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# A highly convergent synthesis of an N-linked glycopeptide presenting the H-type 2 human blood group determinant

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We dedicate this paper to the magnificent accomplishments of Raymond Lemieux

**Abstract**—The total synthesis of an H-type blood group determinant in a model biological setting is described. The construct is comprised of a high mannose core structure with projecting lactose spacers, culminating in a two-copy presentation of the H-type blood group determinant itself. Key reactions that were used in this construction include sulfonamidohydroxylation (see **15**→**18**) and benzoate-directed glycosylation via an activated thiophenyl donor (see **34**→**36**). Another key strategic element involved the epimerization of an interior core glucoside to reach the β-mannoside (see **37**→**38**) required in the ring C sugar of the high mannose core.


## 1. Introduction

Among the great variety of functions assumed by carbohydrates in biological systems, the role of the carbohydrate domain of glycoproteins in molecular recognition has been a focus of particular attention for many scientific disciplines.[1] Indeed, the growing field of glycobiology is devoted to defining the effects of protein glycosylation on a range of phenomena, including fertilization,[2] tumor metastasis,[3] and immune response.[4] Specific glycosylation patterns also serve as markers for tumorigenesis and are used in blood profiling.[5] A major challenge facing the field of glycobiology is the limited natural accessibility of homogeneous glycopeptides, which can be attributed to difficulties in isolation, compounded by their often microheterogeneic nature. Certainly, the development of technologies to allow access to homogeneous glycopeptides would be of significant value in probing critical interactions at the interface of glycochemistry and glycobiology. Despite the daunting challenges associated with the preparation of glycopeptides in the laboratory, we envisioned that chemical synthesis could yet prove to be the most viable way to provide the field of glycobiology with 'precision tools' for the study of glycoproteins.[6,7]

An ongoing focus of our laboratory has been the development of methodologies that enable the preparation of complex, fully synthetic glycopeptides.[8] An early goal of this research program was the assembly of pentacyclic glycopeptide, **1**, in which the carbohydrate domain is N-linked to the peptide through an asparagine γ-carboxamide, not dissimilar to the way Nature combines such structures. We describe, herein, the design and execution of the synthesis of **1**.[9] We note that, for reasons discussed below, we also accomplished the synthesis of **2**, which differs from **1** only in the absolute stereochemistry of the peptide domain.[10]

As a further demonstration of the advances in the field of glycopeptide synthesis, we set for ourselves the rather ambitious ultimate goal of synthesizing an N-linked, 15-ring glycan construct (**3**), containing *a mature H-type 2 blood group determinant* at its non-reducing end. Nothing of this scope had previously been undertaken in synthesizing N-linked glycopeptides. The manner in which we accomplished the synthesis of this complex target compound is described herein.[11]

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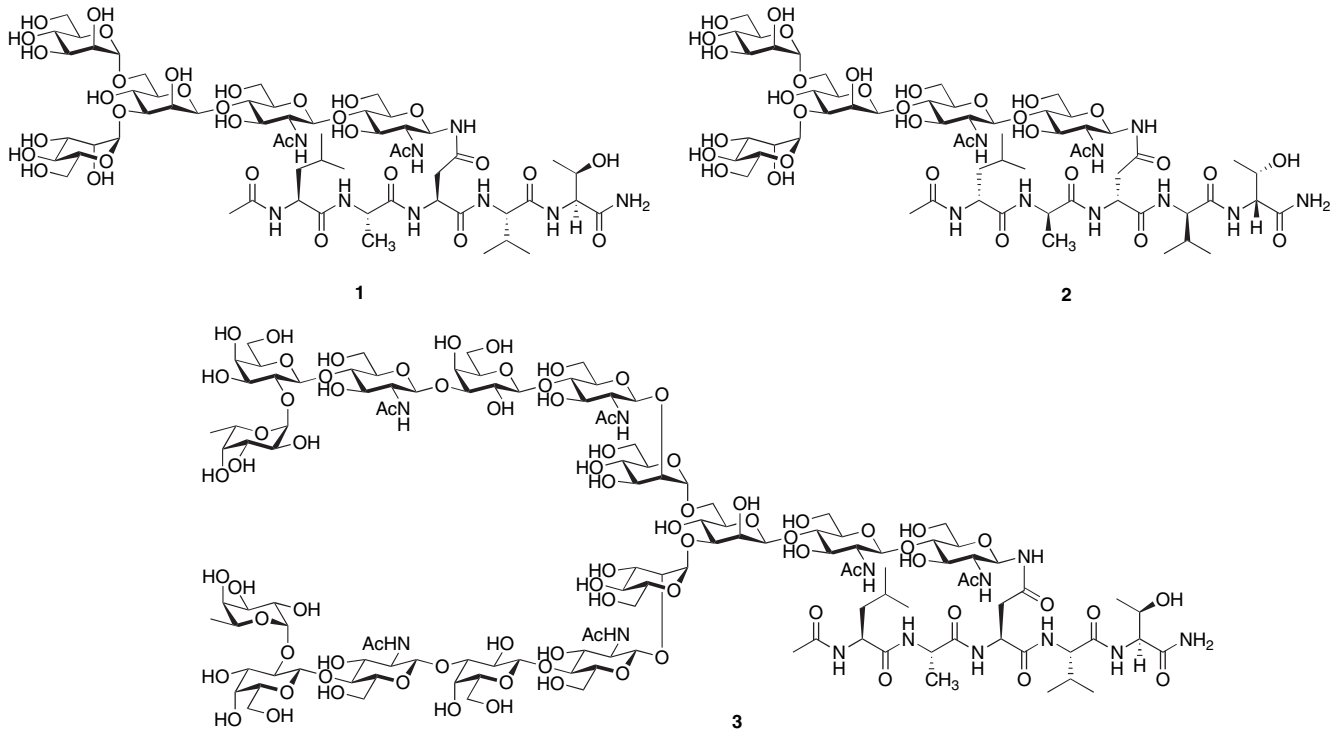


## 2. Results and discussion

In charting a program for glycopeptide synthesis, provisions must be made for assembling the glycan domain. To the extent that the ultimate goal is that of simulating the motifs of Nature, the glycan synthesis itself may well be quite complex. In addition, a polypeptide domain must be assembled. Depending on the situation, the polypeptide domain may be introduced as a single block, or in the form of segments to be subsequently ligated. In either case, the need for the glycan to be coupled to an appropriate peptide chain in the final stages of the synthesis requires careful selection of appropriate glycosylation and protection strategies, with due planning from the outset. Indeed, the implementation of an efficient global deprotection sequence is critical to the success of the enterprise.

In the case of N-linked glycopeptides, the anomeric amine required for eventual merger with a peptide-based acyl donor can be generated in several forms (Fig. 1). Coupling of the 'per protected' glycosylamine produces a glycopeptide, which is then subjected to global deprotection. While the coupling step can be high yielding, maintenance of anomeric configurational homogeneity of the N-linked peptide may be problematic.[12] Indeed, coupling of the anomeric amino group of a perbenzylated glycan (path *a*) or peracetylated glycan (path *b*) to the γ-carboxyl of an aspartyl residue on the peptide chain can produce a difficult

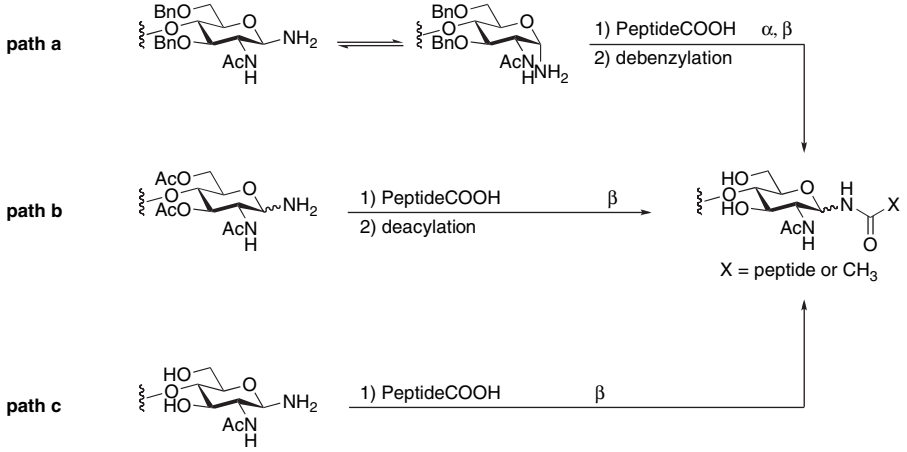
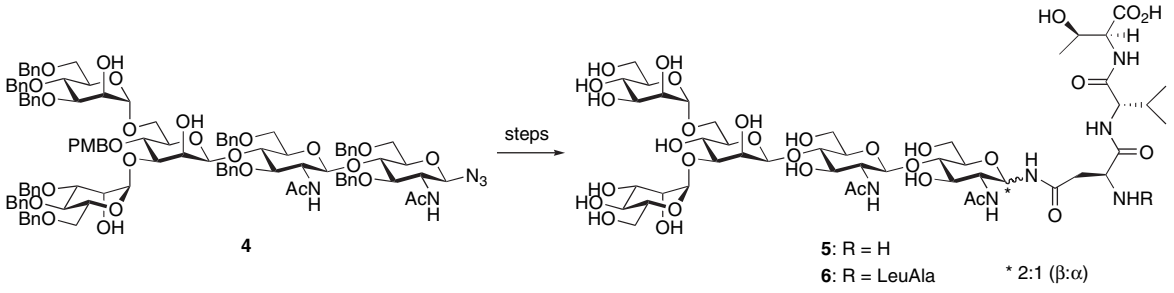



**Figure 1.** Comparison of glycopeptide assembly: (a) O-benzylated (electron-donating) glycosylamine, was coupled with peptide acid, followed by debenzylation. In a complex glycosylamine system, peptide conjugation led to α/β mixture of glycopeptide due to anomerization of benzylated glycosylamine; (b) O-acetylated glycosylamine (electron-deficient) was acylated with peptide acid. In this system, potential migration of acetyl from $O \rightarrow N$ led to the formation of glycosyl acetamide (X=$CH_3$) in addition to the desired β-glycopeptide; (c) selective N-acylation of a polyhydroxyl glycosylamine can be achieved with peptide acid utilizing minimum protection.

to separate mixture of *N*-aspartyl anomers or anomeric acetamide.

In this connection, we note that in an earlier initiative in our laboratory, we had accomplished the maximally convergent synthesis of an N-linked glycopeptide.[12] A pentacyclic glycan was prepared using methodology instituted under our paradigm of glycal assembly. Even in this complex setting, the glycal linkage was convertible to the required terminal glucosamine linkage. In the final stage of that effort, reduction of β-anomeric azide **4**, in the context of a substantially protected glycan, produced the glycosylamine. Following aspartylation with tripeptide and pentapeptide donors and then global deprotection, high mannose tripeptide **5** and pentapeptide **6** were each isolated as 2:1 (β:α) anomeric mixtures. The precise point at which anomeric configurational integrity was compromised (cf. inter alia, reduction of the azide, acylation or deprotection) is not clear.




Fortunately, as demonstrated by Lansbury[13] fully deprotected glycans bearing β-configured anomeric amino functions can be efficiently acylated, taking advantage of the significantly higher nucleophilicity of the amino group (Fig. 1, path *c*). The stereochemical integrity of the reducing terminus is preserved in the case of fully deprotected glycans. Furthermore, use of fully deprotected free glycans and glycosylamines obviates the need for late stage global deprotection after formation of the glycopeptide.

In planning the synthesis of the five-ring glycopeptide **1**, we anticipated that the required glycan might be assembled through various glycal-based methods.[14,15] Following global deprotection, the glycan would be aminated at its reducing end, using the procedure developed by Kochetkov,[16] and then subjected to conjugation with the peptide (vide infra) as reported by Lansbury.[13]

As in our previously reported syntheses of **5** and **6**,[12] we hoped that a terminal glycal (see structure type **10**) would prove suitable for the eventual presentation of the 1-β-amino function of the A ring (*N*-acetylglucosamine) to the peptide, as required under path *c* (Fig. 1). The pentasaccharide glycal would be obtained with high convergence by coupling of a donor derived from 'pre-trimannose glycal' **7** with prechitobiose glycal **8**. Both **7** and **8** can be accessed in short order from D-glucal.[12,15]

However, a significant issue presents itself in this otherwise attractive coupling strategy. The glycal assembly method, which we favored, was well suited to fashion the C-ring as a β-glucoside (cf. **9**) rather than as the requisite β-mannoside. The high convergence of our proposed synthesis seemed sufficiently attractive to justify, if necessary, an indirect route to reach the β-mannoside BC connection. The thought was to exploit the expected ease of first appending the C-ring as a β-glucoside. This would be followed by epimerization of the C2 center of the C-ring (see asterisk, structure **9**), thereby producing the mannoside (**10**). In essence, the question was whether the efficiency of the assembly phase, enabled by glycal logic, would vindicate the awkwardness of the need for a late stage gluco→manno epimerization sequence (Fig. 2).

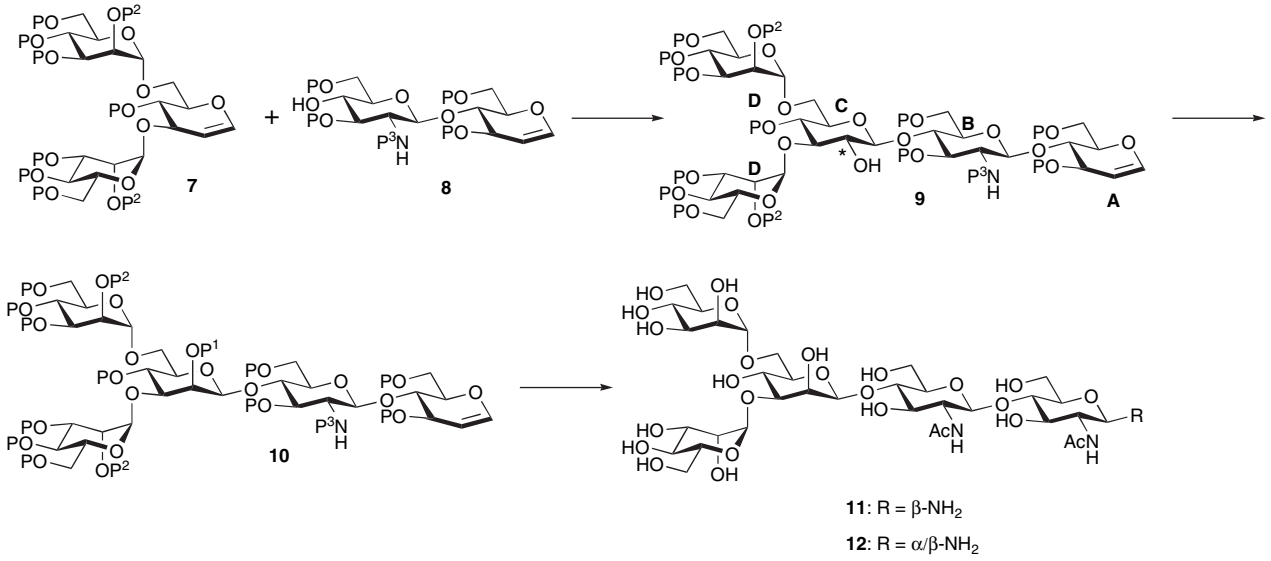



**Figure 2.** Synthetic plan for the core pentasaccharide glycosylamine. *P*, $P^1$, $P^2$, and $P^3$ represent protecting groups.

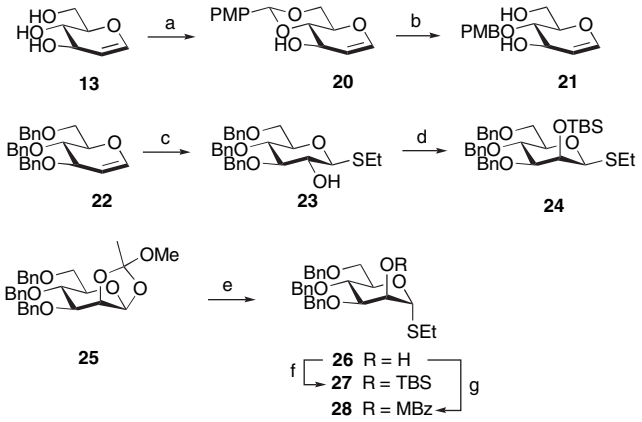
## 2.1. Discussion of results

Our venture commenced with the preparation of the building blocks from which the core pentasaccharide glycan (cf. **11**) could be synthesized. From the outset, we recognized that it would be important to develop a program that would allow for maximum diversification during the assembly of the mature glycan. Hopefully the strategy would not only encompass this endeavor, but would also establish a framework for future projects. As shown in Schemes 1 and 2, D-glucal (**13**) would serve as the common starting material for each building block. Additionally, the synthesis would make use of a tactic for azaglycosylation that had been developed in our laboratory some 15 years ago.[17]

The synthesis of the pre-chitobiose glycal (cf. **19**) commenced with 3,6-dibenzylglucal **14**, which had in turn been synthesized from D-glucal (**13**) as previously reported (Scheme 1).[17] Acetylation of the free alcohol in **14** provided the fully protected glucal **15**. Iodosulfonamidation followed by thiolysis of the intermediary aziridine at the anomeric center gave rise to thiodonor **17**. The original glucal **14** then served as an acceptor in the methyl triflate promoted glycosylation with **17**. Following methanolysis of the C4′ acetate, the pre-chitobiose glycal acceptor **19** was in hand.

Our most convergent route to the requisite pre-trimannose glycal of the type **7** would be through the selective introduction of α-mannosyl residues at C3 and C6 of glycal **21**. The synthesis of **21** was accomplished in a two-step sequence starting, once again, from D-glucal (**13**). Thus, engagement of the C4 and C6 alcohols of **13** as a *p*-methoxybenzylidene derivative gave rise to **20** (Scheme 2). Reductive cleavage of the acetal linkage afforded the appropriately protected glycal **21**.

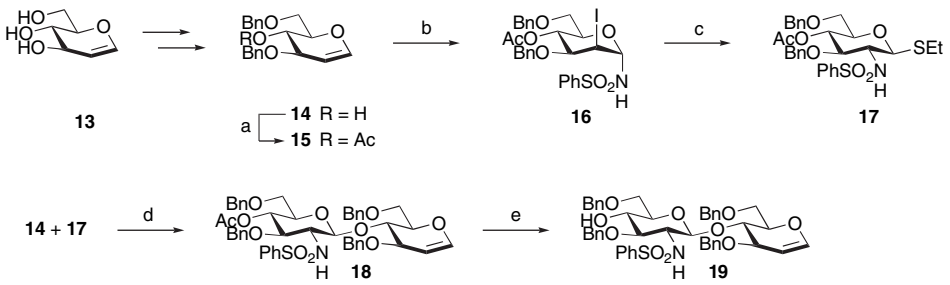
The required α-mannoside donor, **24**, was synthesized from commercially available tri-*O*-benzyl-D-glucal (**22**). Thus, stereoselective epoxidation with dimethyldioxirane (DMDO), followed by selective ring opening with ethanethiol, in the presence of trifluoroacetic anhydride, gave rise to thioglycoside **23**. Inversion of the alcohol at C2 was accomplished by Moffat-like oxidation[18] followed by stereoselective reduction of the resulting ketone with sodium borohydride. The now inverted alcohol was then protected to afford thiodonor **24**.



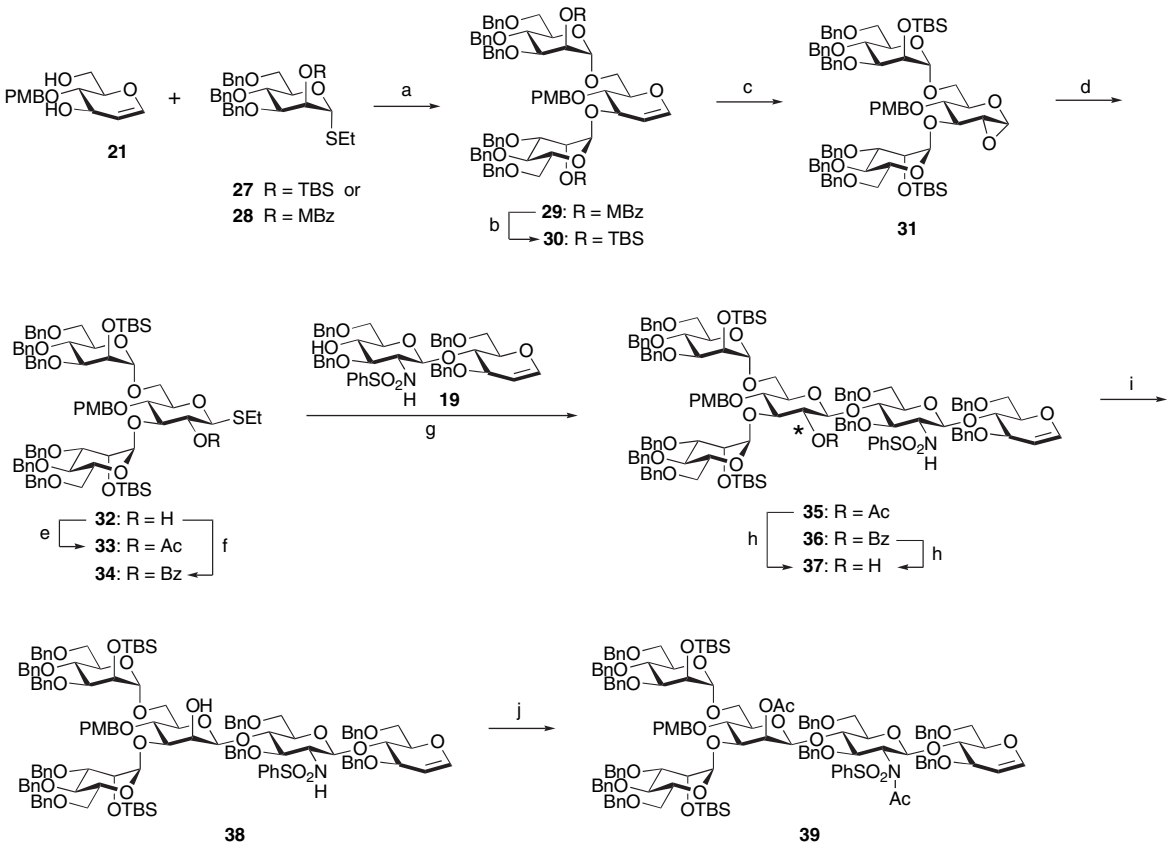

**Scheme 2.** Synthesis of the α-mannoside donor and acceptor from D-glucal: (a) *p*-anisaldehyde dimethyl acetal, PPTS, THF, 48%; (b) Dibal-H, DCM, 75%; (c) 1. DMDO, DCM, 2. EtSH, TFAA, 89% in two steps; (d) 1. DMSO, $Ac_2O$, 2. $NaBH_4$, DCM, MeOH, 76% in two steps, 3. TBSOTf, DCM, $Et_3N$, 93%; (e) 1. EtSH, $HgBr_2$, $CH_3CN$, 95%, 2. NaOMe, MeOH; (f) TBSOTf, DCM, $Et_3N$, 93%; (g) *p*-toluoyl chloride, DCM, $Et_3N$, 75%.

Alternatively, monosaccharides of this type may be prepared from the commercially available orthoester **25** in three steps, beginning with mercuric bromide-mediated ring opening with ethanethiol followed by methanolysis of the resulting acetate to afford **26**. The free alcohol of **26** can be reprotected with TBS triflate or *p*-toluoyl chloride (4-methylbenzoyl chloride) to afford **27** or **28**, respectively. It should be noted that this alternate route is more attractive due to its amenability to the production of large quantities of the α-mannoside donor.

With the mono- and disaccharide building blocks in hand, we began assembly of the pentasaccharide (cf. **11**), and ultimately construction of the target pentasaccharide–pentapeptides **1** and **2**. This exercise would serve to field-test and, indeed, validate our plan for synthesizing complex glycopolypeptides carrying biological information (cf. **3**). Thus, the construction of the α-trimannoside donor commenced with di-α-mannosylation of the glycal diol acceptor **21** (Scheme 3). Diglycosylation with 2-*O*-TBS protected thiomannoside **27**, using methyl triflate as the activating agent, afforded trisaccharide glycal **30** in 54% yield. The same transformation proceeded in somewhat higher yield (63%) to afford **29** when ester-protected thiomannoside **28** was used as the donor. However, because this ester group would later interfere with the epimerization sequence required at




**Scheme 1.** Synthesis of the pre-chitobiose glycal acceptor building block from D-glucal: (a) $Ac_2O$, pyridine, DMAP, 94%; (b) IDCP (Iodonium-di-*sym*-collidine perchlorate), $PhSO_2NH_2$, DCM, 0 °C, 94%; (c) EtSH, LHMDS, DMF, −40 °C–0 °C, 62%; (d) MeOTf, DTBP (2,6-di-*tert*-butylpyridine), DCM, 0 °C to rt, 70%, β/α 6:1; (e) NaOMe, MeOH, 90%.



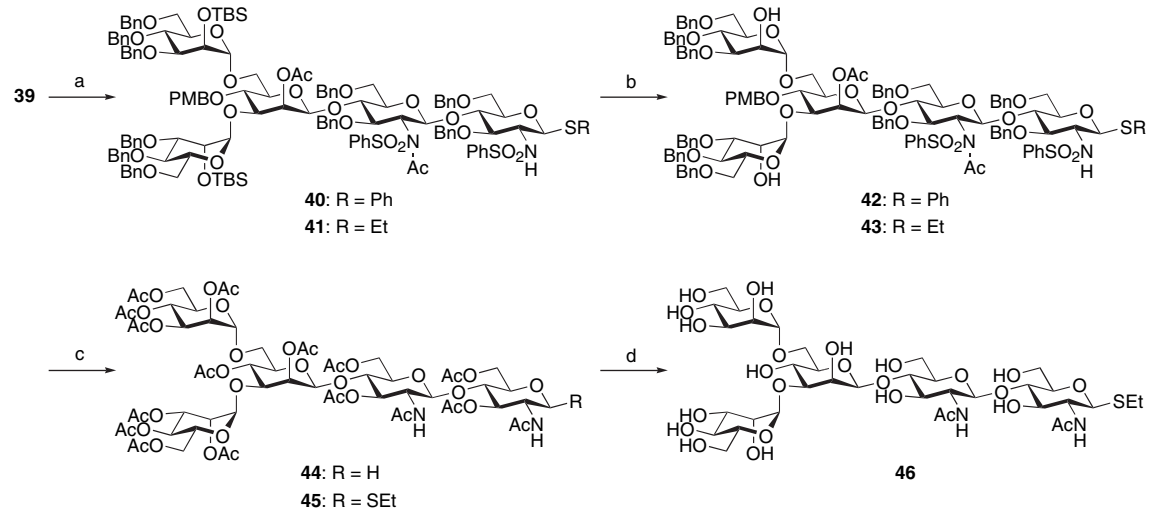

**Scheme 3.** Synthesis of the core pentasaccharide glycal: (a) **27** or **28**, MeOTf, DTBP, DCM, 54% for **30**, 63% for **29**; (b) 1. NaOMe, MeOH, 77%, 2. TBSOTf, $Et_3N$, DCM, 95%; (c) DMDO, DCM, crude yield >99%; (d) EtSH, TFAA, DCM, 76%; (e) $Ac_2O$, $Et_3N$, DCM, 91%; (f) BzCl, pyridine, DCM, 90%; (g) **19**, MeOTf, DTBP, DCM, 64% for **35**, 71% for **36**; (h) $LiAlH_4$, $Et_2O$; (i) 1. Dess–Martin periodinane, DCM, 2. L-Selectride, THF; (j) $Ac_2O$, pyridine, DMAP, DCM, 82% in four steps.

the pentasaccharide stage, the ester protecting groups in **29** had to be converted to silyl groups (**29**→**30**). While this two-step sequence could easily be achieved, the overall route was not highly efficient.

In either case, the double bond of the trisaccharide **30** was epoxidized with DMDO to afford **31**. Unfortunately, the direct coupling between this epoxide as a donor, and the pre-chitobiose acceptor **19** was never successful. Despite significant efforts to optimize this type of transformation, only trace amounts of pentasaccharide product could be detected by this seemingly straightforward approach. Fortunately we had earlier developed a modality to deal with such a contingency.[9,19] Applied to the case at hand, epoxide **31** was transformed into the thioglycoside alcohol **32**, which was subsequently protected as either an acetate (**33**) or benzoate (**34**). Both the β-glucoside bond donors were evaluated in coupling with the acceptor **19**. Coupling with trisaccharide acetate **33** provided corresponding pentasaccharide **35**, albeit in modest yield (64%). In practice, glycosylation was significantly complicated by orthoester formation. By contrast, upon using benzoate thioglucoside **34** as donor, the reaction was quite reproducible, providing pentasaccharide **36** in a more acceptable 71% yield. Not surprisingly, the benzoate proved to be a superior *trans*-glycosylation directing group.

The proposal had indeed been realized in a highly convergent fashion. However, it would now be necessary to convert the C-ring from gluco to the manno stereochemistry, by overall epimerization at C2 (compound **37**, see asterisk).[20] The overall inversion at this center would require gaining access to a C-ring uloside (i.e., 2-keto) functionality which could subsequently be reduced to provide, hopefully, a C2 axial alcohol (cf. **38**). In the event, deprotection of neither the acetate in **35** or the benzoate in **36** could be achieved under Zemplén conditions.[21] However, either acyl group could easily be cleaved reductively with LAH, yielding alcohol **37**. The equatorial alcohol in **37** was then oxidized with Dess–Martin periodinane,[22] giving rise to an unstable ketone that was immediately reduced using L-Selectride.[23] In this way, alcohol **38**, with the C2-axial hydroxyl group, was obtained in 82% yield over four steps. Subsequent acylation of the alcohol afforded diacetate **39**.

At this point, appropriate functionalization of the reducing end glycal and global deprotection had to be achieved prior to introduction of the peptide chain. Toward this end, glucal **39** was subjected to iodosulfonamidation and thiolysis, thereby giving rise to phenyl thioglycoside **40** in 85% yield (Scheme 4). The latter was subjected to TBAF-mediated desilylation, and the resultant crude product, **42**, was reacted with sodium in liquid ammonia, followed by peracetylation of the fully deprotected glycan. Unfortunately, as spectroscopic analysis of the product demonstrated, the erstwhile thiophenyl group was no longer present, having presumably been cleaved during the dissolving metal reduction. The observed product of this sequence, **44**, contained two anomeric protons.




**Scheme 4.** Upgrading of the pentasaccharide terminal glycal: (a) 1. IDCP, $PhSO_2NH_2$, DCM, 2. PhSH, LHMDS, DMF, 85% in two steps for **40**, or EtSH, LHMDS, DMF, 81% in two steps for **41**; (b) TBAF, THF, 80%; (c) 1. $Na/NH_3$, THF, 2. $Ac_2O$, pyridine, DMAP, 70% for **44**, 77% for **45**; (d) NaOMe, MeOH, 86%.

We reasoned that the phenyl ring of the anomeric thiophenyl function could well have facilitated the reduction of the C–S linkage. Accordingly, it was surmised that an alkyl thioglycoside might be more stable under the same reductive conditions. In the event, when ethylthioglycoside **43** (obtained from glucal **39** in a similar sequence) was subjected to the action of sodium in liquid ammonia followed by peracetylation and purification, the desired product **45**, containing an intact C–S bond, was isolated in 77% yield. The *O*-esters in **45** were then saponified to provide the fully deprotected pentasaccharide **46** in 86% yield.

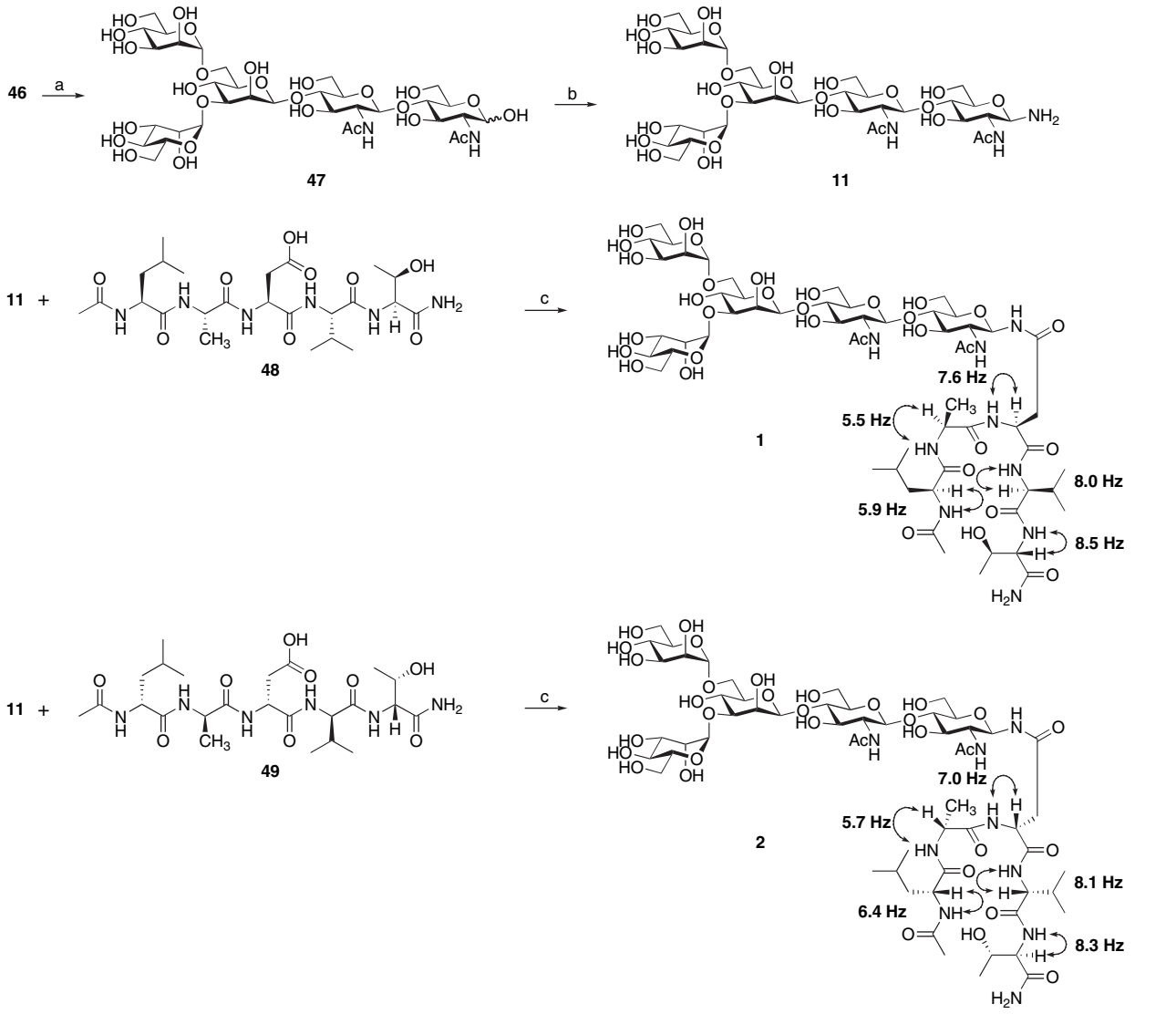
In order to implement a Kochetkov–Lansbury sequence for building the glycopeptide domain, it would be necessary to liberate, in some as yet unspecified fashion, an anomeric hydroxyl group. Thus, overall hydrolysis of the thioglycoside functionality in **46** would perhaps lead us to **11**. Though reaction of **46** with NBS in aqueous THF turned out to be difficult to reproduce, hydrolysis with $HgCl_2$ proceeded efficiently[24] to afford the reducing saccharide **47** in 95% yield, after gel filtration. Finally, this alcohol was converted into β-anomeric amine **11** using the Kochetkov protocol;[16] no α-anomer was detected in the $^1H$ NMR spectrum of the product.

Two pentapeptide enantiomers, one composed entirely of L-amino acids (**48**) and the other of D-amino acids (**49**), were prepared using an automatic Fmoc-synthesizer on a Rink amide MBHA resin. Each of the peptides was conjugated with the amine **11** using HOBt/HBTU to activate the aspartic acid. The diastereomeric β-N-linked glycopeptides **1** and **2** were isolated in 40% yield, with no observable formation of the α-anomer (Scheme 5).

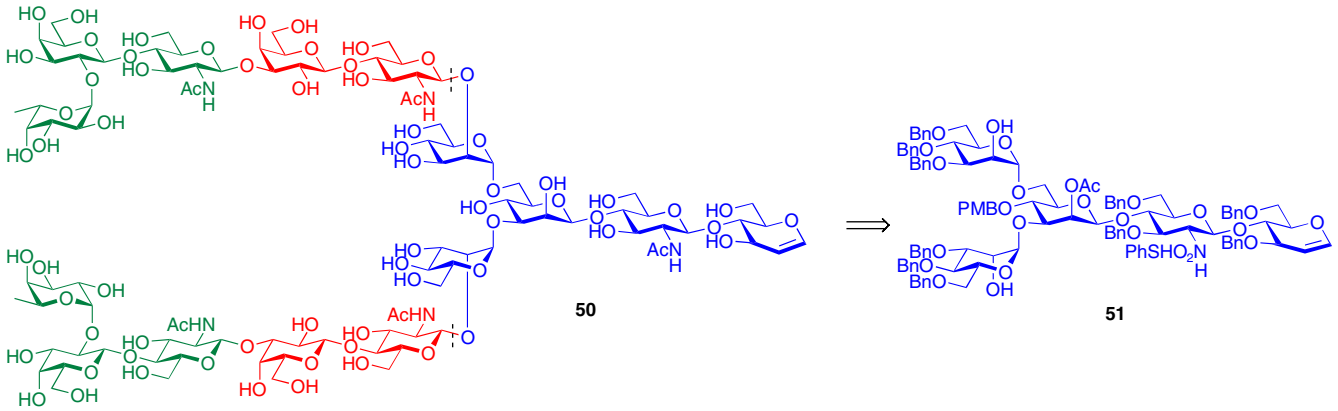
At this point, it is appropriate to discuss our reasons for preparing not only glycopeptide **1**, but also glycopeptide **2**, which differs only in the absolute stereochemistry of the peptide domain. An important area of study in the field of glycobiology is the evaluation of the structural configurations of glycopeptides. Early work in this field has focused on the evaluation of structural interactions within carbohydrate or peptide domains; however, little is known about communication across domains.[25] This gap can be attributed, in large part, to the very limited accessibility of homogeneous glycopeptide constructs. Given our interest in assembling such constructs through convergent chemical synthesis, we chose to prepare **1** and **2** in order to probe questions of stereochemical communication between the carbohydrate and peptide domains.[10]

Indeed, the results of high-field NMR studies performed on **1** and **2** strongly indicate the existence of stereochemical interactions between the peptide and carbohydrate domains of these N-linked glycopeptides. Although the NOESY patterns of compounds **1** and **2** were quite similar, as expected, distinct shift differences were observed for the protons at the central amino acid residues, as indicated in Figure 3. Thus, distinct shifts were observed for the amide NH protons of asparagine (to which the carbohydrate is linked) and the adjacent valine residue. Since amide proton chemical shifts are good indicators of peptide backbone conformations, these results clearly demonstrated that a global change in peptide stereochemistry impacts the structural conformation of the glycopeptide.[26] Importantly, the results of this exercise show that cross-domain communication cannot be solely attributed to the bulk of the carbohydrate moiety.[10]

Having field-tested the technologies required for glycal assembly and peptide conjugation in the syntheses of glycopeptides **1** and **2**, we were prepared to take on the challenge of synthesizing our ultimate target compound: a pentadecasaccharide glycan construct linked to a mature H-type 2 blood determinant (**3**). As will be seen, many new difficulties had to be overcome to meet this challenge. We turn first to our approach to the synthesis of the glycal portion of the molecule (**50**). In this system, the H-type 2 trisaccharides (shown in green) are appended to the common pentasaccharide domain (shown in blue) through two lactosamine spacer units (shown in red).




**Scheme 5.** Construction of glycopeptides: (a) $HgCl_2$, $CaCO_3$, $H_2O$, 95%; (b) $NH_4HCO_3$, $H_2O$, >99%; (c) **48** or **49**, HOBt, HBTU, $^{i}Pr_2NEt$, DMSO, 40%.




A logical disconnection, shown in structure **50** (see lines), would lead us to the common pentasaccharide domain as a key building block. The appropriately protected pentasaccharide intermediate (**51**) would be easily obtained through desilylation of the previously synthesized **39** (Scheme 3).

At this stage, two approaches for the preparation of the pentadecasaccharide **50** were considered. In the first, the di- and trisaccharide precursors **52** and **53** would be assembled to form the H-type 2-lactosamine donor pentasaccharide **54**. The latter would then be joined with **51** through

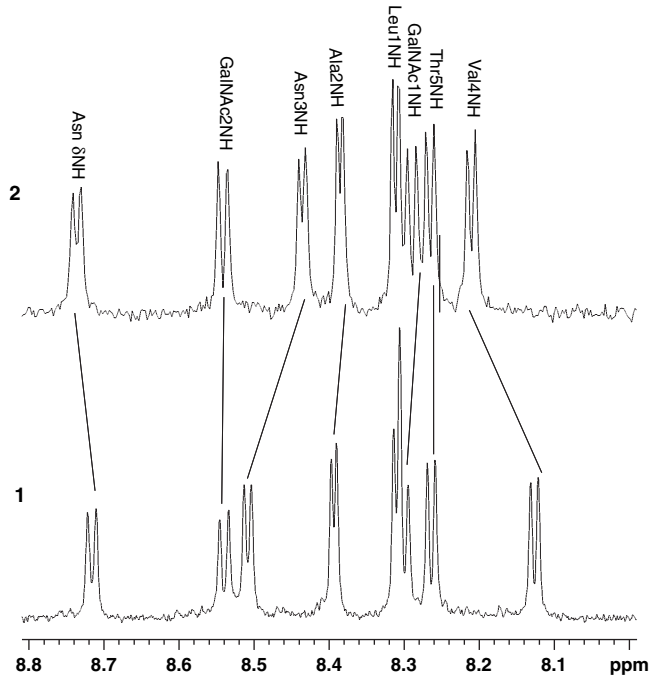


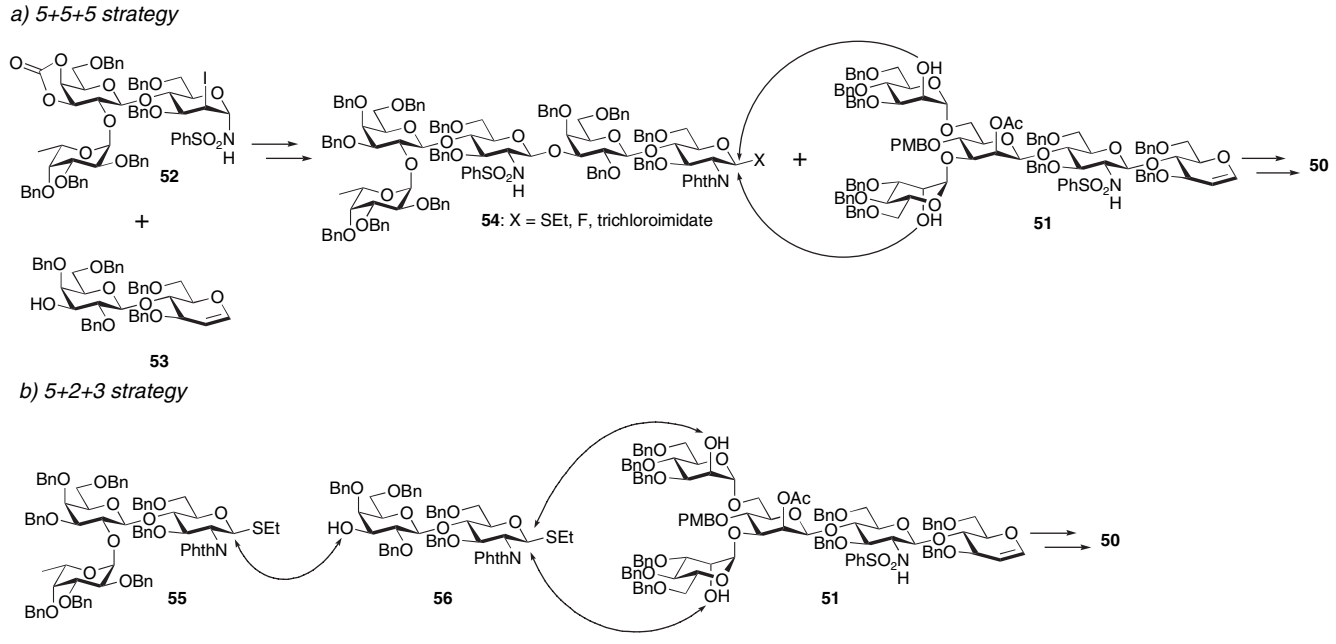

**Figure 3.** Amide proton spectra of **1** and **2** recorded at 800 MHz at 5 °C. Samples were dissolved in 90% $H_2O$/10% $D_2O$ ([**1**]=5 mM, [**2**]=1 mM) and the pH was adjusted with a 10 mM phosphate buffer (**1**: pH 3.5, **2**: pH 4.2). The $H_2O$ signal was suppressed using the Watergate method.

diglycosylation, as shown, in a highly convergent '5+5+5' coupling reaction (Scheme 6a). A second, more linear, approach would involve sequential coupling of the di- and trisaccharide precursors to the initial pentasaccharide unit (**51**). Thus, an appropriately protected lactosamine derivative **56** would be coupled to **51**. Then, following selective deprotection, diglycosylation with the H-type 2 trisaccharide would occur to afford the glycal **50** (Scheme 6b).

The more convergent '5+5+5' approach was investigated first. Unfortunately, the projected 5+5+5 coupling reaction did not occur cleanly upon activation of either the thioethyl-, fluoro- or trichloroacetimidate derivatives of donor **54**. However, on the basis of mass spectral analysis, it appeared that 5+5 coupling did in fact occur at each of the acceptor sites in **51**. The stereochemistry of these glycosylations could not be determined in the context of the inhomogeneous product. However, in no fraction could we claim mass spectral evidence that the 15-mer had arisen from an actual 5+5+5 coupling.

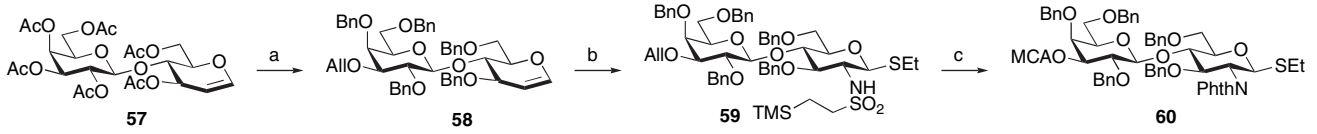
Since at least one glycosylation had occurred, we surmised that each of the glycosylation acceptor sites in **51** was, in principle, a competent acceptor. Perhaps with a more reactive glycosyl donor, two-fold glycosylation could be achieved. We reasoned that smaller, properly activated lactosamine donors, such as **56**, might allow for two-fold glycosylation of **51**. With proper selection of the lactosamine protecting groups, two new acceptor sites could be unveiled subsequent to coupling with **51**, at which point two activated H-type 2 donors, such as **55**, could be concurrently installed, producing the pentadecasaccharide via a '5+2+3' coupling strategy (Scheme 6b).

With these considerations in mind, we chose compound **60** as our lactosamine donor. This compound incorporates an orthogonally protected hydroxyl group at C3′ as well as a strongly β-directing phthalamide at C2. The synthesis of **60** commenced with the hexaacetyllactal **57** (Scheme 7). This compound was subjected to deacetylation with ammonia in methanol, and the resulting hexaol was selectively allylated at C3′ through the intermediacy of dibutyltin acetal formation.[27] Benzylation of the remaining hydroxyl groups afforded lactal **58** in 50% yield over four steps. The reducing end was then functionalized using the previously developed silylethylsulfonamide (SES) methodology.[28]



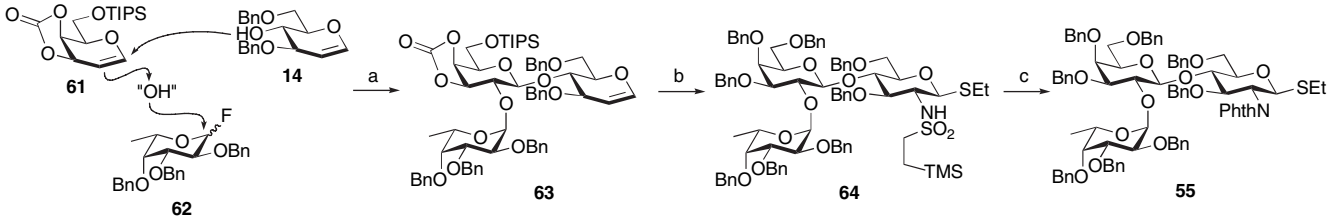

**Scheme 6.** Assembly of divalent H-type 2 antigen-encoded 15-mer glycal: comparison of the 5+5+5 and 5+2+3 strategies.

Thus, iodosulfonamidation with 2-trimethylsilylethylsulfonamide, followed by thiolysis, with concurrent migration of sulfonamide from C-1 to C-2 and ejection of iodide, provided thioglycoside **59** in 85% yield (two steps). Finally, the sulfonyl and allyl protecting groups were converted to phthaloyl[29] and chloroacetyl, respectively, to provide the desired lactosamine donor **60**.




**Scheme 7.** Synthesis of the lactosamine building block: (a) 1. $NH_3$, MeOH, 2. $Bu_2SnO$, PhH, 3. allyl bromide, $Bu_4NBr$, 4. BnBr, NaH, DMF, 50% in four steps; (b) 1. IDCP, $TMSCH_2CH_2SO_2NH_2$, 75%, 2. EtSH, LiHMDS, DMF, 85%; (c) 1. CsF, DMF, 90 °C, 94%, 2. phthalic anhydride, pyridine, then $Piv_2O$ or $Ac_2O$, >99%, 3. $RhCl(PPh_3)_2$, DABCO, THF; 1 N HCl, 74%; 4. $(ClCH_2CO)_2O$, DTBP, cat. DMAP, DCM, 97%.

Glycal assembly was once again used to prepare the H-type 2 trisaccharide donor **55** (Scheme 8). Galactal **61** was selectively α-epoxidized with DMDO and the resulting epoxide was directly coupled with 3,6-dibenzylglucal **14** in the presence of $ZnCl_2$. The newly formed C2′ hydroxyl in the resulting disaccharide was fucosylated with **62**, to furnish trisaccharide glucal **63** in 50% yield (three steps). Perbenzylation and functionalization of the reducing end, following the procedure described for lactosamine **60** (vide supra), provided the 2-*N*-phthaloyl thioglycoside donor **55** required for the introduction of H-type 2 specificity.



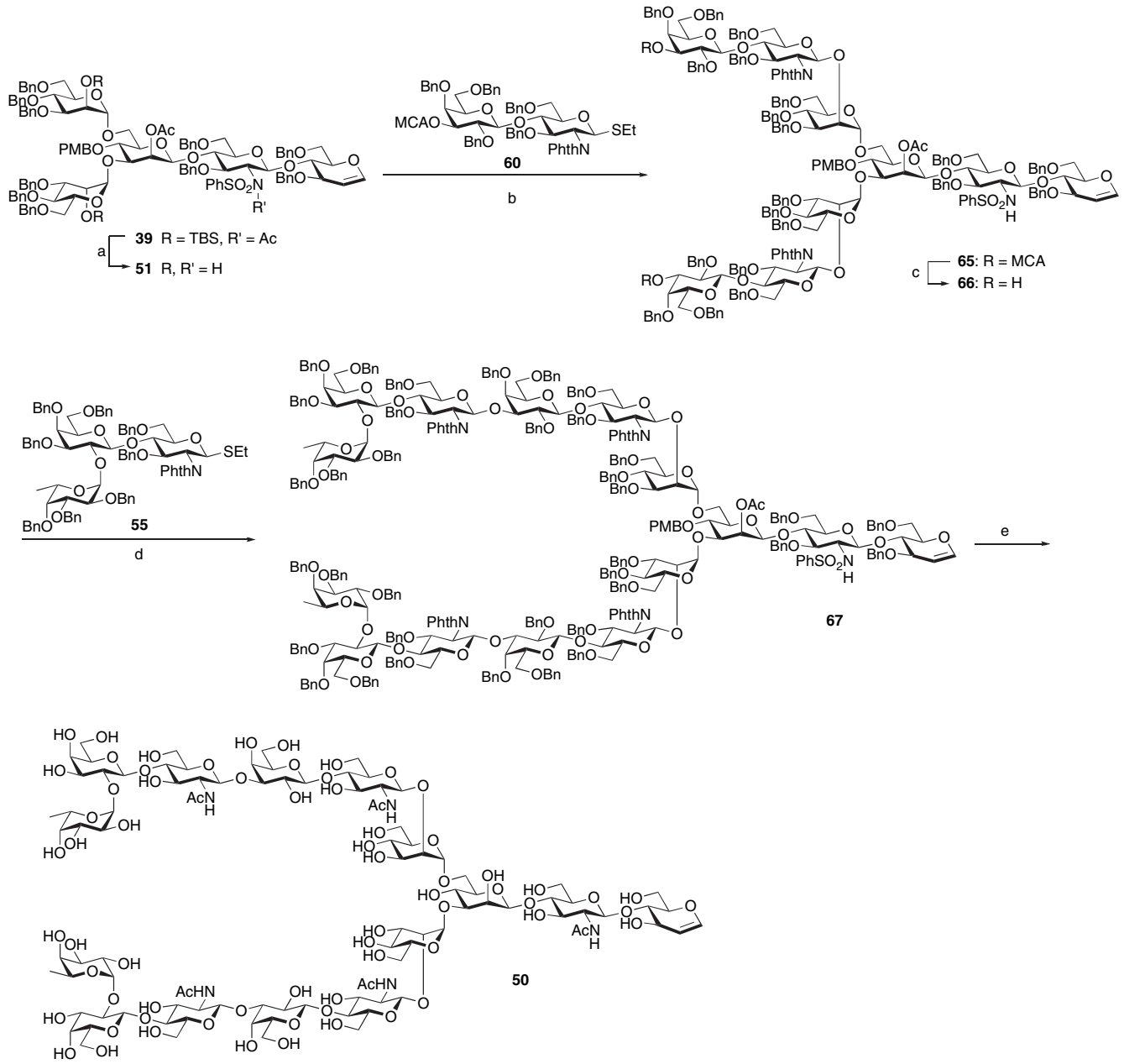

**Scheme 8.** Synthesis of the wing trisaccharide **55**: (a) 1. **61**, DMDO, DCM; 2. **14**, $ZnCl_2$, DCM; 3. **62**, $SnCl_2$, AgOTf, DTBP, 50% (three steps); (b) 1. TBAF, THF, 2 h then $K_2CO_3$, MeOH; 2. BnBr, NaH, DMF, 85% (two steps); 3. IDCP, $TMSCH_2CH_2SO_2NH_2$; 4. EtSH, LiHMDS, DMF −40 °C to 0 °C, 75% (two steps); (c) 1. CsF, DMF, 100 °C, 5 d, 65%; 2. phthalic anhydride, pyridine; 3. $Ac_2O$, 97% (two steps).

With the required subunits and technology in hand, we turned to the assembly of the pentadecasaccharide glycal. Thus, the C2 hydroxyl groups in **39**, previously protected with silyl groups, were now unveiled with TBAF to afford diol **51**. The latter was coupled with excess lactosamine donor **60**, using methyl triflate as a promoter (Scheme 9).[30] Importantly, the diglycosylation of **51**, which had failed with pentasaccharide donor **54**, could be easily achieved with disaccharide donor **60**, to provide the desired nonasaccharide **65** in 62% yield. Next, the chloroacetate groups at C3′ of the lactosamine units in **65** were removed under neutral conditions, while keeping the acetate on the central mannose unit and the terminal glycal intact. Finally, the H-type 2 trisaccharides (**55**) were introduced and, once again, diglycosylation proceeded smoothly to give rise to pentadecasaccharide **67** in 78% yield.

At this stage, the challenge was that of achieving global deprotection of the pentadecasaccharide system. Though our ultimate strategy was to deprotect following functionalization of the reducing end, we field-tested the deprotection sequence on the unfunctionalized glycal **67**. Thus, the phthaloyl and *O*-acetate groups were first removed using ethylenediamine. Next, the sulfonamide, *p*-methoxybenzyl, and benzyl linkages were reductively cleaved with sodium in liquid ammonia. The crude product was peracetylated, purified through normal phase chromatography, and then deacetylated under Zemplén conditions, to afford fully deprotected glycal **50**.

Having established the feasibility of the deprotection sequence with the glycal, **67**, we sought to apply the same logic to the deprotection of the functionalized glycan. Thus, iodosulfonamidation of glycal **67** gave the *trans*–diaxial adduct **68**, which was then subjected to thiolysis with LiSEt in the hope of obtaining the corresponding thioglycoside **69** (Scheme 10). However, this transformation failed to provide **69**; instead, the reducing hemiacetal **70** was isolated from the reaction mixture in 40% yield. In fact, we were never able to gain access to **69**, whereas the hydrolysis product **70** was isolated even under strictly anhydrous conditions.

As we were unable to convert the glycal into the reducing end thioglycoside in the context of the pentadecasaccharide, we decided to investigate the possibility of direct global deprotection of the oligosaccharide hemiacetals under dissolving metal reduction conditions. Such conditions, while clearly too harsh for the hemiacetal in the open aldehyde form, may leave the 'reducing end' intact provided that it is 'protected' via total dominance of the ring closed form in liquid ammonia. The results of our model studies (summarized in Table 1) demonstrate the truly remarkable stability and survivability of the reducing oligosaccharides under these conditions. A series of reducing glycans, including examples with amides and hydroxyls at C-2, were deprotected with yields of isolated peracetylated products typically above 70%.[31]



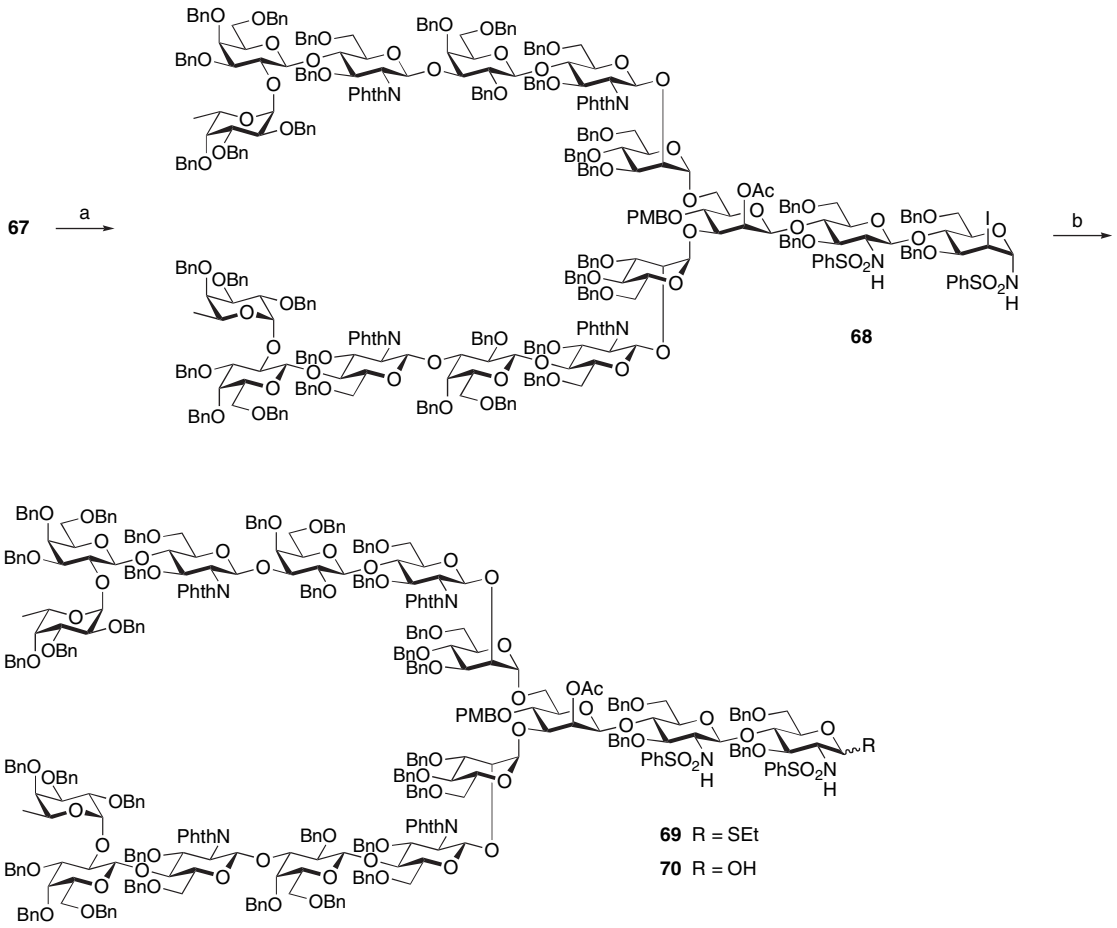

**Scheme 9.** Synthesis of the 15-mer glycal: (a) TBAF, THF, 77%; (b) **60**, MeOTf, DTBP, DCM, 0 °C to rt, 62%; (c) thiourea, $NaHCO_3$, EtOH, 99%; (d) **55**, MeOTf, DTBP, DCM, $Et_2O$, 78%; (e) 1. $NH_2CH_2CH_2NH_2$, EtOH, reflux; 2. $Na/NH_3$, THF; 3. $Ac_2O$, pyridine, DMAP; 4. NaOMe, MeOH (30%, four steps).

This pivotal finding paved the way for a much more efficient and straightforward approach to the preparation of N-linked glycans. Not only is the need for a moisture-sensitive and problematic thiolysis step obviated, but both isomeric sulfonamides can be utilized in the hydrolysis, greatly simplifying the transformation and allowing higher throughput of material.

We were eager to apply this novel approach to our pentadecasaccharide system (Scheme 11). Thus, the phthalimides in **67** were first converted into acetamides (cf. **71**). Then, the glycal was subjected to iodosulfonamidation, followed by basic hydrolysis of the resulting product mixture, to afford pentadecasaccharide **72**. At this point, the stage was set for the critical global deprotection.

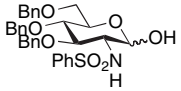
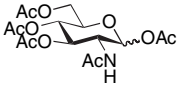
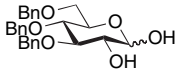
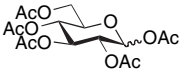
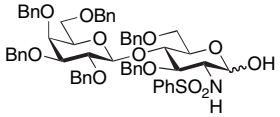
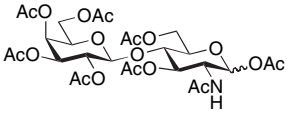
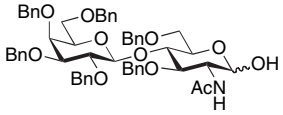
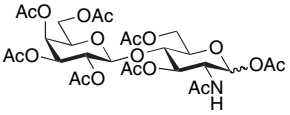
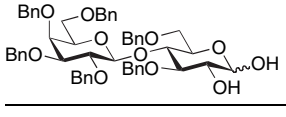
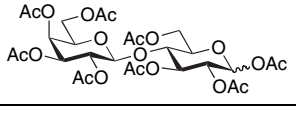
Hemiacetal **72** was subjected to reduction with sodium in liquid ammonia (Scheme 12),[32] and happily, the reducing end hemiacetal proved sufficiently robust; the fully deprotected material, **73**, was isolated in 57% yield over two steps, following partial reacetylation and reverse-phase column chromatography. The reducing alcohol was then converted into anomeric amine **74** under Kochetkov amination conditions,[16] and the latter was then coupled with pentapeptide **48** to furnish the N-linked glycopeptide **3**, presenting the H-type 2 blood group specificity.

The ability to gain access through chemical synthesis to such an advanced N-linked glycopeptide in a homogeneous state provided a unique opportunity to investigate not only its glycoarchitecture but also the biological implications of the



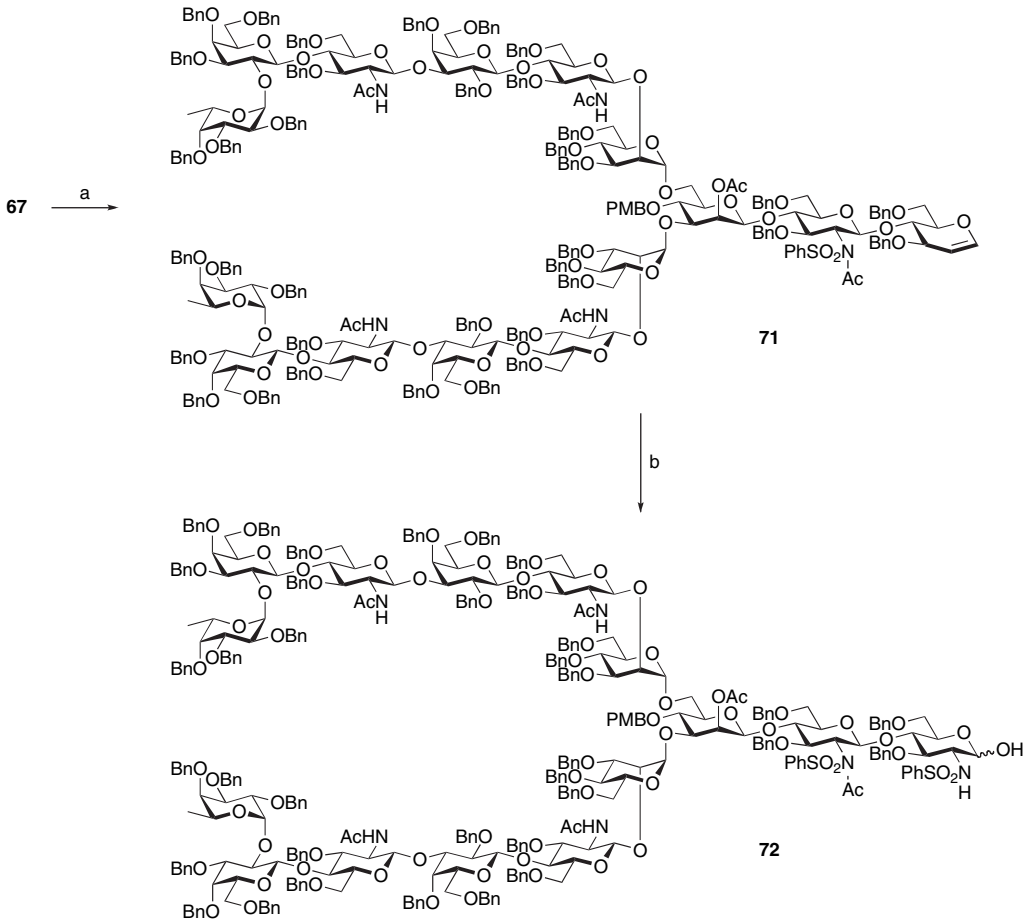

**Scheme 10.** Functionalization of the terminal glycal: (a) $PhSO_2NH_2$, IDCP; (b) EtSH, LiHMDS, DMF, **70** only, 40% (two steps).

**Table 1**. Dissolving metal reduction of glycal-derived 1-hydroxy sugars

| Deprotection substrate | Peracetylated substrate | Time (min) | Yield[a] (%) |
|---|---|---|---|
|  |  | 60 | 64 |
|  |  | 20 | 65 |
|  |  | 60 | 76 |
|  |  | 60 | 72 |
|  |  | 20 | 79 |

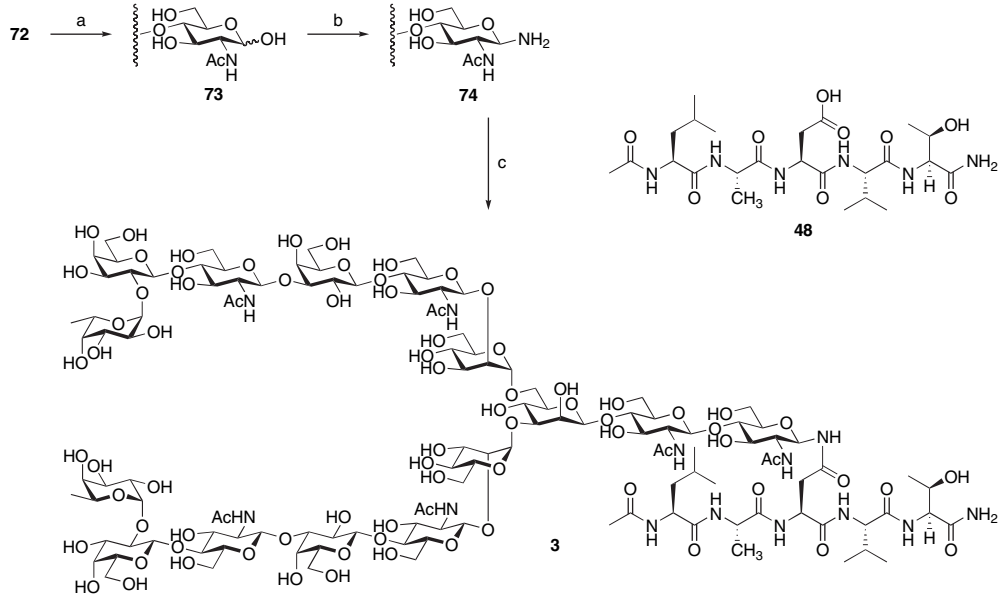
Reactions were performed by the addition of the saccharide in THF to a stirred solution of Na (6 equiv perbenzyl group) in $NH_3$ at −78 °C. After quenching and workup, the crude product was acetylated for analysis.

[a] Isolated yield following column chromatography.




**Scheme 11.** Upgrading of the terminal glycal to a hemiacetal: (a) 1. $NH_2CH_2CH_2NH_2$, EtOH, 2. $Ac_2O$, pyridine, DMAP, 85% (two steps); (b) 1. IDCP, $PhSO_2NH_2$, DCM, 74%; 2. LiHMDS, AgOTf, THF, $H_2O$, 63% or aq $K_2CO_3$, THF, 60%.

attached H-type 2 specificity. Indeed, the $^1H$ NMR spectrum of **3** (Fig. 4) is extremely well resolved, indicative of a high degree of structural order.[25] Moreover, the functionality of the H-type 2 blood group determinants was confirmed in an enzyme-linked immunosorbent assay (ELISA) where glycopeptide **3** reacted with an antibody against the H-type




**Scheme 12.** Stereocontrolled synthesis of a β-N-linked 15-mer glycopeptide containing the H-type 2 blood group determinants: (a) 1. Na/$NH_3$, THF; 2. $Ac_2O$, MeOH, 57% (two steps); (b) $NH_4HCO_3$, $H_2O$, >99%; (c) **48**, HOBt, HBTU, DIPEA, DMSO, 20%.

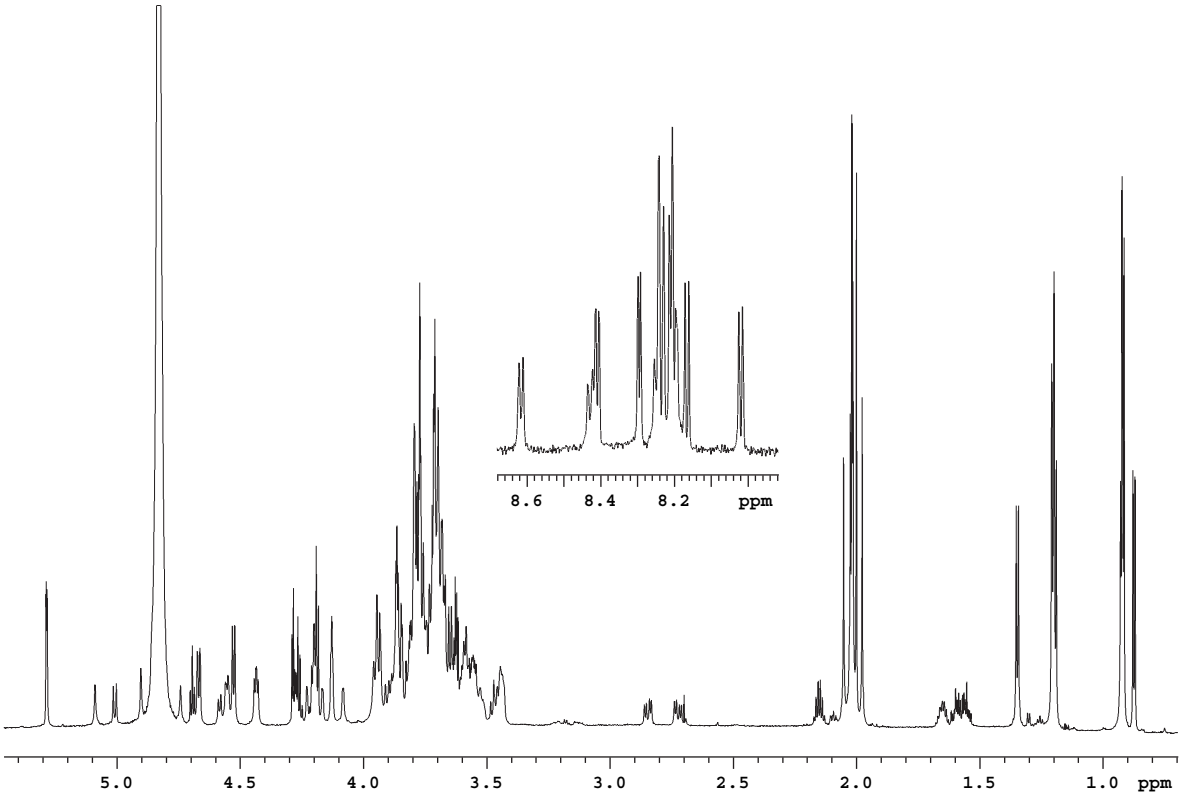



**Figure 4.** The $^{1}$H NMR spectrum (800 MHz) of **3** in $D_2O$ at 20 °C and pH 3.7 (phosphate buffer). The inset shows the secondary NH signals of amides from the peptide backbone, side chain, and GlcNAc sites when the spectrum is taken in $H_2O$ at 5 °C and pH 3.7 (phosphate buffer).

2 determinant (Fig. 5). This antibody is highly specific for the H-type 2 blood group determinant, and does not react with related structures, such as H-type 1, $Le^a$, or $Le^x$.[33,34]

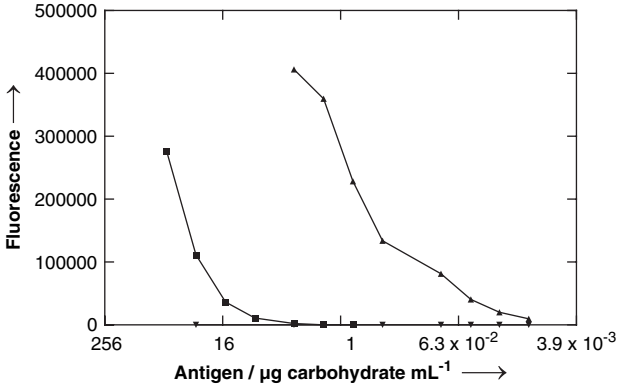



**Figure 5.** Reactivity of glycopeptide **3** (■), H-type 2 active mucin (▲) as a positive control, and $Le^x$/$Le^a$ active mucin (▼) as negative control with the antibody against H-type 2 determinants (mAbSA) as determined with an ELISA. The mucin preparations have been previously reported.[33]

## 3. Conclusion

In conclusion, the goals of our investigation were substantially met. The logic of glycal assembly, honed over a decade, proved equal to the task of synthesizing the complex oligosaccharide **71**. The glycal olefin proved susceptible to our sulfonamidohydroxylation methodology. More importantly, the powerful Birch reduction methodology for the simultaneous cleavage of multiple benzyl groups was appropriate, even in this most difficult context (cf. **72**→**73**). Happily the Kotchetkov–Lansbury amination–aspartylation was also appropriate (cf. **73**→**3**).

With the ability to reach reasonable quantities of homogeneous structures containing this level of complexity in the laboratory come new questions. For instance, is it possible to develop methodology wherein complex glycopeptides can be ligated to create even more biorelevant structures? If so, can the chemistry be conducted in such a fashion that it would allow for the conservation of the fragile glycosidic linkages? One could then consider even more challenging systems as targets for chemical synthesis. One of the most exciting dimensions of synthetic chemistry, in our view, is its capacity to deal with still more biopertinent structures which might illuminate Nature's ways and even, occasionally serve to modulate these processes in constructive directions. Having reached the H-type 2 human blood group structural context by chemical synthesis, we were led to wonder if a system of the complexity of, say, erythropoietin[35] might be accessible via suitable advances in methodology. It is toward such goals that we have recently directed considerable attention.[36]

## 4. Experimental

### 4.1. General

All glassware was dried in an oven at 140 °C before use. All experiments were conducted under an atmosphere of dry

argon unless indicated otherwise. Solvents were dried as follows: methylene chloride, diethyl ether, tetrahydrofuran (THF), benzene, and toluene were obtained from a dry solvent system (alumina) and used without further drying. Pyridine and triethylamine were freshly distilled from $CaH_2$. All NMR spectra were recorded on a Bruker model AMX-400 ($^1$H: 400 MHz, $^{13}$C: 100 MHz) or a Bruker model DRX-500 ($^1$H: 500 MHz, $^{13}$C: 125 MHz) NMR spectrometer. Chemical shifts are reported in parts per million (ppm) from internal tetramethylsilane ($^1$H NMR spectra, $\delta$ 0.00 ppm) or the residual solvent signal of $CDCl_3$ ($^{13}$C NMR spectra, $\delta$ 77.23 ppm). Infrared spectra were taken on a Perkin Elmer 1600 Series FTIR spectrometer using thin film deposition on polished NaCl plates. Peaks are reported in wavenumbers ($cm^{-1}$). Low and high-resolution mass spectra were obtained from a PE Sciex API 100 instrument under EI mode, and are reported in units of *m*/*z*. Column chromatography (low-pressure chromatography) was performed with E. Merck silica gel 60 (40–63 mesh). Thin layer chromatography (TLC) was carried out on Merck silica gel 60 F-254 glass-backed plates. TLC visualization was done with a 254 nm UV lamp and potassium permanganate or phosphomolybdic acid staining solution. All chemicals were purchased from Aldrich Chemical Co. and used as received. While we cannot rule out the possibility of anomeric mixtures during glycosylation reactions, we were unable to observe such mixtures and the yields reported for these reactions are for the pure anomer listed.

**4.1.1. Improved synthesis of 4-*p*-methoxybenzylglucal (21).** To a solution of 4,6-*p*-methoxybenzylidine glucal[15] (**20**) (6.00 g, 23 mmol) in $CH_2Cl_2$ (20 mL) at −78 °C was added Dibal-H (1.5 M solution in toluene, 58 mL, 87 mmol). After removing the cooling bath, the resulting mixture was stirred at room temperature for 4 h and then diluted with $CH_2Cl_2$ (200 mL). The mixture was treated with saturated aq KNa-tartrate (100 mL) and was stirred for 15 h. The organic layer was separated, washed with brine, and dried over $Na_2SO_4$ and then concentrated in vacuo. The residue was purified by silica gel chromatography (hexane/EtOAc=1:1) to afford **21** (4.5 g, 17 mmol, 74%) as a white waxy solid. $[\alpha]_D^{25}$ 15.3 (*c*=2.86; $CH_2Cl_2$); IR (thin film): 3285, 2955, 2933, 2836, 1649, 1613, 1514, 1232, 1173, 1086, 952, 814, 761 $cm^{-1}$; $^1$H NMR (500 MHz, $CDCl_3$): $\delta$=7.26 (d, *J*=8.6 Hz, 2H), 6.85 (d, *J*=8.6 Hz, 2H), 6.30 (dd, *J*=1.3, 6.0 Hz, 1H), 4.75–4.65 (m, 3H), 4.30 (br d, *J*=5.4 Hz, 1H), 3.88–3.77 (m, 3H), 3.75 (s, 3H), 3.57 (dd, *J*=6.7, 8.8 Hz, 1H), 2.79 (br s, 1H), 2.70 (br s, 1H); $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$=159.4, 144.1, 130.2, 129.7, 114.0, 103.3, 77.4, 76.6, 73.4, 71.6, 68.9, 61.7, 55.2.

**4.1.2. Synthesis of 3,4,6-tribenzyl-1-thioethyl-β-mannoside.** 3,4,6-Tribenzyl-1-thioethyl-β-glucopyranoside[12] (**23**) (9.29 g, 18.7 mmol) was treated with DMSO/$Ac_2O$ (100 mL/50 mL) at room temperature for 3 d. The reaction mixture was then diluted with diethyl ether (1 L) and washed with $H_2O$ (5×200 mL), saturated aq $Na_2CO_3$ (3×200 mL), and brine. The crude ketone was dried over $MgSO_4$ and concentrated in vacuo. The residue was dissolved in $CH_2Cl_2$ (60 mL) and MeOH (60 mL) then cooled to 0 °C. $NaBH_4$ (2.13 g, 37.8 mmol) was added in several portions and the reaction mixture was allowed to warm to room temperature then stirred for another 20 min. The reaction was quenched with $H_2O$ (20 mL) and extracted with ether (3×300 mL). The combined extracts were washed with saturated aq $NaHCO_3$ (200 mL) and brine (300 mL) then dried ($MgSO_4$) and concentrated. The crude material was purified by silica gel chromatography (hexane/EtOAc=6.5:1) to afford the desired 3,4,6-tribenzyl thioethyl mannoside (7.05 g, 14.2 mmol, 76%) as a white foam.

**4.1.3. Synthesis of 2-*tert*-butyldimethylsilyl-3,4,6-tribenzyl-1-thioethyl-β-mannoside (24).** To a solution of 3,4,6-tribenzyl-1-thioethyl-β-mannoside (500 mg, 1.01 mmol) in $CH_2Cl_2$ (5 mL) was added triethylamine (2.81 mL, 20.2 mmol) and TBSOTf (464 μL, 2.02 mmol) drop wise at 0 °C. The reaction was slowly warmed to room temperature and stirred for 3 h. The reaction was diluted with EtOAc (100 mL), washed with saturated aq $NaHCO_3$ and brine then dried ($MgSO_4$), and concentrated in vacuo. Purification by silica column chromatography (hexane/EtOAc=10:1) yielded **24** (570 mg, 0.94 mmol, 93%). $[\alpha]_D^{25}$ −36.1 (*c*=4.09, $CH_2Cl_2$); IR (thin film): 3087, 2926, 2916, 1496, 1463, 1362, 1251, 1102, 1027, 966, 834 $cm^{-1}$; $^1$H NMR (400 MHz, $CDCl_3$): $\delta$=7.40–7.21 (m, 15H), 4.90 (d, *J*=8.8 Hz, 1H), 4.81 (d, *J*=11.8 Hz, 1H), 4.69 (d, *J*=4.5 Hz, 1H), 4.62 (d, *J*=4.7 Hz, 1H), 4.58 (d, *J*=5.3 Hz, 1H), 4.51 (d, *J*=4.0 Hz, 1H), 4.49 (s, 1H), 4.22 (d, *J*=2.4 Hz, 1H), 3.93 (t, *J*=9.5 Hz, 1H), 3.75–3.68 (m, 2H), 3.52–3.48 (m, 2H), 2.75–2.72 (m, 1H), 1.33 (t, *J*=7.3 Hz, 3H), 0.98–0.96 (m, 9H), 0.20–0.13 (m, 6H); $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$=137.3, 136.9, 136.8, 127.0, 126.9, 126.8, 126.8, 126.4, 126.3, 126.2, 125.9, 83.9, 82.7, 78.8, 73.7, 73.1, 71.8, 71.3, 71.1, 68.1, 24.8, 24.3, 17.3, 13.8, −4.9, −5.4, −5.7; HRMS calcd for $C_{35}H_{48}O_5S_1Si_1Na$: 631.2889, found: 631.2883.

**4.1.4. Synthesis of 2-*tert*-butyldimethylsilyl-3,4,6-tribenzyl-1-thioethyl-α-mannoside (27).** 2-Acetyl-3,4,6-tribenzyl-1-thioethyl-α-mannoside (14.1 g, 26 mmol) was dissolved in MeOH (50 mL) then treated with NaOMe (25% solution in MeOH, 0.6 mL, 2.62 mmol). The reaction was stirred for 12 h and then was neutralized with Amberlyst 15 and filtered. The resin was washed with MeOH (3×50 mL). The combined filtrates were concentrated under reduced pressure and azeotroped with benzene (3×30 mL) then dried under high vacuum to afford the free alcohol (12.8 g). The crude alcohol was dissolved in $CH_2Cl_2$ (50 mL) and $Et_3N$ (15 mL, 107.6 mmol) then cooled to 0 °C. TBSOTf (7.83 mL, 34 mmol) was added and the mixture was warmed to room temperature over 4 h then diluted with EtOAc (500 mL). The solution was washed with saturated aq $NaHCO_3$ and brine then dried ($Na_2SO_4$) and concentrated in vacuo. The residue was purified by silica gel chromatography (hexane/EtOAc=20:1) to afford **27** (14.7 g, 24.2 mmol, 93% over two steps) as a clear, colorless oil. $^1$H NMR (500 MHz, $CDCl_3$): $\delta$=7.20–7.40 (m, 15H), 5.10 (d, *J*=1.2 Hz, H-2), 4.72 (d, *J*=10.8 Hz, 1H), 4.60 (d, *J*=11.7 Hz, 1H), 4.58 (d, *J*=12.3 Hz, 1H), 4.50 (d, *J*=11.7 Hz, 1H), 4.40 (d, 12.3 Hz, 1H), 4.38 (d, *J*=10.8 Hz, 1H), 4.10 (s, 1H), 4.07 (m, 1H), 3.95 (dd, *J*=9.4, 9.4 Hz, 1H), 3.60–3.80 (m, 3H), 2.52 (m, 2H), 1.25 (t, *J*=7.5 Hz, 3H), 0.85 (s, 9H), 0.09, 0.00, −0.02 (3s, 12H). HRMS calcd for $C_{35}H_{48}O_5S_1Si_1Na$: 631.2889, found: 631.2878.

**4.1.5. Synthesis of 3,4,6-tribenzyl-2-*p*-toloyl-1-thioethyl-α-mannoside (28).** To a solution of 3,4,6-tribenzyl-1-thioethyl-α-mannoside (8.0 g, 16 mmol) in $CH_2Cl_2$ (50 mL) was added triethylamine (25 mL, 18 mmol) followed by *p*-toluoyl chloride (8.5 mL, 63.7 mmol) at 0 °C. The resulting mixture was warmed to room temperature and stirred for 2 d. Saturated aq $NaHCO_3$ (50 mL) was added and the mixture was stirred vigorously for 15 h then diluted with EtOAc (500 mL). After separation, the organic extract was washed with saturated aq $NaHCO_3$ and brine then dried ($Na_2SO_4$) and concentrated in vacuo. Purification over silica gel (hexane/EtOAc=20:1, then 10:1) yielded **28** (7.4 g, 12.1 mmol, 75%) as yellowish syrup. $^1$H NMR (500 MHz, $CDCl_3$): $\delta$=7.89–7.91 (m, 2H), 7.19–7.33 (m, 15H), 7.08–7.10 (m, 2H), 5.65 (dd, *J*=1.4, 3.0 Hz, 1H), 5.38 (d, *J*=1.4 Hz, 1H), 4.79, (d, *J*=10.5 Hz, 1H), 4.71, (d, *J*=11.2 Hz, 1H), 4.69 (d, *J*=11.9 Hz, 1H), 4.47 (d, *J*=11.2 Hz, 1H), 4.44 (d, *J*=11.9 Hz, 1H), 4.40 (d, *J*=10.5 Hz, 1H), 4.14 (ddd, *J*=1.6, 3.4, 9.7 Hz, 1H), 4.07 (dd, *J*=9.2, 9.7 Hz, 1H), 3.93 (dd, *J*=3.0, 9.2 Hz, 1H), 3.87 (dd, *J*=3.4, 10.6 Hz, 1H), 3.69 (dd, 1.6, 10.6 Hz, 1H), 2.50–2.64 (m, 2H), 2.34 (s, 3H), 1.21 (t, *J*=7.4 Hz, 3 Hz); $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$=166.1, 144.2, 138.7, 138.7, 138.1, 131.2, 130.3, 129.4, 128.6, 128.4, 128.3, 128.0, 127.9, 127.9, 127.8, 127.5, 126.5, 82.9, 79.0, 76.0, 74.8, 73.7, 72.3, 71.8, 70.9, 69.3, 25.9, 22.0, 15.3.

**4.1.6. Synthesis of disilyl dimannosyl trisaccharide glycal 30.** *Method A (p-toluoyl protecting group)*: Thiodonor **28** (7.4 g 12.1 mmol) and glucal acceptor **21** (1.0 g, 4 mmol) were mixed and azeotroped with toluene (3×40 mL). The residue was dissolved in $CH_2Cl_2$ (20 mL) and $Et_2O$ (20 mL) followed by the addition of 2,6-di-*tert*-butylpyridine (13.45 mL, 60 mmol) and freshly dried 4 Å molecular sieves (10 g). The resulting slurry was stirred for 30 min at room temperature then cooled to 0 °C. MeOTf (5.42 mL, 48 mmol) was added and the mixture was slowly warmed to room temperature and stirred for 15 h at which point $Et_3N$ (5 mL, 35.8 mmol) was added. After an additional 15 min of stirring, the solution was filtered through a pad of Celite and concentrated. The residue was purified over silica gel (hexane/EtOAc=5:1) to yield **29** (3.3 g, 2.41 mmol, 63%) as a thick, colorless oil. $^1$H NMR (500 MHz, $CDCl_3$): $\delta$=8.05 (m, 4H), 7.20–7.50 (m, 23H), 6.80 (d, *J*=8.7 Hz, 2H), 6.30 (d, *J*=6.4 Hz, 1H), 5.70 (d, *J*=1.0 Hz, 1H), 5.60 (d, *J*=2.4 Hz, 1H), 5.22 (d, *J*=1.7 Hz, 1H), 5.05 (d, *J*=1.6 Hz, 1H), 4.50–5.00 (m, 10H), 4.50–4.70 (m, 10H), 3.70–4.30 (m, 7H), 3.58 (s, 3H), 2.45 (s, 3H), 2.43 (s, 3H).

The trisaccharide glycal **29** (3.3 g, 2.4 mmol) was dissolved in MeOH (20 mL) and toluene (5 mL) and NaOMe (25% solution in MeOH, 0.2 mL, 0.87 mmol) was added. The mixture was stirred at room temperature for 15 h then concentrated in vacuo. The residue was purified over silica gel (hexane/EtOAc=2:1, then 1:1) to afford the dihydroxylated glycal (2.2 g, 1.94 mmol, 77%) as a thick syrup.

The dihydroxy glycal (2.0 g, 1.76 mmol) was dissolved in $CH_2Cl_2$ (20 mL) and cooled to 0 °C. Triethylamine (1.22 mL, 8.75 mmol) and TBSOTf (1.2 mL, 5.27 mmol) were added and the resulting mixture was stirred at room temperature for 3 h then diluted with EtOAc (200 mL). The organic layer was washed with saturated aq $NaHCO_3$ and brine then dried ($Na_2SO_4$) and concentrated. The residue was purified over silica gel (hexane/EtOAc=20:1, then 10:1) to afford **30** (2.3 g, 1.69 mmol, 95%) as a thick syrup. $[\alpha]_D^{25}$ 24.4 (*c*=0.6, $CHCl_3$); IR (thin film) 3030, 2926, 2855, 1648, 1514, 1454, 1380, 1249, 1043, 835, 777 cm$^{-1}$; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$=7.40–7.12 (m, 32H), 6.79 (dd, *J*=2.0, 6.4 Hz, 2H), 6.21 (dd, *J*=1.2, 6.4 Hz, 1H), 5.01 (dd, *J*=2.2, 6.1 Hz, 1H), 4.94 (d, *J*=1.9 Hz, 1H), 4.84 (d, *J*=10.8 Hz, 2H), 4.79 (d, *J*=2.0 Hz, 1H), 4.78 (d, *J*=11.7 Hz, 1H), 4.68–4.60 (m, 6H), 4.56–4.45 (dd, *J*=3.0, 11.9 Hz, 4H), 4.51 (dd, *J*=3.6, 10.8 Hz, 1H), 4.36 (dd, *J*=1.7, 7.1 Hz, 1H), 4.13 (t, *J*=2.4 Hz, 1H), 4.02–3.82 (m, 8H), 3.78–3.63 (m, 7H), 3.73 (s, 3H), 0.89 (b, 18H), 0.10 (s, 3H), 0.06 (s, 3H), 0.05 (s, 3H), 0.01 (s, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$=159.3, 144.2, 138.6, 138.5, 138.4, 130.1, 129.2, 128.3, 128.2, 128.2, 128.2, 128.2, 128.1, 127.6, 127.6, 127.4, 127.4, 127.3, 127.2, 113.9, 102.6, 102.3, 101.2, 80.0, 79.9, 79.2, 75.1, 75.0, 74.9, 74.7, 74.0, 73.1, 73.0, 72.6, 72.4, 72.1, 70.4, 69.6, 69.4, 69.2, 65.7, 55.2, 25.8, 25.7, 18.1, 0.0, −4.5, −4.8, −4.8; HRMS calcd for $C_{80}H_{102}O_{15}Si_2Na$: 1381.6650, found: 1381.6670.

*Method B (TBS protecting group)*: Thiodonor **27** (8 g, 13 mmol) and glucal acceptor **21** (1.15 g, 4.38 mmol) were mixed and azeotroped with benzene (3×30 mL), and then further dried under high vacuum. To the residue was added $CH_2Cl_2$ (100 mL) followed by 2,6-di-*tert*-butylpyridine (14.7 mL, 65 mmol) and freshly dried 4 Å molecular sieves (10 g). The slurry was stirred at room temperature for 30 min then cooled to 0 °C and MeOTf (5.94 mL, 52.5 mmol) was added. The reaction was slowly warmed to room temperature over 4 h with vigorous stirring then quenched with saturated aq $NaHCO_3$ (10 mL) and diluted with EtOAc (500 mL). The mixture was filtered through a pad of Celite, and the filtrate was washed with saturated aq $NaHCO_3$ (50 mL) and brine (50 mL) then dried ($Na_2SO_4$) and concentrated in vacuo. The residue was purified over silica gel (hexane/EtOAc=1:0, 10:1, then 5:1) to yield **30** (3.2 g, 2.36 mmol, 53%) as a thick syrup.

**4.1.7. Synthesis of dimannosyl trisaccharide alcohol thioglycoside 32.** To a solution of **30** (1.0 g, 0.73 mmol) in $CH_2Cl_2$ (10 mL) was added 4 Å MS (flame-dried, 1 g). The resulting slurry was stirred at room temperature for 30 min then cooled to 0 °C. Dimethyldioxirane (0.072 M in acetone, 12.2 mL, 0.876 mmol) was added *very slowly* (*important*) under vigorous stirring over 1 h. After the addition was complete, the mixture was stirred for 10 min and the volatiles were removed under a stream of dry $N_2$. The crude epoxide (**31**) was azeotroped with benzene (×3) then dissolved in $CH_2Cl_2$ (5 mL) and cooled to −78 °C. Ethanethiol (2.5 mL, 33.75 mmol) and trifluoroacetic anhydride (10 μL, 0.07 mmol) were added and the reaction was stirred for 10 min then quenched with $Et_3N$ (100 μL, 0.72 mmol). After removing the volatiles under a $N_2$ stream, the residue can be azeotroped with benzene (×2) for direct acylation or can be purified over silica gel (hexane/EtOAc=5:1) to afford the thioglycoside **32** (0.80 g, 0.58 mmol, 76%) as a thick syrup. $[\alpha]_D^{24}$ 12.8 (*c*=0.7, $CHCl_3$); IR (thin film): 3472, 2927, 2856, 1613, 1514, 1454, 1360, 1250, 1137, 1092, 1049, 978, 835, 777 cm$^{-1}$; $^1$H NMR (400 MHz, $CDCl_3$): $\delta$=7.40–7.10 (m, 32H), 6.80 (d, *J*=8.4 Hz, 1H), 4.90 (d, *J*=1.8 Hz, 1H), 4.84 (d, *J*=10.8 Hz, 2H), 4.79 (d, *J*=11.8 Hz, 1H), 4.65

(d, $J$=11.0 Hz, 2H), 4.58–4.45 (m, 9H), 4.39 (d, $J$=10.6 Hz, 1H), 4.34 (d, $J$=9.6 Hz, 1H), 4.08 (dd, $J$=2.2 Hz, 2H), 4.04–4.01 (m, 1H), 3.91 (dd, $J$=9.0, 18.0 Hz, 2H), 3.84 (d, $J$=2.4 Hz, 1H), 3.84–3.66 (m, 11H), 3.59 (t, $J$=8.1 Hz, 1H), 3.43–3.32 (m, 3H), 2.75–2.60 (m, 2H), 1.23 (t, $J$=7.6 Hz, 3H), 0.90 (s, 9H), 0.88 (s, 9H), 0.10 (s, 3H), 0.07 (s, 3H), 0.05 (s, 3H), 0.01 (s, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$=159.3, 138.6, 138.6, 138.2, 138.2, 138.1, 128.8, 128.3, 128.3, 128.2, 128.1, 128.0, 128.0, 127.7, 127.6, 127.5, 127.5, 127.4, 127.4, 127.3, 127.2, 114.0, 102.8, 100.9, 90.8, 84.8, 78.1, 76.3, 75.1, 74.9, 74.9, 74.6, 73.2, 72.9, 72.7, 72.1, 72.0, 70.1, 69.4, 69.3, 69.1, 65.7, 55.2, 18.1, 15.0, −4.5, −4.5, −4.7, −4.9; FAB(+)MS: 1460, 1439, 1357, 963; HRMS calcd for $C_{82}H_{108}O_{16}SSi_2Na$: 1459.6790, found: 1459.6760.

**4.1.8. Synthesis of dimannosyl trisaccharide acetate thioglycoside 33.** To a room temperature solution of **32** (3.01 g, 2.09 mmol) in $CH_2Cl_2$ was added $Ac_2O$ (1.07 g, 10.4 mmol), $Et_3N$ (2.11 g, 20.9 mmol), and a catalytic amount of DMAP. After stirring for 2 h the reaction was concentrated in vacuo and the remaining residue was purified directly by silica gel chromatography to afford **33** (2.81 g, 1.90 mmol, 91%). $[\alpha]_D^{24}$ 24.6 ($c$=1.0, $CHCl_3$); IR (thin film): 2927, 2855, 1750, 1613, 1514, 1454, 1361, 1249, 1225, 1050, 835, 777 cm$^{-1}$; $^1$H NMR (400 MHz, $CDCl_3$): $\delta$=7.40–7.05 (m, 30H), 6.75 (d, $J$=8.4 Hz, 2H), 5.06 (br s, 1H), 4.98–4.72 (m, 6H), 4.70–4.40 (m, 8H), 4.29 (d, $J$=8.5 Hz, 1H), 4.08–4.06 (br m, 3H), 3.98–3.55 (m, 14H), 3.68 (s, 3H), 3.48–3.42 (m, 1H), 3.39–3.35 (m, 1H), 2.65–2.60 (m, 1H), 2.23 (s, 3H), 0.91 (s, 9H), 0.84 (s, 9H), 0.12 (s, 3H), 0.08 (s, 3H), 0.02 (s, 3H), −0.06 (s, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$=169.9, 166.3, 159.1, 138.9, 138.7, 138.6, 138.5, 138.4, 129.7, 128.3, 128.2, 128.2, 128.1, 128.1, 128.0, 127.7, 127.5, 127.3, 127.3, 127.2, 127.2, 127.2, 113.9, 102.1, 101.2, 83.5, 79.9, 79.5, 78.4, 74.9, 74.7, 74.6, 74.0, 73.2, 73.0, 72.9, 72.3, 72.2, 72.0, 71.1, 69.4, 69.2, 65.5, 60.3, 55.2, 25.7, 25.7, 24.0, 22.1, 21.2, 18.1, 18.1, 14.8, −4.5, −4.5, −4.8, −5.1; HRMS calcd for $C_{84}H_{110}O_{17}SSi_2Na$: 1501.6900, found: 1501.6900.

**4.1.9. Synthesis of trisaccharide benzoate thioglycoside 34.** To a solution of **32** (3.28 g, 2.34 mmol) and pyridine (5 mL, 61.8 mmol) in $CH_2Cl_2$ (50 mL) was added benzoyl chloride (1.08 mL, 9.36 mmol) and the resulting mixture was stirred at room temperature for 4 d. At that point, propanol (1 mL) was added and stirring was continued for an additional hour. The mixture was diluted with EtOAc (200 mL) and washed with saturated aq $NaHCO_3$ and brine. The organic layer was dried over $Na_2SO_4$ and then concentrated in vacuo. The residue was purified over silica gel (hexane/EtOAc=3:1) to afford **34** (3.2 g, 2.11 mmol, 90%) as a white foam. $^1$H NMR (500 MHz, $CDCl_3$): $\delta$=7.80 (d, $J$=7.0 Hz, 2H), 7.36 (m, 2H), 6.90–7.30 (m, 18H), 6.70 (d, $J$=7.0 Hz, 2H), 5.20 (dd, $J$=8.0, 8.0 Hz, 1H), 5.00 (d, $J$=1.8 Hz, 1H), 4.76 (d, $J$=10.5 Hz, 1H), 4.70 (d, $J$=2.0 Hz, 1H), 4.65 (d, $J$=12.5 Hz, 1H), 4.63 (d, $J$=12.5 Hz, 1H), 4.54 (d, $J$=12.5 Hz, 1H), 3.98–4.05 (m, 2H), 3.85 (m, 2H), 3.75 (dd, $J$=2.0, 12.0 Hz, 1H), 3.63 (s, 3H), 3.57 (m, 1H), 3.40 (br d, 1H), 3.20 (d, $J$=12.0 Hz, 1H), 3.60 (m, 2H), 1.20 (t, $J$=7.0 Hz, 3H), 0.82 (s, 9H), 0.72 (s, 9H), 0.03 (s, 3H), 0.00 (s, 3H), −0.12 (s, 3H), −0.18 (s, 3H); LRMS calcd for $C_{89}H_{112}O_{17}SSi_2$: 1540.72, found: 1542 (M+H).

**4.1.10. Synthesis of pentasaccharide glycal acetate 35.** Trisaccharide donor **33** (1.03 g, 0.695 mmol) and disaccharide acceptor **19** (561 mg, 0.695 mmol) were combined and azeotroped with benzene (×2). Freshly activated 4 Å molecular sieves (4.0 g) were added, followed by $CH_2Cl_2$ (8 mL) and 2,6-di-*tert*-butylpyridine (1.09 mL, 4.9 mmol) and the mixture was stirred for 45 min at room temperature. The reaction was cooled to −10 °C and MeOTf (0.47 mL, 4.2 mmol) was slowly added. The reaction mixture was stirred at −8 °C for 10 h, at −5 °C for 6 h and finally at 5 °C for 6 h then quenched with $Et_3N$ (2.0 mL, 14.3 mmol), filtered through a plug of silica, washed with $NaHCO_3$, and extracted with EtOAc. The combined organic extracts were washed with water and brine, dried ($MgSO_4$), and concentrated in vacuo. Purification by silica gel chromatography (hexane/EtOAc=3:1) yielded **35** (994 mg, 0.45 mmol, 64%) as a white foam. $[\alpha]_D^{24}$ −5.3 ($c$=1.31, $CH_2Cl_2$); IR (thin film) 2247, 1650, 1612, 1586, 1571, 1514, 1498, 1454, 1361, 1249, 910, 836 cm$^{-1}$; $^1$H NMR (400 MHz, $CDCl_3$): $\delta$=7.87 (d, $J$=7.7 Hz, 2H), 7.45–7.19 (m, 45H), 6.76 (d, $J$=8.8 Hz, 2H), 6.34 (d, $J$=6.0 Hz, 1H), 5.05 (br d, $J$=1.6 Hz, 1H), 4.95–4.77 (m, 6H), 4.75–4.35 (m, 24H), 4.23 (d, $J$=6.2 Hz, 1H), 4.16–3.95 (m, 7H), 3.90–3.70 (m, 14H), 3.75 (s, 3H), 3.70–3.35 (m, 14H), 3.24–3.19 (m, 1H), 1.98 (s, 3H), 0.94 (s, 9H), 0.88 (s, 9H), 0.10 (s, 3H), 0.06 (s, 6H), −0.03 (s, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$=170.3, 159.0, 144.4, 141.8, 138.8, 138.7, 138.7, 138.6, 138.6, 138.5, 138.4, 137.9, 137.9, 137.8, 133.5, 128.6, 128.4, 128.4, 128.3, 128.3, 128.2, 128.1, 128.1, 128.1, 128.1, 128.0, 127.9, 127.7, 127.6, 127.4, 127.4, 127.3, 127.3, 127.2, 127.2, 127.2, 113.8, 101.6, 101.3, 100.0, 99.8, 99.4, 79.8, 79.5, 77.9, 75.4, 75.0, 74.7, 74.5, 74.3, 74.2, 74.0, 73.6, 73.4, 73.3, 73.3, 73.2, 73.0, 72.9, 72.8, 72.5, 72.5, 72.2, 72.1, 71.7, 70.2, 69.9, 69.1, 69.0, 67.7, 65.5, 56.2, 55.2, 25.7, 25.6, 21.1, 18.1, −4.4, −4.5, −4.6, −4.7, −5.1; HRMS calcd for $C_{128}H_{153}NO_{27}SSi_2Na$: 2246.9786, found: 2246.9840.

**4.1.11. Synthesis of pentasaccharide glycal benzoate 36.** Trisaccharide donor **34** (2.2 g, 1.45 mmol) and disaccharide acceptor **19** (1.5 g, 1.85 mmol, 1.3 equiv) were combined and azeotroped with benzene (3×20 mL). The residue was dissolved in dry $CH_2Cl_2$ (20 mL) then activated 4 Å molecular sieves (5 g) and 2,6-di-*tert*-butylpyridine (1.30 mL, 5.78 mmol) were added. The slurry was stirred at room temperature for 30 min then cooled to 0 °C. MeOTf (0.40 mL, 3.53 mmol) was added and the resulting mixture was stirred at a temperature between 0 °C and room temperature for 2 d then quenched by the addition of saturated aq $NaHCO_3$ (1 mL) and diluted with EtOAc (200 mL). After filtration through a short pad of Celite, the filtrate was washed with saturated aq $NaHCO_3$ (20 mL) and brine (20 mL) then dried ($Na_2SO_4$) and concentrated in vacuo. The residue was purified over silica gel (hexane/EtOAc=3:1) to afford **36** (2.36 g, 1.03 mmol, 71%) as a white foam. $R_f$ 0.5 (hexane/EtOAc=3:1), $[\alpha]_D^{25}$ −5.0 ($c$=1.0, $CHCl_3$); IR (thin film): 3468, 3030, 2933, 2858, 1651, 1496, 1092 cm$^{-1}$; $^1$H NMR (500 MHz, $CDCl_3$): $\delta$=7.70 (d, $J$=8.0 Hz, 2H), 7.20–7.50 (m, 62H), 6.78 (d, $J$=8.0 Hz, 1H), 6.40 (d, $J$=6.2 Hz, 1H), 4.40–4.59 (m, 22H), 4.60 (d, $J$=8.0 Hz, 1H), 3.95–4.15 (m, 6H), 0.93 (s, 9H), 0.92 (s, 9H), 0.00 (s, 3H), 0.01 (s, 3H), 0.04 (s, 3H), 0.06 (s, 3H); $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$=166.3, 159.5, 144.7, 142.5, 139.0, 139.0,

138.9, 130.3, 128.9, 128.8, 128.7, 128.6, 128.6, 128.5, 128.4, 128.1, 128.1, 127.9, 127.8, 127.8, 127.6, 127.6, 127.3, 114.7, 102.7, 101.7, 101.2, 100.2, 99.2, 81.9, 80.1, 80.0, 74.9, 74.4, 74.2, 74.1, 73.8, 73.7, 73.3, 73.3, 72.7, 72.6, 72.2, 70.4, 69.5, 68.3, 65.8, 57.5, 55.6, 26.2, 26.0, −4.4, −4.1, −4.2, −4.8; LRMS calcd for $C_{133}H_{155}NO_{27}SSi_2Na$: 2310, found: 2310 (M+Na).

**4.1.12. Synthesis of pentasaccharide dimannosyl glycal alcohol (37).** Pentasaccharide glucal **36** (330 mg, 0.144 mmol) was azeotropically dried with benzene (3×25 mL) then placed under high vacuum for 15 min. $Et_2O$ (20 mL) was added and the solution was cooled to −40 °C. Lithium aluminum hydride (1 M solution in $Et_2O$, 0.58 mL, 0.58 mmol) was added and the solution was stirred at 0 °C for 1 h. After diluting the solution with saturated aq $NaHCO_3$ (10 mL) and extracting with EtOAc (3×15 mL), the combined organic extracts were washed with brine (10 mL), dried ($NaSO_4$), and concentrated in vacuo. The residue was placed on high vacuum to afford the crude product as a white foam (330 mg, >95%), which was used in the next reaction without any further purification. For analytical data, the crude product was purified over a short column of silica gel ($R_f$ 0.50; EtOAc/hexane=1:4). $[\alpha]_D^{24}$ 0.9 ($c$=1.73, $CH_2Cl_2$); IR (thin film) 3468, 3351, 3063, 3030, 2927, 2856, 1650, 1612, 1586, 1514, 1498, 1453, 1360, 1249, 1208, 1093, 910, 835, 735, 698 $cm^{-1}$; $^1H$ NMR (500 MHz, $CDCl_3$): $\delta$=7.75 (d, $J$=7.5 Hz, 2H), 7.45–7.18 (m, 45H), 6.81 (d, $J$=8.6 Hz, 2H), 6.31 (d, $J$=6.1 Hz, 1H), 4.93–4.84 (m, 6H), 4.70–4.41 (m, 24H), 4.29 (d, $J$=7.6 Hz, 1H), 4.14–4.01 (m, 7H), 3.98–3.71 (m, 14H), 3.79 (s, 3H), 3.67–3.62 (m, 2H), 3.53–3.36 (m, 9H), 3.28–3.19 (m, 2H), 0.96 (s, 9H), 0.92 (s, 9H), 0.12 (s, 3H), 0.06 (s, 3H), 0.03 (s, 3H), 0.02 (s, 3H); $^{13}C$ NMR (125 MHz, $CDCl_3$): $\delta$=159.1, 144.3, 141.7, 138.9, 138.6, 138.6, 138.5, 138.3, 138.2, 138.1, 137.5, 132.1, 130.0, 128.6, 128.5, 128.4, 128.4, 128.3, 128.2, 128.2, 128.1, 128.1, 128.1, 128.0, 127.9, 127.7, 127.6, 127.5, 127.4, 127.4, 127.4, 127.3, 127.3, 127.2, 127.1, 113.8, 103.0, 102.3, 101.1, 100.7, 100.3, 80.0, 79.1, 79.0, 76.8, 75.8, 75.0, 75.0, 74.8, 74.5, 74.5, 74.0, 73.8, 73.4, 73.1, 73.0, 72.9, 72.5, 72.0, 71.8, 70.4, 70.0, 69.2, 69.1, 69.0, 68.7, 67.5, 66.0, 58.7, 55.2, 25.7, 25.7, 18.0, −4.6, −4.7, −4.8, −5.0; FAB(+)MS: 2205, 2192, 2130, 2115, 2110, 1988; HRMS calcd for $C_{126}H_{151}NO_{26}SSi_2Na$: 2204.9681, found: 2204.9680.

**4.1.13. Synthesis of pentasaccharide trimannosyl glycal alcohol 38.** Glycal alcohol **37** (330 mg, 0.144 mmol) was azeotropically dried with toluene (3×5 mL) then placed under high vacuum for 15 h. The residue was dissolved in $CH_2Cl_2$ (9 mL) and pyridine (106 μL, 1.23 mmol) was added. In an argon-filled glove bag, Dess–Martin reagent (330 mg, 0.77 mmol) was added in one portion. The resulting mixture was stirred at room temperature until the starting material had been consumed (about 1 h). At that point, the reaction was quenched by adding saturated aq $Na_2S_2O_3$ (1 mL) and diluted with EtOAc (100 mL). The organic layer was washed with saturated aq $Na_2S_2O_3$ (10 mL) and brine (10 mL) then dried ($Na_2SO_4$) and concentrated to afford the intermediate keto-compound (330 mg) pure enough for further use. After azeotropically drying the residue with toluene (3×), it was dissolved in dry THF (8 mL) and cooled to −40 °C. L-Selectride (1 M solution in THF, 0.35 mL, 0.35 mmol) was added drop wise and the reaction was stirred at −40 °C, then warmed to room temperature and stirred for an additional 2 h. The reaction was quenched by adding saturated aq $NaHCO_3$ and $H_2O_2$ (33% in water, 2.4 mL). After stirring for 30 min, the mixture was diluted with EtOAc (100 mL) and the combined organic layers were washed with saturated aq $NaHCO_3$, saturated aq $Na_2S_2O_3$, and brine then dried ($Na_2SO_4$) and concentrated to afford a residue (320 mg) of suitable purity for the next reaction. For analytical data, the residue was purified over silica gel (hexane/EtOAc=4:1). ($R_f$ 0.50; EtOAc/hexane=1:4); $[\alpha]_D^{24}$ −2.6 ($c$=2.05, $CH_2Cl_2$); IR (thin film) 3473, 3276, 1650, 1612, 1586, 1514, 1498, 1470, 1454, 1361, 1328, 1249, 1094, 910, 836, 778 $cm^{-1}$; $^1H$ NMR (500 MHz, $CDCl_3$): $\delta$=7.79 (d, $J$=7.5 Hz, 2H), 7.38–7.18 (m, 54H), 6.84 (d, $J$=8.5 Hz, 2H), 6.36 (d, $J$=6.1 Hz, 1H), 4.96 (br s, 1H), 4.93 (br s, 1H), 4.88 (br s , 1H), 4.81 (br s, 1H), 4.90–4.39 (m, 24H), 4.33–4.30 (m, 2H), 4.28 (br s, 1H), 4.19 (br s, 1H), 4.10–3.50 (m, 30H), 3.80 (s, 3H), 3.49–3.45 (m, 3H), 0.99 (s, 18H), 0.16 (s, 3H), 0.12 (s, 6H), 0.11 (s, 3H); $^{13}C$ NMR (125 MHz, $CDCl_3$): $\delta$=159.1, 144.3, 141.7, 138.8, 138.7, 138.6, 138.5, 138.2, 138.1, 138.1, 138.0, 137.7, 137.6, 132.1, 130.1, 128.7, 128.5, 128.4, 128.3, 128.2, 128.2, 128.1, 128.1, 128.0, 128.0, 128.0, 127.9, 127.6, 127.4, 127.4, 127.3, 127.3, 127.2, 127.1, 113.7, 102.2, 101.1, 100.6, 100.5, 100.1, 84.2, 80.1, 79.7, 75.9, 75.7, 75.3, 74.9, 74.5, 74.5, 73.4, 73.4, 73.1, 73.1, 73.0, 72.9, 72.8, 72.3, 70.3, 70.2, 69.8, 69.2, 69.0, 67.6, 66.5, 58.4, 55.1, 25.8, 18.1, −4.6, −4.7, −4.8, −5.0; FAB(+)MS: 2207, 2132, 2115, 1990; HRMS calcd for $C_{126}H_{151}NO_{26}SSi_2Na$: 2204.9680, found: 2204.9700.

**4.1.14. Synthesis of acetylated pentasaccharide trimannosyl glycal alcohol 39.** The crude alcohol (320 mg) was dissolved in $CH_2Cl_2$ (8 mL) then treated with $Ac_2O$ (1.2 mL, 12.7 mmol), $Et_3N$ (1.2 mL, 14.3 mmol), and DMAP (5 mg, 0.04 mmol) at room temperature for 15 h. The reaction mixture was diluted with EtOAc (100 mL), and the organic layer was washed with saturated aq $NaHCO_3$ (15 mL) and brine (15 mL) then dried ($Na_2SO_4$) and concentrated under reduced pressure. The residue was purified over silica gel to afford **39** (270 mg, 0.119 mmol, 82% in four steps) as a white foam. $R_f$ 0.5 (hexane/EtOAc=1:1); $^1H$ NMR (500 MHz, $CDCl_3$): $\delta$=7.98 (d, J 7.5 Hz, 2H), 7.20–7.50 (m, 57H), 6.74 (d, $J$=6.5 Hz, 2H), 6.35 (d, $J$=6.5 Hz, 1H), 5.44 (s, 1H), 5.40 (d, $J$=8.0 Hz, 1H), 4.40–5.00 (m, 36H), 3.60–3.80 (m, 22H), 3.58 (m, 4H), 2.08 (s, 3H), 1.97 (s, 3H), 0.89 (s, 9H), 0.86 (s, 9H), 0.02 (s, 3H), 0.01 (s, 3H), 0.00 (s, 3H), −0.02 (s, 3H); LRMS calcd for $C_{130}H_{155}NO_{28}SSi_2Na$: 2289, found: 2289 (M+Na).

**4.1.15. Synthesis of ethylthio pentasaccharide 41.** Pentasaccharide glycal **39** (353 mg, 0.155 mmol) and benzenesulfonamide (98 mg, 0.62 mmol) were combined and azeotropically dried with benzene (3×5 mL). The residue was dissolved in $CH_2Cl_2$ (15 mL), mixed with freshly dried 4 Å MS (1.5 g), and stirred at room temperature for 30 min. Iodonium-di-*sym*-collidine perchlorate (583 mg, 1.25 mmol) in $CH_2Cl_2$ (5 mL) was added via cannula to the mixture, and the resulting suspension was stirred at 0 °C for 30 min at which point it was warmed to room temperature and filtered through a short pad of Celite. The solids

were washed with EtOAc and the combined filtrates were washed with saturated $Na_2S_2O_3$ (×2), $CuSO_4$ (×4), brine, $Na_2S_2O_3$, and brine. The organic layer was dried ($Na_2SO_4$) and concentrated in vacuo. Purification over silica gel (hexane/EtOAc=2:1) yielded the intermediate iodosulfonamide, which was taken on crude. The iodosulfonamide was subsequently dissolved in DMF (5 mL) and cooled to −40 °C. A solution of LiHMDS (1 M in THF, 467 μL, 0.47 mmol) and ethanethiol (80 μL, 1.08 mmol) was cooled to −40 °C and added via cannula to the solution containing the iodosulfonamide. The mixture was stirred at −40 °C for 3 h and subsequently diluted with ether (150 mL), washed with saturated aq $NaHCO_3$ and brine then dried ($Na_2SO_4$) and concentrated. The residue was purified over silica gel (hexane/EtOAc=2:1) to afford the desired thioglycoside **41** (293 mg, 0.12 mmol, 81%) as white foam. $[\alpha]_D^{24}$ 10 ($c$=1.3, $CHCl_3$); IR (thin film): 3275, 2925, 2855, 1747, 1453, 1361, 1328, 1247, 1157, 1092, 736 $cm^{-1}$; $^1H$ NMR (500 MHz, $CDCl_3$): $\delta$=7.00–8.00 (m, 64H), 6.75 (d, $J$=6.4 Hz, 2H), 5.60 (d, $J$=9.0 Hz, 1H), 5.35 (d, $J$=2.8 Hz, 1H), 5.15 (s, 2H), 4.90 (s, 1H), 4.40–4.85 (m, 33H), 4.20 (d, $J$=7.1 Hz, 1H), 3.72 (s, 3H), 3.40–3.80 (m, 27H), 2.50 (m, 2H), 2.10 (s, 3H), 1.10 (t, $J$=7.6 Hz, 3H), 0.90 (s, 9H), 0.88 (s, 9H), 0.02 (s, 6H), 0.01 (s, 6H), 0.00 (s, 6H); HRMS calcd for $C_{138}H_{166}N_2O_{30}S_3Si_2$: 2483.0225, found: 2483.0264 (M+H).

**4.1.16. Synthesis of the desilylated pentasaccharide thioglycoside 43.** The thioglycoside **41** (170 mg, 0.07 mmol) in THF (5 mL) was stirred with TBAF (1 M solution in THF, 0.7 mL, 0.70 mmol) at room temperature for 36 h. At that point, the solution was concentrated and the residue was diluted with EtOAc. The organic layer was washed with saturated aq $NH_4Cl$ and brine then dried ($Na_2SO_4$) and concentrated. The residue was purified over silica gel (hexane/EtOAc=1:1, then 2:3) to afford the desired diol **43** (120 mg, 0.054 mmol, 80%) as a white foam. $R_f$ 0.25 (hexane/EtOAc=1:1); $[\alpha]_D^{24}$ −6 ($c$=1.5, $CHCl_3$); $^1H$ NMR (500 MHz, $CDCl_3$): $\delta$=7.86 (d, $J$=7.4 Hz, 2H), 7.76 (d, $J$=7.4 Hz, 2H), 7.00–7.40 (m, 60H), 6.75 (d, $J$=8.5 Hz, 2H), 5.70 (d, $J$=6.4 Hz, 1H), 5.25 (s, 1H), 5.10 (s, 1H), 4.90 (s, 1H), 4.30–4.60 (m, 33H), 4.10–4.20 (m, 2H), 3.40–4.00 (m, 27H), 3.35 (m, 2H), 3.20 (m, 2H), 2.95 (d, $J$=7.0 Hz, 2H), 2.4 (m, 2H), 2.08 (s, 3H), 1.10 (t, $J$=6.4 Hz, 3H); $^{13}C$ NMR (125 MHz, $CDCl_3$): $\delta$=169.2, 158.3, 140.5, 137.4, 137.2, 137.1, 136.9, 136.8, 128.2, 127.5, 127.4, 127.3, 127.3, 127.2, 127.1, 127.0, 126.9, 126.8, 126.8, 126.8, 126.7, 126.6, 126.3, 126.9, 100.7, 98.9, 98.5, 97.0, 93.0, 86.6, 81.7, 79.3, 78.6, 78.6, 78.0, 77.7, 72.4, 72.3, 72.3, 71.0, 70.6, 70.4, 67.8, 67.6, 67.0, 43.8, 37.8, 23.8, 18.8, 13.7; LRMS calcd for $C_{124}H_{136}N_2O_{29}S_3$: 2213, found: 2236 (M+Na) positive, 2248 (M+Cl) negative.

**4.1.17. Synthesis of peracetylated thioglycoside pentasaccharide 45.** To a deep blue solution of sodium metal (58 mg, 2.52 mmol) in $NH_3$ (~10 mL) at −78 °C was added a solution of thioglycoside **43** (58 mg, 0.03 mmol) in THF (2 mL). The solution was stirred at −78 °C for 45 min then quenched by the addition of solid $NH_4Cl$ (96 mg, 1.79 mmol) and MeOH (1 mL). The volatiles were removed under a stream of $N_2$ and the residue was treated with $Ac_2O$ (2 mL, 21.2 mmol), pyridine (2 mL, 24.7 mmol), and DMAP (10 mg, 0.08 mmol). The resulting mixture was stirred at room temperature for 15 h then concentrated at reduced pressure. The residue was dissolved in EtOAc (100 mL), washed with saturated aq $NaHCO_3$ and brine then dried ($Na_2SO_4$) and concentrated. Purification over silica gel (EtOAc/MeOH=95:5) yielded the peracetylated thioglycoside **45** (31 mg, 0.020 mmol, 77%) as a slightly yellow foam. $R_f$ 0.5 (EtOAc/MeOH=95:5); $[\alpha]_D^{24}$ −15 ($c$=2.2, $CHCl_3$); IR (thin film): 2900, 1750, 1420, 1280, 1050 $cm^{-1}$; $^1H$ NMR (500 MHz, $CDCl_3$): $\delta$=6.11 (d, $J$=9.3Hz, 1H), 5.59 (d, $J$=9.7 Hz, 1H), 5.58–5.20 (m, 6H), 5.20–4.90 (m, 8H), 4.76 (s, 1H), 4.57 (s, 1H), 4.42–3.90 (m, 19H), 2.62 (m, 2H), 2.59–1.76 (16s, 48H), 1.20 (t, $J$=7.3 Hz, 3H); $^{13}C$ NMR (500 MHz, $CDCl_3$): $\delta$=171.0, 170.8, 170.7, 170.6, 170.5, 170.4, 170.3, 170.2, 170.1, 169.9, 169.8, 169.7, 113.7, 101.2, 98.6, 97.1, 96.7, 84.4, 77.3, 77.0, 76.8, 76.0, 74.1, 72.7, 72.5, 72.0, 69.7, 69.3, 69.2, 69.2, 68.8, 68.3, 68.2, 66.8, 65.6, 65.5, 62.4, 62.2, 62.1, 60.4, 54.2, 52.9, 24.3, 23.2, 23.0, 21.0, 20.9, 20.9, 20.8, 20.8, 20.7, 20.6, 20.6, 19.7, 14.7, 14.2; HRMS calcd for $C_{64}H_{90}N_2O_{39}S$: 1542.4840, found: 1542.4863.

**4.1.18. Synthesis of deacetylated thioglycoside 46.** To a solution of the peracetylated pentasaccharide **45** (30 mg, 0.02 mmol) in MeOH (2 mL) was added NaOMe (25% solution in MeOH, 30 μL, 0.13 mmol). The mixture was stirred at room temperature for 15 h then purified directly using lipophilic LH-20 Sephadex. The appropriate fractions were combined and concentrated in a lyophilizer to afford the thioglycoside **46** (16 mg, 16.7 μmol, 86%). $R_f$ 0.80 (EtOAc/$^i$PrOH/$H_2O$=1:1:1); $^1H$ NMR (500 MHz, $D_2O$, 24 °C): $\delta$=5.12 (s, 1H), 4.93 (s, 1H), 4.68 (d, $J$=10.7 Hz, 1H), 4.62 (d, $J$=8.9 Hz, 1H), 4.27 (s, 1H), 4.08 (s, 1H), 3.99 (s, 1H), 3.52–3.96 (m, 30H), 2.75 (m, 2H), 2.10 (s, 3H), 2.05 (s, 3H), 1.25 (t, $J$=7.4 Hz, 3H); $^{13}C$ NMR (125 MHz, $D_2O$, 24 °C): $\delta$=174.8, 173.6, 104.4, 103.3, 102.2, 101.3, 85.9, 82.8, 81.3, 80.8, 76.8, 75.8, 75.4, 74.7, 73.9, 72.7, 72.2, 71.9, 69.4, 69.1, 68.1, 63.4, 63.2, 62.0, 61.6, 56.7, 55.8, 50.1, 25.2, 23.5, 23.1, 15.4; HRMS calcd for $C_{36}H_{62}N_2O_{25}S$: 954.3362, found: 954.3382.

**4.1.19. Synthesis of free pentasaccharide 47.** To a solution of ethylthioglycoside **46** (16 mg, 16.7 μmol) in $H_2O$ (5 mL) was added $HgCl_2$ (30 mg, 83.3 μmol) and then $CaCO_3$ (30 mg, 167 μmol). The mixture was stirred for 15 h at room temperature and then purified via size-exclusion chromatography. The appropriate fractions were collected and lyophilized to afford **47** (16 mg, 17.5 μmol, 95%) as a α/β mixture of anomers (2:3 or 3:2). $R_f$ 0.70 (EtOAc/$^i$PrOH/$H_2O$=1:1:1); $^1H$ NMR (500 MHz, $D_2O$, 24 °C): $\delta$=5.20 (s, 1H), 5.11 (s, 1H), 4.99 (s, 1H), 4.26 (s, 2H), 4.08–3.43 (m, 30H), 2.08 (s, 3H), 2.04 (s, 3H); LRMS calcd for $C_{34}H_{58}N_2O_{26}$: 910, found: 933 (M+Na), 915 (M−$H_2O$+Na).

**4.1.20. Synthesis of glycosylamine 11.** To the solution of free sugar **47** (16 mg, 0.018 mmol) in $H_2O$ (14 mL) was added solid $NH_4HCO_3$ (9.0 g, 114 mmol). The resulting mixture was stirred at room temperature until all the starting material was completely converted (monitored by $^1H$ NMR and TLC, EtOAc/$^i$PrOH/$H_2O$=1:1:1). During this period, $NH_4HCO_3$ needs to be continually added to keep the solution saturated. After completion of the reaction, the stirring bar was removed and the solution was lyophilized. The solid

material was several times redissolved in $H_2O$ (5 mL) and lyophilized until the residue exhibited a constant weight. A white powder (17 mg, 18.7 μmol, >99%) was obtained. $R_f$ 0.34 (EtOAc/$^i$PrOH/$H_2O$=1:1:1); $^1$H NMR (500 MHz, $D_2O$, 24 °C): $\delta$=5.12 (s, 1H), 4.93 (d, $J$=1.4 Hz, 1H), 4.62 (d, $J$=7.8 Hz, 1H), 4.27 (s, 1H), 4.16 (d, $J$=9.0 Hz, 1H), 4.08 (m, 1H), 3.98 (m, 3H), 3.48–3.94 (br m, 28H), 2.09 (s, 3H), 2.05 (s, 3H); HRMS calcd for $C_{34}H_{59}N_3O_{25}Na$: 932.3437, found: 932.3468 (M+Na).

**4.1.21. Synthesis of the N-linked pentasaccharide–pentapeptide adduct 1.** Pentasaccharide glycosylamine **11** (4 mg, 4.40 μmol) and L-pentapeptide **48**[37] (3.68 mg, 6.60 μmol) were mixed in anhydrous DMSO (0.5 mL) and HOBt (2.97 mg, 22 μmol), HBTU (9.48 mg, 22 μmol), and Hünig's base (15 μL, 8.8 μmol) were added. After stirring for 2 d at room temperature, the reaction mixture was lyophilized and purified on a reverse-phase Vydac C18-column (4.6×250 mm) to afford **1** (2.5 mg, 1.72 μmol, 40%). HPLC gradient: A: 0.1% TFA/$H_2O$, B: 0.09% TFA/70% $CH_3CN/H_2O$, gradient 5→50% B over 23 min; 50→100% B over 5 min; glycopeptide elutes at 21 min. $^1$H NMR (800 MHz, $H_2O$, Watergate method, 24 °C): 8.52 (d, $J$=8.9 Hz, 1H), 8.33 (d, $J$=9.6 Hz, 1H), 8.33 (d, $J$=7.3 Hz, 1H), 8.22 (d, $J$=5.9 Hz, 1H), 8.12 (d, $J$=8.2 Hz, 2H), 8.08 (d, $J$=8.0 Hz, 1H), 7.92 (d, $J$=8.0 Hz, 1H), 7.42 (s, 1H), 7.04 (s, 1H); (500 MHz, $D_2O$, 30 °C) $\delta$=5.24 (s, 1H, H-1c), 5.15 (d, $J$=9.7 Hz, 1H, H-1a), 5.04 (s, 1H, H-1d), 4.90 (s, 1H, H-1d), 4.84 (t, $J$=6.3 Hz, 1H), 4.76 (d, $J$=12.6 Hz, 1H, H-1b), 4.33–4.48 (m, 22H), 4.19 (s, 1H), 3.70–4.10 (m, 18H), 3.00 (dd, $J$=6.4, 16.4 Hz, 1H), 2.87 (dd, $J$=6.4, 16.7 Hz, 1H), 2.30 (m, 1H), 2.20 (s, 3H, Ac), 2.15 (s, 3H, Ac), 2.13 (s, 3H, Ac), 1.76 (m, 1H), 1.50 (d, $J$=7.2 Hz, 3H, $CH_3$), 1.34 (d, $J$=6.4 Hz, 3H, $CH_3$), 1.07 (d, $J$=6.5 Hz, 6H, $2CH_3$), 1.02 (d, $J$=6.4 Hz, 3H, $CH_3$); $^{13}$C (200 MHz, $H_2O$, Watergate method, 24 °C): $\delta$=105.2 (C-1), 104.2 (C-1), 103.3 (C-1), 102.5 (C-1), 81 (C-1a); HRMS (FAB) calcd for $C_{58}H_{99}N_9O_{33}Na$: 1472.6237, found: 1472.6282.

**4.1.22. Synthesis of the N-linked pentasaccharide–pentapeptide adduct 2.** Same procedure as in the synthesis of **1**. $^1$H NMR (500 MHz, $D_2O$, 36 °C): 5.01 (s, 1H), 4.93 (d, $J$=9.5 Hz, 1H), 4.81 (s, 1H), 4.67 (s, 1H), 4.60 (t, $J$=7.0 Hz, 1H), 4.52 (d, $J$=7.5 Hz, 1H), 4.18–4.25 (m, 2H), 4.19 (m, 2H), 3.98 (s, 1H), 3.88 (s, 1H), 2.75 (dd, $J$=6.5, 15.0 Hz, 1H), 2.60 (m, 2H), 2.10 (m, 1H), 1.97 (s, 3H), 1.93 (s, 3H), 1.90 (s, 3H), 1.78 (br s, 1H), 1.58 (m, 1H), 1.50 (m, 2H), 1.28 (d, $J$=7.0 Hz, 3H), 1.12 (d, $J$=7.0 Hz, 3H), 0.87 (s, 6H), 0.81 (d, $J$=6.4 Hz, 3H).

**4.1.23. Synthesis of allyl lactal 58.** 3′-Allyl lactal[38] (6.50 g, 18.68 mmol) was suspended in DMF (160 mL) and cooled to 0 °C. NaH (60% in oil, 5.98 g, 149.44 mmol) was added in one portion and the reaction was maintained at 0 °C for 30 min. Benzyl bromide (22.2 mL, 186.8 mmol) was added drop wise and the reaction was stirred at room temperature for 24 h. The reaction was cooled to 0 °C and quenched by drop wise addition of glacial AcOH (2.24 g, 37 mmol). Most of the solvent (DMF) was removed by evaporation under high vacuum and the residue was diluted with saturated aq $NaHCO_3$ (100 mL) and washed with EtOAc (5×100 mL). The organic layer was dried over $Na_2SO_4$ and concentrated in vacuo. The residue was purified by silica gel chromatography (hexane/EtOAc=10:1) to afford **58** (13.58 g, 17 mmol, 91%) as a white foam. $[\alpha]_D^{25}$ −9.8 ($c$=3.9, $CHCl_3$); IR (thin film): 3028, 2865, 1650, 1453, 1097 cm$^{-1}$; $^1$H NMR (500 MHz, $CDCl_3$): $\delta$=7.71–7.24 (m, 25H), 6.45 (d, $J$=6.2 Hz, 1H), 5.93 (m, 1H), 5.28 (dd, $J$=1.6, 12.3 Hz, 1H), 5.18 (dd, $J$=1.5, 10.4 Hz, 1H), 4.97 (d, $J$=12.3 Hz, 1H), 4.87 (m, 1H), 4.74 (m, 2H), 4.66–4.44 (m, 7H), 4.38 (m, 2H), 4.26 (m, 1H), 4.17 (m, 4H), 3.85–3.31 (m, 8H); $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$=144.4, 138.8, 138.7, 138.1, 137.9, 128.4, 128.3, 128.2, 128.2, 128.1, 127.9, 127.7, 127.6, 127.5, 127.4, 127.3, 82.0, 79.3, 75.9, 75.1, 73.6, 73.5, 73.3, 73.2, 72.3, 71.8, 70.3, 68.6, 68.0; HRMS (FAB) calcd for $C_{50}H_{54}O_9Na$: 821.3666, found: 821.3666.

**4.1.24. Synthesis of iodosulfonamide.** To a flask containing lactal **58** (6.0 g, 7.51 mmol), 2-(trimethylsilyl)ethanesulfonamide (5.44 g, 30.0 mmol), and powdered 4 Å molecular sieves (3.0 g, freshly flame-dried) was added $CH_2Cl_2$ (30 mL) and the mixture was stirred at room temperature for 30 min. The mixture was cooled to 0 °C and I(*sym*-collidine)$_2ClO_4$ (2.23 g, 4.76 mmol), prepared in situ from Ag(collidine)$_2ClO_4$ (10.13 g, 22.53 mmol) and 5.52 g $I_2$ (21.78 mmol) in the presence of powdered 4 Å molecular sieves (3.0 g, freshly flame-dried) in $CH_2Cl_2$ (130 mL) was added via cannula. The reaction was stirred for 1.5 h, then diluted with $CH_2Cl_2$ (150 mL) and filtered. The filtrate was washed with saturated aq $Na_2S_2O_3$ (3×75 mL), saturated aq $CuSO_4$ (5×75 mL), saturated aq $Na_2S_2O_3$ (1×75 mL), and brine (1×75 mL). The organic layer was dried over $Na_2SO_4$ and concentrated in vacuo. The residue was purified by silica gel chromatography (hexane/EtOAc=4:1 to 3:1) to afford the desired diaxial isomer (6.26 g, 5.65 mmol, 75%) as well as a small amount of the diequatorial isomer (0.589 g, 0.90 mmol, 12%). $[\alpha]_D^{25}$ −12.6 ($c$=0.9, $CHCl_3$); IR (thin film): 3258, 3029, 2867, 1455, 1337, 1107 cm$^{-1}$; $^1$H NMR (500 MHz, $CDCl_3$): $\delta$=7.46–7.22 (m, 25H), 5.93 (m, 1H), 5.34 (dd, $J$=1.6, 17.2 Hz, 1H), 5.20 (m, 2H), 4.93–4.37 (m, 12H), 4.19 (m, 4H), 4.08 (m, 1H), 3.85–3.34 (m, 10H), 3.04 (m, 1H), 1.06 (m, 2H), 0.07 (s, 9H); $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$=138.5, 138.3, 137.9, 137.7, 137.1, 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 128.0, 127.9, 127.9, 127.8, 127.8, 127.7, 127.7, 127.6, 127.5, 127.4, 116.8, 103.7, 81.8, 79.6, 79.1, 79.0, 78.2, 75.3, 74.6, 74.4, 73.9, 73.6, 73.5, 73.4, 73.1, 72.4, 71.9, 68.9, 67.4, 51.0, 29.8, 10.4, −2.1; HRMS (FAB) calcd for $C_{55}H_{68}INO_{11}SSi$: 1105.3286, found: 1105.3290.

**4.1.25. Synthesis of sulfonamidoethylthioglycoside 59.** The iodosulfonamide (6.26 g, 5.65 mmol) was dissolved in DMF (20 mL) and added drop wise to a solution of ethanethiol (2.09 mL, 28.25 mmol, 5 equiv) and LiHMDS (1 M solution in THF, 17 mL, 17 mmol) in DMF (80 mL) and cooled to −40 °C. The reaction was allowed to slowly warm to room temperature and stirred for 12 h. Most of the solvent (DMF) was removed by evaporation under high vacuum and the residue was diluted with saturated aq $NaHCO_3$ (100 mL) and washed with EtOAc (3×100 mL). The organic layer was dried over $Na_2SO_4$ and concentrated in vacuo. The residue was purified by silica gel chromatography (hexane/EtOAc=3.5:1) to afford **59** (5.0 g,

4.80 mmol, 85%) as a white glass. $[\alpha]_D^{25}$ −10.2 ($c$=3.1, $CHCl_3$); IR (thin film): 3268, 3029, 2868, 1454, 1091 $cm^{-1}$; $^1H$ NMR (500 MHz, $CDCl_3$): $\delta$=7.38–7.18 (m, 25H), 5.92 (m, 1H), 5.34 (d, $J$=17.5 Hz, 1H), 5.19 (d, $J$=10.5 Hz, 1H), 4.93 (d, $J$=11.8 Hz, 2H), 4.77–4.36 (m, 12H), 4.16 (m, 2H), 3.97–3.37 (m, 13H), 3.13 (m, 2H), 2.71 (m, 2H), 1.28 (t, $J$=7.4 Hz, 3H), 1.05 (m, 2H), −0.01 (s, 9H); $^{13}C$ NMR (125 MHz, $CDCl_3$): $\delta$=138.7, 138.6, 138.2, 137.9, 134.8, 128.3, 128.3, 128.2, 128.1, 128.1, 128.0, 127.9, 127.9, 127.8, 127.8, 127.7, 127.7, 127.7, 127.6, 127.6, 127.5, 127.5, 127.4, 127.4, 127.3, 116.3, 103.1, 83.8, 82.0, 81.2, 79.8, 79.7, 76.6, 75.2, 74.5, 73.8, 73.4, 73.2, 73.0, 71.6, 68.3, 67.9, 58.0, 24.0, 14.8, −2.0.

**4.1.26. Synthesis of ethylthioglycoside lactosamine.** To the solution of thiosulfonamide **59** (5.00 g, 4.80 mmol) in DMF (500 mL) was added powdered CsF (3.64 g, 24.0 mmol) and the mixture was stirred at 90 °C for 2 d. The solution was cooled to room temperature and most of the solvent (DMF) was removed by evaporation under high vacuum. The remaining residue was diluted with saturated aq $NaHCO_3$ (100 mL) and washed with $Et_2O$ (3×100 mL). The organic layer was dried over $Na_2SO_4$ and concentrated in vacuo. The residue was purified by silica gel chromatography (hexane/EtOAc=1:1 to 1:2 with 2% of $Et_3N$ v/v) to afford the title compound (3.96 g, 3.75 mmol, 94%) as a white glass. $[\alpha]_D^{25}$ −12.2 ($c$=1.0, $CHCl_3$); IR (thin film): 3029, 2921, 2867, 1453, 1090 $cm^{-1}$; $^1H$ NMR (500 MHz, $CDCl_3$): $\delta$=7.48–7.22 (m, 25H), 5.92 (m, 1H), 5.34 (dd, $J$=1.7, 7.2 Hz, 1H), 5.19 (m, 2H), 4.97 (d, $J$=11.1 Hz, 1H), 4.81 (m, 2H), 4.57–4.28 (m, 8H), 4.18 (d, $J$=5.3 Hz, 2H), 3.99–3.17 (m, 5H), 3.53–3.33 (m, 6H), 2.95 (m, 1H), 2.72 (m, 2H), 1.29 (t, $J$=7.5 Hz, 3H); $^{13}C$ NMR (125 MHz, $CDCl_3$): $\delta$=139.0, 138.9, 138.8, 138.0, 134.9, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 127.8, 127.7, 127.6, 127.5, 127.4, 127.3, 119.4, 102.7, 86.5, 84.8, 82.3, 79.9, 79.8, 76.2, 75.3, 75.2, 74.6, 73.4, 73.0, 71.6, 68.4, 68.1, 55.7, 24.1, 15.4; HRMS (FAB) calcd for $C_{52}H_{61}NO_9S$: 876.4145, found: 876.4156.

**4.1.27. Synthesis of phthalamidoethylthioglycoside.** To a solution of the lactosamine (3.70 g, 4.22 mmol) in pyridine (60 mL) was added phthalic anhydride (0.94 g, 6.33 mmol) and the reaction was stirred at room temperature for 45 min. Pivalic anhydride (4.72 g, 25.32 mmol) was added and the reaction was stirred at 85 °C for 5 d. The solution was cooled to room temperature and the pyridine was removed by evaporation. The remaining residue was dissolved in EtOAc (150 mL) and washed with saturated aq $NaHCO_3$ (3×50 mL). The organic layer was dried over $Na_2SO_4$ and concentrated in vacuo. The residue was purified by silica gel chromatography (hexane/EtOAc=10:1) to afford the title compound (4.25 g, 4.22 mmol, >99%) as a white foam. $[\alpha]_D^{25}$ 22.2 ($c$=0.7, $CHCl_3$); IR (thin film): 3029, 3026, 2923, 2867, 1774, 1713, 1386, 1086 $cm^{-1}$; $^1H$ NMR (500 MHz, $CDCl_3$): $\delta$=7.81 (m, 1H), 7.67 (m, 3H), 7.37–7.21 (m, 20H), 6.97 (m, 2H), 6.85 (m, 3H), 5.94 (m, 1H), 5.35 (dd, $J$=1.7, 7.2 Hz, 1H), 5.25 (d, $J$=10.0 Hz, 1H), 5.19 (dd, $J$=1.1, 10.5 Hz, 1H), 4.92–4.81 (m, 4H), 4.56–4.17 (m, 10H), 4.16 (m, 2H), 3.86 (m, 1H), 3.76 (m, 2H), 3.47–3.32 (m, 7H), 2.68 (m, 2H), 1.18 (t, $J$=8.4 Hz, 3H); $^{13}C$ NMR (125 MHz, $CDCl_3$): $\delta$=168.1, 167.5, 139.1, 138.8, 138.5, 138.1, 134.9, 133.8, 133.7, 131.7, 128.4, 128.2, 128.2, 128.1, 128.0, 127.8, 127.7, 127.7, 127.6, 127.5, 127.4, 127.3, 127.2, 126.8, 123.4, 123.2, 116.4, 102.9, 82.2, 80.9, 80.0, 79.7, 78.1, 77.8, 75.4, 74.5, 74.3, 73.6, 73.4, 73.0, 71.5, 68.3, 68.2, 54.8, 23.7, 14.9; HRMS (FAB) calcd for $C_{60}H_{63}NO_{11}SNa$: 1028.4019, found: 1028.4040.

**4.1.28. Synthesis of 3′-hydroxy ethylthioglycoside.** The phthalamidoethylthioglycoside (4.25 g, 4.22 mmol) was dissolved in EtOH/$H_2O$ (80 mL, 1:1 v/v). $RhCl(PPh_3)_2$ (0.39 g, 0.42 mmol) and DABCO (0.947 g, 8.44 mmol) were added and the reaction was heated to 90 °C for 3 h. At that point, the reaction was cooled to room temperature and diluted with THF (50 mL) and 1 N HCl (40 mL). The mixture was stirred at room temperature for 48 h. The organic solvents and water were removed by evaporation and the residue was dissolved in EtOAc (150 mL) then washed with saturated aq $NaHCO_3$ (3×50 mL), dried over $Na_2SO_4$, and concentrated in vacuo. The residue was purified by silica gel chromatography (hexane/EtOAc=5:1) to afford the title compound (3.0 g, 3.1 mmol, 74%) as a white foam. $[\alpha]_D^{25}$ 22.1 ($c$=0.6, $CHCl_3$); IR (thin film): 3472, 3029, 2868, 1774, 1713, 1307, 1078 $cm^{-1}$; $^1H$ NMR (500 MHz, $CDCl_3$): $\delta$=7.79 (m, 1H), 7.67 (m, 3H), 7.36–7.22 (m, 20H), 6.98 (m, 2H), 6.86 (m, 3H), 5.23 (d, $J$=10.1 Hz, 1H), 4.87 (m, 2H), 4.73 (m, 2H), 4.58 (m, 2H), 4.47–4.30 (m, 7H), 4.07 (m, 1H), 3.82 (m, 2H), 3.51 (m, 6H), 2.68 (m, 2H), 1.15 (t, $J$=7.4 Hz, 3H); $^{13}C$ NMR (125 MHz, $CDCl_3$): $\delta$=168.1, 167.5, 138.8, 138.7, 138.4, 138.3, 137.9, 131.7, 128.5, 128.4, 128.3, 128.2, 127.9, 127.8, 127.8, 127.7, 127.7, 127.6, 127.5, 127.5, 127.4, 126.9, 123.5, 102.9, 80.9, 80.7, 79.6, 78.0, 77.9, 75.9, 74.9, 74.4, 74.0, 73.4, 73.3, 73.1, 68.2, 68.1, 54.7, 23.8, 14.9; HRMS (FAB) calcd for $C_{57}H_{59}NO_{11}SNa$: 988.3706, found: 988.3744.

Propyl phthalamidoethylthioglycoside (product of allylic double bond reduction) was isolated as a by-product (0.62 g, 15%). If DABCO is excluded, the yield of desired product is much higher (91%), but isomerization required 3 d to reach completion.

**4.1.29. Synthesis of ethylthioglycoside donor 60.** To a solution of 3′-hydroxy ethylthioglycoside (3.0 g, 3.1 mmol) in $CH_2Cl_2$ (10 mL) was added chloroacetic anhydride (2.65 g 15.5 mmol), DMAP (cat.), and di-*tert*-butylpyridine (DTBP, 1.78 g, 9.3 mmol) and the reaction was stirred at room temperature for 24 h. The solution was diluted with $CH_2Cl_2$ (100 mL) and washed with saturated aq $NaHCO_3$ (3×50 mL) and brine. The organic layer was dried over $Na_2SO_4$ and concentrated in vacuo. The residue was purified by silica gel chromatography (hexane/EtOAc=10:1) to afford **60** (3.142 g, 3.02 mmol, 97%) as a white foam. $[\alpha]_D^{25}$ 49.5 ($c$=0.2, $CHCl_3$); IR (thin film): 2922, 1771, 1713, 1386, 1076 $cm^{-1}$; $^1H$ NMR (500 MHz, $CDCl_3$): $\delta$=7.83 (m, 1H), 7.71 (m, 3H), 7.37–7.22 (m, 20H), 6.98 (m, 2H), 6.87 (m, 3H), 5.23 (d, $J$=10.1 Hz, 1H), 4.84 (m, 3H), 4.65–4.26 (m, 12H), 4.10 (m, 1H), 3.93 (d, $J$=3.2 Hz, 1H), 3.87 (m, 1H), 3.77–3.42 (m, 7H), 2.69 (m, 2H), 1.19 (t, $J$=7.5 Hz, 3H); $^{13}C$ NMR (125 MHz, $CDCl_3$): $\delta$=168.1, 167.5, 166.6, 138.3, 138.2, 138.1, 137.9, 137.8, 133.8, 133.7, 131.6, 128.5, 128.4, 128.3, 128.3, 128.2, 128.0, 127.9, 127.8, 127.7, 127.7, 127.7, 127.6. 127.5, 127.4, 126.9, 123.5, 123.2, 102.7, 80.9, 79.5, 77.9, 77.8, 77.6,

75.1, 74.9, 74.7, 74.5, 73.3, 73.1, 72.6, 67.9, 67.4, 54.6, 40.5, 23.7, 14.9; HRMS (FAB) calcd for $C_{59}H_{60}ClNO_{12}SNa$: 1064.3422, found: 1064.3386.

**4.1.30. Synthesis of perbenzylated H-type 2 trisaccharide.** To a solution of glycal **63**[39] (9.9 g, 9.07 mmol) in THF (110 mL) was added TBAF (1 M solution in THF, 108 mL, 108 mmol) and the reaction was stirred at room temperature for 2 h. At that point MeOH (110 mL) and $K_2CO_3$ (15.0 g, 108 mmol) were added and the reaction was continued to stir at room temperature for an additional 12 h. The reaction was quenched by the addition of $NH_4Cl$, filtered through a pad of Celite, dried ($Na_2SO_4$), and concentrated. The crude triol was passed through a short plug of silica gel using EtOAc as eluent then concentrated. The resulting yellow oil was diluted with DMF (130 mL) then cooled to 0 °C and NaH (60% suspension in oil, 2.92 g, 72.9 mmol) was added in a few portions. The reaction was maintained at 0 °C for 30 min then benzyl bromide (12.8 mL, 108 mmol) was added drop wise and the reaction was stirred at room temperature for 14 h. The reaction was cooled to 0 °C and quenched by drop wise addition of MeOH. The solution was diluted with saturated aq $NaHCO_3$ (100 mL) and washed with EtOAc (3×300 mL). The organic layer was dried over $Na_2SO_4$ and concentrated in vacuo. The residue was purified by silica gel chromatography (hexane/EtOAc=10:1) to afford the H-type trisaccharide (9.05 g, 7.71 mmol, 85%) as a white foam. $[\alpha]_D^{25}$ −22.0 ($c$=2.7, $CHCl_3$); IR (thin film): 3029, 2869, 1453, 1098 $cm^{-1}$; $^1H$ NMR (500 MHz, $CDCl_3$): $\delta$=7.42–7.01 (m, 40H), 6.28 (d, $J$=6.3 Hz, 1H), 5.69 (d, $J$=2.9 Hz, 1H), 4.94 (d, $J$=11.6 Hz, 1H), 4.87–4.80 (m, 5H), 4.60–4.38 (m, 14H), 4.23 (m, 1H), 4.14 (m, 1H), 4.04–3.95 (m, 4H), 3.80 (m, 1H), 3.72–3.57 (m, 7H), 1.19 (d, $J$=6.4 Hz, 3H); $^{13}C$ NMR (125 MHz, $CDCl_3$): $\delta$=143.9, 139.0, 138.9, 138.5, 138.4, 138.3, 138.0, 137.9, 137.7, 128.4, 128.4, 128.3, 128.3, 128.2, 128.2, 128.1, 128.1, 128.0, 128.0, 127.9, 127.8, 127.7, 127.6, 127.6, 127.4, 127.4, 127.3, 127.2, 126.3, 101.3, 99.2, 97.0, 84.5, 79.5, 78.1, 75.5, 75.3, 74.7, 74.4, 73.6, 73.5, 73.2, 72.8, 72.6, 72.0, 71.3, 71.1, 70.3, 70.1, 68.7, 67.7, 66.2, 16.6; HRMS (FAB) calcd for $C_{74}H_{78}O_{13}Na$: 1197.5340, found: 1197.5389.

**4.1.31. Synthesis of iodosulfonamide.** To a solution of the H-type 2 trisaccharide glycal (6.64 g, 5.65 mmol) in $CH_2Cl_2$ (160 mL) was added 2-(trimethylsilyl)ethanesulfonamide (1.53 g, 8.45 mmol) and powdered 4 Å molecular sieves (5 g, freshly flame-dried). The mixture was stirred at room temperature for 20 min and then cooled to 0 °C. Iodonium-di-*sym*-collidine perchlorate (5.3 g, 11.32 mmol) was added in one portion and the reaction was stirred for 1 h and subsequently diluted with $CH_2Cl_2$ (100 mL) and filtered. The filtrate was washed with saturated aq $Na_2S_2O_3$ (2×75 mL), saturated aq $CuSO_4$ (1×75 mL), saturated aq $Na_2S_2O_3$ (1×75 mL), and brine (1×75 mL). The organic layer was dried over $Na_2SO_4$ and concentrated in vacuo. The residue (8.5 g) was used without further purification. $[\alpha]_D^{25}$ −20.2 ($c$=0.7, $CHCl_3$); IR (thin film): 3260, 3029, 2949, 1453, 1331, 1139, 1068 $cm^{-1}$; $^1H$ NMR (500 MHz, $CDCl_3$): $\delta$=7.37–7.00 (m, 40H), 5.65 (d, $J$=3.8 Hz, 1H), 5.09 (t, $J$=10.4 Hz, 1H), 4.92–4.17 (m, 21H), 4.15 (m, 3H), 4.00 (m, 1H), 3.89 (m, 2H), 3.77–3.62 (m, 5H), 3.51 (m, 4H), 1.15 (d, $J$=6.4 Hz, 3H), 1.05 (m, 2H), −0.04 (s, 9H); $^{13}C$ NMR (125 MHz, $CDCl_3$): $\delta$=138.7, 138.1, 138.0, 137.5, 137.1, 128.5, 128.4, 128.4, 128.3, 128.3, 128.2, 128.1, 128.1, 128.0, 127.9, 127.8, 127.7, 127.4, 127.3, 126.4, 102.9, 97.3, 84.2, 80.0, 79.6, 78.2, 77.9, 77.9, 75.4, 75.0, 74.8, 74.6, 74.4, 74.0, 73.7, 73.6, 73.5, 73.0, 72.7, 72.1, 72.0, 71.6, 69.3, 67.5, 66.2, 51.0, 30.5, 17.2, 10.4, −1.9; HRMS (FAB) calcd for $C_{79}H_{92}INO_{15}SSi$: 1504.4899, found: 1504.4963.

**4.1.32. Synthesis of sulfonamidoethylthioglycoside 64.** The iodosulfonamide (8.5 g) was suspended in DMF (5 mL) and cooled to −40 °C. To this solution was added a mixture of ethanethiol (2.5 mL, 33.7 mmol) and LiHMDS (1 M solution in THF, 16.95 mL, 16.95 mmol) in DMF (30 mL). The reaction was warmed to room temperature then stirred for 12 h. At that point, the reaction was diluted with saturated aq $NaHCO_3$ (75 mL) and washed with EtOAc (3×500 mL). The organic layer was dried over $Na_2SO_4$ and concentrated under a stream of $N_2$. The residue was purified by silica gel chromatography (hexane/EtOAc=10:1) to afford **64** (6 g, 4.24 mmol, 75% over two steps) as a white glass. $[\alpha]_D^{25}$ −33.6 ($c$=0.5, $CHCl_3$); IR (thin film): 3261, 3029, 2869, 1453, 1326, 1098 $cm^{-1}$; $^1H$ NMR (500 MHz, $CDCl_3$): $\delta$=7.38–7.05 (m, 40H), 5.70 (d, $J$=3.7 Hz, 1H), 4.95 (m, 2H), 4.92–4.33 (m, 21H), 3.96 (m, 3H), 3.73 (m, 1H), 3.70–3.42 (m, 9H), 3.02 (m, 2H), 2.71 (m, 2H), 1.30 (t, $J$=7.4 Hz, 3H), 1.06 (d, $J$=6.5 Hz, 3H), 1.06 (m, 2H), 0.02 (s, 9H); $^{13}C$ NMR (125 MHz, $CDCl_3$): $\delta$=138.8, 138.7, 138.6, 138.4, 138.1, 138.0, 137.9, 128.6, 128.4, 128.4, 128.3, 128.2, 128.2, 128.1, 128.1, 128.0, 127.9, 127.7, 127.6, 127.5, 127.5, 127.3, 127.3, 127.2, 127.2, 126.1, 101.5, 97.4, 83.9, 83.6, 81.2, 80.2, 79.2, 77.8, 76.3, 75.7, 74.7, 74.5, 73.7, 73.4, 73.3, 73.2, 72.7, 72.4, 72.1, 70.9, 68.9, 68.1, 66.3, 57.8, 51.4, 23.9, 16.7, 14.9, 10.4, −1.9; HRMS (FAB) calcd for $C_{81}H_{97}NO_{15}S_2SiNa$: 1438.5966, found: 1438.5990.

**4.1.33. Synthesis of ethylthioglycoside amine.** The sulfonamidoethylthioglycoside **64** (2.80 g, 1.97 mmol) was suspended in DMF (15 mL) and CsF (1.5 g, 9.86 mmol) was added. The reaction was stirred at 100 °C for 5 d then cooled to room temperature, diluted with saturated aq $NaHCO_3$ (75 mL), and washed with EtOAc (3×75 mL). The organic layer was dried over $Na_2SO_4$ and concentrated in vacuo. The residue was purified by silica gel chromatography (hexane/EtOAc=5:1) to afford the free amine (1.63 g, 1.30 mmol, 65%) as a white glass. IR (thin film): 3382, 3029, 2868, 1453, 1099 $cm^{-1}$; $^1H$ NMR (500 MHz, $CDCl_3$): $\delta$=7.32–7.07 (m, 40H), 5.71 (d, $J$=3.7 Hz, 1H), 5.22 (d, $J$=10.3 Hz, 1H), 4.95 (d, $J$=11.6 Hz, 1H), 4.85 (d, $J$=11.3 Hz, 1H), 4.77–4.20 (m, 18H), 4.05–3.25 (m, 14H), 2.90 (m, 1H), 2.72 (m, 2H), 1.32 (t, $J$=7.5 Hz, 3H), 1.21 (d, $J$=6.5 Hz, 3H); $^{13}C$ NMR (125 MHz, $CDCl_3$): $\delta$=138.8, 138.6, 138.5, 138.4, 138.3, 138.0, 137.9, 128.5, 128.3, 128.3, 128.2, 128.1, 128.1, 128.0, 127.9, 127.8, 127.7, 127.6, 127.5, 127.4, 127.3, 127.2, 127.1, 127.0, 126.1, 100.7, 97.3, 86.2, 84.5, 84.1, 79.1, 77.8, 75.6, 75.5, 75.2, 74.7, 74.6, 73.5, 73.3, 73.1, 72.6, 72.2, 72.1, 70.8, 68.3, 68.0, 66.3, 55.5, 23.9, 16.7, 15.4; HRMS (FAB) calcd for $C_{76}H_{85}NO_{13}SNa$: 1274.5639, found: 1274.5596.

**4.1.34. Synthesis of phthalamidoethylthioglycoside 55.** To a solution of the ethylthioglycoside amine (800 mg,

0.639 mmol) in pyridine (5 mL) was added phthalic anhydride (142 mg, 0.958 mmol) and the reaction was stirred at room temperature for 45 min. Acetic anhydride (1.2 mL, 12.7 mmol) was added and the reaction was stirred at 85 °C for 5 h. The solution was cooled to room temperature, diluted with 50 mL of saturated aq $NaHCO_3$, and washed with EtOAc (3×50 mL). The organic layer was dried over $Na_2SO_4$ and concentrated in vacuo. The residue was purified by silica gel chromatography (hexane/EtOAc=10:1) to afford **55** (860 mg, 0.626 mmol, 98%) as a white foam. $[\alpha]_D^{25}$ −37.5 ($c$=0.4, $CHCl_3$); IR (thin film): 3028, 2926, 2867, 1801, 1714, 1453, 1100 $cm^{-1}$; $^1H$ NMR (500 MHz, $CDCl_3$): $\delta$=8.00 (m, 1H), 7.67 (m, 3H), 7.33–7.04 (m, 35H), 6.88–6.75 (m, 5H), 5.70 (d, $J$=3.8 Hz, 1H), 5.19 (d, $J$=10.0 Hz, 1H), 4.95 (d, $J$=11.6 Hz, 1H), 4.86 (d, $J$=12.0 Hz, 1H), 4.75–4.62 (m, 14H), 4.04 (m, 2H), 3.92 (m, 1H), 3.86 (dd, $J$=2.5, 10.2 Hz, 1H), 3.77 (m, 2H), 3.58 (dd, $J$=2.7, 9.7 Hz, 1H), 3.47 (m, 4H), 2.69 (m, 2H), 1.33 (d, $J$=6.5 Hz, 3H), 1.19 (t, $J$=7.4 Hz, 3H); $^{13}C$ NMR (125 MHz, $CDCl_3$): $\delta$=167.9, 167.5, 138.9, 138.8, 138.7, 138.6, 138.5, 138.3, 138.1, 138.0, 133.9, 133.8, 131.7, 131.6, 128.4, 128.4, 128.3, 128.2, 128.1, 128.0, 128.0, 127.9, 127.8, 127.7, 127.6, 127.5, 127.5, 127.4, 127.4, 127.3, 127.2, 127.1, 126.8, 126.1, 123.4, 123.2, 100.7, 97.5, 83.9, 81.1, 80.3, 79.2, 78.1, 77.7, 77.7, 76.4, 75.6, 74.8, 74.5, 74.4, 73.5, 73.4, 73.1, 72.5, 72.3, 72.2, 70.8, 68.3, 66.4, 54.7, 23.7, 16.7, 15.0; HRMS (FAB) calcd for $C_{84}H_{87}NO_{15}SNa$: 1404.5964, found: 1404.5696.

**4.1.35. Synthesis of core mannose pentasaccharide acceptor 51.** To a solution of glycal **39** (90 mg, 41 μmol) in THF (3 mL) was added TBAF (1 M solution in THF, 0.4 mL, 0.4 mmol) and the reaction was stirred at room temperature for 48 h. At that point, the reaction mixture was concentrated and purified over silica gel (hexane/EtOAc=1:1) to afford **51** (63 mg, 31.5 μmol, 77%) as a white foam. $R_f$ 0.42 (EtOAc/hexane=2:1); $^1H$ NMR (500 MHz, $CDCl_3$): $\delta$=7.68 (d, $J$=7.4 Hz, 2H), 7.08–7.32 (m, 57H), 6.72 (d, $J$=8.6 Hz, 2H), 6.21 (d, $J$=6.1 Hz, 1H), 5.19 (d, $J$=2.9 Hz, 1H), 5.03 (s, 1H), 4.87 (s, 1H), 4.20–4.72 (m, 29H), 3.30–3.95 (m, 32H), 3.22 (m, 1H), 2.98 (br d, $J$=6.0 Hz, 1H), 2.19 (s, 3H), $^{13}C$ NMR (125 MHz, $CDCl_3$): $\delta$=159.9, 145.0, 142.0, 139.0, 138.8, 138.5, 138.4, 130.4, 129.1, 129.0, 128.9, 128.8, 128.6, 128.5, 128.4, 128.3, 128.3, 128.2, 128.1, 128.1, 114.5, 102.3, 101.1, 100.9, 100.6, 98.1, 80.3, 80.2, 76.5, 75.6, 75.3, 75.2, 74.8, 73.9, 73.8, 73.6, 72.6, 72.1, 72.0, 71.0, 69.4, 68.5, 66.5, 60.9, 58.3, 55.8, 21.7; LRMS (FAB) calcd for $C_{116}H_{125}NO_{27}S$: 1996, found: 2019 (M+Na); 2031 (M+Cl) and 1995 (M−H).

**4.1.36. Synthesis of nonasaccharide glycal 65.** Lactosamine donor **60** (1.18 g, 1.12 mmol) and pentasaccharide acceptor **51** (0.37 g, 0.18 mmol) were combined and azeotropically dried with toluene (3×20 mL). The residue was dissolved in $CH_2Cl_2$ (10 mL) and 2,6-di-*tert*-butylpyridine (1 mL, 4.46 mmol) then freshly dried 4 Å MS (1.0 g) was added. The resulting slurry was stirred at room temperature for 30 min then cooled to 0 °C. MeOTf (0.41 mL, 3.63 mmol) was added drop wise, and the mixture was slowly warmed to room temperature then stirred for 2 d. The reaction was quenched with saturated aq $NaHCO_3$ (5 mL), diluted with EtOAc (100 mL), and filtered through Celite. The filtrate was washed with saturated aq $NaHCO_3$ (20 mL) and brine then dried ($Na_2SO_4$) and concentrated. The residue was purified over silica gel (EtOAc/hexane=1:4, then EtOAc/toluene=4:1) to afford the nonasaccharide glycal **65** (440 mg, 0.111 mmol, 62%) as a white foam. $^1H$ NMR (500 MHz, $CDCl_3$): $\delta$=7.68 (d, $J$=7.4 Hz, 2H), 6.70–7.50 (m, 115H), 6.60 (d, $J$=8.6 Hz, 2H), 6.19 (d, $J$=6.1 Hz, 1H), 5.20 (d, $J$=8.0 Hz, 1H), 4.99 (d, $J$=8.0 Hz, 1H), 4.98 (s, 1H), 4.79 (s, 1H), 4.50–4.78 (m, 17H), 4.25–4.49 (m, 48H), 3.20–4.20 (m, 70H), 1.98 (s, 3H); $^{13}C$ NMR (125 MHz, $CDCl_3$): $\delta$=170.9, 166.9, 159.1, 144.7, 141.8, 139.3, 138.8, 138.7, 138.6, 138.5, 138.2, 128.8, 128.7, 128.6, 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 127.8, 127.7, 127.6, 123.5, 114.0, 103.2, 100.7, 100.6, 98.2, 97.1, 80.3, 78.3, 75.3, 75.2, 74.6, 74.5, 73.7, 73.6, 73.5, 73.0, 72.7, 70.8, 67.8, 55.9, 55.3, 40.8, 40.7, 21.4; LRMS (FAB) calcd for $C_{230}H_{233}Cl_2N_3O_{51}S$: 3954, found: 2000 (M+2Na)/2e.

**4.1.37. Synthesis of dechloroacetylated nonasaccharide 66.** To a solution of the nonasaccharide glycal **65** (430 mg, 0.11 mmol) in toluene (2 mL) and ethanol (30 mL) were added thiourea (42 mg, 0.55 mmol) and solid $NaHCO_3$ (140 mg, 1.66 mmol). The mixture was heated at 75 °C for 15 h, cooled to room temperature, and concentrated. The residue was purified over silica gel (toluene/EtOAc=4:1) to afford diol **66** (410 mg, 0.11 mmol, >99%) as a white foam. $^1H$ NMR (500 MHz, $CDCl_3$): $\delta$=7.80 (d, $J$=8.4 Hz, 2H), 6.80–7.60 (m, 115H), 6.70 (d, $J$=8.5 Hz, 2H), 6.30 (d, $J$=6.2 Hz, 1H), 5.35 (d, $J$=7.9 Hz, 1H), 5.25 (d, $J$=8.3 Hz, 1H), 5.15 (s, 1H), 4.95 (s, 1H), 4.00–4.93 (m, 71H), 3.20–3.95 (m, 52H), 2.95 (br d, 3H), 2.85 (br, 1H), 2.74 (d, $J$=6.0 Hz, 1H), 2.20 (s, 1H), 2.10 (s, 1H), 2.08 (s, 3H); $^{13}C$ NMR (125 MHz, $CDCl_3$): $\delta$=170.9, 159.2, 144.8, 141.9, 139.3, 139.2, 138.9, 138.8, 138.6, 138.4, 128.9, 128.8, 128.7, 128.6, 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 127.8, 127.7, 127.6, 127.5, 125.7, 123.5, 114.2, 103.8, 103.4, 100.8, 100.6, 99.4, 97.2, 81.0, 80.8, 78.4, 76.3, 75.3, 75.2, 74.6, 74.5, 74.4, 73.7, 73.6, 73.5, 73.4, 73.3, 73.0, 72.8, 70.9, 69.8, 68.5, 55.9, 55.4, 21.4; LRMS (FAB) calcd for $C_{226}H_{231}N_3O_{49}S$: 3802.5, found: 1925 [M+2Na]/2, 1936 [M+2Cl]/2.

**4.1.38. Synthesis of pentadecasaccharide glycal 67.** The H-type 2 trisaccharide donor **55** (1.0 g, 0.72 mmol) and nonasaccharide diol acceptor **66** (410 mg, 0.108 mmol) were azeotropically dried with toluene (3×20 mL) and then dried under high vacuum for 15 h. The residue was dissolved in $CH_2Cl_2$ (10 mL), ether (20 mL), and 2,6-di-*tert*-butylpyridine (DTBP, 658 μL, 2.94 mmol, 27 equiv) and then freshly dried 4 Å molecular sieves (3 g) were added. After stirring for 30 min at room temperature, the mixture was cooled to 0 °C and treated with MeOTf (306 μL, 2.71 mmol). The reaction was slowly warmed to room temperature and stirred for 48 h. The reaction was quenched with saturated aq $NaHCO_3$ and then filtered through a pad of Celite. The filtrate was washed with saturated aq $NaHCO_3$ and brine then dried ($Na_2SO_4$) and concentrated. The residue was purified over silica gel (toluene/EtOAc=4:1) to afford glycal **67** (560 mg, 0.09 mmol, 78%) as a white foam. $R_f$ 0.5 (toluene/EtOAc=4:1); $^1H$ NMR (500 MHz, $CDCl_3$): $\delta$=7.70 (d, $J$=8.2 Hz, 2H), 6.60–7.60 (m, ~200H), 6.48 (d, $J$=8.5 Hz, 2H), 6.25 (d, $J$=6.2 Hz, 1H), 5.65 (s, 2H), 5.30 (d,

$J$=6.8 Hz, 2H), 4.75–5.05 (m, 14H), 4.50–4.70 (m, 20H), 4.05–4.50 (m, 92H), 3.60–4.00 (m, 50H), 3.00–3.60 (m, 70H), 1.88 (s, 3H), 1.38 (d, $J$=6.3 Hz, 6H); $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$=172.5, 169.5, 168.8, 159.5, 144.9, 142.8, 139.8, 139.7, 138.6, 138.5, 138.3, 136.5, 134.5, 124.8, 116.5, 114.5, 104.5, 103.7, 102.8, 101.5, 98.0, 97.5, 96.0, 84.0, 78.0, 77.4, 76.5, 76.4, 76.3, 76.2, 75.6, 75.4, 75.2, 74.8, 74.6, 74.5, 74.2, 74.1, 73.7, 72.5, 71.9, 71.7, 68.7, 67.2, 66.8, 57.5, 54.8, 37.0, 31.2, 22.0, 18.7; LRMS (FAB) calcd for $C_{390}H_{393}N_5O_{79}S$: 6442, found: 2171 (M+3Na)/3; 1634 (M+4Na)/4.

**4.1.39. Synthesis of deprotected pentadecasaccharide 50.** To a solution of **67** (100 mg, 0.02 mmol) in toluene (2 mL) and EtOH (15 mL) was added ethylenediamine (0.70 mL). The mixture was heated at 80 °C for 24 h then the volatiles were removed at reduced pressure. The residue was purified over silica gel ($MeCl_2$/MeOH=20:1) to yield the free amine intermediate (90 mg).

A solution of free amine (42 mg, 0.01 mmol) in THF (2 mL) was cooled to 78 °C. Gaseous $NH_3$ (~10 mL) was condensed into the reaction vessel and solid sodium (95 mg, 4.1 mmol) was added under vigorous stirring. The resulting blue solution was stirred at −78 °C for 1 h then quenched with solid $NH_4Cl$ and MeOH (5 mL) until the solution turned clear. The resulting mixture was stirred for additional 60 min at −78 °C then warmed to room temperature. The $NH_3$ was removed under stream of nitrogen then further dried under high vacuum for 2 h. Acetic anhydride (1 mL), pyridine (1 mL), and DMAP (20 mg) were added to the dry glycan. The mixture was stirred at room temperature for 24 h then the volatiles were removed in vacuo. The residue was purified over silca gel ($MeCl_2$/MeOH=20:1). To the residue was added MeOH (0.2 mL) and NaOMe (10 μL, 0.5 M in MeOH). The reaction was stirred at room temperature overnight then concentrated in vacuo. The residue was purified by size-exclusion chromatography (LH-20 column) using MeOH/$H_2O$ (1:1) as eluent to afford pentadecasaccharide **50** as a white powder (5.5 mg 30%). $^1$H NMR (500 MHz, $D_2O$, 40 °C): 6.55 (d, $J$=6 Hz, 1H), 5.40 (s, 2H, 2Fuc-1), 5.25 (s, 1H), 5.05 (s, 1H), 4.50–4.95 (m, 17H), 4.45 (br s, 1H), 4.18–4.40 (m, 6H), 3.60–4.20 (m, 78H), 2.25 (s, 3H), 2.20 (m, 12H), 1.35 (m, 6H); LRMS (FAB) calcd for $C_{100}H_{165}N_5O_{72}$: 2589, found: 1317 (M+2Na)/2, 1329 (M+2Cl)/2.

**4.1.40. Synthesis of acetylated 15-mer glycal 71.** To a solution of glycal **67** (53 mg, 8.22 μmol) in ethanol (15 mL) and toluene (2 mL) was added ethylenediamine (0.26 mL, 3.88 mmol). The resulting mixture was heated at 85 °C for 2 d then cooled and concentrated. The residue was stirred at room temperature with acetic anhydride (1 mL, 10.59 mmol), pyridine (2 mL, 25 mmol), and DMAP (5 mg, 0.04 mmol) for 15 h and then concentrated. The residue was purified over silica gel ($CHCl_3$/MeOH=20:1, containing 1% $Et_3N$) to afford the *N*-acetylated product **71** (43 mg, 7.19 μmol, 85%) as a white foam. $^1$H NMR (500 MHz, $CDCl_3$): $\delta$=8.20 (d, $J$=8.0 Hz, 2H), 7.20–7.60 (m, ~180H), 6.83 (d, $J$=8.5 Hz, 2H), 6.54 (d, $J$=8.0 Hz, 1H), 5.90 (d, $J$=2.0 Hz, 2H), 5.65 (d, $J$=6.0 Hz, 1H), 5.50 (m, 3H), 3.50–5.30 (m, ~170H), 2.17 (s, 3H), 2.10 (s, 3H), 1.87 (s, 3H), 1.82 (s, 3H), 1.64 (s, 3H), 1.62 (s, 3H), 1.45 (d, $J$=5.5 Hz, 6H); $^{13}$C NMR (500 MHz, $CDCl_3$, selected peaks): $\delta$=168.9, 137.7, 137.4, 137.0, 136.8, 136.7, 127.9, 127.4, 127.3, 127.2, 127.1, 127.0, 126.9, 126.8, 126.6, 126.5, 126.4, 126.1, 124.4, 112.7, 101.6, 96.5, 82.9, 73.8, 73.5, 73.4, 72.4, 72.1, 72.0, 71.5, 71.3, 71.2, 67.4, 67.1, 65.3, 24.7, 22.0, 20.4, 15.8; LRMS calcd for $C_{368}H_{395}N_5O_{76}S$: 6136, found: 2046 (M+3H)/3e, 2068 (M+3Na)/3e.

**4.1.41. 15-mer Iodosulfonamidation product.** The pentadecasaccharide glycal **71** (130 mg, 21.2 μmol) and benzenesulfonamide (35 mg, 0.222 mmol) were mixed and azeotropically dried with toluene (3×5 mL). After addition of freshly dried 4 Å MS (500 mg) and $CH_2Cl_2$ (8 mL), the suspension was cooled to −15 °C. Under an atmosphere of argon, iodonium-di-*sym*-collidine perchlorate (80 mg, 0.17 mmol) in $CH_2Cl_2$ (2 mL) was added and the reaction was stirred at 0 °C for 30 min. After diluting with EtOAc (150 mL), the reaction mixture was filtered through a pad of Celite, which was thoroughly washed with EtOAc (20 mL). The combined filtrates were successively washed with saturated aq $Na_2S_2O_3$ (3×20 mL), saturated aq $CuSO_4$ (2×20 mL), saturated aq $Na_2S_2O_3$ (20 mL), and brine (20 mL) then dried ($Na_2SO_4$) and concentrated in vacuo. The residue was purified over silica gel (4% MeOH in $CH_2Cl_2$) to afford the intermediate iodosulfonamide (100 mg, 15.6 μmol, 74%) as a slightly yellow foam. $^1$H NMR (500 MHz, $CDCl_3$): $\delta$=6.80–7.60 (m, ~190H), 6.60 (d, $J$=6.8 Hz, 2H), 5.60 (s, 2H), 5.20–5.40 (bm, 5H), 3.20–5.00 (m, ~100H), 1.85 (s, 3H), 1.83 (s, 3H), 1.62 (s, 3H), 1.61 (s, 3H), 1.58 (s, 3H), 1.38 (s, 3H), 1.20 (d, $J$=6.4 Hz, 6H), LRMS (FAB) calcd for $C_{374}H_{401}IN_6O_{78}S_2$: 6414, found: 2162 (M+3Na)/3e.

**4.1.42. Hydrolysis of iodosulfonamide: synthesis of 2-sulfonamido-1-hydroxy pentadecasaccharide 72.** *Procedure A*: To a solution of iodosulfonamide (100 mg, 15.6 μmol) in THF (2 mL) was added saturated aq $K_2CO_3$ (2 mL) and the reaction was stirred at room temperature for 15 h. The reaction mixture was diluted with EtOAc (100 mL) and then washed with saturated aq $NH_4Cl$ (10 mL) and brine (10 mL), dried over $Na_2SO_4$ and concentrated. Chromatography over silica gel (hexane/EtOAc=1:1→1:5) afforded the desired hydrolysis product **72** (60 mg, 9.5 μmol, 60%) as a white foam.

*Procedure B*: A solution of the iodosulfonamide (60 mg, 9.35 μmol) in THF (2 mL) was cooled to −78 °C and then LiHMDS (1 M solution in THF, 60 μL, 60 μmol) and AgOTf (4 mg, 15.6 μmol) in $H_2O$ (1 mL) were added. The resulting mixture was allowed to warm gradually to room temperature and stirred for 15 h. Upon complete consumption of starting material, saturated aq $NaHCO_3$ (5 mL) was added. The reaction was diluted with EtOAc (100 mL) and washed with brine (10 mL) then dried ($Na_2SO_4$) and concentrated. Chromatography over silica gel afforded **72** (38 mg, 6.0 μmol, 63%). $^1$H NMR (500 MHz, $CDCl_3$): $\delta$=7.87 (d, $J$=2.0 Hz, 2H), 7.75 (d, $J$=2.0 Hz, 2H), 6.80–7.30 (m, ~190H), 6.61 (d, $J$=6.8 Hz, 2H), 6.60 (d, $J$=3.0 Hz, 2H), 3.20–5.00 (m, ~105H), 1.91 (s, 3H), 1.85 (s, 3H), 1.58 (s, 3H), 1.55 (s, 3H), 1.53 (s, 3H), 1.46 (s, 3H), 1.20 (d, $J$=6.0 Hz, 6H); LRMS calcd for $C_{374}H_{402}N_6O_{79}S_2$: 6306, found: 2125 (M+3Na)/3e.

**4.1.43. Global Birch-type deprotection: synthesis of 1-hydroxy-pentadecasaccharide 73.** A solution of **72** (62 mg, 9.83 μmol) in THF (1 mL) was cooled to −78 °C. Gaseous $NH_3$ (~10 mL) was condensed into the reaction vessel and solid sodium (55 mg, 2.39 mmol) was added, under vigorous stirring. The resulting blue solution was stirred at −78 °C for 1 h and then quenched with solid $NH_4Cl$ and MeOH (5 mL). The, now colorless, solution was stirred for an additional 60 min at −78 °C then warmed to room temperature. The $NH_3$ was evaporated under a stream of nitrogen and then placed under high vacuum for 2 h. The resulting residue was dissolved in MeOH (1 mL) and stirred with acetic anhydride (0.2 mL, 211 μmol) at room temperature for 2 h. Upon completion of the reaction, the mixture was purified via size-exclusion chromatography. The volatiles were removed at reduced pressure until the residue exhibited constant weight to give the desired saccharide **73** (15 mg, 5.64 μmol, 57%). $^1$H NMR ($D_2O$, 500 MHz, 24 °C): $\delta$=5.32 (s, 2H, 2H-1Fuc), 5.20 (s, 1H, H-1 Man), 5.12 (s, 1H, H-1 Man), 4.96 (s, 1H, H-1 Man), 4.70 (d, $J$=8.2 Hz, 2H, 2H-1), 4.59 (d, $J$=7.6 Hz, 2H, 2H-1), 4.55 (d, $J$=7.8 Hz, 3H, 3H-1), 4.47 (d, $J$=8.0 Hz, 2H, 2H-1), 4.11–4.24 (m, 5H), 3.49–3.99 (m, br, ~85H), 2.09 (s, 9H, 3Ac), 2.05 (s, 9H, 3Ac), 1.24 (d, $J$=6.4 Hz, 6H, $2CH_3$). LRMS calcd for $C_{103}H_{170}N_6O_{74}$: 2664, found: 2688 (M+Na), 1355 (M+2Na)/2, 2698 (M+Cl).

**4.1.44. Synthesis of pentadecasaccharide glycosylamine 74.** To a room temperature solution of pentadecasaccharide **73** (15 mg, 5.64 μmol) in water (4 mL) was added a large excess of solid $NH_4HCO_3$. The saturated solution was stirred at room temperature for 3 d. It is important to keep the solution saturated by continually adding $NH_4HCO_3$. After complete consumption of the starting material, the reaction mixture was lyophilized. The white solid was redissolved in water and again lyophilized. This procedure was repeated until the weight remained constant. Pentadecasaccharide glycosylamine **74** (15 mg, 5.64 μmol, >99%) was obtained as a white powder. $^1$H NMR ($D_2O$, 500 MHz, 24 °C): $\delta$=5.32 (d, $J$=3.2 Hz, 2H, 2H-1Fuc), 5.13 (s, 1H, H-1 Man), 4.94 (s, 1H, H-1 Man), 4.72 (d, $J$=8.0 Hz, 2H, 2H-1), 4.60 (d, $J$=8.0 Hz, 2H, 2H-1), 4.56 (d, $J$=8.0 Hz, 3H, 3H-1), 4.48 (d, $J$=7.8 Hz, 2H, 2H-1), 4.12–4.28 (m), 3.48–4.00 (m, ~90H), 2.09, 2.05, 2.01 (s, 15H, 5Ac), 1.95 (s, 3H, Ac), 1.25 (d, $J$=6.5 Hz, 6H, $2CH_3$); LRMS calcd for $C_{102}H_{171}N_7O_{73}$: 2662, found: 2684 (M+Na).

**4.1.45. Pentadecasaccharide pentapeptide conjugate (3).** To a solution of glycosylamine **74** (10 mg, 3.75 μmol) in anhydrous DMSO (0.5 mL) at room temperature was added Hünig's base (100 μL DIEA/DMSO, prepared from 12.8 μL DIEA in 1 mL of DMSO, 7.34 μmol), pentapeptide **48**[37] (4 mg, 7.2 μmol), 1-hydroxybenzotriazole (100 μL HOBt/DMSO, prepared from 25.2 mg HOBt in 1 mL of DMSO, 18.8 μmol), and HBTU (100 μL HBTU/DMSO, prepared from 80.4 mg HBTU in 1 mL of DMSO, 18.8 μmol). The reaction was stirred at room temperature for 36 h (suspension turned into solution) and then lyophilized and purified on a reverse-phase Vydac C18-column (4.6×250 mm) to afford glycopeptide **3** (2.3 mg, 20%) as a white powder. HPLC gradient: A: 0.1% TFA/$H_2O$, B: 0.09% TFA/70% $CH_3CN/H_2O$, gradient 5→50% B over 23 min; 50→100% B over 5 min. $^1$H NMR (***$H_2O$***, 800 MHz, 22 °C): $\delta$=8.74 (d, $J$=8.8 Hz, 1H, NH), 8.54 (d, $J$=9.6 Hz, 1H, NH), 8.52 (d, $J$=7.2 Hz, 2H, 2NH), 8.42 (d, $J$=5.6 Hz, 2H, 2NH), 8.36 (d, $J$=5.6 Hz, 1H, NH), 8.35 (d, $J$=9.6 Hz, 1H, NH), 8.34 (d, $J$=12.8 Hz, 1H, NH), 8.32 (d, $J$=6.4 Hz, 1H, NH), 8.31 (d, $J$=8.8 Hz, 1H, NH), 8.29 (d, $J$=8.8 Hz, 2H, 2NH), 8.14 (d, $J$=8.8 Hz, 2H, 2NH), 7.60 (s, 1H, NH, C-terminal), 7.24 (s, 1H, NH, C-terminal); $^1$H NMR (***$D_2O$***, 800 MHz, 22 °C): $\delta$=5.18 (d, $J$=3.2 Hz, 2H, 2H-1Fuc), 4.99 (s, 1H, H-1 Man), 4.91 (d, $J$=9.6 Hz, 1H, NH), 4.80 (s, 1H, H-1 Man), 4.64 (s, 1H, H-1 Man), 4.60 (dd, $J$=6.4, 7.2 Hz, 1H), 4.57 (d, $J$=8.0 Hz, 2H, 2H-1), 4.49 (d, $J$=8.0 Hz, 1H, H-1b), 4.46 (d, $J$=8.0 Hz, 2H, 2H-1), 4.44 (d, $J$=8.0 Hz, 2H, 2H-1), 4.34 (d, $J$=7.2 Hz, 1H, H-1), 4.33 (d, $J$=7.2 Hz, H-1), 4.15–4.20 (m), 4.06–4.14 (m), 3.40–4.00 (m), 2.85 (dd, $J$=6.4, 16.1 Hz, 1H), 2.64 (dd, $J$=6.7, 16.1 Hz, 1H), 2.06 (m, 1H, $CH(CH_3)_2$, 1.95 (s, 3H, Ac), 1.93 (s, 3H, Ac), 1.92 (s, 6H, 2Ac), 1.91 (s, 3H, Ac), 1.90 (d, $J$=7.3 Hz, 6H, $2CH_3$Fuc), 1.09 (d, $J$=7.7 Hz, 3H, $CH_3$), 0.82 (d, $J$=5.9 Hz, 3H, $CH_3$), 0.81 (d, $J$=6.4 Hz, 6H, $2CH_3$), 0.77 (d, $J$=8.0 Hz, $CH_3$); LRMS calcd for $C_{126}H_{211}N_{13}O_{81}$: 3202, found: 3225 (M+Na), 1624 (M+2Na)/2e, 1637 (M+2Cl)/2 in FAB mode.

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## References and notes

1. (a) Burda, P.; Aebi, M. *Biochem. Biophys. Acta* **1999**, *1426*, 239; (b) Dwek, R. A. *Chem. Rev.* **1996**, *96*, 683; (c) Dwek, R. A. *Science* **1995**, *269*, 1234; (d) Varki, A. *Glycobiology* **1993**, *3*, 97.
2. Shevinsky, L. H.; Knowles, B. B.; Damjanov, I.; Solter, D. *Cell* **1982**, *30*, 697.
3. (a) Kobata, A. *Acc. Chem. Res.* **1993**, *26*, 319; (b) Feizi, T. *Nature* **1985**, *314*, 53.
4. (a) Van Dijk, W.; Turner, G. A.; Mackiewicz, A. *Glycosyl. Dis.* **1994**, *1*, 5; (b) Van Dijk, W.; Van der Stelt, M. E.; Salera, A.; Dente, L. *Eur. J. Cell Biol.* **1991**, *55*, 143.
5. (a) Beitter, J. N.; Means, R. E.; Desrosiers, R. C. *Nat. Med.* **1998**, *4*, 678; (b) Lloyd, K. O. *Am. J. Clin. Pathol.* **1987**, *87*, 129.
6. For a review on the syntheses of O- and N-linked glycopeptides, see: (a) Arsequell, G.; Valencia, G. *Tetrahedron: Asymmetry* **1997**, *8*, 2839; (b) Arsequell, G.; Valencia, G. *Tetrahedron: Asymmetry* **1999**, *10*, 3045; (c) Pratt, M. R.; Bertozzi, C. R. *Chem. Soc. Rev.* **2005**, *34*, 58; (d) Hölemann, A.; Seeberger, P. H. *Curr. Opin. Biotechnol.* **2004**, *15*, 615.

7. For the all-chemical and chemoenzymatic synthesis of high mannose-containing N-linked glycopeptides, see, for example: (a) Matsuo, I.; Nakahara, Y.; Ito, Y.; Nukada, T.; Nakahara, Y.; Ogawa, T. *Bioorg. Med. Chem.* **1995**, *3*, 1455; (b) Ito, Y.; Ogawa, T. *J. Am. Chem. Soc.* **1997**, *119*, 5562; (c) Guo, Z. W.; Nakahara, Y.; Ogawa, T. *Angew. Chem.* **1997**, *109*, 1527 *Angew. Chem., Int. Ed.* **1997**, *36*, 1464; (d) Unverzagt, C. *Carbohydr. Res.* **1998**, *305*, 423; (e) Unverzagt, C. *Angew. Chem.* **1997**, *109*, 2078; *Angew. Chem., Int. Ed.* **1997**, *36*, 1989; (f) Unverzagt, C. *Angew. Chem.* **1996**, *108*, 2507; *Angew. Chem., Int. Ed.* **1997**, *35*, 2350; (g) Unverzagt, C. *Angew. Chem.* **1994**, *106*, 1170; *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 1102; (h) Mitzuno, M.; Handa, K.; Iguchi, R.; Muramoto, I.; Kawakami, T.; Aimoto, S.; Yamamoto, K.; Inazu, T. *J. Am. Chem. Soc.* **1999**, *121*, 284; (i) Schmidt, R. R.; Kinzy, R. R. *Adv. Carbohydr. Chem. Biochem.* **1994**, *50*, 21.
8. (a) Dudkin, V. Y.; Miller, J. S.; Danishefsky, S. J. *J. Am. Chem. Soc.* **2004**, *126*, 736; (b) Mandal, M.; Dudkin, V. Y.; Geng, X.; Danishefsky, S. J. *Angew. Chem., Int. Ed.* **2004**, *43*, 2557; (c) Geng, X.; Dudkin, V. Y.; Mandal, M.; Danishefsky, S. J. *Angew. Chem., Int. Ed.* **2004**, *43*, 2562.
9. Wang, Z.-G.; Zhang, X.; Live, D.; Danishefsky, S. J. *Angew. Chem., Int. Ed.* **2000**, *39*, 3652.
10. Live, D. H.; Wang, Z.-G.; Iserloh, U.; Danishefsky, S. J. *Org. Lett.* **2001**, *3*, 851.
11. Wang, Z.-G.; Zhang, X.; Visser, M.; Live, D.; Zatorski, A.; Iserloh, U.; Lloyd, K. O.; Danishefsky, S. J. *Angew. Chem., Int. Ed.* **2001**, *40*, 1728.
12. Danishefsky, S. J.; Hu, S.; Cirillo, P. F.; Eckhardt, M.; Seeberger, P. H. *Chem. Eur. J.* **1997**, *3*, 1617.
13. Cohen-Anisfeld, S. T.; Lansbury, P. T. *J. Am. Chem. Soc.* **1993**, *115*, 10531.
14. Danishefsky, S. J.; Bilodeau, M. T. *Angew. Chem.* **1996**, *108*, 1482; *Angew. Chem., Int. Ed.* **1996**, *35*, 1380.
15. For the general strategy we employ in reaching the 'high mannose' core system, see: Seeberger, P. H.; Cirillo, P. F.; Hu, S.; Beebe, X.; Bilodeau, M. T.; Danishefsky, S. J. *Enantiomer* **1996**, *1*, 311.
16. Likhosherstov, L. M.; Novikova, O. S.; Derevitskaja, V. A.; Kochetkov, N. K. *Carbohydr. Res.* **1986**, *146*, C1.
17. Griffith, D. A.; Danishefsky, S. J. *J. Am. Chem. Soc.* **1990**, *112*, 5811.
18. Pfitzer, K. E.; Mofatt, J. G. F. *J. Am. Chem. Soc.* **1965**, *87*, 5661.
19. Seeberger, P.; Eckhardt, M.; Gutteridge, C.; Danishefsky, S. J. *J. Am. Chem. Soc.* **1997**, *119*, 10064.
20. With modification of the original procedure from: Shaban, M. A. E.; Jeanloz, R. W. *Carbohydr. Res.* **1976**, *46*, 138.
21. The catalytic use of sodium methoxide in methanol is referred to as the Zemplén de-O-acetylation, see: Greene, T. W.; Wuts, P. G. M. *Protective Groups in Organic Synthesis*; Wiley: New York, NY, 1999; p 154.
22. Dess, D. B.; Martin, J. C. *J. Am. Chem. Soc.* **1991**, *113*, 7277.
23. Amann, A.; Ourisson, G.; Luu, G. B. *Synthesis* **1987**, 2002.
24. Meyers, A. I.; Comins, D. L.; Roland, D. M.; Henning, R.; Shimizu, K. *J. Am. Chem. Soc.* **1979**, *101*, 7104.
25. (a) Imperiali, B.; O'Connor, S. E. *Curr. Opin. Chem. Biol.* **1999**, *3*, 643; (b) Imperiali, B. *Acc. Chem. Res.* **1997**, *30*, 452; (c) Imperiali, B.; O'Connor, S. E. *Chem. Biol.* **1996**, *3*, 803.
26. (a) O'Connor, S. E.; Imperiali, B. *J. Am. Chem. Soc.* **1997**, *119*, 2295; (b) Davis, J. T.; Hirani, S.; Bartlett, C.; Reid, B. R. *J. Biol. Chem.* **1994**, *269*, 3331; (c) O'Connor, S. E.; Imperiali, B. *Chem. Biol.* **1998**, *5*, 427.
27. (a) Gigg, R. *Am. Chem. Soc. Symp. Ser.* **1977**, *39*, 253; (b) Wang, Z. G.; Ito, Y.; Nakahara, Y.; Ogawa, T. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 2805.
28. Danishefsky, S. J.; Koseki, K.; Griffith, D. A.; Gervay, J.; Peterson, J. M.; McDonald, F. E.; Oriyama, T. *J. Am. Chem. Soc.* **1992**, *114*, 8331.
29. $Piv_2O$ (or $Ac_2O$) is needed to dehydrate the initial adduct (amide) into the phthalimide.
30. For a brilliant example featuring the simultaneous removal of 17 benzyl groups, see: Frick, W.; Bauer, A.; Bauer, S.; Wied, S.; Müller, G. *Biochem.* **1998**, *37*, 13421.
31. Iserloh, U.; Dudkin, V.; Wang, Z.-G.; Danishefsky, S. J. *Tetrahedron Lett.* **2002**, *43*, 7027.
32. Frick, W.; Bauer, A.; Bauer, J.; Wied, S.; Müller, G. *Biochemistry* **1998**, *37*, 13421.
33. For analysis of the fine specificities of 11 mouse monoclonal antibodies reactive with H-type 2 blood group determinants see: Furukawa, K.; Welt, S.; Yin, B. W. T.; Feickert, H.-J.; Takahashi, T.; Ueda, R.; Lloyd, K. O. *Mol. Immunol.* **1990**, *27*, 723.
34. Actually, these data demonstrate that, in the very least, one of the two H-type 2 determinants in **3** is immunorecognized. They do not prove that *both* subunits are recognized, although this is presumably the case.
35. (a) Krantz, S. B.; Jacobsen, L. O. *Erythropoietin and the Regulation of Erythropoiesis*; University of Chicago Press: Chicago, 1970; (b) Goldwasser, E.; Kung, C. K.-H. *Proc. Natl. Acad. Sci. U.S.A.* **1971**, *68*, 697; (c) Miyake, T.; Kung, C. K.-H.; Goldwasser, E. *J. Biol. Chem.* **1977**, *252*, 5558.
36. Warren, J. D.; Miller, J. S.; Keding, S. J.; Danishefsky, S. J. *J. Am. Chem. Soc.* **2004**, *126*, 6576.
37. Both the L-amino acid-containing peptide (**48**) and D-amino acid-containing peptide (**49**) were synthesized by San San Yi of the Microchemistry Laboratory, Sloan-Kettering Institute for Cancer Research, using a peptide synthesizer and were purified by standard reverse-phase HPLC using an $H_2O$/TFA–$CH_3CN$/TFA gradient. The first C-terminal amino acid was attached to the superacid-lable Fmoc-Rink Amide MBHA resin. The subsequent amino acids, which were introduced were protected as Thr(OtBu) and Asp(Dmab ester). Standard TFA-cleavage, Dmab- and Fmoc-removal (2% hydrazine in MeOH), and acetylation of the N-terminus yielded the desired peptides.
38. Prepared from lactal according to the procedure published by Schaubach, R.; Hemberger, J.; Kinzy, W. *Liebigs Ann. Chem.* **1991**, *7*, 607–614.
39. Danishefsky, S. J.; Behar, V.; Randolph, J. T.; Lloyd, K. O. *J. Am. Chem. Soc.* **1995**, *117*, 5701.