JOC*Article*

Glucuronic Acid Derivatives as Branching Units for the Synthesis of Glycopeptide Mimetics

Niels Röckendorf and Thisbe K. Lindhorst*

Institut für Organische Chemie, Christian-Albrechts-Universität zu Kiel,
Otto-Hahn-Platz 3, 24098 Kiel, Germany

tklind@oc.uni-kiel.de

Received February 20, 2004

Natural glycopeptides and glycoproteins exhibit a large structural diversity, which can be mimicked by synthetic glycopeptide derivatives to assist the investigation of biological functions and structure−activity relationships. Here, dendronized saccharides were synthesized to provide glycosyl amino acids, equipped with a branching element for the preparation of branched glycopeptide mimetics. An optimized Staudinger-type reaction served as key reaction en route to the complex glycopeptide **14**, in which three mannose moieties were connected to the branched glucuronyl scaffold.

Introduction

The glycan moieties of glycoproteins are implicated in a large number of important biological processes such as correct protein folding, protein secretion, or cell−cell recognition.[1] Understanding of these processes is hampered by difficulties associated with the "microheterogeneity" of glycoproteins[2] and the limited availability of glycopeptides and glycoproteins for biological studies, their chemical synthesis being a demanding and time-consuming task.[3]

As a consequence of these problems, chemists have sought novel methods for the synthesis of structural analogues of glycopeptides, so-called glycopeptide mimetics,[4] which are easier to synthesize as well as more easily structurally varied than their natural counterparts. Literature-known examples for the synthesis of glycopeptide mimetics regard variation at the anomeric linkage, such as S- and C-glycosides,[5] glycosyl oximes,[6] or glycosyl ureas.[7] Other approaches have utilized the 6-position of the sugar ring for further derivatization, such as in the case of oxime[8] or peptide linkages.[9]

It was our goal to provide glycosyl amino acids equipped with a dendron moiety to allow an easy and flexible access to branched glycopeptide mimetics of variable sugar composition. We decided to utilize the 6-position of a glucuronic acid derivative for the attachment of a dendritic partial structure. The synthetic concept applies peptide coupling chemistry and avoids tedious glycosylation protocols at an advanced stage of the synthesis. Figure 1 illustrates the general architecture of the targeted glycopeptide mimetics.

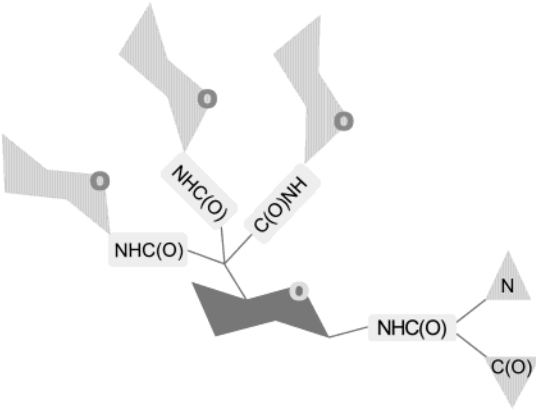


FIGURE 1. General architecture of the targeted glycopeptide mimetics. A glycodendron moiety is attached to the 6-position of a glucuronic acid core molecule, while the anomeric position of the glucuronic acid core can be used for further functionalization such as peptide coupling to amino acids.

Results and Discussion

The synthesis started from glucuronic acid, which was first acetylated following a well-established procedure[10] leading to the fully protected glucopyranuronic acid derivative **1**. The carboxyl function at the 6-position of **1** could be connected to the readily available aminotri-

---

(1) Parodi, A. J. *Annu Rev. Biochem.* **2000**, *69*, 69−93. Aebi, M. *Science* **2001**, *291*, 2364−2369.

(2) Rush, R. S.; Derby, P. L.; Smith, D. M.; Merry, C.; Rogers, G.; Rohde, M. F.; Katta V. *Anal. Chem.* **1995**, *67*, 1442−1452.

(3) Kunz, H.; Schulz, M. In *Oligosaccharides in Chemistry and Biology−A Comprehensive Handbook*; Wiley VCH: Weinheim, 2000.

(4) Marcaurelle, L. A.; Bertozzi, C. R. *Chem. Eur. J.* **1999**, *5*, 1384−1390.

(5) Sparks, M. A.; Williams, K. W.; Whitesides, G. M. *J. Med. Chem.* **1993**, *36*, 78−82.

(6) Zhao, Y.; Kent S. B. H.; Chait B. T. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 1629−1633. Cervigni, S: E.; Dumy P.; Mutter, M. *Angew. Chem.* **1996**, 108 1325−1327; *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 1230−1232.

(7) Ichikava, Y.; Nishiyama, T.; Isobe, M. *Synlett* **2000**, 1253−1256.

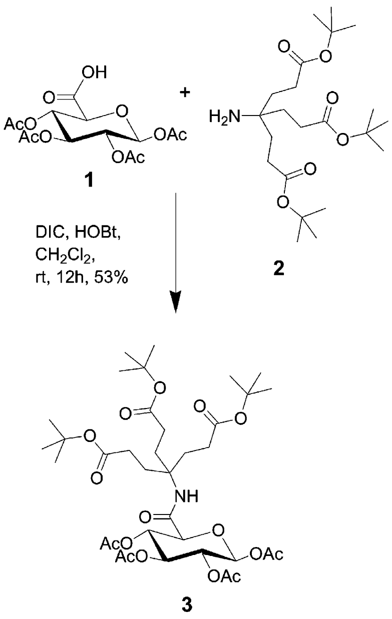
(8) Rodriguez, E. C.; Winans, K. A.; King, D. S.; Bertozzi C. R. *J. Am. Chem. Soc.* **1997**, *119*, 9905−9909.

(9) Gervay-Hague, J.; Weathers, T. M. In *Glycochemistry, Principles Synthesis and Applications*; Marcel Dekker: New York, 2001.

(10) Malkinson, J. P.; Falconer, R. A.; Toth, I. *J. Org. Chem.* **2000**, *65*, 5249−5252.

10.1021/jo049708c CCC: $27.50

SCHEME 1. Synthesis of Dendron-Modified Glucuronic Acid Derivative 3



ester **2**,[11] which was used as branching element. Peptide coupling, which was performed under standard conditions using DIC/HOBt as the coupling reagents gave the dendron-functionalized carbohydrate derivative **3** even on a multigram scale after an easy and inexpensive purification step on silica gel.

To allow the synthesis of a variety of more complex glycoconjugates, further functionalization of the dendronized saccharide **3** at the anomeric center was investigated. First, compound **3** was converted into a glycosyl donor in the form of the trichloroacetimidate **5**. This was accomplished in two steps, by regioselective cleavage of the anomeric acetyl group to yield the reducing derivative 2,3,4-tri-*O*-acetyl-6-{3-cascade:amidomethane[3]:propanoic-acid-*tert*-butylester}-α-D-glucuronic-acid **4**, followed by reaction with trichloroacetonitrile, which led to the glycosyl donor **5** in 71% yield over two steps, again on a multigram scale. Trichloroacetimidate **5** is produced under basic conditions, which do not affect the *tert*-butylester protecting groups of the dendritic moiety of the molecule.

Unfortunately, all of our attempts to accomplish *O*-glycoside synthesis using trichloroacetimidate **5** as the glycosyl donor were not successful, although the preparation of *tert*-butylester-protected glycopeptide building blocks using the same strategy has been reported in the literature.[12] In our hands *O*-glycoside synthesis with **5** employing Lewis acids such as TMSOTf, $BF_3{\cdot}Et_2O$, or $SnCl_4$ failed as a result of partial cleavage of the *tert*-butylester protecting groups. Even glycosyl transfer under neutral conditions using chloral as the catalyst[13] did not lead to the desired product.

On the other hand, introduction of an anomeric azido group to activate glucuronic acid for *N*-glycoside formation started a successful route to complex glycopeptide mimetics. Treatment of the glucuronic acid derivative **1** with $TMSN_3$ under Lewis acid catalysis using $SnCl_4$ led to the literature-known[10] glycosyl azide **6**. The procedure reported in the literature was slightly modified in that the reaction was carried out at 0 °C to prevent warming up of the reaction mixture during $SnCl_4$ addition. At higher temperatures the yield of the desired glycosyl azide **6** decreased while the starting material **1** underwent cyclization to give the corresponding ϵ-lactone.[14]

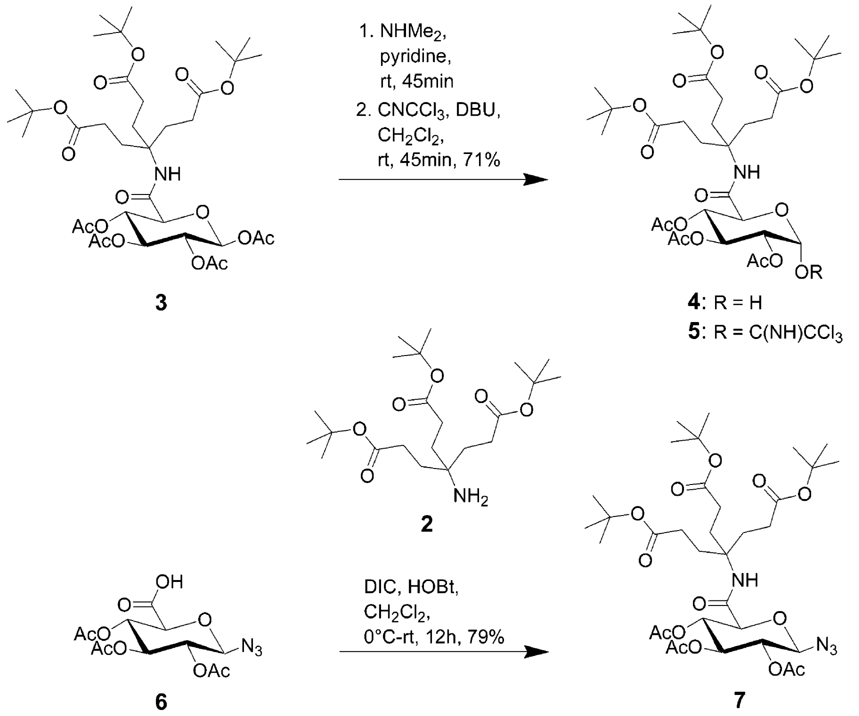
The glycosyl azide **6** that was obtained under these reaction conditions could be coupled to the dendritic amine **2** in analogy to the synthesis of **3**, using DIC/HOBt in CH_2Cl_2. An optimized purification procedure led to a significantly improved yield of **7**. The dendronized glycosyl azide **7** represents a building block, which is suited for *N*-glycopeptide synthesis and furthermore provides three protected carboxylic acid functions for the attachment of carbohydrate moieties, e.g., via peptide coupling.

Commonly, *N*-glycopeptides are prepared from the corresponding glycosylamines, which can be obtained by aminolysis of unprotected carbohydrates in saturated ammonium hydrogen solution[15] or by catalytic reduction of glycosyl azides.[16] Both of these strategies suffer from the disadvantage that glycosylamines are relatively unstable; they are prone to anomerization and tend to dimerize under formation of diglycopyranosylamines. To circumvent this problem we decided to convert azide **7** directly into the corresponding glycosyl amide without previous reduction to an amine. According to a modified Staudinger reaction,[17] glycosyl azides can be coupled to an activated carboxylic acid function in the presence of a trialkyl- or triarylphosphines, respectively, to give glycosyl amides, a method that has been applied to *N*-glycoside synthesis in a variety of examples.[18-20] In this study we have elaborated this method with the dendronized azide **7** using different Fmoc-protected amino acid derivatives under a variety of reaction conditions. When, according to the literature,[20] glycosyl azide **7** was treated with the β-amino acid derivative **8** and tributylphosphine in acetonitrile at −30 °C, no product formation was observed. When the reactivity of the amino acid **8** was enhanced by using the corresponding acid chloride[19] or active ester derivatives,[10] only traces of the desired glycosyl amide were observed using triphenylphosphine in acetonitrile or THF at −30 °C. Even when tributylphosphine was used with activated **8**, the glycosyl azide **7** proved to be unreactive in the Staudinger reaction, even at ambient temperatures, possibly because of steric hindrance by the *tert*-butylester groups. Finally, we found reaction conditions allowing a successful synthesis, as shown for two examples. The desired glycopeptide mimetics **9** and **11** were obtained in good yields

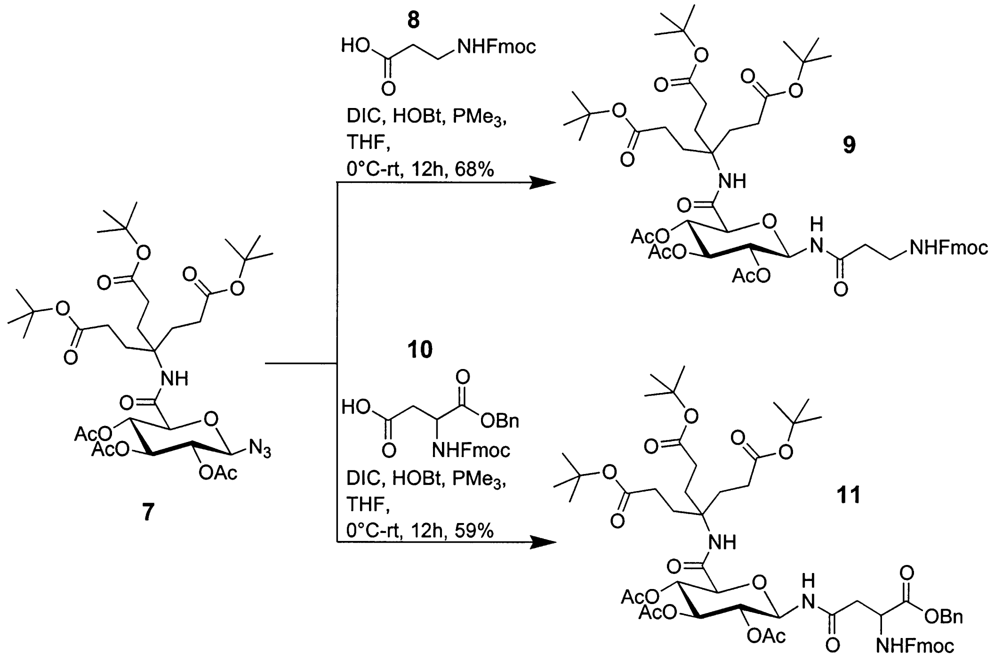
---

[11] Newkome, G. R.; Weis, C. D. *OPPI Briefs* **1996**, *28*, 495−498.
[12] Mathieux, N.; Paulsen, H.; Meldal, M.; Bock, K. *J. Chem. Soc., Perkin Trans. 1* **1997**, 2359−2368.
[13] Schmidt, R. R.; Gade, H.; Jatzke, H. *Tetrahedron Lett.* **1990**, *31*, 3, 327−330.
[14] Lee, B. Y.; Thurmon, T. F. *Clin. Chim. Acta* **1993**, *218*, 215−222.
[15] Monsigny, M.; Quétard, C.; Bougerie, S.; Delay, D.; Pichon, C.; Midoux, P.; Mayer, R.; Roche, A. C. *Biochimie* **1998**, *80*, 99−108.
[16] Van Ameijde, J.; Bauke Albada, H.; Liskamp, R. M. J. *J. Chem. Soc., Perkin Trans. 1* **2002**, 1042−1049.
[17] Gololobov, Y. G.; Kasukhin, L. F. *Tetrahedron* **1992**, *48*, 1353−1406.
[18] Inazu, T.; Kobayashi, K. *Synlett* **1993**, 869−870.
[19] Maunier, V.; Boullanger, P.; Lafont, D. *J. Carbohydr. Chem.* **1997**, *16*, 231−235.
[20] Mizuno, M.; Muramoto, I.; Kobayashi, K.; Yaginuma, H.; Inazu, T. *Synthesis* **1999**, 162−165.

SCHEME 2. Generation of Dendron-Modified Glycosyl Donors for *O*- and *N*-Glycoside Formation



SCHEME 3. Conversion of Dendron-Functionalized Glycosyl Azide 7 into *N*-Glycosides via a Modified Staudinger Reaction



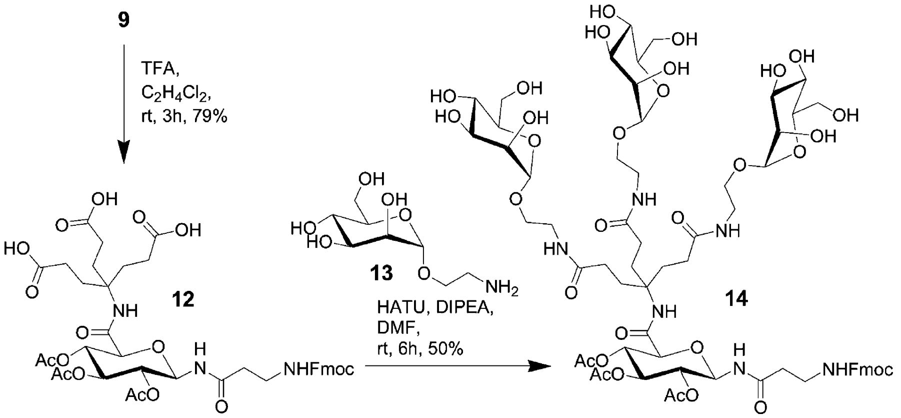
when the amino acid derivatives **8** and **10** were employed as their HOBt esters and trimethylphosphine was used at a temperature of 0 °C. Formation of a stable trialkyliminophosphorane derivative, which inhibits amide bond formation as reported earlier,[10,20] was not observed using trimethylphosphine, even at higher temperatures.

With the building blocks **9** and **11** in hand, the field is open for a variety of further functionalizations at the branched triester moiety attached at the 6-position of the sugar ring, such as peptide coupling to biologically relevant carbohydrate epitopes. This was exemplified with glycopeptide **9**, which was subjected to 95% aqueous TFA to cleave the *tert*-butylester groups to obtain the triacid **12**, which had to be purified by preparative reversed phase HPLC. Then 2-aminoethyl mannoside **13**[21] was selected for peptide coupling with the triacid **12**. The highly reactive guanidinium coupling reagent HATU was used in this conversion in order to prevent structural defects in this step, which were observed when

---

[21] Kieburg, C.; Sadalapure, K.; Lindhorst, T. K. *Eur. J. Org. Chem.* **2000**, *11*, 2035–2040.

SCHEME 4. Synthesis of Branched Glycopeptide Mimetic 14 via Peptide Coupling



the carbodiimide method was applied instead. HATU-promoted peptide coupling allowed the synthesis of the complex, branched glycopeptide mimetic **14**. Moderate yields around 50% were due to the tedious purification procedure, which was necessary. First, the reaction mixture had to be subjected to GPC on a sephadex column to remove small-molecule impurities, and in a second purification step reversed phase HPLC was used to provide the pure target molecule **14** after lyophilization.

Characterization of all glycopetide mimetics reported herein was achieved using ESI mass spectrometry and two-dimensional NMR specroscopy. Purities were determined by reversed phase HPLC.

Conclusions

Concluding our work described herein, it is important to emphasize the Staudinger reaction envolving the stable glycosyl azide **7** as a key step of the synthetic route. This reaction could be optimized for the synthesis of dendronized glycopeptide mimetics such as **9** and **11**, resembling valuable oligofunctional building blocks of an A_3B- and A_3BC-type, respectively. These provide an entry into an array of more complex glycopeptide mimetics including an option for solid-phase synthesis to oligomerize or further elaborate the branched monomers into multivalent glycoconjugates, suitable for glycobiological investigations.

Experimental Section

1,2,3,4-Tetra-*O*-acetyl-6-{3-cascade:amidomethane[3]:propanoic-acid-*tert*-butylester}-*β*-D-glucuronic-acid 3. Tetraacetyl-glucuronic acid **1** (9.08 g, 25.08 mmol) was mixed with 10.42 g (25.08 mmol) of amine **2**, and 3.59 g (26.61 mmol) of HOBt was added. The mixture was dissolved in 150 mL of dry CH_2Cl_2 under an argon atmosphere, and 5.01 mL (32.17 mmol) DIC was added at 0 °C. The mixture was stirred overnight at room temperature, the solvent was partly removed under reduced pressure, and the precipitate formed was filtered off. The filtrate was concentrated under reduced pressure and purified by MPLC (A = cyclohexane, B = ethyl acetate, 30% B 10 min → 30% B → 40% B 60 min, 20 mL/min) to yield a foamy solid (10.14 g (13.3 mmol, MW = 759.83 g mol) 53%). ^1H NMR (300 MHz, $CDCl_3$): δ 6.25 (br. s, 1H), 5.75 (d, 1H, $J_{1,2} = 8.1$ Hz), 5.26 (dd $\approx$ t, 1H, $J_{2,3} = 9.2$ Hz), 5.15 (dd $\approx$ t, 1H, $J_{4,5} = 9.7$ Hz), 5.12 (dd, 1H, $J_{2,3} = 9.2$ Hz $J_{1,2} = 8.1$ Hz), 3.92 (d, 1H, $J_{4,5} = 9.7$ Hz), 2.2 (m_c, 9H), 2.05, 2.04, 2.01 (s, each 3H), 1.93 (m_c, 6H), 1.45 (s, 27H) ppm. ^{13}C NMR (75.47 MHz, $CDCl_3$): δ 172.6, 172.6, 169.8, 169.3, 168.7, 164.9, 91.2, 80.7, 73.5, 72.0, 70.2, 69.0, 57.7, 29.5, 29.4, 28.1, 20.8, 20.6. 20.6, 20.4 ppm. ESI-MS: *m/z* 782.3499 [M + Na^+] (782.3570 calcd for $C_{36}H_{57}NO_{16}$ + Na).

2,3,4-Tri-*O*-acetyl-6-{3-cascade:amidomethane[3]:propanoic-acid-*tert*-butylester}-α-D-glucuronyl-trichloroacetimidate 5. Acetate **3** (2.29 g, 3.01 mmol) was dissolved in 30 mL of pyridine, 15 mL of dimethylamine (33% solution in ethanol) was added, and the solution was stirred for 30 min. Next, 100 mL of toluene was added, and the mixture was washed with 150 mL of brine and 5 times with 100 mL of water. The organic layer was dried over $MgSO_4$. After filtration the solvent was removed under reduced pressure, and the residue was filtered through silica gel (cyclohexane/ethyl acetate 3:1). The solvent was removed under reduced pressure, and the residual alcohol **4** (1.51 g, 2.11 mmol) was dissolved in 30 mL of CH_2Cl_2. Then, 2.5 mL (25.22 mmol) of trichloroacetonitrile and 0.1 mL of DBU were added, and the solution was stirred overnight at room temperature. The mixture was concentrated under reduced pressure, and the residue was purified by MPLC (A = cyclohexane, B = ethyl acetate, 25% B, 20 mL/min, 60 min) to yield a colorless amorphous solid (1.47 g (1.71 mmol, MW = 862.18 g mol) 71%). ^1H NMR (300 MHz, $CDCl_3$): δ 8.75 (s, 1H), 6.6 (d, 1H, $J_{1,2} = 3.6$ Hz), 6.58 (br. s, 1H), 5.58 (dd $\approx$ t, 1H, $J_{2,3} = 9.9$ Hz), 5.23 (dd, 1H, $J_{4,5} = 9.9$ Hz), 5.12 (dd, 1H, $J_{2,3} = 9.9$ Hz $J_{1,2} = 3.6$ Hz), 4.29 (d, 1H, $J_{4,5} = 9.9$ Hz), 2.2 (m_c, 6H), 2.06, 2.04, 2.03 (s, each 3H), 1.95 (m_c, 6H), 1.44 (s, 27H) ppm. ^{13}C NMR (75.47 MHz, $CDCl_3$): δ 172.6, 169.9, 169.6, 165.4, 160.7, 92.2, 90.4, 80.6, 71.3, 69.4, 67.0, 57.6, 29.7, 29.5, 28.0, 20.6. 20.6, 20.4 ppm; ESI-MS: *m/z* 883.3665 [M + Na^+] (883.2560 calcd for $C_{36}H_{55}Cl_3N_2O_{15}$ + Na).

1-Azido-1-deoxy-2,3,4-tri-*O*-acetyl-6-{3-cascade:amidomethane[3]:propanoic-acid-*tert*-butylester}-*β*-D-glucuronic-acid 7. Azide **6** (1.88 g, 5.4 mmol) was mixed with 2.25 g (5.45 mmol) of amine **2** and 0.75 g (5.51 mmol) of HOBt. The mixture was dissolved in 50 mL of dry CH_2Cl_2, and under an argon atmosphere 0.86 mL (5.51 mmol) of DIC was added at 0 °C. The reaction mixture was allowed to warm to room temperature and was stirred overnight. The solvent was partly removed under reduced pressure, and the precipitate formed was filtered off. The filtrate was concentrated under reduced pressure and purified by MPLC on silica gel (A = cyclohexane, B = ethyl acetate, 30% B, 20 mL/min, 60 min) to yield a colorless amorphous solid (3.19 g (4.3 mmol, MW = 742.81 g mol^{-1}) 79%). ^1H NMR (300 MHz, $CDCl_3$): δ 6.64 (br s, 1H), 5.25 (dd $\approx$ t, 1H, $J_{2,3} = 9.4$ Hz), 5.13 (dd $\approx$ t, 1H, $J_{4,5} = 9.6$ Hz), 4.96 (dd, 1H, $J_{2,3} = 9.3$ Hz $J_{1,2} = 8.9$ Hz), 4.72 (d, 1H, $J_{1,2} = 8.9$ Hz), 3.88 (d, 1H, $J_{4,5} = 9.8$ Hz), 2.23 (m_c, 6H), 2.09, 2.03, 2.01 (s, each 3H), 1.95 (m_c, 6H), 1.45 (s, 27H) ppm. ^{13}C NMR (75.47 MHz, $CDCl_3$): δ 172.6, 172.6, 172.6, 169.8, 169.2, 164.9, 87.7, 80.6, 75.1, 71.8, 70.5, 69.0, 57.7, 29.7, 29.5, 28.0, 20.5, 20.5, 20.5 ppm. ESI-MS: *m*/*z* 765.3156 [M + Na^+] (765.3529 calcd for $C_{34}H_{54}N_4O_{14}$ + Na).

1-*N*-[(9-Fluorenyl-methoxycarbonyl)-*β*-alanyl]-1-deoxy-2,3,4-tri-*O*-acetyl-6-{3-cascade:amidomethane[3]:propanoic-acid-*tert*-butylester}-*β*-D-glucuronic-acid 9. Azide **7** (120 mg, 0.16 mmol) was mixed with 93 mg (0.3 mmol) of *N*-Fmoc-*β*-alanine **8** and 40 mg (0.3 mmol) of HOBt. The mixture was dissolved in 20 mL of dry THF, 46.7 *μ*L (0.3 mmol) of DIC was added under an argon atmosphere, and the solution was stirred for 10 min at 0 °C. Next, 200 *μ*L of a 1 M solution of trimethylphosphine in dry THF (2 mmol) was added, and the mixture was stirred for 1 h at 0 °C and finally was stirred overnight at room temperature. Water (50 mL) was added, and the solution was extracted three times with 50 mL of ethyl acetate. The combined organic phases were washed with 50 mL of brine and subsequently dried over $MgSO_4$. After filtration, the solvent was removed under reduced pressure, and the residue was purified by MPLC on silica gel (A = cyclohexane, B = ethyl acetate, 33% B, 20 mL/min, 60 min). Remaining impurities were removed via GPC on Sephadex LH-20 (MeOH) to yield a colorless glass (110 mg (0.11 mmol, MW = 1010.13 g mol^{-1}) 68%). ^1H NMR (500 MHz, $CDCl_3$): δ 7.75, 7.55, 7.36, 7.29 (m_c, each 8H), 6.47 (br. d, 1H), 6.38 (br s, 1H), 5.48 (br. s, 1H), 5.33 (dd ≈ t, 1H), 5.29 (dd ≈ t, 1H), 5.09 (dd ≈ t, 1H), 4.91 (dd ≈ t, 1H), 4.38 (m_c, 2H), 4.21 (m_c, 1H), 3.91 (d, 1H, $J_{4,5}$ = 9.9 Hz), 3.49 (m_c, 2H), 2.49 (m_c, 2H), 2.17 (m_c, 6H), 2.07, 2.02, 1.99 (s, each 3H), 1.92 (m_c, 6H), 1.43 (s, 27H) ppm. ^{13}C NMR (125.47 MHz, $CDCl_3$): δ 172.7, 171.7, 171.0, 169.6, 169.3, 165.3, 156.4, 143.9, 141.3, 127.7, 127.0, 125.1, 125.0, 120.0, 80.6, 77,8, 74.7, 71.9, 70.62, 69.5, 66.8, 57.7, 47.2, 36.6, 36.1, 29.7, 29.6, 28.1, 20.6, 20.5, 20.5 ppm. ESI-MS: *m*/*z* 1032.5989 [M + Na^+] (1032.4676 calcd for $C_{52}H_{71}N_3O_{17}$ + Na).

1-*N*-[(9-Fluorenyl-methoxycarbonyl)-l-benzyl-asparagyl]-1-deoxy-2,3,4-tri-*O*-acetyl-6-{3-cascade:amidomethan[3]:propanoic-acid-*tert*-butylester}-*β*-D-glucuronic-acid 11. Azide **7** (250 mg, 0.56 mmol) was mixed with 300 mg (0.41 mmol) of *N*-Fmoc-benzyl-L-asparagine **10** and 81 mg (0.6 mmol) of HOBt. The mixture was dissolved in 50 mL of dry THF, 90 *μ*L (0.6 mmol) DIC was added under an argon atmosphere, and the solution was stirred for 10 min at 0 °C. Next. 600 *μ*L of a 1 M solution of trimethylphosphine in dry THF (2 mmol) was added, and the mixture was stirred for 1 h at 0 °C and finally was stirred overnight at room temperature. Water (100 mL) was added, and the solution was extracted three times with 100 mL of ethyl acetate. The combined organic phases were washed with 100 mL of brine and subsequently dried over $MgSO_4$. After filtration the solvent was removed under reduced pressure. The residue was purified by MPLC on silica gel (A = cyclohexane, B = ethyl acetate, 40% B, 20 mL/min, 60 min), remaining impurities were removed in a GPC on Sephadex LH-20 (MeOH) to yield a colorless glass (277 mg (0.24 mmol, MW = 1144.26 g mol^{-1}) 59%). ^1H NMR (500 MHz, $CDCl_3$): δ 7.73, 7.55, 7.36, 7.28 (m_c, 13H, aryl-H), 6.56 (br d, 1H), 6.41 (br s, 1H), 6.14 (br d, 1H), 5.33 (dd ≈ t, 1H, $J_{2,3}$ = 9.6 Hz), 5.27 (dd ≈ t, 1H, $J_{1,2}$ = 9.2 Hz), 5.17 (m_c, 2H), 5.08 (dd ≈ t, 1H, $J_{3,4}$ = 9.7 Hz), 4.88 (dd ≈ t, 1H), 4.69 (m_c, 1H), 4.45−4.25 (m, 2H), 4.17 (m_c, 1H), 3.88 (d, 1H, $J_{4,5}$ = 9.9 Hz), 2.91 (dd, 1H, *J* = 4.2, 16.2 Hz), 2.79 (dd, 1H, *J* = 3.3, 16.1 Hz), 2.19 (m_c, 6H), 2.01, 2.00, 1.99 (s, each 3H), 1.89 (m_c, 6H), 1.41 (s, 27H) ppm. ^{13}C NMR (125.47 MHz, $CDCl_3$): δ 172.7, 172.5, 169.6, 169.3, 165.2, 156.2, 143.8, 143.7, 141.2, 135.2, 128.6, 128.3, 128.2, 127.9, 127.8, 127.1, 125.1, 119.9, 80.6, 77.8, 74.8, 71.8, 70.4, 69.5, 67.4, 67.4, 57.7, 50.7, 47.0, 37.7, 29.9, 29.7, 29.5, 28.0, 20.6, 20.5, 20.4 ppm. ESI-MS: *m*/*z* 1166.6105 [M + Na^+] (1166.5043 calcd for $C_{60}H_{77}N_3O_{19}$ + Na).

1-*N*-[(9-Fluorenyl-methoxycarbonyl)-*β*-alanyl]-1-deoxy-2,3,4-tri-*O*-acetyl-6-{3-cascade:amidomethan[3]:propanecarboxylic-acid}-*β*-D-glucuronic-acid 12. *tert*-Butylester **9** (308 mg, 0.3 mmol) was dissolved in 20 mL of 95% TFA (aq) and stirred for 1 h at room temperature. Toluene (50 mL) was added, and the solvent was removed under reduced pressure at room temperature. The residue was purified by preparative HPLC on RP-8 silica gel (A = water, B = acetonitrile, 20% B → 70% B, 10 mL/min, 70 min, RZ 39 min), and pure fractions were lyophylized to yield the target triacid as a white lyophylizate (177 mg (0.21 mmol, MW = 841.81 g mol^{-1}) 69%). ^1H NMR (300 MHz, MeOH-d_4): δ 7.84, 7.67, 7.42, 7.34 (m_c, 8H), 5.36 (d, 1H, $J_{1,2}$ = 9.3 Hz), 5.35 (dd ≈ t, 1H), 5.26 (dd ≈ t, 1H), 5.08 (dd ≈ t, 1H), 4.38 (m_c, 2H), 4.23 (m_c, 1H), 4.15 (d, 1H, $J_{4,5}$ = 9.7 Hz), 3.41 (m_c, 2H), 2.48 (m_c, 2H), 2.28 (m_c, 6H), 2.06 (m_c, 6H), 2.05, 2.01, 1.99 (s, each 3H), ppm. ^{13}C NMR (75.47 MHz, MeOH-d_4): δ 178.3, 174.5, 171.5, 171.3, 171.0, 168.0, 158.7, 145.3, 142.6, 128.8, 128.2, 126.2, 120.9, 79.1, 76.7, 74.6, 71.7, 70.7, 67.7, 59.3, 48.4, 37.9, 37.0, 31.1, 30.2, 20.7, 20.6, 20.5 ppm. ESI-MS: *m*/*z* 864.3613 [M + Na^+] (864.2798 calcd for $C_{36}H_{55}Cl_3N_2O_{15}$ + Na).

1-*N*-[(9-Fluorenyl-methoxycarbonyl)-*β*-alanyl]-1-deoxy-2,3,4-tri-*O*-acetyl-6-{3-cascade:amidomethane[3]:propane-(1-amidoethyl-*β*-D-mannopyranoside)}-*β*-D-glucuronic-acid 14. Amine **13** (45 mg, 0.2 mmol) was mixed with 76 mg (0.2 mmol) of HATU and 50 mg (0.06 mmol) of the triacid **12** and dissolved in 10 mL of dry, strictly amine-free DMF. Next, 68 *μ*L of DIPEA was added ,and the reaction mixture was stirred for 6 h at room temperature. The solvent was removed under reduced pressure, and the residue was taken up in methanol and then partially purified via GPC on Sephadex LH-20. Product-containing fractions were collected, the solvent was removed under reduced pressure, and the residue was taken up in water for further purification via preparative HPLC on RP-8 silica gel (A = water, B = acetonitrile, 5% B → 60% B, 60 min, RZ 52 min). After lyophlization of the Product fractions the target glycoconjugate was obtained as a white lyophylizate (43 mg (29 *μ*mol, MW = 1457.44 g mol^{-1}) 50%). ^1H NMR (500 MHz, MeOH-d_4): δ 7.83, 7.68, 7.43, 7.34 (m_c, 8H), 5.02 (d, 1H, $J_{1,2}$ = 8.9 Hz), 4.74 (m, 3H), 4.37 (d, 2H), 4.24 (t, 1H), 3.88 (m_c, 6H), 3.85 (d, 1H, $J_{4,5}$ = 9.8 Hz), 3.79 (m_c, 3H), 3.76 (m_c, 6H), 3.65 (m ≈ t, 3H), 3.57 (m_c, 7H), 3.49 (m_c, 1H), 3.44 (m_c, 8H), 3.37 (m_c, 1H), 2.54 (m_c, 2H), 2.25 (m_c, 6H), 2.05 (s, 9H), 2.02 (m_c, 6H) ppm. ^{13}C NMR (125.47 MHz, MeOH-d_4): δ 176.0, 174.9, 173.4, 170.6, 158.8, 145.3, 142.6, 128.8, 128.2, 126.2, 120.9, 101.7, 81.2, 78.8, 78.5, 74.8, 73.5, 72.9, 72.5, 72.1, 68.7, 67.8, 67.2, 63.0, 59.7, 48.7, 40.4, 38.0, 37.2, 31.8, 31.2, 20.4 ppm. ESI-MS: *m*/*z* 1479.6866 [M + Na^+] (1479.5648 calcd for $C_{64}H_{92}N_6O_{32}$ + Na).

Acknowledgment. Support of this work by FCI and BMBF is gratefully acknowledged.

Supporting Information Available: ^1H and ^{13}C NMR spectra for compounds **3**, **5**, **7**, **9**, **11**, **12**, and **14**. This material is available free of charge via the Internet at http://pubs.acs.org.

JO049708C