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Mannosazide Methyl Uronate Donors. Glycosylating Properties and Use in the Construction of β-ManNAcA-Containing Oligosaccharides

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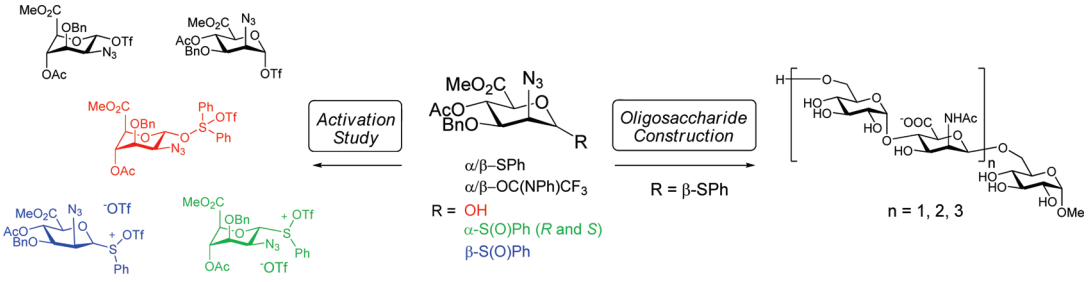

Mannosazide methyl uronate donors equipped with a variety of anomeric leaving groups (β- and α-*S*-phenyl, β- and α-*N*-phenyltrifluoroacetimidates, hydroxyl, β-sulfoxide, and (R_s)- and (S_s)-α-sulfoxides) were subjected to activating conditions, and the results were monitored by ^{1}H NMR. While the *S*-phenyl and imidate donors all gave a conformational mixture of anomeric α-triflates, the hemiacetal and β- and α-sulfoxides produced an oxosulfonium triflate and β- and α-sulfonium bis-triflates, respectively. The β-*S*-phenyl mannosazide methyl uronate performed best in both activation experiments and glycosylation studies and provided the 1,2-*cis* mannosidic linkage with excellent selectivity. Consequently, an α-Glc-(1→4)-β-ManN_3A-SPh disaccharide, constructed by the stereoselective glycosylation of a 6-*O*-Fmoc-protected glucoside and β-*S*-phenyl mannosazide methyl uronate, was used as the repetitive donor building block in the synthesis of tri-, penta-, and heptasaccharide fragments corresponding to the *Micrococcus luteus* teichuronic acid.

Introduction

N-Acetyl-D-mannosaminuronic acid (ManNAcA) is a common constituent of numerous bacterial acidic polysaccharides. It is found in Gram-positive and Gram-negative cell wall glycopolymers,[1] bacterial (surface) antigens,[2] and the enterobacterial common antigen (ECA).[3] Within these bacterial glycans, ManNAcA is primarily β-1,3 or β-1,4 linked to a wide variety of other hexapyranosides. The capsular polysaccharide, teichuronic acid from *Micrococcus luteus*, for example, is composed of alternating ManNAcA and glucose residues, both linked in a 1,2-*cis* fashion (Figure 1).[4]

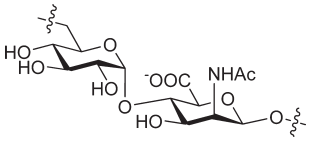

FIGURE 1. *Micrococcus luteus* teichuronic acid displaying the repetitive motif [→6)-α-D-Glc*p*-(1→4)-β-D-Man*p*NAcA-(1→].

(1) *Haemophilus influenzae*: Tsui, F.-P.; Schneerson, R.; Boykins, R. A.; Karpas, A. B.; Egan, W. *Carbohydr. Res.* **1981**, *97*, 293–306. *Bacillus subtilis*: Yoneyama, T.; Araki, Y.; Ito, E. *Eur. J. Biochem.* **1984**, *141*, 83–89.

(2) *Escherichia coli*: Tsui, F.-P.; Boykins, R. A.; Egan, W. *Carbohydr. Res.* **1982**, *102*, 263–271. *Staphylococcus aureus*: Jones, C. *Carbohydr. Res.* **2005**, *340*, 1097–1106. *Neisseria meningitidis*: van der Kaaden, A.; Gerwig, G. J.; Kamerling, J. P.; Vliegenthart, J. F. G.; Tiesjema, R. H. *Eur. J. Biochem.* **1985**, *152*, 663–668. Michon, F.; Brisson, J. R.; Roy, R.; Ashton, F. E.; Jennings, H. J. *Biochemistry* **1985**, *24*, 5592–5598.

(3) (a) Lugowski, C.; Romanowska, E.; Kenne, L.; Lindberg, B. *Carbohydr. Res.* **1983**, *118*, 173–181. (b) Mannel, D.; Mayer, H. *Eur. J. Biochem.* **1978**, *86*, 361–370.

(4) (a) Perkins, H. R. *Biochem. J.* **1963**, *86*, 475–483. (b) Hase, S.; Matsushima, Y. *J. Biochem.* (*Tokyo*) **1972**, *72*, 1117–1128. (c) Nasir-ud-Din; Jeanloz, R. W. *Carbohydr. Res.* **1976**, *47*, 245–260.

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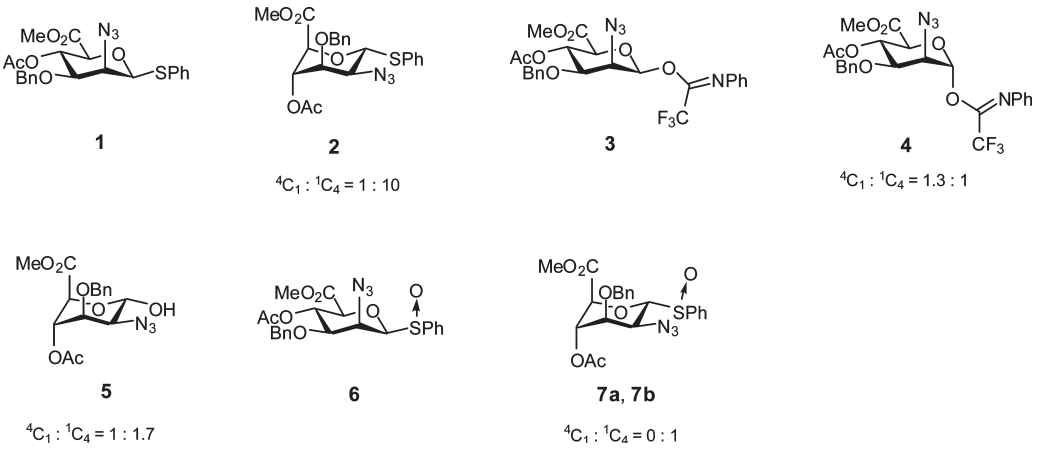


FIGURE 2. Mannosazide methyl uronate donors **1**−**7**. The reported $^4C_1/^1C_4$ ratios were determined by ^1H NMR spectroscopy at −80 °C.

M. luteus has been implicated to play a role in recurrent bacteremia,[5] septic shock,[6] and meningitis.[7] Interestingly, whereas the peptidoglycan part of the *M. luteus* cell wall lacks immunomodulatory activity, its teichuronic acid component induces the production of inflammatory cytokines.[8] Additionally, it was shown that reduction of the carboxylic acids to the primary alcohols led to elimination of the immunostimulating activity.[8]

To date, only a few research papers detail the synthesis of ManNAcA-containing oligosaccharide fragments,[9] and no general protocol exists. We previously described the synthesis of the β-mannosaminuronic acid-containing acidic trisaccharide, β-D-Glc*p*NAc-(1→4)-β-D-Man*p*NAcA-(1→3)-α-L-GalNAcA(4-OAc), of the bacteriolytic complex of lysoamidase.[10] The β-mannosamine linkage in this trimer was constructed using a 4,6-*O*-benzylidene mannosazide thioglycoside[11] following the pioneering work of Crich and coworkers on β-mannoside synthesis.[12] However, compared to the 2,3-*O*-benzyl-protected 4,6-*O*-benzylidene mannopyranoside, the 2-azido-3-*O*-benzyl mannopyranoside showed reduced β-stereoselectivity. As an alternative strategy, we disclosed that appropriately derivatized donor mannuronates (ManA) can be condensed with a variety of acceptor glycosides to produce 1,2-*cis* ManA linkages with good efficiency and high stereoselectivity.[13] In a recent preliminary report,[14] we demonstrated that 2-azido-2-deoxymannuronate ($ManN_3A$) donors also have the propensity to form β-glycosidic bonds. In this study, we made the intriguing observation that activation of such mannosazide methyl uronates using the Ph_2SO-Tf_2O system[12a,15,16] gives rise to the formation of a triflate intermediate which preferentially adopts a $^1C_4$ conformation, placing the anomeric triflate in an equatorial position. We present here our in-depth studies on the activation of a variety of $ManN_3A$ donors, the nature of the activated species formed with emphasis on both structural and conformational aspects, and their glycosylating properties. We also present our results in the application of the outcome of these studies in the first synthesis of a series of tri-, penta-, and heptasaccharide fragments corresponding to the *Micrococcus luteus* teichuronic acid.

Results and Discussion

In the first instance, we synthesized several $ManN_3A$ donors and examined their behavior upon activation, their reactivity, and their stereoselectivity in glycosylation reactions.[17] The eight $ManN_3A$ donors used in this study are β- and α-*S*-phenyl mannosides **1** and **2**,[18] β- and α-*N*-phenyltrifluoroacetimidates **3** and **4**,[19] 1-hydroxyl mannuronate **5**,[20] β-sulfoxide **6**, and the α-sulfoxides **7a/b** (Figure 2).[21] The sulfoxide moieties in **6** and **7a/b** were obtained in diastereomerically pure but undefined form[22,23] (see the Supporting Information for a full description of the synthesis of donors **1**−**7**). Interestingly, the ^1H NMR spectra of the α-sulfoxides

(5) Von Eiff, C.; Kuhn, N.; Herrmann, M.; Weber, S.; Peters, G. *Pediatr. Infect. Dis. J.* **1996**, *15*, 711–713.
(6) Albertson, D.; Natsios, G. A.; Gleckman, R. *Arch. Intern. Med.* **1978**, *138*, 487–488.
(7) Fosse, T.; Peloux, Y.; Granthil, C.; Toga, B.; Bertrando, J.; Sethian, M. *Infection* **1985**, *13*, 280–281.
(8) Yang, S.; Sugawara, S.; Monodane, T.; Nishijima, M.; Adachi, Y.; Akashi, S.; Miyake, K.; Hase, S.; Takada, H. *Infect. Immun.* **2001**, 2025–2030.
(9) For the synthesis of an ECA fragment, see: Paulsen, H.; Lorentzen, J. P. *Carbohydr. Res.* **1984**, *133*, C1–C4. For the synthesis of a *Micrococcus luteus* fragment, see: Osa, Y.; Kaji, E.; Takahashi, K.; Hirooka, M.; Zen, S.; Lichtenthaler, F. W. *Chem. Lett.* **1993**, 1567–1570. For the synthesis of a *Haemophilus influenzae* fragment, see: Classon, B.; Garegg, P. J.; Oscarson, S.; Tidén, A.-K. *Carbohydr. Res.* **1991**, *216*, 187–196.
(10) Litjens, R. E. J. N.; den Heeten, R.; Timmer, M. S. M.; Overkleeft, H. S.; van der Marel, G. A. *Chem.—Eur. J.* **2005**, *11*, 1010–1016.
(11) (a) Litjens, R. E. J. N.; van den Bos, L. J.; Codée, J. D. C.; van den Berg, R. J. B. H. N.; Overkleeft, H. S.; van der Marel, G. A. *Eur. J. Org. Chem.* **2005**, 918–924. (b) Litjens, R. E. J. N.; Leeuwenburgh, M. A.; van der Marel, G. A.; van Boom, J. H. *Tetrahedron Lett.* **2001**, *42*, 8693–8696.
(12) (a) Crich, D.; Sun, S. *Tetrahedron* **1998**, *54*, 8321–8348. (b) Crich, D.; Smith, M. *J. Am. Chem. Soc.* **2001**, *123*, 9015–9020. (c) Crich, D.; Lim, L. B. L. *Org. React.* **2004**, *64*, 115–251.
(13) Codée, J. D. C.; van den Bos, L. J.; de Jong, A. R.; Dinkelaar, J.; Lodder, G.; Overkleeft, H. S.; van der Marel, G. A. *J. Org. Chem.* **2009**, *74*, 38–47.
(14) Walvoort, M. T. C.; Lodder, G.; Mazurek, J.; Overkleeft, H. S.; Codée, J. D. C.; van der Marel, G. A. *J. Am. Chem. Soc.* **2009**, *131*, 12080–12081.
(15) Crich, D.; Sun, S. *J. Org. Chem.* **1996**, *61*, 4506–4507.
(16) Codée, J. D. C.; van den Bos, L. J.; Litjens, R. E. J. N.; Overkleeft, H. S.; van Boeckel, C. A. A.; van Boom, J. H.; van der Marel, G. A. *Tetrahedron* **2004**, *60*, 1057–1064.
(17) (a) Zhu, X.; Schmidt, R. R. *Angew. Chem., Int. Ed.* **2009**, *48*, 2–37. (b) Mydock, L. K.; Demchenko, A. V. *Org. Biomol. Chem.* **2010**, *8*, 497–510. (c) Boltje, T. J.; Buskas, T.; Boons, G.-J. *Nature Chem.* **2009**, *1*, 611–622.
(18) Codée, J. D. C.; Litjens, R. E. J. N.; van den Bos, L. J.; Overkleeft, H. S.; van der Marel, G. A. *Chem. Soc. Rev.* **2005**, *34*, 769–782.
(19) (a) Yu, B.; Tao, H. *Tetrahedron Lett.* **2001**, *42*, 2405–2407. (b) Yu, B.; Sun, J. *Chem. Commun.* **2010**, *46*, 4668–4679.
(20) Gin, D. *J. Carbohydr. Chem.* **2002**, *21*, 645–665.
(21) (a) Kahne, D.; Walker, S.; Cheng, Y.; van Engen, D. *J. Am. Chem. Soc.* **1989**, *111*, 6881–6882. (b) Aversa, M. C.; Barattucci, A.; Bonaccorsi, P. *Tetrahedron* **2008**, *64*, 7659–7683.

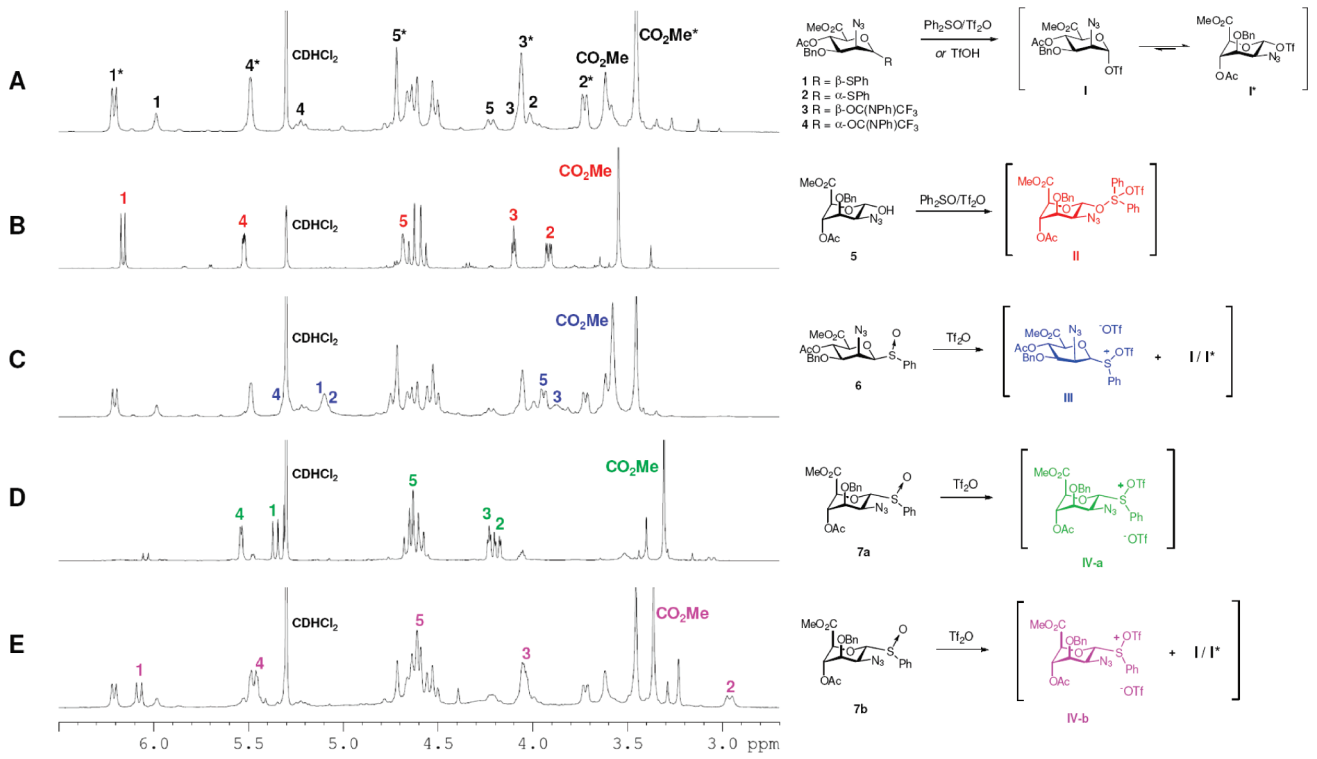


FIGURE 3. Part of the ^{1}H NMR spectra obtained after activation of donors **1**−**4** (A) at −80 °C, hemiacetal donor **5**, (B) at −30 °C, *β*-sulfoxide donor **6**, (C) at −80 °C and α-sulfoxide donors **7a**, (D) at −50 °C and **7b**, and (E) at −80 °C.

7a/b show that both donors exist exclusively in the $^1C_4$ conformation. The ^{1}H NMR spectra of the other α-configured donors **2**, **4**, and **5** recorded at room temperature show coupling constants for the signals belonging to the ring protons having a value in between the typical values obtained for $^1C_4$ and $^4C_1$ chair protons. At −80 °C, interconversion of the $^1C_4$ and $^4C_1$ chairs was slowed down sufficiently to allow detection of resonance sets for both conformers, and from these ^{1}H NMR spectra the conformer ratios were extracted, as reported in Figure 2 (for NMR spectra, see the Supporting Information). α-*S*-Phenyl mannoside **2** predominantly exists as a $^1C_4$ chair, whereas the two chair conformers are more evenly distributed for the imidate **4** ($^4C_1/^1C_4$ = 1.3:1) and 1-hydroxyl mannuronate **5** ($^4C_1/^1C_4$ = 1:1.7).

Activation of the Donors. To investigate the behavior of the ManN_3A donors upon activation and subsequent glycosylation with MeOH-d_4, a series of low-temperature NMR experiments was conducted. We recently reported[14] on the activation of *β*-thiodonor **1** using Ph_2SO-Tf_2O as the activator system and *β*-imidate donor **3** using stoichiometric TfOH and revealed that both donors were rapidly converted at −80 °C into the same mixture of anomeric α-triflates **I**/**I***.[24] The ^{1}H NMR spectrum of this conformational triflate mixture is depicted in Figure 3A and shows that the equatorial anomeric $^1C_4$ triflate **I*** prevails over its $^4C_1$ counterpart **I** (**I***:**I** = 3:1). Structure **I*** arranges three substituents in sterically unfavorable axial positions and does not benefit from a stabilizing anomeric effect. This conformation is in line with the structural preference of the related mannuronate ester oxacarbenium ion, which preferentially adopts a $^3H_4$ half chair or closely related conformation.[14,17b,25,26] Because the anomeric carbon is quite electron depleted, the α-triflate **I*** takes up a structure closely mimicking the structure of the $^3H_4$-like oxacarbenium ion, which is best stabilized by an equatorial substituent at C-2 and by axial substituents at C-3, C-4, and C-5. Treatment of the conformational mixture of anomeric α-triflates **I**/**I*** with a 25-fold excess of MeOH-d_4 at −80 °C rapidly provided methyl mannoside **8** with high *β*-selectivity (see Table 1, entries 1 and 3). The selective formation of the *β*-linked products from the mannosaziduronic acid donors can be explained by the S_N2-like substitution on the α-triflate. Alternatively, the selective attack of the $^3H_4$-like oxacarbenium

(22) Oxidation of *β*-thio donor **1** yielded a single diastereomeric sulfoxide **6**. On the other hand, oxidation of α-thio donor **2** resulted in a mixture of diastereomeric α-sulfoxides **7a** and **7b**. Although readily separable, the absolute configuration of the diastereomers, which were obtained as oils after purification, could not be assigned. Empirical assignment of the configuration based on axial α-sulfoxides as described by Crich et al. was deemed unfeasible since the anomeric moiety is placed equatorially in donor **2**. Crich, D.; Mataka, J.; Zakharov, L. N.; Rheingold, A. L.; Wink, D. J. *J. Am. Chem. Soc.* **2002**, *124*, 6028–6036.

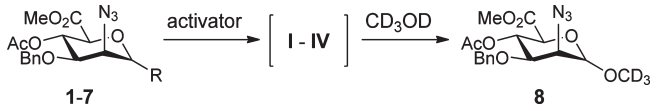
(23) The diastereoselective oxidation of *β*-thio glycosides has been reported before: Khiar, N.; Fernández, I.; Araújo, C. S.; Rodríguez, J.-A.; Suárez, B.; Álvarez, E. *J. Org. Chem.* **2003**, *68*, 1433–1442.

(24) Anomeric triflates have been observed before; see, for example: (a) Crich, D.; Sun, S. *J. Am. Chem. Soc.* **1997**, *46*, 11217–11223. (b) Crich, D. *J. Carbohydr. Chem.* **2002**, *21*, 667–690.

(25) (a) Lucero, C. G.; Woerpel, K. A. *J. Org. Chem.* **2006**, *71*, 2641–2647. (b) Romero, J. A. C.; Tabacco, S. A.; Woerpel, K. A. *J. Am. Chem. Soc.* **2000**, *122*, 168–169. (c) Dinkelaar, J.; de Jong, A. R.; van Meer, R.; Somers, M.; Lodder, G.; Overkleeft, H. S.; Codée, J. D. C.; van der Marel, G. A. *J. Org. Chem.* **2009**, *74*, 4982–4991. (d) Codée, J. D. C.; de Jong, A. R.; Dinkelaar, J.; Overkleeft, H. S.; van der Marel, G. A. *Tetrahedron* **2009**, *65*, 3780–3788.

(26) For recent reviews on oxacarbenium ions, see: (a) Walvoort, M. T. C.; Dinkelaar, J.; van den Bos, L. J.; Lodder, G.; Overkleeft, H. S.; Codée, J. D. C.; van der Marel, G. A. *Carbohydr. Res.* **2010**, *345*, 1252–1263. (b) Smith, D. M.; Woerpel, K. A. *Org. Biomol. Chem.* **2006**, *4*, 1195–1201. (c) Satoh, H.; Hansen, H. S.; Manabe, S.; van Gunsteren, W. F.; Hünenberger, P. H. *J. Chem. Theory Comput.* **2010**, *6*, 1783–1797. (d) Whitfield, D. M. *Adv. Carbohydr. Chem. Biochem.* **2009**, *62*, 83–159. (e) Horenstein, N. A. *Adv. Phys. Org. Chem.* **2006**, *41*, 275–314. (f) Bohé, L.; Crich, D. *C. R. Chim.* **2010**, DOI: 10.1016/j.crci.2010.03.016.

TABLE 1. Results of the Activation of Donors 1−7 and Coupling to MeOH-d_4



1-7 8

entry	compd	leaving group	T (°C)	intermediates	α/β ratio of **8**[a]
1	**1**	β-SPh	−80	**I**/**I***	1/5[b]
2	**2**	α-SPh	−80 → −40	**I**/**I***	1/6[b]
3	**3**	β-C(NPh)CF_3	−80	**I**/**I***	1/>10[b]
4	**4**	α-C(NPh)CF_3	−80	**I**/**I***	1/>10[b]
5	**5**	α−OH	−80 → +10	**II**	0/1[c]
6	**6**	β-S(O)Ph	−80	**I**/**I***, **III**	1/5[d]
7	**7a**	α-S(O)Ph (*R or S*)	−80 → −20	**IV-a** (*R or S*)	nd
8	**7b**	α-S(O)Ph (*R or S*)	−80 → −60	**I**/**I***, **IV-b** (*R or S*)	1/5[e]

[a]As determined by NMR. [b]Full conversion of the activated species. [c]Mixture of **5**/**8** ∼ 3/1. [d]Mixture of **6**/**8** ∼ 2/3 . [e]Mixture of **7b**/**8**/**2** ∼ 4/5/1.

ion from the β-face in an S_N1-like process, can also account for the observed selectivity. Both pathways can contribute to the β-selectivity observed, and the amount of S_N1 and S_N2 character depends on the reactivity of the nucleophile and steric interactions of the donor and acceptor at hand.[14]

In similar activation experiments, donors **2**, **4**, **5**, **6**, and **7a/b** were assessed, and the results of these experiments are summarized in Figure 3 and Table 1. First, a mixture of α-thiodonor **2** and Ph_2SO (1.3 equiv) in DCM-d_2 (0.05 M) at −80 °C was treated with Tf_2O (1.3 equiv), and a ^{1}H NMR spectrum was recorded. Upon activation, several new signals appeared, indicating the formation of the conformational mixture of α-triflates **I**/**I***. However, unlike the rapid consumption of β-thiodonor **1**, α-thiodonor **2** remained present, and a prolonged reaction time (∼1 h) at −80 °C did not lead to more conversion.[27] Raising the temperature to −40 °C eventually gave complete conversion of donor **2** into the mixture of α-triflates also observed after activation of β-donors **1** and **3**. Above −40 °C decomposition was observed. Cooling down to −80 °C and addition of MeOH-d_4 to the activation mixture of donor **2** generated mainly β-methyl mannopyranoside **8**, as expected (Table 1, entry 2).

To monitor the activation of α-imidate **4**, a solution of donor **4** in DCM-d_2 (0.05 M) was treated with TfOH (1.3 equiv) at −80 °C. As with β-imidate donor **3**, compound **4** was quickly consumed, and the spectrum obtained was identical to the one displayed in Figure 3A and the one obtained from activation of donor **3**. Thus, both imidate donors produce upon preactivation the same conformational mixture of α-anomeric triflates. Addition of MeOH-d_4 to the activation mixture gave rapid conversion to the β-methyl mannopyranoside **8** with excellent selectivity (Table 1, entry 4).[28]

Next, hemiacetal donor **5** was subjected to activating conditions (1.3 equiv of Tf_2O, 1.3 equiv of Ph_2SO, 0.05 M in DCM-d_2). The donor was completely consumed at −40 °C resulting in a single set of signals as displayed in Figure 3B. The anomeric proton (δ = 6.16 ppm) appeared as a doublet with a coupling constant of 8.3 Hz, in analogy to the large coupling constant ($^3J_{H1-H2}$ = 8.8 Hz) observed for the anomeric proton in equatorial triflate **I***. The activated species generated from donor **5** proved to be stable up to +10 °C. Given the anomeric chemical shift values reported by Garcia and Gin[29] for oxosulfonium triflates and the similarity between the ^{1}H spectrum from activation of **5** and the resonance set belonging to the equatorial triflate **I***, the intermediate formed upon activation of hemiacetal **5** was assigned oxosulfonium triflate structure **II** residing in the $^1C_4$ chair conformation. Upon addition of MeOH-d_4 (25 equiv at −80 °C) the activated mixture of donor **5** remained unchanged, in contrast to the fast conversion of anomeric triflates **I/I***. Only after warming of the mixture to +10 °C was full consumption of intermediate **II** observed. Next to β-coupled product **8**, which was formed in 25% yield, regenerated donor **5** was found as the main product (Table 1, entry 5).

When the β-sulfoxide donor **6** was treated with Tf_2O at −80 °C, the ^{1}H NMR spectrum showed full consumption of the donor, with the conformational mixture of α-triflates **I**/**I*** as the major product alongside a second product (Figure 3C). On the basis of the relatively small chemical shift of H-1 (δ = 5.22 ppm), the chemical shift of C-1 (δ = 91.4 ppm), and the activation experiments of the α-sulfoxides **7a/b** (vide infra), we presume that this latter species corresponds to the β-sulfonium bistriflate species **III**.[30] Addition of MeOH-d_4 resulted in a mixture of products containing the methyl mannoside product **8** (α:β = 1:5, ∼60%) and regenerated donor **6**.

Activation of α-sulfoxide diastereomer **7a** (1.3 equiv of Tf_2O) at −80 °C led to the rapid formation of one predominant species (Figure 3D). However, the signals did not correspond to the peaks assigned to the (conformational mixture of) anomeric triflates **I**/**I***. Since an overall downfield shift was observed for the pyranosyl protons, the

(27) Although we previously established that thioglycosyl methyl uronates require relatively high (pre)activation temperatures in a sulfonium-based activation protocol (activation of the uronate donors generally proceeds at −65 to −55 °C, as opposed to the activation of "non-oxidized" thioglycosides which can be effected at −78 °C), in the $ManN_3A$-case at hand, the activation temperature seems to be largely dependent on the anomeric configuration. Van den Bos, L. J.; Litjens, R. E. J. N.; van den Berg, R. J. B. H. N.; Overkleeft, H. S.; van der Marel, G. A. *Org. Lett.* **2005**, *7*, 2007–2010.

(28) The difference in stereoselectivities between glycosylation of MeOH-d_4 with the thio- and the imidate donors may result from slight experimental variations caused during the mixing of the NMR samples outside the spectrometer. Furthermore, the reaction mixtures of the activated thioglycosides contain different (sulfonium) species, generated upon expulsion of the anomeric thiophenyl moiety, and unreacted diphenylsulfoxide, which potentially affect the stereochemical outcome of the glycosylations.

(29) It has been shown that an anomeric triflate is instantaneously converted to the oxosulfonium triflate species by the addition of diphenyl sulfoxide, indicating that this is the more stable intermediate. Garcia, B. A.; Gin, D. Y. *J. Am. Chem. Soc.* **2000**, *122*, 4269–4279.

(30) The existence of a sulfonium bistriflate species has been postulated before (ref 24a).

doublet assigned to H-1 at δ 5.38 ppm displayed a coupling constant of $J_{H1-H2} = 10.8$ Hz, and the chemical shift of C-1 was indicative for an anomeric thio functionality (δ 86.2 ppm), the activated species was considered to be the equatorial α-anomeric sulfonium bistriflate **IV-a**.[31] Because the stereochemistry of the parent sulfoxide **7a** could not be determined, the stereochemistry of the sulfonium bistriflate cannot be determined either. Prolonged reaction time and warming of the reaction mixture to −20 °C did not lead to transformation of this species into the anomeric triflate **I**/**I***. Treatment of the activated mixture with MeOH-d_4 resulted in a complex mixture of compounds, which contained a substantial amount of recovered donor **7a**. Interestingly, activation of the other α-sulfoxide diastereomer **7b** in a similar NMR experiment led to different intermediates. The α-triflates **I**/**I*** were formed as well as a new species, which did not correspond to the sulfonium bistriflate **IV-a**. On the basis of the similarity of the ^{1}H-resonances of this species and **IV-a**, and the chemical shift of C-1 (δ 85.4 ppm), again indicative of an anomeric thio group, we speculate that this species is the other diastereomeric sulfonium bistriflate **IV-b** (Figure 3E). Gradual warming of the reaction mixture to −60 °C led to further conversion of **IV-b** into anomeric triflates **I**/**I*** (**I**/**I***:**IVb** ∼ 4:3). The addition of MeOH-d_4 resulted in a mixture of products containing methyl mannoside **8** (α:β = 1:5, ∼50%), together with regenerated donor **7b** and α-thio mannuronate **2** (Table 1, entry 8).[32]

The activation experiments described above provide a detailed picture of the behavior of donors **1**−**7** upon activation. The reactivity boundaries of the activation protocols used and the influence of the anomeric configuration are apparent. While β-thio donor **1** is rapidly activated at −80 °C, its α-counterpart **2** requires a higher temperature (−40 °C) in order to be fully consumed. The reactivity difference between two anomers is often attributed to a stabilizing anomeric effect in the α-anomer. However, both α- and β-mannoside **1** and **2** exist in a conformation in which the sulfur aglycon is positioned equatorially, thereby lacking anomeric stabilization.[33] We therefore attribute the reactivity difference between **1** and **2** to a difference in stability caused by the (stereo)electronic repulsion between the substituents on C-1, C-2, and the ring-oxygen (the destabilizing Δ2-effect).[34] The reactivity difference between the α- and β-thiomannosides **1** and **2** was not observed for the imidate anomers **3** and **4**. Under the influence of a stoichiometric amount of TfOH, both donors were rapidly transformed into a mixture of α-triflate conformers, which gave an identical β-selectivity in the ensuing substitution by MeOD-d_4. Hemiacetal donor **5** was fully converted to the relatively stable oxosulfonium triflate **II** upon activation. Treatment of this activated intermediate with a nucleophile did not result in effective glycosylation. Instead, mainly hemiacetal **5** was regenerated. This result shows that the oxosulfonium triflate is not easily expelled from the mannuronate donor and that a competing attack at either of the sulfonium centers in **II** can take place. Although glycosyl sulfoxides are generally regarded to be among the most powerful glycosyl donors, the results obtained with the sulfoxide donors **6** and **7a**,**b** show a reactivity limit for the sulfoxide method. Because of the unreactivity of the mannosaziduronic acid core, reactivity differences became apparent not only between the α- and β-anomers but also between the two different sulfoxide diastereomers, which provided different reactive species upon Tf_2O activation.[35] Although the existence of pyranosyl sulfonium bistriflates has been postulated before,[24a] such species have not been experimentally observed, since they rapidly collapse to the corresponding anomeric triflates.[36]

Glycosylations with Glucoside Acceptors. To assess the glycosylating properties of mannosazide methyl uronates with a glycosyl acceptor, the donors **1**−**4**, which provided a productive glycosylation with MeOH-d_4 as described above, were further examined.

First, β-thiodonor **1** was preactivated with the Ph_2SO−Tf_2O reagent combination for 15 min during which time the temperature was raised from −65 to −55 °C. Then acceptor **9** was added, and disaccharide **11** was produced in high yield and selectivity (Table 2, entry 1). In contrast, when α-thiodonor **2** was preactivated from −80 to −40 °C, as deduced from the NMR experiments to be the optimal activation temperature, and subsequently condensed with acceptor **9**, the yield of disaccharide **11** was significantly lower, while the stereoselectivity remained intact (Table 2, entry 2). This poor coupling efficiency may be attributed to the fact that the preactivation temperature (−40 °C) is close to the temperature at which decomposition of the anomeric triflate sets in, as observed in the NMR experiments. Optimization of the glycosylation of donor **2** proved to be precarious; monitoring of the activation progress was troublesome and slight adjustments to the experimental procedure resulted in considerable differences in glycosylation outcome. The best conditions found involved activation of thiomannoside **2** with Ph_2SO−Tf_2O for 15 min at −65 to −55 °C prior to addition of acceptor **9** and led to the stereoselective formation of disaccharide **11** in 75% yield (Table 2, entry 3). The imidate donors **3** and **4** were coupled with acceptor **9** under the agency of a catalytic amount of triflic acid (TfOH). The α-imidate **4** provided predominantly the β-linked disaccharide, whereas the use of β-imidate **3** led to the formation of a substantial amount of the α-linked disaccharide (Table 2, entries 5 and 4, respectively). Since NMR analysis of imidate donors **3** and **4** showed that both form the same mixture of α-triflate intermediates under preactivation conditions with a equimolar amount of TfOH and that both provided excellent β-selectivity in the glycosylation of MeOH-d_4, the significant amount of α-product **11** generated from β-imidate **3** must arise from S_N2-displacement of the anomeric imidate by the

(31) The chemical shift of the anomeric carbon atom in ^{13}C NMR, the recovery of unreacted sulfoxide donor and the reduction of the sulfoxide moiety in donor **7b** (ref 32). advocate against the intermediacy of an anomeric sulfenate. Gildersleeve, J.; Pascal, R. A., Jr.; Kahne, D. *J. Am. Chem. Soc.* **1998**, *120*, 5961–5969.

(32) The formation of mannuronate **2** from donor **7b** can be explained by a Swern-like oxidation of methanol by the intermediate sulfonium bistriflate **IV-b**.

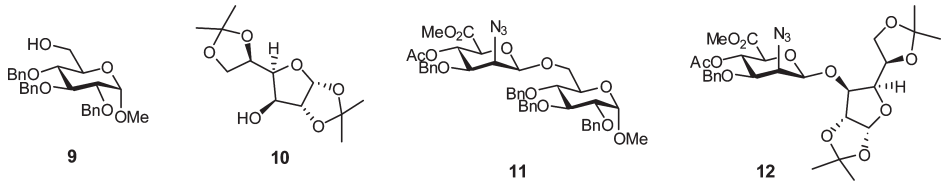
(33) (a) Deslongchamps, P. *Stereoelectronic Effects in Organic Chemistry*; Pergamon, New York, 1983; Chapter 6. (b) Juaristi, E.; Cuevas, G. *Tetrahedron* **1992**, *48*, 5019–5087.

(34) (a) Reeves, R. E. *J. Am. Chem. Soc.* **1950**, *72*, 1499–1506. (b) Lii, J.-H.; Chen, K.-H.; Allinger, N. L. *J. Comput. Chem.* **2003**, *24*, 1504–1513.

(35) Different reactivities for sulfoxide diastereomers have been acknowledged before: (a) Ferrières, V.; Joutel, J.; Boulch, R.; Roussel, M.; Toupet, L.; Plusquellec, D. *Tetrahedron Lett.* **2000**, *41*, 5515–5519. (b) Khiar, N.; Alonso, I.; Rodriguez, N.; Fernandez-Mayoralas, A.; Jimenez-Barbero, J.; Nieto, O.; Cano, F.; Foces-Foces, C.; Martin-Lomas, M. *Tetrahedron Lett.* **1997**, *38*, 8267–8270.

(36) Callam, C. S.; Gadikota, R. R.; Krein, D. M.; Lowary, T. L. *J. Am. Chem. Soc.* **2003**, *125*, 13112–13119.

TABLE 2. Condensations of the Mannosazide Methyl Uronate Donors 1−4 with Acceptors 9 and 10



9 **10** **11** **12**

entry	donor	acceptor	(pre)activation	yield (%) (α/β)
1	**1**	**9**	Ph_2SO, Tf_2O, −65 → −55 °C (15 min), then add **9**	90 (1:7)
2	**2**	**9**	Ph_2SO, Tf_2O, −80 → −40 °C (1 h), then add **9**	45 (1:5)
3	**2**	**9**	Ph_2SO, Tf_2O, −65 → −55 °C (15 min), then add **9**	75 (1:6)
4	**3**	**9**	0.2 equiv of TfOH	84 (1:2)
5	**4**	**9**	0.2 equiv of TfOH	53 (1:5)
6	**1**	**10**	Ph_2SO, Tf_2O, −65 → −55 °C (15 min), then add **10**	85 (0:1)[a]
7	**2**	**10**	Ph_2SO, Tf_2O, −65 → −55 °C (15 min), then add **10**	58 (0:1)

[a]The yield includes 45% of the β-linked disaccharide bearing one isopropylidene group on C-1 and C-2 because of cleavage of the C5,6-isopropylidene functionality under the coupling conditions.

nucleophile, already present in the reaction mixture.[37] Because the thiomannosides **1** and **2** performed best in terms of yield and β-selectivity, these donors were further probed with the secondary acceptor 1,2:3,4-diisopropylideneglucofuranose (**10**). Under the optimal preactivation conditions, the condensations of **1** and **2** with **10** gave the β-linked dimer **12** as the sole product (Table 2, entries 6 and 7). Also in this case, the β-configured donor was shown to be superior to its α-linked equivalent.

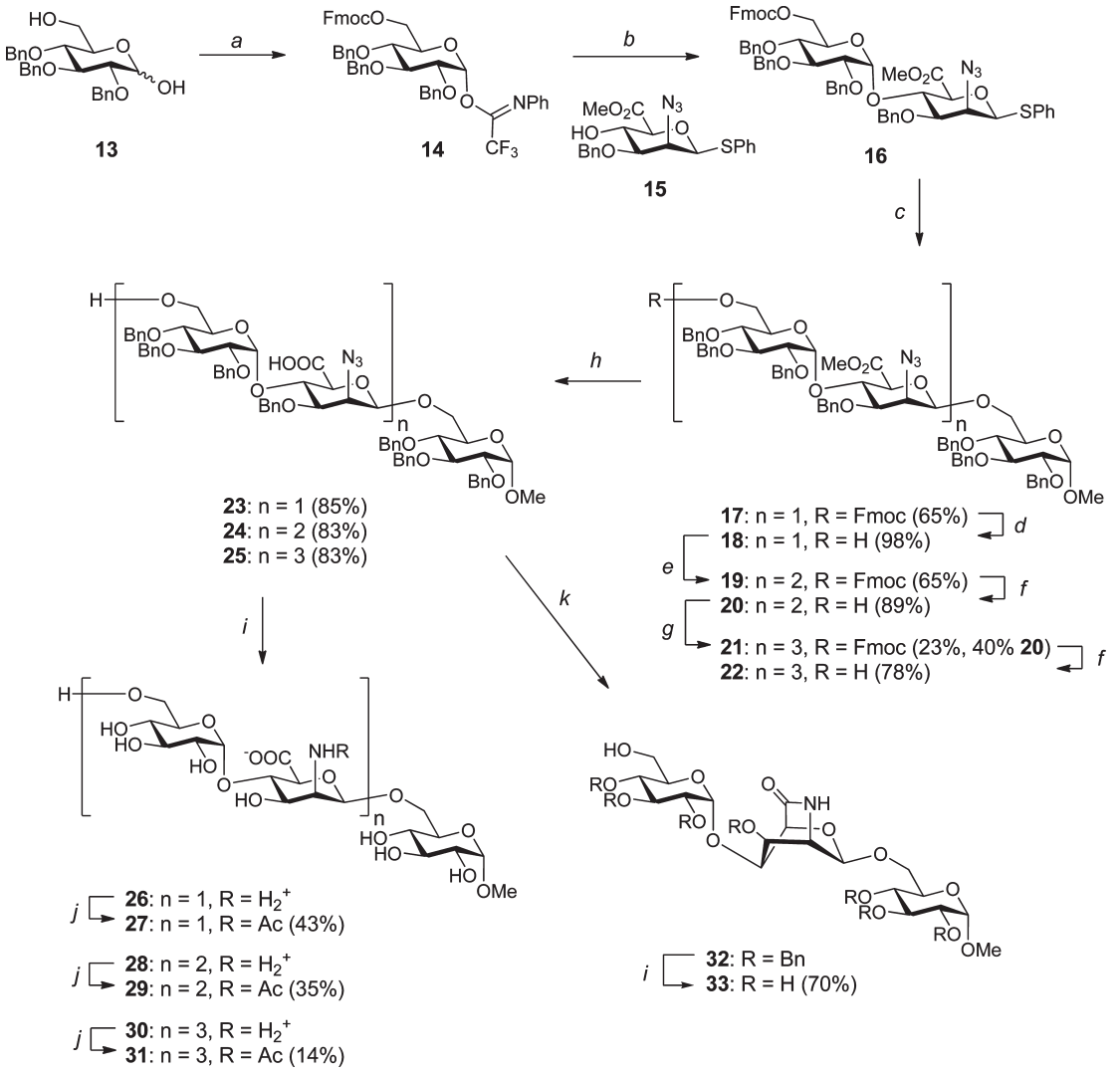
Oligosaccharide Assembly. Having established that mannosaziduronic acid donors provide highly β-selective condensations, the construction of an anionic *M. luteus* oligosaccharide containing the [→6)-α-D-Glc*p*-(1→4)-β-D-Man-*p*NAcA-(1→] repetitive motif (Figure 1) was undertaken. The alternating character of the Glc and ManNAcA building blocks allows for a disaccharide block coupling strategy. Guided by the excellent β-selectivities obtained with ManN_3A β-thio donor **1**, the Glc-ManN_3A thiophenyl dimer **16** was selected to serve as an iterative building block (Scheme 1).

For the stereoselective installation of the α-*gluco* linkage in compound **16**, several strategies may be followed.[38−42] For us,[43] the best method turned out to be the protocol described by Adinolfi and co-workers, who reported that a glucosyl trifluoroimidate carrying a large C-6 dimethoxytrityl protecting group is an effective α-glucosyl donor in etherous solvents.[42] We explored the use of glucose imidate **14** having the bulky Fmoc-protecting group[44] at C-6. This donor was readily obtained from 2,3,4-tri-*O*-benzyl-α/β-D-glucopyranose (**13**)[45] by regioselective *N*-(phenyl)trifluoroacetimidate[46] formation and subsequent Fmoc installation at the C-6 hydroxyl. Disaccharide **16** was efficiently produced by treating a mixture of donor **14** and acceptor **15** with a catalytic amount of TfOH in diethyl ether (0.05 M) at −35 to −15 °C. The α-coupled product **16** was formed as the sole product in 90% yield. To investigate how the α-glucosyl appendage in **16** affects the glycosylation properties of the ManN_3A donor, disaccharide **16** was subjected to activation conditions and the progress of the activation reaction was monitored using low-temperature NMR spectroscopy as described above. After addition of Tf_2O at −80 °C, donor **16** was immediately consumed producing a conformational mixture of anomeric triflates, in which the $^1C_4$ chair product dominates ($^1C_4$: $^4C_1$ = 4: 1). The H-1 signal, characteristic of the equatorial triflate, resides at δ 6.22 ppm with a coupling constant of J_{H1-H2} = 8.8 Hz (C-1 δ 100.5 ppm), and the axial triflate appeared as a singlet at δ 5.99 ppm. Addition of MeOH-d_4 to this mixture resulted in clean conversion to the β-fused methyl disaccharide. Encouraged by this result, the construction of *M. luteus* teichuronic acid fragments was commenced. Thus, dimer **16** was activated (Ph_2SO-Tf_2O, −65 °C to −55 °C for 15 min) and reacted with glucosyl acceptor **9** at −60 °C to provide trisaccharide **17** as a single stereoisomer in 65%. Liberation of the C6″-OH was accomplished by treatment of compound **17** with a catalytic amount of TBAF in THF to give trisaccharide acceptor **18** in quantitative yield. In the ensuing glycosylation event, dimer **16** and trimer **18** were combined under analogous conditions to provide the *all-cis*-linked product **19** as the sole isomer in 42% yield. To improve the yield, the reaction temperature and time were adjusted, and when **16** and **18** were condensed overnight at −80 °C, pentasaccharide **19** was obtained in 65% yield.[47]

Removal of the Fmoc group in **19** with a catalytic amount of TBAF in THF proceeded sluggishly to yield compound **20** in 83% yield after 3 days. The use of excess triethylamine in pyridine improved both the yield (89%) and reaction time

(37) Schmidt, R. R.; Hoffmann, M. *Tetrahedron Lett.* **1982**, *23*, 409–412.
(38) Lemieux, R. U.; Hendriks, K. B.; Stick, R. V.; James, K. *J. Am. Chem. Soc.* **1975**, *97*, 4056–4062.
(39) (a) Hadd, M. J.; Gervay, J. *Carbohydr. Res.* **1999**, *320*, 61–69. (b) Lam, S. N.; Gervay-Hague, J. *Org. Lett.* **2002**, *4*, 2039–2042.
(40) Kim, J.-H.; Yang, H.; Park, J.; Boons, G.-J. *J. Am. Chem. Soc.* **2005**, *127*, 12090–12097.
(41) Crich, D.; de la Mora, M.; Vinod, A. U. *J. Org. Chem.* **2003**, *68*, 8142–8148.
(42) (a) Adinolfi, M.; Barone, G.; Iadonisi, A.; Shiattarella, M. *Tetrahedron Lett.* **2002**, *43*, 5573–5577. (b) Adinolfi, M.; Iadonisi, A.; Schiattarella, M. *Tetrahedron Lett.* **2003**, *44*, 6479–6482.
(43) Initial attempts at achieving an α-selective glucosylation using 4,6-*O*-benzylidene or 6-*O*-dimethoxytrityl thiophenyl glucosyl donors and various ManN_3A acceptors met with limited success.
(44) Roussel, F.; Knerr, L.; Grathwohl, M.; Schmidt, R. R. *Org. Lett.* **2000**, *5*, 3043–3046.
(45) Zhang, G.-t.; Guo, Z.-w.; Hui, Y.-z. *Synth. Commun.* **1997**, *27*, 1907–1917.
(46) Adinolfi, M.; Iadonisi, A.; Ravidà, A. *Synlett* **2006**, *4*, 583–586.
(47) Careful analysis of the crude glycosylation mixture revealed the presence of de-Fmocylated oligosaccharide. A reduction of the amount of pyridine to quench the glycosylation reaction did not prevent cleavage of the Fmoc. The reported yields only include Fmoc-protected product.

SCHEME 1. Synthesis of *M. luteus* Tri-, Penta-, and Heptasaccharides 27, 29, and 31[a]



[a]Reagents and conditions: (a) (i) $CF_3C(NPh)Cl$, K_2CO_3, acetone; (ii) Fmoc-Cl, pyridine, DCM (**14**: 83% over two steps); (b) **15**, TfOH, Et_2O, −35 → −15 °C (**16**: 90%); (c) Ph_2SO, Tf_2O, TTBP, DCM, −65 → −55 °C, then **9** (**17**: 65%); (d) TBAF, THF (**18**: 98%); (e) **16**, Ph_2SO, Tf_2O, TTBP, DCM, −70 → −60 °C, then **18**, −80 °C, o.n. (**19**: 65%); (f) Et_3N, pyridine (**20**: 89%, **22**: 78%); (g) **16**, Ph_2SO, Tf_2O, TTBP, DCM, −70 → −55 °C, then **20**, −80 °C, 2 days (**21**: 23%); (h) H_2O_2, aq KOH (**23**: 85%, **24**: 83%, **25**: 83%); (i) Na (s), liq NH_3, THF, −60 °C (**33**: 70% over two steps); (j) Ac_2O, $NaHCO_3$, H_2O/THF (**27**: 43%, **29**: 35%, **31**: 14%, over two steps); (k) H_2S, pyridine/H_2O, 2 days.

(3 h) of this deprotection step. Finally, to construct heptasaccharide **21**, disaccharide donor **16** was activated and reacted with pentasaccharide **20** over 2 days at −80 °C. Heptamannuronate **21** was obtained in 23% yield, reflecting the lower reactivity of the bulky pentasaccharide acceptor,[47] alongside 40% of unreacted pentasaccharide **20**. Cleavage of the Fmoc group using TEA/pyridine proceeded uneventfully to give heptasaccharide **22** in 78% yield.

Global deprotection of oligosaccharides **18**, **20**, and **22** started with saponification of the methyl esters. Reaction of trisaccharide **18** with KOH in THF/H_2O gave the desired uronic acid **23** together with side products generated by β-elimination in the $ManN_3A$ moiety. The use of a more nucleophilic and less basic reagent mixture (H_2O_2 in aqueous KOH) reduced the undesired β-elimination, and mannuronic acid **23** was obtained in 85% yield. Application of these conditions to substrates **20** and **22** delivered di- and triacid **24** and **25**, respectively, in good yields. Simultaneous reduction of the azide functionality and the benzyl ethers in trisaccharide **23** with H_2 and Pd/C proved to be troublesome and led to an inseparable product mixture. A stepwise approach in which the azide was transformed into the free amine using H_2S in pyridine/H_2O prior to reduction of the benzyl groups also failed because reduction of the azide was accompanied by cyclization to provide lactam **32**. Formation of this amide probably results from attack of the free amine to the thiol acid, generated from the carboxylic acid and H_2S.[48] In the end, direct Birch reduction of trisaccharide **23** proved to be the most efficient protocol, and anionic trisaccharide **27** was obtained after acetylation of the free amine in 43%. When pentasaccharide **24** was treated under similar conditions,

(48) Keller, M.; Blöchl, E.; Wächtershäuser, G.; Stetter, K. O. *Nature* **1994**, *368*, 836–838.

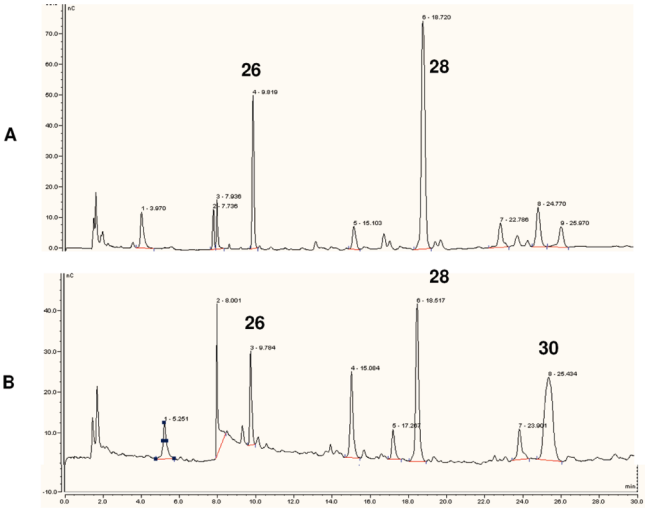


FIGURE 4. HPAEC traces of the crude reaction mixture of the Birch reduction of pentasaccharide **24** (A) and heptasaccharide **25** (B).

target pentamer **29** was formed in 35% yield. Unfortunately, we observed that fragmentation of the oligosaccharide occurred during the Birch reduction. High-performance anion exchange chromatography (HPAEC, see Figure 4A) and LC−MS indicated that a substantial amount of trisaccharide **26** next to pentamer **28** was formed. Formation of the trisaccharide cannot be explained by β-elimination of the mannuronic acid residue but must have occurred via the unexpected cleavage of the β-mannosyl glycosidic bond.[49,50] Finally, heptamer **25** was subjected to the reduction conditions, and after subsequent acetylation and purification target compound **31** was obtained. The reduction of the heptamer was also accompanied by fragmentation, and HPAEC analysis revealed the formation of zwitterionic tri- and pentasaccharide **26** and **28**, next to the desired product **30** (Figure 4B). Gel filtration (HW-40) of the product mixture was hampered by poor separation of heptamer **30** from the smaller fragments; however, pure **30** was obtained, which yielded heptasaccharide **31** in 14% yield after *N*-acetylation.

Conclusion

We have described a thorough evaluation of the glycosylation properties of a series of mannosaziduronic methyl ester donors. Depending on the anomeric leaving group and the preactivation conditions, reactive intermediates with various stabilities are formed: anomeric triflates from the α- and β-*S*-phenyl and *N*-phenyltrifluoro imidates, an oxosulfonium triflate from the hemiacetal, and sulfonium bistriflates from the α- and β-sulfoxides. Interestingly, the intermediates formed from the sulfoxides, generally regarded to be very powerful glycosyl donors, did not provide productive glycosylations. The high β-stereoselectivity and good coupling efficiency of the β-*S*-phenyl $ManN_3A$ were exploited in the synthesis of *M. luteus* teichuronic acid fragments. An α-stereoselective glycosylation between a glucosyl *N*-phenyltrifluoro imidate and an *S*-phenyl mannosaziduronic acid acceptor provided the key α-Glc-(1→4)-β-$ManN_3A$-SPh building block, which was used in the assembly of tri-, penta-, and heptasaccharide fragments. Final deprotection of the oligomers under Birch reduction conditions, which was accompanied by partial fragmentation of the oligosaccharide chain, yielded the anionic tri-, penta-, and heptasaccharide *M. luteus* teichuronic acid fragments. The results presented in this paper may facilitate the synthesis of other complex (uronic acid-containing) oligosaccharides. Moreover, this research illustrates the importance of a comprehensive survey of the behavior in glycosylations when unreactive carbohydrate moieties are the building blocks of interest.

Experimental Section

General Procedure for the Low-Temperature NMR Experiments. Ph_2SO/Tf_2O activation: A mixture of the donor (30 μmol) and Ph_2SO (39 μmol) was coevaporated with toluene (2×). The residue was dissolved in DCM-d_2 (0.6 mL) and transferred to an NMR tube under an argon atmosphere. The tube was stoppered and sealed. The NMR magnet was cooled to −80 °C, locked, and shimmed. In an acetone bath (−80 °C), the sample was treated with Tf_2O (39 μmol), shaken three times, and placed back in the NMR magnet. The first ^{1}H spectrum was immediately recorded. Further temperature changes were executed depending on the spectra recorded, but always with multiples of 10 °C. Ultimately, the sample was placed in the acetone bath (−80 °C), and MeOH-d_4 (25 μL), which was used for its invisibility in ^{1}H NMR, was added. After the sample was shaken three times, it was placed back in the NMR magnet at −80 °C and immediately a ^{1}H spectrum was recorded. Then the temperature was raised to rt, and a final ^{1}H spectrum was recorded. TfOH activation: The donor (39 μmol) was coevaporated with dry toluene (2×), dissolved in DCM-d_2 (0.6 mL), and transferred to an NMR tube under an argon atmosphere. At −80 °C in an acetone bath TfOH (39 μmol) was added, the sample was transferred to the precooled NMR magnet, and the first ^{1}H spectrum was immediately recorded. Further temperature changes were executed depending on the spectra recorded, but always with multiples of 10 °C. Ultimately, the sample was placed in the acetone bath (−80 °C), and MeOH-d_4 (25 μL) was added. After the sample was shaken three times, it was placed back in the NMR magnet at −80 °C and immediately a ^{1}H spectrum was recorded. The temperature was then raised to rt, and a final ^{1}H spectrum was recorded.

General Procedure for the Ph_2SO/Tf_2O-Mediated Glycosylations. A mixture of the donor (1 equiv), Ph_2SO (1.3 equiv), and TTBP (2.5 equiv) was coevaporated twice with toluene. While the mixture was under an argon atmosphere, freshly distilled DCM (0.05 M) was added, followed by the addition of activated molecular sieves (3 Å). The resulting mixture was stirred for 30 min at room temperature and cooled to the activation temperature. Tf_2O (1.3 equiv) was added in one portion, and the activation progress was monitored by TLC analysis. The mixture was then cooled to the indicated reaction temperature, and a solution of the acceptor (0.3−0.5 M in DCM) was slowly added via the wall of the flask. The mixture was allowed to warm to 0 °C, after which Et_3N or pyridine was added to quench the reaction. Aqueous workup, passage of the residue through a column of Sephadex LH-20 (eluted with DCM/MeOH, 1/1, v/v), and purification using flash column chromatography (silica gel) gave the coupled product.

General Procedure for the TfOH-Mediated Glycosylations. A mixture of the donor (1 equiv) and the acceptor (1.5 equiv) was

(49) Birch reduction is commonly applied in the global deprotection of oligosaccharides and has been used in the synthesis of β-mannans: Nitz, M.; Purse, B. W.; Bundle, D. R. *Org. Lett.* **2000**, *2*, 2939–2942. For the use of Birch reduction in a PSA glycopeptide, see: Dudkin, V. Y.; Miller, J. S.; Danishefsky, S. J. *J. Am. Chem. Soc.* **2004**, *126*, 736–738.

(50) Seeberger and co-workers have previously noted the fragmentation of a β-mannosazide-containing oligosaccharide under Birch reduction conditions: Oberli, M. A.; Bindschädler, P.; Werz, D. B.; Seeberger, P. H. *Org. Lett.* **2008**, *10*, 905–908.

coevaporated with toluene (2×). While the mixture was under an argon atmosphere, freshly distilled DCM (0.05 M) was added, followed by the addition of activated molecular sieves (3 Å). The resulting mixture was stirred for 30 min at room temperature and cooled to the activation temperature. TfOH (0.2 equiv) was added, and the reaction mixture was warmed to the desired temperature. The reaction was then quenched by the addition of Et_3N or pyridine. After aqueous workup, the product was purified using Sephadex LH-20 (eluted with DCM/MeOH, 1/1, v/v) and flash column chromatography (silica gel).

Methyl (Phenyl-4-*O*-[2,3,4-tri-*O*-benzyl-6-*O*-[9-fluorenylmethoxycarbonyl]-α-D-glucopyranosyl]-2-azido-3-*O*-benzyl-2-deoxy-1-thio-β-D-mannopyranosyl uronate) (16). Imidate **14** (1.70 g, 2.02 mmol) and acceptor **15** (1.89 g, 1.5 mmol) were coevaporated with dry toluene (2×). Et_2O (40 mL, dried over 4 Å MS prior to use) was added, and the mixture was cooled to −35 °C. TfOH (40 μL, 0.45 mmol) was added, and the mixture was allowed to warm to −15 °C over 90 min. Pyridine (1 mL) was added, and the mixture was diluted with EtOAc and washed with satd aq NaCl (2×). The organic layer was dried over Na_2SO_4, concentrated in vacuo, and purified using column chromatography (silica gel, 20% EtOAc in PE) to yield the title compound as a white foam (1.44 g, 1.35 mmol, 90%): TLC R_f 0.47 (PE/EtOAc, 3/1, v/v); $[\alpha]^{20}_D$ +34.4 (*c* 1, DCM); IR (neat, cm^{-1}) 725, 905, 1070, 1452, 1747, 2110; ^1H NMR ($CDCl_3$, 400 MHz, HH−COSY, HSQC) δ 7.74 (d, 2H, *J* = 7.5 Hz, CH_{arom}), 7.60 (dd, 2H, *J* = 7.5, 11.1 Hz, CH_{arom}), 7.00−7.50 (m, 29H, CH_{arom}), 5.31 (d, 1H, *J* = 3.4 Hz, H-1′), 4.97 (d, 1H, *J* = 10.8 Hz, C*H*H Bn), 4.89 (d, 1H, *J* = 10.9 Hz, C*H*H Bn), 4.83 (d, 1H, *J* = 10.8 Hz, CH*H* Bn), 4.72 (d, 1H, *J* = 0.9 Hz, H-1), 4.69 (d, 2H, *J* = 6.4 Hz, CH_2 Bn), 4.57−4.63 (m, 3H, CH*H* Bn, CH_2 Bn), 4.32−4.46 (m, 5H, H-4, H-6, H-6, CH_2 Fmoc), 4.23 (t, 1H, *J* = 7.2 Hz, CH Fmoc), 4.05 (d, 1H, *J* = 2.5 Hz, H-2), 3.94 (t, 1H, *J* = 9.2 Hz, H-3′), 3.86 (d, 1H, *J* = 9.4 Hz, H-5), 3.76 (dd, 1H, *J* = 3.6, 9.1 Hz, H-3), 3.73 (s, 3H, CH_3 CO_2Me), 3.65−3.72 (m, 1H, H-5′), 3.58−3.64 (m, 1H, H-4′), 3.52 (dd, 1H, *J* = 3.5, 9.8 Hz, H-2′); ^{13}C NMR ($CDCl_3$, 100 MHz, HSQC) δ 167.4 (C=O CO_2Me), 154.9 (C=O Fmoc), 143.4, 143.1, 141.2 (C_q Fmoc), 138.4, 137.9, 137.2 (C_q Bn), 133.9 (C_q SPh), 131.0, 128.5, 128.3, 127.9, 127.8, 127.6, 127.1, 125.1, 125.0, 120.0 (CH_{arom}), 98.5 (C-1′), 86.6 (C-1), 81.3 (C-3, C-3′), 79.7 (C-2′), 79.1 (C-5), 76.7 (C-4′), 75.5, 75.1 (CH_2 Bn), 74.8 (C-4), 73.0, 72.9 (CH_2 Bn), 69.8 (CH_2 Fmoc), 69.7 (C-5′), 65.8 (C-6′), 63.2 (C-2), 52.8 (CH_3 CO_2Me), 46.6 (CH Fmoc); ^{13}C-GATED (100 MHz, $CDCl_3$) δ 98.5 ($J_{C1,H1}$ = 172 Hz, C-1′), 86.6 ($J_{C1,H1}$ = 154 Hz, C-1); HRMS $[M + Na]^+$ calcd for $C_{62}H_{59}N_3O_{12}SNa$ 1092.37117, found 1092.37178.

Methyl 6-*O*-(Methyl 4-*O*-[2,3,4-tri-*O*-benzyl-6-*O*-[9-fluorenylmethoxycarbonyl]-α-D-glucopyranosyl]-2-azido-3-*O*-benzyl-2-deoxy-β-D-mannopyranosyl uronate)-2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (17). Disaccharide **16** (123 mg, 0.12 mmol), Ph_2SO (30 mg, 0.15 mmol), and TTBP (71 mg, 0.29 mmol) were coevaporated with dry toluene (2×), dissolved in freshly distilled DCM (2.3 mL), and cooled to −65 °C. Tf_2O (25 μL, 0.15 mmol) was added, and the mixture was warmed to −55 °C during 15 min. The reaction was cooled back to −60 °C, and a solution of acceptor **9** (80 mg, 0.17 mmol, coevaporated twice with dry toluene prior to use) in distilled DCM (1 mL) was slowly added. The mixture was warmed to −40 °C over 1 h, quenched with pyridine (0.2 mL), diluted with EtOAc (20 mL), and washed with satd aq NaCl (2 × 30 mL). The organic fraction was dried over Na_2SO_4, concentrated in vacuo, and purified by passing the residue through a column of Sephadex LH-20 (eluted with DCM/MeOH, 1/1, v/v) followed by column chromatography (silica gel, 25% EtOAc in PE) to afford the title compound as a colorless oil (107 mg, 75 μmol, 65%): TLC R_f 0.47 (PE/EtOAc, 2/1, v/v); $[\alpha]^{20}_D$ +39.8 (*c* 1, DCM); IR (neat, cm^{-1}) 698, 739, 1028, 1072, 1257, 1749, 2110, 2910; ^1H NMR ($CDCl_3$, 400 MHz, HH−COSY, HSQC) δ 7.74 (d, 2H, *J* = 7.5 Hz, CH_{arom} Fmoc), 7.60 (t, 2H, *J* = 8.6 Hz, CH_{arom} Fmoc), 7.10−7.50 (m, 39H, CH_{arom}), 5.28 (d, 1H, *J* = 3.5 Hz, H-1″), 4.99 (d, 1H, *J* = 10.8 Hz, C*H*H Bn), 4.98 (d, 1H, *J* = 10.9 Hz, C*H*H Bn), 4.88 (d, 1H, *J* = 10.9 Hz, C*H*H Bn), 4.80−4.86 (m, 3H, CH*H* Bn, CH_2 Bn), 4.77 (d, 2H, *J* = 11.9 Hz, C*H*H Bn, CH*H* Bn), 4.47−4.70 (m, 7H, CH_2 Bn, H-1), 4.26−4.45 (m, 6H, H-1′, H-4′, H-6″, H-6″, CH_2 Fmoc), 4.23 (t, 1H, *J* = 7.3 Hz, CH Fmoc), 4.04−4.10 (m, 1H, H-6), 3.98 (t, 1H, *J* = 9.2 Hz, H-3), 3.93 (t, 1H, *J* = 9.3 Hz, H-3″), 3.83 (d, 1H, *J* = 8.6 Hz, H-5′), 3.73−3.80 (m, 1H, H-5), 3.65−3.73 (m, 1H, H-5″), 3.68 (s, 3H, CH_3 CO_2Me), 3.56−3.65 (m, 3H, H-2′, H-3′, H-4″), 3.52 (dd, 1H, *J* = 3.5, 9.8 Hz, H-2″), 3.38−3.50 (m, 2H, H-2, H-6), 3.32 (t, 1H, *J* = 9.4 Hz, H-4), 3.28 (s, 3H, CH_3 OMe); ^{13}C NMR ($CDCl_3$, 100 MHz, HSQC) δ 168.0 (C=O CO_2Me), 154.9 (C=O Fmoc), 143.4, 143.2, 141.2 (C_q Fmoc), 138.7, 138.5, 138.2, 138.1, 137.9, 137.9, 137.5 (C_q Bn), 127.1−128.5 (CH_{arom} Bn), 125.2, 125.1, 120.0 (CH_{arom} Fmoc), 99.8 (C-1′), 98.1 (C-1″), 97.7 (C-1), 82.0 (C-3), 81.3 (C-3″), 79.9 (C-2), 79.6 (C-2″), 78.7 (C-4″), 77.6 (C-4), 76.8 (C-3′), 75.7, 75.6 (CH_2 Bn), 75.2 (C-5′), 75.1, 74.7 (CH_2 Bn), 74.4 (C-4′), 73.3, 72.9, 72.2 (CH_2 Bn), 69.9 (CH_2 Fmoc), 69.7 (C-5), 69.6 (C-5″), 68.7 (C-6), 65.9 (C-6″), 60.7 (C-2′), 55.0 (OMe), 52.7 (CH_3 CO_2Me), 46.7 (CH Fmoc); ^{13}C-GATED ($CDCl_3$, 100 MHz) δ 99.8 ($J_{C1′,H1′}$ = 162 Hz, C-1′), 98.2 ($J_{C1″,H1″}$ = 170 Hz, C-1″), 97.7 ($J_{C1,H1}$ = 164 Hz, C-1); HRMS $[M + Na]^+$ calcd for $C_{84}H_{85}N_3O_{18}Na$ 1446.57203, found 1446.57310.

Methyl 6-*O*-(Methyl 4-*O*-[2,3,4-tri-*O*-benzyl-α-D-glucopyranosyl]-2-azido-3-*O*-benzyl-2-deoxy-β-D-mannopyranosyl uronate)-2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (18). A solution of compound **17** (1.26 g, 0.89 mmol) in THF (18 mL) was cooled to 0 °C under an argon atmosphere. TBAF (1 M solution in THF, 89 μL, 89 μmol) was added, and the reaction was stirred at 4 °C for 24 h. The mixture was quenched with satd aq $NaHCO_3$, diluted with EtOAc, washed with satd aq NaCl (2×), dried over Na_2SO_4, and concentrated in vacuo. Purification using flash column chromatography (silica gel, 50% EtOAc in PE) afforded the title product as a colorless oil (1.0 g, 0.87 mmol, 98%): TLC R_f 0.50 (PE/EtOAc, 1/1, v/v); $[\alpha]^{20}_D$ +42.5 (*c* 1, DCM); IR (neat, cm^{-1}) 696, 729, 1026, 1069, 1751, 2110, 2882; ^1H NMR ($CDCl_3$, 400 MHz, HH−COSY, HSQC) δ 7.20−7.40 (m, 35H, CH_{arom}), 5.19 (d, 1H, *J* = 3.6 Hz, H-1″), 4.98 (d, 1H, *J* = 10.2 Hz, C*H*H Bn), 4.96 (d, 1H, *J* = 9.3 Hz, CH*H* Bn), 4.85−4.90 (m, 3H, C*H*H Bn, CH_2 Bn), 4.80 (d, 1H, *J* = 11.0 Hz, CH*H* Bn), 4.76 (d, 1H, *J* = 12.1 Hz, C*H*H Bn), 4.57−4.70 (m, 6H, CH_2 Bn), 4.54 (s, 1H, H-1), 4.76 (d, 1H, *J* = 12.1 Hz, CH*H* Bn), 4.28 (s, 1H, H-1′), 4.28 (t, 1H, *J* = 8.4 Hz, H-4′), 4.04−4.13 (m, 1H, H-6), 3.99 (t, 1H, *J* = 9.2 Hz, H-3), 3.91 (t, 1H, *J* = 9.2 Hz, H-3″), 3.83 (d, 1H, *J* = 8.7 Hz, H-5′), 3.73−3.80 (m, 2H, H-5, H-6″), 3.68 (s, 3H, CH_3 CO_2Me), 3.64 (bd, 2H, *J* = 3.4 Hz, H-2′, H-6″), 3.59 (dd, 1H, *J* = 3.6, 8.5 Hz, H-3′), 3.40−3.56 (m, 5H, H-2, H-2″, H-4″, H-5″, H-6), 3.34 (t, 1H, *J* = 9.4 Hz, H-4), 3.29 (s, 3H, CH_3 OMe), 1.97 (bs, 1H, 6″−OH); ^{13}C NMR ($CDCl_3$, 100 MHz, HSQC) δ 168.1 (C=O CO_2Me), 138.5, 138.4, 138.1, 138.0, 137.9, 137.8, 137.4 (C_q Bn), 128.3, 128.2, 128.0, 127.9, 127.8, 127.7, 127.6, 127.4 (CH_{arom}), 99.7 (C-1′), 97.8 (C-1), 97.6 (C-1″), 81.9 (C-3″), 81.1 (C-3), 79.8, 79.6 (C-2, C-2″), 78.7 (C-3′), 77.4 (C-4), 77.1 (C-4″), 75.6, 75.4 (CH_2 Bn), 75.2 (C-5′), 74.9, 74.5 (CH_2 Bn), 73.9 (C-4′), 73.2, 72.9, 72.3 (CH_2 Bn), 72.0 (C-5″), 69.5 (C-5), 68.6 (C-6), 61.4 (C-6″), 60.9 (C-2′), 54.9 (OMe), 52.5 (CH_3 CO_2Me); ^{13}C-GATED (100 MHz, $CDCl_3$) δ 99.7 ($J_{C1′,H1′}$ = 160 Hz, C-1′), 97.8 ($J_{C1,H1}$ = 169 Hz, C-1), 97.6 ($J_{C1″,H1″}$ = 167 Hz, C-1″); HRMS $[M + Na]^+$ calcd for $C_{69}H_{75}N_3O_{16}Na$ 1224.50395, found 1224.50511.

Methyl 6-*O*-(Methyl 4-*O*-[6-*O*-[methyl 4-*O*-(2,3,4-tri-*O*-benzyl-6-*O*-[9-fluorenylmethoxycarbonyl]-α-D-glucopyranosyl)-2-azido-3-*O*-benzyl-2-deoxy-β-D-mannopyranosyl uronate]-2,3,4-tri-*O*-benzyl-α-D-glucopyranosyl]-2-azido-3-*O*-benzyl-2-deoxy-β-D-mannopyranosyl uronate)-2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (19). Disaccharide **16** (214 mg, 0.2 mmol), Ph_2SO (53 mg, 0.26 mmol), and

TTBP (124 mg, 0.5 mmol) were coevaporated with dry toluene (2×), dissolved in freshly distilled DCM (2.3 mL), and cooled to −70 °C. Tf_2O (37 μL, 0.22 mmol) was added, the reaction was allowed to warm to −60 °C over 30 min and then cooled to −80 °C, and a solution of acceptor **18** (172 mg, 0.14 mmol, coevaporated twice with dry toluene prior to use) in distilled DCM (1 mL) was slowly added. The reaction was allowed to stir at −80 °C overnight (cryostat). Pyridine (0.2 mL) was added, and the mixture was diluted with EtOAc, washed with satd aq NaCl (2×), dried over Na_2SO_4, concentrated in vacuo, and purified by passing the residue through a column of Sephadex LH-20 (eluted with DCM/MeOH, 1/1, v/v) to yield the title compound as a white foam (200 mg, 92 μmol, 65%): TLC R_f 0.26 (PE/EtOAc, 2/1, v/v); $[\alpha]^{20}_D$ +36.4 (*c* 1, DCM); IR (neat, cm^{-1}) 696, 727, 907, 1028, 1258, 1452, 1749, 2110; ^1H NMR ($CDCl_3$, 400 MHz, HH−COSY, HSQC) δ 7.74 (d, 2H, J = 7.6 Hz, CH_{arom}), 7.60 (dd, 2H, J = 7.5, 10.8 Hz, CH_{arom}), 7.19−7.40 (m, 59H, CH_{arom}), 5.35 (d, 1H, J = 3.5 Hz, H-1_{Glc}), 5.21 (d, 1H, J = 3.5 Hz, H-1_{Glc}), 4.99 (d, 1H, J = 11.1 Hz, C*H*H Bn), 4.98 (d, 1H, J = 10.9 Hz, C*H*H Bn), 4.96 (d, 1H, J = 12.2 Hz, CH*H* Bn), 4.88 (d, 1H, J = 10.7 Hz, C*H*H Bn), 4.74−4.86 (m, 7H, CH_2 Bn), 4.49−4.72 (m, 12H, CH_2 Bn, H-1_{Glc}), 4.26−4.44 (m, 8H, H-1_{Man}, H-1_{Man}, H-4_{Man}, H-4_{Man}, H-6_{Glc}, H-6_{Glc}, CH_2 Fmoc), 4.23 (t, 1H, J = 7.5 Hz, CH Fmoc), 4.10 (d, 1H, J = 9.4 Hz, H-6_{Glc}), 3.98 (t, 2H, J = 8.8 Hz, H-3_{Glc}, H-6_{Glc}), 3.87−3.94 (m, 2H, H-3_{Glc}, H-3_{Glc}), 3.85 (d, 1H, J = 8.2 Hz, H-5_{Man}), 3.82 (m, 1H, H-2_{Man}), 3.79 (d, 1H, J = 9.3 Hz, H-5_{Man}), 3.72−3.77 (m, 1H, H-5_{Glc}), 3.69 (s, 3H, CH_3 CO_2Me), 3.64 (bs, 5H, H-3_{Man}, H-5_{Glc}, CH_3 CO_2Me), 3.54−3.62 (m, 5H, H-2_{Man}, H-3_{Man}, H-4_{Glc}, H-5_{Glc}, H-6_{Glc}), 3.50 (dd, 1H, J = 3.7, 10.0 Hz, H-2_{Glc}), 3.37−3.49 (m, 4H, H-2_{Glc}, H-2_{Glc}, H-4_{Glc}, H-6_{Glc}), 3.32 (t, 1H, J = 9.6 Hz, H-4_{Glc}), 3.26 (CH_3 OMe); ^{13}C NMR ($CDCl_3$, 100 MHz, HSQC) δ 168.2, 168.0 (C=O CO_2Me), 154.9 (C=O Fmoc), 143.4, 143.2, 141.2, 141.2 (C_q Fmoc), 138.6, 138.5, 138.5, 138.4, 138.2, 138.0, 138.0, 137.9, 137.9, 137.5, 137.4 (C_q Bn), 128.4, 128.3, 127.8, 127.7, 127.5, 127.3, 127.1 (CH_{arom}), 125.2, 125.1, 119.9 (CH_{arom} Fmoc), 99.7, 99.7 (C-1_{Man}), 97.9, 97.7, 97.7 (C-1_{Glc}), 82.0, 81.4, 81.3 (C-3_{Glc}), 79.9, 79.6 (C-2_{Glc}), 79.4, 79.4 (C-2_{Glc}, C-3_{Man}), 78.6 (C-3_{Man}), 77.7 (C-4_{Glc}), 76.9, 76.7 (C-4_{Glc}), 75.7, 75.5, 75.4 (CH_2 Bn), 75.3 (C-5_{Man}), 75.0 (CH_2 Bn), 74.9 (C-5_{Man}), 74.7, 74.7 (CH_2 Bn), 74.0, 73.6 (C-4_{Man}), 73.3, 73.1, 72.7, 72.1, 71.8 (CH_2 Bn), 70.9 (C-5_{Glc}), 69.8 (CH_2 Fmoc), 69.6, 69.5 (C-5_{Glc}), 68.7, 67.6, 65.8 (C-6_{Glc}), 60.8, 60.3 (C-2_{Man}), 55.0 (OMe), 52.6, 52.6 (CH_3 CO_2Me), 46.6 (CH Fmoc); ^{13}C-HMBC (150 MHz, $CDCl_3$) δ 99.7 ($J_{C1,H1}$ = 161 Hz, C-1_{Man}), 99.7 ($J_{C1,H1}$ = 160 Hz, C-1_{Man}), 97.9 $J_{C1,H1}$ = 171 Hz, C-1_{Glc}), 97.7 ($J_{C1,H1}$ = 171 Hz, C-1_{Glc}), 97.7 ($J_{C1,H1}$ = 168 Hz, C-1_{Glc}); HRMS $[M + NH_4]^+$ calcd for $C_{125}H_{132}N_7O_{28}$ 2179.91484, found 2179.91016.

Methyl 6-*O*-(Methyl 4-*O*-[6-*O*-[methyl 4-*O*-(2,3,4-tri-*O*-benzyl-α-D-glucopyranosyl)-2-azido-3-*O*-benzyl-2-deoxy-β-D-mannopyranosyl uronate]-2,3,4-tri-*O*-benzyl-α-D-glucopyranosyl]-2-azido-3-*O*-benzyl-2-deoxy-β-D-mannopyranosyl uronate)-2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (20). A solution of compound **19** (133 mg, 62 μmol) in dry pyridine (1.3 mL) was treated with Et_3N (0.13 mL, 0.9 mmol) at rt. After 3 h, TLC analysis indicated complete consumption of the starting material, and the reaction was diluted with EtOAc (10 mL), washed with satd aq NaCl (2×), dried over Na_2SO_4, and concentrated in vacuo. Purification using flash column chromatography (silica gel, 50% EtOAc in PE) afforded the title compound as a colorless oil (106 mg, 55 μmol, 89%): TLC R_f 0.73 (PE/EtOAc, 1/1, v/v); $[\alpha]^{20}_D$ +35.9 (*c* 1, DCM); IR (neat, cm^{-1}) 696, 733, 1026, 1070, 1751, 2108, 2880; ^1H NMR ($CDCl_3$, 400 MHz, HH−COSY, HSQC) δ 7.19−7.37 (m, 55H, CH_{arom}), 5.24 (d, 1H, J = 3.6 Hz, H-1_{Glc}), 5.21 (d, 1H, J = 3.5 Hz, H-1_{Glc}), 4.98 (d, 1H, J = 7.4 Hz, C*H*H Bn), 4.96 (d, 1H, J = 7.5 Hz, C*H*H Bn), 4.74−4.89 (m, 7H, CH_2 Bn), 4.70 (d, 1H, J = 12.0 Hz, C*H*H Bn), 4.54−4.68 (m, 10H, CH_2 Bn), 4.49−4.54 (m, 3H, CH_2 Bn, H-1_{Glc}), 4.34 (s, 1H, H-1_{Man}), 4.21−4.30 (m, 2H, H-4_{Man}, H-4_{Man}), 4.24 (s, 1H, H-1_{Man}), 4.10 (dd, 1H, J = 1.1, 10.4 Hz, H-6_{Glc}), 3.98 (bt, 2H, J = 9.1 Hz, H-3_{Glc}, H-6_{Glc}), 3.90 (bt, 2H, J = 9.5 Hz, H-3_{Glc}, H-3_{Glc}), 3.85 (d, 1H, J = 8.2 Hz, H-5_{Man}), 3.79 (bd, 2H, J = 9.2 Hz, H-2_{Man}, H-5_{Man}), 3.73−3.76 (m, 2H, H-5_{Glc}, H-6_{Glc-OH}), 3.69 (s, 3H, CH_3 CO_2Me), 3.64 (s, 3H, CH_3 CO_2Me), 3.54−3.63 (m, 6H, H-2_{Man}, H-3_{Man}, H-3_{Man} H-5_{Glc}, H-6_{Glc-OH}, H-6_{Glc}), 3.38−3.49 (m, 7H, H-2_{Glc}, H-2_{Glc}, H-2_{Glc}, H-4_{Glc}, H-4_{Glc}, H-5_{Glc}, H-6_{Glc}), 3.32 (t, 1H, J = 9.4 Hz, H-4_{Glc}), 3.26 (s, 3H, CH_3 OMe), 1.93 (s, 1H, 6-OH_{Glc}); ^{13}C NMR ($CDCl_3$, 100 MHz, HSQC) δ 168.2 (C=O CO_2Me), 138.6, 138.5, 138.5, 138.3, 138.1, 138.1, 138.0, 138.0, 137.9, 137.5, 137.4 (C_q Bn), 128.4, 128.3, 128.0, 127.9, 127.8, 127.7, 127.6, 127.5, 127.3 (CH_{arom}), 99.7, 99.7 (C-1_{Man}), 97.7, 97.6 (C-1_{Glc}), 82.0, 81.4, 81.2 (C-3_{Glc}), 79.8 (C-2_{Glc}), 79.6, 79.5, 79.4 (C-2_{Glc}, C-2_{Glc}, C-3_{Man}), 78.6 (C-3_{Man}), 77.7, 77.1, 76.8 (C-4Glc), 75.7, 75.5, 75.5 (CH_2 Bn), 75.4 (C-5_{Man}), 75.0 (CH_2 Bn), 74.9 (C-5_{Man}), 74.7, 74.7 (CH_2 Bn), 73.7, 73.5 (C-4_{Man}), 73.3, 73.1, 72.8, 72.1, 72.1 (CH_2 Bn), 72.0, 70.9 (C-5_{Glc}), 68.7, 67.6 (C-6_{Glc}), 61.6 (C-6_{Glc-OH}), 61.1, 60.3 (C-2_{Man}), 55.0 (OMe), 52.7, 52.6 (CH_3 CO_2Me); ^{13}C-HMBC (100 MHz, $CDCl_3$) δ 99.7 ($J_{C1,H1}$ = 160 Hz, C-1_{Man}), 99.7 ($J_{C1,H1}$ = 160 Hz, C-1_{Man}), 97.7 ($J_{C1,H1}$ = 168 Hz, C-1_{Glc}), 97.7 ($J_{C1,H1}$ = 170 Hz, C-1_{Glc}), 97.6 ($J_{C1,H1}$ = 170 Hz, C-1_{Glc}); HRMS $[M + NH_4]^+$ calcd for $C_{110}H_{122}N_7O_{26}$ 1956.84340, found 1956.84289.

Methyl 6-*O*-(Methyl 4-*O*-[6-*O*-[methyl 4-*O*-(6-*O*-[methyl 4-*O*-[2,3,4-tri-*O*-benzyl-6-*O*-(9-fluorenylmethoxycarbonyl)-α-D-glucopyranosyl]-2-azido-3-*O*-benzyl-2-deoxy-β-D-mannopyranosyl uronate]-2,3,4-tri-*O*-benzyl-α-D-glucopyranosyl)-2-azido-3-*O*-benzyl-2-deoxy-β-D-mannopyranosyl uronate]-2,3,4-tri-*O*-benzyl-α-D-glucopyranosyl]-2-azido-3-*O*-benzyl-2-deoxy-β-D-mannopyranosyl uronate)-2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (21). Disaccharide **16** (230 mg, 0.22 mmol), Ph_2SO (43 mg, 0.22 mmol), and TTBP (53 mg, 0.22 mmol) were coevaporated with dry toluene (2×), dissolved in freshly distilled DCM (1.4 mL), and cooled to −70 °C. Tf_2O (35 μL, 0.21 mmol) was added, the reaction was allowed to warm to −55 °C in 15 min and cooled to −80 °C, and a solution of acceptor **20** (139 mg, 72 μmol, coevaporated twice with dry toluene prior to use) in distilled DCM (1 mL) was slowly added. The reaction was allowed to stir at −80 °C over two nights (cryostat). Then pyridine (0.02 mL) was added, and the mixture was diluted with EtOAc, washed with satd aq NaCl (2×), dried over Na_2SO_4, concentrated in vacuo, and purified by passing the residue through a column of Sephadex LH-20 (eluted with DCM/MeOH, 1/1, v/v), and subsequent flash column chromatography (silica gel, 33% EtOAc in PE) to yield the title compound as a colorless oil (47 mg, 16.4 μmol, 23%). Acceptor **20** was recovered in 40% yield: TLC R_f 0.37 (PE/EtOAc, 2/1, v/v); $[\alpha]^{20}_D$ +33.6 (*c* 1, DCM); IR (neat, cm^{-1}) 698, 739, 1028, 1072, 1749, 2108, 2956; ^1H NMR ($CDCl_3$, 400 MHz, HH−COSY, HSQC, tentatively assigned based on ^1H NMR of compound **19**) δ 7.76 (d, 2H, J = 7.5 Hz, CH_{arom}), 7.62 (dd, 2H, J = 7.5, 11.4 Hz, CH_{arom}), 7.20−7.43 (m, 79H, CH_{arom}), 5.38 (d, 1H, J = 3.5 Hz, H-1_{Glc}), 5.33 (d, 1H, J = 3.4 Hz, H-1_{Glc}), 5.22 (d, 1H, J = 3.4 Hz, H-1_{Glc}), 5.01 (d, 1H, J = 10.8 Hz, C*H*H Bn), 4.99 (d, 1H, J = 10.6 Hz, C*H*H Bn), 4.97 (d, 1H, J = 10.8 Hz, C*H*H Bn), 4.75−4.92 (m, 10H, CH_2 Bn), 4.58−4.72 (m, 15H, CH_2 Bn), 4.50−4.58 (m, 3H, CH_2 Bn, H-1_{Glc}), 4.32−4.45 (m, 7H, H-1_{Man}, H-6_{Glc}, H-6_{Glc}, H-6_{Glc}, H-6_{Glc}, CH_2 Fmoc), 4.22−4.32 (m, 6H, H-1_{Man}, H-1_{Man}, H-4_{Man}, H-4_{Man}, H-4_{Man}, CH Fmoc), 4.11 (d, 1H, J = 9.8 Hz, H-6_{Glc}), 3.90 (t, 2H, J = 9.1 Hz, H-3_{Glc}, H-6_{Glc}), 3.82−3.95 (m, 4H, H-3_{Glc}, H-3_{Glc}, H-3_{Glc}, H-5_{Man}), 3.73−3.82 (m, 5H, H-2_{Man}, H-2_{Man}, H-5_{Glc}, H-5_{Man}, H-5_{Man}), 3.71 (s, 3H, CH_3 CO_2Me), 3.70 (s, 3H, CH_3 CO_2Me), 3.64 (s, 3H, CH_3 CO_2Me), 3.55−3.63 (m, 7H, H-2_{Man}, H-3_{Man}, H-3_{Man}, H-3_{Man}, H-4_{Glc}, H-5_{Glc}, H-6_{Glc}), 3.39−3.55 (m, 8H, H-2_{Glc}, H-2_{Glc}, H-2_{Glc}, H-2_{Glc}, H-4_{Glc}, H-4_{Glc}, H-5_{Glc}, H-6_{Glc}), 3.33 (t, 1H, J = 9.4 Hz, H-4_{Glc}), 3.27 (s, 3H, CH_3 OMe); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC, tentatively assigned based on ^{13}C NMR of compound **19**) δ 168.2, 168.2, 168.0 (C=O CO_2Me),

154.9 (C=O Fmoc), 143.5, 143.2, 141.2, 141.2 (C_q Fmoc), 138.6, 138.6, 138.5, 138.5, 138.4, 138.2, 138.0, 138.0, 138.0, 137.9, 137.5, 137.5, 137.4 (C_q Bn), 128.4, 127.9, 127.8, 127.7, 127.6, 127.5, 127.4, 127.3, 127.2, 127.1 (CH_{arom}), 125.2, 125.1, 120.0 (CH_{arom} Fmoc), 99.8, 99.7, 99.7 (C-1_{Man}), 97.9, 97.8, 97.7, 97.5 (C-1_{Glc}), 82.0, 81.4, 81.3 (C-3_{Glc}), 79.8, 79.6, 79.5, 79.4, 78.6 (C-2_{Glc}, C-3_{Man}), 77.7, 77.2, 76.9, 76.8 (C-4_{Glc}), 75.7, 75.6, 75.5, 75.4 (CH_2 Bn), 75.3, 75.2 (C-5_{Man}), 75.1 (CH_2 Bn), 75.0 (C-5_{Man}), 74.7, 74.7, 74.6 (CH_2 Bn), 74.0, 73.6 (C-4_{Man}), 73.4 (CH_2 Bn), 73.3 (C-4_{Man}), 73.1, 72.8, 72.7, 72.1, 71.9, 71.7 (CH_2 Bn), 70.9, 70.8 (C-5_{Glc}), 69.9 (CH_2 Fmoc), 69.6, 69.5 (C-5_{Glc}), 68.7, 67.6, 67.6, 65.9 (C-6_{Glc}), 60.9, 60.7, 60.3 (C-2_{Man}), 55.0 (OMe), 52.7, 52.7, 52.6 (CH_3 CO_2Me), 46.7 (CH Fmoc); ^{13}C-HMBC (150 MHz, $CDCl_3$) δ 99.8 ($J_{C1,H1}$ = 161 Hz, C-1_{Man}), 99.7 ($J_{C1,H1}$ = 161 Hz, C-1_{Man}), 99.7 ($J_{C1,H1}$ = 161 Hz, C-1_{Man}), 97.9 ($J_{C1,H1}$ = 172 Hz, C-1_{Glc}), 97.8 ($J_{C1,H1}$ = 170 Hz, C-1_{Glc}), 97.7 ($J_{C1,H1}$ = 169 Hz, C-1_{Glc}), 97.5 ($J_{C1,H1}$ = 171 Hz, C-1_{Glc}); HRMS $[M + Na]^+$ calcd for $C_{166}H_{171}N_9O_{38}Na$ 2922.16508, found 2922.15435.

Methyl 6-*O*-(Methyl 4-*O*-[6-*O*-[methyl 4-*O*-(6-*O*-[methyl 4-*O*-[2,3,4-tri-*O*-benzyl-α-D-glucopyranosyl]-2-azido-3-*O*-benzyl-2-deoxy-β-D-mannopyranosyl uronate]-2,3,4-tri-*O*-benzyl-α-D-glucopyranosyl)-2-azido-3-*O*-benzyl-2-deoxy-β-D-mannopyranosyl uronate]-2,3,-4-tri-*O*-benzyl-α-D-glucopyranosyl]-2-azido-3-*O*-benzyl-2-deoxy-β-D-mannopyranosyl uronate)-2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (22). Compound **21** (48 mg, 16.5 μmol) was dissolved in dry pyridine (1 mL) followed by the addition of Et_3N (8 μL, 54 μmol), and the resulting solution was stirred at rt overnight. The mixture was diluted with EtOAc and washed with satd aq NaCl (3×). The combined aqueous layers were extracted with EtOAc, and the combined organic layers were dried over Na_2SO_4, filtered, and concentrated in vacuo. Purification using flash column chromatography (silica gel, 40% EtOAc in PE) yielded the title compound as a colorless oil (35 mg, 13 μmol, 78%): TLC R_f 0.30 (PE/EtOAc, 3/2, v/v); $[\alpha]^{20}_D$ +35.6 (*c* 1, DCM); IR (neat, cm^{-1}) 698, 1028, 1072, 1751, 2108, 2954; ^{1}H NMR ($CDCl_3$, 400 MHz, HH−COSY, HSQC) δ 7.20−7.37 (m, 75H, CH_{arom}), 5.31 (d, 1H, *J* = 3.4 Hz, H-1_{Glc}), 5.24 (d, 1H, *J* = 3.5 Hz, H-1_{Glc}), 5.20 (d, 1H, *J* = 3.4 Hz, H-1_{Glc}), 4.98 (d, 1H, *J* = 10.7 Hz, C*H*H Bn), 4.96 (d, 2H, *J* = 10.8 Hz, C*H*H Bn), 4.87 (d, 2H, *J* = 10.8 Hz, C*H*H Bn), 4.79−4.84 (m, 4H, CH_2 Bn), 4.77 (d, 2H, *J* = 11.8 Hz, CH*H* Bn), 4.53−4.69 (m, 16H, CH_2 Bn), 4.49−4.53 (m, 4H, CH_2 Bn, H-1_{Glc}), 4.33 (s, 1H, H-1_{Man}), 4.27 (s, 1H, H-1_{Man}), 4.22−4.27 (m, 3H, H-4_{Man}, H-4_{Man}, H-4_{Man}), 4.21 (s, 1H, H-1_{Man}), 4.09 (d, 1H, *J* = 9.4 Hz, H-6_{Glc}), 3.88−4.01 (m, 6H, H-3_{Glc}, H-3_{Glc}, H-3_{Glc}, H-3_{Glc}, H-6_{Glc}, H-6_{Glc}), 3.84 (d, 1H, *J* = 8.2 Hz, H-5_{Man}), 3.71−3.82 (m, 6H, H-2_{Man}, H-2_{Man}, H-5_{Man}, H-5_{Man}, H-5_{Glc}, H-6_{Glc}), 3.69 (s, 3H, CH_3 CO_2Me), 3.67 (s, 3H, CH_3 CO_2Me), 3.63 (bs, 4H, H-6_{Glc}, CH_3 CO_2Me), 3.54−3.62 (m, 7H, H-2_{Man}, H-3_{Man}, H-3_{Man}, H-3_{Man}, H-5_{Glc}, H-6_{Glc}, H-6_{Glc}), 3.38−3.53 (m, 10H, H-2_{Glc}, H-2_{Glc}, H-2_{Glc}, H-2_{Glc}, H-4_{Glc}, H-4_{Glc}, H-4_{Glc}, H-5_{Glc}, H-5_{Glc}, H-6_{Glc}), 3.32 (t, 1H, *J* = 9.4 Hz, H-4_{Glc}), 3.25 (s, 3H, CH_3 OMe), 1.91 (bs, 1H, 6-OH_{Glc}); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC) δ 168.2, 168.2 (C=O CO_2Me), 138.6, 138.6, 138.6, 138.5, 138.5, 138.4, 138.2, 138.1, 138.0, 138.0, 137.9 137.6, 137.5 137.4 (C_q Bn), 128.5, 128.4, 128.3, 128.1, 128.0, 127.9, 127.8, 127.7, 127.6, 127.5, 127.4 127.3 (CH_{arom}), 99.8, 99.7 99.7 (C-1_{Man}), 97.8, 97.7, 97.7, 97.5 (C-1_{Glc}), 82.0, 81.4, 81.2 (C-3_{Glc}), 79.9, 79.6, 79.6, 79.5, 79.4 (C-2_{Glc}, C-3_{Man}), 78.6 (C-3_{Man}), 77.7, 77.2, 77.2, 76.9 (C-4_{Glc}), 75.7, 75.5, 75.6 (CH_2 Bn), 75.4, 75.2 (C-5_{Man}), 75.0 (CH_2 Bn), 75.0 (C-5_{Man}), 74.7, 74.7, 74.6 (CH_2 Bn), 73.8, 73.6 (C-4_{Man}), 73.4 (CH_2 Bn), 73.3 (C-4_{Man}), 73.1, 72.9, 72.1 (CH_2 Bn), 72.0 (C-5_{Glc}), 71.8 (CH_2 Bn), 70.9, 70.7, 69.7 (C-5_{Glc}), 68.7, 67.6, 67.6, 61.6 (C-6_{Glc}), 61.1, 60.7, 60.3 (C-2_{Man}), 55.0 (OMe), 52.7, 52.7 (CH_3 CO_2Me); ^{13}C-HMBC (150 MHz, $CDCl_3$) δ 99.8 ($J_{C1,H1}$ = 162 Hz, C-1_{Man}), 99.7 ($J_{C1,H1}$ = 161 Hz, C-1_{Man}), 99.7 ($J_{C1,H1}$ = 160 Hz, C-1_{Man}), 97.8 ($J_{C1,H1}$ = 170 Hz, C-1_{Glc}), 97.7 ($J_{C1,H1}$ = 171 Hz, C-1_{Glc}), 97.7 ($J_{C1,H1}$ = 168 Hz, C-1_{Glc}), 97.5 ($J_{C1,H1}$ = 172 Hz, C-1_{Glc}); HRMS $[M + NH_4]^+$ calcd for $C_{151}H_{165}N_{10}O_{36}$ 2695.14160, found 2695.13146.

General Procedure for the KOOH-Mediated Saponification. A mixture of KOH and H_2O_2 was freshly prepared: aq KOH (0.5 M, 4.86 mL, 2.5 mmol) was added to H_2O_2 (50 wt % in H_2O, 0.28 mL, 5 mmol). A solution of the methyl uronate (1 equiv) in THF (0.05 M) was cooled to 0 °C, and the KOH−H_2O_2 solution was dropwise added. The resulting mixture was stirred at rt until full conversion of the starting material was indicated by TLC analysis. When an emulsion was observed, THF was dropwise added to obtain a clear solution. The reaction was quenched by the addition of 1 M HCl until pH ~6. Subsequently, the mixture was partitioned between EtOAc and H_2O, the organic layer was washed with satd aq NaCl (2×), dried over Na_2SO_4, and concentrated in vacuo. The product was obtained after passing the residue through a column of Sephadex LH-20 (eluted with DCM/MeOH, 1/1, v/v) to remove any eliminated side products.

Methyl 6-*O*-(4-*O*-[2,3,4-Tri-*O*-benzyl-α-D-glucopyranosyl]-2-azido-3-*O*-benzyl-2-deoxy-β-D-mannopyranosyl uronate)-2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (23). Compound **18** (76 mg, 63 μmol) was saponified using the general procedure (0.25 mL of KOH−H_2O_2 solution) to produce the title compound as a colorless oil (63 mg, 53 μmol, 85%): TLC R_f 0.38 (PE/EtOAc, 1/3, v/v + 1% AcOH); $[\alpha]^{20}_D$ +30.6 (*c* 1, DCM); IR (neat, cm^{-1}) 698, 1028, 1070, 1736, 2110, 2854, 2923; ^{1}H NMR ($CDCl_3$, 400 MHz, HH−COSY, HSQC) δ 7.18−7.36 (m, 35H, CH_{arom}), 5.10 (d, 1H, *J* = 3.5 Hz, H-1″), 4.97 (d, 1H, *J* = 10.9 Hz, C*H*H Bn), 4.96 (d, 1H, *J* = 10.9 Hz, C*H*H Bn), 4.82−4.87 (m, 2H, CH_2 Bn), 4.74−4.80 (m, 3H, CH_2 Bn), 4.68 (d, 1H, *J* = 11.9 Hz, C*H*H Bn), 4.57−4.63 (m, 4H, CH_2 Bn), 4.52−4.57 (m, 3H, CH_2 Bn, H-1), 4.47 (d, 1H, *J* = 11.4 Hz, CH*H* Bn), 4.42 (s, 1H, H-1′), 4.33 (t, 1H, *J* = 7.0 Hz, H-4′), 3.89−4.02 (m, 4H, H-3, H-3″, H-5′, H-6), 3.81 (app d, 1H, *J* = 10.2 Hz, H-6″), 3.70−3.76 (m, 2H, H-5, H-5″), 3.59−3.67 (m, 4H, H-2′, H-3′, H-6, H-6″), 3.43−3.52 (m, 3H, H-2, H-2″, H-4″), 3.37 (t, 1H, *J* = 9.4 Hz, H-4), 3.28 (s, 3H, CH_3 OMe); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC) δ 171.0 (C=O CO_2H), 138.6, 138.5, 138.1, 137.9, 137,9, 137.8, 137.3 (C_q Bn), 128.4, 128.3, 128.1, 128.0, 127.9, 127.8, 127.7, 127.5 (CH_{arom}), 99.7 (C-1′), 98.4 (C-1″), 97.8 (C-1), 81.8, 81.2 (C-3, C-3″), 79.8, 79.7 (C-2, C-2″), 77.4, 77.2, 77.1 (C-3′, C-4, C-4″), 75.7 (C-5′), 75.6, 75.5 (CH_2 Bn), 75.4 (C-4′), 75.1, 74.6 (CH_2 Bn), 73.3, 73.0, 72.6 (CH_2 Bn), 72.0 (C-5″), 69.4 (C-5), 69.2 (C-6), 61.4 (C-6″), 59.9 (C-2′), 55.2 (OMe); ^{13}C-GATED ($CDCl_3$, 100 MHz) δ 99.7 ($J_{C1,H1}$ = 163 Hz, C-1′), 98.4 ($J_{C1,H1}$ = 171 Hz, C-1″), 97.8 ($J_{C1,H1}$ = 170 Hz, C-1); HRMS $[M + NH_4]^+$ calcd for $C_{68}H_{77}N_4O_{16}$ 1205.53291, found 1205.53387.

Methyl 6-*O*-(4-*O*-[6-*O*-[4-*O*-(2,3,4-Tri-*O*-benzyl-α-D-glucopyranosyl)-2-azido-3-*O*-benzyl-2-deoxy-β-D-mannopyranosyl uronate]-2,3,4-tri-*O*-benzyl-α-D-glucopyranosyl]-2-azido-3-*O*-benzyl-2-deoxy-β-D-matnnopyranosyl uronate)-2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (24). Compound **20** (116 mg, 60 μmol) was saponified using the general procedure (0.36 mL KOH−H_2O_2 solution) to yield the title compound as a colorless oil (96 mg, 50 μmol, 83%): TLC R_f 0.60 (PE/EtOAc, 1/3, v/v + 5% AcOH); $[\alpha]^{20}_D$ +35.9 (*c* 1, DCM); IR (neat, cm^{-1}) 696, 731, 1026, 1067, 1742, 2108, 2955; ^{1}H NMR ($CDCl_3$, 400 MHz, HH−COSY, HSQC) δ 7.10−7.38 (m, 55H, CH_{arom}), 5.37 (s, 1H, H-1_{Glc}), 5.19 (s, 1H, H-1_{Glc}), 4.90−4.99 (m, 3H, CH_2 Bn), 4.73−4.85 (m, 7H, CH_2 Bn), 4.43−4.68 (m, 14H, CH_2 Bn, H-1_{Glc}, H-1_{Man}), 4.36−4.42 (m, 1H, H-4_{Man}), 4.33 (s, 1H, H-1_{Man}), 4.25−4.32 (m, 1H, H-4_{Man}), 4.05 (app d, 1H, *J* = 6.5 Hz, H-5_{Man}), 3.82−3.43 (m, 9H, H-3_{Glc}, H-3_{Glc}, H-3_{Glc}, H-5_{Glc}, H-5_{Glc}, H-5_{Glc}, H-5_{Man}, H-6_{Glc}, H-6_{Glc}), 3.70−3.76 (m, 2H, H-6_{Glc}, H-6_{Glc}), 3.63−3.67 (m, 1H, H-3_{Man}), 3.52−3.63 (m, 5H, H-2_{Man}, H-2_{Man}, H-3_{Man}, H-6_{Glc}, H-6_{Glc}), 3.41−3.52 (m, 4H, H-2_{Glc}, H-2_{Glc}, H-2_{Glc}, H-4_{Glc}), 3.33−3.37 (m, 1H, H-4_{Glc}), 3.28 (bs, 4H, H-4_{Glc}, CH_3 OMe); ^{13}C NMR ($CDCl_3$, 150 MHz, HSQC) δ 172.0, 169.7 (C=O CO_2H), 138.6, 138.5, 138.2, 138.1, 138.0, 137.4, 137.4

(C_q Bn), 128.4, 128.3, 128.2, 128.0, 127.9, 127.8, 127.7, 127.6, 127.4 (CH_{arom}), 99.8, 99.5 (C-1_{Man}), 98.7, 97.7, 97.7 (C-1_{Glc}), 81.9, 81.3 (C-3_{Glc}), 79.8, 79.7, 79.7 (C-2_{Glc}), 78.1, 77.9, 77.2, 75.6, 75.5, 75.4, 74.9, 74.6, 74.6, 74.3, 73.3, 73.1, 72.8, 72.7, 72.0, 71.7, 70.2, 69.5, 69.0, 68.4 (CH_2 Bn, C-4_{Glc}, C-5_{Glc}, C-3_{Man}, C-4_{Man}, C-5_{Man}), 63.9 (C-6_{Glc}), 61.8 (C-6_{Glc}), 60.3, 59.7 (C-2_{Man}), 55.1 (OMe); ^{13}C-HMBC ($CDCl_3$, 150 MHz) δ 99.8 ($J_{C1,H1}$ = 162 Hz, C-1_{Man}), 99.57 ($J_{C1,H1}$ = 163 Hz, C-1_{Man}), 98.7 ($J_{C1,H1}$ = 169 Hz, C-1_{Glc}), 97.7 ($J_{C1,H1}$ = 173 Hz, C-1_{Glc}), 97.7 ($J_{C1,H1}$ = 169 Hz, C-1_{Glc}); HRMS $[M + NH_4]^+$ calcd for $C_{108}H_{118}N_7O_{26}$ 1929.8155, found 1929.8157.

Methyl 6-*O*-(4-*O*-[6-*O*-[4-*O*-(6-*O*-[4-*O*-[2,3,4-Tri-*O*-benzyl-α-D-glucopyranosyl]-2-azido-3-*O*-benzyl-2-deoxy-*β*-D-mannopyranosyl uronate]-2,3,4-tri-*O*-benzyl-α-D-glucopyranosyl)-2-azido-3-*O*-benzyl-2-deoxy-*β*-D-mannopyranosyl uronate]-2,3,4-tri-*O*-benzyl-α-D-glucopyranosyl]-2-azido-3-*O*-benzyl-2-deoxy-*β*-D-mannopyranosyl uronate)-2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (25). Compound **22** (35 mg, 13 μmol) was saponified using the general procedure (0.2 mL of KOH−H_2O_2 solution) to yield the title compound as a colorless oil (29 mg, 10.8 μmol, 83%). The presence of three uronic acid moieties resulted in such broadening of the NMR signals that accurate assignment was impossible; however, the disappearance of the CO_2Me signals was confirmed: TLC R_f 0.65 (PE/EtOAc, 1/3, v/v + 5% AcOH); IR (neat, cm^{-1}) 698, 1028, 1607, 2112, 3414; HRMS $[M + NH_4]^+$ calcd for $C_{148}H_{159}N_{10}O_{36}$ 2653.0946, found 2653.0844.

General Procedure for the Birch Reduction and Subsequent Acetylation. THF was distilled over Na/benzophenone prior to use. A three-necked 50-ml round-bottom flask was equipped with a cooling condenser (−40 °C) and a bubbler and charged with a solution of the oligosaccharide (1 equiv) in THF (0.1 M). A glass stir bar and *t*-BuOH (16 equiv) were added, and the mixture was cooled to −65 °C. A small piece of sodium was added, and liquid ammonia was collected (1−2 mL) by passing ammonia gas through the system. Extra sodium was added until the solution remained dark blue in color. The resulting mixture was stirred for 30 min while the temperature was kept below −40 °C, quenched with satd aq NH_4Cl (~1 mL), and warmed to rt. After evaporation of the ammonia, the mixture was concentrated in vacuo and desalted using size-exclusion chromatography (HW40, eluted with Et_3NHOAc). The crude zwitterionic oligosaccharide was redissolved in H_2O/THF (0.01 M, 10/1, v/v). Ac_2O (5 equiv per free amine) was added, and the pH was adjusted to ~9 by the addition of solid $NaHCO_3$. After being stirred for 1 h, the mixture was neutralized by the addition of 1 M HCl. After concentration in vacuo, the crude product was purified by size-exclusion chromatography (HW40, eluted with Et_3NHOAc).

Methyl 6-*O*-(4-*O*-[α-D-Glucopyranosyl]-2-acetamido-2-deoxy-*β*-D-mannopyranosyl uronate)-α-D-glucopyranoside (27). Compound **23** (99 mg, 84 μmol) was deprotected using the general protocol for Birch reduction and subsequent acetylation to yield compound **27** as a white amorphous solid (24.2 mg, 36 μmol, 43%): IR (neat, cm^{-1}) 619, 1132, 1406, 1558, 2340, 3298; ^{1}H NMR (D_2O, 600 MHz, T = 288 K, HH−COSY, HSQC) δ 5.34 (d, 1H, J = 3.9 Hz, H-1″), 4.75 (s, 1H, H-1′), 4.68 (d, 1H, J = 3.7 Hz, H-1), 4.39 (d, 1H, J = 4.1 Hz, H-2′), 4.04 (d, 1H, J = 10.2 Hz, H-6), 4.00 (dd, 1H, J = 4.3, 9.6 Hz, H-3′), 3.81 (t, 1H, J = 9.6 Hz, H-4′), 3.66−3.74 (m, 5H, H-5, H-5′, H-6, H-6″, H-6″), 3.58−3.63 (m, 2H, H-3″, H-5″), 3.56 (t, 1H, J = 9.4 Hz, H-3), 3.46 (dd, 1H, J = 3.8, 9.8 Hz, H-2), 3.42 (dd, 1H, J = 3.9, 9.9 Hz, H-2″), 3.33 (t, 1H, J = 9.8 Hz, H-4″), 3.31 (s, 3H, CH_3 OMe), 3.29 (t, 1H, J = 9.4 Hz, H-4), 2.00 (s, 3H, CH_3 NHAc); ^{13}C-APT NMR (D_2O, 150 MHz, T = 288K, HSQC) δ 176.4, 176.2 (C=O NHAc, CO_2H), 100.5 (C-1′), 99.9 (C-1), 99.1 (C-1″), 78.0 (C-5′), 74.4 (C-4′), 73.8 (C-3), 73.5 (C-3″), 73.3 (C-3′), 72.5 (C-5″), 72.4 (C-2″), 72.0 (C-2), 71.1 (C-5), 70.3 (C-4), 69.9 (C-4″), 69.6 (C-6), 60.7 (C-6″), 55.7 (OMe), 54.4 (C-2′), 22.8 (CH_3 NHAc); ^{13}C-HMBC (D_2O, 150 MHz, T = 288K) δ 100.5 ($J_{C1,H1}$ = 163 Hz, C-1′), 99.9 ($J_{C1,H1}$ = 170 Hz, C-1), 99.1 ($J_{C1,H1}$ = 173 Hz, C-1″); HRMS $[M + H]^+$ calcd for $C_{21}H_{36}NO_{17}$ 574.1978, found 574.1975.

Methyl 6-*O*-(4-*O*-[6-*O*-[4-*O*-(α-D-Glucopyranosyl)-2-acetamido-2-deoxy-*β*-D-mannopyranosyl uronate]-α-D-glucopyranosyl]-2-acetamido-2-deoxy-*β*-D-mannopyranosyl uronate)-α-D-glucopyranoside (29). Compound **24** (63 mg, 33 μmol) was deprotected using the general protocol for Birch reduction and subsequent acetylation to yield compound **29** as a white amorphous solid (13.2 mg, 11.4 μmol, 35%): IR (neat, cm^{-1}) 1034, 1369, 1603, 3285; ^{1}H NMR (D_2O, 600 MHz, T = 280K, HH−COSY, HSQC) δ 5.34 (d, 1H, J = 3.8 Hz, H-1$_{Glc}$), 5.30 (d, 1H, J = 3.7 Hz, H-1$_{Glc}$), 4.77 (s, 2H, H-1$_{Man}$, H-1$_{Man}$), 4.68 (d, 1H, J = 3.6 Hz, H-1$_{Glc}$), 4.38−4.43 (m, 2H, H-2$_{Man}$, H-2$_{Man}$), 4.04 (d, 1H, J = 10.7 Hz, H-6$_{Glc}$), 3.99−4.02 (m, 2H, H-3$_{Man}$, H-3$_{Man}$), 3.93 (d, 1H, J = 10.8 Hz, H-6$_{Glc}$), 3.78−3.85 (m, 5H, H-4$_{Man}$, H-4$_{Man}$, H-5$_{Man}$, H-5$_{Man}$, H-6$_{Glc}$), 3.65−3.75 (m, 4H, H-5$_{Glc}$, H-6$_{Glc}$, H-6$_{Glc}$, H-6$_{Glc}$), 3.60−3.65 (m, 1H, H-5$_{Glc}$), 3.53−3.60 (m, 4H, H-3$_{Glc}$, H-3$_{Glc}$, H-3$_{Glc}$, H-5$_{Glc}$), 3.45 (dd, 1H, J = 3.8, 9.9 Hz, H-2$_{Glc}$), 3.42 (dd, 1H, J = 3.9, 10.0 Hz, H-2$_{Glc}$), 3.38 (dd, 1H, J = 4.2, 9.7 Hz, H-2$_{Glc}$), 3.31−3.37 (m, 2H, H-4$_{Glc}$, H-4$_{Glc}$), 3.30 (s, 3H, CH_3 OMe), 3.28 (t, 1H, J = 9.5 Hz, H-4$_{Glc}$), 2.01 (s, 3H, CH_3 NHAc), 2.00 (s, 3H, CH_3 NHAc); ^{13}C-APT NMR (D_2O, 150 MHz, T = 280K, HSQC) δ 176.2, 176.2, 175.6, 175.2 (C=O NHAc, CO_2H), 100.5, 100.5 (C-1$_{Man}$), 99.8, 99.2, 99.2 (C-1$_{Glc}$), 77.0, 76.9 (C-5$_{Man}$), 74.4, 74.3 (C-4$_{Man}$), 73.7, 73.3, 73.2 (C-3$_{Glc}$), 73.0, 73.0 (C-3$_{Man}$), 72.5 (C-5$_{Glc}$), 72.2, 72.1, 71.9 (C-2$_{Glc}$), 71.5, 71.0 (C-5$_{Glc}$), 70.1, 69.7 (C-4$_{Glc}$), 69.6 (C-6$_{Glc}$), 69.3 (C-4$_{Glc}$), 68.7, 60.5 (C-6$_{Glc}$), 55.6 (OMe), 54.1, 54.1 (C-2$_{Man}$), 22.8, 22.7 (CH_3 NHAc); ^{13}C-HMBC (D_2O, 150 MHz, T = 280 K) δ 100.5 ($J_{C1,H1}$ = 163 Hz, C-1$_{Man}$), 100.5 ($J_{C1,H1}$ = 163 Hz, C-1$_{Man}$), 99.8 ($J_{C1,H1}$ = 171 Hz, C-1$_{Glc}$), 99.2 ($J_{C1,H1}$ = 176 Hz, C-1$_{Glc}$), 99.2 ($J_{C1,H1}$ = 174 Hz, C-1); HRMS $[M + H]^+$ calcd for $C_{35}H_{57}N_2O_{28}$ 953.30924, found 953.31039.

Methyl 6-*O*-(4-*O*-[6-*O*-[4-*O*-(6-*O*-[4-*O*-[α-D-glucopyranosyl]-2-acetamido-2-deoxy-*β*-D-mannopyranosyl uronate]-α-D-glucopyranosyl)-2-acetamido-2-deoxy-*β*-D-mannopyranosyl uronate]-α-D-glucopyranosyl]-2-acetamido-2-deoxy-*β*-D-mannopyranosyl uronate)-α-D-glucopyranoside (31). Compound **25** (32 mg, 12 μmol) was deprotected using the general protocol for Birch reduction and subsequent acetylation to yield compound **31** as a white amorphous solid (2.8 mg, 1.7 μmol, 14%): ^{1}H NMR (D_2O, 600 MHz, T = 280 K, HH−COSY, HSQC) δ 5.35 (d, 1H, J = 3.9 Hz, H-1$_{Glc}$), 5.33 (d, 1H, J = 4.1 Hz, H-1$_{Glc}$), 5.32 (d, 1H, J = 4.1 Hz, H-1$_{Glc}$), 4.75 (s, 1H, H-1$_{Man}$), 4.73 (s, 2H, H-1$_{Man}$, H-1$_{Man}$), 4.67 (d, 1H, J = 3.7 Hz, H-1$_{Glc}$), 4.35−4.40 (m, 3H, H-2$_{Man}$, H-2$_{Man}$, H-2$_{Man}$), 3.96−4.05 (m, 4H, H-3$_{Man}$, H-3$_{Man}$, H-3$_{Man}$, H-6$_{Glc}$), 3.87−3.93 (m, 2H, H-6$_{Glc}$, H-6$_{Glc}$), 3.83−3.87 (m, 2H, H-6$_{Glc}$, H-6$_{Glc}$), 3.76−3.87 (m, 3H, H-4$_{Man}$, H-4$_{Man}$, H-4$_{Man}$), 3.63−3.75 (m, 9H, H-5$_{Man}$, H-5$_{Man}$, H-5$_{Man}$, H-5$_{Glc}$, H-5$_{Glc}$, H-5$_{Glc}$, H-6$_{Glc}$, H-6$_{Glc}$, H-6$_{Glc}$), 3.53−3.63 (m, 5H, H-3$_{Glc}$, H-3$_{Glc}$, H-3$_{Glc}$, H-3$_{Glc}$, H-5$_{Glc}$), 3.43−3.47 (m, 2H, H-2$_{Glc}$, H-2$_{Glc}$), 3.40 (dd, 1H, J = 4.0, 10.0 Hz, H-2$_{Glc}$), 3.34−3.39 (m, 3H, H-2$_{Glc}$, H-4$_{Glc}$, H-4$_{Glc}$), 3.31−3.33 (m, 1H, H-4$_{Glc}$), 3.30 (s, 3H, CH_3 OMe), 3.28 (t, 1H, J = 9.5 Hz, H-4$_{Glc}$), 2.00 (s, 6H, CH_3 NHAc), 1.99 (s, 3H, CH_3 NHAc); ^{13}C-APT NMR (D_2O, 150 MHz, T = 280 K, HSQC) δ 176.4, 176.3, 176.3, 176.2, 176.2 (C=O NHAc, CO_2H), 100.5, 100.4 (C-1$_{Man}$), 99.8 (C-1$_{Glc}$), 98.9, 98.9, 98.8 (C-1$_{Glc}$), 77.9, 77.8, 77.8 (C-5$_{Man}$), 74.1, 73.9, 73.9 (C-4$_{Man}$), 73.7, 73.4, 73.3, 73.2 (C-3$_{Man}$, C-3$_{Glc}$), 72.3 (C-5Glc), 72.3, 72.2, 71.9 (C-2$_{Glc}$), 71.3, 71.3, 71.0 (C-5$_{Glc}$), 70.1, 69.8 (C-4$_{Glc}$), 69.5 (C-6$_{Glc}$), 69.2, 69.1 (C-4$_{Glc}$), 68.6, 68.6, 60.5 (C-6$_{Glc}$), 55.6 (OMe), 54.5, 54.4, 54.4 (C-2$_{Man}$), 22.8, 22.7, 22.7 (CH_3 NHAc); HRMS $[M + Na]^+$ calcd for $C_{49}H_{77}N_3O_{39}Na$ 1354.4026, found 1354.4035.

Methyl 6-*O*-(4-*O*-[α-D-Glucopyranosyl]-2-deoxy-*β*-D-mannopyranosylurono-6,2-lactam)-α-D-glucopyranoside (33). Compound **23** (13.7 mg, 11.6 μmol) was dissolved in pyridine/H_2O (2 mL, 3/1, v/v), and the resulting solution was purged with H_2S for 10 min at rt. The three-necked flask was stoppered and

stirred overnight. Then the solution was again purged with H_2S for 10 min and stirred overnight, after which time the mixture was transferred with toluene/EtOAc, concentrated in vacuo, and co-concentrated with toluene (3×) to remove any traces of pyridine/H_2O. Product **32** was used crude in the next reaction step. Analytical data are reported for the crude lactam intermediate **32**: IR (neat, cm^{-1}) 698, 1028, 1070, 1454, 1705, 2855, 2922; ^1H NMR ($CDCl_3$, 400 MHz, HH−COSY, HSQC) δ 6.34 (bs, 1H, NH), 4.89 (m, 1H, H-1), 4.55 (m, 1H, H-1″), 4.54 (m, 1H, H-1′); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC) δ 175.6 (C═O NHCO), 98.4 (C-1), 97.7 (C-1″), 97.1 (C-1′), 54.5 (C-2′); ^{13}C-HMBC ($CDCl_3$, 150 MHz) δ 98.4 ($J_{C1,H1}$ = 166 Hz, C-1), 97.7 ($J_{C1,H1}$ = 169 Hz, C-1″), 97.1 ($J_{C1,H1}$ = 173 Hz, C-1′); HRMS $[M + NH_4]^+$ calcd for $C_{68}H_{73}N_1O_{15}$ 1161.53185, found 1161.53286. Compound **32** was coevaporated with toluene (2×) and transferred to a three-necked flask using freshly distilled THF (3 mL). *t*-BuOH (30 μL) was added, and the solution was cooled to −60 °C. A piece of Na was added, and liquid NH_3 (~5 mL) was collected. When the blue color disappeared an extra piece of Na was added. The blue solution was stirred at −50 °C for 15 min and quenched with AcOH. After evaporation of the NH_3, the solution was transferred with H_2O and concentrated in vacuo. Purification using gel filtration (HW-40, eluted with NH_4HCO_3) afforded the title compound as a white solid (4.2 mg, 8.1 μmol, 70% over two steps): ^1H NMR (D_2O, 600 MHz, T = 290K, HH−COSY, HSQC) δ 5.14 (d, 1H, J = 0.7 Hz, H-1′), 5.08 (d, 1H, J = 3.8 Hz, H-1″), 4.71 (d, 1H, J = 3.8 Hz, H-1), 4.44 (d, 1H, J = 1.5 Hz, H-5′), 4.01 (d, 1H, J = 11.1 Hz, H-6), 3.92 (s, 2H, H-2′, H-3′), 3.69−3.82 (m, 6H, H-4′, H-5, H-5″, H-6, H-6″, H-6″), 3.67 (t, 1H, J = 9.6 Hz, H-3″), 3.58 (t, 1H, J = 9.4 Hz, H-3), 3.52 (dd, 1H, J = 3.8, 9.9 Hz, H-2″), 3.49 (dd, 1H, J = 3.8 Hz, H-2), 3.38 (t, 1H, J = 9.5 Hz, H-4″), 3.33 (s, 3H, OMe), 3.28 (t, 1H, J = 9.4 Hz, H-4); ^{13}C-APT NMR (D_2O, 150 MHz, T = 290K, HSQC) δ 173.0 (C═O CONH), 100.0 (C-1), 99.3 (C-1″), 98.7 (C-1′), 81.9 (C-4′), 76.0 (C-5′), 73.8 (C-3), 73.6 (C-3″), 73.2 (C-5″), 72.1 (C-2″), 72.0 (C-2), 71.5 (C-5), 71.2 (C-3′), 70.6 (C-4), 70.1 (C-4″), 68.6 (C-6), 61.1 (C-6″), 56.7 (C-2′), 55.8 (OMe); ^{13}C-HMBC (D_2O, 150 MHz, T = 290 K) δ 100.0 ($J_{C1,H1}$ = 170 Hz, C-1), 99.3 ($J_{C1,H1}$ = 170 Hz, C-1″), 98.7 ($J_{C1,H1}$ = 175 Hz, C-1′);[51−53] HRMS $[M + NH_4]^+$ calcd for $C_{19}H_{35}N_2O_{15}$ 531.20319, found 531.20313.

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Supporting Information Available: Experimental data for donors **1**−**7**, spectroscopic data of all compounds, and NMR spectra of the low-temperature experiments. This material is available free of charge via the Internet at http://pubs.acs.org.

(51) The conformational restriction of the lactam in mannopyranoside **33** forces the ring in a boat-like conformation, resulting in a parallel orientation of the axial C1−H1 bond with respect to the ring oxygen lone pair.[52] The large $J_{C1,H1}$ coupling constant of 175 Hz of compound **33** is analogous to the coupling constant observed with β-mannofuranosides ($J_{C1,H1}$ = 175 Hz),[53] which also place the C1−H1 bond parallel to the ring oxygen lone pair.

(52) Kalinowski, H.-O.; Berger, S.; Braun, S. *Carbon-13 NMR Spectroscopy*; John Wiley & Sons: New York, 1988; pp 508−509.

(53) Cyr, N.; Perlin, A. S. *Can. J. Chem.* **1979**, *57*, 2504–2511.