DMT-MM Mediated Functionalisation of the Non-Reducing End of Glycosaminoglycans

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Supporting Information

General Experimental

All reactions involving air- or water-sensitive reagents were carried out under an atmosphere of nitrogen using flame- or oven-dried glassware. Unless otherwise noted, starting materials and reagents were obtained from commercial suppliers and were used without further purification. CH_2Cl_2 and Et_3N were distilled from calcium hydride. Anhydrous methanol was used as supplied by Acros. Purification by flash column chromatography was carried out using Merck Kieselgel 60 silica gel as the stationary phase. Analytical high performance chromatography (HPLC) was performed using a Waters 600E instrument fitted with a variable wavelength UV detector monitoring at 232 nm, using either a Dionex IonPac AS17 column (250 mm × 4 mm i.d.) fitted with an IonPac AG17 guard column (50 mm × 4 mm i.d.), or an Amersham Biosciences Superdex Peptide 10/300 column. ^{1}H and ^{13}C NMR spectra were measured on a Bruker AC250, Bruker DPX360, or Bruker AVA600 instrument; *J*-values are in Hz. Assignments were made using COSY and HSQC experiments. Melting points were determined on a Gallenkamp Electrothermal Melting Point apparatus and are uncorrected. Optical rotations were measured on an AA-1000 polarimeter with a path length of 1.0 dm, at the sodium D-line at room temperature. Fast atom bombardment (FAB) mass spectra were obtained using a Kratos MS50TC mass spectrometer at The University of Edinburgh. Electrospray (ES) mass spectra were obtained using a MicroMass ZMD mass spectrometer. A Smith Synthesiser (Personal Chemistry AB) was used for microwave irradiation.

Methyl (methyl 2,3,4-tri-*O*-acetyl-β-D-glucopyranosid)uronate 2

Bromide **1**[1] (5.00 g, 12.6 mmol) was dissolved in dry methanol (5 ml) and 3Å molecular sieves added to the solution. The suspension was stirred under nitrogen at room temperature for 0.5 h and then cooled to – 20 ºC. Silver oxide (3.20 g, 13.9 mmol) was added and the reaction mixture allowed to reach room temperature in the absence of light. After 2 h the reaction mixture was filtered through a Celite pad and the filtrate was concentrated under reduced pressure. The residue obtained was crystallized from absolute ethanol to give the methyl glycoside **2** as needles (3.36 g, 76%), **MP** 148-150 ºC (lit.,[2] 149-150 ºC).

Methyl (methyl β-D-glucopyranosid)uronate 3

Peracetylated methyl glycoside **2** (2.06 g, 5.92 mmol) was dissolved in dry methanol (10 ml) and the solution cooled to 0 ºC. Methanolic sodium methoxide (1 M, 50 μl) was then added and the reaction mixture allowed to slowly reach room temperature. After 24 h the mixture was again cooled to 0 ºC and a second addition of sodium methoxide (1 M, 50 μl) was made. After 48 h a third addition of sodium methoxide (1 M, 50 μl) was made in the same manner and after 72 h the reaction mixture was neutralized with Bio-Rad AG50-X8 (H^+) resin. The resin was removed by filtration and the filtrate concentrated under reduced pressure. The residue obtained was purified by flash chromatography (CH_2Cl_2:MeOH, 9:1) to afford the methyl ester **3** (1.23 g, 94%). Spectroscopic data were in agreement with the literature.[3]

Methyl β-D-glucopyranosiduronic acid 4

Methyl glucuronide **3** (1.20 g, 5.40 mmol) was dissolved in methanol-water 1:1 (20 ml) and sodium hydroxide (1.10 g, 27.5 mmol) was added to the solution. The reaction mixture was stirred at room temperature overnight and Bio-Rad AG50-X8 (H^+) resin was added to adjust the pH to ~2. The resin was removed by filtration and the filtrate concentrated under reduced pressure. Residual water was removed by lyophilization to give crude **4** (1.20 g) as a glass, which was used without further purification.

Methyl (methyl 2,3-di-*O*-acetyl-4-deoxy-α-L-threo-hex-4-enopyranosid)uronate 5

Peracetylated glycoside **2** (3.36 g, 9.65 mmol) was dissolved in dry dichloromethane (10 ml) and the solution cooled to 0 ºC under nitrogen. DBU (2.20 ml, 14.7 mmol) was then added and the reaction mixture allowed to reach room temperature. The reaction was stirred at room temperature overnight and then quenched with cold saturated ammonium chloride. The organic phase was then shaken with water, dried and concentrated under reduced pressure. The residue obtained was purified by flash chromatography (toluene-ethyl acetate 4:1) to give **5** (2.10 g, 75%). The material obtained on a smaller scale (starting from 340 mg of **2**) was crystallized from absolute ethanol to give prisms, **MP** 91-93 ºC (lit.,[4] 90.5-91 ºC). **^{1}H NMR** (360 MHz, $CDCl_3$): δ 6.20 (1 H, dd, *J* 4.7 & 1.4 Hz, H-4), 5.18 (1 H, ddd, *J* 4.7, 2.2 & 0.7 Hz, H-3), 5.11 (1 H, d, *J* 2.9 Hz, H-1), 5.06 (1 H, m, H-2), 3.83 (3 H, s, COOC*H*$_3$), 3.50 (3 H, s, OC*H*$_3$), 2.09 (3 H, s, C*H*$_3$CO), 2.07 (3 H, s, C*H*$_3$CO). **^{13}C NMR** (62.9 MHz, $CDCl_3$): δ 170.1, 169.6 (CH_3CO), 162.4 ($COOCH_3$), 142.4 (C-5), 107.4 (C-4), 97.9 (C-1), 68.6 (C-2), 64.5 (C-3), 57.2 (OCH_3), 52.8 ($COOCH_3$), 21.1, 20.9 (CH_3CO).

Methyl (methyl 4-deoxy-α-L-threo-hex-4-enopyranosid)uronate 6

Unsaturated methyl glycoside **5** (2.10 g, 7.29 mmol) was dissolved in dry methanol (10 ml) and methanolic sodium methoxide (1 M, 100 µl) was added. The reaction mixture was stirred at room temperature for 12 h and neutralized with Bio-Rad AG50-X8 (H^+) resin. The resin was removed by filtration and the filtrate concentrated under reduced pressure. The residue obtained was purified by flash chromatography (CH_2Cl_2:MeOH, 19:1) to afford the methyl ester **6** (1.1 g, 74%). **^{1}H NMR** (250 MHz, $CDCl_3$): δ 6.25 (1 H, dd, *J* 4.2 & 1.5 Hz, H-4), 5.11 (1 H, dd, *J* 3.2 & 1.2 Hz, H-1), 4.02-3.95 (2 H, m, H-2 & H-3), 3.83 (3 H, s, COOC*H*$_3$), 3.51 (3 H, s, OC*H*$_3$), 2.83 (1 H, d, *J* 10.0 Hz, O*H*), 2.49 (1 H, d, *J* 6.5 Hz, O*H*). **^{13}C NMR** (62.9 MHz, $CDCl_3$): δ 162.6 ($COOCH_3$), 139.7 (C-5), 111.9 (C-4), 100.9 (C-1), 69.0 (C-2), 65.4 (C-3), 57.0 (OCH_3), 52.5 ($COOCH_3$).

Methyl 4-deoxy-α-L-threo-hex-4-enopyranosiduronate 7

Methyl hexenuronide **6** (1.10 g, 5.40 mmol) was dissolved in ethanol-water 1:1 (20 ml) and sodium hydroxide (1.10 g, 27.5 mmol) was added to the solution. The reaction mixture was stirred at room temperature overnight and Bio-Rad AG50-X8 (H^+) resin was added to adjust the pH to ~2. The resin was removed by filtration and the filtrate concentrated under reduced pressure. Residual water was removed by lyophilization to give crude **7** (1.10 g) as a pale yellow glass, which was used without further purification.

1-*N*-Acetyl-2,2,6,6-tetramethyl-4-aminopiperidine 8b

2,2,6,6-Tetramethyl-4-aminopiperidine (2.00 g, 12.8 mmol) was dissolved in dry dichloromethane (20 ml). Benzyl chloroformate (2.00 ml, 14.0 mmol) was then added dropwise over 0.5 h with vigorous stirring. The reaction mixture was stirred vigorously for 3 h and diluted with diethyl ether (20 ml). The white precipitate was removed by filtration and thoroughly rinsed with diethyl ether to give 4-benzyloxycarbonylamino-2,2,6,6-tetramethylpiperidine

hydrochloride salt (4.08 g, 98%) as a white solid, **MP** 275-277 ºC. **^{1}H NMR** (250 MHz, CD_3OD): δ 7.36-7.29 (5 H, m, Ar*H*), 5.09 (2 H, s, CH_2Ph), 4.90 (3 H, br s, N*H*), 4.01 (1 H, tt, *J* 12.5 & 3.8 Hz, H-4), 2.03 (2 H, dd, *J* 14.0 & 3.8 Hz, H-3a & H-5a), 1.59-1.48 (14 H, m, CH_3, H-3b & H-5b). **^{13}C NMR** (62.9 MHz, CD_3OD): δ 158.0 (*C*=O), 138.3 (ArC-1), 129.5, 129.0, 128.9 (ArC-2-6), 67.5 (*C*H_2Ph), 58.6 (C-2), 43.0 (C-4), 42.4 (C-3), 30.6, 25.1 (*C*H_3). ***m/z*** (FAB, THIOG.) 291 ([M]$^+$, 95 %), 201 (40), 157 (29). **HRMS** (FAB, 3-NOBA) [M]$^+$ found 291.2076, $C_{17}H_{27}N_2O_2$ requires 291.2072.

4-benzyloxycarbonylamino-2,2,6,6-tetramethylpiperidine (1.45 g, 5.00 mmol), which was obtained upon extraction with diethyl ether of a basified aqueous solution of the hydrochloride salt, was dissolved in triethylamine (1 ml) and acetic anhydride (945 µl, 10.0 mmol) was added. The reaction mixture was then stirred and heated by microwave irradiation at 160 ºC for 1 h. It was then concentrated under reduced pressure and the residue obtained purified by flash chromatography (Hexanes:EtOAc, 2:3) to give 1-*N*-acetyl-4-benzyloxycarbonylamino-2,2,6,6-tetramethylpiperidine (1.60 g, 96%) as a white solid, **MP** 110-111 ºC. **^{1}H NMR** (250 MHz, $CDCl_3$): δ 7.33-7.20 (5 H, m, ArH), 5.02 (2 H, br s, CH_2Ph), 4.91 (1 H, d, *J* 7.2 Hz, N*H*), 4.04-3.92 (1 H, m, H-4), 2.15-2.05 (5 H, m, H-3a, H-5a & CH_3CO), 1.63 (2 H, dd, *J* 14.5 & 7.0 Hz, H-3b & H-5b), 1.38 (6 H, s, CH_3), 1.21 (6 H, s, CH_3). **^{13}C NMR** (62.9 MHz, $CDCl_3$): δ 174.0 (CH_3*C*O), 155.8 ($PhCH_2O$*C*O), 136.4 (ArC-1), 128.6, 128.3 (ArC-2-6), 66.9 (*C*H_2Ph), 56.4 (C-2), 45.3 (C-3), 41.9 (C-4), 31.3, 29.4 (*C*H_3), 28.8 (*C*H_3CO). ***m/z*** (FAB, THIOG.) 333 ([M+H]$^+$, 65 %), 291 (46), 199 (42). **HRMS** (FAB, 3-NOBA) [M+H]$^+$ found 333.2182, $C_{19}H_{29}N_2O_3$ requires 333.2178.

1-*N*-acetyl-4-benzyloxycarbonylamino-2,2,6,6-tetramethylpiperidine (1.18 g, 3.96 mmol) was dissolved in methanol (10 ml) and a catalytic amount of palladium on charcoal was added. The reaction mixture was stirred under hydrogen for 4 h and then filtered through a Celite pad. The filtrate was concentrated under reduced pressure and the resulting residue purified by flash chromatography (CH_2Cl_2:MeOH:NH_4OH(15.4 M), 190:9:1) to give the title compound **8b** (0.67 g, 85%) as an oil. **^{1}H NMR** (250 MHz, $CDCl_3$): δ 3.19 (1 H, qt, *J* 7.3 Hz, H-4), 2.13 (3 H, s, CH_3CO), 1.97 (2 H, dd, J 14.1 & 7.1 Hz, H-3a & H-5a), 1.59-1.38 (16 H, m, H-3b, H-5b, CH_3 & NH_2). **^{13}C NMR** (62.9 MHz, $CDCl_3$): δ 174.0 (CH_3*C*O), 56.9 (C-2), 49.4 (C-3), 41.3 (C-4), 31.8, 29.3 (*C*H_3), 29.0 (*C*H_3CO). ***m/z*** (FAB, THIOG.) 199 ([M+H]$^+$, 72 %). **HRMS** (FAB, 3-NOBA) [M+H]$^+$ found 199.1814, $C_{11}H_{23}N_2O$ requires 199.1810.

General Procedure for coupling Uronic Acid models 4 and 7

50 mg of crude acid (0.22 mmol of **4** or 0.24 mmol of **7**) was dissolved in methanol-water (9:1, 5 ml) together with the appropriate amine (1.2 eq.) and the mixture was stirred at room temperature for 10 min. DMT-MM (1.5 eq.) was then added and the reaction stirred at room temperature until complete as judged by tlc (5-14 h). The solvent was removed under reduced pressure and the residue co-evaporated with absolute ethanol to remove traces of water. Flash chromatography (dichloromethane-methanol, 15:1) afforded the corresponding amide.

Methyl *N*-propargyl-β-D-glucopyranosiduronamide 9a

(94%). **MP** 181-182 ºC. **^{1}H NMR** (360 MHz, CD_3OD): δ 4.25 (1 H, d, *J* 7.8 Hz, H-1), 4.02 (2 H, d, J 2.4 Hz, CH_2C≡C), 3.73 (1 H, d, *J* 9.5 Hz, H-5), 3.54 (3 H, s, OCH_3), 3.54-3.44 (1 H, m, H-4), 3.40 (1 H, t, *J* 9.0 Hz, H-3), 3.23 (1 H, t, *J* 8.4 Hz, H-2), 2.60 (1 H, t, *J* 2.5 Hz, C≡C*H*). **^{13}C NMR** (62.9 MHz, CD_3OD): δ 171.4 (*C*ONH), 105.5 (C-1), 80.4 (CH_2*C*≡C), 77.5 (C-3), 76.2 (C-5), 74.5 (C-2), 73.4 (C-4), 72.3 (C≡*C*H), 57.6 (O*C*H_3), 29.3 (*C*H_2C≡C). ***m/z*** (FAB, THIOG.) 246 ([M+H]$^+$, 27 %), 215 (43). **HRMS** (FAB, 3-NOBA) [M+H]$^+$ found 246.0976, $C_{10}H_{16}NO_6$ requires 246.0978. **$[\alpha]_D$** –60 (*c* 1.0 in MeOH).

Methyl *N*-(1-*N*-Acetyl-2,2,6,6-tetramethyl-piperidin-4-yl)-β-D-glucopyranosiduronamide 9b

(88%). **^{1}H NMR** (360 MHz, CD_3OD): δ 4.27-4.21 (2 H, m, H-1_{GlcA} & H-4_{pip}), 3.73 (1 H, d, *J* 9.6 Hz, H-5_{GlcA}), 3.55-3.50 (4 H, m, H-4_{GlcA} & OCH_3), 3.40 (1 H, t, *J* 9.1 Hz, H-3_{GlcA}), 3.24 (1 H, dd, *J* 9.2 & 7.8 Hz, H-2_{GlcA}), 2.21-2.14 (5 H, m, H-$3a_{pip}$, H-$5a_{pip}$ & CH_3CO), 1.90 (1 H, t, *J* 7.2 Hz, H-$3b_{pip}$), 1.86 (1 H, t, *J* 7.0 Hz, H-$5b_{pip}$), 1.56 (6 H, s, 2 × CH_{3pip}), 1.49 (3 H, s, CH_{3pip}), 1.48 (3 H, s, CH_{3pip}). **^{13}C NMR** (62.9 MHz, CD_3OD): δ 176.5 (CH_3CO), 170.9 (*C*ONH), 105.7 (C-1_{GlcA}), 77.6 (C-3_{GlcA}), 76.6 (C-5_{GlcA}), 74.6 (C-2_{GlcA}), 73.1 (C-4_{GlcA}), 58.0 (C-2_{pip} & C-6_{pip}), 57.6 (O*C*H_3), 45.1, 44.9 (C-3_{pip} & C-5_{pip}), 41.5 (C-4_{pip}), 31.4, 31.3, 29.7 & 29.5 (*C*H_{3pip}), 28.8 (*C*H_3CO). ***m/z*** (FAB, THIOG.) 389 ([M+H]$^+$, 89 %). **HRMS** (FAB, 3-NOBA) [M+H]$^+$ found 389.2281, $C_{18}H_{33}N_2O_7$ requires 389.2288. **[α]$_D$** –33 (*c* 1.0 in MeOH).

Methyl *N*-propargyl-4-deoxy-α-L-threo-hex-4-enopyranosiduronamide 10a

(81%). **^{1}H NMR** (250 MHz, CD_3OD): δ 5.99 (1 H, dd, *J* 3.9 & 0.7 Hz, H-4), 4.95 (1 H, dd, *J* 5.0 & 0.7 Hz, H-1), 4.06-4.02 (3 H, m, H-3 & CH_2C≡C), 3.71 (1 H, t, *J* 4.3 Hz, H-2), 3.55 (3 H, s, OCH_3), 2.56 (1 H, t, J 2.5 Hz, C≡C*H*). **^{13}C NMR** (62.9 MHz, CD_3OD): δ 163.9 (*C*ONH), 143.5 (C-5), 109.4 (C-4), 103.6 (C-1), 80.5 (CH_2C≡C), 71.9 (C≡*C*H), 71.7 (C-2), 67.9 (C-3), 57.3 (O*C*H_3), 29.4 (*C*H_2C≡C). ***m/z*** (FAB, THIOG.) 228 ([M+H]$^+$, 32 %). **HRMS** (FAB, 3-NOBA) [M+H]$^+$ found 228.0871, $C_{10}H_{14}NO_5$ requires 228.0872. **[α]$_D$** –40 (*c* 1.0 in MeOH).

Methyl *N*-(1-*N*-Acetyl-2,2,6,6-tetramethyl-piperidin-4-yl)-4-deoxy-α-L-threo-hex-4-enopyranosiduronamide 10b

(85%). **MP** 203-205 ºC. **^{1}H NMR** (250 MHz, CD_3OD): δ 5.99 (1 H, dd, *J* 4.0 & 0.8 Hz, H-$4_{ΔUA}$), 4.98 (1 H, dd, *J* 4.7 & 0.7 Hz, H-$1_{ΔUA}$), 4.30 (1 H, qt, *J* 7.5 Hz, H-4_{pip}), 4.03 (1 H, t, *J* 4.0 Hz, H-$3_{ΔUA}$), 3.74 (1 H, t, *J* 4.7 Hz, H-$2_{ΔUA}$), 3.53 (3 H, s, OCH_3), 2.23-2.10 (5 H, m, H-$3a_{pip}$, H-$5a_{pip}$ & CH_3CO), 1.95 (1 H, t, *J* 7.5 Hz, H-$3b_{pip}$), 1.89 (1 H, t, *J* 7.5 Hz, H-$5b_{pip}$), 1.57 (6 H, s, 2 × CH_{3pip}), 1.50 (6 H, s, 2 × CH_{3pip}). **^{13}C NMR** (62.9 MHz, CD_3OD): δ 176.5 (CH_3CO), 163.5 (*C*ONH), 143.5 (C-$5_{ΔUA}$), 109.1 (C-$4_{ΔUA}$), 103.4 (C-$1_{ΔUA}$), 71.6 (C-$2_{ΔUA}$), 67.7 (C-$3_{ΔUA}$), 58.2 (C-2_{pip} & C-6_{pip}), 57.2 (O*C*H_3), 45.5, 45.3 (C-3_{pip} & C-5_{pip}), 41.7 (C-4_{pip}), 31.6, 31.5, 29.5 & 29.3 (*C*H_{3pip}), 28.9 (*C*H_3CO).). ***m/z*** (FAB, THIOG.) 371 ([M+H]$^+$, 49 %). **HRMS** (FAB, 3-NOBA) [M+H]$^+$ found 371.2190, $C_{18}H_{31}N_2O_6$ requires 371.2182. **[α]$_D$** –20 (*c* 0.5 in MeOH).

Methyl *N*-(1-*N*-Acetyl-2,2,6,6-tetramethyl-piperidin-4-yl)-4-deoxy-α-L-threo-hex-4-enopyranosiduronamide 10c

(77%). ***m/z*** (FAB, 3-NOBA) 345 ([M+H]$^+$, 33 %). **HRMS** (FAB, 3-NOBA) [M+H]$^+$ found 345.2025, $C_{16}H_{29}N_2O_6$ requires 345.2026. **[α]$_D$** –23 (*c* 1 in MeOH).

General Procedure for coupling disaccharide 11

(4-deoxy-2-*O*-sulfato-α-L-threo-hex-4-enopyranosyluronic acid)-(1→4)-2-deoxy-2-sulfamido-6-*O*-sulfato-D-glucose **11** (200 μg, 0.30 μmol) was dissolved in 20 μl MeOH-water (1:1) together with 1.2 eq. of the appropriate amine and stirred at rt for 5 min. DMT-MM (250 μg, 0.92 μmol) dissolved in 10 μl MeOH was then added and the reaction stirred at room temperature for the first 3-4 hours and at -20 ºC thereafter. The reaction mixture was purified by strong anion-exchange HPLC according to the following protocol: after a brief (5 min) wash with pH 3.5 water, the column was eluted with a linear gradient of 0-1.0 M NaCl, pH 3.5, over 60 min at a flow rate of 1 ml min^{-1}. Fractions were lyophilized and desalted by gel filtration chromatography on a Superdex Peptide column eluted with 0.25 M NH_4HCO_3 at a flow rate of 0.5 ml min^{-1}. In both cases, elution was monitored on-line by UV absorption at 232 nm. GPC fractions were extensively lyophilized to remove excess ammonium bicarbonate.

TEMPO-analogue-linked disaccharide 12b

R_t 34.6 min. 74% conversion. **^{1}H NMR** (600 MHz, D_2O): δ 6.08 (1 H, d, *J* 4.2 Hz, H-$4_{\Delta UA}$), 5.54 (1 H, d, *J* 3.1 Hz, H-$1_{\Delta UA}$), 5.42 (1 H, d, *J* 3.1 Hz, H-1_{GlcNS}), 4.58 (1 H, bs, H-$2_{\Delta UA}$), 4.39 (1 H, bs, H-$3_{\Delta UA}$), 4.36-4.14 (3 H, m, H-5-6_{GlcNS}), 4.09 (1 H, dd, *J* 13, 6.6 Hz, H-4_{pip}), 3.84 (1 H, t, *J* 9.6 Hz, H-4_{GlcNS}), 3.74 (1 H, t, *J* 10.2 Hz, H-3_{GlcNS}) 3.26 (1 H, dd, *J* 10.2, 3.3 Hz, H-2_{GlcNS}), 2.26-2.21 (5 H, m, H-$3a_{pip}$, H-$5a_{pip}$ & CH_3CO), 1.97-1.94 (2 H, m, H-$3b_{pip}$ & H-$5b_{pip}$), 1.56, 1.55, 1.47, 1.46 (12 H, 4 s, 4 × CH_{3pip}). ***m/z*** (ESI–) 252 ($[M–3H]^{3–}$, 100 %).

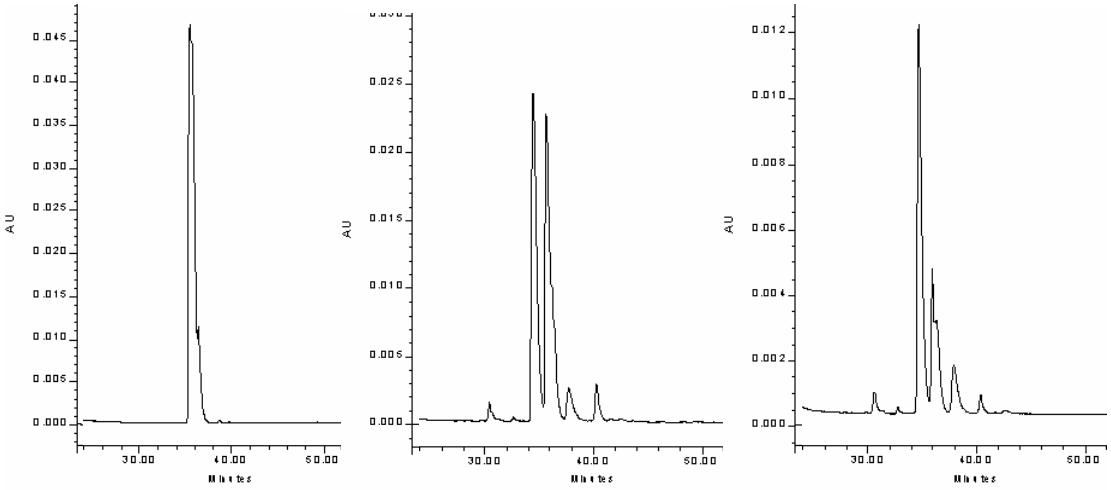

Figure 1. SAX-HPLC analysis of the DMT-MM promoted reaction of **11** and **8b** at time = 0, 1 and 17 hours respectively. Absorbance was measured at 232 nm and times are given in minutes.

TEMPO-linked disaccharide 12c

R_t 34.3 min. 68% conversion. ***m/z*** (ESI–) 364 ($[M–2H]^{2–}$, 55 %).

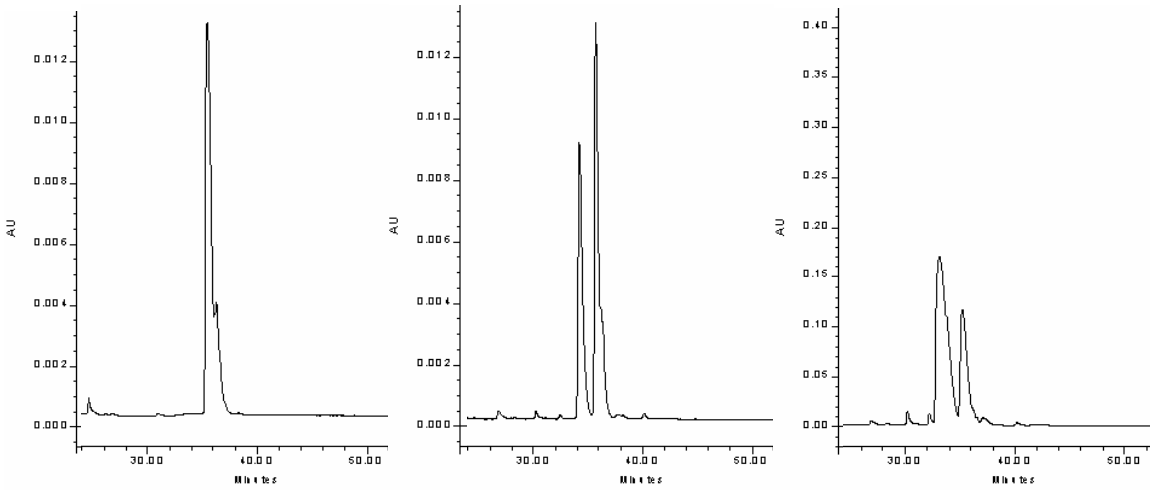

Figure 2. SAX-HPLC analysis of the DMT-MM promoted reaction of **11** and **8c** at time = 0, 2 and 18 hours respectively. Absorbance was measured at 232 nm and times are given in minutes.

Amber Modelling of the TEMPO-disaccharide.

Parameterization of the AMBER force field for GAGs. Parm99 force field in AMBER7[5] was amended by inclusion of the GLYCAM_2000a[6] parameter set for carbohydrates. The parameters for sulfates and sulfamates were applied according to Huige and Altona[7] and GLYCAM_2000a was modified to include a new atom type, SO. Parameters for sulfate and sulfamate groups that were not available from the work of Huige and Altona were approximated by the Parm99 parameters of phosphates. The compatibility issues arising from the presence of the double-bond contained in the iduronic ring were resolved. The resulting modified force field was loaded together with the original Parm99 force field into XLEAP sessions for molecular dynamics.

Molecular modelling The structure of the TEMPO-disaccharide was built within XLEAP by adding 1-oxyl-4-carboxamido-2,2,6,6,-tetra-methyl-piperidine to the carboxyl group of the uronic acid using previously refined structure of the fully sulfated disaccharide. Partial atom charges were calculated with RESP procedure using Gaussian98 and 6-31G* basis set. The single point charge calculation was carried out with the convergence criteria of 1.00D-02 and 1.00D-04 for the maximal and rmsd change of the density matrix elements, respectively. The net charge of the molecule was set to be -3. Both $^{1}H_{2}$ and $^{2}H_{1}$ conformations of the uronic ring were considered as starting structures.

The TEMPO-disaccharide molecule was dissolved in a cubic 32.683 Å water box with a spacing of 8 Å containing 981 triangulated 3-point water molecules. The water box was first energy minimized using tight restraints on the solute atoms, followed by energy minimization of the complete system without restraining the solute. The temperature of the system was then increased from 0 K to 300 K in 0.002 ns and stabilized at 300 K for 0.02 ns at a constant volume using SHAKE for bonds involving H-atoms. The position of the solute was weakly restrained. This step was followed by a 0.1 ns equilibration molecular dynamic at 300 K and constant pressure (1 atm) without any restraints on the position of the solute. Finally, a constant temperature free molecular dynamics with SHAKE option at 300 K was performed for 1 ns. Both initial $^{1}H_{2}$ and $^{2}H_{1}$ conformations converged to the $^{2}H_{1}$ form during the simulation. The following table compares the glycosidic dihedral angles of the optimized TEMPO-disaccharide structure with those observed for the disaccharide alone.

	Ring D	Ring A	Φ	ψ
TEMPO	$^{2}H_{1}$	$^{4}C_{1}$	51.0	4.8
X-ray[8]	$^{1}H_{2}$	$^{4}C_{1}$	42.3	18.3
Solution[9]	$^{1}H_{2}$	$^{4}C_{1}$	45.7	13.2

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