A Modular Approach for the Synthesis of Oligosaccharide Mimetics

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To allow modular syntheses of oligosaccharide mimetics, the potentially trifunctional glycoside **7** was synthesized and used as a scaffold for the successive attachment of further monosaccharide derivatives to lead to the di-, tri-, and tetrasaccharide mimetics **11**, **13**, and **16**. This synthetic strategy can also be used to prepare oligovalent neoglycoconjugates, e.g., **18**, which contains nine mannosyl units. The applied concept implies numerous options for the synthesis of a wide array of structural variations, biolabeling, or solid-phase synthesis as well as combinatorial approaches.

Introduction

Complex oligosaccharides as part of glycoconjugates play an important role in biological communication processes.[1] They store information in the form of their three-dimensional structures which is "decoded" in molecular recognition processes involving carbohydrate-recognizing proteins called lectins and selectins.[2] However, the exact structures of optimal lectin ligands are often not known, and therefore, the synthesis of carbohydrate ligands that are necessary for biological investigations cannot generally be based on a rational design. While chemical and enzymatic methods for the preparation of oligoantennary carbohydrates according to the natural example structures has been developed enormously during the last years,[3] the synthesis of every target molecule remains an individual and difficult task. Consequently, efforts have been directed toward the synthesis of structurally modified sugar-containing molecules, "glycomimetics".[4] These do not necessarily have to be assembled by glycosidation reactions, while the biological properties of their natural counterparts might be preserved or even surpassed. No strict definition of the term glycomimetic has yet been made. It seems to be generally accepted that the approach to obtain biologically active carbohydrate-containing molecules by artificial design bears the potential to implement a number of relevant advantages. These include (i) simplified synthesis, (ii) greater flexibility with regard to possible structural modifications, (iii) feasible access to libraries of structurally related mimetics, (iv) improvement of stability and specificity, (v) improvement of affinity by the incorporation of additional functional groups, and/or (vi) increasing affinity by designing glycomimetics as multivalent molecules.[5]

Various molecular scaffolds have been used for the synthesis of oligosaccharide mimetics, such as branched, oligofunctional non-carbohydrate core molecules[6] or monosaccharides as oligofunctional scaffolds for the synthesis of oligosaccharide mimetics.[7] Herein, we introduce a trifunctional monosaccharide scaffold that can be used for carbohydrate clustering while preserving some of its hydroxyl groups for molecular recognition. In Figure 1 the principal structure of such a derivative is shown, designed as an ABC-type building block, carrying three potentially reactive sites, namely A, B, and C. These can be subsequently addressed in coupling reactions leading to disaccharide mimetics of type II or trisaccharide mimetics of type III, carrying the remaining functional group C. Eventually, C can be used in many different ways, such as for the attachment of a further monosaccharide unit to obtain tetrasaccharide mimetics, attachment to the solid phase, biolabeling, or incorporation of

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† Dedicated to Prof. Dr. Joachim E. Thiem on the occasion of his 60th birthday.

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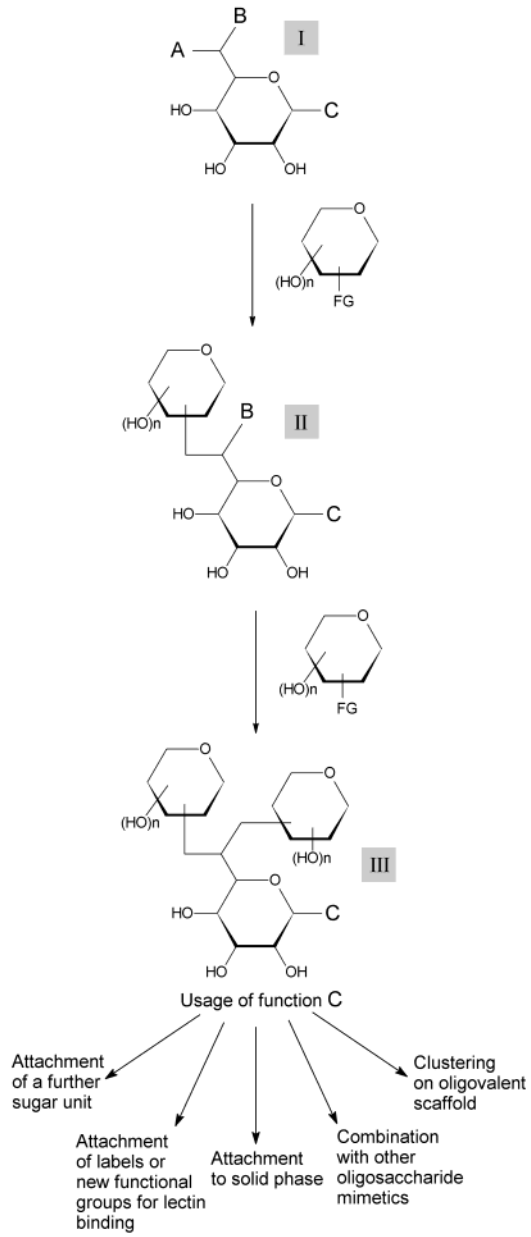
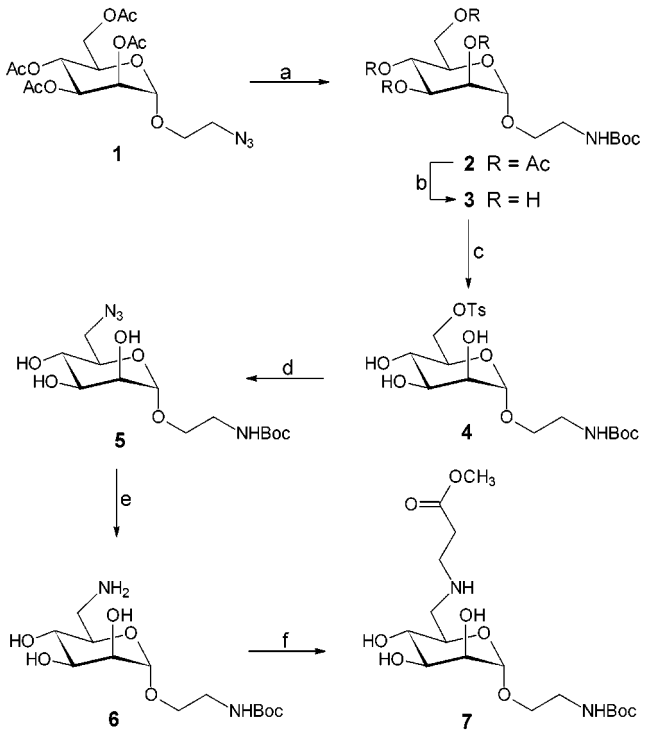


Figure 1. ABC-type carbohydrate building block I , carrying the three functions A, B, and C. These can be consecutively functionalized with various sugar moieties to lead to disaccharide mimetics of type II and in the next step to trisaccharide mimetics of type III. The remaining function C in building block III can be utilized in different ways, e.g., for the attachment of further carbohydrate units, for biolabeling or solid-phase synthesis, for the combination with other oligosaccharide mimetics, or for clustering of III to obtain the respective multivalent analogues. ABC building block I was realized as mannoside **7**, II as compound **11**, and III as the derivative **13**. Further enlargement of **13** was demonstrated with the synthesis of **16** and **18**.

a further group to add affinity. Furthermore, coupling to other oligosaccharide mimetics or clustering on an oligovalent scaffold is possible in order to obtain oligovalent oligosaccharide mimetics. This concept also implies the possibility to synthesize oligosaccharide analogues with mixed carbohydrate configurations as well as combinatorial applications.

As we have been especially interested in the development of mannose-containing glycoclusters for the inhibition of mannose-specific adhesion of *Escherichia coli* bacteria to their host cells,[8] the concept outlined in Figure 1 was demonstrated on the basis of mannose as the principal sugar. Thus, building block I was realized as mannoside **7**, II as the disaccharide analogue **11**, and III as **14**. Furthermore, enlargement of **14** to the tetrasaccharide analogue **16** was shown as well as its oligomerization to glycocluster **18**, containing nine mannose moieties. The prepared glycoclusters will eventually be tested as inhibitors of mannose-specific bacterial adhesion.

Scheme 1[a]



[a] Key: (a) Pd−C, H_2, $(Boc)_2O$, ethyl acetate, rt, 12 h, 91%; (b) NH_3−MeOH, 0 °C, 12 h, 94%; (c) TsCl, pyridine, 0 °C → rt, 2 h, 68%; (d) NaN_3, DMF, 60 °C, 8 h, 78%; (e) Pd−C, H_2, MeOH, rt, 6 h, 80%; (f) methyl acrylate, MeOH, 0 °C → rt, 15 h, 70%.

Synthesis

The key steps in the synthetic route to the potentially trifunctional building block **7** starting with D-mannose are glycosidation, regioselective protection of the 6-position of the sugar ring, and controlled Michael-analogue addition reaction as the last step (Scheme 1). First, the Lewis acid-catalyzed mannosylation of 2-bromoethanol[9] with peracetylated mannose followed by nucleophilic displacement of the bromo substituent in the aglycon part of the resulting mannoside with sodium azide gave rise to the known 2-azidoethyl mannoside derivative **1**.[10] Then, catalytic hydrogenation of the azide group in **1** succeeded in order to obtain the respective amine. This reaction was carried out in the presence of Boc_2O to avoid

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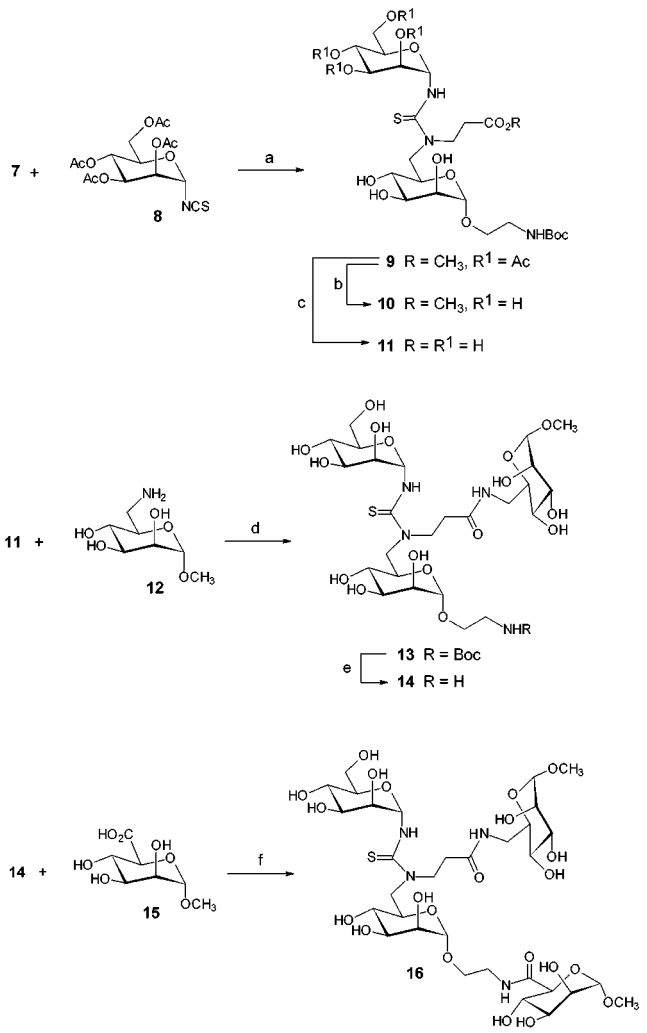
O → N acetyl group migration and to achieve in situ protection of the resulting free amine in the same reaction vessel. This procedure furnished the acetylated *N*-Boc-protected mannoside **2** in 91% yield. For **2** an upfield shift of the protons adjacent to the NHBoc group (3.36 compared to 3.46 ppm for the azide **1**) and a singlet for the Boc group at 1.46 ppm were observed in its ^{1}H NMR spectrum. Mannoside **2** was easily deacetylated to **3**, which was regioselectively tosylated to the 6-activated glycoside **4**. A nucleophilic substitution reaction using sodium azide afforded the 6-azido-6-deoxy-mannoside **5**, which was reduced to the corresponding amine **6** by catalytic hydrogenation in 92% overall yield.

The exhaustive Michael-analogue addition of primary amines to methyl acrylate is known as one of two synthetic steps in the construction of PAMAM dendrimer generations.[11] While in this reaction an excess of methyl acrylate is used to obtain the bis-addition product, a stoichiometric amount of methyl acrylate allowed the conversion of the amine **6** into the mono-Michael adduct **7** in 70% yield. To avoid the formation of polymeric side products resulting from photochemical reactions of methyl acrylate, the reaction was carried out under the exclusion of light. The structure of **7** was easily confirmed by ^{1}H and ^{13}C NMR spectroscopy, which displayed the signals for the methoxycarbonylethyl spacer as expected. The chemical shifts for singlets of methoxycarbonyl groups in ^{1}H NMR are normally obtained in the range of 3.6−4.0 ppm, and a corresponding chemical shift of 3.63 ppm was recorded for the methoxycarbonyl group of **7** when the ^{1}H NMR spectrum was recorded in acetone-d_6. However, when **7** was dissolved in methanol-d_4 or D_2O, respectively, the CH_3-singlet was observed at 3.33 ppm after 12 h at ambient temperature, whereas the chemical shifts of the other signals remained unchanged. NMR studies revealed that this change was due to ester hydrolysis of the methoxycarbonyl group by water, which was also present in the methanol-d_4 used. The observed singlet at 3.33 ppm corresponds to the CH_3 group of resulting methanol. In the ^{13}C NMR spectrum of **7**, two peaks were observed for the carbonyl carbon of the Boc group (at 158.9 and 158.8 ppm), thus revealing the presence of *E*,*Z* isomers that result from the partial double-bond character of the Boc C−N bond. As a result of this isomerism, broadening of the signal for the anomeric proton is observed in the ^{1}H NMR spectrum.

Mannoside **7** represents the desired building block of type I as suggested in Figure 1. It carries three potentially reactive functions, an unprotected secondary amine, a methyl ester that is cleavable under basic conditions, and a Boc-protected amine that can be liberated under mild acidic conditions, which leave the glycosidic bond intact. The secondary amino group in **7** can be functionalized by thiourea bridging[12] or peptide coupling, for example, and was addressed first (Scheme 2). Thus, **7** was reacted with acetylated mannosyl isothiocyanate **8**,[13] allowing the formation of the partially protected thiourea derivative **9**. The formation of the thiourea bridge was confirmed by downfield shifts (in the range of 1 ppm) of the signals for both diastereotopic H-6 as well as the

Scheme 2[a]



[a] Key: (a) CH_2Cl_2, rt, 6 h, 89%; (b) NH_3−MeOH, rt, 2 h, 91%; (c) LiOH·H_2O, MeOH−H_2O (2:1), 0 °C, 12 h, quant; (d) HATU, DIPEA, DMF, rt → 40 °C, 12 h, 43%; (e) Me_2S−CF_3CO_2H (1:2), 0 °C, 4 h, quant; (f) HATU, DIPEA, DMF, rt → 40 °C, 12 h, 71%.

NCH_2 protons in the ^{1}H NMR spectrum. Furthermore, the structure of **9** was confirmed by the appearance of a peak for the thiocarbonyl group (C═S) in the ^{13}C NMR spectrum; MALDI-TOF analysis gave the expected peak (m/z = 798.6 for $[M + H]^+$). Compound **9** can be regarded as a disaccharide mimetic in which two monosaccharide units are connected by nonglycosidic linkages. Treatment of **9** under mild basic conditions afforded the OH-unprotected methyl ester **10** in quantitative yield. Using an excess of lithium hydroxide in aqueous methanol led to concomitant hydrolysis of the acetates and the methyl ester providing the propanoic acid derivative **11**. This was subjected to a peptide coupling reaction in order to prepare the trisaccharide mimetic of type III (Figure 1). Methyl 6-amino-6-deoxy-α-D-mannopyranoside (**12**)[14] was used as the amino component and uronium salt HATU[15] as coupling reagent. This reaction provided the desired glycopeptide mimetic **13**, which was purified by two subsequent size-exclusion chromatography steps. First gel permeation chromatography (GPC) using Sephadex

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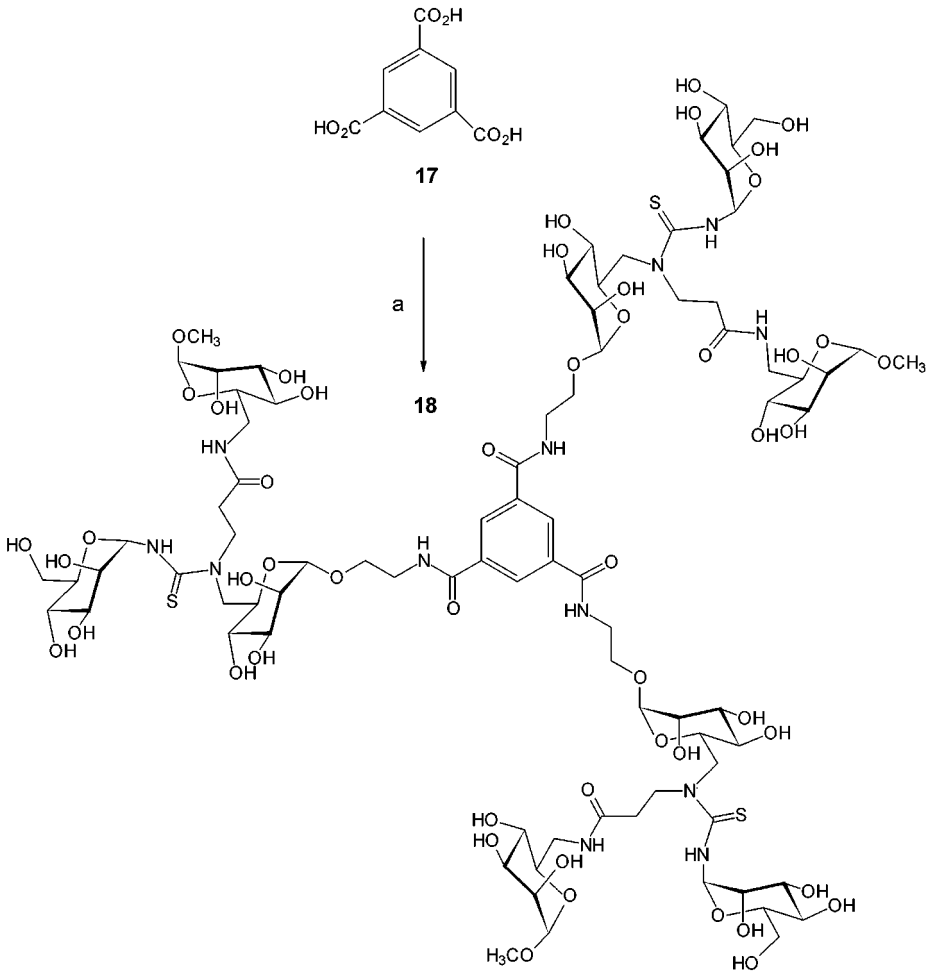
Scheme 3[a]



[a] Key: (a) **14**, HATU, DIPEA, DMF, rt → 40 °C, 12 h, 30%.

LH-20 and methanol as the eluent allowed the isolation of a mixture of **13** and **11**, which was separated from reagent impurities. Then GPC on Bio-Gel P-2 using 15mM NH_4HCO_3 buffer as the eluent was suited to separate **13** from **11** and furnished **13** in 43% yield and 35% recovered carboxylic acid **11**. The NMR spectra of **13** showed the expected signals of the additional monomeric mannoside unit with its methyl aglycone as a useful NMR reporter group. The MALDI-TOF spectrum showed the correct $[M + H]^+$ peak at $m/z = 791.2$.

The obtained trisaccharide mimetic **13** carries a Boc-protected amino group that was regarded as "function C" in Figure 1. According to the possibilities outlined in Figure 1, **13** can be modified in a variety of ways. Here, we demonstrate the functionalization of **13** with another mannose moiety leading to the tetrasaccharide mimetic **16** and, furthermore, the utilization of **13** as a molecular wedge leading to the trivalent conjugate **18** (Scheme 3).

To synthesize the tetrasaccharide mimetic **16**, **13** was subjected to Boc deprotection in an anhydrous mixture of trifluoroacetic acid and dimethyl sulfide (2:1)[16] to yield the respective free amine **14**. In this reaction after concentration of the reaction mixture it was important to neutralize the last traces of TFA with aqueous ammonia in order to avoid trifluoroacetylation of the product amine **14** in the next step. The amine **14** was then subjected to a peptide-coupling reaction using the mannouronic acid derivative **15**. Analogous reaction conditions as used previously for synthesis of compound **13** afforded the mannose cluster **16** in 70% yield. The anomeric hydrogen and carbon atoms resonated as distinct signals both in the ^1H as well as the ^{13}C NMR spectrum. The MALDI-TOF mass spectrum showed $[M + H]^+$ at $m/z = 881.8$.

For clustering of **14**, trimesic acid (**17**) was used as a trivalent core (Scheme 3). Deprotection of **13** to **14**, followed by HATU-mediated peptide-coupling reaction with **17**, afforded the complex glycocluster **18** in 30% yield. Compound **18** resembles a trimeric derivative of the trisaccharide mimetic **14**. Its purification was problematic and succeeded best when it was carried out using GPC on Bio-gel P-2 on a small scale. NMR spectra of **18** were very similar to those of **13** except for the missing signals for the Boc group and the appearance of those for the aromatic core moiety, which were detected as expected (8.34 ppm in ^1H NMR). A clean MALDI-TOF mass spectrum ($m/z = 2227.8$ for $[M + H]^+$) further confirmed the structure of **18**.

Discussion and Conclusions

En route to the oligosaccharide mimetics **13**, **16**, and **18**, respectively, three peptide-coupling reactions were elaborated. Interestingly, the coupling step proceeded

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with yields around 70%, when **14** was used as the amine component. Consequently, cluster **18**, in which three peptide linkages had to be formed, was obtained in 30% overall yield. On the other hand, peptide coupling under the same conditions proceeded with modest yields when the carboxylic function of **11** was addressed as significant amounts of **11** did not react in this step. A possible reason for the limited reactivity of this system may be the formation of an intramolecular hydrogen bond between the carboxylic acid proton and the nitrogen atom resulting in a stable six-membered-ring arrangement.

For the structural characterization of the OH-unprotected compounds **9**, **10**, **13**, **16**, and **18** with molecular weights 797.8, 629.7, 790.8, 880.8, and 2228.7, respectively, extensive NMR-spectroscopic experiments were necessary. While some parts of their ^{1}H NMR spectra are rather poorly resolved, the anomeric protons of the contained sugar moieties are always separated from the other signals and can be used as immediate indicators for the success of the reaction. ^{13}C NMR spectra of these compounds, on the other hand, were well resolved, and thus, together with 2D NMR experiments all hydrogen and carbon atoms of each derivative could be detected and all signals unequivocally assigned. The structures of all glycoclusters were confirmed by MALDI-TOF mass spectrometry, which was also an excellent and important tool for monitoring the reactions described herein.

On the basis of the presented building block system, a wide array of oligosaccharide mimetics is accessible without utilizing the notoriously difficult glycosylation chemistry. The suggested concept implies synthetic routes for biolabeling, combinatorial approaches, or synthesis of oligovalent neoglycoconjugates, for example. It is important to note that the orthogonal functional group pattern in **7** allows the successive attachment of very different (sugar) moieties including pharmacophores and the preparation of "mixed" glycoclusters, representing various three-dimensional ensembles of functional groups that are otherwise assembled by the oligosaccharide parts of complex glycoconjugates. This might be of relevance when bacterial adhesion of wild-type strains has to be inhibited, which normally carry pili of different specificities. We will explore the presented concept to optimize ligand structures for various lectins and adhesion systems.

Experimental Section

General Methods and Materials. TLC as well as flash chromatography were performed on silica gel, 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 was used with methanol as the eluent and Biogel P-2 with 15 mM aqueous NH_4HCO_3 buffer (pH = 7.8−8.0) as the eluent. Organic solutions were concentrated using a rotary evaporator at bath temperatures <45 °C. Aqueous solutions were concentrated by lyophilization. ^{1}H and ^{13}C NMR spectra were recorded at 298 K at 400 MHz (for ^{1}H, 100.67 MHz for ^{13}C NMR) or 500 MHz (for ^{1}H, 125.84 MHz for ^{13}C NMR). Chemical shifts are given in ppm relative to internal TMS (0.00 ppm for ^{1}H and ^{13}C NMR), and when the samples were measured in D_2O the spectra were calibrated referring to internal HOD (4.63 ppm for ^{1}H NMR) and in the case of ^{13}C NMR to MeOH-d_4 (49.30 ppm for ^{13}C NMR) which was added to the solution. *J* values 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. Melting points are uncorrected. For MALDI-TOF measurements the samples were prepared as solutions in TFA−acetonitrile−water, 0.1:2:1, with a concentration of 1 mg of compound in 1 mL of solution. Dihydroxybenzoic acid (DHB) was used as the matrix for the crystallization of compounds **9**, **11**, **13**, **16**, and **18**. The mass peaks obtained with these 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 $[2M + H]^+$ peak of α-cyano-4-hydroxycinamic acid (380.02). Optical rotations were recorded at the Na D line, 589 nm, 20 °C, cell length 10 cm. Elemental analyses were carried out in the microanalytical laboratory of the Institut für Organische Chemie der Universität Hamburg. Methyl α-D-mannopyranuronic acid **15** was synthesized from methyl α-D-mannopyranoside according to a literature procedure.[17]

2-*tert*-Butyloxycarbonylamidoethyl 2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranoside (2). A suspension of the azide **1** (7.10 g, 17.02 mmol), activated Pd catalyst (10% on charcoal, 100 mg), and di-*tert*-butyl dicarbonate (5.57 g, 25.53 mmol) in ethyl acetate (50 mL) was hydrogenated under atmospheric pressure for 6 h at rt. Then the reaction mixture was filtered through a thin Celite bed, and the filtrate was concentrated. The crude product was purified by flash chromatography (ethyl acetate−petroleum ether, 1:2) to afford the Boc-protected mannoside **2** in the form of a white solid (7.60 g, 15.47 mmol, 91%): $[\alpha]_D = +36°$ ($c = 1.10$ in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.33 (dd, $J_{3,4} = 9.7$ Hz, 1H, H-3), 5.28 (dd, $J_{4,5} = 9.2$ Hz, 1H, H-4), 5.26 (dd, $J_{2,3} = 3.1$ Hz, 1H, H-2), 4.93 (s, 1H, NH), 4.83 (d, $J_{1,2} = 1.5$ Hz, 1H, H-1), 4.28 (dd, $J_{6a,6b} = 12.2$ Hz, 1H, H-6a), 4.10 (dd, $J_{6a,6b} = 12.2$, $J_{5,6b} = 5.1$ Hz, 1H, H-6b), 3.98 (ddd, $J_{5,6} = 2.5$, $J_{5,6b} = 5.1$ Hz, 1H, H-5), 3.76 (ddd, 1H, OCH_a), 3.54 (ddd, 1H, OCH_b), 3.36 (m_c, 2H, CH_2N), 2.18, 2.12, 2.05, 2.01 (each s, each 3H, 4 $COCH_3$), 1.46 (s, 9H, *t*-Bu); ^{13}C NMR (100.67 MHz, $CDCl_3$) δ 172.7, 172.0, 171.9, 171.8 (4 $COCH_3$), 158.8 (BocCO), 99.3 (C-1), 80.6 ($C(CH_3)_3$), 71.2 (C-2), 71.1 (C-3), 70.3 (C-5), 68.6 (OCH_2), 67.7 (C-4), 63.9 (C-6), 41.4 (CH_2N), 29.2 ($C(CH_3)_3$), 21.1, 21.07, 21.02 (4 $COCH_3$). Anal. Calcd for $C_{21}H_{33}NO_{12}$ (491.49): C, 51.32; H, 6.77; N, 2.85. Found: C, 51.30; H, 6.84; N, 2.83.

2-*tert*-Butyloxycarbonylamidoethyl α-D-mannopyranoside (3). To a solution of the tetraacetylated mannoside **2** (7.60 g, 15.47 mmol) in methanol (50 mL), was added saturated NH_3−MeOH (10 mL) at 0 °C and the reaction mixture was stirred at 0 °C for 2 h. Then it was concentrated and the crude product was purified by flash chromatography (MeOH−CH_2Cl_2, 1:10) to yield **3** in the form of a white solid (4.67 g, 14.45 mmol, 94%): $[\alpha]_D = +60°$ ($c = 1.21$ in MeOH); ^{1}H NMR (400 MHz, [D$_4$]-MeOH): $\delta = 4.76$ (d, $J_{1,2} = 1.5$ Hz, $^1J_{C1,H1} = 168.5$, 1H, H-1), 3.82 (dd, $J_{6a,5} = 2.6$ Hz, 1H, H-6a), 3.81 (dd, $J_{2,3} = 3.1$ Hz, 1H, H-2), 3.75−3.68 (m, 2H, H-6b, OCH_a), 3.69 (dd, $J_{3,4} = 9.7$ Hz, 1H, H-3), 3.61 (dd, $J_{4,3} = 9.7$ Hz, 1H, H-4), 3.56−3.44 (m, 2H, H-5, OCH_b), 3.24 (m_c, 2H, NCH_2), 1.44 (s, 9H, *t*-Bu); ^{13}C NMR (100.62 MHz, [D$_4$]-MeOH): $\delta = 158.3$ (BocCO), 101.7 (C-1), 80.1 ($C(CH_3)_3$), 74.7 (C-5), 72.5 (C-3), 72.1 (C-2), 68.6 (C-4), 67.6 (OCH_2), 62.9 (C-6), 41.2 (CH_2N), 28.8 ($C(CH_3)_3$). Anal. Calcd for $C_{13}H_{25}NO_8 \times 1H_2O$ (341.36): C, 45.74; H, 7.97; N, 4.10. Found: C, 45.68; H, 7.74; N, 3.89.

2-*tert*-Butyloxycarbonylamidoethyl 6-*O*-(toluene-4-sulfonyl)-α-D-mannopyranoside (4). To a solution of the *O*-unprotected mannoside **3** (4.67 g, 14.45 mmol) in pyridine (20 mL) was added tosyl chloride (4.13 g, 21.68 mmol) at 0 °C under argon atmosphere. Then the reaction mixture was allowed to attain rt and was further stirred for 2 h. The reaction was quenched with MeOH at 0 °C and the solvent was removed. The crude product so obtained was purified by flash chromatography (CH_2Cl_2−MeOH, 10:1) to give the 6-*O*-tosylated derivative **4** (4.60 g, 9.64 mmol, 68%) as a white solid: $[\alpha]_D = +36°$ ($c = 1.96$ in MeOH); ^{1}H NMR (400 MHz, [D$_4$]-MeOH): $\delta = 7.80$ (d, $J = 8.1$ Hz, 2H, aryl-H), 7.40 (d, $J = 8.1$ Hz, 2H, aryl-H), 4.66 (s, 1H, H-1), 4.32 (dd, $J_{5,6b} = 1.5$, $J_{6a,6b} = 10.7$ Hz, 1H, H-6b), 4.17 (dd, $J_{6a,6b} = 10.7$ Hz, 1H,

(17) Davis, N. J.; Flitsch, S. L. *Tetrahedron Lett.* **1993**, *34*, 1181−1184.

H-6a), 3.78 (dd, $J_{2,3}$ = 3.1 Hz, 1H, H-2), 3.66 (m_c, $J_{5,6a}$= 6.6, $J_{5,6b}$ = 1.5 Hz, 1H, H-5), 3.64 (dd, $J_{3,4}$ = 9.7 Hz, 1H, H-3), 3.6 (m_c, 1H, OCH_a), 3.52 (dd, 1H, H-4), 3.4(m_c, 1H, OCH_b), 3.2 (m_c, 2H, CH_2N), 2.45 (s, 3H, $TsCH_3$), 1.43 (s, 9H, *t*-Bu); ^{13}C NMR (100.62 MHz, [D$_4$]-MeOH): δ = 158.1 (BocCO), 146.84 (aryl-C_q), 134.81 (aryl-C_q), 131.4 (aryl-CH), 131.2 (aryl-CH), 129.6 (2 aryl-CH), 102.1 (C-1), 80.1 ($C(CH_3)_3$), 72.8 (C-5), 72.6 (C-3), 72.2 (C-2), 71.7 (C-6), 68.5 (C-4), 68.1 (OCH_2), 41.5 (CH_2N), 29.2 ($C(CH_3)_3$, 22.0 ($TsCH_3$). Anal. Calcd for $C_{20}H_{31}NO_{10}S \times 1H_2O$ (495.54): C, 48.48; H, 6.71; N, 2.83, S, 6.47. Found: C, 48.82; H, 6.51; N, 3.22, S, 6.34.

2-*tert*-Butyloxycarbonylamidoethyl 6-azido-6-deoxy-α-D-mannopyranoside (5). A suspension of the tosylated mannoside **4** (4.60 g, 9.64 mmol), NaN_3 (1.90 g, 29.22 mmol) and a catalytic amount of tetra-*n*-butylammonium iodide (100 mg) in dry DMF (25 mL) was stirred at 60 °C for 8 h. After completion of the reaction (monitored by TLC with CH_2Cl_2−MeOH, 10:1) DMF was removed under high vacuum and the residual solid was dissolved in ethyl acetate and filtered through a thin Celite bed to remove the excess of NaN_3 from the product mixture. Then the solvent was removed and the crude product was purified by flash chromatography (CH_2Cl_2−MeOH, 10:1) to yield the azide **5** (2.60 g, 7.47 mmol, 78%) as a colorless syrup: $[\alpha]_D$ = +29° (c = 4.43 in MeOH); ^{1}H NMR (400 MHz, [D$_4$]-MeOH): δ = 4.77 (s, 1H, H-1), 3.82 (dd, $J_{2,1}$ = 1.5, $J_{2,3}$ = 3.1 Hz, 1H, H-2), 3.74 (m_c, 1H, OCH_b), 3.68 (dd, $J_{3,4}$ = 9.2 Hz, 1H, H-3), 3.66 (m_c, 1H, H-5), 3.57 (dd, 1H, H-4), 3.43 (dd, $J_{6a,5}$ = 6.6, $J_{6a,6b}$ = 13.2 Hz, 1H, H-6a), 3.43−3.53 (m, $J_{6a,6b}$ = 13.2 Hz, 2H, H-6b, OCH_a), 3.24 (m_c, 2H, CH_2N), 1.45 (s, 9H, *t*-Bu); ^{13}C NMR (100.62 MHz, [D$_4$]-MeOH): δ = 158.9 (BocCO), 102.2 (C-1), 80.6 ($C(CH_3)_3$), 74.3 (C-5), 72.7 (C-3), 72.4 (C-2), 69.8 (C-4), 68.2 (OCH_2), 53.3 (C-6), 41.6 (CH_2N), 29.2 ($C(CH_3)_3$). Anal. Calcd for $C_{13}H_{24}N_4O_7 \times 1H_2O$ (366.1): C, 42.61; H, 7.10. Found: C, 42.82; H, 6.98.

2-*tert*-Butyloxycarbonylamidoethyl 6-amino-6-deoxy-α-D-mannopyranoside (6). To a solution of the azide **5** (2.60 g, 7.47 mmol) in MeOH (20 mL) was added activated Pd-catalyst (10% on charcoal, 50 mg), and the reaction mixture was hydrogenated under atmospheric pressure for 6 h at rt. Then it was filtered through a thin Celite bed and the filtrate was concentrated. The crude product was purified by flash chromatography (MeOH−CH_2Cl_2, 1:1) to give **6** (1.93 g, 5.99 mmol, 80%) as a white solid: $[\alpha]_D$ = +45° (c = 0.80 in MeOH); ^{1}H NMR (400 MHz, [D$_4$]-MeOH): δ = 4.74 (s, 1H, H-1), 3.8 (s, 1H, H-2), 3.6−3.8 (m, 2H, OCH_b, H-3), 3.55−3.4 (m, 2H, OCH_a, H-5), 3.5 (dd, 1H, H-4), 3.3 (d, 1H, H-6b), 3.22 (m_c, 2H, CH_2N), 2.8 (m_c, 1H, H-6a), 1.5 (s, 9H, *t*-Bu); ^{13}C NMR (100.62 MHz, [D$_4$]-MeOH): δ = 158.4 (BocCO), 101.6 (C-1), 79.1 ($C(CH_3)_3$), 74.3 (C-5), 72.3 (C-3), 72.0 (C-2), 69.8 (C-4), 67.3 (OCH_2), 43.5 (C-6), 41.2 (CH_2N), 28.7 ($C(CH_3)_3$). A correct elemental analysis for $C_{13}H_{26}N_2O_7$ (322.36) was not obtained.

2-*tert*-Butyloxycarbonylamidoethyl 6-deoxy-6-[*N*-(2-methoxycarbonylethyl)amino]-α-D-mannopyranoside (7). To a solution of the amino-functionalized mannoside **6** (1.93 g, 5.99 mmol) in dry MeOH (8 mL) was added freshly distilled methyl acrylate (0.54 mL, 5.99 mmol) at 0 °C under argon atmosphere and the reaction mixture was stirred in the dark for 15 h at rt. Then it was concentrated and the residue was purified by flash chromatography (CH_2Cl_2−MeOH, 1:1) to afford the Michael-adduct **7** (1.70 g, 4.16 mmol, 70%) as a white solid: $[\alpha]_D$ = +35° (c = 1.50 in MeOH); ^{1}H NMR (400 MHz, [D$_4$]-MeOH): δ = 4.74 (s, 1H, H-1), 3.80 (s, 1H, H-2), 3.76−3.6 (m, 3H, H-5, OCH_a, H-3), 3.68 (s, 3H, CO_2CH_3), 3.53−3.43 (m, 2H, H-4, OCH_b), 3.34−3.15 (m, 2H, CH_2NHBoc), 2.98 (dd, 1H, H-6a), 2.90 (m_c, 2H, NCH_2), 2.76 (dd, 1H, H-6b), 2.57 (m_c, 2H, CH_2CO), 1.43 (s, 9H, *t*-Bu); ^{13}C NMR (100.62 MHz, [D$_4$]-MeOH): δ = 174.9 (CO_2CH_3), 158.9 (BocCO), 102.1 (C-1), 80.5 ($C(CH_3)_3$), 72.8 (C-3), 72.5 (C-5), 72.4 (C-2), 71.2 (C-4), 68.0 (OCH_2), 52.6 (CO_2CH_3), 52.2 (C-6), 46.3 (NCH_2), 41.6 (CH_2-NBoc), 35.0 (CH_2CO), 29.26 ($C(CH_3)_3$). Anal. Calcd for $C_{17}H_{32}N_2O_9 \cdot x\ 1H_2O$ (426.46): C, 47.88; H, 8.04; N, 6.57. Found: C, 48.34; H, 7.91; N, 6.17.

2-*tert*-Butyloxycarbonylamidoethyl 6-deoxy-6-*N*-[(2′,3′,4′,6′-tetra-*O*-acetyl-α-D-mannopyranosyl)thiocarbamoyl]-*N*-[2-(methoxycarbonyl)ethyl]amino-α-D-mannopyranoside (9). Mannoside **7** (1.10 g, 2.69 mmol) and the mannosyl isothiocyanate derivative **8** (1.15 g, 2.96 mmol) were dissolved in dry CH_2Cl_2 (10 mL) and stirred at rt for 12 h. When the reaction was complete (monitored by TLC with MeOH−CH_2Cl_2, 1:9) it was concentrated and the crude product was purified by flash chromatography (MeOH−CH_2Cl_2, 1:9) to give the thiourea-bridged disaccharide mimetic **9** as a white solid (1.90 g, 2.38 mmol, 89%): $[\alpha]_D$ = +7° (c = 1.30 in MeOH); ^{1}H NMR (500 MHz, [D$_4$]-MeOH): δ = 6.20 (d, $J_{1',2'}$ = 1.9 Hz, 1H, H-1′), 5.36−5.25 (m, 3H, H-2′, H-4′, H-3′), 5.02 (s, 1H, H-1), 4.35 (dd, $J_{6',5'}$ = 4.1, $J_{6a',6b'}$ = 12.0 Hz, 1H, H-6a′), 4.3−4.0 (m, $J_{6',5'}$ = 4.1, $J_{6a',6b'}$ = 12.0 Hz, 4H, H-5′, H-6b′, NCH_2), 3.96−3.85 (m, 3H, H-5, H-6a, H-6b), 3.83 (dd, $J_{1,2}$ = 1.6, $J_{2,3}$ = 2.8 Hz, 1H, H-2), 3.73 (dd, $J_{3,4}$ = 9.2 Hz, 1H, H-3), 3.68 (s, 3H, CO_2CH_3), 3.66 (m_c, 1H, OCH_a), 3.60−3.50 (m, 2H, H-4, OCH_b), 3.23 (m_c, 2H, CH_2NHBoc), 2.90 (t, 2H, CH_2CO), 1.90, 2.00, 2.10, 2.20 (each s, each 3H, 4 $COCH_3$), 1.44 (s, 9H, *t*-Bu); ^{13}C NMR (125.84 MHz, [D$_4$]-MeOH): δ = 186.0 (C=S), 172.9, 172.1, 171.9, 171.7 (4 $COCH_3$, CO_2CH_3), 158.8 (BocCO), 102.5 (C-1), 82.9 ($C(CH_3)_3$), 80.6 (C-1′), 72.7 (C-3), 72.1 (C-2, C-5), 71.5 (C-5′), 71.4 (C-3′), 71.1 (C-2′), 69.4 (C-4), 68.5 (OCH_2), 67.9 (C-4′), 63.7 (C-6′), 53.43 (NCH_2), 52.7 (CO_2CH_3), 51.5 (C-6), 41.5 (CH_2NHBoc), 32.8 (CH_2CO), 29.2 ($C(CH_3)_3$), 21.16 (4 $COCH_3$); MALDI-TOF MS: m/z = 798.6 $[M+H]^+$, 820.7 $[M+Na]^+$ and 836.6 $[M+K]^+$ observed for $C_{32}H_{51}N_3O_{18}S$ (797.28). Anal. Calcd for $C_{32}H_{51}N_3O_{18}S \times 1H_2O$ (815.84): C, 47.11; H, 6.55; N, 5.15; S, 3.93. Found: C, 47.19; H, 6.46; N, 5.09; S, 4.14.

2-*tert*-Butyloxycarbonylamidoethyl 6-deoxy-6-*N*-[(α-D-mannopyranosyl)thiocarbamoyl]-*N*-[2-(methoxycarbonyl)ethyl]amino-α-D-mannopyranoside (10). A solution of compound **9** (0.142 g, 0.178 mmol) in MeOH (2 mL) was treated with saturated NH_3−MeOH solution (2 mL) at 0 °C for 2 h. Then the solvent was evaporated and the crude product was purified by flash chromatography (MeOH−CH_2Cl_2, 1:1) providing **10** (0.102 g, 0.161 mmol, 91%) as a light yellow solid, which showed hygroscopic properties: $[\alpha]_D$ = −41° (c = 0.97 in H_2O); ^{1}H NMR (500 MHz, D_2O): δ = 5.97 (d, $J_{1',2'}$ = 1.9 Hz, 1H, H-1′), 4.85 (bs, 1H, H-1), 4.18 (m_c, 1H, NCH_a), 4.0 (m_c, 1H, NCH_b), 3.96−3.91 (m, 2H, H-2, H-2′), 3.81−3.73 (m, $J_{5,6a}$ = 4.1 Hz, $J_{5',6a'}$ = 2.5 Hz, $J_{6a',6b'}$ = 13.6 Hz, 4H, H-6a, H-6b, H-6a′, H-6b′), 3.74 (m_c, 1H, H-5), 3.71−3.67 (m, 2H, H-3′, H-3), 3.66 (s, 3H, CO_2CH_3), 3.58−3.45 (m, 4H, OCH_2, H-4′, H-4), 3.41 (m_c, 1H, H-5′), 3.23 (m_c, 2H, CH_2NHBoc), 2.83 (m_c, 2H, CH_2CO), 1.39 (s, 9H, *t*-Bu); ^{13}C NMR (125 MHz, D_2O): δ = 183.2 (C=S), 174.9 (CO_2CH_3), 158.4 (BocCO), 100.5 (C-1), 83.5 (C′-1), 81.3 ($C(CH_3)_3$), 77.9 (C′-5), 74.5 (C′-4, C-4), 71.3 (C-5), 70.3, 70.2 (C′-2, C-2), 67.4 (OCH_2), 67.2 (C-3, C′-3), 61.2 (C′-6), 52.8 (CO_2CH_3), 52.0 (C-6), 49.7 (NCH_2), 39.9 (CH_2NHBoc), 32.1 (CH_2CO), 28.1 ($C(CH_3)_3$); MALDI-TOF MS m/z = 630.8 $[M + H]^+$, 652.8 $[M + Na]^+$ and 668.8 $[M + K]^+$ obsd for $C_{24}H_{43}N_3O_{14}S$ (629.24). Anal. Calcd for $C_{24}H_{43}N_3O_{14}S \cdot 1H_2O$ (647.69): C, 44.51; H, 7.00; N, 6.49; S, 4.95. Found: C, 44.3; H, 6.94; N, 6.63; S, 5.00.

2-*tert*-Butyloxycarbonylamidoethyl 6-Deoxy-6-*N*-[(α-D-mannopyranosyl)thiocarbamoyl]-*N*-[2-[(6″-deoxy-1″-*O*-methyl-α-D-mannopyranos-6″-yl)carbamoyl]ethyl]amino-α-D-mannopyranoside (13). The thiourea derivative **9** (500 mg, 0.627 mmol) was dissolved in MeOH−H_2O (1:2; 5 mL) and treated with $LiOH \cdot 1H_2O$ (131.6 mg, 3.135 mmol) at 0 °C for 12 h. Then the pH of the basic reaction mixture was neutralized at 0 °C to a value of 6 by the careful addition of 2 N HCl solution. The clear solution that was obtained was freeze-dried to afford the crude carboxylic acid **11** as a white solid (385 mg), which was subjected to peptide coupling without purification. It was dissolved in dry DMF (10 mL), and the 6-amino-modified methyl mannoside **12** (144 mg, 0.751 mmol), HATU (285 mg, 0.751 mmol), and Hünig's base (DIPEA, 0.22 mL, 1.25 mmol) were added at rt under argon atmosphere. The reaction mixture was stirred at 40 °C for 12 h, and then DMF was removed in vacuo. The resulting crude product was a pale yellow syrup that was subjected to gel permeation chromatography on Biogel P-2. This afforded the title compound **13** (215 mg, 0.272 mmol, 43%) with 145 mg (0.235 mmol, 37%) of the intermediate acid **11** being recovered in pure form. Product **13** was obtained as a white lyophilisate that showed hygro-

scopic properties: $[\alpha]_D = -30°$ ($c = 1.15$ in H_2O); ^1H NMR (500 MHz, D_2O) δ 5.5 (bs, 1H, H-1′), 4.86 (bs, 1H, H-1), 4.68 (d, $J_{1'',2''} = 1.6$ Hz, 1H, H-1″), 4.2 (bs, 1H, NCH_a), 3.93 (bs, 1H, H-2′), 3.90 (bs, 1H, NCH_b), 3.88 (bs, 1H, H-2), 3.87 (dd, $J_{2'',3''} = 3.2$ Hz, 1H, H-2″), 3.83 (bs, 1H, H-6a′), 3.76−3.70 (m, 3H, H-6a, H-3, H-6b), 3.70−3.64 (m, 4H, H-5, H-3″, H-3′, H-6b′), 3.61 (d, 1H, H-6a″), 3.60−3.45 (m, 6H, H-4″, OCH_a, H-4, H-4′, H-5″, OCH_b), 3.40 (m_c, $J_{5',6a'} = 3.8$, $J_{5',6b'} = 6.0$, $J_{4',5'} = 9.8$ Hz, 1H, H-5′), 3.35 (m_c, 1H, H-6b″), 3.33 (s, 3H, OCH_3), 3.20 (m_c, 2H, C*H*$_2$NHBoc), 2.72 (m_c, 2H, CH_2CO), 1.4 (s, 9H, *t*-Bu); ^{13}C NMR (125.84 MHz, D_2O, MeOH-d_4) δ 183.5 (C═S), 174.5 (CONH), 158.8 (BocCO), 101.57 (C-1″), 100.32 (C-1), 83.87 (C-1′), 81.65 (*C*$(CH_3)_3$), 78.23 (C-5′), 74.18 (C-3′), 71.4 (C-2′), 71.37 (C-4″), 71.1 (C-5, C-3″), 70.66 (C-3), 70.59 (C-2, C-2″), 68.82 (C-4′, C-5″), 68.3 (C-4), 67.73 (OCH_2), 61.95 (C-6′), 55.44 (OCH_3), 52.29 (C-6), 51.3 (NCH_2), 40.82 (C-6″), 40.29 (CH_2-NHBoc), 34.22 (*C*H_2CO), 28.47 (C(*C*$H_3)_3$); MALDI-TOF MS *m*/*z* = 791.2 $[M + H]^+$, 813.2 $[M + Na]^+$ and 829.2 $[M + K]^+$ observed for $C_{30}H_{54}N_4O_{18}S$ (790.31). Anal. Calcd for $C_{30}H_{54}N_4O_{18}S\cdot 2H_2O$ (826.87): C, 43.58; H, 7.07; N, 6.78; S, 3.88. Found: C, 43.25; H, 7.14; N, 6.90; S, 3.98.

2-Aminoethyl 6-Deoxy-6-*N*-[(α-D-mannopyranosyl)-thiocarbamoyl]-*N*-[2-[(6″-deoxy-1″-*O*-methyl-α-D-mannopyranos-6″-yl)carbamoyl]ethyl]amino-α-D-mannopyranoside (14). The Boc-protected trisaccharide mimetic **13** (120 mg, 0.151 mmol) was dissolved in a freshly prepared solution of $Me_2S-CF_3CO_2H$ (1:2; 2 mL) at 0 °C and stirred at 0 °C for 3 h. Then the reaction mixture was concentrated. Traces of trifluoroacetic acid which were left in the reaction mixture were carefully neutralized at 0 °C with saturated NH_3-H_2O solution (5 mL). Then lyophilization of the mixture afforded crude amine **14** (104 mg, quant) as a pale yellow solid, which was used for the synthesis of **16** and **18** without purification.

2-[(6‴-Deoxy-1‴-*O*-methyl-α-D-mannopyranos-6‴-yl)-carbamoyl]ethyl 6-Deoxy-6-*N*-[(α-D-mannopyranosyl)-thiocarbamoyl]-*N*-[2-[(6″-deoxy-1″-*O*-methyl-α-D-mannopyranos-6″-yl)carbamoyl]ethyl]amino-α-D-mannopyranoside (16). The amine **14** (32 mg, 0.0463 mmol), the mannouronic acid derivative **15** (18 mg, 0.086 mmol), HATU (34.35 mg, 0.090 mmol), and Hünig's base (DIPEA, 0.025 mL, 0.15 mmol) were dissolved in dry DMF (5 mL) at rt under argon atmosphere and stirred at 40 °C for 12 h. Then the reaction mixture was concentrated, and the resulting pale yellow syrup was subjected to gel permeation chromatography on Bio-gel P-2 to afford **16** (29 mg, 0.032 mmol, 71%) as a white lyophilisate that showed hygroscopic properties: $[\alpha]_D = -3°$ ($c = 1.23$ in H_2O); ^1H NMR (500 MHz, D_2O) δ 5.51 (bs, 1H, H-1′), 4.87 (bs, 1H, H-1), 4.77 (s, 1H, H-1‴), 4.68 (s, 1H, H-1″), 4.16 (bs, 1H, NCH_a), 4.5−3.8 (m, 7H, NCH_b, H-5‴, H-2′, H-2, H-2″, H-2‴, H-6a′), 3.8−3.64 (m, 9H, H-6a, H-3, H-4‴, H-6b, H-3‴, H-6b′, H-5, H-3″, H-3′), 3.64−3.42 (m, 8H, H-6b″, OCH_a, H-4″, OCH_b, H-4′, H-4, H-5″, CH_bN), 3.42−3.32 (m, 3H, H-5′, H-6a″, CH_aN), 3.36, 3.32 (2s, 6H, 2 OCH_3), 2.72 (m, 2H, CH_2CO); ^{13}C NMR (125.84 MHz, D_2O) δ 183.43 (C═S), 174.39 (CONH), 171.77 (NHCO), 101.78 (C-1‴), 101.26 (C-1″), 100.03 (C-1), 83.57 (C-1′), 77.93 (C-5′), 73.85 (C-3″), 72.56 (C-5‴, C-2′), 71.06 (C-4″), 70.78 (C-5), 70.76 (C-3′), 70.55(C-3‴), 70.28 (C-2), 70.26 (C-2″), 70.04 (C-2‴), 68.74 (C-3, C-4‴), 68.48 (C-5″, C-4), 67.02 (C-4′), 66.26 (OCH_2), 61.57 (C-6′), 55.63, 55.11 (2 OCH_3), 52.01 (C-6), 50.92 (NCH_2), 40.47 (C-6″), 39.14 (CH_2N), 33.89 (*C*H_2-CO); MALDI-TOF MS *m*/*z* 881.8 $[M + H]^+$, 903.2 $[M + Na]^+$, 919.2 $[M + K]^+$ obsd for $C_{32}H_{56}N_4O_{22}S$ (880.31). A correct elemental analysis for $C_{32}H_{56}N_4O_{22}S$ (880.87) was not obtained.

1,3,5-[Tris-*N*-[2-(6-deoxy-6-*N*-[(α-D-mannopyranosyl)-thiocarbamoyl]-*N*-[2-((6″-deoxy-1″-*O*-methyl-α-D-mannopyranos-6″-yl)carbamoyl)ethyl]amino-α-D-mannopyranosyloxyethyl]]benzenetriamide (18). The amino-functionalized trisaccharide mimetic **14** (8.10 mg, 0.0117 mmol), trimesic acid **17** (1.70 mg, 0.008 mmol), HATU (15.30 mg, 0.04 mmol), and Hünig's base (DIPEA, 8 μL, 0.048 mmol) were dissolved in dry DMF (3 mL) at rt under argon atmosphere. The reaction mixture was stirred at 40 °C for 12 h and then concentrated to dryness. The resulting crude product was a pale yellow syrup that was subjected to gel permeation chromatography on Bio-gel P-2 to afford the title cluster **18** (7.80 mg, 0.0035 mmol, 30%) as a white lyophilisate that showed hygroscopic properties: $[\alpha]_D = -9°$ ($c = 0.8$ in H_2O); ^1H NMR (500 MHz, D_2O) δ 8.34 (s, 3H, 3 aryl-H), 5.48 (bs, 3H, 3 H-1′), 4.9 (bs, 3H, 3 H-1), 4.67 (s, 3H, 3 H-1″), 3.95 (bs, 3H, 3 H-2), 3.90−3.82 (m, 12H, 3 NCH_a, 3 H-2″, 3 H-6a′, 3 H-2′), 3.82−3.70 (m, 6H, 3 H-3, 3 OCH_a), 3.72−3.60 (m, 27H, 3 OCH_b, 3 C*H*$_a$NHCO, 3 H-6b, 3 H-6b′, 3 H-3′, 3 H-5, 3 H-3″, 3 H-6a, 3 C*H*$_b$NHCO), 3.60−3.45 (m, 18H, 3 H-6a″, 3 NCH_b, 3 H-5″, 3 H-4″, 3 H-4′, 3 H-4), 3.45−3.36 (m, 3H, 3 H-5′), 3.36−3.3 (m, 3H, 3 H-6b″), 3.3 (s, 9H, 3 OCH_3), 2.67−2.47 (m, 6H, 3 CH_2CO); ^{13}C NMR (125.84 MHz, D_2O): δ = 183.40 (3 C═S), 175.94 (3 aryl-CONH), 174.24 (3 NHCO), 129.75 (3 aryl-C), 101.22 (3 C-1″), 100.00 (3 C-1), 83.51 (3 C-1′), 77.92 (3 C-5′), 73.81 (3 C-3″, 3 C-5), 71.01 (3 C-5″, 3 C-4″), 70.98 (3 C-3′), 70.76 (3 C-3), 70.33 (3 C-2″), 70.26 (3 C-2, 3 C-2′), 68.45 (3 C-4′, 3 C-4), 67.07 (3 OCH_2), 61.82 (3 C-6′), 55.07 (3 OCH_3), 52.36 (3 C-6), 51.4 (3 NCH_2), 40.43 (3 C-6″), 40.1 (3 CH_2NH), 33.78 (3 *C*H_2CO); MALDI-TOF MS *m*/*z* 2227.8 $[M + H]^+$, 2250.0 $[M + Na]^+$, 2265.9 $[M + K]^+$, obsd for $C_{84}H_{138}N_{12}O_{51}S_3$ (2226.77). A correct elemental analysis for $C_{84}H_{138}N_{12}O_{51}S_3$ (2228.25) was not obtained.

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Abbreviations used: HATU, *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluroniumhexafluorophosphate; DIPEA, diisopropylethylamine.

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