Solid-Phase Synthesis of Carbohydrate Derivatives

Submitted by

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A thesis submitted for the degree of

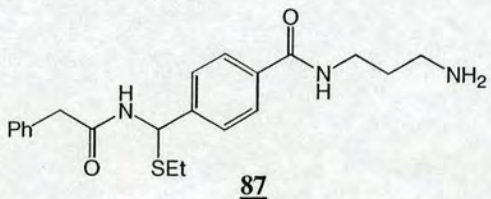
Doctor of Philosophy

University of Edinburgh

2000

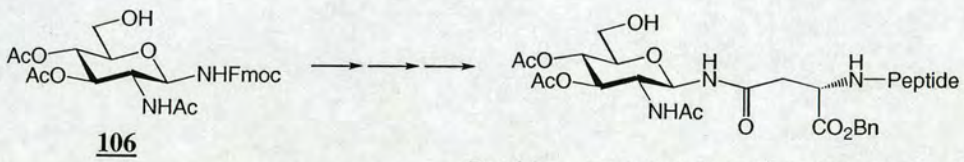
Abstract

Novel linker *N*-({[4-(3-aminopropylcarbamoyl)phenyl]ethylsulphanyl}methyl)-2-phenylacetamide **87** has been developed which is compatible with a range of reactions (e.g. base, oxidation, alkylation), can be cleaved under mild conditions and has the ability to release alcohols and amines. An efficient synthesis of the linker has been devised and attachment to a variety of solid supports (Tentagel®, polystyrene) has been achieved.



87

As a general building block 2-acetamido-3, 4-di-*O*-acetyl-2-deoxy-*N*-(fluoren-9-yl-methoxycarbonyl)-β-D-glucopyranosylamine **106** was synthesised in gram quantities from *N*-acetylglucosamine in 8 steps. The saccharide was linked through the 6-hydroxyl group onto carboxy-Tentagel® *via* linker **87** in excellent yield and extension of the glycosidic amino group provides a route towards the synthesis of glycopeptides.



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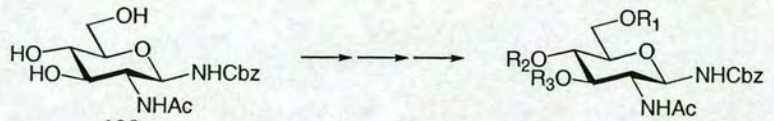
Peptide = -L-Phe-L-Ala-L-Thr-L-Ile-L-Val-NHFmoc

As an alternative, sugar building block 2-acetamido-2-deoxy-*N*-(benzyloxycarbonyl)-β-D-glucopyranosylamine **129** was synthesised from *N*-acetylglucosamine in 5 steps. The saccharide was attached onto carboxy-Tentagel® *via* linker **87** and has been used in initial studies into esterification reactions to generate a small library of compounds.



129

R_1 = H; R_2 = H or COR; R_3 = H or COR

Acknowledgements

Firstly, I wish to thank my supervisor Prof. Sabine L. Flitsch for her constant source of support, encouragement and enthusiasm for which I am very grateful. I would like to acknowledge all the members of the Turner-Flitsch group (past and present) who have helped me throughout this work. In particular, I would like to thank Dr's Ian Archer, James Dowden and Greg Watt for all their technical advice in the laboratory. I would also like to thank the Edinburgh Centre for Protein Technology for providing financial support throughout my three years of study.

Many thanks to all the technical staff at Edinburgh University who provided structural and analytical services. In particular, I would like to thank John Millar for the NMR spectral service; Adam Goldsworthy, Mark Scott and Sally Shirran for mass spectrometry analysis and Lorna Eades for CHN analysis. My gratitude also goes out to Derek, Raymond and Tim who work at the stores and all the staff in the glassblowing, electrical and mechanical workshops.

Lastly, I would like to thank my Mother and Father for providing love, support and encouragement throughout my many years at university. I also wish to thank Inga who has not only provided all these but has helped to proof read my thesis.

Abbreviations

AA	Amino acid
Ac	Acetyl
Ala	Alanine
All	Allyl
AMPS	Aminomethylpolystyrene
Anth	Anthracene
APCI	Atmospheric pressure chemical ionisation
Ara	Arabinose
Arg	Arginine
Asn	Asparagine
Asp	Aspartic acid
aq	Aqueous
Bn	Benzyl
Boc	Butoxycarbonyl
BOP	Benzotriazolyloxy-tris(dimethylamino)-phosphonium hexafluorophosphate
bp	Boiling point
Bt	Benzotriazole
Bu	Butyl
Bz	Benzoyl
c	Concentration
C_{ar}	Aromatic carbon
Cbz	Benzyloxycarbonyl
2-ClTrt	2-Chlorotrityl
CMP	Cytidine monophosphate
Coll	Collidine
Cp	Cyclopentyl
CPG	Controlled pore glass
Cys	Cysteine
δ	Chemical shift

d	Doublet
DCE	Dichloroethane
DCC	*N*, *N*′-Dicyclohexylcarbodiimide
DCM	Dichloromethane
DDQ	2, 3-Dichloro-5, 6-dicyano-1, 4-benzoquinone
Dhbt	3, 4-Dihydro-4-oxo-1, 2, 3-benzotriazole
DIC	*N*, *N*′-Diisopropylcarbodiimide
DIEA	*N*, *N*′-Diisopropylethylamine
DMAP	4-Dimethylaminopyridine
DMF	*N*, *N*′-Dimethylformamide
DMSO	Dimethylsulfoxide
DTBP	2, 6-Di-*tert*-butylpyridine
DVB	Divinylbenzene
EDCI	1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride
EDT	Ethanedithiol
EEDQ	2-Ethoxy-1-ethoxycarbonyl-1, 2-dihydroquinoline
EI	Electron impact
eq	Equivalent
ES	Electrospray
Et	Ethyl
ether	Diethylether
FAB	Fast atom bombardment
Fmoc	Fluorenylmethoxycarbonyl
Fuc	Fucose
g	Gram
GalNAc	*N*-Acetylgalactosamine
GalT	Galactosyltransferase
GlcNAc	*N*-Acetylglucosamine
Gln	Glutamine
Glu	Glutamic acid
Gly	Glycine

h	Hour
H_{ar}	Aromatic proton
HATU	2-(1H-9-Azabenzotriazolyloxy)-1,1,3,3-tetramethyluronium hexafluorophosphate
HBTU	2-(1H-9-Benzotriazolyloxy)-1, 1, 3, 3-tetramethyluronium hexafluorophosphate
HEPES	4-(2-Hydroxyethyl)-1-piperazineethanesulfonic acid
His	Histidine
HMP	4-Hydroxymethylphenoxy
HOBt	N-Hydroxybenzotriazole
HPLC	High performance liquid chromatography
Hyl	Hydroxylysine
Hyp	Hydroxyproline
IIDQ	2-Isobutoxy-1-isobutoxycarbonyl-1, 2-dihydroquinoline
Ile	Isoleucine
IR	Infrared
J	Coupling constant
LC-MS	Liquid chromatography mass spectrometry
Le^x	Lewis X
Lit.	Literature
Lys	Lysine
Lyx	Lyxose
m	Multiplet
M	Moles per Litre
M^+	Molecular ion
MALDI	Matrix assisted laser desorption ionisation
Man	Mannose
ManNAc	*N*-Acetylmannosamine
Me	Methyl
Met	Methionine
MHC	Major Histocompatibility complex
MHz	MegaHertz

mp	Melting point
ms	Molecular sieves
MS	Mass spectrometry
Mtr	4-Methoxy-2, 3, 6-trimethylbenzenesulfonyl
m/z	Mass-to-charge
Neu	Neuraminic acid
NIS	*N*-Iodosuccinimide
Nle	Norleucine
nm	Nanometre
NMM	*N*-Methylmorpholine
NMP	*N*-Methylpyrrolidinone
NMR	Nuclear magnetic resonance
PEG	Polyethyleneglycol
PEGA	Polyethyleneglycolacrylamide
Pfp	Pentafluorophenyl
Ph	Phenyl
Phe	Phenylalanine
Phth	Phthaloyl
Piv	Pivaloyl
ppm	Parts per million
PMB	*p*-Methoxybenzyl
PPTS	Pyridinium *p*-toluenesulfonate
Pr	Propyl
Pro	Proline
PS	Polystyrene
pyr	Pyridine
RP	Reverse phase
RT	Room temperature
s	Singlet
Sat.	Saturated
Ser	Serine
SPS	Solid-phase synthesis

Su	Succinimide
Sug	Sugar
TBAF	Tetrabutylammonium fluoride
TBAHS	Tetrabutylammonium hydrogen sulphate
TBDMS	*Tert*-Butyldimethylsilyl
TBDPS	*Tert*-Butyldiphenylsilyl
TBTU	2-(1H-9-benzotriazolyloxy)-1, 1, 3, 3-tetramethyluronium tetrafluoroborate
TFA	Trifluoroacetic acid
TfOH	Trifluoromethanesulfonic acid (Triflic acid)
TG	Tentagel®
THF	Tetrahydrofuran
THP	Tetrahydropyran
Thr	Threonine
TIPS	Triisopropylsilyl
TLC	Thin layer chromatography
TOF	Time-of-flight
Trt	Trityl
TsOH	*p*-Toluenesulfonic acid (Tosic acid)
Tyr	Tyrosine
UDP	Uridine diphosphate
UV	Ultraviolet
Val	Valine
Xyl	Xylose

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Chapter One; Introduction

Preface

Carbohydrates are widely found in nature and perform a variety of important functions [1]. For example, oligosaccharides on the surface of cells mediate many fundamental cellular processes including embryogenesis, tissue differentiation, inflammation and metastasis. Cell surface carbohydrates also function as receptors for bacteria, viruses and toxins [2]. Prokaryotic cells produce a variety of *O*-linked glycoconjugates with potent antitumour or antibiotic activity [3]. Some examples of natural carbohydrates are shown in Figure 1. A detailed understanding of the interactions between carbohydrates and their various receptors could lead to the ability to influence important recognition events and develop novel therapeutics.

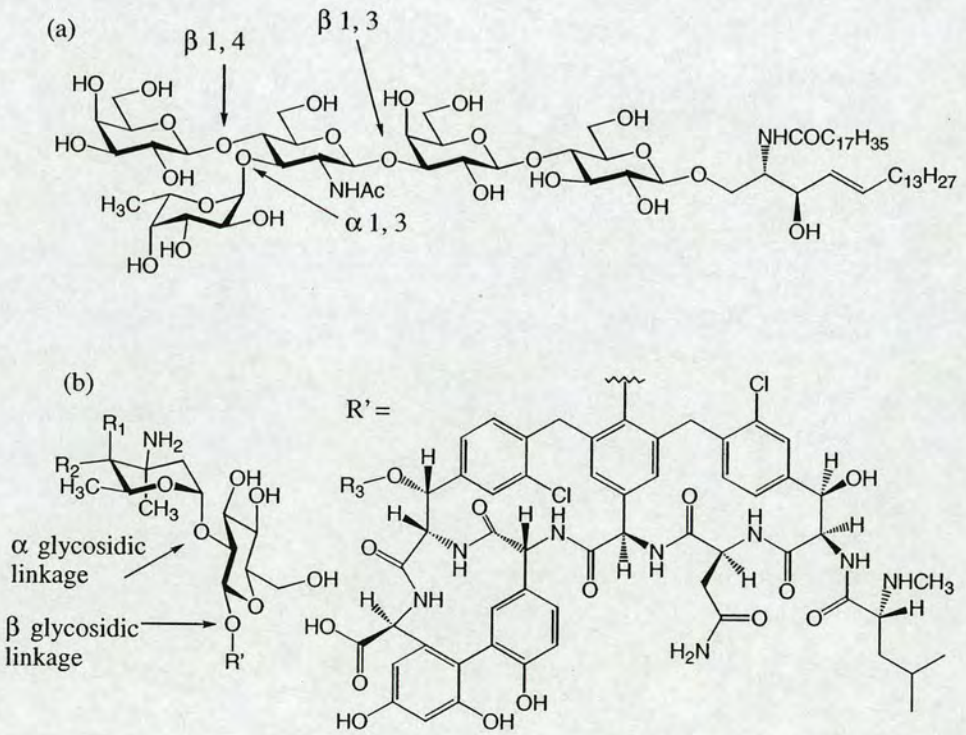


Figure 1: *Examples of carbohydrates. (a) Structure of Le^x, a natural cell surface oligosaccharide involved in both normal and pathogenic cell recognition processes. The shape of the molecule is largely determined by the glycosidic linkage conformation, anomeric stereochemistry and point of attachment. (b) Structure of vancomycin, a glycopeptide antibiotic. The attached oligosaccharide, which contains both α- and β-linkages, is critical for biological activity.*

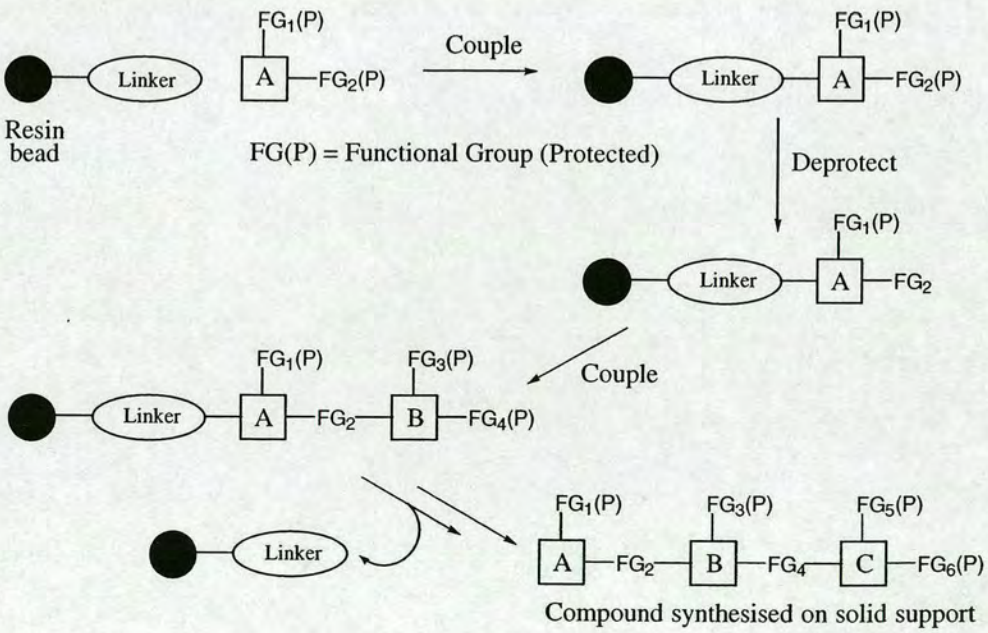
One major difficulty in determining an overall understanding of carbohydrates is that carbohydrate derivatives are extremely complex to synthesise. This is a result of a great number of possibilities for the regioselective combination of monomeric sugar units as well as the possible formation of two anomeric linkages, the α or β linkage. Biochemical studies on carbohydrate recognition also require access to both the natural carbohydrate and a set of related analogues that can be used to probe the role of particular structural features in binding. Unfortunately, conventional strategies for carbohydrate synthesis are so time consuming that progress in studies on carbohydrate recognition has been painstakingly slow. A solid-phase approach could dramatically accelerate the synthesis of these important compounds.

1.1. Solid Phase Synthesis

1.1.1 General Methodology

The techniques for solid-phase synthesis (SPS) are based on the pioneering work of Merrifield [4], who was the first to utilise substituted resins as the solid-phase for the synthesis of peptides. Solid-phase naturally lends itself to the production of peptides because of the limited range of synthetic transformations that are required for synthesis. Each of the key reactions required for peptide synthesis has been optimised to allow the production of peptides of sizeable length.

To be successful, solid-phase organic synthesis relies on three interconnected requirements. Firstly, the polymeric support used has to be inert to the conditions of synthesis. Secondly, there has to be some means of linking the substrate to this support that permits selective cleavage of product from the support without affecting the product structure in any way and this linkage unit is invariably called the linker. Lastly, a chemical protection strategy must be used to allow selective orthogonal protection and deprotection of reactive groups of monomers so that reactions can be performed on the solid phase. This is summarised in Scheme 1.



Scheme 1: *A schematic overview of solid-phase synthesis as used in peptide and oligonucleotide synthesis.*

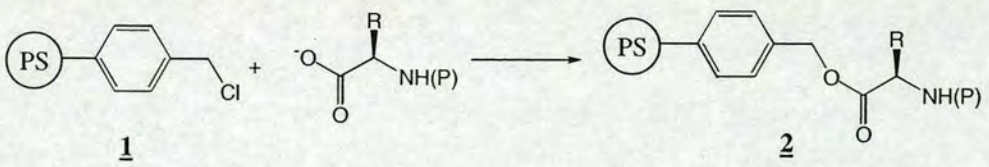
Solid-phase routes often allow the use of excess reagents to force reactions to completion. Other benefits often quoted when compared to solution phase methods are the ease of isolation at each step by simple filtration, the ease of automation and also the pseudo dilution effect [5], which can be synthetically useful in crosslinking or cyclisation reactions.

The main disadvantages of solid-phase chemistry are the extra labour required to develop a solid-phase route, the limitations of the current range of commercially available supports and linkers as well as limited means of monitoring reactions in real time. Solid-phase routes also necessitate additional steps to link and cleave to and from the support and are generally used to prepare less than 100 milligram of final product.

1.1.2 Solid Supports

The earliest forms of resin used were partially crosslinked polystyrene beads (styrene is copolymerised with 1 % divinylbenzene to give strength and insolubility whilst still permitting flexibility apparent during swelling) in a wide variety of sizes, prepared by light- or radical-catalysed polymerisation in an aqueous/organic mixture. Polymerisation takes place in micro droplets giving beads of approximately spherical shape. Sieving ensures the consistency of size.

The earliest form of polystyrene resin (Merrifield resin, **1**) used for peptide synthesis was derivatised with a chloromethyl group to which amino acids could be coupled by nucleophilic displacement (Scheme 2). The resulting ester bond **2** was stable to the conditions of peptide synthesis and was cleaved to give carboxylic acid products under strong acidic conditions (hydrogen fluoride).



1 **2**

Scheme 2: *Merrifield chloromethyl resin used for the attachment of carboxylic acids.*

Many alternative linking chains on polystyrene have been designed and described which are more labile to acid cleavage and these resins were used extensively for many years. As more and more groups have used them, it has become apparent that the resin has a significant effect on the rate and extent of reactions. The hydrophobic nature of the polystyrene and the hydrophilic nature of the growing peptide chain causes the latter to fold up. Rather than being solvated, internal hydrogen bonding of the peptide limits access to the exposed growing end chain, which results in variable reaction yields. Alternative resins have been investigated to alleviate these problems. The polyamide resin [6] developed by Sheppard *et al* is more hydrophilic like the growing peptide and the resin can be readily solvated by aprotic solvents such as DMF. For molecules other than peptides, there has been much use of the Tentagel® resin [7] (Rapp Polymere GmbH), which consists of about 80 % polyethylene glycol, grafted to cross-linked polystyrene. This resin is considered much more closely related to ether and tetrahydrofuran and consequently it has the potential for compatibility with a large range of reactions that are currently being investigated.

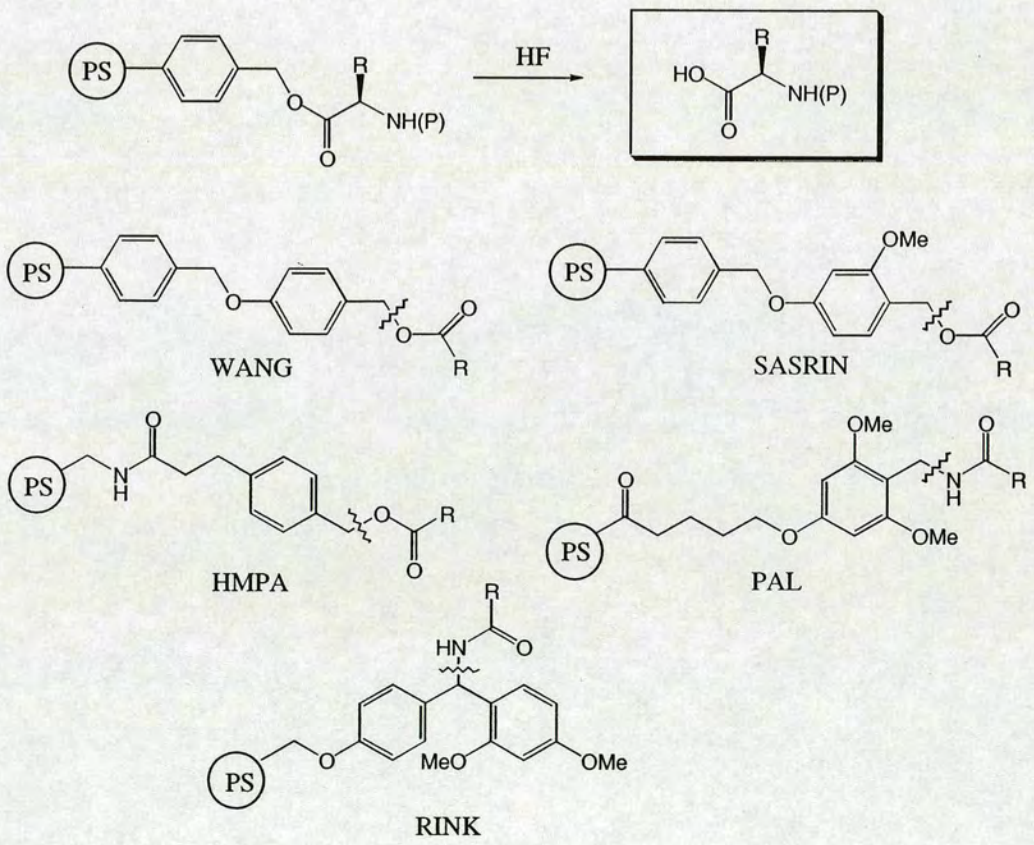
Other materials have been used for SPS including cellulose in the form of 'Perloza' beads [8], paper [9] and cotton [10]. Controlled pore glass (CPG) has also been reported to be a good alternative support [11] because no preswelling is required and has proven to be successful in solid-phase synthesis of oligonucleotides and light-induced spatially addressable parallel synthesis.

1.1.3 The Linker

SPS requires a covalent linker group to attach the small molecule onto the polymeric resin. This linker bears many similarities to protecting groups in solution phase synthesis in as much as it needs to be stable to the reaction conditions used during the elaboration of the small molecule. It needs to be cleaved selectively at the end of the synthesis thus releasing the small molecule from the resin into solution. It should be noted that compared to solution phase synthesis this requirement for a linker often adds two additional synthetic steps to a solid-phase route. Properties of a linker, which may assist SPS, are listed below.

- Stable to the reaction conditions required for synthesis
- Cleaved selectively at the end of synthesis
- Re-useable
- Facilitate reaction monitoring
- Sequential/Partial release
- Asymmetric induction

Historically, classical solid-phase peptide synthesis [4] has made use of an ester group (mentioned earlier) as a linker stable to peptide coupling conditions but cleaved by acid (HF) promoted hydrolysis thus liberating a carboxylic acid (Scheme 3). Other acid labile linkers have been developed for peptide synthesis and these can be cleaved using less harsh conditions and can liberate groups other than carboxylic acids (Scheme 3).



Scheme 3: *Acid cleavable linkers.*

The growing desire to synthesise increasingly diverse, non-peptide small molecules has led to a requirement for linkers which do not necessarily lead to a polar carboxylic acid group upon cleavage and currently a wide variety of these linkers exist [12]. The selection of the most appropriate linker for a particular class of target molecule is a key factor in designing a solid phase synthesis.

1.1.4 Reaction Monitoring [13]

Once the solid support and synthetic route has been selected, the next problem concerns the monitoring of reactions. In solution this would normally be performed using some form of chromatography (i.e. TLC). However, once the molecule is bound to an insoluble support, chromatographic monitoring can only be made possible after cleavage and work up procedures. For many supports this is very labour intensive and is normally only an option for linker functionalised supports which allow clean and fast release of substrate into a suitable solvent for trace analysis.

Useful techniques for on-resin monitoring are non-destructive techniques such as IR [14] and gel-phase NMR [15] which give useful results with standard laboratory instrumentation. However, the success of such techniques relies heavily on the loading of the substrate. Fully solvated resins give good quality ^{13}C NMR spectra under standard acquisition conditions and both PS-DVB and PEG-PS-DVB resins give similar quality spectra. More specialised equipment such as a high-resolution magic angle spinning probe is necessary to obtain good quality proton spectra and ^{1}H-^{13}C correlation spectra from polymer supported molecules [16], although the results can sometimes justify the expense.

Mass analysis using MALDI-TOF MS has been demonstrated as a useful analytical method for bead analysis [17]. There are also a range of classical analytical techniques which can give useful information on the progress of solid-phase reactions, such as titration of functional groups (amine, acids, thiols, etc); elemental analysis; gravimetric analysis; colour tests [18] (Ellman-thiols, Kaiser-amines, bromophenol blue-basic nitrogen, chloroanil- secondary vs. tertiary amine).

1.1.5 Experimental Conditions (vessels and agitation)

Typical glassware used for solution phase synthesis can also be suited to solid-phase reactions. Disposable fritted polypropylene vessels (isolute tubes) [19] are useful for carrying out solid-phase reactions at ambient temperature, although some solvents/reagents will leach plasticiser from the reaction vessel on cleavage of the product from the solid support.

Solid supports show varying levels of fragmentation during agitation and this breakdown is more pronounced at elevated temperatures. Hence, great care must be taken when deciding on methods of agitation [20]. Resins can successfully be stirred magnetically, but intermittent stirring and very low stirrer speeds must be used to avoid breakdown of beads and subsequent loss of material. Mechanical stirring (low speeds), gas sparging, vortexing and shaking are all less destructive methods of agitation than magnetic stirring, and sonification for short periods can also be used without detrimental effects with many supports. It is also generally accepted that allowing reactions to stand without agitation gives good results when using swelling solvents and high yielding reaction.

1.2. Polymer-Supported Syntheses of Glycopeptides

1.2.1 Overview

Linkages between carbohydrates and polypeptide chains can be divided into two principal groups: (1) those bearing an *N*-glycosidic linkage to L-asparagine and (2) those bearing an *O*-glycosidic linkage to L-serine, L-threonine, 4-hydroxy-L-proline or δ-hydroxyl-L-lysine (Figure 2).

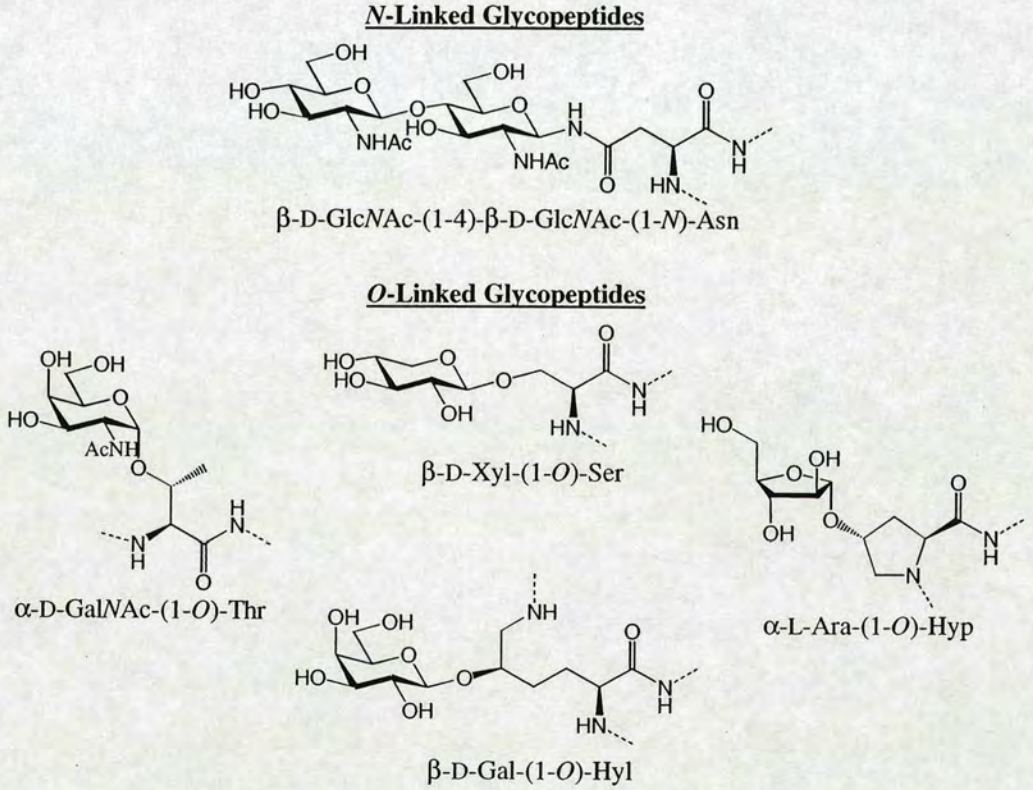


Figure 2: *Glycopeptide linkages.*

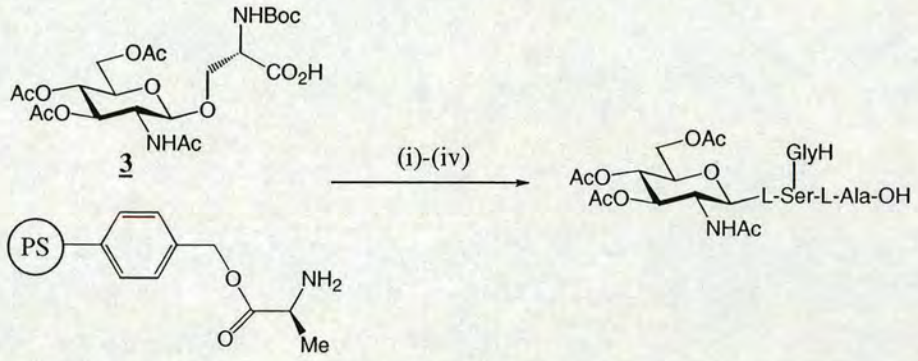
Both *N*-linked and *O*-linked glycopeptides and various structural analogues thereof are important for pharmaceutical, immunological, medical and biological research [21]. Various functional roles for carbohydrate moieties in glycoproteins have been postulated [1], including molecular recognition processes, control of membrane permeability and trafficking, protection from proteolytic attack, immunological masking, as well as the initiation and control of protein folding [22]. However, much information, pivotal to understanding the precise functions of these oligosaccharide moieties in living systems, is still not available.

Despite the tremendous amount of research that has been committed to unveil the biosynthetic pathways and biological effects of protein glycosylation, even for the more extensively studied classes of glycoproteins, the biological roles of the saccharide moiety of these compounds is not well understood [23]. As stated by J.C. Paulson [23(a)]: "Multidisciplinary approaches involving biologists, chemists, molecular biologists and geneticists will ultimately be required to unravel the emerging roles of carbohydrate-recognition signals in complex biological systems governing 'social' interactions of cells." This implies that for a more thorough understanding of the role of the carbohydrate portions featured in glycoproteins, elaborate synthesis and structural analysis of simpler glycopeptides are required [24]. This could be undertaken by the use of solid-phase techniques.

1.2.2 Solid-Phase Synthesis of O-Linked Glycopeptides

Recent developments have almost transformed the preparation of small glycopeptides carrying simple saccharides into matter of routine and have even put glycoproteins within reach of the synthetic chemist. At present, glycosylation of protected oligopeptides by chemical means does not constitute a feasible route to *O*-linked glycopeptides, despite attempts by Hollósi *et al* [25] involving the coupling of an oxazoline sugar derivative to resin bound protected peptides which had a serine residue present with a free hydroxyl group. However, yields for *O*-glycosylation were low. Instead, the most general synthetic route employs glycosylated amino acids for stepwise assembly of *O*-glycopeptides, preferably on solid-phase. Synthetic routes to suitably protected glycosylated amino acids therefore constitute a key success in the synthesis of glycopeptides.

The first method for the solid-phase synthesis of *O*-linked glycopeptides reported by Guilleman *et al* [26] involved attachment of Merrifield resin to alanine, which was subsequently coupled with an *N*-Boc-*O*-glycosyl serine derivative **3**. Subsequent removal of the *N*-Boc group on serine with TFA allowed formation of a new amine ready for subsequent iterative coupling to the required amino acids using the solid-phase technique (Scheme 4).



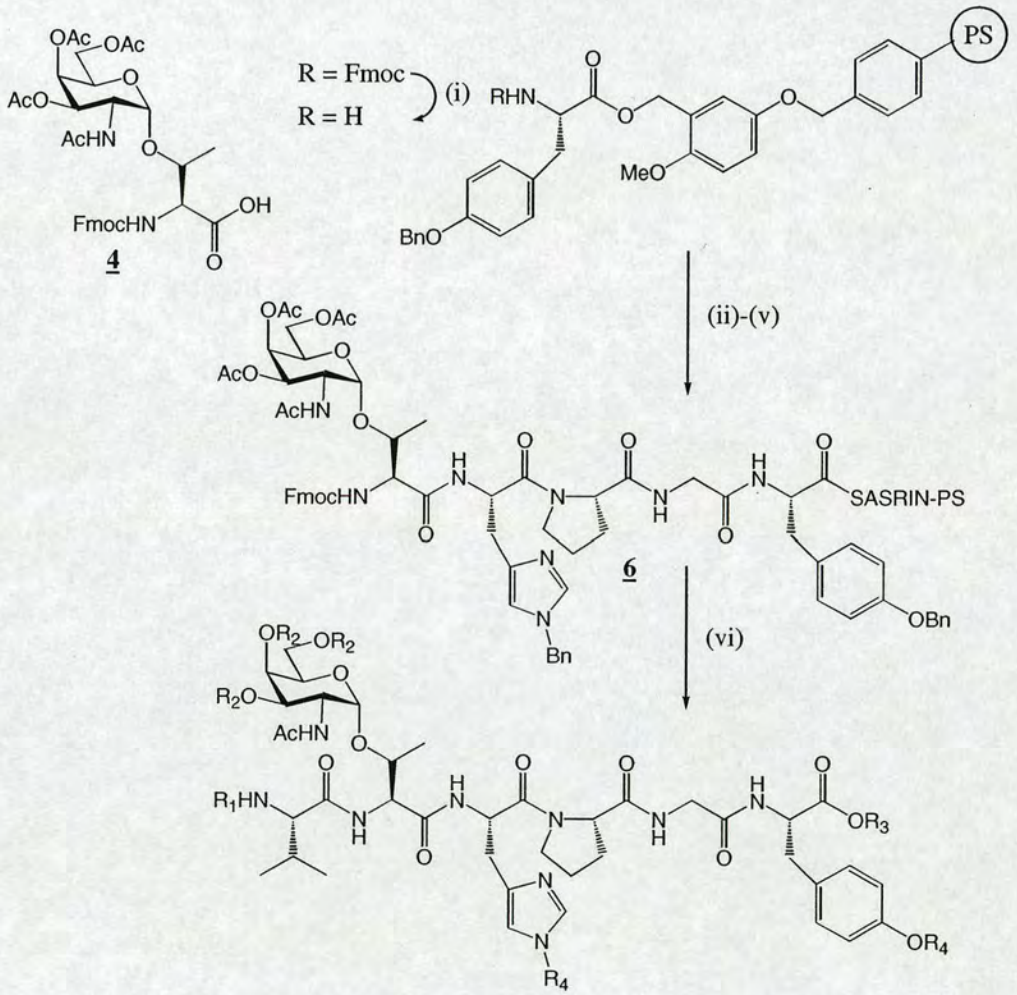
(i) DCC, HOBt, DCM:DMF (3:1), RT, 2h; (ii) TFA:DCM; Boc-Gly-OH, DCC, DCM, RT, 2h;
(iv) Anisole, HF, -20 °C for 0.5h then 0 °C for 0.3h.

Scheme 4: *Guilleman et al general strategy for the synthesis of O-linked glycopeptides.*

Since *O*-glycosyl-β-hydroxyl carboxyl acid derivatives are sensitive to both strong acid (bond cleavage and anomerisation) and strong base (β-elimination and racemization) suitable acid- and base-labile protecting groups are required. This early approach, reported by Guilleman *et al*, to solid-phase synthesis of *O*-linked glycopeptides relies on the *tert*-butyloxycarbonyl (Boc) group for α-amino group protection. This process, however, requires repeated N^{α}-deprotection with trifluoroacetic acid and final cleavage from the solid phase with a strong acid, which are likely to cleave glycosidic bonds. Protection of the α-amino group with the base labile fluoren-9-ylmethoxycarbonyl (Fmoc) group (Scheme 5) has shown to be attractive in this respect due to its mild sensitivity to weak organic bases such as morpholine [27] and piperidine [28] which was later reported to be the base of choice. This group thus allows the use of protective groups for amino acid side chains and linkers on the solid phase, which are cleaved by moderately strong acid such as trifluoroacetic acid [29].

Work reported by Lüning *et al* [30] illustrates the use of the Fmoc group and reiterates the general strategy towards *O*-glycopeptides. The glycosylated amino acid building block was synthesised in an 8-step synthesis using Fmoc-threonine phenacyl ester, which after glycosylation was de-esterified with zinc in acetic acid to give the free acid **4** (Scheme 5). The oncofetal sequence of fibronectin attached to glycosylated-threonine **5** was chosen as the target molecule. Fmoc-*O*-benzyltyrosine functionalised SASRIN resin was first treated with 50 % piperidine in DMF to remove the Fmoc group and then Fmoc–glycine, Fmoc-proline and Fmoc-histidine

were successively coupled either as their symmetrical anhydride or 1-hydroxybenzotriazole ester. Peracetylated Fmoc-*O*-(α-D-GalNAc*p*)-threonine **4** was then coupled to give resin bound glycopeptide **6**. Fmoc-Valine was coupled last and after Fmoc removal, the resin was treated with 1 % TFA in DCM to give the desired glycopeptide **5**. Paulsen *et al* have also reported [31] using peracetylated Fmoc-*O*-(α-D-GalNAc*p*)-threonine **4** in the solid-phase synthesis of two glycopeptide sequences.



R_1 = Fmoc; R_2 = Ac; R_3 = SASRIN-PS; R_4 = Bn

$R_1 = R_2 = R_3 = R_4 = H$, **5**

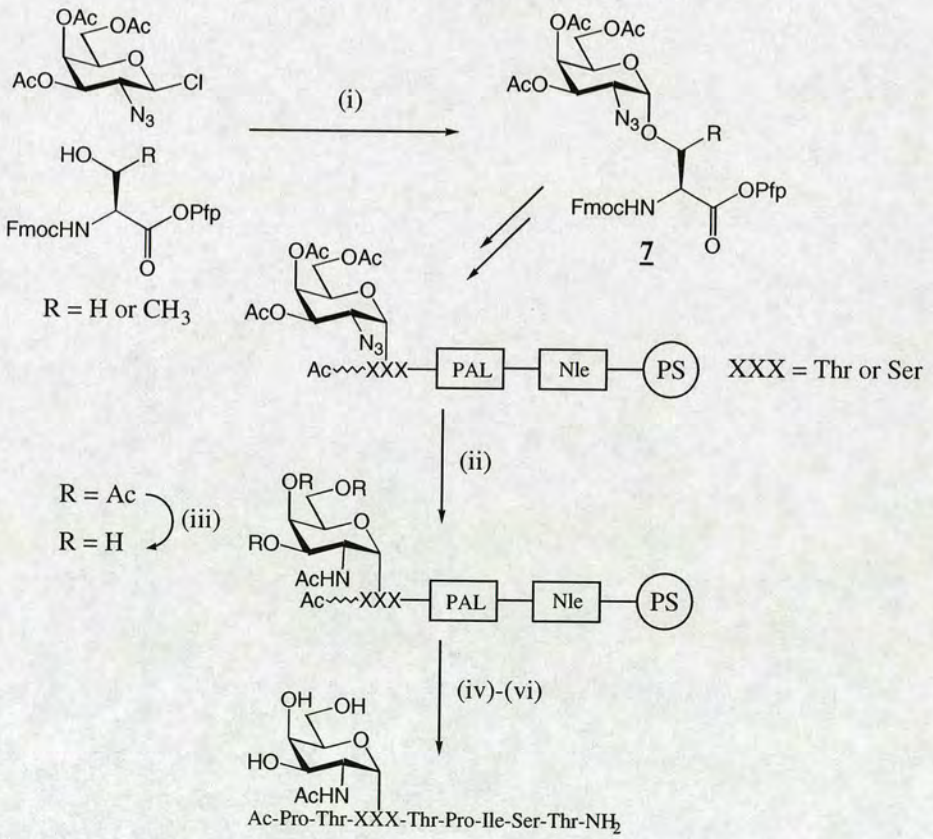
(vii)-(x)

(i) 50% piperidine in DMF; (ii) Fmoc-Gly-OH, DCC, HOBt, DMF, repeat (i);(iii) Fmoc-Pro anhydride, DMF, repeat (i); (iv) Fmoc-His(Bn)-OH, DCC, HOBt, DMF, repeat (i); (v) **7**, DCC, HOBt, DMF, repeat (i); (vi) Fmoc-Val anhydride, DMF, repeat (i); (vii) 1% TFA, DCM; (viii) 0.1M NaOMe, MeOH; (ix) 10% Pd/C, H_2.

Scheme 5: *Lüning et al synthesis of the oncofetal sequence of fibronectin* **5**.

It was later reported that for temporary protection of the α-carboxyl group, pentafluorophenyl (Pfp) esters **7** (Scheme 6) have several advantages in the building block synthesis as they serve the dual purpose of protecting the carboxylic acid during glycosylation and at the same time activating the carboxyl group for the subsequent amide bond formation [32]. The use of such groups therefore avoids elaborate orthogonal protection schemes, however, the recoveries of these glycosidic amino acids still require a number of steps.

Meldal *et al* reported [33] a new strategy towards the synthesis of *O*-glycopeptides in which sugar building block **7** (Scheme 6) is used in the peptide synthesis. The transformation of the azido group into acetamido functionality can take place after completion of the peptide chain on solid support and deacetylation of all sugar residues was also possible on the polymer-bound peptide. In the last step the final glycopeptide was cleaved off the resin. Synthesis of sugar building block **7** can be achieved in 2 steps less than the 8 steps reported by Lüning *et al.*



i) Ag_2CO_3, $AgClO_4$; (ii) CH_3COSH; (iii) $N_2H_4.H_2O$, MeOH; (iv) Fmoc-AA-OPfp, Dhbt-OH; (v) TFA, H_2O; (vi) HPLC.

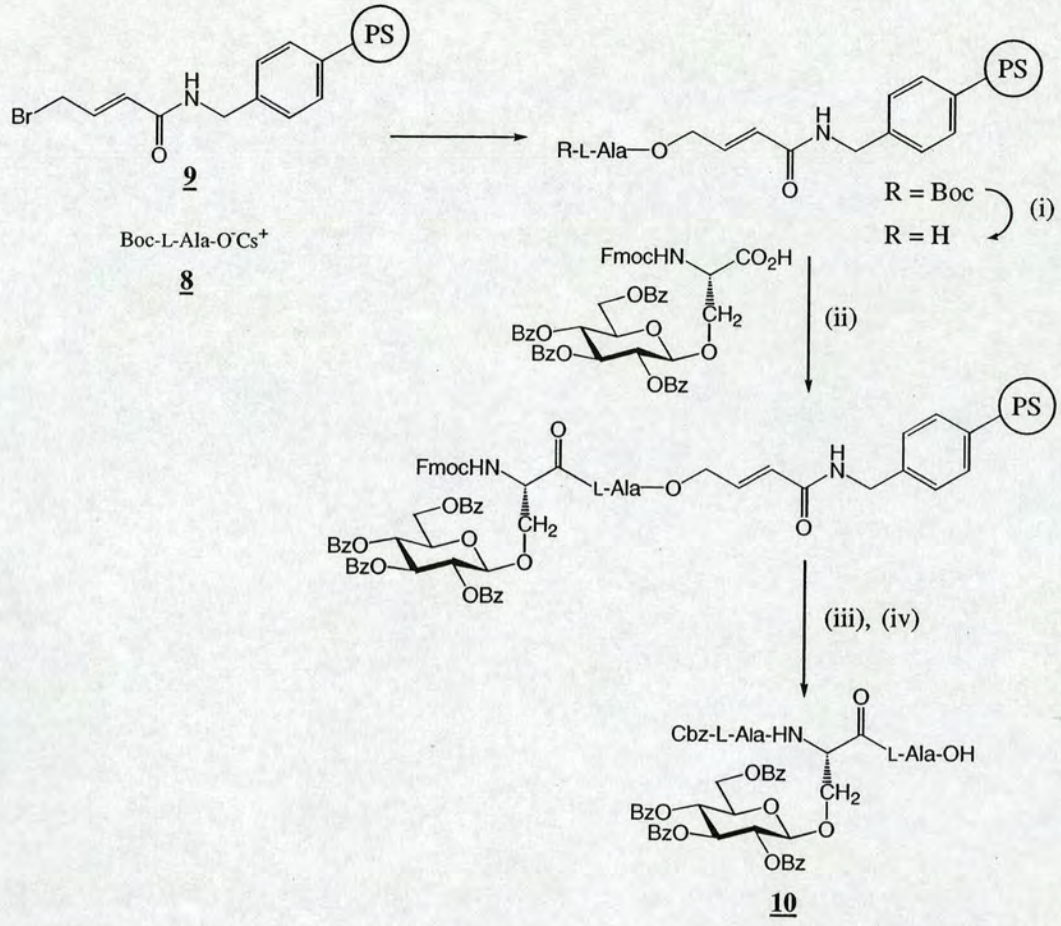
Scheme 6: *Meldal et al synthesis of O-linked glycopeptide using* **7** *as the sugar building block.*

Meldal *et al* have further illustrated this strategy in the synthesis of highly glycosylated *O*-glycopeptides with T^n-antigenic structures corresponding to human glycophorin A^N [34]. It has also been demonstrated that this methodology can be used with disaccharide building blocks [35]. It was later reported by Meldal *et al* [36] that the synthesis of oligosaccharides on glycopeptides bound to a solid support could also be achieved successfully. Glycopeptides with linear and branched trisaccharide side chains were obtained.

The groups of Schmidt [37], Nakahara [38] and Kihlberg [28, 39] have all been productive in this area demonstrating the syntheses of many *O*-glycopeptides using a variety of threonine and serine building blocks. Many alternative solid supports and linkers have been adopted from procedures developed for the solid-phase syntheses of peptides.

A new HYCRAM™ linker compatible with the reaction conditions necessary for *N*- and *O*-deprotection of amino acids was introduced by Kunz *et al* [40] for improved polymer supported syntheses of glycopeptides [41]. Amino acids are easily attached to the polymer by reaction of the caesium salt of the *N*-protected amino acid, for example **8**, with 2-(4-bromo-2-butenamido)methyl-polystyrene (HYCRAM™) **9** (Scheme 7).

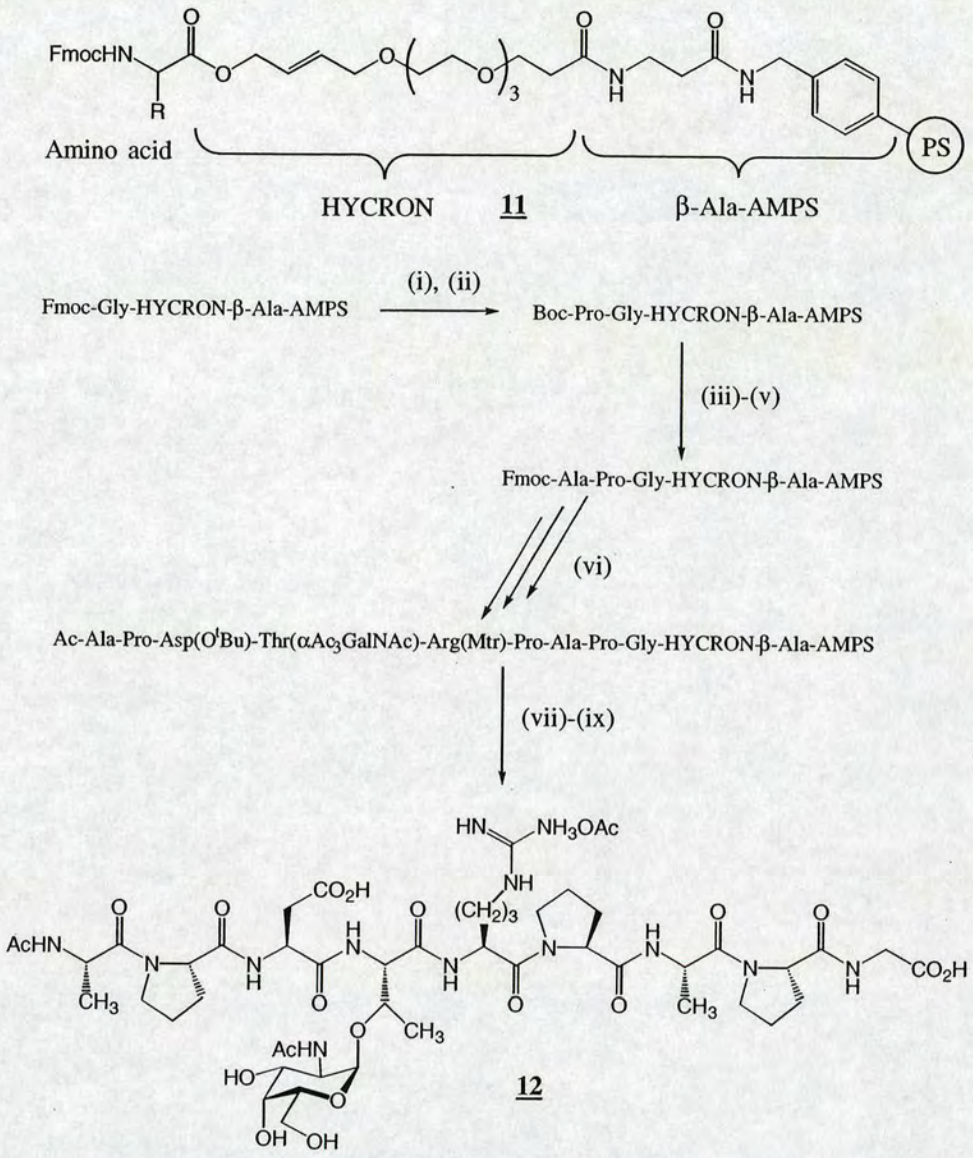
The glycotripeptide **10** was readily removed from the resin using palladium (0)-catalysed allyl ester cleavage [40]. Under the essentially neutral conditions employed, the other protecting groups, as well as the *O*-glycosidic bond, remained intact. Complex glycopeptides have been synthesised successfully using this method [42].



(i) TFA; (ii) DCC, HOBt; (iii) Morpholine; (iv) Cbz-Ala-OH, DCC, HOBt; (v) $[Ph_3P]_4Pd$, morpholine.

Scheme 7: *Kunz et al syntheses of O-linked glycopeptides using HYCRAM™ linker* **9**.

While glycopeptides could be detached from the resin in high yields when the Boc strategy was used, reduced yields were observed using the much-preferred Fmoc group. Studies showed that upon exposure to morpholine for 3 days, 12 % of the allyl ester was aminolysed. As in many solid-phase syntheses, losses can occur at the dipeptide stage (formation of diketopiperazine) and due to steric hindrance, during the cleavage step itself. A new anchor of the allyl ester type was therefore designed **11** (Scheme 8) [43], which incorporated a flexible, polar spacer in order to reduce steric hindrance and associations with the polystyrene matrix [44]. This anchor also replaced the α, β-unsaturated carbonyl structure, which in turn increased stability against nucleophilic attack. This newly developed linker **11** was used in the synthesis of glycononapeptide **12** [45] as outlined in Scheme 8. Peptide **12** represents a partial sequence of the repeating unit of MUC-1 mucin.

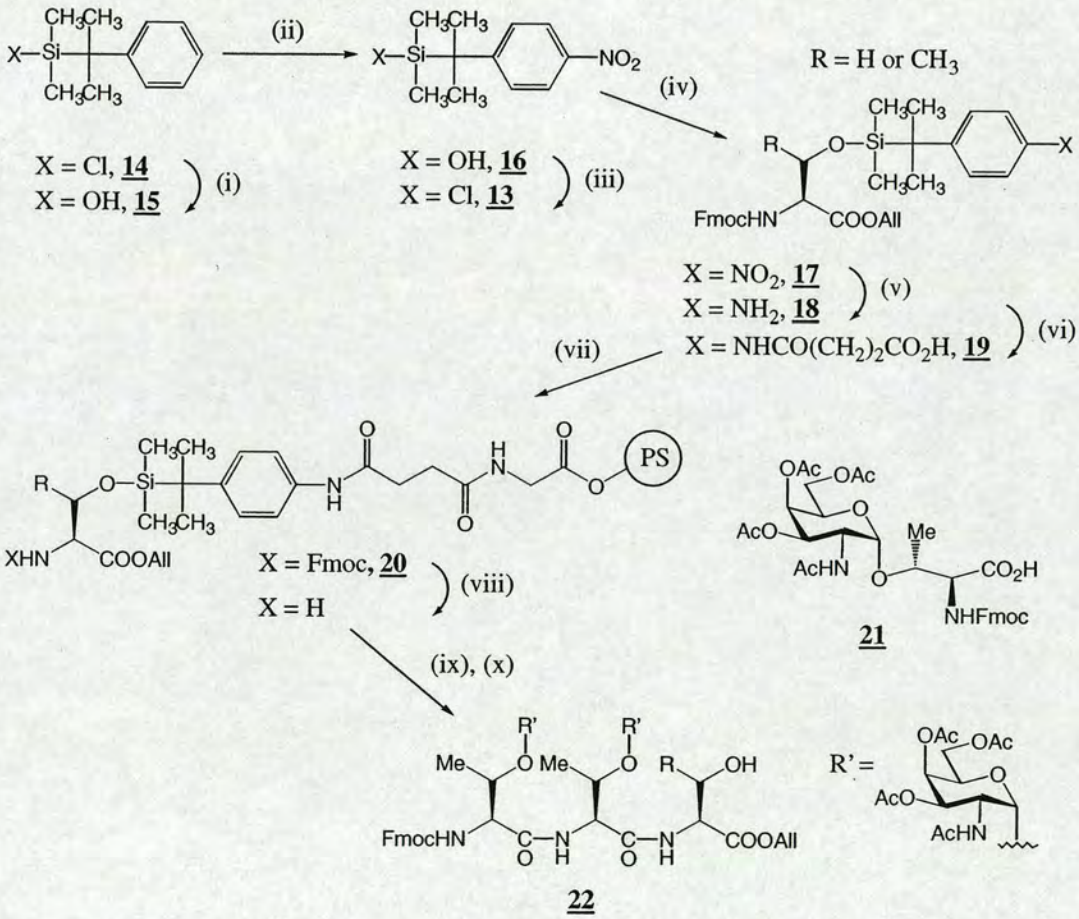


(i) DMF, morpholine; (ii) Boc-Pro-OH, DIC, HOBt; (iii) DCM/TFA [1/1]; (iv) DIEA/DCM [1/10]; (v) Fmoc-Ala-OH, DIC, HOBt; (vi) Fmoc-AA-OH, TBTU, DIC, HOBt; (vii) [$(PPh_3)_4Pd$], morpholine, DMF/DMSO [1/1]; (viii) TFA/MeSEt/MeOPh [40/1/1]; (ix) NaOMe, MeOH.

Scheme 8: *Kunz et al syntheses of MUC-1 repeating unit glyconanapeptide* ***12*** *using the HYCRON™ anchor* ***11***.

Recently, Nakahara *et al* have reported [46] a novel silyl linker **13** for the synthesis of glycopeptides, which enables the side-chain hydroxyl groups of the peptide or glycopeptide to bind to the solid support. A key feature of this approach is the feasibility of peptide chain-elongation at both *N*- and *C*-termini. Synthesis of this linker can be achieved in 3 steps (Scheme 9).

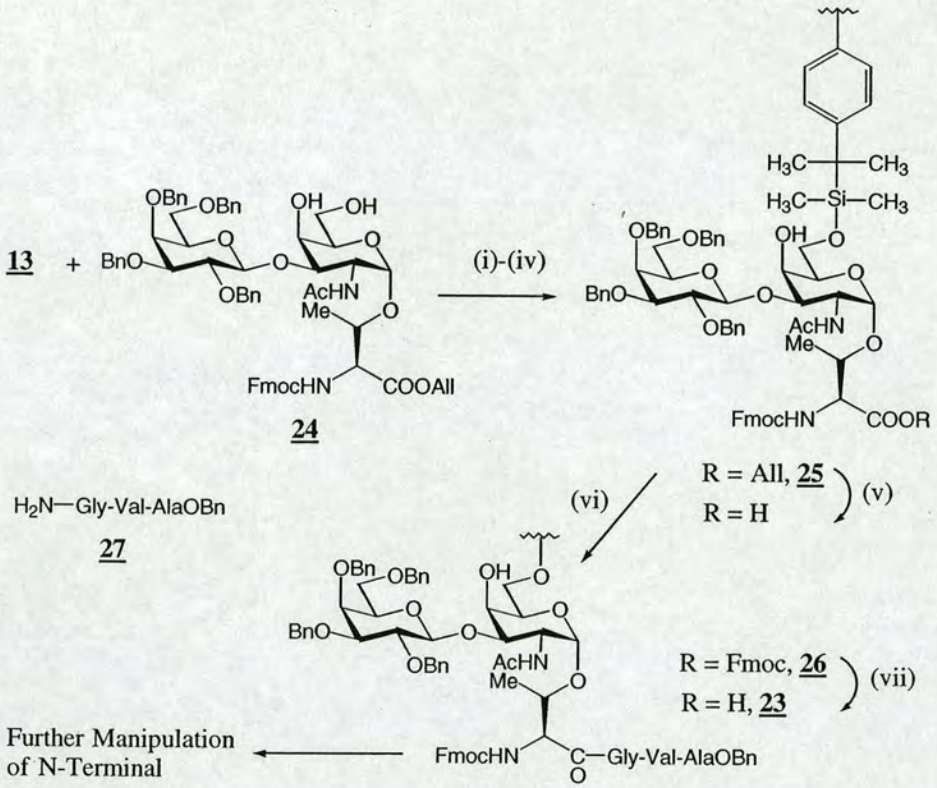
Chloro(α, α-dimethylbenzyl)dimethylsilane **14** was first hydrolysed with aqueous KOH to give silanol **15**. Nitration of this compound with ammonium nitrate-trifluoroacetic anhydride afforded *p*-nitro compound **16**, after which chlorination with oxalyl chloride gave linker **13**. β-Hydroxy amino acids can then be attached using **13**, NaI and NMM in DMF which gave the most consistent yields towards **17**. Reduction of the nitro group was achieved using Zn-AcOH to give the aniline derivative **18**, which was treated with succinic anhydride to afford **19**. The carboxylic acid of **19** was then activated and attached to Gly-preloaded Wang resin to give **20**, which can then be further derivatised at the *N*-termini using **21** to give **22** [47]. Isolation of the synthesised glycopeptide **22** was readily accomplished by fluoride ion-mediated hydrolysis and simple chromatographic purification.



(i) KOH; (ii) NH_4NO_3, $(CF_3CO)_2O$, CH_3CN; (iii) $(COCl)_2$, DMF, DCM; (iv) NaI, NMM, DMF; (v) Zn-AcOH; (vi) succinic anhydride, DCM; (vii) polymer-Wang-Gly-NH_2, HBTU, HOBt, DIEA, NMP; (viii) 50% piperidine in NMP; (ix) **21**, HBTU, HOBt, NMP; (x) CsF, AcOH, DMF.

Scheme 9: *Nakahara et al syntheses of glycopeptides using silyl linker **13** (N-terminal).*

This methodology was also shown to be useful in *C*-terminal peptide chain elongation as demonstrated with the synthesis of **23** (Scheme 10) [47]. Attachment of **24** was achieved using linker **13**, *O*-glycosyl-β-hydroxyl carboxyl acid derivative **24** and imidazole to give **25**, which can be further manipulated to glycopeptide oligomer **26** by coupling **27**. Removal of the Fmoc group yields **23** which can be further manipulated at the *N*-terminus.



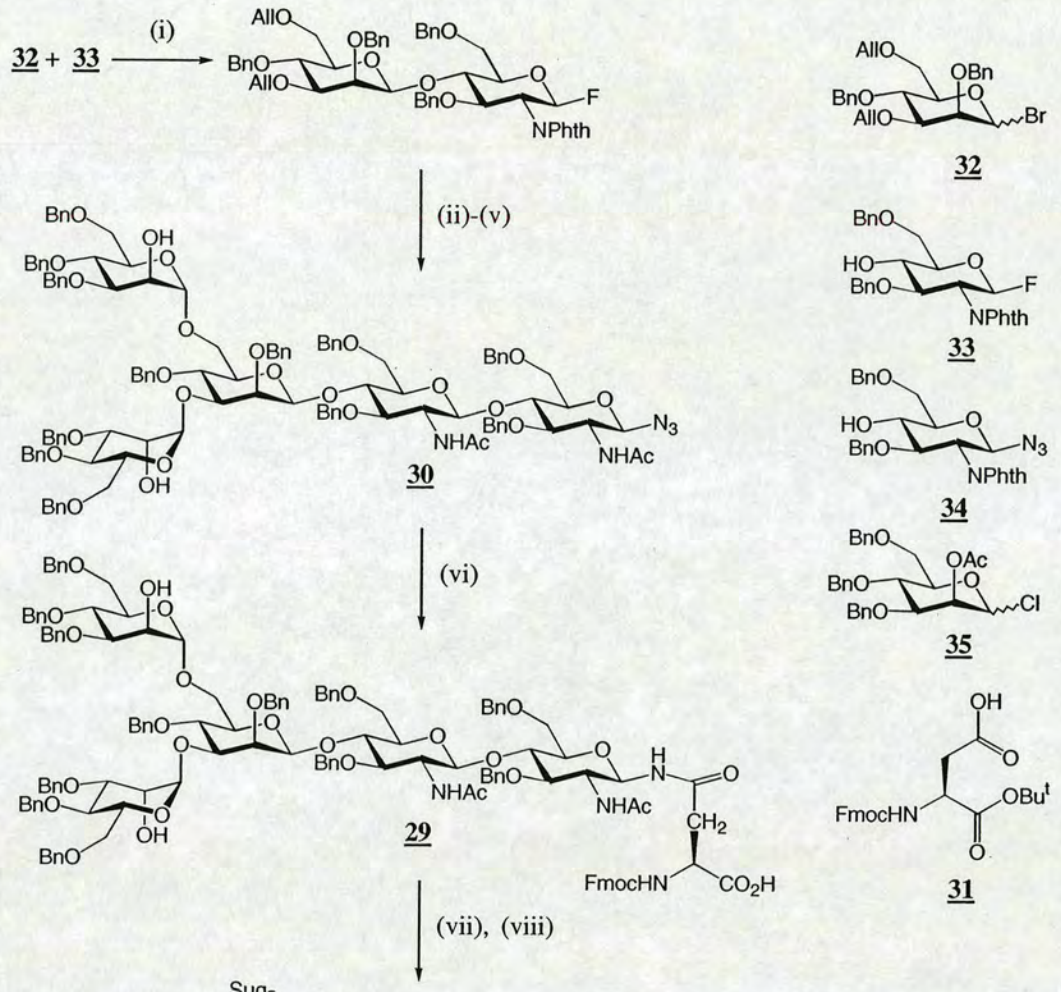
(i) NaI, NMM, DMF; (ii) Zn-AcOH; (iii) succinic anhydride, DCM; (iv) polymer-HMP-Gly-NH_2, HBTU, HOBt, DIEA, NMP; (v) [(PPh_3)Pd], dimedone, THF; (vi) **27**, HBTU, HOBt, DIEA, NMP; (vii) 50% piperidine in NMP.

Scheme 10: *Nakahara et al syntheses of glycopeptides using silyl linker **13** (C-terminal).*

1.2.3 Solid-Phase Synthesis of N-Linked Glycopeptides

Synthesis of *N*-glycopeptides have been carried out most often by the stepwise approach, in which a β-glycosyl amine is coupled to a suitably-protected Asp derivative to give an Asn(Sug) derivative. β-Glycosyl amines have generally been synthesised by reduction of the corresponding azides [48] and can be illustrated by the synthesis of CD52 glycopeptide **28** (Scheme 11) that was reported by Nakahara *et al* [49]. The first step of the synthesis was the preparation of the key building

block **29**. Once the core pentasaccharide azide **30** was achieved in 5 steps, the next key reaction was reduction of the azide to the corresponding amine. This was achieved successfully using a Lindlar catalyst under a hydrogen atmosphere followed by selective acylation of the resulting oligosaccharyl amine by activated aspartic acid **31** using the standard HOBt/DCC activation method. The final step towards the core pentasaccharide-Asn conjugate **29** was deprotection of the carboxylic acid using TFA in DCM in an overall yield of 23 %. The synthetic strategy involved the automated synthesis of Asp^{4}-Ser^{12} peptide on an HMP resin using the traditional Fmoc method. Manual coupling to the Asn pentasaccharide **29** was achieved using HOBt and DCC in 98 % yield. The glycopeptide was then reapplied to the automatic synthesiser and elongated to afford the target glycopeptide **28** in 94 %.

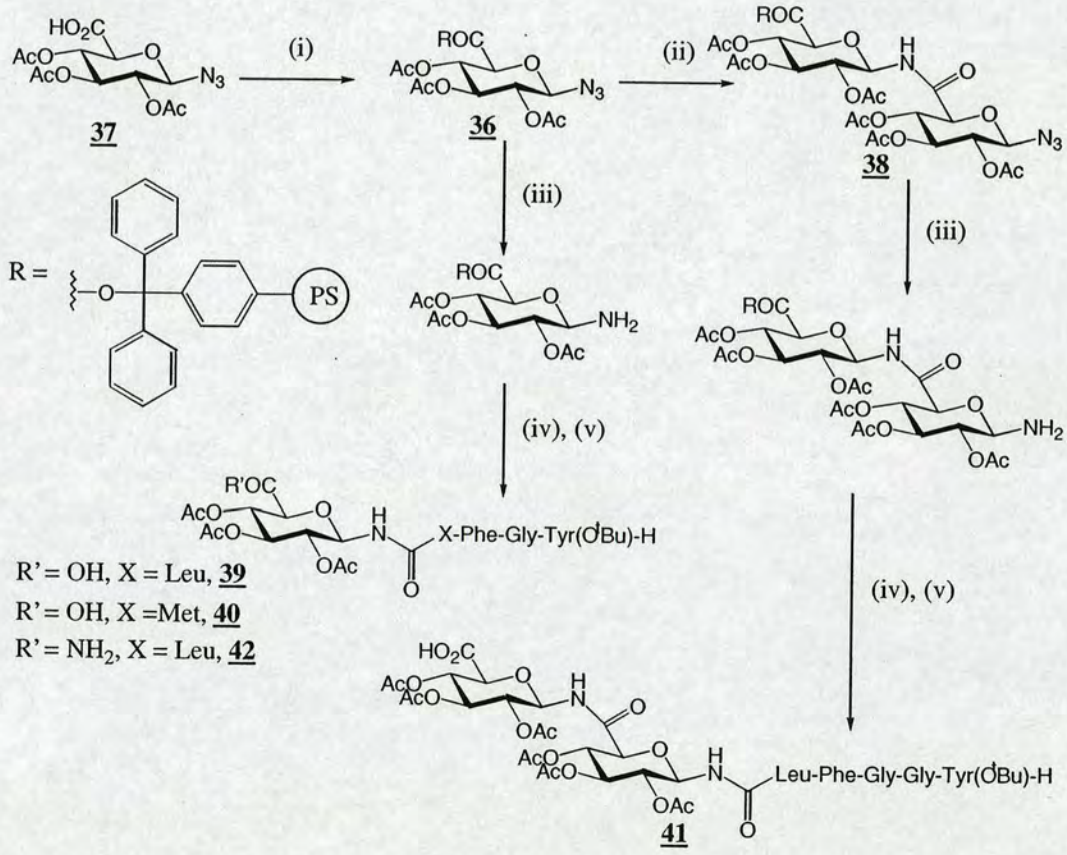


(i) Silver alumina silicate, 4Å ms, DCM, 0 °C-RT, 3h; (ii) 4Å ms, Cp_2HfCl_2, $AgClO_4$, **34**, DCM, -20 °C-RT, 16h; (iii) $[Ir(COD)(PMePh_2)_2]PF_6$, THF, RT, 1h; $HgO/HgCl_2$, $(CH_3)_2CO$, RT, 1h; (iv) AgOTf, 4Å ms, **35**, DCM, -40 °C-RT, 16h; (v) a) $NH_2(CH_2)_2NH_2$, *n*-BuOH, 90 °C, 44h; b) Ac_2O, MeOH, 0 °C, 2h; (vi) a) Lindlar cat., H_2, MeOH, RT, 16h; b) **31**, DCC, HOBt, DCM, RT, 16h; c) TFA, DCM, RT, 2h; (vii) a) Peptide-HMP-Resin, HOBt, DCC, RT, 1h; b) 20 % piperidine in NMP; c) Fmoc-AA-OH, HOBt, DCC, RT, 2h; (viii) a) 95 % TFA,/ 2.5 % EDT/H_2O, RT, 2h; b) 20 % $Pd(OH)_2/C$, H_2, EtOH, H_2O, RT.

Scheme 11: *Nakahara et al synthesis of CD52 glycopeptide **28** using core pentasaccharide-Asn conjugate **30**.*

In 1997 Toth *et al* synthesised unnatural *N*-linked glycopeptides in order to synthesise *C*-terminal carbohydrate enkephalins [50]. In this work a modified glucuronic acid was immobilised on a polystyrene based support *via* a 2-chlorotrityl linker **36** (Scheme 12). Reduction of the resin bound sugar-azide was achieved by treatment with a mixture of triethylamine and propane-1, 3-dithiol to generate the free amine in *situ* [51] which was coupled to monosaccharide **37** using HBTU and

HOBt as the coupling reagents to give resin bound dimer **38**. **36** and **38** were used in peptide synthesis using standard coupling procedures. Upon completion of the syntheses, the *N*-terminal Fmoc group was removed followed by hydrazine-mediated deprotection of the sugar acetates. Acidolytic resin cleavage and concomitant side chain deprotection relinquished the carbohydrate-modified enkephalins **39**, **40** and **41**. The carboxamide analogue **42** of glycoconjugates **39** was also synthesised by initial immobilisation of the glycosyl azide onto NovaSyn TGR resin modified with the Rink linker.

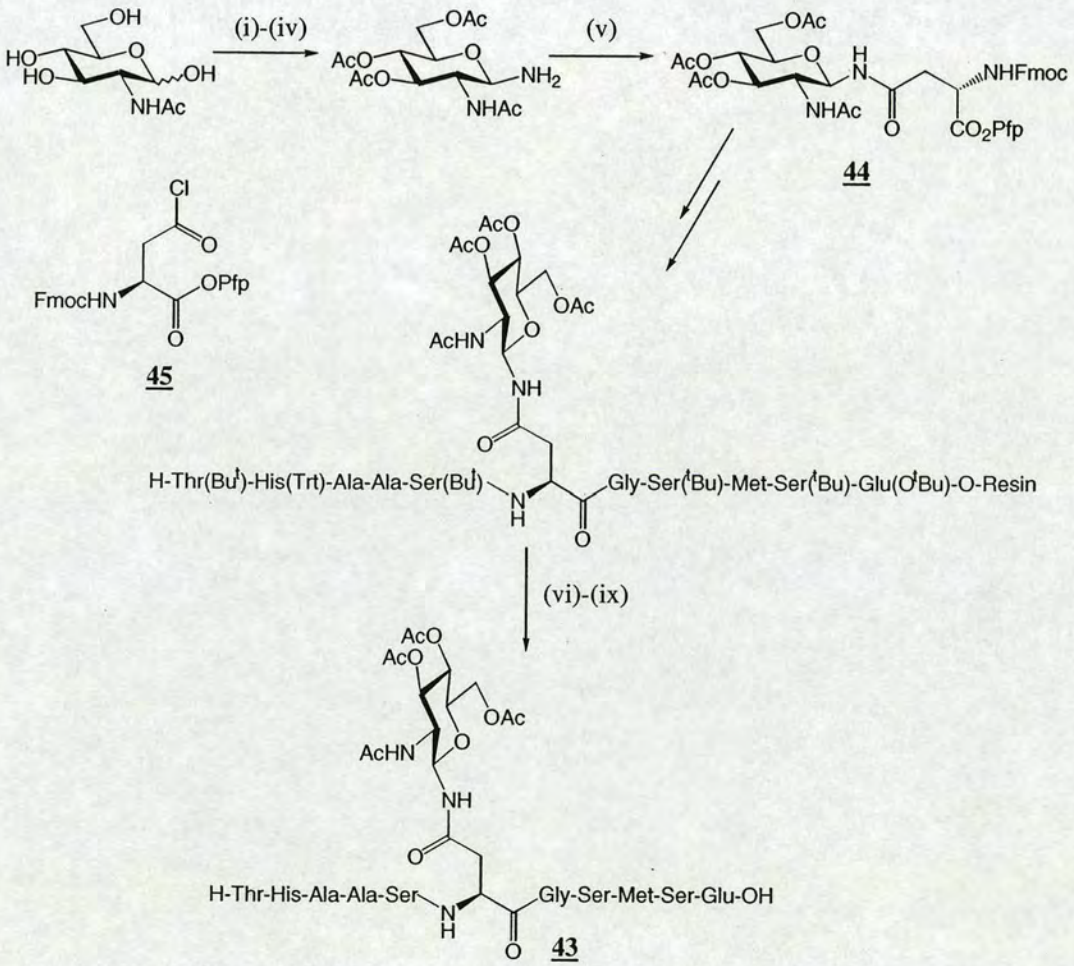


(i) 2-Clt resin, DIEA, DCM; (ii) a) EDT, Et_3N; b) HBTU, HOBt, DIEA, DMF; (iii) 20% piperidine in DMF;
(iv) Fmoc-AA-OH: Leu or Met, Phe, Gly, and Tyr(O^{t}Bu), HBTU, HOBt, DIEA;
(v) X = Leu, TFA/H_2O/TIPS (95/2.5/2.5) or X = Met, TFA/H_2O/EDT/TIPS (92.5/2.5/2.5/2.5).

Scheme 12: *Toth et al synthesis of C-terminal carbohydrate enkephalins.*

More recently, several groups [52] have begun to use the much simpler approach introduced by Kochetkov *et al* [53], in which the reducing oligosaccharide is treated for an extended period of time with saturated aqueous ammonium bicarbonate to afford exclusively the β-isomer of the corresponding amine. The

Asn(Sug) derivative synthesised is then deprotected and elongated to give the desired glycopeptide. There are several solid-phase methods available which utilise this approach [52(a), 54] and this methodology can be illustrated by the work of Meldal *et al* [55] in the synthesis of *N*-glycopeptide **43** (Scheme 13). Synthesis of the sugar building block **44** can be then achieved in 5 steps using **45**, which can then used in the solid-phase assembly of *N*-linked glycopeptide **43** employing Dhbt esters conducted on a fully automated peptide synthesiser. The glycopeptide was cleaved off the resin using TFA and purified using HPLC.

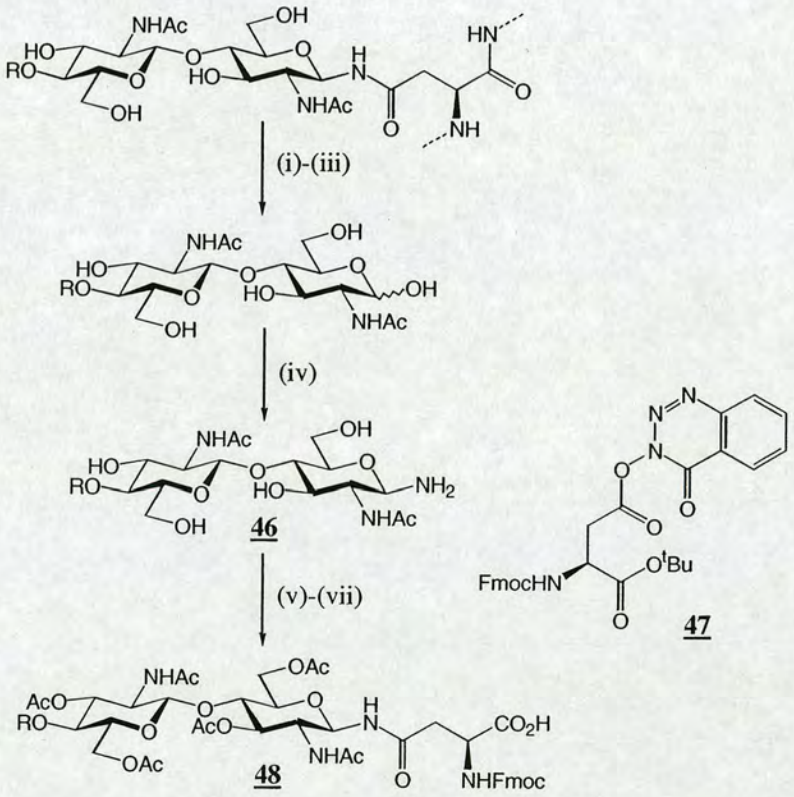


(i) Satd. aq. NH_4HCO_3; (ii) Fmoc-OSu, pyr; (iii) Ac_2O, pyr; (iv) 20% piperidine in THF; (v) **45**, *N*-Ethyl-morpholine, THF; (vi) TFA, DCM; (vii) HPLC; (viii) NaOMe, MeOH; (ix) HPLC.

Scheme 13: *Meldal et al syntheses of N-linked glycopeptide* ***43***.

This methodology was later used in the synthesis of a D-Ala^1 peptide-T amide analogue [56] utilizing the Rink linker.

Meldal *et al* later reported [57] that the carbohydrate building block can not only be prepared synthetically but can be released in a suitable form from natural glycoproteins using routine hydrazinolysis techniques as illustrated in Scheme 14. For example, a series of *N*-linked oligosaccharides have been released intact in their unreduced form from fetuin and ribonuclease B using hydrazinolysis. The respective glycosylamine **46** was then obtained by the Kochetkov method and coupled to the side chain of activated aspartic acid derivative **47** to give **48**, thus allowing their incorporation into the peptide synthesis.

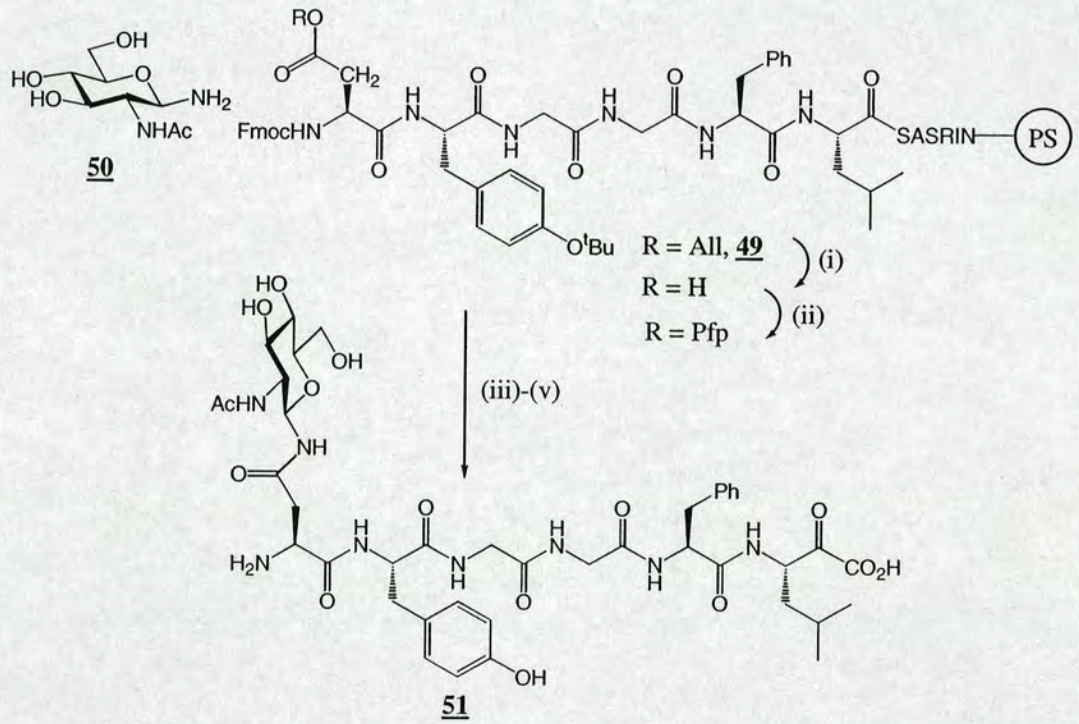


(i) NH_2NH_2, 85°C, 12h; (ii) Ac_2O, aq. $NaHCO_3$; (iii) $Cu(OAc)_2$, 30min; (iv) sat. $(NH_4)HCO_3$; (v) DIEA, DMSO; (vi) Ac_2O, pyr, 6h; (vii) TFA, 1h.

Scheme 14: *Meldal et al alternative route towards sugar building blocks for N-linked glycopeptides.*

An alternative route towards *N*-linked glycopeptides is illustrated in Scheme 15 which is methodology reported by Vetter *et al* [58], although similar procedures were reported previously by Lansbury *et al* [59] and Albericio *et al* [60]. The peptide chain was synthesised on SASRIN resin as normal; however, an allyl-asparagine

derivative was incorporated into the synthesis instead of a sugar-asparagine derivative. After peptide chain elongation was complete the allyl group on **49** was removed using Pd (0) to give the carboxylic acid, which was then subsequently activated by preparation of the pentafluorophenyl (Pfp) ester derivative. The glycosylamine **50** was coupled to the polymer-bound peptide using standard coupling reagents (Pfp esters) to give glycopeptide **51**.

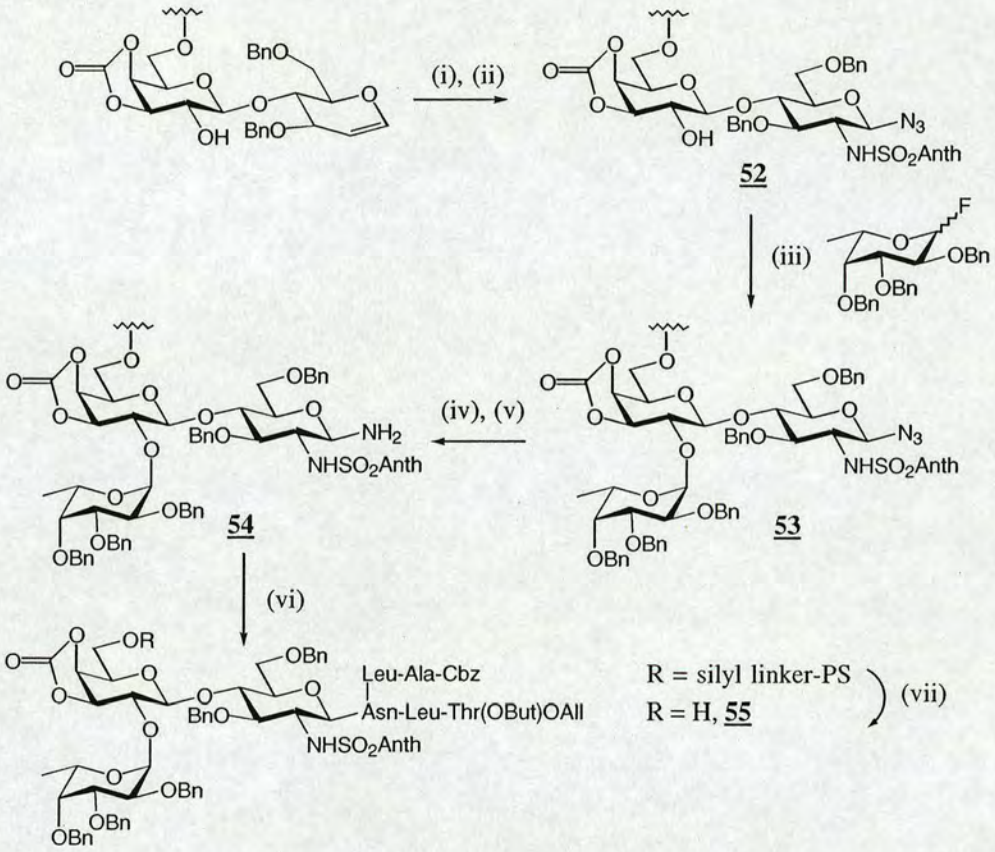


(i) $Pd(PPh_3)_4$, NMM, AcOH, $CHCl_3$; (ii) $CF_3COOC_6F_5$, DMF; (iii) **50**, DIEA, HOBt, DMSO; (iv) 20% piperidine in DMF; (v) 50% TFA in DCM.

Scheme 15: *Vetter et al synthesis of N-linked glycopeptides using allyl asparagine derivatives.*

One strategy that involves the construction of the oligosaccharide on a solid support was reported by Danishefsky *et al* and utilises the glycal methodology [61]. Both linear and branched oligosaccharides can be constructed in this manner and are subsequently modified with anthracenesulfonamide in an azasulfonamidation sequence of reactions to afford, for example, polymer bound disaccharide **52** (Scheme 16). Fucosylation in the 2-position of the galactose moiety gave the polymer-bound H-type II blood group determinant trisaccharide derivative **53**. Acetylation and reduction gave trisaccharidyl amine **54**, which was subsequently coupled to the protected pentasaccharide using standard coupling procedures.

Retrieval of the construct and removal from the polymer support provided the branched trisaccharide pentapeptide **55** in a 10 % overall yield. An added advantage of this route is that since the glycopeptide contains orthogonal protecting groups on the *C*- and *N*-termini of the peptide, the chain itself can be extended with the glycopeptide bound to the polymer.



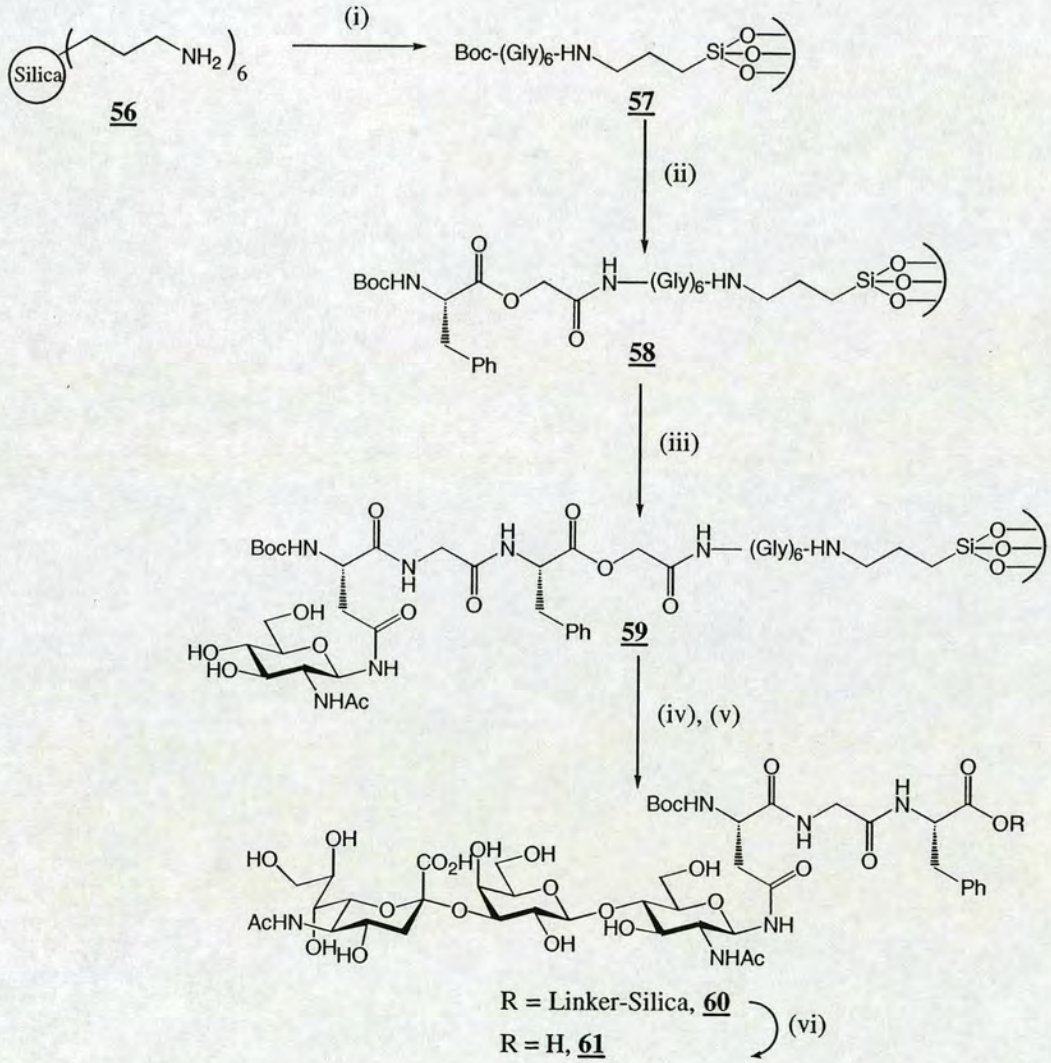
(i) $I(coll)_2ClO_4$, $AnthSO_2NH_2$, THF, -10 to 0 °C; (ii) Bu_4NN_3, THF; (iii) $Sn(OTf)_2$, DTBP, THF; (iv) Ac_2O, DMAP, THF; (v) 1, 3-propanedithiol, DIEA, DMF; (vi) peptide, IIDQ, DCM; (vii) HF.Pyr, anisole, DCM, -10 °C.

Scheme 16: *Danishefsky et al synthesis of N-linked glycopeptides using glycal methodology.*

1.2.4 Solid-Phase Chemical-Enzymatic Synthesis of Glycopeptides

Wong *et al* reported a new strategy towards branched glycopeptides [62], which enables a rapid iterative formation of peptide bonds chemically and glycosidic bonds enzymatically with glycosyl transferases on a silica-based solid support compatible with both organic and aqueous solvents. The major advantage offered by the use of enzymes is that they initiate highly regio- and stereoselective reactions without employing protecting groups. This results in reducing the number of

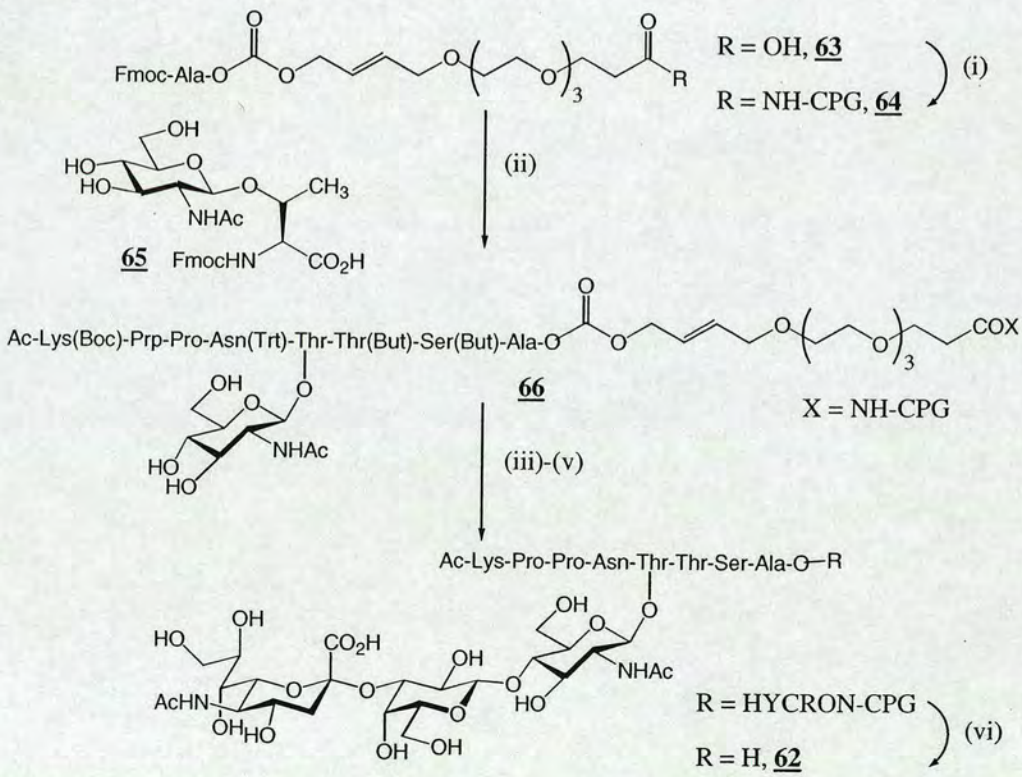
synthetic steps required to synthesise oligosaccharide derivatives. In the first step, a hexaglycine spacer was attached to aminopropyl silica **56** to give **57** (Scheme 17). Excess amino groups were capped using acetic anhydride. In the second step, a selective cleavage site was implemented for the release of intermediates and final products from the support under mild conditions by introduction of a α-chymotrypsin-sensitive phenylalanyl ester bond [63] **58**. Addition of a glycopeptide gave acceptor **59** which can then be further manipulated in enzymatic synthesis reactions using several glycosyl transferases to give **60**. α-Chymotrypsin-catalysed hydrolysis gave **61** in 35 % yield.



(i) a) Boc-Gly_3-OH, DCC, HOBt, DIEA; b) Ac_2O, pyr; c) 25% TFA, DCM; d) Boc-Gly_3-OH, DCC, HOBt; (ii) a) 25% TFA, DCM; b) *O*-(*N*-Boc-Phenylalanyl)glycolic acid, BOP, HOBt, DIEA; (iii) a) 25% TFA, DCM; b) Boc-Gly-OH, BOP, HOBt, DIEA; c) 25% TFA, DCM; d) Boc-Asn(Glc*N*Acβ)-OH, BOP, HOBt, DIEA; (iv) β-1,4-Gal T, UDP-Gal, HEPES, $MnCl_2$; (v) α-2,3-Sialyl T, CMP-NeuAc, HEPES, $MnCl_2$; (vi) α-chymotrypsin, H_2O.

Scheme 17: *Wong et al chemoenzymatic synthesis of glycopeptides.*

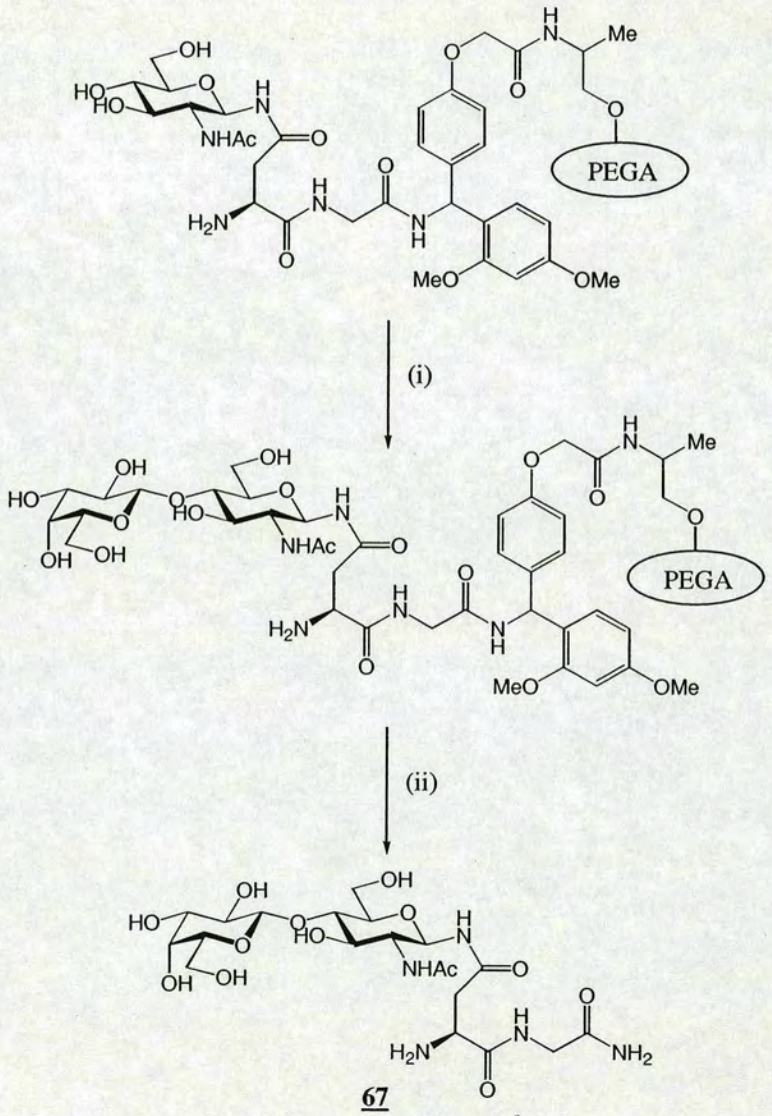
Wong *et al* later reported [64] similar work in the chemoenzymatic synthesis of the 227-234 sequence of the Mucin domain of MAdCAM-1 carrying an *O*-linked SLe^x-ligand **62** (Scheme 18). The Fmoc-Ala-HYCRON conjugate **63** was employed as the linker and attached to aminopropyl-CPG **64**. Removal of the Fmoc groups was achieved using morpholine and amino acids were subsequently attached using HBTU and NMM as the coupling reagents and the carbohydrate was incorporated as the *O*-unprotected amino acid glycoside Fmoc-Thr(*β*-GlcNAc)-OH **65**. Enzymatic synthesis was then performed on **66** to give the desired glycopeptide on solid support. Cleavage from the solid support was achieved using Pd (0) and morpholine to give an overall yield of 9 % (based on the initial amino acid loading of the resin), which was achieved after workup of the supernatant. Although it was reported that the solution-phase synthesis of **62** gives higher yields, the complete on-resin assembly is performed in less than 9 days and offers new opportunities for automation and diversification in glycopeptide synthesis.



(i) HBTU, NMM, HOBt, DMF; (ii) a) morpholine, DMF; b) Fmoc-AA-OH, HBTU, NMM, HOBt, DMF; c) a) and b) repeated, *N*-acylation: AcOH, HBTU, NMM, HOBt, DMF; (iii) TFA/EDT/H_2O [40/1/1]; (iv) UDP-Gal, Gal T; (v) CMP-Neu*N*Ac, Sialyl T; (vi) $Pd(PPh_3)_4$, morpholine, DMF, DMSO.

Scheme 18: *Wong et al chemoenzymatic synthesis of the 227-234 sequence of the Mucin domain of MAdCAM-1 carrying an O-linked SLe^x-ligand* **62**.

Meldal *et al* have also reported similar work, which involved the use of a new resin consisting of a beaded polyethylene glycol polyacrylamide copolymer ($PEGA_{1900}$) [65]. The resin was reported to be mechanically stable, yet highly swelling in both organic solvents and aqueous buffers. Scheme 19 illustrates the methodology for the chemical-enzymatic synthesis of glycopeptide **67**.



67

(i) UDP-Gal, β(1, 4)GalT; (ii) TFA, 23 °C, 2h.

Scheme 19: *Meldal et al chemical-enzymatic synthesis of glycopeptide* **67**.

1.3. Carbohydrates as Scaffolds

1.3.1 Overview

Modern organic/medicinal chemistry is currently undergoing a cultural revolution in the way new drugs are discovered. Instead of the time consuming, traditional synthesis and screening of one compound at a time, combinatorial chemistry can rapidly provide large collections of compounds (i.e. libraries) in a short time. These libraries can then be evaluated using high-throughput screening.

In contrast to the rapid development of combinatorial synthesis in the area of small-molecule libraries and biopolymers such as polypeptides, the development of combinatorial carbohydrate libraries has been slow in part because of the inherent difficulties presented by the polyfunctionality of the compounds. Nevertheless, because of their biological significance, strategies to overcome these complications have been devised and several reports on carbohydrate libraries have begun to appear during the last five years.

Recently, the field of combinatorial carbohydrate chemistry has been further extended by the recognition that carbohydrates can act as highly functionalised scaffolds or platforms for projecting pharmacophore groups. Nicolaou *et al* [66] first demonstrated that carbohydrates have the potential as highly functionalised and rigid scaffolds. Non-peptide peptidomimetics of the peptide hormone somatostatin (SRIF) **68** were designed and synthesised utilising β-D-glucose as novel scaffolding **69**. As illustrated in Figure 3, such compounds resemble conventional peptide analogues (i.e. **69** for **70**) in that they retain critical amino acid chains but differ in that they are devoid of both the peptide backbone and amide surrogates. Nicolaou *et al* later reported [67] similar work synthesising mimetics **71** of the potent peptidic antagonist of $\alpha_v\beta_3$ and $\alpha_v\beta_5$ pentapeptide cRGDFV **72** (Figure 4).

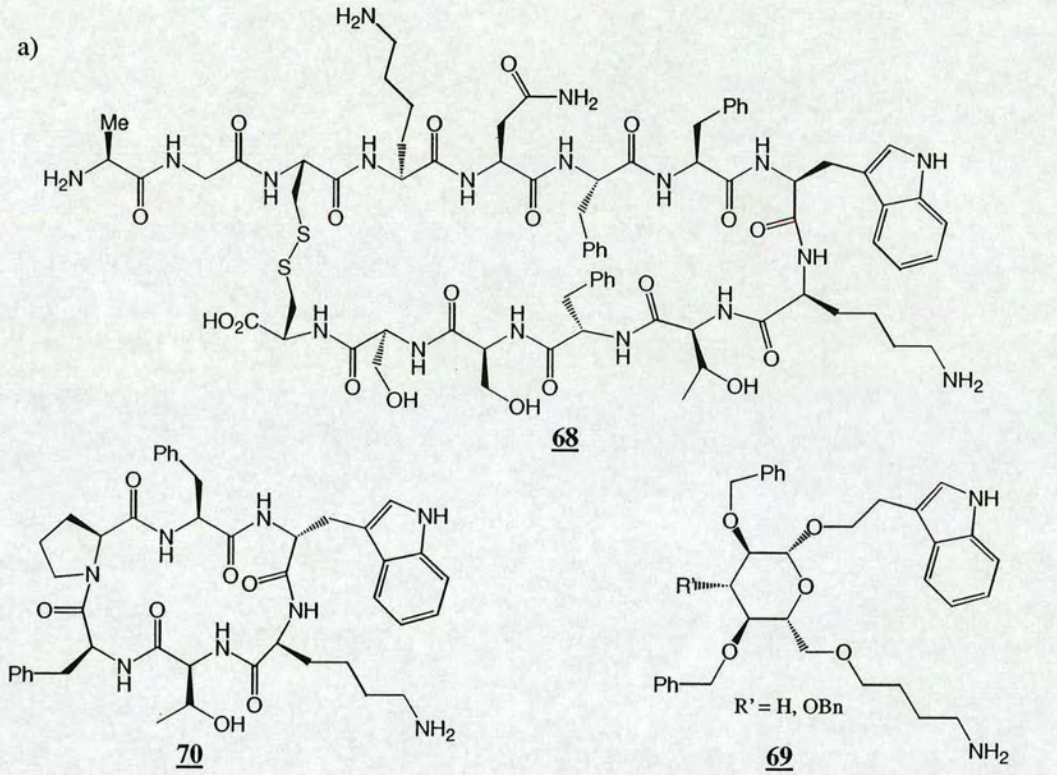


Figure 3: *Nicolaou et al non-peptidal peptidomimetics* ***69*** *of the peptide hormone somatostatin (SRIF)* ***68***.

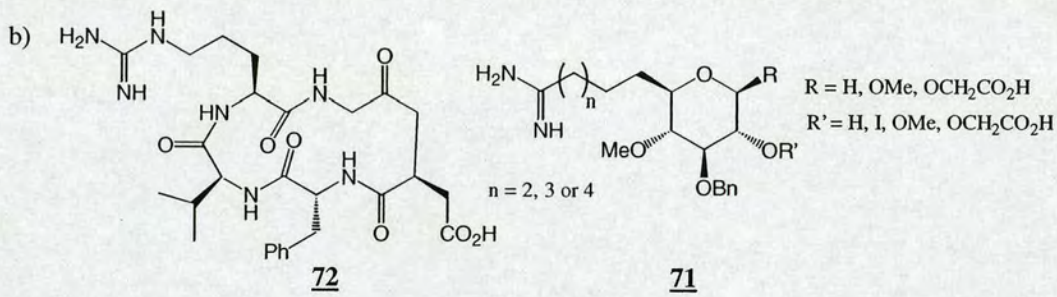


Figure 4: *Nicolaou et al non-peptidal peptidomimetics* ***71*** *of the potent peptidic antagonist of* $\alpha_v\beta_3$ *and* $\alpha_v\beta_5$ *pentapeptide cRGDFV* ***72***.

In 1998 Kessler *et al* also reported [68] the design and synthesis of new non-peptide peptidomimetics utilising carbohydrate-based amino acids such as **73** (Figure 5) as a peptide building block. These sugars carry an amino acid and a carboxylic functional group and have specific conformational effects when incorporated into a polypeptide sequence.

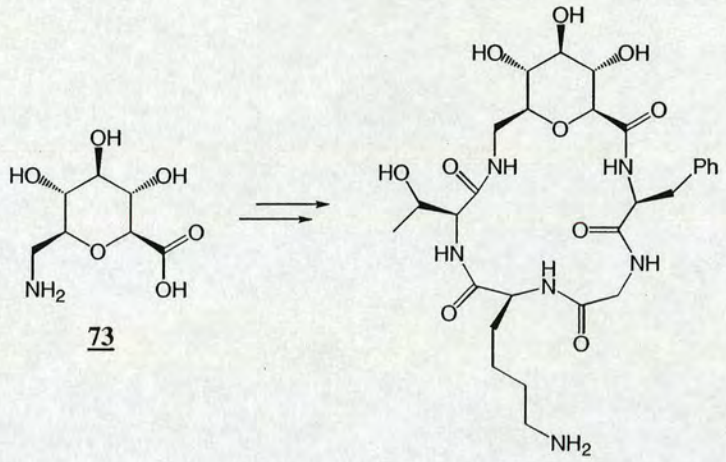


Figure 5: *Kessler et al synthesis and design of new non-peptide peptidomimetics utilising carbohydrate based amino acids such as* **73**.

1.3.2 Oligosaccharide Libraries

Using glycosyl sulfoxide chemistry Kahne *et al* [69] prepared the first carbohydrate library on the solid phase. This library of approximately 1300 1→3 linked di- and trisaccharides was constructed using the split-mix strategy. Employing six distinct monosaccharide acceptors linked to amino functionalised Tentagel® resin through an anomeric thioether linker **74** (Scheme 20), 72 different di- and trisaccharides were produced by first removal of an acetate protecting group to reveal a free hydroxyl group followed by glycosylation with 10 monosaccharide and 2 disaccharide sulfoxide donors (see Scheme 20). In each of the 72 different di- and trisaccharides, the glycosidic bonds were formed with control of both α and β anomeric stereochemistry. Many of the sugar donors and acceptors were azido-containing sugars. Therefore, further structural diversity was introduced by first reduction of the azido sugars to their corresponding amines and then acylation of each of the 72 oligosaccharides with 18 different acylating agents. A single step protecting group removal provided the targeted library of derivatised di- and trisaccharides.

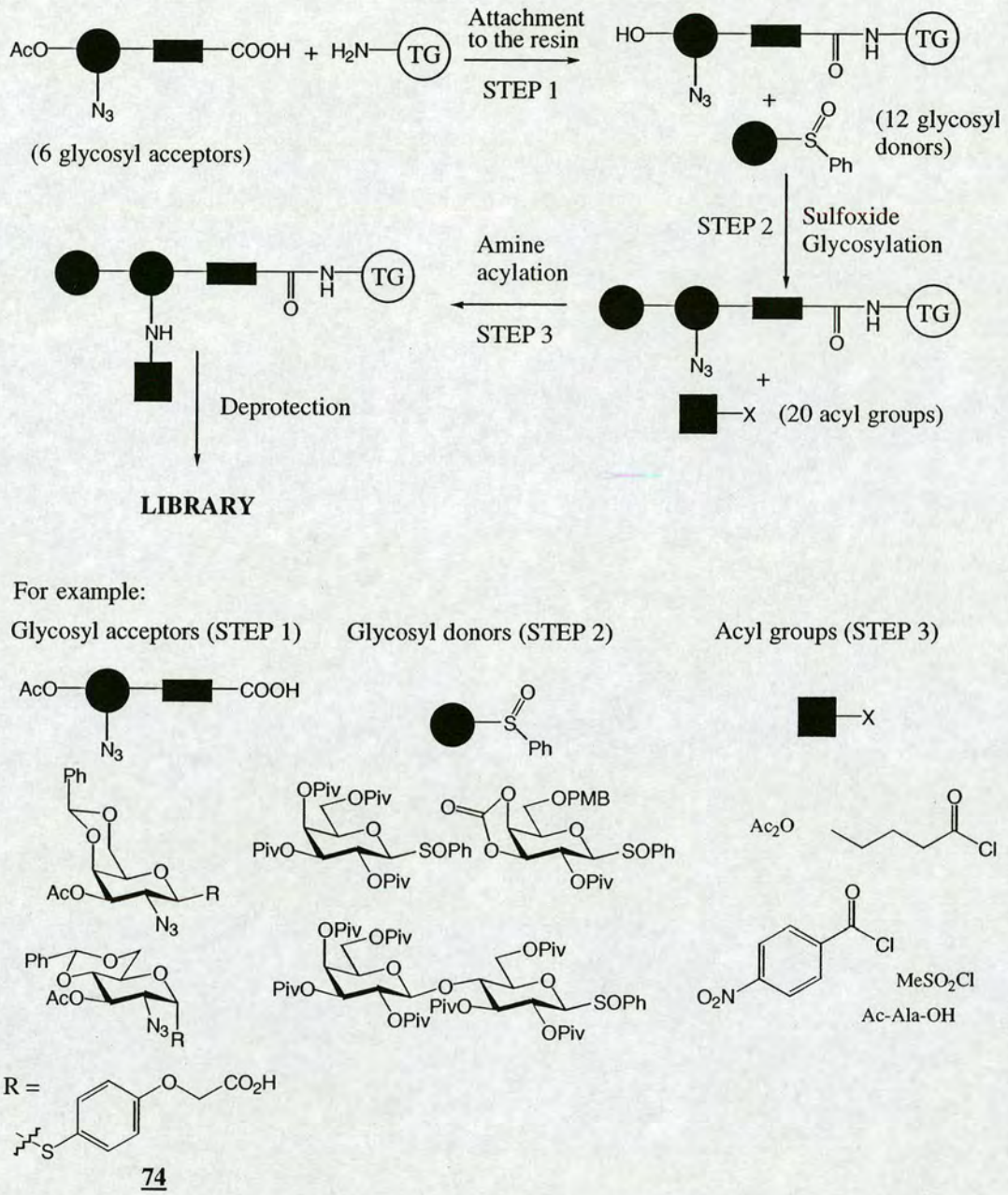
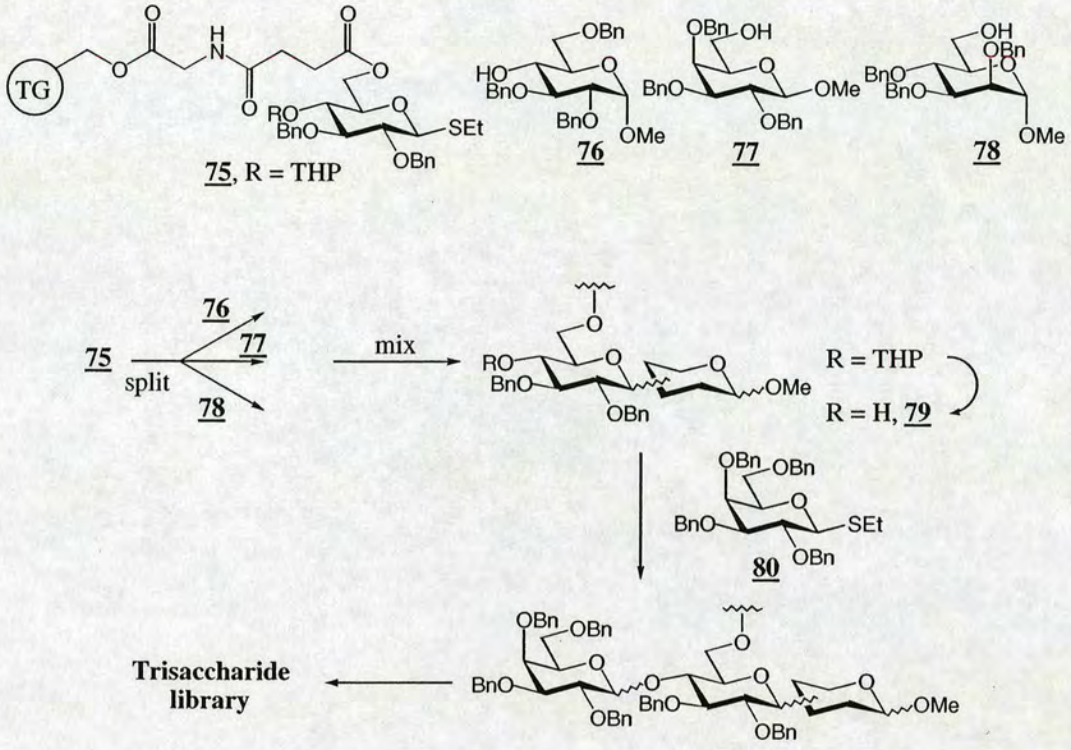


Figure 20: *Kahne et al Oligosaccharide library strategy.*

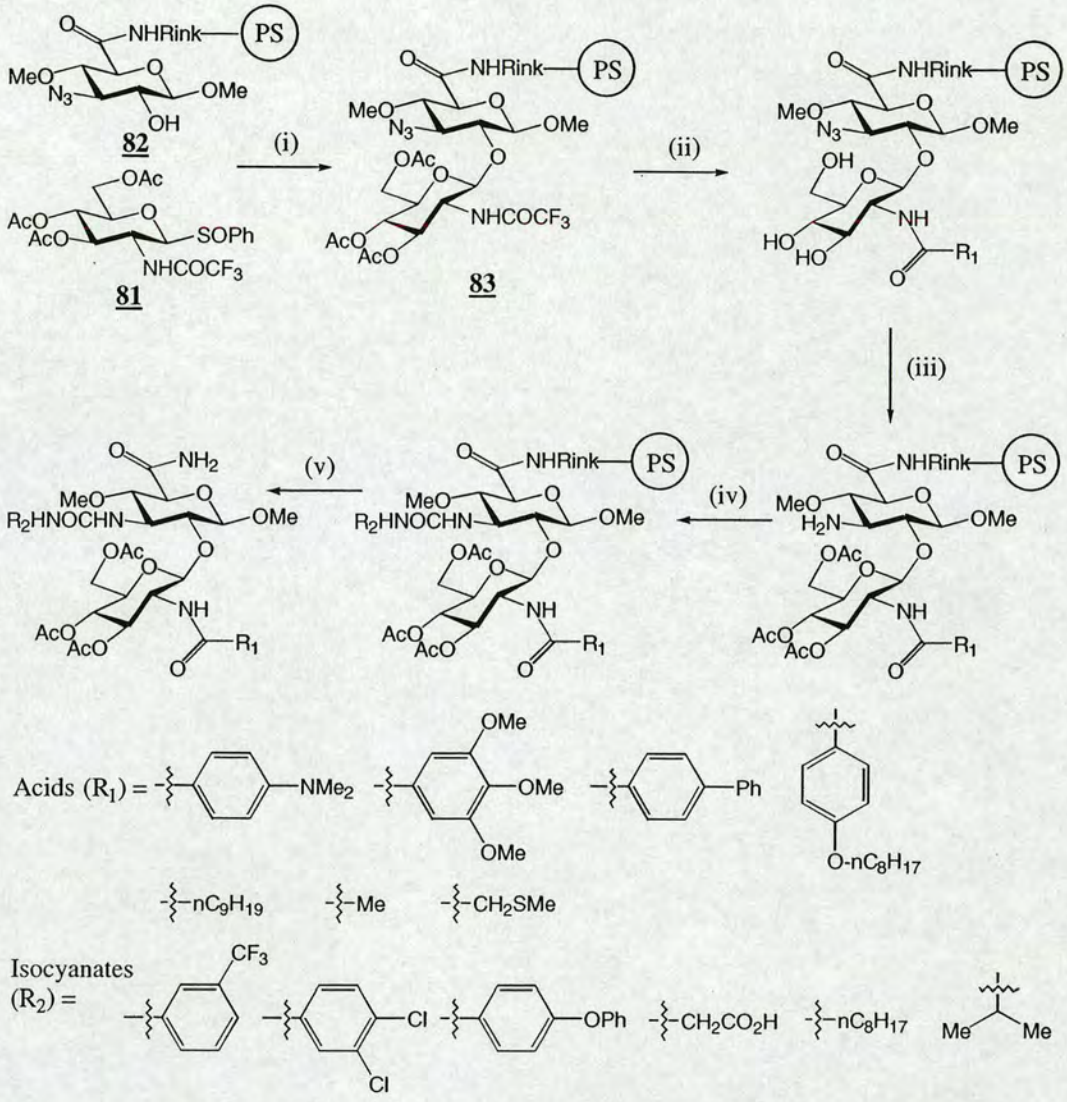
Boons *et al* later reported [70] a 'two-directional approach' for the synthesis of trisaccharide libraries. Initially, a benzyl and tetrahydropyranyl (THP)-protected thioglycoside donor was immobilised on a glycine-derivatised Tentagel hydroxyl resin *via* a succinimidyl linker **75** (Scheme 21). The donor was reacted with three different glycosyl acceptors (**76**, **77**, **78**) to give, after pooling and splitting, a mixture of six different disaccharides (including anomers). After removal of the THP group the immobilised disaccharide acceptors **79** were glycosylated with a benzyl-protected

thioglycoside donor **80**. Cleavage from the resin and deprotection (hydrogenation) afforded a library of 12 different trisaccharides.



Scheme 21: *Boons et al oligosaccharide library strategy.*

More recently Sofia *et al* [71] have constructed disaccharide libraries using phenylsulfenyl 2-deoxy-2-trifluoroacetamido glycopyranoside **81** as the glycosyl donor (Scheme 22). Solid-phase glycosidations of glycosyl donor **81** with glucuronic acid acceptor immobilised on Rink amide resin **82** afforded the corresponding β-glycoside **83** exclusively in high yield. The trifluoroacetamido group was removed under mild basic conditions and a 48-member combinatorial library was designed around disaccharide **83** using 6 different isocyanates and 8 different carboxylic acids as elements of diversity. After cleavage using TFA in DCM the library products were characterised by LC-MS. The desired derivatised disaccharides were all obtained as major products in greater than 85 % purity.



(i) DTBP, DCM, EtOAc, Tf_2O; (ii) a) LiOH, MeOH, THF; b) R_1COOH, HATU, DIEA, DMF;
(iii) Ac_2O, pyr, DMAP, Toluene, DCM; (iv) a) PMe_3, THF, EtOH, H_2O; b) R_2NCO, Toluene; (v) 20 % TFA, DCM.

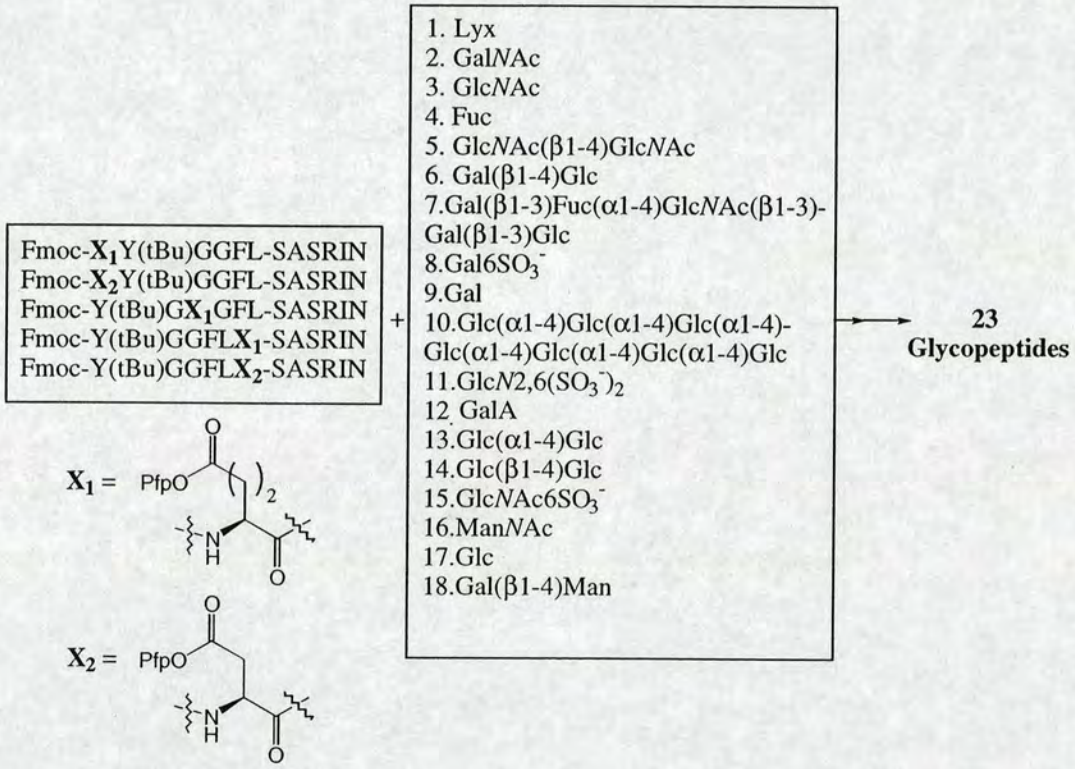
Scheme 22: *Sofia et al disaccharide library synthesis.*

Other recent solid-supported oligosaccharide syntheses that will have an impact on future oligosaccharide library design were reported by the groups of Nicolaou [72], Danishefsky [73], Schmidt [74] Kobayashi [75] and Wong [62].

1.3.3 Glycopeptide Libraries

Vetter *et al* constructed a small glycopeptide library (23 members) [58] implementing the same procedure as mentioned in section 1.2.3 (Scheme 15). Five peptides with Glu(OAll) or Asp(OAll) and permutations of the motif Tyr(^{t}Bu)-Gly-

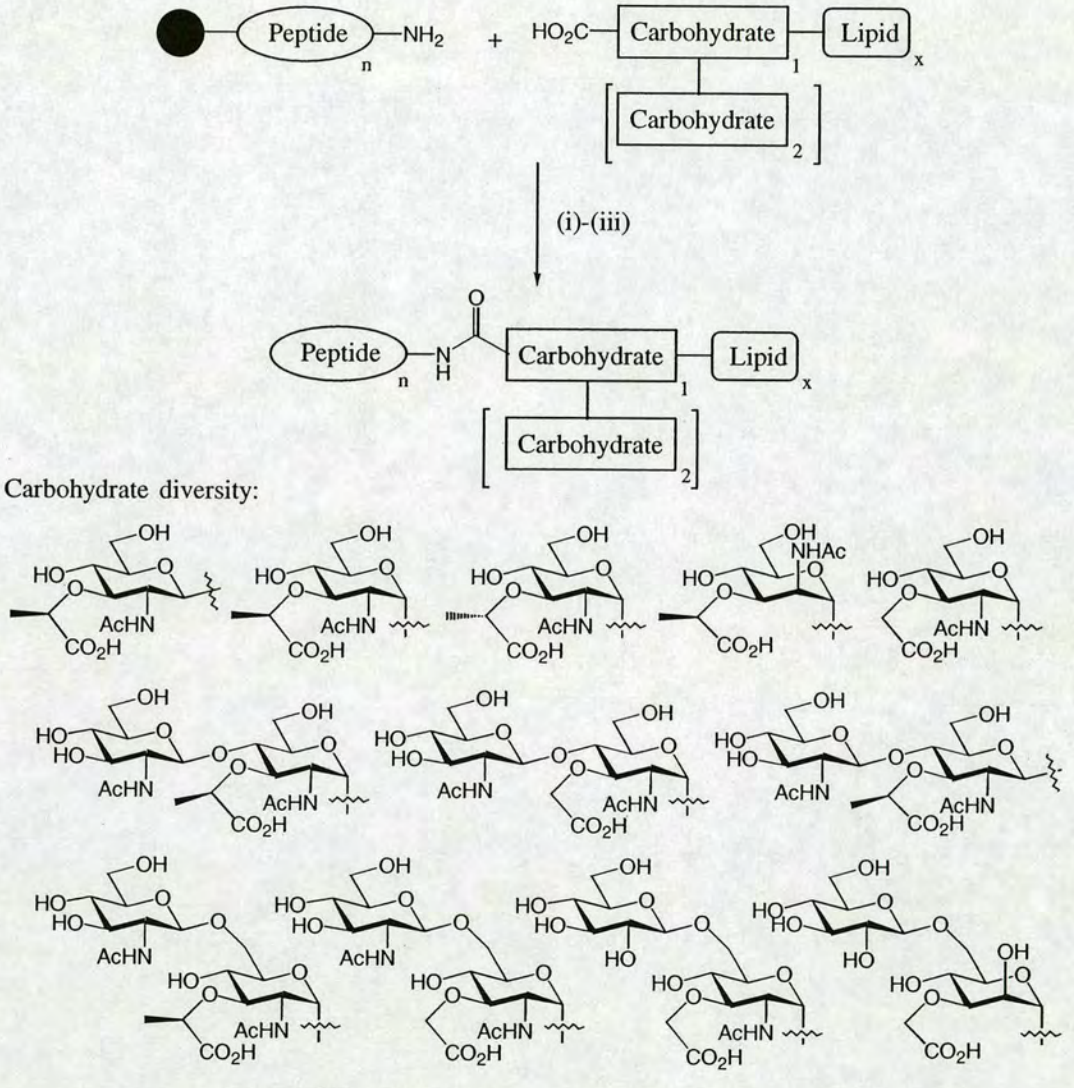
Gly-Phe-Leu were synthesised and subsequently coupled to 1-amino-1-deoxy derivatives of 18 commercially available saccharides *via* amide bond construction (Scheme 23). Coupling yields of 50-80 % were observed with uncharged oligosaccharides and yields of 30-50 % were observed when charged oligosaccharides were used. Cleavage of the products from the solid support with 50 % TFA provided the desired 23-component glycopeptide library.



Scheme 23: *Vetter et al 23-component glycopeptide library.*

Another solid-phase glycopeptide library was reported by Chan *et al* for the construction of a library of approximately 350 peptidoglycan monomer analogues for screening as inhibitors of bacterial peptidoglycan biosynthesis [76]. The building of this peptidoglycan library is strategically different from previously reported solid-phase glycopeptide syntheses. Unlike typical *N*-linked glycopeptide syntheses, in this construction the carboxylic acid group involved in amide bond formation was not part of an aspartic acid or glutamic acid amino acid residue unit and the link to the peptide occurred at the peptide terminal amino group (Scheme 24). In this solid

phase library synthesis a series of 12 preconstructed mono- and disaccharide building blocks, each containing a muramic acid sugar derivative, were coupled to the terminal amino group of each peptide of a small library of 22 peptides linked through a chlorotrityl linker.



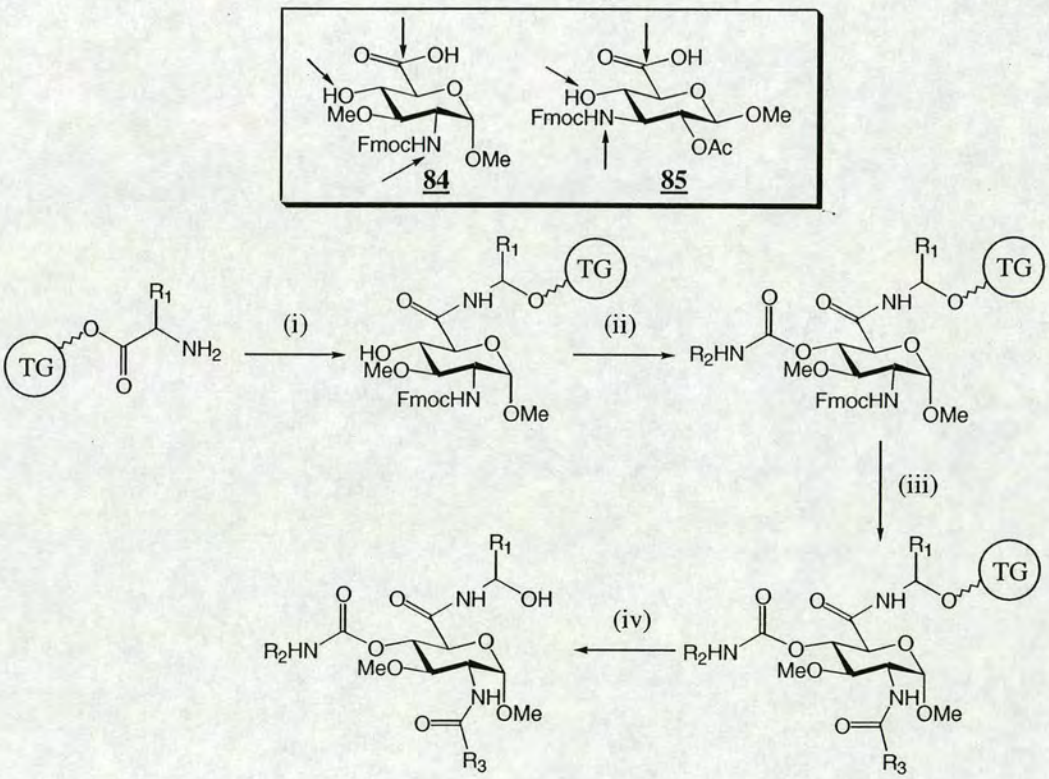
(i) HATU, DIEA, DMF; (ii) NaOMe, MeOH, DMF; (iii), 95 % aq. TFA.

Scheme 24: *Chan et al 350 peptidoglycan library synthesis.*

Other groups that have reported the synthesis of glycopeptide libraries include the groups of Meldal [77] and Armstrong [78].

1.3.4 Monosaccharide libraries

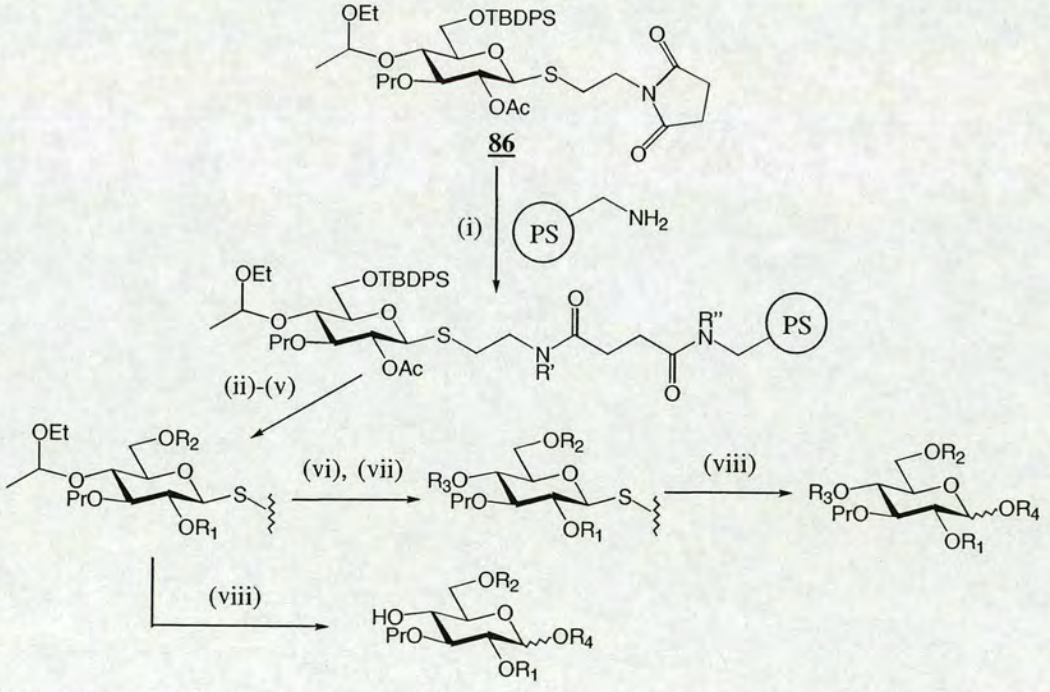
Sofia *et al* [79] in 1998 reported on the combinatorial synthesis of encoded trifunctionalised saccharide scaffolds termed universal pharmacophore mapping libraries (Scheme 25). Construction of the library employed the two-sugar building blocks **84** and **85** having a three-point attachment motif comprising a carboxylic acid moiety, a free hydroxyl group and a protected amino group. The free carboxylic acid was first reacted with eight amino-acid functionalised trityl Tentagel resins, followed by carbamate formation at the free hydroxyl site with six isocyanates. Finally, the deprotected amino function was acylated with eight different carboxylic acids. Deacetylation and cleavage from the resin gave 16 x 48 sub libraries of high purity.



(i) **84** or **85**, HATU, DIEA, DMF; (ii) R_2NCO, DMF Cu(I)Cl;
(iii) a) 20% piperidine in DMF; b) R_3COOH, HATU, DIEA, DMF; (iv) 10% TFA, DCE.

Scheme 25: *Sofia et al construction of universal pharmacophore mapping libraries using carbohydrates as scaffolds.*

Kunz *et al* [80] more recently developed an orthogonal protection procedure using the *tert*-butyldiphenylsilyl, acetate and ethoxyethyl protecting groups, as well as a special thioglycoside linker **86** that allowed the individual derivatisation of all hydroxyl groups (including those in the anomeric position) in a monosaccharide (Scheme 26). The thioglycoside linker served two functions. It allowed the immobilisation of the starting material on the solid phase (aminomethyl polystyrene) and secondly it could be used as a glycosyl donor for further functionalisation at the anomeric position. Initially, the acetate-protecting group was selectively removed and the hydroxyl group alkylated with a variety of primary alkyl halides, followed by removal of the silyl protecting group and a second alkylation step. Cleavage from the resin in the presence of a large excess of alcohol (primary and secondary) lead to triply derivatised combinatorial carbohydrate scaffolds as anomeric mixtures (route 1). Alternatively, the ethoxyethyl protecting group could be removed and the resulting hydroxyl function reacted with the alkyl halides or isocyanates. Cleavage from the resin in the presence of alcohols led to diverse polyether systems (route 2).



(i) a) LiOH, THF, H_2O; b) DIEA, HOSu, DCM; (ii) $H_2NNH_2.H_2O$, DMF; (iii) KO^{t}Bu, DMF; (iv) R_1X, DMF; (v) a) TBAF, THF; b) KO^{t}Bu, DMF; c) R_2X, DMF; (vi) PPTS, MeOH, dioxane; (vii) a) KO^{t}Bu, DMF; b) R_3X, DMF or a) R_3NCO, DMAP, dioxane; (viii) a) Br_2, DCM, DTBP; b) R_4OH, Et_4NBr, DCM, cyclohexane.

Scheme 26: *Kunz et al combinatorial synthesis using carbohydrates as multifunctional chiral scaffolds.*

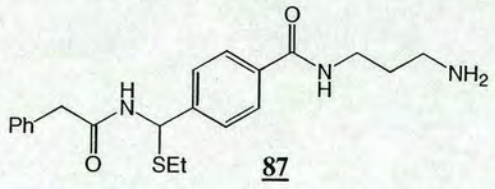
1.4. A novel Linker for the Attachment of Alcohols to Solid Support

1.4.1 Background

As mentioned earlier, carbohydrates are well known in many biological systems and consequently there has been much interest in developing new methodologies towards the synthesis of carbohydrate molecules, in particular, by the use of solid-phase strategies. However, one of the major problems in solid-phase carbohydrate synthesis is selective release from the solid support. Linkers are required, which are suitable for the attachment and release of substrates and can be cleaved under mild and selective conditions.

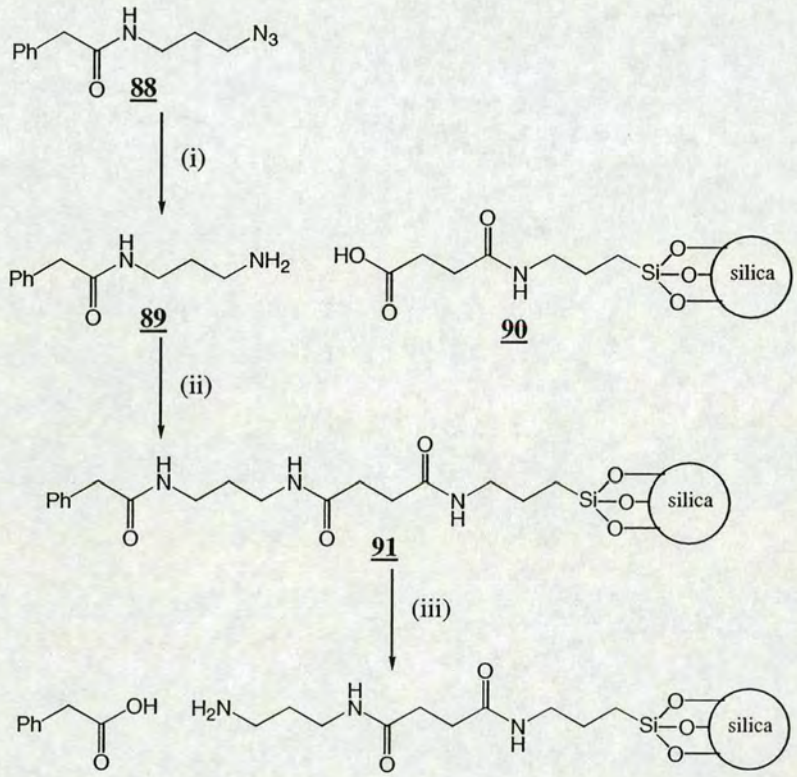
Enzyme-cleavable linkers are therefore particularly attractive because cleavage might be achieved under mild, neutral and aqueous conditions. Two reports have demonstrated that enzymes can be used to cleave molecules from the solid supports using phosphodiesterase [81] and an endopeptidase (chymotrypsin) [62]. A major drawback of both methods is that the compound released from the solid support retains part of the recognition site of the enzyme, i.e. a phosphate ester [81] and a peptide with *C*-terminal phenylalanine residues [62]. Although both 'tags' could in principle be cleaved with phosphatases or peptidases respectively, this would introduce several additional synthetic steps into the reaction sequences.

Linker **87** was developed [82] to provide mild, selective release of alcohols, in particular carbohydrates, from the solid support. The linker has a pendant phenylacetamide, which is the recognition for the enzyme penicillin amidase (EC 3.5.1.11), a commercially available and widely used enzyme [83]. Penicillin amidase is known to catalyse the hydrolysis of a wide range of amines protected as the corresponding phenylacetyl derivatives and has been used in synthesis for the cleavage of cysteine groups [84].



87

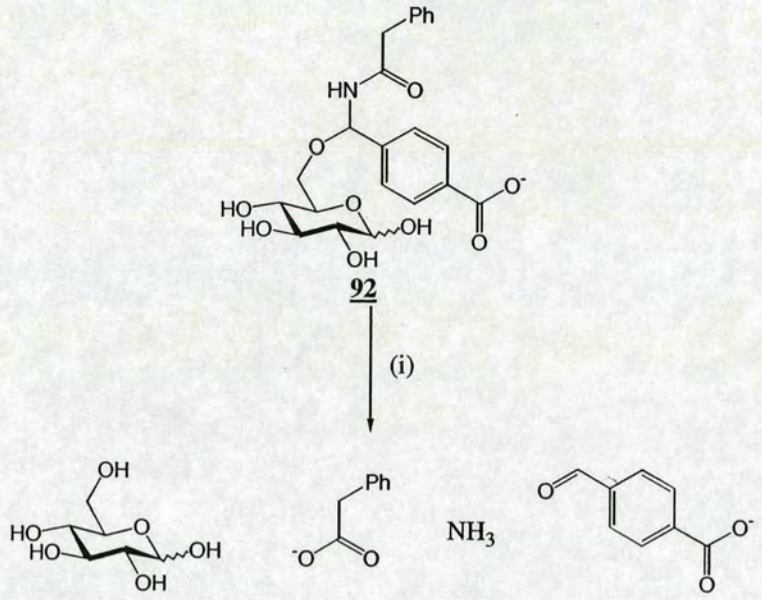
In development of linker **87** it was first established that penicillin amidase was able to cleave phenylacetic acid from a solid support. Model compound **88** was synthesised, reduced to compound **89** and was coupled to derivatised aminopropyl silica **90** [62, 85] as illustrated in Scheme 27 to give **91**. Treatment of this compound with penicillin amidase in phosphate buffer released the expected phenylacetic acid, which indicated the potential for this enzyme in solid-phase chemistry.



(i) H_2, PtO_2, THF; (ii) EEDQ, DMF; (iii) Penicillin amidase, phosphate buffer.

Scheme 27: *Initial studies of using penicillin amidase for solid-phase chemistry.*

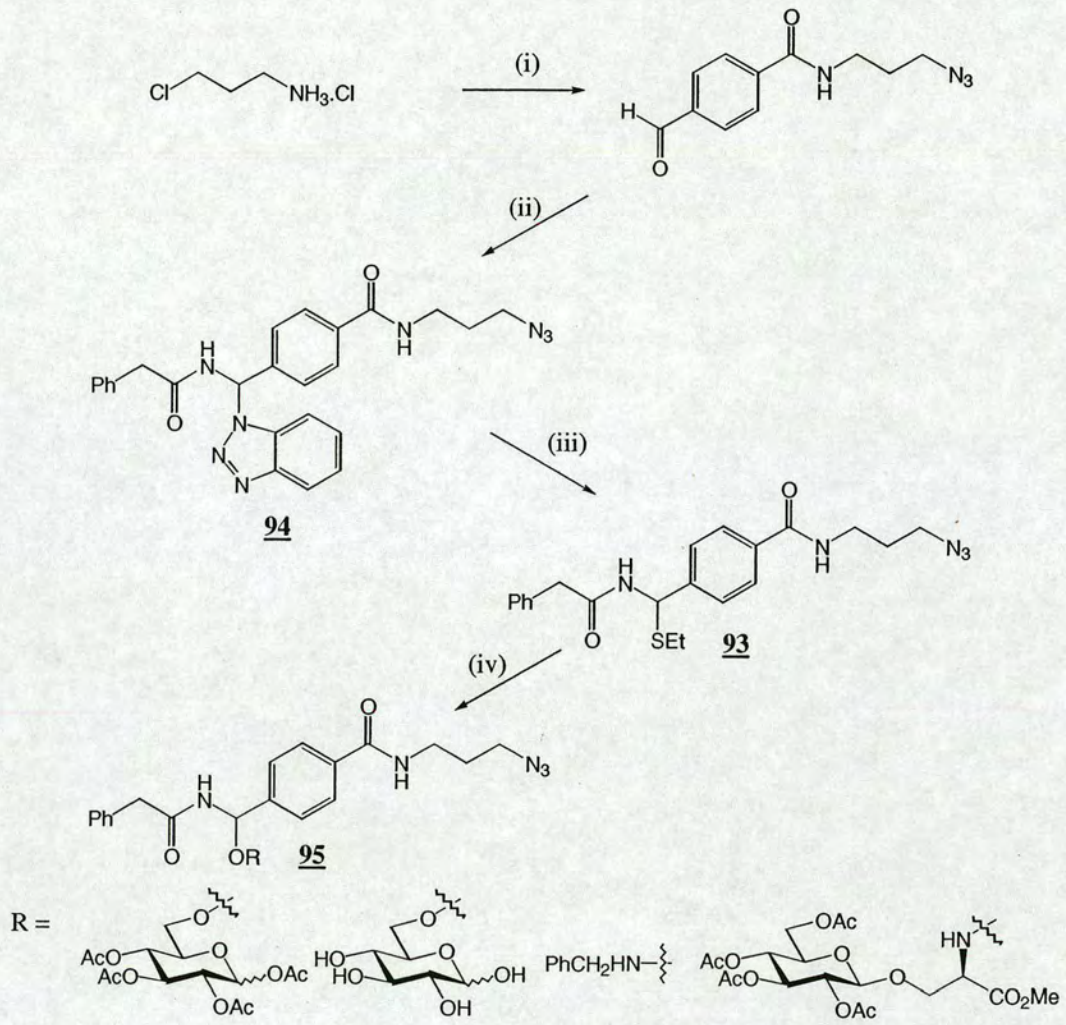
By way of further illustration using the same enzyme, a soluble form of the linker **92** (see Scheme 28) was hydrolysed by penicillin amidase to give the expected three products (glucose, phenyl acetic acid and 4-carboxybenzaldehyde, ammonia was not monitored). A carbohydrate was attached to the linker exemplifying the enzyme hydrolysis of this complex molecule and unnatural substrate for penicillin amidase. It was also found that non-enzymatic hydrolysis of **92**, using acidic conditions, was found to give phenylacetamide rather than phenylacetic acid, thus indicating that the linker may also complement the existing range of alcohol linkers.



(i) Penicillin amidase, phosphate buffer.

Scheme 28: *Treatment of penicillin amidase to an unnatural substrate and complex molecule* ***92***.

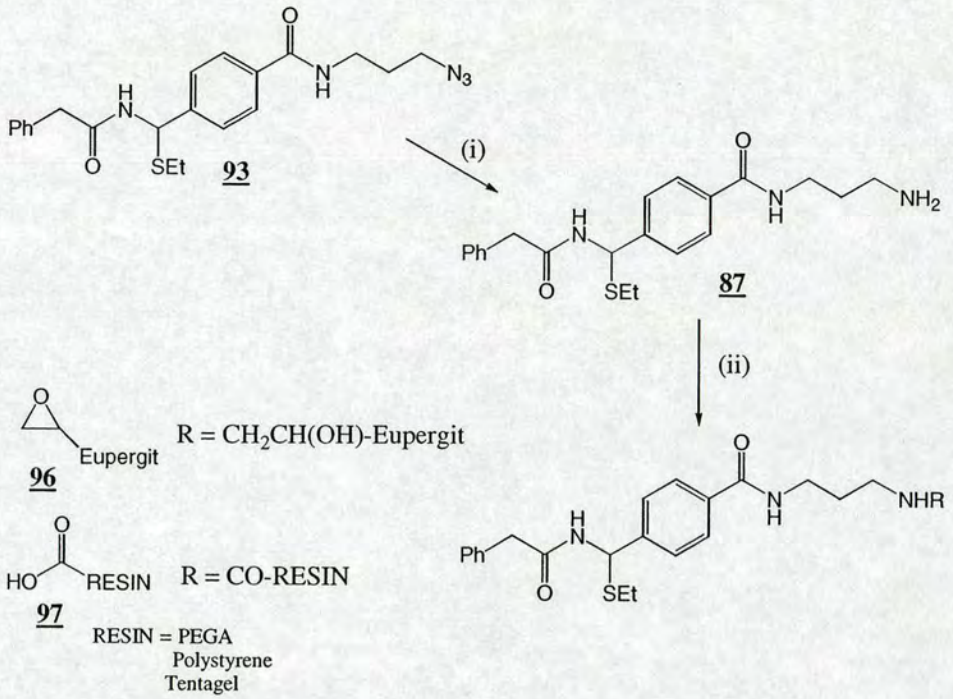
The synthesis of linker **93** is illustrated in Scheme 29. Suitably substituted benzaldehyde could be achieved in several steps, which upon reflux with phenylacetamide and benzotriazole in toluene yields benzotriazole adduct **94**. It was found that displacement of the benzotriazole group could be performed by a good nucleophile such as sodium ethyl thiolate, to generate thioamidal **93**. The thioether could then be displaced by weaker nucleophiles in the presence of thiophilic reagents, such as *N*-iodosuccinimide (NIS), to lead to a variety of structures in good yield. Direct displacement of the benzotriazole group with weaker nucleophiles did not give similar compounds in acceptable yield. It was also shown that the attached R group on **95** could be further modified after coupling. For example, the peracetylated glucoside was deacetylated by treatment with triethylamine in methanol. The thioethyl group could also be easily displaced by amines, as these compounds should equally be susceptible to penicillin amidase hydrolysis, regenerating the corresponding amine.



(i) a) NaN_3, H_2O, 120°C; b) KOH, Et_2O; c) *p*-$CHOC_6H_4COCl$, Et_3N, THF; (ii) $PhCH_2CONH_2$, BtH, Toluene, reflux; (iii) NaSEt, THF; (iv) RH, NIS, DCM.

Scheme 29: *Synthesis of Linker* ***93***.

Attachment of the linker onto solid supports could also be achieved. Reduction of the azide moiety on **93** to the corresponding amine **87** could be easily performed using triphenylphosphine and water in THF. The amine functionality can then be used to couple to the commercially available support 'Eupergit' **96** and various other supports **97** (see Scheme 30).



(i) PPh_3, H_2O, THF; (ii) Various coupling conditions.

Scheme 30: *Attachment of Linker 87 to Solid Support.*

A number of Tentagel® and PEGA® resins were investigated [86] because of their compatibility with aqueous reaction conditions and they were reported as being suitable for enzyme-catalysed reactions [65]. This identified Tentagel® to be the most promising support as this had optimal success in enzymatic cleavage studies, although this was found to be only 50 % cleavage at best with typical yields being a lot lower.

1.4.2 Aim of the present project

The aim of this work was to continue to use and develop linker **87** in the synthesis of carbohydrate derivatives. The work would not only provide an investigation into the value of linker **87**, but will provide a novel route towards biologically important carbohydrate derivatives. The synthetic route to linker **87**, mentioned previously, had not been performed on a large scale and a major aim of this work was to improve and develop the synthesis of linker **87** so that gram quantities could be obtained. Similarly the attachment of the linker **87** onto solid

supports was achieved with typical yields of only 50 % and so it was hoped that other procedures would be found to increase coupling yields.

In order to investigate the value of linker **87** for the solid-phase synthesis of carbohydrate derivatives, appropriate sugar building blocks must also first be designed and synthesised which are compatible with the chemical properties of the linker. The sugar must be selectively protected to tolerate attachment onto solid support (*via* a free hydroxyl group) and once bound will allow selective deprotection so that chemistry can be performed. Our general design for the sugar building blocks is shown in Figure 6.

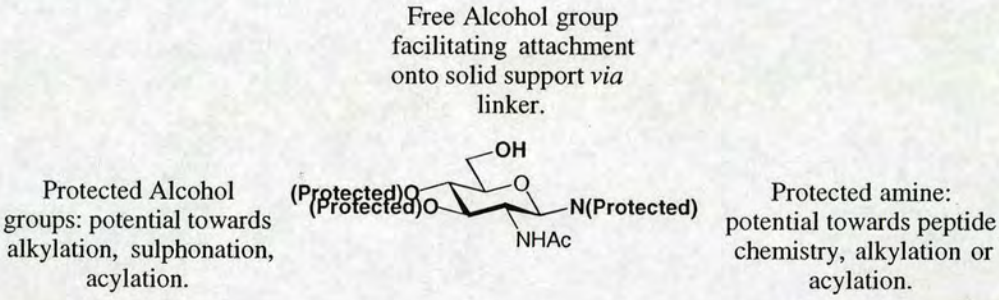


Figure 6: *Framework of the desired sugar building block for Solid-Phase Synthesis.*

The alcohol group at the 6-position was the choice of attachment onto solid support since the primary alcohol is the most reactive and has greater availability. An amino group at the anomeric centre would allow selective deprotection and *N*-acylation or alkylation and also give a possible alternative route towards glycopeptides.

It has been envisaged that hydroxyl groups at the 3 and 4-position would be protected during the coupling step with groups orthogonal to the amine protection to allow further elaboration of the carbohydrate ring with alkylation, sulfation, sulfonation and acylation reactions. As mentioned earlier, the use of combinatorial chemistry provides a route towards synthesising large collections of compounds quickly and it is hoped that this strategy, utilising a suitable sugar building block and linker **87**, may provide a route towards a library of sugar derivatives.

Chapter Two; Results and Discussion

2.1. Synthesis of Sugar Building Block 106

2.1.1 Introduction

In order to investigate the value of linker **87** an appropriate sugar building block must first be designed and synthesised. As discussed in section 1.4.2, the sugar must be selectively protected to tolerate attachment onto solid support and once bound will allow selective deprotection so that chemistry can be performed. The desired sugar building block **98** is shown in Figure 7 for which *N*-acetylglucosamine **99** could be a suitable starting material. The azido group was chosen for the anomeric carbon as this can be easily incorporated into the molecule but would also give the desired amine functionality, in one easy step by the action of a number of reducing agents [87] including H_2 and a catalyst. Acetate protection was chosen as protection for the 3, 4-hydroxyl groups in view of the fact that initial studies have shown that acetate groups can be removed from sugar substrates whilst attached to linker **87** as mentioned in section 1.4.1. It was also envisaged that the selective free hydroxyl group at the 6-position would be introduced by the use of a bulky protecting group and so sugar **100** was an important synthetic target.

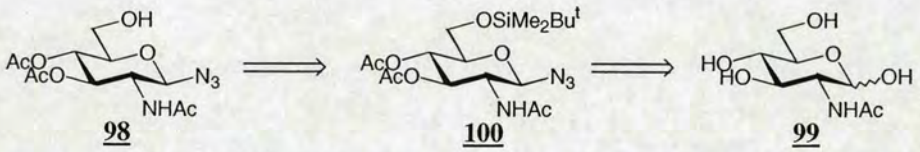


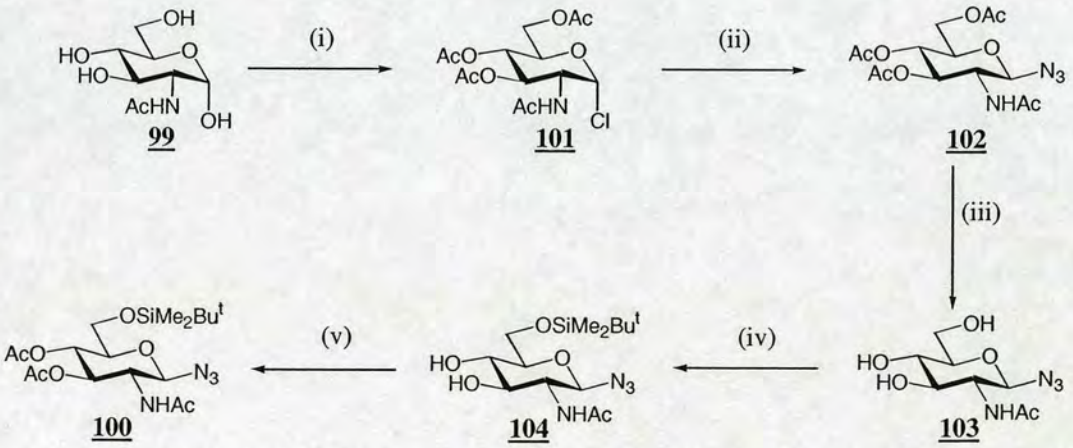
Figure 7: *Desired sugar building block **98**.*

*2.1.2 Synthesis of Sugar **98***

Sugar **100** was synthesised as illustrated in Scheme 31. *N*-Acetylglucosamine **99** was treated with acetyl chloride [88] to give selectively the α-glycosyl chloride **101** by consequence of the anomeric effect [89] in 46 %. During this reaction it is thought that the α-peracetylated glucoside is generated first after which the chloride displaces the C-1 acyl group to give the β-glycosyl halide (kinetic product) which then undergoes anomerisation to the α-glycosyl chloride **101** (thermodynamic

product) exclusively. The introduction of the azide moiety was achieved [90] by displacement of the chloride with sodium azide under phase transfer catalysis conditions to give the desired β-glycosyl azide **102** in 90 %.

Deprotection of the acetate groups on **102** was achieved by the Zemplén procedure [89] which involved using a catalytic amount of sodium methoxide in methanol to give **103** in 92 % yield. The primary 6-position was then selectively protected using the large bulky *tert*-butyldimethylsilyl group yielding **104**. This step, however, was a little more problematic than expected. Conditions first adopted involved using *tert*-butyldimethylsilyl chloride and imidazole as the base in pyridine [91]. Surprisingly this proved to be unsuccessful. More forcing conditions were therefore attempted using *tert*-butyldimethylsilyl chloride, triethylamine and dimethylaminopyridine in acetonitrile [92] at an elevated temperature as shown in Scheme 1. This reaction was successful yielding the expected glycoside **104** in 79 %. ^{1}H NMR, ^{13}C NMR and mass spectrometry confirmed that the protection at the 6-position was selective. The 3, 4-hydroxyl groups were again protected as acetate groups using acetic anhydride with cold pyridine [93] as the solvent and catalyst to give the fully protected β-glycosyl azide **100** in 98 %.

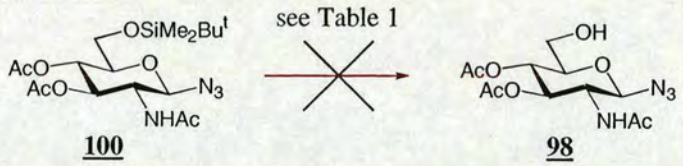


(i) AcCl, RT, 16h; (ii) NaN_3, TBAHS, $NaHCO_3$, DCM, RT, 2h; (iii) NaOMe, MeOH, RT, 1h; (iv) TBDMSCl, DMAP, Et_3N, CH_3CN, 60 °C, 3h; (v) Ac_2O, pyr, RT, 16h.

Scheme 31: *Synthesis of fully protected* β*-glycosyl azide* ***100***.

The next step of the synthesis was the removal of the silyl-protecting group (Scheme 32). Many different reagents have been reported [91, 94-96] to remove

such a group and a number were attempted. Table 1 summarises the methods employed and the results obtained.



100 **98**

Scheme 32: *Silyl deprotection of β-glycosyl azide **100**.*

De-silylation conditions	Observation
TBAF, THF	Decomposition indicated by TLC (several spots)
AcOH:THF:H_2O [3:1:1]	Acetate migration (confirmed by ^{1}H NMR) to free 6-position
DDQ, 10 % H_2O in CH_3CN	Acetate migration (confirmed by ^{1}H NMR) to free 6-position

Table 1: *De-silylation conditions attempted and results obtained.*

Employment of a fluoride ion is a well-established method, as the silicon-fluorine bond is very strong and so a solution of tetrabutylammonium fluoride (TBAF) in THF was therefore attempted, adopting literature procedures [91, 94, 95]. However, under these conditions the fluoride ion is very basic and the reaction revealed several spots upon TLC analysis indicating that decomposition of the sugar was occurring.

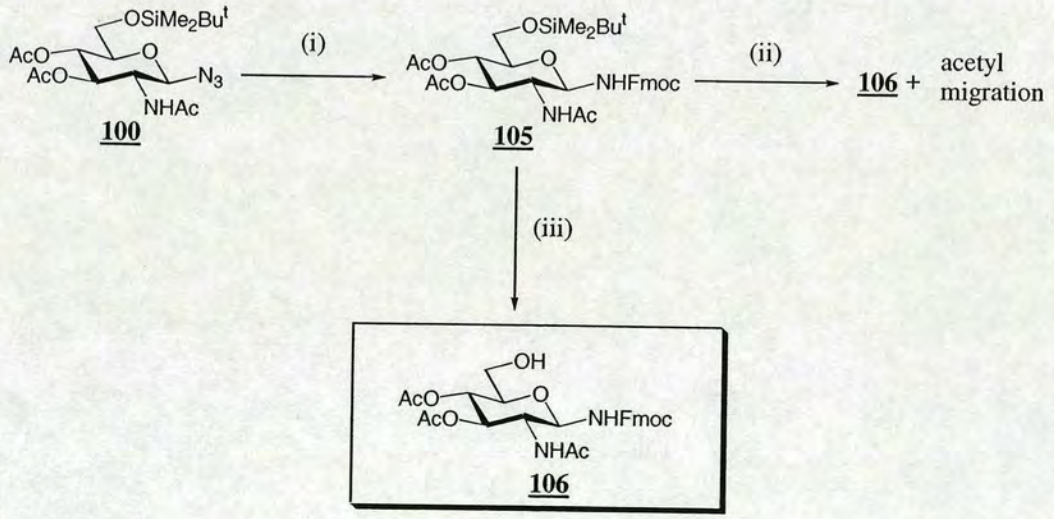
A mixture of acetic acid, THF and water in the ratio of 3:1:1 [91] was also used and this looked more promising upon TLC analysis as only one spot was observed. After purification ^{1}H NMR analysis of this compound showed a more complex spectrum than expected and indicated that acetate migration was occurring. The acidic condition implemented was successful in removing the silyl-protecting group, but was also promoting acetate migration at the 4-position to the less sterically hindered 6-position. This was apparent by the signal shift of the H-4 proton in the ^{1}H NMR spectra to 3.65 ppm.

Due to lack of success, a far milder method was attempted using DDQ which was reported [96] to have success in removing the silyl group. This reaction looked hopeful but ^{1}H NMR again indicated that acetate migration was occurring and so the sugar building block target that was first intended had to be re-addressed.

2.1.3 Synthesis of Fmoc Protected Sugar ***106***

It was decided that since the synthesis of sugar **98** was unsuccessful the reduction of the azide group and protection of the corresponding amine was the best solution to the problem. The protecting group of the amine could be sterically hindering for the 6-postion hydroxyl thus reducing the prospect of acetate migration. It was also decided that the *N*-(9-fluorenylmethoxycarbonyloxy) group was the best choice of protecting group as this would not only provide steric bulk but would also be easily removed. In addition, this group would provide an easy route towards calculating the sugar loading on solid-phase by UV detection, which would be useful for later studies on solid-phase.

Reduction of the azide moiety on **100** (see Scheme 33) was therefore performed using platinum oxide under an atmosphere of hydrogen [97], which yields the corresponding amine. In order to remove the platinum oxide the mixture solution was passed through Celite, however, this was unsuccessful. The crude amino mixture was therefore dried and used directly in the protection step using *N*-(9-fluorenylmethoxycarbonyloxy) succinimide [98] in pyridine to give the fully protected glycosylamine **105** in 87 % yield for the 2 steps. Column chromatography of glycosylamine **105** removed the platinum oxide retained from the previous step.



(i) a) PtO_2, H_2, THF, RT, 2h; b) FmocOSu, pyr, RT, 16h; (ii) AcOH:THF:H_2O (3:1:1), RT, 96h; (iii) DDQ, CH_3CN:H_2O (9:1), RT, 48h.

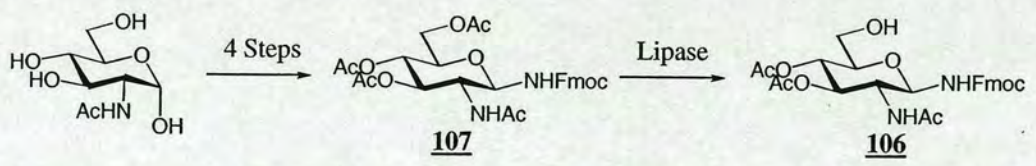
Scheme 33: *Synthesis of 2-acetamido-3, 4-di-O-acetyl-2-deoxy-N-(fluoren-9-yl-methoxycarbonyl)-β-D-glucopyranosylamine* ***106***.

Deprotection of the 6-position hydroxyl was again attempted using acetic acid, THF and water in a ratio of 3:1:1 to give the desired sugar building block **106**. However, it was noticed by TLC that occasionally a secondary similar spot just below the original product spot could be observed and this could not be removed. ^{1}H NMR of this mixture showed no difference to the desired spectra as this impurity was only a trace and it proved very difficult to retrieve. Enough was eventually obtained for a ^{1}H NMR which indicated that the impurity was in fact the acetate migrated product. Reassuringly, the idea of the bulky amino protecting group reduced the acetate migration problem as only a trace was seen if at all, but the acidic removal still proved to be a little problematic. DDQ was therefore again attempted adopting the same method as before to give the desired sugar building block **106** in 85 %.

The desired sugar building block **106** was easily synthesised with the last step showing no acetate migrated product whatsoever. This reaction also proved to be significantly quicker especially in large-scale reactions. The overall synthesis of sugar building block **106** can be achieved from *N*-acetylglucosamine in 23 % and was used for synthesising 5-6 gram quantities.

2.1.4 Summary and Conclusions

Sugar **106** was synthesised in 8 steps from *N*-acetylglucosamine in an overall yield of 23 % and has proven to be effective enough to generate gram quantities. Since this route involves a number of steps, work for the future would be to investigate alternative routes which may reduce the number of steps. One possible route would be to use lipases to selectively remove the 6-position acetate on sugar **107** (Scheme 34).



Scheme 34: *Future work: A possible alternative route towards Sugar* **106**.

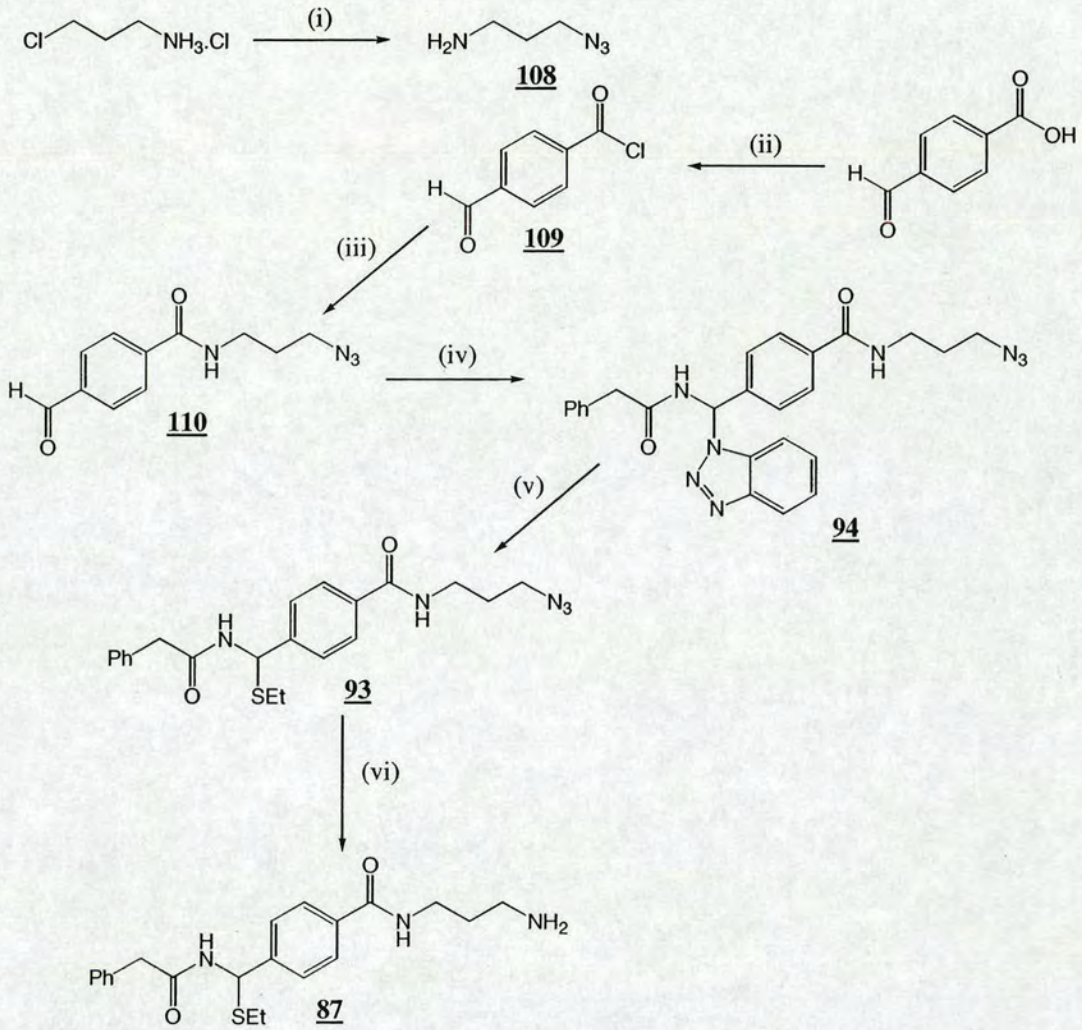
2.2. Synthesis of Linker 87

2.2.1 Introduction

As shown in section 1.4.1, linker **87** has been developed for the attachment of alcohols onto solid support and it was the aim of this work to use and develop linker **87** in the solid-phase synthesis of carbohydrate derivatives. A synthetic route was previously developed and used by many, although it had not been performed on a large scale (10 g). Since large amounts of the linker were required it was thought best to generate as much material as possible and hence develop a synthesis for linker **87** that was practical for a larger scale.

2.2.2 Syntheses and Development of Linker 87

3-Azidopropylamine **108** (Scheme 35) was synthesised in 81 % as the literature procedure described [99] and was carried out on a comparatively large scale (~10 g) with no problems. It should be noted that organic azides are extremely unstable or explosive under appropriate conditions of initiation and so reactions were performed behind blast shields as a precautionary measure. Low molecular weight azides are generally more unstable and so **108** was used immediately where possible. Generation of the acid chloride **109** from 4-carboxybenzaldehyde proved more problematic as it was difficult to remove excess thionyl chloride completely. The consistency of the mixture produced after the reaction was a very thick liquid which seemed to retain a lot of the excess thionyl chloride. Although previously no yield was quoted for this transformation as the acid chloride was used directly, the yield of the subsequent step (i.e. the generation of **110**) was only 37 %. An alternative route towards the generation of this amide bond was therefore required [100].

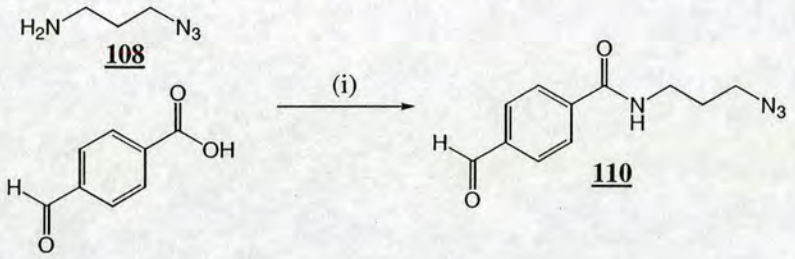


(i) a) NaN_3, H_2O, 80 °C, 16h; b) KOH, Et_2O, 0 °C; (ii) $SOCl_2$, THF, RT; (iii) Et_3N, THF, RT, 3h;
(iv) $PhCH_2CONH_2$, BtH, toluene, 140 °C, 18h; (v) NaSEt, THF, RT, 16h; (vi) PPh_3, H_2O, THF, RT, 16h.

Scheme 35: *Initial synthesis of* N-*({[4-(3-azidopropylcarbamoyl)phenyl]ethylsulphanyl}methyl)-2-phenylacetamide* ***87***.

It was decided for convenience that an amide-coupling reagent would be useful rather than generating an activated carboxylic acid. This would therefore reduce the number of steps involved and improve the synthesis as a whole. The use of a coupling reagent would also have a greater potential to accommodate a large scale, which was clearly not the case in the acid chloride route. 2-Ethoxy-1-ethoxycarbonyl-1, 2-dihydro-quinoline (EEDQ) was chosen as a promoter for the coupling reaction (Scheme 36) [101]. 4-Carboxybenzaldehyde was used directly with 3-azidopropylamine **108** to generate the desired amide bond *via* a mixed

anhydride mechanism, which after purification using gradient elution flash chromatography gives the pure aldehyde **110** as a white solid in 61 % yield.

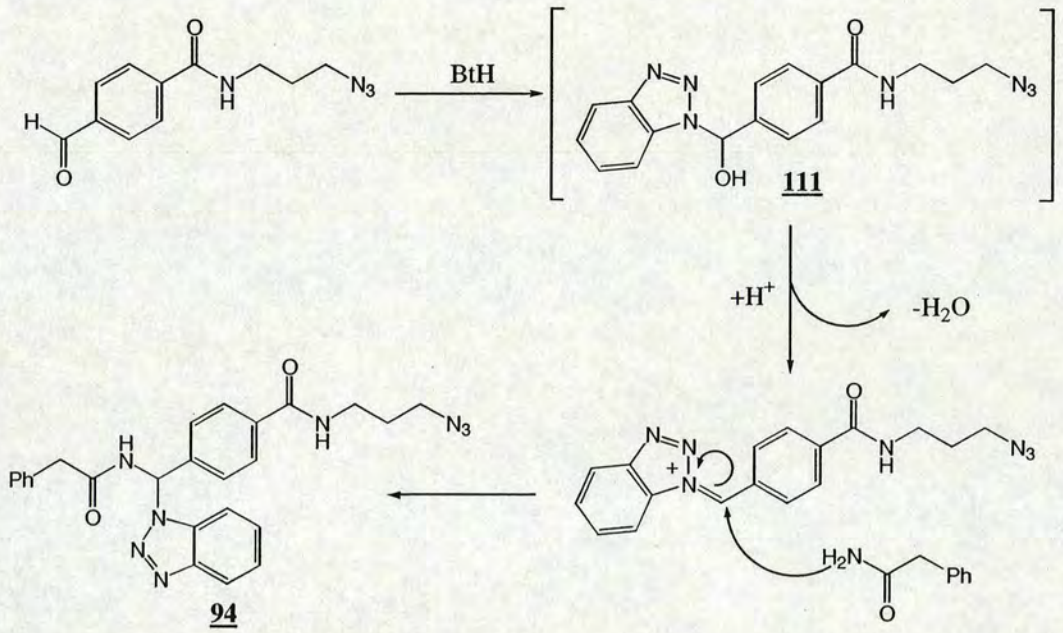


(i) EEDQ, THF, 35 °C, 24h.

Scheme 36: *Generation of the amide bond using EEDQ as the coupling reagent.*

The next step of the synthesis is the generation of the unsymmetrical aminal **94** and this was achieved using chemistry reported by Katritzky *et al* [102]. In a one pot reaction, aldehyde **110**, benzotriazole and phenylacetamide, in equimolar amounts, are refluxed in dry toluene for 18 hours. The reaction was presumed to follow the mechanism involving formation of a hydroxyalkylbenzotriazole intermediate **111** (Scheme 37) that subsequently reacts with the amide *via* an SN1 mechanism. The water formed as a side product was removed azeotropically with toluene using a Dean-Stark apparatus. TLC analysis of the reaction mixture indicated 3 spots corresponding to the starting materials and one new spot, but the reaction never went to completion. Increased reaction times were tried in an attempt to drive the reaction to completion with no improvement. It was found that the increased reaction time had, in fact, a detrimental effect on the reaction resulting in decomposition of the product. This was observed by TLC analysis as the product spot became less intense.

As it has already been mentioned, water is formed as a side product resulting from the protonation of the hydroxyalkylbenzotriazole intermediate **111** (Scheme 37) with the benzotriazole providing the catalytic amount of protons to induce the removal of a water molecule. Given that benzotriazole is not a very strong acid, it was decided that an addition of a catalytic amount of further acid be added to the reaction in order to catalyse the reaction and hopefully increase yield. Tosic acid was therefore added to the reaction mixture in a trace amount resulting in better and more reproducible yields of 50-60 % (typical yields are around 50 %).



Scheme 37: *Proposed mechanism for the formation of* ***94***.

An additional problem to this reaction step was the purification of **94**. Due to the many different functional groups present on **94** only a limited range of solvents dissolved the product and unfortunately were not suitable for the use in column chromatography. Previous purification steps were performed using ethyl acetate and petroleum ether 40-60 °C, in a ratio of 2:1, as the solvent system. As these reactions were on a much smaller scale, the purification worked with greater success. However, on a larger scale this solvent system proved to be unsuccessful with poor separation of products. After some time and effort it was found that the best solvent system to use was dichloromethane and methanol in a ratio of 98:2. It should be noted however, that purification by column chromatography is a limiting factor in the synthesis of the linker.

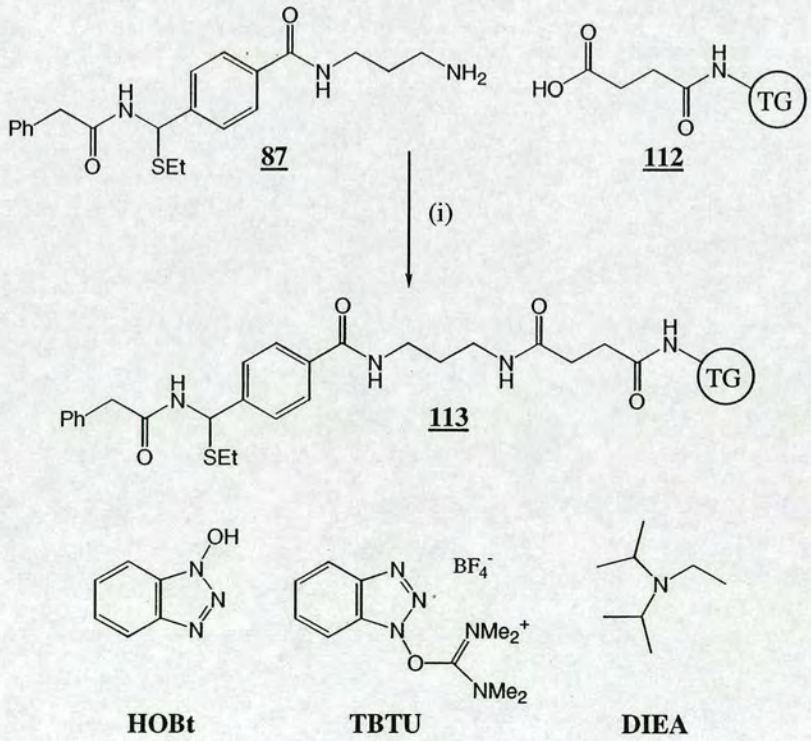
Displacement of the benzotriazole ring using sodium ethyl thiolate was accomplished in 95 % yield to give **93**, which can then be treated with triphenylphosphine and water in THF to reduce the azide to the corresponding amine **87**. Purification of this compound can be easily achieved by trituration with ether. The solid formed is filtered and dried to give a white solid, which is then stirred in ether overnight to remove any further impurities. Filtration and drying of the solid generates a fine white powder in 88 % yield that is sufficiently pure to use in solid-phase reactions. Previously, the solid was used after the initial trituration; however,

it was found that a second treatment with ether gives purer material and better yields in the coupling of the linker onto solid-supports.

The overall synthesis of linker **87** can be achieved in 21 % from 3-chloropropylamine hydrochloride and was used in synthesising gram quantities (5-6 g).

2.2.3 Attachment of Linker 87 onto Solid-Support

The optimal conditions for the attachment of linker **87** on to carboxy-Tentagel® **112** were employed using 2-(1H-benzotriazole-1-yl)-1, 1, 3, 3-tetramethyluronium tetraborate (TBTU), 1-hydroxybenzotriazole (HOBt) and *N*, *N*-diisopropylethylamine (DIEA) (see Scheme 38) [87]. These three reagents, as well as linker **87** and carboxy-Tentagel® **112**, were placed in an isolute tube using DMF as the solvent. After 16 h the resin is filtered, washed and dried under *vacuo* to give the linker-functionalised Tentagel® **113**.

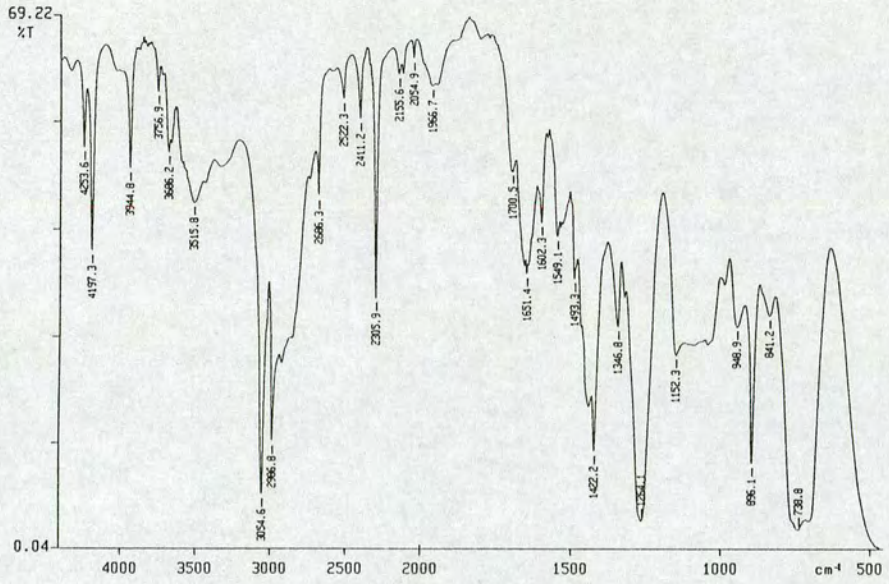


(i) TBTU, HOBt, DIEA, DMF, RT, 16h.

Scheme 38: *Attachment of Linker 87 onto Carboxy-Tentagel® using TBTU.*

The loading of linker-functionalised Tentagel® **113** was determined by treating a known amount of resin with acid or base which releases either phenylacetamide or phenylacetic acid respectively. As Tentagel® can be used with aqueous media, 2M hydrochloric acid and sodium hydroxide were used. The resin is treated with either reagent for 16 h after which the supernatant is neutralised and a known amount is injected into the HPLC for analysis. The amount of phenylacetamide or phenylacetic acid released is measured using standard calibration curves and this value can be then used to calculate the loading of the linker, which can be as high as 0.14 mmol/g (75 % of initial loading, 0.20 mmol/g), although is typically around 0.09 mmol/g (50 %). IR and gel-^{13}C NMR spectra of the resin were obtained and are shown in Figure 8.

a)



b)

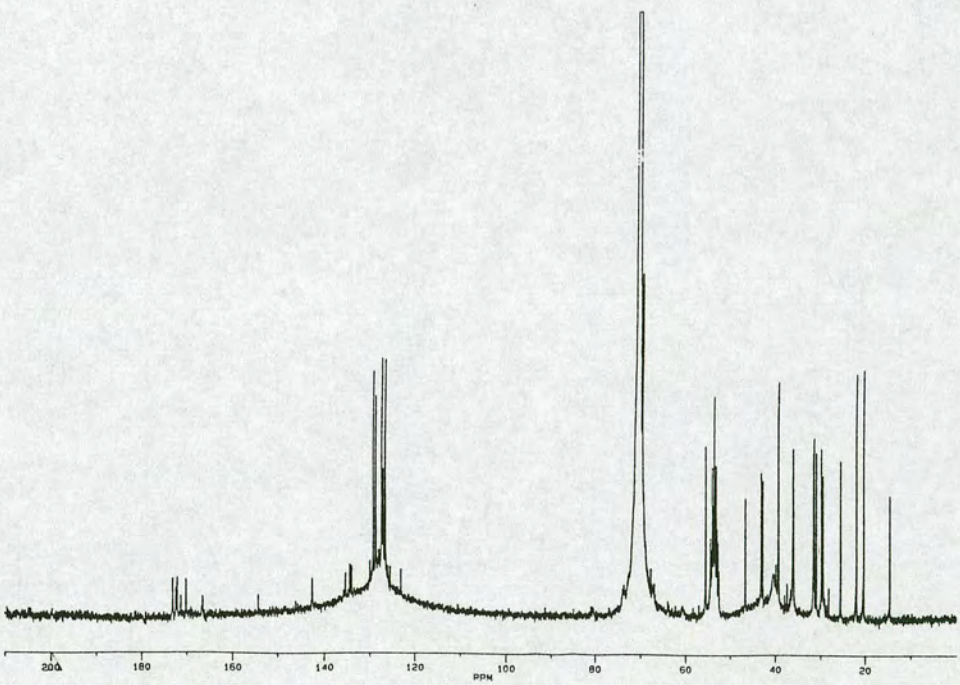
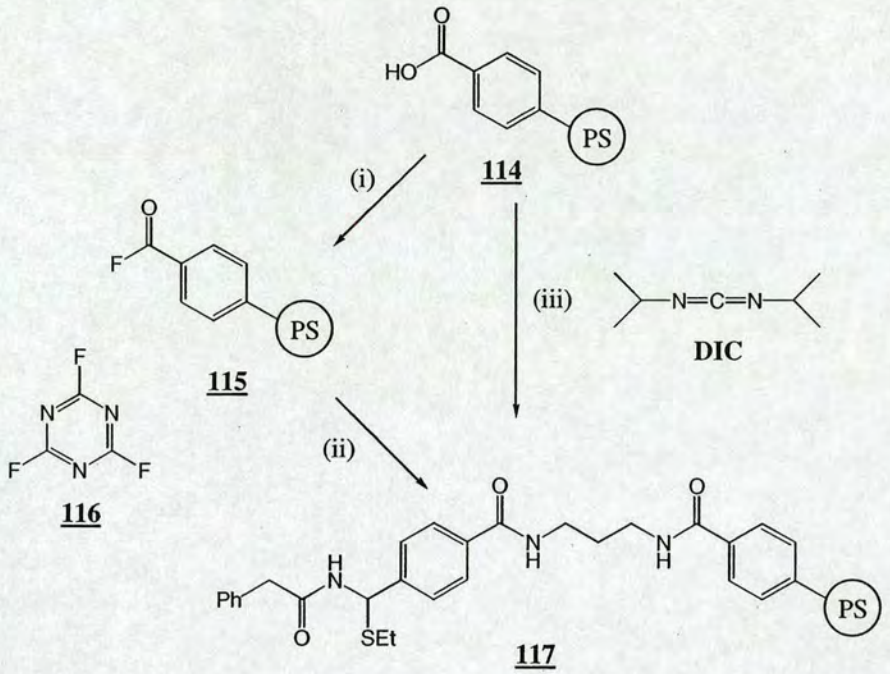


Figure 8: a) *IR and b) gel-^{13}C NMR analysis of linker-functionalised Tentagel® **113** using TBTU as the coupling reagent.*

It was later discovered that polystyrene could also be derivatised with linker **87** using two different coupling methods (Scheme 39) [103]. This resin would provide value when the linker is used for non-aqueous chemistry, since the loading is generally higher (0.98-1.24 mmol/g).



(i) **116**, pyr, DCM, RT, 16h; (ii) **87**, DIEA, DMF, RT, 16h; (iii) **87**, DIC, HOBt, DIEA, DMF, RT, 16h.

Scheme 39: *Coupling of Linker **87** onto carboxy-polystyrene.*

The first coupling method involves activating carboxy-polystyrene **114** as the acylfluoride **115**. This is easily prepared using cyanuric fluoride [104] and after washing and drying the resin, IR analysis of the resin shows an intense peak at approximately 1800 cm^{-1} corresponding to the acylfluoride stretch. This activated resin can then be used to couple linker **87** to give linker-functionalised polystyrene **117** with excellent success and loadings are typically around 0.66 mmol/g (92 % of initial loading, 0.98 mmol/g).

The other method involves the use of 1, 3-diisopropylcarbodiimide (DIC), 1-hydroxybenzotriazole (HOBt) and *N*, *N*-diisopropylethylamine (DIEA) [105]. This method is a one-step reaction and loadings obtained are approximately 0.60 mmol/g (84 % of initial loading, 0.98 mmol/g). Since polystyrene is unsuitable for aqueous media, loadings for the linker-functionalised polystyrene **117** were not determined using 2M hydrochloric acid and sodium hydroxide as performed for the linker-functionalised Tentagel® **113**. Alternatively, a known amount of resin was treated with a solution of TFA, DCM and water in a ratio of 9:10:1. The liquid after cleavage is filtered off, the solvent removed to dryness and the residue obtained

dissolved up in the HPLC solvent. The amount of phenylacetamide is measured and as before is used in calculating the loading.

Since these methods were so successful for the attachment of linker **87** onto carboxy-polystyrene it was thought that these should also be attempted on carboxy-Tentagel® as the yields obtained using TBTU were still a concerning factor. Carboxy-Tentagel® was therefore treated with cyanuric fluoride in the hope of generating the acylfluoride derivatised resin. An IR of the resin was obtained, showing a peak at approximately 1800 cm^{-1}, however, this was not very intense (see Figure 9). This was then treated with linker **87** as mentioned for polystyrene to give an overall loading of 0.09 mmol/g (38 % of the initial loading, 0.26 mmol/g). After several attempts no increase in yield was obtained and so this method was no longer pursued for Tentagel®.

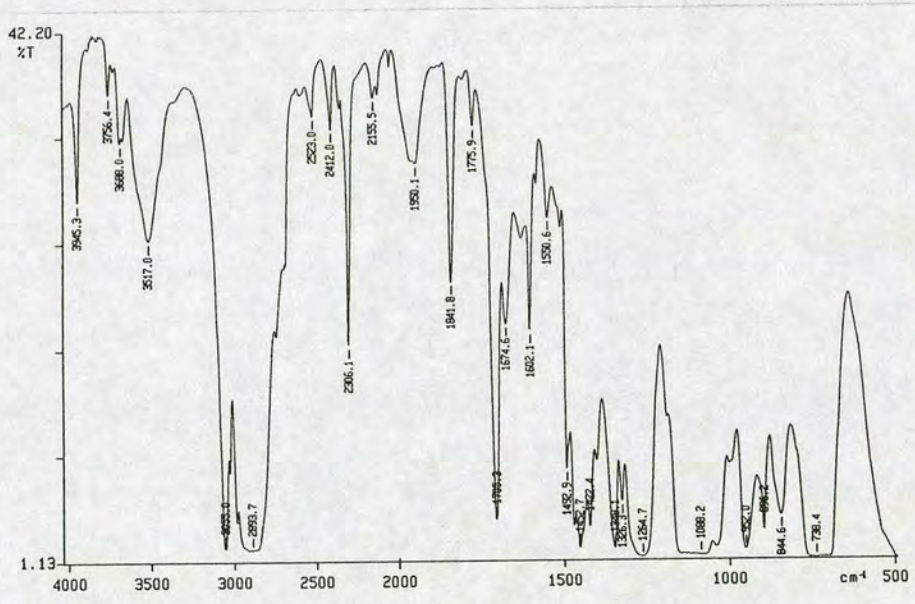
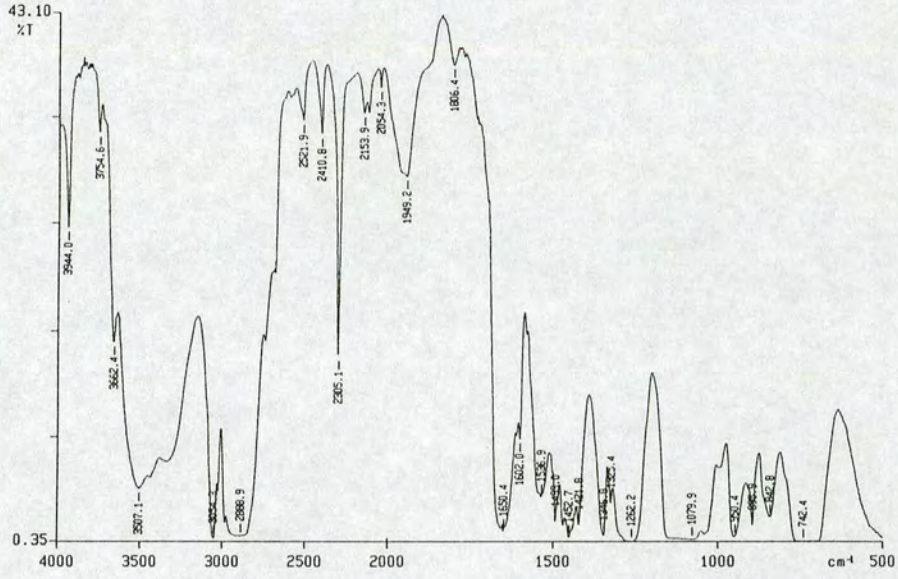


Figure 9: *IR spectra of acylfluoride Tentagel®.*

Given the success with the DIC coupling for polystyrene, this method was also attempted for Tentagel® with excellent results. Carboxy-Tentagel®, linker **87**, DIC, HOBt and DMF were all placed on a blood rotator in an isolute tube for 5 minutes prior to adding DIEA. After 16 h the resin was filtered, washed and dried before treating a known amount of resin with TFA solution to determine the loading. The loading achieved was found to be 0.22 mmol/g (92 % of initial loading, 0.26

mmol/g), which indicated a far superior method of attachment. IR and gel-^{13}C NMR spectra of the resin were obtained and are shown in Figure 10.

a)



b)

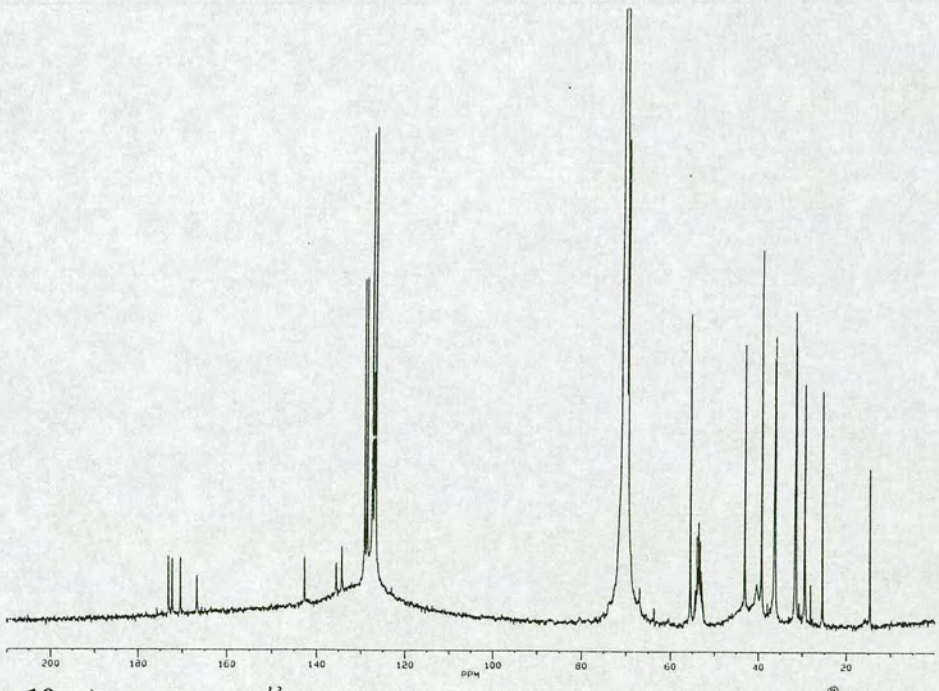


Figure 10: a) *IR and b) gel-^{13}C NMR analysis of linker-functionalised Tentagel® <u>17</u> using DIC as the coupling reagent.*

Comparing both sets of spectra, it can be seen that there are fewer impurities in the material obtained from the DIC coupling. By comparison of the peak at approximately 1650 cm^{-1}, which is consequence of the amide bond formed in the

coupling step, it can be seen that the material obtained from the DIC coupling shows a much more intense peak than that of the TBTU coupling. The gel-^{13}C NMR spectrum also shows a major difference between the two methods. The spectrum obtained from the TBTU coupling method has many spurious peaks, which have not been assigned and are probably due to impurities formed during the poor coupling step.

2.2.4 Summary and Conclusions

Linker **87** was successfully synthesised in 5 steps from 3-chloropropylamine in an overall yield of 21 % in gram quantities. Improvements to the synthesis include the use of EEDQ in the amide bond formation step; increased yields in the Katritzky reaction by using a catalytic amount of tosic acid and an improved purification step of the benzotriazole adduct **94** using DCM:MeOH (98:2). **87** was then attached successfully to a both carboxy-Tentagel® and carboxy-polystyrene using several different reagents with DIC giving the highest yields (92 % for Tentagel and 84-92 % for polystyrene).

2.3. Syntheses of *N*-Glycopeptides

2.3.1 Introduction

Many synthetic routes towards glycopeptides and analogues have been reported in the literature. The most general route employed uses glycosylated amino acids for stepwise assembly of glycopeptides and so synthetic routes towards these building blocks therefore constitute a key success. Recently, an alternative route towards *O*-glycopeptides was reported by Nakahara *et al* (see section 1.2.2, Schemes 9 and 10), which involved the attachment of a sugar molecule to the solid support *via* a novel silyl linker. A key feature of this approach was the feasibility of peptide chain-elongation at both the *N*- and *C*-termini. It has been envisaged that our methodology would provide a complementary route towards *N*-glycopeptides with the direct attachment of sugar building block **106** onto solid support *via* linker **87** (Figure 11).

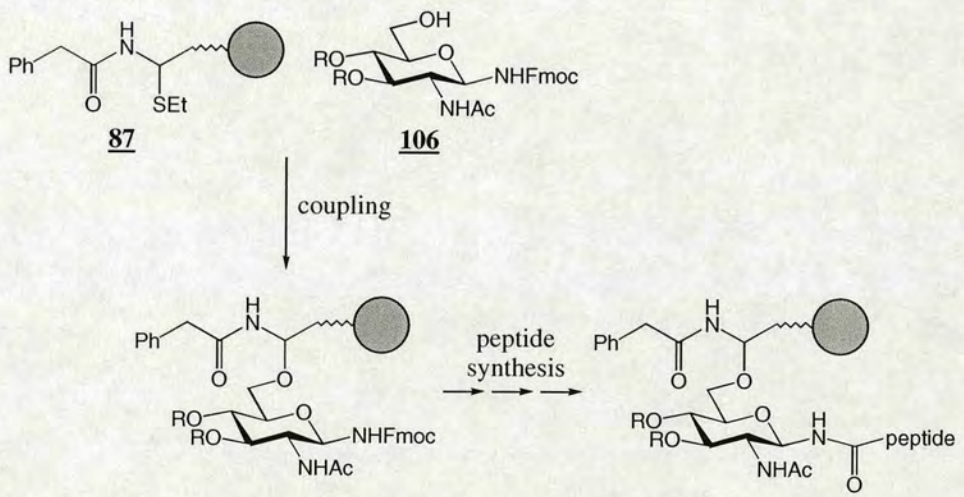
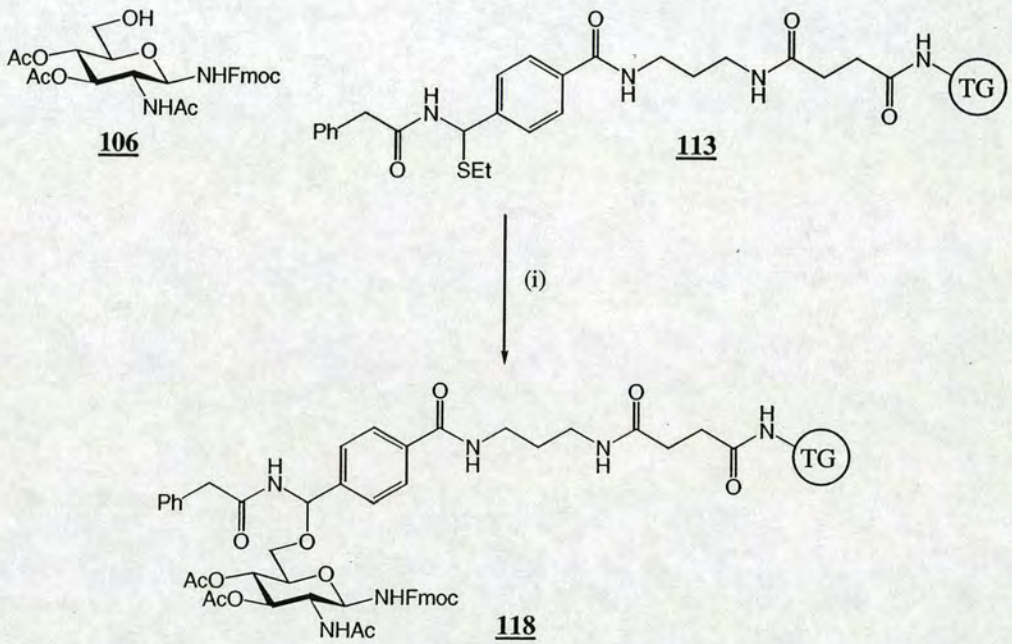


Figure 11: *Alternative strategy for the synthesis of N-glycopeptides using sugar* ***106*** *and linker* ***87***.

2.3.2 Attachment of Sugar Building Block ***106*** *onto Linker-functionalised Tentagel®* ***113***

Initial studies into the attachment of sugar building block **106** onto linker-functionalised Tentagel® **113**, illustrated in Scheme 40, adopted procedures already developed by others in the group [82, 86]. Linker-functionalised Tentagel® **113** and

sugar **106** were placed in an isolute tube with DMF and *N*-iodosuccinimide (NIS) was then added. The mixture was left overnight to give sugar-functionalised Tentagel® **118** after which the resin was filtered, washed and dried. By consequence of the sugar furnishing the Fmoc group, the loading of the sugar was determined by UV analysis and was found to be a poor 0.02 mmol/g (18 % of initial loading, 0.1 mmol/g). This method was attempted several times trying to optimise the conditions by varying the concentrations and reaction times but no increase in yield was achieved.



(i) NIS, Solvent, RT, 16h.

Scheme 40: *Coupling of sugar **106** onto Linker-functionalised Tentagel® **113**.*

A systematic investigation was therefore required and undertaken varying several factors involved in the sugar coupling. Many small-scale reactions were systematically attempted to try and identify the most promising conditions. In each reaction approximately 10-20 mg of linker-functionalised Tentagel® **113** (1 eq) and sugar **106** (10 eq) were place into an isolute tube with dry solvent and 4Å molecular sieves. The mixture was left for 30 minutes after which a solution of *N*-iodosuccinimide (4 eq) in the chosen solvent was added. The reaction mixture was then left for 16 hours after which the resin was filtered, washed and dried. Again loadings were measured by Fmoc analysis. These reactions were repeated several

times and Table 2 summarises the conditions attempted quoting the average % coupling obtained for each.

Solvent	TfOH used	Temp (°C)	Time (h)	% Coupling
DMF	N	24	16	18
DMF	Y	24	16	24
DCM	N	24	16	18
DCM	Y	24	16	10
$CHCl_3$	N	24	16	2
$CHCl_3$	Y	24	16	3
CH_3CN	N	24	16	16
CH_3CN	Y	24	16	13
THF	N	24	16	10
THF	Y	24	16	40

Table 2: *Results for the coupling of Sugar **106** onto Linker-functionalised Tentagel® **113** varying conditions.*

As the initial results using *N*-iodosuccinimide solution alone did not look promising it was decided to try more forcing conditions. Thioglycosides are frequently used for glycosidation reactions [106] by activation of the thioethyl group followed by displacement with an alcohol. This glycosidation reaction is very similar to the coupling being attempted and so these procedures were studied. The addition of trifluoromethanesulfonic acid (triflic acid) [107] has often been used in these reactions as this reagent favours the generation of the iodonium ion required for the activation and release of the thiolate group. The addition of such a reagent was therefore also attempted in the sugar coupling reactions. As before, the same scale and conditions were tried except a solution of *N*-iodosuccinimide (4 eq) and triflic acid (0.125 eq) was added. Table 2 gives the average % coupling obtained.

As it can be seen from Table 2, best results were obtained with THF as the solvent with a fair 40 % coupling and so it was decided to further optimise this coupling. Several different reactions were attempted differing the concentration of resin and sugar by varying the amount of THF used. Table 3 summarises the results.

Resin (mg)	Sugar (mg)	TfOH used	THF (ml)	% Coupling
11.1	10.3	Y	2.0	22
9.0	9.4	Y	1.0	29
7.5	9.1	Y	0.7	40
9.4	11.2	Y	0.5	62

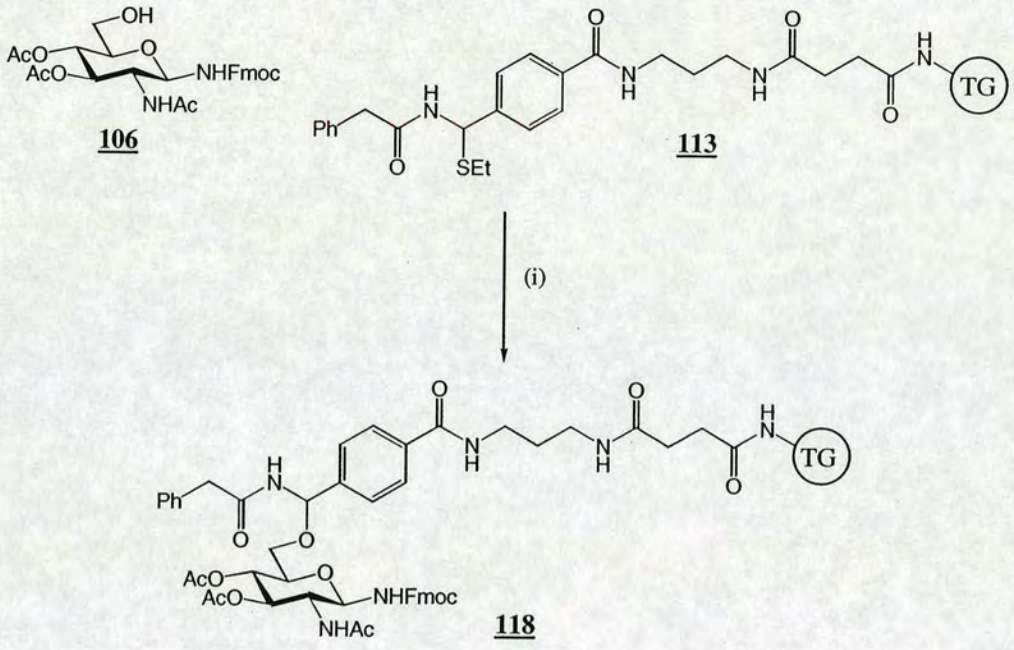
Table 3: *Results for the coupling of Sugar* ***106*** *onto Linker-functionalised Tentagel® 113 varying concentrations.*

As it can be seen from Table 3, the coupling of sugar **106** onto linker-functionalised Tentagel® **113** was achieved in good yield using minimal solvent and so these conditions were adopted. Unfortunately, the yield of this coupling reaction proved to be erratic upon repeated experiments.

The reaction protocol was therefore investigated in more detail to make the outcome of the coupling reaction more reproducible. Whilst looking at the procedure, it was noticed that the temperature of the molecular sieves was an important factor since the original coupling used sieves direct from the oven. This therefore sparked the idea that an increase of temperature was required as this was aiding in solubilising sugar **106**. As a result of this, the coupling of sugar **106** onto linker-functionalised resin **113** was performed at a slightly elevated temperature of 45 °C to give startlingly consistent results with yields ranging from 60-90 % with typical yields of approximately 70 %.

It was later found that the reaction time for the coupling could be reduced considerably to only 2 hours thus reducing the time to generate material for reactions to follow. Scheme 41 shows the overall coupling conditions for the reaction which will provide consistently good results with slightly elevated yields at larger scales. However, it should be noted that the purity of linker-functionalised resin **113** and sugar **106** is very important to achieve these yields. Couplings have been performed on linker-functionalised resin **113** that has been stored for some time with reduction of yields and so the use of freshly prepared resin was preferred. Both the linker-functionalised resin **113** and the sugar **106** should also be dried in *vacuo* for

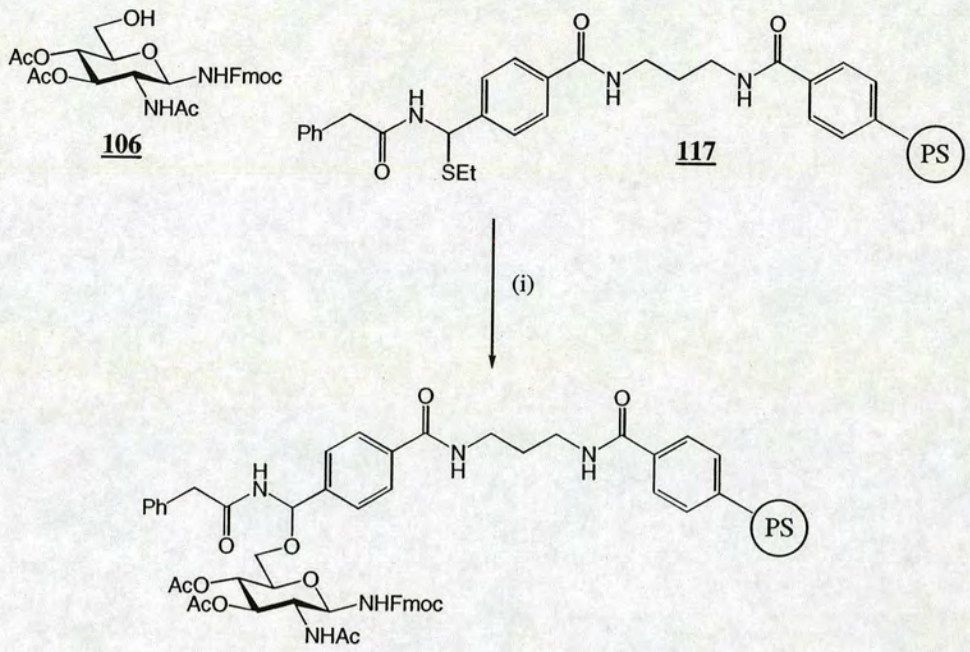
approximately 1 hour prior to use and the THF if possible should be obtained freshly distilled.



(i) NIS, TfOH, THF, 4Å ms, 45 °C, 2h (60-90%).

Scheme 41: *Overall conditions for the coupling of Sugar* ***106*** *onto Linker-functionalised Tentagel®* ***113***.

As mentioned earlier the use of polystyrene would also provide value in non-aqueous chemistry since the loading of the resin is generally higher and because of this the coupling of sugar **106** onto linker-functionalised polystyrene **117** (Scheme 42) was also investigated. Table 4 summarises the conditions attempted, which gave poor yields, the highest at about 28 %. Conditions adopted from the Tentagel® coupling were also unsuccessful and it is postulated that the poor yields are due to the difference in the polymer used. Tentagel® resin consists of about 80 % polyethylene glycol grafted to cross-linked polystyrene and it is thought that this backbone may aid in solubilising sugar **106**. The polystyrene resin does not have such a backbone and this may be a possible reason for the poor couplings obtained. Due to lack of time, this work was not further pursued.



(i) NIS, TfOH, solvent (see table), 4Å.

Scheme 42: *Coupling of sugar **106** onto Linker-functionalised polystyrene **117**.*

Solvent	Temp (°C)	Time	% Coupling
THF	24	16	28
THF	45	16	15
DCM	24	16	9
$CHCl_3$	24	16	12
CH_3CN	24	16	9
DMF	24	16	7
Et_2O/DCE	24	16	3
DCM/CH_3CN	24	16	6
Dioxane	24	16	20

Table 4: *Results for the coupling of Sugar **106** onto Linker-functionalised polystyrene **117**.*

Once sugar **106** had been coupled successfully onto Tentagel® *via* linker **87**, work on sugar-functionalised Tentagel® **118** began. Since sugar **106** was designed with an Fmoc group the most logical step forward was removal of the group and further manipulation at the anomeric amine. The importance of different strategies towards glycopeptides and the need to synthesise them for further study into their biological importance was mentioned in section 1.2. It was therefore decided to demonstrate that this methodology has the potential to synthesise glycopeptides and by example, the synthesis of *N*-glycopeptide **119** (Figure 12) was undertaken. The peptide attached to the sugar is a fragment of a decapeptide sequence, which is known to bind well to the Major Histocompatibility Complex (MHC) class II E^k molecule [55].

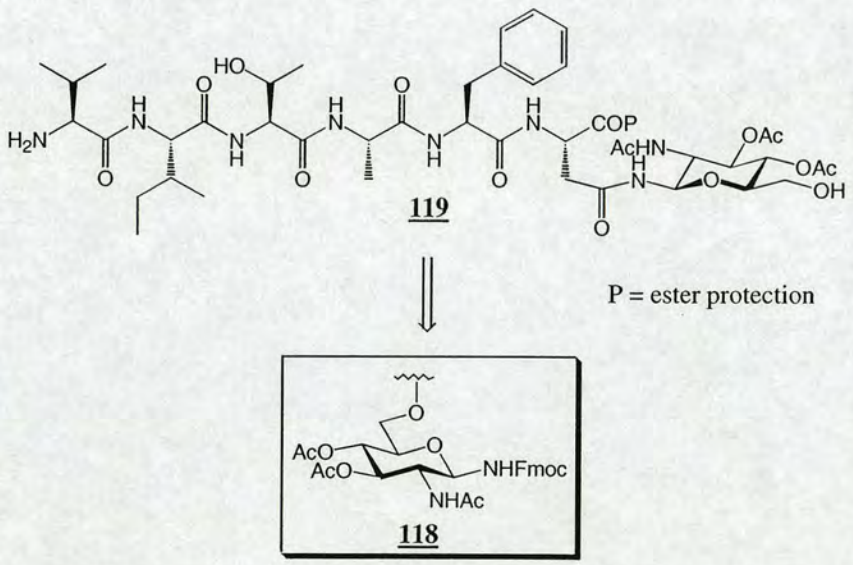
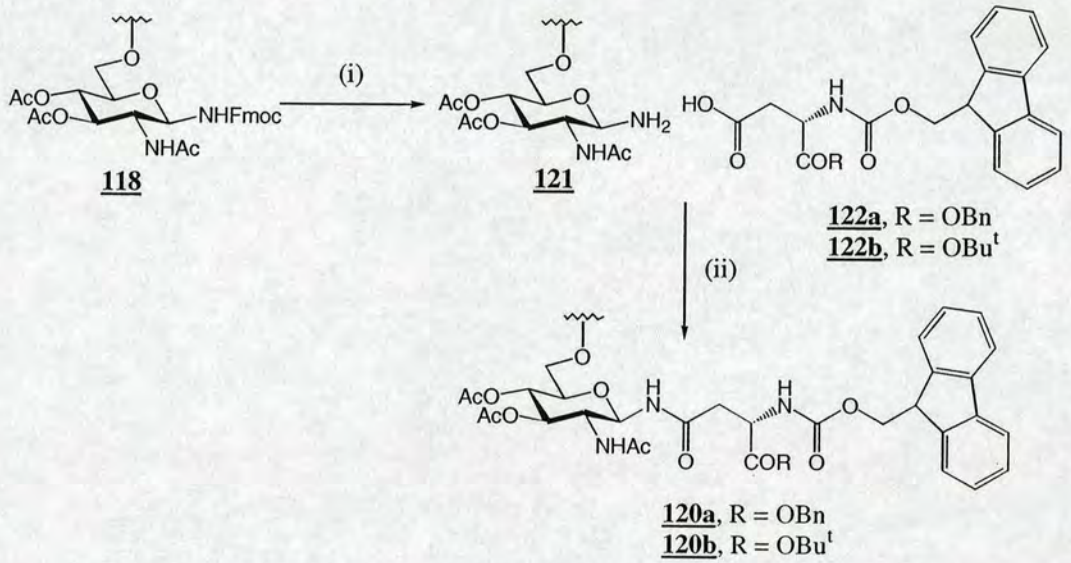


Figure 12: *Synthetic target N-glycopeptide **119** from sugar-functionalised Tentagel® **118**.*

The first important step in the synthesis of *N*-glycopeptide **119** is the attachment of a selectively protected aspartic acid onto the anomeric amine thus generating *N*-acetylglucosamine covalently attached to an asparagine (Asn) side chain *via* a β-glycosidic linkage **120** (Scheme 43). The Fmoc group on **118** is first removed by treatment with 20 % piperidine in DMF for 15 minutes after which the

amino-resin **121** is filtered, washed and dried under suction for several minutes. TBTU, HOBt and aspartic acid **122a** were then placed in the same isolute tube as the amino resin **121** and DMF was added. The mixture was left for approximately 5-10 minutes after which *N*-methylmorpholine was added and the mixture left on a blood rotator for 16 hours. The resin was then filtered, washed, dried and subsequently tested by UV analysis to determine the loading of **120a**, which was found to be approximately 0.10 mmol/g (60 %).



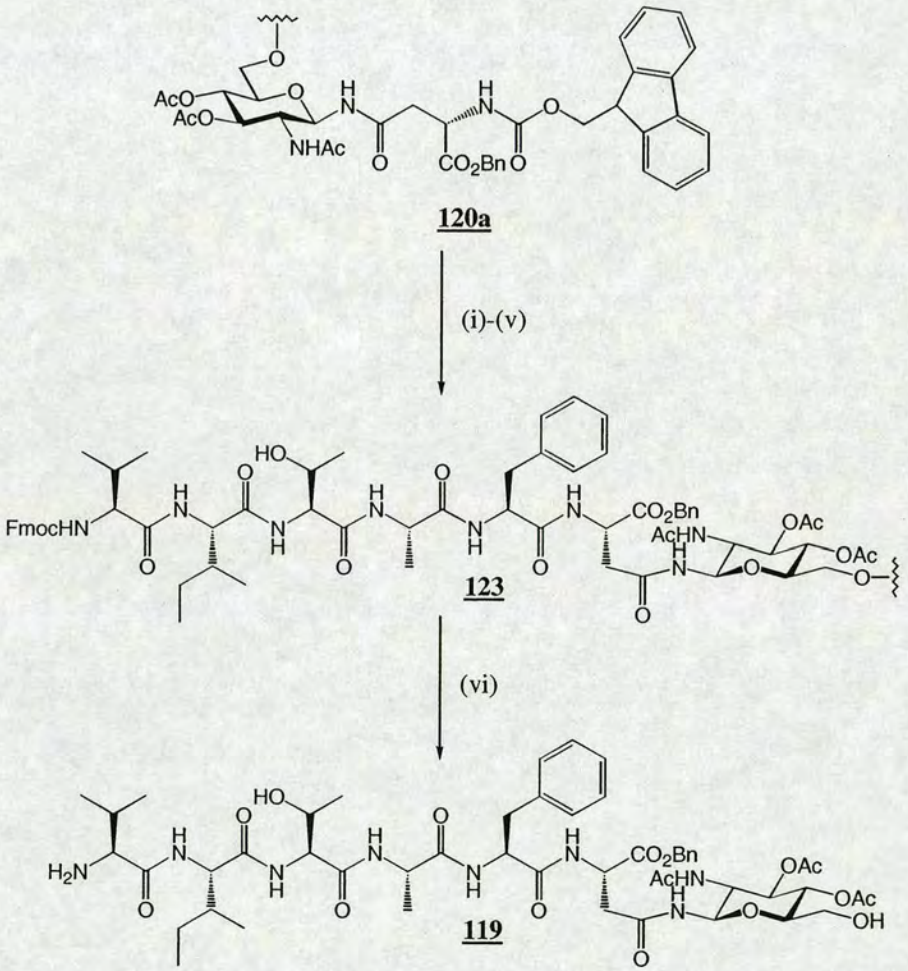
(i) 20% piperidine in DMF, RT, 15 min; (ii) TBTU, HOBt, NMM, DMF, RT, 16h.

Scheme 43: *Synthesis of* N-*acetylglucosamine covalently attached to an asparagine (Asn) side chain via a β-glycosidic linkage* ***120***.

This reaction has been performed several times throughout this work and typical yields of 60 % were usually obtained with slightly elevated yields when the reaction was performed at a larger scale. This yield was accepted to be reasonable since it is believed that the anomeric amine is less nucleophilic than compared to free amino acids. Again it should be noted that linker **87** is very sensitive to impure reagents and so all reagents including the solvents must be extremely pure. When reactions were performed with reagents that had been stored for several weeks yields were considerably lower. Smaller reagent equivalents with double and triple couplings were attempted but also resulted in low yields suggesting decomposition during reactions. The *tert*-butyl ester **122b** (Scheme 43) was also coupled using these reagents giving comparable results. **120b** is very useful as the *tert*-butyl group

present can be removed during the acid cleavage step hence further manipulation at the *C*-terminal would be possible.

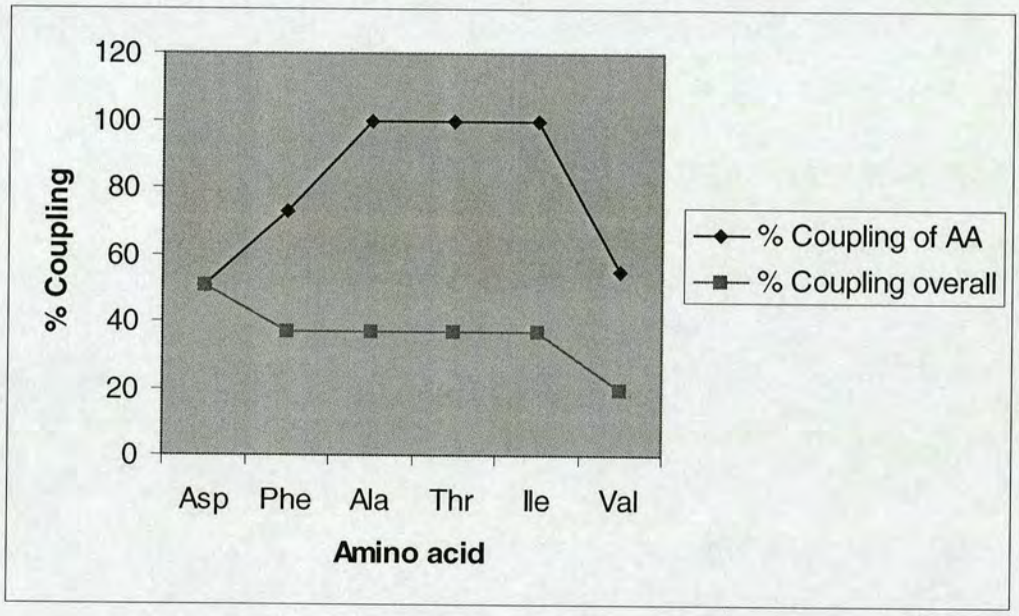
120a was then used as a starting material for the synthesis of glycopeptide **119**. The Fmoc group as previously mentioned was removed using 20 % piperidine in DMF (Scheme 44) and the next amino acid, phenylalanine, was coupled using the same reagents. Further coupling with Fmoc-protected alanine, threonine, isoleucine and valine furnished polymer-bound *N*-glycopeptide **123**.



(i) a) 20% piperidine in DMF, RT, 15 min; b) Fmoc-Phe-OH, TBTU, HOBt, NMM, DMF, RT, 16h; (ii) b) a) 20% piperidine in DMF, RT, 15 min; b) Fmoc-Ala-OH, TBTU, HOBt, NMM, DMF, RT, 16h; (iii) a) 20% piperidine in DMF, RT, 15 min; b) Fmoc-Thr(OBu^t)-OH, TBTU, HOBt, NMM, DMF, RT, 16h; (iv) a) 20% piperidine in DMF, RT, 15 min; b) Fmoc-Ile-OH, TBTU, HOBt, NMM, DMF, RT, 16h; (v) a) 20% piperidine in DMF, RT, 15 min; b) Fmoc-Val-OH, TBTU, HOBt, NMM, DMF, RT, 16h; (vi) a) 20% piperidine in DMF, RT, 15 min; b) TFA:DCM:H_2O (9:10:1), RT, 16h.

Scheme 44: *Synthesis of* N*-glycopeptide* ***119*** *using TBTU and HOBt as the coupling reagents.*

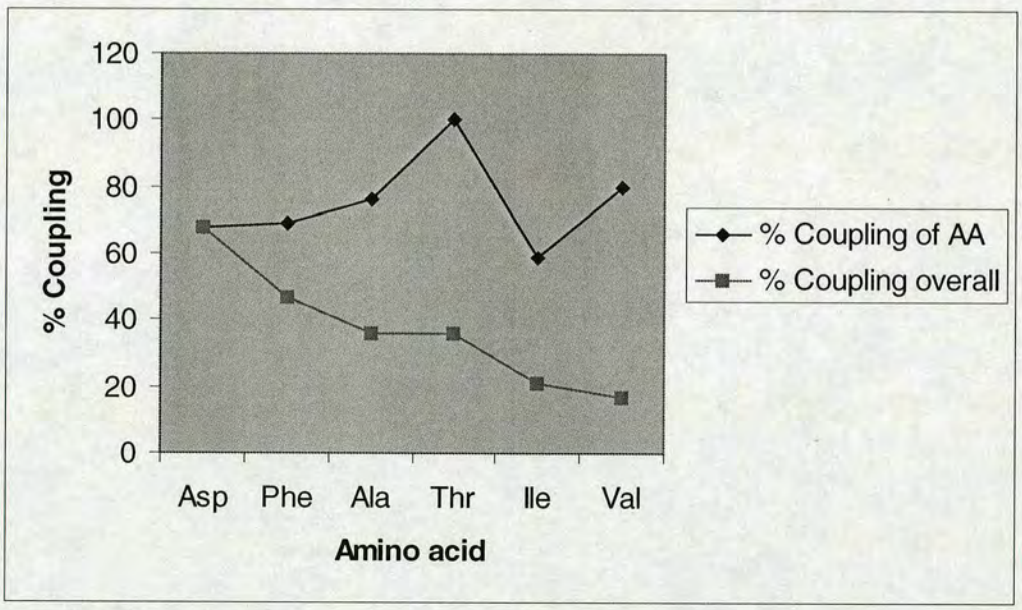
The synthesis of *N*-glycopeptide **123** was repeated twice using TBTU and HOBt as the coupling reagents. The first attempt was performed on a 60 mg scale, which gave only 15 mg of resin at the end of the synthesis with an overall loading of 0.03 mmol/g for the 6 couplings. The % yield for each coupling and the overall yield for the glycopeptide synthesis are given in Graph 1. The synthesis was encouraging as the couplings were good to excellent, however the last coupling, which was only 55 %, was disappointing.



Graph 1: *% Couplings obtained in the synthesis of N-glycopeptide **123** using TBTU (1st attempt).*

The product obtained on solid support was treated with 20 % piperidine in DMF and then cleaved off using TFA:DCM:H_2O. The cleavage mixture was analysed by electrospray mass spectrometry and indicated a peak at 1041 m/z, which corresponds to the desired product **119**. Removal of the Fmoc group was performed prior to the cleavage since it was predicted that the hydrophobic character of the peptide would cause problems in the ionising step in the mass spectrometer. The cleavage mixture was also analysed by HPLC but unfortunately did not show any significant peaks, which was consequence of removing the Fmoc group. It was therefore hoped that the synthesis of *N*-glycopeptide **123** be repeated on a larger scale which would provide enough material for a 600 MHz ^{1}H NMR. The Fmoc group would not be removed as this would aid in the HPLC purification step.

The synthesis of *N*-glycopeptide **123** was therefore repeated on a 100 mg scale, which was enough resin to provide material for a ^{1}H NMR. As seen on Graph 2, the % couplings were much lower than previously obtained and the overall loading for the 6 couplings was a poor 0.02 mmol/g (NB started with higher initial loading). This synthesis did not provide sufficient amount of glycopeptide and was therefore not used for further analysis. It was believed that the main problem of this route was the quality of the *N*-methylmorpholine used. It can be comprehended that this reagent in time will decompose to other amines and this may have a detrimental effect on linker **87**. Purification of this reagent was also very time-consuming which proved to be inappropriate in this reaction strategy. A much more reliable coupling method was therefore required which would give higher yields and would also provide an easier route using reagents that would have less chance of affecting linker **87**.

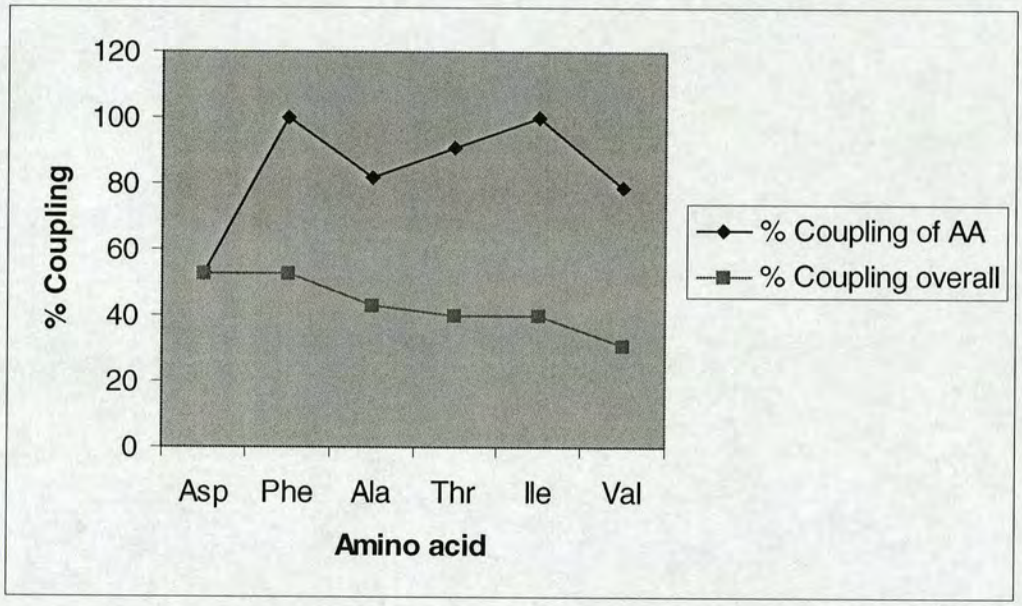


Graph 2: *% Couplings obtained in the synthesis of N-glycopeptide **123** using TBTU (2nd attempt).*

In the literature Meldal *et al* [33] reported that the use of pentafluorophenyl esters are very suitable for solid-phase glycopeptide synthesis and show very fast coupling rates in the presence of 3, 4-dihydro-3-hydroxy-4-oxo-1, 2, 3-benzotriazine (Dhbt-OH). The progress of the acylation can also be followed visually by the

displacement of the yellow ion pair formed between Dhbt-OH and resin bound amino groups [33].

The first synthesis (Graph 3) was performed on a 60 mg scale to test if the procedure was successful. This resulted in an overall yield of 59 % (loading = 0.06 mmol/g), which is considerably higher than that of the TBTU coupling. The experimental method is a much easier method and double and triple couplings were attempted which showed no detrimental effects towards the synthesis with the result of increased yields.

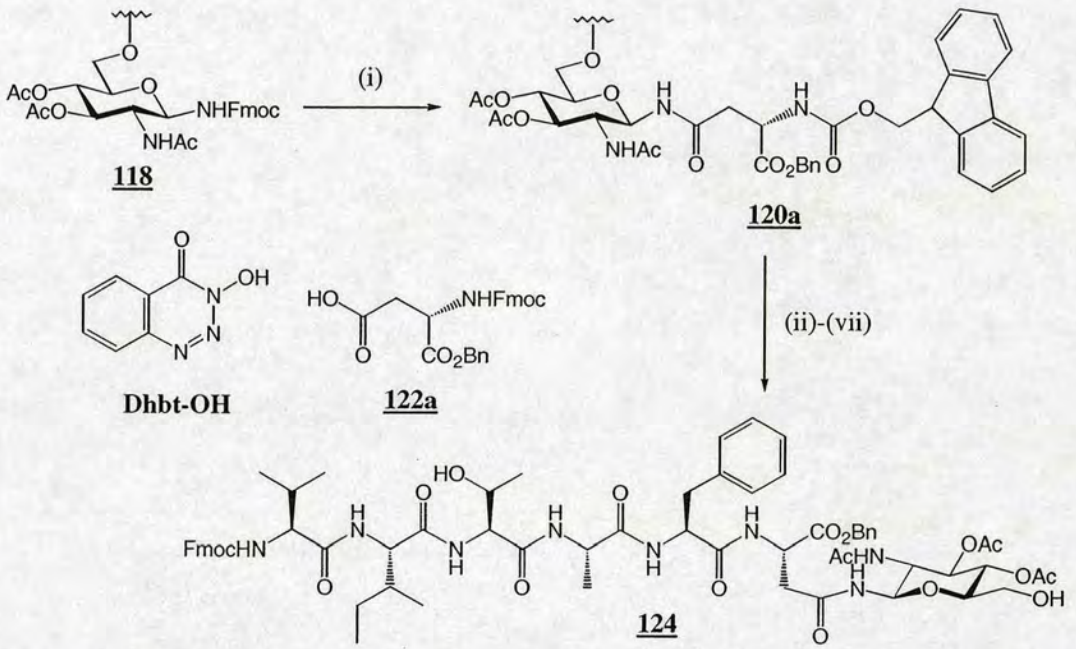


Graph 3: *% Couplings obtained in the synthesis of N-glycopeptide **124** using pentafluorophenyl esters (1st attempt).*

This method therefore proved to be far more beneficial than that of the TBTU coupling and so was adopted on a large scale. An acetate capping procedure was also introduced so that mixtures of peptide chains were reduced. The overall route taken towards *N*-glycopeptide **124** is shown in Scheme 45.

The scale used for this synthesis was approximately 0.6 g of sugar-functionalised Tentagel® **118**, which had a loading of 0.11 mmol/g. The initial aspartic acid residue was coupled using TBTU as the aspartic acid pentafluorophenyl ester was not commercially available and the synthesis of the compound proved to be a little problematic. However, the reaction proved successful and aspartic acid **122a**

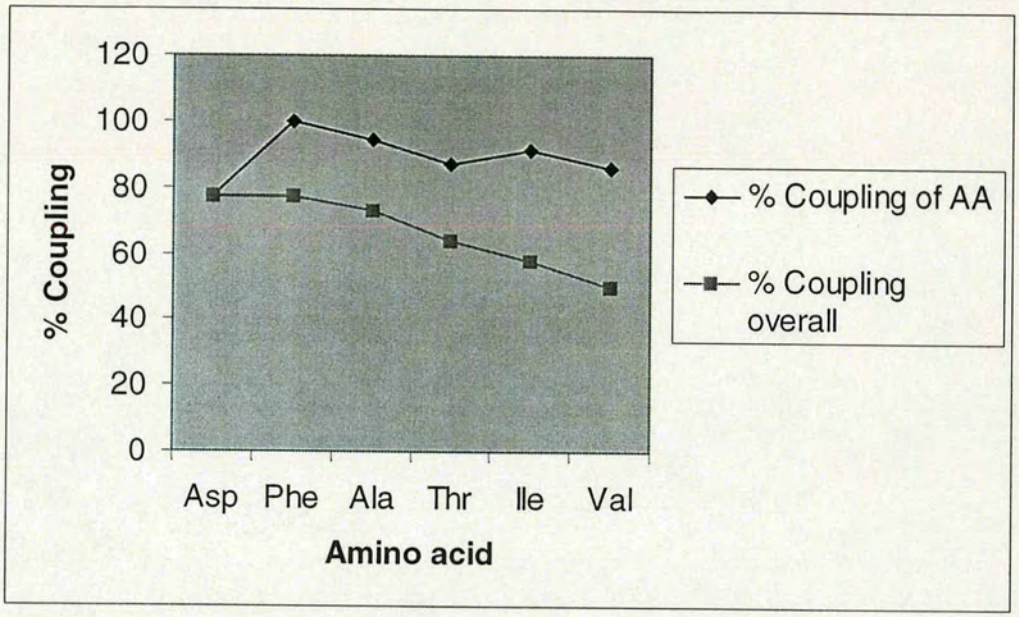
coupled to sugar-functionalised Tentagel® **118** in 78 % (Loading = 0.086 mmol/g). The acetate capping procedure was then performed and an additional loading was measured to check that the linker was stable to the conditions. The Fmoc group was then removed and the next amino acid coupled using a solution of Fmoc-amino acid pentafluorophenyl ester and Dhbt-OH in DMF. This process was repeated until all the amino acids were coupled and graph 4 shows the % couplings obtained for each and the overall % coupling.



(i) a) 20% piperidine in DMF, RT, 15 min; b) **122a**, TBTU, HOBt, NMM, DMF, RT, 16h; c) Ac_2O, DMF, RT, 7h; (ii) a) 20% piperidine in DMF, RT, 15 min; b) Fmoc-Phe-OPfp, Dhbt-OH, DMF, RT, 16h; c) Ac_2O, DMF, RT, 7h; (iii) a) 20% piperidine in DMF, RT, 15 min; b) Fmoc-Ala-OPfp, Dhbt-OH, DMF, RT, 16h; c) Ac_2O, DMF, RT, 7h; (iv) a) 20% piperidine in DMF, RT, 15 min; b) Fmoc-Thr(OBu^t)-OPfp, Dhbt-OH, DMF, RT, 16h; c) Ac_2O, DMF, RT, 7h; (v) a) 20% piperidine in DMF, RT, 15 min; b) Fmoc-Ile-OPfp, Dhbt-OH, DMF, RT, 16h; c) Ac_2O, DMF, RT, 7h; (vi) a) 20% piperidine in DMF, RT, 15 min; b) Fmoc-Val-OPfp, Dhbt-OH, DMF, RT, 16h; c) Ac_2O, DMF, RT, 7h; (vii) TFA:DCM:H_2O (9:10:1), RT, 16h.

Scheme 45: *Overall synthesis of* N-*glycopeptide* ***124***.

The overall synthesis was performed in 50 % yield giving a final loading of 0.06 mmol/g. The amount of resin collected at the end of the synthesis was approximately 0.54 g, which had theoretically 40 mg of *N*-glycopeptide **124** on solid support.



Graph 4: *% Couplings obtained in the synthesis of N-glycopeptide **124** using pentafluorophenyl esters (2nd attempt).*

Approximately 13 mg of resin was then treated with TFA:DCM:H_2O and the cleavage mixture was analysed by HPLC. The HPLC trace obtained is shown in Figure 13. The addition of the Fmoc group helped to identify the glycopeptide.

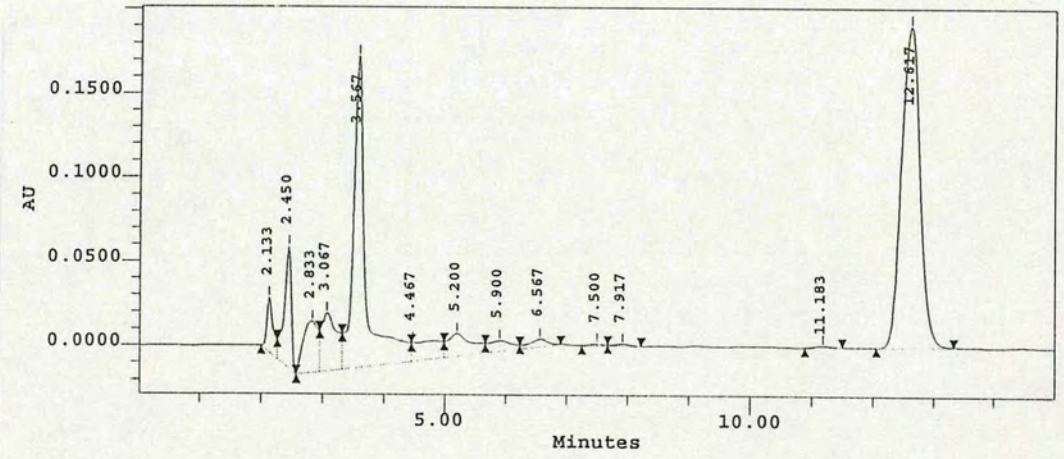


Figure 13: *HPLC trace of the cleavage mixture from **123**.*

An intense peak at 12-13 minutes was observed and presumed to be the desired product. The purification looked to be relatively easy and it was therefore decided that a 1 ml injection would be tried, collecting the solvent at routine intervals. The solvent fractions were collected and re-injected (20 µl) into the HPLC to identify the

pure glycopeptide fractions. These were combined, concentrated and re-injected (20 µl) into the HPLC to give the trace shown in Figure 14.

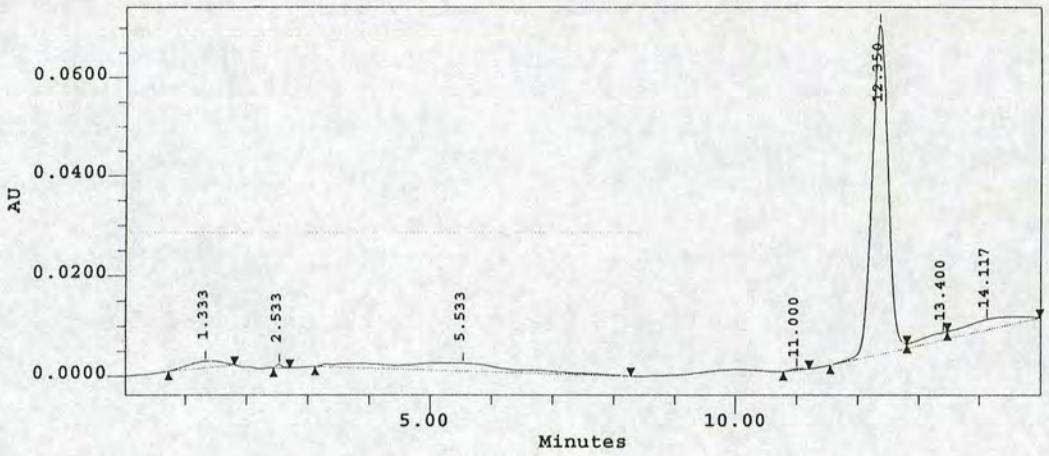


Figure 14: *HPLC trace of the purified N-glycopeptide* ***124***.

This was then repeated on a larger scale (70 mg), which theoretically gives 5.3 mg. The cleavage mixture was concentrated to dryness and approximately 8 ml of the HPLC solvent was added. Eight (1 ml) HPLC injections were then performed collecting the solvent around 10-15 minutes of each. The solvent was combined and removed in *vacuo* to give 3.4 mg of glycopeptide. Problems then arose when trying to dissolve the glycopeptide in deuterated solvent. Many solvents were tried ($CDCH_3$, CD_2Cl_2, CD_3COCD_3, CD_3CN, CD_3OD, D_2O) including several mixed solvents ($CDCH_3$:CD_3OD, CD_2Cl_2:CD_3OD, CD_3CN:D_2O), but the glycopeptide would not dissolve and therefore dimethylsulfoxide had to be used. Unfortunately the ^1H NMR obtained showed that the product obtained was not pure and the spectrum could not be used for characterisation. This could be due to either the inefficiency of the purification step or the purity of the dimethylsulfoxide used.

A similar amount of resin (79 mg) was also treated with the TFA solution so that the purification may be performed again, but unfortunately the glycopeptide cleavage mixture would not dissolve up in the HPLC solution as it did before. Extra solvent was added to help dissolve the solid but had little effect. This therefore indicated that the HPLC purification adopted using H_2O and acetonitrile was not reliable and so an alternative solvent system must be found. This could not be achieved due to time constraints. A mass spectrum of the pure glycopeptide was

obtained (Figure 15) however, indicating a peak at 1263 m/z that corresponds to the Fmoc-glycopeptide **124** with sodium and potassium adducts at 1285 and 1301 m/z respectively. Other significant peaks are 1221 [MH^+-OAc], 1155 [MH^+-OBn] and 1113 [MH^+-OBn-OAc]. Sodium and potassium adducts are also present for these.

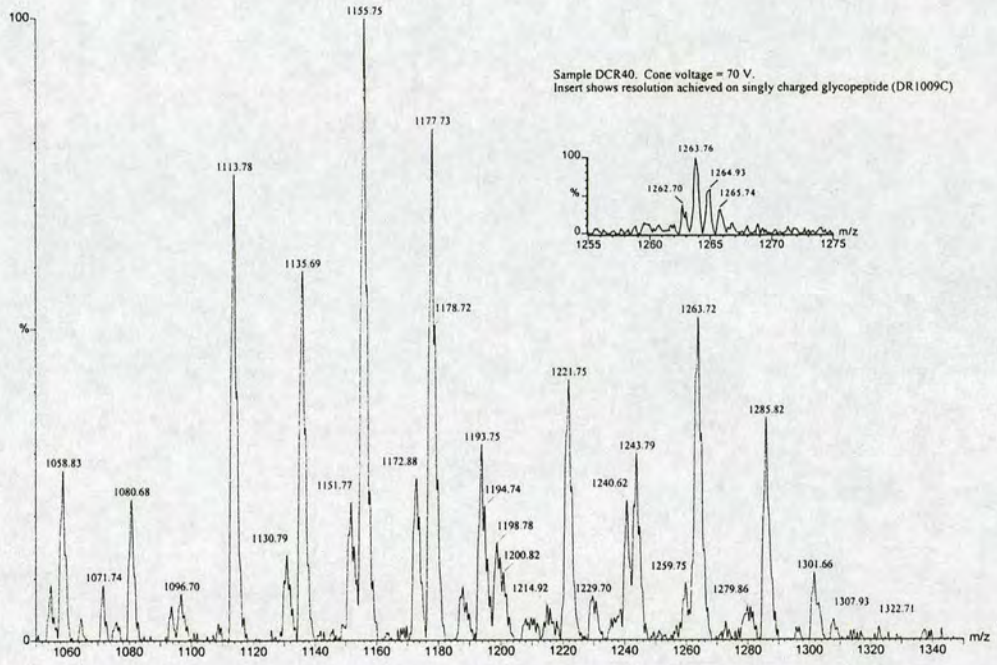


Figure 15: *Mass spectrum of purified* N*-glycopeptide* ***124***.

2.3.4 Summary and Conclusions

Sugar **106** was successfully linked through the 6-hydoxyl group onto carboxy-Tentagel® *via* linker **87** in 60-90 % yield. Extension of the glycosidic amino group on **118** provides a route towards the synthesis of *N*-glycopeptides and has been demonstrated in the synthesis of *N*-glycopeptide **124** using two different routes. The use of pentafluorophenyl esters and Dhbt-OH has proved to be the most successful route towards **124** with excellent couplings being obtained. A mass spectrum of *N*-glycopeptide **124** did indicate that the synthesis was successful.

Future work would be to synthesise sugar-functionalised resin **120** and demonstrate that this methodology provides a route towards manipulation of the *C*-terminal of glycopeptides. Particularly useful should be the *C*-terminal *tert*-butyl protecting group **120b**.

2.4. Library Syntheses

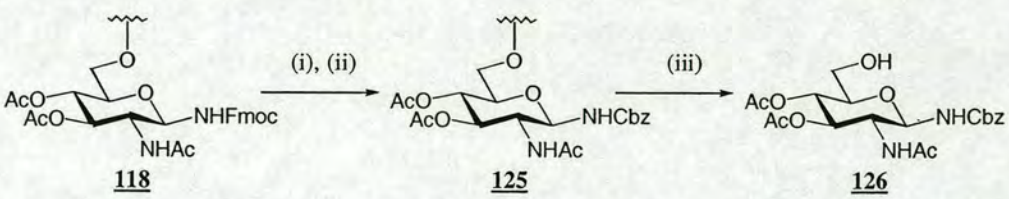
2.4.1 Introduction

The use of combinatorial chemistry provides a route towards synthesising large collections of compounds quickly and thus has the potential to speed up biological testing and identifying new drug candidates. It has been envisaged that removal of the acetate groups on sugar **118** and further elaboration of the free hydroxyl groups produced with alkylation, sulfonation, sulfation or acylation reactions, is a potential strategy to synthesise such a library.

2.4.2 Library Synthesis

Previous work in our group indicated that the acetate groups on a sugar can be successfully removed using triethylamine [87] and linker **87** was stable to the conditions adopted. It was therefore hoped that sugar-functionalised Tentagel® **118** (Scheme 46) would be treated with triethylamine to give the free hydroxyl groups which would then be used in esterification reactions to generate a small library. Before this could be performed the base labile Fmoc group had to be exchanged to a more robust protecting group. A *N*-benzyloxycarbonyl (Cbz) protecting group was the chosen amino protection, as this group is known to be relatively acid and base stable but may be cleaved by catalytic hydrogenolysis [108]. The *N*-benzyloxycarbonyl protecting group also contained a benzene ring which would aid in detection.

Sugar-functionalised Tentagel® **125** was synthesised using **118** (Scheme 46). Deprotection of the amine was first undertaken using 20 % piperidine in DMF and then the amine generated was treated with *N*-benzyloxycarbonyl succinimide and triethylamine in DMF to give **125**.

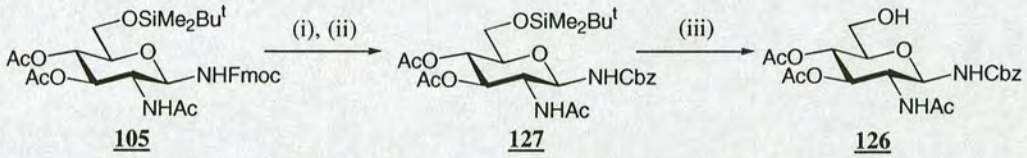


118 **125** **126**

(i) 20% piperidine in DMF, RT, 15 min; (ii) Cbz-OSu, Et_3N, DMF, RT, 16h; (iii) TFA:DCM:H_2O (9:10:1), RT, 16h.

Scheme 46: *Synthesis of sugar-functionalised Tentagel® **125**.*

Determination of the loading was achieved by comparing HPLC standards with the cleavage mixture and Scheme 47 shows the synthetic route towards the HPLC standard **126**. Sugar **105**, which was previously synthesised, was used as the starting material and first treated with 20 % piperidine in acetonitrile to remove the Fmoc group. The corresponding free amine was then treated with *N*-benzyloxycarbonyl succinimide in pyridine [109] to give **127**. The last step is removal of the silyl-protecting group which was achieved using DDQ to give the desired HPLC standard **126** in an overall yield of 73 % from saccharide **105**.



105 **127** **126**

(i) 20% piperidine in CH_3CN, RT, 5 min; (ii) Cbz-OSu, pyr, RT, 16h; (iii) DDQ, DCM:H_2O (20:1), RT, 16h.

Scheme 47: *Synthesis of HPLC standard **126**.*

Sugar-functionalised Tentagel® **125** was treated with TFA:DCM:H_2O solution and the cleavage mixture analysed by HPLC using water (plus 0.1 % TFA) and acetonitrile in a ratio of 60:40 as the solvent system. A typical trace of the cleavage mixture is shown in Figure 16. The peak at approximately 3.4 minutes corresponds to the phenylacetamide, produced by consequence of the linker, and the desired peak at 4.2 minutes was also observed. The loading of the sugar-functionalised Tentagel® **125** was determined to be approximately 0.09 mmol/g (70 % of initial loading, 0.13 mmol/g).

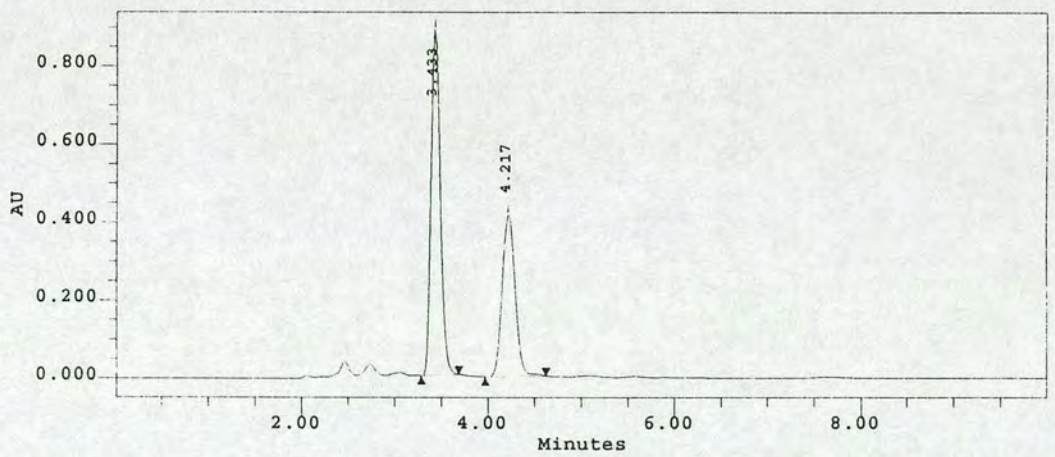
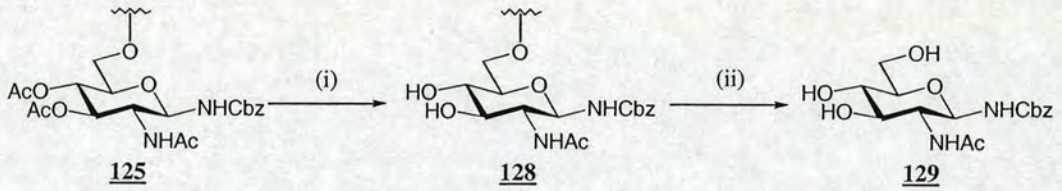


Figure 16: *HPLC trace of the cleavage mixture from **125**.*

The next stage was to remove the acetate groups on sugar-functionalised Tentagel® **125** (Scheme 46), which had previously been shown to be successful using a solution of triethylamine in methanol. As shown in Scheme 48, sugar-functionalised Tentagel® **125** was treated with a solution of triethylamine in methanol overnight and was then filtered, washed and dried to yield **128**.

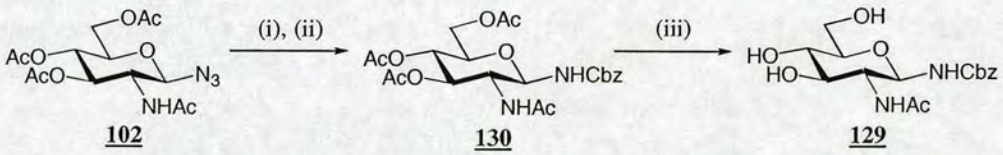


125 **128** **129**

(i) Et_3N, MeOH, RT, 16h; (ii) TFA:DCM:H_2O (9:10:1), RT, 16h.

Scheme 48: *Synthesis of sugar-functionalised Tentagel® **128**.*

Again an HPLC standard had to be synthesised in order to determine the loading of the sugar and Scheme 49 shows the synthetic route adopted towards sugar **129**. Previously synthesised sugar **102** was used as the starting material and was treated with platinum oxide under an atmosphere of hydrogen to reduce the azide to the corresponding amine. The amine produced was then treated with *N*-benzyloxycarbonyl succinimide in pyridine to give **130**, which was then treated with a solution of sodium methoxide in methanol to give the desired HPLC standard **129**.



102 **130** **129**

(i) H_2, PtO_2, THF, RT, 2h; (ii) Cbz-OSu, pyr, RT, 16h; (iii) NaOMe, MeOH, RT, 1h.

Scheme 49: *Synthesis of HPLC standard sugar **129**.*

128 which was then treated with TFA:DCM:H_2O solution and the cleavage mixture was then analysed by HPLC (Figure 17). The loading determined to be 0.04 mmol/g (44 %).

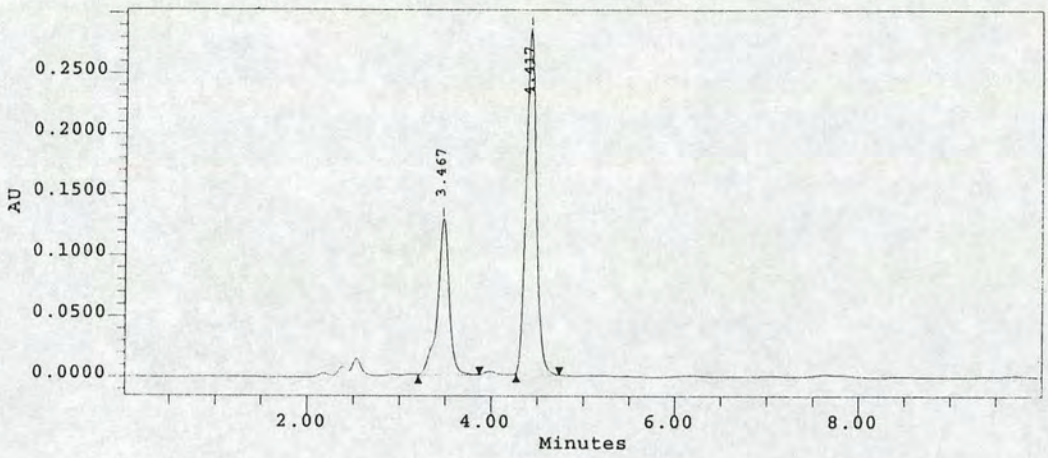
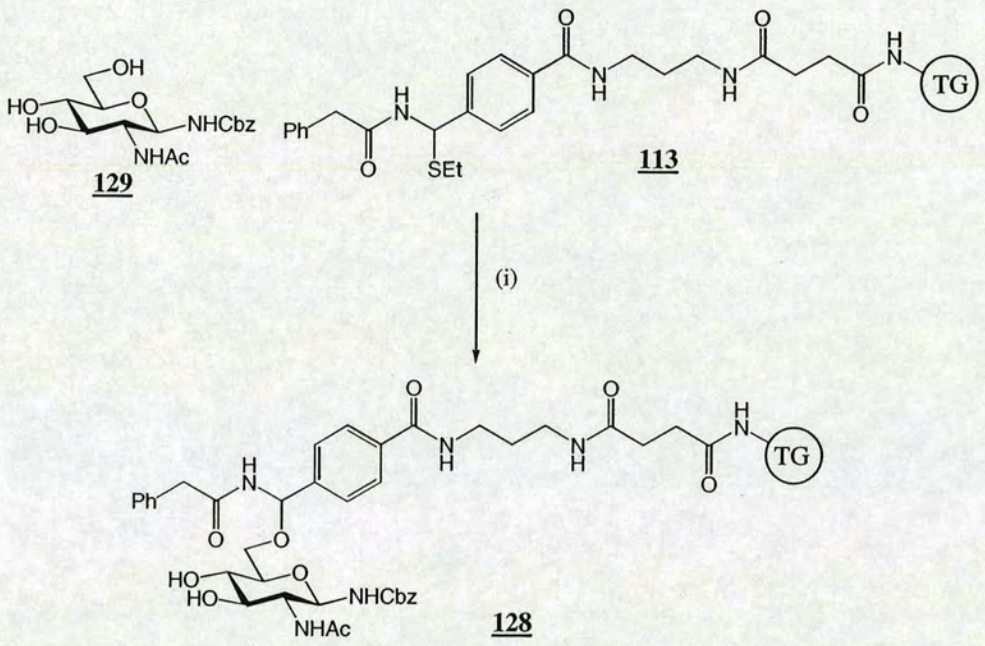


Figure 17: *HPLC trace of the cleavage mixture from* ***128***.

As the synthesis of sugar-functionalised Tentagel® **128** looked promising, the reaction was repeated several times so that sufficient material was obtained and esterification reactions could be performed. Unfortunately, during these reactions it became apparent that the reactions performed gave very erratic results and so were unreliable. Typical yields were found to be 30-50 %, which were not efficient loadings for our purposes. It was therefore decided that as an alternative route towards sugar-functionalised Tentagel® **128,** sugar **129** be coupled directly to linker-functionalised Tentagel® **113** in the hope of achieving elevated loadings. It was presumed that the free sugar would couple preferentially to the primary hydroxyl group since this has the highest availability and is the most reactive site.

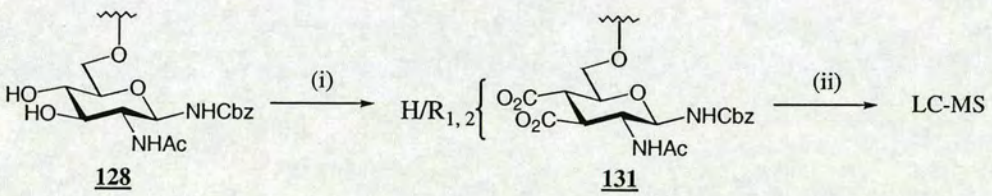
Sugar **129** was therefore coupled onto linker-functionalised Tentagel® **113** to give **128** adopting the same coupling protocol mentioned for sugar **106** and is shown in Scheme 50. THF was again used as the solvent. The loading however had to be determined by HPLC analysis of the cleavage mixture. Typical yields for this coupling were found to be 30-50 % (0.06-0.10 mmol/g), and this was sufficient to perform esterification reactions. It should be noted however, that this reaction has not been optimised due to time constraints but has the potential to give better yields than actually quoted.



(i) NIS, TfOH, THF, 45 °C, 2h.

Scheme 50: *Coupling of sugar **129** onto linker-functionalised Tentagel® **113**.*

The sugar-functionalised Tentagel® **128** generated was then used to perform esterification reactions to produce a small library of compounds. The conditions adopted have previously been successful in generating esterified libraries [110] and Scheme 51 illustrates the synthetic approach implemented towards **131**.



$R_{1,2}$	Compound
$PhCH_2$	**131a**
$MeOC_6H_4CH_2$	**131b**
$HOC_6H_4CH_2$	**131c**
$MeC_6H_4CH_2$	**131d**
$ClC_6H_4CH_2$	**131e**

(i) RCO_2H, DMAP, EDCI, DCM, RT, 16h; (ii) TFA:DCM:H_2O (9:10:1), RT, 16h.

Scheme 51: *Esterification reaction used in the synthesis of the small library.*

A solution of carboxylic acid, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI) and dimethylaminopyridine (DMAP) in DCM was first prepared and left for 10 minutes after which was added to 20-30 mg of dried resin **128** and the mixture was placed on a blood rotator overnight. The resin was then filtered, washed and dried and then treated with TFA:DCM:H_2O solution. The cleavage mixture of each reaction was analysed by LC-MS, which provided enough information to indicate if the reactions were successful. Table 5 summarises the details and results.

RCO_2H	HPLC Peaks (mins)	MS data (m/z)	Observation
$PhCH_2CO_2H$[a]	12.07	473.19, 495.31 ($M + Na^+$), 518.37 ($M + K^+$).	Monosubstituted
	4.26	135.55, 176.62 ($M + K^+$).	Phenylacetamide
	3.26	355.17, 377.30 ($M + Na^+$).	Unsubstituted
$MeOC_6H_4CH_2CO_2H$[b]	12.63	503.31, 525.30 ($M + Na^+$), 548.49 ($M + K^+$).	Monosubstituted
	4.25	135.55, 176.62 ($M + K^+$).	Phenylacetamide
	3.18	355.05, 377.18 ($M + Na^+$).	Unsubstituted
$HOC_6H_4CH_2CO_2H$[c]	6.90	624.59.	Disubstituted adduct
	5.75	489.33, 511.20 ($M + Na^+$), 534.63 ($M + K^+$).	Monosubstituted
	4.23	135.55, 176.62 ($M + K^+$).	Phenylacetamide
	3.18	355.17, 377.18 ($M + Na^+$).	Unsubstituted
$CH_3C_6H_4CH_2CO_2H$[d]	19.70	487.30, 509.29 ($M + Na^+$), 532.47 ($M + K^+$).	Monosubstituted
	4.24	135.55, 176.62 ($M + K^+$).	Phenylacetamide
	3.18	355.17, 377.18 ($M + Na^+$).	Unsubstituted
$ClC_6H_4CH_2CO_2H$[e]	4.23	135.55, 176.74 ($M + K^+$).	Phenylacetamide
	3.18	355.17, 377.18 ($M + Na^+$).	Unsubstituted

[a] see Figure 18, [b] see Figure 19, [c] see Figure 20, [d] see Figure 21, [e] see Figure 22 (see appendix A3).

Table 5: *LC-MS data*: *Reaction products of **128** with different acids RCO_2H.*

None of the reactions performed had gone to completion (i.e. diesterification) with substantial amounts of starting material being observed. It can also be concluded that for most of the reactions only the monosubstituted products were

obtained with substitution probably occurring at the 3-position as this is the more reactive hydroxyl group. Another possibility for this monosubstitution is that the 4-position hydroxyl is sterically hindered. This is only speculation and has not been proven as there was not sufficient material to obtain a ^{1}H NMR. Optimisation of these reactions was also attempted using double couplings but HPLC analysis indicated that this had a detrimental effect with loss of product. However, this work does provide a good example of the utility of LC-MS in solid-phase reactions and this methodology may be a possible route towards selective esterifications.

2.4.3 Summary and Conclusions

While initial studies have shown the potential of sugar **118**, attempts to synthesise suitable sugar-functionalised resins from **128** for library synthesis have proved problematic. This is thought to be due to the linker instability. As an alternative route, sugar **129** was synthesised from *N*-acetylglucosamine in 5 steps. The saccharide was attached onto carboxy-Tentagel® *via* linker **87** although the coupling has not been optimised. Resin bound **128** was then used in initial studies into esterification reactions by means of a small library. The material generated on solid-support was cleaved off and analysed by LC-MS. Initial work has shown that esterifications on sugar-functionalised Tentagel® **128** were possible although were not high yielding. It has also been observed that the method adopted only produced monoesterified products which may prove significant.

Work for the future would be to optimise the coupling of sugar **129** to linker-functionalised Tentagel® **113** and determine fully the point of attachment onto the linker. Further investigation into the esterification reaction would also be undertaken as to establish if the reaction is selective with other carboxylic acids.

Chapter Three; Experimental

3.1. General Experimental

3.1.1 Instrumentation

^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker AC200, Bruker AC250, Varian Gemini 200 and Varian Inova 600 instruments.

Fast atom bombardment (FAB) mass spectroscopy was performed using a Kratos MS50TC instrument and electron impact (EI) mass spectroscopy was carried out on a Finnigan 4500 instrument. Electrospray (ESI) and atmospheric pressure chemical ionisation (APCI) mass spectrometry was carried out on a Micromass Platform II instrument.

Infrared spectroscopy was performed on a Perkin Elmer Paragon 1000 FT-IR spectrometer.

UV/Visible spectroscopy was performed on either a Hewlett Packard 8453 or Unicam UV2-100 UV/Visible spectrometer.

Optical rotations were performed on an Optical Activity Ltd. Polarimeter.

Elemental analysis was carried out using a Perkin Elmer 2400 CHN Elemental Analyser.

Melting points were measured on a Gallenkamp melting point apparatus and were uncorrected.

A Stuart Scientific SB1 360° blood rotator or a New Brunswick Scientific G76 gyrotary shaker was used to agitate solid supported samples at room temperature.

3.1.2 Solvents and Reagents

All reagents were standard laboratory grade and used as supplied unless otherwise stated. Where a solvent is described as dry either it was purchased as anhydrous grade or was distilled prior to use.

Carboxy-Tentagel® resin and carboxy-polystyrene resin were purchased from NovaBiochem and amino acids were purchased from Bachem.

3.1.3 Chromatography

Analytical TLC was performed on aluminium-backed plates coated with silica gel $60F_{254}$ (Merck: layer thickness of 0.2 mm). For flash chromatography, silica gel C60 (Merck: 40-60 μm) was used with a variety of different sized columns. The components were identified by UV light (254 nm); a solution of 1 % sulphuric acid, 2 % *p*-anisaldehyde in ethanol was used for sugar derivatives; a solution of 0.3 % ninhydrin, 3 % acetic acid in butan-1-ol was used for amino derivatives.

3.1.4 High Performance Liquid Chromatography (HPLC)

The HPLC system used consisted of a Waters 486 Tuneable Absorbance Detector and a Waters 600E Pump and Controller together with the Waters Millennium Chromatography manager. The column employed was a Phenomenex® Sphereclone RP-18 (5 μm particle size) with dimensions 25 cm by 4.6 mm. A Spherisorb ODS 2 (5 μm particle size) pre-column of length 1 cm was also used. Samples were injected via either a 20 μl loop (analytical) or 1000 μl (preparation) and a flow rate of 1 ml/min was used for elution. A wavelength of 215 nm was used for sample detection.

Phenylacetic acid and phenylacetamide were initially monitored using a gradient elution (Method 1, Table 6). Four standards were run for phenylacetic acid and phenylacetamide and a calibration curve constructed for each.

This method was later replaced by an isocratic system (Method 2; 0.1 % TFA in H_2O: MeCN [70:30] for 15 min). Analysis of sugar derivatives was performed using either Method 2 or Method 3, 0.1 % TFA in H_2O: MeCN [60:40] for 10 min. Method 3 was used for the purification of glycopeptide **124**. When using the isocratic systems two standards were run before and one after for each compound and a standard concordancy test was carried out.

Mins	25mM, pH6.5 buffer sol^{n}	Acetonitrile
0	100	0
15	85	15
20-30	60	40
40-55	100	0

Table 6: *Method 1, HPLC gradient elution.*

3.2. General Protocols

3.2.1 Resin washing protocol

A wash cycle for *c.a.* 20-50 mg of resin typically consisted of THF (5-10 ml x 2), DMF (5-10 ml x 2), DMF:MeOH (1:1) (5-10 ml x 2), DMF (5-10 ml x 2), THF (5-10 ml x 2) and DCM (5-10 ml x 2).

3.2.2 Fmoc analysis

Dry resin (c.a. 5-15 mg) was accurately weighed in a 5 or 10 ml volumetric flask. Freshly prepared 20 % piperidine in DMF solution was added up to the mark and the mixture sonicated for 10 min. The supernatant was transferred to a UV cell and the absorbance at λ = 301 nm recorded. The loading was then calculated using Equation 1 derived from the Beer-Lambert Law using the extinction coefficient (ε @ 301 nm = 7800 M^{-1} cm^{-1}) quoted by Sabatier *et al* [111].

Equation 1: $L = A \times V/7.8 \times m$,

Where: L = loading of Fmoc in mmol/g resin

A = absorbance at λ = 301 nm

V = volume in ml

m = mass of resin in mg

3.2.3 IR Spectroscopy

Where an IR spectrum of the resin has been taken several drops of DCM have been added to facilitate swelling and the resin placed between two NaCl plates after which the spectra are run as normal.

3.2.4 Gel Phase ^{13}C NMR

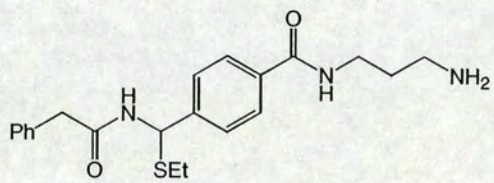
Gel Phase ^{13}C NMR were carried out by swelling the resin in DCM-D_2. Where ^{13}C NMR data is quoted in the experimental section, diagnostic peaks have been assigned sometimes with the aid of relevant solution spectra. These peaks may also have been observed in the presence of other peaks that were not assigned and may be due to the resin impurities generated by reaction on solid support or the desired compound itself.

3.2.5 Cleavage Protocol for Compounds Synthesised on Solid Support

Dry resin (c.a. 5-15 mg) was accurately weighed out and treated with a solution of TFA:DCM:H_2O [9:10:1] (0.5-1.5 ml) and the mixture was left for 16 h after which the resin was filtered off and washed with DCM (2 ml x 2) or successively with DCM (2 ml), MeOH:DCM 1:1 (2 ml) and DCM (2 ml). The filtrate and washings were combined and the solvent was removed *in vacuo* to dryness.

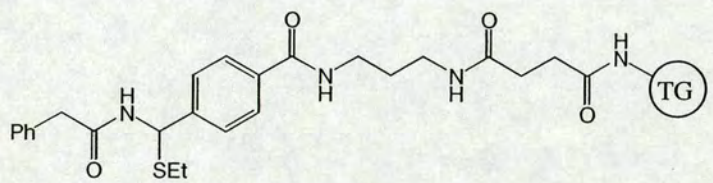
3.3. Nomenclature of Resin Bound Compounds [112]

When naming compounds in accordance with IUPAC recommendations it is necessary to determine the characteristic group to be cited as a suffix or as a functional class name. For example, in the case of **87** the amide functional group takes precedence over the thioether and amine functional groups. The amide is therefore the characteristic group and the other groups are substituents that are described using prefixes in alphabetical order. Hence, **87** is called *N*-({[4-(3-aminopropylcarbamoyl) phenyl] ethylsulphanyl}methyl)-2-phenylacetamide.



87

When naming resin bound compounds the resin has been considered to be the functional class name. For example, 4-{[ethylsulphanyl-*N*-(2-phenylacetyl)] aminomethyl}-benzamidopropylamido Tentagel® **113**.



113

3.4. Calculations of Yields for Resin Bound Compounds

3.4.1 Calculation of the loading for the attachment of **87** *to carboxy resin*

The residue collected after the cleavage protocol was dissolved up in the HPLC solvent (method 2) and injected into the HPLC. The area of the peak obtained is compared to standard phenylacetamide sample areas and the loading is calculated (see appendix A1 for example calculation).

3.4.2 Calculation of the loading for the attachment of sugar **106** *and amino acids*

The loading was determined using Fmoc analysis (see section 5.1.2) and was calculated directly using equation 1 (see appendix A2 for example calculation).

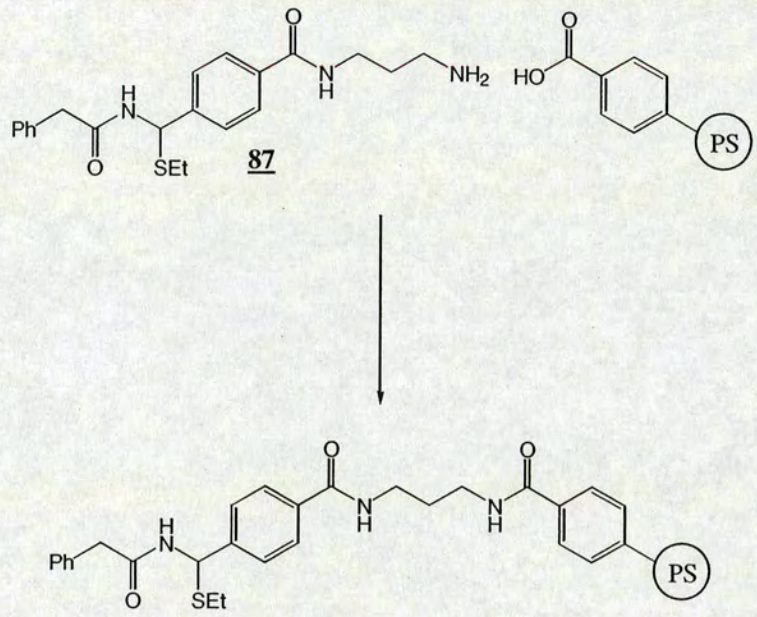
3.4.3 Calculation of the loading for the generation of sugar **125** *and* **128** *on Tentagel®*

The residue collected after the cleavage protocol was dissolved in the HPLC solvent (method 2 for **125** and method 3 for **128**) and injected into the HPLC. The area of the peak obtained is compared to areas of **126** and **129** standards and the loading is calculated (see appendix A1 for example calculation).

3.4.4 Calculation of weight gain or loss

When calculating the yield for resin bound compounds the weight increase or loss incurred during the reaction is taken into account, however this is more significant for polystyrene than Tentagel®, but is carried out nonetheless. For example, in the case of coupling **87** to carboxy-polystyrene (Scheme 53), the loading of the carboxy functionality on carboxy-polystyrene was typically 0.98 mmol/g. If complete coupling is achieved 1 g of resin will increase in weight by (0.98×10^{-3} x molecular weight of linker **87** – weight of 1 x O and 2 x H =) 0.36 g. The loading of linker would therefore be (1/1.36) x 0.98 = 0.72 mmol/g. If cleavage from the resin

indicates a loading of 0.68 mmol/g then (0.68/0.72 x 100) % coupling has been achieved.



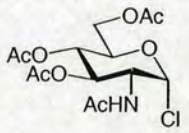
Scheme 53: *Coupling of* ***87*** *to carboxy-polystyrene.*

3.5. Safety Notice

Please note "many compounds of both organic and inorganic derivation, which contain the azide function, are unstable or explosive under appropriate conditions of initiation" [113]. Appropriate precautions should be taken when handling such compounds. Organic azides synthesised in this project were reduced using PPh_3/H_2O in THF prior to disposal.

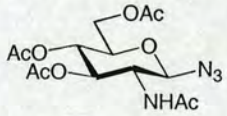
3.6. Synthesis of Sugar Building Block 106

3.6.1 2-Acetamido-3, 4, 6-tri-O-acetyl-2-deoxy-α-D-glucopyranosyl chloride **101** [88]



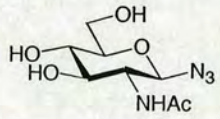
Dried 2-acetamido-2-deoxy-α-D-glucopyranose (10.09g, 45.6 mmol) was added gradually to acetyl chloride (20 ml, 281.3 mmol) with good stirring and the mixture stirred at RT for 16 h after which chloroform (80 ml) was added and the solution poured with vigorous stirring onto ice (80 g) and water (20 ml). The organic layer was treated with ice-cold saturated sodium bicarbonate solution (80 ml) and the mixture separated. The organic layer was dried over anhydrous $MgSO_4$, filtered, concentrated in *vacuo* and dry diethyl ether (100 ml) added. After 12 h the solid was collected, washed with dry diethyl ether (30 ml x 2) and dried under suction for 5 min to yield the crude product. The solid was purified using flash chromatography (EtOAc) to yield *title compound* **101** (7.61 g, 46 %) as a white solid, mp 124-126 °C (Lit. mp 127-128 °C [88]) (Found: C, 46.22; H, 5.62; N, 3.68. $C_{14}H_{20}ClNO_8$ requires C, 45.97; H, 5.51; N, 3.83 %); $[\alpha]_D$ +114.6 (c 1.02, $CHCl_3$) (Lit. $[\alpha]_D$ +109.7-118.0° [88]); υ_{max} (Nujol)/cm^{-1} 3241.7 (NH), 1741.2 (CO), 1642.9 (NH*CO*), 723.1 (CCl); δ_H (250 MHz; $CDCl_3$) 6.16 (1H, d, *J* 3.7, 1-H), 5.94 (1H, d, *J* 8.8, NH), 5.31 (1H, dd, *J* 9.4 and 10.1, 3-H), 5.18 (1H, dd, *J* 9.4 and 9.7, 4-H), 4.51 (1H, ddd, *J* 3.7 8.8 and 10.1, 2-H), 4.25 (2H, m. 5-H, 6a-H), 4.09 (1H, dd, *J* 3.5 and 13.6, 6b-H), 2.07 (3H, s, OCOCH_3), 2.02 (6H, s, OCOCH_3), 1.96 (3H, s, NHCOCH_3); δ_C (63 MHz) 171.3 170.4 170.0 169.0 (CO), 93.5 (C1), 70.7 (C5), 69.9 (C3), 66.8 (C4), 61.0 (C6), 53.2 (C2), 22.9 (NHCO*C*H_3), 20.5 20.4 (OCO*C*H_3); m/z (FAB) 368 (MNa^+, 14.4 %), 366 (MH^+, 21.2 %), 330 (MH^+-Cl, 22.8 %) (Found: MH^+ 366.09510. $C_{13}H_{21}ClNO_8$ requires 366.09557).

3.6.2 2-Acetamido-3, 4, 6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl azide **102** [90]



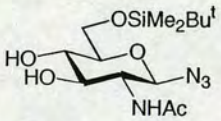
To a solution of **101** (5.08 g, 13.9 mmol), tetrabutylammonium hydrogen sulphate (4.72 g, 13.9 mmol) and sodium azide (3.61 g, 55.5 mmol) in DCM (50 ml) was added saturated sodium bicarbonate solution (50 ml). The two-phase mixture was stirred vigorously at RT for 2 h after which TLC (EtOAc) indicated complete transformation of the starting material. Ethyl acetate (500 ml) was added, the organic phase separated and successively washed with saturated sodium bicarbonate solution (50 ml), water (50 ml x 2) and brine (50 ml). The combined organic extracts were dried over anhydrous Na_2SO_4, filtered and evaporated under reduced pressure to yield the crude product. The solid was recrystallised using chloroform and hexane to afford *the title compound* **102** (4.65 g, 90 %) as a white solid, mp 165-166 °C (Lit. mp 166-167 °C [90]) (Found: C, 45.40; H, 5.51; N, 14.90. $C_{14}H_{21}N_4O_8$ requires C, 45.16; H, 5.41; N, 15.05 %); $[\alpha]_D$ –40.0 (c 1.09, $CHCl_3$) (Lit. $[\alpha]_D$ –43.0° [90]); υ_{max} (Nujol)/cm^{-1} 3275.3 (NH), 2127.7 (N_3), 1748.6 (CO), 1657.0 (NH*CO*); δ_H (250 MHz; $CDCl_3$) 6.12 (1H, d, *J* 9.0, NH), 5.25 (1H, dd, *J* 9.4 and 10.5, 3-H), 5.08 (1H, dd, *J* 9.4 and 10.0, 4-H), 4.78 (1H, d, *J* 9.2, 1-H), 4.25 (1H, dd, *J* 4.8 and 12.4, 6b-H), 4.13 (1H, dd, *J* 2.4 and 12.4, 6a-H), 3.90 (1H, ddd, *J* 9.0 9.2 and 10.5, 2-H), 3.80 (1H, ddd, *J* 2.4 4.8 and 10.0, 5-H), 2.07 (3H, s, OCOC*H*$_3$), 2.01 (3H, s, OCOC*H*$_3$), 2.00 (3H, s, OCOC*H*$_3$), 1.95 (3H, s, NHCOC*H*$_3$); δ_C (63 MHz) 170.8 170.6 170.5 169.2 (CO), 88.2 (C1), 73.7 (C5), 72.0 (C3), 68.0 (C4), 61.7 (C6), 53.9 (C2), 23.1 (NHCOC*H*$_3$), 20.6 20.5 20.4 (OCOC*H*$_3$); m/z (FAB) 373 (MH^+, 31.4 %), 330 (MH^+-N_3, 34.1 %), 150 (MH^+-N_3-OAc x 3, 46.0 %), 43 (Ac, 86.8 %) (Found: MH^+ 373.13475. $C_{14}H_{22}N_4O_8$ requires 373.13594).

3.6.3 2-Acetamido-2-deoxy-β-D-glucopyranosyl azide **103**



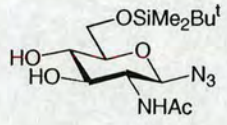
To a solution of **102** (4.51 g, 12.1 mmol) in dry methanol (40 ml) was added sodium methoxide in methanol (0.5 M, 7 ml) and the mixture stirred at RT for 1 h after which TLC ($CHCl_3$:MeOH:H_2O [3:1:1]) indicated complete transformation of the starting material. The reaction mixture was filtered through cation ion-exchange resin and the methanol evaporated in *vacuo* to yield the crude product. The solid was recrystallised using acetone to afford *the title compound* **103** (2.75 g, 92 %) as a white solid, mp 145-147 °C (Found: C, 36.64; H, 5.80; N, 21.04. $C_8H_{14}N_4O_5.H_2O$ requires C, 36.36; H, 6.10; N, 21.20 %); $[\alpha]_D$ –41.5 (c 1.01, MeOH); υ_{max} (Nujol)/cm^{-1} 3280.5 (NH OH), 2116.4 (N_3), 1640.0 (NH*CO*); δ_H (250 MHz; CD_3OD) 4.60 (1H, d, *J* 9.2, 1-H), 4.02 (1H, dd, *J* 1.8 and 12.1, 6a-H), 3.80 (1H, dd, *J* 5.5 and 12.1, 6b-H), 3.77 (1H, dd, *J* 9.2 and 10.1, 2-H), 3.47-3.38 (3H, m, 3-H 4-H 5-H), 2.09 (3H, s, NHCOC*H*$_3$); δ_C (63 MHz) 169.6 (CO), 88.6 (C1), 79.5 (C5), 74.0 (C3), 70.2 (C4), 60.9 (C6), 54.9 (C2), 23.1 (NHCO*C*H$_3$); m/z (FAB) 247 (MH^+, 100.0 %), 204 (MH^+-N_3, 50.6 %), 186 (MH^+-N_3-H_2O, 43.6 %) (Found: MH^+ 247.10524. $C_8H_{15}N_4O_5$ requires 247.10424).

*3.6.4 2-Acetamido-6-*tert-O*-butyldimethylsilyl-2-deoxy-β-D-glucopyranosyl azide* **104**



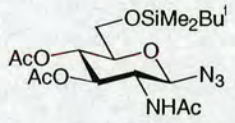
To a solution of **103** (0.10 g, 0.4 mmol) and *tert*-butyldimethylsilyl chloride (0.08 g, 0.5 mmol) in pyridine (3 ml) was added imidazole (0.05 g, 0.8 mmol) and the mixture left stirring for 18 h after which TLC (EtOAc:MeOH [9:1]) indicated no transformation of starting material. DMAP (0.05 g, 0.4 mmol) was then added and the mixture was left for a further 16 h after which TLC indicated no reaction.

*3.6.5 2-Acetamido-6-*O*-tert-butyldimethylsilyl-2-deoxy-*β*-D-glucopyranosyl azide* **104**



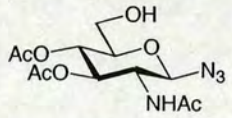
A mixture of **103** (2.30 g, 9.3 mmol), *tert*-butyldimethylsilyl chloride (2.80 g, 18.6 mmol), triethylamine (2.6 ml, 18.6 mmol), and DMAP (0.68 g, 5.6 mmol) in acetonitrile (46 ml) was heated to 60 °C for 3 h after which TLC (EtOAc) indicated all the starting material was consumed. Water (100 ml) was then added and the product extracted using chloroform (100 ml x 3). The organic layer was dried over anhydrous $MgSO_4$ and evaporated in *vacuo* to yield yellow oil. The product was purified using flash-column chromatography (EtOAc:MeOH [9:1]) to yield *the title compound* **104** as a white solid (2.65 g, 79 %), mp 141-142 °C (Found: C, 46.46; H, 8.06; N, 15.25. $C_{14}H_{28}N_4O_5Si$ requires C, 46.65; H, 7.83; N, 15.54 %); $[\alpha]_D$ –53.3 (c 1.04, $CHCl_3$); υ_{max} (Nujol)/cm^{-1} 3276.0 (NH OH), 2116.8 (N_3), 1659.8 (NH*CO*), 1075.2 779.0 (SiO), 837.1 ($Si(CH_3)_2$); δ_H (250 MHz; $CDCl_3$) 7.06 (1H, d, *J* 7.5, NH), 5.19 (1H, d, *J* 3.4, 3-OH), 4.60 (1H, d, *J* 8.8, 1-H), 4.37 (1H, d, *J* 3.4, 4-OH), 3.92 (1H, dd, *J* 4.0 and 11.5, 6a-H), 3.85 (1H, dd, *J* 4.5 and 11.5, 6b-H), 3.61 (2H, m, 2-H 3-H), 3.42 (2H, m, 4-H 5-H), 2.02 (3H, s, $NHCOCH_3$), 0.89 (9H, s, $C(CH_3)_3$), 0.09 (6H, s, $Si(CH_3)_2$); δ_C (63 MHz) 172.3 (CO), 88.2 (C1), 77.3 (C5), 74.3 (C3), 71.6 (C4), 63.5 (C6), 55.4 (C2), 25.7 ($C(CH_3)_3$), 23.2 ($NHCOCH_3$), 18.2 ($C(CH_3)_3$), -5.4 ($Si(CH_3)_2$); m/z (FAB) 361 (MH^+, 95.4 %), 318 (MH^+-N_3, 75.4 %), 187 (MH^+-N_3-OTBDMS, 37.6 %), 131 ($OTBDMS^+$, 21.7 %), 115 ($TBDMS^+$, 79.3 %), 42 (N_3, 81.1 %) (Found: MH^+ 361.19145. $C_{14}H_{29}N_4O_5Si$ requires 361.19072).

*3.6.6 2-Acetamido-3, 4-di-*O*-acetyl-6-*O*-*tert*-butyldimethylsilyl-2-deoxy-β-D-glucopyranosyl azide* **100**



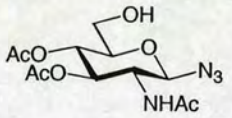
To a cooled solution of **104** (3.14 g, 8.7 mmol) in pyridine (70 ml, 870 mmol) was added drop wise acetic anhydride (82 ml, 870 mmol). After 1 h the mixture was allowed to warm to RT and the mixture left stirring for 16 h after which TLC (EtOAc) indicated complete transformation of starting material. The reaction mixture was then poured onto ice (200 g) and chloroform (500 ml) and separated. The organic layer was washed with saturated sodium bicarbonate solution (150 ml x 3) and water (200 ml x 3), dried over anhydrous $MgSO_4$ and the solvent azeotroped with toluene in *vacuo* to yield the crude product. The product was purified using flash-column chromatography (EtOAc:Hex [7:3]) to yield *the title compound* **100** (3.79 g, 98 %) as a white solid, mp 175-176 °C (Found: C, 48.93; H, 7.52; N, 12.35. $C_{18}H_{32}N_4O_7Si$ requires C, 48.63; H, 7.26; N, 12.60 %); $[\alpha]_D$ –30.0 (c 1.04, $CHCl_3$); υ_{max} (Nujol)/cm^{-1} 3261.3 (NH), 2118.5 (N_3), 1750.2 (CO), 1656.6 (NH*CO*), 1037.5 779.6 (SiO), 838.2 ($Si(CH_3)_2$); δ_H (250 MHz; $CDCl_3$) 5.99 (1H, d, *J* 9.1, NH), 5.19 (1H, dd, *J* 9.4 and 9.4, 3-H), 5.04 (1H, dd, *J* 9.4 and 9.5, 4-H), 4.64 (1H, d, *J* 9.3, 1-H), 3.92 (1H, ddd, *J* 9.1 9.3 and 9.4, 2-H), 3.73 (1H, dd, *J* 2.6 and 11.5, 6a-H), 3.70 (1H, dd, *J* 4.7 and 11.5, 6b-H), 3.61 (1H, ddd, *J* 2.6 4.7 and 9.5, 5-H), 2.01 (6H, s, $OCOCH_3$), 1.99 (3H, s, $OCOCH_3$), 1.95 (3H, s, $NHCOCH_3$), 0.86 (9H, s, $C(CH_3)_3$), 0.03 (6H, s, $Si(CH_3)_2$); δ_C (63 MHz) 171.1 170.4 169.0 (CO), 88.2 (C1), 76.6 (C5), 72.5 (C3), 68.4 (C4), 62.1 (C6), 53.8 (C2), 25.6 ($C(CH_3)_3$), 23.1 ($NHCOCH_3$), 20.5 ($OCOCH_3$), 18.2 ($C(CH_3)_3$), -5.5 ($Si(CH_3)_2$); m/z (FAB) 446 (MH^+, 16.9 %), 402 (MH^+-N_3, 69.1 %), 282 (MH^+-N_3-OAc x 2, 56.0 %), 115 ($TBDMS^+$, 78.2 %), 43 (Ac, 100 %), 42 (N_3, 10.5 %) (Found: MH^+ 445.21040. $C_{18}H_{33}N_4O_7Si$ requires 445.21185).

3.6.7 2-Acetamido-3, 4-di-O-acetyl-2-deoxy-β-D-glucopyranosyl azide **98**



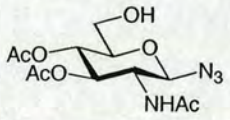
To a stirred solution of **100** (0.10 g, 0.2 mmol) in dry THF (2 ml) was added drop wise TBAF in THF (1 M, 0.5 ml) and the mixture was left stirring at RT for 2 h after which TLC (EtOAc) indicated many spots forming.

3.6.8 2-Acetamido-3, 4-di-O-acetyl-2-deoxy-β-D-glucopyranosyl azide **98**



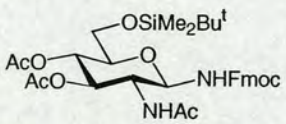
To **100** (90.0 mg, 0.2 mmol) was added a solution of AcOH:THF:H_2O [3:1:1] (3 ml) and the mixture left stirring at RT for 2 h after which TLC (EtOAc) indicated complete transformation of the starting material. Water (20 ml) was then added and the product extracted using chloroform (20 ml x 3). The combined organic extracts were washed with saturated sodium bicarbonate solution (10 ml x 2) and water (10 ml x 2), dried over anhydrous $MgSO_4$ and concentrated to give an oil which was purified using flash-column chromatography (EtOAc:Hex [9:1]) to yield a white solid which was identified as 2-acetamido-3, 6-di-O-acetyl-2-deoxy-β-D-glucopyranosyl azide (0.05 g, 78 %); δ_H (250 MHz; $CDCl_3$ CD_3OD) 5.10 (1H, dd, *J* 9.4 and 9.7, 3-H), 4.81 (1H, d, *J* 9.4, 1-H), 4.54 (1H, dd, *J* 2.0 and 12.1, 6a-H), 4.35 (1H, dd, *J* 5.3 and 12.1, 6b-H), 3.88 (1H, dd, *J* 9.4 and 9.4, 2-H), 3.77 (1H, m, 5-H), 3.65 (1H, dd, *J* 9.7 and 10.0, 4-H), 2.11 (6H, s, OCOCH_3), 2.07 (3H, s, OCOCH_3), 2.02 (3H, s, NHCOCH_3); δ_C (63 MHz) 171.8 171.7 170.7 (CO), 88.4 (C1), 75.9 (C5), 74.6 (C3), 68.4 (C4), 62.9 (C6), 53.7 (C2), 23.1 (NHCOCH_3), 20.8 20.7 20.5 (O$COCH_3$).

*3.6.9 2-Acetamido-3, 4-di-*O*-acetyl-2-deoxy-*β*-D-glucopyranosyl azide* **98**



To a stirred solution of **100** (88.9 mg, 200.0 μmol) in 10 % water in acetonitrile (1 ml) was added a solution of DDQ (4.5 mg, 19.8 μmol) in 10 % water in acetonitrile (1 ml) and the mixture was stirred at RT for 16 h after which TLC (EtOAc) indicated that all the starting material had been consumed. The solvent was then removed in *vacuo* to yield the crude product and was purified using flash-column chromatography (EtOAc) to yield white solid which was identified as 2-acetamido-3, 6-di-O-acetyl-2-deoxy-β-D-glucopyranosyl azide (59.8 mg, 83 %); δ_H (250 MHz; $CDCl_3$ CD_3OD) 5.10 (1H, dd, *J* 9.4 and 9.7, 3-H), 4.81 (1H, d, *J* 9.4, 1-H), 4.54 (1H, dd, *J* 2.0 and 12.1, 6a-H), 4.35 (1H, dd, *J* 5.3 and 12.1, 6b-H), 3.88 (1H, dd, *J* 9.4 and 9.4, 2-H), 3.77 (1H, m, 5-H), 3.65 (1H, dd, *J* 9.7 and 10.0, 4-H), 2.11 (6H, s, $OCOCH_3$), 2.07 (3H, s, $OCOCH_3$), 2.02 (3H, s, $NHCOCH_3$); δ_C (63 MHz) 171.8 171.7 170.7 (CO), 88.4 (C1), 75.9 (C5), 74.6 (C3), 68.4 (C4), 62.9 (C6), 53.7 (C2), 23.1 ($NHCOCH_3$), 20.8 20.7 20.5 ($OCOCH_3$).

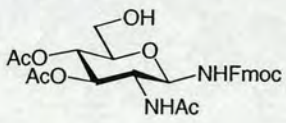
*3.6.10 2-Acetamido-3, 4-di-*O*-acetyl-6-*O*-tert-butyldimethylsilyl-2-deoxy-*N*-(fluoren-9-yl-methoxycarbonyl)-*β*-D-glucopyranosylamine* **105**



Under an atmosphere of hydrogen, **100** (2.76 g, 6.2 mmol), platinum (IV) oxide (0.23 g, 1.0 mmol) and dry THF (65 ml) were stirred vigorously at RT for 2 h after which TLC (EtOAc) indicated that all the starting material had been consumed and the reaction mixture was concentrated in *vacuo* to yield yellow oil that contained platinum oxide. This crude amino sugar mixture and *N*-(9-fluorenylmethoxy carbonyloxy) succinimide (2.50 g, 7.4 mmol) were then dissolved in anhydrous

pyridine (40 ml) and the reaction was stirred at RT for 16 h after which TLC (EtOAc) indicated complete transformation of the amino starting material. The solvent was azeotroped with toluene in *vacuo* and the product purified using flash-column chromatography ($CHCl_3$:EtOAc [8:2]) to yield *the title compound* **105** (3.44 g, 87 %) as a white solid, mp 182-183 °C (Found: C, 61.56; H, 6.85; N, 4.32. $C_{33}H_{44}N_2O_9Si$ requires C, 61.85; H, 6.92; N, 4.37 %); $[\alpha]_D$ –6.3 (c 1.03, $CHCl_3$); υ_{max} (Nujol)/cm^{-1} 3306.0 (NH), 1745.0 (CO), 1703.0 (NH*CO*O), 1658.6 (NH*CO*), 1047.3 740.2 (SiO), 838.4 ($Si(CH_3)_2$); δ_H (250 MHz; $CDCl_3$) 7.74 (2H, d, *J* 7.3, H_{ar}), 7.55 (2H, d, *J* 7.3, H_{ar}), 7.37 (2H, dd, *J* 7.3, H_{ar}), 7.28 (2H, dd, *J* 7.3, H_{ar}), 6.37 (1H, d, *J* 8.9, N*H*Fmoc), 6.17 (1H, d, *J* 7.4, N*H*Ac), 5.13 (1H, dd, *J* 9.5 and 9.6, 3-H), 5.07 (1H, dd, *J* 9.5 and 9.6, 4-H), 4.88 (1H, dd, *J* 8.9 and 9.5, 1-H), 4.29 (1H, t, *J* 8.0, C*H*CH_2O), 4.19 (2H, d, *J* 8.0, CHC*H*$_2$O), 4.14 (1H, m, 2-H), 3.76 (1H, dd, *J* 2.5 and 11.3, 6a-H), 3.66 (1H, dd, *J* 3.8 and 11.3, 6b-H), 3.59 (1H, m, 5-H), 2.06 (3H, s, OCOC*H*$_3$), 2.01 (3H, s, OCOC*H*$_3$), 1.95 (3H, s, NHCOC*H*$_3$), 0.86 (9H, s, C(C*H*$_3$)$_3$), 0.02 (6H, s, Si(C*H*$_3$)$_2$); δ_C (63 MHz) 171.9 171.5 169.0 155.8 (CO), 143.7 143.4 141.1 (C_{ar}), 127.6 127.0 125.0 119.8 (CH_{ar}), 82.4 (C1), 75.8 (C5), 73.4 (C3), 68.4 (C4), 67.4 (CH*C*H$_2$O), 62.2 (C6), 53.1 (*C*HCH$_2$O), 46.7 (C2), 25.7 (C(*C*H$_3$)$_3$), 23.0 (NHCO*C*H$_3$), 20.6 (OCO*C*H$_3$), 18.2 (*C*(CH$_3$)$_3$), -5.5 (Si(*C*H$_3$)$_2$); m/z (APCI+) 641 (MH^+, 100.0 %), 419 (MH^+-Fmoc, 84.2 %), 402 (MH^+-NHFmoc, 80.4 %), 281.3 (MH^+-NHFmoc-OTBDMS, 74.0%); m/z (FAB) 641 (MH^+, 1.3 %) (Found: MH^+ 641.28788. $C_{33}H_{44}N_2O_9Si$ requires 641.28944).

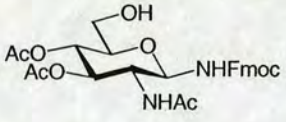
*3.6.11 2-Acetamido-3, 4-di-*O*-acetyl-2-deoxy-*N*-(fluoren-9-yl-methoxycarbonyl)-*β*-D-glucopyranosylamine* **106**



To **105** (0.26 g, 0.4 mmol) was added a solution of AcOH:THF:H_2O [3:1:1] (10 ml) and the mixture left stirring at RT for up to 96 h until TLC (EtOAc) indicated complete transformation of the starting material. Water (50 ml) was then added and the product extracted using chloroform (50 ml x 3). The combined organic extracts

were washed with saturated sodium bicarbonate solution (50 ml x 2) and water (50 ml x 2), dried over anhydrous $MgSO_4$ and concentrated to give the crude product which was purified using flash-column chromatography (EtOAc:Hex [9:1]) to yield a white solid (0.19 g, 91 %); δ_H (250 MHz; $CDCl_3$ CD_3OD) 7.64 (2H, d, *J* 7.4, H_{ar}), 7.46 (2H, d, *J* 7.4, H_{ar}), 7.28 (2H, dd, *J* 7.4, H_{ar}), 7.19 (2H, dd, *J* 7.4, H_{ar}), 5.06 (1H, dd, *J* 9.5 and 9.6, 3-H), 4.93 (1H, dd, *J* 9.3 and 9.5, 4-H), 4.82 (1H, d, *J* 9.8, 1-H), 4.23 (2H, d, *J* 7.5, $CHCH_2O$), 4.11 (1H, t, *J* 7.5, $CHCH_2O$), 4.03 (1H, m, 2-H), 3.53 (3H, m, 5-H 6a-H 6b-H), 1.94 (6H, s, $OCOCH_3$), 1.84 (3H, s, $NHCOCH_3$).

*3.6.12 2-Acetamido-3, 4-di-*O*-acetyl-2-deoxy-*N*-(fluoren-9-yl-methoxycarbonyl)-*β*-D-glucopyranosylamine* **106**

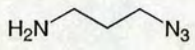


To a stirred solution of **105** (1.35 g, 2.1 mmol) in 10 % water in acetonitrile (16 ml) was added a solution of DDQ (0.05 g, 0.2 mmol) in 10 % water in acetonitrile (2 ml) and the mixture was stirred at RT for up to 48 h until TLC (EtOAc) indicated that all the starting material had been consumed. The solvent was then removed in *vacuo* to yield the crude product and was purified using flash-column chromatography ($CHCl_3$:MeOH [98:2]) to yield *the title compound* **106** (0.94 g, 85 %) as a white solid, mp 227-228 °C (Found: C, 61.56; H, 5.90; N, 4.95. $C_{27}H_{30}N_2O_9$ requires C, 61.59; H, 5.74; N, 5.23 %); $[\alpha]_D$ +6.8 (c 1.01, DMSO); υ_{max} (Nujol)/cm^{-1} 3313.8 (NH OH), 1744.2 (CO), 1707.2 ($NHCOO$), 1659.7 ($NHCO$); δ_H (250 MHz; $CDCl_3$ CD_3OD) 7.64 (2H, d, *J* 7.4, H_{ar}), 7.46 (2H, d, *J* 7.4, H_{ar}), 7.28 (2H, dd, *J* 7.4, H_{ar}), 7.19 (2H, dd, *J* 7.4, H_{ar}), 5.06 (1H, dd, *J* 9.5 and 9.6, 3-H), 4.93 (1H, dd, *J* 9.3 and 9.5, 4-H), 4.82 (1H, d, *J* 9.8, 1-H), 4.23 (2H, d, *J* 7.5, $CHCH_2O$), 4.11 (1H, t, *J* 7.5, $CHCH_2O$), 4.03 (1H, m, 2-H), 3.53 (3H, m, 5-H 6a-H 6b-H), 1.94 (6H, s, $OCOCH_3$), 1.84 (3H, s, $NHCOCH_3$); δ_C (63 MHz) 172.0 170.9 169.9 156.5 (CO), 143.3 143.2 140.9 (C_{ar}), 127.5 126.7 124.7 119.6 (CH_{ar}), 81.2 (C1), 75.4 (C5), 73.1 (C3), 68.5 (C4), 67.1 ($CHCH_2O$), 60.7 (C6), 52.2 ($CHCH_2O$), 46.5 (C2), 22.3 ($NHCOCH_3$), 20.2 ($OCOCH_3$); m/z (APCI+) 527 (MH^+, 100.0 %), 305 (MH^+-Fmoc,

40.2 %), 288 (MH^+-NHFmoc, 72.5%), 83 ($C_5H_5O^+$, 100 %); m/z (FAB) 527 (MH^+, 4.1 %) (Found: MH^+ 527.20082. $C_{27}H_{31}N_2O_9$ requires 527.20296).

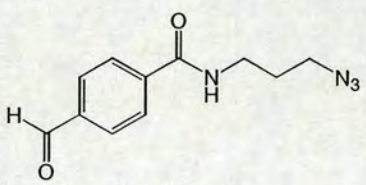
3.7. Synthesis of linker 87

3.7.1 3-Azidopropylamine **108** [99]



A solution of 3-chloropropylamine hydrochloride (10.01 g, 77.0 mmol) and sodium azide (11.99 g, 184.8 mmol) in water (75 ml) was stirred at 80 °C for 16 h after which the solution was cooled to 0 °C and diethyl ether (100 ml) added. Potassium hydroxide (5.18 g, 92.4 mmol) pellets were then added gradually so that the solution temperature did not exceed 10 °C. The aqueous layer was washed with diethyl ether (100 ml x 2), saturated with sodium chloride (20.04 g, 0.34 mol) and then washed again with diethyl ether (50 ml x 3). The combined organic extracts were then dried over anhydrous K_2CO_3 and the solvent evaporated in *vacuo* using a cold-water bath to yield **108** as a pale yellow oil (6.21 g, 80 %), bp 50-52 °C @ 20 mmHg (Lit. bp 45-50 °C @ 15 mmHg [99]); υ_{max} (neat)/cm^{-1} 3370.0 (NH_2), 2939.0 2869.3 (CH), 2098.3 (N_3); δ_H (250 MHz; $CDCl_3$) 3.41 (2H, t, *J* 6.8, CH_2N_3), 2.77 (2H, t, *J* 6.8, CH_2NH_2), 1.70 (2H, tt, *J* 6.8, $CH_2CH_2CH_2$), 1.57 (2H, s, NH_2); δ_C (63 MHz) 48.9 (CH_2N_3), 39.1 (CH_2NH_2), 32.1 ($CH_2CH_2CH_2$); m/z (EI) 101 (MH^+, 3.6 %), 84 (MH^+-NH_2, 6.5 %), 71 ($N_3CH_2CH_2^+$, 20.5 %), 56 ($N_3CH_2^+$, 27.2 %), 44 ($NH_2CH_2CH_2^+$, 82.7 %), 42 (N_3, 41.4 %), 30 ($NH_2CH_2^+$, 100.0%).

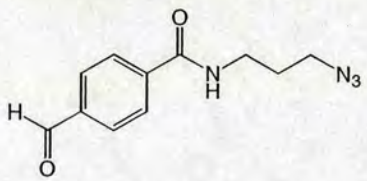
3.7.2 N-*(3-Azidopropyl)-4-formylbenzamide* **110** [82]



To a stirred solution of 4-carboxybenzaldehyde (9.98 g, 66.5 mmol) in dry THF (60 ml.) was added thionyl chloride (15 ml, 199.5 mmol) and the reaction mixture was left stirring until HCl gas evolution ceased. Once cooled to RT the solvent was removed in *vacuo* to yield the crude acid chloride. This was then

dissolved in dry THF (40 ml) and added to a cooled solution of **108** (6.20 g, 61.9 mmol), triethylamine (11.1 ml, 80.0 mmol) and dry THF (40 ml) and the reaction mixture left stirring at RT (EtOAc:Hex [2:1]) until TLC indicated complete transformation of starting material. Water (200 ml) was then added and the aqueous layer was washed with chloroform (200 ml x 2), saturated with sodium chloride (20.5 g, 0.15 mmol) and then washed again with chloroform (100 ml x 2). The combined organic extracts were then dried over anhydrous Na_2SO_4 and the solvent evaporated in *vacuo* to yield pale yellow oil. The product was purified using flash-column chromatography (EtOAc:Hex [2:1]) to yield *the title compound* **110** (5.74 g, 37 %) as a pale yellow syrup; υ_{max} (Nujol)/cm^{-1} 3286.6 (NH), 2162.4 (N_3), 1697.9 (COH), 1633.2 (NH*CO*); δ_H (250 MHz; $CDCl_3$) 10.00 (1H, s, CO*H*), 7.87 (4H, s, CH_{ar}), 7.02 (1H, s, CON*H*CH_2), 3.53 (2H, dt, *J* 6.5, CONHCH_2), 3.41 (2H, t, *J* 6.5,CH_2N_3), 1.87 (2H, tt, *J* 6.5, $CH_2CH_2CH_2$).

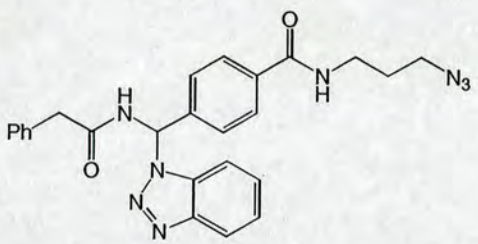
3.7.3 N-*(3-Azidopropyl)-4-formylbenzamide* **110**



To a solution of **108** (3.70 g, 37.0 mmol) and 4-carboxybenzaldehyde (5.55 g, 37.0 mmol) in dry THF (60 ml) was added EEDQ (10.98 g, 44.4mmol) in the course of a few minutes. The reaction mixture was stirred at 35 °C for 24 h after which TLC (EtOAc:Hex [2:1]) indicated complete transformation of the starting material and water (150 ml) was added. The product was extracted using chloroform (150 x 3 ml), the combined organic extracts washed successively with 1 M hydrochloric acid (100 ml x 2), water (100 ml), brine (100 ml x 2) and again water (100 ml x 2), dried over anhydrous $MgSO_4$ and the solvent removed in *vacuo* to yield pale yellow oil containing solid. The product was purified using flash-column chromatography (gradient elution used starting with Hex:EtOAc [9:1] increasing to Hex:EtOAc [1:1]) to yield *the title compound* **110** (5.24 g, 61 %) as a white solid, mp 41-42 °C (Found: C, 56.90; H, 4.98; N, 23.88. $C_{11}H_{12}N_4O_2$ requires C, 56.89; H, 5.21; N, 24.12 %);

υ_{max} (Nujol)/cm^{-1} 3286.6 (NH), 2162.4 (N_3), 1697.9 (*C*OH), 1633.2 (NH*C*O); δ_H (250 MHz; $CDCl_3$) 10.00 (1H, s, CO*H*), 7.87 (4H, s, CH_{ar}), 7.02 (1H, s, CON*H*CH_2), 3.53 (2H, dt, *J* 6.5, CONHCH_2), 3.41 (2H, t, *J* 6.5,CH_2N_3), 1.87 (2H, tt, *J* 6.5, $CH_2CH_2CH_2$); δ_C (63 MHz) 191.5 (*C*OH), 166.5 (*C*ONH), 139.4 137.9 (C_{ar}), 129.6 127.5 (*C*H$_{ar}$), 49.3 (*C*H_2N_3), 37.8 (CONH*C*H_2), 28.4 ($CH_2CH_2CH_2$); m/z (FAB) 233 (MH^+, 5.6 %), 105 (C_6H_4COH, 7.1 %) (Found: MH^+ 233.10274. $C_{11}H_{13}N_4O_2$ requires 233.103851).

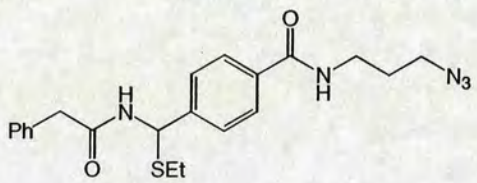
3.7.4 N-*({[4-(3-Azidopropylcarbamoyl)phenyl]benzotriazol-1-yl}methyl)-2-phenylacetamide* **94** [82]



A solution of **110** (5.50 g, 23.7 mmol), benzotriazole (2.82 g, 23.7 mmol), phenylacetamide (3.20 g, 23.7 mmol) and tosic acid (0.10 g, 0.5 mmol) in dry toluene (150 ml) was refluxed for 18 h after which TLC (EtOAc:Hex [2:1]) indicated complete transformation of starting material. Dean-Stark apparatus was employed to remove the water side-product. The toluene was removed in *vacuo* to yield yellow oil containing solid and the product purified using flash-column chromatography (DCM:MeOH [98:2]) to yield *the title compound* **94** (5.54 g, 50 %) as a white solid, mp 163-164 °C (Found: C, 64.38; H, 4.93; N, 23.86. $C_{25}H_{24}N_8O_2$ requires C, 64.09; H, 5.16; N, 23.92 %); υ_{max} (Nujol)/cm^{-1} 3275.1 (NH), 2112.1 (N_3), 1649.5 (NH*C*O), 1627.2 (NH*C*O); δ_H (250 MHz; DMSO-D_6) 10.06 (1H, d, *J* 7.0, N*H*CHBt), 8.57 (1H, t, *J* 6.8, CON*H*CH_2), 8.08 (1H, d, *J* 8.3, CH_{ar} of bt), 8.00 (1H, d, *J* 8.3, C*H*Bt), 7.89 (1H, d, *J* 8.3, CH_{ar} of bt), 7.87 (2H, d, *J* 8.3, CH_{ar}), 7.54 (1H, ddd, *J* 1.0 7.0 and 8.3, CH_{ar} of bt), 7.48 (2H, d, *J* 8.3, CH_{ar}), 7.42 (1H, ddd, *J* 1.0 7.0 and 8.3, CH_{ar} of bt), 7.24 (5H, m, *Ph*CH_2), 3.68 (1H, d, *J* 14.2, PhC*H*), 3.59 (1H, d, *J* 14.2, PhC*H*), 3.38 (2H, dt, *J* 6.8, CONHCH_2), 3.31 (2H, t, *J* 6.8,CH_2N_3), 1.76 (2H, tt, *J* 6.8,

$CH_2CH_2CH_2$); δ_C (63 MHz) 170.9 165.8 (*C*O), 145.3 139.2 135.6 135.1 132.0 (C_{ar}), 129.2 128.3 127.8 127.7 126.9 126.7 124.5 119.4 111.2 (CH_{ar}), 65.0 (NH*C*HBt), 48.6 (PhCH_2), 41.7 (CH_2N_3), 36.8 (CONHCH_2), 28.5 ($CH_2CH_2CH_2$); m/z (FAB) 469 (MH^+, 22.2 %), 350 (MH^+-Bt, 100.0 %), 232 (MH^+-Bt-$PhCH_2CO$, 44.9 %), 223 (MH^+-$CONH(CH_2)_3N_3$, 12.6 %), 120 (BtH, 19.4 %) (Found: MH^+ 469.21080. $C_{25}H_{25}N_8O_2$ requires 469.21080).

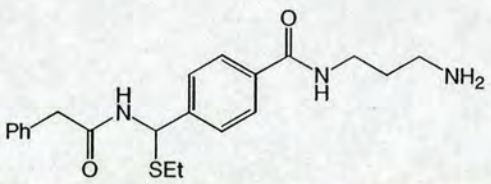
3.7.5 N-*({[4-(3-Azidopropylcarbamoyl)phenyl]ethylsulphanyl}methyl)-2-phenylacetamide* **93** [82]



A solution of **94** (2.34 g, 5.0 mmol) in dry THF (50 ml) was added drop wise to a stirred suspension of ethylthiolate sodium salt (0.90 g, 10.5 mmol) in THF (10 ml) and the overall solution was left stirring at RT for 2 h after which TLC (EtOAc:Hex [2:1]) indicated that all the starting material was consumed. Water (150 ml) was then added and the product extracted using chloroform (200 x 3 ml), the aqueous layer saturated with sodium chloride (40.04 g, 0.68 mol) and the product again extracted with chloroform (100 x 2 ml). The combined organic extracts were dried over Na_2SO_4 and the solvent removed in *vacuo* to yield *the title compound* **93** (1.96 g, 95 %) as a white solid, mp 172-173 °C (Found: C, 61.29; H, 6.12; N, 17.02. $C_{21}H_{25}N_5O_2S$ requires C, 61.58; H, 6.10; N, 16.77 %); υ_{max} (Nujol)/cm^{-1} 3285.7 (NH), 2095.6 (N_3), 1639.7 (NH*CO*); δ_H (250 MHz; $CDCl_3$) 9.15 (1H, d, *J* 9.5, N*H*CHSEt), 8.52 (1H, t, *J* 6.8, CON*H*CH_2), 7.81 (2H, d, *J* 8.4, CH_{ar}), 7.52 (2H, d, *J* 8.4, CH_{ar}), 7.28 (5H, m, *Ph*CH_2), 6.21 (1H, d, *J* 9.5, C*H*SEt), 3.58 (1H, d, *J* 13.7, PhC*H*), 3.52 (1H, d, *J* 13.7, PhC*H*), 3.43-3.28 (4H, m, CH_2N_3 and CONHCH_2), 2.58 (1H, dt, *J* 7.4 and 12.7, SC*H*CH_3), 2.42 (1H, dt, *J* 7.4 and 12.7, SC*H*CH_3), 1.77 (2H, tt, *J* 6.8, $CH_2CH_2CH_2$), 1.15 (3H, t, *J* 7.4, SCH_2CH_3); δ_C (63 MHz) 170.0 166.0 (*C*O), 143.0 136.3 134.0 (C_{ar}), 131.0 129.5 129.1 128.3 127.4 126.6 (CH_{ar}), 54.6

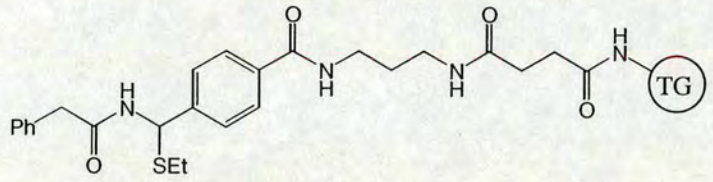
(*C*HSEt), 48.6 (Ph*C*H_2), 42.2 (*C*H_2N_3), 36.7 (CONH*C*H_2), 28.5 ($CH_2CH_2CH_2$), 24.8 (S*C*H_2CH_3), 14.7 (SCH_2CH_3); m/z (FAB) 412 (MH^+, 5.1 %), 350 (MH^+-SEt, 32.7 %), 307 (MH^+-SEt-N_3, 22.1 %), 232 (MH^+-SEt-$PhCH_2CO$, 15.6 %) (Found: MH^+ 412.18251. $C_{21}H_{26}N_5O_2S$ requires 412.18072).

3.7.6 N-*({[4-(3-Aminopropylcarbamoyl)phenyl]ethylsulphanyl}methyl)-2-phenylacetamide* **87** [82]



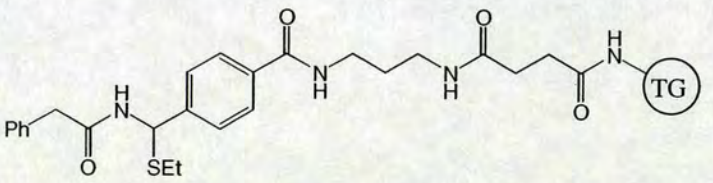
To a solution of **93** (2.10 g, 5.1 mmol) in THF (60 ml) and water (1 ml) was added triphenylphosphine (1.61 g, 6.1 mmol) and the overall mixture stirred at RT for up to 72 h until TLC (EtOAc) indicated complete transformation of starting material. The reaction mixture was then concentrated in *vacuo* to give an oil after which diethyl ether (100 ml) was added and the walls of the flask scratched to facilitate crystal nucleation. The solid formed was filtered, stirred in ether (100 ml) for 16 h, filtered again and the solid dried to yield *the title compound* **87** (1.73 g, 88 %) as a white powder, mp 133-135 °C (Found: C, 65.17; H, 6.91; N, 10.62. $C_{21}H_{27}N_3O_2S$ requires C, 65.43; H, 7.06; N, 10.90 %); υ_{max} (Nujol)/cm^{-1} 3282.8 (NH), 1641.5 (NH*CO*); δ_H (250 MHz; CD_3OD), 7.86 (2H, d, *J* 8.3, C*H*$_{ar}$), 7.60 (2H, d, *J* 8.3, C*H*$_{ar}$), 7.39 (5H, m, *Ph*CH_2), 6.34 (1H, s, C*H*SEt), 3.71 (1H, d, *J* 13.9, PhC*H*), 3.65 (1H, d, J 13.9, PhC*H*), 3.52 (2H, t, *J* 6.8, CONHC*H*$_2$), 2.79 (2H, t, *J* 6.8, C*H*$_2NH_2$), 2.70 (1H, dt, *J* 7.4 and 20.3, SC*H*CH_3), 2.54 (1H, dt, *J* 7.4 and 20.3, SC*H*CH_3), 1.84 (2H, tt, *J* 6.8, $CH_2CH_2CH_2$), 1.30 (3H, t, *J* 7.4, SCH_2CH_3); δ_C (63 MHz) 164.0 160.4 (*C*O), 135.1 127.5 125.8 (C$_{ar}$), 120.6 120.2 119.1 118.5 (*C*H$_{ar}$), 47.1 (*C*HSEt), 34.3 (Ph*C*H_2), 30.3 (CONH*C*H_2), 28.9 (CH_2NH_2), 23.9 ($CH_2CH_2CH_2$), 16.9 (S*C*H_2CH_3), 5.6 (SCH_2CH_3); m/z (FAB) 386 (MH^+, 26.5 %), 307 (MH^+-SEt-NH_2, 29.6 %) (Found: MH^+ 386.18958. $C_{21}H_{28}N_3O_2S$ requires 386.19023).

*3.7.7 Preparation of 4-{[Ethylsulphanyl-*N*-(2-phenylacetyl)]aminomethyl}-benzamidopropylamido Tentagel® **113** using TBTU* [86]



Carboxy-Tentagel® (0.50 g, 0.20 mmol/g), **87** (0.23 g, 0.6 mmol), TBTU (0.42 g, 1.3 mmol), HOBt (0.18 g, 1.3 mmol) and DMF (3 ml) were placed on a blood rotator in an isolute tube (10 ml) for 5 min after which DIEA (0.23 ml, 1.4 mmol) was added and the mixture left on the blood rotator for 16 h. The resin was then filtered, washed and dried in *vacuo* yield *the title compound* **113** (50 %, 0.09 mmol/g); υ_{max} (DCM)/cm^{-1} 3515.8 (NH), 2954.6 (CH), 1651.4 (NH*CO*); δ_C (63 MHz, DCM-D_2) 170.2 (CO), 142.7 135.4 134.5 (C_{ar}), 129.3 128.8 127.5 127.1 126.7 (CH_{ar}), 55.6 (*C*HSEt), 42.9 (Ph*C*H_2), 36.2 (CONH*C*H_2), 36.1 (*C*H_2NHCO), 29.6 ($CH_2CH_2CH_2$), 25.5 (SCH_2CH_3), 14.7 (SCH_2CH_3).

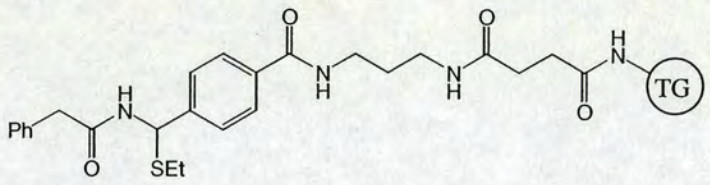
3.7.8 *Preparation of 4-{[Ethylsulphanyl-*N*-(2-phenylacetyl)]aminomethyl}-benzamidopropylamido Tentagel® **113** using Cyanuric Fluoride*



Carboxy-Tentagel® (0.19 g, 0.26 mmol/g), DCM (1 ml) and pyridine (8.0 µl, 0.1 mmol) were placed on a blood rotator in an isolute tube (3 ml) for 5 min after which cyanuric fluoride (20.7 µl, 0.2 mmol) was added and the mixture left on the blood rotator for 16 h. The resin was then filtered, washed and dried in *vacuo* to give acyl fluoride functionalised Tentagel®; υ_{max} (DCM)/cm^{-1} 1841.6 (COF). To this was added **87** (0.08 g, 0.2 mmol), DIEA (17.4 µl, 0.1 mmol) and DMF (1 ml) and the mixture left on the blood rotator for 16 h after which the resin was filtered, washed

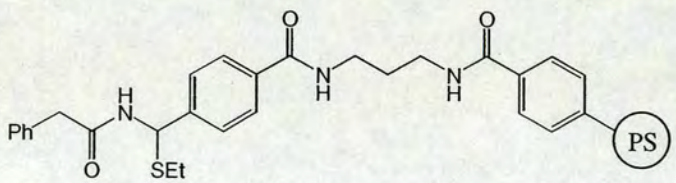
and dried in *vacuo* to yield *the title compound* **113** (38 %, 0.09 mmol/g); (υ_{max} (DCM)/cm^{-1} 3513.2 (NH), 2892.9 (CH), 1659.9 (*CO*NH).

*3.7.9 Preparation of 4-{[Ethylsulphanyl-*N*-(2-phenylacetyl)]aminomethyl}-benzamidopropylamido Tentagel® **113** using DIC*



Carboxy-Tentagel® (0.46 g, 0.26 mmol/g), **87** (0.28 g, 0.7 mmol), DIC (0.25 ml, 1.6 mmol), HOBt (0.21 g, 1.6 mmol) and DMF (3 ml) were placed on a blood rotator in an isolute tube (10 ml) for 5 min after which DIEA (0.29 ml, 1.7 mmol) was added and the mixture left on the blood rotator for 16 h. The resin was then filtered, washed and dried in *vacuo* to yield *the title compound* **113** (92 %, 0.22 mmol/g); υ_{max} (DCM)/cm^{-1} 3507.1 (NH), 2888.9 (CH), 1650.4 (NHCO); δ_C (63 MHz, DCM-D_2) 170.3 166.7 (CO), 142.6 135.4 134.2 (C_{ar}), 129.1 128.6 127.4 126.9 126.6 (*C*H_{ar}), 55.4 (*C*HSEt), 43.0 (Ph*C*H_2), 36.2 (CONH*C*H_2), 36.1 (*C*H_2NHCO), 29.3 ($CH_2CH_2CH_2$), 23.4 (SCH_2CH_3), 14.5 (SCH_2CH_3).

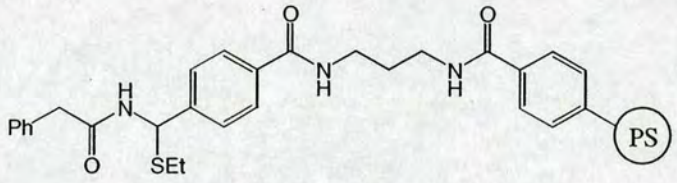
*3.7.10 Preparation of 4-{[Ethylsulphanyl-*N*-(2-phenylacetyl)]aminomethyl}-benzamidopropylamido Polystyrene **117** using DIC* [103]



Carboxy-polystyrene (0.16 g, 0.98 mmol/g), **87** (0.35 g, 0.9 mmol), DIC (0.32 ml, 2.0 mmol), HOBt (0.27 g, 2.0 mmol) and DMF (3 ml) were placed on a blood rotator in an isolute tube (10 ml) for 5 min after which DIEA (0.38 ml, 2.2 mmol) was added and the mixture left on the blood rotator for 16 h. The resin was then

filtered, washed and dried in *vacuo* to yield *the title compound* **117** (84 %, 0.60 mmol/g); υ_{max} (DCM)/cm^{-1} 3290.0 (NH), 3053.5 2985.8 (CH), 1673.9 (*CO*NH); δ_C (63 MHz, DCM-D_2) 55.8 (*C*HSEt), 43.5 (Ph*C*H_2), 36.4 (CONH*C*H_2), 31.3 (*C*H_2NHCO), 29.9 ($CH_2CH_2CH_2$), 25.7 (S*C*H_2CH_3), 14.8 (SCH$_2$*C*H_3).

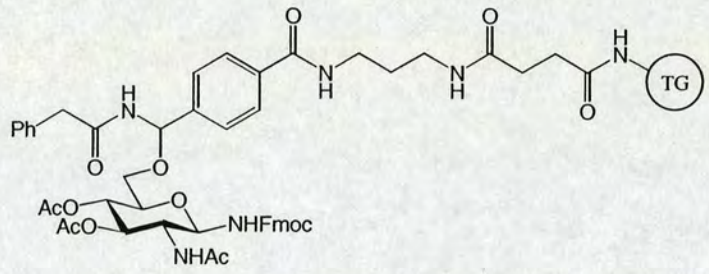
*3.7.11 Preparation of 4-{[Ethylsulphanyl-*N*-(2-phenylacetyl)]aminomethyl}-benzamidopropylamido Polystyrene* **117** *using Cyanuric Fluoride* [103]



Carboxy-polystyrene (0.18 g, 0.98 mmol/g), DCM (1.5 ml) and pyridine (28.5 µl, 0.4 mmol) were placed on a blood rotator in an isolute tube (5 ml) for 5 min after which cyanuric fluoride (75.6 µl, 0.9 mmol) was added and the mixture left on the blood rotator for 16 h. The resin was then filtered, washed and dried in *vacuo* to give acyl fluoride functionalised polystyrene **115**; υ_{max} (DCM)/cm^{-1} 1806.8 (COF). To this was added **87** (0.31 g, 0.8 mmol), DIEA (61.5 µl, 0.4 mmol) and DMF (3 ml) and the mixture left on the blood rotator for 16 h after which the resin was filtered, washed and dried in *vacuo* to give yield *the title compound* **117** (92 %, 0.66 mmol/g); υ_{max} (DCM)/cm^{-1} 3308.1 (NH), 2918.3 (CH), 1651.3 (*CO*NH); δ_C (63 MHz, DCM-D_2) 55.7 (*C*HSEt), 43.4 (Ph*C*H_2), 36.2 (CONH*C*H_2), 36.1 (*C*H_2NHCO), 30.8 ($CH_2CH_2CH_2$), 25.8 (S*C*H_2CH_3), 14.8 (SCH$_2$*C*H_3).

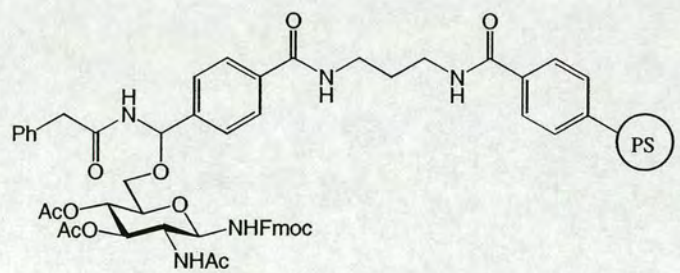
3.8. Synthesis of *N*-Glycopeptide 124

3.8.1 Coupling of 2-Acetamido-3, 4-di-O-acetyl-2-deoxy-N-(fluoren-9-yl-methoxy carbonyl)-β-D-glucopyranosylamine **106** *to 4-{[Ethylsulphanyl-N-(2-phenylacetyl)] aminomethyl}-benzamidopropylamido Tentagel®* **113**



A solution of NIS (0.24 g, 1.07 mmol), dry THF (10 ml) and triflic acid (3 µl, 31.9 µmol) was allowed to stand for 30 min. Meanwhile, 4 Å molecular sieves (0.1 g) were added to a solution of **113** (0.21 g, 0.24 mmol/g) and **106** (0.27 g, 0.50 mmol) in dry THF (3 ml) and heated to 45 °C. An aliquot of NIS solution (1.9 ml) was then added and the mixture left at 45 °C for 2 h after which the resin was filtered, washed and dried in *vacuo* to give the desired resin (79 %, 0.17 mmol/g); cleavage mixture: m/z (APCI+) 549 (MNa^+, 38.8 %), 527 (MH^+, 100.0 %), 136 ($PhCH_2CONH_3^+$, 100.0 %).

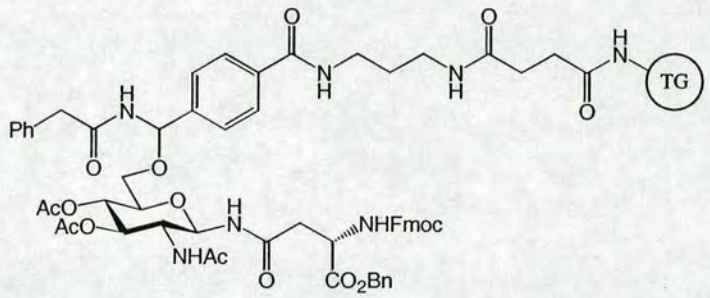
3.8.2 Coupling of 2-Acetamido-3, 4-di-O-acetyl-2-deoxy-N-(fluoren-9-yl-methoxy carbonyl)-β-D-glucopyranosylamine **106** *to 4-{[Ethylsulphanyl-N-(2-phenylacetyl)] aminomethyl}-benzamidopropylamido Polystyrene* **117**



A solution of NIS (0.24 g, 1.07 mmol), dry THF (10 ml) and triflic acid (3 µl, 31.9 µmol) was allowed to stand for 30 min. Meanwhile, 4 Å molecular sieves (30.0

mg) were added to a solution of **117** (15.4 mg, 0.60 mmol/g) and **106** (48.7 mg, 92.4 μmol) in dry THF (0.5 ml) and heated to 45 °C. An aliquot of NIS solution (0.3 ml) was then added and the mixture left at 45 °C for 2 h after which the resin was filtered, washed and dried in *vacuo* to give the desired resin (15 %, 0.07 mmol/g).

3.8.3 Coupling of N^{α}-(fluoren-9-yl-methoxycarbonyl)-L-aspartic-4-acid-1-Benzyl Ester **122a** *to 4-{[2-Acetamido-3, 4-di-O-acetyl-2-deoxy-N-(fluoren-9-yl-methoxycarbonyl)-β-D-glucopyranosylamine-N-(2-phenylacetyl)]aminomethyl}-benzamidopropylamido Tentagel®* **118**



In an isolute tube (10 ml), **118** (0.6 g, 0.11 mmol/g) was treated with 20 % piperidine in DMF (5 ml) for 15 min after which the resin was filtered, washed successively with DMF (20 ml), THF (15 ml) and DCM (20 ml) and dried under suction for 5 min. Fmoc-Asp-OBzl **122a** (0.31, 0.7 mmol), HOBt (0.11 g, 0.8 mmol), TBTU (0.26 g, 0.8 mmol) and DMF (7.5 ml) were then added and the mixture placed on a shaker for 5 min after which NMM (55.0 μl, 0.5 mmol) was added and the mixture left on the shaker for a further 16 h. The resin was then filtered, washed and dried in *vacuo* to give the desired resin (78 %, 0.09 mmol/g); cleavage mixture: m/z (APCI+) 754 (MNa^+, 24.2 %), 732 (MH^+, 100.0 %), 624 (M^+-$PhCH_2O$, 33.3 %), 402 (M^+-$PhCH_2O$-Fmoc, 22.4 %), 136 ($PhCH_2CONH_3^+$, 100.0 %).

3.8.4 Coupling of Fmoc-Amino Acids using TBTU

In an isolute tube (3 ml), Fmoc-functionalised resin (c.a. 50.0 mg, 1 eq) was treated with 20 % piperidine in DMF (1 ml) for 15 min after which the resin was filtered, washed successively with DMF (10 ml), THF (10 ml) and DCM (20 ml) and dried under suction for 5 min. Fmoc-AA-OH (10 eq), HOBt (12 eq), TBTU (12 eq) and DMF (0.3 ml) were then added and the mixture placed on a blood rotator for 5 min after which NMM (8 eq) was added and the mixture left on a blood rotator for 16 h. The resin was then filtered, washed and dried in *vacuo* to yield Fmoc-amino acid functionalised resin (36-100 %).

3.8.5 Coupling of Fmoc-Amino Acids using Fmoc-Amino Acid Pentafluorophenyl Esters and DhbtOH

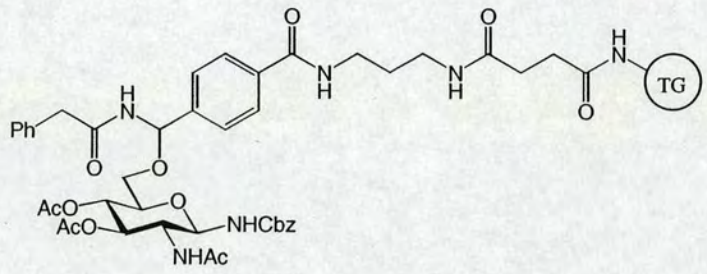
In an isolute tube (10 ml), Fmoc-functionalised resin (c.a. 0.50-0.60 g, 0.10-0.06 mmol/g, 1 eq) was treated with 20 % piperidine in DMF (5 ml) for 15 min after which the resin was filtered, washed with DMF (20 ml x 3) and dried under suction for 5 min. A solution of Fmoc-AA-OPfp (10-15 eq), DhbtOH (5 eq) and DMF (4 ml) was then added and the mixture placed on a shaker for 16 h after which the resin was filtered and washed with DMF (20 ml x 2). A second addition of fresh solution was added for a further 8 h and the resin was then filtered, washed and dried in *vacuo* to yield Fmoc-amino acid functionalised resin (87-100 %).

3.8.6 Acetate Capping Procedure

A solution of acetic anhydride (0.20 ml, 2.1 mmol) in DMF (7ml) was added to Fmoc-amino acid functionalised resin (c.a. 0.50-0.60 g) in an isolute tube (10 ml) and was placed on a shaker for 8 h after which the resin was then filtered, washed and dried in *vacuo*.

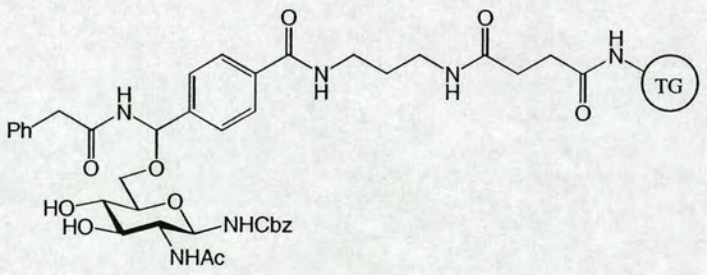
3.9. Library Syntheses

*3.9.1 Synthesis of 2-acetamido-3, 4-di-*O*-acetyl-*N*-(benzyloxycarbonyl)-2-deoxy-*β*-D-glucopyranosylamine on Tentagel®* **125**



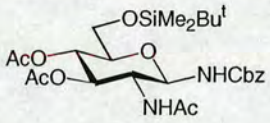
In an isolute tube (3 ml), **118** (27.3 mg, 0.14 mmol/g) was treated with 20 % piperidine in DMF (2 ml) for 15 min after which the resin was filtered, washed and dried in *vacuo* for 1 h. *N*-benzyloxycarbonyloxy succinimide (27.4 mg, 0.11 mmol), triethylamine (2.1 µl, 15.3 µmol) and DCM (0.6 ml) were then added and placed on a blood rotator for 16 h after which the resin was filtered, washed and dried in *vacuo* to give **125** (35 %, 0.05 mmol/g).

*3.9.2 Synthesis of 2-acetamido-*N*-(benzyloxycarbonyl)-2-deoxy-*β*-D-glucopyranosyl amine on Tentagel®* **128**



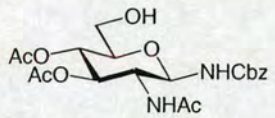
A mixture of **125** (27.7 mg, 0.05 mmol/g), methanol (0.1 ml), DMF (0.1 ml) and triethylamine (19.5 µl, 0.14 mmol) were placed on a blood rotator in an isolute tube (3 ml) for 16 h after which the resin was filtered, washed and dried in *vacuo* to yield the desired resin (40 %, 0.02 mmol/g).

3.9.3 2-Acetamido-3, 4-di-O-acetyl-N-(benzyloxycarbonyl)-6-O-tert-butyldimethylsilyl-2-deoxy-β-D-glucopyranosylamine **127**



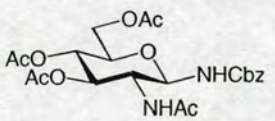
105 (0.31g, 0.5 mmol) was treated with 20 % piperidine in acetonitrile (3 ml) for 5 min after which the solvent was removed in *vacuo* to give a white solid. This crude amino sugar and *N*-(benzyloxycarbonyloxy) succinimide (0.15 g, 0.6 mmol) were then dissolved in anhydrous pyridine (4 ml) and the reaction was stirred at RT for 16 h after which TLC (EtOAc) indicated complete transformation of the amino starting material. The solvent was azeotroped with toluene in *vacuo* and the product was purified using flash-column chromatography (EtOAc:Hex [1:1]) to yield *the title compound* **127** (0.22 g, 82 %) as a white solid, mp 177-178 °C; υ_{max} (Nujol)/cm^{-1} 3266.7 (NH), 1751.4 (CO), 1710.7 (NH*CO*O), 1657.6 (NH*CO*), 1093.4 778.7 (SiO), 836.0 (Si(CH$_3$)$_2$); δ_H (250 MHz; CDCl$_3$) 7.29 (5H, s, *Ph*CH$_2$), 6.20 (1H, d, *J* 9.2, N*H*Cbz), 6.16 (1H, d, *J* 8.5, N*H*Ac), 5.11 (1H, d, *J* 12.3, PhC*H*), 5.05 (1H, d, *J* 12.3, PhC*H*), 5.11-5.05 (2H, m, 3-H 4-H), 4.85 (1H, dd, *J* 9.2 and 10.1, 1-H), 4.07 (1H, ddd, *J* 8.5 10.1 and 10.5, 2-H), 3.73 (1H, dd, *J* 2.5 and 11.5, 6a-H), 3.64 (1H, dd, *J* 4.4 and 11.5, 6b-H), 3.55 (1H, m, 5-H), 2.03 (3H, s, OCOC*H*$_3$), 2.00 (3H, s, OCOC*H*$_3$), 1.87 (3H, s, NHCOC*H*$_3$), 0.85 (9H, s, C(C*H*$_3$)$_3$), 0.00 (3H, s, Si(C*H*$_3$)$_2$); δ_C (63 MHz) 171.8 171.4 169.1 155.8 (CO), 136.0 (C$_{ar}$), 128.3 128.0 127.7 (*C*H$_{ar}$), 82.2 (C1), 75.7 (C5), 73.3 (C3), 68.4 (C4), 66.8 (Ph*C*H$_2$), 62.2 (C6), 53.0 (C2), 25.9 (C(*C*H$_3$)$_3$), 22.9 (NHCO*C*H$_3$), 20.6 (OCO*C*H$_3$), 18.2 (*C*(CH$_3$)$_3$), -5.5 (Si(*C*H$_3$)$_2$); m/z (FAB) 553 (MH$^+$, 40.7 %), 403 (MH$^+$-NHCbz, 1.9 %), 91 (PhCH$_2^+$, 100 %), 73 (Ph, 52.7 %), 43 (Ac, 29.6 %) (Found: MH$^+$ 553.25786. $C_{26}H_{41}N_2O_9Si$ requires 553.25814).

3.9.4 2-Acetamido-3, 4-di-O-acetyl- N-(benzyloxycarbonyl)-2-deoxy-β-D-glucopyranosylamine **126**



To a stirred solution of **127** (0.28 g, 0.5 mmol) in 0.05 % water in DCM (3 ml) was added a solution of DDQ (0.02 g, 0.1 mmol) in 0.05 % water in DCM (1 ml) and the mixture was stirred at RT for up to 120 h until TLC (EtOAc) indicated that all the starting material had been consumed. The solvent was then removed in *vacuo* to yield the crude product and was purified using flash-column chromatography ($CHCl_3$:MeOH [97:3]) to yield *the title compound* **126** (0.20 g, 89 %) as a white solid, mp 237-238 °C, υ_{max} (Nujol)/cm^{-1} 3296.7 (NH OH), 1742.4 (CO), 1701.1 (NH*CO*O), 1656.8 (NH*CO*); δ_H (250 MHz; $CDCl_3$ CD_3OD) 7.25 (5H, s, *Ph*CH_2), 5.05 (1H, dd, *J* 9.5 and 9.8, 3-H), 5.03 (2H, m, PhC*H*$_2$), 4.92 (1H, dd, *J* 9.4 and 9.5, 4-H), 4.79 (1H, d, *J* 10.0, 1-H), 3.98 (1H, dd, *J* 9.8 and 10.0, 2-H), 3.62-3.49 (2H, m, 5-H 6a-H), 3.48 (1H, dd, *J* 4.7 and 12.4, 6b-H), 1.98 (3H, s, OCOC*H*$_3$), 1.96 (3H, s, OCOC*H*$_3$), 1.83 (3H, s, NHCOC*H*$_3$); δ_C (63 MHz) 172.0 171.1 170.0 (CO), 135.6 (C_{ar}), 128.3 128.0 127.7 (*C*H$_{ar}$), 81.5 (C1), 75.5 (C5), 72.9 (C3), 68.4 (C4), 67.0 (Ph*C*H$_2$), 60.8 (C6), 52.3 (C2), 22.5 (NHCO*C*H$_3$), 20.4 (OCO*C*H$_3$); m/z (FAB) 423 (MH^+, 38.7 %), 217 (MH^+-NHCbz, 21.0 %), 109 ($PhCH_2O$, 15.5 %), 91 ($PhCH_2^+$, 100 %) (Found: MH^+ 439.17145. $C_{20}H_{27}N_2O_9$ requires 439.17166).

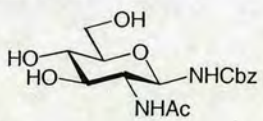
3.9.5 2-Acetamido-3, 4, 6-tri-O-acetyl-N-(benzyloxycarbonyl)-2-deoxy-β-D-glucopyranosylamine **130**



Under an atmosphere of hydrogen, **102** (1.01 g, 2.7 mmol), platinum (IV) oxide (0.10 g, 0.4 mmol) and THF (25 ml) were stirred vigorously at RT for 2 h after

which TLC (EtOAc) indicated that all the starting material had been consumed. The reaction mixture was concentrated in *vacuo* to yield yellow oil that contained platinum oxide. This crude amino sugar mixture and *N*-(benzyloxycarbonyloxy) succinimide (0.81 g, 3.3 mmol) were then dissolved in anhydrous pyridine (20 ml) and the reaction was stirred at RT for 16 h after which TLC (EtOAc) indicated complete transformation of the amino starting material. The solvent was azeotroped with toluene in *vacuo* and the product was purified using flash-column chromatography ($CHCl_3$:MeOH [98:2]) to yield *the title compound* **130** (0.85 g, 65 %) as a white solid, mp 220-221 °C; $[\alpha]_D$ –23.0 (c 1.45, $CHCl_3$); υ_{max} (Nujol)/cm^{-1} 3334.2 (NH), 1747.5 (CO), 1699.1 (NH*CO*O), 1657.5 (NH*CO*); δ_H (250 MHz; $CDCl_3$) 7.29 (5H, s, *Ph*CH_2), 6.28 (1H, d, *J* 9.1, N*H*Cbz), 6.18 (1H, d, *J* 8.9, N*H*Ac), 5.13-5.00 (4H, m, 3-H 4-H PhCH_2), 4.89 (1H, dd, *J* 9.1 and 9.3, 1-H), 4.27 (1H, dd, *J* 4.3 and 12.5, 6b-H), 4.14 (1H, ddd, *J* 8.9 8.9 and 9.3, 2-H), 4.07 (1H, dd, *J* 2.2 and 12.5, 6a-H), 3.71 (1H, m, 5-H), 2.06 (3H, s, OCOCH_3), 2.03 (3H, s, OCOCH_3), 2.01 (3H, s, OCOCH_3), 1.88 (3H, s, NHCOCH_3); δ_C (63 MHz) 171.6 171.4 170.6 169.1 155.8 (CO), 135.7 (C_{ar}), 128.3 128.0 127.8 (*C*H_{ar}), 82.5 (C1), 73.1 (C5), 72.9 (C3), 67.7 (C4), 67.0 (Ph*C*H_2), 61.6 (C6), 52.8 (C2), 22.9 (NHCO*C*H_3), 20.6 20.5 20.4 (OCO*C*H_3); m/z (FAB) 481 (MH^+, 100 %), 330 (MH^+-NHCbz, 90.0 %) (Found: MH^+ 481.18172. $C_{22}H_{29}N_2O_{10}$ requires 481.18222).

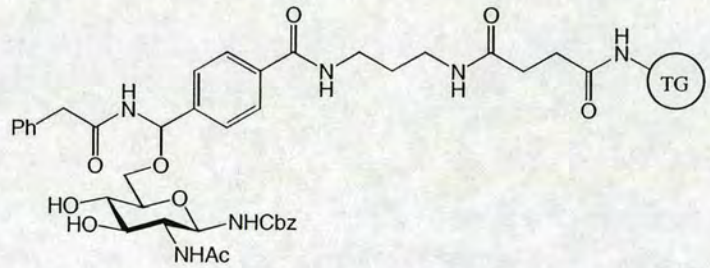
*3.9.6 2-Acetamido-*N*-(benzyloxycarbonyl)-2-deoxy-*β*-D-glucopyranosylamine* **129**



To a solution of **130** (0.50 g, 1.0 mmol) in dry methanol (3 ml) was added sodium methoxide in methanol (0.5 M, 0.6 ml) and the mixture stirred at RT for 1 h after which TLC (EtOAc) indicated complete transformation of the starting material. The reaction mixture was filtered through cation ion-exchange resin and the methanol evaporated in *vacuo* to afford *the title compound* **129** (0.35 g, 95 %) as a white solid, mp 224-226 °C; $[\alpha]_D$ +10.4 (c 1.09, MeOH); υ_{max} (Nujol)/cm^{-1} 3271.4

(NH OH), 1709.6 (NH*CO*O), 1651.6 (NH*CO*); δ_H (250 MHz; CD_3OD) 7.42 (5H, s, *Ph*CH_2), 5.20 (1H, d, *J* 12.4, PhC*H*), 5.15 (1H, d, *J* 12.4, PhC*H*), 4.87 (1H, d, *J* 9.8, 1-H), 3.93 (1H, dd, *J* 1.7 and 12.0, 6a-H), 3.81 (1H, dd, *J* 8.7 and 9.8, 2-H), 3.76 (1H, dd, *J* 4.9 and 12.0, 6b-H), 3.56 (1H, dd, *J* 8.7 10.1, 4-H), 3.43-3.39 (2H, m, 3-H 5-H), 2.02 (3H, s, NHCOCH_3); δ_C (63 MHz) 172.5 156.4 (CO), 136.0 (C_{ar}), 127.6 127.2 127.0 (CH_{ar}), 81.1 (C1), 77.6 (C5), 74.4 (C3), 69.9 (C4), 65.8 (PhCH_2), 60.7 (C6), 54.2 (C2), 20.8 (NHCOCH_3); m/z (FAB) 355 (MH^+, 91.3 %), 217 (MH^+-NHCbz, 48.0 %), 91 ($PhCH_2^+$, 100 %) (Found: MH^+ 355.15111. $C_{16}H_{23}N_2O_7$ requires 355.15053).

3.9.7 *Coupling of 2-acetamido-*N*-(benzyloxycarbonyl)-2-deoxy-*β*-*D*-glucopyranosylamine* **129** *to 4-{[Ethylsulphanyl-*N*-(2-phenylacetyl)] aminomethyl} -benzamidopropylamido Tentagel®* **113**



A solution of NIS (0.26 g, 1.16 mmol), dry THF (10 ml) and triflic acid (3.2 µl, 36.2 µmol) was allowed to stand for 30 min. Meanwhile, 4 Å molecular sieves (0.1 g) were added to a solution of **113** (0.20 g, 0.22 mmol/g) and **129** (0.23 g, 0.44 mmol) in dry THF (3 ml) and heated to 45 °C. An aliquot of NIS solution (1.5 ml) was then added and the mixture left for 2 h after which the resin was filtered, washed and dried in *vacuo* to give the desired resin (50 %, 0.10 mmol/g).

3.9.8 General Procedure for Esterification Library [110]

A solution of Carboxylic acid (0.38 mmol), EDCI (72.8 mg, 0.38 mmol) and dimethylaminopyridine (46.4 mg, 0.38 mmol) in dry DCM (10 ml) was allowed to stand for 10 min after which was added (2 ml) to **128** (c.a. 25.0 mg, 0.10 mmol/g) in

an isolute tube (3 ml). The mixture was then left on a blood rotator for 16 h after which the resin was filtered, washed and dried in *vacuo* to yield esterified resin.

Appendix

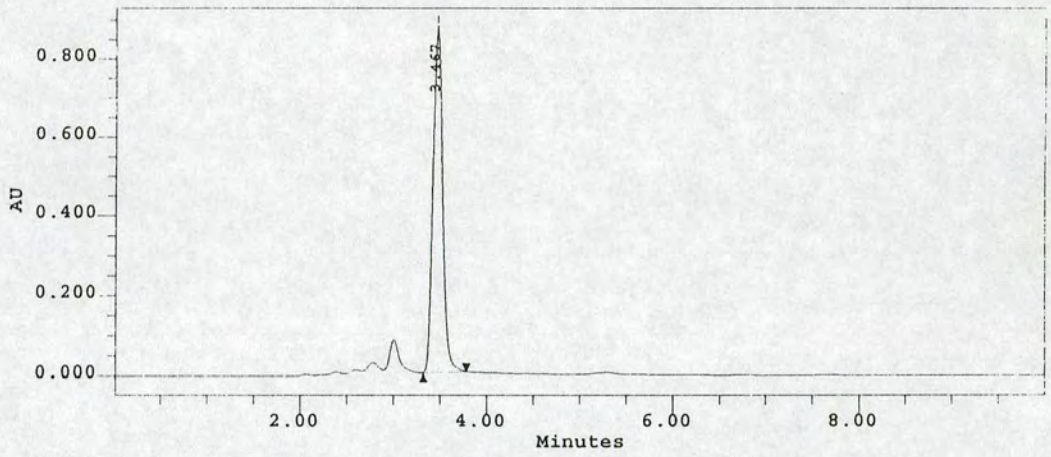
A1 Calculation for the loading of resins using Standard Samples

For example, calculating the loading for the attachment of Linker **87** onto carboxy-Tentagel®.

Two standards run: 1) 20 µl injection of 0.125 mg/ml gave response 3726503.
2) 20 µl injection of 0.250 mg/ml gave response 6685999.

Therefore, Average 20 µl injection 1mg/ml gives response 28278010.

7.5 mg of linker-functionalised Tentagel® **113** was treated with TFA:DCM:H_2O solution.
HPLC trace from 20 µl injection:



Ret. Time = 3.465, Area (uV* sec) = 6070798.
Therefore 6070798/28278010 = 0.22 mg/ml

Theoretically, 100 % coupling from carboxy-Tentagel® (0.26 mmol/g) = 0.237 mmol/g and so 7.5 mg @ 0.237 mmol/g should give a response of

$$(7.5 \times 10^{-3}) \times (0.237 \times 10^{-3}) \times 135.162 = 0.24 \text{ mg/ml.}$$

Therefore,

$$\% \text{ Yield} = (0.22/0.24) \times 100 = \underline{92\ \%}$$

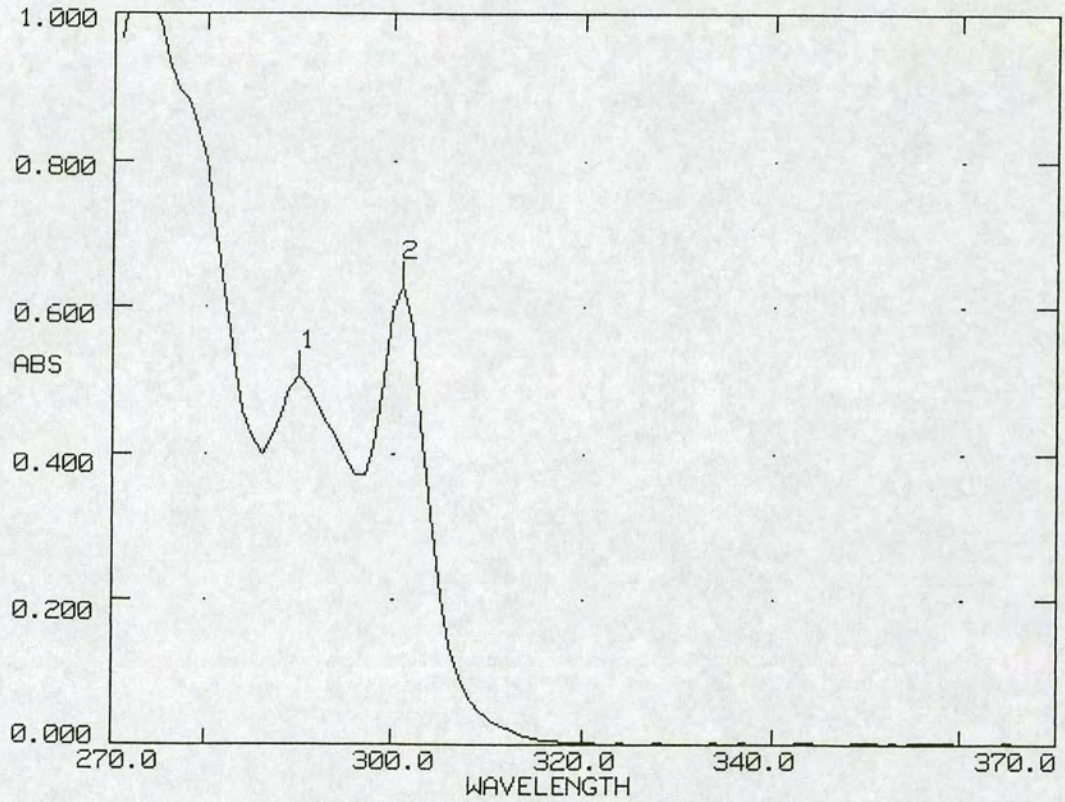
$$\text{Loading} = \underline{0.212 \text{ mmol/g}}.$$

Sugar-functionalised resins **125** and **128** are calculated in the same way.

A2 Calculation of the loading for the attachment of Sugar 106 and amino acids using UV analysis

5.8 mg of resin (0.20 mmol/g) was treated with 20 % piperidine in DMF in a 10 ml graduated flask.

UV trace obtained:



Absorbance at 301 nm = 0.626.

Therefore using equation 1,

Loading = (0.629 x 10)/(7.8 x 5.8) = 0.14 mmol/g.

% Yield = (0.14/0.18) x 100 = 78 %.

A3 Library LC-MS data

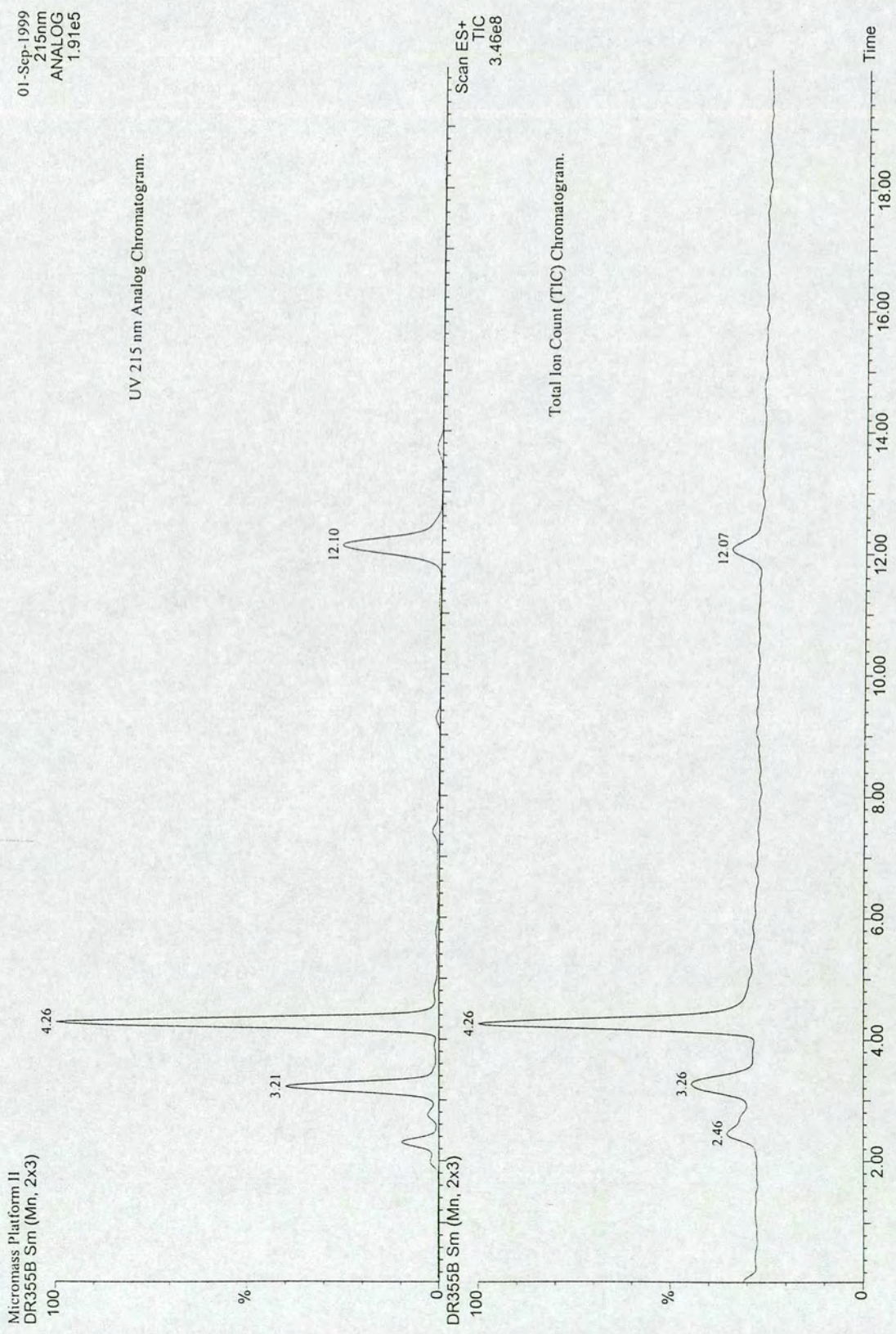


Figure 18a: *LC-MS chromatogram data for $PhCH_2CO_2H$.*

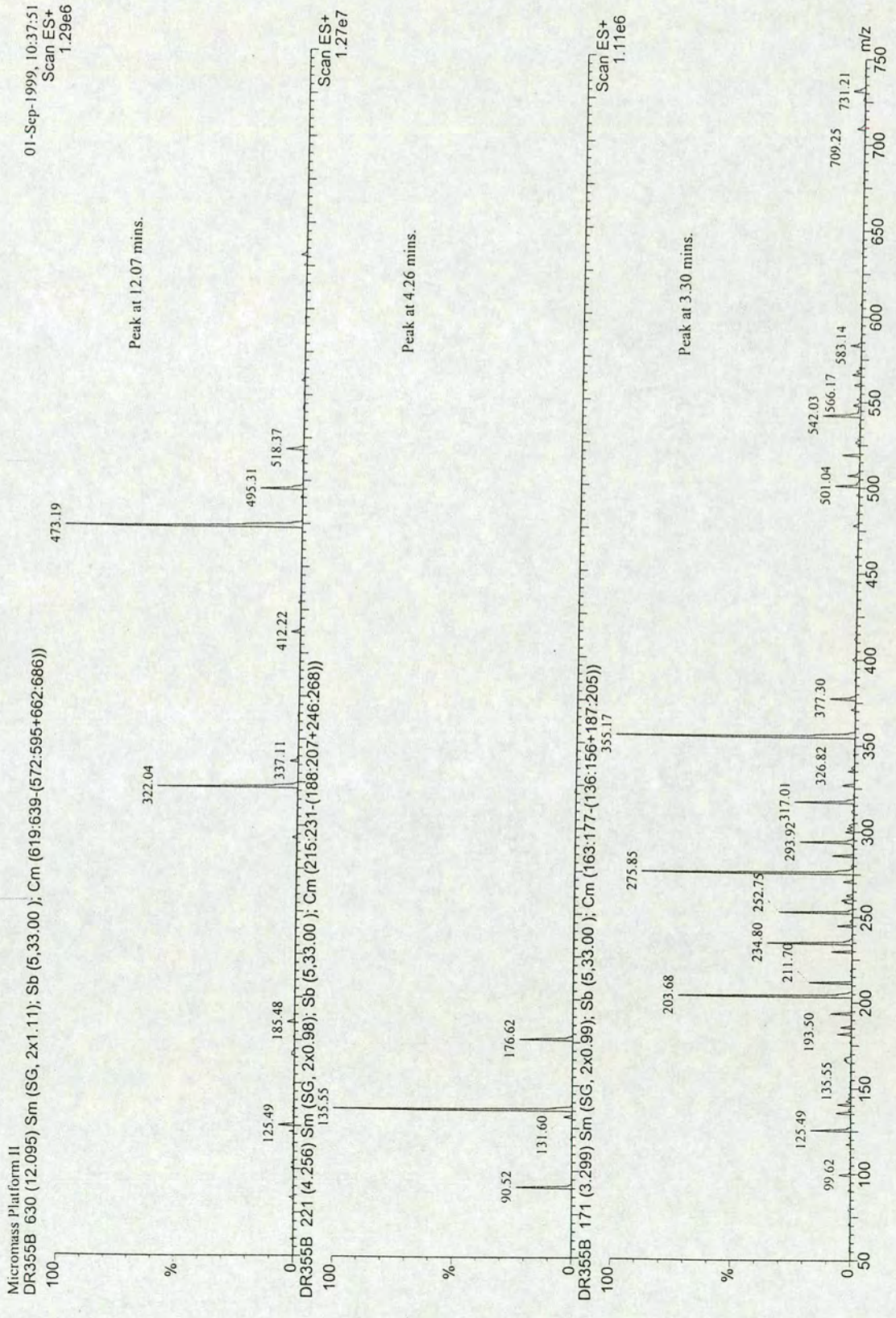


Figure 18b: *Mass spectrometry data for $PhCH_2CO_2H$.*

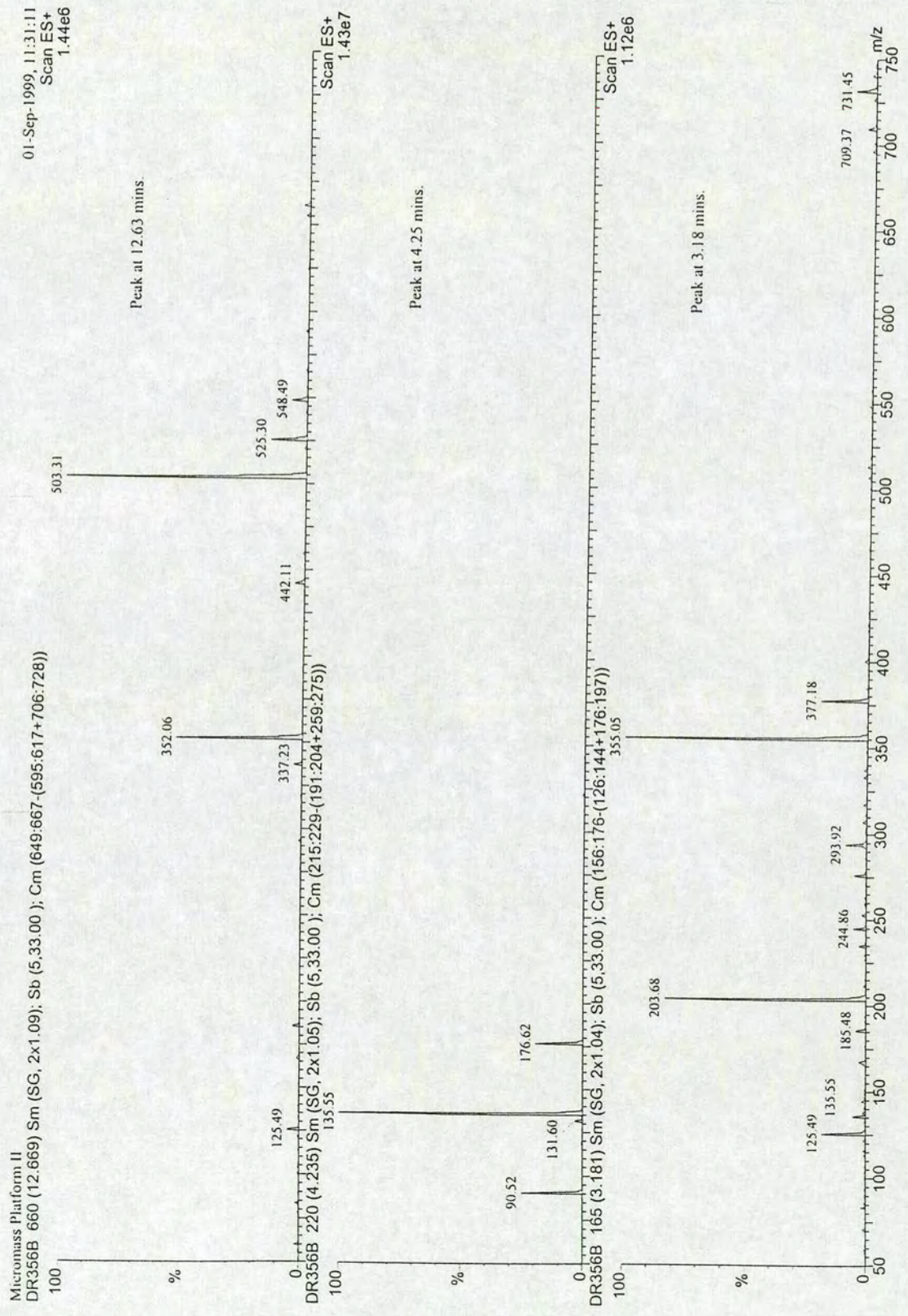


Figure 19b: *Mass spectrometry data for $MeOC_6H_4CH_2CO_2H$.*

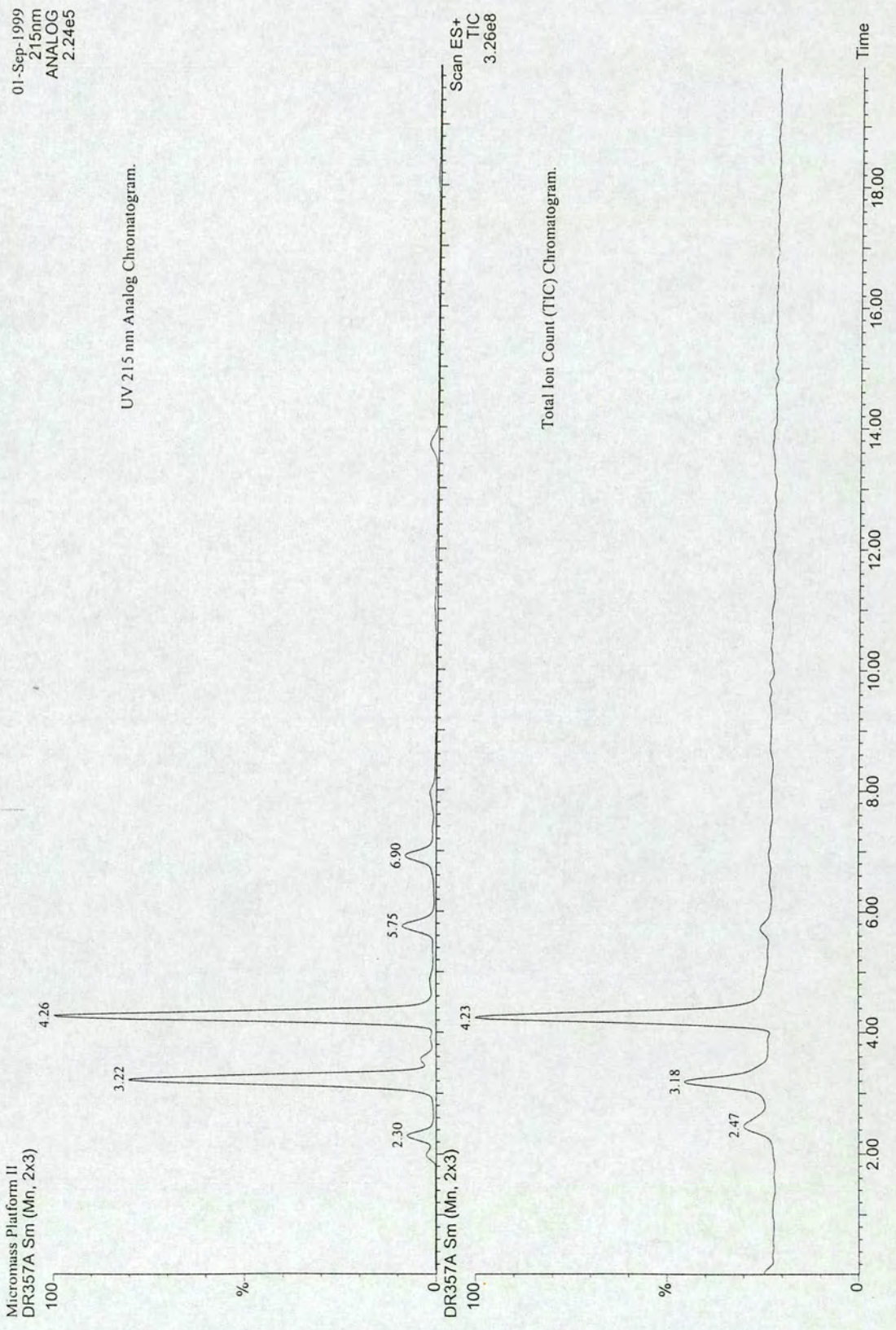


Figure 20a: *LC-MS chromatogram data for* $HOC_6H_4CH_2CO_2H$.

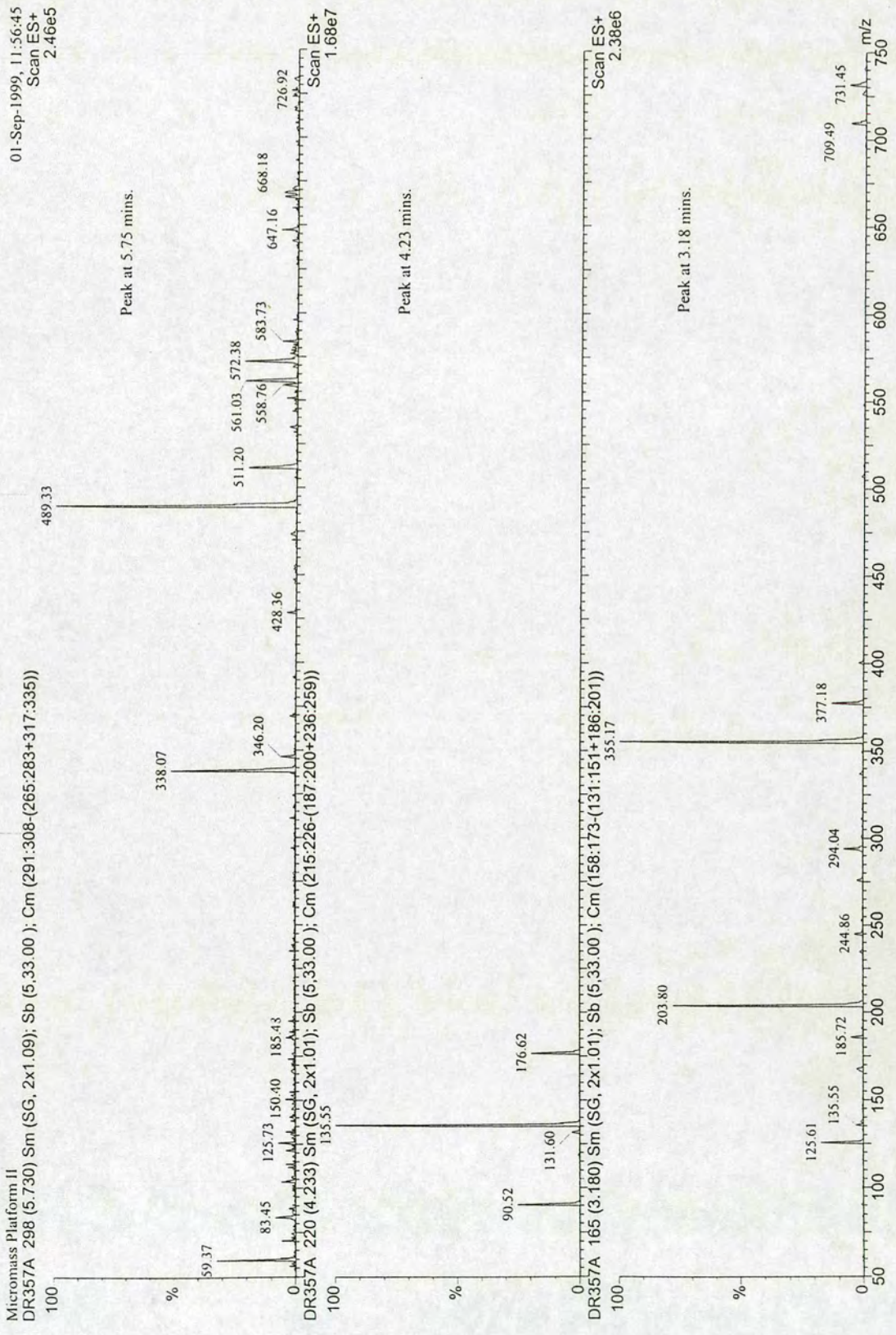


Figure 20b: *Mass spectrometry data for $HOC_6H_4CH_2CO_2H$.*

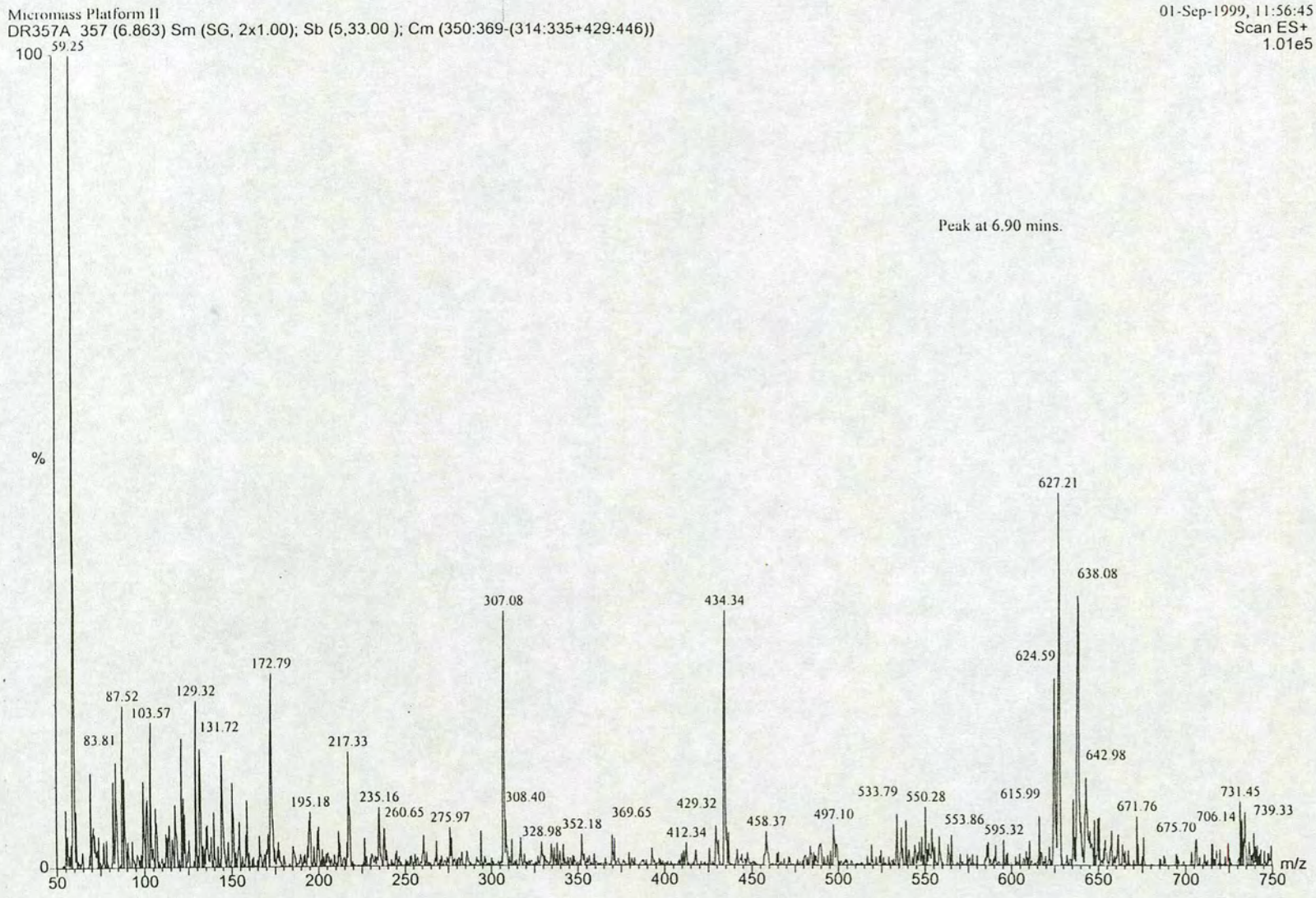


Figure 20c: *Mass spectrometry data for* $HOC_6H_4CH_2CO_2H$.

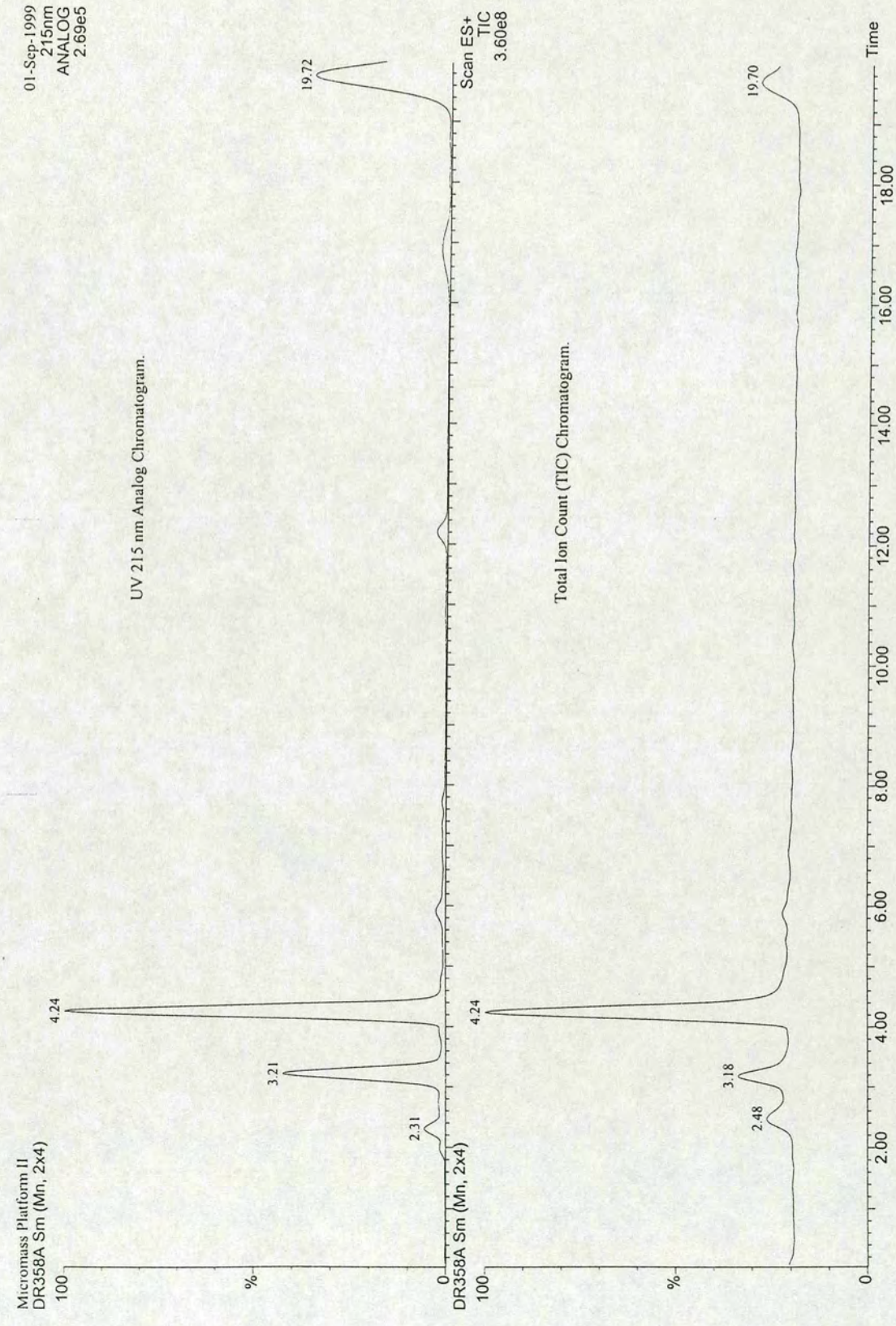


Figure 21a: *LC-MS chromatogram data for $CH_3C_6H_4CH_2CO_2H$.*

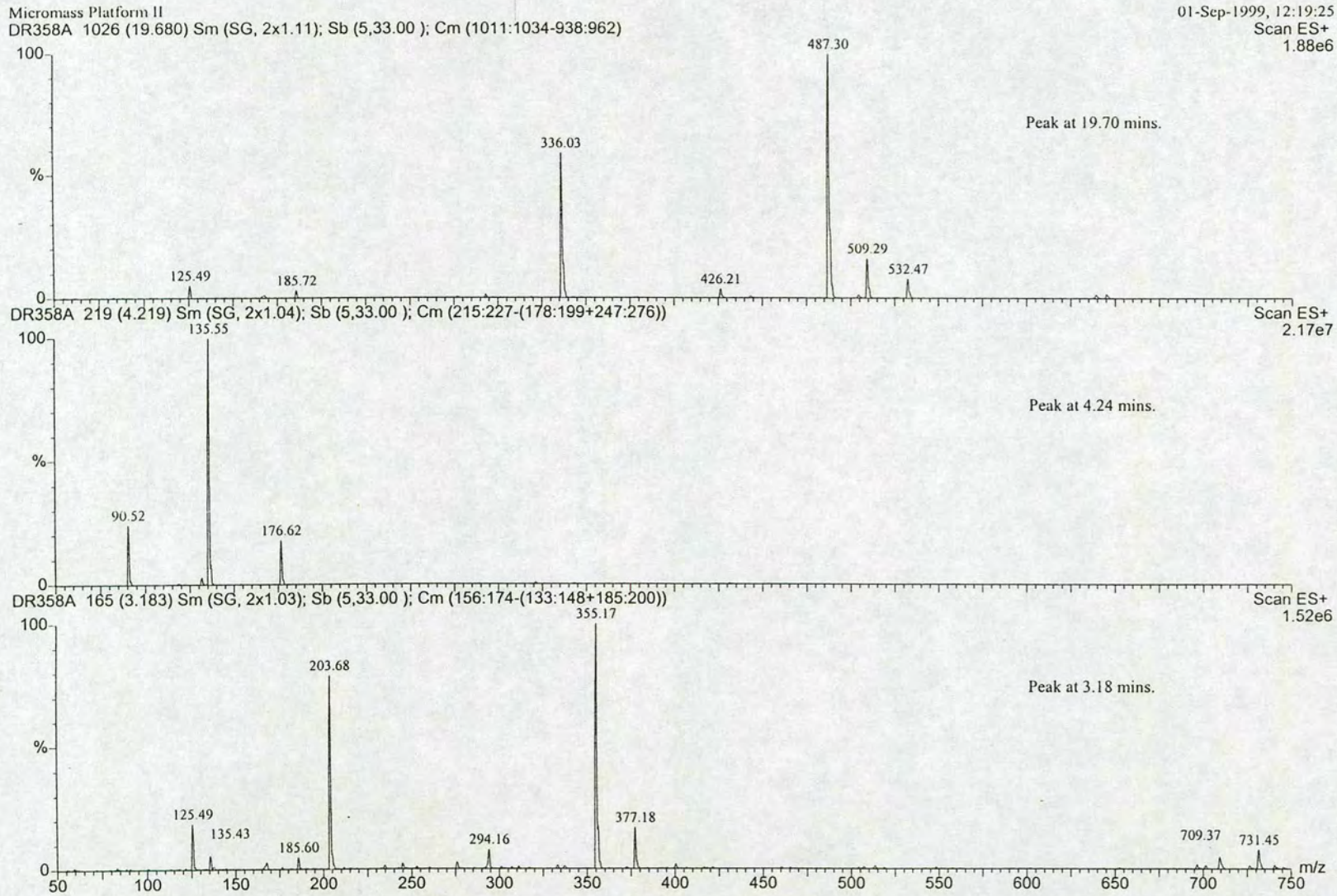


Figure 21b: *Mass spectrometry data for* $CH_3C_6H_4CH_2CO_2H$.

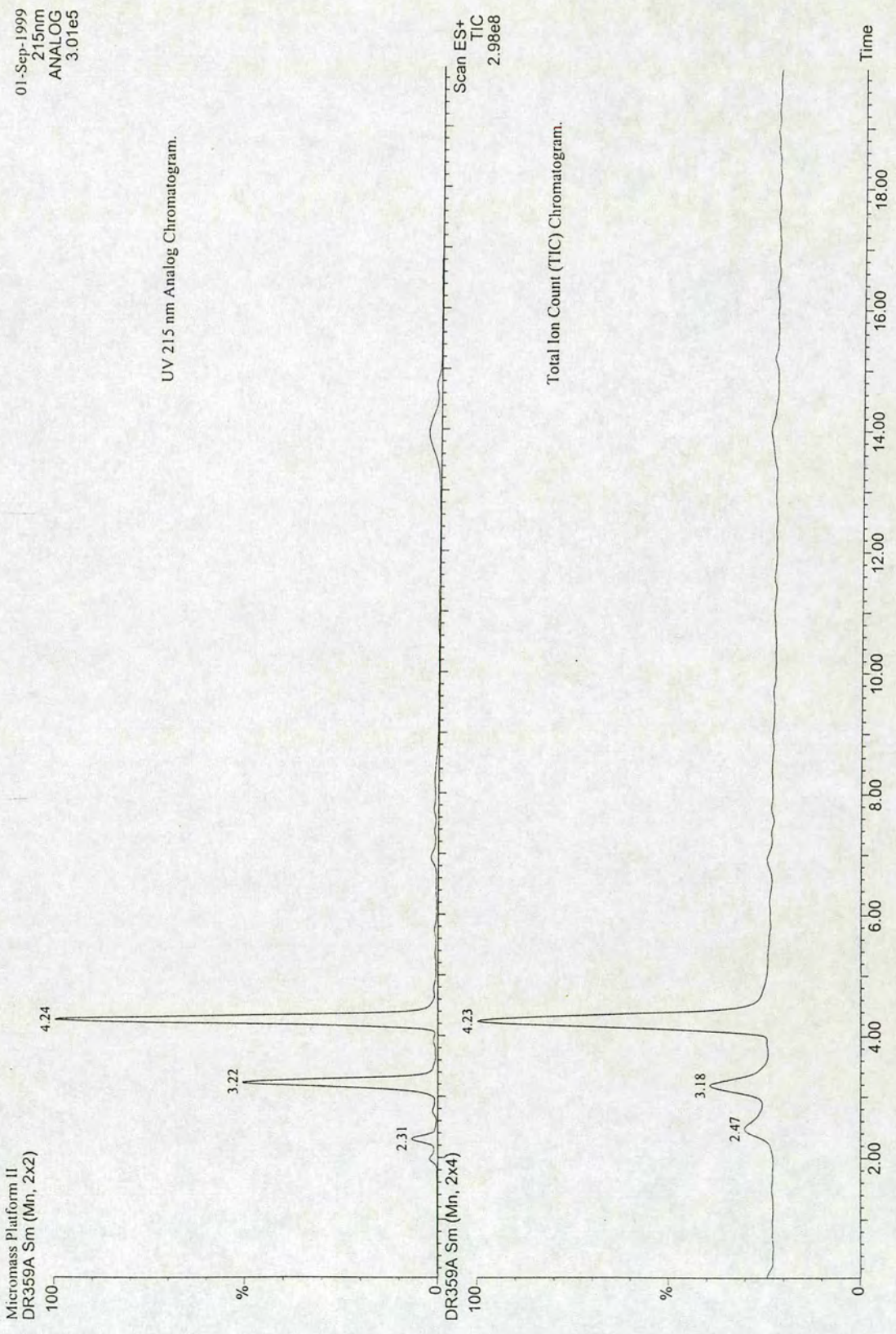


Figure 22a: *LC-MS chromatogram data for $ClC_6H_4CH_2CO_2H$.*

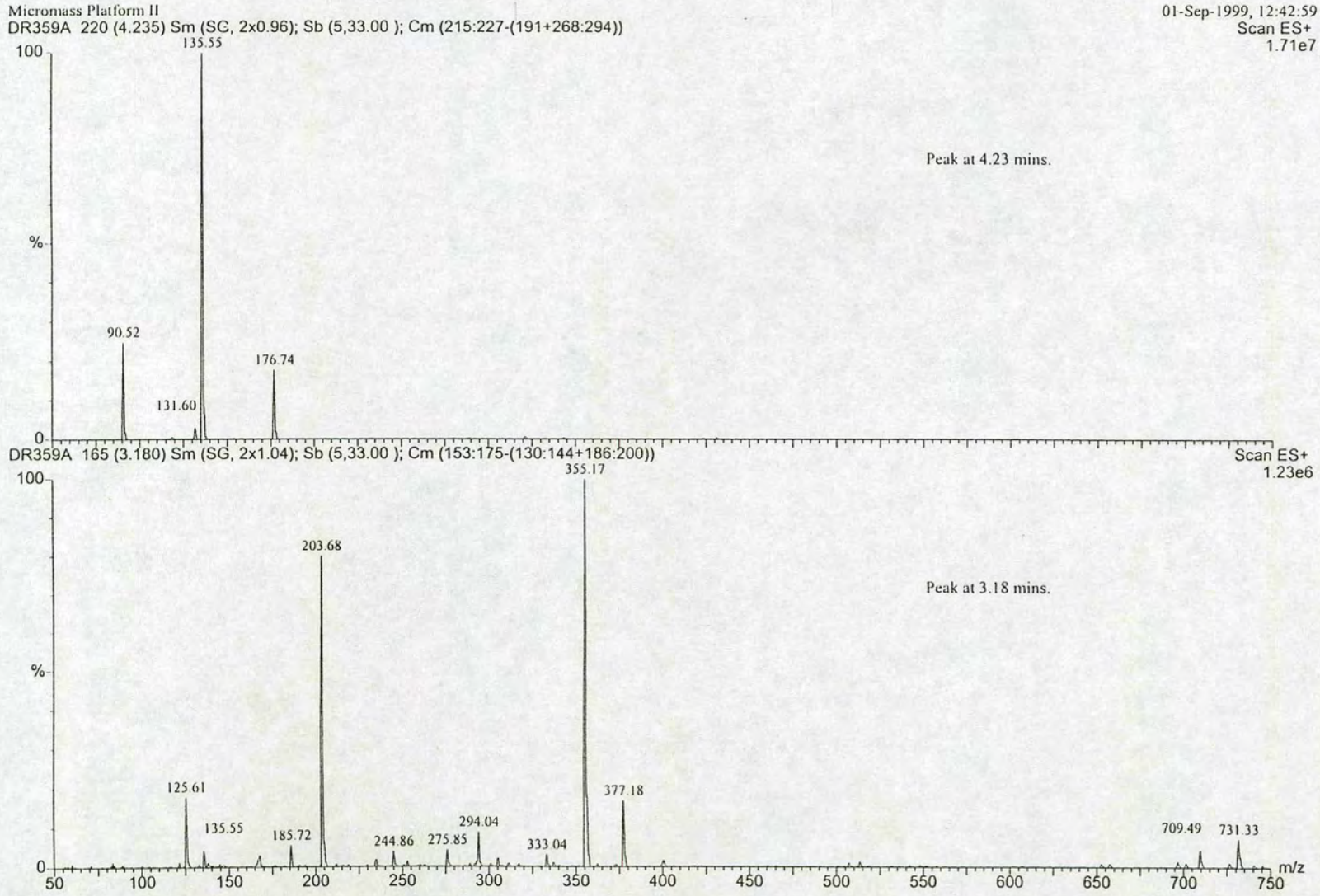


Figure 22b: *Mass spectrometry data for* $ClC_6H_4CH_2CO_2H$.

Pergamon

Tetrahedron Letters 39 (1998) 3819–3822

TETRAHEDRON LETTERS

A novel linker for the attachment of alcohols to solid supports

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Received 25 February 1998; accepted 13 March 1998

Abstract

A novel linker for the immobilisation of alcohols on solid support is described. This linker can be cleaved either enzymatically using penicillin amidase, or by very mild acid hydrolysis using 10% TFA.

Keywords: supported reactions; enzymes; alcohols, carbohydrates.

The recent surge of interest in solid-phase organic synthesis has resulted in the need for new solid-phase methodologies, in particular new linkers for functional groups other than amines and carboxylic acids [1]. Enzyme-cleavable linkers are particularly attractive because cleavage might be achieved under mild, neutral and aqueous conditions. Two reports have demonstrated that enzymes can be used to cleave molecules from solid supports using a phosphodiesterase [2] and an endopeptidase (chymotrypsin) [3]. A major drawback of both methods is that the compound released from the solid support retains part of the recognition site of the enzyme, *i.e.* a phosphate ester [2] and a peptide with C-terminal phenylalanine residues [3]. Although both 'tags' could in principle subsequently be cleaved with phosphatases or peptidases respectively, this would introduce several additional synthetic steps into the reaction sequences. Here we describe the design and synthesis of a more general linker, which can be cleaved either with penicillin amidase (EC 3.5.1.11), a commercially available and widely used enzyme [4] or by the use of dilute TFA, thereby complementing the existing range of alcohol linkers.

Penicillin amidase is known to catalyse the hydrolysis of a wide range of amines protected as the corresponding phenylacetyl derivatives and has also been used in peptide synthesis for the cleavage of cysteine protecting groups [5]. Thus, in order to incorporate the enzyme recognition site, the linker **1** was designed as shown in Scheme 1, in which -OR

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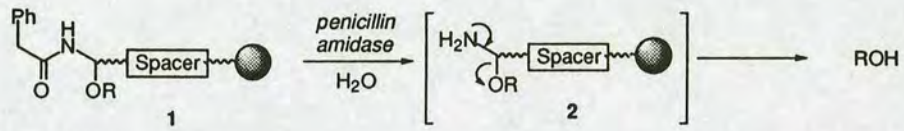
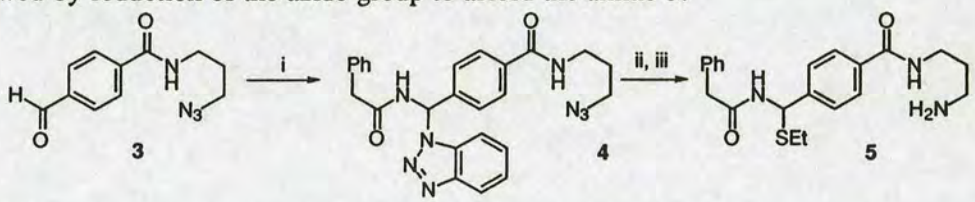
represents the alcohol group. It was envisaged that cleavage would be initiated by hydrolysis of the phenylacetamide moiety, generating the hemiaminal **2** which should easily fragment in aqueous medium releasing the alcohol ROH.



Scheme 1

An activated form of the linker was accessible using methodology developed by Katritzky *et al.* [6-8]. The benzotriazole derivative **4** was prepared in 67% yield by refluxing aldehyde **3** with benzotriazole and phenylacetamide in a Dean-Stark apparatus (Scheme 2). Although the benzotriazole is a good leaving group, and can be replaced by strong nucleophiles, we have found that the thioethyl group is more convenient for our purposes. The latter can be activated with a thiophilic reagent, such as *N*-iodosuccinimide, and is then susceptible to displacement by relatively poor nucleophiles such as secondary alcohols. The thioethyl derivative **5** was easily obtained in excellent yield by reaction of **4** with sodium ethanethiolate followed by reduction of the azide group to afford the amine **5**.



Scheme 2
(i) Phenylacetamide (1 eq), benzotriazole (1 eq), TsOH (0.2eq), toluene, reflux, Dean-Stark, 16h, 67%; (ii) sodium ethanethiolate (10 eq.), THF, RT, 4h, 96%; (iii) triphenylphosphine (1.2 eq), H_2O, THF, RT, 16h, 96%.

Amine **5** was then coupled to a variety of solid supports in reasonable to excellent yields (Scheme 3)[1]. A number of Tentagel® and PEGA® resins were investigated because of their compatibility with aqueous reaction conditions and because they had been reported to be suitable for enzyme-catalysed reactions [9]. Coupling yields were between 75% (TentaGel®-COOH) and 100% (PEGA®-COOH). Polystyrene was also successfully derivatised in quantitative yield by activating the resin as the acylfluoride[2] and should be useful when the

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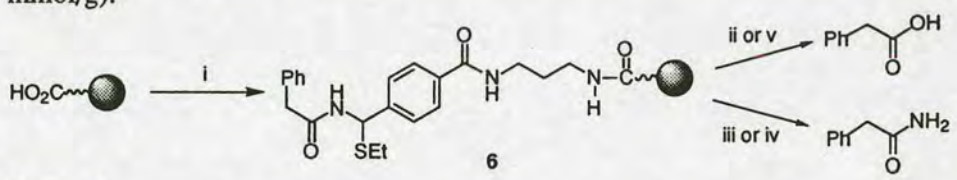
[1]**Typical procedure for the coupling of the linker 5 to solid supports** (for Tentagel and PEGA resins): A mixture of the resin (100 mg; loading 0.2 mmol/g), the linker **5** (50 mg, 0.13 mmol), TBTU (77 mg, 0.24 mmol), HOBt (32 mg, 0.24 mmol) and N,N-diisopropylethylamine (31 mg, 0.24 mmol) in DMF (2ml) was shaken for 16h at 25 °C. The resin was washed twice with 10 ml aliquots of THF, DMF, DMF:MeOH (1:1), DMF,THF and CH_2Cl_2 and dried.

[2]**Typical procedure for the coupling of the linker 5 to solid supports** (for polystyrene resin): Cyanuric fluoride (0.837 g, 6.2 mmol) was added (with caution) to a suspension of carboxypolystyrene resin (1.00 g; loading 1.24 mmol/g), and pyridine (0.200 ml, 2.48 mmol) in dichloromethane (5 ml) and the mixture was agitated on a blood rotator for 16h at 25 °C. The resin was washed with 10 ml aliquots of THF, DMF, THF and CH_2Cl_2 and dried in a vacuum oven (ν_{max} 1805 cm^{-1}). N,N-Diisopropylethylamine (0.432 ml, 2.48 mmol) was added to a suspension of the linker **5** (2.40 g, 6.23 mmol) and the acyl fluoride resin (1.00 g,1.24 mmol/g) in DMF (5ml) and was agitated on a blood rotator for 16h at 25 °C. The resin was washed twice with 10 ml aliquots of THF, DMF, DMF:MeOH (1:1), DMF,THF and CH_2Cl_2 and dried.

linker is used for non-aqueous chemistry, since the loading is generally higher (1.24 mmol/g).



Scheme 3
(i) **5** (6.5 eq), 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (12 eq), HOBt (12 eq), DIEA (12 eq), DMF, 16h, 25°C; (ii) 2M NaOH; (iii) 2M HCl; (iv) TFA:DCM:H_2O 9:10:1; (v) penicillin amidase.

The loading values of the linker on the resin were determined by hydrolytic cleavage[3] of the phenylacetamide group using either strong acid or base and quantitative determination by HPLC of the amount of phenylacetamide or phenylacetic acid released, respectively[4]. These loading values were then used to determine the efficiency of cleavage of the linker **6** using penicillin amidase[5]. It was found that penicillin amidase was indeed able to effect cleavage of **6**, although the yield of enzyme cleavage was strongly dependent on the resin used, ranging from 25% for PEGA® to a maximum yield of 50% for TentaGel®. We are currently attempting to improve these yields by varying the spacer arm of the linker and also by using tailor-made resins that are more compatible with enzyme catalysed reactions [10]. In view of the susceptibility of the linker to cleavage under mild acid conditions (aqueous TFA)[6], this protocol was used in subsequent experiments involving attachment of a range of alcohols.

The thioethyl group in **6** was activated by treatment with *N*-iodosuccinimide followed by displacement with a variety of alcohols **7** to **11** (Scheme 4)[7]. Fmoc-protected serine methylester **7** was found to couple in excellent yield to the polystyrene-linker and could also be cleaved quantitatively, as judged by HPLC analysis after acid release. Fmoc analysis of the resin bound **7** was in good agreement with the values derived from cleavage reactions. Secondary alcohols **8** and **9** gave good yields of coupling. These could possibly be improved by double-coupling methods, since analysis of phenylacetamide loading revealed that some of the linker had remained intact on the resin.

---

[3]**Chemical cleavage of the linker from solid support and determination of the loading:** (for Tentagel and PEGA resins): A suspension of the resin (2 mg) in 2M HCl (300 µl) or 2M NaOH (300 µl) was shaken for 16h at 25 °C. An aliquot of the supernatant solution was neutralised and analysed by HPLC by comparison with standard solutions.

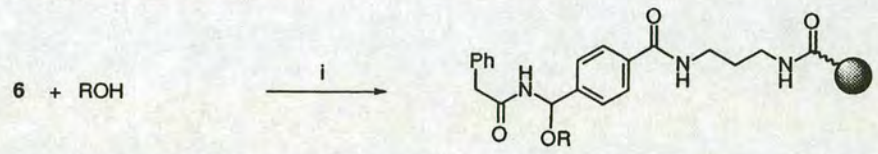
[4]**HPLC-analysis:** HPLC was carried out on a Waters HPLC system with a Phenomenex Sphereclone (5µm) ODS2 column (250 mm x 4.6 mm) using a gradient of two eluents (25 mM potassium phosphate buffer pH 6.5; acetonitrile).

[5]**Enzymatic cleavage of the linker from solid support:** A solution of penicillin amidase (700 units) in 0.1M potassium phosphate buffer (pH 7.5; 0.4 ml) was added to the resin (2 mg) and the mixture was shaken for 16h at 25 °C. The solution was removed, treated with 2 M HCl (0.5 ml) and extracted with CH_2Cl_2 (2 x 5 ml). The combined organic layers were concentrated, the remaining residue was dissolved in 300 µl methanol and analysed by HPLC.

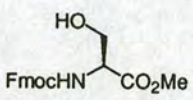
[6]**Cleavage of linker using mild acid conditions** (for polystyrene resins): A suspension of the resin (10 mg, 0.01 mmol) in CH_2Cl_2:TFA:H_2O (10:9:1 v/v, 4ml) was agitated for 3h. An aliquot (300 µl) was removed, concentrated under reduced pressure, dissolved into the same volume of MeOH and analysed by HPLC.

[7]**Typical procedure for the coupling of alcohols to 6:** A suspension of the resin bound linker **6** (50 mg, 0.05 mmol), alcohol (**7-11**) (0.25 mmol) and 4 Å molecular sieves in CH_2Cl_2 (2ml) was agitated on a blood rotator for 30 min. *N*-Iodosuccinimide (28 mg, 0.13 mmol) (and in the case of alcohols **8**, **10** and **11** 4µmol of triflic acid) was added and the suspension agitated for a further 16h. The resin was washed with twice with 3 ml aliquots of THF, DMF, DMF:MeOH (1:1), DMF, THF, CH_2Cl_2 and was separated from molecular sieves by decantation.

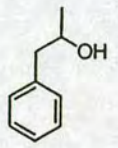
Since we were particularly interested in applying the linker to solid phase carbohydrate synthesis, the protected glucosides **10** and **11** were coupled to linker **6**. Yields of coupling were markedly improved to 75% and 80% respectively, by adding catalytic amounts of triflic acid (0.125 eq) to the NIS solution.



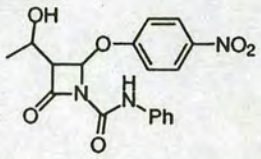
ROH (resin used; combined yields of coupling to **6** and subsequent cleavage) :



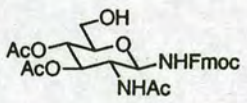
7 (Polystyrene; 70%. Tentagel; quantitative)



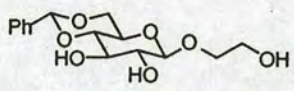
8 (Tentagel; 80%)



9 (Polystyrene; 62%)



10 (Tentagel; quantitative)



11 (Polystyrene; 80%)

Scheme 4
(i) ROH, *N*-Iodosuccinimide, CH_2Cl_2, RT, 16h.

In summary, we have developed an efficient synthesis of the linker **6** and have shown that a variety of alcohols can be coupled under mild conditions. The linker can be cleaved by penicillin amidase, although yields are currently restricted to 50%, or alternatively by quantitative mild acid cleavage. Both attachment to, and cleavage from, the linker can be achieved under much milder conditions than methodology based on dihydropyran-functionalised resins [11]. It should therefore be particularly useful for more acid labile compounds, such as carbohydrates and acid-labile protecting groups.

Acknowledgements This work was funded by the BBSRC and Genzyme Ltd. I.B. was supported by an EPSRC/GlaxoWellcome CASE award, A.H. by a BBSRC studentship and D.C.R. by The Edinburgh Centre for Protein Technology.

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FCR/ik/sep99.076
9 September 1999

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