Chemoenzymatic Solution- and Solid-Phase Synthesis of *O*-Glycopeptides of the Mucin Domain of MAdCAM-1. A General Route to *O*-LacNAc, *O*-Sialyl-LacNAc, and *O*-Sialyl-Lewis-X Peptides

Oliver Seitz and Chi-Huey Wong*

Contribution from the Department of Chemistry and the Skaggs Institute for Chemical Biology, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, California 92037

Received April 30, 1997[⊗]

Abstract: An efficient and general method for the solid-phase synthesis of glycopeptides containing an *O*-linked sialyl-Lewis-X (SLe^x) tetrasaccharide is described. Using a combined chemoenzymatic approach, the first synthesis of an unnatural β-*O*-linked SLe^x attached to a partial sequence of the mucin domain of the L-selectin ligand MAdCAM-1, was demonstrated. A resin-bound *O*-glycoconjugate was synthesized from a new Fmoc-threonine building block which carries an *O*-unprotected β-linked *N*-acetylglucoseamine (GlcNAc) moiety. The acid- and base-stable HYCRON-linker enabled the complete removal of all protecting groups on solid phase. Glycosyltransferases were employed to extend the glycan on supported and unsupported *O*-GlcNAc-octapeptide substrates. The acid- and base-sensitive *O*-glycopeptides were released under practically neutral conditions, taking advantage of the palladium(0)-catalyzed cleavage of the allylic linkage. Studies toward the selective *O*-deacetylation of ester-linked glycopeptides possessing *O*-acetyl-protected carbohydrates are also reported.

Introduction

The phenomenon of cell−cell adhesion[1] is a subject of current intensive research. This intercellular recognition event is involved in processes such as cell growth, cell differentiation, and cell targeting. One example is the adhesion of leukocytes to the endothelium in the early phase of an inflammatory response.[2] The interactions between selectins[3] and their ligands lead to "rolling" of the leukocyte on the endothelial cell surface followed by extravasation to the site of injury.[4] It has been shown that selectins bind to adhesion molecules which carry the tetrasaccharide moities SLe^x [5] and SLe^a [6] as well as their sulfated derivatives.[7] The ligands of P- and L-selectin, namely PSGL-1,[8] GlyCAM-1,[9] CD-34,[10] and MAdCAM-1,[11] possess SLe^x and SLe^a motifs on *O*-linked carbohydrates, which are presented in various glycoforms.

Chemical syntheses of partial sequences with known glycoform are of high interest, since they allow the investigation of cell−cell adhesion in a well-defined system.[12] Solid-phase-based methods[13] enable a rapid and, if desired, combinatorial access[14] to complex structures. A crucial step of solid-phase glycopeptide synthesis[15] is introduction of the carbohydrate part. Most commonly, preformed glycosyl amino acids are employed

[⊗] Abstract published in *Advance ACS Abstracts,* September 15, 1997.

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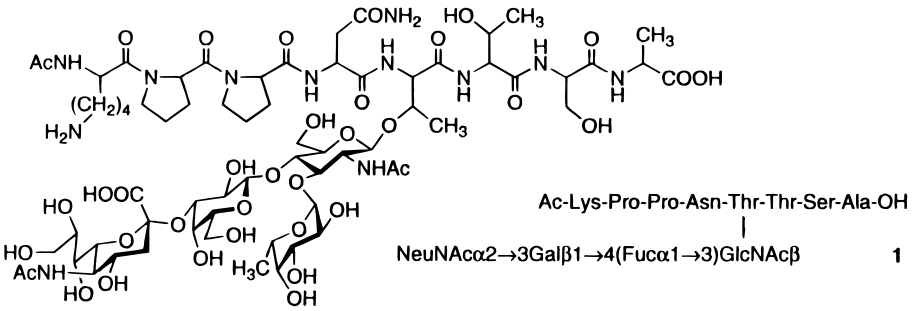
S0002-7863(97)01383-8 CCC: $14.00

Scheme 1. The 227−234 Sequence of the Mucin Domain of MAdCAM-1 Carrying an *O*-Linked SLe^x-Ligand



in the stepwise assembly of the peptide backbone. The excess equivalents needed for quantitative coupling[16] cannot be recycled without risking the incorporation of partially racemized material into the next reaction. Although some progress has been made in the chemical synthesis of oligosaccharides on solid phase,[17] these methods were applied only occasionally to glycopeptide synthesis.[18−20] One reason for the lack of a general method for on-resin carbohydrate synthesis is the necessity to differentiate numerous protecting groups of the supported glycopeptides, a methodology which can be omitted if enzymatic protocols[21] are used. Glycosyltransferases have shown to be versatile tools in oligosaccharide synthesis, especially in the synthesis of naturally occurring oligosaccharides. Since these enzymes typically use unprotected glycopeptide substrates, their application in solid-phase synthesis requires a linkage enabling the removal of protecting groups without detaching the supported substrates. Following the Fmoc/tBu strategy[22] the linker must provide stability under the conditions for both removal of the Fmoc groups (morpholine, piperidine, or DBU in DMF solutions) and the side chain protecting groups (TFA). Most of the commonly used linker groups are either acid- or base-labile, and cleavage of the linker often requires conditions, which are too harsh for the synthesis of acid- and base-sensitive *O*-glycopeptides. We wish to report a new efficient and general method for the solid phase synthesis of *O*-glycopeptides using a combined chemoenzymatic[23] approach.

The mucosal adressin cell adhesion molecule-1 (MAdCAM-1), a 58−66 K glycoprotein expressed by the high endothel venules (HEV) of lymph nodes and mucosal lymphoid tissues, was found to be a ligand for L-selectin on leukocytes and is therefore involved in leukocyte trafficking.[24] It consists of three immunoglobulin-like sequences, which support integrin-mediated adhesion,[25] and one mucin-like sequence.[26] It was suggested that the mucin-like domain serves as a scaffold to present *O*-glycosidically linked carbohydrates containing SLe^x motifs to lymphocytes.[27]

To illustrate the feasibility for the synthesis of *O*-linked SLe^x glycopeptides, glycoconjugate **1** (Scheme 1), in which one threonine carries the SLe^x tetrasaccharide, was chosen as target. The peptide backbone is part of the mucin domain and spans the 227−234 sequence of MAdCAM-1. The glycan contains labile glycosidic linkages such as the α-fucosidic bond.[28] In contrast to numerous publications concerning the synthesis of *N*-glycosidically linked SLe^x conjugates,[29] the synthesis of glycopeptides with *O*-linked SLe^x structures has not been reported. Although the GlcNAc-Thr linkage is not a typical mucin-type structure, *O*-glycopeptide **1** is perfectly suited to demonstrate the versatility of new methods for the synthesis of complex glycoconjugates.

The peptide backbone of *O*-SLe^x glycopeptide **1** can be assembled employing the established techniques of solid-phase peptide synthesis via the Fmoc/tBu strategy. Glycosyltransferases have been successfully used to extend the *N*-acetylglucosamine (GlcNAc) moiety to SLe^x structures.[30] Little has been published with regard to the application of the same set of enzymes to resin-bound substrates.[31,32]

The hydroxyl groups of the glycosyl amino acid building block Fmoc-Thr(*β*GlcNAc) may be free or protected in order to exclude any side reactions. Acyl protecting groups are preferred since *O*-acyl groups increase the acid stability of the

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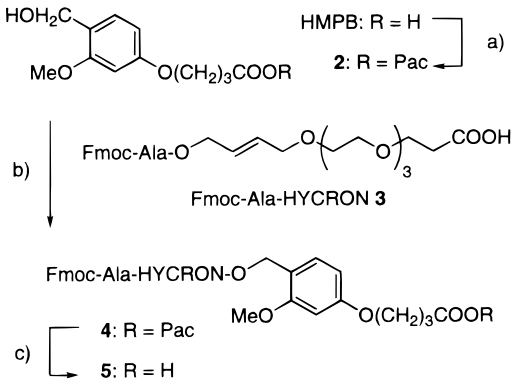
Scheme 2[a]



[a] (a)Pac-Br, NEt_3, EtOAc, 16 h, 70%; (b) Pyr, 2,6-Cl_2-PhCOCl, DMF, 2 d, 64%; (c) Zn, AcOH, 6 h, 89%. Pac = CH_2COPh.

O-glycosidic linkage,[33] but the incorporation of an *O*-protected carbohydrate derivative such as Fmoc-Thr(βAc_3GlcNAc) would necessitate an on-resin removal of the acetyl groups if further reactions have to be performed on the supported carbohydrate moiety. One aim of this study was to investigate whether removal of *O*-acetyl groups is possible on a solid support without cleaving the *C*-terminal ester linkage. After having performed the chemical peptide synthesis, the removal of the side chain protecting groups and the enzymatic glycosyltransfer reactions, the *O*-SLe^x octapeptide has to be detached under conditions which would not affect the acid- and base-labile glycoconjugate. Linkers of the allyl ester type[34] in particular the acid- and base-stable HYCRON anchor[20,35] satisfy the demanding properties required in the outlined chemoenzymatic solid-phase synthesis.

The solid support itself also has to meet several criteria. It has to be suitable for chemical synthesis in organic solvents, and for enzymatic synthesis in aqueous solvents. That is, it must swell not only in organic solvents, but in water to a degree that allows the enzymes to react with the substrate presented through a spacer group on the polymeric network. These properties were described for the PEGA resin.[36] Alternatively, a rigid support with a sufficiently large pore size such as aminopropyl controlled-pore glass (CPG) can be employed.[21] The use of both of these supports was exploited in the chemoenzymatic synthesis of the *O*-SLe^x octapeptide **1**.

Results and Discussion

Syntheses of Building Blocks. To assemble the fully protected glycoconjugates on a solid support, an *N*-protected starting amino acid−HYCRON handle was attached to a super acid labile linker such as the HMPB linker [4-[4′-(hydroxymethyl)-3′-methoxyphenoxy]butyric acid],[37] which can be cleaved by 1% TFA in dichloromethane. For the synthesis of a Fmoc-Ala-HYCRON-HMPB conjugate the HMPB linker was derivatized as phenacyl (Pac) ester **2** (Scheme 2). Fmoc-Ala-HYCRON **3**[20] was esterified with **2** followed by reductive Pac ester removal to yield the Fmoc-Ala-HYCRON-HMPB conjugate **5**.

The glycosyl amino acid building block **9** was synthesized using the thioglycosyl donor **6**[38] (Scheme 3). Upon activation with DMTST[39] the donor reacted smoothly with the Fmoc-Thr-OBn acceptor.[40] Reductive cleavage of the [(trichloroethyl)-oxy]carbonyl (Troc) group and subsequent acetylation of the liberated amino group gave the fully protected amino acid glycoside **8**. Hydrogenolysis of the benzyl ester yielded the building block **9**.

For the preparation of the *O*-unprotected GlcNAc amino acid building blocks **12a** and **12b**, which have not been described, Z-Thr and Z-Ser were glycosylated using peracetylated *N*-acetylglucosamine **10** in the presence of the promotor BF_3-etherate[40] (Scheme 4). Glycosides **11a** and **11b** were obtained exclusivly in β-configuration. The acetyl groups were removed by a sodium methylate-catalyzed transesterification. After neutralization, the amino group was released by hydrogenolysis and blocked by reaction with Fmoc-OSu. All protecting group manipulations can be performed in one pot giving yields of 78% and 60% of the building blocks **12a** and **12b**, respectively.

Solid-Phase Synthesis of *O*-GlcNAc Octapeptide. The amino acid linker derivative **5** was attached to PEGA resin (Scheme 5). Treatment with DMF/morpholine (1:1) for 50 min removed the Fmoc groups.[41] All coupling reactions were performed in DMF using the HBTU method with NMM as base.[42] *O*-protected and *O*-unprotected carbohydrates were introduced by coupling the preformed glycosyl amino acids Fmoc-Thr(βAc_3GlcNAc)-OH (**9**) or Fmoc-Thr(βGlcNAc)-OH (**12a**), respectively. For cleavage of the protected glycooctapeptides the resins were treated with highly diluted TFA in dichloromethane. The isolation of the *O*-unprotected compound **14** required HPLC purification, resulting in a substantially lower overall yield. HPLC analysis of the material obtained after the final peptide cleavage showed that protection of the carbohydrate hydroxyl groups leads to crude products of higher purity.

The primary step in the synthesis of the *O*-Ac_3GlcNAc octapeptide **15** is the attachment of the Fmoc-Ala-HYCRON **3** conjugate to PEGA resin (Scheme 5). The solid-phase synthesis was performed as described above. Liberation of the protected *O*-glycosylated octapeptide **15** was accomplished by suspending the resin in DMF/DMSO with a catalytic amount of the palladium(0) catalyst and a 10−15-fold excess of the scavenger nucleophile morpholine under exclusion of oxygen.

Compounds **13** and **14** were treated with TFA in the presence of ethanedithiol and anisole as scavengers (Scheme 5). The very high yields of **16** and **17** obtained after size-exclusion chromatography reveal the acid stability of the HYCRON

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(40) A one-pot-preparation of the Fmoc-Thr(βAc_3GlcNAc)-OH building block **9** via a BF_3-etherate-promoted glycosylation of Fmoc-Thr-OH using peracetylated *N*-acetylglucosamine as glycosyl donor has been described: Arsequell, G.; Krippner, L.; Dwek, R. A.; Wong, S. Y. C. *J. Chem. Soc., Chem. Commun.* **1994**, 2383. However, attempts to reproduce the glycosylation under the conditions described failed. Instead, a compound with identical molecular mass but slightly different ^1H- and ^{13}C-NMR spectra (compared to authentic reference, see Scheme 3, **9**) was isolated. The high-field shift of the anomeric proton as well as the anomeric carbon indicated that the carboxyl group and not the hydroxyl group had been glycosylated. (For a similar observation see: Salvador, L. A; Elofsson, M.; Kihlberg, J. *Tetrahedron* **1995**, *51*, 5643.)

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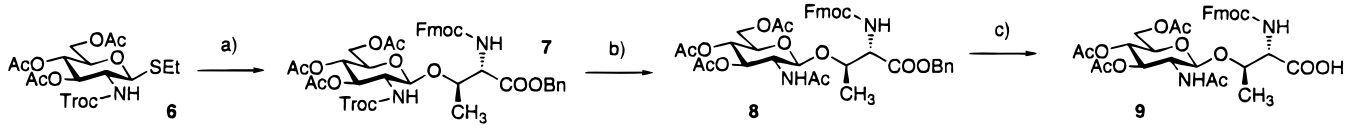
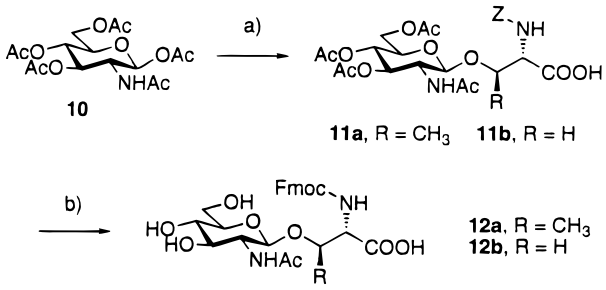
Scheme 3[a]



[a] (a) Fmoc-Thr-OBn, DMTST, MS 4 Å, DCM, 1.5 h, 78%; (b) *i*, Zn, AcOH, 20 h; *ii*, Pyr, Ac_2O, 3 h, 77%; (c) Pd/C (5%), EtOAc, EtOH, 1.5 h, 88%.

Scheme 4[a]



[a] (a) Z-Thr, $BF_3{\cdot}Et_2O$, DCM, MS 4 Å, 5 d, 41% (R = CH_3) or Z-Ser, $BF_3{\cdot}Et_2O$, DCM, MS 4 Å, 5 d, 49%, (R = H); (b) *i*, NaOMe, MeOH, pH 8.8, 1.5 h; *ii*, Pd/C (10%), MeOH, 1 h; *iii*, Fmoc-OSu, MeOH, 17 h, 78% (**12a**), 60% (**12b**).

linkage, which is neccessary for the removal of acid-labile protecting groups on solid supports. For complete deprotection glycopeptide **15** was deacetylated by a sodium methoxide-catalyzed transesterification[43] followed by TFA cleavage of the side chain protecting groups to yield **18**.

Experiments on the Selective *O*-Deacetylation of 13. In order to examine whether selective *O*-deacetylation of the fully protected ester-linked glycopeptide could be achieved, the model compound **13** was treated with several reagents known for their mild and selective deacetylations.The reagents not only removed the *O*-acetyl groups but also cleaved the *C*-terminal ester bond.[44] The best result was obtained with the α-nucleophile LiOOH,[45] which released the desired compound **14** in 56% yield (Scheme 6a). This was accompanied by the formation of products **19** and **20**, which were formed in 28% and 16% yields respectively. Interestingly lipase WG (Scheme 6b), which was reported to remove the acetyl groups of *O*-acetylated glycosyl amino acid (methoxyethoxy)ethyl esters,[46] selectively cleaved the HYCRON-linker in 76% yield after complete conversion of the starting material.

Since the selective removal of the *O*-acetyl groups of the fully protected glycopeptide conjugate **13** was not successful, the application of enzymatic deacetylations of a side chain unprotected substrate was investigated.

Acetylesterase was reported to selectively remove *O*-acetyl groups from carbohydrate substrates at neutral pH.[47] The application of this reaction to conjugate **16** resulted in a complete hydrolysis of all ester bonds to give **18** (Scheme 7). Treatment of **16** with lipase WG gave **21** in 77% yield. As in the case of **13**, lipase WG cleaved the amino acid ester prior to removal of the acetyl groups.

In conclusion, the selective removal of *O*-acetyl groups could not be accomplished for the solid-phase strategy, although it is useful for preparing the unprotected glycopeptide for further enzymatic glycosylation. The best result, which was obtained with LiOOH, showed no satisfactory selectivity in the potential application on supported glycopeptides. Thus, an unprotected carbohydrate as used in the solid-phase synthesis of **14** needs to be incorporated.

Enzymatic Solution-Phase Synthesis of *O*-SLe^x Octapeptides. In the chemoenzymatic solid-phase synthesis of **1**, a supported form of **17** would be used as substrate for the corresponding glycosyltransferases. Therefore it is of interest to examine the substrate specificity of conjugate **17** in the enzymatic glycosyltransfer reactions.

HPLC analysis of the galactosyltransferase-catalyzed galactosylation using UDP galactose as glycosyl donor showed that the substrate **17** was efficiently glycosylated to give product **22** (Scheme 8a). Unexpectedly, at longer reaction times the formation of the ester-cleaved product **23** was observed. After 27 h the ester-cleaved *O*-LacNAc octapeptide **23** was formed in 30% yield. In the preparative galactosylation (Scheme 8b) the *O*-LacNAc octapeptide conjugate **22** was isolated in 87% yield.

In the sialyltransferase-catalyzed sialylation of conjugate **22**, satisfactory yields were more difficult to obtain. Sialyltransferase has a significantly lower specific activity than the galactosyltransferase, so that under low concentrations of this enzyme the rate of the enzyme-independent ester hydrolysis is comparable to the rate of glycosylation. Scheme 9 shows the yields obtained with a low enzyme concentration (method a). After 20 h sialylated compounds **24** and **25** were formed in 19% yield (Table 1) and both starting material **22** and product **24** were ester hydrolyzed to 16% overall yield. After 42 h the hydrolysis proceeded and glycooctapeptides **23** and **25** were obtained in 83% yield.

The rate of sialylation can be accelerated by increasing the enzyme concentration. To overcome product inhibition by CMP alkaline phosphatase was added.[48] It was speculated that the sensitivity of the ester bond toward hydrolysis might be induced by a specific conformation. Therefore, Triton X-100, known to randomize peptide conformations, was included in the buffer. After 24 h only the sialylated compounds **24** and **25** were detected (methods b and c). The product ratio is affected by the concentrations of substrate, CMP-NeuNAc, and Triton X-100. Lower concentrations seem to increase the rate of the ester hydrolysis (method c). The preparative sialylation was performed at high concentrations of substrate, CMP-NeuNAc, and Triton X-100 (method d). The *O*-sialyl-LacNAc octapeptide conjugate **24** was isolated in quantitative yield. At this stage, it is not clear how the detergent affects the system.

The sensitivity of the allylic ester bond of conjugates **17**, **22**, and **24** toward hydrolysis under neutral conditions is difficult to understand since the same ester bond is stable against TFA/scavenger, DMF/morpholine, and DMF/piperidine mixtures. Experiments in which conjugates **17** and **22** were treated with various enzyme-free buffer media revealed that ester hydrolysis

(43) Peters, S.; Bielefeldt, T.; Meldal, M.; Bock, K.; Paulsen, H. *J. Chem. Soc., Perkin Trans.1.* **1992**, 1163.

(44) A complete description of the reagents used and the selectivities obtained is available as Supporting Information.

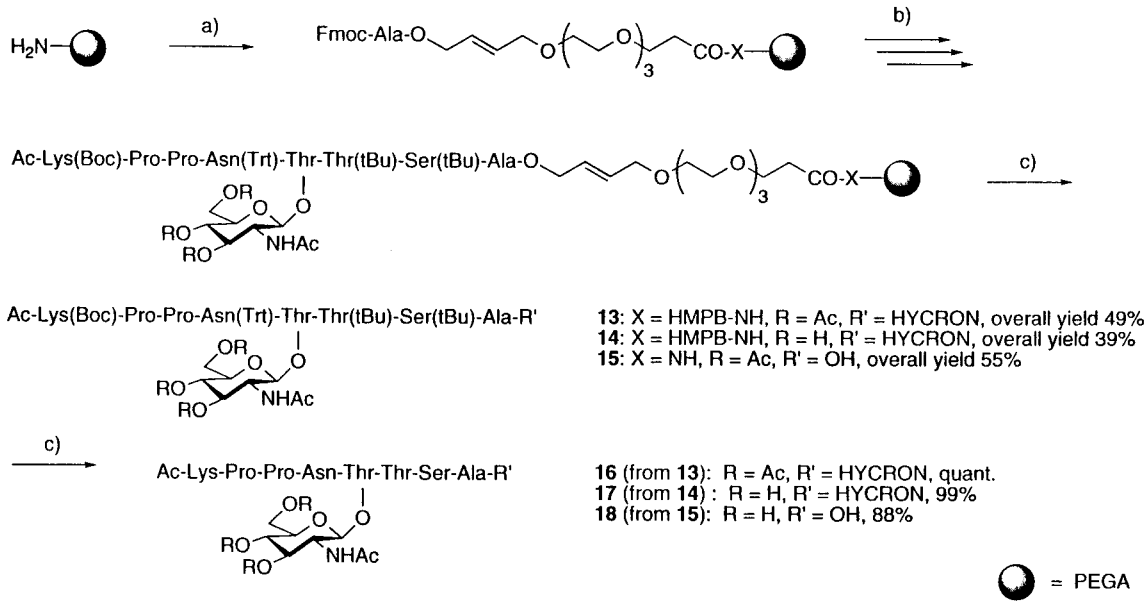
(45) Evans, D. A.; Britton, T. C.; Ellman, J. A. *Tetrahedron Lett.* **1987**, *28*, 6141.

(46) Eberling, J,; Braun, P.; Kowalczyk, D.; Schultz, M.; Kunz, H. *J. Org. Chem.* **1996**, *61*, 2638.

(47) (a) Waldmann, H.; Heuser, A. *Bioorg. Med. Chem.* **1994**, *2*, 477. (b) Waldmann, H.; Heuser, A.; Reidel, A. *Synlett* **1994**, 65.

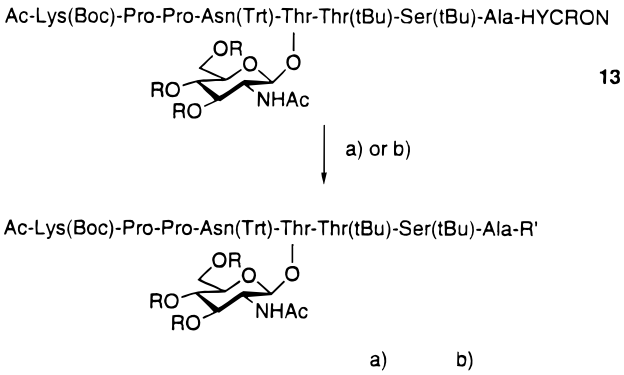
(48) Unverzagt, C.; Kunz, H.; Paulson, J. C. *J. Am. Chem. Soc.* **1990**, *112*, 9308.

Scheme 5[a]



[a] (a) *i*, **5** (X = HMPB-NH) or **3** (X = NH), HBTU, NMM, HOBt, DMF, 24 h; *ii*, Pyr/Ac_2O, 5 min; (b) *i*, DMF/morpholine (1:1), 54 min; *ii*, Fmoc-AA, HBTU, NMM, HOBt, DMF, 3−5 h; *iii*, Pyr/Ac_2O, 5 min (if R=Ac); *iv*, i−iii repeated, *N*-acetylation: Pyr, Ac_2O (R = Ac) or AcOH, HBTU, NMM, HOBt, DMF (R = H); (c) 1% TFA/DCM, 7 × 2 min (X = HMPB-NH) or $Pd(PPh_3)_4$, morpholine, DMF, DMSO, 14 h (X = NH); (d) TFA/ethanedithiol/anisol (40:1:1), 1.5 h (if **10** or **11**) or *i*, NaOMe, MeOH, pH 8.5, 2 h; *ii*, TFA/ethanedithiol/anisol (40:1:1), 1.5 h (if **12**).

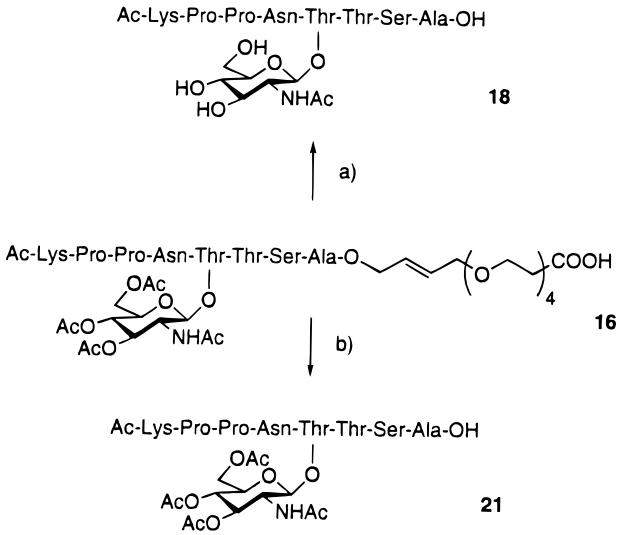
Scheme 6[a]



	a)	b)
14: R = H, R' = HYCRON	56%	11%
19: R = Ac, R' = OH	28%	76%
20: R = H, R' = OH	16%	13%

[a] (a) 0.01 M LiOOH/THF, 4 h; (b) lipase WG, 0.2 M phosphate buffer (10% acetone), pH 7.0, 37 °C, 6 d. Yields determined by HPLC.

Scheme 7[a]



[a] (a) acetylesterase (EC 3.1.1.6, 14 U/mL), 0.1 M phosphate buffer, 8 mM substrate, pH 6.8, 1 d, 37 °C; (b) lipase WG, 0.2 M phosphate buffer, pH 7.0, 37 °C, 2 d, 77%.

was independent of metal ion and Triton X-100 concentration.[49] The rate of hydrolysis was decreased by lowering the pH. An esterase contamination was excluded since both **22** and the chemically synthesized **17** were hydrolyzed equally well. The model compound Ac-Ala-HYCRON[50] showed no sign of any ester hydrolysis. Thus, the remarkable sensitivity of conjugates **17**, **22**, and **24** toward ester hydrolysis must be regarded a sequence-specific phenomenon.

The enzymatic fucosylation[51] proceeded at pH 6.0 without concomitant ester hydrolysis (Scheme 10). During the long reaction time a second addition of GDP fucose[52] and fucosyltransferase was necessary. However, it was not possible to drive the reaction to completion. Neither the addition of more transferase nor the use of lower substrate concentrations allowed a complete conversion of the starting material. After 5 d the *O*-SLe^x octapeptide conjugate **26** was isolated in 55% yield. Interestingly, dissolving the purified product **26** in water led to complete hydrolysis of the ester bond after 3 days, whereas the same ester bond was stable under the fucosylation conditions.

For the solution synthesis of the *O*-SLe^x octapeptide **1**, glycopeptide **18** was enzymatically galactosylated to furnish the *O*-LacNAc peptide **23** in quantitative yield (Scheme 11). The sialylation of **23**, however, proceeded much slower than the reaction with the corresponding conjugate **22**. A second portion of CMP-NeuNAc and sialyltransferase had to be added. After 4 d the *O*-sialyl-LacNAc octapeptide **25** was obtained in 76% yield. The fucosylation proceeded as described previously (see **26**) giving the *O*-SLe^x octapeptide **1** in 59% yield.

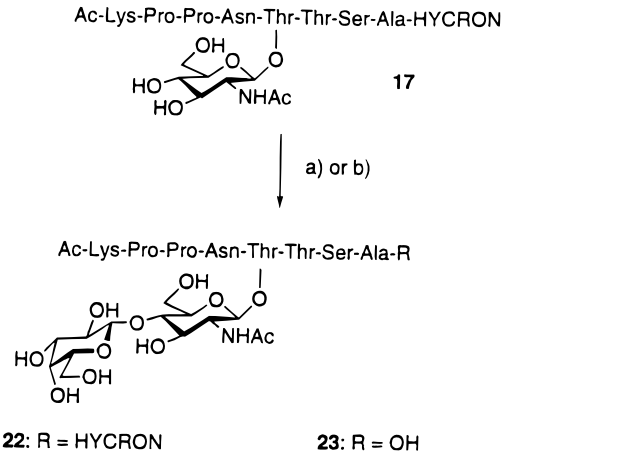
(49) Data available as Supporting Information.

(50) Ac-Ala-HYCRON was synthesized from conjugat **3** by cleaving the Fmoc group with morpholine and acetylating the liberated amino group with Ac_2O/Pyr/H_2O. This conjugate was stable for weeks upon treatment with buffers which caused ester hydrolysis of conjugates **14**, **19**, and **21**. Data available as Supporting Information.

(51) Wong, C.-H.; Dumas, D. P.; Ichikawa, Y.; Koseki, K.; Danishefsky, S. J.; Weston, B. W.; Lowe, J. B. *J. Am. Chem. Soc.* **1992**, *114*, 7321.

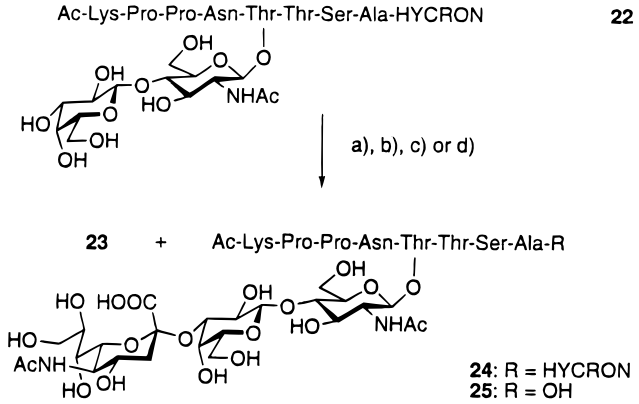
(52) Wittmann, V.; Wong, C.-H. *J. Org. Chem.* **1997**, *62*, 2114.

Scheme 8[a]



[a] (a) 1.4 equiv of UDP-Gal (19 mM), GalTase (1.7 U/mL), 50 mM HEPES, 5 mM $MnCl_2$, pH 7.2, 37 °C, yields determined by HPLC; (b) 1.25 equiv of UDP-Gal (15 mM), GalTase (1.7 U/mL), 50 mM HEPES, 5 mM $MnCl_2$, pH 7.0, 37 °C.

Scheme 9[a]



[a] (a) 1.5 equiv of CMP-NeuNAc (10 mM), SialTase (0.021 U/mL), 100 mM HEPES, 5 mM $MnCl_2$; (b) 1.6 equiv of CMP-NeuNAc (7.5 mM), SialTase (0.05 U/mL), 100 mM HEPES, 2.5 mM $MnCl_2$, 0.17% Triton X-100, alk. phosphatase (46 U/mL); (c) 1.6 equiv of CMP-NeuNAc (3.75 mM), SialTase (0.05 U/mL), 100 mM HEPES, 2.5 mM $MnCl_2$, 0.085% Triton X-100, alk. phosphatase (46 U/mL); (d) 1.3 equiv of CMP-NeuNAc (9 mM), SialTase (0.054 U/mL), 100 mM HEPES, 2.5 mM $MnCl_2$, 0.2% Triton X-100, alk. phosphatase (2.8 U/mL). All reactions at pH 7.0, 37 °C.

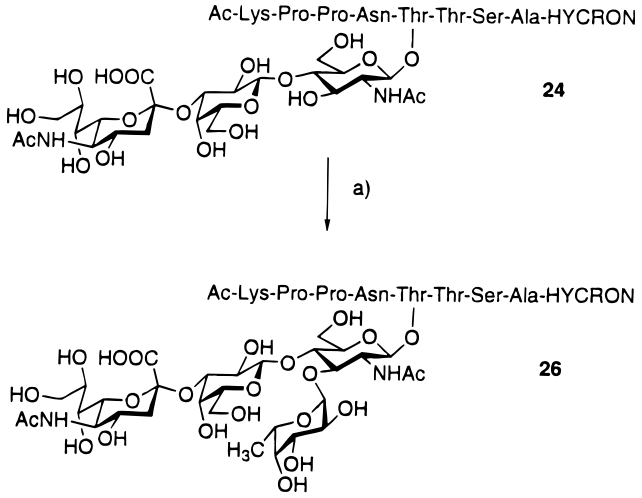
Table 1. Glycosylation and Ester Hydrolysis During the Enzymatic Sialylation of **22** (See Scheme 9)

method	**22**, % yield	**23**, % yield	**24**, % yield	**25**, % yield
a[a]	69 (20 h) 15 (47 h)	12 (20 h) 67 (47 h)	15 (20 h) 2 (47 h)	4 (20 h) 16 (47 h)
b[a]	0	0	89	11
c[a]	0	0	74	26
d[b]	nd	nd	quant	nd

[a] Yields determined by HPLC. [b] Isolated yield.

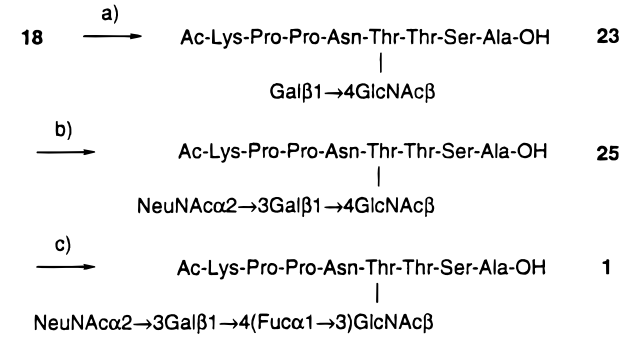
Chemoenzymatic Solid-Phase Synthesis of the *O*-Sialyl-LacNAc Octapeptide 25. Initial experiments studying lipase reactions with PEGA-supported peptides showed that the PEGA resin shrank after contact with the aqueous enzyme solution. For that reason, CPG was employed as a support in the solid-phase synthesis of the glycopeptide. The starting amino acid was attached to aminopropyl-CPG using the HYCRON linkage (Scheme 12). The carbohydrate was incorporated as the *O*-unprotected amino acid glycoside Fmoc-Thr(βGlcNAc)-OH.

Scheme 10[a]



[a] (a) GDP-Fuc, FucTase, 50 mM MES, 15 mM $MnCl_2$, alk. phospatase, pH 6.0, 37 °C, 4 d, 55%.

Scheme 11[a]



[a] (a) UDP-Gal, GalTase, 50 mM HEPES, 5 mM $MnCl_2$, pH 7.0, 37 °C, 21 h, quant.; (b) CMP-NeuNAc, SialTase, 100 mM HEPES, 5 mM $MnCl_2$, alk. phosphatese, pH 7.0, 37 °C, 5 d, 77%; (c) GDP-Fuc, FucTase, 50 mM MES, 15 mM $MnCl_2$, pH 6.0, 37 °C, 6 d, 59%.

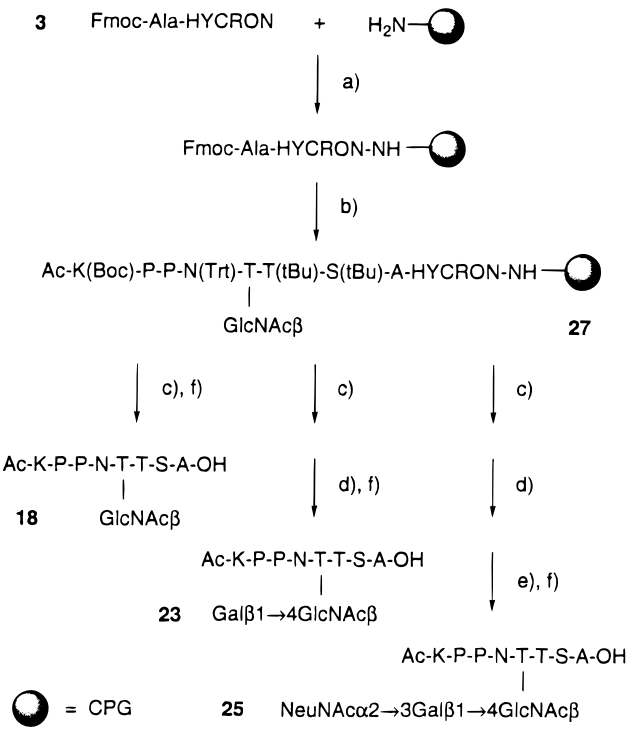
The resin-linked *O*-GlcNAc octapeptide **27** was treated with TFA in order to remove the amino acid side chain protecting groups. Treatment of the product with palladium(0) in the presence of the allyl scavenger morpholine released the glycopeptide **18**, which was isolated in an overall yield of 20%. Compared to the synthesis of **14**, the use of the CPG resin rather than the PEGA resin led to a 50% decrease of the overall yield. Hence, CPG is not the optimal support for chemical peptide synthesis, an observation reported before by Giralt and coworkers;[53] however, CPG is most suitable for performing enzymatic reactions.[54]

The supported glycopeptide **27** was submitted to the enzymatic galactosyltransfer reaction after protecting group removal. Since the previous experiments revealed the sensitivity of the octapeptide ester linkage toward hydrolysis, it was not unexpected that HPLC analysis showed the presence of the *O*-LacNAc peptide **23** in the supernatant. After 4 days, **23** was isolated in an overall yield of 8%. However, the cleavage of the linker bond was not completed. The palladium(0)-catalyzed cleavage reaction furnished a 2:1 mixture of the *O*-LacNAc peptide **23** and the unreacted *O*-GlcNAc peptide **18** in 10% yield. Overall, the *O*-LacNAc peptide **23** was synthesized in 15% yield, based on the loaded starting amino acid. Compared

(53) Albericio, F.; Pons, M.; Pedroso, E.; Giralt, E. *J. Org. Chem.* **1989**, *54*, 360.

(54) Slomczynska, U.; Albericio, F.; Cardenas, F.; Giralt, E. *Biomed. Biochim. Acta* **1991**, *50*, 67.

Scheme 12[a]



[a] (a) HBTU, NMM, HOBt, DMF, 20 h; (b) *i*, DMF/morpholine (1:1), 54 min; *ii*, Fmoc-AA, HBTU, NMM, HOBt, DMF, 3−5 h; *iii*, i−ii repeated, *N*-acetylation: AcOH, HBTU, NMM, HOBt, DMF; (c) TFA/ethanedithiol/H_2O (40:1:1), 2 × 45 min; (d) UDP-Gal, GalTase, 50 mM HEPES, 5 mM $MnCl_2$, pH 7.0, 37 °C; (e) CMP-NeuNAc, SialTase, 100 mM HEPES, 5 mM $MnCl_2$, alk. phosphatase, pH 7.0, 37 °C; (f) $Pd(PPH_3)_4$, morpholine, DMF, DMSO.

to the solid-phase synthesis of **18**, 75% of the supported glycopeptide had been galactosylated.

For the synthesis of the *O*-sialyl-LacNAc octapeptide **25** the permanent protecting groups of the resin-linked glycopeptide **27** were removed and the galactose was transferred enzymatically as decribed. The enzyme-catalyzed sialylation was performed subsequently. As in the solution synthesis of **25**, a second addition of the sugar nucleotide and the silayltransferase was carried out. An overall yield of 9% (based on the initial amino acid load of the resin) was achieved after workup of the supernatant. Interestingly, the product released into the supernatant showed a higher purity than the product obtained by the subsequent palladium(0)cleavage. This indicates that the hydrolysis of the allylic ester bond might be induced by a specific conformation, which is not preferred in the side products.

It has to be emphasized that although the solution-phase synthesis of **25** gives higher yields the complete on-resin assembly of this complex structure is performed in less then 9 days and offers new opportunities for automation and diversification in glycopeptide synthesis.

Summary

The synthesis of a glycopeptide with an *O*-glycosidically linked SLe^x structure has been accomplished for the first time. Glycosyltransferases were used to extend the glycan on a glycopeptide substrate in solution and on solid support. The glycopeptide substrate was synthesized in solid phase by using the HYCRON-linker, which enables the removal of acid labile amino acid side-chain-protecting groups while the glycopeptide remains supported. The Pd(0)-catalyzed cleavage of the HYCRON linkage provides a mild and efficient method to liberate acid- and base-sensitive compounds such as the *O*-sialyl-LacNAc octapeptide **25**. It is noted, however, that some unexpected side reaction may occur with certain glycopeptide sequences which facilitate the release of the glycopeptide from the ester-linked group. This interesting problem requires further study as glycosylation may effect the peptide conformation in this case study to affect its stability and chemoenzymatic reactivity. In any case, the solid-phase methodology elaborated in these studies should be applicable to the rapid synthesis of any *O*-SLe^x peptide in general.

Experimental Section

Generals. All amino acid derivatives were L-amino acids. Reactions were carried out at room temperature if no specifications are given. Solid-phase synthesis was performed manually using a reaction vessel similiar to the Merrifield reactor. Photometric determination of the Fmoc loadings was performed by treating aliquots of the resin with 2.00 mL of DMF/morpholine (1:1) for 1 h. This solution was diluted and subjected to UV analysis (300.5 nm). Chromatography (silica gel 60, 230−400 mesh, Mallinckrodt) was performed on open columns if no further explanations are given. Analytical and semipreparative HPLC was performed on a Hitachi system (L-6200 A Pump, L-4000 Detector, D-2500 Integrator) using columns and gradients as specified in the text.

Solid-Phase Synthesis of the *O*-GlcNAc-Octapeptides. Ac-Lys(Boc)-Pro-Pro-Asn(Trt)-Thr(βAc_3GlcNAc)-Thr(tBu)-Ser(tBu)-Ala-HYCRON (13). Loading. To 1.5 g of PEGA (swollen in methanol, ∼0.12 mmol NH_2/g), which was washed extensively with MeOH and DMF, was added a solution of **5** (1.24 g, 1.53 mmol) in DMF (20 mL). To the suspension HOBt (297 mg, 2.2 mmol) were added NMM (426 μL, 3.06 mmol) and HBTU (580 mg, 1.53 mmol). After 24 h of shaking at room temperature, the solution was filtered off and the polymer washed with DMF and methanol to yield 1.17 g of wet polymer. The loading was determined by photometric detection of the Fmoc cleavage product. Loading: 0.12 mmol Fmoc/g. **Fmoc Removals.** Shaking in DMF/morpholine (1:1, 10 mL) for 54 min was followed by filtration and washing with DMF. **Couplings.** The resin was treated with a 5-fold excess of the Fmoc-amino acid as a 0.06 M solution in DMF, which contains 1.5 equiv of HOBt, 2.0 equiv of NMM, and 1.0 equiv of HBTU per equivalent Fmoc-amino acid. After 3−5 h of shaking the reactants were removed by filtration. The support was washed with DMF. The following amino acid derivatives have been used: Fmoc-Ser(tBu), Fmoc-Thr(tBu), Fmoc-Thr(βAc_3GlcNAc) **9**, Fmoc-Asn(Trt), Fmoc-Pro, Fmoc-Lys(Boc). **Capping.** The resin was suspended in Pyr/Ac_2O (3:1, 8 mL) and shaken for 6 min. Reactants were removed by filtration and the resin was thoroughly washed with DMF. **Acetylation of the *N*-terminus.** The Fmoc-group of the *N*-terminal lysine was removed according to the described protocol. Acetylation was accomplished by reacting the supported octapeptide in Pyr/Ac_2O (3:1, 8 mL) for 30 min. After filtration the resin was washed thoroughly with DMF followed by dichloromethane. **Peptide Cleavage.** The resin was shaken in 5 mL of 1% TFA in dichloromethane for 2 min. The supernatant was filtered into 1 mL of MeOH/Pyr (9:1) before the resin was washed once with 5 mL of dichloromethane. This procedure was repeated 7 times. **Purification.** The filtrate was concentrated before 20% acetic acid (10 mL) was added. After addition of water (10 mL), the mixture was extracted with dichloromethane (30 mL). The organic layer was separated and the aqueous layer extracted twice with dichloromethane (10 mL). To the combined organic layers pyridine (1 mL) was added before the volatiles were removed by distillation *in vacuo*. Twofold silica gel chromatography ($CHCl_3$/MeOH, 90:10 and 95:5) gave a material, which was subjected to two subsequent gel permeation chromatographies (Sephadex LH-20, $CHCl_3$/MeOH, 1:1). After lyophilization from dichloromethane/i-PrOH/benzene 130 mg of a white fluffy powder were obtained. Yield: 49% (based on initial loading of polymer with amino acid), t_R: 11.8 min [Microsorb, C8, 5μ, 250 × 4 mm, 0 min (50% B), 2 min (50% B), 42 min (80% B), A = 0.1% TFA in H_2O, B = 0.1% TFA in MeCN)]. ^{1}H NMR (500 MHz, ^{1}H,^{1}H-COSY, DMSO-d_6): 8.65 (s, 1H, $N^{\beta-NH}$), 8.19−8.18 (m, 2H, A^{NH}, $N^{\alpha-NH}$), 8.02 (d, 1H, $K^{\alpha-NH}$, J = 7.8), 7.90 (d, 1H, $C2'^{NH}$, J = 8.5), 7.57 (d, 1H, T^{NH}, J = 7.2), 7.53 (d, 1H, S^{NH}, J = 8.2), 7.35−7.14 (m, 16H, T^{NH}, Trt^{ar-H}), 6.77 (t, 1H, $K^{\epsilon-NH}$, J = 5.5), 5.84−

5.79 (m, 1H, HYCRON^{H-15}), 5.76−5.71 (m, 1H, HYCRON^{H-16}), 5.19 (t, 1H, H-3′, $J_{3',2'} = J_{3',4'} = 9.9$), 4.85 (d, 1H, H-1′, $J_{1',2'} = 8.3$), 4.78 (t, 1H, H-4′, $J_{4',3'} = J_{4',5'} = 9.7$), 4.69 (m, 1H, T$^{\alpha}$), 4.55−4.50 (m, 4H, HYCRON^{H-17}, P$^{\alpha}$, N$^{\alpha}$), 4.43−4.38 (m, 3H, S$^{\alpha}$, P$^{\alpha}$, K$^{\alpha}$), 4.32−4.27 (m, 2H, T$^{\alpha}$, A$^{\alpha}$), 4.15−4.12 (m, 1H, H-6′^{a}), 4.01−3.93 (m, 5H, HYCRON^{H-14}, 2 × T$^{\beta}$, H-6′^{b}), 3.81 (m, 1H, H-5′), 3.68 (m, 1H, P$^{\epsilon a}$), 3.62−3.56 (m, 4H, HYCRON^{H-3}, H-2′, P$^{\epsilon a}$), 3.51−3.46 (m, 18H, HYCRON$^{(CH2CH2O)3}$, 2 × P$^{\epsilon b}$), 2.86 (m, 2H, K$^{\epsilon}$), 2.67 (m, 2H, N$^{\beta}$), 2.42 (t, 2H, HYCRON^{H-2}, $J_{2,3} = 6.3$), 2.09−1.71 (m, 23H, 5 × Ac, 2 × P$^{\beta}$, 2 × P$^{\gamma}$), 1.58 (m, 1H, K$^{\beta a}$), 1.45 (m, 1H, K$^{\beta b}$), 1.36−1.29 (m, 14H, Boc, K$^{\gamma}$, K$^{\delta}$, A$^{\beta}$), 1.16 (s, 9H, tBu), 1.10 (s, 9H, tBu), 0.99, 0.97 (2 × d, 6H, 2 × T$^{\gamma}$, $J_1 = 6.3$, $J_2 = 6.1$). ESI-MS (neg): 1914 (M (1 × ^{13}C)) − H$^+$), calcd 1914.1.

Ac-Lys(Boc)-Pro-Pro-Asn(Trt)-Thr(βGlcNAc)-Thr(tBu)-Ser(tBu)-Ala-HYCRON (14). Loading. To 3.0 g of PEGA (swollen in methanol, ∼0.12 mmol NH_2/g), which was washed with MeOH and DMF, was added a solution of the HMPB anchor (216 mg, 0.9 mmol), HOBt (180 mg, 1.26 mmol), NMM (250 μL, 1.8 mmol), and HBTU (340 mg, 0.9 mmol) in DMF (8 mL). After 3 h of shaking the solution was filtered off and the polymer washed with DMF. The resin was suspended in a solution of Fmoc-Ala-HYCRON (530 mg, 0.9 mmol), pyridine (145 μL, 1.8 mmol) and 2,6-dichlorobenzoylchloride (120 μL, 0.9 mmol) in DMF (8 mL) and shaken for 22 h. After filtration the resin was washed with DMF, with methanol, and with DMF again. Unreacted hydroxyl groups were blocked by shaking the support for 1.5 h in benzoyl chloride/pyridine (1:3, 8 mL). Filtration and washing with DMF and methanol gave 2.49 g of wet polymer. The loading was determined by photometric detection of the Fmoc cleavage product. Loading: 25 μmol Fmoc/g. **Fmoc removals.** See **13**. **Couplings.** See **13**; Fmoc-Thr(βGlcNAc) **12a** was used instead of Fmoc-Thr(βAc$_3$-GlcNAc) **9**. **Capping.** After the first two couplings capping was performed as described (see **13**). Capping was omitted after introduction of the *O*-unprotected amino acid glycoside. **Acetylation of the *N*-Terminus.** The Fmoc group of the *N*-terminal lysine was removed according to the described protocol. Acetylation was accomplished by adding a solution of acetic acid (17 μL, 0.3 mmol), HOBt (64 mg, 0.45 mmol), NMM (83 μL, 0.6 mmol), and HBTU (114 mg, 0.3 mmol) in DMF (5 mL) to the supported glycooctapetide. After 1 h of shaking, filtration and washing with DMF (5×), the procedure was repeated once more. **Peptide cleavage.** See **13**. **Purification.** The filtrate was concentrated before 20% acetic acid (10 mL) was added. After addition of water (10 mL), the mixture was extracted with dichloromethane (30 mL). The organic layer was separated, and the aqueous layer was extracted twice with dichloromethane (10 mL). To the combined organic layers was added pyridine (1 mL) before the volatiles were removed by distillation *in vacuo*. Silica gel chromatography ($CHCl_3$/MeOH, 90:10) followed by gel permeation chromatography (Sephadex LH-20, $CHCl_3$/MeOH, 1:1) gives a material which was subjected to preparative HPLC [Vydac C18, 250 × 20 mm, 0 min (35% MeCN in H_2O) and 30 min (60% MeCN in H_2O)] to yield 44 mg of a white solid. Yield: 39% (based on initial loading of polymer with amino acid). t_R: 26.9 min [Microsorb, C8, 5μ, 250 × 4 mm, 0 min (30% B) and 50 min (70% B), A = 2% MeCN, 0.1% TFA in H_2O, B = 2% H_2O, 0.1% TFA in MeCN)]. ESI-MS (neg): 1790 (M (3 × ^{13}C) − H$^+$), calcd 1790.1, 1789 (M (2 × ^{13}C)) − H$^+$), calcd 1789.1, 1788 (M (1 × ^{13}C) − H$^+$), calcd 1788.1, 1787 (M − H$^+$), calcd 1787.1.

Ac-Lys(Boc)-Pro-Pro-Asn(Trt)-Thr(βAc$_3$GlcNAc)-Thr(tBu)-Ser(tBu)-Ala-OH (15). Loading. To 1.5 g of PEGA (swollen in methanol, ∼0.12 mmol NH_2/g), which was washed thoroughly with MeOH and DMF, was added a solution of Fmoc-Ala-HYCRON-OH (264 mg, 0.45 mmol) in DMF (8 mL). To the suspension HOBt (90 mg, 0.63 mmol) were added NMM (125 μL, 0.9 mmol) and HBTU (170 mg, 0.45 mmol). After 20 h of shaking at room temperature, the solution was filtered off and the polymer washed with DMF. Pyridine (6 mL) and aceticanhydride (2 mL) were added to the resin. The suspension was shaken for 6 min. Filtration followed by washing with DMF and methanol gave 0.94 g of wet polymer. The loading was determined by photometric detection of the Fmoc cleavage product. Loading: 92 μmol Fmoc/g. **Fmoc removals.** See **13**, except for shaking was in 8 mL DMF/morpholine (1:1). **Couplings.** See **13**. **Capping.** See **13**. **Acetylation of the *N*-Terminus.** See **13**. **Peptide Cleavage.** The resin was suspended in DMF/DMSO (1:1, 8 mL) and morpholine (0.3 mL) was added. The reaction vessel was evacuated until bubbling of the suspension occurred and then streamed by argon. After this step was repeated 5 times, tetrakis(triphenylphosphine)-palladium (10 mg) was added. The mixture was shaken in argon under exclusion of light for 14 h. The resin was repeatedly washed with DMF. **Purification.** The combined filtrates were concentrated *in vacuo*. The residue was dissolved in MeOH. Precipitated palladium was removed by filtration. The filtrate was concentrated *in vacuo*. Twofold silica gel chromatography ($CHCl_3$/MeOH, 90:10 and 95:5) gave a material, which was subjected to gel permeation chromatography (Sephadex LH-20, $CHCl_3$/MeOH, 1:1). The remaining inpurity was removed by preparative HPLC [Vydac C18, 250 × 20 mm, 0 min (50% MeCN in H_2O) and 30 min (80% MeCN in H_2O)] to yield 78 mg of a white solid. Yield: 55% (based on initial loading of polymer with amino acid). t_R: 29.5 min (Microsorb, C8, 5μ, 250 × 4 mm, 0 min (30% B) and 2 min (30% B) and 42 min (80% B), A = 2% MeCN, 0.1% TFA in H_2O, B = 2% H_2O, 0.1% TFA in MeCN)). ^{1}H NMR (500 MHz, DMSO-d_6): 8.64 (s, 1H, N$^{\beta-NH}$), 8.17 (d, 1H, N$^{\alpha-NH}$, J = 7.7), 8.01 (d, 1H, K$^{\alpha-NH}$, J = 7.6), 7.95 (d, 1H, A^{NH}, J = 7.1), 7.90 (d, 1H, C2′^{NH}, J = 8.5), 7.56 (d, 1H, T^{NH}, J = 7.1), 7.49 (d, 1H, S^{NH}, J = 7.9), 7.35−7.14 (m, 16H, T^{NH}, Trt^{ar-H}), 6.75 (t, 1H, K$^{\epsilon-NH}$), 5.20 (t, 1H, H-3′, $J_{3',2'} = J_{3',4'} = 9.9$), 4.85 (d, 1H, H-1′, $J_{1',2'} = 8.3$), 4.77 (t, 1H, H-4′, $J_{4',3'} = J_{4',5'} = 9.7$), 4.69 (m$_c$, 1H, T$^{\alpha}$), 4.52−4.49 (m, 2H, P$^{\alpha}$, N$^{\alpha}$), 4.41−4.33 (m, 3H, S$^{\alpha}$, P$^{\alpha}$, K$^{\alpha}$), 4.29−4.27 (m, 1H, T$^{\alpha}$), 4.22 (m, 1H, A$^{\alpha}$), 4.14 (dd, 1H, H-6′^{a}), 4.01−3.97 (m, 3H, 2 × T$^{\beta}$, H-6′^{b}), 3.81 (m$_c$, 1H, H-5′), 3.67−3.43 (m, 5H, 2 × P$^{\epsilon}$, H-2′), 2.84 (m, 2H, K$^{\epsilon}$), 2.65 (m, 2H, N$^{\beta}$), 2.08−1.71 (m, 23H, 5 × Ac, 2 × P$^{\beta}$, 2 × P$^{\gamma}$), 1.56 (m$_c$, 1H, K$^{\beta a}$), 1.47−1.22 (m, 15H, K$^{\beta b}$, Boc, K$^{\gamma}$, K$^{\delta}$, A$^{\beta}$), 1.17 (s, 9H, tBu), 1.10 (s, 9H, tBu), 0.99, 0.97 (2 × d, 6H, 2 × T$^{\gamma}$, J_1 = 6.3, J_2 = 6.1). ESI-MS (neg): 1639 (M − H$^+$), calcd 1639.0. ESI-MS (pos): 1666 (M (3 × ^{13}C) + Na$^+$), calcd 1666.0, 1665 (M (2 × ^{13}C) + Na$^+$), calcd 1665.0, 1664 (M (1 × ^{13}C) + Na$^+$), calcd 1664.0, 1663 (M + Na$^+$), calcd 1663.0.

Ac-Lys-Pro-Pro-Asn-Thr(βAc$_3$GlcNAc)-Thr-Ser-Ala-HYCRON (16). A solution of **13** (40 mg, 20.9 μmol) in a mixture of 0.05 mL ethanedithiol, 0.05 mL anisol and 2 mL TFA was stirred for 1.5 h. The solvent was removed *in vacuo*, and the residue was dissolved in MeOH (3 mL). Addition of of ether (30 mL) followed by cooling to −78 °C leads to precipitation of the product. The precipitate was collected, and the filtrate was concentrated to dryness. The precipitation was repeated once more. The combined precipitates were purified by GPC (Sephadex LH-20, $CHCl_3$/MeOH, 1:1) to yield 33 mg of a white solid. Yield: 100%. t_R: 19.6 min [Vydac C18, 250 × 4 mm, 0 min (1% B) and 2 min (1% B) and 42 min (80% B), A = 0.1% TFA in H_2O, B = 0.1% TFA in MeCN)]. ^{1}H NMR (500 MHz, D_2O): 5.90−5.87 (m, 2H, HYCRON^{H-15}, HYCRON^{H-16}), 5.23 (t, 1H, H-3′, $J_{3',2'} = J_{3',4'} = 9.9$), 5.00 (t, 1H, H-4′, $J_{4',3'} = J_{4',5'} = 9.7$), 4.76−4.71 (m, 2H, P$^{\alpha}$, N$^{\alpha}$), 4.67 (d, 2H, HYCRON^{H-17}, J = 3.7), 4.60 (d, 1H, T$^{\alpha}$, J = 3.5), 4.57 (dd, 1H, K$^{\alpha}$, $J_1 = 5.2$, $J_2 = 8.8$), 4.48 (t, 1H,S$^{\alpha}$, $J_{\alpha,\beta} = 5.0$), 4.44−4.37 (m, 4H, H-1′, P$^{\alpha}$, T$^{\alpha}$, A$^{\alpha}$), 4.34−4.27 (m, 2H, 2 × T$^{\beta}$), 4.16−4.13 (m, 1H, H-6′^{a}), 4.08 (d, 2H, HYCRON^{H-14}, J = 3.7), 3.98−3.89 (m, 2H), 3.87−3.81 (m, 3H), 3.80−3.71 (m, 3H), 3.68−3.59 (m, 16H), 3.00 (t, 2H, K$^{\epsilon}$, $J_{\epsilon,\delta} = 7.5$), 2.84 (dd, 1H, N$^{\beta a}$, $J_{\beta a,\beta b} = 15.5$, $J_{\beta a,\alpha}$ = 6.9), 2.76 (dd, 1H, N$^{\beta b}$, $J_{\beta b,\beta a} = 15.6$, $J_{\beta b,\alpha} = 6.1$), 2.64 (t, 2H, HYCRON^{H-2}, $J_{2,3} = 6.1$), 2.39−2.24 (m, 2H, 2 × P$^{\beta a}$), 2.10−1.98 (m, 19H, 5 × Ac, 2 × P$^{\gamma}$), 1.97−1.88 (m, 2H, 2 × P$^{\beta b}$), 1.82−1.76 (m, 1H, K$^{\beta a}$), 1.71−1.64 (m, 3H, K$^{\delta}$, K$^{\beta b}$), 1.49−1.41 (m, 5H, K$^{\gamma}$, A$^{\beta}$, $J_{\beta,\alpha}$ = 7.3), 1.24 (d, 3H, T$^{\gamma}$, $J_{\gamma,\beta} = 6.4$), 1.18 (d, 3H, T$^{\gamma}$, $J_{\gamma,\beta} = 6.4$). FAB-MS (pos): 1592 (M + Cs$^+$), calcd 1592.7.

Ac-Lys-Pro-Pro-Asn-Thr(βGlcNAc)-Thr-Ser-Ala-HYCRON (17). A solution of **14** (10 mg, 7.6 μmol) in TFA/ethanedithiol/anisole (40:1:1) was stirred for 1.5 h. The volatiles were removed *in vacuo*, and the residue was dissolved in MeOH (3 mL). The product was precipitated with ether and subjected to GPC (Biogel P2, 0.1M NH_4HCO_3). After lyophilization 10 mg of a white powder were obtained. Yield: 99%. t_R: 24.3 min [Vydac C18, 250 × 4 mm, 0 min (0% B) and 2 min (0% B) and 30 min (20% B), A = 2% MeCN, 0.1% TFA in H_2O, B = 2% H_2O, 0.1% TFA in MeCN)]. ^{1}H NMR (500 MHz, D_2O): 5.89−5.82 (m, 2H, HYCRON^{H-15}, HYCRON^{H-16}), 4.70−4.67 (m, 2H, P$^{\alpha}$, N$^{\alpha}$), 4.63 (m, 2H, HYCRON^{H-17}), 4.54−4.51 (m, 3H, T$^{\alpha}$, K$^{\alpha}$, H-1′), 4.43−4.34 (m, 3H, S$^{\alpha}$, P$^{\alpha}$, T$^{\alpha}$), 4.25−4.17 (m, 2H, 2 × T$^{\beta}$), 4.05−4.03 (m, 3H, HYCRON^{H-14}, A$^{\alpha}$), 3.89−3.62 (m,

24H), 3.49 (m, 1H, H-3′), 3.38−3.36 (m, 1H, H-4′, H-5′), 2.96 (t, 2H, K^{ϵ}, $J_{\epsilon,\delta} = 7.5$), 2.82 (dd, 1H, $N^{\beta a}$, $J_{\beta a,\beta b} = 15.5$, $J_{\beta a,\alpha} = 6.5$), 2.73 (dd, 1H, $N^{\beta b}$, $J_{\beta b,\beta a} = 15.5$, $J_{\beta b,\alpha} = 7.0$), 2.42 (t, 2H, $HYCRON^{H-2}$, $J_{2,3} = 6.7$), 2.34−2.21 (m, 2H, 2 × $P^{\beta a}$), 2.01−1.91 (m, 12H, 2 × Ac, 2 × P^{γ}, 2 × $P^{\beta b}$), 1.78−1.71 (m, 1H, $K^{\beta a}$), 1.67−1.62 (m, 3H, K^{δ}, $K^{\beta b}$), 1.43−1.37 (m, 5H, K^{γ}, A^{β}, $J_{\beta,\alpha} = 7.2$), 1.19 (d, 3H, T^{γ}, $J_{\gamma,\beta} = 6.3$), 1.12 (d, 3H, T^{γ}, $J_{\gamma,\beta} = 6.3$). ESI-MS (pos): 1357 (M + Na^+) calcd 1356.8, 1335 (M + H^+) calcd 1334.8.

Ac-Lys-Pro-Pro-Asn-Thr(*β*GlcNAc)-Thr-Ser-Ala-OH (18). A solution of **15** (18.0 mg, 10.99 μmol) in dry methanol (1 mL) was adjusted to pH 8.5 by addition of a 0.1 M solution of sodium methylate in methanol. After 2 h of stirring, the solution was acidified to pH 6 by addition of acetic acid. The solution was concentrated *in vacuo* to give 17.6 mg of material which was dissolved in TFA/ethanedithiol/anisole (40:1:1, 6.3 mL) and stirred for 1.5 h. The mixture was concentrated *in vacuo*, and the residue was dissolved in MeOH (3 mL). The product precipitates upon addition of ether. The precipitate was collected and purified by GPC (Biogel P2, 0.1M NH_4HCO_3). Lyophilization of the product fraction gave 10.2 mg of a white fluffy powder. Yield: 88%. t_R: 16.0 min (Vydac C18, 250 × 4 mm, 0 min (0% B) and 2 min (0% B) and 30 min (10% B), A = 2% MeCN, 0.1% TFA in H_2O, B = 2% H_2O, 0.1% TFA in MeCN)]. ^{1}H NMR (500 MHz, ^{1}H,^{1}H-COSY, D_2O): 4.71−4.68 (m, 2H, N^{α}, P^{α}), 4.56−4.54 (m, 3H, H-1′, T^{α}, K^{α}), 4.45−4.37 (m, 3H, S^{α} (4.44), T^{α} (4.38), P^{α}), 4.30 (m_c, 1H, T^{β}), 4.24 (m_c, 1H, T^{β}), 4.11 (q, 1H, A^{α}, $J_{\alpha,\beta} = 7.2$), 3.88−3.77 (m, 5H, H-6′^{a}, S^{β}, $P^{\epsilon a}$), 3.72−3.60 (m, 4H, H-6′^{b}, H-2′, $P^{\epsilon b}$), 3.51 (m_c, 1H, H-3′), 3.39 (m_c, 2H, H-4′, H-5′), 2.97 (t, 2H, K^{ϵ}, $J_{\epsilon,\delta} = 7.5$), 2.83 (dd, 1H, $N^{\beta a}$, $J_{\beta a,\beta b} = 15.6$, $J_{\beta a,\alpha} = 6.6$), 2.74 (dd, 1H, $N^{\beta b}$, $J_{\beta b,\beta a} = 15.7$, $J_{\beta b,\alpha} = 7.2$), 2.34 (m_c, 1H, $P^{\beta a}$), 2.26 (m_c, 1H, $P^{\beta a}$), 2.03−1.93 (m, 10H, 2 × Ac, 2 × P^{γ}), 1.90−1.87 (m, 2H, 2 × $P^{\beta b}$), 1.75 (m_c, 1H, $K^{\beta a}$), 1.66 (m_c, 3H, $K^{\beta b}$, K^{δ}), 1.45 (m_c, 2H, K^{γ}), 1.30 (d, 3H, A^{β}, $J_{\beta,\alpha} = 7.2$), 1.21 (d, 3H, T^{γ}, $J_{\gamma,\beta} = 6.2$), 1.14 (d, 3H, T^{γ}, $J_{\gamma,\beta} = 6.2$). ESI-MS (neg): 1058 (M − H^+) calcd 1058.6.

Lipase WG-Catalyzed Removal of the HYCRON Linker. Ac-Lys-Pro-Pro-Asn-Thr(*β*Ac$_3$GlcNAc)-Thr-Ser-Ala-OH (21). The glycopeptide **16** (12.6 mg, 8.0 μmol) was dissolved in a solution of lipase WG (Sigma, EC 3.1.1.3, 10 U/mL) in phosphate buffer (0.2 M NaH_2PO_4, 10 U/mL, pH 7.0, 3.1 mL) and shaken at 37 °C for 2 d. After lyophilization the crude was purified by GPC (50 mM NH_4OAc). The product fractions were lyophilized to yield 7.3 mg of **20**. Yield: 77%. t_R: 18.3 min [Microsorb C18, 250 × 4 mm, 0 min (0% B) and 1 min (0% B) and 30 min (20% B), A = 2% MeCN, 0.1% TFA in H_2O, B = 2% H_2O, 0.1% TFA in MeCN)]. ^{1}H NMR (500 MHz, D_2O): 5.21 (t, 1H, H-3′, $J_{3',2'} = J_{3',4'} = 9.6$), 4.97 (t, 1H, H-4′, $J_{4',3'} = J_{4',5'} = 9.7$), 4.71−4.67 (m, 2H, P^{α}, N^{α}), 4.57 (d, 1H, T^{α}, $J = 3.7$), 4.54 (dd, 1H, K^{α}, $J_1 = 5.3$, $J_2 = 8.6$), 4.45 (t, 1H,S^{α}, $J_{\alpha,\beta} = 5.3$), 4.41−4.36 (m, 3H, H-1′, T^{α}, P^{α}), 4.31−4.24 (m, 2H, 2 × T^{β}), 4.13−4.19 (m, 2H, A^{α}, H-6′^{a}), 3.94−3.76 (m, 7H), 3.65−3.58 (m, 2H), 2.97 (t, 2H, K^{ϵ}, $J_{\epsilon,\delta} = 7.5$), 2.82 (dd, 1H, $N^{\beta a}$, $J_{\beta a,\beta b} = 15.7$, $J_{\beta a,\alpha} = 6.7$), 2.73 (dd, 1H, $N^{\beta b}$, $J_{\beta b,\beta a} = 15.7$, $J_{\beta b,\alpha} = 7.2$), 2.36−2.22 (m, 2H, 2 × $P^{\beta a}$), 2.07−1.95 (m, 19H, 5 × Ac, 2 × P^{γ}), 1.92−1.86 (m, 2H, 2 × $P^{\beta b}$), 1.80−1.72 (m, 1H, $K^{\beta a}$), 1.69−1.59 (m, 3H, K^{δ}, $K^{\beta b}$), 1.49−1.39 (m, 5H, K^{γ}), 1.30 (d, 3H, A^{β}, $J_{\beta,\alpha} = 7.1$), 1.21 (d, 3H, T^{γ}, $J_{\gamma,\beta} = 6.4$), 1.15 (d, 3H, T^{γ}, $J_{\gamma,\beta} = 6.4$). MALDI-MS (α-cyanocinnamic acid): 1186 (M + H^+), calcd 1186.7.

Enzymatic Solution-Phase Synthesis of O-SLe^x Octapeptides. Ac-Lys-Pro-Pro-Asn-Thr(Gal*β*1→4GlcNAc*β*)-Thr-Ser-Ala-HYCRON (22). A solution of **17** (9.5 mg, 7.1 μmol), UDP-Gal (5.3 mg, 9.0 μmol), and β1,4-galactosyltransferase (Sigma, 1 mg, 1 U) in 600 μL of buffer (50 mM HEPES, 5 mM $MnCl_2$, pH 7.0) was shaken for 16 h at 37 °C. After lyophilization the residue was purified by GPC (Biogel P4, 0.05M NH_4OAc). Lyophilization of the product fractions furnishes 9.3 mg of a white fluffy powder. Yield: 87%. t_R: 20.2 min [Vydac C18, 250 × 4 mm, 0 min (0% B) and 1 min (0% B) and 30 min (20% B), A = 2% MeCN, 0.1% TFA in H_2O, B = 2% H_2O, 0.1% TFA in MeCN)]. ESI-MS (neg): 1495 (M − H^-), calcd 1494.9.

Ac-Lys-Pro-Pro-Asn-Thr(Sialα2→3Gal*β*1→4GlcNAc*β*)-Thr-Ser-Ala-HYCRON (24). To a solution of **22** (3.7 mg, 2.5 μmol) and CMP-NeuNAc (2.1 mg, 3.2 μmol) in 350 μL of buffer (0.1 M HEPES, 5 mM $MnCl_2$, 0.2% Triton X-100, pH 7.0) α2,3-sialyltransferase (Cytel, 3 U/mL, 6.5 μL) and alkaline phosphatase (Boehringer Mannheim, 1 U/mL, 1 μL) were added. The mixture was shaken at 37 °C for 16 h and directly subjected to GPC (Biogel P4, 0.05M NH_4OAc). After lyophilization 4.4 mg of a white fluffy powder were obtained. Yield: 99%. t_R: 19.3 min [Vydac C18, 250 × 4 mm, 0 min (0% B) and 1 min (0% B) and 30 min (20% B), A = 2% MeCN, 0.1% TFA in H_2O, B = 2% H_2O, 0.1% TFA in MeCN)]. ESI-MS (pos): 1789 (M (1 × ^{13}C) + H^+), calcd 1789.0.

Ac-Lys-Pro-Pro-Asn-Thr(Sialα2→3Gal*β*1→4(Fucα1→3)GlcNAc*β*)-Thr-Ser-Ala-HYCRON (26). To a solution of **24** (2.5 mg, 1.3 μmol) and GDP-Fuc (1.2 mg, 1.9 μmol) in 200 μL of buffer (0.05 M MES, 15 mM $MnCl_2$, pH 6.0), α1,3-Fucosyltransferase (Cytel, 2.16 U/mL, 13 μL) and alkaline phosphatase (Boehringer Mannheim, 1 U/mL, 0.5 μL) were added. The mixture was shaken at 37 °C for 1 d before GDP-Fuc (1.0 mg, 1.6 μmol) and fucosyltransferase (10 μL, 22 mU) were added a second time. The mixture was shaken for 3 more days at 37 °C. Purification was achieved by GPC using a Biogel P2 column (0.05 M NH_4OAc) followed by a second GPC with a Biogel P4 column (0.05 M NH_4OAc). The product fractions were lyophilized to yield 1.4 mg of a white fluffy powder. Yield: 55%. t_R: 30.5 min [Microsorb, C18, 5μ, 250 × 4 mm, 0 min (0% B) and 2 min (0% B) and 30 min (10% B) and 40 min (40% B), A = 2% MeCN, 0.1% TFA in H_2O, B = 2% H_2O, 0.1% TFA in MeCN)]. ^{1}H NMR (500 MHz, D_2O, characteristic signals): 5.86 (m, 2H, $HYCRON^{H-15}$, $HYCRON^{H-16}$), 5.05 (m_c, 1H, Fuc^{H-1}), 2.96 (t, 2H, K^{ϵ}, $J_{\epsilon,\delta} = 7.5$), 2.87−2.81 (m, 1H, $N^{\beta a}$), 2.77−2.70 (m, 2H, $N^{\beta b}$, $Sial^{H-3a}$), 2.42 (t, 2H, $HYCRON^{H-2}$, $J_{2,3} = 6.7$), 2.34−2.21 (m, 2H, 2 × $P^{\beta a}$), 2.00, 1.98, 1.97 (3 × s, 3 × Ac), 1.39, 1.28, 1.21, 1.13 (4 × d, 2 × T^{γ}, A^{β}, Fuc^{H-6}). ESI-MS (neg): 1954 (M (1 × ^{13}C) + Na^+ − 2H^+), calcd 1954.1), 1933 (M (1 × ^{13}C) − H^+), calcd 1933.1, 1641 (M − Sial − H^+), calcd 1641.0.

Ac-Lys-Pro-Pro-Asn-Thr(Gal*β*1→4GlcNAc*β*)-Thr-Ser-Ala-OH (23). A solution of **18** (6.8 mg, 6.4 μmol), UDP-Gal (5.1 mg, 8.2 μmol) and 1 mg (1 U) of β1,4-galactosyltransferase (Sigma) in 550 μL of buffer (50 mM HEPES, 5 mM $MnCl_2$, pH 7.0) was shaken for 21 h at 37 °C. After lyophilization the residue was purified by GPC (Biogel P2, 0.1M NH_4HCO_3). Lyophilization of the product fractions gave 7.8 mg of a white fluffy powder. Yield: 100%. t_R: 14.4 min [Vydac C18, 250 × 4 mm, 0 min (0% B) and 2 min (0% B) and 30 min (10% B), A = 2% MeCN, 0.1% TFA in H_2O, B = 2% H_2O, 0.1% TFA in MeCN)]. ESI-MS (pos): 1245 (M + Na^+), calcd 1245.7, 1223 (M + H^+), calcd 1223.7.

Ac-Lys-Pro-Pro-Asn-Thr(Sialα2→3Gal*β*1→4GlcNAc*β*)-Thr-Ser-Ala-OH (25). α 2,3-Sialyltransferase (Cytel, 3 U/mL, 15 μL) and CMP-NeuNAc (4.9 mg, 7.4 μmol) were added to a solution of **23** (7.1 mg, 5.8 μmol) in 820 μL of buffer (0.1 M HEPES, 5 mM $MnCl_2$, 0.2% Triton X-100, pH 7.0). The mixture was shaken at 37 °C for 29 h before alkaline phosphatase (Boehringer Mannheim, 1 U/mL, 2.5 μL) was added. A second addition of CMP-NeuNAc (3.6 mg, 5.4 μmol) and sialyltransferase (15 μL, 45 mU) was added after 46 h. The mixture was lyophilized after two more days. Twofold GPC (Biogel P4, 0.1M NH_4HCO_3) and lyophilization of the product fractions gave 6.7 mg of product and 1.4 mg (20%) of starting material as white fluffy powders. Yield: 77%. t_R: 13.1 min [Vydac C18, 250 × 4 mm, 0 min (0% B) and 2 min (0% B) and 30 min (10% B), A = 2% MeCN, 0.1% TFA in H_2O, B = 2% H_2O, 0.1% TFA in MeCN)]. ^{1}H NMR (500 MHz, D_2O): 4.72−4.68 (m, 2H, N^{α}, P^{α}), 4.58−4.50 (m, 4H, $GlcNAc^{H1}$, Gal^{H1}, T^{α}, K^{α}), 4.45 (t, 1H, S^{α}, $J_{\alpha,\beta} = 5.4$), 4.41 (dd, 1H, P^{α}, $J_{\alpha,\beta 1} = 8.4$, $J_{\alpha,\beta 2} = 5.3$), 4.38 (d, 1H, T^{α}, $J_{\alpha,\beta} = 4.5$), 4.29 (dq, 1H, T^{β}, $J_{\beta,\gamma} = 6.3$, $J_{\beta,\alpha} = 4.0$), 4.24 (dq, 1H, T^{β}, $J_{\beta,\gamma} = 6.3$, $J_{\beta,\alpha} = 4.6$), 4.11 (q, 1H, A^{α}, $J_{\alpha,\beta} = 7.2$), 4.08 (dd, 1H, $J_1 = 9.9$, $J_2 = 3.0$), 3.96−3.76 (m, 10H), 3.72−3.52 (m, 14H), 2.98 (t, 2H, K^{ϵ}, $J_{\epsilon,\delta} = 7.5$), 2.84 (dd, 1H, $N^{\beta a}$, $J_{\beta a,\delta b} = 15.6$, $J_{\beta a,\alpha} = 6.6$), 2.78−2.69 (m, 2H, $N^{\beta b}$, $Sial^{H3a}$), 2.35 (m_c, 1H, $P^{\beta a}$), 2.27 (m_c, 1H, $P^{\beta a}$), 2.03−1.96 (m, 13H, 3 × Ac, 2 × P^{γ}), 1.94−1.86 (m, 2H, 2 × $P^{\beta b}$), 1.79−1.74 (m, 2H, $K^{\beta a}$, $Sial^{H3b}$), 1.68−1.65 (m, 3H, $K^{\beta b}$, K^{δ}), 1.47−1.43 (m_c, 2H, K^{γ}), 1.32 (d, 3H, A^{β}, $J_{\beta,\alpha} = 7.2$), 1.22 (d, 3H, T^{γ}, $J_{\gamma,\beta} = 6.4$), 1.14 (d, 3H, T^{γ}, $J_{\gamma,\beta} = 6.4$). MALDI-MS (α-cyanocinnamic acid): 1536 (M + Na^+), calcd 1536.8, 1514 (M + H^+), calcd 1513.8.

Ac-Lys-Pro-Pro-Asn-Thr(Sialα2→3Gal*β*1→4(Fucα1→3)GlcNAc*β*)-Thr-Ser-Ala-OH (1). GDP-Fuc (3.2 mg, 6.0 μmol) was dissolved in a solution of glycopeptide **25** (6.5 mg, 4.3 μmol) in buffer (660 μL, 50 mM MES, 15 mM $MnCl_2$, pH 6.0). α1,3-Fucosyltransferase (Cytel, 2.16 U/mL, 43 μL) and alkaline phosphatase (Boehringer Mannheim,

1 U/μL, 1.5 μL) were added. After 2 d of shaking at 37 °C GDP-Fuc (3.0 mg, 5.6 μmol) and fucosyltransferase (43 μL) were added. The mixture was shaken for 2 more days at 37 °C prior to a third addition of GDP-Fuc (2.0 mg, 3.8 μmol), fucosyltransferase (20 μL), and alkaline phosphatase (1.0 μL). After 43 h at 37 °C the mixture was lyophilized. The residue was dissolved in water and filtered through cotton. The residue obtained after lyophilization was purified by 2-fold GPC (Biogel P4, 0.1 M NH_4HCO_3) to yield a lyophilisate (6.2 mg) which still contains starting material. Further purification was achieved by preparative HPLC [Vydac C18, 250 × 20 mm, 2 mL/min, 0 min (0% MeCN in H_2O) and 5 min (0% MeCN in H_2O) and 40 min (5% MeCN in H_2O)] to yield a material (4.2 mg) that according to analytical HPLC contains 7% of starting material. Yield: 59%. t_R: 13.0 min [Vydac C18, 250 × 4 mm, 0 min (0% B) and 2 min (0% B) and 30 min (10% B), A = 2% MeCN, 0.1% TFA in H_2O, B = 2% H_2O, 0.1% TFA in MeCN)]. ^{1}H NMR (600 MHz, ^{1}H,^{1}H TOCSY, ^{1}H,^{1}H COSY, D_2O): 5.08 (Fuc^{H-1}), 4.80 (Fuc^{H-5}), 4.72 (N^{α}, P^{α}), 4.59 ($GlcNAc^{H-1}$), 4.58 (L^{α}), 4.55 (T^{α}), 4.50 (Gal^{H-1}), 4.42 (P^{α}), 4.39 (T^{α}), 4.30 (T^{β}), 4.25 (T^{β}), 4.15 (A^{α}), 4.07 (Gal^{H-3}), 3.88 (Fuc^{H-3}), 3.87 ($P^{\delta a}$, $HlcNAc^{H-2}$), 3.84 ($Sial^{H-5}$), 3.80 ($P^{\delta a}$), 3.76 (Fuc^{H-4}), 3.66 (FucH-2, SialH-4, $P^{\delta b}$), 3.64 ($Sial^{H-6}$), 3.61 ($P^{\delta b}$), 3.54 ($GlcNAc^{H-3}$), 3.50 (Gal^{H-2}), 3.00 (L^{ϵ}), 2.85 ($N^{\beta a}$), 2.77 ($N^{\beta b}$), 2.75 ($Sial^{H-3a}$), 2.36 ($P^{\beta a}$), 2.29 ($P^{\beta a}$), 2.04 (P^{γ}), 2.03 (P^{γ}), 1.92 ($P^{\beta b}$), 1.91($P^{\beta b}$), 1.78 ($L^{\beta a}$), 1.69 (L^{γ}, $L^{\beta a}$), 1.47 (L^{γ}), 1.33 (A^{β}), 1.23 (T^{γ}), 1.15 (Fuc^{H-6}, T^{γ}). MALDI-MS (α-cyanocinnamic acid): 1705 (M − H^+ + 2Na^+), calcd 1704.9, 1683 (M + Na^+), calcd 1682.9, 1661 (M + H^+), calcd 1660.9, 1536 (M − Fuc + Na^+), calcd 1536.8, 1514 (M − Fuc + H^+), calcd 1514.8, 1391 (M − Sial + Na^+), calcd 1390.8, 1369 (M − Sial + H^+), calcd 1368.8.

Chemoenzymatic Solid-Phase Synthesis of 18, 23, and 25. Loading of the Aminopropyl-CPG. To aminopropyl-CPG (Sigma, 700 Å, 5.0 g, 77 μmol NH_2/g) was added a solution of Fmoc-Ala-HYCRON **3** (902 mg, 1.54 mmol) in DMF (13 mL). To the suspension HOBt (328 mg, 2.3 mmol) were added NMM (429 μL, 3.1 mmol) and HBTU (584 mg, 1.54 mmol). After 20 h of shaking at room temperature the solution was filtered off and the polymer washed with DMF. The support was treated with pyridine/Ac_2O (3:1, 15 mL) for 10 min. After filtration and washing with dichloromethane the solid support was dried *in vacuo* to give 5.11 g of material. The loading was determined by photometric detection of the Fmoc cleavage product. Loading: 61 μmol Fmoc/g.

Ac-Lys(Boc)-Pro-Pro-Asn(Trt)-Thr(β-GlcNAc)-Thr(tBu)-Ser(tBu)-Ala-HYCRON-CPG (27). The synthesis starts from 3.16 g (193 μmol) of Fmoc-Ala-HYCRON-CPG (61 μmol Fmoc/g). **Fmoc Removals.** Shaking in DMF/morpholine (1:1, 16 mL) for 54 min was followed by filtration and washing with DMF. **Couplings.** The resin was treated with a 4-fold excess of the Fmoc-amino acid in a 0.08 M solution in DMF, which contains 1.5 equiv of HOBt, 2.0 equiv of NMM, and 1.0 equiv of HBTU per equivalent Fmoc-amino acid. After 3−5 h of shaking the reactants were removed by filtration. The support was washed with DMF. The following amino acid derivatives have been used in the following order: Fmoc-Ser(tBu) and Fmoc-Thr(tBu). **Capping.** The resin was suspended in Pyr/Ac_2O (3:1, 8 mL) and shaken for 6 min. Reactants were removed by filtration and washing with DMF.

After coupling of Fmoc-Thr(tBu) and subsequent capping, the resin was washed with dichloromethane and dried *in vacuo* to give 3.17 g of solid support. 1.55 g of the resin were used for the completion of the synthesis. The Fmoc removals were performed by using DMF/morpholine (1:1, 10 mL) according to the decribed protocol. **12a** was coupled in a 3-fold excess, the following amino acid derivatives (Fmoc-Asn(Trt), 2 × Fmoc-Pro, Fmoc-Lys(Boc)) in a 5-fold excess. No capping was performed after the introduction of the unprotected carbohydrate. After coupling of the *N*-terminal Fmoc-Lys the support was washed with dichloromethane and dried *in vacuo* to give 1.55 g. The loading of the support was determined photometrically to be 50 μmol Fmoc/g. **Acetylation of the *N*-Terminus.** See **14**.

Ac-Lys-Pro-Pro-Asn-Thr(β-GlcNAc)-Thr-Ser-Ala-OH (18). Removal of Side Chain Protective Groups. To **27** (0.2 g) was added TFA/ethanedithiol/anisol (40:1:1, 2.1 mL). The suspension was shaken for 1 h. After washing with dichloromethane the support was resuspended in TFA/ethanedithiol/anisole (40:1:1, 4.2 mL) and shaken for 30 min. The support was washed with dichloromethane and DMF. **Peptide Cleavage.** The resin was suspended in DMF/DMSO (1:1, 4 mL) and morpholine (0.5 mL) was added. Degassing was achieved by streaming argon through the fritted disk of the reaction vessel. After 5 min, a microspatula of tetrakis(triphenylphosphine)palladium was added. The mixture was shaken in argon under exclusion of light for 16 h. The resin was repeatedly washed with DMF followed by water. **Purification.** The filtrate was conctrated *in vacuo*. The residue was dissolved in MeOH. The product was precipitated through addition of ether. The precipitate was dissolved in water and filtered. The crude was purified by GPC (Biogel P2, 0.05 M NH_4OAc) and preparative HPLC [Vydac C18, 250 × 20 mm, 0 min (0% B) and 5 min (0%B) and 30 min (15% B), A = 2% MeCN, 0.1% TFA in H_2O, B = 2% H_2O, 0.1% TFA in MeCN]. After lyophilization 2.9 mg of a white fluffy powder were obtained. Yield: 20% (based on the TFA salt of **18** and the initial loading of the solid support with amino acid).

Ac-Lys-Pro-Pro-Asn-Thr(Galβ1→4GlcNAcβ)-Thr-Ser-Ala-OH (23). Removal of Side Chain Protective Groups. To **27** (0.15 g) was added TFA/ethanedithiol/anisole (40:1:1, 4.2 mL). The suspension was shaken for 45 min. After washing with TFA and dichloromethane the procedure was repeated once more. The support was dried *in vacuo*. **Galactosylation.** The support was transferred into an Eppendorf vial and washed with buffer (50 mM HEPES, 5 mM $MnCl_2$, pH 7.0) by repeated vortexing, centrifuging, and removing of the supernatant. UDP-Gal (10.0 mg, 16.4 μmol) and galactosyltransferase (Sigma, 1 mg, 1U) in 0.6 mL of buffer (50 mM HEPES, 5 mM $MnCl_2$, pH 7.0) were added. The suspension was shaken for 4 d at 37 °C. After centrifugation the supernatant was removed and the support washed by repeated vortexing, centrifuging, and removing of the supernatant. The supernatants were collected and worked up (see purification). **Peptide Cleavage.** See **18**. The resin was repeatedly washed with DMF and water. **Purification.** The combined supernatants collected after the galactosylation were lyophilized. The residue was purified by GPC (Biogel P2, 0.05 M NH_4OAc) followed by preparative HPLC [Vydac C18, 250 × 20 mm, 0 min (0% B) and 5 min (0%B) and 30 min (5% B), A = 2% MeCN, 0.1% TFA in H_2O, B = 2% H_2O, 0.1% TFA in MeCN]. Lyophilization gave 1.0 mg of the product. Yield: 8% (based on the TFA salt of **23** and the initial loading of the solid support with amino acid).

The filtrate obtained after the peptide cleavage was concentrated *in vacuo*. The residue was dissolved in MeOH. The product was precipitated through addition of ether. The precipitate was dissolved in water and filtered. The crude was purified by GPC (Biogel P2, 0.05 M NH_4OAc) and preparative HPLC [Vydac C18, 250 × 20 mm, 0 min (0% B) and 5 min (0%B) and 30 min (15% B), A = 2% MeCN, 0.1% TFA in H_2O, B = 2% H_2O, 0.1% TFA in MeCN]. After lyophilization 1.2 mg of a white fluffy powder was obtained. According to analytical HPLC the material was a 2.1:1 mixture of the LacNAc octapeptide **23** and the GlcNAc octapeptide **18**. Yield: 7% **23**, 3% **18** (based on the TFA salts and the initial loading of the solid support with amino acid).

Ac-Lys-Pro-Pro-Asn-Thr(Sialα2→3Galβ1→4GlcNAcβ)-Thr-Ser-Ala-OH (25). Removal of Side Chain Protective Groups. To **27** (0.15 g) was added TFA/ethanedithiol/water (40:1:1, 6.0 mL). The suspension was shaken for 45 min. After washing with TFA the procedure was repeated once more. The support was washed with dichloromethane and dried *in vacuo*. **Galactosylation.** The support was transferred into an Eppendorf vial and washed with buffer (50 mM HEPES, 5 mM $MnCl_2$, 0.2 Triton X-100, pH 7.0) by repeated vortexing, centrifuging, and removing of the supernatant. UDP-Gal (7.0 mg, 11.5 μmol) and galactosyltransferase (1 mg, 1 U) in 375 μL of buffer (50 mM HEPES, 5 mM $MnCl_2$, pH 7.0) were added. The suspension was shaken for 40 h at 37 °C before UDP-Gal (4.0 mg, 6.5 μmol) and galactosyltransferase (Sigma, 50 U/mL 25 mM HEPES in H_2O/glycerol 1:1, pH 7.0, 13 μL, 650 mU) were added. After shaking the suspension for 26 h at 37 °C alkaline phosphatase (1 μL, 1U) was added. The suspension was shaken for one more hour. **Sialylation.** To the suspension were added 1.3 M HEPES (25 μL, 32.5 μmol), CMP-NeuNAc (5.3 mg, 8.3 μmol), and sialyltransferase (Cytel, 3 U/mL, 20 μL, 60 mU). The suspended support was shaken for 2 d at 37 °C prior to a second addition of CMP-NeuNAc (3.0 mg, 4.7 μmol) and sialyltransferase (10 μL, 30 mU). After 2 d the resin was filtered off and repeatedly washed with water. The filtrates were collected and

worked up (see purification). **Peptide Cleavage.** See **18**. The resin was repeatedly washed with DMF and water. **Purification.** The combined filtrates collected after the sialylation were lyophilized. The residue was dissolved in water and filtered through a nylon cartridge (Gelman, 0.2 μm). Preparative HPLC [Vydac C18, 250 $\times$ 20 mm, 0 min (0% MeCN in H_2O) 5 min (0% MeCN in H_2O) 30 min (5% MeCN in H_2O)] gave 1.5 mg of a material which was subjected to GPC (Biogel P4, 0.15 M NH_4HCO_3) for further purification. Lyophilization furnished 1.3 mg of the product **25** and 0.2 mg of the unsialylated **23**. Yield: 9% **25**, 2% **23** (based on the initial loading of the solid support with amino acid).

The filtrate obtained after the peptide cleavage was concentrated *in vacuo*. The residue was dissolved in MeOH. The product was precipitated through addition of ether. The precipitate was dissolved in water and filtered through a nylon cartridge (Gelman, 0.2 μm). Analytical HPLC shows a small amount of the desired product **25** as part of a complicated mixture.

Acknowledgment. This work was supported by the NIH and NSF. O.S. is grateful for a postdoctoral fellowship provided by the Deutsche Forschungsgemeinschaft.

Supporting Information Available: The synthesis and characterization of compounds **2**−**12b** (8 pages). See any current masthead page for ordering and Internet access instructions.

JA971383C