Synthesis and Molecular Recognition Studies of the HNK-1 Trisaccharide and Related Oligosaccharides. The Specificity of Monoclonal Anti-HNK-1 Antibodies as Assessed by Surface Plasmon Resonance and STD NMR

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Supporting information 1

1. Synthetic procedures and spectral data

General: NMR spectra were recorded on Bruker AMX-400, Bruker DRX-500, and Bruker Avance 600 instruments. The spectra of protected carbohydrate derivatives were measured for solutions in $CDCl_3$ or CD_3OD, and ^1H NMR chemical shifts were referenced to residual signal of $CHCl_3$ (δ_H 7.27) or CH_3OH (δ_H 3.33). ^{13}C chemical shifts were referenced to the central resonance of $CDCl_3$ (δ_C 77.0) and CD_3OD (δ_C 49.0). NMR spectra of free oligosaccharides were measured for solutions in D_2O using acetone (δ_H 2.225, δ_C 31.45) as an internal standard. Signal assignment was made using COSY, TOCSY, and HSQC experiments. Monosaccharide residues in oligosaccharides are numbered by Roman numerals starting from the reducing end. Data for aromatic carbons in ^{13}C NMR spectra are not given. HRMS (ESI) were obtained on a MicrOTOF II (Bruker Daltonics) instrument. Optical rotations were measured using a JASCO DIP-360 polarimeter at 18–22 °C in solvents specified. Melting points were determined using a Koffler apparatus. TLC was performed on Silica Gel 60 F254 plates (E. Merck), and visualization was accomplished using UV light or by charring at ~150 °C with 10% (v/v) H_3PO_4 in ethanol or Mostain reagent (ceric sulfate (1% w/v) and ammonium molybdate (2.5% w/v) in 10% (v/v) aqueous H_2SO_4). Column chromatography was carried out on Silica gel 60 (40–63 μm, E. Merck). Gel-permeation chromatography of free oligosaccharides was performed on a Sephadex G-15 column (2.5×50 cm) in water. Gel-permeation chromatography of biotin-tagged saccharides was carried out on a TSK HW-40(S) column (2×80 cm) in 0.05 M NH_4HCO_3. Refractive Index Detector K-2401 (Knauer) was used to monitor gel-permeation chromatography. All air- or moisture-sensitive reactions were carried out using dry solvents under dry argon. Chemicals were purchased from Acros, Fluka, or Aldrich and used without further purification.

2-Azidoethyl 2-acetamido-3-*O*-benzyl-2-deoxy-β-D-glucopyranoside (12)

A solution of benzylidene acetal **11** (2.04 g, 5.40 mmol) in 80% aqueous AcOH (60 mL) was stirred at 40 ºC for 36 h, then concentrated, and residual AcOH was removed by coevaporation with toluene. The residue was purified by column chromatography (toluene/MeOH 27:1) to give diol **12** (1.34 g, 86%) as an amorphous solid. ^{1}H NMR (500 MHz, $CDCl_3$ + CD_3OD): δ = 7.35–7.22 (m, 5H; Ph), 4.87, 4.66 (2d, J_{gem} = 11.4 Hz, 2H; C*H*$_2$Ph), 4.54 (d, $J_{1,2}$ = 8.1 Hz, 1H; H-1), 4.03 (m, 1H; OC*Ha*HbCH_2N_3), 3.90 (dd, $J_{6a,5}$ = 2.3 Hz, $J_{6a,6b}$ = 11.0 Hz, 1H; H-6a), 3.77 (t, $J_{2,3}$ = 9.0 Hz, 1H; H-2), 3.71 (dd, $J_{6b,5}$ = 5.9 Hz, 1H; H-6b), 3.67 (m, 1H; OCHa*Hb*CH_2N_3), 3.52 (m, 2H; H-3, H-4), 3.43 (m, 1H; OCH_2C*Ha*HbN_3), 3.31 (m, 2H; H-5, OCH_2CHa*Hb*N_3) 1.87 ppm (c, 3H; CH_3CO). ^{13}C NMR (125 MHz, $CDCl_3$ + CD_3OD): δ = 173.56 (CO), 102.43 (C-1), 84.08 (C-3), 78.09 (C-5), 75.74 (*C*H_2Ph), 72.10 (C-4), 69.36 (O*C*$H_2CH_2N_3$), 62.76 (C-6), 56.28 (C-2), 51.89 (OCH_2*C*H_2N_3), 23.30 ppm (*C*H_3CO); HRMS (ESI) *m/z* calcd for $C_{17}H_{24}N_4O_6$ + H^+: 381.1769 $[M+H]^+$; found: 381.1767.

2-Azidoethyl 2-acetamido-3-*O*-benzyl-2-deoxy-6-*O*-tosyl-β-D- glucopyranoside (13)

p-Toluenesulfonyl chloride (0.57 g, 3.00 mmol) was added to a chilled (4 ºC) solution of **12** (0.71 g, 2.46 mmol) in pyridine (7 mL), the resulting mixture was stirred for 10 min, then cooling was removed and stirring was continued for 24 h at room temperature. Water (1 mL) was added, the mixture was diluted with $CHCl_3$ (120 mL), washed successively with water, 1 M HCl, water, and satd $NaHCO_3$, and concentrated. Column chromatography of the residue (toluene/EtOAc 1:1) produced **13** (0.82 g, 75%), m. p. = 226-227 ºC (EtOAc/light petroleum), $[\alpha]_D$ = +32 (*c* = 1, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.79–7.22 (m, 9H; Ph, C_6*H*$_4CH_3$), 5.84 (d, $J_{2,NH}$ = 7.7 Hz, 1H; NH), 4.88 (d, $J_{1,2}$ = 8.2 Hz, 1H; H-1), 4.69 (s, 2H; C*H*$_2$Ph), 4.28 (dd, $J_{6a,5}$ = 1.8 Hz, $J_{6a,6b}$ = 10.9 Hz, 1H; H-6a), 4.21 (dd, $J_{6b,5}$ = 5.3 Hz, 1H; H-6b) 4.03 (dd, $J_{3,2}$ = 8.5 Hz, $J_{3,4}$ = 9.8 Hz, 1H; H-3), 3.90, 3.60 (2 m, 2H; OC*H*$_2CH_2N_3$), 3.53 (m, 1H; H-5), 3.47 (t, $J_{4,5}$ = 9.7 Hz, 1H; H-4), 3.41 (m, 1H,

$OCH_2CHaHbN_3$), 3.24 (m, 1H; H-2), 3.18 (m, 1H; $OCH_2CHaHbN_3$), 2.85 (br. s, 1H; 4-OH), 2.42 (s, 3H; $C_6H_4CH_3$), 1.89 ppm (s, 3H, CH_3CO). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 171.00 (CO), 99.55 (C-1), 79.97 (C-3), 74.45 (CH_2Ph), 73.37 (C-5), 70.52 (C-4), 68.90 (C-6), 68.34 ($OCH_2CH_2N_3$), 57.12 (C-2), 50.54 ($OCH_2CH_2N_3$), 23.52 (CH_3CO), 21.59 ppm ($C_6H_4CH_3$); elemental analysis calcd (%) for $C_{24}H_{30}N_4O_8S$ (534.58): C 53.92, H 5.66, N 10.48; found: C 53.88, H 5.69, N 10.32.

2-Azidoethyl 2-acetamido-3-*O*-benzyl-2-deoxy-6-*O*-(*p*-methoxyphenyl)-β-D-glucopyranoside (14)

A solution of tosylate **13** (1.42 g, 3.21 mmol) in DMF (7 mL) was added to a mixture of *p*-methoxyphenol (437 mg, 3.52 mmol) and NaH (155 mg of 60% suspension in mineral oil, 3.88 mmol) in DMF (3 mL). The resulting mixture was stirred at 60 ºC for 72 h under Ar, then cooled, diluted with CH_2Cl_2 (300 mL), and washed with water and satd $NaHCO_3$. The organic solution was dried, concentrated, and the residue was purified by column chromatography (toluene/EtOAc 1:1) to provide **14** (1.31 g, 91%) as an amorphous solid; $[\alpha]_D$ = +38 (*c* = 1, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$ + CD_3OD): δ = 7.33–7.22 (m, 5H, Ph), 6.88, 6.80 (2 d, *J* = 9.0 Hz 4H; $C_6H_4OCH_3$), 4.84, 4.66 (2 d, J_{gem} = 11.4 Hz, 2H; CH_2Ph), 4.61 (d, $J_{1,2}$ = 8.5 Hz, 1H; H-1), 4.28 (br. d, $J_{6a,6b}$ = 10.5 Hz, 1H; H-6a), 4.11 (dd, $J_{6b,5}$ = 5.4 Hz, 1H; H-6b), 3.95 (m, 1H; $OCHaHbCH_2N_3$), 3.74 (m, 4H; H-2, CH_3O), 3.62 (m, 4H, H-3, H-4, H-5, $OCHaHbCH_2N_3$), 3.42 (m, 1H; $OCH_2CHaHbN_3$), 3.25 (m, 1H; $OCH_2CHaHbN_3$), 1.88 ppm (s, 3H; CH_3CO). ^{13}C NMR (125 MHz, $CDCl_3$ + CD_3OD): δ = 171.17 (CO), 100.59 (C-1), 81.91 (C-3), 74.69 ($PhCH_2$), 74.17 (C-5), 70.42 (C-4), 68.11 (C-6), 67.84 OCH_2CH_2N), 55.16, 54.82 (C-2, CH_3O), 50.29 (OCH_2CH_2N), 22.19 ppm (CH_3CO); elemental analysis calcd (%) for $C_{24}H_{30}N_4O_7$ (486.52): C 59.25, H 6.22, N 11.52; found: C 58.96, H 6.29, N 11.35.

Allyl [methyl(3-*O*-acetyl-2,4-di-*O*-benzoyl-β-D-glucopyranosyl)uronate]-(1→3)-2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-galactopyranoside (18)

A mixture of imidate **9** (1.47 g, 2.33 mmol), acceptor **16** (1.12 g, 2.70 mmol) and mol. sieves AW 300 (2.00 g) in toluene (190 mL) was stirred at room temperature under Ar for 1 h, cooled to 0 ºC, and $BF_3{\cdot}Et_2O$ (173 µL, 1.40 mmol) was added. The mixture was stirred at +4 ºC for 48 h, filtered through a Celite layer, and the solids were thoroughly washed with a mixture of CH_2Cl_2 – EtOAc (3:1). The filtrate was washed with aq satd $NaHCO_3$, dried, and concentrated. The residue was crystallized from EtOAc/light petroleum to give **18** (1.24 g, 64%); m. p. = 267 ºC; $[\alpha]_D$ = +15 (*c* 1, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.92–7.10 (m, 20H; 4 Ph), 5.63 (m, 1H; OCH_2CH=CH_2), 5.55 (dd, $J_{2,3}$ = 10.0 Hz, 1H; H-2^{I}), 5.52 (s, 1H; PhC*H*), 5.49 (t, $J_{4,5}$ = 8.9 Hz, 1H; H-3^{II}), 5.44 (t, $J_{3,4}$ = 9.4 Hz, 1H; H-4^{II}), 5.29 (t, $J_{2,3}$ = 8.4 Hz, 1H; H-2^{II}), 5.09 (d *J* = 17.6 Hz, 1H; OCH_2CH=C*Ha*Hb), 5.06 (d, $J_{1,2}$ = 7.3 Hz, 1H; H-1^{II}), 4.96 (d, *J* = 10.5 Hz, 1H; OCH_2CH=CHa*Hb*), 4.57 (d, $J_{1,2}$ = 8.0 Hz, 1H; H-1^{I}), 4.43 (d, 1H; H-4^{I}), 4.28 (d, $J_{6a,6b}$ = 12.3 Hz, 1H; H-6a^{I}), 4.25 (dd, *J* = 8.9 Hz, *J* = 13.3 Hz, 1H; OC*Ha*HbCH=CH_2), 4.21 (d, 1H; H-5^{II}), 4.13 (dd, $J_{3,4}$ = 3.4 Hz, 1H; H-3^{I}), 4.07 (d, 1H; H-6b^{I}), 4.01 (dd, *J* = 6.2 Hz, 1H; OCHa*Hb*CH=CH_2), 3.57 (s, 3H; CH_3O), 3.47 (s, 1H; H-5^{I}), 1.72 ppm (s, 3H; CH_3CO); ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.06, 167.39, 165.23, 164.73 (CO), 137.57 (OCH_2CH=CH_2), 117.05 (OCH_2CH=CH_2), 100.85 (2C; C-1^{II}, Ph*C*H), 99.93 (C-1^{I}), 77.63 (C-3^{I}), 75.85 (C-4^{I}), 72.38 (C-5^{II}), 71.68 (C-3^{II}), 71.56 (C-2^{II}), 70.24 (C-2^{I}), 69.91 (C-4^{II}), 69.11 (OCH_2CH=CH_2), 68.76 (C-6^{I}), 66.64 (C-5^{I}), 52.72 (CH_3O), 20.19 (CH_3CO); elemental analysis calcd (%) for $C_{46}H_{44}O_{16}$ (852.83): C 64.78, H 5.20; found: C 64.96, H 5.29.

Allyl [methyl(3-*O*-acetyl-2,4-di-*O*-benzoyl-β-D-glucopyranosyl)uronate]-(1→3)-2,4,6-tri-*O*-benzoyl-β-D-galactopyranoside (19)

PPTS (1.15 g, 4.60 mmol) was added to a solution of **18** (1.10 g, 1.29 mmol) in 90% aqueous acetonitrile (170 mL) and the resulting mixture was kept at 80 ºC for 36 h. Acetonitrile was evaporated, and the remaining aqueous suspension of the product was extracted with CH_2Cl_2 (3×80 mL). The combined extracts were dried and concentrated to afford 4,6-diol (1.00 g, 98%). To a solution the diol (1.00 g, 1.31 mmol) in pyridine (18 mL) was added benzoyl chloride (1.10 mL, 9.55 mmol) at –10 ºC, the resulting mixture was stirred at this temperature for 3 h, then quenched by adding water and diluted with CH_2Cl_2 (80 mL). The solution was successively washed with 1 M HCl, water, and satd $NaHCO_3$, dried and concentrated. Column chromatography of the residue (toluene/EtOAc 5:1 → 2:1) provided **19** (1.17 g, 92%) as an amorphous solid. $[\alpha]_D$ +24 (*c* 1, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.15–7.17 (m, 25H; 5 Ph), 5.94 (d, $J_{4,3}$ = 2.9 Hz, 1H; H-4^I), 5.71 (m, 1H; OCH_2CH=CH_2), 5.67 (dd, $J_{2,3}$ = 9.7 Hz, 1H; H-2^I), 5.53 (t, $J_{4,5}$ = 9.3 Hz, 1H; H-4^{II}), 5.45 (t, $J_{3,4}$ = 9.1 Hz, 1H; H-3^{II}), 5.18 (t, $J_{2,3}$ = 9.8 Hz, 1H; H-2^{II}), 5.14 (d, *J* = 17.2 Hz, 1H; OCH_2CH=C*Ha*Hb), 5.05 (d, *J* = 11.1 Hz, 1H; OCH_2CH=CHa*Hb*), 5.02 (d, $J_{1,2}$ = 7.0 Hz, 1H; H-1^{II}), 4.70 (d, $J_{1,2}$ = 7.9 Hz, 1H; H-1^I), 4.54 (m, 2H; H-6a^I, H-6b^I), 4.40 (dd, 1H; H-3^I), 4.30 (dd, *J* = 4.7 Hz, *J* = 13.1 Hz, 1H; OC*Ha*HbCH=CH_2), 4.21 (d, 1H; H-5^{II}), 4.19 (t, $J_{5,6}$ = 7.2 Hz, 1H; H-5^I), 4.10 (dd, *J* = 6.3 Hz, 1H; OCHa*Hb*CH=CH_2), 3.65 (s, 3H, CH_3O), 1.73 ppm (s, 3H, CH_3CO); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 169.76, 166.86, 166.06, 165.57, 164.75, 164.45, 164.13 (CO), 117.56 (OCH_2CH=*C*H_2), 100.39 (C-1^{II}), 99.81 (C-1^I), 76.26 (C-3^{II}), 72.74 (C-5^{II}), 71.66 (C-5^I), 71.60 (C-2^I), 71.49 (C-2^{II}), 71.33 (C-3^{II}), 69.68 (C-4^I, O*C*H_2CH=CH_2), 69.54 (C-4^{II}), 62.63 (C-6^I), 52.70 (CH_3O), 20.16 ppm (*C*H_3CO); elemental analysis calcd (%) for $C_{53}H_{48}O_{18}$ (972.94): C 65.43, H 4.97; found: C 65.56, H 5.11.

[Methyl(3-*O*-acetyl-2,4-di-*O*-benzoyl-β-D-glucopyranosyl)uronate]-(1→3)-2,4,6-tri-*O*-benzoyl-D-galactopyranose (20)

$PdCl_2$ (243 mg, 1.37 mmol) and AcONa (238 mg, 2.90 mmol) were added to a solution of **19** (1.17 g, 1.20 mmol) in 95% aqueous AcOH (40 mL) and the mixture was stirred at 70 ºC for 3 h. After dilution with $CHCl_3$ (80 mL), the mixture was filtered through a Celite layer, the solids were washed with $CHCl_3$, and the combined filtrates were concentrated. Residual AcOH was removed by coevaporation with toluene. Column chromatography of the residue (light petroleum/EtOAc 2:1) afforded hemiacetal **20** (716 mg, 64%) as a mixture of anomers (α,β-ratio ~5:1). $[\alpha]_D$ = +29 (*c* 1, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.10–7.07 (m, 30H, Phαβ), 5.96 (d, $J_{4,3}$ = 3.0 Hz, 1H; H-4α^I), 5.92 (d, $J_{4,3}$ = 3.1 Hz, 0.2H; H-4β^I), 5.65 (t, $J_{1,2}$ = 3.4 Hz, 1H; H-1α^I), 5.51 (t, $J_{4,3}$ = 9.4 Hz, 1.2H; H-4αβ^{II}), 5.45 (m, 2.4H; H-2αβ^I, H-3αβ^{II}), 5.14 (m, 2.2H; H-1α^{II}, H-2αβ^{II}), 5.07 (d, $J_{1,2}$ = 6.8 Hz, 0.2H; H-1β^{II}), 4.79 (t, $J_{1,2}$ = 8.7 Hz, 0.2H; H-1β^I), 4.72 (dd, $J_{5,6}$ = 6.2 Hz, 1H; H-5α^I), 4.52–4.37 (m, 2.6H; H-6αβ^I, H-3β^I), 4.26 (d, $J_{5,4}$ = 9.4 Hz, 1H; H-5α^{II}), 4.24 (d, $J_{5,4}$ = 9.6 Hz, 0.2H; H-5β^{II}), 4.17 (t, 0.2H; H-5β^I), 3.82 (d, $J_{OH,1}$ = 9.1 Hz, 0.2H; 1-OHβ), 3.64 (s, 3H; CH_3Oα), 3.62 (s, 0.6H; CH_3Oβ), 3.40 (d, $J_{OH,1}$ = 2.9 Hz, 1H; 1-OHα), 1.67 ppm (s, 3.6H; CH_3COαβ); ^{13}C NMR (125 MHz, $CDCl_3$): δ 166.38, 166.19, 165.67, 165.54, 165.40, 164.89, 164.26 (CO), 100.90 (C-1α^{II}), 100.53 (0.2C; C-1β^{II}), 96.17 (0.2C; C-1β^I), 90.65 (C-1α^I), 75.72 (0.2C; C-3β^I), 74.32 (0.2C; C-2β^I), 72.83 (C-5α^{II}), 72.42 (C-3α^I), 72.01 (0.2C; C-5β^I), 71.71, 71.63, 71.54 (C-2α^{II}, C-2α^I, C-3α^{II}), 71.32 (0.2C; C-3β^{II}), 70.99 (C-4α^I), 69.74 (C-4α^{II}), 69.49 (0.2C; C-4β^{II}), 67.36 (C-5α^I), 62.91 (C-6α^I), 62.69 (0.2C; C-6β^I), 52.82 (CH_3O), 20.26 ppm (*C*H_3CO); elemental analysis calcd (%) for $C_{50}H_{44}O_{18}$ (932.87): C 64.38, H 4.75; found: C 64.56, H 5.01.

{[Methyl(3-*O*-acetyl-2,4-di-*O*-benzoyl-β-D-glucopyranosyl)uronate]-(1→3)-2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl}trichloroacetimidate (21)

Trichloroacetonitrile (161 µL, 1.61 mmol) and DBU (7 µL, 0.05 mmol) were added to a solution of **20** (150 mg, 0.16 mmol) in CH_2Cl_2 (2 mL) at –30 ºC. The mixture was allowed to attain room temperature (2 h) and then applied on a silica gel column. Elution with light petroleum/EtOAc (3:4) containing 1% (v/v) of Et_3N afforded imidate **21** (130 mg, 75%) as a foam; $[\alpha]_D$ = +51 (*c* 1, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.50 (s, 1H, NH), 8.15–7.14 (m, 25H, 5 Ph), 6.77 (d, $J_{1,2}$ = 3.7 Hz, 1H; H-1^I), 6.09 (d, 1H; H-4^I), 5.77 (dd, $J_{2,3}$ = 10.2 Hz, 1H; H-2^I), 5.55 (t, $J_{4,5}$ = 9.3 Hz, 1H; H-4^{II}), 5.50 (t, $J_{3,4}$ = 9.2 Hz, 1H; H-3^{II}), 5.19 (dd, $J_{2,3}$ = 8.1 Hz, 1H; H-2^{II}), 5.15 (d, $J_{1,2}$ = 6.9 Hz, 1H; H-1^{II}), 4.73 (dd, $J_{3,4}$ = 3.3 Hz, 1H; H-3^I), 4.71 (m, 1H; H-5^I), 4.54 (dd, $J_{6a,5}$ = 4.7 Hz, $J_{6a,6b}$ = 11.7 Hz, 1H; H-6a^I), 4.44 (dd, $J_{6b,5}$ = 7.4 Hz, 1H; H-6b^I), 4.32 (d, 1H; H-5^{II}), 3.70 (s, 3H; CH_3O), 1.71 ppm (s, 3H; CH_3CO); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 169.84, 166.01, 165.53, 164.85 (CO), 160.35 (C=NH), 100.75 (C-1^{II}), 93.65 (C-1^I), 73.26 (C-3^I), 72.96 (C-5^{II}), 71.72 (C-2^{II}), 71.31 (C-3^{II}), 70.37 (C-5^I), 69.94 (2C, C-2^I, C-4^I), 69.56 (C-4^{II}), 62.87 (C-6^I), 52.89 (CH_3O), 20.27 ppm (CH_3CO); HRMS (ESI) *m/z* calcd for $C_{52}H_{44}Cl_3NO_{18}$ + Na^+: 1098.1516 $[M+Na]^+$; found: 1098.1521

Ethyl [methyl(3-*O*-acetyl-2,4-di-*O*-benzoyl-β-D-glucopyranosyl)uronate]-(1→3)-2,4,6-tri-*O*-benzoyl-1-thio-β-D-galactopyranoside (22)

A mixture of **21** (932 mg, 0.87 mmol), ethanethiol (109 µL, 1.47 mmol) and mol. sieves 4 Å in CH_2Cl_2 (10 mL) was stirred for 40 min at room temperature under Ar and cooled to 4 ºC. TMSOTf (55 µL, 0.31 mmol) was added and stirring was continued for 2 h. The mixture was filtered through a Celite layer, the solids were washed with CH_2Cl_2 (80 mL), the filtrate was washed with aq satd $NaHCO_3$, and water, and concentrated. The residue was purified by column chromatography (toluene/acetone 6:1) to provide crystalline **22** (830 mg, 98%); m. p. = 78 ºC; $[\alpha]_D$ = +17 (*c* = 1, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.12–7.14 (m, 25H; 5 Ph), 5.94 (d, 1H; H-4^I), 5.64 (t, $J_{2,3}$ = 9.8 Hz, 1H; H-2^I), 5.47 (t, $J_{4,5}$ = 9.5 Hz, 1H; H-4^{II}), 5.40 (t, $J_{3,4}$ = 9.2 Hz, 1H; H-3^{II}), 5.13 (dd, $J_{2,3}$

= 8.8 Hz, 1H; H-2^{II}), 4.95 (d, $J_{1,2}$ = 7.1 Hz, 1H; H-1^{II}), 4.63 (d, $J_{1,2}$ = 9.9 Hz, 1H; H-1^{I}), 4.49–4.42 (m, 2H; 2H-6^{I}), 4.35 (dd. $J_{3,4}$ = 3.3 Hz, 1H; H-3^{I}), 4.18 (m, 1H; H-5^{I}), 4.15 (d, 1H; H-5^{II}), 3.62 (s, 3H; CH_3O), 2.77–2.61 (m, 2H; SCH_2CH_3), 1.67 (s, 3H, CH_3CO), 1.17 ppm (t, J = 7.2 Hz, 3H; SCH_2CH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 167.00, 166.17, 165.66, 164.86, 164.71, 164.21 (CO), 100.56 (C-1^{II}), 83.98 (C-1^{I}), 77.58 (C-3^{I}), 75.64 (C-5^{II}), 72.79 (C-5^{I}), 71.58 (C-2^{II}), 71.41 (C-3^{II}), 70.21 (C-4^{I}), 69.98 (C-2^{I}), 69.63 (C-4^{II}), 62.90 (C-6^{I}), 52.81 (CH_3O), 24.15 (SCH_2CH_3), 20.23 (CH_3CO), 14.81 ppm (SCH_2CH_3); elemental anal. calcd (%) for $C_{52}H_{48}O_{17}S$ (976.99): C 63.93, H 4.95; found: C 63.56, H 5.01.

2-Azidoethyl [methyl(3-*O*-acetyl-2,4-di-*O*-benzoyl-β-D-glucopyranosyl)uronate]-(1→3)-(2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside (23)

A mixture of **22** (97 mg, 0.10 mmol), **5** (29 mg, 0.06 mmol) and molecular sieves AW 300 (300 mg) in CH_2Cl_2 (3 mL) was stirred at room temperature under Ar for 20 min, then cooled to –25 ºC. NIS (31 mg, 0.14 mmol) and, after 5 min, TfOH (13 μL, 0.15 mmol) were added and the resulting mixture was stirred for 2.5 h, while the temperature was gradually increased to –10 ºC. The reaction was quenched by pyridine (0.1 mL), the mixture was diluted with CH_2Cl_2 (80 mL) and solids were filtered off. The filtrate was washed with 1 M $Na_2S_2O_3$ solution, satd $NaHCO_3$, dried, and concentrated. Column chromatography (toluene/acetone 4:1) of the residue provided **23** (59 mg, 69%) as an amorphous solid. $[\alpha]_D$ = –10 (c = 1, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.13–7.12 (m, 35H; 7 Ph), 5.87 (d, $J_{NH,2}$ = 8.3 Hz, 1H; NH), 5.85 (d, $J_{4,3}$ = 3.3 Hz, 1H; H-4^{II}), 5.56 (dd, $J_{2,3}$ = 10.0 Hz, 1H; H-2^{II}), 5.51 (t, $J_{4,5}$ = 9.5 Hz, 1H, H-4^{III}), 5.43 (t, $J_{3,4}$ = 8.9 Hz, 1H; H-3^{III}), 5.01 (dd, $J_{2,3}$ = 8.8 Hz, 1H; H-2^{III}), 4.96 (d, $J_{1,2}$ = 7.0 Hz, 1H; H-1^{III}), 4.78, 4.63 (2 d, J_{gem} = 11.7 Hz, 2H; CH_2Ph), 4.62 (d, $J_{1,2}$ = 7.9 Hz, 1H; H-1^{II}), 4.56, 4.28 (2 d, J_{gem} = 12.0 Hz, 2H; CH_2Ph), 4.53 (d, $J_{1,2}$ = 7.0 Hz,

1H; H-1^{I}), 4.36 (dd, $J_{6a,5}$ = 6.0 Hz, $J_{6a,6b}$ = 11.5 Hz, 1H; H-6a^{II}), 4.30 (dd, $J_{6b,5}$ = 5.2 Hz, 1H; H-6b^{II}), 4.24 (d, 1H; H-5^{III}), 4.17 (dd, 1H; H-3^{II}), 4.02 (t, $J_{4,5}$ = 6.7 Hz, 1H; H-4^{I}), 3.94 (t, 1H; H-5^{II}), 3.87 (t, $J_{3,4}$ = 6.4 Hz, 1H; H-3^{I}), 3.74 (m, 1H; OC*Ha*HbCH$_2$N$_3$), 3.65 (s, 3H; CH$_3$O), 3.61 (m, 1H, H-2^{I}), 3.58 (dd, $J_{6a,5}$ = 3.8 Hz, $J_{6a,6b}$ = 10.4 Hz, 1H; H-6a^{I}), 3.49 (dd, $J_{6b,5}$ = 4.6 Hz, 1H; H-6b^{I}), 3.35 (m, 1H; H-5^{I}), 3.30 (m, 2H; OCHa*Hb*CH$_2$N$_3$, OCH$_2$C*Ha*HbN$_3$), 3.12 (m, 1H; OCH$_2$CHa*Hb*N$_3$), 1.89 (s, 3H; CH$_3$CON), 1.68 ppm (s, 3H; CH$_3$COO); ^{13}C NMR (125 MHz, CDCl$_3$): δ = 170.31, 169.89, 166.99, 166.07, 165.50, 164.85, 164.73, 164.20 (CO), 100.64 (C-1^{III}), 100.25 (C-1^{I}), 99.62 (C-1^{II}), 79.04 (C-3^{I}), 76.19 (C-3^{II}), 75.00 (C-4^{I}), 74.76 (C-5^{I}), 73.47, 73.25 (2 *C*H$_2$Ph), 73.87 (C-5^{III}), 72.07 (C-2^{II}), 71.71 (C-5^{II}, C-2^{III}), 71.35 (C-3^{III}), 69.71 (C-4^{II}), 69.53 (C-4^{III}), 68.50 (C-6^{I}), 67.86 (O*C*H$_2$CH$_2$N$_3$), 62.22 (C-6^{II}), 54.10 (C-2^{I}), 52.85 (CH$_3$O), 50.42 (OCH$_2$*C*H$_2$N$_3$), 23.29 (*C*H$_3$CON), 20.24 ppm (*C*H$_3$COO); elemental analysis calcd (%) for C$_{74}$H$_{72}$N$_4$O$_{23}$ (1385.38): C 64.16, H 5.24, N 4.04; found: C 64.33, H 5.09, N 4.24.

2-Azidoethyl [methyl(3-*O*-acetyl-2,4-di-*O*-benzoyl-β-D-glucopyranosyl)uronate]-(1→3)-(2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-2-acetamido-3-*O*-benzyl-2-deoxy-6-*O*-(*p*-methoxyphenyl)-β-D-glucopyranoside (24)

Donor **22** (66 mg, 0.068 mmol) was allowed to react with acceptor **14** (18 mg, 0.038 mmol) in the presence of mol. sieves AW 300 (200 mg), NIS (21 mg, 0.095 mmol) and TfOH (9 µL, 0.1 mmol) in CH$_2$Cl$_2$ (2 mL) as described for **23**. After column chromatography (toluene/acetone 5:1) trisaccharide **24** (31 mg, 60%) was obtained as an amorphous solid, [α]$_D$ = +8 (*c* = 0.5, CHCl$_3$); ^{1}H NMR (500 MHz, CDCl$_3$): δ = 8.09–7.11 (m, 30H; 6 Ph), 6.75, 6.66 (2 d, *J* = 9.2 Hz, 4H; C$_6$*H*$_4$OCH$_3$), 5.99 (d, $J_{NH,2}$ = 8.6 Hz, 1H; NH), 5.86 (d, $J_{4,3}$ = 3.2 Hz, 1H; H-4^{II}), 5.59 (dd, $J_{2,3}$ = 9.8 Hz, $J_{2,1}$ = 8.3 Hz, 1H; H-2^{II}), 5.47 (t, $J_{4,5}$ = 9.5 Hz, 1H; H-4^{III}), 5.40 (t, $J_{3,4}$ = 9.1 Hz, 1H; H-3^{III}), 5.10 (dd, $J_{2,3}$ = 8.6 Hz, 1H, H-2^{III}), 4.95 (d, $J_{1,2}$ = 6.9 Hz, 1H; H-1^{III}), 4.64 (m, 3H; C*H*$_2$Ph, H-1^{II}), 4.47 (d,

$J_{1,2}$ = 6.0 Hz, 1H, H-1^{I}), 4.40 (dd, $J_{6a,5}$ = 5.6 Hz, $J_{6a,6b}$ = 10.7 Hz, 1H; H-6a^{II}), 4.34 (dd, $J_{6b,5}$ = 7.2 Hz, 1H; H-6b^{II}), 4.22 (dd, 1H; H-3^{II}), 4.18 (d, 1H; H-5^{III}), 4.09 (t, $J_{4,5}$ = 5.4 Hz, 1H; H-4^{I}), 4.05 (t, 1H; H-5^{II}), 4.03 (dd, $J_{6a,5}$ = 5.5 Hz, $J_{6a,6b}$ = 9.8 Hz, 1H; H-6a^{I}), 3.94 (dd, $J_{6b,5}$ = 4.6 Hz, 1H, H-6b^{I}), 3.87 (t, $J_{3,4}$ = 5.7 Hz, 1H; H-3^{I}), 3.80 (m, 1H, H-2^{I}), 3,75 (s, 3H; C*H*$_3$OC$_6$H$_4$), 3.68 (m, 1H; OC*Ha*HbCH$_2$N$_3$), 3.64 (m, 1H; H-5^{I}), 3.62 (s, 3H; CH$_3$O), 3.23 (m, 2H; OCHa*Hb*CH$_2$N$_3$, OCH$_2$C*Ha*HbN$_3$), 3.09 (m, 1H; OCH$_2$CHa*Hb*N$_3$), 1.92 (s, 3H; CH$_3$CON), 1.67 ppm (s, 3H; CH$_3$COO); ^{13}C NMR (125 MHz, CDCl$_3$): δ = 170.15, 169.82, 166.86, 166.04, 165.50, 164.95, 164.79, 164.18 (CO), 100.71 (C-1^{III}), 100.27 (C-1^{I}), 99.68 (C-1^{II}), 77.10 (C-3^{I}), 76.38 (C-3^{II}), 74.25 (C-4^{I}), 73.90 (C-5^{I}), 72.77 (*C*H$_2$Ph, C-5^{III}), 72.05, 71.98 (C-2^{II}, C-5^{II}), 71.60 (C-2^{III}), 71.25 (C-3^{III}), 69.65 (C-4^{II}), 69.44 (C-4^{III}), 67.78 (O*C*H$_2$CH$_2$N$_3$), 67.28 (C-6^{I}), 62.36 (C-6^{II}), 55.69 (*C*H$_3$OC$_6$H$_4$), 52.80 (CH$_3$O), 52.52 (C-2^{I}), 50.34 (OCH$_2$*C*H$_2$N$_3$), 23.20 (*C*H$_3$CON), 20.21 ppm (*C*H$_3$COO); elemental analysis calcd (%) for C$_{74}$H$_{72}$N$_4$O$_{24}$ (1401.38): C 63.42, H 5.18, N 4.00; found: C 63.43, H 5.11, N 4.14.

2-Azidoethyl [methyl(2,4-di-*O*-benzoyl-β-D-glucopyranosyl)uronate]-(1→3)-(2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside (26)

Trisaccharide **23** (49 mg, 0.04 mmol) was dissolved in a solution of anhydrous HCl in MeOH that was obtained by adding AcCl (0.2 mL) to MeOH (5 mL) upon cooling with an ice-bath. The resulting solution was kept for 16 h at 4 ºC, diluted with CH_2Cl_2 (50 mL), and washed with satd $NaHCO_3$ solution. The organic layer was dried, concentrated and the residue was purified by column chromatography (toluene/acetone 2:0.6) to give compound **26** (41 mg, 86%) as an amorphous solid. $[\alpha]_D$ = –12 (*c* = 0.5, $CHCl_3$); NMR (500 MHz, $CDCl_3$): δ = 8.07–7.12 (m, 35H; 7 Ph), 5.87 (d, $J_{NH,2}$ = 8.6 Hz, 1H; NH), 5.84 (d, $J_{4,3}$ = 2.9 Hz, 1H; H-4^{II}), 5.59 (dd, $J_{2,3}$ = 9.3 Hz, 1H;

H-2^{II}), 5.48 (t, $J_{4,5}$ = 9.0 Hz, H-4^{III}), 5.01 (d, $J_{1,2}$ = 5.9 Hz, 1H; H-1^{III}), 4.94 (t, $J_{2,3}$ = 7.0 Hz, 1H; H-2^{III}), 4.78, 4.65 (2 d, J_{gem} = 11.7 Hz, 2H; C*H*$_2$Ph), 4.65 (d, $J_{1,2}$ = 7.6 Hz, 1H; H-1^{II}), 4.55, 4.31 (2 d, J_{gem} = 12.0 Hz, 2H; C*H*$_2$Ph), 4.52 (d, $J_{1,2}$ = 6.6 Hz, 1H; H-1^{I}), 4.33 (m, 3H; H-3^{II}, 2 H-6^{II}), 4.23 (d, 1H; H-5^{III}), 4.03 (t, $J_{4,5}$ = 6.3 Hz, 1H; H-4^{I}), 3.96 (br. t, 1H; H-5^{II}), 3.90 (t, $J_{3,4}$ = 7.6 Hz, 1H; H-3^{III}), 3.87 (t, $J_{3,4}$ = 6.4 Hz, 1H; H-3^{I}), 3.73 (m, 1H; OC*Ha*HbCH$_2$N$_3$), 3.64 (m, 4H; H-2^{I}, CH$_3$O), 3.58 (dd, $J_{6a,5}$ = 3.9 Hz, $J_{6a,6b}$ = 10.3 Hz, 1H; H-6a^{I}), 3.51 (dd, $J_{6b,5}$ = 4.5 Hz, 1H; H-6b^{I}), 3.38 (m, 1H; H-5^{I}), 3.29 (m, 2H; OCHa*Hb*CH$_2$N$_3$, OCH$_2$C*Ha*HbN$_3$), 3.12 (m, 1H; OCH$_2$CHa*Hb*N$_3$), 2.98 (br. s, 1H; OH), 1.90 ppm (s, 3H; CH$_3$CON); ^{13}C NMR (125 MHz, CDCl$_3$): δ = 170.29, 167.73, 166.01, 165.79, 165.72, 165.28, 164.88 (CO), 100.25 (C-1^{I}), 99.80 (C-1^{III}), 99.62 (C-1^{II}), 77.55 (C-3^{I}), 75.31 (C-3^{II}), 74.91 (C-4^{I}), 74.78 (C-5^{I}), 74.08 (C-2^{III}), 73.48, 73.16 (2 *C*H$_2$Ph), 72.74 (C-5^{III}), 72.44 (C-2^{II}), 72.19 (C-3^{III}), 72.00 (C-4^{III}), 71.64 (C-5^{II}), 69.79 (C-4^{II}), 68.58 (C-6^{I}), 67.84 (O*C*H$_2$CH$_2$N$_3$), 62.08 (C-6^{II}), 53.87 (CH$_3$O), 52.81 (C-2^{I}), 50.41 (OCH$_2$*C*H$_2$N$_3$), 23.28 ppm (*C*H$_3$CON); elemental analysis calcd (%) for C$_{72}$H$_{70}$N$_4$O$_{22}$ (1343.34): C 64.37, H 5.25, N 4.17; found: C 64.33, H 5.09, N 4.24.

2-Azidoethyl [methyl(2,4-di-*O*-benzoyl-β-D-glucopyranosyl)uronate]-(1→3)-(2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-2-acetamido-3-*O*-benzyl-2-deoxy-β-D-glucopyranoside (27)

CAN (150 mg, 0.3 mmol) was added to a solution of trisaccharide **24** (127 mg, 0.091 mmol) in a mixture of acetonitrile (8 mL) and water (1 mL) at –5 °C. After being stirred at 4 °C for 1 h, the mixture was diluted with CH_2Cl_2 and washed with satd $NaHCO_3$ solution. The organic layer was concentrated and the residue was subjected to flash-chromatography (toluene/EtOAc 2:0.6) to give 99 mg of 6-OH product. It was dissolved in methanolic hydrogen chloride (5 mL of MeOH + 0.2 mL of AcCl), the solution was kept for 8 h at 4 °C, diluted with CH_2Cl_2 (50 mL) and washed with a

mixture of satd NaCl and satd $NaHCO_3$ (1:1, 50 mL). The organic layer was concentrated and the residue was subjected to column chromatography ($CHCl_3$/MeOH 20:3) to provide diol **27** (81 mg, 71% over two steps) as an amorphous solid. $[\alpha]_D = -18$ (c = 1, $CHCl_3$); NMR (500 MHz, $CDCl_3$ + CD_3OD): δ = 8.09–6.96 (m, 30H; 6 Ph), 5.87 (d, $J_{4,3}$ = 3.2 Hz, 1H; H-4^{II}), 5.63 (dd, $J_{2,3}$ = 9.8 Hz, 1H; H-2^{II}), 5.25 (t, $J_{4,5}$ = 9.8 Hz, 1H; H-4^{III}), 5.02 (t, $J_{2,3}$ = 9.0 Hz, 1H; H-2^{III}), 4.93 (d, $J_{1,2}$ = 7.7 Hz, 1H; H-1^{III}), 4.91 (d, $J_{1,2}$ = 7.9 Hz, 1H, H-1^{II}), 4.90, 4.60 (2d, J_{gem} = 11.3 Hz, 2H; CH$_2$Ph), 4.40 (dd, 1H; H-3^{II}), 4.38 (d, $J_{1,2}$ = 7.7 Hz, 1H; H-1^{I}), 4.28 (dd, $J_{6a,5}$ = 5.5 Hz, $J_{6a,6b}$ = 10.9 Hz, 1H; H-$6a^{II}$), 4.17 (d, 1H; H-5^{III}), 4.14 (t, 1H; H-5^{II}), 4.09 (dd, $J_{6b,5}$ = 6.7 Hz, 1H; H-$6b^{II}$), 3.95 (t, $J_{4,5}$ = 9.1 Hz, 1H, H-4^{I}), 3.87 (t, $J_{3,4}$ = 9.3 Hz, 1H; H-3^{III}), 3.82 (m, 1H; OCHaHbCH$_2$N$_3$), 3.67 (dd, $J_{2,3}$ = 9.5 Hz, 1H; H-2^{I}), 3.60 (t, $J_{3,4}$ = 9.6 Hz, 1H; H-3^{I}), 3.57 (m, 2H; 2 H-6^{I}), 3.55 (s, 3H; CH_3O), 3.47 (m, 1H, OCHaHbCH$_2$N$_3$), 3.32 (m, 1H; OCH$_2$CHaHbN$_3$), 3.19 (m, 1H; OCH$_2$CHaHbN$_3$), 3.04 (m, 1H; H-5^{I}), 1.82 ppm (s, 3H; CH_3CON); ^{13}C NMR (125 MHz, $CDCl_3$ + CD_3OD): δ = 173.20, 169.18, 167.85, 167.31, 167.19, 166.53, 166.39 (CO), 102.47 (C-1^{III}), 102.20 (C-1^{I}), 101.84 (C-1^{II}), 80.64 (C-3^{I}), 78.45 (C-3^{II}), 77.26 (C-4^{I}), 76.73 (C-5^{I}), 75.26 (CH$_2$Ph), 75.11 (C-2^{III}), 74.19 (C-5^{III}), 73.50 (C-3^{III}, C-4^{III}), 73.39 (C-2^{II}), 72.86 (C-5^{II}), 71.71 (C-4^{II}), 69.29 (OCH$_2$CH$_2$N$_3$), 63.69 (C-6^{II}), 61.21 (C-6^{I}), 56.00 (C-2^{I}), 53.82 (CH_3O), 51.84 (OCH$_2C$H$_2$N$_3$), 23.80 ppm (CH$_3$CON); elemental analysis calcd (%) for $C_{65}H_{64}N_4O_{22}$ (1253.22): C 62.30, H 5.15, N 4.47; found: C 62.24, H 5.07, N 4.25.

2-Azidoethyl [methyl(2,4-di-*O*-benzoyl-3-*O*-sulfo-β-D-glucopyranosyl)uronate]-(1→3)-(2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside, sodium salt (28)

To a solution of **26** (22 mg, 16 μmol) in DMF (1 mL) was added SO_3·Py (21 mg, 0.13 mmol) and the mixture was stirred for 1.5 h at room temperature. Solid $NaHCO_3$ (54 mg, 0.64 mmol) was added to destroy the excess of the reagent, the resulting mixture was stirred for 1 h, filtered, and

solids were washed with MeOH (10 mL). The combined filtrates were concentrated, and to a solution of the residue in aqueous MeOH (10:1, 5.5 mL) was added Amberlite IRA-120 (Na^+) (2 mL). After being kept for 20 min, the mixture was filtered, the resin was washed with MeOH (10 mL), and the combined filtrates were concentrated. Column chromatography ($CHCl_3$/MeOH 10:1) of the residue provided sulfate **28** (17 mg, 86%) as an amorphous solid. $[\alpha]_D = -12$ (*c* = 1, MeOH); NMR (500 MHz, CD_3OD): δ = 8.11–6.93 (m, 35H; 7 Ph), 5.87 (d, $J_{4,3}$ = 2.8 Hz, 1H; H-4^{II}), 5.59 (dd, $J_{2,3}$ = 9.9 Hz, 1H; H-2^{II}), 5.36 (t, $J_{4,5}$ = 9.9 Hz, 1H; H-4^{III}), 5.15 (t, $J_{2,3}$ = 8.9 Hz, 1H; H-2^{III}), 5.12 (d, $J_{1,2}$ = 8.0 Hz, 1H; H-1^{III}), 4.97, 4.63 (2 d, J_{gem} = 11.1 Hz, 2H; CH_2Ph), 4.95 (t, $J_{3,4}$ = 9.8 Hz, 1H; H-3^{III}), 4.74 (d, $J_{1,2}$ = 8.0 Hz, 1H; H-1^{II}), 4.57, 4.31 (2 d, J_{gem} = 12.0 Hz, 2H; CH_2Ph), 4.47 (d, 1H; H-5^{III}), 4.37 (d, $J_{1,2}$ = 8.4 Hz, 1H; H-1^{I}), 4.35 (dd, $J_{6a,5}$ = 4.7 Hz, $J_{6a,6b}$ = 11.3 Hz, 1H; H-$6a^{II}$), 4.26 (dd, 1H; H-3^{II}), 4.05–3.95 (m, 3H; H-4^{I},5^{II},$6b^{II}$) 3.86 (m, 1H; OC*Ha*H*b*CH_2N_3), 3.81 (t, $J_{2,3}$ = 9.2 Hz, 1H; H-2^{I}), 3.62–3.52 (m, 6H; H-3^{I},$6a^{I}$, CH_3O, OCH*a*H*b*CH_2N_3), 3.43 (br. d, $J_{6b,6a}$ = 10.7 Hz, 1H; H-$6b^{I}$), 3.37, 3.22 (2 m, 2H; $OCH_2CH_2N_3$), 3.14 (br. d, $J_{5,4}$ = 9.6 Hz, 1H; H-5^{I}), 1.84 ppm (s, 3H; CH_3CON); ^{13}C NMR (125 MHz, CD_3OD): δ = 173.33, 169.17, 167.69, 167.34, 167.22, 166,67, 166.33, 102.47 (C-1^{I}), 102.18 (C-1^{III}), 101.52 (C-1^{II}), 81.30 (C-3^{I}), 78.97 (C-3^{II}), 78.53 (C-3^{III}), 77.32 (C-4^{I}), 75.72 (C-5^{I}), 75.21, 74.45 (2 *C*H_2Ph), 73.81 (C-5^{III}), 73.48 (C-2^{III}), 73.05 (C-2^{II}), 72.84 (C-5^{II}), 71.97 (C-4^{III}), 71.71 (C-4^{II}), 69.38 (O*C*$H_2CH_2N_3$), 68.83 (C-6^{I}), 63.86 (C-6^{II}), 55.91 (C-2^{I}), 53.28 (CH_3O), 51.73 ($OCH_2CH_2N_3$), 23.11 ppm (*C*H_3CON); HRMS (ESI) *m/z* calcd for $C_{72}H_{70}N_4O_{25}S - H^+$: 1421.3977 $[M-H]^-$; found: 1421.3959.

2-Azidoethyl [methyl(2,4-di-*O*-benzoyl-3-*O*-sulfo-β-D-glucopyranosyl)uronate]-(1→3)-(2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-2-acetamido-3-*O*-benzyl-2-deoxy-6-*O*-sulfo-β-D-glucopyranoside, disodium salt (29)

To a solution of diol **27** (82 mg, 68 μmol) in DMF (6 mL) was added SO_3·Py (100 mg, 0.68 mmol) and the mixture was stirred for 2 h at room temperature. Solid $NaHCO_3$ (154 mg, 1.84 mmol) was added, the mixture was stirred for 1 h, filtered, and the solids were washed with MeOH (10 mL). The combined filtrate and washings were concentrated, the residue was dissolved in aqueous MeOH (10:1, 5.5 mL), and Amberlite IRA-120 (Na^+) (2 mL) was added. After being kept for 20 min, the mixture was filtered, the resin was washed with MeOH (10 mL), and the combined filtrates were concentrated. Amorphous **29** (77 mg, 81%) was isolated by column chromatography ($CHCl_3$/MeOH 10:1). $[\alpha]_D$ = +5 (*c* = 1, MeOH); ^{1}H NMR (400 MHz, CD_3OD): δ = 8.11–6.95 (m, 30H; 6 Ph), 5.94 (d, $J_{4,3}$ = 3.1 Hz, 1H; H-4^{II}), 5.63 (dd, $J_{2,3}$ = 9.8 Hz, 1H; H-2^{II}), 5.32 (t, $J_{4,5}$ = 9.8 Hz, 1H; H-4^{III}), 5.22 (d, $J_{1,2}$ = 8.0 Hz, 1H; H-1^{II}), 5.14 (d, $J_{1,2}$ = 7.9 Hz, 1H; H-1^{III}), 5.12 (t, $J_{2,3}$ = 8.6 Hz, 1H; H-2^{III}), 4.94, 4.60 (2 d, J_{gem} = 11.1 Hz, 2H; C*H*$_2$Ph), 4.89 (t, $J_{3,4}$ = 8.9 Hz, 1H; H-3^{III}), 4.55 (dd, 1H; H-3^{II}), 4.46 (d, 1H; H-5^{III}), 4.36 (d, $J_{1,2}$ = 7.8 Hz, 1H; H-1^{I}), 4.33 (m, 2H; H-5^{II}, H-6a^{II}), 4.28 (dd, $J_{6a,5}$ = 2.2 Hz, $J_{6a,6b}$ = 11.3 Hz, 1H; H-6a^{I}), 4.14 (dd, $J_{6b,5}$ = 4.0 Hz, $J_{6b,6a}$ = 10.7 Hz, 1H; H-6b^{II}), 4.05 (t, $J_{4,5}$ = 9.5 Hz, 1H; H-4^{I}), 3.94 (br. d, 1H; H-6b^{I}), 3.86 (m, 1H, OC*Ha*HbCH$_2$N$_3$), 3.72 (dd, $J_{2,3}$ = 9.9 Hz, 1H; H-2^{I}), 3.57 (s, 3H; CH_3O), 3.53 (t, $J_{3,4}$ = 9.0 Hz, 1H; H-3^{I}), 3.51 (m, 1H, OCHa*Hb*CH$_2$N$_3$), 3.32, 3.21 (2 m, 2H; OCH$_2$C*H*$_2$N$_3$), 3.16 (br. d, 1H, H-5^{I}), 1.82 ppm (CH_3CON); ^{13}C NMR (100 MHz, CD_3OD): δ = 173.21, 169.39, 167.76, 167.46, 167.30, 166.86, 166.75 (CO), 102.57 (C-1^{III}), 102.31 (C-1^{I}), 101.37 (C-1^{II}), 81.05 (C-3^{I}), 79.15 (C-3^{II}), 78.62 (C-3^{III}), 76.64 (C-4^{I}), 74.95 (*C*H$_2$Ph), 74.66 (C-5^{I}), 73.70 (C-5^{III}), 73.42 (C-2^{III}), 73.14 (C-2^{II}), 72.89 (C-5^{II}), 72.58 (C-4^{II}), 72.19 (C-4^{III}), 69.16 (O*C*H$_2$CH$_2$N$_3$), 66.11 (C-6^{I}), 63.97 (C-6^{II}), 55.69 (C-2^{I}), 53.13 (CH_3O), 51.69 (OCH$_2$*C*H$_2$N$_3$), 23.04 ppm (*C*H$_3$CON); HRMS (ESI): calcd for $C_{65}H_{62}N_4Na_2O_{28}S_2$ + Na^+: 1479.2680 $[M+Na]^+$; found: 1479.2674.

2-Trifluoroacetamidoethyl [methyl(2,4-di-*O*-benzoyl-3-*O*-sulfo-β-D-glucopyranosyl)uronate]-(1→3)-(2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranoside, sodium salt (30)

To a solution of sulfate **28** (54 mg, 38 μmol) in MeOH (2 mL) were added 10% PdO/C (30mg), AcOH (10 μmol), and the mixture was stirred in a hydrogen atmosphere for 1 h. The catalyst was filtered off through a Celite layer, washed with MeOH (30 mL) and toluene (30 mL) and the combined filtrates were concentrated. To a solution of the residue in MeOH (2 mL) were added CF_3CO_2Et (30 μL) and Et_3N (22 μL, 0.15 mmol). After 4 h, the mixture was concentrated and the residue was passed through a layer of silica gel in $CHCl_3$/MeOH (5:1) to give *N*-trifluoroacetylated product (55 mg). It was dissolved in MeOH (2 mL) and hydrogenated over PdO/C (30 mg) in the presence of AcONa (5 mg) for 72 h. The catalyst was filtered off through a Celite layer, washed with MeOH (30 mL), and the combined filtrates were concentrated. The residue was dissolved in aqueous MeOH (3:1, 2 mL) and treated with Amberlite IRA-120 (Na^+) (2 mL) for 20 min. The resin was filtered off, washed with MeOH (10 mL), and the filtrate was concentrated. Column chromatography ($CHCl_3$/MeOH 5:1) of the residue gave amorphous sulfate **30** (22 mg, 45%). $[\alpha]_D$ = –8 (*c* = 1, MeOH); NMR (500 MHz, CD_3OD): δ = 8.13–7.20 (m, 25H; 5 Ph), 5.91 (d, $J_{4,3}$ = 2.9 Hz, 1H; H-4^{II}), 5.60 (dd, $J_{2,3}$ = 9.9 Hz, 1H; H-2^{II}), 5.32 (t, $J_{4,5}$ = 9.5 Hz, 1H; H-4^{III}), 5.16 (d, $J_{1,2}$ = 7.6 Hz, 1H; H-1^{III}), 5.12 (t, $J_{2,3}$ = 8.6 Hz, 1H; H-2^{III}), 4.97 (d, $J_{1,2}$ = 8.1 Hz, 1H; H-1^{II}), 4.89 (t, $J_{3,4}$ = 8.9 Hz, 1H; H-3^{III}), 4.65 (dd, $J_{6a,5}$ = 3.9 Hz, $J_{6a,6b}$ = 11.8 Hz, 1H; H-$6a^{II}$), 4.54 (dd, 1H, H-3^{II}), 4.43 (d, 1H; H-5^{III}), 4.40 (br. t, 1H; H-5^{II}), 4.27 (d, $J_{1,2}$ = 8.3 Hz, 1H; H-1^{I}), 4.25 (dd, $J_{6b,5}$ = 7.4 Hz, 1H; H-$6b^{II}$), 3.79 (m, 1H; OC*Ha*HbCH$_2$N), 3.71 (t, $J_{2,3}$ = 9.4 Hz, 1H; H-2^{I}), 3.60–3.51 (m, 6H; H-3^{I},4^{I}, CH_3O, OCHa*Hb*CH$_2$N), 3.39 (m, 3H; H-$6a^{I}$, OCH_2CH_2N), 3.33 (m, 1H, H-$6b^{I}$), 3.13 (m, 1H; H-5^{I}), 1.87 ppm (CH_3CON); ^{13}C NMR (125 MHz, CD_3OD): δ = 173.71, 169.16, 167.76, 167.59, 167.19,

166.62, 166.51 (CO), 102.68 (C-1^{I}), 102.56 (C-1^{II}), 102.24 (C-1^{III}), 81.38 (C-4^{I}), 78.97 (C-3^{II}), 78.49 (C-3^{III}), 75.96 (C-5^{I}), 73.82 (C-3^{I}), 73.70 (C-5^{III}), 73.58 (C-5^{II}), 73.32 (C-2^{III}), 72.42 (C-2^{II}), 72.06 (C-4^{III}), 71.98 (C-4^{II}), 68.05 (O*C*$H_2$$CH_2$N), 64.47 (C-$6^{II}$), 61.13 (C-$6^{I}$), 56.36 (C-$2^{I}$), 53.18 ($CH_3O$), 40.83 (OC$H_2$*C*$H_2$N), 22.88 ppm (*C*$H_3$CON); HRMS (ESI) calcd for $C_{60}H_{58}F_3N_2NaO_{26}S$ + Na^+: 1357.2741 $[M+Na]^+$; found: 1357.2727.

2-Trifluoroacetamidoethyl [methyl(2,4-di-*O*-benzoyl-3-*O*-sulfo-β-D-glucopyranosyl)uronate]-(1→3)-(2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-2-acetamido-2-deoxy-6-*O*-sulfo-β-D-glucopyranoside, disodium salt (31)

10% PdO/C (30 mg) was added to a solution of disulfate **29** (77 mg, 53 μmol) in MeOH (2 mL) and the mixture was stirred in a hydrogen atmosphere for 2 h. The catalyst was filtered off through a Celite layer, washed with MeOH (30 mL), and the filtrate was concentrated. The residue was dissolved in MeOH (2 mL), CF_3CO_3Et (30 μL, 0.25 mmol) and Et_3N (22 μL, 0.15 mmol) were added, the mixture was kept for 4 h and evaporated. The residue was applied on a short silica gel column; elution with $CHCl_3$/MeOH (5:1) afforded trifluoroacetamide (67 mg). To a solution of this compound in MeOH (2 mL) was added 10% PdO/C (30 mg), the mixture was stirred under a hydrogen atmosphere for 2 h, and filtered through a Celite layer. The solids were washed with MeOH (30 mL) and the combined filtrates were concentrated. The residue was treated with Amberlite IRA-120 (Na^+) (2 mL) in aqueous MeOH (3:1, 2 mL) for 20 min, the resin was filtered off, washed with MeOH (10 mL), and the combined filtrates was concentrated. The residue was purified by column chromatography ($CHCl_3$/MeOH 5:1) to provide amorphous disulfate **31** (57 mg, 77%). $[\alpha]_D$ = –11 (*c* = 1, MeOH); NMR (400 MHz, CD_3OD): δ = 8.11–7.10 (m, 25H; 5 Ph), 5.95 (d, $J_{4,3}$ = 3.2 Hz, 1H; H-4^{II}), 5.59 (dd, $J_{2,3}$ = 9.9 Hz, 1H; H-2^{II}), 5.32 (t, $J_{4,5}$ = 9.8 Hz, 1H; H-4^{III}), 5.17 (d, $J_{1,2}$ = 7.9 Hz, 1H; H-1^{II}), 5.15 (d, $J_{1,2}$ = 7.6 Hz, 1H, H-1^{III}), 5.09 (T, $J_{2,3}$ = 8.8 Hz, 1H; H-2^{III}),

4.88 (t, $J_{3,4}$ = 9.1 Hz, 1H; H-3^{III}), 4.57 (dd, 1H; H-3^{II}), 4.56 (dd, $J_{6a,5}$ = 5.1 Hz, $J_{6a,6b}$ = 11.3 Hz, 1H, H-6a^{II}), 4.46 (d, 1H, H-5^{III}), 4.42 (br. t, 1H, H-5^{II}), 4.35 (dd, $J_{6b,5}$ = 6.3 Hz, 1H, H-6b^{II}), 4.27 (d, $J_{1,2}$ = 8.4 Hz, 1H; H-1^{I}), 4.16 (br. d, $J_{6a,6b}$ = 10.9 Hz, 1H; H-6a^{I}), 3.85 (br. d, 1H, H-6b^{I}), 3.76 (m, 1H; OC*Ha*HbCH$_2$N), 3.74 (t, $J_{4,5}$ = 9.6 Hz, 1H; H-4^{I}), 3.68 (t, $J_{2,3}$ = 10.1 Hz, 1H; H-2^{I}), 3.57 (s, 3H; CH$_3$O), 3,57 (t, $J_{3,4}$ = 9.5 Hz, H-1; H-3^{I}), 3.54 (m, 1H; OCHa*Hb*CH$_2$N), 3.39 (t, J = 5.4 Hz, 2H; OCH$_2$C*H*$_2$N), 3.21 (br.d, 1H; H-5^{I}), 1.90 ppm (s, 3H; CH$_3$CON); ^{13}C NMR (100 MHz, CD$_3$OD): δ = 173.68, 169.38, 167.83, 167.69, 167.32, 166.84, 166.75 (CO), 102.54 (C-1^{I}), 102.46 (C-1^{III}), 101.78 (C-1^{II}), 79.03 (C-4^{I}), 78.79 (C-3^{II}), 78.62 (C-3^{III}), 74.15 (C-5^{I}), 73.69 (C-5^{III}), 73.40 (C-2^{III}, C-3^{I}, C-5^{II}), 72.66 (C-4^{II}), 72.47 (C-2^{II}), 72.15 (C-4^{III}), 67.91 (O*C*H$_2$CH$_2$N), 65.93 (C-6^{I}), 64.04 (C-6^{II}), 56.37 (C-2^{I}), 53.17 (CH$_3$O), 40.79 (OCH$_2$*C*H$_2$N), 22.97 ppm (*C*H$_3$CON); HRMS (ESI): calcd for $C_{60}H_{57}F_3N_2Na_2O_{29}S_2$ + Na^+: 1459.2128 $[M+Na]^+$; found: 1459.2107.

2-Aminoethyl (3-*O*-sulfo-β-D-glucopyranosyluronic acid)-(1→3)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranoside, disodium salt (32)

a) To a solution of **30** (48 mg, 36 μmol) in aqueous THF (10:1, 5 mL) was added 2 M LiOH (0.2 mL) at –10 °C and the mixture was stirred for 3 h, while the temperature was gradually increased to ambient. The mixture was diluted with aqueous MeOH (5:3, 5 mL), 2 M NaOH (0.4 mL) was added, and the mixture was kept at room temperature for 48 h, made neutral with AcOH, and concentrated. The residue was subjected to gel chromatography on the Sephadex G-15 column in water, and appropriate fractions were freeze-dried to give sulfate **32** (19 mg, 72%) as a fluffy solid. $[\alpha]_D$ = –10 (*c* = 1, H$_2$O); NMR (500 MHz, D$_2$O): δ = 4.78 (d, $J_{1,2}$ = 7.9 Hz, 1H; H-1^{III}), 4.59 (d, $J_{1,2}$ = 8.2 Hz, 1H; H-1^{I}), 4.54 (d, $J_{1,2}$ = 7.8 Hz, 1H; H-1^{II}), 4.34 (t, $J_{3,4}$ = 9.0 Hz, 1H; H-3^{III}), 4.19 (d, $J_{4,3}$ = 2.7 Hz, 1H, H-4^{II}), 4.06 (m, 1H, OC*Ha*HbCH$_2$N), 4.02 (br. d, $J_{6a,6b}$ = 11.8 Hz, H-6a^{I}), 3.90 (m, 1H; OCHa*Hb*CH$_2$N), 3.87 (dd, $J_{6b,5}$ = 5.3 Hz, 1H; H-6b^{I}), 3.84 (dd, $J_{3,2}$ = 10.2 Hz, 1H; H-3^{II}), 3.82–

3.68 (m, 8H; H-2^{I},3^{I},4^{I},2^{II}, 2 H-6^{II}, H-4^{III},5^{III}), 3.63 (m, 1H; H-5^{I}), 3.61 (t, $J_{2,3}$ = 8.6 Hz, 1H; H-2^{III}), 3.21 (m, 2H; OCH_2CH_2N), 2.07 ppm (CH_3CON); ^{13}C NMR (125 MHz, D_2O): δ = 176.49, 176.00 (C-6^{III}, CH_3CON), 104.30 (C-1^{III}), 103.65 (C-1^{I}), 102.30 (C-1^{II}), 84.79 (C-3^{III}), 83.37 (C-3^{II}), 79.48 (C-4^{I}), 77.24 (C-5^{III}), 76.24 (C-5^{II}), 75.84 (C-5^{I}), 73.38 (C-3^{I}), 73.14 (C-2^{III}), 71.55, 71.20 (C-2^{II}, C-4^{III}), 69.26 (C-4^{II}), 67.14 (OCH_2CH_2N), 62.18 (C-6^{II}), 61.13 (C-6^{I}), 56.04 (C-2^{I}), 40.61 (OCH_2CH_2N), 23.31 ppm (CH_3CON); HRMS (ESI) *m/z* calcd for $C_{22}H_{38}N_2O_{20}S - H^+$: 681.1666 $[M–H]^–$; found: 681.1673.

b) Compound **38** (34 mg, 22 μmol) was subjected to saponification as described in the run a). According to the NMR and HRMS data, sulfate **32** (12 mg, 75%) obtained was completely identical to that described in the run a).

2-Aminoethyl (3-*O*-sulfo-β-D-glucopyranosyluronic acid)-(1→3)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-6-*O*-sulfo-β-D-glucopyranoside, trisodium salt (33)

To a solution of **31** (50 mg, 35 μmol) in aqueous THF (10:1, 5 mL) was added 2 M LiOH (0.2 mL) at –10 °C and the mixture was stirred for 3 h, while the temperature was gradually increased to ambient. Then the mixture was diluted with aqueous MeOH (5:3, 5 mL) and 2 M NaOH (0.4 mL) was added. After being kept at room temperature for 48 h, the resulting solution was made neutral with AcOH, and concentrated. Gel-permeation chromatography of the residue on the Sephadex G-15 column in water followed by freeze-drying afforded disulfate **33** (21 mg, 83%) as a fluffy solid. $[\alpha]_D$ = –14 (*c* = 1, water); NMR (500 MHz, D_2O): δ = 4.78 (d, $J_{1,2}$ = 7.9 Hz, 1H; H-1^{III}), 4.62 (d, $J_{1,2}$ = 8.4 Hz, 1H; H-1^{I}), 4.58 (d, $J_{1,2}$ = 7.9 Hz, 1H; H-1^{II}), 4.45 (br. d, $J_{6a,6b}$ = 10.7 Hz, 1H; H-$6a^{I}$), 4.35 (t, $J_{3,4}$ = 9.0 Hz, 1H; H-3^{III}), 4.34 (br. d; 1H; H-$6b^{I}$), 4.20 (d, $J_{4,3}$ = 3.0 Hz, 1H; H-4^{II}), 4.04, 3.97 (2 m, 2H; OCH_2CH_2N), 3.87–3.68 (m, 9H; H-2^{I}, 3^{I},4^{I}, H-2^{II},3^{II}, 2 H-6^{II}, H-4^{III}, 5^{III}), 3.60 (t, $J_{2,3}$ = 8.7

Hz, 1H; H-2^{III}), 3.26, 3.19 (2 m, 2H; OCH_2CH_2N), 2.07 ppm (CH_3CON); ^{13}C NMR (125 MHz, D_2O): δ = 176.90, 176.35 (C-6^{III}, CH_3CON), 104.70 (C-1^{III}), 103.72 (C-1^{II}), 102.49 (C-1^{I}), 85.14 (C-3^{III}), 83.97 (C-3^{II}), 78.93 (C-4^{I}), 77.56 (C-5^{III}), 76.56 (C-5^{II}), 74.00 (C-5^{I}), 73.64 (C-3^{I}), 73.51 (C-2^{III}), 71.98 (C-4^{III}), 71.53 (C-2^{II}), 69.62 (C-4^{II}), 67.91 (C-6^{I}), 67.62 (OCH_2CH_2N), 62.58 (C-6^{II}), 56.34 (C-2^{I}), 41.03 (OCH_2CH_2N), 23.68 ppm (CH_3CON); HRMS (ESI) *m/z* calcd for $C_{22}H_{38}N_2O_{23}S_2 - 2H^+$: 380.0581 $[M–2H]^{2–}$; found: 380.0584.

2-Trifluoroacetamidoethyl [methyl(3-*O*-acetyl-2,4-di-*O*-benzoyl-β-D-glucopyranosyl)uronate]-(1→3)-(2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranoside (35)

To a solution of **23** (107 mg, 77 μmol) in MeOH (4 mL) were added 10% PdO/C (50mg), AcOH (20 μmol), and the mixture was stirred in a hydrogen atmosphere for 1 h. The catalyst was filtered off through a Celite layer, washed with MeOH (50 mL) and toluene (30 mL) and the combined filtrates were concentrated. To a solution of the residue in MeOH (4 mL) were added CF_3CO_2Et (150 μL) and Et_3N (100 μL, 0.72 mmol). After 6 h, the mixture was concentrated and the residue was passed through a layer of silica gel in $CHCl_3$/MeOH (5:1) to give *N*-trifluoroacetylated product (59 mg). It was dissolved in MeOH (4 mL) and hydrogenated over PdO/C (50 mg) for 12 h. The catalyst was filtered off through a Celite layer, washed with MeOH (30 mL), and the combined filtrates were concentrated. Column chromatography ($CHCl_3$/MeOH 5:1) of the residue gave amorphous **35** (50 mg, 51%). $[\alpha]_D$ = +9 (*c* = 1, MeOH); NMR (600 MHz, $CDCl_3$): δ = 8.13–7.20 (m, 25H; 5 Ph), 5.90 (d, $J_{4,3}$ = 3.0 Hz, 1H; H-4^{II}), 5.70 (t, $J_{2,3}$ = 9.1 Hz, 1H; H-2^{II}), 5.60 (d, $J_{2,NH}$ = 7.4 Hz, 1H; NH^{I}), 5.49 (t, $J_{4,5}$ = 9.3 Hz, 1H; H-4^{III}), 5.43 (t, $J_{3,4}$ = 9.3 Hz, 1H; H-3^{III}), 5.15 (t, $J_{2,3}$ = 8.6 Hz, 1H; H-2^{III}), 4.98 (d, $J_{1,2}$ = 7.4 Hz, 1H; H-1^{III}), 4.82 (d, $J_{1,2}$ = 8.1 Hz, 1H; H-1^{II}), 4.75 (br. d, $J_{6a,6b}$ = 9.5 Hz, 1H; H-$6a^{II}$), 4.56 (d, $J_{1,2}$ = 8.2 Hz, 1H; H-1^{I}), 4.38 (dd, 1H, H-3^{II}), 4.31 (m, 2H; H-5^{II},$6b^{II}$), 4.22 (d, 1H; H-

5^{III}), 3.85 (m, 1H; OC*Ha*HbCH$_2$N), 3.73 (t, $J_{3,4}$ = 9.2 Hz, 1H; H-3^{I}), 3.70 (s, 3H; CH$_3$O); 3.63 (t, $J_{4,5}$ = 9.2 Hz, 1H; H-4^{I}), 3.61-3.55 (m, 3H; H-2^{I}, OCHa*Hb*CH$_2$N, OCH$_2$C*Ha*HbN), 3.42 (m, 2H; H-$6a^{I}$, OCH$_2$CHa*Hb*N), 3.33 (br. d, $J_{6a,6b}$ = 12.8 Hz, 1H; H-$6b^{I}$); 3.23 (br. d, $J_{5,4}$ = 9.3 Hz, 1H; H-5^{I}); 1.95 (s, 3H; CH$_3$CON); 1.71 (s, 3H; CH$_3$COO); ^{13}C NMR (150 MHz, CDCl$_3$): δ = 176.88, 171.00, 169.72, 166.67, 166.09, 165.51, 164.74, 164.33, 164.06 (CO), 101.47 (C-1^{II}), 100.70 (C-1^{I}), 100.65 (C-1^{III}), 80.71 (C-4^{I}), 76.36 (C-3^{II}), 74.15 (C-5^{I}), 72.73 (C-5^{II}), 72.64 (C-5^{III}), 71.82 (C-3^{I}), 71.37 (C-2^{III}), 71.21 (C-2^{II}), 71.08 (C-3^{III}), 69.60 (C-4^{II}), 69.45 (C-4^{III}), 66.42 (O*C*H$_2$CH$_2$N), 62.91 (C-6^{II}), 60.24 (C-6^{I}), 55.85 (C-2^{I}), 52.89 (CH$_3$O), 39.33 (OCH$_2$*C*H$_2$N), 23.23 ppm (*C*H$_3$CON), 20.13 ppm (*C*H$_3$CO); elemental analysis calcd (%) for C$_{62}$H$_{61}$F$_3$N$_2$O$_{24}$ (1274.36): C 58.40, H 4.82, N 2.20; found: C 58.27, H 4.64, N 2.27; HRMS (ESI) *m/z* calcd for C$_{62}$H$_{61}$F$_3$N$_2$O$_{24}$ + H$^+$: 1275.3645 [M+H]$^+$; found: 1275.3639.

Trifluoroacetamidoethyl [methyl(3-*O*-acetyl-2,4-di-*O*-benzoyl-β-D-glucopyranosyl)uronate]-(1→3)-(2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-2-acetamido-3,6-di-*O*-benzoyl-2-deoxy-β-D-glucopyranoside (36)

To a solution of **35** (50 mg, 39 μmol) in pyridine (3 mL) was added benzoic anhydride (88 mg, 0.39 mmol) and DMAP (2.3 mg, 19 μmol). The reaction mixture was kept for 24 h at 45 °C and then diluted with ethyl acetate (30 mL). The solution was washed with 1 M H$_2$SO$_4$ (20 mL) satd aq NaHCO$_3$ (50 mL), and satd aq NaCl (10 mL), dried, and concentrated. Column chromatography of the residue (toluene/acetone 2:0.6 → 2:1) provided **36** (56 mg, 96%). [α]$_D$ +2.4 (*c* 1, CHCl$_3$); NMR (400 MHz, CDCl$_3$): δ = 8.13–6.90 (m, 35H; 7 Ph), 6.37 (d, $J_{NH,2}$ = 8.6 Hz, 1H; NH^{I}), 5.60 (d, $J_{4,3}$ = 3.4 Hz, 1H; H-4^{II}), 5.39 (dd, $J_{2,3}$ = 10.1 Hz, 1H; H-2^{II}), 5.33 (m, 3H; H-3^{I}, H-3^{III}, H-4^{III}), 4.98 (dd, $J_{2,3}$ = 9.0 Hz, 1H; H-2^{III}), 4.73 (d, $J_{1,2}$ = 7.2 Hz, 1H; H-1^{III}), 4.57 (d, $J_{1,2}$ = 8.1 Hz, 1H; H-1^{I}), 4.55 (d,

$J_{1,2}$ = 7.9 Hz, 1H; H-1^{II}), 4.33 (dd, $J_{6a,5}$ = 2.1 Hz, $J_{6a,6b}$ = 12.2 Hz, 1H; H-6a^{I}), 4.29 (m, $J_{2,3}$ = 9.3 Hz, 1H; H-2^{I}), 4.15 (dd, $J_{6b,5}$ = 3.9 Hz, 1H; H-6b^{I}), 4.05 (t, $J_{4,5}$ = 9.1 Hz, 1H; H-4^{I}), 4.03 (d, $J_{5,4}$ = 9.3 Hz, 1H; H-5^{III}), 4.00 (dd, 1H; H-3^{II}); 3.92 (dd, 1H; $J_{6a,5}$ = 5.6 Hz, $J_{6a,6b}$ = 11.7 Hz, 1H; H-6a^{II}), 3.88 (m, 1H; OC*Ha*HbCH$_2$N), 3.67 (m, 2H; H-5^{II}, OCHa*Hb*CH$_2$N), 3.53 (s, 3H; CH$_3$O), 3.50 (m, 1H; H-5^{I}), 3.48-3.34 (m, 3H, H-6b^{II}, OCH$_2$C*H*$_2$N), 1.73 (s, 3H; CH$_3$CON), 1.58 (s, 3H; CH$_3$COO); ^{13}C NMR (100 MHz, CDCl$_3$): δ = 171.08, 170.63, 169.91, 167.05, 166.70, 165.95, 165.14, 164.41, 164.29 (CO), 101.63 (C-1^{III}), 101.20 (C-1^{I}), 100.76 (C-1^{II}), 77.30 (C-3^{II}), 74.94 (C-4^{I}), 73.15 (C-3^{I}), 73.00 (C-5^{I}), 72.87 (C-5^{III}), 72.11 (C-5^{II}), 71.61 (C-3^{III}), 71.48 (C-2^{III}), 71.44 (C-2^{II}), 69.59 (C-4^{II}), 69.49 (C-4^{III}), 66.88 (O*C*H$_2$CH$_2$N), 62.33 (C-6^{I}), 61.88 (C-6^{II}), 55.54 (C-2^{I}), 52.88 (CH$_3$O), 39.84 (OCH$_2$*C*H$_2$N), 23.35 ppm (*C*H$_3$CON), 20.28 ppm (*C*H$_3$COO); HRMS (ESI): *m/z* calcd for C$_{76}$H$_{69}$F$_3$N$_2$O$_{26}$ + Na$^+$: 1505.3988 [M+Na]$^+$; found: 1505.3983.

2-Trifluoroacetamidoethyl [methyl(2,4-di-*O*-benzoyl-β-D-glucopyranosyl)uronate]-(1→3)-(2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-2-acetamido-3,6-di-*O*-benzoyl-2-deoxy-β-D-glucopyranoside (37)

Trisaccharide **36** (40 mg, 27 μmol) was dissolved in a solution of anhydrous HCl in MeOH that was obtained by adding AcCl (0.2 mL) to MeOH (5 mL) upon cooling with an ice-bath. The resulting solution was kept for 10 h at 4 ºC, diluted with CH_2Cl_2 (30 mL), and washed with satd $NaHCO_3$ solution. The organic layer was dried, concentrated and the residue was purified by column chromatography (toluene/acetone 2:0.6) to give compound **37** (33 mg, 85%) as an amorphous solid. $[\alpha]_D$ = –1.4 (*c* = 1, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.07–6.92 (m, 35H; 7 Ph), 5.87 (d, $J_{NH,2}$ = 9.1 Hz, 1H; NH), 5.60 (d, $J_{4,3}$ = 3.3 Hz, 1H; H-4^{II}), 5.41 (dd, $J_{2,3}$ = 9.8 Hz, 1H; H-2^{II}), 5.37 (t, $J_{3,4}$ = 9.6 Hz, 1H; H-3^{I}), 5.27 (t, $J_{4,5}$ = 9.3 Hz, H-4^{III}), 4.85 (dd, $J_{2,3}$ = 9.0 Hz, 1H; H-2^{III}), 4.78 (1, $J_{1,2}$ = 6.5 Hz, 1H; H-1^{III}), 4.59 (d, $J_{1,2}$ = 8.9 Hz, 1H; H-1^{I}), 4.55 (d, $J_{1,2}$ = 8.0 Hz, 1H; H-1^{II}), 4.30 (m,

2H; H-2^{I}, H-6a^{I}), 4.17 (dd, $J_{6b,5}$ = 3.7 Hz, $J_{6a,6b}$ = 12.4 Hz, 1H; H-6b^{I}), 4.08 (dd, 1H; H-3^{II}), 4.05 (d, 1H; H-5^{III}), 4.03 (t, $J_{4,5}$ = 8.9 Hz, 1H; H-4^{I}), 3.87 (m, 2H; H-6a^{II}, OC*Ha*HbCH$_2$N), 3.76 (m, 1H; H-3^{III}), 3.68 (m, 2H; H-5^{II}, OCHa*Hb*CH$_2$N H-6b^{II}), 3.56 (s, 3H; CH$_3$O), 3.52 (m, 1H; H-5^{I}), 3.45 (m, 2H; OCH$_2$C*H*$_2$N), 3.38 (m, H-6b^{II}), 2.76 (d, $J_{3,OH}$ = 6.4 Hz, 1H; OH), 1.72 ppm (s, 3H; CH$_3$CON); ^{13}C NMR (100 MHz, CDCl$_3$): δ = 170.91, 167.34, 165.96, 165.87, 165.83, 165.33, 164.53 (CO), 101.60 (C-1^{I}), 100.81 (C-1^{II}), 100.54 (C-1^{III}), 76.88 (C-3^{II}), 75.07 (C-4^{I}), 73.96 (C-2^{III}), 73.21 (C-3^{I}), 73.11 (C-5^{I}), 72.80 (C-5^{III}), 72.65 (C-3^{III}), 72.15 (C-4^{III}), 72.02 (C-5^{II}), 71.88 (C-2^{II}), 69.50 (C-4^{II}), 66.97 (O*C*H$_2$CH$_2$N), 62.33 (C-6^{I}), 61.75 (C-6^{II}), 53.50 (C-2^{I}), 52.84 (CH$_3$O), 39.86 (OCH$_2$*C*H$_2$N), 23.36 ppm (*C*H$_3$CON); HRMS (ESI): *m/z* calcd for $C_{74}H_{67}F_3N_2O_{25}$ + Na^+: 1463.3883 $[M+Na]^+$; found: 1463.3877.

2-Trifluoroacetamidoethyl [methyl(2,4-di-*O*-benzoyl-3-*O*-sulfo-β-D-glucopyranosyl)uronate]-(1→3)-(2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-2-acetamido-3,6-di-*O*-benzoyl-2-deoxy-β-D-glucopyranoside, sodium salt (38)

To a solution of **37** (33 mg, 23 μmol) in DMF (1 mL) was added SO$_3$·Py (33 mg, 0.21 mmol) and the mixture was stirred for 1.5 h at room temperature. Solid NaHCO$_3$ (54 mg, 0.64 mmol) was added to destroy the excess of the reagent; the resulting mixture was stirred for 1 h, filtered, and solids were washed with MeOH (10 mL). The combined filtrates were concentrated, and to a solution of the residue in aqueous MeOH (10:1, 7 mL) was added Amberlite IRA-120 (Na^+) (3 mL). After being kept for 20 min, the mixture was filtered, the resin was washed with MeOH (10 mL), and the combined filtrates were concentrated. Column chromatography (EtOAc/MeOH 10:1) of the residue provided sulfate **38** (34 mg, 96%) as an amorphous solid. $[\alpha]_D$ = +0.9 (*c* = 1, MeOH); NMR (400 MHz, CD$_3$OD): δ = 8.00–6.78 (m, 35H; 7 Ph), 5.55 (d, $J_{4,3}$ = 3.4 Hz, 1H; H-4^{II}), 5.37 (dd, $J_{2,3}$ =

10.0 Hz, 1H; H-2^{II}), 5.28 (dd, $J_{3,4}$ = 9.0 Hz, 1H; H-3^{I}), 5.17 (t, $J_{4,5}$ = 9.8 Hz, 1H; H-4^{III}), 4.97 (t, $J_{2,3}$ = 8.8 Hz, 1H; H-2^{III}), 4.91 (d, $J_{1,2}$ = 7.8 Hz, 1H; H-1^{III}), 4.75 (t, $J_{3,4}$ = 9.0 Hz, 1H; H-3^{III}), 4.63 (d, $J_{1,2}$ = 8.4 Hz, 1H; H-1^{II}), 4.47 (d, $J_{1,2}$ = 8.5 Hz, 1H; H-1^{I}), 4.28 (dd, $J_{6a,5}$ = 2.0 Hz, $J_{6a,6b}$ = 12.3 Hz, 1H; H-6a^{I}), 4.22 (m, 3H; H-3^{II}, H-5^{III}, H-6b^{I}), 4.04 (dd, $J_{2,3}$ = 10.7 Hz, 1H; H-2^{I}), 3.96 (t, $J_{4,5}$ = 9.2 Hz, 1H; H-4^{I}), 3.85 (dd, $J_{6a,5}$ = 5.1 Hz, $J_{6a,6b}$ = 11.4 Hz, 1H; H-6a^{II}), 3.77 (dd, 1H; H-5^{II}), 3.68 (m, 1H; OC*Ha*HbCH$_2$N); 3.55 (m, 2H; H-5^{I}, OCHa*Hb*CH$_2$N), 3.39 (s, 3H; CH$_3$O); 3.29 (m, 2H; OCH$_2$C*H*$_2$N), 3.05 (dd, $J_{6b,5}$ = 7.8 Hz, 1H; H-6b^{II}), 1.63 ppm (s, 3H; CH$_3$CON); ^{13}C NMR (100 MHz, CD$_3$OD): δ = 173.51, 169.08, 167.34, 167.31, 167.25, 167.17, 167.07, 166.68, 166.16, 102.17 (C-1^{III}), 102.10 (C-1^{II}), 102.02 (C-1^{III}), 79.38 (C-3^{II}), 78.45 (C-3^{III}), 77.11 (C-4^{I}), 74.62 (C-3^{I}), 74.09 (C-5^{I}), 73.72 (C-5^{III}), 73.27 (C-2^{III}), 73.09 (C-5^{II}), 72.66 (C-2^{II}), 71.96 (C-4^{III}), 71.32 (C-4^{II}), 68.14 (O*C*H$_2$CH$_2$N), 63.91 (C-6^{I}), 63.54 (C-6^{II}), 54.94 (C-2^{I}), 53.14 (CH$_3$O), 40.65 (OCH$_2$*C*H$_2$N), 22.59 ppm (*C*H$_3$CON); HRMS (ESI) *m/z* calcd for $C_{74}H_{66}F_3N_2NaO_{28}S + Na^+$: 1565.3270 $[M+Na]^+$; found: 1565.3265.

2-Aminoethyl (β-D-glucopyranosyluronic acid)-(1→3)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranoside, sodium salt (39)

Trisaccharide **35** (14 mg, 11 μmol) was subjected to saponification as described for compound **32**. After gel-permeation chromatography on the Sephadex G-15 column and liophylization, trisaccharide **39** (5.4 mg, 82%) was obtained. ^{1}H NMR (500 MHz, D$_2$O): δ = 4.67 (d, $J_{1,2}$ = 7.8 Hz, 1H; H-1^{III}), 4.57 (d, $J_{1,2}$ = 8.3 Hz, 1H; H-1^{I}), 4.52 (d, $J_{1,2}$ = 7.8 Hz, 1H; H-1^{II}), 4.18 (d, $J_{4,3}$ = 2.9 Hz, 1H; H-4^{II}), 4.06 (m, 1H; OC*Ha*HbCH$_2$N), 4.00 (dd, $J_{6a,5}$ = 2.1 Hz, $J_{6a,6b}$ = 12.3 Hz, 1H; H-6a^{I}), 3.90 (m, 1H; OCHa*Hb*CH$_2$N), 3.85 (dd, $J_{6b,5}$ = 5.1 Hz, 1H; H-6b^{I}), 3.81 (dd, $J_{3,2}$ = 9.8 Hz, 1H; H-3^{II}), 3.80 (dd, $J_{2,3}$ = 10.0 Hz, 1H; H-2^{I}), 3.78–3.71 (m, 6H; H-3^{I},4^{I},5^{II},5^{III}, 2 H-6^{II}), 3.69 (dd, $J_{2,3}$ = 9.8 Hz, 1H; H-2^{II}), 3.62 (m, 1H; H-5^{I}), 3.52 (m, 2H; H-3^{III},4^{III}), 3.41 (dd, $J_{2,3}$= 9.2 Hz, 1H; H-2^{III}), 3.22 (m,

2H, OCH_2CH_2N), 2.05 ppm (CH_3CON); HRMS (ESI): *m/z* calcd for $C_{22}H_{38}N_2O_{17}$ + H^+: 603.2243 $[M+H]^+$; found: 603.2242.

2-Trifluoroacetamidoethyl [methyl(3-*O*-acetyl-2,4-di-*O*-benzoyl-β-D-glucopyranosyl)uronate]-(1→3)-(2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-2-acetamido-2-deoxy-6-*O*-(*p*-methoxyphenyl)-β-D-glucopyranoside (40)

Trisacchartide **24** (120 mg, 85 μmol) was hydrogenated in MeOH (5 mL) in the presence of PdO/C (50 mg) and AcOH (20 μL) and the amine formed was N-trifluoroacetylated with CF_3CO_2Et (50 μL, 0.42 mmol) in the presence of Et_3N (42 μL, 0.28 mmol) as described for compound **30**. Filtration of the reaction mixture through a short silica gel column in toluene/acetone (2:1) afforded the corresponding trifluoroacetamide (101 mg). To its solution in MeOH (4 mL) was added PdO/C (30 mg) and the mixture was stirred under a hydrogen atmosphere for 2 h. The catalyst was filtered off, washed with MeOH (30 mL) and the combined filtrates were concentrated. The residue was purified by column chromatography (toluene/acetone 2:1) to give **40** (67 mg, 57%) as an amorphous solid. $[\alpha]_D$ = +39 (*c* = 1, $CHCl_3$); NMR (500 MHz, $CDCl_3$): δ = 8.13–7.15 (m, 25H; 5 Ph), 6.64, 6.39 (2 d, *J* = 9.1 Hz, 4H; $C_6H_4OCH_3$), 5.85 (d, $J_{4,3}$ = 3.1 Hz, 1H; H-4^{II}), 5.62 (dd, $J_{2,3}$ = 9.8 Hz, 1H; H-2^{II}), 5.48 (d, $J_{NH,2}$ = 7.5 Hz, 1H; NH), 5.43 (t, $J_{4,5}$ = 9.6 Hz, 1H; H-4^{III}), 5.35 (t, $J_{3,4}$ = 9.1 Hz, 1H; H-3^{III}), 5.07 (dd, $J_{2,3}$ = 8.8 Hz, 1H; H-2^{III}), 4.86 (d, $J_{1,2}$ = 7.0 Hz, 1H; H-1^{III}), 4.73 (dd, $J_{6a,5}$ = 2.7 Hz, $J_{6a,6b}$ = 11.5 Hz, 1H; H-$6a^{II}$), 4.69 (d, $J_{1,2}$ = 8.3 Hz, 1H; H-1^{I}), 4.24 (m, 3H; H-3^{II},5^{II},$6b^{II}$), 4.14 (d, 1H, H-5^{III}), 3.78 (m, 1H: $OCHaHbCH_2N$), 3.75 (s, 3H; $CH_3OC_6H_4$), 3.73 (m, 3H, H-3^{I},4^{I},$6a^{I}$), 3.64 (s, 3H, CH_3), 3.60 (m, 3H; H-2^{I},$6b^{I}$, $OCHaHbCH_2N$), 3.50 (m, 2H; C-5^{I}, $OCH_2CHaHbN$), 1.90 ppm (s, 3H, CH_3CON); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 170.96, 169.77, 166.75, 166.18, 165.56, 164.78, 164.28, 164.08 (CO), 101.60 (C-1^{II}), 100.67 (C-1^{III}), 100.48 (C-1^{I}), 81.19 (C-4^{I}), 76.33 (C-3^{II}), 72.92 (C-5^{I}), 72.72 (C-5^{II},5^{III}), 72.13 (C-3^{I}), 71.37 (C-2^{III}), 71.17 (C-2^{II},3^{III}), 69.59 (C-4^{II}), 69.43 (C-

4^{III}), 66.52 (C-6^{I}), 66.14 (O*C*H_2CH_2N), 62.97 (C-6^{II}), 55.87 (C-2^{I}), 55.67 (*C*H_3OC_6H_4), 52.88 (CH_3O), 39.44 (OCH_2*C*H_2N), 23.37 (*C*H_3CON), 20.19 ppm (*C*H_3COO); elemental analysis calcd (%) for $C_{69}H_{67}F_3N_2O_{25}$ (1381.26): C 60.0, H 4.89, N 2.03; found: C 60.23, H 5.09, N 2.24.

Ethyl 3,4-di-*O*-benzoyl-2-*O*-benzyl-1-thio-β-L-fucopyranoside (41)

Benzoyl chloride (0.24 mL, 2.05 mmol) was added to a chilled (4 °C) solution of ethyl 2-*O*-benzyl-1-thio-β-L-fucopyranoside (152 mg, 0.51 mmol) in pyridine (3 mL). The mixture was allowed to attain to room temperature and stirred for 5 h. After adding water (0.1 mL), the mixture was diluted with $CHCl_3$ (50 mL) and washed successively with water (50 mL), 1 M HCl (100 mL), satd aq $NaHCO_3$ (100 mL), and water (50 mL). The organic layer was dried and the solvent was evaporated. Column chromatography of the residue (toluene/EtOAc 95:5 → 9:1) gave thiofucoside **41** (248 mg, 96%) as a colorless foam. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.14- 7.12 (15H, 3 Ph), 5.67 (d, 1H. H-4), 5.45 (dd, $J_{3,4}$ = 3.4 Hz, 1H, H-3), 4.87 (d, J_{gem} = 10.7 Hz, 1H, PhCH_2) 4.67 (d, $J_{1,2}$ = 9.5 Hz, 1H, H-1), 4.64 (d, J_{gem} = 10.7 Hz, 1H, PhCH_2), 3,98 (q, $J_{5,6}$ = 7.8 Hz, 1H, H-5), 3.93 (t, $J_{2,3}$ = 9.6 Hz, 1H, H-2), 2.86 (m, 2H, SCH_2CH$_3$), 1.38 (t, *J* = 7.5 Hz, 3H, SCH$_2$CH_3), 1.30 (d, 3H, H-6) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 165.96, 165.56 (CO), 85.27 (C-1), 76.26 (C-2), 75.48, 75.12, 73.32, 71.85 (C-3, C-4, C-5, Ph*C*H_2), 25.14 (S*C*H_2CH$_3$), 16.69 (C-6), 15.03 (SCH$_2$*C*H_3) ppm; HRMS (ESI) *m/z* calcd for $C_{29}H_{30}O_9S$ + Na^+: 529.1655 $[M+Na]^+$; found: 529.1652.

2-Trifluoroacetamidoethyl [methyl(3-*O*-acetyl-2,4-di-*O*-benzoyl-β-D-glucopyranosyl)uronate]-(1→3)-(2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-[3,4-di-*O*-benzoyl-2-*O*-benzyl-α-L-fucopyranosyl-(1→3)]-2-acetamido-2-deoxy-6-*O*-(*p*-methoxyphenyl)-β-D-glucopyranoside (42)

To a solution of thioglycoside **41** (31 mg, 60 μmol) and acceptor **40** (35 mg, 30 μmol) in CH_2Cl_2 (3 mL) was added MS 4 Å (200 mg), the mixture was stirred for 20 min at room temperature, and

cooled to –25 °C. Then NIS (19 mg, 80 μmol) and TfOH (6.3 μL, 70 μmol) were added, and the mixture was stirred for 3 h, while the temperature was gradually increased to –10 °C. The reaction was quenched by adding pyridine (0.1 mL), the mixture was diluted with CH_2Cl_2 (30 mL) and filtered through a Celite layer. The filtrate was washed with 1 M aqueous $Na_2S_2O_3$, satd $NaHCO_3$, and concentrated. The residue was subjected to column chromatography (toluene/acetone 5:1) to provide amorphous tetrasaccharide **42** (24 mg, 52%). $[\alpha]_D$ = –76 (*c* = 1, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.15–7.11 (35H; 7 Ph), 6.83 (m, 4H; $C_6H_4OCH_3$), 6.52 (d, $J_{NH,2}$ = 9.5 Hz, 1H; NH), 5.94 (d, $J_{4,3}$ = 3.5 Hz, 1H; H-4^{II}), 5.63 (dd, $J_{2,3}$ = 9.9 Hz, 1H; H-2^{II}), 5.51 (t, $J_{4,5}$ = 9.4 Hz, 1H; H-4^{III}), 5.45 (t, $J_{3,4}$ = 8.7 Hz, 1H; H-3^{III}), 5.43 (dd, $J_{3,4}$ = 3.3 Hz, 1H; H-3^{IV}), 5.31 (d, $J_{1,2}$ = 3.4 Hz, 1H; H-1^{IV}), 5.13 (t, $J_{2,3}$ = 8.4 Hz, 1H, H-2^{III}), 5.06 (d, $J_{1,2}$ = 6.6 Hz, 1H; H-1^{III}), 5.04 (br. s, 1H; H-4^{IV}), 4.72 (d, $J_{1,2}$ = 8.1 Hz, 1H; H-1^{II}), 4.66, 4.55 (2 d, J_{gem} = 11.8 Hz, 2H; CH_2Ph), 4.54 (dd, $J_{6a,5}$ = 7.6 Hz, $J_{6a,6b}$ = 11.9 Hz, 1H; H-6a^{II}), 4.48 (dd, $J_{6b,5}$ = 4.9 Hz, 1H; H-6b^{II}), 4.40 (dd, 1H; H-3^{II}), 4.22 (m, 4H; H-3^{I},5^{II},5^{III},6a^{I}), 4.18 (m, 1H; H-2^{I}), 4.11 (dd, $J_{6b,5}$ = 5.0 Hz, $J_{6b,6a}$ = 9.5 Hz, 1H; H-6b^{I}), 4.01 (dd, 1H; H-2^{IV}), 4.00 (m, 2H; H-1^{I},4^{I}), 3.86 (m, 1H; H-5^{I}), 3.76 (q, $J_{5,6}$ = 6.4 Hz, H-5^{IV}), 3.73 (s, 3H; CH_3OC$_6$H$_4$), 3.65 (s, 3H; CH_3O), 3.28 (m, 1H, OC*Ha*HbCH_2N), 3.13 (m, 2H; OCH_2CH_2N), 2.87 (m, 1H; OCHa*Hb*CH_2N), 2.07 (s, 3H; CH_3CON), 1.79 (s, 3H; CH_3COO), 0.72 ppm (d, 3H; H-6^{IV}); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 170.41, 169.82, 166.97, 166.01, 165.76, 165.70, 165.63, 165.37, 164.81, 164.28 (CO), 101.20 (C-1^{I}), 100.81 (C-1^{III}), 98.60 (C-1^{II}), 96.14 (C-1^{IV}), 76.10 (C-3^{II}), 75.39 (C-4^{I}), 74.33 (C-5^{I}), 73.10 (C-2^{IV}), 72.83 (C-5^{III}), 72.55 (C-3^{I}), 72.32 (*C*H$_2$Ph), 71.95 (C-2^{II},5^{II}), 71.82 (C-4^{IV}), 71.76 (C-2^{III}), 71.16 (C-3^{III}), 69.88 (C-3^{IV}), 69.69 (C-4^{II}), 69.31 (C-4^{III}), 67.60 (C-6^{I}), 67.06 (O*C*H$_2$CH$_2$N), 65.68 (C-5^{IV}), 62.78 (C-6^{II}), 55.69 (*C*H$_3$OC$_6$H$_4$), 52.86 (CH_3O), 50,23 (C-2^{I}), 39.44 (OCH$_2$*C*H$_2$N), 22.91 (*C*H$_3$CON), 20.22 (*C*H$_3$COO), 15.68 ppm (C-6^{IV}); elemental

analysis calcd (%) for $C_{96}H_{91}F_3N_2O_{31}$ (1825.74): C 63.15, H 5.02, N 1.53; found: C 63.08, H 5.09, N 1.27.

2-Trifluoroacetamidoethyl [methyl(3-*O*-acetyl-2,4-di-*O*-benzoyl-β-D-glucopyranosyl)uronate]-(1→3)-(2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-[3,4-di-*O*-benzoyl-α-L-fucopyranosyl-(1→3)]-2-acetamido-2-deoxy-β-D-glucopyranoside (43)

10% PdO/C (30 mg) was added to a solution of **42** (27 mg, 15 μmol) in MeOH (2 mL) and the mixture was stirred under hydrogen for 2 h. The catalyst was removed by filtration, washed with MeOH (30 mL), and the filtrate and washings were concentrated. The residue was dissolved in aqueous MeCN (8:1, 2 mL), CAN (22 mg, 40 μmol) was added, and the resulting mixture was stirred for 1 h at 4 °C, diluted with CH_2Cl_2 (50 mL), and washed with satd $NaHCO_3$. The organic layer was concentrated and the residue was purified by column chromatography (toluene/acetone 3:1) to produce diol **43** (15 mg, 64%) as an amorphous solid. $[\alpha]_D$ = –102 (*c* = 1, $CHCl_3$); NMR (500 MHz, $CDCl_3$): δ = 8.13–7.13 (m, 35H; 7 Ph), 6.64 (d, $J_{NH,2}$ = 8.8 Hz, 1H; NH), 5.92 (d, $J_{4,3}$ = 3.2 Hz, 1H; H-4^{II}), 5.59 (dd, $J_{2,3}$ = 9.7 Hz, 1H; H-2^{II}), 5.50 (t, $J_{4,5}$ = 9.5 Hz, 1H; H-4^{III}), 5.43 (t, $J_{3,4}$ = 9.0 Hz, 1H; H-3^{III}), 5.35 (dd, $J_{3,4}$ = 3.0 Hz, 1H; H-3^{IV}), 5.25 (d, 1H; H-4^{IV}), 5.16 (d, $J_{1,2}$ = 3.6 Hz, 1H; H-1^{IV}), 5.13 (dd, $J_{2,3}$ = 8.5 Hz, 1H; H-2^{III}), 5.05 (d, $J_{1,2}$ = 6.8 Hz, 1H; H-1^{III}), 4.72 (d, $J_{1,2}$ = 8.1 Hz, 1H; H-1^{II}), 4.57 (dd, $J_{6a,5}$ = 7.6 Hz, $J_{6a,6b}$ = 11.6 Hz, 1H; H-$6a^{II}$), 4.46 (dd, $J_{6b,5}$ = 5.0 Hz, 1H; H-$6b^{II}$), 4.42 (dd, 1H, H-3^{II}), 4.35 (br. s, 1H; H-1^{I}), 4.23 (d, 1H; H-5^{III}), 4.19 (m, 1H, H-5^{II}), 4.16–4.05 (m, 5H; H-2^{I},2^{IV}, H-3^{I},4^{I},5^{IV}), 3.97 (m, 1H; H-$6a^{I}$), 3.77 (m, 1H; H-$6b^{I}$), 3.71 (m, 1H; OC*Ha*HbCH_2N), 3.66 (m, 1H; H-5^{I}), 3.63 (s, 3H; CH_3O), 3.38 (m, 2H; OCH_2CH_2N), 3.30 (m, 2H; OCHa*Hb*CH_2N, OH), 2.07 (CH_3CON), 1.70 (s, 3H; CH_3COO), 0.98 ppm (d, 3H; H-6^{IV}); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 170.44, 169.79, 166.95, 166.67, 165.94, 165.71, 165.53, 164.82, 164.25 (CO), 100.66 (C-1^{III}), 99.96 (C-1^{I}), 99.35 (C-1^{II}), 97.75 (C-1^{IV}), 75.93 (C-3^{II}), 74.90 (C-5^{I}), 72.84

(C-5^{III}), 72.53 (C-5^{II}), 72.25 (C-4^{I}), 71.96 (C-2^{II}), 71.89 (C-4^{IV}), 71.70 (C-2^{III}, C-3^{IV}), 71.33 (C-3^{I}), 71.18 (C-3^{III}), 69.60 (C-4^{II}), 69.37 (C-4^{III}), 67.88 (O*C*H$_2$CH$_2$N), 67.51 (C-2^{IV}), 66.54 (C-5^{IV}), 62.68 (C-6^{II}), 62.23 (C-6^{I}), 52.84 (CH$_3$O), 47.58 (C-2^{I}), 39.49 (OCH$_2$*C*H$_2$N), 22.78 (*C*H$_3$CON), 20.22 (*C*H$_3$COO), 15.90 ppm (C-6^{IV}); elemental analysis calcd (%) for $C_{96}H_{91}F_3N_2O_{31}$ (1629.50): C 60.44, H 4.89, N 1.72; found: C 60.27, H 5.09, N 1.53.

2-Trifluoroacetamidoethyl [methyl(3-*O*-acetyl-2,4-di-*O*-benzoyl-β-D-glucopyranosyl)uronate]-(1→3)-(2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-[2,3,4-tri-*O*-benzoyl-α-L-fucopyranosyl-(1→3)]-2-acetamido-6-*O*-benzoyl-2-deoxy-β-D-glucopyranoside (44)

To a solution of diol **43** (15 mg, 9 μmol) in pyridine (1 mL) was added BzCl (6.4 μL, 60 μmol) upon cooling with an ice-bath. After 10 min, the bath was removed; the mixture was stirred for 4 h at room temperature, diluted with $CHCl_3$ (10 mL), and washed successively with 1 M HCl (30 mL), water (50 mL), satd $NaHCO_3$ (50 mL), and water. The organic layer was concentrated and the residue was purified by column chromatography (toluene/acetone 4:1) to give tetrasaccharide **44** (16 mg, 95%). $[\alpha]_D$ = –43 (*c* = 1, $CHCl_3$); NMR (500 MHz, $CDCl_3$): δ = 8.12–7.10 (m, 45H; 9 Ph), 6.22 (d, $J_{NH,2}$ = 8.8 Hz, NH), 5.85 (d, $J_{4,3}$ = 3.3 Hz, 1H; H-4^{II}), 5.79 (dd, $J_{3,4}$ = 3.1 Hz, 1H; H-3^{IV}), 5.70 (d, $J_{1,2}$ = 3.8 Hz, 1H; H-1^{IV}), 5.60 (dd, $J_{2,3}$ = 9.8 Hz, 1H; H-2^{II}), 5,49 (dd, $J_{2,3}$ = 10.7 Hz, 1H; H-2^{IV}), 5.46 (t, $J_{4,5}$ = 9.2 Hz, 1H; H-4^{III}), 5.42 (t, $J_{3,4}$ = 9.2 Hz, 1H; H-3^{III}), 5.41 (br. s, 1H; H-4^{IV}), 5.11 (t, $J_{2,3}$ = 8.7 Hz, 1H; H-2^{IV}), 5.00 (d, $J_{1,2}$ = 7.0 Hz, 1H; H-1^{III}), 4.62 (d, $J_{1,2}$ = 8.1 Hz, 1H; H-1^{II}), 4.57 (m, 3H; H-6a^{I}, 6a^{II}, 6b^{II}), 4.46 (dd, $J_{6b,5}$ = 5.9 Hz, $J_{6a,6b}$ = 11.7 Hz, 1H; H-6b^{I}), 4.42 (q, 1H; H-5^{IV}), 4.31 (dd, 1H; H-3^{II}), 4.21 (d, 1H; H-5^{III}), 4.15 (br. d, $J_{1,2}$ = 5.2 Hz, 1H; H-1^{I}), 4.11 (br. t, $J_{3,4}$ = 4.7 Hz, 1H; H-3^{I}), 4.07 (br. t, $J_{4,5}$ = 4.8 Hz, 1H; H-4^{I}), 3.99 (br. t, *J* = 6.5 Hz, H-5^{II}), 3.88 (m, 1H; H-2^{I}), 3.77 (m, 1H; H-5^{I}), 3.63 (s, 3H; CH$_3$O), 3.14 (m, 2H; OC*Ha*HbCH$_2$N, OCH$_2$C*Ha*HbN), 2.88 (m, 2H; OCHa*Hb*CH$_2$N, OCH$_2$CHa*Hb*N), 2.03 (s, 3H; CH$_3$CON), 1.67 (s, 3H; CH$_3$COO), 1.11 ppm (d,

$J_{6,5}$ = 6.3 Hz, H-6^{IV}); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 170.43, 169.82, 166.88, 166.12, 166.05, 165.95, 165.49, 165.16, 164.80, 164.23 (CO), 100.82 (C-1^{III}), 100.52 (C-1^{I}), 99.47 (C-1^{II}), 94.33 (C-1^{IV}), 76.24 (C-3^{II}), 73.51 (C-5^{I}), 72.85 (C-4^{I},5^{III}), 72.75 (C-3^{I},5^{II}), 71.77 (C-4^{IV}), 71.61 (C-2^{III}), 71.51 (C-2^{II}), 71.17 (C-3^{III}), 69.65 (C-4^{II}), 69.37 (C-4^{III}), 69.22 (C-2^{IV}), 68.42 (C-3^{IV}), 66.95 (O*C*H$_2$CH$_2$N), 65.79 (C-5^{IV}), 63.95 (C-6^{I}), 62.42 (C-6^{II}), 52.86 (CH_3O), 51.17 (C-2^{I}), 39.27 (OCH$_2$*C*H$_2$N), 22.91 (*C*H$_3$CON), 20.22 (*C*H$_3$COO), 15.93 ppm (C-6^{IV}); elemental analysis calcd (%) for $C_{96}H_{87}F_3N_2O_{32}$ (1837.71): C 62.74, H 4.77, N 1.52; found: C 62.91, H 4.59, N 1.67.

2-Trifluoroacetamidoethyl [methyl(2,4-di-*O*-benzoyl-β-D-glucopyranosyl)uronate]-(1→3)-(2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-[2,3,4-tri-*O*-benzoyl-α-L-fucopyranosyl-(1→3)]-2-acetamido-6-*O*-benzoyl-2-deoxy-β-D-glucopyranoside (45)

Monoacetate **44** (15 mg, 8 μmol) was treated with a solution of anhydrous HCl in MeOH as described for compound **26**. Column chromatography (toluene/acetone 7:3) afforded compound **45** (11 mg, 75%). $[\alpha]_D$ = –61 (*c* = 1, $CHCl_3$); NMR (500 MHz, $CDCl_3$): δ = 8.06–7.12 (m, 45H; 9 Ph), 6.25 (d, $J_{NH,2}$ = 8.8 Hz, 1H; NH), 5.86 (br. s, 1H; H-4^{II}), 5.81 (dd, $J_{3,4}$ = 3.2 Hz, 1H; H-3^{IV}), 5.70 (d, $J_{1,2}$ = 3.9 Hz, 1H; H-1^{IV}), 5.62 (t, $J_{2,3}$ = 8.6 Hz, 1H; H-2^{II}), 5.51 (dd, $J_{2,3}$ = 10.7 Hz, 1H; H-2^{IV}), 5.45 (d, 1H; H-4^{IV}), 5.42 (t, $J_{4,5}$ = 9.4 Hz, 1H; H-4^{III}), 5.04 (d, $J_{1,2}$ = 6.1 Hz, 1H; H-1^{III}), 4.95 (t, $J_{2,3}$ = 7.5 Hz, 1H; H-2^{III}), 4.65 (br. d, $J_{1,2}$ = 7.6 Hz, 1H; H-1^{II}), 4.61 (dd, $J_{6a,5}$ = 5.2 Hz, $J_{6a,6b}$ = 11.9 Hz, 1H; H-6a^{I}), 4.55 (m, 2H; 2 H-6^{II}), 4.48 (dd, $J_{6b,5}$ = 5.9 Hz, 1H; H-6b^{I}), 4.43 (m, 2H; H-3^{II},5^{IV}), 4.22 (d, 1H; H-5^{III}), 4.19 (br. d, $J_{1,2}$ = 4.8 Hz, 1H; H-1^{I}), 4.13 (br. t, $J_{3,4}$ = 6.3 Hz, 1H; H-3^{I}), 4.08 (br. t, 1H; H-4^{I}), 4.01 (br. t, 1H; H-5^{II}), 3.91 (t, $J_{3,4}$ = 8.3 Hz, 1H; H-3^{III}), 3.86 (m, 1H; H-2^{I}), 3.79 (m, 1H; H-5^{I}), 3.63 (s, 3H; CH_3O), 3.13 (m, 2H; OC*Ha*HbCH$_2$N, OCH$_2$C*Ha*HbN), 2.90 (m, 2H; OCHa*Hb*CH$_2$N, OCH$_2$CHa*Hb*N), 2.03 (s, 3H; CH_3CON), 1.13 ppm (d, $J_{6,5}$ = 6.4 Hz, H-6^{IV}); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 170.61, 167.51, 166.13, 166.06, 165.90, 165.69, 165.52, 165.25, 162.49 (CO), 100.49

(C-1^{I}), 100.17 (C-1^{III}), 99.58 (C-1^{II}), 94.41 (C-1^{IV}), 75.54 (C-3^{II}), 74.07 (C-2^{III}), 73.52 (C-5^{I}), 72.94 (C-3^{I}), 72.84 (C-4^{I},5^{III}), 72.69 (C-5^{II}), 72.26 (C-3^{III}), 71.96 (C-4^{III}), 71.81 (C-2^{II},4^{IV}), 69.73 (C-4^{II}), 69.26 (C-2^{IV}), 68.44 (C-3^{IV}), 66.98 (O*C*H$_2$CH$_2$N), 65.75 (C-5^{IV}), 63.95 (C-6^{I}), 62.29 (C-6^{II}), 52.80 (CH$_3$O), 51.20 (C-2^{I}), 39.28 (OCH$_2$*C*H$_2$N), 22.93 (*C*H$_3$CON), 15.95 ppm (C-6^{IV}); elemental analysis calcd (%) for $C_{94}H_{85}F_3N_2O_{31}$ (1795.67): C 62.87, H 4.77, N 1.56; found: C 62.61, H 4.59, N 1.67.

2-Aminoethyl (3-*O*-sulfo-β-D-glucopyranosyluronic acid)-(1→3)-(β-D-galactopyranosyl)-(1→4)-[α-L-fucopyranosyl-(1→3)]-2-acetamido-2-deoxy-β-D-glucopyranoside, disodium salt (47)

To a solution of monohydroxy derivative **45** (11 mg, 6 μmol) in a mixture of DMF and pyridine (3:1, 1 mL) was added SO_3·Py (48 mg, 0.3 mmol), the resulting mixture was stirred for 42 h at room temperature, and then worked-up as described for compound **28**. Column chromatography ($CHCl_3$/MeOH 10:1) afforded sulfate **46** (10 mg, 85%) which was subjected to two-steps saponification as described for compound **33**. Gel-permeation chromatography on the Sephadex G-15 column in water followed by liophylization gave free sulfate **47** (4.3 mg, 92%). $[\alpha]_D$ = –32 (*c* = 1, H_2O); NMR (500 MHz, D_2O): δ = 5.12 (d, $J_{1,2}$ = 3.9 Hz, 1H; H-1^{IV}), 4.82 (q, $J_{5,6}$ = 6.7 Hz, 1H; H-5^{IV}), 4.76 (d, $J_{1,2}$ = 7.8 Hz, 1H; H-1^{III}), 4.60 (d, $J_{1,2}$ = 8.3 Hz, 1H; H-1^{I}), 4.53 (d, $J_{1,2}$ = 7.8 Hz, 1H; H-1^{II}), 4.34 (t, $J_{3,4}$ = 9.1 Hz, 1H; H-3^{III}), 4.15 (d, $J_{4,3}$ = 2.6 Hz, 1H; H-4^{II}), 4.07–3.95 (m, 4H; H-2^{I},4^{I},6a^{I}, OC*Ha*HbCH$_2$N), 3.93–3.85 (m, 4H; H-3^{I},3^{IV},6b^{I}, OCHa*Hb*CH$_2$N), 3.84–3.77 (m, 3H; H-3^{II},4^{IV},5^{III}), 3.76–3.68 (m, 4H; 2 H-6^{II}, H-2^{IV},4^{III}), 3.78–3.68 (m, 4H; H-2^{II},2^{III},5^{I},5^{II}), 3.19 (m, 2H; OCH$_2$C*H*$_2$N), 2.05 (s, 3H; CH$_3$CON), 1.18 ppm (d, 3H; H-6^{IV}); ^{13}C NMR (125 MHz, D_3O): δ = 175.44, 174.63 (C-6^{III}, CH$_3$*C*ON), 103.16 (C-1^{III}), 191.41 (C-1^{II}), 100.82 (C-1^{I}), 98.63 (C-1^{IV}), 83.67 (C-3^{III}), 82.63 (C-3^{II}), 76.26 (C-5^{III}), 75.25 (C-5^{II}), 74.79 (C-3^{I},5^{I}), 73.19 (C-4^{I}), 72.02 (C-2^{III}), 71.90 (C-4^{IV}), 70.47 (C-4^{III}), 70.08 (C-2^{II}), 69.22 (C-3^{IV}), 67.97 (C-4^{II}), 67.68 (C-2^{IV}), 66.77 (C-5^{IV}), 66.19

(O*C*H_2CH_2N), 61.56 (C-6^{II}), 59.68 (C-6^{I}), 55.65 (C-2^{I}), 39.52 (OCH_2*C*H_2N), 22.27 (*C*H_3CON), 15.30 ppm (C-6^{IV}); HRMS (ESI) *m/z* calcd for $C_{28}H_{46}N_2Na_2O_{24}S$ + Na^+: 895.1854 $[M+Na]^+$; found: 895.1844.

Methyl (2-trifluoroacetamidoethyl 3-*O*-acetyl-2,4-di-*O*-benzoyl-β-D-glucopyranosid)uronate (48)

A solution of BF_3·Et_2O (6.3 µL, 50 µmol) in toluene (150 µL) was added to a solution of imidate **9** (50 mg, 83 µmol) and 2-trifluoroacetamidoethanol (15 mg, 95 µmol) in CH_2Cl_2 (3 mL) at 0 °C, the mixture was stirred at 0 °C for 2 h, then pyridine (50 µL) was added. The mixture was diluted with $CHCl_3$ (50 mL), washed with water (100 mL) and concentrated. Column chromatography of the residue (toluene/acetone 95:5) provided glucuronide **48** (31 mg, 62%); m. p. 139-141 °C (EtOAc/light petroleum); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.98, 7.59, 7.44 (3 m, 10H; 2 Ph), 6.98 (br. s, 1H; NH), 5.67 (t, $J_{3,4}$ = 9.4 Hz, 1H; H-3), 5.54 (t, $J_{4,5}$ = 9.5 Hz, 1H; H-4), 5.34 (dd, $J_{2,3}$ = 9.2 Hz, 1H; H-2), 4.80 (d, $J_{1,2}$ = 7.2 Hz, 1H; H-1), 4.29 (d, 1H; H-5), 3.97, 3.83 (2 m, 2H; OCH_2CH$_2$N), 3.69 (s, 3H; CH_3O), 3.57 (m, 2H; OCH$_2$CH_2N) 1.87 ppm (CH_3CO); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 169.98, 167.36, 165.28, 165.23 (4 CO), 101.19 (C-1), 72.94 (C-5), 71.82 (C-2), 71.30 (C-3), 69.83 (C-4), 68.30 (O*C*H_2CH_2N), 53.10 (CH_3O), 39.91 (OCH$_2$*C*H$_2$N), 20.55 ppm (*C*H_3CO); HRMS (ESI) *m/z* calcd for $C_{27}H_{26}F_3NO_{11}$+Na^+: 620.1350 $[M+Na]^+$; found: 620.1355.

Methyl (2-trifluoroacetamidoethyl 2,4-di-*O*-benzoyl-β-D-glucopyranosid)uronate (49)

To a solution of acetate **48** (36 mg, 60 µmol) in a mixture of $CHCl_3$ (0.5 mL) and MeOH (1 mL) was added AcCl (12 µL). The resulting solution was kept at room temperature for 30 h, then diluted with CH_2Cl_2 (50 mL), washed with satd $NaHCO_3$ (2×50 mL), and concentrated. The residue was dissolved in THF (1 mL) and treated with CF_3CO_2Et (100 µL) for 1 h. The solvent was evaporated,

and the residue was subjected to column chromatography (toluene/acetone 9:1) to give monohydroxy derivative **49** (28 mg, 84%) as a glassy solid; ^{1}H NMR (600 MHz, $CDCl_3$): δ = 8.05, 7.60, 7.44 (3 m, 10H; 2 Ph), 7.18 (br. s, 1H; NH), 5.49 (t, $J_{3,4}$ = 9.2 Hz, 1H; H-4), 5.28 (t, $J_{2,3}$ = 8.2 Hz, 1H; H-2), 4.78 (d, $J_{1,2}$ = 7.1 Hz, 1H; H-1), 4.27 (d, $J_{4,5}$ = 9.4 Hz, 1H; H-5), 4.18 (t, 1H; H-3), 3.97, 3.83 (2 m, 2H; OC*H*$_2$CH$_2$N), 3.69 (s, 3H; CH_3O), 3.54 (m, 2H; OCH_2CH_2N), 3.24 ppm (br. s, 1H; OH); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 167.81, 166.09, 165.96 (3 CO), 100.86 (C-1), 74.16 (C-2), 72.73, 72.75 (C-3, C-5), 72.26 (C-4), 68.11 (O*C*H$_2$CH$_2$N), 52.94 (CH_3O), 39.84 ppm (OCH_2CH_2N); HRMS (ESI): *m/z* calcd for $C_{25}H_{24}F_3NO_{10}$+Na^+: 578.1250 $[M+Na]^+$; found: 578.1244.

Methyl (2-trifluoroacetamidoethyl 2,4-di-*O*-benzoyl-3-*O*-sulfo-β-D-glucopyranosid)uronate, sodium salt (50)

SO_3·Py (40 mg. 0.25 mmol) was added to a solution of monohydroxy derivative **49** (28 mg, 50 μmol) in pyridine (1 mL) and the mixture was stirred at room temperature for 4 h. The excess of the reagent was destroyed by adding MeOH (100 μL), the solvent was evaporated, and a solution of the residue in MeOH (2 mL) was treated with Amberlite IRA-120 (Na^+) (2 mL) for 30 min. The resin was filtered off, washed with MeOH (10 mL) and the combined filtrates were concentrated. The residue was chromatographed ($CHCl_3$/MeOH 9:1) to afford sulfate **50** (30 mg, 91%) as an amorphous solid. ^{1}H NMR (500 MHz, $CDCl_3$ + CD_3OD): δ = 7.92, 7.45, 7.30 (3 m, 10H; 2 Ph), 5.49 (t, $J_{4,5}$ = 8.9 Hz, 1H; H-4), 5.24 (t, $J_{2,3}$ = 7.3 Hz, 1H; H-2), 4.90 (t, $J_{3,4}$ = 8.1 Hz, 1H; H-3), 4.77 (d, $J_{1,2}$ = 6.9 Hz, 1H; H-1), 4.29 (d, 1H; H-5), 3.88, 3.66 (2 m, 2H; OC*H*$_2$CH$_2$N), 3.58 (s, 3H; CH_3O), 3.49 ppm (m, 2H; OCH_2CH_2N); ^{13}C NMR (125 MHz, $CDCl_3$ + CD_3OD): δ = 168.14, 166.39, 166.11 (3 CO), 100.32 (C-1), 76.51 (C-3), 72.27 (C-5), 72.07 (C-2), 69.90 (C-4), 67.99

(O*C*H$_2$CH$_2$N), 52.79 (CH$_3$O), 39.55 ppm (OCH$_2$*C*H$_2$N); HRMS (ESI): *m/z* calcd for $C_{25}H_{24}F_3NO_{13}S$ – H^+: 634.0848 $[M–H]^–$; found: 634.0840.

2-Aminoethyl 3-*O*-sulfo-β-D-glucopyranosiduronic acid, disodium salt (51)

To a solution of sulfate **50** (30 mg, 46 μmol) in 90% aqueous THF (2 mL) was added 2 M LiOH (0.25 mL) at –10 °C, the mixture was stirred at this temperature for 3 h, then diluted with aqueous MeOH (5:3, 3 mL). 2 M aqueous NaOH (0.3 mL) was added, the mixture was kept at room temperature for 40 h, and made neutral by adding AcOH. The solvents were evaporated and the residue was subjected to gel-chromatography on the Sephadex G-15 column in water to give, after liophylization from water, sulfate **51** (14 mg, 84%) as a fluffy solid. ^{1}H NMR (500 MHz, D_2O): δ = 4.63 (d, $J_{1,2}$ = 7.9 Hz, 1H; H-1), 4.35 (t, $J_{3,4}$ = 9.1 Hz, 1H; H-3), 4.13, 4.02 (2 m, 2H, OC*H*$_2$CH$_2$N), 3.83 (d, $J_{5,4}$ = 10.0 Hz, 1H; H-5), 3.72 (t, 1H; H-4), 3.59 (dd, $J_{2,3}$ = 8.9 Hz, 1H; H-2), 3.29 ppm (m, 2H, OCH$_2$C*H*$_2$N); ^{13}C NMR (125 MHz, D_2O): δ = 176.70 (C-6), 102.99 (C-1), 85.13 (C-3), 77.31 (C-5), 73.21 (C-2), 71.82 (C-4), 67.38 (O*C*H$_2$CH$_2$N), 40.98 ppm (OCH$_2$*C*H$_2$N); HRMS (ESI): *m/z* calcd for $C_8H_{15}NO_{10}S$ – H^+: 316.0344 $[M–H]^–$; found: 316.0348.

Compound 55. 60% Suspension of NaH in mineral oil (13 mg, 0.27 mmol) was added to a solution of hexaethylenglycol **52** (6.50 g, 23 mmol) in THF (9 mL), the mixture was stirred at room temperature for 10 min, then *tert*-butyl acrylate (1 mL, 6.90 mmol) was added. The mixture was kept for 48 h at room temperature, AcOH (0.1 mL) was added, and the solvent was evaporated. The residue was dissolved in water (100 mL), the solution was extracted with EtOAc (3×100 mL) and the combined extracts were dried and concentrated to give crude *tert*-butyl ester **53** (2.20 g, 78%), which was used in the next step without further purification.

Methanesulfonyl chloride (1 mL) was added to a solution of *tert*-butyl ester **53** (2.20 g, 5.36 mmol) in CH_2Cl_2 (15 mL) and Et_3N (2 mL), the resulting mixture was stirred for 24 h at room temperature, diluted with CH_2Cl_2 (50 mL), washed with satd $NaHCO_3$ (2×50 mL), and concentrated to provide crude sulfonate **54**. It was dissolved in DMF (5mL), NaN_3 (1.89 g) was added, the mixture was stirred for 72 h at room temperature, diluted with EtOAc (100 mL), washed with water (3×50 mL), and the organic layer was concentrated. Column chromatography of the residue (ethyl acetate /methanol 95:5) afforded azide **55** (1.75 g, 75%) as a colorless oil. ^{1}H NMR (500 MHz, CD_3OD): 3.68 (t, *J* = 6.5 Hz, 2H; OC*H*$_2$CH$_2$COOBu-*tert*), 3.67–3.58 (m, 22H; $N_3CH_2CH_2O$, OCH_2CH_2O), 3.37 (t, *J* = 4.9 Hz, 2H; $N_3CH_2CH_2O$), 2.48 (t, *J* = 6.5 Hz, 2H; OCH_2CH_2COOBu-*tert*), 1.46 ppm (s, 9H, ($(CH_3)_3CO$). ^{13}C NMR (125 MHz, CD_3OD): δ = 172.70 (*C*OOBu-*tert*), 81.65 ($(CH_3)_3CO$), 71.62-71.13 (11C; $N_3CH_2CH_2O$, 5 OCH_2CH_2O), 67.88 (O*C*H$_2$CH$_2$COOBu-*tert*), 51.75 ($N_3CH_2CH_2O$), 37.22 (OCH_2CH_2COOBu-*tert*), 28.37 ppm ($(CH_3)_3CO$). HRMS (ESI) *m/z* calcd for $C_{19}H_{37}N_3O_8 + Na^+$: 458.2478 $[M+Na]^+$; found: 458.2489

Compound 56. 10% PdO/C (150 mg) and AcCl (15μL) were added to a solution of **55** (211 mg, 0.485 mmol) in MeOH (2.5 mL) and the mixture was stirred under hydrogen for 2 h. The catalyst was filtered off, washed with MeOH (50 mL), and the combined filtrate and washings were concentrated to yield amine **56** (191 mg, 96%) as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$): 3.68 (t, *J* = 6.5 Hz, 2H; OC*H*$_2$CH$_2$COOBu), 3.66–3.58 (m, 20H; OCH_2CH_2O), 3.48 (t, *J* = 5.2 Hz, 2H; $H_2NCH_2CH_2O$), 2.84 (t, *J* = 5.2 Hz, 2H; $NH_2CH_2CH_2O$), 2.47 (t, *J* = 6.5 Hz, 2H; OCH_2CH_2COOBu-*tert*), 1.41 ppm (s, 9H; ($(CH_3)_3CO$). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 170.82 (*C*OOBu-*tert*), 80.46 ($(CH_3)_3CO$), 73.42 ($H_2NCH_2CH_2O$), 70.54-70.25 (10C; 5 OCH_2CH_2O), 66.85 (O*C*H$_2$CH$_2$COOBu-*tert*), 41.77 ($H_2NCH_2CH_2O$), 36.22 (OCH_2CH_2COOBu-*tert*), 28.06 ppm ($(CH_3)_3CO$). HRMS (ESI) *m/z* calcd for $C_{19}H_{39}NO_8 + H^+$: 410.2754 $[M+H]^+$; found: 410.2759.

Compound 58. Et_3N (10 μL) was added to a solution of amine **56** (47.5 mg, 0.116 mmol) and active ester **57** (47 mg, 0.139 mmol) in DMF (0.7 ml) and the mixture was kept for 18 h at room temperature and concentrated. Column chromatography of the residue ($CHCl_3$/MeOH 8:1) afforded amide **58** (56 mg, 76%) as an amorphous solid. ^{1}H NMR (500 MHz, $CDCl_3$): 6.87 (br. t, 1H; CON*H*CH_2CH_2O); 6.68 (br. s, 1H; H-3 biotin); 5.78 (br. s, 1H; H-1 biotin); 4.48 (dd, $J_{6a,3a}$ = 7.6 Hz, $J_{6a,6}$ = 5.1 Hz, 1H; H-6a biotin); 4.29 (dd, $J_{3a,4}$ = 5.1 Hz, 1H; H-3a biotin); 3.68 (t, J = 6.4 Hz, 2H; O$CH_2$$CH_2$COOBu-*tert*), 3.65–3.56 (m, 20H; O$CH_2$$CH_2$O), 3.55 (t, J = 4.8 Hz, 2H; NHCH$_2$$CH_2$O), 3.41 (m, J = 5.2 Hz, 2H; NHCH_2CH$_2$O), 3.12 (m, 1H; H-4 biotin), 2.88 (dd, $J_{6,6'}$ 12.8 Hz, 1H, H-6 biotin), 2.74 (d, 1H; H-6′ biotin), 2.48 (t, J = 6.5 Hz, 2H; OCH$_2$CH_2COOBu-*tert*), 2.21 (m, 2H; (CH$_2$)$_3$CH_2C(O) biotin), 1.68 (m, 2H; CH_2(CH$_2$)$_3$C(O) biotin), 1.64 (m, 2H; (CH$_2$)$_2$CH_2CH$_2$C(O) biotin), 1.41 ppm (m, 11H; (CH_3)$_3$CO, CH$_2$CH_2(CH$_2$)$_2$C(O) biotin). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 173.30 (*C*ON), 170.85 (*C*OOBu-*tert*), 164.07 (C-2 biotin), 80.46 ((CH$_3$)$_3$*C*O), 70.52–69.94 (11C; 5 O$CH_2$$CH_2O, NHCH_2CH_2$O), 66.86 (O$CH_2CH_2$COOBu-*tert*), 61.76 (C-3a biotin), 60.20 (C-6a biotin), 55.06 (C-4 biotin), 40.48 (C-6 biotin), 39.13 (NHCH_2CH$_2$O), 36.25 ((CH$_2$)$_2$CH$_2$$CH_2$C(O) biotin), 35.92 (OCH$_2$$CH_2$COOBu-*tert*), 28.24–28.07 (5C; CH$_2$$CH_2$(CH$_2$)$_2$C(O) biotin, (CH$_2$)$_2$$CH_2CH_2$C(O) biotin, (*C*H$_3$)$_3$CO), 25.57 ppm (*C*H$_2$(CH$_2$)$_3$C(O) biotin); HRMS (ESI) *m/z* calcd for $C_{29}H_{53}N_3O_{10}S + Na^+$: 658.3349 $[M+Na]^+$; found: 658.3348.

Compound 59. Trifluoroacetic acid (130 μL) was added to a solution of amide **57** (78 mg, 0.123 mmol) in CH_2Cl_2 (0.7 mL), the mixture was kept for 1 h at room temperature, the solvents were removed, and toluene (2×15 mL) was evaporated from the residue. Column chromatography ($CHCl_3$/MeOH 5:1) gave acid **59** (64 mg, 90%) as an amorphous solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 6.78 (br. t, 1H; CON*H*CH_2CH_2O); 6.72 (br. s, 1H; H-3 biotin); 5.78 (br. s, 1H; H-1 biotin); 4.52 (dd, $J_{6a,3a}$ 7.6 Hz, $J_{6a,6}$ 5.1 Hz, 1H; H-6a biotin), 4.33 (dd, $J_{3a,4}$ = 5.1 Hz, 1H; H-3a

biotin), 3.75 (t, J = 6.4 Hz, 2H; OCH_2CH$_2$COOH), 3.68–3.61 (m, 20H; OCH_2CH_2O), 3.57 (t, J = 4.8 Hz, 2H; NHCH$_2$CH_2O), 3.43 (m, J = 5.2 Hz, 2H; NHCH_2CH$_2$O), 3.18 (m, 1H, H-4 biotin), 2.91 (dd, $J_{6,6'}$ = 12.8 Hz, 1H; H-6 biotin); 2.74 (d, 1H; H-6′ biotin), 2.58 (t, J = 6.5 Hz, 2H; OCH$_2$CH_2COOH), 2.23 (t, 2H; (CH$_2$)$_3$CH_2C(O) biotin), 1.70 (m, 2H; CH_2(CH$_2$)$_3$C(O) biotin), 1.68 (m, 2H; (CH$_2$)$_2$CH_2CH$_2$C(O) biotin), 1.47 ppm (m, 2H; CH$_2$CH_2(CH$_2$)$_2$C(O) biotin); ^{13}C NMR (125 MHz, CDCl$_3$): δ = 174.77 (*C*OOH), 174.60 (*C*(O)N), 164.92 (C-2 biotin), 70.31–69.96 (10C; 5 O*C*H$_2$*C*H$_2$O), 69.49 (NHCH$_2$*C*H$_2$O), 66.49 (O*C*H$_2$CH$_2$COOH), 62.23 (C-3a biotin), 60.65 (C-6a biotin), 55.23 (C-4 biotin), 40.24 (C-6 biotin), 39.41 (NH*C*H$_2$CH$_2$O), 35.46 ((CH$_2$)$_2$CH$_2$*C*H$_2$C(O) biotin), 34.89 (OCH$_2$*C*H$_2$COOH), 27.97 (CH$_2$*C*H$_2$(CH$_2$)$_2$C(O) biotin), 27.79 (*C*H$_2$(CH$_2$)$_3$C(O) biotin), 25.32 ppm ((CH$_2$)$_2$*C*H$_2$CH$_2$C(O) biotin). HRMS (ESI): *m/z* calcd for C$_{25}$H$_{45}$N$_3$O$_{10}$S + H$^+$: 580.2898 [M+H]$^+$; found: 580.2896.

Compound 60. Pyridine (58 µL, 0.71 mmol) and pentaflourophenyl trifluoroacetate (19 µL, 111 µmol) were added to a solution of acid **59** (21.5 mg, 37 µmol) in CH$_2$Cl$_2$ (0.5 mL). After being stirred for 1 h at room temperature, the mixture was diluted with cold CHCl$_3$ (30 mL) and washed with ice-cold 1 M HCl (20 mL) and ice-cold water (2×10 mL). The organic solution was dried, the solvent was evaporated, and the residue was dried in vacuum of an oil pump for 3 h to give ester **60** (25 mg, 92%) as a syrup, which was homogeneous on TLC (EtOAc/MeOH 2:1), R_f 0.38. It was dissolved in DMF (0.5 mL) to provide a 77 µM stock solution of **60** that was directly used for the preparation of biotin-tagged saccharides. Ester **60** was stable in the DMF solution upon storage at -20 °C at least for 1 month.

Synthesis of biotin-tagged saccharides. General procedure.

To a solution/suspension of a saccharide 2-aminoethyl glycoside (4-7 μmol) in DMF (250-400 μL) were added Et_3N (20-30 μL) and the stock solution of active ester **60** (1.3 equiv.). The resulting mixture was stirred for 16-20 h at room temperature and the solvent was removed in vacuum of an oil pump. The residue was subjected to gel-permeation chromatography, appropriate fractions were collected and freeze-dried to provide a biotin-tagged saccharide in 70-75% yield as a fluffy solid.

Compound 66. ^{1}H NMR (500 MHz, D_2O, selected data); oligosaccharide moiety: δ = 4.79 (d, $J_{1,2}$ = 7.9 Hz, 1H; H-1^{III}), 4.54 (d, $J_{1,2}$ = 8.1 Hz, 1H; H-1^{I}), 4.53 (d, $J_{1,2}$ = 7.8 Hz, 1H; H-1^{II}), 4.35 (t, J = 9.9 Hz, 1H; H-3^{III}), 4.17 (d, $J_{4,3}$ = 3.1 Hz, 1H; H-4^{II}), 2.05 ppm (s, 3H; CH_3CON); biotin moiety: δ = 4.61 (dd, $J_{6a,6}$ = 5.0 Hz, $J_{6a,3a}$ = 7.9 Hz, 1H; H-6a), 4.43 (dd, $J_{3a,4}$ = 4.5 Hz, 1H; H-3a), 3.34 (m, 1H, H-4), 3.00 (dd, $J_{6,6'}$ = 12.9 Hz, 1H; H-6), 2.79 ppm (d, 1H; H-6′); HRMS (ESI) *m/z* calcd for $C_{47}H_{81}N_5O_{29}S_2 - H^+$: 1242.4386 $[M–H]^–$; found: 1242.4399.

Compound 67. ^{1}H NMR (600 MHz, D_2O, selected data); oligosaccharide moiety: δ = 4.78 (d, $J_{1,2}$ = 7.9 Hz, 1H; H-1^{III}), 4.61 (d, $J_{1,2}$ = 8.0 Hz, 1H; H-1^{I}), 4.58 (d, $J_{1,2}$ = 7.8 Hz, 1H; H-1^{II}), 4.44 (br. d, $J_{6a,6b}$ = 11.0 Hz, 1H; H-6a^{I}), 4.35 (t, J = 9.1 Hz, 1H; H-3^{III}), 4.33 (dd, $J_{6b,5}$ = 3.8 Hz, 1H; H-6b^{I}), 4.21 (d, $J_{4,3}$ = 3.0 Hz, 1H; H-4^{II}) 2.05 ppm (s, 3H; CH_3CON); biotin moiety: δ = 4.63 (dd, $J_{6a,6}$ = 5.1 Hz, $J_{6a,3a}$ = 7.9 Hz, 1H; H-6a), 4.45 (dd, $J_{3a,4}$ = 4.6 Hz, 1H; H-3a), 3.36 (m, 1H, H-4), 3.02 (dd, $J_{6,6'}$ = 13.0 Hz, 1H; H-6), 2.81 ppm (d, 1H; H-6′); HRMS (ESI) *m/z* calcd for $C_{47}H_{80}N_5NaO_{32}S_3 - H^+$: 1344.3773 $[M–H]^–$; found: 1344.3773.

Compound 68. ^{1}H NMR (500 MHz, D_2O, selected data); oligosaccharide moiety: δ = 4.81 (d, $J_{1,2}$ = 7.9 Hz, 1H; H-1^{II}), 4.53 (d, $J_{1,2}$ = 7.7 Hz, 1H; H-1^{I}), 4.36 (t, J = 9.9 Hz, 1H; H-3^{II}), 4.19 ppm (d, $J_{4,3}$ = 3.1 Hz, 1H; H-4^{I}); biotin moiety: δ = 4.63 (dd, $J_{6a,6}$ = 4.9 Hz, $J_{6a,3a}$ = 7.9 Hz, 1H; H-6a), 4.45 (dd,

$J_{3a,4}$ = 4.5 Hz, 1H; H-3a), 3.37 (m, 1H, H-4), 3.03 (dd, $J_{6,6'}$ = 13.1 Hz, 1H; H-6), 2.81 ppm (d, 1H; H-6′); HRMS (ESI) *m/z* calcd for $C_{39}H_{68}N_4O_{24}S_2 - H^+$: 1039.3592 $[M–H]^–$; found: 1039.3588.

Compound 69. ^{1}H NMR (600 MHz, D_2O, selected data); oligosaccharide moiety: δ = 5.10 (d, $J_{1,2}$ = 4.0 Hz, 1H; H-1^{IV}), 4.80 (q, $J_{5,6}$ = 6.7 Hz, 1H; H-5^{IV}), 4.73 (d, $J_{1,2}$ = 7.9 Hz, 1H; H-1^{III}), 4.54 (d, $J_{1,2}$ = 8.2 Hz, 1H; H-1^{I}), 4.51 (d, $J_{1,2}$ = 7.8 Hz, 1H; H-1^{II}), 4.32 (t, J = 9.1 Hz, 1H; H-3^{III}), 4.14 (d, $J_{4,3}$ = 2.9 Hz, 1H; H-4^{II}), 2.02 (s, 3H; CH_3CON), 1.17 ppm (d, 3H; H-6^{IV});); biotin moiety: δ = 4.61 (dd, $J_{6a,6}$ = 5.0 Hz, $J_{6a,3a}$ = 7.9 Hz, 1H; H-6a), 4.42 (dd, $J_{3a,4}$ = 4.6 Hz, 1H; H-3a), 3.34 (m, 1H, H-4), 3.00 (dd, $J_{6,6'}$ = 13.1 Hz, 1H; H-6), 2.78 ppm (d, 1H; H-6′); HRMS (ESI) *m/z* calcd for $C_{53}H_{91}N_5O_{33}S_2 - H^+$: 1388.4965 $[M–H]^–$; found: 1388.4919.

Compound 70. ^{1}H NMR (600 MHz, D_2O, selected data); oligosaccharide moiety: δ = 4.80 (d, $J_{1,2}$ = 7.9 Hz, 1H; H-1^{V}), 4.73 (d, $J_{1,2}$ = 8.0 Hz, 1H; H-1^{III}), 4.55 (d, $J_{1,2}$ = 7.9 Hz, 1H; H-1^{IV}), 4.51 (d, $J_{1,2}$ = 7.9 Hz, 1H; H-1^{I}), 4.45 (d, $J_{1,2}$ = 7.6 Hz. 1H; H-1^{II}), 4.35 (t, J = 9.2 Hz, 1H; H-3^{V}), 4.19 (d, $J_{4,3}$ = 3.1 Hz, 1H; H-4^{IV}), 4.17 19 (d, $J_{4,3}$ = 3.1 Hz, 1H; H-4^{II}), 2.05 ppm (s, 3H; CH_3CON); biotin moiety: δ = 4.63 (dd, $J_{6a,6}$ = 5.0 Hz, $J_{6a,3a}$ = 8.0 Hz, 1H; H-6a), 4.45 (dd, $J_{3a,4}$ = 4.7 Hz, 1H; H-3a), 3.36 (m, 1H, H-4), 3.02 (dd, $J_{6,6'}$ = 13.0 Hz, 1H; H-6), 2.80 ppm (d, 1H; H-6′); HRMS (ESI) *m/z* calcd for $C_{59}H_{101}N_5O_{39}S_2 - 2H^+$: 782.7685 $[M–2H]^{2–}$; found: 782.7685.

Compound 71. ^{1}H NMR (600 MHz, D_2O, selected data); oligosaccharide moiety: δ = 4.73 (d, $J_{1,2}$ = 8.1 Hz, 1H; H-1^{III}), 4.51 (d, $J_{1,2}$ = 7.8 Hz, 1H; H-1^{I}), 4.50 (d, $J_{1,2}$ = 7.8 Hz, 1H; H-1^{IV}), 4.45 (d, $J_{1,2}$ = 7.5 Hz, 1H; H-1^{II}), 4.17 (d, $J_{4,3}$ = 3.0 Hz, 1H; H-4^{II}), 3.95 (d, $J_{4,3}$ = 3.2 Hz, 1H; H-4^{IV}); 2.05 ppm (s, 3H; CH_3CON); biotin moiety: δ = 4.63 (dd, $J_{6a,6}$ = 4.9 Hz, $J_{6a,3a}$ = 7.8 Hz, 1H; H-6a), 4.44 (dd, $J_{3a,4}$

= 4.8 Hz, 1H; H-3a), 3.35 (m, 1H, H-4), 3.02 (dd, $J_{6,6'}$ = 13.1 Hz, 1H; H-6), 2.81 ppm (d, 1H; H-6′); HRMS (ESI) *m/z* calcd for $C_{53}H_{93}N_5O_{30}S + Na^+$: 1334.5524 $[M+Na]^+$; found: 1334.5518.

Compound 72. ^{1}H NMR (600 MHz, D_2O, selected data); oligosaccharide moiety: δ = 4.69 (d, $J_{1,2}$ = 7.8 Hz, 1H; H-1^{III}), 4.56 (d, $J_{1,2}$ = 8.3 Hz, 1H; H-1^{I}), 4.54 (d, $J_{1,2}$ = 7.9 Hz, 1H; H-1^{III}), 4.21 (d, $J_{4,3}$ = 3.0 Hz, 1H; H-4^{II}), 2.05 ppm (s, 3H; CH_3CON); biotin moiety: δ = 4.63 (dd, $J_{6a,6}$ = 5.0 Hz, $J_{6a,3a}$ = 7.9 Hz, 1H; H-6a), 4.45 (dd, $J_{3a,4}$ = 4.5 Hz, 1H; H-3a), 3.36 (m, 1H, H-4), 3.02 (dd, $J_{6,6'}$ = 13.0 Hz, 1H; H-6), 2.80 ppm (d, 1H; H-6′); HRMS (ESI) *m/z* calcd for $C_{47}H_{81}N_5O_{26}S - H^+$: 1162.4818 $[M–H]^–$; found: 1162.4825.

Compound 73. ^{1}H NMR (600 MHz, D_2O, selected data); saccharide moiety: δ = 4.57 (d, $J_{1,2}$ = 8.0 Hz, 1H; H-1), 4.34 (t, J = 9.2 Hz, 1H; H-3); biotin moiety: δ = 4.63 (dd, $J_{6a,6}$ = 5.0 Hz, $J_{6a,3a}$ = 7.8 Hz, 1H; H-6a), 4.45 (dd, $J_{3a,4}$ = 4.5 Hz, 1H; H-3a), 3.35 (m, 1H, H-4), 3.02 (dd, $J_{6,6'}$ = 13.1 Hz, 1H; H-6), 2.80 ppm (d, 1H; H-6′); HRMS (ESI) *m/z* calcd for $C_{33}H_{58}N_4O_{19}S_2 - H^+$: 877.3064 $[M–H]^–$; found: 877.3063.

Compound 74. ^{1}H NMR (600 MHz, D_2O, selected data); oligosaccharide moiety: δ = 4.76 (d, $J_{1,2}$ = 7.9 Hz, 1H; H-1^{III}), 4.53 (d, $J_{1,2}$ = 7.9 Hz, 1H; H-1^{I}), 4.52 (d, $J_{1,2}$ = 7.8 Hz, 1H; H-1^{II}), 4.33 (m, 2H; H-3^{III},6a^{III}), 4.23 (d, $J_{4,3}$ = 3.0 Hz, 1H; H-4^{II}), 4.20 (dd, $J_{6b,5}$ = 5.8 Hz, $J_{6b,6a}$ = 11.2 Hz, 1H; H-6b^{III}), 2.03 ppm (s, 3H; CH_3CON); biotin moiety: δ = 4.61 (dd, $J_{6a,6}$ = 4.9 Hz, $J_{6a,3a}$ = 7.8 Hz, 1H; H-6a), 4.42 (dd, $J_{3a,4}$ = 4.4 Hz, 1H; H-3a), 3.34 (m, 1H, H-4), 2.99 (dd, $J_{6,6'}$ = 13.1 Hz, 1H; H-6), 2.78 ppm (d, 1H; H-6′); HRMS (ESI) *m/z* calcd for $C_{47}H_{83}N_5O_{31}S_3 - 2H^+$: 653.7044 $[M–2H]^{2–}$; found: 653.7050.

2. The study of the oligosaccharide – antibody binding by SPR

Sensorgrams of interactions of oligosaccharides with HNK-1 related antibodies. Smooth curves represent fitted data (two states kinetic model)

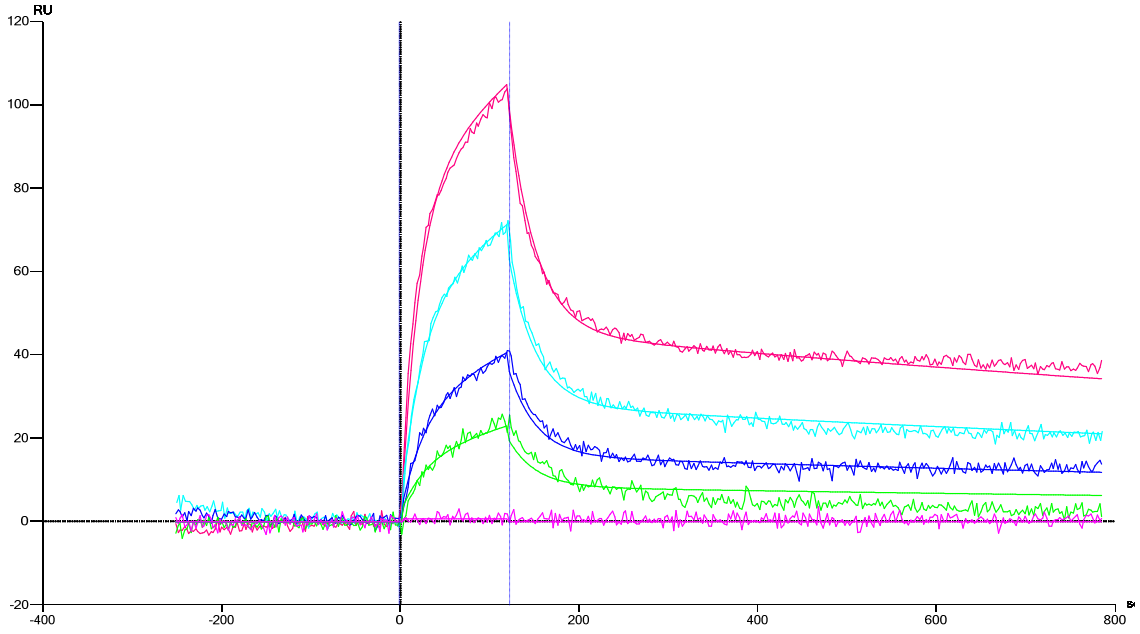

Figure 1. Interaction of trisaccharide **67** loaded at a concentration of 2 nM with the HNK-1 412 antibody (10, 5, 2.5, and 1.25 nM).

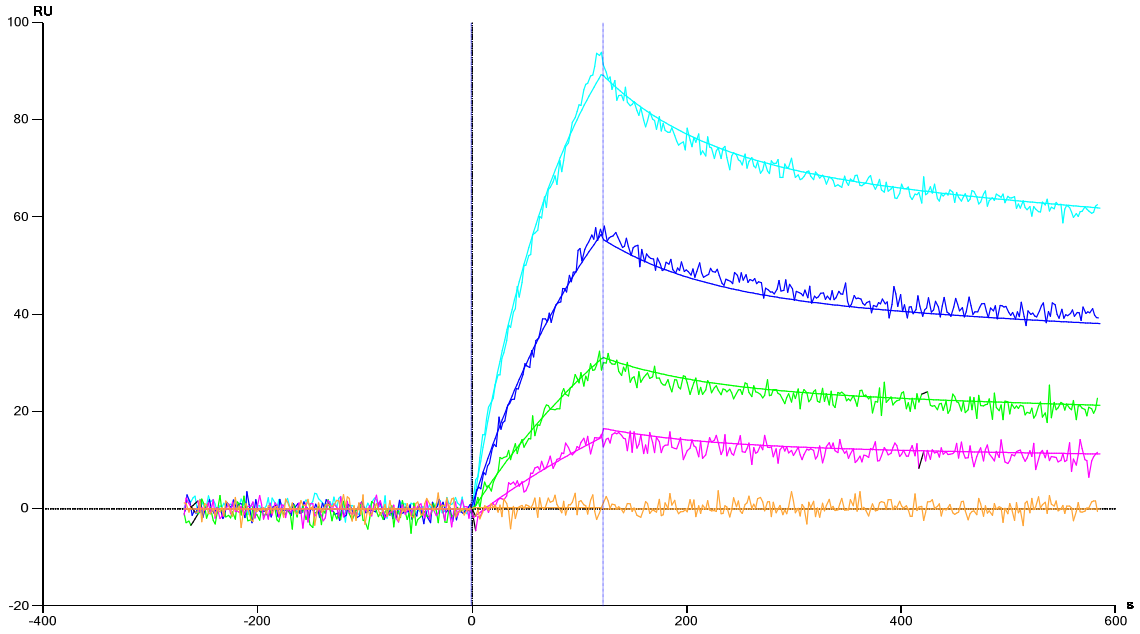

Figure 2. Interaction of trisaccharide **67** loaded at a concentration of 10 nM with the HNK-1 antibody (0.5, 0.25, 0.125, and 0.0625 nM).

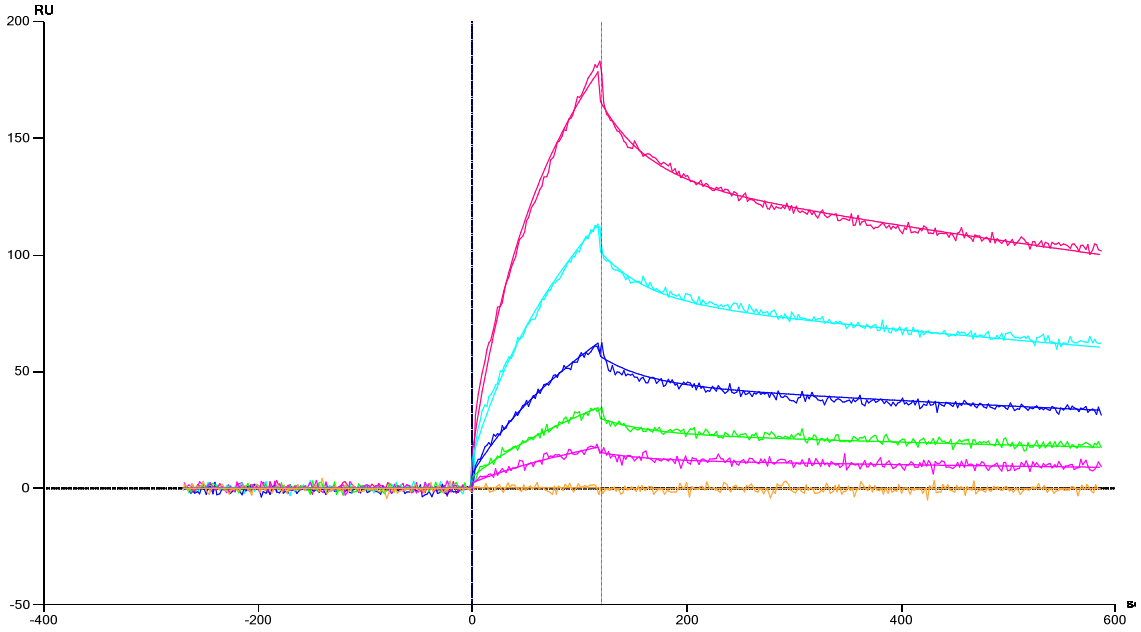

Figure 3. Interaction of disaccharide **68** loaded at a concentration of 10 nM with the HNK-1 412 antibody (100, 50, 25, 12.5, and 6.25 nM).

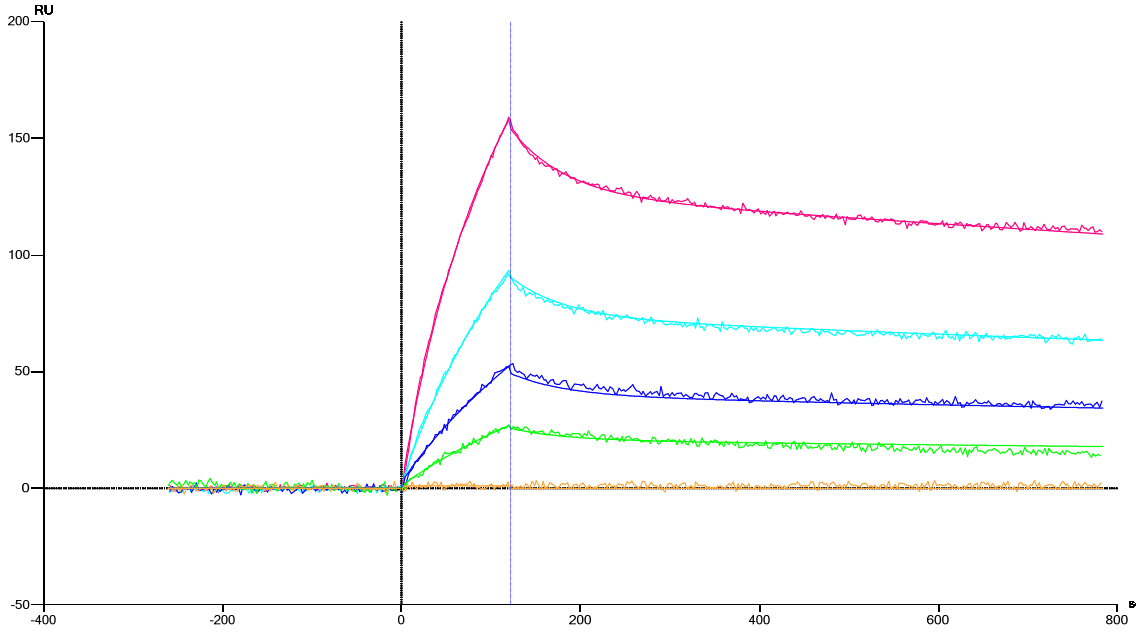

Figure 4. Interaction of disaccharide **68** loaded at a concentration of 2 nM with the HNK-1 antibody (1, 0.5, 0.25, and 0.125 nM).

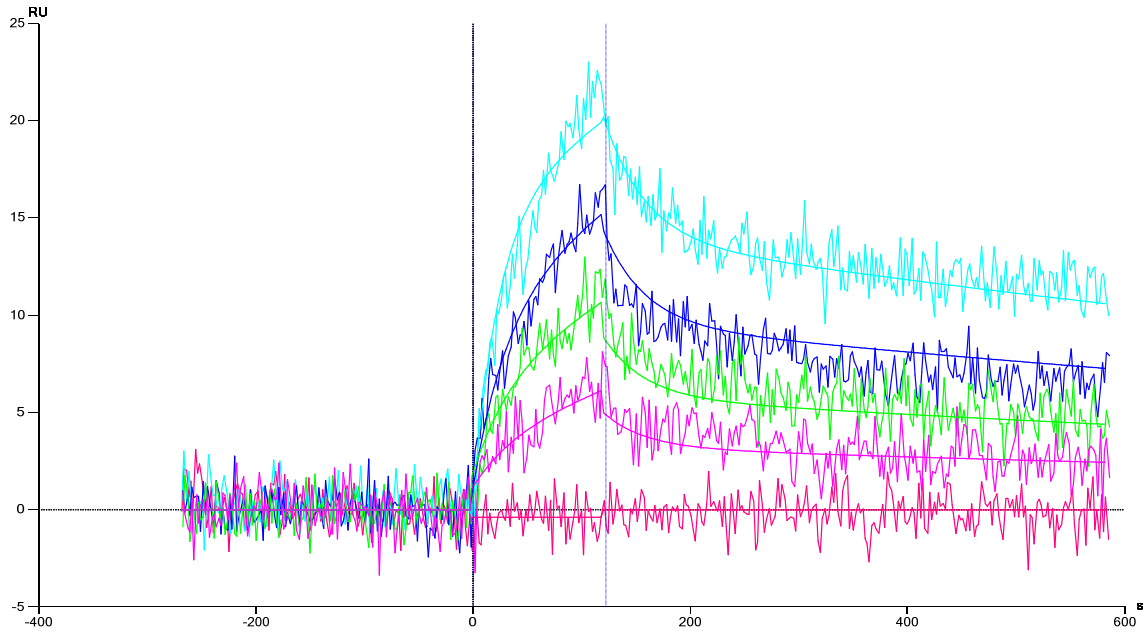

Figure 5. Interaction of tetrasaccharide **69** loaded at a concentration of 10 nM with the HNK-1 412 antibody (100, 50, 25, and 12.5 nM).

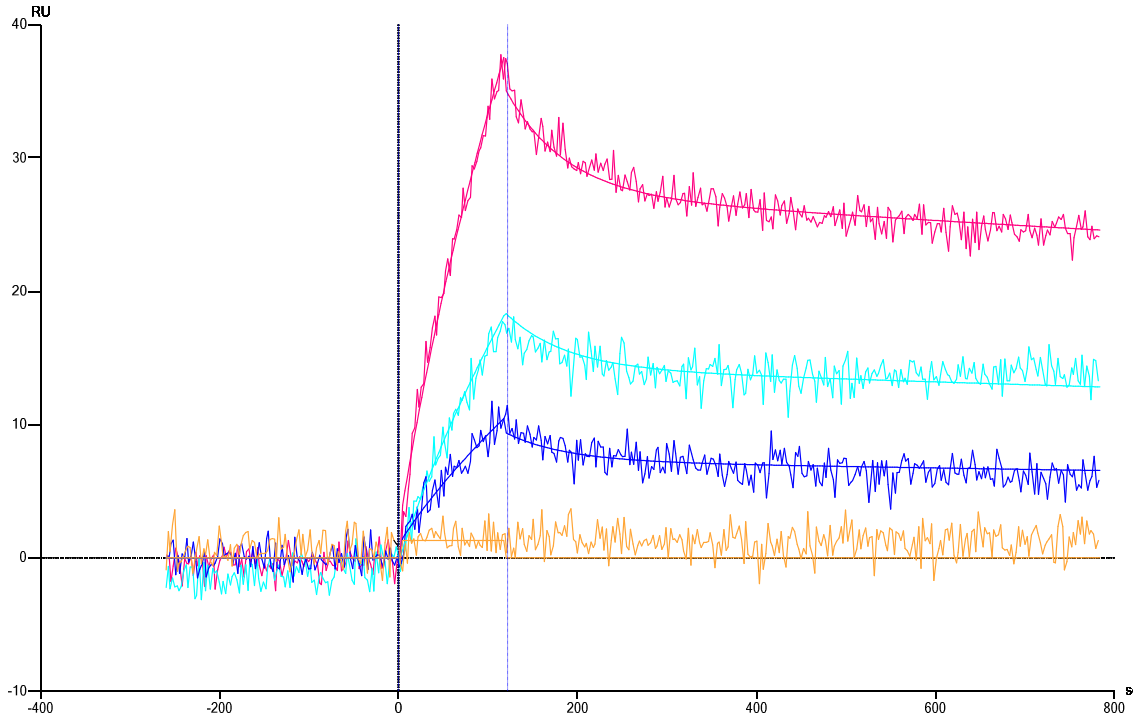

Figure 6. Interaction of tetrasaccharide **69** loaded at a concentration of 2 nM with the HNK-1 antibody (1, 0.5, and 0.25 nM).

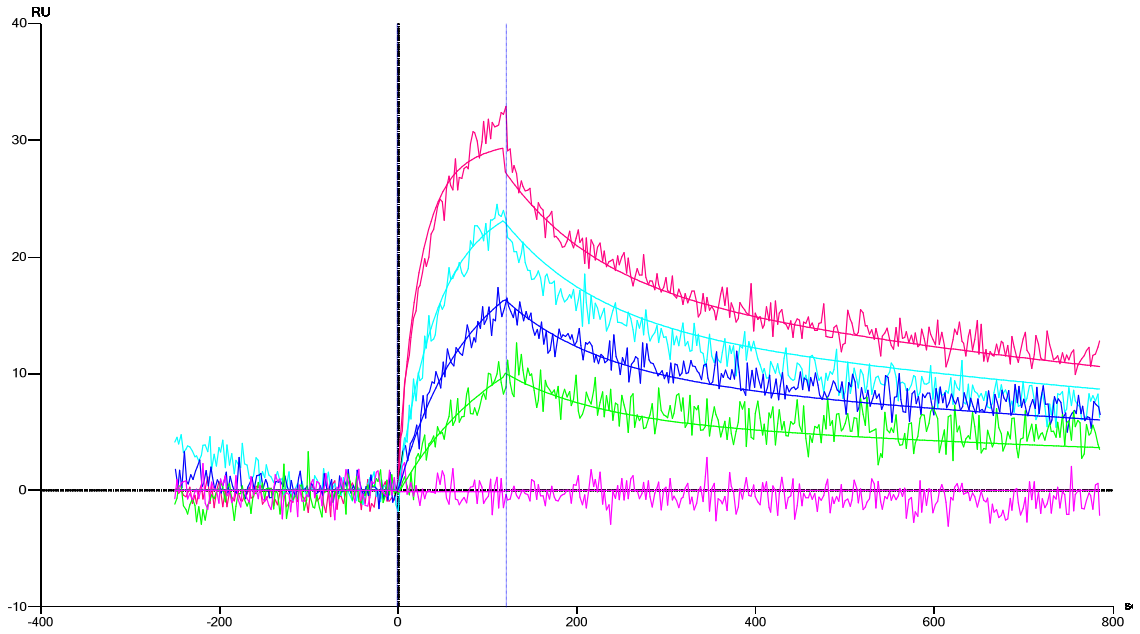

Figure 7. Interaction of pentasaccharide **70** loaded at a concentration of 0.5 nM with HNK-1 412 antibody (10, 5, 2.5, and 1.25 nM).

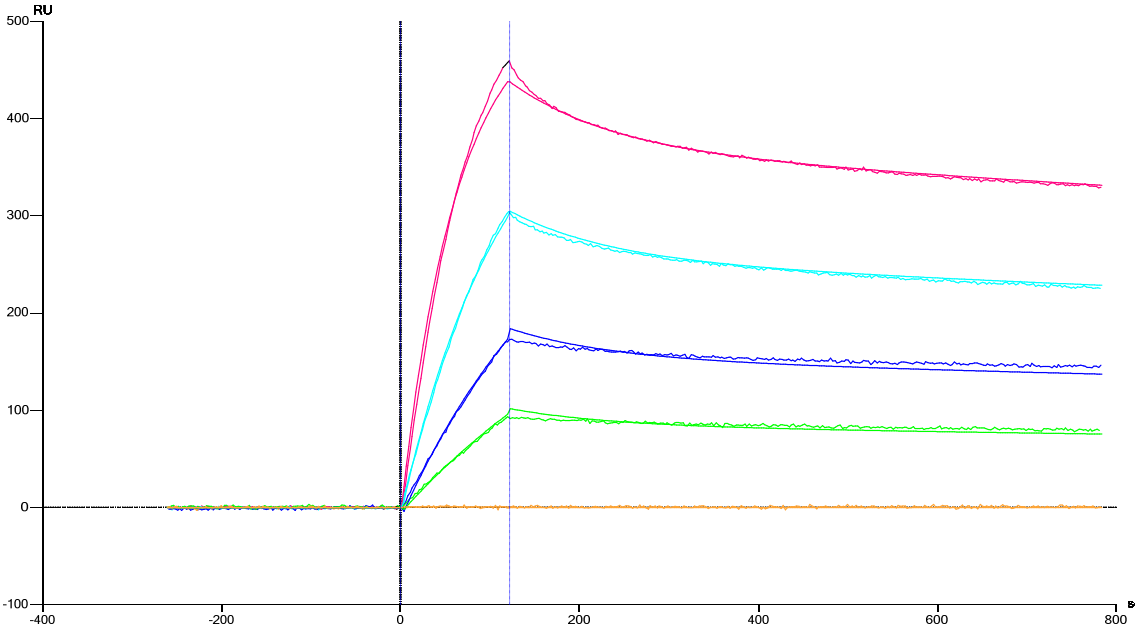

Figure 8. Interaction of pentasaccharide **70** loaded at a concentration of 2 nM with HNK-1 antibody (1, 0.5, 0.25, and 0.125 nM).

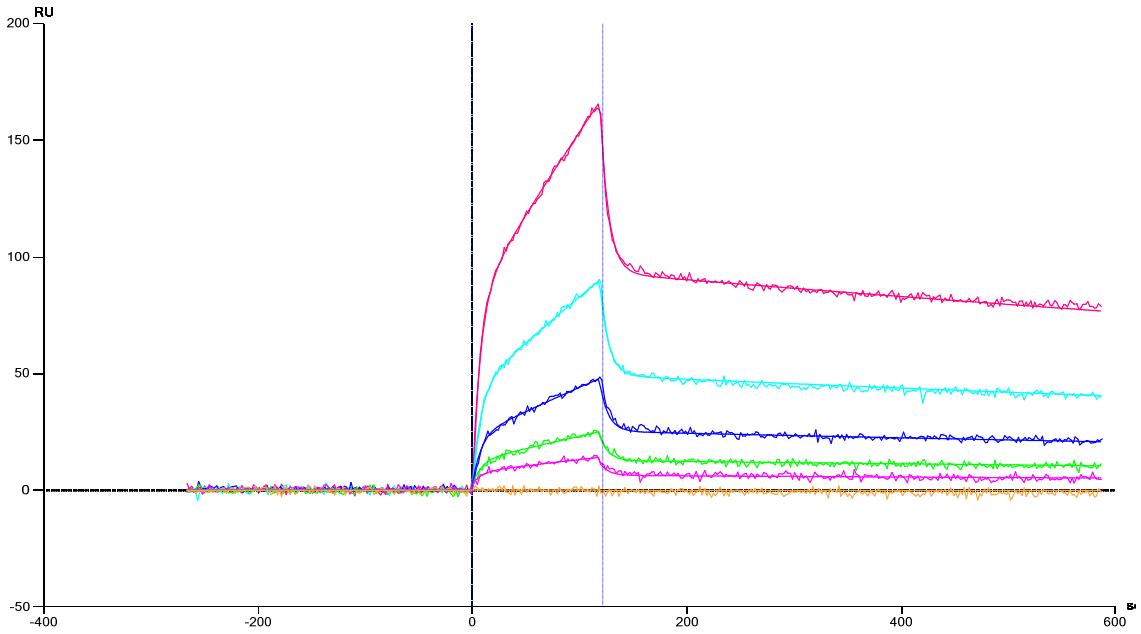

Figure 9. Interaction of trisaccharide **72** loaded at a concentration of 100 nM with HNK-1 412 antibody (20, 10, 5, 2.5, and 1.25 nM).

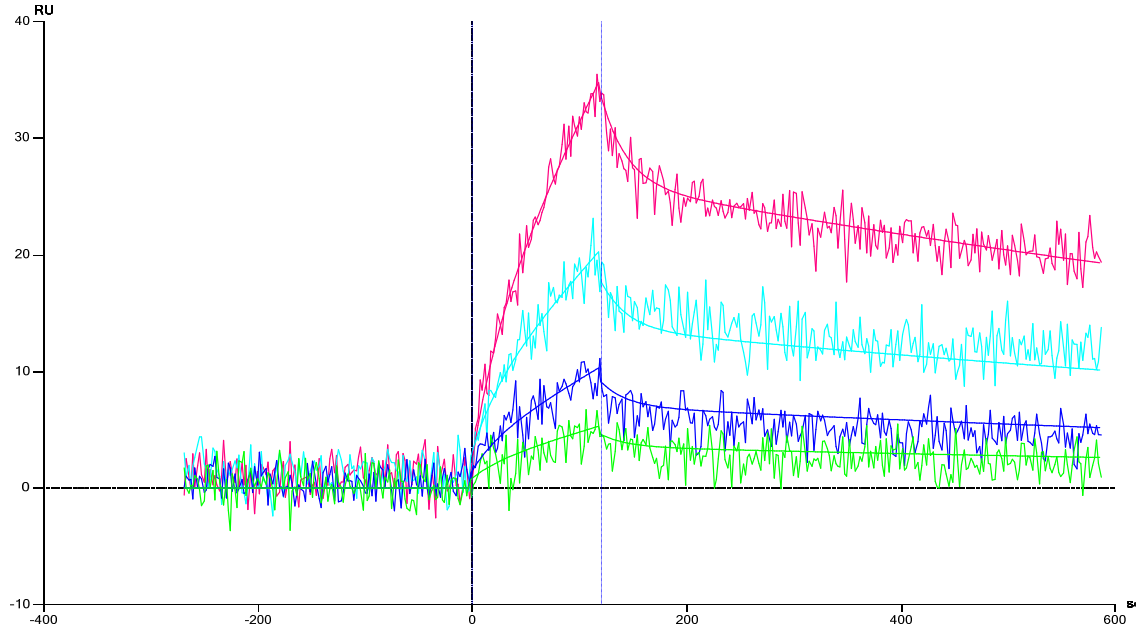

Figure 10. Interaction of monosaccharide **73** loaded at a concentration of 100 nM with HNK-1 412 antibody (100, 50, 25, and 12.5 nM).

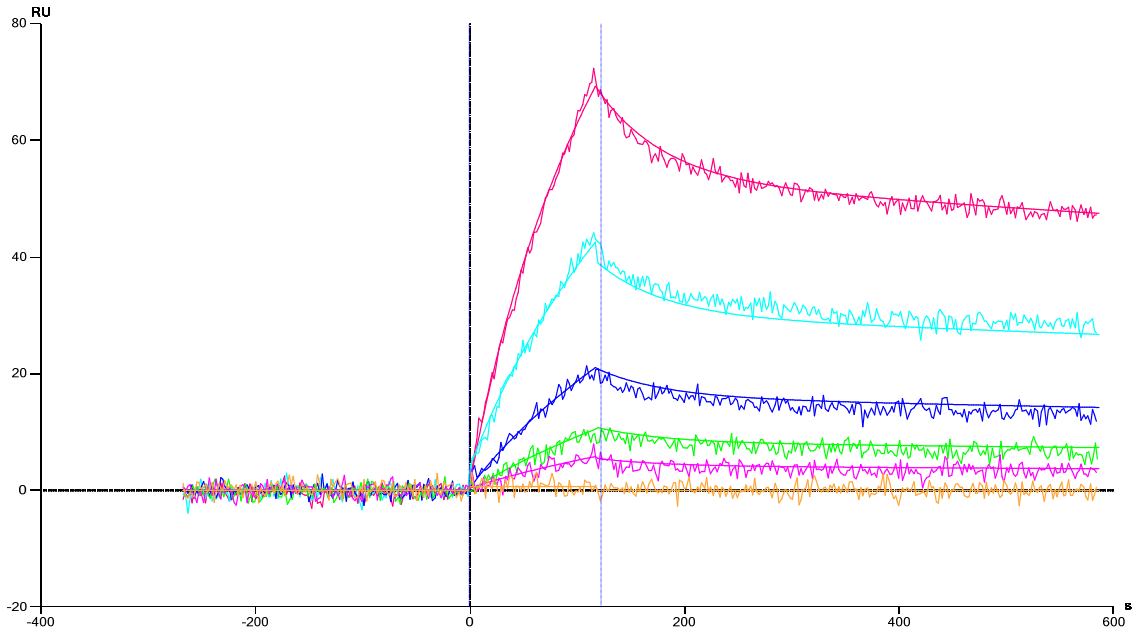

Figure 11. Interaction of monosaccharide **73** loaded at a concentration of 100 nM with HNK-1 antibody (1, 0.5, 0.25, and 0.125, and 0.0625 nM).

Table 1. Kinetic data of interactions of saccharides **66**-**70**, **72**, **73** with the HNK-1 412 antibody

Oligosaccharide	$k_a(1)\times10^5$ $M^{-1}s^{-1}$	$k_d(1)\times10^{-3}$ s^{-1}	$K_D(1)\times10^{-8}$ M	$k_a(2)\times10^{-3}$ $M^{-1}s^{-1}$	$k_d(2)\times 10^{-3}$ s^{-1}	$K_D(2)$ M
66	24.1±0.1	8.95±0.08	0.371	3.04±0.03	1.12±0.02	0.37
67	19.2±0.3	30.0±0.4	1.45	4.85±0.04	0.50±0.01	0.10
68	0.971±0.09	10.0±0.4	10.9	10.0±0.2	1.36±0.02	0.14
69	2.47±0.09	20.0±1.5	7.25	8.68±0.39	0.90±0.07	0.10
70	36.1±0.5	5.53±0.30	0.153	3.79±0.27	1.39±0.10	0.37
72	5.35±0.10	120.0±2.2	22.2	8.86±0.05	0.45±0.05	0.05
73	0.288±0.03	30.0±3.2	91.5	20.0±0.7	1.03±0.05	0.05

Table 2. Kinetic data of interactions of saccharides **66**-**70**, **73** with the HNK-1 antibody

Oligosaccharide	$k_a(1)\times10^6$ $M^{-1}s^{-1}$	$k_d(1)\times10^{-3}$ s^{-1}	$K_D(1)\times10^{-10}$ M	$k_a(2)\times10^{-3}$ $M^{-1}s^{-1}$	$k_d(2)\times 10^{-3}$ s^{-1}	$K_D(2)$ M
66	36.6±0.2	1.79±0.09	0.490	5.38±0.31	0.83±0.06	0.15
67	18.0±0.2	3.68±0.23	2.04	6.06±0.45	0.74±0.12	0.12
68	6.76±0.04	6.02±0.19	8.92	10.0±0.2	0.59±0.01	0.06
69	1.73±0.12	5.93±0.52	34.2	9.00±0.51	0.40±0.04	0.04
70	14.80±0.04	2.38±0.08	1.61	6.01±0.19	0.60±0.03	0.10
73	5.32±0.09	6.83±0.39	12.8	9.04±0.38	0.58±0.05	0.06

3. Complete references

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