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An efficient synthesis of a hexasaccharide—the repeating unit of the exopolysaccharide from *Cryptococcus neoformans* serovar A

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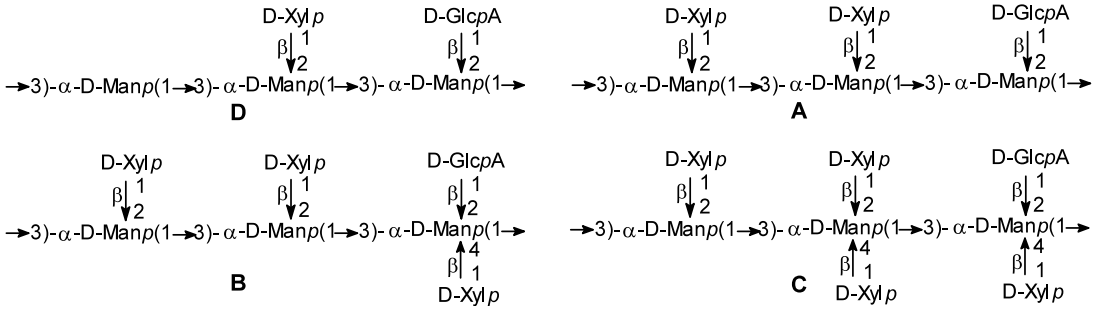
Abstract—A general method for the synthesis of 2-xylose or glucuronic acid branched (1→3)-linked mannose oligosaccharides has been developed. As a typical example, the synthesis of the methyl glycoside of β-D-Glc*p*A-(1→2)-α-D-Man*p*-(1→3)-[β-D-Xyl*p*-(1→2)-]α-D-Man*p*-(1→3)-[β-D-Xyl*p*-(1→2)-]α-D-Man*p*, the repeating unit of the exopolysaccharide from *Cryptococcus neoformans* serovar A, was achieved in a regio- and stereoselective manner.

Cryptococcus neoformans has been a primary cause of opportunistic infections in patients diagnosed with AIDS.[1] The yeast *C. neoformans* is a pulmonary pathogen and can disseminate to the central nervous system causing meningoencephalitis.[2] *C. neoformans* produces large amounts of a polysaccharide composed of mannose, xylose, and glucuronic acid. This polysaccharide, termed glucuronoxylomannan (GXM),[3] is the major constituent of the cryptococcal capsule.[4]

GXM occurs as four major serotypes,[5] designated A–D (Scheme 1). All the four serotypes have a linear α-1,3-linked mannosyl backbone with β-glucopyranosyluronic acid and β-xylopyranosyl substituents.[6–9] In addition, the backbone is substituted with variable amounts of 6-*O*-acetyl groups, with serotype D being the most heavily *O*-acetylated and serotype C the least *O*-acetylated.[10] However, the *O*-acetyl groups are not essential for binding, though they have a significant contribution.[3]

Synthesis of the repeating units of the polysaccharide from *C. neoformans* serotypes A–D is of interest since it can afford synthetic samples in sufficient amounts for studies on the relationship of oligosaccharide structure-bioactivity. However, this synthesis is not easy because of the relatively poor reactivity of the 2-axial hydroxy group of mannose, and the steric hindrance caused by the 2,3-substitution of the mannose residues. The synthesis of trisaccharide and tetrasaccharide fragments corresponding to structures in capsular polysaccharides of *C. neoformans* has been reported[11,12] and the synthesis of a pentasaccharide—the repeating unit of the polysaccharide in *C. neoformans* serovar D has appeared.[13] In these syntheses, multiple steps and orthogonal masking groups were involved making the



Scheme 1. Model structures of GXM of *C. neoformans* serotypes A–D.

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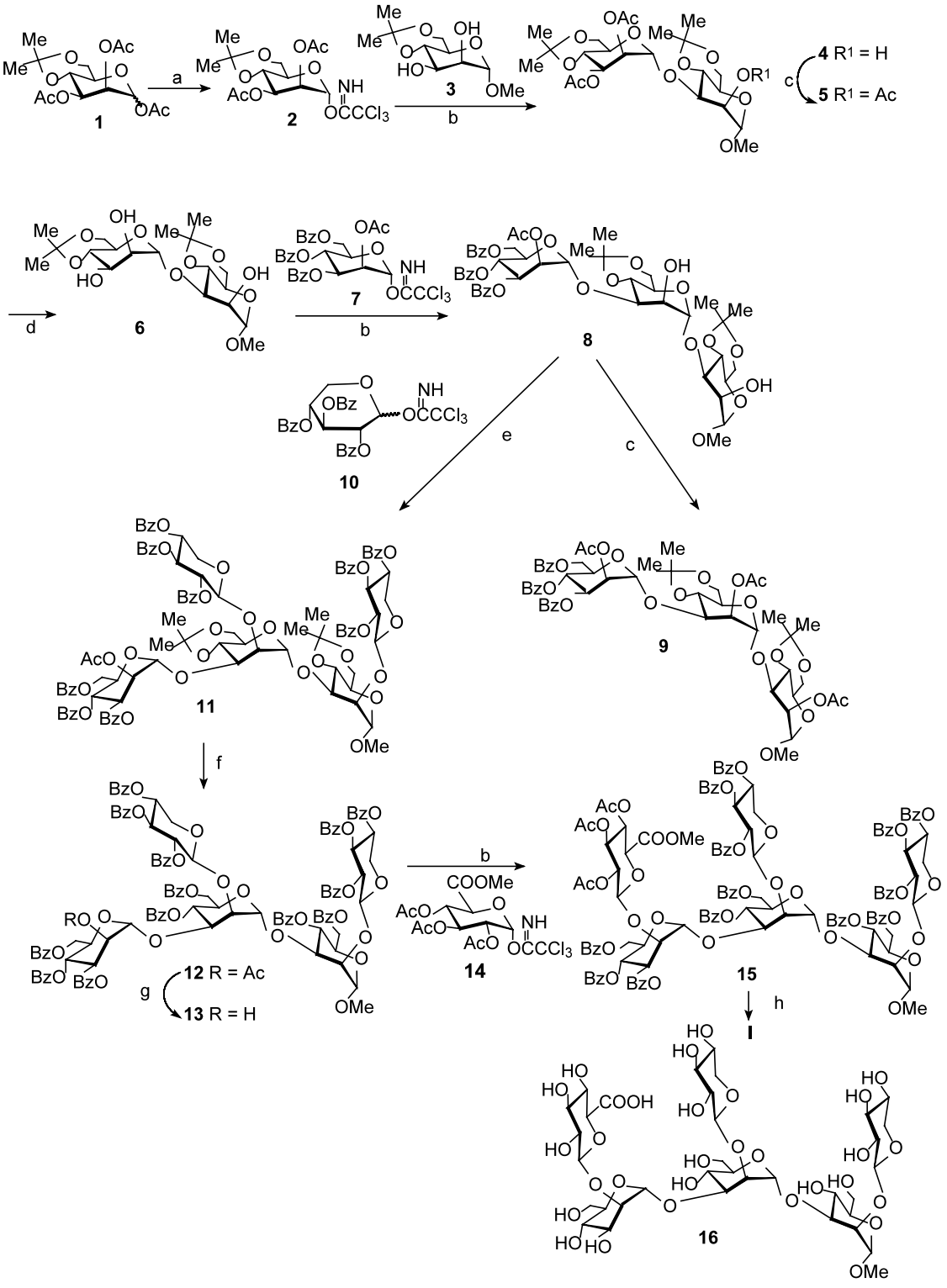

Scheme 2. *Reagents and conditions*: (a) (i) THF–CH_3OH, 1.5 N NH_3, rt, 2–3 h; (ii) CH_2Cl_2, CCl_3CN (2.0 equiv.), K_2CO_3 (2.0 equiv.), rt, 12 h, 71%; (b) TMSOTf (0.01 equiv.), 4 Å MS, CH_2Cl_2, −20°C, 2–4 h (74% for **4**, 67% for **8**, 88% for **15**); (c) Ac_2O–pyridine, 100%; (d) CH_3OH saturated with ammonia, rt, 12 h, 100%; (e) **10** (2 equiv.), TMSOTf (0.1 equiv.), 4 Å MS, CH_2Cl_2, −0°C, 0.5 h; then TMSOTf (1.0 equiv.), 0.5 h, and further **10** (2 equiv.) was added, 42%; (f) (i) 90% HOAc, 60°C, 20 h; (ii) BzCl–pyridine, rt, 10 h, 91%; (g) 2% CH_3COCl in CH_2Cl_2–CH_3OH, rt, 20 h, 67%; (h) methanol saturated with ammonia, rt, 36 h, then water (2 equiv.) was added, 5 h, 65%.

procedure rather complex. As a result, it would be difficult to synthesize by the reported methods, the higher oligosaccharides—the repeating units of *C. neoformans* serotypes A, B, and C. Previously, we have reported the regio- and stereoselective synthesis of oligosaccharides with un- or lightly protected mannose[14] and rhamnose[15] as the glycosyl acceptors and glycosyl trichloroacetimidates as the donors giving satisfactory results. We present herein, for the first time, the regio- and stereoselective synthesis of a frame-shifted hexasaccharide repeating unit[16] **I** (structure **16** in Scheme 2) of *O*-deacetylated GXM of *C. neoformans* serotype A with lightly protected mannose derivatives as the acceptors.

As shown in Scheme 2, 1,2,3-tri-*O*-acetyl-4,6-*O*-isopropylidene-D-mannopyranose **1**, obtained from selective 4,6-*O*-isopropylidenation of mannose[17] with 2-methoxypropene followed by acetylation, was chosen as the starting material. Selective 1-*O*-deacetylation with ammonia in THF–MeOH followed by trichloroacetimidation[18] with trichloroacetonitrile in the presence of potassium carbonate gave the donor 2,3-di-*O*-acetyl-4,6-*O*-isopropylidene-α-D-mannopyranosyl trichloroacetimidate **2**. Condensation of **2** with the acceptor methyl 4,6-*O*-isopropylidene-α-D-mannopyranoside **3** selectively afforded the (1→3)-linked disaccharide **4** (74%). The regioselectivity of the coupling was confirmed by acetylation of **4** to give **5**, and the ^{1}H NMR spectrum of **5** showed a newly emerged downfield doublet of doublets at δ 5.34 ppm with $J_{1,2}=1.5$ and $J_{2,3}=3.0$ Hz for H-2 compared to that of **4**. Deacetylation of **4** or **5** in a solution of ammonia in methanol furnished the disaccharide triol acceptor **6** quantitatively. Again, 3-*O*-selective glycosylation of **6** with the donor 2-*O*-acetyl-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl trichloroacetimidate **7** yielded (1→3)-linked trisaccharide **8** (67%). Confirmation of the 3-regioselectivity was carried out by acetylation of **8** to give the trisaccharide **9** which showed two characteristic signals at δ 5.40 ppm with $J_{1,2}=1.3$, $J_{2,3}=3.4$ Hz for H-2, and 5.22 ppm with $J_{1,2}=1.3$, $J_{2,3}=2.9$ Hz for H-2′, respectively. The trisaccharide **8** was an ideal acceptor since it contained two free hydroxyl groups at the positions where the xylosyl residue should be attached. Thus, TMSOTf promoted xylosylation of **8** with 2,3,4-tri-*O*-benzoyl-D-xylopyranosyl trichloroacetimidate **10** was carried out. It was noted that at the initial stage of the coupling, formation of the tetrasaccharide intermediate was quite fast and little pentasaccharide was obtained. The quantity of pentasaccharide did not increase along with the reaction time. For completion of the reaction, an 'inverse Schmidt' strategy was used. Thus, further TMSOTf was added, and after stirring the reaction mixture for 20 min, further 2 equiv. of donor **10** was added giving the pentasaccharide **11** in fair yield (42%). De-isopropylidenation of **11** in 60% aqueous acetic acid at 60°C followed by benzoylation with benzoyl chloride in pyridine gave the protected pentasaccharide **12**. Selective deacetylation of **12** with 2% acetyl chloride–methanol[19] was accompanied by some decomposition, perhaps caused by breaking of xylosyl linkage, giving the pentasaccharide acceptor **13** in 67% yield. Coupling of **13** with methyl 2,3,4-tri-*O*-acetyl-α-D-glucopyranosyluronate trichloroacetimidate **14** went smoothly affording the protected hexasaccharide in good yield (88%). The ^{1}H NMR spectrum of **15** showed two methyl signals (δ 3.69 ppm and 3.23 ppm, respectively), thirteen benzoyl C=O signals (δ 165.9, 165.9, 165.9, 165.4, 165.4, 165.4, 165.3, 165.2, 165.1, 165.0, 164.9, 164.6, 164.6 ppm), four C=O signals (δ 167.0, 168.5, 168.5, 168.3 ppm), six anomeric C signals (100.9, $J_{C1,H1}=175$ Hz, Man*p*; 100.3, $J_{C1,H1}=163$ Hz, GluA*p*; 99.9, $J_{C1,H1}=164$ Hz, Xyl*p*; 99.5, $J_{C1,H1}=163$ Hz, Xyl*p*; 98.5, $J_{C1,H1}=172$ Hz, Man*p*; 95.2, $J_{C1,H1}=176$ Hz, Man*p*).[20] Deprotection of **15** was carried out in a saturated solution of ammonia in methanol for 36 h, then water (2 equiv.) was added to cleave the methyl ester. After standing at room temperature for 5 h, the reaction mixture was concentrated and purified on a Bio-Gel P2 column (eluent: water), affording the target hexasaccharide **16** as a foamy solid.

In summary, an efficient synthesis of a hexasaccharide repeat unit of *O*-deacetylated GXM of *C. neoformans* serotype A with lightly protected mannose derivatives as the acceptors was achieved. The strategy presented here also provides a route to the synthesis of more complex repeating units of GXM of *C. neoformans* serotype B and C.

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20. All new compounds gave satisfactory elemental analysis results. Selected physical data for some key compounds are as follows, for **8**: $[\alpha]_D$ +91.7 (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz): δ 7.95–7.34 (m, 15 H, 3 Ph*H*), 5.94 (dd, 1 H, $J_{3,4}=J_{4,5}=10.0$ Hz, H-4), 5.75 (dd, 1 H, $J_{2,3}=3.3$ Hz, $J_{3,4}=10.0$ Hz, H-3), 5.53 (dd, 1 H, $J_{1,2}=1.8$ Hz, $J_{2,3}=3.3$ Hz, H-2), 5.43 (d, 1 H, $J_{1,2}=1.8$ Hz, H-1), 5.31 (d, 1 H, $J_{1,2}=1.0$ Hz, H-1), 4.71 (d, 1 H, $J_{2,1}=1.0$ Hz, H-1), 4.66–4.58 (m, 3 H), 4.26–4.18 (m, 4 H), 4.04–3.99 (m, 2 H), 3.90–3.82 (m, 4 H), 3.68–3.63 (m, 2 H), 3.36 (s, 3 H, OC*H*$_3$), 2.17 (s, 3 H, COC*H*$_3$), 1.56, 1.45 (2s, 6 H, isopropylidene), 1.37 (s, 6 H, isopropylidene). ^{13}C NMR (100 MHz, $CDCl_3$): 169.5 (*C*OCH$_3$), 166.3, 165.6, 163.6 (5 C, 5 *C*OPh), 101.2, 100.8 (2 C, 2 Me$_2$*C*), 99.9, 99.4, 98.3 (3 C, 3 C-1), 54.8 (O*C*H$_3$), 29.1, 28.9 (2 C, *C*H$_3$C*C*H$_3$), 20.6 (CO*C*H$_3$), 19.2, 19.0 (2 C, *C*H$_3$C*C*H$_3$). Anal. calcd for $C_{48}H_{56}O_{20}$: C, 60.50; H, 5.92. Found: C, 60.45; H, 5.66. For **9**: $[\alpha]_D$ +82.6 (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz): δ 8.10–7.23 (m, 15 H, 3 Ph*H*), 6.04 (dd, 1 H, $J_{3,4}=J_{4,5}=10.0$ Hz, H-4), 5.64 (dd, 1 H, $J_{2,3}=3.2$ Hz, $J_{3,4}=10.0$ Hz, H-3), 5.45 (dd, 1 H, $J_{1,2}=2.0$ Hz, $J_{2,3}=2.9$ Hz, H-2), 5.40 (dd, 1 H, $J_{1,2}=1.3$ Hz, $J_{2,3}=3.4$ Hz, H-2), 5.32 (d, 1 H, $J_{1,2}=1.7$ Hz, H-1), 5.22 (dd, 1 H, $J_{1,2}=1.3$ Hz, $J_{2,3}=2.9$ Hz, H-2), 5.10 (d, 1 H, $J_{1,2}=1.2$ Hz, H-1), 4.67 (dd, 1 H, $J_{2,3}=2.9$ Hz, $J_{3,4}=12.1$ Hz, H-3), 4.63 (d, 1 H, $J_{1,2}=1.2$ Hz, H-1), 4.46–4.38 (m, 2 H), 4.09–4.05 (m, 4 H), 3.87–3.81 (m, 4 H), 3.70–3.60 (m, 2 H), 3.36 (s, 3 H, OC*H*$_3$), 2.31, 2.18, 2.07 (3s, 9 H, 3 COC*H*$_3$), 1.56, 1.52 (2s, 6H, isopropylidene), 1.37 (s, 6H, isopropylidene). Anal. calcd for $C_{52}H_{60}O_{22}$: C, 60.22; H, 5.83. Found: C, 60.47; H, 5.61. For **12**: $[\alpha]_D$ −58.6 (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz): δ 8.11–7.34 (m, 65 H, 13 Ph*H*), 5.94 (dd, 1 H, $J_{3,4}=J_{4,5}=10.0$ Hz, H-4, Man*p*), 5.77 (dd, 1 H, $J_{2,3}=3.2$ Hz, $J_{3,4}=10.0$ Hz, H-3, Man*p*), 5.71 (dd, $J_{1,2}=J_{2,3}=6.3$ Hz, H-2, Xyl*p*), 5.59 (dd, 1 H, $J_{3,4}=J_{4,5}=10.0$ Hz, H-4, Man*p*), 5.54 (dd, 1 H, $J_{3,4}=J_{4,5}=9.9$ Hz, H-4, Man*p*), 5.47–5.40 (m, 3 H), 5.31–5.27 (m, 2 H), 5.18 (d, 1 H, $J_{1,2}=0.7$ Hz, H-1, Man*p*), 5.16 (dd, 1 H, $J_{1,2}=1.0$ Hz, $J_{2,3}=3.0$ Hz, H-2, Man*p*), 4.99 (d, 1 H, $J_{1,2}=0.8$ Hz, H-1, Man*p*), 4.80 (d, 1 H, $J_{1,2}=4.8$ Hz, H-1, Xyl*p*), 4.53 (d, 1 H, $J_{1,2}=1.0$ Hz, H-1, Man*p*), 4.45 (d, 1 H, $J_{1,2}=6.1$ Hz, H-1, Xyl*p*), 3.19 (s, 3 H, OC*H*$_3$), 1.90 (s, 3 H, COC*H*$_3$). ^{13}C NMR (100 MHz, $CDCI_3$): 168.9 (*C*OCH$_3$), 166.0, 165.9, 165.8, 165.4, 165.3, 165.3, 165.3, 165.2, 165.1, 165.0, 164.9, 164.7, 164.6 (13 C, 13 *C*OPh), 99.7, 99.7, 99.7, 99.0, 98.5 (5 C, 5 C-1), 54.8 (O*C*H$_3$), 20.3 (CO*C*H$_3$); Anal. calcd for $C_{122}H_{104}O_{38}$: C, 67.27; H, 4.81. Found: C, 67.45; H, 5.96. For **15**: $[\alpha]_D$ −22.5 (*c* 0.5, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz): δ 8.13–7.30 (m, 65 H, 13 Ph*H*), 6.00 (dd, 1 H, $J_{3,4}=J_{4,5}=10.0$ Hz, H-4, Man*p*), 5.71 (dd, 1 H, $J_{2,3}=3.2$ Hz, $J_{3,4}=10.0$ Hz, H-3, Man*p*), 5.15 (d, 1 H, $J_{1,2}=1.0$ Hz, H-1, Man*p*), 4.96 (d, 1 H, $J_{1,2}=0.9$ Hz, H-1, Man*p*), 4.75 (d, 1 H, $J_{1,2}=4.9$ Hz, H-1, Xyl*p*), 4.60 (d, 1 H, $J_{1,2}=4.8$ Hz, H-1, Xyl*p*), 4.56 (d, 1 H, $J_{1,2}=1.0$ Hz, H-1, Man*p*), 4.04 (d, 1 H, $J_{1,2}=6.6$ Hz, H-1, GluA*p*), 3.69 (s, 3 H, COOC*H*$_3$), 3.23 (s, 3 H, OC*H*$_3$), 1.96, 1.92, 1.31 (3s, 9 H, 3 COC*H*$_3$). ^{13}C NMR (100 MHz, $CDCl_3$): 167.0, 168.5, 168.5, 168.3 (3 C, 3 *C*OCH$_3$, *C*OOMe), 165.9, 165.9, 165.9, 165.4, 165.4, 165.4, 165.3, 165.2, 165.1, 165.0, 164.9, 164.6, 164.6 (13 C, 13 *C*OPh), 100.9 (C-1, $J_{C1,H1}=175$ Hz, Man*p*), 100.3 (C-1, $J_{C1,H1}=163$ Hz, GluA*p*), 99.9 (C-1, $J_{C1,H1}=164$ Hz, Xyl*p*), 99.5 (C-1, $J_{C1,H1}=163$ Hz, Xyl*p*), 98.5 (C-1, $J_{C1,H1}=172$ Hz, Man*p*), 95.2 (C-1, $J_{C1,H1}=176$ Hz, Man*p*), 54.8 (O*C*H$_3$), 52.3 (COO*C*H$_3$), 20.5, 20.3, 20.2 (CO*C*H$_3$); Anal. Calcd for $C_{133}H_{118}O_{46}$: C, 65.13; H, 4.85. Found: C, 65.45; H, 4.66. For **16**: $[\alpha]_D$ +99.6 (*c* 0.5, H_2O); ^1H NMR (D_2O, 400 MHz): δ 5.11 (s, 1 H, H-1, Man*p*), 4.75 (s, 1 H, H-1, Man*p*), 4.31 (s, 1 H, H-1, Man*p*), 4.29 (d, 1 H, $J_{1,2}=8.0$ Hz, H-1, GluA*p*), 4.12 (d, 1 H, $J_{1,2}=8.9$ Hz, H-1, Xyl*p*), 4.10 (d, 1 H, $J_{1,2}=9.2$ Hz, H-1, Xyl*p*), 3.33 (s, 3 H, OC*H*$_3$); ^{13}C NMR (100 MHz, D_2O): 174.1 (–COONH$_4$), 103.4, 103.3, 103.2, 102.5, 100.3, 100.2 (6 C-1), 79.0, 78.5, 78.4, 78.3, 78.3, 76.4, 76.2, 75.9, 75.8, 73.6, 73.4, 73.2, 72.9, 72.9, 70.7, 70.3, 69.5, 69.5, 68.4, 67.0, 66.7, 66.5, 65.4, 65.3, 61.5, 60.6, 60.6, 56.6 (O-*C*H$_3$). MALDI–TOF MS calcd for the ammonium salt of **16**, $C_{35}H_{61}O_{30}N$: 975.8 [M]. found: 975.8 (M); 980.9 (M–NH$_4^+$+Na$^+$).