Convergent Synthesis of *N*-Linked Glycopeptides on a Solid Support

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Abstract: Solid-supported synthesis can be conducted to produce a variety of glycopeptides in good overall yields. The carbohydrates are formed by the glycal assembly method. The polymer-bound construct terminates in a glycal. The terminal double bond can be functionalized to provide a C2−*N*-acetyl glucosamine linkage with an amino group in the anomeric position. The latter can be coupled, in a convergent manner, to the γ-carboxyl group of an aspartyl residue on a preformed peptide. Iodosulfonamidation of the polymer-bound glucal to the *N*-acetyl glucosamine using anthracenesulfonamide was crucial for the success of the solid-phase synthesis. This general method was employed in the formation of a variety of glycopeptides.

Introduction

The automated solid-phase synthesis of oligonucleotides and polypeptides has had a major impact on the fields of molecular biology and pharmacology by providing access to needed molecular tools. Among these invaluable synthetic products are oligonucleotide primers for application of the polymerase chain reaction (PCR),[1] labeled probes for gene detection, synthetic mutagenic agents to alter DNA sequences, and potential therapeutic agents.[2] Correspondingly, solid-phase peptide synthesis is widely used for drug lead discovery, for the preparation of synthetic vaccines, and for peptide bioscreening[3,4] including the generation of combinatorial peptide libraries.[5,6] Similarly, it is likely that synthetic oligosaccharides and glycopeptides could find considerable application (vide infra).

However, the synthesis of oligosaccharides and glycopeptides is intrinsically a more challenging problem than is the case with oligonucleotides or oligopeptides. First, there is greater structural diversity in the potential linkage sites for joining the saccharide units. Further complicating the goal of oligosaccharide synthesis is the need to control the stereochemistry of each anomeric linkage. The laboratory synthesis of glycopeptides faces additional difficulties stemming from the chemical sensitivity of the bonds connecting the first amino acid to the carbohydrate domain. Needless to say, any attempt to bring to bear the advantages of polymer-bound synthesis protocols on the glycopeptide problem would be dependent on the use of blocking groups which are appropriate to both the carbohydrate and peptidal domains.[7]

The surge of interest in glycoproteins[8,9] arises from heightened awareness of their importance in diverse biochemical processes including cell growth regulation, binding of pathogens to cells,[10] and intercellular communication and metastasis.[11] Glycoproteins serve as cell differentiation markers and assist in protein folding and transport, possibly by providing protection against proteolysis.[12]

Improved isolation techniques and structural elucidation methods[13] have revealed significant levels of microheterogeneity in naturally produced glycopeptides.[14] Single eukaryotic cell lines often produce many glycoforms of any given protein sequence. For instance, erythropoietin (EPO), a clinically useful red blood cell stimulant for anemia, is glycosylated by more than 13 known types of oligosaccharide chains when expressed in Chinese hamster ovary cells (CHO).[15] The efficacy of erythropoietin is heavily dependent on the type and extent of glycosylation.[16]

The naturally occurring *N*-asparagine glycopeptides are β-*N*-linked to an *N*-acetylglucosamine segment of a chitobiose, which is part of the high mannose antennary structure (Figure 1). This pentasaccharide core carbohydrate is the medium to which virtually all *N*-asparagine glycoproteins are attached. The specific role of *N*-linked proteins is not well understood. However, the necessity of such arrays has been demonstrated in that mice which lack the *N*-acetylglucosamine transferase gene die at midgestation.[17] It is possible that the glycosylation

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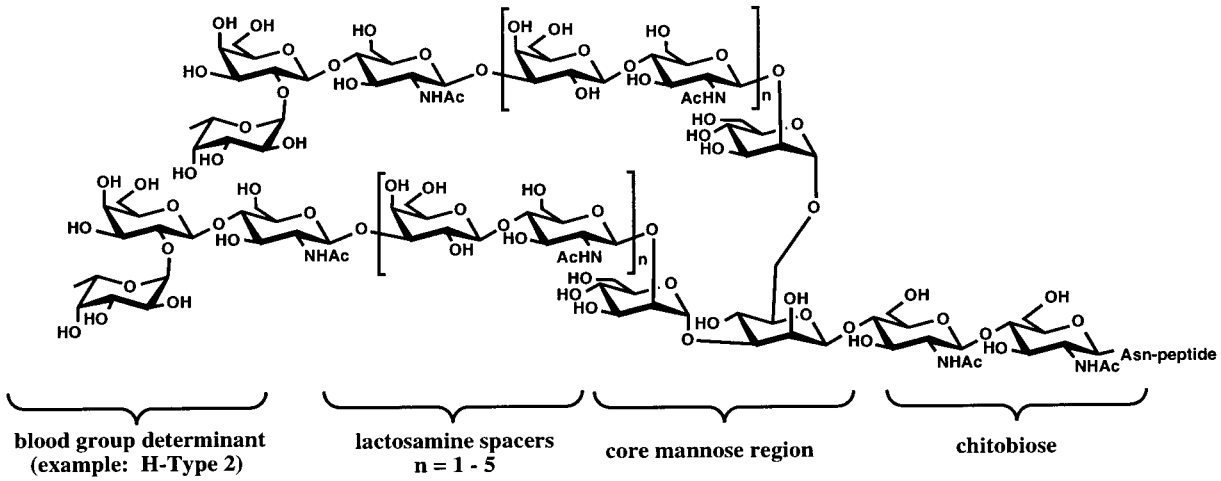

Figure 1. Complex type biantennary structure.

has a conformational effect on the protein which influences the rate or even energetics of protein folding.[18] Indeed, NMR studies on the glycopeptide **48** synthesized herein has shown that glycosylation has a definite effect on limiting the conformational possibilities of the peptide.[19]

Elucidation of the biological relevance of particular glycoprotein oligosaccharide chains would benefit from isolation of homogeneous entities. Currently, glycoprotein heterogeneity renders this process particularly labor intensive. Some relief may be in sight in that particular cell lines can be selected to produce more homogeneous glycoproteins for structure−activity relationship studies.[20] However, the problem of isolation of pure glycoproteins from natural sources remains daunting.

Fortunately, receptors normally recognize only a small fraction of a given macromolecular glycoconjugate. Consequently, synthesis of smaller but well-defined putative glycopeptide ligands could emerge as functionally competitive with isolation as a source of critical structural information.[15] Indeed, prior to the work described herein, important progress in glycopeptide synthesis pioneered by Kunz and others had matured to the point where synthetic access to homogeneous target systems could be achieved both in solution and in the solid phase.[7,21−29] An important advance in this area was achieved by Cohen-Anisfeld and Lansbury who described a highly convergent solution-based coupling of selected already available saccharides with peptides.[27]

Herein, we report a new and efficient solid-phase synthesis of *N*-linked glycopeptides based on our solid phase glycal assembly method. We also demonstrate the capacity to extend the peptide domain while the synthetic ensemble is polymer-bound. The method we are describing here allows for fashioning the carbohydrate domain of choice and also benefits from the advantages associated with solid-phase synthesis in the all critical carbohydrate−peptide coupling step.

Synthetic Strategy

The most successful approach to solid phase *N*-linked glycopeptide synthesis which is currently practiced in several variations in other laboratories is summarized in Scheme 1A. A peptide segment bearing a terminal amine equivalent residue is built on a solid support (see **2** → **3** → **4**). A solution-based glycosylated asparagine is assembled (see structure **7**) by coupling an adequately protected aspartic acid **6** with 1β-glucosylamine **5**. The latter is prepared using Koenig's Knorr chemistry or via anomeric amination of a naturally occurring anomeric saccharide.[23,24] The α-amino function of structure **7** is most often protected as its 9-fluorenylmethoxycarbonyl (Fmoc) derivative and the α-carboxyl as a pentafluorophenyl (Pfp) ester. Coupling of the two domains affords **8**. Deprotection of the α-amino linkage generates **9**. The next Fmoc protected amino acid **1** is coupled to **9**. After retrieval from the support and deprotection of the resultant blocking groups, target system **10** is obtained.

By contrast, we propose an alternate method which is illustrated under Scheme 1B.[30] An oligosaccharide terminating in a glycal is constructed on the solid support (see structure **11**). As shown earlier, **11** can be an extended linear structure, or can contain branching as desired.[31−35] Through chemistry which we were projecting (vide infra Scheme 2) **11** would be converted to the solid-phase bound **12**, bearing a terminal 2-*N*-acetyl-1β-aminoglucosamine residue (GlcNAc). Peptide **13** would be readily assembled through standard solution-phase peptide synthesis methodology or by a solid-phase assembly

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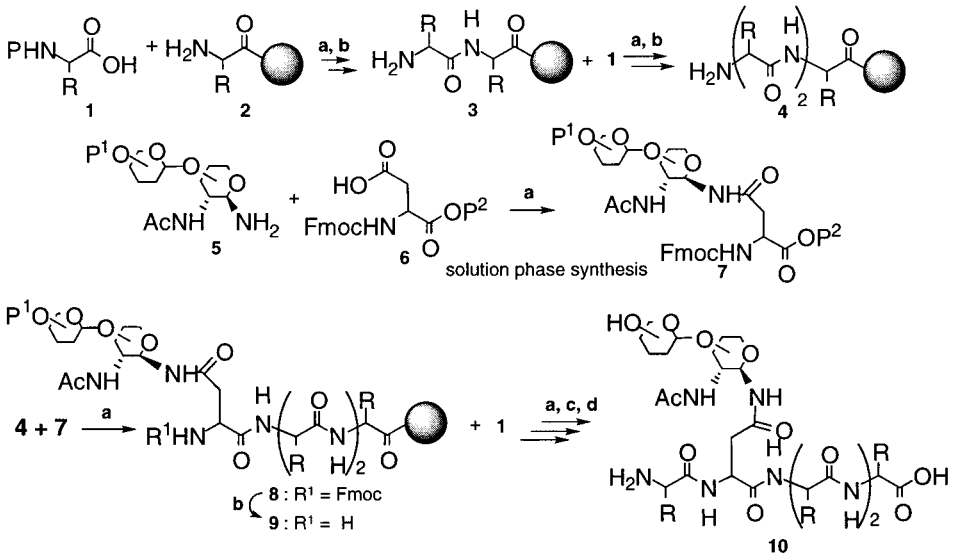
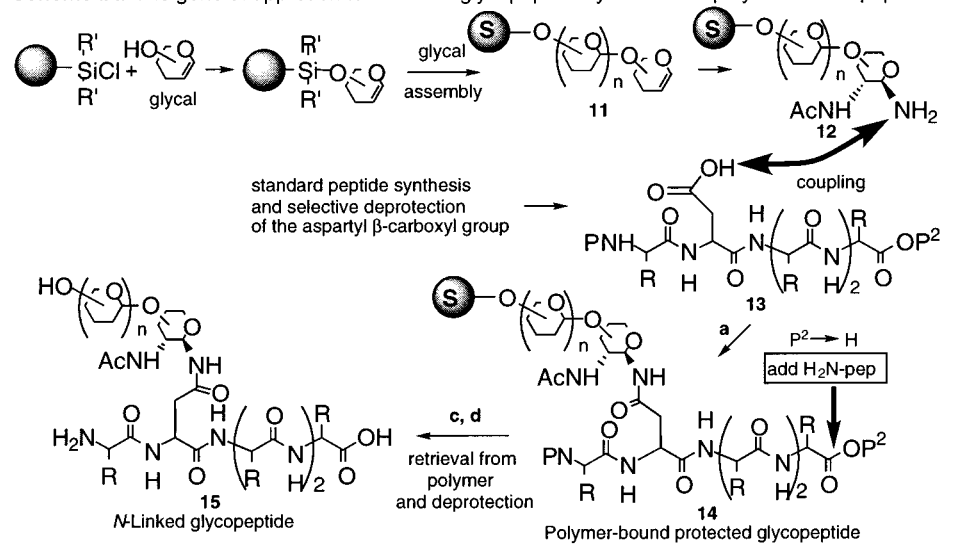
Scheme 1



Scheme IA: The general approach to *N*-Linked glycopeptide synthesis *via* polymer-bound peptides.



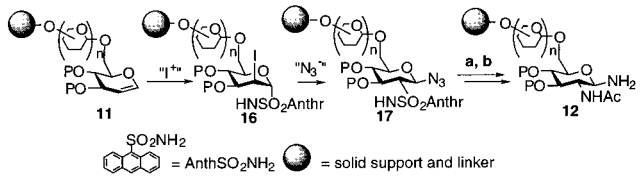
Scheme IB: A new approach to *N*-linked glycopeptide synthesis *via* polymer-bound glycals.

Legend: = solid support; = solid support and linker

P = amino protecting group; P^1 = saccharide protecting groups or hydrogen; P^2 = carboxyl protecting group or activated ester; R = amino acid side chains; Fmoc = *N*-(9-fluorenylmethoxycarbonyl)

[a] activation of the carboxylic group and amide bond formation; **[b]** amine group deprotection; **[c]** cleavage from the solid support; **[d]** removal of all resident protecting groups.

Scheme 2. Preparation of Polymer-Bound 2-*N*-Acetyl-1-β-glucosylamine *via* the Azasulfonamidation Reaction



Legend: P = saccharide protecting group; "I^+" = iodonium bis(collidine) perchlorate ($I(coll)_2ClO_4$); "N_3^-" = tetrabutylammonium azide; **[a]** acylation with acetic anhydride and 4-*N*,*N*-dimethylaminopyridine (DMAP); **[b]** reduction with 1,3-propanedithiol and *N*,*N*-diisopropyl-*N*-ethylamine.

retrieval sequence. Coupling of **12** + **13** should lead to solid-phase bound **14**. Retrieval and global deprotection would provide **15**. In addition, targeted deprotection of the *C*-terminus and coupling to peptide containing free *N*-terminus would allow for elongation of the peptide chain while the glycopeptide **14** is still bound to the solid support.

The advantages, in principle, of convergence and the streamlining of protection requirements inherent in our proposal are obvious on inspection. However, to implement the method, a sound protocol to convert a polymer-bound glycal of the type **11** to **12** which bears the β-disposed anomeric amine would be necessary. Fortunately, a simple sequence was devised as shown schematically in Scheme 2. Crucial to the success of the method was the use of the anthracenesulfonamide[36] in the azasulfonamidation sequence.[37] Thus, the addition step (see

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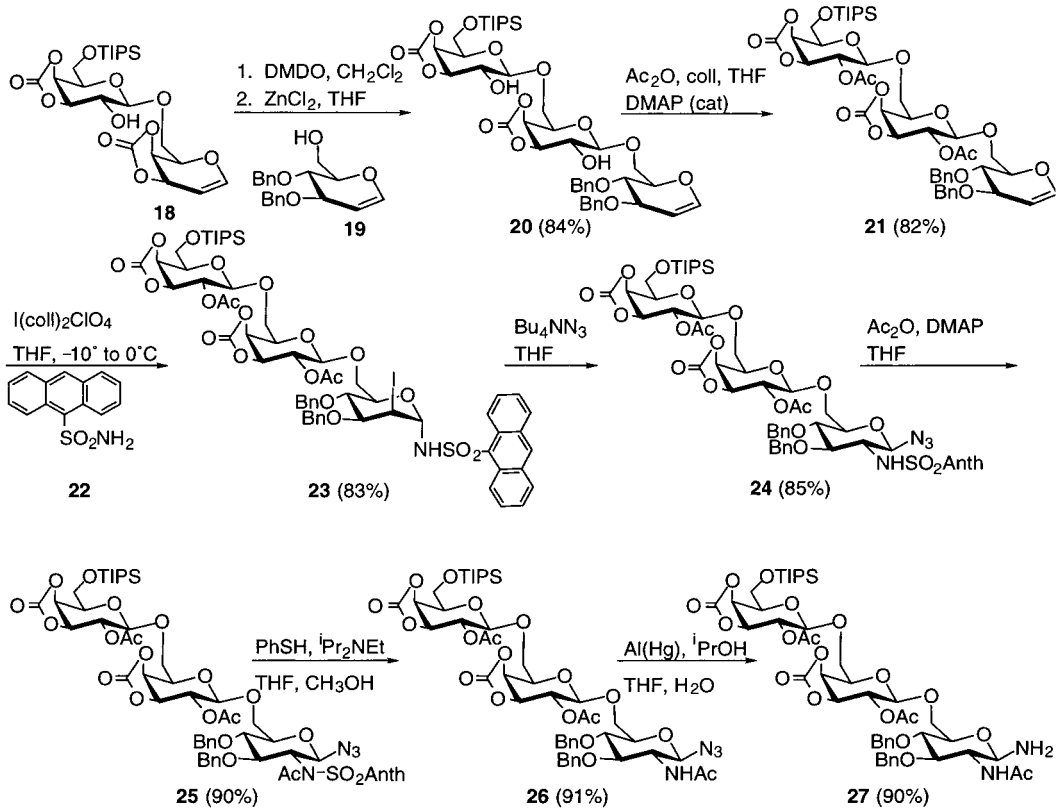
Scheme 3. Solution Synthesis of the Trisaccharide



structure **16**), the azide-induced rearrangement (see structure **17**) and the fashioning of the solid-phase bound GlcNAc residue bearing a 1β-amino function (see structure **12**) all occur smoothly as will be demonstrated.

Results and Discussion

We first explored the projected glycopeptide synthesis in solution phase in order to demonstrate the feasibility and selectivity of each projected step in the reaction sequence. The use of anthracenesulfonamide in the iodosulfonamidation reaction was pivotal in the synthesis of the β-*N*-linked glycopeptides. Other sulfonamides such as benzensulfonamide, *p*-toluenesulfonamide, and β-(trimethylsilyl)ethanesulfonamide were employed but resulted in a variety of problems including poor solubility and a low yield in the subsequent rearrangement (vide infra). Most disabling to the use of these sulfonamides was our inability to achieve late stage deprotection without concomitant destruction of the glycopeptide. The anthracenesulfonamide group proved to be unique in that it could be discharged under a variety of mild conditions which we envisioned to be compatible with polymer-supported synthesis and glycopeptide stability. These expectations were realized. The required reagent is also highly soluble in THF. This solvent is optimal for the iodosulfonamidation reaction and also a good polystyrene swelling solvent. Thus, the use of the anthracene-based agent results in a more efficient and complete iodosulfonamidation reaction.

The trisaccharide with the necessary glucosamine anomeric amine was synthesized using the glycal assembly method as shown in Scheme 3. The trisaccharide **21**, prepared as shown in Scheme 3 was chosen to test the feasibility of convergent glycopeptide synthesis because of the excellent β glycosylation selectivity of the galactal carbonate derivatives and the ease of placement of the desired terminal glucal moiety. The carbonate-protected galactoses would also provide a polar linker arm from the polystyrene used in the polymer-supported synthesis. The linkage to the polymer support would correspond to the 6-*O*-TIPS position of the starting galactal **18**.

In practice, the known disaccharide **18** was extended with the necessary glucal in the usual way. Following epoxidation with dimethyldioxirane, subsequent Lewis acid mediated epoxide opening with the glycosyl acceptor 3,4-di-*O*-benzyl glucal and acetylation, the trisaccharide **21** was in hand. The terminal glucal was to be transformed into the β-*N*-linked glucosamine glycopeptide.

Iodosulfonamidation of **21** with iodonium bis(*sym*-collidine) perchlorate[38] and anthracene sulfonamide (**22**) gave the trans-diaxial iodosulfonamide **23** in good yield. Upon treatment of this compound with the highly soluble tetrabutylammonium azide[39] the iodosulfonamide linkage underwent the expected C1α → C2α rearrangement with capture at C1 to give the β anomeric azide (see compound **24**). Acetylation of the nitrogen atom of the sulfonamide allowed for subsequent deprotection of the anthracene sulfonyl group using thiophenol and Hunig's base[35] (see **25** → **26**). These extremely mild conditions gave the stable trisaccharide *N*-acetylglucosamine **26** with the anomeric azide intact. Reduction of the azide with aluminum amalgam provided the anomeric amine **27**. This amine was unstable to silica gel and was used immediately in the peptide coupling reaction.

Synthesis of the Peptides. The peptide sequence dictated by nature for the β-*N*-linked glycopeptides requires a specific AsnXSer/Thr where the asparagine is the site of glycosylation and X corresponds to any amino acid except proline. In the cellular machinery, glycosylation takes place on the internal

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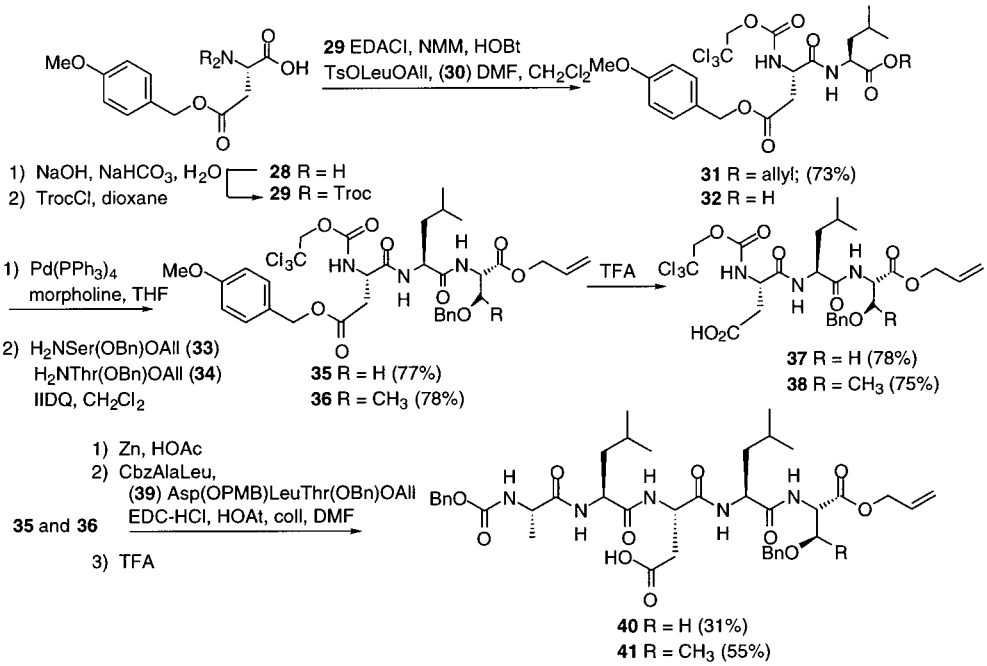
Scheme 4. Synthesis of the Peptide



surface of the rough endoplasmic reticulum of the golgi apparatus where *N*-glycosylation enzymes recognize the serine or threonine and glycosylates at the asparagine one amino acid away. The glycopeptide is then transported to the surface of the cell by vesicles that break off of the golgi apparatus.

For our model purposes, relevant tripeptides and pentapeptides were synthesized using standard solution-phase peptide synthesis as described in Scheme 4. The *p*-methoxybenzyl ester of aspartic acid (see compound **28**) was *N*-protected with a Troc (1,1,1-trichloroethyl carbamate) group. The free carboxyl was then coupled with the tosylate salt of leucine using standard peptide-coupling conditions. The allyl ester of the leucine was then cleaved by Pd° and the resulting acid **32** was coupled to the allyl esters of serine (**33**) and threonine (**34**) to give tripeptides **35** and **36**, respectively. Removal of the *p*-methoxybenzyl blocking group from the aspartic acid was accomplished by the action of neat TFA to give the tripeptides **37** and **38**. It proved to be crucial to remove all traces of TFA from these peptides to provide complete coupling with the valuable anomeric amine trisaccharide. The pentapeptides were prepared from the tripeptides by removal of the Troc group with zinc dust and subsequent coupling with the commercially available dipeptide CbzAlaLeu. Removal of the *p*-methoxybenzyl group as described provided the free aspartic acid residues **40** and **41** to be coupled to the anomeric amine of the trisaccharide.

As will be demonstrated (vide infra), orthogonal protecting groups of the type pioneered by Kunz[21,26] proved to be compatible with glycopeptide synthesis and also allowed for extension of the peptide part of the glycopeptide from the *C*-terminus while it is still bound to the polymer support.

The agenda next called for fully convergent coupling of amine-terminating saccharide **27**, with our synthetic peptides. We started with the goal of coupling tripeptide **38** and pentapeptide **40** each of which contains a single differentiated free γ-aspartamine type carbohydrate. Standard peptide-coupling conditions under the action of IIDQ[40] using these free aspartic acid residues afforded glycopeptides **42** and **43** in reasonable yield (Scheme 5). However, purification of these glycopeptides by column chromatography proved to be tedious partly due to the precipitation of the unreacted peptide on the column. The silyl protecting group (and envisioned model for the eventual point of attachment to the polymer-support) was removed by HF·pyridine[41] to give the glycopeptide alcohols **44** and **45**. Subsequent deprotection of the C-terminal allyl group of each glycopeptide with Pd° and dimethylbarbituric acid[42] gave the acids **46** and **47**. The Troc group of each glycopeptide was removed with Zn°,[41] the benzyl protecting groups were removed by catalytic hydrogenation,[43,44] and the acetates and carbonates were cleaved by potassium cyanide in methanol,[45,46] to give the fully deprotected trisaccharide tripeptide **48** and trisaccharide pentapeptide **49**. These fully deprotected glycopeptides provided among the largest synthetic glycopeptides available by synthetic means.

Polymer-Supported Synthesis of Trisaccharide Tripeptide and Trisaccharide Pentapeptide. While we did not seek to optimize the solution-phase synthesis of glycopeptides, the successful realization of the basic plan was certainly of great value as we began to evaluate extensions to solid-phase synthesis. The polymer-supported assembly of the trisaccharide glycopeptides followed the general logic of the solution-phase assembly as shown in Scheme 6. Epoxidation of the known[30,31] polymer-bound disaccharide glycal **50** with 2,2-dimethyldioxirane and subsequent glucosylation with 3,4-di-*O*-benzyl glucal gave the polymer-bound trisaccharide **51** which was acetylated to provide **52**. Iodosulfonamidation of this system with iodonium bis(*sym*-collidine) perchlorate and anthracenesulfonamide (**23**) proceeded, as expected, to give **53**. Azide-induced sulfonamide rearrangement with the highly soluble tetra-*n*-

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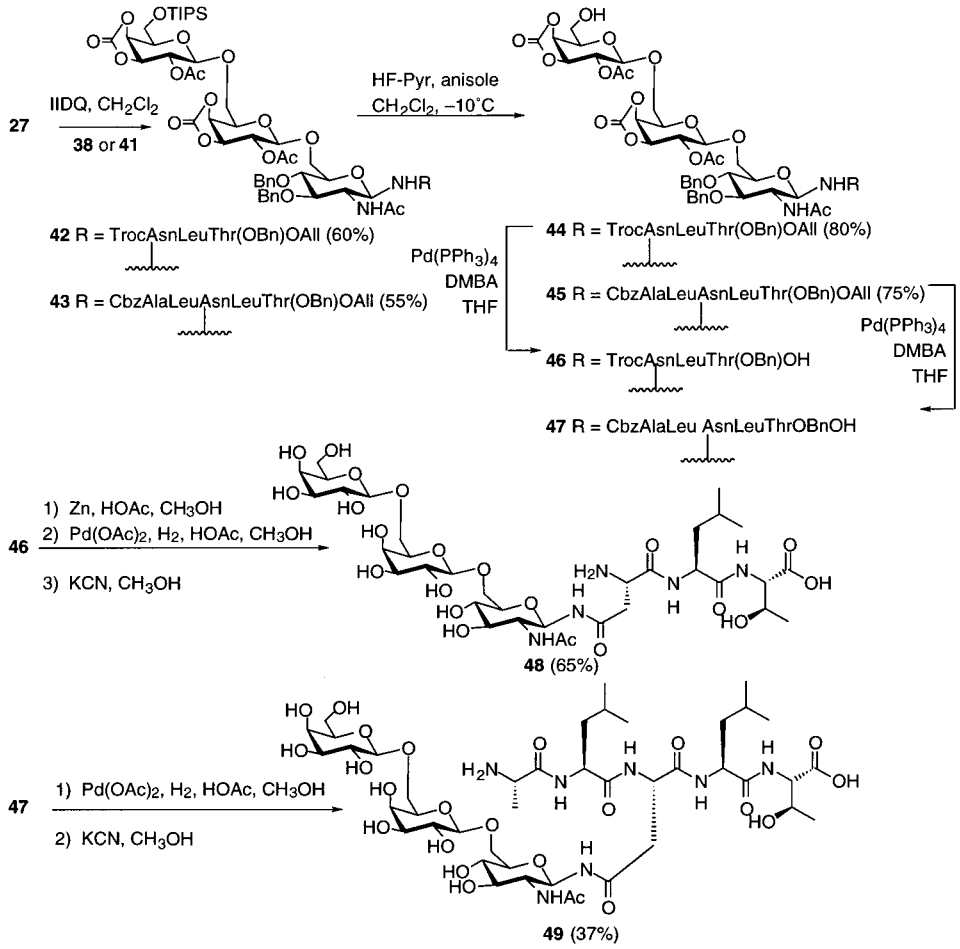
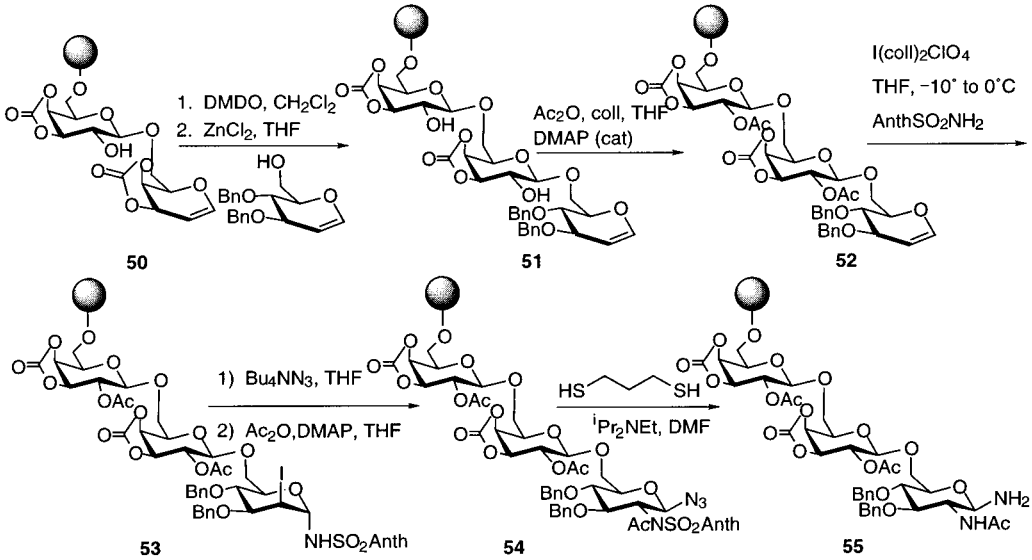
Scheme 5. Solution Coupling of the Peptides and Protecting Group Removal



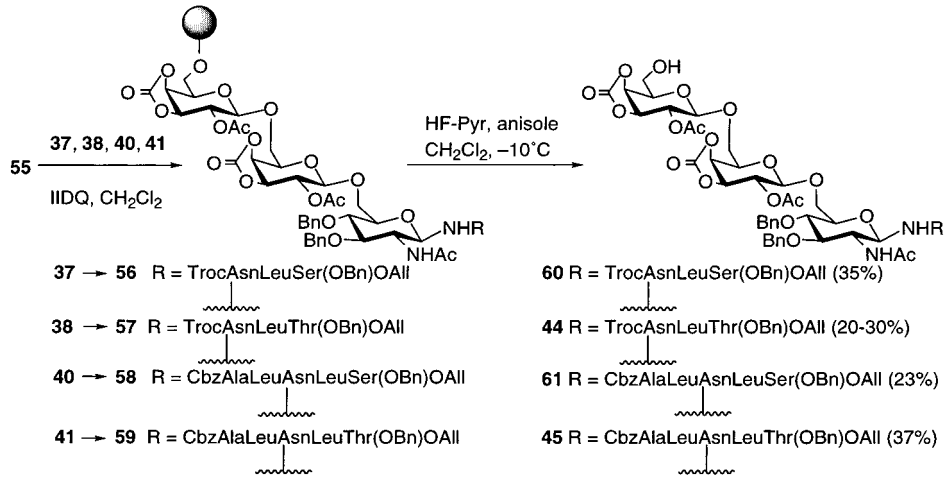
Scheme 6. Solid-Phase Glycal Assembly and Anomeric Amine Synthesis



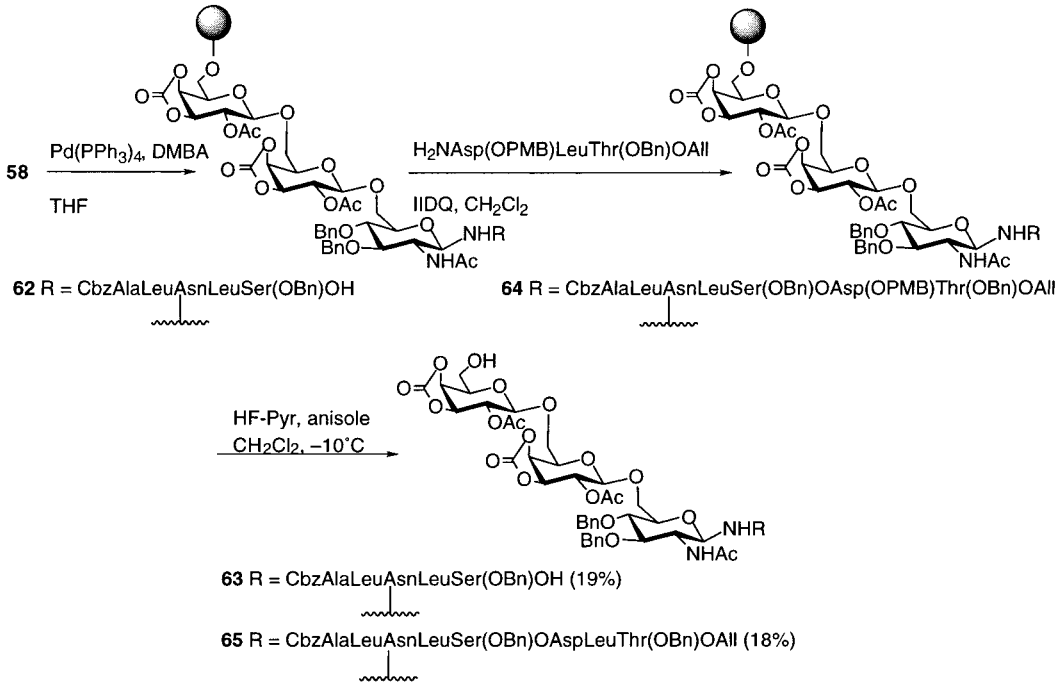
butylammonium azide followed by presumed *N*-acetylation provided the anomeric azide **54**. The sulfonamide could now be removed either stepwise with thiophenol and Hunig's base, or inundation with 1,3-propanedithiol and Hunig's base. Each of these conditions effected concomitant reduction of the azide to give polymer-bound *N*-acetyl glucosamine **55** with the anomeric amine in place for coupling with a peptide. The propanedithiol reduction of the anomeric amine is particularly well suited for the polymer-supported work due to the ease of workup compared with solution-phase synthesis.

In solution phase the convergent coupling of carbohydrates and mature peptides is difficult. Separation of the product from the unreacted components and byproducts is not a trivial matter. This process is greatly simplified when conducted with carbohydrate bound to a solid support. Most of the excess peptide is recovered by washing the polymer and readily purified by

Scheme 7. Solid-Phase Glycopeptide Synthesis



Scheme 8



chromatography. Small amounts of peptide are lost due to cyclization of activated aspartate. The coupling of the polymer-bound trisaccharide **55** with the peptides shown in Scheme 7 proceeded smoothly. The peptides were usually used in 2−3 equiv excess to the polymer-bound amine. Retrieval from the polymer-support with HF·pyridine gave the freed glycopeptides **44**, **45**, **60**, and **61** in 20−35% overall yield based on the initial loading of polymer-bound galactal carbonate. These compounds had previously been obtained from the solution-phase work and fully deprotected.

In practice, protected trisaccharide−pentapeptide **45** was obtained in 37% overall yield based on the initial loading of galactal carbonate. *Thus, the average yield for each of the 10 steps of the sequence to this compound was 91%.* Chromatography on a short column of reverse phase silica (C-18) was sufficient to obtain this compound in pure form. This rather straightforward purification capability arises from the previously described "self-policing"[34,35] feature of the solid-phase glycal assembly method and underscores the efficiency in the conversion of the terminal glycal to the terminal glucosylamine.

Extension of the Peptide Portion while Bound to the Polymer-Support. Another feature of our method is that the presence of orthogonal protecting groups on the *C*- and *N*-termini of the peptide provides the opportunity to extend the peptide chain while it is still bound to the solid support, either one amino acid at a time or, convergently, with larger fragments. Alternatively, after removal from the support, the freed peptide terminus may provide suitable functionality for linking to a carrier molecule to generate other glycoconjugates.[47] This potentiality has been reduced to practice. Thus the peptide portion of the polymer-bound glycopeptide **58** (Scheme 8) was extended. The *C*-terminus was deprotected as shown to give the acid, **62**. Solid support bound **62** was then coupled to tripeptide Asp(OPMB)LeuThr(OBn)OAll with a free *N*-terminus to give polymer-bound trisaccharide octapeptide **64**. Retrieval from the solid support afforded trisaccharide octapeptide **65** in an 18% overall yield from polymer-bound galactal carbonate. There is the possibility of racemization associated with the use of IIDQ in this peptide coupling. Racemized product was not

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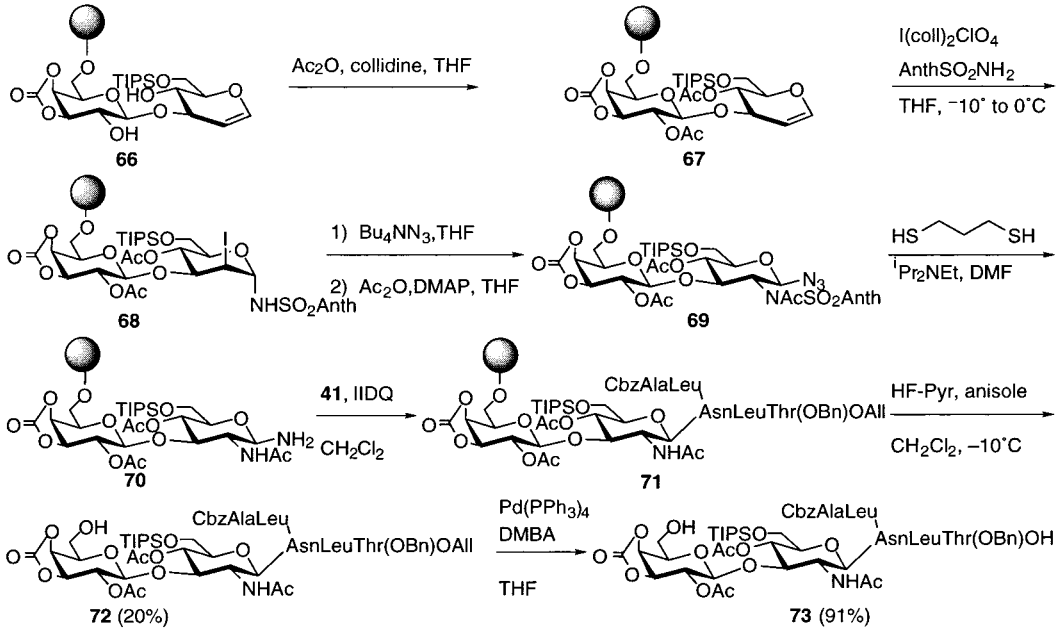
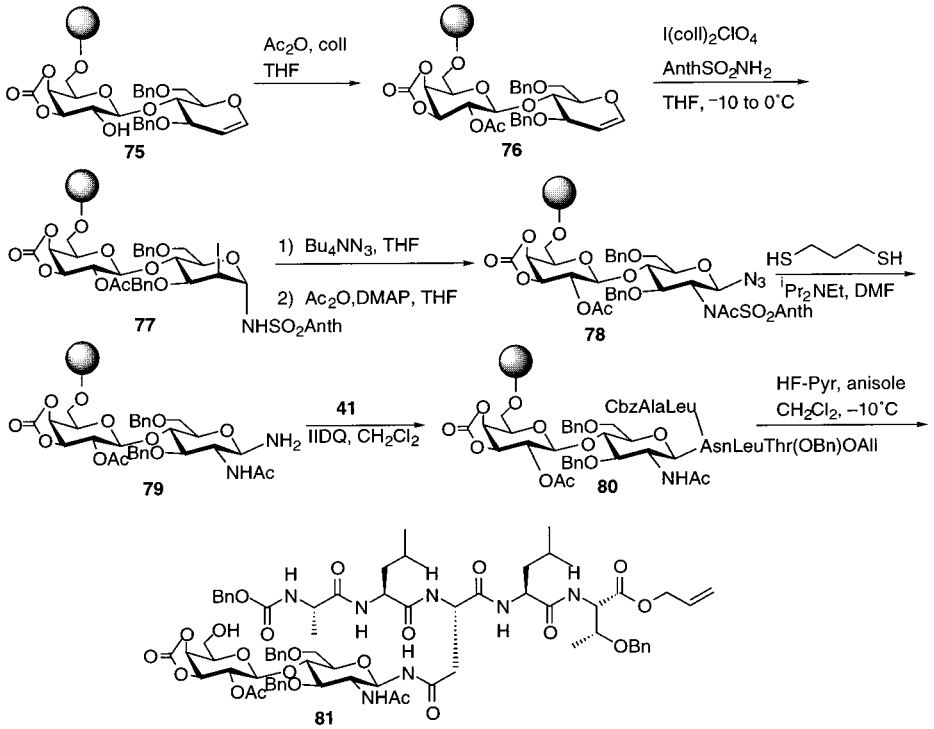
Scheme 9



Scheme 10. Solid-Phase Lactosamine−Pentapeptide Synthesis



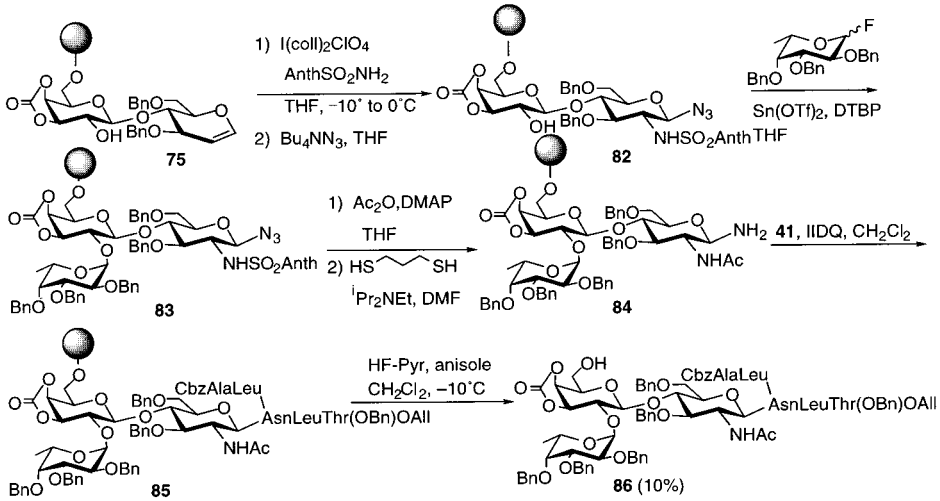
observed in the crude ^{1}H NMR of the final glycopeptide cleaved from the solid support.

Disaccharide Pentapeptides and a Branched Trisaccharide Pentapeptide. The methodology described above has proven to be effective for other glycosylation sequences. Thus the polymer-bound disaccharide **66** (Scheme 9) with a β-1−3 glycosylation pattern could be acetylated to give disaccharide **67**. Subsequent iodosulfonamiation (see system **68**), azide rearrangement (see system **69**), sulfonamide acetylation, and reduction provided the polymer-bound anomeric amine **70**. The latter was coupled to the previously described pentapeptide **41** and yielding, upon removal from the polymer-support, the disaccharide pentapeptide **72** in 20% overall yield based on the initial loading of polymer-bound galactal carbonate.

Similarly, the polymer-bound lactal derivative **75** could be transformed into a lactosamine-linked glycopeptide where C6 of the glucosamine residue is protected as its benzyl ether as shown in Scheme 10 in a 24% overall yield. This methodology had proven compatible with more difficult glycosylation strategies.

Attempts to form unnatural glycopeptides from the blood group antigen carbohydrates by fucosylation at the glycal stage of **75** proved difficult. However, this problem was alleviated by first performing the iodosulfonamidation on the disaccharide **75** with the 2-position free to give iodosulfonamide (Scheme 11). Subsequent azide-induced rearrangement (see compound **83**) allowed for fucosylation in the 2-position of the galactose moiety to give the polymer-bound H-type II blood group

Scheme 11. Solid-Phase Synthesis of a Branched Glycopeptide



determinant trisaccharide derivative **84**. Acetylation, reduction, peptide coupling led to **85**. Retrieval of this construct and removal from the polymer support provided the branched trisaccharide pentapeptide **86** in a 10% overall yield. The lactosamine pentapeptide **81** was also recovered from the polymer support in a 6% overall yield. This indicated a fucosylation yield, in the context of the solid-phase bound glycopeptide, of ca. 60%. It is possible that the HF·pyridine deprotection also cleaved the acid labile fucosyl residue, thus lowering the yield of the desired glycopeptide. This would give a product similar to **81** but lacking the 2-*O*-acetyl group on the galactose. This type of compound was not detected in the crude ^{1}H NMR of the glycopeptide obtained from the solid support.

Summary

In considering the future of approaches to glycopeptides by chemical synthesis, it is also well to recall the excellent method of Wong which involves enzymatically mediated elaboration of a solid-phase-bound glycosylated peptide construct using glycosyl transferases to append unprotected nucleoside phosphate activated monosaccharides in the elongation phase.[48] The Wong method also partakes of a most admirable feature in that retrieval from the solid support is mediated with controllable action of protease.

Obviously, there is merit in either the chemo- and or chemoenzymatic methodologies. Clearly, the relief from protecting groups in enzymatically mediated chemistry is a massive advantage. Both methods allow for the use of unnatural amino acids and non-amino acids. We note, however, that the method described herein is, in principle, totally general in that it does not presuppose the existence of the transferases and the availability of nucleoside activated hexoses. *It will accommodate the inclusion of unnatural (artificial) sugars in the scheme.* Such building blocks are available from the Lewis acid-catalyzed diene−aldehyde cyclocondensation reaction.[49,50] Clearly, all workable approaches, whether purely chemical or chemoenzymatic, are complementary and serve to foster the advent of carefully designed, fully synthetic glycopeptides.

In terms of our own program, the next milestone must involve elongation of the terminal GlcNac residue to a chitobiose moiety as well as interpolation of the suitable β-linked mannose in turn branched with α-mannose residues.[31,51−53] The logic by which these linkages can be implemented from glycals has been described in previous publications from our laboratory.[54] The translation of these findings to solid methodology represents a not unsubstantial developmental goal.

Experimental Section

General Methods. Melting points are not corrected. Infrared spectra were recorded on a Perkin-Elmer 1600 series FTIR. ^{1}H NMR spectra were obtained on a Bruker AMX-400 NMR (400 MHz) and are reported in parts per million (δ) relative to $SiMe_4$ (0.00 ppm) as an internal reference, with coupling constants (J) reported in hertz. ^{13}C NMR spectra were obtained at 100 MHz and are reported in δ relative to $CDCl_3$ (77.00 ppm) as an internal reference, with coupling constants (J) reported in hertz. High-resolution mass spectra were recorded on a JOEL JMS-DX-303 HF mass spectrometer. Optical rotations were recorded on a Jasco DIP-370 polarimeter using a 1 dm cell at the reported temperatures and concentrations.

Chemicals used were reagent grade and used as supplied except where noted. Pyridine, benzene, and dichloromethane (CH_2Cl_2) were distilled from calcium hydride under N_2. Tetrahydrofuran (THF) was distilled from sodium/benzophenone ketyl under N_2. Analytical thin-layer chromatography was performed on E. Merck silica gel 60 F_{254} plates (0.25 mm) and E. Merck HPTLC RP-18 WF_{254}s plates 0.20 mm. Compounds were visualized by dipping the plates in a cerium sulfate−ammonium molybdate solution followed by heating. Liquid column chromatography was performed using forced flow of the indicated solvent on Sigma H-Type silica gel (10−40 μm) for normal phase and EM Science LiChroprep RP-18 (15−25 μm) for reverse phase.

Trisaccharide 20. To a cooled (0 °C) solution of the disaccharide glycal **18** (1.282 g, 2.48 mmol) in 25 mL of CH_2Cl_2 was added 74 mL of dimethyldioxirane (≈0.08 M) in acetone. After 30 min, the starting glycal was not visible by TLC and the solvent was removed under a stream of N_2 and then under high vacuum for 5 h. To a solution of the resulting epoxide in 10 mL of THF was added a solution of 3,4-dibenzyl glucal **19** (1.303 g, 3.99 mmol) in 10 mL of THF. The mixture was cooled to − 78 °C, and $ZnCl_2$ (3.7 mL of 1 M in Et_2O, 3.7 mmol) was added. The reaction was allowed to warm to room temperature overnight and was quenched with $NaHCO_3$ (30 mL satd aq), the aqueous layer was extracted with EtOAc (3 × 70 mL), and combined organics

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(52) Griffith, D. A.; Danishefsky, S. J. *J. Am. Chem. Soc.* **1990**, *112*, 5811.
(53) Griffith, D. A.; Danishefsky, S. J. *J. Am. Chem. Soc.* **1991**, *113*, 5863.
(54) Danishefsky, S. J.; Bilodeau, M. T. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 1381.

were washed with brine and dried (Na_2SO_4). The solvent was removed in vacuo and the crude material purified using flash chromatography (5:4:1 EtOAc:hexanes:CH_2Cl_2) to give **20** (1.782 g, 84%) as a white foam: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34 (10H, m), 6.40 (1H, d, J = 6.1 Hz), 4.89 (1H, dd, J = 2.9, 6.1 Hz), 4.86−4.83 (2H, m), 4.68−4.63 (4H, m), 4.58−4.54 (2H, m), 4.49 (1H, dd, J = 4.6, 7.8 Hz), 4.38 (1H, d, J = 7.6 Hz), 4.18−3.86 (10H, m), 3.82−3.69 (3H, m), 3.55 (1H, dd, J = 4.5, 9.5 Hz), 3.22 (1H, d, J = 4.3 Hz), 2.85 (1H, d, J = 3.1 Hz), 1.06 (21H, m).

Trisaccharide 21. To a solution of the trisaccharide glycal **20** (1.274 g, 1.48 mmol) in 20 mL of THF was added 1.40 mL (14.82 mmol) of acetic anhydride followed by 2.0 mL (14.82 mmol) of collidine and a catalytic amount of DMAP. The reaction was stirred at room temperature for 2.5 h and then quenched with $NaHCO_3$ (30 mL satd aq), the aqueous layer was extracted with EtOAc (3 × 50 mL), and combined organics were washed with $CuSO_4$ (2 × 20 mL satd aq) and brine (2 × 30 mL) and dried (Na_2SO_4). The solvent was removed in vacuo and the crude material purified using flash chromatography (gradient 3:7 to 4:6 EtOAc:hexanes) to give **21** (1.152 g, 82%) as a white foam: $[\alpha]^{23}_D$ −38.8 (CH_2Cl_2, c = 0.71); FTIR (NaCl, neat) 2943, 1814, 1756, 1648, 1454, 1371, 1225, 1172, 1066 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.29 (10H, m), 6.39 (1H, d,J = 6.2 Hz), 5.08−5.03 (3H, m), 4.93 (1H, m), 4.89−4.85 (2H,m), 4.82−4.79 (2H, m), 4.76−4.72 (2H, m), 4.66 (1H, d, J = 11.5 Hz), 4.61 (1H, d, J = 11.8 Hz), 4.53 (1H, d, J = 11.8 Hz), 4.14−3.89 (7H, m), 3.86 (1H, d, J = 9.2 Hz), 3.81 (1H, dd, J = 6.2, 11.2 Hz), 3.74 (1H, dd, J = 5.7, 7.8 Hz), 3.65 (1H, ddd, J = 5.0, 5.5, 10.5 Hz), 2.13 (3H, s), 2.09 (3H, s), 1.08 (21H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.9, 168.7, 154.0, 153.2, 144.5, 138.4, 138.2, 128.4, 127.8, 127.7, 127.6, 99.6, 98.6, 96.6, 75.9, 74.7, 74.0, 73.7, 73.1, 73.0, 72.0, 71.5, 70.2, 69.5, 69.4, 68.9, 67.8, 67.2, 67.1, 61.7, 20.7, 17.9, 11.8; HRMS (FAB) calcd for $C_{47}H_{62}O_{18}$-SiNa (M + Na^+) 965.3604, found 965.3613.

Trisaccharide 23. To a cooled (−10 °C ice−acetone bath) solution of the trisaccharide glycal **21** (532 mg, 0.564 mmol), 9-anthracenesulfonamide **22** (507 mg, 1.971 mmol), and 0.887 g of powdered 3 Å molecular sieves in 50 mL of THF was added freshly prepared iodonium bis(*sym*-collidine) perchlorate (544 mg, 1.16 mmol) in one portion, and the mixture was gently stirred for 10 min at this temperature. The yellow suspension was warmed to 0 °C and stirred for an additional 45 min before being cooled to −10 °C. A cold (−10 °C) solution of ascorbic acid (2.75 g) in THF:water (10:1, 60 mL) was added. The reaction was filtered, and the filtrate was diluted with 35 mL of H_2O and the aqueous layer extracted with EtOAc (2 × 100 mL). The combined organics were washed with satd $CuSO_4$(aq) (2 × 50 mL) and brine (2 × 50 mL) and dried (Na_2SO_4). The solvent was removed in vacuo, and the crude material was purified by column chromatography with 10−50 μm silica gel (3:1:6 EtOAc:CH_2Cl_2:hexanes) to give (619 mg, 83%) the iodo sulfonamide **23**: $[\alpha]^{23}_D$ −28.9 (CH_2Cl_2, c = 1.04); FTIR (NaCl, neat) 3280, 2942, 1808, 1756, 1623, 1454, 1371, 1336, 1223, 1167, 1068 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.31 (2H, d, J = 9.3 Hz), 8.72 (1H, s), 8.06 (2H, d, J = 8.4 Hz), 7.71 (2H, dd, J = 9.3, 6.6 Hz), 7.55 (2H, dd, J = 8.4, 6.6 Hz), 7.32−7.24 (8H, m), 7.16 (2H, m), 6.29 (1H, m), 5.15 (1H, t, J = 73.6 Hz), 5.09 (2H, m), 4.97 (1H, d, J = 8.6 Hz), 4.86 (2H, d, J = 14.6 Hz), 4.76−4.69 (3H, m), 4.62 (2H, m), 4.39 (1H, d, J = 11.5 Hz), 4.26 (1H, d, J = 11.5 Hz), 4.08−3.96 (4H, m), 3.93−3.86 (2H, m), 3.78 (2H, m), 3.47 (1H, dd, J = 5.7, 9.7 Hz), 3.37 (1H, m), 3.17 (2H, m), 2.17 (3H, s), 2.11 (3H, s), 1.10 (m, 21H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.8, 168.8, 154, 137.9, 137.3, 136.0, 131.2, 130.4, 129.4, 129.2, 128.3, 127.9, 127.8, 127.7, 127.6, 125.5, 125.0, 96.7, 96.5, 83.7, 74.7, 74.2, 73.7, 72.6, 72.2, 71.9, 71.4, 70.8, 69.4, 68.2, 67.4, 67.2, 67.1, 61.6, 31.9, 20.8, 20.7, 17.9, 11.8.

Trisaccharide 24. To a solution of the iodosulfonamide **23** (548 mg, 0.413 mmol) in 25 mL of THF was added tetrabutylammoium azide (294 mg, 1.240 mmol) in one portion. The reaction was stirred at room temperature for 40 min and then filtered through silica gel, the solvent removed in vacuo, and the crude material purified by column chromatography using 10−50 μm silica gel and 4:1:5 EtOAc:CH_2Cl_2:hexanes to give 434 mg (85%) of the anomeric azide trisaccharide **24**: $[\alpha]^{23}_D$ −26.4 (CH_2Cl_2, c = 0.975); FTIR (NaCl, neat) 3308, 2942, 2117, 1814, 1757, 1454, 1371, 1331, 1224, 1162, 1147, 1085 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.19 (2H, d, J = 9.3 Hz), 8.57 (1H, s), 7.98 (2H, d, J = 8.2 Hz), 7.60 (2H, dd, J = 9.3, 6.6 Hz), 7.49 (2H, dd, J = 8.2, 6.6 Hz), 7.31−7.25 (6H, m), 7.19 (2H, m), 7.08 (2H, m), 5.38 (1H, d, J = 7.4 Hz), 5.03 (2H, m), 4.92 (1H, t apparent, J = 3.5 Hz), 4.85 (2H, m), 4.70−4.61 (4H, m), 4.47 (3H, m), 4.34 (1H, d, J = 7.7 Hz), 3.98−3.83 (6H, m), 3.56−3.42 (6H, m), 2.12 (3H, s), 2.03 (3H, s), 1.09 (m, 21H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.8, 153.9, 153.3, 137.5, 137.4, 135.4, 131.2, 129.9, 129.5, 128.8, 128.5, 128.4, 127.9, 127.6, 125.2, 124.9, 97.8, 96.5, 88.8, 80.3, 74.1, 73.9, 72.99, 72.7, 71.9, 71.4, 69.5, 68.9, 68.0, 67.7, 67.2, 67.1, 61.6, 57.8, 20.7, 20.6, 17.9, 11.8; HRMS (FAB) calcd for $C_{61}H_{72}O_{20}N_4SiSNa$ (M + Na^+) 1263.4130, found 1263.4150.

Trisaccharide 25. To a solution of the azide **24** (368 mg, 0.296 mmol) in 20 mL of THF was added 0.70 mL (7.40 mmol) of acetic anhydride followed by 0.7266 g (5.92 mmol) of DMAP. The reaction was protected from light and stirred at room temperature for 1 h and then quenched with $NaHCO_3$ (50 mL satd aq), the aqueous layer was extracted with EtOAc (3 × 70 mL), and combined organics were washed with $NaHCO_3$ (50 mL satd aq), NH_4Cl (2 × 50 mL satd aq), and brine (50 mL) and dried (Na_2SO_4). The solvent was removed in vacuo and the crude material purified using column chromatography with 10−50 μm silica gel and a gradient of 3.5:1:4.5 EtOAc:CH_2Cl_2:hexanes to give 343 mg (90%) of **25** as a yellow solid: $[\alpha]^{23}_D$ +27.3 (CH_2Cl_2, c = 1.02); FTIR (NaCl, neat) 2942, 2118, 1815, 1758, 1701, 1370, 1224, 1165, 1086 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$, rotamers at room temperature) δ 9.40 (1H, d, J = 9.4 Hz), 9.29 (1H, d, J = 9.4 Hz), 8.81 (0.5H, s), 8.73 (0.5H, s), 8.07 (2H, d, J = 8.4 Hz), 8.03 (2H, d, J = 8.4 Hz), 7.71 (2H, m), 7.54 (2H, m), 7.37−7.17 (10H, m), 6.65 (1H, d, J = 9.0 Hz), 5.48 (0.5H, d, J = 8.1 Hz), 5.36 (0.5H, d, J = 10.5 Hz), 5.13−4.87 (6H, m), 4.78−4.71 (4H, m), 4.57 (0.5H, d, J = 11.1 Hz), 4.54 (0.5H, d, J = 11.3 Hz), 4.40 (1H, m), 4.33−3.58 (8H, m), 3.45 (1H, m), 2.17 (1.5H, s), 2.15 (1.5H, s), 2.13 (1.5H, s), 2.12 (1.5H, s), 2.07 (1.5H, s), 2.05 (1.5H, s), 1.06 (21H, m); ^{13}C NMR (100 MHz, $CDCl_3$, rotamers at room temperature) δ 174.2, 172.4, 169.0, 168.8, 168.7, 154.1, 153.3, 138.2, 137.9, 137.1, 131.7, 131.3, 131.2, 129.6, 129.5, 129.2, 128.6, 128.4, 128.3, 128.1, 127.9, 127.7, 127.5, 125.9, 125.7, 125.6, 125.3, 98.1, 96.7, 96.3, 86.8, 86.6, 80.6, 80.3, 79.9, 78.8, 75.3, 74.7, 73.4, 72.8, 72.4, 72.0, 71.8, 71.6, 71.5, 69.6, 69.5, 69.3, 68.5, 67.8, 67.6, 67.4, 66.3, 65.2, 63.1, 61.6, 26.6, 24.1, 20.7, 17.9, 11.8.

Trisaccharide 26. To a cooled (0 °C) solution of the trisaccharide **25** (282 mg, 0.220 mmol) in 7 mL of THF was added 89 μL (0.87 mmol) of thiophenol followed by stepwise addition of 72 μL (0.41 mmol) diisopropylethylamine. The reaction was stirred for 1 h, and then 1 mL of CH_3OH was added and the reaction slowly lightened in color. The reaction was stirred 30 min more and then quenched with a solution of 15 mL of NH_4Cl combined with 6 drops of 1 N HCl and diluted with 50 mL of EtOAc. The organic layer was removed and the aqueous layer extracted with EtOAc (2 × 50 mL). The combined organics were washed with H_2O (15 mL), $NaHCO_3$ (15 mL of satd aq), and brine and dried (Na_2SO_4). The crude material was filtered through silica gel and the solvent removed in vacuo. The product was purified by column chromatography with 10−50 μm silica gel using a gradient of 9:1 to 6:4 CH_2Cl_2:EtOAc to give 208 mg (91%) of the desired *N*-acetyl azide trisaccharide **26**: $[\alpha]^{23}_D$ −30.6 (CH_2Cl_2, c = 6.16); FTIR (NaCl, neat) 3293, 2943, 2115, 1813, 1757, 1665, 1545, 1455, 1371, 1225, 1087 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31 (10H, m), 5.90 (1H, m), 5.06 (2H,m), 5.02 (1H, t apparent, J = 3.5 Hz), 4.94 (1H, m), 4.88−4.81 (4H, m), 4.79 (1H, d, J = 3.5 Hz), 4.73, (2H, m), 4.64 (1H, d, J = 11.5 Hz), 4.61 (1H, d, J = 11.5 Hz), 4.08−3.85 (7H, m), 3.70−3.57 (4H, m), 3.51 (1H, t apparent, J = 8.4 Hz), 2.13 (3H, s), 2.10 (3H, s), 1.85 (3H, s), 1.08 (21H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.5, 168.9, 168.8, 153.9, 153.3, 138.2, 137.8, 128.4, 128.2, 128.0, 127.8, 127.7, 98.0, 96.8, 87.7, 80.6, 78.0, 76.3, 74.5, 73.2,

(55) Iodonium bis(*sym*-collidine) perchlorate: Under nitrogen, iodine (775 mg, 3 mmol) was added to a suspension of silver bis(*sym*-collidine) perchlorate and *sym*-collidine (45 μL, 0.34 mmol) in 12 mL of chloroform (dried over activated basic alumina). The mixture was vigorously stirred for 15 min and the bright yellow suspension was filtered over flame-dried Celite under dry nitrogen. Ether (10 mL) was added and the faintly green precipitate was filtered over sintered glass under dried nitrogen, washed with ether and dried under reduced pressure. Yield ∼1 g.

72.9, 72.0, 71.7, 69.6, 69.1, 68.3, 67.7, 67.3, 61.5, 55.4, 23.3, 20.7, 20.6, 17.8, 11.9.

Trisaccharide 27. To a solution of the trisaccharide azide **26** (93.5 mg, 0.090 mmol) in 1.7 mL of THF and 0.7 mL of 2-propanol was added freshly prepared aluminum amalgam (from 48 mg, 1.79 mmol Al and $HgCl_2$ (10% aq)) followed by 130 μL (7.17 mmol) of H_2O. A precipitate formed immediately upon addition of the H_2O. The reaction was stirred at room temperature for 16 h, and no more starting material was identified by TLC. The crude material was filtered through Celite and the solvent removed in vacuo to give 82.2 mg (90%) of crude **27** which was used immediately: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31 (10H, m), 5.19 (1H, d, J = 7.7 Hz), 5.09−5.04 (2H, m), 4.95 (1H, m), 4.90 (2H, dd, J = 1.7, 8.5 Hz), 4.87−4.80 (3H, m), 4.75−4.73 (2H,m), 4.66−4.59 (2H, m), 4.08−3.85 (7H, m), 3.68−3.45 (6H, m), 2.14 (3H, s), 2.12 (3H, s), 1.84 (3H, s), 1.57 (2H, br s), 1.08 (21H, m).

Glycopetide 42. To a solution of the trisaccharide amine **27** (22 mg, 0.022 mmol) and the tripeptide **38** (TrocAspLeuThr(OBn)OAll, 31 mg, 0.048 mmol) in 1 mL of dichloromethane was added IIDQ (13 mL, 0.043 mmol). The mixture was stirred at room temperature for 24 h. The mixture was poured into 10 mL of 0.05 N hydrochloric acid and was extracted with 3 × 20 mL of ethyl acetate. The combined organic fractions were washed with brine, dried with sodium sulfate, and concentrated. The product was purified by column chromatography with fine silica gel using a mixture of 3:2:5 acetone:CH_2Cl_2:hexane to give 25.4 mg of slightly impure trisaccharide tripeptide. The mixture was purified by column chromatography with fine silica gel using a gradient of 97.5:2.5 to 95:5 CH_2Cl_2/methanol to give 21.4 mg (60%) of the desired product **42**: $[\alpha]^{23}_D$ −9.4° (*c* 1.15, CH_2Cl_2); FTIR (NaCl, neat) 3286, 2948, 1813, 1753, 1719, 1644, 1536, 1371, 1224, 1078 cm-^{1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31 (16H, m), 7.07 (1H, d, J = 7.7 Hz), 6.63 (1H, d, J = 8.2 Hz), 6.56 (1H, d, J = 9 Hz), 5.82 (1H, m),5.29 (1H, d, J = 15 Hz), 5.22 (1H, d, J = 10 Hz), 5.15−4.30 (20H, m), 4.08−3.50 (13H, m), 2.72−2.60 (2H, m), 2.13 (3H, s), 2.10 (3H, s), 1.76 (3H, s), 1.55 (2H, m), 1.52 (1H, m), 1.40−1.00 (27H, m) 0.99−0.80 (6H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.15, 172.00, 171.56, 170.11, 169.97, 168.97, 168.84, 154.41, 154.17, 153.28, 137.99, 137.79, 131.52, 128.79, 128.68, 128.53, 128.36, 128.30, 128.00, 127.94, 127.80, 127.78, 118.98, 98.29, 96.53, 95.27, 80.54, 79.63, 78.17, 77.20, 76.30, 74.99, 74.66, 74.29, 74.19, 74.11, 73.37, 71.97, 71.36, 70.71, 69.74, 69.31, 67.29, 66.99, 66.10, 61.62, 56.66, 54.01, 52.08, 51.34, 40.91, 37,15, 29.67, 29.24, 24.57, 23.19, 22.89, 22.07, 20.79, 20.73, 17.91, 17.87, 16.19, 16.19, 11.78; HRMS calcd for $C_{76}H_{103}Cl_3N_5O_{27}Si$ 1650.5674, found 1650.5646.

Glycopeptide 46. Dimethylbarbituric acid (17 mg, 0.11 mmol) and tetrakis(triphenylphosphine)palladium (2 mg, 0.0018 mmol) were successively added to a solution of alcohol **44** (27.2 mg, 0.018 mmol) in 2.5 mL of THF, and the dark orange mixture was stirred at room temperature for 2 h. The solvent was removed under reduced pressure, and the residue was dissolved in a minimum amount of MeOH (∼0.3 mL) and applied over reverse phase silica [3 g, C-18, 1:1 H_2O (0.1% TFA):MeOH → MeOH] to give **46** (29 mg). The product was contaminated with byproducts of the tetrakis(triphenylphosphine)-palladium and was used for the next step.

Glycopeptide 43. To a solution of the pentapeptide **41** (80.3 mg, 0.0789 mmol) in 2 mL of CH_2Cl_2 was added the trisaccharide **27** (132.8 mg, 0.1455 mmol) in 3 mL of CH_2Cl_2 via Teflon cannula. To this mixture was added IIDQ (25 μL, 0.0789 mmol), and the reaction was stirred at room temperature for 10.5 h. The reaction was quenched with a mixture of 20 mL of water and 4 mL of 0.1 N HCl, and the aqueous phase was extracted with 6 × 30 mL of ethyl acetate. The organics were washed with brine (30 mL) and dried (Na_2SO_4). The solvent was removed in vacuo and the crude glycopeptide purified by flash chromatography using 95:5 CH_2Cl_2:CH_3OH to give 83.6 mg (55%) of the desired glycopeptide **43**: $[\alpha]^{24}_D$ −27.0° (*c* 2.29, CH_2Cl_2); FTIR (neat) 3284, 2942, 1812, 1754, 1636, 1539, 1371, 1224, 1073 cm-^{1}; ^{1}H NMR (400 MHz, CD_3OD) δ 7.30 (20H, m), 5.86, (1H, ddd, J = 5.5, 10.5, 16.5 Hz), 5.30 (1H, dd, J = 1.2, 16.5 Hz), 5.19 (1H, d, J = 1.2, 10.5 Hz), 5.06−5.13 (3H, m), 5.01−5.03 (2H, m), 4.84−4.95 (6H, m), 4.73−4.79 (4H, m), 4.65−4.70 (3H, m), 4.51−4.62 (5H, m), 4.47−0.39 (2H, m), 4.30−4.34 (1H, m), 4.17−3.86 (12H, m), 3.69−3.65 (4H, m), 3.51−3.47 (2H, m), 2.72 (1H, d, J = 5.0, 16.0 Hz), 2.64 (1H, dd, J = 7.0, 16.0 Hz), 2.09 (3H, s), 2.06 (3H, s), 1.85 (3H, s), 1.64 (6H, m), 1.35 (3H, d, J = 7.0 Hz), 1.20 (3H, d, J = 6.0 Hz), 1.09 (21H, m), 0.93 (6H, d, J = 6.0 Hz), 0.88 (6H, d, J = 5.0 Hz); ^{13}C NMR (CD_3OD) δ 175.4, 174.6, 174.1, 173.7, 172.3, 171,1, 170.6, 170.5, 158.1, 155.8, 155.2, 139.4, 139.2, 139.1, 137.2, 132.8, 129.3, 129.2, 128.9, 128.8, 128.7, 128.6, 128.5, 119.1, 998, 98.6, 84.3, 79.5, 79.1, 77.2, 76.4, 75.9, 75.6, 75.4, 75.2, 74.1, 73.9, 71.8, 71.2, 70.9, 69.1, 68.6, 67.7, 66.9, 63.0, 58.0, 55.3, 53.2, 53.1, 51.9, 50.9, 41.7, 41.2, 37.7, 25.6, 25.5, 23.5, 23.2, 22.1, 21.8, 20.9, 18.4, 18.2, 16.5, 12.8; HRMS (FAB) calcd for $C_{90}H_{124}N_7O_{29}Si$ 1794.8216, found 1794.8200.

Glycopeptide 44. To a cold (−10 °C to −15 °C) solution of the fully protected trisaccharide tripeptide **42** (19.1 mg, 0.012 mmol) and anisole (2.5 μL, 0.023 mmol) in CH_2Cl_2 (3 mL) was added HF·pyridine (16.5 μL, 0.58 mmol). The mixture was stirred for 50 min at −10 °C to −15 °C and then water (5 mL) was added and the mixture was warmed to room temperature. The mixture was extracted with ethyl acetate (3 × 30 mL) and washed a basic mixture of brine and saturated bicarbonate (2 × 5 mL), and the organic alyers were dried with sodium sulfate. After filtration and concentration, **44** was obtained as a crude amorphous solid, essentially pure by proton NMR. The mixture was purified by column chromatography with fine silica gel using a gradient of 97.5:2.5 to 95:5 CH_2Cl_2 methanol to give 14.6 mg (84%) of the desired product. This product still had traces of impurities and was used directly in the next deprotection step.

Glycopeptide 48. The acid **46** (26.2 mg, 0.018 mmol) was dissolved in a mixture of 2.5 mL of MeOH and 0.6 mL of acetic acid, and zinc dust (Aldrich, 30 mg, 0.45 mmol) was added. The suspension was stirred at room temperature for 15 h. The gray mixture was filtered over Celite, the solids were washed successively with ethanol (15 mL) and MeOH (15 mL), and the solvents were combined and evaporated. The residue was applied on reverse phase silica [3 g, C-18, 7:3 H_2O (0.1% TFA):MeOH → 9:1] to give the TFA salt (20.7 mg, ∼83%). R_f = 0.23 [7:3 H_2O (0.1% TFA):MeOH]; FTIR (KBr pellet); 3306 (OH), 2928, 1804, 1752, 1670 (C═O), 1534, 1372, 1083; HRMS (FAB) calcd for $C_{61}H_{78}N_5O_{25}$ 1280.4990, found 1280.5000. The product was still slightly impure by ^{1}H NMR and was used for the next step. The material from the previous reaction (20.7 mg, 0.014 mmol) was dissolved in 2 mL of MeOH and 2 mL of acetic acid and palladium-(II) acetate (12 mg, 0.053 mmol) was added. The yellow mixture was kept under a positive pressure of hydrogen (balloon) for 17 h with efficient stirring. At the end of this period, the black suspension was sonicated for 5 min and filtered over Celite, and the solids were washed with MeOH and acetic acid. The solvents were removed under reduced pressure. The completion of the reaction was monitored by observing the disappearance of the aromatic signals in the ^{1}H NMR spectra of the crude product in D_2O. The solvent was removed under reduced pressure, and the residue was dissolved 4 mL of MeOH. Potassium cyanide (2.6 mg, 0.04 mmol) was added, and the pH was observed to be between 7 and 8 (moist pH paper). After 1 h, additional potassium cyanide was added (1 mg, 0.015 mmol), and the mixture was stirred at pH ∼8 for 9 h. A 100 μL volume of acetic acid was added, most of the solvents were removed under a flow of nitrogen in an efficient fume hood, and the residue was kept under vacuum for 30 min. The glassy solid was applied on reverse phase silica [1 g, C-18, H_2O (0.1% TFA) → 9:1 H_2O (0.1% TFA):MeOH] to give **48** as its TFA salt (10 mg, 65% overall yield from **46**). R_f = 0.55 [H_2O (0.1% TFA)]; $[\alpha]^{23}_D$ −0.6° (*c* 0.48, H_2O); FTIR (KBr pellet); 3424 (OH), 2928, 1653 (C═O), 1552, 1388, 1077 cm^{-1}; ^{1}H NMR (400 MHz, D_2O) δ 5.10 (d, 1H, J = 10 Hz, H1 GlcNAc), 4.46 (d, 1H, J = 9 Hz, H1 Gal), 4.44 (d, 1H, J = 8 Hz, H1 Gal), 4.39 (dd, 1H, J = 9 Hz, J = 4 Hz, Hα Asn), 4.30−4.22 (m, 2H, Hβ Thr, Hα Leu), 4.10−4.03 (m, 1H), 4.00−3.50 (m, 18H), 3.05 (dd, 1H, J = 18 Hz, J = 4 Hz, Hβ Asn), 2.90 (dd, 1H, J = 18 Hz, J = 9 Hz, Hβ Asn), 2.01 (s, 3H, NHAc), 1.75−1.63 (m, 3H, Hβ, Hγ Leu), 1.17 (d 3H, J = 6 Hz, Me Thr), 0.95 (d 3H, J = 6 Hz, Me Leu), 0.91 (d 3H, J = 6 Hz, Me Leu); ^{13}C (D_2O) δ 177.40, 176.21, 175.21, 172.57, 169.96, 164.90 ($^2J_{CF}$ = 37 Hz), 115.50 ($^1J_{CF}$ = 286 Hz), 104.71, 104.63, 79.72, 77.95, 76.58, 75.61, 75.33, 74.11, 73.91, 72.15, 72.08, 70.81, 70.39, 70.10, 70.06, 70.02, 69.37, 62.39, 61.46, 55.35, 54.51, 50.88, 41.05, 36.77, 25.75, 23.52 (2C), 22.12, 20.60; HRMS (FAB, Na^+) *m/z* 874.3821 (MH^+), calcd for $C_{34}H_{60}N_5O_{21}$ 874.3780.

Glycopeptide 45. To a cooled (−10 °C) solution of glycopeptide **43** (17.3 mg, 0.0090 mmol) and anisole (2 μL, 0.018 mmol) in 2 mL of CH_2Cl_2 was added 0.2 mL of HF·pyridine, and the reaction was stirred for 2 h. The reaction was quenched with a cooled (−10 °C) mixture of 5 mL of 31.5 mM phosphate buffer and 10 mL of ethyl acetate. The aqueous layer was extracted with 4 × 20 mL of ethyl acetate, the combined organics were washed with pH 8 brine (9 mL of brine and 1 mL of satd bicarbonate) and dried (Na_2SO_4), and the solvent removed in vacuo. The resulting white powder was purified by RP-18 column chromatography, eluting with a gradient of 7:3 to 9:1 CH_3OH:H_2O to give 9.6 mg (65%) of glycopeptide **45** as a white powder: $[\alpha]^{24}_D$ −14.2° (*c* 1.38, CH_2Cl_2); FTIR (neat) 3289, 2956, 1807, 1750, 1637, 1533, 1454, 1371, 1229, 1171, 1073 cm^{-1}; ^1H NMR (400 MHz, CD_3OD) δ 7.31 (20H, m), 5.88, (1H, ddd, *J* = 5.5, 10.5, 16.5 Hz), 5.31 (1H, dd, *J* = 1.0, 16.5 Hz), 5.19 (1H, d, *J* = 1.2, 10.5 Hz), 5.14−5.00 (5H, m), 4.96 (1H, t, *J* = 4.0 Hz), 4.92−4.89 (6H, m), 4.81−4.68 (7H, m), 4.66−4.57 (7H, m), 4.49−4.41 (2H, m), 4.34 (1H, d, *J* = 6.0, 9.0 Hz), 4.19−4.00 (7H, m), 3.92 (1H, t, *J* = 10.0 Hz), 3.82−3.67 (6H, m), 3.52 (2H, d, *J* = 5.0 Hz), 2.76 (1H, d, *J* = 5.0, 16.0 Hz), 2.64 (1H, dd, *J* = 7.0, 16.0 Hz), 2.09 (3H, s), 2.06 (3H, s), 1.85 (3H, s), 1.74−1.60 (6H, m), 1.35 (3H, d, *J* = 7.0 Hz), 1.22 (3H, d, *J* = 6.5 Hz), 0.94 (6H, d, *J* = 6.0 Hz), 0.89 (6H, d, *J* = 6.0 Hz); ^{13}C NMR (100 MHz, CD_3OD) δ 175.6, 174.9, 174.3, 173.6, 172.5, 172.4, 171.2, 170.7, 168.8, 161.8, 155.6, 155.4, 139.7, 139.4, 139.3, 133.1, 129.4, 129.3, 128.9, 128.8, 128.7, 128.6, 119.1, 102.0, 99.2, 84.4, 79.7, 79.1, 76.6, 76.1, 75.8, 75.6, 75.4, 73.3, 71.9, 71.2, 70.6, 69.8, 68.6, 68.4, 68.3, 67.7, 67.0, 61.8, 58.2, 55.5, 53.3, 53.2, 52.1, 51.1, 41.9, 41.4, 37.8, 25.8, 25.6, 23.5, 23.2, 22.1, 21.9, 21.2, 20.8, 18.1, 16.5; HRMS (FAB) calcd for $C_{81}H_{103}N_7O_{29}$ 1638.6881, found 1638.6840.

Glycopeptide 47. Dimethylbarbituric acid (2.1 mg, 0.013 mmol) and tetrakis(triphenylphosphine)palladium (1.0 mg, 0.0009 mmol) were successively added to a solution of alcohol **45** (3.6 mg, 0.0022 mmol) in 2.0 mL of THF, and the dark orange mixture was stirred at room temperature for 5 h. The solvent was removed under reduced pressure, and the residue was dissolved in a minimum amount of MeOH (∼0.3 mL) and applied over reverse phase silica, C-18, 7:3 H_2O (0.1% TFA):MeOH → MeOH] to give trisaccharide pentapeptide **47** (3.5 mg, 100%): $[\alpha]^{24}_D$ −14.3° (*c* 0.46, CH_2Cl_2); FTIR (neat) 3298, 1812, 1754, 1651, 1514, 1454, 1371, 1221, 1168, 1069 cm^{-1}; ^1H NMR (400 MHz, CD_3OD) δ 8.25 (1H, d, *J* = 9.0 Hz), 8.16 (1H, d, *J* = 8.0 Hz), 8.07 (1H, d, *J* = 7.5 Hz), 7.91−7.87 (2H, m), 7.56−7.51 (2H, m), 7.31 (20H, m), 5.13−4.99 (4H, m), 4.96 (1H, t, *J* = 3.5 Hz), 4.91−4.89 (5H, m), 4.79−4.69 (5H, m), 4.66−4.60 (3H, m), 4.57−4.54 (1H, m), 4.50−4.45 (2H, m), 4.31−4.38 (1H, m), 4.21−4.00 (6H, m), 3.92 (1H, t, *J* = 10.0 Hz), 3.86−3.64 (6H, m), 3.52 (2H, d, *J* = 5.5 Hz), 2.75 (1H, dd, *J* = 5.0, 16.0 Hz), 2.67 (1H, dd, *J* = 9.0, 16.0 Hz), 2.09 (3H, s), 2.05 (3H, s), 1.87 (3H, s), 1.73−1.57 (6H, m), 1.35 (3H, d, *J* = 7.0 Hz), 1.21 (3H, d, *J* = 6.5 Hz), 0.94 (6H, d, *J* = 6.0 Hz), 0.89 (6H, d, *J* = 6.0 Hz); ^{13}C NMR (CD_3OD) δ 199.2, 175.8, 174.9, 174.0, 173.4, 172.7, 172.6, 170.9, 155.8, 155.6, 139.9, 139.6, 129.5, 129.4, 129.1, 129.0, 128.9, 128.8, 128.7, 100.1, 99.5, 84.8, 79.9, 79.3, 77.4, 76.9, 76.1, 75.8, 75.6, 75.2, 75.0, 72.3, 72.2, 71.8, 71.4, 70.1, 68.9, 68.6, 67.8, 62.0, 58.1, 55.6, 53.4, 52.2, 51.3, 41.9, 41.6, 37.9, 25.9, 25.7, 23.6, 23.2, 22.1, 21.9, 20.8, 18.2, 16.7.

Glycopeptide 49. In a sealed tube, a mixture of $Pd(OAc)_2$ and glycopeptide **47** (4.7 mg, 0.0027 mmol) in 4 mL of CH_3OH and 2 drops of acetic acid was charged and evacuated with 4 × H_2 and filled to a pressure of 60 psi and stirred overnight. The reaction was then sonicated and filtered through Celite, and the solvent was removed in vacuo to give a crude white powder, the ^1H NMR of which showed no more benzyl groups in the aromatic region. The debenzylated glycopeptide was redissolved in 1 mL of CH_3OH, and 50 μL of 0.1 M KCN/CH_3OH was added. The reaction was pH 8−9 by pH paper. It was stirred at room temperature for 4 h and then quenched with 2 drops of trifluoroacetic acid, and the solvent was removed under a stream of N_2. The crude material was purified by RP-18 column chromatography, eluting with a gradient of 1:9 to 9:1 CH_3OH:H_2O to give 1.2 mg (0.0010 mmol, 37%) of the glycopeptide **49** as a white powder: FTIR (neat) 3326, 1671, 1538, 1204 cm^{-1}; ^1H NMR (400 MHz, D_2O) δ 5.08 (1H, d, *J* = 9.8 Hz), 4.75 (1H, m), 4.49 (1H, d, *J* = 7.8 Hz), 4.45 (1H, d, *J* = 7.9 Hz), 4.40−4.24 (3H, m), 4.07 (1H, dd, *J* = 3.0, 9.5 Hz), 3.98−3.52 (11H, m), 2.90 (1H, dd, *J* = 5.5, 16.5 Hz), 2.77 (dd, *J* = 7.5, 16.5 Hz), 2.03 (3H, s), 1.70−1.56 (6H, m), 1.39 (3H, d, *J* = 7.0 Hz), 1.20 (3H, d, *J* = 6.5 Hz), 0.96 (6H, d, *J* = 6.0 Hz), 0.90 (6H, d, *J* = 6.0 Hz); HRMS (FAB) calcd for $C_{43}H_{75}N_7O_{23}Na$ 1080.4792, found 1080.4800.

Trisaccharide 51. In a polymer synthesis flask, the polymer-bound disaccharide glycal **50** (981 mg, ∼0.77 mmol) was suspended in 15 mL of CH_2Cl_2 for 1 h at room temperature. The mixture was cooled to 0 °C, 3,3-dimethyldioxirane (30 mL in acetone, ∼2.4 mmol) was added, and the mixture was gently stirred for 1 h 20 min. The solvents were filtered, and the resin was kept under reduced pressure for 7 h. A solution of 3,4-dibenzyl glucal (dried by azeotropic distillation with benzene, 1.67 g, 5.12 mmol) in 10 mL of THF was added at 0 °C to the polymer-bound epoxide and a solution of zinc chloride (1.53 mL, 1 M in ether, 1.53 mmol). The mixture was gently stirred at room temperature for 16 h. The solvents were filtered, and the resin was successively washed with THF (4 × 50 mL) and CH_2Cl_2 (3 × 50 mL) and was kept under vacuum overnight to give 1.23 g of polymer-bound trisaccharide **51**.

Trisaccharide 52. In a polymer synthesis flask, the polymer-bound trisaccharide glycal **51** (1.23 g, ∼0.76 mmol) was suspended in 20 mL of THF for 1 h at room temperature. Acetic anhydride (1.44 mL, 15.2 mmol) and *sym*-collidine (2.0 mL, 15.2 mmol) were successively added, and the mixture was gently stirred for 12 h. The solvents were filtered, and the resin was successively washed with THF (4 × 50 mL) and CH_2Cl_2 (3 × 50 mL) and was kept under vacuum overnight to give 1.22 g of polymer-bound trisaccharide **52**.

Trisaccharide 53. In a polymer synthesis flask, the polymer-bound trisaccharide glycal **52** (559 mg, ∼0.212 mmol) and 9-anthracenesulfonamide (381 mg, 1.5 mmol) were suspended in 15 mL of THF for 1 h at room temperature. The mixture was cooled to −10 °C (ice−acetone bath) and freshly prepared iodonium bis(*sym*-collidine) perchlorate (261 mg, 1.1 mmol) was added in one portion, and the mixture was gently stirred for 10 min at this temperature. The yellow suspension was warmed to 0 °C and stirred for an additional 1 h before being cooled to −10 °C. A cold (−10 °C) solution of ascorbic acid (2 g) in THF:water (10:1, 40 mL) was added, turning the solution almost colorless while the polymer remained bright yellow. After 5 min at −10 °C, the mixture was warmed to room temperature (∼1 h). The solvents were filtered, and the resin was successively washed with THF (4 × 50 mL) and CH_2Cl_2 (3 × 50 mL) and was kept under vacuum overnight to give 643 mg of polymer-bound trisaccharide **53**. This resin fluoresced blue at 365 nm.

Trisaccharide 54. In a polymer synthesis flask, the polymer-bound iodo sulfonamide **53** (553 mg, ∼0.18 mmol) was suspended in 12 mL of THF for 1 h at room temperature. Tetrabutylammonium azide (261 mg, 1.1 mmol) was added, and the mixture was gently stirred for 5 h. The solvent was filtered, and the resin was successively washed with THF (4 × 50 mL) and CH_2Cl_2 (3 × 50 mL) and was kept under vacuum overnight to give 520 mg of polymer-bound trisaccharide with the anomeric azide and 2-sulfonamide. This resin also fluoresced blue at 365 nm. In a polymer synthesis flask, the polymer-bound azide sulfonamide (469 mg, ∼0.16 mmol) was suspended in 15 mL of THF for 1 h at room temperature. Acetic anhydride (752 μL, 8 mmol) and 4-(*N*,*N*-dimethylamino)pyridine (779 mg, 6.4 mmol) were successively added, and the mixture was gently stirred for 3 h. The solvent was filtered, and the resin was successively washed with THF (3 × 50 mL) and CH_2Cl_2 (3 × 50 mL) and was kept under vacuum overnight to give 480 mg of polymer-bound trisaccharide **54**. This resin fluoresced green at 365 nm.

Trisaccharide 55. In a polymer synthesis flask, the polymer-bound trisaccharide azide sulfonamide **54** (51 mg, ∼0.018 mmol) was suspended in 5 mL of DMF for 1 h at room temperature. 1,3-Propanedithiol (105 μL, 1.04 mmol) and *N*,*N*-diisopropyl-*N*-ethylamine (109 μL, 0.63 mmol) were added, and the suspension was gently stirred at room temperature for 6 h. The resin was successively washed with DMF (3 mL) and THF (2 × 4 mL), and an aliquot of the resin was examined by IR (KBr pellet). The reaction was incomplete as was indicated by the presence of an azide stretch at 2115 cm^{-1}. The resin was suspended in 2 mL of DMF, 1,3-propanedithiol (220 μL, 2.19 mmol) and *N*,*N*-diisopropyl-*N*-ethylamine (220 μL, 1.26 mmol) were

added, and the suspension was gently stirred at room temperature for 12 h. The resin was successively washed with DMF (3 mL), THF (2 × 5 mL), and CH_2Cl_2 (2 × 5 mL). The resin was dried under reduced pressure to give 51 mg of solid **55**. This resin did not substantially fluoresce at 365 nm.

Glycopeptide 59. In a polymer synthesis flask, the polymer-bound trisaccharide **55** (120.9 mg, ∼0.014 mmol) was swollen in 4 mL of CH_2Cl_2 for 1 h at room temperature. The pentapeptide CbzAlaLeuAspLeuThr(OBn)OAll **41** (96.4 mg, 0.106 mmol) and IIDQ (Aldrich, 30 μL, 0.101 mmol) were successively added. After 25 h, the solvent was filtered, and the resin was washed with CH_2Cl_2 (5 × 5 mL), THF (1 × 5 mL), and CH_2Cl_2 (3 × 5 mL), and the resin was dried under reduced pressure to give 133.3 mg of resin **59**.

Glycopeptide 45. In a Teflon flask, the polymer-bound trisaccharide pentapeptide **59** (94.3 mg, ∼0.0273 mmol) and anisole (8.5 μL, 0.78 mmol) were suspended in 5 mL of CH_2Cl_2 for 1 h at room temperature. The mixture was cooled to −10 °C (acetone/ice), and HF·pyridine (∼50 μL, 1.7 mmol) was slowly added. After 2 h, H_2O (5 mL) was added at −10 °C, and the mixture was extracted with ethyl acetate (4 × 30 mL). The combined organic layers were washed twice with a mixture of brine (10 mL) and saturated $NaHCO_3$ (1−2 mL, pH 7). The organic extracts were dried over sodium sulfate, filtered over a medium porosity glass filter, and concentrated. The residue was purified over RP-18 silica, eluting with 7:3 MeOH:H_2O (0.1% TFA) → 9:1 to give trisaccharide pentapeptide **45** (17.9 mg, ∼37%).

Glycopeptide 62. In a polymer synthesis flask, the polymer-bound trisaccharide pentapeptide **58** (125.4 mg, ∼0.030 mmol) was swollen in 6 mL of THF for 1 h at room temperature. To this mixture, dimethylbarbituric acid (140 mg, 0.90 mmol) and tetrakis(triphenylphosphine)palladium (17 mg, 0.015 mmol) were successively added, and the dark orange mixture was stirred at room temperature. After 14 h, the solvent was filtered, the resin was washed with CH_2Cl_2 (4 × 10 mL), and the resin was dried under reduced pressure to give 113.5 mg of resin **62**.

Trisaccharide 83. To the polymer-supported azide **82** (102 mg, ∼0.05 mmol) swollen in 1.0 mL of THF was added tri-*O*-benzyl fucosyl fluoride (269 mg, 0.511 mmol) as a 0.5 M solution in THF. To this cooled (−10 °C) mixture was added 2,6-di-*tert*-butylpyridine (46 μL, 0.204 mmol) followed by stannous(II)trifluoromethanesulfonate (170 mg, 0.40 mmol). The reaction was allowed to warm to room-temperature overnight and then diluted with CH_2Cl_2 and filtered, and the polymer-support was washed with CH_2Cl_2 (3 × 20 mL), DME (3 × 20 mL), and CH_2Cl_2 (3 × 20 mL) and dried under vacuum to give 130.3 mg of polymer-bound trisaccharide **83**.

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Supporting Information Available: Experimental descriptions for **29, 31, 32, 34−41, 56−58, 60, 61, 63−65, 67−73, 76−82, 84−86** (20 pages). See any current masthead page for ordering and Internet access instructions. See any current masthead page for ordering and Web access instructions.

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