Synthesis of "Mixed Type" Oligosaccharide Mimetics Based on a Carbohydrate Scaffold

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In multivalent glycoligands including cluster glycosides, one type of sugar epitope is normally clustered. Such molecules have been valuable tools for investigation and inhibition of cell adhesion processes. They have, for example, served as in vitro antiadhesives in mannose-specific bacterial adhesion. Wild-type bacteria, however, utilize a number of different sugar epitopes for adhesion, involving bacterial lectins of different specificity. The synthesis of glycomimetics containing sugar derivatives from different sugar series is therefore of interest, with the eventual goal of providing multivalent glycoligands of a more complex type. The synthesis of novel "mixed" glycoclusters, starting from the trifunctional galactoside **10** that serves as a carbohydrate scaffold, is hence presented here. Compound **10** has three potential reactive sites, which were addressed successively. Firstly, peptide coupling with the carboxy-functionalized mannoside **11** furnished the disaccharide mimetic **13**, which in turn provided the trisaccharide mimetic **19** after peptide coupling with the amino-functionalized fucoside **17**. Finally, a pentasaccharide mimetic **23** was obtained after thiourea-bridging with acetylated lactosyl isothiocyanate.

Introduction

Naturally occurring glycoconjugates contain complex oligosaccharides of high structural diversity, which are able to encode biological information.[1−3] This is decoded in specific molecular recognition events[4] involving saccharide portions as the carbohydrate ligands and proteins as receptors; these are called lectins and selectins, respectively.[5] Carbohydrate−protein interactions mediate such important cellular processes as signal transduction, immune response, or adhesion of pathogens to their host cells.[6] Synthetic glycoligands have been valuable tools with which to investigate possible interference with these interactions and to obtain a better understanding of their molecular details.[7,8] They have been designed as oligosaccharide mimetics or as structurally simplified mimetics of multivalent glycoconjugates,[9] as shown with, for example, neoglycopolymers or glycodendrimers.[10,11] In all cases, the principal architecture of these synthetic glycoligands has comprised a core molecule serving as an oligovalent scaffold, carbohydrate epitopes, and spacers to link the sugar epitopes to the core molecule. Recently, carbohydrate derivatives − termed "octopus glycosides" − have been introduced[12] and used as core molecules for the synthesis of cluster glycosides:[13] as pentavalent ligands for shiga-like toxins, for example.[14] All the approaches so far chosen for the synthesis of multivalent glycoligands have in common that it has always been one type of glycoligand presented on a multivalent scaffold.[10] The orthogonal derivatization of a carbohydrate scaffold would allow successive attachment of different sugar moieties, implying the synthesis of more complex oligosaccharide mimetics. Orthogonally protected carbohydrate derivatives were introduced as core molecules for combinatorial chemistry,[15−17] and carbohydrate derivatives have only recently been derivatized to serve as scaffold molecules.[18]

In our group, an ABC-type building block has been synthesized; it is based on mannose and bears three orthogonally protected functions A, B, and C, with three free hydroxy groups retained in the sugar ring.[19] We have used this building block as a core molecule for the synthesis of oligomannoside mimetics, including a dendritic high-molecular-weight derivative, as we are especially interested in the inhibition of mannose-specific adhesion of *Escherichia coli*[20,21] by cluster mannosides and similar glycomimetics.[22] In this contribution we have extended this concept to access glycomimetics of a "mixed" type. The use of carbohydrate moieties from different sugar series in a successive manner should make oligosaccharide mimetics of the complex and hybrid types available, similarly to the high-mannose-type oligosaccharides synthesized earlier.[19] Such mixed-type glycomimetics should be of great utility for the synthesis of lectin ligands as well as for the provision of antiadhesives for a "multi-lectin"-mediated adhesion scenario.

To fulfil the outlined concept, an ABC-type galactoside (**10**; Scheme 2) was chosen as the core saccharide and subsequently subjected to coupling reactions, firstly with a

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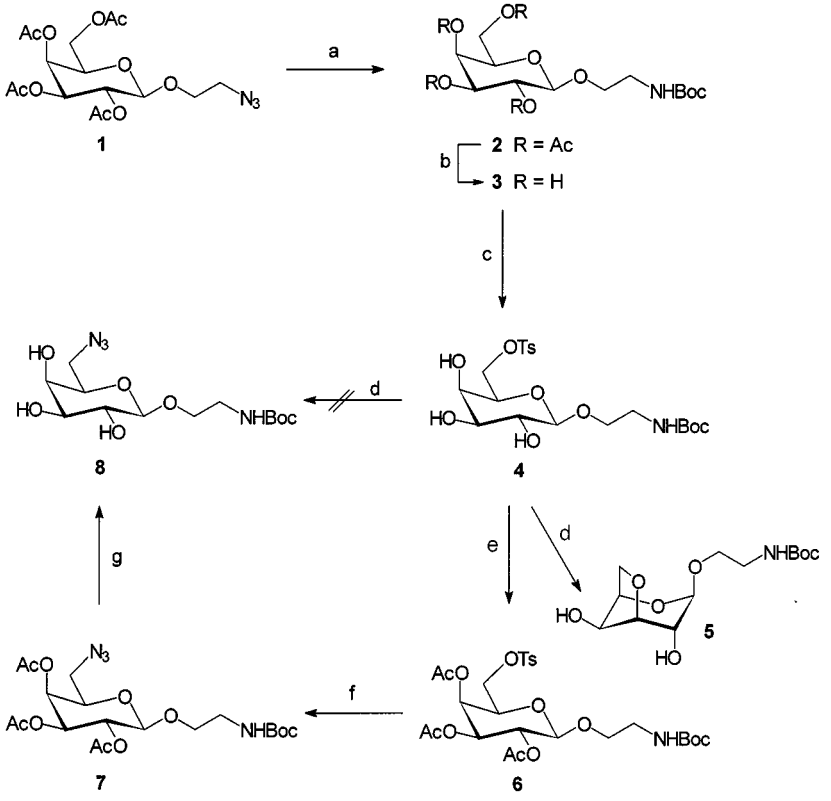
mannoside derivative, then an L-fucoside moiety, and finally a lactose derivative, to provide a pentasaccharide mimetic in high yield with a minimum of protecting group chemistry.

Results and Discussion

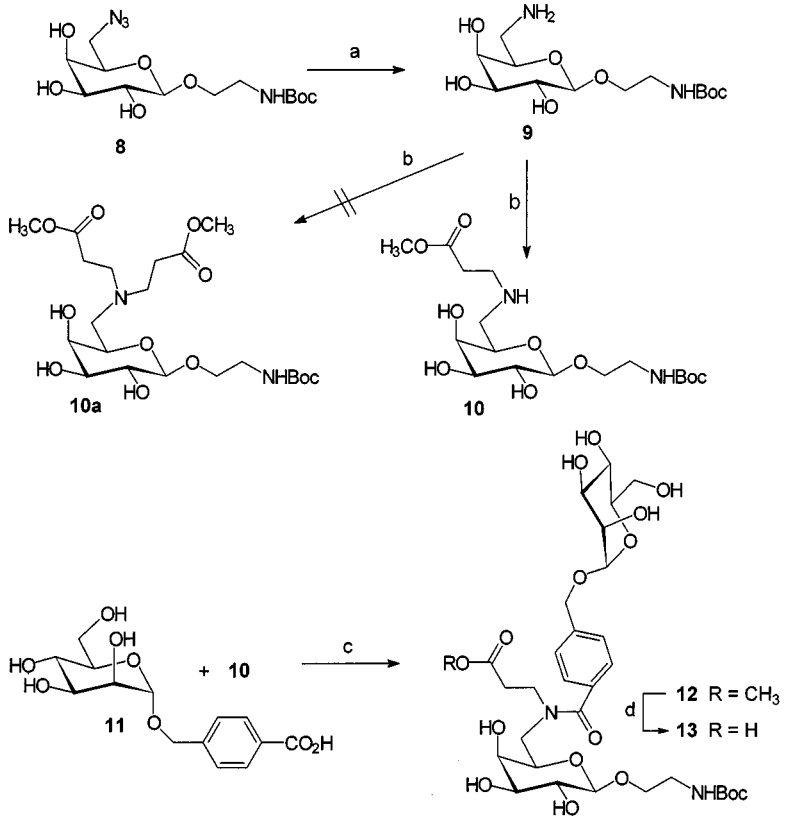
Galactoside **10** (Scheme 2) was chosen as the central building block, with three (potential) reactive sites. It was synthesized starting from the known 2-azidoethyl β-D-galactopyranoside **1** (Scheme 1),[23] which was subjected to Pd-catalyzed hydrogenation in the presence of Boc_2O in order to protect the resulting free amine in situ, thus avoiding *O*- → *N*-acetyl group migration. This procedure furnished the acetylated *N*-Boc-protected galactoside **2** in 81% yield. Zemplén deprotection[24] afforded the tetraol **3**, which was in turn regioselectively tosylated to give **4** in 61% overall yield. Surprisingly, when the 6-*O*-tosylate **4** was subjected to a nucleophilic displacement reaction using sodium azide in DMF, the 3,6-anhydro derivative **5** was obtained in high yield, instead of the desired azide **8**. A singlet for the anomeric proton and a large downfield shift of the 3-H signal from δ = 3.45 to δ = 3.92 in the ^{1}H NMR spectrum of **5** indicated formation of the anhydro ring,[25] which may occur through a $^1C_4$ conformation of **4**, in equilibrium with the more favored $^4C_1$ conformation. MS data (306.1 for [M + H]$^+$) further confirmed the structure of **5**. Sodium azide obviously reacts in this case as a base rather than a nucleophile, which has not been observed in the analogous case in the mannose series.[19]

In order to avoid anhydro ring formation, the free hydroxy groups of **4** were protected prior to the nucleophilic displacement reaction. Tosylate **4** was carefully treated at −10 °C with acetic anhydride in pyridine, to provide the tri-*O*-acetylated galactoside **6** in 97% yield. This allowed the synthesis of the unprotected 6-azido-6-deoxy galactoside derivative **8** without problems, after deacetylation of **7**. Catalytic hydrogenation of **8** with palladium on charcoal yielded the corresponding amine **9**, which was then subjected to a Michael-type reaction with methyl acrylate to afford the Michael-type mono-adduct **10** in 60% overall yield (Scheme 2). Interestingly, the bis-addition product **10a** could not be obtained even when an excess of methyl acrylate[26] at elevated reaction temperatures (ca. 40 °C) was used in the treatment of the amine **9**. This may be attributable to steric hindrance caused by the axial 4-hydroxy group in **10**.

Galactoside **10** represents a trifunctional carbohydrate scaffold molecule in which the three functionalities can be successively addressed in further reactions. Thus, the secondary amino function in **10** was subjected to peptide coupling with *p*-(α-D-mannosyloxy)methylbenzoic acid (**11**)[27] by using the onium salt based coupling reagent HATU.[28] This resulted in the mixed disaccharide mimetic **12** (Scheme 2), bearing a galactosyl and a mannosyl residue, in

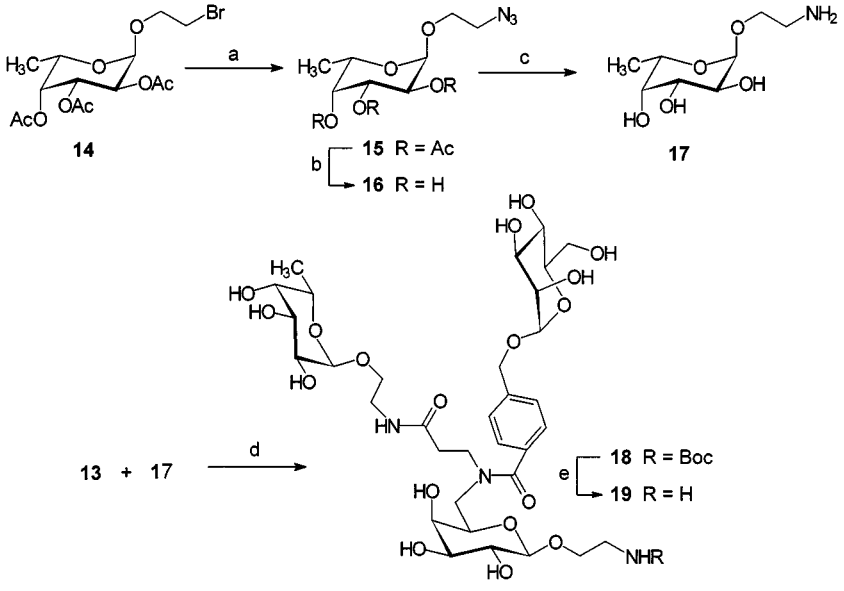


Scheme 1. a) Pd/C, H_2, $(Boc)_2O$, ethyl acetate, room temp., 12 h, 81%; b) NaOMe/MeOH, 0 °C → room temp., 1 h, 96%; c) TsCl, pyridine, 0 °C → room temp., 2 h, 64%; d) NaN_3, DMF, 80 °C, 24 h, (**5**: 82%; **8**: 0%); e) Ac_2O, pyridine, −10 °C → +4 °C, 12 h, 97%; f) NaN_3, DMF, 80 °C, 24 h, 72%; g) NaOMe/MeOH, 0 °C → room temp., 1 h, 98%

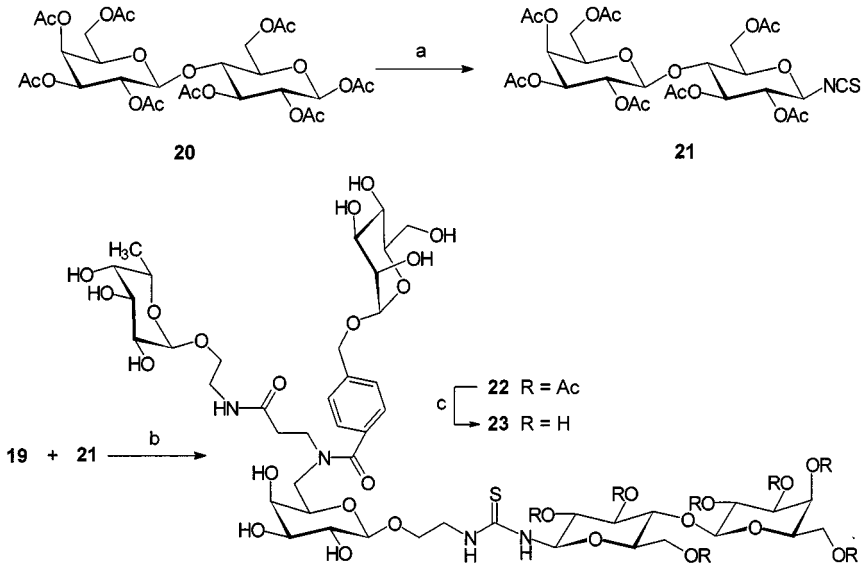


Scheme 2. a) Pd/C, H_2, MeOH, room temp., 6 h; b) methyl acrylate (excess), MeOH, room temp. → 40 °C, 15 h, 60% over two steps; c) HATU, DIPEA, DMF, room temp.→ 45 °C, 12 h, 79%; d) LiOH·H_2O, MeOH/H_2O (2:1), 0 °C, 12 h, quant.

79% yield. Interestingly, it was observed that the galactoside **10** and the disaccharide mimetic **12** are prone to undergo ester hydrolysis in deuterated protic solvents. When NMR samples of **10** and **12** in $[D]_4$MeOH were stored overnight at ambient temperature and then recorded, their ^1H and ^{13}C NMR spectra revealed the disappearance of those signals corresponding to the protons (δ = 3.63) and carbon atoms (δ = 52.6) of the methyl ester. Concomitantly, additional signals appeared at δ = 3.33 and δ = 49.84, representing liberated methanol and thus indicating methyl ester hydro-



Scheme 3. a) NaN_3, TBABr, DMF, 60 °C, 30 min; b) NaOMe/MeOH, 0 °C → room temp., 1 h, 70% over two steps; c) Pd/C, H_2, MeOH, room temp., 6 h, quant.; d) HATU, DIPEA, DMF, room temp. → 45 °C, 12 h, 56%; e) Me_2S/CF_3CO_2H (1:2), 0 °C, 4 h, 88%



Scheme 4. a) $SnCl_4$ (1 M in CH_2Cl_2), TMSNCS, DCE (dichloroethane), 50 °C, 3 h, 75% (β/α = 2:1); b) DMF, DIPEA, 50 °C, 12 h; c) NH_3/MeOH, 0 °C, 12 h, 83% over two steps

lysis by the trace amount of water present in [D_4]methanol. This observation with **10** and **12** was only made in [D]$_4$MeOH and D_2O, and not in [D]$_6$acetone.

In order to synthesize a trisaccharide mimetic based on galactoside **10**, compound **12** was saponified to the corresponding acid **13**, which was carried forward into a second peptide coupling reaction, with 2-aminoethyl α-L-fucoside (**17**) as the amine component. This could easily be obtained from the known bromo derivative **14**[29] in three steps (Scheme 3). HATU-assisted peptide coupling of the amine **17** and the disaccharide mimetic **13** gave the trisaccharide mimetic **18** in 56% yield. The Boc-protected amino function in **18** was then liberated in an anhydrous mixture of trifluoroacetic acid and dimethyl sulfide (2:1)[30] to yield the corresponding free amine **19**, without affecting the glycosidic bond. To increase the yield in the next step, it was necessary to neutralize final traces of TFA carefully with aqueous ammonia during the workup and to desalt the crude amine on a short Bio-gel P-2 column.

Finally, amine **19** was ligated to the lactosyl isothiocyanate **21** by thiourea bridging. The disaccharide isothiocyanate could easily be obtained from the corresponding peracetate by using trimethylsilyl isothiocyanate (TMSNCS)[31] in the presence of $SnCl_4$ in preference to other methods (Scheme 4).[32] Thus, the pentasaccharide mimetic **22** was obtained when the amine **19** was treated with the lactosyl isothiocyanate **21** in dry DMF, and deacetylation of the crude product with methanolic ammonia gave the fully deprotected glycocluster **23**. After purification by GPC on Bio-gel P-2 with ammonium hydrogen carbonate buffer as the eluent, pure **23** was obtained in 83% yield over two steps.

The structures of all the synthesized oligosaccharide mimetics could be clearly confirmed by their NMR and MALDI-TOF-MS data. The ^{1}H NMR spectra of the di- and trisaccharide analogues **12** and **18**, respectively, showed double and broad signals for 1-H, and less pronounced signals for 2-H and 3-H, an effect caused by (*E*)/(*Z*) isomerism occurring in the NHBoc group, due to the partial double-bond character of the Boc C−N bond.[33] In compound **23** a similar multiplet pattern due to the presence of (*E*) and (*Z*) stereoisomers resulting from the thiourea linkage was observed.

Conclusion

Our goal was to achieve easy and quick access to complex oligosaccharide mimetics based on an orthogonally protected oligofunctional core saccharide. It was demonstrated that, starting from the trifunctional carbohydrate scaffold **10**, oligosaccharide mimetics of a "mixed" type (compounds **12**, **18**, **23**) containing carbohydrate moieties of differently configured sugar series, could be produced easily and quickly by employing ligation chemistries alternative to glycosidation. The synthetic route demonstrated here can be applied for the synthesis of a large number of different oligosaccharide mimetics and glycoconjugates. The concept is open to a variety of modifications, such as solid-phase synthesis, combinatorial approaches, biolabeling, or multivalent presentation. Exploration of the different arrangements of functional groups provided by such types of oligosaccharide mimetics may also provide new lead structures for the design of potent antiadhesives. In addition, if it is considered that adhesion of wild-type bacteria is dependent on more than only one type of fimbriae, but rather utilizes sugar epitopes of different configuration, as is also the case in many other natural adhesion processes, "mixed" glycoclusters may offer great potential in this context.

Experimental Section

Abbreviations: HATU: *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate; DIPEA: diisopropylethylamine; CCA: α-Hydroxy-4-cyanocinnamic acid; DHB: Dihydroxybenzoic acid; gal: galactose; man: mannose; fuc: fucose; glc: glucose; gal′: galactoside of lactose moiety; *i*PrOH: 2-propanol.

General Remarks: For TLC, Merck silica gel 60 F_{254} plates were used. Flash chromatography was performed on silica gel 60 (230−400 mesh, 40−63 μm, Merck) and detection was carried out by charring with 20% ethanolic sulfuric acid solution containing 5% of α-naphthol and under UV light when applicable. For size exclusion chromatography, Sephadex LH-20 with methanol as the eluent and Bio-gel P-2 and Bio-gel P-6 with 15 mM aq. NH_4HCO_3 buffer (pH = 7.8−8.0) as the eluent were used. Organic solutions were concentrated in a rotary evaporator at bath temperatures < 45 °C. Aqueous solutions were concentrated by lyophilization. ^{1}H and ^{13}C NMR spectra were recorded with Bruker ARX 300 and DRX 500 instruments at 298 K, at 300 MHz (for ^{1}H), 75.46 MHz (for ^{13}C NMR), and at 500 MHz (for ^{1}H), 125.84 MHz (for ^{13}C NMR). Chemical shifts are given in ppm relative to internal TMS (δ = 0.00 for ^{1}H and ^{13}C NMR) and, when the samples were measured in D_2O, the spectra were calibrated relative to internal HOD (δ = 4.63) for ^{1}H NMR and, in the case of ^{13}C NMR, to [D_4]MeOH (δ = 49.30 for ^{13}C NMR), which was added to the solution. Coupling constants (*J*) are given in Hz. Two-dimensional ^{1}H-^{1}H and ^{1}H-^{13}C-COSY (HMQC) experiments were performed for complete signal assignments wherever necessary. IR spectra were recorded with FT-IR ATI Mattson Instruments (USA). MALDI-TOF mass spectra were recorded with Bruker Biflex and EI-MS with Finnigan MAT 8230 machines. For MALDI-TOF measurements, the samples were prepared as solutions in acetonitrile/water/TFA (2:1:0.1) with a concentration of 1 mg/mL solution. Compounds were co-crystallized with either DHB or CCA. The mass peaks obtained with all the samples were calibrated in reference to the $[M + H]^+$ peaks of angiotensin II (1046.54), angiotensin I (1296.69), bombesin (1619.82), and to the $[2 M + H]^+$ peak of α-cyano-4-hydroxycinnamic acid (380.02). Optical rotations were recorded at the Na-D line, 589 nm, 20 °C, cell length 10 cm and 1 cm in special cases. Elemental analyses were carried out in the Institute of Inorganic Chemistry, Christian-Albrechts-University, Kiel.

2-(*tert*-Butyloxycarbonylamido)ethyl 2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranoside (2): A suspension of 2-azidoethyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside (**1**;[23] 4.00 g, 9.59 mmol), activated Pd catalyst (10% on charcoal; 100 mg) and di-*tert*-butyl dicarbonate (3.14 g, 14.38 mmol) in ethyl acetate (30 mL) was hydrogenated under atmospheric pressure for 6 h at room temp. The reaction mixture was then filtered through a thin Celite bed and the filtrate was concentrated. The crude product was purified by flash chromatography (ethyl acetate/toluene, 1:1) to afford the Boc-protected galactoside **2** (3.80 g, 7.73 mmol, 81%) in the form of a white solid. $[\alpha]_D$ = +2.4 (*c* = 1.23 in $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.40 (d, $J_{3,4}$ = 3.1 Hz, 1 H, 4-H), 5.20 (dd, $J_{2,3}$ = 10.7, $J_{1,2}$ = 8.2 Hz, 1 H, 2-H), 5.02 (dd, $J_{2,3}$ =10.7, $J_{3,4}$= 3.5 Hz, 1 H, 3-H), 4.93 (s, 1 H, NH), 4.47 (d, $J_{1,2}$ = 7.7 Hz, 1 H, 1-H), 4.17 (dd, $J_{6a,6b}$ = 11.2, $J_{5,6a}$ = 6.7 Hz, 1 H, 6-H_a), 4.13 (dd, $J_{6a,6b}$ = 11.2 Hz, 1 H, 6-H_b), 3.95−3.85 (m, 2 H, 5-H, OCH_a), 3.65 (m_c, 1 H, OCH_b), 3.33 (m_c, 2 H, CH_2N), 2.16, 2.08, 2.05, 1.99 (each s, each 3 H, 4 $COCH_3$), 1.44 (s, 9 H, *t*Bu). ^{13}C NMR (100.67 MHz, $CDCl_3$): δ = 170.7, 170.6, 170.5, 169.9 (4 *C*OCH_3), 156.3 (BocCO), 102.0 (C-1), 79.8 [*C*$(CH_3)_3$], 71.2 (C-5, C-3), 70.2 (OCH_2), 69.3 (C-2), 67.4 (C-4), 61.7 (C-6), 40.8 (CH_2N), 28.8 [C(*C*$H_3)_3$], 21.2, 21.1, 20.9 (4 CO*C*H_3). $C_{21}H_{33}NO_{12}$ (491.49): calcd. C 51.32, H 6.77, N 2.85; found C 50.93, H 6.94, N 2.93.

2-(*tert*-Butyloxycarbonylamido)ethyl β-D-Galactopyranoside (3): A freshly prepared solution of NaOMe in MeOH (1 M, 1 mL) was added at 0 °C to a solution of the Boc-protected galactoside **2** (3.80 g, 7.73 mmol) in methanol (40 mL), and the mixture was stirred at room temp. for 1 h. The basic reaction mixture was then neutralized with methanolic HCl solution (10%) and concentrated. The crude product was purified by flash chromatography (MeOH/CH_2Cl_2, 1:10) to yield tetraol **3** (2.40 g, 7.42 mmol, 96%) as a white amorphous solid. $[\alpha]_D$ = −1.5 (*c* = 1.08 in MeOH). ^{1}H NMR (400 MHz, [D_4]MeOH): δ = 4.22 (d, $J_{1,2}$ = 7.1 Hz, 1 H, 1-H), 3.88 (m_c, 1 H, OCH_a), 8.82 (d, 1 H, 4-H), 3.78−3.68 (m, 2 H, 3-H, 6-H_a), 3.61 (m_c, 1 H, OCH_b), 3.55−3.44 (m, $J_{1,2}$ = 7.1, $J_{5,6b}$ = 6.1 Hz, 3 H, 2-H, 6-H_b, 5-H), 3.25 (m_c, 2 H, CH_2N), 1.45 (s, 9 H, *t*Bu). ^{13}C NMR (100.62 MHz, [D_4]MeOH): δ = 158.5 (BocCO), 105.1 (C-1), 80.1 [*C*$(CH_3)_3$], 76.7 (C-4), 74.8 (C-3), 72.5 (C-2), 70.2 (C-5), 70.0 (OCH_2), 62.5 (C-6), 41.5 (CH_2N), 28.8 [C(*C*$H_3)_3$]. $C_{13}H_{25}NO_8$ (323.34): calcd. C 48.29, H 7.79, N 4.33; found C 47.98, H 7.80, N 4.30.

2-(*tert*-Butyloxycarbonylamido)ethyl 6-*O*-(4-Tolylsulfonyl)-β-D-galactopyranoside (4): The tetraol **3** (2.33 g, 7.20 mmol) was dissolved in pyridine (10 mL) and treated with tosyl chloride (1.51 g, 7.92 mmol) at 0 °C under argon. The reaction mixture was then allowed to attain room temp. and was further stirred for 2 h. Excess tosyl chloride was quenched by adding MeOH at 0 °C and then the solvents were evaporated. The crude product thus obtained was purified by flash chromatography (CH_2Cl_2/MeOH, 10:1) to give the 6-*O*-tosylated derivative **4** (2.20 g, 4.61 mmol, 64%) as a white amorphous solid. $[\alpha]_D$ = −7.0 (*c* = 1.42 in MeOH). ^{1}H NMR (500 MHz, [D_4]MeOH): δ = 7.80 (d, *J* = 8.4 Hz, 2 H, 2 aryl-H), 7.45 (d, *J* = 8.7 Hz, 2 H, 2 aryl-H), 4.24−4.14 (m, 3 H, 1-H, 6-H_a, 6-H_b), 3.83−3.61 (m, 3 H, 2-H, 4-H, OCH_a), 3.52 (m_c, 1 H, OCH_b), 3.48−3.42 (m, 2 H, 3-H, 5-H), 3.21 (m_c, 2 H, CH_2N), 2.45 (s, 3 H, $TsCH_3$), 1.42 (s, 9 H, *t*Bu). ^{13}C NMR (125.75 MHz, [D_4]MeOH): δ = 158.2 (BocCO), 146.6 (aryl-C_q), 134.3 (aryl-C_q), 131.2 (2 aryl-CH), 129.0 (2 aryl-CH), 104.8 (C-1), 80.0 [*C*$(CH_3)_3$], 74.3 (C-3), 74.0 (C-4, C-2), 72.2 (C-5), 70.7 (C-6), 70.0 (OCH_2), 41.5 (CH_2N), 28.8 [C(*C*$H_3)_3$, 21.6 ($TsCH_3$). $C_{20}H_{31}NO_{10}S$ (477.53): calcd. C 50.31, H 6.54, N 2.93 found C 50.43, H 6.79, N 2.92.

2-(*tert*-Butyloxycarbonylamido)ethyl 3,6-Anhydro-β-D-galactopyranoside (5): A suspension of the 6-*O*-tosylated mannoside **4** (1.56 g, 3.27 mmol) and NaN_3 (1.60 g, 24.52 mmol) in dry DMF (20 mL) was stirred at 60 °C for 12 h. DMF was then removed in vacuo and the residual solid was dissolved in ethyl acetate and filtered through a thin Celite bed to remove insoluble salts from the product mixture. The filtrate was concentrated and the crude product was purified by flash chromatography (CH_2Cl_2/MeOH, 9:1) to yield the anhydro-galactoside **5** (0.82 g, 2.68 mmol, 82%) as a colorless syrup. $[\alpha]_D$ = −70.4 (*c* = 3.8 in MeOH). ^{1}H NMR (500 MHz, [D_4]MeOH): δ = 4.52 (s, 1 H, 1-H), 4.33 (d, $J_{5,6a}$ = 1.9 Hz, 1 H, 6-H_a), 4.18−4.14 (m, 2 H, 6-H_b, 4-H), 4.11 (d, $J_{2,3}$ = 4.8 Hz, 1 H, 2-H), 3.92 (d, $J_{2,3}$ = 4.8 Hz, 1 H, 3-H), 3.90 (m_c, 1 H, 5-H), 3.76 (m_c, 1 H, OCH_a), 3.37 (m_c, 1 H, OCH_b), 3.23 (m_c, 2 H, CH_2N), 1.43 (s, 9 H, *t*Bu). ^{13}C NMR (125.75 MHz, [D_4]MeOH): δ = 158.3 (BocCO), 103.5 (C-1), 82.5 (C-2), 80.1 [*C*$(CH_3)_3$], 79.1 (C-4), 74.0 (C-3), 72.3 (C-5), 71.2 (C-6), 68.2 (OCH_2), 41.1 (CH_2N), 28.8 [C(*C*$H_3)_3$]. EI-MS: *m*/*z* = 306.1 $[M + H]^+$ found for $C_{13}H_{23}NO_7$ (calcd. 305.14).

2-(*tert*-Butyloxycarbonylamido)ethyl 2,3,4-Tri-*O*-acetyl-6-*O*-(4-tolylsulfonyl)-β-D-galactopyranoside (6): The unprotected tosylated **5**

(2.10 g, 4.40 mmol) was dissolved in pyridine and the solution was cooled to −10 °C. Ice-cold acetic anhydride (2 mL) was then added slowly while stirring. The reaction mixture was kept at 4 °C for 12 h and was then concentrated, and the crude product was purified by flash chromatography (*n*-hexane/acetone, 2.5:1) to afford the acetylated derivative **6** (2.57 g, 4.25 mmol, 97%) as a colorless syrup. $[\alpha]_D = -2.7$ ($c = 1.14$ in $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$): δ = 7.75 (d, $J = 8.4$ Hz, 2 H, 2 aryl-H), 7.35 (d, $J = 8.6$ Hz, 2 H, 2 aryl-H), 5.35 (dd, $J_{3,4} = 3.4$, $J_{4,5} = 1.2$ Hz, 1 H, 4-H), 5.14 (dd, $J_{1,2} = 7.9$, $J_{2,3} = 10.5$ Hz, 1 H, 2-H), 4.97 (dd, $J_{2,3} = 10.5$, $J_{3,4} = 3.4$ Hz, 1 H, 3-H), 4.82 (s, 1 H, NH), 4.42 (d, $J_{1,2} = 7.9$ Hz, 1 H, 1-H), 4.11 (dd, $J_{5,6a} = 6.6$, $J_{6a,6b} = 12.8$ Hz, 1 H, 6-H_a), 4.00 (dd, $J_{5,6b} = 6.3$, $J_{6a,6b} = 12.8$ Hz, 1 H, 6-H_b), 3.93 (ddd, $J_{5,6} = 7.6$, $J_{5,6b} = 6.4$, $J_{4,5} = 1.2$ Hz, 1 H, 5-H), 3.82 (m_c, 1 H, OCH_a), 3.58 (m_c, 1 H, OCH_b), 3.29 (m_c, 2 H, CH_2N), 2.44 (s, 3 H, $TsCH_3$), 2.05, 2.03, 1.96 (each s, each 3 H, 3 $COCH_3$), 1.43 (s, 9 H, *t*Bu). ^{13}C NMR (125.75 MHz, $CDCl_3$): δ = 170.0, 169.9, 169.6 (3 $COCH_3$), 156.2 (BocCO), 145.3 (aryl-C_q), 132.3 (aryl-C_q), 130.0 (2 aryl-CH), 128.1 (2 aryl-CH), 101.5 (C-1), 80.0 [$C(CH_3)_3$], 70.7 (C-5), 70.5 (C-3), 69.7 (OCH_2), 68.7 (C-2), 66.8 (C-4), 66.1 (C-6), 40.2 (CH_2N), 28.4 [$C(CH_3)_3$], 21.7 ($TsCH_3$), 20.53, 20.52, 20.51 (3 $COCH_3$).

2-(*tert*-Butyloxycarbonylamido)ethyl 2,3,4-Tri-*O*-acetyl-6-azido-6-deoxy-β-D-galactopyranoside (7): A suspension of the acetyl-protected galactoside **6** (2.47 g, 4.09 mmol) and NaN_3 (5.32 g, 81.83 mmol) in dry DMF (30 mL) was stirred at 80 °C for 24 h. DMF was removed in vacuo and the crude product was dissolved in ether. The organic layer was washed with water to remove inorganic salts and brine. Drying with anhydrous Na_2SO_4, concentration, and purification by flash chromatography (*n*-hexane/acetone, 2.5:1) afforded the 6-deoxy-6-azido derivative **7** (1.40 g, 2.95 mmol, 72%) as a colorless syrup. $[\alpha]_D = -3.4$ ($c = 1.10$ in $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$): δ = 5.35 (dd, $J_{3,4} = 3.5$, $J_{4,5} = 1.2$ Hz, 1 H, 4-H), 5.21 (dd, $J_{1,2} = 7.9$, $J_{2,3} = 10.5$ Hz, 1 H, 2-H), 5.02 (dd, $J_{2,3} = 10.5$, $J_{3,4} = 3.4$ Hz, 1 H, 3-H), 4.88 (s, 1 H, NH), 4.50 (d, $J_{1,2} = 7.9$ Hz, 1 H, 1-H), 3.93 (m_c, 1 H, OCH_a), 3.85 (ddd, $J_{4,5} = 1.2$, $J_{5,6a} = 8.1$, $J_{5,6b} = 4.4$ Hz, 1 H, 5-H), 3.64 (m_c, 1 H, OCH_b), 3.55 (dd, $J_{5,6a} = 8.1$, $J_{6a,6b} = 12.9$ Hz, 1 H, 6-H_a), 3.34 (m_c, 2 H, CH_2N), 3.16 (dd, $J_{5,6b} = 4.4$, $J_{6a,6b} = 12.9$ Hz, 1 H, 6-H_b), 2.20, 2.10, 2.00 (each s, each 3 H, 3 $COCH_3$), 1.44 (s, 9 H, *t*Bu). ^{13}C NMR (125.75 MHz, $CDCl_3$): δ = 170.2, 170.0, 169.6 (3 $COCH_3$), 155.8 (BocCO), 101.5 (C-1), 79.4 [$C(CH_3)_3$], 72.9 (C-5), 70.7 (C-3), 69.7 (OCH_2), 68.8 (C-2), 67.9 (C-4), 50.5 (C-6), 40.2 (CH_2N), 28.4 [$C(CH_3)_3$], 20.9, 20.7, 20.6 (3 $COCH_3$).

2-(*tert*-Butyloxycarbonylamido)ethyl 6-Azido-6-deoxy-β-D-galactopyranoside (8): The acetylated galactoside **7** (1.27 g, 2.67 mmol) was dissolved in methanol (10 mL) and a freshly prepared solution of NaOMe in MeOH (1 M, 0.5 mL) was then added at 0 °C. The mixture was stirred at room temp. for 1 h. The basic reaction mixture was neutralized with methanolic HCl solution (10%) and concentrated. The crude product thus obtained was purified by flash chromatography (MeOH/CH_2Cl_2, 1:10) to yield triol **8** (0.91 g, 2.62 mmol, 98%) as a white hygroscopic solid. $[\alpha]_D = -29.5$ ($c = 1.15$ in MeOH). ^1H NMR (500 MHz, [D$_4$]MeOH): δ = 4.26 (d, $J_{1,2} = 7.5$ Hz, 1 H, 1-H), 3.90 (m_c, 1 H, OCH_a), 3.74 (dd, $J_{4,5} = 1.1$, $J_{3,4} = 3.0$ Hz, 1 H, 4-H), 3.69 (dd, $J_{4,5} = 1.2$, $J_{5,6b} = 4.8$ Hz, 1 H, 5-H), 3.65−3.58 (m, 2 H, 6-H_a, OCH_b), 3.55−3.48 (m, 2 H, 2-H, 3-H), 3.27 (m_c, 2 H, CH_2N), 3.22 (dd, $J_{6a,6b} = 11.0$, $J5,_{6b} = 4.2$ Hz, 1 H, 6-H_b), 1.43 (s, 9 H, *t*Bu). ^{13}C NMR (125.75 MHz, [D$_4$]MeOH): δ = 158.5 (BocCO), 104.9 (C-1), 80.1 [$C(CH_3)_3$], 75.7 (C-5), 74.5 (C-3), 72.3 (C-2), 70.7 (C-4), 70.0 (OCH_2), 52.5 (C-6), 41.4 (CH_2N), 28.8 [$C(CH_3)_3$].

2-(*tert*-Butyloxycarbonylamido)ethyl 6-Deoxy-6-{[2-(methoxycarbonyl)ethyl]amino}-β-D-galactopyranoside (10): A mixture of the azide **8** (0.85 g, 2.44 mmol) and activated Pd catalyst (10% on charcoal, 50 mg) in MeOH (10 mL) was hydrogenated under atmospheric pressure for 10 h at room temp. The suspension was filtered through a short pad of Celite and the filtrate was concentrated. The crude amino-functionalized derivative **9** thus obtained was further treated with freshly distilled methyl acrylate (1.91 g, 22.23 mmol) in dry MeOH (10 mL) at 0 °C under argon. The reaction mixture was then heated at 40 °C in darkness for 18 h. Concentration and purification of the crude product by flash chromatography (CH_2Cl_2/MeOH, 2.5:1) yielded the Michael mono-adduct **10** (0.60 g, 1.47 mmol, 60% over two steps) as a white, hygroscopic solid. $[\alpha]_D = -1.1$ ($c = 1.58$ in MeOH). ^1H NMR (500 MHz, [D$_4$]MeOH): δ = 4.27 (d, $J_{1,2} = 7.6$ Hz, 1 H, 1-H), 3.90 (m_c, 1 H, OCH_a), 3.81 (dd, $J_{4,5} = 1.6$ Hz, 1 H, 4-H), 3.76 (m_c, 1 H, 5-H), 3.71 (s, 3 H, CO_2CH_3), 3.62 (m_c, 1 H, OCH_b), 3.53−3.48 (m, 2 H, 2-H, 3-H), 3.31 (m_c, 1 H, CH_aNHBoc), 3.27−3.19 (m, 2 H, CH_bNHBoc, 6-H), 3.13 (m_c, 2 H, NCH_2), 3.08 (dd, $J_{6a,6b} = 12.7$, $J_{5,6b} = 3.4$ Hz, 1 H, 6-H_b), 2.71 (t, $J = 6.6$ Hz, 2 H, CH_2CO), 1.44 (s, 9 H, *t*Bu). ^{13}C NMR (125.75 MHz, [D$_4$]MeOH): δ = 173.7 (CO_2CH_3), 158.5 (BocCO), 105.1 (C-1), 80.2 [$C(CH_3)_3$], 74.5 (C-3), 72.6 (C-5), 72.2 (C-2), 71.6 (C-4), 70.1 (OCH_2), 52.5 (CO_2CH_3), 50.4 (C-6), 45.3 (NCH_2), 41.5 (CH_2NBoc), 32.7 (CH_2CO), 28.8 [$C(CH_3)_3$]. $C_{17}H_{32}N_2O_9 \cdot H_2O$ (426.46): calcd. C 47.88, H 8.04, N 6.57, found C 48.21, H 7.95, N 6.49.

2-(*tert*-Butyloxycarbonylamido)ethyl 6-Deoxy-6-({*p*-[(α-D-mannosyloxy)methyl]benzoyl}[2-(methoxycarbonyl)ethyl]amino)-β-D-galactopyranoside (12): The galactoside **10** (120 mg, 0.29 mmol), the mannoside **11** (170 mg, 0.58 mmol), HATU (246 mg, 0.65 mmol), and DIPEA (0.25 mL, 1.47 mmol) were dissolved in DMF (5 mL) under argon. The reaction mixture was stirred at 45 °C for 6 h and DMF was then removed in vacuo. The resulting crude product was a pale yellow syrup, which was subjected to gel permeation chromatography on Bio-gel P-2. This afforded the title compound **12** (160 mg, 0.23 mmol, 79%) as a white, hygroscopic lyophilizate. $[\alpha]_D = +37$ ($c = 1.14$ in MeOH). ^1H NMR (500 MHz, [D$_4$]MeOH): δ = 7.50−7.37 (m, 4 H, 4 aryl-H), 4.84 (d, $J_{1,2} = 1.8$ Hz, 1 H, man 1-H), 4.79 (d, $J = 12.2$ Hz, 1 H, $PhCH_a$), 4.57 (d, $J = 11.8$ Hz, 1 H, $PhCH_b$), 4.24 (d, $J_{1,2} = 7.8$ Hz, 1 H, gal 1-H), 3.96−3.76 (m, 7 H, OCH_a, man 6-H_a, NCH_a, man 2-H, gal 3-H, gal 4-H, gal 5-H), 3.75−3.65 (m, 5 H, man 6-H_b, gal 6-H_a, OCH_b, NCH_b, man 3-H), 3.71 (s, 3 H, CO_2CH_3), 3.64−3.51 (m, 3 H, man 4-H, gal 6-H_b, man 5-H), 3.50 (m_c, 1 H, gal 2-H), 3.28 (m_c, 2 H, CH_2NHBoc), 2.68 (m_c, 2 H, CH_2CO), 1.44 (s, 9 H, *t*Bu). ^{13}C NMR (125.84 MHz, [D$_4$]MeOH): δ = 174.8 (CO_2CH_3), 173.5 (NCO), 158.5 (BocCO), 141.4 (aryl-C_q), 137.7 (aryl-C_q), 129.1, 128.4, 127.8 (4 aryl-CH), 105.1 (gal C-1), 101.0 (man C-1, $^1J_{C1,H1} = 168.3$ Hz), 80.2 [$C(CH_3)_3$], 75.0 (man C-5), 74.6 (gal C-2), 74.2 (gal C-3), 72.7 (man C-3), 72.4 (gal C-5), 72.2 (man C-2), 71.0 (gal C-4), 70.0 (OCH_2), 69.4 ($PhCH_2$), 68.7 (man C-4), 63.0 (man C-6), 52.5 (gal C-6), 52.3 (CO_2CH_3), 47.8 (NCH_2), 41.5 (CH_2NHBoc), 33.1 (CH_2CO), 28.8 [$C(CH_3)_3$]. MALDI-TOF MS: *m*/*z* = 727.5 [M + Na]$^+$ and 743.5 [M + K]$^+$ found for $C_{31}H_{48}N_2O_{16}$ (calcd. 704.3). $C_{31}H_{48}N_2O_{16}$ (704.73): calcd. C 52.83, H 6.87, N 3.98, found C 52.02, H 6.56, N 4.15.

2-*tert*-Butyloxycarbonylamidoethyl 6-Deoxy-6-[{*p*-[(α-D-mannosyloxy)methyl]benzoyl}(2-propanoyl)amino]-β-D-galactopyranoside (13): The disaccharide analogue **12** (156 mg, 0.22 mmol) was dissolved in MeOH/H_2O (1:2; 2 mL) and treated with LiOH·H_2O (11 mg, 0.26 mmol) at 0 °C for 12 h. The basic reaction mixture was then neutralized at 0 °C to a value of pH = 6 by

careful addition of HCl (2 N). The obtained clear solution was freeze-dried to afford the crude carboxylic acid **13** as a white, amorphous solid (152 mg, 0.22 mmol, quant.), which was used in the next step without purification.

2-Azidoethyl α-L-Fucoside (16): The bromoethyl fucoside **14**[29] (2.00 g, 5.03 mmol) was treated with NaN_3 (1.63 g, 25.17 mmol) in DMF (5 mL) at 60 °C for 1 h. After removal of DMF in vacuo, the residual syrup was suspended in ethyl acetate and the suspension was filtered through a thin Celite bed. The filtrate was washed with water to remove inorganic salts and brine and the organic layer was then dried with anhydrous Na_2SO_4. After filtration and concentration, the triacetylated azido derivative **15** was obtained as a white solid. Without further purification, **15** was then dissolved in methanol and treated with a freshly prepared NaOMe solution (0.5 mL, 1 M) for 1 h at room temp. The reaction was quenched with diluted HCl solution and the solvent was removed. Subsequent chromatographic purification (CH_2Cl_2/MeOH, 9:1) of the crude product afforded the title fucoside **16** (0.82 g, 3.51 mmol, 70% over two steps) as a colorless syrup. $[\alpha]_D = -112.8$ ($c = 1.25$ in MeOH). ^{1}H NMR (500 MHz, [D$_4$]MeOH): δ = 4.78 (d, $J_{1,2}$ = 3.44 Hz, 1 H, 1-H), 4.01 (m_c, 1 H, 5-H), 3.84 (m_c, 1 H, OCH_a), 3.75 (m_c, 2 H, 3-H, 2-H), 3.67 (dd, 1 H, $J_{3,4}$ = 1.32, $J_{4,5}$ = 3.15 Hz, 4-H), 3.62 (m_c, 1 H, OCH_b), 3.55 (m_c, 1 H, OCH_aN_3), 3.36 (m_c, 1 H, CH_bN_3), 1.22 (d, $J_{1,2}$ = 3.44 Hz, 3 H, 6-CH_3). ^{13}C NMR (125.75 MHz, [D$_4$]MeOH): δ = 100.8 (C-1), 73.6 (C-4), 71.5 (C-3), 69.81 (C-2), 68.1 (OCH_2), 67.8 (C-5), 51.9 (CH_2N_3), 16.6 (6-CH_3).

2-(*tert*-Butyloxycarbonylamido)ethyl 6-Deoxy-6-[{*p*-[(α-D-mannocarbamoyl}syloxy)methyl]benzoyl}(2-{[2-(α-L-fucosyloxy)ethyl]-ethyl)amino]-β-D-galactopyranoside (18): The azidoethyl fucoside **16** (0.10 g, 0.42 mmol) and Pd catalyst (10% on charcoal; 50 mg) were dissolved in MeOH (10 mL) and the mixture was hydrogenated under atmospheric pressure for 3 h at room temp. The suspension was filtered through a short pad of Celite and the filtrate was concentrated. The obtained crude amino derivative **17** was used without further purification in the subsequent coupling reaction. A mixture of carboxylic acid **13** (140 mg, 0.20 mmol), 2-aminoethyl α-L-fucoside **17** (63 mg, 0.30 mmol), HATU (100 mg, 0.26 mmol), and DIPEA (0.10 mL, 0.61 mmol) in dry DMF (2 mL) was stirred at 45 °C for 12 h under argon. The reaction mixture was then concentrated in vacuo and the resulting pale yellow syrup was subjected to gel permeation chromatography on Bio-gel P-2 to provide the title compound **18** (100 mg, 0.11 mmol, 56%) as a white, hygroscopic lyophilizate. $[\alpha]_D = -11.5$ ($c = 1.03$ in MeOH). ^{1}H NMR (500 MHz, [D$_4$]MeOH): δ = 7.50−7.38 (m, 4 H, 4 aryl-H), 4.84 (d, $J_{1,2}$ = 1.8 Hz, 1 H, man 1-H), 4.81−4.74 (m, 2 H, $PhCH_a$, fuc 1-H), 4.58 (d, J = 11.8 Hz, 1 H, $PhCH_b$), 4.24 (d, $J_{1,2}$ = 7.7 Hz, 1 H, gal 1-H), 3.98−3.78 (m, 7 H, $OOCH_a$, man 6-H_a, gal 3-H, man 2-H, gal 4-H, gal 5-H, fuc 5-H), 3.76−3.67 (m, 8 H, man 6-H_b, gal 6-H_a, NCH_2, man 3-H, fuc 2-H, OCH_2), 3.66−3.42 (m, 7 H, gal 6-H_b, man 4-H, man 5-H, gal 2-H, OCH_b, fuc 3-H, fuc 4-H), 3.28 (m_c, 2 H, CH_2NHBoc), 2.65 (m_c, 2 H, CH_2CONH) 2.45 (m_c, 2 H, CONHCH_2), 1.44 (s, 9 H, *t*Bu), 1.20 (s, 3 H, fuc 6-CH_3). ^{13}C NMR (125.75 MHz, [D$_4$]MeOH): δ = 174.7 (CONH), 173.3 (NCOPh), 158.5 (BocCO), 141.2, 137.5 (2 aryl-C_q), 129.2, 129.0, 128.5, 127.9 (4 aryl CH), 105.1, 104.9 (gal C-1), 101.0 (man C-1), 100.6 (fuc C-1), 80.2 [$C(CH_3)_3$], 75.1 (man C-5), 74.6 (gal C-2), 73.9 (gal C-3), 73.6 (fuc C-4), 72.7 (man C-3), 72.4 (gal C-5), 72.2 (man C-2), 71.7 (fuc C-5), 71.0 (gal C-4), 70.0 (fuc C-3), 69.9 (OCH_2), 69.4 ($PhCH_2$), 68.7 (man C-4), 67.7 (fuc C-2), 67.8 (OCH_2), 63.0 (man C-6), 52.3 (gal C-6), 48.6 (NCH_2), 41.5 (CH_2NHBoc), 35.9 (CONHCH_2), 35.1 (CH_2CO), 28.8 [C(CH_3)$_3$], 16.7 (fuc 6-CH_3). MALDI-TOF MS: m/z = 902.3 [M + Na]$^+$ and 918.3 [M + K]$^+$ found for $C_{38}H_{61}N_3O_{20}$ (calcd. 879.38). $C_{38}H_{61}N_3O_{20}$ (879.91): calcd. C 51.87, H 6.99, N 4.78, found C 50.98, H 6.80, N 4.93.

2-Aminoethyl 6-Deoxy-6-[{*p*-[(α-D-mannosyloxy)methyl]benzoyl}(2-{[2-(α-L-fucosyloxy)ethyl]carbamoyl}ethyl)amino]-β-D-galactopyranoside (19): The trisaccharide analogue **18** (45 mg, 0.05 mmol) was dissolved in a freshly prepared solution of $Me_2S-CF_3CO_2H$ (1:2; 1 mL) and stirred for 3 h at 0 °C. The reaction mixture was then concentrated in a rotary evaporator in a closed fume hood and final traces of CF_3CO_2H were quenched with satd. NH_3/H_2O solution (1 mL) at 0 °C. After lyophilization of the aqueous mixture, it was desalted with Bio-gel P-2 to give the corresponding amino derivative **19** [35 mg, 0.05 mmol, 88%; TLC (*i*PrOH/H_2O/NH_3, 7:2:1): R_f = 0.1 (UV, α-naphthol)].

2,3,6,2′,3′,4′,6′-Hepta-*O*-acetyl-β-D-lactopyranosyl Isothiocyanate (21): A solution of tin(IV) chloride in CH_2Cl_2 (1 M, 0.60 mL, 0.60 mmol) was added to a solution of lactose octaacetate (**20**; 270 mg, 0.40 mmol) in dry DCE (dichloroethane) (4 mL). Trimethylsilyl isothiocyanate (0.09 mL, 0.60 mmol) was then added slowly at 0 °C under argon and the reaction mixture was heated at 50 °C. After 3 h, the reaction mixture was allowed to cool to room temp., diluted with CH_2Cl_2 (5 mL), and washed successively with water, satd. $NaHCO_3$, and brine. The organic layer was then dried with anhydrous Na_2SO_4, filtered, and concentrated. TLC analysis of the crude product showed the presence of an anomeric mixture, which was subjected to flash chromatography (*n*-hexane/ethyl acetate, 1.5:1) to yield the title β anomer **21** (135 mg, 0.20 mmol) as a white solid, together with the corresponding α anomer (70 mg, 0.10 mmol) in the ratio of 2:1 (75%). IR: $\tilde{\nu}$ = 2029.3 cm^{-1}(NCS). M.p. 75−77 °C. $[\alpha]_D = -1.0$ ($c = 1.14$ in $CHCl_3$) (ref.[32] syrup). $[\alpha]_D = +5.5$ ($c = 1$ in CH_2Cl_2). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 5.35 (dd, $J_{4,5}$ = 1.3, $J_{3,4}$ = 3.5 Hz, 1 H, gal 4-H), 5.20 (dd, $J_{2,3}$ = 9.2, $J_{3,4}$ = 10.0 Hz, 1 H, glc 3-H), 5.10 (dd, $J_{1,2}$ = 7.9, $J_{2,3}$ = 10.4 Hz, 1 H, gal 2-H), 5.02 (dd, $J_{1,2}$ = 8.0, $J_{2,3}$ = 9.2 Hz, 1 H, glc 2-H), 5.01 (d, $J_{1,2}$ = 8.0 Hz, 1 H, glc 1-H), 4.96 (dd, $J_{2,3}$ = 10.4, $J_{3,4}$ = 3.5 Hz, 1 H, gal 3-H), 4.49 (d, $J_{1,2}$ = 7.9 Hz, 1 H, gal 1-H), 4.48 (dd, $J_{5,6}$ = 2.1, $J_{6a,6b}$ = 12.2 Hz, 1 H, glc 6-H_a), 4.13 (dd, $J_{5,6b}$ = 6.5, $J_{6a,6b}$ = 12.2 Hz, 1 H, glc 6-H_b), 4.13−4.06 (m, 2 H, gal 6-H_a, 6b), 3.88 (ddd, $J_{4,5}$ = 1.3, $J_{5,6a}$ = 7.4, $J_{5,6b}$ = 13.6 Hz, 1 H, gal 5-H), 3.82 (dd, $J_{4,5}$ = 9.0, $J_{3,4}$ = 10.0 Hz, 1 H, glc 4-H), 3.66 (m_c, 1 H, glc 5-H), 2.16, 2.15, 2.10, 2.07, 2.06, 2.05, 1.96 (each s, each 3 H, 7 $COCH_3$). ^{13}C NMR (125.84 MHz, $CDCl_3$): δ = 170.3, 170.2, 170.0, 169.9, 169.6, 169.3, 169.0 (7 *C*OCH_3), 144.0 (C=S), 101.0 (gal C-1), 83.2 (glc C-1), 75.6 (glc C-4), 74.7 (glc C-5), 72.4 (glc C-3), 72.1 (gal C-2), 70.8 (gal C-3), 70.7 (gal C-5), 69.0 (gal C-2), 66.5 (gal C-4), 61.6 (glc C-6), 60.7 (gal C-6), 20.70, 20.63, 20.57, 20.56, 20.54, 20.50, 20.40 (7 CO*C*H_3).

2-[(β-D-Lactopyranosyl)thioureido]ethyl 6-Deoxy-6-[{*p*-[(α-D-mannosyloxy)methyl]benzoyl}(2-{[2-(α-L-fucosyloxy)ethyl]carbamoyl}-ethyl)amino]-β-D-galactopyranoside (23): The amino-functionalized trisaccharide analogue **19** (35 mg, 0.05 mmol) was treated with the lactosyl isothiocyanate **21** (45 mg, 0.07 mmol) in dry DMF (1 mL) at 50 °C for 12 h. The reaction mixture was then concentrated in vacuo to afford the crude partially protected thiourea derivative **22**. This was treated with NH_3/MeOH (7 N, 2 mL) at 0 °C and stored in the refridgerator for 12 h. Concentration of the reaction mixture yielded a pale yellow syrup, which was subjected to gel permeation chromatography on Bio-gel P-2 to afford the fully deprotected pentasaccharide mimetic **23** (43 mg, 0.04 mmol, 83% over two steps) as a white, hygroscopic lyophilizate. $[\alpha]_D = -15.4$ ($c = 1.30$ in H_2O). ^{1}H NMR (500 MHz, D_2O): δ = 7.55−7.46 (m, 2 H, 2 aryl-H), 7.45−7.35 (m, 2 H, 2 aryl H), 4.98 (s, 1 H, man 1-H), 4.86−4.74 (m, 3 H, glc 1-H, $PhCH_a$, fuc 1-H), 4.63 (m_c, 1 H,

$PhCH_b$), 4.45 (d, $J_{1,2}$ = 7.7 Hz, 1 H, gal′ 1-H), 4.40 (d, $J_{1,2}$ = 7.8 Hz, 1 H, gal 1-H), 4.07−3.87 (m, 5 H, $OOCH_a$, man 6-H_a, gal 3-H, man 2-H, fuc 5-H), 3.86−3.61 (m, 25 H, gal 4-H, gal 5-H, gal′ 6-H_a, gal′ 5-H, man 6-H_b, gal′ 4-H, man 3-H, fuc 4-H, glc 6-H_a, gal ′ 6-H_b, NCH_2, OCH_a, fuc 2-H, glc 2-H, fuc 3-H, gal 6-H_a, glc 6-H_b, man 4-H, glc 3-H, gal′ 3-H, glc 4-H, man 5-H, 2 OCH_b), 3.60−3.21 (m, 6 H, gal 2-H, gal′ 2-H, glc 5-H, CH_2NHCO, gal 6-H_b), 2.67 (m_c, 2 H, CH_2CONH) 2.45 (m_c, 2 H, CONHCH_2), 1.16 (d, J = 7.7 Hz, 3 H, fuc 6-CH_3). ^{13}C NMR (125.75 MHz, D_2O): δ = 181.8 (C=S, weak signal), 176.3 (CONH), 175.3 (NCOPh), 141.6, 136.8 (2 aryl-C_q), 131.1, 131.0 (2 aryl CH), 129.5, 129.3 (2 aryl CH), 105. 3 (gal C-1), 105.1 (gal′ C-1), 101.9 (man C-1, fuc C-1), 100.8 (glc C-1), 80.3 (glc C-3, gal C-5), 77.4 (gal C-2, C-3), 75.4 (glc C-5), 74.9 (glc C-2, gal′ C-2), 74.2 (fuc C-4), 73.3 (man C-3), 73.0 (gal C-5, man C-2), 72.4 (fuc C-5), 72.0 (fuc C-3), 71.7 (gal C-4), 71.4 (gal′ C-4), 71.2 (OCH_2, $PhCH_2$), 71.0 (glc C-4), 70.4 (man C-4), 69.1 (fuc C-2), 68.7 (OCH_2), 63.5 (man C-6), 63.2 (glc C-6), 62.3 (gal′ C-6), 53.2 (gal C-6), 48.0 (NCH_2), 41.7 (CH_2NHCO), 36.9 (CONHCH_2), 36.2 (CH_2CO), 17.7 (fuc 6-CH_3). MALDI-TOF MS: *m/z* = 1183.4 $[M + Na]^+$ found for $C_{47}H_{76}N_4O_{27}S$ (calcd. 1160.44). A correct elemental analysis for $C_{47}H_{76}N_4O_{27}S$ (1161.19) was not obtained.

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[1] A. Varki, *Glycobiology* **1993**, *3*, 97−130.
[2] A. Kobata, *Glycoconjugate J.* **2000**, *17*, 443−464.
[3] C. R. Bertozzi, L. L. Kiessling, *Science* **2001**, *291*, 2357−2364.
[4] T. Muramatsu, *Glycoconjugate J.* **2000**, *17*, 577−595.
[5] H. Lis, N. Sharon, *Chem. Rev.* **1998**, *98*, 637−674.
[6] K.-A. Karlsson, *Curr. Opin. Struct. Biol.* **1995**, *5*, 622−635.
[7] P. Sears, C.-H. Wong, *Angew. Chem.* **1999**, *111*, 2446−2471; *Angew. Chem. Int. Ed.* **1999**, *38*, 2300−2324.
[8] Y. C. Lee, R. T. Lee, *Acc. Chem. Res.* **1995**, *28*, 321−327.
[9] R. T. Lee, Y. C. Lee, *Glycoconjugate J.* **2000**, *17*, 543−551.
[10] Th. K. Lindhorst, *Top. Curr. Chem.* **2002**, *218*, 201−235.
[11] N. Röckendorf, Th. K. Lindhorst, *Top. Curr. Chem.* **2001**, *217*, 201−238.
[12] M. Dubber, Th. K. Lindhorst, *Carbohydr. Res.* **1998**, *310*, 35−41.
[13] M. Dubber, Th. K. Lindhorst, *J. Org. Chem.* **2000**, *65*, 5275−5281.
[14] P. I. Kitov, J. M. Sadowska, G. Mulvey, G. D. Armstrong, H. Ling, N. S. Pannu, R. J. Read, D. R. Bundle, *Nature* **2000**, *403*, 669−672.
[15] C.-H. Wong, X.-S. Ye, Z. Zhang, *J. Am. Chem. Soc.* **1998**, *120*, 7137−7138.
[16] T. Wunberg, C. Kallus, T. Opatz, S. Henke, W. Schmidt, H. Kunz, *Angew. Chem.* **1998**, *110*, 2620−2622; *Angew. Chem. Int. Ed.* **1998**, *37*, 2503−2505.
[17] R. Hirschmann, L. Ducry, A. B. Smith III, *J. Org. Chem.* **2000**, *65*, 8307−8316.
[18] M. Ghosh, R. G. Dulina, R. Kakarla, M. J. Sofia, *J. Org. Chem.* **2000**, *65*, 8387−8390.
[19] A. Patel, Th. K. Lindhorst, *J. Org. Chem.* **2001**, *66*, 2674−2680.
[20] N. Sharon, *FEBS Lett.* **1987**, *217*, 145−157.
[21] S. Kötter, U. Krallmann-Wenzel, S. Ehlers, Th. K. Lindhorst, *J. Chem. Soc., Perkin Trans. 1* **1998**, 2193−2200.
[22] Th. K. Lindhorst, M. Dubber, U. Krallmann-Wenzel, S. Ehlers, *Eur. J. Org. Chem.* **2000**, 2027−2034.
[23] A. Y. Chernyak, G. V. M. Sharma, L. O. Kononov, P. Radha Krishna, A. B. Levinsky, N. K. Kochetkov, A. V. Ramarao, *Carbohydr. Res.* **1992**, *223*, 303−309.
[24] G. Zemplén, E. Pacsu, *Ber. Dtsch. Chem. Ges.* **1929**, *62*, 1613−1614.
[25] R. L. Whistler, M. L. Wolfrom (Eds.), *Methods in Carbohydrate Chemistry. Reactions of Carbohydrates*, Academic Press, New York, **1963**, vol. II, p. 172−187.
[26] C. Kieburg, K. Sadalapure, Th. K. Lindhorst, *Eur. J. Org. Chem.* **2000**, 2035−2040.
[27] Th. K. Lindhorst, S. Kötter, J. Kubisch, U. Krallmann-Wenzel, S. Ehlers, V. Křen, *Eur. J. Org. Chem.* **1998**, 1669−1674.
[28] L. A. Carpino, *J. Am. Chem. Soc.* **1993**, *115*, 4397−4398.
[29] J. Dahmen, T. Frejd, G. Grönberg, T. Lave, G. Magnusson, G. Noori, *Carbohydr. Res.* **1983**, *116*, 303−307.
[30] K. Katano, H. A. Y. Aoyagi, M. Overhand, S. J. Sucheck, W. C. Stevens, Jr., C. D. Hess, X. Zhou, S. M. Hecht, *J. Am. Chem. Soc.* **1998**, *120*, 11285−11296.
[31] H. Paulsen, Z. Györgydeák, M. Friedmann, *Chem. Ber.* **1974**, *107*, 1590−1613.
[32] Th. K. Lindhorst, C. Kieburg, *Synthesis* **1995**, 1228−1230.
[33] G. Fischer, *Chem. Soc. Rev.* **2000**, *29*, 119−127.

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