A Highly Convergent Total Synthetic Route to Glycopeptides Carrying a High-Mannose Core Pentasaccharide Domain *N*-linked to a Natural Peptide Motif

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Abstract: *N*-Linked glycopeptides were synthesized by condensation of a high-mannose anomeric amine bearing a pentasaccharide with aspartic-acid-containing tri- and pentapeptides through the agency of IIDQ. The pentasaccharide portion, corresponding to the "core" region of all asparagine-linked glycoproteins, was assembled by means of glycal-derived thioethyl donors and glycal acceptors. The central mannose residue was established by inversion of the C 2 hydroxyl of a glucosyl precursor in the pentasaccharide. The protecting-group scheme employed allows the extension of the pentasaccharide through the terminal mannose units. While a fully convergent coupling of the high-mannose carbohydrate to the peptide domain has thus been accomplished for the first time with a fully synthetic sugar, the stereochemical integrity of the anomeric center of the carbohydrate domain was not maintained and a mixture of glycopeptides was obtained.

Keywords
glycals · glycopeptides · glycosylations · β-mannosides · oligosaccharides

Introduction

The elaborate machinery involved in the biosynthesis of glycosylated proteins,[1] and their widespread occurrence, suggest that these bidomainal structures are assembled by cells in a purposeful and regulated way. The characteristic carbohydrate moieties present in cell adhesion molecules,[2] tumor-associated antigens,[3] targets for viral or bacterial invasion,[4] and blood group determinants[5] are most commonly presented to recognition systems in the form of glycoproteins. The role of glycosylation in influencing protein folding is also a matter of great current interest.[6]

There are two abundant natural types of glycoproteins which merit particular attention. These are the *N*-asparagine-linked systems (motif **A**) and the *O*-serine/threonine-linked ensembles (pattern **B**, Scheme 1). Homogeneous and structurally defined glycopeptides could serve as models for the study of how the carbohydrate and polypeptide domains in each of these classes influence one another in terms of conformation and presentation to complimentary ligands, receptors, or antibodies (structure **C**).[7]

Considering the complexity of the problem, progress in the synthesis of glycopeptides has been remarkable.[8] Accomplishments from the laboratories of Paulsen, Kunz, Meldal, T. Ogawa and Unverzagt have been of particular significance in fostering this progress.[9] The elegant interplay of chemically and enzymatically mediated couplings pioneered by Wong[10] constitutes a most promising direction. An impressive demonstration of the feasibility of accomplishing a convergent union of carbohydrate and peptide domains was provided by Lansbury,[11] who used naturally derived anomeric glycosylamines and minimally protected synthetic peptides. We have demonstrated the stereospecific and convergent coupling of fully synthetic polymer-bound model sugar to solution-based peptide.[12] Spectroscopic analysis of a deprotected system has revealed an unusual degree of structure in the peptide segment.[13]

Our intention in the present work was the achievement of a highly convergent synthesis of a naturally occurring high-mannose "core" carbohydrate domain terminating in an anomeric amine. With this accomplished, we would then address the feasibility of convergent coupling to a synthetic peptide carrying the Asn–X–Ser/Thr signature sequence (see constructs **5** and **6**).[14] We would thus gain access to free *N*-glycopeptides wherein the carbohydrate domain corresponds to a natural motif. NMR spectroscopic investigations of such systems should be even more revealing than was the case with our model probe structures.[13] As is our practice, we planned to first work out the chemistry in solution, and then attempt to translate our findings to assembly on a polymer support.

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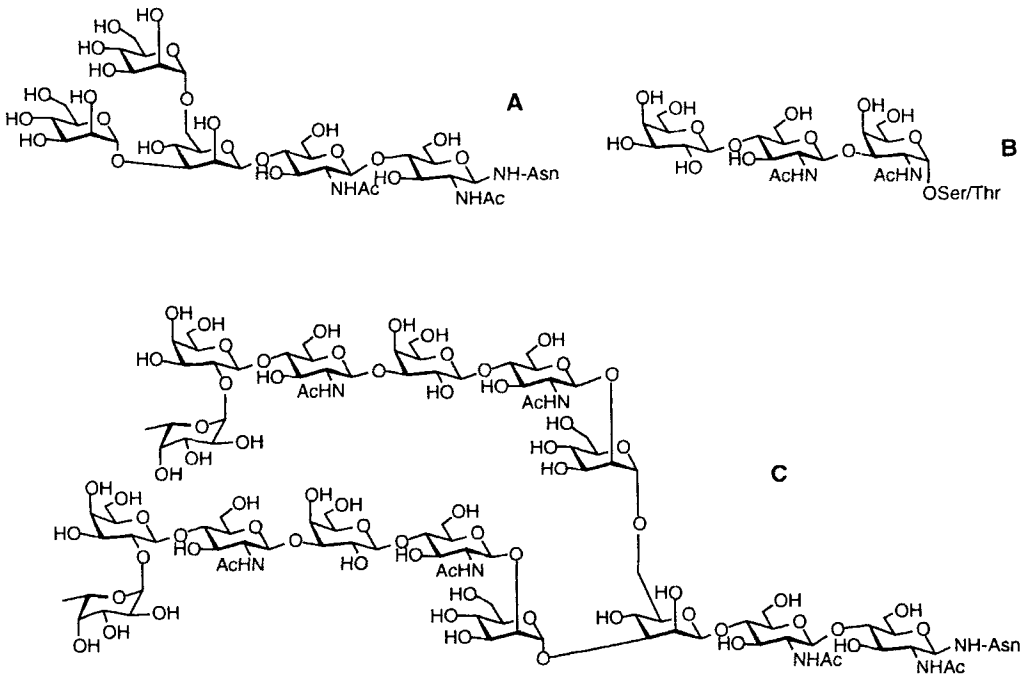
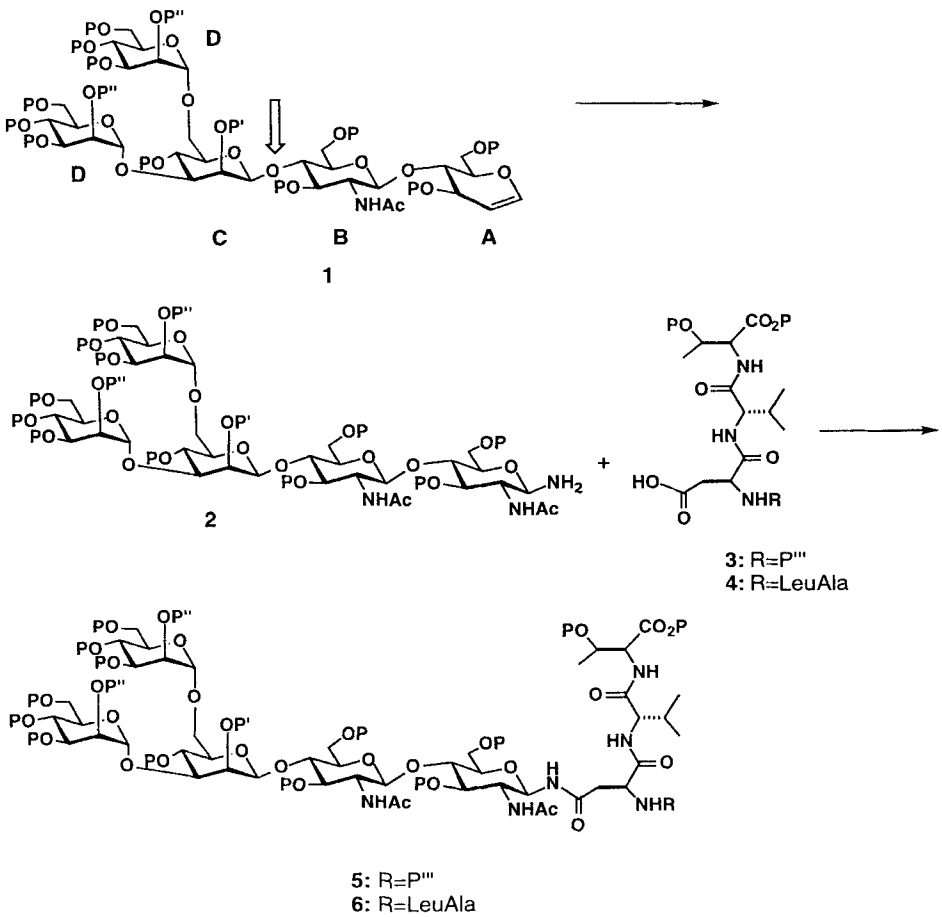


Scheme 1. Naturally occurring glycopeptide motifs. **A**: Asn-linked core pentasaccharide glycoprotein; **B**: *O*-linked glycoprotein; **C**: Asn-linked glycoprotein containing a core pentasaccharide, a lactosamine spacer, and H-type 2 blood group determinants.

Synthetic Planning: We identified a pentacyclic glycal corresponding to our key interim target, system **1** (Scheme 2). Several methods corresponding to overall "acetamidoamination" of the terminal glycal double bond, which would be necessary to progress from **1** to **2**, had already been developed in our laboratory.[15] Coupling of the anomeric amino linkage of system **2** with a complete peptide construct (see **3** or **4**) would lead to **5** or **6** and thence to deprotected glycopeptide. At this highly tentative and preliminary level of planning, we deliberately avoid specification of the precise character of the myriad of protecting groups. However, we do draw attention to one feature of the protecting group problem, which, while not critical per se to the construction of the high-mannose core, has considerable implications for building more ambitious biorelevant domains from this signature core (see Scheme 1, structure **C**). The two mannose moieties in **1** are, ideally, equipped with unique protecting groups at their C2 axial hydroxyl groups. It is at these hydroxyl groups that important extensions such as blood group determinants or tumor antigens are to be mounted to mimic the natural presentation, as illustrated in Scheme 1.



Scheme 2. Strategy for the planned synthesis of the pentasaccharide moiety.

Not surprisingly,[16] glycal building blocks would also be employed as our components en route to high-mannose glycal **1**. From the standpoint of synthesis, the most interesting union point in target **1** is the one which joins the B and C rings. The "central" mannose, ring C, is β-linked to the 4′ hydroxyl of the prechitobiose AB system (see bold arrow). Provision for placement of a

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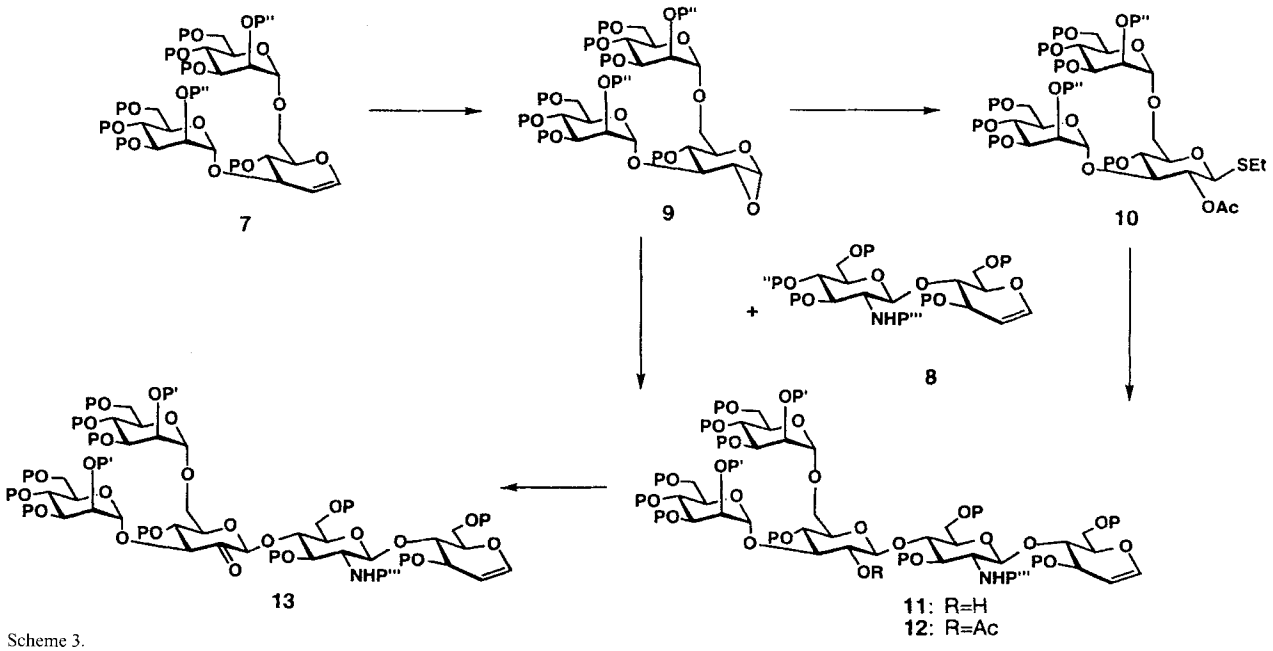
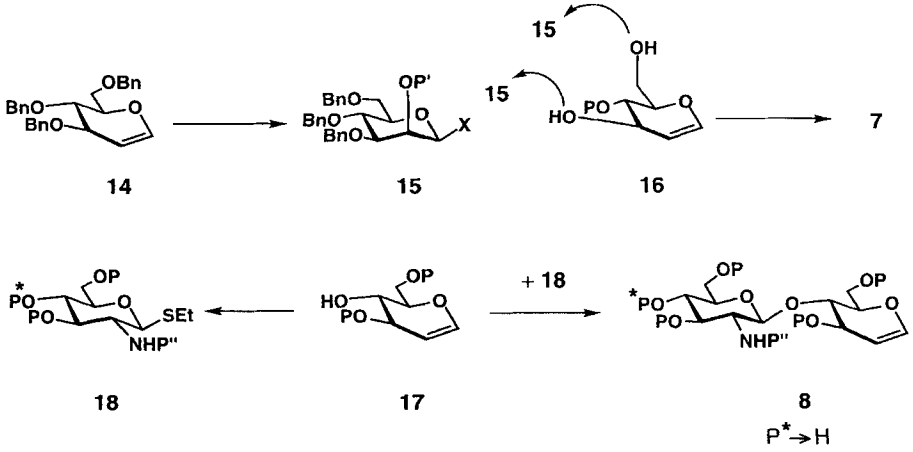


Scheme 3.

β-mannose linkage in the midst of a complex carbohydrate ensemble through a convergent coupling protocol loomed as the defining challenge of the synthesis.

The C–D β-mannose problem can be formulated with somewhat greater specificity by recourse to hypothetical subunits **7** and **8** (Scheme 3). The former corresponds to a dimannosylated glucal (which is of course precursor to a dimannosylated "mannal"). Hypothetical building block **8** corresponds to a "chitobiose glucal" with the C4′ hydroxyl acceptor site suitably differentiated. The prospect of convergent assembly of **1** through the melding of fragments such as **7** and **8** seemed so attractive that we were prepared to assume some risks in attempting it. The idea was to convert **7** to a reliable β-glucoside donor. The donor capacity would have to be equal to the task of glycosylating what appeared to be a seriously hindered C4′ acceptor site of **8** (see asterisk in Scheme 4). Another condition also had to be met. Even if coupling to a β-glucoside could be achieved, a free C2 hydroxyl of the ring C glucose had to be clearly distinguished (see **11**) and had to be responsive to overall inversion en route to **1**. We were not optimistic as to the possibility of introducing a C2 axial hydroxyl group required for **1** through direct displacement of the equatorial hydroxyl function of **11** (see asterisk in **42**, Scheme 8). Rather, we were drawn to the possibility of oxidation of this single hydroxyl group and reduction of the resultant ketone **13** to approach the structure of **1**. Still on the level of thought experiments, we wondered about the usefulness of glycal epoxide **9** as a glucosidation partner for **8**. On careful consideration of precedents,[16] we had little reason to be sanguine about the prospects of such a coupling. Here, however, we retained an obvious backup option, the conversion of **9** to a donor of proven value with hindered acceptors (thioacetate donor **10**).[17] It was appreciated that coupling product **11**, which would arise from **9**, would already have the differentiated hydroxyl at C2 of glucose unit C (**9** → **13** → **1**). Alternatively, a unique C2 acetate function in a coupling product of the type **12** arising from a "thioacetate" donor **10** could be exploited to reach **11** on the way to **13** and **1**.

Several attractive routes to **7** and **8** from glycals could be entertained. Perhaps commercially available tribenzylglucal (**14**) could be converted to a suitable mannosyl-type donor (**15**, wherein the precise anomeric and C2 protecting functions are, for the moment, unspecified). Coupling of **15** with a monoprotected glucal **16** would give rise to the desired **7**. To fashion the chitobiose glycal **8** we would employ 3,6-protected glucal **17** in two stages. We felt confident that glucal **17** would be converted to **18** bearing a uniquely dischargeable group at C4. Coupling of **18** with **17** followed by deprotection to give the C4′ hydroxyl would lead to a new acceptor system corresponding to **8**, poised to couple to **7** as discussed above.



Scheme 4. The asterisk denotes the hindered C4′ acceptor site of **8**.

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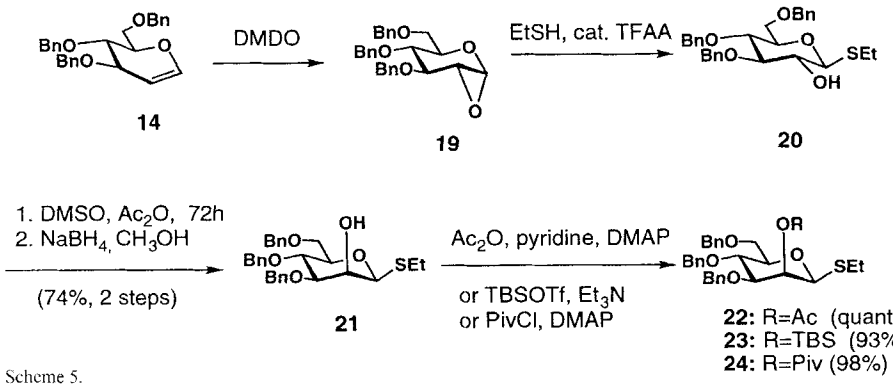
Results and Discussion

We first describe efforts which were directed toward the synthesis of a specified and viable version of **7**. We began by developing a route from the commercially available tribenzyl glucal **14** to an α-mannosyl donor (Scheme 5). Reaction of **14** with dimethyldioxirane generated epoxide **19**. The latter afforded the β-thioethylglucoside **20** upon treatment with ethanethiol in the presence of a catalytic amount of trifluoroacetic anhydride. It will be noted that the efficiency and stereoselectivity of this transformation were both much improved relative to our original protocol.[18] Moffat-like oxidation of **20**[19] followed by reduction of the resultant ketone with sodium borohydride afforded **21** and thence the acetate **22**, the silyl ether **23**, or the pivaloate **24**. As will be appreciated from the previous discussion (see also Scheme 1) donor **23** was the most attractive agent in terms of the overall design of the synthesis. The course which was followed will be explained as we describe the synthesis and use of our ring C construct.

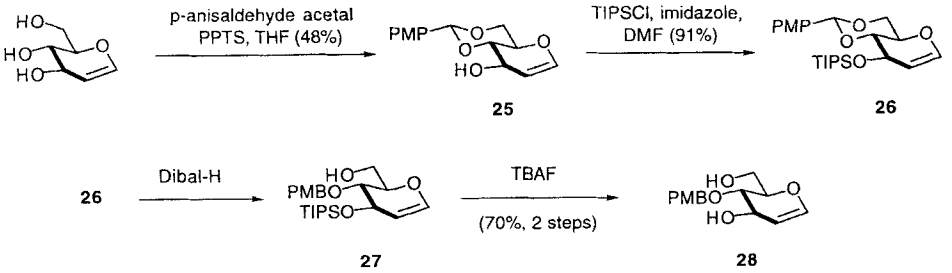


Scheme 5.

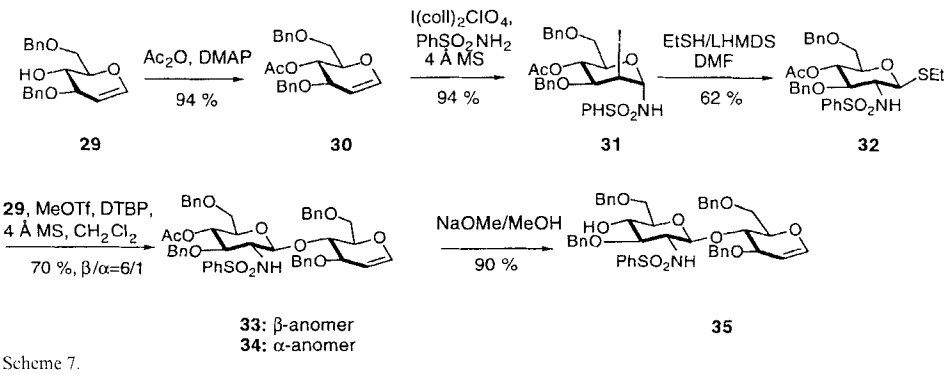
We next turned to the preparation of a specific version of **16** (Scheme 6). This pyranose would accept two α-mannose units at carbons 3 and 6, and eventually act as a β-mannose donor. We began with glucal itself, as previously described. This compound could be converted to its *p*-methoxybenzylidene derivative **25** (70%) and thence to the triisopropylsilyl derivative **26**. The acetal linkage was exposed to reductive cleavage by Dibal-H[20] to afford **27** cleanly, which upon desilylation gave rise to **28**.

We then turned to the synthesis of a specific chitobiose-equivalent glycal corresponding to the generalized structure **8** (Scheme 7). For this purpose, we started with 3,6-dibenzylglucal **29** as our specific version of **17**. Acetylation of the free hydroxyl of **29** gave **30**. This compound gave rise to **31** upon iodosulfonamidation as shown; **31**, after loss of iodide, rearrangement of the sulfonamide and thiolysis at the anomeric center, provided **32**. The original glucal, **29**, was to serve as the acceptor function in a coupling reaction with donor **32**. In the event, glycosylation was achieved with methyl triflate as the promoter, as shown, to provide chitobiose-equivalent glycal **33**. This reaction also produced the α-AB glycoside **34** in ca. 10% yield. Removal of the C4′ protecting group yielded the chitobiose acceptor **35**. The stage was set for us to obtain high-mannose-equivalent glycal type **1** by assembling building blocks **23**, **28**, and **35**.

Twofold coupling of **23** with diol **28** was achieved under mediation with methyl triflate to provide a 54% yield of the desired product **36**; in addition, other stereoisomers were formed during the glycosylation reaction. Use of donor **22** resulted in low yields of the desired product (30%) and undesired orthoester products (cf. 50%). Much improved yields of the dimannosylation reaction were achieved (63% of **37**) by the use of pivaloate donor **24**, in which the neighboring group can participate and which does not form orthoesters.[21] However, for the purposes of the synthesis described here, we carried on with trisaccharide **36** (from **23**), in which the blocking groups were more easily differentiated. We thus had in hand a compound carrying the two flanking α-mannosyl groups, properly joined to a latent core β-linked mannose matrix. It will not escape notice that the flanking mannose residues carry uniquely deprotectable silyl groups at the respective C2 axial hydroxyl centers. As discussed earlier, and as seen in Scheme 1, smooth access to these two axial alcohols is necessary to equip the high-mannose region with its biological determinants (see structure **C**).

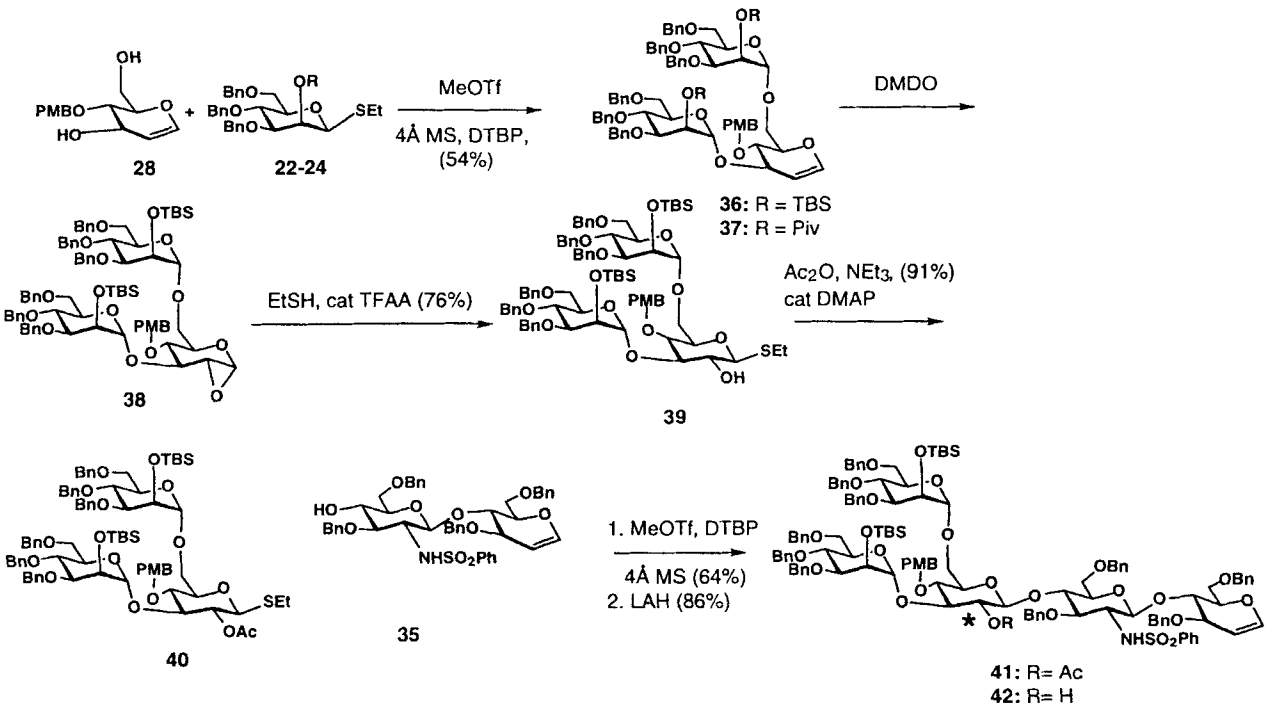


Scheme 6.



Scheme 7.

0947-6539/97/0310-1620 $ 17.50+.50/0 *Chem. Eur. J.* **1997**, *3*, No. 10

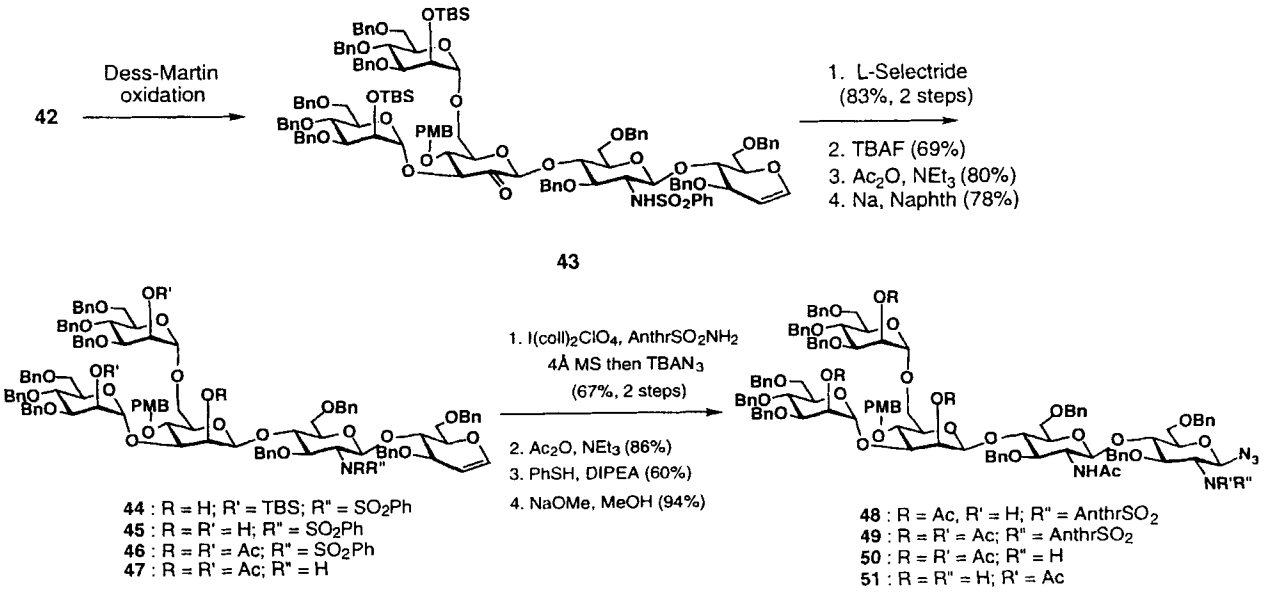


Scheme 8. The asterisk denotes the C2 site to be inverted.

We now confronted the key issue in our approach, construction of the β-mannoside linkage connecting rings B and C by the merger of a donor derived from **36** with **35**. Epoxidation of the glycal double bond of **36** was smoothly accomplished with dimethyldioxirane to afford **38** (Scheme 8). We expended a great deal of effort on the attempted direct coupling of **35** and **38**. The occurrence of coupling to only a minute extent, if at all, was suggested by examination of the crude products arising from such efforts. These negative findings were not surprising, since direct coupling of α-epoxides of type **38** with complex C4-based hindered acceptors like **35** has always been problematic.

Fortunately, the means to solve this problem were already known to us from other studies.[16] In this particular case, thiolysis of **38** as shown afforded **39** and thence, by acetylation, **40**. It was hoped that the acetate group at C2 would direct a glycosyl acceptor such as **35** to produce a β-glucoside. Indeed, coupling was accomplished under mediation by methyl triflate, as shown, to give **41** in 64% yield.

We could reductively deacetylate the unique ester at C2 of the C-ring (asterisk in **41**) to afford **42**. Moreover, Dess–Martin oxidation[22] of the unique equatorial alcohol afforded the unstable ketone **43** (Scheme 9). Reduction of this ketone function with L-selectride[23] gave rise to **44** in 83% yield. To our knowl-



Scheme 9.

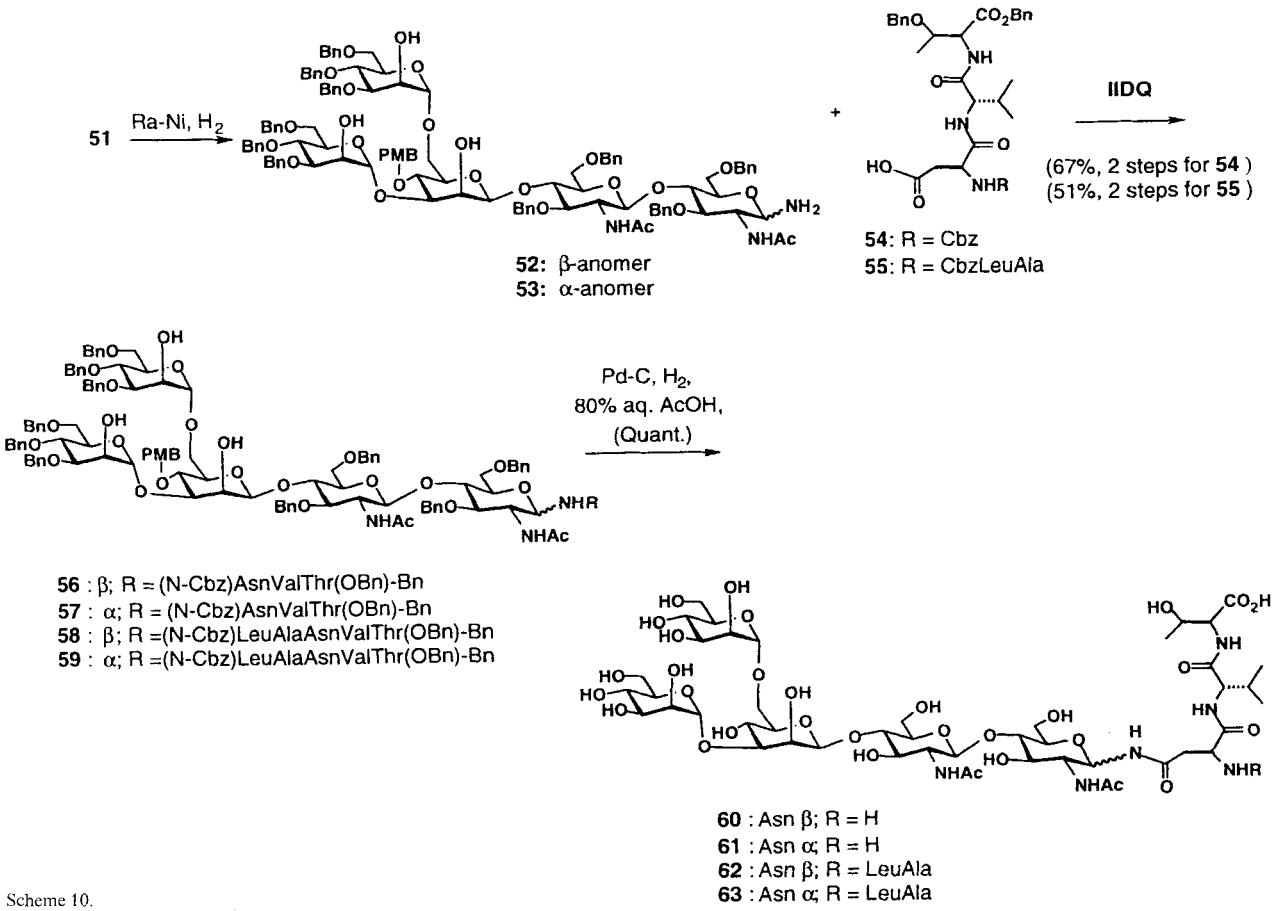
edge *this is the most complex setting in which a β-mannoside has been fashioned by inversion (through an oxidation–reduction sequence) of a β-glycoside.*[24]

Having demonstrated that we could distinguish the two axial centers on the flanking mannoses (rings D and E) from the one in the core C ring, we decided to regularize these blocking groups for the more limited purpose at hand. The two silyl protecting groups of **44** were cleaved and the resulting triol **45** was triacetylated to give rise to **46** (Scheme 9).

Compound **45** was to function in the capacity envisioned for the generalized system **1**. The next phase called for progression to a functional version of **2** en route to convergent coupling to mature peptide. In this regard, efforts were first directed to the benzenesulfonamido group of the B ring. The NH group of this linkage underwent ready acetylation with acetic anhydride to give compound **46** in 80% yield. This acetylation enabled smooth reductive removal of the sulfonamide, resulting in a 78% yield of **47**.

Attention could now be focused on upgrading the terminal glycal of **47**. Iodoanthracenesulfonamidation[16] of this linkage was followed by azidolytic rearrangement[15] generating **48** in 67% overall yield. Acetylation of the sulfonamido NH group gave rise to **49**, which was subjected to the reducing action of thiophenol in the presence of diisopropylethylamine. This sequence resulted in the formation of **50** in 60% yield. The three acetate groups were cleaved through the action of sodium methoxide leading to **51**. Assuming successful coupling to peptide could be accomplished, only debenzylation would be required to reveal the full carbohydrate domain of the resultant glycopeptide construct.

Efficient reduction of an anomeric azido sugar in a complex setting such as a high-mannose domain has long been an unsolved problem in glycopeptide synthesis.[25] Among the competing processes are anomerization of the resultant amine and *trans* acylation through the neighboring acetamido linkage. Raney nickel was recently reported by Kunz and coworkers[26] to act as a mild hydrogenation catalyst for reducing *O*-acetyl-2-acetamido-2-deoxy-β-azido sugars to yield, exclusively, β-anomeric aminosugars in quantitative yield. Using simpler model donors, we had been able to achieve reduction of anomeric azide and coupling to mature tri- or pentapeptides **54** and **55** (Scheme 10) quite smoothly with highly selective formation of β-asparagine-linked glycopeptide.[27] Furthermore, this type of goal had been accomplished very smoothly in solid-phase work.[12] However, in the complex case at hand, serious complications were to arise. Reduction of azidopentasaccharide **51** was conducted under Raney-nickel conditions with or without triethylamine. The presence of triethylamine had been believed to be useful in curtailing anomerization of the amino sugar.[28] We next undertook convergent coupling of the amino sugar, which was not fully characterized, with either tripeptide **54** or pentapeptide **55** under the agency of 2-isobutoxy-1-isobutoxycarbonyl-1,2-dihydroquinoline (IIDQ).[29] Coupling occurred smoothly, but slowly. Unfortunately, the products were glycopeptide mixtures **56**:**57** and **58**:**59** in 67 and 51% yields, respectively. Thus, either in the course of reduction of azide **51**



Scheme 10.

0947-6539/97/0310-1622 $ 17.50+.50/0 *Chem. Eur. J.* **1997**, *3*, No. 10

or during the course of coupling of what we had expected to be the resultant amine **52** with peptide, equilibration at the reducing end had occurred, leading to a mixture of **52** and **53**. On acylation with the γ-carboxyl group of the aspartic acid function of **54** or **55**, mixtures **56**/**57** and **58**/**59** were obtained.

Following separation of mixture **56**/**57** to its homogeneous components, global deprotection of **56** and **57** afforded glycopeptides **60** and **61**. The structures of both anomers were confirmed by ^{1}H NMR spectroscopy and high-resolution mass spectrometry. The region from $\delta = 5.2-4.5$ reveals 5 resonances corresponding to the anomeric protons, containing two doublets of the β-GlucNac linkages. The set of two multiplets at $\delta = 2.8$ and 2.6 confirms the presence of β-Asn protons. The ^{1}H NMR spectrum of β-pentasaccharide glycopeptide **60** was compared to data obtained by Ogawa and coworkers[8c] for an identical glycopeptide synthesized by a different strategy. The magnetic resonance spectral data are virtually identical, and confirm that the structure of **60** is that shown.

Global deprotection of **58**/**59** led to the fully deprotected material **62**/**63**. Unfortunately, these compounds could not be separated, but mass spectral and high-field NMR analysis (500 MHz) revealed the presence of approximately a 2:1 (β:α) ratio of the components.

Summary

In conclusion, a novel and reasonably efficient synthesis of the high-mannose carbohydrate domain of glycopeptides has been accomplished (see compound **51**). This construction owed much of its convergence to the melding of key building blocks through the logic of glycal assembly.[16] A key feature of the synthesis was the coupling of AB acceptor glycal **35** with CDE thioethyl donor **40**, itself derived from high-mannose trisaccharide glycal **36**. Coupling of **35** and **40** cleanly provided the β-glucoside, which was subsequently converted to the C ring β-mannoside (compound **44**) by inversion through oxidation–reduction of the hydroxylic center (**42** → **44**). Another important finding was that the terminal glycal linkage of compound **47** could be brought to the stage of azidoacetamide **51** in anticipation of glycopeptide formation. The closing phase of the synthesis underscored what is still a serious problem in the field, reduction of a β-linked anomeric azide in a highly complex high-mannose-type setting. We have not yet produced functionally pure anomeric amine corresponding to **54** with survival times consistent with coupling to a complex peptide.

This setback should not obscure the accomplishment of the first totally convergent union of a synthetically derived complex carbohydrate with fully mature peptide. Arrangements allowing for global deprotection were achieved (see compound **57**). As such, these experiments provide the basis for future efforts directed at solution of the remaining stereochemical problem, as well as the extension of the carbohydrate domain to encompass inclusion of important biological markers. It is anticipated that such studies will progress both by solution and solid-phase methodology. Earlier work[12] suggests that the anomerization problem of systems such as **52** may be more readily dealt with in a context where they are linked to an insoluble support.

Experimental Procedure

Infrared spectra were recorded on a Perkin–Elmer 1600 series FTIR. ^{1}H NMR spectra were obtained on a Bruker AMX 400 (400 MHz) and are reported in parts per million (δ) relative to either trimethylsilane ($\delta = 0.00$) or $CHCl_3$ ($\delta = 7.26$) for spectra run in $CDCl_3$. Coupling constants (J) are reported in Hertz. ^{13}C NMR spectra were obtained on a Bruker AMX 400 (100 MHz) and are reported in δ relative to $CDCl_3$ ($\delta = 77.00$) or CD_3OD ($\delta = 49.05$) as internal reference. Mass spectra were recorded on a Perkin–Elmer SCIEX API 100 mass spectrometer. High-resolution mass spectra were recorded on a JEOL JMS-DX-303 HF mass spectrometer. Optical rotations were measured on a Jasco DIP-370 polarimeter using a 0.5 dm cell at the reported temperatures and concentrations (g dL^{-1}).

Chemicals were reagent grade and used without further purification unless otherwise noted. Tetrahydrofuran (THF) was distilled from sodium benzophenone ketyl under N_2 atmosphere. Dichloromethane (CH_2Cl_2) and benzene were distilled from calcium hydride under N_2. Analytical thin-layer chromatography was performed on E. Merck silica gel 60 F_{254} plates (0.25 mm thickness). Compounds were visualized by dipping the plates in a cerium sulfate/ammonium molybdate solution followed by heating. Column chromatography was performed with forced flow of the indicated solvents on Sigma H-type silica (10–40 μm).

Synthesis of 3,4,6-tribenzyl-1-thioethyl β-glucopyranoside (20): Tribenzyl glucal **14** (10.0 g, 24.0 mmol) was dissolved in CH_2Cl_2 (50 mL) and cooled to 0 °C. Dimethyldioxirane (360 mL in acetone, 28.8 mmol) was added and stirred for 20 min. The solvent was removed in a stream of N_2. After drying for 30 min under vacuum, the epoxide was dissolved in CH_2Cl_2 (20 mL), and EtSH (17.8 mL, 240 mmol) was added. The mixture was cooled to −78 °C and trifluoroacetic acid anhydride (300 μL) was added dropwise. The reaction mixture was stirred at −78 °C for 20 min and then warmed up to room temperature. The solvents were removed in a stream of N_2 and the residue was purified by silica gel chromatography to afford 9.29 g (78%) of **20**. $[\alpha]_D^{24} = -12.8$ (c 2.05, CH_2Cl_2); IR (thin film): $\tilde{\nu}$ = 3458, 1496, 1453, 1359, 1054 cm^{-1}; ^{1}H NMR ($CDCl_3$): $\delta = 7.31-7.16$ (m, 13H), 7.11–7.08 (m, 2H), 4.86 (d, $J = 11.3$ Hz, 1H), 4.77 (d, $J = 11.3$ Hz, 1H), 4.76 (d, $J = 12.1$ Hz, 1H), 4.22 (d, $J = 9.1$ Hz, 1H), 3.67 (dd, $J = 1.8$, 10.9 Hz, 1H), 3.61 (dd, $J = 4.5$, 10.9 Hz, 1H), 3.56–3.39 (m, 4H), 2.70–2.60 (m, 2H), 1.24 (d, $J = 4.0$ Hz, 3H); ^{13}C NMR ($CDCl_3$): δ = 138.6, 138.2, 138.0, 128.4, 128.3, 127.9, 127.9, 127.7, 127.7, 127.5, 86.1, 86.0, 79.4, 75.2, 75.0, 73.4, 73.2, 69.0, 24.2, 15.4; HRMS (FAB) calcd for $C_{29}H_{34}O_5S$: 494.2127, found: 494.2136.

Synthesis of 2-acetyl 3,4,6-tribenzyl thioethyl mannoside (22), 2-*tert*-butyldimethylsilyl 3,4,6-thioethyl mannoside (23) and **2-pivaloyl 3,4,6-tribenzyl thioethyl mannoside (24)**: Thioglucoside **20** (9.29 g, 18.7 mmol) was treated with DMSO/Ac_2O (100 mL/50 mL) for three days at room temperature. It was then diluted with ether (100 mL) and washed with H_2O (5 × 200 mL), saturated aqueous Na_2CO_3 (3 × 200 mL) and saturated aqueous NaCl. The crude ketone was dried with $MgSO_4$ and concentrated to dryness. It was then dissolved in CH_2Cl_2/MeOH (60 mL/60 mL) and cooled to 0 °C. $NaBH_4$ (2.13 g, 37.8 mmol) was added in several portions. The reaction mixture was allowed to warm up to room temperature and 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 aqueous $NaHCO_3$ (200 mL), saturated aqueous NaCl (300 mL) and dried ($MgSO_4$). The crude material was purified by silica gel chromatography (15% EtOAc/hexanes) to afford 7.05 g (76%) of the desired 3,4,6-tribenzyl thioethyl mannoside (**21**).

Compound **21** (770 mg, 1.56 mmol) was treated for 1.5 h with pyridine/Ac_2O (4 mL/2 mL) in the presence of DMAP (50 mg). After evaporation to dryness, the residue was purified by silica gel chromatography (20% EtOAc/hexanes) to afford 834 mg (99.8%) of **22**. $[\alpha]_D^{24} = -73.6$ (c 5.94, CH_2Cl_2); IR (thin film): $\tilde{\nu}$ = 1743, 1496, 1453, 1372, 1229, 1109 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.35-7.27$ (m, 13H), 7.19–7.17 (m, 2H), 5.65 (dd, $J = 0.9$, 3.1 Hz, 1H), 4.86 (d, $J = 11$ Hz, 1H), 4.77 (d, $J = 11$ Hz, 1H), 4.67 (d, $J = 0.9$ Hz, 1H), 4.62 (d, $J = 12.0$ Hz, 1H), 4.56 (d, $J = 12.0$ Hz, 1H), 4.52 (d, $J = 10$ Hz, 1H), 4.49 (d, $J = 10$ Hz, 1H), 3.79 (dd, $J = 1.9$, 10.9 Hz, 1H), 3.75–3.67 (m, 3H), 3.52 (ddd, $J = 1.9$, 5.6, 8.9 Hz, 1H), 2.75 (dd, $J = 7.5$, 14.7 Hz, 1H), 2.74 (dd, $J = 7.5$, 14.7 Hz, 1H), 2.20 (s, 3H), 1.30 (t, $J = 7.5$ Hz, 3H); ^{13}C NMR ($CDCl_3$): δ = 170.4, 138.2, 138.1, 137.5, 128.3, 128.2, 128.1, 127.8, 127.7, 127.6, 127.4, 82.1, 81.4, 79.8, 75.1, 74.1, 73.3, 71.6,

  0947-6539/97/0310-1623 $ 17.50+.50/0

69.6, 69.5, 25.5, 20.8, 15.0; HRMS (FAB) calcd for $C_{31}H_{36}O_6S$: 536.2232, found: 536.2238.

Compound **21** (500 mg, 1.01 mmol) was dissolved in 5 mL CH_2Cl_2, cooled to 0 °C, triethylamine (2.81 mL, 20.2 mmol) and TBS triflate (464 μL, 2.02 mmol) were added dropwise. The reaction was warmed up and stirred at room temperature for 3 h. The reaction was diluted with EtOAc (100 mL), washed with saturated aqueous $NaHCO_3$ and brine, dried over $MgSO_4$, and concentrated. Purification by silica column chromatography yielded 570 mg (93%) of **23**. $[\alpha]_D^{24} = -36.1$ (*c* 4.09, CH_2Cl_2); IR (thin film): $\tilde{\nu}$ = 3087, 2926, 2916, 1496, 1463, 1362, 1251, 1102, 1027, 966, 834 cm^{-1}; ^1H NMR ($CDCl_3$): δ = 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, 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* = 7.4, 7.3 Hz, 3H), 0.98–0.96 (m, 9H), 0.20–0.13 (m, 6H); ^{13}C NMR ($CDCl_3$): δ = 137.31, 136.93, 136.76, 126.96, 126.94, 126.82, 126.77, 126.41, 126.29, 126.22, 125.93, 83.93, 82.65, 78.84, 73.73, 73.09, 71.77, 71.33, 71.05, 68.07, 24.76, 24.29, 17.32, 13.83, −4.93, −5.35, −5.722; HRMS calcd for $C_{35}H_{48}O_5S_tSi_1Na$: 631.2889, found: 631.2883.

Compound **21** (500 mg, 1.01 mmol) was dissolved in 5 mL CH_2Cl_2, and DMAP (0.24 g, 2.02 mmol) and pivaloyl chloride (190 μL, 1.53 mmol) were added. The reaction mixture was stirred at room temperature for 30 min, diluted with EtOAc (100 mL), washed with saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4 and concentrated. Purification by silica column chromatography yielded 574 mg (98%) of **24**. $[\alpha]_D^{24} = -0.32$ (*c* 1.04, CH_2Cl_2); IR (thin film): $\tilde{\nu}$ = 2968, 2868, 1732, 1454, 1363, 1281, 1154, 1109, 736 cm^{-1}; ^1H NMR ($CDCl_3$): δ = 7.41–7.25 (m, 13H), 7.22–7.18 (m, 2H); 5.66 (d, *J* = 3.1 Hz, 1H), 4.87 (d, *J* = 10.8 Hz, 1H), 4.79 (d, *J* = 11.81 Hz, 1H),4.70 (s, 1H), 4.69 (d, *J* = 11.2 Hz, 1H), 4.61, (d, *J* = 12.1 Hz, 1H), 4.57 (d, *J* = 10.8 Hz, 1H), 4.51 (d, *J* = 11.1 Hz, 1H), 3.85–3.67 (m, 4H), 3.54 (m, 1H), 2.77 (q, *J* = 7.4 Hz, 1H), 1.33 (t, *J* = 7.4 Hz, 3H), 1.30 (s, 9H); ^{13}C NMR ($CDCl_3$): δ = 177.6, 138.5, 138.1, 137.8, 128.3, 128.2, 128.2, 128.1, 127.7, 127.6, 127.4, 82.3, 81.6, 79.7, 75.2, 74.0, 73.2, 71.4, 69.3, 39.1, 27.2, 25.7, 15.0.

Synthesis of 4-*p*-methoxybenzyl-3-triisopropylsilyl-D-glucal (27): Protected glucal **26**[18] (5.030 g, 11.96 mmol) was dissolved in 100 mL anh. CH_2Cl_2 and cooled to −20 °C. Dibal-H solution (1.0 M in toluene, 47.8 mL, 47.8 mmol) was added slowly and stirred for 3 h. The reaction was quenched by addition of 3 mL methanol. The reaction mixture was diluted with 50 mL EtOAc, and 50 mL 20% aqueous K–Na tartrate was added. The mixture was stirred for 2 h and extracted with EtOAc (4 × 30 mL). The combined organic fractions were washed with brine, dried over Na_2SO_4, and concentrated. Purification by silica column chromatography yielded 4.260 g (84.3%) of **27**. $[\alpha]_D^{24} = -40.2$ (*c* 5.93; CH_2Cl_2); IR (thin film): $\tilde{\nu}$ = 3441, 2941, 2865, 1646, 1613, 1513, 1463, 1247, 1098, 1036, 881, 819, 680 cm^{-1}; ^1H NMR ($CDCl_3$): δ = 7.26 (d, *J* = 8.6 Hz, 2H), 6.86 (d, *J* = 8.6 Hz, 2H), 6.37 (d, *J* = 6.2 Hz, 1H), 4.79 (m, 1H), 4.67 (AB q, *J* = 11.2 Hz, 2H), 4.34 (m, 1H), 4.06 (m, 1H), 3.82 (m, 1H), 3.79 (s, 3H), 3.64 (m, 1H), 1.06 (s, 9H); ^{13}C NMR ($CDCl_3$): δ = 159.35, 143.55, 130.00, 129.53, 113.85, 102.59, 76.44, 76.42, 72.60, 66.77, 61.84, 55.20, 18.04, 12.69; FAB(+)MS: 448, 441, 421, 391, 369, 301, 285, 249, 241; HRMS calcd for $C_{23}H_{38}O_5NaSi$: 445.2386, found: 445.2377.

Synthesis of 4-*p*-methoxybenzyl-D-glucal (28): Glucal **27** (3.350 g, 7.927 mmol) was dissolved in 70 mL anh. THF, and a 1.0 M solution of TBAF in THF was added (11.89 mL, 11.89 mmol) and stirred for 14 h at room temperature. The solvent was evaporated and the remaining residue was purified by silica column chromatography to afford 1.675 g (79.3%) of **28** as a white waxy solid. $[\alpha]_D^{24} = +15.3$ (*c* 2.86; CH_2Cl_2); IR (thin film): $\tilde{\nu}$ = 3285, 2955, 2933, 2836, 1649, 1613, 1514, 1232, 1173, 1086, 952, 814, 761 cm^{-1}; ^1H NMR ($CDCl_3$): δ = 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 ($CDCl_3$): δ = 159.38, 144.05, 130.23, 129.67, 114.00, 103.34, 77.42, 76.59, 73.40, 71.57, 68.92, 61.67, 55.24; HRMS calcd for $C_{13}H_{16}O_5Na$: 275.0895, found: 275.0889.

Synthesis of disilyl trisaccharide glycal 36: A mixture of 2-*tert*-butyldimethylsilyl 3,4,5-tribenzyl thioethyl mannoside (**23**, 9.33 g, 15.32 mmol) and acceptor **28** (1.13 g, 4.26 mmol) was azeotroped with benzene (3 × 50 mL) and dried under vacuum for 1 h. Freshly dried 4 Å molecular sieves (20 g) were added in a glove bag; CH_2Cl_2 and di-*tert*-butylpyridine (14.1 g, 73.5 mmol) were added to the mixture, which was then stirred at room temperature for 30 min, after which MeOTf (10 g, 61.3 mmol) was added slowly. After being stirred at RT for 4 h, the reaction mixture was diluted with EtOAc (500 mL) and washed with saturated aqueous $NaHCO_3$ (100 mL) saturated aqueous NaCl (100 mL) and dried ($MgSO_4$). Purification by silica gel chromatography (10% EtOAc/hexanes) afforded 3.11 g (54%) of **36** plus the other three anomeric isomers. $[\alpha]_D^{24} = +24.4$ (*c* 0.55, $CHCl_3$); IR (film): $\tilde{\nu}$ = 3030, 2926, 2855, 1648, 1514, 1454, 1380, 1249, 1043, 835, 777 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 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* = 30.0, 11.9 Hz, 4H), 4.51 (dd, *J* = 10.8, 3.6 Hz, 1H), 4.36 (dt, *J* = 7.1, 1.7 Hz, 1H), 4.13 (t, *J* = 2.4 Hz, 1H), 4.02–3.82 (8H, m), 3.78–3.63 (m, 7H), 3.73 (s, 3H), 0.897 (br s, 18H), 0.10 (s, 3H), 0.06 (s, 3H), 0.05 (s, 3H), 0.01 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 159.28, 144.17, 138.62, 138.48, 138.37, 130.05, 129.22, 128.31, 128.24, 128.20, 128.18, 128.15, 128.05, 127.60, 127.55, 127.40, 127.36, 127.32, 127.24, 113.87, 102.64, 102.27, 101.24, 79.99, 79.91, 79.16, 75.08, 75.04, 74.85, 74.65, 74.00, 73.12, 73.02, 72.62, 72.35, 72.11, 70.35, 69.55, 69.41, 69.22, 65.66, 55.23, 25.75, 25.73, 18.13, −0.01, −4.49, −4.78, −4.82; FAB(+)MS 1400, 1382, 804, 728; HRMS calcd for $C_{80}H_{102}O_{15}NaSi_2$: 1381.6650, found: 1381.6670.

Synthesis of dipivaloyl trisaccharide glycal 37: A mixture of 2-pivaloyl 3,4,5-tribenzyl thioethyl mannoside (**24**, 310 mg, 0.536 mmol) and acceptor **28** (46 mg, 0.172 mmol) was azeotropically distilled with benzene (3 × 50 mL) and dried under vacuum for 1 h. Freshly dried 4 Å molecular sieves (300 mg) were added in a glove bag. Into the mixture was added CH_2Cl_2 and di-*tert*-butylpyridine (0.48 mL, 12.4 mmol). The mixture was stirred at room temperature for 30 min, and MeOTf (0.24 mL, 12.4 mmol) was added slowly. After being stirred at 0 °C for 24 h, the reaction mixture was diluted with EtOAc (500 mL) and washed with saturated aqueous $NaHCO_3$ (100 mL) and saturated aqueous NaCl (100 mL), and dried ($MgSO_4$). Purification by silica gel chromatography afforded 140 mg (63%) of **37**. $[\alpha]_D^{24} = +0.3$ (*c* 2.72, $CHCl_3$); IR (film): $\tilde{\nu}$ = 3030, 2970, 2870, 1732, 1649, 1514, 1454, 1363, 1279, 1047, 835, 777 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 7.38–7.12 (m, 32H), 6.81 (d, *J* = 8.6 Hz, 1H), 6.21 (d, *J* = 6.1 Hz, 1H), 5.45 (m, 1H), 5.36 (m, 1H), 5.10 (s, 1H), 4.92–4.82 (m, 4H), 4.84 (d, *J* = 10.8 Hz, 2H), 4.79 (d, *J* = 2.0 Hz, 1H), 4.78 (d, *J* = 11.7 Hz, 1H), 4.76–4.62 (m, 5H), 4.57–4.46 (m, 7H), 4.49–4.34 (m, 1H), 4.05–3.66 (m, 18H), 1.22 and 1.21 (2s, 17H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 177.50, 159.3, 144.5, 138.4, 138.3, 138.1, 129.8, 129.7, 129.5, 128.3, 128.2, 128.2, 128.1, 128.1, 128.0, 127.6, 127.5, 127.4, 127.3, 127.3, 127.2, 114.0, 101.5, 99.1, 98.3, 78.2, 79.9, 79.1, 75.0, 75.0, 74.8, 74.6, 74.0, 73.1, 73.0, 72.6, 72.3, 72.1, 68.5, 67.9, 65.8, 55.2, 38.9, 27.1, 25.4.

Synthesis of trisaccharide alcohol thioglycoside 39: Disilyl trisaccharide glycal **36** (3.82 g, 2.80 mmol) was dissolved in CH_2Cl_2 (20 mL) and cooled to 0 °C. Dimethyldioxirane (102 mL in acetone, 3.36 mmol) was added slowly over 40 min. The reaction mixture was maintained at 0 °C for 20 min and concentrated at low temperature (≈10 °C). The residue was azeotropically distilled with benzene (2 × 50 mL) and further dried under vacuum for 20 min. It was then dissolved in CH_2Cl_2 (8 mL), and EtSH (6.96 g, 112 mmol) was added. The mixture was cooled down to −78 °C and trifluoroacetic anhydride (59 mg, 0.28 mmol) was added. The reaction mixture was then allowed to warm up to room temperature and the solvent was removed in a stream of N_2. The residue was purified by silica gel chromatography to afford 3.06 g (76%) of **39**. $[\alpha]_D^{24} = 12.8$ (*c* 0.70, $CHCl_3$); IR (film): $\tilde{\nu}$ = 3472, 2927, 2856, 1613, 1514, 1454, 1360, 1250, 1137, 1092, 1049, 978, 835, 777 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 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, 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, $CDCl_3$): δ = 159.29, 138.62, 138.59, 138.18, 138.16, 138.10, 128.84, 128.32, 128.27, 128.19, 128.14, 128.04, 127.98, 127.67, 127.56, 127.49, 127.47, 127.41, 127.35, 127.32, 127.20, 113.92, 102.81, 100.90, 90.83, 84.76, 78.14, 76.28, 75.05, 74.91, 74.86, 74.58, 73.17, 72.88, 72.72, 72.08, 72.01, 70.12, 69.35, 69.26, 69.08, 65.71, 55.22,

0947-6539/97/0310-1624 $ 17.50+.50/0 *Chem. Eur. J.* **1997**, *3*, No. 10

18.11, 14.97, −4.50, −4.54, −4.72, −4.90; FAB(+)MS: 1460, 1439, 1357, 963; HRMS calcd for $C_{82}H_{108}O_{16}NaSSi_2$: 1459.6790, found: 1459.6760.

Synthesis of trisaccharide acetate thioglycoside 40: Trisaccharide alcohol thioglycoside **37** (3.01 g, 2.09 mmol) was treated in CH_2Cl_2 with Ac_2O (1.07 g, 10.4 mmol), Et_3N (2.11 g, 20.9 mmol) and catalytic amount of DMAP at room temperature for 2 h. After concentration by rotary evaporation, the residue was purified by silica gel chromatography to afford 2.81 g (91%) of **40**. $[\alpha]_D^{24} = +51.7$ (*c* 0.75; CH_2Cl_2), $[\alpha]_D^{24} = +24.6$ (*c* 1.01, $CHCl_3$); IR (thin film): $\tilde{\nu} = 2927, 2855, 1750, 1613, 1514, 1454, 1361, 1249, 1225, 1050, 835, 777\ cm^{-1}$; ^1H NMR ($CDCl_3$): $\delta = 7.40-7.05$ (m, 30H), 6.75 (d, $J = 8.4$ Hz, 2H), 5.06 (brs, 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 (brm, 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 ($CDCl_3$): $\delta = 169.91, 166.33, 159.14, 138.88, 138.74, 138.60, 138.52, 138.43, 129.70, 128.30, 128.19, 128.15, 128.12, 128.07, 127.98, 127.68, 127.45, 127.33, 127.30, 127.23, 127.19, 127.16, 113.84, 102.07, 101.16, 83.47, 79.85, 79.52, 78.43, 74.89, 74.71, 74.55, 73.98, 73.20, 73.00, 72.88, 72.29, 72.20, 71.95, 71.05, 69.36, 69.21, 65.52, 60.32, 55.16, 25.72, 25.65, 23.94, 22.10, 21.19, 18.09, 18.05, 14.82, −4.45, −4.52, −4.78, −5.09; HRMS calcd for $C_{84}H_{110}O_{17}NaSSi_2Na$: 1501.6900, found: 1501.6900.

Synthesis of pentasaccharide glycal 41: Trisaccharide donor **40** (1.03 g, 0.695 mmol) and disaccharide acceptor **35**[18] (561 mg, 0.695 mmol) were combined and dried by azeotropic distillation with benzene. Activated 4 Å molecular sieves (4.0 g) were added, followed by CH_2Cl_2 (8 mL). Di-*tert*-butylpyridine (1.09 mL, 4.9 mmol) was added and stirred for 45 min. The reaction was cooled to −10 °C, and methyl triflate (0.47 mL, 4.2 mmol) was added slowly. 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. The reaction was quenched with triethylamine (2.0 mL), filtered through a SiO_2 plug, washed with $NaHCO_3$ and extracted with EtOAc. The combined organic extracts were washed with water and brine, dried over $MgSO_4$ and concentrated. Purification by silica column chromatography yielded 994 mg (64.0%) of the desired pentasaccharide **41**. $[\alpha]_D^{24} = -5.3$ (*c* 1.31; CH_2Cl_2); IR (thin film): $\tilde{\nu} = 2247, 1571, 1650, 1612, 1586, 1514, 1498, 1454, 1361, 1249, 910, 836\ cm^{-1}$; ^1H HMR ($CDCl_3$): $\delta = 7.87$ (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 (brd, $J = 1.6$ 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 HMR ($CDCl_3$): $\delta = 170.29, 159.00, 144.43, 141.84, 138.84, 138.73, 138.69, 138.62, 138.57, 138.45, 138.39, 137.92, 137.89, 137.75, 133.50, 128.61, 128.44, 128.38, 128.34, 128.25, 128.19, 128.14, 128.12, 128.09, 128.06, 128.01, 127.94, 127.68, 127.61, 127.43, 127.42, 127.34, 127.30, 127.23, 127.20, 127.18, 113.76, 101.58, 101.25, 99.99, 99.79, 99.39, 79.82, 79.52, 77.94, 75.40, 74.99, 74.74, 74.45, 74.34, 74.20, 74.03, 73.61, 73.41, 73.31, 73.26, 73.22, 73.01, 72.88, 72.81, 72.54, 72.47, 72.21, 72.13, 71.70, 70.21, 69.92, 69.07, 68.97, 67.72, 65.45, 56.19, 55.15, 25.74, 25.64, 21.14, 18.05, −4.40, −4.54, −4.60, −4.71, −5.11; FAB(+)MS: 2264, 2248, 2172, 2157, 2100; HRMS calcd for $C_{128}H_{153}NO_{27}NaSSi_2$: 2246.9786, found: 2246.9840.

Synthesis of pentasaccharide dimannosyl glycal alcohol 42: Pentasaccharide glucal acetate **41** (994 mg; 0.447 mmol) was dried by azeotroping with benzene (3 × 25 mL), then placed under high vacuum for 15 min, then dissolved in 20.0 mL of dry Et_2O and cooled to 0 °C. Lithium aluminum hydride (68 mg, 1.8 mmol) was added to the solution while it was stirred and maintained at 0 °C for 1 h. It was then quenched with saturated aqueous $NaHCO_3$ solution (10 mL) and extracted with EtOAc (3 × 15 mL). The combined organic extracts were washed with brine (10 mL), dried over $MgSO_4$, and filtered. The solvent was removed under reduced pressure and the product mixture chromatographed on SiO_2 to afford **42** as a colorless foam (842.5 mg; 86%). $R_f = 0.50$ (1:4 EtOAc/hex); $[\alpha]_D^{24} = +0.9$ (*c* 1.73; CH_2Cl_2); IR (thin film): $\tilde{\nu} = 3468, 3351, 3063, 3030, 2927, 2856, 1650, 1612, 1586, 1514, 1498, 1453, 1360, 1249, 1208, 1093, 910, 835, 735, 698\ cm^{-1}$; ^1H NMR ($CDCl_3$): $\delta = 7.75$ (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 ($CDCl_3$): $\delta = 159.13, 144.34, 141.72, 138.88, 138.62, 138.60, 138.49, 138.31, 138.17, 138.10, 137.49, 132.05, 130.00, 128.55, 128.47, 128.44, 128.38, 128.25, 128.22, 128.19, 128.14, 128.11, 128.07, 128.02, 127.92, 127.65, 127.58, 127.49, 127.43, 127.41, 127.36, 127.34, 127.28, 127.18, 127.13, 113.82, 102.97, 102.29, 101.11, 100.71, 100.30, 80.01, 79.09, 79.04, 76.83, 75.84, 75.04, 74.96, 74.79, 74.54, 74.47, 74.00, 73.82, 73.44, 73.07, 72.97, 72.92, 72.45, 72.04, 71.81, 70.41, 70.00, 69.20, 69.11, 68.98, 68.65, 67.54, 65.98, 58.65, 55.17, 25.70, 25.65, 18.03, −4.58, −4.69, −4.83, −4.95; FAB(+)MS: 2205, 2192, 2130, 2115, 2110, 1988; HRMS calcd for $C_{126}H_{151}NO_{26}NaSSi_2$: 2204.9681, found: 2204.9680.

Synthesis of pentasaccharide trimannosyl glycal alcohol 44: Dimannosyl glycal alcohol **42** (992 mg, 0.454 mmol) was azeotropically distilled with benzene (3 × 50 mL) and dried under vacuum overnight. It was then dissolved in CH_2Cl_2 (31 mL) and freshly redistilled pyridine was added (431 mg, 5.45 mmol). Dess–Martin reagent (770 mg, 1.81 mmol) was added in one portion in a glove bag. The reaction was carefully monitored by TLC and quenched with saturated aqueous NaS_2O_3 (30 mL) as soon as the reaction went to completion (4 h). The mixture was extracted with EtOAc (3 × 100 mL). The combined extract was thoroughly washed with saturated aqueous $Na_2S_2O_3$ (3 × 50 mL), followed by saturated aqueous $NaHCO_3$ (50 mL) and saturated aqueous NaCl (100 mL). After drying (Na_2SO_4) and concentration, the crude ketone **43** was azeotropically distilled with benzene (3 × 20 mL) and further dried under vacuum for 20 min. It was then dissolved in THF, and cooled to −42 °C. L-selectride (1 M in THF, 1.36 mmol) was added slowly. The reaction mixture was then allowed to warm up to room temperature and further stirred for 2 h. It was quenched with saturated aqueous $NaHCO_3$ (10 mL) and extracted with EtOAc (3 × 50 mL). The combined extracts were washed with saturated aqueous NaCl and dried ($MgSO_4$). Purification by silica gel chromatography (22% EtOAc/hexanes) afforded 817 mg (83%) of **44**. $R_f = 0.50$ (1:4 EtOAc/hex); $[\alpha]_D^{24} = -2.6$ (*c* 2.05; CH_2Cl_2); IR (thin film): $\tilde{\nu} = 3473, 3276, 1650, 1612, 1586, 1514, 1498, 1470, 1454, 1361, 1328, 1249, 1094, 910, 836, 778\ cm^{-1}$; ^1H NMR ($CDCl_3$): $\delta = 7.79$ (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 (brs, 1H), 4.93 (brs, 1H), 4.88 (brs, 1H), 4.81 (brs, 1H), 4.90–4.39 (m, 24H), 4.33–4.30 (m, 2H), 4.28 (brs, 1H), 4.19 (brs, 1H), 4.10–3.50 (m, 30H), 3.80 (s, 3H), 3.49–3.45 (m, 3H), 3.80–3.30 (m, 3H), 0.99 (s, 18H), 0.16 (s, 3H), 0.12 (s, 6H), 0.11 (s, 3H); ^{13}C NMR ($CDCl_3$): $\delta = 159.05, 144.31, 141.74, 138.80, 138.72, 138.61, 138.54, 138.19, 138.14, 138.05, 138.00, 137.74, 137.57, 132.10, 130.05, 128.67, 128.53, 128.39, 128.28, 128.21, 128.16, 128.13, 128.07, 128.04, 128.02, 127.97, 127.91, 127.55, 127.41, 127.37, 127.32, 127.26, 127.17, 127.07, 113.70, 102.20, 101.14, 100.57, 100.47, 100.13, 84.20, 80.12, 79.74, 75.88, 75.72, 75.30, 74.85, 74.52, 74.50, 73.40, 73.36, 73.14, 73.07, 72.99, 72.88, 72.76, 72.26, 70.33, 70.19, 69.78, 69.15, 69.00, 67.62, 66.48, 58.40, 55.08, 25.79, 18.05, −4.57, −4.69, −4.79, −4.96; FAB(+)MS: 2207, 2132, 2115, 1990; HRMS calcd for $C_{126}H_{151}NO_{26}NaSSi_2$: 2204.9680, found: 2204.9700.

Synthesis of pentasaccharide glycal triol 45: Silyl-protected pentasaccharide glycal **44** (82.0 mg; 0.0375 mmol) was dried by azeotropic distillation with benzene (3 × 10 mL). It was then dissolved in dry THF (3.0 mL) and treated at room temperature with TBAF (1.0 M solution in THF, 0.15 mL; 0.15 mmol) while stirring under inert atmosphere. After 14 h the solvent was removed under reduced pressure and the residue was chromatographed on SiO_2 using 60%, then 80% EtOAc in hexanes. Compound **43** was isolated as a white foam (51.0 mg; 69%). $R_f = 0.80$ (3:2 EtOAc/hex); $[\alpha]_D^{24} = -1.0$ (*c* 1.0; CH_2Cl_2); IR (thin film): $\tilde{\nu} = 3463, 1649, 1611, 1586, 1513, 1496, 1453, 1363, 1329, 1248, 1209, 1093, 910, 821\ cm^{-1}$; ^1H NMR ($CDCl_3$): $\delta = 7.78$ (d, $J = 7.5$ Hz, 2H), 7.38–7.18 (m, 54H), 6.83 (d, $J = 8.6$ Hz, 2H), 6.31 (d, $J = 6.0$ Hz, 1H), 5.07–4.96 (m, 3H), 4.89–4.83 (m, 2H), 4.77–4.40 (m, 26H), 4.27–4.09 (m, 3H), 4.06 (brs, 1H), 4.00 (t, $J = 5.9$ Hz, 1H), 3.97–3.84 (m, 6H), 3.80–3.50 (m, 23H), 3.76 (s, 3H), 3.42 (dd, $J = 2.8$, 10.8 Hz, 1H), 3.35 (brs, 1H), 3.22 (brd, $J = 7.6$ Hz, 1H), 2.71 (d, $J = 2.5$ Hz, 1H), 2.53 (s, 1H); ^{13}C NMR ($CDCl_3$): $\delta = 159.19, 144.31, 140.41, 138.77, 138.55, 138.29, 138.26, 138.06, 137.96, 137.88, 137.68, 137.52, 137.48, 132.10, 129.21, 128.45, 128.39, 128.32, 128.26, 128.24, 128.18, 128.14, 127.88, 127.84, 127.79, 127.74, 127.70, 127.47, 127.38, 127.17, 113.78, 100.59, 100.58, 100.35, 100.30, 99.74, 83.44, 79.99, 79.89, 79.57, 77.19, 75.80, 75.79, 75.32, 74.95, 74.70, 74.34, 74.28, 73.31, 73.23, 73.20, 73.16, 72.95, 72.92, 72.86, 72.00, 71.66, 71.34, 71.20, 70.35, 69.80, 69.46, 69.14, 68.74, 67.65, 67.52, 66.29, 57.86, 55.13; FAB(+)MS: 2161, 2091, 2067, 2015, 2001, 1924, 1873; HRMS calcd for $C_{114}H_{123}NO_{26}NaS$: 1976.7591, found: 1976.7890.

  0947-6539/97/0310-1625 $ 17.50+.50/0

Synthesis of pentasaccharide tetraacetate glycal sulfonamide 46: Pentasaccharide triol glycal **45** (175 mg; 0.0896 mmol) was dried by azeotropic distillation with benzene (3 × 10 mL). It was then dissolved in dry CH_2Cl_2 (3.0 mL) and treated at room temperature with DMAP (approx 6 mg), triethylamine (0.25 mL; 1.8 mmol) and acetic anhydride (85 mL; 0.9 mmol) while stirred under an inert atmosphere. The mixture was stirred at room temperature for 3 h, then quenched with water (10 mL) and extracted with CH_2Cl_2 (3 × 8 mL). The combined organic extracts were washed once with brine (8 mL). The organic layer was then dried over $MgSO_4$ and filtered, and the solvent was evaporated under reduced pressure to afford a yellow oil that was chromatographed on SiO_2 with EtOAc/hexanes as eluant. Peracetylated pentasaccharide **46** (152 mg; 80%) was isolated as a colorless foam. $R_f = 0.50$ (2:3 EtOAc/hex); $[\alpha]_D^{24} = +8.6$ (*c* 1.0; CH_3CN); IR (thin film): $\tilde{\nu} = 1746$, 1697, 1651, 1612, 1514, 1496, 1454, 1364, 1236, 1043, 1088, 911 cm^{-1}; ^1H NMR ($CDCl_3$): $\delta = 8.00$ (d, $J = 7.8$ Hz, 2H), 7.45–7.18 (m, 55H), 6.84 (d, $J = 8.5$ Hz, 2H), 6.39 (d, $J = 6.2$ Hz, 1H), 5.53 (s, 1H), 5.46 (s, 1H), 5.39 (d, $J = 2.8$ Hz, 1H), 5.32 (d, $J = 7.6$ Hz, 1H), 5.15 (brs, 1H), 5.02 (brs, 1H), 4.91–4.85 (m, 5H), 4.80–4.42 (m, 26H), 4.29 (t, $J = 8.4$ Hz, 1H), 4.19–3.90 (m, 11H), 3.84–3.57 (m, 21H), 3.34 (brd, $J = 9.6$ Hz, 1H), 3.03 (brd, $J = 9.4$ Hz, 1H), 2.16 (s, 3H), 2.14 (s, 3H), 1.95 (s, 3H), 1.92 (s, 3H); ^{13}C NMR ($CDCl_3$): $\delta = 170.75$, 170.06, 169.95, 169.87, 159.14, 144.19, 140.11, 138.60, 138.54, 138.40, 138.30, 138.24, 138.07, 138.02, 137.94, 137.86, 137.78, 133.27, 129.91, 129.16, 128.95, 128.81, 128.47, 128.42, 128.34, 128.26, 128.24, 128.20, 128.18, 128.13, 128.06, 127.87, 127.83, 127.71, 127.64, 127.62, 127.53, 127.49, 127.47, 127.42, 127.36, 127.33, 113.70, 99.68, 99.52, 99.33, 98.20, 98.03, 80.36, 78.95, 77.74, 77.72, 77.20, 75.17, 75.11, 75.07, 74.64, 74.56, 74.36, 74.24, 74.19, 73.96, 73.36, 73.34, 73.19, 73.09, 73.07, 72.28, 71.83, 71.22, 70.97, 70.61, 70.22, 68.83, 68.29, 68.19, 67.66, 64.91, 63.13, 55.13, 25.68, 20.97, 20.91, 20.72; FAB(+)MS: 2146, 2104, 2055, 2016, 1888, 1798, 1696, 1657; HRMS calcd for $C_{122}H_{131}NO_{30}NaS$: 2144.8373, found: 2144.8400.

Synthesis of pentasaccaride triacetate glycal 47: A solution of sodium naphthalenide was made by addition of sodium metal (26.2 mg; 1.14 mmol) to naphthalene (175.3 mg; 1.37 mmol) in dry DME (5.0 mL) and stirring at room temperature under inert atmosphere for 1.5 h. The sufonamide glycal **46** (484 mg; 0.228 mmol) was dried by azeotroping with benzene (3 × 20 mL). It was then dissolved in 10.0 mL dry DME and cooled while stirring under inert atmosphere to −60 °C. The solution of sodium naphthalenide was then added dropwise from a syringe onto a stirred solution of glycal **46**. This addition was continued until a green color persisted (approx. 0.9 mL). The reaction was then immediately quenched with a saturated aqueous solution of $NaHCO_3$ and extracted with EtOAc (3 × 10 mL). The combined organic extracts were washed once with brine (8 mL). The organic layer was then dried over $MgSO_4$, filtered and the solvent evaporated under reduced pressure to afford a yellow oil which was chromatographed on SiO_2 with EtOAc/hexanes as eluant. Pentasaccharide **47** (351.5 mg; 78%) was isolated as a colorless foam. $R_f = 0.12$ (2:5 EtOAc/hex); $[\alpha]_D^{24} = +16.8$ (*c* 0.56; CH_3CN); IR (thin film): $\tilde{\nu} = 3425$, 1745, 1672, 1612, 1514, 1496, 1454, 1369, 1236, 1078, 911 cm^{-1}; ^1H NMR ($CDCl_3$): $\delta = 7.37–7.15$ (m, 52H), 6.85 (d, $J = 8.6$ Hz, 2H), 6.42 (d, $J = 6.2$ Hz, 1H), 5.48 (brs, 1H), 5.39 (brs, 1H), 5.34 (d, $J = 2.6$ Hz, 1H), 5.13 (brs, 1H), 5.00–4.98 (m, 2H), 4.93 (d, $J = 1.1$ Hz, 1H), 4.87–4.78 (m, 4H), 4.72–4.34 (m, 22H), 4.18 (brs, 1H), 4.04–3.85 (m, 10H), 3.81–3.53 (m, 17H), 3.42–3.39 (m, 1H), 3.27–3.17 (m, 3H), 2.15 (s, 3H), 2.03 (s, 3H), 2.01 (s, 3H), 1.57 (s, 3H); ^{13}C NMR ($CDCl_3$): $\delta = 170.26$, 170.21, 170.17, 170.04, 159.36, 144.39, 139.11, 138.70, 138.67, 138.60, 138.45, 138.30, 138.15, 137.98, 137.89, 129.74, 129.36, 128.55, 128.39, 128.36, 128.32, 128.26, 128.24, 128.20, 128.04, 127.91, 127.84, 127.71, 127.66, 127.63, 127.56, 127.53, 127.49, 127.45, 127.43, 127.34, 113.87, 99.90, 99.64, 99.36, 97.86, 97.46, 78.86, 78.22, 77.71, 77.24, 76.02, 75.87, 75.42, 74.80, 74.69, 74.68, 74.25, 73.96, 73.92, 73.80, 73.39, 73.33, 73.31, 72.38, 72.06, 71.86, 71.70, 71.51, 71.13, 70.11, 68.92, 68.75, 68.58, 67.96, 65.70, 56.76, 55.23, 29.69, 25.71, 21.03, 20.94; FAB(+)MS: 2006, 1983, 1914, 1876, 1748, 1658, 1567, 1549, 1537; HRMS calcd for $C_{116}H_{127}NO_{28}Na$: 2004.8442, found: 2004.8500.

Synthesis of pentasaccharide azidosulfonamide 48: The pentasaccharide glycal **47** (112 mg; 0.0565 mmol) was dried by azeotropic distillation with benzene (3 × 6 mL). It was then mixed with anthracene sulfonamide (dried in vacuo over P_2O_5 for 2 h) and activated 4 Å molecular sieves (500 mg) in dry THF (6.0 mL) and stirred at room temperature for 30 min in the dark under inert atmosphere. The mixture was then cooled to 0 °C and freshly prepared iodonium biscollidine perchlorate (132 mg; 0.283 mmol) was then added in one portion. The reaction mixture was stirred in the dark at 0 °C for 3 h, then saturated aqueous sodium thiosulfate solution (10 mL) was added and the mixture shaken vigorously until all red color disappeared. The mixture was extracted with EtOAc (3 × 8 mL). The combined organic extracts were washed with a saturated aqueous solution of sodium thiosulfate (5 mL), with water (5 mL), and finally with brine (5 mL). The organic layer was then dried over $MgSO_4$ and filtered, and the solvent evaporated under reduced pressure to afford a yellow oil. The crude product was immediately dissolved in 3.0 mL dry THF and stored in the dark under inert atmosphere at room temperature, where dry tetrabutylammonium azide (160 mg; 0.565 mmol) was added in one portion. The mixture was stirred for 30 min and then saturated aqueous sodium thiosulfate solution (10 mL) was added and the mixture extracted with EtOAc (2 × 10 mL). The organic extracts were washed with brine, dried over $MgSO_4$, and filtered. The solvent was evaporated under reduced pressure and the residue chromatographed on SiO_2 with EtOAc/hexanes as eluant to afford azidopentasaccharide **48** (83 mg; 67%) as a yellow oil. $R_f = 0.26$ (1:1 EtOAc/hex); $[\alpha]_D^{24} = -4.0$ (*c* 0.75; $CHCl_3$); IR (thin film): $\tilde{\nu} = 3030$, 2868, 2116, 1745, 1668, 1514, 1496, 1454, 1370, 1236, 1078, 910 cm^{-1}; ^1H NMR ($CDCl_3$): $\delta = 9.33$ (d, $J = 8.9$ Hz, 2H), 8.61 (s, 1H), 7.97 (d, $J = 8.3$ Hz, 2H), 7.65–7.57 (m, 2H), 7.48–7.42 (m, 2H), 7.30–7.10 (m, 60H), 7.0–7.05 (m, 2H), 6.83 (d, $J = 8.7$ Hz, 2H), 6.29 (d, $J = 9.1$ Hz, 1H), 5.49 (brs, 1H), 5.44 (dd, $J = 2.8$, 2.0 Hz, 1H), 5.33 (d, $J = 3.0$ Hz, 1H), 5.11 (d, $J = 1.4$ Hz, 1H), 5.04 (d, 1H, $J = 1.6$ Hz), 4.88–4.77 (m, 5H), 4.69–4.35 (m, 22H), 4.28–4.22 (m, 3H), 4.12 (d, $J = 10.6$ Hz, 1H), 4.05–3.62 (m, 23H), 3.60–3.38 (m, 10H), 3.37–3.33 (m, 1H), 3.26–3.21 (m, 1H), 3.14 (brd, $J = 9.5$ Hz, 1H); 2.15 (s, 3H); 2.13 (s, 3H); 1.93 (s, 3H); 1.66 (s, 3H); ^{13}C NMR ($CDCl_3$): $\delta = 170.31$, 170.18, 170.13, 159.29, 138.57, 138.49, 138.44, 138.30, 138.23, 137.93, 137.86, 137.83, 137.78, 137.64, 131.18, 130.31, 129.87, 129.22, 128.76, 128.59, 128.55, 128.36, 128.31, 128.28, 128.24, 128.20, 128.05, 127.99, 127.93, 127.88, 127.79, 127.74, 127.71, 127.67, 127.62, 127.56, 127.52, 127.47, 127.44, 125.52, 125.19, 113.83, 99.84, 99.36, 99.10, 89.00, 78.83, 78.36, 77.76, 77.58, 76.54, 75.25, 74.91, 74.78, 74.76, 74.48, 74.37, 74.27, 73.95, 73.39, 73.26, 73.24, 72.57, 72.44, 71.94, 71.83, 71.49, 71.22, 69.07, 68.83, 68.38, 65.37, 55.46, 55.20, 54.62, 23.27, 21.03, 20.80; FAB(+)MS: 2298.5, 2281.7, 1657.8, 1549.8, 1183.9; HRMS calcd for $C_{130}H_{137}N_5O_{30}NaS$: 2302.8966, found: 2302.8940.

Synthesis of pentasaccharide azide 50: The anthracene sulfonamide-containing azido sugar **48** (80 mg; 0.035 mmol) was dissolved in dry CH_2Cl_2 (1.5 mL) and treated with DMAP (2 mg), triethylamine (0.10 mL; 0.73 mmol) and acetic anhydride (0.04 mL; 0.37 mmol). The mixture was stirred at room temperature, in the dark, under an inert atmosphere for 2 h. Water (5 mL) was then added and the mixture extracted with CH_2Cl_2 (3 × 4 mL). The combined organic extracts were dried ($MgSO_4$), filtered and the solvent evaporated under reduced pressure. Column chromatography on SiO_2 afforded 70 mg (0.030 mmol; 86%) of **49**. $R_f = 0.65$ (3:2 EtOAc/hex). The light-sensitive compound **49** (69 mg; 0.030 mmol) was dissolved in 3.5 mL dry THF, cooled to 0 °C under inert atmosphere and treated with Hünig's base (82 mL; 0.47 mmol) and thiophenol (113 mL; 1.10 mmol). The mixture was stirred in the dark for 30 min, then quenched with dilute aqueous ammonium chloride solution (8 mL) and extracted with EtOAc (3 × 5 mL). The combined organic extracts were dried ($MgSO_4$) and the solvent removed under reduced pressure. The product was purified by chromatography on SiO_2 to afford **50** (37 mg; 60%) as a colorless glass. $R_f = 0.09$ (3:2 EtOAc/hex); $[\alpha]_D^{24} = -6.1$ (*c* 3.6; $CDCl_3$); IR (thin film): $\tilde{\nu} = 3287$, 2113, 1745, 1657, 1514, 1453, 1369, 1235, 1077 cm^{-1}; ^1H NMR ($CDCl_3$): $\delta = 7.36–7.14$ (m, 52H), 6.83 (d, $J = 8.7$ Hz, 2H), 6.26 (d, $J = 8.8$ Hz, 1H), 5.48 (brs, 1H), 5.41 (brs, 1H), 5.31 (d, $J = 2.9$ Hz, 1H), 5.09 (brs, 1H), 5.00 (brs, 1H), 4.87–4.74 (m, 5H), 4.69–4.34 (m, 20H), 4.27 (d, $J = 8.0$ Hz, 1H); 4.03–3.83 (m, 8H), 3.81–3.50 (m, 19H); 3.40 (dd, $J = 8.5$ Hz, 1H), 3.26–3.20 (m, 1H), 3.07 (brd, $J = 9.1$ Hz, 1H), 2.15 (s, 3H), 2.12 (s, 3H), 1.91 (s, 3H), 1.89 (s, 3H), 1.66 (s, 3H); ^{13}C NMR ($CDCl_3$): $\delta = 170.34$, 170.17, 170.15, 170.11, 159.30, 138.53, 138.45, 138.34, 138.30, 138.24, 138.00, 137.85, 137.77, 137.74, 129.84, 129.21, 128.75, 128.58, 128.54, 128.43, 128.34, 128.31, 128.28, 128.27, 128.20, 128.07, 127.94, 127.87, 127.72, 127.71, 127.68, 127.66, 127.61, 127.56, 127.50, 127.48, 127.46, 113.83, 99.83, 99.81, 98.07, 88.23, 78.85, 77.88, 77.81, 77.73, 77.20, 76.36, 75.26, 74.85, 74.77, 74.46, 74.38, 74.29, 73.96, 73.38, 73.32, 73.21, 72.59, 72.44, 71.81, 71.55, 71.19, 69.02, 68.83, 68.76, 68.41, 65.40, 55.19, 54.72, 51.61, 29.66, 23.35, 23.06, 21.01, 20.78; FAB(+)MS: 2105, 2083, 2040; HRMS calcd for $C_{118}H_{131}N_5O_{29}Na$: 2104.8827, found: 2104.8870.

0947-6539/97/0310-1626 $ 17.50+.50/0 *Chem. Eur. J.* **1997**, *3*, No. 10

Synthesis of pentasaccharide azide triol 51: The azido sugar **50** (65 mg; 0.031 mmol) was dissolved in anhydrous MeOH (2.5 mL) and treated with a 25% NaOMe solution in MeOH (20 μL). The mixture was stirred at room temperature under an inert atmosphere for 6 h. The solvent was then evaporated under reduced pressure. Column chromatography on SiO_2 afforded 58 mg (94%) of **51** as a colorless glass. $R_f = 0.31$ (4:1 EtOAc/hex); $[\alpha]_D^{24} = +7.2$ (*c* 1.1; CH_3CN); IR (thin film): $\tilde{\nu} = 3303$, 2923, 2869, 2114, 1659, 1514, 1496, 1453, 1367, 1304, 1248, 1074, 910 cm^{-1}; ^1H NMR ($CDCl_3$): $\delta = 7.35-7.14$ (m, 52H), 6.81 (d, $J = 8.6$ Hz, 2H), 6.29 (d, $J = 8.7$ Hz, 1H), 5.24 (d, $J = 8.4$ Hz, 1H), 4.98 (brs, 1H), 4.95 (brs, 1H), 4.85–4.78 (m, 3H), 4.72 (d, $J = 6.8$ Hz, 1H), 4.66–4.36 (m, 24H), 4.27 (brt, $J = \sim 8$ Hz, 1H), 4.13 (d, $J = 3.4$ Hz, 1H), 4.02–3.88 (m, 7H), 3.85–3.53 (m, 26H), 3.43 (dd, 1H, $J = 2.5$, 9.4 Hz); 3.35–3.31 (m, 1H); 3.22 (brdd, 1H, $J = 3.0$, 9.7 Hz), 1.88 (s, 3H), 1.71 (s, 3H); ^{13}C NMR ($CDCl_3$): $\delta = 170.52$, 170.44, 159.27, 138.68, 138.52, 138.36, 138.14, 137.95, 137.92, 137.81, 137.57, 137.50, 130.24, 129.17, 128.53, 128.51, 128.45, 128.39, 128.38, 128.28, 128.26, 128.03, 127.97, 127.91, 127.87, 127.85, 127.81, 127.77, 127.73, 127.64, 127.54, 127.49, 113.85, 100.74, 99.87, 99.80, 99.65, 88.30, 84.08, 79.94, 79.84, 78.50, 77.69, 77.30, 76.47, 76.27, 75.07, 75.01, 74.83, 74.71, 74.46, 74.33, 73.49, 73.42, 73.32, 73.31, 73.05, 73.04, 72.40, 72.05, 71.58, 71.57, 71.16, 70.00, 69.23, 69.07, 68.98, 68.82, 67.92, 66.60, 55.23, 54.79, 51.61, 23.39, 23.06; FAB(+)MS: 1974.8, 1957.7, 1533.8, 1099.9; HRMS calcd for $C_{112}H_{125}N_5O_{26}Na$: 1978.8510, found: 1978.8510.

Synthesis of tripeptide 54 and pentapeptide 55: Peptides **54** and **55** were assembled by coupling of Fmoc-amino acids in the presence of IIDQ. The C-terminal threonine residue was protected by a benzyl ester. After each coupling and deprotection step the products were purified by flash column chromatography. A complete reaction scheme is available with the supplementary material.

Synthesis of protected *N*-linked pentasaccharide–tripeptide 56/57: Raney nickel (50% slurry in H_2O, 75 mg) was washed intensively with deionized H_2O (pH 7.0), followed by ethanol washes. It was then transferred with ethanol to a flask containing azidopentasaccharide **51** (14.6 mg, 7.5 μmol). Reduction was carried out at room temperature for 30 min under H_2 (1 atm). The crude aminosugar was then filtered through filter paper with ethanol, concentrated, and dried for 20 min under vacuum. Tripeptide **54** (19.4 mg, 30 μmol) was mixed with IIDQ (8.7 mg, 29 μmol) in CH_2Cl_2 (1 mL) at room temperature for 2 min. The solution was then added to the crude aminosugar **52/53**. After stirring for 2 h, the crude protected glycopeptide **56/57** was purified directly on a silica column (EtOAc:hexanes:MeOH 60:40:3.5) to afford 12.0 mg (67%) of a 1:1 mixture (^1H NMR) of **56** and **57**. The mixture was further separated on analytic TLC plates (E. Merck silica gel 60 F_{254}, 0.25 mm, development system: EtOAc:hexanes:MeOH 60:40:3.5) to afford homogeneous material. Data for protected *N*-β-linked pentasaccharide–tripeptide **56**: IR (film): $\tilde{\nu} = 3300$, 3062, 3030, 2925, 2870, 1705, 1650, 1538, 1514, 1454, 1371, 1248, 1074, 1050 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): $\delta = 7.42$ (brd, $J = 6.8$ Hz, 1H), 7.32–7.10 (m), 6.78 (d, $J = 8.8$ Hz, 2H), 6.46 (brd, $J = 8.8$ Hz, 1H), 6.40 (d, $J = 7.6$ Hz, 1H), 5.13–5.07 (m, 5H), 4.95–4.90 (m, 3H), 4.85–4.10 (m), 4.05–3.95 (m, 4H), 3.90–3.40 (m), 3.72 (s, 3H), 3.40–3.28 (m, 2H), 3.20 (m, 1H), 2.70 (dd, 1H), 2.52 (dd, 1H), 2.05 (m, 1H), 1.731 (s, 3H), 1.63 (s, 5H), 1.15 (d, $J = 6.0$ Hz, 3H), 0.91–0.80 (d, d, $J = 6.8$, 7.2 Hz, 6H); HRMS (FAB) calcd for $C_{147}H_{166}N_6O_{36}Na$: 2582.1340, found: 2582.1401. Data for protected *N*-α-linked pentasaccharide–tripeptide **57**: IR (film): $\tilde{\nu} = 3331$, 3030, 2925, 2870, 1732, 1660, 1514, 1454, 1371, 1315, 1249, 1075, 1046 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): $\delta = 7.35-7.05$ (m), 6.81 (d, $J = 8.4$ Hz, 2H), 6.52 (d, $J = 8.8$ Hz, 1H), 6.31 (d, $J = 7.6$ Hz, 1H), 5.54 (m, 1H), 5.10–5.05 (m, 4H), 4.98 (d, 2H), 4.72–4.40 (m), 4.35–4.20 (m, 4H), 4.15 (m, 2H), 4.14–3.50 (m), 3.72 (s, 3H), 3.25, (m, 2H), 2.80 (brd, 1H), 2.57 (dd, 1H), 2.11 (s, 3H), 1.77 (s, 3H), 1.16 (d, $J = 6.0$ Hz, 3H), 0.90 (dd, $J = 6.8$, 6.8 Hz, 6H); HRMS (FAB) calcd for $C_{147}H_{166}N_6O_{36}Na$: 2582.1340, found: 2582.1335.

Synthesis of asparagine *N*-linked pentasaccharide–tripeptides 60 and 61: The global deprotection of *N*-β-linked glycopeptide **56** (4.6 mg, 1.8 μmol) was carried out in 80% aqueous acetic acid (2 mL). Pd/C (10%, 20 mg) was added and H_2 (1 atm) was bubbled through for 4 h at room temperature. The reaction mixture was then filtered through filter paper. The filtrate was evaporated to dryness and further dried under high vacuum overnight. The thin film was purified by reverse-phase gel chromatography (RP-18, pure H_2O) to afford 2.2 mg (quantitative) of **60**. ^1H NMR (500 MHz, D_2O, 50 °C): $\delta = 5.10$ (brs, 1H, H-$1^{4\alpha}$), 5.05 (d, $J = 9.3$ Hz, 1H, H-$1^{1\beta}$), 4.90 (brs, 1H, H-1, H-$1^{4\alpha}$), 4.76 (brs, 1H, H-$1^{3\beta}$), 4.61 (d, $J = 7.1$ Hz, 1H, H-$1^{2\beta}$), 4.25–4.15 (m), 4.10–3.5 (m), 2.9–2.6 (brd, 2H), 2.15 (m, 1H), 2.06 (s, 3H), 2.00 (s, 3H), 1.15 (d, $J = 5.5$ Hz, 3H), 0.96 (brs, 6H). The global deprotection of *N*-α-linked glycopeptide **57** was carried out in the same way to yield 59% of **61**. ^1H NMR (500 MHz, D_2O, 50 °C): $\delta = 5.69$ (brs, 1H, H-$1^{1\alpha}$), 5.11 (s, 1H, H-$1^{4\alpha}$), 4.91 (s, 1H, H-$1^{4\alpha}$), 4.76 (s, 1H, H-$1^{3\beta}$), 4.60 (d, 1H, $J = 7.2$ Hz), 4.25–4.16 (m), 4.05–3.60 (m), 2.85–2.70 (brd, 2H), 2.04 (s, 3H), 2.00 (s, 3H), 1.15 (brd, 3H, $J = 5.5$ Hz), 0.97 (m, 6H). HRMS (FAB) calcd for $C_{47}H_{80}O_{31}N_6Na$: 1247.4700; found: 1247.4800.

Synthesis of asparagine-*N*-linked pentasaccharide–pentapeptide 62/63: Raney nickel (50% slurry in H_2O, 75 mg) was washed intensively with deionized H_2O (pH 7.0) followed by ethanol. It was then transferred with ethanol to a flask containing azidopentasaccharide **51** (13.5 mg, 6.9 μmol). Reduction was carried out at room temperature for 1 h under H_2 (1 atm). The crude aminosugar was then filtered through filter paper with ethanol, concentrated and dried under vacuum for 20 min. Pentapeptide **55** (24.5 mg, 28 μmol) was mixed with IIDQ (8.0 mg, 26 μmol) in CH_2Cl_2 (1 mL), and after 2 min the partially soluble mixture was added to the crude aminosugar. After stirring overnight, the crude mixture was purified directly on analytical silica TLC plates (E. Merck silica gel 60 F_{254}, 0.25 mm, development solvent system: EtOAc:hexanes:MeOH 60:40:3.5) to afford 9.7 mg (51%) of a mixture of β- and α-protected glycopeptides **62/63**. HRMS (FAB) calcd for $C_{156}H_{182}O_{36}N_8Na$: 2766.2550, found: 2766.2540. 3.7 mg of the above mixture underwent global deprotection in 80% aqueous acetic acid (2 mL) under H_2 (1 atm) in the presence of Pd/C (10%, 20 mg). After being stirred for 4 h at room temperature, the mixture was filtered through filter paper with 20% aqueous acetic acid. The filtrate was evaporated to dryness and further dried under high vacuum overnight. The thin film was purified by reverse-phase gel chromatography (RP-18, pure H_2O, followed by 20% MeOH/H_2O) to afford 1.9 mg (quantitative) of the free glycopeptide **62/63** as a mixture. No further separation was achieved. ^1H NMR (500 MHz, D_2O, 50 °C) of the mixture. δ 5.73 (d, $J = 5.0$ Hz, 1H, H-$1^{1\alpha}$), 5.18 (brs, 2H, H-$1^{4\alpha}$), 5.10 (d, $J = 9.7$ Hz, 1H, H-$1^{1\beta}$), 4.98 (ds, 2H, H-$1^{4\alpha}$), 4.83 (brs, 2H, H-$1^{13\beta}$), 4.79(d, $J = 6.4$ Hz, 1H, H-$1^{2\beta}$), 4.68 (d, $J = 7.4$ Hz, 1H, H-$1^{2\beta}$), 4.30–4.20 (m), 4.10–3.5 (m), 3.0–2.8 (m, 4H), 2.20 (m, 2H), 2.14 (s, 6H), 2.07 (s, 6H), 1.75–1.60 (m, 6H), 1.45 (m, 6H), 1.22 (d, $J = 6.2$ Hz, 6H), 1.05–0.95 (m, 24H); HRMS (FAB) calcd for $C_{56}H_{96}O_{33}N_8Na$: 1431.5980, found: 1431.6000.

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