Supporting Information

Probing the Substrate Specificity of Golgi α-mannosidase II using Synthetic Oligosaccharides and a Catalytic Nucleophile Mutant

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Experimental Procedures

Synthetic Procedures

General synthetic procedures: ^{1}H-NMR spectra were recorded in $CDCl_3$ or D_2O on a Varian Merc-300, Varian Inova-500, Inova-600 or Inova-800 spectrometers equipped with Sun workstations at 300K. TMS (δ_H=0.00) or D_2O (δ_H=4.67) was used as the internal reference. ^{13}C-NMR spectra were recorded in $CDCl_3$ or D_2O at 75 MHz on Varian Merc-300 spectrometer, using the central resonance of $CDCl_3$ (δ_C=77.0) as the internal reference. COSY, HSQC, HMBC and TOCSY experiments were used to assist assignment of the signals. Mass spectra were obtained on Bruker Daltonics 9.4T (FTICR, external calibration with BSA). Optical rotations was obtained on Jasco P-1020 polarimeter at 300 K. Chemicals were purchased from Aldrich or Fluka and used without further purification. DCM was distilled from

calcium hydride; THF from sodium; MeOH from magnesium and iodine. Aqueous solutions are saturated unless otherwise specified. Molecular sieves were activated at 350 • for 3 hrs *in vacuo*. All the reactions were performed under anhydrous conditions under argon and monitored by TLC on Kieselgel 60 F254 (Merck). Detection was by examination under UV light (254 nm) and by charring with 10 % sulfuric acid in methanol. Silica gel (Merck, 70-230 mesh) was used for column chromatography. Iatrobeads 6RS-8060 was purchased from Bioscan. Bio-Gel P-2 Gel was purchased from Bio-Rad Laboratories.

General procedure for glycosidations with thioglycosides 6, 8 and 16. A mixture of glycosyl acceptor (1 eq.), glycosyl donor (1.1 eq.) and 4Å powdered molecular sieves in DCM (0.06 mol/L) was stirred at 0° for 1h. Subsequently, NIS (1.1 eq.) and TfOH (0.2 eq.) were added. The reaction mixture was stirred at 0° for 1 hr, and then neutralized with triethylamine. The solution was filtered through celite, washed with MeOH/DCM (5:95, v/v), and the combined filtrates were concentrated to dryness. The residue was dissolved in DCM, and the solution was washed with $Na_2S_2O_3$ (1 M) and water. The organic layer was dried ($MgSO_4$), filtered, and concentrated to dryness. Purification of the crude product by column chromatography on silica gel afforded compounds **9**, **11**, **17** and **20**.

General procedure for synthesis of glycosyl trichloroimidate 18 and 22. To a solution of **17** or **21** (1 eq.) in DCM (0.12 mol/L) was added trifluoacetic acid under argon. The reaction mixture was stirred at room temperature for 5 hrs, and then concentrated to dryness. The residue was co-evaporated with EtOAc/toluene (1:1, *v*/*v*) and toluene (twice). To the resulting residue in anhydrous DCM (0.06 mol/L) under

argon was added trichloroacetonitrile (10 eq.) and DBU (0.2 eq.). The reaction mixture was stirred at room temperature for 2 h, and then concentrated to dryness. Purification of the crude product by fast column chromatography on silica gel afforded the trichloroimidates **18** or **22** as colorless oils.

General procedure for glycosidations with glycosyl trichloroimidates 18 and 22. A mixture of glycosyl acceptor (1 eq.), glycosyl donor (1.1 eq.) and 4Å powdered molecular sieves in DCM (0.06 mol/L) was stirred at 0° for 1 hr, and then cooled to -20°. Subsequently, TMSOTf (0.2 eq.) was added and the reaction mixture was allowed to warm to room temperature and stirred for 3 hrs. After neutralized with triethylamine, the mixture was filtered through celite, and the filtrate was washed with MeOH/DCM (5:95, v/v). The combined filtrates were concentrated *in vacuo*, and the residue was purified by column chromatography over silica gel to afford **23** or **25** as colorless oils.

General procedure for phthalimido removal followed by acetylation. To a solution of **23** or **25** (1 eq.) in EtOH (0.02 mol/L) was added $H_2NNH_2 \cdot H_2O$ (80 eq.). The reaction mixture was stirred at 90° for 24 hrs, and then concentrated to dryness. The residue was dissolved in Ac_2O (40 eq.) and pyridine (40 eq.). The reaction mixture was stirred at room temperature for 12 hrs, and then concentrated to dryness. Purification of the residue by column chromatography over silica gel afforded **24** or **26** as colorless oils.

General procedure for global deprotection. To a solution of **24**, **26**, **29**, **31** and **33** (1 eq.) in dry MeOH (0.06 mol/L) was added NaOMe (pH=8-10). The reaction

mixture was stirred for 18 hrs, and then neutralized by the addition of Dowex 650 H^+. The suspension was filtered through celite, and the filtrate was washed with MeOH/DCM (1:1, *v*/*v*). The combined filtrates were concentrated to dryness. Purification of the residue by Iatrobeads afforded the deacetylated product. A solution of the partially deprotected compound (1 eq.) in THF (0.02 mol/L) was added to NH_3 (l) at -78°. Small pieces of sodium were added until the reaction mixture remained blue. The reaction mixture was stirred at -78° for 20 min, and then added ammonium chloride until the blue color disappeared. The NH_3 (l) was allowed to evaporate slowly and the remaining solution was concentrated to dryness. The residue was dissolved with milli-Q water. The aqueous solution was loaded on a P2 column to afford **1**, **2**, **3**, **4** or **5** as white solids.

Methyl *O*-(2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→3)-2-*O*-benzyl-4, 6-di-*O*-benzylidene-α-D-mannopyranoside (9). Compound **9** was synthesized according to the general procedure for the glycosidation with thioglycoside **6**. Glycosyl donor **6** (1.70 g, 3.17 mmol), glycosyl acceptor **7** (1.07 g, 2.87 mmol) and 4Å powdered molecular sieves (2.80 g) in DCM (36 mL) in the presence of NIS (0.79 g, 3.49 mmol) and TfOH (58 μL, 0.64 mmol) gave **9** as colorless oil (1.95 g, 80%). R_f 0.42 (Hexane/EtOAc, 2:1, *v*/*v*); $[\alpha]_D^{27}$ +24.1 (*c* 0.34, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.02 (s, 3H, OC(O)CH_3), 3.26 (m, 1H, H-5_a), 3.44 (s, 3H, OCH_3), 3.60-3.74 (m, 4H, H-4_b, H-5_b, H-6_b, H-$6_{b'}$), 3.80-3.88 (m, 4H, H-2_a, H-3_a, H-6_a, H-3_b), 4.12(t, 1H, $J_{3,4}$=9.6, $J_{4,5}$=9.6 Hz, H-4_a), 4.25 (dd, 1H, $J_{5,6}$=4.8, $J_{6,6'}$=10.5 Hz, H-$6_{a'}$), 4.27 (s, 1H, H-1_a), 4.33-4.41 (m, 3H, PhCH*H*), 4.49 (d, 1H, *J*=12.0 Hz, PhCH*H*), 4.59 (d, 1H, *J*=11.4 Hz, PhCH*H*), 4.71 (d, 1H, *J*=12.3 Hz, PhCH*H*), 4.80 (d, 1H, *J*=12.3 Hz, PhCH*H*), 4.82 (d, 1H, *J*=10.5 Hz, PhCH*H*), 5.23 (d,

1H, $J_{1,2}$=1.8 Hz, H-1_b), 5.54 (s, 2H, H-1_b, PhC*H*), 7.08-7.37 (m, 25H, H_{arom}); ^{13}C NMR (75 MHz, $CDCl_3$): δ 21.0 (OC(O)*C*H_3), 57.4 (O*C*H_3), 67.3 (C-5_a), 68.1 (C-2_b), 68.5 (C-6_a), 69.0 (C-6_b), [71.6, 73.3, 74.9, 75.1 (Ph*C*H_2)], 72.0 (C-5_b), 74.2 (C-4_b), 75.0 (C-3_a), 77.9 (C-2_a), 78.0 (C-3_b), 78.7 (C-4_a), 99.8 (C-1_b), 101.1 (Ph*C*H), 103.2 (C-1_a), [125.9, 127.5, 127.6, 127.7, 128.0, 128.1, 128.2, 128.3, 128.4, 128.7, 137.2, 137.8, 138.2, 138.3, 138.5 (C_{arom})], 169.9 (O*C*(O)CH_3); MALDI-FTICR/MS: *m/z*: found $[M+Na]^+$ 869.3503, $C_{50}H_{54}O_{12}$ calcd for $[M+Na]^+$ 869.3513.

Methyl *O*-(3, 4, 6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→3)-2-*O*-benzyl-4, 6-di-*O*-benzylidene-β-D-mannopyranoside (10): To a solution of **9** (0.58 g, 0.67 mmol) in DCM/MeOH (6 mL: 6 mL, *v*/*v*) was added NaOMe (pH=8-10). The mixture was stirred at room temperature for 4 hrs, and then concentrated *in vacuo*. Purification of the residue by column chromatography over silica gel (Hexane/EtOAc, 3:1, *v*/*v*) afforded **10** as a white solid (0.49 g, 89%). R_f 0.31 (Hexane/EtOAc, 2:1, *v*/*v*). $[\alpha]_D^{27}$ -34.2 (*c* 0.31, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.27 (m, 1H, H-5_a), 3.42 (s, 3H, OCH_3), 3.59-3.76 (m, 4H, H-3_b, H-4_b, H-6_b, H-$6_{b'}$), 3.80-3.87 (m, 2H, H-2_a, H-6_a), 3.93 (dd, 1H, $J_{2,3}$=2.7, $J_{3,4}$=9.9 Hz, H-3_a), 4.03-4.09 (m, 2H, H-4_a, H-2_b), 4.22 (d, 1H, $J_{5,6}$=4.5 Hz, H-$6_{a'}$), 4.26 (s, 1H, H-1_a), 4.44 (d, 2H, *J*=11.4 Hz, PhCH*H*), 4.47 (d, 1H, *J*=12.0 Hz, PhCH*H*), 4.53 (d, 1H, *J*=11.4 Hz, PhCH*H*), 4.56 (d, 1H, *J*=11.4 Hz, PhCH*H*), 4.72 (d, 1H, *J*=12.0 Hz, PhCH*H*), 4.74 (d, 1H, *J*=11.1 Hz, PhCH*H*), 4.83 (d, 1H, *J*=12.3 Hz, PhCH*H*), 5.25 (d, 1H, $J_{1,2}$=1.5 Hz, H-1_b), 5.50 (s, 1H, PhC*H*), 7.06-7.39 (m, 25H, H_{arom}); ^{13}C NMR (75 MHz, $CDCl_3$): δ 57.4 (O*C*H_3), 67.3 (C-5_a), 68.0 (C-2_b), 68.6 (C-6_a), 69.2 (C-6_b), [71.8, 73.4, 74.9, 75.1 (Ph*C*H_2)], 71.9 (C-5_b), 74.3 (C-4_b), 75.2 (C-3_a), 77.8 (C-2_a), 78.7 (C-4_a), 79.9 (C-3_b), 100.3 (C-1_b), 101.5 (Ph*C*H), 103.3 (C-1_a), [125.9, 127.5, 127.6, 127.7, 127.8, 127.9, 128.1, 128.2, 128.3, 128.5,

128.9, 137.3, 137.7, 138.3, 138.4, 138.5 (C_{arom})]; MALDI-FTICR/MS: *m/z*: found $[M+Na]^+$ 827.3392, $C_{48}H_{52}O_{11}$ calcd for $[M+Na]^+$ 827.3407.

Methyl *O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→2)-*O*-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→3)-2-*O*-benzyl-4,6-di-*O*-benzylidene-β-D-mannopyranoside (11). Compound **11** was synthesized according to the general procedure for the glycosidation of thioglycosides. Glycosyl donor **8** (0.32 g, 0.67 mmol), glycosyl acceptor **10** (0.49 g, 0.61 mmol) and 4Å powdered molecular sieves (0.81 g) in DCM (20 mL) in the presence of NIS (0.17 g, 0.74 mmol) and TfOH (12 μL, 0.13 mmol) gave, after silica gel purification, **11** as colorless oil (0.56 g, 76%). R_f 0.57 (Hexane/EtOAc, 1:1, *v*/*v*). $[\alpha]_D^{27}$ -33.7 (*c* 0.31, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ [1.79, 1.93, 2.00 (3×s, 9H, OC(O)C*H*$_3$)], 2.19 (m, 1H, H-5_c), 2.61 (dd, 1H, $J_{5,6}$=6.6, $J_{6,6'}$=10.8 Hz, H-6_b), 3.20-3.44 (m, 4H, H-5_a, H-4_b, H-5_b, H-$6_{b'}$), 3.42 (s, 3H, OC*H*$_3$), 3.67-4.00 (m, 8H, H-2_a, H-3_a, H-4_a, H-6_a, H-2_b, H-3_b, H-6_c, H-$6_{c'}$), 4.24 (s, 1H, H-1_a), 4.21-4.27 (m, 4H, H-$6_{a'}$, H-2_c, PhCH*H*), 4.31 (d, 1H, *J*=11.7 Hz, PhCH*H*), 4.35 (d, 1H, *J*=11.4 Hz, PhCH*H*), 4.56 (d, 1H, *J*=12.0 Hz, PhCH*H*), 4.65 (d, 1H, *J*=13.5 Hz, PhCH*H*), 4.71 (d, 1H, *J*=12.6 Hz, PhCH*H*), 4.74 (d, 1H, *J*=10.8 Hz, PhCH*H*), 4.92-4.99 (m, 3H, H-1_b, H-1_c, H-4_c), 5.48 (s, 1H, PhC*H*), 5.52 (t, 1H, $J_{2,3}$=9.9, $J_{3,4}$=9.9 Hz, H-3_c), 6.96-7.67 (m, 29H, H_{arom}); ^{13}C NMR (75 MHz, $CDCl_3$): δ [20.5, 20.6, 20.7 (OC(O)*C*H$_3$)], 54.2 (C-2_c), 57.5 (O*C*H$_3$), 61.3 (C-6_c), 67.1 (C-5_a), 68.7 (C-4_c), 68.8 (C-6_a), 69.8 (C-6_b), 70.3 (C-3_c), 70.7 (C-5_c), [71.1, 74.8×3 (Ph*C*H$_2$)], 71.8 (C-2_b), 71.9 (C-5_b), 72.5 (C-3_a), 74.0 (C-4_b), 77.2 (C-2_a), 77.8 (C-3_b), 78.9 (C-4_a), 95.5 (C-1_c), 97.7 (C-1_b), 102.6 (Ph*C*H), 103.2 (C-1_a), [123.5, 123.6, 127.0, 127.2, 127.3, 127.5, 127.6, 127.8, 128.0, 128.1, 128.2, 128.3, 128.7, 130.1, 133.9, 137.6, 138.0, 138.3, 138.4, 138.7 (C_{arom})], [167.2, 167.8 (*C*=O, Phth)],

[169.2, 170.1, 170.7 (O*C*(O)CH_3)]; MALDI-FTICR/MS: *m/z*: found $[M+Na]^+$ 1244.4464, $C_{68}H_{71}NO_{20}$ calcd for $[M+Na]^+$ 1244.4467.

Methyl *O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→2)-*O*-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→3)-2,4-di-*O*-benzyl-β-D-mannopyranoside (12). To a flask containing **11** (0.21 g, 0.17 mmol) was added a solution of 1 M BH_3 in THF (1.7 mL) at 0℃, and the resulting solution was stirred for 5 min. Next, a solution of 1 M Bu_2BOTf in DCM (0.17 mL) was then added dropwise. After stirring for 30 min at 0℃, TLC analysis (Hexane/EtOAc, 1:1, *v*/*v*) showed the disappearance of starting material. Triethylamine (0.1 mL) was then added followed by careful addition of methanol until evolution of H_2 had ceased. The mixture was co-evaporated with methanol three times. Purification of the residue by column chromatography over silica gel afforded **12** as white solid (0.14 g, 67%). R_f 0.3 (Hexane/EtOAc, 1:1, *v*/*v*). $[\alpha]_D^{27}$ -135.0 (*c* 0.14, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 300 MHz): δ [1.78, 1.93, 1.97 (3×s, 9H, OC(O)C*H*$_3$)], 2.55 (m, 1H, H-5$_c$), 2.68 (dd, 1H, $J_{5,6}$=6.9, $J_{6,6'}$=10.8 Hz, H-6$_b$), 3.21-3.36 (m, 3H, H-5$_a$, H-4$_b$, H-6$_{b'}$), 3.35 (s, 3H, OC*H*$_3$), 3.49-3.97 (m, 10H, H-2$_a$, H-3$_a$, H-4$_a$, H-6$_a$, H-6$_{a'}$, H-2$_b$, H-3$_b$, H-5$_b$, H-6$_c$, H-6$_{c'}$), 4.28 (s, 1H, H-1$_a$), 4.26-4.33 (m, 2H, H-2$_c$, PhCH*H*), 4.42 (d, 1H, *J*=11.4 Hz, PhCH*H*), 4.52-4.78 (m, 8H, PhCH*H*), 4.92 (s, 1H, H-1$_b$), 4.98 (t, 1H, $J_{3,4}$=9.3, $J_{4,5}$=9.9 Hz, H-4$_c$), 5.08 (d, 1H, $J_{1,2}$=8.4 Hz, H-1$_c$), 5.55 (t, 1H, $J_{2,3}$=10.8, $J_{3,4}$=9.3 Hz, H-3$_c$), 6.98-7.54 (m, 29H, H_{arom}); ^{13}C NMR (75 MHz, $CDCl_3$): δ [20.5, 20.6, 20.7 (OC(O)*C*H$_3$)], 54.3 (C-2$_c$), 57.2 (O*C*H$_3$), 61.6 (C-6$_c$), 61.8 (C-6$_a$), 68.6 (C-4$_c$), 69.9 (C-6$_b$), 70.4 (C-3$_c$), [71.2, 73.9, 74.2, 74.6, 74.7 (Ph*C*H$_2$)], 72.2 (C-5$_c$), 72.7 (C-2$_b$, C-5$_b$), 73.3 (C-3$_a$), 74.1 (C-4$_b$), 74.6 (C-2$_a$), 75.7 (C-5$_a$), 77.7 (C-3$_b$), 80.6 (C-4$_a$), 96.0 (C-1$_c$), 98.8 (C-1$_b$), 102.6 (C-1$_a$), [123.5, 123.6, 126.3, 127.1, 127.3, 127.6, 127.7, 128.1, 128.2, 128.7,

131.5, 133.9, 138.0, 138.3, 138.4, 138.5, 138.6 (C_{arom})], [167.2, 167.8 (*C*=O, Phth)], [169.3, 170.1, 170.6 (O*C*(O)CH_3)]; MALDI-FTICR/MS: *m/z*: found $[M+Na]^+$ 1246.4624, $C_{68}H_{73}NO_{20}$ calcd for $[M+Na]^+$ 1246.4624.

2-(Trimethylsilyl)ethyl 2,3,4-tri-*O*-acetyl-6-*O*-triphenylmethyl-α-D-mannopyranoside (14). To a solution of **13** (0.62 g, 2.21 mmol) in pyridine (5 mL) was added TrCl (0.93 g, 3.34 mmol). The reaction mixture was stirred at 80℃ for 2 hrs, and then concentrated to dryness. To the solution of this residue in pyridine (7 mL) was added acetic anhydride (1.25 mL). The reaction mixture was stirred at room temperature for 6 hrs, and then concentrated to dryness. The residue was co-evaporated with methanol (3×20 mL) and toluene (3×20 mL). The residue was dissolved in DCM (30 mL), and then washed with 1N HCl (1×50 mL). The organic layer was dried ($MgSO_4$), filtered, and concentrated *in vacuo*. Purification of the residue by column chromatography over silica gel (Hexane/EtOAc, 3:1, *v*/*v*) afforded **14** as yellow oil (1.37 g, 96%). R_f 0.20 (Hexane/EtOAc, 3:1, *v*/*v*). $[\alpha]_D^{27}$ +30.1 (*c* 1.05, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.04-0.07 (3×s, 9H, SiCH_3), 0.98-1.06 (m, 2H, SiCH_2), 1.74, 1.97, 2.17 (3×s, 9H, OC(O)CH_3), 3.15-3.23 (m, 2H, H-6, H-6'), 3.63 (m, 2H, OCH_2), 3.88-3.97 (m, 2H, H-5, $OCH_{2'}$), 4.89 (s, 1H, H-1), 5.18-5.25 (m, 2H, H-2, H-4), 5.32 (dd, 1H, *J*=3.3, 10.2 Hz, H-3), 7.20-7.47 (m, 15H, H_{arom}); ^{13}C NMR (75 MHz, $CDCl_3$): δ -1.3, 1.0 (Si*C*H_3), 17.8 (*C*H_2Si), [20.6, 20.7, 20.9 (OC(O)*C*H_3)], 62.8 (C-6), 65.4 (O*C*H_2), 66.8 (C-4), 69.5 (C-3), 70.1 (C-2), 70.2 (C-5), 86.6 (Ph_3*C*), 96.6 (C-1), [126.9, 127.8, 128.7, 143.8 (C_{arom})], [169.4, 170.0, 170.2 (O*C*(O)CH_3)]; MALDI-FTICR/MS: *m/z*: found $[M+Na]^+$ 671.2636, $C_{36}H_{44}O_9Si$ calcd for $[M+Na]^+$ 671.2652.

2-(Trimethylsilyl)ethyl 2,3,4-tri-*O*-acetyl-α-D-mannopyranoside (15). To a solution of **14** (0.70 g, 1.08 mmol) in DCM (10 mL) was added $FeCl_3 \cdot 6H_2O$ (1.02 g, 3.77 mmol). The reaction mixture was stirred at room temperature for 1 hr, and then diluted with DCM (20 mL), and washed with water (30 mL).The organic layer was dried ($MgSO_4$), filtered, and concentrated *in vacuo*. Purification of the residue by column chromatography over silica gel (Hexane/EtOAc, 2:1, *v*/*v*) afforded **15** as white solid (0.36 g, 82%). R_f 0.35 (Hexane/EtOAc, 1:1, *v*/*v*). $[\alpha]_D^{27}$ +27.3 (*c* 0.12, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ -0.01-0.05 (3×s, 9H, SiC*H*$_3$), 0.92-0.98 (m, 2H, SiC*H*$_2$), [1.98, 2.06, 2.13 (3×s, 9H, OC(O)C*H*$_3$)], 3.47-3.82 (m, 5H, H-5, H-6, H-6', OCH_2), 4.82 (s, 1H, H-1), 5.18-5.25 (m, 2H, H-2, H-4), 5.38 (dd, 1H, $J_{2,3}$=3.3, $J_{3,4}$=10.2 Hz, H-3); ^{13}C NMR (75 MHz, $CDCl_3$): δ [-1.5×3 (Si*C*H$_3$)], 17.8 (*C*H$_2$Si), [20.6, 20.7, 20.8 (OC(O)*C*H$_3$)], 61.3 (C-6), 65.7 (O*C*H$_2$), 66.5 (C-4), 68.9 (C-3), 69.8 (C-2), 70.5 (C-5), 97.0 (C-1), [169.8, 170.1, 170.8 (O*C*(O)CH$_3$)]; MALDI-FTICR/MS: *m/z*: found $[M+Na]^+$ 429.1555, $C_{17}H_{30}O_9Si$ calcd for $[M+Na]^+$ 429.1557.

2-(Trimethylsilyl)ethyl *O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-(1→6)-2,3,4-tri-*O*-acetyl-α-D-mannopyranoside (17). Compound **17** was synthesized according to the general procedure for the glycosidation of thioglycosides. Glycosyl donor **16** (0.24 g, 0.61 mmol), glycosyl acceptor **15** (0.21 g, 0.52 mmol) and 4Å powdered molecular sieves (0.45 g) in DCM (8 mL) in the presence of NIS (0.15 g, 0.67 mmol) and TfOH (12 μL, 0.12 mmol) gave, after silica gel purification, **17** as colorless oil (0.29 g, 76%). R_f 0.31 (Hexane/EtOAc, 1:1, *v*/*v*). $[\alpha]_D^{27}$ +48.3 (*c* 0.16, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ [0.02×2, 0.05 (2×s, 9H, SiC*H*$_3$)], 0.92-0.99 (m, 2H, SiC*H*$_2$), [1.95, 1.97, 2.02, 2.04, 2.09, 2.10, 2.13, 2.14 (7×s, 21H, OC(O)C*H*$_3$)], 3.48-3.58 (m, 2H, H-6$_a$, OCH_2), 3.73-3.85 (m, 2H, H-6$_{a'}$, $OCH_{2'}$), 3.94 (m, 1H, H-5$_a$),

4.04-4.11 (m, 2H, H-5_b, H-6_b), 4.24 (dd, 1H, $J_{5,6}$=5.1, $J_{6,6'}$=12.0 Hz, H-$6_{b'}$), 4.78 (s, 1H, H-1_a), 4.84 (s, 1H, H-1_b), 5.18-5.36 (m, 6H, H-2_a, H-3_a, H-4_a, H-2_b, H-3_b, H-4_b); ^{13}C NMR (75 MHz, $CDCl_3$): δ [-1.5×2, 1.0 (Si*C*H_3)], 17.8 (*C*H_2Si), [20.6, 20.7, 20.9 (OC(O)*C*H_3)], 62.4 (C-6_b), 65.8 (O*C*H_2), 66.0 (C-4_b), 66.7 (C-4_a), 66.8 (C-3_b), 68.6 (C-6_a), 69.0 (C-5_b), 69.2 (C-3_a, C-5_a), 69.4 (C-2_b), 69.8 (C-2_a), 96.8 (C-1_a), 97.7 (C-1_b), [169.5, 169.9, 170.3, 170.6 (O*C*(O)CH_3)]; MALDI-FTICR/MS: *m/z*: found $[M+Na]^+$ 759.2487, $C_{31}H_{48}O_{18}Si$ calcd for $[M+Na]^+$ 759.2508.

Trichloroacetimidate *O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-(1→6)-2,3,4-tri-*O*-acetyl-α-D-mannopyranoside (18): Compound **18** was synthesized according to the general procedure for synthesis of glycosyl trichloroacetimidates. Treatment of **17** (0.25 g, 0.34 mmol) in DCM (3 mL) with trifluoacetic acid (5.9 mL, 79.43 mmol) followed by treatment of the residue in DCM (8 mL) with trichloroacetonitrile (0.34 mL, 3.4 mmol) and DBU (10 µL, 0.068 mmol) gave, after silica gel purification, **18** as colorless oil (0.22 g, 83%). R_f 0.45 (Hexane/EtOAc, 1:1, *v*/*v*). $[\alpha]_D^{27}$ +30.9 (*c* 0.75, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ [1.91, 1.95, 1.99, 2.02, 2.04, 2.08, 2.14 (7×s, 21H, OC(O)C*H*$_3$)], 3.58 (dd, 1H, $J_{5,6}$=2.4, $J_{6,6'}$=10.8 Hz, H-6_a), 3.73 (dd, 1H, $J_{5,6}$=5.7, $J_{6,6'}$=10.8 Hz, H-$6_{a'}$), 4.00-4.15 (m, 3H, H-5a, H-5b, H-6b), 4.24 (dd, 1H, $J_{5,6}$=4.8, $J_{6,6'}$=12.6 Hz, H-$6_{b'}$), 4.77 (d, 1H, $J_{1,2}$=1.5 Hz, H-1_b), 5.19-5.41 (m, 6H, H-2_a, H-3_a, H-4_a, H-2_b, H-3_b, H-4_b), 6.16 (d, 1H, $J_{1,2}$=1.5 Hz, H-1_a), 8.76 (s, 1H, OC(N*H*)CCl_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ [20.6, 20.7, 20.8, 20.9 (OC(O)*C*H_3)], 62.3 (C-6_b), 65.7 (C-4_a), 66.0 (C-4_b), 66.2 (C-6_a), 67.9 (C-2_a), 68.5 (C-5_b), 68.8 (C-3_a), 68.9 (C-2_b), 69.4 (C-3_b), 71.9 (C-5_a), 90.5 (OC(NH)*C*Cl_3), 94.3 (C-1_a), 97.5 (C-1_b), 159.7 (O*C*(NH)CCl_3), [169.6, 169.7, 169.8, 169.9, 170.0, 170.6 (O*C*(O)CH_3)].

2-(Trimethylsilyl)ethyl *S*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-(1→6)-2,3, 4-tri-*O*-acetyl-α-D-mannopyranoside (21): To a solution of **15** (0.35 g, 0.81 mmol) and 2,6-lutidine (0.13 ml, 1.08 mmol) in DCM (4 mL) was added trifluoromethanesulfonic anhydride (0.22 ml, 1.29 mmol) The reaction mixture was stirred at -40℃ for 1 hr, and then quenched by the addition of saturated aqueous $NaHCO_3$(5 mL). The resulting mixture was extracted with diethyl ether (3×10 mL). The combined organic phases were dried ($MgSO_4$), filtered, and the filtrate was concentrated *in vacuo* below 30℃. A solution of the residue and **20** (0.25 g, 0.62 mmol) in DMF (7 mL) was cooled (0℃) and placed under an atmosphere of argon. Liquid diethyl amine (0.3 mL) was added over a period of 5 min. The reaction mixture was stirred at 0℃ for 24 hrs and at room temperature for 5 hrs, and then concentrated to dryness. The residue was dissolved in EtOAc (20 mL), and the solution was washed with H_2O (20 mL) and brine (20 mL). The organic layer was dried ($MgSO_4$), filtered, and the filtrate was concentrated *in vacuo*. Purification of the residue by column chromatography over silica gel afforded **21** as colorless oil (0.34 g, 73%). R_f 0.29 (Hexane/EtOAc, 1:1, *v*/*v*). $[\alpha]_D^{27}$ +65.5 (*c* 0.67, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.04-0.07 (2×s, 9H, SiCH_3), 0.93-1.00 (m, 2H, SiCH_2), [1.99×2, 2.05×2, 2.12, 2.15, 2.16 (5×s, 21H, OC(O)CH_3)], 2.72 (dd, 1H, $J_{5,6}$=7.2, $J_{6,6'}$=13.8 Hz, H-6_a), 2.87 (dd, 1H, $J_{5,6}$=3.0, $J_{6,6'}$=13.8 Hz, H-$6_{a'}$), 3.55 (m, 1H, OCH_2), 3.85 (m, 1H, $OCH_{2'}$), 3.97 (m, 1H, H-5_a), 4.10 (dd, 1H, $J_{5,6}$=1.8, $J_{6,6'}$=12.0 Hz, H-6_b), 4.31 (dd, 1H, $J_{5,6}$=4.8, $J_{6,6'}$=12.0 Hz, H-$6_{b'}$), 4.39 (m, 1H, H-5_b), 4.78 (s, 1H, H-1_a), 5.18-5.36 (m, 7H, H-2_a, H-3_a, H-4_a, H-1_b, H-2_b, H-3_b, H-4_b); ^{13}C NMR (75 MHz, $CDCl_3$): δ [-1.4×2, 1.0 (SiCH_3)], 17.8 (CH_2Si), [20.6, 20.7, 20.8, 20.9 (OC(O)CH_3)], 32.0 (C-6_a), 62.3 (C-6_b), 65.9 (OCH_2), 66.2 (C-4_a), [68.8, 69.0, 69.1 (C-3_a, C-3_b, C-4_b)], 69.3 (C-

5_b), 69.5 (C-5_a), 69.8 (C-2_a), 70.8 (C-2_b), 82.6 (C-1_b), 96.8 (C-1_a), [169.6, 169.7, 169.8, 169.9, 170.0, 170.2, 170.6 (O*C*(O)CH_3)]; MALDI-FTICR/MS: *m/z*: found $[M+Na]^+$ 775.2271, $C_{31}H_{48}O_{17}SSi$ calcd for $[M+Na]^+$ 775.2279.

Trichloroacetimidate *S*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-(1→6)-2,3,4-tri-*O*-acetyl-α-D-mannopyranoside (22): Compound **22** was synthesized according to the general procedure for synthesis of glycosyl trichloroacetimidates. Treatment of **21** (0.21 g, 0.28 mmol) in DCM (2.5 mL) with trifluoacetic acid (4.9 mL, 65.97 mmol) followed by reaction of the residue with trichloroacetonitrile (0.28 mL, 2.8 mmol) and DBU (8.2 µL, 56 µmol) in DCM (6 mL) gave, after silica gel purification, **22** as colorless oil (0.19 g, 86%). R_f 0.70 (Hexane/EtOAc, 3:2, *v*/*v*). $[\alpha]_D^{27}$ +65.2 (*c* 0.95, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ [1.91, 1.94, 1.98, 2.00, 2.04, 2.09, 2.13 (7×s, 21H, OC(O)C*H*$_3$)], 2.64 (dd, 1H, $J_{5,6}$=6.0, $J_{6,6'}$=14.4 Hz, H-6_a), 2.88 (dd, 1H, $J_{5,6}$=2.7, $J_{6,6'}$=14.4 Hz, H-$6_{a'}$), 4.04 (dd, 1H, $J_{5,6}$=4.5, $J_{6,6'}$=11.7 Hz, H-6_b), 4.11 (m, 1H, H-5_a), 4.22 (dd, 1H, $J_{5,6}$=4.8, $J_{6,6'}$=11.7 Hz, H-$6_{b'}$), 4.30 (m, 1H, H-5_b), 5.16-5.38 (m, 7H, H-2_a, H-3_a, H-4_a, H-1_b, H-2_b, H-3_b, H-4_b), 6.17 (d, 1H, $J_{1,2}$=1,5 Hz, H-1_a), 8.72 (s, 1H, OC(N*H*)CCl_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ [20.6, 20.7, 20.9 (OC(O)*C*H_3)], 31.5 (C-6_a), 62.3 (C-6_b), 66.2 (C-4_a), [67.7, 67.8, 68.6 (C-3_a, C-3_b, C-4_b)], 69.1 (C-5_b), 69.3 (C-2_b), 70.8 (C-2_a), 72.2 (C-5_a), 82.5 (C-1_b), 90.5 (OC(NH)*C*Cl_3), 94.2 (C-1_a), 159.6 (O*C*(NH)CCl_3), [169.7, 169.8, 169.9, 170.6 (O*C*(O)CH_3)].

Methyl *O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→2)-*O*-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→3)-*O*-[(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-(1→6)-*O*-(2,3,4-tri-*O*-acetyl-α-D-mannopyranosyl)-(1→6)]-

2,4-di-*O*-benzyl-β-D-mannopyranoside (23): Compound **23** was synthesized according to the general procedure for the glycosidation with glycosyl trichloroacetimidates. Glycosyl donor **18** (68.0 mg, 87.1 μmol), glycosyl acceptor **12** (96.8 mg, 79.1 μmol) and 4Å powdered molecular sieves (0.17 g) in DCM (4 mL) in the presence of TMSOTf (3.2 μL, 17.7 μmol) gave, after silica gel purification, **23** as colorless oil (116.7 mg, 80%). R_f 0.28 (Hexane/EtOAc, 2:3, *v*/*v*). $[\alpha]_D^{27}$ +24.9 (*c* 0.78, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$): δ [1.90, 1.93×2, 1.94, 1.96×3, 2.02, 2.07, 2.08 (7×s, 30H, OC(O)C*H*$_3$)], 2.45 (m, 1H, H-5$_c$), 2.70 (dd, 1H, $J_{5,6}$=7.5, $J_{6,6'}$=10.5 Hz, H-6$_b$), 3.27 (d, 1H, $J_{6,6'}$=10.5 Hz, H-6$_{b'}$), 3.36 (s, 3H, OC*H*$_3$), 3.31-3.40 (m, 2H, H-4b, H-5a), 3.44-3.50 (m, 2H, H-5b, H-6d), 3.56-3.75 (m, 6H, H-2$_a$, H-4$_a$, H-6$_a$, H-6$_{a'}$, H-3$_b$, H-6$_{d'}$), 3.78-3.89 (m, 4H, H-3$_a$, H-2$_b$, H-6$_c$, H-5$_d$), 3.91-3.95 (m, 2H, H-6$_{c'}$, H-5$_e$), 4.00 (d, 1H, $J_{6,6'}$=12.0 Hz, H-6$_e$), 4.12 (s, 1H, H-1a), 4.21 (dd, 1H, $J_{5,6}$=5.0, $J_{6,6'}$=12.0 Hz, H-6$_{e'}$), 4.26-4.31 (m, 2H, H-2$_c$, PhCH*H*), 4.30 (d, 1H, *J*=11.0 Hz, PhCH*H*), 4.53-4.57 (m, 5H, PhCH*H*), 4.68 (d, 1H, *J*=13.0 Hz, PhCH*H*), 4.76 (s, 1H, H-1$_d$), 4.77-4.79 (m, 2H, PhCH*H*), 4.83 (s, 1H, H-1$_e$), 4.89 (s, 1H, H-1$_b$), 4.97 (t, 1H, J3, 4=9.5, J4, 5=10.0 Hz, H-4c), 5.02 (d, 1H, J1, 2=8.5 Hz, H-1c), 5.19-5.31 (m, 6H, H-2$_d$, H-3$_d$, H-4$_d$, H-2$_e$, H-3$_e$, H-4$_e$), 5.53 (t, 1H, $J_{2,3}$=10.5, $J_{3,4}$=9.5 Hz, H-3$_c$), 6.98-7.54 (m, 29H, H$_{arom}$); ^{13}C NMR (75 MHz, $CDCl_3$): δ [20.5, 20.6, 20.8 (OC(O)*C*H$_3$)], 54.2 (C-2$_c$), 57.1 (O*C*H$_3$), 61.6 (C-6$_c$), 62.4 (C-6$_e$), [66.0, 66.2 (C-4$_d$, C-4$_e$), [66.2, 66.4 (C-6$_a$, C-6$_d$)], 68.5 (C-4$_c$), [68.6, 68.9 (C-5$_d$, C-5$_e$)], [69.0, 69.2, 69.3, 69.4 (C-2$_d$, C-3$_d$, C-2$_e$, C-3$_e$)], 69.8 (C-6$_b$), 70.4 (C-3$_c$), 71.2 (C-5$_c$), 72.2 (C-5$_b$), 72.7 (C-2$_b$), 73.4 (C-3$_a$), 74.1 (C-4$_b$), [71.3, 73.9, 74.0×2, 74.7 (Ph*C*H$_2$×5)], 75.0 (C-5$_a$), 75.1 (C-2$_a$), 77.7 (C-3$_b$), 80.8 (C-4$_a$), 96.0 (C-1$_c$), 97.2 (C-1$_e$), 97.8 (C-1$_d$), 98.9 (C-1$_b$), 102.6 (C-1$_a$), [126.3, 127.1, 127.2, 127.3, 127.7, 128.1, 128.2, 128.3, 128.7, 138.0, 138.3, 138.4, 138.5, 138.7 (C$_{arom}$)], [169.3, 169.5, 169.7, 169.9, 170.0, 170.1, 170.6, 170.7

(O*C*(O)CH_3)]. MALDI-FTICR/MS: *m/z*: found $[M+Na]^+$ 1864.6423, $C_{94}H_{107}NO_{37}$ calcd for $[M+Na]^+$ 1864.6420.

Methyl *O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-acetamido-β-D-glucopyranosyl)-(1→2)-*O*-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→3)-*O*-[(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-(1→6)-*O*-(2,3,4-tri-*O*-acetyl-α-D-mannopyranosyl)-(1→6)]-2,4-di-*O*-benzyl-β-D-mannopyranoside (24): Compound **24** was synthesized according to the general procedure for the phthalimido removal. Treatment of **23** (88.2 mg, 47.9 μmol) in EtOH (2 mL) with $H_2NNH_2 \cdot H_2O$ (0.2 mL, 4.12 mmol) followed by acetylation with Ac_2O (0.5 mL) and pyridine (0.5 mL) gave, after silica gel purification, **24** as colorless oil (68.1 mg, 81%). R_f 0.22 (Hexane/EtOAc, 1:5, *v*/*v*). $[\alpha]_D^{27}$ +62.2 (*c* 0.1, $CHCl_3$,). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.73 (s, 3H, NHC(O)C*H*$_3$), [1.92, 1.95×2, 1.98×2, 1.96×2, 2.03, 2.07×2 (6×s, 30H, OC(O)C*H*$_3$)], 2.70 (m, 1H, H-6$_b$), 3.41 (s, 3H, OC*H*$_3$), 3.31-3.67 (m, 7H), 3.77-4.11 (m, 10H), 4.18-4.22 (m, 2H), 4.31-4.46 (m, 5H), 4.59-4.85 (m, 11H), 4.93-4.98 (m, 3H), 5.18-5.32 (m, 6H), 7.05-7.37 (m, 25H); MALDI-FTICR/MS: *m/z*: found $[M+Na]^+$ 1776.6477, $C_{88}H_{107}NO_{36}$ calcd for $[M+Na]^+$ 1776.6471.

Methyl *O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→2)-*O*-(3, 4, 6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→3)-*O*-[(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-(1→6)-*S*-(2,3,4-tri-*O*-acetyl-α-D-mannopyranosyl)-(1→6)]-2,4-di-*O*-benzyl-β-D-mannopyranoside (25). Compound **25** was synthesized according to the general procedure for the glycosidation with glycosyl trichloroacetimidates. Glycosyl donor **22** (86.0 mg, 107.9 μmol), glycosyl acceptor **12** (120.1 mg, 98.1 μmol) and 4Å powdered molecular sieves (0.21 g) in DCM (4 mL) in

the presence of TMSOTf (3.9 µL, 21.5 µmol) gave, after silica gel purification, **25** as colorless oil (151.3 mg, 83%). R_f 0.24 (Hexane/EtOAc, 2:3, *v/v*). $[\alpha]_D^{27}$ +36.0 (*c* 0.6, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ [1.92×2, 1.94, 1.96, 1.97×2, 1.98, 2.03, 2.05, 2.07 (8×s, 30H, OC(O)C*H*$_3$)], 2.44 (m, 1H, H-5$_c$), 2.57 (dd, 1H, J5, 6=5.7, J6, 6'=13.8 Hz, H-6d), 2.68 (dd, 1H, $J_{5,6}$=7.2, $J_{6,6'}$=10.8 Hz, H-6$_b$), 2.77 (d, 1H, J6, 6'=13.8 Hz, H-6d'), 3.27 (d, 1H, $J_{6,6'}$=10.8 Hz, H-6$_{b'}$), 3.36 (s, 3H, OC*H*$_3$), 3.29-3.38 (m, 2H, H-4b, H-5a), 3.46-4.02 (m, 12H, H-2$_a$, H-3a, H-4$_a$, H-6$_a$, H-6$_{a'}$, H-2b, H-3$_b$, H-5b, H-6c, H-6c', H-5d, H-6$_e$), 4.11 (s, 1H, H-1a), 4.23-4.31 (m, 4H, H-2$_c$, H-5e, H-6$_{e'}$, PhCH*H*), 4.44 (d, 1H, *J*=11.1 Hz, PhCH*H*), 4.53-4.58 (m, 5H, PhCH*H*), 4.68 (d, 1H, *J*=13.2 Hz, PhCH*H*), 4.75 (s, 1H, H-1$_d$), 4.76-4.82 (m, 2H, PhCH*H*), 4.89 (s, 1H, H-1$_b$), 4.96 (t, 1H, J3, 4=9.3, J4, 5=10.2 Hz, H-4c), 5.01 (d, 1H, J1, 2=8.1 Hz, H-1c), 5.17-5.29 (m, 7H, H-2$_d$, H-3$_d$, H-4$_d$, H-1e, H-2$_e$, H-3$_e$, H-4$_e$), 5.52 (t, 1H, $J_{2,3}$=10.5, $J_{3,4}$=9.3 Hz, H-3$_c$), 6.84-7.54 (m, 29H, H_{arom}); ^{13}C NMR (125 MHz, $CDCl_3$): δ [20.5, 20.6, 20.7, 20.8, 20.9, 21.0 (OC(O)*C*H$_3$)], 32.2 (C-6$_d$), 54.2 (C-2$_c$), 57.1 (O*C*H$_3$), 62.3 (C-6$_c$), 62.4 (C-6$_e$), [66.2×2, 66.3 (C-6$_a$, C-4$_d$, C-4$_e$)], 68.4 (C-4$_c$), [68.6, 68.9 (C-5$_d$, C-5$_e$)], [69.0, 69.2, 69.4, 69.5 (C-2$_d$, C-3$_d$, C-2$_e$, C-3$_e$)], 70.4 (C-6$_b$), 70.8 (C-3$_c$), 71.2 (C-5$_c$), 72.3 (C-5$_b$), 72.7 (C-2$_b$), 73.4 (C-3$_a$), 74.1 (C-4$_b$), [71.4, 73.9, 74.0×2, 74.7 (Ph*C*H$_2$×5)], 74.9 (C-5$_a$), 75.2 (C-2$_a$), 77.7 (C-3$_b$), 80.8 (C-4$_a$), 82.5 (C-1$_e$), 96.0 (C-1$_c$), 97.2 (C-1$_d$), 98.9 (C-1$_b$), 102.6 (C-1$_a$), [126.3, 127.1, 127.2, 127.3, 127.7, 128.1, 128.2, 128.3, 128.7, 138.0, 138.3, 138.4, 138.5, 138.7 (C_{arom})], [169.3, 169.7, 169.8, 169.9, 170.0, 170.1, 170.6, 170.7, 170.8, 171.1 (O*C*(O)CH$_3$)]. MALDI-TOF/MS: *m/z*: found $[M+Na]^+$ 1880.7, MALDI-FTICR/MS: *m/z*: found $[M+Na]^+$ 1880.6178, $C_{94}H_{107}NO_{36}S$ calcd for $[M+Na]^+$ 1880.6191.

Methyl *O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-acetamido-β-D-glucopyranosyl)-(1→2)-*O*-

(3, 4, 6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→3)-*O*-[(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-(1→6)-*S*-(2,3,4-tri-*O*-acetyl-α-D-mannopyranosyl)-(1→6)]-2,4-di-*O*-benzyl-β-D-mannopyranoside (26). Compound **26** was synthesized according to the general procedure for the phthalimido removal. Treatment of **25** (96.3 mg, 51.8 μmol) in EtOH (2 mL) with $H_2NNH_2 \cdot H_2O$ (0.2 mL, 4.12 mmol) followed by acetylation with Ac_2O (0.5 mL) and pyridine (0.5 mL) gave, after silica gel purification, **26** as colorless oil (81.6 mg, 89%). R_f 0.18 (Hexane/EtOAc, 1:3, *v*/*v*). $[\alpha]_D^{27}$ +94.9 (*c* 0.28, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.73 (s, 3H, NHC(O)CH_3), [1.92×4, 1.95, 1.96, 1.98, 2.03, 2.05, 2.06 (7×s, 30H, OC(O)CH_3)], 2.58 (dd, 1H, $J_{5,6}$=6.3, $J_{6,6'}$=13.8 Hz, H-6_d), 2.66-2.76 (m, 2H, H-6_b, H-$6_{d'}$), 3.42 (s, 3H, OCH_3), 3.38-3.82 (m, 10H), 3.88-4.02 (m, 5H), 4.12 (d, 1H, J=7.8 Hz), 4.22-4.46 (m, 7H), 4.59-4.71 (m, 5H), 4.81-4.86 (m, 4H), 4.95-5.02 (m, 3H), 5.18-5.31 (m, 7H), 7.04-7.40 (m, 25H); MALDI-FTICR/MS: *m/z*: found $[M+Na]^+$ 1792.6227, $C_{88}H_{107}NO_{35}S$ calcd for $[M+Na]^+$ 1792.6242.

Methyl *O*-(2-deoxy-2-acetamido-β-D-glucopyranosyl)-(1→2)-*O*-(α-D-mannopyranosyl)-(1→3)-*O*-[(α-D-mannopyranosyl)-(1→6)-*S*-(α-D-mannopyranosyl)-(1→6)]-β-D-mannopyranoside (4). Compound **4** was synthesized according to the general procedure for the global deprotection. Treatment of **26** (78.8 mg, 44.5 μmol) in MeOH/DCM (0.5 mL: 0.5 mL, v/v) with NaOMe (pH=8-10) gave deacetylated product (60.1 mg, quantitive). Treatment of the partially deprotected compound (60.1 mg, 44.5 μmol) in THF (1 mL) with Na (s) in NH_3 (l) gave **4** as white solid (34.4 mg, 86%). ^{1}H NMR (800 MHz, D_2O): δ 1.92 (s, 3H, NHC(O)CH_3), 2.64 (dd, 1H, $J_{5,6}$=8.8, $J_{6,6'}$=14.4 Hz, H-6_d), 3.06 (d, 1H, $J_{6,6'}$=14.4 Hz, H-$6_{d'}$), 3.32-3.34 (m, 2H), 3.39 (m, 1H), 3.40 (s, 3H, OCH_3), 3.43-3.45 (m, 2H, H-4_a, H-3_c), 3.49-

3.52 (m, 2H), 3.55-3.58 (m, 2H, H-2_c, H-4_d), 3.61-3.64 (m, 4H), 3.66-3.72 (m, 6H), 3.77-3.80 (m, 3H), 3.88 (s, 1H, H-2_d), 3.89-3.93 (m, 2H), 3.94 (s, 1H, H-2_e), 4.02 (d, 1H, *J*=3.2 Hz, H-2_a), 4.07 (s, 1H, H-2_b), 4.42 (d, 1H, $J_{1,2}$=8.8 Hz, H-1_c), 4.47 (s, 1H, H-1_a), 4.76 (s, 1H, H-1_d), 5.00 (s, 1H, H-1_b), 5.22 (s, 1H, H-1_e); ^{13}C NMR (75 MHz, D_2O): δ 22.5 (NHC(O)*C*H_3), 31.5 (C-6_d), 55.5 (C-2_c), 57.0 (O*C*H_3), 60.8, 61.0, 61.8, 65.5, 66.0, 67.3 (C-4_d), 67.4, 69.6, 69.7, 70.0 (C-4_c), 70.1 (C-2_d), 70.2 (C-2_a), 70.7, 71.0, 71.2 (C-2_e), 73.3, 73.5, 73.6, 74.1, 76.0, 76.6 (C-2_b), 80.7, 84.3 (C-1_e), [99.5, 99.6 (C-1_b, C-1_d)], 99.8 (C-1_c), 101.2 (C-1_a), 175.0 (NH*C*(O)CH_3); 1D Coupling HSQC (500 MHz, D_2O): $J_{H\text{-}1a\text{-}C\text{-}1a}$=159.8 Hz, $J_{H\text{-}1b\text{-}C\text{-}1b}$=171.0 Hz, ($J_{H\text{-}1d\text{-}C\text{-}1d}$, $J_{H\text{-}1e\text{-}C\text{-}1e}$ =172.0, 168.3 Hz). MALDI-FTICR/MS: *m/z*: found $[M+Na]^+$ 922.2837, $C_{25}H_{44}NO_{20}S$ calcd for $[M+Na]^+$ 922.2838.

Methyl *O*-(2-deoxy-2-acetamido-β-D-glucopyranosyl)-(1→2)-*O*-(α-D-mannopyranosyl)-(1→3)-*O*-[(α-D-mannopyranosyl)-(1→6)-*O*-(α-D-mannopyranosyl)-(1→6)]-β-D-mannopyranoside (5). Compound **5** was synthesized according to the general procedure for the global deprotection. Treatment of **24** (67.1 mg, 38.2 μmol) in MeOH/DCM (0.5 mL: 0.5 mL, v/v) with NaOMe (pH=8-10) gave deacetylated product (51.0 mg, quantitative). Treatment of the partially deprotected compound (51.0 mg, 38.2 μmol) in THF (1 mL) with Na (s) in NH_3 (l) gave **5** as white solid (29.4 mg, 87%). ^{1}H NMR (600 MHz, D_2O): δ 1.94 (s, 3H, NHC(O)C*H*$_3$), 3.34-3.40 (m, 2H), 3.42 (s, 3H, OC*H*$_3$), 3.43-3.50 (m, 2H), 3.50-3.75 (m, 14H), 3.78-3.87 (m, 6H), 3.90 (m, 1H), 4.04 (d, 1H, *J*=3.0 Hz, H-2_a), 4.09 (s, 1H, H2-$_b$), 4.44 (d, 1H, $J_{1,2}$=8.4 Hz, H-1_c), 4.48 (s, 1H, H-1_a), [4.79, 4.80 (2×s, 2H, H-1_d, H-1_e)], 5.01 (s, 1H, H-1_b); ^{13}C NMR (125 MHz, D_2O): δ 22.5 (NHC(O)*C*H_3), 55.5 (C-2_c), 57.0 (O*C*H_3), 60.8, 61.1, 61.8, 65.5, 65.8, 66.0, 66.7, 66.9, 67.4, 69.6, 70.0, 70.1, 70.2×2,

70.7, 70.9, 71.0, 72.9, 73.5, 73.6, 74.1, 76.0, 76.7 (C-2_b), 80.8, [99.5, 99.6×2 (C-1_b, C-1_d, C-1_e)], 99.8 (C-1_c), 101.2 (C-1_a), 175.0 (NH*C*(O)CH_3); 1D Coupling HSQC (500 MHz, D_2O): $J_{H\text{-}1a\text{-}C\text{-}1a}$=160.3 Hz, $J_{H\text{-}1b\text{-}C\text{-}1b}$=172.0 Hz, ($J_{H\text{-}1d\text{-}C\text{-}1d}$, $J_{H\text{-}1e\text{-}C\text{-}1e}$ =171.5, 173.1 Hz). MALDI-FTICR/MS: *m/z*: found $[M+Na]^+$ 906.3064, $C_{25}H_{44}NO_{20}S$ calcd for $[M+Na]^+$ 906.3067.

Methyl 2-*O*-benzyl-4,6-di-*O*-benzylidene-α-D-altropyranoside (28). To a solution of **27** (0.93 g, 2.5 mmol) in DMSO (5 mL) was added Ac_2O/DMSO (10 mL, 1:2, *v*/*v*) and the mixture was stirred for 16 hrs, and then concentrated to dryness. To a solution of the residue in DCM/MeOH (60 mL 1:1, *v*/*v*) was added $NaBH_4$ (4.60 g, 0.12 mol). The reaction mixture was stirred at 5-10 ℃ for 5 hrs, and then concentrated to dryness. The residue was dissolved in DCM (60 mL), and the solution was washed with 5% citric acid solution (60 mL), saturated $NaHCO_3$ (60 mL) and water (60 mL). The organic layer was dried ($MgSO_4$), filtered, and the filtrate was concentrated to dryness. Purification of the crude product by column chromatography over silica gel afforded **28** as colorless oil (0.70 g, 75%). R_f 0.30 (Hexane/EtOAc, 3:1, *v*/*v*). ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.81 (d, 1H, *J*=7.2 Hz, OH), 3.35 (s, 3H, OCH_3), 3.66 (d, 1H, $J_{2,3}$=3.0 Hz, H-2), 3.76 (t, 1H, $J_{5,6}$=10.2, $J_{6,6'}$=10.2 Hz, H-6), 3.92 (dd, 1H, $J_{3,4}$=3.0, $J_{4,5}$=9.6 Hz, H-4), 4.07-4.15 (m, 2H, H-3, H-5), 4.27 (dd, 1H, $J_{5,6}$=4.8, $J_{6,6'}$=10.2 Hz, H-6'), 4.58 (dd, 2H, *J*=12.0 Hz, PhCH*H*), 4.66 (s, 1H, H-1), 5.57 (s, 1H, PhC*H*), 7.18-7.44 (m, 10H, H_{arom}); ^{13}C NMR (75 MHz, $CDCl_3$): δ 5 5.8 (O*C*H_3), [58.5, 67.3 (C-3, C-5)], 69.4 (C-6), 72.8 (Ph*C*H_2), 76.8 (C-4), 76.9 (C-2), 100.3 (C-1), 102.5 (Ph*C*H), [126.2, 127.8, 128.1, 128.3, 128.6, 129.1, 137.2, 137.3 (C_{arom})]; MALDI-TOF/MS: *m/z*: found $[M+Na]^+$ 395.1, $C_{21}H_{24}O_6$ calcd for $[M+Na]^+$ 395.1471.

Methyl *S*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-(1→3)-2-*O*-benzyl-4,6-di-*O*-benzylidene-α-D-mannopyranoside (29). To a solution of **27** (0.14 g, 0.38 mmol) and freshly distilled pyridine (2.9 mL) in dry DCM (5.8 mL) was added trifluoromethanesulfonic anhydride (0.1 ml, 0.57 mmol) at -20℃. The reaction mixture was stirred for 2 hrs at room temperature, and then quenched by the addition of H_2O (0.2 mL). The resulting mixture was extracted with EtOAc (3×80 mL) and washed with 1 N HCl (70 mL), saturated $NaHCO_3$ (70 mL) and H_2O (70 mL). The combined organic phases were dried ($MgSO_4$), filtered, and the filtrate was concentrated to dryness below 30℃. To a solution of this residue and **20** (0.13 g, 0.32 mmol) in DMF (3 mL) at 0℃ under an atmosphere of argon was added liquid diethyl amine (0.15 mL) over a period of 5 min. The reaction mixture was stirred at 0℃ for 24 hrs and at room temperature for 5 hrs, and then concentrated to dryness. The residue was dissolved in EtOAc (30 mL), and the solution was washed with H_2O (30 mL) and brine (30 mL). The organic layer was dried ($MgSO_4$), filtered, and the filtrate was concentrated to dryness. Purification of the crude product by column chromatography over silica gel (toluene/acetone, 10:1, *v*/*v*) afforded the **29** as colorless oil (0.14 g, 61%). R_f 0.33 (toluene/acetone, 9:1, *v*/*v*). ^{1}H NMR (300 MHz, $CDCl_3$): δ [1.90, 1.95, 1.98, 2.01 (4×s, 12H, OC(O)CH_3)], 3.28 (s, 3H, OCH_3), 3.42 (dd, 1H, $J_{2,3}$=3.0, $J_{3,4}$=10.8 Hz, H-3_a), 3.64 (d, 1H, $J_{2,3}$=3.0 Hz, H-2_a), 3.67-3.77 (m, 2H, H-5_a, H-6_a), 3.97-4.05 (m, 2H, H-4_a, H-6_b), 4.12-4.25 (m, 3H, H-$6_{a'}$, H-5_b, H-$6_{b'}$), 4.60 (s, 1H, H-1_a), 4.55-4.64 (dd, 2H, *J*=12.0 Hz, PhCH*H*), 5.13-5.15 (m, 2H, H-3_b, H-4_b), 5.37 (s, 1H, H-2_b), 5.53 (s, 1H, PhC*H*), 5.59 (s, 1H, H-1_b), 7.19-7.34 (m, 10H, H_{arom}); ^{13}C NMR (75 MHz, $CDCl_3$): δ [20.9 (OC(O)*C*H_3)], 45.2 (C-3_a), 55.0 (O*C*H_3), 62.7 (C-6_b), 65.4 (C-5_a), 66.9 (C-4_b), [68.9 (C-6_a, C-5_b)], 69.5 (C-3_b), 70.8 (C-2_b), 73.8 (Ph*C*H_2), 79.9 (C-2_a), 80.3 (C-4_a), 83.1 (C-1_b), 98.3 (C-1_a), 101.7 (Ph*C*H), [126.1,

128.1, 128.3 128.7, 129.1, 137.5 (C_{arom})], [170.0, 170.1 (O*C*(O)CH_3)]; MALDI-FTICR/MS: *m/z*: found $[M+Na]^+$ 741.2190, $C_{35}H_{42}O_{14}S$ calcd for $[M+Na]^+$ 741.2193.

Methyl *S*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-(1→3)-2,4-di-*O*-benzyl-α-D-mannopyranoside (30). Compound **30** was synthesized according to the procedure for compound **9**. Treatment of **29** (48 mg, 0.067 mmol) with a solution of 1 M BH_3 in THF (0.66 mL) and a solution of 1 M Bu_2BOTf in DCM (66 μL) gave, after silica gel purification, **30** as white solid (31 mg, 63%). R_f 0.25 (toluene/acetone, 4:1, *v*/*v*). ^{1}H NMR (300 MHz, $CDCl_3$): δ [1.90, 1.94, 1.98, 2.00 (4×s, 12H, OC(O)C*H*$_3$)], 3.24 (s, 3H, OC*H*$_3$), 3.26 (dd, 1H, $J_{2,3}$=3.5, $J_{3,4}$=10.5 Hz, H-3$_a$), 3.51-3.56 (m, 2H, H-2$_a$, H-5$_a$), 3.70 (dd, 1H, $J_{5,6}$=4.0, $J_{6,6'}$=12.0 Hz, H-6$_a$), 3.76 (d, 1H, $J_{6,6'}$=12.0 Hz, H-6$_{a'}$), 3.83 (t, 1H, $J_{3,4}$=10.5, $J_{4,5}$=9.5 Hz, H-4$_a$), 3.94 (d, 1H, $J_{6,6'}$=10.5 Hz, H-6$_b$), 4.10-4.16 (m, 2H, H-5$_b$, H-6$_{b'}$), 4.77 (d, 1H, *J*=11.0 Hz, PhCH*H*), 4.52-4.64 (m, 3H, PhCH*H*), 5.10 (m, 2H, H-3$_b$, H-4$_b$), 5.31 (s, 1H, H-2$_b$), 5.40 (s, 1H, H-1$_b$), 7.10-7.35 (m, 10H, H_{arom}); ^{13}C NMR (75 MHz, $CDCl_3$): δ [20.5, 20.6, 20.7, 20.8 (OC(O)*C*H$_3$)], 47.9 (C-3$_a$), 54.6 (O*C*H$_3$), 62.2 (C-6$_a$), 62.5 (C-6$_b$), 66.6 (C-4$_b$), 68.6 (C-5$_b$), 69.4 (C-3$_b$), 70.3 (C-2$_b$), 72.9 (C-5$_a$), [73.1, 75.6 (2×Ph*C*H$_2$)], 76.2 (C-4$_a$), 79.5 (C-2$_a$), 83.4 (C-1$_b$), 97.2 (C-1$_a$), [127.8, 127.9, 128.0, 128.2, 128.4, 137.3, 137.7 (C_{arom})], [169.5, 169.7, 169.8, 170.7 (O*C*(O)CH_3)]; MALDI-FTICR/MS: *m/z*: found $[M+Na]^+$ 743.2347, $C_{35}H_{44}O_{14}S$ calcd for $[M+Na]^+$ 743.2349.

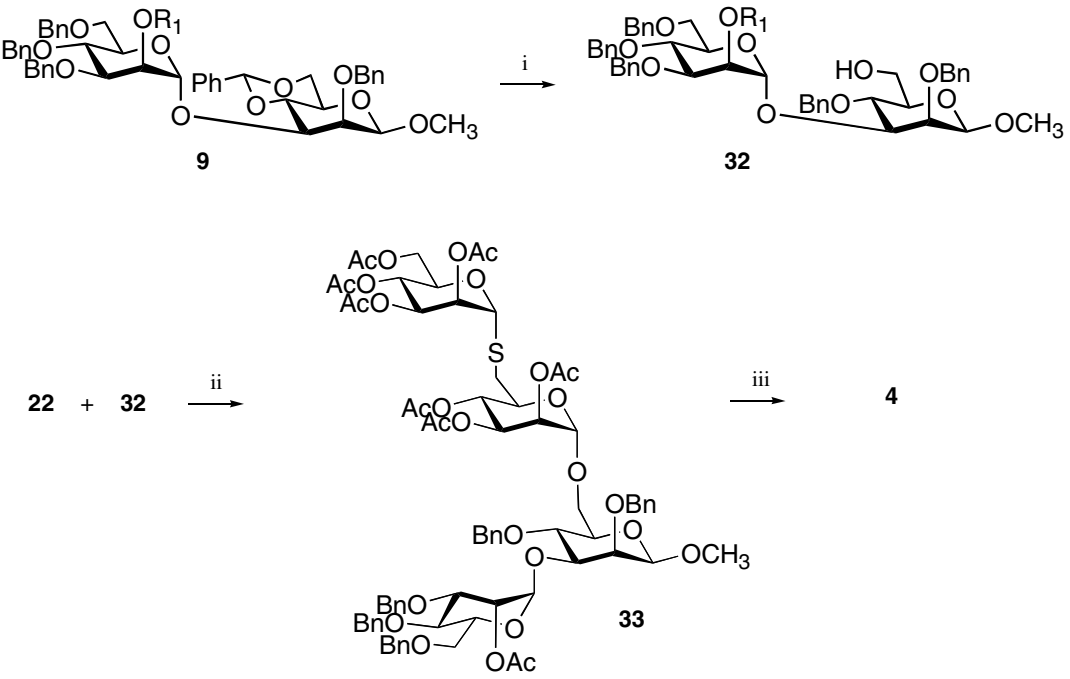
Methyl *S*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-(1→3)-[*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-(1→6)]-2,4-di-*O*-benzyl-α-D-mannopyranoside (31). Compound **31** was synthesized according to the general procedure for the glycosidation of thioglycosides. Glycosyl donor **16** (18 mg, 0.046 mmol), glycosyl

acceptor **30** (27 mg, 0.038 mmol) and 4Å powdered molecular sieves (0.05 g) in DCM (1 mL) in the presence of NIS (11 mg, 0.051 mmol) and TfOH (1 μL, 0.01 mmol) gave, after silica gel purification, **31** (31 mg, 77%) as colorless oil. R_f 0.5 (toluene/acetone, 3:1, *v*/*v*). ^{1}H NMR (500 MHz, $CDCl_3$): δ [1.90, 1.93, 1.96, 1.97, 1.99, 2.00, 2.07 (8×s, 24H, OC(O)C*H*$_3$)], 3.27 (m, 4H, H-3$_a$, OC*H*$_3$), 3.49 (d, 1H, $J_{2,3}$=2.0 Hz, H-2$_a$), 3.69 (m, 4H, H-4$_a$, H-5$_a$, H-6$_a$, H-6$_{a'}$), 3.92 (d, 1H, $J_{6,6'}$=13.5 Hz, H-6$_b$), 4.00-4.15 (m, 5H, H-5$_b$, H-6$_{b'}$, H-5$_c$, H-6$_c$, H-6$_{c'}$), 4.50 (d, 1H, *J*=11.5 Hz, PhCH*H*), 4.55 (d, 1H, *J*=11.0 Hz, PhCH*H*), 4.57 (s, 1H, H-1$_a$), 4.65 (d, 1H, *J*=11.5 Hz, PhCH*H*), 4.80 (d, 1H, *J*=11.0 Hz, PhCH*H*), 4.86 (s, 1H, H-1$_c$), 5.09 (m, 2H, H-3$_b$, H-4$_b$), 5.19 (m, 2H, H-2$_c$, H-4$_c$), 5.28 (m, 2H, H-2$_b$, H-3$_c$), 5.36 (s, 1H, H-1$_b$), 7.10-7.35 (m, 10H, H$_{arom}$); ^{13}C NMR (125 MHz, $CDCl_3$): δ 20.7 (OC(O)*C*H$_3$), 48.1 (C-3$_a$), 54.5 (O*C*H$_3$), [62.3, 62.4 (C-6$_b$, C-6$_c$)], [66.7, 66.8, 67.6, 68.6, 68.7, 68.8, 69.5, 70.1, 70.3 (C-5$_a$, C-2$_b$, C-3$_b$, C-4$_b$, C-5$_b$, C-2$_c$, C-3$_c$, C-4$_c$, C-5$_c$)], 72.9 (C-4$_a$), [73.5, 76.5 (2×Ph*C*H$_2$)], 77.8 (C-6$_a$), 79.3 (C-2$_a$), 83.3 (C-1$_b$), 96.8 (C-1$_a$), 97.5 (C-1$_c$), [127.8, 128.0, 128.2, 128.3, 128.4, 137.4, 137.5 (C$_{arom}$)], [169.4, 169.6, 169.8, 169.9, 170.7, 170.8 (O*C*(O)CH$_3$)]; MALDI-FTICR/MS: *m/z*: found [M+Na]$^+$ 1073.3295, $C_{49}H_{62}O_{23}S$ calcd for [M+Na]$^+$ 1073.3300.

Methyl *S*-(α-D-mannopyranosyl)-(1→3)-α-D-mannopyranoside (1). Compound **1** was synthesized according to the general procedure for the global deprotections. Treatment of **29** (0.021 g, 0.029 ㎜ol) in MeOH/DCM (0.5 mL: 0.5 mL, *v*/*v*) with NaOMe (pH=8-10) gave deacetylated product (14mg, 0.025 mmol, 87%). Treatment of the partially deprotected compound (12 mg, 0.022 mmol) in THF (1 mL) with Na (s) in NH_3 (l) at -78℃ gave **1** as white solid (7.7 mg, 95%). ^{1}H NMR (500 MHz, D_2O): δ 3.13 (t, 1H, $J_{2,3}$=3.0, $J_{3,4}$=7.5 Hz, H-3$_a$), 3.31 (s, 3H, OC*H*$_3$), 3.53-3.58 (m,

3H, H-6_a, H-3_b, H-6_b), 3.78 (m, 2H, H-5_a, H-$6_{b'}$), 3.85 (d, 1H, $J_{2,3}$=3.0 Hz, H-2_a), 3.90 (m, 1H, H-5_b), 4.02 (d, 1H, $J_{2,3}$=2.0 Hz, H-2_b), 4.58 (s, 1H, H-1_a), 5.41 (s, 1H, H-1_b); ^{13}C NMR (75 MHz, D_2O): δ 52.6 (C-3_a), 57.3 (O*C*H_3), 63.6 (C-5_a), 63.8 (C-6_b), 73.6 (C-3_b), 73.8 (C-2_a), 74.3 (C-2_b), [69.4, 69.8, 76.1 (C-4_a, C-6_a, C-4_b)], 76.3 (C-5_b), 89.0 (C-1_b), 102.6 (C-1_a); 1D Coupling HSQC (500 MHz, D_2O): $J_{H\text{-}1a\text{-}C\text{-}1a}$=172 Hz, $J_{H\text{-}1b\text{-}C\text{-}1b}$=170 Hz. MALDI-FTICR/MS: *m/z*: found $[M+Na]^+$ 395.0987, $C_{13}H_{24}O_{10}S$ calcd for $[M+Na]^+$ 395.0988.

Methyl *S*-(α-D-mannopyranosyl)-(1→3)-[*O*-(α-D-mannopyranosyl)-(1→6)]-α-D-mannopyranoside (2). Compound **2** was synthesized according to the general procedure for the global deprotections. Treatment of **31** (0.018 g, 0.017 ㎜ol) in MeOH/DCM (0.5 mL: 0.5 mL, *v*/*v*) with NaOMe (pH=8-10) gave deacetylated product (9 mg, 0.013 mmol, 77%). Treatment of the partially deprotected compound (9 mg, 0.013 mmol) in THF (1 mL) with Na (s) in NH_3 (l) at -78℃ gave **2** as white solid (6 mg, 91%). ^{1}H NMR (500 MHz, D_2O): δ 3.23 (dd, 1H, $J_{2,3}$=2.5, $J_{3,4}$=10.0 Hz, H-3_a), 3.41 (s, 3H, OC*H*$_3$), 3.62-3.89 (m, 12H, H-4_a, H-5_a, H-6_a, H-$6_{a'}$, H-3_b, H-4_b, H-6_b, H-$6_{b'}$, H-3_c, H-4_c, H-5_c, H-6_c), 3.98-4.01 (m, 4H, H-2_a, H-5_b, H-2_c, H-$6_{c'}$), 4.12 (dd, 1H, $J_{1,2}$=1.5, $J_{2,3}$=3.5 Hz, H-2_b), 4.68 (s, 1H, H-1_a), 4.89 (s, 1H, H-1_c), 5.50 (s, 1H, H-1_b); ^{13}C NMR (75 MHz, D_2O): δ 50.3 (C-3_a), 54.8 (O*C*H_3), [61.0, 61.1, 65.8, 66.5, 66.9, 67.3, 70.1, 70.7, 71.1, 71.3, 71.7, 71.8, 72.8, 73.5 (C-2_a, C-4_a, C-5_a, C-6_a, C-2_b, C-3_b, C-4_b, C-5_b, C-6_b, C-2_c, C-3_c, C-4_c, C-5_c, C-6_c)], 86.4 (C-1_b), 99.6 (C-1_c), 100.4 (C-1_a); 1D Coupling HSQC (500 MHz, D_2O): $J_{H\text{-}1a\text{-}C\text{-}1a}$=172 Hz, $J_{H\text{-}1b\text{-}C\text{-}1b}$=170 Hz, $J_{H\text{-}1c\text{-}C\text{-}1c}$=173 Hz. MALDI-FTICR/MS: *m/z*: found $[M+Na]^+$ 557.1513, $C_{19}H_{34}O_{15}S$ calcd for $[M+Na]^+$ 557.1516.



Scheme S1. Reagents and conditions: i)BH_3 in THF, Bu_2BOTf (70%); ii)TMSOTf, DCM, 0 ^oC (81%); v) NaOMe, MeOH then $Na/NH_3(l)$, -78 ^oC (90%)

Methyl *O*-(2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→3)-2,4-di-*O*-benzyl-β-D-mannopyranoside (32). To a flask containing **9** (0.13 g, 0.15 mmol) was added a solution of 1 M BH_3 in THF (1.5 mL) at 0℃, and the resulting solution was stirred for 5 min. Next, a solution of 1 M Bu_2BOTf in DCM (0.15 mL) was then added dropwise. After stirring for 30 min at 0℃, TLC analysis (Hexane/EtOAc, 1:1, *v*/*v*) showed the disappearance of starting material. Triethylamine (0.1 mL) was then added followed by careful addition of methanol until evolution of H_2 had ceased. The mixture was co-evaporated with methanol three times. Purification of the residue by column chromatography over silica gel afforded **32** as colorless oil (0.091 g, 70%). R_f 0.3 (Hexnae/EtOAc, 1:1, *v*/*v*). $[\alpha]_D^{27}$ -23.3 (*c* 0.58, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$): δ 2.00 (s, 3H, OC(O)C*H*$_3$), 3.23 (m, 1H, H-5_a), 3.40 (s, 3H, OCH_3), 3.56 (m, 2H, H-6_b, H-$6_{b'}$), 3.67-3.71 (m, 4H, H-2_a, H-6_a, H-4_b, H-$5_{b'}$), 3.79-3.86 (m, 3H, H-3_a, H-$6_{a'}$, H-3_b), 3.89 (t, 1H, $J_{3,4}$=9.6, $J_{4,5}$=9.6 Hz, H-4_a), 4.23 (s, 1H, H-1_a), 4.35-4.40 (m,

3H, PhCH*H*), 4.45 (d, 1H, *J*=12.3 Hz, PhCH*H*), 4.55 (d, 1H, *J*=11.1 Hz, PhCH*H*), 4.56 (d, 1H, *J*=11.4 Hz, PhCH*H*), 4.67 (d, 1H, *J*=12.6 Hz, PhCH*H*), 4.70 (d, 1H, *J*=11.1 Hz, PhCH*H*), 4.76 (d, 1H, *J*=11.4 Hz, PhCH*H*), 4.85 (d, 1H, *J*=12.0 Hz, PhCH*H*), 5.13 (s, 1H, H-1_b), 5.41 (s, 1H, H-2_b), 7.08-7.30 (m, 25H, H_{arom}); ^{13}C NMR (75MHz, $CDCl_3$): δ 21.0 (OC(O)*C*H_3), 57.3 (O*C*H_3), 62.1 (C-6_a), 68.7 (C-2_b), 69.1 (C-6_b), [71.9, 73.4, 74.3, 74.8, 75.2 (Ph*C*H_2)], 72.1 (C-5_b), 74.2 (C-4_b), 75.1 (C-4_a), 75.8 (C-5_a), 77.2 (C-3_a), 77.9 (C-3_b), 79.9 (C-2_a), 99.7 (C-1_b), 102.7 (C-1_a), [127.3, 127.4, 127.5, 127.6, 127.7, 127.8, 127.9, 128.0, 128.1, 128.2, 128.3, 128.4, 128.9, 137.7, 137.8, 138.1, 138.5 (C_{arom})], 170.0 (O*C*(O)CH_3); MALDI-FTICR/MS: *m/z*: found $[M+Na]^+$ 871.3662, $C_{50}H_{56}O_{12}$ calcd for $[M+Na]^+$ 871.3669.

Methyl *O*-(2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→3)-*O*-[(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-(1→6)-*S*-(2,3,4-tri-*O*-acetyl-α-D-mannopyranosyl)-(1→6)]-2,4-di-*O*-benzyl-β-D-mannopyranoside (33). Compound **33** was synthesized according to the general procedure for the glycosidation with glycosyl trichloroacetimidates. Glycosyl donor **22** (91.1 mg, 114.3 μmol), glycosyl acceptor **32** (88.0 mg, 103.7 μmol) and 4Å powdered molecular sieves (0.18 g) in DCM (5 mL) in the presence of TMSOTf (4.2 μL, 23.2 μmol) gave, after silica gel purification, **33** as colorless oil (124.6 mg, 81%). R_f 0.18 (Hexane/EtOAc, 3:2, *v*/*v*). $[\alpha]_D^{27}$ +11.1 (*c* 0.14, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$): δ [1.90, 1.91, 1.94, 1.97, 1.98, 2.03, 2.05, 2.06 (8×s, 24H, OC(O)C*H*$_3$)], 2.59 (dd, 1H, $J_{5,6}$=6.0, $J_{6,6'}$=14.0 Hz, H-6_c), 2.80 (dd, 1H, $J_{5,6}$=3.0, $J_{6,6'}$=14.0 Hz, H-$6_{c'}$), 3.34 (m, 1H, H-5_a), 3.41 (s, 3H, OC*H*$_3$), 3.52 (m, 2H, H-6_b, H-$6_{b'}$), 3.65-3.85 (m, 8H, H-2_a, H-3_a, H-4_a, H-6_a, H-$6_{a'}$, H-3_b, H-4_b, H-5_b), 3.97 (m, 1H, H-5_c), 4.02 (dd, 1H, $J_{5,6}$=2.0, $J_{6,6'}$=12.0 Hz, H-6_d), 4.20 (s, 1H, H-1_a), 4.26 (dd, 1H, $J_{5,6}$=5.0, $J_{6,6'}$=12.0 Hz, H-$6_{d'}$), 4.30 (m, 1H, H-5_d), 4.37

(d, 1H, *J*=12.0 Hz, PhCH*H*), 4.38 (d, 1H, *J*=10.5 Hz, PhCH*H*), 4.41 (d, 1H, *J*=11.5 Hz, PhCH*H*), 4.46 (d, 1H, *J*=12.0 Hz, PhCH*H*), 4.53 (d, 1H, *J*=11.5 Hz, PhCH*H*), 4.58 (d, 1H, *J*=10.5 Hz, PhCH*H*), 4.65 (d, 1H, *J*=12.5 Hz, PhCH*H*), 4.76 (s, 1H, H-1_c), 4.77 (d, 1H, *J*=11.0 Hz, PhCH*H*), 4.81 (d, 1H, *J*=12.5 Hz, PhCH*H*), 4.85 (d, 1H, *J*=11.0 Hz, PhCH*H*), 5.09 (s, 1H, H-1_b), 5.19-5.31 (m, 7H, H-2_c, H-3_c, H-4_c, H-1_d, H-2_d, H-3_d, H-4_d), 5.39 (dd, 1H, $J_{1,2}$=1.0, $J_{2,3}$=3.5 Hz, H-2_b), 7.05-7.28 (m, 25H, H_{arom}); ^{13}C NMR (75MHz, $CDCl_3$): δ [20.5, 20.6, 20.7, 20.8, 20.9 (OC(O)*C*H_3)], 31.7 (C-6_c), 57.1 (O*C*H_3), 62.2 (C-6_d), 66.2 (C-6_a), 66.4 (C-4_c), [68.4, 68.7, 68.9, 69.0×2, 69.2, 69.3, 69.4 (C-2_b, C-6_b, C-2_c, C-3_c, C-5_c, C-3_d, C-4_d, C-5_d)], 70.8 (C-2_d), [71.9, 72.0, 73.3, 73.9, 74.2, 74.7, 74.9, 75.0, 75.4 (C-4_a, C-3_b, C-4_b, C-5_b, Ph*C*H_2×5)], 77.2 (C-3_a), 77.9 (C-3_b), 80.1 (C-2_a), 82.6 (C-1_d), 97.1 (C-1_c), 99.7 (C-1_b), 102.5 (C-1_a), [127.2, 127.4, 127.5, 127.6, 127.7, 127.8, 128.1, 128.2, 128.3, 128.4, 137.7, 137.8, 138.1, 138.5, 138.6 (C_{arom})], [169.6, 169.7, 169.8, 169.9, 170.0, 170.6 (O*C*(O)CH_3)]. MALDI-FTICR/MS: *m/z*: found $[M+Na]^+$ 1505.5233, $C_{76}H_{90}O_{28}S$ calcd for $[M+Na]^+$ 1505.5237.

Methyl *O*-(α-D-mannopyranosyl)-(1→3)-*O*-[(α-D-mannopyranosyl)-(1→6)-*S*-(α-D-mannopyranosyl)-(1→6)]-β-D-mannopyranoside (3). Compound **3** was synthesized according to the general procedure for the global deprotection. Treatment of **33** (55.1 mg, 37.1 μmol) in MeOH/DCM (0.5 mL: 0.5 mL, v/v) with NaOMe (pH=8-10) gave deacetylated product (42.6 mg, quantitive). Treatment of the partially deprotected compound (42.6 mg, 37.1 μmol) in THF (1 mL) with Na (s) in NH_3 (l) gave **3** as white solid (23.2 mg, 90%). ^{1}H NMR (500 MHz, D_2O): δ 2.72 (dd, 1H, $J_{5,6}$=8.5, $J_{6,6'}$=13.5 Hz, H-6_c), 3.14 (d, 1H, $J_{6,6'}$=13.5 Hz, H-$6_{c'}$), 3.47 (s, 3H, OCH_3), 3.49-3.52 (dd, 1H, *J*=3.5, 10.0 Hz), 3.55-3.65 (m, 3H), 3.67-3.79 (m, 9H), 3.82-3.85

(m, 3H), 3.95 (s, 1H, H-2_c), 3.97-4.00 (m, 2H), 4.01 (s, 2H, H2-$_b$, H-2_d), 4.09 (d, 1H, *J*=2.5 Hz, H-2_a), 4.53 (s, 1H, H-1_a), 4.82 (s, 1H, H-1_c), 5.05 (s, 1H, H-1_b), 5.29 (s, 1H, H-1_d); ^{13}C NMR (75MHz, D_2O): δ 31.5 (C-6_c), 57.0 (O*C*H_3), 61.0, 61.1, 65.5, 65.9, 66.9, 67.3, 69.7, 70.0, 70.1, 70.2, 70.5, 70.7, 71.1, 71.2, 71.8, 73.3, 73.5, 74.2, 80.9, 84.3 (C-1_d), 99.5 (C-1_c), 101.2 (C-1_a), 102.6 (C-1_b); 1D Coupling HSQC (500 MHz, D_2O): $J_{H\text{-}1a\text{-}C\text{-}1a}$=160.3 Hz, $J_{H\text{-}1b\text{-}C\text{-}1b}$=172.5 Hz, $J_{H\text{-}1c\text{-}C\text{-}1c}$=172.0 Hz, $J_{H\text{-}1d\text{-}C\text{-}1d}$=168.3 Hz. MALDI-FTICR/MS: *m/z*: found $[M+Na]^+$ 719.2037, $C_{25}H_{44}NO_{20}S$ calcd for $[M+Na]^+$ 719.2044.

X-ray Crystallography

Crystallization. Purified D204A_dGMII was diluted in Tris-buffer to a concentration of approximately 15 mg/ml. Crystals of unliganded D204A or dGMII were grown using the hanging-drop vapor diffusion method using 1 ml of Tris-reservoir-buffer (0.1 M Tris pH 7.0, 8.5 w/v% PEG 6K, 2.5 v/v% methylpentane diol (MPD) in each well of the crystal tray. A total of 3 µl protein solution was combined with 3 µl of native dGMII crystal seeds in reservoir buffer to form the crystallization drop. Preparation of the seeds followed the Hampton protocol for the Seed Bead Kit. Crystals were grown for 16-18 h at 20°C

Crystals were washed in a phosphate-buffered reservoir solution (PBSR) (0.1 M sodium phosphate pH 7.0, 8.5 w/v% PEG 6K 2.5 v/v% MPD) followed by a 24 h soak in PBSR containing 2 mM of the saccharides. Crystals were then passed through solutions of PBSR with 1 mM of saccharide and increasing concentrations of MPD (10, 15, 20, 25% v/v) for cryoprotection. Crystals were flash frozen in liquid nitrogen. D204A-**5** complexes were also obtained by soaking. Crystals were washed in MOPS buffered reservoir solution (MBSR) (0.1 M MOPS pH 7.0, 8.5 w/v% PEG 6K 2.5 v/v% MPD) followed by a 24 h soak in MBSR containing 2 mM of **5**. Crystals were then passed through solutions of MBSR with 1 mM **5** and increasing concentrations of MPD (10, 15, 20, 25% v/v) for cryoprotection. Crystals were flash frozen in liquid nitrogen.

Data collection. X-ray diffraction data were collected at 100 K on a Rigaku or Bruker home source or at the Cornell High Energy Synchrotron Source. Data were integrated and scaled using HKL2000, or the Bruker program suite. A constant set of R_{free} data was created by reading the data into CCP4[S1] and merging with a previous R_{free} data

set. The data was then written in mtz, cns, and hklf4 formats for use in subsequent refinement programs.

Refinement of complexes. The structure of the complexes were initially phased by molecular replacement using the software program CNS.[S2] Rigid body refinement was carried out against the published structure of native dGMII (PDB: 1HTY) with Tris and waters in the region of the active site removed. This was followed by simulated annealing to 3500 K, group B-factor refinement, and individual B-factor refinement. At this stage, the R-factors were close to 20% and the F_0-F_C density clearly indicated the presence or absence of bound compound. Additional water molecules were picked using the Arp/Warp routine in CCP4.[S3] Ligand molecule dictionaries and starting coordinate files were generated using the ProDrg server (http://davapc1.bioch.dundee.ac.uk/programs/prodrg/). Ligand fitting and verifying proper fit to the electron density was performed using the program Coot 0.31.[S4] REFMAC5[S5] refinement was then iterated with Coot fitting. For high resolution refinement SHELX97[S6] was used. CGLS refinement to increasing resolution was followed by refinement of anisotropic B-factors. This was followed by a number of iterative rounds of model building using Coot and SHELX97 refinement where clear alternative conformations of side chains were inserted into the model, water molecules were added, and the geometry of the side chains was corrected. Hydrogen atoms were added and the occupancy of the alternative conformations was refined in later rounds of SHELX97 refinement. Statistics for data collection and refinement are presented in Table S1. The model was prepared for PDB deposition using shelxpro which removes the hydrogen atoms from the deposited molecule. The quality of the final models were assessed using a number of structure validation programs including

MolProbity (http://molprobity.biochem.duke.edu/) and the Validation Suite (http://biotech.ebi.ac.uk). Protein overlays were carried out using the SSM Superpose subroutines[S7] of Coot. Graphics were generated using PyMOL (http://pymol.sourceforge.net).

Results

Enzyme	native	D204A	D204A	D341N	D204A
Compound	**Water**	**Water**	**Tris**	**Mannose**	**Mannose**
PDB Accession No.	3BUB	3BUD	3BUI	3BUP	3BUQ
DATA COLLECTION					
Xray Source (λ(Å))	CHESS A1(0.977)	Rigaku (1.54)	CHESS F1(0.912)	Rigaku (1.54)	Rigaku (1.54)
Cell dimensions (Å)	68.8X109.6 X138.6	68.9X109.5 X138.5	68.3X109.0 X137.6	68.7X109.5 X138.6	68.6X109.5 X138.6
Resolution (Å) overall	20-1.38	30-1.85	30-1.25	20-2.03	20-2.01
Resolution (Å) high resolution shell	1.40-1.38	1.89-1.85	1.30-1.25	2.08-2.03	2.08-2.01
% Completeness (overall/hi_res)	98.5/82.0	99.8/98.6	93.0/62.1	96.0/79.8	92.0/63.2
Unique Reflections	211715	89372	263155	66283	65465
Redundancy (overall/hi_res)	6.4/2.3	7.3/4	6.9/3.5	7.8/3	3.9/2
I/sigma (overall/hi_res)	20.7/3.5	31/4.3	15.5/4.7	27/4.1	17.3/3.4
R merge (overall/hi_res)	0.06/0.33	0.054/0.31	.079/0.30	0.068/0.34	0.068/0.32
REFINEMENT	Refmac	Refmac	ShelX	Refmac	Refmac
R/Rfree for all reflections	0.18/0.204	0.157/0.192	0.121/0.159	0.152/0.195	0.154/0.174
R/Rfree for Fo>4sigma	-	-	0.113/0.15	-	-
Atoms in Model	10102	9963	10090	9429	9352
Amino Acids	1016	1016	1016	1016	1016
Alternate Conformers	52	32	50	13	14
Water Molecules	1493	1509	1505	1105	1034
rmsd bonds (Å)	0.014	0.013	0.012	0.012	0.014
rmsd angles (°)	1.5	1.32	2.2	1.4	1.39
Average BFactor Å^2					
Overall	14.7	19	16.7	19.3	14.1
Protein Main Chain	11	16.3	11.7	17.7	12.7
Protein Side Chain	13.5	17.7	16.2	18.9	13.9
Water	27.9	29.2	31.3	26.4	19.7
Bound Compound	_	_	15.1	23.8	16.9
Others (MPD,NAG,PO4,Zn)	36.1	39.5	38.3	44.4	41.3

Enzyme	D204A	D204A	D204A	D204A	D204A
Compound, Ligand code	**1**, WZ1	**2**, WZ2	**3**, WZ3	**4,** WZ4	**5**, WZ5
PDB Accession No.	3BVT	3BVU	3BVV	3BVW	3BVX
DATA COLLECTION					
Xray Source (λ(Å))	CHESS A1(0.977)	CHESS A1(0.977)	CHESS A1(0.972)	CHESS F1(0.918)	CHESS A1(0.978)
Cell dimensions (Å)	69.0X109.8 X138.6	69.0 X109.8 X139.0	69.1X109.8 X139.3	68.74X109.5 X138.4	68.8X109.6 X138.5
Resolution (Å) overall	20-1.30	20-1.12	20-1.30	20-1.20	30-1.10
Resolution (Å) high resolution shell	1.33-1.30	1.15-1.12	1.32-1.30	1.24-1.20	1.13-1.10
%Completeness (overall/hi_res)	99.8/99.6	99.2/98.0	97.8/78.4	98.2/96.5	98.3/94.3
Unique Reflections (I>0)	256264	400361	254755	318631	416374
Redundancy (overall/hi_res)	6.7/5.5	9.0/5.0	7.6/3.6	11.6/6.2	11/3.4
I/sigma (overall/hi_res)	22.8/2.6	14/2.5	17.2/4.8	19.1/4.2	13.4/2.1
R merge (overall/hi_res)	0.07/0.88	0.077/0.63	0.08/0.26	0.07/0.38	0.09/0.50
REFINEMENT	Refmac	ShelX	Refmac	ShelX	ShelX
R/Rfree for all reflections	0.174/0.192	0.114/0.155	0.152/0.173	0.133/0.170	0.153/0.190
R/Rfree for Fo>4sigma	-	0.099/0.140	-	0.122/0.158	0.115/0.150
Atoms in Model	9987	10001	10336	9943	10186
Amino Acids	1016	1015	1016	1015	1016
Alternate Conformers	38	42	55	35	45
Water Molecules	1466	1515	1657	1403	1526
rmsd bonds (Å)	0.014	0.014	0.012	0.014	0.015
rmsd angles (°)	1.52	2.2	1.46	2.3	2.4
Average BFactors (Å^2)					
Overall	17	19.6	16.4	19.3	20.2
Protein Main Chain	13.2	14.0	12.6	14.7	14.8
Protein Side Chain	15.6	19.3	14.8	19.1	19.6
Water	31.2	35.5	29.4	33.5	36.3
Bound Compound	19.8	15.5	16.8*	28.6	32.5
Others (MPD,NAG,PO4,Zn)	35.4	43.6	24.8	30.2	30.7

Table 1S: Data collection and refinement statistics for *Drosophila* Golgi Mannosidase II complexes reported in this study. The asterisk indicates that compound **3** was refined with the occupancy set at 0.7.

Binding of Tris to D204A [PDB: 3BUI]

As expected, the absence of the catalytic aspartate changed the environment of the active site. One of the principal changes is that the active site zinc, which normally coordinates with four amino acid side chains (Asp204, His90, Asp92, and His471), now has only three side chains for coordination and this change in the environment of the active site is reflected in the way Tris, extracted from the crystallization buffer, complexes with the mutant protein (Figure S1). Interestingly, the density observed in the structure can be accounted for by two different conformations of the Tris. In conformation 1, the active site zinc is penta-coordinated to the three aforementioned amino acids as well as the hydroxyl and the amino group of Tris, while in the second conformation the zinc becomes hexa-coordinated as a second hydroxyl (O1) comes into close proximity to the zinc (2.4 Å). The position of O1 in conformation 2 is similar to the position of the Oδ1 of Asp204 in the native enzyme (0.5Å difference in superposed structures). In conformation 2, O1 also makes interactions with amino acid side chains His90 Nε2 (3.1Å) and Tyr269 OH (3.0 Å) while in conformation 1 it was in close proximity to two water molecules (2.6 and 2.9 Å) and Arg228 NH1 (3.1 Å). Arg228, which in the native protein interacts with Asp204 (Arg228 NH2 and Nε are less than 3 Å away from Asp204 Oδ1), now also demonstrates two alternate conformations. In conformation 1, which seems to be the slightly predominant position, the Arg228 NH1 is close to both the O1 of the Tris and the main chain carbonyl of Ala204 (2.9 Å). In this position it would clash with the Oδ1 of Asp204 of the native enzyme (~ 2 Å away in the superposed structure). In conformation 1, the Arg228 NH2 interacts with a water molecule, while in conformation 2 it interacts with water and Tyr407 OH (3.0 Å). The Arg228 Nε in conformation 1 interacts with

Tyr407 OH (3.0 Å) while in conformation 2 the interaction is with the main chain carbonyl of Ala204 (3.2 Å).

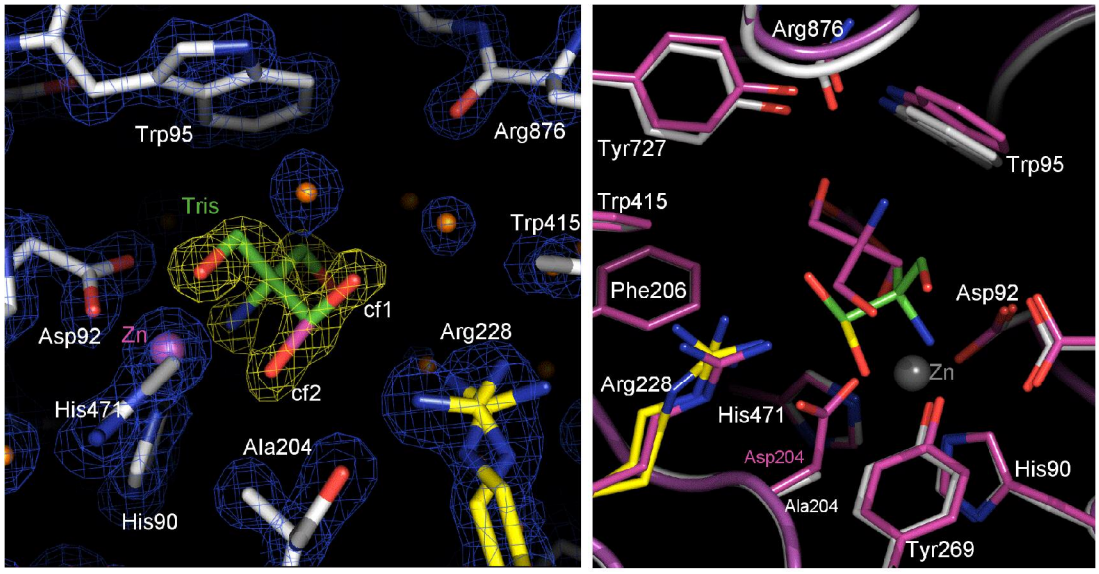


Figure S1. Binding of Tris in the active site of D204A. Tris-HCl is used in the crystallization buffer and is found in dGMII crystals unless displaced by washing the crystals with a Tris-free buffer or in the presence of tight binding compounds. (Left panel) The electron density in the active site of D204A in the presence of Tris. The 2Fo-Fc density (contoured at 1.8 sigma/0.97 e/Å^3) is colored blue, while the Fo-Fc omit density (contoured at 3 sigma/0.32 e/Å^3) is colored gold. The alternate position of the Tris oxygen is indicated by a magenta bond and the two alternate positions of Arg228 are colored yellow. Water molecules are colored orange. (Right panel) Comparison of the Tris binding site in D204A (white) to that in wild-type dGMII (from [PDB:1HTY][S8] magenta). Alternate conformations in D204A are colored yellow. There is an obvious movement of the interacting residues towards the Tris in D204A (including the pi-stacking Trp95) while the hydrophobic pocket at the left (Trp415 and Phe206) shows no movement.

Binding of water to dGMII [PDB:3BUB] and D204A [PDB:3BUD]

Tris can be removed from the active site by washing the crystals in crystallization buffer containing phosphate or MOPS as buffer (see Materials and Methods). The washing does not alter the diffraction quality of the crystals but results in replacement of the Tris by water molecules. The positions of the water molecules in the active site of the wild-type enzyme and D204A are different and reflect the changes in the active site (Figure S2). Of particular interest is the additional water molecule in D204A, (w2 in figure) which is bound to the zinc close to the position occupied by the Oδ2 of

Asp204 in the native enzyme (1.1 Å difference in superposed structures). This water maintains the penta-coordinated nature of the zinc. The zinc itself moves slightly (0.3 Å difference in superposed structures) being pulled towards Asp92. The distance between the zinc and Asp92 Oδ1 decreases from 2.2 Å in the native enzyme to 2.0 Å in the D204A structure. Arg228 also shows a major change in position as the NH2 now interacts with Tyr407 OH (2.9 Å) and the Nε interacts with the backbone carbonyl of Ala204 (3.1 Å). The overall effect is a shift in the position of the waters in proximity to the active site zinc and a movement of Trp95 (the residue that forms the cap on the binding pocket) about 0.5 Å upwards in D204A resulting in a slightly more spacious pocket.

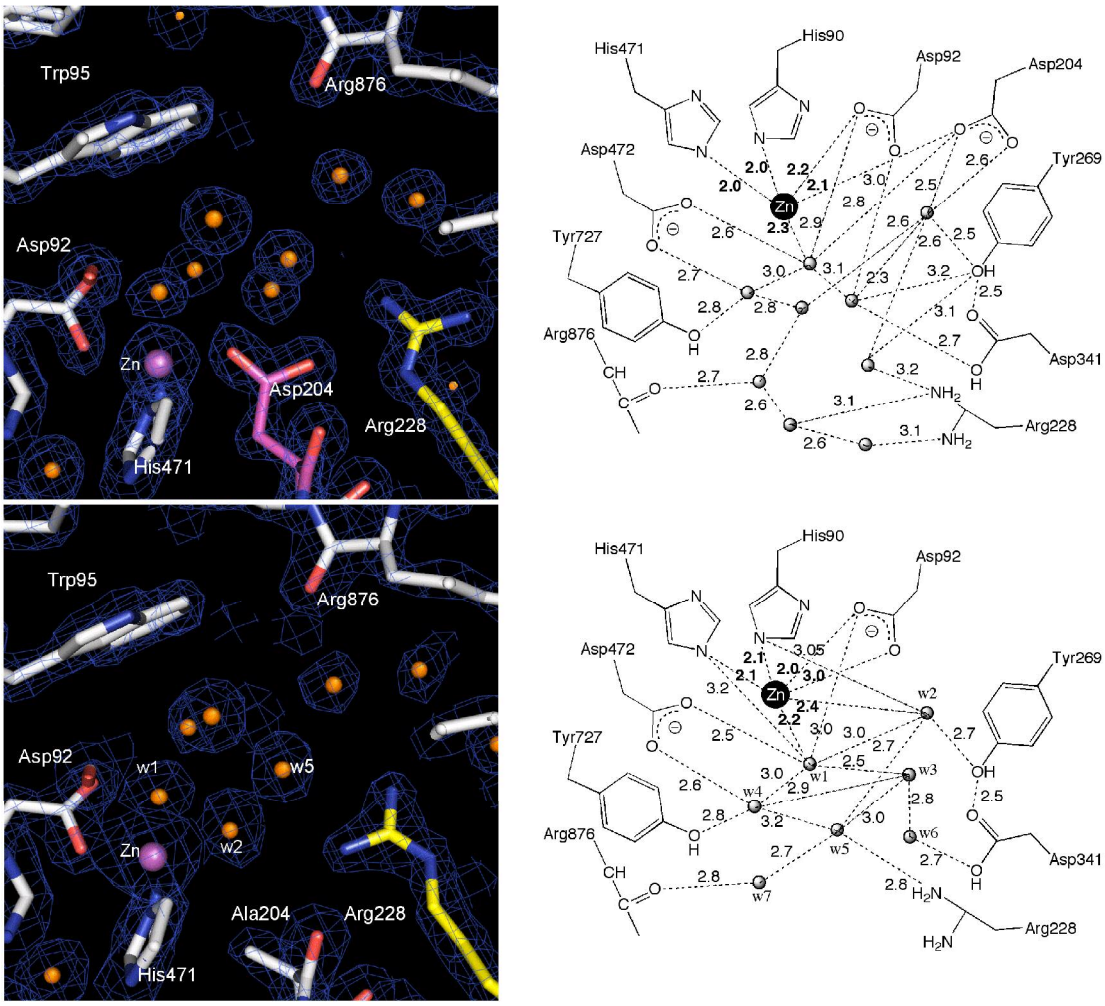


Figure S2. Water interactions in the active site (-1) of dGMII [PDB:3BUB](top) and D204A [PDB:3BUD](bottom). Crystals of dGMII and D204A were washed with a phosphate-buffered

crystallization buffer to displace Tris from the active site. Left hand panels: The 2Fo-Fc electron density map is shown in blue (contoured at 1.4 sigma, 0.64 e/A^3). Water molecules are represented as orange spheres while the active site zinc is magenta. Amino acids showing major changes are colored to highlight the differences. Right hand panels: Close interactions between the water residues and the amino acid side chains of the active site (< 3.2Å) are shown in the right hand panels. Distances in Å. Water molecules are represented as grey spheres.

Interactions of D341N and D204A mutants with mannose

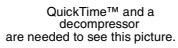


Figure S3. Interactions made with mannose in the active site of GMII with mutations in the acid-base catalyst (D341N, left, from [PDB:3BUP]) or catalytic nucleophile (D204A, right, from [PDB:3BUQ]). Interaction distances of less than 3.2 Å are shown (distances in Å). Interactions with the zinc (black ball) are indicated in bold. Water molecules are depicted as grey spheres.

Co-complex structures of dGMII with 1 [PDB: 3BVT]. Compound **1** is an α(1,3) linked di-mannoside in which the exocyclic oxygen of the non-reducing mannoside is replaced by sulfur (Figure 1). The position of the mannoside compexed to zinc is clearly defined in the electron density (Figure S4) of the D204A complex. The electron density for the second sugar was poorly defined but of sufficient quality to assign its position. The poor quality of the density for the reducing mannoside and its higher B-factors (average of 27.7 *vs.* 10.9 for terminal mannoside) indicates that it is not binding tightly and is probably somewhat mobile. In contrast to the many interactions made between the mannoside residue bound to the zinc and the amino acid residues of the protein, water molecules mediate most of the contacts of the

reducing mannoside and only the O6 makes a hydrogen bond with the hydroxyl group of Ser268. Interestingly, the O1 hydrogen bonds with a water molecule, which in turn bonds to O6 of the first ring and the NH2 of Arg228 in conformation B. There is also close contact (3.1 Å) between the sulfur of the saccharide, where cleavage would be expected to occur in the natural substrate, and Oδ2 of the presumptive acid-base catalyst Asp341.

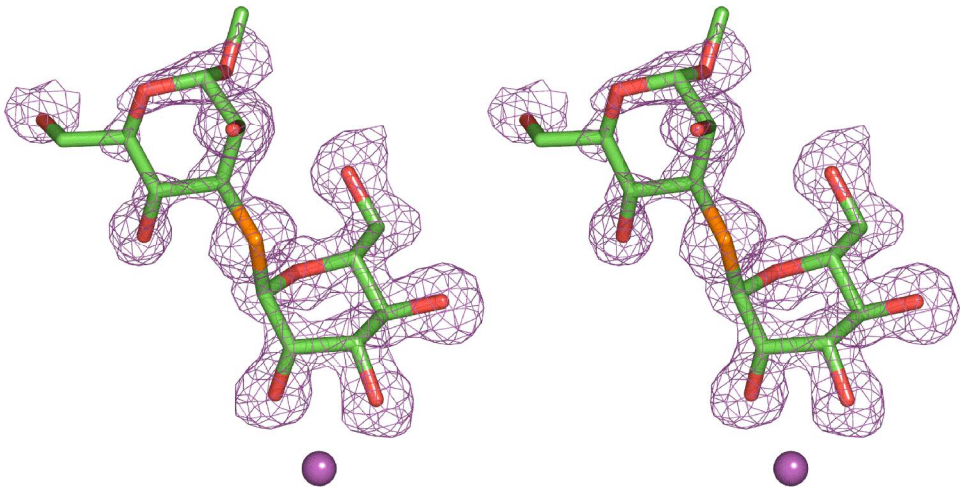


Figure S4. Stereoview of the electron density for the thio-linked α(1,3)-di-mannoside **1** in the active site of D204A. The Fo-Fc omit map for the compound is shown. The thio-linkage is colored orange and the active site zinc is represented by a magenta ball. Contour level is 2σ/0.25 e/Å^3.

Co-complex structures of dGMII with 2 [PDB: 3BVV] and 3[PDB: 3BVV].

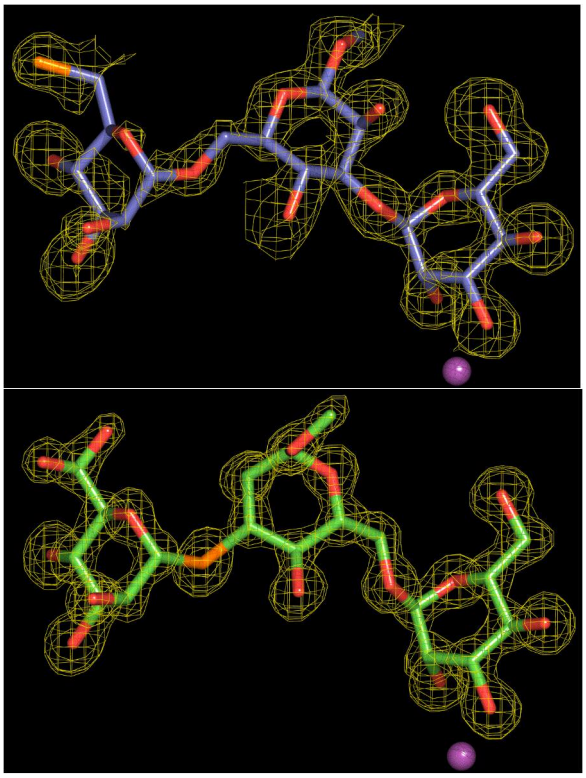


Figure S5. Identification of a secondary mannoside binding site in GMII. Binding **2** and **3** to phosphate washed D204A crystals shows the presence of a second mannoside binding site. The electron density (F_o-F_c omit maps contoured at 3 sigma) for **2** (bottom/green) or **3** (top/slate). Compound **2** has an α(1,6)-linked mannoside bound in the active site while **3** has an α(1,3)-linked mannoside in the active site. Compound **3** is a tetra-mannoside but only three mannosides are visible in the electron density, the terminal thio-linked mannose cannot be assigned.

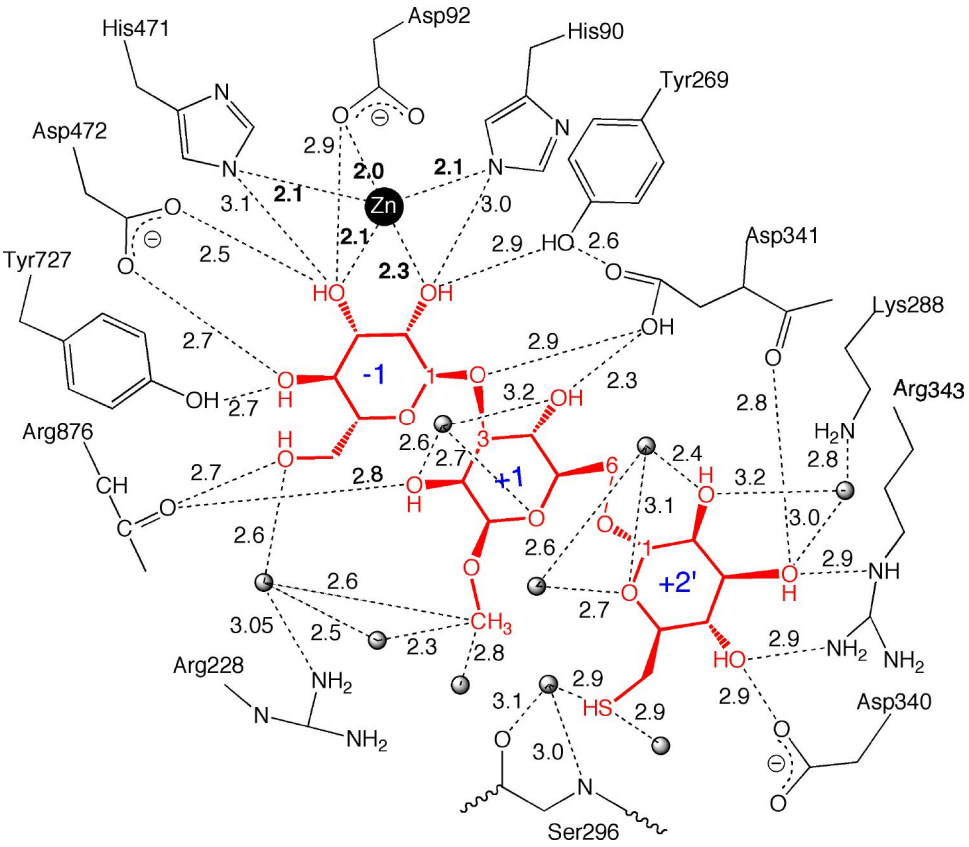


Figure S6. Interactions made with **3** in the active site of D204A from [PDB:3BVV]). Interaction distances of less than 3.2 Å are shown (distances in Å). Interactions with the zinc (black ball) are indicated in bold. Water molecules are depicted as grey spheres.

Co-complex structures of dGMII with pentasaccharide 4. The addition of an *N*-acetyl glucosamine residue to the α(1,3)-linked mannoside of **3** results in a penta-saccharide which formed a complex with dGMII, which was very different from that the complex formed with the tetrasaccharide, and which revealed the presence of a third sugar binding site. In this case, the thio α(1,6)-linked mannoside was complexed to the zinc in the -1 site. The density for the second ring (+1) was ill-defined despite completeness of the data and the highly refined nature of the model (Figure S7). This probably reflects the unfavorable nature of the thio-mannoside for complexation.

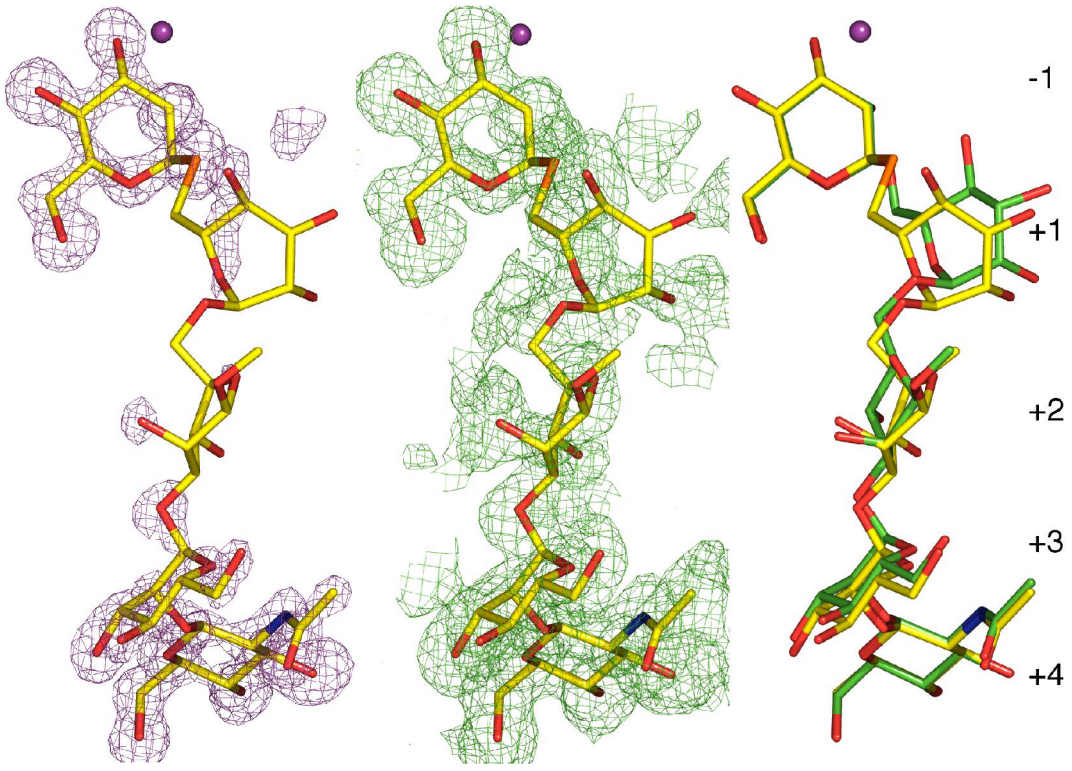


Figure S7. The electron density for the central section of the α(1,2)-GlcNAc containing thio-linked pentasaccharide **4** is too weak for unambiguous assignment. Omit (Fo-Fc) maps of the density in the active site of the D204A:**3** complex show excellent density for the mannoside in the -1 cleavage pocket and good density for the GlcNAc (+4) and +3 mannoside when contoured at 3 sigma (0.32 e/Å^3) (magenta, left panel) but the two intermediate mannoside residues have negligible density. Contouring at 1 sigma (0.1 e/Å^3) (green, center panel) allows a plausible fitting of the +1 and +2 mannosides which differ in position from those of compound **5** (right panel).

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