Glycero-*diitol*-1-*yl*)-3-*phenyl*-2-*isoxazoline* (249) to 1-*phenyl*-2-*deoxy*-D-*xylose* (255)

According to the general procedure given above, isoxazoline (**249**) (50 mg, 0.24 mmol) yielded after 4 hr, 1-*phenyl*-2-*deoxy*-D-*xylose* (**255**) as a pale yellow oil (19 mg, 37%); δ_H [360 MHz, $(CD_3)_2CO$] see Table 123; δ_C [90 MHz, $(CD_3)_2CO$] 200.7 (C_3), 137.1 (*C*Ph), 133.2, 128.6, 128.3, 126.8, 126.4 (5 × CH*Ph*), 74.4 (C_5), 68.7 (C_6), 62.9 (C_7), 41.9 (C_4); m/z (FAB) 212.10485 (M^++2), $C_{11}H_{16}O_4$ requires 212.10483.

[a] Best results were obtained when Raney-nickel was washed with water (stir, decant × 20) then stored under methanol in a freezer for 3-4 weeks prior to use

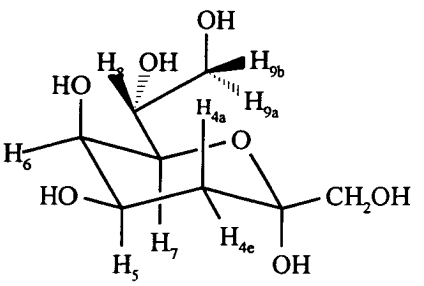


Resonance	δ_H/ppm (255)	Coupling	*J*/Hz (255)
4a	3.17	4a,4b	16.5
4b	3.39	4a,5	8.7
5	4.22	4b,5	3.1
6	3.61	5,6	6.7
7a	3.77	6,7a	5.5
7b	3.68	6,7b	4.1
		7a,7b	10.6

Table 123

3.15.2 Hydrogenolysis of 5R-5-(D-arabino-*tetritol*-1-*yl*)-3-*hydroxymethyl*-2-*isoxazoline* (307) to 3-*deoxy*-D-*manno*-2-*octulose* (339)

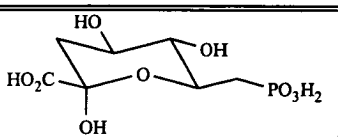
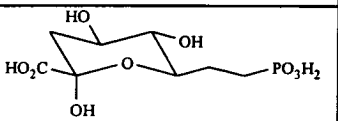
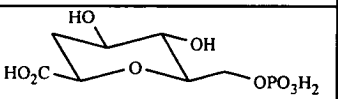
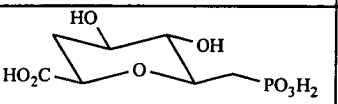
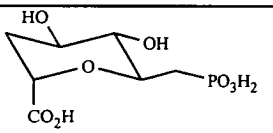
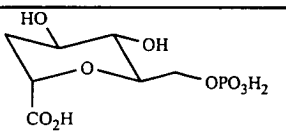
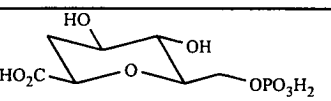
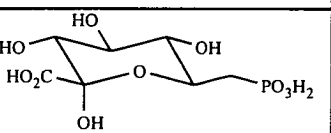
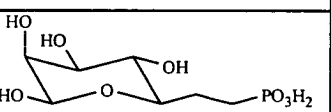
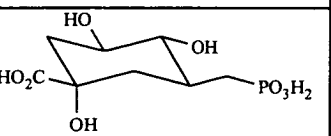
According to the general procedure given above, isoxazoline (**307**) (50 mg, 0.23mmol) yielded after 2 hr, 3-*deoxy*-D-*manno*-2-*octulose* (**339**) as a pale yellow oil (22 mg, 43%); δ_H (360 MHz, D_2O) see Table 124; δ_C (90 MHz, D_2O) 80.4 (C_3), 73.3, 70.8, 70.0, 69.4 (C_5, C_6, C_7, C_8), 63.0 (C_9), 36.7 (C_4), 56.5 (CH_2OH); m/z (FAB) 222.07395 (M^+-2), $C_8H_{14}O_4$ requires 222.07424.

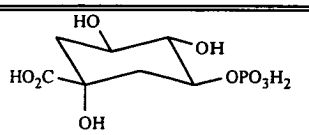
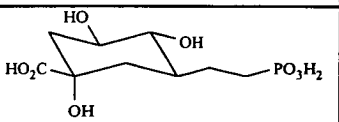
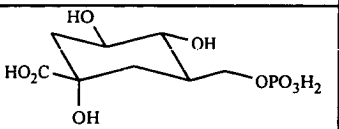
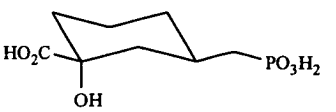
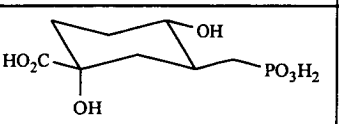
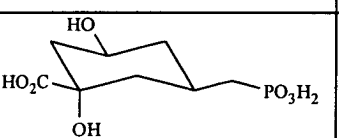
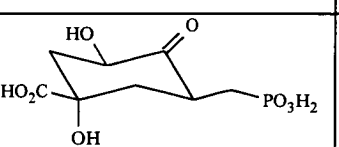
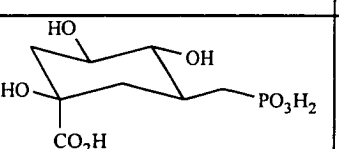
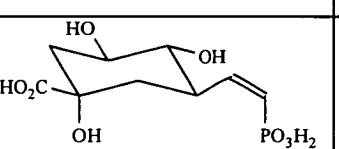
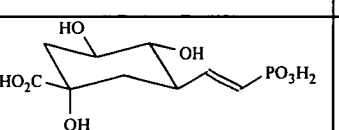


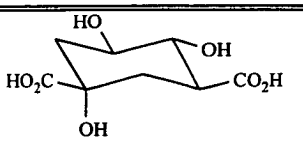
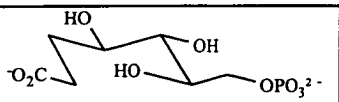
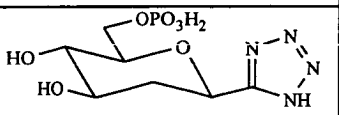
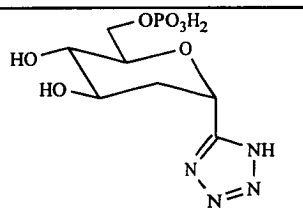
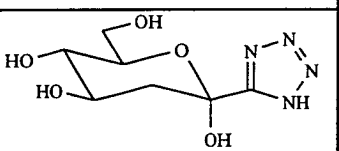
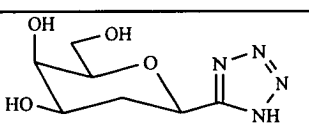
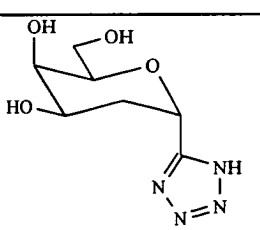
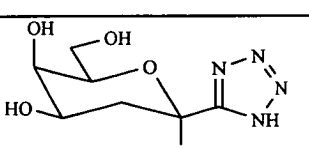
Resonance	δ_H/ppm (339)	Coupling	*J*/Hz (339)
4a	1.78	4a,4b	13.0
4e	1.83	4a,5	9.0
5	4.09	4e,5	6.0
6	3.87-3.80	5,6	3.0
7	3.87-3.60	6,7	nd
8	3.87-3.80	7,8	nd
9a	4.02	8,9a	2.6
9b	3.63	8,9b	5.5
CH_2OH	3.87-3.60	9a,9b	11.8

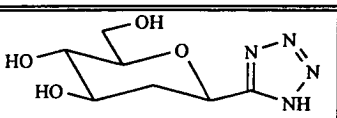
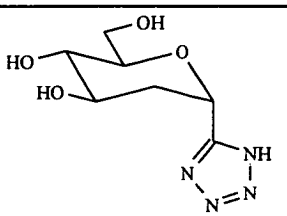
Table 124

Appendix 1 : DHQ Synthase Inhibitors

Compound Number	Compound	Inhibition	$K_i/\mu M$[a]	K_m/K_i (pH 7.5)
29		Competitive inhibitor, but less than (**34**)	1.1[58] 2.5[65,72]	16.4[58] 54[61]
33		Inactive[58] No inhibition even at 500μM. Weak competitive inhibitor[65,72]	260[65]	0.07[61]
40		Competitive inhibitor, but less than (**29**)	35[62]	nd
41		Inactive[58]	-	-
32		Competitive inhibitor, but weaker than (**40**)	129[58]	0.14
42		Competitive inhibitor, but less than (**29**)	33[58]	nd
43		Competitive inhibitor, but much weaker than (**42**)	193[58]	nd
44		Strong competitive inhibitor	5[72]	nd
45		Very weak competitive inhibitor	1200[72]	nd
34		Competitive slow binding inhibitor. Important *in vivo* inhibitor.	0.0054[68] 0.0008[73]	5000[61]

Compound Number	Compound	Inhibition	$K_i/\mu M$[a]	K_m/K_i (pH 7.5)
46		Poor inhibitor	nd	2[61]
47		Competitive inhibitor	1.7[12]	2[61]
35		Competitive inhibitor	0.12[62] 0.16[69]	nd
48		Inactive[66] No inhibition even at mM conc.	-	-
30		Slowly reversible	0.22[63,66]	nd
37		Slowly reversible	0.54[63,66]	nd
31		Irreversible inhibitor. Enzyme inhibition time dependent	0.15[67] (short incub. time)	nd
49		Potent competitive inhibitor	0.0073[68]	nd
36		Competitive inhibitor, but less than (**50**)	25[73]	25[61]
50		Competitive inhibitor	0.16[73]	0.16[61]

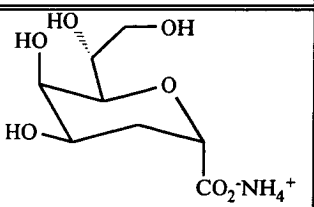
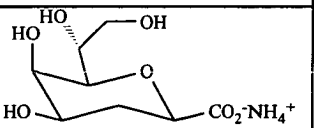
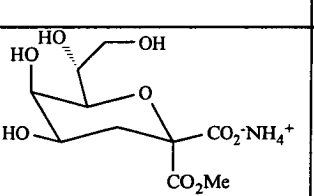
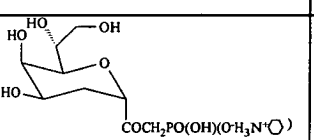
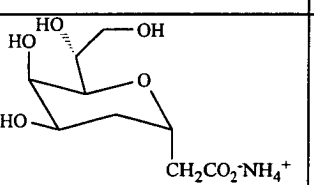
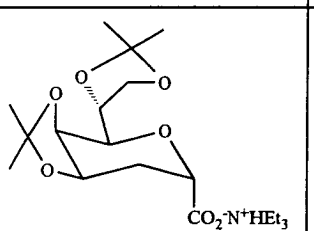
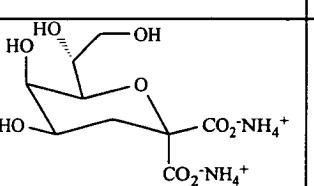
Compound Number	Compound	Inhibition	$K_i/\mu M^a$	K_m/K_i (pH 7.5)
51		Modest inhibitor	100[69]	-
38		Neither substrate or inhibitor of enzyme[62]	-	-
52		No significant inhibition at sub mM conc.[70]	-	-
39		No significant inhibition at sub mM conc.[70]	-	-
53		No significant inhibition at sub mM conc. No significant herbicidal or anti-bacterial activity[70]	-	-
54		No significant inhibition at sub mM conc. No significant herbicidal or anti-bacterial activity[70]	-	-
55		No significant inhibition at sub mM conc. No significant herbicidal or anti-bacterial activity[70]	-	-
56		No significant inhibition at sub mM conc. No significant herbicidal or anti-bacterial activity[70]	-	-

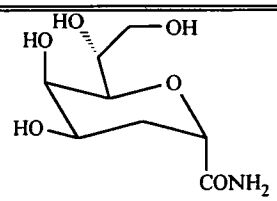
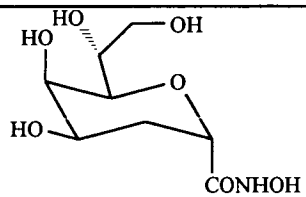
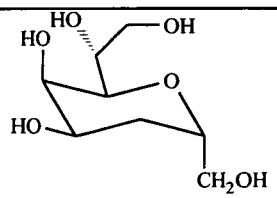
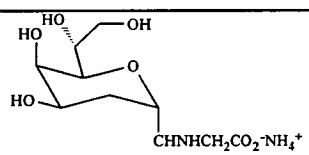
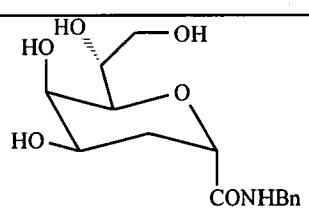
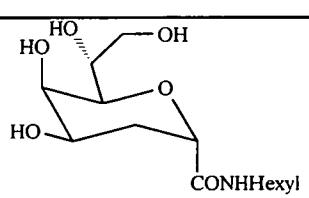
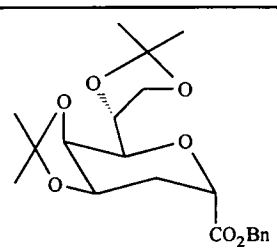
Compound Number	Compound	Inhibition	$K_i/\mu M^a$	K_m/K_i (pH 7.5)
57		No significant inhibition at sub mM conc. No significant herbicidal or anti-bacterial activity[70]	-	-
58		No significant inhibition at sub mM conc. No significant herbicidal or anti-bacterial activity[70]	-	-

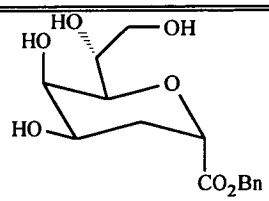
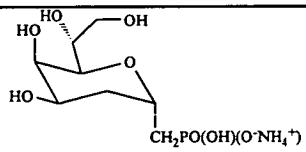
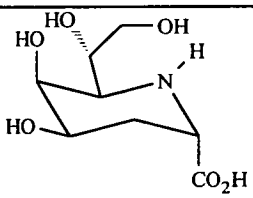
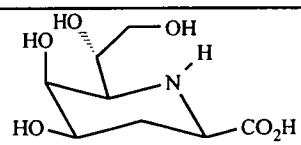
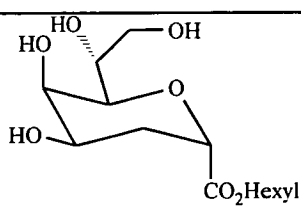
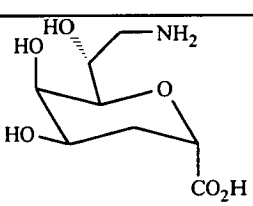
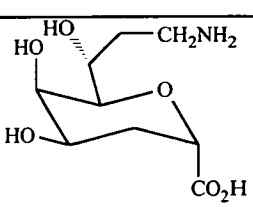
a Inhibition against enzyme purified from *Escherichia coli*

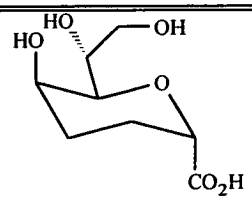
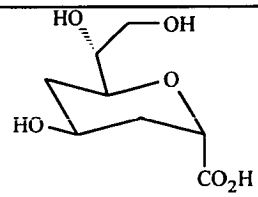
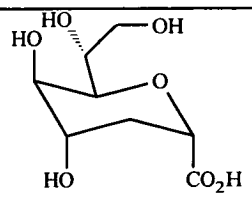
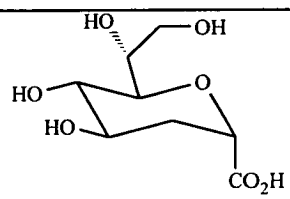
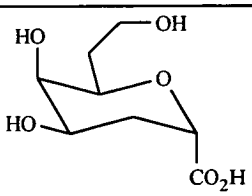
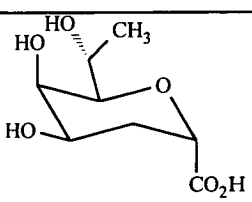
Differences in K_i values quoted may be due to purity of enzyme used and assay method used for kinetic measurements.

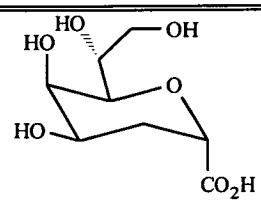
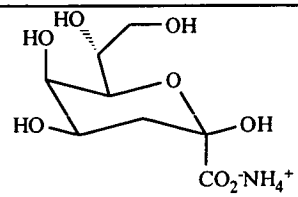
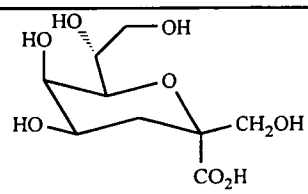
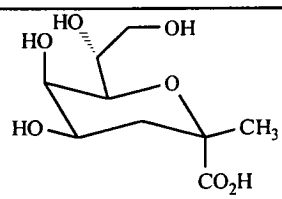
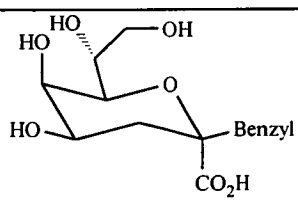
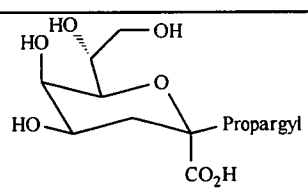
Appendix 2 : CMP-KDO Inhibitors

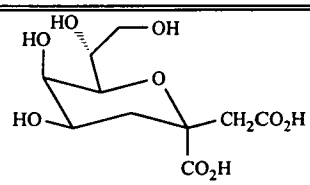
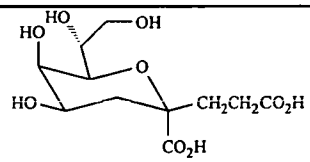
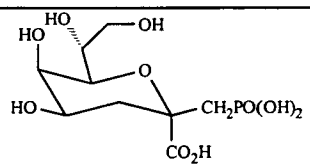
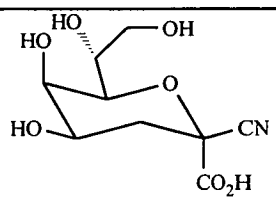
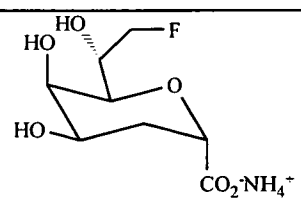
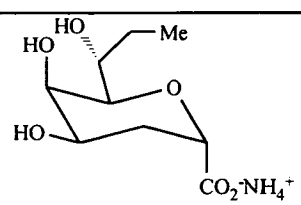
Compound Number	Compound	Inhibition	Relative Activity %	K_i/μM	IC_{50}/μM
96		Potent competitive inhibitor, but inactive against Gram negative bacteria[102,113]	100[102]	3.9[113]	10.5[138]
97		Inactive[102,113]	-	-	-
112		Inactive[102]	-	-	-
113		Weak inhibitor	<50[102]	nd	nd
114		Inactive[102]	-	-	-
100		Inactive[102] No antibacterial effect	-	-	-
115		Inactive[102]	-	-	-

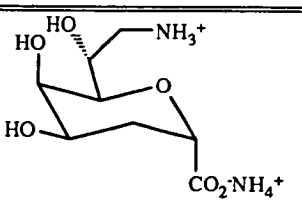
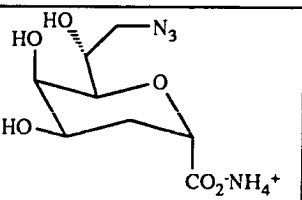
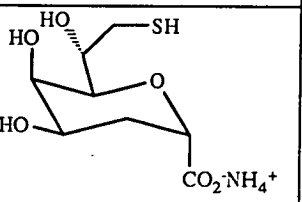
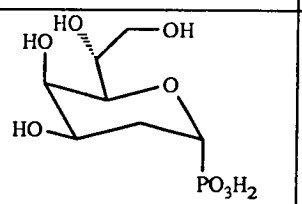
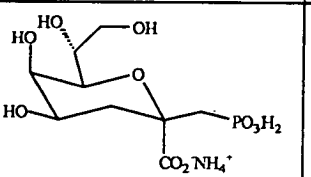
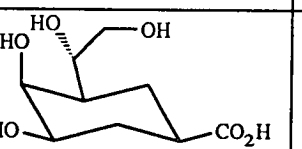
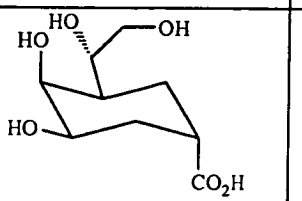
Compound Number	Compound	Inhibition	Relative Activity %	$K_i/\mu M$	$IC_{50}/\mu M$
116		Good inhibitor. No antibacterial effect	50-80[102]	nd	nd
117		Good inhibitor	80-90[102]		
118		Inactive[102]	-	-	-
119		Weak inhibitor No antibaterial effect	<50[102]	nd	nd
120		Inactive[102]	-	-	-
121		Inactive[102]	-	-	-
122		Inactive[102]	-	-	-

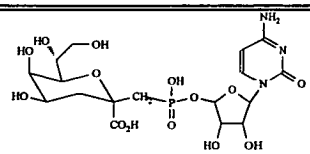
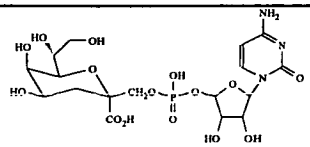
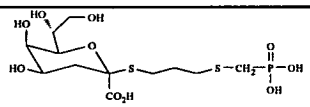
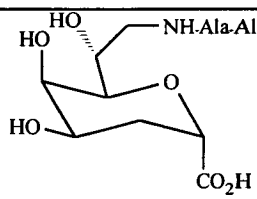
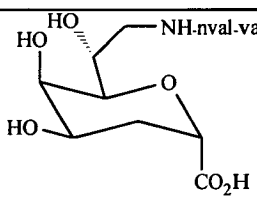
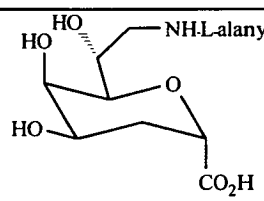
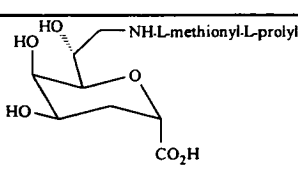
Compound Number	Compound	Inhibition	Relative Activity %	K_i/μM	IC_{50}/μM
123		Inactive[102]	-	-	-
124		Inactive[102]	-	-	-
102		Modest/weak inhibitor[109,129]	nd	nd	250
125		Inactive[102]	-	-	-
126		Inactive[102]	-	-	-
127		Potent competitive inhibitor.[96,110,126,127,129,133,138] Limited antibacterial activity	nd	4	4.2
128		Potent competitive inhibitor.[96,138] Limited antibacterial activity	nd	2.5	2.0

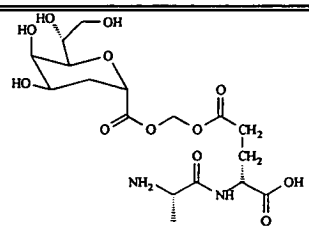
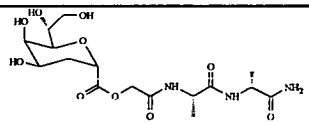
Compound Number	Compound	Inhibition	Relative Activity %	K_i/μM	IC_{50}/μM
129		Weak inhibitor. No significant antibacterial activity	nd	nd	1200[96]
130		Weak inhibitor. No significant antibacterial activity	nd	nd	1150[96]
131		Very weak inhibitor. No significant antibacterial activity	nd	nd	1860[96]
132		Very weak inhibitor. No significant antibacterial activity	nd	nd	1770[96]
133		Very weak inhibitor	nd	nd	3100[96]
134		Competitive inhibitor, much stronger than compound (**133**).	nd	nd	4.7[96]

Compound Number	Compound	Inhibition	Relative Activity %	K_i/μM	IC_{50}/μM
135		Extremely weak inhibitor[96]	nd	nd	>10000
136		Moderate inhibitor[130]	nd	nd	nd
103		Very weak inhibitor	<15[131] (at equimol. conc. of inhibitor and KDO)	nd	nd
104		Very weak inhibitor	<15[131] (at equimol. conc. of inhibitor and KDO)	nd	nd
137		Very weak inhibitor	<15[131] (at equimol. conc. of inhibitor and KDO)	nd	nd
138		Very weak inhibitor	<15[131] (at equimol. conc. of inhibitor and KDO)	nd	nd

Compound Number	Compound	Inhibition	Relative Activity %	K_i/μM	IC_{50}/μM
139		Very weak inhibitor	<15[131] (at equimol. conc. of inhibitor and KDO)	nd	nd
140		Very weak inhibitor	<15[131] (at equimol. conc. of inhibitor and KDO)	nd	nd
141		Very weak inhibitor	<15[131] (at equimol. conc. of inhibitor and KDO)	nd	nd
142		Very weak inhibitor	<15[131] (at equimol. conc. of inhibitor and KDO)	nd	nd
105		Potent inhibitor (As potent as 2-deoxy-β-KDO **(96)** No antibacterial activity.	nd	nd	24[111]
106		Potent inhibitor (As potent as 2-deoxy-β-KDO **(96)** No antibacterial activity.	nd	nd	22[111]

Compound Number	Compound	Inhibition	Relative Activity %	K_i/μM	IC_{50}/μM
107		Potent inhibitor (As potent as 2-deoxy-β-KDO (96) Weak antibacterial activity against some strains.	nd	nd	c[111] 4.0[138]
99		Very weak inhibitor	nd	nd	c[111]
108		Potent inhibitor, but less potent than 2-deoxy-β-KDO (96)	nd	nd	c[111] 27.0[138]
143		Inhibition still to be determined[110]	nd	nd	nd
144		Weak inhibitor. No significant antibacterial activity	nd	nd	>10 mM[107]
145		Inactive[132]	-	-	-
98		Weak inhibitor.[132] No antibacterial effect	nd	nd	nd

Compound Number	Compound	Inhibition	Relative Activity %	$K_i/\mu M$	$IC_{50}/\mu M$
101		Very weak inhibitor.[96,107,125] No significant antibacterial activity.	nd	nd	4100
109		Weak inhibitor[125]	nd	nd	nd
146		Inactive[125]	-	-	-
111		No *in vitro* activity.[96,106,127,128,133] Potent antibacterial against Gram -ve bacteria, but not Gram +ve bacteria.	-	-	-
147		No *in vitro* activity.[96] Potent antibacterial against Gram -ve bacteria, but not Gram +ve bacteria. Better than (**111**)	-	-	-
148		No *in vitro* activity.[133] Potent antibacterial against Gram -ve bacteria, but not Gram +ve bacteria.	-	-	-
149		No *in vitro* activity.[133] Potent antibacterial against Gram -ve bacteria.	-	-	-

Compound Number	Compound	Inhibition	Relative Activity %	$K_i/\mu M$	$IC_{50}/\mu M$
110		Potent antibacterial against Gram -ve bacteria.[127]	-	-	-
150		Potent antibacterial against Gram -ve bacteria.[127]	-	-	-

c Compound gave rise to strongly interfering absorption in thiobarbituric acid assay.

Appendix 3 : π-Facial Selectivities for Nitrile Oxide Cycloadditions

	Alkene	Nitrile Oxide	*erythro* : *threo*
Six-carbon Analogues	**(195)**	EtO_2CCNO	77 : 23
		PhCNO	79 : 21
		$(EtO)_2P(O)CH_2CNO$	81 : 19
		MeCNO	82 : 18
	(226)	EtO_2CCNO	75 : 25
		PhCNO	72 : 28
Seven-carbon Analogues	**(260)**	EtO_2CCNO	53 :47
		PhCNO	52 : 48
		$(EtO)_2P(O)CH_2CNO$	50 :50
	(271)	EtO_2CCNO	59 : 41
		PhCNO	63 : 37
	(276)	EtO_2CCNO	55 : 45
		PhCNO	62 :38
	(286)	EtO_2CCNO	83 : 17
		PhCNO	81 : 19
Eight-carbon Analogues	**(293)**	EtO_2CCNO	62.5 : 37.5
		PhCNO	67 : 33
		$(EtO)_2P(O)CH_2CNO$	63 : 37
	(317)	EtO_2CCNO	82 : 18
		PhCNO	81 : 19
		$(EtO)_2P(O)CH_2CNO$	88 :12

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