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# Combining Weak Affinity Chromatography, NMR Spectroscopy and Molecular Simulations in Carbohydrate-Lysozyme Interaction Studies

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## List of compounds in Table 1

**1** *N*-Acetyl-D-glucosamine

**2** Methyl 2-acetamido-2-deoxy-α-D-glucopyranoside

**3** Methyl 2-acetamido-2-deoxy-β-D-glucopyranoside

**4** Methyl 2-acetamido-2-deoxy-1-seleno-β-D-glucopyranoside

**5** *N*-Acetyl-D-galactosamine

**6** *N*-Acetyl-muraminic acid

**7** NAG-thiazoline

**8** NAG-selenazoline

**9** *N*,*N*'-diacetylchitobiose

**10** *N*-Acetyl-lactosamine

**11** β-D-Gal*p*-(1→4)-β-D-Glc*p*NAc-OMe

**12** β-D-Glc*p*NAc-(1→4)-β-D-Glc*p*NAc-OMe

**13** β-D-Glc*p*NAc-(1→6)-α-D-Man*p*-OMe

**14** β-D-Glc*p*NAc-(1→3)-α-D-Glc*p*-OMe

**15** β-D-Glc*p*NAc-(1→3)-β-D-Gal*p*-OMe

**16** α-D-Gal*p*-(1→3)-α-D-Glc*p*NAc-OMe

**17** β-D-Glc*p*NAc-(1→4)-β-D-Gal*p*-OMe

## Weak Affinity Chromatography

*Materials.* Kromasil® silica was kindly provided by Akzo Nobel (EKA chemicals AB, Bohus, Sweden). Hen egg white lysozyme (HEWL), sodium cyanoborohydride, *N*-acetyl-D-glucosamine (GlcNAc), *N*-acetyl-D-galactosamine (GalNAc), and *N-N'*-diacetylchitobiose, β-D-Gal-(1→4)-D-GlcNAc (LacNAc) were purchased from Sigma-Aldrich (St Louis, MO, USA). Compounds were also available at the Department of Organic Chemistry, Stockholm University, or from previous studies (1-3).

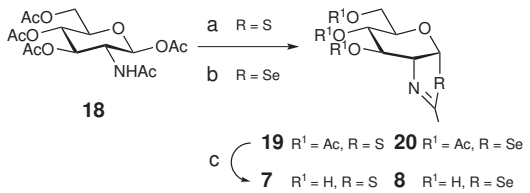
*Immobilization of HEWL to porous silica.* The yield of the immobilization was 67%, calculated from the difference in absorbance of the protein solution before and after immobilization. This is considerably less than what is normally achieved using the reductive amination coupling procedure; the expected yield is about 95% provided that the immobilization capacity of the silica is not exceeded. The reason for the low yield is probably that the coupling was performed at pH 7 (optimized for the coupling procedure *per se*), but at this pH lysozyme exists as dimers (4) that dissociate after immobilization. Consequently, a low coupling yield is obtained for the HEWL protein, but as it is inexpensive this does not pose a problem.

*Frontal chromatography.* The amount (moles) of the saccharide that was needed to reach equilibrium with the binding sites in the affinity column was indicated by the midpoint of the "front" or break-through curve expressed as the retention volume ($\Delta v$). The retention volume was calculated from the retention time subtracted by the void time, and the flow rate of the separation. Changing the saccharide concentration ([A]) influences the front position and by plotting $\Delta v \times$ [A] versus [A] a one-site binding hyperbola according to Eq. S1 was obtained. The number of binding sites ($B_{max}$) on the column and the dissociation constant ($K_D$) of the analyzed compound was obtained via non-linear regression analysis using GraphPad Prism 5 (SanDiego, CA, USA).

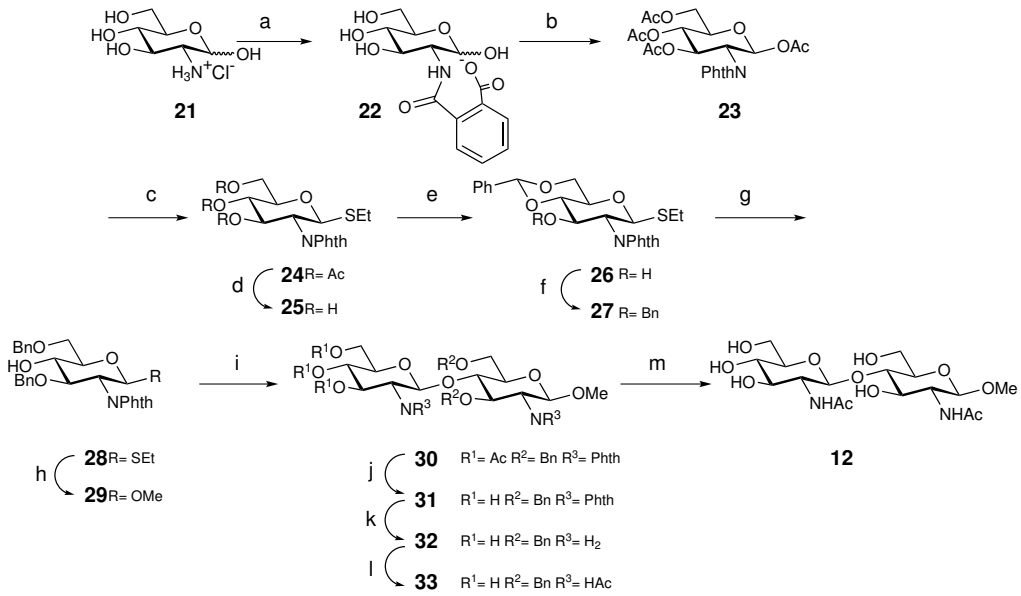
$$\Delta v \times [A] = \frac{B_{max} \times [A]}{K_D + [A]} \qquad \text{(S1)}$$

*Weak affinity chromatography (zonal chromatography).* As the concentrations of the injected analytes were low compared to the expected $K_D$ values, the simplified version of Eq. S1 ($K_D = B_{max}/\Delta v$) was used in order to calculate the $K_D$ value of each compound. The number of binding sites of each column ($B_{max}$) was known from the frontal chromatography evaluation and the $K_D$ values of the analytes were calculated from the retention volume ($\Delta v$).

## Syntheses of 7, 8, 11 and 12

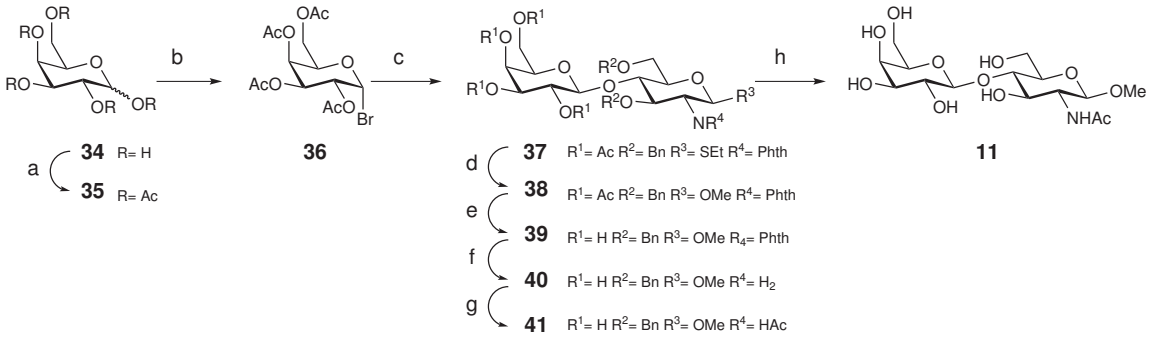



**Scheme S1.** *Reagents and conditions:* a) Lawesson's reagent, Toluene, 80 °C, 85%; b) Woollins' reagent, Toluene, 80 °C, 40%; c) NaOMe, MeOH, 90-95%.




**Scheme S2.** *Reagents and conditions:* a) $Phth_2O$, NaOH (aq); b) $Ac_2O$, pyridine, 90% over two steps; c) EtSH, $BF_3{\cdot}Et_2O$, $CHCl_3$, 78%; d) NaOMe, MeOH; e) *p*-TsOH, DMF, benzaldehyde dimethylacetal, 42% over two steps; f) NaH, BnBr, DMF, 89%; g) $NaCNBH_3$, $HCl/Et_2O$, THF, 72%; h) MeOH, NIS/AgOTf, $CH_2Cl_2$, 70%; i) **24**, NIS/AgOTf, $CH_2Cl_2$, 85%; j) NaOMe, MeOH; k) EDA, EtOH, 85% over two steps; l)

$Ac_2O$, MeOH, quantitative; m) $Pd(OH)_2/C$, $MeOH/H_2O$, $H_2$, 100 psi, 42%.




**Scheme S3.** *Reagents and conditions:* a) $Ac_2O$, $HClO_4$, 93%; b) AcBr, MeOH, 60%; c) **29**, AgOTf/Toluene, $CH_2Cl_2$, 62%; d) MeOH, NIS/AgOTf, $CH_2Cl_2$, 74%; e) NaOMe, MeOH; f) EDA, EtOH, 94% over two steps; g) $Ac_2O$, MeOH, 75%; h) $Pd(OH)_2/C$, $MeOH/H_2O$, $H_2$, 100 psi, 40%.

TLC was carried out on Merck® precoated $F_{254}$ plates that were visualized in UV-light and developed in 10% $H_2SO_4$, AMC or Ninhydrin solutions. For column chromatography, Davisil® silica gel 60 (230-400 mesh) was used.

NMR spectra were recorded in $CDCl_3$ using internal TMS (0 ppm) standard as $^1H$ reference and solvent resonance (77.16 ppm) as $^{13}C$ reference, MeOD-d4 using solvent residual resonance (3.31 ppm) as $^1H$ reference and solvent resonance (49.00 ppm) as $^{13}C$ reference and $D_2O$ using internal TSP (0 ppm) standard as $^1H$ reference and external dioxane in $D_2O$ (67.4 ppm) as $^{13}C$ reference. A Bruker Avance II 400 MHz spectrometer equipped with a BBO probe was used for all NMR assignments of the synthetic intermediates. $^1H$ NMR chemical shifts and proton-proton coupling constants for all characterized species were refined with the PERCH NMR software.

FPLC purifications on the target compounds were performed on an ÄKTA™ system equipped with a Superdex™ column. Detection was made with UV and RI detectors. The eluent system was $H_2O$ with 1% BuOH and the flowrate 2 mL/min.

Electrospray ionization high resolution mass spectra (ESI-HRMS) were recorded in positive mode using a Bruker MicroTOF 125 spectrometer with 2-propanol/$H_2O$ (1:1) as solvent and sodium formiate as internal calibrant.

**Methyl 2-acetamido-2-deoxy-1-seleno-β-D-glucopyranoside (4).** Methyl 2-acetamido-2-deoxy-1-seleno-β-D-glucopyranoside (**9**) was synthesized as described in reference 5.

**(3a*R*,5*R*,6*S*,7*R*,7a*R*)-5-(hydroxymethyl)-2-methyl-5,6,7,7a-tetrahydro-3a*H*-pyrano[3,2-*d*]thiazole-6,7-diol (7).** The NAG-thiazoline (**7**) was synthesized from **18** as described in reference 6.

**(3a*R*,5*R*,6*S*,7*R*,7a*R*)-5-(hydroxymethyl)-2-methyl-5,6,7,7a-tetrahydro-3a*H*-pyrano[3,2-*d*]selenazole-6,7-diol (8).** The same procedure as for **7** was used for the NAG-selenazoline except that Woollins' reagent (7) was used instead of Lawesson's.

(3a*R*,5*R*,6*S*,7a*R*)-5-(acetoxymethyl)-2-methyl-5,6,7,7a-tetrahydro-3a*H*-pyrano[3,2-*d*]oxazole-6,7-diyl diacetate (**20**)

$^1$H NMR ($CDCl_3$, 25 °C, 400 MHz): $\delta$ 6.76 (d, 1H, $J$ = 6.7 Hz, H1), 4.43 (m, 1H, $J$ = 6.7, 4.0, 2.2, 1.1 Hz, H2) 5.59 (dd, 1H, $J$ = 4.0, 2.6 Hz, H3), 4.99 (m, 1H, $J$ = 1.1, 2.6, 9.6 Hz, H4), 3.62 (m, 1H, $J$ = 9.6, 5.9, 2.7 Hz, H5), 4.15 (dd, 1H, $J$ = 5.9, -12.4 Hz, $H6_R$), 4.13 (dd, 1H, $J$ = 2.7, -12.4 Hz, $H6_S$), 2.40 (d, 1H, $J$ = 2.2 Hz, Me) 2.09, 2.09, 2.14 (s, 9H, Ac 3,4,6).

(3a*R*,5*R*,6*S*,7*R*,7a*R*)-5-(hydroxymethyl)-2-methyl-5,6,7,7a-tetrahydro-3a*H*-pyrano[3,2-*d*]selenazole-6,7-diol (**8**)

$^1$H NMR ($D_2O$, 25 °C, 400 MHz): $\delta$ 6.85 (d, 1H, $J$ = 6.4 Hz, H1), 4.36 (m, 1H, $J$ = 6.4, 5.1, 2.0, 1.0 Hz, H2) 4.25 (dd, 1H, $J$ = 5.1, 3.9 Hz, H3), 3.61 (m, 1H, $J$ = 1.0, 3.9, 9.0 Hz, H4), 3.43 (m, 1H, $J$ = 9.0, 7.1, 2.4 Hz, H5), 3.69 (dd, 1H, $J$ = 7.1, -12.3 Hz, $H6_R$), 3.85 (dd, 1H, $J$ = 2.4, -12.3 Hz, $H6_S$), 2.41 (d, 1H, $J$ = 2.0 Hz, Me). ESI-HRMS: calcd for $C_8H_{13}NO_4Se$ $[M + Na]^+$ 289.9902, found 289.9889.

**Ethyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (24).** D-Glucosamine hydrochloride (**21**) (29.99 g, 139 mmol) was dissolved in a solution of 1 M NaOH (aq) (9.44 g, 236 mmol) (240 mL). Phthalic anhydride was added (30.98 g, 209 mmol) and the reaction mixture was stirred at rt over night. The clear yellow aqueous solution that had formed was washed with 3 × EtOAc. The water was removed under reduced pressure to afford 2-(2-carboxybenzamido)-2-deoxy-D-glucopyranose (**22**), as a yellow foam. Crude compound **22** was dissolved in pyridine (200 mL). At 0 °C, acetic anhydride (140 mL, 1.5 mol) was added. The mixture was left to attain ambient temperature and was stirred over night. TLC indicated one product and the reaction was quenched with MeOH at 0 °C. Solvents were evaporated and co-evaporation with toluene was performed. The brown suspension was dissolved in EtOAc and washed with brine, 1 M HCl, brine, satd. $NaHCO_3$ (aq) and brine again. The solution was dried over $MgSO_4$ and evaporation of solvent under reduced pressure gave 59.5 g of the α/β mixture of the product as a white foam. Yield: 90% over two steps. $R_f$ = 0.6 (β), 0.5 (α) (Toluene/EtOAc 1:1). NMR-characterisation of 1,3,4,6-Tetra-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranose (**23**): $^1$H NMR ($CDCl_3$, 25 °C, 400 MHz): $\delta$ 6.52 (d, 1H, $J$ = 8.9 Hz, H1), 4.48 (dd, 1H, $J$ = 8.9, 10.6 Hz, H2), 5.89 (dd, 1H, $J$ = 9.1, 10.6 Hz, H3) 5.22 (dd, 1H, $J$ = 9.1, 10.2 Hz, H4), 4.03 (ddd, 1H, $J$ = 2.1, 4.4, 10.2 Hz, H5), 4.37 (dd, 1H, $J$ = 4.4, -12.5 Hz, $H6_R$), 4.15 (dd, 1H, $J$ = 2.1, -12.5 Hz, $H6_S$), 1.87, 2.00, 2.04, 2.12 (s, 12H, Ac 1,3,4,6), 7.87 (m, 1H, Phth H1A), 7.87 (m, 1H, Phth H1B), 7.76 (m, 1H, Phth H2A), 7.76 (m, 1H, Phth H2B). $^{13}$C NMR ($CDCl_3$, 25 °C, 100 MHz): $\delta$ 89.92 (C1), 53.65 (C2), 70.66 (C3), 68.44 (C4), 72.79 (C5), 61.68 (C6), 168.79, 169.62, 170.18, 170.82 (Ac 1,3,4,6 C=O), 20.55, 20.76, 20.89, 20.92 (Ac 1,3,4,6), 167.54 (Phth C=O), 131.37 (Phth C-*ipso*), 123.95 (Phth C1), 134.63 (Phth C2). ESI-HRMS: calcd for $C_{22}H_{23}NO_{11}$ $[M + Na]^+$ 500.1163, found 500.1164. Compound **23** (5.86 g, 12.3 mmol) was dissolved in dry $CHCl_3$ (50 mL). At 0 °C, $BF_3{\cdot}OEt_2$ (7.8 mL, 62 mmol) was added dropwise and the mixture was stirred for 10 min in inert atmosphere and was allowed to attain rt. EtSH (2.7 mL, 37 mmol) was added and the reaction was stirred at rt and inert atmosphere for 2 h. TLC analysis indicated completion of the reaction and quenching was done with $NaHCO_3$ (s) at 0 °C. Removal of solvent was done under reduced pressure and toluene was twice added for the purpose of co-evaporation. Extraction with DCM, washing with

brine, 2 × satd. $NaHCO_3$ (aq) and brine again and drying over anhydrous $MgSO_4$ followed by solvent evaporation, yielded a white foam. The crude product was re-crystallised from 99.7% EtOH to afford 4.60 g of colourless crystals of **24** (9.6 mmol). Yield: 78%. $R_f$ = 0.65 (Toluene/EtOAc 1:1). ESI-HRMS: calcd for $C_{22}H_{25}NO_9S$ $[M + Na]^+$ 502.1142, found 502.1143.

$^1$H NMR ($CDCl_3$, 25 °C, 400 MHz): $\delta$ 5.49 (d, 1H, *J* = 10.6 Hz, H1), 4.40 (dd, 1H, *J* = 10.3, 10.6 Hz, H2), 5.83 (dd, 1H, *J* = 9.2, 10.3 Hz, H3), 5.18 (dd, 1H, *J* = 9.2, 10.2 Hz, H4), 3.90 (ddd, 1H, *J* = 2.3, 5.0, 10.2 Hz, H5), 4.18 (dd, 1H, *J* = 2.3, -12.3 Hz, $H6_S$), 4.31 (dd, 1H *J* = 5.0, -12.3 Hz, $H6_R$), 2.65 (dq, 1H, *J* = 7.5, -12.6 Hz, SEt H1a), 2.71 (dq, 1H, *J* = 7.4, -12.6 Hz, SEt H1b), 1.22 (dd, 3H, *J* = 7.4, 7.5 Hz, SEt H2), 7.85 (m, 1H, Phth H1A), 7.88 (m, 1H, Phth H1B), 7.75 (m, 2H, Phth H2), 1.87, 2.04, 2.11 (s, 9H Ac 3,4,6).

$^{13}$C NMR ($CDCl_3$, 25 °C, 100 MHz): $\delta$ 81.32 (C1), 53.80 (C2), 71.68 (C3), 69.01 (C4), 76.05 (C5), 62.43 (C6), 24.50 (SEt C1), 15.02 (SEt C2), 167.30, 167.91 (Phth C=O), 131.30, 131.73 (Phth C-*ipso*), 123.84 (Phth C1) 134.43, 134.57 (Phth C2), 169.61, 170.24, 170.84 (Ac 3,4,6 C=O), 20.59, 20.76, 20.90 (Ac 3,4,6).

**Ethyl 4,6-*O*-benzylidene-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (26).** Compound **24** (3.7 g, 7.7 mmol) was dissolved in a small amount of MeOH. A few drops of a 1 M solution of NaOMe in MeOH was added until pH 9 was obtained and the mixture was stirred at rt for 90 min. TLC analysis indicated completion of the reaction and the mixture was neutralised with Dowex-$H^+$. Solvent was evaporated yielding crude product, ethyl 2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (**25**), as a white foam. $R_f$ = 0.8 (EtOAc/MeOH/$H_2O$ 7:2:1). Crude compound **25** was dissolved in DMF (30 mL). To the solution, 99% benzaldehyde dimethylacetal (2.5 mL, 17 mmol) and *p*-TsOH (0.4 g, 2.3 mmol) were added. The reaction mixture was stirred over night at rt. To obtain full conversion the reaction flask was put on a rotavapor that maintained a constant, reduced pressure at 50 °C for 6 h and 3×1 mL of 99% benzaldehyde dimethylacetal was added at 2 h intervals. TLC indicated disappearance of starting material and the reaction was quenched with $NEt_3$ (5 mL). Solvents were then evaporated

and a brown oil was formed which was extracted with EtOAc. The solution was washed with brine, satd. $NaHCO_3$ (aq), brine and then dried over anhydrous $MgSO_4$. Solvents were evaporated and the crude product was re-crystallised from 99.7% EtOH affording white crystals of compound **26** (8) (1.46g, 3.3 mmol). Yield: 42% over two steps. $R_f$ = 0.7 (Toluene/EtOAc 2:1). ESI-HRMS: calcd for $C_{23}H_{23}NO_6S$ $[M + Na]^+$ 464.1138, found 464.1117.

$^1$H NMR ($CDCl_3$, 25 °C, 400 MHz): $\delta$ 5.40 (d, 1H, *J* = 10.6 Hz, H1), 4.32 (dd, 1H, *J* = 10.1, 10.6 Hz, H2), 4.65 (ddd, 1H, *J* = 3.3, 9.1, 10.1 Hz, H3), 3.60 (dd, 1H, *J* = 9.1, 9.4 Hz, H4), 3.68 (ddd, 1H, *J* = 5.0, 9.4, 10.1 Hz, H5), 4.39 (dd, 1H, *J* = 5.0, -10.5 Hz, $H6_S$), 3.80 (dd, 1H, *J* = 10.1, -10.5 Hz, $H6_R$), 2.71 (dq, 1H, *J* = 7.4, -12.5 Hz, SEt H1a), 2.66 (dq, 1H, *J* = 7.5, -12.5 Hz, SEt H1b), 1.19 (dd, 3H, *J* = 7.4, 7.5 Hz, SEt H2), 7.86 (m, 1H, Phth H1A), 7.85 (m, 1H, Phth H1B), 7.72 (m, 1H, Phth H2A), 7.72 (m, 1H, Phth H2B), 2.66 (d, 1H, *J* = 3.3 Hz, HO3), 5.57 (s, 1H, benzylidene H1), 7.49 (m, 2H, benzylidene H2), 7.37 (m, 2H, benzylidene H3), 7.37 (m, 1H, benzylidene H4).

$^{13}$C NMR ($CDCl_3$, 25 °C, 100 MHz): $\delta$ 81.99 (C1), 55.58 (C2), 69.66 (C3), 82.24 (C4), 70.47 (C5), 68.75 (C6), 24.31 (SEt C1). 14.99 (SEt C2), 167.83, 168.33 (Phth C=O), 131.68, 131.83 (Phth C-*ipso*), 123.46, 123.99 (Phth C1), 134.33 (Phth C2), 102.06 (benzylidene C1), 137.06 (benzylidene C-*ipso*), 126.43 (benzylidene C2), 128.51 (benzylidene C3) 129.50 (benzylidene C4 ).

**Ethyl 3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (27).** Compound **26** (1.46 g, 3.3 mmol) was dissolved in DMF (20 mL). To the solution, 60% NaH in mineral oil (330 mg, 8.3 mmol) was added and the mixture was stirred in argon atmosphere for 30 min. Benzyl bromide (1.4 mL, 12 mmol) was added and stirring at rt was continued over night. TLC analysis the following day revealed disappearance of starting material the reaction was quenched with MeOH at 0 °C. After solvent evaporation, the residue was extracted with EtOAc, washed with brine, satd. $NaHCO_3$ (aq), brine and dried over anhydrous $MgSO_4$. Purification of the crude product was carried out with flash chromatography (Toluene/EtOAc, 30:1). The reaction yielded the desired compound **27** (1.57 g, 2.9 mmol) as a white foam. Yield:

89%. $R_f$ = 0.6 (Toluene/EtOAc 5:1). ESI-HRMS: calcd for $C_{30}H_{29}NO_6S$ $[M + Na]^+$ 554.1608, found 554.1590.

$^1$H NMR ($CDCl_3$, 25 °C, 400 MHz): $\delta$ 5.34 (d, 1H, *J* = 10.7 Hz, H1), 4.30 (dd, 1H, *J* = 9.9, 10.7 Hz, H2), 4.46 (dd, 1H, *J* = 9.0, 9.9 Hz, H3), 3.82 (dd, 1H, *J* = 9.0, 9.5 Hz, H4), 3.71 (ddd, 1H, *J* = 5.0, 9.5, 10.1 Hz, H5), 4.42 (dd, 1H, *J* = 5.0, -10.5 Hz, $H6_S$) 3.83 (dd, 1H, *J* = 10.1, -10.5 Hz, $H6_R$), 2.68 (dq, 1H, *J* = 7.4, -12.5 Hz, SEt H1a), 2.63 (dq, 1H, *J* = 7.5, -12.5 Hz, SEt H1b), 1.16 (dd, 3H, *J* = 7.4, 7.5 Hz, SEt H2), 7.85 (m, 1H, Phth H1A), 7.65 (m, 1H, Phth H1B), 7.72 (m, 1H, Phth H2A), 7.70 (m, 1H, Phth H2B), 4.79 (d, 1H, *J* = -12.3 Hz, Bn H1A) 4.51 (d, 1H, *J* = -12.3 Hz, Bn H1B), 6.99 (m, 2H, Bn H2), 6.92 (m, 2H, Bn H3), 6.88 (m, 1H, Bn H4), 5.63 (m, 1H, benzylidene H1), 7.53 (m, 2H, benzylidene H2), 7.40 (m, 2H, benzylidene H3), 7.38 (m, 1H, benzylidene H4).

$^{13}$C NMR ($CDCl_3$, 25 °C, 100 MHz): $\delta$ 81.92 (C1), 54.80 (C2), 75.55 (C3), 83.19 (C4), 70.58 (C5), 68.71 (C6), 24.21 (SEt C1), 14.99 (SEt C2), 167.55, 167.87 (Phth C=O), 131.70, 131.76 (Phth C-*ipso*), 123.69, 123.46 (Phth C1), 133.98, 134.09 (Phth C2), 74.34 (Bn C1), 128.36 (Bn C-*ipso*), 128.18 (Bn C2, C4), 127.56 (Bn C3), 101.45 (benzylidene C1), 137.44 (benzylidene C-*ipso*), 126.19 (benzylidene C2), 128.44 (benzylidene C3, C4).

**Ethyl 3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (28).** Compound **27** (1.54 g, 2.9 mmol) was dissolved in dry THF (30 mL) and 4 Å powdered MS was added. The mixture was stirred for 30 min at rt. To the solution, 95% $NaCNBH_3$ (2.38 g, 38 mmol) was added. Dropwise at 0 °C, a satd. sol. of HCl (g) in $Et_2O$ (15 mL) was added until cease of gas development. The reaction was left to attain rt and it was stirred for 5 h, until completion. The reaction flask was put on a rotavapor to remove solvent until only a few mL were left. The solution was diluted with EtOAc and filtered through a layer of celite on a sintered glass funnel. The filtered solution was extracted with an iced satd. $NaHCO_3$ (aq) solution. The aqueous phase was washed twice with EtOAc and the pooled org. phases were then washed twice with brine. The solution was dried over anhydrous $MgSO_4$ and the crude product was upon evaporation of solvent purified by flash chromatography (Toluene/EtOAc, 7:1) to afford compound **28** (9) (1.12

g, 2.0 mmol). Yield: 72%. $R_f$ = 0.55 (P-eter/EtOAc 3:1). ESI-HRMS: calcd for $C_{30}H_{31}NO_6S$ $[M + Na]^+$ 556.1764, found 556.1754.

$^1$H NMR ($CDCl_3$, 25 °C, 400 MHz): $\delta$ 5.28 (d, 1H, *J* = 10.4 Hz, H1), 4.24 (dd, 1H, *J* = 10.3, 10.4 Hz, H2), 4.28 (dd, 1H, *J* = 8.5, 10.3 Hz, H3), 3.83 (ddd, 1H, *J* = 1.8, 8.5, 9.6 Hz, H4), 3.27 (d, 1H, *J* = 1.8 Hz, OH4), 3.68 (ddd, 1H, *J* = 4.8, 5.2, 9.6 Hz, H5), 3.77 (dd, 1H, *J* = 5.2, -10.2 Hz, H6R), 3.84 (dd, 1H, *J* = 4.8, -10.2 Hz, H6S), 2.59 (dq, 7.5, -12.5 Hz, SEt H1a), 2.66 (dq, 1H, *J* = 7.4, -12.5 Hz, SEt H1b), 1.16 (dd, 3H, *J* = 7.4, 7.5 Hz, SEt H2), 7.81 (m, 1H, Phth H1A), 7.66 (m, 1H, Phth H1B), 7.68 (m, 1H, Phth 2A), 7.68 (m, 1H, Phth 2B), 4.54 (d, 1H, *J* = -12.2 Hz, Bn3 H1A), 4.75 (d, 1H, *J* = -12.2 Hz, Bn3 H1B), 7.04 (m, 2H, Bn3 H2), 6.94 (m, 2H, Bn3 H3), 6.94 (m, 1H, Bn3 H4), 4.58 (d, 1H, *J* = -12.0 Hz, Bn6 H1A), 4.63 (d, 1H, *J* = -12.0 Hz, Bn6 H1B), 7.35 (m, 2H, Bn6 H2), 7.35 (m, 2H, Bn6 H3), 7.31 (m, 1H, Bn6 H4).

$^{13}$C NMR ($CDCl_3$, 25 °C, 100 MHz): $\delta$ 81.21 (C1), 54.47 (C2), 79.65 (C3), 74.42 (C4), 77.81 (C5), 70.85 (C6), 24.03 (SEt C1), 14.98 (SEt C2), 167.59, 168.12 (Phth C=O), 131.67 (Phth C-*ipso*), 123.57 (Phth C1A), 123.33 (Phth C1B), 133.89, 133.98 (Phth C2), 74.52 (Bn3 C1), 138.17 (Bn3 C2), 127.96 (Bn3 C3), 128.20 (Bn3 C4), 127.48 (Bn3 C5), 73.81 (Bn6 C1), 137.68 (Bn6 C2), 127.86 (Bn6 C3), 128.56 (Bn6 C4), 127.95 (Bn6 C5).

**Methyl 3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (29).** Compound **28** (0.59 g, 1.1 mmol) was dissolved in dry DCM (15 mL). MeOH (4.6 mL, 0.11 mol), NIS (0.37g, 1.7 mmol) and AgOTf (0.12 g, 0.48 mmol) were added. The reaction mixture was stirred at rt and under argon atmosphere for 10 min. The reaction was quenched with $NEt_3$ at 0 °C. Solvent evaporation was followed by extraction with EtOAc, washing with satd. $Na_2S_2O_3$ (aq), brine, satd. $NaHCO_3$ (aq), brine and drying over anhydrous $MgSO_4$. Solvent was evaporated and the crude product was purified by flash chromatography (Toluene/EtOAc, 5:1). The pure product **29** (0.390 g, 0.78 mmol) was obtained in a 70% yield. $R_f$ = 0.7 (P-eter/EtOAc 1:1). ESI-HRMS: calcd for $C_{29}H_{29}NO_7$ $[M + Na]^+$ 526.1836, found 526.1830.

$^1$H NMR ($CDCl_3$, 25 °C, 400 MHz): $\delta$ 5.07 (d, 1H, *J* = 8.5 Hz, H1), 4.14 (dd, 1H, *J* = 8.5, 10.8 Hz, H2), 4.23 (dd, 1H, *J* = 8.5, 10.8 Hz, H3), 3.83 (ddd, 1H, *J* = 2.3, 8.5, 9.6 Hz, H4), 3.65 (ddd, 1H, *J* = 4.9, 5.1, 9.6 Hz, H5), 3.80 (dd, 1H, *J* = 5.1, -10.1 Hz, $H6_R$), 3.85 (dd, 1H, *J* = 4.9, -10.1 Hz, $H6_S$), 3.38 (s, 3H, OMe), 7.80 (m, 2H, Phth H1), 7.69 (m, 2H, Phth H2), 4.74 (d, 1H, *J* = -12.2 Hz, Bn3 H1A), 4.53 (d, 1H, *J* = -12.2 Hz, Bn3 H1B), 7.05 (m, 2H, Bn3 H2), 6.96 (m, 2H, Bn3 H3), 6.94 (m, 2H, Bn3 H4), 2.91 (d, 1H, *J* = 2.3 Hz, HO4), 4.60 (d, 1H, *J* = -12.0 Hz, Bn6 H1A), 4.66 (d, 1H, *J* = -12.0 Hz, Bn6 H1B), 7.36 (m, 2H, Bn6 H2), 7.37 (m, 2H, Bn6 H3), 7.31 (m, 1H, Bn6 H4).

$^{13}$C NMR ($CDCl_3$, 25 °C, 100 MHz): $\delta$ 99.37 (C1), 55.40 (C2), 78.85 (C3), 74.67 (C4), 73.65 (C5), 70.84 (C6), 56.80 (OMe), 171.28 (Phth C=O), 123.50 (Phth C1), 133.91 (Phth C2), 74.46 (Bn3 C1), 128.67 (Bn3 C-*ipso*), 127.95 (Bn3 C2), 127.99 (Bn3 C3), 127.56 (Bn3 C4), 74.46 (Bn6 C1), 137.75 (Bn6 C-*ipso*), 128.06 (Bn6 C2, C4), 128.36 (Bn6 C3).

**Methyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (30).** Donor **24** (0.14 g, 0.29 mmol) and acceptor **29** (0.120, 0.24 mmol) were mixed together with 4 Å powdered MS and put under high vacuum for an hour. Dry DCM was added and the mixture was stirred at rt and under inert atmosphere. NIS (85 mg, 0.38 mmol) and AgOTf (33 mg, 0.13 mmol) were added and the solution turned dark red. The reaction had gone to completion after 15 min and it was then quenched at 0 °C with $NEt_3$. Solvents were evaporated and the dry mixture was dissolved in EtOAc and filtered through a layer of celite. The organic phase was washed with brine, satd. $Na_2S_2O_3$ (aq), brine, satd. $NaHCO_3$ (aq), brine and then dried over $MgSO_4$. The product was purified by flash chromatography (5:1 Toluene/EtOAc) yielding 0.19 g (0.2 mmol) of pure disaccharide **30**. Yield: 85%. $R_f$ = 0.6 (Peter/EtOAc 1:1).

$^1$H NMR ($CDCl_3$, 25 °C, 400 MHz): $\delta$ 4.88 (d, 1H, *J* = 8.6 Hz, H1), 4.10 (dd, 1H, *J* = 8.6, 10.7 Hz, H2), 4.18 (dd, 1H, *J* = 8.5, 10.7 Hz, H3), 4.25 (dd, 1H, *J* = 8.5, 9.9 Hz, H4), 3.36 (ddd, 1H, *J* = 1.5, 3.6, 9.9 Hz, H5), 3.50 (dd, 1H, *J* = 3.6, -11.2 Hz, $H6_R$), 3.60 (dd, 1H, *J* = 1.5, -11.2 Hz, $H6_S$), 3.29 (s, 3H, OMe), 7.89 (m, 2H, Phth H1), 7.76 (m, 2H,

Phth H2), 4.47 (d, 1H, *J* = -12.6 Hz, Bn3 H1A), 4.84 (d, 1H, *J* = -12.6 Hz, Bn3 H1B), 7.02 (m, 2H, Bn3 H2), 6.85 (m, 2H, Bn3 H3), 6.84 (m, 1H, Bn3 H4), 4.55 (d, 1H, *J* = -11.9 Hz, Bn6 H1A), 4.58 (d, 1H, *J* = -11.9 Hz, Bn6 H1B), 7.38 (m, 2H, Bn6 H2), 7.37 (m, 2H, Bn6 H3), 7.30 (m, 1H, Bn6 H4), 5.53 (d, 1H, *J* = 8.4 Hz, H1'), 4.33 (dd, 1H, *J* = 8.4, 10.7 Hz, H2'), 5.79 (dd, 1H, *J* = 9.0, 10.7 Hz, H3'), 5.12 (dd, 1H, *J* = 9.0, 10.2 Hz, H4'), 3.45 (ddd, 1H, *J* = 2,2, 4.1, 10.2 Hz, H5'), 4.22 (dd, 1H, *J* = 4.1, -12.3 Hz, $H6_R$'), 3.94 (dd, 1H, *J* = 2.2, -12.3 Hz, $H6_S$'), 7.92 (m, 2H, Phth' H1), 7.75 (m, 2H, Phth' H2), 1.84, 1.97, 2.00 (s, 9H, Ac3', 4', 6').

$^{13}$C NMR ($CDCl_3$, 25 °C, 100 MHz): $\delta$ 99.19 (C1), 55.65 (C2), 77.01 (C3), 76.33 (C4), 74.56 (C5), 68.16 (C6), 56.60 (OMe), 168.23, 168.46 (Phth C=O), 131.55, 131.85 (Phth C-*ipso*), 133.75, 134.59 (Phth C1), 123.87 (Phth C2), 74.33 (Bn3 C1), 138.67 (Bn3 C-*ipso*), 128.03 (Bn3 C2), 128.05 (Bn3 C3), 127.16 (Bn3 C4), 72.87 (Bn6 C1), 138.54 (Bn6 C-*ipso*), 128.45 (Bn6 C2), 127.59 (Bn6 C3), 127.63 (Bn6 C4), 96.96 (C1'), 55.45 (C2'), 70.81 (C3'), 68.97 (C4'), 71.65 (C5'), 61.66 (C6'), 168.23, 168.46 (Phth C=O), 131.55, 131.85 (Phth C-*ipso*), 133.75, 134.59 (Phth C1), 123.87 (Phth C2), 169.65, 170.26, 170.85 (Ac 3',4',6' C=O), 20.59, 20.78, 21.60 (Ac 3', 4', 6').

**Methyl 2-amino-2-deoxy-β-D-glucopyranosyl-(1→4)-2-amino-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside (32).** To a solution of compound **30** (0.19 g, 0.2 mmol) in MeOH (5 mL), a 1 M solution of NaOMe in MeOH was added drop wise until pH 9-10 was attained. The mixture was stirred in rt for 30 min until disappearance of starting material. The mixture was neutralized with Dowex-$H^+$ and filtered in a sintered glass funnel. The solvent was then evaporated under reduced pressure affording crude methyl 2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (**31**) as a white foam. $R_f$ = 0.85 (EtOAc/MeOH/$H_2O$ 7:2:1). Compound **31** was dissolved in 99.7% EtOH. To the solution, EDA (0.57 mL, 8.5 mmol) was added and the mixture was brought to reflux for 90 min after which TLC indicated full consumption of starting material. Solvents were evaporated and co-evaporation was performed with EtOH/Toluene mixtures. The crude product was purified by flash chromatography (EtOAc/MeOH/$H_2O$ 7:2:1) to give pure compound **32** (0.09 g,

0.17 mmol). Yield: 85% over two steps. $R_f$ = 0.1 (EtOAc/MeOH/$H_2O$ 7:2:1). ESI-HRMS: calcd for $C_{27}H_{38}N_2O_9$ $[M + Na]^+$ 557.2470, found 557.2486.

$^1$H NMR (MeOD, 25 °C, 400 MHz): $\delta$ 4.19 (d, 1H, *J* = 8.0 Hz, H1), 2.71 (dd, 1H, *J* = 8.0, 10.1 Hz, H2), 3.48 (dd, 1H, *J* = 9.1, 10.1 Hz, H3), 4.16 (dd, 1H, *J* = 9.1, 9.7 Hz, H4), 3.56 (ddd, 1H, *J* = 1.9, 3.4, 9.7 Hz, H5), 3.93 (dd, 1H, *J* = 3.4, -11.4 Hz, $H6_R$), 3.80 (dd, 1H, *J* = 1.9, -11.4 Hz, $H6_S$), 3.51 (s, 3H, OMe), 5.10 (d, 1H, *J* = -10.6 Hz, Bn3 H1A), 4.65 (d, 1H, *J* = -10.6 Hz, Bn3 H1B), 7.46 (m, 2H, Bn3 H2), 7.36 (m, 2H, Bn3 H3), 7.38 (m, 1H, Bn6 H4), 4.61 (d, 1H, *J* = -11.9 Hz, Bn6 H1A), 4.65 (d, 1H, *J* = -11.9 Hz, Bn6 H1B), 7.39 (m, 2H, Bn6 H2), 7.30 (m, 2H, Bn6 H3), 7.34 (m, 1H, Bn6 H4), 4.45 (d, 1H, *J* = 8.0 Hz, H1'), 2.66 (dd, 1H, *J* = 8.0, 9.9 Hz, H2'), 3.23 (dd, 1H, *J* = 8.4, 9.9 Hz, H3'), 3.21 (dd, 1H, *J* = 8.4, 9.7 Hz, H4'), 3.18 (ddd, 1H, *J* = 2.1, 5.6, 9.7 Hz, H5'), 3.51 (dd, 1H, *J* = 5.6, -12.1 Hz, $H6_R$'), 3.78 (dd, 1H, *J* = 2.1, -12.1 Hz, $H6_S$').

$^{13}$C NMR (MeOD, 25 °C, 100 MHz): $\delta$ 105.24 (C1), 57.57 (C2), 75.56 (C3), 83.89 (C4), 76.21 (C5), 69.31 (C6), 57.35 (OMe), 76.66 (Bn3 C1), 139.54 (Bn3 C-*ipso*), 129.88 (Bn3 C2), 129.47 (Bn3 C3), 129.11 (Bn3 C4), 74.09 (Bn6 C1), 139.39 (Bn6 C-*ipso),* 128.89 (Bn6 C2), 129.45 (Bn6 C3), 128.69 (Bn6 C4), 102.54 (C1'), 59.26 (C2'), 77.34 (C3'), 72.29 (C4'), 78.77 (C5'), 63.04 (C6').

**Methyl 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside (33).** Disaccharide **32** (0.07 g, 0.13 mmol) was dissolved in MeOH. At 0 °C, acetic anhydride (30 µl, 0.32 mmol) was added. The reaction mixture was allowed to attain rt and was stirred over night. Upon solvent evaporation under reduced pressure, the crude product was purified by flash chromatography (EtOAc/MeOH/$H_2O$ 7:2:1) to afford **33** (0.08 g, 0.13 mmol). Yield: quantitative. $R_f$ = 0.6 (EtOAc/MeOH/$H_2O$ 7:2:1). ESI-HRMS: calcd for $C_{31}H_{42}N_2O_{11}$ $[M + Na]^+$ 641.2681, found 641.2707.

$^1$H NMR (MeOD, 25 °C, 400 MHz): $\delta$ 4.58 (d, 1H, *J* = 8.3 Hz, H1), 3.71 (dd, 1H, *J* = 8.3, 10.5 Hz, H2), 3.44 (dd, 1H, *J* = 8.7, 10.5 Hz, H3), 3.22 (dd, 1H, *J* = 8.7, 9.9 Hz, H4), 3.16 (ddd, 1H, *J* = 2.2, 7.0, 9.9 Hz, H5), 3.47 (dd, 1H, *J* = 7.0, -12.3 Hz, $H6_R$), 3.76

(dd, 1H, $J$ = 2.2, -12.3 Hz, H6$_S$), 3.44 (s, 3H, OMe), 1.98 (s, 3H, Ac), 4.63 (d, 1H, $J$ = -11.1 Hz, Bn3 H1A), 4.99 (d, 1H, $J$ = -11.1, Bn3 H1B), 7.39 (m, 2H, Bn3 H2), 7.34 (m, 2H, Bn3 H3), 7.37 (m, 1H, Bn3 H4), 4.61 (d, 1H, $J$ = -11.8 Hz, Bn6 H1A), 4.66 (d, 1H, $J$ = -11.8 Hz, Bn6 H1B), 7.36 (m, 2H, Bn6 H2), 7.28 (m, 2H, Bn6 H3), 7.33 (m, 1H, Bn6 H4), 4.31 (d, 1H, $J$ = 8.2 Hz, H1'), 3.84 (dd, 1H, $J$ = 8.2, 10.0 Hz, H2'), 3.59 (dd, 1H, $J$ = 8.6, 10.0 Hz, H3'), 4.03 (dd, 1H, $J$ = 8.6, 9.2 Hz, H4'), 3.47 (ddd, 1H, $J$ = 1.6, 3.8, 9.2 Hz, H5'), 3.79 (dd, 1H, $J$ = 3.8, -8.2 Hz, H6$_R$'), 3.81 (dd, 1H, $J$ = 1.6, -8.2 Hz, H6$_S$'), 1.90 (s, 3H, Ac').

$^{13}$C NMR (MeOD, 25 °C, 100 MHz): $\delta$ 101.52 (C1), 58.08 (C2), 75.54 (C3), 82.45 (C4), 78.57 (C5), 62.98 (C6), 56.97 (OMe), 173.74 (Ac C=O), 23.11 (Ac), 76.56 (Bn3 C1), 139.67 (Bn3 C-*ipso*), 129.52 (Bn3 C2), 129.33 (Bn3 C3), 128.87 (Bn3 C4), 74.34 (Bn6 C1), 139.61 (Bn6 C-*ipso*), 129.49 (Bn6 C2), 128.81 (Bn6 C3), 128.70 (Bn6 C4), 103.47 (C1'), 55.70 (C2'), 72.50 (C3'), 76.27 (C4'), 76.45 (C5'), 69.61 (C6'), 173.21 (Ac' C=O), 22.96 (Ac')

**Methyl 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranoside (12).** Compound **33** (0.07 g, 0.11 mmol) was dissolved in a mixture of MeOH/$H_2O$ 1:1 (2 mL). A catalytic amount of 20% $Pd(OH)_2$ on carbon was added together with a drop of conc. HCl. The reaction flask was connected to equipment for catalytic hydrogenation and the reaction mixture was stirred at 100 psi $H_2$ over night. The reaction had gone to completion and the mixture was filtered through a layer of celite. Solvents were evaporated and purification was performed with a C-18 reversed-phase cartridge. The product was subsequently purified with an FPLC apparatus equipped with a size-exclusion agarose/dextran column. This afforded target compound **12** (0.02 g, 0.05 mmol) in a 42% yield. $R_f$ = 0.2 (EtOAc/MeOH/$H_2O$ 7:2:1). ESI-HRMS: calcd for $C_{17}H_{30}N_2O_{11}$ $[M + Na]^+$ 461.1742, found 461.1756.

$^1$H and $^{13}$C NMR chemical shifts and $J_{H,H}$ are given in Table S2.

**2,3,4,6-Tetra-*O*-acetyl-α-D-galactopyranosyl bromide (36).** Compound **34** (0.10 g, 0.6 mmol) was dissolved in acetic anhydride (2 mL, 20 mmol). Two small drops of $HClO_4$

was added at 0 °C. After 3 h, the reaction had gone to completion and the reaction mixture was extracted with EtOAc. The organic phase was washed with iced solutions of brine, satd. $NaHCO_3$ (aq) and brine again. The solution was dried over $MgSO_4$ and solvents were evaporated under reduced pressure to afford crude product 1,2,3,4,6-penta-*O*-acetyl-D-galactopyranose (**35**) (0.20 g, 0.5 mmol) in a 92% yield and $^1$H NMR revealed a quite pure α/β mixture. ESI-HRMS: calcd for $C_{16}H_{22}O_{11}$ $[M + Na]^+$ 413.1054, found 413.1066. Compound **35** (7.10 g, 18 mmol) was dissolved in dry DCM. At 0 °C, AcBr (7.6 mL 0.1 mol) together with dry MeOH (3 mL, 70 mmol) was added and the reaction was stirred under inert atmosphere for 5 h. TLC indicated full consumption of starting material and the reaction was quenched with satd. $NaHCO_3$ (aq). The mixture was diluted with DCM and iced satd. $NaHCO_3$ (aq) and the organic phase was washed with brine. The solution was dried over $MgSO_4$ and the solvents were concentrated under reduced pressure. The crude product was purified by flash chromatography (Toluene/EtOAc 5:1) and re-crystallisation from $Et_2O$/Pentane, yielding **36** as white crystals, (4.52 g, 11 mmol). Yield: 60%. $R_f$ = 0.6 (Toluene/EtOAc 2:1).

$^1$H NMR ($CDCl_3$, 25 °C, 400 MHz): $\delta$ 6.70 (d, 1H, $J$ = 4.0 Hz, H1), 5.05 (dd, 1H, $J$ = 10.6 Hz, H2), 5.41 (dd, 1H, $J$ = 3.3, 10.6 Hz, H3), 5.52 (dd, 1H, $J$ = 1.4, 3.3 Hz, H4), 4.49 (ddd, 1H, $J$ = 1.4, 6.4, 6.7 Hz, H5), 4.12 (dd, 1H, $J$ = 6.7, -11.4 Hz, $H6_R$), 4.19 (dd, 1H, $J$ = 6.4, -11.4 Hz, $H6_S$), 2.02, 2.06, 2.12, 2.16 (s, 12H, Ac 2,3,4,6).

**Ethyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (37).** Acceptor **29** (0.15 g, 0.28 mmol) and donor **36** (0.14 g, 0.34 mmol) were dissolved in dry DCM. Powdered MS (4 Å) were added and the mixture was stirred under argon atmosphere at -40 °C. The reaction flask was equipped with a case of aluminium foil. Re-crystallised AgOTf (0.22 g, 0.86 mmol) was dissolved in dry toluene. Through a drop funnel the AgOTf solution was added to the reaction during 1 h. After another 3.5 h, TLC showed full consumption of acceptor and the reaction mixture was allowed to attain rt. The reaction was quenched with $Na_2S_2O_3$ (s) and the mixture was diluted with toluene. It was then filtered through a layer of celite and satd. $Na_2S_2O_3$ (aq) was added after which it was shaken and the phases were

subsequently separated. The organic phase was dried over $MgSO_4$ and solvents were then removed under reduced pressure. The disaccharide was purified by flash chromatography (Toluene/EtOAc, 6:1), affording 0.18 g (0.21 mmol) of pure **37**. Yield: 61%. $R_f$ = 0.3 (Peter/EtOAc 3:1). ESI-HRMS: calcd for $C_{44}H_{49}NO_{15}S$ $[M + Na]^+$ 886.2715, found 886.2725.

$^1$H NMR ($CDCl_3$, 25 °C, 400 MHz): $\delta$ 5.21 (d, 1H, *J* = 10.5 Hz, H1), 4.23 (dd, 1H, *J* = 10.3, 10.5 Hz, H2), 4.28 (dd, 1H, *J* = 8.6, 10.3 Hz, H3), 4.09 (dd, 1H, *J* = 8.6, 9.9 Hz, H4), 3.55 (ddd, 1H, *J* = 1.8, 3.0, 9.9 Hz, H5), 3.79 (dd, 1H, *J* = 3.0, -12.2, $H6_R$), 3.79 (dd, 1H, *J* = 1.8, -12.2 Hz, $H6_S$), 2.60 (dd, 1H, *J* = 7.5, -12.5 Hz, SEt H1a), 2.67 (dd, 1H, *J* = 7.4, -12.5 Hz, SEt H1b), 1.17 (dd, 3H, *J* = 7.4, 7.5 Hz, SEt H2), 7.85 (m, 2H, Phth H1), 7.67 (m, 2H, Phth H2), 4.80 (d, 1H, *J* = -12.1 Hz, Bn3 H1A), 4.44 (d, 1H, *J* = -12.1 Hz, Bn3 H1B), 7.02 (m, 2H, Bn3 H2), 6.89 (m, 2H, Bn3 H3), 6.86 (m, 1H, Bn3 H4), 4.79 (d, 1H, *J* = -12.0 Hz, Bn6 H1A), 4.50 (d, 1H, *J* = -12.0 Hz, Bn6 H1B), 7.39 (m, 2H, Bn6 H2), 7.41 (m, 2H, Bn6 H3), 7.17 (m, 1H, Bn6 H4), 4.62 (d, 1H, *J* = 8.0 Hz, H1'), 5.15 (dd, 1H, *J* = 8.0, 10.4 Hz, H2'), 4.86 (dd, 1H, *J* = 3.5, 10.4 Hz, H3'), 5.27 (dd, 1H, *J* = 1.2, 3.5 Hz, H4'), 3.64 (ddd, 1H, *J* = 1.2, 6.2, 7.5 Hz, H5'), 3.95 (dd, 1H, *J* = 7.5, -11.1 Hz, $H6_R$'), 3.98 (dd, 1H, *J* = 6.2, -11.1 Hz, $H6_S$'), 1.97, 2.02, 2.02, 2.06 (s, 12H, Ac 2, 3, 4, 6).

$^{13}$C NMR ($CDCl_3$, 25 °C, 100 MHz): $\delta$ 81.23 (C1), 54.82 (C2), 77.75 (C3), 77.98 (C4), 79.21 (C5), 67.87 (C6), 24.00 (SEt C1), 15.03 (SEt C2), 131.75 (Phth C-*ipso*), 123.58 (Phth C1), 133.83 (Phth C2), 74.68 (Bn3 C1), 128.00 (Bn3 C2), 128.03 (Bn3 C3), 127.24 (Bn3 C4), 138.02, 138.62 (Bn3/Bn6 C-*ipso*), 73.74 (Bn6 C1), 128.16 (Bn6 C2), 128.73 (Bn6 C3), 129.17 (Bn6 C4), 100.43 (C1'), 69.65 (C2'), 71.14 (C3'), 67.04 (C4'), 70.58 (C5'), 60.83 (C6'), 169.33, 170.19, 170.36, 170.44 (Ac 2, 3, 4, 6 C=O), 20.71, 20.75, 20.81, 20.95 (Ac 2, 3, 4, 6).

**Methyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (38).** Compound **37** (0.07 g, 0.08 mmol) was dissolved in dry DCM. MeOH (0.3 mL, 0.8 mmol) was added followed by addition of NIS (2 equiv.) and AgOTf (0.5 equiv.). The reaction mixture turned dark red shortly after the addition of AgOTf and it was stirred at rt under inert atmosphere. After 1 h, TLC

indicated disappearance of starting material and the reaction was quenched with $NEt_3$ at 0 °C. Solvents were evaporated followed by extraction with EtOAc. The organic phase was washed with brine, satd. $Na_2S_2O_3$ (aq), brine, satd. $NaHCO_3$ (aq) and brine again, followed by drying over $MgSO_4$. Solvents were evaporated and the crude product was purified by flash chromatography (Toluene/EtOAc, 7:1→3:1) yielding pure **38** (0.05 g, 0.06 mmol). Yield: 75%. $R_f$ = 0.6 (P-eter/EtOAc 1:1). ESI-HRMS: calcd for $C_{43}H_{47}NO_{16}$ $[M + Na]^+$ 856.2787, found 856.2788.

$^1$H NMR ($CDCl_3$, 25 °C, 400 MHz): $\delta$ 5.02 (d, 1H, *J* = 8.6 Hz, H1), 4.14 (dd, 1H, *J* = 8.6, 10.8 Hz, H2), 4.24 (dd, 1H, *J* = 8.6, 10.8 Hz, H3), 4.08 (dd, 1H, *J* = 8.6, 9.9 Hz, H4), 3.53 (ddd, 1H, *J* = 1.5, 2.9, 9.9 Hz, H5), 3.8 (dd, 1H, *J* = 2.9, -11.4 Hz, $H6_R$), 3.79 (dd, 1H, *J* = 1.5, -11.4 Hz, $H6_S$), 3.38 (s, 3H, OMe), 7.69 (m, 2H, Phth H1), 7.66 (m, 2H, Phth H2), 4.80 (d, 1H, *J* = -12.3 Hz, Bn3 H1A), 4.43 (d, 1H, *J* = -12.3 Hz, Bn3 H1B), 7.01 (m, 2H, Bn3 H2), 6.88 (m, 2H, Bn3 H3), 6.85 (m, 1H, Bn3 H4), 4.51 (d, 1H, *J* = -12.1 Hz, Bn6 H1A), 4.83 (d, 1H, *J* = -12.1 Hz, Bn6 H1B), 7.40 (m, 2H, Bn6 H2), 7.41 (m, 2H, Bn6 H3), 7.35 (m, 1H, Bn6 H4), 4.58 (d, 1H, *J* = 8.0 Hz, H1'), 5.14 (dd, 1H, *J* = 8.0, 10.4 Hz, H2'), 4.84 (dd, 1H, *J* = 3.5, 10.4 Hz, H3'), 5.27 (dd, 1H, *J* = 1.1, 3.5 Hz, H4'), 3.63 (ddd, 1H, *J* = 1.1, 6.3, 7.4 Hz, H5'), 3.94 (dd, 1H, *J* = 7.4, -11.1 Hz, $H6_R$'), 3.98 (dd, 1H, *J* = 6.3, -11.1 Hz, $H6_S$'), 1.97, 2.02, 2.02, 2.06 (s, 12H, Ac 2, 3, 4, 6).

$^{13}$C NMR ($CDCl_3$, 25 °C, 100 MHz): $\delta$ 99.38 (C1), 55.62 (C2), 76.78 (C3), 78.12 (C4), 74.91 (C5), 67.59 (C6), 56.76 (OMe), 131.82 (Phth C*ipso*), 123.36 (Phth C1), 133.79 (Phth C2), 74.53 (Bn3 C1), 127.92 (Bn3 C2), 128.00 (Bn3 C3), 127.18 (Bn3 C4), 137.98, 138.65 (Bn3/6 *C-ipso*), 73.76 (Bn6 C1), 128.20 (Bn6 C2), 128.73 (Bn6 C3), 128.18 (Bn6 C4), 100.43 (C1'), 69.64 (C2'), 71.11 (C3'), 67.02 (C4'), 70.56 (C5'), 60.83 (C6'), 169.30, 170.14, 170.32, 170.42 (Ac2, 3, 4, 6 C=O), 20.68, 20.71, 20.77, 20.91 (Ac 2, 3, 4, 6).

**Methyl β-D-galactopyranosyl-(1→4)-2-amino-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside (40).** To a solution of compound **38** (0.10 g, 0.12 mmol) dissolved in MeOH (15 mL), 20 drops of 1 M NaOMe in MeOH was added until pH 9. The mixture was stirred for 30 min and TLC then indicated complete *O*-deacetylation. The reaction

was quenched with Dowex-$H^+$ (pH 6) and the mixture was filtered in a glass-sintered funnel. Solvent evaporation yielded crude product methyl β-D-galactopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (**39**). $R_f$ = 0.7 (EtOAc/MeOH/$H_2O$ 7:2:1). Crude compound **39** was dissolved in 99.7% EtOH (15 mL). To the solution, EDA (0.25 mL, 3.7 mmol) was added and the reaction mixture was brought to reflux. After 1 h of reaction the solvent was concentrated under reduced pressure and the crude product was purified by flash chromatography (EtOAc/MeOH/$H_2O$ 7:2:1) to yield pure **40** (0.06g, 0.12 mmol). Yield = 93%. $R_f$ = 0.6 (EtOAc/MeOH/$H_2O$ 7:2:1).

$^1$H NMR ($CDCl_3$, 25 °C, 400 MHz): $\delta$ 4.16 (d, 1H, *J* = 7.9 Hz, H1), 2.86 (dd, 1H, *J* = 7.9, 9.5 Hz, H2), 3.53 (dd, 1H, *J* = 8.8, 9.5 Hz, H3), 4.04 (dd, 1H, *J* = 8.8, 9.6 Hz, H4), 3.53 (ddd, 1H, *J* = 2.1, 3.6, 9.6 Hz, H5), 3.93 (dd, 1H, *J* = 3.6, -11.1 Hz, $H6_R$), 3.80 (dd, 1H, *J* = 2.1, -11.1 Hz, $H6_S$), 3.51 (s, 3H, OMe), 5.12 (d, 1H, *J* = -11.1 Hz, Bn3 H1A), 4.64 (d, 1H, *J* = -11.1 Hz, H1B), 7.30 (m, 2H, Bn3 H2), 7.17 (m, 2H, Bn H3), 7.25 (m, 1H, Bn H4), 4.53 (d, 1H, *J* = -12.0 Hz, Bn6 H1A), 4.69 (d, 1H, *J* = -12.0 Hz, Bn6 H1B), 7.34 (m, 2H, Bn6 H2), 7.33 (m, 2H, Bn6 H3), 7.24 (m, 1H, Bn6 H4), 4.40 (d, 1H, *J* = 7.7 Hz, H1'), 3.63 (dd, 1H, *J* = 7.7, 9.5 Hz, H2'), 3.35 (dd, 1H, *J* = 3.3, 9.5 Hz, H3'), 3.85 (d, 1H, *J* = 3.3 Hz, H4'), 3.20 (dd, 1H, *J* = 4.2, 5.4 Hz, H5'), 3.71 (dd, 1H, *J* = 5.4, -12.1 Hz, $H6_R$'), 3.64 (dd, 1H, *J* = 4.2, -12.1 Hz, $H6_S$'), 2.58 (broad, 6H, OH2', 3',4',6', $NH_2$).

**Methyl β-D-galactopyranosyl-(1→4)-2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside (41).** Disaccharide **40** (0.026 g, 0.05 mmol) was dissolved in MeOH. At 0 °C, acetic anhydride (7 µl, 0.07 mmol) was added. The reaction mixture was allowed to attain rt and was stirred over night. Upon solvent evaporation under reduced pressure, the crude product was purified by flash chromatography (EtOAc/MeOH 9:1) to afford **41** (0.021g, 0.04 mmol). Yield: 75%. $R_f$ = 0.25 (EtOAc/MeOH 9:1).

$^1$H NMR (MeOD, 25 °C, 400 MHz): $\delta$ 4.37 (d, 1H, *J* = 8.4 Hz, H1), 3.79 (dd, 1H, *J* = 8.4, 10.4 Hz, H2), 3.66 (dd, 1H, *J* = 8.9, 10.4 Hz, H3), 4.02 (dd, 1H, *J* = 8.9, 9.7 Hz, H4), 3.54 (ddd, 1H, *J* = 1.9, 3.8, 9.7 Hz, H5), 4.01 (dd, 1H, *J* = 3.8, -11.1 Hz, $H6_R$), 3.85 (dd, 1H, *J* = 1.9, -11.1 Hz, $H6_S$), 3.45 (s, 3H, OMe), 1.91 (s, 3H, Ac), 4.65 (d, 1H, *J* =

-12.0 Hz, Bn3 H1A) 4.57 (d, 1H, *J* = -12.0 Hz, Bn3 H1B), 7.38 (m, 2H, *J* = 0.6, 1.2, 1.9, 7.7 Hz, Bn3 H2), 7.34 (m, 2H, *J* = 0.6, 1.4, 7.5, 7.7 Hz, Bn3 H3), 7.27 (m, 1H, *J* = 1.2, 7.5 Hz, Bn3 H4), 4.56 (d, 1H, *J* = -10.8 Hz, Bn6 H1A), 5.08 (d, 1H, *J* = -10.8 Hz, Bn6 H1B), 7.40 (m, 2H, *J* = 0.6, 1.3, 1.8, 7.7 Hz, Bn6 H2), 7.31 (m, 2H, *J* = 0.6, 1.4, 7.4, 7.7 Hz, Bn6 H3), 7.26 (m, 1H, *J* = 1.3, 7.4 Hz, Bn6 H4), 4.39 (d, 1H, *J* = 7.7 Hz, H1'), 3.59 (dd, 1H, *J* = 7.7, 9.7 Hz, H2'), 3.38 (dd, 1H, *J* = 3.5, 9.7 Hz, H3'), 3.78 (dd, 1H, *J* = 1.0, 3.5 Hz, H4'), 3.34 (ddd, 1H, *J* = 1.0, 4.3, 7.4 Hz, H5'), 3.75 (dd, 1H, *J* = 7.4, -11.8 Hz, $H6_R$'), 3.56 (dd, 1H, *J* = 4.3, -11.8 Hz, $H6_S$').

$^{13}$C NMR (MeOD, 25 °C, 100 MHz): $\delta$ 103.49 (C1), 56.11 (C2), 82.50 (C3), 77.40 (C4), 76.31 (C5), 69.29 (C6), 57.00 (OMe), 173.21 (Ac C=O), 23.00 (Ac), 76.52 (Bn3 C1), 139.63 (Bn3 C-*ipso*), 129.83 (Bn3 C2), 129.44 (Bn3 C3), 128.77 (Bn3 C4), 74.24 (Bn6 C1), 139.67 (Bn6 C-*ipso*), 128.86 (Bn6 C2), 129.20 (Bn6 C3), 128.64 (Bn6 C4), 104.22 (C1'), 72.99 (C2'), 74.83 (C3'), 70.39 (C4'), 77.45 (C5'), 62.98 (C6').

**Methyl β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranoside (11).** Compound **41** (0.048 g, 0.09 mmol) was dissolved in a mixture of MeOH/$H_2O$ 1:1 (4 mL). A catalytic amount of 20% $Pd(OH)_2$ on carbon was added. The reaction flask was connected to a hydrogenation equipment and the reaction mixture was stirred at 100 psi $H_2$ over night. After this period of time the reaction had gone to completion and the mixture was filtered through a layer of celite. Solvents were evaporated and purification was performed with a C-18 reversed-phase cartridge. The product was subsequently purified with an FPLC apparatus equipped with a size-exclusion agarose/dextran column. This afforded target compound **11** (0.013 g, 0.03 mmol) in a 40% yield. $R_f$ = 0.15 (EtOAc/MeOH/$H_2O$ 7:2:1). ESI-HRMS: calcd for $C_{15}H_{27}NO_{11}$ $[M + Na]^+$ 420.1476, found 420.1478.

$^1$H and $^{13}$C NMR chemical shifts and $J_{H,H}$ are given in Table S1.

## $^{1}$H and $^{13}$C NMR chemical shift assignments of 11 and 12

**Table S1.** $^{1}$H and $^{13}$C chemical shifts (ppm) and proton-proton coupling constants (Hz) for **11** at 278 and 343 K. $^{1}$H chemical shifts and coupling constants were refined with the PERCH NMR software.

| Residue | 343 K | 1 | 2 | 3 | 4 | 5 | $6_{pro\text{-}S}$ | $6_{pro\text{-}R}$ | OMe | CO | Me |
|---|---|---|---|---|---|---|---|---|---|---|---|
| β-D-Gal*p*-(1→ | $^{1}$H | 4.48 | 3.57 | 3.67 | 3.95 | 3.73 | 3.77 | 3.79 | | | |
| | $J_{H,H}$ | 7.9 | 9.9 | 3.5 | 1.1 | 4.4 7.8 | -11.8 | | | | |
| | $^{13}$C | 103.80 | 71.89 | 73.55 | 69.48 | 76.18 | 61.80 | | | | |
| →4)-β-D-Glc*p*NAc-OMe | $^{1}$H | 4.49 | 3.72 | 3.73 | 3.69 | 3.6 | 4.00 | 3.84 | 3.51 | | 2.04 |
| | $J_{H,H}$ | 8.3 | 10.0 | 8.6 | 9.8 | 2.4 5.3 | -12.3 | | | | |
| | $^{13}$C | 102.66 | 55.92 | 73.37 | 79.87 | 75.66 | 61.22 | | 57.80 | 175.33 | 23.04 |
| | 278 K | | | | | | | | | | |
| β-D-Gal*p*-(1→ | $^{1}$H | 4.48 | 3.54 | 3.68 | 3.93 | 3.73 | 3.75 | 3.78 | | | |
| | $J_{H,H}$ | 7.8 | 10.0 | 3.3 | 1.2 | 3.6 8.6 | -12.1 | | | | |
| →4)-β-D-Glc*p*NAc-OMe | $^{1}$H | 4.47 | 3.76 | 3.70 | 3.74 | 3.61 | 4.01 | 3.85 | 3.52 | 2.04 | |
| | $J_{H,H}$ | 8.5 | 10.6 | 8.8 | 10.0 | 2.2 5.1 | -12.3 | | | | |

**Table S2.** $^{1}$H and $^{13}$C chemical shifts (ppm) and proton-proton coupling constants (Hz) for **12** at 278 and 343 K. $^{1}$H chemical shifts and coupling constants were refined with the PERCH NMR software.

| Residue | 343 K | 1 | 2 | 3 | 4 | 5 | $6_{pro\text{-}S}$ | $6_{pro\text{-}R}$ | OMe | CO | Me |
|---|---|---|---|---|---|---|---|---|---|---|---|
| β-D-Glc*p*NAc-(1→ | $^{1}$H | 4.60 | 3.74 | 3.59 | 3.48 | 3.51 | 3.92 | 3.75 | | | 2.07 |
| | $J_{H,H}$ | 8.5 | 10.5 | 8.9 | 9.9 | 2.4 5.9 | -12.3 | | | | |
| | $^{13}$C | 102.27 | 56.54 | 74.43 | 70.80 | 76.79 | 61.58 | | | 175.28 | 23.02 |
| →4)-β-D-Glc*p*NAc-OMe | $^{1}$H | 4.45 | 3.71 | 3.71 | 3.59 | 3.52 | 3.86 | 3.67 | 3.50 | | 2.03 |
| | $J_{H,H}$ | 8.4 | 10.0 | 8.4 | 9.8 | 2.2 5.6 | -12.1 | | | | |
| | $^{13}$C | 102.65 | 55.78 | 73.47 | 80.61 | 75.42 | 61.17 | | 57.82 | 175.34 | 23.02 |
| | 278 K | | | | | | | | | | |
| β-D-Glc*p*NAc-(1→ | $^{1}$H | 4.58 | 3.76 | 3.56 | 3.47 | 3.50 | 3.92 | 3.76 | | | 2.08 |
| | $J_{H,H}$ | 8.5 | 10.5 | 9.0 | 10.1 | 2.2 5.8 | -12.3 | | | | |
| →4)-β-D-Glc*p*NAc-OMe | $^{1}$H | 4.43 | 3.73 | 3.68 | 3.63 | 3.52 | 3.88 | 3.68 | 3.50 | | 2.03 |
| | $J_{H,H}$ | 8.6 | 10.5 | 8.6 | 9.8 | 2.1 5.5 | -12.1 | | | | |

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## Glycosidic torsion angles of 11 and 12 in complex with HEWL

**Table S3.** Glycosidic torsion angle averages of **11** and **12** in complex with HEWL from 175 and 50 ns MD simulations, respectively. RMSD in parenthesis.

| Compound | $\phi_{0\text{-}175\ ns}$ | $\psi_{0\text{-}175\ ns}$ | $\phi_{0\text{-}50\ ns}$ | $\psi_{0\text{-}50\ ns}$ | $\phi_{50\text{-}175\ ns}$ | $\psi_{50\text{-}175\ ns}$ |
|---|---|---|---|---|---|---|
| **11** | 35 (11) | –20 (14) | 43 (10) | –7 (14) | 31 (9) | –25 (10) |
| **12** | | | 51 (9) | –6 (13) | | |

## STD NMR effects for 11 and 12 in complex with HEWL

**Table S4.** Absolute STD NMR effects in % for disaccharide **11** (1 mM) in complex with HEWL (20 µM) at 278 K. Irradiation at 0.5 and 7.0 ppm with four saturation times 0.5-4 s.

| Time (s) | Irradiation (ppm) | H1 | H2 | H3 | H4 | H5 | $H6_{pro\text{-}S}$ | $H6_{pro\text{-}R}$ | OMe | Me |
|---|---|---|---|---|---|---|---|---|---|---|
| β-D-Gal*p*-(1→ | | | | | | | | | | |
| 0.5 | 0.5 | 0.25 | 0.21 | 0.5 | 0.17 | | | 0.50 | | |
| | 7.0 | 0.37 | 0.15 | 0.5 | 0.29 | | | 1.0 | | |
| 1 | 0.5 | 0.57 | 0.40 | 1 | 0.41 | | | 1.1 | | |
| | 7.0 | 0.72 | 0.40 | 1 | 0.63 | | | 1.9 | | |
| 2 | 0.5 | 0.95 | 0.77 | 1 | 0.82 | | | 2.0 | | |
| | 7.0 | 1.2 | 0.99 | 1 | 1.2 | | | 2.0 | | |
| 4 | 0.5 | 1.3 | 1.2 | 2 | 1.1 | | | 2.5 | | |
| | 7.0 | 1.6 | 1.2 | 2 | 1.5 | | | 3.1 | | |
| →4)-β-D-Glc*p*NAc-OMe | | | | | | | | | | |
| 0.5 | 0.5 | 0.25 | | 0.30 | | 0.30 | 0.12 | 0.24 | 0.17 | 1.2 |
| | 7.0 | 0.21 | | 0.40 | | 0.44 | 0.18 | 0.30 | 0.12 | 0.49 |
| 1 | 0.5 | 0.57 | | 1.3 | | 0.72 | 0.30 | 0.30 | 0.30 | 2.3 |
| | 7.0 | 0.42 | | 1.1 | | 0.64 | 0.40 | 0.50 | 0.23 | 1.2 |
| 2 | 0.5 | 1.1 | | 2.3 | | 1.4 | 0.67 | 0.76 | 0.70 | 4.7 |
| | 7.0 | 0.80 | | 1.1 | | 1.2 | 0.65 | 0.79 | 0.61 | 2.6 |
| 4 | 0.5 | 1.5 | | 3.3 | | 1.9 | 0.68 | 0.90 | 1.0 | 6.3 |
| | 7.0 | 1.1 | | 2.7 | | 1.7 | 0.90 | 0.81 | 0.90 | 3.6 |

**Table S5.** Absolute STD NMR effects in % for disaccharide **12** (1 mM) in complex with HEWL (20 µM) at 278 K. Irradiation at 0.5 and 7.0 ppm with four saturation times 0.5-4 s.

| Time (s) | Irradiation (ppm) | H1 | H2 | H3 | H4 | H5 | $H6_{pro\text{-}S}$ | $H6_{pro\text{-}R}$ | OMe | Me |
|---|---|---|---|---|---|---|---|---|---|---|
| β-D-Glc*p*NAc-(1→ | | | | | | | | | | |
| 0.5 | 0.5 | 0.49 | | 0.90 | 1.2 | 1 | 0.72 | | | 0.40 |
| | 7.0 | 0.32 | | 0.25 | 0.41 | 0.4 | 0.81 | | | 0.05 |
| 1 | 0.5 | 0.96 | | 1.60 | 2.1 | 2 | 1.4 | | | 0.52 |
| | 7.0 | 0.78 | | 0.64 | 0.55 | 0.7 | 1.5 | | | 0.09 |
| 2 | 0.5 | 1.6 | | 2.45 | 4.0 | 3 | 2.2 | | | 0.68 |
| | 7.0 | 1.2 | | 1.15 | 1.3 | 1 | 2.1 | | | 0.12 |
| 4 | 0.5 | 1.9 | | 3.07 | 4.7 | 4 | 2.5 | | | 0.81 |
| | 7.0 | 1.3 | | 1.46 | 1.6 | 1 | 2.2 | | | 0.20 |
| →4)-β-D-Glc*p*NAc-OMe | | | | | | | | | | |
| 0.5 | 0.5 | 0.17 | 0.30 | | 0.35 | 0.3 | 0.24 | | 0.40 | 1.2 |
| | 7.0 | 0.12 | 0.30 | | 0.26 | 0.2 | 0.26 | | 0.14 | 0.40 |
| 1 | 0.5 | 0.39 | 1.0 | | 0.83 | 0.6 | 0.35 | | 0.72 | 2.4 |
| | 7.0 | 0.31 | 0.85 | | 0.68 | 0.5 | 0.34 | | 0.31 | 1.1 |
| 2 | 0.5 | 0.75 | 1.9 | | 1.5 | 1 | 0.60 | | 1.1 | 4.2 |
| | 7.0 | 0.60 | 0.89 | | 1.2 | 1 | 0.60 | | 0.58 | 2.1 |
| 4 | 0.5 | 1.0 | 2.8 | | 2.2 | 1 | 0.55 | | 1.4 | 5.6 |
| | 7.0 | 0.76 | 1.3 | | 1.7 | 1 | 0.50 | | 0.80 | 2.9 |

## $K_D$ from NMR titrations

Compound **11** was titrated into a solution of 30 µM HEWL and $T_2$ relaxation measurements were made for each of the eight concentrations of **11** with the $^1$H NMR CPMG spin-echo experiment. The ligand resonance at 2.04 ppm was monitored.

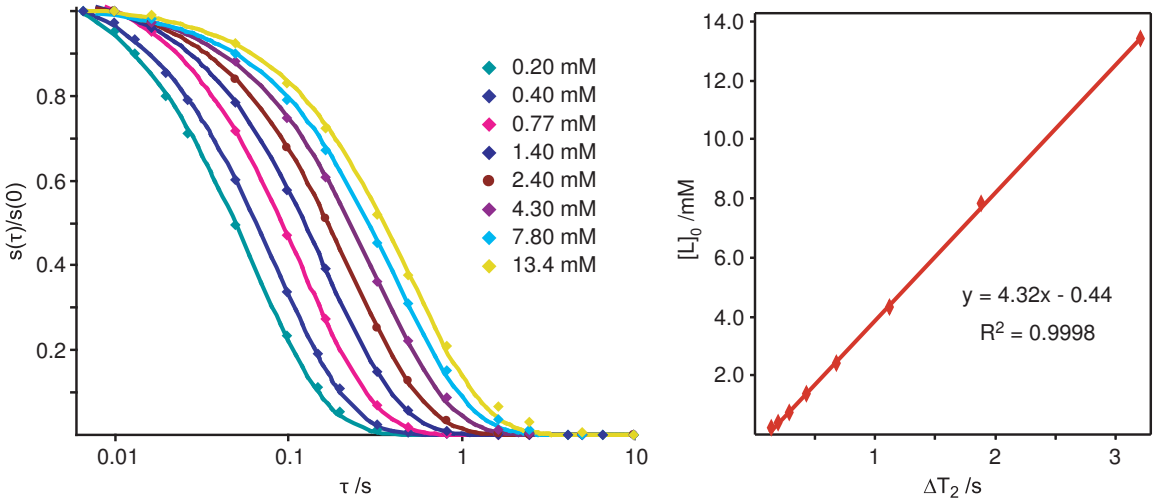



**Figure S1**. To the left: plot with S(τ)/S(0) versus τ for the eight concentrations of **11** together with the fitted curves, from which $T_2$ was extracted. To the right: plot with $[L]_0$ versus $\Delta T_2$, giving the $-K_D$ as the intercept on the $[L]_0$ axis. The observed changes in spin-spin relaxation rates, $\Delta R_2$, for a given concentration were calculated relative to that of the free ligand, where $T_2^{free}$ = 1.47 s.

## References

1. Jansson, P.-E., Widmalm, G. (1992) NMR Studies of some 1,2- and 1,3-linked disaccharides. *J. Chem. Soc. Perkin Trans. 2.* 1085-1090.

2. Olsson, U., Säwén, E., Stenutz, R., Widmalm, G. (2009) Conformational flexibility and dynamics of two (1→6)-linked disaccharides related to an oligosaccharide epitope expressed on malignant tumour cells. *Chem. Eur. J. 15*, 8886-8894.

3. Roslund, M. U., Säwén, E., Landström, J., Rönnols, J., Jonsson, H. M., Lundborg, M., Svensson, M. V., Widmalm, G. (2011) Complete $^{1}$H and $^{13}$C NMR chemical shift assignments of mono-, di-, and trisaccharides as basis for NMR chemical shift predictions of polysaccharides using the computer program CASPER. *Carbohydr. Res. 346*, 1311-1319.

4. Sophianopoulos, A. J., and Van Holde, K. E. (1964) Physical studies of muramidase (lysozyme). II. pH-dependent dimerization. *J. Biol. Chem. 239*, 2516-2524.

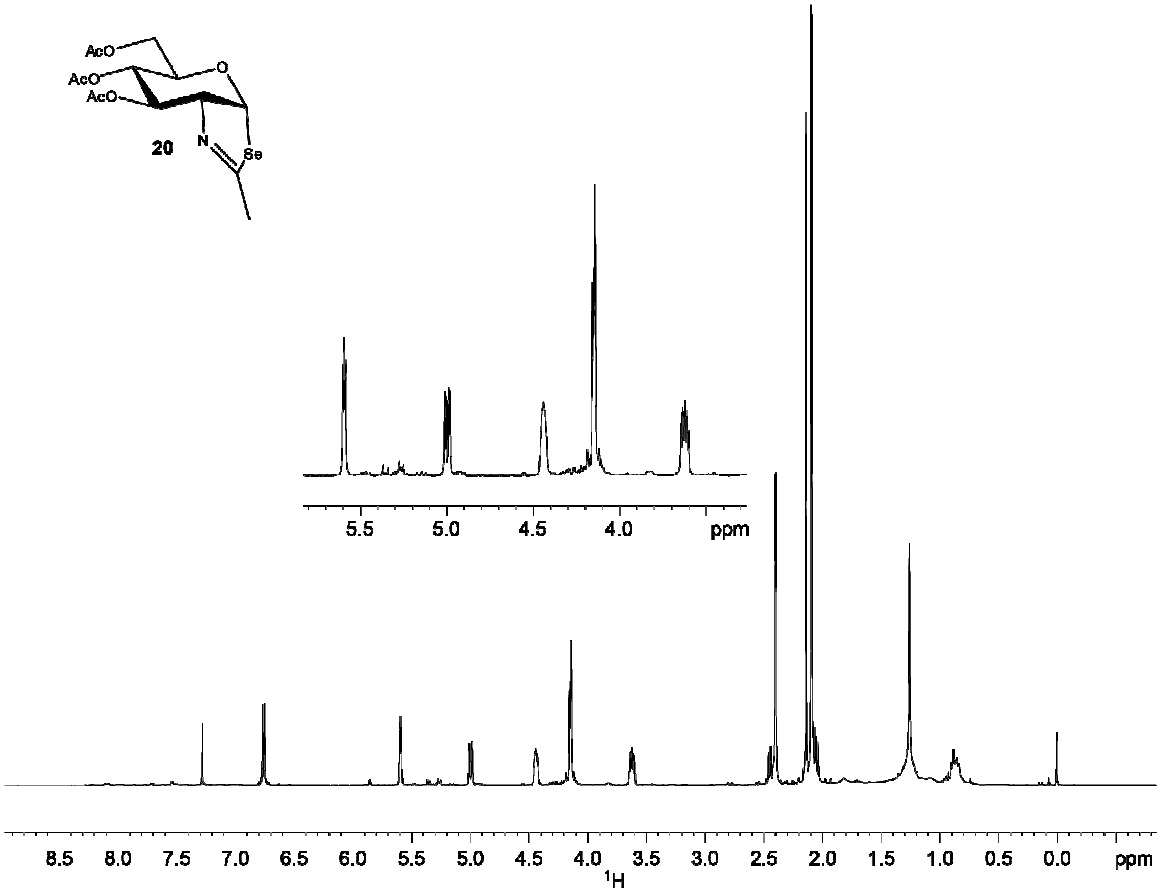
5. Ogra, Y., Ishiwata, K., Takayama, H., Aimi, N., and Suzuki, K. T. (2002) Identification of a novel selenium metabolite, Se-methyl-*N*-acetylselenohexosamine, in rat urine by high-performance liquid chromatography-inductively coupled plasma mass spectrometry and -electrospray ionization tandem mass spectrometry. *J. Chromatogr. B 767*, 301-312.

6. Knapp, S., Vocadlo, D., Gao, Z. N., Kirk, B., Lou, J. P., and Withers, S. G. (1996) NAG-thiazoline, an *N*-acetyl-β-hexosaminidase inhibitor that implicates acetamido participation. *J. Am. Chem. Soc. 118*, 6804-6805.

7. Bhattacharyya, P., and Woollins, J. D. (2001) Selenocarbonyl synthesis using Woollins reagent. *Tetrahedron Lett. 42*, 5949-5951.

8. Hamark, C., Landström, J., Eriksson L., Widmalm, G. (2010) Ethyl 4,6-*O*-benzylidene-2-deoxy-*N*-phthalimido-1- thio-β-D-glucopyranoside. *Acta Cryst. E66*, o3249.

9. Hamark, C., Landström, J., Eriksson L., Widmalm, G. (2010) Ethyl 3,6-di-*O*-benzyl-2-deoxy-*N*-phthalimido-1- thio-β-D-glucopyranoside. *Acta Cryst. E66*, o3250-o3251.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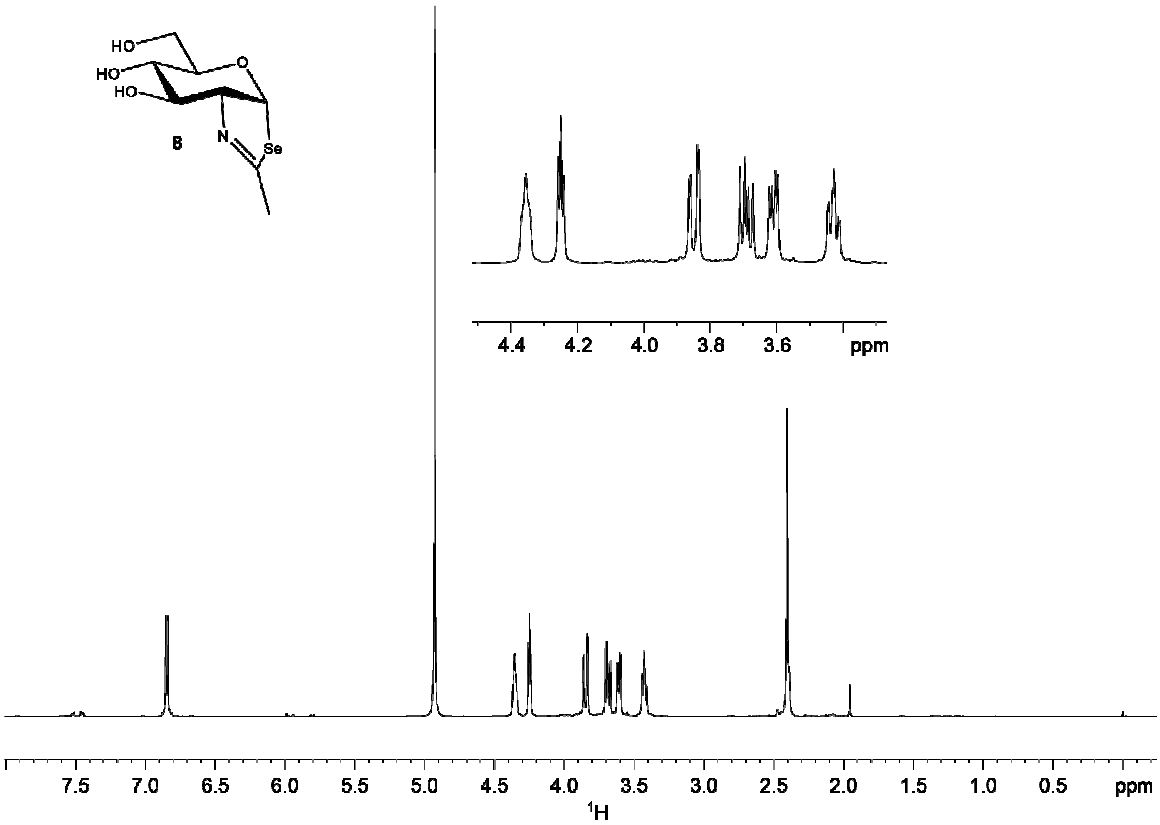
AcO
AcO
O
20
N
Se
5.5
5.0
4.5
4.0
ppm
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
ppm
1H


HO
HO
HO
O
8
N
Se
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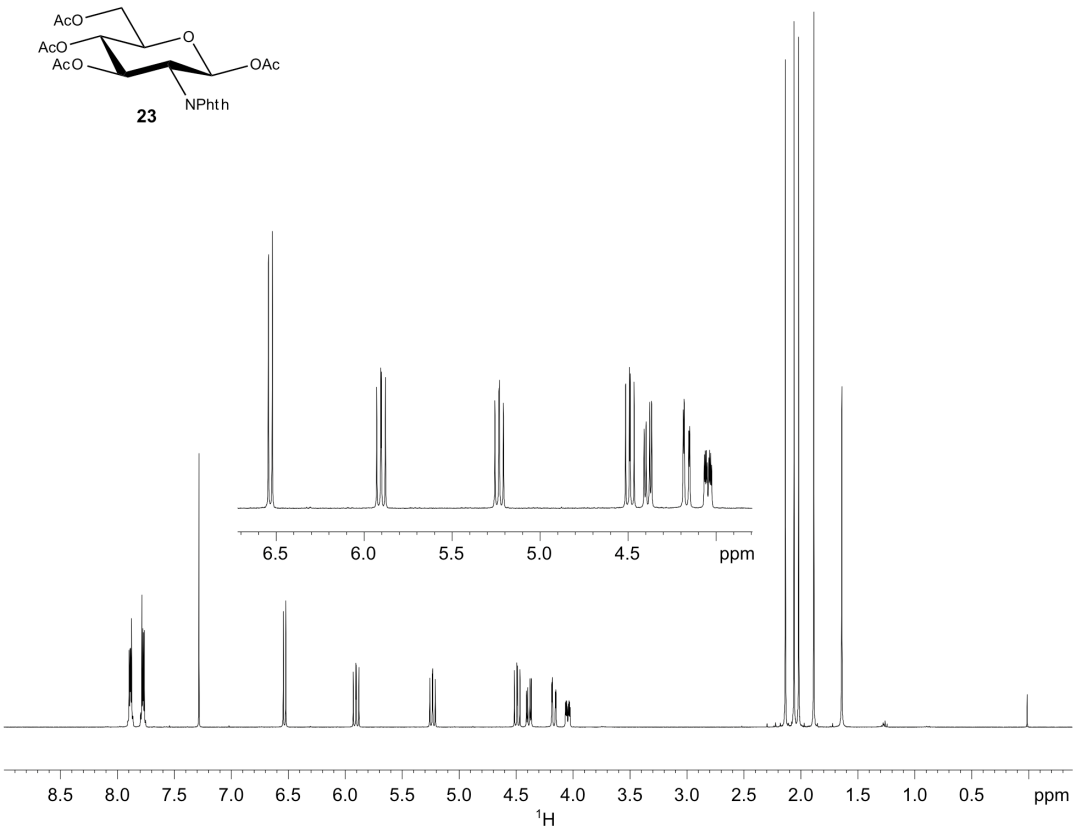
4.2
4.0
3.8
3.6
ppm
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm
1H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AcO
AcO
O
OAc
NPhth
23
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6.0
5.5
5.0
4.5
ppm
8.5
8.0
7.5
7.0
6.5
6.0
5.5
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4.0
3.5
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1.5
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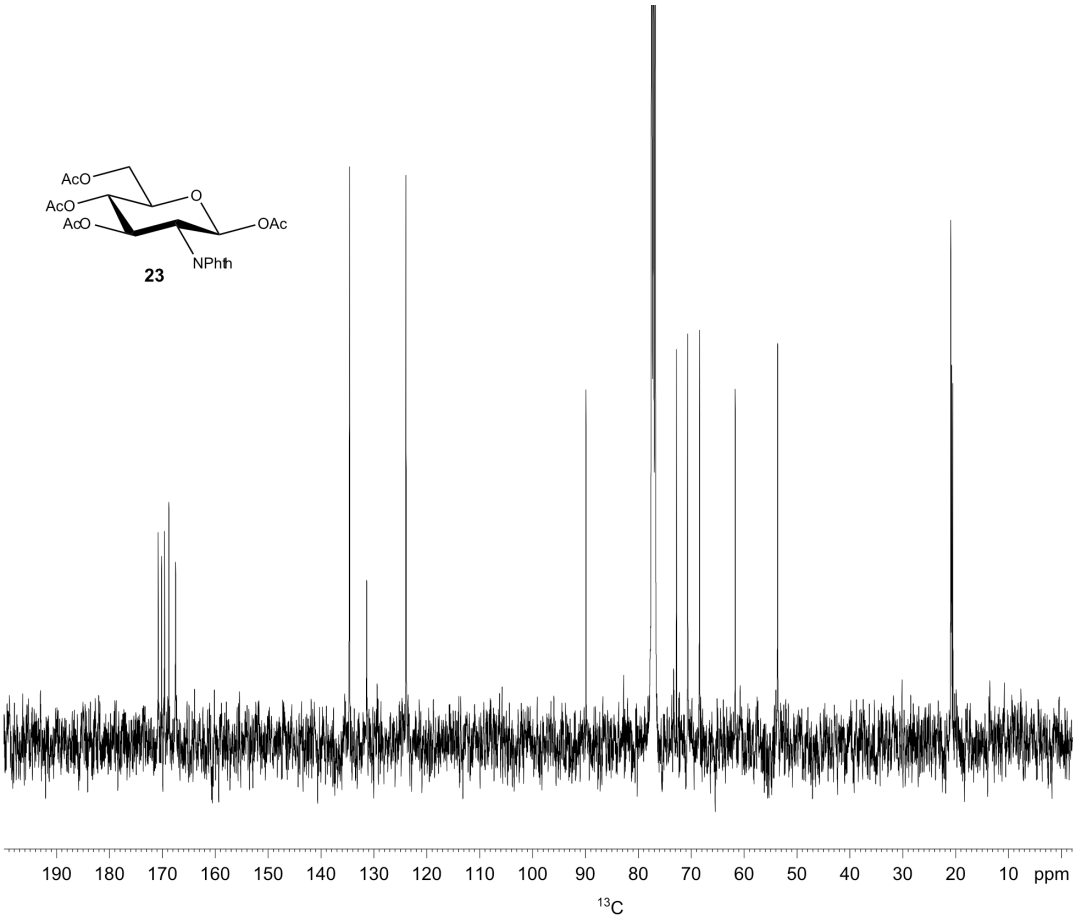
0.5
ppm
1H


AcO
AcO
AcO
O
OAc
NPhth
23
190
180
170
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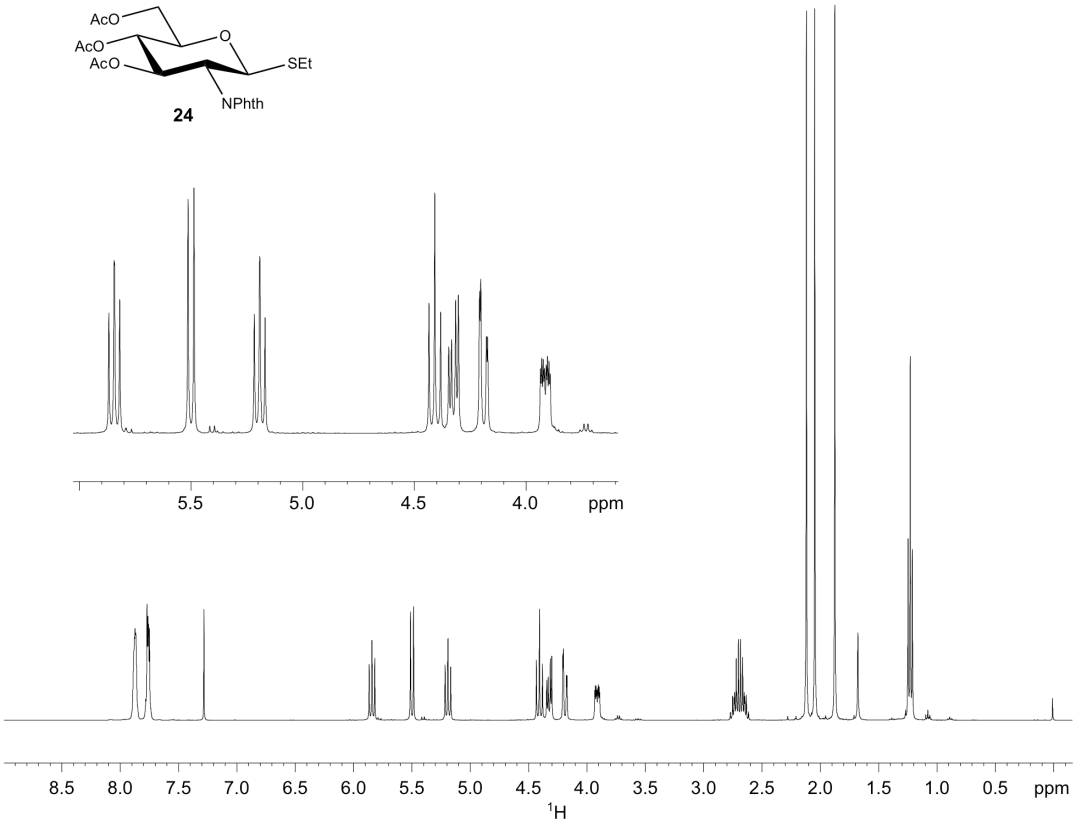
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm
13C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AcO
AcO
O
SEt
NPhth
24
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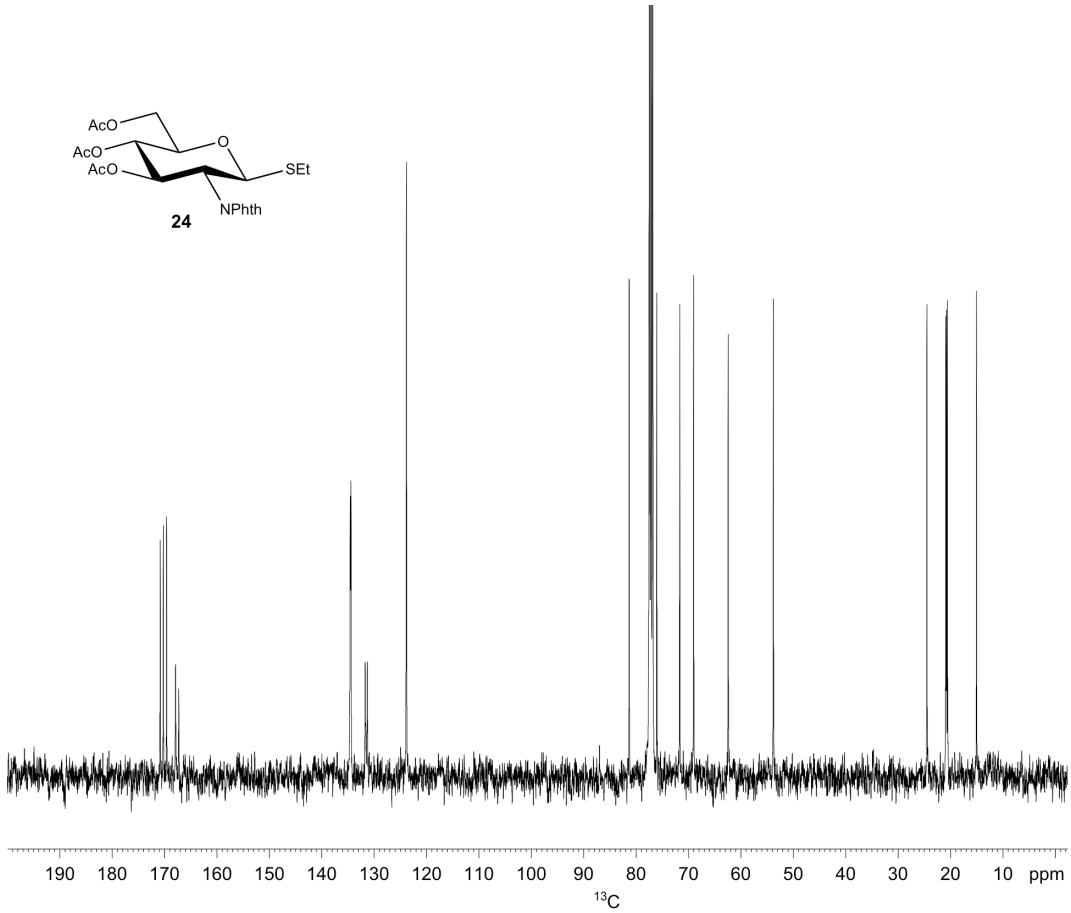
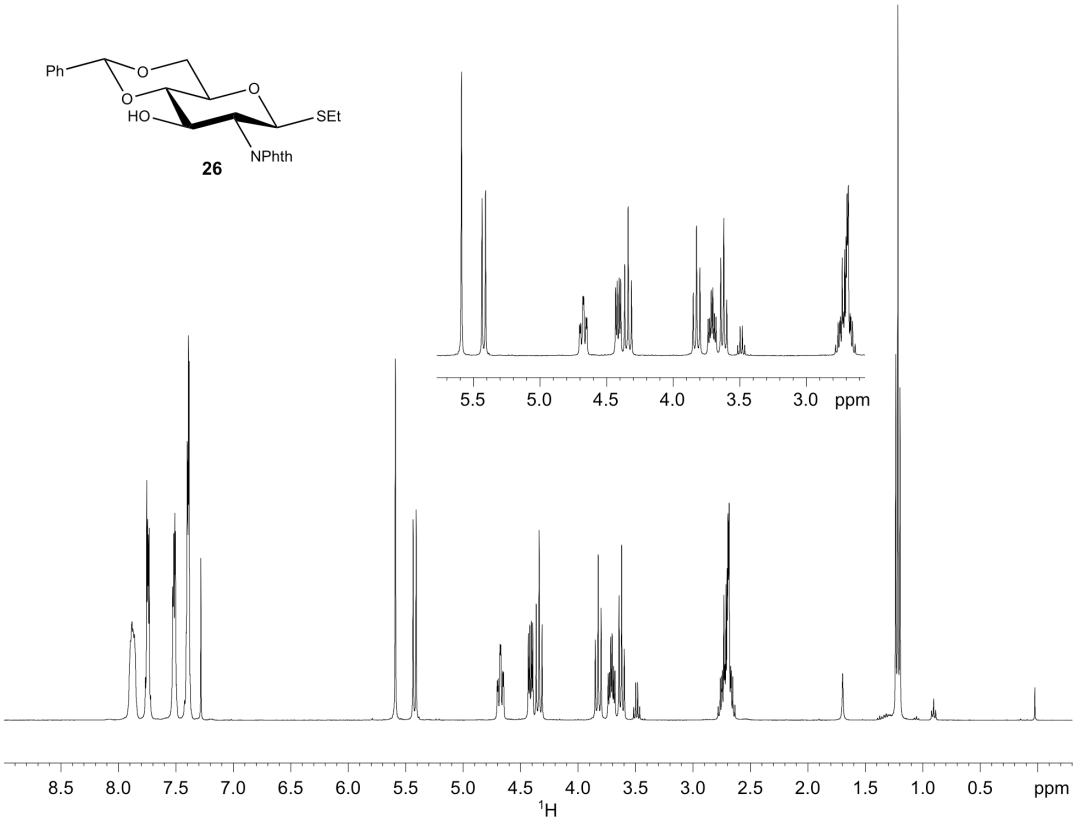
5.0
4.5
4.0
ppm
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm
1H


AcO
AcO
AcO
O
SEt
NPhth
24
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm
13C


Ph
O
O
O
HO
SEt
NPhth
26
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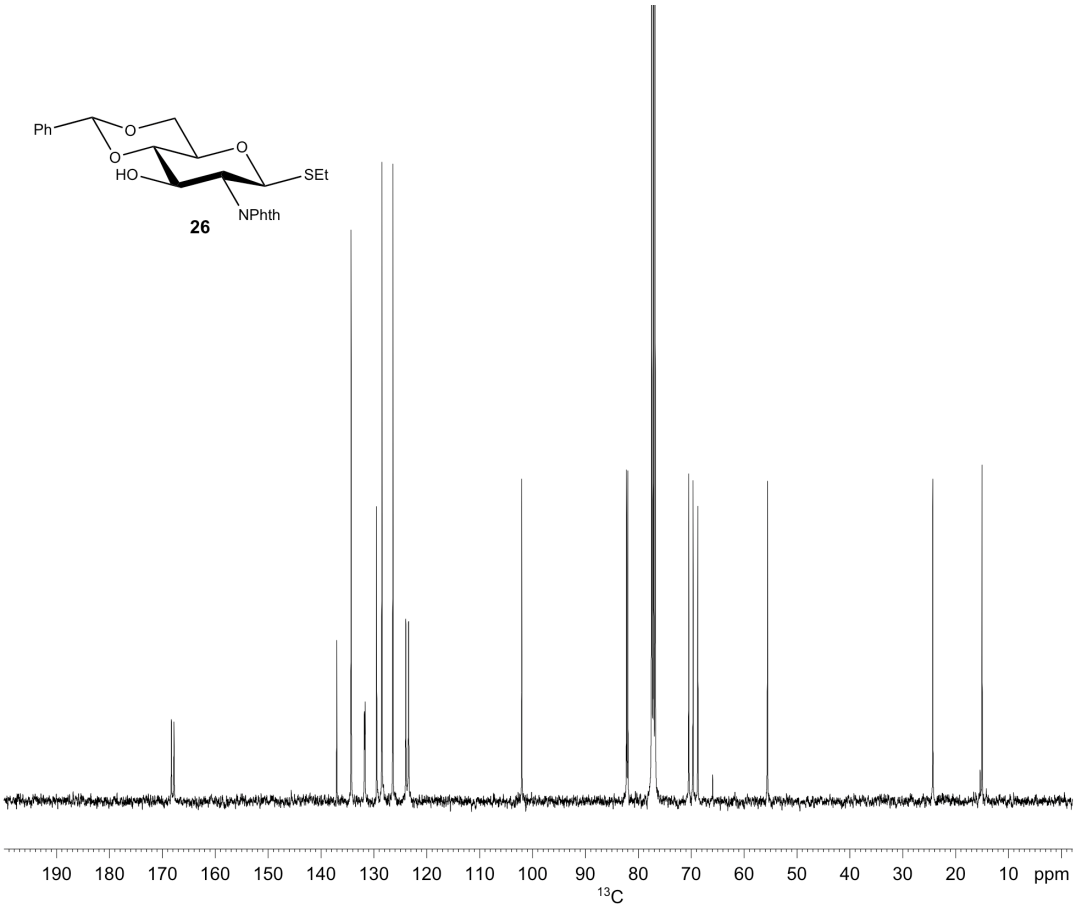
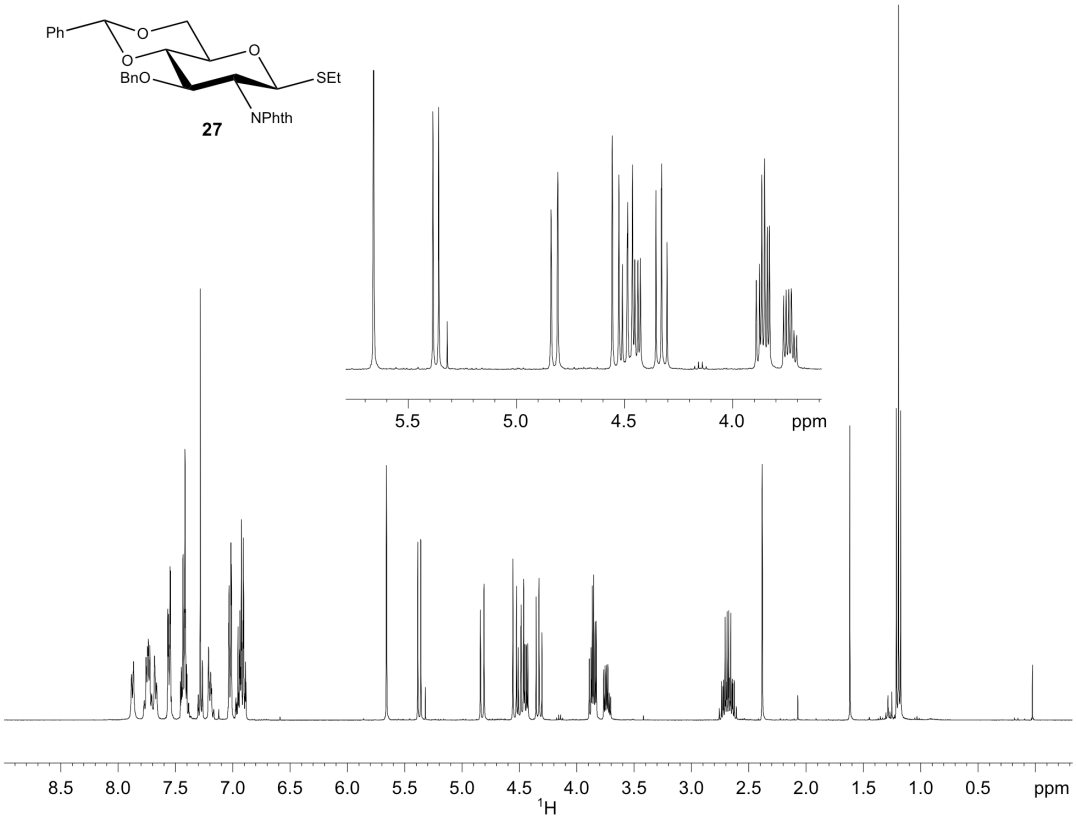
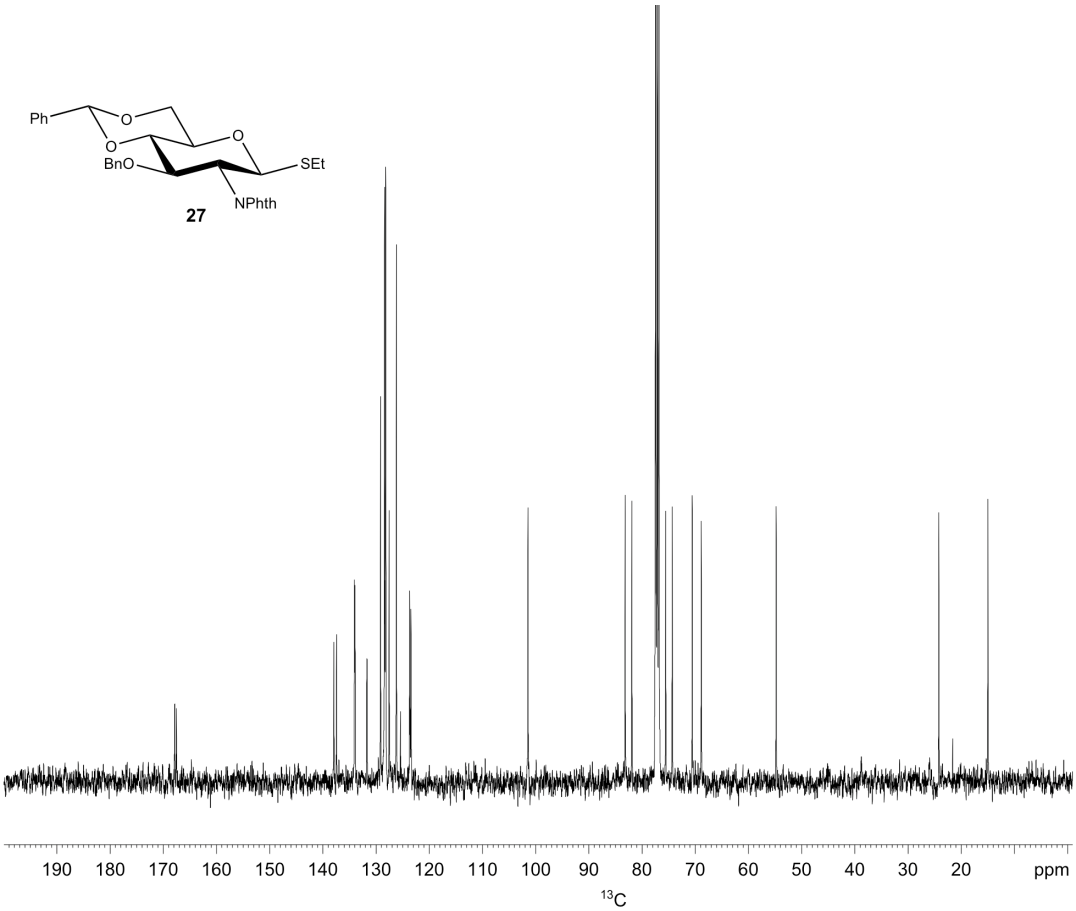
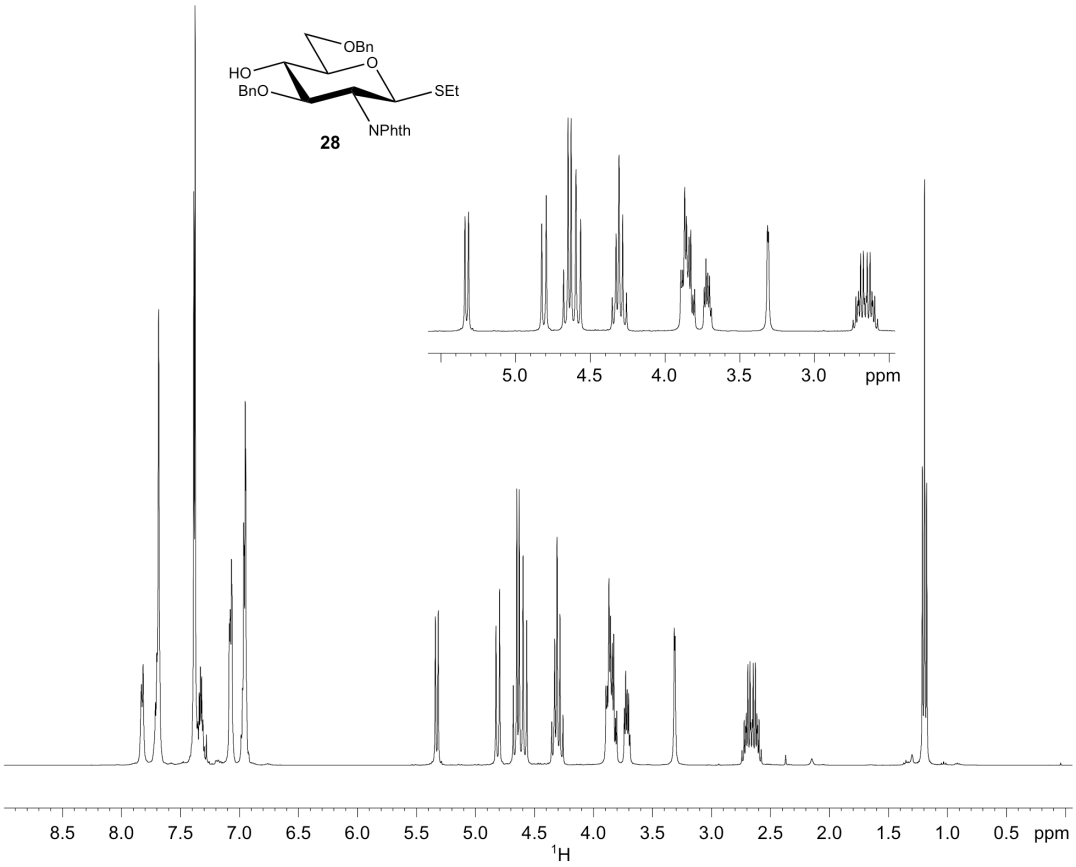
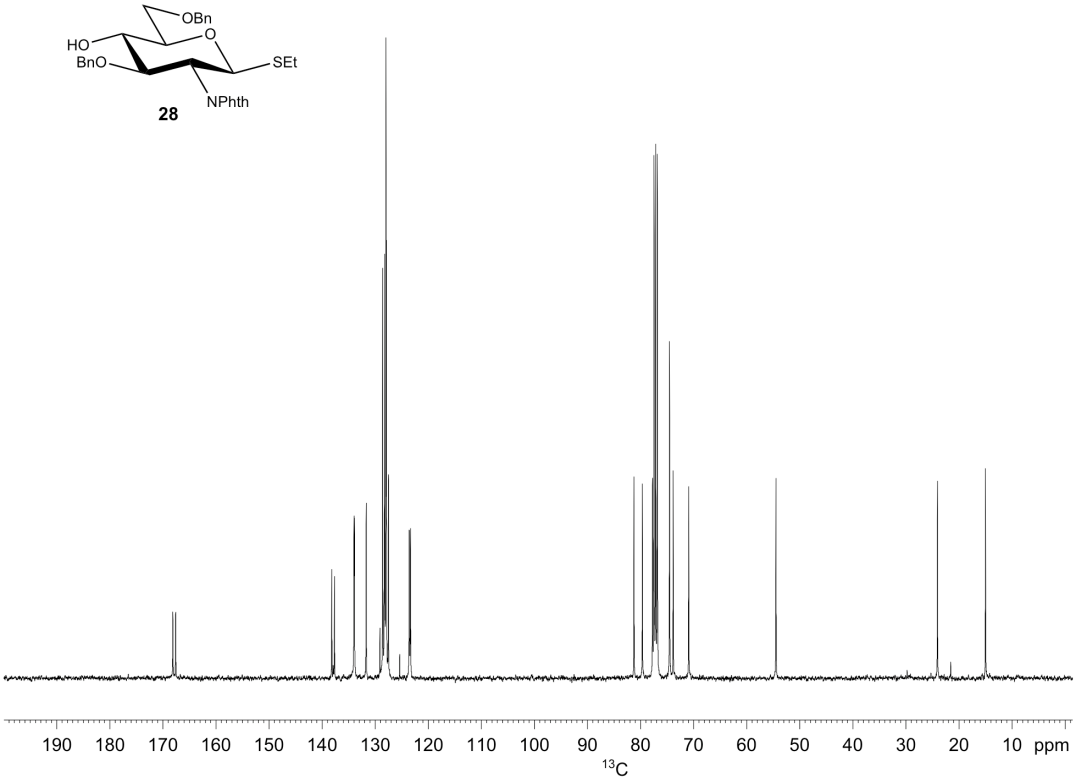
1H

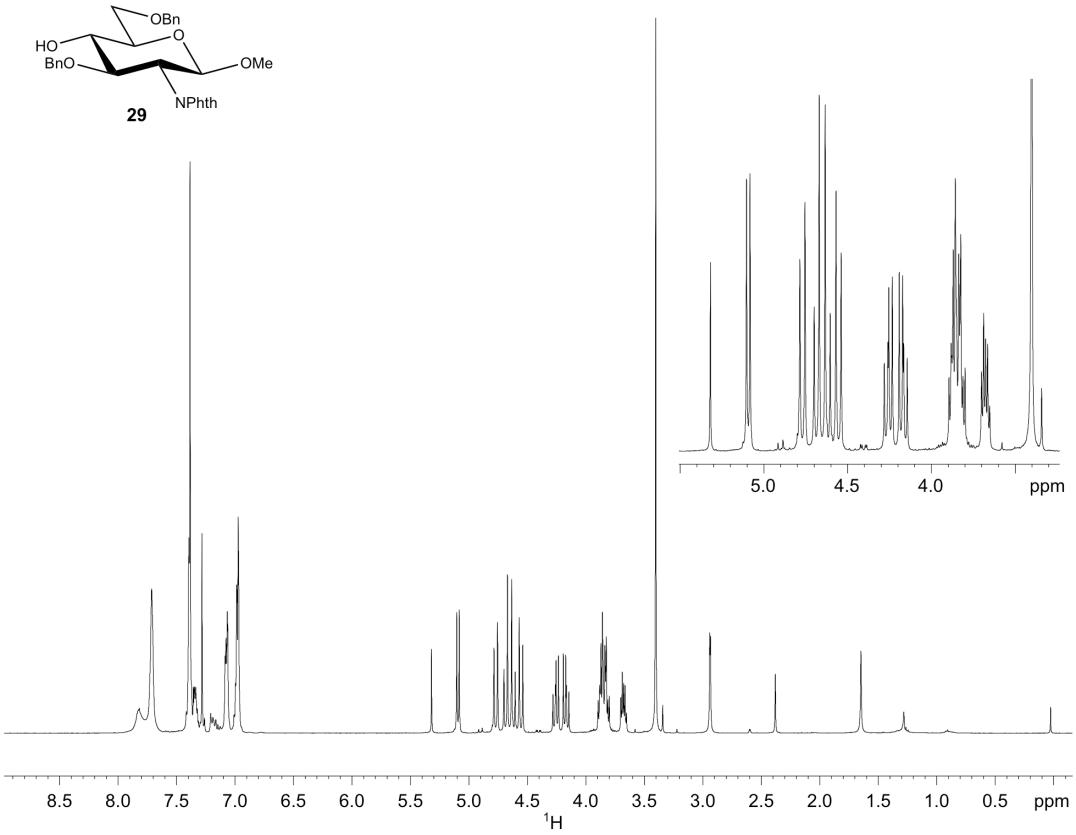

Ph
O
O
O
HO
SEt
NPhth
26
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 ppm
13C


Ph
O
O
O
BnO
SEt
NPhth
27
5.5
5.0
4.5
4.0
ppm
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm
1H


Ph
O
O
O
BnO
SEt
NPhth
27
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
ppm
13C


OBn
HO
O
BnO
SEt
NPhth
28
5.0 4.5 4.0 3.5 3.0 ppm
8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 ppm
1H


OBn
HO
O
BnO
SEt
NPhth
28
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 ppm
13C


OBn
O
HO
BnO
OMe
NPhth
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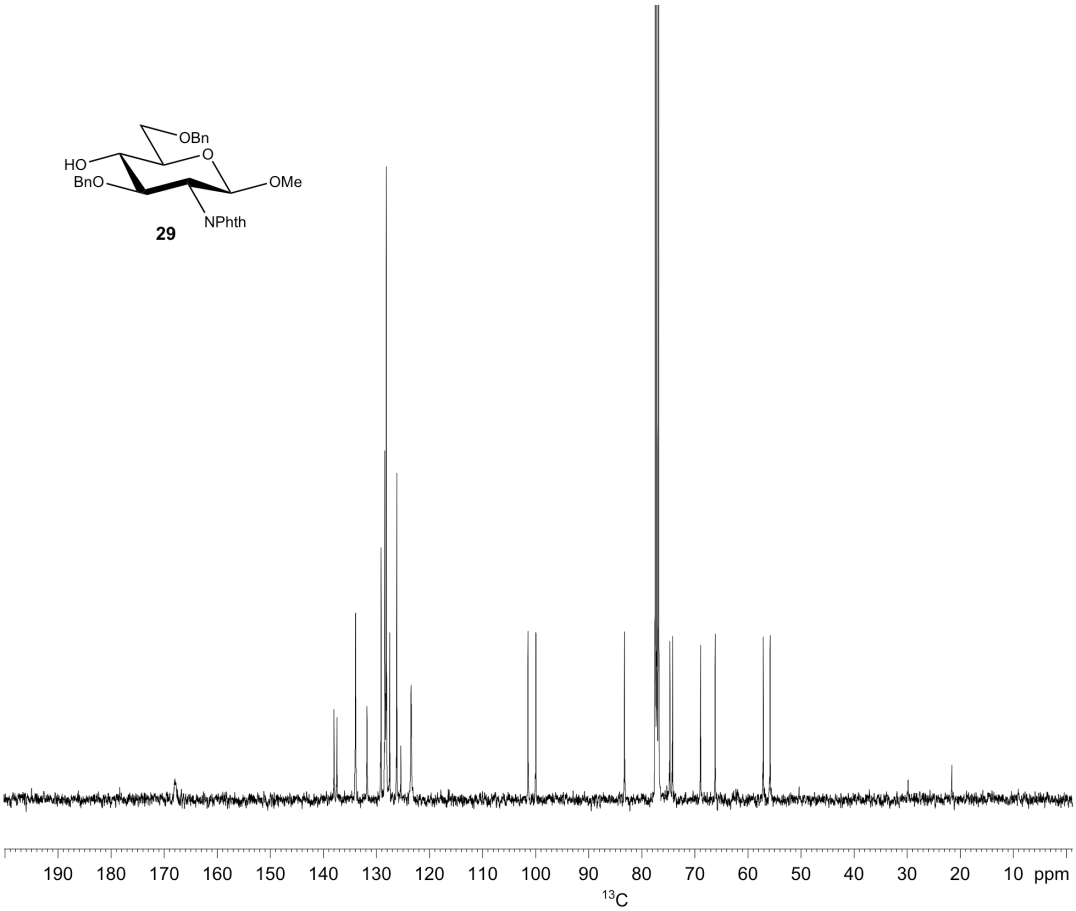
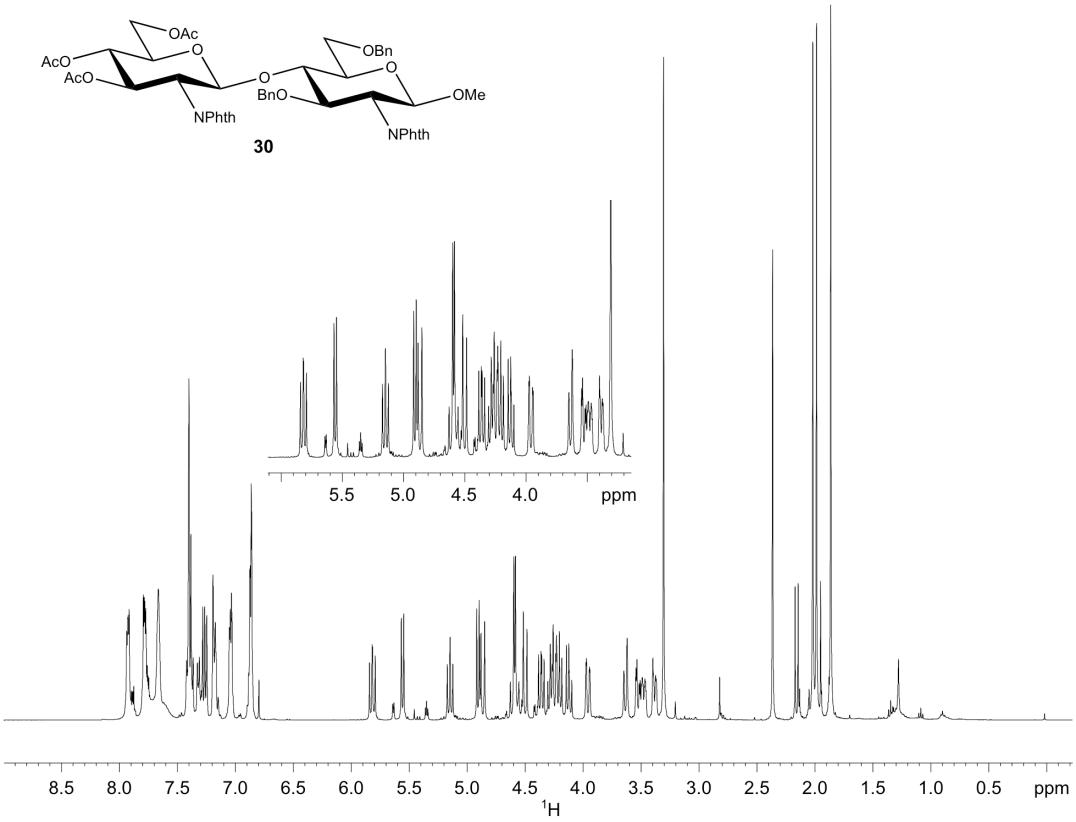
5.0
4.5
4.0
ppm
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm
1H


OBn
O
HO
BnO
OMe
NPhth
29
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm
13C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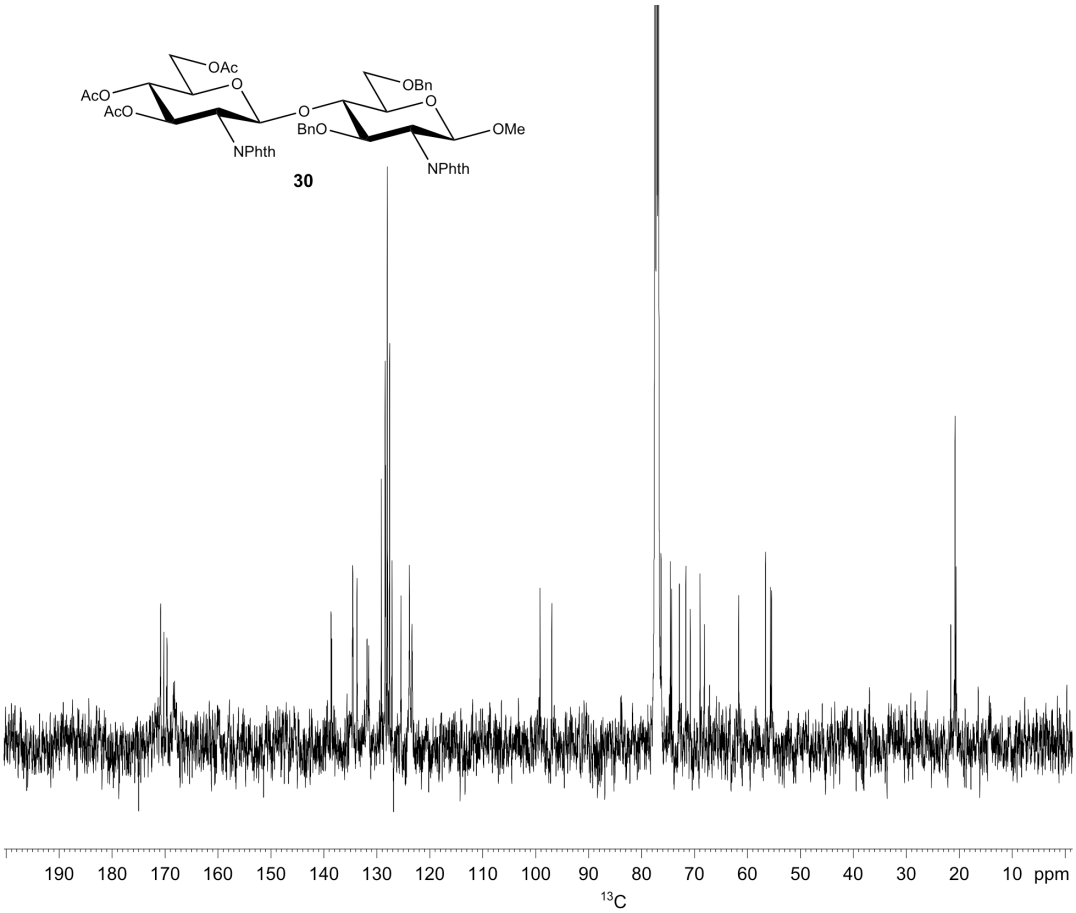
AcO
AcO
O
NPhth
O
BnO
OBn
O
OMe
NPhth
30
5.5
5.0
4.5
4.0
ppm
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm
1H


OAc
AcO
AcO
O
NPhth
O
BnO
OBn
O
OMe
NPhth
30
190
180
170
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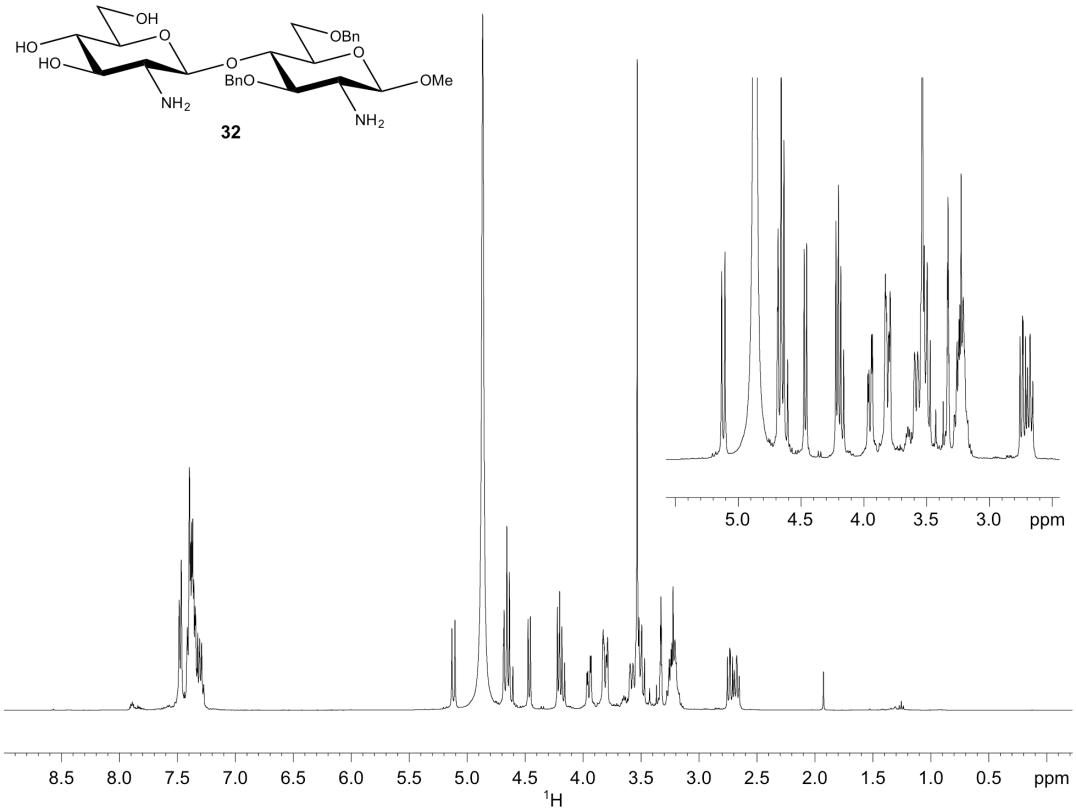
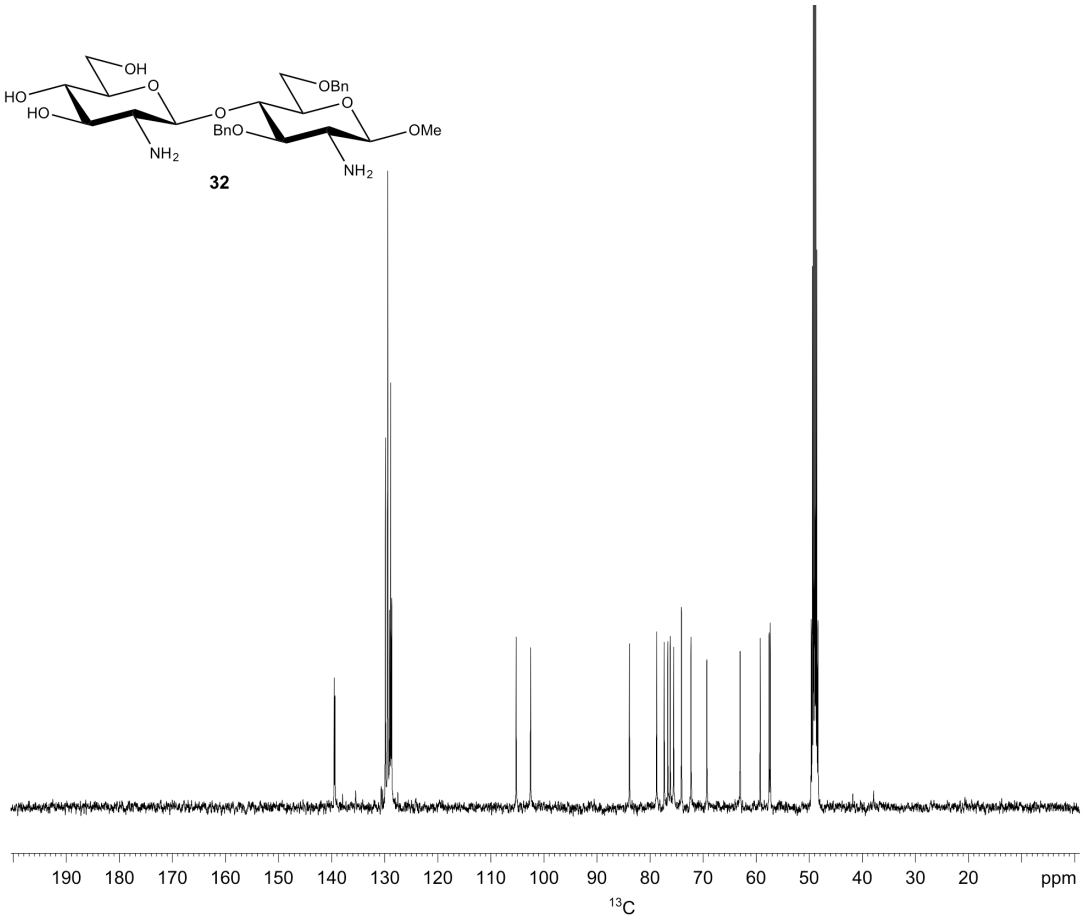
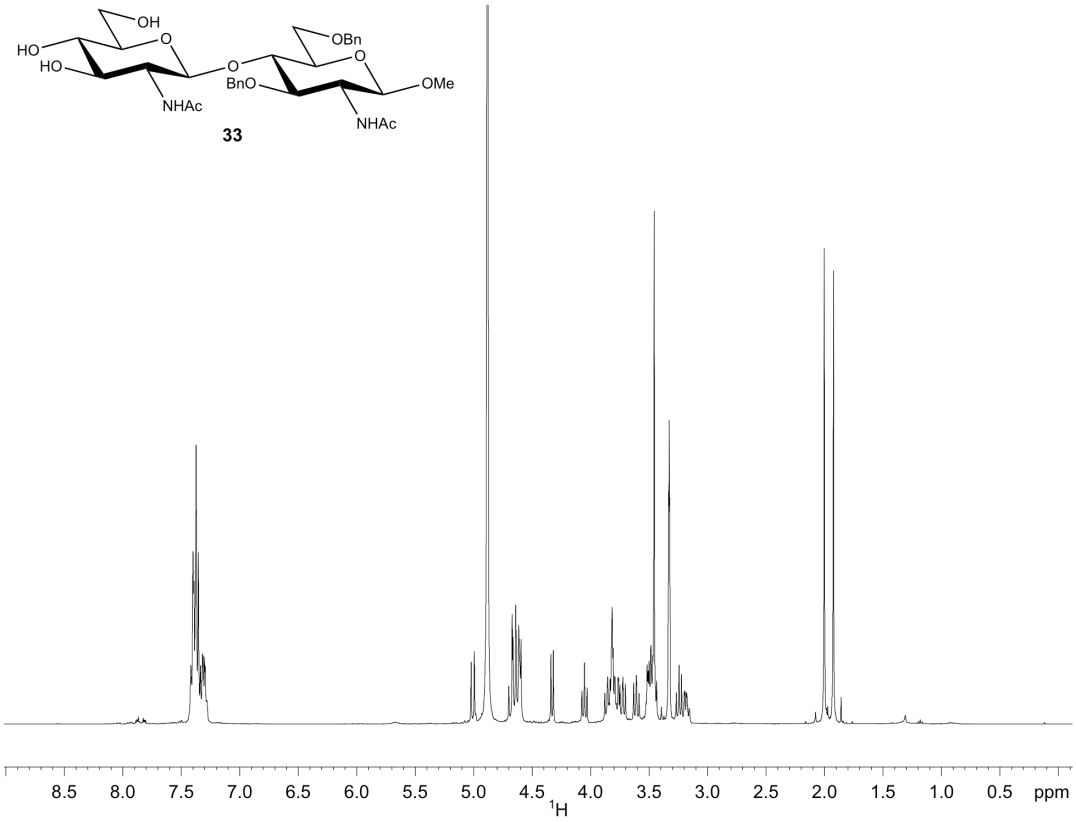
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm
13C


OH
HO
HO
O
NH2
O
BnO
OBn
O
OMe
NH2
32
5.0 4.5 4.0 3.5 3.0 ppm
8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 ppm
1H


OH
HO
HO
O
NH2
O
BnO
OBn
O
OMe
NH2
32
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 ppm
13C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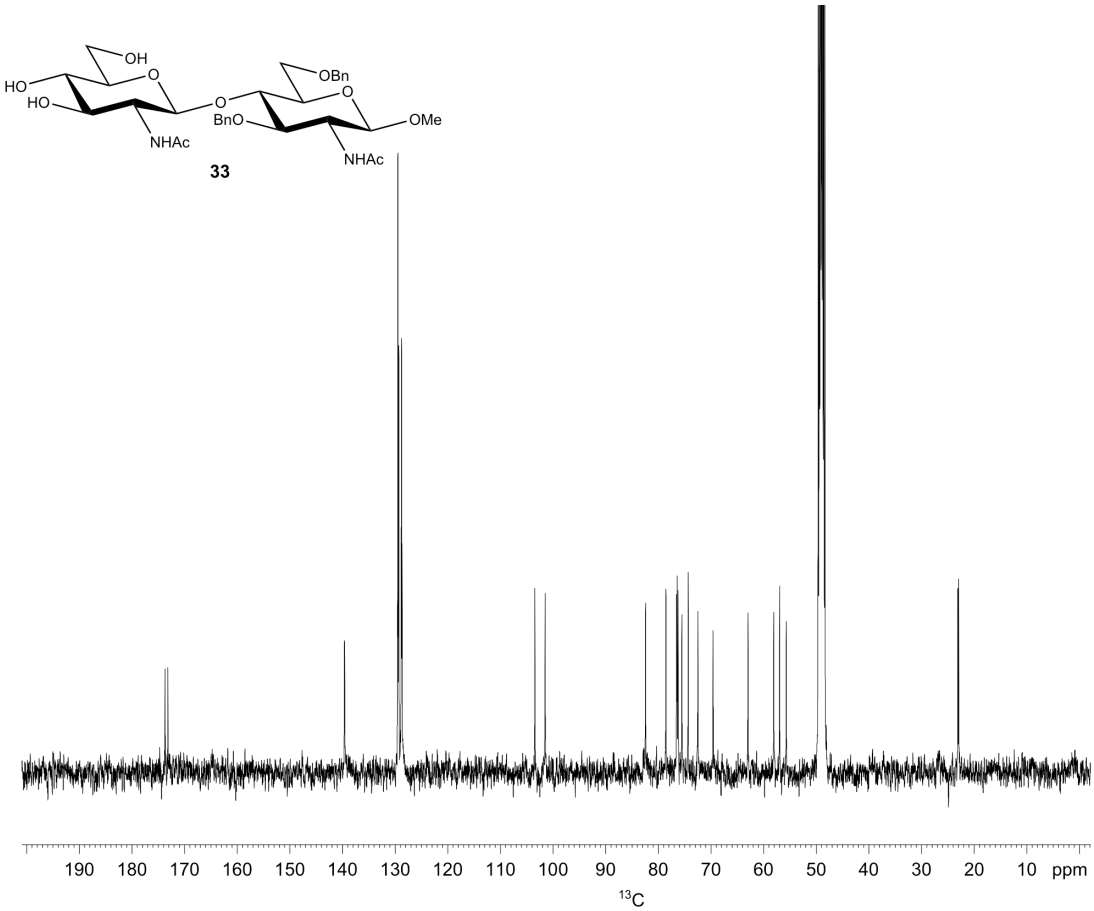
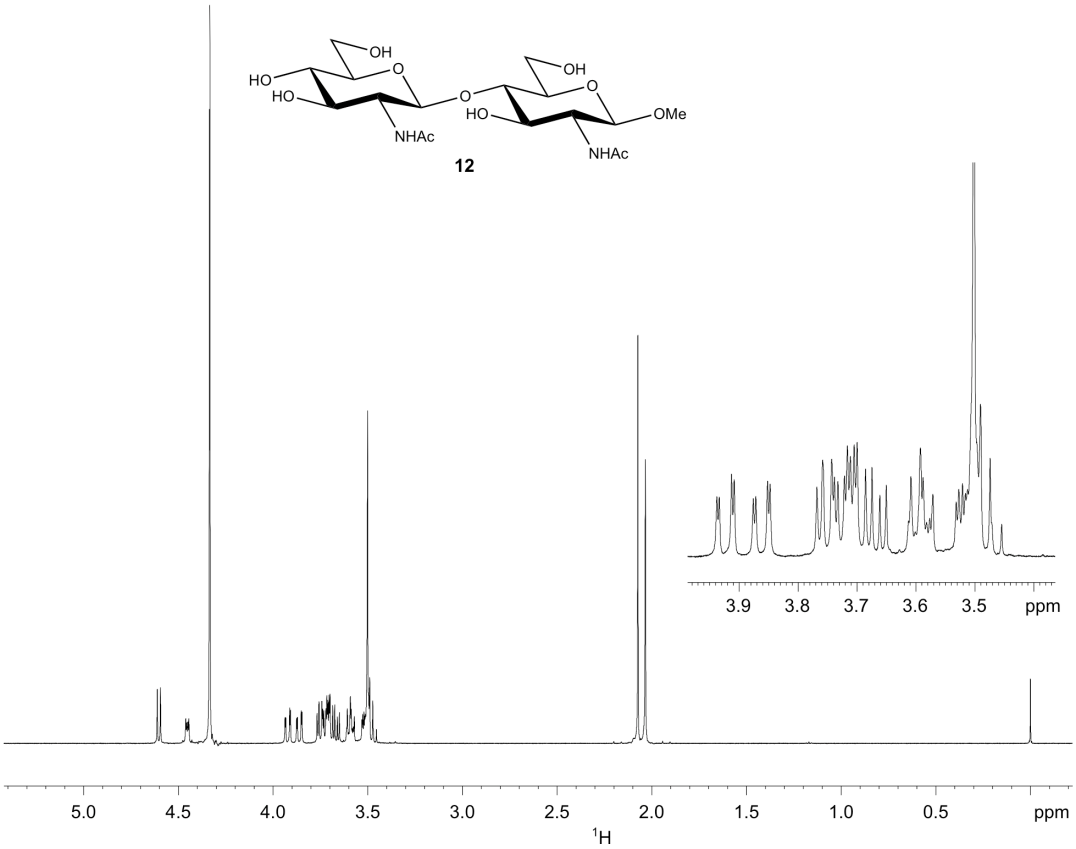
HO
HO
O
NHAc
O
BnO
OBn
O
OMe
NHAc
33
8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 ppm
1H


OH
HO
HO
O
NHAc
O
BnO
OBn
O
OMe
NHAc
33
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 ppm
13C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OMe
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12
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3.8
3.7
3.6
3.5
ppm
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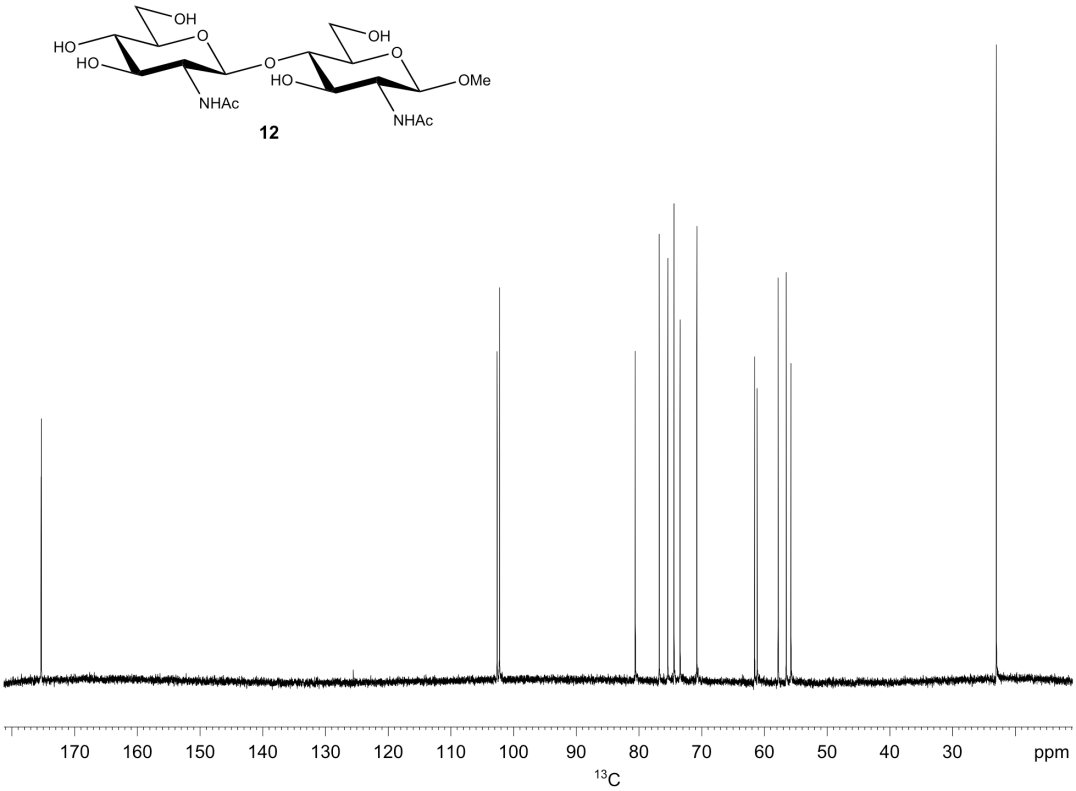
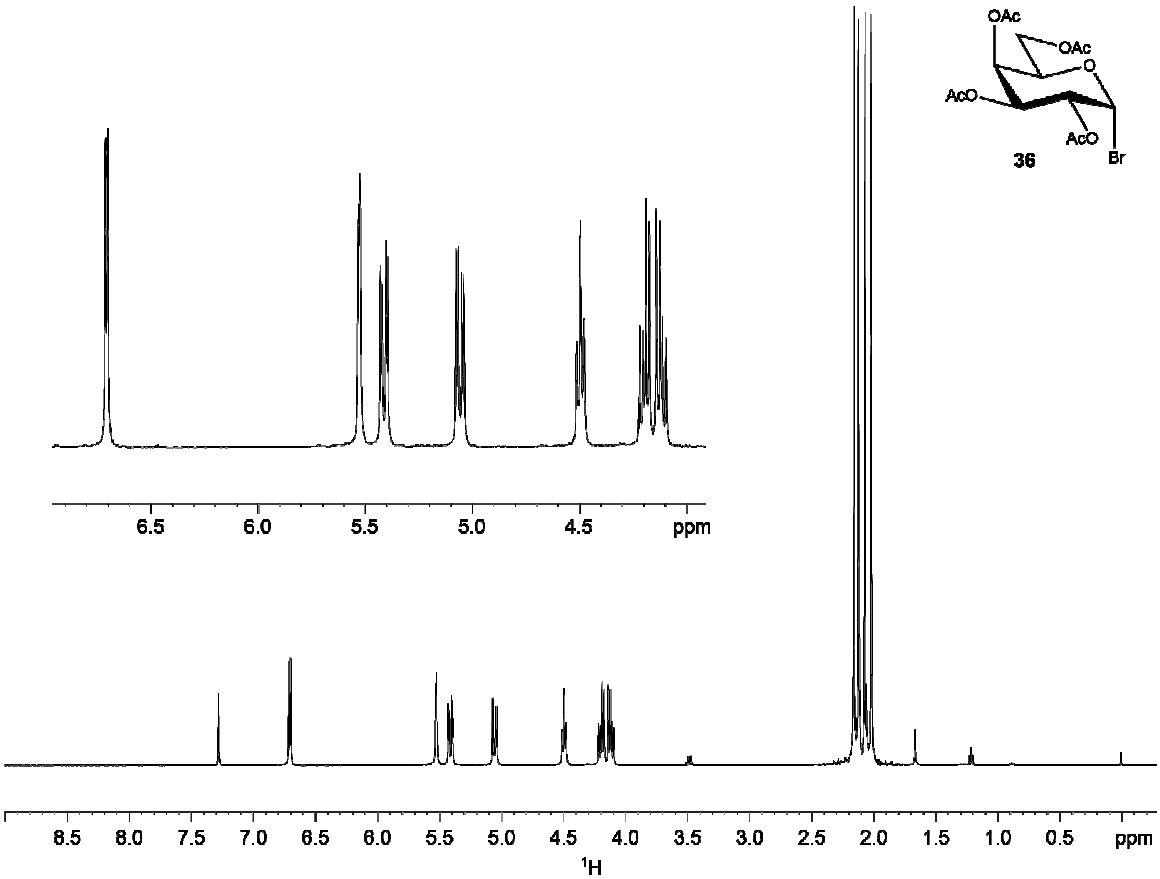
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm
1H


OH
HO
HO
O
NHAc
O
OH
O
HO
OMe
NHAc
12
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
ppm
13C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OAc
O
AcO
AcO
Br
36
6.5
6.0
5.5
5.0
4.5
ppm
8.5
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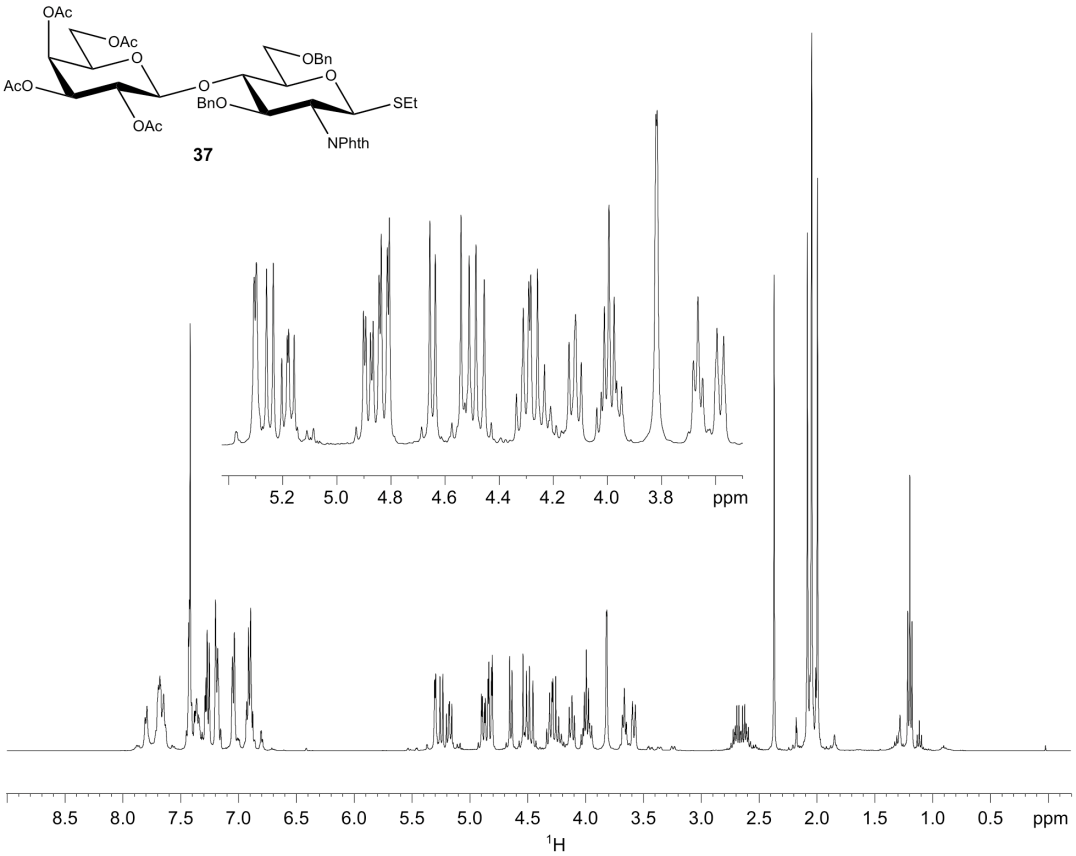
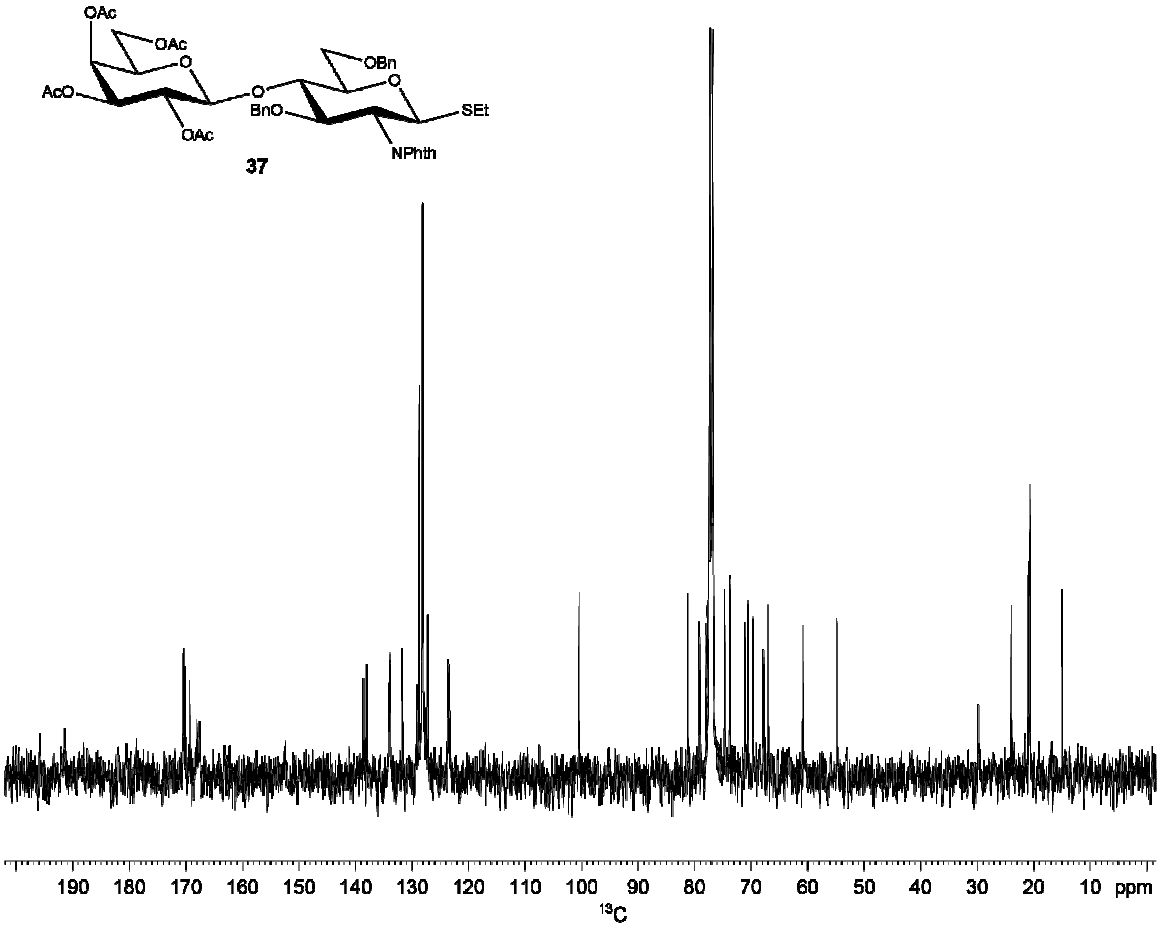
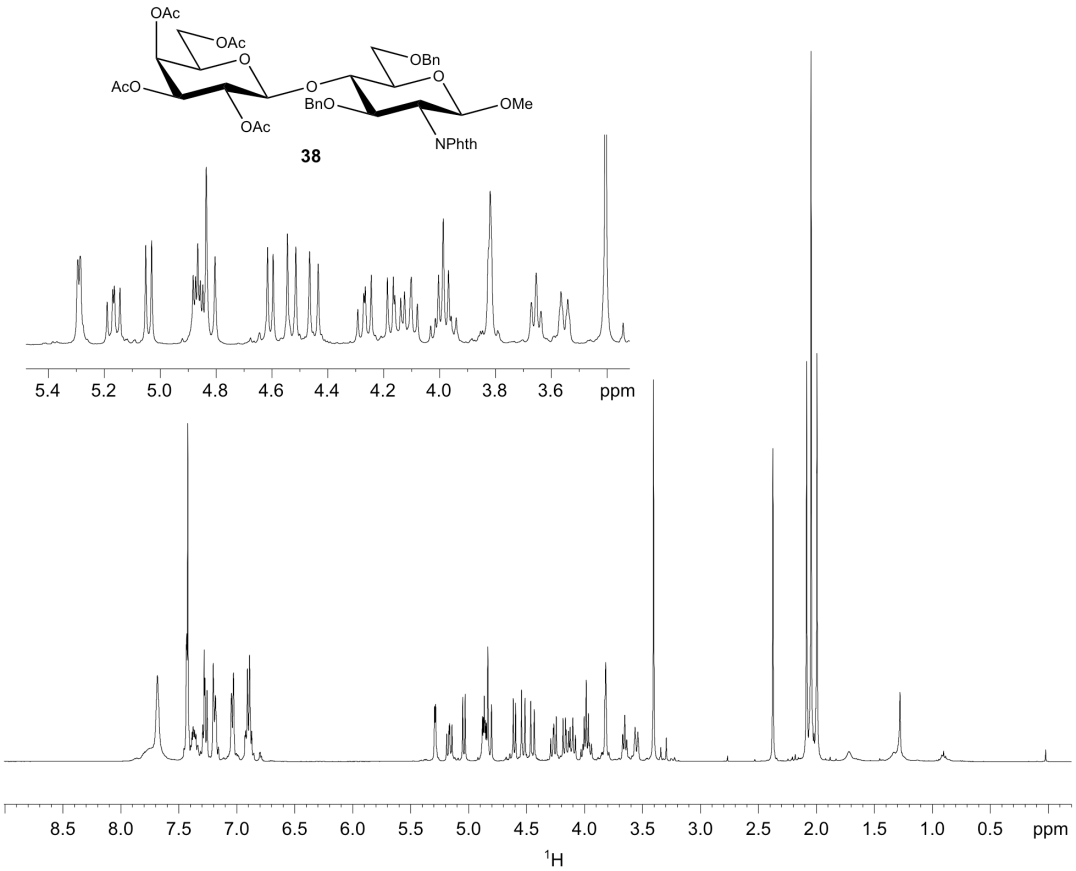
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm
$^1$H


OAc
OAc
O
AcO
OAc
O
BnO
OBn
O
SEt
NPhth
37
5.2 5.0 4.8 4.6 4.4 4.2 4.0 3.8 ppm
8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 ppm
1H


OAc
OAc
O
AcO
OAc
O
BnO
OBn
O
SEt
NPhth
37
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 ppm
13C


OAc
OAc
O
AcO
OAc
O
BnO
OBn
O
OMe
NPhth
38
5.4 5.2 5.0 4.8 4.6 4.4 4.2 4.0 3.8 3.6 ppm
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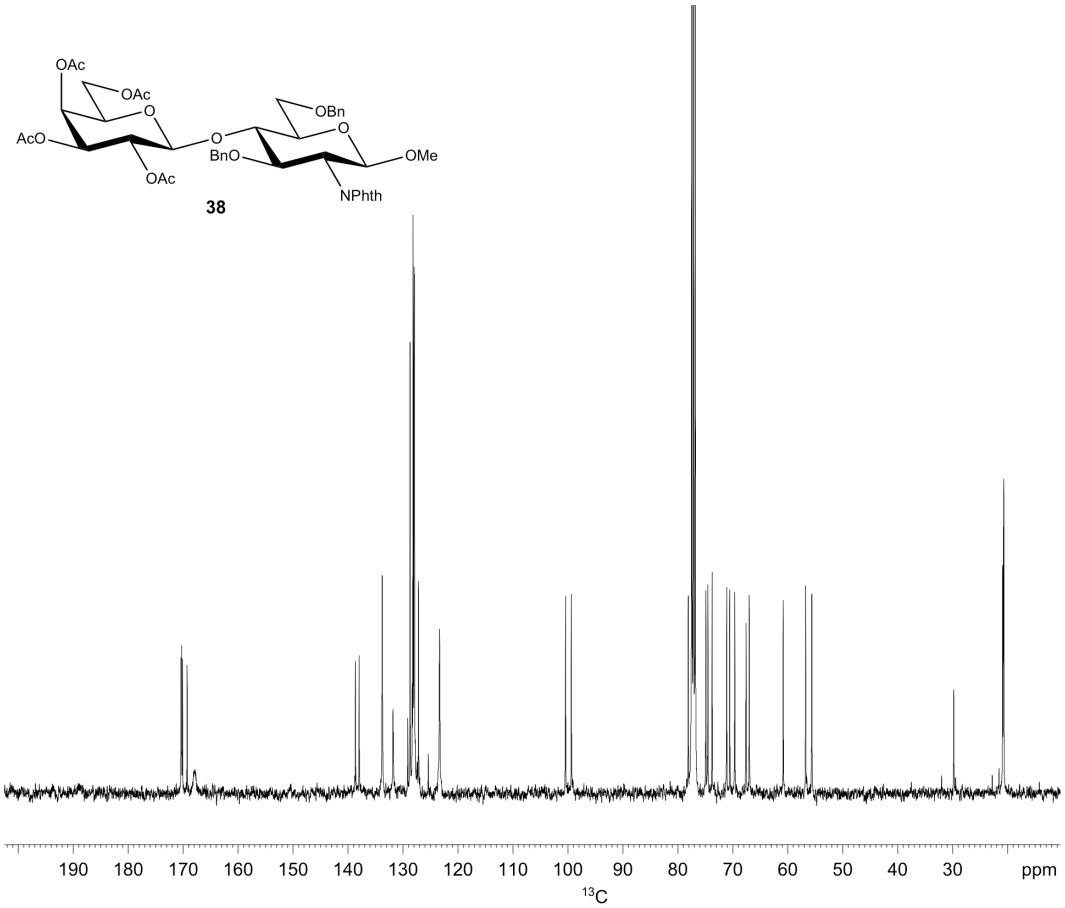
1H


OAc
OAc
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OAc
O
BnO
OBn
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OMe
NPhth
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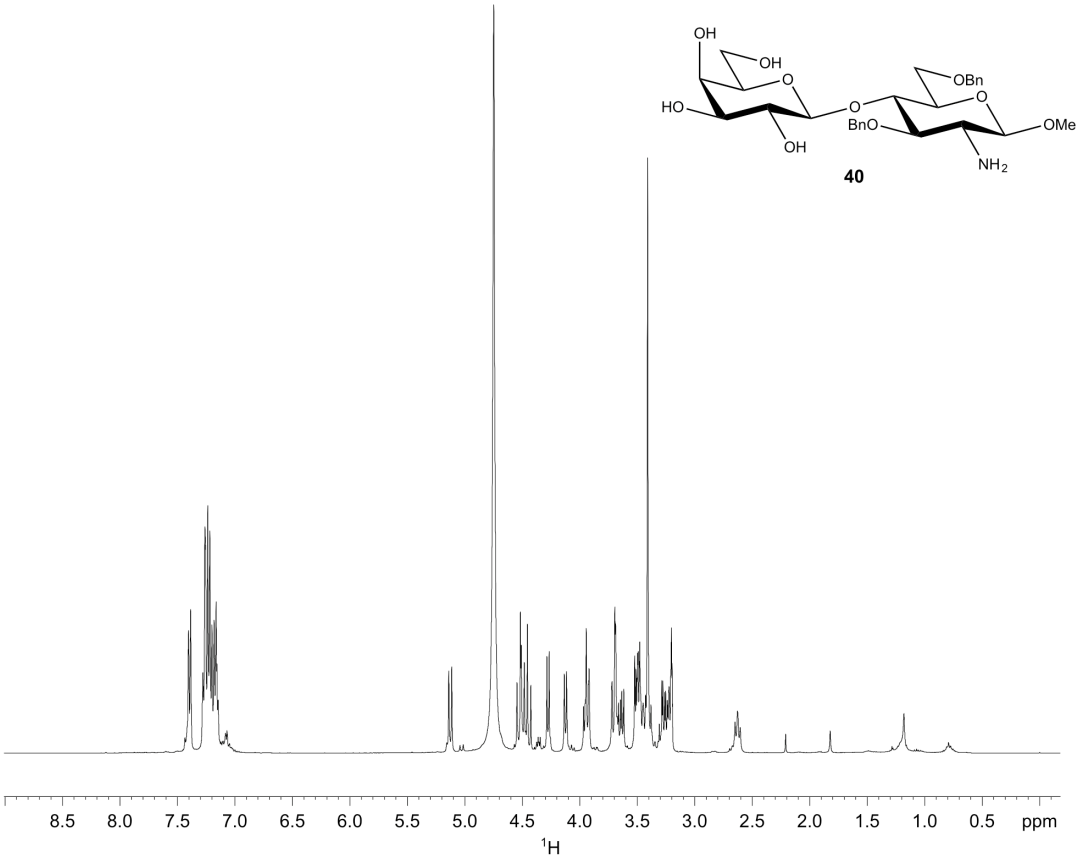
13C


OH
OH
O
HO
OH
O
BnO
OBn
O
OMe
NH2
40
8.5
8.0
7.5
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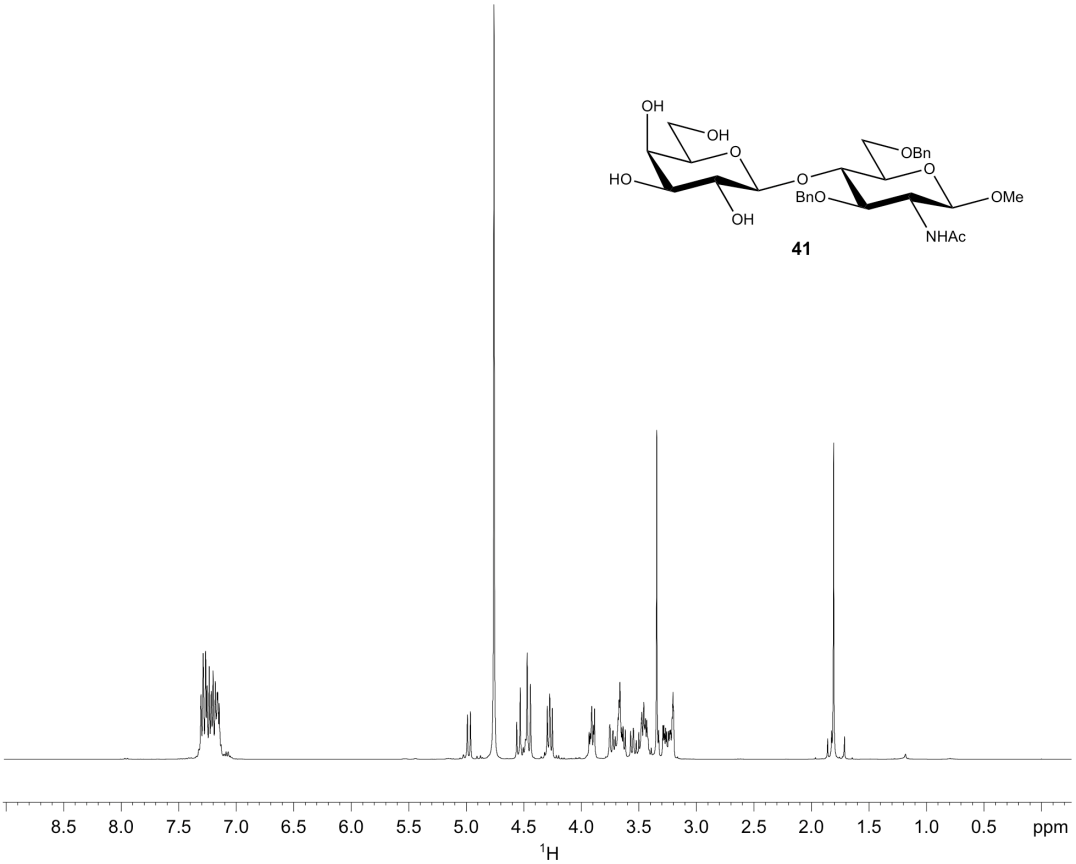
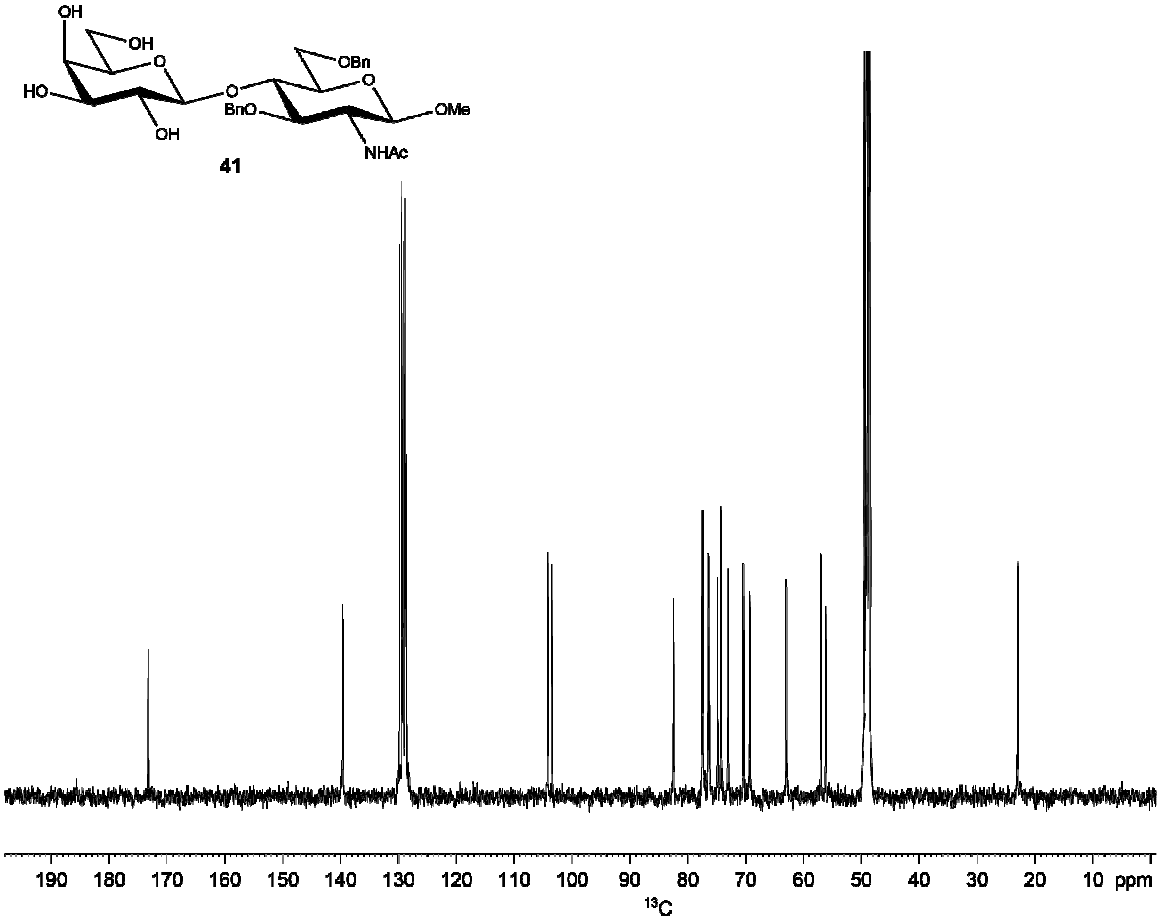
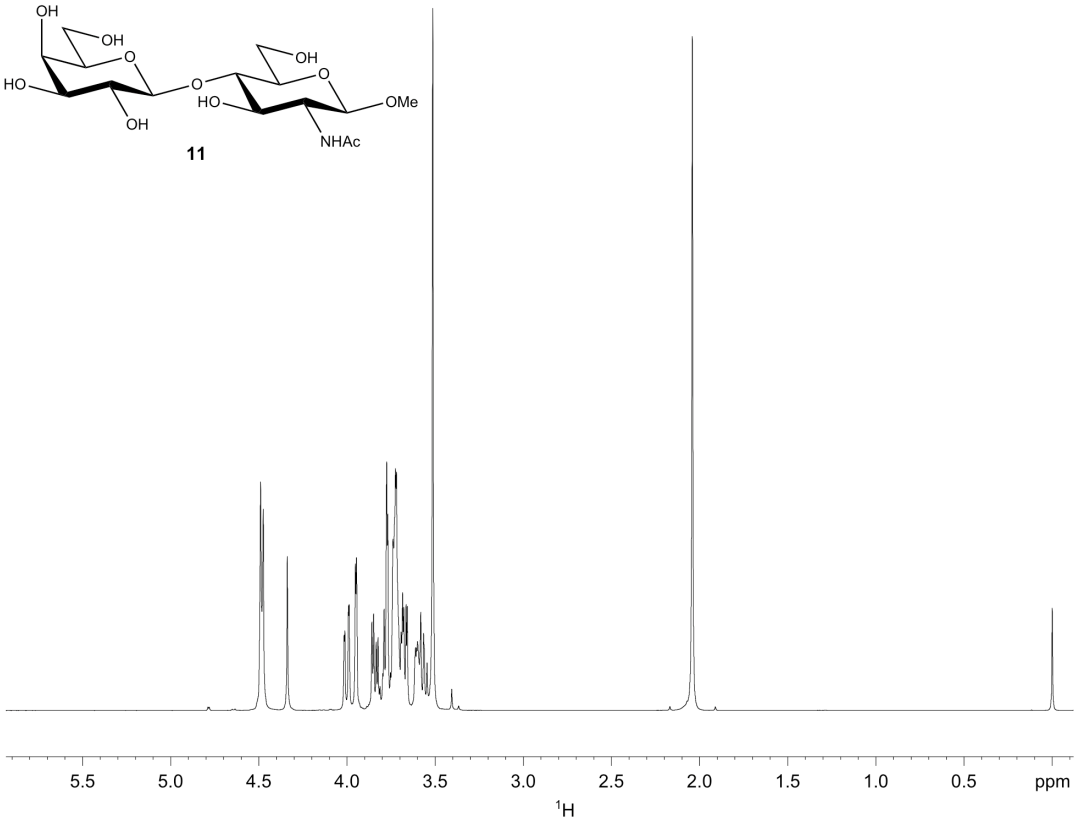
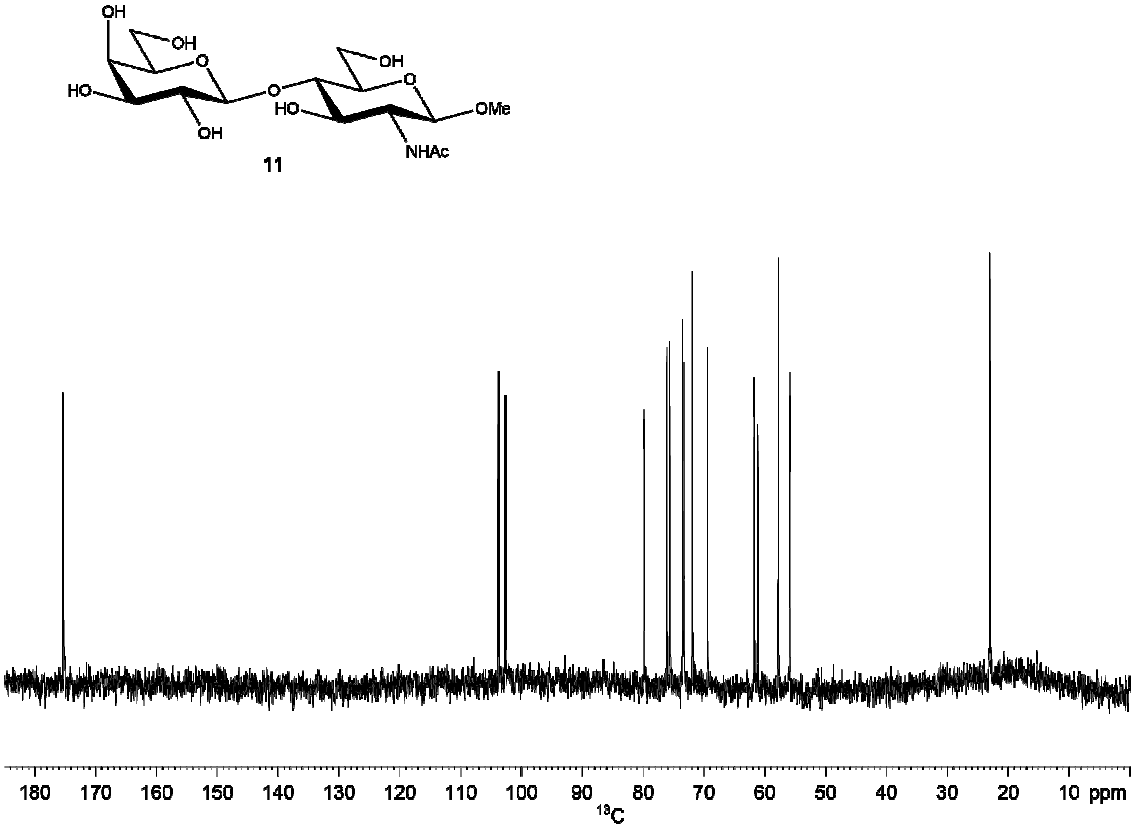
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm
1H


OH
OH
O
HO
OH
O
BnO
OBn
O
OMe
NHAc
41
8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 ppm
1H


OH
OH
O
HO
OH
O
BnO
OBn
O
OMe
NHAc
41
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 ppm
13C


OH
OH
O
HO
O
OH
OH
HO
OMe
NHAc
11
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm
1H


OH
OH
O
HO
O
OH
OH
HO
OMe
NHAc
11
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10 ppm
13C