Development of Novel Chiral Stationary Phases for use in Chromatography

by

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To all my family, my nearly family, all my friends
.....and flying subaseals

CONTENTS

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DECLARATION

The work described in this thesis is the original work of the author, except where acknowledgement has been made to results and ideas previously published. The work was carried out in the Department of Chemistry, University of Warwick, between October 1st 1995 and December 20th 1998 and has not been previously submitted for a degree at any institution.

ABSTRACT

The aim of this project was to prepare novel chiral stationary phases that could be investigated analytically by chromatography with a view to increasing to a preparative scale.

A summary of the different types of compounds used as chiral stationary phases as well as an introduction to chromatography as a method of separation is given, and a description of silica and PGC used as solid supports in chromatography. The suitability of PGC as a solid support is described, using a known stationary phase containing a phenyl carbamate derivative of cellulose.

The preparation of novel chiral stationary phases based on the polymerisation of chiral monomer units based on tartaric acid with diisocyanate linkers is described. Some of these compounds were tested using HPLC. However the reaction between monomer and diisocyanate linker resulted in a wide range of products such as macrocycles of different sizes which reduced the efficiency of the CSP.

The synthesis of a 1,4-functionalised sugar unit is described with a view to its polymerisation to form a saccharide polymer that could be used as a CSP.

The synthesis of novel macromolecules based on saccharide units linked with non-saccharide linkers is described. The saccharide units used (glucose, cellobiose, maltose and maltotriose) were linked by a glycosidation reaction coupling the linker diol (1,4-*trans*-cyclohexanediol, 1,4-*cis*-2-butenediol and 1,4-butynediol) with the peracetylated sugar. Phenyl carbamate derivatives of these saccharide mimics were then prepared and tested for enantioselectivity by HPLC. Analysis by UV and CD spectroscopy was also carried out to investigate the secondary structures of these novel macromolecules.

An alternative method of loading the CSP onto the solid support was also investigated as well as attempts at a larger scale separation.

ABBREVIATIONS

α	Selectivity
APC	Amylose tris(phenyl carbamate)
Ar	Aryl
Bn	Benzyl
bp	Boiling point
br	Broad
c	Concentration
°C	Degrees centigrade
CA	α-Hydroxy cinnamic acid
CD	Circular dichroism
CDMPC	Cellulose tris(3,5-dimethyl phenyl carbamate)
CI	Chemical ionization
CPC	Cellulose tris(phenyl carbamate)
CSA	Chiral solvating agent
CSP	Chiral stationary phase
CTA	Cellulose triacetate
CTB	Cellulose tris(benzoate)
d	Doublet
dd	Doublet of doublets
DHB	2,5-Dihydroxybenzoic acid
DMAP	4-(Dimethylamino)pyridine
DMF	Dimethylformamide
DMSO	Dimethylsulfoxide

EI	Electron impact
eq.	Equivalent
Et	Ethyl
et al	And co-workers
EtOH	Ethanol
FAB	Fast atom bombardment
g	Gram
GC	Gas chromatography
GPC	Gel permeation chromatography
HPLC	High performance liquid chromatography
Hz	Hertz
IR	Infra-red
J	Coupling constant
k'	Capacity factor
LC	Liquid chromatography
LEC	Ligand exchange chromatography
m	Multiplet
MALDI-MS	Matrix assisted laser desorption-ionization mass spectrometry
Me	Methyl
MeOH	Methanol
mg	Milligrams
mL	Millilitres
mm	Millimetres
M_n	Number average molecular weight

mol	Mole
mp	Melting point
MS	Mass spectrum
MW	Molecular weight
N	Plate number
$NaHCO_3$	Sodium Hydrogen Carbonate
NMR	Nuclear magnetic resonance
N,N-DMA	N,N-dimethylacetamide
ODS	Octadecylated silica
pet. ether	Petroleum ether, bp 40-60°C
PGC	Porous graphitic carbon
p-TsOH	*p*-Toluenesulphonic acid
Ph	Phenyl
ppm	Parts per million
psi	Pounds per square inch
RI	Refractive index
R_s	Resolution
s	Singlet
SEC	Size exclusion chromatography
SFC	Supercritical fluid chromatography
t	Triplet
t_a	Retention time for analyte a
THF	Tetrahydrofuran
TLC	Thin layer chromatography

TMS	Trimethylsilyl
TOF	Time of flight
UV	Ultra-violet
% w/w	Percentage by weight

CHAPTER 1

INTRODUCTION

1.1 Chirality

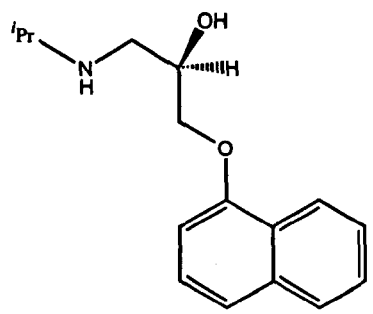
1.1.1 Introduction

As far back as Pasteur, who in 1848[1] worked on the enantioseparation of sodium ammonium tartrate crystals, there has been interest in the separation of enantiomers. Most naturally occurring compounds are sensitive to chirality. Interactions between chiral biologically active compounds and receptor proteins for example often show high, or complete, enantiomer specificity. This sensitivity to chirality has been used by many industries, in particular the pharmaceutical industry, in a move forward in developing new drugs. Chiral chromatography as a tool to separate enantiomers has thus developed into a well established field. Not only have different methods of chromatography been developed but an insight into the prerequisites required for the chiral recognition needed for efficient enantioseparation have been studied.

There are different reasons for wanting to separate enantiomers. It may be for scientific curiosity or to determine, or confirm, a reaction mechanism. The majority of reasons, however, relate to the different properties of the enantiomers. Many chiral compounds display different pharmacodynamic, pharmacokinetic and toxicological effects due to their stereochemistry. Different enantiomers can have different physiological properties. One example is Propranolol[2] as the (*R*)-(+) isomer **1** has contraceptive properties whereas the (*S*)-(-) isomer **2** is used for the treatment of heart

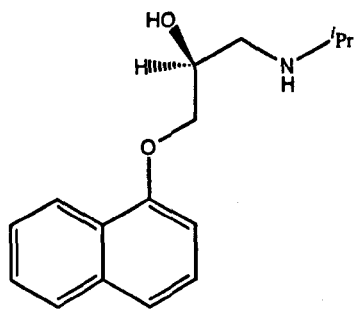
disease.[3] Hormones possess chiral discrimination in physiological reactions, for example, adrenaline (epinephrine); (*R*)-(-)-epinephrine **3** is 20 times as active as (*S*)-(+)-epinephrine.

The thalidomide tragedy illustrates the need for in-depth information about a compound before release as a prescription drug. Racemic *n*-phthalylglutamic acid imide (thalidomide) was prescribed as a sedative and to treat nausea and in particular was given to pregnant women in the 1960's. It was not until a high number of malformed babies were born to these women that the drug was investigated further. It was discovered that only the (*R*)-(+) enantiomer had the sedative properties and the (*S*)-(⁻) isomer **4** was in fact teratogenic.[4]



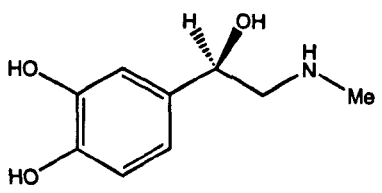
(*R*)-(+)-Propranolol

1



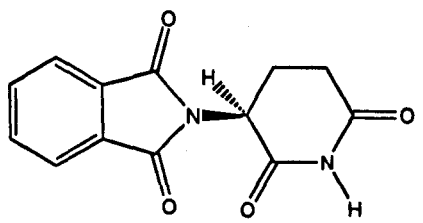
(*S*)-(⁻)-Propranolol

2



(*R*)-(-)-Epinephrine

3

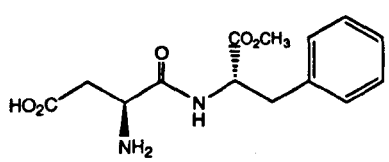


(*S*)-(⁻)-Thalidomide

4

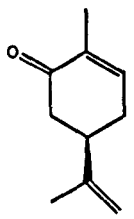
Figure 1.1: Examples of enantiomers with different physiological properties

Industries other than the pharmaceutical industry are also interested in the separation of enantiomers. The agrochemical industry is investigating the potential of single enantiomers as more efficient fertilisers and weed killers that are economical to manufacture without being toxic. In the food industry, different enantiomers are investigated as they may have different odours and tastes. One example of commercial interest is N-aspartylphenylalanine methyl ester (aspartame) which is used as a sweetener. This compound exists as four stereoisomers but it is only the *L,L*-isomer **5** that is marketed as the synthetic sweetening agent as it is more than 100 times as sweet as sucrose and, for example, the *L,D*-diastereomer is bitter.[5] Other compounds may have enantiomers with different odours, such as carvone **6** and limonene **7**,[6] which highlights the consideration of the possibility of racemization of some enantiomers as this may result in a change in taste or odour of the product.



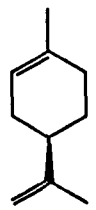
L,L-(-)-Aspartame

5



(*R*)-(-)-Carvone

6



(*R*)-(+)-Limonene

7

Figure 1.2: Examples of compounds which possess enantiomers with different tastes and odours

1.1.2 Definition of Chirality

A molecule is chiral if it is asymmetric, existing in two different structural forms, each being a non-superimposable mirror image of the other. They have identical physical and chemical properties in their gaseous, liquid and solid states and when in solution with an achiral solvent. Chirality can only be observed when the molecule is subjected to a chiral influence. A chiral compound may be asymmetric, where the molecule lacks any symmetry element or dissymmetric where the molecule does not possess any S_n symmetry element but instead has a simple axis of rotation or C_n axis. There are three general categories separating chiral molecules into those possessing central, axial or planar symmetry.

Enantiomers are related to each other by reflection symmetry and have identical internal energies. Diastereomers however are not related to each other by reflection symmetry and have non-identical internal energies.

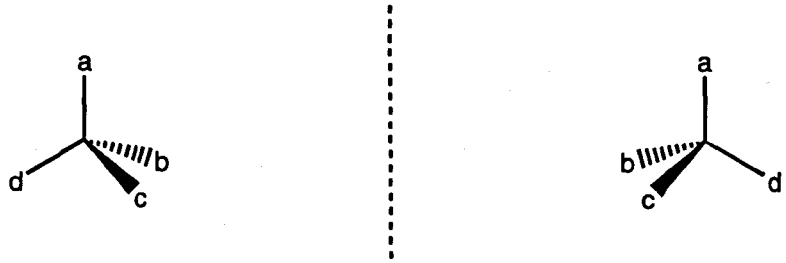
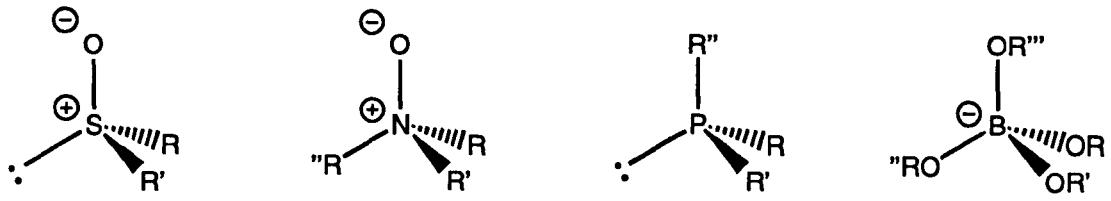


Figure 1.3: Enantiomers are non-superimposable mirror images

Enantiomers can also be referred to as optical isomers, optical antipodes or enantiomorphs. Each optical isomer rotates the plane of plane-polarized light as it passes through a crystal, liquid or solution. If there is a one to one ratio of enantiomers the mixture is said to be racemic or referred to as a racemate and there will be no net rotation

of the polarised light. To indicate the direction of the polarized light the prefixes *d* (dextrorotary) and *l* (laevorotary) are used with *dl* representing the racemate. Alternatively the prefixes (+), (-) and (±) are used, respectively.



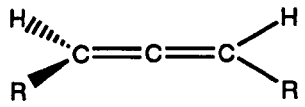
a sulphoxide | an amine oxide | a phosphine | a borate anion

Figure 1.4: Examples of chiral molecules based on a chiral centre[7]

A molecule will exist as two enantiomers if it contains four different groups that can be arranged tetrahedrally around a central atom. The central atom, which includes atoms such as carbon, sulphur, nitrogen, phosphorus or boron, is said to be the stereochemical centre. The examples shown above (**Fig. 1.4**) indicate the presence of four different groups or the situation where a lone pair may act as the fourth group forcing the tetrahedral shape. Molecules that possess chiral axes, such as allenes **8** also show optical activity. This type of chirality is similar to the optical isomerism found in metal-ion co-ordination complexes in metallocenes **9**. Optical activity is also observed in molecules with a chiral plane, such as hexahelicene, (steric crowding in a molecule gives rise to molecular distortion and hence chirality). Also molecules with restricted rotation around a single bond, such as biphenyls **10** can be referred to as chiral. An example of induced chirality is polarized alkenes. If one end of the double bond has two electron-withdrawing groups attached and the other end has two electron-donating groups the result is a

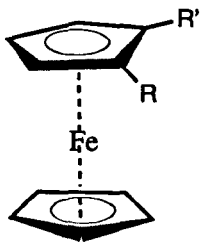
polarized bond, with a weakened π-bond. This allows the structure to twist and if the substituents are sterically hindered two enantiomers are possible.

It is also important to consider the stability of the chiral structure when considering the existence of enantiomers. The energy barrier between interconversion between a given molecular structure and its mirror image may be small and there is then the possibility that the enantiomers will interconvert.



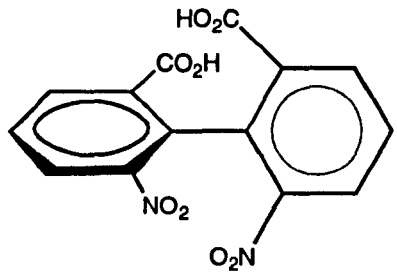
an allene

8



a ferrocene derivative

9



o,o'-dinitrophenic acid

10

Figure 1.5: Examples of enantiomers without a chiral centre

Regulatory bodies, such as the FDA, have now recognised the different properties of enantiomers and no longer allow the registration of racemic compounds, unless with good justification. Each enantiomer must be isolated, characterized and evaluated independently. This not only ensures safety but usually improves efficiency, with a possible reduction in unwanted effects.

1.2 Determination of Optical Purity[7]

There are many methods for determining the enantiomeric purity of a compound (that is its enantiomeric purity and not the purity of the compound as determined by common analytical procedures). These can be considered as methods that require either physical separation of the enantiomers or not. Methods that do not require the physical separation of the enantiomers include polarimetry, nuclear magnetic resonance (NMR), isotopic dilution, calorimetry and enzyme techniques. These techniques will be briefly discussed. All of these methods, apart from those derived from NMR, require information from one pure enantiomer for comparison as they measure a net effect. NMR either uses a technique of converting the enantiomers into diastereomers (by chiral derivatisation e.g. Mosher's esters[8]) or measuring diastereomeric interactions (e.g. chiral solvating agents). Methods that involve the separation of the enantiomers include crystallization and chromatography. Separation by chromatography will be discussed in more detail later on in this chapter, as it is more frequently used than recrystallization and of relevance to this thesis.

1.2.1 Polarimetry

Polarized light is composed of two vectors that can be regarded as chiral objects that do not behave identically on interaction with chiral molecules. Therefore as these vectors pass through a chiral medium (the chiral compound in solution) they are propagated at different speeds. This results in a rotation of the plane of the polarized light, referred to as the optical rotation, α.

As this method is only comparative, knowledge of the optical rotation of the pure enantiomer is required. Identical measurement conditions are also needed for an accurate comparison as optical rotation is dependent on concentration, optical path length, solvent, temperature and the wavelength of the light used. Specific rotation, $[\alpha]_D$ is calculated using **equation 1.1** and concentration, temperature, wavelength and solvent should be stated.

Equation 1.1: Calculation of specific optical rotation

$$[\alpha]_D^T = \frac{100\alpha}{lc}$$

where α = measured optical rotation

D = wavelength of the light

c = concentration (g/100 ml)

l = cell path length (dm)

T = temperature (°C)

The optical purity (P%) can be calculated using **equation 1.2** so that comparisons between samples can be made.

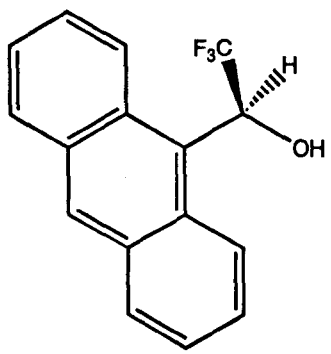
Equation 1.2: Calculation of percentage optical purity

$$P = \frac{100[\alpha]}{[\alpha]_{max}}$$

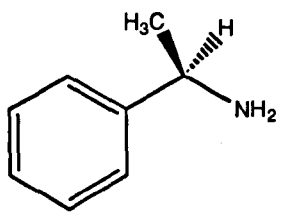
where $[\alpha]_{max}$ is the optical rotation of the pure enantiomer

1.2.2 NMR

This method requires the transformation of the enantiomers into diastereomers or by measuring diastereomeric interactions. The former can be done by reaction of the enantiomers with a suitable reagent to form diastereomers (e.g. the conversion developed by Mosher[8]) or a diastereomeric environment can be achieved by complexation with a chiral solvating agent (CSA). Examples of CSAs include (*R*)-(-)-2,2,2-trifluoro-1-(anthryl) ethanol (TFAE) **11**[9] and (-)-α-methylbenzylamine (PEA) **12**.[10] The spectrum shows a chemical shift difference between the two diastereomeric complexes and integration of the peaks can be used to calculate the ratio of the enantiomers.



11 (*S*)-(+)-TFAE



12 (*R*)-(+)-PEA

Figure 1.6: PEA and TFAE, used in chiral NMR

The advantage of using a chiral solvent is that the optical purity of the solvent is not important. The purity of the solvent only affects the separation of the peaks and not their integration. However, the peak separation, caused by different interactions between each enantiomer and the solvent is often too small to be of practical use.

Optically active lanthanide shift reagents[11] can also be used, resulting in a significant downfield or upfield shift for groups near to the functional group that is complexing with the metal. The use of chiral lanthanide-shift reagents can produce an increase in spectral resolution, which means that the resonance lines corresponding to the two diastereomeric interactions are better separated and the enantiomer composition can be more accurately determined, again by integration.

1.2.3 Isotope Dilution

The basic principle involved in isotope dilution is to mix the sample of unknown enantiomeric purity with an isotopically labelled racemate of the same compound. The mixture is then recrystallized and the isotope content determined. From this, the maximum specific rotation, [α], of the compound can be calculated and used to determine the optical purity of the original sample. This method therefore requires the specific rotation of the sample, obtained by polarimetry, its isotope content, obtained by mass spectrometry or by liquid scintillation, and a sample of isotopically labelled racemate.

1.2.4 Calorimetry

This method is only applicable to solid compounds and collects data such as melting point, enthalpy of fusion of the racemate and the termination of fusion temperature of the sample, from which the enantiomeric composition can be calculated. Essentially, the energy absorbed or evolved by a sample is determined as a function of the temperature using differential scanning calorimetry (DSC).

1.2.5 Enzyme Techniques

Enzymes are highly stereoselective and especially in the case of amino-acids, can discriminate completely between a pair of enantiomers. Enzyme techniques of determining optical purity involve transformation of the contaminating enantiomer to determine its percentage composition. Using such techniques it is possible to detect 0.1% of an enantiomer in the presence of 99.9% of its antipode, by studying its reaction with the enzyme. The use of this technique is limited by the reactions that can be catalysed using enzymes and the enantiomer specificity of the enzyme.

1.3 Strategies for Obtaining Enantiomerically Pure Compounds

The increased demand for the isolation of enantiomers for both analysis and preparation has led to improved methods of chiral separation and an insight into the relevant mechanisms required for separation. There are two possible methods for obtaining single enantiomers; asymmetric synthesis where only the desired enantiomer predominates or resolution of a racemate. Both methods are often used and both have their own merits and drawbacks.

High performance liquid chromatography (HPLC) is a very efficient method of enantioseparation and many chiral columns have been developed. However, chiral HPLC columns are expensive and difficult to use when large-scale preparations are required. Chiral flash chromatography is beginning to be accepted as a method for enantioseparation, as it is cheaper and easier to use for large-scale preparations. Work is now being done to improve many chiral phases to allow them to be applicable to separate as wide a variety of chiral compounds as possible. Asymmetric synthesis uses a variety of

approaches to furnish the chirality and these include the use of chiral starting materials (sometimes called the 'chiral pool') or using chiral reagents, chiral auxiliaries or chiral catalysts.

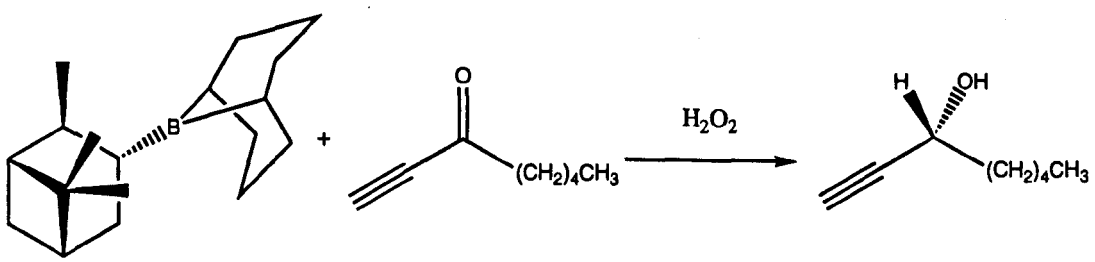
1.3.1 Asymmetric Synthesis[12]

1.3.1.1 Chiral Pool

This approach involves using the chiral pool of molecules that contain natural chirality such as amino acids and sugars as starting materials for further synthesis. Unfortunately this method is restricted by the small number of existing chiral compounds that are commercially available thus limiting the generality and efficiency of these syntheses.

1.3.1.2 Chiral Reagent

A chiral reagent can introduce chirality at any step in a synthesis, an example is the use of α-pinene BBN **13** in the reduction of acetylenic ketones (**scheme 1.1**).

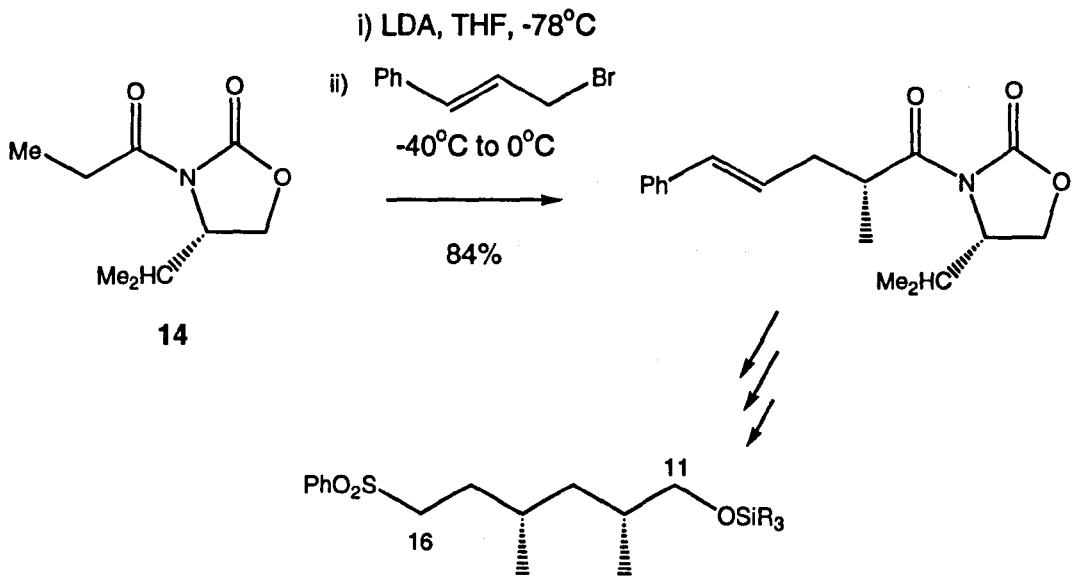


13 92% ee

Scheme 1.1: Enantioselective reduction of a ketone using a chiral reagent[13], where enantiomeric excess is the (% major enantiomer minus % minor enantiomer)

1.3.1.3 Chiral Auxiliary

The use of chiral auxiliaries in asymmetric syntheses is a versatile and broadly applicable method. The chiral auxiliary that transfers its chiral information in further synthetic steps can often be recycled and these reactions are often highly predictable and reliable. The diastereomeric products obtained are normally purified by conventional techniques. The disadvantage of this approach is that stoichiometric amounts of the chiral auxiliary are needed and the purity of the auxiliary will dictate the diastereomeric excess of the desired product. Another major disadvantage is the increase in the number of steps required in the synthesis of the target (due to the attaching and removing of the auxiliary). Examples of such auxiliaries include oxazolines,[14] enones[15] and sulphoxides.[16]



14

C_{11}-C_{16} fragment

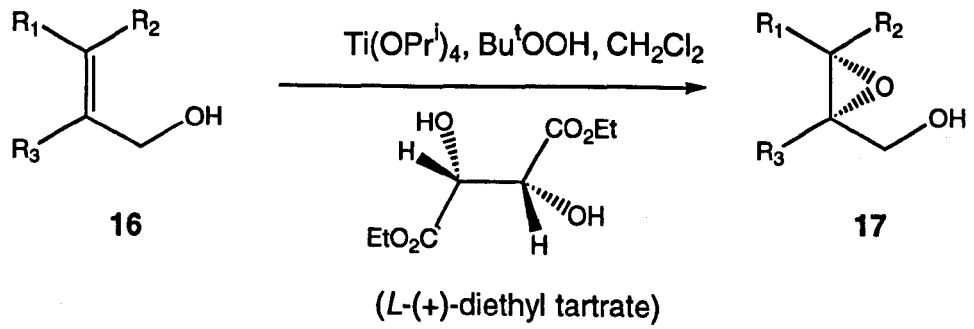
15

Scheme 1.2: Use of a chiral auxiliary by Evans[17]

Chiral auxiliaries have often been used in the synthesis of natural products. Evans *et al.* used many chiral auxiliaries in the synthesis of the polyether antibiotic Ionomycin.[17] The stereochemical directing effect of the chiral oxazolidinone auxiliary **14** is shown in **scheme 1.2,** one step in the synthesis of the C_{11}-C_{16} fragment **15** of Ionomycin.

1.3.1.4 Chiral Catalyst

This is the ideal approach to the synthesis of chiral molecules as the limits imposed on the chiral species become less important when considering small and potentially recoverable catalytic amounts. Biological examples include enzymes such as lipases and esterases. Synthetic examples of chiral catalysis include the Sharpless-Katsuki epoxidation,[18] (**scheme 1.3**) a versatile method for the epoxidation of allylic alcohols, e.g. **16**. The chiral catalyst is formed from the reaction of *tert*-butyl hydroperoxide with titanium tetraisopropoxide and a diester of (+)- or (-)- tartaric acid. Different ratios of tartrate or tartramide to alkoxide have been studied but the standard reaction employs a ratio of tartrate to titanium alkoxide of 1.2:1 to achieve optimal enantioselectivity, although as little as 5 mol% can be effective.



Scheme 1.3: Sharpless-Katsuki epoxidation using chiral catalyst

1.4 Basic Principles of Chromatography

As this project is concerned with the development of stationary phases (SP) for chromatography a more detailed description of this method of separation will be given.

Chromatography is a method of separating substances in a mixture. This is done by passing the sample over the surface of a fixed, stationary phase. The separation may be due to selective adsorption, volatility, solubility, charge, diffusion, sedimentation or ligand binding of different mixture components. It relies on the different distribution of each analyte between the two phases, one of which is moving, referred to as the mobile phase (MP), with respect to another one, the stationary phase (SP). The SP is usually packed into a column and the MP, containing the mixture of analytes, is passed through the column. As the sample passes through the column it undergoes some form of interaction with the SP. There are different forms of chromatography that utilise various types of SP and MPs. The MP can be gaseous or liquid and the SP may be solid or liquid (usually liquid on a solid support). Gas chromatography (GC) requires the sample to be volatile enough to get into the gaseous phase and consequently of sufficient stability at elevated temperatures. Liquid chromatography includes high performance liquid chromatography (HPLC) and flash chromatography, using a wide variety of solvents, referred to as the eluent, depending on the analyte.

1.4.1 History of Chromatography

Tswett, in 1903,[19] separated compounds by elution through a bed of adsorbent and attributed this to the compounds having different affinities for the adsorbent. However, it was not until 1941 when Martin and Synge[20] treated the peaks quantitatively

that a theoretical, rather than empirical, basis for chromatography was established. They also compared the process of chromatography with fractional distillation to provide a method of assessing efficiency in terms of height equivalent to a theoretical plate. Since then many developments have been made in understanding the principles involved in chromatography as well as manipulation of the data and the equipment used.

1.4.2 Detection Methods

After a mixture has been separated on a column the separated individual components must be detected in order for them to be collected. The four most common types of detector are those based on refractive index (RI), UV, fluorescence or electrochemical detection systems. RI is a universal method as it measures a bulk property but has low sensitivity. This method also requires constant conditions such as solvent composition, temperature and flow rate. UV is used in most liquid chromatography systems but sensitivity is dependent on the solute. Fluorescence detectors are highly selective and often sensitive. If the analyte does not contain a fluorophore then derivatives can be prepared by either pre or post column reactions. Electrochemical detectors are highly selective and sensitive to compounds susceptible to electro-oxidation or reduction.

1.4.3 Equations used to Describe Chromatography

There are a number of equations used in chromatography that enable a comparison of different separation methods. A typical chromatogram obtained from a column is shown in **Fig. 1.7**.

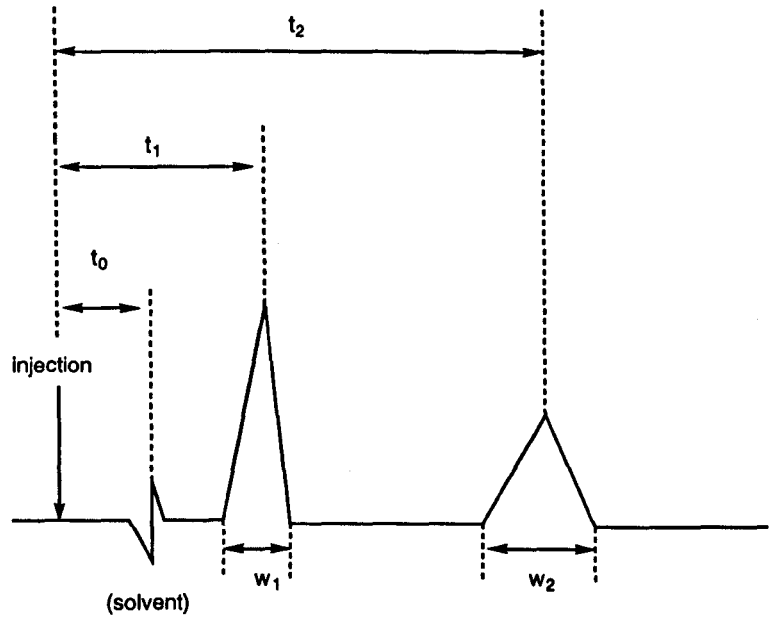


Figure 1.7: A schematic diagram representing a chromatogram of 2 analytes

Ideally the profile of a chromatographic peak should have a Gaussian distribution, as suggested by Van Deemter in 1958,[21] resulting in a completely symmetrical peak. To monitor the shape of the peak there needs to be a suitable detection system the form of which will depend on the analyte and the MP. However, as the compound moves through the column there are dispersion effects that broaden the band. There are 3 types of factor that contribute to this broadening of the peaks; eddy diffusion, longitudinal diffusion and mass transfer resistance. Eddy diffusion occurs as some molecules travel through the column at different rates. This can be reduced by small, even sized particles that are well packed into the column. Longitudinal diffusion occurs as sample molecules disperse in the MP and so are affected by the choice of MP and flow rate of the column. Mass transfer occurs if the molecules are able to remain in stagnant pores in the SP for too long.

The quantities t_0, t_1 and t_2 are the absolute retention times of the three solutes with t_0 representing the solvent flow marker or an unretained analyte. They can be measured in time, volume of solvent or distance on the chart recorder. There are a number of factors such as flow rate and length of the column, that can affect the values of these quantities so the capacity factor, k', of each analyte is usually calculated (**equation 1.3**).

Equation 1.3: Calculation of capacity factor

$$k' = (t_r - t_0)/t_0 = (V_r - V_0)/V_0$$

where k' = capacity factor

t_r = retention time of analyte, t_0 = retention time of solvent flow marker

V_r = retention volume of analyte, V_0 = retention volume of solvent flow marker

The capacity factor, k' (or capacity ratio), is related to the equilibrium constant (K) of the analyte in the SP and MP as shown in **equation 1.4** and should be in the range 2-10. If it is too small the peaks will be difficult to separate and if it is too big then the column will take a long time and the separation will be affected by other factors. The choice of solvent can be changed to include the use of solvent mixtures to optimise the capacity factor.

Equation 1.4: Alternative definition of capacity ratio, k'

$$k' = A_s/A_m = K$$

where A_s = amount of a substance in the SP

A_m = amount of a substance in the MP

K = equilibrium constant

The capacity factors of the two retained analytes can be used to calculate the selectivity or retention factor, α, as in **equation 1.5**. It is a measure of the relative peak separation of the two analytes and is constant under constant conditions.

Equation 1.5: Calculation of separation factor

$$\alpha = k'_2/k'_1 = (t_{R2}-t_0)/t_{R1}-t_0)$$

where α = separation factor

k'_1 = capacity factor of first eluting analyte

k'_2 = capacity factor of second eluting analyte

t_{R1} = retention time of first eluting analyte

t_{R2} = retention time of second eluting analyte

t_0 = solvent flow marker

Resolution, R_S, of two peaks is a function of both column efficiency and the separation factor and is defined in **equation 1.6**. A resolution of 1.5 will give essentially complete separation of the two peaks. It is desirable to obtain a balance between the resolution and the time taken for the separation.

Equation 1.6: Calculation of the resolution, R_s

$$R_s = 2(t_{R2}-t_{R1})/(w_1-w_2)$$

where R_s = resolution

t_{R1} = retention time of first eluting analyte

t_{R2} = retention time of second eluting analyte

w_1 = peak width of first eluting analyte at base of peak

w_2 = peak width of second eluting analyte at base of peak

The efficiency of a column is a measure of its ability to transport a compound with little peak broadening. It can be defined by **equations 1.7** and **1.8** where H is the plate height, or height equivalent to one theoretical plate (see section 1.4.1). For maximum efficiency H will be as close to zero as possible. Factors affecting the column efficiency therefore include flow rate, column dimensions, particle size and the column packing.

Equation 1.7: Calculation of plate number

$$N=16(t_R/w)^2$$

where N = number of theoretical plates, or plate number

t_R = retention time of peak

w = baseline peak width

Equation 1.8: Calculation of plate height

$$H = L/N$$

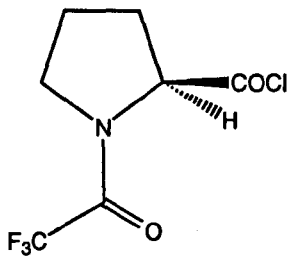
where H = plate height

N = number of theoretical plates, or plate number

L = length of column

1.5 The Background of Chiral Chromatography

The separation of enantiomers by chromatography can be categorised into two methods. Indirect methods involve separating diastereomeric derivatives of enantiomers using achiral columns and direct methods which involve the separation of enantiomers directly using a chiral column. Chiral columns consist of a SP that is usually an optically active compound, or an optically active compound coated onto a solid support. The chiral SP interacts with both enantiomers differently causing the separation.



18

Figure 1.8: The acid chloride of (*S*)-(-)-N-trifluroacetyl proline used to derivatise enantiomers prior to column chromatography using an achiral column

When an achiral column is used to separate diastereomeric compounds the diastereomers can be formed prior to chromatography by derivatisation. Resolution of various amines and alcohols by GC and HPLC has been achieved by derivitisation using the acid chloride of (*S*)-(⁻)-N-trifluoroacetyl proline **18**.[22] After resolution, the derivitization needs to be reversed, in this case by hydrolysis of the ester or amide bond. Enantiomers can also be separated by adding CSAs (chiral solvating agents) to the eluent and again using an achiral column. Additives include camphor sulphonic acid, quinine and α, β and γ cyclodextrins[23] and although they need to be separated from the

enantiomers after chromatography this is usually easier than removal of a derivitising agent.

Direct methods are better regarded both analytically and preparatively, as the formation of diastereomeric derivatives has a number of disadvantages. Although the use of achiral columns is cheaper and simpler the synthesis of the diastereomeric complexes may be difficult or expensive, there is the possibility of racemization or decomposition of the analytes and the addition of additives may interfere with the detection system. In addition for preparative applications the additives need to be removed from the analyte once resolved.

1.5.1 Mode of Action

To understand the reason why there is a difference in retention times of enantiomers in chiral columns, the molecular interactions of the analytes with the chiral stationary phase (CSP) need to be considered. There are many types of possible interactions, depending on the functional groups present in the SP and in the molecules being separated. These might be binding interactions, which include hydrogen bonding, electrostatic, dipole-dipole, charge-transfer and, in aqueous systems, hydrophobic interactions, or steric and dipole-dipole interactions which are repulsive interactions.

Charge-transfer interactions require π-electron systems (e.g. aromatic rings) with two components; a π-donor and a π-acceptor. Good π-acceptors are groups such as nitroaromatics or compounds with electron-withdrawing substituents whereas aromatic systems with electron donating substituents, such as amino groups, can act as π-donors. Although π-π interactions can be the sole source of retention for some molecules, e.g.

condensed aromatic hydrocarbons of planar chirality, it is more usual to have other bonding interactions as well.

Some phases have a structure that enables them to include guest molecules.[23] One of these is urea which can form channel like cavities which allows unbranched alkanes inside but excludes branched alkanes. Another is α-cyclodextrin which has a cavity that will fit around benzene but will not allow bromobenzene inside. This inclusion phenomenon can be extended to the inclusion and exclusion of enantiomers.

1.6 Theories of Molecular Recognition in Chiral Chromatography

In 1952, Dalgliesh[24] separated amino acids using paper chromatography. In order to rationalise his theory of enantioseparation by selective adsorption by the optically active cellulose molecules he proposed a 'three point-of-attachment rule'. He suggested that three points of attachment were required for stereochemical specificity in adsorption. These interactions could be hydrogen bonding or steric in origin and, in the example of the amino acids used, included interactions of the amine, the acid and the structural component of the side chain.

Pirkle later re-stated this rule by clarifying that these 3 interactions must be simultaneous between the CSP and at least one of the enantiomers.[25] Also at least one of these interactions must be stereochemically dependant.

Chiral chromatography is based on the idea of forming transient diastereomeric complexes between the SP (the selector) and the chiral analyte (the selectand). It is a difference in stability, or difference in free energy of formation, of these complexes between each enantiomer that causes a difference in retention time. Therefore for

enantioselectivity to occur there must either be a difference in the interactions or the energy gained by the interactions.

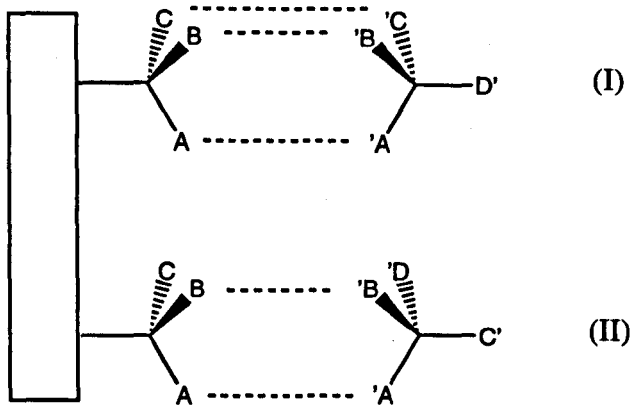


Figure 1.9: Difference in interactions of analyte with CSP

The possible interactions between an analyte and the chiral selector of a SP are shown in **Fig. 1.9**. In situation (I) the possible interactions that could occur are the A-A', B-B' and C-C' interactions while in situation (II) the C-C' interaction is missing. If the C-C' interaction stabilizes transient complex (I) then this enantiomer will be retained for longer on the column than the enantiomer in situation (II). However, if the interaction C-C' destabilizes transient complex (I) then it will be eluted first. If the C-C' interaction is minimal or non-existent then there will be no difference in binding between situation (I) and (II) so there will be no enantioselectivity.

In general there will be three interactions and they may all be attractive or one of them may be repulsive but at least one of them has to be stereochemically dependent. The three interactions also need to be different in nature, such as H-bonding, π-π stacking and steric, so there is no possibility of alternative arrangements forming another stable transient complex. Steric interactions are usually repulsive in nature but may also be

attractive, as in the situation where there is a perfect steric fit the van der Waals forces can be bonding. Steric repulsion can also have the secondary effect of weakening other bonds such as acid-base and π-π attractions.

One important factor is the frequency of chiral units attached to the CSP. If there are too many chiral selectors they may occur too close together in the CSP and then the analyte may be able to interact with more than one chiral selector at the same time. This may affect the enantioselectivity of the interactions. It has been shown that less densely bonded silicas have increased enantioselectivity, attributed to the association of enantiomers with only one receptor molecule at a time.[23]

Another consideration that affects enantioselectivity is the flexibility of both the analyte and the chiral selector. If one or either can easily change conformation then different enantiomers may be able to form stable complexes of similar energy which would reduce the enantioselectivity.

Evidence of multiple interactions in chiral recognition can be obtained from intermolecular nuclear Overhauser effects in NMR and molecular mechanics calculations of analytes and CSPs. Although the application of the information is limited as former studies involve the investigation of interactions in solution while in reality recognition in chromatography is occurring at the solid-liquid interface.

It may be possible to understand the interactions occurring that cause retention but it may not be possible to fully understand the mechanism behind chiral discrimination, as it is far more complex. Chiral recognition comes from a weighted time average of all the contributions of all the possible complexes. Each enantiomer can approach from different directions, in different conformers and there will be different combinations of at least 3 simultaneous interactions. It is sensitive to changes in free energy of association which

reflect structure, dynamics and solvation. It is not possible to represent the complicated process by a simple model but increased understanding of the factors discussed have helped improve the existing CSPs and continued study will help further.

1.7 Types of Chiral Stationary Phases

Many materials have been used as the SP in chiral chromatography. These range from natural chiral polymers, to synthetic polymers that are either (i) made from the polymerization of asymmetric monomers, (ii) have a chiral secondary structure, or (iii) are derivitized with chiral units. Chiral ion-exchange resins (e.g. quinine and polyacryloyl chloride) and surface coated solid supports, such as alumina and silica gel, have also been used as CSPs.

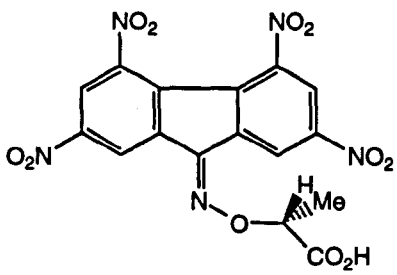
There are different ways of categorising CSPs. One classification is to separate them by their mechanism of chiral discrimination[26] while an alternative classification is based on the type of chiral selector.

1.7.1 Type 1: Ligand Exchange Chromatography (LEC)

This type of chromatography requires an analyte than can act as a ligand to a metal ion and therefore form a metal complex by co-ordination. Separation of the two analytes depends on the difference in thermodynamic stabilities of adsorbates that are formed. The chiral selector may be an immobilized amino acid and a divalent cation with the choice of metal cation greatly affecting the stability of the complexes. The choice of solid support can also affect the efficiency (with silica-gel having shown more suitability than resin packings).[27] In addition the MP is normally a complicated mixture of buffers

which maintain the correct pH and concentration of the metal ion (maintaining saturation of the SP). Commercial packings of this type are available (e.g. Chiralpak W).

1.7.2 Type 2: Charge Transfer Packing



19

(*R*)-(-)-TAPA

Figure 1.10: Tetranitrofluorenylideneaminooxypropionic acid (TAPA) used as a charge transfer CSP when bonded onto silica

Charge transfer SPs have been developed by Gil-Av,[28] to resolve helicene enantiomers. The main difficulty with resolution of these types of enantiomers is their lack of acidic or basic functionality. Resolution can be achieved by bonding tetranitrol-9-fluorenylidene-amino-oxypropionic acid (TAPA) **19** to aminopropylated silica gel. This chiral phase can then be used to resolve polyaromatics by charge transfer.

1.7.3 Type 3: Asymmetric Strand Packings

Pirkle is considered to be the first to have developed such asymmetric strand packings as CSPs,[29, 30] hence they are sometimes referred to as Pirkle brush-type phases.[31] They generally consist of organic molecules capable of multiple interactions, attached to

a solid support such as silica gel. They contain functional groups containing at least one of the following near, or at, the stereogenic centre; π-acidic or π-basic aromatic group; hydrogen bond donor or acceptor; dipolar bond capable of dipole-dipole interactions such as stacking; bulky, non-polar groups providing potential for steric repulsion, van der Waals interactions and/or conformational control.

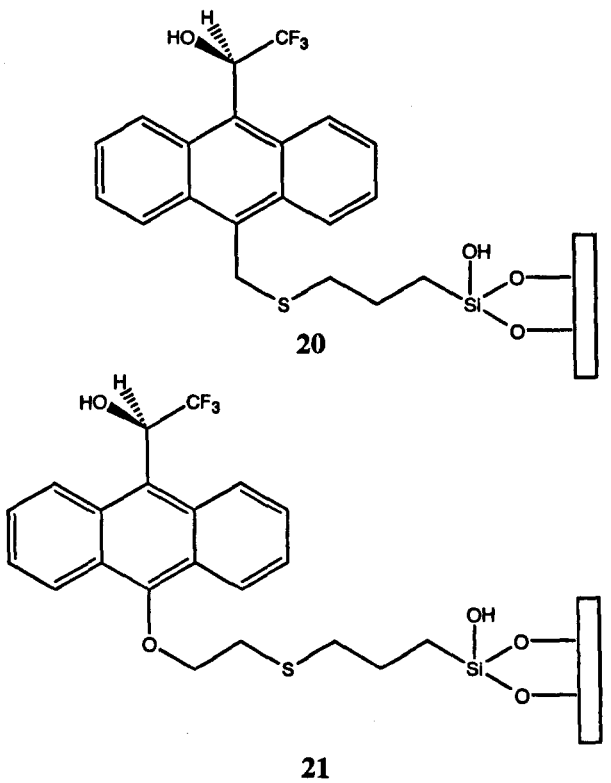


Figure 1.11: Pirkle brush type CSPs[30]

For enantioselectivity, the analytes must have corresponding, complementary groups. A large number of analytes, of a wide variety of types, have been resolved on such phases. The first phase to be developed by Pirkle, **20**, during the 1970's was inspired by NMR studies on enantiomers complexing with a range trifluoromethylaryl carbinols, showing chemical shift non-equivalence.[32] Resolution was improved by increasing the π-

donating nature of the anthryl system **21** indicating the importance of the π-π interactions in chiral discrimination.

Computer assisted models have been used in the study of the interactions involved with these CSPs but the exact mechanism seems to vary with each analyte and there are many considerations such as the several conformations of both strand CSP and analyte.

Work on such phases, including synthesis and investigation of the possible mechanisms of chiral recognition has been done by many groups around the world.[33] Another example of a strand chiral phase is a CSP developed by Hara and Dobashi[34] which is synthesised from (*R,R*)-tartaric acid **22** and was shown to be useful for the resolution of a broad category of enantiomers.

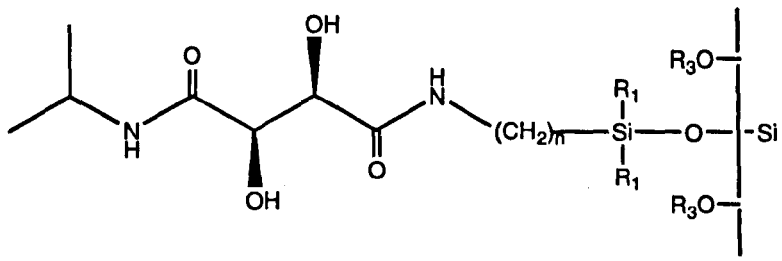


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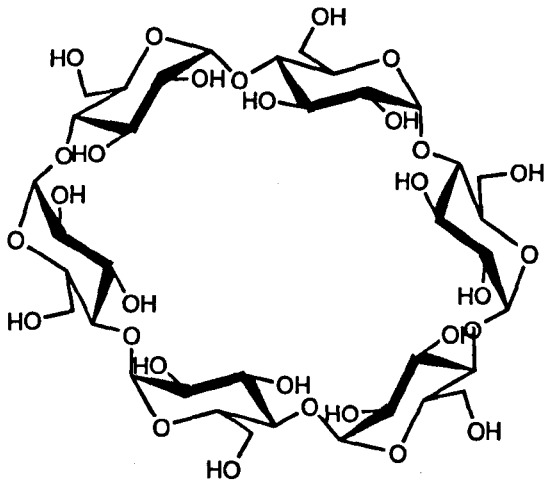
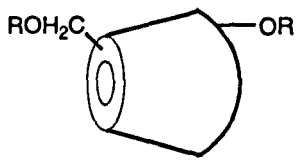
Figure 1.12: CSP designed by Hara *et al.*[34]

There are so many phases of this type available that the main problem is no longer if separation of a chosen analyte is possible but which column to choose to suit the problem.

1.7.4 Type 4: Chiral Cavity Packings





23

Figure 1.13: α-Cyclodextrin

Chiral cavity packings were first demonstrated by Cram and workers[35] in America, using chiral crown ethers bound to silica gel. A lot of interest has also been generated by the use of cyclodextrins. In this type of SP differential inclusion of enantiomers in the cavities has to be responsible for chiral recognition of cyclodextrins. Cyclodextrins are α-1,4-*D*-glucosides that have cyclized into rings of 6-12 units (**Fig. 1.13** shows hydroxylated α-cyclodextrin **23** with 6 glucose units) and have a wide scope due to their potential for derivitization. In aqueous systems the conformations of cyclodextrins is assumed to be that of a truncated cone, where molecules can fit inside the cavity. The interior surface is relatively hydrophobic allowing complexation with non-polar compounds but the exterior is hydrophilic making it suitable for use with aqueous MPs. There is also the additional interaction of the analyte with the functional groups around the rim of the cavity and the fact that the interior is in fact chiral and of varying

sizes depending on the cyclodextrin used. Therefore, if there is a difference in stability of the reversible guest-host complex there is the potential of enantioselectivity. The cyclodextrins are usually bound to a solid support such as silica gel by means of an alkyl chain and there are commercial phases of this kind available (Cyclobond, J. T. Baker and Technicol Ltd).

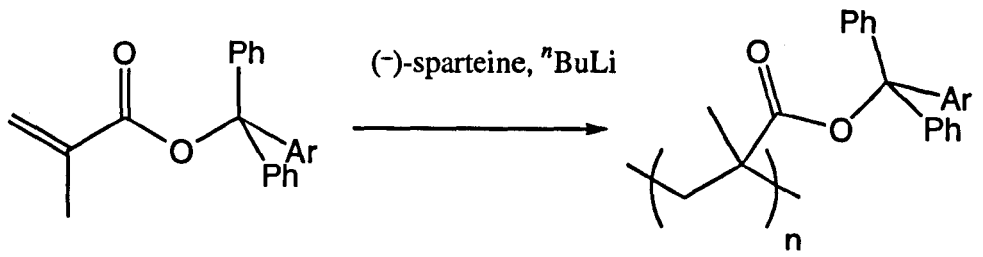
1.7.5 Type 5: Chiral Polymers

These chiral polymers include those of natural origin, such as cellulose, or of synthetic origin, such as those based upon polyacrylates and polyacrylamides.

Much work has been done using cellulose or cellulose derivatives,[36-39] either as the solid phase or on a solid support. Many phases, including different polymers with many different functional groups, are commercially available (mainly from the Japanese company Diacel). Polysaccharides other than cellulose have been studied[36] i.e. amylose, chitosin, xylan, curdlan, dextran and inulin. The mechanism of action of these polysaccharide derivatives is complicated and many suggestions have been made, usually indicating the necessity of attractive interactions, such as dipole-stacking, but with an important contribution from inclusion into chiral cavities or ravines, formed due to their ordered secondary structure.

Chiral synthetic polymers can either be synthesised (i) by the polymerisation of chiral monomer units, (ii) polymerisation of monomers using a chiral catalyst or (iii) by molecular imprinting of chiral interactions. An example of using a chiral environment to induce chirality was developed by Okamoto[40] who produced an isotactic polymer, which was chiral due to its helical secondary structure, starting from triphenylmethyl

methacrylate **24** with a chiral sparteine-*n*-butyl lithium catalyst. Generally, lower molecular weight polymers immobilized on silica gel are more useful than very high molecular weight polymers as CSPs.



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Scheme 1.4: Chiral polymerisation of a methacrylate[40]

Polyacrylamide based CSPs depend on the chirality of the individual units rather than any macrostructural chirality. This strategy allows for the development of a great number of phases and such phases have generally a broad applicability, high capacity, good efficiency and can be used with a variety of MPs.

1.7.6 Type 6: Silica Bound Proteins

One advantage of silica bound proteins as CSPs is the use of a chiral selector that is likely to be suited to molecules of biological activity. Early classes of this type of CSP were based on bovine serum albumin[41] or acid glycoprotein,[42] though recent more reliable and reproducible phases have been based on second-generation silica-bound α_1-acid glycoprotein (AGP).[43] They are generally unsuitable for cationic analytes but have been used to separate a number of different types of anionic and neutral analytes, in

particular amino acids. The efficiency of these columns also depends on the MP, as they are often sensitive to pH and the concentration changes of any buffer in solution.

Bound enzymes also fit into this category, being biopolymers they normally exhibit stereoselective interactions, however, they are very specific to just a few substrates. Generally, they can be used to distinguish between certain amino acids.[44]

1.8 Polysaccharides as Chiral Stationary Phases

By far the most successful commercial chiral phases are those derived from polysaccharides. Many CSPs are based on polysaccharides or polysaccharide derivatives coated onto solid supports. The first separation of aromatic acids using paper chromatography by Kotake[45] and later by Dalgliesh[24] involved interactions between the analytes and the cellulose and/or the water bound to the cellulose units. Since then many other sugars and their derivatives have been studied as chiral phases.[36]

Cellulose is a naturally occurring polymer consisting of 1,4-linked *D*-(+)-glucose units. These chains form mutual hydrogen bonds that result in chiral helical structures[23] consisting of up to 1500 units in length producing molecules of mass 2.5×10^5 to 1×10^6 or more. Cellulose fibres consist of parallel bundles held together by hydrogen bonding between the hydroxy groups. This results in areas of crystallinity, the degree of which can be increased if subjected to partial hydrolysis using dilute acid. As a CSP itself it has poor mechanical properties with poor resolving abilities due to its porous structure slowing down mass transfer and diffusion. There is also the possibility of many non-stereoselective interactions with the many polar hydroxyl groups present. To overcome some of these problems derivatives were synthesised. Hesse and Hagel in 1973[46]

prepared microcrystalline cellulose triacetate (MCTA) **25** by heterogenous acetylation of native microcrystalline cellulose in benzene.

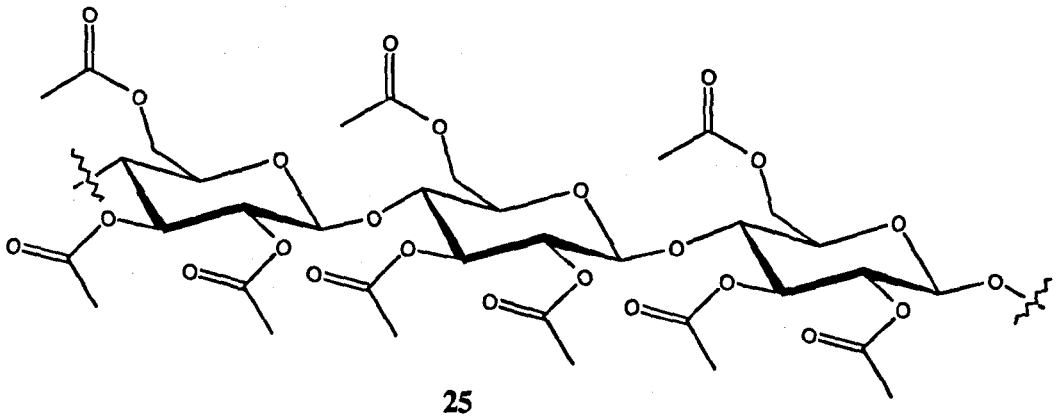

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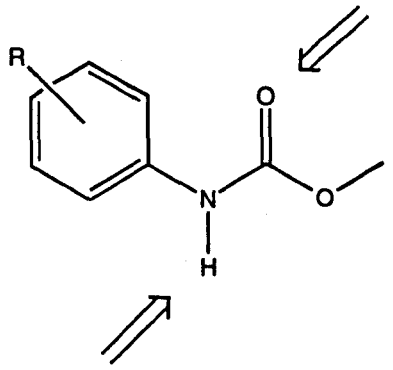
Figure 1.14: Cellulose triacetate (CTA)

Microcrystalline cellulose is obtained by subjecting cellulose to partial hydrolysis using dilute acid. Hydrolysis occurs in the amorphous regions first with the resulting cellulose having approximately 200 glucose units per chain. Their method of preparation of acetylated cellulose preserved the microcrystalline structure of the cellulose. This gave an enantioselective CSP with a high loading capacity, however, it was unable to withstand high pressures (necessary to pack the columns and run them at suitable flow rates in HPLC).

Acetates and other ester derivatives (e.g. benzoates) were prepared by Okamoto *et al.*.[47] They prepared CSPs by supporting the polysaccharide on a silica support. This led to enantioselectivity by a different mechanism but with greater column efficiency, durability and a greater choice of solvents for the MP. Another alternative to silica supports was developed by Mannschreck *et al.*[48] and Francotte *et al.*[49] who prepared spherical beads of cellulose tribenzoate (CTB) and other derivatives to afford CSPs with

high loading capacities. Although the different derivatives showed complementary enantioselectivities they were in general comparable to that of CTB-silica columns, making them useful on a preparative scale.



H-bonding with NH and C=O

dipole-dipole on C=O

π-π stacking with Ar

Figure 1.15: Sites for possible interactions with analyte

These different derivatives are likely to have different mechanisms for enantioseparation. The crystallinity of cellulose and MCTA was thought to be an important factor in separation primarily because enantioselectivity was reduced or lost on dissolution and reprecipitation.[50] However, examination of CTA coated on silica shows it to be amorphous.[51] In general, most proposals for mechanisms include the formation of diastereomeric complexes. This may be through hydrogen bonding between, for example, an alcohol group on the analyte and an ester of the SP, or by inclusion of an aromatic portion into a chiral cavity. Derivatisation of the polysaccharide alcohol functionality as phenyl carbamates has produced perhaps the most useful of CSPs (**Fig. 1.15**). Ichida *et al.*[52] showed the importance of the urethane group in these phases in that they can interact with polar alcohol or amino groups of the analytes as well as be involved with π-π interactions with phenyl groups. Chiral discrimination is then achieved due to differences

in steric fit of analytes into the chiral cavities of the CSP or by interaction of substituents near the chiral centres.

The different chiral recognition abilities of CTA and CTA coated on a silica support highlights the importance of various factors that affect enantioselectivity. The choice of functional groups on the polysaccharide SP is important as well as the higher order structure of the polysaccharide, which can be affected by its mode of preparation. The choice of solvent used to coat the polysaccharide onto the silica and the molecular weight of the cellulose also influences the chiral recognition of the CSP.[51]

1.8.1 Studies of Esters and Carbamates

Work on different derivatives of cellulose show that the important sites for enantioselective discrimination are the carbonyl of the ester or carbamate and the NH of the carbamate. The strength of these interactions with the analyte is affected by the R group of the ester or carbamate (**Fig. 1.16**). In general phenyl rings with electron withdrawing substituents, such as halogens, increases the acidity of the NH protons in the carbamate group making a hydrogen-bond (H-bond) with for example a C=O in the analyte stronger. Electron donating substituents on the phenyl ring, such as alkyl groups, increase the electron density of the carbonyl group strengthening its H-bond accepting characteristics. For esters this is the important factor.

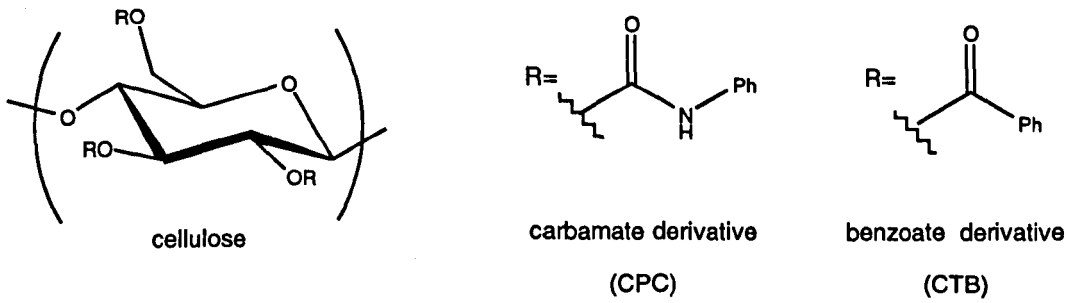


Figure 1.16: Different derivatives of polysaccharides used as CSPs

The choice of aryl substituents can have additional effects. Halogens such as chlorine can make the compound more soluble in certain solvents restricting the choice of MP. Methoxy and nitro groups can interact with the solute directly and as this interaction is far removed from the chiral glucose units it is likely to be non-stereoselective and therefore the enantioselectivity and resolution will be reduced. The position of the substituents on the aryl group is also important. Okamoto[36] showed that any substituent at the 2 and/or 6 position of the aromatic ring showed poor chiral recognition ability. They suggested that the reason for this was due to a disruption of the regular helical structure of the cellulose which results in a reduced range of chiral adsorbing sites and therefore reduced resolution. There will also be reduced intramolecular H-bonds resulting in more free amine groups available for H-bond formation with the analyte.

In general, the best substituents were found to be chlorine and methyl groups in positions 3 and 5 or 4. Though there were some anomalies such as 3,5-dimethyl benzoate cellulose not being soluble enough to coat onto the silica support. Other derivatives such as methyl carbamate showed little enantioselectivity highlighting the importance of the phenyl groups.

1.9 Solid Supports in Chromatography

1.9.1 Silica

One of the most common solid supports in liquid chromatography is silica gel. It can be used as the SP itself or as the base for bonded or coated phases. The silica gel used in chromatography is amorphous, non-crystalline and porous. Certain characteristics, such as the porosity and the size and shape of the particles, can be controlled in the manufacturing process and these properties affect the packing and efficiency of the column.

Porosity is characterized by the width of the pores, their shape and their distribution within the solid particles where a pore is a hole, cavity or channel open to the surface of the solid. The pore size defines the diameter of the pore opening, whereas the pore volume defines the volume inside the pore.

The mean pore diameter, D, may cover a range of several orders of magnitude. If the pore width is less than 2 nm these are referred to as micropores, if greater than 50 nm they are macropores whereas those of intermediate size ($2<D<50$nm) are called mesopores.[53] The shape of the pore affects the pore volume. Due to irregularities in most porous solids the real shape is never known. To overcome this various models have been used for approximation of size.[54] Generally there is an inverse relationship between the specific surface area of the solid and the average pore diameter of a porous solid, where specific surface area is the total of the internal and external surface areas. For most porous silicas the internal surface area is several orders of magnitude larger than the exterior surface area. A compromise in porosity is required as silica gels with a high porosity will have poor mechanical strength and are therefore unsuitable for HPLC.

Column efficiency in liquid chromatography is improved by using small particles. Studies have shown that 5-10 μm are optimal with respect to column efficiency and for practical use.[53, 55] The shape of the particle will also affect the packing density and the geometry of the interstitial voids (between the particles). Spherical particles, with a narrow size distribution, produce a more homogeneous and dense column packing resulting in improved column efficiency.

The surface of silica gel is covered with hydroxyl groups attached to silica atoms, referred to as silanol groups. This produces a very polar SP that is usually used with a non-polar MP such as heptane or hexane (termed normal-phase chromatography). Reversed phase chromatography is possible by modification of these surface silanols. Heterogeneous reaction with a suitable reactant forms an immobile chemically bonded surface layer. Classification of these 'bonded' phases[54] is based on the type of bond by which the functional group is attached to the silicon atoms. Si-R can involve a Si-C bond directly but these are generally prone to hydrolysis restricting the use of aqueous solvents. Alternatively, the functional group can be attached via a bridge such as Si-O-R, Si-O-Si-R or Si-NH-R. These either form a stronger bond or reduce the problem of hydrolysis due to steric reasons. The choice of functional groups produces a range of SPs of different polarities and increases the range of possible interactions with the analyte. Reversed-phase chromatography uses a MP consisting of a polar solvent such as water mixed with a polar water-miscible organic solvent or buffer as required for resolution. This situation usually occurs when the SP is hydrophobic such as non-polar *n*-alkyl bonded phases.

The surface of silica can also be modified by adsorbing a compound onto the surface. This process is referred to as physisorption and involves short-range intermolecular attraction of the solid support with the adsorbent and repulsion of the

adsorbent with solvent. The disadvantage of this method is that care needs to be taken not to leach the compound from the surface during chromatography.

1.9.2 Porous Graphitic Carbon

Reverse phase columns usually consist of a hydrocarbonaceous SP made from chemically bonded porous silica, with a Si-O-Si linkage of *n*-alkyl groups joined to the silica matrix. These are versatile and many alkyl groups can be used (e.g. methyl, *n*-butyl, *n*-octyl and *n*-octadecyl) but there are often problems due to their solubility and thus have a limited pH range of 2-7.5. Another problem often encountered is the effect of residual silanol groups present due to incomplete coverage or gradual hydrolysis of the bonded phase. These can affect retention mechanisms and therefore reduce efficiency and resolution by a broadening of the peak shape although recent improvements in methodology have been made to reduce the number of residual silanol groups. The pH range of reversed phase columns has been extended from 7.5 to 13 mainly by using polymer-based reversed phases, although these are unstable at high pressures and may shrink or swell in certain solvents used as MP.

Carbon is an alternative to silica as a solid support and many different types of carbon have been produced and investigated as phases for HPLC,[56-60] with the most suitable of these being porous graphitized carbon (PGC). Porous glassy carbon was first described by Knox and Gilbert in 1979[61] and used in GC and HPLC.[62] Knox *et al.* in 1986[62] improved the manufacture process and probed the structure by X-ray analysis and electron microscopy as well as investigated its use as a SP. Based on these results PGC was renamed as porous graphitized carbon.

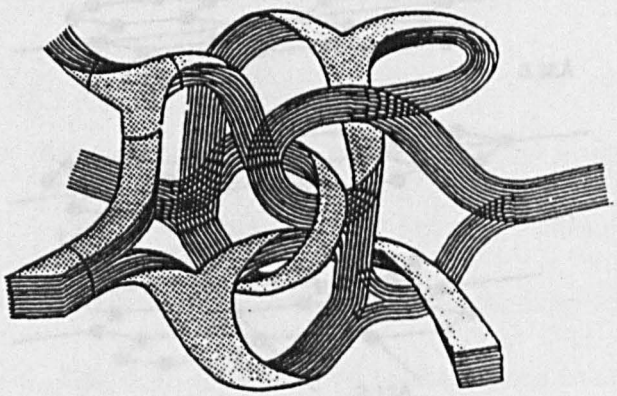


Figure 1.17: Diagram illustrating random porous structure of PGC

PGC is a 2-D graphite with a continuous sponge like structure. At a molecular level it can be considered to have a flat, crystalline surface of intertwining graphitic ribbons held together by covalent bonds to form a dense carbon network.[63] It is a spherical microparticulate material with a surface area of 150-200 m^2/g, porosity of 60-80% and a similar mechanical strength to silica gels with a similar porosity, being able to withstand pressures up to 7000 psi (470bar). Hexagonal graphite consists of layers of hexagonally arranged carbon atoms with no layer registration. In perfect graphite, as described by Bernal,[64] there is alignment with layers above and below but the activation energy required for this to occur from randomly orientated sheets is very high. The distance between 2 layers of carbon atoms in perfect graphite is 3.35 Å,[56] whereas in turbostratic graphite, suggested by Warren,[65] the distance is 3.40-3.43 Å.[62] The distance between carbon atoms within the sheet is 1.42 Å, similar to that in anthracene.

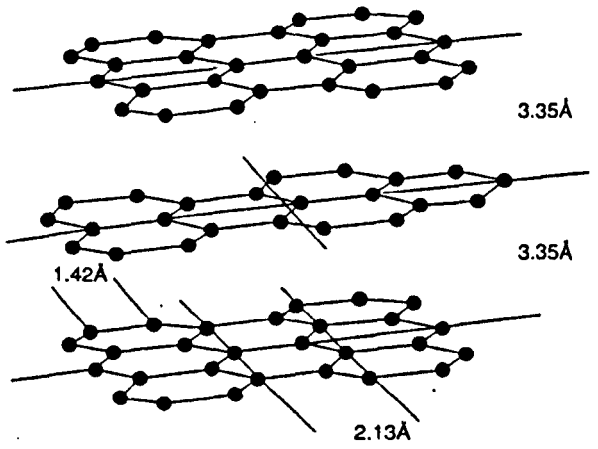


Figure 1.18: 2-Dimensional structure of graphitic PGC

The retention mechanism on graphite is unique. In terms of optimum MP it behaves as a reversed-phase material. In terms of selectivity towards isomeric compounds it behaves like silica gel and alumina (with normal phase MP). For example, graphite shows the *ortho* effect, where the retention of aromatic substances with substituents in the *ortho* position is greater than that of other positional isomers. Phases such as silica gel and alumina also show the *ortho* effect whereas octadecyl silica (ODS) phases exhibit a reverse-*ortho* effect.[66] In comparison with other hydrophobic phases such as ODS a larger organic content in the MP is needed (typically only 50% MeOH compared with 95% in typical reverse phase silica columns).

The carbon atoms in PGC have a full valency satisfied by σ and π bonds resulting in virtually no surface functional groups, with minimal active sites occurring at the edges of the graphitic sheet.[67] PGC has a delocalized band of electrons that can interact with solutes and solvents. Electronic interactions with solutes may be donor-acceptor (charge transfer) and/or direct π-electron overlapping. Due to these interactions organic and

electronic modifiers are often used with PGC to help increase efficiency and reduce peak broadening. The surface can also be modified by prior adsorption of high molecular weight substances or detergents onto the surface.[68] This reduces the hydrophobic interactions which may cause long retention times. Care needs to be taken to avoid contamination, as some solutes can be very strongly retained on the PGC, though regeneration of the surface can usually be achieved by flushing with a strong solvent such as THF or dioxan. The flat, energetically homogeneous surface of PGC makes it particularly suitable for the separation of geometrical and positional isomers. Suggestions for possible mechanisms of retention have been proposed based on studies of various disubstituted benzenes on PGC at various pHs and temperatures.[66, 67, 69, 70] Other solutes have been separated on PGC using both HPLC and supercritical fluid chromatography (SFC)[71] such as alprenolol, metoprolol and related substances,[72] small ionizable compounds of biomedical interest including oxalic acid[73] and analogues related to a cephalosporin antibiotic and steroids such as cortisone and hydrocortisone.[74] Separation of the stereoisomers of a potential anti-asthma agent LY170680 **26** was achieved on PGC with better resolution and in less time than on an ODS column.[75]

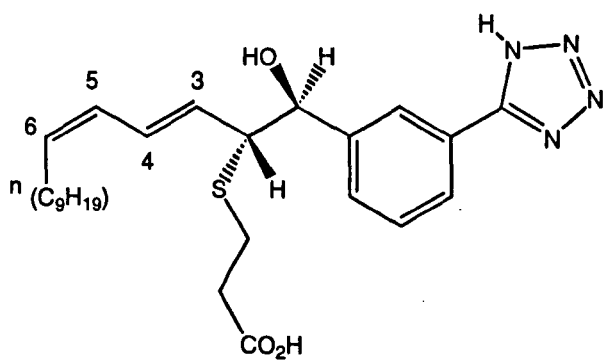


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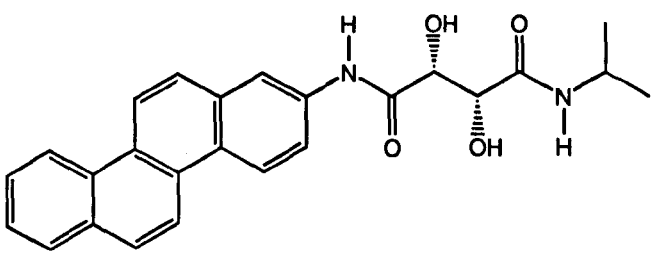
Figure 1.19: LY170680[75]

PGC as a solid support for HPLC provides a complement to existing reversed-phase silica columns, with the biggest advantage being the resistance to acids and bases allowing the use of pH 0-14.

1.9.2.1 PGC in Chiral Chromatography

PGC has been used as the solid support in chiral chromatography. PGC has no functional groups that can be derivatised but chiral molecules can be adsorbed on to the surface or added to the MP. Cyclodextrins, used in reverse phase chromatography with ODS columns,[76] can also be used for optical resolutions on PGC. Optical resolution of a series of amino alcohol β-blockers was achieved using dichloromethane as MP containing 0.4 mM triethylamine and 2.5 mM carbobenzoxyglycyl-*L*-proline.[77]



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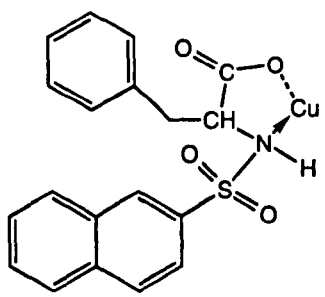

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Figure 1.20: CSPs using aromatic anchors[78, 80]

If a CSP is to be used then it must be able to withstand the conditions employed during chromatography. One way to ensure the molecule is retained on the surface of the PGC is to use an aromatic anchor. Anthracene has been used to anchor a tartramide based chiral selector onto PGC in supercritical fluid chromatography (SFC).[71] HPLC phases

have used a chrysene anchor with a similar tartramide moiety **27**[78] and napthalene with bonded amino acids **28** using copper in the MP have afforded a method of chiral ligand exchange chromatography[79, 80] on PGC.

Another strategy is to use polymers that have the correct solubility profile. A monolayer of tris(phenyl carbamate)cellulose (CPC) on PGC resulted in a phase suitable for the separation of heterocyclic N-containing sulfoxide enantiomers.[77] This phase was consequently studied by Matlin and Grieb, investigating other racemates and refining the chromatographic conditions as well as conditions of preparation of the CSP.[55] Other polymers, such as poly-*L*-leucine coated onto PGC, have been studied and shown to separate racemic epoxides.[81]

CHAPTER 2

PGC AS A SOLID SUPPORT FOR CHIRAL CHROMATOGRAPHY

2.1 Introduction

As previously mentioned PGC has various advantages over silica when used as a solid support in chromatography, such as stability to the full pH range and no surface functional groups that could interact with analytes. To establish the suitability of PGC as a solid support in chiral chromatography and identify any differences with silica we investigated efficiency of one CSP on both supports. This was achieved by coating cellulose tris(3,5-dimethyl phenyl)carbamate (CDMPC) onto PGC and testing with a range of analytes. These results were then compared with a commercially available Diacel OD column, which contains CDMPC coated onto silica.

2.2 Comparison of CSP Derived from Cellulose

2.2.1 Preparation of CDMPC

CDMPC was prepared by reaction of cellulose with 3,5-dimethyl phenyl isocyanate in pyridine according to a method established by Crawford.[53] Cellulose (Avicel) was dried over phosphorus pentoxide under vacuum for 24 hours. It was then refluxed in pyridine for 24 hours in order to 'wet' the surface of the polysaccharide, allowing the reagents in the next step to penetrate more easily. After cooling, 3.5 equivalents of 3,5-dimethyl phenyl isocyanate were added and the mixture refluxed with stirring for á further 72 hours. After cooling the solution was poured into a large quantity

of methanol. The white precipitate that formed was filtered off, washed with methanol and dried under vacuum.

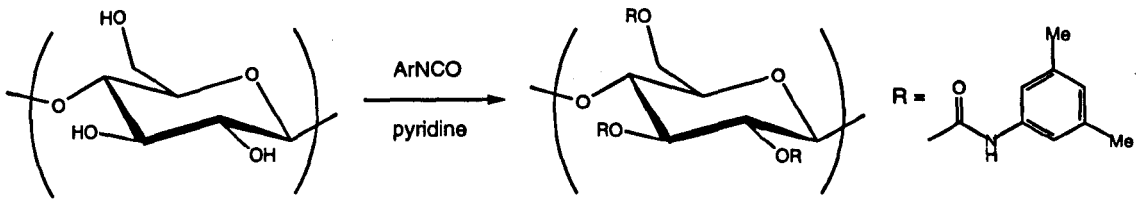


Figure 2.1: Preparation of CDMPC

The product of this reaction was analysed by IR and elemental analysis to confirm the extent of derivatisation. Elemental analysis indicated that approximately 67% of the free hydroxyl groups were functionalised. The polymer derivative that was produced was insoluble in a range of solvents such as alcohols and hydrocarbons but was partially soluble in THF and DMF solvents. The presence of an IR peak at 1724 cm^{-1} indicative of the carbonyl stretch of an urethane link and an absence of any absorbance at 3600 cm^{-1} (due to free hydroxyl groups) indicated a successful reaction. The IR did have a small peak at 1615 cm^{-1} which may correspond to the formation of urea as a by-product of the reaction, resulting from the reaction of isocyanate with any residual water in the system **(Fig. 2.2)**.

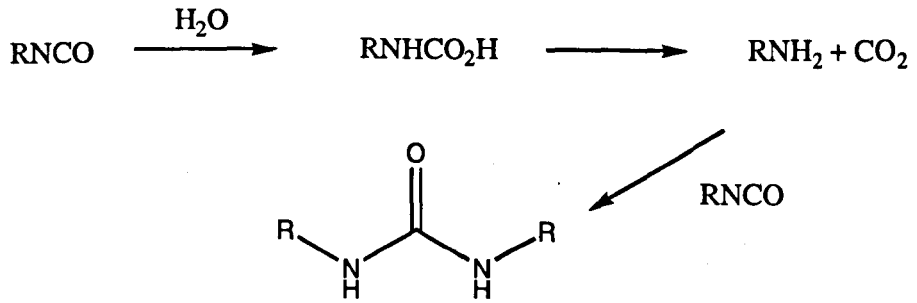


Figure 2.2: General scheme for the formation of urea

2.2.2 Coating of the Solid Support by the Batch Method

With the chiral phase in hand attention was turned to coating it onto PGC (particle size 7μm). This was achieved using a 'batch method' that involves adding the compound in portions in an attempt to ensure even coverage of the solid support.[55] The amount of CDMPC coated onto the silica used in the Diacel columns is not known (Daicel only provide information about the type of derivative and particle size of the silica). We chose an initial loading of 25% w/w of CDMPC:PGC as a reasonable amount as work using CDMPC coated onto silica by Okamoto had shown this percentage to be optimum.

The material to be coated onto the PGC was separated into 3-4 batches, dissolving each in a small volume of THF (each batch was typically 0.1 g and dissolved in approximately 10 ml). These were then added one at a time to the PGC, which had been previously refluxed in THF for 30 minutes. The solvent was then removed slowly *in vacuo* using a rotary evaporator set at low rotation speed. A baffled flask (a round bottom flask with four indents) was used to also ensure even coverage of the solid by tumbling (although it was found that the solid often stuck to the walls of the flask and had to be removed physically in between additions). When all of the compound had been added the temperature of the water bath was elevated to 40°C to ensure complete removal of the solvent. The solid was then pushed through a 38 μm sieve to provide a free flowing powder that was ready to be packed into HPLC columns.

2.2.3 Testing by HPLC

The sample of PGC coated with CDMPC was packed (at Hypersil, UK), using a high pressure slurry method, into a stainless steel HPLC column (4.6 mm diameter) and

evaluated using a range of analytes (appendix 1). These results were compared with those obtained from a Chiralcel OD column, which consists of CDMPC coated onto silica (**Fig. 2.3**).

		CDMPC coated on PGC			Chiralcel OD column		
Racemate		Eluent	K'	α	Eluent	K'	α
CP55940	**B**	95/05	13.93	1.34 f	92/08	3.50	1.25 r
SCH50911	**C**	95/05	3.11	1.31 f	90/10	1.68	1.48 r
CS469	**D**	90/10	8.56	1.35 f	85/15	3.26	1.36 r
1-Naphthoxylactic acid	**F**	90/10 [a]	12.14	1.20 f	80/20 [a]	2.41	1.95 r
Propranolol Glycol	**G**	85/15			80/20	2.98	1.22 r
Phenyl Propionic Acid	**J**	99/01 [a]	6.42	1.21 f	98/02 [a]	1.02	1.40 r
Phenyl Propanol	**K**	99/01	5.93	1.15 f	90/10	0.83	1.07 f
Mandelic acid	**L**	96/04	7.41	1.14 f	80/20 [a]	0.76	1.53 r
Benzoin	**M**	97/03	8.19	1.37 r	90/10	2.70	1.67 r
Phenyl-1,2-ethanediol	**N**	98/02	17.88	1.10 f	90/10	1.98	1.14 r

Figure 2.3: Comparison of separation of various analytes (appendix 1) using CDMPC coated onto PGC and silica (Chiralcel OD)

eluent: Hexane/Isopropranol; (a) 0.1% trifluoroacetic acid (TFA) added; r = peaks were resolved, f = peaks were fused

The column showed good chiral selectivity under normal phase conditions for a variety of compounds although complete resolution of the two enantiomers was not

always achieved. The α-values were sometimes better than those obtained on the commercial OD column (e.g. CP 55940 **B** and phenyl propanol **K**), although as the eluent was less polar this indicates a less efficient column. All of the compounds tested were retained for longer on the CDMPC coated PGC column than on the Chiralcel OD column. The peak shape on the CDMPC coated PGC was also poorer than for the OD column, with many of the peaks overlapping, becoming fused. Although this may be a consequence of the decreased polarity of the solvent there may also be a contributing factor from the PGC. Interactions with the PGC surface would be non-enantioselective which would increase the retention time of the analytes and reduce the effect of any chiral separation achieved by the CSP due to peak broadening.

2.3 Conclusions

The successful development of novel solid supports in chiral chromatography is important to improve existing techniques. PGC has been shown to be suitable for the use as a solid support for CDMPC in chiral analytical scale chromatography. However, there are differences between the use of silica and PGC as a solid support. This difference may arise from the preparation of the CSP coated solid supports (it is not known how Diacel coat their columns or the %w/w loading). It also indicates the necessity for further work to investigate the optimum loading weights for PGC (as these are likely to be different to the optimum loading weights for silica) and the method used to coat the solid support (to ensure that the surface is completely covered preventing non-enantioselective interactions between the analyte and PGC). However, the PGC columns showed partial separation of 90% of the analytes and showed better resolution in 20% of the cases that the commercial

Diacel column. The fact that the PGC analytical column was successful in separating a wide range of enantiomers encouraged us to utilise this support in further investigations. Hence we next turned our attention to developing novel chiral phases to complement the use of PGC as a solid support.

CHAPTER 3

NOVEL CHIRAL POLYMERS AS CHIRAL PHASES

3.1 Introduction

Chromatographic enantioseparation is a practical and useful method for the analysis of enantiomers as well as their purification. There is an abundance of different CSPs that are commercially available ranging from the Pirkle type strand phases[30] (consisting of discrete chiral molecules tethered to a solid support) to helical polymers (which are coated on to the solid support). The phases which have found most practical use however include those of polymeric form such as phenyl carbamate derivatives of polysaccharides[36, 39] (as mentioned in chapter 2) and the one-handed helical polymer poly(triphenylmethyl methacrylate).[81]

Separation of enantiomers on a preparative scale currently uses preparative chiral HPLC. However, as commercial preparative chiral HPLC columns are exceedingly expensive it would be more convenient if chiral flash chromatography could be developed. In order to develop flash chiral chromatography it will be necessary to develop cheap chiral phases that can be easily prepared on a large scale. Due to existing patents phases based upon polysaccharides were unavailable thus we chose to prepare a range of novel phases and examine their efficiencies in chiral chromatography. In order to lower costs further we decided that our new chiral phases should be coated onto (and not covalently attached) to our chosen solid support as this would shorten the number of steps in the preparation of the chiral column and hence lower the cost of any new product. With these objectives in mind it was important that any phase that was coated

onto a solid support should not be washed off under normal chromatographic conditions. To prepare a chiral phase that would not readily dissolve in conventional chromatographic solvents (e.g. hexane, isopropanol) we chose to prepare a range of novel polyurethanes. The polymeric nature coupled with the insolubility of multifunctional carbamate derivatives of these phases should result in molecules with a suitable solubility profile, together with additional functional groups containing the necessary functionality to interact with analytes.

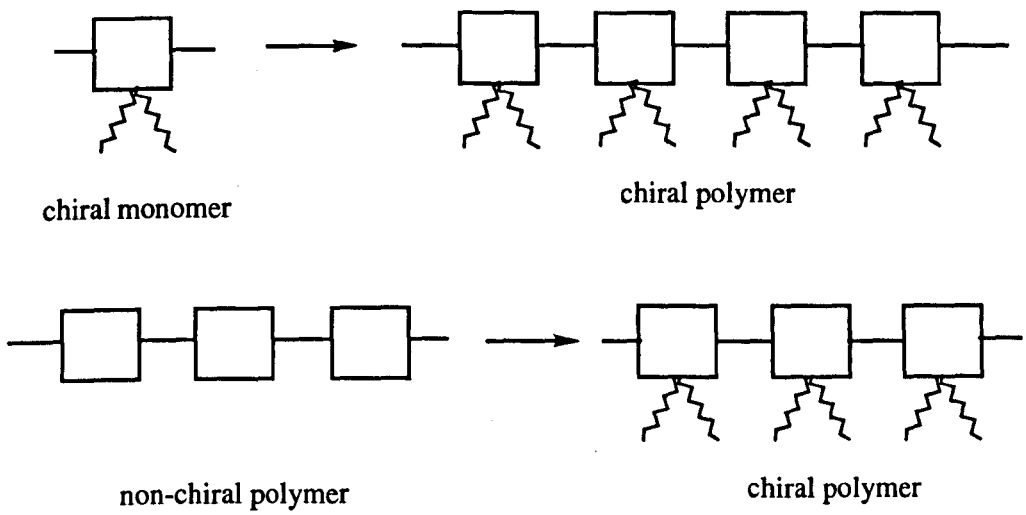


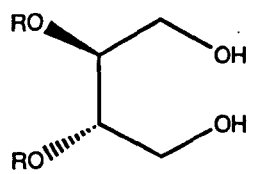
Figure 3.1: Schematic diagram of 2 different approaches to forming a chiral polymer, where ~~~ represents chiral groups

Chiral polymers can be obtained in two ways i) polymerisation of a chiral monomer unit or ii) chiral derivitisation of a pre-made polymer (**Fig. 3.1**). During polymerisation of a chiral monomer the polymer must retain the chirality of the monomer unit. This approach has the advantage that uniform functionalization of the polymer is obtained. On the other hand there is the possibility that with the chiral derivatisation approach not all of the functional groups in the polymer will react with the chiral reagent,

thus our chosen strategy was to polymerise a chiral monomer unit. This unit was designed to be a symmetrical diol (with a centre of symmetry, rather than plane of symmetry) to ensure the formation of a regular macromolecule. Reaction with a diisocyanate linker would then result in a chiral polyurethane polymer with a suitable solubility profile.

A chiral diol which could be linked via urethane linkages using diisocyanates would allow the use of the range of diisocyanates that are commercially available, allowing us to change the secondary structure and solubility profile of the resulting polymers. The resulting macromolecules would be characterised by MALDI-TOF MS and their secondary structure examined by the use of circular dichroism spectroscopy (CD). Their efficiency of chiral separation would then be investigated by coating onto a solid support that would be packed into HPLC columns to be tested with a range of racemic analytes to investigate any chiral selectivity. The chosen solid support was carbon (PGC) as it has various advantages over silica, as previously mentioned. The initial target chiral monomer diol units (**29-32**) are shown below (**Fig. 3.2**).

3.2 Synthesis of the Chiral Monomer Units

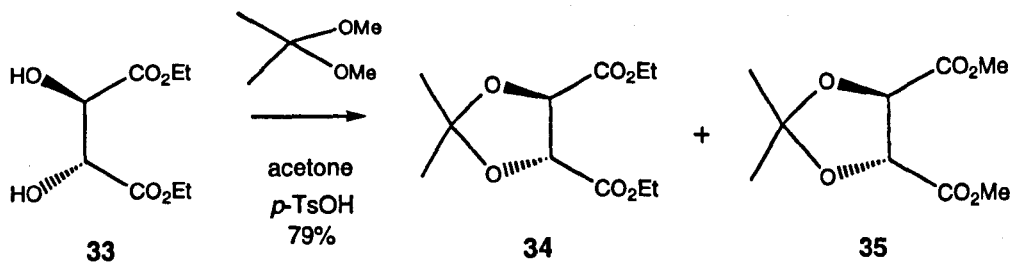


29 R = Ac
30 R = Bz
31 R = phenyl carbamate
32 R = (3,5-dimethyl)phenyl carbamate

Figure 3.2: Target monomer units

Tartaric acid was chosen as the starting material for the chiral monomer units (**29-32**) as it is cheap and commercially available, in both enantiomers as well as in acid and

ester forms. It also has the potential for manipulation to a diol suitable for polymerization by reaction with a diisocyanate. In order that there were appropriate functional groups suitable to interact with potential analytes the secondary hydroxyl groups of tartaric acid were to be functionalised as acetate **29**, benzoate **30** and phenyl carbamate **31** and **32** derivatives and the acid groups reduced to the primary alcohols necessary for polymerisation. These derivatives were chosen as the functional groups have H-bonding acceptor (C=O) and donor sites (NH) as well as sites capable of π-π interactions (Ph) with analytes. In addition these type of functional groups have been used to derivatise polysaccharides and are the derivatives among the most successful CSPs used in HPLC (e.g. Diacel OA = acetyl cellulose; Diacel OB = benzoyl cellulose; Diacel OC = phenyl carbamate cellulose; Diacel OD = (3,5-dimethyl)phenyl carbamate cellulose)



33 **34** **35**

Scheme 3.1: Addition of acetal protecting group

The diethyl ester of *L*-tartaric acid **33** was the chosen starting material being readily commercially available and cheaper than the corresponding diacid. Initially, the two secondary hydroxyl groups of diethyl *L*-tartaric acid **33** were protected using an acetal group to yield **34**. This was accomplished by reaction of **33** with 2,2-dimethoxypropane in acetone using *p*-toluenesulphonic acid as catalyst. In this way the acetonide **34** was obtained in 79% yield after purification by vacuum distillation. A small

amount of the methyl ester transesterification product, **35**, was also detected by NMR. Although the mixture of both the methyl ester **35** and the ethyl ester **34** were not separated and the mixture was used in the next step.

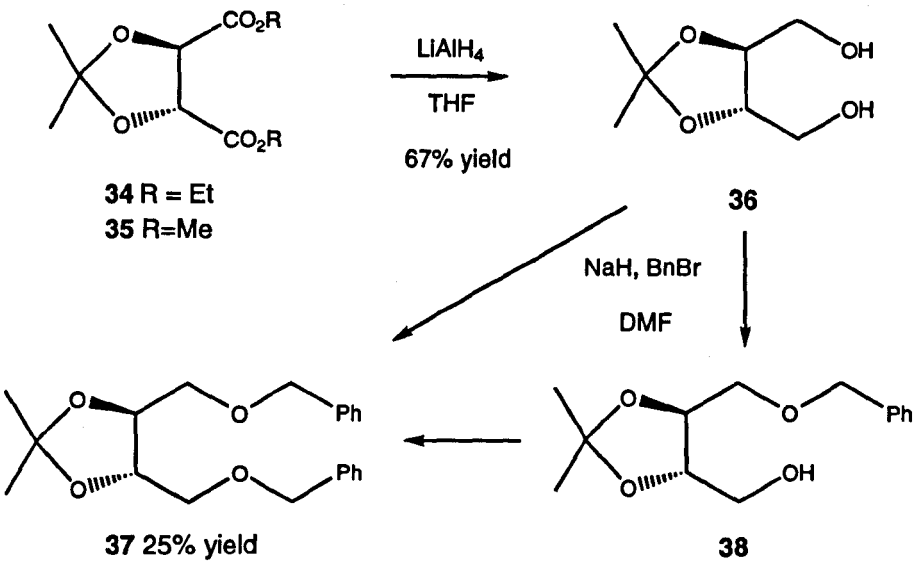


Figure 3.3: Reduction of ester groups and the protection of the resulting hydroxyl groups using benzyl groups

Reduction of the mixture of ester products **34** and **35** with lithium aluminium hydride[82] in THF furnished the desired alcohol **36** in 67% yield. Some problems were experienced in separating the aqueous phase during the work-up due to the formation of an emulsion caused by insoluble aluminium salts. Protection of the newly formed primary alcohol groups as benzyl ethers was achieved using standard benzylation conditions[83] (NaH, BnBr, DMF). Reaction at room temperature for 12 hours furnished the monobenzylation product **38** (43%) as the major product with a small amount of the dibenzylated product **37** (12%). The desired di-protected product **37** could be obtained in higher yield (25%) if the reaction time was increased to 24 hours. Reaction of the mono-

benzylated product with benzyl bromide also resulted in the formation of the desired di-protected product **37**, in 71% yield.

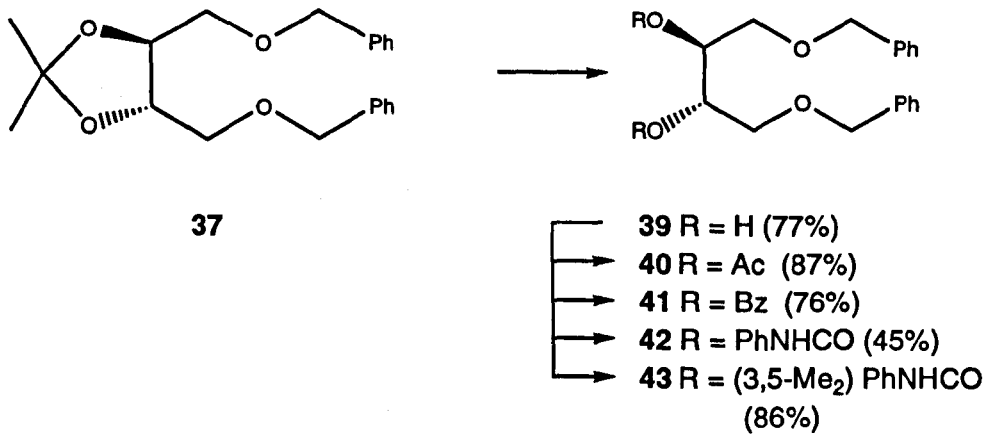
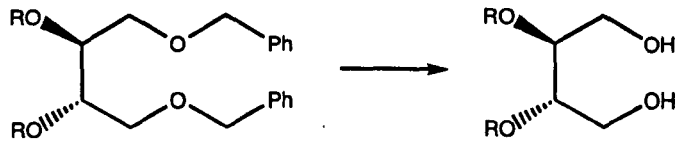


Figure 3.4: Removal of acetal group and addition of functional groups (yields%)

Removal of the acetal protecting group using hydrochloric acid in aqueous THF furnished the diol **39** in 77% yield after column chromatography. With this protected diol **39** in hand attention was turned to its derivatisation. Hence reactions with either acetyl chloride, benzoyl chloride, phenyl isocyanate or (3,5-dimethyl)phenyl isocyanate in pyridine furnished the acetate **40**, benzoate **41** and carbamate **42** and **43** derivatives respectively in the yields shown. Traditionally the removal of benzyl groups by hydrogenolysis is carried out in polar solvents such as alcohols or ethyl acetate. Catalytic hydrogenation in ethanol was therefore employed to remove the benzyl protecting groups to furnish the diol monomers **29-32**, using a palladium/carbon catalyst. The phenyl carbamate derivatives **42** and **43** were only sparingly soluble in ethanol. Although they were soluble in THF the deprotection reaction using H_2 and palladium on carbon did not occur in this solvent and hence the complete removal of the benzyl groups could only be achieved using dilute reaction concentrations in ethanol and long reaction times.



40 R = Ac
41 R = Bz
42 R = PhNHCO
43 R = (3,5-Me_2) PhNHCO

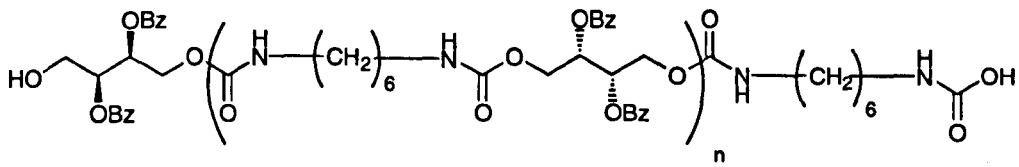
29 R = Ac (96%)
30 R = Bz (90%)
31 R = PhNHCO (90%)
32 R =(3,5-Me_2)PhNHCO (98%)

Scheme 3.2: Preparation of functionalized diols (yields%)

With the desired monomers in hand attention was turned to their polymerisation to make a range of chiral polymers. We initially chose to use the reactive hexamethylene diisocyanate as the urethane linker.

3.3 Polymerisation of the Benzoate Monomer

3.3.1 Coupling of Benzoate Monomer (30) with Hexamethylene Diisocyanate



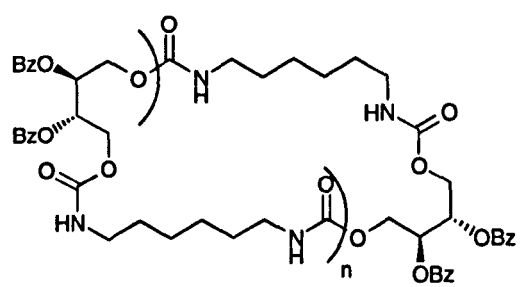
44

Figure 3.5: Target polymer from reaction of benzoate monomer with hexamethylene diisocyanate, where n is the number of repeating units

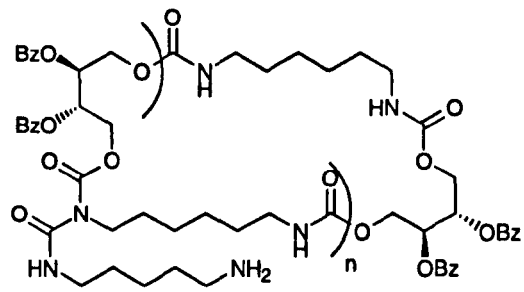
Initially the benzoate diol monomer **30** was reacted with a stoichiometric amount of hexamethylene diisocyanate in anhydrous pyridine at a concentration of 0.2 M. The reaction mixture was refluxed for 12 hours and then stirred at room temperature for 48

hours. The resulting white solid obtained after taking the reaction mixture up in ether was analysed by NMR and MALDI-MS.

The ^{1}H NMR signals were broad suggesting the presence of large molecules, or many similar molecules, with peaks that would be expected from the desired polymer. MALDI-MS, however, detected the presence of cyclic molecules corresponding to **45**, where n = 0, 1 and 2. Cyclic molecules with an additional hexamethylene unit were also detected, corresponding to **46** where n = 0 and 1.



45



46

Figure 3.6: Formation of cyclic molecules

Gel permeation chromatography (GPC) data also showed the presence of large molecules with the spectrum showing the presence of two discrete peaks merging into a broad peak extending to the high limit of 5 minutes with a polydispersity of the entire range of 7.6 (**Fig. 3.7**). There are problems associated with determination of molecular masses by the use of GPC. The standards used to calibrate GPC columns are linear polystyrene polymers. Differences in size and shape of the polymer affect the time of elution from the column and therefore accurate information about the molar masses of the novel polymers formed cannot be obtained.

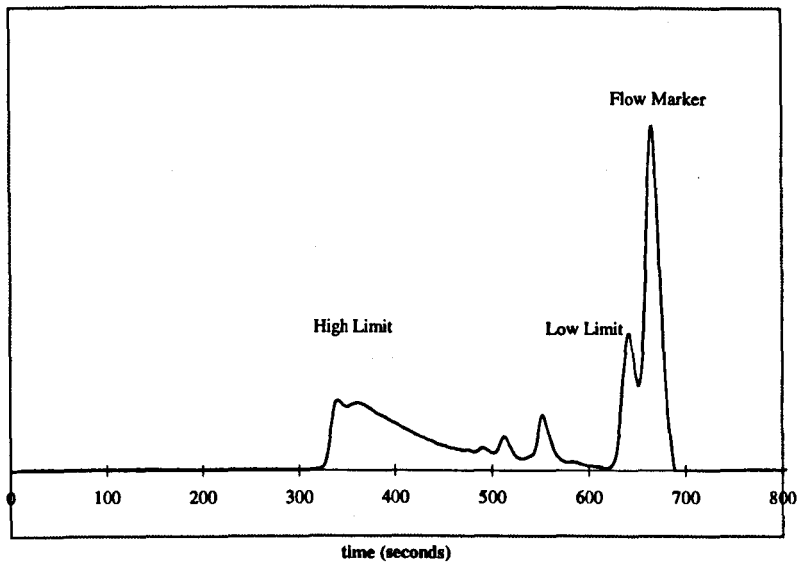
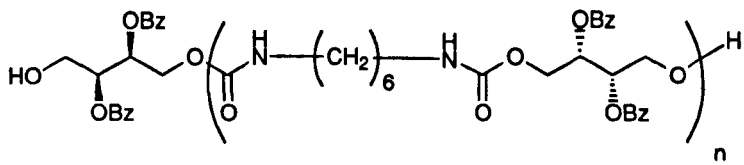


Figure 3.7: GPC using method one in THF, high limit at 325 seconds low limit 600 seconds



47

Figure 3.8: Formation of linear polymer with extra benzoate units

As the formation of cyclic molecules may be a consequence of the concentration of the reaction mixture the reaction between **30** and hexamethylene diisocyanate was repeated at a higher concentration of 2.2 M. Again the reaction was refluxed for 12 hours but the solvent was then removed immediately and the residue taken up in dichloromethane, so that it could be washed with aqueous hydrochloric acid to remove any remaining pyridine. MALDI-MS of the product of this reaction showed an increase in the size of the cyclic molecules with **45**, where n = 0, 1, 2, 3, 4 and 5, being detected.

Cyclic molecules resulting from the addition of an extra hexamethylene diisocyanate, **46**, were also detected, where n = 0, 1 and 2. However, the desired straight chain macromolecule **47** was also formed. The GPC data shows a broad peak ranging from the lower limit almost to the higher limit of 325 seconds with a polydispersity of 3.6.

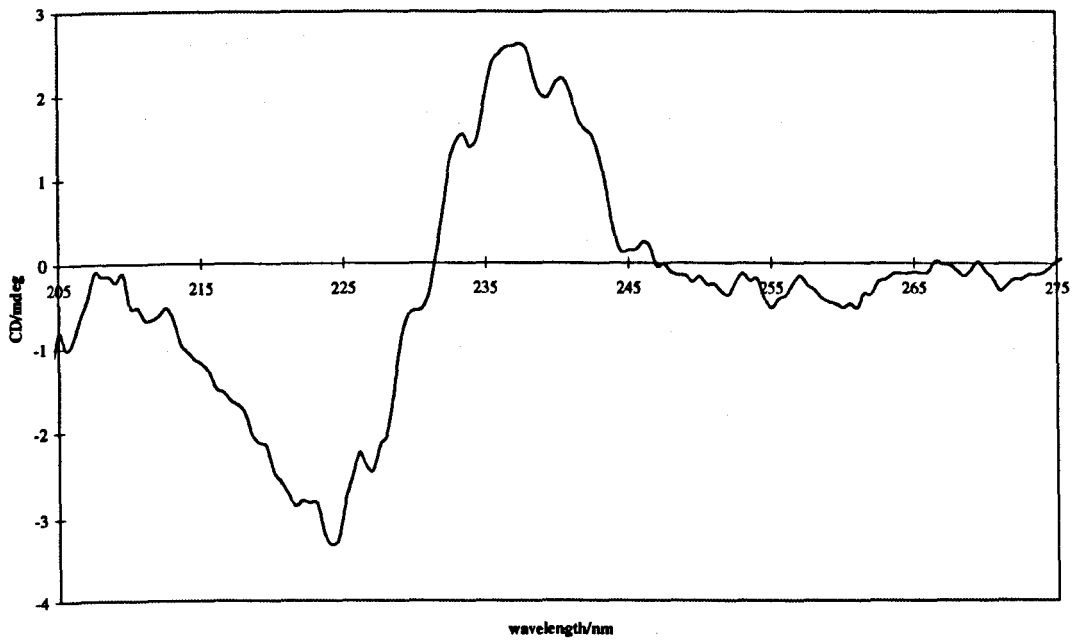


Figure 3.9: CD spectrum for the product of the reaction between **30** and hexamethylene diisocyanate, 1 mg/mL, path length 0.1 mm, in THF

The secondary structure of the macromolecules produced in the reaction between **30** and hexamethylene diisocyanate in pyridine was studied using circular dichroism. The only solvent suitable for UV studies in which the substrate was soluble was THF. The UV cut-off point of THF is 200 nm so the CD spectrum was collected from 200 to 300 nm. The spectrum shows a negative peak at 224 nm, crosses the x-axis at 232 nm and has a positive peak at 237 nm (**Fig. 3.9**). This shows that there are two types of phenyl groups present in chiral environments. As the phenyl groups are not themselves chiral then this

chirality is assumed to arise from the secondary structure of the macromolecules formed. The exact nature of the secondary structure can not be determined from the CD spectrum obtained but comparisons can be made with the other novel CSPs produced.

To investigate the effect of solvent on the polymerisation reaction, the coupling of **30** and hexamethylene diisocyanate was repeated using toluene instead of pyridine as the solvent. The catalyst dibutyl tin dilaurate was also employed to facilitate the reaction. Hence the monomer **30** at a concentration of 1.11 M, hexamethylene diisocyanate and tin catalyst (0.01 equivalents) in toluene was refluxed for 9 hours and then stirred for 16 hours at room temperature. The products obtained were analysed by MALDI-MS which indicated the presence of the cyclic molecule **45** (**Fig. 3.6**) where n = 0 and 1 as had been obtained before, but also included various peaks that could not be assigned. The GPC spectrum showed the presence of molecules with a high molecular weight indicated by a broad peak (maximum at 14 minutes on the scale 18 minutes to 9 minutes) with 5 individual peaks visible.

The CD spectrum was collected from 200 to 300 nm and the spectrum shows a negative peak at 224 nm, crosses the x-axis at 232 nm and has a positive peak at 237 nm, which is identical, within experimental error, to the spectra of the product obtained from the previous reaction using pyridine as solvent. Therefore the secondary structures of the macromolecules formed can be assumed to be very similar.

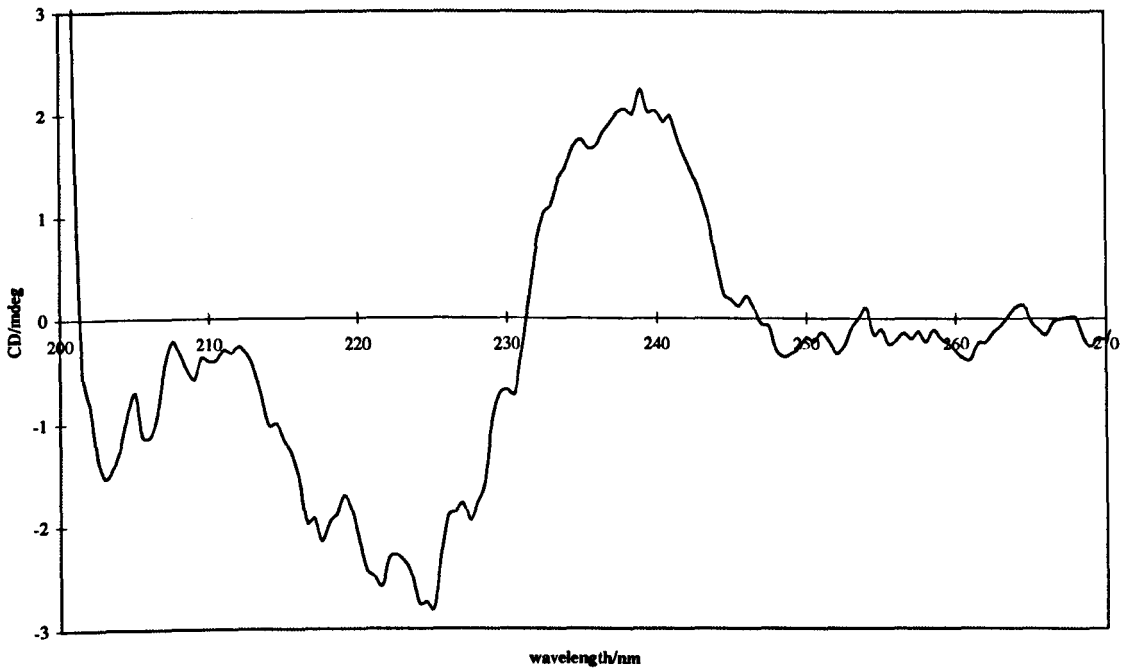


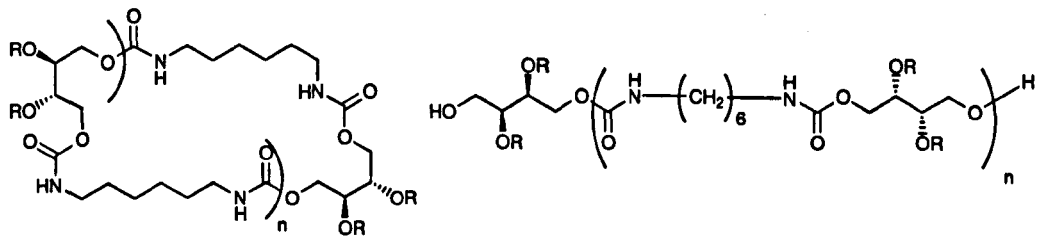
Figure 3.10: CD spectrum for product of the reaction between **30** and hexamethylene diisocyanate in toluene. 1 mg/mL, 0.1 mm path length in THF

3.4 Polymerisation of the Phenyl Carbamate Monomers

3.4.1 Coupling of Phenyl Carbamate Monomer (31) with Hexamethylene Diisocyanate

Reaction of phenyl carbamate diol **31** with hexamethylene diisocyanate was carried out at concentration of 1.1 M in pyridine. After refluxing the solution for 20 hours a small amount of *tert*-butylamine was added, in order to react with any remaining isocyanate groups. The ^{1}H NMR spectrum shows peaks for the protons of the phenyl groups, amines and hexamethylene protons as expected. The GPC shows the presence of molecules with a high molecular weight with a broad peak stretching to the higher limit of 325 seconds with a few, small peaks near the lower limit. However, it was not possible

to characterise the products using MALDI-MS and so no accurate structure determination was possible. The inability to obtain good MALDI spectra may be due to a number of factors including the insolubility of the product in solvents compatible with the preparation of MALDI-MS slides (i.e. of suitable volatility).



48 49

Figure 3.11: Formation of phenyl carbamate macromolecules, where R = CONHPh

The coupling of the phenyl carbamate diol **31** with hexamethylene diisocyanate was repeated at a slightly higher concentration of 1.4 M in pyridine with the addition of 0.01 equivalents of dibutyl tin dilaurate as catalyst. The mixture was allowed to reflux for only 1 hour and was then left stirring at room temperature for 12 hours. The solution was poured into methanol and the precipitate collected by filtration. Analysis by MALDI showed the presence of some cyclic molecules, **48** where n = 0, 1 and 2, similar to those seen with the benzoate diol but linear polymers corresponding to **49** where n = 1, 2 and 3, were also detected. The GPC spectrum showed many peaks (approximately 7, though very close together) lying in the range of 400 to 600 seconds.

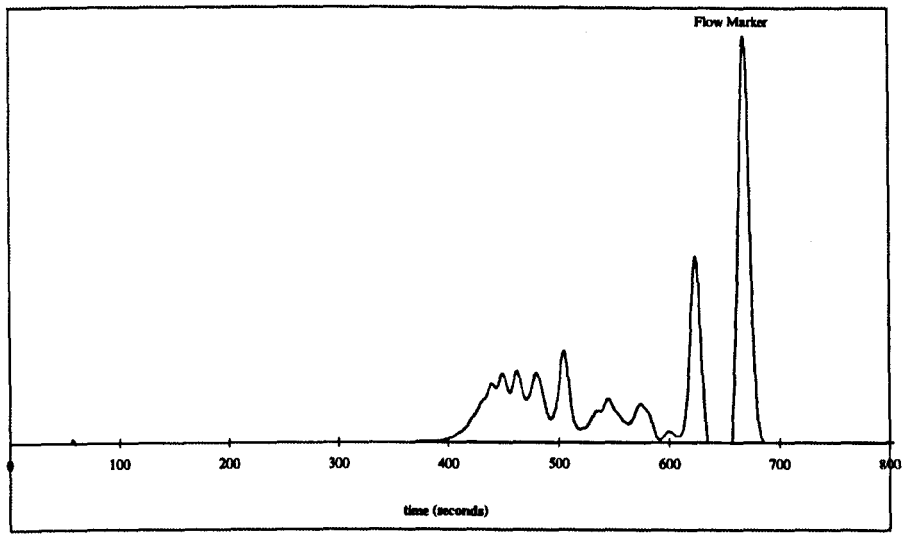


Figure 3.12: GPC for phenyl carbamate and hexamethylene, higher limit at 325 seconds and lower limit at 600 seconds

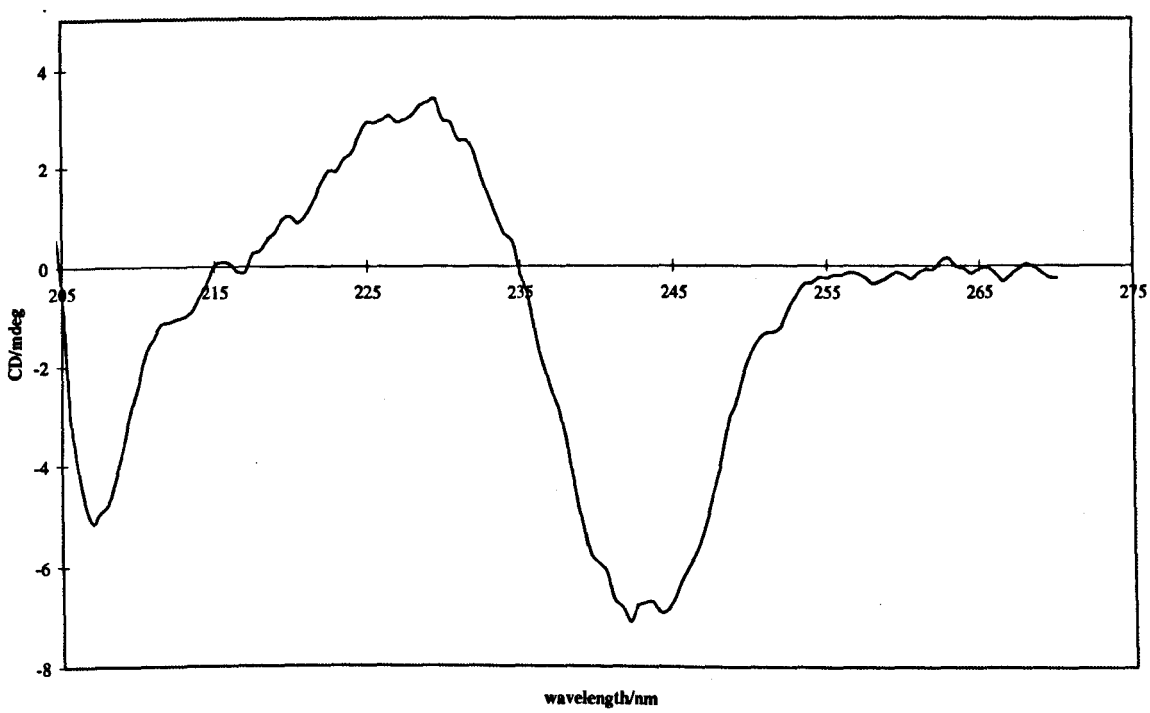


Figure 3.13: CD spectrum of product of the reaction between **31** and hexamethylene diisocyanate in pyridine with tin catalyst, 1 mg/mL, 0.1 mm path length in THF

The CD spectra of the reaction between the phenyl carbamate diol **31** and hexamethylene diisocyanate in pyridine shows a negative peak at 244 nm, crosses the x-axis at 235 nm and has a positive peak at 229 nm. As with the benzoate macromolecules there are two different types of phenyl groups in different chiral environments but there is an inversion of sign of the two peaks. This indicates a different secondary structure of the macromolecules with phenyl carbamate groups compared to benzoate groups resulting in different interactions between the phenyl groups. The amplitude of the negative peak at longer wavelengths is greater than that of the positive peak, which may mean that there are more phenyl groups in a more hydrophobic environment. This may arise from a more enclosed secondary structure that would exclude polar solvent molecules providing a more hydrophobic environment.

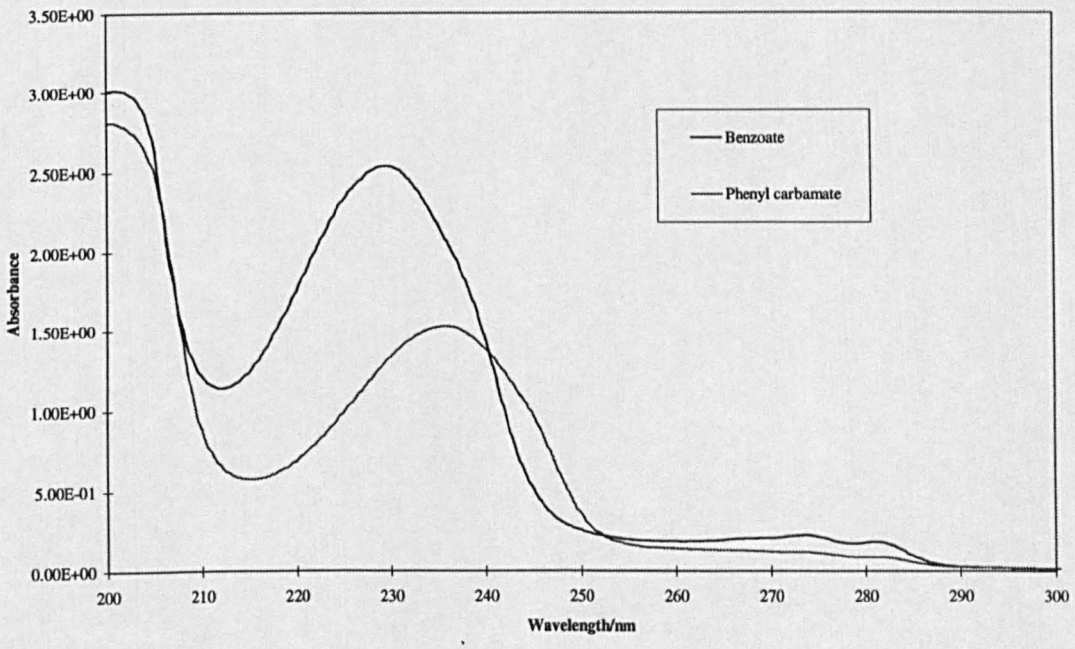


Figure 3.14: UV Spectra of macromolecules obtained from the reaction of the benzoate monomer and phenyl carbamate monomer with hexamethylene diisocyanate. 1 mm cell path length in THF, benzoate 5 mg/mL, phenyl carbamate 1 mg/mL

There is a slight shift in wavelength of the maximum absorbance and this can be attributed to the different electronic nature of the macromolecules with the benzoate group compared to the phenyl carbamate group. This can be seen by comparing their UV spectra, which shows a shift of the main peak obtained from 230 nm to 238 nm for the phenyl carbamate group (**Fig 3.14**).

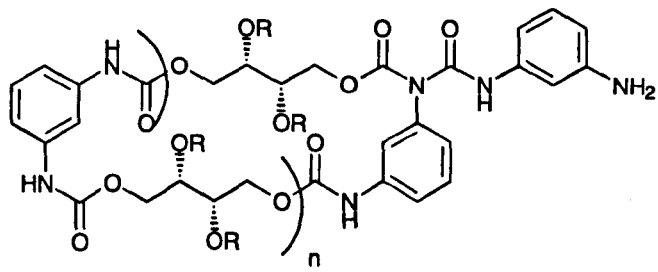
3.4.2 Coupling of (3,5-dimethyl)Phenyl Carbamate Monomer (32) with Hexamethylene Diisocyanate

The coupling of (3,5-dimethyl)phenyl carbamate **32** with hexamethylene diisocyanate was attempted in pyridine at a concentration of 1.2 M by refluxing the reagents together for 24 hours and then pouring into methanol. The ^{1}H NMR of the product shows clearly the presence of 3 types of methylene protons but the rest of the spectrum is not clear. MALDI-MS was attempted but the peaks obtained could not be interpreted. The product of the reaction between the phenyl carbamate diol **32** and hexamethylene diisocyanate had no CD spectrum (at a concentration of 1 mg/mL with 0.1 mm path length in THF the signal was practically zero).

3.4.3 Coupling of (3,5-dimethyl)Phenyl Carbamate Monomer (32) with Phenylene Diisocyanate

The reaction of (3,5-dimethyl)phenyl carbamate diol **32** with 1,3-phenylene diisocyanate was attempted in pyridine at 1.0 M concentration. After the mixture was refluxed for 19 hours more pyridine was added, as the solution had become very viscous. The solution was poured into methanol but as no precipitate formed the solvent was

removed *in vacuo*. The resulting brown solid was analysed by MALDI-MS and revealed the presence peaks corresponding to cyclic molecules with additional phenylene units, **50** where n = 0, 1 and 2 only.



50

Figure 3.15: Cyclic macromolecules formed from phenylene diisocyanate R = (3,5-Me_2)PhNHCO

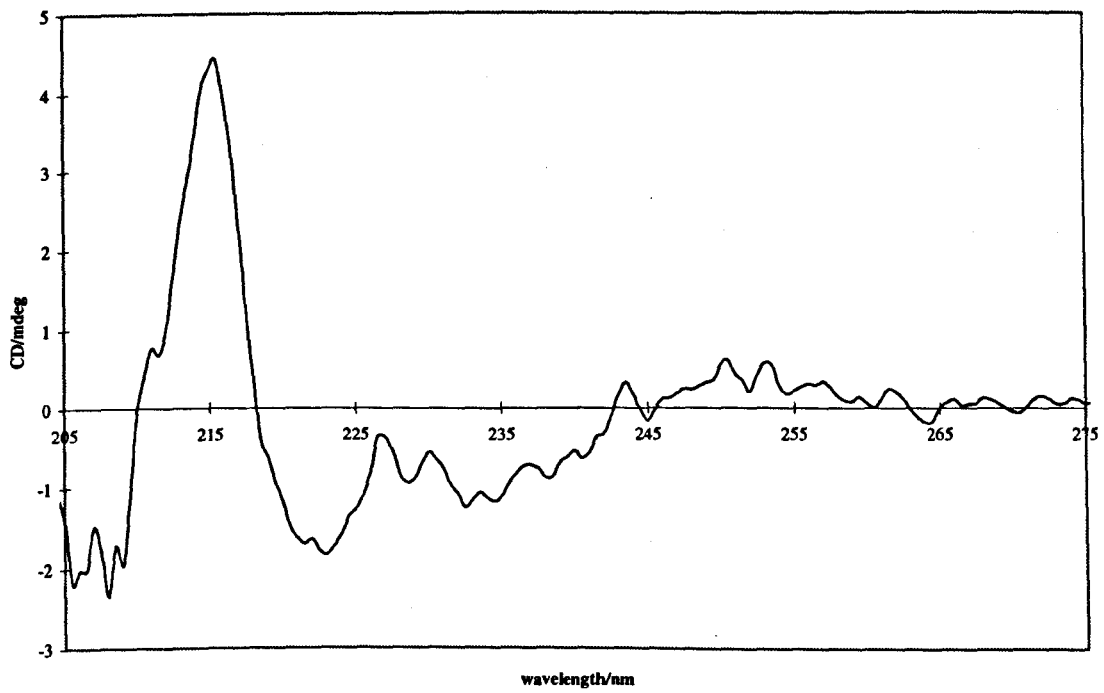


Figure 3.16: CD spectrum for the product of the reaction between **32** and 1,3-phenylene diisocyanate, 1 mg/mL 0.1 mm cell path length in THF

The product of the reaction between the phenyl carbamate diol **32** and 1,3-phenylene diisocyanate showed a different CD spectrum compared to the macromolecules containing hexamethylene linkers. There appears to be a slight positive peak at 254 nm, a small negative peak at 227 nm and a large positive peak at 215 nm. This shows that the influence of a phenylene linker has a large effect on the secondary structure of the macromolecules formed. The large absorbance at 215 nm is likely to arise from interactions of the phenylene linker, which may be able to interact with the phenyl carbamate groups resulting in a CD absorption, depending on the actual secondary structure formed.

3.5 Discussion

Under the right conditions the reaction between monomer diol and diisocyanate is quick, shown by the formation of linear molecules in the reaction of one hour. However, the reaction does not produce only the desired linear polymers and under similar conditions different products were obtained.

An increase in concentration helps the formation of linear polymers but also increases the size of cyclic molecules that are also formed. Problems caused by the formation of such cyclic molecules is that once the ring is formed further reaction is stopped, apart from reaction with additional isocyanate units forming side chains.

There seems to be slightly conflicting data when comparing the GPC data with MALDI spectra. This may be due partly to the problem of MALDI-MS being mass sensitive. It is not a quantitative technique and has the additional disadvantage of the detector becoming less sensitive to molecules with a higher mass. This can mean that

higher mass products may have gone undetected in these reaction mixtures. GPC analysis indicated the presence of large molecules but no accurate mass determination was possible due to the use of polystyrene polymers to calibrate the columns. The shape as well as mass of a polymer can affect its passage through a GPC column and therefore affects the time taken for it to reach the detector.

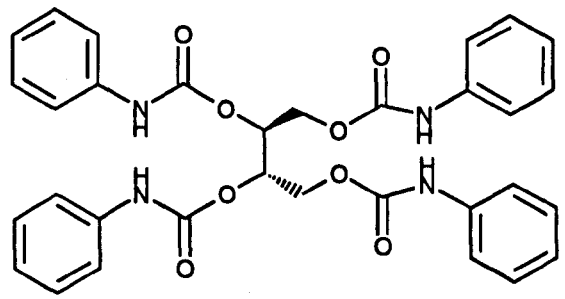
Benzoate and phenyl carbamate diol monomers gave products on reaction with hexamethylene diisocyanate with different secondary structures, as shown by their CD spectra. The shift in the wavelength of absorbance is likely to be due to the different electronic nature of the phenyl groups in these molecules as shown by their UV and the inversion of sign indicates that the nature of the groups affects the chiral secondary structure. The CD spectra of the macromolecule obtained from (3,5-dimethyl)phenyl carbamate coupled with phenylene diisocyanate showed yet again a different secondary structure to those of the macromolecules obtained with hexamethylene linkers. The influence of the phenylene linker is shown by the large absorbance at shorter wavelengths.

Even though the intended straight chain polymer was not the sole product of the coupling reactions discussed, many of the products exhibited a chiral secondary structure. They were therefore used in the next stage to test for enantioselectivity by preparing CSPs to be used in HPLC columns.

3.6 Formation of Discrete Phenyl Carbamate Molecules

In addition to the polymeric phases we also prepared the discrete tetra(phenyl carbamate) derivative of *D*-threitol (butanetetrol) **51** by the reaction of *D*-threitol with

phenyl isocyanate in pyridine. This molecule would have the same functional groups present as in the polymers however would contain no induced chirality from a secondary structure. This would help us to determine what factors were important in chiral recognition.



51

Figure 3.17: Tetra(phenyl carbamate) butanetetrol

3.7 Preparation of Chiral Stationary Phases

The compounds produced from the coupling reactions which showed a chiral secondary structure as discussed earlier as well as the discrete tetra(phenyl carbamate) butanetetrol **51** were coated onto PGC and packed into HPLC columns using the batch method (section 2.2.2).

The coated samples of solid were then packed, using a slurry method, into stainless steel HPLC columns (4.6 mm diameter) and tested under various conditions with a variety of analytes. The analytes chosen to test the enantioselectivity of these novel phases are listed with their structures in appendix 1. They cover a range of functional groups, including acidic, basic and neutral molecules and include compounds that are known to be separated by commercially available phases, such as the Diacel OD column.

3.7.1 Testing for Enantioselectivity of Chiral Columns by HPLC

The columns **52-55** were tested with different mobile phases not only to evaluate the chiral selectivity but also to test the stability of the columns. Initially, normal phase conditions were used with varying proportions of hexane and isopropanol. Trifluoroacetic acid and diethylamine were added to the mobile phase for the acidic and basic racemates respectively in an attempt to minimise the peak tailing effect. Having completed all the tests under normal conditions reverse phase conditions were used. For the neutral samples a simple water and methanol phase was used. For the acidic analytes a dilute acid was used in place of the water to suppress the ionisation of the compound. Two acids were tried: 0.1 M acetic acid and pH 2 perchloric acid. An aqueous salt solution and methanol were used for the basic compounds consisting of 0.2 M ammonium acetate or 0.2 M sodium perchlorate.

Product from reaction of:	Column number
Benzoate diol **30** and hexamethylene diisocyanate	**52**
Phenyl carbamate diol **31** and hexamethylene diisocyanate	**53**
(3,5-dimethyl)Phenyl carbamate diol **32** and phenylene diisocyanate	**54**
Butanetetrol and phenyl isocyanate (**51**)	**55**

Figure 3.18: The compounds coated onto PGC to be tested by HPLC

3.7.1.1 Results of the HPLC Tests for Benzoate Derivative

Column **52** (PGC coated with macromolecules formed from benzoate monomer) showed no chiral separation when tested with analytes in appendix 1, under either normal

or reverse phase conditions. The solvent for normal phase conditions had to be changed to hexane to try and increase retention of the analytes. Peak shape was also very broad, even with the addition of TFA (for acidic compounds). The addition of DEA improved the peak shape but reduced the retention time considerably.

3.7.1.2 Results of the HPLC Tests for Aryl Carbamate Derivatives

3.7.1.2.1 Phenyl Carbamate Derivatives

Racemate		Mobile Phase (solvent ratio)	K'	α
Benzoin	**M**	normal (98/2)	3.52/3.84	1.09
trans-stilbene oxide	**P**	normal (100)	1.71/2.00	1.17
CS469	**D**	reverse (40/60)	13.78/17.58	1.28

Figure 3.19: Table showing enantioselectivity of column **53**

Normal Phase: Hexane/Isopropanol; Reverse Phase: 0.2 M $NaClO_4$/Methanol

The product of the reaction between the phenyl carbamate monomer and hexamethylene diisocyanate (column **53**) was tested with the analytes listed in appendix 1. Under normal phase conditions a separation of benzoin (**M**) was observed, although the peak shape was still broad (**Fig. 3.20**). To try and improve the peak shape the analysis was repeated using a lower injection concentration. The injection concentration was reduced from 0.5 mg mL^{-1} to 0.05 mg mL^{-1} and the α value was improved from 1.07 to 1.13. Separation of *trans*-stilbene oxide (**P**) under normal phase conditions was also

achieved, obtaining an α value of 1.17, but again peak shape was still broad. Under reverse phase conditions only one compound showed signs of being separated. An α value of 1.28 was obtained for CS469 (**D**) using a MP of 0.2 M sodium perchlorate and methanol.

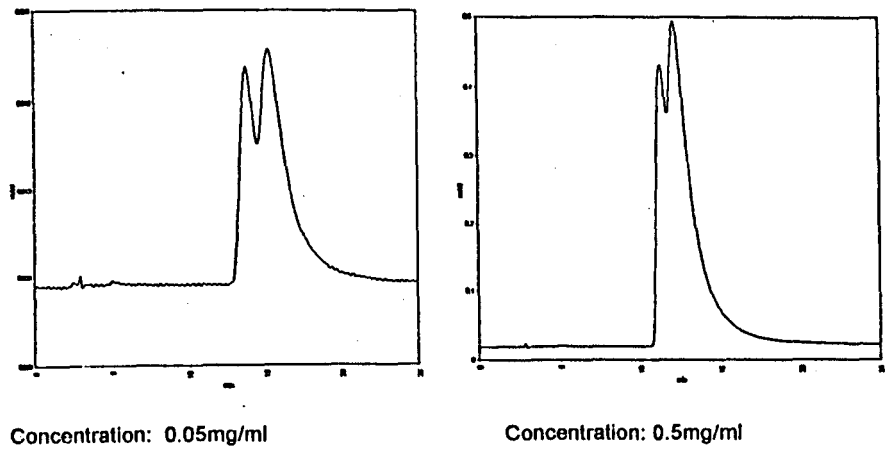


Figure 3.20: Separation of benzoin using column **53**

3.7.1.2.2 (3,5-Dimethyl)phenylcarbamate Derivatives

The product of the reaction between the (3,5-dimethyl)phenyl carbamate monomer and 1,3-phenylene diisocyanate (column **54**) was evaluated with 12 compounds (structures given in appendix 1), under various solvent conditions. Under normal phase conditions (95/5 hexane/isopropanol) a separation of benzoin was observed with an α value of 1.12. However, the resolution was poor and no separation was possible for the other analytes. The capacity factors (relating to the time taken for the analyte to pass through the column), however were generally higher for the CSP containing the phenylene linker **54** compared to that with the hexamethylene linker **53**. This may indicate stronger interactions with the analytes, without discrimination between the

analytes. Another possibility is that the covering of the support was not even, allowing non-selective interactions with the PGC to increase the retention of the analytes, reducing any chiral selectivity achieved by the CSP.

Racemate		53		54	
		eluent	K'	eluent	K'
CP 55940	**B**	95/5	13.8	95/5	19.9
Propranolol Glycol	**G**	95/5	12.1	95/5	18.2
2-Phenylpropionic Acid	**J**	99/1	2.3	98/2 [b]	1.1
1-Phenyl-1-Propanol	**K**	hexane	9.4	99/1	3.9
Mandelic Acid	**L**	98/2 [b]	13.0	98/2 [b]	10.7
Benzoin	**M**	98/2	3.5	95/5	7.8
1-Phenyl-1,2-Ethanediol	**N**	95/5	4.1	98/2	22.2
Stilbene Oxide	**P**	hexane	1.7	hexane	1.9
Oxprenolol	**Q**	98/2 [a]	4.7	98/2 [a]	7.8
Flavanone	**R**	90/10	2.2	99/1	8.5
Trifluoro-1-(9-Anthryl)-Ethanol	**S**	90/10	6.4	98/2	4.9
Homatropine	**T**	98/2 [a]	12.0	98/2 [a]	18.3

Figure 3.21: Table comparing capacity factors for phenyl carbamate monomer with hexamethylene linker (**53**) and (3,5-dimethyl)phenyl carbamate monomer with phenylene linker (**54**)

(a) 0.1% DEA added; (b) 0.1% TFA added; K' capacity factor of first eluting peak

Column **55** (PGC coated with 1,2,3,4-tetra(3,5-dimethyl phenyl carbamate) butanetetrol **51**) showed no signs of chiral selectivity with any racemate. A very high pressure was observed and the flow rate had to be dropped to 0.3 mL/min to keep the pressure below 4000 psi. With a flow rate of 1 mL/min a pressure of between 500 and 700 psi is usually observed.

3.8 Conclusions

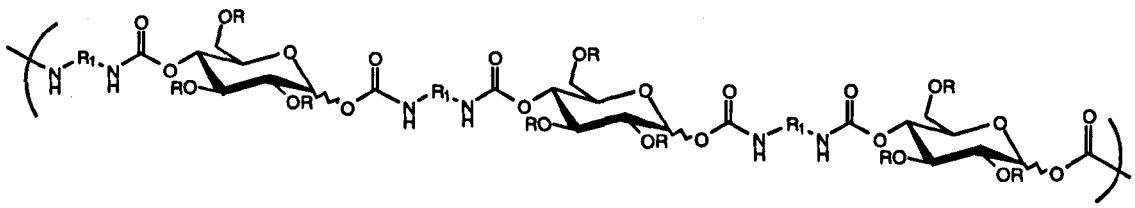
The reaction between the chosen monomers and diisocyanate linkers produced chiral molecules with suitable solubility profiles to be coated onto solid support for use as a stationary phase in HPLC. The molecules formed exhibited a chiral secondary structure, as shown by their CD spectra, although the products of the reactions consisted of a mixture of linear and cyclic molecules. When the benzoate derivatives were coated onto PGC and tested on analytical scale HPLC they showed no chiral selectivity. However, the phenyl carbamate derivatives did show signs of chiral recognition, although for a limited number of analytes.

Even though these macromolecules showed encouraging results improvements need to be made on the isolation of the different macromolecules present. The existence of a range of different molecule types may be producing the poor conflicting results when being tested for enantioselectivity on a HPLC column as each has the potential for different modes of interactions with analytes. Therefore work needs to be done to separate the different products formed or prepare discrete molecules with some degree of chiral secondary structures. The importance of secondary structure was shown by the lack of any chiral selectivity with the phenyl carbamate derivative of butanetetrol.

CHAPTER 4

NOVEL MACROMOLECULES BASED ON SACCHARIDE MONOMERS

4.1 Introduction



56

Figure 4.1: New target polyurethane polymer containing sugar moieties

Due to the disappointing results obtained using tartrate derived polyurethanes attention was turned to the synthesis of other chiral polyurethanes to be used as CSPs. The success of commercial phases based upon saccharides prompted our decision to investigate novel polyurethane carbohydrate derivatives as possible suitable phases for chiral chromatography. Thus the objective was to synthesise molecules containing sugar moieties connected by urethane linkages that could be used as a CSP. The use of saccharide monomers would mimic cellulose by the individual unit possessing a 6-membered carbohydrate heterocycle with the three hydroxyl groups capable of functionalization to give a variety of derivatives capable of various interactions with analytes. However, as the structure would be interrupted by non-sugar linkers (e.g. sugar-linker-sugar-linker etc.) the macromolecules would not be defined as polysaccharides and thus would avoid existing patent restrictions. These molecules could be investigated by

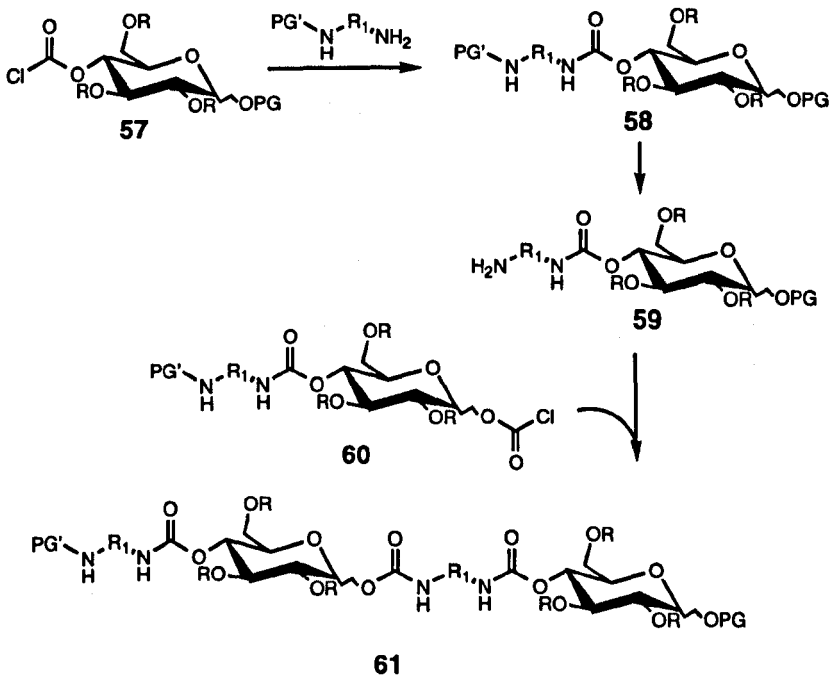
HPLC to identify the necessary requirements of the CSP for enantioselectivity such as the importance of the individual units (i.e. whether they need to be mono- or di-saccharides). Further investigation could be made by comparing different functionalization of the hydroxyl groups (R) and by altering the nature of the urethane linker (R_1), thus affecting the overall shape and the secondary structure of the molecule.

Two possible approaches were considered to prepare macromolecules based on saccharide monomers: a stepwise approach and a polymerisation approach, both will be described here. The final product itself however needs to be prepared in very few steps in high yields with little by-products as multigram quantities will be required. This restricts the approaches and type of chemistry that can be utilised.

4.2 Stepwise strategy

The stepwise approach (**Scheme 4.1**) involves the synthesis of a sugar unit with the anomeric position protected with one protecting group, positions 2,3 and 6 protected with another group and the 4-position activated (**57**). This molecule could then be reacted with a diamine, having one of the amines protected with another different protecting group to form the di-protected molecule **58**. The amine would then be selectively deprotected (**59**) and then reacted with the molecule that has had the anomeric position selectively deprotected and activated (**60**) to form a new, larger di-protected species **61**. This step could be repeated to sequentially add more units increasing the chain length of the final compound **56** (**Fig. 4.1**). However, potential problems with this approach include the many different protection and deprotection steps, selective deprotection of the amine and the activation of the anomeric position. In addition, another major

disadvantage with this approach is the length of the synthesis as it contains many steps with the initial saccharide not being straightforward to prepare. The subsequent number of reactions is high and they would therefore all need to be high yielding with easy purification to satisfy the criteria set for the preparation of a CSP. Consequently it was felt this strategy was too complicated to liberate gram quantities of CSP rapidly and other strategies were considered.

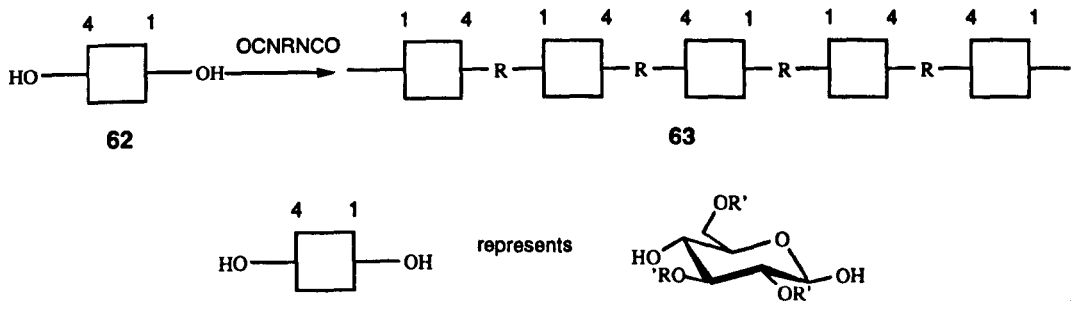


Scheme 4.1: Overall scheme for stepwise formation of macromolecules

4.3 Polymerisation Strategy

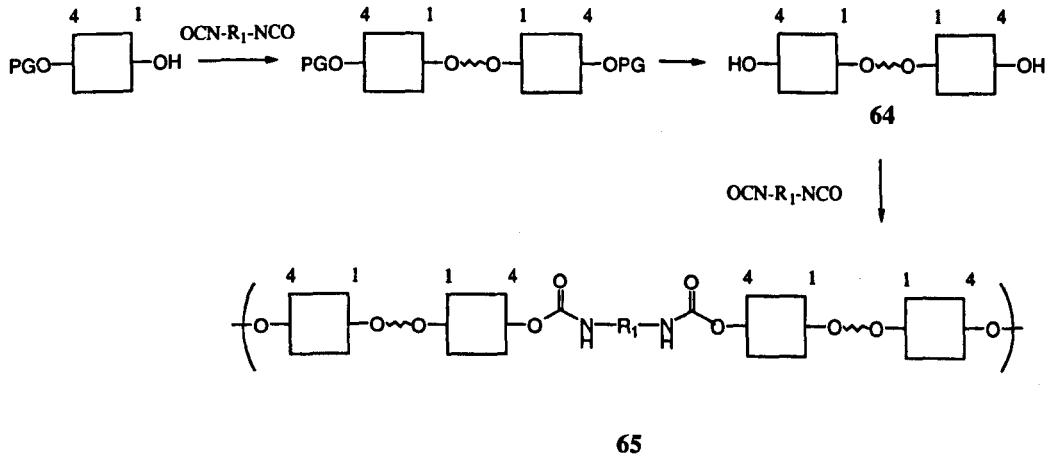
A polymerisation approach to the coupling of sugar units with non-saccharide linkers (diisocyanates) would reduce the overall number of steps required to make similar CSP structures compared to the stepwise approach and could be achieved by two possible routes. The simplest route would be to synthesise a sugar unit containing two free hydroxyl groups at the 1- and 4-positions, with the 2-, 3- and 6-positions protected **62.**

This unit could then be reacted with a diisocyanate to yield a chain polymer **63** (**scheme 4.2**).



Scheme 4.2: Polymerisation of sugar unit with two free hydroxyls

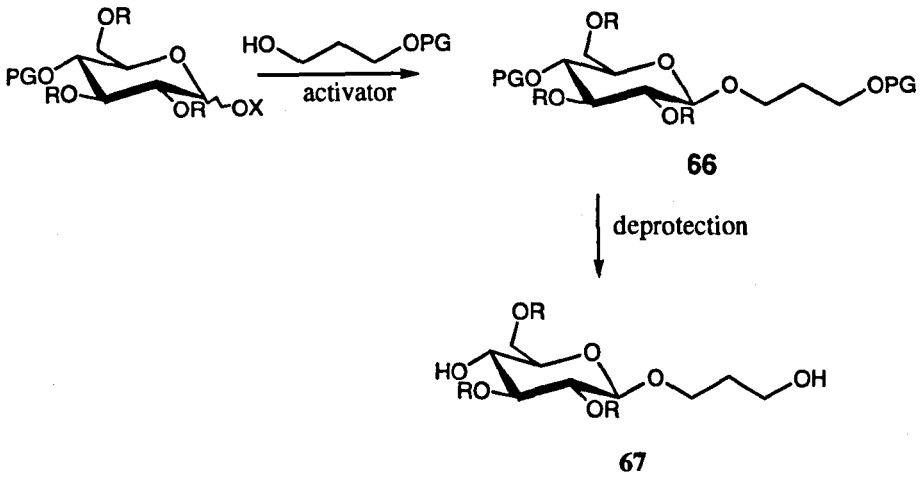
If a lack of control in the polymerisation step was a problem then a prepolymer could be prepared. This would also allow purification before linking the regular repeating unit **64** with the diisocyanate to form **65** (**scheme 4.3**) and may also form a more regular macromolecule by ensuring the reaction of identical monomer units (with the same orientation of sugar moieties).



Scheme 4.3: Formation of a regular polymer via a pre-polymer

4.4 Stereochemistry of Anomeric Centre

An ongoing problem through these syntheses is the stereoselectivity of reactions at the anomeric centre. Reactions taking place at the anomeric centre can produce either the α or β product, or mixtures of both. If a mixture of anomers were formed then the polymer would not have an identical repeating unit which would lead to a lack of secondary structure. One solution to this problem would be to attach a tether at the anomeric position in a fixed orientation (**66**), which would fix the stereochemistry throughout subsequent reactions. If this tether included a protected alcohol then it may be possible to reduce the number of protecting steps used in the total synthesis thus lowering the total number of steps. The stereochemistry at the anomeric centre can be controlled by the choice of activating groups and activators.

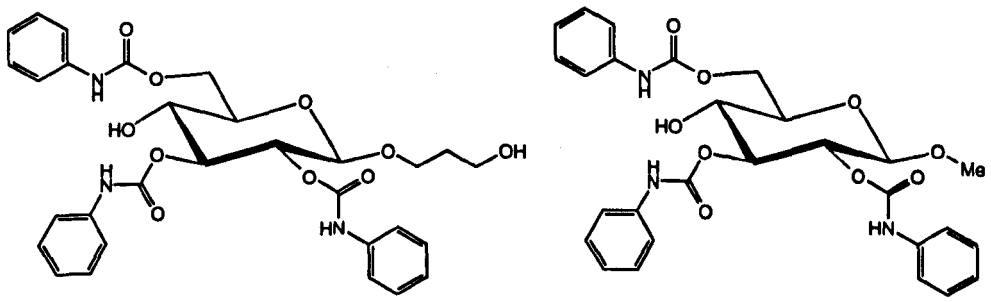


Scheme 4.4: Use of a tether to fix β-stereochemistry at the anomeric centre

4.5 Synthesis of the Individual Sugar Unit

Due to the above considerations our initial saccharide targets are shown in **Fig. 4.2**. We initially studied the preparation of the methyl glucopyranoside **68** as a model to

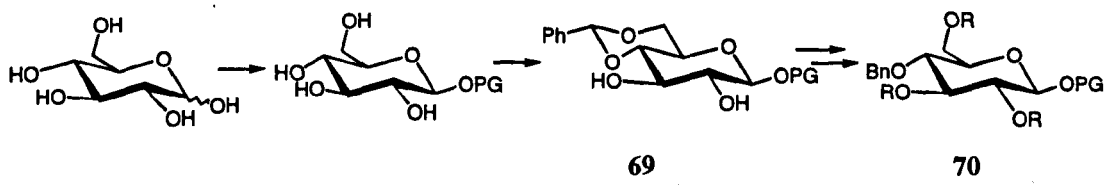
investigate the manipulation of the remaining hydroxyl groups. The 2-, 3- and 6-positions need to be derivatised as phenyl carbamate groups while the 1- and 4-positions need to be free to allow functionalization in the polymer reaction. The selective reaction of the hydroxyl groups at position 4 is not possible so protecting groups need to be used to result in a free hydroxyl group at this position.



67 target **68** model

Figure 4.2: Target and model saccharide units

One method of distinguishing between the four hydroxyl groups (at positions 2, 3, 4 and 6) is the use of acetal protecting groups. A benzylidene acetal, for example, prefers the conformation of a six-membered ring so can be used to selectively protect the 4 and 6 positions simultaneously **69**. This acetal group can then be opened in either the 4 or 6 direction and therefore can be used in this system to yield a benzyl protecting group at the 4-position. Positions 2, 3 and 6 would all then be functionalised by reaction with phenyl isocyanate to form phenyl carbamate groups (**70**) and the benzyl group at the 4-position can then be removed to furnish the free hydroxylated compound **68**.

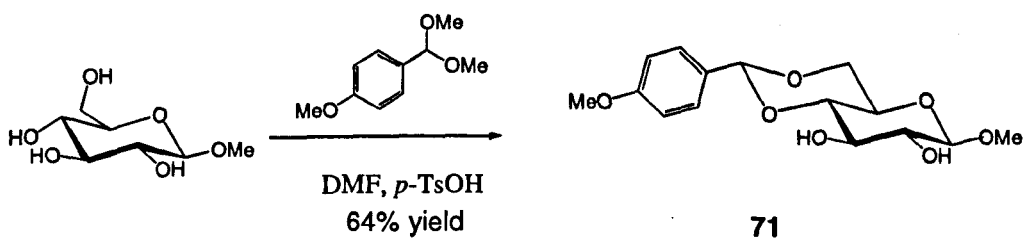


Scheme 4.5: General scheme for the synthesis of 1,4-protected sugar unit

4.5.1 Reaction at the Anomeric Centre

The anomeric position is the most reactive in saccharide moieties so in the synthesis of the target this would be the first hydroxyl group to be protected or tethered. There are many literature methods that could be applied to this system and it was expected to follow standard procedures. Therefore to probe the chemistry needed for the manipulation of the remaining hydroxyl groups 1-methoxy glucopyranoside was used as a model as it is commercially available allowing easy study of the conditions and techniques required for subsequent steps.

4.5.2 Addition of the Methoxybenzylidene Acetal



Scheme 4.6: Protection of 4- and 6-positions with a methoxybenzylidene acetal

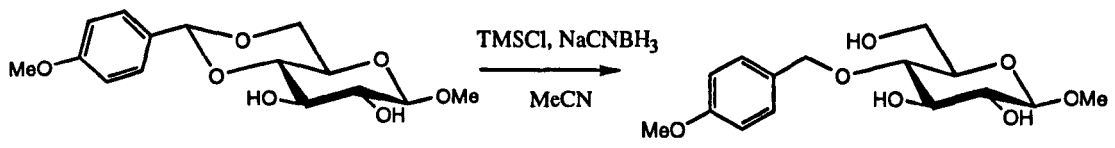
A methoxybenzylidine acetal was used to simultaneously protect the 4- and 6-positions of methyl *D*-glucopyranoside. This was achieved using 4-methoxybenzaldehyde

acetal in DMF with *p*-toluenesulphonic acid[84] to form **71**. This reaction proceeded smoothly in 64% yield and **71** was purified by recrystallization with ethyl acetate. The 4-methoxybenzaldehyde was the preferred choice as it can be cleaved more selectively than the benzylidene acetal. Also different conditions are used to cleave a 4-methoxybenzylidene acetal than those for a benzylidene acetal allowing the use of such a different protecting group if needed later on in the synthesis.

4.5.3 Cleavage of the Methoxybenzylidene Acetal

The next step was to cleave the benzylidene acetal selectively in the 4-direction. This would then leave free hydroxyl groups at the 2-, 3-, and 6-positions, which could then be functionalised at the same time. There are a range of conditions that can be used to cleave a benzylidene acetal[84, 85] and these result in complete cleavage or partial cleavage (opening). Opening of the benzylidene acetal can occur in either the 4- or 6-direction, leaving a free hydroxyl and a hydroxyl group protected by a benzyl ether or benzoyl ester depending on the conditions employed. The reductive cleavage of benzylidene acetals of carbohydrates has been studied using different conditions. Lithium aluminium hydride-aluminium chloride[86, 87] is one example of reagents that give the major product as the 6-O benzyl ether derivative whereas the use of sodium cyanoborohydride-hydrogen chloride[84, 88] gives the opposite 4-O-benzyl regioselectivity.

4.5.3.1 Reductive Cleavage



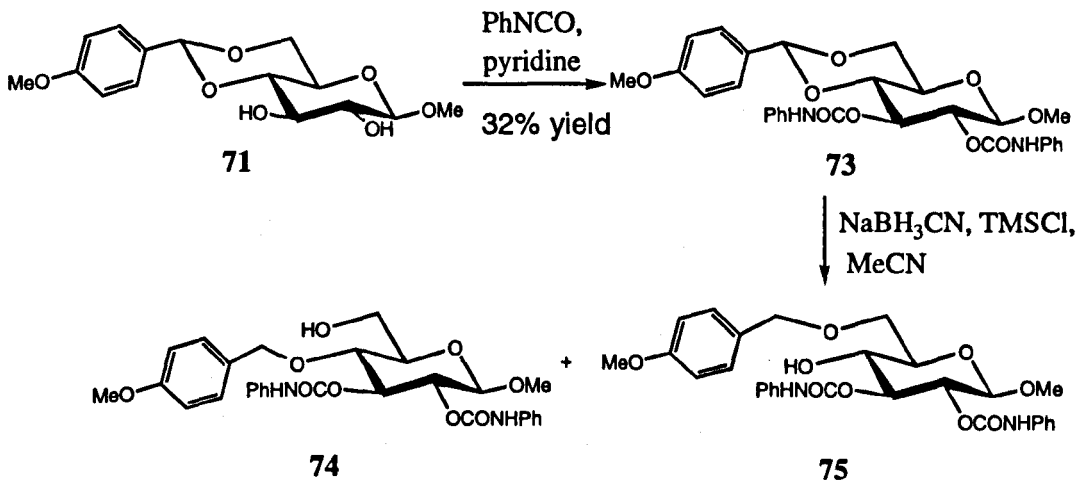
71 **72** 2%

Scheme 4.7: Attempted cleavage of the methoxybenzylidene acetal

Initial attempts at opening the benzylidene acetal used sodium cyanoborohydride following a method used by Johansson and Samuelsson[84] with the intention of obtaining the 4-O-benzyl derivative. Reaction of **71** with sodium cyanoborohydride and trimethylsilyl chloride in acetonitrile yielded very little product (**72**) after 20 hours. TLC indicated the starting material **71** had disappeared and therefore it was assumed that the majority of the product had been lost in the aqueous work-up. So that the product was not lost due to its solubility in water it was decided to functionalise the hydroxyl groups at positions 2 and 3 as phenyl carbamate groups before cleavage was attempted. These would be suitable functional groups in the final CSP and thus some steps would be saved.

Hence reaction of **71** with phenyl isocyanate in pyridine afforded the disubstituted phenyl carbamate product **73**[89] which was purified by recrystallization with an overall yield of 32%. As no reports of benzylidene ring opening with phenyl carbamate functionalised sugars has appeared in the literature we were unsure if this step would be successful. Cleavage using sodium cyanoborohydride and trimethylsilyl chloride in acetonitrile was attempted and afforded the desired 4-methoxybenzyl ether **74** as the major product although only in 22% yield. The low yield may be attributed to the

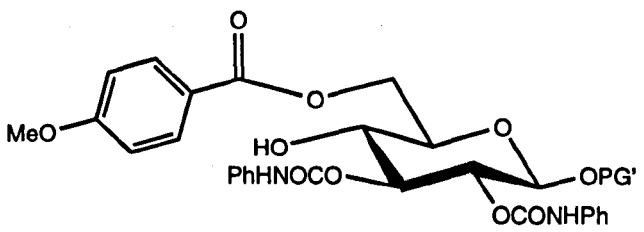
presence of unreacted starting material and cleavage of the methoxybenzylidene acetal in the 6-direction, to form **75** (as determined by NMR as they were not isolated by column chromatography).



Scheme 4.8: Cleavage of methoxybenzylidene acetal of **73**

Alternative conditions for cleavage of benzylidene acetals include the use of diisobutylaluminium hydride (DIBAL) in toluene.[90, 91] Therefore this method was attempted on the methoxybenzylidene acetal **73** to see if the yield and regioselectivity could be improved. The addition of DIBAL to a solution of **73** at -40°C resulted in a mixture of compounds being produced with no desired compound **74**. The reduction in reactivity and regioselectivity of the reaction to open the benzylidene acetal may be due to the bulkiness of the phenyl carbamate at position 3 influencing the opening mechanism.[91]

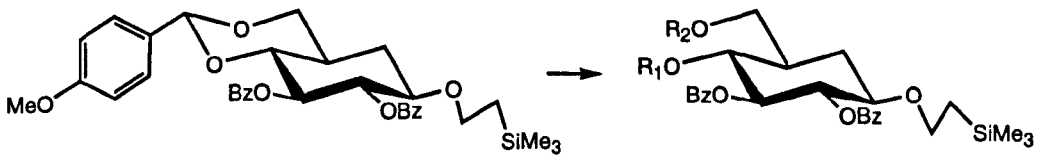
4.5.3.2 Oxidative Cleavage



76

Figure 4.3: The saccharide unit with different functional groups at positions 2, 3 and 6

As these reductive methods of benzylidene cleavage had resulted in a range of products and the yields were low, especially after complicated purification by column chromatography, attention was turned to methods of oxidative cleavage[92, 93] of the benzylidene acetal. As there is no literature precedence for such reactions with carbohydrates containing carbamate moieties at positions 2 and 3 it was not possible to predict the outcome of the reaction. As the cleaved product contains an ester and a free hydroxyl group it may be possible to utilise the 6-methoxybenzoyl ester **76** if it is the major product of the reaction.



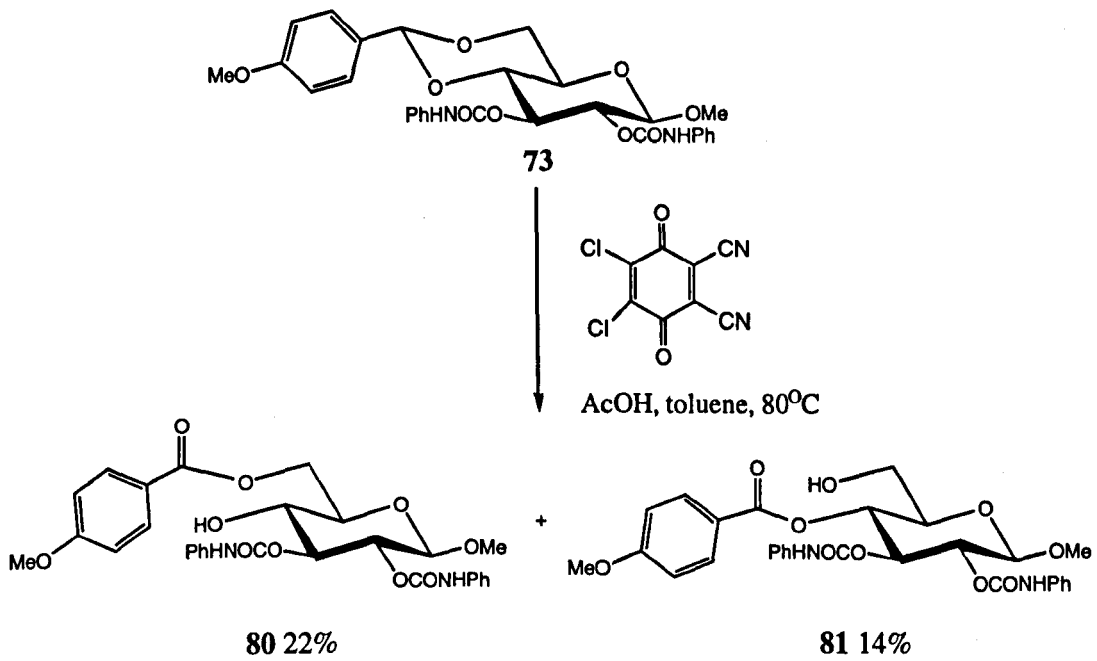
77

78 R_1=H, R_2=$COC_6H_4OCH_3$

79 R_1=$COC_6H_4OCH_3$, R_2=H

Figure 4.4: Example of cleavage of methoxybenzylidene acetal by Zhang and Magnusson[93]

Zhang and Magnusson[93] used 2,3-dichloro-5,6-dicyano-*p*-benzoquinone (DDQ) to selectively open carbohydrates with 4,6-acetals and ester functionality at positions 2 and 3, such as **77** (**Fig. 4.4**) to give up to a 6:1 ratio in favour of the 6-O ester product **78**. This system was therefore applied to the methyl glucopyranoside **73** which was heated to 80°C in toluene with DDQ, a small amount of acetic acid and non-activated molecular sieves (**scheme 4.9**). The crude product obtained was purified by column chromatography to reveal the presence of both regioisomers **80** and **81**. The 6-O benzoyl ester **80** was the major product (22%) compared to the 4-O benzoyl ester **81** (14%) but the ratio of 3:2 was lower than the selectivity for the ester derivatives.[93]



80 22% **81** 14%

Scheme 4.9: Oxidative cleavage of the benzylidene acetal

The regioisomers were identified by NMR experiments. The benzylidene ester is more electron withdrawing than the acetal and this is shown by a shift in the signal for the protons at positions 4 and 6 respectively. The 6-O-benzoyl derivative **80** shows a shift

in the signal for the protons at C-6 from 4.3 and 3.84 ppm to 4.69 and 4.51 ppm. In contrast the 4-benzoyl ester **71** has a signal at 3.70 ppm for the protons at the 6-position and the proton at the 4-position had shifted from 3.80 to 5.20 ppm compared to the starting material **73**. Decoupling and D_2O experiments for 6-O benzoyl compound showed the doublet at 5.05 ppm, corresponding to the hydroxyl proton, coupled with the multiplet at 3.82 ppm for the proton at position 4.

These reactions showed that the standard methodology for cleavage of benzylidene acetals could be applied to the chosen system containing phenyl carbamate groups at positions 2 and 3 but with reduced efficiency, both in respect to the regioselectivity and the overall yield of product.

4.6 Conclusions

The synthesis of a desired saccharide diol model unit with positions 2, 3 and 6 functionalised as phenyl carbamates and a free hydroxyl at position 4 was achieved. However, the three steps to get to this sugar unit had an overall yield, at most, of 4%. It was also possible to synthesis the saccharide diol model containing phenyl carbamate groups at positions 2 and 3, a methoxybenzoyl group at the 6 position and a free hydroxyl at position 4, but again in very low overall yield. The synthesis overall is therefore not very efficient and it is likely that this would also be similar for the molecule with a tether at the anomeric position leading to the target **67**.

The cleavage of the methoxybenzylidene acetal was not greatly selective in the presence of phenyl carbamate groups at position 2 and 3 with a reduction in selectivity of opening of the acetal compared with benzyl ethers and benzoyl esters. Therefore, due to

the low yields of most of the reactions and complications with purification (due to the formation of similar by-products) attention was turned to alternative strategies to prepare a CSP in fewer steps.

CHAPTER 5

SACCHARIDE MACROMOLECULES AS STATIONARY PHASES: THEIR SYNTHESIS

5.1 Introduction

As mentioned previously any new compound that is to be used as a CSP needs to satisfy various criteria. It must be capable of interactions with the analyte and these interactions must be able to differentiate between enantiomers of the analyte. Ideally the one CSP would be able to differentiate between many different analytes with different functional groups. Practically the CSP must have the correct solubility profile where it is insoluble in solvents that are used as eluents in chromatography but soluble enough to be purified and coated onto or attached to the solid support.

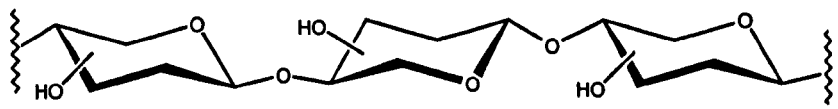
In commercial polysaccharide phases the interactions arise from the functional groups attached to the hydroxyl groups. As the derivatives used as polysaccharide phases are usually esters or carbamates[39] then the functional groups include carbonyl groups or amides and aromatic rings, that are capable of hydrogen donating, accepting and dipole or π-π stacking. The secondary structure of polysaccharides is important as it induces more chirality to the compound. Cellulose and amylose in particular form helices[84] where chiral ravines form into which analytes may selectively enter. However not all CSPs are polymeric as CSPs containing small discrete molecules have been designed for certain analytes by choosing molecules with functional groups that specifically complement those of the analyte and by attaching these molecules to a solid support efficient enantioseparations have been obtained.[30]

A compromise between these two extremes would be to form large, discrete molecules with a similar structure to polysaccharides. One way to achieve this would be to connect two saccharides together with a non-saccharide linker. These new compounds would contain functional groups allowing the same range of interactions with the analytes found in commercial saccharide phases and depending on the choice of saccharide may be large enough to have a suitable solubility profile. The regular structure of the saccharide portions combined with the linker will hopefully mimic the secondary structure of larger polysaccharide polymers making them versatile in their enantioselectivity. In addition, the preparation of large monodisperse molecules means it would be easier to study and model any CSP/analyte interactions than for conventional polymeric phases. Another aspect of this strategy is that the secondary structure could be manipulated by the choice of the non-saccharide linker thus allowing structure activity relationships to be studied.

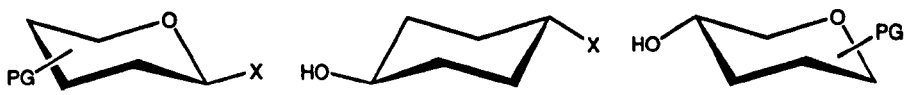
If successful as CSPs these new phases would not be covered by existing patents covering the use of polysaccharides in chiral HPLC columns allowing the potential for commercial use. It would also establish the requirements of CSPs showing the importance of secondary structure and establish the required number of sugar units before the necessary secondary structure has been built up. Investigation into the suitability of various solid supports may be possible, in particular the use of PGC as a solid support investigating its effect on the secondary structure of the compounds coated on to it.

5.1.1 Strategies of Synthesis of Polysaccharide Mimics

There are two approaches to linking sugar units together to mimic the polymeric cellulose and amylose phases. The first involves linking a 4-hydroxy sugar component **83** with the anomeric position of a second sugar component **82** by a difunctionalised linker (**Fig. 5.1**). The second approach involves linking 2 sugar units at the anomeric position (**82**) thus forming a dimer **84** (**Fig. 5.2**).



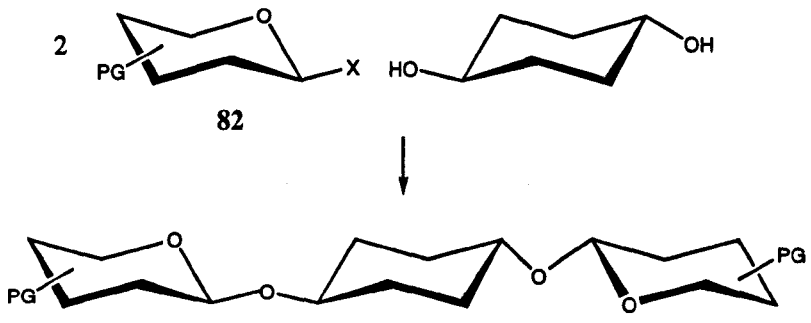
cellulose



82 **83**

Figure 5.1: Reaction of two sugar units with a difunctionalised linker, such as cyclohexane, one at its 1-position **82** the other at the 4-position **83**. Where PG = protecting group and X = activating group

While the first approach is more likely to mimic the secondary structures of the natural polysaccharides better than the second approach it suffers from the disadvantage that a larger number of steps would be required to make the sugar component with a free 4-hydroxyl group (**82**). As multigram quantities of the CSPs will ultimately be required we initially investigated the second approach, as it would lead to derivatised phases in only 3 steps.



84

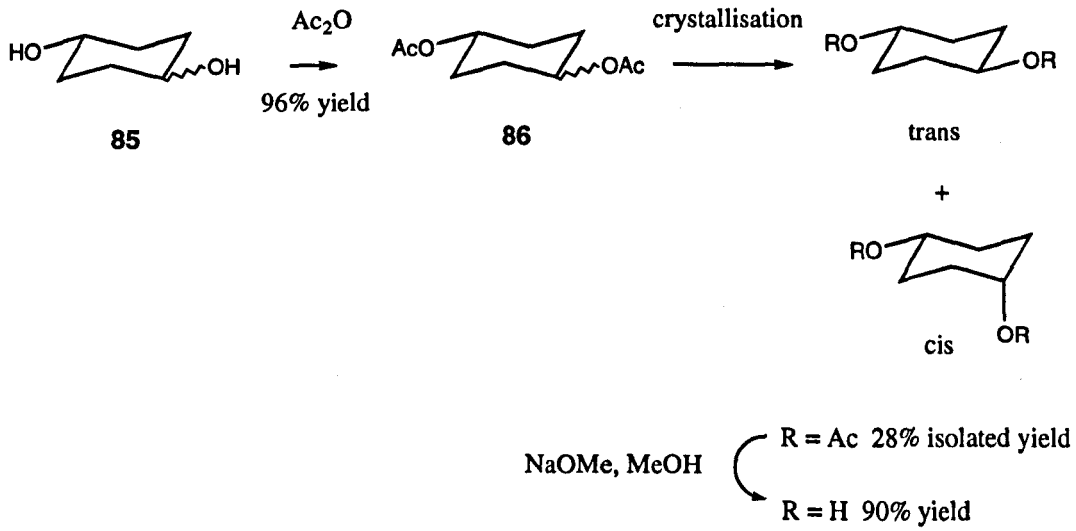
Figure 5.2: Reaction of difunctionalised linker e.g. cyclohexane, with two identical sugar units (**82**) at their anomeric positions. Where PG = protecting group and X = activating group

5.2 Linking the Saccharide Units

The site of reaction should be the same on each saccharide unit so one hydroxyl group needs to be activated while the others need to be protected. The anomeric site is the obvious choice for such a reaction as it is the most reactive and accessible due to the oxygen in the ring. There is a range of different glycosidation reaction conditions that use a variety of activating groups and promoters for the reaction of an anomeric group with an alcohol. The reaction we investigated therefore required coupling of a diol linker with two identical sugar units via a glycosidation reaction scheme.

There are many diols that are commercially available which can be used as a possible linker for the two saccharide units. The closest replacement for a sugar unit is 1,4-cyclohexanediol with respect to size and shape although the influence of size and shape can be investigated by using diols such as 1,3-propanediol, 1,4-butenediol and 1,4-butynediol. Of the many cyclohexanediols commercially available some can be obtained

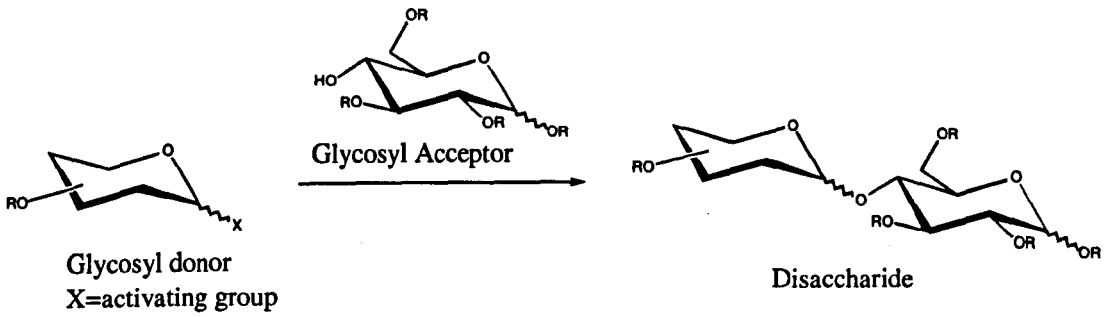
diastereomerically pure although 1,4-cyclohexanediol can only be bought as a mixture of *cis* and *trans* isomers. Isolation of the pure *trans* diastereomer however could be achieved by reaction with acetic anhydride at reflux to form the diacetate **86**, obtained as a 1:2 mixture of *cis/trans* derivatives. Recrystallization from hexane resulted in preferential crystallization of the *trans* compound in 28% yield thus the *trans* compound could be obtained pure. The *cis* isomer that remains in the mother liquor was always contaminated with the *trans* compound and could not be obtained pure. Even though the isolated yield of the *trans* cyclohexanediol was low the reaction could be done on a large scale (up to 20 g) and the starting material is cheap. The acetates were easily removed using $^{2}/_{15}$ equivalents of sodium methoxide in methanol at room temperature to yield the *trans*-1,4-cyclohexanediol (90% yield from diacetate).



Scheme 5.1: Isolation of *trans* cyclohexanediol

5.3 Glycosidation reactions

Reactions involving functionalisation of the anomeric position of a sugar with an alcohol are called glycosidation reactions. Linking of the two units together involves a donor and an acceptor and the coupling reaction is usually catalysed by an activator. The glycosyl donor contains an activated group at the anomeric centre (C-1) with all the other hydroxyl groups protected while the glycosyl acceptor has one free hydroxyl group (**scheme 5.2**). Regioselectivity is therefore achieved by the use of selective protecting groups and activating groups.

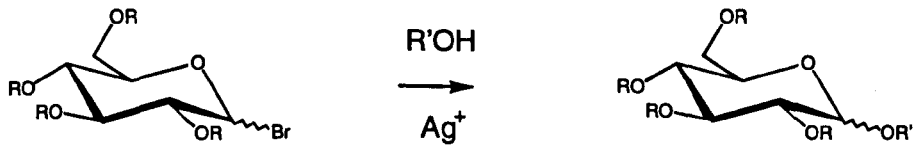


Scheme 5.2: General scheme for glycosidation reaction between two saccharides

To promote the reaction between a sugar and an alcohol many methods have been developed which include a variety of different groups at the anomeric centre and the use of different activators. The classic Koenigs-Knorr reaction[94] is an example of a glycosidation reaction where a glycosyl halide (the glycosyl donor, X = chloride or bromide) is reacted with an alcohol (the glycosyl acceptor) in the presence of heavy metal salts (the activators). Other schemes may introduce a group other than a halogen at the anomeric centre that can then either be activated directly or converted to the halide *in situ*. Consideration needs to be given to the stereochemistry of the reaction, which can result in the formation of α or β anomers.

Even though our strategy involves the coupling of a non-sugar alcohol with the anomeric position of two sugar units some of the known glycosidation techniques were applied to our system.

5.3.1 Halide Donors

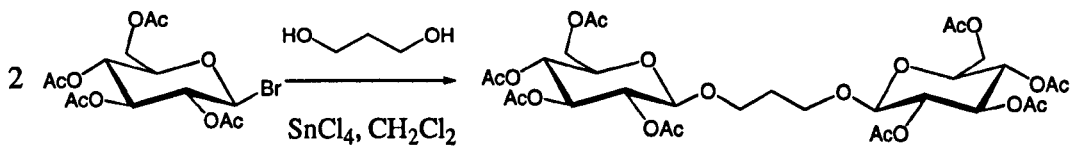


Scheme 5.3: Koenigs-Knorr method

The Koenigs-Knorr Method[94] is a useful and widely applicable method of glycosidation. It involves the formation of a glycosyl halide, usually the bromide or chloride, followed by reaction with a nucleophilic acceptor. The activators for the glycosidation step were initially heavy metal salts such as silver carbonate and the neighbouring group effect (an ester group at position 2 should lead to the 1,2-*trans* product whereas an ether will preferentially form the 1,2-*cis* product) is often used to control stereochemistry. Later work used a variety of different activators[95-100] but they were often toxic (such as mercuric salts),[95] dangerous (silver perchlorate is explosive)[97] or expensive.

Glycosyl bromides can be synthesised using hydrogen bromide in acetic acid with the peracetylated sugar,[101] although care needs to be taken to ensure rigorously dry conditions and the length of time of the reaction needs to be monitored to prevent loss of the acetate groups. Glucosyl bromide **87**, however, is commercially available and we

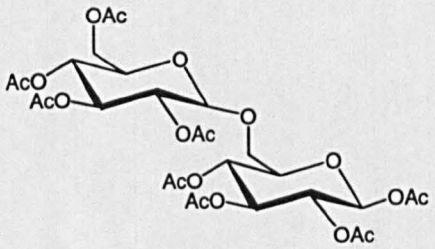
therefore used this in initial glycosidation reactions with symmetrical diols. We first investigated the use of both propanediol (a primary alcohol) and *trans*-1,4-cyclohexanediol (a mimic of the saccharide ring) as linkers between two glucose units.



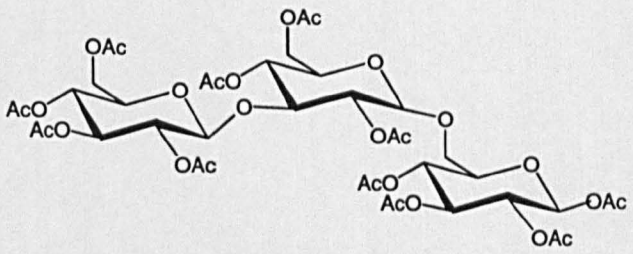
87 **88**

Scheme 5.4: Glucosidation of glucosyl bromide **87** with propanediol using tin tetrachloride

The initial attempt at glycosidation of glucosyl bromide **87** with propanediol was in dichloromethane with tin tetrachloride as the activator[102] (**scheme 5.4**). However, the diol was not sufficiently soluble in the solvent and the reaction produced a complicated mixture of products. To overcome solubility problems the reaction was repeated using THF as solvent. This reaction yielded a crude waxy solid which, according to MALDI-MS analysis (**Fig. 5.4**) did not include any products resulting from the addition of a propanediol unit (**88**) but consisted mainly of disaccharides (peaks labelled A, e.g. **89**) and trisaccharides (peaks labelled B, e.g. **90**, **Fig. 5.3**) with varying number of acetate groups removed. Due to the complicated nature of the reaction these products were not isolated so stereochemistry and regiochemistry were not determined.



89



90

Figure 5.3: Possible structures of a disaccharide and a trisaccharide (exact regiochemistry and stereochemistry not known)

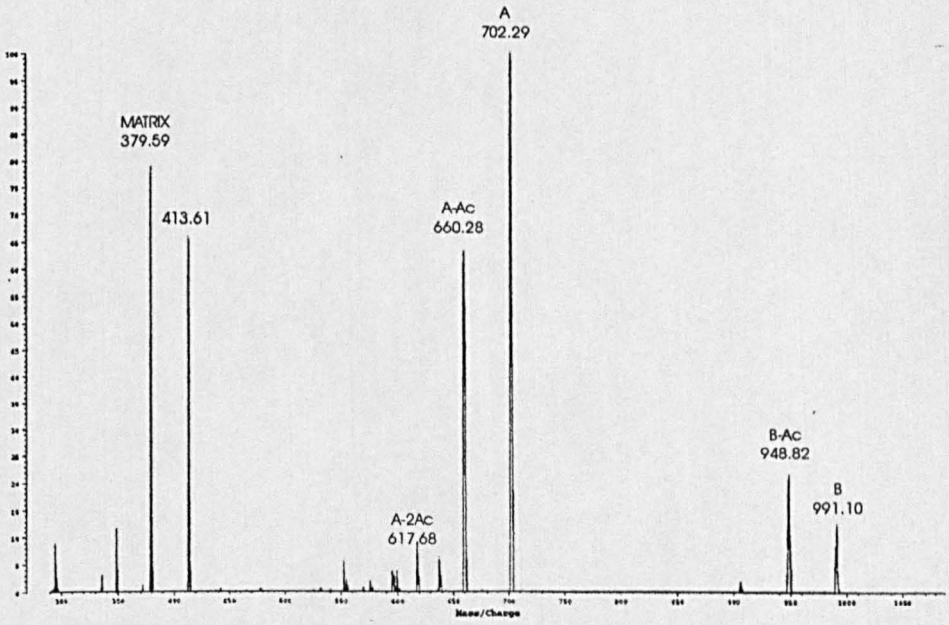
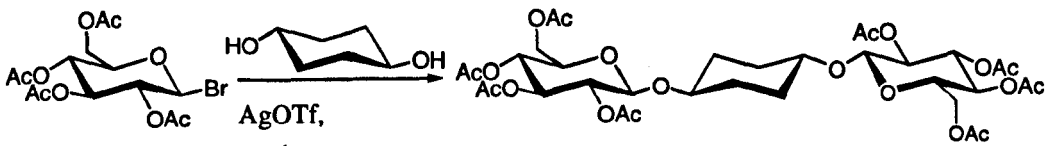


Figure 5.4: MALDI for reaction between **87** and propanediol (α-hydroxy cinnamic acid as matrix). A: disaccharide (e.g. **89**); B: trisaccharide (e.g. **90**)

Alternative activators for the glycosidation of the glucosyl bromide **87** were then utilised. Glycosidation of bromides has been achieved using silver trifluromethanesulfonate in the presence of 2,6-di-*tert*-butyl pyridine[103] or 1,1,3,3-tetramethyl urea.[104] The solvent for such reactions was a 1:1 v/v mixture of

dichloromethane and toluene, improving the solubility of the propanediol. However, reaction of glucopyranosyl bromide **87** with propanediol in the presence of 2.3 equivalents of silver trifluoromethanesulfonate and 1 equivalent of 2,6-di-*tert*-butyl pyridine resulted in a range of products. Analysis by MALDI-MS showed the formation of disaccharides (e.g. **89**) and trisaccharides (e.g. **90**) shown in **Fig. 5.3**, including compounds having lost various numbers of acetate groups as before and again there was no addition of propanediol to the sugar unit or any dimer **88** being detected.

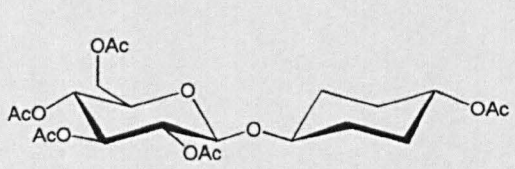


87 **91**

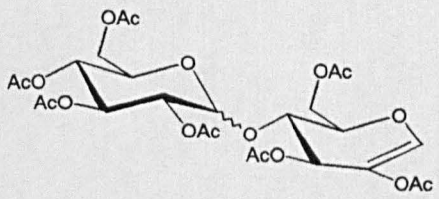
Scheme 5.5: Activation of glycosidation by silver triflate with the bromide derivative **87**

Despite the lack of success with propanediol the reaction was repeated using the more soluble *trans*-1,4-cyclohexanediol the glucosyl bromide **87** (**scheme 5.5**). Using the same conditions as the reaction with propanediol the range of products included the desired disubstituted product **91**, as determined by MALDI-MS of the crude sample (**Fig. 5.6**). Purification by column chromatography was attempted however only the monosubstituted product **92** was isolated (9% yield) while the disubstituted product **91** could not be fully separated from the numerous disaccharide, trisaccharide and higher analogues present in the crude reaction mixture (**Fig. 5.6**). Under the reaction conditions the acetate protecting groups were removed leaving a free hydroxyl groups which can

undergo subsequent glycosidation reactions to give di- (e.g. **89**) and trisaccharides (e.g. **90**, stereochemistry and regiochemistry not known).



92



93

Figure 5.5: Additional products in reaction with cyclohexanediol

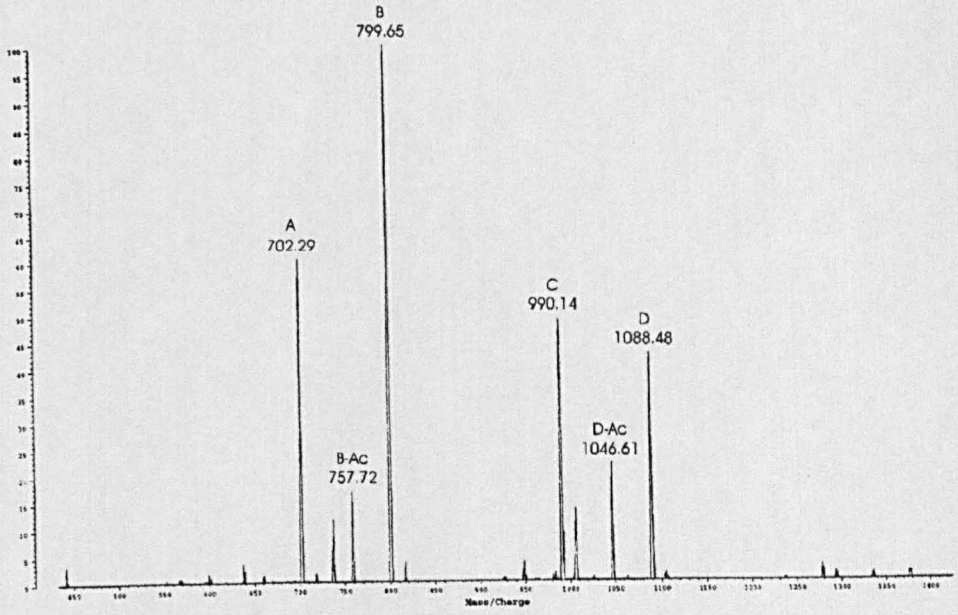
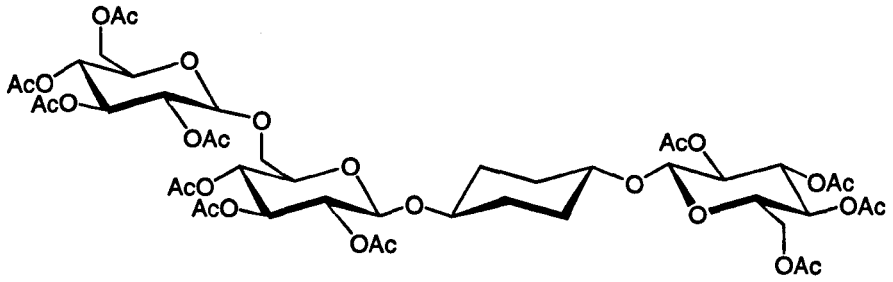


Figure 5.6: MALDI for reaction between **87** and cyclohexanediol

A: disaccharide (e.g. **89**); B: dimer (**91**); C: trisaccharide (e.g. **90**); D: trisaccharide and cyclohexane (e.g. **94**)



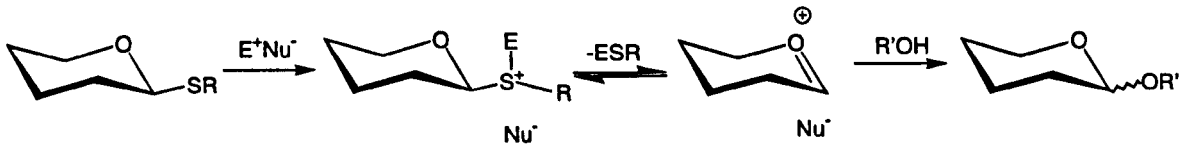
94

Figure 5.7: Possible structure of a trisaccharide with cyclohexane

Another by-product as determined by MALDI-MS seen as a small peak corresponding to a loss of mass 63, may correspond to the formation of a glycal **93** by elimination of HBr[105] (**Fig. 5.5**). Magnusson *et al*[106] have detected glycals when acetate protecting groups have been used in glycosidation reactions using glycosyl halides as donors. In their reactions using chloride donors only trace amounts of the desired product were obtained with the major product being the glycal formed by HCl elimination. They found using thioglycosyl donors instead of halide donors could reduce the formation of glycals consequently we decided to study the use of thioglycosyl donors as possible alternatives.

5.3.2 Thioether Donors

Thioglycosides are useful glycosyl donors because sulphur is a soft nucleophile, whereas oxygen, both in the ring and in the hydroxyl groups, is a hard nucleophile. This increases the potential for manipulation of the saccharide unit by functionalization of the hydroxyl groups in the presence of the thiol.



Scheme 5.6: Use of sulphur activating groups

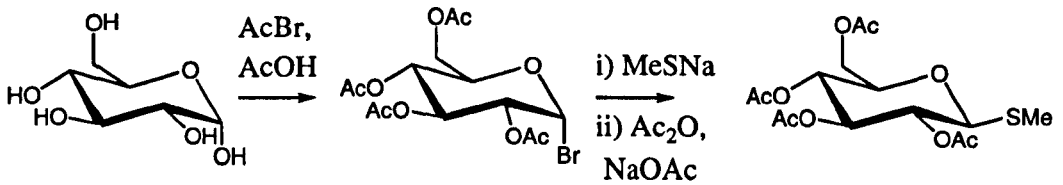
Thioglycosides themselves are not usually very stable and are not normally used as donors directly. They are either activated by electrophilic addition to form a better leaving group or replaced by a halide. Lönn[107] used methyl triflate to activate thioglycosides where the methylated sulphonium ion acts as a good leaving group. Other activators that have been developed[108] include dimethyl(methylthio)sulfonium triflate (DMTST)[109] which shows improved selectivity. Another advantage of using thioethers as glycosyl donors is that their synthesis is usually simpler than glycosyl halides[110] and their stereochemistry can be more easily controlled.

5.3.2.1 Preparation of Thioethers

We thus decided to investigate the use of thioglycoside donors in the synthesis of the desired CSPs. Hence we required a thioether of glucose so literature methods were followed to prepare the thiomethyl and thiophenol derivatives of glucose.

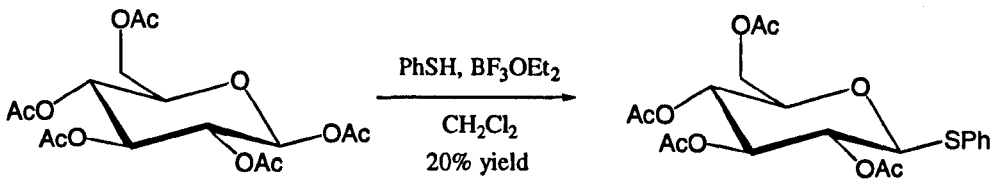
The thiomethylether of glucopyranoside **95** was prepared according to the process by Koto[111] (**scheme 5.7**). Acetobromination of *D*-glucose was achieved by treatment with acetyl bromide in acetic acid at 0°C. Evaporation of excess acetyl bromide and acetic acid gave 2,3,4,6-tetra-*O*-acetyl α-*D*-glucopyranoside bromide **87α** that was immediately reacted with aqueous sodium methanethiolate in acetone. To ensure de-acetylation did not occur acetic anhydride and sodium acetate were added when the reaction appeared to

be near completion by TLC. The whole process was done in a single vessel yielding a brown oil (50%) that was, however, difficult to purify.



87 95

Scheme 5.7: Formation of thiomethyl ether of glucose



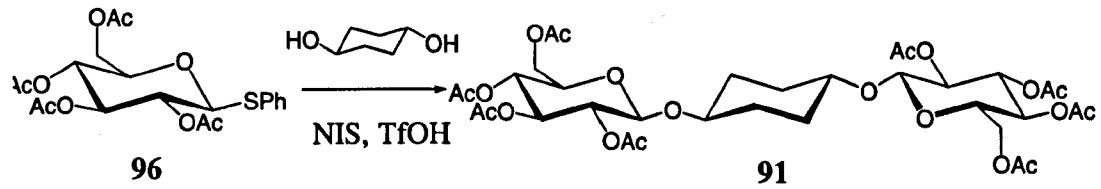
96

Scheme 5.8: Formation of the thioether phenyl 2,3,4,6-tetra-*O*-acetyl 1-thio-β-*D*-glucopyranoside

The thiophenol ether of glucose **96** was obtained in better yield by reaction of the peracetylated sugar with a Lewis acid and thiophenol (**scheme 5.8**). Reaction of glucose pentaacetate with thiophenol in the presence of 0.4 equivalents of boron trifluoride etherate in dry dichloromethane yielded the desired compound **96** as a white solid in 67% yield. Purification by recrystallization from ethyl acetate isolated the pure product in a quite low yield (20%) but the reaction could be easily scaled up without loss in yield of crude product. NMR indicated the presence of only the β-anomer of the carbohydrate as the doublet at 4.68 ppm (250 MHz; $CDCl_3$) had a coupling constant *J* 9.8 Hz (the α

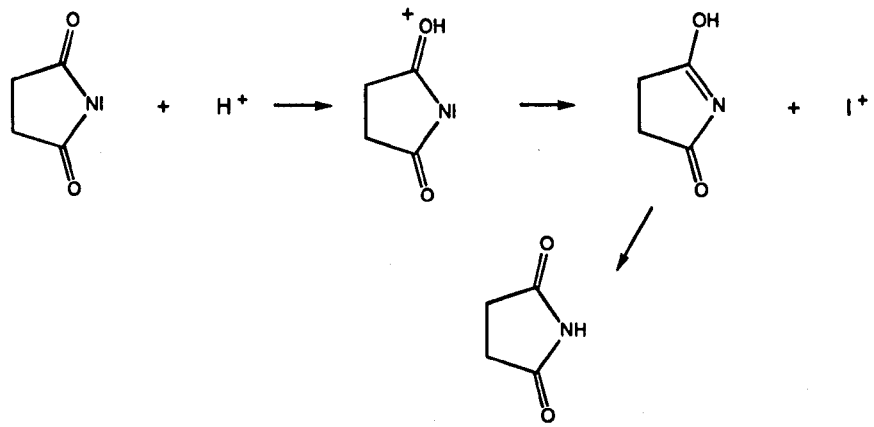
anomer would be expected to have a coupling constant of about 4 Hz). This would be consistent with the group-participation of the acetate ester at carbon-2.

5.3.2.2 Reaction of the Thioether Donors



Scheme 5.9: Glycosidation using thiophenol glucopyranoside as the donor

Initial attempts at glycosidation of the thiophenol glucoside **96** with *trans*-1,4-cyclohexanediol as the linker between the two sugar units was via a halide intermediate (**scheme 5.9**). The source of the halide ion was N-iodosuccinimide (NIS) which releases an iodine cation in the presence of triflic acid (**scheme 5.10**).[112]



Scheme 5.10: Generation of iodinium ions from NIS[112]

The reactions were repeated changing the amount of acid added to generate the iodine cation from the NIS. Too much resulted in a complex mixture of products but too

little only resulted in recovered starting material. The addition of 3 equivalents of triflic acid over a couple of hours at room temperature resulted in a very dark solution, which was found to contain a complex mixture of products. The addition of 0.4 equivalents of triflic acid at room temperature yielded only starting material. However, if the solvent was changed to acetonitrile then the addition of 0.12 equivalents of triflic acid to a solution of 1-thiophenol glucopyranoside **96** at –30°C produced the desired product **91**. Purification by column chromatography afforded the product **91** as a yellow solid in 23% yield although a relatively large amount of starting material was also recovered (33%). Many other compounds were also produced corresponding to mono-substituted product **92**, the addition of extra saccharide units to the mono- and disubstituted products (e.g. **94**, **97**) as well as those compounds having lost acetate groups (**Fig. 5.8** and **5.9**).

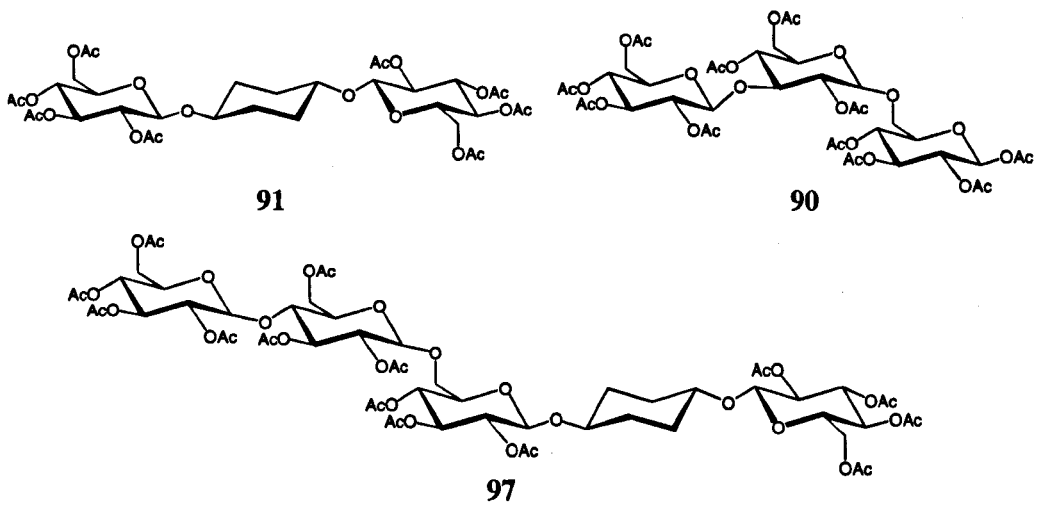


Figure 5.8: Products of the reaction with **95** and cyclohexandiol using triflic acid

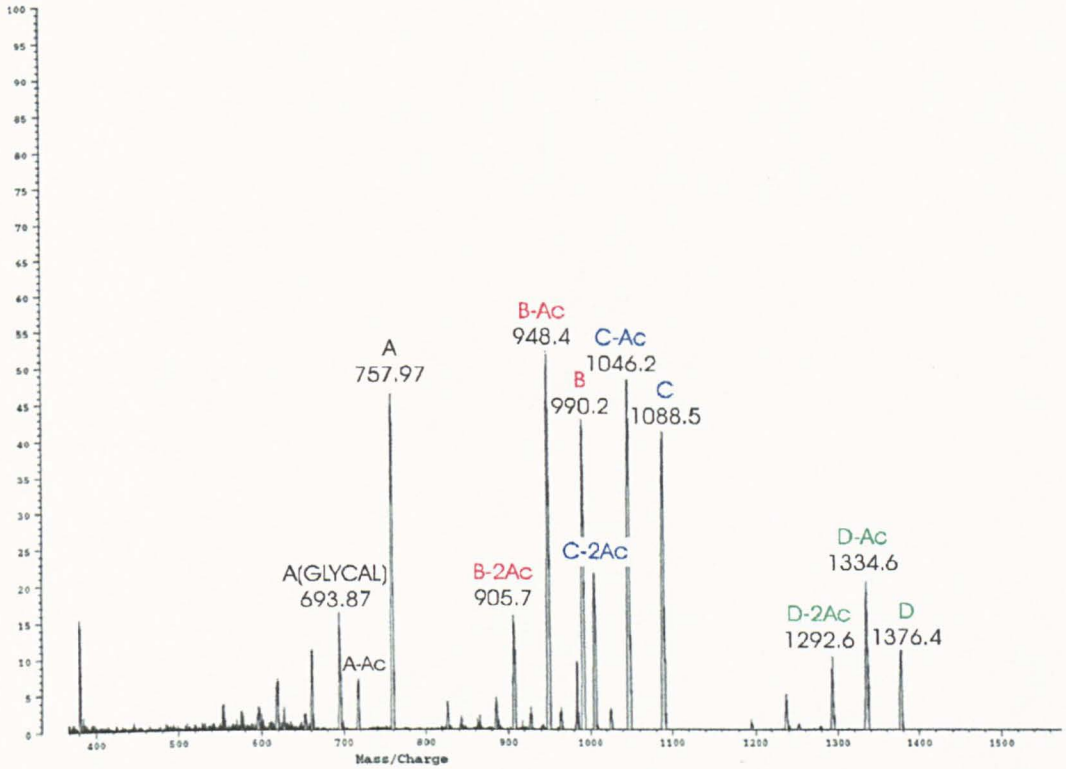
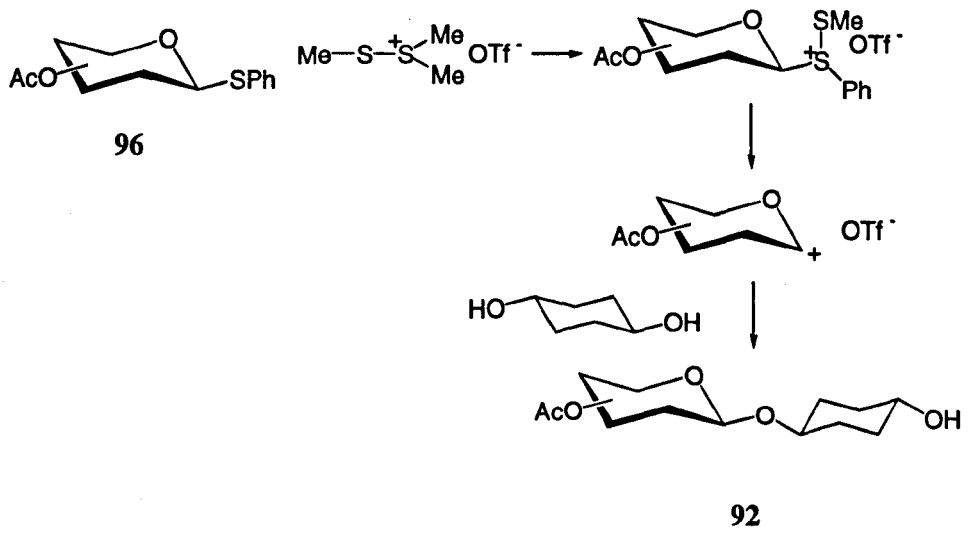


Figure 5.9: MALDI of the reaction between **95** with cyclohexanediol

A: dimer (**91** minus one acetate group); B: trisaccharide (e.g. **90**); C: extra saccharide (e.g. **94**); D: extra disaccharide (e.g. **97**)

Another direct method of activation of thioether glycosides can be achieved by using dimethyl(methylthio)sulfonium triflate (DMTST). It forms a reactive intermediate by alkylsulfenylating the thioglycoside and it is this species, or the decomposition product, that acts as the glycosylation species. DMTST[113] can be easily prepared from methyl triflate and dimethyl disulfide by simply mixing in dichloromethane, cooling and filtering of the crystalline product. It is hygroscopic and if it is to be stored should be done so under dry and cold conditions. In order to check the application of DMTST to the system under investigation 1.2 equivalents of thiophenol glucoside **96** was reacted with 1.0 equivalents of *trans*-1,4-cyclohexanediol in the presence of 5 equivalents of DMTST according to a method used by Fügedi and Garegg.[109, 114] This would hopefully establish

the necessary conditions for this system even though the chance of forming mono-substituted cyclohexane **92** was increased (previous reaction had used 1.0 equivalents of donor to 0.6 equivalents of cyclohexanediol). Freshly prepared DMTST was added to a solution of thiophenol glucopyranoside **96** and *trans*-1,4-cyclohexanediol in dichloromethane (**scheme 5.11**) and after 24 hours the reaction was quenched and the crude product subjected to column chromatography but no desired product (**91** or **92**) was obtained.



96

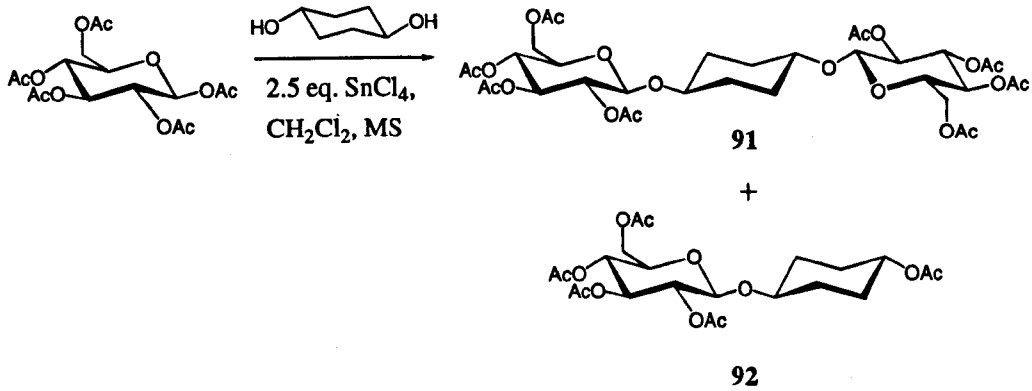
92

Scheme 5.11: Glycosidation promoted by DMTST

5.3.3 Activation of Peracetylated Sugars

While it was possible to obtain some of the desired product **91** using the thioglucoside method the need to prepare the appropriate thioglucoside led to an extra step in the overall synthesis of the CSPs. We therefore investigated whether we could perform glycosidation reactions with the diol acceptor and the peracetylated sugars themselves (which are either commercially available or easily prepared using standard

conditions in high yields without any need for purification). Stannic chloride has frequently been used as a promoter of glycosidation with acetylated sugars.[102] Reaction of glucose pentaacetate and *trans*-1,4-cyclohexanediol was therefore attempted with the aid of tin tetrachloride as the activator and was successful in producing the desired compound **91** (**scheme 5.12**). However purification by chromatography was unable to isolate the disubstituted product **91** from traces of by-products such as analogues with extra saccharide units attached (e.g. **88**) and compounds with acetate groups removed.



Scheme 5.12: Activation by tin tetrachloride on peracetylated sugar

The length of reaction time dictated the extent to which these by-products formed. Two reactions involving identical amounts of glucose pentaacetate, tin tetrachloride and cyclohexanediol in dichloromethane were left for different lengths of time. The results were analysed by MALDI-MS (**Figs 5.10** and **5.11**) and even though this is not a quantitative method of analysis it does show the presence of different products and gives an indication of their different ratios. The longer the reaction time the greater the number of acetate groups that were removed resulting in the formation of oligosaccharides. These oligosaccharides included molecules with a cyclohexane unit with the largest compound detected corresponding to 4 sugar units and one cyclohexanediol **97** (**Fig. 5.13**). The

products from this reaction were also hygroscopic and the extent of formation of the by-products made the separation of the desired disubstituted product **91** near impossible.

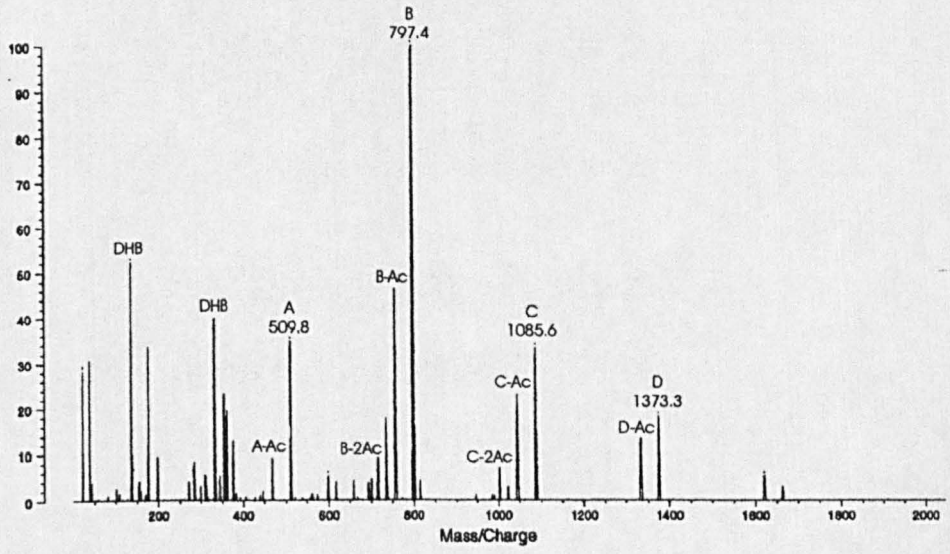


Figure 5.10 MALDI for reaction between glucose pentaacetate and cyclohexanediol, after 22 hours

A: mono-substituted cyclohexane (**92**); B: dimer (**91**); C: extra saccharide (e.g. **94**); D: extra disaccharide (e.g. **97**)

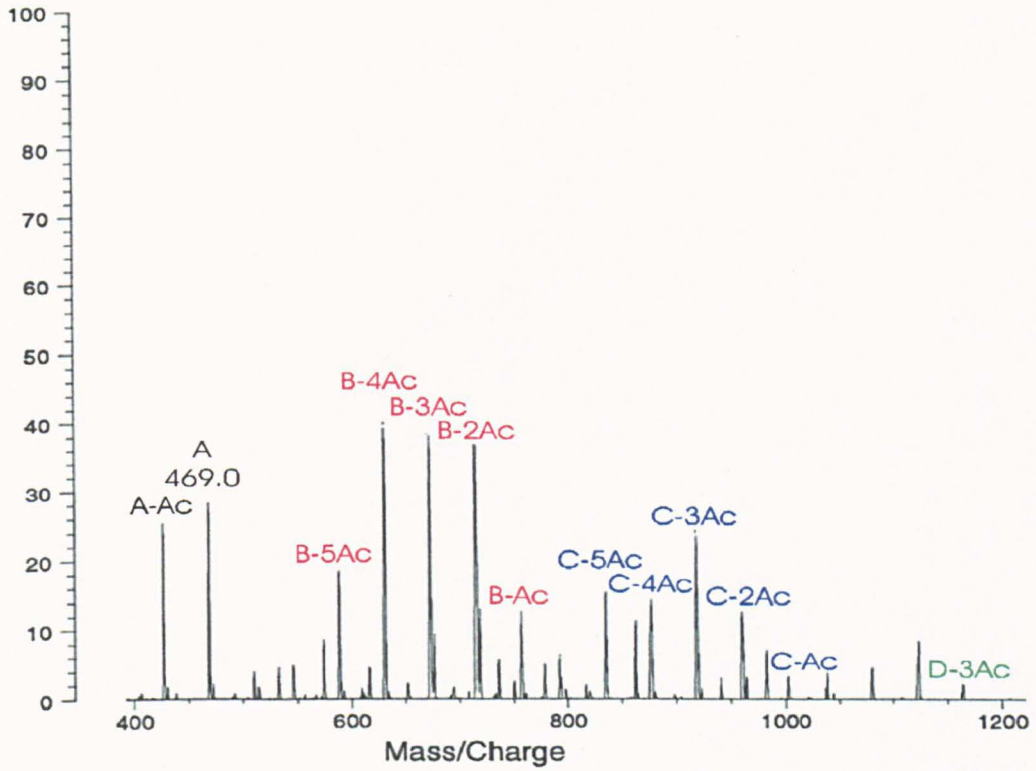
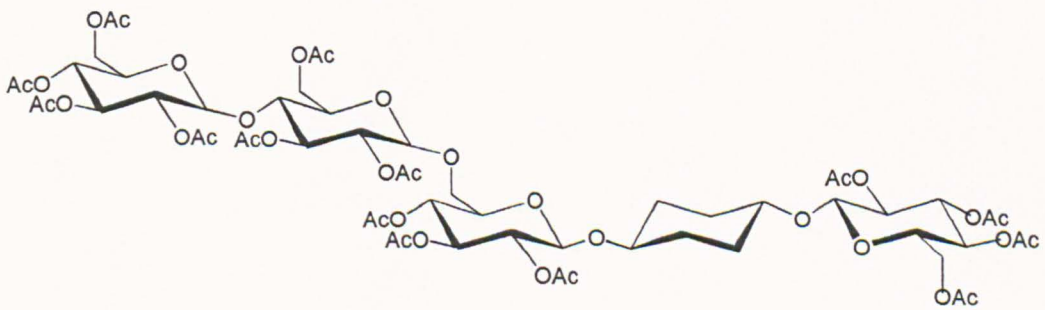


Figure 5.11: MALDI for reaction between glucose pentaacetate and cyclohexanediol, after 5 days

A: mono-substituted cyclohexane (**92**); B: dimer (**91**); C: extra saccharide (e.g. **94**); D: extra disaccharide (e.g. **97**)



97

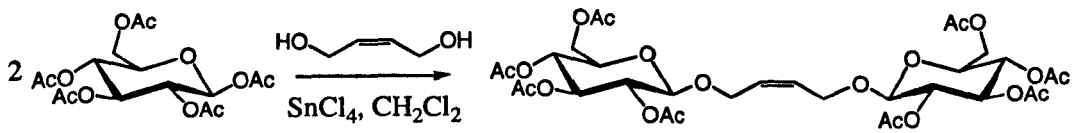
Figure 5.12: Possible structure of substituted cyclohexane with additional disaccharide unit (stereochemistry and regiochemistry not known)

	92		**91**		**94**		**97**	
	MW	found	MW	found	MW	found	MW	found
+Na	511.4		799.7		1088.0		1376.3	
-1Ac	469.4	468.0	757.7	755.7	1046.0	1044.0	1334.3	
-2Ac	427.3	425.8	715.6	713.5	1003.9	1001.9	1292.2	
-3Ac	385.3	383.6	673.6	671.7	961.9	959.0	1250.2	
-4Ac	343.3		631.6	629.9	919.9	917.4	1208.2	
-5Ac	301.2		589.5	588.1	877.8	876.2	1166.1	1163.3
-6Ac			547.5	545.6	835.8	833.8	1124.1	1122.3
-7Ac			505.4		793.7	791.2	1082.0	1079.3
-8Ac			463.4		751.7	748.6	100.0	1042.1

Figure 5.13: Product range after a reaction time of 5 days

An alternative activator was therefore used to try and improve the ratio of the desired product **91** to other products. An alternative activator for the glycosidation reaction between acetylated sugars and alcohols is TMSOTf.[115] Roush *et al*[115] used a variety of activators and glycosyl donors in their strategy to synthesise 2-deoxy-β-glucosides. They showed activation using TMSOTf resulted in the loss of the anomeric acetate and if the alcohol was unhindered then the β-glucoside was obtained with good selectivity. Reaction of glucose pentaacetate and 1,4-cyclohexanediol with TMSOTf in THF however resulted in a complex mixture of products as similarly obtained with tin tetrachloride as the activator. As an excess of reagent was required (up to 2 equivalents)

with secondary alcohols this may explain the failure to produce any disubstituted diol **91** when applied to this system.



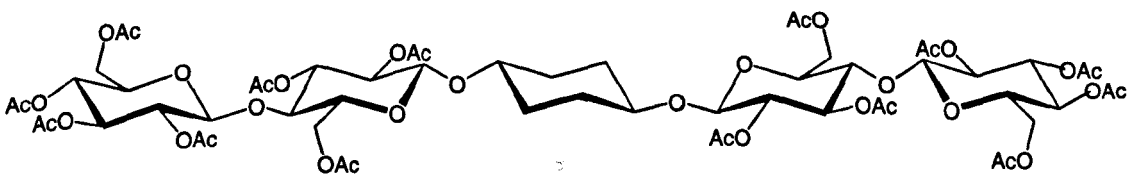
98

Scheme 5.13: Reaction of glucose pentaacetate with *cis*-butenediol

A change in the diol linker also resulted in a similar a shift in reaction products. When glucose pentaacetate was reacted with 2-*cis*-butene-1,4-diol in the presence of tin tetrachloride (**scheme 5.13**) no disubstituted product **98** was observed. Instead a range of oligosaccharides were detected by MALDI-MS corresponding to di- tri- and tetra saccharides, plus the loss of acetates. It was not possible to separate these compounds so neither the site of linkage nor stereochemistry of these oligosaccharides could be established.

5.3.4 Formation of Linked Disaccharides

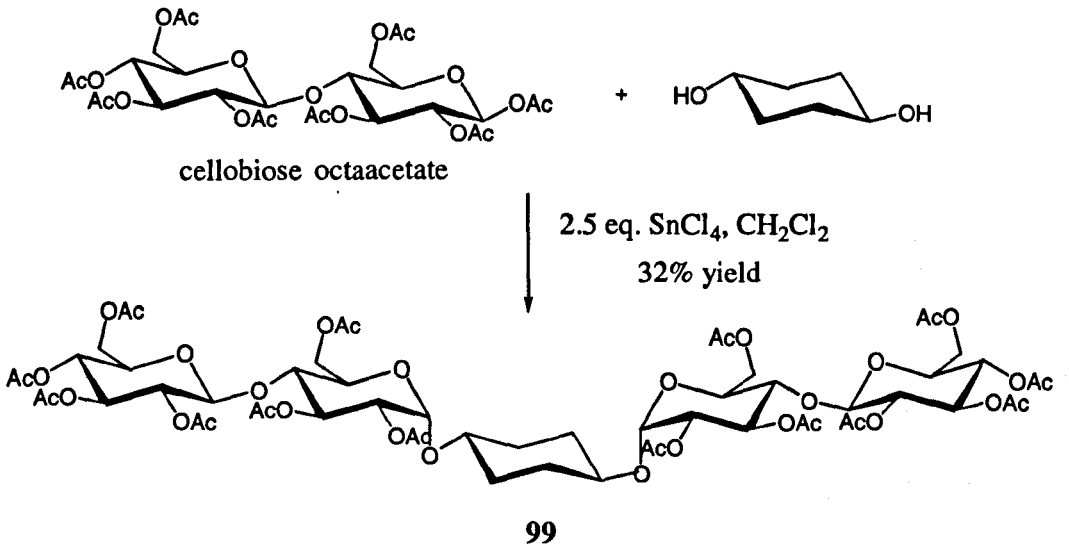
5.3.4.1 Cellobiose Linked with Cyclohexane



99

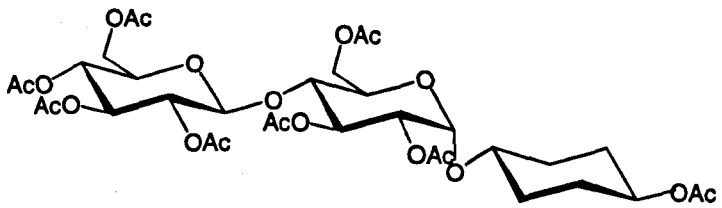
Figure 5.14: Cellobiose with cyclohexane (intended target)

Since all the products from the reaction between glucose and a diol were difficult to separate by chromatography an increase in chain length may make them more distinguishable. Therefore attempts to link sugar units with a diol were continued using the disaccharide cellobiose to produce the dimer **99** (**Fig. 5.14**). Cellobiose was chosen as it consists of β (1,4) linked glucose units, as in cellulose, and has the added advantage of the acetylated compound being commercially available.



Scheme 5.14: Formation of β-linked cellobiose units with cyclohexane

All reactions involving cellobiose octaacetate and trimethylsilyl triflate as the activator resulted in recovered starting material however the desired product **99** was obtained using tin tetrachloride as the activator and *trans*-1,4-cyclohexanediol in 32% isolated yield (**scheme 5.14**).



100 9% yield

Figure 5.15: Mono-substituted cyclohexanediol

In contrast to the glucose products it was possible to separate the products from the cellobiose reaction with cyclohexandiol by column chromatography with samples of mono- (**100**) and di-substituted (**99**) products being obtained (5 g of cellobiose octaacetate gave 4.3 g of crude product which contained 1.6 g of disubstituted product **99** (32%) and 0.9 g of the mono-substituted product **100** (9%)). Elemental analysis of the disubstituted compound **99** was satisfactory and NMR was used to assign stereochemistry. As the protecting groups of the hydroxyl groups are acetates the expected product would be the 1,2-*trans* β-anomer due to participation of the ester at C-2. This would result in a doublet with a large coupling constant (8-10 Hz) appearing in the NMR for H-1 and a triplet for H-2. This was not the case for **99** which had a doublet at 4.70 ppm with a coupling constant of 3.9 Hz and a doublet of doublets at 4.92 ppm with coupling constants of 3.9 and 9.8 Hz, indicating the formation of the α-anomer (**Fig. 5.16**). This is likely to be due to anomerization under the reaction conditions, to form the α-anomer which is more thermodynamically stable. This is a consequence of the length of reaction time required for the desired product to be formed leading to the thermodynamic product. Another contributing factor may be the lability of the acetate groups. As shown previously the acetate groups can readily be lost and if this were to occur at position 2

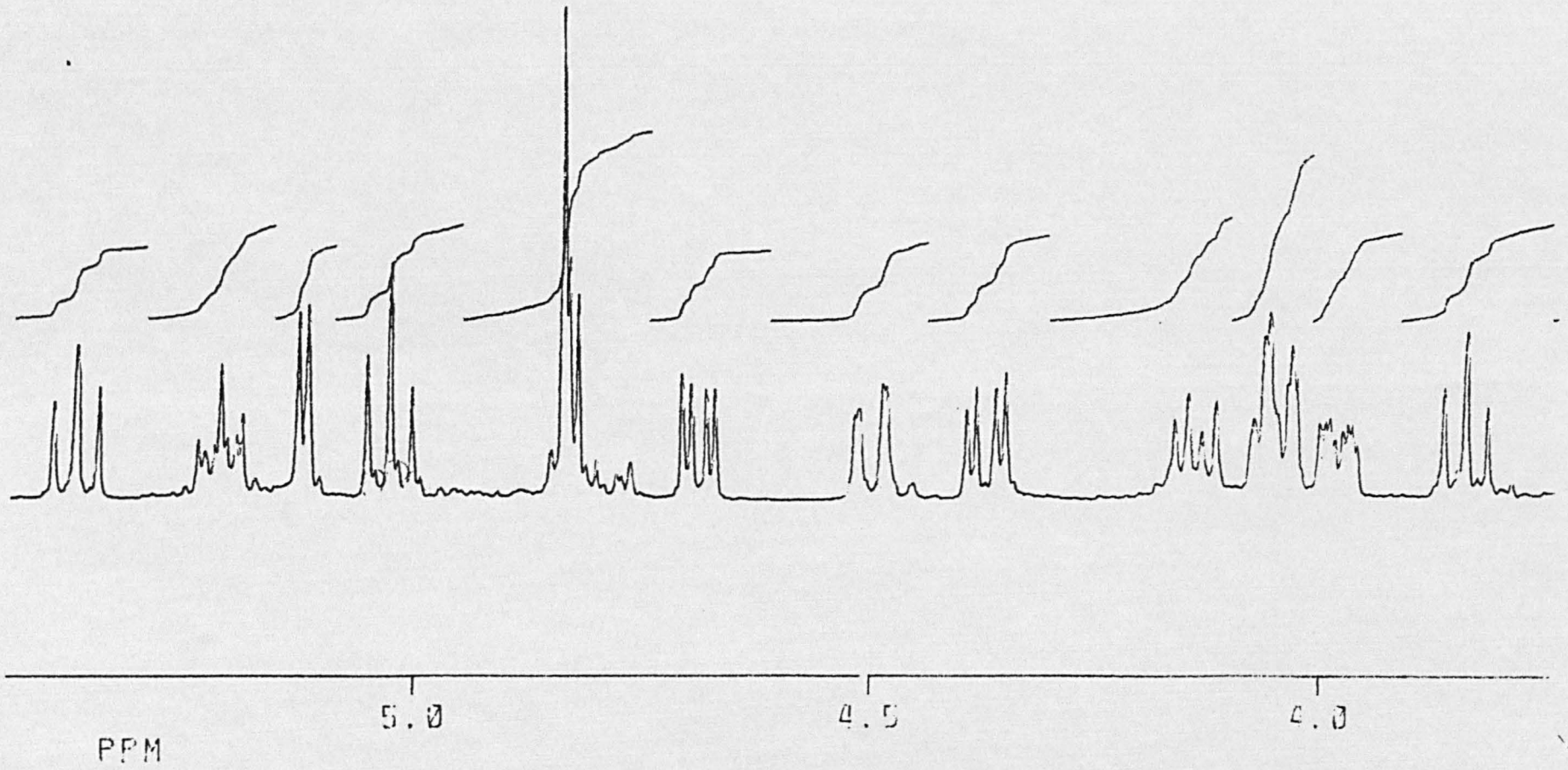
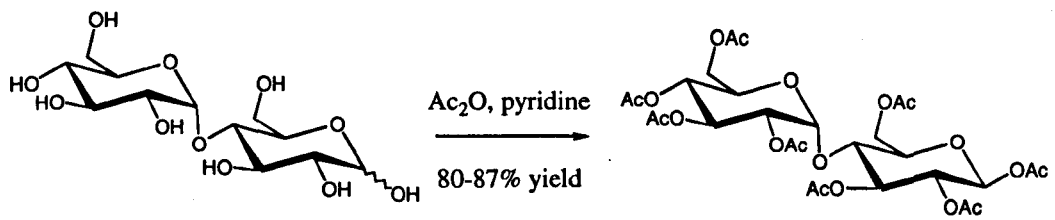


Figure 5.16: NMR for **99** showing the formation of an α-linkage with cyclohexanediol

then there would no longer be any participation in the reaction involving the addition of the alcohol allowing the formation of the α-anomer directly.

An increased amount of activator was added in an attempt to reduce the reaction time so that the β-anomer would be formed and isolated before anomerization. Unfortunately the addition of 6 equivalents of tin tetrachloride had no effect on the reaction and after 1 hour starting material was recovered. This suggested that the reaction is quite slow and if the β-anomer did form the rate of epimerization to the α-anomer is competitive.

5.3.4.2 Maltose linked with Cyclohexane



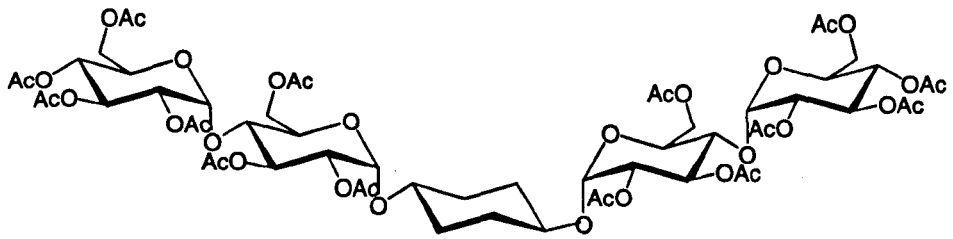
101

Scheme 5.15: Acetylation of Maltose

Having determined that the reaction proceeded to give the α-anomer with cellobiose our attention was turned to the formation of the corresponding product for the α-linked disaccharide maltose so as to maintain a regular linkage in the structure. Maltose octaacetate **101** can be easily prepared from maltose using a 1:1 v/v mixture of acetic anhydride and pyridine in good yields of 80-87% (**scheme 5.15**). The reaction goes to completion, the length of time taken depending on the scale of the reaction (18.5 g took 2

days at room temperature and yielded 30 g (86%) of white solid β-maltose octaacetate **101**).

Ideally to maintain an α-linked polysaccharide the *cis*-1,4-cyclohexanediol would be used to mimic an α-sugar unit but as this was not obtainable pure so the *trans*-1,4-cyclohexanediol was used.

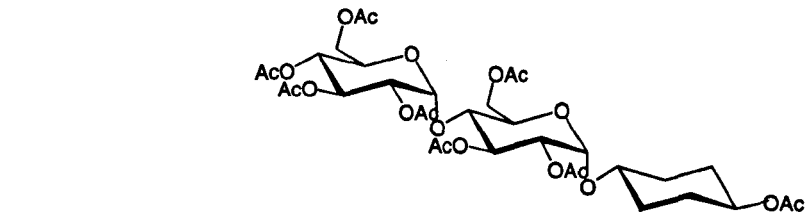


102

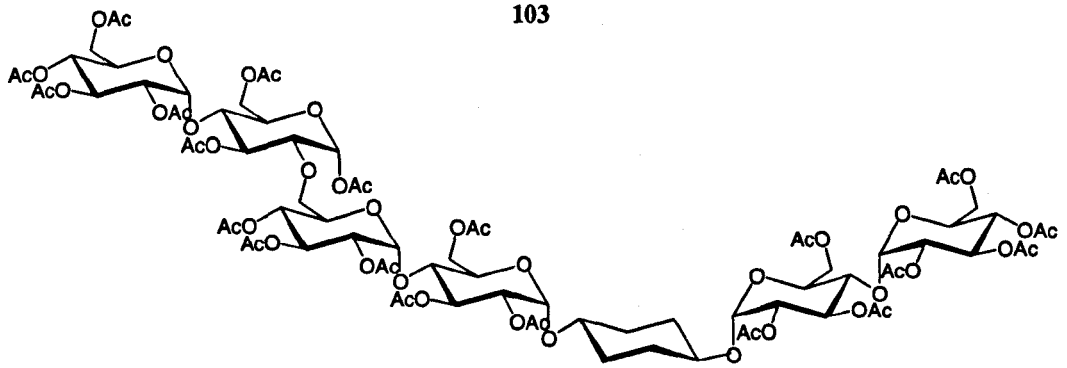
Figure 5.17: Maltose and cyclohexanediol with α-linkage

Coupling of the maltose octaacetate and *trans*-1,4-cyclohexanediol was achieved using tin tetrachloride under anhydrous conditions to give the desired product **102** in 20-30% yield. This reaction could be scaled up (to 10 g) with no loss in yield of the desired disubstituted product **102** (20-28%) although the range of by-products was wider, including the mono-substituted compound **103** and higher analogues such as **104**.

Isolation of the desired compound was a little easier compared with the glucose derivatives however the desired product **102** often contained trace amounts of the mono-substituted compound **103** minus one acetate, both compounds having very similar R_f values. After repeated column chromatography the isolated yield of **102** was quite low (often 10-15%) so **102** was used in the next step of the reaction scheme without further attempts at purification.



103



104

Figure 5.18: Additional products from the reaction of maltose octaacetate with cyclohexane

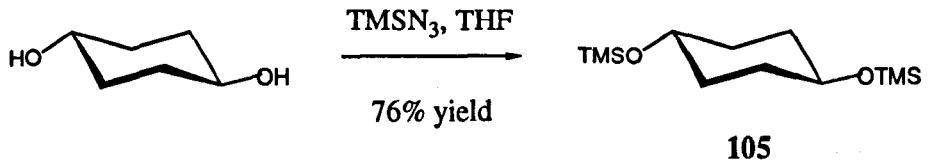
5.3.5 Activation of Acceptor

The reactions between the cyclohexanediol and the various glycosyl donors studied were shown to be quite slow. In addition the detection of products which contain only one sugar unit attached to the linker (such as **103**) with the end of the linker being acetylated suggested that the free hydroxyl groups in the 1,4-cyclohexanediol could be acetylated under the reaction conditions. This would be a major contributing factor to the low yield of the reaction.

Trimethylsilyl ethers are usually used as protecting groups for alcohols and have also been used as glycosyl acceptors in the synthesis of 2-amino-2-deoxy-β-glucopyranosides. A range of activators were studied by Mukaiyama and Matsubara[116, 117] and they found an active species generated from tin tetrachloride and silver triflate gave

glycosides with high β selectivity. We thus speculated that if we used the disilylated 1,4-cyclohexanediol (**105**) we may be able to increase the yield of the desired dimer **99** or **102**.

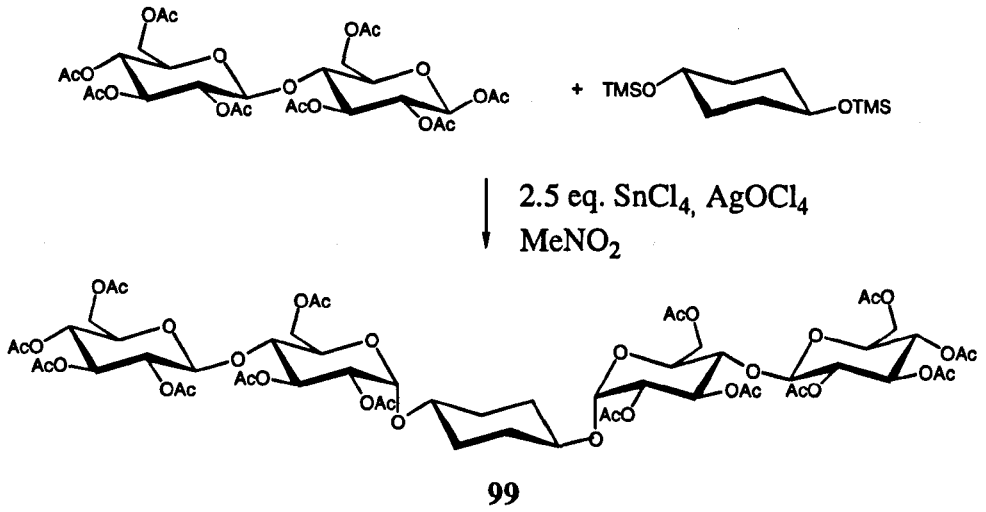
The best method for forming the trimethylsilyl ether of cyclohexanediol **105** was found to be with trimethylsilyl azide (**scheme 5.16**).[118] The use of trimethylsilyl chloride[119] in pyridine or DMF was not highly yielding but reaction of *trans*-1,4-cyclohexanediol with an excess of trimethylsilyl azide in THF furnished the desired disubstituted product **105** after 45 minutes in 76% yield. The white solid produced could be used without purification as NMR and elemental analysis indicated it was pure.



105

Scheme 5.16: Formation of trimethylsilyl protected cyclohexandiol

Mukaiyama and Matsubara found that the solvent used could affect the rate of α/β anomerization with nitromethane furnishing the more thermodynamically stable α products whereas toluene gave predominantly the β-product. In an attempt to make the β-linked product, cellobiose octaacetate was reacted with bis(trimethylsilyl) cyclohexanediol **105** in the presence of an active species produced from tin tetrachloride and silver perchlorate in toluene. The reaction was monitored by TLC but after 2 hours no product (**99**) was detected.



99

Scheme 5.17: Reaction of cellobiose octaacetate with activated cyclohexanediol

Therefore the reaction was repeated using nitromethane as solvent in an effort to obtain the α-linked product (**scheme 5.17**). The oily solid produced contained the desired dimer **99** as well as mono-substituted compound **100** as before with additional compounds with lost acetates also being present. However, this result could not be repeated with maltose octaacetate so this strategy was not continued any further.

5.3.6 Application to Bigger Saccharides

5.3.6.1 Coupling of Maltotriose

106

Figure 5.19: Acetylated maltotriose disubstituted cyclohexane

With the failure to get good results in the coupling of glucose derivatives but with the better success with maltose and cellobiose derivatives we next investigated the use of higher sugars in the coupling reaction. This would also allow us to investigate the effect of increasing the helical nature of the saccharide chain. Acetylated maltotriose was easily attainable by acetylation of the commercially available hydroxylated trisaccharide in high yield (85-92%) and this was then coupled with *trans*-1,4-cyclohexanediol using tin (IV) chloride in dichloromethane to successfully produce the disubstituted product **106** (**Fig. 5.20**). The yield was overall a little lower than with maltose octaacetate (23% average isolated yield), although the relative amount of mono-substituted product **107** was slightly higher in comparison (16% yield, ratio of mono:di for maltotriose 1:1.4 compared to maltose mono:di ratio was 1:1.7). As the availability of higher oligosaccharides are not readily commercially available at reasonable prices another source of such longer chains sugars was necessary.

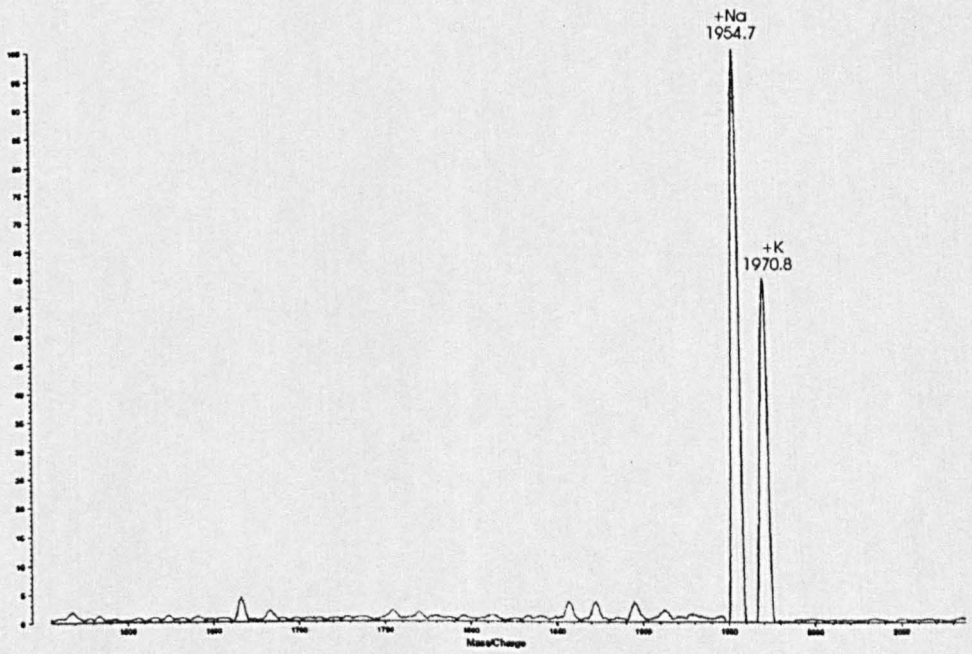
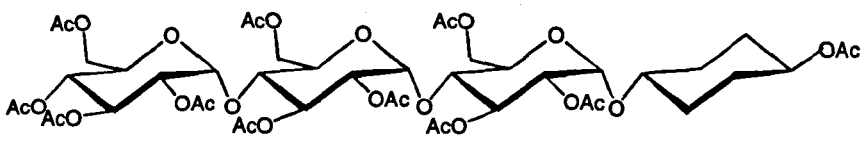


Figure 5.20: MALDI for the coupling of maltotriose with cyclohexanediol to form **106**

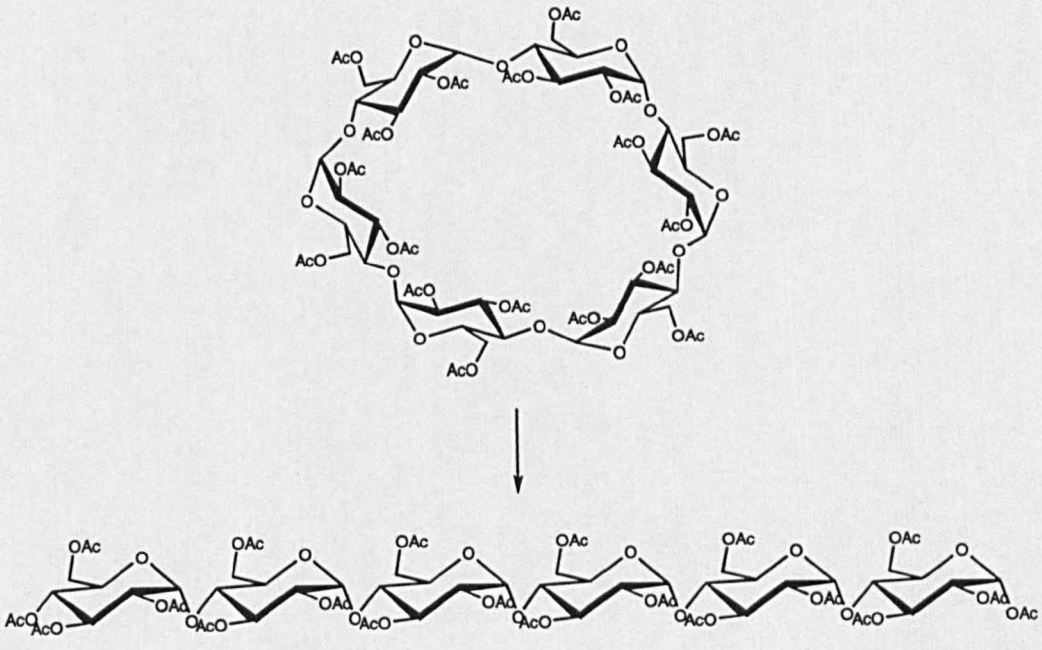


107

Figure 5.21: Maltotriose mono-substituted cyclohexanediol

5.3.6.2 Application to Synthesis of Larger Oligosaccharide Dimers (Maltohexaose)

Larger oligosaccharides are commercially available however at extremely high prices. Consequently we turned our attention to the synthesis of other oligosaccharides by other means such as the preparation of maltohexaose by the cleavage of α-cyclodextrin. Fellow colleagues had been working on the selective cleavage by hydrolysis of cyclodextrins and if the method could be controlled then discrete oligosaccharides would be produced. The acetylated α-cyclodextrin was dissolved in acetic anhydride, stirred at 0°C and perchloric acid (70%) was added dropwise under nitrogen (**scheme 5.18**). Extreme care had to be taken with the work up however, as the addition of sodium hydrogen carbonate resulted in an exothermic reaction. If the base was added too quickly the heat generated accelerated the cleavage of the glycosidic bonds resulting in the crude reaction mixture containing sugar units from 2 up to the desired 6 units (**Fig. 5.22**). When the sodium hydrogen carbonate was added dropwise while stirring after the reaction had been left for 2 hours there was still starting material though the major product was the maltohexaose **108**. Among the other products included the loss of acetates which made isolation of the desired hexaose difficult.



108

Scheme 5.18: Cleavage of acetylated α-cylodextrin to yield acetylated maltohexaose

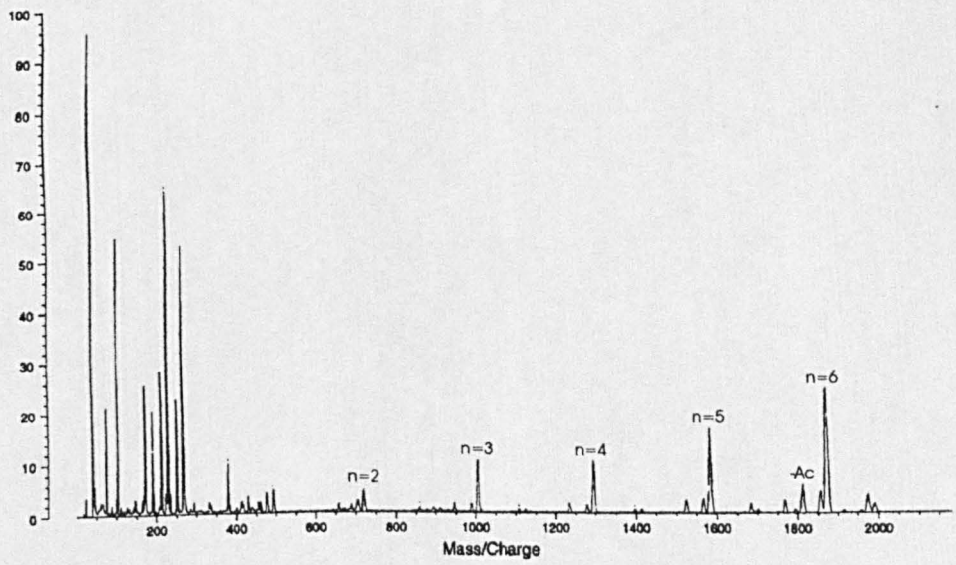
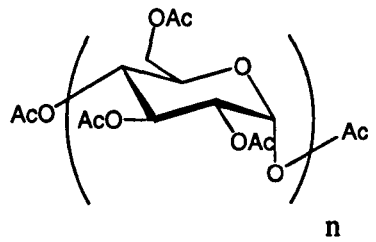


Figure 5.22: MALDI for the cleavage of α-cyclodextrin using perchloric acid (where n = number of glucose units in the chain, for **108** n = 6)



n = 6 maltohexaose **108**
n = 5 pentamaltose
n = 4 tetramaltose
n = 3 maltotriose
n = 2 maltose

Figure 5.23: Cleavage of α-cyclodextrin resulting in saccharides of different sizes

An alternative to perchloric acid is the use of sulphuric acid in acetic anhydride which was a preferable method[120] as even though the reaction was heated to 50°C there was little loss of acetate groups (**Fig. 5.24**). Column chromatography was used to isolate the desired hexaose **108** in 24% yield from the remaining starting material with only a trace of maltopentaose. If the reaction time was increased to 32 hours (from 20 hours) then the amount of remaining starting material decreased but the number of shorter chain oligosaccharides increased. The desired hexaose **108** could still be separated although in only 17% yield.

The MALDI spectra shown in **Fig. 5.22** and **5.24** show the effect of the different acids on the hydrolysis reaction. With perchloric acid the cleavage to give penta- (n = 5), tetra- (n = 4), tri- (n = 3) and disaccharides (n = 2) can easily be seen whereas with sulphuric acid the crude reaction mixture can be seen to be much cleaner to yield maltohexaose **108**.

With the acetylated maltohexaose **108** prepared attention was turned to its coupling. However, using similar conditions to those already described with *trans*-1,4-cyclohexanediol (i.e. tin (IV) chloride in dichloromethane) led to no desired product.

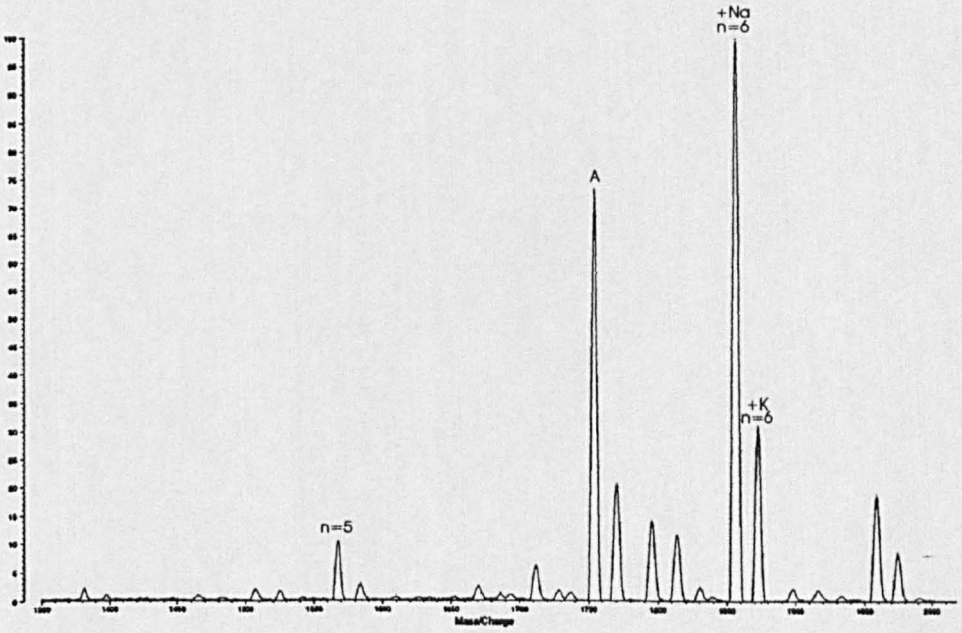


Figure 5.24: MALDI for the cleavage of α-cyclodextrin using sulphuric acid (where n = number of glucose units in the chain, for **108** n = 6) A: α-cyclodextrin

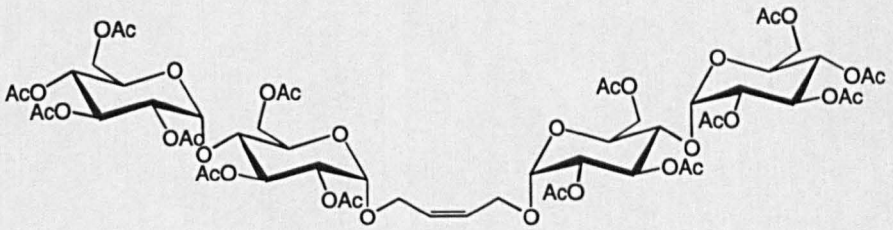
5.3.7 Investigation of Different Linkers

To investigate the effect of the non-saccharide linker on the secondary structure of the macromolecules and consequently their enantioselectivity the cyclohexane linker was replaced by 2-*cis*-butene and 2-butyne linkers.

5.3.7.1 Coupling of Maltose with Butenediol and Butynediol

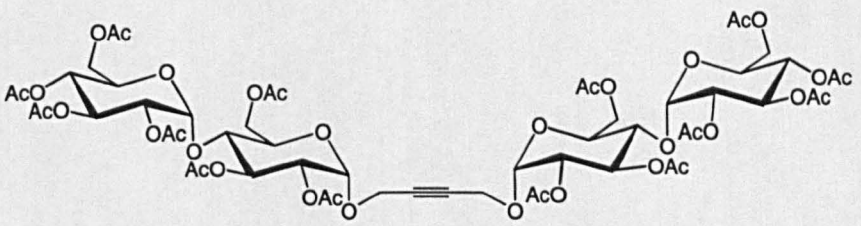
The coupling between maltose octaacetate and 2-butene-1,4-diol was carried out under the same conditions as with cyclohexanediol using tin (IV) chloride in dichloromethane and the desired **109** was produced in 83% crude yield (isolated yield after chromatography 9-42%). The range of products obtained was analogous with those obtained with cyclohexanediol and maltose octaacetate, including the mono- and di-

substituted (**109**) linked products and molecules with additional saccharide units as well as those missing acetate groups (**Fig. 5.27**). The coupling between maltose octaacetate and the 2-butyne-1,4-diol was carried out under the same conditions and yielded **110** in 55% yield. The range of products again included the mono- and di-substituted linker (**110**), molecules with additional saccharide units and all of those with different numbers of missing acetate groups. The reaction time was reduced and this seemed to have the consequence of reducing the loss of acetate groups from the molecules although separation of the mono-substituted linker was still difficult to remove from the desired di-substituted linker **110**.



109 83% yield

Figure 5.25: Acetylated maltose with butene linker



110 55% yield

Figure 5.26: Coupled maltose and butyne diol

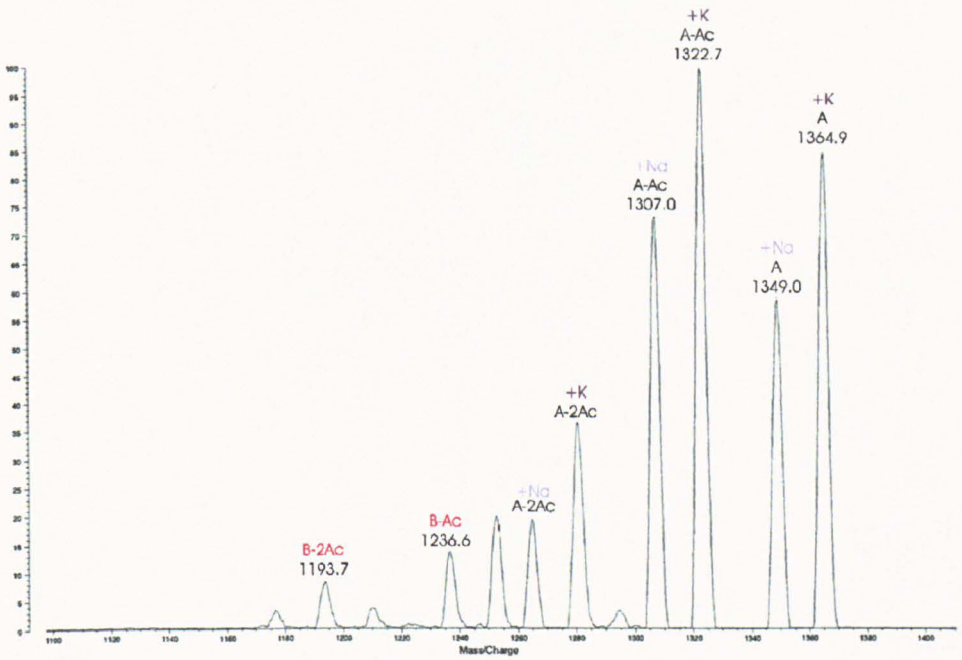
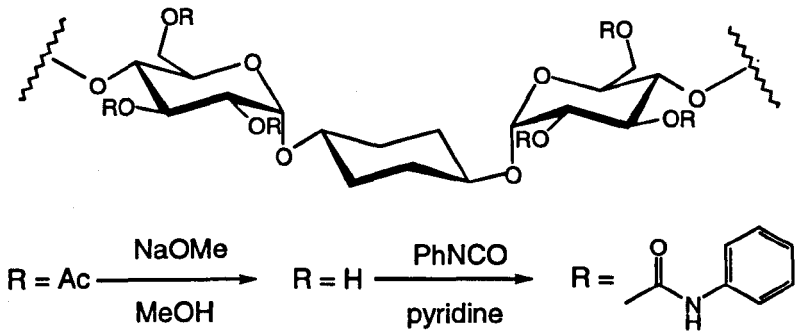


Figure 5.27: MALDI for the reaction between maltose octaacetate and butene, after chromatography, A: dimer (**109**); B: tetrasaccharide

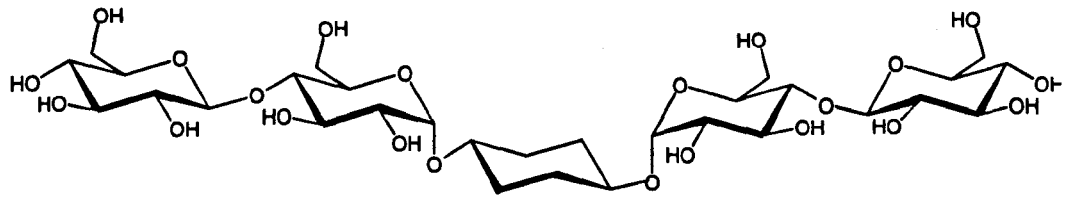
5.3.8 Overall Scheme for the Formation of Phenyl Carbamate Derivatives

With a range of functionalised peracetylated sugar dimers in hand attention was turned to producing different derivatives of these compounds. As previously mentioned most successful carbohydrate CSPs are phenyl carbamate derivatives and we thus set about preparing these. In addition, the presence of many carbamate functional groups would lead to molecules which should have a reduced solubility in the solvents used in chromatography. Firstly the acetate protecting groups needed to be removed from the compounds **99**, **102**, **106**, **109** and **110** and then the free hydroxyl groups would be reacted with phenyl isocyanate forming the carbamate functional groups (**scheme 5.19**).



Scheme 5.19: Functionalization of the disaccharide linked by cyclohexane to form phenyl carbamate derivative

5.3.8.1 Removal of the Acetate Protecting Groups



111 100% yield

Figure 5.28: Hydroxylated derivative of cellobiose with cyclohexane

The acetate protecting groups of all the sugar dimers produced were easily removed using sodium methoxide in methanol with a work-up involving the ion exchange resin Dowex or Amberlyst[15]. A typical procedure is illustrated by the deacetylation of the cellobiose/cyclohexane derivative **99**. The acetates were removed from cellobiose/cyclohexane **99** by stirring with $^{14}/_{15}$ equivalents of sodium methoxide ($^{1}/_{15}$ equivalents per acetate group) in methanol at room temperature and produced the hydroxylated compound **111** in quantitative yield. The hydroxylated compound was analysed by mass spectrometry and infra-red (IR) spectroscopy. IR was used to determine

the complete removal of the acetate groups by an absence of the characteristic absorbance around 1700 cm^{-1} and the appearance of a broad absorbance by the hydroxyl groups at 3400 cm^{-1}. While the NMR spectra was complicated due to all of the protons having similar shifts it did show the removal of all the acetate groups, by an absence of any singlet signals at 2.05 ppm. Elemental analysis was not conclusive as compound **111** was slightly hygroscopic but MALDI-MS and FT-ICR were able to identify the correct molecular weight. The accurate mass electrospray FT-ICR spectrum for **111** is shown in **Fig. 5.29**.

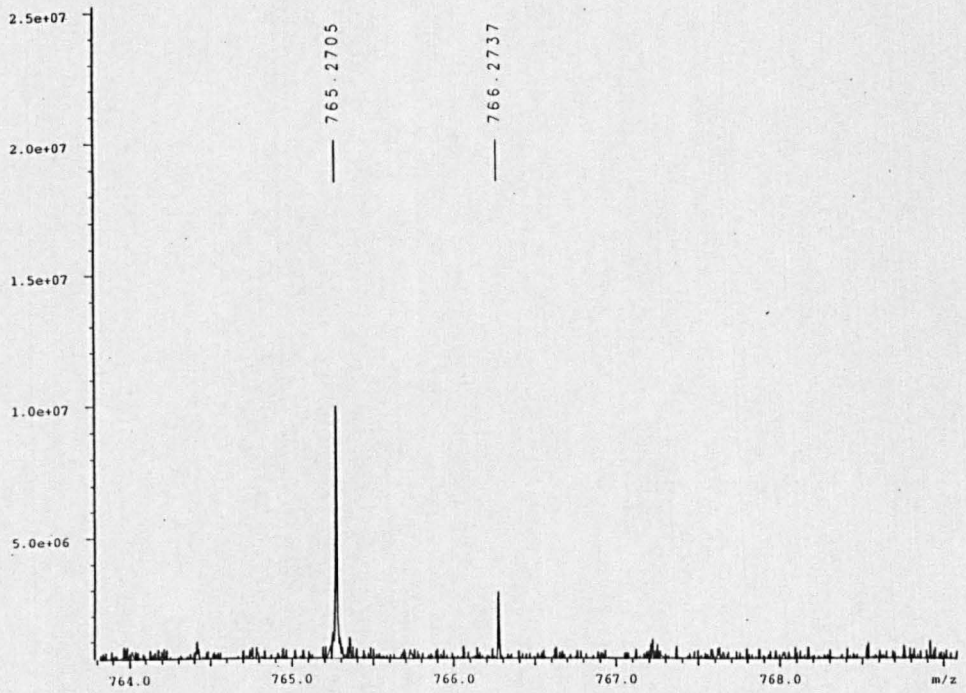


Figure 5.29: FT-ICR spectrum for compound **111** (requires 765.3028)

Saccharide Unit	Linker	Acetylated Starting Material	Product Number	Reaction time (hours)	Yield
Cellobiose	Cyclohexane	**99**	**111**	4	0.28 g (100%)
Maltose	Cyclohexane	**102**	**112**	46	0.45 g (99%)
Maltose	Cyclohexane	**103**	**113**	24	0.45 g (99%)
Maltotriose	Cyclohexane	**106**	**114**	22	0.30 g (99%)
Maltotriose	Cyclohexane	**107**	**115**	24	0.23 g (71%)
Maltose	Butene	**109**	**116**	96	0.24 g (100%)
Maltose	Butyne	**110**	**117**	168	0.48 g (85%)

Figure 5.30: Table of yields of hydroxylated products obtained from reaction of the acetylated compounds with sodium methoxide in methanol

The acetates were also removed from the compounds **102, 103, 106, 107, 109** and **110** in the same way, with sodium methoxide in methanol (**Fig. 5.30**), and were also analysed using IR and MALDI-MS or FT-ICR.

5.3.8.2 Reaction with Phenyl Isocyanate and Saccharide Mimics

The functionalization to form the phenyl carbamate derivatives was achieved by reaction of the hydroxylated derivatives **111-117** with phenyl isocyanate in anhydrous pyridine heated to 50°C. Analysis of the products as determined by MALDI-MS indicated that temperature and concentration were important in controlling the extent of the functionalization. An increase in temperature resulted in an increased degree of

functionalization. Again the chemistry will be illustrated using the reaction of the cellobiose/cyclohexane derivative **111** with phenyl isocyanate but similar conditions were applied to the other macromolecules (**Fig. 5.31**). When the reaction between the cellobiose/cyclohexane **111** and phenyl isocyanate in pyridine (concentration of 0.034 mol/dm^3) was left at room temperature for 24 hours the number of phenyl carbamate groups added was between 6-12 (**Fig. 5.32**). Increasing the temperature and concentration of the reaction to 50-55°C and 0.042 mol/dm^3 resulted in a more highly functionalised product (n = 11-14 carbamate groups, **Fig. 5.33**). The temperature was kept under 90°C however to prevent side reactions such as the formation of allophanates (**Fig. 5.34**). An increase in concentration generally reduced the reaction time, although for the maltotriose derivative **114** the reaction times were longer than for the maltose derivatives **112** and **113**.

Starting Material	Product number	reaction time (hours)	concentration (mol/dm^3)	yield (%)	n/o PhNHCO groups product
111	**118**	48	0.033	62	6-12
		24	0.049	58	11-14
112	**119**	20	0.042	96	12-14
113	**120**	45	0.088	98	7-8
114	**121**	50	0.022	96	20
116	**122**	36	0.066	97	14
117	**123**	48	0.035	92	12-14

Figure 5.31: Formation of the phenyl carbamate derivatives of linked saccharides

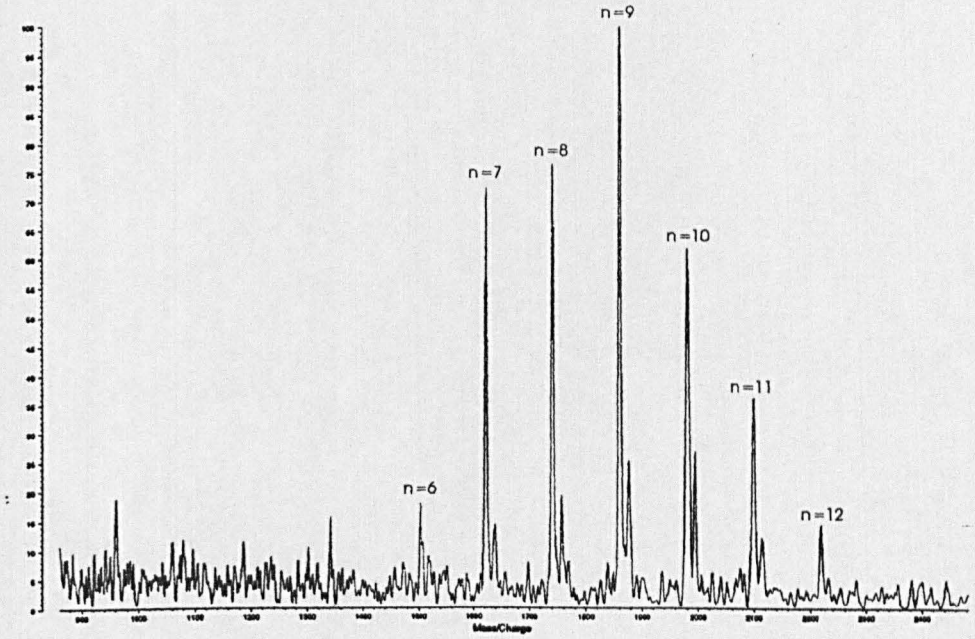


Figure 5.32: MALDI for the reaction of **111** with phenyl isocyanate at room temperature, where n = number of phenyl carbamate groups

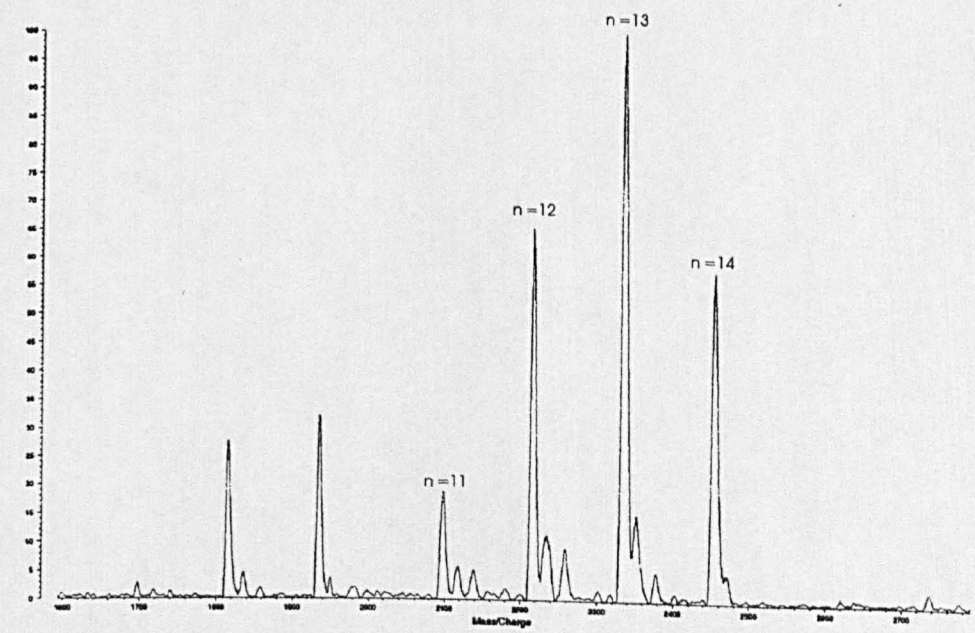


Figure 5.33: MALDI for the reaction of **111** with phenyl isocyanate at 55°C, where n = number of phenyl carbamate groups

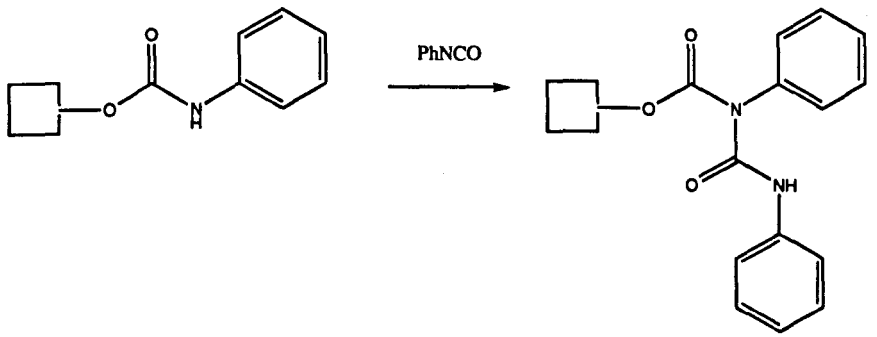
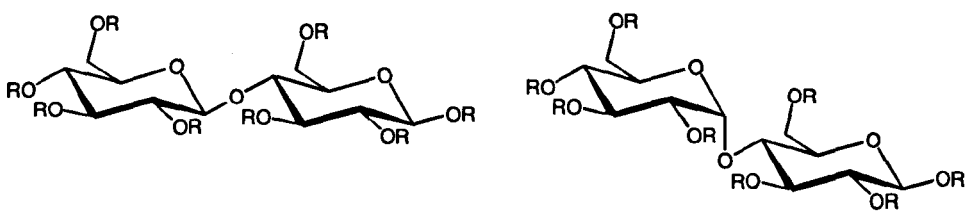


Figure 5.34: Possible formation of allophanates at higher reaction temperatures

5.3.8.3 Reaction with Phenyl Isocyanate and Natural Saccharides

The phenyl carbamate derivatives of the natural saccharides cellobiose, maltose and maltotriose were also prepared so that their secondary structure and enantioselectivity could be compared to the dimers just produced. The fully functionalised derivative (n = 8) of cellobiose **124** was obtained in 46% yield by heating at 50-60°C with phenyl isocyanate in pyridine for 4 days.



124 46% yield

R = CONHPh

125 95% yield

Figure 5.35: Formation of phenyl carbamate derivatives of cellobiose and maltose

In an attempt to reduce the reaction time with maltose and phenyl isocyanate a catalyst was employed. The initial reaction between maltose and phenyl carbamate with dibutyl tin dilaurate (1 mol%) in pyridine was heated at 40-45°C and resulted in the

addition of 4-6 phenyl carbamate groups in the molecule **125**. However, when the reaction was repeated in rigorously dry conditions all 8 hydroxyl groups reacted with isocyanate to form phenyl carbamate groups in only an hour **125** (although the MALDI spectrum (**Fig. 5.36**) also indicated the presence of molecules with 7 phenyl carbamate groups). Maltotriose was also successfully derivatised with phenyl isocyanate in pyridine to form the trisaccharide **126** with 9, 10 and 11 phenyl carbamate groups.

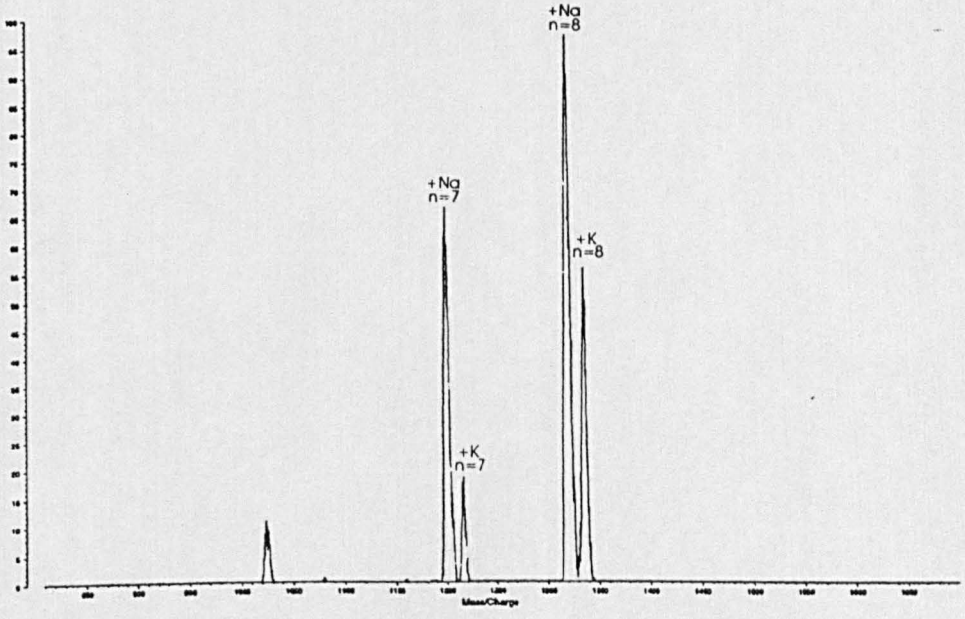
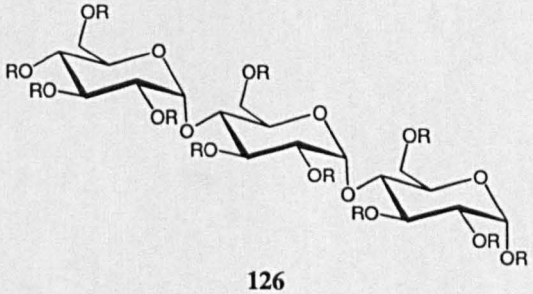


Figure 5.36: MALDI for the reaction between maltose and phenyl isocyanate after 1 hour, where n = number of phenyl carbamate groups



126

Figure 5.37: Phenyl carbamate functionalised maltotriose. R = CONHPh

5.3.8.4 Purification of the Phenyl Carbamate Derivatives

The intention was to synthesise molecules that were not soluble in solvents used as eluents in HPLC therefore purification was always going to be difficult. In general the compounds prepared were soluble in THF, pyridine and DMSO although partially soluble in acetone. Attempts at column chromatography therefore had to use acetone in the MP and even though the desired product was usually obtained after column chromatography the recovered yields were very low, as the compound was likely to be retained on the column. Therefore some of the compounds were used crude if attempted purification was not successful.

5.4 Conclusions

A range of polysaccharide mimics have been successfully prepared that can now be investigated as CSPs in HPLC. The range includes molecules with a range of different saccharide units (cellobiose, maltose and maltotriose), non-saccharide linkers (*trans*-1,4-cyclohexandiol, 1,4-*cis*-2-butene diol and 1,4-butyne diol) and different functional groups (acetate esters and phenyl carbamate groups).

Many techniques were utilised in their synthesis with the most successful being the use of the activator tin tetrachloride with the peracetylated sugars. There were problems with all of the syntheses caused by the loss of acetate groups and acetylation of the diol linker. An additional problem was the insolubility of the final phenyl carbamate derivatives that hindered attempts at their purification.

Further analysis of these novel CSPs was undertaken by coating them onto solid supports and their enantioselectivity tested by HPLC (chapter 6). Their secondary structure was also investigated by using CD spectroscopy (chapter 7).

CHAPTER 6

SACCHARIDE MACROMOLECULES AS STATIONARY PHASES: HPLC TESTS FOR ENANTIOSELECTIVITY

6.1 Introduction

We chose to evaluate the range of novel CSPs prepared in chapter 5 by coating them onto a solid support and packing into HPLC columns. Their enantioselectivity could then be investigated by testing with a range of analytes. The compounds chosen to be tested for enantioselectivity on an analytical scale included an example of an acetate derivative and the phenyl carbamate derivatives of the linked saccharides as well as one example of a natural saccharide.

6.2 Preparation of the Stationary Phase

Acetylated cellobiose/cyclohexane **99** was coated onto PGC at a 20 and 25% loading using the batch method (as described in section 2.2.2) using THF as the solvent. The cellobiose/cyclohexane phenyl carbamate **118**, maltose/cyclohexane phenyl carbamate **119** and maltotriose/cyclohexane phenyl carbamate **121** were coated onto PGC also using the batch method using THF. A loading weight of 20% was initially chosen as a starting point for these HPLC tests as the optimum loading weight for PGC nor for these phases was known. Also in order to be able to compare our new phases with a natural saccharide the phenyl carbamate derivatised maltotriose **126** was coated onto PGC. These stationary phases were then slurry packed into stainless steel HPLC columns

(4.6 mm diameter) and tested with a range of analytes, the structures of which are shown in appendix 1.

Compound	Elemental analysis			%w/w loading[a]	Column number
	%C	%H	%N		
99	92.18	0.97	-	16	**127**
99	89.94	1.37	-	22	**128**
118	94.25	1.07	1.35	17	**129**
119	92.70	1.04	1.84	21	**130**
119	93.01	1.05	0.91	18	**131**
121	91.47	1.29	0.83	22	**132**
126	93.71	0.98	0.92	17	**133**

Figure 6.1: Loading of the PGC for columns **127-133**

a) as determined by elemental analysis

6.3 Results of HPLC tests for Enantioselectivity

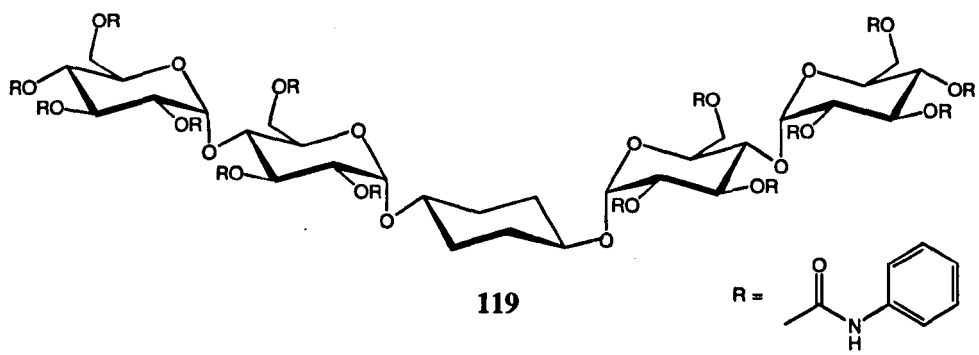
6.3.1 Acetylated Maltose Derivatives

Columns **127** and **128** containing the peracetylated derivatives of cellobiose/cyclohexane showed no chiral selectivity with any of the racemates tested under normal phase conditions. Column **128** did show a very high pressure that was observed with all of the racemates. This resulted in the flow rate being reduced so that the pressure could be maintained below 4000 psi. The main difference between columns **127**

and **128** was the % w/w loading of the PGC. Column **128** had a higher w/w of 22% which may be the reason for the high pressure shown and give an indication of the maximum weighting of coating for PGC with this phase.

6.3.2 Phenyl Carbamate Cellobiose and Maltose Derivatives

Column **129** (containing the phenyl carbamate derivative of cellobiose/cyclohexane **118**) showed some chiral selectivity with 2,2,2-trifluoro-1-(-anthryl)-ethanol **S**, with capacity factor, K', of 11.7 and a selectivity, α, of 1.13, although the peaks were broad while column **130** (containing the phenyl carbamate derivative of maltose/cyclohexane **119**) showed better chiral selectivity under normal phase conditions when tested with 10 analytes (**A, C, G, L, M, P–T**, structures given in appendix 1). Signs of chiral selectivity were seen for five out of these ten racemates (**Fig. 6.3**). The effect of loading of this more successful phase was then briefly examined.



119

Figure 6.2: Phenyl carbamate derivative of maltose/cyclohexane

Racemate		Column **130**		Column **131**	
		K'	α	K'	α
MK801	**A**	2.97	1.12	2.48	1.23
CP 55940	**B**	-	-	13.6	1.08
SCH50911	**C**	2.04		-	-
Napthoxylactic Acid	**F**	-	-	10.08	1.22
Propranolol Glycol	**G**	14.90	1.35	16.32	1.30
Mandelic Acid	**L**	4.72		0.15	
Benzoin	**M**	2.93	1.12	1.99	1.11
trans-Stilbene Oxide	**P**	1.16	1.08	2.62	
Oxprenolol	**Q**	4.77	1.07	2.87	
Flavanone	**R**	5.17		3.97	1.12
Trifluoro Anthryl Ethanol	**S**	8.80		4.67	
Homatropine	**T**	5.05		4.20	
Pindolol	**U**	-	-	5.44	
Propranolol	**V**	-	-	5.51	1.20
Methyl propranolol	**W**	-	-	6.32	1.18
Pronethalol	**X**	-	-	4.01	

Figure 6.3: Chiral selectivity for columns **130** and **131,** maltose and cyclohexane phenyl carbamate derivatives. Eluent: hexane/isopropanol in normal phase conditions

K' capacity factor of first eluting peak; α selectivity; (-) not tested

Column **131** contains the same CSP, **119**, as column **130** coated onto PGC but with a slightly lower % w/w loading of 18%. This column was tested with a slightly different range of analytes (**A, B, F, G, L–X**) (**Fig. 6.3**). While column **130** was packed using hexane/isopropanol 70:30 as solvent column **131** was packed using a different mixture of 99:1 hexane/isopropanol. As the eluent used to test each racemate was changed to improve any separation that was observed care needs to be taken when comparing K'.

There is an improvement in enantioselectivity for the CSPs derived from the phenyl carbamate derivatives of maltose/cyclohexane although no definite conclusions can be made to relate the structure of the racemate to the chiral selectivity shown by this CSP. Separation of the neutral molecules CP 55940 (**B**), propranolol glycol (**G**) (**Fig. 6.4**), benzoin (**M**) and flavanone (**R**) were all achieved with the only neutral molecule not separable being 2,2,2-trifluoro-1-(9-anthryl) ethanol (**S**). There was little difference between the ability of the two columns **130** and **131** to separate acidic or basic molecules although almost baseline separation was obtained with MK801 (**A**) with column **131** (**Fig. 6.4**). The number of functional groups capable of hydrogen bonding in the analyte may be of importance to selectivity. Of the 4 molecules with 2 or more hydroxyl groups 3 were separated. Of the 10 racemates with 2 or more H-donating functional groups (OH and NH) 5 were separated. In addition π–π stacking interactions may also be important. Of the 10 racemates with 2 aromatic rings included in their structure 7 were separated. Four of the five racemates with a naphthalene moiety showed signs of separation though the only molecule with an anthracene functionality was not resolvable. The inclusion of an naphthalene moiety in the analyte also seems to have the effect of increasing the retention time on the column, especially for napthoxylactic acid (**F**) (**Fig. 6.4**) and

propranolol glycol (**J**) which were run in high polarity solvents (90/10 v/v hexane/isopropanol).

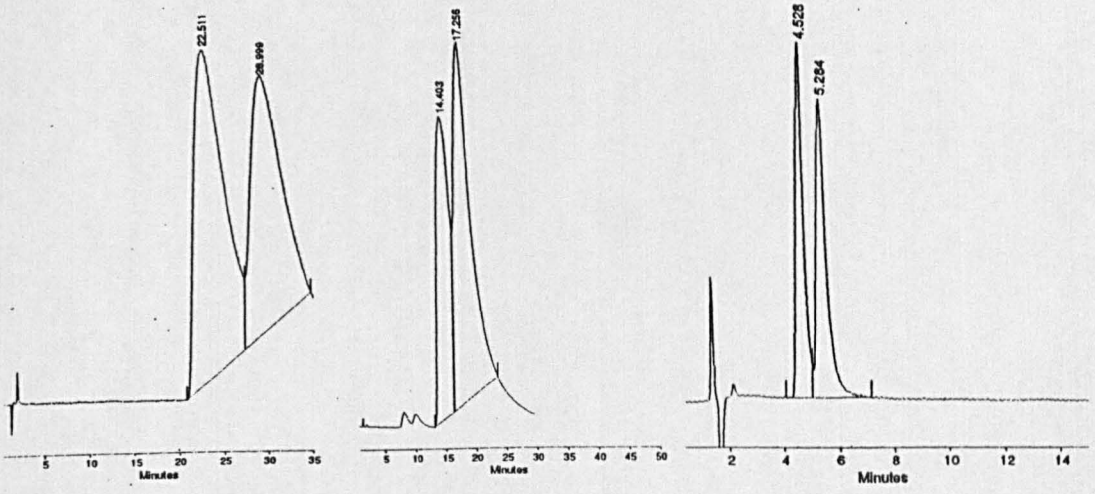


Figure 6.4: HPLC data for three analytes separated on column **131** using hexane/isopropanol (v/v) at flow rate of 1 mL/min: i) propranolol glycol (**G**) 90/10; ii) napthoxylactic acid (**F**) 90/10; iii) MK 801 (**A**) 98/2

In general these results were encouraging showing that for the maltose/cyclohexane phase **119** chiral selectivity was possible with a range of analytes. Retention of the larger aromatic molecules may suggest that non-chiral interactions are occurring with the PGC as well as chiral interactions with the coated phase. This would result in an increase in the retention of the compound on the column with loss of any chiral selectivity obtained by the chiral phase. The decrease in % w/w loading of the CSP did not seem to affect the enantioselectivity achieved by columns **130** and **131** in the HPLC tests.

6.3.3 Phenyl Carbamate Maltotriose Derivatives

Having shown that the maltose derivatives were useful as CSPs we next examined the effect of introducing extra carbohydrates to the CSP hence we investigated those derived from maltotriose/cyclohexane **121** and the natural sugar maltotriose **126** as a comparison. Columns **132** (containing **121** coated onto PGC) and **133** (containing **126** coated onto PGC) were tested with a range of racemates (**A, B, F, G, L–X**) under normal phase conditions (**Fig. 6.6**).

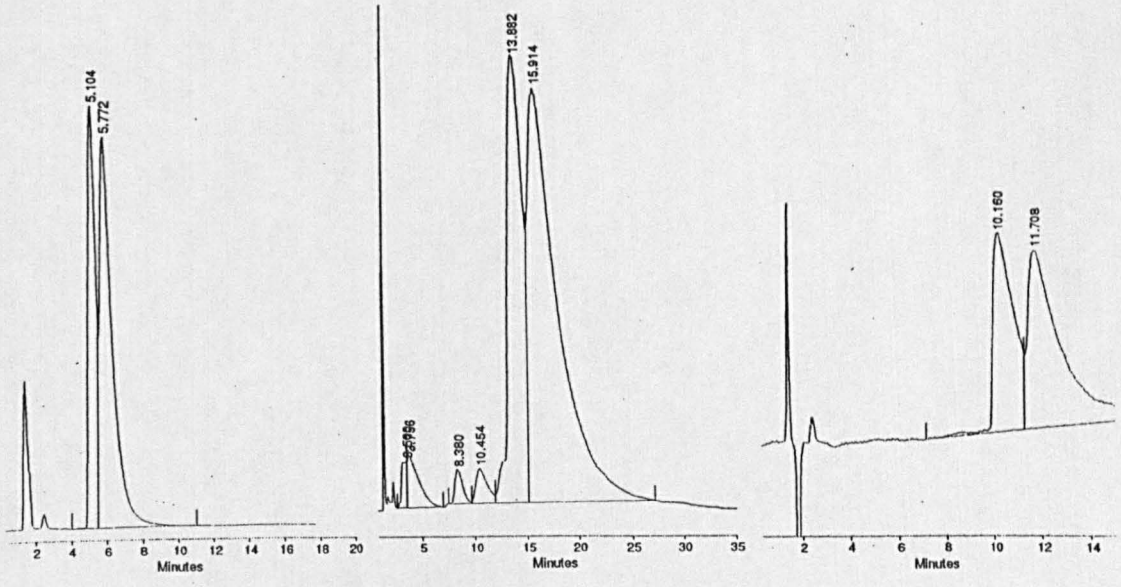


Figure 6.5: HPLC data for the separation of three analytes on column **132** using hexane/isopropanol (v/v) at flow rate of 1 mL/min: i) benzoin (**M**) 95/5; ii) CP 55,940 (**B**) 95/5; iii) homatropine (**T**) 98/2

Racemate		Column **132**		Column **133**	
		K'	α	K'	α
MK801	**A**	3.87	1.15	2.18	
CP 55940	**B**	9.68	1.26	9.69	
Napthoxylactic Acid	**F**	5.66		12.59	1.18
Propranolol Glycol	**G**	14.75	1.18	12.92	1.42
Mandelic Acid	**L**	0.40		0.1	
Benzoin	**M**	2.93	1.17	1.63	1.13
trans-Stilbene Oxide	**P**	0.97		0.99	
Oxprenolol	**Q**	4.13		4.11	
Flavanone	**R**	4.76		3.44	1.15
Trifluoro Anthryl Ethanol	**S**	5.92		5.34	
Homatropine	**T**	6.82	1.17	3.47	
Pindolol	**U**	4.67		3.83	
Propranolol	**V**	5.75		6.33	1.2
Methyl propranolol	**W**	5.36		7.78	1.23
Pronethalol	**X**	4.53		5.49	

Figure 6.6: Chiral selectivity for columns **132** and **133** showing the comparison of two maltotriose units linked with cyclohexane (**121**) with maltotriose (**126**)

Column **132** (containing maltotriose/cyclohexane **121**) showed chiral selectivity with 5 of the racemates tested (**Fig. 6.6**) although compared to the maltose/cyclohexane column **131** (**Fig. 6.3**) this column is slightly less efficient. Also the α values for **132** are

generally lower than for **131**, taking in to account differences in capacity factor. (A smaller capacity factor is likely to mean that the solvent is more polar and if the compound is on the column for less time the difference between enantiomers is going to be less). This may be attributed to the higher loading of the PGC for **132** which at 22% is making the column less efficient. However, the separation of homatropine (**T**) with the trisaccharide linked phase **121** (**Fig. 6.5**) was possible which had not been seen on any of the other phases which may suggest a different retention mechanism for this CSP. This column also produced the best resolution of benzoin (**M**) and CP 55,940 (**B**) (**Fig. 6.5**).

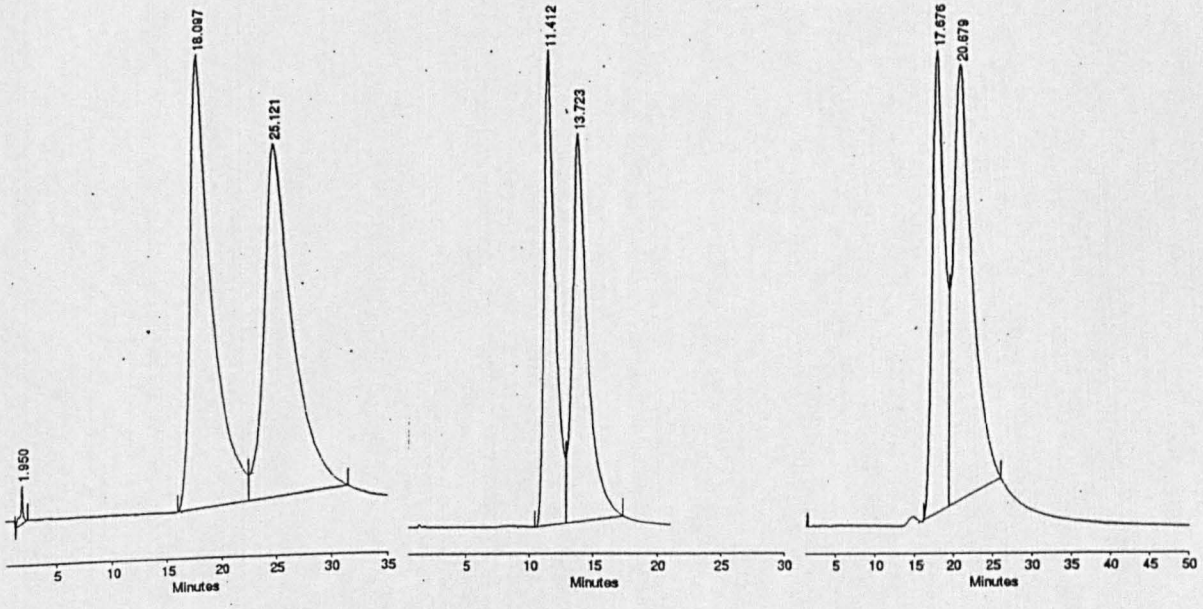


Figure 6.7: HPLC data for the separation of three analytes on column **133** using hexane/ispropanol (v/v) at a flow rate of 1 mL/min: i) propranolol glycol (**G**) 90/10; ii) methylpropranolol (**W**) 80/20; iii) naphthoxylactic acid (**F**) 90/10

We then compared the novel dimeric phases to that produced from maltotriose phenyl carbamate **126**. In general columns **131** (containing maltose/cyclohexane **119**) and **133** (containing maltotriose **126**) showed similar chiral selectivities for the racemates

tested with the K' and α values for naphthoxylactic acid (**F**), propanolol glycol (**G**), benzoin (**M**), flavanone (**R**), propranolol (**V**) and 4-methylpropranolol (**W**) being comparable (**Fig. 6.7**). Column **133** however did not show any selectivity between the enantiomers of CP 55940 (**B**) nor MK801 (**A**), which were separated on column **131** and **132**. The separation on these dimeric phases (column **131** and **132**) again suggesting different possible retention mechanisms. The major similarity between the dimeric phase in column **131** and column **133** (containing maltotriose) is the ability to separate molecules that contain 1,2-hydrogen bond donating groups attached to a naphthalene moiety by an ether linkage (**F, G, V** and **W**). The hydrogen donating groups are either hydroxyl groups or amines and are present in 4 of the 6 racemates that showed chiral selectivity with both columns **131** and **133** (**Fig. 6.8**).

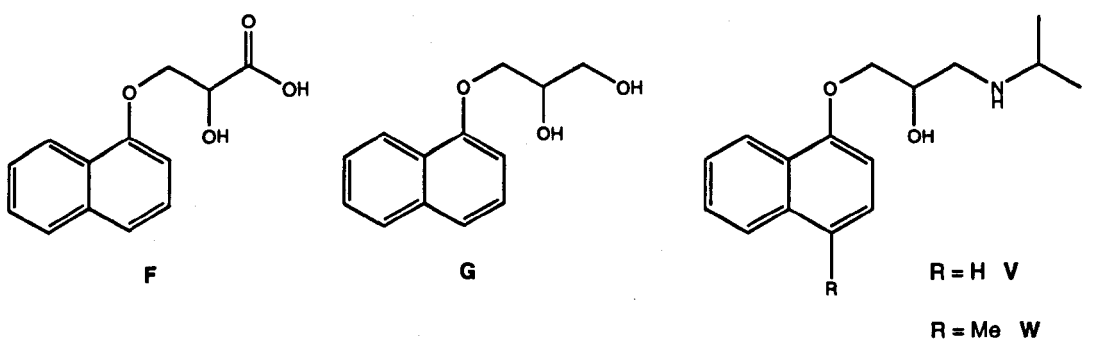


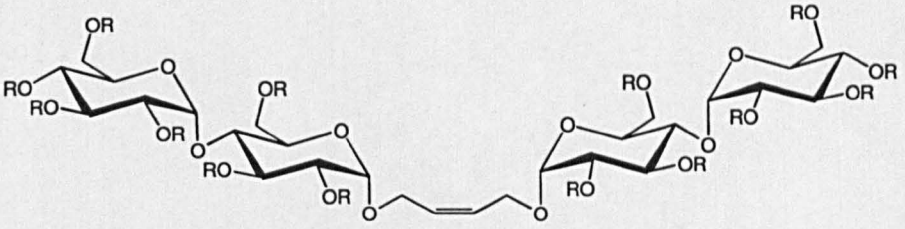
Figure 6.8: Racemates that showed chiral selectivity with column **133**

6.4 Leaching of CSP from the Surface of the Solid Support

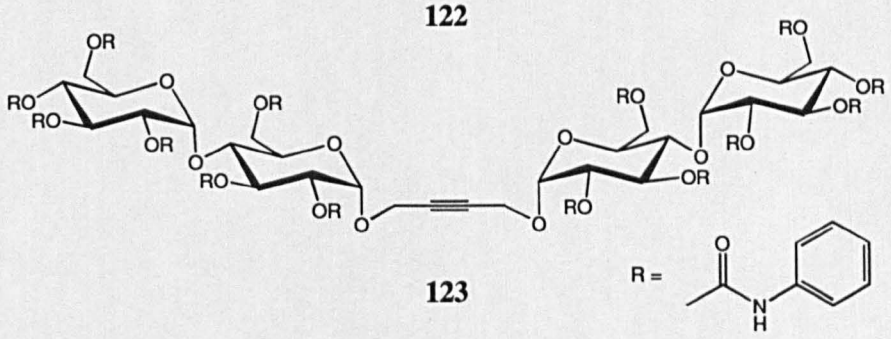
It was noticed through out the testing of the columns **131, 132** and **133** the chiral phase component was constantly being leached from the columns observed as a white precipitate that formed in the collection flask and there was also a high background UV

absorbance. This indicated that the chiral phases were not staying on the surface of the PGC causing contamination of the separated enantiomers resulting in increased UV absorbance observed by the detector. This means that the solubility profile these phases on PGC was not acceptable. We thus examined the effect of the solid support by changing from PGC to silica.

Therefore the saccharide mimics containing maltose linked with cyclohexane **119**, butene **122** and butyne **123** linkers were coated onto silica. The results for the maltose/cyclohexane phenyl carbamate derivative **119** on silica could then also be compared to the results obtained on PGC (**Fig. 6.3**).



122



123

Figure 6.9: Phenyl carbamate derivatives of maltose linked with *cis*-butene and butyne linkers

6.4.1 Silica as a Solid Support

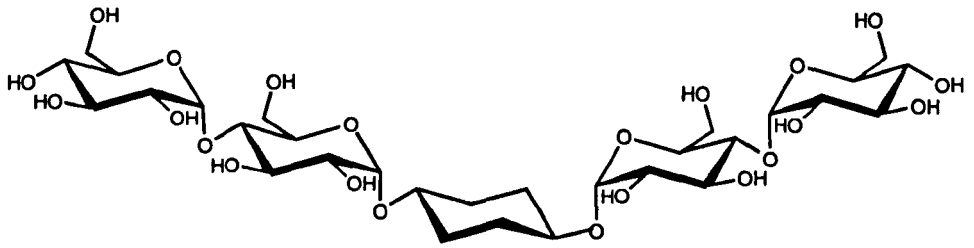
Compound	%C	%H	%N	% w/w loading	Column number
119	11.50	1.27	1.30	16	**134**
122	13.75	1.41	1.82	22	**135**
123	13.24	1.43	1.73	22	**136**

Figure 6.10: Loading percentages of the CSPs coated onto silica for columns **134-136**

The coated samples of silica were packed into HPLC columns using 1% isopropanol in hexane. Solvent (90/10 hexane/isopropanol) was then pumped through the columns for three hours at 0.8 ml/min to determine if the phases would be leached off the silica solid support using conventional chromatographic conditions. The UV absorbancy of the eluent passing through the column reaching the detector was off the scale during the evaluation and showed no signs of dropping. It was assumed the chiral component was being leached from the surface of the silica and any further testing of the columns was inappropriate.

In an effort to prevent the loss of the CSP from the surface of the silica we chose to examine a method of covalently attaching the CSP to the surface of the solid support. This is not the ideal situation as involves extra steps in the preparation of the CSP but would hopefully allow investigation of the phases without them being washed from the surface.

6.4.2 Attachment of the CSP to Silica



112

Figure 6.11: Underivatised maltose/cyclohexane compound

The method chosen to attach the saccharide mimics to the surface of the silica followed the preparation of a CSP in which amylose was attached to silica using a diisocyanate.[121] The free hydroxylated derivative maltose/cyclohexane **112** was coated onto aminopropylated silica (APS) using the batch method (section 2.2.2) with a mixed solvent of 1:1 THF:MeOH. This solid was then suspended in toluene containing methylene bis(phenyl isocyanate) and pyridine. After heating at 90-100°C for 5 hours phenyl isocyanate was added to functionalise the remaining hydroxyl groups of the saccharide mimic. After heating for a further 24 hours at 90-100°C the solid was filtered off and washed with THF to remove any unattached CSP. A schematic representation of this approach is shown in **Fig. 6.12.**

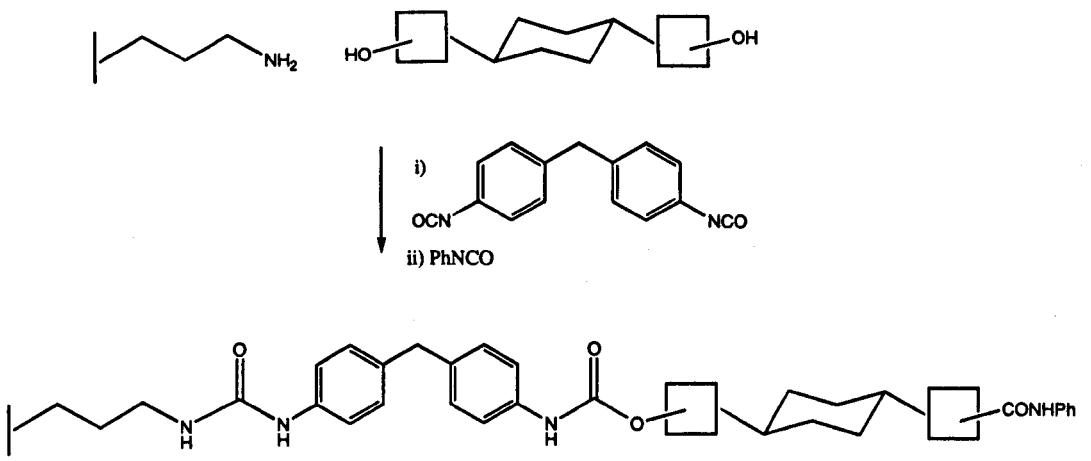


Figure 6.12: Attachment of maltose/cyclohexane **112** to APS silica and derivatisation to form phenyl carbamate CSP

The solid produced was packed into a stainless steel HPLC column using 1% isopropanol in hexane. The column (**137**) was then washed through with hexane/ isopropanol (90/10) and a stable baseline for the UV absorbance was achieved. The column was then tested with 15 racemates (**A, B, F, G, L, M, P-X**) using normal phase solvent conditions but disappointingly showed no signs of chiral selectivity with any of the analytes.

6.5 Conclusions

Of the phases tested for enantioselectivity by HPLC most showed some signs of chiral selectivity. Acetate derivatives were shown not to be suitable for the CSPs in this system. The best CSPs were shown to be the phenyl carbamate derivatives in particular the phase derived from 2 maltose saccharide units linked with a cyclohexane **119**, being capable of separating 53% of the analytes tested. Promising results were also seen with

the maltotriose linked with cyclohexane **121** separating 33% of the analytes and maltotriose **126** which separated 40% of the analytes tested.

The importance of the number of hydrogen-bond donating groups present in the analyte was highlighted by the selection of analytes that were separated. However, to increase the understanding of the relationship between the structure of the analyte and enantioselective interactions with the CSP more analytes would have to be investigated.

The difference in enantioselectivity of the cellobiose and maltose macromolecules shows that there is a relationship between the secondary structure and enantioselectivity, resulting in different modes of enantioseparation. Unfortunately the effect of the non-saccharide linker could but be investigated as the phases linking maltose with butene and butyne linkers were washed from the surface of the solid support. However, the secondary structure of the maltose units with butene and butyne linkers were still analysed and compared with those obtained for the other saccharides and saccharide mimics as discussed in the next chapter.

PGC was therefore shown to be a superior solid support for these phases shown by the instability of the stationary phase consisting of the macromolecules coated onto silica.

CHAPTER 7

SACCHARIDE MACROMOLECULES AS STATIONARY PHASES: THEIR ANALYSIS BY CIRCULAR DICHROISM SPECTROSCOPY

7.1 Investigation of Secondary Structure by Circular Dichroism and Ultra-Violet Spectroscopy

In order to gain some information about the secondary structure of our novel CSPs we examined them by circular dichroism (CD) spectroscopy which also required analysis by ultra-violet (UV) spectroscopy.

7.2 Background to Circular Dichroism Spectroscopy

CD spectroscopy is a form of light absorption spectroscopy that measures the difference of absorption of right- and left-circularly polarized light by a substance. It is sensitive to the secondary structure of macromolecules such as polypeptides, proteins and polysaccharides. CD occurs within a normal absorption band and requires either an inherently asymmetric chromophore or a symmetric one in an asymmetric environment.

CD data can be analysed either qualitatively or quantitatively. The method used in this discussion involves comparison of spectra of molecules of known structures with the spectra for the new systems synthesised in this work as well as comparisons between the spectra of new systems. Absolute structural content for our systems is difficult to determine as standards for carbohydrates of known structure are difficult to obtain.

7.3 UV and CD spectroscopy of Amylose and Cellulose

The molecules synthesised for this work were designed to mimic the oligosaccharides cellulose and amylose, therefore the UV and CD spectra of cellulose tris(phenyl carbamate) (CPC) and amylose tris(phenyl carbamate) (APC) were collected as standards for analysis. The CD spectra for CPC and APC show different shaped spectra with different intensities of the negative and positive peaks. CPC shows a small negative peak at 245 nm and a large peak at 214 nm where as APC has a negative peak at 242 nm and a slightly smaller positive peak at 226 nm. X-ray analysis by Zugenmaier[84] has shown the secondary structures of APC and CPC are both helical, although of different sizes (with respect to the number of units per turn).

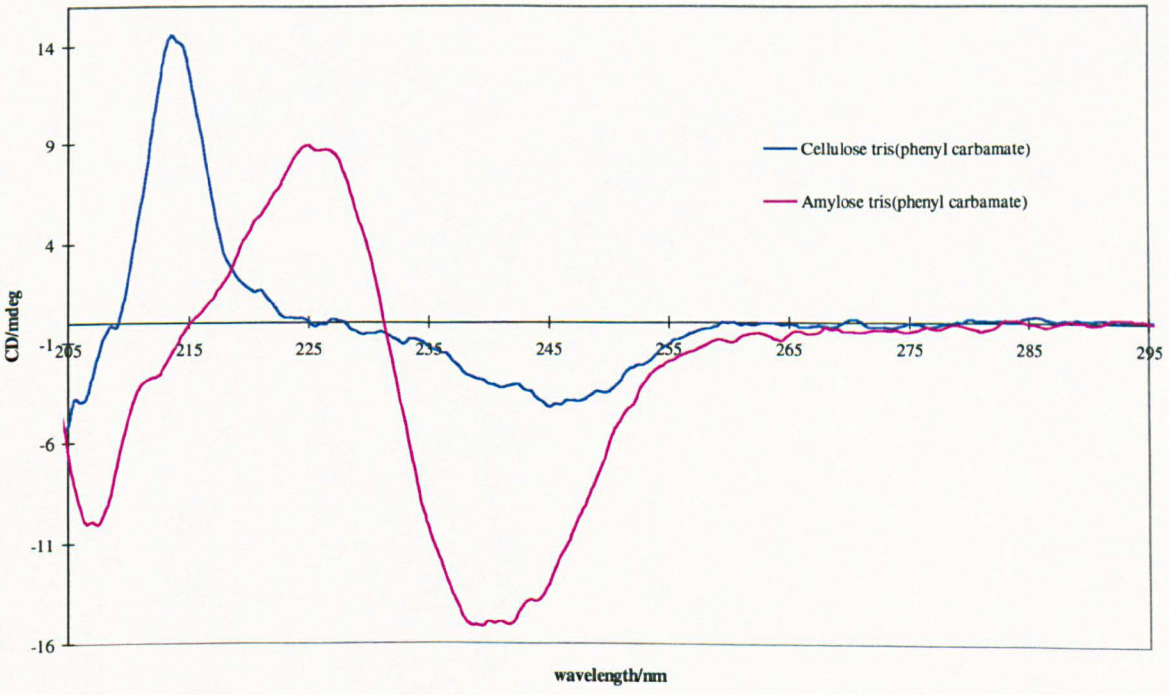


Figure 7.1: CD spectra of CPC and APC measured in THF at a concentration of 1 mg/mL, 0.1 mm cell path length

7.4 Analysis of Saccharide and Saccharide Mimics

UV and CD spectra of the natural saccharides and the saccharide mimics were obtained in order to gain some information about their secondary structure and conformation in solution. It was hoped that a comparison with each other and with CPC and APC would then give an indication of their solution conformations. Due to solubility problems the spectra were run in THF. This is not an ideal solvent as THF has a cut-off of 205 nm and interferes with any absorbance of the carbonyl functionality. However, all the phenyl carbamate derivatives possess phenyl groups which could be investigated using UV and therefore CD spectroscopy in THF. Both the UV and CD spectra were collected between 200 and 300 nm. In each case the extinction coefficient of the wavelength of maximum absorbance in the UV spectrum, ε_{max}, (where ε_{max} = A/cl and A = maximum absorbance, c = concentration in moles/dm^3 and l = path length in cm), was calculated and could be used to compare the absorbance and CD intensities of different compounds. However, a comparison of the ε-values is made difficult by the presence of a different number of phenyl groups in each molecule for each compound. A greater number of phenyl groups would result in a larger absorbance per molecule giving a higher ε_{max} value. Thus variation in absorbance due to different phenyl environments may not be apparent.

7.4.1 Analysis by UV Spectroscopy

Initially the UV spectra of natural saccharide derivatives based on glucose, maltose and maltotriose were obtained so that they could be compared to the saccharide mimics (the molecules with a non-saccharide linker replacing one of the sugar units). The

UV spectrum of methyl 2,3,4,6-*O*-tetra(phenyl carbamate) α-*D*-glucopyranoside **138** (**Fig. 7.3**) has a single peak at 236 nm, with a slight shoulder at approximately 245 nm. This suggests that this absorbance represents more than one type of phenyl group, the 2 transitions being due to the phenyl groups being in slightly different environments. This may be the result of some phenyl groups being protected from the solvent by the rest of the molecule. The UV spectra of maltose **125** and maltotriose **126** (**Fig. 7.3**) show the presence of 3 types of phenyl groups or 3 transitions. A main peak at 236 nm with a shoulder at approximately 242 nm and another peak at 257 nm. For the peak at 257 nm to be due to phenyl groups they must be in a significantly more hydrophobic environment as they are shielded from the solvent by a coiled secondary structure of the oligosaccharides.

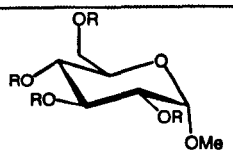
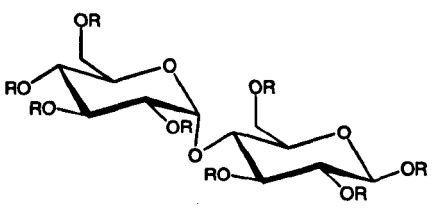
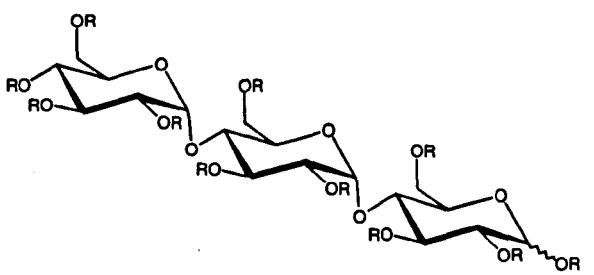
Saccharide unit	Structure	Compound Number
Glucose		**138**
Maltose		**125**
Maltotriose		**126**

Figure 7.2: Structures of the phenyl carbamate derivatives of the natural saccharides glucose, maltose and maltotriose, where R = CONHPh

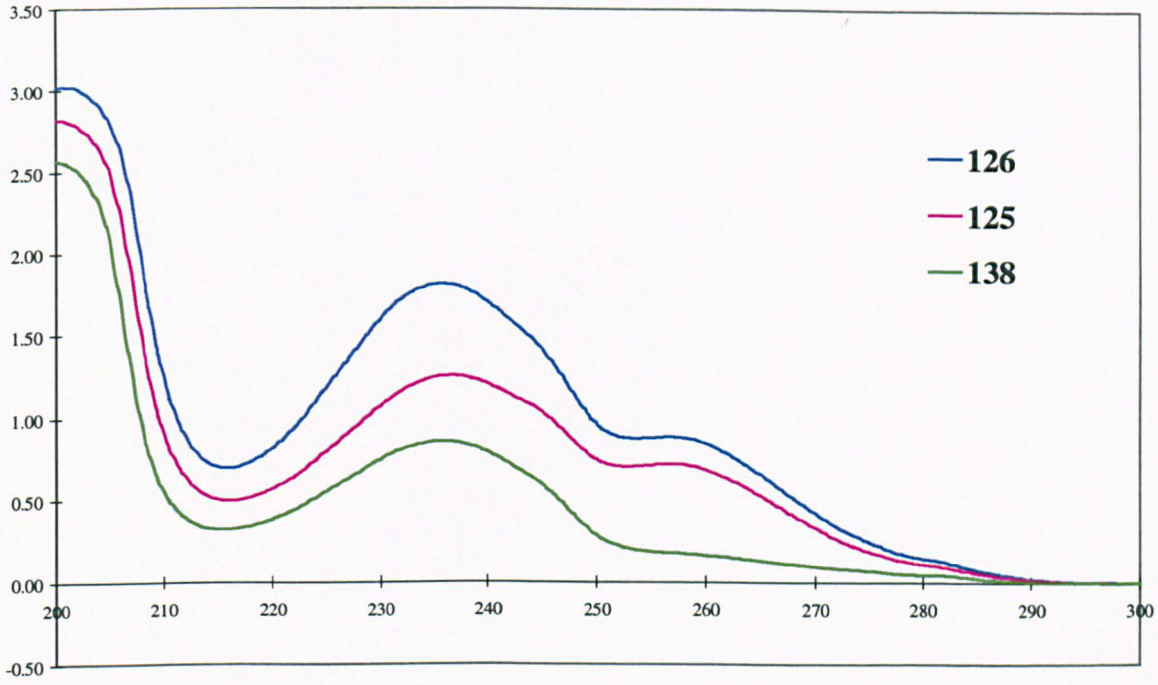


Figure 7.3: UV spectra of maltotriose undeca(phenyl carbamate) **126**, maltose octa(phenyl carbamate) **125** and methyl 2,3,4,6-*O*-tetra(phenyl carbamate) α-*D*-glucopyranoside **138**. All at $1x10^{-4}$ M in THF, 1.0 mm path length

The UV spectrum of APC has a peak at 232 nm only, with no absorbance around 257 nm (**Fig. 7.4**). This may mean that an alternative explanation for the peak at 257 nm is that it arises from an effect of the terminal glucose units that contain four carbamate moieties, which are essentially absent in the polysaccharide amylose.

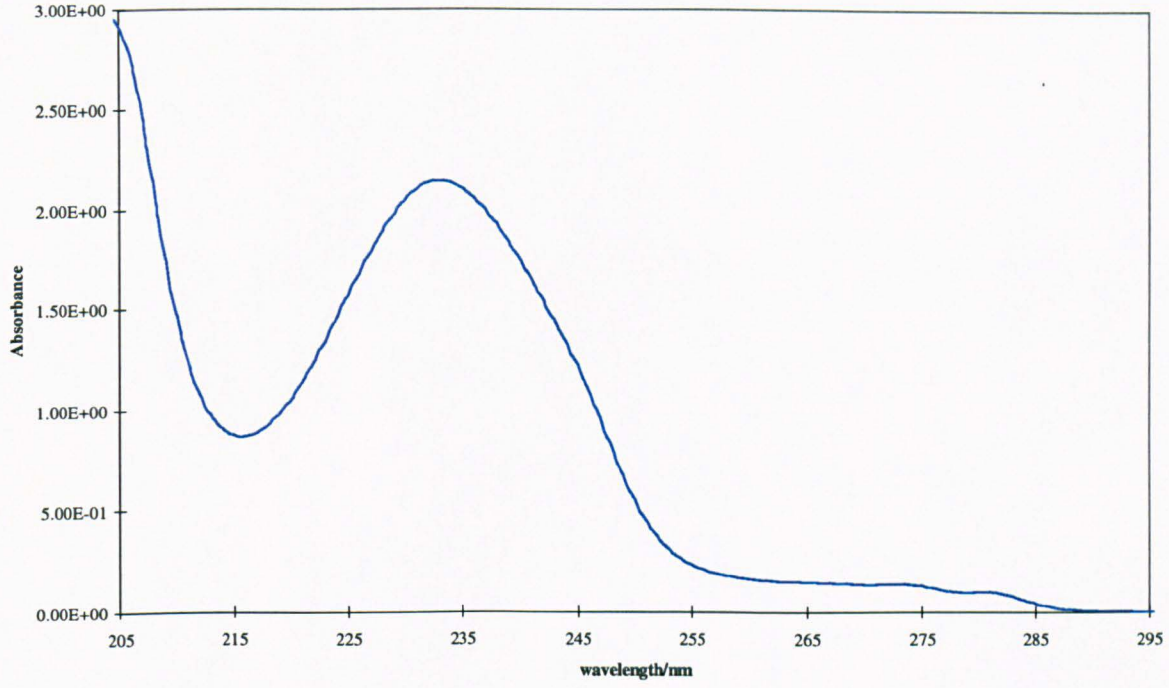
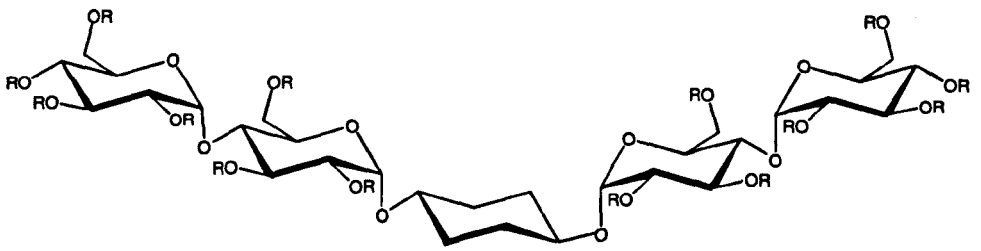


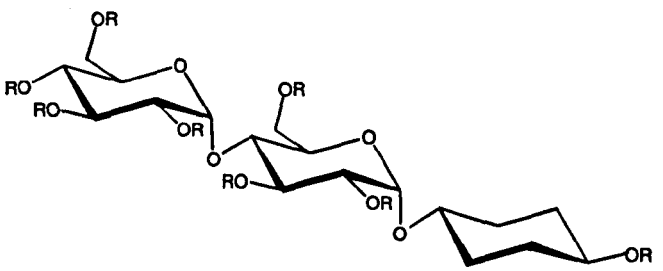
Figure 7.4: UV spectrum of APC in THF at ~ 0.5 mg/mL, 1.0 mm path length

The replacement of one of the sugar units with a cyclohexane ring produces UV spectra that look similar to those of the natural saccharide derivatives. The structures of the saccharide mimics are given again in **Fig. 7.5**. The UV spectra of maltose/monosubstituted cyclohexane **120**, maltose/cyclohexane **119** and maltotriose/cyclohexane **121** all possess a peak at 236 nm with a shoulder at 242 nm and a peak at 257 nm (**Fig. 7.6**). The peak at 257 nm is smaller than in the natural saccharide derivatives **125**, **126** and **138**. This may indicate a less enclosed structure compared with the natural saccharides.

Saccharide unit	Linker	Compound Number
Maltose	Cyclohexane	**119**



Maltose	Cyclohexane	**120**



Maltotriose	Cyclohexane	**121**

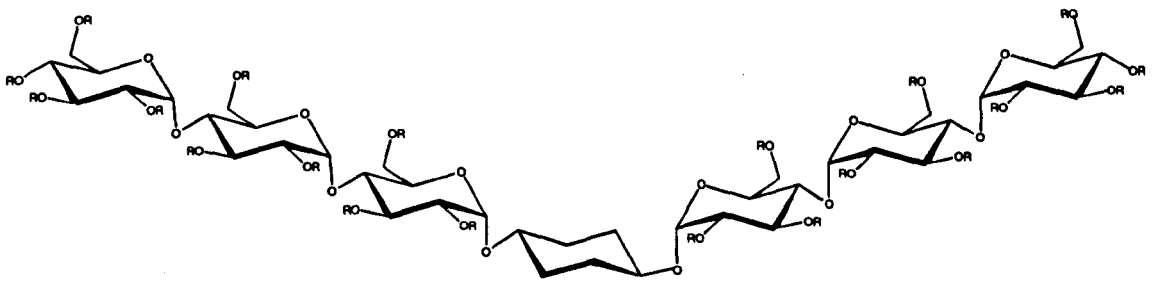


Figure 7.5: Structures of the phenyl carbamate derivatives of maltose and maltotriose units linked with cyclohexane, where R = CONHPh

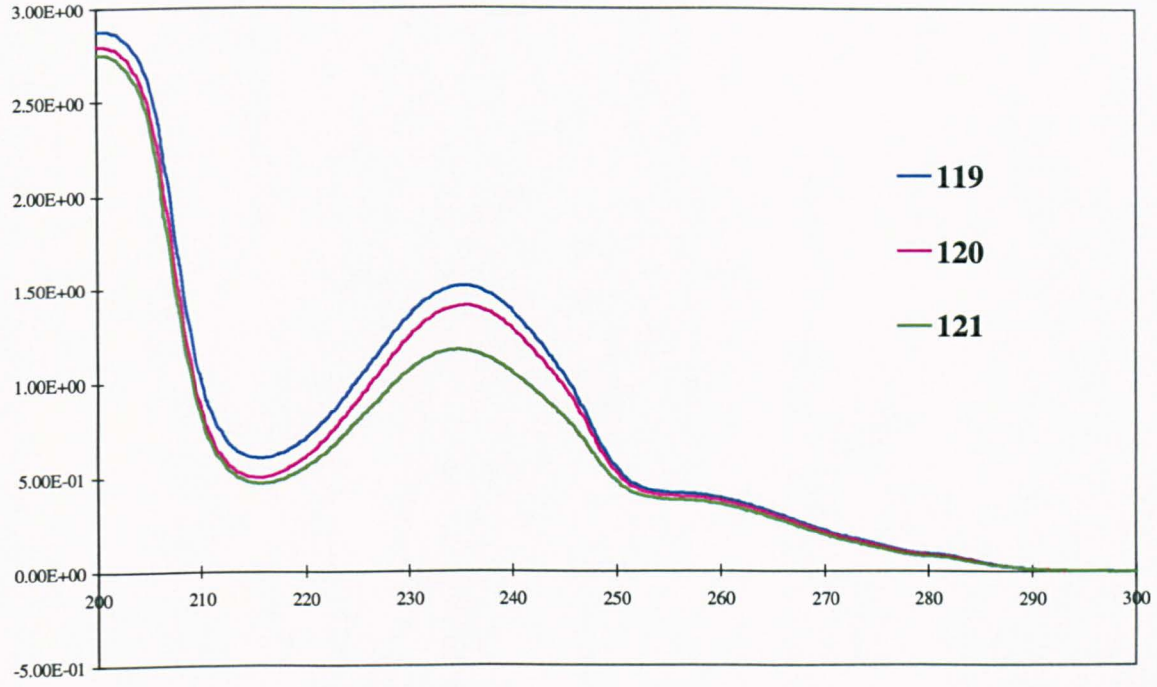


Figure 7.6: UV spectra of maltose/cyclohexane **119** (8.2 x 10^{-4} M), maltose/monosubstituted cyclohexane **120** (1.0 x 10^{-4} M) and maltotriose/cyclohexane **121** (3.8 x 10^{-5} M), in THF, 1.0 mm path length

7.4.2 Analysis by CD Spectroscopy

The CD spectra of the natural saccharide derivatives and saccharide mimics were collected (**Fig. 7.7-7.9**). The CD spectrum for methyl 2,3,4,6-*O*-tetra(phenyl carbamate) α-*D*-glucopyranoside **138** was practically non-existent but CD spectra for maltose octa(phenyl carbamate) **125** and maltotriose undeca(phenyl carbamate) **126** were obtained (**Fig. 7.7**). The CD spectra **125** and **126** are the same shape consisting of a negative peak at 243 nm and a positive peak at 228 nm, intercepting the x-axis at 234 nm however, the peaks have different intensities. At the same molar concentrations, maltotriose **126** gives a much larger signal that is partly due to the extra phenyl groups but also indicates that

more of these phenyl groups are in a chiral environment, caused by a more ordered secondary structure.

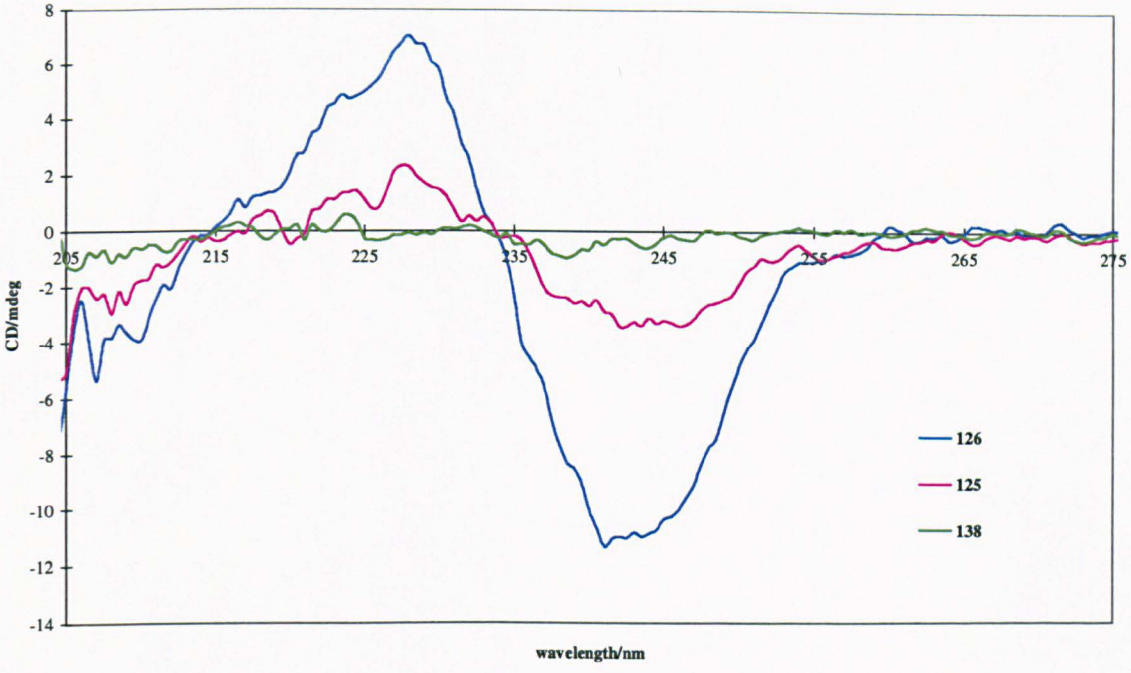


Figure 7.7: CD spectra of maltotriose **126**, maltose **125** and methyl glucoside **138**, at 5 x 10^{-4} M in THF, 1.0 mm path length

It should be noted that there is little CD intensity at 257 nm suggesting that the phenyl groups giving rise to this absorbance are not held in a chiral conformation. Also, the fact that the mono-saccharide **138** does not exhibit any CD absorbance suggests that the chiral environment shown by the di- (**125**) and tri-saccharide (**126**) is due to intramolecular interactions and not intermolecular interactions. This conclusion is supported by the fact that there was no concentration dependence of the shape of the spectra, which would have suggested intermolecular interactions with different molecules at higher concentrations.

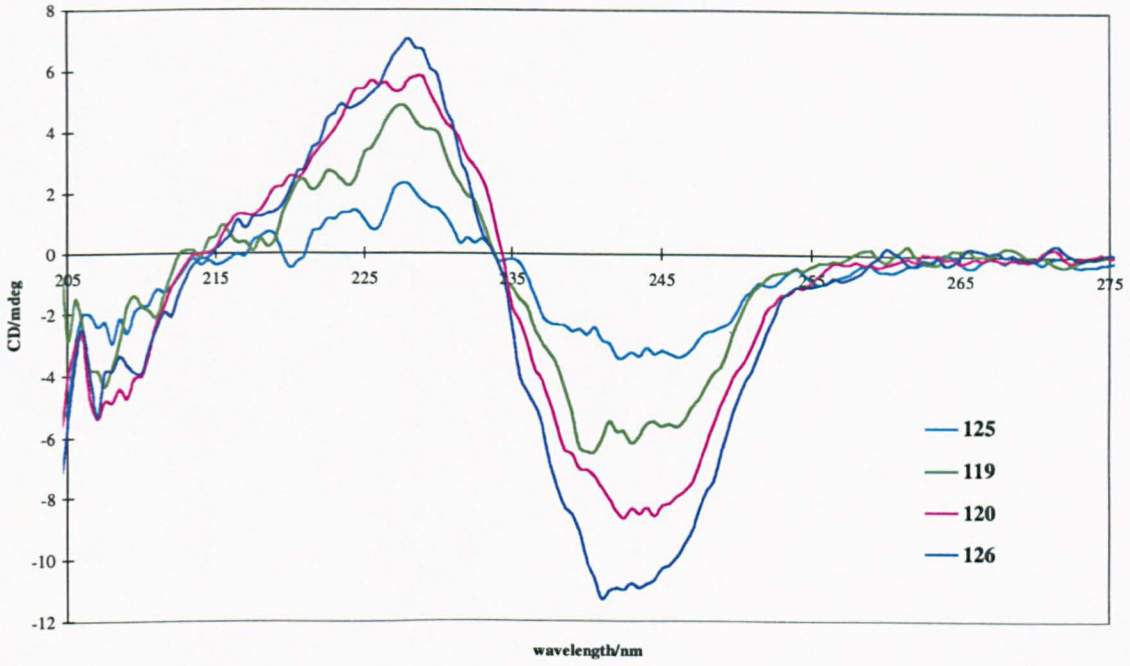


Figure 7.8: CD spectra of maltose/cyclohexane **119** (4.1 x 10^{-4} M), maltose **125** (5.7 x 10^{-4} M), maltotriose **126** (5.3 x 10^{-4} M) and maltose/mono-substituted cyclohexane **120** (5.0 x 10^{-4} M), in THF, 0.1 mm path length

When a cyclohexane unit is used to replace one of the sugar units the CD spectra closely resemble those of the phenyl carbamate derivatives of the natural saccharides. The CD spectrum for maltose/cyclohexane **119**, for example, has a negative peak at 242 nm, a positive peak at 226 nm and crosses the x-axis at 235 nm. The same increase in the size of the peaks is observed upon the addition of a cyclohexane unit as was seen with an additional sugar unit. At the same concentration the peaks for maltose/mono-substituted cyclohexane **120** are greater than maltose which can be attributed to a more ordered secondary structure as the number of phenyl groups per molecule is the same (hence the absorbance due to phenyl groups is the same).

A comparison of the CD spectra for APC and maltotriose/cyclohexane **121** shows a slight shift in wavelength of the x-intercept (**Fig. 7.9**). The CD spectrum of APC crosses the x-axis at 232 nm whereas the CD spectrum of maltotriose/cyclohexane **121** crosses at 234 nm. This may indicate that as the number of sugar units is increased the secondary structure of amylose is slowly attained, indicated by a shift in the wavelength of the intercept of the x-axis to shorter wavelengths. A comparison of maltotriose/cyclohexane and APC also shows a slight shift in wavelength for the negative peak from 242 to 240 nm.

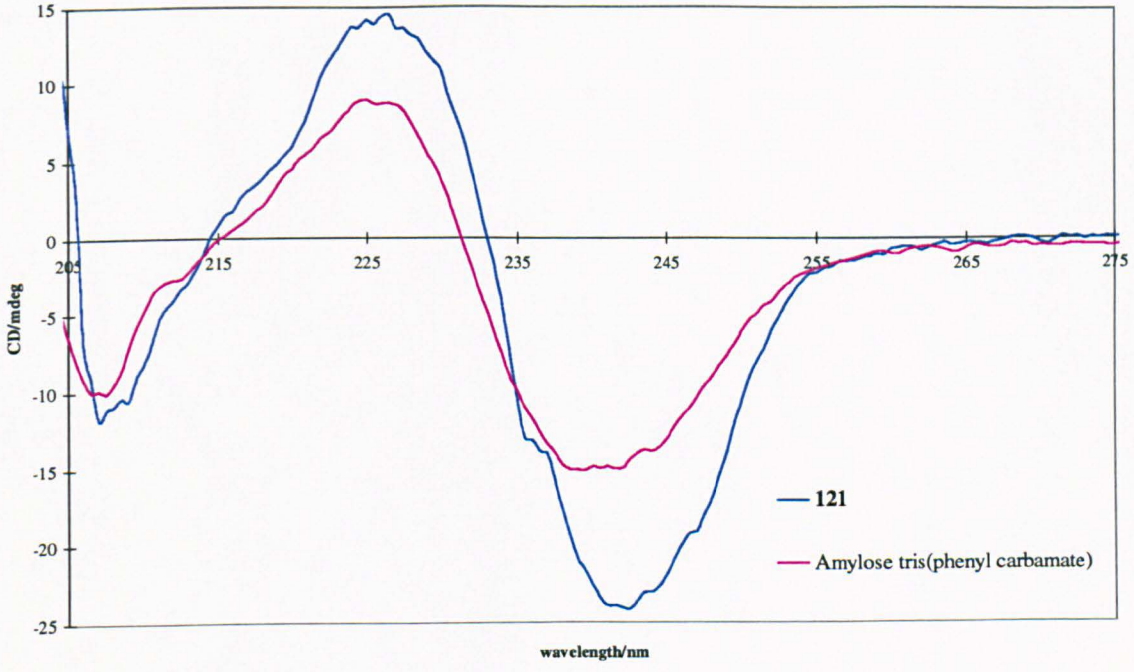


Figure 7.9: CD spectra of APC and maltotriose/cyclohexane **121** ~1 mg/mL in THF, 0.1 mm path length

7.4.3 The Effect of Solvent

As we are designing HPLC phases we were interested in any solvent induced conformation effects as this would dramatically influence the choice of solvents for coating the molecules onto the solid support as well as for MP considerations. If the UV and CD spectra are sensitive to the conformations of the molecules in which the absorbing phenyl groups are located then the choice of solvent will have an effect on such absorbance spectra. Unfortunately most of the solvents that these compounds are soluble in are not suitable for UV or CD analysis due to their UV cut-offs. Acetonitrile was the only solvent with good UV characteristics as well as sufficient solubility for the compounds to be analysed.

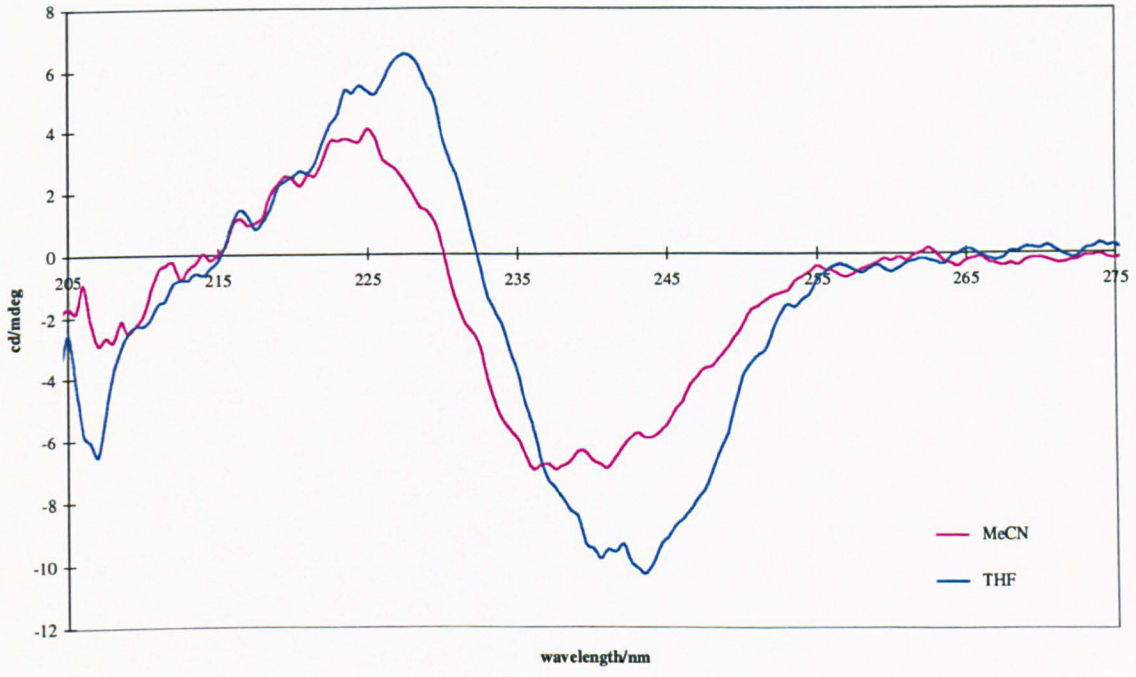


Figure 7.10: CD spectra of maltotriose/cyclohexane **121** in acetonitrile and THF 1 mg/mL, 0.1 mm path length

Three compounds were chosen to study the effect of solvent on conformation. Maltotriose/cyclohexane **121**, maltose/cyclohexane **119** and maltotriose **126** provide examples of a natural saccharide and saccharide mimics with increasing numbers of saccharide units. All three compounds showed a shift of absorbance maxima to shorter wavelengths in the more polar acetonitrile. For example, in THF maltotriose/cyclohexane **121** had a negative maximum at 242 nm, crossed the x-axis at 232 nm and a positive peak at 229 nm. In acetonitrile both the negative and positive peaks shift 4 nm to shorter wavelengths. This indicates that the phenyl groups are in a more polar environment. A comparison with APC was not possible as it was not sufficiently soluble in acetonitrile. Interestingly, another observation was made, namely the area under each peak of the CD spectrum of maltotriose/cyclohexane **121** in acetonitrile was reduced when compared with the CD spectrum run in THF. This suggests that the phenyl groups are in a less chiral environment. This may be because the molecule is opening up allowing more solvent molecules to enter increasing the polarity of the surroundings and interrupting any intramolecular bonding. However, it should be noted that the UV absorbance is also reduced in the more polar solvent (**Fig. 7.11**).

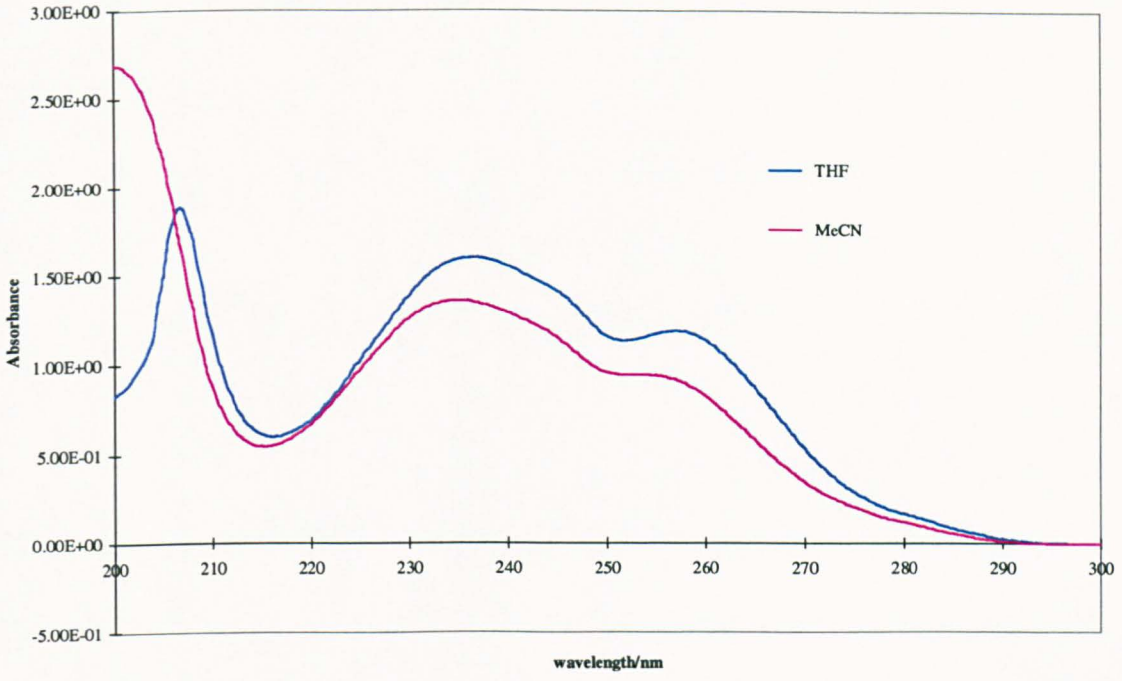


Figure 7.11: UV spectra of maltotriose/cyclohexane **121** in acetonitrile and THF at 1 mg/mL, 1.0 mm path length (with the respective solvents subtracted from each spectra)

7.4.4 The Effect of Changing the Linking Unit

The effect of the non-saccharide unit was investigated by comparing 2 maltose units linked by cyclohexane **119**, butene **122** and butyne **123** (derivatised with different numbers of phenyl carbamate groups).

The UV spectra of the alkyne derivative **123** with n = 5–10 (partially derivatised, where n = number of phenyl groups) has a large absorbance at 257 nm (ε_{max} = 232,000 $mol^{-1}dm^3cm^{-1}$) and just a shoulder at shorter wavelengths (244 nm). The more derivatised alkyne **123** derivative (n = 11–14) appears to absorb at 236 nm only (ε_{max} = 232,000 $mol^{-1}dm^3cm^{-1}$) with no absorbance at 257 nm (though this may be obscured by light

scattering caused be un-dissolved compound). This shows that the phenyl groups are controlling the secondary structure of these molecules. The conformation of the molecule changes with the number of phenyl groups present and is therefore important when considering the molecules as CSPs. The reason for the change in UV absorbance is likely to be due to strong interactions between the residual hydroxyl groups and the carbonyl group of the carbamate groups causing an enclosed structure. This may cause a hydrophobic environment for the phenyl groups present, resulting in them absorbing at longer wavelengths. The alkene derivative **122** shows the same absorbance at 232 nm as the fully derivatised alkyne derivative **123**, which is comparable to the main absorbance of the maltose/cyclohexane **119**.

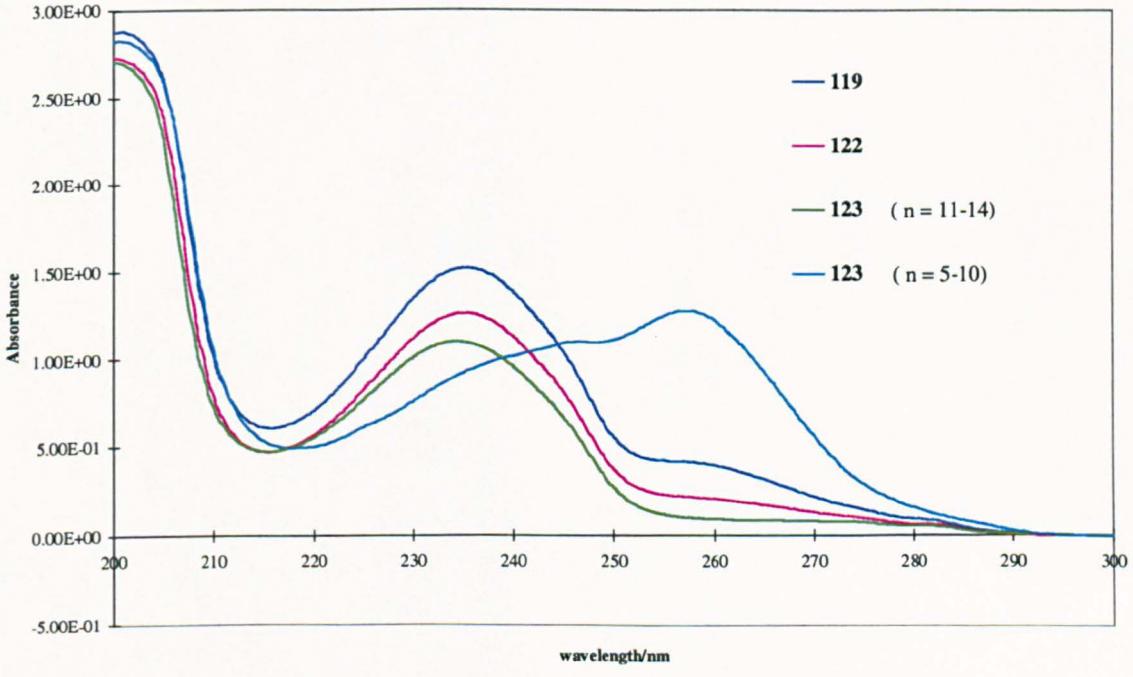


Figure 7.12: UV spectra comparing maltose linked with alkene **122** (5.6 x 10^{-5} M), cyclohexane **119** (8.2 x 10^{-4} M), alkyne **123** (n = 5–10, 4.9 x 10^{-5} M) and partially functionalised alkyne **123** (n = 11–14, 5.6 x 10^{-5} M) derivatives, in THF 1.0 mm path length

The CD spectra of the partially derivatised alkyne derivative **123** (n = 5–10) is practically non-existent. The CD spectra of the more functionalised (n = 11–14) alkyne **123** has a negative peak at 242 nm, crosses the x-axis at 234 nm and a positive peak at 226 nm. This is comparable to the CD spectra of the cyclohexane derivative **119**, although the amplitude of the peaks is slightly reduced. The CD spectra of the maltose/alkene **122** shows the same shape as the cyclohexane derivative **119**, with a slightly enhanced negative absorbance at 242 nm.

The existence of CD spectra for the alkene **122** and alkyne **123** derivatives supports the suggestion that the absorbance at 257 nm in the UV does not relate to the chiral secondary structure of the final molecule.

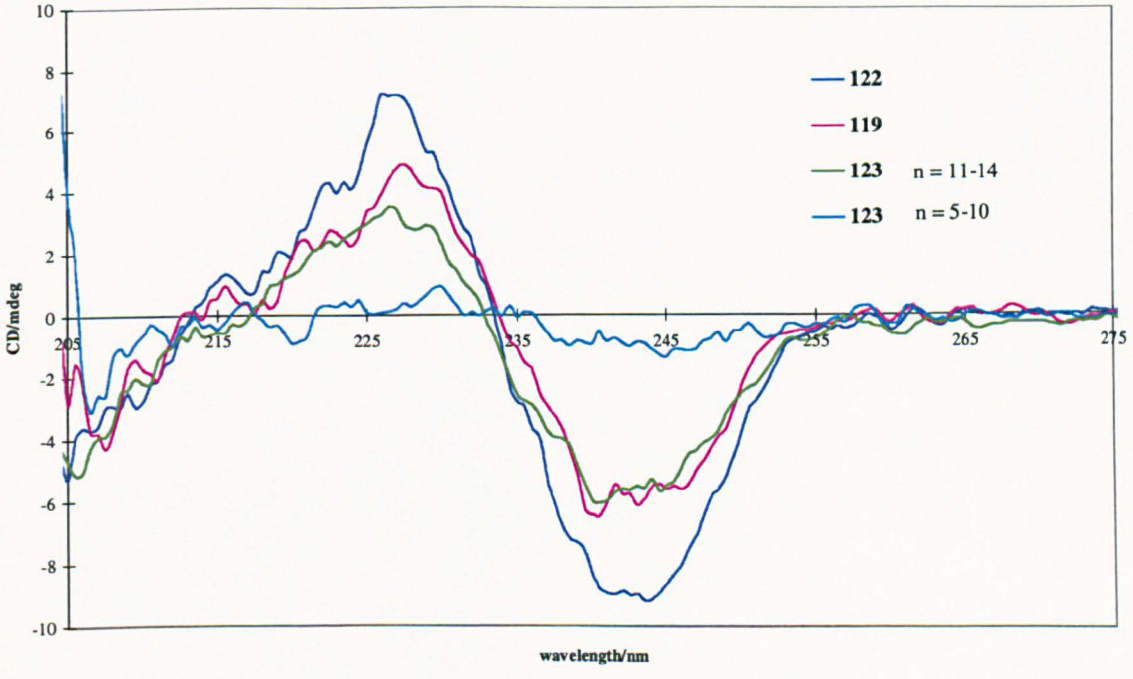


Figure 7.13: CD spectra of maltose/cyclohexane **119** (8.2 x 10^{-4} M)), maltose/butene **122** (4.2 x 10^{-4} M), maltose/butyne **123** (n = 5–10, 5.0 x 10^{-4} M) and maltose/butyne **123** (n = 11–14, 2.1 x 10^{-4} M) in THF, 0.1 mm path length

7.5 Conclusions

The non-zero CD signals for the saccharide mimics indicates that they all have a chiral secondary structure. This secondary structure has shown to be similar for the natural di- and tri-saccharide derivatives as well as the derivatives containing cyclohexane, butene and butyne as linker replacing the sugar unit. Therefore, these can be considered successful saccharide mimics, with respect to secondary structure compared with the oligosaccharides maltose and maltotriose.

The number of functional groups present in the molecule is very important. The conformation of the molecule is affected by the number of phenyl groups present and consequently the number of remaining hydroxyl groups. This is due to the different hydrogen bonds that are formed affecting the secondary structure of the molecule (possible making it more coiled, enclosing the phenyl groups that are present within the molecule). The effect of this conformation change could be investigated further by using the partially functionalised derivatives as a CSP as their different conformation may result in different enantioselectivity.

The solvent also affects the conformation of the molecule. A more polar solvent is capable of interrupting hydrogen bonds that hold the secondary structure in place. This may have the effect of opening up the structure, affecting the chiral secondary structure. This may affect the choice of solvent used to coat the molecules on to a solid support in the preparation of a CSP, though the conformation of the molecules may be different once at the interface of the solid, out of solution.

CHAPTER 8

INVESTIGATION OF THE EFFECT OF THE COATING METHOD AND APPLICATION OF PGC TO FLASH CHROMATOGRAPHY

8.1 Introduction

It has been shown that PGC can be used as a support for CSPs and is superior than silica for the novel CSPs prepared in chapter 5. However, in a comparison between the known CSP cellulose tris(dimethyl phenyl carbamate (CDMPC) coated onto PGC and silica there was reduced efficiency on PGC (chapter 2). There are many reasons that may account for this difference. One important factor is likely to be the incomplete covering of the surface of the PGC as this would allow non-enantioselective interactions with analytes thus reducing column efficiency. There are two aspects which affect the coverage of the solid support and therefore two approaches to resolving the problem. The first would be to investigate the % w/w loading of the CSP onto the support, as insufficient compound would allow regions of the PGC to be uncovered. The second consideration is to investigate the type of method used to achieve homogeneous coverage of the solid support as different methods may produce different results.

We also wished to tell if it was possible to use existing CSPs in a flash chiral chromatography system. The method of coating would be an important consideration when applied to flash chromatography as larger samples of packing material would need to be prepared. Other methods than the batch method already described for coating the solid support may be more suitable when the scale is increased and may be preferable in a commercial environment (e.g. with consideration to the choice of solvents).

8.2 Coating by the Precipitation Method

As the loading of the CSP onto the solid support may be affected by the method of coating it was decided to investigate a different approach to coating the PGC. An alternative to the batch method is to use precipitation to coat the solid support. This involves dissolution of the compound in solvent and stirring with PGC in suspension. A solvent in which the compound is insoluble, such as hexane, is added gradually so the compound precipitates out on to the PGC. This was achieved by dissolving the commercial phase CDMPC in boiling THF (100 mL THF per 5 g CDMPC) and adding PGC (25% w/w with CDMPC i.e. 20 g per 5 g CDMPC). The mixture was allowed to cool to room temperature and then hexane was added dropwise over a period of 3 days. The suspension was filtered and the solid washed with hexane. The coated solid support was then packed into an HPLC column (**139**) and tested with a range of analytes (structures given in appendix). These results were compared with a column containing CDMPC coated onto PGC (**140**) using the batch method (section 2.2.2) and a Chiralcel OD column (containing CDMPC coated onto silica).

8.3 Results of HPLC Tests

Results from the column consisting of PGC coated with cellulose tris(phenyl carbamate) coated using the precipitation method **139** showed a general improvement in chromatography. Similar separations were achieved compared with the batch method **140** of preparation but with reduced capacity factors. This means the time taken for the analysis has been reduced indicating that a superior CSP has been prepared using the precipitation method. This could be attributed to either a more even coating of the solid

support or maybe a higher loading of compound. In most cases the chiral selectivity was still better for the Chiralcel OD column, with the exception of MK801 (**A**) and homatropine (**T**), indicating that there are other influences in enantioselectivity and efficiency of separation of the analytes, such as the PGC support influencing the chiral secondary structure.

		Chiralcel OD		PGC **139** (precipitation)		PGC **140** (batch)	
Racemate		K'	α	K'	α	K'	α
MK801	**A**	5.31	1.14	4.67	1.2		a
SCH50911	**C**	1.68	1.48	2.33	1.33	3.11	1.31
Propranolol glycol	**G**	2.98	1.22	12	1.17		a
Mandelic acid	**L**	0.76	1.53	6.1	1.18	7.41	1.14
Benzoin	**M**	2.7	1.67	3.29	1.36	8.19	1.37
Stilbene oxide	**P**	0.81	1.94	3.1	1.68	-	-
Oxprenolol	**Q**	0.95	5.38	3.11	4.54	-	-
Flavanone	**R**	1.53	1.47	4.94	1.19	-	-
Trifluoroanthryl ethanol	**S**	2.56	2.74	8.09	1.52	-	-
Homatropine	**T**	2.7	2.08	3.05	2.41	-	-

Figure 8.1: Table showing enantioselectivity for CSP prepared by precipitation and batch method compared with a commercial phase

(a) not separated; - not tested

8.4 Use of PGC as a Solid Support for Flash Chromatography

Another area of investigation was the suitability of PGC as a solid support for flash chromatography. CDMPC was loaded on to PGC by the precipitation method by dissolving the CDMPC (25 g) in boiling THF (500 ml) and adding PGC (100 g). This mixture was allowed to cool to room temperature and hexane was added dropwise over a period of 3 days until the total volume was 4.5 dm^3. The suspension was then filtered and the solid washed with hexane. Evaporation and weighing of the filtrate showed that the PGC was loaded to an extent of 23%. A 45 mL flash column was packed with the coated PGC as a slurry. SCH50911 ethyl ester (**C**) was chosen as the analyte for this investigation for two reasons. Firstly this racemate had shown good separation on an analytical scale but also had very good solubility in the chosen solvent system. Other racemates that showed good separation on an analytical scale were not soluble enough to allow a reasonable load volume in the chosen system for flash scale medium pressure chromatography. The separation of the racemate was effected using 10% isopropranol/hexane with a flow rate of 3.5 ml per minute.

The fractions that contained SCH50911 ethyl ester were collected in 1 ml fractions and submitted for HPLC analysis. They showed an encouraging imbalance in enantiomer content that changed as the fractions progressed (**Fig. 8.2**). However, there was no clear separation of the racemate, shown by the graph where absorption *vs* fraction number for each enantiomer is represented (**Fig. 8.3**).

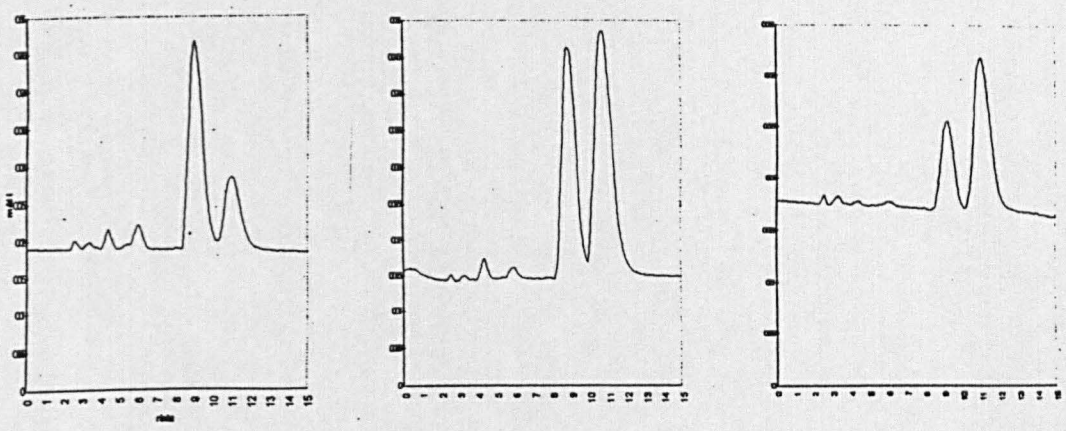


Figure 8.2: Results from preparative separation of SCH 50911 (**C**), fractions 1, 6 and 12 as analysed by HPLC

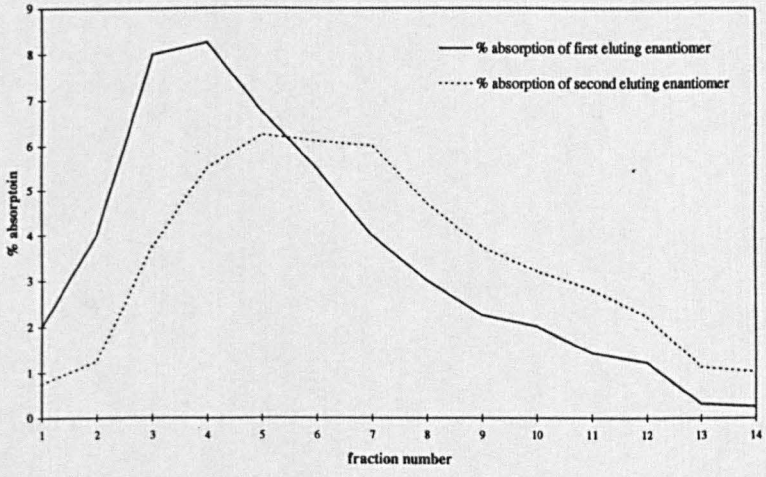


Figure 8.3: Graph showing percentage absorption *vs* fraction number

8.5 Conclusions

The use of PGC as solid support in HPLC was verified by analysis of a CSP consisting of cellulose tris(dimethyl phenyl carbamate). The column using PGC coated with cellulose tris(dimethyl phenyl carbamate) showed good chiral selectivity under

normal phase conditions although complete resolution of the two peaks was not always achieved. The results were comparable to those of a CSP consisting of cellulose tris(dimethyl phenyl carbamate) coated onto silica although further investigation into the optimum loading weighting is still needed. All of the compounds tested on this column were resolvable on the Chiralcel OD column indicating a difference between silica and carbon as solid support such as the effect on the secondary structure.

The best method of CSP preparation for cellulose tris(dimethyl phenyl carbamate) was shown to be the precipitation method, where similar separations compared with cellulose tris(phenyl carbamate) coated onto PGC using the batch method were obtained but with reduced capacity factors.

It was also been shown that PGC has the potential for use as a solid support in flash chromatography. Some enantioselectivity was seen although no separation was possible. The conditions for this column were not optimised so it may be possible to improve the separation by reducing the amount loaded on to the column, by slowing the flow rate even more, increasing the length of the column or possibly by increasing the loading of CSP on the PGC.

The drastic reduction in analyte separation when the same CSP had been used on a flash scale than on an analytical scale means more investigation into the process is needed. A direct comparison with analytical scale separation is not possible although the use of different conditions may improve the situation such as the optimum loading and the method of coating.

CHAPTER 9

EXPERIMENTAL

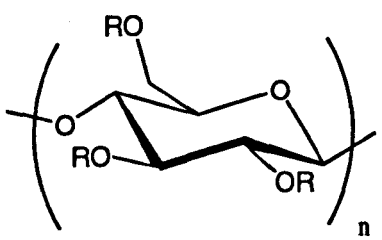
9.1 General experimental

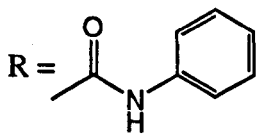
Melting points were recorded on a Stuart scientific SMP1 melting point apparatus and are uncorrected. Boiling points were determined by distillation with pressures being quoted in mmHg. Accurate masses were performed on a Kratos MS80 spectrometer at the University of Warwick or by Swansea Mass Spectrometry Service. Microanalyses were recorded at the University of Warwick on a Leeman Labs Inc. CE440 Elemental Analyser. Infra-red spectra were recorded in a solution cell, as Nujol mulls, using hexachlorobutadiene (HCB) or neat, as stated in the text, on a Perkin-Elmer 1720X Fourier transform spectrometer, with only selected absorbances (υ_{max}) being reported. Ultra-violet spectra were recorded in solution in a quartz cell on a Pye-Unicam 8720 UV/VIS scanning spectrometer, with peak values (λmax) being quoted in nm and accompanying ε_{max} values being quoted in $dm^3mol^{-1}cm^{-1}$. Optical rotations were recorded on an AA-1000 polarimeter, Optical Activity Ltd. using a 20 cm cell path length recorded at 589 nm (D). Shown in the form $[\alpha]^T$ (concentration *c*, solvent) where temperatures, T, are quoted in °C and concentration in g per 100 mL. ^{1}H NMR spectra were recorded on either 250 MHz, 300 MHz or 400 MHz on a Bruker ACF 250, Bruker DPX 300 or Bruker ACP 400 instruments respectively. Chemical shifts (δ) are quoted in parts per million (ppm) and referenced to the appropriate solvent peak. Coupling constants, *J*, are quoted in Hertz (Hz). ^{13}C NMR spectra were recorded at 62.9 MHz, 75.5 MHz or 100.6 MHz on a Bruker ACF 250, DPX 300 or ACP 400 instrument. Chemicals used in the

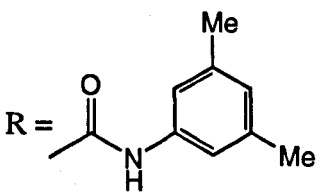
experimental were obtained from Sigma-Aldrich, Lancaster, Avocado or Fluka. All solvents were purchased from Fisons Scientific Equipment at SLR grade and purified when needed by literature methods. Flash chromatography was carried out on silica gel (Merck Kieselgel $60F_{254}$ 230-400 mesh). TLC was carried out using aluminium backed plates precoated with silica (0.2 mm, $60F_{254}$) and were developed using UV fluorescence (254 nm), phosphomolybdic acid, potassium permanganate solution or dilute sulphuric acid (in methanol). Mass spectra obtained using matrix assisted laser desorption-ionization mass spectrometry (MALDI-MS) were on a Kratos KOMPACT with a time-of-flight (TOF) detector. Irradiation was at 257 nm from a nitrogen laser source using 2,5-dihydroxybenzoic acid (DHB) or α-hydroxy cinnamic acid as matrix. For GPC analysis a Polymer Laboratories™ modular system was used. This utilises a 5 μm, 15 cm mixed-E column with PL Caliber™ GPC software with THF as eluent at flow rate of 1 mL/min. calibration was with Polymer Laboratories polystyrene narrow molecular weight standards.

9.2 Experimental for Chapter 2

General Method for the Preparation of Polysaccharide Carbamates







Cellulose :β-linked polymer (CPC)

Amylose :α-linked polymer (APC)

Method: All glassware was dried in an oven overnight and the polysaccharide was dried for 24 hours in a vacuum dessicator over phosphorus pentoxide prior to use. The polysaccharide (6.3 g) was refluxed for 24 hours in dry pyridine. The mixture was allowed to cool to room temperature before the phenyl isocyanate (20 mL) or 3,5-dimethyl phenyl isocyanate was added by syringe and the mixture heated at reflux for a further 72 hours. After 72 hours the mixture was allowed to cool to room temperature and the resulting brown liquid was poured slowly into a round bottom flask containing 1.5 L of AR methanol. After stirring for 2 hours the suspension was filtered and the cream precipitate washed until no pyridine odour was left. Placing in a vacuum oven at 50°C dried the product further.

Cellulose tris(3,5-dimethyl phenyl carbamate)

67% of hydroxyl groups derivatised (according to equation y = 15.633logz + 58.305, where y = %C and %OH derivatised = 100z/3).[55] Found C 63.01, H 6.05, N 6.84. $C_{33}H_{43}N_3O_8$ (individual glucose unit) requires C 65.66, H 6.18, N 6.96%; υ_{max} (nujol mull)/cm^{-1} 1724, 1615, 1539, 1273, 1223, 1163, 1083 and 723.

Amylose tris(phenyl carbamate)

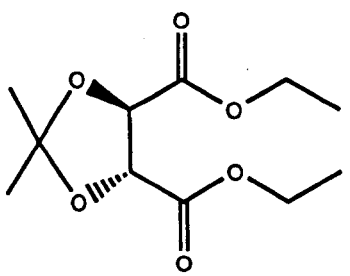
60% of hydroxyl groups derivatised (according to equation y = 14.526logz + 55.549).[55] Found C 59.26, H 5.25, N 7.89. $C_{27}H_{25}N_3O_8$ (individual glucose unit) requires C 62.42, H 4.85, N 8.09%; υ_{max} (nujol mull)/cm^{-1} 3058, 2956, 1745, 1444, 1273, 1314, 1229, 1169 and 990.

General Procedure for Coating CSPs onto PGC

PGC (1 g) was refluxed in THF (100 mL) in a 250 mL round bottomed flask with four indents for 30 mins and left to cool. The compound (0.26 g) to be coated onto the solid support was split into 5 portions (0.06 g) and dissolved in THF (10 mL). The first portion of compound in solution was added to the PGC and the solvent removed at room temperature using a rotary evaporator set at low rotation speed. This procedure was repeated until all of the compound had been added and the dry solid was then brushed through a 38 μm sieve to ensure a free flowing powder ready for packing into an HPLC column.

9.3 Experimental for Chapter 3

(*R,R*)-4,5-Bis(ethoxycarbonyl)-2,2-dimethyl-1,3-dioxolan[122]

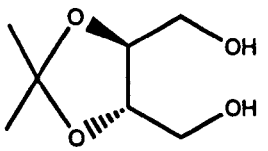


34

Method: To a solution of diethyl-*L*-tartrate **33** (48.9 g, 0.237 mol) in acetone (20 mL), 2,2-dimethoxypropane (3eq, 74.1 g, 0.71 mol) and *p*-toluenesulfonic acid (1/200eq, 0.206 g, 1.08 mmol) were added. The solution was stirred at reflux for 24 hours and then the solvent removed *in vacuo*. Purification by vacuum distillation (82-92°C, 0.3mm Hg (lit.,[122] 90-100°C 0.5mm Hg)) gave the product **34** as a yellow oil (46.0 g, 79%). R_f 0.55 (1:1 PE:EtOAc); $[\alpha]^{23}$ -41.3 (*c* 0.9, $CHCl_3$); υ_{max} (liquid cell)/cm^{-1} 2991, 2939, 1746,

1438, 1385, 1210 and 1100; δ_H (250 MHz; $CDCl_3$) 4.73 (2 H, s, 2 x C*H*CO_2Et), 4.21 (4 H, q, *J* 7.3, 2 x C*H*$_2$$CH_3$), 1.45 (6H, s, 2 x CC*H*$_3$), 1.27 (6H, t, *J* 7.3, 2 x CH_2C*H*$_3$); δ_C (62.9 MHz; $CDCl_3$) 169.5 (2 x s), 113.6 (s), 76.5 (2 x d), 62.2 (2 x t), 26.1 (2 x q), 13.9 (2 x q); *m/z* (CI) 247.1182 (MH^+ $C_{11}H_{19}O_6$ requires 247.1182), 231 (47%), 217 (45), 203 (30), 173 (42), 159 (35), 115 (34), 87 (55) and 43 (100).

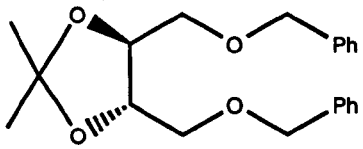
(*S*,*S*)-4,5-Bis(hydroxymethyl)-2,2-dimethyl-1,3-dioxolan[122]



36

Method[123]: The ester **34** (31.5 g, 0.127 mol) in THF (20 mL) was added slowly to a suspension of lithium aluminium hydride (1.3eq, 6.27 g, 0.165 mol) in THF (150 mL) at 0°C. The mixture was left to warm to room temperature overnight and then carefully quenched with water (7 mL). After stirring for 20 minutes, 10% aqueous sodium hydroxide solution (7 mL) was carefully added, and stirred for a further 20 minutes. More water (20 mL) was added and the mixture filtered, washed with ethyl acetate (20 mL). The combined filtrates were dried with magnesium sulphate and the solvent removed *in vacuo*, to yield **36** as a pale yellow oil (13.78 g, 67%). R_f 0.13 (1:1 PE:EtOAc) $[\alpha]^{24}$ -3.3 (*c* 1.3, $CHCl_3$); υ_{max} ($CHCl_3$)/cm^{-1} 3418, 2990, 2935, 2882, 1456, 1372 and 1010; δ_H (250 MHz; $CDCl_3$) 3.95 (2 H, m, 2 x C*H*CH_2), 3.72 (4 H, dd, *J* 4.1, 11.9, 2 x C*H*$_2$OH),), 3.00 (2 H, br s, 2 x OH), 1.40 (6 H, s, 2 x Me); δ_C (250 MHz; $CDCl_3$) 109.2 (s), 78.1 (2 x d), 62.0 (2 x t), 26.8 (2 x q); *m/z* (CI) 180 (8%, MNH_4^+) 163 (100, MH^+), 147 (30) and 131 (10).

(4*S*, 5*S*)-2,2-Dimethyl-4,5(benzyloxymethyl)-1,3-dioxolane

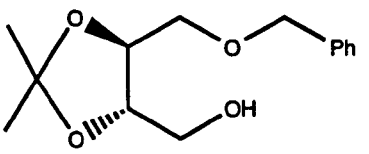


37

Method: A cold solution of diol **36** (1.00 g, 6.2 mmol) in DMF (10 mL) was added to a flask containing washed sodium hydride (2.4eq, 0.36 g, 14.8 mmol, 60% in mineral oil). After stirring for 30 minutes, benzyl bromide (2.2eq, 2.32 g, 13.6 mmol) was added slowly. The reaction was stirred overnight and then quenched with water (5 mL) and extracted with dichloromethane (3 x 10 mL). The combined organic extract was dried and concentrated to give a pale yellow oil (79%) which was purified by column chromatography (5:1 PE:EtOAc) to yield **37** (0.53 g, 25%) and mono-benzylated product **38** (0.67 g, 43%).

Data for **37**: R_f 0.79 (1:1 PE:EtOAc), $[\alpha]^{24}$ -0.4 (*c* 0.4, $CHCl_3$); υ_{max} ($CHCl_3$)/cm^{-1} 3688, 3601, 3462, 2990, 2935, 1602, 1490 and 1010; δ_H (250 MHz; $CDCl_3$) 7.34 (10 H, m, Ph), 4.58 (4 H, apparent s, 2 x C*H*$_2$Ph), 4.05 (2 H, m, 2 x OC*H*CH$_2$), 3.61 (4H, apparent dd, *J* 11.5, 3.2, 2 x CHC*H*$_2$), 1.47 (6 H, s, 2 x Me); δ_C (62.9 MHz; $CDCl_3$) 137.8 (2 x s), 128.3 (4 x d), 127.6 (6 x d), 109.1 (s), 79.4 (2 x d), 73.4 (2 x t), 70.6 (2 x t), 27.0 (2 x q); *m/z* (CI) 343.1901 (MH^+ $C_{21}H_{27}O_4$ requires 343.1910), 327 (32), 251 (39), 216 (42), 195 (71), 181 (70), 108 (88) and 91 (100).

(4*S*, 5*S*)-2,2-Dimethyl-4-(benzyloxy)methyl-5-(hydroxy)methyl-1,3-dioxolan



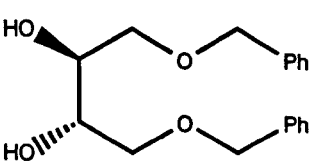
38

δ_H (250 MHz; $CDCl_3$) 7.3 (5 H, m, Ph), 4.59 (2 H, apparent s, C*H*$_2$Ph), 4.03 (1 H, dt, *J* 4.3, 8.5, C*H*CH$_2$), 3.92 (1 H, dt, *J* 4.3, 8.5, C*H*CH$_2$), 3.76 (1 H, dd, *J* 4.3, 11.8, CHC*H*$_2$) 3.66 (2 H, m, 2 x CHC*H*$_2$), 3.55 (1 H, dd, *J* 5.6, 9.9, CHC*H*$_2$), 2.40 (1 H, br s, OH), 1.42 (6 H, s, 2 x Me); δ_C (62.9 MHz; $CDCl_3$) 137.4 (s), 128.3 (2 x d), 127.7 (d), 127.6 (2 x d), 109.2 (s), 79.4 (d), 76.4 (d), 73.5 (t), 70.2 (t), 62.2 (t), 26.8 (2 x q).

(4*S*, 5*S*)-2,2-Dimethyl-4,5(benzyloxymethyl)-1,3-dioxolane

A cold solution of **38** (0.67 g, 2.6 mmol) in DMF (10 mL) was added to a flask containing washed NaH (1.2eq, 0.13 g, 3.19 mmol 60% in mineral oil). After stirring for 30 minutes, benzyl bromide (1.1eq, 2.92 mmol, 0.35 mL) was added slowly. The reaction was stirred overnight and then quenched with water (5 mL) and extracted with dichloromethane (3 x 10 mL). The combined organic extract was dried and concentrated to give **37** as a pale yellow oil (0.64 g, 71%).

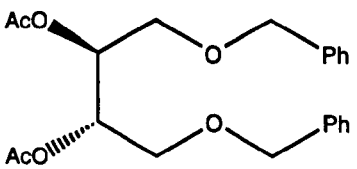
(2*S*, 3*S*)-1,4-Bis(benzyl)butanetetrol



39

Method: A solution of **37** (1.0 g, 2.92 mmol) in aqueous 10% HCl in THF (1:1 v/v, 100 mL) was stirred at room temperature for 2 hours. The reaction was then diluted with water (50 mL) and extracted with diethyl ether (3 x 50 mL). After washing with sodium carbonate and brine the organic extracts were dried with magnesium sulphate and the solvent removed *in vacuo*. Purification by column chromatography (1:1 PE:EtOAc) yielded a white solid **39** (0.52 g, 77%). On a larger scale, purification was by filtration through a celite bed using 20:1 PE:EtOAc initially, followed by elution with EtOAc to obtain the pure product. R_f 0.27 (1:1 PE: EtOAc). (Melting point 53-55°C (lit.,[124] 58-59°C), $[\alpha]^{25}$ -6.7 (*c* 1.7, $CHCl_3$ (lit.,[124] $[\alpha]_D$ -5.0 (*c* 5.0, $CHCl_3$)); υ_{max} ($CHCl_3$)/cm^{-1} 3560, 3009, 2867, 1710, 1496, 1453, 1391, 1362 and 1090; δ_H (250 MHz; $CDCl_3$) 7.30 (10 H, m, Ph), 4.57 (2 H, d, *J* 11.9, 2 x C*H*HPh), 4.52 (2 H, d, *J* 11.9, 2 x C*H*HPh), 3.88 (2 H, m, 2 x C*H*CH_2), 3.62 (2 H, dd, *J* 4.6, 9.8, 2 x CH*H*OBn), 3.57 (2 H, dd, *J* 5.7, 9.8, 2 x CH*H*OBn), 2.80 (2 H, br s, 2 x OH); δ_C (62.9 MHz; $CDCl_3$) 137.7 (2 x s), 128.4 (4 x d), 127.7 (6 x d), 73.4 (2 x t), 71.9 (2 x d), 70.5 (2 x t); *m/z* (CI) 303.1580 (MH^+ $C_{18}H_{23}O_4$ requires 303.1597), 181 (25%), 93 (62) and 91 (100).

Acetic acid (2*S*, 3*S*)-2-acetoxy-3-benzyloxy-1-benzyloxymethyl propyl ester

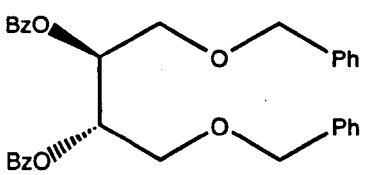


40

Method: Acetyl chloride (3eq, 2.13 mL, 29.8 mmol) was added to a solution of the diol **39** (3.2 g, 9.9 mmol) in pyridine (20 mL) at 0°C. After stirring for 15 hours the reaction was quenched with water (20 mL) and extracted with ether (3 x 20 mL). The organic

extracts were washed with aqueous 10% HCl, sodium carbonate (3 x 20 mL) and dried with magnesium sulphate. The solvent was removed *in vacuo* to yield **40** as a yellow oil (3.48 g, 87%). R_f 0.3 (4:1 PE: EtOAc), $[\alpha]^{26}$ -16.8 (*c* 1.9, $CHCl_3$); υ_{max} (thin film)/cm^{-1} 3062, 3030, 2867, 1743, 1496, 1453, 1371, 1201, 1010 and 1002; δ_H (250 MHz; $CDCl_3$) 7.30 (10 H, m, 2 x Ph), 5.36 (2 H, m, 2 x C*H*CH_2), 4.55 (2 H, d, *J* 11.9, 2 x C*H*HPh), 4.43 (2 H, d, *J* 11.9, 2 x C*H*HPh), 3.56 (4 H, m, 2 x CHCH_2), 2.05 (6 H, s, 2 x Me); δ_C (62.9 MHz; $CDCl_3$) 170.1 (2 x s), 137.5 (2 x s), 128.3 (4 x d), 127.6 (6 x d), 73.4 (2 x t), 70.6 (2 x d), 67.9 (2 x t), 20.8 (2 x q); *m/z* (CI) 404 (9%, MNH_4^+), 387.1802 (MH^+ $C_{22}H_{27}O_6$ requires 387.1808), 327 (21), 279 (24), 237 (22), 189 (35), 108 (64), 91 (100), 74 (52) and 58 (21).

Benzoic acid, (2*S*, 3*S*)-2-benzoyloxy-3-benzyloxy-1-benzyloxymethyl propyl ester

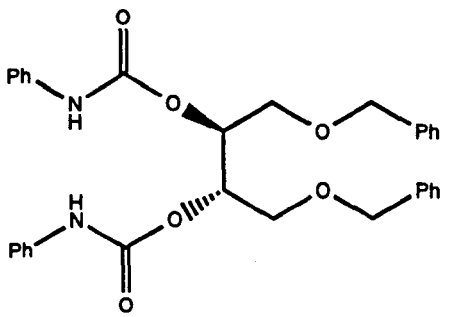


41

Method: Benzoyl chloride (2.5eq, 1.4 mL, 12.0 mmol) was added to a solution of the diol **39** (1.46 g, 4.8 mmol) in pyridine (20 mL) at 0°C. After stirring for 1 hour, the reaction was quenched with water (20 mL) and extracted with ether (3 x 20 mL). The organic extracts were washed with aqueous 10% HCl, sodium carbonate (2 x 30 mL) and dried with magnesium sulphate. The solvent was removed *in vacuo* to yield **41** as a colourless oil (1.92 g, 76%). $[\alpha]^{24}$ -17.9 (*c* 1.6, $CHCl_3$), Found: C 74.62, H 5.95. $C_{32}H_{30}O_6$ requires C 75.29, H 5.88%, R_f 0.77 (4:1 PE: EtOAc); υ_{max} (liquid film in $CHCl_3$)/cm^{-1} 3031, 2866, 1787, 1723, 1599, 1492, 1451, 1277, 1213 and 1090; δ_H (250 MHz; $CDCl_3$) 8.00

(4 H, d, *J* 7.0, Ph), 7.54 (2 H, t, *J* 7.0, Ph), 7.41 (2 H, t, *J* 7.0, Ph), 7.29 (12 H, m, Ph), 5.77 (2 H, m, 2 x C*H*CH_2), 4.57 (2 H, d, *J* 11.9, 2 x C*H*HPh), 4.46 (2 H, d, *J* 11.9, 2 x C*H*HPh), 3.78 (4 H, apparent s, 2 x CHCH_2); δ_C (62.9 MHz; $CDCl_3$) 165.7 (2 x s), 137.5 (2 x s), 132.9 (2 x s, 2 x d), 129.7 (4 x d), 128.2 (8 x d), 127.6 (6 x d), 73.2 (2 x t), 71.4 (2 x d), 68.0 (2 x t); *m/z* (CI) 528 (10%, MNH_4^+), 511 (17, MH^+), 389 (70), 122 (78), 105 (100), 91 (91), 77 (11) and 65 (8).

N-Phenylcarbamic acid, (2*S*, 3*S*)-3-benzyloxy-1-benzyloxymethyl-2-N'-phenylcarbamoxy propyl ester

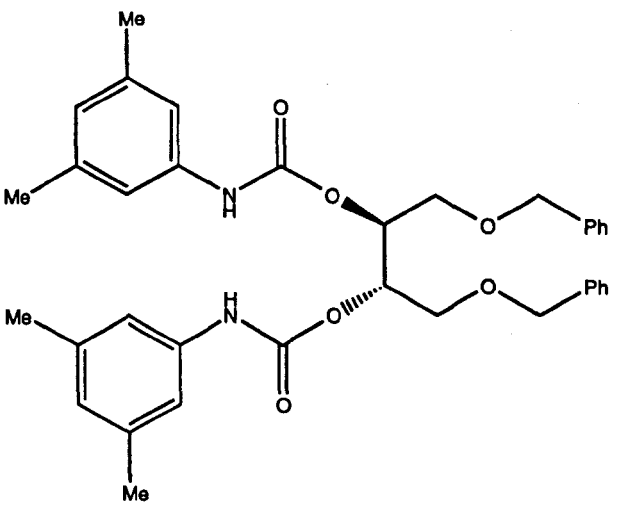


42

Method: Phenyl isocyanate (2.2eq, 2.6 g, 21.8 mmol) was added carefully to the diol **39** (3 g, 9.92 mmol) at reflux in pyridine (15 mL) and heated for a further 12 hours. The reaction was quenched with water (10 mL), extracted with ether (3 x 20 mL), washed with aqueous 10% HCl, sodium carbonate (3 x 30 mL) and dried with magnesium sulphate. After removal of the solvent *in vacuo* the cream solid was purified by recrystallization; initially using THF and pet. ether, to remove one impurity, and then with ethyl acetate and pet. ether to yield **42** as a white solid (8 g, 45%) R_f 0.47 (1:1 PE: EtOAc), melting point 149-154°C; $[\alpha]^{23}$ +13.6 (*c* 0.6, $CHCl_3$); Found: C 70.94, H 5.94, N 5.32. $C_{32}H_{32}N_2O_6$ requires C 71.10, H 5.97, N 5.18%; υ_{max} ($CHCl_3$)/cm^{-1} 3420, 3032,

2869, 1739, 1598, 1527, 1443, 1325, 1200 and 1075; δ_H (400 MHz; DMSO) 9.77 (2 H, s, 2 x NH), 7.50 (4 H, d, *J* 7.6, Ar), 7.29 (14 H, m, Ar), 6.98 (2 H, t, *J* 7.4, Ar), 5.32 (2 H, m, 2 x C*H*CH_2), 4.53 (2 H, d, *J* 11.9, 2 x C*H*HPh), 4.47 (2 H, d, *J* 11.9, 2 x C*H*HPh), 3.70 (4 H, m, 2 x CHC*H*$_2$); δ_C (62.5 MHz; DMSO) 153.1 (2 x s), 139.1 (2 x s), 138.0 (2 x s), 128.8 (4 x d), 128.3 (4 x d), 127.7 (4 x d), 127.6 (4 x d), 122.6 (2 x d), 118.5 (2 x d), 72.4 (2 x t), 71.3 (2 x d), 68.5 (2 x t); *m/z* (CI) 541.2338 (MH^+ $C_{32}H_{33}N_2O_6$ requires 541.2340), 404 (42%), 343 (22), 327 (20), 307 (22), 213 (100), 181 (93), 119 (32), 108 (62) and 91 (98).

N-(3,5-Dimethyl)phenylcarbamic acid, (2*S*, 3*S*)-3-benzyloxy-1-benzyloxymethyl-2-N'-(3,5-dimethyl)phenylcarbamoxy propyl ester



43

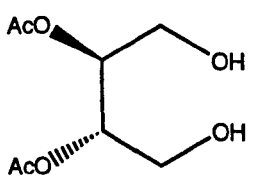
Method: 3,5-Dimethyl phenyl isocyanate (2.2eq, 5 g, 34.0 mmol) was added carefully to the diol **39** (4.67 g, 15.0 mmol) at reflux in pyridine (25 mL) and heated for a further 24 hours. The reaction was quenched with water (20 mL), extracted with ethyl acetate (3 x 40 mL), washed with aqueous 10% HCl (3 x 20 mL), aqueous sodium carbonate (3 x 20 mL) and dried with magnesium sulphate. Removal of the solvent *in vacuo* yielded **43** as a

cream solid (7.7 g, 86% crude yield). R_f 0.9 (1:1 PE:EtOAc); $[\alpha]^{25}$ -14.3 (*c* 1.1, $CHCl_3$); Found: C 72.47, H 6.77, N 4.84. $C_{36}H_{40}N_2O_6$ requires C 72.40, H 6.70, N 4.70%; δ_H (250MHz; DMSO) 7.28 (10 H, m, Ar), 6.96 (4 H, m, Ar), 6.70 (2 H, m, Ar), 6.64 (s, 2H, 2 x NH), 5.37 (2 H, m, 2 x C*H*CH_2), 4.56 (2 H, d, *J* 11.9, 2 x C*H*HAr), 4.44 (2 H, d, *J* 11.9, 2 x C*H*HAr), 3.69 (2 H, dd, *J* 3.5, 11.9, 2 x C*H*HOBn), 3.65 (2 H, dd, *J* 3.5, 11.9, 2 x C*H*HOBn), 2.27 (12 H, s, 4 x Me); δ_C (62.5MHz; DMSO) 152.6 (2 x s), 138.6 (4 x s), 137.5 (2 x s), 128.3 (4 x d), 127.8 (6 x d), 125.2 (2 x d), 116.3 (4 x d), 73.2 (2 x t), 72.0 (2 x d), 68.4 (2 x t), 21.2 (4 x q); *m/z* (EI) 597 ($M-H^+$), 432 (11%), 342 (5), 234 (4), 181 (9), 165 (20) and 91 (100).

General method of debenzylation of substrates 40-43

Palladium on carbon 10% (0.045 g 10-50% w/w substrate:catalyst) was added to ethanol (100 mL) in a hydrogenation flask. The substrate (0.5 mmol) was then added to the suspension. The flask was filled with H_2 to approximately 30 psi and the reaction mixture was shaken for 24-72 hours. The suspension was then filtered through celite (previously washed with water) and the ethanol removed to yield the deprotected monomers **29**, **30**, **31** and **32**.

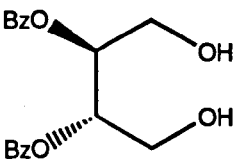
Acetic acid, (2*S*, 3*S*)-2-acetoxy-3-hydroxy-1-hydroxymethyl propyl ester[125]



29

Palladium on carbon (0.045 g) was added to **40** (0.093 g, 0.45 mmol) to give **29** as a yellow oil (0.048 g, 96%). R_f 0.10 (1:2 PE:EtOAc); $[\alpha]^{25}$ +18.1 (*c* 1.0, $CHCl_3$); υ_{max} ($CHCl_3$)/cm^{-1} 3400, 2345, 1731, 1372 and 1250; δ_H (250 MHz; $CDCl_3$) 5.12 (2 H, br s, 2 x C*H*CH_2), 3.69 (4 H, br s, 2 x CHC*H*$_2$), 3.20 (2 H, br s, OH), 2.09 (6 H, s, 2 x Me); δ_C (62.5MHz; $CDCl_3$) 171.0 (2 x s), 72.4 (2 x d), 60.6 (2 x t), 20.8 (2 x q).

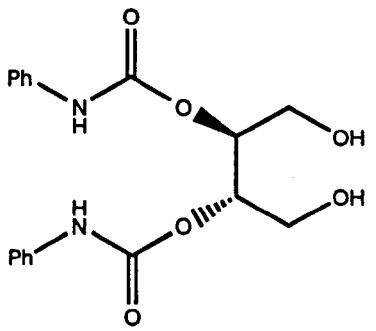
Benzoic acid, (2*S*, 3*S*)-2-benzoyloxy-3-hydroxy-1-hydroxymethyl propyl ester[126]



30

Palladium on carbon (0.097 g) was added to **41** (0.21 g, 4.11 mmol) to give **30** as a colourless oil (0.122 g, 90%). R_f 0.62 (1:4 PE:EtOAc); $[\alpha]^{24}$ +0.32 (*c* 0.8, $CHCl_3$); υ_{max} ($CHCl_3$)/cm^{-1} 3401, 1716, 1521, 1475, 1422 and 1250; δ_H (250 MHz; $CDCl_3$) 7.42 (10 H, m, Ph), 6.30 (2 H, s, 2 x OH), 5.61 (2 H, m, 2 x C*H*CH_2) 3.98 (4 H, m, 2 x CHC*H*$_2$); δ_C (62.5 MHz $CDCl_3$) 166.5 (2 x s), 133.4 (2 x s), 130.0 (4 x d), 129.4 (2 x d), 128.3 (4 x d), 73.2 (2 x d), 60.9 (2 x t); *m/z* (EI) 279 (10%), 167 (20), 149 (55), 119 (65), 107 (60), 105 (29) and 91 (100).

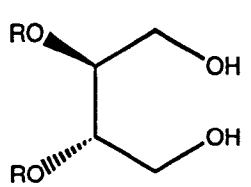
N-Phenylcarbamic acid, (2*S*, 3*S*)-2-N'-phenyl-carbamoxy-3-hydroxy-1-hydroxymethyl propyl ester

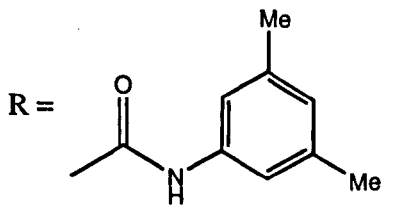


31

Palladium on carbon (0.021 g) was added to **42** (0.107 g, 0.19 mmol) to give **31** as a white solid (0.071 g, 90.2%). R_f 0.06 (1:1 PE:EtOAc); $[\alpha]^{25}$ +11.1 (*c* 0.6, THF); melting point 82-84°C; Found: C 60.29, H 5.79, N 7.34. $C_{18}H_{20}N_2O_6$ requires C 59.99, H 5.59, N 7.77%; υ_{max} (nujol mull)/cm^{-1} 3421, 2923, 2854, 1707, 1598, 1536, 1446, 1376, 1312, 1200 and 1010; δ_H (400 MHz; DMSO) 9.67 (2 H, s, 2 x NH), 7.48 (4 H, d, *J* 7.7, Ph), 7.25 (4 H, t, *J* 7.7, Ph), 6.96 (2 H, t, *J* 7.7, Ph), 5.05 (2 H, m, 2 x C*H*CH_2), 4.96 (2 H, t, *J* 5.4, 2 x OH), 3.59 (4 H, m, 2 x CHC*H*$_2$); δ_C (100.5 MHz; DMSO) 153.6 (2 x s), 139.5 (2 x s), 129.0 (4 x d), 122.4 (2 x d), 118.4 (4 x d), 73.3 (2 x d), 59.9 (2 x t); *m/z* (CI) 361 (6%, M-H^+), 242 (8), 119 (42), 105 (35), 91 (32), 77 (24) and 35 (100).

N-(3,5-Dimethyl)phenylcarbamic acid, (2*S*, 3*S*)-2-N'-(3,5-dimethyl)phenyl-carbamoxy-3-hydroxy-1-hydroxymethyl propyl ester

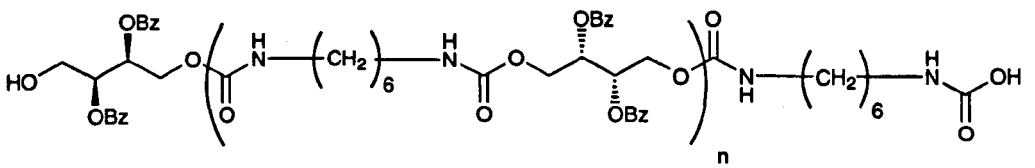




32

Palladium on carbon (0.1 g) was added to **43** (1.0 g, 1.68 mmol) in 250 mL of ethanol to give **32** as a white solid (0.68 g, 98%). melting point >200°C; $[\alpha]^{25}$ +27.5 (*c* 0.3, acetone); Found C 63.06, H 6.72, N 6.63. $C_{22}H_{28}N_2O_6$ requires C 63.45, H 6.78, N 6.73%. δ_H (300 MHz; DMSO) 9.50 (2 H, s, 2 x NH), 7.48 (2 H, s, Ar), 7.05 (2 H, s, Ar), 6.64 (2 H, s, Ar), 4.95 (2 H, s, 2 x C*H*CH_2), 4.92 (2 H, m, 2 x OH), 3.61 (4 H, m, 2 x CHC*H*$_2$), 2.15 (12 H, s, 4 x Me); δ_C (75.5 MHz; DMSO) 152.1 (2 x s), 137.9 (2 x s), 136.3 (4 x s), 122.8 (2 x d), 115.0 (4 x d), 72.1 (2 x d), 58.6 (2 x t), 19.9 (4 x q); *m/z* (CI) 417 (11%, MH^+), 270 (40), 252 (15), 147 (61), 122 (86), 35 (100).

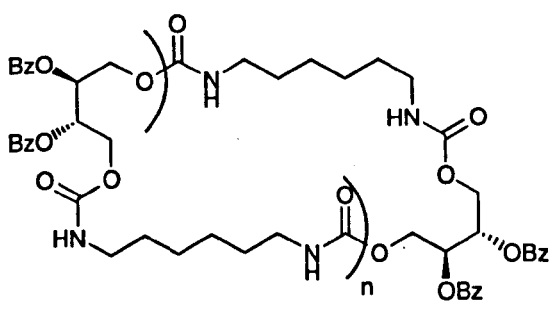
Coupling of (2*S*, 3*S*)-2-benzoyloxy-3-hydroxy-1-hydroxymethyl propyl ester of benzoic acid and hexamethylene diisocyanate



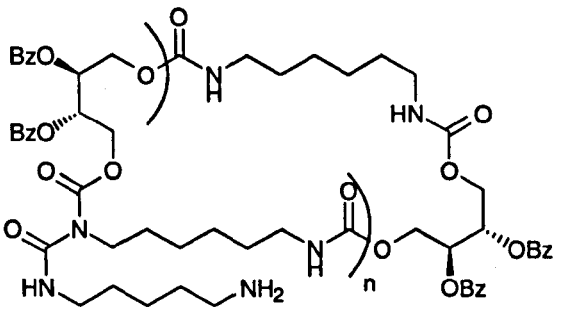
44

Method 1: Hexamethylene diisocyanate (1eq., 0.4 mmol, 66μL) was added to the benzoate diol **30** (0.13 g, 0.4 mmol) in dry pyridine (2 mL) under an atmosphere of nitrogen. The solution was heated at reflux for 12 hours and then left stirring at room temperature for 48 hours. Ether (5 mL) was added, washed with aqueous 10% HCl (3 x 10 mL) and left to evaporate leaving a white solid (0.02 g). υ_{max} ($CHCl_3$)/cm^{-1} 3450, 2932, 2858, 1724, 1518, 1452, 1262, 1100 and 1050; δ_H (250 MHz; d_6-pyridine) 8.00 (4 H, d, *J* 7.5, Ph), 7.50 (2 H, t, *J* 7.5, Ph), 7.38 (4 H, t, *J* 7.5, Ph), 5.66 (2 H, s, 2 x NH), 5.00 (2 H, br m, 2 x C*H*CH_2), 4.38 (4 H, br m, 2 x CHC*H*$_2$), 3.05 (4 H, br m, 2 x NHC*H*$_2$), 1.28 (12 H, br m, 4 x C*H*$_2$$CH_2$); δ_C (62.5 MHz; $CDCl_3$) 165.5 (2 x s), 155.6 (2

x s), 133.3 (2 x s), 129.7 (4 x d), 129.2 (2 x d), 128.4 (4 x d), 70.3 (2 x d), 62.4 (2 x t), 40.7 (2 x t), 29.5 (2 x t), 25.9 (2 x t); *m/z* (MALDI-TOF MS) 521.6 (Na^+, **45** n=0, 521.5), 1020.3 (Na^+, **45** n=1, 1020.1), 1518.3 (Na^+, **45** n=2, 1518.6) plus 663.3 (Na^+, **46** n=0, 662.7), 1162.0 (Na^+, **46** n=0, 1161.3), 1660.5 (Na^+, **46** n=1, 1659.8); GPC 1, THF, M_p 52691, M_n 2165, M_w 16395, polydispersity 7.571.



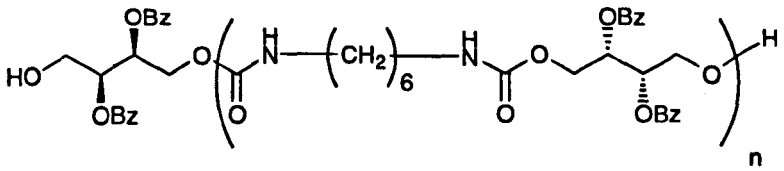
45



46

Method 2: Hexamethylene diisocyanate (1eq., 1.99 mmol, 0.32 mL) was added to a solution of benzoate diol **30** (0.657 g, 1.99 mmol) in dry pyridine (0.9 mL) under an atmosphere of nitrogen. The solution was heated at reflux for 12 hours and the pyridine then removed *in vacuo*. The residue was taken up in dichloromethane and poured in to MeOH. Removal of the solvent *in vacuo* yielded an off-white solid (0.69 g). υ_{max} ($CHCl_3$)/cm^{-1} 3445, 2930, 2854, 1736, 1517, 1451, 1282, 1100 and 1040; δ_H (250 MHz; d_6-pyridine) 8.00 (4 H, d, *J* 7.4, Ph), 7.50 (2 H, t, *J* 7.4, Ph), 7.38 (4 H, t, *J* 7.4, Ph), 5.66 (2 H, s, 2 x NH), 5.00 (2 H, br m, 2 x C*H*CH_2), 4.38 (4 H, br m, 2 x CHC*H*$_2$), 3.05 (4 H, br m, 2 x NHC*H*$_2$), 1.28 (8 H, br m, 4 x C*H*$_2$$CH_2$); δ_C (62.5 MHz; $CDCl_3$) 165.5 (2 x s), 155.6 (2 x s), 133.3 (2 x s), 129.7 (4 x d), 129.2 (2 x d), 128.4 (4 x d), 70.3 (2 x d), 62.4 (2 x t), 40.7 (2 x t), 29.5 (2 x t), 25.9 (2 x t); *m/z* (MALDI) 535.3 (K^+, **45** n=0, 537.5), 1033.8 (K^+, **45** n=1, 1036.1), 1532.8 (K^+, **45** n=2, 1534.6), 2031.5 (K^+, **45** n=3, 2033.1),

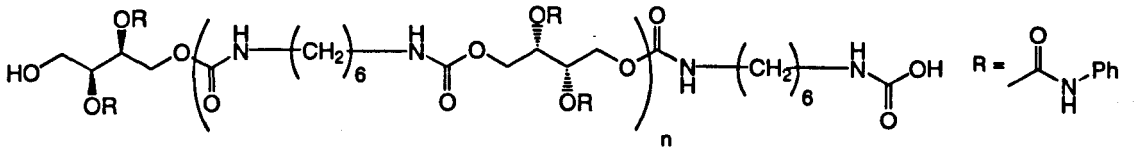
2530.0 (K^+, **45** n=4, 2531.5), 3029.2 (K^+, **45** n=5, 3030.1) and 677.5 (K^+, **46** n=0, 678.7), 1176.3 (K^+, **46** n=1 1177.3), 1674.2 (K^+, **46** n=2, 1675.9) and 851.3 (K^+, **47** n=0, 851.9) and 1349.3 (K^+, **47** n=1, 1349.4); GPC, THF, M_n 5358, polydispersity 3.59.



47

Method 3: Hexamethylene diisocyanate (1eq, 1.67 mmol, 0.27 mL) was added to a solution of benzoate diol **30** (0.55 g, 1.67 mmol) and dibutyl tin dilaurate (0.01eq, $1.67x10^{-2}$ mmol, 10μL) in dry toluene (1.5 mL) under an atmosphere of nitrogen. After heating for 9 hours at 110°C the reaction mixture was left stirring overnight at room temperature. The toluene was removed *in vacuo* and the residue taken up in dichloromethane (10 mL). The resulting solution was poured into methanol and removal of the solvent *in vacuo* yielded an off-white solid (0.20 g). Found C 62.05, H 6.80, N 5.28. $C_{26}H_{30}N_2O_8$ (repeating unit) requires C 62.64, H 6.07, N 5.62%. υ_{max} (Nujol Mull)/cm^{-1} 3250, 2925, 2724, 1733, 1302 and 1153; δ_H (250 MHz; d_6-pyridine) 8.00 (4 H, d, *J* 7.3, Ph), 7.52 (2 H, m, Ph), 7.39 (4 H, t, *J* 7.3, Ph), 5.67 (2 H, s, 2 x NH), 4.99 (2 H, br m, 2 x C*H*CH_2), 4.38 (4 H, br m, 2 x CHC*H*$_2$), 3.05 (4 H, br m, 2 x NHC*H*$_2$), 1.28 (16 H, br m, 4 x C*H*$_2$CH$_2$); δ_C (62.5 MHz; $CDCl_3$) 165.5 (2 x s), 155.6 (2 x s), 133.3 (2 x s), 129.7 (4 x d), 129.2 (2 x d), 128.4 (4 x d), 70.3 (2 x d), 62.5 (2 x t), 40.7 (2 x t), 29.5 (2 x t), 26.0 (2 x t); *m/z* (MALDI-TOF MS) 538.9 (K^+, **45** n=0, 537.5), 1038.7 (K^+, **45** n=1, 1036.1), 1538.8 (K^+, **45** n=2, 1534.6) and other peaks 771.8, 1271.9, 1771.2, 2271.1, 2771.0, 3270.8, 3775.5; GPC, THF, M_n 3068, polydispersity 2.26.

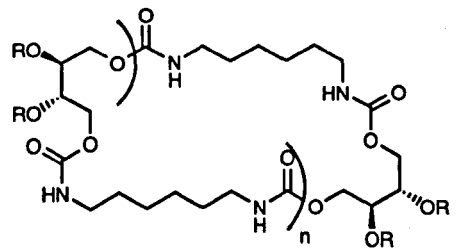
Coupling of (2*S*, 3*S*)-2-N'-phenyl-carbamoxy-3-hydroxy-1-hydroxymethyl propyl ester of N-phenylcarbamic acid and hexamethylene diisocyanate



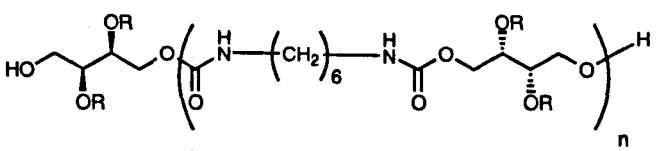
Method 1: Hexamethylene diisocyanate (1eq, 1.99 mmol, 0.32 mL) was added to a solution of phenylcarbamate diol **31** (0.30 g, 0.85 mmol) in dry pyridine (0.8 mL) under an atmosphere of nitrogen. After 20 hours at 110°C the solution was cooled to room temperature and ^{t}butylamine (1 mL) was added. The solution was then poured into stirring MeOH and after two hours filtered through a sinter to yield an off-white solid (0.27 g). Found C 55.71, H 6.19, N 10.24. $C_{24}H_{24}N_4O_8$ (repeating unit) requires C 59.08, H 6.10, N 10.60%. υ_{max} (nujol mull)/cm^{-1} 3321, 2922, 1693, 1460, 1376, 1010 and 1002; δ_H (250 MHz; DMSO) 9.79 (2 H, s, 2 x N*H*Ph), 7.49 (4 H, d, *J* 7.7, Ph), 7.25 (4 H, t, *J* 7.9, Ph), 6.98 (2 H, t, *J* 7.9, Ph), 5.24 (2 H, s, 2 x NH), 4.33 (2 H, m, C*H*CH$_2$), 4.10 (4 H, m, CHC*H*$_2$), 2.91 (6 H, br m, C*H*$_2$NH), 1.25 (12 H, m, C*H*$_2$CH$_2$); δ_C (62.5 MHz; $CDCl_3$) 155.8, 152.9, 139.0, 128.7, 122.7, 118.5, 70.6, 62.4, 29.4, 26.0; *m/z* (MALDI) 758.

Method 2: Hexamethylene diisocyanate (1eq, 1.39 mmol, 0.224 mL) was added to a solution of N-phenylcarbamate diol **31** (0.5 g, 1.39 mmol) and dibutyl tin dilaurate (0.01eq, 1.39x10^{-2} mmol, 8.2 μL) in dry pyridine (1.0 mL) under an atmosphere of nitrogen. After refluxing for 1 hour the solution was left stirring at room temperature overnight. More pyridine (5 mL) was added in an attempt to dissolve the residue which was then poured into stirring MeOH (100 mL). The white precipitate was filtered through a sinter and dried in a vacuum oven at 70°C for 24 hours to yield a white solid (0.44 g).

Found C 58.21, H 6.41, N.8.37 $C_{26}H_{32}N_4O_8$ repeating unit requires C 59.09, H 6.06, N 10.60%; υ_{max} (nujol mull)/cm^{-1} 3321, 2922, 1693, 1460 and 1376; δ_H (250 MHz; DMSO) 9.80 (2 H, s, 2 x PhN*H*), 7.49 (4 H, d, *J* 7.6, Ph), 7.26 (4 H, t, *J* 7.6, Ph), 6.99 (2 H, t, *J* 7.6, Ph), 5.26 (2 H, m, CH_2NH), 4.32 (2 H, m, $CHCH_2$), 4.13 (2 H, m, $CHCH_2$), 2.91 (4 H, m, $NHCH_2$), 1.25 (8 H, m, CH_2CH_2); δ_C (62.5 MHz; $CDCl_3$) 155.8 (2 x s), 152.9 (2 x s), 139.0 (2 x d), 128.7 (4 x d), 122.7 (2 x d), 118.5 (4 x d), 70.6 (2 x d), 62.4 (2 x t), 40.6 (2 x t), 29.4 (2 x t), 26.0 (2 x t); *m/z* (MALDI) 552.1 (Na^+, **48** n=0, 551.6), 569.7 (K^+, **48** n=0, 567.6), 1080.9 (Na^+, **48** n=1, 1080.1), 1097.2 (K^+, **48** n=1, 1096.1), 1609.7 (Na^+, **48** n=2, 1608.7) 1626.9 (K^+, **48** n=2, 1624.7), and 912.1, (Na^+, **49** n=1, 911.9), 929.2 (K^+, **49** n=1, 927.9), 1440.5 (Na^+, **49** n=2, 1440.5), 1458.5 (K^+, **49** n=2, 1456.5), 1969.5 (Na^+, **49** n=3, 1969.1); GPC, THF, M_n 102, polydispersity 12.35.

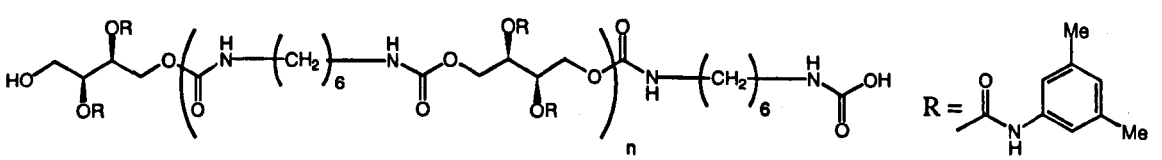


48



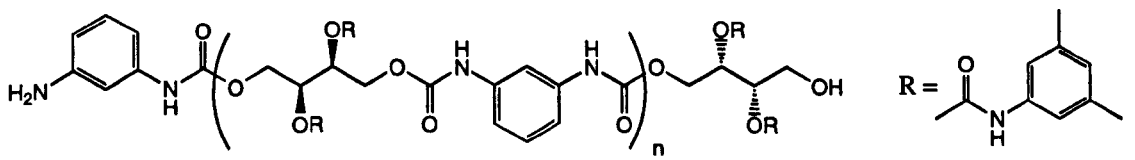
49

Coupling of (2*S*, 3*S*)-2-N'-(3,5-dimethyl)phenylcarbamoxy-3-hydroxy-1-hydroxymethyl propyl ester of N-(3,5-dimethyl)phenylcarbamic acid and hexamethylene diisocyanate



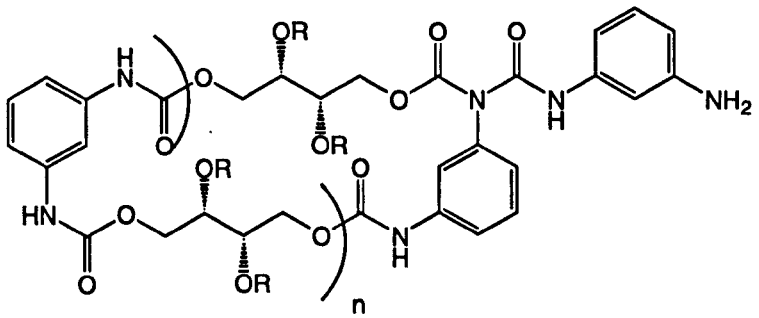
Method 1: Hexamethylene diisocyanate (1eq, 1.17 mmol, 189 μL) was added to a solution of (3,5-dimethyl)phenylcarbamate diol **32** (0.49 g, 1.17 mmol) and dibutyl tin dilaurate (0.01eq, 7.2 x 10^{-3} mmol, 7 μL) in dry pyridine (1.0 mL) under an atmosphere of nitrogen. After heating at 110°C for 24 hours the solution was poured into stirring MeOH (150 mL). The solvent was removed *in vacuo* to yield a white solid (0.66 g). υ_{max} (nujol mull)/cm^{-1} 3289 , 2923, 1805, 1713, 1613, 1459, 1376, 1290 and 1016; δ_H (250 MHz; d_6-acetone) 8.94 (2 H, s, 2 x NH), 8.28 (2 H, m), 7.43 (6 H, m), 6.88 (2 H, m), 4.2-5.51 (10 H, m), 3.38 (4 H, m, 2 x NHCH_2), 2.50 (12 H, s, 4 x Me), 1.75 (4 H, m, 2 x CH_2CH_2), 1.58 (4 H, m, 2 x CH_2CH_2); *m/z* (MALDI) 331.0 (Na^+, **32**-Ar 333.4), 459.8 (K^+, **32** 455.5.), 620.6 and 767.5.

Coupling of (2*S*, 3*S*)-2-N'-(3,5-dimethyl)phenyl-carbamoxy-3-hydroxy-1-hydroxymethyl propyl ester of N-(3,5-dimethyl)phenylcarbamic acid and phenylene diisocyanate



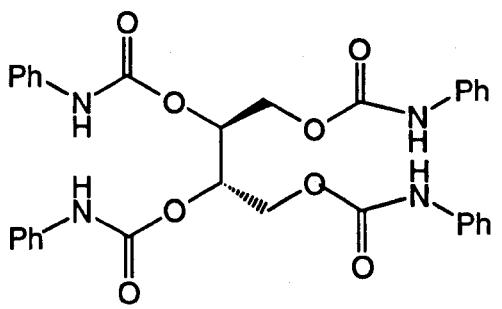
Method: A solution of (3,5-dimethyl)phenylcarbamate diol **32** (0.398 g, 0.96 mmol), 1,3-phenylene diisocyanate (1.0eq, 0.96 mmol, 0.153 g) in dry pyridine (1 mL) was heated at reflux for 19 hours. More pyridine (10 mL) was added to dissolve the resulting viscous mixture and then the solvent removed *in vacuo* to yield a pale brown solid (0.57 g). Found C 55.71, H 6.19, N 10.24. $C_{24}H_{24}N_4O_8$ requires C 57.08, H 6.10, N 10.60%. υ_{max} (nujol mull)/cm^{-1} 3321, 2922, 1713, 1613, 1537, 1457, 1376, 1215, 1095 and 1072; δ_H (250 MHz; d_6-acetone) 9.30 (2 H, s, NH), 9.06 (2 H, s, NH), 8.13 (4 H, m, Ph), 7.54 (4 H,

m, Ph), 7.03 (2 H, m, Ph), 5.76 (2 H, m, 2 x C*H*CH_2), 4.94 (2 H, m, CHCH_2), 4.72 (2 H, m, CHCH_2), 2.17 (12 H, s, 4 x Me); δ_C (62.5 MHz; d_6-acetone) 154.2 (2 x s), 153.9 (2 x s), 140.8 (2 x d), 139.9 (2 x d), 139.3 (4 x d), 130.2 (2 x d), 117.6 (2 x d), 71.8 (2 x d), 63.9 (2 x t), 21.8 (4 x q);; *m/z* MALDI 732.4 (Na^+, **50** n=0, 733.0), 752.3 (K^+, **50** n=0, 749.7), 1331.0 (K^+, **50** n=1, 1326.3), 1909.6 (K^+, **50** n=2, 1902.9); (EI) 975, 725, 711 (**50**-H^+ n=0, 711), 546, electrospray 586.2, 1149.5 and 733.3045 (Na^+, **50** n=0, 733.2602), 1296.5, 1859.7



50

Tetra-(N-phenylcarbamoxy) butanetetrol



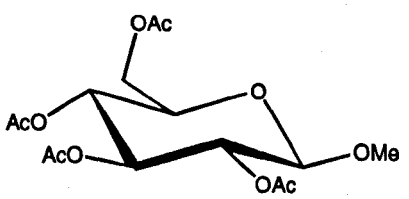
51

Method: Phenyl isocyanate (4eq., 0.014 mol, 1.55 mL,) was added carefully to *DL*-threitol (0.43 g, 3.56 mmol) and heated at reflux in pyridine (5 mL) for 2 hours, under an atmosphere of nitrogen. The white precipitate that was formed was filtered to yield the

desired product **51** as a white solid (1.24 g, 58%). R_f 0.9 (1:1 PE:EtOAc); melting point 230-232°C; Found C 64.09, H 5.02, N 9.32. $C_{32}H_{30}N_4O_8$ requires C 64.21, H 5.02, N 9.36%; δ_H (250 MHz; DMSO) 9.87 (2 H, s, 2 x NH), 9.78 (2 H, s, 2 x NH), 7.47 (8 H, t, *J* 7.5, Ph), 7.27 (4 H, t, *J* 7.5, Ph), 7.26 (4 H, t, *J* 7.5, Ph), 7.00 (2 H, t, *J* 7.5, Ph), 6.99 (2 H, t, *J* 7.5, Ph), 5.41 (2 H, m, 2 x C*H*CH_2), 4.59 (2 H, dd, *J* 2.8, 12.1, 2 x CHC*H*H), 4.30 (2 H, dd, *J* 6.3, 12.1, 2 x CHC*H*H); δ_C (62.5 MHz; DMSO) 153.4 (2 x s), 153.1 (2 x s), 139.2 (4 x s), 129.1 (8 x d), 122.9 (4 x d), 118.9 (8 x d), 70.8 (2 x d), 61.0 (2 x t); *m/z* (EI) 460 (M-$C_7H_6NO_2$ 460), 307 (53%), 289 (12), 277 (38), 185 (80), 149 (34), 110 (51) and 93 (100).

9.4 Experimental for Chapter 4

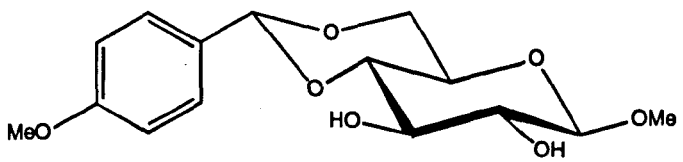
Methyl 2, 3, 4, 6-tetra-O-acetyl-β-*D*-glucopyranoside



Method: Acetyl chloride (8.26 mL, 0.16 mol, 4.5eq) was added to a cooled solution (0°C) of methyl β-*D*-glucopyranoside (5 g, 25.7 mmol) in pyridine (40 mL), that had been stirring for 4 hours under nitrogen. The mixture was left to warm up to room temperature and after 19 hours the reaction was quenched by adding water (20 mL). The aqueous phase was extracted with diethyl ether (2 x 100 mL) and washed with aqueous 10% HCl (3 x 50 mL) and $NaHCO_3$ (100 mL). The organic layer was dried with $MgSO_4$ and the solvent removed *in vacuo* to yield a pale yellow oil (1.1 g, 21%). (R_f 0.55, 1:1 EtOAc:PE); $[\alpha]^{25}$ –16.6 (*c* 1.0, $CHCl_3$); υ_{max} (HCB)/cm^{-1} 2924, 1758, 1446 and 1376; δ_H

(250 MHz; $CDCl_3$) 5.18 (1 H, t, *J* 9.5, H_3), 5.03 (1 H, t, *J* 9.5, H_4), 4.96 (1 H, dd, *J* 7.9, 9.5, H_2), 4.40 (1 H, d, *J* 7.9, H_1), 4.26 (1 H, dd, *J* 4.7, 12.5, H_{6a}), 4.12 (1 H, dd, *J* 2.6, 12.5, H_{6b}), 3.7 (1 H, ddd, *J* 2.6, 4.7, 9.5, H_5), 3.47 (3 H, s, OCH_3), 2.03, 2.03, 2.00, 1.98 (12 H, s, 4 x Me); δ_C (62.5 MHz; $CDCl_3$) 170.5 (s), 170.1 (s), 169.3 (2 x s), 101.5 (s), 72.7 (d), 71.6 (d), 71.1 (d), 68.2 (d), 61.7 (t), 56.9 (q), 20.6 (4 x q).

Methyl 4,6-O-methoxybenzylidene-β-*D*-glucopyranoside[84]

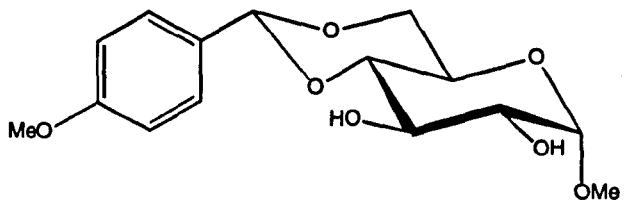


71β

Method: A solution of methyl β-*D*-glucopyranoside (2.5 g, 12.9 mmol), 4-methoxybenzaldehyde acetal (1.5eq., 19.3 mmol, 3.52 g) and *p*-toluenesulphonic acid (0.01eq, 0.13 mmol, 0.03 g) in DMF (12 mL) was rotated on a rotary evaporator, under reduced pressure, at 50°C overnight (10 hours). The temperature was then elevated to 70°C to reduce the volume of DMF to approximately half. The remaining solution was poured into a solution of cold water (50 mL) containing sodium hydroxide (5 mg) which was stirred overnight and then filtered. The solid recovered was dried under vacuum at 50°C to yield **71** (2.47 g, 64%). R_f 0.46 (1:1 PE:EtOAc); melting point 158-165°C; $[\alpha]^{24}$ –74.1 (*c* 1.0, MeOH); Found C 57.28, H 6.45. $C_{15}H_{20}O_7$ requires C 57.69, H 6.45%. υ_{max} (HCB)/cm^{-1} 3353, 2924, 2876 and 1372; δ_H (250 MHz; DMSO) 7.36 (2 H, d, *J* 8.5, Ar), 6.91 (2 H, d, *J* 8.5, Ar), 5.51 (1 H, s, C*H*Ar), 5.36 (1 H, d, *J* 4.8, OH, determined by D_2O exchange), 5.31 (1 H, d, *J* 4.8, OH, determined by D_2O exchange), 4.24 (1 H, d, *J* 8.9, H_1), 4.18 (1 H, t, *J* 8.9, H_2), 3.75 (3 H, s, ArOCH_3), 3.70 (1 H, m, H_4), 3.4 (6 H, m, Me,

H_3, H_{6a}, H_{6b}), 3.09 (1 H, m, H_5); δ_C (62.5 MHz; DMSO) 159.6 (s), 130.2 (s), 127.8 (2 x d), 113.2 (2 x d), 104.6 (d), 100.5 (d), 80.9 (d), 74.3 (d), 72.9 (d), 68.2 (t), 65.9 (d), 56.5 (q), 55.2 (q); *m/z* (FAB) 313 (12%, MH^+), 217 (31), 176 (22), 154 (83), 136 (100), 107 (45) and 89 (63); [literature data for methyl 4,6-O-benzylidene-β-*D*-glucopyranoside: $[\alpha]_D$ -65.2 (*c* 0.96, MeOH); melting point 198-200°C[127]].

Methyl 4,6-O-methoxybenzylidene-α-*D*-glucopyranoside

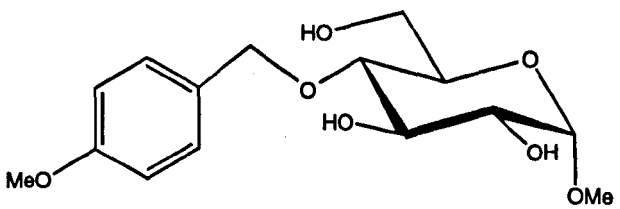


71α

Method[84]: A solution of methyl α-*D*-glucopyranoside (5.0 g, 25.7 mmol), 4-methoxybenzaldehyde acetal (1.5eq., 38.6 mmol, 7.0 g) and *p*-toluenesulphonic acid (0.01eq, 0.26 mmol, 0.05 g) in DMF (25 mL) was rotated on a rotary evaporator, under reduced pressure, at 50°C for 2½ hours. The temperature was elevated to 70°C and the volume of DMF reduced to approximately half. The remaining solution was poured into ice (12 g), saturated aqueous $NaHCO_3$ (25 mL) and diethyl ether (25 mL) and a slurry was formed due to the formation of a white precipitate. The water was removed using a freeze drier and the solid recrystallized using methanol and ether to yield **71α** as a white solid (5.7 g, 71%). R_f 0.06 (1:1 PE:EtOAc) melting point 196-200°C; $[\alpha]^{25}$ +82.5 (*c* 1.0, d_4-MeOH); Found C 57.57, H 6.47. $C_{15}H_{20}O_7$ requires C 57.69, H 6.45%; υ_{max} (HCB)/cm^{-1} 3355, 2922, 2866 and 1371; δ_H (250 MHz; DMSO) 7.36 (2 H, d, *J* 8.9, Ar), 6.91 (2 H, d, *J* 8.9, Ar), 5.51 (1 H, s, C*H*Ar), 5.13 (2 H, broad s, 2 x OH), 4.63 (1 H, d, *J* 4.0, H_1), 4.14 (1 H, dd, *J* 4.0, 9.5, H_2), 3.75 (3 H, s, ArOCH_3), 3.57 (3 H, m, H_4, H_{6a},

H_{6b}), 3.36 (2 H, m, H_3, H_5), 3.31 (3 H, s, OMe); δ_C (100.5 MHz; DMSO) 160.9 (s), 130.8 (s), 129.5 (2 x d), 114.6 (2 x d), 103.2 (d), 101.9 (d), 79.6 (d), 73.5 (d), 73.3 (d), 68.9 (t), 67.0 (d), 57.4 (q), 55.5 (q); *m/z* (EI) 313 (29%, MH^+), 273 (12), 154 (80), 136 (100), 107 (40) and 89 (59); [lit.,[89] for methyl 4,6-O-benzylidene-α-*D*-glucopyranoside: melting point 163-164°C; $[\alpha]_D$ +119.0 (*c* 1.1, $CHCl_3$)].

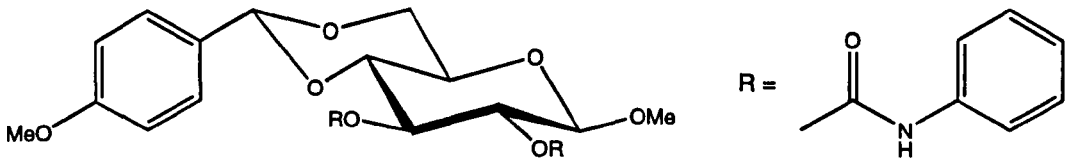
Methyl 4-O-methoxybenzyl-α-*D*-glucopyranoside[84]



72

Method: A cooled solution (0°C) of trimethylsilylchloride (6eq, 9.6 mmol, 1.22 mL) in acetonitrile (5 mL) was added to a solution of **71α** (0.5 g, 1.6 mmol), sodium cyanoborohydride (6eq, 9.6 mmol, 0.6 g) and molecular sieves (3 Å) in dry acetonitrile (10 mL). After stirring at room temperature for 18 hours the reaction mixture was filtered into sodium hydrogen carbonate (30 mL). The filtrate was extracted with dichloromethane (3 x 25 mL) and the combined organic layers washed with $NaHCO_3$ (3 25 mL), water (1 x 50 mL) and dried with $MgSO_4$. The solvent was removed *in vacuo* to yield **72** as a yellow oil that was purified by column chromatography using 9:1 CH_2Cl_2:PE as eluent (0.02 g, 2%). δ_H (300 MHz; D_2O) 7.41 (2 H, d, *J* 8.5, Ar), 7.22 (2 H, d, *J* 8.5, Ar), 4.72 (1 H, d, *J* 3.6, H_1), 4.18 (2 H, s, ArCH_2), 3.76 (1 H, dd, *J* 2.1, 12.3, H_{6a}), 3.64 (1 H, dd, *J* 5.3, 12.3, H_{6b}), 3.56 (2 H, m, H_3 ,H_5), 3.45 (1 H, dd, *J* 3.6, 9.6, H_2), 3.31 (4 H, m, H_4, ArOCH_3), 3.24 (3 H, s, OMe).

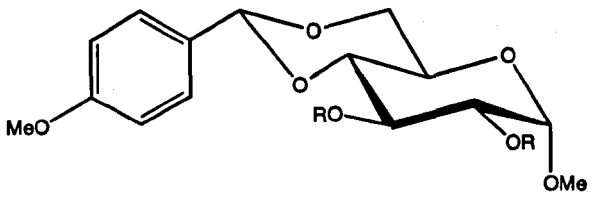
Methyl 2,3-di-O-(phenyl carbamate)-4,6-O-methoxybenzylidene-β-*D*-glucopyranoside[84]

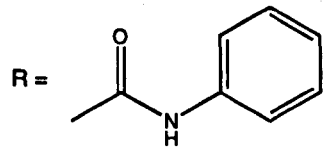


73β

Method: Phenyl isocyanate (1.04 mL, 9.6 mmol, 3eq) was added to a solution of **71β** (1.0 g, 3.2 mmol) in dry pyridine (5 mL), under nitrogen, and was heated at 100°C for 16 hours. The reaction was quenched by adding methanol (2 mL) and stirring for 10 minutes. On addition of ethyl acetate (20 mL) and water (20 mL) a precipitate was formed and filtered off to yield a white solid (0.12 g, 72%). The filtrate was dried and recrystallized from methanol to yield a white solid **73β** (0.06 g, 32%). $[\alpha]^{26}$ –93.5 (*c* 1.0, THF); melting point 210-212°C; Found C 63.35, H 5.52, N 5.24. $C_{29}H_{30}N_2O_9$ requires C 63.27, H 5.51, N 5.09%; υ_{max} (HCB)/cm^{-1} 3291, 2923, 1712, 1227, 880 and 830; δ_H (400 MHz; d_6-acetone) 8.79 (1 H, s, NH), 8.75 (1 H, s, NH), 6.80-7.60 (8 H, m, Ar), 5.60 (1 H, s, C*H*Ar), 5.31 (1 H, t, *J* 9.6, H_3), 4.94 (1 H, dd, *J* 9.6, 7.9, H_2), 4.69 (1 H, d, *J* 7.9, H_1), 4.30 (1 H, dd, *J* 4.9, 10.2, H_{6a}), 3.84 (1 H, t, *J* 10.2, H_{6b}), 3.80 (1 H, t, *J* 9.6, H_4), 3.74 (3 H, s, ArOCH_3), 3.67 (1 H, m, H_5), 3.40 (3 H, s, OMe); δ_C (62.5 MHz; d_6-acetone) 161.0 (s), 153.6 (s), 153.3 (s), 140.8 (s), 139.8 (s), 130.9 (s), 129.5 (4 x d), 128.4 (2 x d), 123.6 (2 x d), 119.3 (4 x d), 114.1 (2 x d), 103.2 (d), 102.0 (d), 79.6 (d), 73.5 (d), 73.2 (d), 68.9 (t), 67.0 (d), 57.0 (q), 55.4 (q); *m/z* (EI) 551 (6%, MH^+), 433 (7, $M\text{-}R^+$), 307 (32), 213 (31), 154 (100), 136 (91), 107 (69) and 105 (46).

Methyl 2,3-di-O-(phenyl carbamate)-4,6-O-methoxybenzylidene-α-*D*-glucopyranoside

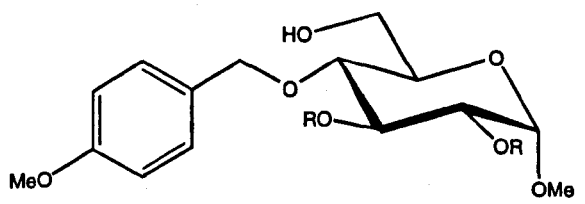


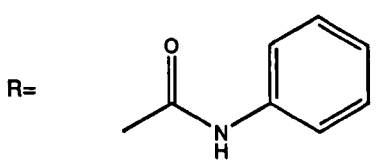


73α

See method for synthesis of **73β** using **71α** as the starting material. Found C 62.48, H 5.50, N.5.13. $C_{29}H_{30}N_2O_9$ requires C 63.27, H 5.47, N 5.09%; δ_H (300 MHz; $CDCl_3$) 9.10 (1 H, s, NH), 8.74 (1 H, s, NH), 7.4-6.7 (14 H, m, Ar), 5.59 (1 H, s, *CH*Ar), 5.50 (1 H, t, *J* 9.8, H_3), 5.06 (1 H, d, *J* 3.8, H_1), 4.97 (1 H, dd, *J* 3.8, 9.8, H_2), 4.34 (1 H, m, H_{6a}), 3.98 (1 H, m, H_5), 3.78 (5 H, m, H_4, H_{6b}, ArOCH_3), 3.47 (3 H, s, OMe); δ_C (75.5 MHz; $CDCl_3$) 160.5 (2 x s), 129.5 (4 x s), 129.4 (4 x d), 128.0 (2 x d), 123.9 (2 x d), 119.1 (4 x d), 114.1 (2 x d), 102.1 (d), 98.5 (d), 79.6 (d), 72.7 (d), 70.7 (d), 69.2 (t), 62.9 (d), 56.0 (q), 55.7 (q).

Methyl 2,3-di-O-(phenyl carbamate)-6-O-(*p*-methoxybenzyl)-α-*D*-glucopyranoside



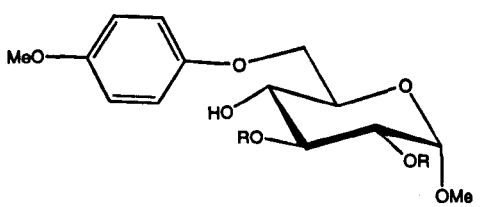


74

Method[91]: To a solution of **73** (0.2 g, 0.36 mmol) in dichloromethane (30 mL), which had been cooled to -40°C, a solution of diisobutylaluminium hydride (DIBAL) in hexane (5eq, 1.8 mL of 1.0 M solution) was added. After stirring for 2 hours the mixture was

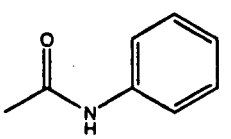
added to methanol (50 mL). The aqueous layer was extracted with ether (3 x 25 mL) and the combined organic extracts washed with $NaHCO_3$ (2 x 25 mL) and water (1 x 50 mL). The organic layer was dried using $MgSO_4$ and the solvent removed *in vacuo* to yield a white solid (0.15 g, 78%), which was purified by flash chromatography (eluent 10:1 $CHCl_3$:EtOAc).

Methyl 2,3-di-O-(phenyl carbamate)-4-O-(*p*-methoxy)benzyl-α-*D*-glucopyranoside **74**, R_f 0.29 (28% yield); δ_H (300 MHz; $CDCl_3$) 7.28-6.63 (16 H, m, Ar, NH), 5.53 (1 H, t, *J* 9.8, H_3), 5.42 (1 H, s, ArC*H*), 4.98 (1 H, d, *J* 3.8, H_1), 4.92 (1 H, dd, *J* 3.8, 9.8, H_2), 4.23 (1 H, dd, *J* 4.6, 10.1, H_{6a}), 3.91 (1 H, m, H_5), 3.70 (2 H, m, H_4, H_{6b}), 3.59 (3 H, s, ArOCH_3), 3.36 (3 H, s, OMe); δ_C (75.5 MHz; $CDCl_3$) 160.5 (s), 153.0 (2 x s), 137.9 (2 x s), 130.1 (s), 129.6 (4 x d), 128.0 (2 x d), 124.1 (2 x d), 120.0 (4 x d), 114.0 (2 x d), 102.2 (d), 98.5 (d), 79.6 (d), 72.8 (d), 70.7 (t), 69.1 (t), 62.9 (d), 55.9 (q), 55.6 (q).



75

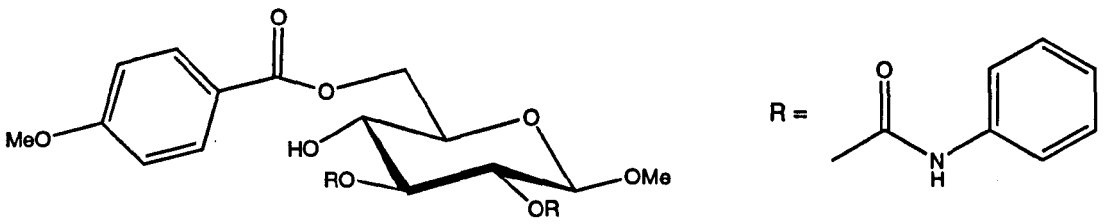
R=



Methyl 2,3-di-O-(phenyl carbamate)-6-O-(*p*-methoxybenzyl)-α-*D*-glucopyranoside **75**, R_f 0.21 (19% yield); δ_H (300 MHz; $CDCl_3$) 7.36-6.75 (14 H, m, Ar), 5.43 (1 H, s, ArC*H*), 4.97 (1 H, d, *J* 3.8, H_1), 4.74 (1 H, dd, *J* 3.8, 9.6, H_2), 4.20 (2 H, m, H_3, H_{6a}), 3.81 (5 H, m, ArOCH_3, H_5, H_{6b}), 3.53 (1 H, m, H_4), 3.34 (3 H, s, OMe); δ_C (75.5 MHz; $CDCl_3$) 160.7 (s), 154.3 (2 x s), 138.0 (2 x s), 129.8 (s), 129.4 (4 x d), 128.1 (2 x d), 124.1 (2 x

d), 119.1 (4 x d), 114.0 (2 x d), 102.4 (d), 100.7 (d), 81.7 (d), 74.5 (d), 69.3 (t), 69.2 (t), 63.2 (d), 56.0 (q), 55.7 (q).

Methyl 2,3-di-O-(phenyl carbamate)-6-O-(*p*-methoxybenzoyloxy)-β-*D*-glucopyranoside

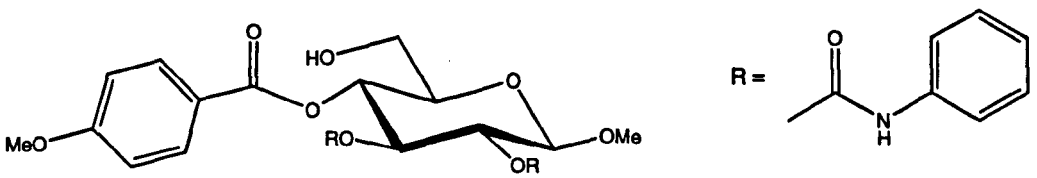


80

Method[93]: A suspension of **73β** (0.1 g, 0.18 mmol) in toluene (5 mL) and acetic acid (0.25 mmol, 14 μl) was stirred at room temperature for 5 minutes. 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ) (0.25 mmol, 0.058 g, 1.5eq.) was then added and the temperature was raised to 80°C. After 24 hours ethyl acetate (50 mL) was added and the solution was filtered through prewashed Celite. The filtrate was washed with sodium hydrogen carbonate (3 x 50 mL), water (1 x 50 mL) and dried with $MgSO_4$ before removing the solvent in *vacuo* to yield a pink solid (0.10 g, 99%). Purification was attempted using column chromatography, eluent PE:EtOAc 4:1 v/v.

Methyl 2,3-di-O-(phenyl carbamate)-6-O-(*p*-methoxy)benzoyloxy-β-*D*-glucopyranoside **80**, R_f 0.34 (22% yield); $[\alpha]^{25}$ +15.6 (*c* 0.35, $CHCl_3$); υ_{max} (HCB)/cm^{-1} 3519, 3344, 2941, 2869, 1709, 1539, 1446, 1318, 1254 and 1098; δ_H (400 MHz; d_6-acetone) 8.72 (2 H, s, NH), 8.00 (2 H, d, *J* 8.3, Ar), 7.53 (4 H, d, *J* 8.3, Ar), 7.25 (4 H, t, *J* 8.3, Ar), 7.03 (2 H, m, Ar), 6.97 (2 H, m, Ar), 5.14 (1 H, t, *J* 9.7, H_3), 5.05 (1 H, d, *J* 5.5, OH), 4.86 (1 H, dd,

J 8.0, 9.7, H_2), 4.69 (1 H, dd, *J* 2.0, 11.9, H_{6a}), 4.62 (1 H, d, *J* 8.0, H_1), 4.51 (1 H, dd, *J* 5.4, 11.9, H_{6b}), 3.88-3.84 (4 H, m, ArOCH_3, H_5), 3.82 (1 H, m, H_4), 3.45 (3 H, s, OMe); δ_C (62.5 MHz; d_6-acetone) 164.5 (s), 154.3 (2 x s), 153.6 (2 x s), 140.2 (2 x s), 132.6 (4 x d), 129.8 (2 x d), 123.8 (2 x d), 119.6 (4 x d), 114.9 (2 x d), 102.9 (d), 77.4 (d), 75.2 (d), 73.3 (d), 70.5 (t), 64.4 (d), 57.0 (q), 56.2(q); *m/z* (EI) 567 (12 %, MH^+), 535 (23, M-OMe^+), 307 (42), 289 (30), 154 (93), 135 (100), 120 (60) and 90 (74).

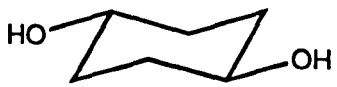


81

Methyl 2,3-di-O-(phenyl carbamate)-4-O-(*p*-methoxy)benzoyloxy-β-*D*-glucopyranoside **81**, R_f 0.23 (14% yield); $[\alpha]_D$ -37.1 (*c* 0.26, $CHCl_3$); υ_{max} (HCB)/cm^{-1} 3464, 3320, 2937, 2850, 1711, 1445, 1247 and 1086; δ_H (400 MHz; d_6-acetone) 8.77 (2 H, s, NH), 7.96 (2 H, d, *J* 9.2, Ar), 7.38 (12 H, m, Ar), 5.46 (1 H, t, *J* 9.8, H_3), 5.20 (1 H, t, *J* 9.8, H_4), 4.96 (1 H, dd, *J* 7.9, 9.8, H_2), 4.70 (1 H, d, *J* 7.9, H_1), 3.88 (2 H, m, H_5, OH), 3.83 (3 H, s, ArOCH_3), 3.70 (2 H, m, H_{6a}, H_{6b}), 3.50 (3 H, s, OMe); *m/z* 567 (8%, MH^+), 535 (12, M-OMe^+), 307 (59), 289 (32), 154 (100), 135 (92), 120 (81) and 90 (74).

9.5 Experimental for Chapters 5, 6 and 7

Trans-1,4-Cyclohexanediol

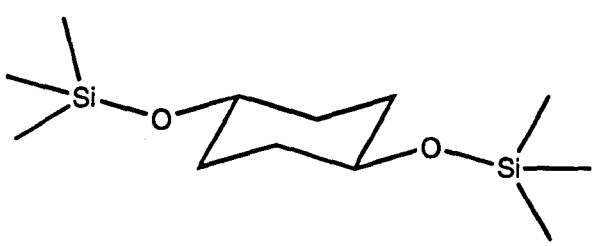


85

Method: A solution of *trans*-1,4-diacetoxycyclohexanediol **86** (9.6 g, 47.5 mmol) in methanol (50 mL) was stirred at room temperature with sodium methoxide (0.34 g, 6.34 mmol, 2/15 eq.) for approximately 3 days, until no starting material was visible by TLC (using EtOAc as eluent and $KMnO_4$ as visualiser, product R_f 0.16). When the reaction had reached completion, activated Dowex was added to the reaction mixture and stirred until neutral pH was achieved. The solution was then filtered through fluted filter paper and the solvent removed *in vacuo* to yield **85** as a white solid (3.2 g, 90%). υ_{max} (HCB)/cm^{-1} 3279, 2937, 2860, 1451 and 1335; δ_H (250 MHz; D_2O) 3.61 (2 H, m, 2 x C*H*CH_2), 1.87 (4 H, m, 4 x CHCH*H*), 1.28 (4 H, m, 4 x CHC*H*H); δ_C (62.5MHz; D_2O) 70.0 (2 x d), 32.4 (4 x t); m/z (EI) 116 (3%, M^+), 98.0730 (100, M-H_2O^+ $C_6H_{10}O$ requires 98.0732), 83 (65), 69 (85), 58 (85) and 41 (71).

Trans-1,4-bis(trimethylsilyl)cyclohexanediol

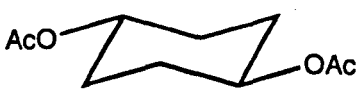
Trans-1,4-Di[trimethylsiloxy]cyclohexane



105

Method[118]: Trimethylsilylazide (1.28 mL, 9.64 mmol, 2.8eq) was added to a solution of *trans*-1,4-cyclohexanediol (0.4 g, 3.44 mmol) in anhydrous THF (10 mL) under an atmosphere of argon. After stirring at room temperature for $1^1/_2$ hours TLC analysis (ethyl acetate as eluent, $KMnO_4$ as visualizer, R_f product 0.61) showed the reaction to be almost complete. The solvent was removed *in vacuo* and the product (0.68 g, 76%) was purified by flash chromatography (2:1 hexane:EtOAc) to afford a white solid (0.62 g, 70%). Melting point 44-45°C [lit.,[127] 53-54°C]; Found C 55.04, H 10.65. $C_{12}H_{28}O_2Si_2$ requires C 55.34, H 10.83%; υ_{max} ($CDCl_3$ liquid cell)/cm^{-1} 2944, 2862, 1469, 1382; δ_H (250 MHz; $CDCl_3$) 3.54 (2 H, m, 2 x C*H*CH_2), 1.8 (4 H, m, 4 x CHCH*H*), 1.32 (4 H, m, 4 x CHCH*H*), 0.09 (18 H, s, 6 x Me); δ_C (62.5 MHz; $CDCl_3$) 70.7 (2 x d), 34.0 (4 x t), 0.54 (6 x q).

***Trans*-1,4-diacetoxycyclohexanediol**

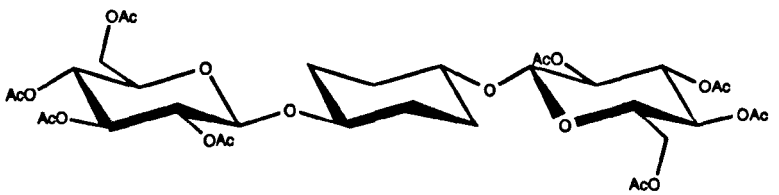


86

Method: A solution of 1,4-cyclohexanediol (20 g, 0.17 mol, mixture of *cis* and *trans*) in 1:1 v/v mixture of pyridine:acetic anhydride (total volume 200 mL) was stirred at room temperature for 21 hours, until no starting material was visible by TLC (using EtOAc as eluent and H_2SO_4 in MeOH as visualiser, R_f product 0.65). The solution was reduced *in vacuo* and the syrup taken up in dichloromethane (50 mL) which was washed with water (2 x 50 mL), 10% aqueous HCl (3 x 25 mL), water (1 x 50 mL) and brine (1 x 50 mL). After drying with $MgSO_4$ the solvent was removed *in vacuo* to give a white solid **86** (33.6 g, 96%). The *cis* and *trans* products were separated by recrystallization with warm

hexane, the first crop of crystals having a melting point of 93°C (lit 95°C) in 28% yield. $[\alpha]^{26}$ -0.12 (*c* 1.8, CH_2Cl_2); Found C 59.97, H 8.07. $C_{10}H_{16}O_4$ requires C 60.00 H 8.06%; υ_{max} (CH_2Cl_2)/cm^{-1} 2954, 1726 and 1351; δ_H (250 MHz; $CDCl_3$) 4.38 (2 H, m, 2 x $CHCH_2$), 1.99 (6 H, s, 2 x Me), 1.94 (4 H, m, $CHCH_2$), 1.48 (4 H, m, $CHCH_2$), δ_C (62.5 MHz; $CDCl_3$) 170.7 (2 x s), 71.2 (2 x d), 28.4 (4 x t), 21.5 (2 x q).

Bis(2,3,4,6-tetra-*O*-acetyl-β-*D*-glucopyranosyl) cyclohexanediol



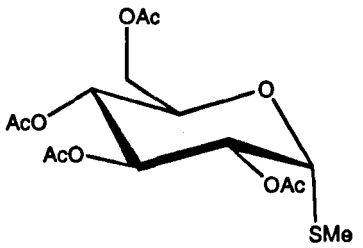
91

Attempted methods

Triflic acid (1 mL of 0.15 M solution) was added to a solution of phenyl 2,3,4,6-tetra-*O*-acetyl 1-thio-β-*D*-glucopyranoside (0.20 g, 0.41 mmol), 1,4-*trans*-cyclohexanediol (0.6eq., 0.03 g, 0.24 mmol) and NIS (2.5eq., 0.23 g, 0.10 mmol) in dry dichloromethane (3 mL). There was a colour change from colourless to pale pink and after 10 minutes the solution was dark red in colour. TLC indicated no starting material was left so the reaction was diluted with dichloromethane and washed with $NaHCO_3$ (3 x 10 mL) and NaS_2O_4 10% w/v in water (2 x 10 mL). After drying with $MgSO_4$ the solvent was removed *in vacuo* to yield an orange oil (0.27 g, 99%) which was subjected to column chromatography but isolation of the desired product was not possible. m/z MALDI-MS 1376.4 (GGG-C-G +Na 1376.3), 1334.6 (GGG-C-G-1Ac +Na 1334.3), 1292.6 (GGG-C-G-2Ac +Na 1292.2), 1088.5 (GG-C-G +Na 1088.0), 1046.2 (GG-C-G-1Ac +Na 1046.0), 1002.2 (GG-C-G-2Ac +Na 1003.9), 990.2 (GGG +Na 990.0), 948.4 (GGG-1Ac +Na

948.0), 905.7 (GGG-2Ac +Na 905.9), 799.4 (G-C-G +Na 799.7), 758.0 (G-C-G-1Ac +Na 757.7), 716.6 (G-C-G-2Ac +Na 715.6), 701.4 (GG +Na 701.6), 511.6 (G-C-Ac +Na 511.4).

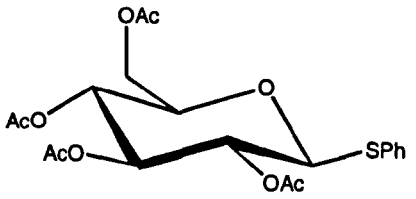
Methyl 2,3,4,6-tetra-*O*-acetyl 1-thio-α-glucopyranoside[111]



95

Method: Cooled acetic acid (0.5 mL) was added to a suspension of *D*-glucose (0.18 g, 1 mmol) in acetyl bromide (1 mL), while being stirred. The mixture was then stirred for 4 hours at room temperature. The excess reagents were evaporated *in vacuo* at 20-30°C, followed by evaporation with toluene (5 mL) to yield an orange oil. Cold aqueous MeSNa (2 mL 15%) was added into a solution of the acetobromo sugar in acetone (3.5 mL) under stirring at 0°C. The mixture was then stirred for 40 hours at room temperature. Removal of the solvent *in vacuo* at 50°C gave a solid material which was heated in acetic anhydride (3 mL) containing sodium acetate (0.1 g) under stirring at 100°C. The solvent was removed *in vacuo* after 18 hours to yield **95** as a brown solid (0.20 g, 50%). R_f 0.52 (1:1 PE:EtOAc) $[\alpha]^{25}$ –10.2 (*c* 0.5, $CHCl_3$) (lit.,[111] $[\alpha]_D$ –15 (*c* 0.9, $CHCl_3$)); δ_H (250 MHz; $CDCl_3$) 5.15 (1 H, t, *J* 9.6, H_3), 5.03 (2 H, m, H_2, H_4), 4.36 (1 H, d, *J* 8.8, H_1), 4.22 (1 H, m, H_{6a}), 4.11 (1 H, m, H_{6b}), 3.70 (1 H, m, H_5), 2.12, 2.04, 2.02, 1.99, 1.97 (15 H, s, 5 x Me); δ_C (62.5 MHz; $CDCl_3$) 170.9 (s), 170.5 (s), 169.8 (s), 169.9 (s), 83.6 (d), 74.7 (d), 72.1 (d), 68.5 (d), 67.7 (d), 61.6 (t), 21.0 (5 x q).

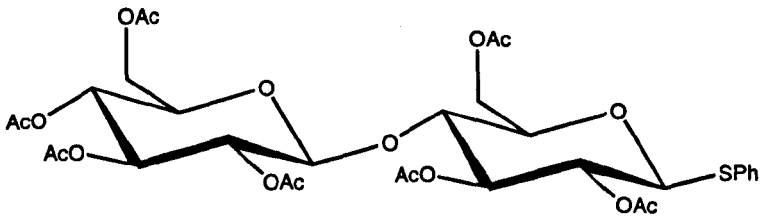
Phenyl 2,3,4,6-tetra-*O*-acetyl 1-thio-β-*D*-glucopyranoside



96

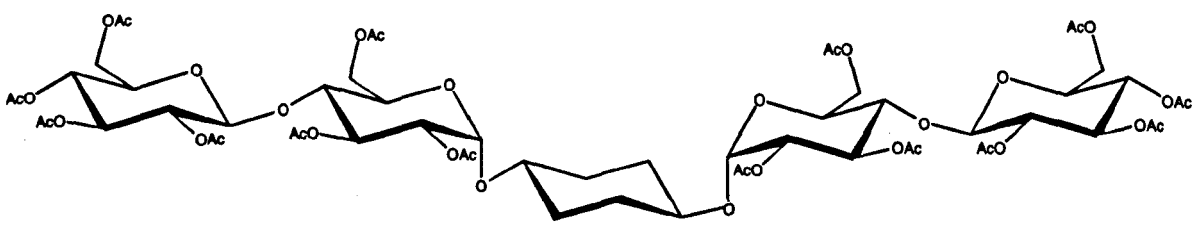
Method[128]: To a solution of β-*D*-glucose pentaacetate (2.0 g, 5.12 mmol) in dry dichloromethane (5 mL) was added thiophenol (1.5eq., 0.85 g, 7.69 mmol), followed by boron trifluoride etherate (0.4eq., 0.25 mL, 2.05 mmol). After stirring at room temperature for 25 hours the solution was diluted with dichloromethane and washed with $NaHCO_3$ (2 x 20 mL) and water (1 x 20 mL). The organic layer was dried using $MgSO_4$ and the solvent removed *in vacuo* to yield **96** as a white solid (1.59 g, 63%). The product was purified by recrystallization with ethyl acetate affording white, needle-like crystals. Rf 0.58 (1:1 PE:EtOAc); $[\alpha]^{27}$ –13.9 (*c* 1.0, $CHCl_3$); Found C 54.65, H 5.47. $C_{20}H_{24}O_9S$ requires C 54.55, H 5.45%; δ_H (250 MHz; $CDCl_3$) 7.48 (2 H, m, Ph), 7.30 (3 H, m, Ph), 5.20 (1 H, t, *J* 9.9, H_3), 5.02 (1 H, t, *J* 9.9, H_4), 4.95 (1 H, t, *J* 9.9, H_2), 4.68 (1 H, d, *J* 9.9, H_1), 4.18 (2 H, m, H_{6a}, H_{6b}), 3.72 (1 H, m, H_5), 2.07-1.97 (12 H, s, 4 x Me); δ_C (62.5 MHz $CDCl_3$) 170.9 (s), 170.5 (s), 169.8 (s), 169.9 (s), 133.4 (2 x d), 131.9 (s), 129.3 (2 x d), 128.8 (d), 86.1 (d), 76.2 (d), 74.3 (d), 70.3 (d), 68.7 (d), 62.5 (t), 21.0 (4 x q); m/z (MALDI) 463.4 (Na^+ 463.5).

Phenyl (2,3,4,6-tetra-*O*-acetyl-β-*D*-glucopyranosyl)-2,3,6-tri-*O*-acetyl-1-thio-β-*D*-glucopyranoside



Method[128]: To a solution of β-*D*-cellobiose octaacetate (1.0 g, 1.5 mmol) in dry dichloromethane (5 mL) was added thiophenol (1.5eq., 0.23 mL, 2.4 mmol), followed by boron trifluoride etherate (0.4eq., 0.11 mL, 0.8 mmol). After stirring at room temperature for 20 hours the solution was diluted with dichloromethane and washed with $NaHCO_3$ (2 x 25 mL) and water (2 x 25 mL). The organic layer was dried using $MgSO_4$ and the solvent removed *in vacuo* to yield a white solid (0.9 g, 83%) containing a mixture of α and β anomers as well as starting material. m/z (MALDI) 751.4 (+Na 751.6), 701.4 (starting material +Na 701.6), 717.0 (starting material +K 717.7), 637.0.

***Trans*-1,4-bis(2,3,4,6-tetra-*O*-acetyl-β-*D*-glucopyranosyl)-(1→4)-(2,3,6-tri-*O*-acetyl-α-*D*-glucopyranosyl) cyclohexanediol**



99

Method: Tin tetrachloride (2.1 mL, 18 mmol, 2.5eq) was added to a cooled solution (0°C) of cellobiose octaacetate (5.0 g, 7.4 mmol) and *trans*-1,4-cyclohexandiol (0.52 g, 4.42

mmol, 0.6eq) in anhydrous dichloromethane (20 mL) under an atmosphere of nitrogen. The reaction mixture was allowed to warm up to room temperature and after stirring for 35 hours was quenched by pouring into aqueous $NaHCO_3$ (80 mL) and dichloromethane (50 mL) and stirred vigorously for 30 minutes. The organic phase was separated and the aqueous phase extracted with more dichloromethane (2 x 100 mL). The organic extractions were combined and washed with water (3 x 100 mL), brine (1 x 100 mL) and dried with $MgSO_4$ to yield a brown solid (4.33 g, 85%). Purification by flash chromatography (2:1 EtOAc:PE R_f product 0.27) afforded **99** as a pale yellow crystalline solid (1.6 g, 32%) and the mono-substituted cyclohexanediol **100** (0.9 g, 20%).

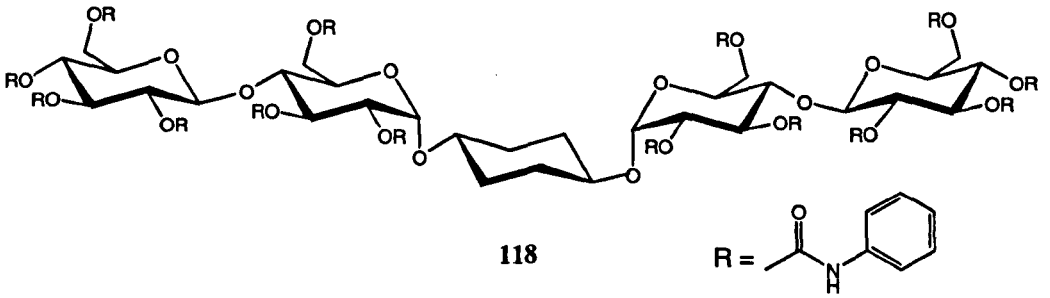
Data for **99**: melting point 213-215°C; $[\alpha]^{26}$ +57.8 (*c* 0.17, acetone); Found C 51.28, H 6.05. $C_{58}H_{80}O_{36}$ requires C 51.50, H 5.96%; υ_{max} (Nujol Mull)/cm^{-1} 2916, 1743, 1459, 1375, 1226 and 1030; δ_H (250 MHz; $CDCl_3$) 5.37 (2 H, t, *J* 9.8, H_3), 5.21 (2 H, m, $H_{3'}$), 5.12 (2 H, d, *J* 3.9, H_1), 5.03 (2 H, t, *J* 9.8, $H_{4'}$), 4.82 (4 H, m, $H_{1'}$, $H_{2'}$), 4.69 (2 H, dd, *J* 3.9, 9.8, H_2), 4.50 (2 H, dd, *J* 1.5, 12.1, H_{6a}), 4.37 (2 H, dd, *J* 4.3, 12.1, $H_{6'a}$), 4.15 (2 H, m, H_{6b}), 4.04 (4 H, m, H_5, $H_{6'b}$), 3.98 (2 H, m, $H_{5'}$), 3.84 (2 H, t, *J* 9.8, H_4), 3.66 (2 H, broad m, 2 x C*H*CH_2) 2.07, 2.03, 2.02, 1.99, 1.98, 1.96, 1.91 (42 H, 14 x CH_3), 1.51 (4 H, m, 4 x CHCH*H*), 1.37 (4 H, m, 4 x CHCH*H*); δ_C (62.5 MHz; $CDCl_3$) 170.8 (2 x s), 170.7 (2 x s), 170.6 (2 x s), 170.3 (4 x s), 170.1 (2 x s), 169.7 (2 x s), 101.4 (2 x d), 94.9 (2 x d), 77.8 (2 x d), 76.0 (2 x d), 73.6 (2 x d), 73.3 (2 x d), 72.4 (2 x d), 72.3 (2 x d), 71.8 (2 x d), 70.2 (2 x d), 68.8 (2 x d), 62.9 (2 x t), 62.4 (2 x t), 28.3 (4 x t), 20.7-20.4 (14 x q); m/z (MALDI) 1376.1 (+Na 1376.3), 1392.2 (+K 1392.3).

***Trans*-1,4-bis[(β-*D*-glucopyranosyl)-(1→4)-(α-*D*-glucopyranosyl)] cyclohexanediol**

111

Method: A solution of **99** (0.5 g, 0.36 mmol) in methanol was stirred at room temperature with a catalytic amount of sodium methoxide (0.06eq, 3 mg, 2.4×10^{-2} mmol). After 4 hours the reaction was worked up by adding a spatula of Amberlyst 15 and stirring for 5 minutes. The resin was removed by filtration and the solvent removed *in vacuo* to yield a yellow oil, which was titurated with pet. ether to give a hygroscopic solid **111** (0.28 g, 100%). R_f 0.11 (5:2 EtOAc:MeOH); $[\alpha]^{25}$ +54.4 (*c* 0.3, MeOH), +73.9 (*c* 0.2, MeOH); υ_{max} (Nujol)/cm^{-1} 3372, 2920 and 1709; δ_H (250 MHz; d_4-MeOH) 4.45 (2 H, d, *J* 7.9, H_1), 3.21-3.90 (28 H, m), 2.07 (4 H, m, 4 x CHCH*H*), 1.51 (4 H, m, 4 x CHC*H*H); δ_C (62.5 MHz; MeOH) 104.6 (2 x d), 98.3 (2 x d), 81.1 (2 x d), 78.1 (2 x d), 77.8 (2 x d), 76.2 (2 x d), 74.9 (2 x d), 73.4 (2 x d), 73.2 (2 x d), 72.2 (2 x d), 71.4 (2 x d), 62.4 (2 x t), 61.8 (2 x t), 29.1 (4 x t); m/z (MALDI) 787.6 (+Na 787.7); FAB (electrospray) 765.2705 ($C_{30}H_{53}O_{22}$ requires 765.3028).

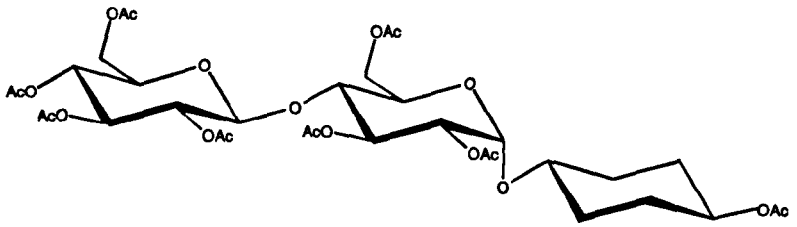
***Trans*-1,4-bis[(2,3,4,6-tetra-*O*-(phenylcarbamoxy)-β-*D*-glucopyranosyl)-(1→4)-(2,3,6-tri-*O*-(phenylcarbamoxy)-α-*D*-glucopyranosyl)] cyclohexanediol**



118

Method: Phenyl isocyanate (15eq, 1.17 mL, 10.8 mmol) was added to a solution of **111** (0.54 g, 0.72 mmol) in dry pyridine (15 mL). After stirring at room temperature for 48 hours. MeOH (10 mL) was added and the reaction mixture stirred for a further 10 minutes. The reaction mixture was extracted using dichloromethane (3 x 25 mL) and the combined organic layers washed with water (3 x 20 mL), 10% aqueous HCl (3 x 20 mL), water (1 x 20 mL) and dried with $MgSO_4$. The solvent was removed *in vacuo* to yield **118** as a brown solid (1.08g, 62%). R_f 0.79 (1:1 PE:EtOAc); melting point decomposes 215°C; $[\alpha]^{24}$ +23.0 (*c* 0.2, THF), Found C 62.36, H 5.41, N.7.95. $C_{128}H_{122}N_{14}O_{36}$ requires C 63.20, H 5.05, N 8.06%; υ_{max} (Nujol)/cm^{-1} 3319, 1647, 1596, 1530 and 1313; δ_H (250 MHz; d_6-acetone) 8.86 (m), 7.57 (m), 7.25 (m), 6.95 (m), 3.20-5.3 (m), 2.04 (m), 1.28 (m); δ_C (100.5 MHz; d_6-acetone) 155.0-154.9 (14 x s), 154.4-153.2 (14 x s), 140.8-139.4 (14 x s), 129.6 (14 x d), 123.8-123.0 (14 x d), 119.6 (28 x d), 95.4 (2 x d), 95.3 (2 x d), 73.7-70.3 (16 x d), 64.0 (2 x d), 32.0 (4 x t); m/z (MALDI) with a reaction time of 28 hours and concentration of 0.049 mol/dm^3: 2097.7 (n = 11, +Na 2096.7), 2215.4 (n = 12, +Na 2215.7), 2334.7 (n = 13, +Na 2334.7), 2453.9 (n = 14, +Na 2453.8); with a reaction time of 48 hours at a concentration of 0.033 mol/dm^3 n = 6-12.

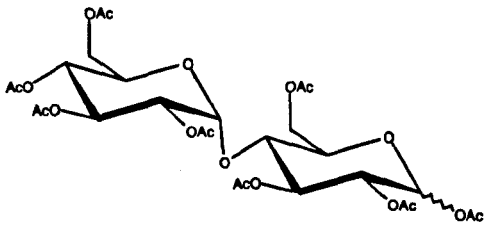
***Trans*-1-*O*-[(2,3,4,6-tetra-*O*-acetyl-β-*D*-glucopyranosyl)-(1→4)-(2,3,6-tri-*O*-acetyl-α-*D*-glucopyranosyl)]-4-acetoxy cyclohexanediol**



100

Column chromatography of product mixture from reaction to prepare **99** yielded **100**. (R_f 0.36 2:1 EtOAc:PE); melting point 130-133°C; $[\alpha]^{24}$ +54.2 (*c* 1.0, $CHCl_3$) υ_{max} (Nujol Mull)/cm^{-1} 2926, 2845, 2131, 1651-1800, 1457 and 1376; δ_H (250 MHz $CDCl_3$) 5.39 (1 H, t, *J* 10.0, H_3), 5.20 (1 H, m, $H_{3'}$), 5.12 (1 H, d, *J* 3.8, H_1), 5.02 (1 H, t, *J* 9.8, $H_{4'}$), 4.82 (2 H, m, $H_{1'}$, $H_{2'}$), 4.69 (1 H, dd, *J* 3.8, 10.0, H_2), 4.50 (1 H, m, H_{6a}), 4.6 (1 H, dd, *J* 4.6, 12.4, $H_{6'a}$), 4.14 (1 H, m, H_{6b}), 4.05 (2 H, m, H_5, $H_{6'b}$), 3.98 (1 H, m, $H_{5'}$), 3.83 (1 H, t, *J* 10.0, H_4), 3.66 (1 H broad m, C*H*CH_2) 2.07-1.91 (28 H, 8 x s, 8 x CH_3, 4 x CHCH*H*), 1.43 (4 H, m, 4 x CHCH*H*); δ_C (75.5 MHz; d_6-acetone) 170.7 (2 x s), 170.3 (2 x s), 169.9 (2 x s), 169.6 (2 x s), 101.4 (d), 95.1 (d), 77.7 (d), 76.7 (d), 73.6 (d), 72.4 (d), 72.3 (d), 71.7 (d), 70.2 (d), 69.4 (d), 68.8 (d), 63.0 (t), 62.4 (t), 30.2 (2 x t), 29.9 (2 x t), 21.2 (d), 20.9-20.4 (8 x q); m/z (MALDI) 799.1 (+Na 799.7), 815.2 (+K 815.7); (FAB) 799 (7%), 619 (16), 331 (96), 169 (100), 137 (36), 109 (42).

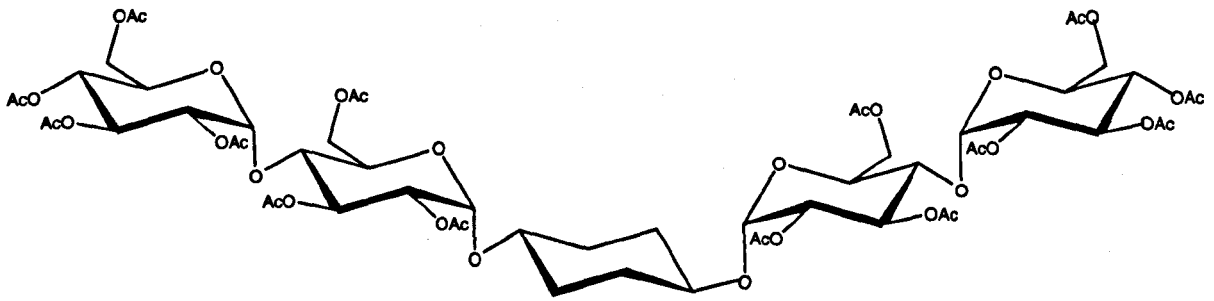
Maltose octaacetate



101

Method: A solution of maltose monohydrate (5.0 g, 13.8 mmol) in 1:1 v/v pyridine:acetic anhydride (total volume 40 mL) was stirred at room temperature until no starting material was visible by TLC (using ethyl acetate as eluent and H_2SO_4 in MeOH (10%) as visualiser, 24-48 hours). The solution was reduced *in vacuo* and the remaining syrup taken up in dichloromethane (50 mL) and washed successively with water (3 x 30 mL), 10% aqueous HCl (3 x 30 mL), water (3 x 30 mL) and brine (30 mL). After drying with $MgSO_4$ the solvent was removed *in vacuo* to give a white crystalline solid in 80-87% yield (R_f 0.6 (EtOAc)) which was used without further purification.; melting point 164-165°C; $[\alpha]^{26}$ +74.3 (*c* 1.0, $CHCl_3$); Found C 48.74, H 5.58. $C_{28}H_{38}O_{19}.H_2O$ requires C 48.28 H 5.75%; υ_{max} (HCB)/cm^{-1} 3485, 3014, 2960, 1758 and 1368; δ_H (250 MHz; $CDCl_3$) 5.7 (1 H, d, *J* 8.7, H_1), 5.36 (1 H, d, *J* 4.2, $H_{1'}$), 5.31 (1 H, t, *J* 10.2, $H_{3'}$), 5.25 (1 H, t, *J* 8.7, H_3), 5.02 (1 H, t, *J* 10.2, $H_{4'}$), 4.94 (1 H, t, *J* 8.7, H_2), 4.82 (1 H, dd, *J* 4.2, 10.2, $H_{2'}$), 4.41 (1 H, dd, *J* 2.5, 12.0, H_{6a}), 4.2 (2 H, m, H_{6b}, $H_{6'a}$), 3.99 (2 H m, H_4, $H_{6'b}$), 3.89 (1 H, m, $H_{5'}$), 3.79 (1 H, m, H_5); δ_C (100.5 MHz; $CDCl_3$) 170.1-168.8 (8 x s), 95.7 (d), 91.3 (d), 75.2 (d), 72.9 (d), 72.4 (d), 70.9 (d), 70.0 (d), 69.3 (d), 68.6 (d), 67.9 (d), 62.5 (t), 61.4 (t), 21.1-20.6 (8 x q); m/z (MALDI) 702.4 (+Na 701.6), 718.6 (+K 717.6).

***Trans*-1,4-bis[(2,3,4,6-tetra-*O*-acetyl-α-*D*-glucopyranosyl)-(1→4)-(2,3,6-tri-*O*-acetyl-α-*D*-glucopyranosyl)] cyclohexanediol**



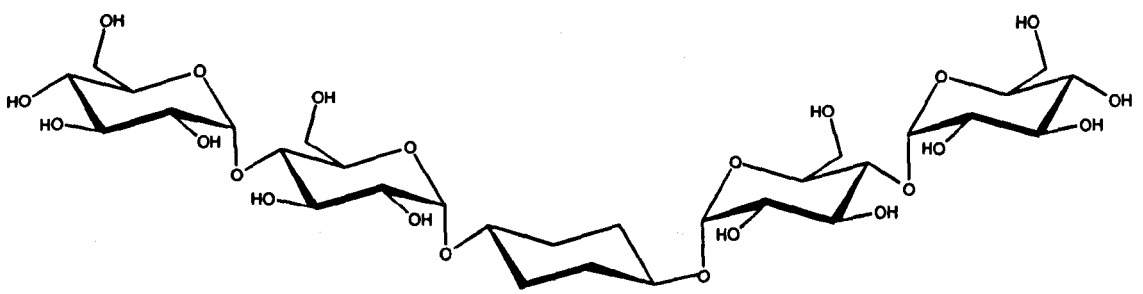
102

Method: Tin tetrachloride (2.1 mL, 18 mmol, 2.5eq) was added to a cooled solution (0°C) of maltose octaacetate (5.0 g, 7.4 mmol) and *trans*-1,4-cyclohexandiol (0.51 g, 4.42 mmol, 0.6eq) in anhydrous dichloromethane under an atmosphere of nitrogen. The ice was then removed and the reaction mixture was allowed to warm up to room temperature. After 21-120 hours the reaction was quenched by pouring into aqueous $NaHCO_3$ (80 mL) and dichloromethane (50 mL) and stirred for 30 minutes. The organic phase was separated and the aqueous phase extracted with more dichloromethane (2 x 100 mL). The organic extractions were combined and washed with water (3 x 100 mL), brine (1 x 100 mL) and dried with $MgSO_4$ to yield a brown solid (3.84-4.67 g, 77-96%). Purification by flash chromatography (2:1 EtOAc: $CHCl_3$ R_f product 0.3) afforded a pale yellow crystalline solid **102** (0.78-2.3 g, 20-30%) and the monosubstituted cyclohexane **103** (0.7-1.4 g, 12-22%).

Data for **102**: melting point 76-80°C; $[\alpha]^{25}$ +153.2 (*c* 0.2, $CHCl_3$); +13.8 (*c* 1.2, THF); +103.8 (*c* 0.2, Acetone); Found C 50.89, H 6.01. $C_{58}H_{80}O_{36}.H_20$ requires C 50.80 H 6.03%; υ_{max} (nujol)/cm^{-1} 2920, 2850, 1747, 1228 and 1028; δ_H (250 MHz; $CDCl_3$) 5.52 (2 H, dd, *J* 9.8, 10.2, H_3), 5.38 (2 H, d, *J* 3.8, $H_{1'}$), 5.37 (2 H, t, *J* 10.2, $H_{3'}$), 5.14 (2 H, d, *J*

3.9, H_1), 5.06 (2 H, t, *J* 10.2, $H_{4'}$), 4.87 (2 H, dd, *J* 3.8, 10.2, $H_{2'}$), 4.69 (2 H, dd, *J* 3.9, 10.2, H_2), 4.50 (4 H, dd, *J* 2.4, 11.8, H_{6a}, $H_{6'a}$), 4.37-3.97 (10 H, m, H_4, H_5, $H_{5'}$, H_{6b}, $H_{6'b}$), 3.71 (2 H, broad m, 2 x C*H*CH_2), 2.07, 2.03, 2.02, 1.99, 1.98, 1.96, 1.91 (42 H, 7 x s, 14 x C*H*$_3$), 1.51 (4 H, m, 4 x CHCH*H*), 1.37 (4 H, m, 4 x CHCH*H*); δ_C (62.5 MHz; $CDCl_3$) 170.8 (2 x s), 170.7 (2 x s), 170.6 (2 x s), 170.3 (2 x s), 170.1 (4 x s), 169.7 (2 x s), 95.6 (2 x d), 93.9 (2 x d),75.1 (2 x d), 72.9 (2 x d), 72.5 (2 x d), 71.5 (2 x d), 69 (2 x d), 69 (2 x d), 68 (2 x d), 67 (2 x d), 67 (2 x d), 62 (2 x t), 61.8 (2 x t), 29.5 (4 x t), 20.5 (14 x q); m/z (MALDI) 1392.0 (K^+ 1392.3), 1375.9 (Na 1376.3); (EI) 1371 (1%, M+H_2O), 619 (10), 331 (37), 169 (100), 154 (63), 136 (69), 109 (97), 89 (40).

***Trans*-1,4-bis[(β-*D*-glucopyranosyl)-(1→4)-(α-*D*-glucopyranosyl)] cyclohexanediol**

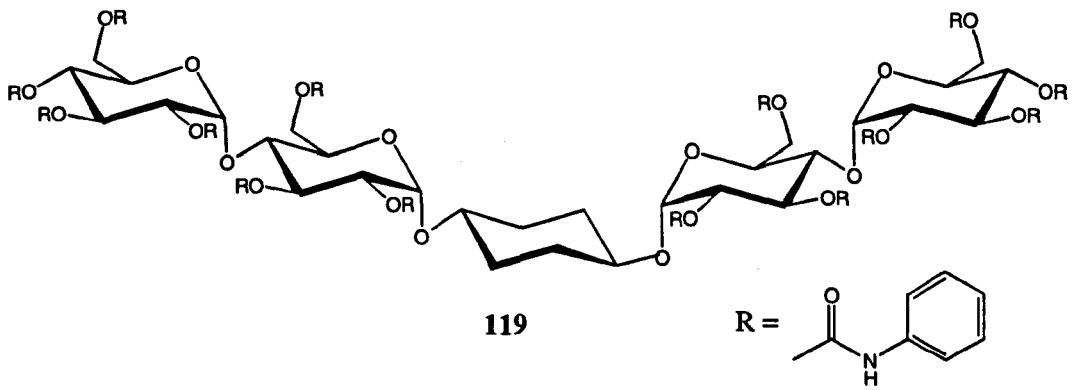


112

Method: A solution of **102** (0.8 g, 0.59 mmol) and sodium methoxide (0.93eq, 0.55 mmol, 30 mg) in MeOH (20 mL) was stirred at room temperature for 46 hours. A spatula of washed Dowex was added and stirred until neutral pH attained. The Dowex was removed by filtration and the solvent removed *in vacuo* to yield a pale yellow solid **112** (0.45 g, 99.6%). R_f 0.5 (MeOH); melting point 161-163°C; $[\alpha]^{24}$ +150.5 (*c* 0.1, MeOH); Found C 45.00, H 7.03. $C_{30}H_{52}O_{22}.2H_2O$ requires C 45.00, H 7.05%; υ_{max} (HCB)/cm^{-1} 3382, 2929, 1368 and 1168; δ_H (250 MHz; MeOH) 5.18 (2 H, d, *J* 3.4, H_1), 3.90-3.30 (30

H, m), 2.08 (4 H, m, 4 x CHC*H*H), 1.43 (4 H, m, 4 x CHC*H*H); δ_C (62.5 MHz; d_4-MeOH) 102.9 (2 x d), 98.4 (2 x d), 81.9 (2 x d), 76.2 (2 x d), 75.1 (2 x d), 74.9 (2 x d), 74.8 (2 x d), 74.2 (2 x d), 73.1 (2 x d), 72.4 (2 x d), 71.5 (2 x d), 62.8 (2 x t), 62.1 (2 x t), 31.5 (4 x t); m/z (MALDI) 787.8 (+Na 787.7); (ES+) 787.1 (M+Na^+ 787.7), (ES-) 763.1 (M-H^- 763.3).

***Trans*-1,4-bis[(2,3,4,6-tetra-*O*-(phenylcarbamoxy)-α-*D*-glucopyranosyl)-(1→4)-(2,3,6-tri-*O*-(phenylcarbamoxy)-α-*D*-glucopyranosyl)] cyclohexanediol**

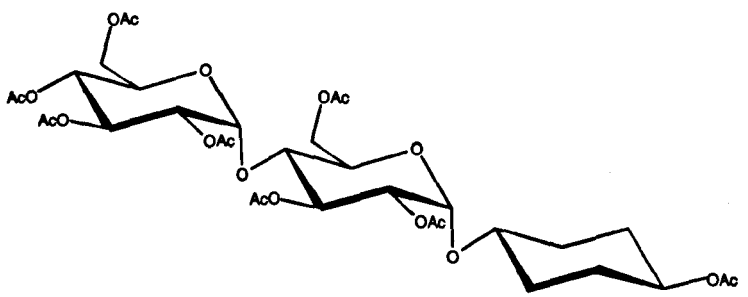


119

Method: Phenyl isocyanate (15eq, 1.24 mL, 11.0 mmol) was added to a solution of **112** (0.58 g, 0.76 mmol) in anhydrous pyridine (15 mL) under N_2. After heating at 50°C for 20 hours methanol (5 mL) was added and stirred at room temperature for 30 minutes. The reaction mixture was extracted with ethyl acetate (45 mL) which was then washed with water (3 x 50 mL), 10% aqueous HCl (3 x 50 mL), water (1 x 50 mL) and dried with $MgSO_4$. Removal of the solvent *in vacuo* yielded **119** as a pale yellow solid (1.78 g, 96%). R_f 0.79 1:1 PE:EtOAc; melting point decomposes ~230°C; $[\alpha]^{26}$ +132.7 (*c* 1.3, MeCN), +112.7 (*c* 1.0, formic acid), +50.4 (*c* 0.1, THF); Found C 62.76, H 5.32, N 7.88. $C_{128}H_{122}N_{14}O_{36}$ requires C 63.20, H 5.05, N 8.06%; υ_{max} (HCB)/cm^{-1} 3323, 3058, 2952, 1713, 1444 and 1313; δ_H (250 MHz; DMSO) 9.73 (m, 14 x NH), 6.97-7.49 (m, Ph), 4.05-

5.62 (m), 2.01 (m), 1.3 (m); δ_C (100.5 MHz; $CDCl_3$) 153.7-153.1 (14 x s), 139.5 (14 x s), 129.4 (28 x d), 123.4 (14 x d), 118.0 (28 x d), 95.5 (4 x d), 74.9 (2 x d), 74.2 (2 x d), 71.9 (2 x d), 71.4 (2 x d), 70.6 (2 x d), 70.4 (2 x d), 69.1 (2 x d), 68.8 (2 x d), 63.5 (2 x d), 30.4 (4 x t); m/z (MALDI) 2469.6 (n = 14, +K 2469.9), 2453.4 (n = 14, +Na 2453.8), 2350.7 (n = 13, +K 2350.9), 2334.1 (n = 13, +Na 2334.8), 2231.7 (n = 12, +K 2231.8).

***Trans*-1-*O*-(2,3,4,6-tetra-*O*-acetyl-α-*D*-glucopyranosyl)-(1→4)-(2,3,6-tri-*O*-acetyl-α-*D*-glucopyranosyl)-4-*O*-acetoxy cyclohexanediol**

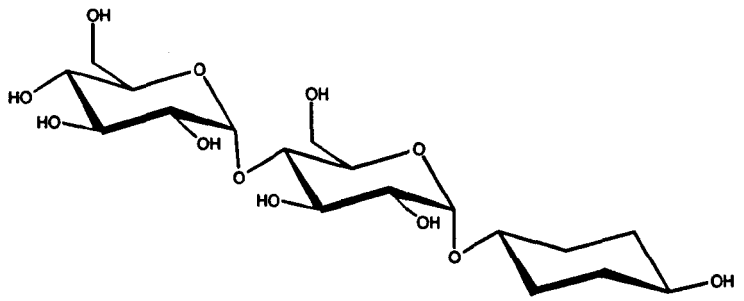


103

Obtained after column chromatography of the reaction mixture from the synthesis of **102**. R_f 0.48 (1:1 EtOAc:$CHCl_3$); $[\alpha]^{26}$ +122 (*c* 0.1, acetone); +126 (*c* 0.1, $CHCl_3$); Found C 51.03, H 5.98. $C_{34}H_{48}O_{20}$ requires C 52.58, H 6.23. $C_{34}H_{48}O_{20}.H_2O$ requires C 51.38, H 6.34%.; υ_{max} (HCB)/cm^{-1} 2955, 1757, 1433, 1368, 1229 and 1063; δ_H (250 MHz; d_6-acetone) 5.52 (1 H, t, *J* 10.0, H_3), 5.38 (2 H, m, $H_{1'}$, $H_{3'}$), 5.15 (1 H, d, *J* 3.7, H_1), 5.06 (1 H, t, *J* 9.1, $H_{4'}$), 4.86 (1 H, dd, *J* 3.9, 10.7, $H_{2'}$), 4.70 (1 H, dd, *J* 3.7, 10.0, H_2), 4.5 (1 H, m, H_1), 4.16 (6 H, m, H_4, H_5, $H_{5'}$, H_{6b}, $H_{6'a}$, $H_{6'b}$), 3.70 (1 H, broad s, C*H*CH_2), 2.09-1.95 (25 H, m, 8 x Me, C*H*CH_2), 1.43 (8 H, broad m, 8 x CHC*H*H); δ_C (70.5 MHz; $CDCl_3$) 171.8 (s), 170.4 (s), 170.3 (2 x s), 169.9 (2 x s), 169.4 (2 x s), 169.4 (s), 96.1 (d), 94.4 (d), 75.5 (d), 73.2 (d), 72.1 (d), 71.5 (d), 70.3 (d), 69.7, (d) 68.3 (d), 63.1 (t), 60.7 (t), 31.3 (d),

29.7 (2 x t), 28.3 (2 x t), 21.2 (8 x q); m/z (MALDI) 799.6 (+Na 799.7), 815.8 (+K 815.7).

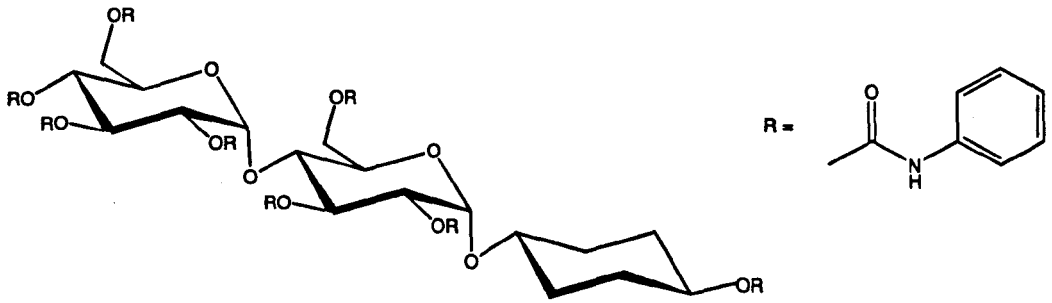
***Trans*-1-[(α-*D*-glucopyranosyl)-(1→4)-(α-*D*-glucopyranosyl)] cyclohexanediol**



113

Method: A solution of **103** (1.2 g, 1.54 mmol) and sodium methoxide (8/15eq, 0.82 mmol, 44 mg) in MeOH (20 mL) was stirred at room temperature for 24 hours. A spatula of washed Dowex was added and stirred until neutral pH attained. The Dowex was removed by filtration and the solvent removed *in vacuo* to yield a pale yellow hygroscopic solid **113** (0.45 g, 99.6%). $[\alpha]^{24}$ +79.9 (*c* 7.4, MeOH); Found C 43.68, H 7.30. $C_{18}H_{30}O_{12}.3H_2O$ requires C 43.9, H 7.32%; υ_{max} (HCB)/cm^{-1} 3300, 2929, 1368 and 1168; δ_H (300 MHz; MeOH) 5.2 (m), 4.95 (1 H, d, *J* 3.4, H_1), 4.1-3.05 (m), 2.0 (m), 1.3 (m); δ_C (75.5 MHz; MeOH) 103.1 (d), 98.8 (d), 82.1 (d), 77.1 (d), 75.5 (d), 74.6 (d), 73.8 (d), 72.8 (d), 70.6 (d), 70.4 (d), 63.1 (t), 62.6 (t), 57.9 (d), 34.1 (2 x t), 32.2 (2 x t), 30.3 (d); m/z (MALDI) 464 (+Na 461).

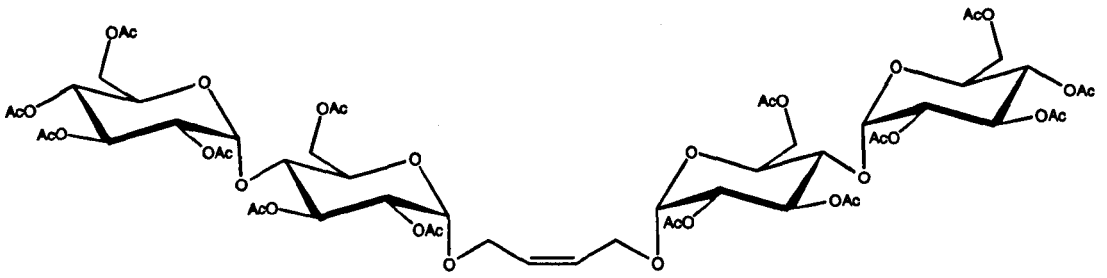
***Trans*-1-[(2,3,4,6-tetra-*O*-(phenylcarbamoxy)-α-*D*-glucopyranosyl)-(1→4)-(2,3,6-tri-*O*-(phenylcarbamoxy)-α-*D*-glucopyranosyl)]-4-(phenylcarbamoxy) cyclohexanediol**



120

Method: Phenyl isocyanate (8.8eq, 0.76 mL, 7 mmol) was added to a solution of **113** (0.35 g, 0.79 mmol) in anhydrous pyridine (9 mL) under nitrogen. After heating at 40-50°C for 45 hours methanol (2 mL) was added and stirred at room temperature for 30 minutes. The reaction mixture was extracted with ethyl acetate (30 mL) which was then washed with water (3 x 50 mL), 10% aqueous HCl (3 x 50 mL), water (1 x 50 mL) and dried with $MgSO_4$. Removal of the solvent *in vacuo* yielded a pale yellow solid (1.12 g, 98%). R_f 0.79 1:1 PE:EtOAc; $[\alpha]^{25}$ +45.0 (*c* 0.5, acetone); Found C 64.05, H 5.23, N 8.67. $C_{74}H_{72}N_8O_{20}$ requires C 63.77, H 5.21, N 8.04%; υ_{max} (HCB)/cm^{-1} 3317, 3057, 2952, 1712, 1445, 1230 and 1170; δ_C (100.5 MHz; DMSO) 154.4-152.0 (8 x s), 139.1 (8 x s), 129.1 (16 x d), 122.8 (8 x d), 118.8 (16 x d), 95.0 (d), 94.6 (d), 75.5 (d), 73.0 (d), 71.7 (d), 71.0 (d), 69.7 (2 x d), 68.9 (d), 67.9 (d), 61.8 (t), 60.1 (t), 51.9 (d), 30.3 (2 x t), 28.8 (2 x t), 21.1 (d).

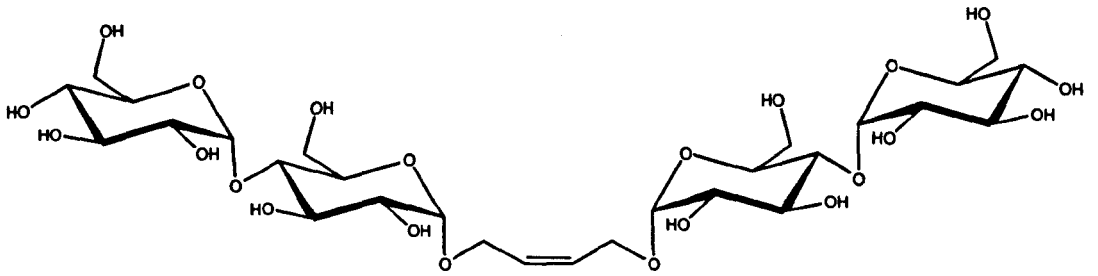
***Trans*-1,4-bis(2,3,4,6-tetra-*O*-acetyl-α-*D*-glucopyranosyl)-(1→4)-(2,3,6-tri-*O*-acetyl-α-*D*-glucopyranosyl) butenediol**



109

Method: Tin tetrachloride (4.14 mL, 3.53 mmol, 3eq) was added to a cooled solution (0°C) of maltose octaacetate (8.0 g, 11.8 mmol) and *cis*-2-butene-1,4-diol (98% *cis*, 0.58 mL, 7.07 mmol, 0.6eq) in anhydrous dichloromethane, with 4 Å molecular sieves, under an atmosphere of nitrogen (which had been stirring for 10 minutes). The reaction mixture was allowed to warm room temperature and after 16-40 hours the reaction was quenched by pouring into aqueous $NaHCO_3$ (350 mL) and dichloromethane (400 mL) and stirred for a further 30 minutes. The organic phase was separated and the aqueous phase extracted with dichloromethane (2 x 100 mL). The combined organic extracts were washed with water (3 x 100 mL), brine (1 x 100 mL) and dried with $MgSO_4$ to yield 109 as a brown solid (6.5 g, 83%). Purification by flash chromatography (4:1 EtOAc:PE R_f product 0.34); melting point 89-92°C; $[\alpha]^{25}$ +127.0 (*c* 4.8, acetone); υ_{max} (HCB)/cm^{-1} 2962, 1748, 1433, 1370, 1225 and 1010; δ_C (75.5 MHz; $CDCl_3$) 170.6-169.6 (14 x s), 129.4 (2 x d), 99.3 (2 x d), 95.8 (2 x d), 78.1 (2 x d), 75.1 (2 x d), 73.1 (2 x d), 72.3 (2 x d), 70.6 (2 x d), 70.1 (4 x d), 69.6 (2 x d), 63.8 (2 x t), 63.0 (2 x t), 61.7 (2 x t), 21.2 (2 x q), 21.1 (2 x q), 21.0 (2 x q), 20.9 (4 x q), 20.8 (2 x q), 20.7 (2 x q); m/z (MALDI) 1349.3 (+Na^+ 1348), 1365.0 (+K^+ 1364); (FAB) 1347 (4%), 619 (24), 331 (51), 169 (100), 109 (46).

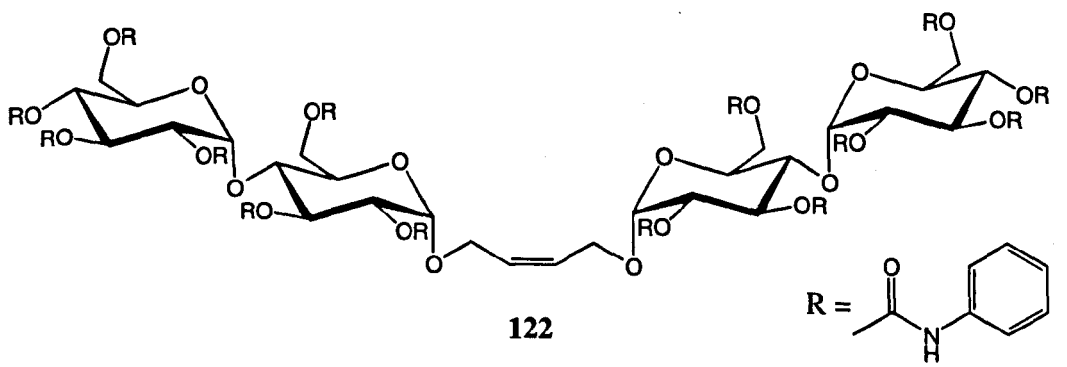
***Trans*-1,4-bis[(α-*D*-glucopyranosyl)-(1→4)-(α-*D*-glucopyranosyl)] butenediol**



116

Method: A solution of **109** (0.5 g, 0.38 mmol) and sodium methoxide (0.93eq, 0.35 mmol, 18 mg) in MeOH (20 mL) was stirred at room temperature for 22 hours. A spatula of washed Dowex was added and stirred until neutral pH attained. The Dowex was removed by filtration and the solvent removed *in vacuo* to yield a pale yellow hygroscopic solid **116** (0.30g, 99.6%). $[\alpha]^{25}$ +112.6 (*c* 1.0, MeOH); υ_{max} (nujol)/cm^{-1} 3330, 2922, 1461, 1376 and 1152; δ_H (300 MHz; MeOH) 6.00-3.1 (m); δ_C (62.5 MHz; d_4-MeOH) 103.2 (2 x d), 99.6 (2 x d), 81.9 (2 x d), 76.9 (2 x d), 75.4 (2 x d), 74.6 (2 x d), 73.8 (2 x d), 72.8 (2 x d), 72.2 (2 x d), 64.6 (2 x d), 63.1 (2 x t), 62.7 (2 x t), 50.4 (2 x t); m/z (MALDI) 759.5 (+Na 759.67); (ES-) 735.1 ($M-H^-$ 735.3), (ES+) 759.2526 (M+Na 759.2535).

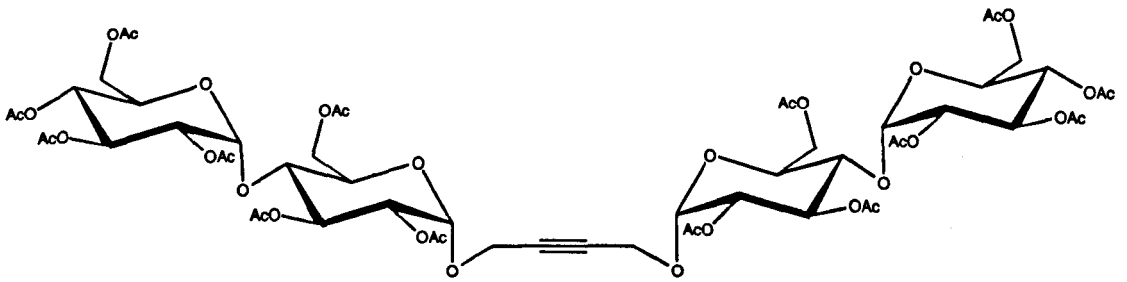
***Trans*-1,4-bis[(2,3,4,6-tetra-*O*-(phenylcarbamoxy)-α-*D*-glucopyranosyl)-(1→4)-(2,3,6-tri-*O*-(phenylcarbamoxy)-α-*D*-glucopyranosyl)] butenediol**



122

Method: Phenyl isocyanate (15.4eq, 0.56 mL, 5 mmol) was added to a solution of **116** (0.25 g, 0.33 mmol) in anhydrous pyridine (5 mL) under N_2. After heating at 75°C for 36 hours methanol (2 mL) was added and stirred at room temperature for 30 minutes. The reaction mixture was extracted with ethyl acetate (75 mL) which was then washed with water (3 x 20 mL), 10% aqueous HCl (3 x 40 mL), water (1 x 50 mL) and dried with $MgSO_4$. Removal of the solvent *in vacuo* yielded **122** as a pale brown solid (0.78 g, 97%). R_f 0.67 1:1 PE:Acetone; Found C 62.93, H 5.23, N.8.30. $C_{126}H_{118}N_{14}O_{36}$ requires C 62.92, H 4.95, N 8.16%; melting point decomposes ~245°C; υ_{max} (HCB)/cm^{-1} 3323, 3058, 2952, 1713, 1444, 1313, 1229 and 1066; υ_{max} (nujol)/cm^{-1} 3313, 2922, 2853, 1712, 1601, 1541, 1314 and 1231; δ_H (300 MHz; d_6-acetone) 8.9 (m, NH), 6.8-7.6 (m, Ph), 4.05-5.9 (m), 3.7 (m), 2.9 (m); m/z (MALDI) 2443.4 (n = 14, +K 2443.4), 2427.2 (n = 14, +Na 2427.4).

1,4-bis[(2,3,4,6-tetra-*O*-acetyl-α-*D*-glucopyranosyl)-(1→4)-(2,3,6-tri-*O*-acetyl-α-*D*-glucopyranosyl)] butynediol

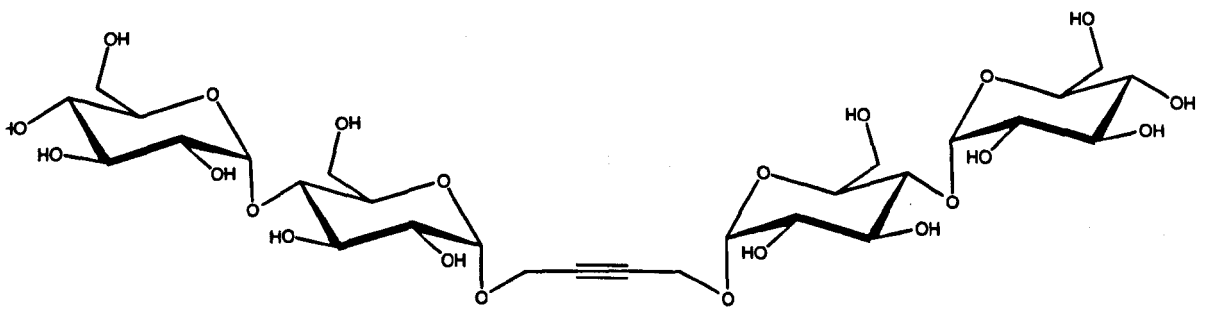


110

Method: Tin tetrachloride (2.6 mL, 22 mmol, 3eq) was added to a cooled solution (0°C) of maltose octaacetate (5.0 g, 7.4 mmol) and butyne-1,4-diol (0.4 g, 4.42 mmol, 0.6eq) in anhydrous dichloromethane under an atmosphere of nitrogen, the ice removed and left stirring at room temperature. After 12 hours the reaction was quenched by pouring into

aqueous $NaHCO_3$ (80 mL) and dichloromethane (50 mL) and stirred for 30 minutes. The organic phase was separated and the aqueous phase extracted with more dichloromethane (2 x 100 mL). The combined organic extractions were washed with water (3 x 100 mL), brine (1 x 100 mL) and dried with $MgSO_4$ to yield 110 as a brown solid (2.6 g, 54-55%). Purification by flash chromatography (2:1 EtOAc:PE R_f 0.51 product; melting point 83-84°C; $[\alpha]^{26}$ +61.3 (*c* 10.3, acetone); Found C 50.24, H 5.60. $C_{56}H_{74}O_{36}$ requires C 50.82, H 5.64, $C_{56}H_{74}O_{36}.H_2O$ requires C 50.15, H 5.67%; υ_{max} (HCB)/cm^{-1} 2963, 1754, 1440, 1371 and 1230; δ_H (300 MHz; $CDCl_3$) 5.39 (2 H, d, *J* 3.9, H_1), 5.33 (2 H, t, *J* 10.1, $H_{3'}$), 5.25 (2 H, t, *J* 9.0, H_3), 5.03 (2 H, t, *J* 9.9, $H_{4'}$), 4.80 (4 H, m, H_2, $H_{2'}$), 4.71 (2 H, d, *J* 7.9, $H_{1'}$), 4.69 (2 H, m, $H_{6'a}$), 4.36 (4 H, m, 2 x $CH_2C{\equiv}C$), 4.21 (4 H, m, H_{6a}, $H_{6'b}$), 4.01 (6 H, m, H_4, H_5, H_{6b}), 3.68 (2 H, m, H_5), 2.19-2.01 (42 H, m, 14 x CH_3); δ_C (75.4 MHz; $CDCl_3$) 170.6-169.6 (14 x s), 98.0 (2 x d), 95.7 (2 x d), 82.0 (2 x s), 75.4 (2 x d), 72.9 (2 x d), 72.4 (2 x d), 72.1 (2 x d), 70.2 (2 x d), 69.5 (2 x d), 68.7 (2 x d), 68.2 (2 x d), 62.9 (2 x t), 61.7 (2 x t), 56.2 (2 x t), 20.8 (14 x q); m/z 1362 (+K 1362.2), 1346 (+Na 1346.2).

1,4-Bis[(α-*D*-glucopyranosyl)-(1→4)-(α-*D*-glucopyranosyl)] butynediol

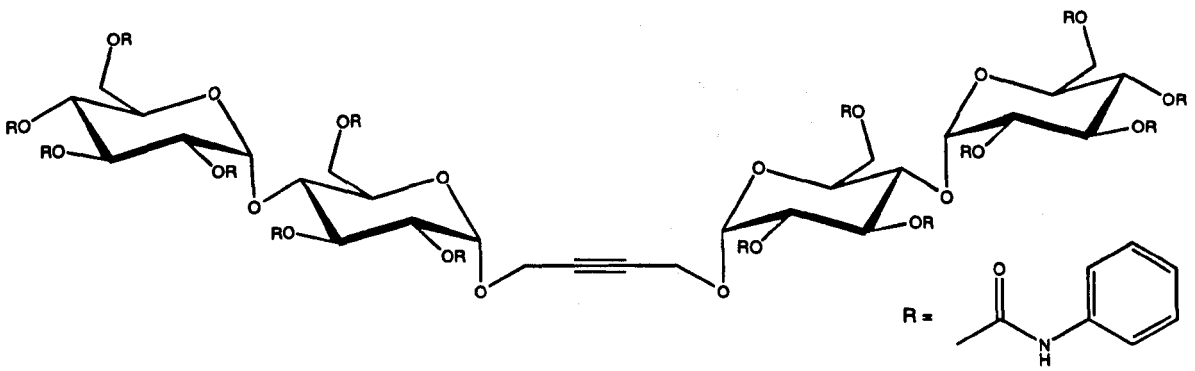


117

Method: A solution of **110** (0.6 g, 0.45 mmol) and sodium methoxide (0.93eq, 0.42 mmol, 22 mg) in MeOH (20 mL) was stirred at room temperature for 24 hours. A spatula of washed Dowex was added and stirred until neutral pH attained. The Dowex was

removed by filtration and the solvent removed *in vacuo* to yield a pale yellow solid **117**, which was taken up in a water/methanol mixture and washed with dichloromethane. The solvent was removed using a freeze drier to yield **117** (0.23 g, 71%). melting point 75-79°C; $[\alpha]^{24}$ +69.4 (*c* 1.0, MeOH); Found C 42.65, H 6.22. $C_{28}H_{46}O_{22}.2H_2O$ requires C 43.64, H 6.54%; υ_{max} (HCB)/cm^{-1} 3404 and 2925; δ_H (300 MHz; d_4-MeOH) 6.00-3.1 (m); δ_C (75.5 MHz; d_4-MeOH) 103.2 (2 x d), 102.4 (2 x d), 83.8 (2 x s), 81.4 (2 x d), 78.2 (2 x d), 77.1 (2 x d), 75.5 (2 x d), 75.2 (2 x d), 74.9 (2 x d), 74.5 (2 x d), 71.9, (2 x d) 63.1 (2 x t), 62.6 (2 x t), 57.3 (2 x t); m/z (MALDI) 773.7 (+K 773.7), 757.8 (+Na 757.63); (ES-) 733.1 (M-H^- 733.2), (ES+) 757.2379 ($C_{28}H_{46}O_{22}Na$ requires 757.2378).

1,4-Bis[(2,3,4,6-tetra-*O*-(phenylcarbamoxy)-α-*D*-glucopyranosyl)-(1→4)-(2,3,6-tri-*O*-(phenylcarbamoxy)-α-*D*-glucopyranosyl)] butynediol

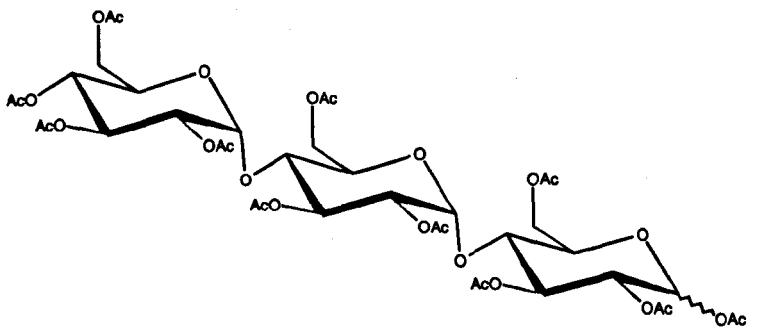


123

Method: Phenyl isocyanate (15.4eq, 0.6 mL, 5.4 mmol) was added to a solution of **117** (0.26 g, 0.35 mmol) in anhydrous pyridine (10 mL) under nitrogen. After heating at 50°C for 48 hours the reaction mixture was extracted with ethyl acetate (50 mL) which was then washed with 10% aqueous HCl (2 x 50 mL), water (1 x 50 mL), brine (1 x 50 mL) and dried with $MgSO_4$. Removal of the solvent *in vacuo* yielded **123** as a yellow solid (0.94 g, 112%). R_f 0.58 1:1 PE:Acetone; Found C 63.85, H 5.41, N 8.76. $C_{126}H_{116}N_{14}O_{36}$

requires C 62.98, H 4.87, N 8.17%; melting point decomposes ~235°C; $[\alpha]^{25}$ +24.4 (*c* 0.5, MeCN), +25.9 (*c* 0.5, THF); υ_{max} (HCB)/cm^{-1} 3314, 3057, 2954, 1713, 1444, 1315, 1230, 1020 and 1010; δ_H (300 MHz; d_6-acetone) 8.9 (m, NH), 6.8-7.6 (m, Ph), 4.05-5.9 (m), 3.7 (m), 2.9 (m); δ_C (62.5 MHz; DMSO) 153.6-152.0 (14 x s), 139.3-138.7 (14 x s), 129.1-128.6 (28 x d), 123.0-122.8 (14 x d), 119.2-118.7 (28 x d), 98.5 (2 x d), 94.7 (2 x d), 80.1 (2 x s), 72.3-69.3 (16 x d), 61.5 (2 x t), 60.1 (2 x t), 57.9 (2 x t); m/z (MALDI) 2469.6 (n = 14, +K 2469.9), 2453.4 (n = 14, +Na 2453.8), 2350.7 (n = 13, +K 2350.9), 2334.1 (n = 13, +Na 2334.8), 2231.7 (n = 12, +K 2231.8).

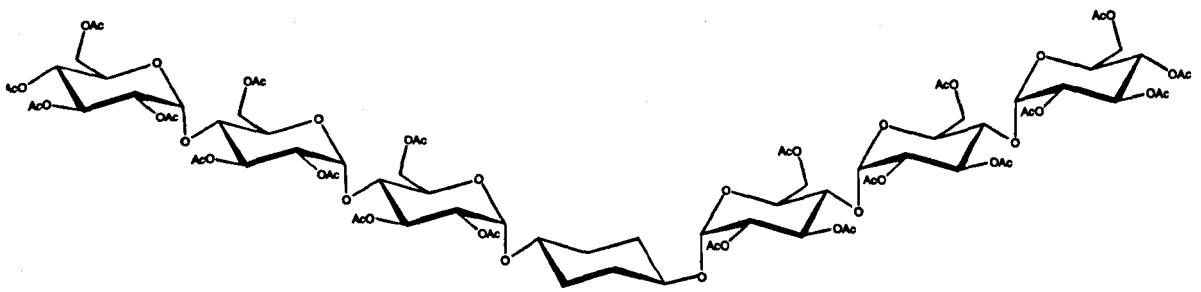
Maltotriose undecaacetate



Method: A solution of maltotriose (4.0 g, 7.9 mmol) in 1:1 v/v pyridine:acetic anhydride (total volume 40 mL) and a few crystals of DMAP was stirred at room temperature until no starting material was visible by TLC (using EtOAc as eluent and 10% H_2SO_4 in MeOH (as visualiser, 21-48 hours). The solution was reduced using a rotary evaporator and the syrup taken up in dichloromethane (50 mL). This solution was washed successively with water (3 x 50 mL), 10% aqueous HCl (3 x 50 mL), water (3 x 50 mL) and brine (3 x 50 mL). After drying with $MgSO_4$ the solvent was removed *in vacuo* to give a white crystalline solid (85-92%, R_f 0.55 (EtOAc)) which was used without further purification. NMR indicated the presence of both anomers in an approximate ratio of 1:1.

$[\alpha]^{27}$ +101.7 (*c* 1.0, acetone), +90.7 (*c* 0.2, acetone), +113.8 (*c* 1.6, acetone); Found C 48.85, H 5.64. $C_{40}H_{54}O_{27}$ requires C 49.70, H 5.63, $C_{40}H_{54}O_{27}.H_2O$ requires C 48.78, H 5.73; υ_{max} (Nujol)/cm^{-1} 2923, 2854, 1747, 1462 and 1377; (HCB)/cm^{-1} 3411, 2938, 1753, 1370 and 1230; δ_H (400 MHz; $CDCl_3$) 6.1 (d, ½H, *J* 3.4, H_1-α), 5.63 (d, ½H, *J* 7.8, H_1-β), 5.36 (dd, ½H, *J* 7, 9.3, H_3-β), 5.45 (4½ H, m, $H_{1''}$, $H_{3'}$, H_3-α, $H_{3''}$, $H_{1'}$), 4.95 (1 H, dt, *J* 2.7, 10.4, $H_{4''}$), 4.8 (1 H, m, H_2), 4.72 (1 H, dd, *J* 3.9, 10.8, $H_{2''}$), 4.6 (1 H, dt, *J* 4.4, 9.8, $H_{2'}$), 4.35 (2 H, m, H_{6a}, $H_{6'a}$), 4.2-3.79 (9 H, m, H_4, $H_{4'}$, $H_{5''}$, H_{6b}, $H_{6'b}$, $H_{6''a}$, $H_{6''b}$); δ_C (100.5 MHz; $CDCl_3$) 169.5-168.7 (11 x s), 95.8 (d), 95.5 (d), 91.1 (d), 88.7 (d), 74.9 (d), 72.8 (d), 72.5 (d), 72.3 (d), 72.0 (d), 71.6 (d), 70.9 (d), 70.1 (d), 69.6 (d), 68.9 (d), 68.4 (d), 62.6 (t), 62.2 (t), 61.3 (t), 20.9-20.3 (11 x q); m/z (MALDI) 989.8 (+Na 989.7), 1005.8 (+K 1005.8); (FAB) 989 (84%), 947 (16),887 (7), 331 (21), 169 (60), 115 (100).

***Trans*-1,4-bis(2,3,4,6-tetra-*O*-acetyl-α-*D*-glucopyranosyl)-(1→4)-(2,3,6-tri-*O*-acetyl-α-*D*-glucopyranosyl)-(1→4)-(2,3,6-tri-*O*-acetyl-α-*D*-glucopyranosyl) cyclohexanediol**



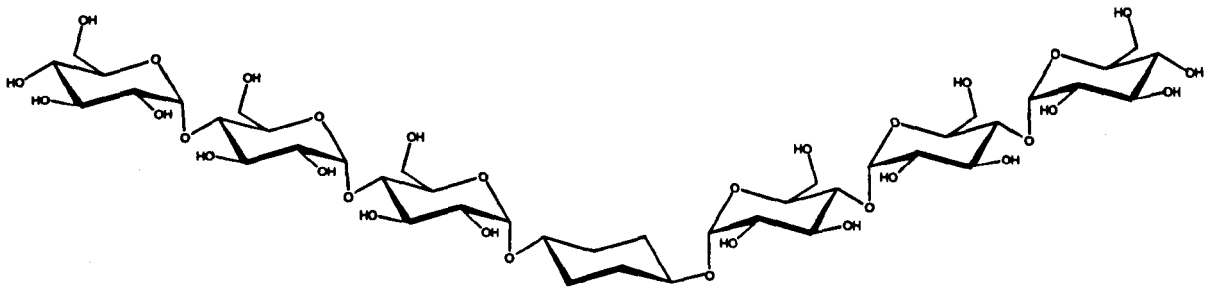
106

Method: Tin tetrachloride (1.28 mL, 10.9 mmol, 3eq) was added to a cooled solution (0°C) of maltose undecaacetate (3.52 g, 3.64 mmol) and *trans*-1,4-cyclohexandiol (0.25 g, 2.19 mmol, 0.6eq) in anhydrous dichloromethane (30 mL) under an atmosphere of nitrogen, the ice removed and left stirring at room temperature. After 22-144 hours the

reaction was quenched by pouring into aqueous $NaHCO_3$ (150 mL) and dichloromethane (50 mL) and stirred for 30 minutes. The organic phase was separated and the aqueous phase extracted with more dichloromethane (2 x 125 mL). The organic extracts were combined and washed with water (3 x 100 mL), brine (1 x 100 mL) and dried with $MgSO_4$ to yield a brown solid (2.5 g, 44-93%). Purification by flash chromatography (R_f 0.18 1:1 EtOAc: $CHCl_3$) afforded a pale yellow crystalline solid **106** (0.58-1.4 g, 11-24%) and the mono-substituted product **107** (0.5-1.10 g, 14-21%).

Data for **106:** melting point 100-103°C; $[\alpha]^{25}$ +141.5 (*c* 0.2, $CHCl_3$), +190 (*c* 0.1, acetone); Found C 50.49, H 5.97. $C_{82}H_{112}O_{52}$ requires C 51.02, H 5.85; $C_{82}H_{112}O_{52}.H_2O$ requires C 50.57, H 5.90%; υ_{max} (nujol)/cm^{-1} 3742, 2920, 2850, 1747, 1458, 1376, 1228 and 1028; δ_H (250 MHz; $CDCl_3$) 5.52 (2 H, t, *J* 10.0, H_3), 5.35 (8 H, m, $H_{1''}$, $H_{3''}$, $H_{3'}$), 5.25 (2 H, d, *J* 3.9, H_1), 5.07 (2 H, d, *J* 3.6, $H_{1'}$), 5.05 (2 H, t, *J* 9.8, $H_{4''}$), 4.82 (2 H, dd, *J* 3.9, 10.3, $H_{2''}$), 4.72 (2 H, dd, *J* 3.9, 10.0, H_2), 4.65 (2 H, dd, *J* 3.6, 10.1, $H_{2'}$), 4.43 (4 H, m, H_{6a}, $H_{6'a}$), 4.29-3.87 (16 H, m, H_4, $H_{4'}$, H_5, $H_{5'}$, $H_{5''}$, H_{6b}, $H_{6'b}$, $H_{6''a}$, $H_{6''a}$), 3.64 (2 H, broad m, C*H*CH_2), 2.16-1.96 (60 H, 20 x s, 20 x CH_3), 1.86, (4 H, m, 4 x CHC*H*H), 1.37 (4 H, m, 4 x CHC*H*H); δ_C (101.5 MHz; $CDCl_3$) 170.7-169.5 (20 x s), 105.2 (2 x d), 95.8 (2 x d), 95.5 (2 x d), 91.1 (2 x d), 88.7 (2 x d), 75.3 (2 x d), 73.9 (2 x d), 72.4 (2 x d), 71.8 (2 x d), 70.5 (2 x d), 70.1 (2 x d), 69.4 (2 x d), 68.9 (2 x d), 68.4 (2 x d), 67.9 (2 x d), 67.7 (2 x d), 63.0 (2 x t), 62.3 (2 x t), 61.4 (2 x t), 27.4 (4 x d), 20.9-20.6 (20 x q); m/z (MALDI) 1952.2 (+Na 1951.6), (ES+) 1951.7 (10%), 1045 (18), 887 (20), 331 (12), 169 (55) and 109 (100); (FAB) 1952.

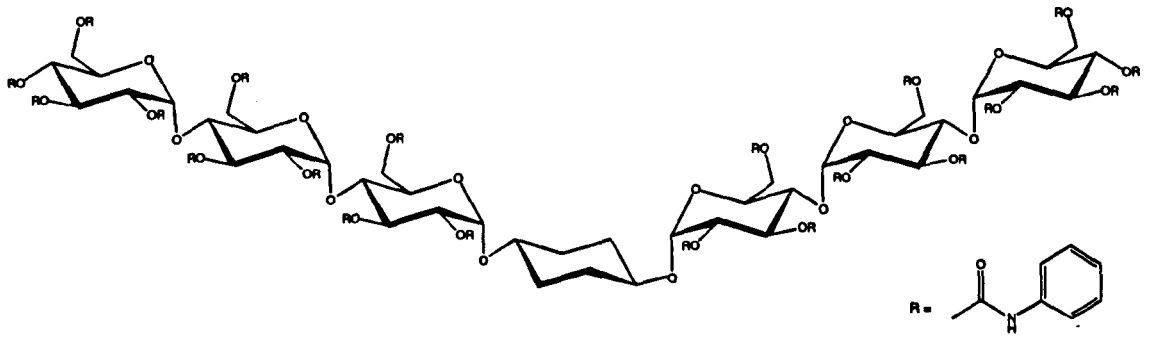
***Trans*-1,4-bis[(α-D-glucopyranosyl)-(1→4)-(α-D-glucopyranosyl)-(1→4)-(α-D-glucopyranosyl)] cyclohexanediol**



114

Method: A solution of **106** (0.43g, 0.223 mmol) and sodium methoxide (1.33eq, 0.297 mmol, 16 mg) in MeOH (15 mL) was stirred at room temperature for 96 hours. A spatula of washed Dowex was then added and stirred until neutral pH attained. The Dowex was removed by filtration and the solvent removed *in vacuo* to yield **114** as a pale yellow hygroscopic solid (0.24g, 100%). $[\alpha]^{24}$ +156.5 (*c* 0.1, MeOH); υ_{max} (HCB)/cm^{-1} 3300, 2929, 1368 and 1168; δ_H (250 MHz; d_4-MeOH) 5.19 (m), 3.91-3.34 (m), 2.07 (m), 1.4 (m); δ_C (62.5 MHz; d_4-MeOH) 103.2 (2 x d), 98.8 (2 x d), 98.6 (2 x d), 82.3 (2 x d), 81.6 (2 x d), 76.6 (2 x d), 75.5 (2 x d), 75.4 (2 x d), 75.3 (2 x d), 75.1 (2 x d), 74.6 (2 x d), 74.3 (2 x d), 73.7 (2 x d), 73.4 (2 x d), 72.8 (2 x d), 71.9 (2 x d), 63.1 (2 x t), 62.6 (2 x t), 62.3 (2 x t), 35.5 (4 x t); m/z (ES+) 1111.3 (+Na 1111.4); (ES–) 1087.3 (M–H 1087.4), (MALDI) 1112.8, 1129.7.

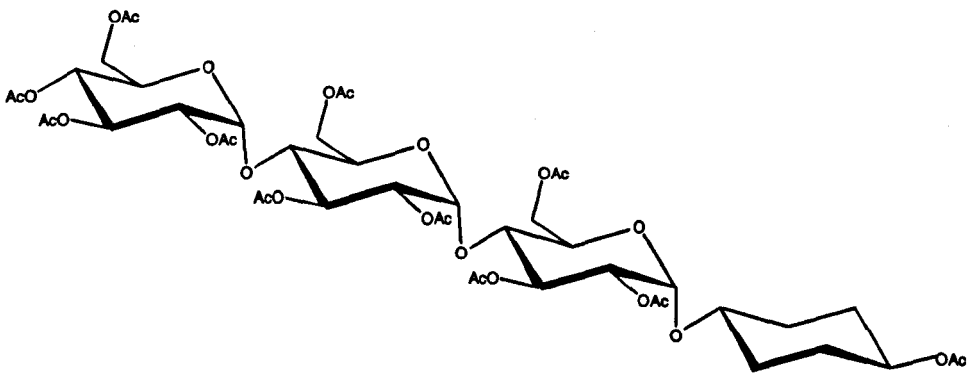
***Trans*-1,4-bis[(2,3,4,6-tetra-O-(phenylcarbamoxy)-α-*D*-glucopyranosyl)-(1→4)-(2,3,4,6-tetra-O-(phenylcarbamoxy)-α-*D*-glucopyranosyl)-(1→4)-(2,3,4,6-tetra-O-(phenylcarbamoxy)-α-*D*-glucopyranosyl)] cyclohexanediol**



121

Method: Phenyl isocyanate (22eq, 0.53 mL, 4.84 mmol) was added to a solution of **114** (0.40g, 0.22 mmol) in anhydrous pyridine (10 mL) under nitrogen. After heating at 45°C for 50 hours methanol (2 mL) was added and stirred at room temperature for 30 minutes. The reaction mixture was extracted with ethyl acetate (45 mL) which was then washed with water (3 x 50 mL), 10% aqueous HCl (3 x 50 mL), water (1 x 50 mL) and dried with $MgSO_4$. Removal of the solvent *in vacuo* yielded **121** as a pale yellow solid (0.42 g, 96%). R_f 0.68 EtOAc; $[\alpha]^{25}$ +26.5 (*c* 0.1, THF); melting point decomposes ~230°C; Found C 63.56, H 5.22, N.8.47 $C_{182}H_{172}N_{20}O_{52}$ requires C 62.98, H 4.99, N 8.07; υ_{max} (HCB)/cm^{-1} 3323, 2956, 1713 and 1443; δ_H (250 MHz; d_5-pyridine) 10.8 (m, NH), 7.8-7.04 (m, Ar), 6.12-3.20 (m), 2.01 (m), 1.3 (m) ;δ_C (62.5 MHz; d_5-pyridine), 148.2-147.8 (20 x s), 134.8-133.5 (20 x s), 123.5-122.8 (40 x d), 117.8-116.5 (20 x d), 113.6-113.2 (40 x d), 93.9 (2 x d), 91.2 (2 x d), 89.2 (2 x d), 69.4-62.1 (24 x d), 59.3 (2 x t), 57.6 (2 x t), 56.7 (2 x t), 54.2 (4 x t), 20.2 (2 x d); m/z (MALDI) 3494.6 (n = 20, +Na 3494.4).

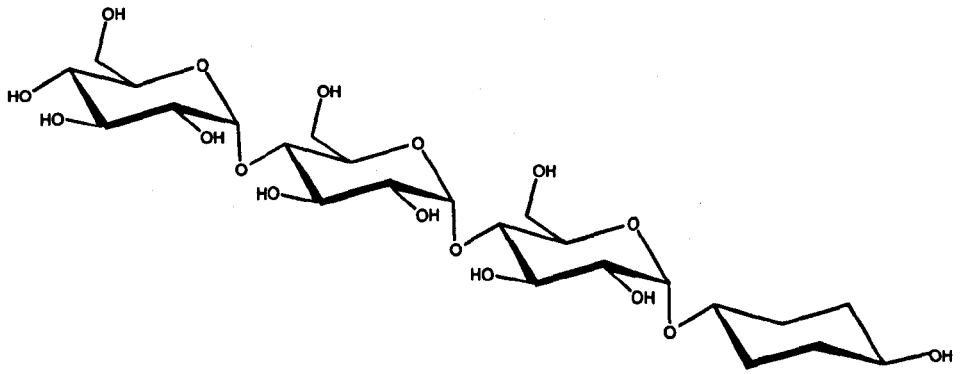
***Trans*-1-[(2,3,4,6-tetra-*O*-acetyl-α-*D*-glucopyranosyl)-(1→4)-(2,3,6-tri-*O*-acetyl-α-*D*-glucopyranosyl)-(1→4)-(2,3,6-tri-*O*-acetyl-α-*D*-glucopyranosyl)]-4-acetoxy cyclohexanediol**



107

Obtained after column chromatography of the reaction mixture for **107**. R_f 0.35 (2:1 EtOAc:PE); $[\alpha]_D$ +127.7 (*c* 0.5, acetone); melting point 80-82°C; Found C 51.61, H 5.87. $C_{46}H_{64}O_{28}$ requires C 51.88, H 6.06; υ_{max} (thin film)/cm^{-1} 3629, 2950 and 1739; δ_H (400 MHz; d_6-acetone) 5.51-5.38 (4 H, m, H_3, $H_{3'}$, $H_{3''}$, H_1), 5.30 (1 H, d, *J* 3.9, $H_{1'}$), 5.14 (1 H, d, *J* 3.9, $H_{1''}$), 5.05 (1 H, t, *J* 9.6, $H_{4''}$), 4.85 (1H, dd, *J* 3.9, 10.3, $H_{2'}$), 4.77 (1 H, dd, *J* 3.9, 10.3, H_2), 4.68 (1 H, dd, *J* 3.9, 10.3, $H_{2''}$), 4.5 (2 H, m, H_{6a}, $H_{6'a}$), 4.33-3.96 (8 H, m, H_4, $H_{4'}$, H_5, $H_{5'}$, $H_{5''}$, H_{6b}, $H_{6'b}$, $H_{6''a}$, $H_{6''b}$), 3.73 (1H, m, C*H*CH_2), 2.11-1.96 (38 H, C*H*CH_2, 4 x CHCH*H*, 11 x CH_3), 1.5 (4 H, m, 4 x CHCH*H*); δ_C (101.5 MHz; $CDCl_3$) 170.0-169.9 (11 x s), 95.8 (d), 95.6 (d), 94.0 (d), 88.8 (d), 75.1 (d), 73.8 (d), 72.4 (d), 71.8 (d), 71.6 (d), 71.3 (d), 70.4 (d), 70.1 (d), 69.4 (d), 68.9 (d), 67.9 (d), 67.7 (d), 62.9 (t), 62.1 (t), 61.3 (t), 29.5 (2 x t), 27.8 (2 x t), 21.3-20.4 (d, 11 x q); m/z (MALDI) 1087.6 (+Na 1087.3), 1102.2 (+K 1104.1).

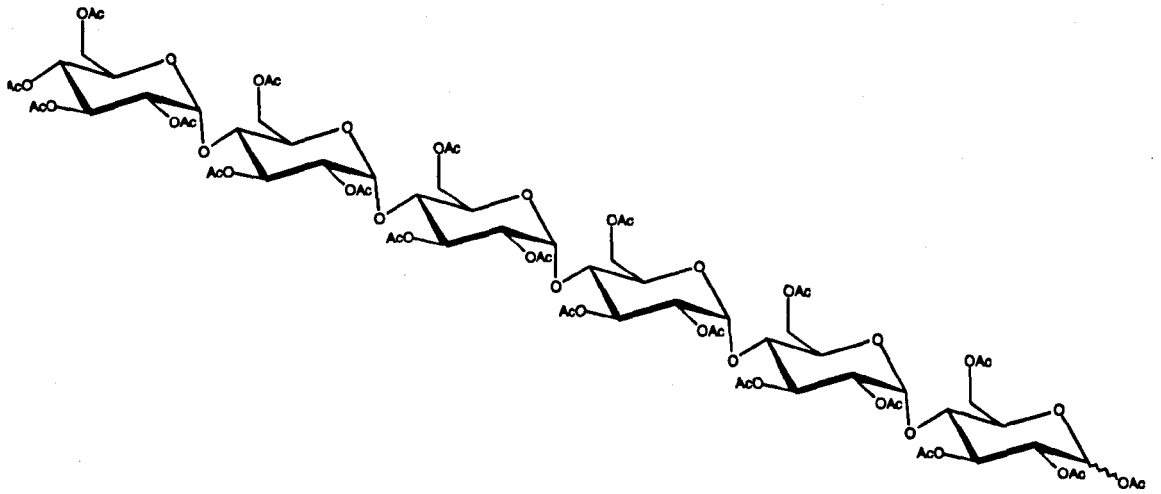
***Trans*-1-[(α-*D*-glucopyranosyl)-(1→4)-(α-*D*-glucopyranosyl)-(1→4)-(α-*D*-glucopyranosyl)] cyclohexanediol**



115

Method: A solution of **107** (1g, 0.94 mmol) and sodium methoxide (0.73eq, 0.69 mmol, 36 mg) in methanol (10 mL) was stirred at room temperature for 168 hours. A spatula of washed Dowex was added and stirred until neutral pH attained. The Dowex was removed by filtration and the solvent removed *in vacuo* to yield **115** as a pale yellow hygroscopic solid (0.48g, 85%). $[\alpha]^{25}$ +127.7 (*c* 0.5, acetone), +123.35 (*c* 1.0, MeOH); υ_{max} (HCB)/cm^{-1} 3404, 2927, 1230, 1168 and 1015; δ_H (400 MHz; d_4-MeOH) 5.20 (m), 4.95-3.29 (m), 2.04 (m), 1.36 (m); δ_C (101 MHz; d_4-MeOH) 102.7 (d), 98.4 (d), 98.1 (d), 81.9 (d), 81.3 (d), 76.7 (d), 75.1 (d), 74.7 (d), 74.2 (d), 73.9 (d), 73.3 (d), 73.0 (d), 72.4 (d), 71.4 (d), 70.2 (d), 68.8 (d), 62.7 (t), 62.2 (t), 60.9 (t), 31.8 (4 x t), 29.8 (d); m/z (MALDI) 626.6 (+Na 626.6).

Icosa-O-acetyl maltohexaose



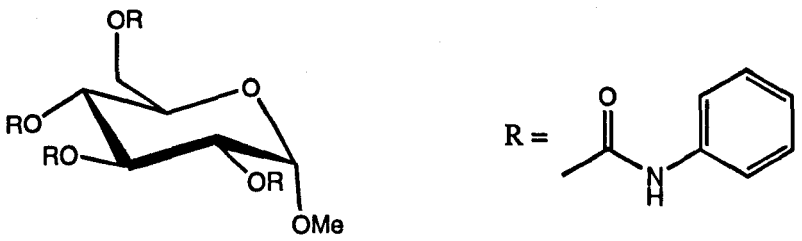
108

Method[120]: A solution of peracetylated α-cyclodextrin (1.0 g, 0.58 mmol) in acetic anhydride (10 mL) and concentrated H_2SO_4 (0.2 mL) was heated at 50°C for 24 hours. The resulting dark red solution was poured into iced water (25 mL) containing sodium acetate (0.58 g) and left to stir at room temperature overnight. The solution was extracted using chloroform (3 x 50 mL) and the combined organic extracts were washed with water (50 mL), $NaHCO_3$ (2 x 50 mL), water (1 x 50 mL) and brine (1 x 50 mL). After drying with $MgSO_4$ the solvent was removed *in vacuo* to yield a dark brown solid (0.82g, 77%) which was purified by flash chromatography (2:1 $CHCl_3$:EtOAc) to yield **108** as a pale brown solid (17%). R_f 0.19 (2:1 $CHCl_3$:EtOAc); $[\alpha]^{26}$ +118.5 (*c* 0.1, acetone); Found C 48.94, H 5.52. $C_{76}H_{102}O_{51}$ requires C 49.84, H 5.61%; υ_{max} (HCB)/cm^{-1} 2958, 1747, 1371, 1230 and 1168; δ_H (250 MHz; d_6-acetone) 6.58 (1 H, d, *J* 3.7, H_1), 5.9-5.6 (11 H, m), 5.04-4.77 (24 H, m), 2.19-1.96 (60 H, Me); m/z (MALDI) 1871 (+K 1870.7), 1855.6 (+Na 1854.6).

Phenyl carbamate derivative of saccharides

Method: Phenyl isocyanate (1.1eq. per hydroxyl group) was added to a solution of hydroxylated sugar (previously dried under vacuum, approx 0.3 mol/dm^3) in anhydrous pyridine under N_2. After heating at 45-55°C, for 20-48 hours, methanol was added and stirred at room temperature for 30 minutes. The reaction mixture was extracted with ethyl acetate which was then washed with water, 10% aqueous HCl, water and dried with $MgSO_4$. Removal of the solvent *in vacuo* yielded a pale yellow or white solid.

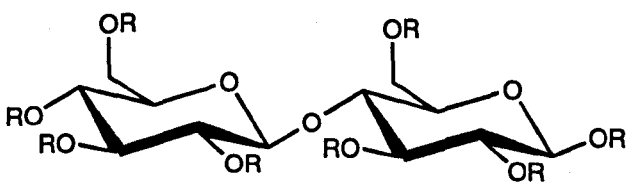
Methyl 2,3,4,6-O-tetra(phenyl carbamate) α-*D*-glucopyranoside

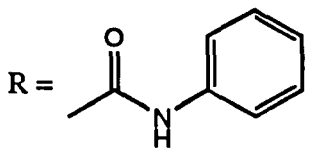


138

α-Methoxy glucopyranoside (1.2 g, 6.18 mmol) was heated at 55°C with phenyl isocyanate (3.02 mL, 27.8 mmol) in pyridine (20 mL) to yield **138** as a white solid (4.1 g, 98%). R_f 0.74 (acetone); $[\alpha]^{25}$ +58.1 (*c* 0.9, acetone); Found C 61.83, H 5.12, N 8.20. $C_{35}H_{34}N_4O_{10}$ requires C 62.67, H 5.11, N 8.36; υ_{max} (solid)/cm^{-1} 3350, 2356, 1707, 1534 and 1230; δ_H (300 MHz; DMSO) 9.95 (1 H, s, NH), 9.81 (2 H, s, NH), 9.73 (1 H, s, NH), 7.45 (20 H, m, Ar), 5.53 (1 H, t, *J* 10.3, H_3), 5.08 (2 H, m, H_1, H_4), 4.96 (1 H, dd, *J* 3.4, 10.3, H_2), 4.29 (2 H, m, H_{6a}, H_{6b}), 4.07 (1 H, m, H_5), 3.44 (3 H, s, OMe);δ_C (75.5 MHz; DMSO) 153.5 (s), 153.0 (s), 152.9 (s), 152.5 (s), 139.4 (4 x s), 129.1 (8 x d), 122.9 (4 x d), 118.9 (8 x d), 96.9 (d), 70.9 (d), 70.3 (d), 69.4 (d), 68.1 (d), 62.6 (t), 55.3 (q); m/z (FAB) 693 (M+Na 693), 670 (M^+ 670), 639 (M-OMe 639).

Cellobiose octa(phenyl carbamate)

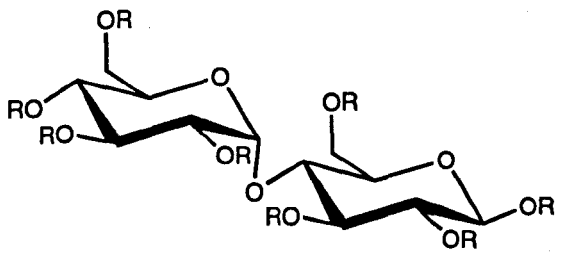


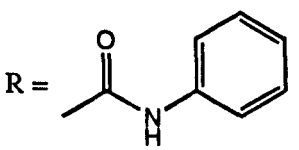


124

Cellobiose (1.2 g, 6.18 mmol) was heated at 55°C with phenyl isocyanate (3.02 mL, 27.8 mmol) in pyridine (50 mL) to yield **124** as a white solid (4.1 g, 98%). R_f 0.47 1:2 PE:EtOAc; υ_{max} (nujol)/cm^{-1} 3309, 2922, 1712, 1601 and 1537; δ_H (250 MHz; DMSO) 9.11, 8.99, 8.92, 8.82, 8.79, 8.72, 8.60, 8.53 (8 H, 8 x s, 8 x NH), 7.60-6.85 (40 H, m, Ar), 5.93 (1 H, d, *J* 8.7, H_1), 5.40 (1 H, t, *J* 9.3, $H_{3'}$), 5.29 (1 H, t, *J* 9.2, H_3), 5.02 (2 H, m), 4.88 (3 H, m), 4.30 (3 H, m), 3.99 (3 H, m); m/z (MALDI) 1333.9 (+K 1334.4), 1318.1 (+Na 1318.3).

Maltose octa(phenyl carbamate)



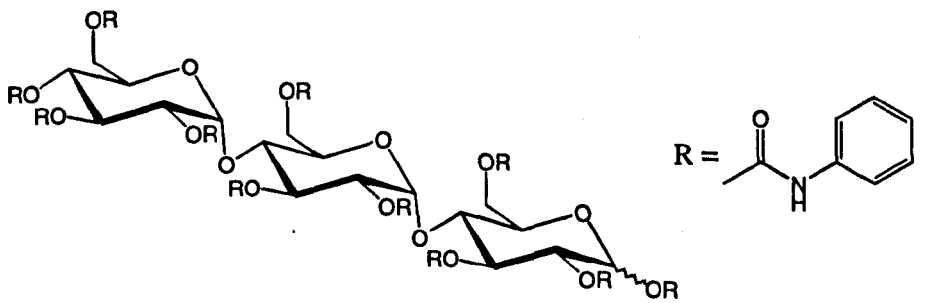


125

Maltose (1.0 g, 2.77 mmol) was heated at 50-55°C with phenyl isocyanate (2.72 mL, 25.0 mmol) in pyridine (10 mL) to yield **125** as a white solid (3.7 g, 98%). R_f 0.74 (acetone); melting point decomposes >230°C; $[\alpha]^{26}$ +31.5 (*c* 0.1, THF); Found C 63.51, H 5.14, N 9.23. $C_{68}H_{62}N_8O_{19}$ requires C 63.03, H 4.83, N 8.65%. υ_{max} (HCB)/cm^{-1} 3323, 3059, 1713, 1647 and 1445; δ_H (250 MHz; DMSO) 9.73 (8 H, m, 8 x NH), 7.48 (16 H, d, *J* 8.1, Ar), 7.28 (16 H, t, *J* 8.1, Ar), 6.97 (8 H, t, *J* 8.1, Ar), 6.1-3.7 (m), 4.05-5.62 (m), 2.01 (m),

1.3 (m); δ_C (62.5 MHz; DMSO) 153.6-152.1 (8 x s), 138.9 (16 x d), 128.8 (8 x s), 123.0 (16 x d), 118.7 (8 x d), 95.1 (d), 92.2 (d), 75.8 (d), 72.7 (d), 71.7 (d), 70.7 (d), 70.2 (d), 69.7 (d), 69.4 (d), 68.8 (d), 63.5 (t), 61.7 (t); m/z (MALDI) 1335.8 (n = 8, +K 1334.4), 1319.0 (n = 8, +Na 1318.3), 1216.4 (n = 7, +K 1215.3), 1201.0 (n = 7, +Na 1199.2).

Maltotriose undeca(phenyl carbamate)



126

Maltotriose undecaacetate (0.5 g, 0.9 mmol) reacted with phenyl isocyanate (10.9 mmol, 1.3 mL) in pyridine (15 mL) to yield **126** as a pale yellow solid (1.7 g, 95%). R_f 0.83 1:2 PE:EtOAc; $[\alpha]^{24}$ +27.5 (*c* 0.1, THF); Found C 63.58, H 5.09, N.9.04. $C_{95}H_{87}N_{11}O_{27}$ requires C 62.87, H 4.83, N 8.49%; υ_{max} (HCB)/cm^{-1} 3324, 3061, 2959, 1713, 1649, 1445 and 1314; δ_H (250 MHz; DMSO) 9.84 (11 H, 11 x s, 11 x NH), 7.48 (22 H, d, *J* 7.6, Ar), 7.28 (22 H, t, *J* 7.6, Ar), 6.1-4.1 (m); m/z (MALDI) 1854.1 (n = 11, +K 1853.8), 1838.3 (n = 11, +Na 1837.8), 1734.6 (n = 10, +K 1734.7), 1718.6 (n = 10, +Na 1718.7), 1614.5 (n = 9, +K 1615.5), 1598.4 (n = 9, +Na 1599.5), 1496.2 (n = 8, +K 1496.4), 1480.7 (n = 8, +Na 1480.4).

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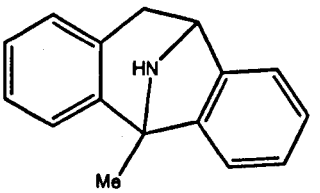
APPENDIX 1

MK801

A



CPP 55,940

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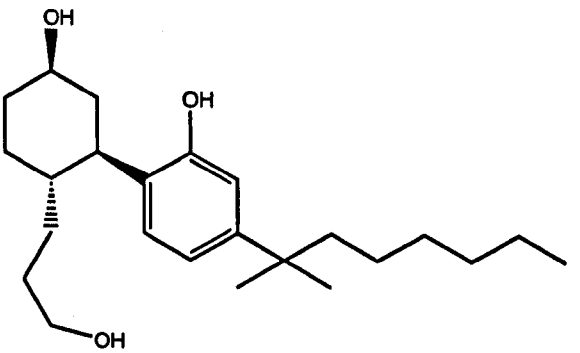

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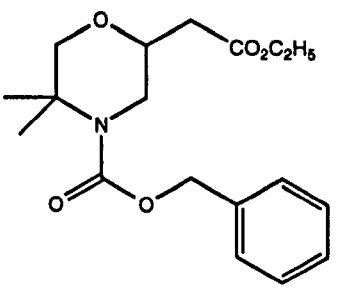

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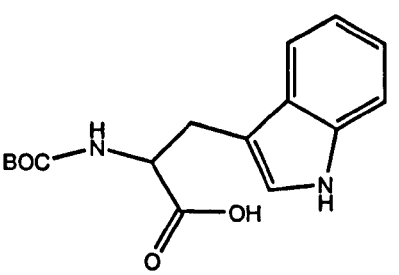

U-50488

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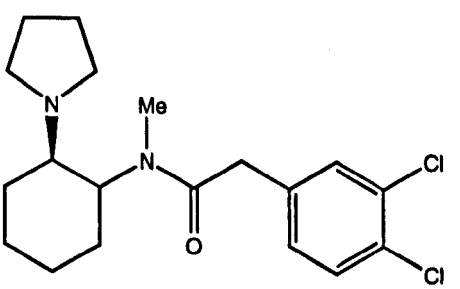
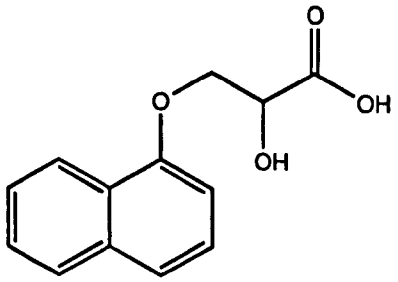
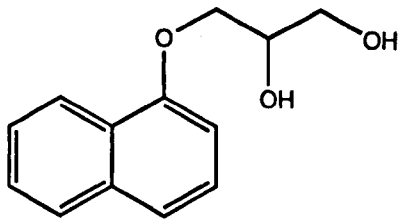


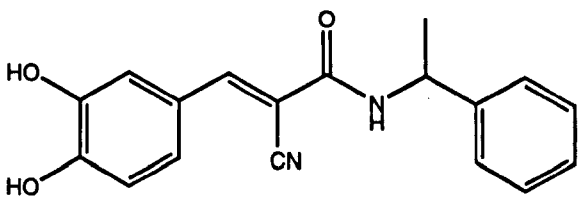
1-Napthoxylactic Acid **F**



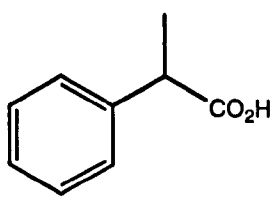
Propranolol Glycol **G**



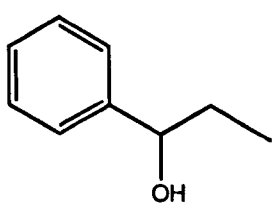
Tyrphostin B44 **H**



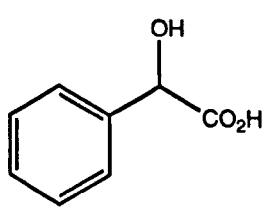
2-Phenylpropionic Acid **J**



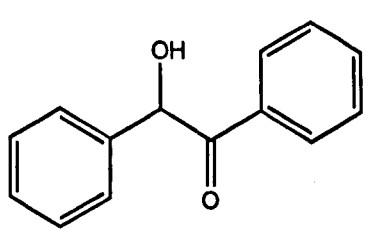
1-Phenyl-1-propanol **K**



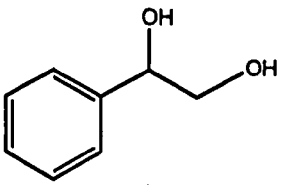
Mandelic Acid **L**



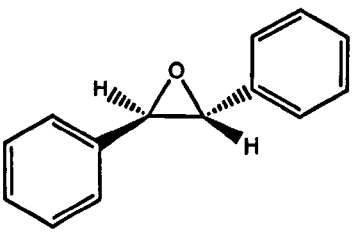
Benzoin **M**



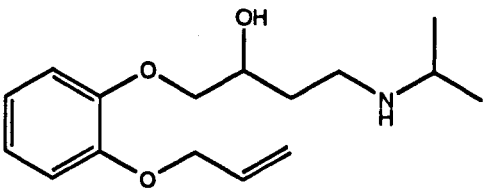
1-Phenyl-1,2-ethanediol **N**



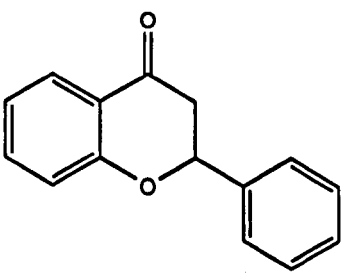
trans-Stilbene Oxide **P**



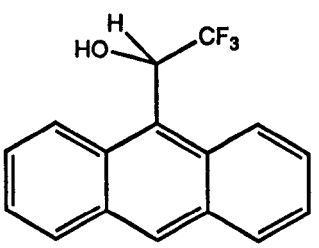
Oxprenolol **Q**



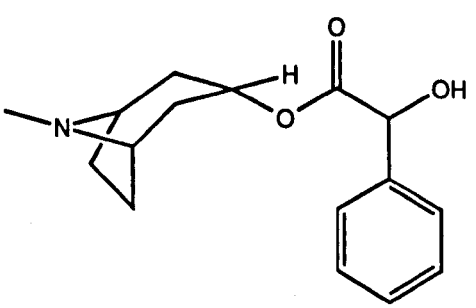
Flavanone **R**



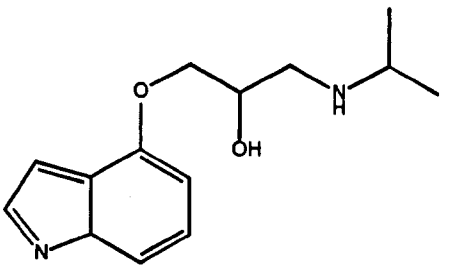
2.2.2-trifluoro-1-(9-anthryl) ethanol **S**



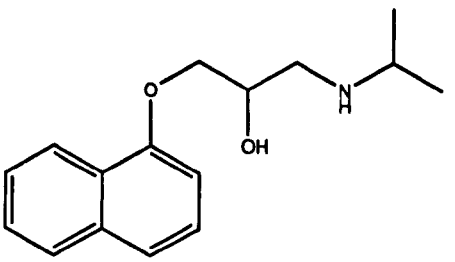
Homatropine **T**



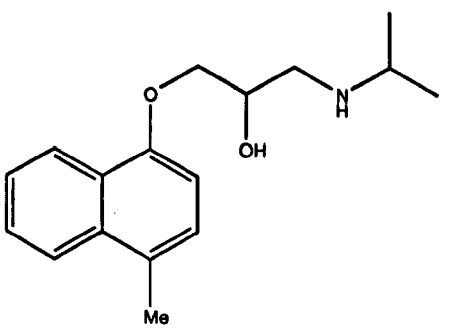
Pindolol **U**



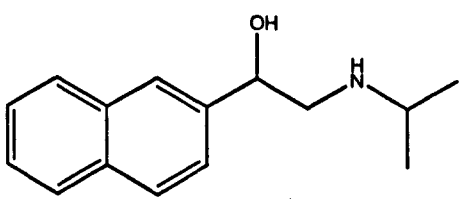
Propranolol **V**



4-Methylpropranolol **W**



Pronethalol **X**

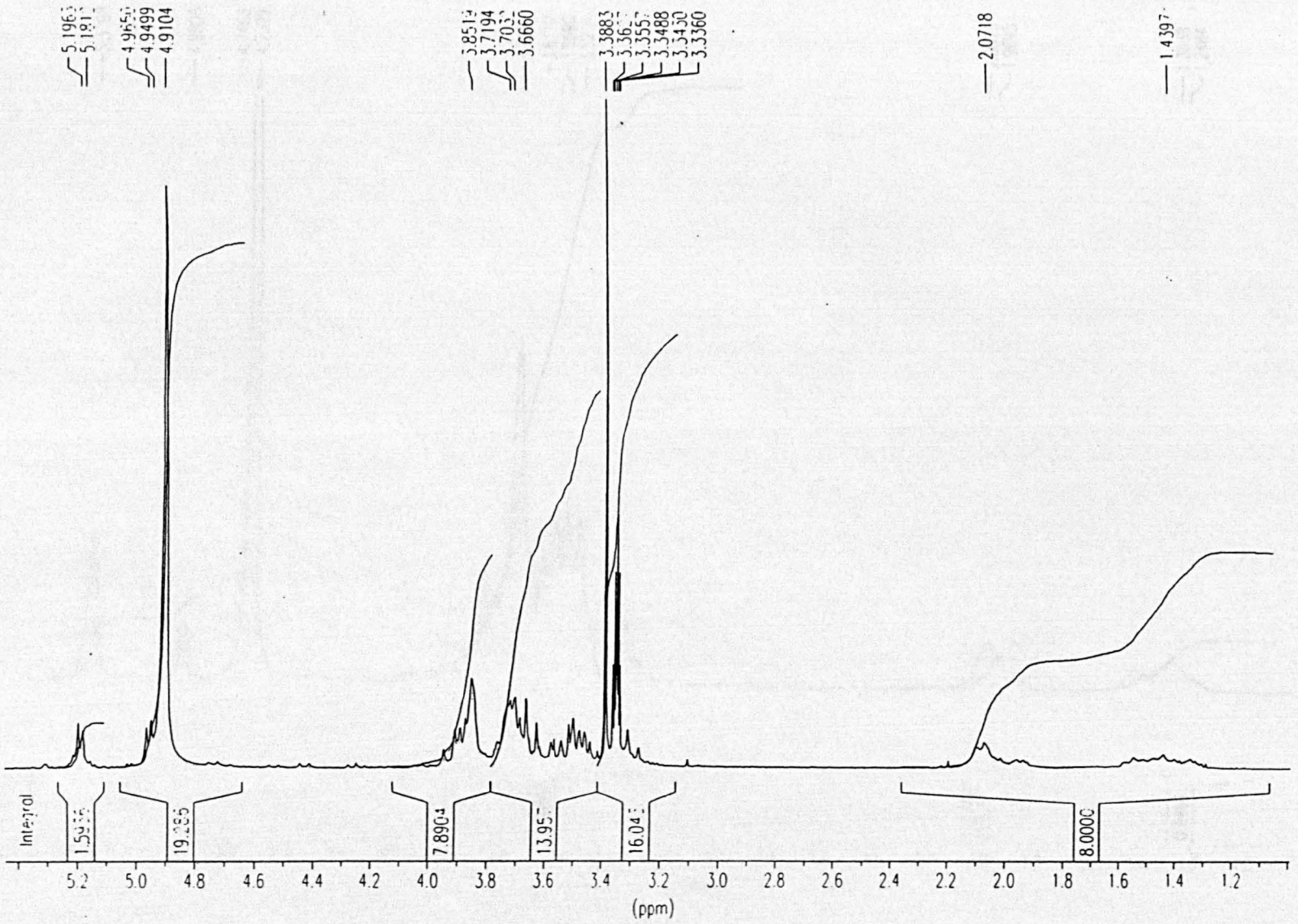


APPENDIX 2

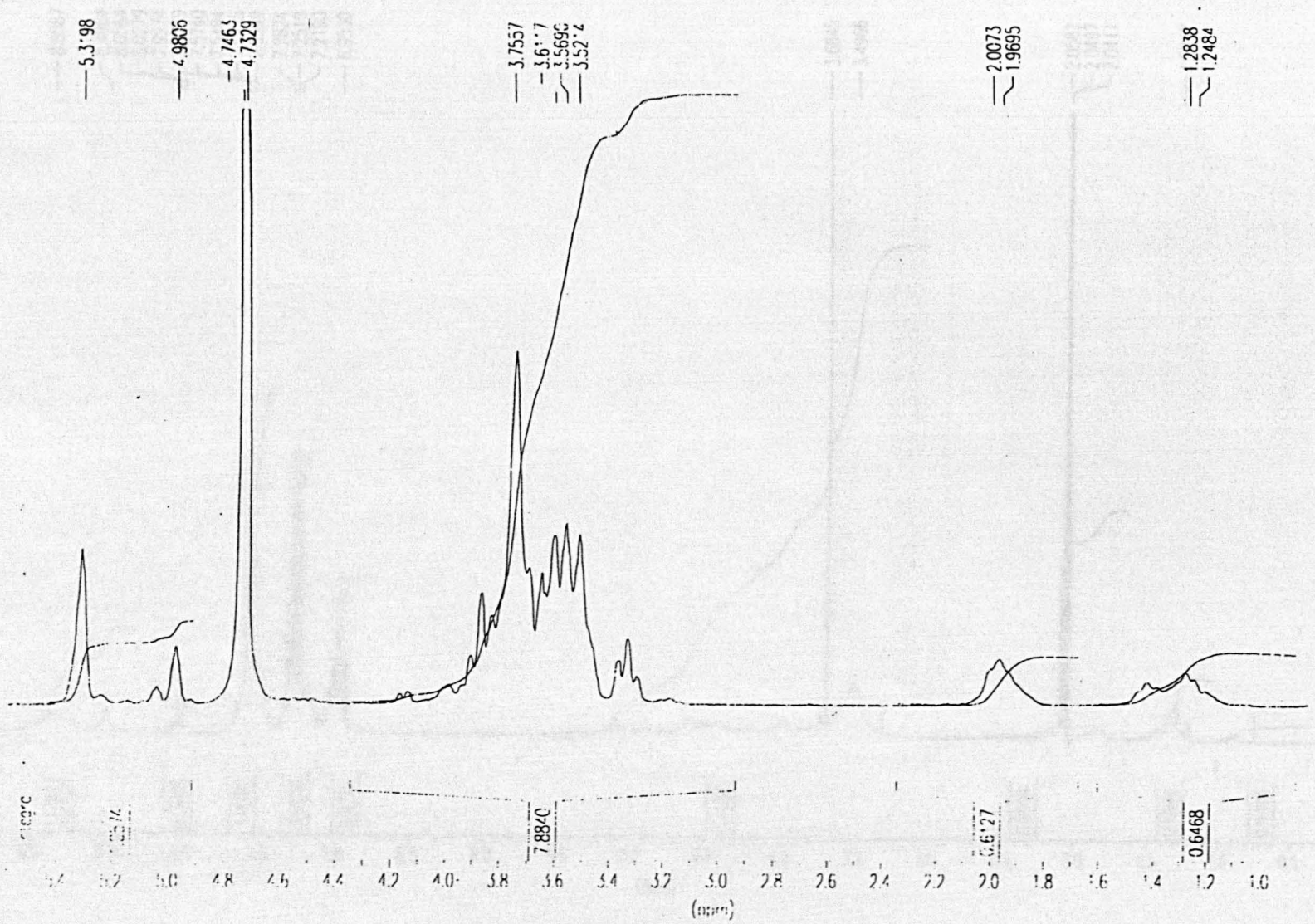
This appendix contains a selection of the NMR spectra for compounds in section 9.5 illustrating the difficulty in assignment including calculating the integrals for the respective peaks.

Compound 111

Compound 112



Compound 114



Compound 118

Compound 119

Compound 121