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SYNTHESIS OF GLYCOPEPTIDOMIMETICS OF SIALYL LEWIS ^{X} AND INVESTIGATIONAL STUDIES OF NEW PROCESSES

Bradley J. Schmor

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ABSTRACT

The solution and solid-phase synthesis of *N*-linked fucopeptidomimetics of Sialyl Lewis^x is described. The syntheses were successful to varying extents. It was determined that *t*-butyl deprotections are not compatible with the *N*-glycosidic linkage. This was overcome by the use of benzyl groups. A second series of syntheses were undertaken, involving tartaric acid as the hydroxyacid tail of the molecules. This convergent route was much more rapid, and molecular modelling studies suggested a good congruence with known binding conformations of the natural ligand. *C*-linked mimetics were also attempted, employing tartaric acid again as the polar tail source. Olefin cross-metathesis was also explored. The work in this area ultimately led to the discovery of a new palladium catalyzed phenyl transfer reaction from antimony.

Glycosyl 1,2,3-triazoles were synthesized as glycosylproline analogues. The relative ratios of isomers were determined, and the structure of one analogue proven by Xray crystallography. Deprotection protocols were compatible with the triazolyl moiety.

α/β selectivity was observed in coupling protected fucosylamine with amino acids. The scope of the selectivity was examined in related systems. In addition, α/β anomerization as catalyzed by protic acid was examined, and some insights about the mechanism can be gleaned from the results.

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LIST OF ABBREVIATIONS

Note: The three-letter codes for the 20 common amino acids are used throughout this work. Their abbreviations are not listed.

α_D optical rotation

Ac acetyl

Alloc allyloxycarbonyl

Ar aryl

Bn benzyl

Boc *t*-butoxycarbonyl

bs broad singlet

CBz carbobenzyloxy

CI chemical ionization

CSA camphor sulfonic acid

d doublet

dd doublet of doublets

DBU 7,11-diazabicycloundecene

DCC dicyclohexylcarbodiimide

dec. decomposes

DCM dichloromethane

DEPT Distortionless Enhancement of NMR signals by Polarization Transfer

DIBAL-H diisobutyl aluminum hydride

DIC diisopropylcarbodiimide

DIPEA diisoproylethylamine

DMAP 4-dimethylaminopyridine

DMF *N,N*-dimethylformamide

DMSO dimethyl sulfoxide

dq doublet of quartets

EDC 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide

EI electron impact

eq. equivalent

ESI electrospray ionization

FAB fast atom bombardment

Fmoc 9-fluorenylmethoxycarbonyl

FT-IR Fourier transform infrared spectroscopy

Fuc D-fucose

GlcNAc D-*N*-acetyl-2-amino-2-deoxyglucose

HATU *O*-(7-Azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate

HMRS high resolution mass spectroscopy

Hz Hertz

IC_{50} concentration for 50% inhibition

Imid. imidizolyl

J coupling constant

M molecular ion

m/z mass to charge ratio

Man mannose

mol moles

m.p. melting point

M.S. mass spectroscopy

NeuAc *N*-acetylneuraminic acid

NHS *N*-hydroxysuccinimide

NIS *N*-iodosuccinimide

NMM *N*-methylmorpholine

NMR nuclear magnetic resonance spectroscopy

ppm parts per million

PTC phase-transfer catalysis

pyr pyridine

q quartet

s singlet

sLe^x Sialyl $Lewis^x$

t triplet

tart tartarate

TEA triethylamine

Tf trifluoromethane sulfonate (triflate)

TFA trifluoroacetic acid

THF tetrahydrofuran

TLC thin layer chromatography

TMS trimethylsilyl or tetramethylsilane

Trt triphenylmethyl (trityl)

1. Introduction

1.1 Selectins, Sialyl Lewis^x and their Roles in Inflammation

Sialyl Lewis^x (sLe^x, Figure **1.1**) is a tetrasaccharide that is central to inflammatory response, acting to bring leukocytes to points of injury and cause extravasation. This process is common to many conditions, involving such widely separated sources of inflammation as infection, injury, and autoimmune diseases.[1]

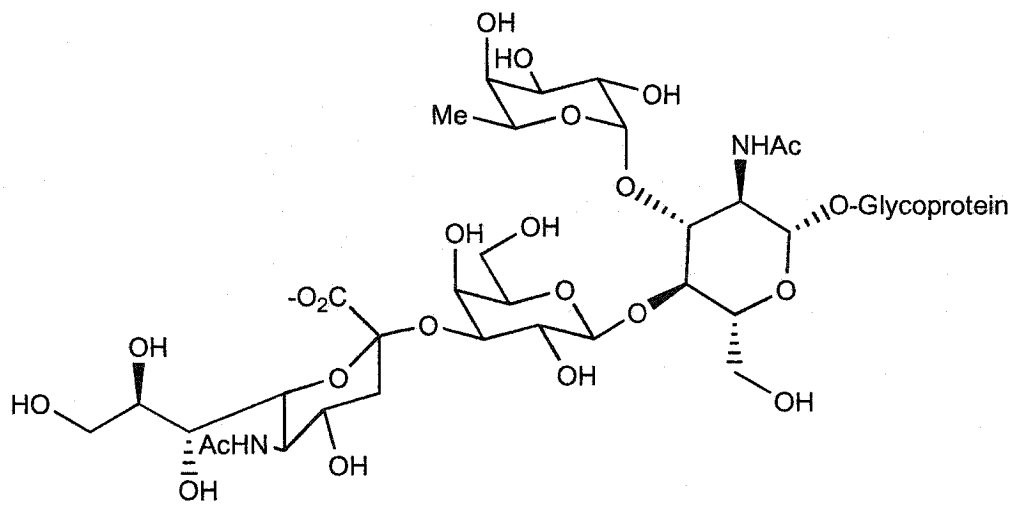

Figure **1.1**: Glycoprotein-Bound Sialyl Lewis^x

Mimetics of sLe^x which are intended to have increased *in vivo* stability, higher activity and simplified structure have been a major research area in recent years,[2,3] with possible therapeutic applications. Herein is discussed the role played by sLe^x and the efforts to afford useful mimetics thereof.

1.1.1 Carbohydrates: Biological Roles and Therapeutic Potential

Carbohydrate research has experienced a renaissance in recent decades, as their role has expanded well beyond the traditional conception, which confined carbohydrates to roles as energy sources and structural material. They have been found to mediate a wide array of biological functions,[1,4,5] as depicted in Figure **1.2**.

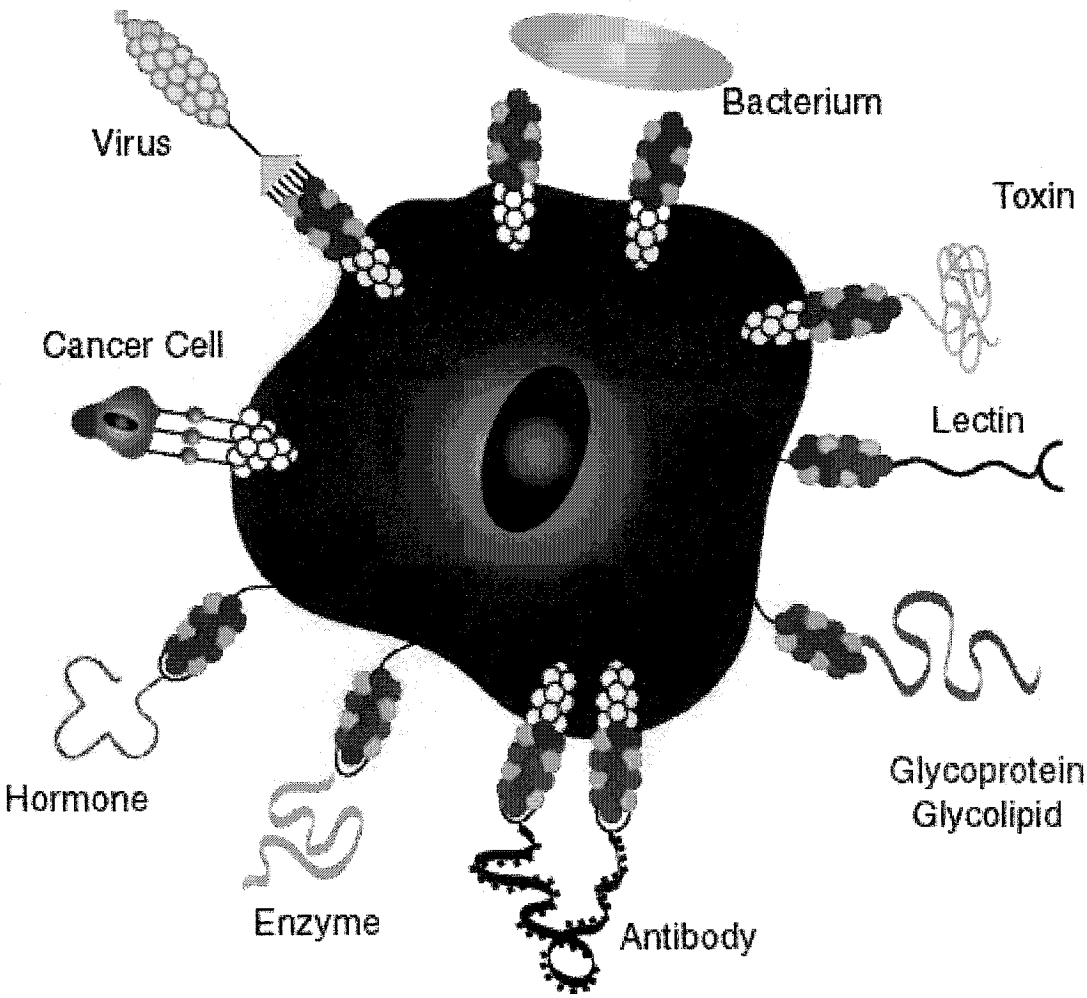

Figure **1.2**: Carbohydrates are Involved in Many Biological Functions

Cellular adhesion processes are largely governed by carbohydrate-carbohydrate or carbohydrate-protein interactions.[1,6,7] To either enhance or prevent certain adhesion processes would be of great benefit; if carbohydrate binding is a key element to a particular condition or disease, it thus follows that control of binding is control of the condition or disease in question.

Carbohydrates themselves are not good candidates for drugs.[8] They are difficult to synthesize,[4] even more difficult to purify, and have poor binding affinity and bioavailability properties.[2] In addition, carbohydrates are not normally stable *in vivo*, being rapidly degraded by a host of enzymes.[2] Hence, therapeutics mimicking the pharmacophore of carbohydrates are desirable synthetic targets.

1.1.2 The Inflammatory Cascade

Selectins are glycoproteins that bind to sLe^x and other carbohydrate structures.[2,9] They, in addition to leukocytes, are the primary players in the process that leads to acute and chronic inflammatory response.[2] Ultimately, inflammation results from overrecruitment of leukocytes to the site of injury. The process by which this occurs is known as the inflammatory cascade. Selectin-carbohydrate interactions dominate the process.[10-12]

The inflammatory cascade (Figure **1.3**) begins with release of cytokines, which instruct for the expression of E-[13] and P-selectin[14] on the endothelium surface. Leukocytes, bearing surface-bound sLe^x, are bound by selectins causing adherence to

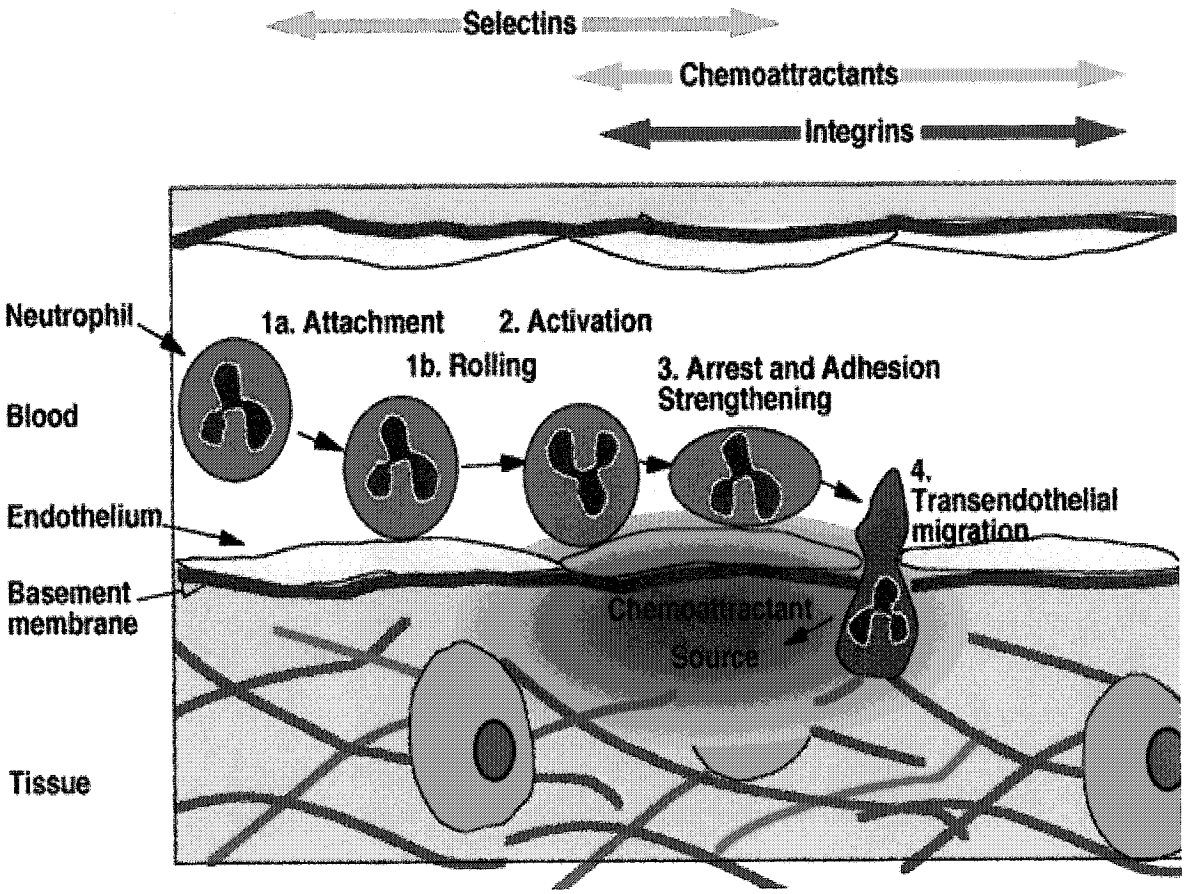

Figure **1.3**: The Inflammatory Cascade

the endothelial lining (**1a**).[10,11] L-selectin is expressed on the surface of the leukocytes, and binds to similar ligands on the endothelium.[15] Rolling (**1b**) of the leukocytes occurs as integrins on the surface of the leukocytes bind to an endothelial protein ICAM-1.[16] As the leukocytes come into closer proximity to the endothelium,

binding interactions become stronger (**2b**). L-Selectin is cleaved from the leukocyte (**3**) and extravasation occurs (**4**).[17] The accumulation of leukocytes is the principle cause of inflammation.[2]

sLe^x serves as the principle carbohydrate-based portion of binding which compliments the Selectins.[2,3,18] The sLe^x-E-Selectin binding is the general value by which potential therapeutic potencies are measured, since E-Selectin tends to be the most specific for sLe^x.[2] Disruption of the sLe^x-Selectin binding is sufficient to interrupt the cascade of events leading to inflammation.[2,19]

1.1.3 Sialyl Lewis^x as a Therapeutic

The design of selectin-binding molecules to confuse and thus prevent binding of leukocytes is a principle research area.[2,3] The concept has been proven; free sLe^x has seen use as an antiinflammatory and functions as predicted.[19] However, sLe^x suffers from many drawbacks as a drug:

1) Cost: Although sLe^x can be synthesized on a kilogram scale in a single reaction vessel via a complex 10-enzyme system,[20] it remains very expensive.

2) Low binding affinity: sLe^x (IC_{50} = 1 mM) has a poor binding affinity;[21] thus, large dosage is required.

3) Bioavailability and Degradation: sLe^x must be administered intravenously. The *in vivo* lifetime is short, and repeated injections are necessary.[2]

Therefore, sLe^x in itself is not a suitable drug; compounds that duplicate its biological role while resisting enzymatic degradation are considered worthy targets. This field of work has been enormous.[2]

1.2 Sialyl Lewis^x-Based Inhibitors

Several other syntheses of sLe^x have appeared,[22-26] although none are commercially viable. A great deal of work has been undertaken to understand the structural features responsible for biological activity (the pharmacophore).[27,28]

1.2.1 Structure-Activity Relationship Studies of Sialyl Lewis^x

Structure-activity relationship studies have produced a map, outlining the key functional groups necessary for binding (Figure **1.4**).[29,30] The calcium-dependant binding involves the fucose ring, the 6-OH group of galactose, and the acid tail of *N*-acetylneuraminic acid (often called sialic acid). Most attempts that disrupt the fucose moiety lead to total loss of binding.[2]

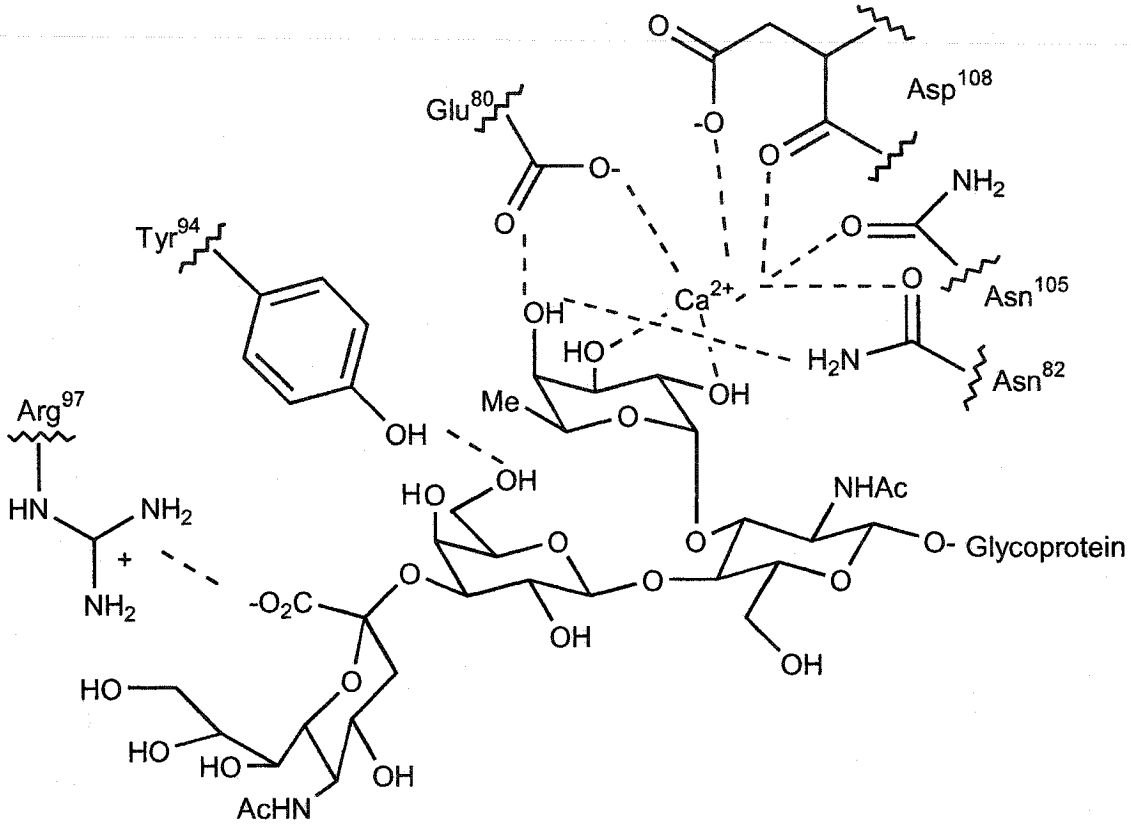

Figure **1.4**: The E-Selectin Binding Domain of sLe^x

1.2.2 Oligosaccharides

Replacement of carbohydrate residues with simple functional groups has been explored. One simple substitution is the NeuAc residue for another anionic functional group. Work in this area[31] has demonstrated that replacement of the 3-OH group of galactose with a sulfate group leads to a molecule with binding affinity similar to sLe^x. Replacement with a phosphate group leads to a 10 x gain in binding affinity towards E-selectin. Replacement with a methylene carboxylate lead to complete loss of activity (Figure **1.5**).

R = NeuAc (sLe^x) IC_{50} = 1 mM
SO_3^- IC_{50} = 0.28 mM
PO_4^{2-} IC_{50} = 0.10 mM
$CH_2CO_2^-$ IC_{50} >10 mM

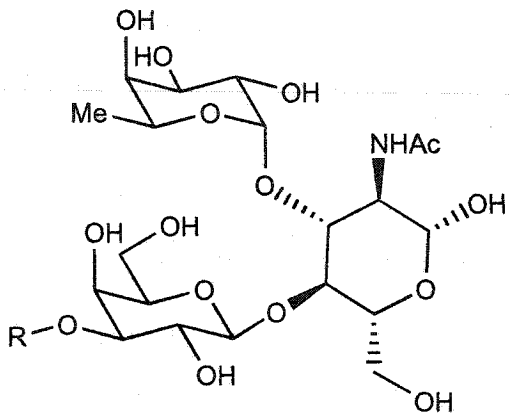

Figure **1.5**: Effect of NeuAc Replacement of sLe^x [31]

Other studies have been undertaken which replace the *N*-acetylglucosamine with a suitable *trans*- ring junction.[32] Most attempts that do not have a 1,2-trans-cyclohexane ring show dramatic drops in binding affinity.[8]

1.2.3 Glycopeptides

A vast number of glycopeptide or glycopeptide-like molecules linked to (L)-fucose have been reported.[2,33-35,] A handful of mimetics have also been reported using much less expensive and more reliably manipulated (D)-mannose,[26,37] which bears an array of hydroxyl groups similarly arranged to (L)-fucose (Figure **1.6**).

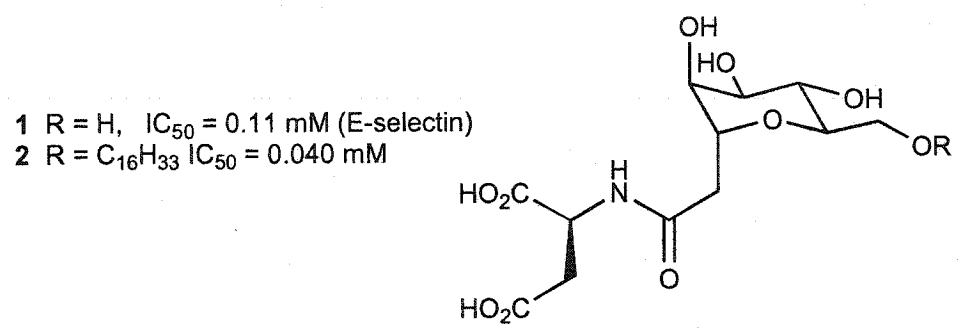

Figure **1.6** A (D)-Mannose Based Glycopeptidomimetic

Compounds **1** and **2** also demonstrate a commonly encountered effect: addition of a hydrophobic tail onto the molecule increases affinity. This hydrophobic effect can be explained as evidence of multivalent potency: hydrophobic interactions lead to clustering of molecules.[2,3] When in close proximity, multiple bindings can occur, leading to a great increase in binding. This is explored further in section **1.2.4.**

Fucose-based mimetics are the most common type. Fucopeptidomimetics sporting a *C*, *O*, or *S* linkage are all known.[2,33,34,38] Discussion of the differing glycosidic linkage can be found in **1.3.** Wong et al have reported some of the most potent sLe^x mimetics (Figure **1.7**).[39] Many are more potent than sLe^x itself. Interestingly, the β-configured anomer of **3** is more potent than its α-counterpart.

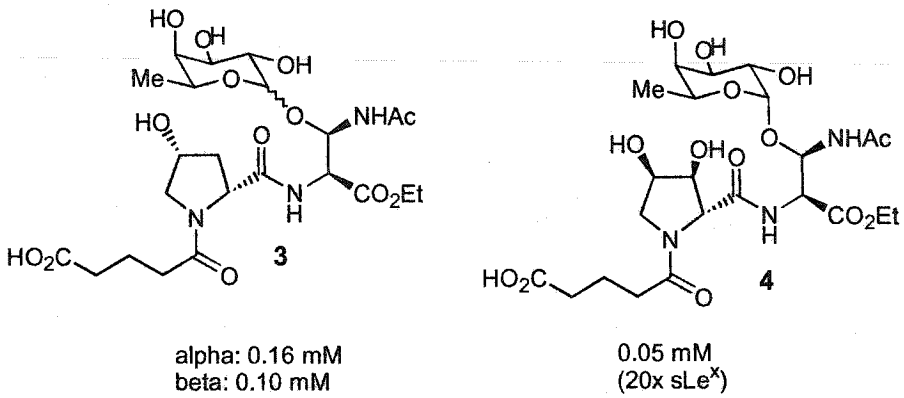

Figure **1.7** Some of the Most Potent sLe^x Mimetics

1.2.4 Polyvalency

The use of polyvalency by Nature to overcome low binding affinities is well established.[40] Attachment of active molecules to a polymeric scaffold can increase binding affinity by many times, measured in a *per residue* manner. This has been exploited by a number of research groups.[2,41-45] Roy has polymerized 3′-sulfo $Lewis^x$ monomer units incorporating a glucose residue in place of *N*-acetylglucosamine from the corresponding acrylamide-linked monomer[46] (Figure **1.8**).

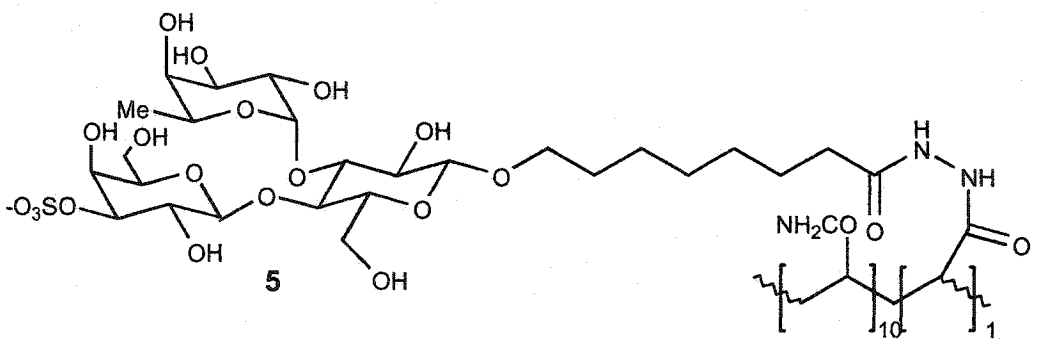

Figure **1.8**: Polyacrylamide-Bearing sLe^x

1.3 Different Approaches to Sial yl Lewis^x Mimetics: The Glycosidic Linkage

The literature contains many examples of fucopeptidomimetics based on α-*O*-fucosyl linkages.[2,33,38] Sialyl Lewis^x (sLe^x) itself contains this type of linkage; however, this linkage has been demonstrated to be prone to rapid enzymatic hydrolysis *in vivo*, leading to cleavage of the fucose residue from the remainder of the molecule in a matter of minutes, thus terminating activity.[47] In spite of this, many research groups continue to pursue this avenue, due to the ease of introduction of the linkage and the degree to which the linkage imparts geometry similar to the natural ligand.[48]

The introduction of *C*-linked mimetics[34,49] solved most of these problems, since the linkage has many available methods of introduction, is chemically stable, and impervious to most enzymatic processes (see Chapter **1.3.4** for a more thorough treatment of this topic). Tremendous work has been performed following this line of mimetics, and very high activities have been reported.[2] However, one could imagine that molecules having very long *in vivo* lifetimes could potentially accumulate, possibly causing toxicity problems. Hence, a class of compounds with an intermediate lifetime under physiological conditions is in need of development.

1.3.1 *N*-Linkages as an Alternative to *O*, *C* and *S* Linkages

N-glycosides have curiously been neglected from the vast field of research that sLe^x mimetics has become. To date, many hundreds (if not thousands)[2] of

fucopeptidomimetics of sLe^x have been reported in the literature. However, not a single example reported to date contains an *N*-fucosyl linkage. There are several reasons why we would wish to pursue *N*-glycosidic linkages instead of *C*, *O*, or *S* linkages, but there are also reasons why this strategy may have been avoided in the past.

1.3.2 *N*-Glycosides in Nature

N-glycosides are a peculiarity in carbohydrate chemistry.[50] Practically all known examples (outside of nucleotides) in natural systems involve an *N*-linkage to glucose or *N*-acetylglucosamine (GlcNAc) to the side chain of asparagine (Asn) in proteins or oligopeptides, and in nearly all cases, this linkage is only observed in systems where Asn is involved in the sequence Asn-Xxx-Ser/Thr.[51] Only a few exceptions are known, one example being nephritogenoside **6**, where the required Ser/Thr residue is replaced with a Leu residue (see Figure **1.9**).[52]

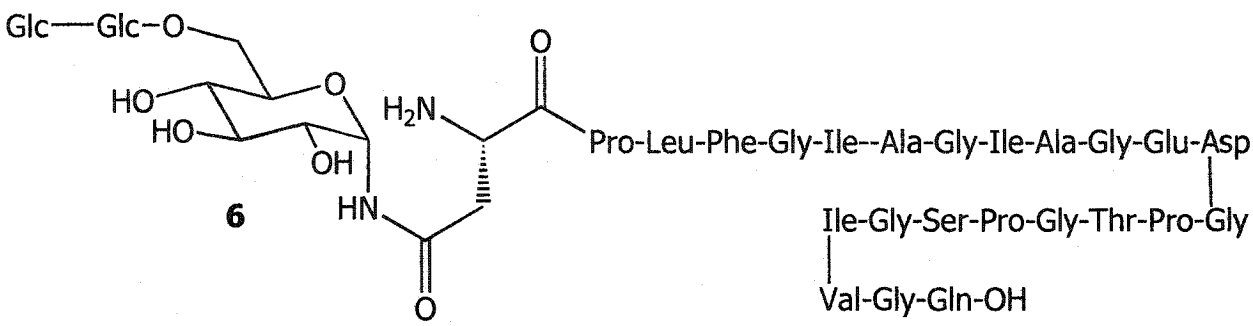

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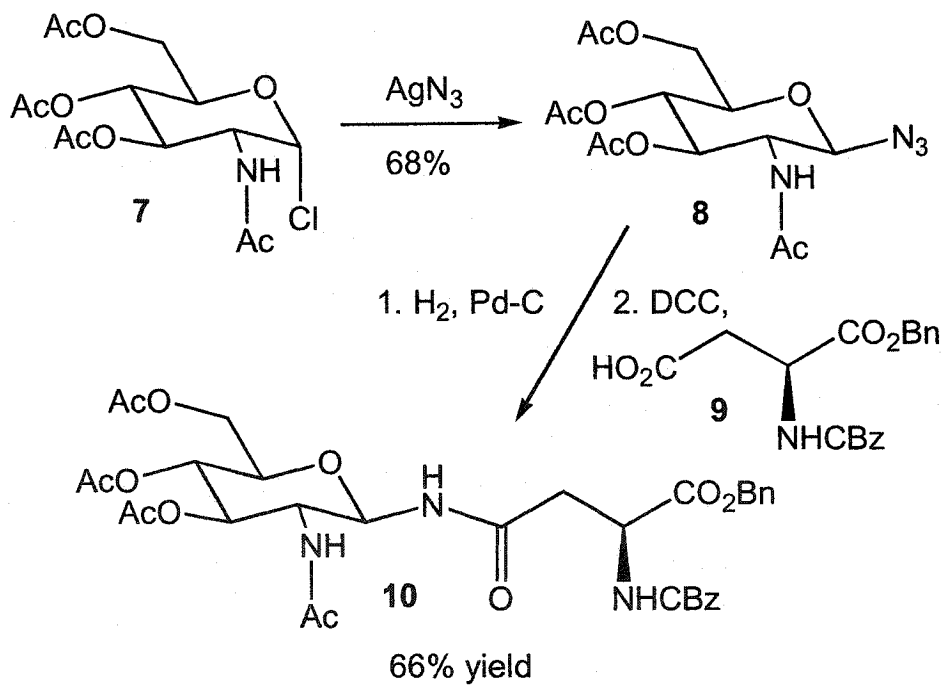
Figure **1.9**: Nephritogenoside, an *N*-linked glycopeptide.

Other examples are known, but are rare.[53,54]

1.3.3 Synthetic Approaches to *N*-Glycosides

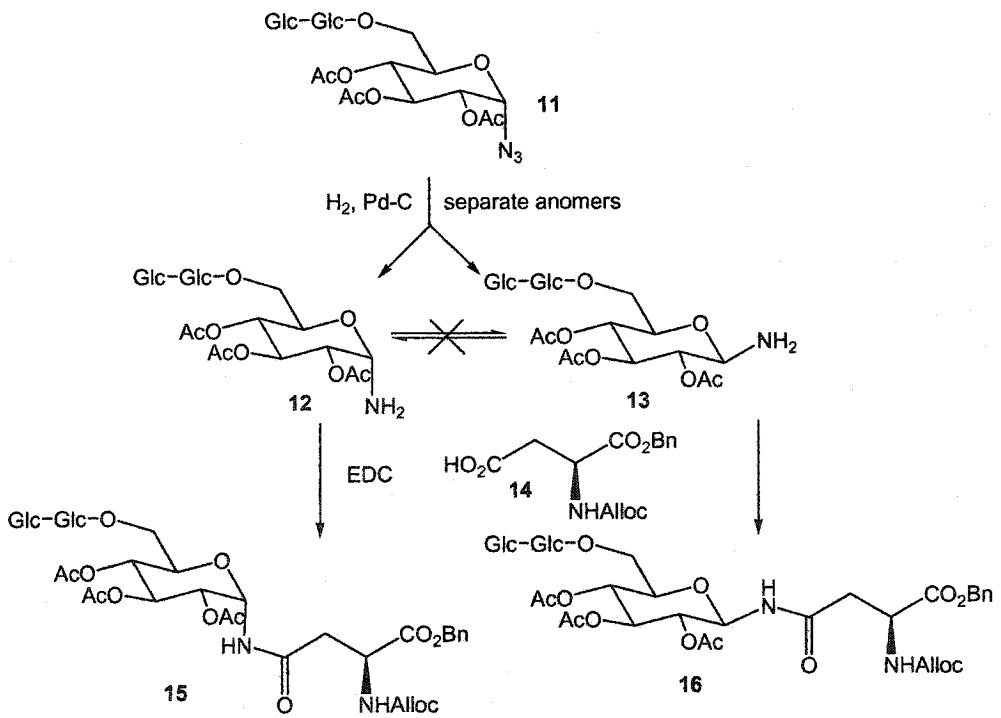
Several methods for the preparation of *N*-glycosides are reported in the literature.[51,55] The most common strategy is to prepare and subsequently reduce a glycosyl azide, which can be produced through displacement of glycosyl halides with azide ion, and then acylate the glycosyl amine with varying active species. Early examples employed silver azide to give the β-azide compound **8**; reduction and coupling using dicyclohexylcarbodiimide (DCC) gave exclusively the β-amide **10** in reasonable yield (Scheme **1.1**).[56,57]



Scheme **1.1**: Traditional Formation of *N*-Glycosidic Linkages.

However, this method suffers from a drawback. Anomerization of the intermediate glycosyl amine tends to occur concomitantly with reduction, and anomeric mixtures

normally result. This normally favors the β-anomer, irrespective of the stereochemistry of the azide.[52] This would appear at first glance to be analogous to the anomerization of free sugars, but it has been revealed that anomerization occurs during the reduction, and not subsequently, at least in some cases.[58,59] Takeda et al[58] found that reduction of a glycosyl azide gave both anomers **12** and **13**, as expected, but the two anomers were separable by column chromatography and remained anomerically stable during purification and subsequent exposure to coupling conditions. Each purified anomer gave only its respective amide, demonstrating that anomerization does not occur after the reduction (Scheme **1.2**). However, in other cases, reduction in the presence of an acid anhydride has prevented anomerization, suggesting that in such cases the scrambling occurs *after* the reduction step.[60]



Scheme **1.2** Separated Glycosyl Amines do not Interconvert, and can be Cleanly Acylated without Anomerization.

1.3.4 The Reverse-Anomeric Effect

As opposed to the well-established anomeric effect[61], the hypothetical reverse-anomeric effect states that the equilibrium position of *N*-linked glycosides can be shifted dependent on pH. Lemieux[62] showed that the equilibrium position of an imidazole-substituted (D)-xylose derivative was dependent on pH of the system; basic pH (at which the ring bears no charge) lead to anomeric effect-inducing axial configurations (Figure **1.10**), but acidic pH (with ring protonation) push the equilibrium to the equatorial configuration. Since there are no steric changes to the system, only stereoelectronic arguments are available to explain the phenomenon.

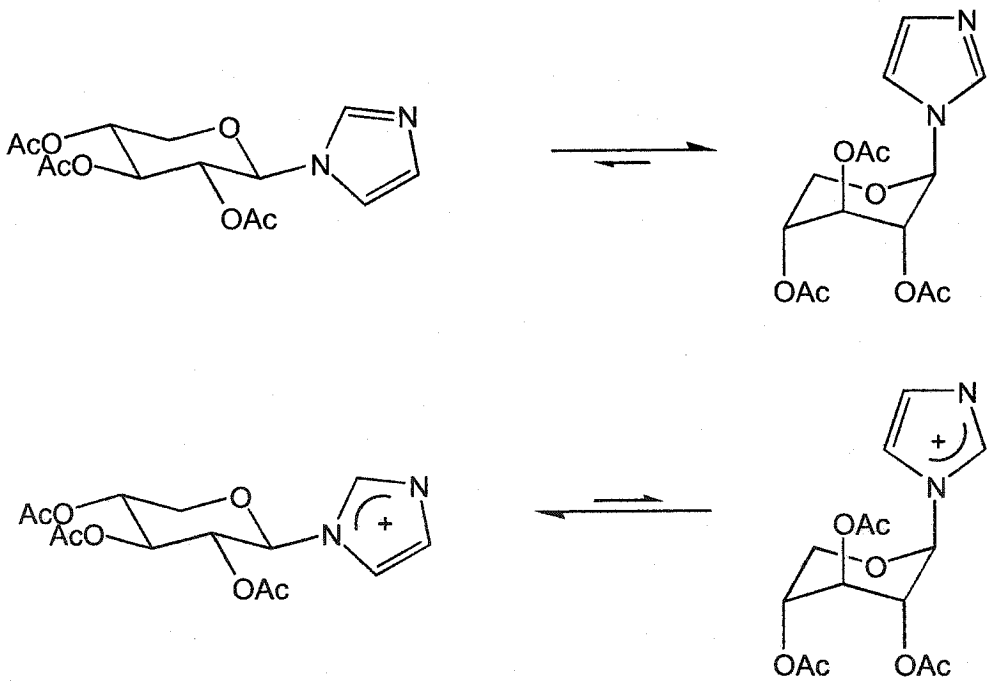
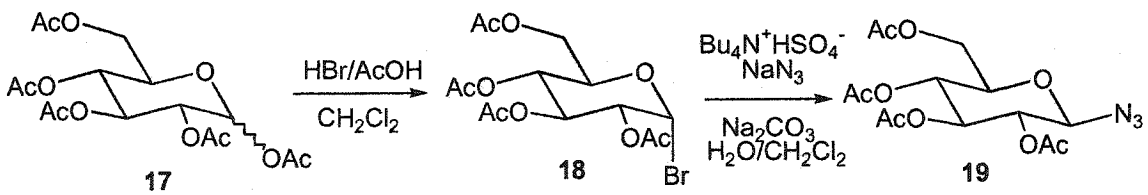


Figure **1.10**: The Reverse-Anomeric Effect is Postulated to Explain the Preference for the Equatorial Configuration of *N*-Glycosides at Acidic pH.[62]

However, exceptions and anomalies continue to exist, and there is doubt about the reality of the reverse anomeric effect.[63,64]

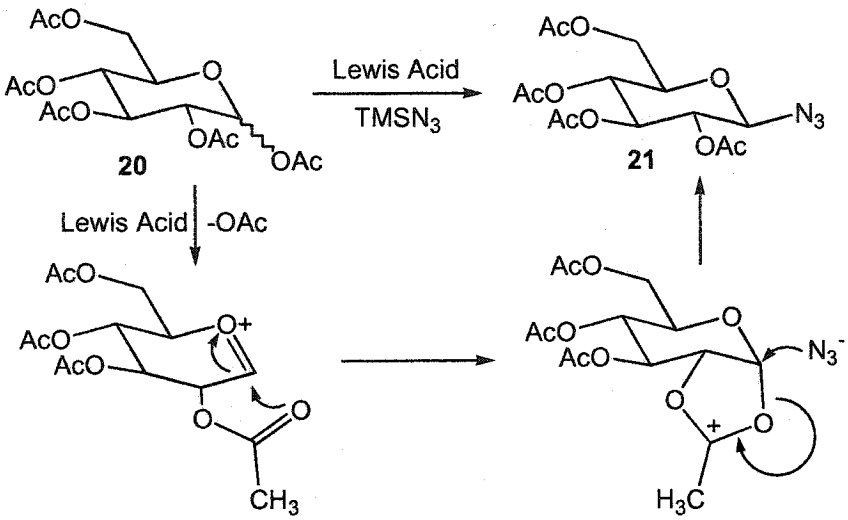
1.3.5 Modern *N*-Glycoside Synthesis

More efficient methods of introduction of the 1-azido group have arisen since these early efforts. Phase-transfer catalysis (PTC) using glycosyl halides with sodium azide affords β-glycosyl azides in high yields[65-67] (Scheme **1.3**). Alternatively, 1-*O*-acyl sugars can be treated with Lewis acids such as $SnCl_4$ or trimethylsilyltriflate (TMSOTf) and azidotrimethylsilane ($TMSN_3$) to yield glycosyl azides.[68,69] The stereochemistry of the azide obtained depends on the functionality and configuration at C-2, and neighboring group participation is a major contributing factor (Scheme **1.4**),[67] favoring a 1,2-*trans* configuration.[70] C-2 axial sugars such as mannose give rise to α-azides, while C-2 equatorial sugars such as glucose or galactose give β-azides.



Scheme **1.3**: Reaction of Glycosyl Bromides with Sodium Azide under PTC Conditions.

The most common method for reduction of azides is catalytic hydrogenation.[55]

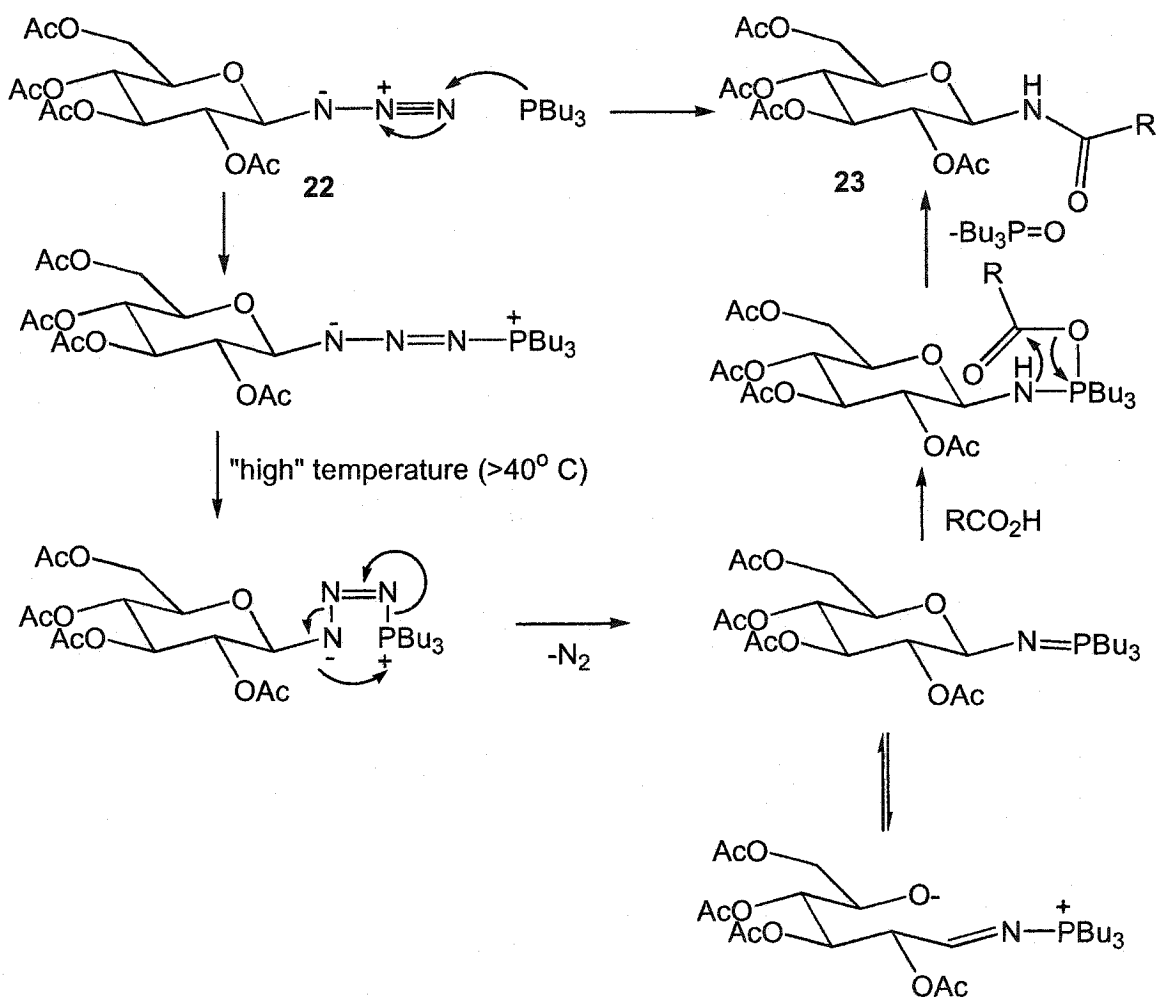


Scheme **1.4**: Neighboring Group Participation Determines Anomeric Configuration when Forming Glycosyl Azides under Lewis Acid Conditions.

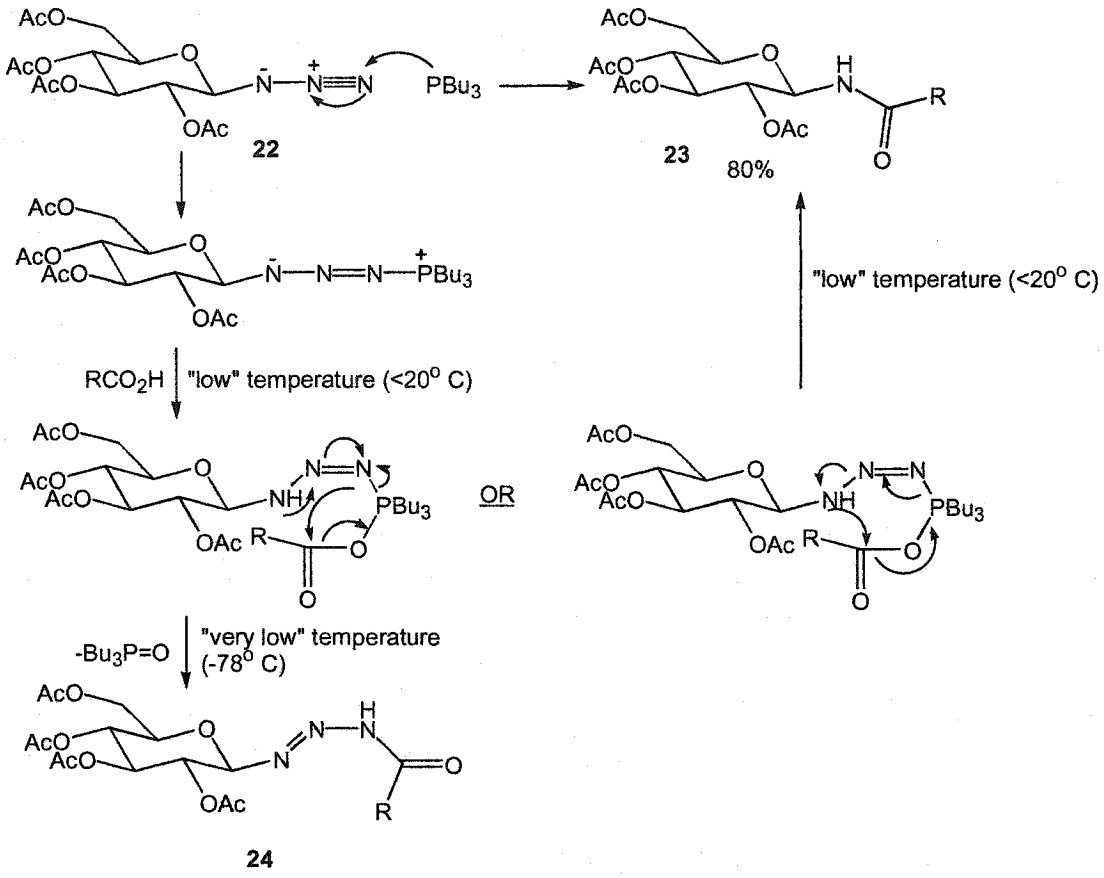
Other methods are less direct, but offer certain advantages over the more common protocols. One such example is a Staudinger reaction of a glycosyl azide with tributyl or triethylphosphine and a carboxylic acid, giving an amide.[72] However, the glycosylphosphorazene intermediate is known to anomerize through an open-chain structure[73] (see Scheme **1.5**), limiting its usefulness.

A solution to this problem was put forth by Inazu et al, using low-temperature reaction conditions with a suitably protected Asp or Glu residue.[74] This reaction proceeds with complete retention of anomeric configuration, at least when β-azides are employed (Scheme **1.6**). There is strong evidence for the postulated mechanism, since the triazene derivative **24** can be obtained if reaction temperatures are very low

(-78 °C) and workup is carefully performed. This triazene readily eliminates nitrogen and rearranges to give the amide **23** if exposed to heating or trace acid.



Scheme **1.5** Staudinger Reaction of Glycosyl Azides



Scheme **1.6** Inazu's Modified Staudinger Reaction Gives Anomerically Pure *N*-Glycosides with High Yields.[74]

Another strategy is to generate a glycosyl isothiocyanate, which can be accomplished either through treatment of glycosyl bromides with potassium thiocyanate under PTC conditions,[75] or oxazolidines with potassium thiocyanate with tetrafluoroboric acid and 18-crown-6.[76]

1.3.6 Synthetic Approaches to *C*-Glycosides

Very few examples of *C*-glycosides exist in nature. One known example[77] has been shown to exist as a post-translational modification of tryptophan found in RNase 2, isolated from human urine and erythrocytes (Figure **1.11**).

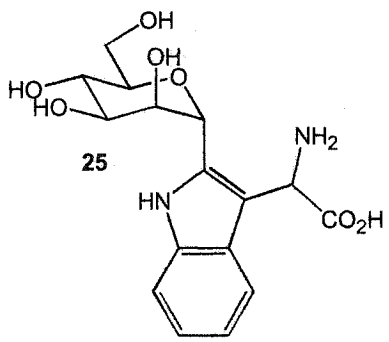
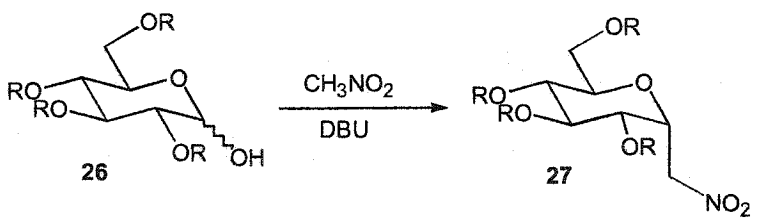
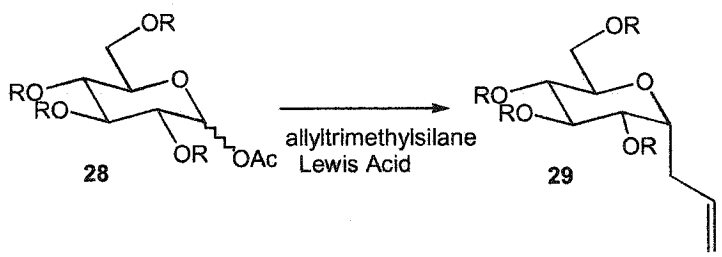


Figure **1.11** A Naturally Occurring *C*-Glycoside

Many entry points exist for *C*-glycosides.[51,78] Most examples involve C-1 as an electrophile, such as the Henry condensation[79] (Scheme **1.7**) or reaction with an allyl silane (Scheme **1.8**)



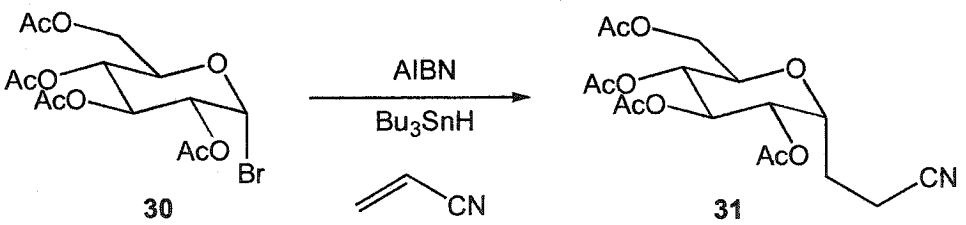
Scheme **1.7** The Henry Condensation



Scheme **1.8** Allyl Glycosides are a Convenient Entry Route[80]

In most cases, allyl compounds like **29** are the main entry points; the terminal olefin is available for oxidation, hydroformylation, ozonolysis, radical additions, and many transition-metal catalyzed processes such as the Heck reaction or olefin metathesis.

Other entry points exist, such as the reaction of glycosyl halides with organometallic species[81,82] and malonyl anions;[83] reaction of the open-chain carbonyl form of C-1 unprotected species with Wittig reagents;[84] and the formation of radical species from glycosyl halides with subsequent addition onto acrylo-type species[85,86] (Scheme **1.9**).



Scheme **1.9** Radical Addition onto Acrylonitrile Leads to *C*-Glycosides[86]

1.4 The Goal of the Thesis

The goal of this thesis is to generate novel *N*-linked and *C*-linked glycopeptidomimetics of sLe^x by a variety of strategies for the purposes of evaluation

as potential therapeutics. The plan is to attempt to minimize the length of routes involved, improve yields, control anomeric configuration, and introduce novel structural features, avoiding moieties which are potential enzymatic substrates.

2. *N*-Linked Fucopeptides as Glycopeptidomimetics of Sialyl-Lewis^x

2.1 Introduction

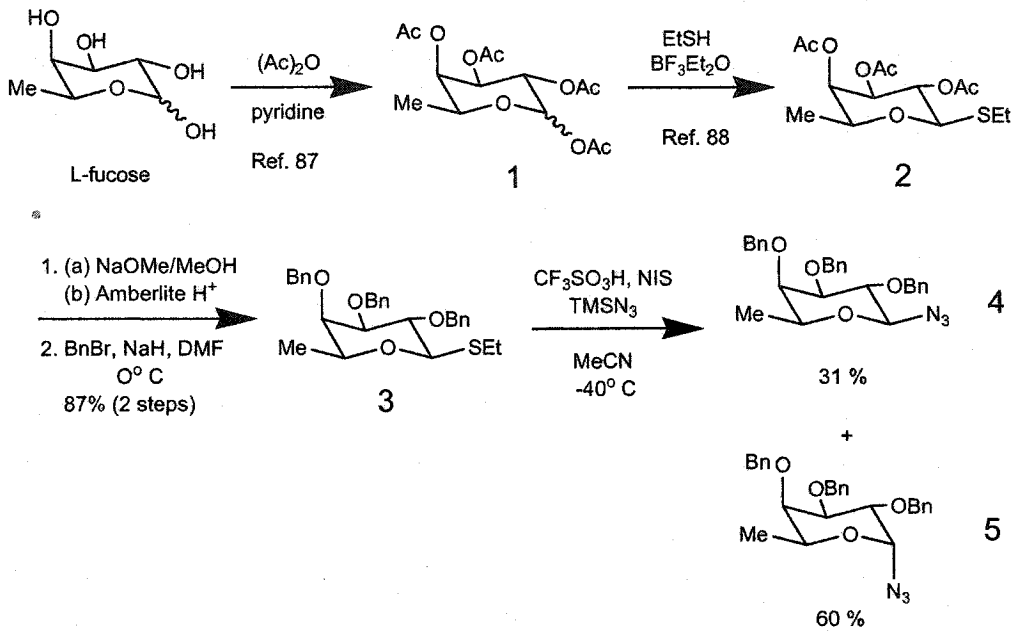
The literature is devoid of examples of *N*-linked fucopeptidomimetics of Sialyl Lewis^x. The principle reason for this may be the anticipation of difficult syntheses. In order to investigate this as a possibility for therapeutics, several strategies were embarked upon, encompassing both solution and solid-phase approaches.

2.2 Synthesis of *N*-Linked Fucopeptidomimetics

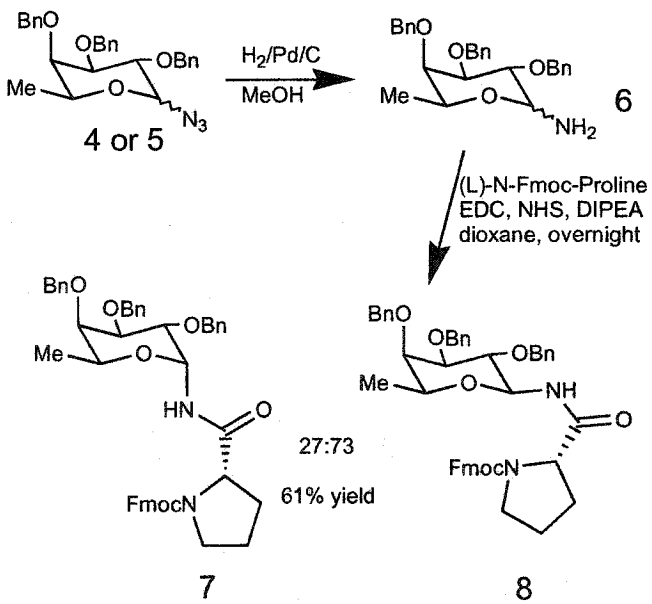
2.2.1 Solution-Phase Synthesis

A major entry point to *N*-glycosides is through an azide functionality (Scheme **2.1**). L-fucose was peracetylated, converted to β-thioethyl compound **2**,[87,88] and the acetyl protecting groups were removed by Zemplen conditions and replaced with benzyl groups[89] to afford **3**.[90] Treatment of **3** with azidotrimethylsilane, trifluoromethanesulfonic acid and *N*-iodosuccinimide at –40 °C provided azides **4** and **5** in excellent combined yield. The easily separated azides could be stored indefinitely without epimerization. Although the azides are easily separated, amine **6**, formed from the reduction of azides **4** and **5**, very quickly anomerized, and coupling of amine **6** with Fmoc-proline afforded an identical α/β mixture, irrespective of the configuration of the starting azide, which could be separated by column

chromatography (Scheme **2.2**), although the separation was by no means trivial. Very difficult chromatography using comparatively large columns was necessary to achieve separation.

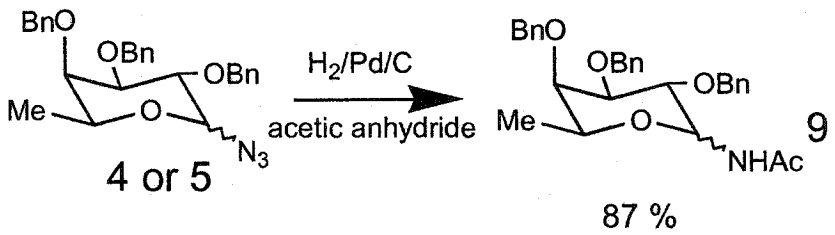


Scheme **2.1**: Preparation of Fucosyl Azides.[87-90]



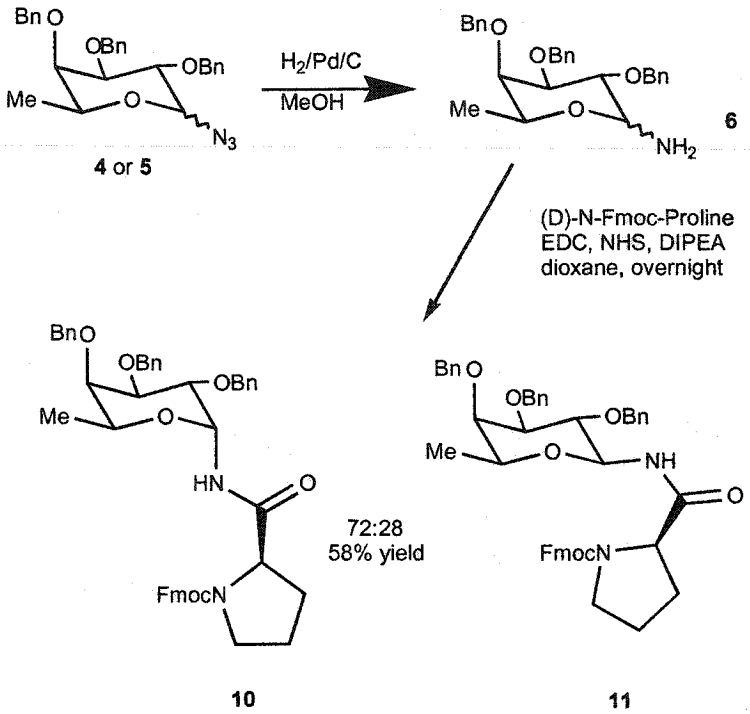
Scheme **2.2**: Reduction and Coupling of Fucosyl Azides.

The ratio observed (27:73 in favor of β) did not noticeably change when changes were made with respect to azide configuration or coupling conditions. Reduction of the azide in acetic anhydride (Scheme **2.3**) afforded a mixture of 25:75 α/β anomers (as determined by ^{1}H NMR). Although the ^{1}H NMR spectrum was poorly resolved, the α/β ratio could be inferred from the relative integration of the H-1 peaks at ~5.6 and ~5.0 ppm, respectively. The purpose of this experiment was to determine the point at which anomerization occurs; it would seem it occurs either during the reduction, or very quickly thereafter; the amines cannot be isolated as single anomers.



Scheme **2.3**: Reduction in Acetic Anhydride Affords a Mixture of Anomers

Interestingly, when coupling of amine **6** was conducted using (D)-proline, anomeric selectivity was reversed (Scheme **2.4**). Further investigation into this phenomenon is discussed in Chapter **5**.

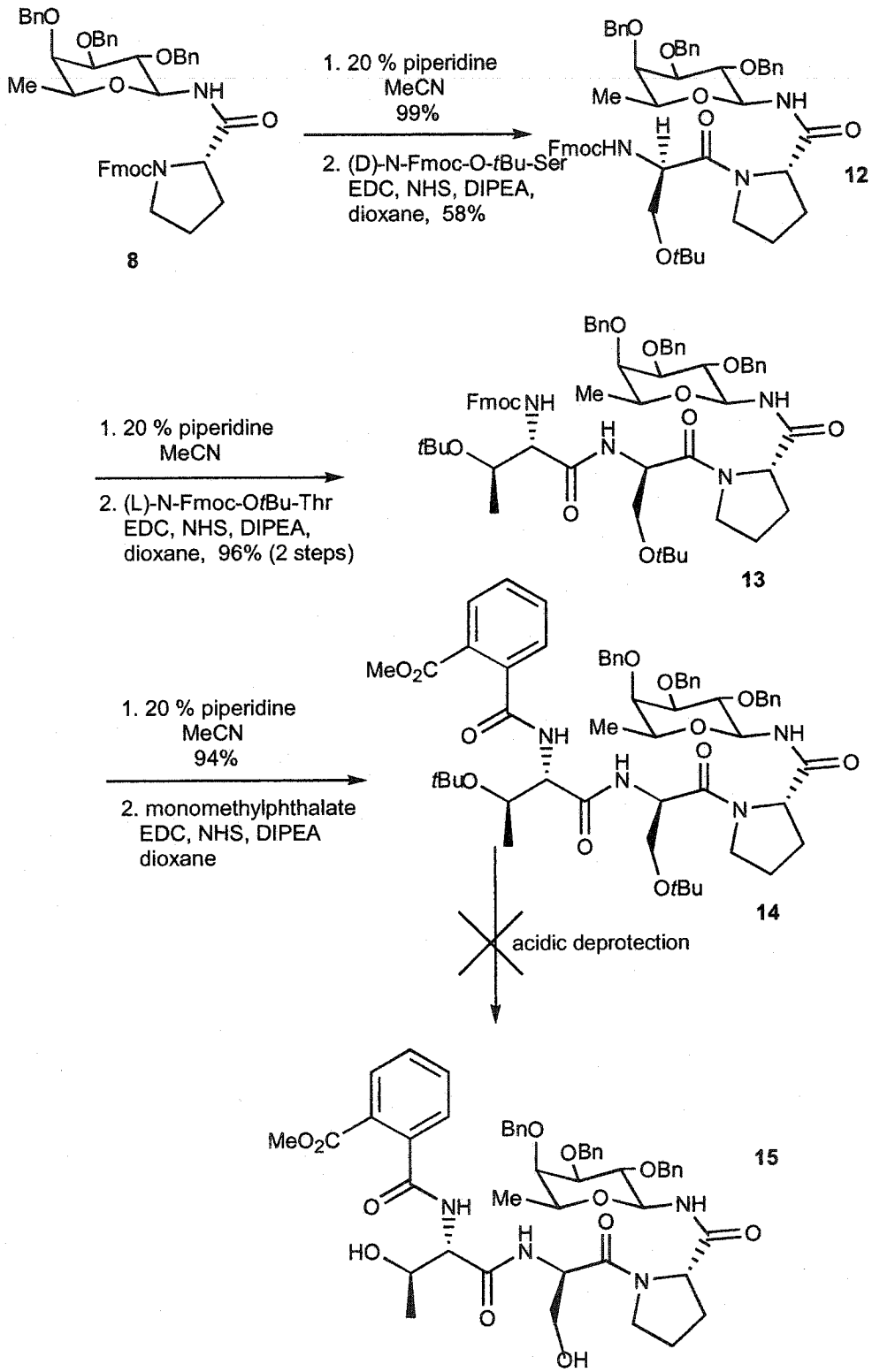


Scheme **2.4**: Reduction and Coupling of Fucosyl Azides with (D)-Proline

Attempts were made at the synthesis of glycopeptides via *t*-butyl protecting groups. Scheme **2.5** depicts the attempted synthesis of fucopeptide **15** by this strategy.

Compound **8** was deprotected and coupled with a protected (D)-serine residue to afford **12** in modest yield. Subsequent Fmoc deprotection and coupling with a protected (L)-threonine residue provided **13** in excellent yield. Another deprotection and attachment of *mono*-methylphthalate afforded penultimate compound **14**, which had such a poorly resolved ^{1}H NMR spectrum that it could not be characterized. The ^{1}H NMR of **12** can be seen in Figure **2.10**, and the FAB-MS of **13** can be seen in Figure **2.11**. However, the synthesis could not be completed, due to the incompatibility of the *N*-glycosidic linkage with conditions needed to remove *t*-butyl ethers and esters. Several methods of deprotection were attempted, including

trifluoroacetic acid (neat and in dichloromethane), 3 N HCl in dioxane, and titanium tetrachloride for 1 minute at 0 °C.[91] Treatment of fully protected **14** did not afford the desired compound **15**. Conditions could not be found that cleaved the protecting groups while preserving the labile *N*-glycosidic linkage. The strategy had to be abandoned in favor of *N*-Fmoc protected compounds; however, valuable experience was obtained with respect to the development of proper coupling, workup, and purification protocols which ultimately would be extended to most other areas of this body of work.

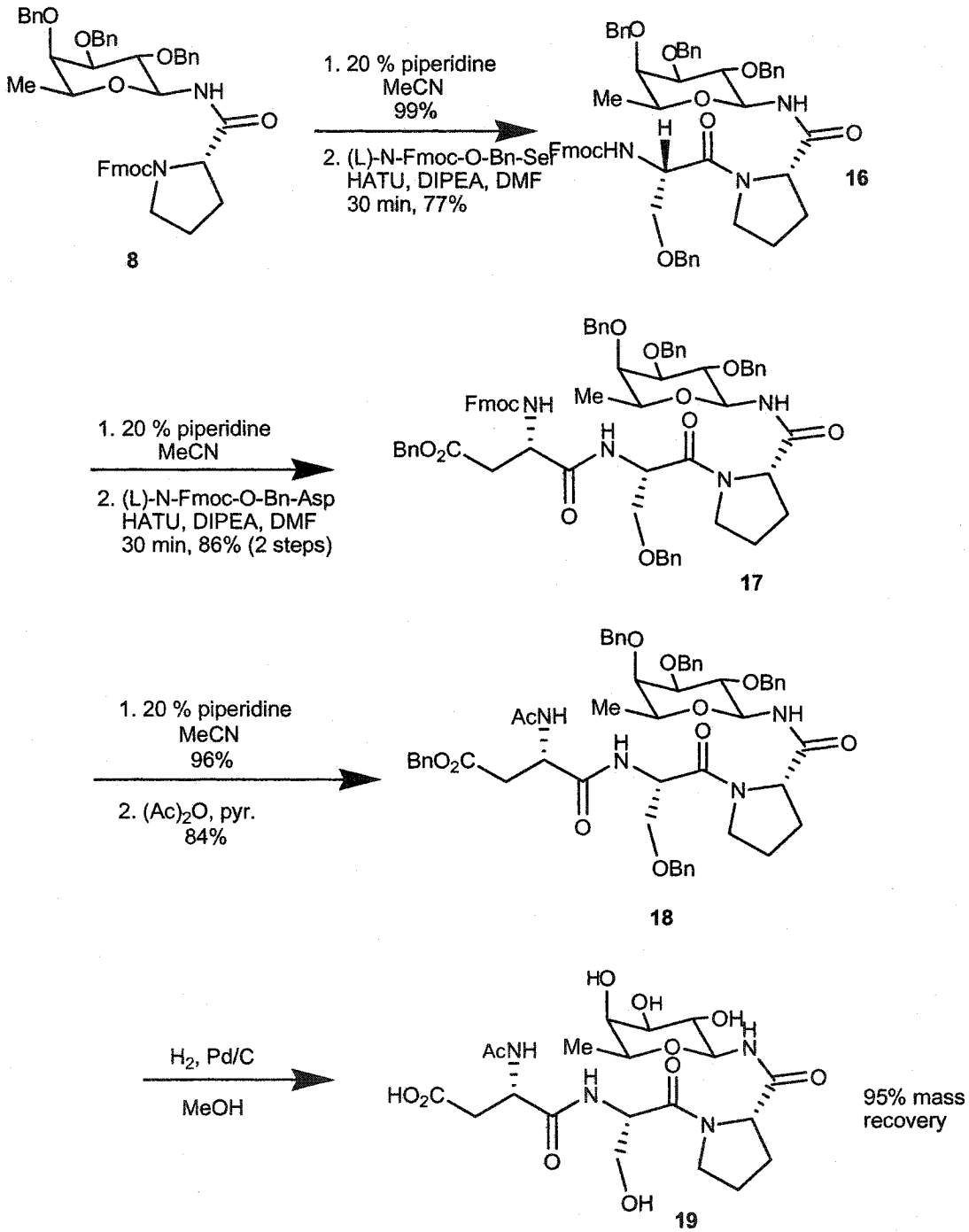


Scheme **2.5**: Synthesis of *N*-Linked Glycopeptidomimetics via *t*-Butyl Protection

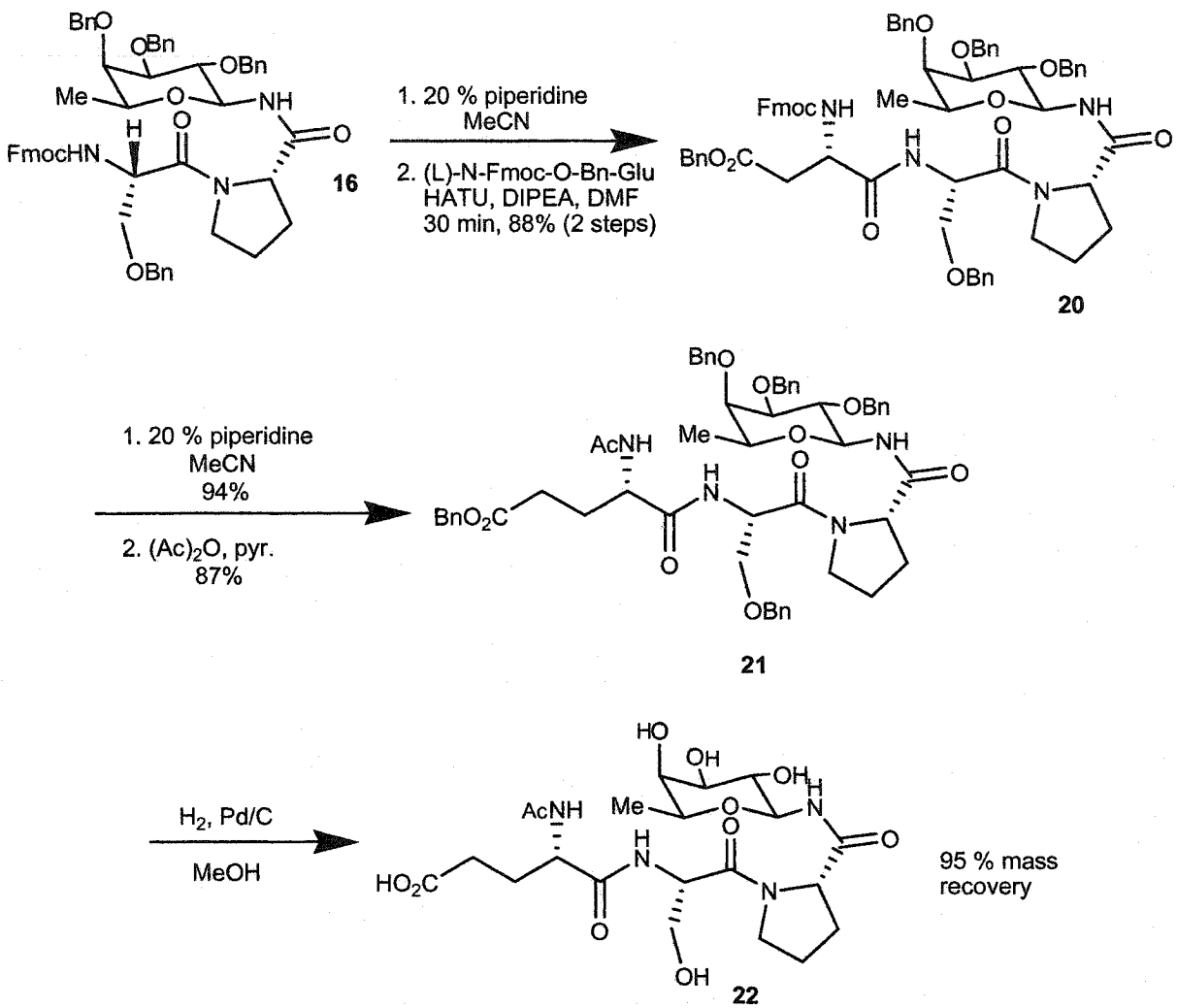
The synthesis of the first glycopeptide **19** is shown in Scheme **2.6**. Deprotection of the Fmoc-group of **8** followed by HATU-mediated coupling afforded **16**, which was subjected to another deprotection and coupling to provide **17**. Fmoc-deprotection and *N*-acetylation afforded fully protected glycopeptide **18** (see Figures **2.12**, **2.13** and **2.14** for spectral data). Global deprotection using catalytic hydrogenation gave **19** as a hygroscopic white solid in 95 % mass recovery, which was of questionable purity.

Use of the same strategy was applied to a second example **22**, which included a glutamate residue in place of the aspartate (see Scheme **2.7**), which proceeded in good overall mass recovery. The ^{1}H NMR and ^{13}C NMR can be found in Figures **2.15** and **2.16**, respectively.

Overall, the strategy afforded low milligram amounts of β-*N*-L-fucosyltripeptides. Compounds **19** and **22** were synthesized from L-fucose in 14 steps each. Although comparatively successful, the strategy was quite long, with, at times, daunting separations by column chromatography. The compounds seemed to be stable over a period of 30 days by TLC; however, this approach to these compounds was ended in hopes for a more rapid, simple strategy.



Scheme **2.6**: Synthesis of the First *N*-Fucopeptidomimetic of Sialyl-Lewis^x



Scheme **2.7**: Synthesis of Glutamate-Containing Fucopeptide **22**

2.2.2 Solid-Phase Synthesis

Ideally, combinatorial chemistry strategies must rely on steps that are high-yielding, with minimal side reactions with concomitant impurities.[92] Chief advantages of solid-phase synthesis are speed, and (hopefully) easy purification. It would be ideal to be able to construct a complete *N*-linked fucopeptide as shown previously (such as **22**) by such a strategy. The motivation for such a change in strategy is to attempt to increase analogue throughput and improve yields, and to introduce a novel methodology for the synthesis for such compounds. However, a route could not be conceived which would allow for complete glycopeptide synthesis on the solid support using an *N*-Fmoc protecting group strategy (common to the synthesis of peptides on solid-phase supports), nor could a carboxylate-protecting group strategy be envisaged. Easily removed carboxylate-protecting groups that do not cleave simultaneously with the resin linkage are difficult to develop.[91] Hence, a different, convergent strategy was in need of development. Using trityl chloride resin (Figure **2.1**), a methodology was developed which produced peptidic chains (Scheme **2.8**) which could then be coupled to the previously synthesized amine **27**, available from the Fmoc-deprotection of **8** (described in Scheme **2.11**).

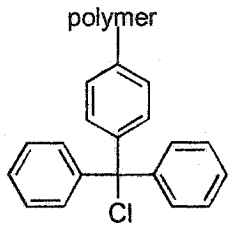
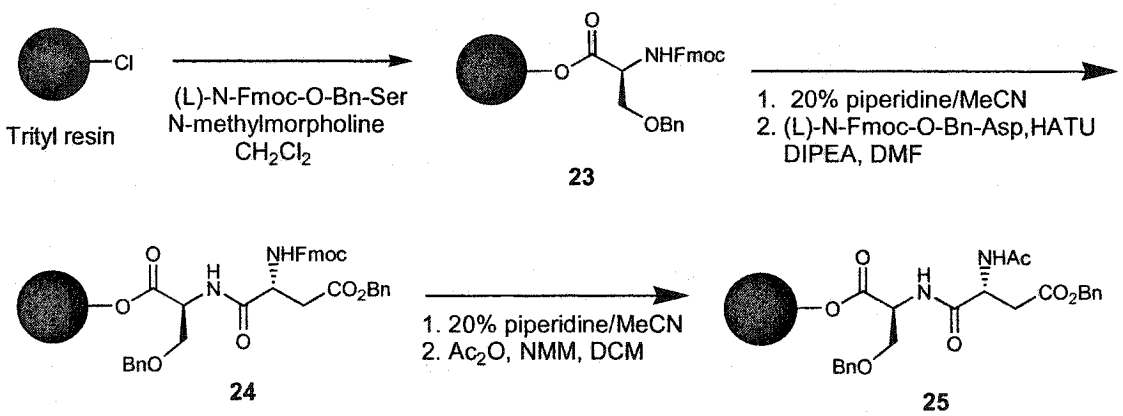


Figure **2.1**: Trityl Chloride Resin (polymer = polystyrene)

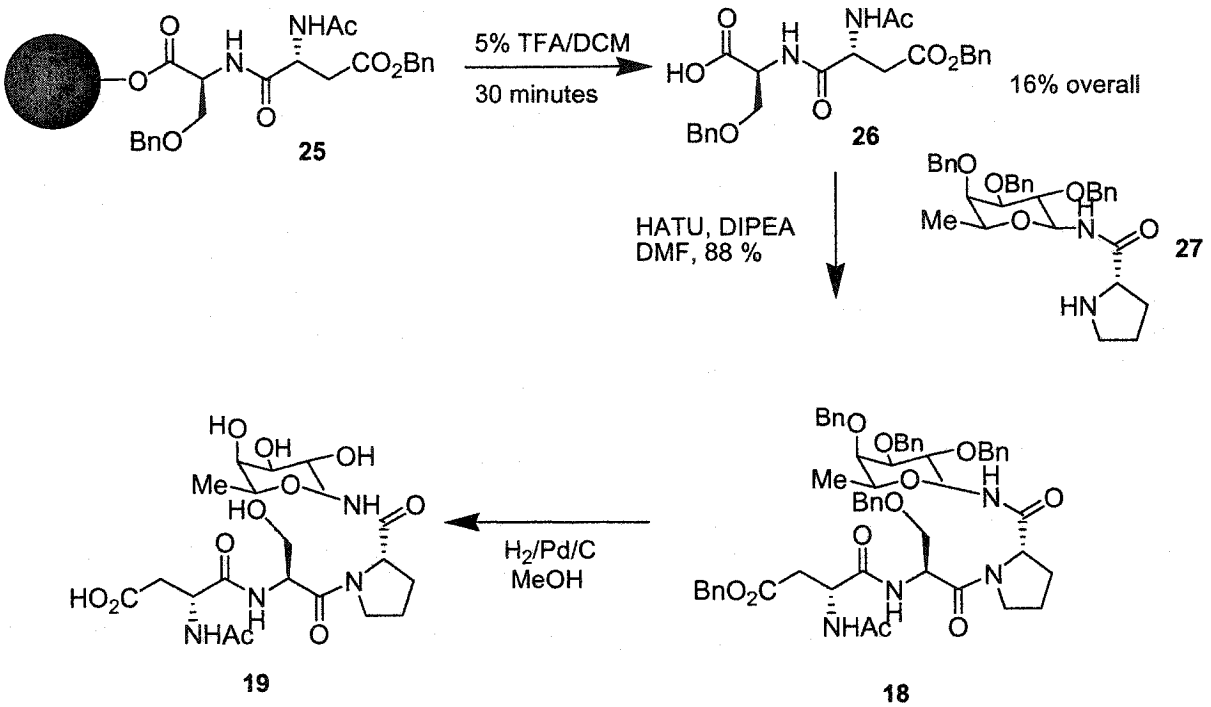
Attachment of suitably protected amino acids onto the trityl chloride resin via a S_N1 displacement, followed by deprotection and coupling of the next amino acid (in this case, aspartic acid), and deprotection again followed by acetylation afforded resin-bound **25**.



Scheme **2.8**: Buildup of a Peptide Chain on Trityl Resin

With **25** in hand, cleavage could be accomplished with the use of 5% trifluoroacetic acid in dichloromethane for 30 minutes (Scheme **2.9**). Product **26** required purification by column chromatography, and was isolated in 16% overall yield. Subsequent coupling onto amine **27** using HATU with DIPEA in DMF afforded fully

protected **28** in 88% yield. Global deprotection using hydrogen with palladium on carbon afforded previously synthesized **19** which had spectral properties identical to the previously obtained material.



Scheme **2.9**: Cleavage of Peptide from Trityl Resin and Completion of the Synthesis

The methodology was convergent in nature, but required solution-phase steps at the beginning and end of the synthesis. Purity did not appreciably improve as compared to the linear solution-phase strategy. It seems likely that the sugar-proline combination induces conformational properties which make for poor spectral properties, and refinement to a state of high purity was not successful.

2.3 Tartaric Acid-Based Mimetics

2.3.1 Introduction

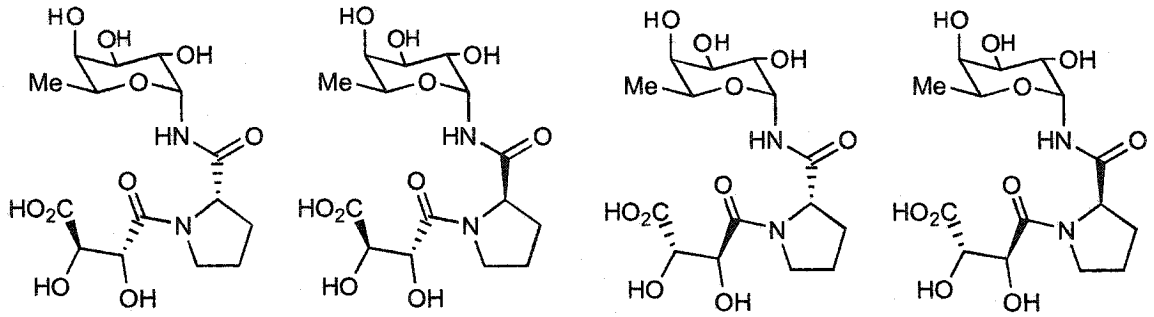
In order to develop syntheses that are both shorter and more convergent, tartaric acid was chosen to provide the hydroxyacid moiety needed at the tail of the molecule. There are two particularly interesting features about this strategy. The first is that the convergent approach improves the overall efficiency. The second is that the two stereoisomers offer interesting conformational arrangements, while at the same time providing all the necessary functional groups for selectin binding. An additional advantage is that compounds bearing non-amino acid building blocks would be less susceptible to degradation *in vivo*.

2.3.2 CAChe Modeling of Tartaric-Acid Based Mimetics

Computer-aided drug design has become an important part of drug discovery. Modeling systems geared to fit known active sites or mimic active compounds of known biologically relevant conformations has been of great value in obtaining or optimizing lead structures.[93]

CAChe is a software program that provides lowest-energy conformation calculations, including dihedral angles and relative distances between atoms. The global minimum was determined using molecular mechanics and electronic effects. The known

pharmacophore of Sialyl Lewis^x was "stripped" of non-relevant atoms, and compared to lowest energy conformations of four different α-fucose-proline-tartaric acid conjugates (see Figure **2.2**).



29 L-Pro-L-Tart

30 D-Pro-L-Tart

31 L-Pro-D-Tart

32 D-Pro-D-Tart

Figure **2.2**: The Four Possible α-Fucose-Proline-Tartaric Acid Conjugates

A CAChe representation of the bound structure of Sialyl Lewis^x is shown in Figure **2.3**. This representation was obtained from the crystal structure of the Sialyl Lewis^x – E-selectin bound complex.[94] The "bare bones" structure is shown in Figure **2.4**. Figures **2.5-2.8** show the lowest energy conformations of the four tartaric acid conjugates. Hydrogen atoms are omitted for clarity. The seemingly hexavalent atom seen in Figures **2.3**, **2.4**, and **2.9** results from a chance overlap of two carbon atoms.

The features most worthy of close attention are the overlap of atoms from C-1 of the fucose residue down the chain, into the first atom beyond the proline ring. This region is much more critical than those regions beyond.[2,29,30] A great deal of conformational flexibility exists for this segment of the molecule, and the differences in energy are small.

The distance between the terminal acid group and the hydroxyl groups has also been shown to not be very important. Atom spacers from two to ten or more atoms have all shown activity.[2]

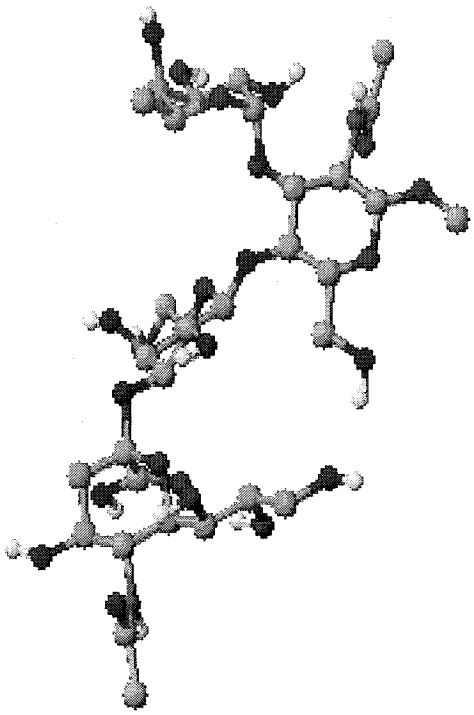


Figure **2.3**: Bound Sialyl Lewis^x

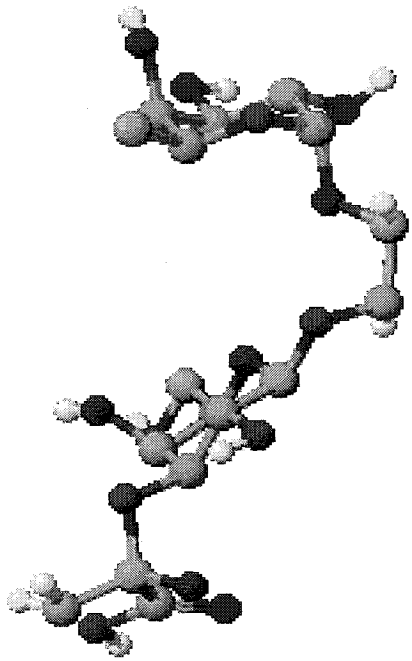


Figure **2.4**: Simplified Sialyl Lewis^x

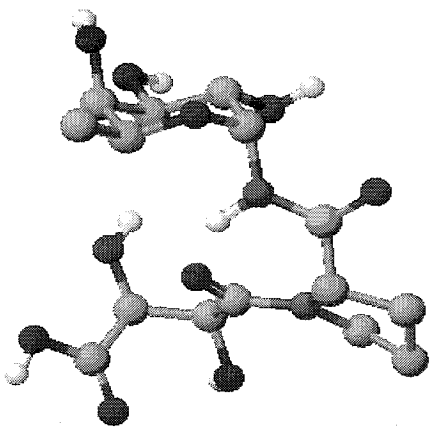


Figure **2.5**: (L)-Pro-(L)-Tartaric Acid **29**

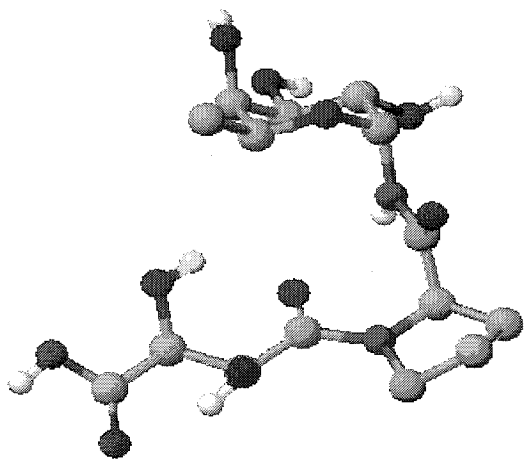
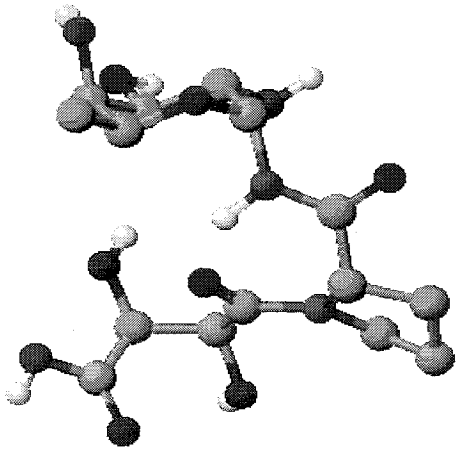


Figure **2.6**: (D)-Pro-(L)-Tartaric Acid **30**



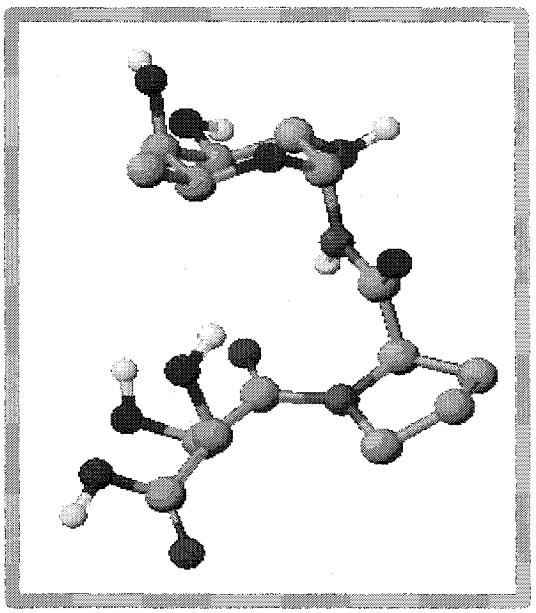
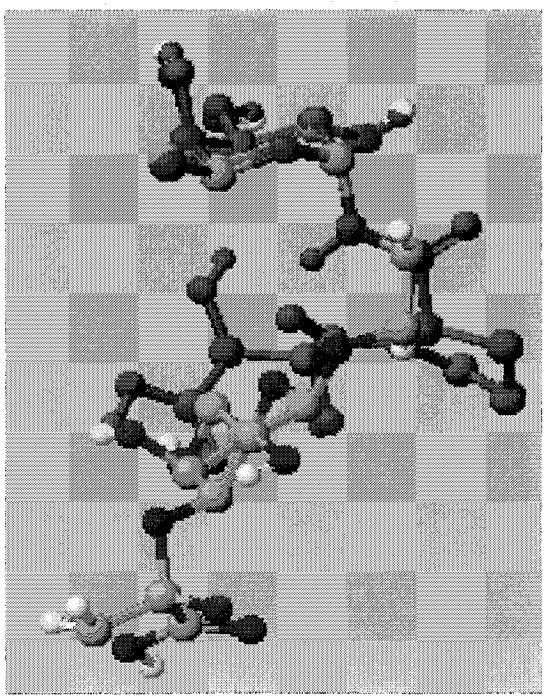


Figure **2.7**: (L)-Pro-(D)-Tartaric Acid **31** Figure **2.8**: (D)-Pro-(D)-Tartaric Acid **32**

The best overlap of the skeletal atoms from fucose-C-1 to the first tartarate carbonyl carbon occurs with **32**, the (D)-pro-(D)-tartaric acid conjugate (see Figure **2.9**).



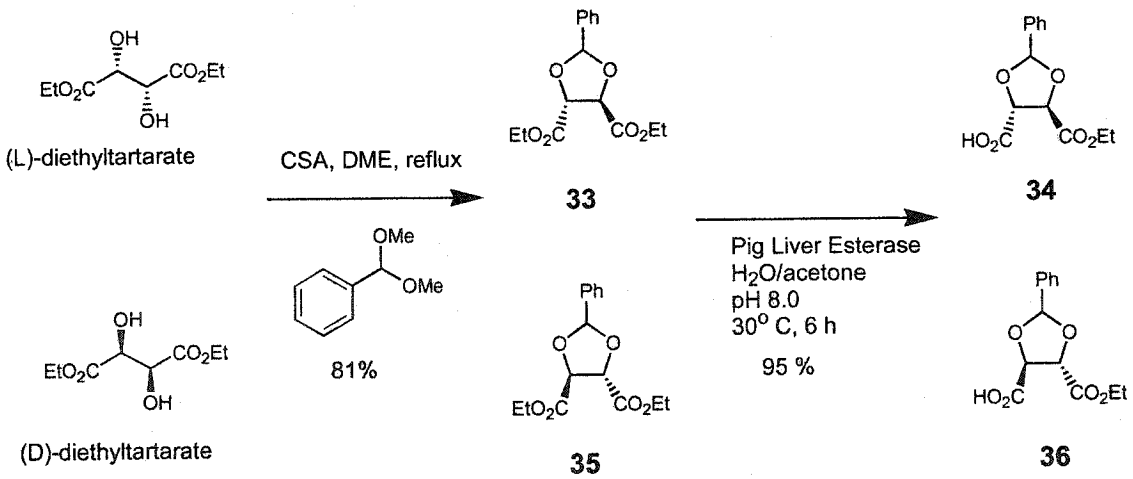
Blue atoms are those of **32**. Overlap from fucose-C-1 to the first carbon of tartaric acid is high. It is especially noteworthy that in all four cases shown above the hydroxyacid tail of the molecule exists in a "cis-like" conformation with respect to the fucose ring, as is the case with Sialyl Lewis^x. In order to investigate such promising modeling studies, a synthesis was employed to furnish the compounds in a convergent fashion.

Figure **2.9**: Sialyl Lewis^x-**32** Comparison

2.3.3 Synthesis of Tartaric Acid-Based Mimetics

The synthesis requires two structural fragments. Members of one set of fragments have either already been prepared or can easily be prepared from materials already described herein. The other set of fragments can be obtained in a facile manner from (L)- or (D)-diethyl tartarate.

Protected tartaric acid fragments are quickly prepared in two steps from commercially available (L)-[95-102] or (D)-[103] diethyl tartarate, as shown in Scheme **2.10**. This strategy is somewhat different from published methods, which have all used benzaldehyde and toluene sulfonic acid in refluxing benzene. Yields are comparable, but reaction time (1 h, vs 20-24 h) is significantly reduced.



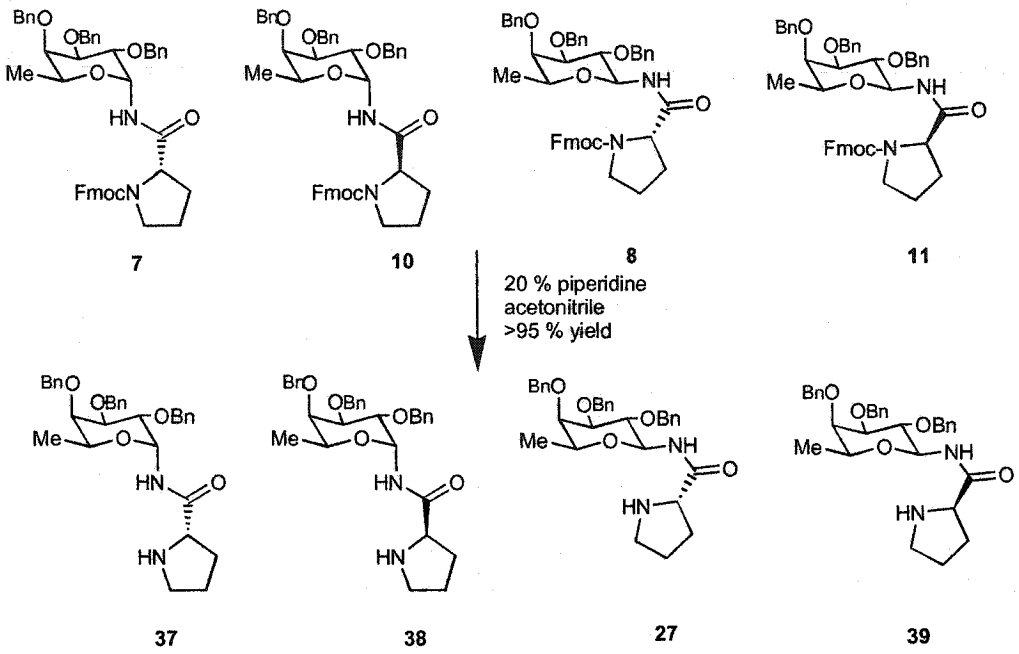
Scheme **2.10**: Synthesis of Protected (L)- and (D)-Tartaric Acid

Formation of benzylidene compounds **33** and **35** proceeded smoothly using catalytic camphorsulfonic acid and dimethoxytoluene in dimethoxyethane at 80 °C. The

choice of solvent proved critical; reaction in dioxane or tetrahydrofuran led to a complex mixture of products, whereas reaction in DME led to formation of a single product. The benzylidene carbon for **33** and **35** is not indicated as a stereocenter because it is not asymmetric.

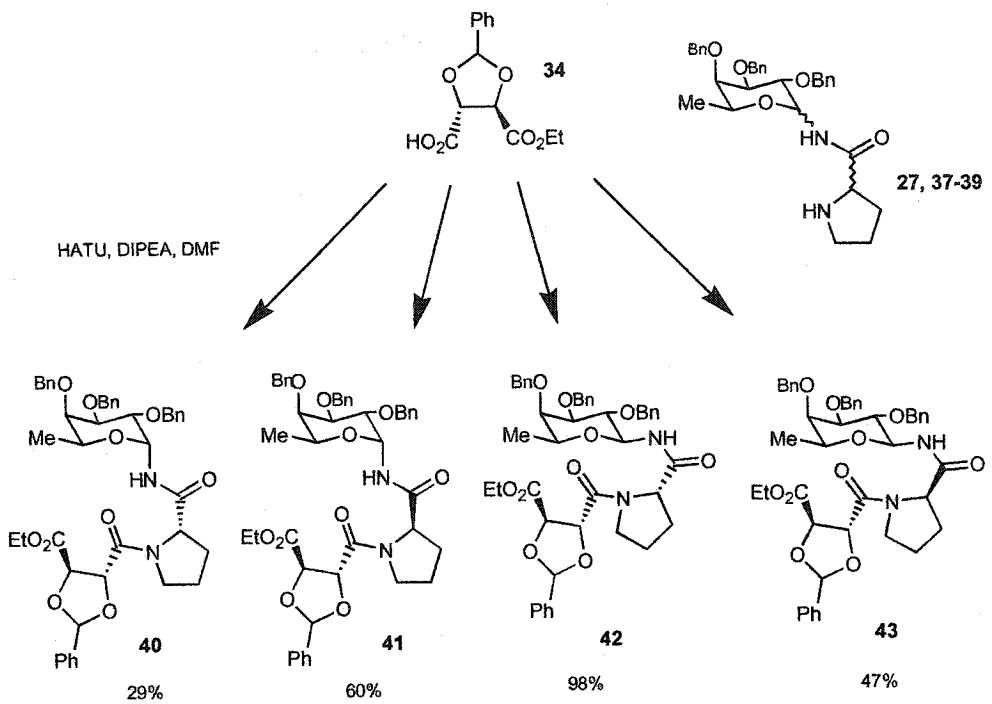
Enzymatic hydrolysis of the diethyl compounds **34** and **36** proceeded with high chemoselectivity. Pig liver esterase contains a large hydrophobic pocket, which often (but not always) provides good stereocontrol, but is most especially useful for symmetrical diesters because the active site will not accept a charged substrate.[93] Hence, the singly hydrolyzed compounds, buffered as their sodium salts, are impervious to further hydrolysis. While stereocontrol was neither good (e.e. ~30 %) nor relevant (the now-chiral benzylidene carbon is removed in the last step), hydrolysis of a single ester group was virtually quantitative. None of the twice-hydrolyzed material was detected. The remaining mass balance was unreacted diester. However, the resultant acids **34** and **36** are best used as prepared. Storage for >24 hours lead to self-catalyzed decomposition.

With **34** and **36** in hand, the necessary proline conjugates **27** and **37 – 39** could be prepared from the Fmoc-compounds **7**, **8**, **10** and **11** by deprotection with piperidine, as shown in Scheme **2.11**. Compound **38** has good spectral characteristics, as can be seen in Figures **2.17** and **2.18**, respectively.

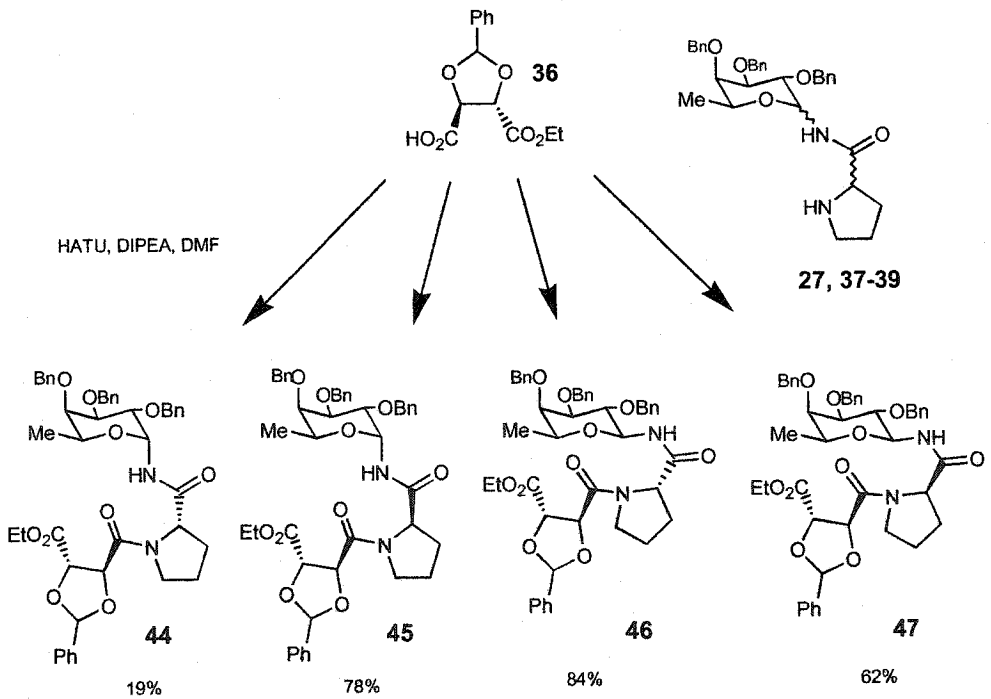


Scheme **2.11**: *N*-Fmoc Deprotection of Proline Conjugates **7, 8, 10** and **11**.

Coupling with (L)-tartarate-derived **34** was achieved using HATU with DIPEA in DMF. Yields varied from poor to high (Scheme **2.12**). Likewise, (D)-tartarate-derived **36** was coupled to the same amines to afford protected conjugates **45 - 48** (Scheme **2.13**). See Figure **2.19** for the mass spectrum of **40**, Figures **2.20** and **2.21** for the ^{1}H and ^{13}C NMR spectra of **41**, and Figure **2.22** for the ^{1}H NMR of **42**. These spectra are well-resolved examples, which are not often encountered with these compounds.

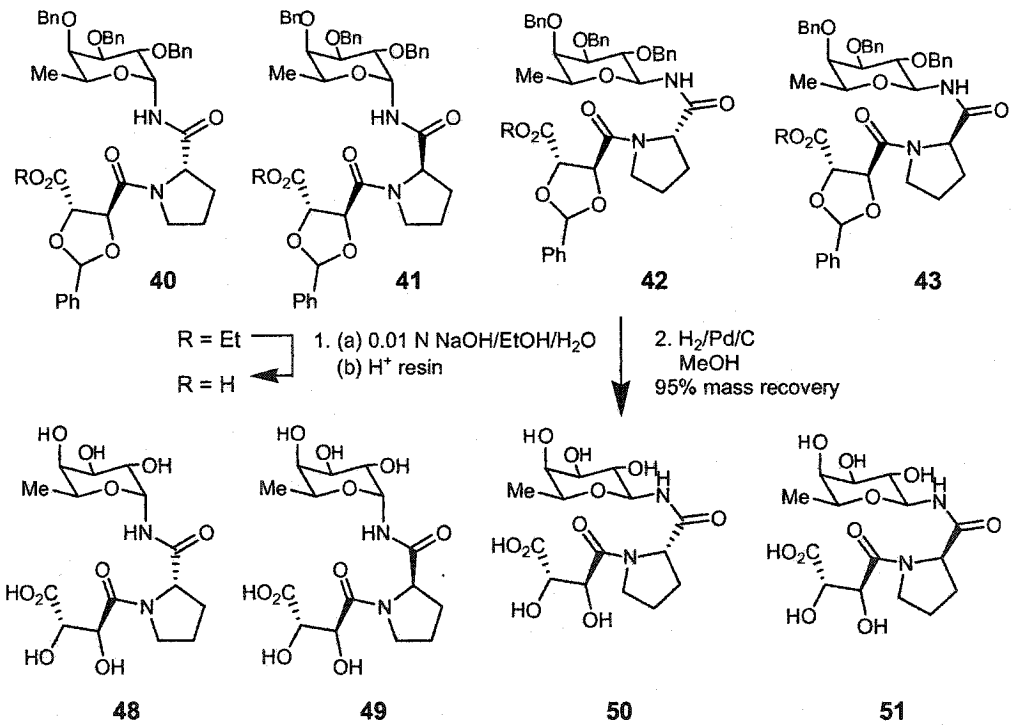


Scheme **2.12**: Convergent Synthesis of Fucosylproline-(L)-Tartarate Conjugates



Scheme **2.13**: Convergent Synthesis of Fucosylproline-(D)-Tartarate Conjugates

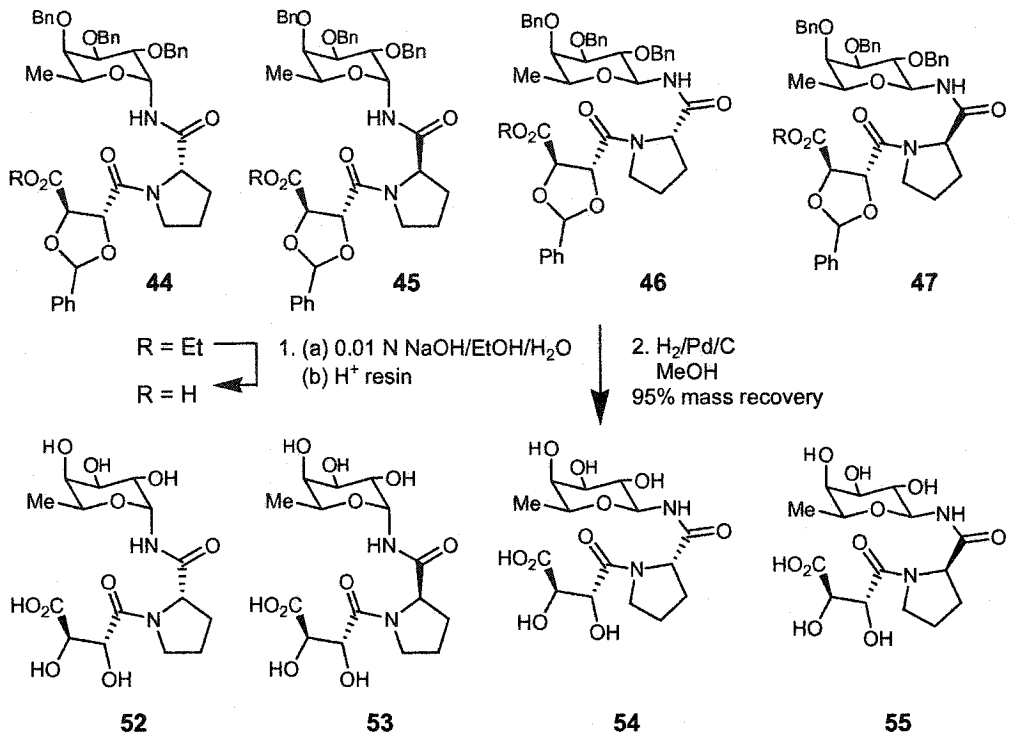
Final deprotection of the (L)-tartarate conjugates **40** – **43** was achieved in two steps; careful hydrolysis with 0.01 M NaOH in aqueous ethanol did not disturb the delicate *N*-glycosidic linkage. Acidification with acidic resin afforded the acid compounds, which could be quantitatively reduced with catalytic hydrogenation to final conjugates **48** – **51**. (Scheme **2.14**). Likewise, (D)-tartarate conjugates **44** – **47** were deprotected, again in nearly quantitative yield. (Scheme **2.15**). However, the compounds formed were clearly impure, and chromatographic purification was unsuccessful. The purity only worsened with time, and very pure materials were never obtained. The materials showed strong molecular ions for negative ESI-MS (see **2.24** for ESI-MS of **50**), but it cannot be concluded with good confidence that the materials were synthesized.



40 **41** **42** **43**

48 **49** **50** **51**

Scheme **2.14**: Deprotection of (L)-Tartarate Conjugates



Scheme **2.15** Deprotection of (D)-Tartarate Conjugates

2.4 Conclusions

Several *N*-linked fucosylpeptidomimetics of Sialyl Lewis^x were synthesized by a variety of strategies, ranging from linear, solution-phase synthesis; solid-phase convergent synthesis; and convergent synthesis of conjugates replacing the acid-alcohol dipeptide moiety with tartaric acid, but the purification of final compounds was unsuccessful, and it cannot be concluded that compounds beyond the protected, penultimate compounds were synthesized. This convergent synthesis would seem to be the most efficient, both in terms of "atom economy" (the information contained with respect to the size of the molecule) and the number of steps involved, if sufficiently scaled syntheses were conducted to facilitate purification. CAChe

molecular modeling software provided evidence of a good conformational overlap between one particular tartarate conjugate and the known bound conformation of Sialyl Lewis^x. Early attempts using more readily available *t*-butyl protected amino acids were not fruitful, as deprotection could not be achieved without cleavage of the glycosidic linkage.

2.5 Spectra Referred to Throughout Chapter 2

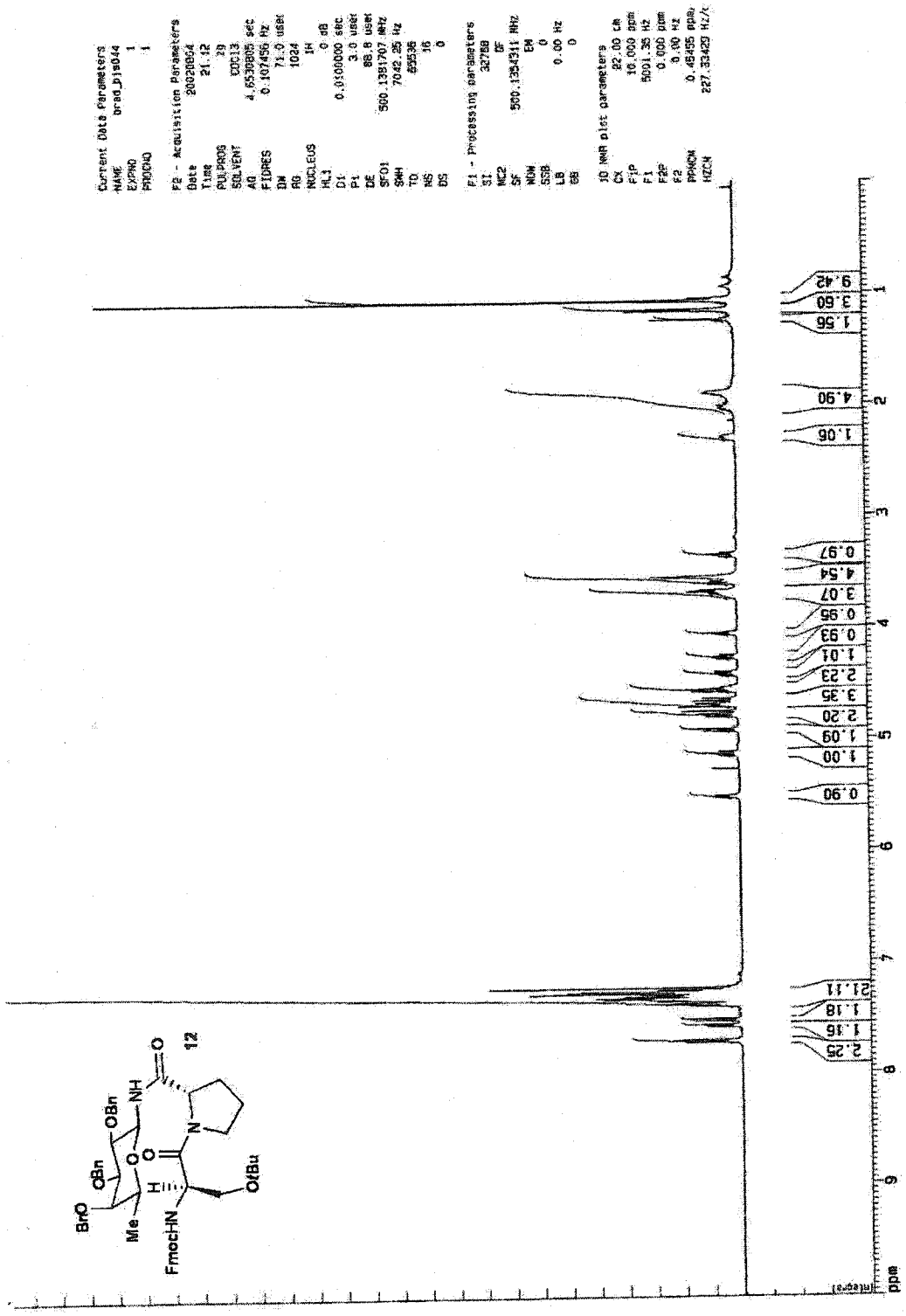


Figure **2.10**: ^{1}H NMR ($CDCl_3$, 500 MHz) of **12**

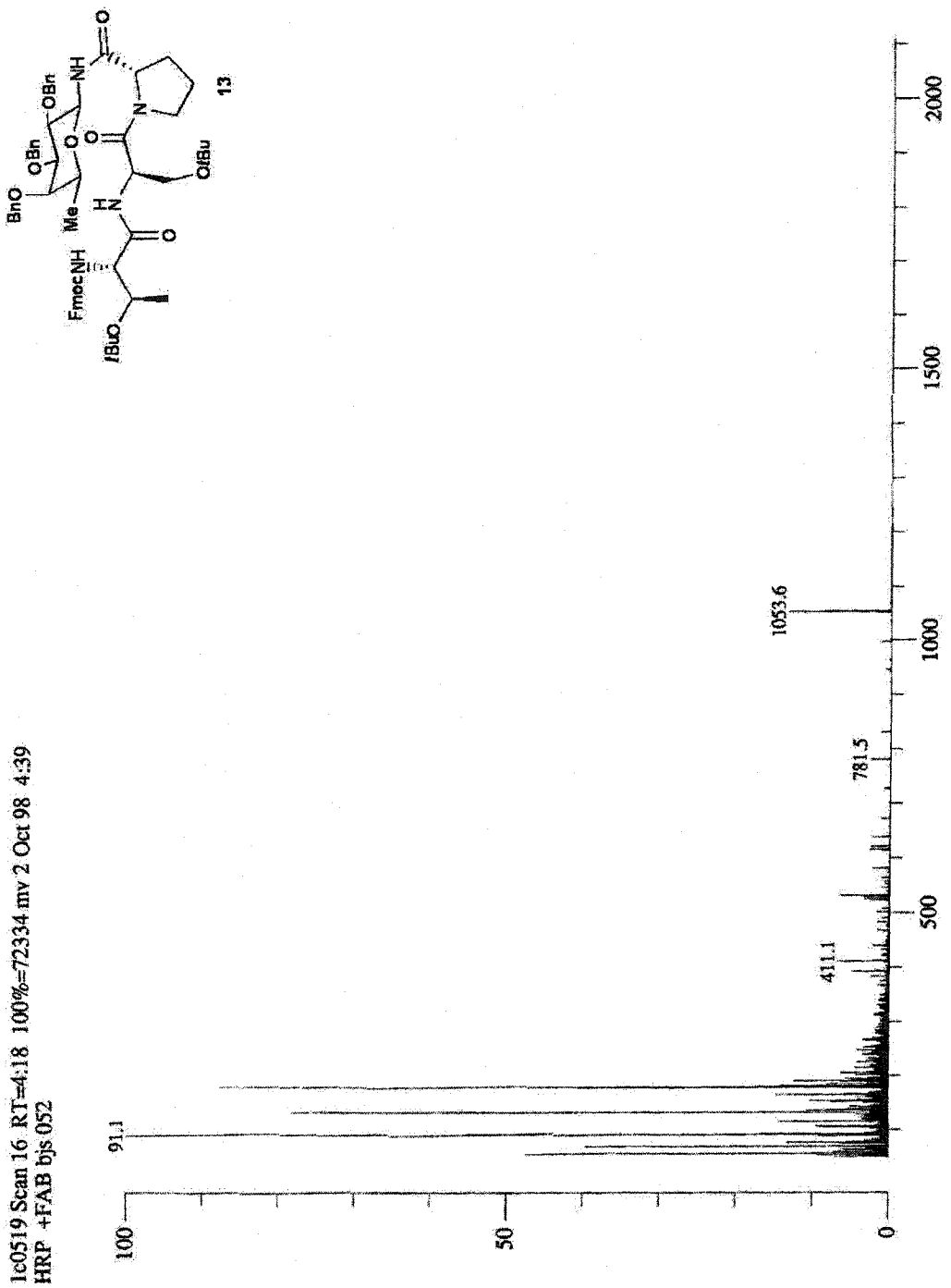


Figure **2.11**: Mass Spectrum (+ESI) of **13**.

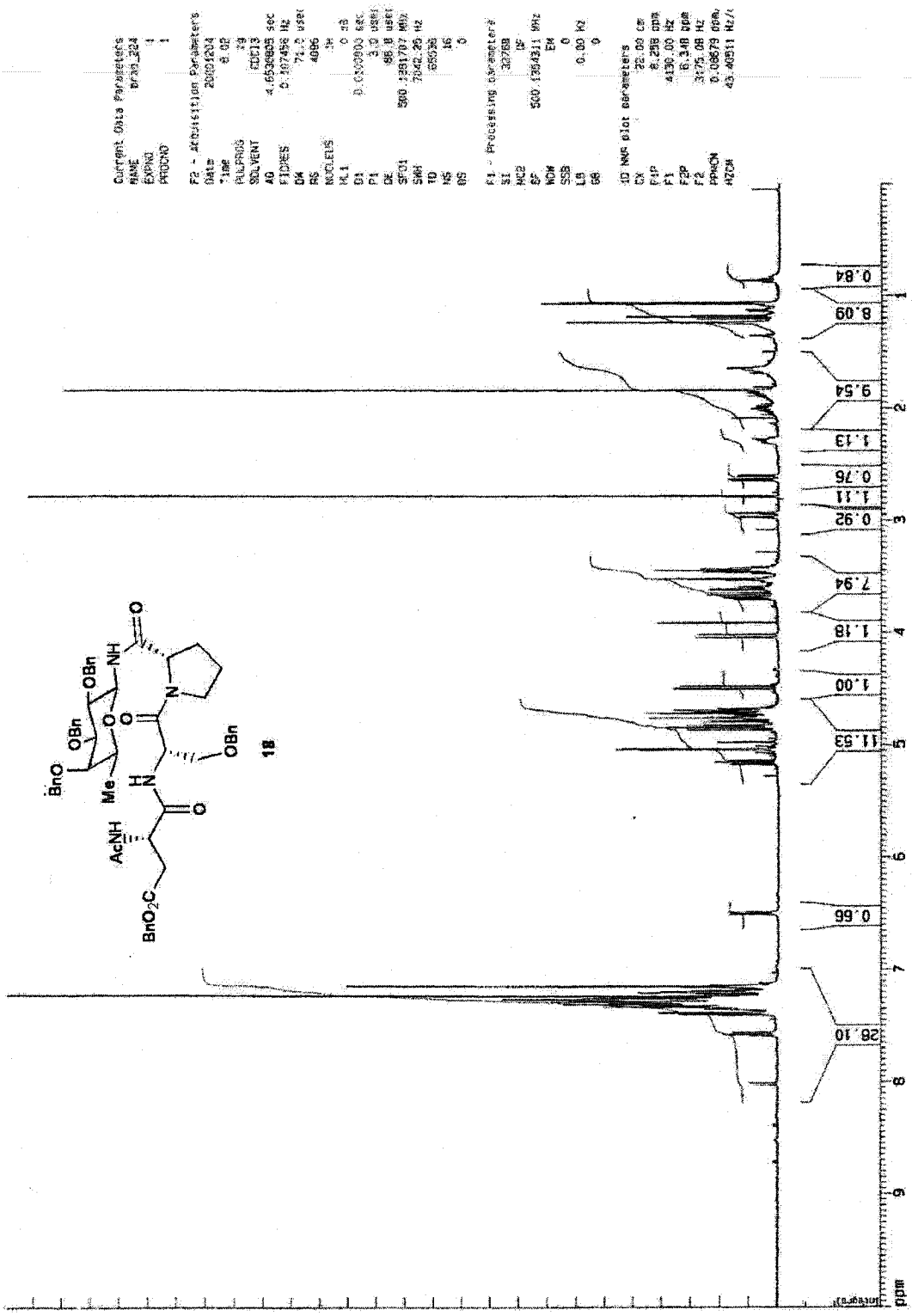


Figure **2.12**: ^{1}H NMR ($CDCl_3$, 500 MHz) of **18**.

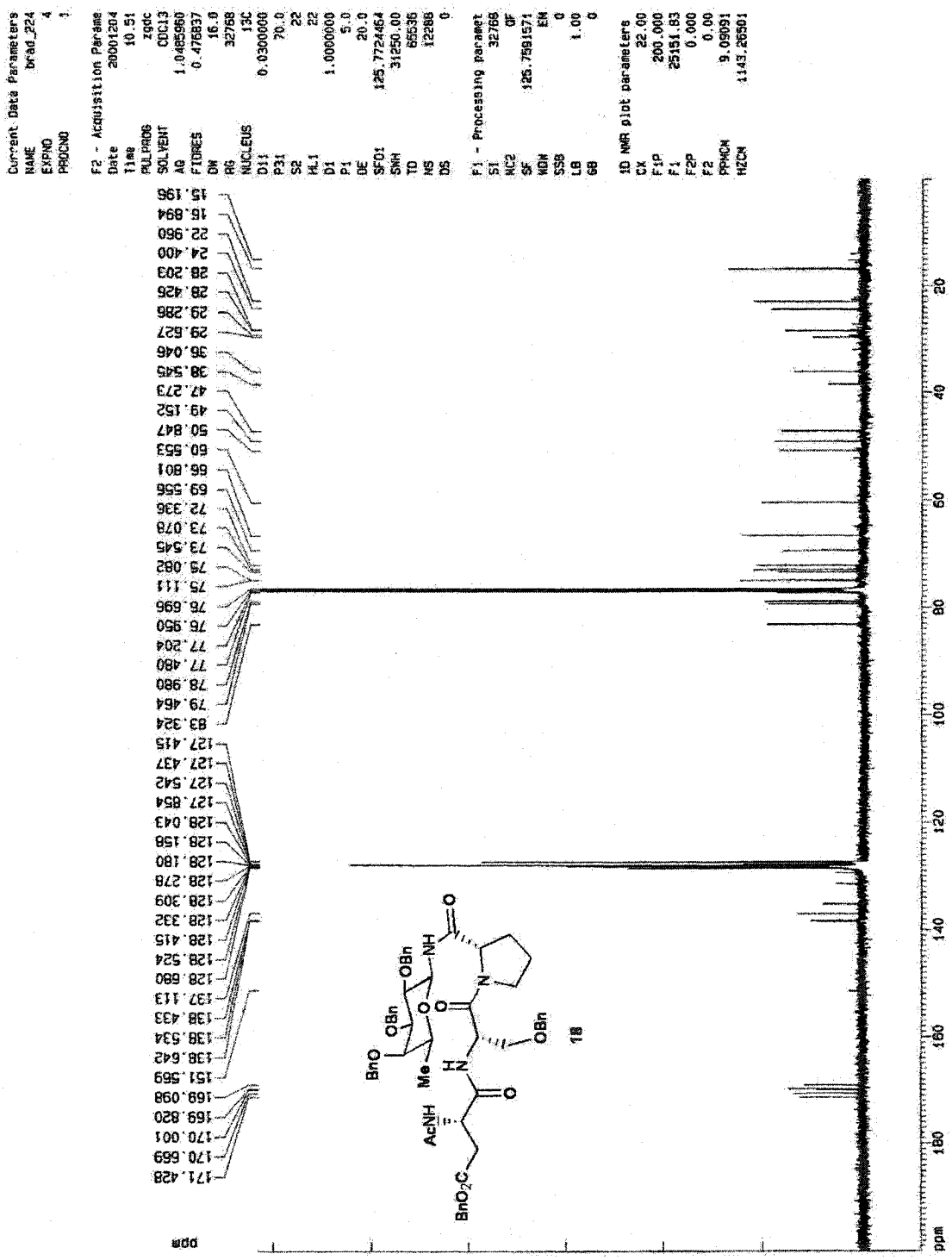


Figure **2.13**: ^{13}C NMR ($CDCl_3$, 125 MHz) of **18**.

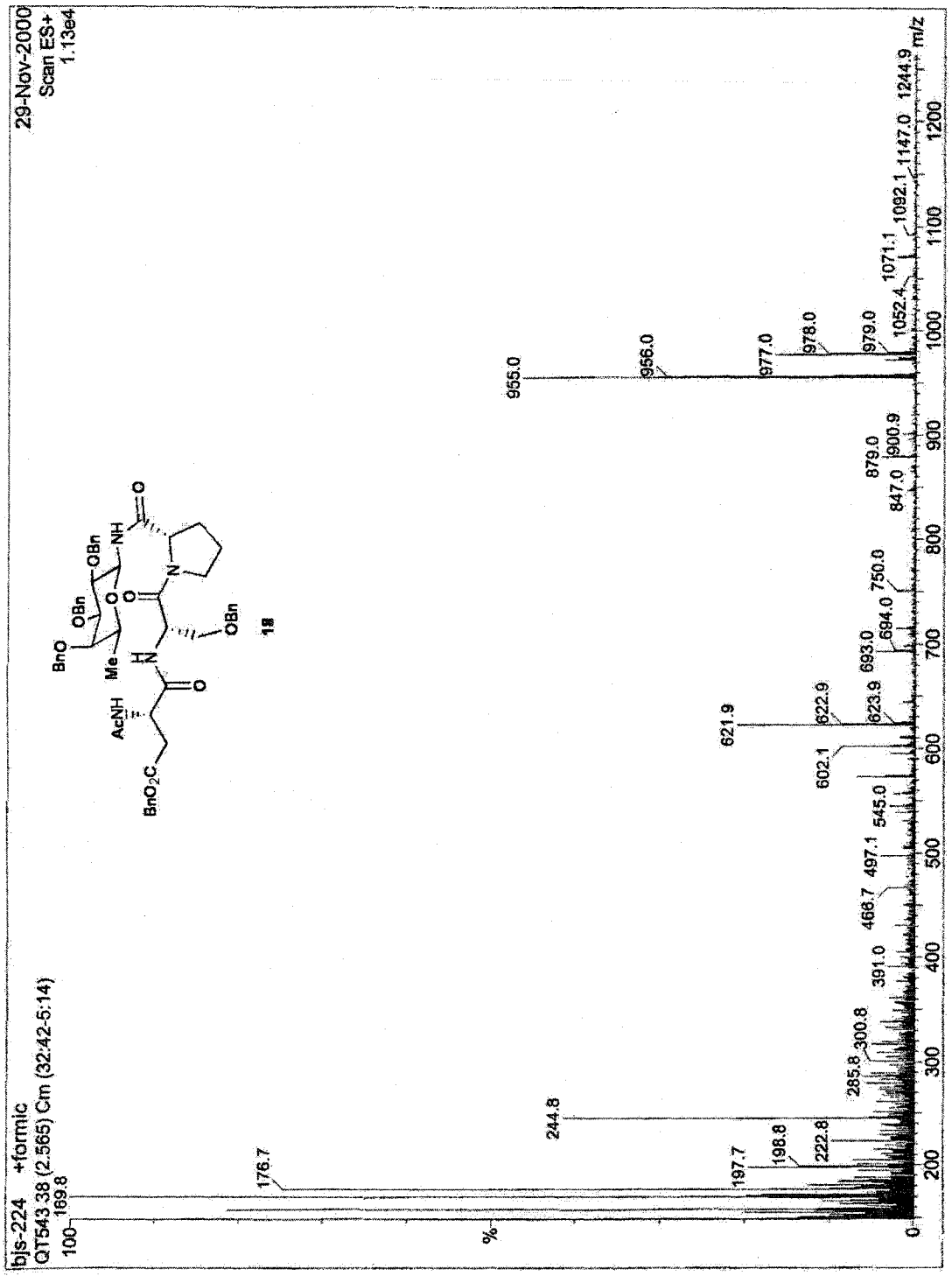


Figure **2.14**: Mass Spectrum (+ESI) of **18**.

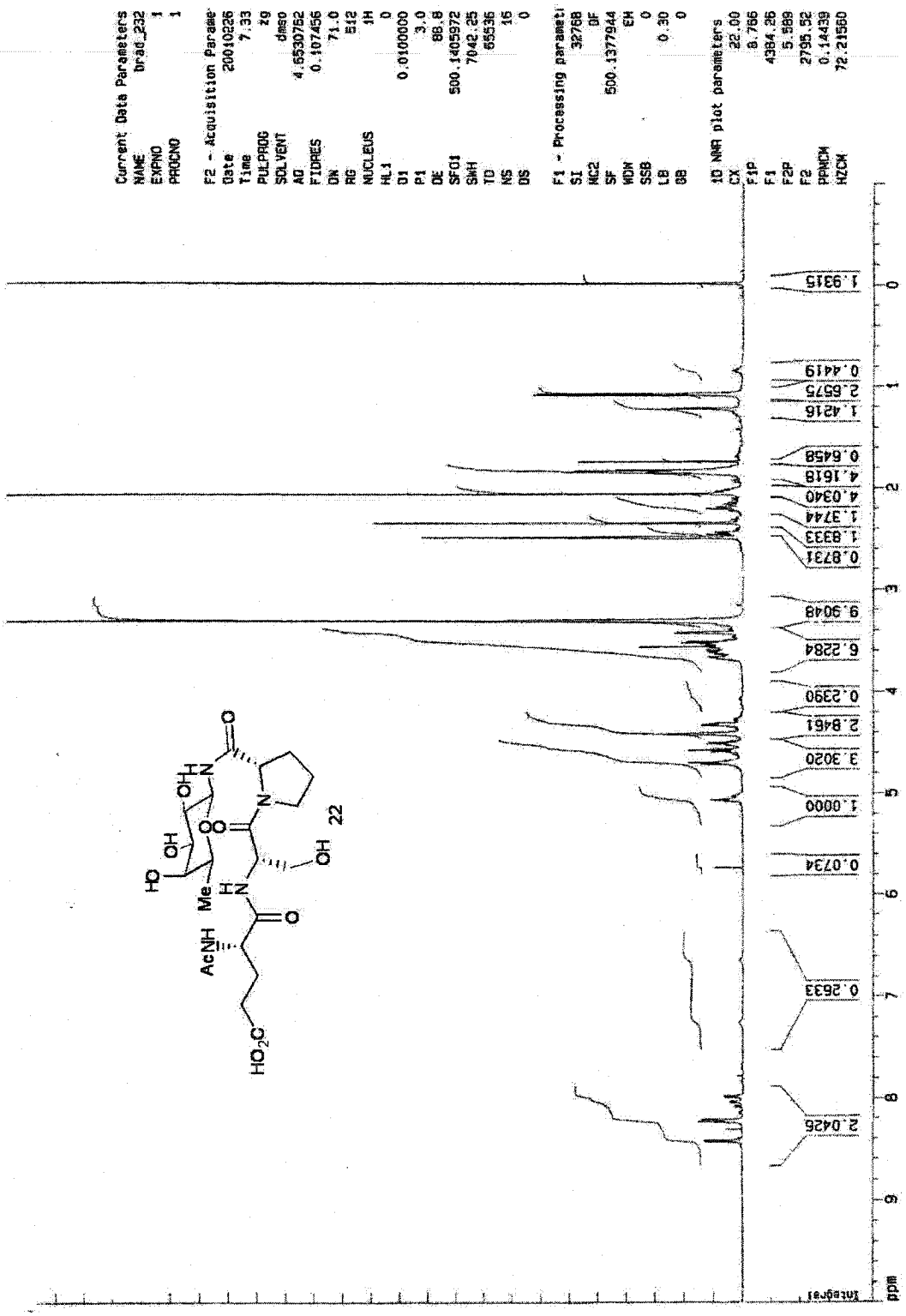


Figure **2.15**: ^{1}H NMR (DMSO-d_6, 500 MHz) of **22**.

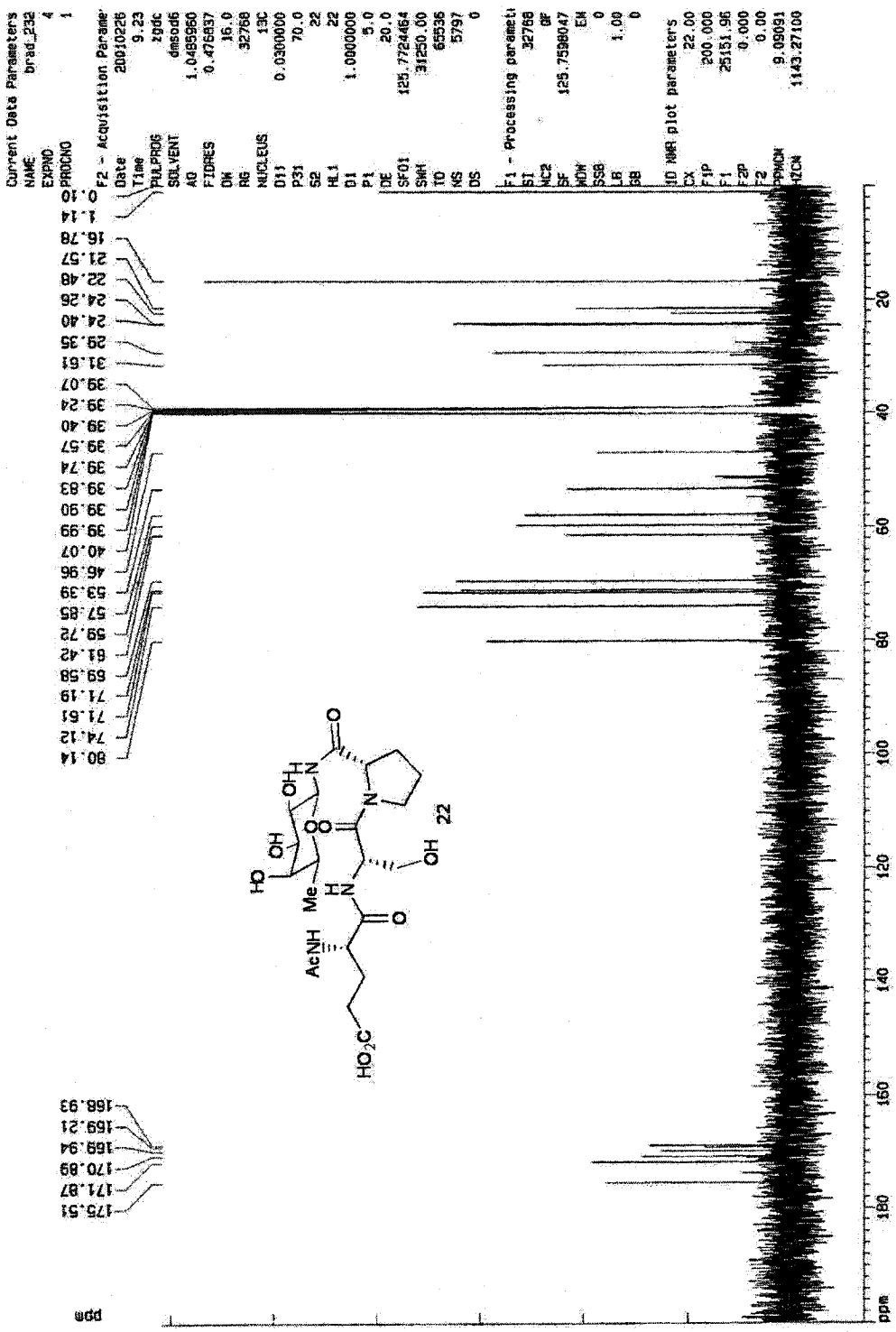


Figure **2.16**: ^{13}C NMR Spectrum (DMSO-d_6, 125 MHz) of **22**.

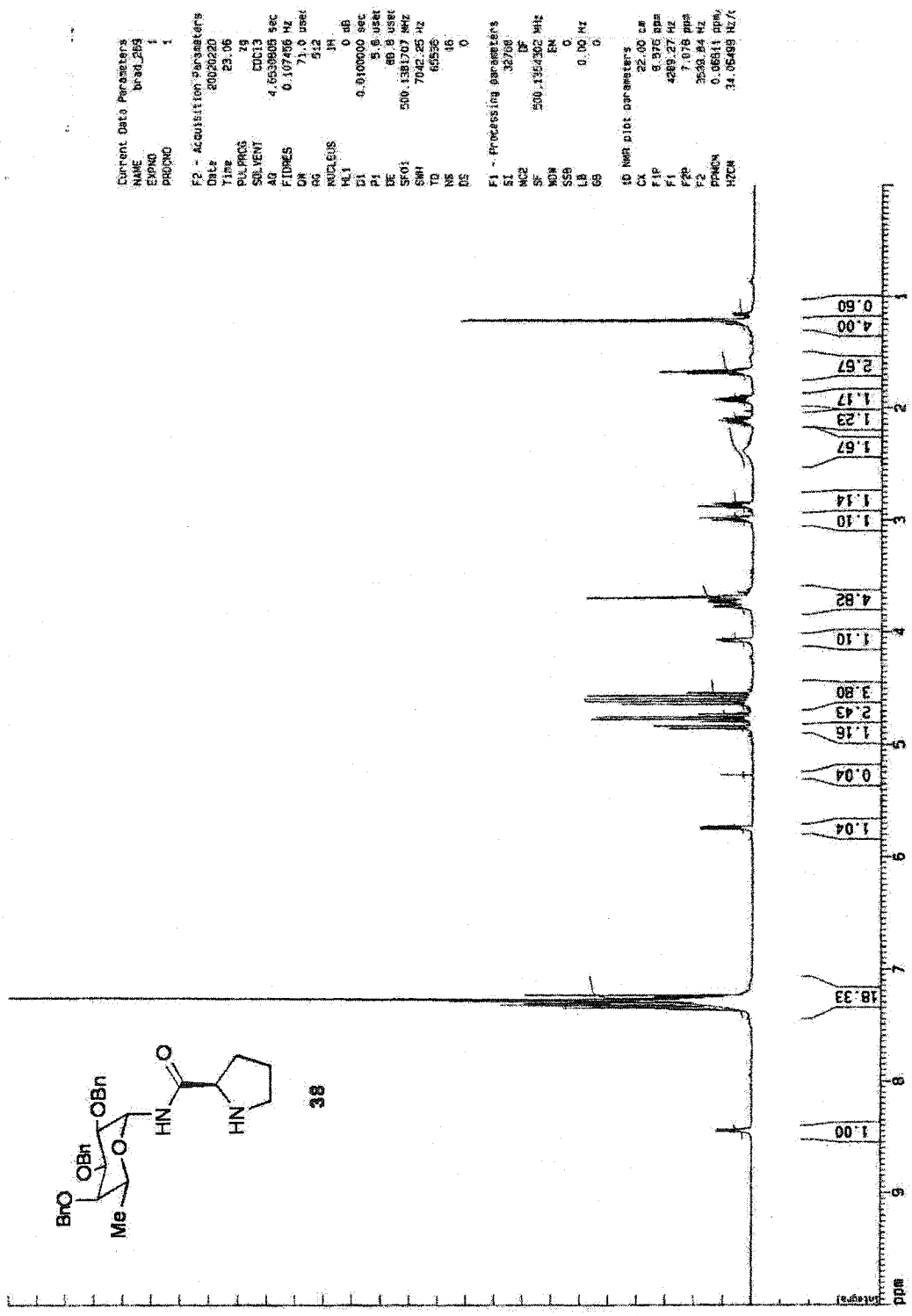


Figure **2.17**: ^{1}H NMR Spectrum ($CDCl_3$, 500 MHz) of **38**.

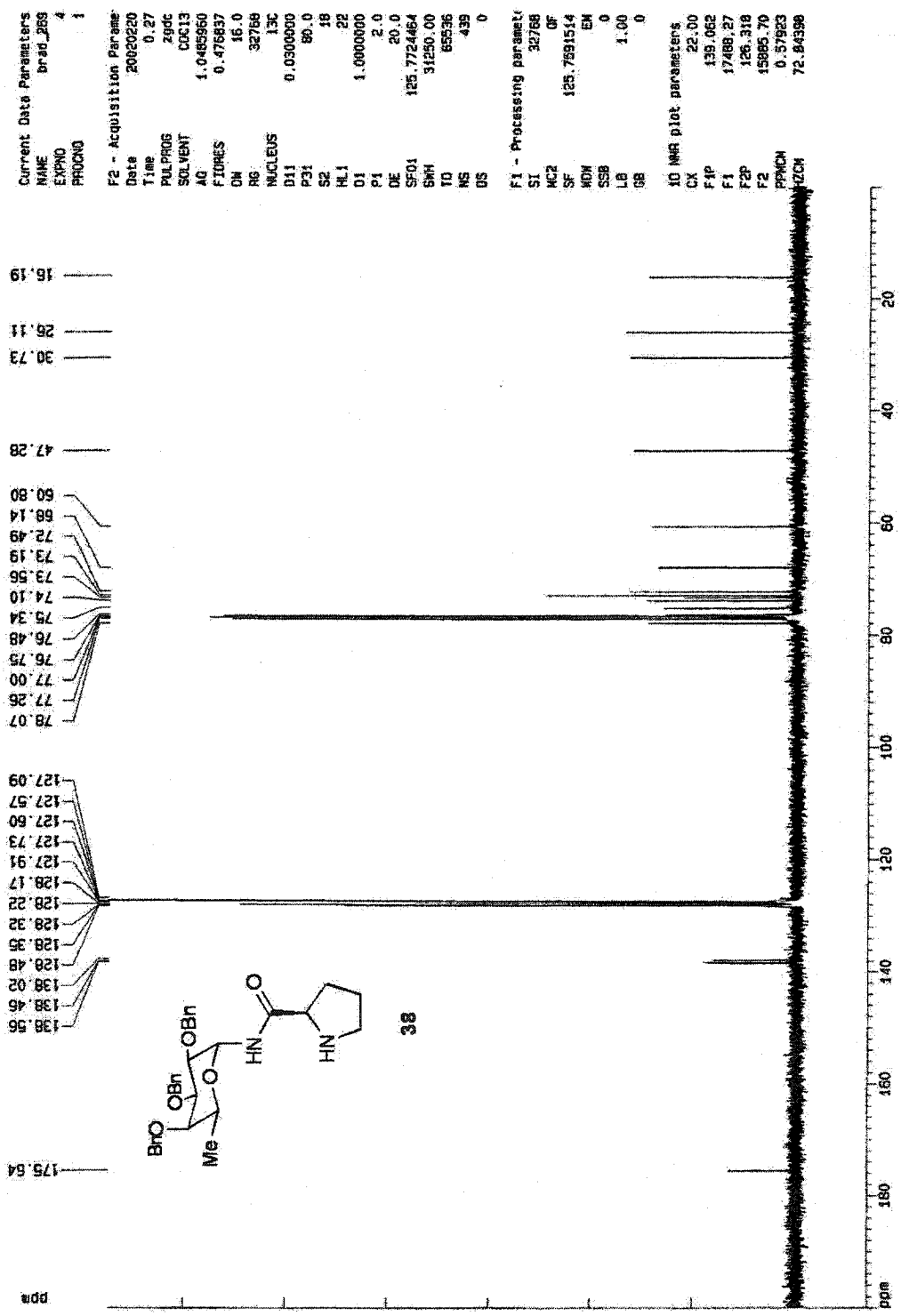


Figure **2.18**: ^{13}C NMR ($CDCl_3$, 125 MHz) of **38**.

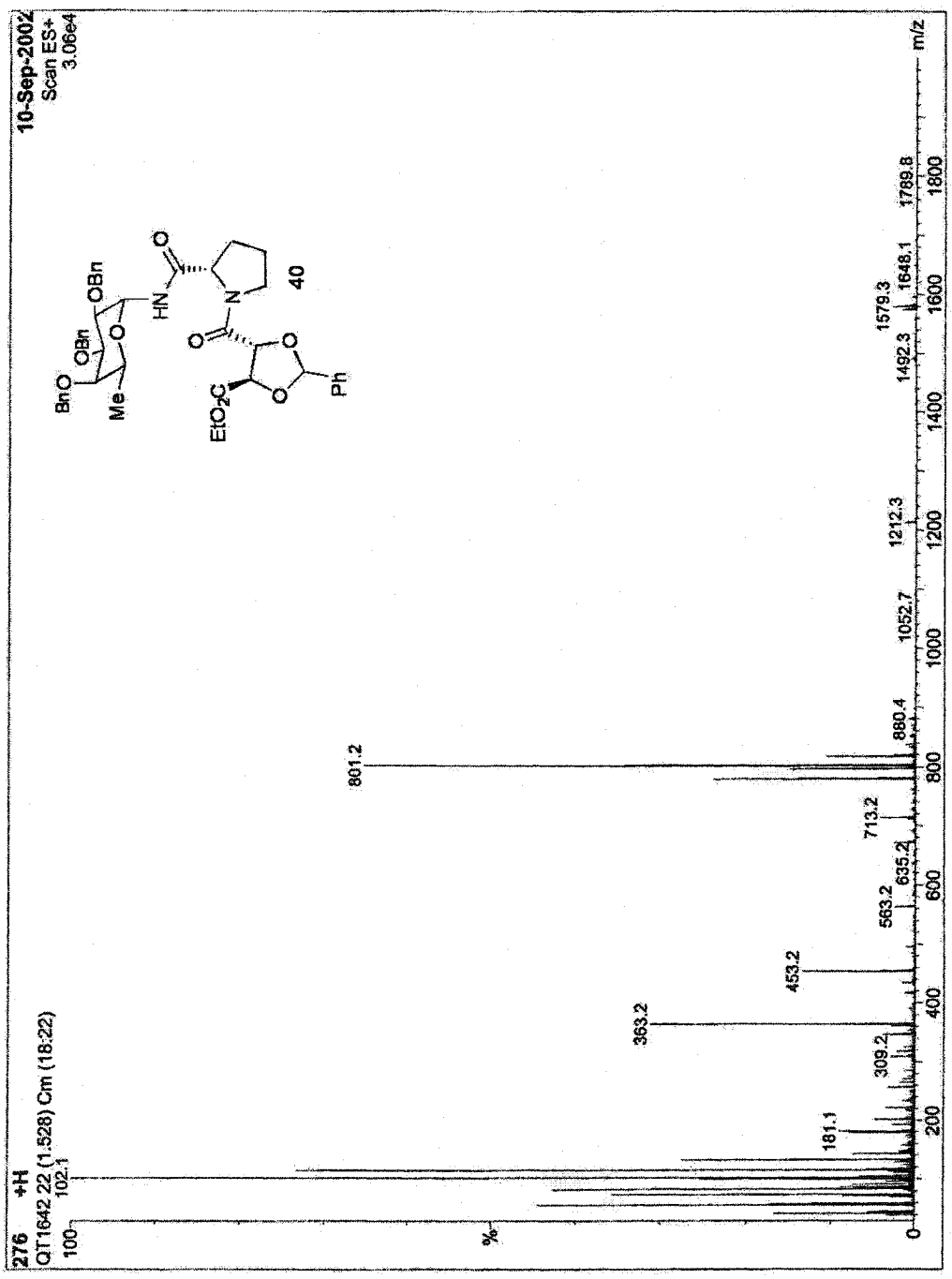


Figure **2.19**: Mass Spectrum (+ESI) of **40**.

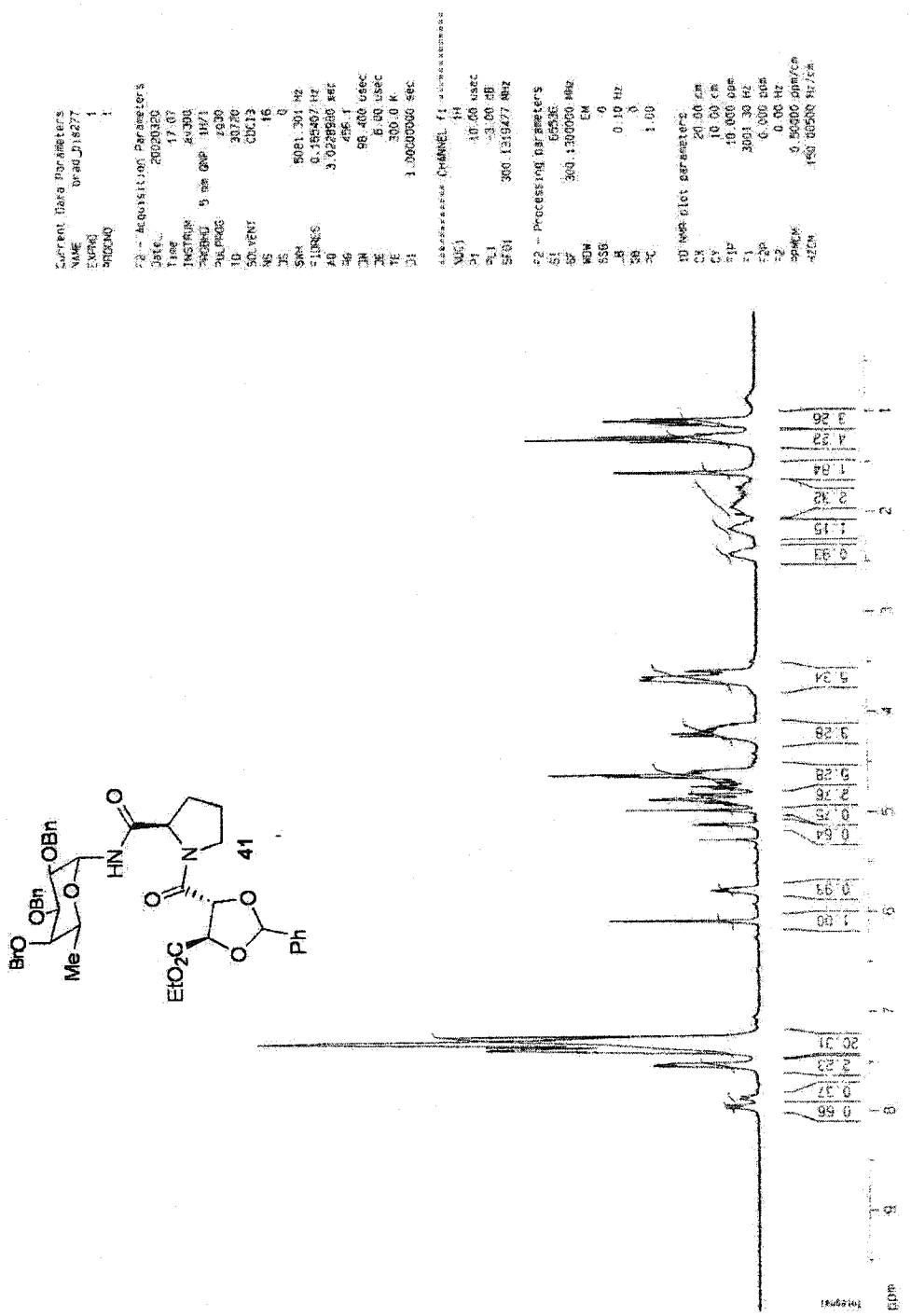


Figure **2.20**: ^{1}H NMR Spectrum ($CDCl_3$, 300 MHz) of **41**.

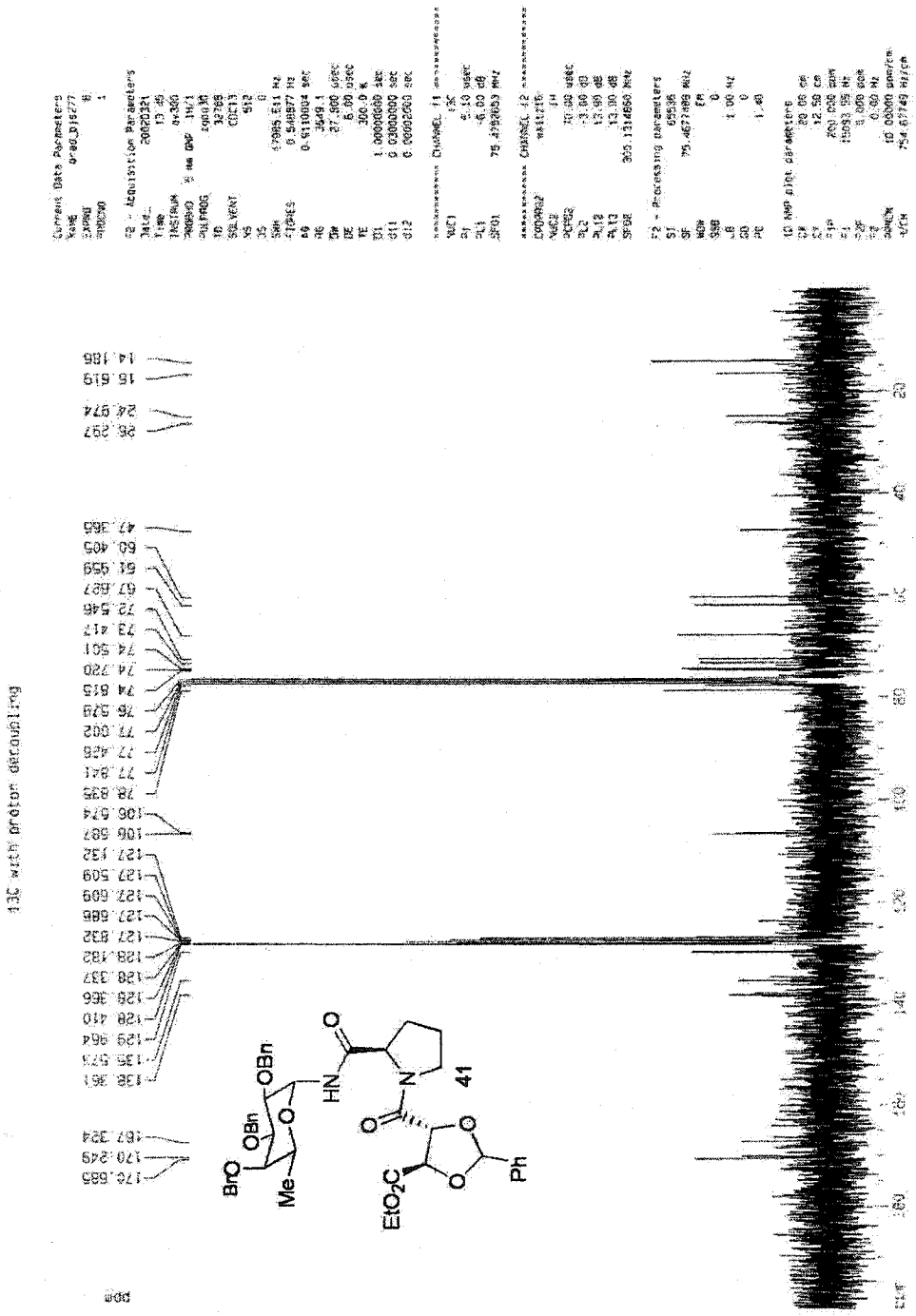


Figure **2.21**: ^{13}C NMR Spectrum ($CDCl_3$, 75 MHz) of **41**.

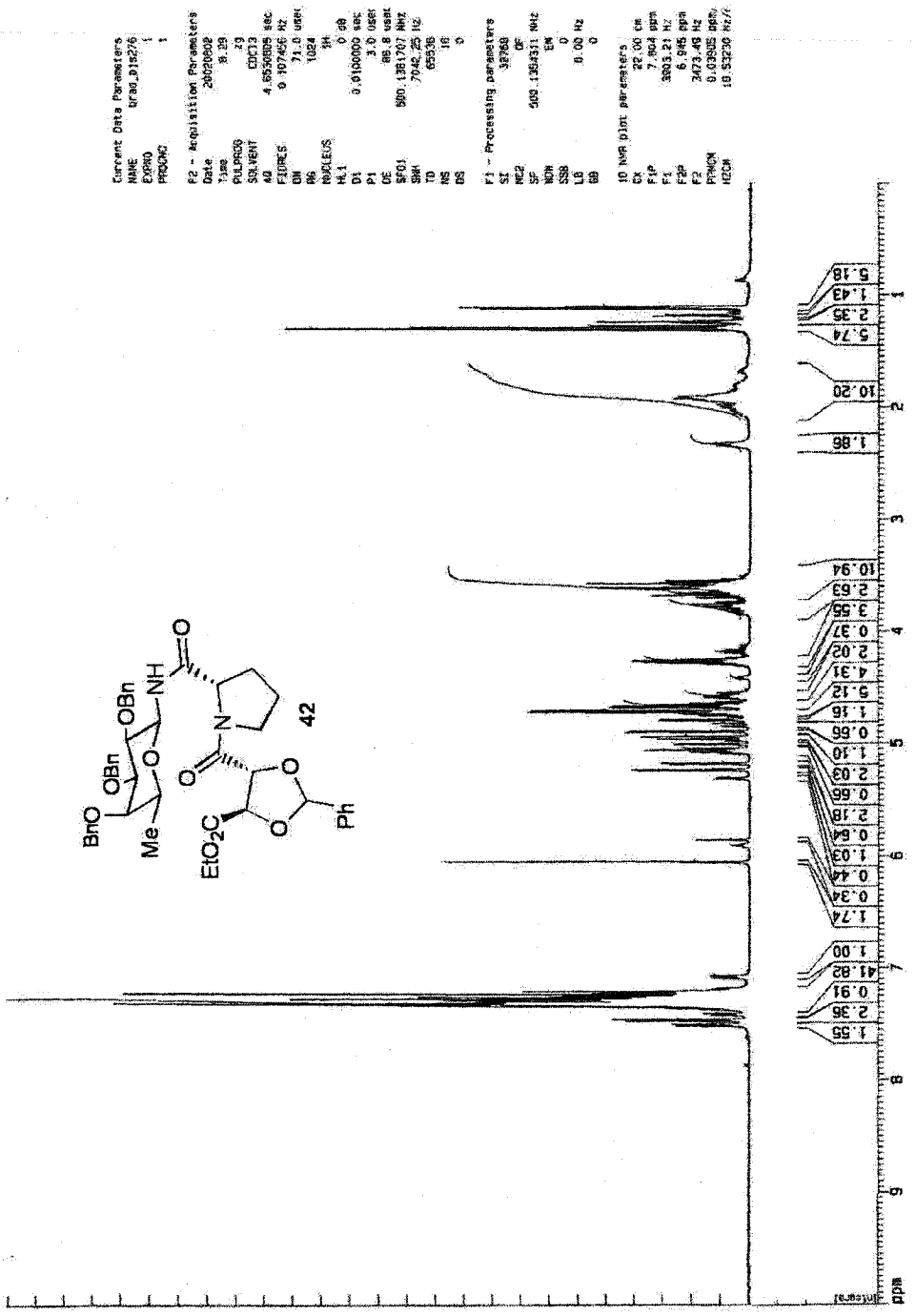


Figure **2.22**: ^{1}H NMR Spectrum ($CDCl_3$, 500 MHz) of **42**.

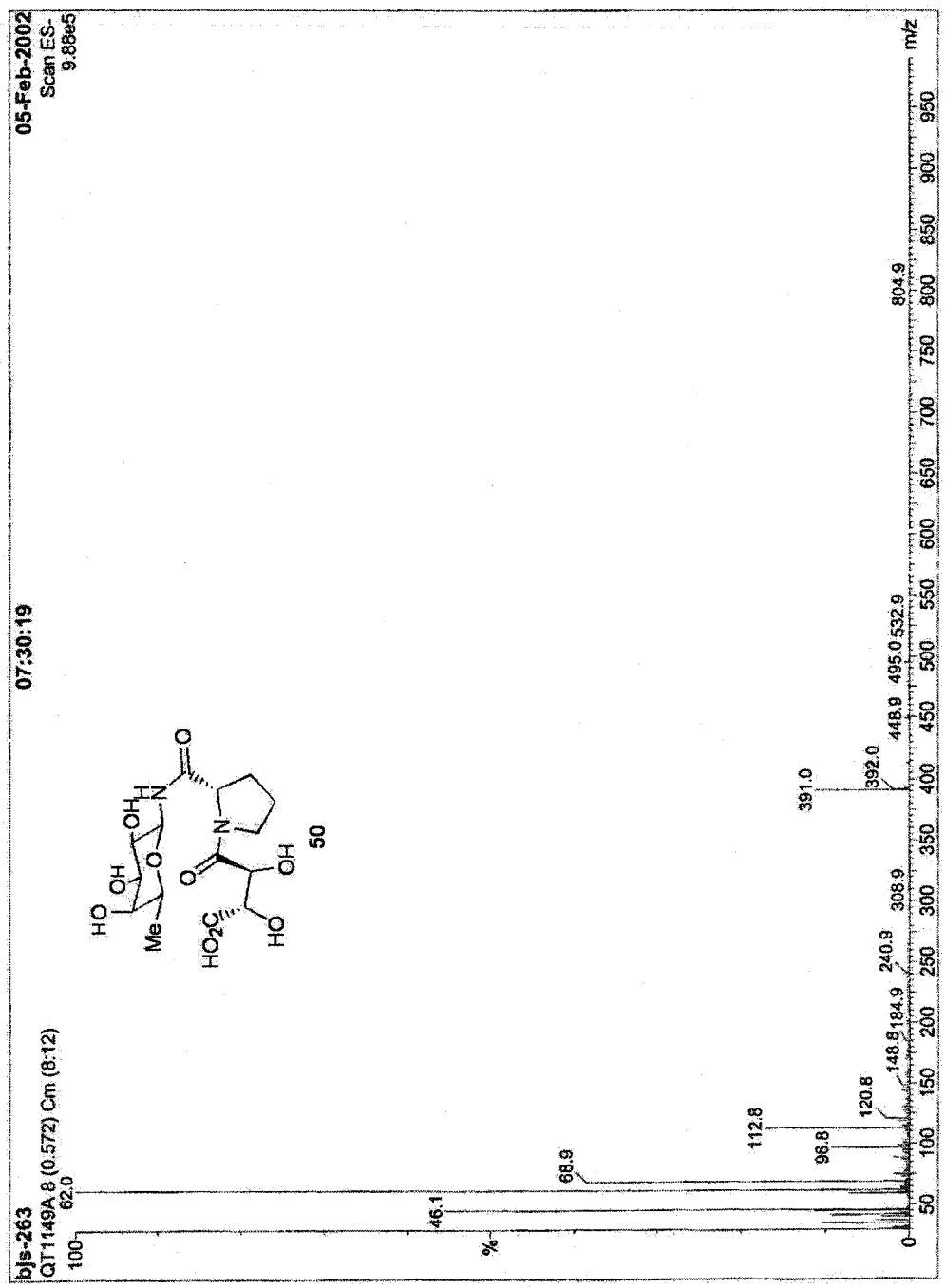


Figure **2.23**: Mass Spectrum (-ESI) of **50**.

2.6 EXPERIMENTAL

General Methods and Protocols Used Throughout the Thesis

These are general remarks encompassing the work performed. Individual protocols and obtained data may be found in the appropriate chapters.

Chemicals used throughout this work were largely obtained from Aldrich Chemical Company and used as received. Solvents were typically obtained from Baker. Grubbs' catalyst was obtained from Strem Chemicals Inc. and stored under nitrogen. Protected amino acids were purchased from Chem-Impex, NovaBioChem, or Advanced ChemTech. Dry benzene, tetrahydrofuran, dioxane and hexane were distilled from sodium-benzophenone ketyl. Dry dichloromethane, acetonitrile, and ethyl acetate were dried over phosphorus pentoxide and distilled. 20 % piperidine in acetonitrile was prepared as needed, as formation of complex products on storage has been observed.

Thin-layer chromatography was performed using glass or aluminum-backed plates containing fluorisil. Visualization was realized using ultraviolet light, cerium ammonium molybdate, ninhydrin spray or an iodine chamber, whichever applied for the particular example. Silica gel chromatography was performed using 60-mesh silica gel (Merck) with a cotton or glass wool plug.

Nuclear magnetic resonance (NMR) spectra were obtained using either a Varian Gemini 200 MHz instrument, a Bruker Avance 300 MHz, or a Bruker AMX 500 MHz spectrometer. Much of the 500 MHz data was obtained by Dr. Glenn Facey, Raj Capoor, or Kim Yach. Spectra were normally referenced to either the solvent peak (for $CDCl_3$ ^1H $\delta = 7.23$ ppm; ^{13}C $\delta = 77.0$; for DMSO-d_6 ^1H $\delta = 2.50$; ^{13}C $\delta = 39.5$) or tetramethylsilane ($\delta = 0.00$ ppm). Complete structural assignment was not always possible, since rotameric mixtures were commonly encountered, leading to either separate peaks or loss of resolution. In many cases, resolution lost at one field strength would be seen in another, and vice versa. Hence, obtaining spectra at multiple field strengths was sometimes beneficial.

Mass spectra were performed by Clem Kazakoff using a Kratos mass spectrometer. Spectra were obtained using EI, CI, FAB and ESI techniques.

Infrared spectra were obtained using a Bomen Fourier transform infrared spectrometer. Solid samples were normally analyzed as thin films deposited on plates of potassium bromide. Liquid samples were normally analyzed as thin films between two KBr plates.

Enzymatic hydrolyses were performed using Pig Liver Esterase obtained from Sigma. A pH of 8.0 was maintained by addition of sodium hydroxide using a Titration 11/Ole Dich autotitrator assembly, and measured using a Radiometer Copenhagen type

PHM26 pH meter fitted with an ORION semi-micro gel-filled combination electrode model 91-15.

Optical rotation was obtained as a solution in a 10 cm path length, using a Perkin-Elmer polarimeter. Melting points were obtained using a Gallenkamp apparatus and are uncorrected.

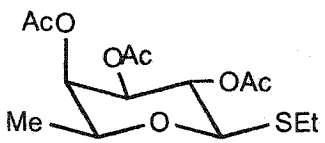
X-ray crystallographic analysis was performed by Dr. Glenn Yap at the University of Ottawa X-Ray Crystallography Center.

Solid-phase syntheses were conducted in BioRad ion-exchange cartridges of either 2 or 10 mL volume (whichever appropriate). Agitation was accomplished by taping the cartridges to a Variac-controlled rotary evaporator and turning slowly. The mixtures were drained by suction applied from the bottom.

2.7 Preparation of Compounds

2.7.1 Solution-Phase Synthesis

1-Thioethyl-2,3,4-tri-*O*-acetyl-β-L-fucopyranose (2)[88]



2

Tetra-*O*-acetyl-L-fucopyranose **1** (5.09 g, 15.3 mmol) of was dissolved in 80 ml dry CH_2Cl_2. This solution was brought to 0 °C on an ice bath under a stream of N_2. To this solution, 22.5 ml of a solution of ethanethiol in $CFCl_3$ (15% w/v, 45.9 mmol, 3 eq.) was added, followed by the slow addition of BF_3OEt_2 (2.52 ml, 19.3 mmol, 1.3 eq.) and allowed to stir for 6 hours. The solution was then quenched with DIPEA (10 ml) and evaporated to a brown semisolid, which was purified by column chromatography using 8:1 hexanes/ethyl acetate as eluent, to give pure **2** as a thick oil. This could be induced to crystallize by treatment with cold hexane, yielding large crystals, mass 3.76 g (74%).

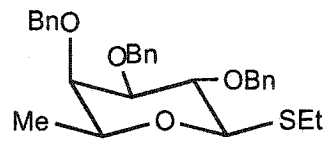
m.p. 78.5 - 79.1° C (Lit.[88] oil).

M.S. (+FAB) (Calc. for $C_{14}H_{22}O_7S$ = 334, Found *m/z* =333 $(C_{14}H_{22}O_7S - H^-)^+$

^1H ($CDCl_3$, 500 MHz) δ (ppm): 5.17 (1H, dd, $J_{3,4}$= 1.0 Hz, $J_{4,5}$ = 3.4 Hz, H-4), 5.11 (1H, t, J = 10.0 Hz, H-2), 4.95 (1H, dd, $J_{2,3}$ = 10.0 Hz, $J_{3,4}$ = 3.4 Hz, H-3), 4.37 (1H, d, $J_{1,2}$ = 10.0 Hz, H-1), 3.74 (1H, dq, $J_{4,5}$ = 1.1 Hz, $J_{5,6}$ = 6.4 Hz, H-5), 2.62 (2H, m, S-C$\underline{H}_2$CH$_3$), 2.07, 1.96, 1.88 (3 x 3H, 3s, 3 x COC$\underline{H}_3$), 1.18 (3H, t, J = 7.4 Hz, S-CH$_2$C$\underline{H}_3$), 1.11 (3H, d, $J_{5,6}$ = 6.4 Hz).

^{13}C (125 MHz) δ (ppm): 170.45, 169.91, 169.47 ($\underline{C}OCH_3$), 83.35 (C-1), 73.03 (C-3), 72.24 (C-2), 70.38 (C-4), 67.24 (C-5), 23.92 (S$\underline{C}H_2CH_3$), 20.68, 20.53, 20.47 (3 x CO$\underline{C}H_3$), 16.28 (C-6), 14.63 (SCH$_2\underline{C}H_3$).

1-Thioethyl-2,3,4-tri-*O*-benzyl-β-L-fucopyranose (3)[89,90]



3

1-Thioethyl-2,3,4-tri-*O*-acetyl-β-L-fucopyranose **2** (3.76 g, 13.0 mmol) was dissolved in CH_2Cl_2 (30 ml) and methanol (20 ml). To it was added NaOMe solution in methanol (~1 M, Zemplen conditions) until pH was indicated to be 10 (pH paper). Thin-layer chromatography at 100 minutes (10:1 $CHCl_3$:MeOH) showed the reaction to be complete. Amberlite IR-120 (H^+ resin) was added until pH 4 was achieved. The solution was filtered and evaporated to give a solid. The crude solid was dissolved in DMF (50 ml) and brought to 0 °C on ice. To it was added 4.9 g NaH (~50% w/w dispersion in mineral oil, 117 mmol, 9 eq.) which had been washed with dry hexane to remove the oil. Benzyl bromide (12.1 ml, 117 mmol, 9 eq.) was added dropwise via syringe, and the solution stirred for 30 minutes, after which the ice was removed and the reaction allowed to reach room temperature, an stirred for 18 hours. Then, 30 ml of dry methanol was carefully added, and the reaction stirred for 30 minutes. The solution was evaporated as much as possible (DMF remaining). The crude product was brought up in CH_2Cl_2/water (200 ml/200 ml), and the aqueous layer extracted 2 x 150 ml CH_2Cl_2. The extracts were dried over Na_2SO_4, filtered and evaporated. The crude sludge was then chromatographed using hexane:ethyl acetate (15:1) to give 4.70 g (87%). Semi-pure fractions can, if necessary, be crystallized from 99% EtOH to avoid secondary chromatography.

m. p. 52.0 - 52.5 °C (Lit.[89] 53 °C)

M.S. (+FAB) : (Calc. for $C_{28}H_{34}O_4S$ = 478, Found *m/z* 477 $(C_{28}H_{34}O_4S - H)^+$

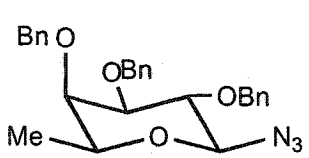
^1H ($CDCl_3$, 500 MHz) δ (ppm): 7.35 - 7.25 (15H, m, Ar-H), 5.00 (1H, d, ^2J = 11.8 Hz, benzylic-H), 4.90 (1H, d, ^2J = 10.2 Hz, benzylic-H), 4.81 (1H, d, ^2J = 10.2 Hz, benzylic-H), 4.76 (2H, dd, ^2J = 11.8 Hz, benzylic-H), 4.70 (1H, d, ^2J = 11.8 Hz, benzylic-H), 4.40 (1H, d, $J_{1,2}$ = 9.6 Hz, H-1), 3.83 (1H, t, J = 9.4 Hz, H-2), 3.62 (1H, d, J = 2.2 Hz, H-4), 3.57 (1H, dd, $J_{3,4}$ = 2.9 Hz, $J_{2,3}$ = 9.3 Hz, H-3), 3.48 (1H, dq, $J_{4,5}$ = 0.7 Hz, $J_{5,6}$ = 6.4 Hz, H-5), 2.75 (2H, m, $SC\underline{H}_2CH_3$), 1.30 (3H, t, J = 7.4 Hz, $SCH_2C\underline{H}_3$), 1.21 (3H, d, J = 6.4 Hz, H-6)

^{13}C (125 MHz) δ (ppm): 138.7, 138.5, 138.4 (4° Ar-C), 128.4, 128.3, 128.1, 128.1, 127.7, 127.6, 127.5, 127.5 (Ar-CH), 84.9 (C-1), 84.5 (C-3), 78.3 (C-2), 76.5 (C-4), 75.6, 74.5*, 72.9 (benzylic-C), 74.5* (C-5), 24.6 ($S\underline{C}H_2CH_3$), 17.2 (C-6), 15.0 ($SCH_2\underline{C}H_3$).

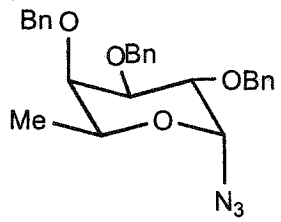
*the C-5 peak at 74.5 ppm overlaps perfectly with the benzylic signal at the same chemical shift; these peaks are only discernable by examination of the DEPT spectrum.

2,3,4-Tri-*O*-benzyl-β-L-fucopyranosyl azide (4)

2,3,4-Tri-*O*-benzyl-α-L-fucopyranosyl azide (5)



4



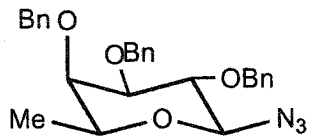
5

1-Thioethyl-2,3,4-tri-*O*-benzyl-β-L-fucopyranose **3** (3.31 g, 6.91 mmol) was dissolved in dry acetonitrile (100 ml) under a nitrogen stream, and brought to –40 °C with a dry ice/acetonitrile bath. To this was added solid *N*-iodosuccinimide (1.80 g, 7.60 mmol, 1.1 eq.), azidotrimethylsilane (1.1 ml, 8.29 mmol, 1.2 eq.), and finally trifluoromethanesulfonic acid (0.64 ml, 7.26 mmol, 1.05 eq.). The deep red-brown solution was stirred for 100 minutes. The reaction was quenched with a solution of $Na_2S_2O_3$ (4 g in 100 ml) to destroy the deep iodine color, and the resulting mixture allowed to warm to room temperature. The acetonitrile was removed by rotary evaporation, and the aqueous suspension was extracted with 2 x 200 ml of CH_2Cl_2. These extracts were dried (Na_2SO_4), filtered, and evaporated to give a thick crude pale yellow oil, mass 3.21 g (101 %). Column chromatography (hexane:ethyl acetate, 6:1) gave the two separated azides **5** (1.90 g, 60%) and **4** (0.99 g, 31%) (total combined yield 91%).

Note: These azides often have trace contamination of ethanethiol remaining, which greatly impedes catalytic hydrogenation, and cannot be removed by recrystallization,

prolonged exposure to high vacuum, or treatment of activated carbon. This can be removed by dissolving the azide in diethylether (1 g/30 ml) and quickly washing 1 x with an equal volume of 30% commercial bleach in water, and 1 x with distilled water followed by drying (Na_2SO_4), filtering and evaporation (>95% recovery).

2,3,4-Tri-*O*-benzyl-β-L-fucopyranosyl azide (4)



4

m.p. (oil)

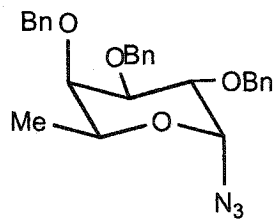
$[\alpha_D]$ (c = 1.58, $CHCl_3$): +8.2°

M.S. (+FAB) : (Calc. for $C_{26}H_{29}N_3O_4$ = 459, Found *m/z* 432 $(C_{26}H_{29}N_3O_4 + H - N_2)^+$

^1H ($CDCl_3$, 500 MHz) δ (ppm): 7.36 - 7.24 (15H, m, Ar-H), 4.96 (1H, d, ^2J = 9.7 Hz, benzylic-H), 4.82 (1H, d, ^2J = 10.3 Hz, benzylic-H), 4.72 (2H, 2 x d, ^2J = 10.8 Hz, ^2J = 9.9 Hz, benzylic-H), 4.69 (1H, d, ^2J = 18.6 Hz, benzylic-H), 4.66 (1H, d, ^2J = 11.7 Hz, benzylic-H), 4.56 (1H, d, $J_{1,2}$ = 8.4 Hz, H-1), 3.74 (1H, dd, $J_{1,2}$ = 8.6 Hz, $J_{2,3}$ = 9.5 Hz, H-2), 3.59 (1H, d, J = 2.3 Hz, H-4), 3.55–3.51 (m, 2H, H-3, H-5), 1.20 (3H, d, J = 6.3 Hz, H-6).

^{13}C (125 MHz) δ (ppm): 138.4, 138.4, 138.0 (4° Ar-C), 128.6, 128.6, 128.5, 128.5, 128.1, 127.7, 127.7, 127.7, 127.3 (Ar-CH), 82.6, 78.9, 77.5, 75.5, 75.1, 73.6, 73.2, 68.9, 16.2 (C-6).

2,3,4-Tri-*O*-benzyl-α-L-fucopyranosyl azide (5)



5

m.p. 74.2 - 74.8 °C

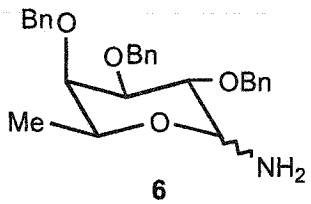
$[\alpha_D]$ (c = 1, $CHCl_3$): -85.4 °

M.S. (+FAB) : (Calc. for $C_{26}H_{29}N_3O_4$ = 459, Found *m/z* 432 $(C_{26}H_{29}N_3O_4 + H - N_2)^+$

^1H ($CDCl_3$, 500 MHz) δ (ppm): 7.37 - 7.24 (15H, m, Ar-H), 5.26 (1H, d, $J_{1,2}$ = 4.3 Hz, H-1), 4.95 (1H, d, ^2J = 11.5 Hz, benzylic-H), 4.82 (2H, 2 x d, ^2J = 11.8 Hz, ^2J = 1.5 Hz, benzylic-H), 4.70 (1H, d, ^2J = 18.6 Hz, benzylic-H), 4.69 (1H, d, ^2J = 18.6 Hz, benzylic-H), 4.62 (1H, d, ^2J = 11.6 Hz, benzylic-H), 4.09 (1H, dd, $J_{1,2}$ = 4.2 Hz, $J_{2,3}$ = 9.9 Hz, H-2), 3.62 (1H, dq, $J_{4,5}$ = 0.5 Hz, $J_{5,6}$ = 6.4 Hz, H-5), 3.78 (1H, dd, $J_{2,3}$ = 9.9 Hz, $J_{3,4}$ = 2.8 Hz, H-3), 3.62 (1H, dd, $J_{3,4}$ = 2.7 Hz, $J_{4,5}$ = 0.9 Hz, H-4), 1.12 (3H, d, J = 6.5 Hz, H-6)

^{13}C (125 MHz) δ (ppm): 138.6, 138.3, 138.1 (4° Ar-C), 128.5, 128.4, 128.4, 128.3, 128.0, 127.9, 127.7, 127.6, 127.5 (Ar-CH), 88.9, 79.2, 77.3, 75.9, 75.0, 73.9, 73.3, 68.9, 16.6 (C-6).

2,3,4-Tri-*O*-benzyl-L-fucopyranosylamine (6)



6

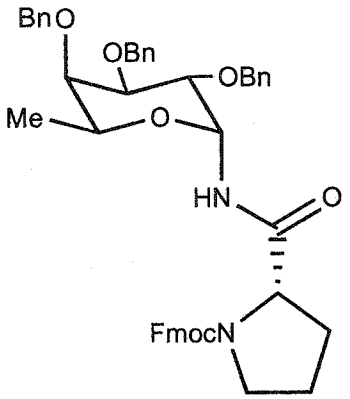
Azide **5** (1.62 g, 3.51 mmol) of was dissolved in 50 ml of dry MeOH. To it was added 136 mg of 10% Pd/C, and the solution set to stir under a stream of hydrogen gas. After 3.5 hours the reaction was complete by TLC (2:1 hexane:ethyl acetate), filtered through a pad of Celite, and evaporated down to a thick oil. This material is then suitable for use in coupling without further purification.

Notes:

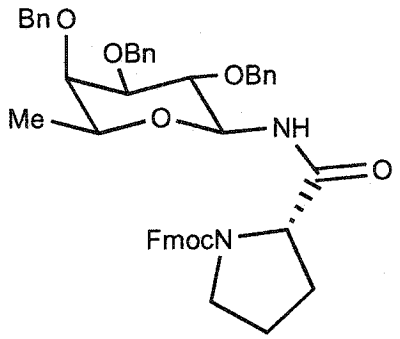
1. Either **4** or **5** give identical results, as the free amino group quickly anomerizes during reduction. Use of other solvents does not seem to have any effect.
2. The azide is not very soluble in methanol; this can be overcome by the addition of small amounts of cosolvents such as THF.
3. See notes for **4** and **5** regarding contaminating thiols.

***N*-Fmoc (*N*'-2,3,4-tri-O-benzyl-α-L-fucopyranosyl)- L-proline (7)**

***N*-Fmoc (*N*'-2,3,4-tri-O-benzyl-α-L-fucopyranosyl)- L-proline (8)**



7



8

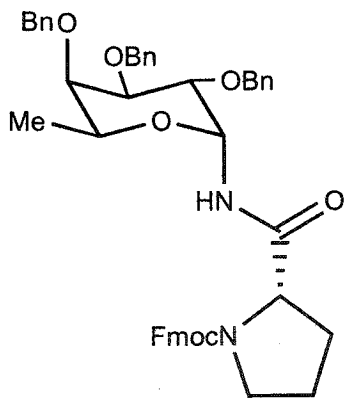
Method A : HATU

Amine **6** (3.07 mmol) was dissolved in 10 ml DMF. To it was added 1.17 g (3.34 mmol, 1.1 eq.) of (L)-*N*-Fmoc-proline, 1.32 g of HATU (3.34 mmol, 1.1 eq.) and DIPEA (1 ml). The resulting deep yellow solution was stirred for 3 hours, and then brought up in CH_2Cl_2/water (200 ml/200 ml) with about 2 g of solid $NaHCO_3$, and the resulting organic phase was removed. The aqueous phase was further extracted with 100 ml CH_2Cl_2. The combined extracts were washed with water (200 ml), dried (Na_2SO_4), filtered and the filtrate was concentrated to give a thick oily solid. This solid was directly chromatographed (1:1 hexane:ethyl acetate) to give 0.43 g (19%) of **7** and 1.43g (62%) of **8** (combined yield 81%, 4:1 β/α ratio).

Method B : EDC

(L)-*N*-Fmoc-proline (475 mg, 1.40 mmol, 1.2 eq.), EDC (269 mg, 1.40 mmol, 1.2 eq.), NHS (162 mg, 1.40 mmol, 1.2 eq.) and 3 drops of DIPEA (catalytic) were dissolved in 20 ml dioxane. To it was added a solution of 1.17 mmol (1 eq.) of amine **6** in dioxane (6 ml). The solution was allowed to stir overnight. Then, the dioxane was evaporated, and the remaining material dissolved in CH_2Cl_2 (40 ml). This layer was washed with 40 ml 5% $NaHCO_3$, which was then back-extracted with CH_2Cl_2 (30 ml). The organic layers were combined, dried (Na_2SO_4), filtered and evaporated, giving 927 mg of crude solid. Column chromatography (hexane:ethyl acetate, 2:1, brought to 1:1) gave 65 mg **7** (7%) and 485 mg of **8** (55%).

_N_-Fmoc (_N'_-2,3,4-tri-O-benzyl-α-L-fucopyranosyl)- L-proline (7)



7

m.p. 142-143° C

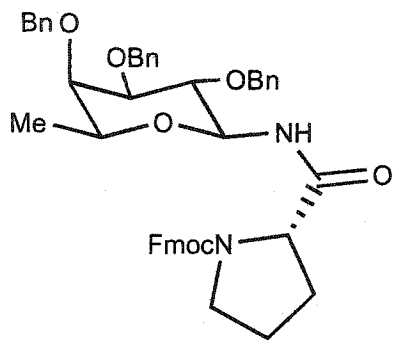
$[\alpha_D]$ (c = 1, $CHCl_3$) -47.7°

M.S. (+FAB) : (Calc. for $C_{46}H_{48}N_2O_7$ = 752, Found *m/z* 753 $(C_{46}H_{48}N_2O_7 + H)^+$.

^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) : 7.82 (1H, d, J = 6.7 Hz, amide N-H), 7.75 (2H, d, J = 6.3 Hz, Fmoc-Ar-H), 7.53 (2H, d, J = 7.0 Hz, Fmoc-Ar-H), 7.37-7.22 (18H, m, Ar-H), 5.25 (1H, m, H-1), 4.80 (1H, d, J = 12.1 Hz, benzylic-H), 4.74-4.55 (5H, m, benzylic-H), 4.35 (2H, m, Fmoc-CH_2), 4.29 (1H, m, Fmoc-CH), 4.26 (1H, m, α-Pro), 4.11 (1H, m, H-2), 3.73 (1H, m, H-5), 3.66 (1H, m, H-3), 3.59 (1H, m, H-4), 3.55 (1H, m, δ-Pro), 3.42 (1H, m, δ-Pro), 2.48 (1H, m, β-Pro), 2.19 (1H, m, γ-Pro), 1.84 (2H, m, γ-Pro, β-Pro), 1.19 (3H, d, J = 6.5 Hz, H-6).

^{13}C (125 MHz) δ (ppm): 170.9 (C=O amide), 155.7 (C=O carbamate), 143.5, 141.5, 138.5, 128.5, 128.3, 127.8, 127.7, 127.7, 127.7, 127.2, 127.1, 119.8 (Ar-C), 78.1 (C-3), 76.6 (C-4), 75.0 (C-2), 74.3 (C-1), 73.9, 73.4, 72.8 (Bn-CH_2), 68.0 (C-5), 67.5 (Fmoc-CH_2), 60.0 (α-Pro), 47.4 (δ-Pro), 29.5 (Fmoc-CH), 27.5 (β-Pro), 24.9 (γ-Pro), 16.4 (C-6).

_N_-Fmoc (_N'_-2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-L-proline (8)



8

m.p. 114-117 °C

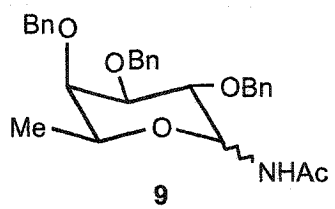
$[\alpha_D]$ (c = 1, $CHCl_3$) –69.6°

M.S. (+FAB) : (Calc. for $C_{46}H_{48}N_2O_7$ = 752, Found *m/z* 753 $(C_{46}H_{48}N_2O_7 + H)^+$.

^1H ($CDCl_3$, 500 MHz) δ (ppm): 7.73 (2H, s, Ar-H), 7.56 (2H, s, Ar-H), 7.35 - 7.28 (19H, m, Ar-H + N-H), 5.11 (1H, d, $J_{1,2}$ = 9.1 Hz, H-1), 4.96 (1H, d, ^2J = 11.6 Hz, benzylic-H), 4.79-4.67 (5H, m, benzylic-H), 4.39 (1H, m, α-Pro), 4.32 (1H, m, δ-Pro), 4.21 (1H, m, δ-Pro), 3.76 (1H, dd, $J_{1,2}$ = 8.9 Hz, $J_{2,3}$ = 8.8 Hz, H-2), 3.63 (1H, dd, $J_{2,3}$ = 8.3 Hz, $J_{3,4}$ = 2.7 Hz, H-3), 3.59 (1H, d, J = 2.6 Hz, H-4), 3.56 (1H, q, $J_{5,6}$ = 6.4 Hz, H-5), 3.51, 3.40 (2 x 1H, 2m, Fmoc-CH_2), 2,40 (1H, m, Fmoc-CH), 1.89 (4H, m, β-, γ-Pro-H), 1.12 (3H, s, H-6)

^{13}C (125 MHz) δ (ppm): 171.8 (C=O amide), 156.1 (C=O carbamate), 143.7, 141.2, 138.3, 128.6, 128.4, 128.2, 128.1, 127.9, 127.7, 127.7, 127.6, 127.6, 127.5, 127.1,, 127.0, 125.1, 119.9 (Ar-C), 83.6 (C-3), 79.7 (C-1), 78.7 (C-2), 76.6 (C-4), 75.1, 74.9, 73.1 (Bn-CH_2), 72.4 (C-5), 67.7 (α-Pro), 61.1 (δ-Pro), 47.3 (Fmoc-CH_2), 47.2 (Fmoc-CH), 28.4 (β-Pro), 24.4 (γ-Pro), 16.9 (C-6).

***N*-Acetamido-2,3,4-tri-O-benzyl-α-L-fucopyranosylamine (9)**



9

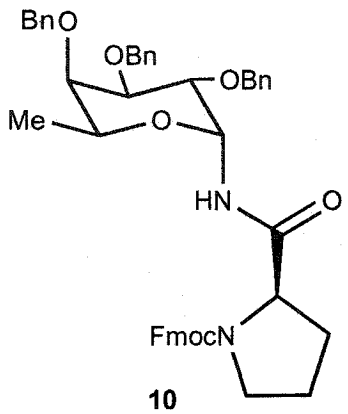
Azide **4** (86 mg, 0.19 mmol) was dissolved in 5 ml of acetic anhydride. To it was added 16 mg of 10% Pd/C, and the solution set to stir under a stream of hydrogen gas. After 5 hours the reaction was complete by TLC (2:1 hexane:ethyl acetate), filtered through a pad of Celite, and evaporated down to a thick oil that solidified upon standing. Purification was accomplished by column chromatography to afford 79 mg (93 %) of a colorless oil. ^{1}H NMR revealed it to be a mixture of 25 % α- anomer and 75 % β-anomer, which could not be separated; fractional integrations represent the contributions of a single anomer.

M.S. (+FAB) : (Calc. for $C_{28}H_{32}NO_5$ = 475, Found *m/z* 476 $(C_{28}H_{32}NO_5 + H)^+$

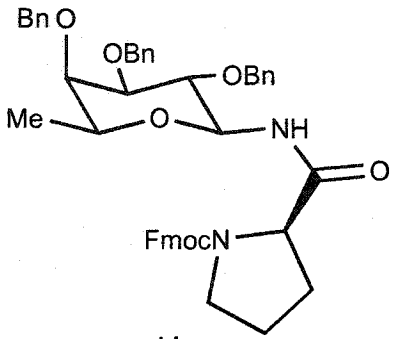
^{1}H ($CDCl_3$, 200 MHz) δ (ppm): 8.01 (0.25H, bs, α-N-H), 7.39 - 7.19 (15H, m, Ar-H), 6.89 (0.75H, bs, β-N-H), 5.31 (0.25H, d, $J_{1,2}$ = 4.4 Hz, α-H-1), 5.00 (0.75H, d, $J_{1,2}$ = 9.1 Hz, β-H-1), 4.94-4.55 (6H, m, benzylic-H), 4.11 (0.25H, dd, $J_{1,2}$ = 4.4 Hz, $J_{2,3}$ = 9.9 Hz, α-H-2), 3.76 (0.75H, dd, $J_{1,2}$ = 4.2 Hz, $J_{2,3}$ = 8.8 Hz, β-H-2), 3.61-3.55 (3H, m, H-3, H-4, H-5), 2.14 (2s, 3H, $COCH_3$), 1.17 (2.25H, d, J = 6.5 Hz, β-H-6), 1.11 (0.75H, d, J = 6.4 Hz, β-H-6).

***N*-Fmoc (*N*'-2,3,4-tri-O-benzyl-α-L-fucopyranosyl)- D-proline (10)**

***N*-Fmoc (*N*'-2,3,4-tri-O-benzyl-α-L-fucopyranosyl)- D-proline (11)**



10



11

See method A and B, for preparation of compounds **7** and **8**. The chromatographic separation of these two compounds was considerably more difficult.

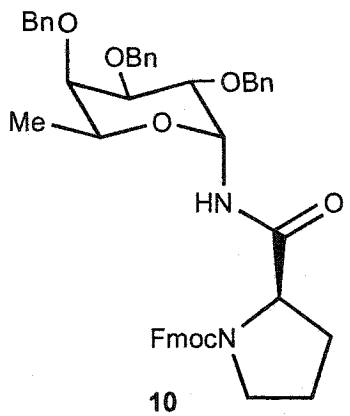
Method A: **10** : 891 mg (57%)

11 : 297 mg (19%) (Total combined: 76%, 1:3 ratio)

Method B: **10**: 344 mg (39%)

11: 158 mg (18%) (Total combined: 57%, 2.2:1 ratio)

***N*-Fmoc (*N'*-2,3,4-tri-O-benzyl-α-L-fucopyranosyl)- D-proline (10)**



10

m.p. 112-113 °C

$[\alpha_D]$ (c = 1, $CHCl_3$) –5.9°

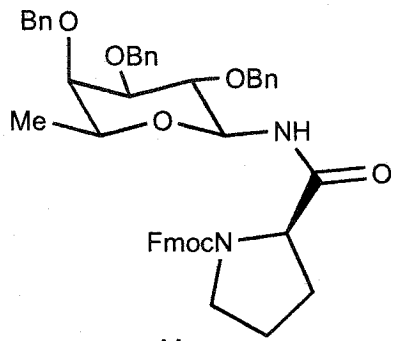
M.S. (+FAB) : (Calc. for $C_{46}H_{48}N_2O_7$ = 752, Found *m/z* 753 $(C_{46}H_{48}N_2O_7 + H)^+$.

^1H NMR (500 MHz, $CDCl_3$) δ (ppm) : 7.83 (1H, d, *J* = 6.9 Hz, amide N-H), 7.76 (2H, d, *J* = 6.3 Hz, Fmoc-Ar-H), 7.53 (2H, d, *J* = 7.0 Hz, Fmoc-Ar-H), 7.39-7.24 (18H, m, Ar-H), 5.27 (1H, m, H-1), 4.81 (1H, d, *J* = 12.0 Hz, benzylic-H), 4.76-4.56 (5H, m, benzylic-H), 4.37 (2H, m, Fmoc-CH_2), 4.28 (1H, m, Fmoc-CH), 4.22 (1H, m, α-Pro), 4.07 (1H, m, H-2), 3.71 (1H, m, H-5), 3.64 (1H, m, H-3), 3.57 (1H, m, H-4), 3.52 (1H, m, δ-Pro), 3.43 (1H, m, δ-Pro), 2.46 (1H, m, β-Pro), 2.18 (1H, m, γ-Pro), 1.90 (2H, m, γ-Pro, β-Pro), 1.12 (3H, d, *J* = 6.3 Hz, H-6).

^{13}C (125 MHz) δ (ppm): 171.7 (C=O amide), 156.5 (C=O carbamate), 143.7, 141.3, 138.6, 128.3, 128.2, 127.8, 127.7, 127.6, 127.5, 127.1, 127.49, 120.0 (Ar-C), 78.3 (C-3), 76.5 (C-4), 75.1 (C-2), 74.1 (C-1), 74.0, 73.3, 72.6 (Bn-CH_2), 68.1 (C-5), 67.9

(Fmoc-CH_2), 60.4 (α-Pro), 47.1 (δ-Pro), 29.7 (Fmoc-CH), 27.3 (β-Pro), 24.6 (γ-Pro), 16.2 (C-6).

_N_-Fmoc (_N'_-2,3,4-tri-O-benzyl-α-L-fucopyranosyl)- D-proline (11)



11

m.p. 92-94 °C

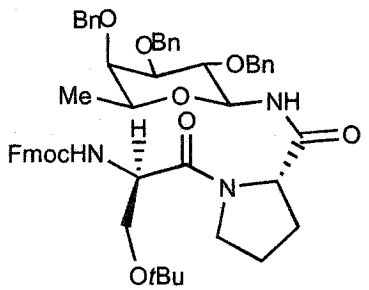
[α_D] (c = 1, $CHCl_3$) +23.9°

M.S. (+FAB) : (Calc. for $C_{46}H_{48}N_2O_7$ = 752, Found *m/z* 753 $(C_{46}H_{48}N_2O_7 + H)^+$.

^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) : 7.72 (2H, d, *J* = 7.8 Hz, Ar-H), 7.60 (1H, d, *J* = 8.7 Hz, amide N-H), 7.48 (1H, d, *J* = 7.0 Hz, Ar-H), 7.36-7.15 (20H, m, Ar-H), 5.14 (1H, t, *J* = 8.7 Hz, H-1), 4.98 (1H, d, *J* = 11.6 Hz, benzylic-H), 4.83 (1H, d, *J* = 11.2 Hz, benzylic-H), 4.70 (4H, m, benzylic-H), 4.35 (1H, d, *J* = 7.4 Hz, α-Pro), 4.19 (1H, m, Fmoc-CH_2), 4.06 (1H, m, Fmoc-CH_2), 3.77 (1H, m, H-2), 3.61 (3H, m, H-3,4,5), 3.37 (2H, m, δ-Pro), 2.40 (1H, m, Fmoc-CH), 2.20-1.75 (4H, m, β-Pro, γ-Pro), 1.17 (3H, d, *J* = 6.0 Hz, H-6).

^{13}C (125 MHz) δ (ppm): 171.3 (C=O amide), 156.4 (C=O carbamate), 144.1, 141.2, 138.6, 138.4, 138.3, 128.4, 128.2, 127.6, 127.4, 127.2, 127.0, 125.1, 124.9, 119.9 (Ar-C), 83.8, 76.7, 72.3 (C-3, C-4, C-5), 79.3 (C-1), 78.4 (C-2), 74.8, 72.9, 68.2 (Bn-CH_2), 67.2 (Fmoc-CH_2), 61.2 (α-Pro), 47.0 (δ-Pro), 27.4 (β-Pro), 24.7 (Fmoc-CH), 23.6 (γ-Pro), 16.9 (C-6).

1-β-[(*N*-Fmoc)-D-(*O*-*t*Bu)-serinyl-L-prolinyl]-amino-1-deoxy-2,3,4-tri-*O*-benzyl-L-fucopyranose (12)



12

Compound **8** (149 mg) was dissolved in 8 mL of a freshly prepared solution of piperidine (20 %) in acetonitrile, and stirred for 10 minutes. The mixture was concentrated by rotary evaporation to provide a white solid. Column chromatography (2:1 hexane:ethyl acetate, until the fulvene derivative is removed, then to 10:1 $CHCl_3$/MeOH) to afford deprotected amine (104 mg, 99 %).

The amine (30 mg, 0.057 mmol), (D)-*N*-Fmoc-*O*-*t*Bu-Ser (24 mg, 0.062 mmol, 1.1 eq.), and diisoproylcarbodiimide (0.01 mL 0.086 mmol) were added to dry THF (5 mL), and stirred for 20 h. TLC (10:1 $CHCl_3$/MeOH) indicated remaining starting material. An additional 0.02 mL of DIC (0.14 mmol, 2.4 eq.) was added, and the

mixture stirred for 24 h. TLC at this time indicated no further starting material. The mixture was evaporated to a crude oil, and then chromatographed on silica gel (2:1 hexanes/ethyl acetate) to afford **12** as a white solid, 36 mg (58 %). For ^{1}H NMR see Figure **2.10**.

m.p. 82-85 °C (dec.)

$[\alpha_D]$ (c = 1, $CHCl_3$) +29.0°

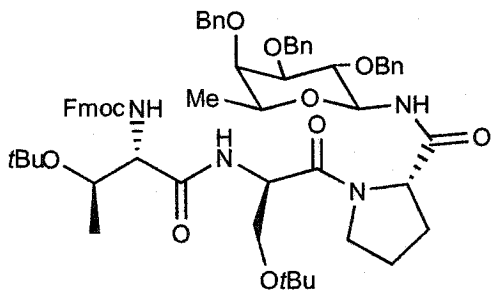
M.S. (+FAB) : (Calc. for $C_{53}H_{61}N_3O_8$ = 895, Found *m/z* 896 $(C_{53}H_{61}N_3O_8 + H)^+$.

^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) : 7.72 (2H, dd, *J* = 2.6 Hz, *J* = 7.6 Hz, Ar-H), 7.58 (1H, d, *J* = 7.4 Hz, Ar-H), 7.53 (1H, d, *J* = 7.5 Hz, Ar-H), 7.37-7.18 (20H, m, Ar-H + amide N-H), 5.52 (1H, d, *J* = 7.7 Hz, Fmoc-NH), 5.14 (1H, t, *J* = 9.2 Hz, H-1), 4.93 (1H, d, *J* = 9.1 Hz, benzylic-H), 4.80 (1H, d, *J* = 10.1 Hz, benzylic-H), 4.77 (1H, d, *J* = 12.0 Hz, benzylic-H), 4.69 (3H, m, 2 x benzylic-H, α-Pro), 4.55 (2H, m, benzylic-H, α-Ser), 4.42 (1H, dd, *J* = 6.8 Hz, *J* = 10.6 Hz, Fmoc-CH_2), 4.06 (1H, dd, *J* = 6.8 Hz, *J* = 10.6 Hz, Fmoc-CH_2), 4.06 (1H, t, *J* = 6.6 Hz, Fmoc-CH), 3.70 (3H, m, H-2, β-Ser, δ-Pro), 3.56 (4H, m, H-3, H-4, H-5, δ-Pro), 3.36 (1H, t, *J* = 9.1 Hz, β-Ser), 2.29 (1H, m, β-Pro), 2.05-1.85 (3H, m, β-Pro, γ-Pro), 1.15 (3H, d, *J* = 6.3 Hz, H-6), 1.07 (9H, s, *t*Bu).

^{13}C (125 MHz) δ (ppm): 171.3, 170.9 (C=O amide), 155.8 (C=O carbamate), 143.8, 143.8, 141.3, 141.3, 138.8, 138.6, 128.5, 128.5, 128.4, 128.2, 128.1, 127.6, 127.1, 127.0, 125.2, 125.0, 119.9, 119.9 (Ar-C), 83.5, 77.6, 72.4 (C-3, C-4, C-5), 79.6 (C-1),

78.9 (C-2), 75.2, 75.0, 73.1 (Bn-CH_2), 66.8 (Fmoc-CH_2), 63.2 (α-Pro), 60.6 (α-Ser), 53.0 (β-Ser), 47.8 (δ-Pro), 47.1 (4° *t*-Bu), 27.4 (β-Pro), 27.2 (1° *t*Bu), 24.7 (Fmoc-CH), 23.6 (γ-Pro), 16.9 (C-6).

1-β-[(*N*-Fmoc)-L-(*O*-*t*Bu)-threoninyl-D-(*O*-*t*Bu)-serinyl-L-prolinyl]-amino-1-deoxy-2,3,4-tri-*O*-benzyl- L-fucopyranose (13)



13

Compound **12** (4.7 mg, 5.25 μmol) of was placed in a 5 mL vial. Acetonitrile (20 drops) and piperidine (5 drops) were added, and the mixture shaken for 5 minutes. The mixture was then evaporated to a white solid, and purified by silica gel chromatography (2:1 hexane:ethyl acetate, until fulvene derivative is removed, then to 10:1 $CHCl_3$/MeOH) to afford deprotected amine (3.4 mg, 97 %). M.S. (FAB) = 674 ($C_{39}H_{51}N_3O_7 + H^+$).

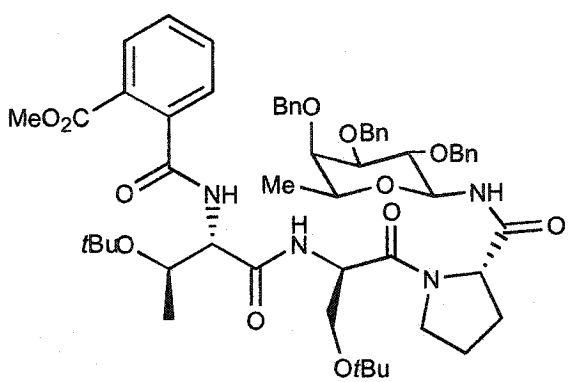
This amine was dissolved in 5 mL dry dioxane, along with DCC (4.0 mg, 9.6 μmol, 1.8 eq.), NHS (2.0 mg, 8.0 μmol, 1.6 eq.) and (L)-*N*-Fmoc-*O*-*t*-Bu-threonine (6.4 mg, 8.0 μmol, 1.6 eq.) and stirred overnight. The mixture was then concentrated and

purified by silica gel chromatography (2:1 hexanes:ethyl acetate) to afford white solid **13** , 5.2 mg (96 %). The mass spectrum can be found in Figure **2.11**.

M.S. (+HMRS-FAB): (Calc. for $C_{62}H_{76}N_4O_{11}$+ H = 1053.5591; Found *m/z* 1053.5589 $(C_{62}H_{76}N_4O_{11} + H)^+$.

^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) : 11.00 (bs, 1H, *N*-H), 7.79 (d, 1H, *J* = 7.3 Hz, , β-Fuc-NH), 7.73 (d, 2H, *J* = 3.0 Hz, Ar-H), 7.69 (d, 1H, *J* = 8.4 Hz, NH), 7.62 (d, 2H, *J* = 3.1 Hz, Ar-H), 7.39-7.15 (m, 19H, Ar-H), 6.00 (d, 1H, *J* = 5.1 Hz, Fmoc-NH), 5.05 (t, 1H, *J* = 9.2 Hz, H-1), 4.91 (d, 1H, *J* = 11.6 Hz, benzylic-H), 4.84 (dd, 1H, *J* = 8.1 Hz, *J* = 13.5 Hz, Ser/Thr α-H), 4.79-4.50 (m, 4H), 4.36 (m 1H), 4.40-4.08 (m, 4H), 3.88 (q, *J* = 6.5 Hz, 1H, H-5), 3.77-3.44 (m, 6H), 1.95-1.55 (m, 8H), 1.23 (s,9H, *t*-Bu), 1.16 (s, 9H, *t*-Bu), 1.09 (d, *J* = 6.5 Hz, 3H, H-6), 1.05 (d, *J* = 6.3 Hz, 3H, Thr-CH_3).

1-β-[(*N*-monomethylphthaloyl)-L-(*O*-*t*Bu)-threoninyl-D-(*O*-*t*Bu)-serinyl-L-prolinyl]-amino-1-deoxy-2,3,4-tri-*O*-benzyl- L-fucopyranose (14)



14

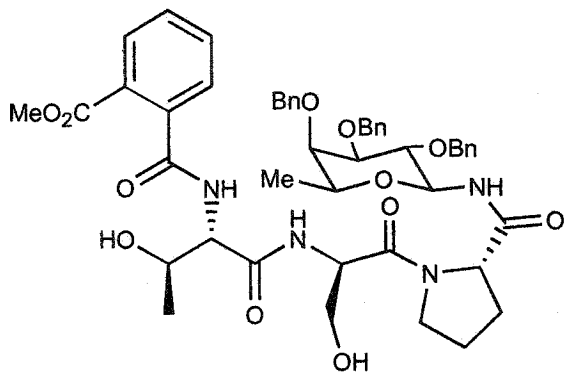
Compound **13** (1.9 mg, 18 μmol) was dissolved in 2 mL of 20 % piperidine in acetonitrile, allowed to stand for 20 minutes, and evaporated to afford a white solid. Column chromatography (2:1 hexane:ethyl acetate, until fulvene derivative is removed, then to 10:1 $CHCl_3$/MeOH) afforded deprotected amine (1.4 mg, 93 %). M.S. (FAB) = 876 ($M + H^+$).

This product was dissolved in dioxane (2 mL) with *mono*-methylphthalic acid (5 mg, 28 μmol, 1.6 eq.), EDC (4.3 mg, 28 μmol, 1.6 eq.), NHS (2.0 mg, 28 μmol, 1.6 eq) and DIPEA (2 drops). The mixture was stirred overnight, and then concentrated and purified by silica gel chromatography (2:1 hexanes:ethyl acetate) to afford off-white solid **14** , 1.2 mg (84 %). Resolution was very poor and the ^1H NMR spectra could not be interpreted for certain, so it is not certain that the material was synthesized. Most of the peak assignments are made with reference to previously synthesized compounds.

^1H NMR (500 MHz, $CDCl_3$) δ (ppm) : 7.73-7.09 (bm, 28H, 25 Ar-H + 3 NH), 5.00 (m, 1H), 4.86-4.44 (m, 6H, benzylic-H), 4.34-4.00 (m, 6H, H-2, H-3, H-4, 3 x α-H), 3.90 (s, 3H, OCH_3), 3.84 (m, 1H, H-5), 3.66-3.50 (m, 2H, δ-Pro), 2.22-1.55 (m, 4H), 1.33 (s, 9H, *t*-Bu), 1.21 (s, 9H, *t*-Bu), 1.11 (bs, 3H), 1.04 (bs, 3H).

M.S. (+FAB) : (Calc. for $C_{56}H_{70}N_4O_{12}$ = 1037, Found *m/z* 1038 $(C_{56}H_{70}N_4O_{12} + H)^+$

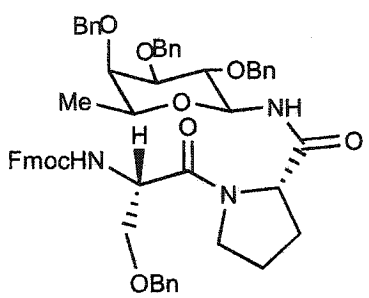
1-β-[(*N*-monomethylphthaloyl)-L-threoninyl-D-serinyl-L-prolinyl]-amino-1-deoxy-2,3,4-tri-*O*-benzyl- L-fucopyranose (15)



15

Compound **14** (1.0 mg) of was dissolved in 1 mL of trifluoroacetic acid and stirred for one hour. TLC at this time (10:1 $CHCl_3$/MeOH) showed a complex mixture of products. Mass spectral analysis of individual spots failed to locate any fragments with *m/z* >300.

1-β-[(*N*-Fmoc)-L-(*O*-Bn)-serinyl-L-prolinyl]-amino-1-deoxy-2,3,4-tri-*O*-benzyl- L-fucopyranose (16)



16

The amine derived from **8** (see **12** for preparation) (290 mg, 0.546 mmol) was dissolved in DMF (1 mL) with (L)-*N*-Fmoc-*O*-*t*Bu-serine (250 mg, 0.601 mmol, 1.1 eq.) and HATU (240 mg, 0.601 mmol, 1.1 eq.), and then DIPEA (0.20 mL, 1.09 mmol, 2.0 eq.) was added. A deep yellow color ensued, and the reaction was stirred for 18 h. TLC at this time (1:1 hexanes/ethyl acetate) indicated no starting material. Column chromatography (2:1 hexanes/ethyl acetate) afforded white solid **16** (410 mg, 82 %).

m.p. 92-93 °C (dec.)

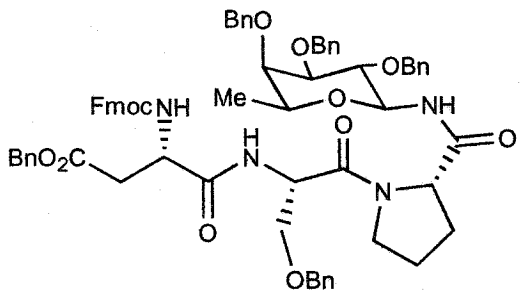
M.S. (+FAB) : (Calc. for $C_{57}H_{59}N_3O_8$ = 914, Found *m/z* 915 $(C_{57}H_{59}N_3O_8 + H)^+$

^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) : 7.71 (2H, d, J = 7.5 Hz, Ar-H), 7.60 (1H, d, J = 7.8 Hz, amide-NH), 7.52 (1H, d, J = 7.2 Hz, Ar-H), 7.50 (1H, d, J = 7.2 Hz, Ar-H), 7.38-7.09 (25H, m, Ar-H), 5.62 (1H, d, J = 7.8 Hz, Fmoc-NH), 5.11 (1H, t, J = 9.0 Hz, H-1), 4.91-4.72 (7H, m, benzylic-H), 4.60 (1H, m, α-Ser), 4.40 (1H, d, J = 12.0 Hz, benzylic-H), 4.39 (1H, m, α-Pro), 4.27 (1H, m, δ-Pro), 4.20 (1H, m, δ-Pro), 4.07 (2H, m, Fmoc-CH_2), 3.72-3.47 (8H, m, Fmoc-CH, β-Ser, H-2, H-3, H-4, H-5, δ-Pro), 2.32 (1H, m, β-Pro), 2.03 (1H, m, β-Pro), 1.80 (2H, m, γ-Pro), 1.10 (3H, d, J = 6.2 Hz, H-6).

^{13}C (125 MHz) δ (ppm): 170.7, 169.9 (amide C=O), 155.7 (carbamate C=O), 143.7, 143.7, 141.2, 141.2, 138.6, 138.5, 137.1, 128.6, 128.5, 128.4, 128.3, 128.2, 128.1, 127.9, 127.6, 127.6, 127.5, 127.0, 127.0, 125.1, 125.0, 119.9 (Ar-C), 83.4, 79.1, 77.6,

72.4, 70.0 (Fmoc-CH, C-2, C-3, C-4, C-5), 79.5 (C-1), 75.2, 75.1, 73.6, 73.1 (benzylic-C), 67.0 (α-Pro), 52.0 (δ-Pro), 47.3 (β-Ser), 47.0 (Fmoc-CH_2), 28.5 (β-Pro), 24.3 (γ-Pro), 16.9 (C-6).

1-β-[(*N*-Fmoc)-L-(*O*-Bn)-aspartyl-L-(*O*-Bn)-serinyl-L-prolinyl]-amino-1-deoxy-2,3,4-tri-*O*-benzyl- L-fucopyranose (17)



17

Compound **16** (29 mg, 32 μmol) of was dissolved in 5 mL of 20 % piperidine in acetonitrile. The mixture stirred for 15 minutes, then evaporated to a white solid, and purified by silica gel chromatography (2:1 hexane:ethyl acetate, until fulvene derivative is removed, then to 10:1 $CHCl_3$/MeOH) to afford deprotected amine (22 mg, 100 %).

M.S. (+FAB) : (Calc. for $C_{42}H_{49}N_3O_7$ = 708, Found *m/z* 709 $(C_{42}H_{49}N_3O_7 + H)^+$

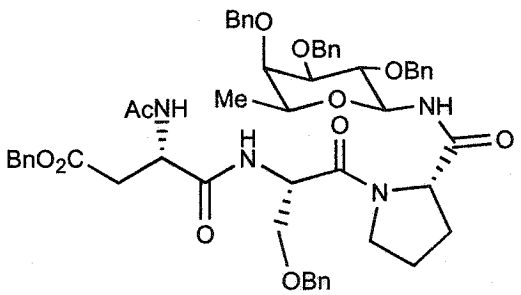
This amine was dissolved in DMF (1 mL) with (L)-*N*-Fmoc-*O*-Bn-aspartic acid (25 mg, 67.2 μmol, 2.1 eq.) and HATU (24 mg, 48 μmol, 1.5 eq.), and then DIPEA (0.20 mL, 320 μmol, 10.0 eq.) was added. A deep yellow color ensued, and the reaction

was stirred for 16 h. TLC at this time (1:1 hexanes/ethyl acetate) indicated no starting material. Column chromatography of the reaction mixture (2:1 hexanes/ethyl acetate, up to 1:1) afforded **17** as a white solid (31 mg, 82 %).

M.S. (+FAB) : (Calc. for $C_{68}H_{70}N_4O_{12}$ = 1135, Found *m/z* 1136 $(C_{68}H_{70}N_4O_{12} + H)^+$

This compound exhibited extremely poor spectral quality and could not be interpreted; however, the synthesis of the next compound was successful, so it can be concluded the material was as claimed.

1-β-[(*N*-Acetyl)-L-(*O*-Bn)-aspartyl-L-(*O*-Bn)-serinyl-L-prolinyl]-amino-1-deoxy-2,3,4-tri-*O*-benzyl- L-fucopyranose (18)



18

Compound **17** (25 mg, 22 μmol) of was dissolved in 1 mL of 20 % piperidine in acetonitrile. The mixture stirred for 10 minutes, then evaporated to a white solid, and purified by silica gel chromatography (2:1 hexane:ethyl acetate, until fulvene derivative is removed, then to 10:1 $CHCl_3$/MeOH) to afford deprotected amine. This

material was found to be unstable, and was carried to the next step without characterization.

The crude amine was dissolved in pyridine (1 mL) and acetic anhydride (0.1 mL) was added. The mixture was stirred for 3 hours and then evaporated, and the mixture was purified by silica gel chromatography (1:1 hexanes/ethyl acetate) to afford 12 mg (84 %, 2 steps) of **18** as a white solid. The ^{1}H and ^{13}C NMR and mass spectrum can be found in Figures **2.12**, **2.13**, and **2.14**, respectively.

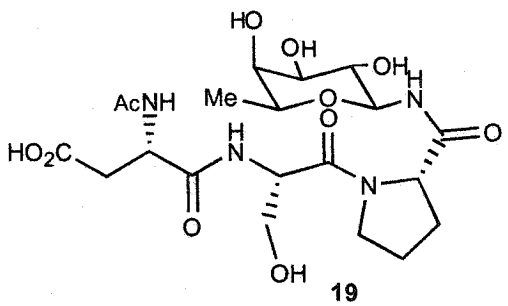
M.S. (+FAB) : (Calc. for = $C_{55}H_{62}N_4O_{11}$ 956, Found *m/z* 957 $(C_{55}H_{62}N_4O_{11} + H)^+$

^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) : 8.02 (1H, d, *J* = 10.6 Hz, Ar-H), 7.58 (1H, dd, *J* = 7.9 Hz, *J* = 8.6 Hz, amide-NH), 7.41-7.12 (25H, m, Ar-H, amide N-H), 6.50 (1H, d, *J* = 8.3 Hz, amide-NH), 5.14 (1H, dd, *J* = 9.5 Hz, *J* = 8.5 Hz, H-1), 5.04 (1H, dd, *J* = 3.2 Hz, benzylic-H), 4.98-4.68 (3H, m, α-Pro, α-Ser, α-Asp), 4.48 (1H, d, *J* = 11.1 Hz, benzylic-H), 4.02 (1H, d, *J* = 12.6 Hz, benzylic-H), 3.67 (2H, m, β-Ser), 3.61 (1H, dd, *J* = 2.9 Hz, *J* = 9.5 Hz, H-3), 3.53 (1H, m, H-5, 3.45 (2H, m, H-2, H-4), 2.95 (1H, dd, *J* = 4.7 Hz, *J* = 17.0 Hz, δ-Pro), 2.62 (1H, dd, *J* = 5.9 Hz, *J* = 16.9 Hz, δ-Pro), 2.27 (1H, m, β-Pro), 2.05-1.80 (4H, m, β-Asp, β-Pro, γ-Pro), 1.91 (3H, s, CO-CH_3), 1.65 (1H, m, γ-Pro), 1.07 (3H, d, *J* = 6.4 Hz).

^{13}C (125 MHz) δ (ppm): 171.4, 170.7, 170.0, 169.8, 169.1 (4 x amide + ester C=O), 138.6, 138.5, 138.4, 137.1, 128.7, 128.5, 128.4, 128.3, 128.3, 128.3, 128.2, 128.2,

128.0, 127.9, 127.5, 127.4, 127.4 (Ar-C), 83.3 (C-3), 79.5 (C-1), 79.0 (C-2), 77.5 (C-5), 75.1, 75.1, 73.5, 73.1, 66.8, (benzylic-C), 72.3 (C-4), 69.6 (β-Ser), 60.6 (α-Pro), 50.8, 49.2 (α-Ser, α-Asp), 47.3 (β-Ser), 38.5 (δ-Pro), 36.0, 29.6, 28.4, 28.2, 27.1, (β-Asp), 24.4 (β-Pro), 23.0 (γ-Pro), 22.6 (*N*-acetyl-CH_3), 16.9 (C-6).

1-β-[(*N*-Acetyl)-L-aspartyl-L-serinyl-L-prolinyl]-amino-1-deoxy-L-fucopyranose (19)



19

Fully protected **18** (8.7 mg, 9.12 μmol) was dissolved in methanol (3 mL). Palladium (10% on carbon) (5 mg) was added, and the mixture flushed with N_2. A hydrogen balloon was added, and the mixture stirred, and the balloon refreshed periodically, for 3 days. After this time, ^{1}H NMR indicated no aromatics present in the molecule. The sample was filtered through Celite, and evaporated to afford a white, hygroscopic solid, 4.5 mg (95%). This molecule exhibited poor spectral qualities, but some peaks could be assigned.

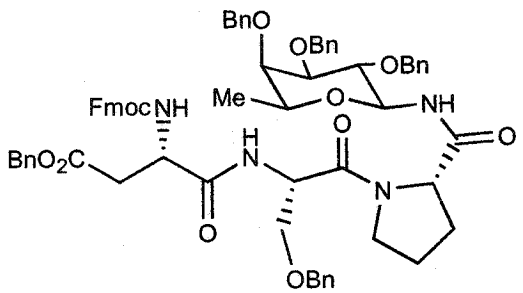
^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 8.41-8.11 (2H, m, amide-NH), 5.03 (1H, m, amide-NH), 4.75 (1H, m, α-Ser), 4.59-4.49 (3H, m), 4.29 (1H, m, α-Pro), 3.73-3.40 (6H, m), 3.36-3.21 (9H, several bs, 4 x OH, H-3, H-5, H_2O(?)), 2.44 (1H, dd, J = 4.0

Hz, J = 5.3 Hz, γ-Glu), 2.11-2.00 (6H, m, 2 x β-Asp, β-Pro, *N*-acetyl-CH_3), 1.80-1.70 (3H, m, β-Pro, 2 x γ-Pro), 1.11 (3H, d, J = 6.5 Hz, H-6).

$[\alpha_D]$ (c = 0.1, H_2O) +77.9°

M.S. (ESI, negative mode) (Calc. for = $C_{20}H_{32}N_4O_{11}$ 504, Found *m/z* 485 ($C_{20}H_{32}N_4O_{11} - H_2O$ - H)$^-$, 503 ($C_{20}H_{32}N_4O_{11}$ – H)$^-$.

1-β-[(*N*-Fmoc)-L-(*O*-Bn)-glutamyl-L-(*O*-Bn)-serinyl-L-prolinyl]-amino-1-deoxy-2,3,4-tri-*O*-benzyl- L-fucopyranose (20)



20

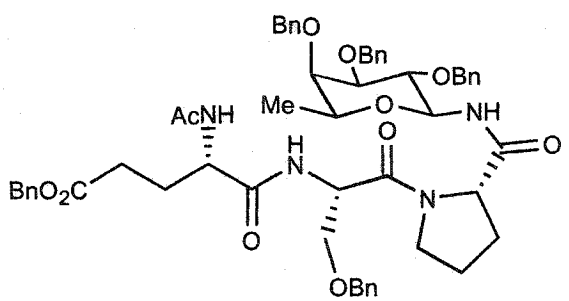
Compound **10** (300 mg, 0.320 mmol) of was dissolved in 3 mL of 20 % piperidine in acetonitrile. The mixture stirred for 20 minutes, then concentrated to a white solid, and purified by silica gel chromatography (hexanes until the fulvene derivative is removed, then to 10:1 $CHCl_3$/MeOH) to afford deprotected amine (159 mg, 70 %). This compound is unstable (cyclization to the diketopiperazine occurs) and was taken directly to the next step without characterization.

The amine was dissolved in DMF (4 mL) with (L)-*N*-Fmoc-*O*-Bn-glutamic acid (150 mg, 352 mmol, 1.1 eq.) and HATU (150 mg, 480 mmol, 1.5 eq.), and then DIPEA (8 drops) was added. A deep yellow color ensued, and the reaction was stirred for 45 minutes. The color faded; TLC (1:1 hexanes/ethyl acetate) indicated the reaction was not complete. Another 10 drops of DIPEA were added, and the mixture stirred for another 90 minutes. TLC at this time showed no starting material. The mixture was poured into 5 % $NaHCO_3$ solution (30 mL) and extracted with ether (50 mL). The ether was washed with water (1 x 30 mL), dried over sodium sulfate, filtered and concentrated to give crude product. Column chromatography (1:1 hexanes/ethyl acetate) afforded **20** as a white solid (212 mg, 82 %).

M.S. (+FAB) : (Calc. for $C_{69}H_{72}N_4O_{12}$ = 1149, Found *m/z* 1150 $(C_{69}H_{72}N_4O_{12} + H)^+$

This compound exhibited extremely poor spectral quality and could not be interpreted; however, the synthesis of the next compound was successful, verifying the identity of the claimed compound.

1-β-[(*N*-Acetyl)-L-(*O*-Bn)-glutamyl-L-(*O*-Bn)-serinyl-L-prolinyl]-amino-1-deoxy-2,3,4-tri-*O*-benzyl- L-fucopyranose (21)



21

Compound **20** (184 mg, 160 μmol) was dissolved in 2 mL of 20 % piperidine in acetonitrile. The mixture stirred for 20 minutes, then concentrated (with no heat) to a white solid. The amino group is set up well for lactamization onto the glutamyl ester, so the material was immediately taken into the next step.

The crude amine was dissolved in pyridine (10 mL) and acetic anhydride (5 mL) was added. The mixture was stirred for 24 hours and then concentrated, and the residue was purified by silica gel chromatography (99:1 chloroform/methanol, to 90:10) to afford 125 mg (80 %, 2 steps) of **21** as a white solid. The ^{1}H NMR can be seen to be a mixture of two rotamers, in approximately a 2:1 ratio. When they can be discerned, the different rotameric components of the NMR analysis are indicated.

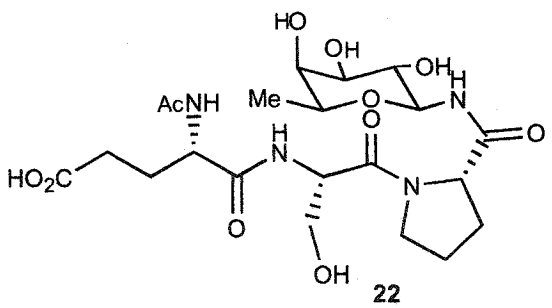
The compound suppreses ionization and gives no useful mass spectral data.

^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) : 7.68 (minor) 7.58 (major) (1H, d, J = 9.5 Hz, amide N-H), 7.32-7.12 (26H, m, Ar-H, amide NH), 6.82 (major) (6.34) minor (1H, d, J = 7.6 Hz, amide-NH), 5.17 (1H, m, H-1), 4.88 (1H, d, J = 11.3 Hz, benzylic-H), 4.84-4.64 (9H, m, α-Ser, α-Glu, 7 x benzylic-H), 4.54 (1H, dd, J = 2.6 Hz, J = 8.8 Hz, α-Pro), 4.48 (1H, d, J = 9.1 Hz, benzylic-H), 4.06 (minor) 3.99 (minor) (1H, d, J = 12.6 Hz, benzylic-H), 3.75 (1H, dd, J = 5.7 Hz, J = 9.3 Hz, β-Ser), 3.65-3.37 (7H, m, H-2, H-3, H-4, H-5, β-Ser, δ-Pro), 2.76 (minor) 2.70 (major) (1H, t, J = 9.9 Hz, γ-

Glu), 2.47 (3H, s, acetyl-CH_3), 2.37 (2H, m, β-Glu, γ-Glu), 2.10-1.95 (6H, m, β-Pro, γ-Pro, β-Glu, γ-Glu), 1.07 (major) 1.04 (minor) (3H, d, J = 6.3 Hz, H-6).

^{13}C (75 MHz) δ (ppm): 174.9, 171.2, 170.6, 170.2, 169.2 (4 x amide + ester C=O), 138.6, 138.5, 138.5, 138.4, 138.3, 137.0, 128.7, 128.5, 128.5, 128.3, 128.3, 128.2, 128.1, 127.9, 127.7, 127.5, 127.5, 127.4 (Ar-C), 83.2 (C-3), 79.4 (C-1), 79.1 (C-2), 77.3 (C-5), 75.2, 75.1, 73.5, 73.4, 66.4, (benzylic-C), 73.0 (C-4), 69.6 (β-Ser), 60.6, 50.3 (α-Ser, α-Glu), 59.7 (α-Pro), 47.3 (δ-Pro), 32.0 (γ-Glu), 24.4 (*N*-acetyl-CH_3), 28.8, 24.3, 21.6 (β-Glu, β-Pro, γ-Pro), 16.9 (C-6).

1-β-[(*N*-Acetyl)-L-glutamyl-L-serinyl-L-prolinyl]-amino-1-deoxy-L-fucopyranose (22)



22

Fully protected **21** (38.2 mg, 39.4 μmol) was dissolved in methanol (5 mL). Palladium (10% on carbon) (17 mg) was added, and the mixture flushed with N_2. A hydrogen balloon was added, and the mixture stirred, with the balloon refreshed periodically, for 3 days. After this time, ^1H NMR indicated no aromatics present in the molecule. The sample was filtered through Celite, and evaporated to afford a

white, hygroscopic solid, 19.2 mg (95%). This material was pure by TLC (1:1:0.1 $CHCl_3$/MeOH/AcOH). The ^1H NMR (500 MHz) (Figure **2.15**) showed a great deal of rotamer activity, as evidenced by the N-H peaks from 8.5-7.8 ppm. The ^{13}C NMR (Figure **2.16**), however, reveals the correct number of peaks.

m.p. 82-85 °C (dec.)

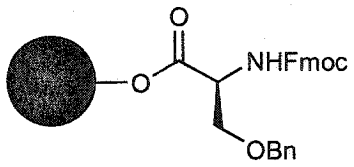
$[\alpha_D]$ (c = 0.1, H_2O) –114.2°

M.S. (ESI, negative mode) (Calc. for = $C_{21}H_{34}N_4O_{11}$ 519, Found *m/z* 499 $(C_{21}H_{34}N_4O_{11} - H_2O - H)^-$, 518 $(C_{21}H_{34}N_4O_{11} - H)^-$.

^1H NMR (500 MHz, DMSO-d_6) δ (ppm): 8.40-7.95 (2H, m, amide-NH), 5.05 (1H, m, amide-NH), 4.80 (1H, dd, J = 2.4 Hz, J = 9.3 Hz, α-Ser), 4.58 (1H, t, J = 8.6 Hz, H-1), 4.51 (2H, m, α-Glu), 4.32 (1H, m, α-Pro), 3.72-3.52 (5H, m, H-2, δ-Pro, β-Ser), 3.43 (1H, bs, H-4), 3.31 (9H, bs, 4 x OH, H-3, H-5, H_2O), 2.44 (1H, dd, J = 4.0 Hz, J = 5.3 Hz, γ-Glu), 2.34 (2H, m, γ-Glu, β-Glu), 2.20 (1H, β-Glu), 2.06 (3H, s, *N*-acetyl-CH_3), 2.04 (1H, m, β-Pro), 1.83 (3H, m, β-Pro, γ-Pro), 1.08 (3H, d, J = 6.4 Hz, H-6).

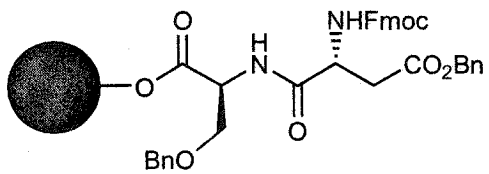
^{13}C (125 MHz) δ (ppm): 175.5, 171.9,170.9, 169.9, 168.9 (4 x amide + ester C=O), 80.1 (C-1), 74.1 (C-3), 71.6 (C-2), 71.2 (C-4), 69.6 (C-5), 61.4, 59.7, 57.9 (α-Pro, α-Ser, α-Glu), 53.4 (β-Ser), 47.0 (δ-Pro), 31.6 (β-Glu), 29.8 (β-Pro), 24.4 (*N*-acetyl-CH_3), 24.3 (γ-Glu), 21.6 (γ-Pro), 16.8 (C-6).

2.7.2 Solid-Phase Synthesis



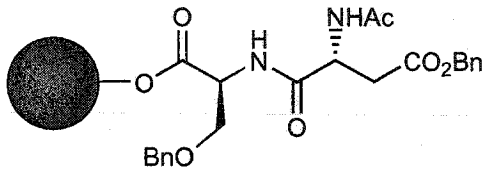
23

Trityl chloride resin 110 mg, 1.3 mmol/g loading, 0.14 mmol, 100-200 mesh, Advanced ChemTech) was suspended in 0.8 mL dichloromethane and allowed to swell for 1 hour. 2 drops of DMF, 2 drops of DIPEA, and 120 mg of (L)-*N*-Fmoc-*O*-Bn-serine (0.28 mmol, 2 eq.) were added, and the mixture agitated for 2 hours. The mixture was drained, washed (3 x DCM/MeOH/DIPEA, 17:2:1), 3 x DCM, 2 x DMF, 2 x DCM, and dried under vacuum for 1 hour. The mixture was then taken on to the next step.



24

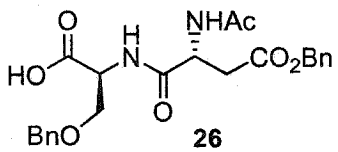
Resin-bound **23** was taken up in 1 mL piperidine (20 %) in DMF, and agitated for 40 minutes. The mixture was drained and washed 1 x DMF, 2 x DCM, 2 x DMF and 2 x DCM, and dried under vacuum for 1 hour. The mixture was then taken up in DMF (1 mL) along with HATU (105 mg, 0.28 mmol, 2 eq.), DIPEA (0.1 mL) and (L)-*N*-Fmoc-γ-*O*-Bn-aspartic acid (122 mg, 0.28 mmol, 2 eq.) and agitated for 2 hours. The mixture was drained, and washed 2 x DMF, 2 x DCM, 2 x DMF and 3 x DCM, and dried overnight under vacuum.



25

Resin-bound **24** was taken up in 1 mL piperidine (20 %) in DMF, and agitated for 60 minutes. The mixture was drained and washed 1 x DMF, 2 x DCM, 2 x DMF and 2 x DCM, and dried quickly (diketopiperazine formation can occur easily at this stage of peptide synthesis). The mixture was then taken up in a mixture of 5 % acetic anhydride, 5 % DIPEA, and a single crystal of DMAP in DCM (1 mL). The mixture was drained, and washed 2 x DCM, 2 x DMF, 3 x DCM. The resin was dried overnight under vacuum.

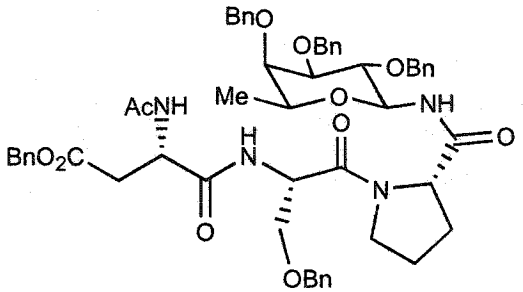
***(N*-Ac-*O*-Bn)- L-Aspartyl-(*O*-Bn)-L-serine (26)**



26

Resin-bound **25** was taken up in a mixture of TFA (5 %) and triisopropylsilane (2 %) in DCM (1 mL), and agitated for 30 minutes. The liquid was collected, and the residue treated a second time with the same mixture (1 mL) for 20 minutes. The isolated liquids were combined and concentrated to afford a sticky white oily solid. TLC (10:1 $CHCl_3$/MeOH) showed it to be composed of mainly 3 compounds. Column chromatography (1:1 hexanes/ethyl acetate, switched to 10:1 $CHCl_3$/MeOH after the first material came out) afforded **26** (10 mg, 16 %) as a white solid which was used in the next step without characterization.

1-β-[(*N*-Acetyl)-L-(*O*-Bn)-aspartyl-L-(*O*-Bn)-serinyl-L-prolinyl]-amino-1-deoxy-2,3,4-tri-*O*-benzyl- L-fucopyranose (18)

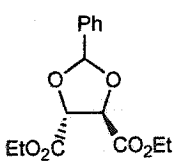


18

Amine **27** from the preparation of **12** (7.6 mg, 1.05 eq., preparation in **2.3.1**), acid **26** (6.0 mg), HATU (10.4 mg) and DIPEA (2 drops) were dissolved in 0.5 mL DMF in a vial, the vial was closed, and agitated periodically for 3 hours. The mixture was concentrated by azeotrope with toluene (20 mL), and then purified by column chromatography (99:1 CH_2Cl_2 in methanol) to afford **18** (11.5 mg, 88 %). The spectral characteristics were identical to **18** synthesized from linear solution-phase methods (**2.5.1**).

2.7.3 Tartaric Acid Based Mimetics

2-Phenyl-[1,3]-dioxolane-(*R,R*)-4,5-dicarboxylic acid diethylester (33)



33

(L)-Diethyltartarate (1.39 g, 6.70 mmol) was dissolved in dimethoxyethane (20 mL). To it was added benzaldehyde dimethylacetal (1.52 mL, 10.1 mmol, 1.5 eq.), and camphorsulfonic acid (0.27 g, 0.67 mmol, 0.1 eq.). The mixture was refluxed for 1 hour. TLC (10:1 $CHCl_3$/MeOH) indicated the reaction was complete. The mixture was boiled down to 5 mL, 20 mL DME added, and the mixture boiled down again. The residue was brought up in ether (40 mL), and washed with 40 mL 5 % $NaHCO_3$, 40 mL water. The ether layer was dried over sodium sulfate, filtered and concentrated. The resulting residue was purified by column chromatography (8:1 hexanes/ethyl acetate, up to 1:1) to afford 1.57 g (76 %) of off-white solid **33**.

m.p. 48-49 °C (Lit.[95] 48.5-49.5 °C)

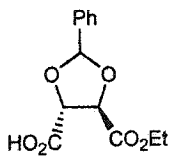
$[\alpha_D]$ (c = 1, $CHCl_3$) –44.5° [Lit. -24.5 (c = 0.1, EtOH)[98]; -29.8 (c = 1, EtOH)[99]]

M.S. (EI): Calc. for $C_{15}H_{18}O_6$ = 294, Found *m/z* 294.

^{1}H NMR (200 MHz, $CDCl_3$) δ (ppm): 7.58 (2H, m, Ar-H), 7.54 (3H, m, Ar-H), 6.13 (1H, s, benzylidene-H), 4.92 (1H, d, J = 4.0 Hz), 4.80 (1H, d, J = 4.0 Hz), 4.40-4.20 (4H, m, $OC\underline{H}_2CH_3$), 1.30 (6H, m, $OCH_2C\underline{H}_3$).

^{13}C NMR (50 MHz, $CDCl_3$) δ (ppm): 169.6, 169.0 (C=O), 135.4, 129.9, 128.3, 127.2 (Ar-C), 106.6 (benzylidene-C), 77.5, 77.2, 62.0 ($O\underline{C}H_2CH_3$), 14.1 ($OCH_2\underline{C}H_3$).

2-Phenyl-[1,3]-dioxolane-(*R*)-4-carboxylic acid ethylester-5-(*R*)-5-carboxylic acid (34)



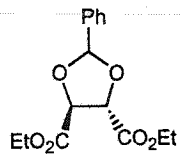
34

Diester **33** (269 mg, 0.911 mmol) was dissolved in acetone (5 mL). To it was added 45 mL of a 0.067 M phosphate buffer, pH 8.0. The product immediately began to cloud out of the mixture. Pig Liver Esterase (167 units) was added, the mixture stirred at 30 °C and pH was maintained at 8.0 by addition of 1M NaOH via syringe pump. After 7.5 h, NaOH consumption ceased, and TLC (10:1 $CHCl_3$/MeOH) showed only trace remaining starting material. The mixture was acidified to pH 4.0 with HCl (12 M), and extracted with diethyl ether (3 x 20 mL). The extracts were combined, dried over sodium sulfate, filtered and concentrated to give colorless oil **34**, 198 mg (81 %). ^{1}H NMR indicates it to be a 2:1 mixture of diastereomers.

M.S. (ESI, negative mode) Calc. for $C_{13}H_{14}O_6 = 266$, Found *m/z* 265 ($C_{13}H_{14}O_6 - H^+$)

^{1}H NMR (200 MHz, $CDCl_3$) δ (ppm): 10.00 (1H, bs, CO_2H), 7.57 (2H, m, Ar-H), 7.37 (3H, m, Ar-H), 6.18 (major) 6.14 (minor) (1H, s, benzylidine-H), 5.00 (1H, m, tart-H), 4.87 (1H, m, tart-H), 4.38-4.20 (4H, m, $OC\underline{H}_2CH_3$), 1.30 (6H, m, $OCH_2C\underline{H}_3$).

2-Phenyl-[1,3]-dioxolane-(*S*,*S*)-4,5-dicarboxylic acid diethylester (35)

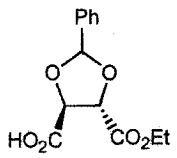


35

Prepared as described for **33**, using 154 mg of (D)-diethyltartarate, to afford **35** as a pale yellow solid, 196 mg (93 %). The physical and spectral characteristics were identical to **33**, excepting optical rotation.

$[\alpha_D]$ (c = 1, $CHCl_3$) +47.1°

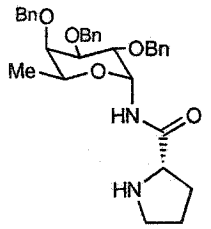
2-Phenyl-[1,3]-dioxolane-(*S*)-4-carboxylic acid ethylester-5-(*S*)-5-carboxylic acid (36)



36

Prepared as described for **34**, using 188 mg (0.64 mmol) of diester **35** to afford **36** as a colorless oil (160 mg, 95%. ^1H NMR indicates a 2.5:1 mixture of diastereomers, with chemical shifts identical to those for the (L)-isomer.

***N*'-2,3,4-tri-O-benzyl-α-L-fucopyranosyl-L-proline (37)**



37

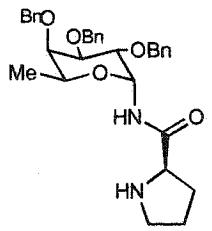
Compound **7** 278 mg (0.373 mmol) of was dissolved in 6 mL of a solution of 20 % piperidine in acetonitrile. The mixture stirred for 10 minutes, then concentrated to a white solid, and purified by silica gel chromatography (hexanes until the fulvene derivative is removed, then to 10:1 $CHCl_3$/MeOH) to afford deprotected amine **37** (197 mg, 99 %). This compound displayed very poor spectral resolution. Compounds **38-39** were much better (see respective preparations).

$[\alpha_D]$ (c = 1, $CHCl_3$) –44.8°

M.S. (+FAB) : (Calc. for $C_{32}H_{38}N_2O_5$ = 530, Found *m/z* 531 $(C_{32}H_{38}N_2O_5 + H)^+$

^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 8.21 (1H, bs, amide-NH), 7.31 (15H, m, Ar-H), 5.66 (1H, m), 5.02 (2H, m), 4.82-4.72 (5H, m), 3.84-3.53 (5H, m), 2.85-2.80 (2H, m), 2.06 (1H, m), 1.88 (1H, m), 1.56 (2H, m), 1.47 (1H, bs, NH), 1.07 (3H, bs, H-6).

N'-2,3,4-tri-O-benzyl-α-L-fucopyranosyl-D-proline (38)



38

Compound **8** (610 mg, 0.808 mmol) of was dissolved in 20 mL of a solution of 20 % piperidine in acetonitrile. The mixture stirred for 20 minutes, then concentrated to a white solid, and purified by silica gel chromatography (4:1 hexanes/ethyl acetate until the fulvene derivative is removed, then to 10:1 $CHCl_3$/MeOH) to afford deprotected amine **38** (439 mg, 99 %). The ^1H and ^{13}C NMR can be found in Figures **2.17** and **2.18**, respectively.

m.p. 112-113 °C

$[\alpha_D]$ (c = 0.24, $CHCl_3$) –14.3°

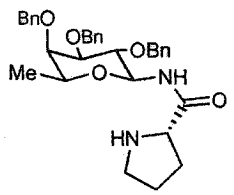
M.S. (+FAB) : (Calc. for $C_{32}H_{38}N_2O_5$ = 530, Found *m/z* 531 $(C_{32}H_{38}N_2O_5 + H)^+$

^1H NMR (500 MHz, $CDCl_3$) δ (ppm): 8.32 (1H, bs, amide-NH), 7.27 (15H, m, Ar-H), 5.77 (1H, d, *J* = 9.2 Hz, H-1), 5.00 (2H, m, benzylic-H), 4.86 (1H, d, *J* = 11.6 Hz, benzylic-H), 4.70 (4H, m, benzylic-H), 3.81 (1H, t, *J* = 9.2 Hz, H-2), 3.69 (1H, bs, α-Pro), 3.64 (1H, dd, *J* = 2.8 Hz, *J* = 9.8 Hz, H-3), 3.61 (1H, dd, *J* = 2.2 Hz, *J* = 5.5 Hz), 3.58 (1H, q, *J* = 6.3 Hz, H-5), 2.88 (1H, m, δ-Pro), 2.81 (1H, m, δ-Pro), 2.10 (1H, m,

β-Pro), 1.85 (1H, m, β-Pro), 1.61 (2H, m, γ-Pro), 1.47 (1H, bs, NH), 1.12 (3H, d, J = 6.4 Hz, H-6).

^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 174.8 (C=O), 138.7, 138.3, 138.3, 128.7, 128.4, 128.3, 128.2, 127.9, 127.8, 127.7, 127.6, 127.4 (Ar-C), 83.9 (H-3), 79.1 (H-1), 78.5 (H-2), 76.6 (H-4), 75.0, 74.9, 73.0 (benzylic-CH_2), 72.4 (H-5), 60.4 (α-Pro), 46.9 (δ-Pro), 30.5 (β-Pro), 25.8 (γ-Pro), 16.9 (C-6).

N'-2,3,4-tri-O-benzyl-β-L-fucopyranosyl-L-proline (27)



27

Compound **10** (210 mg, 0.282 mmol) was dissolved in 5 mL of 20 % piperidine in acetonitrile. The mixture stirred for 20 minutes, then concentrated to a white solid, and the residue was purified by silica gel chromatography (8:1 hexanes/ethyl acetate until the fulvene derivative is removed, then to 10:1 $CHCl_3$/MeOH) to afford deprotected amine **27** (146 mg, 99 %).

m.p. 91.1-91.8 °C

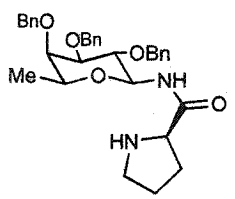
$[\alpha_D]$ (c = 0.5, $CHCl_3$) +9.3°

M.S. (+FAB) : (Calc. for $C_{32}H_{38}N_2O_5$ = 530, Found m/z 531 $(C_{32}H_{38}N_2O_5 + H)^+$

^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 8.44 (1H, d, J = 8.2 Hz, amide-NH), 7.28 (15H, m, Ar-H), 5.04 (1H, dd, J = 4.6 Hz, J = 8.4 Hz), 4.86 (1H, d, J = 11.7 Hz, benzylic-H), 4.79 (1H, d, J = 11.9 Hz, benzylic-H), 4.74 (1H, d, J = 11.9 Hz, benzylic-H), 4.63 (1H, d, J = 12.0 Hz, benzylic-H), 4.60 (1H, d, J = 12.0 Hz, benzylic-H), 4.07 (1H, dd, J = 4.5 Hz, J = 8.3 Hz, α-Pro), 3.77 (1H, dq, J = 1.8 Hz, J = 6.4 Hz, H-5), 3.73 (1H, dd, J = 5.3 Hz, J = 9.1 Hz, H-3), 3.69 (2H, m, H-2, H-4), 2.98 (1H, m, δ-Pro), 2.87 (1H, m, δ-Pro), 2.11 (1H, m, β-Pro), 1.93 (1H, m, β-Pro), 1.68 (2H, m, γ-Pro), 1.50 (1H, bs, NH), 1.21 (3H, d, J = 6.4 Hz, H-6).

^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 175.6 (C=O), 138.6, 138.5, 138.0, 128.5, 128.4, 128.3, 128.2, 128.2, 127.9, 127.6, 127.6 (Ar-C), 78.1, 76.5 (C-3, C-4), 75.3 (C-1), 74.1, 73.2, 72.5 (benzylic-CH_2), 73.6 (C-1), 68.1 (H-5), 60.8 (α-Pro), 47.3 (δ-Pro), 30.7 (β-Pro), 26.1 (γ-Pro), 16.2 (C-6).

_N_'-2,3,4-tri-O-benzyl-β-L-fucopyranosyl-D-proline (39)



39

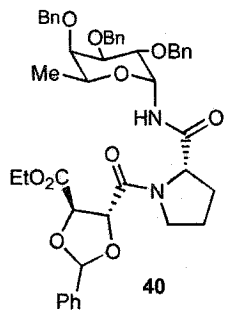
Compound **11** (85 mg, 0.16 mmol) of was dissolved in 3 mL of a solution of 20 % piperidine in acetonitrile. The mixture stirred for 20 minutes, then concentrated to a white solid, and purified by silica gel chromatography (hexanes until the fulvene

derivative is removed, then to 10:1 $CHCl_3$/MeOH) to afford deprotected amine **39** (61 mg, 100 %). This compound exists as rotamers (5:3 ratio as seen in 500 MHz ^1H NMR); integrations listed are those of the combined rotamers.

^1H NMR (500 MHz, $CDCl_3$) δ (ppm): 8.17-8.11 (1H, bs, amide-NH), 7.31 (15H, m, Ar-H), 5.00 (3H, m), 4.85-4.71 (5H, m), 3.82-3.57 (5H, m), 2.85-2.80 (2H, m), 2.06 (1H, m), 1.88 (1H, m), 1.56 (2H, m), 1.45 (1H, bs, NH), 1.09 (3H, bs, H-6).

m.p. 105-109 °C

M.S. (+FAB) : (Calc. for $C_{32}H_{38}N_2O_5 = 530$, Found *m/z* 531 $(C_{32}H_{38}N_2O_5 + H)^+$



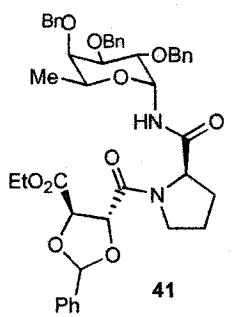
40

Amine **37** (29 mg, 55 μmol) was dissolved in DMF (1.0 mL), along with acid **34** (29 mg, 110μmol, 2 eq.), HATU (42 mg, 66 μmol, 1.2 eq.) and DIPEA (0.1 mL). The deep yellow mixture was stirred overnight, and then partitioned between ethyl acetate and water (20 mL each). The ethyl acetate layer was dried over sodium sulfate, filtered and concentrated. The residue was chromatographed on silica gel (1:1 hexanes/ethyl acetate) to afford a colorless solid, 12.3 mg (29 %). The ^1H NMR (500 MHz) of this material clearly indicated the presence of diastereomers/rotamers;

integrations listed are for the combined species. The ESI-MS can be seen in Figure **2.19**.

^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 8.03-7.80 (m, 1H, N-H), 7.52-7.25 (m, 20H), 6.16 (m, 1H, benzylidine-H), 5.72 (1H, m, H-1), 5.03-4.55 (m, 9H), 4.27 (m, 3H), 3.56 (m, 5H), 2.40 (m, 1H), 2.18 (m, 1H), 1.97-1.66 (m, 2H), 1.31 (m, 4H), 1.10 (m, 3H).

M.S. (+ESI) : (Calc. for $C_{45}H_{50}N_2O_{10}$ = 778, Found *m/z* 779 $(C_{45}H_{50}N_2O_{10} + H)^+$, 801 $(C_{45}H_{50}N_2O_{10} + Na^+)$.



41

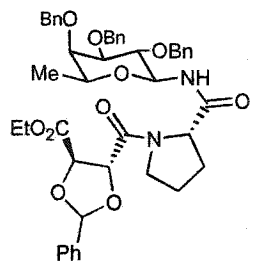
Amine **38** (30.0 mg, 56.5 μmol) was dissolved in DMF (1.0 mL), along with acid **34** (30.0 mg, 113 μmol, 2 eq.), HATU (47 mg, 69 μmol, 1.2 eq) and DIPEA (0.1 mL). The deep yellow mixture was stirred overnight, and then partitioned between ethyl acetate and water (20 mL each). The ethyl acetate layer was dried over sodium sulfate, filtered and concentrated. The residue was chromatographed on silica gel (1:1 hexanes/ethyl acetate) to afford a colorless solid, 26.2 mg (60 %). The ^{1}H and ^{13}C NMR spectra (Figures **2.20** and **2.21**, respectively) of this material showed poor

resolution, and its diastereomeric nature was very complicated, preventing complete deconvolution.

^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 8.00-7.82 (m, 1H, N-H), 7.51 (m, 2H), 7.42-7.22 (m, 18H), 6.20 (s, 1H, benzylidine-H), 5.77 (1H, m, H-1), 5.15-4.51 (m, 9H), 4.22 (m, 3H), 3.52 (m, 5H), 2.42 (m, 1H), 2.15 (m, 1H), 2.01-1.70 (m, 2H), 1.29 (m, 4H), 1.08 (m, 3H).

^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 170.7, 170.2, 167.3 (C=O), 138.4, 135.6, 130.0, 128.4, 128.4, 128.3, 128.2, 127.8, 127.7, 127.6, 127.5, 127.1 (Ar-C), 106.7, 105.6 (benzylidine-CH), 78.8, 77.8, 74.8, 74.7, 74.5, 73.4, 72.5, 67.8, 62.0, 60.4, 47.4 (δ-Pro), 26.3 (β-Pro), 25.0 (γ-Pro), 16.5 (C-6), 14.1 ($CH_2\underline{C}H_3$).

M.S. (+ESI) : (Calc. for $C_{45}H_{50}N_2O_{10}$ = 778, Found *m/z* 779 $(C_{45}H_{50}N_2O_{10} + H)^+$, 801 $(C_{45}H_{50}N_2O_{10} + Na^+)$.



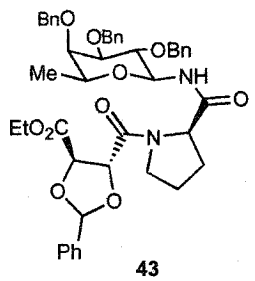
42

Amine **27** (36.4 mg, 6.86 μmol) was dissolved in DMF (1.0 mL), along with acid **34** (36.6 mg, 13.9 μmol, 2 eq.), HATU (52 mg, 8.23 μmol, 1.2 eq.) and DIPEA (0.1 mL). The deep yellow mixture was stirred overnight, and then partitioned between ethyl

acetate and water (20 mL each). The ethyl acetate layer was dried over sodium sulfate, filtered and concentrated. The residue was chromatographed on silica gel (1:1 hexanes/ethyl acetate) to afford a colorless solid, 52.1 mg (98 %). The ^{1}H NMR (Figure **2.22**) (500 MHz) of this material showed excellent line resolution, but due to its diastereomeric/rotameric nature was very complicated, preventing deconvolution.

^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 7.55-7.01 (m, 21H, Ar-H, NH), 6.05-5.96 (m, 1H, benzylidene-H), 5.30-4.22 (m, 13H), 3.77-3.55 (m, 5H), 2.38-1.70 (m, 4H, β-Pro, γ-Pro), 1.35-1.08 (m, 6H, H-6, $CH_2C\underline{H}_3$).

M.S. (+ESI) : (Calc. for $C_{45}H_{50}N_2O_{10}$ = 778, Found *m/z* 779 $(C_{45}H_{50}N_2O_{10} + H)^+$, 801 $(C_{45}H_{50}N_2O_{10} + Na^+)$.



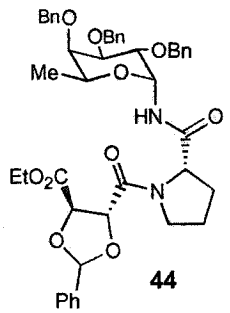
43

Amine **39** (48.9 mg, 92 μmol) was dissolved in DMF (1.0 mL), along with acid **36** (48.0 mg, 180 μmol, 2 eq.), HATU (77 mg, 110 μmol, 1.2 eq.) and DIPEA (0.1 mL). The deep yellow mixture was stirred overnight, and then partitioned between ethyl acetate and water (20 mL each). The ethyl acetate layer was dried over sodium sulfate, filtered and concentrated. The residue was chromatographed on silica gel (1:1 hexanes/ethyl acetate) to afford a colorless solid, 34.0 mg (47 %). The ^{1}H NMR

(500 MHz) of this material showed excellent resolution, but due to its diastereomeric/rotameric nature was very complicated, preventing deconvolution.

^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 7.49-7.06 (m, 21H, Ar-H, NH), 6.06-5.96 (m, 1H, benzylidene-H), 5.20-4.38 (m, 13H), 3.76-3.47 (m, 5H), 2.32-1.71 (m, 4H, β-Pro, γ-Pro), 1.31-1.01 (m, 6H, H-6, $CH_2C\underline{H}_3$).

M.S. (+ESI) : (Calc. for $C_{45}H_{50}N_2O_{10}$ = 778, Found *m/z* 779 $(C_{45}H_{50}N_2O_{10} + H)^+$, 801 $(C_{45}H_{50}N_2O_{10} + Na^+)$.

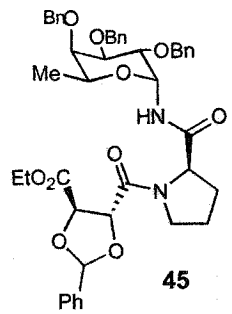


44

Amine **37** (28.1 mg, 53.0 μmol) was dissolved in DMF (1.0 mL), along with acid **36** (28.2 mg, 105 μmol, 2 eq.), HATU (44 mg, 61 μmol, 1.2 eq.) and DIPEA (0.1 mL). The deep yellow mixture was stirred overnight, and then partitioned between ethyl acetate and water (20 mL each). The ethyl acetate layer was dried over sodium sulfate, filtered and concentrated. The residue was chromatographed on silica gel (1:1 hexanes/ethyl acetate) to afford a colorless solid, 8.1 mg (19 %). The ^{1}H NMR (500 MHz) of this material was of very poor quality and could not be fully interpreted.

^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 8.03-7.85 (m, 1H, N-H), 7.45-7.25 (m, 20H), 6.09 (m, 1H, benzylidine-H), 5.61 (1H, m, H-1), 5.00-4.31 (m, 12H), 3.50 (m, 5H), 2.45 (m, 1H), 2.220 (m, 1H), 2.07-1.69 (m, 2H), 1.37 (m, 4H), 1.08 (m, 3H).

M.S. (+ESI) : (Calc. for $C_{45}H_{50}N_2O_{10}$ = 778, Found *m/z* 779 $(C_{45}H_{50}N_2O_{10} + H)^+$, 801 $(C_{45}H_{50}N_2O_{10} + Na^+)$.

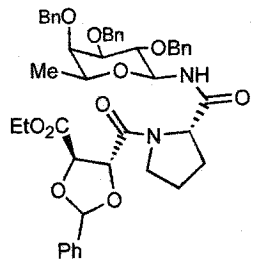


45

Amine **39** (48.2 mg, 90.9 μmol) was dissolved in DMF (1.0 mL), along with acid **36** (48.0 mg, 180 μmol, 2 eq.), HATU (67 mg, 75.5 μmol, 1.2 eq.) and DIPEA (0.1 mL). The deep yellow mixture was stirred overnight, and then partitioned between ethyl acetate and water (20 mL each). The ethyl acetate layer was dried over sodium sulfate, filtered and concentrated. The residue was chromatographed on silica gel (1:1 hexanes/ethyl acetate) to afford a colorless solid, 34.1 mg (78 %). The ^{1}H NMR (500 MHz) of this material showed excellent resolution, but due to its diastereomeric nature was very complicated, preventing deconvolution.

^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 8.01-7.82 (m, 1H, N-H), 7.27-7.15 (m, 20H), 6.06 (m, 1H, benzylidine-H), 5.70 (1H, m, H-1), 4.99-4.38 (m, 12H), 3.53 (m, 5H), 2.51 (m, 1H), 2.22 (m, 1H), 2.17-1.72 (m, 2H), 1.41 (m, 4H), 1.12 (m, 3H).

M.S. (+ESI) : (Calc. for $C_{45}H_{50}N_2O_{10}$ = 778, Found *m/z* 779 $(C_{45}H_{50}N_2O_{10} + H)^+$, 801 $(C_{45}H_{50}N_2O_{10} + Na^+)$.

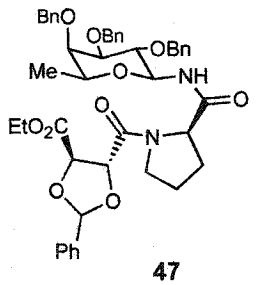


46

Amine **38** (34.4 mg, 6.49 μmol) was dissolved in DMF (1.0 mL), along with acid **36** (35.1 mg, 13.0 μmol, 2 eq.), HATU (56 mg, 7.8 μmol, 1.2 eq.) and DIPEA (0.1 mL). The deep yellow mixture was stirred overnight, and then partitioned between ethyl acetate and water (20 mL each). The ethyl acetate layer was dried over sodium sulfate, filtered and concentrated. The residue was chromatographed on silica gel (1:1 hexanes/ethyl acetate) to afford a colorless solid, 44.6 mg (84 %). The ^{1}H NMR (500 MHz) of this material showed excellent resolution, but due to its diastereomeric nature was very complicated, preventing deconvolution.

^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 7.51-7.07 (m, 21H, Ar-H, NH), 6.02-5.92 (m, 1H, benzylidene-H), 5.32-4.50 (m, 9H), 4.20 (m, 5H), 3.67-3.51 (m, 5H), 2.34-1.72 (m, 4H, β-Pro, γ-Pro), 1.22-1.13 (m, 6H, H-6, $CH_2C\underline{H}_3$).

M.S. (+ESI) : (Calc. for $C_{45}H_{50}N_2O_{10}$ = 778, Found *m/z* 779 $(C_{45}H_{50}N_2O_{10} + H)^+$, 801 $(C_{45}H_{50}N_2O_{10} + Na^+)$.



47

Amine **39** (42.2 mg, 76.9 μmol) was dissolved in DMF (1.0 mL), along with acid **36** (42.0 mg, 151 μmol, 2 eq.), HATU (67 mg, 92 μmol, 1.2 eq.) and DIPEA (0.1 mL). The deep yellow mixture was stirred overnight, and then partitioned between ethyl acetate and water (20 mL each). The ethyl acetate layer was dried over sodium sulfate, filtered and concentrated. The residue was chromatographed on silica gel (1:1 hexanes/ethyl acetate) to afford a colorless solid, 44.9 mg (62 %). The ^{1}H NMR (500 MHz) of this material showed reasonable resolution, but diastereomer/rotamer issues complicated spectral interpretation, and numerous addition peaks appear in the ^{13}C spectrum

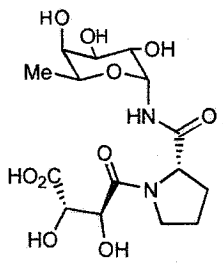
^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 8.11-7.99 (m, 1H, N-H), 7.41 (m, 2H), 7.37-7.20 (m, 18H), 6.15-6.07 (m, 1H, benzylidine-H), 5.11-4.51 (m, 10H), 4.29 (m, 3H), 3.46 (m, 5H), 2.32-2.22 (m, 2H), 2.06-1.82 (m, 2H), 1.33-1.26 (m, 4H), 1.11 (m, 3H).

^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 171.1, 170.1, 170.1, 168.2, 165.3 (C=O), 138.1, 137.7, 137.0, 135.0, 132.2, 128.4, 128.4, 128.4, 128.3, 128.3, 128.3, 128.2,

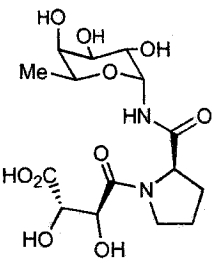
128.1, 128.0, 128.0, 127.8, 127.7, 127.6, 127.5, 127.1, 126.5 (Ar-C), 107.1, 105.9, 105.2 (benzylidine-CH), 79.0, 78.2, 77.8, 77.8, 76.6, 75.2, 74.9, 74.7, 74.5, 73.9, 73.8, 72.5, 71.9, 67.8, 67.4, 62.6, 62.2, 62.0, 61.5, 61.2, 60.6, 48.2, 48.0, 47.0, 26.6, 26.4, 26.3, 25.1, 25.0, 16.5, 14.1, 14.1.

M.S. (+ESI) : (Calc. for $C_{45}H_{50}N_2O_{10}$ = 778, Found *m/z* 779 $(C_{45}H_{50}N_2O_{10} + H)^+$, 801 $(C_{45}H_{50}N_2O_{10} + Na^+)$.

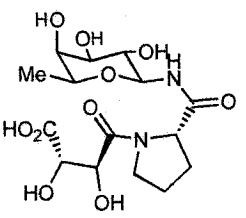
Compounds 48-55



48



49



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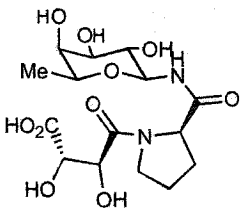
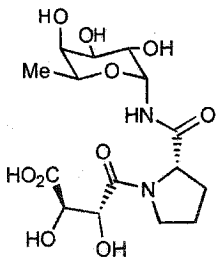
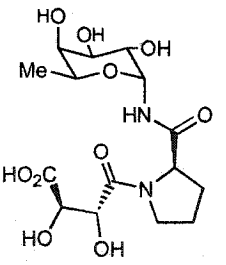


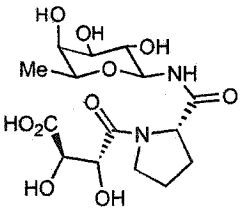
51



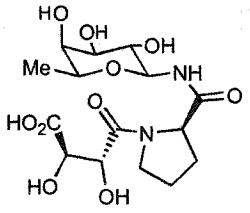
52



53

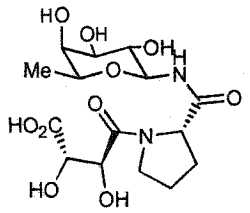


54



55

Each of these compounds exhibited the desired M – H^+ peak for ESI-MS, but did not produce good quality NMR spectra. The materials seem to be quite impure and/or decomposed and cannot be confirmed to be of any quality. A representative procedure is provided.



50

Conjugate **42** (31 mg, 40 μmol) was dissolved in 50 mL of 0.05 N NaOH in EtOH (80 % in water). TLC (3:1 ethyl acetate/hexanes) indicated reaction completion after 25 minutes. Amberlite IR-120 H^+ resin was carefully added until the mixture reached pH 4. The mixture was then filtered and evaporated, and the residue was partitioned between ether and water (20 mL each). The water layer was extracted with ether (2 x 20 mL). The ether extracts were combined, dried over sodium sulfate, filtered and concentrated to afford a white solid. The material was brought up in methanol, and sodium hydroxide (1.6 mg, 40 μmol, 1.0 eq.) was added. Palladium on carbon (41 mg, 10 % Pd) was added, and the mixture flushed with hydrogen for 2 h. TLC (1:1:0.1 $CHCl_3$/MeOH/AcOH) shows the reaction to be complete. The mixture was filtered and concentrated to afford crude material, which did not have useful NMR spectral characteristics. A strong molecular ion was detected, however (see Figure **2.23**). TLC over time (several days) showed the compound to be unstable.

3. *C*-Linked Fucopeptides as Mimetics of Sialyl Lewis^x

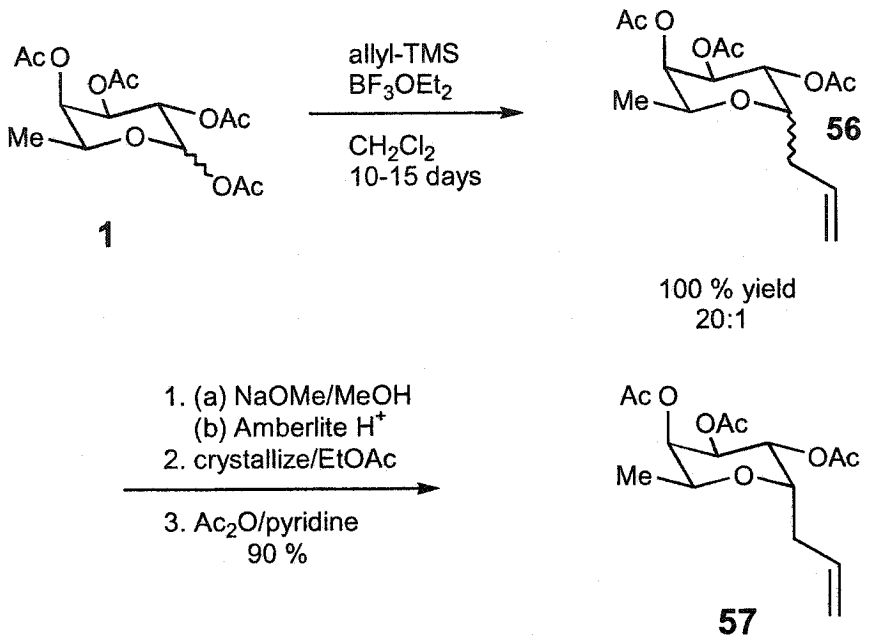
3.1 Introduction

The use of *C*-linked mimetics has been employed in the past as an alternative to *O*-linkages,[1,32,48] largely for reasons of *in vivo* stability, but also for chemical stability.[105] *C*-linked mimetics retain their conformational properties, with (normally) minimal effect on binding affinity and resultant biological activity (refer to section **1.3.6** for more information on synthetic approaches to *C*-glycosides). Kishi[106] has compared *C*-linked to *O*-linked mimetics by conformational NMR studies, and has shown that although they have similar affinities, the *C*-linked mimetics have many more possible conformations. Satoh[49] has developed a series of aryl *C*-glycosides based on a silver triflate/tin (IV) chloride catalyzed electrophilic substitution. Armstrong[35] has shown that a diverse library of *C*-glycosides could be produced using a Ugi reaction on solid support. In the interest of expanding the scope of the work involved here, the synthesis of *C*-linked mimetics of Sialyl Lewis^x was undertaken.

3.2 Synthetic Strategies Leading to *C*-Linked Mimetics

Two separate approaches were followed, both using the known compound **57**[80] as the lead into *C*-linked compounds. An improved procedure for the preparation of **57** is shown in Scheme **3.1**; it was found that prolonged reaction times gave quantitative yields of the alkene **56**, compared with 70-80% as per literature methods.[80]

The first method employed used a Heck coupling to introduce an alkene-aryl bond. The second was olefin cross-metathesis, which introduced a proline spacer at a greater distance than was the case with *N*-linked mimetics.



Scheme **3.1**: Preparation of Allyl Glycoside **57**.[80]

3.2.1 Heck Coupling Sequence

The Heck reaction, first published in 1968,[107] provides a means in which an alkene (normally terminal) is coupled with an aryl or vinyl halide. The reaction is catalyzed by palladium (0). The mechanism for the reaction[108] is detailed in Figure **3.1**.

The reaction involves the use (or *in situ* formation of) tetrakis(triphenyl-phospine)palladium (0) and a tertiary amine base. The cycle begins with the

dissociation of two triphenylphosphine ligands from the palladium center to form species **A**. Oxidative addition of the sp^2-halide bond affords species **B**, which undergoes alkene insertion to form species **C**. Bond rotation is greatly favored to bring the R group from the halide species into a *trans*-like conformation (**D**) with respect to the very large palladium species. β-hydride elimination occurs in a *syn* fashion, and the *trans* alkene (**E**) is expelled. Regeneration of the active catalytic species by removal of HX from species **F** is accomplished with the tertiary amine base.

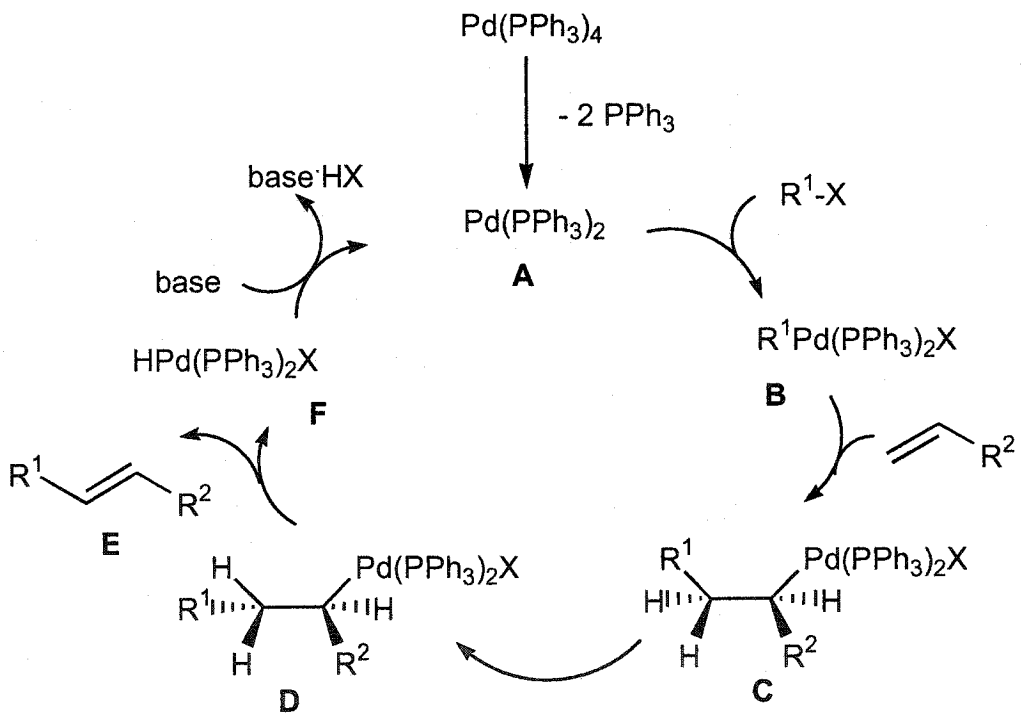
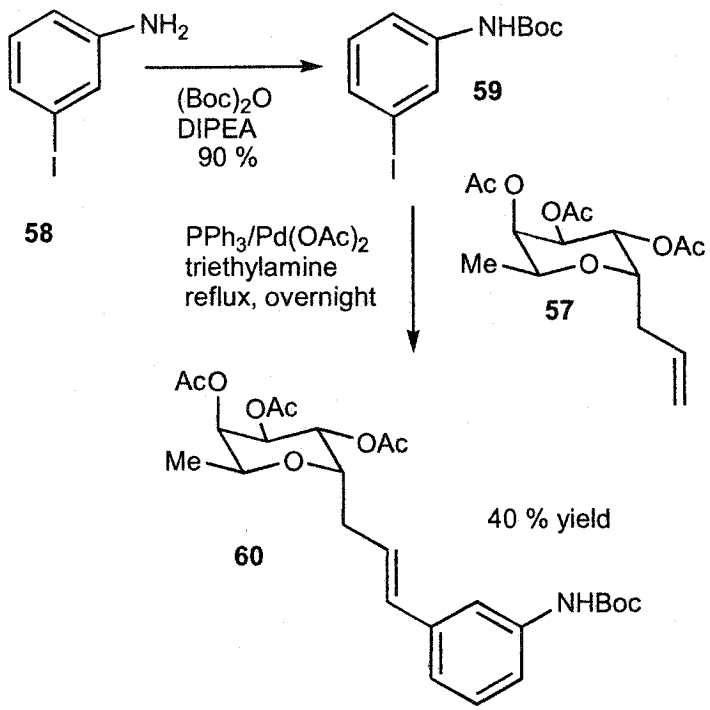


Figure **3.1**: Catalytic Cycle for the Heck Reaction (adapted from Nicolaou[108])

The reaction proceeds best when the halogen is iodide, but also proceeds with bromide.[107,108]

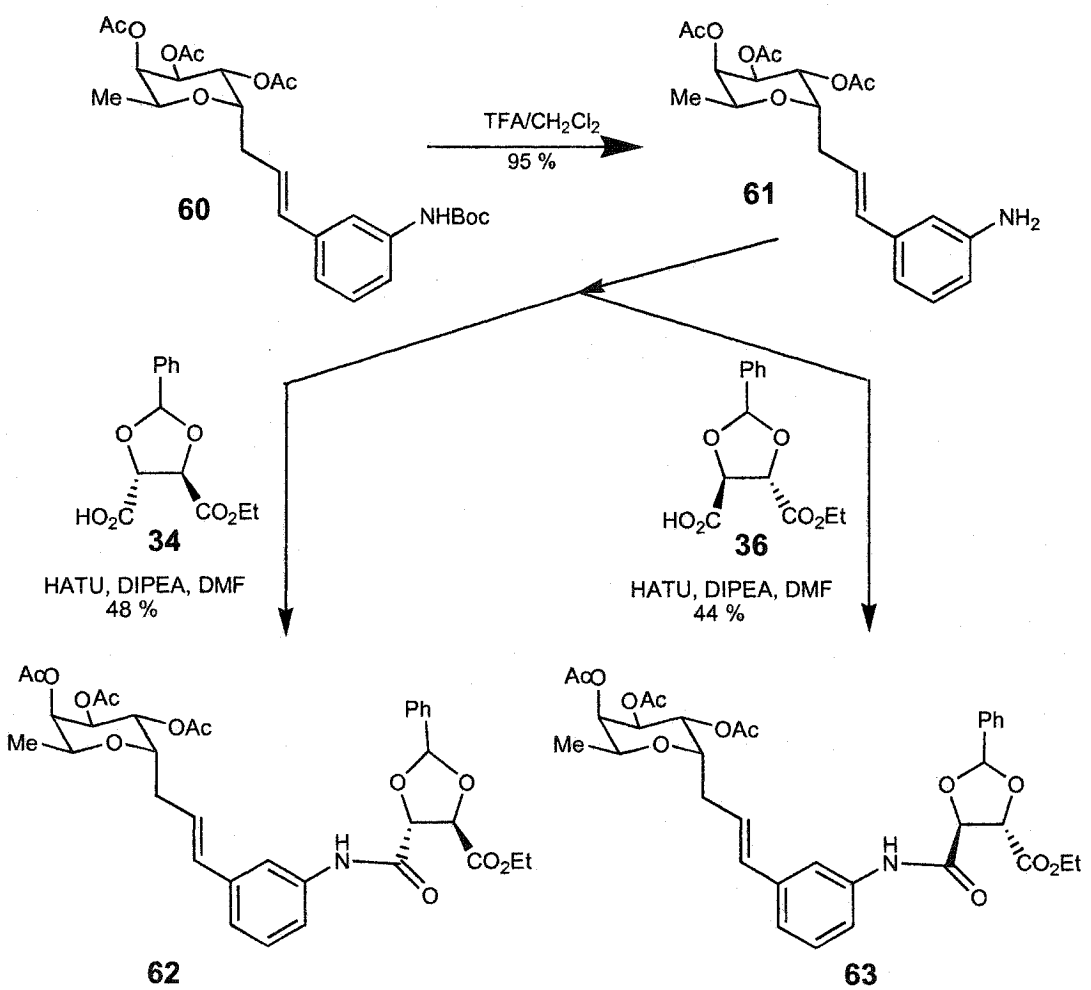
Using *N*-Boc protected 3-iodoaniline **59**,[109] Heck coupling of alkene **57** proceeded in moderate yield (Scheme **3.2**). Attempts to improve the yield by adjustment of temperature, the amount of catalyst, and excess aryl iodide **59** were not successful in terms of the desired product. However, different group V ligands afforded different products, as discussed in section **3.3**.



Scheme **3.2**: Protection of 3-iodoaniline and Heck Coupling with Alkene **57**

The mass balance of material lies with unreacted **57**, which can be recovered by chromatography. The ^{1}H NMR (500 MHz) can be found in Figure **3.5**.

The remainder of the mimetic was constructed using protected as described in Scheme **3.3**.



Scheme **3.3**: Synthesis of Protected *C*-Linked Mimetics **62** and **63**.

Deprotection of the *N*-Boc protecting group from **60** using trifluoroacetic acid proceeded smoothly. Coupling using the protected tartaric acid-derived fragments **34** and **36** afforded protected compounds **62** and **63** (Scheme **3.3**). Deprotection was

attempted using sodium hydroxide in aqueous ethanol to remove the ester groups, followed by acidic hydrolysis to attempt to remove the benzylidine; however, all attempts (0.1 N HCl, acidic resin) led to a complex mixture of products, from which no useful material could be obtained.

3.2.2 Olefin Cross-Metathesis

Olefin metathesis has been in use for limited industrial processes for some time, but for a long period was restricted to simple alkenes, due to poor functional group tolerance.[110] Originally, olefin metathesis used highly reactive metal catalysts (such as **64**) based on tungsten and other transition metals, and was largely limited to the petrochemical and polymer industries.[110] Schrock introduced a molybdenum-based catalyst **65** (Figure **3.2**) which exhibited high activity and some degree of functional group tolerance. However, it is highly sensitive to oxygen and must be handled in a glove box. Although a great improvement, this remained a limitation until the introduction of more air-stable ruthenium-based carbenoids by Grubbs in the later half of the 1990s. Catalysts **66** (known as Grubbs' catalyst)[111] and **67** ("2nd generation" Grubbs' catalyst) sparked a revolution in olefin metathesis (Figure **3.2**).

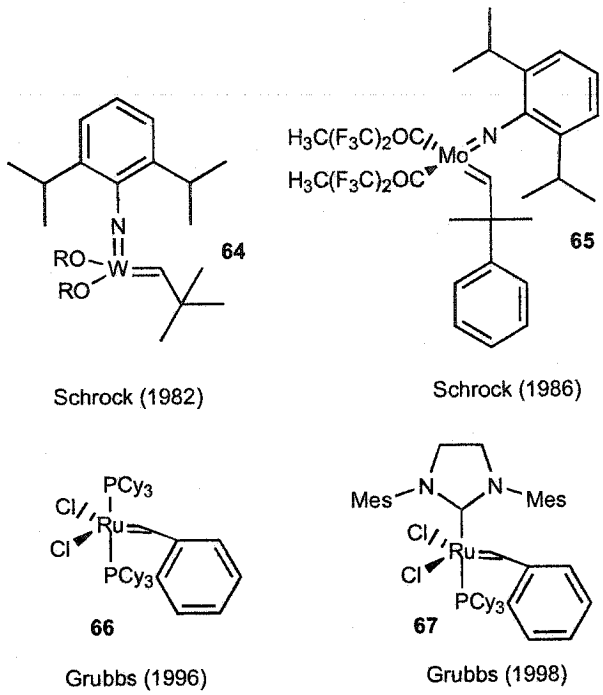


Figure **3.2**: Milestone Catalysts for Olefin Metathesis[112]

Olefin metathesis became a hot research topic in the late 1990s, with many applications developed for industry and total synthesis applications.[112-117] Investigation into the catalytic cycle has suggested a series of [2+2] cycloaddition reactions involving metallocyclobutane complexes (Figure **3.3**).[113] All steps are reversible, and the course of the reaction is driven by the expulsion of volatile ethylene from the reaction.

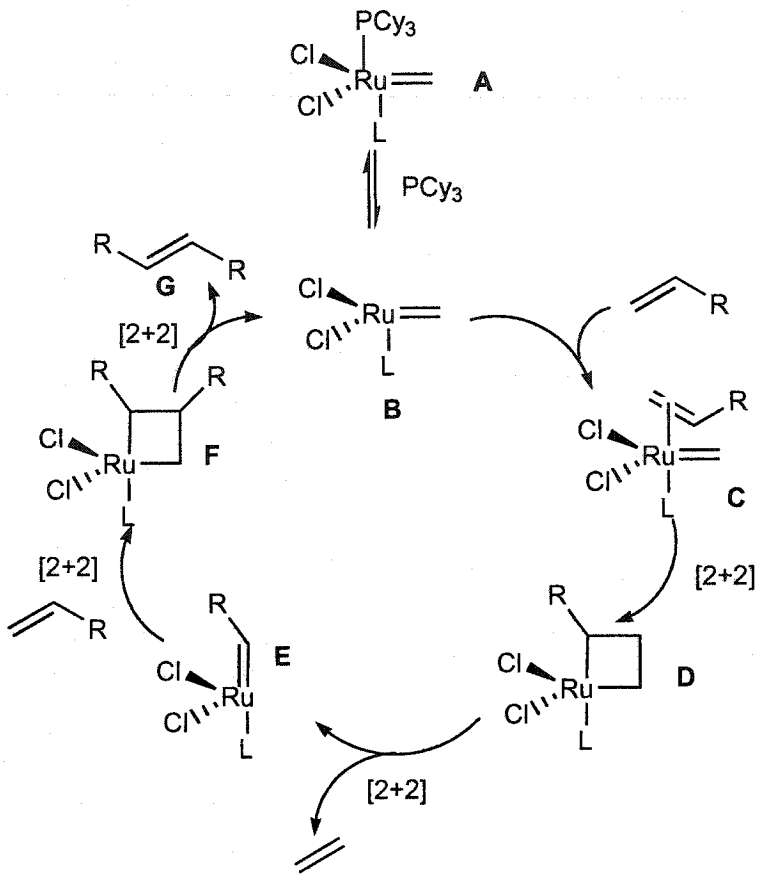


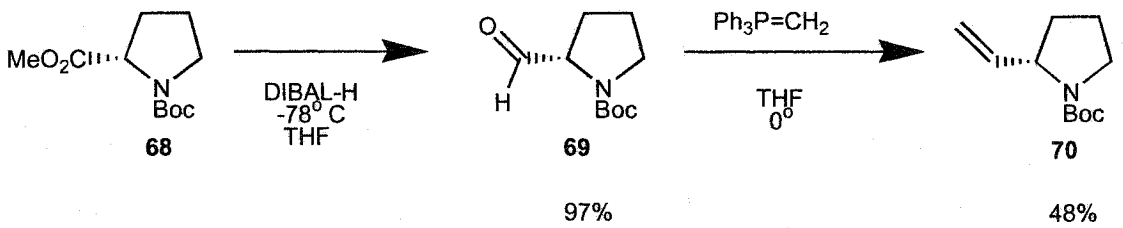
Figure **3.3**: The Catalytic Cycle of Olefin Metathesis[113]

The active catalytic species is actually **A**, formed from **66** after an initial cycle switches a benzylidene for a methylene unit.

Species **A** loses a phosphine ligand to form the complex **B**, containing a vacant coordination site, which ligates with an olefin to form **C**. [2+2] addition forms metallocyclobutane species **D** (reaction producing a distal R group is possible, but is dead-end, reverting back to **C**). Retro-[2+2] addition expels ethylene and forms carbenoid **E**, which can undergo a second ligation, and cycloaddition to form **F**.

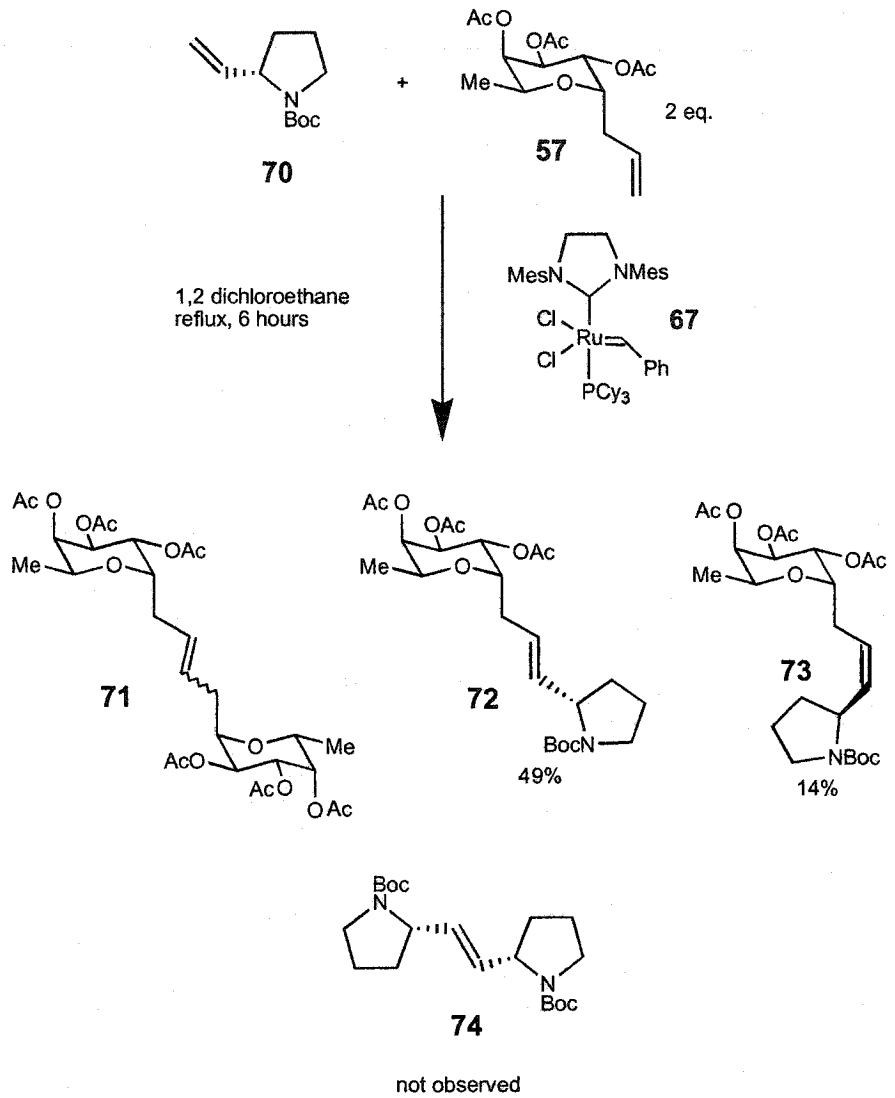
Retro-[2+2] expels olefin **G**, and regenerates the catalyst **B.** The process is driven by the removal of ethylene from the system.

Olefin **57** has been employed in olefin metathesis before,[118] and was used as a monomer for cross-metathesis. The 2nd component was to be a vinylpyrrolidine compound, derived from (L)-proline (Scheme **3.4**). This preparation is very similar to most others appearing in the literature[119-122], but at least two others have appeared using different strategies.[123,124]



Scheme **3.4**: Synthesis of *N*-Boc Protected Vinylpyrrolidine **70**.

(L) *N*-Boc proline methyl ester **68** was reduced to the corresponding aldehyde **69** using diisobutylaluminum hydride, which was subsequently subjected to Wittig conditions with methyltriphenylphosphonium bromide and butyllithium to form the chiral vinylpyrrolidine **70** in satisfactory overall yield. Cross metathesis was performed using "2nd Generation" Grubbs' catalyst **67** (Scheme **3.5**); initial experiments using Grubbs' catalyst **66** afforded only dimer **71**. It has since been reported that olefin **70** undergoes cross-metathesis using 2nd generation Grubb's catalyst using only one equivalent of the cross-coupling partner (in this case methyl-3-butenoate) in high efficiency.[122]

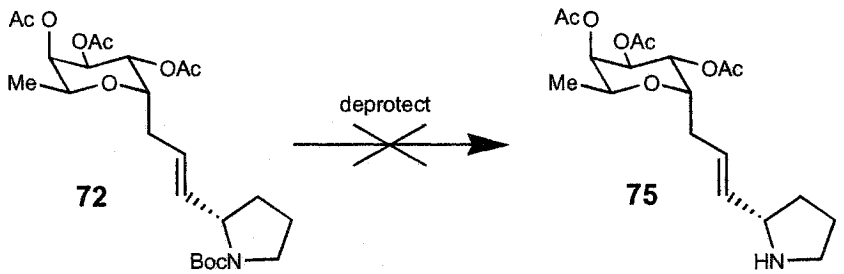


Scheme 3.5: Olefin Cross-Metathesis of **70** and **57**

Cross-metathesis normally gives a statistical mixture of products.[125,126] In many cases, the desired product can be favored by using a large excess of one component. This is not problematic if the reactant is inexpensive. However, the reaction is not normally practical when both components are not readily available in quantity. In this case, it appears that the steric bulk imposed by the *N*-Boc protecting group deters formation

of dimer **74**, and possibly prevents **72** or **73** from re-entering the catalytic cycle. In the aforementioned literature example of cross-metathesis using **70**,[122] no homodimer **74** was observed. In cases such as this, cross-metathesis can be practical when driven by steric or electronic factors to favor a particular outcome.

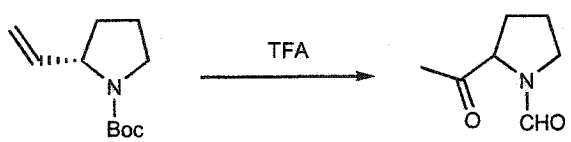
However, removal of the *N*-Boc protecting group proved to be problematic. Treatment of **72** with trifluoroacetic acid did not afford expected amine **75** (Scheme **3.6**).



72 **75**

Scheme **3.6**: Deprotection of **72** is problematic.

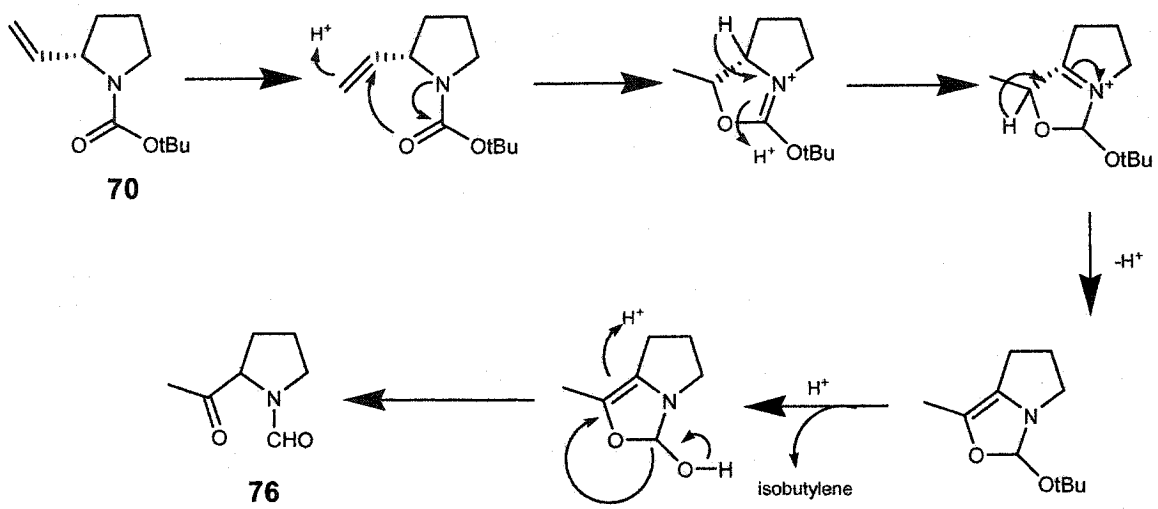
A similar failure involving an *N*-allyl Boc group had been observed in our group before.[127] In order to investigate this failure, parent compound **70** was treated with trifluoroacetic acid and the major product was found to be **76** (Scheme **3.7**).



70 **76**

Scheme **3.7**: Treatment of **70** with Trifluoroacetic Acid Gives an Unexpected Product

The reaction proceeded with a total loss of chirality. A mechanism is therefore proposed which could account for the *N*-formyl ketone **76** (Scheme **3.8**).

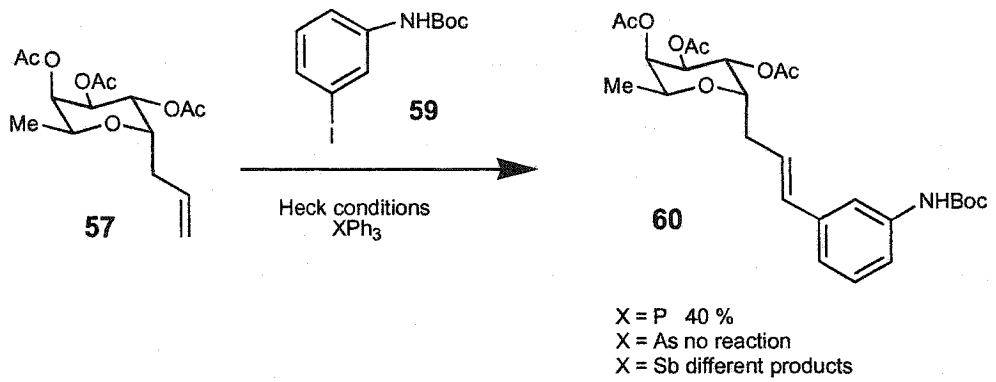


Scheme **3.8**: Proposed Mechanism to Account for the Formation of Racemic **76**.

This work was not investigated further; needless to say, the sequence could not be continued, as *N*-Boc deprotection could not be accomplished.

3.3 Discovery of a New Palladium-Catalyzed Phenyl Transfer Reaction

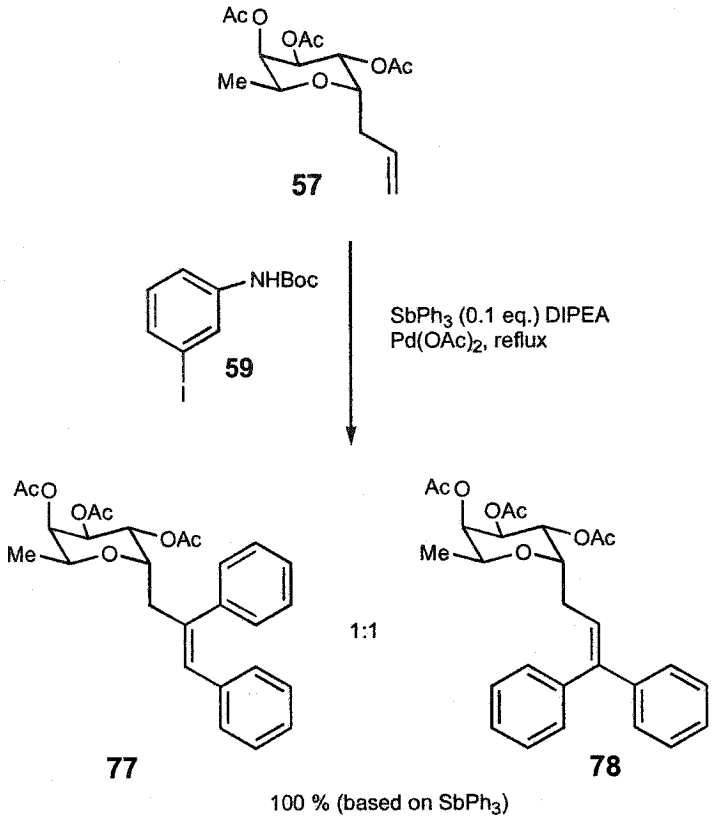
In the course of optimizing the conditions for Heck-coupling as described in **3.2.1**, a switch of triphenylphosphine in favor of other group V element ligands was investigated (Scheme **3.9**).



Scheme **3.9**: Heck Coupling of **57** Using Different Group V Ligands

3.3.1 A Different Outcome Resulting from Heck Conditions

Use of the traditional triphenylphosphine ligand gave a low yield of 40 %. Attempts to improve this yield, even with larger amounts of **59**, were not successful. Switching to triphenylarsenic gave only recovered starting materials. However, when triphenylantimony was used, two entirely different products were obtained (Scheme **3.10**), bearing two phenyl groups. Yield was nearly quantitative based on provided triphenylantimony ligand, suggesting the transfer of all three phenyl groups from the antimony center.



Scheme **3.10**: Triphenylantimony Ligands Give Unexpected Phenyl Transfer[128]

The ^{1}H and ^{13}C NMR and ESI-MS of compounds **77** and **78** can be found in Figures **3.8**, **3.9** and **3.10**, respectively. The identity of **77** was established by an NOE experiment, clearly showing the interaction between the alkene-H and allylic hydrogen atoms.

This reaction is not entirely unprecedented, as literature examples of phenyl transfer from phosphorus,[129] arsenic[130] and antimony[131] have been reported. However, all are under strenuous conditions, involved only terminal alkenes, and required huge (>300

mol %) amounts of "catalytic" palladium species. Yields are always low (<30 %) and are accompanied by rearrangements. Some examples require preformed active pentavalent species.[129]

3.3.2 Investigation of the Reaction

All successful attempts resulted in formation of trisubstituted olefins **77** and **78**. No disubstituted compounds were ever observed, even when triphenylantimony was limiting, suggesting that the addition of one phenyl group activates the alkene towards a second addition. Steric factors presumably prevent addition of a third phenyl group. The third possible *trans* isomer of **77** and **78** was also not observed. It could be due to an initial *trans* configuration of the olefin after a first addition. This would prevent formation of *trans* isomer **80** (Figure **3.4**).

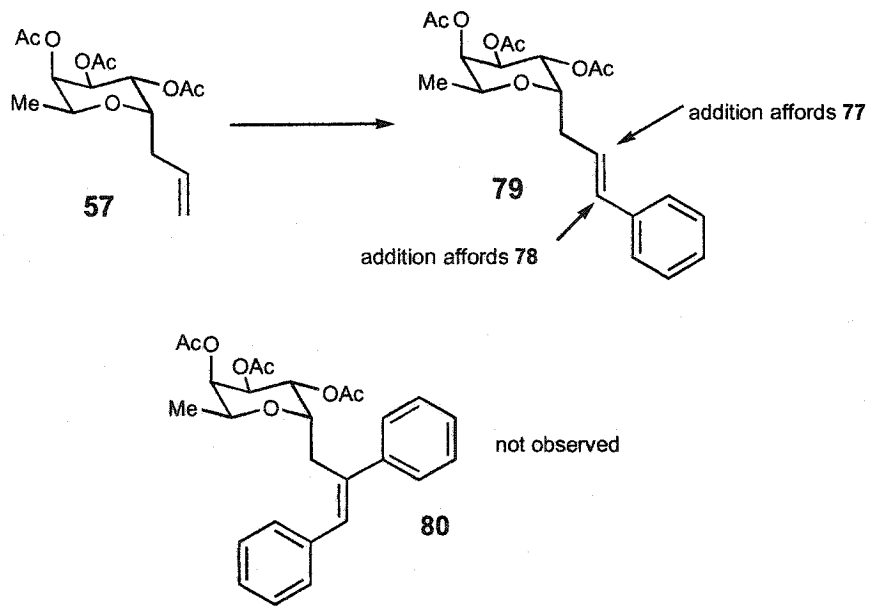
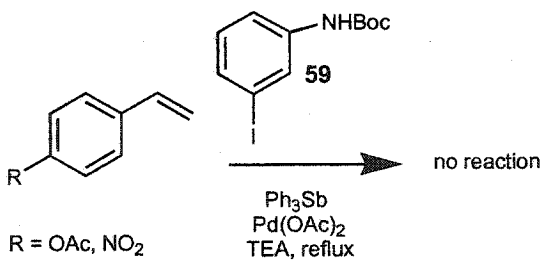


Figure **3.4**: A Possible Explanation for the Formed Products

What is especially unusual is the requirement of the aniline moiety in the catalytic system. In spite of not appearing in the product, compound **59** is consumed in the reaction, and its absence leads to no reaction. Only catalytic amounts (0.1 eq) are required to drive the reaction forward. It could be postulated that **59** is responsible for generating the active catalyst through an aryl-iodine insertion process.

What is known is that palladium (0) is an ultimate product of the reaction. Precipitation of palladium metal as a mirror on the interior of the reaction flask was consistently observed, as well as what appeared to be palladium black (a common precipitate in Heck and other palladium (0) mediated reactions).

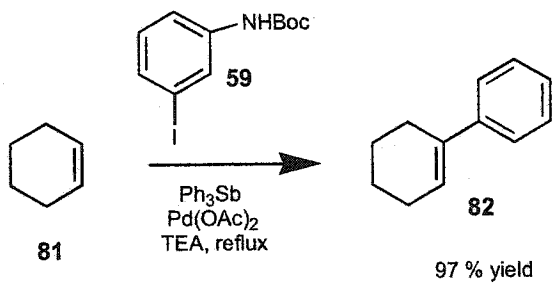
Attempts to extend this reaction to substrates not bearing an α-hydrogen (such as styrenes) were not successful (Scheme **3.11**).



Scheme **3.11**: Styrenes Do Not Give the Reaction

Simple, symmetrical alkenes such as cyclohexene **81** give excellent yields of mono-substituted product (Scheme **3.12**). This reaction would therefore seem useful for

certain symmetry-breaking cases, where a disubstituted, symmetrical olefin should be substituted with a single aryl group.



Scheme **3.12**: Synthesis of 1-Phenylcyclohexene **82**

3.4 Conclusions

Transition-metal catalysis was used to afford protected *C*-linked mimetics of Sialyl Lewis[x]. Heck reaction based strategies involving tartaric acid moieties proceeded well, up to the point of final deprotection. Olefin cross-metathesis of protected vinylpyrrolidine proceeded well, but could not be extended due to side-reactions occurring during deprotection. A new palladium catalyzed transfer of phenyl groups from triphenylantimony to afford trisubstituted olefins was discovered, and some inroads into understanding the nature and scope of the reaction were made.

3.5 Spectra Referred to Throughout Chapter 3

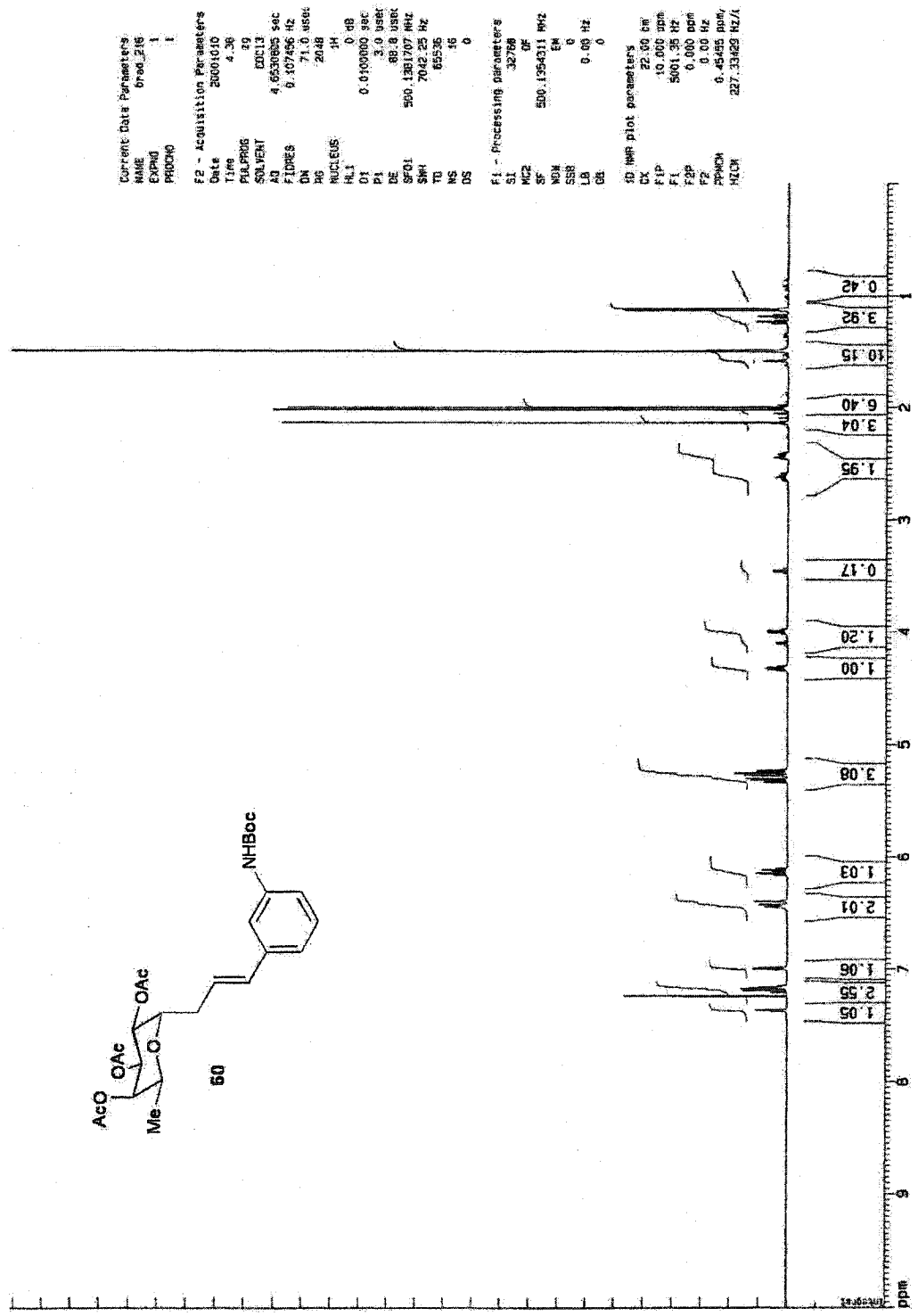


Figure **3.5**: ^{1}H NMR Spectrum ($CDCl_3$, 500 MHz) of **60**.

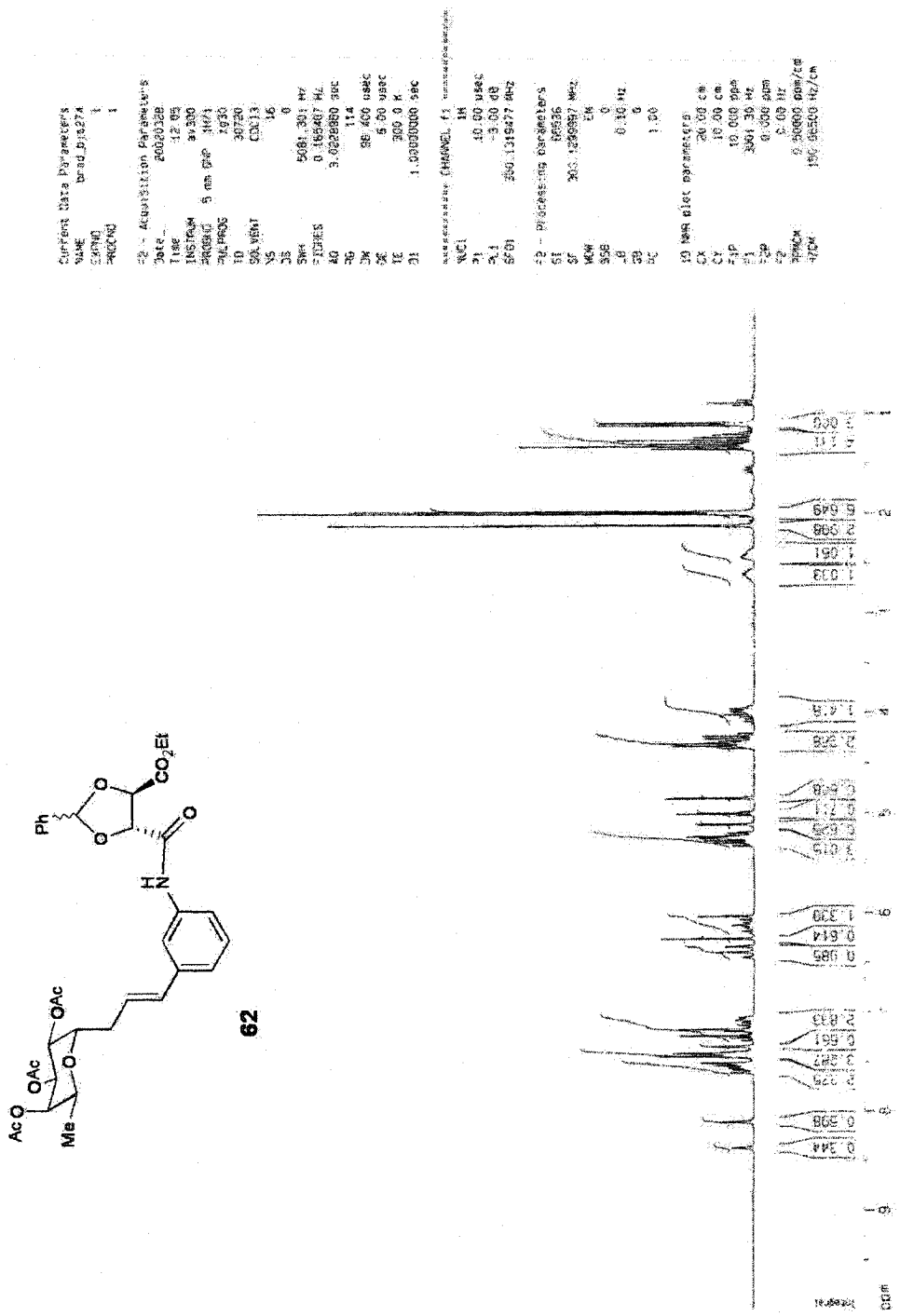


Figure **3.6**: ^{1}H NMR Spectrum ($CDCl_3$, 300 MHz) of Protected Compound **62**.

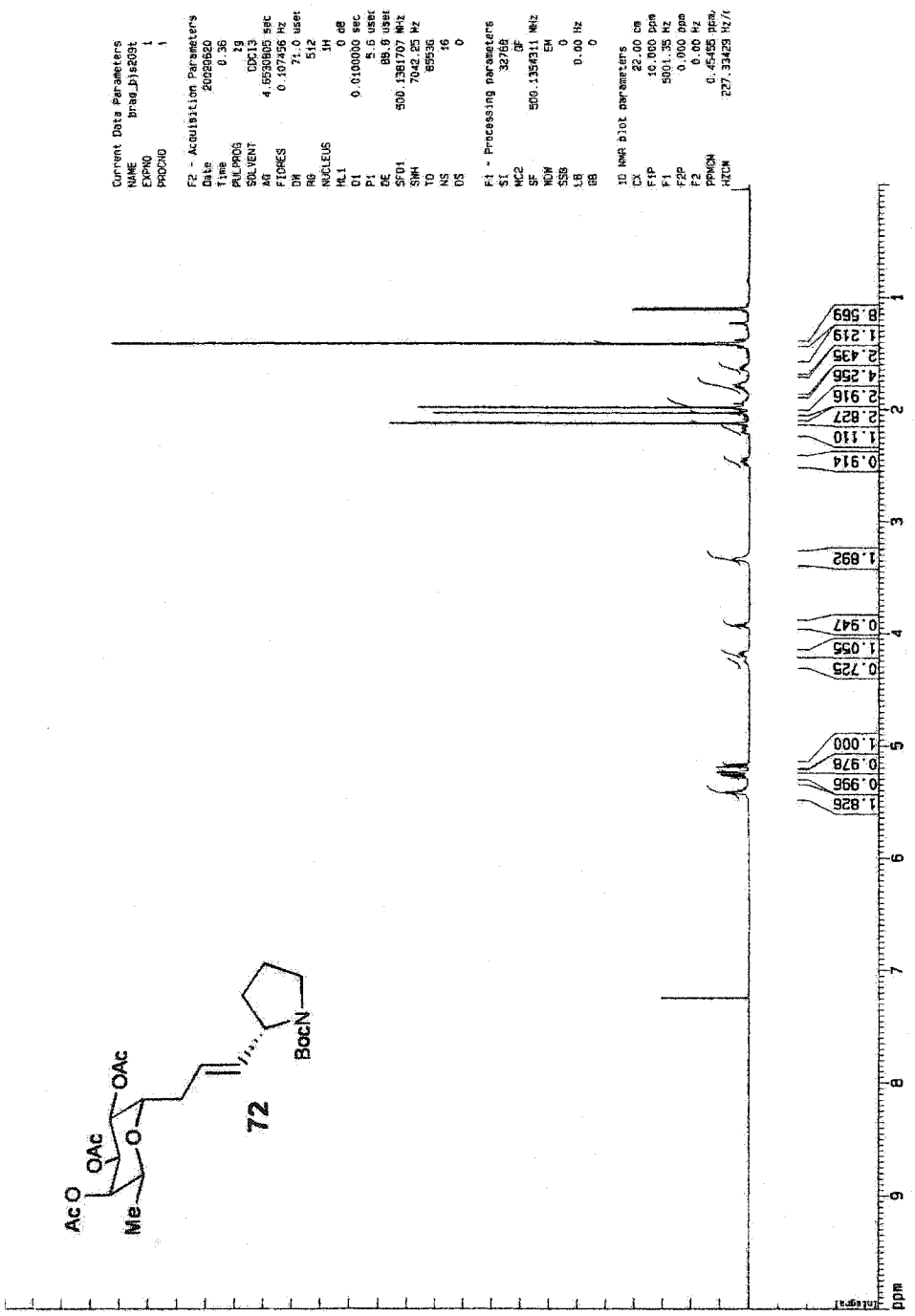


Figure 3.7: ^{1}H NMR ($CDCl_3$, 500 MHz) of Cross Metathesis Product **72**.

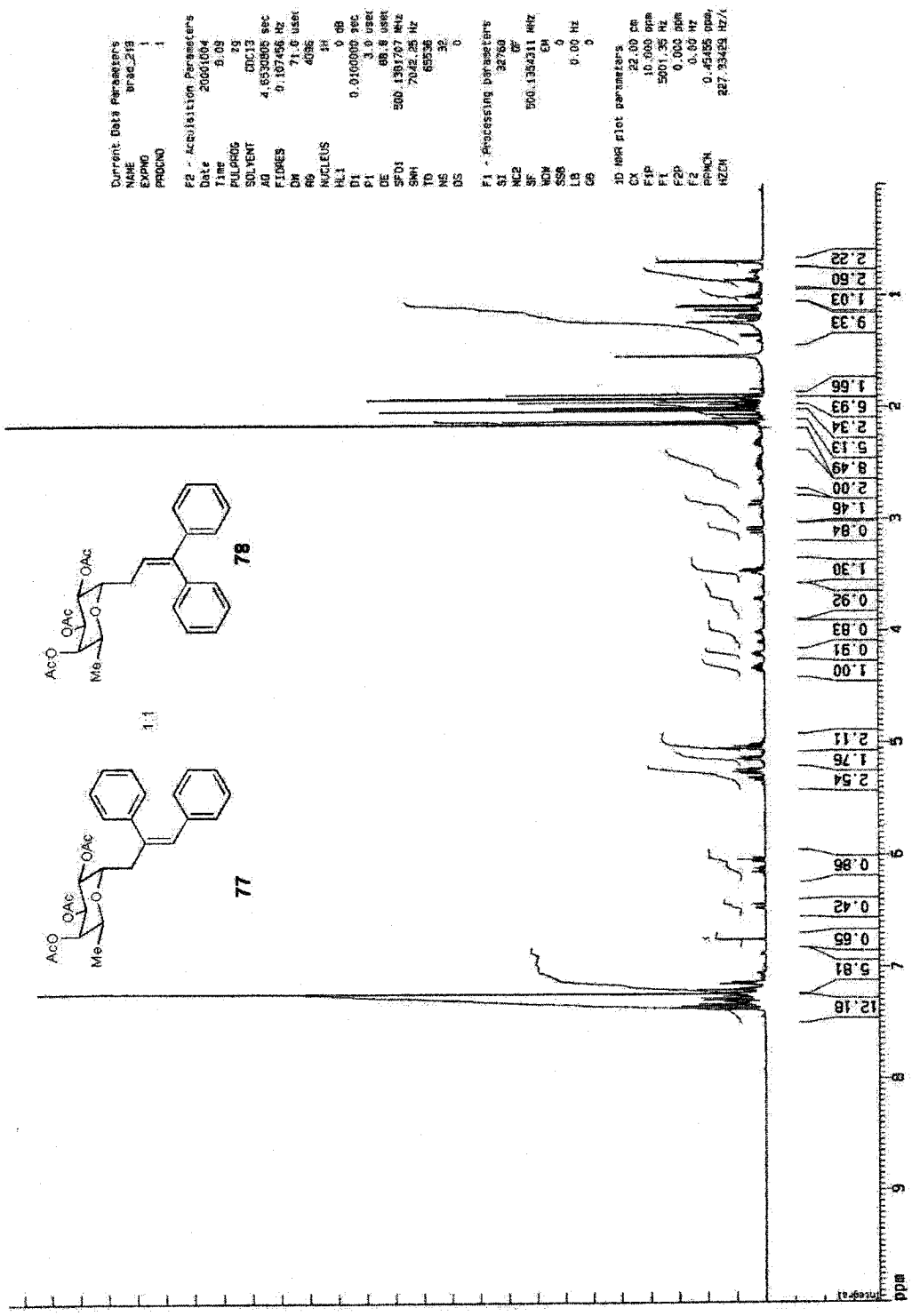


Figure **3.8**: ^{1}H NMR Spectrum ($CDCl_3$, 500 MHz) of **77** and **78.**

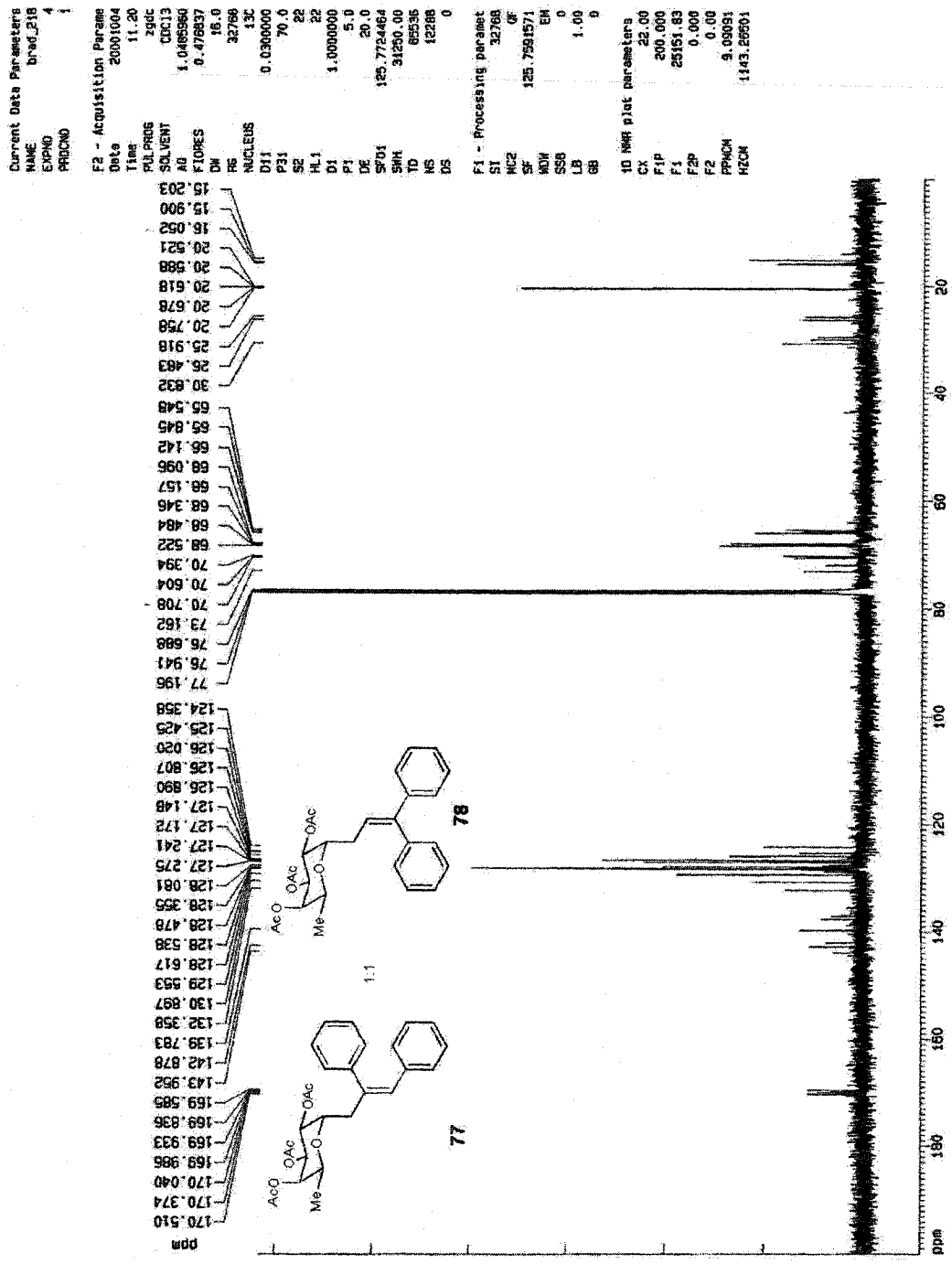


Figure **3.9**: ^{13}C NMR Spectrum ($CDCl_3$, 125 MHz) of **77** and **78**.

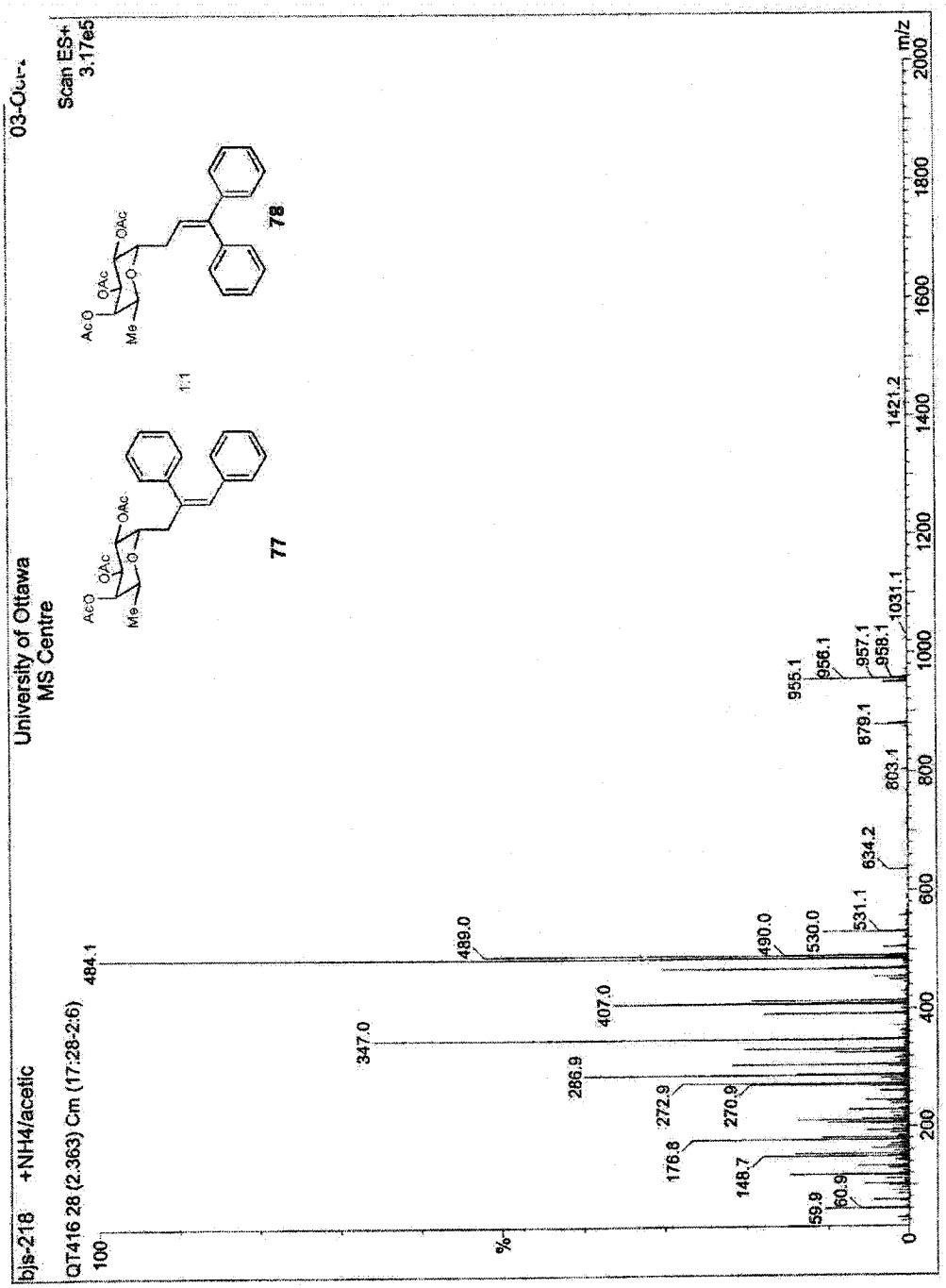
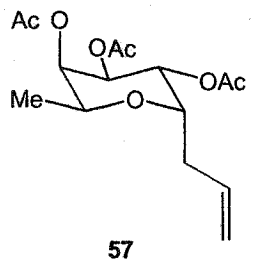


Figure **3.10**: Mass Spectrum (+ESI) of **77** and **78**.

3.6 Preparation of Compounds

3.6.1 Heck Coupling Sequence

3-(Tri-*O*-acetyl-L-fucopyranosyl)-1-propene (57)



57

Peracetylated (L)-fucose **1** (4.03 g, 12.1 mmol) was dissolved in 40 mL dry acetonitrile. To it was added allyltrimethylsilane (5.8 mL, 36.3 mmol, 3 eq.) and boron trifluoride diethyletherate (6.9 mL, 54.5 mmol, 4.5 eq.). The mixture was stirred at room temperature for 15 days (Note 1). The deep yellow reaction mixture contained only product, as judged by TLC (1:1 hexanes/ethyl acetate). The mixture was concentrated to a crude sludge, and partitioned between water and ether (100 mL each). The water was extracted 2 x with ether (40 mL). The extracts were combined, dried over sodium sulfate, filtered and evaporated to give a sticky crude mass (4.34 g). This was purified by filtering through a silica plug (1:1 hexanes/ethyl acetate) and concentrated to afford a thick oil, 3.64 g (98 %). ^{1}H NMR analysis shows this material to be a 20:1 mixture of α to β anomers.

3.60 g was dissolved in 20 mL dichloromethane and 10 mL methanol. Sodium methoxide (1 M in methanol) was added dropwise until the mixture tested for pH 12

(Zemplen conditions). The mixture was stirred for one hour. TLC (1:1 $CHCl_3$:MeOH) indicated total conversion. Amberlite IR-120 H^+ resin was added until the mixture tested for pH 3. The mixture was filtered and evaporated to give a residue that was crystallized from ethyl acetate, giving colorless needles (1.30 g, 60 %).

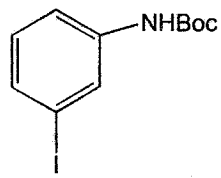
1.25 g of these needles were dissolved in pyridine (3 mL) with dimethylaminopyridine (5 mg) added. Acetic anhydride (5 mL, 109 mmol, 9 eq.) was added, and the mixture stirred overnight. The mixture was then poured into methanol (30 mL) and concentrated to give a thick oil. The residue was brought up in 100 mL satd $NaHCO_3$, and extracted with dichloromethane (3 x 50 mL). The extracts were washed with 2N HCl (3 x 100 mL), satd $NaHCO_3$ (50 mL), and 1 M HCl (50 mL). The organic phase was dried over sodium sulfate, filtered and evaporated to afford **57** as a clear, colorless oil (2.09 g, 100 %). ^1H and ^{13}C NMR appeared identical to that produced by published methods,[80] showed no traces of β-anomer.

^1H NMR (300 MHz, $CDCl_3$)) δ (ppm): 5.76 (1H, m, H-2'), 5.22 (1H, dd, J = 1.5 Hz, J = 9.9 Hz, H-2), 5.18 (1H, dd, J = 1.9 Hz, J = 3.4 Hz, H-4), 5.13 (1H, dd, J = 3.3 Hz, J = 10.0 Hz, H-3), 5.03-4.99 (2H, m, H-3'), 4.19 (1H, m, H-1), 3.89 (1H, dq, J = 3.6 Hz, J = 6.4 Hz, H-5), 2.51 (1H, m, H-1'), 2.20 (1H, m, H-1'), 2.06, 1.96, 1.92 (3 x 3H, 3 s, O_2CCH_3), 1.05 (3H, d, J = 6.4 Hz, H-6).

^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 170.3, 169.9, 169.7, 133.7, , 117.1, 72.5, 70.8, 70.5, 69.3, 65.5, 30.5, 20.7, 20.6, 20.5, 15.7.

Note 1: The time period of 15 days is important; reactions of shorter times (<12 days) give yields in the 65-80 % range, with β-anomer quantities up to 20 %.[80]

_N_-Boc-3-iodoaniline (59)[109]



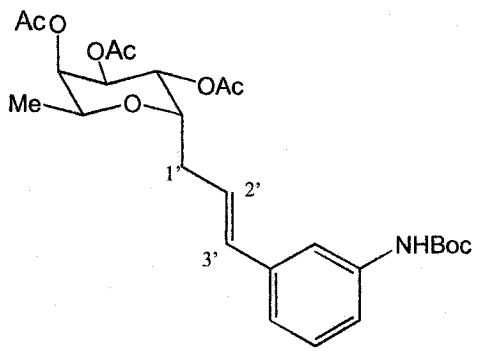
59

3-Iodoaniline **58** (1.08 g, 4.93 mmol) was dissolved in 3 mL of a 2:1 THF/1M NaOH in water mixture. To it was added di-*tert*-butylpyrocarbonate (Boc-anhydride, 1.18 g, 5.42 mmol, 1.1 eq.) and the mixture was stirred for one hour. TLC (1:1 hexanes/ethyl acetate) indicated that the reaction was not complete. Dioxane (1 mL) and water (1 mL) were added, and the mixture became homogenous. Another 0.40 g (1.48 mmol, 0.3 eq.) of di-*tert*-butylpyrocarbonate was added, and the mixture stirred overnight. TLC now indicated the reaction to be complete. The mixture was poured into a mixture of water (80 mL) and dichloromethane (50 mL), titrated to pH 3 with conc. HCl, separated, and the aqueous extracted with dichloromethane (2 x 50 mL). The organics were combined, dried over sodium sulfate, filtered and concentrated to give a thick oil, 1.99 g, which contained some di-*tert*-butylpyrocarbonate as determined by

^{1}H NMR. Column chromatography (2:1 hexanes/ethyl acetate) afforded a pale brown oil (1.47 g, 94 %) which was pure by ^{1}H NMR.

^{1}H NMR (200 MHz, $CDCl_3$) δ (ppm): 7.81 (1H, bs, Ar-H), 7.32 (1H, m, Ar-H), 7.25 (1H, m, Ar-H), 6.95 (1H, t, J = 8.0 Hz, Ar-H), 6.41 (1H, bs, N-H), 1.50 (9H, s, *t*-Bu).

1-*trans*-(*N*-Boc)-3-anilinyl-3-(tri-*O*-acetyl-L-fucopyranosyl)-1-propene (60)



60

Alkene **57** (336 mg, 1.07 mmol), iodo compound **59** (510 mg, 1.60 mmol), palladium acetate (24 mg, 0.11 mmol, 0.1 eq.), triphenylphosphine (56 mg, 0.22 mmol, 0.2 eq.) and triethylamine (5 mL) were mixed and heated at reflux for 24 h. A mirror of palladium metal had precipitated in the flask. The material was evaporated down, and chromatographed on silica gel (4:1 hexanes/ethyl acetate, to 2:1) to afford **60** as a yellow-brown solid, 217 mg (40 %). The ^{1}H NMR can be found in Figure **3.5**.

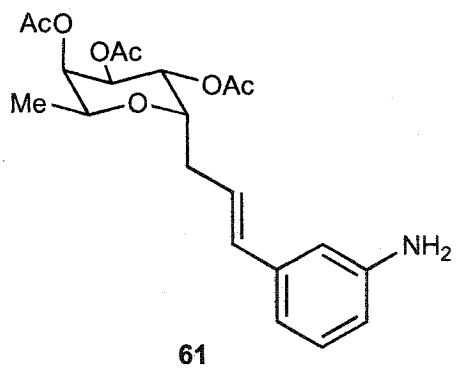
m.p. 114 °C (dec.)

M.S. (+ESI) : (Calc. for $C_{26}H_{35}NO_9$ = 505, Found *m/z* 506 ($C_{26}H_{35}NO_9 + H^+$)

^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 7.37 (1H, s, Ar-H), 7.17 (2H, m, Ar-H), 7.00 (1H, m, Ar-H), 6.44 (1H, s, N-H), 6.41 (1H, d, J = 15.9 Hz, H-3′), 6.13 (1H, m, H-2′), 5.31 (1H, dd, J = 5.5 Hz, J = 9.8 Hz, H-2), 5.27 (1H, dd, J = 2.0 Hz, J = 5.4 Hz, H-4), 5.23 (1H, dd, J = 3.4 Hz, J = 9.8 Hz, H-3), 4.32 (1H, m, H-1), 4.00 (1H, dq, J = 1.9 Hz, J = 6.5 Hz, H-5), 2.61 (1H, m, H-1′), 2.41 (1H, m, H-1′), 2.13, 2.02, 2.00 (3 x 3H, 3 s, O_2CCH_3), 1.50 (9H, s, *t*-Bu), 1.12 (3H, d, J = 6.4 Hz, H-6).

^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 170.5, 170.1, 169.9 (C=O ester), 152.6 (C=O carbamate), 138.6, 138.1, 129.0, 120.8, 117.4, 116.0, (Ar-C), 132.1 (C-3′), 125.9 (C-2′), 80.5 ($\underline{C}(CH_3)_3$) , 72.0 (C-1), 70.6 (C-4), 68.5 (C-2), 68.3 (C-3), 65.9 (C-5), 30.1 (C-2′), 28.3 ($C(\underline{C}H_3)_3$), 20.8, 20.7, 20.6 ($O_2C\underline{C}H_3$), 15.9 (C-6).

1-*trans*-3-Anilinyl-3-(tri-*O*-acetyl-L-fucopyranosyl)-1-propene (61)



61

Compound **60** (19.0 mg, 37.5 μmol) was dissolved in 2 mL trifluoroacetic acid with 10 drops dichloromethane. The mixture was stirred for 4 hours, then evaporated. The product was dissolved in dichloromethane/water (10 mL each), the aqueous phase made basic with NaOH (2 N), and extracted with dichloromethane (2 x 10 mL).

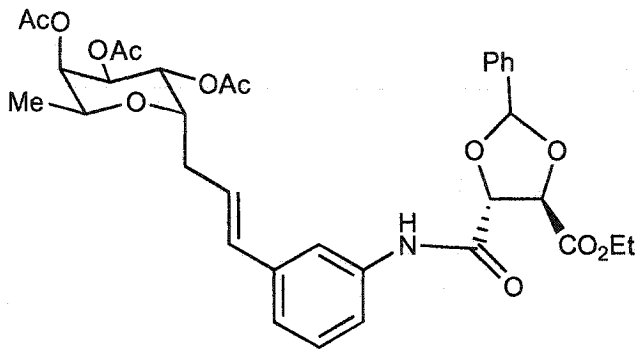
The combined dichloromethane phases were dried over sodium sulfate, filtered and evaporated to afford a yellow solid, 15.2 mg (100 %), pure by TLC (2:1 hexanes/ethyl acetate).

m.p. 64-67 °C.

M.S. (+ESI) : (Calc. for $C_{21}H_{27}NO_7$= 405, Found *m/z* 406 ($C_{21}H_{27}NO_7 + H^+$)

^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 7.07 (1H, t, *J* = 1.6 Hz, Ar-H), 6.75 (1H, m, Ar-H), 6.66 (1H, m, Ar-H), 6.38 (1H, d, *J* = 15.8 Hz, H-3′), 6.07 (1H, m, H-2′), 5.37-5.21 (3H, m, H-2, H-3, H-4), 4.36 (1H, m, H-1), 4.02 (1H, dq, *J* = 1.9 Hz, *J* = 6.4 Hz, H-5), 2.64 (1H, m, H-1′), 2.45 (1H, m, H-1′), 2.13, 2.01, 2.00 (3 x 3H, 3 s, O_2CCH_3), 1.23 (2H, bs, NH_2), 1.12 (3H, d, *J* = 6.5 Hz, H-6).

^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 170.5, 170.1, 170.0 (C=O), 146.5, 138.3, 129.4, 115.1, 114.4, 112.5 (Ar-C), 132.5 (C-3′), 125.2 (C-2′), 70.6 (C-1), 68.5, 68.3, 68.0 (C-2, C-3, C-4), 65.8 (C-5), 30.1 (C-1′), 20.7, 20.7, 20.6 ($O_2C\underline{C}H_3$), 15.9 (C-6).



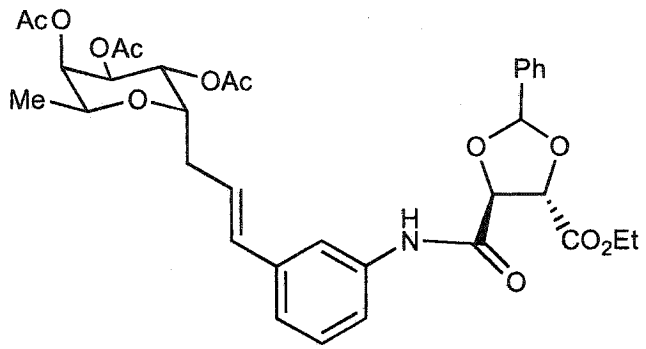
62

Acid **34** (59.1 mg, 0.222 mmol) was dissolved in DMF (2 mL) with amine **60** (100 mg, 1.05 eq.), HATU (0.312 mmol, 120 mg, 1.40 eq.) and DIPEA (0.2 mL). The mixture was stirred for 24 hours, then poured into ether (30 mL) and water (30 mL). The water layer was extracted with ether (3 x 30 mL), and the organic extracts were combined, dried over sodium sulfate, filtered and concentrated to afford a sticky brown solid. The residue was purified on silica gel (2:1 hexanes/ethyl acetate) gave **62** as a white solid (69.7 mg, 48 %). The ^{1}H NMR (Figure **3.3**) indicates the presence of the two diastereomers in a 1.7:1 ratio. Spectral overlap was such that the spectra could not be interpreted. Although not successful, an example of attempted deprotection is given below.

M.S. (+ESI) : Calc. for $C_{34}H_{39}NO_{12}$= 653, Found *m/z* 654 ($C_{34}H_{39}NO_{12} + H^+$)

^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 8.44-8.17 (m, 1H, N-H), 7.61-7.00 (m, 9H, Ar-H), 6.40-6.01 (m, 3H, alkene-C-H, benzylidine-H), 5.33-4.71 (m, 5H, H-2, H-3, H-4, 2 x tart-H), 4.32-3.93 (m, 4H, H-1, H-5, $O_2CC\underline{H}_2CH_3$), 2.61 (m, 1H, H-1'), 2.41 (m,

1H, H-1'), 2.05 (s, 3H, OAc), 2.00 (s, 3H, OAc), 1.95 (s, 3H, OAc), 1.22 (m, 3H, $O_2CCH_2C\underline{H}_3$), 1.04 (d, 3H, J = 6.4 Hz, H-6).



63

Acid **36** (41.4 mg, 0.155 mmol) was dissolved in DMF (2 mL) with amine **60** (70 mg, 0.163 mmol, 1.05 eq.), HATU (85 mg, 0.221 mmol, 1.43 eq.) and DIPEA (0.2 mL). The mixture was stirred overnight, then poured into ether (30 mL) and water (30 mL). The water layer was extracted with ether (3 x 20 mL), and the organic extracts were combined, dried over sodium sulfate, filtered and evaporated to afford a brown solid. The residue was purified on silica gel (2:1 hexanes/ethyl acetate) to afford **63** as a white solid (47.9 mg, 44 %). The ^{1}H NMR indicates the presence of the two diastereomers. Spectral overlap was such that the spectra could not be fully interpreted. Although not successful, an example of attempted deprotection is given below.

M.S. (+ESI) : Calc. for $C_{34}H_{39}NO_{12}$= 653, Found *m/z* 654 ($C_{34}H_{39}NO_{12} + H^+$)

^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 8.36-8.12 (m, 1H, N-H), 7.63-7.03 (m, 9H, Ar-H), 6.31-6.02 (m, 3H, alkene-C-H, benzylidine-H), 5.21-4.67 (m, 5H, H-2, H-3, H-4,

2 x tart-H), 4.45-3.99 (m, 4H, H-1, H-5, $O_2CC\underline{H}_2CH_3$), 2.55 (m, 1H, H-1'), 2.44 (m, 1H, H-1'), 2.02 (s, 3H, OAc), 2.00 (s, 3H, OAc), 1.93 (s, 3H, OAc), 1.17 (m, 3H, $O_2CCH_2C\underline{H}_3$), 1.09 (d, 3H, J = 6.5 Hz, H-6).

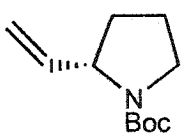
Attempted Deprotection of **62**.

Compound **62** (40 mg, 61 μmol) was dissolved in ethanol (1 mL) and 2N sodium hydroxide was added (100 μL). The mixture was stirred for 90 minutes; TLC (1:1 hexanes/ethyl acetate) indicated the reaction was complete. The mixture was brought to acidic pH with Amberlite IR-120 H^+ resin, filtered and evaporated to give a colorless oil. Methanol was added (1 mL) and Amberlite IR-120 H^+ resin was added until pH 1. The mixture was heated at 60 °C for 2 hours. TLC (1:1 $CHCl_3$/MeOH) indicated no starting material, but a complex mixture had formed. Although ESI-MS showed a good molecular ion peak, the material could not be successfully isolated.

M.S. (+ESI) : Calc. for $C_{19}H_{25}NO_9$= 411, Found *m/z* 434 ($C_{34}H_{39}NO_9 + Na^+$)

3.6.2 Olefin Cross-Metathesis

***(R)*-2-Vinyl-*N*-Boc-pyrollidine (70)**[119-124]



70

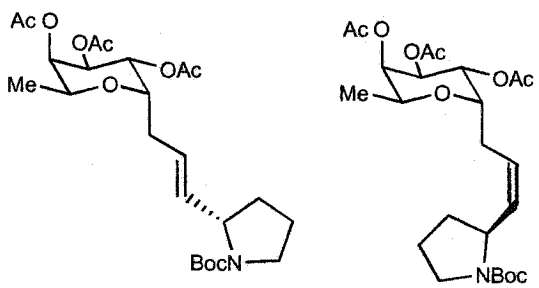
(L)-*N*-Boc-proline methyl ester **68** (9.39 g, 41.0 mmol) was dissolved in dry dichloromethane (40 mL) and the vessel was flushed with nitrogen. The solution was cooled to –78 °C on a dry ice-acetone bath. Diisobutylaluminum hydride (40.5 mL, 1.0 M in hexanes, 40.5 mmol, 0.99 eq.) was transferred via cannula over 10 minutes. The mixture was stirred for 1 h, and satd NH_4Cl solution (20 mL) was cautiously added. The mixture was warmed to room temperature and extracted with dichloromethane (4 x 50 mL). The organics were combined, dried over sodium sulfate, filtered and concentrated to give **69** as a colorless oil, 4.34 g (97 %). TLC (1:1 hexanes/ethyl acetate) shows a small amount of what is likely the alcohol. This material was immediately carried on to the next step.

50 mL of THF was charged with triphenylphosphonium bromide (16.09 g, 45 mmol, 1.10 eq.), and cooled to 0 °C on an ice bath. Butyl lithium (21.5 mL of a 2.0 M solution in cyclohexane, 43 mmol, 1.05 eq.) was added dropwise over 1.5 hours. A deep orange-red solution formed, which was stirred for 1.5 hours. Then, the solution was transferred via cannula to a stirring solution of **69** (40.5 mmol) in THF (30 mL) at 0 °C over 15 min. The mixture was held at 0 °C for 30 minutes, then allowed to warm to room temperature. Water (40 mL) was cautiously added, and the THF removed by evaporation. The residue was brought up to 100 mL with water, and extracted with ether (3 x 100 mL). The ether extracts were dried over sodium sulfate, filtered and concentrated to give a brown oil. This material was purified by column chromatography (1:1 pentane/ether) to afford **70** 4.00 g (48 %) as a volatile, colorless oil; ^1H NMR data agreed well with published values.[119-124]

^{1}H NMR (200 MHz, $CDCl_3$) δ (ppm): 5.70 (1H, m, H-1′), 4.99 (2H, m, H-2′), 4.20 (1H, m, H-2), 3.30 (2H, m, H-5), 2.00-1.50 (4H, m, H-3, H-4), 1.35 (9H, s, *t*Bu).

1-*trans*-[*(R)*-2-*N*-Boc-pyrollidinyl]-3-(tri-*O*-acetyl-L-fucopyranosyl)-1-propene (72)

1-*cis*-[*(R)*-2-*N*-Boc-pyrollidinyl]-3-(tri-*O*-acetyl-L-fucopyranosyl)-1-propene (73)

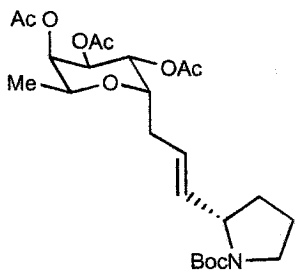


72 **73**

3-(Tri-*O*-acetyl-L-fucopyranosyl)-1-propene **57** (51.4 mg, 164 μmol) along with 64.5 mg (327 μmol, 2.0 eq.) of *N*-butoxycarbonyl-2-(*R*)-vinylpyrrolidine were dissolved in 1.4 ml of dry 1,2-dichloroethane, under a nitrogen atmosphere. To it was added 6.9 mg (8 μmol, 5 mol %) of 2nd generation Grubbs' catalyst. The solution was stirred for 30 minutes at room temperature, and then brought to reflux for 16 hours. The solution was then concentrated, and the residue chromatographed (3:1 hexane/ethyl acetate) to give the *cis*-isomer (11.9 mg, 15% yield) and the *trans*-isomer (39.0 mg, 49% yield) as thick oils with a brown color, which could be removed by a second

chromatography. The samples are very stable, showing no decomposition over many months at room temperature. Product **73** was not sufficiently pure to allow for a good spectral interpretation (contaminating **72**). The ^{1}H NMR of **72** can be found in Figure **3.7**.

1-*trans*-[*(R)*-2-*N*-Boc-pyrollidinyl]-3-(tri-*O*-acetyl-L-fucopyranosyl)-1-propene (72)



72

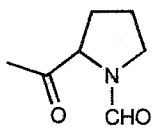
^{1}H: (500 MHz, $CDCl_3$) δ (ppm) : 5.38 (2H, m, C=CH x 2), 5.27 (1H, dd, $J_{1,2}$ = 10.0 Hz, $J_{2,3}$ = 5.6 Hz, H-2), 5.24 (1H, dd, $J_{3,4}$ = 3.4 Hz, $J_{4,5}$ = 2.0 Hz, H-4), 5.17 (1H, dd, $J_{2,3}$ = 10.0 Hz, $J_{3,4}$ = 3.4 Hz, H-3), 4.25 (1H, m, 2-pyrrolidine-H), 4.18 (1H, m, H-1), 3.93 (1H, dq, $J_{5,6}$ = 6.4 Hz, $J_{4,5}$ = 1.8 Hz, H-6), 3.33 (2H, dd, J = 3.7, 7.0, 5-pyrrolidine-H), 2.47 (1H, m, H-1'), 2.17 (1H, m, H-1'), 2.12, 2.03, 1.98 (3 x 3H s, $COCH_3$), 1.95 (1H, m, 3-pyrrolidine-H), 1.80 (2H, m, 4-pyrrolidine-H), 1.64 (1H, m, 3-pyrrolidine-H), 1.41 (9H, s, *t*-butyl-H), 1.10 (3H, d, J = 6.4 Hz, H-6).

^{13}C: (125 MHz) δ (ppm) : 170.5, 170.1, 169.9 (C=OCH_3 x 3), 154.5 (NC=O), 133.6, 124.8 (C=C x 2), 79.0 ($\underline{C}$-$(CH_3)_3$, 72.2 (C-1), 70.7 (C-4), 68.6 (C-2), 68.3 (C-3), 65.6

(C-5), 58.2, (2-pyrrolidine), 46.2 (5-pyrrolidine), 32.0 (3-pyrrolidine), 29.7 (4-pyrrolidine), 28.8 (C-1'), 28.5 (C-($\underline{C}H_3)_3$), 20.8, 20.7, 20.6, ($CO\underline{C}H_3$) 15.9 (C-6).

MS : (+FAB-HRMS) : ($C_{24}H_{38}NO_9^+$) calc'd: 484.2547, found: 484.2559.

_N_-Formyl-2-acetylpyrrolidine (76)



76

A 25 mL roundbottom flask was charged with olefin **70** (501 mg, 2.54 mmol) followed by trifluoroacetic acid (8 mL). The reaction was stirred for 3 hours. TLC (3:1 hexanes/ethyl acetate) indicated disappearance of starting material, but several product spots. The reaction was concentrated and chromatographed (10:1 hexanes/ethyl acetate) on silica gel to afford **76** (232 mg, 65 %) as a colorless oil.

$[\alpha_D]$ (c = 1, $CHCl_3$): 0.0 °

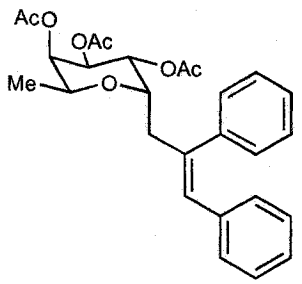
^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 8.30 (1H, s, CHO), 4.36 (1H, dd, J = 2.7 Hz, J = 8.4 Hz, H-2), 2.77 (1H, m, H-4), 2.69 (1H, m, H-4), 2.32-2.19 (4H, m, H-2, H-3), 2.04 (3H, s, CH_3CO).

^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 216.2 (keto-CO), 168.3 (amide-CO), 60.4 (C-2), 46.0 (C-4), 24.8, 24.1 (C-2, C-3), 22.0 ($CO\underline{C}H_3$).

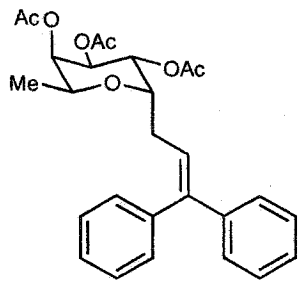
3.6.3 Phenyl Transfer from Antimony

1,2-*cis*-Diphenyl-3-(tri-*O*-acetyl-L-fucopyranosyl)-1-propene (77)

1,1-*bis*-Diphenyl-3-(tri-*O*-acetyl-L-fucopyranosyl)-1-propene (78)



77



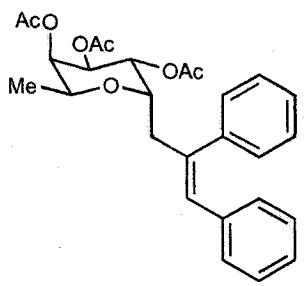
78

Compound **57** (10.5 mg, 33.5 μmol) was treated with triphenylantimony (23.7 mg, 67 mmol, 2 eq.), **59** (16.0 mg, 50.3 mmol, 1.50 eq.), palladium (II) acetate (0.8 mg, 3.5 μmol, 0.11 eq.) and triethylamine (5 mL), and refluxed for 5 hours. The reaction mixture was concentrated under reduced pressure, and the residue chromatographed on silica gel (4:1 hexanes/ethyl acetate) to afford 14.7 mg (94 % based on **57**) of a colorless oily semi-solid. ^1H NMR showed it to be a 1:1 mixture of the 2',3'- and the 3',3'-disubstituted isomers. An excess of **59** was used since this reaction was intended to provide Heck coupling, thus requiring stoichiometric amounts of the aryl halide.

^{1}H and ^{13}C NMR, and ESI-MS can be found in Figures **3.8**, **3.9** and **3.10**, respectively. It must be noted that acetate and phenyl peaks could not be assigned to either isomer.

M.S. (+ESI) : Calc. for $C_{27}H_{30}O_7$= 466, Found *m/z* 467 ($C_{27}H_{30}O_7 + H^+$), 484 $(C_{27}H_{30}O_7 + Na)^+$, 955 $(2\{C_{27}H_{30}O_7\} + Na)^+$

1,2-*cis*-Diphenyl-3-(tri-*O*-acetyl-L-fucopyranosyl)-1-propene (77)



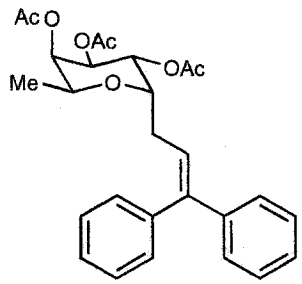
77

^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 7.40-7.00 (10H, m, Ar-H), 6.75 (1H, s, H-3'), 5.26 (1H, dd, $J_{1,2}$ = 8.8 Hz, $J_{2,3}$ = 10.3 Hz, H-2), 5.15 (1H, dd, $J_{3,4}$ = 3.3 Hz, $J_{4,5}$ = 0.8 Hz, H-4), 5.05 (1H, dd, $J_{2,3}$ = 10.3 Hz, $J_{3,4}$ = 3.4 Hz, H-3), 4.21 (1H, m, H-1), 4.02 (1H, dq, $J_{4,5}$ = 0.8 Hz, $J_{5,6}$ = 6.4 Hz, H-5), 3.10 (1H, dd, $J_{1,1'}$ = 10.6 Hz, $^{2}J_{1',1'}$ = 14.7 Hz, H-1'), 2.85 (1H, dd, $J_{1,1'}$ = 3.6 Hz, $^{2}J_{1',1'}$ = 14.8 Hz, H-1'), 2.12, 1.95, 1.91 (9H, 3s, $COCH_3$), 1.13 (3H, d, $J_{5,6}$ = 6.4 Hz)

^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 170.5, 170.0, 169.9 (C=O), 144.0, 142.1, 139.7, 129.6, 128.5, 128.4, 127.3, 127.2, 126.0 (Ar-C), 130.9 (C-3'), 73.2 (C-1), 70.7

(C-2), 70.6 (C-4), 70.4 (C-3), 65.9 (C-5), 26.5 (C-1'), 20.8, 20.6, 20.6 ($COCH_3$), 16.1 (C-6).

1,1-*bis*-Diphenyl-3-(tri-*O*-acetyl-L-fucopyranosyl)-1-propene (78)

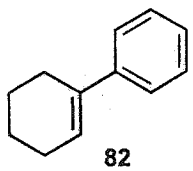


78

^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 7.40-7.00 (10H, m, Ar-H), 6.20 (1H, m, H-2'), 5.26 (1H, dd, $J_{1,2}$ = 8.7 Hz, $J_{2,3}$ = 10.3 Hz, H-2), 5.15 (1H, dd, $J_{3,4}$ = 3.3 Hz, $J_{4,5}$ = 0.8 Hz, H-4), 5.05 (1H, dd, $J_{2,3}$ = 10.3 Hz, $J_{3,4}$ = 3.4 Hz, H-3), 4.33 (1H, m, H-1), 3.63 (1H, dq, $J_{4,5}$ = 0.8 Hz, $J_{5,6}$ = 6.4 Hz, H-5), 2.70-2.40 (2H, m, H-1'), 2.03, 1.92, 1.88 (9H, 3s, $COCH_3$), 1.09 (3H, d, $J_{5,6}$ = 6.4 Hz).

^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 170.4, 170.0, 169.6 (C=O), 142.9, 139.8, 137.7, 128.5, 128.4, 128.1, 127.2, 126.9, 125.4 (Ar-C), 124.4 (C-2'), 71.9 (C-1), 68.5 (C-3), 68.4 (C-2), 68.1 (C-4), 65.6 (C-5), 25.9 (C-1'), 20.7, 20.6, 20.5 ($CO\underline{C}H_3$), 15.2 (C-6).

1-Phenylcyclohexene (82)



82

A screw-cap test tube was charged with cyclohexene (0.50 mL), diisoproylethylamine (0.5 mL), triphenylantimony (63.3 mg, 179 mmol, 537 mmol phenyl groups), palladium acetate (10.1 mg, 45 mmol) and **59** (17.1 mg, 53.5 mmol). The test tube was sealed and heated at 70 °C for ten hours. The resulting black suspension was carefully evaporated under reduced pressure, and the residue brought up in pentane (20 mL). The pentane was washed with 6N HCl (2 x 20 mL), dried over sodium sulfate, filtered and evaporated to afford a yellow oil. The oil was then chromatographed using pentane as eluent to give **82** (83.0 mg, 97 % based on triphenylantimony) as a clear colorless oil. The ^{1}H NMR and mass spectrum matched those in the literature for **82**.[132]

4. Glycosyl Triazoles as Glycosyl Proline Analogues

4.1 Introduction

In order to expand the scope of *N*-linked fucose-based mimetics, the synthesis of fucosyltriazoles as fucosyl proline analogues was undertaken. 1,2,3-triazoles can be formed by [3+2] dipolar cycloaddition reactions of azides and alkynes. Both possible regioisomers are normally formed,[133] as shown in Figure **4.1**. Sharpless[134] has recently shown that 1,5-triazoles can be exclusively formed by the use of magnesiated alkynes.

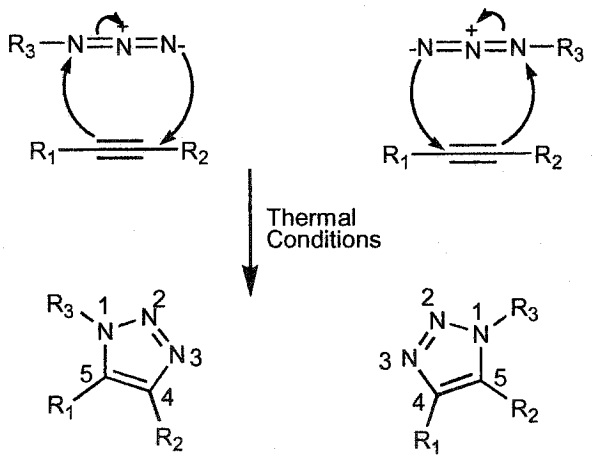


Figure **4.1**: Formation of 1,2,3-Triazoles via 1,3-Dipolar Addition

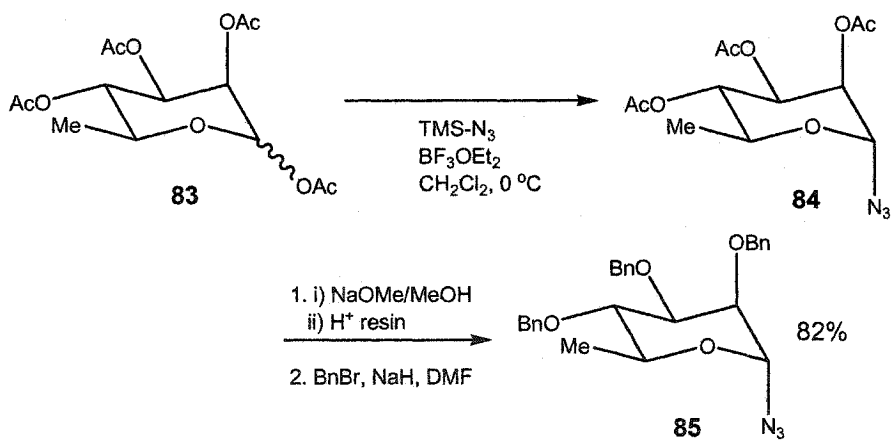
Aromatic heterocycles are commonplace in pharmaceuticals. They are not normally substrates for enzymatic degradation, easy to form, and lack chirality. Hence, substituted glycosyltriazoles would be potential mimics of a glycosyl proline system.

There has been some work done in the area of glucosyl and ribosyl triazoles,[135] but it was felt that not much was known about this potentially useful isostere.

4.2 Formation of Glycosyl Azides

As mentioned in section **1.3.3**, glycosyl azides can be formed by a number of ways. Since anomeric configuration should be preserved, it is necessary to have control of configuration at C-1, or at least be able to separate anomeric mixtures.

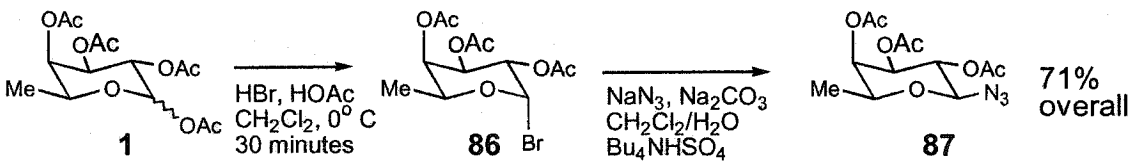
Fucosyl azides **4** and **5** were available from previous work. The α-azide of (L)-rhamnose was synthesized by rather standard methods, as shown in Scheme **4.1**



Scheme **4.1**: Formation of α-Rhamnosyl Azide **85**.

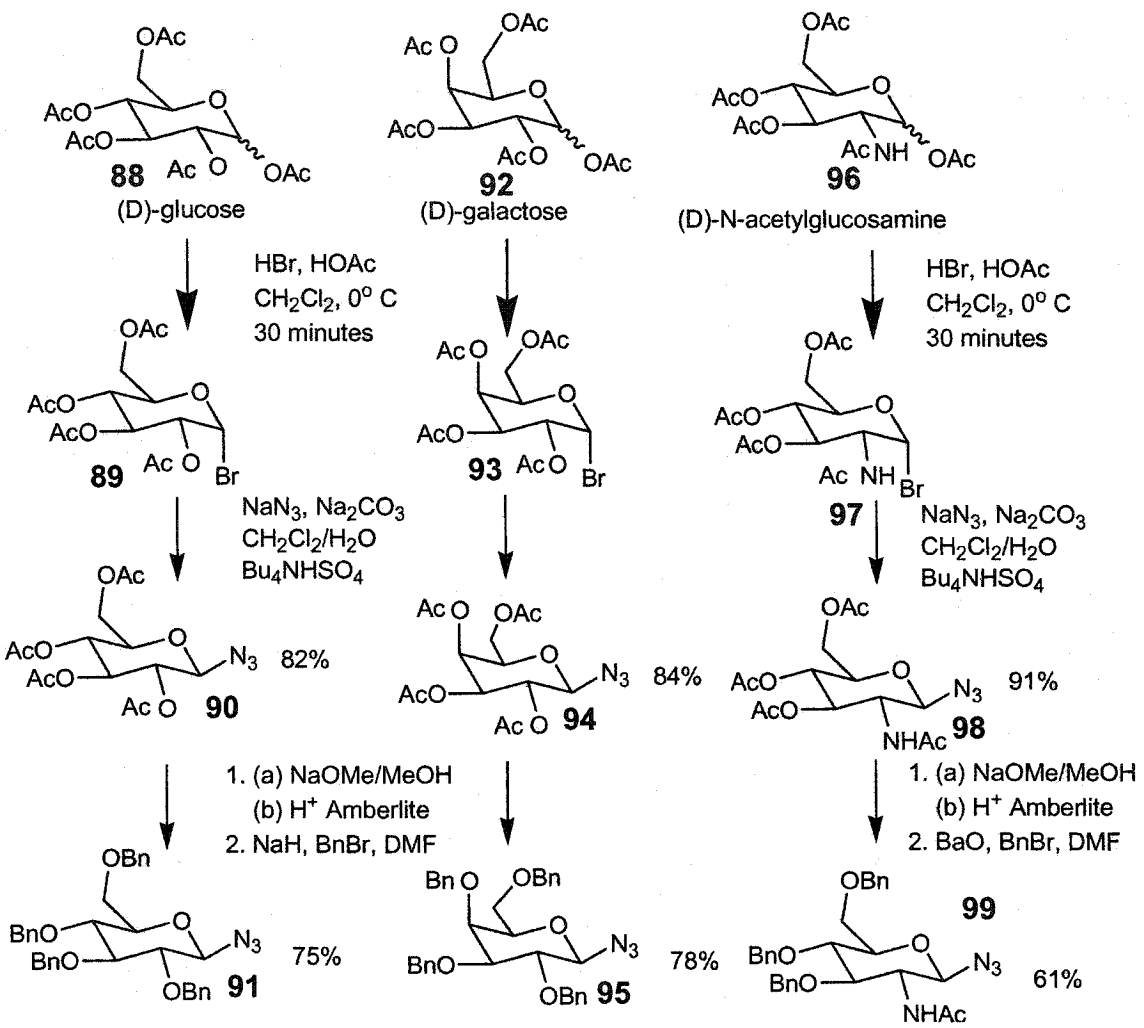
α-Azide **85** was the only azide product isolated from the reaction of peracetylated sugar **83.** Removal of the acetate protecting groups under Zemplen conditions followed by benzylation afforded protected sugar **85.**

β-Azidosugars required a different strategy. α-Azidofucose **87**, protected as the triacetate, was formed via phase-transfer catalysis conditions according to literature procedures[139] (Scheme **4.2**).



Scheme **4.2**: Formation of Peracetylated β-Fucosylazide **87**

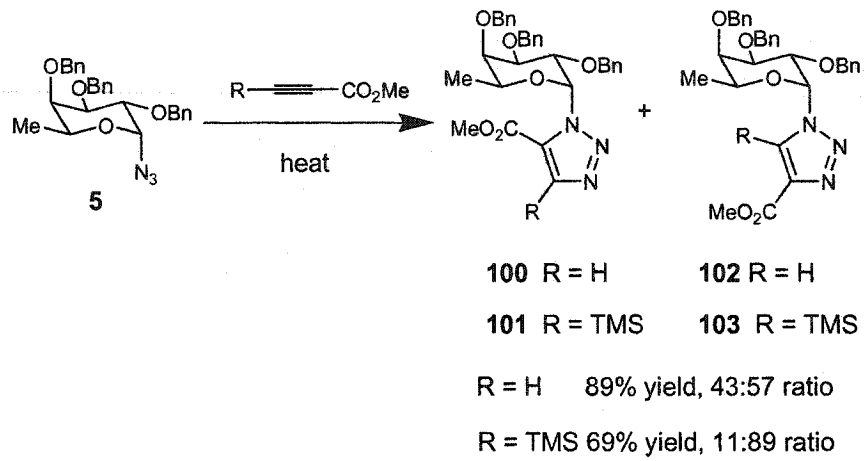
Azides **91**, **95**, and **99** were formed through a similar strategy,[139] but had their protecting groups removed under Zemplen conditions and replaced with benzyl groups.[140-142] In the case of *N*-acetylglucosamine **99**, barium oxide served as base for benzylation without problems (Scheme **4.3**).



Scheme **4.3**: Formation of Glycosyl Azides via Phase-Transfer Catalysis[139-142]

4.3 Formation of Glycosyl Triazoles via 1,3 Dipolar Cycloaddition

An initial comparison for regioselectivity was made with the reaction of azide **5** and methyl propiolate, as well as the trimethylsilyl-derived methyl propiolate (Scheme **4.4**).



Scheme **4.4**: Formation of Fucosyl-1,2,3-Triazoles via Dipolar Cycloaddition

The observed regioselectivity can be explained using a frontier molecular orbital (FMO) approach (Figure **4.2**).

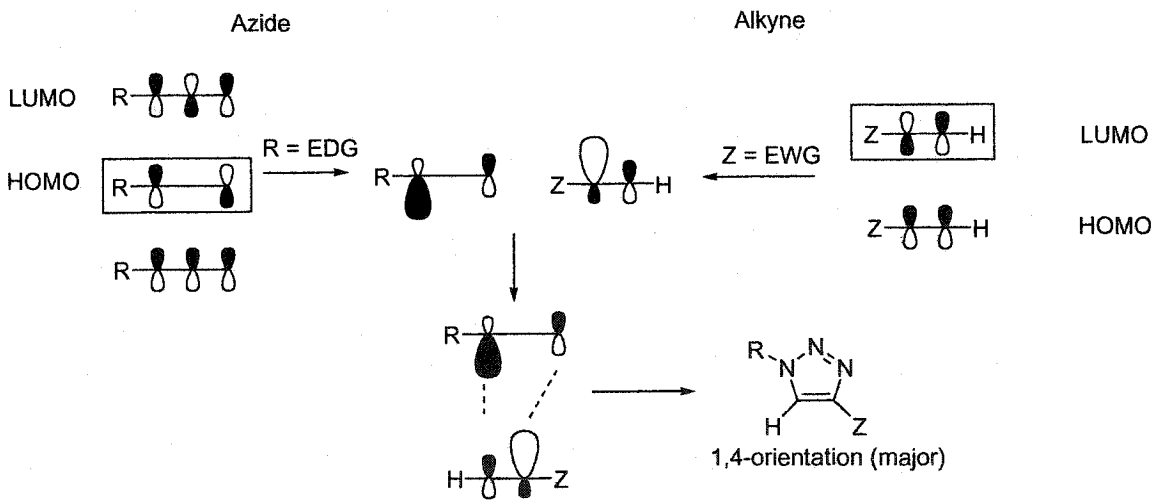
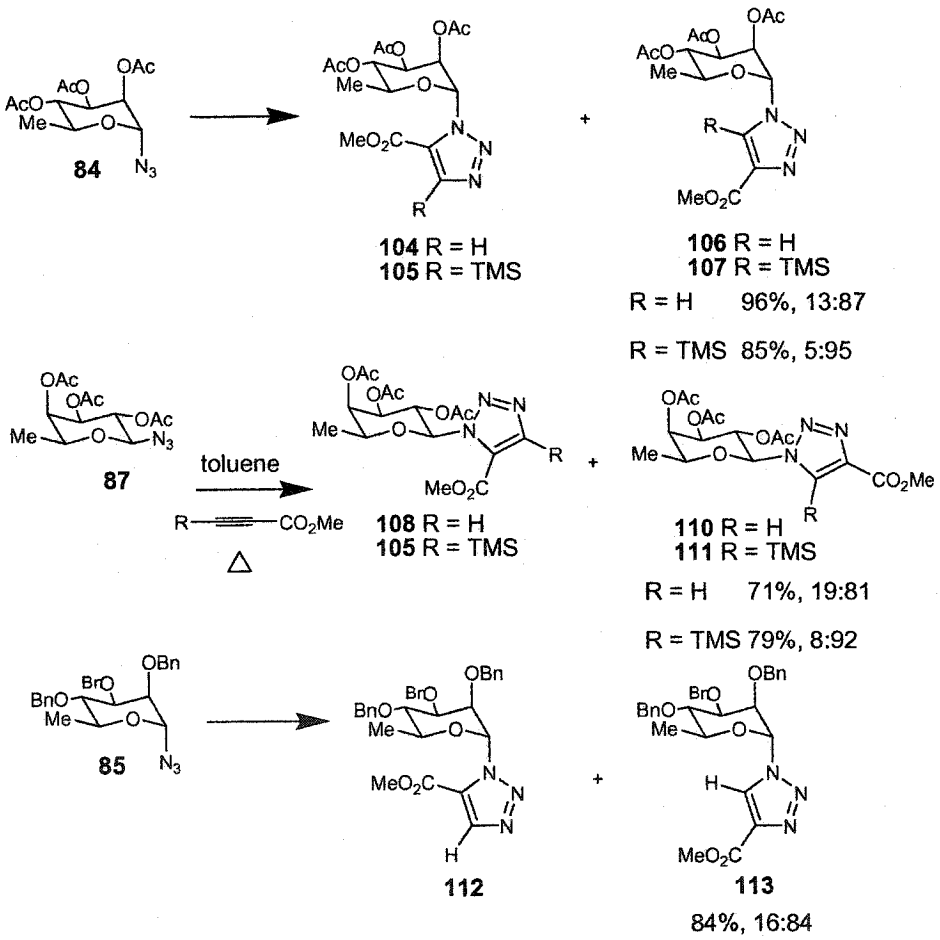


Figure **4.2**: Triazole Regioselectivity is Dominated by Frontier Molecular Orbital Interactions.

The electron-donating or withdrawing properties of the substituents on the reactive components dictate the electron density at each atom; the larger differences in electron density will come together to react. In our case, R (the sugar) is weakly electron donating, and Z (the ester) is electron-withdrawing. Replacement of the alkyne-H with the electron-donating TMS group increases the already present imbalance, thus leading to a greater degree of 1,4-regioselectivity.



Scheme **4.5**: Dipolar Addition of Glycosyl Azides with Methyl Propiolate

Extending this strategy to other azides gave similar results (Scheme **4.5**).

In many cases (especially **104** and **106**) selectivity was already rather good, and the TMS group served only to improve the ratio.

Inseparable mixtures were often observed; given that the 1,4-isomers predominate in nearly all cases in the literature[135-137] it was presumed that major products were likely 1,4-isomers. This was proven in one case, as described below, and it seems very likely in all others based on ^{1}H NMR chemical shift data (see below).

In most cases, unusual ^{1}H NMR data were obtained, particularly for 1,5-isomers. Dramatic deviations in chemical shift were observed at times. Figures **4.7** and **4.8** show the ^{1}H NMR spectra for **104** and **106**; the H-1-δ value for the 1,5-isomer was an incredible 6.8 ppm, and a second sugar proton was over 6 ppm. By contrast, the "normal" shifts were observed for the 1,4-isomer, with a H-1-δ value of 6 ppm. X-ray crystallographic data were obtained for **106** (Figure **4.2**) to confirm the structure.

In addition, significant deviations in coupling constants around the sugar ring were observed for the 1,5-isomers; typically 0.5-1.0 Hz, with respect to typical values (for other fucose compounds). The Karplus relationship[138] describes how the observed coupling constant (*J* value) relates to the dihedral angle between four atoms. Deviations in *J* value mean a change in the geometry of the molecule. This, in addition to the unusual chemical shifts observed, suggests the molecule may adopt a distinctly atypical conformation to relieve strain caused by the 1,5-triazolyl-substructure. This was not investigated further.

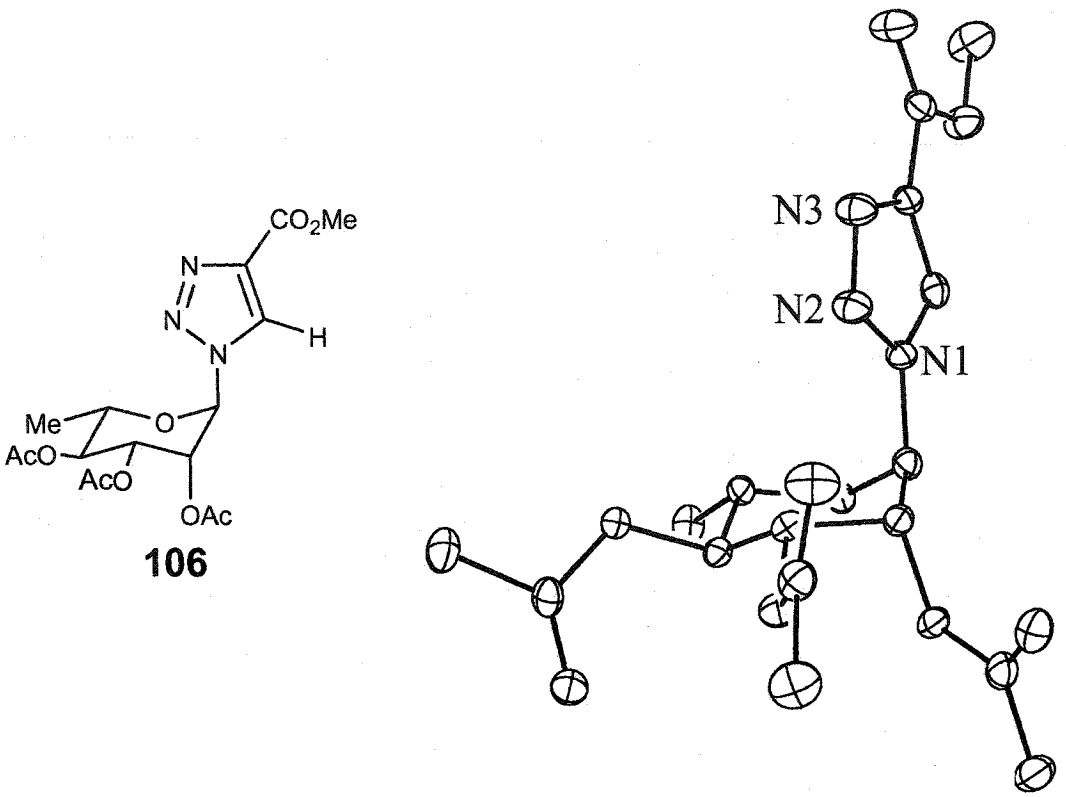


Figure **4.3**: ORTEP Representation of **106** (Right); Structural Representation at Left.

4.4 Copper (I) Catalysis Leads to High Regioselectivity

The problem of regioselectivity can be large, especially in certain cases (such as **100** and **102**) where one isomer is not particularly dominant. Recently however, Meldal[136] and Sharpless[137] have investigated the synthesis of 1,2,3-triazoles via dipolar additions catalyzed by copper (I) (Figure **4.4**). The proposed mechanism is shown in Figure **4.5**.

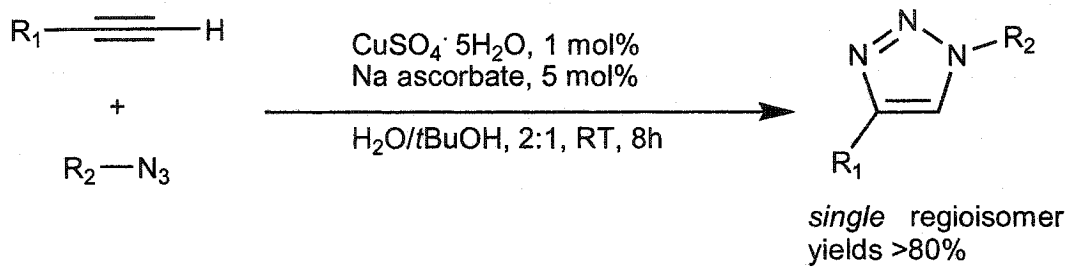


Figure **4.4:** Copper (I) Mediated 1,2,3-Triazole Synthesis

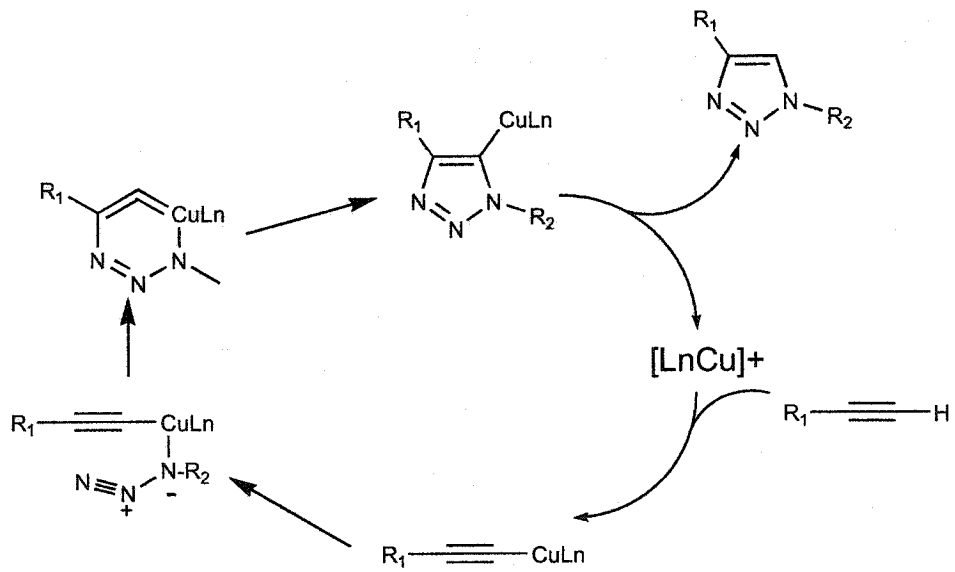
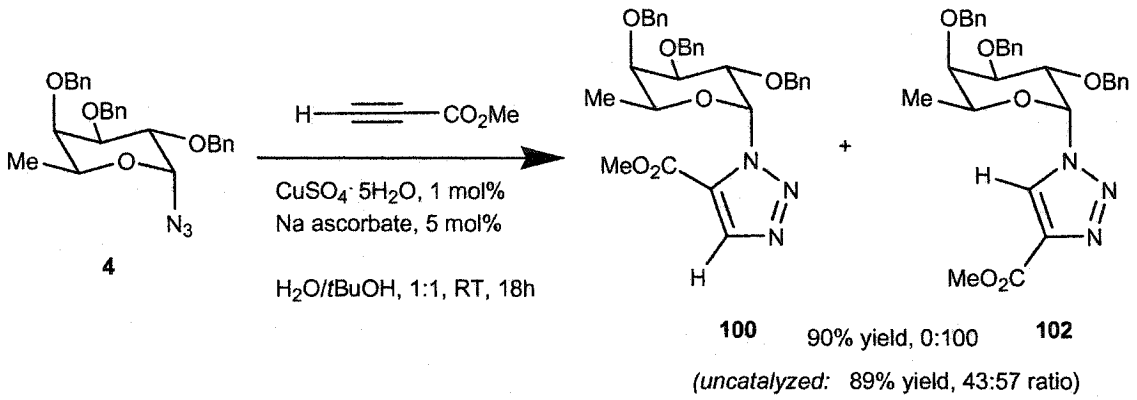


Figure **4.5**: Sharpless' Proposed Catalytic Cycle of Cu(I)-Mediated Cycloaddition[136]

The mechanism proposed is not concerted, but rather step-wise. It begins with the C-H insertion of copper (I) into the acetylenic C-H bond, forming a copper acetylide. This then attacks N^1 of the azido group, which then cyclizes to form a copper carbenoid. Extrusion of copper from the heterocycle, followed by C-Cu bond cleavage, affords one isomer of the 1,2,3-triazole.

An example was attempted (Scheme **4.6**) using **4**, a case which gave particularly poor results with respect to regioselectivity in the uncatalyzed case. A single isomer was obtained at room temperature.

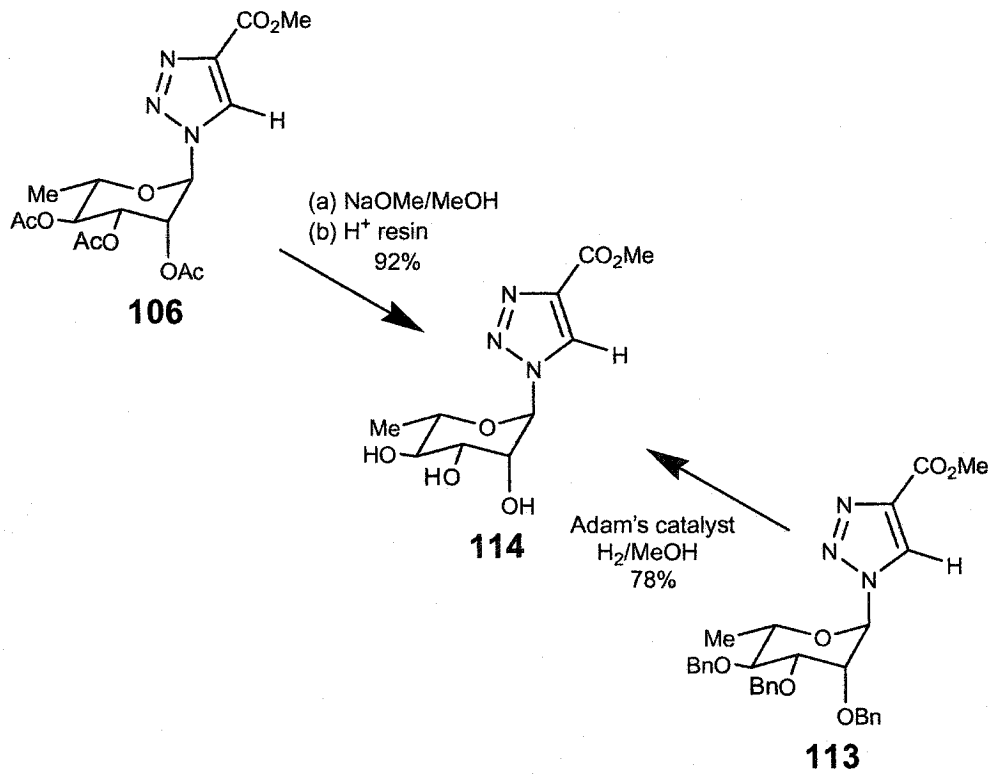


Scheme **4.6**: Copper (I) Mediated Cycloaddition Improves Regioselectivity

4.5 Deprotection Methods

There was some concern that the glycosyltriazole linkage might not be stable to conditions required for deprotection. The linkage is both an aminal linkage, and C-1 of the sugar ring is a "benzylic-type" position, and may not withstand conditions required for debenzylation. Since they would both afford the same compound upon deprotection, **106** and **113** were chosen for examination (Scheme **4.7**).

Triacetylated compound **106** was subjected to Zemplen conditions, and afforded **114** with no necessary purification. Benzylated compound **113** was cleanly hydrogenated using Adam's catalyst (PtO_2) to afford the same compound. Both deprotections proceeded in good to excellent yield. The ^1H NMR of **114** can be seen in Figure **4.9**.



Scheme **4.7**: Deprotection of **106** and **113** Cleanly Afford the Same Product **114**.

4.6 Conclusions

Glycosyl triazoles can be synthesized with good efficiency using standard 1,3-dipolar addition reactions of glycosyl azides and acetylenic compounds. Deprotection can be accomplished without disturbing the triazolyl functionality. Copper (I) greatly facilitates the reaction, enabling reaction at lower temperatures and in aqueous solvents. The structures of glycosyl triazoles were probed to a limited extent, and it was found that the 1,5-isomers typically had unusual chemical shift and coupling constant data, suggesting a distorted conformation. These molecules were not elaborated further, and no biological evalution was performed; however, some good methodology was established for the synthesis, identification, and deprotection chemistry of glycosyl triazoles.

4.7 Spectra Referred to Throughout Chapter 4

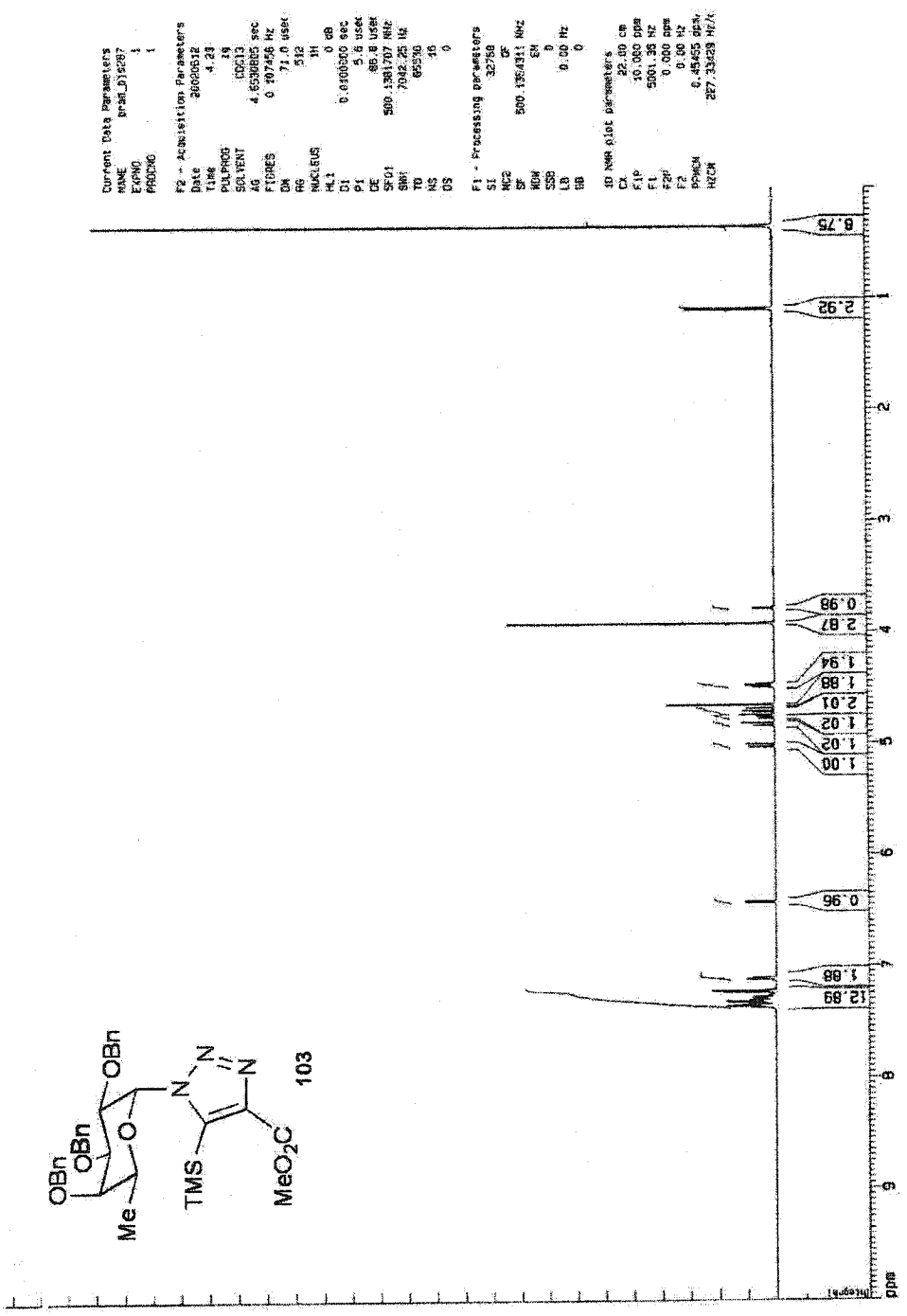


Figure **4.6**: ^{1}H NMR Spectrum ($CDCl_3$, 500 MHz) of **103**.

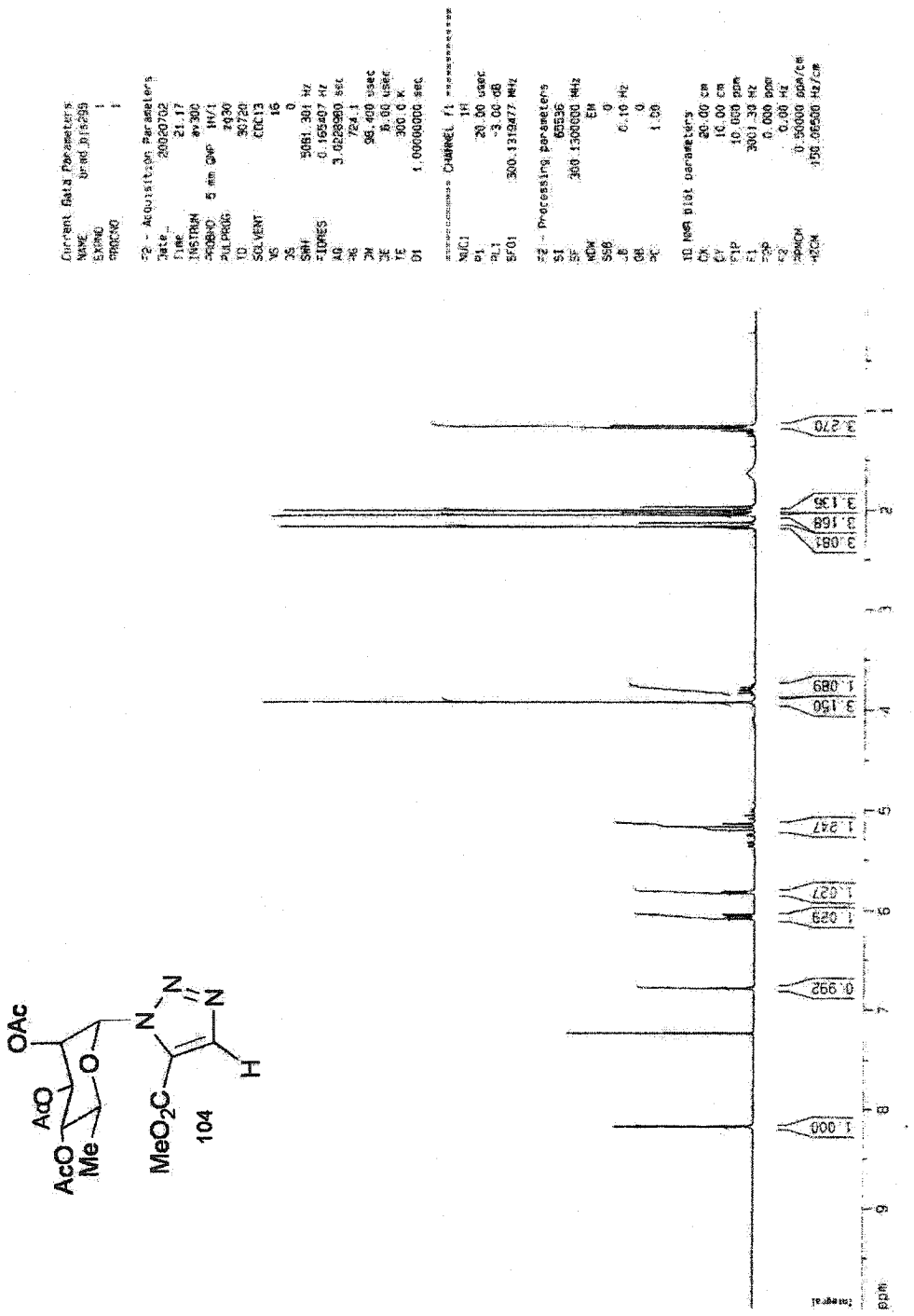


Figure **4.7**: ^{1}H NMR Spectrum ($CDCl_3$, 300 MHz) of Triazole **104**

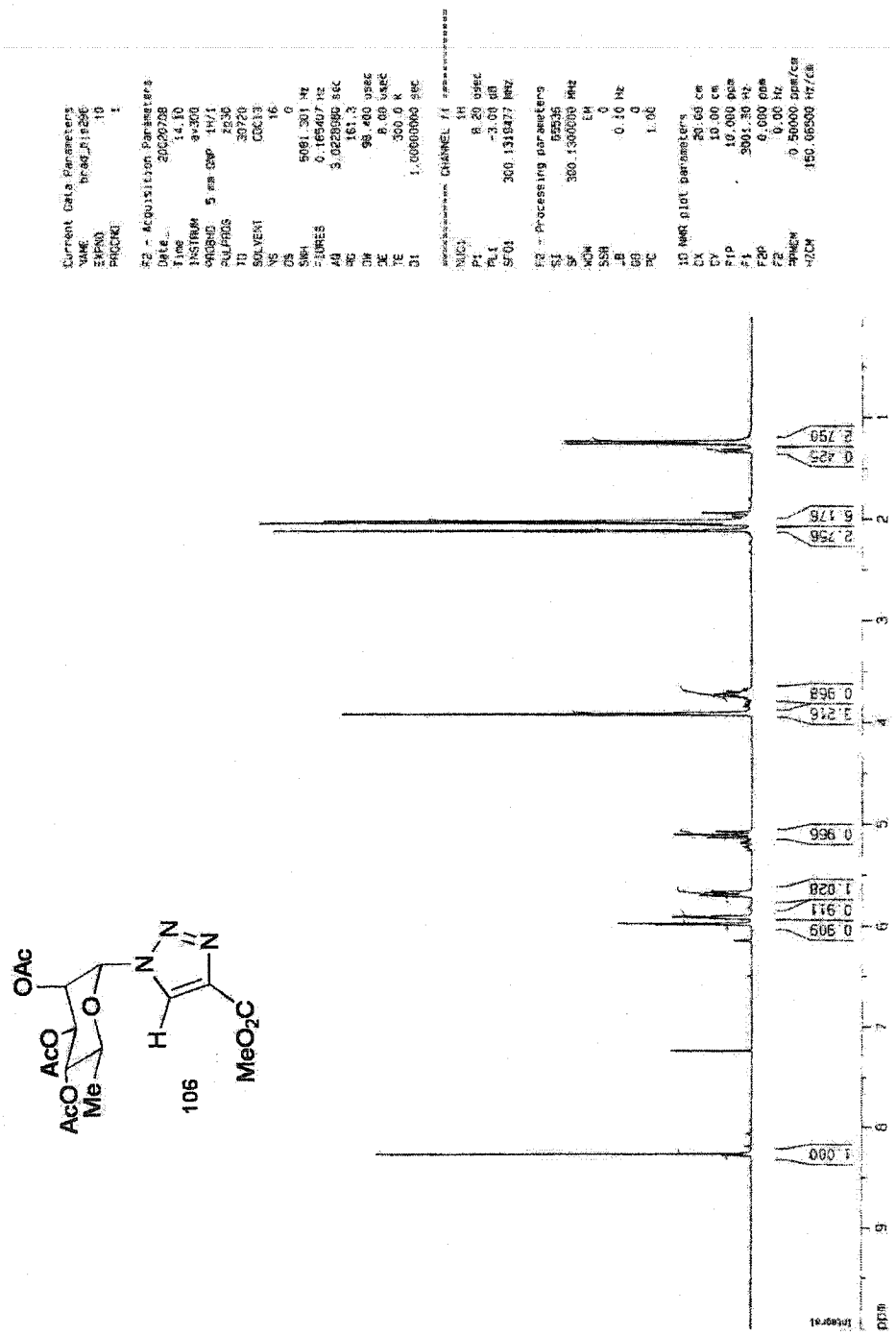


Figure **4.8**: ^{1}H NMR Spectrum ($CDCl_3$, 300 MHz) of Triazole **106**

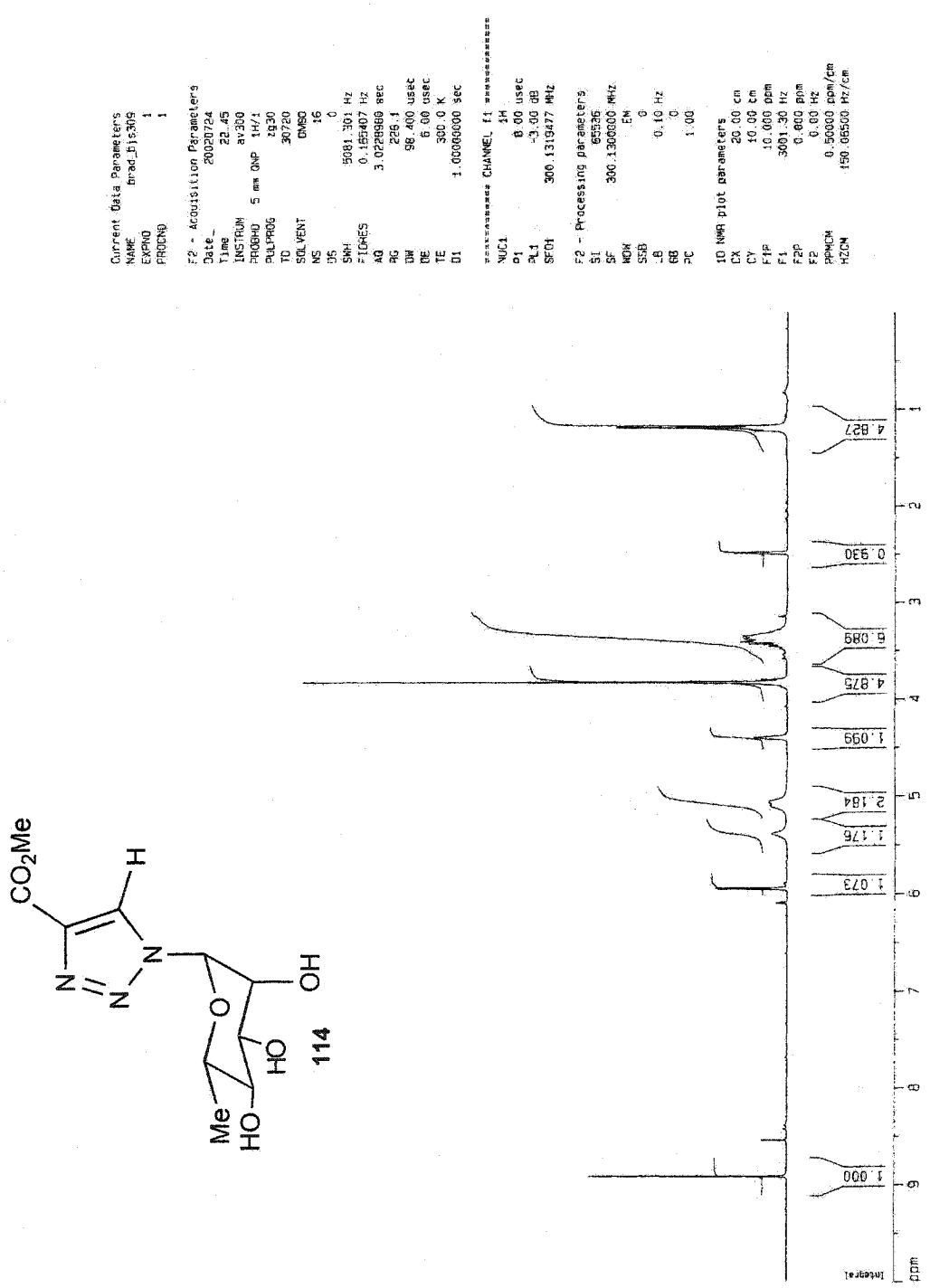
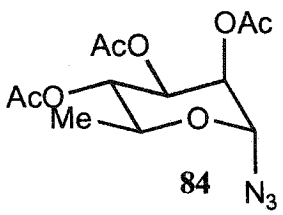


Figure **4.9**: ^{1}H NMR Spectrum (DMSO-d_6, 300 MHz) of **114**.

4.8 Preparation of Compounds

4.8.1 Formation of Glycosyl Azides

2,3,4-Tri-*O*-acetyl-α-L-rhamnopyranosyl azide (84)

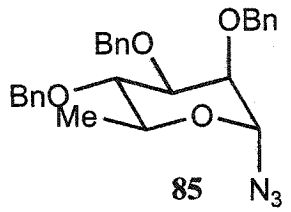


(L)-rhamnose tetraacetate (4.46 g, 13.4 mmol) of was dissolved in dichloromethane (40 mL) and stirred. Azidotrimethylsilane (2.10 mL, 16.1 mmol, 1.2 eq.) and tin tetrachloride (0.31 mL, 2.7 mmol, 0.2 eq) were added, and the mixture was stirred for 3 hours. The mixture was poured into 5 % $NaHCO_3$ (40 mL), shaken, and the organics removed. The water was back-extracted with dichloromethane (2 x 30 mL), and the organics were combined, dried over sodium sulfate, filtered and concentrated to afford a thick colorless oil, 3.79 g (90 %), pure by TLC (1:1 hexanes/ethyl acetate).

FT-IR (KBr) 2120 cm^{-1} (azide stretch)

^1H NMR (200 MHz, $CDCl_3$) δ (ppm): 5.30 (1H, d, J = 1.5 Hz, H-1), 5.15-5.00 (3H, m, H-2, H-3, H-4), 4.00 (1H, dq, J = 6.2 Hz, J = 9.0 Hz, H-5), 2.13, 2.03, 1.98 (9H, 3s, $COCH_3$), 1.23 (3H, d, J = 6.2 Hz, H-6).

2,3,4-Tri-*O*-benzyl-α-L-rhamnopyranosyl azide (85)



L-1-α-azido-1-deoxy-2,3,4-triacetylrhamnose **84** (1.07 g, 3.22 mmol) of was dissolved in methanol (10 mL) with stirring, and 1 mL of sodium methoxide (1 M in methanol) was added. TLC (10:1 $CHCl_3$:MeOH) after 1 h showed the starting material was consumed, and only one product remained. The solution was neutralized with Amberlite 15 H^+ resin to pH 4. The solution was then filtered and concentrated. The concentrate was brought up in 20 mL DMF with stirring, and sodium hydride (1.4 g of a 60% dispersion in mineral oil, 29 mmol, 9 equiv.) was added, followed by benzyl bromide (3.70 mL, 29 mmol, 9 equiv.), and the mixture was stirred for 20 h. Methanol (10 mL) was added, the mixture allowed to stir for 20 min, and then poured into a larger flask with 200 mL toluene. The toluene/DMF azeotrope was removed by rotary evaporation to give a thick oil. This material was brought up in CH_2Cl_2, the inorganic salts filtered off, and the solution concentrated by rotary evaporation. This material was purified by silica gel chromatography (15:1 hexane/ethyl acetate) to give a clear, colorless oil (772 mg, 50% yield, 2 steps).

m.p. (oil)

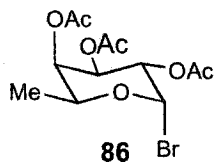
$[\alpha_D]$ (c = 1, $CHCl_3$): +48.4°

M.S. (+ESI) : Calc. for $C_{27}H_{29}N_3O_4$ = 459, Found *m/z* 432 $(C_{27}H_{29}N_3O_4 + H - N_2)^+$

^{1}H ($CDCl_3$, 500 MHz) δ (ppm): 7.50 - 7.39 (15H, m, Ar-H), 5.42 (1H, d, J = 1.8 Hz, H-1), 4.82 (1H, d, ^2J = 12.2 Hz, benzylic-H), 4.78-4.69 (5H, m, benzylic-H), 3.99 (1H, dq, $J_{4,5}$ = 9.2 Hz, $J_{5,6}$ = 6.0 Hz, H-5), 3.92 (1H, dd, $J_{2,3}$ = 3.0 Hz, $J_{3,4}$ = 9.2 Hz, H-3), 3.79 (1H, d, J = 9.3 Hz, H-4), 3.76 (1H, d, $J_{1,8}$ = 1.9 Hz, $J_{2,3}$ = 3.0 Hz, H-2), 1.50 (3H, d, J = 6.2 Hz, H-6)

^{13}C (125 MHz) δ (ppm): 138.3, 138.0, 137.7 (4° Ar-C), 128.2, 128.2, 128.2, 128.0, 127.7, 127.7, 127.7, 127.5, 127.5 (Ar-CH), 87.9 (C-1), 79.7 (C-3), 78.6 (C-2), 75.9 (C-4), 75.4, 75.2, 73.0 (benzylic-C), 68.9 (C-5), 16.6 (C-6).

2,3,4-Tri-*O*-acetyl-α-L-fucopyranosyl bromide (86)[137]



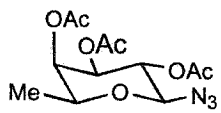
86

L-Fucose tetraacetate **1** (592 mg, 1.78 mmol) was dissolved in 15 mL of dry CH_2Cl_2 at 0°C, and stirred under nitrogen. A solution of 45% HBr in glacial acetic acid (1.0 mL, 0.62 g of HBr, 7.66 mmol, 4.3 eq.) was added dropwise via syringe. TLC at 2.5 h (1:1 hexane/ethyl acetate) showed completion of the reaction. The CH_2Cl_2 was diluted up to 30 mL, and poured into cold 5% $NaHCO_3$ solution (15 mL). The organic layer was removed via a separatory funnel, and the aqueous layer extracted with 20 mL dichloromethane. The organic layers were combined, washed with 10 mL 5% $Na_2S_2O_3$ (to remove traces of bromine) and 10 mL water, with both times the

washing liquids being kept at 0°C prior to introduction into the separatory funnel. The organics were dried over Na_2SO_4 at 0°C for 10 minutes, filtered and concentrated to a crude oil, mass 442 mg (74% yield), pure by TLC. This compound is unstable and should be used within less than one hour, and kept on ice.[139]

^1H (200 MHz, $CDCl_3$) : δ ($CDCl_3$ = 7.24 ppm) : 6.66 (d, *J*= 4.0 Hz, H-1); 5.3 (multiplet, H-3 and H-4); 5.00 (dd, *J*= 3.9 Hz, 10.2 Hz, H-2); 4.37 (q, *J*= 6.3 Hz, H-5); 2.14, 2.08, 1.98 (s, 3H each, 3 acetate CH_3s); 1.18 (d, 3H, *J*= 6.5 Hz, H-6)

2,3,4-Tri-*O*-acetyl-β-L-fucopyranosyl azide (87)[139]



87

Compound **86** (416 mg, 1.23 mmol) was dissolved in 15 mL CH_2Cl_2. To it was added solid NaN_3 (183 mg, 2.81 mmol, 2.3 eq.), tetrabutylammonium hydrogen sulphate (TBAHS) (438 mg of 97 % grade material, 1.25 mmol, 1.02 eq.) and then 10 mL of 1.5 M aqueous sodium carbonate. The reaction was stirred vigorously. After 30 minutes, the reaction is complete by TLC [2:1 hexane/ethyl acetate, R_f 0.3 (slightly lower than the starting bromide)]. The materials were placed in a separatory funnel and the organic phase removed. The aqueous phase was extracted with CH_2Cl_2 (20 mL), and the organic layers combined. The organics were washed with 20 mL of 5% $NaHCO_3$ solution, and 20 mL water. The organics were then dried over Na_2SO_4, filtered, and concentrated by rotary evaporation to a very thick oil which clung quite

tenaciously to traces of solvent. Recrystallization from 99% ethanol gave white flakes, 319 mg (82%), pure by TLC.

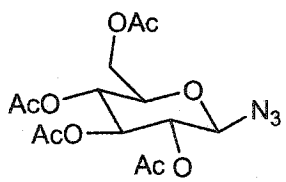
^{1}H (500 MHz, $CDCl_3$) : δ 5.19 (dd, *J*=1.0 Hz, 3.3 Hz, H-4); 5.06 (dd, *J*=8.8 Hz, 10.3 Hz, H-2); 4.97 (dd, *J*=3.4 Hz, 10.3 Hz, H-3); 4.52 (d, *J*=8.7 Hz, H-1); 3.85 (dq, *J*=1.0 Hz, 6.4 Hz, H-5); 2.11, 2.01, 1.91 (s, 3H each, 3 acetate CH_3s); 1.18 (d, *J*=6.4 Hz)

^{13}C (125 MHz, $CDCl_3$): δ 170.4, 169.9, 169.3 (acetate carbonyls); 88.1 (C-1); 71.4, 71.1, 69.9, 68.2 (C-2, C-3, C-4, C-5); 20.6, 20.5, 20.4 (3 acetate methyls); 15.9 (C-6).

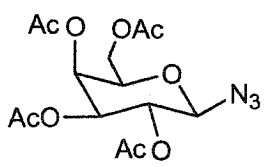
2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl azide (90)

2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl azide (94)

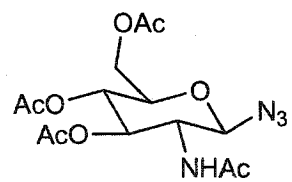
2-Acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl azide (98)



90



94



98

Each of these azides were prepared via known literature methods.[139-142]

2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl azide (90)[139]

^{1}H NMR (500 MHz, $CDCl_3$): 5.09-4.97 (2H, m, H-3, H-4), 4.91-4.84 (2H, m, H-6), 4.78 (1H, d, J = 8.6 Hz, H-1), 4.59 (1H, t, J = 8.8 Hz, H-2), 3.61 (1H, m, H-5), 2.11, 2.08. 2.01, 1.96 (4 x 3H, 4 s, O_2CCH_3).

^{13}C NMR (125 MHz, $CDCl_3$): 170.7, 170.4, 170.3, 170.0, 88.8 (C-1), 84.4, 77.6 (C-3, C-4), 82.3 (C-2), 76.9 (C-5), 70.4 (C-6), 21.9, 20.7, 20.5, 20.3 (O_2CCH_3).

2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl azide (94)[139]

^{1}H NMR (500 MHz, $CDCl_3$): 5.07-4.81 (4H, m, H-3, H-4, H-6), 4.73 (1H, d, J = 8.4 Hz, H-1), 4.52 (1H, dd, J = 2.0 Hz, J = 8.7 Hz, H-2), 3.51 (1H, m, H-5), 2.09, 2.07, 2.00, 1.98 (4 x 3H, 4 s, O_2CCH_3).

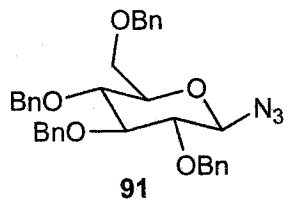
^{13}C NMR (125 MHz, $CDCl_3$): 170.4, 170.3, 170.3, 170.1, 88.4 (C-1), 84.2 (C-3), 82.6 (C-2), 82.1 (C-4), 75.7 (C-5), 68.3 (C-6), 21.1, 20.9, 20.7, 20.1 (O_2CCH_3).

2-Acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl azide (98)[139]

^{1}H NMR (500 MHz, $CDCl_3$): 7.92 (1H, bs, N-H), 5.11-4.91 (4H, m, H-3, H-4, H-6), 4.77 (1H, d, J = 8.9 Hz, H-1), 3.56 (1H, dd, J = 2.1 Hz, J = 8.6 Hz, H-2), 3.52 (1H, m, H-5), 2.04, 2.03, 2.01, 1.91 (4 x 3H, 4 s, $OCCH_3$).

^{13}C NMR (125 MHz, $CDCl_3$): ^{13}C NMR (125 MHz, $CDCl_3$): 170.7, 170.5, 170.3, 166.2, 86.4 (C-1), 84.1 (C-3), 82.3 (C-4), 80.1 (C-2), 75.1 (C-5), 68.0 (C-6), 20.4, 20.3, 20.1, 19.7 ($OCCH_3$).

2,3,4,6-Tetra-*O*-benzyl-β-D-glucopyranosyl azide (91)



91

Azide **90** (341 mg, 0.910 mmol) was dissolved in methanol (5 mL). Sodium methoxide (1M in methanol, Zemplen conditions) was added (0.3 mL) and the mixture stirred for 1 hour. After this time, TLC (1:1 $CHCl_3$/MeOH) indicated only one new product forming. The mixture was neutralized with Amberlite 15 H^+ resin to pH ~3, filtered and evaporated to a solid. The crude material was brought up in DMF (20 mL) with benzyl bromide (1.3 mL, 12 eq.) and sodium hydride (440 mg of a 60 % dispersion in mineral oil, 12 eq.). The mixture was stirred for 20 hours, then methanol (10 mL) was added, and the mixture stirred for 45 minutes. The reaction was then concentrated by azeotrope with toluene (2 x 250 mL) to an oily paste, which was brought up in dichloromethane. The mixture was filtered and concentrated again to afford a yellow oil. Column chromatography (15:1 hexanes/ethyl acetate) afforded **91** as a colorless oil, 370 mg (72 %).

$[\alpha_D]$ (c = 1.0, $CHCl_3$) –14.2° [Lit.[119] (c=0.43, $CHCl_3$)-1.9 °]

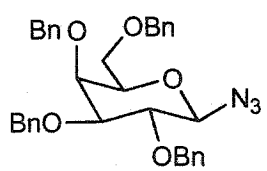
M.S. (+HRMS-FAB) : Calc. for $C_{30}H_{33}N_3O_6 - N_2 + H^+$ = 538.2579; Found: *m/z* 538.2593.

FT-IR (thin film) 2114.5 cm^{-1} (azide stretch) (Lit.[140] 2120 cm^{-1})

^1H NMR (500 MHz, $CDCl_3$): 7.55-7.33 (20H, m, Ar-H), 5.10 (1H, d, *J* = 11.1 Hz, Bn-H), 5.07 (1H, d, *J* = 11.4 Hz, Bn-H), 5.02 (1H, d, *J* = 11.0 Hz, Bn-H), 5.00 (1H, d, *J* = 10.8 Hz, Bn-H), 4.94 (1H, d, *J* = 10.8 Hz, Bn-H), 4.80 (1H, d, *J* = 12.2 Hz, Bn-H), 4.78 (1H, d, *J* = 8.6 Hz, H-1), 4.75 (1H, d, *J* = 11.4 Hz, Bn-H), 4.72 (1H, d, *J* = 12.2 Hz, Bn-H), 3.92 (2H, m, H-6), 3.85 (2H, m, H-3, H-4), 3.68 (1H, m, H-5), 3.59 (1H, t, *J* = 8.8 Hz, H-2).

^{13}C NMR (125 MHz, $CDCl_3$): 138.2, 137.8, 137.6, 128.2, 128.2, 128.0, 127.9, 127.7, 127.6, 127.5, 127.4 (Ar-C), 89.9 (C-1), 84.7, 77.1 (C-3, C-4), 81.5 (C-2), 76.9 (C-5), 75.4, 74.8, 73.3 (benzylic-C), 68.2 (C-6).

2,3,4,6-Tetra-*O*-benzyl-β-D-galactopyranosyl azide (95)



95

Azide **94** (392 mg, 1.05 mmol) was dissolved in methanol (5 mL). Sodium methoxide (1M in methanol, Zemplen conditions) was added (0.3 mL) and the mixture stirred for 1 hour. After this time, TLC (1:1 $CHCl_3$/MeOH) indicated only one new product had formed. The mixture was neutralized with Amberlite 15 H^+ resin to pH ~3, filtered and evaporated to a solid. The crude material was brought up in DMF (20 mL) with benzyl bromide (1.5 mL, 12.1 mmol, 12 eq.) and sodium hydride (510 mg of a 60 % dispersion in mineral oil, 12.1 mmol, 12 eq.). The mixture was stirred for 16 hours, then methanol (10 mL) was added, and the mixture stirred for 90 minutes. The reaction was then concentrated, removing DMF by azeotrope with toluene (2 x 250 mL) to an oily paste, which was brought up in dichloromethane. The mixture was filtered and evaporated again to afford a yellow oil. Column chromatography (15:1 hexanes/ethyl acetate) afforded **95** as a colorless oil, 435 mg (78 %).

M.S. (+HRMS-FAB) : Calc. for $C_{30}H_{33}N_3O_6 - N_2 + H^+$ = 538.2579; Found: *m/z* 538.2583.

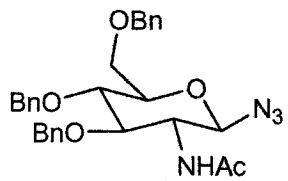
FT-IR (thin film) 2115.1 cm^{-1} (azide stretch)

^1H NMR (500 MHz, $CDCl_3$): 7.57-7.34 (20H, m, Ar-H), 5.12 (1H, d, J = 11.5 Hz, Bn-H), 5.05 (1H, d, J = 11.4 Hz, Bn-H), 5.02 (1H, d, J = 11.5 Hz, Bn-H), 4.98 (1H, d, J = 12.5 Hz, Bn-H), 4.84 (1H, d, J = 12.5 Hz, Bn-H), 4.77 (1H, d, J = 12.0 Hz, Bn-H), 4.75 (1H, d, J = 8.4 Hz, H-1), 4.75 (1H, d, J = 11.4 Hz, Bn-H), 4.72 (1H, d, J =

12.0 Hz, Bn-H), 3.95 (2H, m, H-6), 3.66 (2H, m, H-3, H-4), 3.58 (1H, m, H-5), 3.52 (1H, dd, $J = 8.3$ Hz, $J = 8.6$ Hz, H-2).

^{13}C NMR (125 MHz, $CDCl_3$): 138.5, 138.2, 137.7, 128.4, 128.4, 128.2, 127.9, 127.8, 127.7, 127.6, 127.5, 127.5 (Ar-C), 88.4 (C-1), 81.3, 77.5 (C-3, C-4), 81.0 (C-2), 76.2 (C-5), 75.9, 75.8, 72.0 (benzylic-C), 67.6 (C-6).

2-Acetamido-3,4,6-tri-O-benzyl-2-deoxy-β-D-glucopyranosyl azide (99)



99

Azide **98** (210 mg, 0.410 mmol) was dissolved in methanol (5 mL). Sodium methoxide (1M in methanol, Zemplen conditions) was added (0.3 mL) and the mixture stirred for 1 hour. After this time, TLC (1:1 $CHCl_3$/MeOH) indicated only one new product had formed. The mixture was neutralized with Amberlite 15 H^+ resin to pH ~3, filtered and evaporated to a solid. The crude material was brought up in DMF (20 mL). Barium oxide (385 mg, 2.5 mmol, 6 eq. was added, followed by benzyl bromide (1.0 mL, 8.07 mmol, 20 eq.). The mixture was stirred for 18 hours; methanol (10 mL) and then water (10 mL) were added, and the mixture stirred for 2 hours. The reaction was then concentrated by azeotrope with toluene (2 x 250 mL) to a yellow solid, which was brought up in dichloromethane. The mixture was filtered

and concentrated again to afford a yellow oil. Column chromatography (3:1 hexanes/ethyl acetate) afforded **99** as an off-white solid, 128 mg (61 %).

M.S. (+FAB) : Calc. for $C_{29}H_{32}N_4O_5 = 516$, Found *m/z* 517 $(C_{29}H_{32}N_4O_5 + H)^+$

FT-IR (thin film) 2110.0 cm^{-1} (azide stretch)

^1H NMR (500 MHz, $CDCl_3$): 7.90 (1H, bs, N-H), 7.50-7.30 (15H, m, Ar-H), 5.06 (1H, d, J = 11.0 Hz, Bn-H), 5.02 (1H, d, J = 10.8 Hz, Bn-H), 4.99 (1H, d, J = 12.1 Hz, Bn-H), 4.85 (1H, d, J = 11.0 Hz, Bn-H), 4.81 (1H, d, J = 12.1 Hz, Bn-H), 4.77 (1H, d, J = 8.9 Hz, H-1), 4.71 (1H, d, J = 10.8 Hz, Bn-H), 3.94 (2H, m, H-6), 3.87 (1H, dd, J = 8.7 Hz, J = 9.4 Hz, H-3), 3.81 (1H, dd, J = 8.7 Hz, J = 9.1 Hz, 3.68 (1H, m, H-5), 3.59 (1H, t, J = 8.8 Hz, H-2), 2.01 (3H, s, $COCH_3$).

^{13}C NMR (125 MHz, $CDCl_3$): 165.5 (C=O), 138.2, 137.8, 137.6, 128.2, 128.2, 128.0, 127.9, 127.7, 127.6, 127.5, 127.4 (Ar-C), 89.9 (C-1), 84.7, 77.1 (C-3, C-4), 81.5 (C-2), 76.9 (C-5), 75.4, 74.8, 73.3 (benzylic-C), 68.2 (C-6), 19.0 ($COCH_3$).

4.8.2 Formation of Glycosyl Triazoles via 1,3 Dipolar Addition

General Preparation

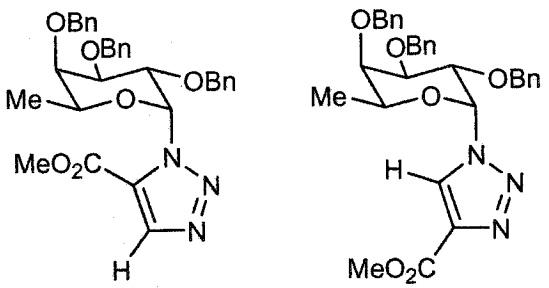
A general preparation is given for compounds **100** and **102**. In some cases, separation was not possible; compounds not listed had their ratios determined by ^1H NMR, and

their identities as 1,4- or 1,5-isomers tentatively assigned by comparison with typical values for pure compounds. All reactions were performed on similar scales (10-80 mg).

X-ray crystallography was performed on compound **106**; see below for details.

1-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-5-carboxymethyl-1,2,3-triazole (100)

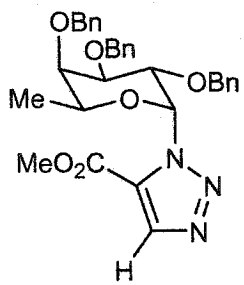
1-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-4-carboxymethyl-1,2,3-triazole (102)



100 **102**

A 4 mL screw-cap vial was charged with **4** (55.7 mg, 12.1 μmol), methyl (3-trimethylsilyl) propiolate (40.8 mg, 24.0 μmol, 2 equiv.) and toluene (0.4 mL). The cap was closed tightly and the mixture shaken. The vial was heated in an oil bath at 120 °C for 16 hours, with occasional shaking. Once heated, the azide became soluble in the toluene. After 36 hours, the toluene was removed by evaporation, and the crude product chromatographed (silica gel, 10:1 hexane/ethyl acetate, stepwise up to 4:1) to give **100** and **102** (71.5 mg, 96%), as a colorless solid. ^{1}H NMR indicated it to be a 13:87 ratio of **100** to **102**. Small amounts were purified by repeated chromatography to obtain pure samples.

1-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-5-carboxymethyl-1,2,3-triazole (100)



100

m.p. (99 °C, dec.)

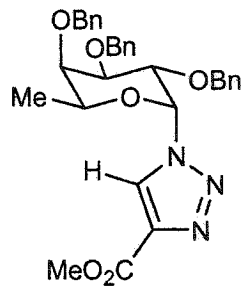
$[\alpha_D]$ (c = 0.24, $CHCl_3$): +129.6°

M.S. (+ESI) : Calc. for $C_{31}H_{33}N_3O_6$ = 553, Found *m/z* 566 $(C_{31}H_{33}N_3O_6 + Na)^+$

^1H ($CDCl_3$, 300 MHz) δ (ppm): 8.09 (1H, s, H-5′), 7.40 – 6.93 (15H, m, Ar-H), 5.60 (1H, d, $J_{1,2}$ = 9.1 Hz, H-1), 5.02 (1H, d, ^2J = 11.8 Hz, benzylic-H), 4.82 (1H, t, $J_{1,2}$ = 9.3 Hz, H-2), 4.76 (4H, m, benzylic-H), 4.35 (1H, d, ^2J = 10.9 Hz, benzylic-H), 3.93 (3H, s, CO_2CH_3), 3.74 (1H, dq, $J_{4,5}$ = 2.8 Hz, $J_{5,6}$ = 6.4 Hz, H-5), 3.72 (1H, d, $J_{2,3}$ = 9.1 Hz, $J_{3,4}$ = 0.8 Hz, H-3), 3.69 (1H, dd, $J_{3,4}$ = 0.7 Hz, $J_{4,5}$ = 2.7 Hz, H-4), 1.20 (3H, d, $J_{5,6}$ = 6.4 Hz, H-6)

^{13}C (75 MHz) δ (ppm): 160.9, (C=O), 140.3, 138.3, 138.0, 137.9(4° Ar-C), 137.4 (C-5′), 128.5, 128.3, 128.2, 128.2, 127.8, 127.7, 127.5 (Ar-CH), 91.6 (C-1), 83.7 (C-2), 76.7 (C-4), 75.7, 75.1, 73.8 (benzylic-C), 74.6 (C-3), 72.9 (C-5), 52.3 (CO_2CH_3), 16.8 (C-6).

1-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-4-carboxymethyl-1,2,3-triazole (102)



102

m.p. (86-88 °C)

$[\alpha_D]$ (c = 0.24, $CHCl_3$): -187.9°

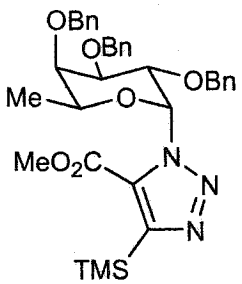
M.S. (+ESI) : Calc. for $C_{31}H_{33}N_3O_6$ = 553, Found *m/z* 566 $(C_{31}H_{33}N_3O_6 + Na)^+$

^1H ($CDCl_3$, 300 MHz) δ (ppm): 8.08 (1H, s, H-4'), 7.40 – 7.12 (15H, m, Ar-H), 6.38 (1H, d, $J_{1,2}$ = 6.0 Hz, H-1), 5.00 (1H, d, ^2J = 11.4 Hz, benzylic-H), 4.86 (1H, d, ^2J = 11.8 Hz, benzylic-H), 4.74 (1H, dd, $J_{2,3}$ = 10.1 Hz, $J_{3,4}$ = 2.0 Hz, H-3), 4.74 - 4.65 (4H, m, benzylic-H), 4.48 (1H, dd, $J_{1,2}$ = 6.0 Hz, $J_{2,3}$ = 10.1 Hz, H-2), 4.44 (1H, q, $J_{5,6}$ = 6.2 Hz, H-5), 3.94 (3H, s, CO_2CH_3), 3.81 (1H, d, $J_{3,4}$ = 2.0 Hz, H-4), 1.08 (3H, d, $J_{5,6}$ = 6.4 Hz, H-6)

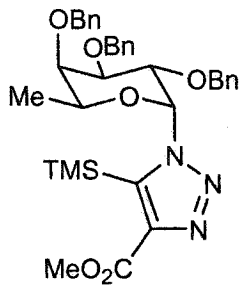
^{13}C (75 MHz) δ (ppm): 161.1 (C=O), 139.6, 138.7, 138.2, 137.5 (4° Ar-C), 136.8 (C-5'), 128.5, 128.4, 128.3, 128.3, 127.9, 127.8, 127.8, 127,6, 127,5 (Ar-CH), 88.8 (C-1), 78.5 (C-2), 77.3 (C-4), 75.1, 73.4, 73.3 (benzylic-C), 74.7 (C-3), 70.9 (C-5), 52.3 (CO_2CH_3), 16.8 (C-6).

1-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-4-trimethylsilyl-5-carboxymethyl-1,2,3-triazole (101)

1-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-4-carboxymethyl-5-trimethylsilyl-1,2,3-triazole (103)



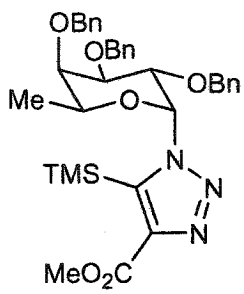
101



103

Prepared as for **100** and **102**. Compound **101** could not be isolated in the pure state (contamination by **103**).

1-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-4-carboxymethyl-5-trimethylsilyl-1,2,3-triazole (103)



103

m.p. 114-116 °C

$[\alpha_D]$ (c = 1.0, $CHCl_3$): -0.3 °

M.S. (+ESI) : Calc. for $C_{34}H_{41}N_3O_6Si$ = 616, Found *m/z* 639 ($C_{34}H_{41}N_3O_6Si + Na^+$)

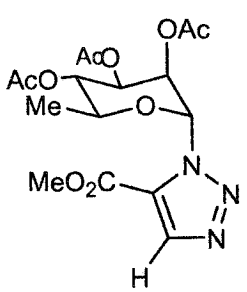
The ^1H NMR can be seen in Figure **4.6**.

^1H ($CDCl_3$, 500 MHz) δ (ppm): 7.40 - 7.11 (15H, m, Ar-H), 6.43 (1H, d, J = 6.0 Hz, H-1), 5.02 (1H, d, ^2J = 11.5 Hz, benzylic-H), 4.83 (1H, d, ^2J = 12.0 Hz, benzylic-H), 4.77 (1H, dd, $J_{2,3}$ = 10.1 Hz, $J_{3,4}$ = 2.9 Hz, H-3), 4.73 (1H, d, ^2J = 11.9 Hz, benzylic-H), 4.70 (1H, d, ^2J = 11.5 Hz), 4.66 (2H, s, benzylic-H), 4.51-4.47 (2H, m, H-2, H-5), 3.93 (3H, s, CO_2CH_3), 3.79 (1H, d, J = 2.4 Hz, H-4), 1.09 (3H, d, J = 6.4 Hz, H-6), 0.35 (9H, s, $Si(CH_3)_3$).

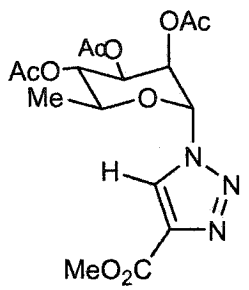
^{13}C (125 MHz) δ (ppm): 162.2 ($\underline{C}O_2CH_3$), 150.8, 144.5 (C-4′, C-5′), 138.7, 138.4, 137.8 (4° Ar-C), 128.6, 128.4, 128.3, 128.2, 127.7, 127.6, 127.6, 127.5 (Ar-CH), 88.2 (C-1), 78.3 (C-2), 77.5 (C-4), 75.1, 73.3, 73.0 (benzylic-C), 74.8 (C-3), 71.0 (C-5), 51.9 ($CO_2\underline{C}H_3$), 16.8 (C-6), -1.6 ($SiCH_3$).

1-(2,3,4-tri-*O*-benzyl-α-L-rhamnopyranosyl)-5-carboxymethyl-1,2,3-triazole (104)

1-(2,3,4-tri-*O*-benzyl-α-L-rhamnopyranosyl)-4-carboxymethyl-1,2,3-triazole (106)

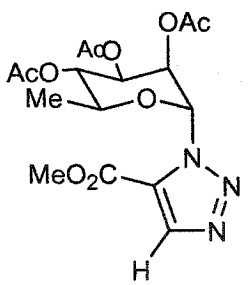


104



106

1-(2,3,4-tri-*O*-benzyl-α-L-rhamnopyranosyl)-5-carboxymethyl-1,2,3-triazole (104)



104

m.p. 114 °C (dec.)

$[\alpha_D]$ (c = 0.34, $CHCl_3$): -47.1°

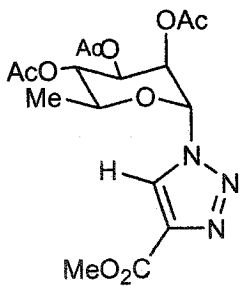
M.S. (+CI) : Calc. for $C_{16}H_{20}N_3O_9$ = 399, Found *m/z* 273 ($C_{16}H_{20}N_3O_9$ – triazole moiety $(C_3H_4N_3O_2)^+$

The ^{1}H NMR spectrum of **104** can be found in Figure **4.7**.

^{1}H ($CDCl_3$, 300 MHz) δ (ppm): 8.18 (1H, s, H-4′), 6.80 (1H, d, J = 2.1 Hz, H-1), 6.07 (1H, dd, $J_{2,3}$ = 4.0 Hz, $J_{3,4}$ = 9.3 Hz, H-3), 5.83 (1H, dd, $J_{1,2}$ = 2.1 Hz, $J_{2,3}$ = 3.9 Hz, H-2), 5.18 (1H, t, J = 9.4 Hz, H-4), 3.93 (3H, s, CO_2CH_3), 3.81 (1H, q, J = 6.2 Hz, H-5), 2.17, 2.06, 2.01 (3 x 3H, 3 x s, $COCH_3$), 1.17 (3H, d, J = 6.2 Hz, H-6).

^{13}C (75 MHz) δ (ppm): 170.0, 169.8, 169.6 ($\underline{C}OCH_3$), 158.3 ($\underline{C}O_2CH_3$), 138.1 (C-4′), 128.2 (C-5′), 82.6 (C-1), 70.9 (C-2), 69.9 (C-4), 69.1 (C-3), 68.8 (C-5), 52.8 ($CO_2\underline{C}H_3$), 20.8, 20.8, 20.6 ($CO\underline{C}H_3$), 17.4 (C-6).

1-(2,3,4-tri-*O*-benzyl-α-L-rhamnopyranosyl)-4-carboxymethyl-1,2,3-triazole (106)



106

m.p. 119 °C (dec.)

[α_D] (c = 0.26, $CHCl_3$): -267.7°

M.S. (+CI) : Calc. for $C_{16}H_{20}N_3O_9$ = 399, Found *m/z* 273 ($C_{16}H_{20}N_3O_9$ – triazole moiety $(C_3H_4N_3O_2)^+$

The ^{1}H NMR spectrum (300 MHz) of **106** (contaminated with **104**) can be found in Figure **4.8**.

^{1}H ($CDCl_3$, 300 MHz) δ (ppm): 8.27 (1H, s, H-5′), 5.98 (1H, d, J = 3.1 Hz, H-1), 5.90 (1H, t, J = 3.5 Hz, H-2), 5.67 (1H, dd, $J_{2,3}$ = 3.6 Hz, $J_{3,4}$ = 8.6 Hz, H-3), 5.10 (1H, t, J = 8.5 Hz, H-4), 3.91 (3H, s, CO_2CH_3), 3.72 (1H, q, J = 6.4 Hz, H-5), 2.10, 2.02, 2.00 (3 x 3H, 3 x 3s, $COCH_3$), 1.23 (3H, d, J = 6.4 Hz, H-6).

^{13}C (75 MHz) δ (ppm): 169.7, 169.5, 169.3 ($COCH_3$), 160.6 (CO_2CH_3), 140.4 (C-4′), 127.5 (C-5′), 83.7 (C-1), 70.6 (C-5), 70.5 (C-4), 68.5 (C-3), 68.1 (C-2), 52.3 (CO_2CH_3), 20.7, 20.6, 20.5 ($COCH_3$), 17.0 (C-6).

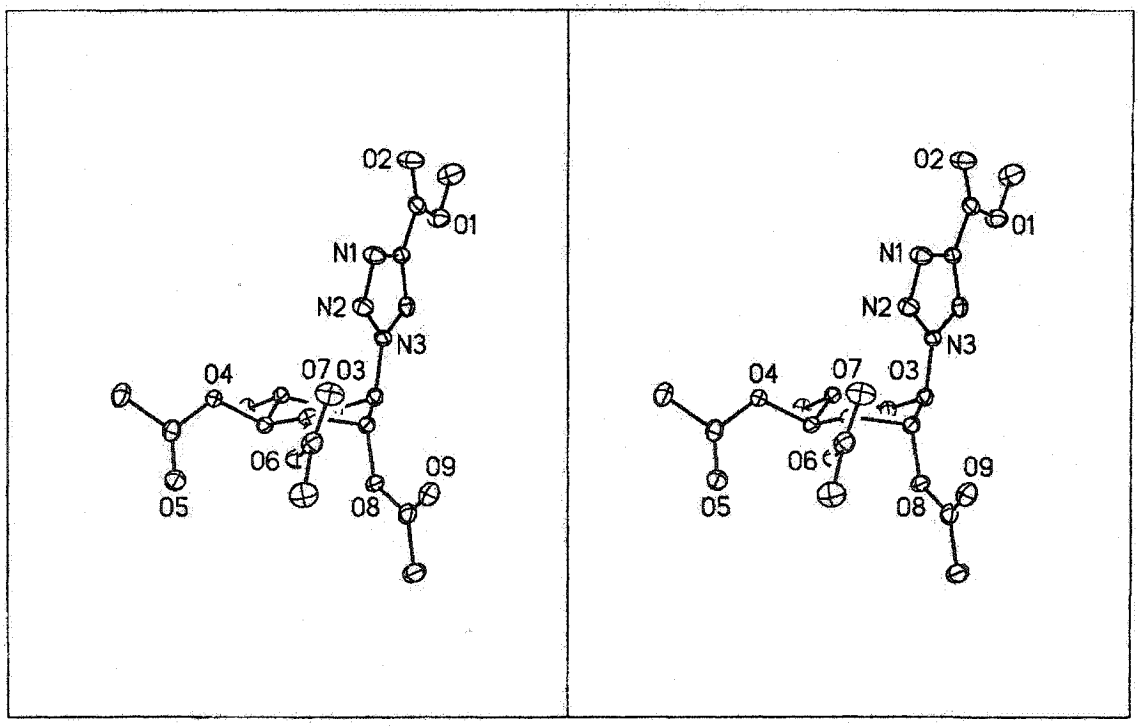
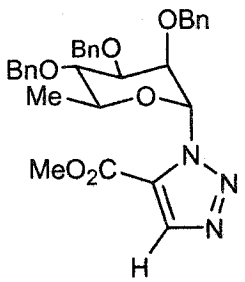


Figure **5.0**: Stereo ORTEP Representation of **106**.

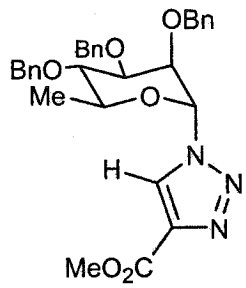
Complete x-ray crystallography data can be found in the Appendix.

1-(2,3,4-tri-*O*-benzyl-α-L-rhamnopyranosyl)-5-carboxymethyl-1,2,3-triazole (112)

1-(2,3,4-tri-*O*-benzyl-α-L-rhamnopyranosyl)-4-carboxymethyl-1,2,3-triazole (113)

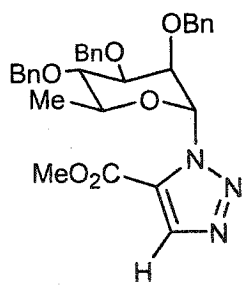


112



113

1-(2,3,4-tri-*O*-benzyl-α-L-rhamnopyranosyl)-5-carboxymethyl-1,2,3-triazole (112)



112

m.p. (109 °C, dec.)

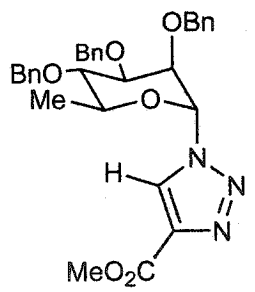
$[\alpha_D]$ (c = 0.34, $CHCl_3$): -36.0°

M.S. (+ESI) : Calc. for $C_{31}H_{33}N_3O_6$ = 553, Found *m/z* 566 $(C_{31}H_{33}N_3O_6 + Na)^+$

^{1}H ($CDCl_3$, 500 MHz) δ (ppm): 8.11 (1H, s, H-4′) 7.48 - 7.29 (15H, m, Ar-H), 6.47 (1H, d, J = 1.9 Hz, H-1), 4.84 (1H, d, ^2J = 11.1 Hz, benzylic-H), 4.80-4.71 (5H, m, benzylic-H), 4.00 (1H, dq, $J_{4,5}$ = 9.0 Hz, $J_{5,6}$ = 6.2 Hz, H-5), 3.93 (1H, dd, $J_{2,3}$ = 3.0 Hz, $J_{3,4}$ = 9.2 Hz, H-3), 3.90 (3H, s, CO_2CH_3), 3.74 (1H, d, J = 9.2 Hz, H-4), 3.73 (1H, d, $J_{1,8}$ = 1.8 Hz, $J_{2,3}$ = 3.0 Hz, H-2), 1.45 (3H, d, J = 6.3 Hz, H-6).

^{13}C (125 MHz) δ (ppm): 160.3 (C=O), 138.3, 137.9, 137.9, 137.7 (4° Ar-C), 135.5 (C-4′), 128.4, 128.4, 128.0, 128.0, 127.9, 127.8, 127.7, 127.5, 127.3 (Ar-CH), 87.9 (C-1), 79.7 (C-3), 78.6 (C-2), 75.9 (C-4), 75.4, 75.2, 73.0 (benzylic-C), 68.9 (C-5), 16.6 (C-6).

1-(2,3,4-tri-*O*-benzyl-α-L-rhamnopyranosyl)-4-carboxymethyl-1,2,3-triazole (113)



113

m.p. (114 °C, dec.)

[α_D] (c = 0.26, $CHCl_3$): +69.2°

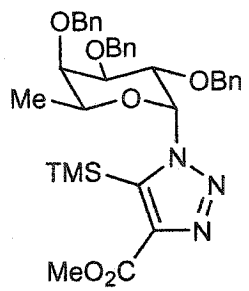
M.S. (+ESI) : Calc. for $C_{31}H_{33}N_3O_6$ = 553, Found *m/z* 566 $(C_{31}H_{33}N_3O_6 + Na)^+$

^{1}H ($CDCl_3$, 500 MHz) δ (ppm): 8.04 (1H, s, H-5′), 7.51 - 7.33 (15H, m, Ar-H), 5.22 (1H, d, J = 1.8 Hz, H-1), 4.80 (1H, d, ^2J = 12.2 Hz, benzylic-H), 4.76-4.68 (5H, m, benzylic-H), 3.97 (1H, dq, $J_{4,5}$ = 9.1 Hz, $J_{5,6}$ = 6.2 Hz, H-5), 3.95 (3H, s, CO_2CH_3), 3.92 (1H, dd, $J_{2,3}$ = 3.1 Hz, $J_{3,4}$ = 9.3 Hz, H-3), 3.81 (1H, d, J = 9.3 Hz, H-4), 3.76 (1H, d, $J_{1,8}$ = 1.7 Hz, $J_{2,3}$ = 3.1 Hz, H-2), 1.43 (3H, d, J = 6.2 Hz, H-6).

^{13}C (125 MHz) δ (ppm): 161.1 (C=O), 140.3, 138.2, 138.2, 137.6 (4° Ar-C), 136.6 (C-5′), 128.3, 128.3, 128.3, 128.2, 128.0, 127.9, 127.7, 127.5, 127.5 (Ar-CH), 90.9 (C-1), 83.5 (C-3), 79.7 (C-2), 75.7 (C-4), 75.0, 74.6, 73.7 (benzylic-C), 67.3 (C-5), 51.1 (CO_2CH_3), 16.0 (C-6).

4.8.3 Copper Catalysis[135,136]

1-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-4-carboxymethyl-5-trimethylsilyl-1,2,3-triazole (103)



103

A 4 mL vial was charged with **4** (51 mg, 0.11 mmol), copper (II) sulfate pentahydrate [27 μg, (1 mL of a 27 μg/mL solution in water), 1.1 μmol, 1 mol %], sodium ascorbate (1.1 mg, 5.5 μmol, 5 mol %), and *t*-butanol (1mL). The mixture was stirred

vigorously for 18 hours at room temperature. After this time, the *t*-butanol was evaporated, and the aqueous phase brought up in diethyl ether (20 mL). The organic phase was washed 2 x 20 mL of water, and the organic phase was dried ($NaSO_4$), filtered and concentrated. This crude material was purified by silica gel chromatography (4:1 hexane/ethyl acetate) to give **103** as a colorless solid (54 mg, 90%). This material was identical to purified **103** isolated previously.

4.8.4 Deprotections

1-α-L-Rhamnopyranosyl)-4-carboxymethyl-1,2,3-triazole (114)

Method A: Deprotection of **106**

A 25 mL roundbottom flask was charged with **106** (30 mg, 74 μmol), methanol (5 mL) and sodium methoxide (1 mL of a 1 M solution in methanol, Zemplen conditions), and set to stir for 1 hour. After this time, the mixture was acidified to pH 4 with Amberlite-15 H^+ resin, filtered, and concentrated, to give pure **114** (19 mg, 92 %) as a colorless semi-solid.

Method B: Deprotection of **113**

A 25 mL roundbottom flask was charged with **113** (34 mg, 63 μmol), palladium hydroxide (Adam's catalyst, 5 mg), and methanol (10 mL). Hydrogen gas was then

bubbled through for 6 hours. The catalyst was removed by filtration, and the solvent evaporated. The material was purified by silica gel chromatography (10:1 $CHCl_3$/methanol) to afford **114** (13 mg, 78%) as a colorless semi-solid.

$[\alpha_D]$ (c = 0.5, H_2O): +41.0 °

M.S. (+ESI) : Calc. for $C_{10}H_{15}N_3O_6$ = 273, Found *m/z* 274 $(C_{10}H_{15}N_3O_6 + H)^+$

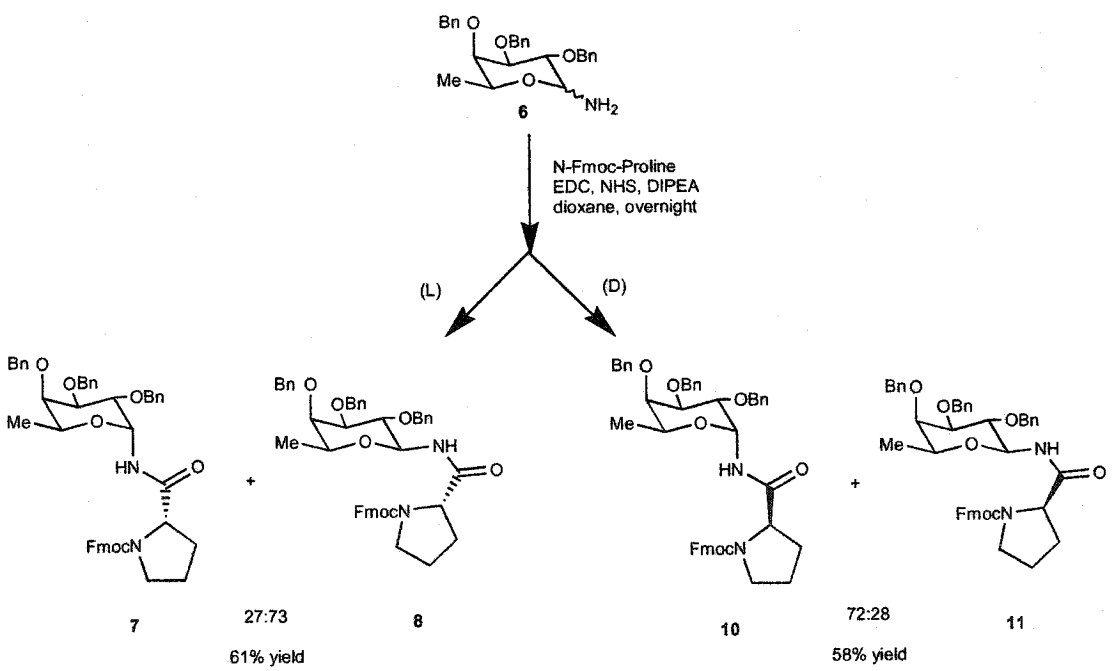
^1H (DMSO-d_6, 500 MHz) δ (ppm): 8.92 (1H, s, H-5′), 5.95 (1H, d, *J* = 3.9 Hz, H-1), 5.39 (1H, s, 2-O*H*), 5.10, 5.09 (2 x 1H, 2 x s, 3-O*H*, 4-O*H*), 4.40 (1H, bs, H-2), 3.83 (3H, s, CO_2CH_3), 3.81 (1H, m, H-3), 3.41 (2H, m, H-4, H-5), 1.19 (3H, d, C-6).

^{13}C (DMSO-d_6, 125 MHz) δ (ppm): 160.7 (C=O), 138.6 (C-4′), 129.2 (C-5′), 86.4 (H-1), 72.8, 72.3 (C-4, C-5), 71.1 (C-3), 68.3 (C-2), 51.9 ($CO_2\underline{C}H_3$), 17.8 (C-6).

5. α/β Selectivity When Forming *N*-Linked Glycopeptides

5.1 Introduction

As discussed in section **1.1**, formation of α-linked *N*-glycosides is not normally easy, although with certain sugars (such as rhamnose and mannose) α-linked products are exclusive.[78] Hence, the formation of the mixture of α- and β-linked products **7** and **8** (Section **2.2.1**) was rather typical. However, the second example (using (D)-proline) afforded predominantly α-product **10** (Scheme **5.1**).

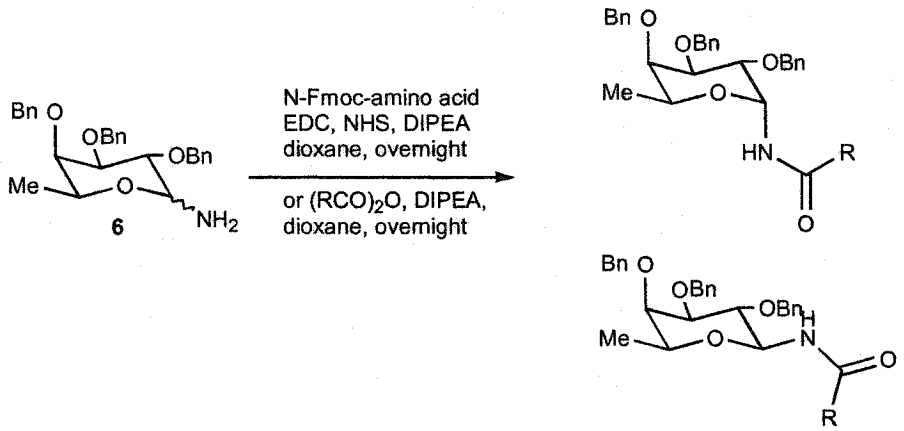


Scheme **5.1**: Coupling of (L)- or (D)-Proline Leads to Different Mixtures.

This type of selectivity is highly unusual, and led to the investigation of some coupling selectivities with fucose and other carbohydrates.

5.2 Selectivity When Forming *N*-Linked Fucopeptides

Fucosyl azide **4** was reduced and coupled with different amino acids or acid anhydrides (Scheme **5.2**). The products were separated by column chromatography and characterized. The results are presented in Table **5.1**.



Scheme **5.2**: Coupling of Fucosyl Amine **6** to Afford α/β Mixtures

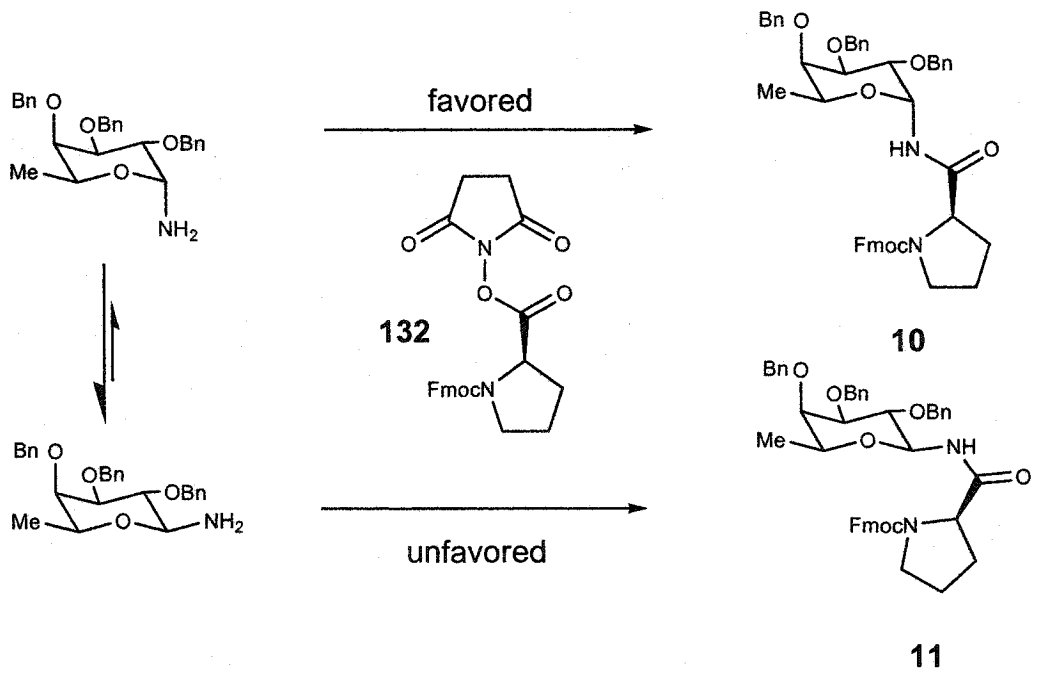
Amino Acid	Yield	α	β	Comp.	#s
N-Fmoc-(L)-Proline	61	27	73	**7**	**8**
N-Fmoc-(D)-Proline	58	78	22	**10**	**11**
N-Fmoc-*O*-*t*Bu-(L)-Tyrosine	60	28	72	**116**	**117**
N-Fmoc-*O*-*t*Bu-(D)-Tyrosine	89	28	72	**118**	**119**
N-Fmoc-(L)-Phenylalanine	79	0	100	**120**	**121**
N-Fmoc-(D)-Phenylalanine	80	39	61	**122**	**123**
N-Fmoc-*N*(Imid)-Trt-(L)-Histidine	24	5	95	**124**	**125**
N-Fmoc-(L)-Alanine	22	20	80	**126**	**127**
N-Fmoc-Glycine	95	14	86	**128**	**129**
Acetic Anhydride	96	19	81	**9 α**	**9β**
Phthalic Anhydride	81	10	90	**130**	**131**

Table **5.1**: Results of Coupling of Amino Acids or Acid Anhydrides With Amine **6**.

In many cases the purifications were not completely efficient and the ratios were determined by ^{1}H NMR. From previous examples (**7**, **8**, **10** and **11**), as well as certain examples from Table **5.1**, it was found that α- products show the H-1 proton at ~5.5-6.0 ppm in the ^{1}H NMR spectrum. When resolution is good, it appears as a clean doublet of doublets; coupling to the amide N-H is always observed. For β-products, H-1 appears from 4.85-5.10, and appears as a triplet. Figures **5.1** and **5.2** show examples of the separated α- and β-products **116** and **117**, with the H-1 protons marked on the spectrum. **115** is not perfectly pure, but the difference is clear. All examples of pure compounds show these characteristic peak shifts. The purpose of these experiments is to observe the α/β- ratios of the formed products, not to obtain the compounds themselves.

Disappointingly, the trend observed with (D)-proline could not be extended to other amino acids, although there were some other deviations from what would normally be expected (15-25 % α-product). Most notable is the case of (L)-phenylalanine, where β-product was formed exclusively.

The appearance of a deviation in α/β ratio would likely be the result of large differences in energy between the two possible "diastereomeric" complexes just prior to bond formation. The active ester of the amino acid would be chiral, and hence exhibit a "match-mismatch" pairing with the incoming amine, where one anomer is sterically less available for coupling than the other (Scheme **5.3**).

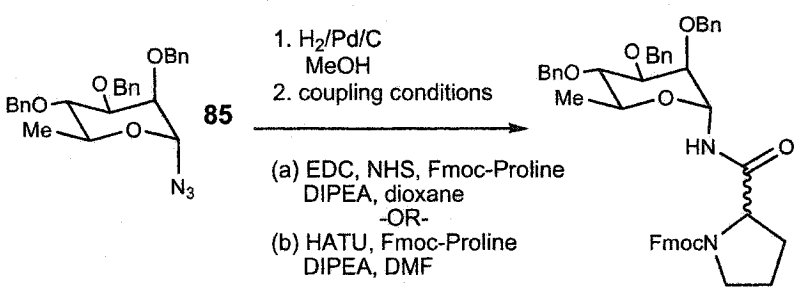


Scheme **5.3**: Match-Mismatch Pairing Leads to Favor of the α-Product

In order to investigate the special case that (D)-proline seems to be, this reaction was extended to other systems.

5.3 Application to Other Carbohydrates

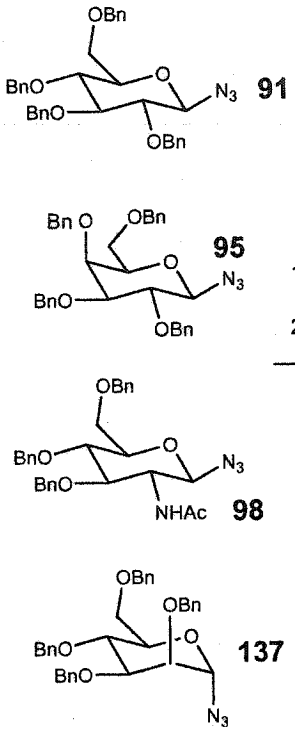
The electronically similar carbohydrate (L)-rhamnose was the first to be explored in this manner. Due to the preferred axial configuration at the 2-position, α-products are normally exclusive.[78] This was the case for both (L)- and (D)-proline. The reaction proceeded in poor yield (~35 %) with EDC/NHS; reaction with the more potent HATU provided an increase in yield but did not change the observed products (Scheme **5.4**).



	Method/Yield		
	a	b	
(L)	34	72	**133/134**
(D)	36	71	**135/136**

Scheme **5.4**: Reduction and Coupling of α-Azido Perbenzylated Rhamnose **85**

The reaction was attempted with other azido sugars available from previous work. However, reaction with EDC/NHS conditions afforded none of the desired *N*-linked glycopeptides. Reaction with HATU afforded coupling, but only in ratios typical for the carbohydrates involved[78] (Scheme **5.5**). The effect exhibited by (D)-proline in the case of fucose never appeared in any of the other carbohydrates involved. Why this case remains unique is not known.



1. H_2/Pd/C, MeOH

2. HATU, Fmoc-Proline DIPEA, DMF

Sugar	(L) or (D)	Yield	alpha	beta	
Glu	L	62	20	80	**138/139**
	D	64	20	80	**140/141**
Gal	L	63	22	78	**142/143**
	D	65	24	76	**144/145**
Glu-NAc	L	58	20	80	**146/147**
	D	57	20	80	**148/149**
Man	L	70	100	0	**150/151**
	D	71	100	0	**152/153**

Scheme **5.5**: Coupling of Sugar Amines with (L)- or (D)-Proline

5.4 Conclusions

A series of *N*-linked sugar-proline conjugates were prepared, and attempts were made to build upon an observed example of match-mismatch pairing with (D)-proline. Other examples with such striking contrast were not found, however. The example with (D)-proline and (L)-fucose remained the only example favoring α-stereochemistry.

5.5 Spectra Referred to Throughout Chapter 5

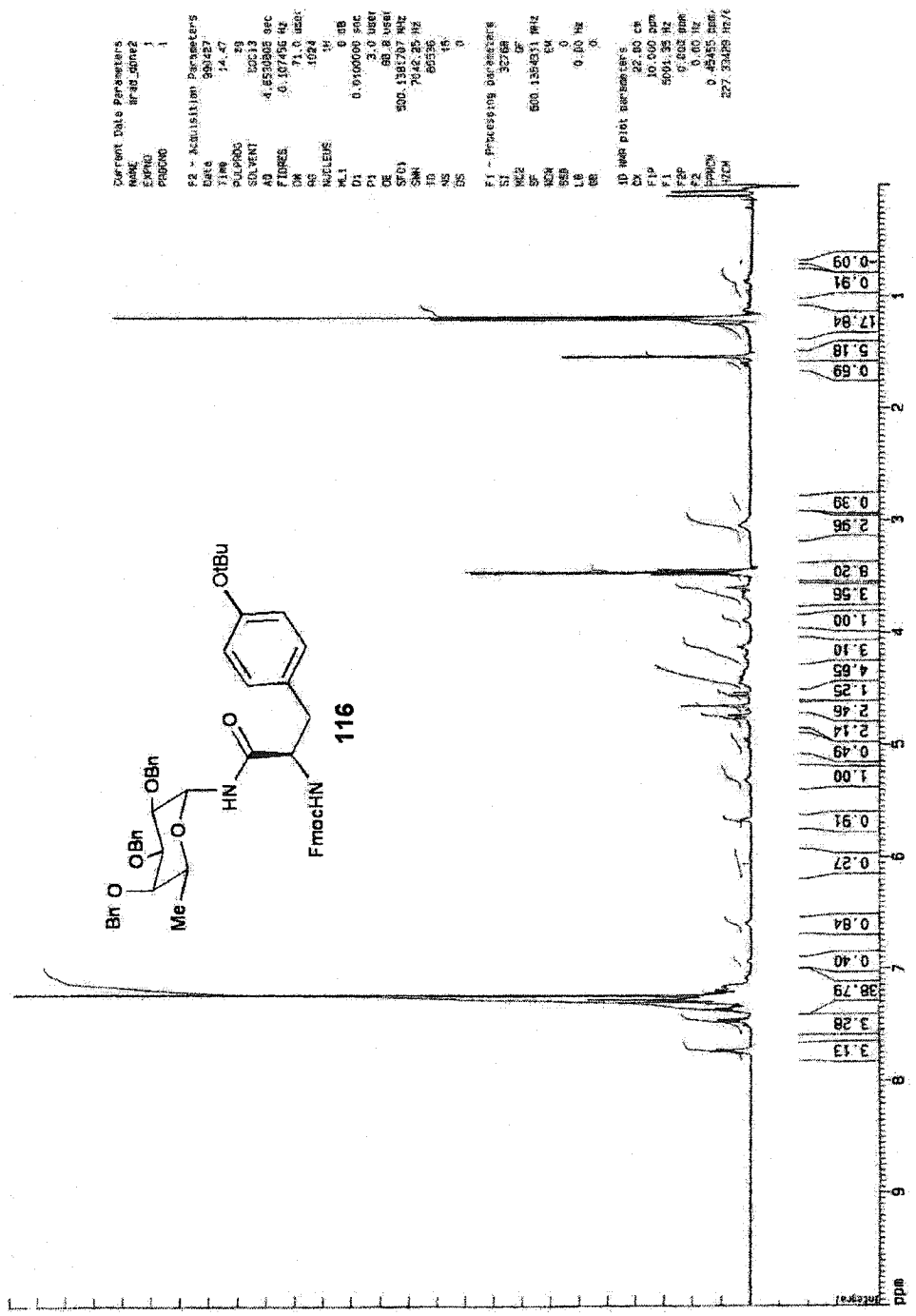


Figure **5.1**: ^{1}H NMR Spectrum ($CDCl_3$, 500 MHz) of **116**.

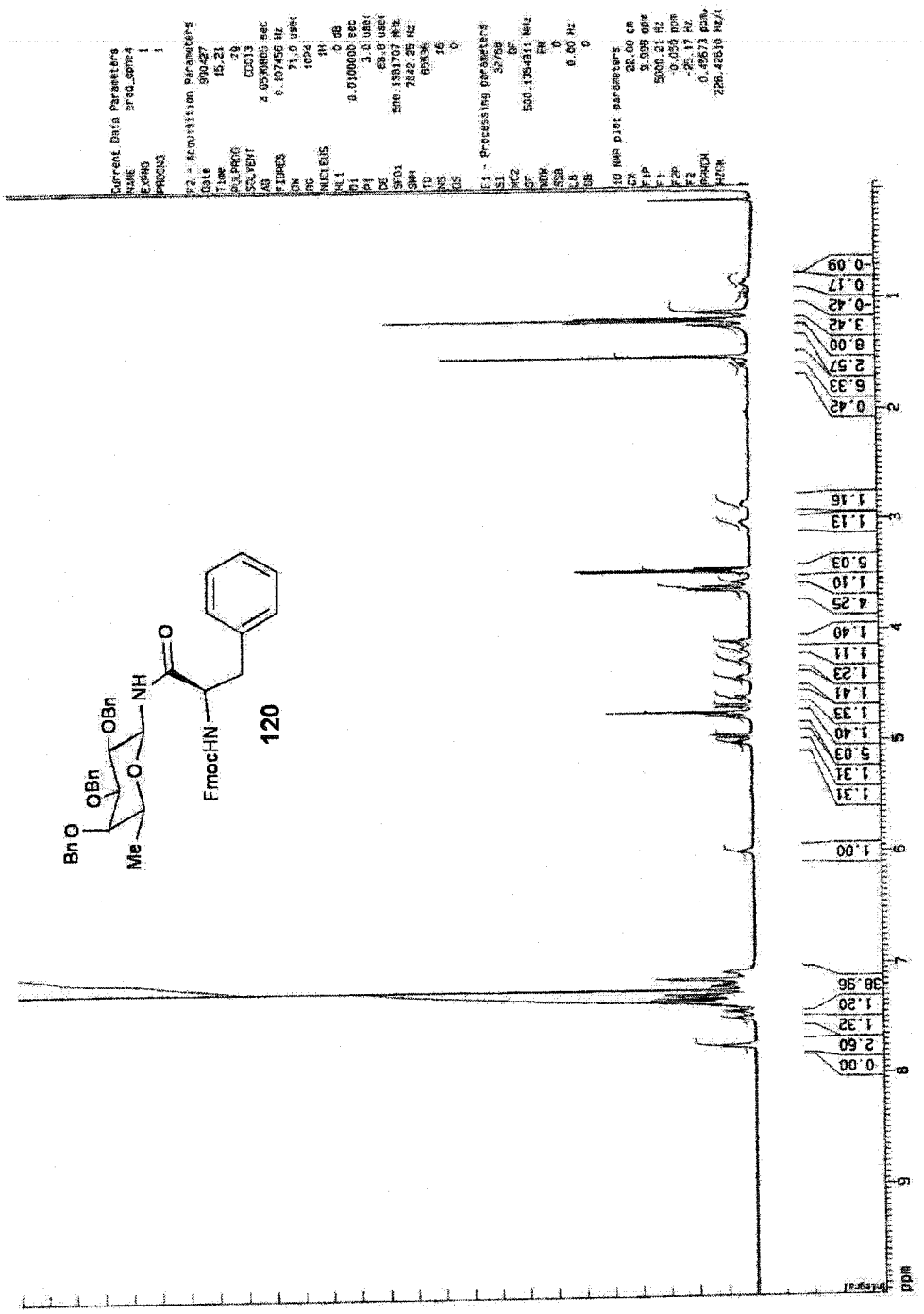


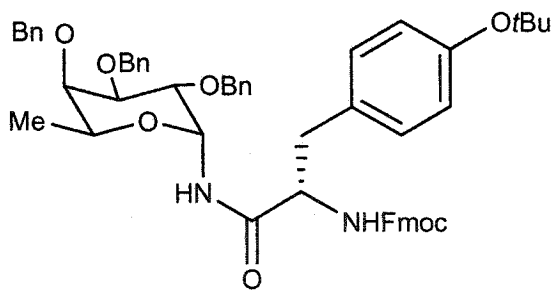
Figure **5.2**: ^{1}H NMR Spectrum ($CDCl_3$, 500 MHz) of **120**.

5.6 Preparation of Compounds

General Procedures:

The preparations of **7** and **8** serve as a model for the reduction of an azido compound followed by either EDC or HATU coupling. Most compounds described in this section were isolated as mixtures and their ratios determined by ^{1}H NMR analysis. Relative amounts of α and β compounds were determined by integration of the H-1 protons in the ^{1}H NMR spectrum. However, some were isolated in pure form (or nearly so) and are listed below.

_N_-Fmoc-_O_-_t_Bu (_N_'-2,3,4-tri-O-benzyl-α-L-fucopyranosyl)- L-tyrosine (116)



116

m.p. 118-121 °C

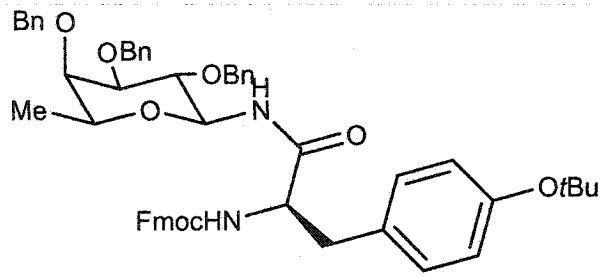
M.S. (+ESI) : Calc. for $C_{55}H_{58}N_2O_8$ = 874, Found *m/z* 875 $(C_{55}H_{58}N_2O_8 + H)^+$

$[\alpha_D]$ (c = 1.0, $CHCl_3$) –31.3°

^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 7.73 (2H, d, J = 7.5 Hz, Tyr-H), 7.51 (2H, m, Ar-H), 7.37 (2H, m, Ar-H), 7.29 (17 H, m, Ar-H), 7.09 (2H, bs, Ar-H), 6.86 (2H, d, J = 7.5 Hz, Tyr-H), 6.47 (1H, bs, amide N-H), 5.65 (1H, t, J = 5.1 Hz, H-1), 5.40 (1H, bs, carbamate N-H), 4.82 (1H, d, J = 11.7 Hz, benzylic-H), 4.65 (1H, d, J = 11.5 Hz, benzylic-H), 4.65 (1H, d, J = 11. Hz, benzylic-H), 4.57 (1H, d, J = 11.7 Hz, benzylic-H), 4.52 (1H, d, J = 11.6 Hz, benzylic-H), 4.47 (1H, d, J = 11.5 Hz, benzylic-H), 4.36 (2H, m, Fmoc-CH_2), 4.25 (1H, m, α-Tyr), 4.15 (1H, m, Fmoc-CH), 4.00 (1H, dd, J = 4.8 Hz, J = 8.8 Hz, H-2), 3.46 (1H, m, H-4), 3.34 (2H, m, H-3, H-5), 3.00 (2H, m, β-Tyr), 1.28 (9H, s, *t*-butyl), 1.14 (3H, d, J = 6.3 Hz, H-6).

^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 171.6 (C=O amide), 154.5 (C=O carbamate), 143.7, 141.3, 138.5, 138.4, 137.6, 129.7, 128.4, 128.4, 128.3, 128.2, 128.0, 127.9, 127.7, 127.6, 127.6, 127.4, 127.1, 125.1, 125.1 (Ar-C), 124.2, 120.0 (Tyr-CH), 78.4, 67.8 (C-3, C-5), 76.25 (C-4), 74.8 (C-2), 74.2 (C-1), 73.1, 72.8, 72.7 (benzylic-C), 65.8 ($\underline{C}(CH_3)_3$), 56.4 (α-Tyr), 47.1 (Fmoc-CH), 34.7 (β-Tyr), 31.6 (Fmoc-CH_2), 28.1 ($C(\underline{C}H_3)_3$), 16.3 (C-6).

_N_-Fmoc-_O_-_t_Bu (_N'_-2,3,4-tri-O-benzyl-β-L-fucopyranosyl)-L-tyrosine (117)



117

m.p. 123.0-123.6 °C

M.S. (+ESI) : Calc. for $C_{55}H_{58}N_2O_8$ = 874, Found *m/z* 875 $(C_{55}H_{58}N_2O_8 + H)^+$

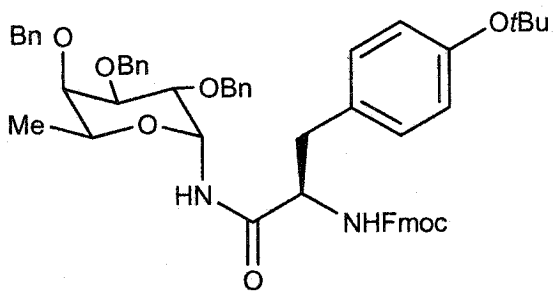
$[\alpha_D]$ (c = 0.25, $CHCl_3$) –11.0°

^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 7.72 (2H, d, *J* = 7.6 Hz, Tyr-H), 7.47 (2H, d, *J* = 7.4 Hz), 7.24 (19H, m, Ar-H), 6.99 (2H, m, Ar-H), 6.82 (2H, d, *J* = 7.3 Hz, Tyr-H), 6.45 (1H, m, amide N-H), 5.10 (1H, t, *J* = 9.2 Hz, H-1), 5.10 (1H, bs, NH-carbamate), 4.94 (1H, d, *J* = 11.5 Hz, benzylic-H), 4.72 (3H, m, benzylic-H), 4.66 (2H, d, *J* = 11.5 Hz, benzylic-H), 4.37 (1H, m, Fmoc-CH_2), 4.32 (1H, m, Fmoc-CH_2), 4.24 (1H, m, Fmoc-CH), 4.10 (1H, m, α-Tyr), 3.70 (1H, t, *J* = 9.2 Hz, H-2), 3.65 (1H, dd, *J* = 2.5 Hz, *J* = 9.4 Hz, H-3), 3.61 (1H, d, *J* = 2.1 Hz, H-4), 3.59 (1H, q, *J* = 6.4 Hz, H-5), 3.07 (1H, m, β-Tyr), 2.95 (1H, m, β-Tyr), 1.27 (9H, s, $C(CH_3)_3$, 1.12 (3H, d, *J* = 6.3 Hz, H-6).

^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 171.2 (C=O amide), 154.4 (C=O carbamate), 143.7, 141.3, 138.2, 138.1, 129.9, 128.5, 128.5, 128.4, 128.3, 128.1, 127.8, 127.8,

127.8, 127.7, 127.6, 127.1, 127.1, 125.1, 125.0 (Ar-C), 124.2, 119.9 (Tyr-CH), 83.9 (C-3), 79.3 (C-1), 77.2 (C-2), 76.4 (C-4), 74.9, 74.7, 72.9 (benzylic-C), 72.3 (C-5), 67.1 ($\underline{C}(CH_3)_3$), 56.0 (α-Tyr), 47.1 (Fmoc-CH), 37.2 (β-Tyr), 37.2 (Fmoc-CH_2), 28.8 ($C(\underline{C}H_3)_3$), 16.9 (C-6).

_N_-Fmoc-_O-t_Bu (_N'_-2,3,4-tri-O-benzyl-α-L-fucopyranosyl)- D-tyrosine (118)



118

m.p. 86-87 °C

M.S. (+ESI) : Calc. for $C_{55}H_{58}N_2O_8$ = 874, Found *m/z* 875 $(C_{55}H_{58}N_2O_8 + H)^+$

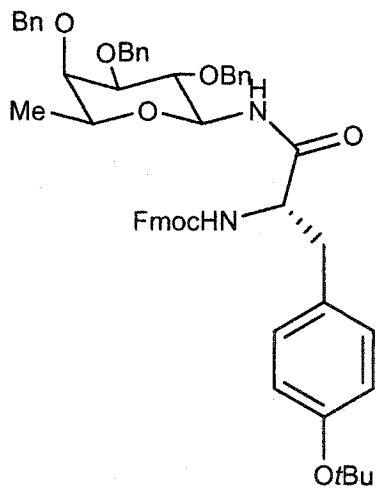
$[\alpha_D]$ (c = 1.0, $CHCl_3$) +41.1 °

^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 7.72 (2H, d, *J* = 7.5 Hz, Tyr-H), 7.51 (2H, m, Ar-H), 7.38 (2H, m, Ar-H), 7.31 (17H, m, Ar-H), 7.05 (2H, m, Ar-H), 6.86 (2H, d, *J* = 7.5 Hz, Tyr-H), 6.39 (1H, bs, amide N-H), 5.67 (1H, dd, *J* = 4.7 Hz, *J* = 6.4 Hz, H-1), 5.34 (1H, bs, carbamate N-H), 4.86 (1H, d, *J* = 11.6 Hz, benzylic-H), 4.67 (1H, d, *J* = 11.6 Hz, benzylic-H), 4.65 (1H, d, *J* = 11.8 Hz, benzylic-H), 4.59 (1H, d, *J* = 11.2 Hz, benzylic-H), 4.51 (1H, d, *J* = 11.2 Hz, benzylic-H), 4.44 (1H, d, *J* = 11.8 Hz, benzylic-H), 4.34 (2H, m, Fmoc-C*H*$_2$), 4.25 (1H, m, α-Tyr), 4.10 (1H, m, Fmoc-H),

4.03 (1H, dd, J = 4.7 Hz, J = 8.5 Hz, H-2), 3.45 (1H, m, H-4), 3.32 (2H, m, H-3, H-5), 2.95 (2H, m, β-Tyr), 1.21 (9H, s, *t*-butyl), 1.18 (3H, d, J = 6.3 Hz, H-6).

^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 171.8 (C=O amide), 154.5 (C=O carbamate), 143.2, 141.8, 138.9, 138.2, 137.0, 129.9, 129.4, 128.5, 128.3, 128.2, 128.1, 127.7, 127.7, 127.6, 127.6, 127.5, 127.0, 125.0, 124.3 (Ar-C), 124.0, 121.5 (Tyr-CH), 77.4, 68.8 (C-3, C-5), 76.1 (C-4), 74.5 (C-2), 74.0 (C-1), 73.5, 72.5, 72.2 (benzylic-C), 64.2 ($\underline{C}(CH_3)_3$), 54.5 (α-Tyr), 46.7 (Fmoc-CH), 34.2 (β-Tyr), 31.1 (Fmoc-CH_2), 29.7 ($C(\underline{C}H_3)_3$), 16.7 (C-6).

_N_-Fmoc-_O_-_t_Bu (_N'_-2,3,4-tri-O-benzyl-β-L-fucopyranosyl)-D-tyrosine (119)



119

m.p. 104.1-104.9 °C

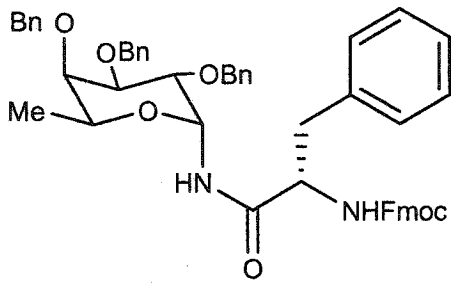
M.S. (+ESI) : Calc. for $C_{55}H_{58}N_2O_8$ = 874, Found *m/z* 875 $(C_{55}H_{58}N_2O_8 + H)^+$

^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 7.70 (2H, d, J = 7.5 Hz, Tyr-H), 7.45 (2H, d, J = 7.5 Hz), 7.26 (19H, m, Ar-H), 7.01 (2H, m, Ar-H), 6.86 (2H, d, J = 7.5 Hz, Tyr-H), 6.70 (1H, m, amide N-H), 5.13 (1H, t, J = 9.0 Hz, H-1), 5.07 (1H, bs, NH-carbamate), 4.97-4.70 (6H, m, benzylic-H), 4.39 (1H, m, Fmoc-CH_2), 4.31 (1H, m, Fmoc-CH_2), 4.27 (1H, m, Fmoc-CH), 4.21 (1H, m, α-Tyr), 3.75 (1H, t, J = 8.8 Hz, H-2), 3.65 (1H, dd, J = 2.1 Hz, J = 9.2 Hz, H-3), 3.59 (2H, m, H-4, H-5), 3.11 (1H, m, β-Tyr), 2.99 (1H, m, β-Tyr), 1.24 (9H, s, $C(CH_3)_3$, 1.15 (3H, d, J = 6.4 Hz, H-6).

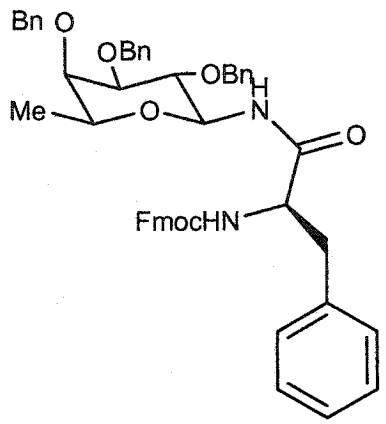
^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 171.0 (C=O amide), 154.1 (C=O carbamate), 143.1, 141.1, 139.2, 138.1, 129.7, 128.7, 128.5, 128.4, 128.1, 128.1, 128.0, 127.7, 127.6, 127.6, 127.6, 127.5, 127.1, 127.0, 125.0, 124.9 (Ar-C), 124.0, 119.7 (Tyr-CH), 83.6 (C-3), 79.1 (C-1), 77.6 (C-2), 76.8 (C-4), 74.2, 74.2, 72.4 (benzylic-C), 72.8 (C-5), 66.6 ($\underline{C}(CH_3)_3$), 57.2 (α-Tyr), 47.7 (Fmoc-CH), 36.5 (β-Tyr), 32.2 (Fmoc-CH_2), 27.9 ($C(\underline{C}H_3)_3$), 16.3 (C-6).

_N_-Fmoc (_N'_-2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-L-phenylalanine (120)

_N_-Fmoc (_N'_-2,3,4-tri-O-benzyl-β-L-fucopyranosyl)-L-phenylalanine (121)



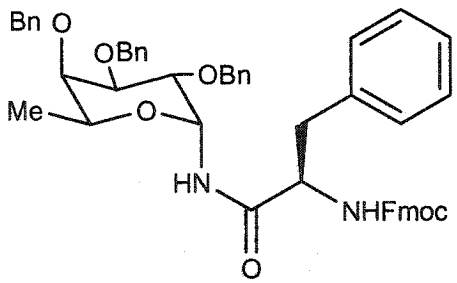
120



121

The compounds were not separable. The ^{1}H NMR spectrum clearly showed the ratio of α to β, but would not allow for full interpretation.

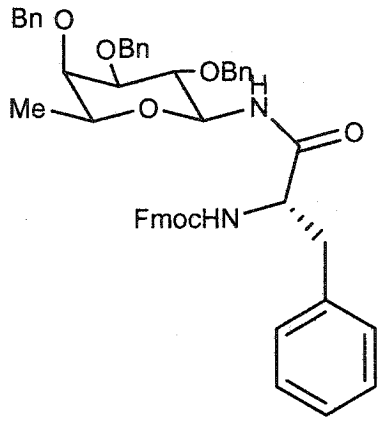
_N_-Fmoc (_N'_-2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-D-phenylalanine (122)



122

This compound was not pure enough to allow for spectral interpretation (contamination by **123**).

_N_-Fmoc (_N'_-2,3,4-tri-O-benzyl-β-L-fucopyranosyl)-D-phenylalanine (123)



123

^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 7.75 (2H, m, Ar-H), 7.50 (1H, d, J = 7.4 Hz, Ar-H), 7.43 (1H, d, J = 7.5 Hz, Ar-H), 7.24 (25 H, m, Ar-H + amide N-H), 6.06 (1H, d, J = 9.2 Hz, carbamate N-H), 5.03 (1H, t, J = 9.2 Hz, H-1), 4.96 (1H, d, J = 11.7 Hz, benzylic-H), 4.75 (3H, m, benzylic-H), 4.68 (1H, d, J = 7.6 Hz, benzylic-H), 4.61 (1H, d, J = 12.0 Hz, benzylic-H), 4.50 (1H, m, Fmoc-CH_2), 4.43 (1H, m, α-Phe), 4.28 (1H, m, Fmoc-CH_2), 4.10 (1H, t, J = 6.5 Hz), 3.66 (3H, m, H-2, H-3, H-4), 3.58 (1H, q, J = 6.4 Hz, H-5), 3.03 (1H, m, β-Phe), 2.87 (1H, m, β-Phe), 1.13 (3H, d, J = 6.4 Hz, H-6).

^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 170.8 (amide CO), 155.8 (carbamate CO), 143.8, 143.7, 141.3, 141.3, 138.3, 138.2, 138.2, 136.1, 129.5, 129.3, 128.5, 128.5, 128.5, 128.3, 127.9, 127.8, 127.8, 127.7, 127.7, 127.6, 127.1, 127.0, 126.9, 125.0, 125.0, 119.9, 119.9 (Ar-C), 84.1 (C-3), 78.9 (C-1), 76.7 (C-2), 76.4 (C-4), 76.3, 74.8,

72.2 (benzylic-C), 72.9 (C-5), 66.6 ($\underline{C}(CH_3)_3$), 55.8 (α-Phe), 47.1 (Fmoc-CH), 37.7 (β-Phe), 29.6 ($C(\underline{C}H_3)_3$), 16.83 (C-6).

Compounds **127** through **131** were not isolated pure; rather, their relative amounts were assessed by ^{1}H NMR. All displayed correct $(M + H)^+$ peaks in the mass spectrum.

Compounds **133** and **135** were purely α, as determined by ^{1}H NMR. However, resolution of these spectra was terrible (see Figure **5.3** for the ^{1}H NMR of **135**).

The same holds true for **138-153**. In each case (excepting mannose) the relative anomeric ratios were determined by ^{1}H NMR. Total integrations of peaks in the NMR spectrum added up to the proper number, using H-1 as a standard (in most cases, H-6 was also clear and could be standardized for 3H). In the case of mannose, the α-material was the sole product; however, having practically the same structure as rhamnose, it should not be surprising that the spectra displayed poor resolution.

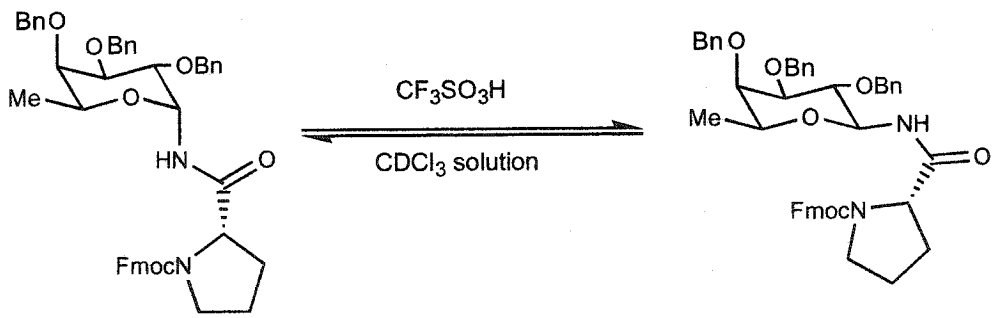
6. Acid-Catalyzed Epimerization of *N*-Linked Fucose-Proline Conjugates

6.1 Introduction

Anomerically pure *N*-linked conjugates have been shown in this work to be stable as neat solids for long periods of time, showing no signs of epimerization.[143] However, in the presence of strong Bronsted acid, an example of equilibration between anomers was observed.

6.2 Observation and Monitoring of Acid-Catalyzed Epimerization

N-linked fucose-proline conjugate **7** was epimerized in $CDCl_3$ using catalytic trifluoromethanesulfonic acid (Scheme **6.1**).



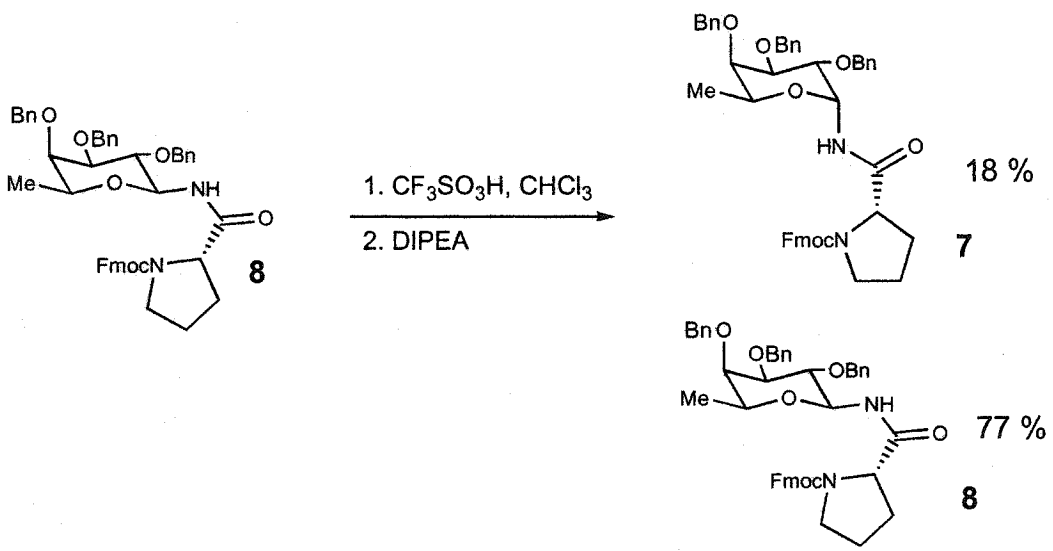
7 8

Scheme **6.1**: Acid-Catalyzed Anomerization of Fucose-Proline Conjugates

This anomerization could be observed by ^{1}H NMR. By integration of the H-1 signals (which are diagnostic and well-separated from other peaks) the ratio can be seen to reach an equilibrium of approximately 20:80 in favor of the β-anomer, irrespective of which anomer of conjugate is used.

6.2.1 Synthetic Studies

An experiment was performed to evaluate the validity of the aforementioned observation (Scheme **6.2**).



Scheme **6.2**: Acid-Catalyzed Epimerization and Quenching

Approximately 100 mg of pure β- **8** was dissolved in chloroform and 0.05 equivalents of trifluoromethanesulfonic acid were added. After stirring for 90 minutes at room temperature, diisopropylethylamine (1 equivalent) was added, and the products isolated by column chromatography.

Pure α-anomer **7** was isolated in 18 % yield, in addition to 77 % of the β-isomer **8**. The missing 5 % of material is presumed to be lost during purification.

6.2.2 NMR Studies

The acid-catalyzed anomerization was performed and followed by ^{1}H NMR for all four fucose-proline conjugates (**7**, **8**, **10**, and **11**). All exhibited the same sort of behaviour, reaching equilibrium at approximately 70 minutes. Figure **6.1** shows ^{1}H NMR behaviour for the relevant spectral regions of **10** as the reaction proceeds.

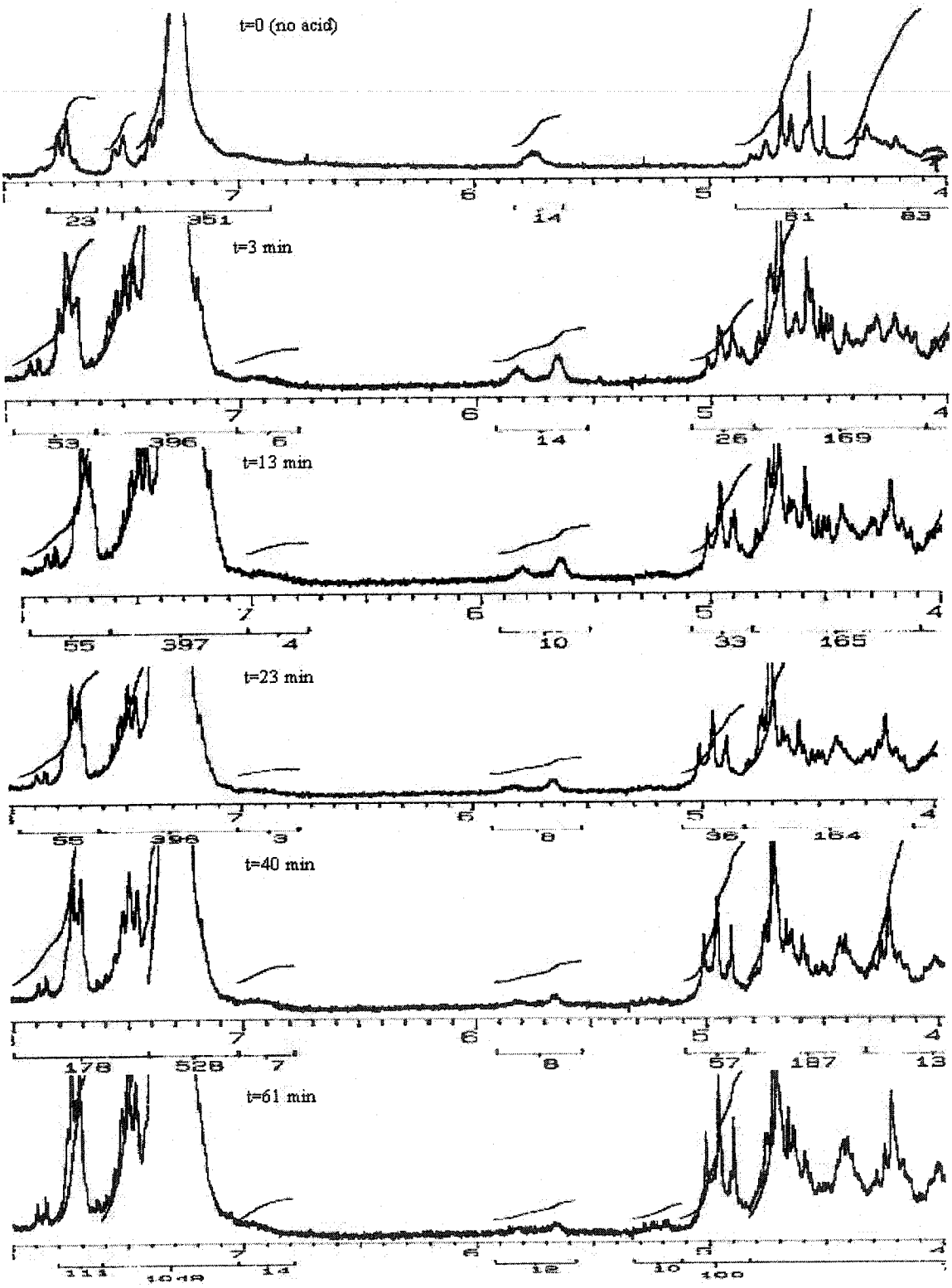


Figure **6.1**: Acid-Catalyzed Epimerization of **10**, Followed by ^{1}H NMR (200 MHz).

α-Proton: 5.75 ppm; β-Proton: 4.95 ppm.

Chart **6.1** shows the relative ratios of each anomer during the course of the reaction.

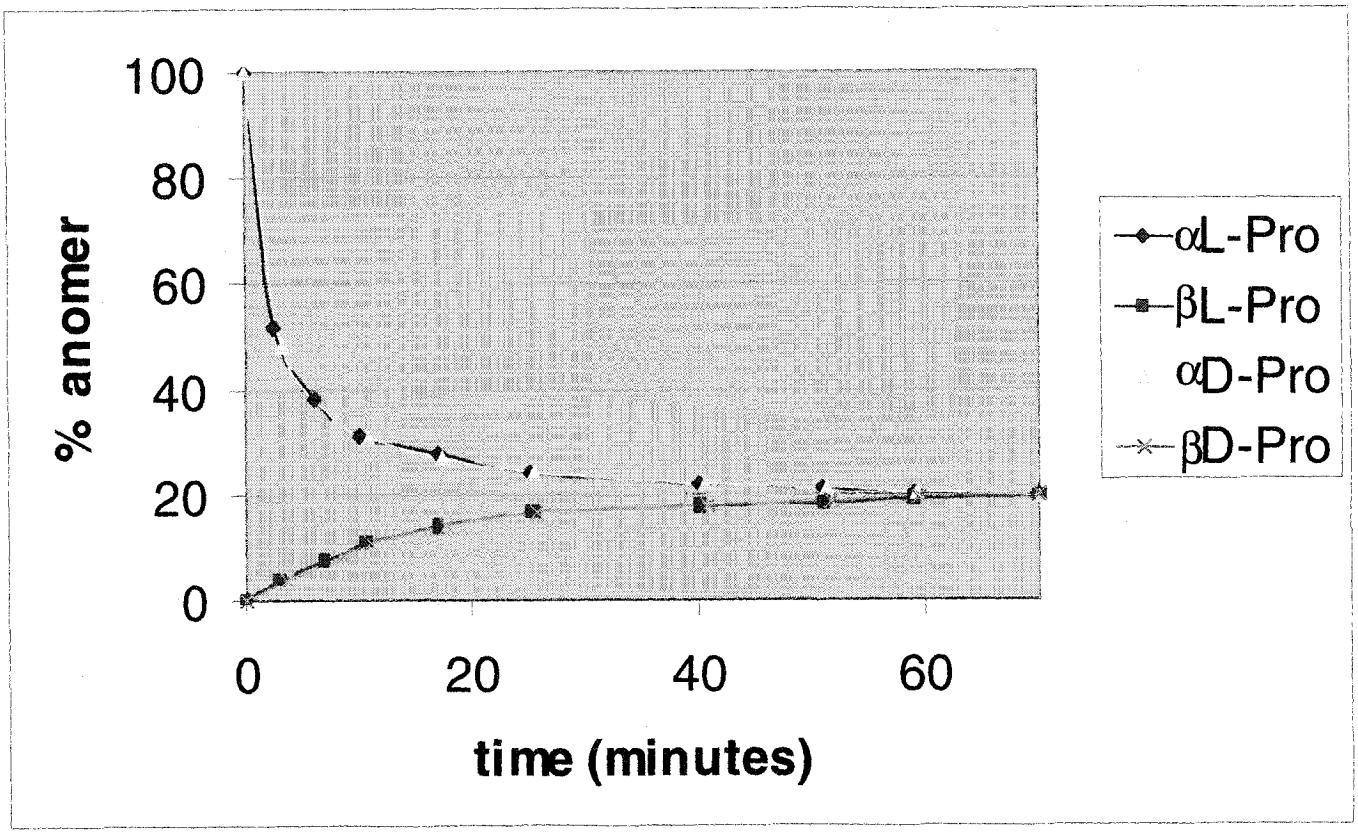
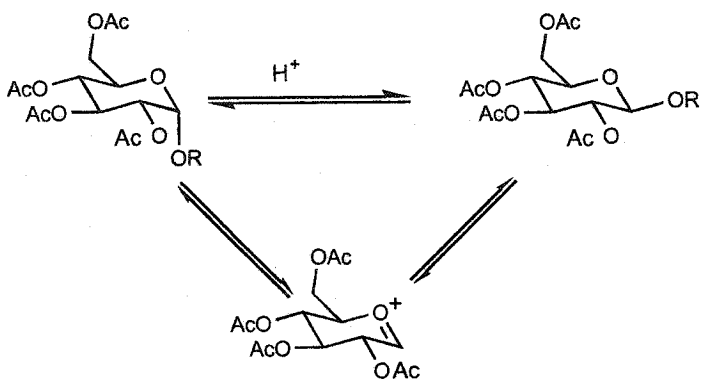


Chart **6.1**: Plot of the % α-Anomer (Y-Axis) vs Time for Each Proline Conjugate

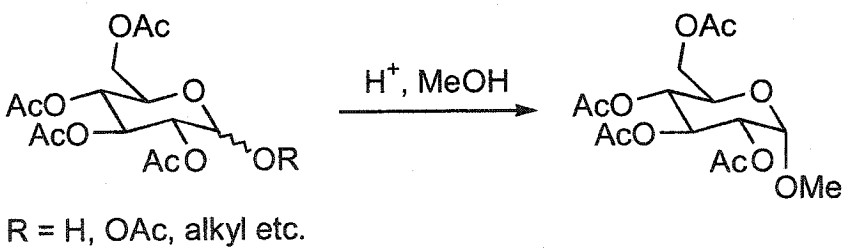
6.3 Mechanistic Insights

Such anomerization of *O*-glycosides is well known (Scheme **6.3**).[144]



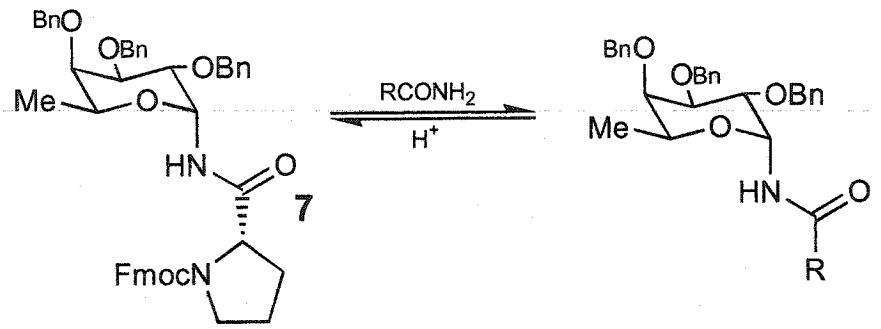
Scheme **6.3**: Acid-Catalyzed Anomerization of *O*-Glycosides

If the reaction is performed in alcoholic solvent, the product is the glycoside of the solvent itself. For example, in methanol, methyl glycosides are formed (Scheme **6.4**).[145]



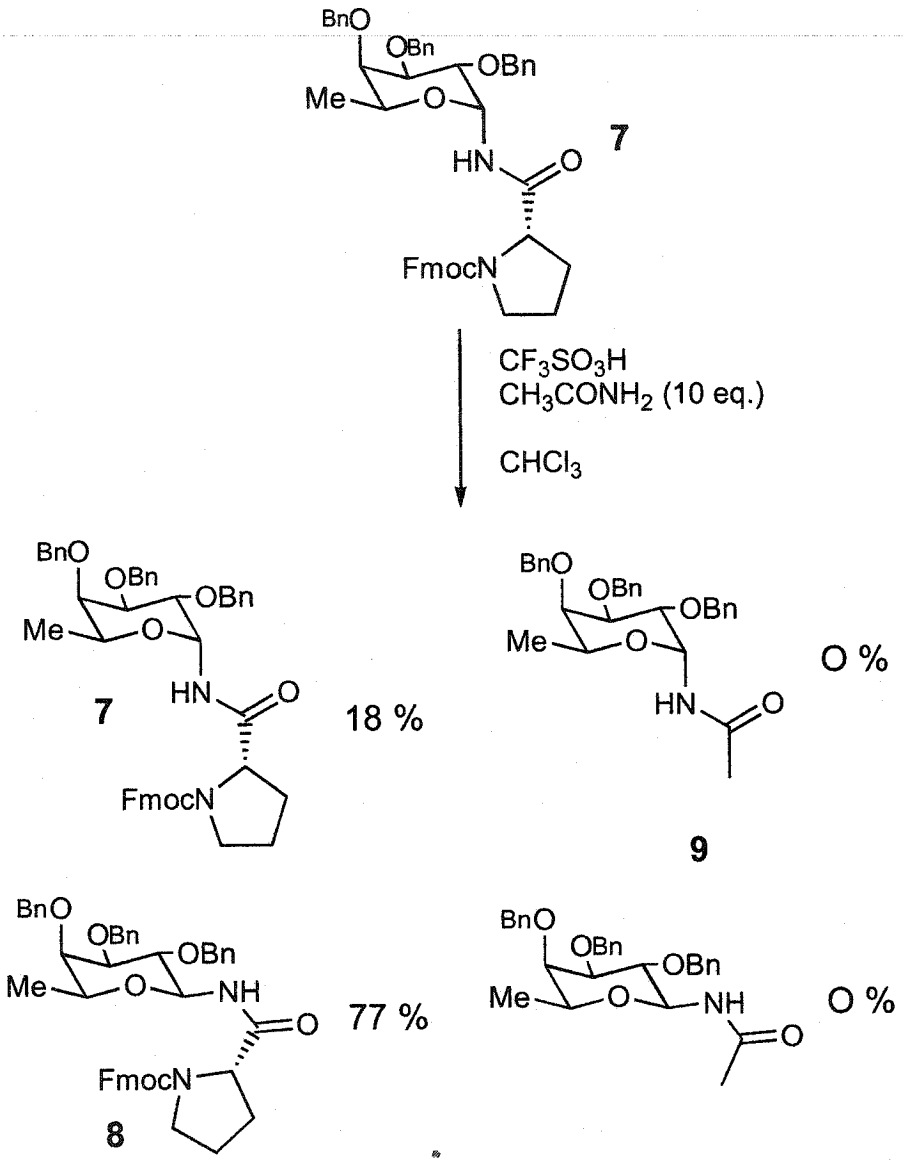
Scheme **6.4**: Formation of Methyl Glycosides by Acid Catalysis

If this sort of mechanism is predominant, then it should follow that amide groups could be exchanged in a similar fashion (Scheme **6.5**).



Scheme **6.5**: An Oxonium Ion-Dissociation Mechanism Should Allow Exchange

An experiment was devised where excess acetamide would be present, which in the event of exchange would afford compound **9,** known from section **2**. The reaction was performed in chloroform initially (Scheme **6.6**) and afforded only anomerized products **7** and **8**. No exchanged product was detected.

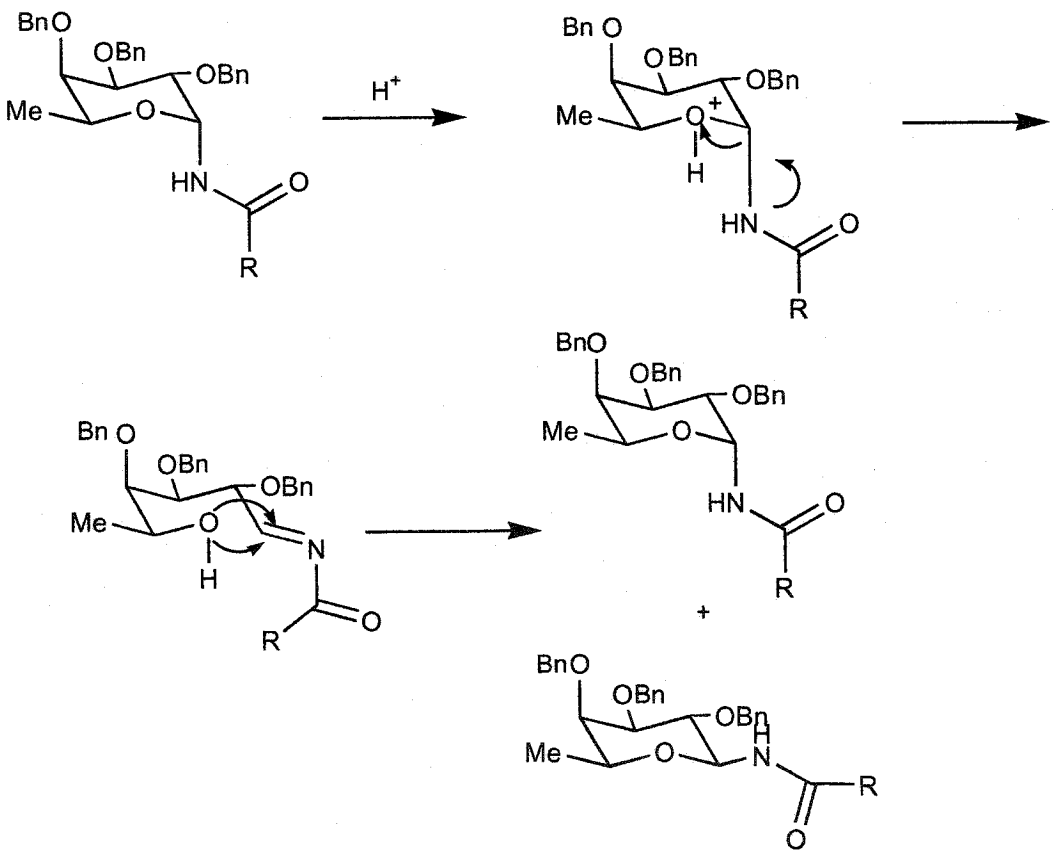


Scheme **6.6**: Acid-Catalyzed Exchange Does Not Occur

This reaction was also performed in other solvents, to exclude the possibility of tight-ion pairing. Reaction in DMF, DMSO and THF lead to different rates of

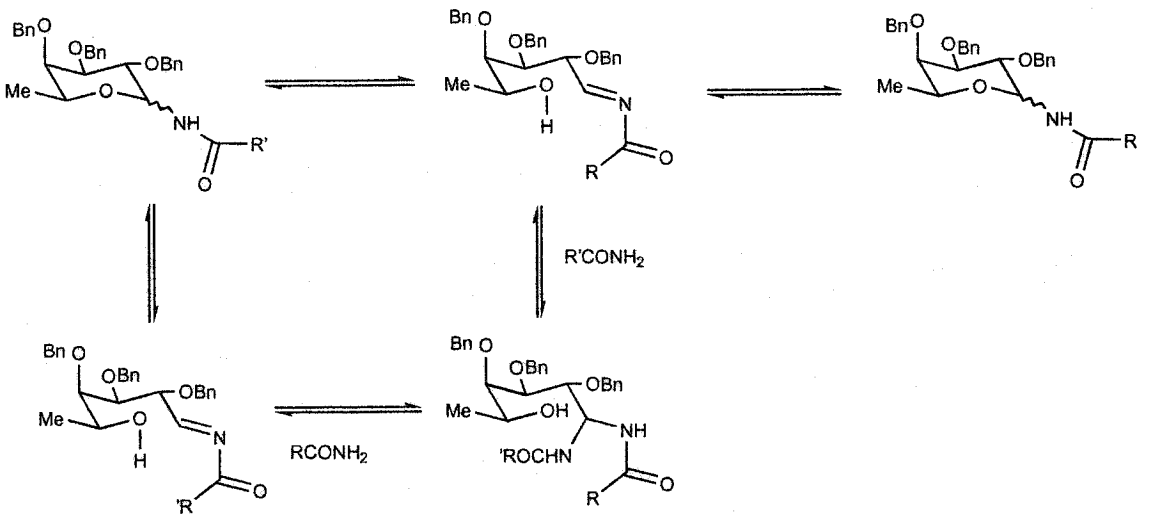
anomerization (as judged by TLC) but did not afford any exchanged product. Even reaction in pure acetamide does not give exchange.

In the absence of an oxonium mechanism, the following ring-opening mechanism is proposed (Scheme **6.7**). Such a mechanism is not known to occur for *N*-linked compounds.



Scheme **6.7**: Proposed Mechanism for Acid-Catalyzed Epimerization of *N*-Glycosides

Since exchange does not occur to any noticeable extent, it can be concluded that a ring-opening mechanism is either the exclusive or the very predominant mechanism for anomerization. One could imagine, however, that exchange may eventually occur even with such a mechanism (Scheme **6.8**). However, it can be presumed that such competition is either not occurring or is sufficiently slow to prevent noticeable exchange.



Scheme **6.8**: Exchange May Still Occur via Ring-Opening Mechanism

6.4 Conclusions

Acid-catalyzed anomerization of *N*-linked fucose-proline conjugates has been observed for all four stereoisomers, followed by ^{1}H NMR, and products isolated and confirmed. A ring-opening mechanism has been proposed which would be congruent with the observation of lack of amide exchange.

6.5 Experimental

^{1}H NMR spectra were measured at 200 MHz, with the reaction occurring in the tube. A solution of the compound (10 mg) in $CDCl_3$ (0.5 mL) was placed in the NMR tube, a spectrum was recorded, and 1 μL of trifluoromethanesulfonic acid was added, the tube shaken, and spectra immediately recorded at regular intervals. If the recovered anomers are desired, 2 drops of diisopropylethylamine are added, and the anomers separated by chromatography (>95% recovery).

All experiments were performed at similar concentrations, irrespective of the solvent(s) used.

CONCLUSIONS

The solution and solid-phase synthesis of *N*-glycopeptidomimetics of Sialyl Lewis^x was accomplished, albeit with questionable purity, and more work devising convergent syntheses of tartaric acid based mimetics were also fruitful.

The development of *C*-linked glycopeptidomimetics led to a convergent sequence involving Heck coupling and protected tartaric acid fragments. The final deprotections proved difficult; however, the penultimate compounds were synthesized with good efficiency.

A novel palladium catalyzed phenyl transfer reaction was discovered whereby triphenylantimony is used to donate phenyl groups onto alkenes until trisubstituted. It was observed that allylic hydrogens were necessary for success, and the 3-iodo-Boc-aniline compound was required for the reaction, but did not appear in the product.

α/β selectivity when reacting glycosyl amines with amino acids was explored. Although initially a good example was found (involving (L)- and (D)-proline) other examples were not found. The rather typical rotamer effects in NMR spectra continued to frustrate efforts to properly characterize the synthesized compounds.

Glycosyl triazoles were synthesized as a glycosyl proline isostere. The general methodology was developed, and the application of copper (I) catalysis to encourage

single isomer formation was performed. The regioselectivity was confirmed through X-ray crystallography.

The acid-catalyzed anomerization of fucose-proline conjugates was observed, and some crude kinetics experiments were monitored. Single anomers clearly give anomeric mixtures, which can be recovered. Some reasonable arguments regarding the mechanism were also postulated.

CLAIMS TO ORIGINAL RESEARCH

1. Previously unknown *N*-linked fucopeptidomimetics of Sialyl Lewis^x were prepared by solution and solid phase strategies.
2. The convergent synthesis of *N*-linked mimetics based on a tartaric acid tail was explored, leading to penultimate compounds.
3. Penultimate *C*-linked mimetics were prepared via Heck coupling onto a *C*-allyl fucoside fragment.
4. A new palladium catalyzed aryl-alkene bond forming reaction was discovered, and some insights into the scope were made.
5. Glycosyl triazoles were prepared, and the known copper catalysis imparting regioselectivity was applied to give high yields of regiocontrolled compounds.
6. Unusual α/β selectivity was observed in the cases of coupling protected fucosylamines with protected proline. The observed ratios of anomers varied depending on the enantiomer of proline used.
7. Acid-catalyzed anomerization of fucose-proline conjugates was observed and some rationalizations made about the observed results.

PUBLICATIONS AND PRESENTATIONS

PUBLICATIONS:

1. Dominique, R; Das, S. K.; Liu, B.; Nahra, J.; **Schmor, B.**; Gan, Z.; Roy, R. "Ruthenium Carbenoids as Catalysts for Olefin Metathesis of Alkenyl Glycosides". *Methods Enzymol.* Lee, Y. C.; Lee, R. T. (Eds.), **2003**, *362*, 17-28.
2. **Schmor, B.**; Roy, R. "A New Palladium Catalyzed Aryl-Alkene Bond Formation". *Molecules* **2002**, 433-436.

CONFERENCE PRESENTATIONS/POSTERS

1. Roy, R.; **Schmor, B. J.**; Dominique, R.; Liu, B.; Hernandez-Mateo, F.; Juarez-Ruiz, J. M.; Das, S. K. *Transition Metal-Catalyzed Cross-Coupling Reactions Toward the Synthesis of Glycomimetics.* Talk presented at the 225th ACS National Meeting, New Orleans, Louisiana, March 2003.
2. Roy, R.; Das, S.K.; Dominique, R.; Liu, B.; Nahra, J.; **Schmor, B.**; Smith, C. *Ruthenium-Carbenoid Complexes as Key Catalysts Toward the Design of Novel Glycoclusters and Glycomimetics of Biological Significance.* Talk presented at the 84th CSC Conference, Montreal, Quebec, May 2001.
3. **Schmor, B.**; Roy, R. *Observation of Anomeric Selectivity in the Coupling of 1-Aminofucosides with Amino Acids and Anomerization of the Fucosylamide*

Product. Poster presented at the 222nd ACS National Meeting, Chicago, Illinois, August 2001.

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APPENDIX

X-Ray Crystallography Data Obtained from Compound 106

Identification code	rr2001
Empirical formula	C16 H21 N3 O9
Formula weight	399.36
Temperature	203(2) K
Wavelength	0.71073 A
Crystal system, space group	Monoclinic, P2(1)
Unit cell dimensions	a = 11.247(5) A alpha = 90 deg. b = 7.666(4) A beta = 108.906(6) deg. c = 11.974(6) A gamma = 90 deg.
Volume	976.8(8) A^3
Z, Calculated density	2, 1.358 Mg/m^3
Absorption coefficient	0.112 mm^-1
F(000)	420
Crystal size	0.20 x 0.20 x 0.10 mm
Theta range for data collection	1.80 to 28.74 deg.
Limiting indices	-15<=h<=11, -10<=k<=8, -13<=l<=16
Reflections collected / unique	4635 / 3598 [R(int) = 0.0363]
Completeness to theta = 28.74	87.6 %
Absorption correction	Semi-empirical from equivalents
Max. and min. transmission	0.9889 and 0.9779
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	3598 / 1 / 254
Goodness-of-fit on F^2	1.007
Final R indices [I>2sigma(I)]	R1 = 0.0616, wR2 = 0.1079
R indices (all data)	R1 = 0.1126, wR2 = 0.1257
Absolute structure parameter	0.0(18)
Largest diff. peak and hole	0.231 and -0.199 e.A^-3

Table A.1: Crystal Data and Structure Refinement for **106**

C(1)-O(1)	1.462(5)
C(2)-O(2)	1.212(5)
C(2)-O(1)	1.347(5)
C(2)-C(3)	1.481(5)
C(3)-C(4)	1.372(5)
C(3)-N(1)	1.380(5)
C(4)-N(3)	1.352(5)
C(5)-O(3)	1.406(5)
C(5)-N(3)	1.484(5)
C(5)-C(9)	1.532(5)
C(6)-O(3)	1.459(4)
C(6)-C(10)	1.523(6)
C(6)-C(7)	1.536(5)
C(7)-O(4)	1.462(4)
C(7)-C(8)	1.531(5)
C(8)-O(6)	1.456(4)
C(8)-C(9)	1.537(5)
C(9)-O(8)	1.457(4)
C(11)-O(5)	1.209(5)
C(11)-O(4)	1.370(5)
C(11)-C(12)	1.502(6)
C(13)-O(7)	1.215(5)
C(13)-O(6)	1.372(5)
C(13)-C(14)	1.502(6)
C(15)-O(9)	1.214(5)
C(15)-O(8)	1.379(5)
C(15)-C(16)	1.505(6)
N(1)-N(2)	1.316(4)
N(2)-N(3)	1.365(4)

O(2)-C(2)-O(1)	124.5(4)
O(2)-C(2)-C(3)	125.9(4)
O(1)-C(2)-C(3)	109.5(3)
C(4)-C(3)-N(1)	108.8(3)
C(4)-C(3)-C(2)	128.4(4)
N(1)-C(3)-C(2)	122.7(3)
N(3)-C(4)-C(3)	104.9(3)
O(3)-C(5)-N(3)	112.1(3)
O(3)-C(5)-C(9)	112.1(3)
N(3)-C(5)-C(9)	111.8(3)
O(3)-C(6)-C(10)	106.0(3)
O(3)-C(6)-C(7)	110.9(3)
C(10)-C(6)-C(7)	112.1(3)
O(4)-C(7)-C(8)	108.4(3)
O(4)-C(7)-C(6)	104.3(3)
C(8)-C(7)-C(6)	111.4(3)
O(6)-C(8)-C(7)	107.8(3)
O(6)-C(8)-C(9)	108.8(3)
C(7)-C(8)-C(9)	112.5(3)
O(8)-C(9)-C(5)	103.8(3)
O(8)-C(9)-C(8)	109.0(3)
C(5)-C(9)-C(8)	112.8(3)
O(5)-C(11)-O(4)	123.7(4)
O(5)-C(11)-C(12)	126.5(4)
O(4)-C(11)-C(12)	109.8(4)
O(7)-C(13)-O(6)	123.0(4)

Table **A.2**: Bond Lengths [Å] and Angles [deg] for **106**

O(7)-C(13)-C(14)	127.0(4)
O(6)-C(13)-C(14)	110.0(4)
O(9)-C(15)-O(8)	123.0(4)
O(9)-C(15)-C(16)	126.3(4)
O(8)-C(15)-C(16)	110.7(4)
N(2)-N(1)-C(3)	108.2(3)
N(1)-N(2)-N(3)	107.6(3)
C(4)-N(3)-N(2)	110.5(3)
C(4)-N(3)-C(5)	126.1(3)
N(2)-N(3)-C(5)	123.3(3)
C(2)-O(1)-C(1)	115.3(3)
C(5)-O(3)-C(6)	113.9(3)
C(11)-O(4)-C(7)	119.1(3)
C(13)-O(6)-C(8)	115.8(3)
C(15)-O(8)-C(9)	115.9(3)

Symmetry transformations used to generate equivalent atoms:

Table **A.2** (cont): Bond Lengths [Å] and Angles [deg] for **106**

	x	y	z	U(eq)
C(1)	560(5)	2231(8)	7979(4)	64(2)
C(2)	562(4)	1608(6)	6037(4)	36(1)
C(3)	1311(4)	1800(5)	5230(3)	30(1)
C(4)	2498(4)	2472(5)	5471(3)	33(1)
C(5)	3842(4)	3159(5)	4194(4)	31(1)
C(6)	2871(4)	5716(5)	3123(3)	29(1)
C(7)	3048(4)	5083(5)	1970(3)	28(1)
C(8)	3133(4)	3092(5)	1939(3)	30(1)
C(9)	4025(3)	2335(5)	3096(3)	30(1)
C(10)	2986(4)	7690(5)	3255(4)	40(1)
C(11)	1983(4)	6258(6)	4(3)	36(1)
C(12)	713(4)	6861(6)	-770(4)	49(1)
C(13)	3206(4)	1045(6)	450(4)	38(1)
C(14)	3857(5)	659(7)	-438(4)	53(1)
C(15)	6028(4)	1456(7)	2969(4)	39(1)
C(16)	7348(4)	2067(7)	3144(4)	48(1)
N(1)	843(3)	1390(5)	4046(3)	40(1)
N(2)	1700(3)	1798(5)	3558(3)	38(1)
N(3)	2712(3)	2461(4)	4422(3)	29(1)
O(1)	1231(3)	2199(4)	7118(3)	48(1)
O(2)	-496(3)	1030(4)	5776(3)	53(1)
O(3)	3838(2)	4992(3)	4147(2)	30(1)
O(4)	1901(2)	5634(4)	1048(2)	35(1)
O(5)	2951(3)	6309(4)	-230(3)	48(1)
O(6)	3601(3)	2625(4)	978(2)	36(1)
O(7)	2430(3)	144(4)	683(3)	51(1)
O(8)	5314(2)	2786(3)	3195(2)	34(1)
O(9)	5621(3)	-2(4)	2685(3)	47(1)

Table **A.3**: Atomic Coordinates (x 10^{-4}) and Equivalent Isotropic Displacement Parameters (A^2 x 10^{-3}) for **106**. U (eq) is defined as one third of the trace of the orthogonalized U_{ij} tensor.

	U11	U22	U33	U23	U13	U12
C(1)	50(3)	103(5)	38(3)	-10(3)	15(2)	-3(3)
C(2)	37(3)	33(3)	37(3)	6(2)	10(2)	-5(2)
C(3)	26(2)	32(2)	30(2)	4(2)	5(2)	-5(2)
C(4)	37(2)	32(2)	26(2)	3(2)	4(2)	1(2)
C(5)	29(2)	32(3)	29(2)	3(2)	5(2)	-2(2)
C(6)	27(2)	26(2)	32(2)	4(2)	8(2)	-1(2)
C(7)	23(2)	34(2)	26(2)	1(2)	6(2)	-1(2)
C(8)	23(2)	35(3)	33(2)	-3(2)	9(2)	0(2)
C(9)	25(2)	25(2)	39(2)	3(2)	9(2)	0(2)
C(10)	44(3)	29(2)	39(2)	-5(2)	4(2)	0(2)
C(11)	38(3)	40(3)	25(2)	-2(2)	4(2)	4(2)
C(12)	41(3)	56(3)	40(3)	5(2)	0(2)	5(2)
C(13)	36(3)	45(3)	32(2)	-4(2)	9(2)	2(2)
C(14)	54(3)	58(3)	50(3)	-15(3)	20(3)	-3(2)
C(15)	36(3)	49(3)	30(2)	8(2)	10(2)	11(2)
C(16)	33(3)	70(4)	42(3)	2(2)	13(2)	6(2)
N(1)	37(2)	51(2)	34(2)	-6(2)	12(2)	-13(2)
N(2)	34(2)	46(2)	31(2)	-4(2)	8(2)	-13(2)
N(3)	25(2)	31(2)	28(2)	1(2)	5(2)	-7(2)
O(1)	36(2)	73(2)	33(2)	-2(2)	9(1)	-9(2)
O(2)	41(2)	77(3)	42(2)	-8(2)	16(2)	-22(2)
O(3)	30(2)	27(2)	29(2)	0(1)	4(1)	-5(1)
O(4)	26(2)	45(2)	31(2)	10(1)	8(1)	2(1)
O(5)	43(2)	67(2)	39(2)	10(2)	21(2)	12(2)
O(6)	34(2)	37(2)	38(2)	-5(1)	15(1)	-3(1)
O(7)	55(2)	50(2)	54(2)	-17(2)	25(2)	-15(2)
O(8)	24(2)	35(2)	42(2)	3(1)	11(1)	1(1)
O(9)	43(2)	43(2)	54(2)	4(2)	15(2)	11(2)

Table **A.4**: Anisotropic Displacement Parameters (A^2 x 10^{-3}) for **106**. The anisotropic displacement factor exponent takes the form: $-2\pi^2[h^2a^2\ U11 + ... + 2\ hka^*b^*U12]$

	x	y	z	U(eq)
H(1A)	1120	2636	8734	95
H(1B)	267	1066	8069	95
H(1C)	-154	3013	7706	95
H(4A)	3043	2857	6203	40
H(5A)	4577	2817	4875	37
H(6A)	2033	5349	3140	35
H(7A)	3797	5629	1855	34
H(8A)	2284	2588	1784	36
H(9A)	3927	1053	3111	36
H(10A)	2887	8032	4000	60
H(10B)	2339	8241	2611	60
H(10C)	3807	8054	3240	60
H(12A)	767	7246	-1523	73
H(12B)	432	7821	-390	73
H(12C)	118	5905	-895	73
H(14A)	3585	-469	-797	80
H(14B)	4759	643	-46	80
H(14C)	3647	1553	-1044	80
H(16A)	7827	1127	2953	72
H(16B)	7741	2413	3960	72
H(16C)	7326	3055	2632	72

Table **A.5**: Hydrogen Coordinates (x 10^{-4}) and Isotropic Displacement Parameters (A^2 x 10^{-3}) for **106**

O(2)-C(2)-C(3)-C(4)	178.7(5)
O(1)-C(2)-C(3)-C(4)	-0.6(6)
O(2)-C(2)-C(3)-N(1)	2.8(7)
O(1)-C(2)-C(3)-N(1)	-176.4(4)
N(1)-C(3)-C(4)-N(3)	0.4(5)
C(2)-C(3)-C(4)-N(3)	-175.9(4)
O(3)-C(6)-C(7)-O(4)	169.8(3)
C(10)-C(6)-C(7)-O(4)	-72.0(4)
O(3)-C(6)-C(7)-C(8)	53.0(4)
C(10)-C(6)-C(7)-C(8)	171.2(3)
O(4)-C(7)-C(8)-O(6)	79.5(4)
C(6)-C(7)-C(8)-O(6)	-166.3(3)
O(4)-C(7)-C(8)-C(9)	-160.7(3)
C(6)-C(7)-C(8)-C(9)	-46.4(4)
O(3)-C(5)-C(9)-O(8)	68.2(4)
N(3)-C(5)-C(9)-O(8)	-164.9(3)
O(3)-C(5)-C(9)-C(8)	-49.7(4)
N(3)-C(5)-C(9)-C(8)	77.2(4)
O(6)-C(8)-C(9)-O(8)	49.1(4)
C(7)-C(8)-C(9)-O(8)	-70.3(4)
O(6)-C(8)-C(9)-C(5)	163.8(3)
C(7)-C(8)-C(9)-C(5)	44.5(4)
C(4)-C(3)-N(1)-N(2)	-0.3(5)
C(2)-C(3)-N(1)-N(2)	176.2(4)
C(3)-N(1)-N(2)-N(3)	0.1(4)
C(3)-C(4)-N(3)-N(2)	-0.3(4)
C(3)-C(4)-N(3)-C(5)	176.2(4)
N(1)-N(2)-N(3)-C(4)	0.1(4)
N(1)-N(2)-N(3)-C(5)	-176.5(3)
O(3)-C(5)-N(3)-C(4)	-75.3(5)
C(9)-C(5)-N(3)-C(4)	157.8(4)
O(3)-C(5)-N(3)-N(2)	100.7(4)
C(9)-C(5)-N(3)-N(2)	-26.2(5)
O(2)-C(2)-O(1)-C(1)	-4.5(7)
C(3)-C(2)-O(1)-C(1)	174.8(4)
N(3)-C(5)-O(3)-C(6)	-68.5(4)
C(9)-C(5)-O(3)-C(6)	58.2(4)
C(10)-C(6)-O(3)-C(5)	177.8(3)
C(7)-C(6)-O(3)-C(5)	-60.3(4)
O(5)-C(11)-O(4)-C(7)	3.6(6)
C(12)-C(11)-O(4)-C(7)	-176.0(3)
C(8)-C(7)-O(4)-C(11)	-100.4(4)
C(6)-C(7)-O(4)-C(11)	140.8(3)
O(7)-C(13)-O(6)-C(8)	6.3(6)
C(14)-C(13)-O(6)-C(8)	-174.5(4)
C(7)-C(8)-O(6)-C(13)	-150.4(3)
C(9)-C(8)-O(6)-C(13)	87.4(4)
O(9)-C(15)-O(8)-C(9)	1.1(6)
C(16)-C(15)-O(8)-C(9)	-177.9(3)
C(5)-C(9)-O(8)-C(15)	135.1(3)
C(8)-C(9)-O(8)-C(15)	-104.4(4)

Symmetry transformations used to generate equivalent atoms:

Table **A.6:** Torsion Angles [deg] for **106**