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Supplementary data

Novel Synthetic (1→6)-α-D-Mannodisaccharide Substrates Support Processive Mannosylation Catalysed by the Mycobacterial Cell Envelope Enzyme Fraction.

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Experimental

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Glycoside **1**,[1] **2**,[1] **3**,[1] or **4**[1] (3 mmol, 1 eq) was dissolved in pyridine (7 mL), and triphenylmethyl chloride (2 eq) was added. The solution was stirred at 55 °C for 24 h. After cooling, the pyridine was evaporated and the residue was co-evaporated with toluene (2 × 20 mL). The crude product was purified by column chromatography (hexane:EtOAc 5:1→0:1) to give 6-*O*-protected compouds **5-8** as an oil.

Octyl 6-*O*-triphenylmethyl-α-D-mannopyranoside (5). (1.22 g, 76%); $[\alpha]_D$ +19 (*c* 1, methanol); lit[2] $[\alpha]_D$ +16.9 (*c* 1.3, $CHCl_3$); ^{1}H NMR (300 MHz, CD_3OD): δ 7.49-7.46 (m, 5H, Ar), 7.30-7.19 (m, 10H, Ar), 4.79 (d, 1H, $J_{1,2}$ = 1.1 Hz, H-1), 3.95 (dt, 1H, *J*= 6.9 Hz, *J* = 9.4 Hz, OC*H*$_2$C$_7$H$_{15}$), 3.84-3.77 (m, 2H, H-2, H-5), 3.65 (dd, 1H, $J_{2,3}$ = 3.4 Hz, $J_{3,4}$ = 9.3 Hz, H-3), 3.54 (dt, 1H, *J*= 6.3 Hz, *J* = 9.5 Hz, OC*H*$_2$C$_7$H$_{15}$), 3.51-3.42 (m, 2H, H-4, H-6a), 3.24 (dd, 1H, $J_{5,6b}$ = 7.8 Hz, $J_{6a,6b}$ = 9.6 Hz, H-6b), 1.73-1.69 (m, 2H, OCH$_2$C*H*$_2$C$_6$H$_{13}$), 1.50-1.22 (m, 10H, O(CH$_2$)$_2$(C*H*$_2$)$_5$CH$_3$), 0.85 (t, 3H, *J* = 6.9 Hz, O(CH$_2$)$_7$C*H*$_3$).^{13}C NMR (75 MHz, CD_3OD): δ 145.7, 130.0, 128.7, 128.0 (Ar), 101.5 (C-1), 87.8 (*C*(Ph)$_3$), 73.8, 72.2 (C-2, C-5), 73.0 (C-3), 69.3 (C-4), 68.5 (O*C*H$_2$C$_7$H$_{15}$), 65.4 (C-6), 33.0, 30.8, 30.6, 30.5, 27.6, 23.8 (OCH$_2$(*C*H$_2$)$_6$CH$_3$),), 14.5 (O(CH$_2$)$_7$*C*H$_3$). HRMS (MALDI): *m/z* 557.2868 MNa$^+$; calcd 557.2879 for $C_{33}H_{42}O_6Na$.

Cyclohexylmethyl 6-*O*-triphenylmethyl-α-D-mannopyranoside (6). (1.27 g, 82%); $[\alpha]_D$ +20 (*c* 1, methanol); ^{1}H NMR (400 MHz, CD_3OD): δ 7.52-7.50 (m, 5H, Ar), 7.32-7.24 (m, 10H, Ar), 4.80 (d, 1H, $J_{1,2}$ = 1.3 Hz, H-1), 3.88-3.80 (m, 3H, H-2, H-5, OC*H*$_2$C$_6$H$_{11}$), 3.68 (dd, 1H, $J_{2,3}$ = 3.5 Hz, $J_{3,4}$ = 9.4 Hz, H-3), 3.48-3.43 (m, 2H, H-4, H-6a), 3.37 (dd, 1H, *J*= 5.9 Hz, *J* = 9.3 Hz, OC*H*$_2$C$_6$H$_{11}$), 3.26 (dd, 1H, $J_{5,6b}$ = 8.0 Hz, $J_{6a,6b}$ = 9.4 Hz, H-6b), 1.84-1.08 (m, 11H, OCH$_2$C$_6$*H*$_{11}$). ^{13}C NMR (100 MHz, CD_3OD): δ 145.8, 130.1, 128.8, 128.1 (Ar), 101.5 (C-1), 87.8 (*C*(Ph)$_3$), 74.0(2x), 72.3 (C-2, C-5, O*C*H$_2$C$_6$H$_{11}$), 73.2 (C-3), 69.4 (C-4), 65.5 (C-6), 39.3, 31.7, 31.4, 27.9, 27.2, 27.1 (OCH$_2$*C*$_6$H$_{11}$). HRMS (MALDI): *m/z* 541.2605 MNa$^+$; calcd 541.2566 for $C_{32}H_{38}O_6Na$.

2-Cyclohexylethyl 6-*O*-triphenylmethyl-α-D-mannopyranoside (7). (1.18 g, 74%); $[\alpha]_D$ +21 (*c* 1, methanol); ^{1}H NMR (600 MHz, CD_3OD): δ 7.51-7.49 (m, 5H, Ar), 7.31-7.23 (m,

10H, Ar), 4.81 (d, 1H, $J_{1,2}$ = 1.2 Hz, H-1), 4.04 (dt, 1H, *J*= 7.1 Hz, *J* = 9.5 Hz, OC*H*$_2$CH$_2$C$_6$H$_{11}$), 3.84-3.80 (m, 2H, H-2, H-5), 3.68 (dd, 1H, $J_{2,3}$ = 3.4 Hz, $J_{3,4}$ = 9.4 Hz, H-3), 3.57 (dt, 1H, *J*= 6.6 Hz, *J* = 9.6 Hz, OC*H*$_2$CH$_2$C$_6$H$_{11}$), 3.48-3.44 (m, 2H, H-4, H-6a), 3.25 (dd, 1H, $J_{5,6b}$ = 7.9 Hz, $J_{6a,6b}$ = 9.6 Hz, H-6b), 1.90-0.96 (m, 13 H, OCH$_2$C*H*$_2$C$_6$*H*$_{11}$).^{13}C NMR (150 MHz, CD$_3$OD): δ 145.8, 130.1, 128.8, 128.1 (Ar), 101.6 (C-1), 87.9 (*C*(Ph)$_3$), 74.0, 72.3 (C-2, C-5), 73.0 (C-3), 69.4 (C-4), 66.6 (OCH$_2$CH$_2$C$_6$H$_{11}$), 65.6 (C-6), 38.4, 36.3, 35.0, 34.6, 27.8, 27.6(2x) (OCH$_2$*C*H$_2$*C*$_6$H$_{11}$). HRMS (MALDI): *m/z* 555.2735 MNa$^+$; calcd 555.2723 for C$_{33}$H$_{40}$O$_6$Na.

Octyl 6-*O*-triphenylmethyl-1-thio-α-D-mannopyranoside (8). (1.21 g, 73%); [α]$_D$ +93 (*c* 1, methanol); ^{1}H NMR (400 MHz, CD$_3$OD): δ 7.51-7.48 (m, 6H, Ar), 7.32-7.22 (m, 9H, Ar), 5.33 (d, 1H, $J_{1,2}$ = 0.9 Hz, H-1), 4.20 (ddd, 1H, H-5), 3.93 (dd, 1H, $J_{2,3}$ = 3.4 Hz, H-2), 3.69 (dd, 1H, $J_{3,4}$ = 9.3 Hz, H-3), 3.54 (t, 1H, $J_{4,5}$ = 9.6 Hz, H-4), 3.48 (dd, 1H, $J_{5,6a}$ = 1.8 Hz, $J_{6a,6b}$ = 9.8 Hz, H-6a), 3.29 (dd, 1H, $J_{5,6b}$ = 7.5 Hz, H-6b), 2.88 (ddd, 1H, *J*= 6.3 Hz, *J*= 8.3 Hz, *J* = 12.9 Hz, SC*H*$_2$C$_7$H$_{15}$), 2.74 (ddd, 1H, *J*= 6.8 Hz, *J*= 8.4 Hz, *J* = 12.8 Hz, SC*H*$_2$C$_7$H$_{15}$), 1.80-1.71 (m, 2H, SCH$_2$C*H*$_2$C$_6$H$_{13}$), 1.46-1.40 (m, 2H, S(CH$_2$)$_2$C*H*$_2$C$_5$H$_{11}$), 1.33-1.19 (m, 8H, S(CH$_2$)$_3$(C*H*$_2$)$_4$CH$_3$), 0.86 (t, 3H, *J* = 7.0 Hz, S(CH$_2$)$_7$C*H*$_3$). ^{13}C NMR (100 MHz, CD$_3$OD): δ 145.7, 130.1, 128.8, 128.1 (Ar), 87.9 (C-1), 85.8 (*C*(Ph)$_3$), 74.1 (C-5), 73.6(2x) (C-2, C-3), 69.6 (C-4), 65.4 (C-6), 33.1, 31.6, 31.0, 30.6, 30.5, 30.3, 23.8 (S(*C*H$_2$)$_7$CH$_3$), 14.6 (S(CH$_2$)$_7$*C*H$_3$). HRMS (MALDI): *m/z* 573.2659 MNa$^+$; calcd 573.2651 for C$_{33}$H$_{42}$O$_5$SNa.

General procedure for the protection of secondary hydroxyl groups. Synthesis of compounds 9-12.

Partially protected mannopyranoside **5**, **6**, **7**, or **8** (1 mmol, 1 eq) was dissolved in DMF (7 mL), the solution was cooled to 0 °C, and sodium hydride (60% in mineral oil, 5 eq) was added during stirring. After 15 min, benzyl bromide (4 eq) was added and the resulting mixture was brought to rt and the stirring was continued for 16 h. The reaction was quenched with methanol (2-3 mL). The reaction mixture was diluted with CH_2Cl_2 (30 mL), washed with water (10 mL). The organic phase was separated and the aqueous phase was extracted with CH_2Cl_2 (3 × 10 mL). The combined organic phases were again washed with water (10 mL), dried (Na_2SO_4), filtered and concentrated. The crude product was purified by column chromatography (hexane:EtOAc 10:1→6:1) and desired products **9-12** were obtained in oil forms.

Octyl 2,3,4-tri-*O*-benzyl-6-*O*-triphenylmethyl-α-D-mannopyranoside (9). (0.67 g, 83%); $[\alpha]_D$ +15 (*c* 1, $CHCl_3$); lit[2] $[\alpha]_D$ +18.7 (*c* 0.3, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.57-7.16 (m, 28H, Ar), 6.88 (m, 2H, Ar), 4.90 (d, 1H, $J_{1,2}$ = 1.3 Hz, H-1), 4.82 (d, 1H, *J* = 12.5 Hz, PhCH_2), 4.73-4.65 (m, 4H, 2x PhCH_2), 4.26 (d, 1H, *J* = 10.4 Hz, PhCH_2), 4.00 (dd, 1H, $J_{3,4}$ = 9.5 Hz, $J_{4,5}$ = 9.5 Hz, H-4), 3.89 (dd, 1H, $J_{2,3}$ = 3.0 Hz, H-3), 3.82-3.79 (m, 2H, H-2, H-5), 3.74 (dt, 1H, *J* =6.9 Hz, *J* = 9.5 Hz, OC$H_2C_7H_{15}$), 3.51 (dd, 1H, $J_{5,6a}$ = 1.3 Hz, $J_{6a,6b}$ = 9.7 Hz, H-6a), 3.41 (dt, 1H, *J* =6.6 Hz, *J* = 9.5 Hz, OC$H_2C_7H_{15}$), 3.26 (dd, 1H, $J_{5,6b}$ = 5.3 Hz, H-6b), 1.59-1.50 (m, 2H, $OCH_2CH_2C_6H_{13}$), 1.33-1.18 (m, 10H, $O(CH_2)_2(CH_2)_5CH_3$), 0.86 (t, 3H, *J* = 7.0 Hz, $O(CH_2)_7CH_3$). ^{13}C NMR (75 MHz, $CDCl_3$): δ 144.2, 138.8, 138.7, 138.2, 128.9-126.8 (Ar), 97.6 (C-1), 86.2 ($C(Ph)_3$), 80.4, 75.8, 75.2, 75.1, 72.7, 72.3, 71.9, 67.4 (C-2, C-3, C-4, C-5, 3x PhCH_2, O$CH_2C_7H_{15}$), 63.1 (C-6), 31.8, 29.5, 29.4, 29.2, 26.2, 22.6 ($OCH_2(CH_2)_6CH_3$), 14.1 ($O(CH_2)_7CH_3$). HRMS (MALDI): *m/z* 827.4269 MNa^+; calcd 827.4287 for $C_{54}H_{60}O_6Na$.

Cyclohexylmethyl 2,3,4-tri-*O*-benzyl-6-*O*-triphenylmethyl-α-D-mannopyranoside (10). (0.67 g, 85%); $[\alpha]_D$ +22 (*c* 1, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.52-7.14 (m, 28H, Ar), 6.88 (m, 2H, Ar), 4.86 (d, 1H, $J_{1,2}$ = 1.3 Hz, H-1), 4.83 (d, 1H, *J* = 12.5 Hz, PhCH_2), 4.73-4.65 (m, 4H, 2x PhCH_2), 4.27 (d, 1H, *J* = 10.5 Hz, PhCH_2), 3.95 (dd, 1H, $J_{3,4}$ = 9.5 Hz, $J_{4,5}$ = 9.6 Hz, H-4), 3.88 (dd, 1H, $J_{2,3}$ = 3.0 Hz, H-3), 3.83-3.78 (m, 2H, H-2, H-5), 3.59 (dd, 1H, *J* =7.3 Hz, *J* = 9.3 Hz, OC$H_2C_6H_{11}$), 3.48 (dd, 1H, $J_{5,6a}$ = 1.3 Hz, $J_{6a,6b}$ = 9.7 Hz, H-6a), 3.27-3.19 (m, 2H, H-6b, OC$H_2C_6H_{11}$), 1.77-0.90 (m, 11 H, $OCH_2C_6H_{11}$). ^{13}C NMR (100 MHz, $CDCl_3$): δ 145.8, 138.9, 138.8, 138.4, 129.1-127.0 (Ar), 97.9 (C-1), 86.5 ($C(Ph)_3$), 80.7, 76.0, 75.5, 75.4, 73.1, 72.9, 72.6, 72.3 (C-2, C-3, C-4, C-5, 3x PhCH_2, O$CH_2C_6H_{11}$), 63.4 (C-6), 38.1, 30.5, 30.2, 26.8, 26.1, 26.0 ($OCH_2C_6H_{11}$). HRMS (MALDI): *m/z* 811.3986 MNa^+; calcd 811.3975 for $C_{53}H_{56}O_6Na$.

2-Cyclohexylethyl 2,3,4-tri-*O*-benzyl-6-*O*-triphenylmethyl-α-D-mannopyranoside (11). (0.69 g, 86%); $[\alpha]_D$ +21 (*c* 1, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.52-7.17 (m, 28H, Ar), 6.89 (m, 2H, Ar), 4.89 (d, 1H, $J_{1,2}$ = 1.5 Hz, H-1), 4.83 (d, 1H, *J* = 12.5 Hz, PhCH_2), 4.72-4.65 (m, 4H, 2x PhCH_2), 4.27 (d, 1H, *J* = 10.5 Hz, PhCH_2), 3.97 (dd, 1H, $J_{3,4}$ = 9.3 Hz, $J_{4,5}$ = 9.4 Hz, H-4), 3.89 (dd, 1H, $J_{2,3}$ = 3.0 Hz, H-3), 3.84-3.78 (m, 3H, H-2, H-5, OC$H_2CH_2C_6H_{11}$), 3.49 (dd, 1H, $J_{5,6a}$ = 1.5 Hz, $J_{6a,6b}$ = 9.8 Hz, H-6a), 3.44 (dt, 1H, *J* =7.0 Hz, *J* = 9.7 Hz,

OC*H*$_2$CH$_2$C$_6$H$_{11}$), 3.25 (dd, 1H, $J_{5,6b}$ = 5.6 Hz, H-6b), 1.71-0.86 (m, 13 H, OCH$_2$C*H*$_2$C$_6$*H*$_{11}$). ^{13}C NMR (100 MHz, CDCl$_3$): δ 144.4, 138.9, 138.8, 138.5, 138.4, 129.1-127.0 (Ar), 97.8 (C-1), 86.5 (*C*(Ph)$_3$), 80.5, 75.9, 75.5, 75.3, 72.9, 72.5, 72.2 (C-2, C-3, C-4, C-5, 3x Ph*C*H$_2$), 65.7 (O*C*H$_2$CH$_2$C$_6$H$_{11}$), 63.5 (C-6), 37.1, 34.9, 33.6, 33.5, 26.8, 26.5(2x) (OCH$_2$*C*H$_2$*C*$_6$H$_{11}$). HRMS (MALDI): *m/z* 825.4133 MNa$^+$; calcd 825.4131 for C$_{54}$H$_{58}$O$_6$Na.

Octyl 2,3,4-tri-*O*-benzyl-6-*O*-triphenylmethyl-1-thio-α-D-mannopyranoside (12). (0.65 g, 79%); [α]$_D$ +49 (*c* 1, CHCl$_3$); ^{1}H NMR (400 MHz, CDCl$_3$): δ 7.52-7.17 (m, 28H, Ar), 6.90-6.88 (m, 2H, Ar), 5.41 (s, 1H, H-1), 4.79 (d, 1H, *J* = 12.2 Hz, PhC*H*$_2$), 4.74-4.61 (m, 4H, 2x PhC*H*$_2$), 4.27 (d, 1H, *J* = 10.8 Hz, PhC*H*$_2$), 4.14-4.07 (m, 2H, H-4, H-5), 3.86-3.83 (m, 2H, H-2, H-3), 3.49 (dd, 1H, $J_{5,6a}$ = 1.5 Hz, $J_{6a,6b}$ = 9.9 Hz, H-6a), 3.28 (dd, 1H, $J_{5,6b}$ = 4.6 Hz, H-6b), 2.70-2.54 (m, 2H, SC*H*$_2$C$_7$H$_{15}$), 1.63-1.56 (m, 2H, SCH$_2$C*H*$_2$C$_6$H$_{13}$), 1.32-1.23 (m, 10H, S(CH$_2$)$_2$(C*H*$_2$)$_5$CH$_3$), 0.86 (t, 3H, *J* = 7.0 Hz, S(CH$_2$)$_7$C*H*$_3$). ^{13}C NMR (100 MHz, CDCl$_3$): δ 144.4, 138.7, 138.5, 138.4, 129.1-127.0 (Ar), 86.5 (*C*(Ph)$_3$), 81.9 (C-1), 80.6, 77.4, 75.5, 75.4,72.5, 72.4(2x) (C-2, C-3, C-4, C-5, 3x Ph*C*H$_2$), 63.1 (C-6), 32.0, 31.3, 29.8, 29.5, 29.4, 29.2, 22.9 (S(*C*H$_2$)$_7$CH$_3$), 14.3 (S(CH$_2$)$_7$*C*H$_3$). HRMS (MALDI): *m/z* 843.4014 MNa$^+$; calcd 843.4059 for C$_{54}$H$_{60}$O$_5$SNa.

General procedure for detritylation. Synthesis of acceptors 13-16.

Compound **9**, **10**, **11** or **12** (0.6 mmol, 1 eq) was dissolved in CH$_2$Cl$_2$:MeOH (2:1, 6 mL), and *p*-TsOH (0.89 eq) was added. The reaction mixture was stirred at rt until TLC indicated that reaction is complete (~35 min). The reaction mixture was diluted with CH$_2$Cl$_2$ (30 mL), washed with satd. NaHCO$_3$ (2 × 10 mL) and water (10 mL), then dried (Na$_2$SO$_4$), filtered and concentrated. Purification by column chromatography (hexane:EtOAc 4:1→2:1) gave the acceptors **13-16** as an oil.

Octyl 2,3,4-tri-*O*-benzyl-α-D-mannopyranoside (13). (0.29 g, 86%); [α]$_D$ +32 (*c* 1, CHCl$_3$); lit[2] [α]$_D$ +27.6 (*c* 0.7, CHCl$_3$); ^{1}H NMR (400 MHz, CDCl$_3$): δ 7.37-7.26 (m, 15H, Ar), 4.93 (d, 1H, *J* = 10.9 Hz, PhC*H*$_2$), 4.79 (d, 1H, *J* = 12.3 Hz, PhC*H*$_2$), 4.78 (d, 1H, $J_{1,2}$ = 1.6 Hz, H-1), 4.70-4.64 (m, 4H, 2x PhC*H*$_2$), 3.98 (dd, 1H, $J_{3,4}$ = 9.3 Hz, $J_{4,5}$ = 9.4 Hz, H-4), 3.92 (dd, 1H, $J_{2,3}$ = 2.7 Hz, H-3), 3.83 (dd, 1H, $J_{5,6a}$ = 2.8 Hz, $J_{6a,6b}$ = 11.7 Hz, H-6a), 3.79-3.75 (m, 2H, H-2, H-6b), 3.63 (m, 1H, H-5), 3.58 (dt, 1H, *J* =6.8 Hz, *J* = 9.5 Hz, OC*H*$_2$C$_7$H$_{15}$), 3.31 (dt, 1H, *J* =6.5 Hz, *J* = 9.6 Hz, OC*H*$_2$C$_7$H$_{15}$), 1.52-1.48 (m, 2H, OCH$_2$C*H*$_2$C$_6$H$_{13}$), 1.30-1.23 (m,

10H, $O(CH_2)_2(CH_2)_5CH_3$), 0.88 (t, 3H, *J* =6.5 Hz, $O(CH_2)_7CH_3$). ^{13}C NMR (100 MHz, $CDCl_3$): δ 138.7, 138.6, 138.5, 129.6-127.8 (Ar), 98.4 (C-1), 80.5, 75.5, 75.3, 75.2, 73.2, 72.5, 72.3, 68.0 (C-2, C-3, C-4, C-5, 3x $PhCH_2$, $OCH_2C_7H_{15}$), 62.7 (C-6), 32.0, 29.6(2x), 29.4, 26.3, 22.9 ($OCH_2(CH_2)_6CH_3$), 14.3 ($O(CH_2)_7CH_3$). HRMS (MALDI): *m/z* 585.3220 MNa^+; calcd 585.3192 for $C_{35}H_{46}O_6Na$.

Cyclohexylmethyl 2,3,4-tri-*O*-benzyl-α-D-mannopyranoside (14). (0.29 g, 89%); $[\alpha]_D$ +36 (*c* 1, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 7.38-7.26 (m, 15H, Ar), 4.93 (d, 1H, *J* = 10.9 Hz, $PhCH_2$), 4.79 (d, 1H, *J* = 12.3 Hz, $PhCH_2$), 4.74 (d, 1H, $J_{1,2}$ = 1.5 Hz, H-1), 4.69-4.63 (m, 4H, 2x $PhCH_2$), 3.97 (dd, 1H, $J_{3,4}$ = 9.3 Hz, $J_{4,5}$ = 9.4 Hz, H-4), 3.91 (dd, 1H, $J_{2,3}$ = 2.8 Hz, H-3), 3.83 (dd, 1H, $J_{5,6a}$ = 3.0 Hz, $J_{6a,6b}$ = 11.6 Hz, H-6a), 3.78-3.74 (m, 2H, H-2, H-6b), 3.64-3.60 (m, 1H, H-5), 3.40 (dd, 1H, *J* =7.0 Hz, *J* = 9.3 Hz, $OCH_2C_6H_{11}$), 3.12 (dd, 1H, *J* =6.0 Hz, *J* = 9.4 Hz, $OCH_2C_6H_{11}$), 1.70-0.84 (m, 11H, $OCH_2C_6H_{11}$). ^{13}C NMR (100 MHz, $CDCl_3$): δ 138.7, 138.6(2x), 129.6-127.8 (Ar), 98.5 (C-1), 80.5, 75.3, 75.2(2x), 73.4, 73.1, 72.5, 72.3 (C-2, C-3, C-4, C-5, 3x $PhCH_2$, $OCH_2C_6H_{11}$), 62.7 (C-6), 38.0, 30.3, 30.0, 26.7, 26.0(2x) ($OCH_2C_6H_{11}$). HRMS (MALDI): *m/z* 569.2855 MNa^+; calcd 569.2879 for $C_{34}H_{42}O_6Na$.

2-Cyclohexylethyl 2,3,4-tri-*O*-benzyl-α-D-mannopyranoside (15). (0.28 g, 85%); $[\alpha]_D$ +34 (*c* 1, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 7.37-7.22 (m, 15H, Ar), 4.93 (d, 1H, *J* = 10.9 Hz, $PhCH_2$), 4.79 (br d, 2H, H-1, $PhCH_2$), 4.70-4.64 (m, 4H, 2x $PhCH_2$), 3.97 (dd, 1H, $J_{3,4}$ = 9.3 Hz, $J_{4,5}$ = 9.4 Hz, H-4), 3.91 (dd, 1H, $J_{2,3}$ = 2.9 Hz, H-3), 3.83 (dd, 1H, $J_{5,6a}$ = 2.9 Hz, $J_{6a,6b}$ = 11.6 Hz, H-6a), 3.78-3.74 (m, 2H, H-2, H-6b), 3.67-3.62 (m, 2H, H-5, $OCH_2CH_2C_6H_{11}$), 3.35 (dt, 1H, *J* =6.8 Hz, *J* = 9.6 Hz, $OCH_2CH_2C_6H_{11}$), 1.69-0.83 (m, 13H, $OCH_2CH_2C_6H_{11}$). ^{13}C NMR (100 MHz, $CDCl_3$): δ 138.7, 138.6(2x), 129.6-127.9 (Ar), 98.4 (C-1), 80.4, 75.5, 75.2 (2x), 73.1, 72.5, 72.3 (C-2, C-3, C-4, C-5, 3x $PhCH_2$), 65.9 ($OCH_2CH_2C_6H_{11}$), 62.7 (C-6), 37.0, 34.8, 33.6, 33.5, 26.7, 26.4(2x) ($OCH_2CH_2C_6H_{11}$). HRMS (MALDI): *m/z* 583.3048 MNa^+; calcd 583.3036 for $C_{35}H_{44}O_6Na$.

Octyl 2,3,4-tri-*O*-benzyl-1-thio-α-D-mannopyranoside (16). (0.27 g, 79%); $[\alpha]_D$ +65 (*c* 1, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 7.38-7.26 (m, 15H, Ar), 5.26 (d, 1H, $J_{1,2}$ = 0.7 Hz, H-1), 4.94 (d, 1H, *J* = 10.9 Hz, $PhCH_2$), 4.69 (d, 1H, *J* = 10.9 Hz, $PhCH_2$), 4.68-4.57 (m, 4H, 2x $PhCH_2$), 4.02-3.97 (m, 2H, H-3, H-5), 3.86-3.74 (m, 4H, H-2, H-4, H-6a, H-6b), 2.58-2.46 (m, 2H, $SCH_2C_7H_{15}$), 1.57-1.52 (m, 2H, $SCH_2CH_2C_6H_{13}$), 1.36-1.21 (m, 10H,

$S(CH_2)_2(CH_2)_5CH_3$), 0.88 (t, 3H, J = 6.9 Hz, $S(CH_2)_7CH_3$). ^{13}C NMR (100 MHz, $CDCl_3$): δ 138.6, 138.4, 138.3, 128.6-127.9 (Ar), 82.9 (C-1), 80.6, 76.8, 75.5, 75.2, 72.6(2x), 72.4 (C-2, C-3, C-4, C-5, 3x $PhCH_2$), 62.6 (C-6), 32.0, 31.7, 29.8, 29.3(2x), 29.0, 22.9 ($S(CH_2)_7CH_3$), 14.3 ($S(CH_2)_7CH_3$). HRMS (MALDI): *m/z* 601.2981 MNa^+; calcd 601.2964 for $C_{35}H_{46}O_5SNa$.

Synthesis of disaccharides 18-21. General procedure for coupling of the donor 17 with acceptors 13-16.

Donor **17** (0.55 mmol, 1.5 eq), acceptor **13**, **14**, **15** or **16** (0.36 mmol, 1 eq) and 4Å MS (0.5 g) in CH_2Cl_2 (6 mL) were cooled at 0 °C and stirred under inert atmosphere for 15 min. After BF_3OEt_2 (0.55 mmol, 1.5 eq) was added, the reaction mixture was stirred at 0 °C until TLC indicated total consuption of starting material (30 min.). The reaction mixture was poured into satd $NaHCO_3$:CH_2Cl_2 (1:1, 30 mL) under stirring. The organic phase was separated, washed with satd $NaHCO_3$ (10 mL), water (10 mL), dried (Na_2SO_4), filtered and concentrated. Purification by column chromatography (hexane:EtOAc 10:1→3:1) yielded disaccharides **18-21** as an oil.

Octyl 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-benzyl-α-D-manno-pyranoside (18). (0.26 g, 83%); $[\alpha]_D$ +32 (*c* 1, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.37-7.26 (m, 15H, Ar), 5.35 (dd, 1H, $J_{3',4'}$ = 10.0 Hz, H-3'), 5.29 (dd, 1H, $J_{2',3'}$ = 3.4 Hz, H-2'), 5.25 (t, 1H, $J_{4',5'}$ = 10.0 Hz, H-4'), 4.98 (d, 1H, J = 11.2 Hz, $PhCH_2$), 4.92 (d, 1H, $J_{1',2'}$ = 1.6 Hz, H-1'), 4.77 (d, 1H, $J_{1,2}$ = 1.7 Hz, H-1), 4.72 (d, 1H, J = 11.5 Hz, $PhCH_2$), 4.69 (d, 1H, J = 12.5 Hz, $PhCH_2$), 4.60 (m, 3H, $PhCH_2$, ½ $PhCH_2$), 4.18 (dd, 1H, $J_{5',6'a}$ = 5.2 Hz, $J_{6'a,6'b}$ = 12.3 Hz, H-6'a), 4.13-4.06 (m, 2H, H-5', H-6'b), 3.91 (dd, 1H, $J_{2,3}$ = 3.0 Hz, $J_{3,4}$ = 9.1 Hz, H-3), 3.85-3.73 (m, 5H, H-2, H-4, H-5, H-6a, H-6b), 3.63 (dt, 1H, J =6.7 Hz, J = 9.7 Hz, $OCH_2C_7H_{15}$), 3.34 (dt, 1H, J =6.5 Hz, J = 9.7 Hz, $OCH_2C_7H_{15}$), 2.13, 2.05, 2.02, 1.96 (each s, each 3H, 4x CH_3CO), 1.57-1.51 (m, 2H, $OCH_2CH_2C_6H_{13}$), 1.30-1.21 (m, 10H, $O(CH_2)_2(CH_2)_5CH_3$), 0.88 (t, 3H, J =6.6 Hz, $O(CH_2)_7CH_3$). ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.0, 170.0 (2x), 169.8 (4x CH_3CO), 138.6, 128.6-127.8 (Ar), 97.8 (C-1), 97.6 (C-1'), 80.7 (C-3), 75.2 ($PhCH_2$), 75.0(2x) (C-2, C-4), 72.9 ($PhCH_2$), 72.3 ($PhCH_2$), 71.4 (C-5), 69.8 (C-2'), 69.3 (C-3'), 68.6 (C-5'), 67.8 ($OCH_2C_7H_{15}$), 67.2 (C-6), 66.5 (C-4'), 62.6 (C-6'), 32.1, 29.6(2x), 29.5, 26.4, 22.9 ($OCH_2(CH_2)_6CH_3$), 21.1, 20.9(2x), 20.8 (4×CH_3CO), 14.3 ($O(CH_2)_7CH_3$). HRMS (MALDI): *m/z* 915.4146 MNa^+; calcd 915.4143 for $C_{49}H_{64}O_{15}Na$.

Cyclohexylmethyl 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-benzyl-α-D-mannopyranoside (19). (0.28 g, 90%); $[\alpha]_D$ +43 (*c* 1, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.37-7.25 (m, 15H, Ar), 5.34 (dd, 1H, $J_{3',4'}$ = 10.0 Hz, H-3'), 5.30 (dd, 1H, $J_{2',3'}$ = 3.3 Hz, H-2'), 5.25 (t, 1H, $J_{4',5'}$ = 10.0 Hz, H-4'), 4.97 (d, 1H, *J* = 11.2 Hz, PhCH_2), 4.91 (d, 1H, $J_{1',2'}$ = 1.6 Hz, H-1'), 4.76 (d, 2H, H-1, PhCH_2), 4.68 (d, 1H, *J* = 12.5 Hz, PhCH_2), 4.61 (m, 3H, PhCH_2, ½ $PhCH_2$), 4.18 (dd, 1H, $J_{5',6'a}$ = 5.3 Hz, $J_{6'a,6'b}$ = 12.3 Hz, H-6'a), 4.14-4.06 (m, 2H, H-5', H-6'b), 3.89 (dd, 1H, $J_{2,3}$ = 3.0 Hz, $J_{3,4}$ = 9.1 Hz, H-3), 3.85-3.71 (m, 5H, H-2, H-4, H-5, H-6a, H-6b), 3.43 (dd, 1H, *J* =7.1 Hz, *J* = 9.4 Hz, OC$H_2C_6H_{11}$), 3.14 (dd, 1H, *J* =5.9 Hz, *J* = 9.4 Hz, OC$H_2C_6H_{11}$), 2.13, 2.04, 2.02, 1.96 (each s, each 3H, 4x CH_3CO), 1.71-0.86 (m, 11H, $OCH_2C_6H_{11}$). ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.0, 170.0 (2x), 169.7 (4x CH_3CO), 138.6, 128.6-127.8 (Ar), 98.0 (C-1), 97.6 (C-1'), 80.6 (C-3), 75.3 (PhCH_2), 75.0(2x) (C-2, C-4), 73.3 ($OCH_2C_6H_{11}$), 72.9 (PhCH_2), 72.4 (PhCH_2), 71.4 (C-5), 69.8 (C-2'), 69.3 (C-3'), 68.6 (C-5'), 67.3 (C-6), 66.4 (C-4'), 62.6 (C-6'), 38.0, 30.3, 30.0, 26.7, 26.1, 26.0 ($OCH_2C_6H_{11}$), 21.1, 20.9(2x), 20.8 (4×CH_3CO). HRMS (MALDI): *m/z* 899.3815 MNa^+; calcd 899.3829 for $C_{48}H_{60}O_{15}Na$.

2-Cyclohexylethyl 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-benzyl-α-D-mannopyranoside (20). (0.26 g, 81%); $[\alpha]_D$ +46 (*c* 1, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.39-7.25 (m, 15H, Ar), 5.34 (dd, 1H, $J_{3',4'}$ = 9.9 Hz, H-3'), 5.30 (dd, 1H, $J_{2',3'}$ = 3.3 Hz, H-2'), 5.25 (t, 1H, $J_{4',5'}$ = 9.9 Hz, H-4'), 4.98 (d, 1H, *J* = 11.2 Hz, PhCH_2), 4.91 (d, 1H, $J_{1',2'}$ = 1.5 Hz, H-1'), 4.76 (d, 1H, $J_{1,2}$ = 1.7 Hz, H-1), 4.72 (d, 1H, *J* = 11.2 Hz, PhCH_2), 4.69 (d, 1H, *J* = 12.5 Hz, PhCH_2), 4.60 (m, 3H, PhCH_2, ½ $PhCH_2$), 4.18 (dd, 1H, $J_{5',6'a}$ = 5.3 Hz, $J_{6'a,6'b}$ = 12.3 Hz, H-6'a), 4.15-4.06 (m, 2H, H-5', H-6'b), 3.90 (dd, 1H, $J_{2,3}$ = 3.0 Hz, $J_{3,4}$ = 9.1 Hz, H-3), 3.85-3.72 (m, 5H, H-2, H-4, H-5, H-6a, H-6b), 3.68 (dt, 1H, *J* =6.9 Hz, *J* = 9.7 Hz, OC$H_2CH_2C_6H_{11}$), 3.36 (dt, 1H, *J* =6.7 Hz, *J* = 9.8 Hz, OC$H_2CH_2C_6H_{11}$), 2.13, 2.05, 2.02, 1.96 (each s, each 3H, 4x CH_3CO), 1.68-0.86 (m, 13H, $OCH_2CH_2C_6H_{11}$). ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.0, 170.0 (2x), 169.7 (4x CH_3CO), 138.6, 128.6-127.8 (Ar), 97.8 (C-1), 97.7 (C-1'), 80.6 (C-3), 75.2 (PhCH_2), 75.0(2x) (C-2, C-4), 72.9 (PhCH_2), 72.4 (PhCH_2), 71.4 (C-5), 69.8 (C-2'), 69.3 (C-3'), 68.6 (C-5'), 67.3 (C-6), 66.4 (C-4'), 65.7 ($OCH_2CH_2C_6H_{11}$), 62.6 (C-6'), 37.0, 34.7, 33.6, 33.4, 26.8, 26.5(2x) ($OCH_2CH_2C_6H_{11}$), 21.1, 20.9(2x), 20.8 (4×CH_3CO). HRMS (MALDI): *m/z* 913.4010 MNa^+; calcd 913.3986 for $C_{49}H_{62}O_{15}Na$.

Synthesis of disaccharide 22.

To a stirred and cooled at 0 °C solution containing disaccharide **21** (0.22 mmol, 1 eq) in CH_2Cl_2 (5 mL), *m*CPBA (0.66 mmol, 3 eq) was added. The reaction mixture was stirred at rt for 2 h, then diluted with CH_2Cl_2 (20 mL), washed with satd $NaHCO_3$ (2 × 20 mL) and water (20 mL). The organic phase was dried (Na_2SO_4), filtered and concentrated. The crude product was purified by flash chromatography (hexane:EtOAc 3:1→1.5:1). To a stirred and at 0 °C precooled solution containing compound (**21**) (0.22 mmol, 1 eq) in CH_2Cl_2 (5 mL) *m*CPBA (0.66 mmol, 3eq, based on 77% peroxide content) was added. The reaction mixture was stirred at rt for 2 h, diluted with CH_2Cl_2 (20 mL), washed with satd $NaHCO_3$ (2 × 15 mL) and water (20 mL). The organic phase was dried (Na_2SO_4), filtered and concentrated. The crude product was purified by flash chromatography (hexane:EtOAc 3:1→1.5:1).

Octyl 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-benzyl-α-D–manno-pyranosyl sulfone (22). (0.17 g, 49% over 2 steps); $[\alpha]_D$ +48 (*c* 1, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.31-7.17 (m, 15H, Ar), 5.24-5.15 (m, 3H, H-2', H-3', H-4'), 4.81 (br d, 2H, H-1', PhC*H*$_2$), 4.77 (d, 1H, $J_{1,2}$ = 2.0 Hz, H-1), 4.62 (d, 1H, *J* = 11.8 Hz, PhC*H*$_2$), 4.57 (d, 1H, *J* = 12.2 Hz, PhC*H*$_2$), 4.54 (d, 1H, *J* = 12.1 Hz, PhC*H*$_2$), 4.50 (d, 1H, *J* = 11.8 Hz, PhC*H*$_2$), 4.46 (d, 1H, *J* = 11.5 Hz, PhC*H*$_2$), 4.40 (dd, 1H, $J_{2,3}$ = 3.3 Hz, H-2), 4.30 (m, 1H, H-5), 4.11-4.06 (m, 2H, H-3, H-6'a), 4.00 (dd, 1H, $J_{5',6'b}$ = 2.2 Hz, $J_{6'a,6'b}$ = 12.2 Hz, H-6'b), 3.87 (m, 1H, H-5'), 3.78 (t, 1H, $J_{3,4}$ = 9.0 Hz, $J_{4,5}$ = 9.0 Hz, H-4), 3.69 (dd, 1H, $J_{5,6a}$ = 5.8 Hz, $J_{6a,6b}$ = 11.3 Hz, H-6a), 3.61 (dd, $J_{5,6b}$ = 1.4 Hz, H-6b), 3.06-2.93 (m, 2H, $SO_2CH_2C_7H_{15}$), 2.07, 1.95(2x), 1.89 (each s, each 3H, 4x CH_3CO), 1.78-1.66 (m, 2H, S $O_2CH_2CH_2C_6H_{13}$), 1.36-1.19 (m, 10H, $SO_2(CH_2)_2(CH_2)_5CH_3$), 0.81 (t, 3H, *J* = 6.3 Hz, $SO_2(CH_2)_7CH_3$).^{13}C NMR (100 MHz, $CDCl_3$): δ 170.9, 170.0, 169.9, 169.7 (4x CH_3CO), 138.2, 138.1, 137.5, 128.7-127.9 (Ar), 97.9 (C-1'), 88.5 (C-1), 79.4 (C-3), 75.7 (C-5), 74.6 (Ph*C*H$_2$), 73.5(2x) (Ph*C*H$_2$, C-4), 72.9 (Ph*C*H$_2$), 70.9 (C-2), 69.7 (C-2'), 69.1 (C-3'), 68.8 (C-5'), 67.4 (C-6), 66.3 (C-4'), 62.5 (C-6'), 50.6 ($SO_2CH_2(CH_2)_6CH_3$), 31.9, 29.3, 29.2, 28.7, 22.8, 21.7 ($SO_2CH_2(CH_2)_6CH_3$), 21.1, 20.9(2x), 20.8 (4×CH_3CO), 14.3 ($SO_2CH_2(CH_2)_6CH_3$). HRMS (MALDI): *m/z* 963.3804 MNa^+; calcd 963.3813 for $C_{49}H_{64}O_{16}SNa$.

General procedure for removal of acetyl groups. Synthesis of disaccharides 23-26.

To a solution of protected disaccharide **18**, **19**, **20** or **22** (0.17 mmol) in MeOH:CH_2Cl_2 (17:1, 4.75 mL) was added 1M MeONa (0.25 mL). After stirring overnight (16 h), the solution was

neutralized with Dowex 50 H^+-form, filtered and concentrated. The crude product was purified by column chromatography (CH_2Cl_2:MeOH 9:1→5:1) to give partially deprotected disaccharides **23-26** as an oil.

Octyl α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-benzyl-α-D-mannopyranoside (23). (0.11 g, 95%); $[\alpha]_D$ +46 (*c* 0.5, $CHCl_3$); ^{1}H NMR (400 MHz, CD_3OD): δ 7.39-7.25 (m, 15H, Ar), 4.89 (d, 1H, $J_{1',2'}$ = 1.2 Hz, H-1'), 4.88 (d, 1H, *J* = 11.0 Hz, PhC*H*$_2$), 4.81 (d, 1H, $J_{1,2}$ = 1.4 Hz, H-1), 4.70-4.62 (m, 3H, PhC*H*$_2$), 4.58 (d, 1H, *J* = 11.6 Hz, PhC*H*$_2$), 4.54 (d, 1H, *J* = 11.6 Hz, PhC*H*$_2$), 3.93-3.58 (m, 13H, H-2, H-3, H-4, H-5, H-6a, H-6b, H-2', H-3', H-4', H-5', H-6'a, H-6'b, OC*H*$_2$C$_7$H$_{15}$), 3.37 (dt, 1H, *J* =6.1 Hz, *J* = 9.7 Hz, OC*H*$_2$C$_7$H$_{15}$), 1.57-1.51 (m, 2H, OCH$_2$C*H*$_2$C$_6$H$_{13}$), 1.38-1.24 (m, 10H, O(CH$_2$)$_2$(C*H*$_2$)$_5$CH$_3$), 0.89 (t, 3H, *J* =6.7 Hz, O(CH$_2$)$_7$C*H*$_3$). ^{13}C NMR (100 MHz, CD_3OD): δ 140.0, 139.8, 132.5, 130.0-128.8 (Ar), 102.1 (C-1'), 99.1 (C-1), 81.3, 76.4, 76.1(2x), 74.6, 73.8, 73.1, 72.9, 72.8, 72.2, 69.2 (C-2, C-3, C-4, C-5, C-2', C-3', C-4', C-5', 3x PhCH$_2$), 68.8 (O*C*H$_2$C$_7$H$_{15}$), 67.5 (C-6), 62.6 (C-6'), 33.1, 30.6(2x), 30.5, 27.5, 23.9 (OCH$_2$(*C*H$_2$)$_6$CH$_3$), 14.6 (O(CH$_2$)$_7$*C*H$_3$). HRMS (MALDI): *m/z* 747.3695 MNa^+; calcd 747.3720 for $C_{41}H_{56}O_{11}Na$.

Cyclohexylmethyl α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-benzyl-α-D-mannopyranoside (24). (0.11 g, 90%); $[\alpha]_D$ +50 (*c* 0.5, $CHCl_3$); ^{1}H NMR (400 MHz, CD_3OD): δ 7.38-7.26 (m, 15H, Ar), 4.89 (d, 1H, $J_{1',2'}$ = 1.3 Hz, H-1'), 4.88 (d, 1H, *J* = 10.9 Hz, PhC*H*$_2$), 4.76 (d, 1H, $J_{1,2}$ = 1.5 Hz, H-1), 4.69 (d, 1H, *J* = 12.2 Hz, PhC*H*$_2$), 4.65-4.62 (m, 2H, PhC*H*$_2$), 4.58 (d, 1H, *J* = 11.6 Hz, PhC*H*$_2$), 4.54 (d, 1H, *J* = 11.7 Hz, PhC*H*$_2$), 3.90-3.59 (m, 12H, H-2, H-3, H-4, H-5, H-6a, H-6b, H-2', H-3', H-4', H-5', H-6'a, H-6'b), 3.44 (dd, 1H, *J* =6.9 Hz, *J* = 9.4 Hz, OC*H*$_2$C$_6$H$_{11}$), 3.16 (dd, 1H, *J* =5.9 Hz, *J* = 9.4 Hz, OC*H*$_2$C$_6$H$_{11}$), 1.73-0.89 (m, 11H, OCH$_2$C$_6$*H*$_{11}$).^{13}C NMR (100 MHz, CD_3OD): δ 139.9, 139.8, 139.7, 133.6, 132.5, 129.5-128.8 (Ar), 101.9 (C-1'), 99.1 (C-1), 81.2, 76.3, 76.1(2x), 74.6, 74.3, 73.8, 73.1, 72.8(2x), 72.2, 68.5 (C-2, C-3, C-4, C-5, C-2', C-3', C-4', C-5', 3x PhCH$_2$, O*C*H$_2$C$_6$H$_{11}$), 67.5 (C-6), 62.8 (C-6'), 39.2, 31.2, 31.1, 27.7, 27.1, 27.0 (OCH$_2$*C*$_6$H$_{11}$). HRMS (MALDI): *m/z* 731.3395 MNa^+; calcd 731.3407 for $C_{40}H_{52}O_{11}Na$.

2-Cyclohexylethyl α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-benzyl-α-D-mannopyranoside (25). (0.11 g, 91%); $[\alpha]_D$ +54 (*c* 0.5, $CHCl_3$); ^{1}H NMR (400 MHz, CD_3OD): δ 7.40-7.24 (m, 15H, Ar), 4.89 (br d, 2H, H-1', PhC*H*$_2$), 4.79 (d, 1H, $J_{1,2}$ = 1.6 Hz, H-1), 4.72-4.62 (m, 3H,

PhCH_2), 4.59 (d, 1H, *J* = 11.7 Hz, PhCH_2), 4.54 (d, 1H, *J* = 11.7 Hz, PhCH_2), 3.91-3.57 (m, 13H, H-2, H-3, H-4, H-5, H-6a, H-6b, H-2', H-3', H-4', H-5', H-6'a, H-6'b, OCH_2CH_2C$_6$H$_{11}$), 3.39 (dd, 1H, *J* =6.9 Hz, *J* = 9.4 Hz, OCH_2CH_2C$_6$H$_{11}$), 1.74-0.86 (m, 13H, OCH$_2$CH_2C$_6$$H_{11}$).^{13}C NMR (100 MHz, CD_3OD): δ 140.0, 139.8(2x), 133.7, 132.5, 129.6-128.8 (Ar), 102.1 (C-1'), 99.1 (C-1), 81.2, 76.4, 76.1(2x), 74.6, 73.8, 73.0(2x), 72.8, 72.2, 68.6 (C-2, C-3, C-4, C-5, C-2', C-3', C-4', C-5'), 67.6 (C-6), 66.7 (O*C*H$_2$CH$_2$C$_6$H$_{11}$), 62.9 (C-6'), 38.1, 36.0, 34.7, 34.4, 27.8, 27.6, 27.5 (OCH$_2$*C*H$_2$*C*$_6$H$_{11}$). HRMS (MALDI): *m/z* 745.3569 MNa$^+$; calcd 745.3564 for $C_{41}H_{54}O_{11}Na$.

Octyl α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-benzyl-α-D-mannopyranosyl sulfone (26). (0.12 g, 89%); $[\alpha]_D$ +52 (*c* 0.5, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.70 (m, 2H, Ar), 7.53 (m, 2H, Ar), 7.36-7.19 (m, 11H, Ar), 4.85 (s, 1H, H-1'), 4.79 (d, 1H, *J* = 11.4 Hz, PhCH_2), 4.76 (d, 1H, $J_{1,2}$ = 2.7 Hz, H-1), 4.65-4.58 (m, 4H, 2x PhCH_2), 4.47-4.44 (m, 2H, H-2, PhCH_2), 4.31 (m, 1H, H-5), 4.11 (d, 1H, $J_{2,3}$ = 3.2 Hz, $J_{3,4}$ = 7.8 Hz, H-3), 3.92-3.89 (m, 2H, H-2', H-4'), 3.85-3.76 (m, 4H, H-4, H-6a, H-3', H-6'a), 3.65-3.60 (m, 2H, H-6b, H-6'b), 3.45 (m, 1H, H-5'), 3.01-2.97 (m, 2H, SO_2C*H*$_2$C$_7$H$_{15}$), 1.77-1.65 (m, 2H, SO_2CH_2C*H*$_2$C$_6$H$_{13}$), 1.41-1.26 (m, 10H, $SO_2(CH_2)_2$(C*H*$_2$)$_5$CH$_3$), 0.92 (t, 3H, *J* = 7.1 Hz, $SO_2(CH_2)_7$*C*H$_3$). ^{13}C NMR (100 MHz, $CDCl_3$): δ 138.2, 138.0, 137.4, 132.6, 131.1, 129.0-127.8 (Ar), 100.3 (C-1'), 88.5 (C-1), 78.9 (C-3), 75.9 (C-5), 74.3 (Ph*C*H$_2$), 73.9 (C-4), 73.4 (Ph*C*H$_2$), 72.9 (Ph*C*H$_2$), 72.4 (C-5'), 71.8 (C-3'), 71.1, 70.9 (C-2, C-2'), 66.8 (C-6), 66.5 (C-4'), 61.1 (C-6'), 50.1 (SO_2*C*H$_2$(CH$_2$)$_6$CH$_3$), 31.9, 29.3, 29.2, 28.7, 22.8, 21.7 (SO_2CH_2(*C*H$_2$)$_6$CH$_3$),14.3 (SO_2CH_2(CH$_2$)$_6$*C*H$_3$). HRMS (MALDI): *m/z* 795.3394 MNa$^+$; calcd 795.3390 for $C_{41}H_{56}O_{12}SNa$.

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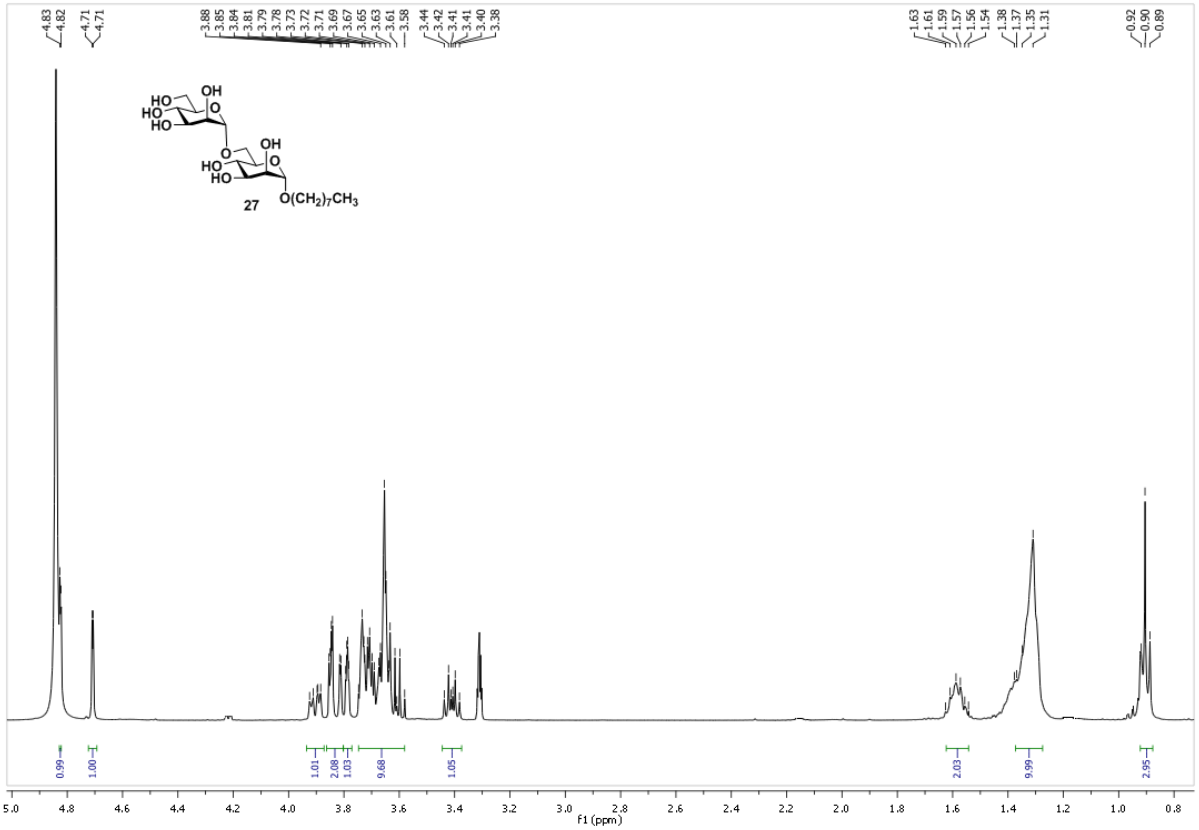
2 V. Subramaniam, S. S. Gurcha, G. S. Besra and T. L. Lowary, *Bioorg. Med. Chem.*, 2005, **13**, 1083.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3.38
1.63
1.61
1.59
1.57
1.56
1.54
1.38
1.37
1.35
1.31
0.92
0.90
0.89
OH
HO
HO
HO
O
OH
O
HO
HO
O(CH2)7CH3
27
0.99
1.00
1.01
2.08
1.03
9.68
1.05
2.03
9.99
2.95
5.0
4.8
4.6
4.4
4.2
4.0
3.8
3.6
3.4
3.2
3.0
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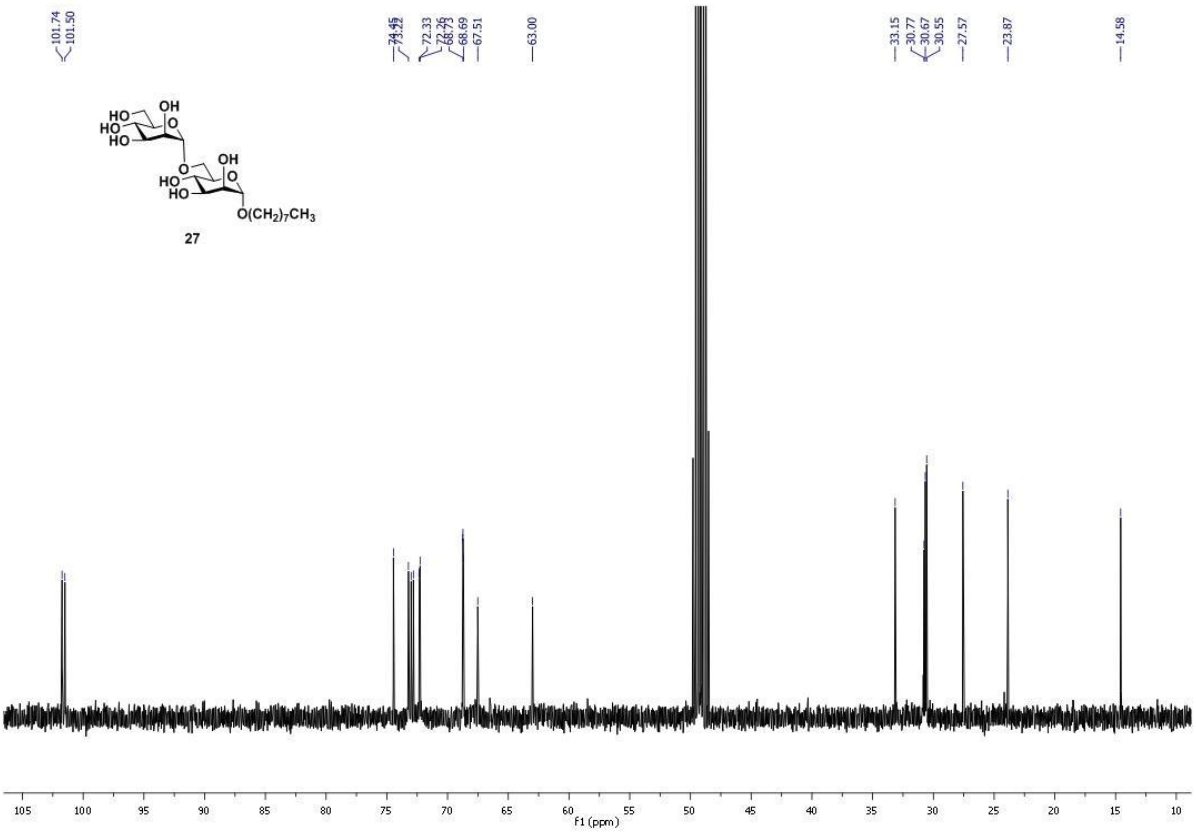
0.8
f1 (ppm)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30.55
27.57
23.87
14.58
OH
HO
HO
HO
O
OH
O
HO
HO
O(CH2)7CH3
27
105
100
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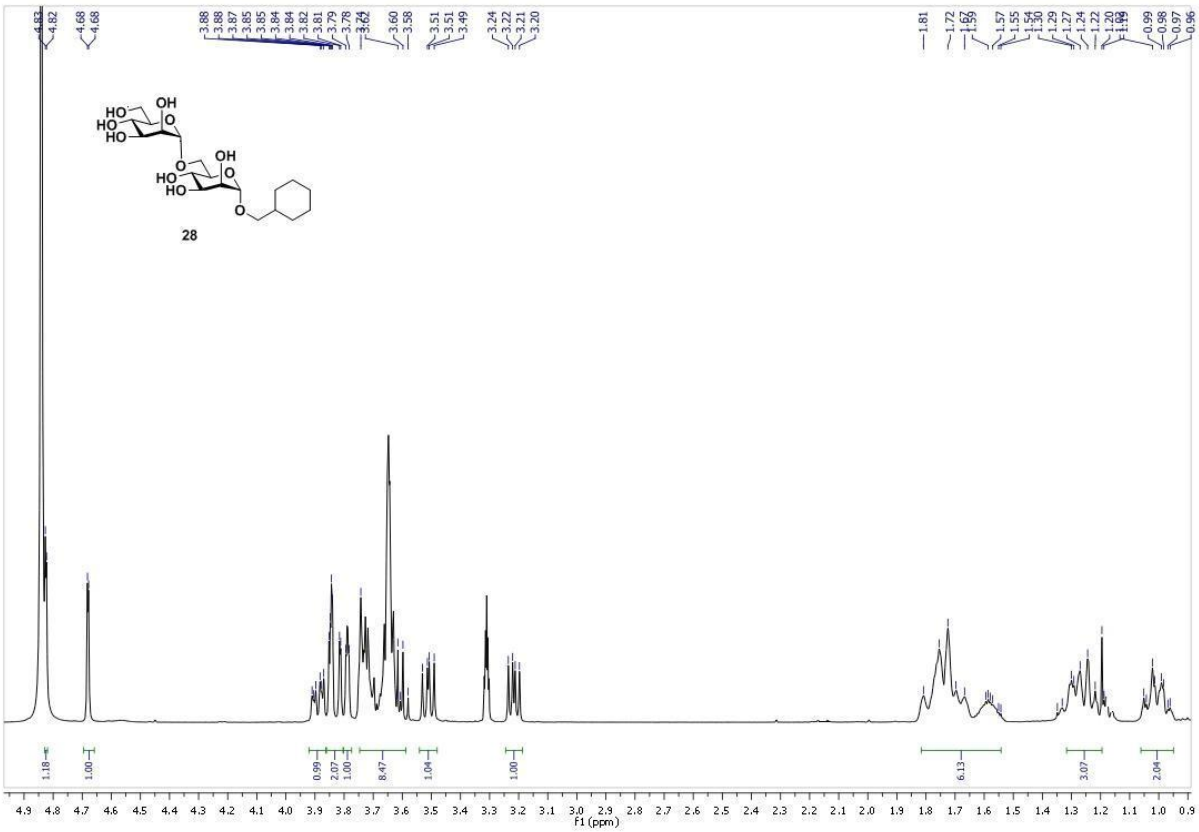

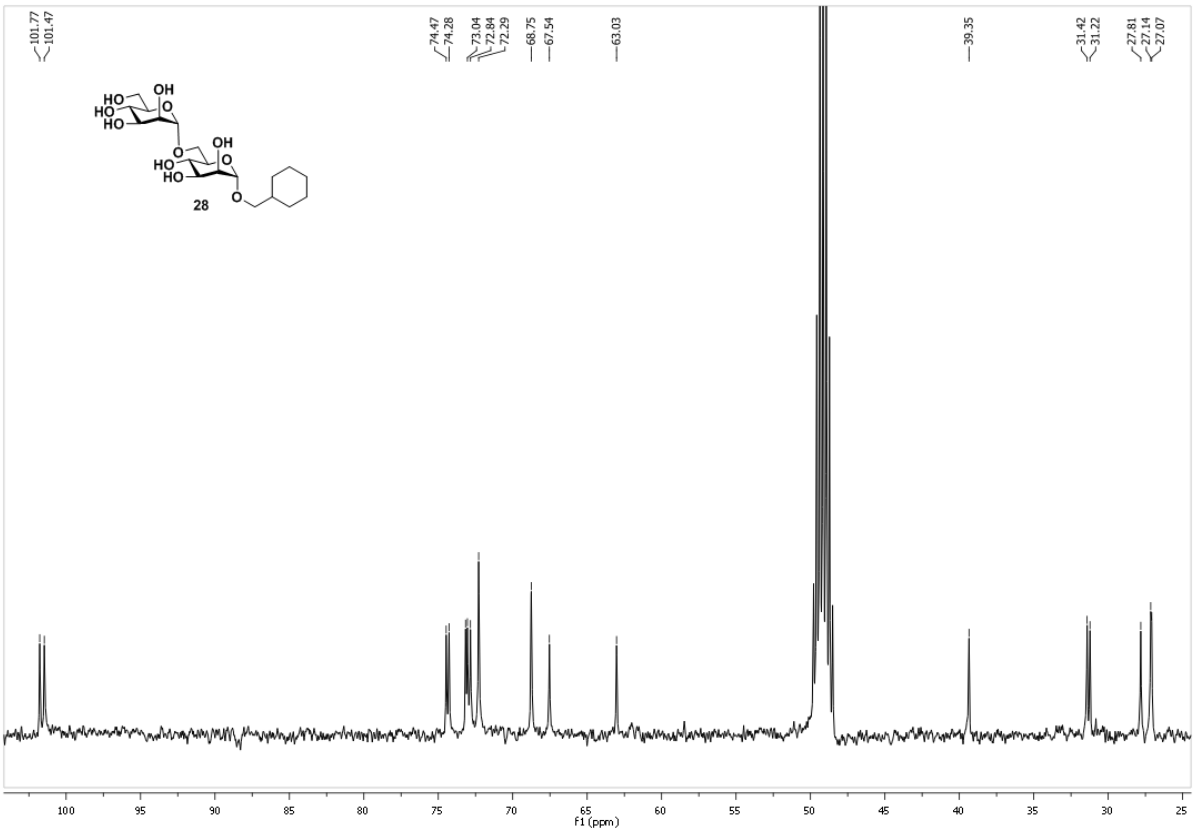

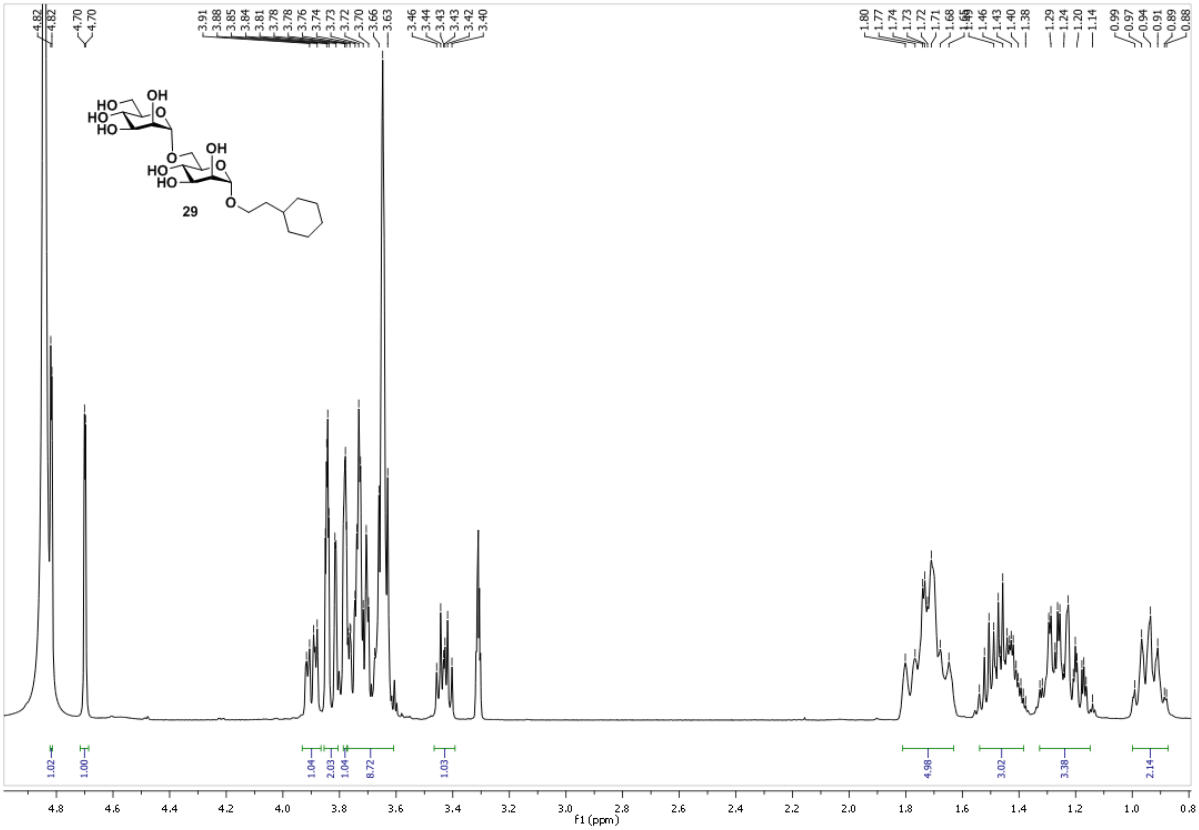
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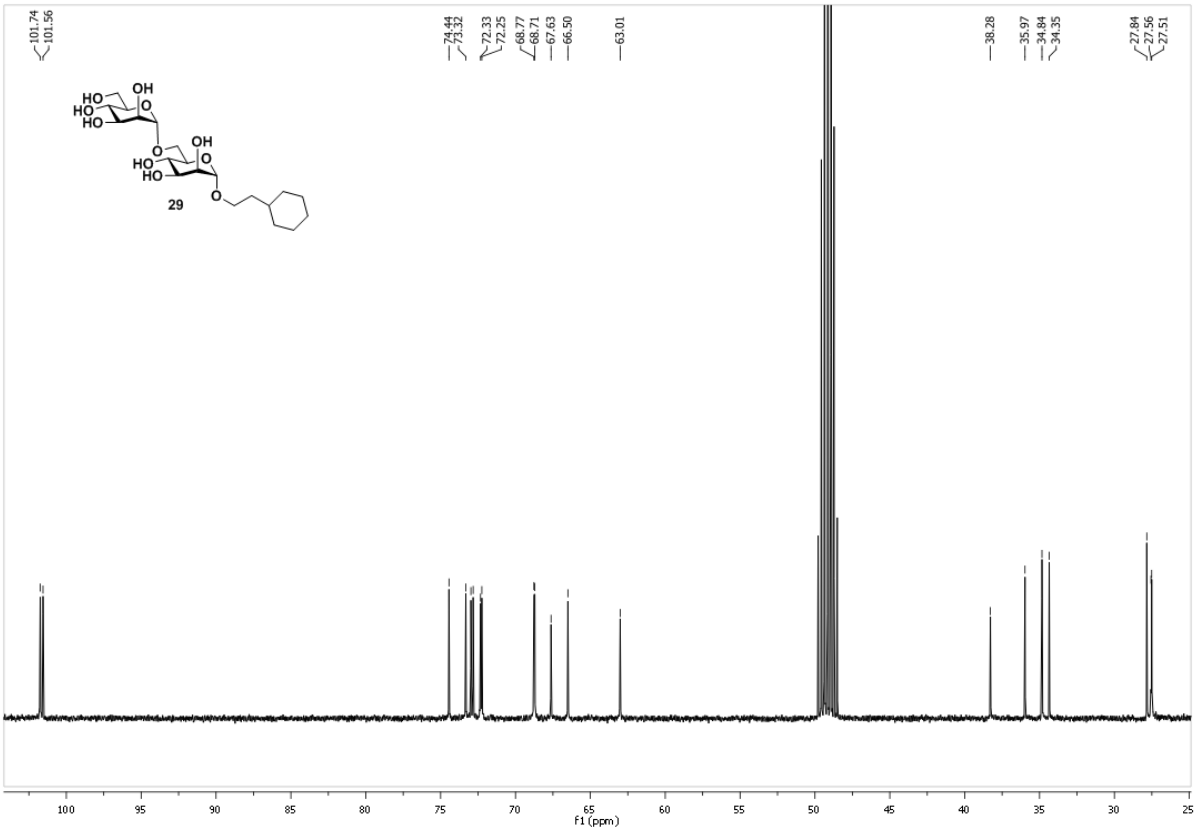
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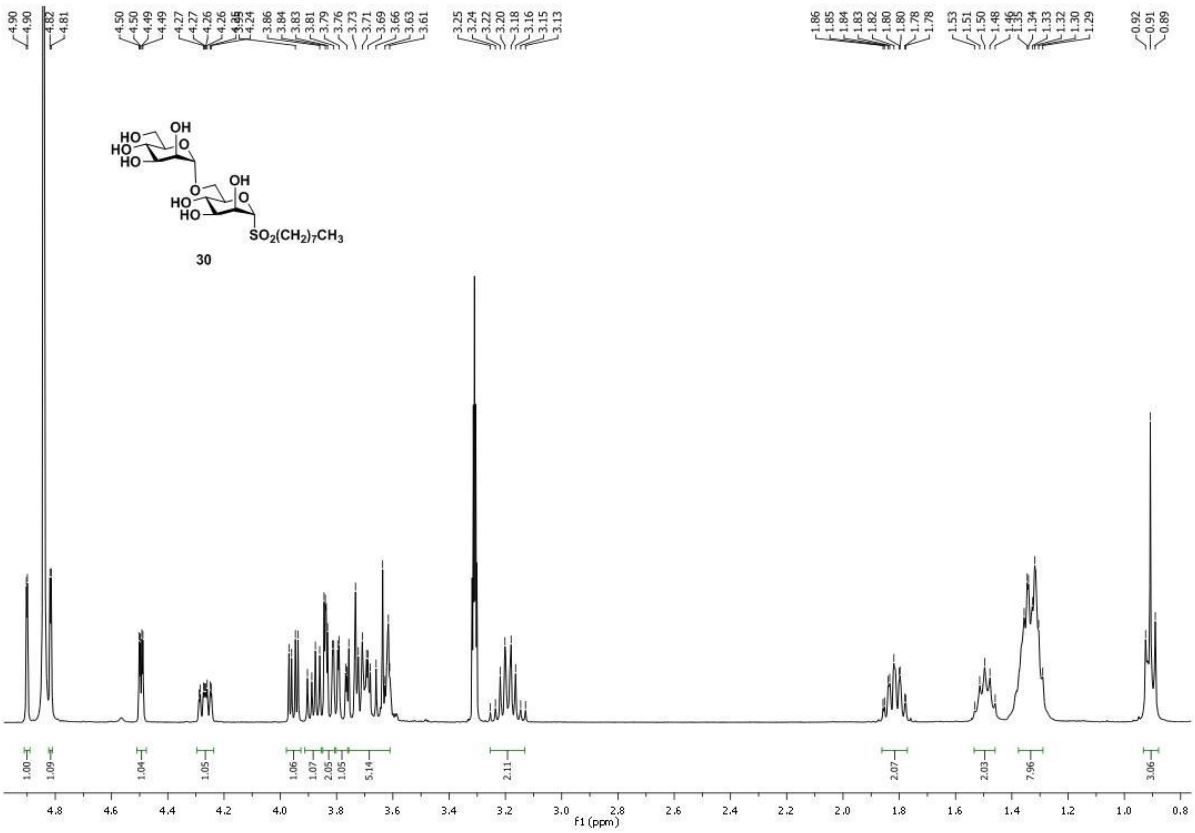
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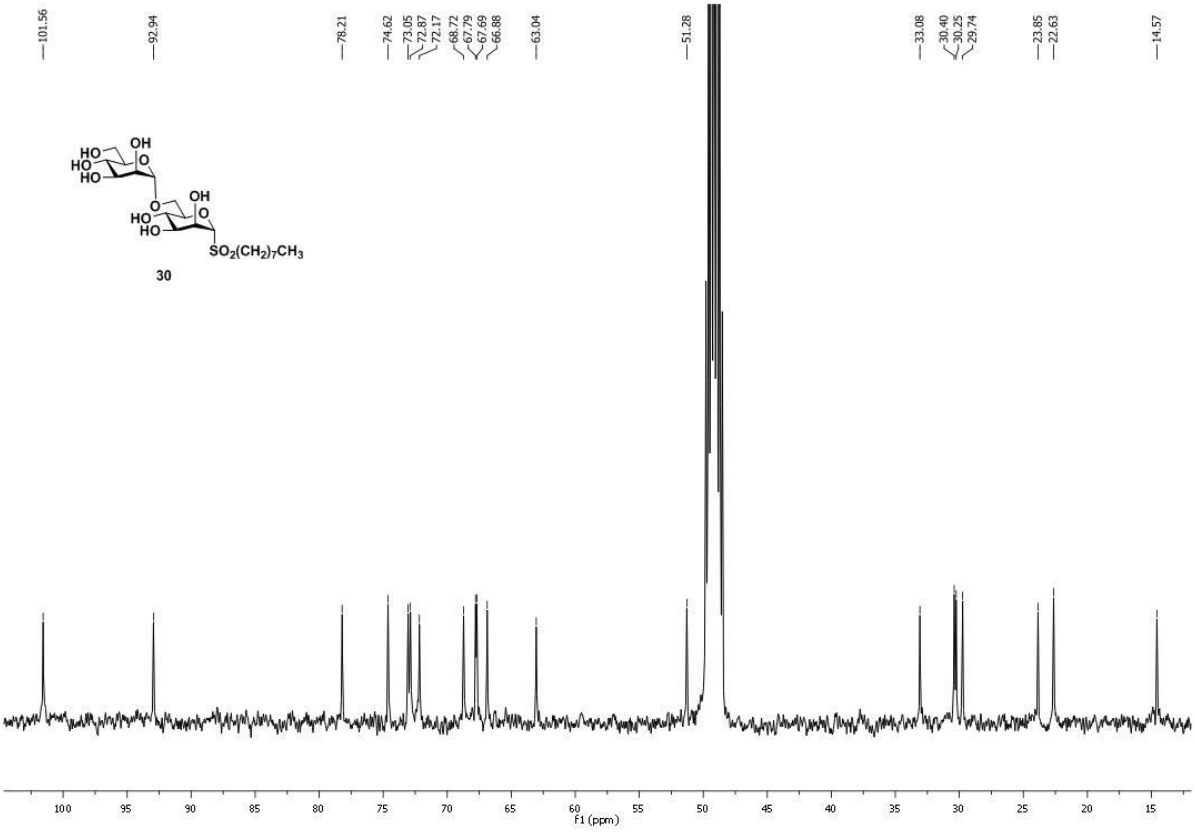
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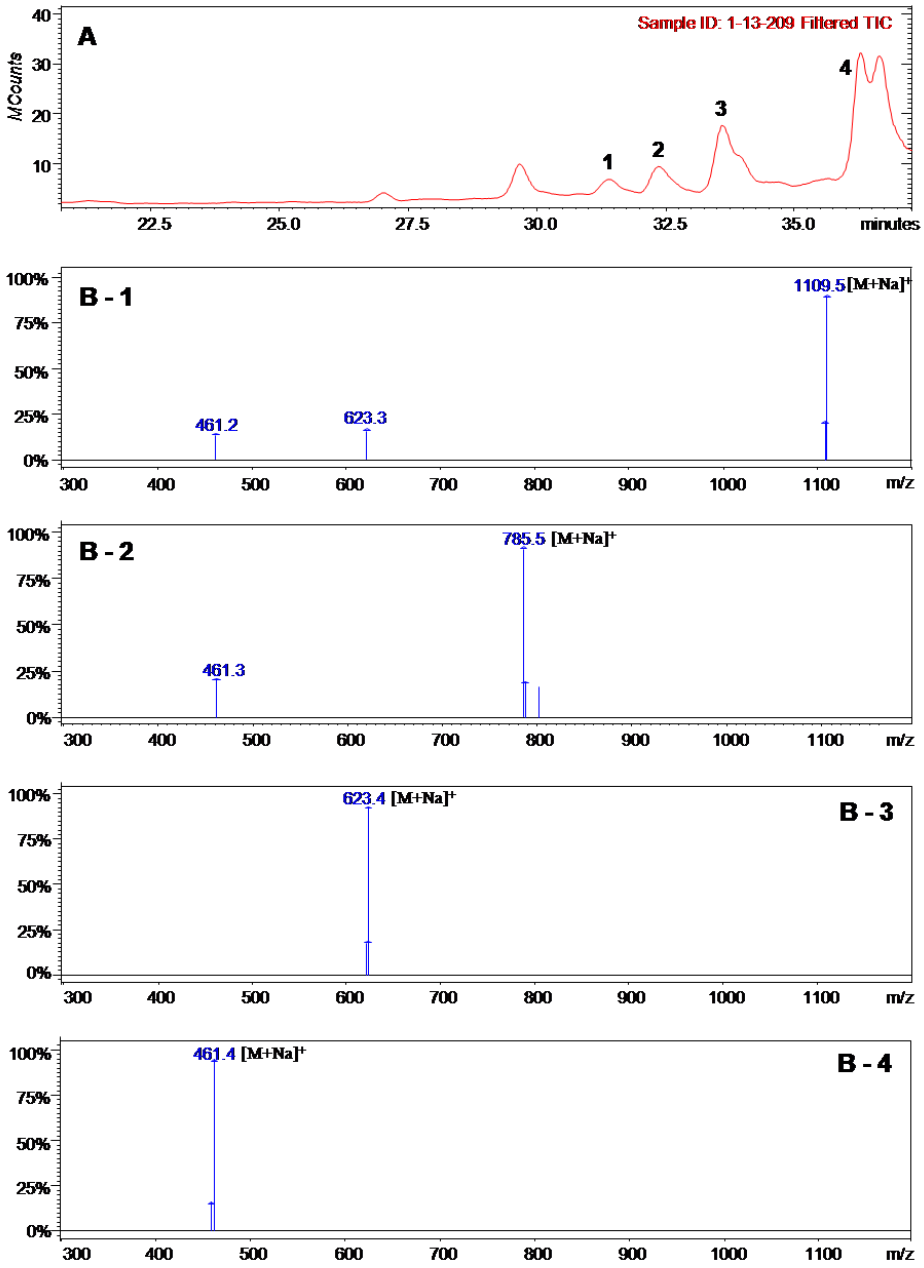

Figure S1. LC-MS analysis of the enzymatic reaction mixture of **28**. Panel (A) - HPLC profile.

Panel (B) - ESI mass spectra obtained from peaks 1-4 in figure (a); all peaks are as $[M+Na]^+$.

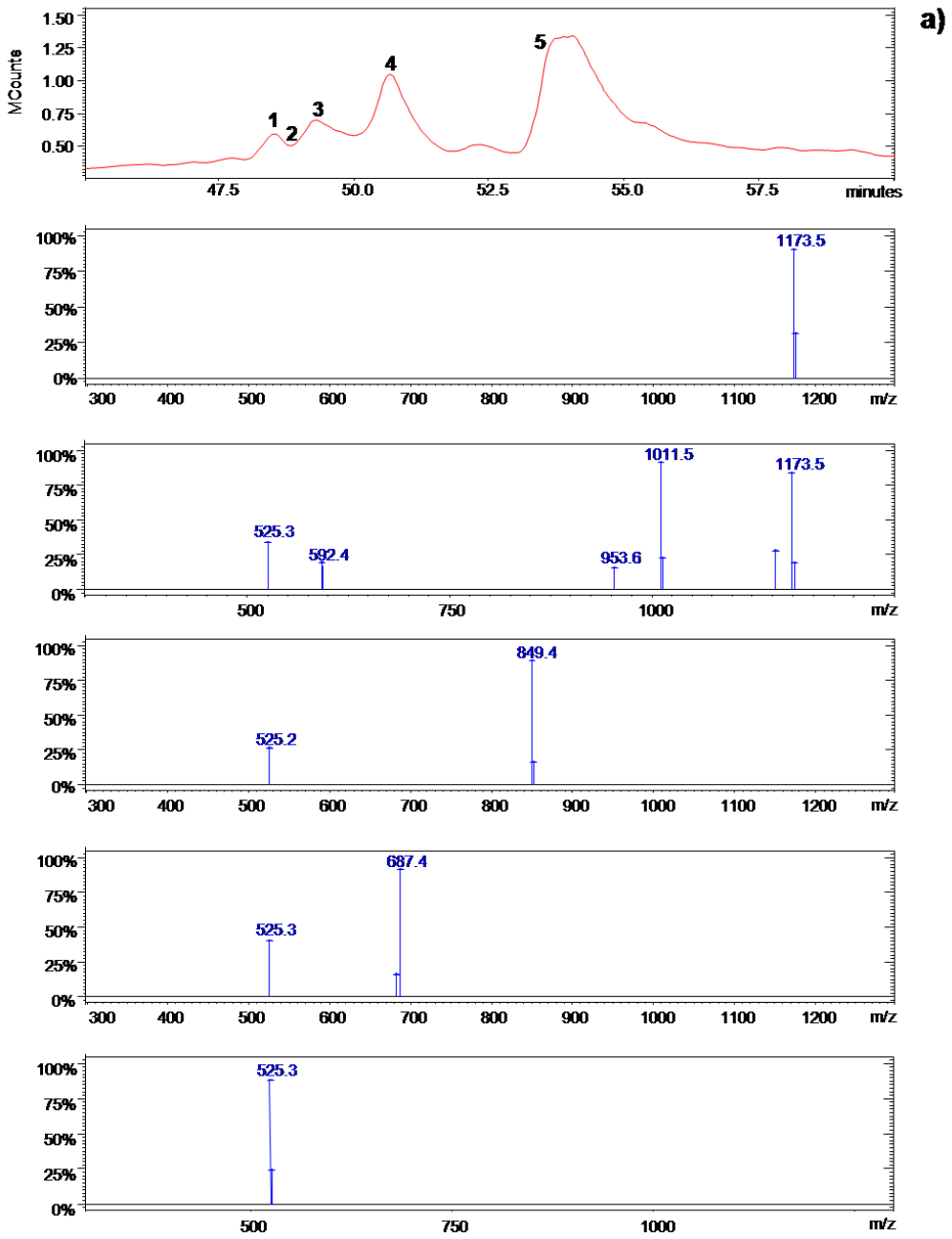

Figure S2. LC-MS analysis of the enzymatic reaction mixture of **30**. Panel (a) – HPLC profile.

Panel (b) - ESI mass spectra obtained from peaks 1-5 in figure (a); all peaks are as $[M+Na]^+$.

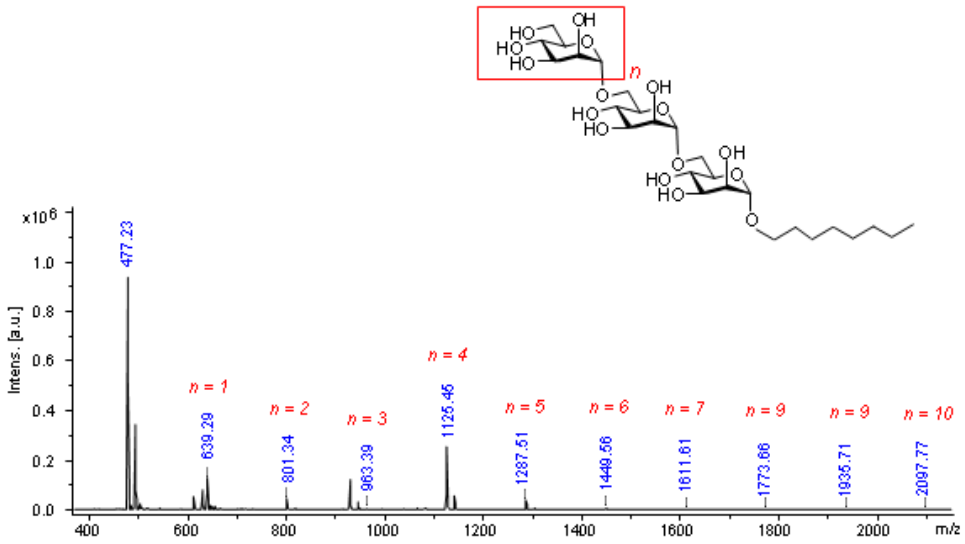

Figure S3. MALDI-MS spectra recorded from the enzymatic sample of reference **27** after SPE fractionation. Peaks corresponding to consecutive mannose attachment are in a blue color and are detected as $[M+Na]^+$. Unlabeled peaks originated from membrane protein of ManT.

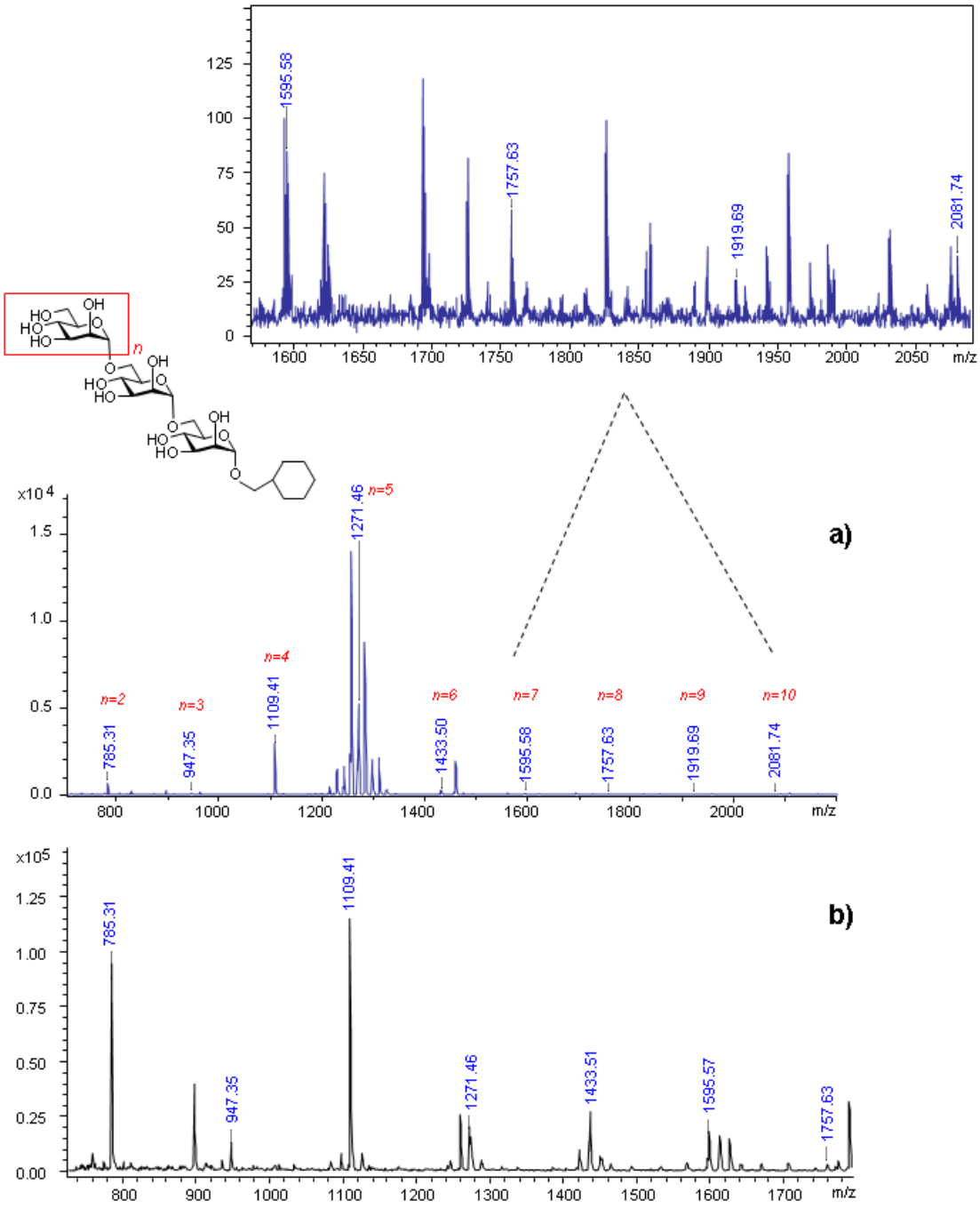

Figure S4. MALDI-MS spectra recorded from the sample **28** after SPE (**a**) and after additional microscale ZipTip fractionation (**b**). Peaks corresponding to consecutive mannose attachment are in a blue color and are detected as $[M+Na]^+$. Unlabeled peaks originated from the cell membrane protein of ManT.

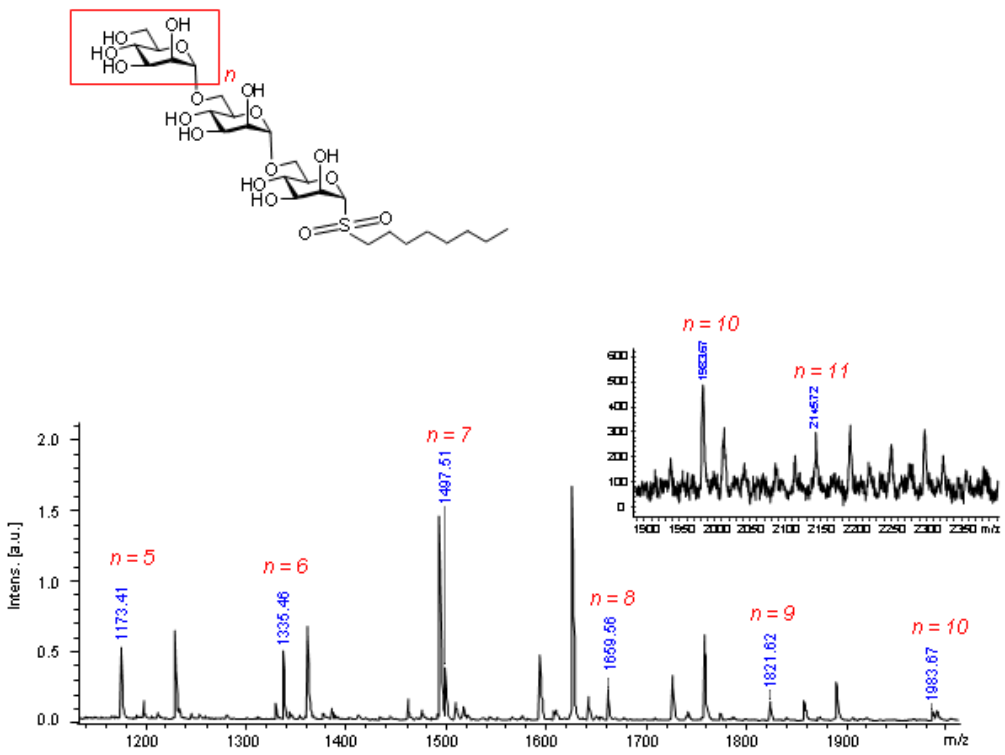

Figure S5. MALDI-MS spectra recorded from the enzymatic sample **30** after SPE fractionation (inset represents a spectrum obtained after additional ZipTip-C18 fractionation). Peaks corresponding to consecutive mannose attachment are in a blue color and are detected as $[M+Na]^+$. Unlabeled peaks originated from membrane protein of ManT.

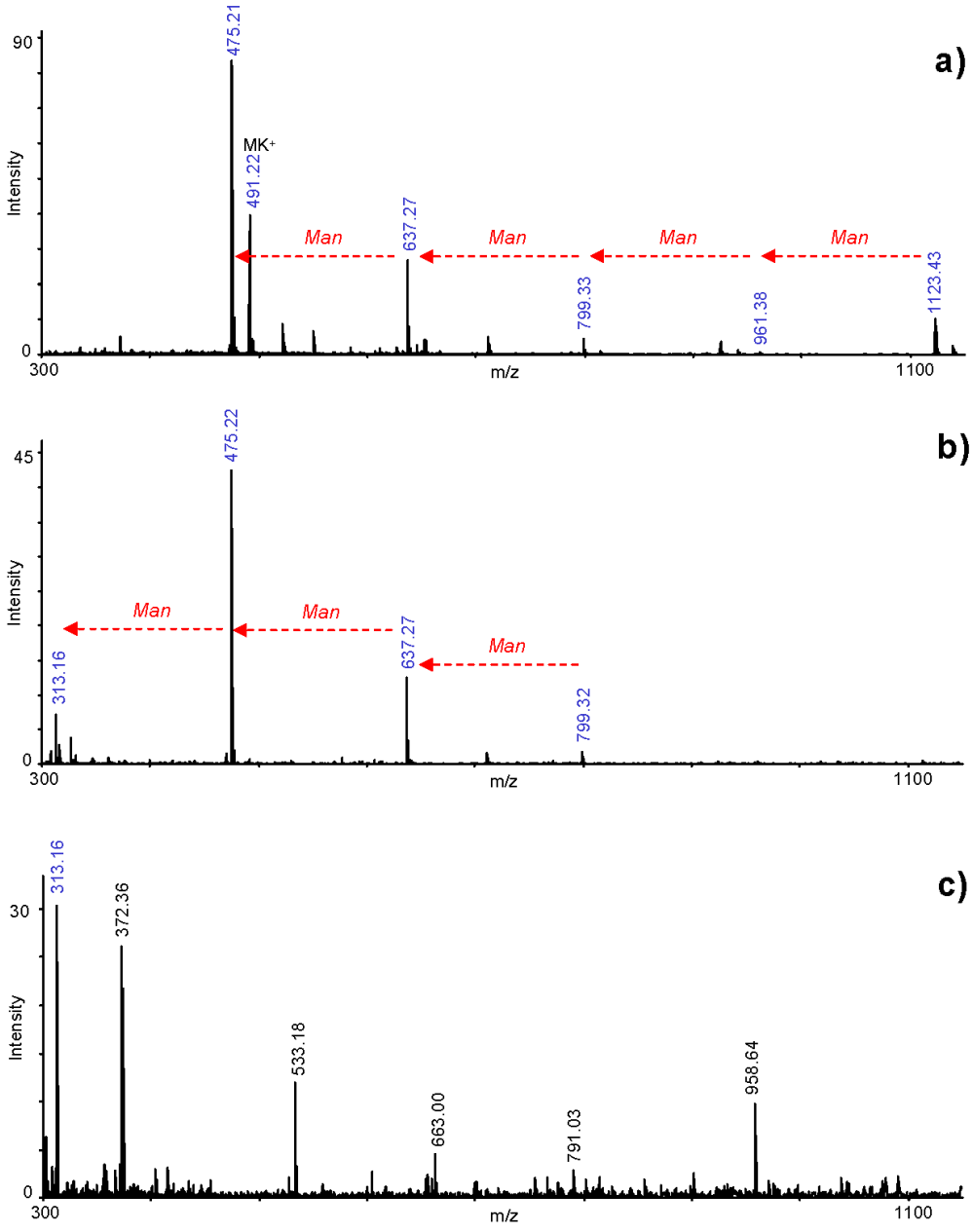

Figure S6. MALDI-MS spectra recorded from the sample **29**: (**a**) before treatment with mannosidase; (**b**) after incubation with mannosidase for 2h; and (**c**) after 8h incubation. All peaks are as $[M+Na]^+$. Peaks corresponding to saccharide adducts are in blue color.

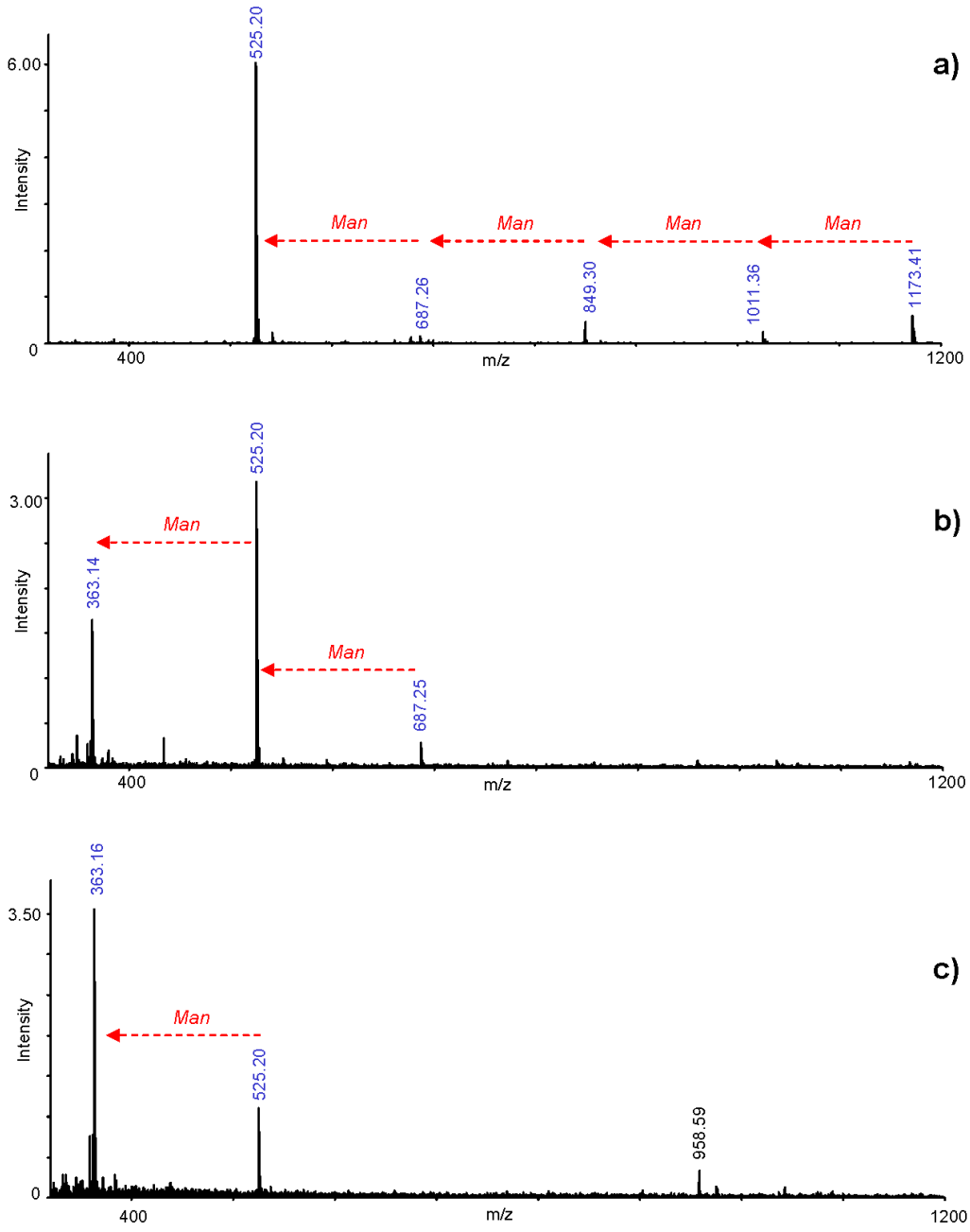

Figure S7. MALDI-MS spectra recorded from the sample **30**: (**a**) before treatment with mannosidase, (**b**) after incubation with mannosidase for 2h; and (**c**) after 8h incubation. All peaks are as $[M+Na]^+$. Peaks corresponding to saccharide adducts are in blue color.