Supporting Information

DNA-programmed spatial screening of carbohydrate-lectin interactions

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Table of contents

Materials and general procedures

All commercially available chemicals were used as received unless otherwise indicated. 3,4,6-Tri-*O*-acetyl-1-thioethyl-*N*-(2,2,2-trichloroethyloxycarbonyl)-β-glucosaminide,[1] (1-thyminyl)-acetic acid,[2] UDP-galactose,[3] and *N*-succinimidyl-3-maleimidopropionate[4, 5] were synthesized following reported procedures. Bovine serum albumin was purchased from Sigma-Aldrich. Calf intestine alkaline phosphatase (EC 3.1.3.1) was purchased from AppliChem GmbH, Germany. β-1,4-Galactosyltransferase from bovine milk (EC 2.4.1.22) was purchased from Calbiochem. PNA monomers were purchased from ASM Research Chemicals, Germany or Panagene, Korea. HCTU, Novasyn® TGR resin and protected amino acids were purchased from Novabiochem. DNA was purchased from BioTeZ Berlin-Buch GmbH, Germany. Sep-Pak® C18 cartridges and Chameleon® C 1-3 mm were purchased from VWR International GmbH, Germany. Water was purified with a Milli-Q Ultra Pure Water Purification System, Membrapure, Germany.

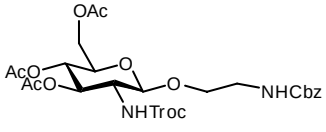
Silica gel 60 (particle size: 0.035 – 0.070 mm) from Acros was used for flash column chromatography. Silica gel 60 F_{254} aluminium sheets from Merck were used for thin layer chromatography. Melting points were determined with a Nagema K8 instrument and are not corrected. Optical rotations were determined with a Perkin Elmer-241 polarimeter with a sodium lamp at wavelength λ = 589 nm.

Analytical HPLC was performed on a Merck-Hitachi Elite LaChrom instrument (column: Varian Polaris C18-A, 250 x 4.6 mm, 5 µm, pore size: 180 Å) using eluents A (98.9% water, 1% acetonitrile, 0.1% TFA) and B (98.9% acetonitrile, 1% water, 0.1 % TFA) in a linear gradient (3→30 % B in 20 min) at 55 °C with a flow rate of 1 mL min^{-1}. Detection of signals was achieved with a photodiode array detector at wavelength λ = 260 nm. Semi-preparative HPLC was performed on an Agilent 1100 series instrument (column: Varian Polaris C18-A, 250 x 10.0 mm, 5 µm, pore size: 180 Å) using eluents A (98.9% water, 1% acetonitrile, 0.1% TFA) and B (98.9% acetonitrile, 1% water, 0.1% TFA) in a linear gradient with a flow rate of 6 mL min^{-1}. Detection of signals was achieved with a UV-detector at wavelength λ = 260 nm.

MALDI-TOF mass spectra were recorded on a Voyager-DE Pro Biospectrometry Workstation from PerSeptive Biosystems (matrix: sinapinic acid). HR-ESI mass spectra were recorded on a Hewlett-Packard GCMS 5995-A instrument. NMR-spectra were recorded on a Bruker DPX 300 or Bruker 400 instrument at 25 °C. Chemical shifts are given in ppm (parts per million). ^{1}H-NMR spectra were referenced to the signal of the residual protonated solvent: $CDCl_3$ 7.26 ppm, D_2O 4.79 ppm, CD_3OD 3.31 ppm. ^{13}C-NMR spectra are proton decoupled and were referenced to the signal of the deuterated solvent: $CDCl_3$ 77.00 ppm, CD_3OD 49.05 ppm. The following abbreviations were used to indicate the multiplicity: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, b = broad.

Synthesis of *N*-acetyllactosamine 14

3,4,6-Tri-*O*-acetyl-1-(*N*-(benzyloxycarbonyl)-2-aminoethyl)-*N*-(2,2,2-trichloroethyloxycarbonyl)-β-glucosaminide 11

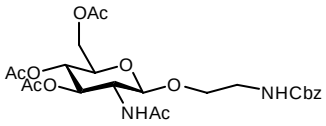


A suspension of 3,4,6-tri-*O*-acetyl-1-thioethyl-*N*-(2,2,2-trichloroethyl-oxycarbonyl)-β-glucosaminide **10**[1] (100.0 mg, 190.5 µmol), benzyl 2-hydroxyethylcarbamate (30.0 mg, 153.7 µmol), and molecular sieves 4 Å in dry dichloromethane (4 mL) was stirred under an atmosphere of

argon for 2 hours. Dimethyl(thiomethyl)sulfonium trifluoromethanesulfonate (132.0 mg, 511.0 µmol) was added and stirring was continued for 65 hours. The suspension was filtered through Celite® 545, the solvent was removed under reduced pressure, and the residue was purified by column chromatography on silica gel (ethyl acetate/cyclohexane 1:3→1:1, v/v) to give a colorless solid (50.4 mg, 50 %).

R_f 0.42 (ethyl acetate/cyclohexane 2:1, v/v); m.p. 102 – 104 °C; ^{1}H-NMR (300 MHz, $CDCl_3$) δ = 2.02 (s, 3 H, CH_3), 2.03 (s, 3 H, CH_3), 2.06 (s, 3 H, CH_3), 3.25 – 3.40 (m, 1 H, CH_{2a}-NHCbz), 3.40 – 3.54 (m, 1 H, CH_{2b}-NHCbz), 3.57 – 3.75 (m, 3 H, 2-C*H*, 5-C*H*, CH_{2a}-CH_2-NHCbz), 3.88 (ddd, ^2J = 9.8 Hz, ^3J = 6.0 Hz, ^3J = 3.5 Hz, 1 H, CH_{2b}-CH_2-NHCbz), 4.07 – 4.18 (m, 1 H, 6-CH_{2a}), 4.24 (dd, ^2J = 12.3 Hz, ^3J = 4.9 Hz, 1 H, 6-CH_{2b}), 4.52 (d, ^2J = 12.5 Hz, 1H, CH_{2a}-CCl_3), 4.58 (d, ^3J = 8.3 Hz, 1H, 1-C*H*), 4.76 (d, ^2J = 11.7 Hz, 1 H, CH_{2b}-CCl_3), 4.99 – 5.16 (m, 3 H, 4-C*H*, CH_2-Ph), 5.16 – 5.29 (m, 3 H, 3-C*H*, N*H*-Cbz, N*H*-Troc), 7.33 (m, 5 H, Ph-H_5); ^{13}C-NMR (75 MHz, $CDCl_3$) δ = 20.6, 20.7 (3x CH_3), 40.7 (CH_2-NHCbz), 56.2 (2-*C*H), 61.9 (CH_2-CH_2-NHCbz), 66.8 (CH_2-Ph), 68.4 (4-*C*H), 69.2 (6-CH_2), 71.8 (3-*C*H, 5-*C*H), 74.4 (CH_2-CCl_3), 95.4 (CCl_3), 101.0 (1-*C*H), 128.1, 128.2, 128.5 (5x Ph-*C*H), 136.4 (Ph-C_q), 154.2 ((C_q=O)-O-Bn), 156.5 ((C_q=O)-O-CH_2-CCl_3), 169.4, 170.6, 170.7 (3x (C_q=O)-CH_3); *m/z* (ESI) 462 [M – $(CH_2)_2$-NHCbz]$^{+}$, 657 [M + H]$^+$, 679 [M + Na]$^+$ ($C_{25}H_{31}Cl_3N_2O_{12}$ requires 656.0943).

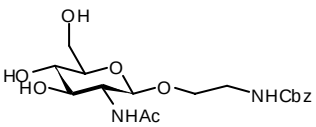
3,4,6-Tri-*O*-acetyl-1-(*N*-(benzyloxycarbonyl)-2-aminoethyl)-β-*N*-acetylglucosaminide



A suspension of 3,4,6-tri-*O*-acetyl-1-(*N*-(benzyloxycarbonyl)-2-aminoethyl)-*N*-(2,2,2-trichloroethyloxycarbonyl)-β-glucosaminide **11** (50.0 mg, 76.0 µmol) and zinc (1.30 g, 19.88 mmol) in acetic acid (2.0 mL, 34.94 mmol) was stirred for 20 hours. The suspension was filtered through Celite® 545 and the solvent was removed under reduced pressure. To the obtained residue were added under an atmosphere of argon pyridine (4.0 mL, 49.46 mmol) and acetic anhydride (2.0 mL, 21.16 mmol). After stirring for 2.5 hours the solvent was removed under reduced pressure and the residue was dissolved in dichloromethane (5 mL). The solution was washed with 1 M hydrochloric acid (3 mL), saturated sodium hydrogencarbonate solution (4 mL), and saturated sodium chloride solution (5 mL), with reextraction of the aqueous phase with dichloromethane (5 mL each). The combined organic phases were dried over magnesium sulfate, the solvent was removed under reduced pressure, and the residue was purified by column chromatography on silica gel (methanol/ chloroform 1:9, v/v) to give a colorless powder (31.0 mg, 78 %).

R_f 0.49 (methanol/chloroform 1:9, v/v); m.p. 169 – 170 °C; $[\alpha]_D^{24}$ = -9.0 ° mL dm^{-1} g^{-1}(*c* = 1.00 g mL^{-1} in acetone); ^{1}H-NMR (300 MHz, $CDCl_3$) δ = 1.88 (s, 3 H, CH_3^{Ac}), 2.01 (s, 3 H, CH_3^{Ac}), 2.02 (s, 3 H, CH_3^{Ac}), 2.05 (s, 3 H, CH_3^{Ac}), 3.15 – 3.50 (m, 2 H, CH_2-NHCbz), 3.60 – 3.73 (m, 2 H, 5-C*H*, CH_{2a}-CH_2-NHCbz), 3.80 – 3.95 (m, 2 H, 2-C*H*, CH_{2b}-CH_2-NHCbz), 4.12 (dd, 1 H, ^2J = 12.2 Hz, ^3J = 2.3 Hz, 6-CH_{2a}), 4.22 (dd, 1 H, ^2J = 12.3 Hz, ^3J = 4.8 Hz, 6-CH_{2b}), 4.59 (d, 1 H, ^3J = 8.3 Hz, 1-C*H*), 4.95 – 5.12 (m, 3 H, 4-C*H*, CH_2-Ph), 5.13 – 5.23 (m, 1 H, 3-C*H*), 5.26 – 5.34 (m, 1 H, N*H*-Cbz), 5.63 – 5.72 (m, 1 H, N*H*-Ac), 7.28 – 7.38 (m, 5 H, Ph-H_5); ^{13}C-NMR (75 MHz, $CDCl_3$) δ = 20.6, 20.7, 20.7, 23.2 (4x CH_3^{Ac}), 40.7 (CH_2-NHCbz), 54.5 (2-*C*H), 62.0 (CH_2-CH_2-NHCbz), 66.7 (CH_2-Ph), 68.3, 69.0, 71.9, 72.3 (3-*C*H, 4-*C*H, 5-*C*H, 6-CH_2), 101.1 (1-*C*H), 128.1, 128.5 (5x Ph-*C*H), 136.5 (Ph-C_q), 162.7 ((C_q=O)-OBn), 169.3, 170.7 (4x C_q^{Ac}); *m/z* (HR-ESI) 525.2076 [M + H]$^+$ ($C_{24}H_{33}N_2O_{11}$ requires 525.2079).

(*N*-(Benzyloxycarbonyl)-2-aminoethyl)-β-*N*-acetylglucosaminide 12

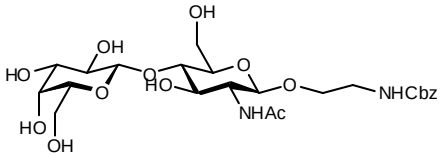


A small amount of sodium methoxide was added to a solution of 3,4,6-tri-*O*-acetyl-1-(*N*-(benzyloxycarbonyl)-2-aminoethyl)- β-*N*-acetylglucos-

aminide (21.1 mg, 40.2 µmol) in dry methanol (2 mL) under an atmosphere of argon. After stirring for 3 hours the solution was neutralized with acetic acid/methanol (1:20, v/v) and the solvent removed under reduced pressure to give a colorless solid (16.0 mg, quantitative).

m.p. 187 – 188 °C; $[\alpha]_D^{24}$ = -22.2 ° mL dm^{-1} g^{-1}(*c* = 1.01 g mL^{-1} in ethanol); ^{1}H-NMR (300 MHz, D_2O) δ = 1.97 (s, 3 H, C*H*$_3$), 3.25 – 3.35 (m, 2 H, 3-C*H*, 5-C*H*), 3.38 – 3.46 (m, 2 H, C*H*$_2$-NHCbz), 3.48 – 3.57 (m, 1 H, C*H*$_{2a}$-CH$_2$-NHCbz), 3.59 – 3.80 (m, 3 H, 2-C*H*, 6-C*H*$_{2a}$, C*H*$_{2b}$-CH$_2$-NHCbz), 3.84 – 3.94 (m, 2 H, 4-C*H*, 6-C*H*$_{2b}$), 4.49 (d, 1 H, ^3J = 8.3 Hz, 1-C*H*), 5.11 (s, 2 H, C*H*$_2$-Ph), 7.34 – 7.50 (m, 5 H, Ph-*H*$_5$); ^{13}C-NMR (75 MHz, D_2O) δ = 22.1 (*C*H$_3$), 40.3 (*C*H$_2$-NHCbz), 55.5 (2-*C*H), 60.7 (*C*H$_2$-CH$_2$-NHCbz), 66.9 (*C*H$_2$-Ph), 68.6 (6-*C*H$_2$), 69.8 (4-*C*H), 73.7 (3-*C*H), 75.8 (5-*C*H), 101.2 (1-*C*H), 127.8, 128.4, 128.8 (5x Ph-*C*H), 136.4 (Ph-*C*$_q$), 158.3 ((*C*$_q$=O)-OBn), 174.6 (*C*$_q^{Ac}$); *m/z* (HR-ESI) 399.1759 $[M + H]^+$, ($C_{18}H_{27}N_2O_8$ requires 399.1762).

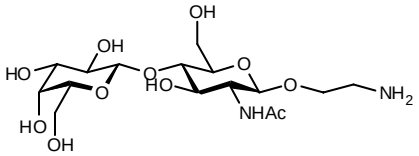
(*N*-(Benzyloxycarbonyl)-2-aminoethyl)-β-*N*-acetyllactosaminide 13



(*N*-(Benzyloxycarbonyl)-2-aminoethyl)-β-*N*-acetylglucosaminide **12** (8.3 mg, 20.8 µmol) and UDP-galactose[3] (48.5 mg, 83.2 µmol) were added to a solution of manganese(II) chloride tetrahydrate (3.5 mg, 17.7 µmol) and bovine serum albumin (0.92 mg) in 0.1 M HEPES pH 7.0 (854 µL). A solution of calf intestine alkaline phosphatase (17.0 U, EC 3.1.3.1) in 0.1 M HEPES pH 7.0 (70.6 µL) was added. After incubation at 37 °C for 20 minutes, a solution of β-1,4-galactosyltransferase from bovine milk (0.83 U, EC 2.4.1.22) in 0.1 M HEPES pH 7.0 (100 µL) was added. The solution was incubated at 37 °C for 2 days. The reaction mixture was loaded on a Sep-Pak® C18 cartridge (equilibrated with water). After elution of the by-products with water, the product was eluted with methanol. The solvent was removed under reduced pressure and the residue was dissolved in water. Lyophilization afforded a colorless foam (10.8 mg, 93 %).

R_f 0.73 (dichloromethane/methanol/water 6:4:1, v/v); ^{1}H-NMR (300 MHz, D_2O) δ = 1.95 (s, 3 H, C*H*$_3$), 3.25 – 3.33 (m, 2 H, C*H*$_2$-NHCbz), 3.51 (dd, ^3J = 9.9 Hz, ^3J = 7.7 Hz, 2 H, 2'-C*H*), 3.49 – 3.58 (m, 1 H, 5-C*H*), 3.60 – 3.81 (m, 9 H, 2-C*H*, 3-C*H*, 4-C*H*, 6-C*H*$_{2a}$, 3'-C*H*, 5'-C*H*, 6'-C*H*$_2$, C*H*$_{2a}$-CH$_2$-NHCbz), 3.82 – 3.98 (m, 3 H, 6-C*H*$_{2b}$, 4'-C*H*, C*H*$_{2b}$-CH$_2$-NHCbz), 4.43 (d, ^3J = 7.7 Hz, 1 H, 1'-C*H*), 4.49 (d, ^3J = 7.2 Hz, 1 H, 1-C*H*), 5.10 (s, 2 H, C*H*$_2$-Ph), 7.34 – 7.47 (m, 5 H, Ph-*H*$_5$); *m/z* (HR-ESI) 561.2295 $[M+H]^+$ ($C_{24}H_{37}N_2O_{13}$ requires 561.2290).

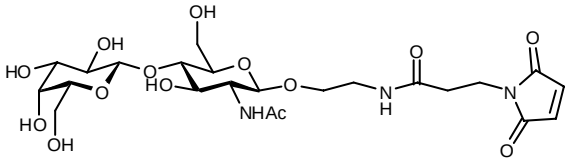
(2-Aminoethyl)-β-*N*-acetyllactosaminide



A suspension of (*N*-(benzyloxycarbonyl)-2-aminoethyl)-β-*N*-acetyllactosaminide **13** (7.29 mg, 13.00 µmol) and palladium on activated charcoal (8 mg, 10 % Pd) in dry, degassed methanol (1 mL) was stirred under an atmosphere of hydrogen for 3 hours. The suspension was filtrated and the solvent was removed under reduced pressure to leave a colorless film (5.41 mg, 98 %).

R_f 0.04 (dichloromethane/methanol/water 6:4:1, v/v); ^{1}H-NMR (300 MHz, D_2O) δ = 2.03 (s, 3 H, C*H*$_3$), 3.02 – 3.22 (m, 2 H, C*H*$_2$-NH$_2$), 3.52 (dd, ^3J = 10.0 Hz, ^3J = 7.6 Hz, 1 H, 2'-C*H*), 3.56 – 3.87 (m, 10 H, 2-C*H*, 3-C*H*, 4-C*H*, 5-C*H*, 6-C*H*$_{2a}$, 3'-C*H*, 5'-C*H*, 6'-C*H*$_2$, C*H*$_{2a}$-CH$_2$-NH$_2$), 3.91 (d, ^3J = 3.9 Hz, 1 H, 4'-CH), 3.94 – 4.05 (m, 2 H, 6-C*H*$_{2b}$, C*H*$_{2b}$-CH$_2$-NH$_2$), 4.46 (d, ^3J = 7.6 Hz, 1 H, 1'-C*H*), 4.55 (d, ^3J = 7.9 Hz, 1 H, 1-C*H*); *m/z* (HR-ESI) 427.1911 $[M+H]^+$ ($C_{16}H_{31}N_2O_{11}$ requires 427.1922).

(*N*-(3'-Maleimidopropanoyl)-2-aminoethyl)-β-*N*-acetyllactosaminide 14

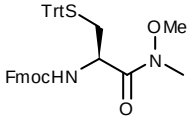


A solution of (2-aminoethyl)-β-*N*-acetyllactosaminide (20.00 µL, 2.000 µmol, 0.1 M in water) and *N*-succinimidyl-3-maleimidopropionate[4, 5] (20.00 µL, 2.000 µmol, 0.1 M in dioxane) in 0.1 M sodium hydrogencarbonate (20.00 µL) was shaken for 1 day. The solvent was removed under reduced pressure, the residue was dissolved in water and loaded on a Sep-Pak® C18 cartridge (equilibrated with water). After elution of the by-products with water, the product was eluted with methanol. The solvent was removed under reduced pressure and the residue was dissolved in water. Lyophilization afforded a colorless foam (0.81 mg, 56 %).

R_f 0.72 (dichloromethane/methanol/water 6:4:1, v/v); ^{1}H-NMR (400 MHz, D_2O) δ = 2.01 (s, 3 H, CH_3), 2.49 (t, ^3J = 6.5 Hz, 2 H, C_q-CH_2-CH$_2$-N), 3.30 (t, ^3J = 5.2 Hz, 2 H, O-CH$_2$-CH_2-NH), 3.48-3.88 (m, 14 H, 2-C*H*, 3-C*H*, 4-C*H*, 5-C*H*, 6-CH_{2a}, 2'-C*H*, 3'-C*H*, 5'-C*H*, 6'-CH_2, O-CH_2-CH$_2$-NH, C_q-CH$_2$-CH_2-N), 3.91 (d, ^3J = 3.2 Hz, 1 H, 4'-C*H*), 3.97 (d, *J* = 12.0 Hz, 1 H, 6-CH_{2b}), 4.45 (d, ^3J = 7.8 Hz, 1 H, 1'-C*H*), 4.51 (d, ^3J = 7.5 Hz, 1 H, 1-C*H*), 6.85 (s, 2 H, C*H*=C*H*); *m/z* (HR-ESI) 578.2177 $[M+H]^+$ ($C_{23}H_{36}N_3O_{14}$ requires 578.2192).

Synthesis of thiol-modified PNA-monomer 1

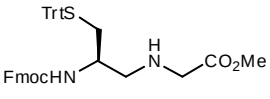
(*R*)-*N*-Fluorenylmethoxycarbonyl-*S*-tritylcysteine-weinrebamide



Under an atmosphere of argon a solution of (*R*)-*N*-fluorenylmethoxycarbonyl-*S*-tritylcysteine (15.00 g, 7.43 mmol) and *N*-methylmorpholine (6.2 mL, 56.34 mmol) in dry dichloromethane (400 mL) was cooled to -10 °C. Isobutylchloroformiat (3.7 mL, 28.17 mmol) was added within 5 minutes. After stirring for 15 minutes at -10 °C *N*,*O*-dimethylhydroxylamine hydrochloride (2.50 mg, 25.61 mmol) was added and stirring was continued for 13 h at room temperature. The solution was washed with 0.2 M potassium hydrogensulfate (180 mL) and the aqueous layer was extracted with dichloromethane (3x 170 mL). The combined organic phases were dried over magnesium sulfate, the solvent was removed under reduced pressure, and the residue was purified by column chromatography on silica gel (ethyl acetate/cyclohexane 1:3→1:2, v/v) to give a colorless foam (11.29 g, 70 %).

R_f 0.53 (ethyl acetate/cyclohexane 1:1, v/v); $[\alpha]_D^{24}$ = -12.5 ° mL dm^{-1} g^{-1}(*c* = 1.00 g mL^{-1} in ethanol); ^{1}H-NMR (300 MHz, $CDCl_3$) δ = 2.47 (dd, ^2J = 12.3 Hz, ^3J = 8.0 Hz, 1 H, CH_{2a}-S), 2.63 (dd, ^2J = 12.3 Hz, ^3J = 4.7 Hz, 1 H, CH_{2b}-S), 3.16 (s, 3 H, N-CH_3), 3.64 (s, 3 H, O-CH_3), 4.23 (t, ^3J = 7.2 Hz, 1 H, 9'-C*H*), 4.36 (dd, ^3J = 7.1 Hz, ^5J = 3.2 Hz, 2 H, CH_2-O), 4.75 – 4.86 (m, 1 H, C*H*-NH), 5.39 (d, ^3J = 9.0 Hz, 1 H, N*H*), 7.17 – 7.45 (m, 19 H, 3x Ph-H_5, 2'-C*H*, 3'-C*H*, 6'-C*H*, 7'-C*H*), 7.62 (dd, ^3J = 7.2 Hz, ^4J = 4.1 Hz, 2 H, 1'-C*H*, 8'-C*H*), 7.77 (dd, ^3J = 7.4 Hz, ^4J = 2.7 Hz, 2 H, 4'-C*H*, 5'-C*H*); ^{13}C-NMR (150 MHz, $CDCl_3$) δ = 32.1 (N-CH_3), 33.9 (CH_2-S), 47.0 (9'-CH), 50.1 (CH-NH), 61.5 (O-CH_3), 66.8 (S-C_q), 67.0 (CH_2-O), 119.9 (4'-CH, 5'-CH), 125.1 (1'-CH, 8'-CH), 126.7 (3x *p*-CH), 127.0, 127.6 (2'-CH, 3'-CH, 6'-CH, 7'-CH), 127.9, 129.5 (6x *o*-CH, 6x *m*-CH), 141.2, 143.7, 143.8 (4x C_q^{Fmoc}), 144.3 (3x C_q^{Trt}), 155.7 (2x C_q=O); *m/z* (HR-ESI) 651.2285 $[M+Na]^+$ ($C_{39}H_{36}N_2NaO_4S$ requires 651.2288).

(*R*)-*N*-Fluorenylmethoxycarbonyl-1-(*S*-tritylthiomethyl)-aminoethylglycine methylester

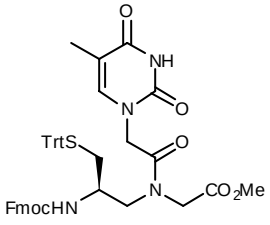


Under an atmosphere of argon and at a temperature of -72 °C lithium aluminium hydride (10.0 mL, 34.99 mmol, 3.5 M in tetrahydrofuran/ toluene) was suspended in dry tetrahydrofuran (80 mL). A solution of (*R*)-*N*-fluorenylmethoxycarbonyl-*S*-tritylcysteine-weinrebamide (11.00 g, 17.50 mmol) in dry tetrahydro-

furan (60 mL) was added dropwise within 15 minutes. After stirring at -72 °C for 1 hour, 0.2 M potassium hydrogensulfate (50 mL) was added slowly. The reaction mixture was extracted with diethyl ether (5x 100 mL). The combined organic phases were washed with saturated sodium chloride solution (50 mL) and the aqueous phase was extracted with diethyl ether (100 mL). The combined organic phases were dried over magnesium sulfate and the solvent was removed under reduced pressure. The obtained residue and glycine methylester hydrochloride (6.59 g, 52.49 mmol) were suspended in dry methanol/tetrahydrofuran (140 mL, 1:3, v/v) under an atmosphere of argon. Sodium cyanoborohydride (1.10 g, 17.50 mmol) was added at 5 °C. After stirring at room temperature for 20 hours, the suspension was concentrated under reduced pressure and the residue was dissolved in ethyl acetate (800 mL). The organic phase was washed with water (100 mL) and saturated sodium chloride solution (100 mL), with reextraction of the aqueous phase with ethyl acetate (150 mL each). The combined organic phases were dried over magnesium sulfate, the solvent was removed under reduced pressure, and the residue was purified by column chromatography on silica gel (ethyl acetate/cyclohexane 1:2→1:1, v/v) to give a colorless foam (4.37 g, 39 %).

R_f 0.16 (ethyl acetate/cyclohexane 1:1, v/v); $[\alpha]_D^{24}$ = +10.7 ° mL dm^{-1} g^{-1}(*c* = 1.00 g mL^{-1} in ethanol); ^{1}H-NMR (300 MHz, $CDCl_3$) δ = 2.33 – 2.44 (m, 1 H, CH_{2a}-S), 2.44 – 2.58 (m, 2 H, CH_{2b}-S, 4-CH_{2a}), 2.64 – 2.78 (m, 1 H, 4-CH_{2b}), 3.33 (d, ^3J = 5.9 Hz, 2 H, 2-CH_2), 3.60 – 3.72 (m, 1 H, 5-C*H*), 3.71 (s, 3 H, CH_3), 4.22 (t, ^3J = 6.8 Hz, 1 H, 9'-C*H*), 4.38 (d, ^3J = 6.6 Hz, 2 H, CH_2-O), 5.13 (d, ^3J = 7.7 Hz, 1 H, N*H*), 7.16 – 7.46 (m, 19 H, 3x Ph-H_5, 2'-C*H*, 3'-C*H*, 6'-C*H*, 7'-C*H*), 7.61 (d, ^3J = 7.4 Hz, 2 H, 1'-C*H*, 8'-C*H*), 7.76 (d, ^3J = 7.6 Hz, 2 H, 4'-C*H*, 5'-C*H*); ^{13}C-NMR (150 MHz, $CDCl_3$) δ = 34.3 (*C*H_2-S), 47.1 (9'-*C*H), 50.3, 51.4 (2-*C*H_2, 4-*C*H_2), 51.8 (*C*H_3), 66.5, 66.8 (S-C_q, *C*H_2-O), 119.8 (4'-*C*H, 5'-*C*H), 125.1 (1'-*C*H, 8'-*C*H), 126.7 (3x *p*-*C*H), 127.0, 127.6 (2'-*C*H, 3'-*C*H, 6'-*C*H, 7'-*C*H), 127.9, 129.5 (6x *o*-*C*H, 6x *m*-*C*H), 141.2, 143.8, 143.9 (4x C_q^{Fmoc}), 144.4 (3x C_q^{Trt}), 155.8 (NH-C_q=O), 172.3 (CH_2-C_q=O); *m/z* (HR-ESI) 643.2621 $[M+H]^+$ ($C_{40}H_{39}N_2O_4S$ requires 643.2625).

(*R*)-*N*-[*N*-Fluorenylmethoxycarbonyl-1-(*S*-tritylthiomethyl)-aminoethyl]-*N*-[(1-thyminyl)-acetyl]-glycine methylester

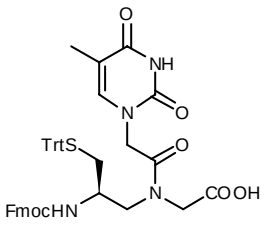


To a suspension of (1-thyminyl)-acetic acid[2] (190.6 mg, 1.04 mmol) in acetonitrile/*N*,*N*-dimethylformamide (15 mL, 1:1, v/v) were added under an atmosphere of argon and at a temperature of -10 °C *N*-methylmorpholine (455 µL, 4.14 mmol) and pivaloyl chloride (191.0 µL, 1.55 mmol). After stirring at -10 °C for 20 minutes, a solution of (*R*)-*N*-fluorenylmethoxy-carbonyl-1-(*S*-tritylthiomethyl)-aminoethylglycine methylester (453.1 mg, 0.69 mmol) in *N*,*N*-dimethylformamide (7.5 mL) was added within 5 minutes and stirring was continued at room temperature for 18 hours. The solution was poured into ethyl acetate (60 mL) and washed with 0.1 M hydrochloric acid (3x 15 mL). The organic phase was dried over magnesium sulfate, the solvent was removed under reduced pressure, and the residue was purified by column chromatography on silica gel (ethyl acetate/cyclohexane 1:4, v/v) to give a colorless foam (252 mg, 48 %).

R_f 0.31 (ethyl acetate); $[\alpha]_D^{24}$ = +7.3 ° mL dm^{-1} g^{-1}(*c* = 1.00 g mL^{-1} in ethanol); ^{1}H-NMR (300 MHz, $CDCl_3$) δ = [2 rotamers] 1.83 (s, 1.5 H, C_q-CH_3), 1.84 (s, 1.5 H, C_q-CH_3), 2.29 – 2.76 (m, 2 H, CH_2-S), 3.00 – 3.20 (m, 1 H, 4'-CH_{2a}), 3.40 – 3.62 (m, 2 H, 4'-CH_{2b}, 5'-C*H*), 3.70 (s, 1.5 H, O-CH_3), 3.76 (s, 1.5 H, O-CH_3), 3.78 – 4.02 (m, 2 H, 2'-CH_2), 4.18 – 4.61 (m, 5 H, CH_2-thymine, CH_2-O, 9''-C*H*), 5.09 (d, ^3J = 7.5 Hz, 0.5 H, 6'-N*H*), 5.43 (d, ^3J = 6.6 Hz, 0.5 H, 6'-N*H*), 6.83 (s, 0.5 H, 6-C*H*), 6.86 (d, ^4J = 1.2 Hz, 0.5 H, 6-C*H*), 7.16 – 7.45 (m, 19 H, 3x Ph-H_5, 2''-C*H*, 3''-C*H*, 6''-C*H*, 7''-C*H*), 7.56 (d, ^3J = 7.3 Hz, 2 H, 1''-C*H*, 8''-C*H*),

7.74 (dd, ^{3}J = 7.3 Hz, ^{4}J = 2.8 Hz, 2 H, 4''-C*H*, 5''-C*H*), 9.03 (s, 1 H, 3-N*H*); ^{13}C-NMR (150 MHz, $CDCl_3$) δ = [2 rotamers] 12.2 (C_q-CH_3), 33.1, 34.1 (*C*H_2-S), 47.0, 47.1 (9''-*C*H), 47.3, 47.5 (*C*H_2-thymine), 48.7, 49.4 (2'-*C*H_2), 49.3, 49.7 (5'-*C*H), 50.4, 50.7 (4'-*C*H_2), 52.3, 52.8 (O-*C*H_3), 66.5, 66.6 (*C*H_2-O), 67.0, 67.4 (S-C_q), 110.4, 110.5 (5-C_q), 119.8, 119.9 (4''-*C*H, 5''-*C*H), 124.9, 125.0, 125.1 (1''-*C*H, 8''-*C*H), 126.7, 126.9, 127.0, 127.6, 127.7, 127.9, 128.0, 129.4, 129.4, 129.6 (2''-*C*H, 3''-*C*H, 6''-*C*H, 7''-*C*H, 3x *o*-*C*H, 3x *m*-*C*H), 141.7, 140.0 (6-*C*H), 141.2, 141.2, 143.7, 143.7, 144.1, 144.3 (4x C_q^{Fmoc}, 3x C_q^{Trt}), 150.7, 150.8 (2-C_q), 155.8, 155.9 (NH-(C_q=O)-O), 164.1, 164.1, 168.2, 169.3, 169.3, 169.3 (4-C_q, 1'-C_q, C_q-CH_2-thymine); *m/z* (HR-ESI) 831.2815 $[M+Na]^+$ ($C_{47}H_{44}N_4NaO_7S$ requires 831.2823).

(*R*)-*N*-[*N*-Fluorenylmethoxycarbonyl-1-(*S*-tritylthiomethyl)-aminoethyl]-*N*-[(1-thyminyl)-acetyl] glycine 1



To a solution of (*R*)-*N*-[*N*-fluorenylmethoxycarbonyl-1-(*S*-tritylthiomethyl)-aminoethyl]-*N*-[(1-thyminyl)-acetyl]-glycine methylester (500 mg, 0.62 mmol) in tetrahydrofuran (6.2 mL) was added lithium hydroxide (2.47 mL, 1.24 mmol, 0.5 M in water) at a temperature of 0 °C. After stirring at room temperature for 2 hours, the solution was acidified to pH 3 with formic acid (25 % in water). The solution was extracted with ethyl acetate (3x 20 mL) and the combined organic phases were washed with saturated sodium chloride solution (5 mL), with reextraction of the aqueous phase with ethyl acetate (10 mL). The combined organic phases were dried over magnesium sulfate, the solvent was removed under reduced pressure, and the residue was purified by column chromatography on silica gel (dichloromethane/methanol/formic acid 95:5:05, v/v) to give a colorless powder (319 mg, 65 %).

R_f 0.30 (dichloromethane/methanol/formic acid 95:5:0.5, v/v); $[\alpha]_D^{24}$ = +6.5 ° mL dm^{-1} g^{-1}(*c* = 1.00 g mL^{-1} in ethanol); ^{1}H-NMR (300 MHz, CD_3OD) δ = [2 rotamers] 1.76 (s, 1.5 H, C_q-CH_3), 1.77 (s, 1.5 H, C_q-CH_3), 2.19 – 2.33 (m, 1 H, CH_{2a}-S, CH_{2b}-S), 2.43 (dd, ^{2}J = 12.8 Hz, ^{3}J = 8.6 Hz, 0.5 H, CH_{2a}-S), 2.62 (dd, ^{2}J = 12.9 Hz, ^{3}J = 8.9 Hz, 0.5 H, CH_{2b}-S), 3.08 – 3.31 (m, 2 H, 4'-CH_2), 3.38 – 3.51 (m, 0.5 H, 5'-C*H*), 3.53 – 3.64 (m, 0.5 H, 5'-C*H*), 3.70 (d, ^{2}J = 17.4 Hz, 0.5 H, 2'-CH_{2a}), 3.93 – 4.04 (m, 1.5 H, 2'-CH_{2a}, 2'-CH_{2b}), 4.13 – 4.22 (m, 1 H, 9''-C*H*), 4.22 – 4.42 (m, 2.5 H, CH_{2a}-thymine, CH_2-O), 4.42 – 4.53 (m, 1 H, CH_{2a}-thymine, CH_{2b}-thymine), 4.67 (d, ^{2}J = 16.5 Hz, 0.5 H, CH_{2b}-thymine), 7.03 (d, ^{4}J = 1.1 Hz, 0.5 H, 6-C*H*), 7.08 (s, 0.5 H, 6-C*H*), 7.13 – 7.43 (m, 19 H, 3x Ph-H_5, 2''-C*H*, 3''-C*H*, 6''-C*H*, 7''-C*H*), 7.57 – 7.65 (m, 2 H, 1''-C*H*, 8''-C*H*), 7.74 (d, ^{3}J = 7.5 Hz, 2 H, 4''-C*H*, 5''-C*H*); ^{13}C-NMR (150 MHz, CD_3OD) δ = [2 rotamers] 12.3 (C_q-CH_3), 35.1, 35.6 (*C*H_2-S), 48.4 (9''-*C*H), 49.2, 49.2 (*C*H_2-thymine), 50.6, 50.7 (2'-*C*H_2), 50.9, 51.3 (5'-*C*H), 52.1, 52.2 (4'-*C*H_2), 67.7, 67.9 (*C*H_2-O), 68.0, 68.4 (S-C_q), 110.8, 110.9 (5-C_q), 120.9 (4''-*C*H, 5''-*C*H), 126.3, 126.4 (1''-*C*H, 8''-*C*H), 127.9, 128.0, 128.2, 128.2, 128.8, 129.0, 129.1, 130.8 (2''-*C*H, 3''-*C*H, 6''-*C*H, 7''-*C*H, 3x *o*-*C*H, 3x *m*-*C*H), 143.4, 143.5 (6-*C*H), 145.4, 145.3, 145.5, 145.6 (4a''-C_q, 4b''-C_q, 8a''-C_q, 9a''-C_q), 146.0, 146.1 (3x C_q^{Trt}), 152.8 (2-C_q), 158.2 (NH-(C_q=O)-O), 166.9, 166.9, 169.7, 170.2, 172.1, 172.2 (4-C_q, 1'-C_q, C_q-CH_2-thymine); *m/z* (HR-ESI) 817.2666 $[M+Na]^+$ ($C_{46}H_{42}N_4NaO_7S$ requires 817.2666).

PNA-synthesis

Linear solid-phase synthesis was performed by using an Intavis ResPep parallel synthesizer equipped with 0.5 mL reactors. Fmoc-Lys loaded (0.11 mmol g^{-1}) NovaSyn® TGR resin (2 µmol) was allowed to swell for 30 min in DMF (200 µL) and transferred to the synthesizer. The resin was washed with DMF (2x 200 µL).

Fmoc cleavage: DMF/piperidine (2x 200 µL, 4:1, v/v) was added for 2 min to the resin. The resin was washed with DMF (11x 200 µL).

Coupling: A preactivation vessel was charged with a 0.6 M HCTU solution in NMP (12 µL), a 4 M NMM solution in DMF (4 µL), and a 0.2 M PNA monomer solution in NMP (40 µL). After 2 min, 50 µL of preactivation solution was transferred to the resin. After 30 min, the resin was washed with DMF (3x 200 µL). Double couplings were performed for modified PNA-monomer **4** and after the tenth coupling step.

Capping: DMF/Ac_2O/2,6-lutidine (200 µL, 89:5:6, v/v) was added for 2 min to the resin. The resin was washed with DMF (5x 200 µL).

Cleavage from the solid support: Non-thiol-containing PNA oligomers were cleaved by the addition of TFA/*m*-cresol/*i*Pr_3SiH/water (850:50:50:50, v/v), thiol-containing PNA oligomers were cleaved by the addition of TFA/EDT/*i*Pr_3SiH/water (850:25:25:50, v/v) to the resin for 90 min. The suspension was filtered and the resin was washed with TFA (2x 300 µL). The combined filtrates were concentrated under reduced pressure to ca. 100 µL. The precipitate formed upon addition of 1 mL cold diethyl ether was washed with 1 mL cold diethyl ether.

Purification and determination of yield: The crude product was dissolved in water/acetonitrile (9:1, v/v) and purified by semi-preparative HPLC (3→17 % B in 30 min). After the eluent was removed under reduced pressure, the product was dissolved in degassed water. An aliquot was diluted with degassed water to 100 µL and the optical density was measured at λ = 260 nm using a quartz cuvette with a 1 cm path length. The sample concentration was calculated using oligo calculation at http://proligo2.proligo.com/Calculation/calculation.html.

H-actt(CH_2SH)acctcacgc-Lys-NH_2 5
Yield: 40.5 OD_{260}, 348.0 nmol (17.4 %); *m/z* (MALDI) 3616; *m/z* (ESI) 3613.28 $[M+H]^+$ ($C_{144}H_{190}N_{73}O_{40}S$ requires 3613.4798); t_R 11.5 min (3→30 % B in 20 min).

H-cagtcatagt(CH_2SH)tcc-Lys-NH_2 6
Yield: 39.9 OD_{260}, 323.0 nmol (16.1 %); *m/z* (MALDI) 3673; *m/z* (ESI) 3668.49 $[M+H]^+$ ($C_{146}H_{191}N_{74}O_{41}S$ requires 3668.4856); t_R 12.0 min (3→30 % B in 20 min).

H-acttacctcacgc-Lys-NH_2 7
Yield: 14.1 OD_{260}, 119.0 nmol (6.0 %), *m/z* (MALDI) 3571; *m/z* (ESI) 3567.27 $[M+H]^+$ ($C_{143}H_{188}N_{73}O_{40}$ requires 3567.4921); t_R 11.2 min (3→30 % B in 20 min).

H-cagtcatagttcc-Lys-NH_2 8
Yield: 22.4 OD_{260}, 181.6 nmol (9.1 %); *m/z* (MALDI) 3626; *m/z* (ESI) 3622.16 $[M+H]^+$ ($C_{145}H_{189}N_{74}O_{41}$ requires 3622.4979); t_R 11.7 min (3→30 % B in 20 min).

H-caatcgtatccag-Lys-NH_2 9
Yield: 29.1 OD_{260}, 220.9 nmol (11.0 %); *m/z* (MALDI) 3630; *m/z* (ESI) 3632.15 $[M+H]^+$ ($C_{145}H_{188}N_{77}O_{39}$ requires 3631.5095); t_R 11.3 min (3→30 % B in 20 min).

Ligation

General procedure for the ligation of *N*-acetyllactosamine 13 to thiol-modified PNA

Aliquots from stock solutions of thiol-modified PNA's **5** or **6** (ca. 350 µM in water) and 3 eq (*N*-(3'-maleimidopropanoyl)-2-aminoethyl)-β-*N*-acetyl-lactosaminide **13** (20 mM in water) were added to freshly degassed buffer (10 mM NaH_2PO_4, pH 6.5, 0.3 mM TCEP) to a final concentration of 100 µM **8** or **9** and 300 µM **13**. After shaking at 30 °C for 1 day, the reaction mixture was purified by semi-preparative HPLC (3→17 % B in 30 min).

H-actt(CH_2S~βLacNAc)acctcacgc-Lys-NH_2 15

Yield: 22.0 OD_{260}, 188.6 nmol (94 %); *m/z* (MALDI) 4196; *m/z* (ESI) 4190.49 $[M+H]^+$ ($C_{167}H_{225}N_{76}O_{54}S$ requires 4190.6917); t_R 11.3 min (3→30 % B in 20 min).

H-cagtcatagt(CH_2S~βLacNAc)tcc-Lys-NH_2 16

Yield: 15.6 OD_{260}, 125.9 nmol (98 %); *m/z* (MALDI) 4249; *m/z* (ESI) 4245.33 $[M+H]^+$ ($C_{169}H_{226}N_{77}O_{55}S$ requires 4245.6975); t_R 11.8 min (3→30 % B in 20 min).

SPR-Experiments

Instrumentation and reagents

The SPR experiments were performed on a BIAcore X instrument (Biacore AB, Uppsala, Sweden) at 25 °C. Sensor chips CM5 (research grade), amine coupling kit and HBS-EP buffer were purchased from GE Healthcare. *Erythrina cristagalli* lectin (ECL) was purchased from Linaris Biologische Produkte GmbH, Germany. Asialofetuin was purchased from Sigma-Aldrich.

Preparation of sensor chips

Sensor chips were prepared at a flow rate of 5 µL min^{-1} using freshly degassed HBS-EP buffer (pH 7.4, 10 mM HEPES, 150 mM NaCl, 3 mM EDTA, and 0.005 % Surfactant P20). After equilibration with buffer, the sensor surface was activated (ca. 140 RU) with a 7 minute pulse of a 1:1 mixture of 0.1 M *N*-hydroxysuccinimide and 0.4 M *N*-ethyl-*N*'-(dimethylaminopropyl)carbodiimide hydrochloride. A solution of ECL (200 µg mL^{-1}) in 10 mM sodium acetate (pH 4.5) was passed over the activated sensor chip until the level of immobilized ECL was approximately 2700 RU or 700 RU, respectively. Blockage of the remaining *N*-hydroxysuccinimide esters was performed by a 7 min pulse of 1.0 M ethanolamine hydrochloride (pH 8.5). The reference channels were prepared in an analogous manner but with denaturation of ECL afterwards by two 4 min pulses of 6.0 M guanidine hydrochloride (pH 1.5). Prepared sensor chips were stored at 4 °C in the presence of silica gel (Chameleon® C 1-3 mm).

Preparation of multivalent LacNAc assemblies

DNA templates:

29 5’-GGA-ACT-ATG-ACT-GGG-AAC-TAT-GAC-TGG-CGT-GAG-GTA-AGT-GCG-TGA-GGT-AAG-T-3’

30 5’-CTG-GAT-ACG-ATT-GCT-GGA-TAC-GAT-TGG-GAA-CTA-TGA-CTG-GCG-TGA-GGT-AAG-T-3’

31 5’-CTG-GAT-ACG-ATT-GGC-GTG-AGG-TAA-GTG-GAA-CTA-TGA-CTG-GCG-TGA-GGT-AAG-T-3’

32 5’-CTG-GAT-ACG-ATT-GGG-AAC-TAT-GAC-TGC-TGG-ATA-CGA-TTG-GCG-TGA-GGT-AAG-T-3’

33 5’-GCG-TGA-GGT-AAG-TGG-AAC-TAT-GAC-TGG-GAA-CTA-TGA-CTG-GCG-TGA-GGT-AAG-T-3’

34 5’-GGA-ACT-ATG-ACT-GCT-GGA-TAC-GAT-TGC-TGG-ATA-CGA-TTG-GCG-TGA-GGT-AAG-T-3’

35 5‘-GGT-AAG-TGC-GTG-A-3‘

A stock solution (10 or 20 µM, respectively) of the appropriate complex in running buffer (pH 7.4, 10 mM Tris, 150 mM NaCl, 2 mM $MgCl_2$, and 2 mM $CaCl_2$) was prepared according to table 1. The solution was incubated at 50 °C for 3 minutes and, after cooling down to room temperature, diluted into samples with concentrations from 0.625 – 10 µM or 2.5 – 20 µM, respectively.

Table 1. Composition of multivalent LacNAc assemblies.

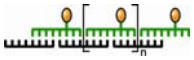
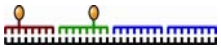
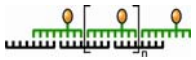
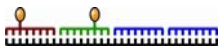
Complex	DNA template	**15**/eq	**16**/eq	**7**/eq	**8**/eq	**9**/eq
36 	**33**	-	-	2	2	-
17 	**31**	-	1	2	-	1
18 	**32**	1	1	-	-	2
19 	**31**	2	1	-	-	1
20 	**33**	2	2	-	-	-
21 	**35**	1	-	-	-	-
22 	**29**	2	-	-	2	-
23 	**30**	1	1	-	-	2
24 	**32**	1	1	-	-	-
25 	**33**	2	-	-	-	-
26 	**33**	2	-	-	2	-
27 	**34**	1	1	-	-	2

Table 2. Kinetic parameters.

		Low ECL concentration						High ECL concentration					
		Langmuir model (1:1 binding)				Steady state affinity		Langmuir model (1:1 binding)				Steady state affinity	
Complex or substrate	Distance/Å	$k_a/M^{-1}\ s^{-1}$	k_d/s^{-1}	$K_D/\mu M$	χ^2	$K_D/\mu M$	χ^2	$k_a/M^{-1}\ s^{-1}$	k_d/s^{-1}	$K_D/\mu M$	χ^2	$K_D/\mu M$	χ^2
Asialofetuin	-	$4.8 \cdot 10^3$	$3.1 \cdot 10^{-3}$	0.6	8.96	[a]	[a]	$2.5 \cdot 10^3$	$1.5 \cdot 10^{-3}$	0.6	199	[a]	[a]
36 	-	[b]	[b]	[b]	[b]	[b]	[b]	n.d.[c]	n.d.	n.d.	n.d.	n.d.	n.d.
17 	-	$4.0 \cdot 10^2$	$3.2 \cdot 10^{-1}$	800	1.58	407	1.25	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
19 	-	$6.0 \cdot 10^3$	$1.5 \cdot 10^{-2}$	2.6	14.7	[a]	[a]	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
20 	-	$6.8 \cdot 10^3$	$7.7 \cdot 10^{-3}$	1.1	22.5	[a]	[a]	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
21 	-	$9.4 \cdot 10^2$	$2.3 \cdot 10^{-3}$	2.5	6.92	[a]	[a]	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
22 	42	$2.0 \cdot 10^3$	$8.7 \cdot 10^{-2}$	43	0.92	1290	6.53	$4.1 \cdot 10^3$	$4.5 \cdot 10^{-2}$	11	2.70	22	1.51
23 	62	$2.3 \cdot 10^3$	$9.4 \cdot 10^{-2}$	40	0.86	159	3.11	$4.9 \cdot 10^3$	$5.6 \cdot 10^{-2}$	12	1.71	20	0.14
18 	104	$3.3 \cdot 10^3$	$6.9 \cdot 10^{-2}$	21	6.33	64	4.66	$4.1 \cdot 10^3$	$5.0 \cdot 10^{-2}$	12	2.94	36	2.01
24 	104	$4.7 \cdot 10^3$	$5.6 \cdot 10^{-2}$	12	1.03	28	0.33	$4.3 \cdot 10^3$	$5.2 \cdot 10^{-2}$	12	1.87	31	0.70
25 	127	$7.8 \cdot 10^3$	$9.5 \cdot 10^{-2}$	12	0.43	21	0.20	$5.1 \cdot 10^3$	$4.6 \cdot 10^{-2}$	9	4.18	21	3.04
26 	127	$4.1 \cdot 10^3$	$7.2 \cdot 10^{-2}$	18	1.17	34	1.34	$5.6 \cdot 10^3$	$5.2 \cdot 10^{-2}$	9	4.32	31	7.43
27 	146	$3.7 \cdot 10^3$	$8.4 \cdot 10^{-2}$	23	0.96	53	2.35	$4.2 \cdot 10^3$	$4.6 \cdot 10^{-2}$	11	3.98	21	1.44

[a] data fitting not feasible [b] no binding was observed [c] not determined.

Kinetic measurements

SPR experiments were performed at a flow rate of 20 µL min^{-1}, using freshly degassed running buffer (pH 7.4, 10 mM Tris, 150 mM NaCl, 2 mM $MgCl_2$, and 2 mM $CaCl_2$). After equilibration with buffer, the samples were injected allowing an association phase of 90 seconds, followed by a dissociation phase of 240 seconds. Regeneration of the sensor surface was performed by a 30 seconds pulse of 10 mM sodium acetate (pH 4.5) for PNA-DNA complexes or a 30 seconds pulse of 20 mM HCl for Asialofetuin. Sensorgrams were obtained after substraction of the reference channel and blank buffer injections. Kinetic parameters were calculated by global fitting of the sensorgram data with BIAevaluation software 4.1. The closeness of fit was judged by the statistical value χ^2 and the form of the fitted curve in relation to the experimental data. Values of χ^2 were below 10, except for complexes **19**, **20**, and Asialofetuin. Since the fitting model sometimes affects the outcome of the kinetic parameters, both the simple Langmuir model (1:1 binding) and the steady state affinity model were applied. For the bivalent complexes **18** and **22** – **27** both models revealed the same tendencies in binding affinity. However, due to the fact that some substrates did not reach equilibrium during the association phase, the steady state affinity model could not be applied to all substrates. As a consequence, we focused on the data obtained by the Langmuir model.

Sensorgrams for high concentration of ECL (ca. 2700 RU)

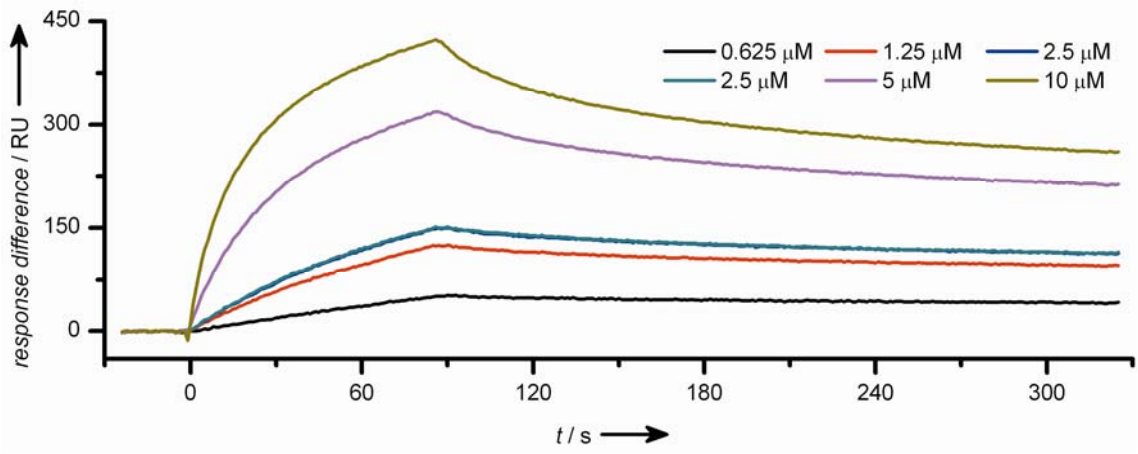


Figure S1. Sensorgrams for Asialofetuin.

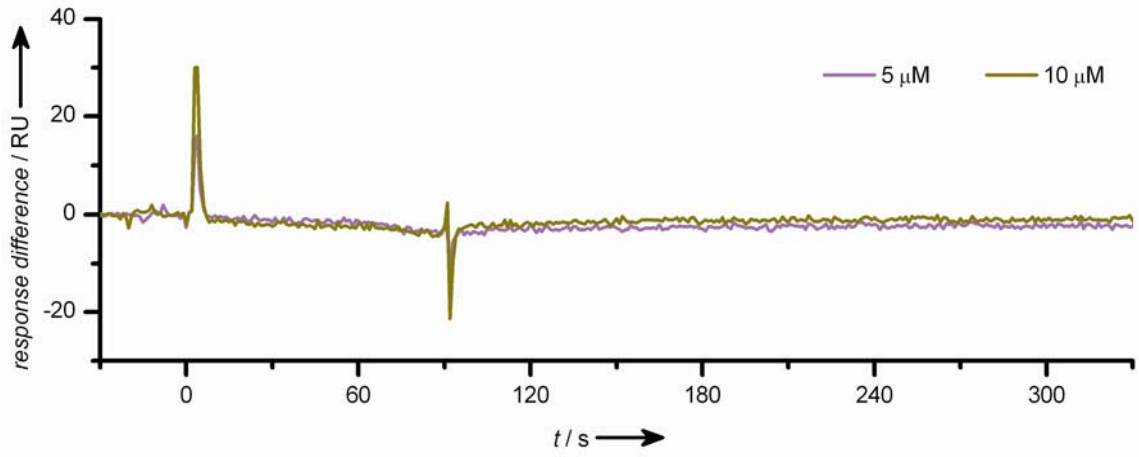


Figure S2. Sensorgrams for **36**.

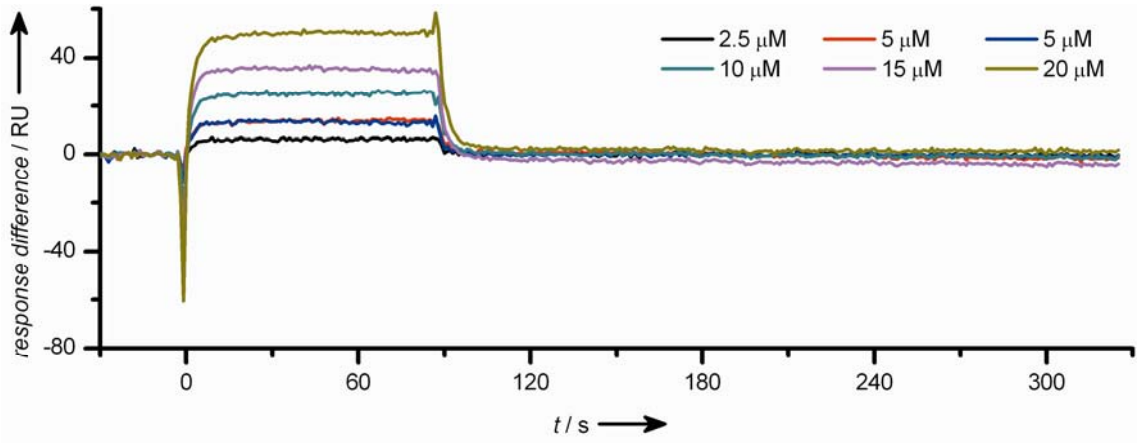


Figure S3. Sensorgrams for **17**.

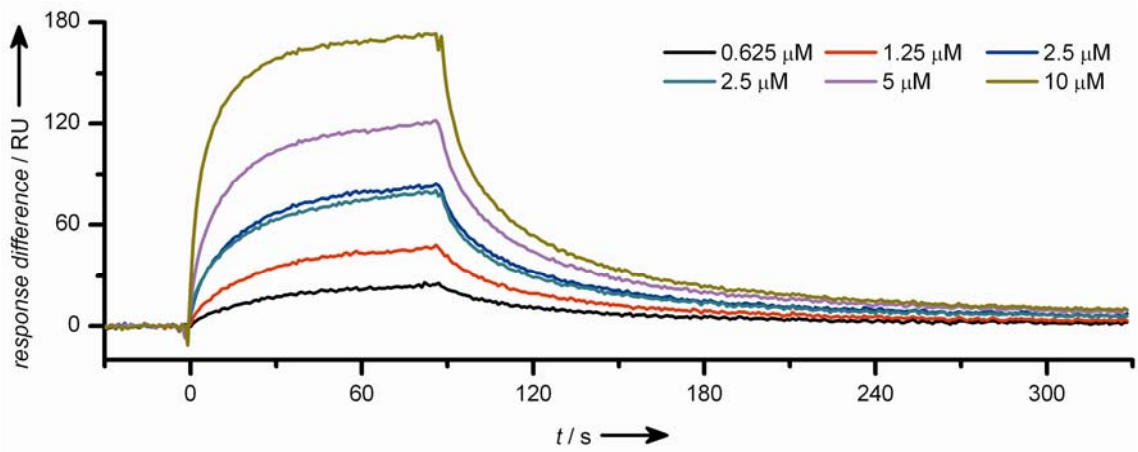


Figure S4. Sensorgrams for **19**.

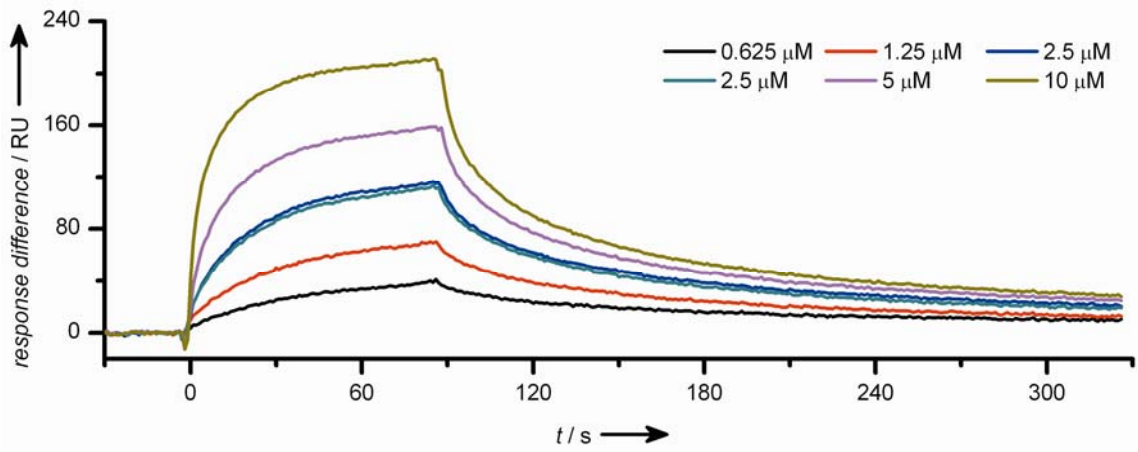


Figure S5. Sensorgrams for **20**.

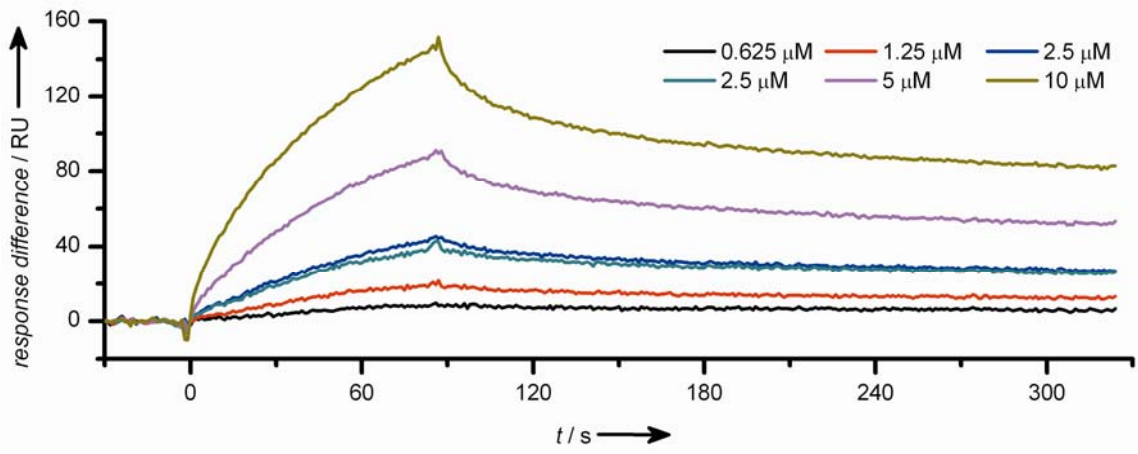


Figure S6. Sensorgrams for **21**.

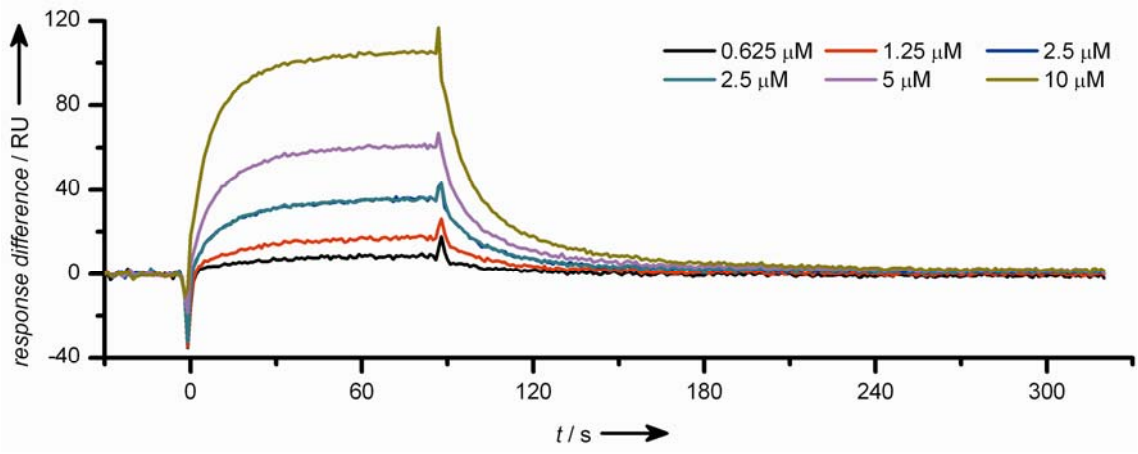


Figure S7. Sensorgrams for **22**.

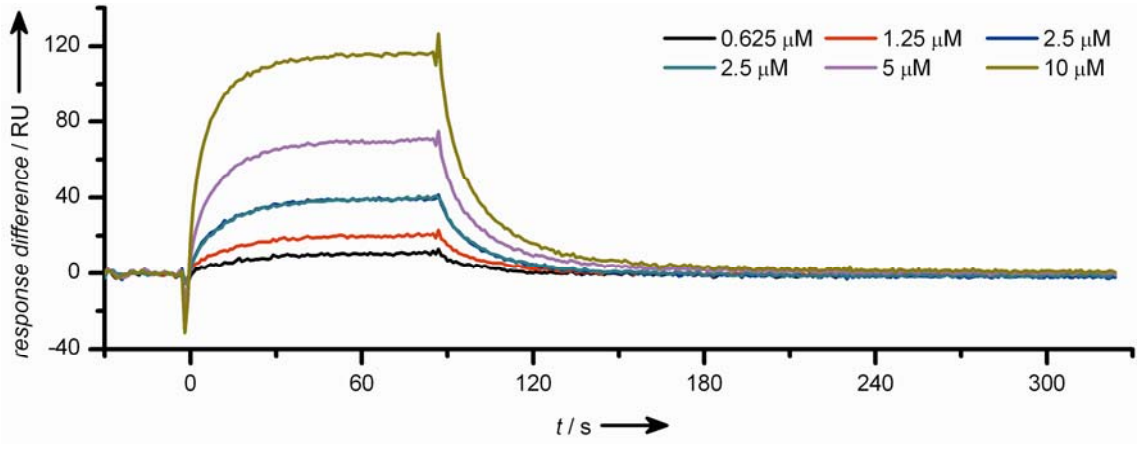


Figure S8. Sensorgrams for **23**.

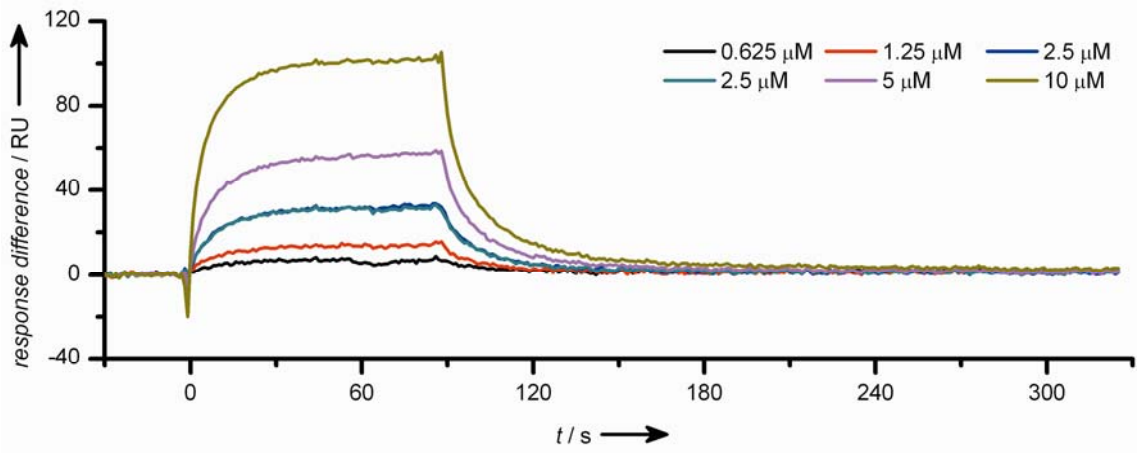


Figure S9. Sensorgrams for **18**.

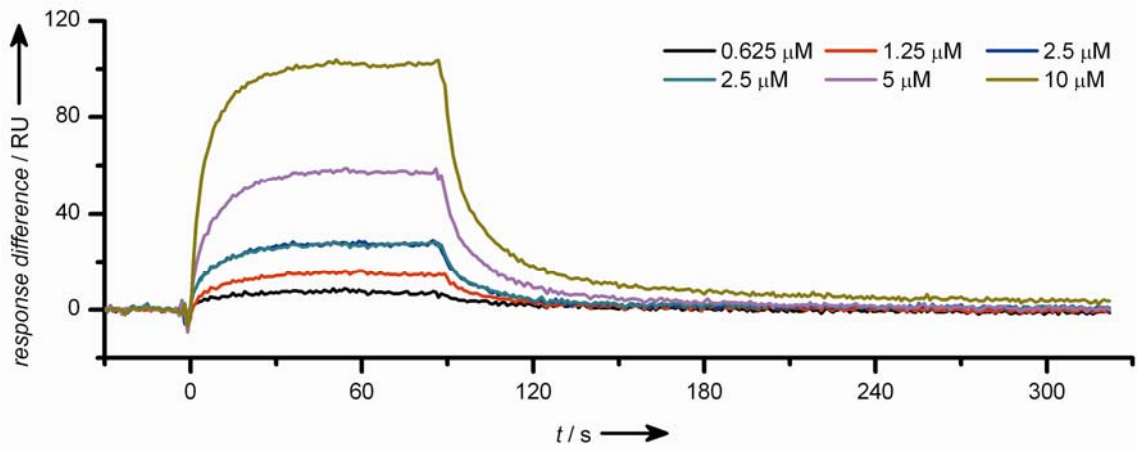


Figure S10. Sensorgrams for **24**.

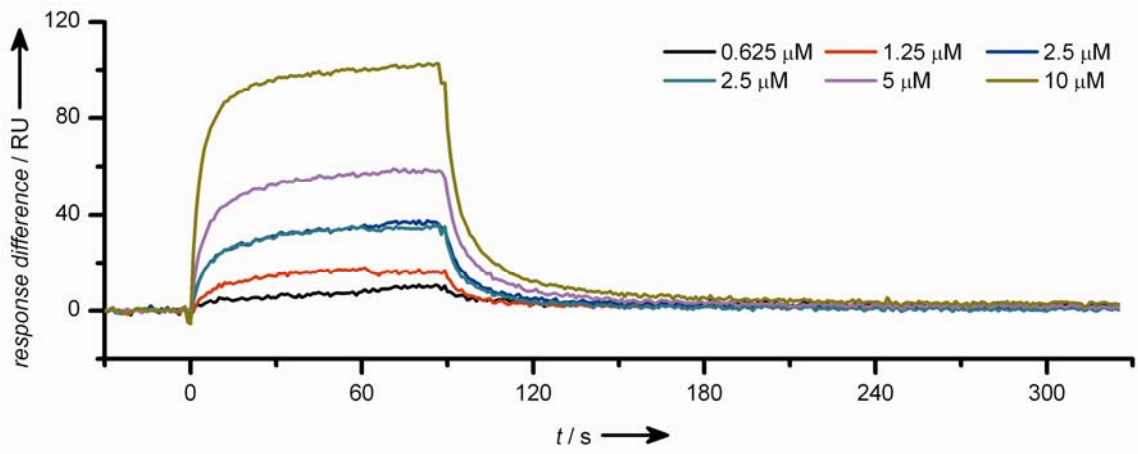


Figure S11. Sensorgrams for **25**.

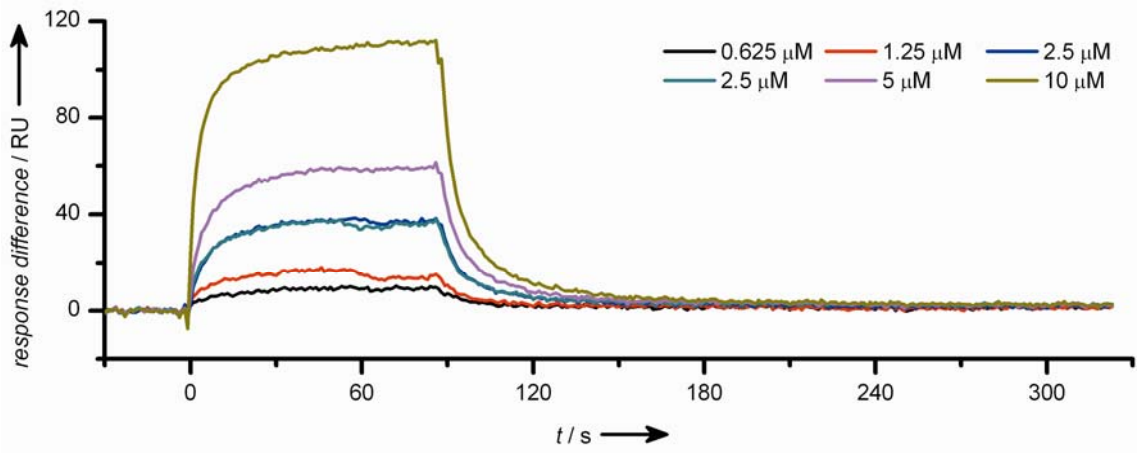


Figure S12. Sensorgrams for **26**.

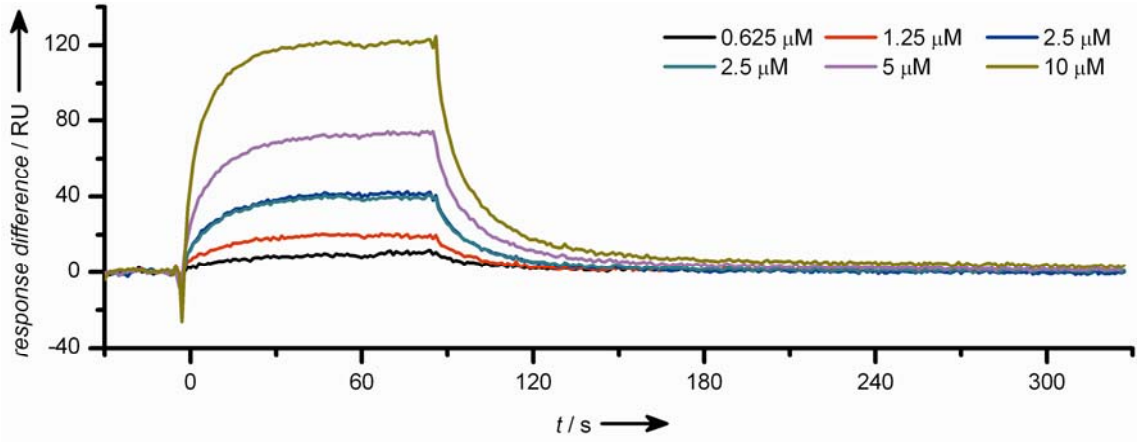


Figure S13. Sensorgrams for **27**.

Sensorgrams for low concentration of ECL (ca. 700 RU)

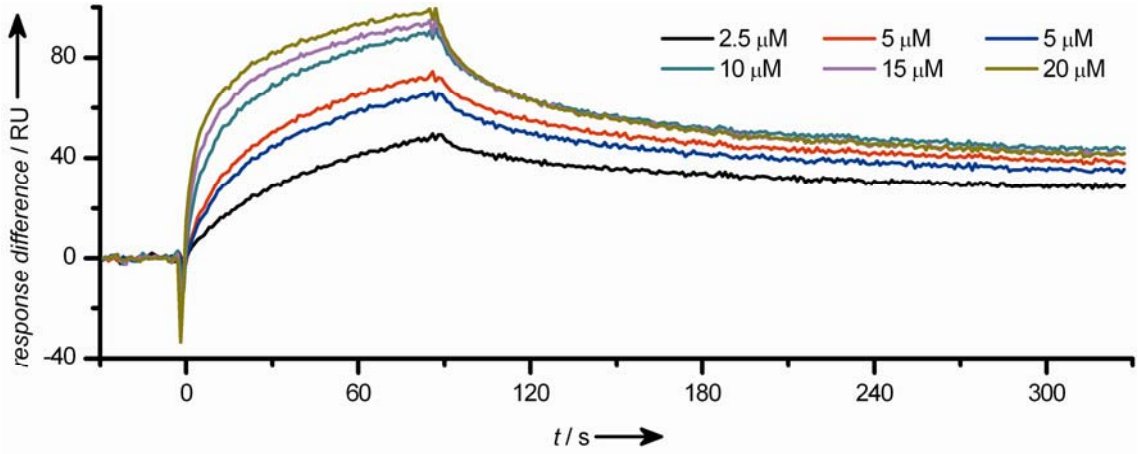


Figure S14. Sensorgrams for Asialofetuin.

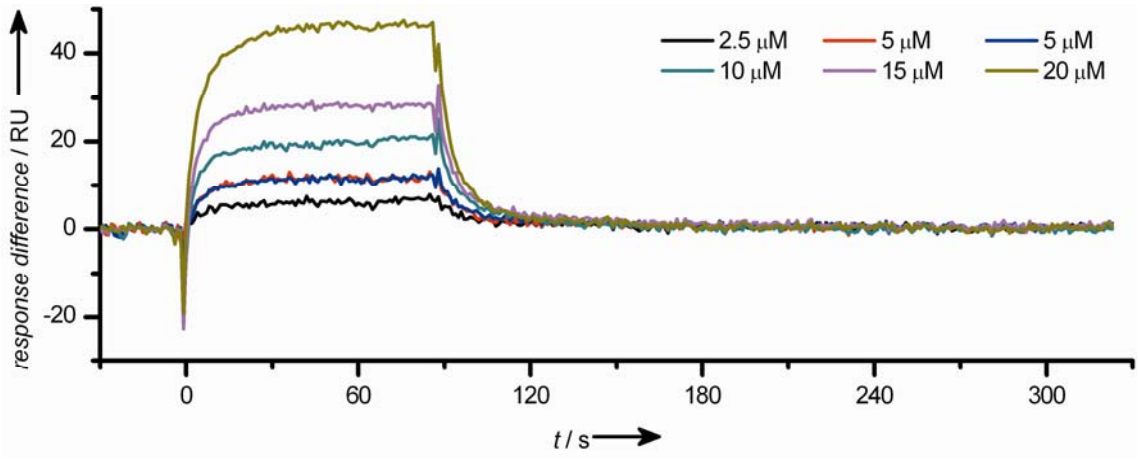


Figure S15. Sensorgrams for **22**.

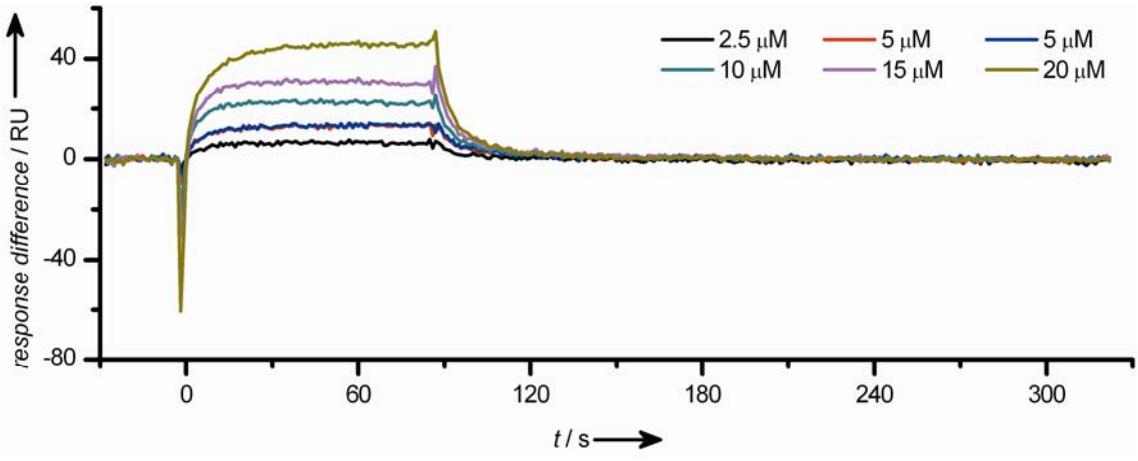


Figure S16. Sensorgrams for **23**.

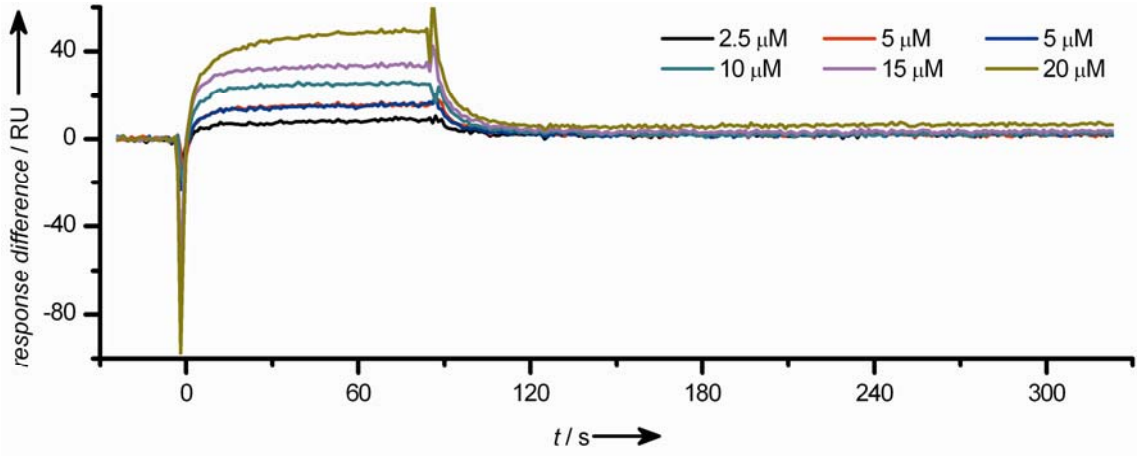


Figure S17. Sensorgrams for **18**.

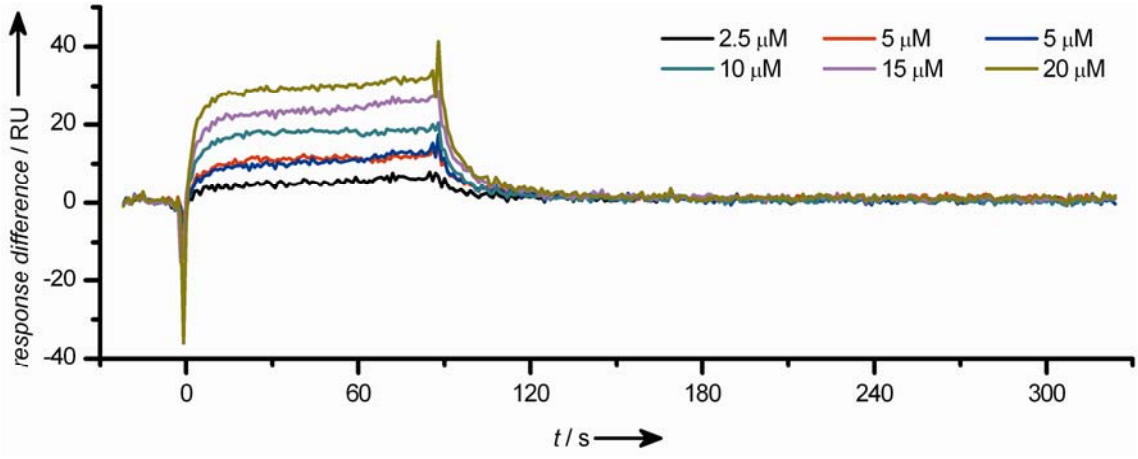


Figure S18. Sensorgrams for **24**.

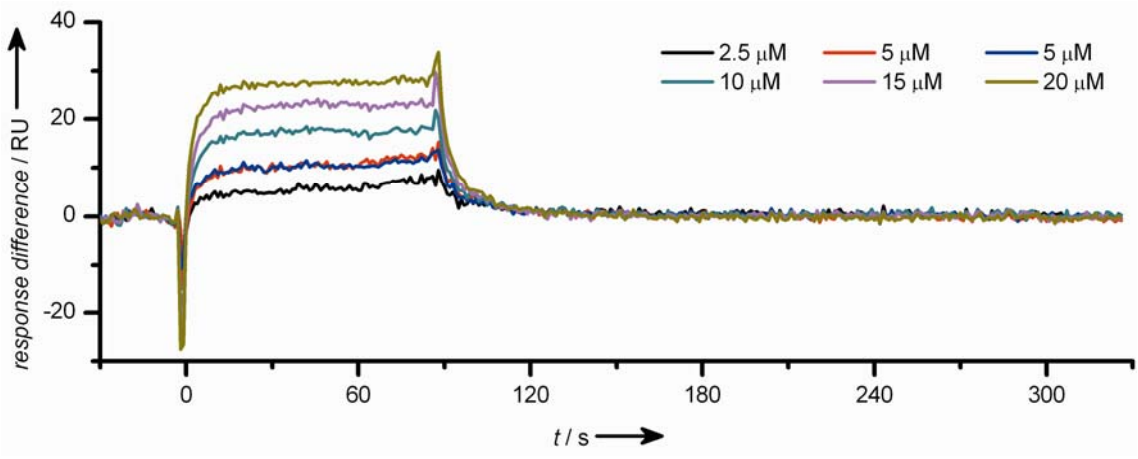


Figure S19. Sensorgrams for **25**.

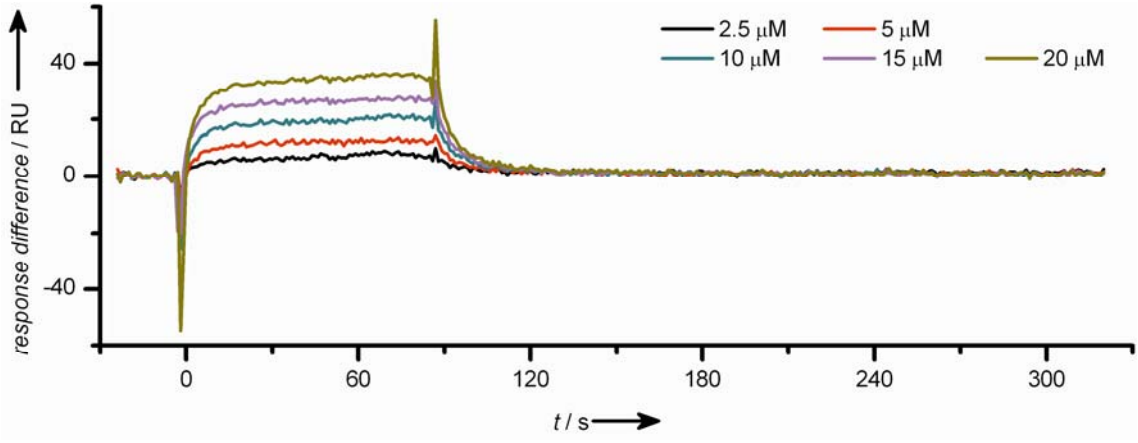


Figure S20. Sensorgrams for **26**.

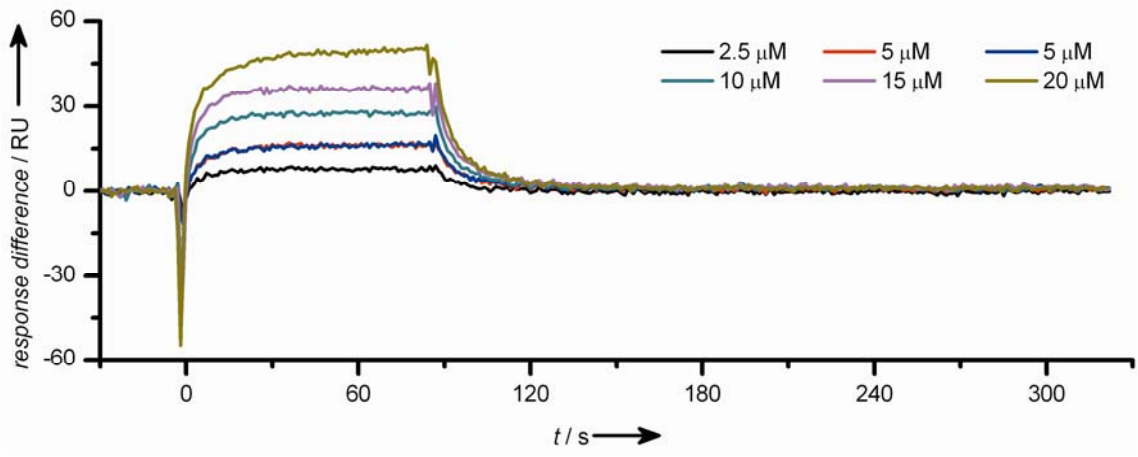


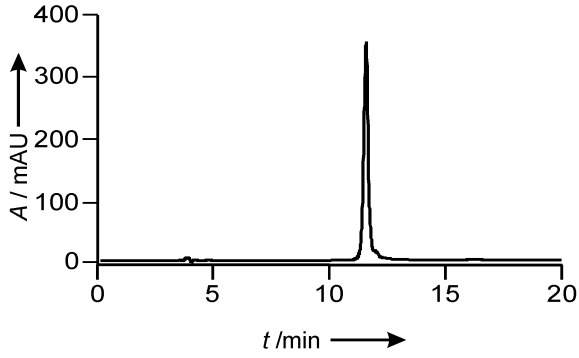
Figure S21. Sensorgrams for **27**.

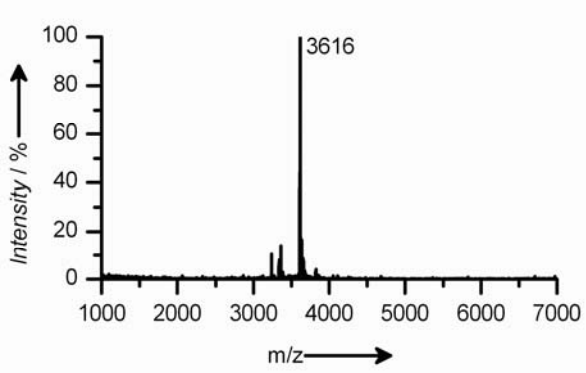
Modeling and simulation

Structures of the complexes were modeled with Amira,[6] based on the solution structure of a PNA-DNA duplex[7] and a generic DNA double helix structure generated with AmberTools 1.2.[8] The modified PNA residue was modeled including the complete linker, but without the ligand. The PNA residues were parameterized for the AMBER 99 SB forcefield[9] using the software Antechamber,[10, 11] with charges calculated by the AM1-BCC method.[12, 13] The protonation state of the PNA-DNA complexes in solvent was the normal one for pH 7. All simulations were performed using the GROMACS 4 software[14] and the according port of the AMBER forcefields, ffAMBER.[15] For the 30 ns simulations, we used the linear complex conformations as starting configurations. The complex structures were solvated with roughly 176,000 water molecules (TIP3P water[16] model) in equally sized rhombic dodecahedron boxes of 19.6 nm side length. To neutralize the overall charge, an adequate amount of potassium ions was added to the simulation boxes. The energy of the systems was minimized with the steepest descent algorithm, and 200 ps position restrained MD simulations were performed to settle the solvent molecules before the unrestrained long time simulations were started. In order to obtain bent complex conformations, we used a constant distance restraining potential to bend the complex structures such that the distance between the linkers was adjusted to the approximate distance of the protein binding sites. This was done using implicit solvent. Afterwards the bent complexes were tranferred into explicit TIP3P solvent, following the protocol described above for the linear complexes, including energy minimization and position restrained MD in order to settle the solvent molecules. These were then used as starting configurations for the force measurements. The forces were measured by applying an harmonic potential restraining the distance between atoms of the two linker attachment sites, and then calculating a 400 ps MD trajectory, yielding mean the force that has to act on the strand to remain in the bent conformation with the correct linker-linker distance. To maintain a constant temperature of 300 K and a pressure of 1 bar, velocity rescaling[17] and Berendsen[18] weak coupling were applied. A twin range cut-off of 1.0/1.4 nm for van der Waals interactions was applied and the smooth particle mesh Ewald algorithm[19] was used for Coulomb interactions, with a switching distance of 1.0 nm. For the 30 ns simulations, bond lengths were constrained using the LINCS algorithm,[20] allowing for an integration step of 2 fs. For the force measurement simulations, we used an integration step of 1 fs and unconstrained bond lenghts.

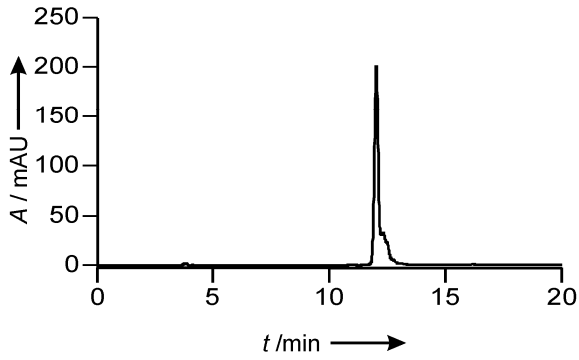
HPLC traces and MALDI-MS spectra of PNA oligomers

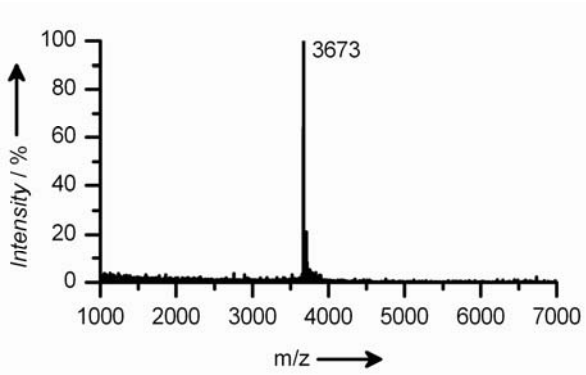
H-actt(CH_2SH)acctcacgc-Lys-NH_2 5



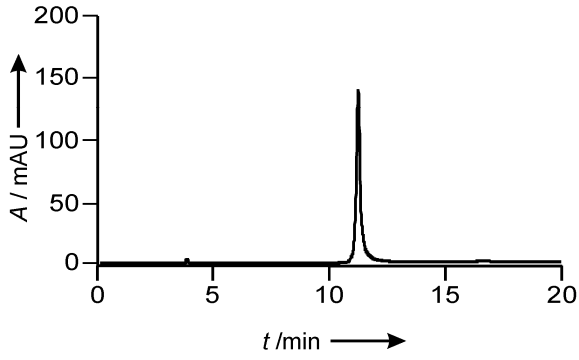


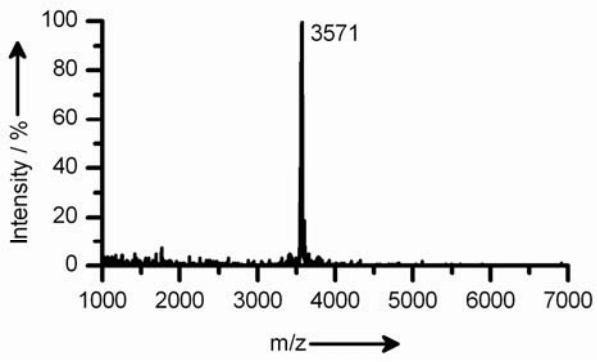
H-cagtcatagt(CH_2SH)tcc-Lys-NH_2 6



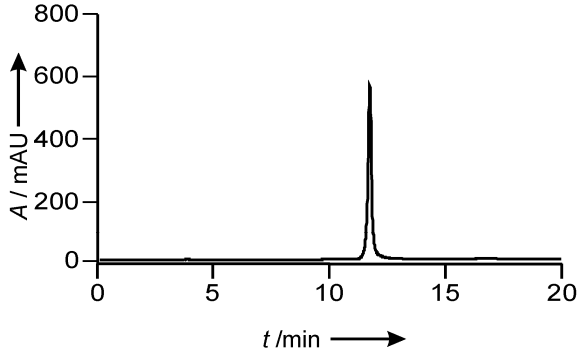


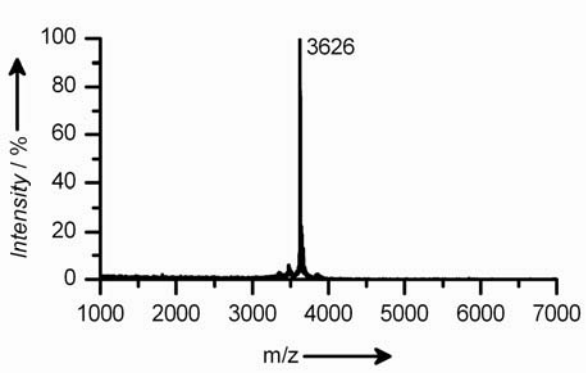
H-acttacctcacgc-Lys-NH_2 7



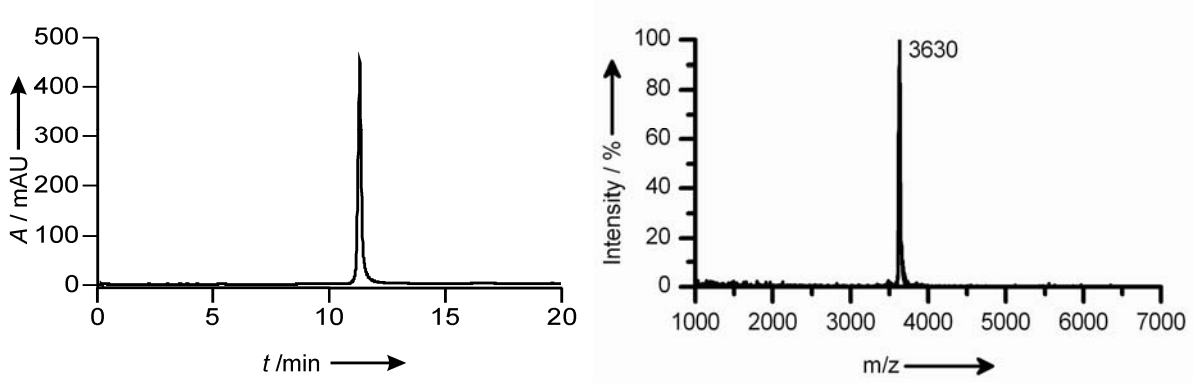


H-cagtcatagttcc-Lys-NH_2 8

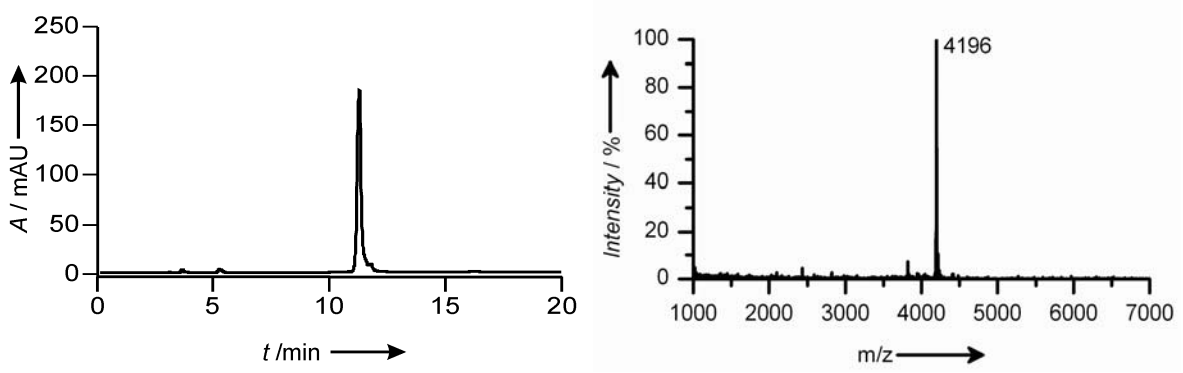




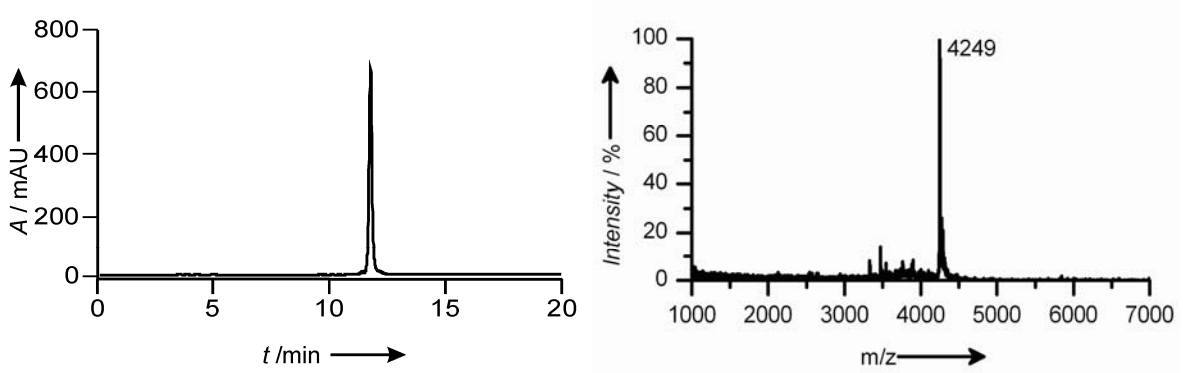
H-caatcgtatccag-Lys-NH_2 9



H-actt(CH_2S~βLacNAc)acctcacgc-Lys-NH_2 15



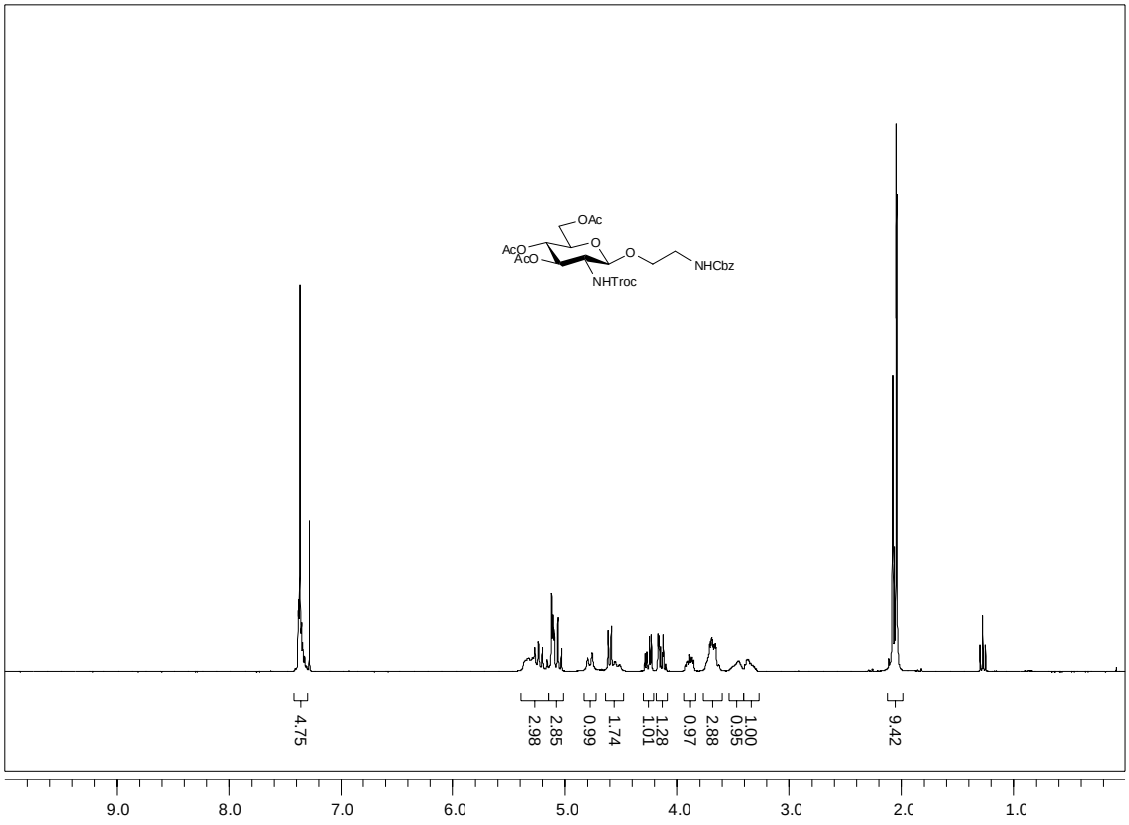
H-cagtcatagt(CH_2S~βLacNAc)tcc-Lys-NH_2 16



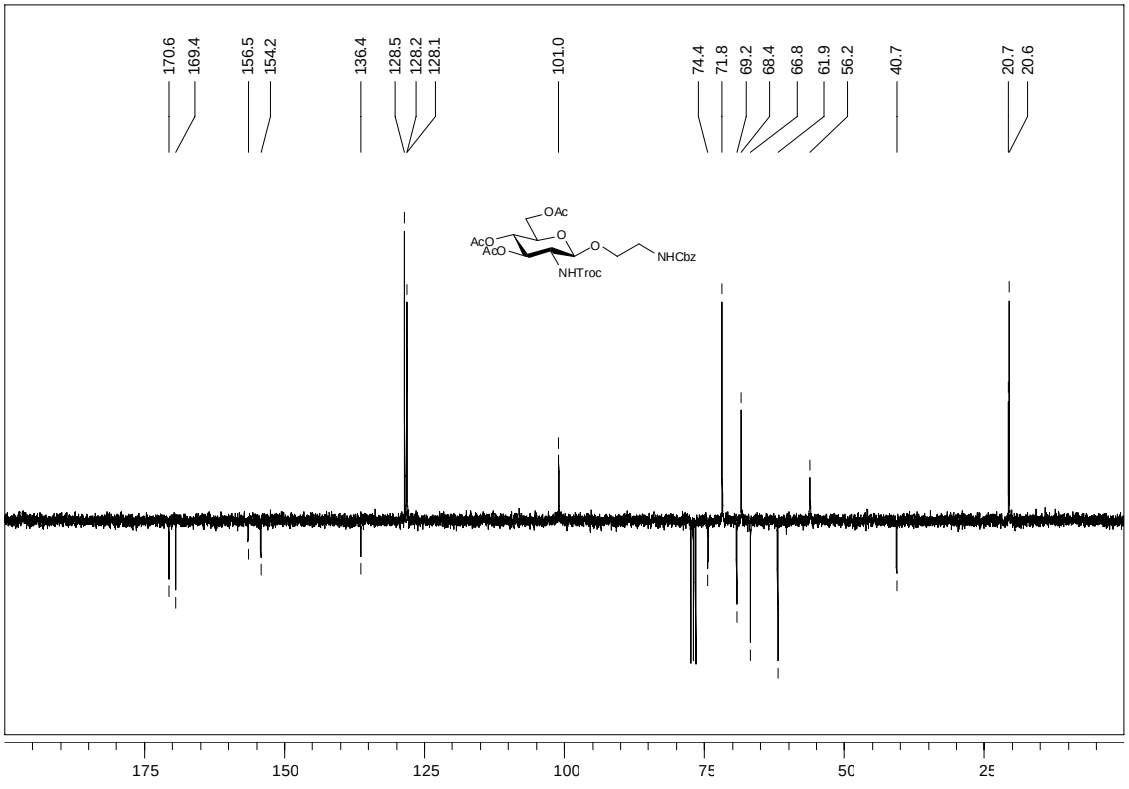
NMR-spectra

3,4,6-Tri-*O*-acetyl-1-(*N*-(benzyloxycarbonyl)-2-aminoethyl)-*N*-(2,2,2-trichloroethyloxycarbonyl)-β-glucosaminide 11

^{1}H-NMR (300 MHz, $CDCl_3$)

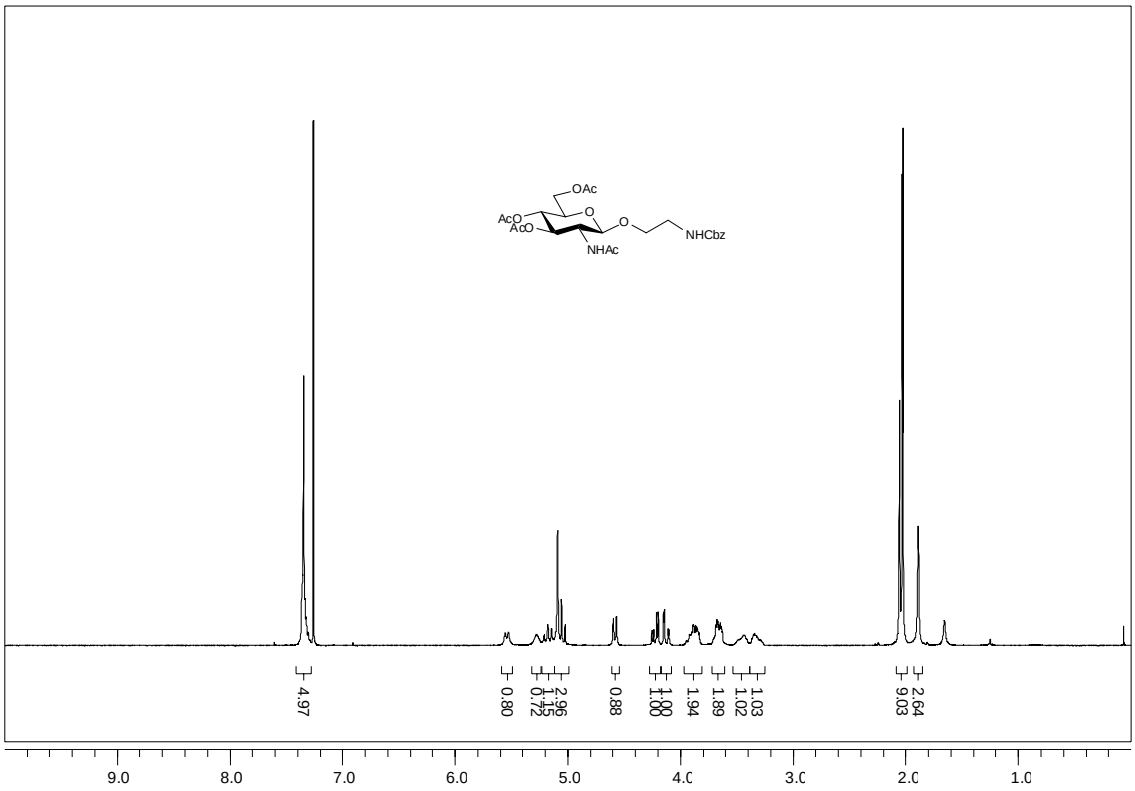


^{13}C-APT-NMR (75 MHz, $CDCl_3$)

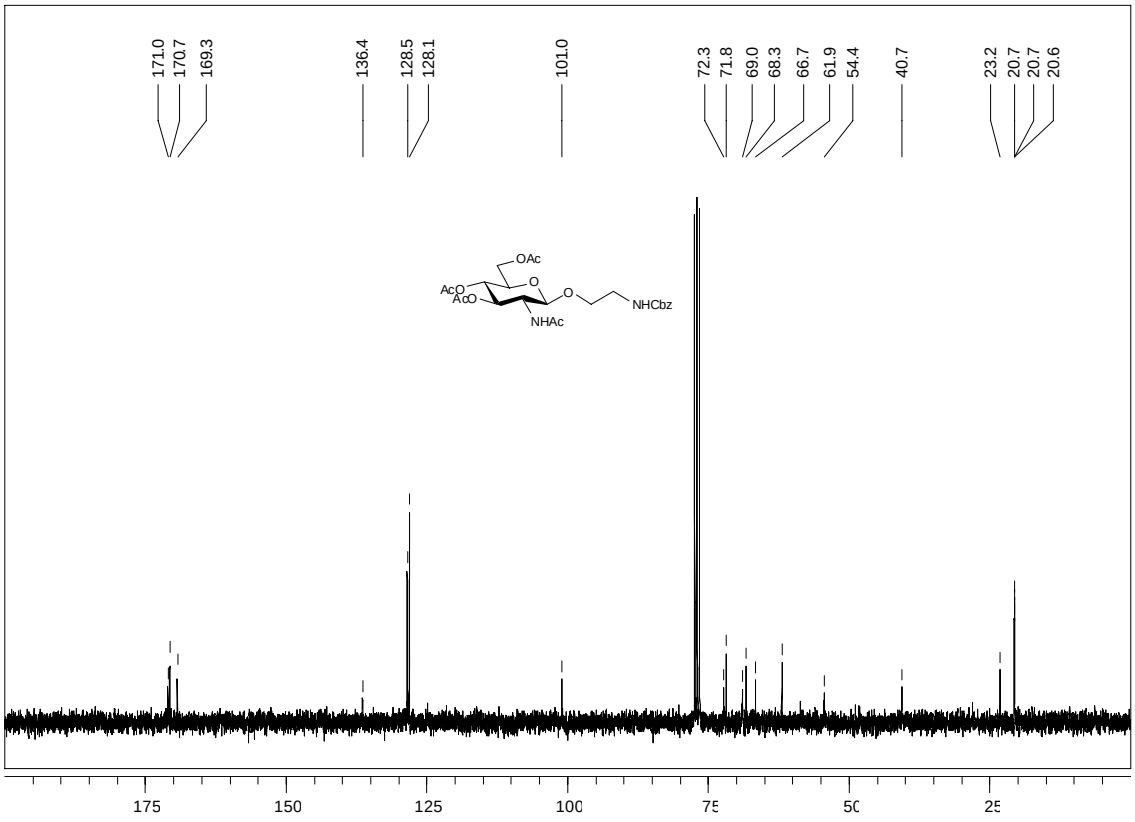


3,4,6-Tri-*O*-acetyl-1-(*N*-(benzyloxycarbonyl)-2-aminoethyl)-β-*N*-acetylglucosaminide

^{1}H-NMR (300 MHz, $CDCl_3$)

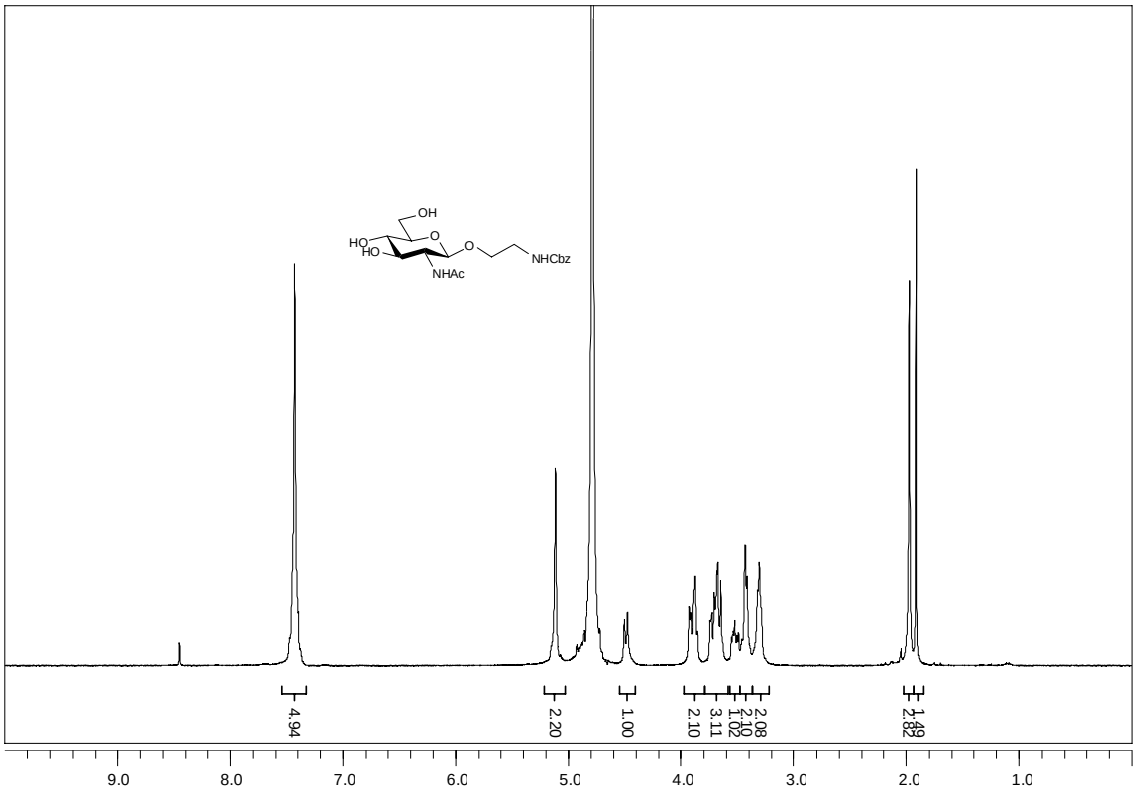


^{13}C-NMR (75 MHz, $CDCl_3$)

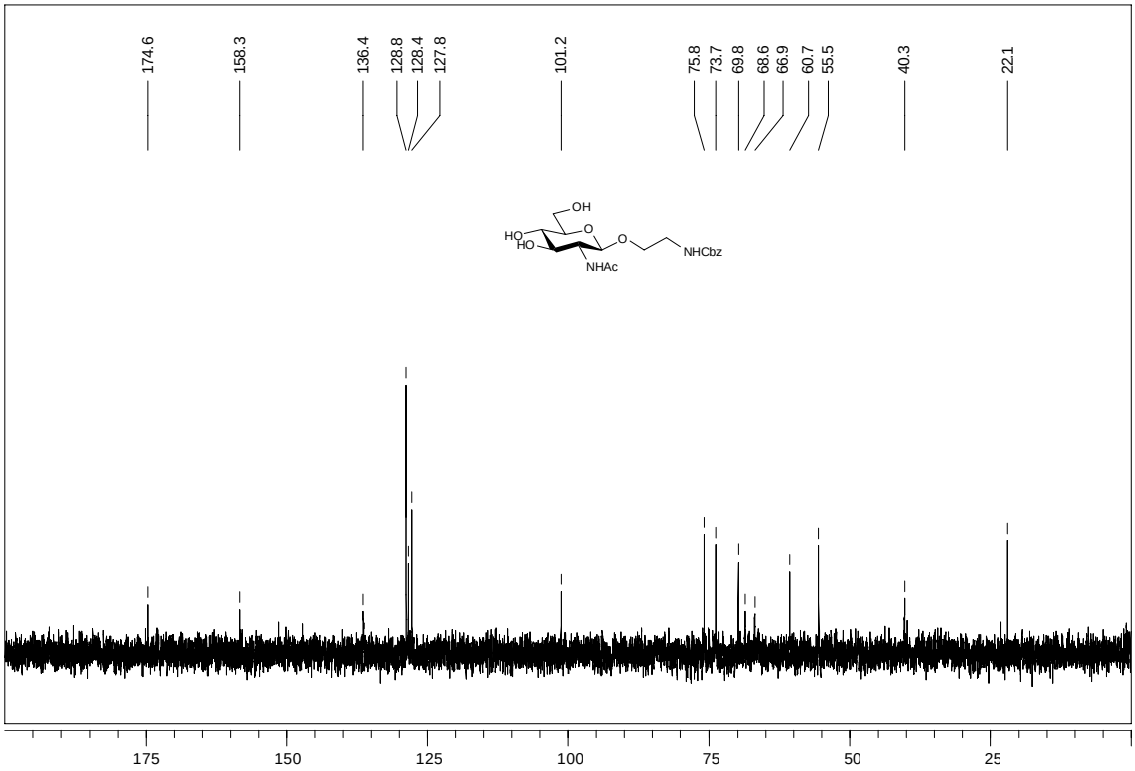


(*N*-(Benzyloxycarbonyl)-2-aminoethyl)-β-*N*-acetylglucosaminide 12

^{1}H-NMR (300 MHz, D_2O)

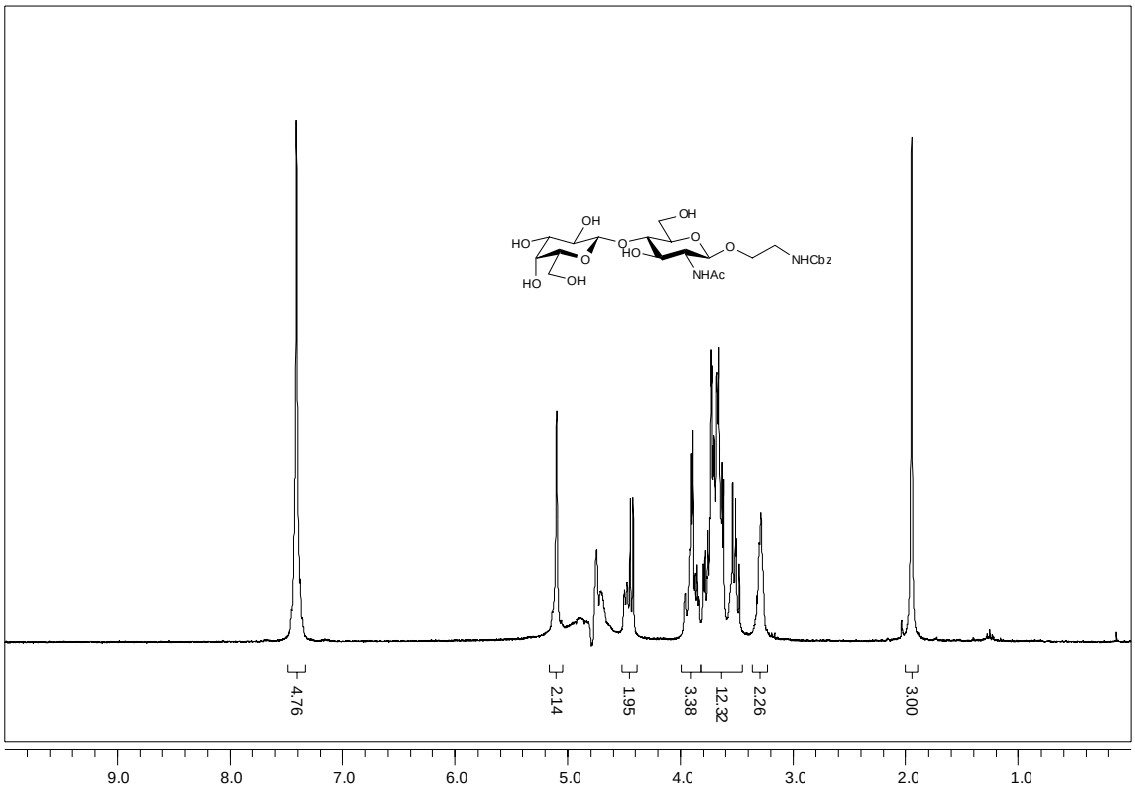


^{13}C-NMR (75 MHz, D_2O)

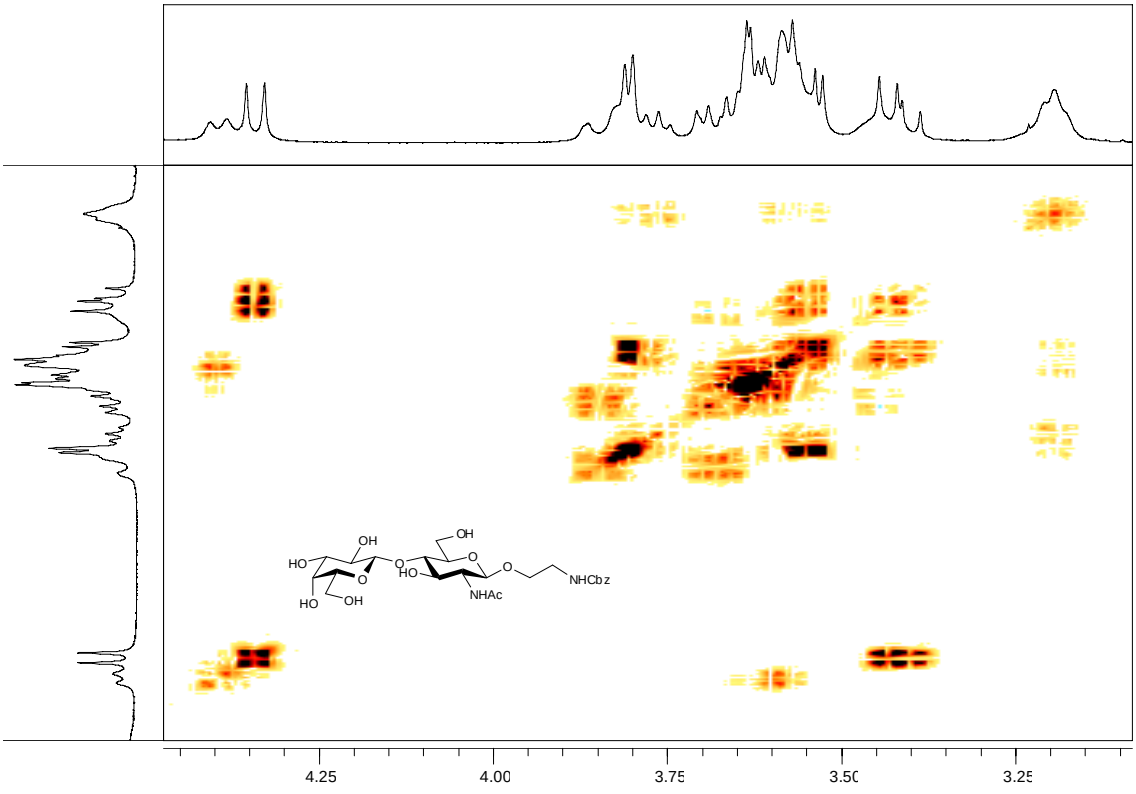


(*N*-(Benzyloxycarbonyl)-2-aminoethyl)-β-*N*-acetyllactosaminide 13

^{1}H-NMR (300 MHz, D_2O)

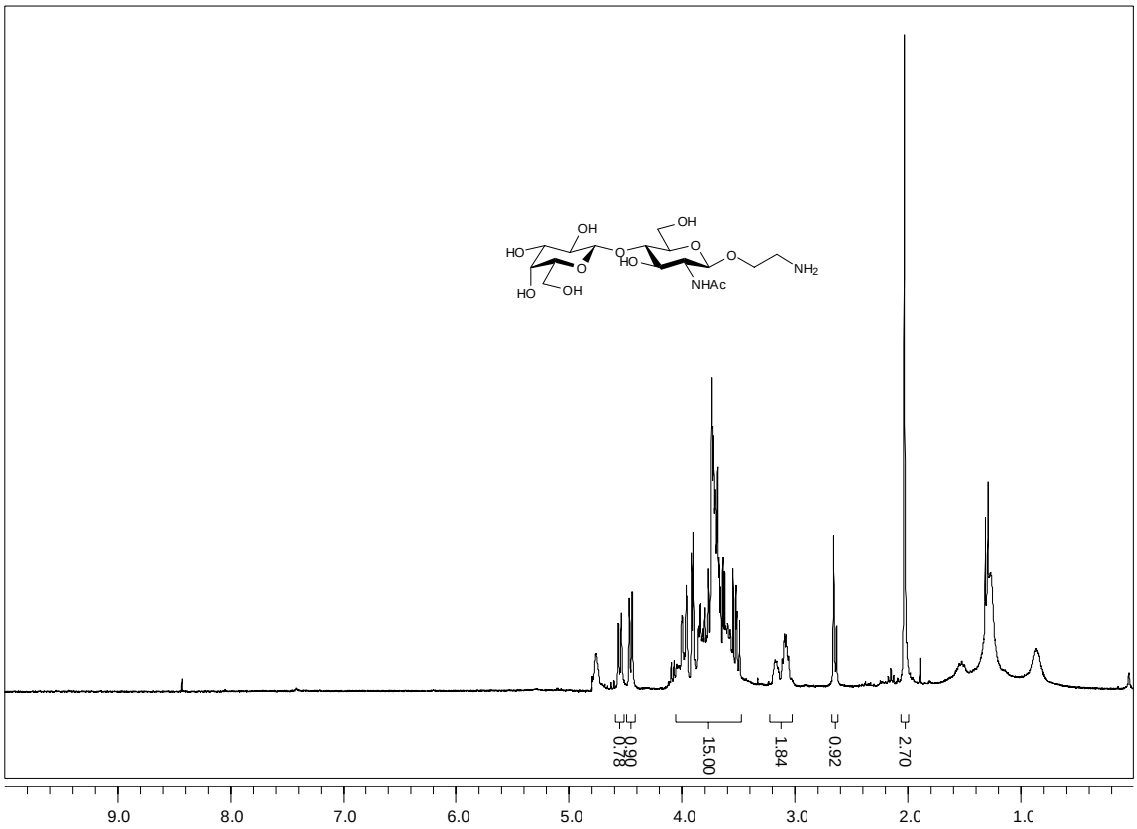


^{1}H,^{1}H-COSY (300 MHz, D_2O)

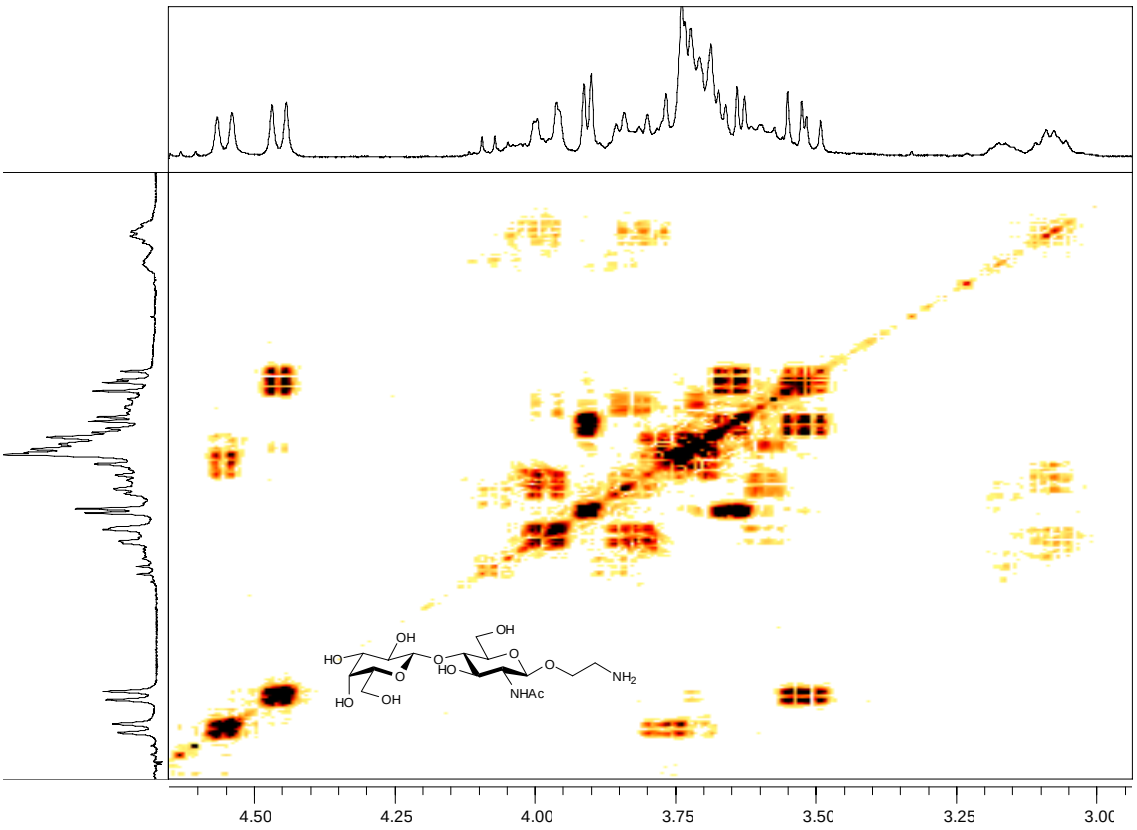


(2-Aminoethyl)-β-*N*-acetyllactosaminide

^{1}H-NMR (300 MHz, D_2O)

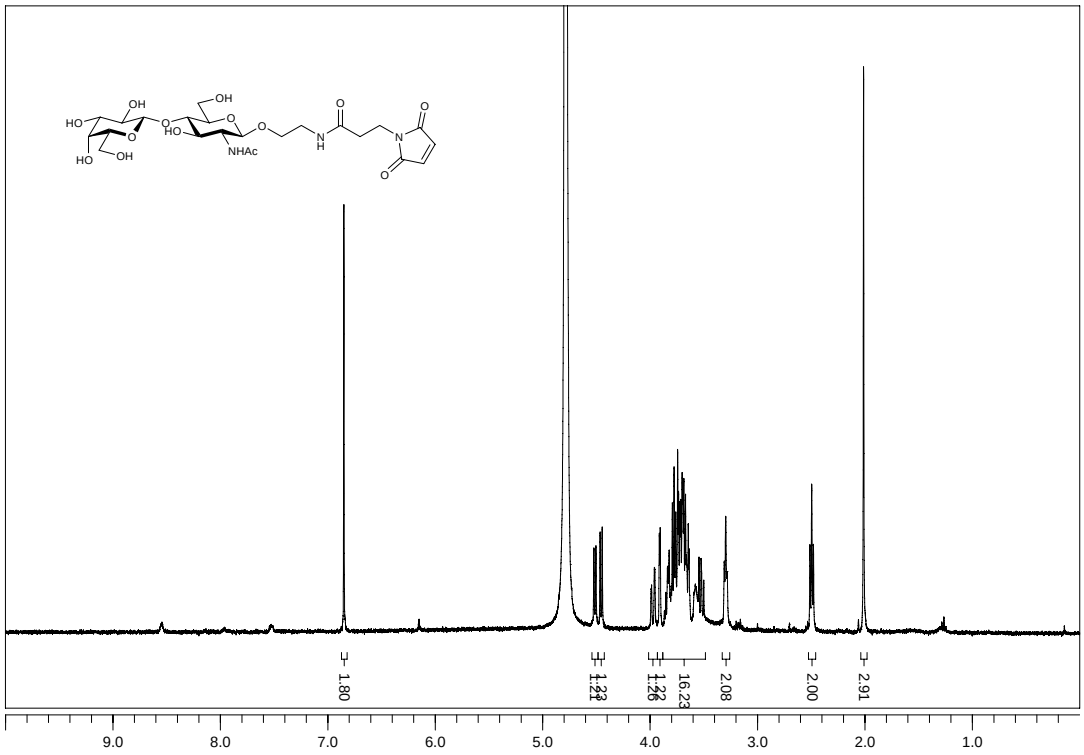


^{1}H,^{1}H-COSY (300 MHz, D_2O)

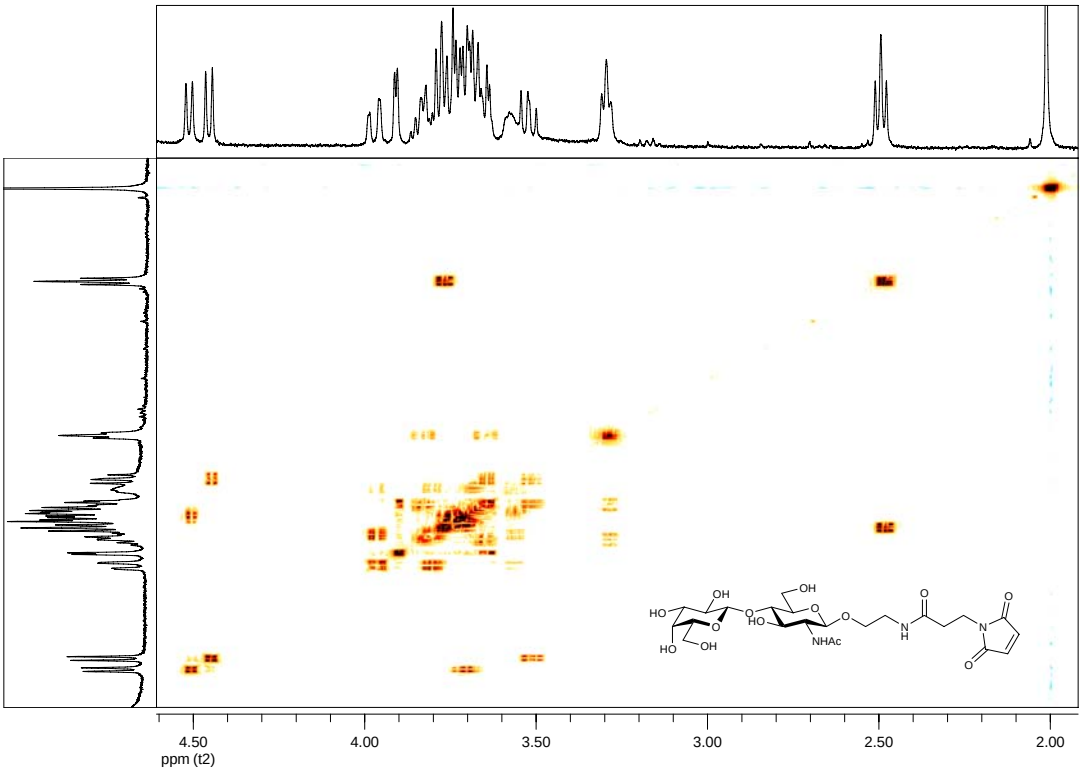


(*N*-(3'-Maleimidopropanoyl)-2-aminoethyl)-β-*N*-acetyllactosaminide 14

^{1}H-NMR (400 MHz, $CDCl_3$)

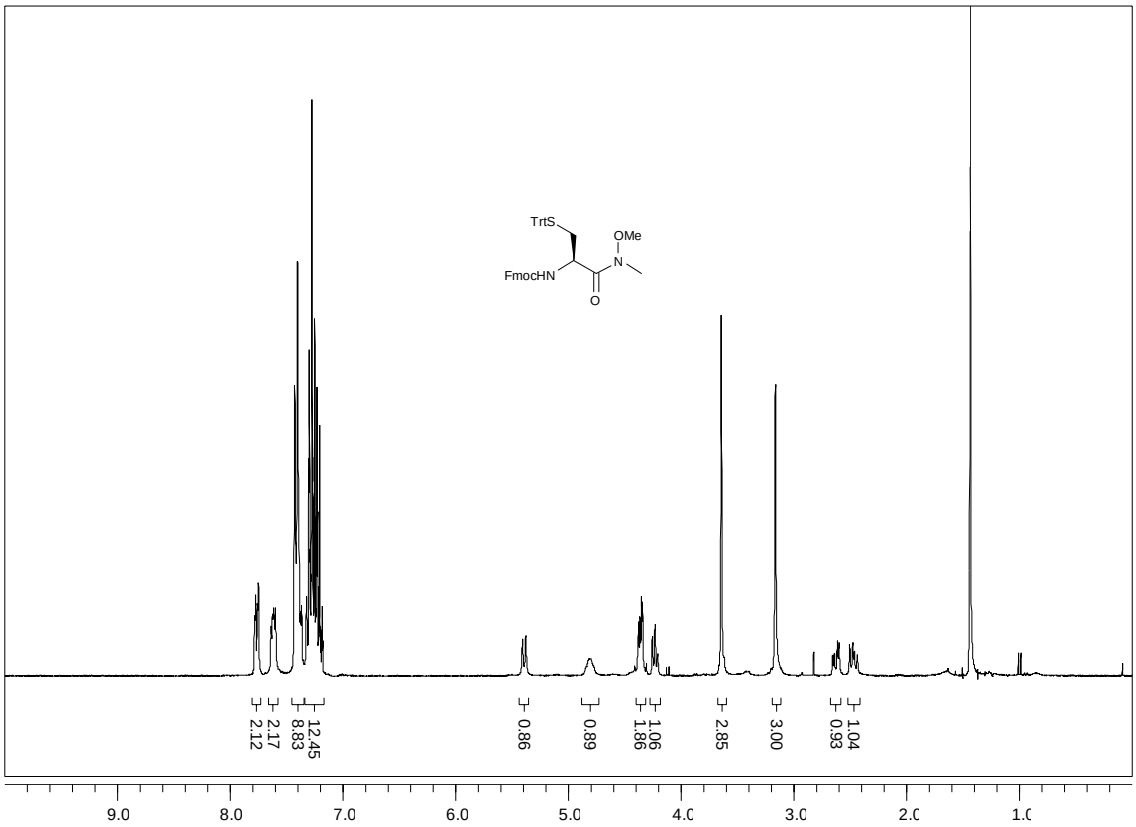


^{1}H,^{1}H-COSY (400 MHz, D_2O)

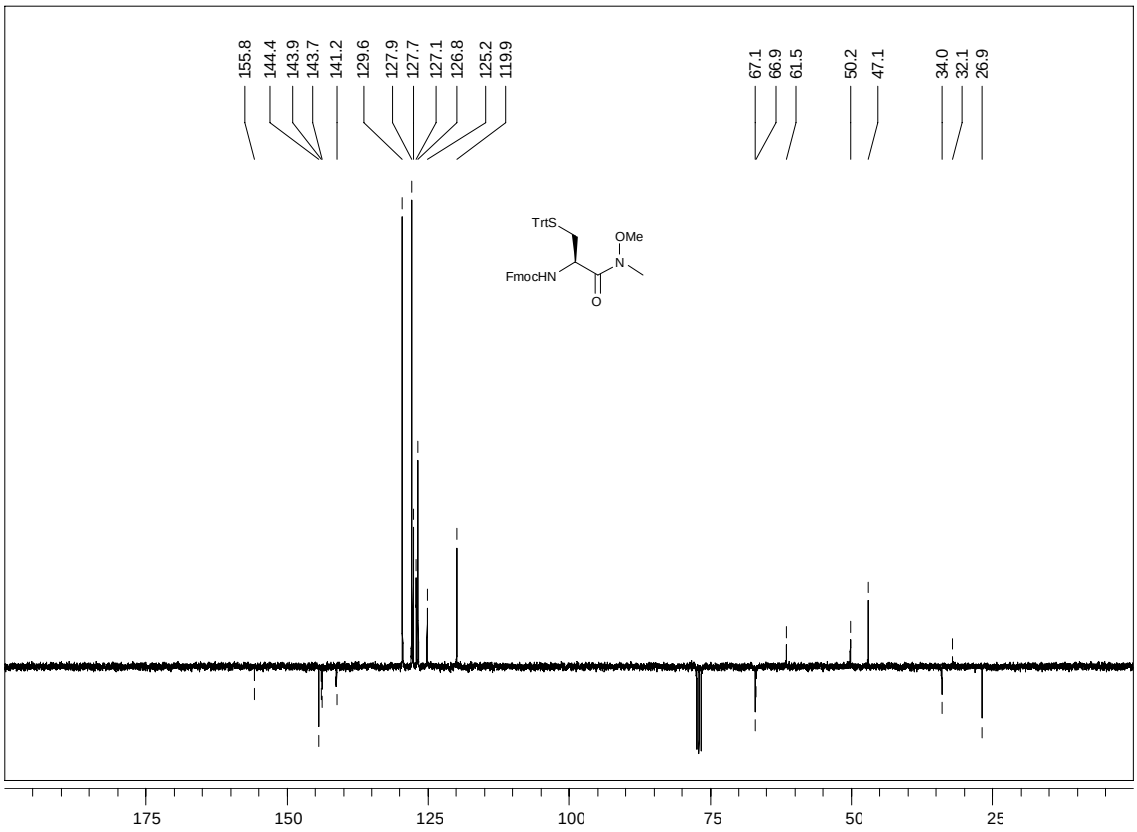


(*R*)-*N*-Fluorenylmethoxycarbonyl-*S*-tritylcysteine-weinrebamide

^{1}H-NMR (300 MHz, $CDCl_3$)

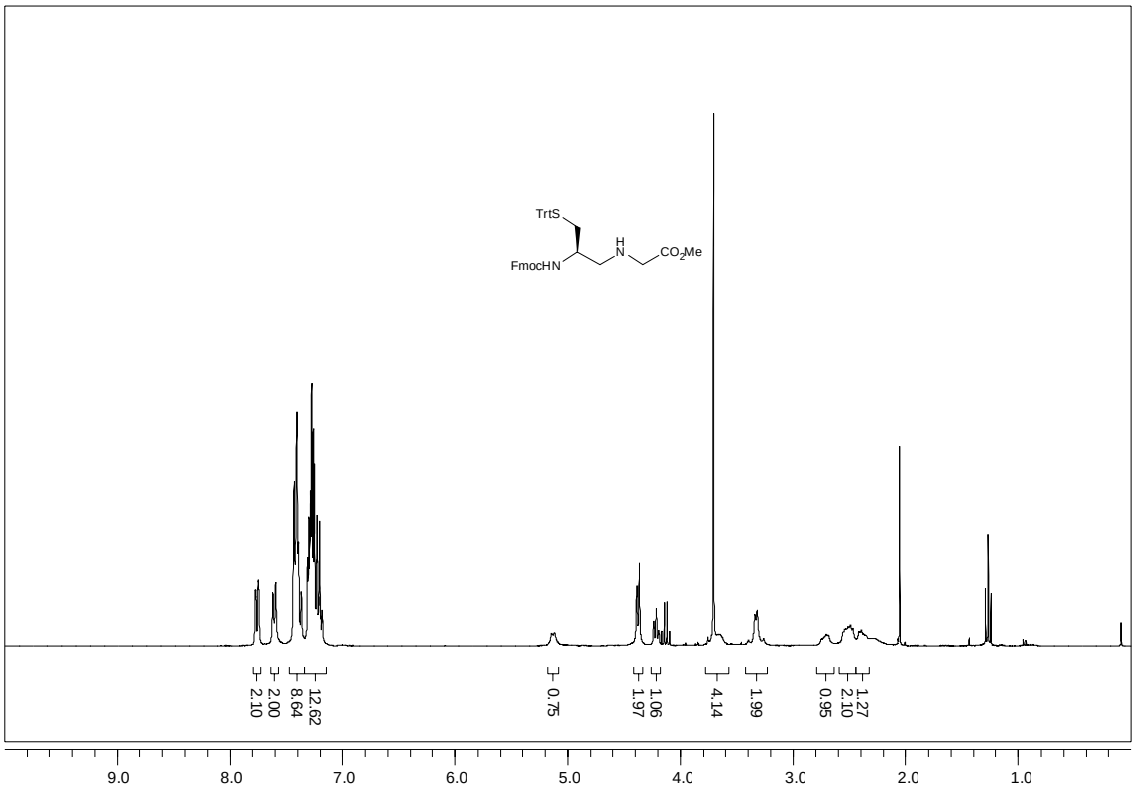


^{13}C-APT-NMR (75 MHz, $CDCl_3$)

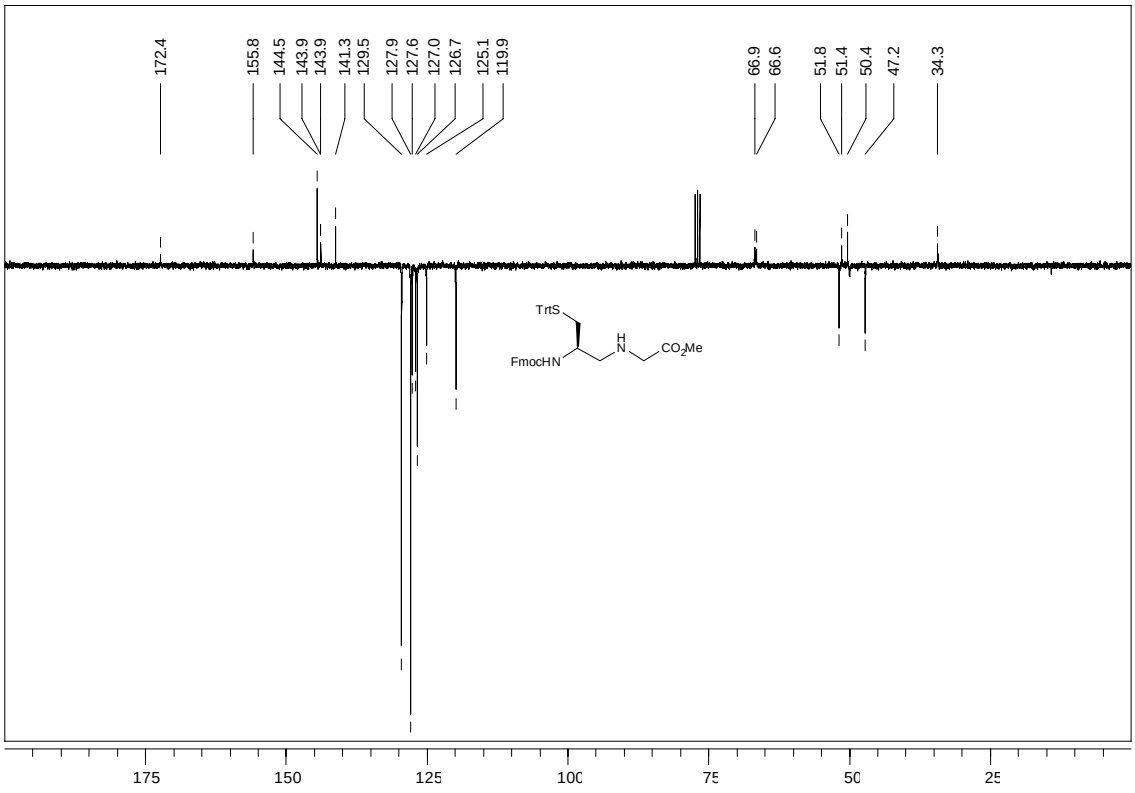


(*R*)-*N*-Fluorenylmethoxycarbonyl-1-(*S*-tritylthiomethyl)-aminoethylglycine methylester

^{1}H-NMR (300 MHz, $CDCl_3$)

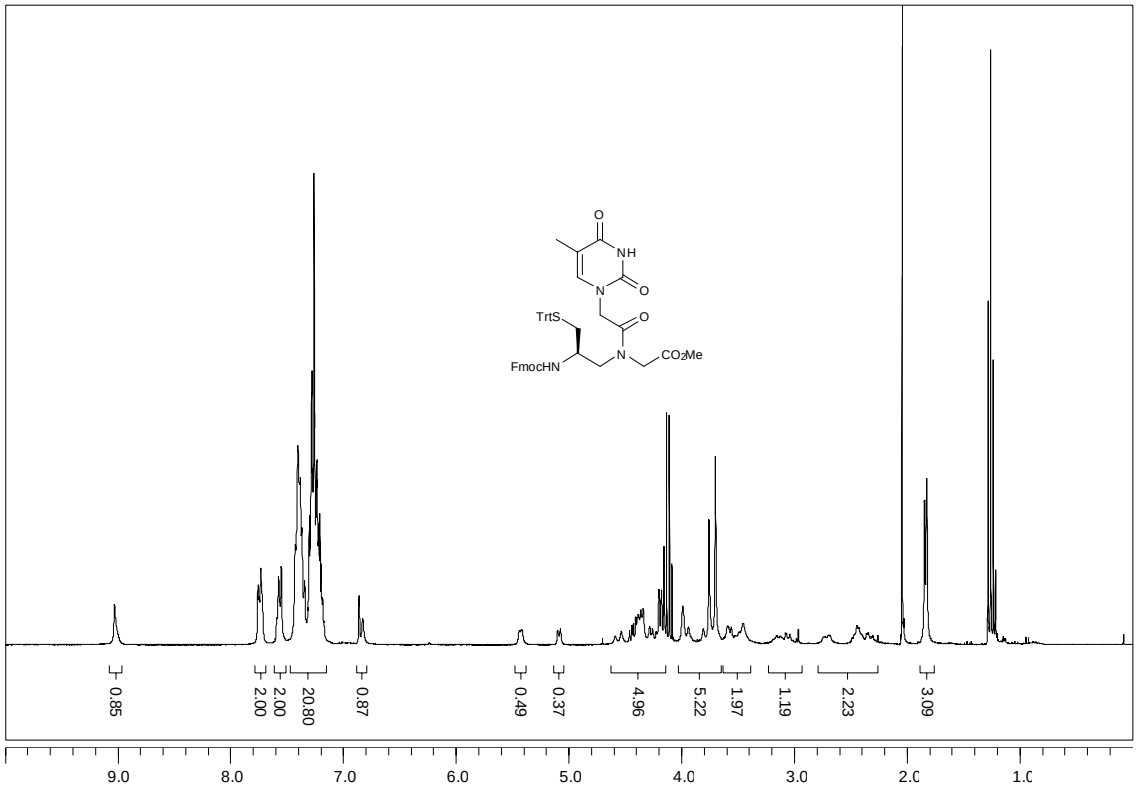


^{13}C-APT-NMR (75 MHz, $CDCl_3$)

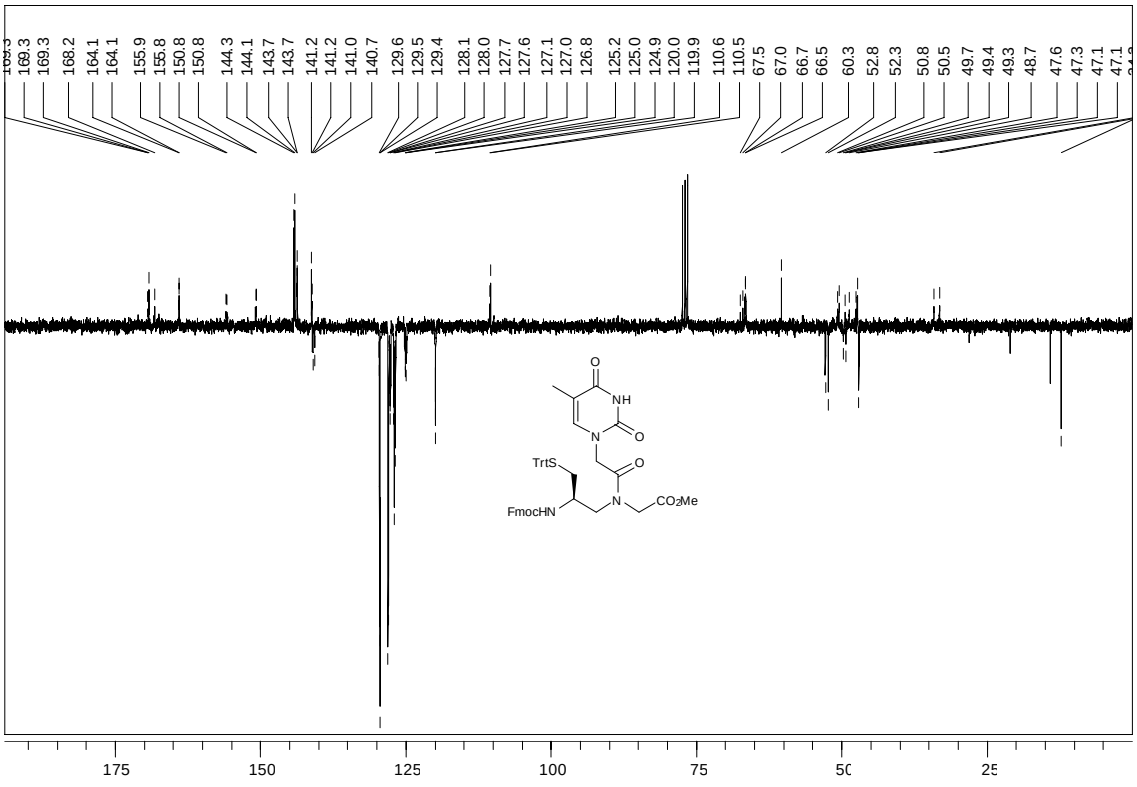


(*R*)-*N*-[*N*-Fluorenylmethoxycarbonyl-1-(*S*-tritylthiomethyl)-aminoethyl]-*N*-[(1-thyminyl)-acetyl]-glycine methylester

^{1}H-NMR (300 MHz, $CDCl_3$)

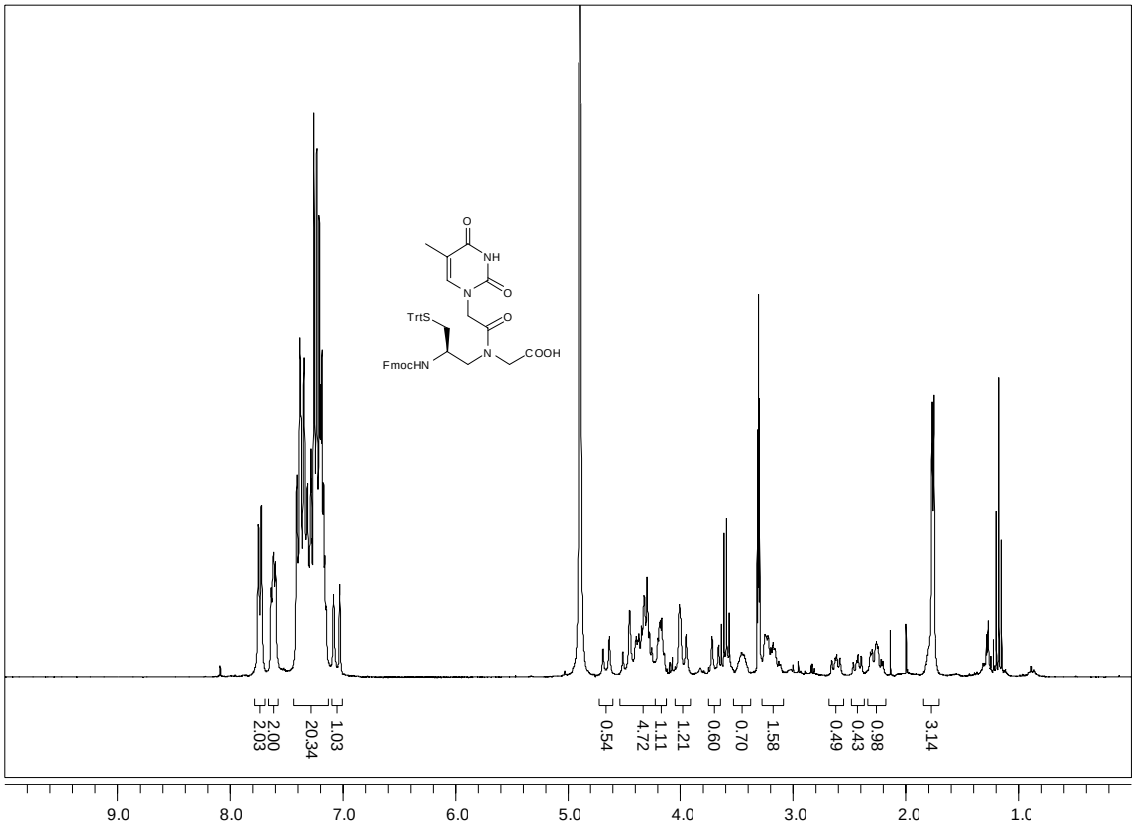


^{13}C-APT-NMR (75 MHz, $CDCl_3$)

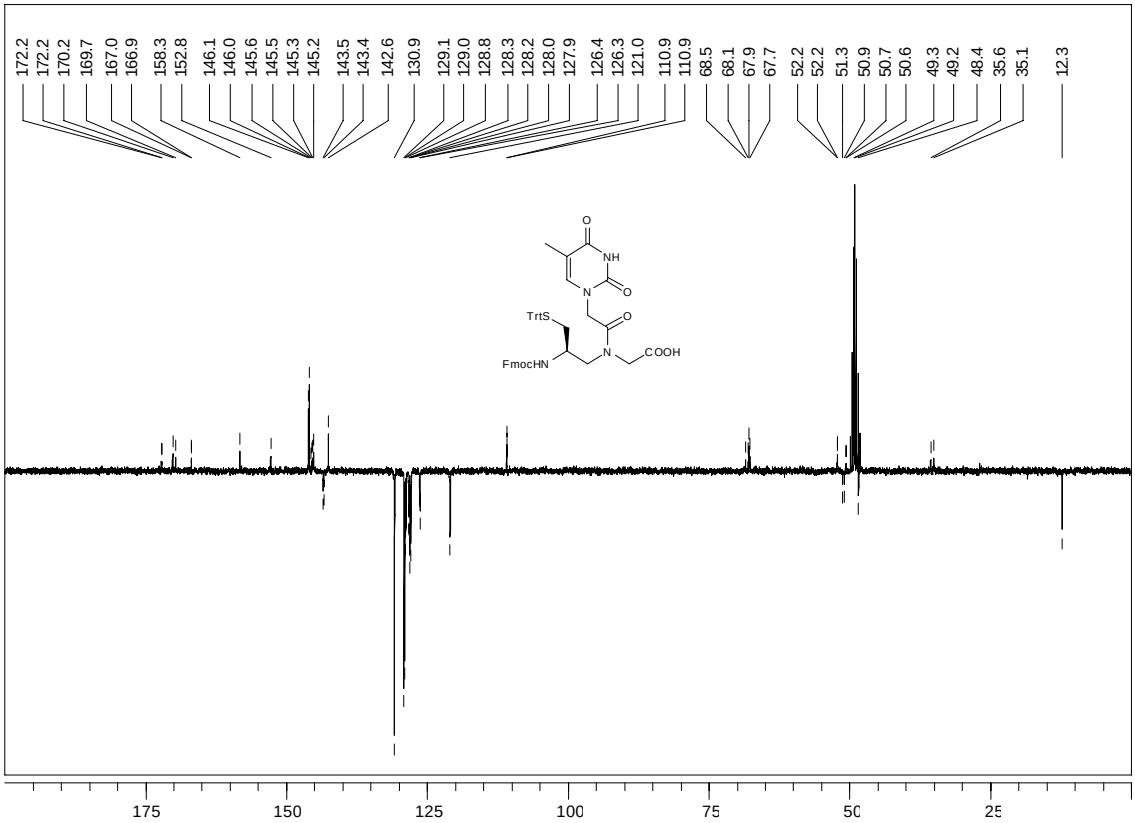


(*R*)-*N*-[*N*-Fluorenylmethoxycarbonyl-1-(*S*-tritylthiomethyl)-aminoethyl]-*N*-[(1-thyminyl)-acetyl] glycine 1

^{1}H-NMR (300 MHz, CD_3OD)



^{13}C-APT-NMR (75 MHz, CD_3OD)



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