ChemBioChem

Supporting Information

Targeting the Central Pocket of the *Pseudomonas aeruginosa* Lectin LecA

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Table of Contents

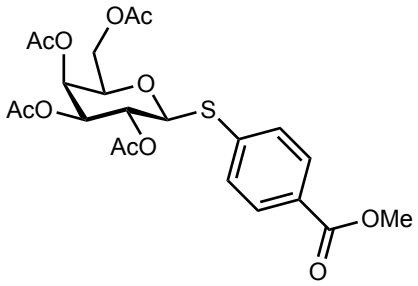
Chemical Synthesis

General information

Chemicals and solvents were obtained from TCI Chemicals, Sigma Aldrich or Carl Roth and deuterated solvents from Eurisotop (Saarbrücken, Germany). Commercial chemicals were used without further purification. Reactions were monitored either by HPLC-MS or by TLC (silica gel 60 Å coated aluminum plates, Macherey-Nagel, Düren, Germany) and visualization with UV light (254 nm) followed by staining with permanganate (1.5 g $KMnO_4$, 10 g, K_2CO_3, 1.25 mL 10% NaOH in 200 mL H_2O) or a molybdate solution (0.02 M $Ce(NH_4)_4(SO_4)_4 \cdot 2H_2O$ and $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ in aq. 10% H_2SO_4). MPLC was used for purification (Teledyne Isco CombiFlash Rf 200) using silica pore size 60 Å, particle size 400 mesh, from Fluka. The final test compounds were purified by preparative HPLC (Waters 2545 Binary Gradient Module, Waters 2489 UV/Visible detector, C18 column (250/21 Nucleodur C18 Gravity-SB, 5 µm, Macherey-Nagel, Germany). Purity of all test compounds was > 95% determined by HPLC-UV and/or ^{1}H-NMR. All synthesized compounds were analyzed by NMR (Bruker Avance III 500 UltraShield spectrometer) at 500 MHz for ^{1}H-NMR and 126 MHz for ^{13}C-NMR. The spectra were analyzed with MestReNova (Version 12.0.2) and chemical shifts were calibrated on residual solvent peaks as internal standard according to literature[1] ($CDCl_3$ = 7.26 ppm and 77.0 ppm, DMSO-d_6 = 2.50 ppm and 39.52 and MeOH-d_4 = 3.31 ppm and 49.00 ppm). Multiplicity is specified as s (singlet), d (doublet), t (triplet), q (quartet) or m (multiplet). Resonance assignment follows the numbering of galactose.

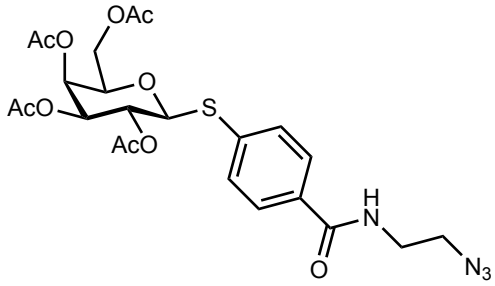
HPLC-MS was performed on a Thermo Dionex UltiMate 3000 HPLC coupled to a Bruker amaZon SL mass spectrometer and UV detection at 254 nm, using a C18 column (100/2 NucleoShell RP 18plus, 2.7 µm Macherey-Nagel, Germany) as stationary phase. High resolution MS was measured on a Bruker maxis 4G hr-QqTOF and analyzed using DataAnalysis (Bruker Daltonics, Bremen, Germany).

4-Methyloxycarbonylphenyl 2,3,4,6-tetra-O-acetyl 1-thio-β-D-galactopyranoside (S3)



The synthesis was performed in analogy to Winssinger *et al*.[2] Galactose pentaacetate (1.16 g, 2.97 mmol, 1.0 eq.) and $BF_3{\cdot}OEt_3$ (4.0 eq.) were dissolved in CH_2Cl_2 and methyl-4-sulfanylbenzoate (3.0 eq.) was added dropwise at 0 °C. The reaction was taken up in CH_2Cl_2 after 18 h, washed with $NaHCO_3$ (2x – strong gas evolution), water and brine, the org. phase was dried over Na_2SO_4, filtered and the volatiles removed in vacuo. The residue was purified by flash chromatography (EtOAc/Tol 1:5, R_f = 0.4). Compound **S3** was obtained as a white solid (1.37 g, 2.75 mmol, 95%). The analytical data match the reported spectra in Kuhaudomlarp *et al*.[3]

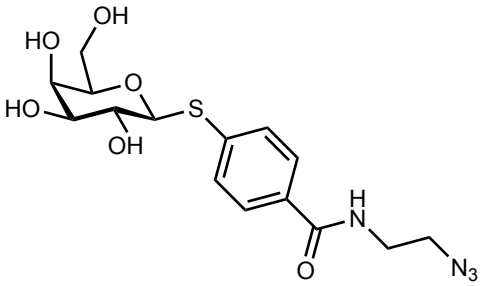
Azidoethyl derivative S5



Azidoethyl derivative **S5** was obtained following the procedure from Kuhaudomlarp *et al*. as a white solid.[3] (324 mg, 0.59 mmol, 57% o2s from **S3**.). The employed 2-azido-ethylamine (**S4**) has an N/C ratio of 2 and is potentially explosive and must be handled with care. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.75 (s, 1H, NH), 7.82 (d, J = 8.6 Hz, 2H, 2x ArCH), 7.52 (d, J = 8.5 Hz, 2H, 2x ArCH), 5.38 (d, J = 10.0 Hz, 1H, H-1), 5.35 (dd, J = 3.5, 1.1 Hz, 1H, H-4), 5.28 (dd, J = 9.9, 3.5 Hz, 1H, H-3), 5.08 (t, J = 9.9 Hz, 1H, H-2), 4.38 (td, J = 6.2, 1.1 Hz, 1H, H-5), 4.07 (d, J = 6.2 Hz, 2H, H-6), 3.50 – 3.44 (m, 4H, $NHCH_2CH_2N_3$), 2.13 (s, 3H, CH_3), 2.04 (s, 2H, CH_3), 2.01 (s, 3H, CH_3), 1.93 (s, 3H, CH_3). ^{13}C NMR (126 MHz, DMSO-d_6) δ 169.95 (C=O), 169.88 (C=O), 169.48 (C=O), 169.31 (C=O), 165.83 (C=O), 137.26 (ArC), 132.52 (ArC), 128.94 (2x ArCH), 127.75 (2x ArCH), 83.09 (C-1), 73.63 (C-5), 70.97 (C-3), 67.57 (C-4), 66.79 (C-2), 61.81 (C-6),

49.77 ($NHCH_2\underline{C}H_2N_3$), 39.02 ($NH\underline{C}H_2CH_2N_3$), 20.52 ($\underline{C}H_3$), 20.50 ($\underline{C}H_3$), 20.42 ($\underline{C}H_3$), 20.35 ($\underline{C}H_3$).

Deprotected azidoethyl galactoside S6

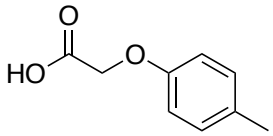


Deprotected azidoethyl galactoside **S6** was obtained as previously reported in good yields (176 mg, 0.45 mmol, 84%). The analytical data match the reported spectra in Kuhaudomlarp *et al.*[3]

General procedure for phenoxy acetic acids

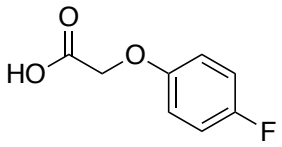
The reactions were performed in analogy to Anbarasa *et al.* and Dewei *et al.*.[4,5] *p*-Cresol or 4-fluorophenol (1 g, 1.0 eq.) and NaOH (849 mg, 21.2 mmol, 2.2 eq.) were dissolved in water (15 mL). Then, a solution of chloroacetic acid (1.04 g, 11.1 mmol, 1.2 eq.) in water (3.5 mL) was added and the mixture was heated to 70 °C. After 16 h, the reaction was stopped by adding 2 M HCl and adjusting the pH to 1–2. The precipitate was collected and dried at 40 °C for 2 h. The products were purified by dissolution under basic conditions (pH = 8–9, K_2CO_3 aq.) and repeated precipitation at pH = 1–2 (HCl aq.).

2-(*p*-tolyloxy)acetic acid (SI-1)



Compound **SI-1** was obtained as a white solid (1.03 g, 6.17 mmol, 67%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.11 (d, *J* = 8.0 Hz, 2H, 2x ArC$\underline{H}$), 6.92 – 6.65 (m, 2H, 2x ArC$\underline{H}$), 4.65 (s, 2H, C$\underline{H}_2$), 2.30 (s, 3H, C$\underline{H}_3$). ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.97 ($\underline{C}$=O), 155.33 (Ar$\underline{C}$), 131.76 (Ar$\underline{C}$), 130.30 (2x Ar$\underline{C}$H), 114.67 (2x Ar$\underline{C}$H), 65.18 ($\underline{C}H_2$), 20.65 ($\underline{C}H_3$).

2-(4-Fluorophenoxy)acetic acid (SI-2)

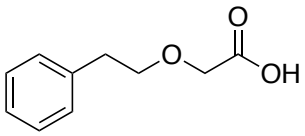


Compound **SI-2** was obtained as a white solid (518 mg, 6.17 mmol, 56%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.06 – 6.95 (m, 2H, 2x ArC<u>H</u>), 6.95-6.77 (2H, 2x ArC<u>H</u>), 4.66 (s, 2H, C<u>H</u>$_2$). ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.51 (C=O), 158.04 (d, J = 240.4 Hz, Ar<u>C</u>F), 153.50 (d, J = 2.5 Hz, Ar<u>C</u>), 116.28 (d, J = 33.4 Hz, 2x Ar<u>C</u>H), 116.02 (d, J = 18.1 Hz, 2x Ar<u>C</u>H), 65.51 (CH_2).

General procedure for phenylalkoxy acetic acids

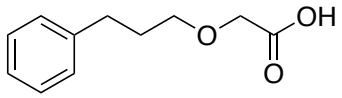
The synthesis was performed in analogy to Machin *et al.* .[6] The alcohol (1.0 g, 1.0 eq) was dissolved in DMF (final conc. = 0.5 M) and treated with NaH (2.0 eq.). This mixture was stirred at 60 °C until gas evolution stopped (15-30 min). Then, chloroacetic acid (1.0 eq.) dissolved in DMF (2 mL) was added carefully. This reaction was stirred at 60 °C for ca. 60 min. The solvent was removed under reduced pressure and the residue was taken up in H_2O and washed with ether. The aq. phase was acidified with 2 N HCl (pH = 1) and extracted with EtOAc. The org. phase was dried over $MgSO_4$, filtered and the solvent was removed in vacuo. The crude was purified by flash chromatography (PE/ EtOAc 7:3) to give the desired ethers (24–46%).

2-Phenylethoxyacetic acid (SI-3)



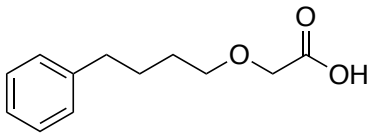
A white solid with fresh flower odor was obtained (684 mg, 3.79 mmol, 46%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.58 (s, 1H, COO<u>H</u>), 7.31 – 7.22 (m, 4H, 4x ArC<u>H</u>), 7.22 – 7.16 (m, 1H, ArC<u>H</u>), 4.00 (s, 2H, COC<u>H</u>$_2$O), 3.67 (t, J = 7.0 Hz, 2H, PhCH$_2$C<u>H</u>$_2$O), 2.83 (t, J = 7.0 Hz, 2H, PhC<u>H</u>$_2$CH$_2$O). ^{13}C NMR (126 MHz, DMSO-d_6) δ 171.66 (<u>C</u>OOH), 138.80 (Ar<u>C</u>), 128.81 (2x Ar<u>C</u>H, 128.21 (2x Ar<u>C</u>H, 126.05 (Ar<u>C</u>H), 71.26 (PhCH$_2$<u>C</u>H$_2$O), 67.38 (<u>C</u>OCH$_2$O), 35.40 (Ph<u>C</u>H$_2$CH$_2$O). HR-MS (ESI): calcd. for **SI-3** $[C_{10}H_{12}O_3 - H]^-$ 179.0714; found 179.0705.

3-Phenylpropoxyacetic acid (SI-4)



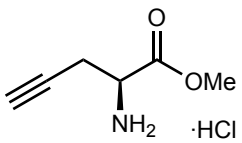
SI-4 was obtained as a white solid (452 mg, 2.23 mmol, 32%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.57 (s, 1H, COOH), 7.27 (t, J = 7.5 Hz, 2H, 2x ArCH), 7.23 – 7.14 (m, 3H, 3x ArCH), 3.98 (s, 2H, $COCH_2O$), 3.45 (t, J = 6.4 Hz, 2H, $PhCH_2CH_2CH_2O$), 2.66 – 2.59 (m, 2H, $PhCH_2CH_2CH_2O$), 1.84 – 1.76 (m, 2H, $PhCH_2CH_2CH_2O$). ^{13}C NMR (126 MHz, DMSO-d_6) δ 171.77 (COOH), 141.77 (ArC), 128.31 (2x ArCH), 128.28 (2x ArCH), 125.72 (ArCH), 69.86 ($PhCH_2CH_2CH_2O$), 67.43 ($COCH_2O$), 31.66 $PhCH_2CH_2CH_2O$, 31.01 ($PhCH_2CH_2CH_2O$). HR-MS (ESI): calcd. for **SI-4** $[C_{11}H_{14}O_3 - H]^-$ 193.0870; found 193.0860.

4-Phenylbutoxyacetic acid (SI-6)



Compound **SI-6** was obtained as an oil/white solid (330 mg, 1.58 mmol, 24%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.55 (s, 1H, COOH), 7.29 – 7.24 (m, 2H, 2x ArCH), 7.21 – 7.13 (m, 3H, 3x ArCH), 3.95 (s, 2H, $COCH_2O$), 3.45 (t, J = 6.4 Hz, 2H, $PhCH_2CH_2CH_2CH_2O$), 2.58 (t, J = 7.6 Hz, 2H, $PhCH_2CH_2CH_2CH_2O$), 1.65 – 1.55 (m, 2H, $PhCH_2CH_2CH_2CH_2O$), 1.52 (tt, J = 8.3, 6.2 Hz, 2H, $PhCH_2CH_2CH_2CH_2O$). ^{13}C NMR (126 MHz, DMSO-d_6) δ 171.78 (COOH), 142.18 (ArC), 128.30 (2x ArCH), 128.23 (2x ArCH), 125.64 (ArCH), 70.23 ($PhCH_2CH_2CH_2CH_2O$), 67.40 ($COCH_2O$), 34.83 ($PhCH_2CH_2CH_2CH_2O$), 28.70 ($PhCH_2CH_2CH_2CH_2O$), 27.61 ($PhCH_2CH_2CH_2CH_2O$). HR-MS (ESI): calcd. for **SI-6** $[C_{12}H_{16}O_3 - H]^-$ 207.1027; found 207.1018.

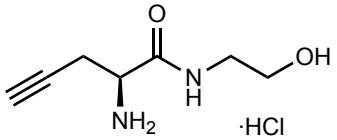
L-H-Pra-OMe *HCl (S8)



The reaction was performed in analogy to Kristensen *et al.*[7] L-H-Pra-OH*HCl (200 mg, 1.77 mmol, 1.0 eq.) was suspended in MeOH (5 mL, final conc. 0.8 M) and thionyl chloride (150 µL, 1.2 eq.) was added dropwise over 15 min at 0 °C. The reaction was warmed to 25 °C and

stirred for 18 h. Volatiles were removed under reduced pressure and the residue was washed with Et_2O and dried to obtain compound **S8** as an oil (283 mg, 1.73 mmol, 98%, R_f = 0.74 CH_2Cl_2/MeOH 9:1). ^1H NMR (500 MHz, $CDCl_3$) δ 8.84 (s, 3H, N$\underline{H}_3$), 4.45 (s, 1H, $\underline{H}_\alpha$), 3.88 (s, 3H, C$\underline{H}_3$), 3.49 (s, 1H, C≡C$\underline{H}$), 3.36 – 2.79 (m, 2H, C$\underline{H}_2$). ^{13}C NMR (126 MHz, $CDCl_3$) δ 168.42 ($\underline{C}$=O), 76.35 ($\underline{C}$≡CH), 74.72 C≡$\underline{C}$H, 54.00 ($\underline{C}H_\alpha$), 52.21 ($\underline{C}H_3$), 20.95 ($\underline{C}H_3$). LR-MS: calcd. **S8** $[C_6H_9NO_2 + H]^+$ 128.07; found 128.49.

L-H-Pra-NH(CH$_2$)$_2$OH*HCl (S9)



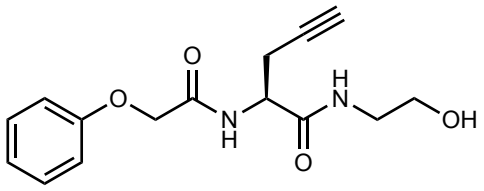
This reaction was performed in analogy to Kristensen *et al*.[7] L-H-Pra-OMe* HCl (**S8**) (283 mg, 1.73 mmol, 1.0 eq.) was dissolved in pure ethanolamine (1.8 mL, final conc. of **S8** = 1 M) and stirred for 18 h at 25 °C. The mixture was diluted with CH_2Cl_2 and washed with aq. K_2CO_3 (satd.), the aq. phase was extracted with CH_2Cl_2 and the combined organic phases were dried over Na_2SO_4, filtered and the solvents were removed under reduced pressure. The obtained yellow oil was washed with Et_2O and dried to give compound **S9** (233 mg, 1.21 mmol, 70%, R_f = 0.56 CH_2Cl_2/MeOH 8:2). ^1H NMR (500 MHz, DMSO-d_6) δ 7.94 (br s, 1H, N$\underline{H}$), 4.68 (br s, 1H, O$\underline{H}$), 3.40 (q, J = 5.9 Hz, 2H, NHCH$_2$C$\underline{H}_2$OH), 3.31 – 3.24 (m, 1H, $\underline{H}_\alpha$), 3.20 – 3.06 (m, 2H, NHC$\underline{H}_2$CH$_2$OH), 2.83 (t, J = 2.6 Hz, 1H, C≡C$\underline{H}$), 2.47 – 2.33 (m, 2H, CC$\underline{H}_2$CH$_\alpha$), 1.92 (s, 2H, N$\underline{H}_2$). ^{13}C NMR (126 MHz, DMSO-d_6) δ 173.26 ($\underline{C}$=O), 81.93 ($\underline{C}$≡CH), 73.43 (C≡$\underline{C}$H), 60.29 (NHCH$_2$$\underline{C}H_2$OH), 53.97 ($\underline{C}H_\alpha$), 41.77 (NH$\underline{C}H_2CH_2$OH), 25.25 (C$\underline{C}H_2CH_\alpha$). HR-MS (ESI): calcd. for **S9** $[C_7H_{12}N_2O_2 + H]^+$ 157.0972; found 157.0972.

General procedure for the (*S*)-*N*-(2-hydroxyethyl)-2-(acetamido)pent-4-ynamides

The peptide couplings were performed in analogy as follows: L-H-Pra-NH(CH$_2$)$_2$OH (**S9**) (50 mg, 0.32 mmol) was dissolved in DMF (final conc. of **S9** = 0.1 M) and EDC*HCl (1.2 eq.), HOBt (1.2 eq.), the individual acids (2.0 eq.) and DIPEA (2.5 eq.) were added. The mixture was stirred at 25 °C for 24 h. The solvent was removed under reduced pressure and the crude was taken up in a mixture of EtOAc and 1 N HCl (1:1) and the phases were separated. The aq. phase was extracted

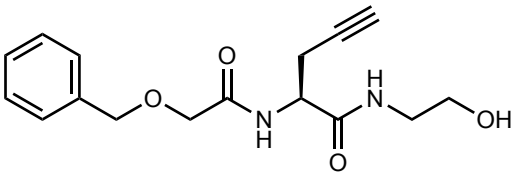
with EtOAc (2x). The combined org. phases were washed with $NaHCO_3$ (aq.), brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (CH_2Cl_2/MeOH) to give the products as white solids (36–71%).

(*S*)-*N*-(2-hydroxyethyl)-2-(2-phenoxyacetamido)pent-4-ynamide (S10-a)



125 mg of **S9** (0.8 mmol) were used for the reaction and 1 eq. of phenoxyacetic acid. Different to the general procedure, the white precipitate containing the product was filtered and washed with Et_2O to give compound **S10-1** (155 mg, 0.53 mmol, 67%, R_f = 0.60 CH_2Cl_2/MeOH 9:1). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.39 (d, J = 7.6 Hz, 1H, CHNHCO), 7.33 (dd, J = 8.7, 7.3 Hz, 2H, 2x ArCH), 7.04 (td, J = 7.4, 1.1 Hz, 1H, ArCH), 6.98 – 6.90 (m, 2H, 2x ArCH), 6.57 (s, 1H, CONHCH$_2$), 4.62 (td, J = 7.5, 5.6 Hz, 1H, CH$_2$CHNHCO), 4.56 (s, 2H, OCH$_2$CO), 3.73 (t, J = 5.0 Hz, 2H, NHCH$_2$CH$_2$OH), 3.45 (t, J = 5.3 Hz, 2H, NHCH$_2$CH$_2$OH), 2.91 – 2.80 (m, 1H, CHCH$_2$C≡CH), 2.69 – 2.60 (m, 1H, CHCH$_2$C≡CH), 2.10 (t, J = 2.6 Hz, 1H, C≡CH). ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.16 (C=O), 168.94 (C=O), 157.13 (ArC), 130.02 (2x ArCH), 122.51 (ArCH), 114.87 (2x ArCH), 79.16 CH$_2$CCH, 72.18 (C≡CH), 67.34 (COCH$_2$O), 61.99 (NHCH$_2$CH$_2$OH), 51.49 (CH$_2$CHNHCO), 42.64 (NHCH$_2$CH$_2$OH), 22.24 (CHCH$_2$CCH). HR-MS (ESI): calcd. for **S10-1** $[C_{15}H_{19}N_2O_4 + H]^+$ 291.1339; found 291.1337.

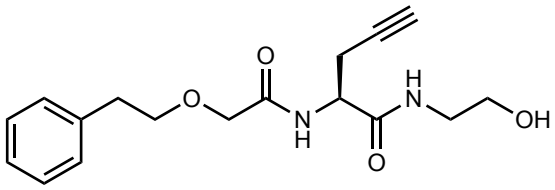
(*S*)-2-(2-(benzyloxy)acetamido)-*N*-(2-hydroxyethyl)pent-4-ynamide (S10-b)



The crude product was purified by flash chromatography to give a yellow oily solid (48.6 mg, 0.16 mmol, 50%, R_f = 0.39; CH_2Cl_2/MeOH 19:1). ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.09 (t, J = 5.7 Hz, 1H, CONHCH$_2$), 7.84 (d, J = 8.3 Hz, 1H, CHNHCO), 7.38 (d, J = 4.4 Hz, 4H, 4x ArCH), 7.35 – 7.29 (m, 1H, ArCH), 4.68 (t, J = 5.5 Hz, 1H, NHCH$_2$CH$_2$OH), 4.57 (d, J = 1.3 Hz, 2H,

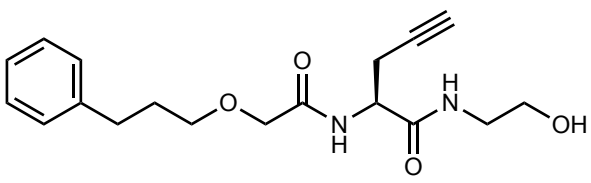
COC$\underline{H}_2$O), 4.49 – 4.43 (m, 1H, $CH_2C\underline{H}NHCO$), 4.04 – 3.91 (m, 2H, $OC\underline{H}_2Ar$), 3.42 – 3.37 (m, 2H, $NHCH_2C\underline{H}_2OH$), 3.20 – 3.09 (m, 2H, $NHC\underline{H}_2CH_2OH$), 2.86 (t, J = 2.6 Hz, 1H, $\underline{H}C{\equiv}C$), 2.65 – 2.53 (m, 2H, $HCCC\underline{H}_2CH$). ^{13}C NMR (126 MHz, DMSO-d_6) δ 169.42 ($\underline{C}$=O), 168.75 ($\underline{C}$=O), 137.56 (Ar$\underline{C}$), 128.37 (2x ArCH), 127.85 (2x Ar$\underline{C}$H), 127.76 (Ar$\underline{C}$H), 80.35 (HC≡$\underline{C}CH_2$), 73.15 (H$\underline{C}$≡C), 72.37 (CO$\underline{C}H_2$O), 69.01 (O$\underline{C}H_2$Ar), 59.64 ($NHCH_2\underline{C}H_2OH$), 50.49 ($CH_2\underline{C}HNHCO$), 41.61 ($NHC\underline{H}_2CH_2OH$), 22.16 (HC≡C$\underline{C}H_2CH$). HR-MS (ESI): calcd. for **S10-2** [$C_{16}H_{20}N_2O_4$ + H]$^+$ 305.1496; found 305.1491.

(*S*)-*N*-(2-hydroxyethyl)-2-(2-phenethoxyacetamido)pent-4-ynamide (S10-c)



The product was obtained as a white powder (37 mg, 0.12 mmol, 36%, R_f = 0.39; CH_2Cl_2/MeOH 9:1). ^{1}H NMR (500 MHz, MeOH-d_4) δ 7.31 – 7.25 (m, 4H, 4x ArC$\underline{H}$), 7.23 – 7.16 (m 1H, ArC$\underline{H}$), 4.53 (td, J = 7.0, 5.8 Hz, 1H, $CH_2C\underline{H}CONH$), 4.06 – 3.92 (m, 2H, $COC\underline{H}_2O$), 3.77 (t, J = 6.8 Hz, 2H, $OC\underline{H}_2CH_2Ph$), 3.60 (td, J = 5.9, 1.5 Hz, 2H, $NHCH_2C\underline{H}_2OH$), 3.33 (d, J = 5.9 Hz, 2H, $NHC\underline{H}_2CH_2OH$), 2.94 (t, J = 6.8 Hz, 1H, $OCH_2C\underline{H}_2Ph$), 2.74 – 2.54 (m, 2H, $CHC\underline{H}_2C{\equiv}CH$), 2.39 (t, J = 2.6 Hz, 1H, C≡C$\underline{H}$). ^{13}C NMR (126 MHz, MeOH-d_4) δ 172.54 ($\underline{C}$=O), 172.07 ($\underline{C}$=O), 140.05 (Ar$\underline{C}$), 130.07 (2x Ar$\underline{C}$H), 129.50 (2x Ar$\underline{C}$H), 127.35 (Ar$\underline{C}$H), 79.93 ($CH_2\underline{C}{\equiv}CH$), 73.78 ($O\underline{C}H_2CH_2Ph$), 72.60 (C≡$\underline{C}$H), 70.84 ($CO\underline{C}H_2O$), 61.40 ($NHCH_2\underline{C}H_2OH$, 52.61 (NH$\underline{C}$HCHCO), 43.05 ($NH\underline{C}H_2CH_2OH$), 37.10 ($OCH_2\underline{C}H_2Ph$), 23.03 ($CH\underline{C}H_2C{\equiv}CH$). HR-MS (ESI): calcd. for **S10-3** [$C_{17}H_{22}N_2O_4$ + H]$^+$ 319.165; found 347.1960.

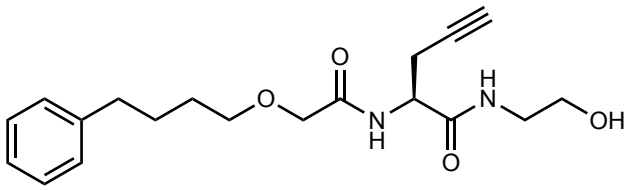
(*S*)-*N*-(2-hydroxyethyl)-2-(2-(3-phenylpropoxy)acetamido)pent-4-ynamide (S10-d)



The product was obtained as a white powder (55 mg, 0.17 mmol, 52%, R_f = 0.42; CH_2Cl_2/MeOH 9:0.25). ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.08 (t, J = 5.7 Hz, 1H, $N\underline{H}CH_2CH_2OH$), 7.74 (d, J =

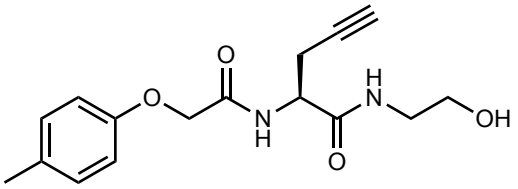
8.3 Hz, 1H, CONHCH), 7.28 (t, J = 7.5 Hz, 2H, 2x ArCH), 7.25 – 7.14 (m, 3H, 3x ArCH), 4.68 (t, J = 5.5 Hz, 1H, NHCH$_2$CH$_2$OH), 4.48 – 4.41 (m, 1H, NHCHCH$_2$CO), 3.95 – 3.85 (m, 2H, COCH$_2$O), 3.47 (tt, J = 6.2, 3.1 Hz, 2H, OCH$_2$CH$_2$CH$_2$Ph), 3.43 – 3.35 (m, 2H, NHCH$_2$CH$_2$OH), 3.19 – 3.09 (m, 2H, NHCH$_2$CH$_2$OH), 2.83 (t, J = 2.6 Hz, 1H, C≡CH), 2.70 – 2.62 (m, 2H, OCH$_2$CH$_2$CH$_2$Ph), 2.62 – 2.56 (m, 2H, CHCH$_2$C≡CH), 1.90 – 1.81 (m, 2H, OCH$_2$CH$_2$CH$_2$Ph). ^{13}C NMR (126 MHz, DMSO-d_6) δ 169.86 (C=O), 169.45 (C=O), 142.11 (ArC), 128.78 (4x ArCH), 126.22 (ArCH, 80.71 (CH$_2$C≡CH), 73.55 (C≡CH), 70.61 (OCH$_2$CH$_2$CH$_2$Ph), 70.03 (COCH$_2$O), 60.10 (NHCH$_2$CH$_2$OH), 50.85 (NHCHCH$_2$CO), 42.07 (NHCH$_2$CH$_2$OH), 32.04 (OCH$_2$CH$_2$CH$_2$Ph), 31.26 (OCH$_2$CH$_2$CH$_2$Ph), 22.65 (CHCH$_2$C≡CH). HR-MS (ESI): calcd. for **S10-4** [$C_{18}H_{224}N_2O_4$ + H]$^+$ 333.1809; found 333.1804.

(*S*)-*N*-(2-hydroxyethyl)-2-(2-(4-phenylbutoxy)acetamido)pent-4-ynamide (S10-e)



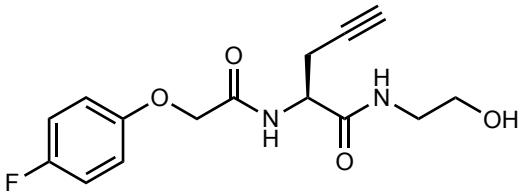
The product was obtained as a white powder (44.3, 0.13 mmol, 40%, R_f = 0.59; CH_2Cl_2/MeOH 19:1). ^{1}H NMR (500 MHz, MeOH-d_4) δ 7.23 (t, J = 7.6 Hz, 2H, 2x ArCH), 7.20 – 7.15 (m, 2H, 2x ArCH), 7.15 – 7.09 (m, 1H, ArCH), 4.54 (t, J = 6.3 Hz, 1H, CH$_2$CHCONH), 4.01 – 3.90 (m, 2H, COCH$_2$O), 3.60 – 3.57 (m, 2H, NHCH$_2$CH$_2$OH), 3.55 (t, J = 6.3 Hz, 2H, OCH$_2$CH$_2$CH$_2$CH$_2$Ph), 3.36 – 3.27 (m, 2H, NHCH$_2$CH$_2$OH), 2.71 – 2.66 (m, 2H, CHCH$_2$C≡CH), 2.63 (t, J = 7.4 Hz, 2H, OCH$_2$CH$_2$CH$_2$CH$_2$Ph), 2.31 (t, J = 2.7 Hz, 1H, C≡CH), 1.77 – 1.68 (m, 2H, OCH$_2$CH$_2$CH$_2$CH$_2$Ph), 1.68 – 1.60 (m, 2H, OCH$_2$CH$_2$CH$_2$CH$_2$Ph). ^{13}C NMR (126 MHz, MeOH-d_4) δ 172.62 (C=O), 172.06 (C=O), 143.59 (ArC), 129.46 (2x ArCH), 129.30 (2x ArCH), 126.75 (ArCH), 79.86 (CH$_2$C≡CH), 72.73 (C≡CH), 72.61 (OCH$_2$CH$_2$CH$_2$CH$_2$Ph), 70.82 (COCH$_2$O), 61.39 (NHCH$_2$CH$_2$OH), 52.52 (CH$_2$CHCONH, 43.05 (NHCH$_2$CH$_2$OH), 36.59 (OCH$_2$CH$_2$CH$_2$CH$_2$Ph), 30.13 (OCH$_2$CH$_2$CH$_2$CH$_2$Ph), 29.05 (OCH$_2$CH$_2$CH$_2$CH$_2$Ph), 23.09 (CHCH$_2$CCH). HR-MS (ESI): calcd. for **S10-e** [$C_{19}H_{26}N_2O_4$ + H]$^+$ 347.1965; found 347.1960.

(*S*)-*N*-(2-hydroxyethyl)-2-(2-(*p*-tolyloxy)acetamido)pent-4-ynamide (S10-f)



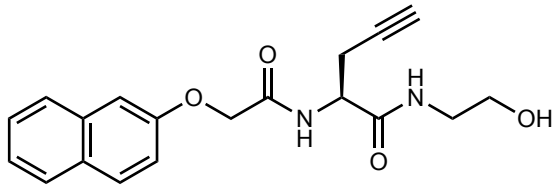
The product was obtained as a white solid (64.1 mg, 0.21 mmol, 71%, R_f = 0.28; CH_2Cl_2/MeOH 19:1). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.39 (d, *J* = 7.6 Hz, 1H, CHNHCO), 7.12 (d, *J* = 8.5 Hz, 2H, 2x ArCH), 6.84 (d, *J* = 8.7 Hz, 2H, 2x ArCH), 6.58 (s, 1H, $CONHCH_2$), 4.61 (td, *J* = 7.6, 5.6 Hz, 1H, $CH_2CHNHCO$), 4.52 (s, 2H, $COCH_2OAr$), 3.72 (t, *J* = 4.9 Hz, 2H, $NHCH_2CH_2OH$), 3.44 (t, *J* = 5.2 Hz, 1H, $NHCH_2CH_2OH$), 2.91 – 2.59 (m, 2H, $HC{\equiv}CCH_2CH$), 2.30 (s, 3H, CH_3), 2.10 (d, *J* = 2.6 Hz, 1H, C≡CH). ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.22 (C=O), 169.19 (C=O), 155.05 (ArCOR), 131.90 ($ArCCH_3$), 130.41 (2x ArCH), 114.69 (2x ArCH), 79.14 ($HC{\equiv}CCH_2$), 72.17 (HC≡C), 67.47 ($COCH_2OAr$), 61.95 ($NHCH_2CH_2OH$), 51.44 $CH_2CHNHCO$, 42.63 $NHCH_2CH_2OH$, 22.17 ($HC{\equiv}CCH_2$), 20.64 (CH_3). HR-MS (ESI): calcd. for **S10-6** [$C_{16}H_{20}N_2O_4$ + H]$^+$ 305.1496; found 305.1491.

L-*p*-Fluorophenoxy-Pra-N(CH_2)$_2$OH (S10-g)



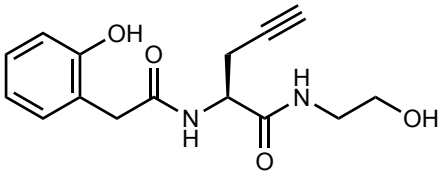
The compounds was obtained as a yellowish powder (64.1 mg, 0.21 mmol, 65%, R_f = 0.31; CH_2Cl_2/MeOH 19:1). ^{1}H NMR (500 MHz, MeOH-d_4) δ 7.09 – 7.01 (m, 4H, 4x ArCH), 4.67 – 4.61 (m, 1H, $CH_2CHNHCO$), 4.59 (d, *J* = 3.8 Hz, 2H, $COCH_2OAr$), 3.62 (td, *J* = 5.9, 1.4 Hz, 2H, $NHCH_2CH_2OH$), 3.36 – 3.34 (m, 2H, $NHCH_2CH_2OH$), 2.81 – 2.66 (m, 2H, $HC{\equiv}CCH_2CH$), 2.40 (t, *J* = 2.7 Hz, 1H, HC≡C). ^{13}C NMR (126 MHz, MeOH-d_4) δ 170.73 (C=O), 169.59 (C=O), 156.96 (ArC), 154.00 (ArC), 115.96 (ArCH), 115.90 (ArCH), 115.60 (ArCH), 115.41 (ArCH), 78.63 $HC{\equiv}CCH_2$, 71.08 (HC≡C), 67.39 ($COCH_2OAr$), 59.98 ($NHCH_2CH_2OH$), 51.58 ($CH_2CHNHCO$), 41.65 ($NHCH_2CH_2OH$, 21.44 ($HC{\equiv}CCH_2CH$). HR-MS (ESI): calcd. for **S10-7** [$C_{15}H_{17}FN_2O_4$ - H]$^-$ 307.1100; found 307.1089.

(*S*)-*N*-(2-hydroxyethyl)-2-(2-(naphthalen-2-yloxy)acetamido)pent-4-ynamide (S10-h)



The product was obtained as a white powder (51.0 mg, 0.15 mmol, 47%, R_f = 0.19; CH_2Cl_2/MeOH 19:1). ^{1}H NMR (500 MHz, MeOH-d_4) δ 7.86 – 7.77 (m, 3H, 3x naphthyl-H), 7.46 (ddd, J = 8.2, 6.8, 1.3 Hz, 1H, naphthyl-H), 7.37 (ddd, J = 8.1, 6.8, 1.3 Hz, 1H, naphthyl-H), 7.32 – 7.28 (m, 2H, 2x naphthyl-H), 4.75 (d, J = 3.4 Hz, 2H, COCH_2OR), 4.70 – 4.62 (m, 1H, CH_2CHNHCO), 3.60 (td, J = 5.9, 1.5 Hz, 2H, NHCH_2CH_2OH), 3.38 – 3.34 (m, 2H, NHCH_2CH_2OH), 2.88 – 2.62 (m, 2H, HC≡CCH_2CH), 2.36 (s, 1H, HC≡C). ^{13}C NMR (126 MHz, MeOH-d_4) δ 170.76 (C=O), 169.66 (C=O), 155.51 (naphthyl-C), 134.49 (naphthyl-C), 129.58 (naphthyl-C), 129.32 (naphthyl-CH), 127.23 (naphthyl-CH), 126.64 (naphthyl-CH), 126.17 (naphthyl-CH), 123.79 (naphthyl-CH), 118.12 (naphthyl-CH), 107.18 (naphthyl-CH), 78.63 HC≡CCH$_2$, 71.06 HC≡C, 66.74 (COCH_2Ar), 59.97 (NHCH_2CH_2OH), 51.63 (CH_2CHNHCO), 41.65 (NHCH_2CH_2OH), 21.45 (HC≡CCH_2CH). HR-MS (ESI): calcd. for **S10-8** $[C_{19}H_{20}N_2O_4 + H]^+$ 341.1496; found 341.1491.

(*S*)-*N*-(2-hydroxyethyl)-2-(2-(2-hydroxyphenyl)acetamido)pent-4-ynamide (S10-i)



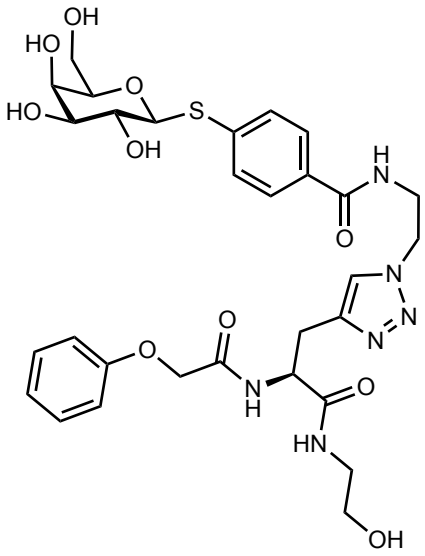
The product was obtained as a white powder (38.7 mg, 0.13 mmol, 42%, R_f = 0.17; CH_2Cl_2/MeOH 19:1). ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.64 (s, 1H, ArOH), 8.17 (d, J = 8.1 Hz, 1H, CHNHCO), 8.00 (t, J = 5.8 Hz, 1H, CONHCH$_2$), 7.11 – 7.05 (m, 1H, ArCH), 7.05 – 7.01 (m, 1H, ArCH), 6.81 – 6.76 (m, 1H, ArCH), 6.76 – 6.70 (m, 1H, ArCH), 4.68 (t, J = 5.3 Hz, 1H, CH$_2$OH), 4.39 (td, J = 7.6, 5.9 Hz, 1H, CH_2CHNHCO), 3.51 – 3.41 (m, 2H, COCH_2Ar), 3.41 – 3.36 (m, 2H, NHCH_2CH_2OH), 3.19 – 3.06 (m, 2H, NHCH_2CH_2OH), 2.81 (t, J = 2.6 Hz, 1H, HC≡C), 2.57 – 2.41 (m, 2H, HC≡CCH_2CH). ^{13}C NMR (126 MHz, DMSO-d_6) δ 171.29 (C=O), 170.16 (C=O), 155.86 (ArC), 131.12 (ArCH), 128.15 (ArCH), 122.95 (ArC), 119.30 (ArCH), 115.58 (ArCH), 80.98 HC≡CCH$_2$, 73.41 (HC≡C), 60.11 (NHCH_2CH_2OH), 51.95 (CH_2CHNHCO), 42.00

(NHCH$_2$CH$_2$OH), 37.49 (COCH$_2$Ar), 22.47 (HCCCH$_2$CH). HR-MS (ESI): calcd. for **S10-9** $[C_{15}H_{18}N_2O_4 - H]^-$ 289.1194; found 289.1185.

General procedure for the copper(I)-catalyzed assembly of LecA inhibitors

Alkyne (1.3 eq) and azide (1.0 eq) were dissolved to 0.1 M in degassed DMF. $CuSO_4$ (0.5 eq. of a 100 mM stock in degassed H_2O) and sodium ascorbate (0.5 eq. of a 100 mM stock in degassed H_2O) were added and the mixture was stirred for 16 h at r.t. . The mixture was diluted with EtOAc and washed with water. The aq. phase was extracted with EtOAc (3x). The combined org. phases were dried over Na_2SO_4, concentrated and the residue was purified by preparative-HPLC (C18, ACN/H_2O with 0.1% formic acid, 5–65% ACN) and fractions containing the product were subsequently lyophilized (yields ranged from 73–92%).

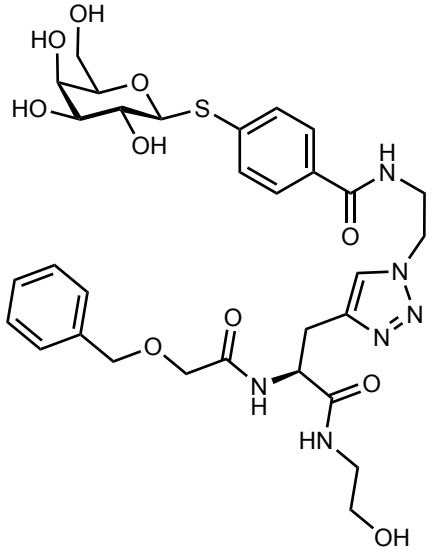
Phenoxy-modified galactoside 1



The product was obtained as a white solid (36 mg, 0.043 mmol, 50%, R_f = 0.53; CH_2Cl_2/MeOH 19:1). ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.56 (t, J = 5.7 Hz, 1H, (NHCH$_2$CH$_2$triazole), 8.18 (d, J = 8.2 Hz, 1H, CHNHCO), 8.01 (t, J = 5.7 Hz, 1H, NHCH$_2$CH$_2$OH), 7.74 (s, 1H, triazole-H), 7.71 (d, J = 8.4 Hz, 2H, 2x SArCH), 7.48 (d, J = 8.5 Hz, 2H, 2x SArCH), 7.28 (dd, J = 8.7, 7.2 Hz, 2H, 2x OArCH), 6.96 (t, J = 7.3 Hz, 1H, OArCH), 6.90 (d, J = 8.2 Hz, 2H, 2x OArCH), 5.22 (d, J = 5.9 Hz, 1H, OH-2), 4.93 (d, J = 5.5 Hz, 1H, OH-3?), 4.69 (d, J = 9.5 Hz, 1H, H-1), 4.66 (s, 1H, OH-4), 4.57 – 4.50 (m, 2H, CH$_2$CHNHCO, OH-4?), 4.49 (d, J = 2.6 Hz, 2H, COCH$_2$O), 4.46 (t, J

= 6.2 Hz, 2H, NHCH$_2$C<u>H$_2$</u>triazol), 3.72 (t, *J* = 3.8 Hz, 1H, H-4?), 3.69 – 3.55 (m, 2H, NHC<u>H$_2$</u>CH$_2$triazol), 3.57 – 3.42 (m, 4H, H-2, H-3?, NHCH$_2$C<u>H$_2$</u>OH), 3.41 – 3.34 (m, 3H, H-5?, H-6), 3.18 – 3.10 (m, 1H, 1x triazoleC<u>H$_2$</u>CH), 3.10 – 3.02 (m, 1H, NHC<u>H$_2$</u>CH$_2$OH), 2.98 (dd, *J* = 14.7, 8.1 Hz, 1H, 1x triazoleC<u>H$_2$</u>CH). ^{13}C NMR (126 MHz, DMSO-*d*$_6$) δ 170.22 (<u>C</u>=O), 167.55 (<u>C</u>=O), 166.08 (<u>C</u>=O), 157.63 OAr<u>C</u>, 142.65 (SAr<u>C</u>), 140.15 (SAr<u>C</u>), 131.13 (triazole-<u>C</u>), 129.49 (2x OAr<u>C</u>H), 127.73 (2x SAr<u>C</u>H), 127.57 (2x SAr<u>C</u>H), 123.14 (triazole-<u>C</u>H), 121.17 (OAr<u>C</u>H), 114.64 (2x OAr<u>C</u>H), 86.67 (C-1), 79.28 (C-5), 74.66 (C-3)), 69.12 (C-2), 68.37 (C-4), 66.60 (CO<u>C</u>H$_2$O), 60.59 (NHCH$_2$<u>C</u>H$_2$OH), 59.60 (C-6), 52.15 (CH$_2$<u>C</u>HNHCO), 48.51 (NHCH$_2$<u>C</u>H$_2$triazole), 41.56 (NH<u>C</u>H$_2$CH$_2$OH), 39.35 (NH<u>C</u>H$_2$CH$_2$triazole), 28.31 (trazole<u>C</u>H$_2$CH). HRMS (ESI) calcd. for **1** [C$_{30}$H$_{38}$N$_6$O$_{10}$S + H]$^+$ 675.2443; found, 675.2437.

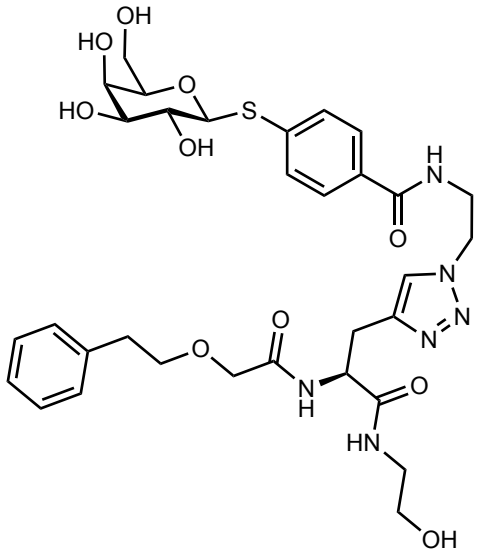
Benzyloxy-modified galactoside 2



The product was obtained as a white powder (21.3 mg, 0.031 mmol, 80%, R_f = 0.23; CH_2Cl_2/MeOH 19:1). ^{1}H NMR (500 MHz, DMSO-*d*$_6$) δ 8.56 (t, *J* = 5.7 Hz, 1H, N<u>H</u>CH$_2$CH$_2$triazole), 8.03 (t, *J* = 5.7 Hz, 1H, N<u>H</u>CH$_2$CH$_2$OH), 7.83 (d, *J* = 8.2 Hz, 1H, CHN<u>H</u>CO), 7.80 (s, 1H, triazole-<u>H</u>), 7.73 – 7.69 (m, 2H, 2x SArC<u>H</u>), 7.50 – 7.46 (m, 2H, 2x SArC<u>H</u>), 7.38 – 7.27 (m, 5H, 5x ArC<u>H</u>), 5.22 (d, *J* = 6.1 Hz, 1H, OH-2), 4.93 (d, *J* = 5.6 Hz, 1H, OH-3), 4.76 – 4.63 (m, 3H, H-1, OH-4, NHCH$_2$CH$_2$O<u>H</u>), 4.56 – 4.51 (m, 2H, OH-6?, CH$_2$C<u>H</u>CO), 4.49 (d, *J* = 0.9 Hz, 2H, COC<u>H$_2$</u>O), 4.47 (t, *J* = 6.3 Hz, 2H, NHCH$_2$C<u>H$_2$</u>triazole), 3.94 – 3.84 (m, 2H, OC<u>H$_2$</u>Ar), 3.72 (t, *J* = 4.0 Hz, 1H, H-4), 3.69 – 3.56 (m, 2H, NHC<u>H$_2$</u>CH$_2$triazole), 3.56 – 3.43 (m, 4H, H-2, H-3, NHCH$_2$C<u>H$_2$</u>OH), 3.40 – 3.36 (m, 3H, H-5?, H-6?), 3.19 – 3.11 (m, 1H, 1x triazoleC<u>H$_2$</u>CH),

3.11 – 3.03 (m, 2H, NHC<u>H</u>$_2$CH$_2$OH), 2.99 (dd, *J* = 14.7, 7.7 Hz, 1H, 1x triazoleC<u>H</u>$_2$CH). ^{13}C NMR (126 MHz, DMSO-d_6) δ 170.25 (<u>C</u>=O), 168.78 NH<u>C</u>OCH$_2$O, 166.12 (<u>C</u>=O), 142.61 (SAr<u>C</u>), 140.20 (SAr<u>C</u>), 137.62 (Ar<u>C</u>), 131.14 (triazole-<u>C</u>), 128.34 (2x Ar<u>C</u>H), 127.78 (2x Ar<u>C</u>H), 127.74 (Ar<u>C</u>H), 127.71 (2x SAr<u>C</u>H), 127.61 (2x SAr<u>C</u>H), 123.24 (triazole-<u>C</u>H), 86.68 (C-1)), 79.30 (C-5), 74.68 (C-3), 72.32 (O<u>C</u>H$_2$Ar), 69.14 (C-2), 69.05 (CO<u>C</u>H$_2$O), 68.41 (C-4), 60.63 (NHCH$_2$<u>C</u>H$_2$OH), 59.63 (C-6), 51.90 (CH$_2$<u>C</u>HNHCO), 48.51 (NHCH$_2$<u>C</u>H$_2$triazole), 41.58 (NH<u>C</u>H$_2$CH$_2$OH), 39.78 (NH$_2$<u>C</u>H$_2$CH$_2$triazole) 28.43 (triazole<u>C</u>H$_2$CH). HRMS (ESI) calcd. for **2** $[C_{31}H_{40}N_6O_{10}S + H]^+$ 689.2599; found, 689.2592.

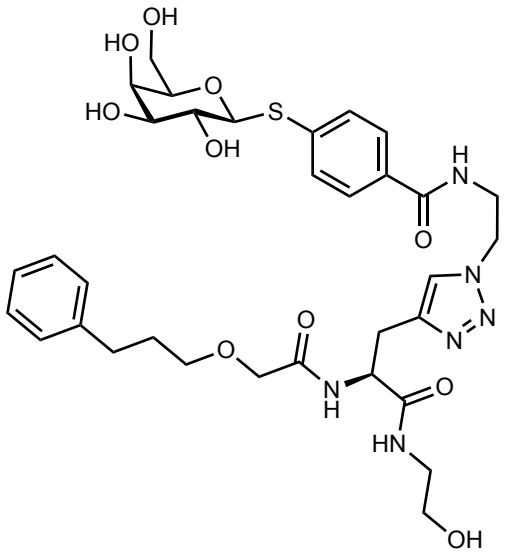
Phenylethoxy-modified galactoside 3



The product was obtained as a white powder (24.5 mg, 0.035 mmol, 90%, R_f = 0.33; CH_2Cl_2/MeOH 19:1). ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.55 (t, *J* = 5.6 Hz, 1H, N<u>H</u>CH$_2$CH$_2$triazole), 8.00 (t, *J* = 5.7 Hz, 1H, N<u>H</u>CH$_2$CH$_2$OH), 7.77 (s, 1H, triazole-<u>H</u>), 7.75 – 7.69 (m, 2H, 2x SArC<u>H</u>), 7.68 (d, *J* = 8.3 Hz, 1H, CON<u>H</u>CH), 7.51 – 7.46 (m, 2H, 2x SArC<u>H</u>), 7.30 – 7.21 (m, 4H, 4x ArC<u>H</u>), 7.21 – 7.16 (m, 1H, ArC<u>H</u>), 4.70 (d, *J* = 9.6 Hz, 1H, H-1), 4.51 (td, *J* = 7.9, 5.4 Hz, 1H, NHC<u>H</u>CH$_2$CO), 4.45 (t, *J* = 6.4 Hz, 2H, NHCH$_2$C<u>H</u>$_2$triazole), 3.90 – 3.79 (m, 2H, COC<u>H</u>$_2$O), 3.72 (d, *J* = 3.2 Hz, 1H, H-4), 3.69 – 3.55 (m, 2H, NHC<u>H</u>$_2$CH$_2$triazole), 3.60 (t, *J* = 7.0 Hz, 2H, PhCH$_2$C<u>H</u>$_2$O), 3.56 – 3.44 (m, 4H, H-2, H-5, H-6), 3.44 – 3.33 (m, 4H, NHCH$_2$C<u>H</u>$_2$OH, H-3), 3.18 – 2.91 (m, 4H, NHC<u>H</u>$_2$CH$_2$OH, CHC<u>H</u>$_2$triazole), 2.83 (t, *J* = 7.0 Hz, 2H, PhC<u>H</u>$_2$CH$_2$O). ^{13}C NMR (126 MHz, DMSO-d_6) δ 170.20 (<u>C</u>=O), 168.94 (<u>C</u>=O), 166.08 (<u>C</u>=O), 142.56 (Ar<u>C</u>), 140.17 (SAr<u>C</u>), 138.66 (triazole-<u>C</u>), 131.13 (SAr<u>C</u>), 128.89 (2x Ar<u>C</u>H),

128.26 (2x ArCH), 127.73 (2x SArCH), 127.58 (2x SArCH), 126.10 (ArCH), 123.17 (triazole-CH), 86.67 (C-1), 79.28 (C-5), 74.66 (C-3), 71.66 ($PhCH_2CH_2O$, 69.51 ($COCH_2O$), 69.12 (C-2), 68.38 (C-4), 60.60 (C-6), 59.61 ($NHCH_2CH_2OH$), 51.81 ($NHCHCH_2CO$), 48.47 ($NHCH_2CH_2$triazole), 41.54 ($NHCH_2CH_2OH$), 39.61 ($NH_2CH_2CH_2$triazole), 35.32 ($PhCH_2CH_2OH$), 28.42 ($CHCH_2$triazole). HR-MS (ESI): calcd. for **3** $[C_{32}H_{42}N_6O_{10}S + H]^+$ 703.2756; found 703.2751.

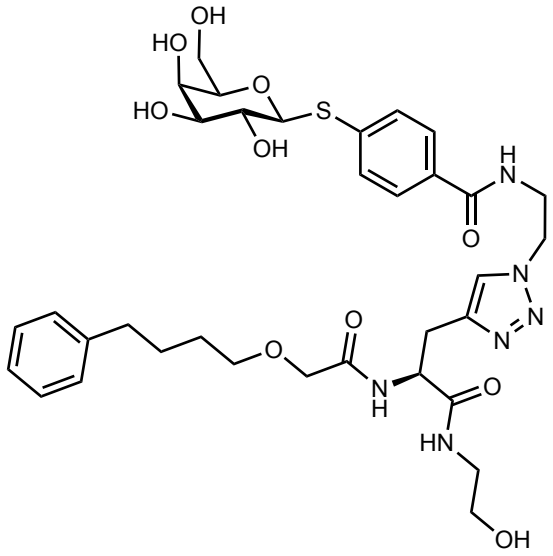
Phenylpropoxy-modified galactoside 4



The product was obtained as a white powder (25.6 mg, 0.036 mmol, 92%, R_f = 0.41; CH_2Cl_2/MeOH 19:1). ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.55 (t, J = 5.7 Hz, 1H, $NHCH_2CH$triazole), 8.00 (t, J = 5.7 Hz, 1H, $NHCH_2CH_2OH$), 7.80 (s, 1H, triazole-H), 7.73 (d, J = 10.7 Hz, 1H, $NHCHCH_2CO$), 7.71 (m, 2H, 2x SArCH), 7.51 – 7.46 (m, 2H, 2x SArCH), 7.27 (t, J = 7.5 Hz, 2H, 2x ArCH), 7.23 – 7.13 (m, 3H, 3x ArCH), 4.70 (d, J = 9.6 Hz, 1H, H-1), 4.52 (td, J = 7.8, 5.4 Hz, 1H, $NHCHCH_2CO$), 4.46 (t, J = 6.4 Hz, 2H, $NHCH_2CH_2$triazole), 3.83 (q, J = 15.1 Hz, 2H, $COCH_2O$), 3.72 (d, J = 3.2 Hz, 1H, H-4), 3.63 (tdd, J = 13.7, 7.6, 5.9 Hz, 2H, $NHCH_2CH_2$triazole), 3.55 – 3.44 (m, 5H, H-2, H-5, H-6), 3.43 – 3.32 (m, 5H, H-3, $NHCH_2CH_2OH$, $PhCH_2CH_2CH_2O$), 3.18 – 2.95 (m, 4H, $CHCH_2$triazole, $NHCH_2CH_2OH$), 2.64 – 2.58 (m, 2H, $PhCH_2CH_2CH_2O$), 1.84 – 1.77 (m, 2H, $PhCH_2CH_2CH_2O$). ^{13}C NMR (126 MHz, DMSO-d_6) δ 170.20 (C=O), 169.00 (C=O), 166.07 (C=O), 142.54 (ArC), 141.69 (SArC), 140.16 (triazole-C), 131.12 (SArC), 128.31 (2x ArCH), 128.29 (2x ArCH), 127.72 (2x SArCH), 127.57 (2x SArCH), 125.72 (ArCH), 123.19 (triazole-CH), 86.67 (C-1), 79.27 (C-5), 74.66 (C-3), 70.13

411 ($PhCH_2CH_2\underline{C}H_2O$), 69.57 ($CO\underline{C}H_2O$), 69.12 (C-2), 68.38 (C-4), 60.59 (C-6), 59.61
412 ($NHCH_2\underline{C}H_2OH$), 51.81 ($NH\underline{C}HCH_2CO$), 48.48 ($NHCH_2\underline{C}H_2$triazole), 41.54 ($NH\underline{C}H_2CH_2OH$),
413 39.78 ($NH\underline{C}H_2CH_2$triazole), 31.54 ($Ph\underline{C}H_2CH_2CH_2O$), 30.80 ($PhCH_2\underline{C}H_2CH_2O$), 28.41
414 ($CH\underline{C}H_2$triazole). HR-MS (ESI): calcd. for **4** $[C_{33}H_{44}N_6O_{10}S + H]^+$ 717.2912; found 717.2902.
415

416 **Phenylbutoxy-modified galactoside 5**

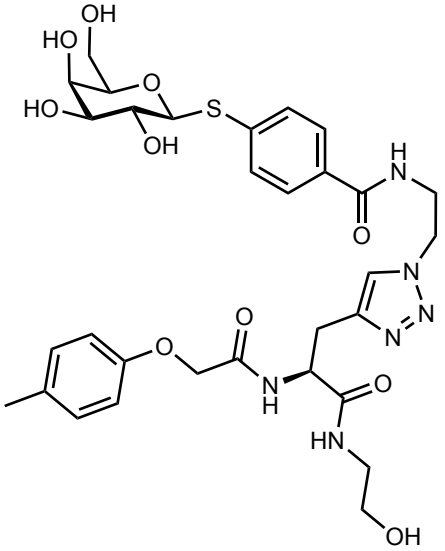


417

418 The product was obtained as a white powder (24.2 mg, 0.033, 85%, R_f = 0.44; CH_2Cl_2/MeOH
419 19:1). ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.55 (t, J = 5.6 Hz, 1H, $N\underline{H}CH_2CH_2$triazole), 8.01 (t, J =
420 5.7 Hz, 1H, $N\underline{H}CH_2CH_2OH$), 7.79 (s, 1H, triazole-$\underline{H}$), 7.71 (d, J = 8.5 Hz, 2H, 2x SArC$\underline{H}$), 7.68
421 (d, J = 8.2 Hz, 1H, $CON\underline{H}CH$), 7.48 (d, J = 8.6 Hz, 2H, 2x SArC$\underline{H}$), 7.26 (t, J = 7.5 Hz, 2H, 2x
422 ArC$\underline{H}$), 7.23 – 7.12 (m, 3H, 3x ArC$\underline{H}$), 5.20 (s, 1H, OH-2), 4.90 (s, 1H, OH-3), 4.70 (d, J = 9.6 Hz,
423 1H, H-1), 4.66 (s, 1H, OH-4), 4.51 (td, J = 7.8, 5.4 Hz, 1H, $NHC\underline{H}CH_2OH$), 4.45 (t, J = 6.3 Hz,
424 2H, $NHCH_2C\underline{H}_2$triazole), 3.88 – 3.75 (m, 2H, $COC\underline{H}_2O$), 3.72 (d, J = 3.2 Hz, 1H, H-4), 3.69 –
425 3.56 (m, 2H, $NHC\underline{H}_2CH_2$triazole), 3.55 – 3.44 (m, 5H, H-2, H-5, H-6), 3.42 – 3.27 (m, 6H,
426 $NHCH_2CH_2O\underline{H}$, $PhCH_2CH_2CH_2C\underline{H}_2O$, H-3), 3.18 – 2.94 (m, 4H, $NHC\underline{H}_2CH_2OH$,
427 $CHC\underline{H}_2$triazole), 2.57 (t, J = 7.5 Hz, 2H, $PhC\underline{H}_2CH_2CH_2CH_2O$), 1.65 – 1.56 (m, 2H,
428 $PhCH_2C\underline{H}_2CH_2CH_2O$), 1.56 – 1.47 (m, 2H, $PhCH_2CH_2C\underline{H}_2CH_2O$). ^{13}C NMR (126 MHz,
429 DMSO-d_6) δ 170.19 ($\underline{C}$=O), 169.02 ($\underline{C}$=O), 166.07 ($\underline{C}$=O), 142.52 (Ar$\underline{C}$), 142.09 (SAr$\underline{C}$), 140.17
430 (triazole-$\underline{C}$), 131.13 (SAr$\underline{C}$), 128.30 (2x Ar$\underline{C}$H), 128.23 (2x Ar$\underline{C}$H), 127.73 (2x SAr$\underline{C}$H), 127.58
431 (2x SAr$\underline{C}$H), 125.64 (ArCH), 123.16 (triazole-$\underline{C}$H), 86.68 (C-1), 79.28 (C-5), 74.66 (C-3), 70.62
432 ($PhCH_2CH_2CH_2\underline{C}H_2O$), 69.58 ($CO\underline{C}H_2O$), 69.12 (C-2), 68.38 (C-4), 60.60 (C-6), 59.60

(NHCH$_2$CH$_2$OH), 51.79 (NHCHCH$_2$CO), 48.47 (NHCH$_2$CH$_2$triazole), 41.55 (NHCH$_2$CH$_2$OH), 39.78 (NHCH$_2$CH$_2$triazole), 34.84 (PhCH$_2$CH$_2$CH$_2$CH$_2$O), 28.59 (PHCH$_2$CH$_2$CH$_2$CH$_2$O), 28.45 (CHCH$_2$triazole), 27.36 (PhCH$_2$CH$_2$CH$_2$CH$_2$O). HR-MS (ESI): calcd. for **5** [$C_{34}H_{46}N_6O_{10}S$ + H]$^+$ 731.3069; found 731.3061.

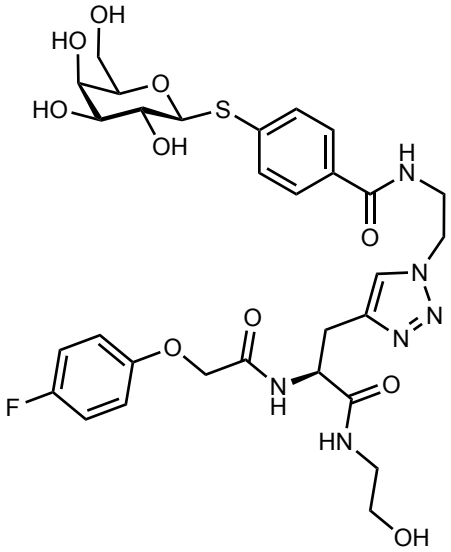
_p_-Tolyloxy-modified galactoside 6



The product was obtained as a white powder (22.9 mg, 0.033 mmol, 85%, R_f = 0.28; CH_2Cl_2/MeOH 19:1). ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.56 (t, J = 5.7 Hz, 1H, NHCH$_2$CH$_2$triazole), 8.13 (d, J = 8.2 Hz, 1H, CHNHCO), 8.00 (t, J = 5.7 Hz, 1H, NHCH$_2$CH$_2$OH), 7.74 (s, 1H, triazole-H), 7.71 (d, J = 8.6 Hz, 2H, 2x SArCH), 7.48 (d, J = 8.6 Hz, 2H, 2x SArCH), 7.10 – 7.05 (m, 2H, 2x OArCH), 6.79 (d, J = 8.6 Hz, 2H, 2x OArCH), 5.22 (d, J = 6.0 Hz, 1H, OH-2), 4.92 (d, J = 5.6 Hz, 1H, OH-3), 4.70 (d, J = 9.2 Hz, 1H, H-1), 4.66 (t, J = 5.8 Hz, 1H, OH-6), 4.55 – 4.49 (m, 2H, CH$_2$CHNH, OH-4), 4.48 – 4.39 (m, 4H, COCH$_2$O, NHCH$_2$CH$_2$triazole), 3.72 (t, J = 4.0 Hz, 1H, H-4), 3.69 – 3.55 (m, 2H, NHCH$_2$CH$_2$triazole), 3.56 – 3.44 (m, 4H, H-2, H-3, NHCH$_2$CH$_2$OH), 3.40 – 3.36 (m, 1H, H-5?), 3.32 (s, 2H, H-6), 3.17 – 3.09 (m, 1H, 1x triazoleCH$_2$CH), 3.09 – 3.03 (m, 2H, NHCH$_2$CH$_2$OH), 2.97 (dd, J = 14.7, 8.1 Hz, 1H, 1x triazoleCH$_2$CH), 2.22 (s, 3H, CH$_3$). ^{13}C NMR (126 MHz, DMSO-d_6) δ 170.21 (C=O), 167.67 (C=O), 166.08 (C=O), 155.56 (OArC), 142.64 (SArC), 140.15 (SArC), 131.13 (triazole-C), 129.90 (OArCCH$_3$), 129.82 (2x OArCH), 127.73 (2x SArCH), 127.57 (2x SArCH), 123.16 (triazole-CH), 114.50 (2x OArCH), 86.67 (C-1), 79.28 (C-5), 74.66 (C-3), 69.12 (C-2), 68.39

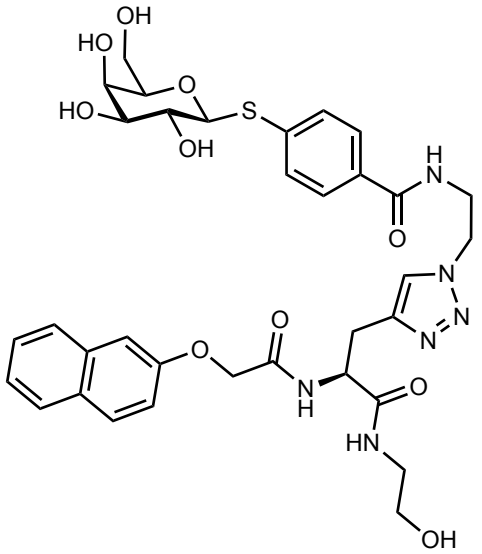
(C-4), 66.81 (CO<u>C</u>H$_2$O, 60.60 (C-6), 59.60 (NHCH$_2$<u>C</u>H$_2$OH, 52.12 (NH<u>C</u>HCH$_2$CO), 48.50 (NHCH$_2$<u>C</u>H$_2$triazole), 41.55 (NH<u>C</u>H$_2$CH$_2$OH), 39.61 (NH<u>C</u>H$_2$CH$_2$triazole), 28.30 (CH<u>C</u>H$_2$triazole), 20.08 (OArC<u>C</u>H$_3$). HRMS (ESI) calcd. For **6** [$C_{31}H_{40}N_6O_{10}S$ +H]$^+$ 689.2599; found, 689.2593.

4-Flurophenyloxy-modified galactoside 7



The product was obtained as a white powder (25.1 mg, 0.036 mmol, 92%, R_f = 0.33; CH_2Cl_2/MeOH 19:1).^{1}H NMR (500 MHz, DMSO-d_6) δ 8.58 (t, J = 5.7 Hz, 1H, N<u>H</u>CH$_2$CH$_2$triazole), 8.20 (d, J = 8.1 Hz, 1H, CHN<u>H</u>CO), 8.02 (t, J = 5.7 Hz, 1H, N<u>H</u>CH$_2$CH$_2$OH), 7.79 (s, 1H, triazole-<u>H</u>), 7.72 (d, J = 8.6 Hz, 1H, 2x SArC<u>H</u>), 7.49 (d, J = 8.6 Hz, 1H, 2x SArC<u>H</u>), 7.17 – 7.10 (m, 2H, 2x OArC<u>H</u>), 6.96 – 6.90 (m, 2H, 2x OArC<u>H</u>), 5.23 (d, J = 6.1 Hz, 1H, OH-2), 4.94 (d, J = 5.7 Hz, 1H, OH-3), 4.74 – 4.67 (m, 3H, H-1, OH-4?, OH-6), 4.57 – 4.51 (m, 2H, CH$_2$C<u>H</u>NHCO, OH-5?), 4.50 – 4.44 (m, 4H, COC<u>H</u>$_2$O, NHCH$_2$C<u>H</u>$_2$triazole), 3.73 (t, J = 4.0 Hz, 1H, H-4), 3.71 – 3.57 (m, 2H, NHC<u>H</u>$_2$CH$_2$triazole), 3.57 – 3.44 (m, 4H, NHCH$_2$C<u>H</u>$_2$OH, H-2, H-3), 3.41 – 3.38 (m, 1H, H-5?, H-6), 3.19 – 3.12 (m, 1H, 1x triazoleC<u>H</u>$_2$CH), 3.12 – 3.05 (m, 2H, NHC<u>H</u>$_2$CH$_2$OH), 3.02 – 2.94 (m, 1H, 1x triazoleC<u>H</u>$_2$CH). ^{13}C NMR (126 MHz, DMSO-d_6) δ 170.25 (<u>C</u>=O), 167.48 (<u>C</u>=O), 166.12 (<u>C</u>=O), 156.82 (d, J = 236.44 Hz, OAr<u>C</u>), 154.02 (d, J = 1.90 Hz, OAr<u>C</u>F), 142.70 (SAr<u>C</u>), 140.19 (SAr<u>C</u>), 131.14 (triazole-<u>C</u>), 127.73 (2x SAr<u>C</u>H), 127.60 (2x SAr<u>C</u>H), 123.21 (triazole-<u>C</u>H), 116.01 (d, J = 14.34 Hz, 2x OAr<u>C</u>H), 115.88 (d, J = 29.25 Hz, 2x OAr<u>C</u>H), 86.68 (C-1), 79.30 (C-5), 74.68 (C-3), 69.13 (C-2), 68.41 (C-4), 67.23 (CO<u>C</u>H$_2$Ar), 60.63 (NHCH$_2$<u>C</u>H$_2$OH), 59.63 (C-6), 52.20 (NH<u>C</u>HCO), 48.54 (NHCH$_2$<u>C</u>H$_2$triazole), 41.58

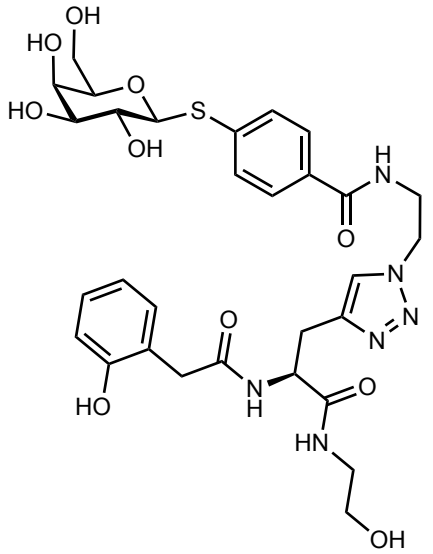
476 (NH<u>C</u>H$_2$CH$_2$OH), 39.77 (NH<u>C</u>H$_2$CH$_2$triazole), 28.31 (triazole<u>C</u>H$_2$CH). HRMS (ESI) calcd. for **7**
477 $[C_{30}H_{37}FN_6O_{10}S + H]^+$ 693.2349; found, 693.2339.
478
479 **Naphthyloxy-modified galactoside 8**



480
481 The product was obtained as a white powder (25.0 mg, 0.034 mmol, 88%, R_f = 0.31;
482 CH_2Cl_2/MeOH 19:1).^{1}H NMR (500 MHz, DMSO-d_6) δ 8.55 (t, J = 5.7 Hz, 1H,
483 N<u>H</u>CH$_2$CH$_2$triazole), 8.28 (d, J = 8.1 Hz, 1H, CHN<u>H</u>CO), 8.04 (t, J = 5.7 Hz, 1H, N<u>H</u>CH$_2$CH$_2$OH),
484 7.84 (dd, J = 8.5, 5.1 Hz, 2H, 2x naphthyl-<u>H</u>), 7.78 (d, J = 8.2 Hz, 1H, naphthyl-<u>H</u>), 7.76 (s, 1H,
485 triazole-<u>H</u>), 7.71 (d, J = 8.3 Hz, 2H, 2x SArC<u>H</u>), 7.49 – 7.44 (m, 3H, 2x SArC<u>H</u>, naphthyl-<u>H</u>), 7.36
486 (ddd, J = 8.1, 6.7, 1.2 Hz, 1H, naphthyl-<u>H</u>), 7.26 (d, J = 2.6 Hz, 1H, naphthyl-<u>H</u>), 7.22 (dd, J = 8.9,
487 2.5 Hz, 1H, naphthyl-<u>H</u>), 4.70 (d, J = 9.6 Hz, 1H, H-1), 4.67 – 4.59 (m, 2H, COC<u>H</u>$_2$O), 4.59 – 4.53
488 (m, 1H, CH$_2$C<u>H</u>NHCO), 4.39 (td, J = 6.3, 2.4 Hz, 2H, NHCH$_2$C<u>H</u>$_2$triazole), 3.72 (d, J = 3.2 Hz,
489 1H, H-4), 3.59 (td, J = 13.8, 7.1 Hz, 2H, NHC<u>H</u>$_2$CH$_2$triazole), 3.55 – 3.43 (m, 4H, H-2, H-3,
490 NHCH$_2$C<u>H</u>$_2$OH), 3.40 – 3.34 (m, 3H, H-5, H-6), 3.19 – 3.10 (m, 1H, 1x triazolC<u>H</u>$_2$CH), 3.10 –
491 3.05 (m, 2H, NHC<u>H</u>$_2$CH$_2$OH), 3.05 – 2.97 (m, 1H, 1x triazolC<u>H</u>$_2$CH). ^{13}C NMR (126 MHz,
492 DMSO-d_6) δ 170.29 (<u>C</u>=O), 167.48 (<u>C</u>=O), 166.10 (<u>C</u>=O), 155.53 (naphthyl-<u>C</u>), 142.72 (Ar<u>C</u>),
493 140.18 (Ar<u>C</u>), 134.07 (naphthyl-<u>C</u>), 131.15 (naphthyl-<u>C</u>), 129.41 (naphthyl-<u>C</u>H), 128.74
494 (triazole-<u>C</u>), 127.73 (2x SAr<u>C</u>H), 127.60 (2x SAr<u>C</u>H), 127.56 (naphthyl-<u>C</u>H), 126.80 (naphthyl-
495 <u>C</u>H) 126.53 (naphthyl-<u>C</u>H), 123.91 (naphthyl-<u>C</u>H), 123.18 (triazole-<u>C</u>H), 118.56 (naphthyl-<u>C</u>H),
496 107.31 (naphthyl-<u>C</u>H), 86.68 (C-1), 79.30 (H-5), 74.67 (C-3), 69.13 (C-2), 68.41 (C-4), 66.76
497 (CO<u>C</u>H$_2$OAr), 60.63 (NHCH$_2$<u>C</u>H$_2$OH), 59.63 (C-6), 52.24 (CH$_2$<u>C</u>HNHCO), 48.48

(NHCH$_2$CH$_2$triazole), 41.59 (NHCH$_2$CH$_2$OH), 39.78 (NHCH$_2$CH$_2$triazole) 28.32 (triazoleCH$_2$CH). HRMS (ESI) calcd. for **8** [$C_{34}H_{40}N_6O_{10}S$ +H]$^+$ 725.2599; found, 725.2591.

o-Hydroxyphenyl-modified galactoside 9



The product was obtained as a white powder (19.1 mg, 0.028 mmol, 73%, R_f = 0.21; CH_2Cl_2/MeOH 19:1). ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.63 (s, 1H, ArOH), 8.58 (t, J = 5.7 Hz, 1H, NHCH$_2$CH$_2$triazole), 8.11 (d, J = 8.2 Hz, 1H, CHNHCO), 7.94 (t, J = 5.7 Hz, 1H, NHCH$_2$CH$_2$OH), 7.72 (d, J = 8.5 Hz, 1H, SArCH), 7.65 (s, 1H, triazole-H), 7.48 (d, J = 8.5 Hz, 1H, SArCH), 7.04 (td, J = 7.7, 1.7 Hz, 1H, ArCH), 6.99 (dd, J = 7.6, 1.7 Hz, 1H, ArCH), 6.78 (dd, J = 8.1, 1.3 Hz, 1H, ArCH), 6.71 (td, J = 7.4, 1.3 Hz, 1H, ArCH), 5.22 (d, J = 6.1 Hz, 1H, OH-2), 4.93 (d, J = 5.7 Hz, 1H, OH-3), 4.70 (d, J = 9.7 Hz, 1H, H-1), 4.68 (s, 1H, OH-6), 4.54 (d, J = 4.4 Hz, 1H, OH-4), 4.49 – 4.41 (m, 3H, NHCH$_2$CH$_2$triazole, CH$_2$CHNHCO), 3.72 (t, J = 3.8 Hz, 1H, H-4), 3.70 – 3.55 (m, 1H, NHCH$_2$CH$_2$triazole), 3.55 – 3.44 (m, 4H, H-2, H-3?, NHCH$_2$CH$_2$OH), 3.41 (s, 2H, COCH$_2$Ar), 3.39 – 3.37 (m, 3H, H-5, H-6), 3.19 – 3.10 (m, 1H, 1x triazoleCH$_2$CH), 3.10 – 2.98 (m, 2H, NHCH$_2$CH$_2$OH), 2.93 – 2.84 (m, 1H, 1x triazoleCH$_2$CH). ^{13}C NMR (126 MHz, DMSO-d_6) δ 170.74 (C=O), 170.52 (C=O), 166.15 (C=O), 155.27 (ArCOH), 142.73 (SArC), 140.22 (SArC), 131.14 (triazole-C), 130.70 (ArCH), 127.78 (ArCH), 127.76 (2x SArCH), 127.63 (2x SArCH), 123.11 (ArC), 122.56 (triazole-CH), 118.99 (ArCH), 115.16 (ArCH), 86.68 (C-1), 79.31 (C-5), 74.68 (C-3), 69.15 (C-2), 68.43 (C-4), 60.65 (NHCH$_2$CH$_2$OH), 59.63 (C-6), 52.51 (CH$_2$CHNHCO), 48.52 (NHCH$_2$CH$_2$triazole), 41.52 (NHCH$_2$CH$_2$OH), 39.78

519 (NH<u>C</u>$H_2$$CH_2$triazole), 37.17 (CO<u>C</u>H_2Ar), 28.55 (triazole<u>C</u>H_2CH). HRMS (ESI) calcd. for **9**
520 $[C_{30}H_{38}N_6O_{10}S + H]^+$ 675.2443; found, 675.2433.
521

Molecular Dynamics

Table S1: Analyzed LecA PDB structures and corresponding co-crystalized ligands, when present.

Ligand	PDB ID	Resolution (Å)
Apo	1l7l	1.5
Apo	1uoj	2.4
β-D-galactose / α-D-galactose	1oko	1.6
Gal α(1→3) Gal β(1→4) Glc	2vxj	1.9
Gal- α(1→2) Gal β-O-Me	2wyf	2.4
4-Hydroxybenzoic acid 4-O-galactoside	3zyb	2.29
4-Nitrophenyl β-D-galactopyranoside	3zyf	1.94
3-(β-D-galactopyranosylthio)propanoic acid	3zyh	1.5
Naphthalen-2-YL-Thio-β-D-galactopyranoside	4a6s	2.15
Melibiose (Gal α(1→6) Glc)	4al9	1.75
(4*S*)-*N*-Ethyl-4-{[*N*-methyl-3-(1-{2-[(4-sulfanylbenzoyl)-aminoethyl}-1H-1,2,3-triazol-4-yl)-L-alanyl]amino}-L-prolinamide-β-D-galactoside	4cp9	1.65
(4S)-N-Ethyl-4-{[N-methyl-3-(1-{2-[(4-sulfanylbenzoyl)-aminoethyl}-1H-1,2,3-triazol-4-yl)-L-alanyl]amino}-L-prolinamide-β-D-galactoside	4cpb	1.57
N-Methyl-3-indolyl β-D-galactopyranoside	4ljh	1.45
Chlorophenol Red-β-D-galactopyranoside	4lk6	2.86
Resorufin-β-D-galactopyranoside	4lk7	1.76
4-Hydroxybenzoic acid 4-O-galactoside	4lkd	2.31
4-Hydroxybenzoic acid 4-O-galactoside	4lke	1.65
4-Hydroxybenzoic acid 4-O-galactoside	4lkf	1.64
N-[(2S)-6-amino-1-oxo-1-(pyrrolidin-1-yl)hexan-2-yl]-4-(beta-D- galactopyranosyloxy)benzamide	4yw6	1.4
(2R,3R,4S,5R,6R,2'R,3'R,4'S,5'R,6'R)-2,2'-([(2R,3R,4S,5S,6S)-3,4- dihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2,5-diyl]bis{1H- 1,2,3-triazole-1,4-diyl[(2S,3R,4S,5S,6S)-3,4-	4yw7	1.82

dihydroxy-6- (hydroxymethyl)tetrahydro-2H-pyran-2,5-diyl]-1H-1,2,3-triazole-1,4- diylpropane-3,1-diyloxy})bis[6-(hydroxymethyl)tetrahydro-2H-pyran- 3,4,5-triol]		
Phenyl 6,7-dideoxy-6,7-epoxy-beta-D-galacto-heptopyranoside(6D)	5mih	1.8

524

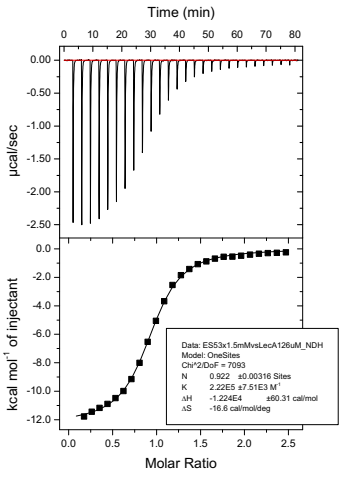
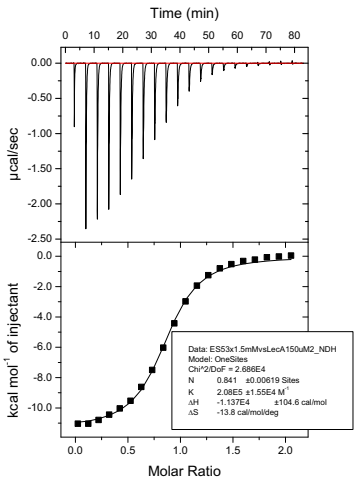
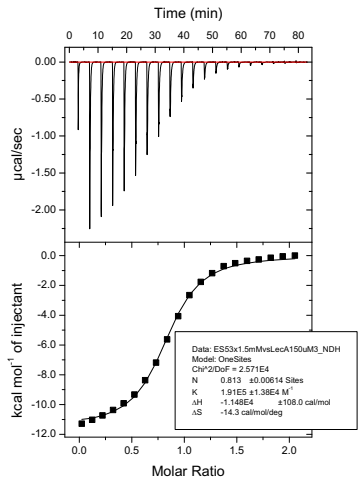
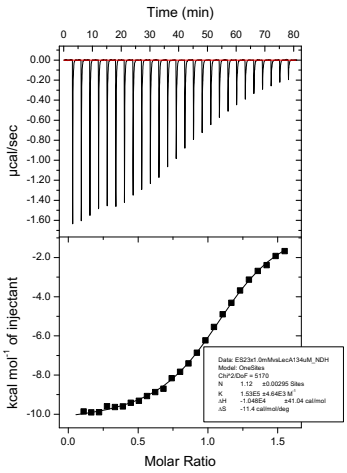
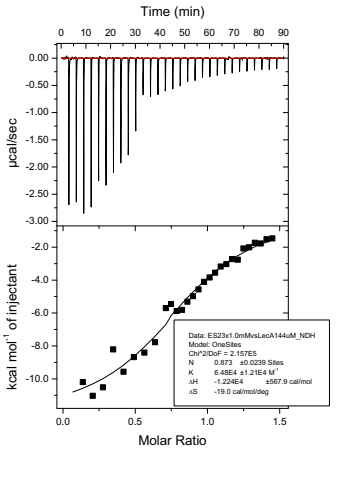
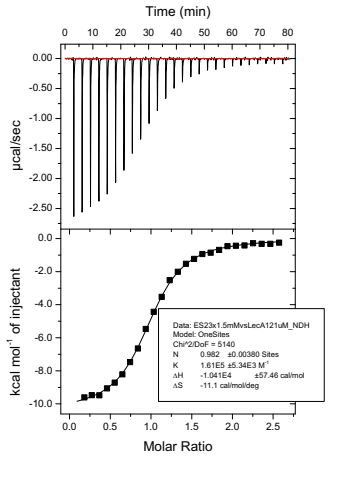
525 **Table S2**: Analyzed dimer LecA structures of different PDB files to determine the volume of the
526 cavity.

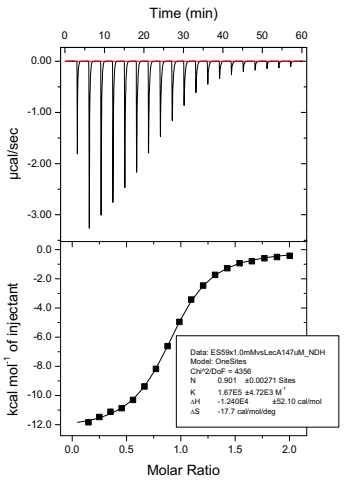
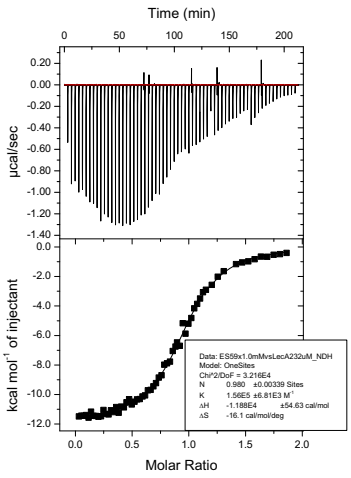
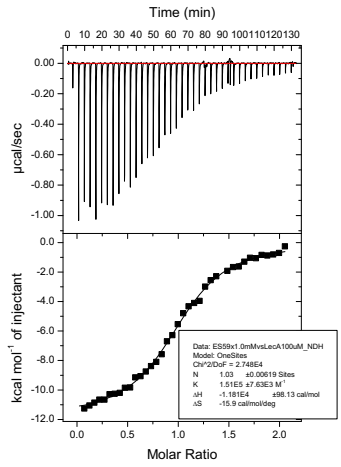
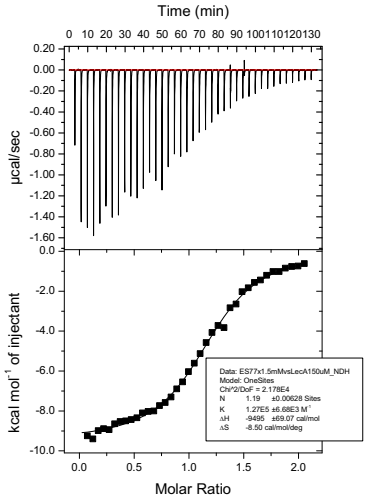
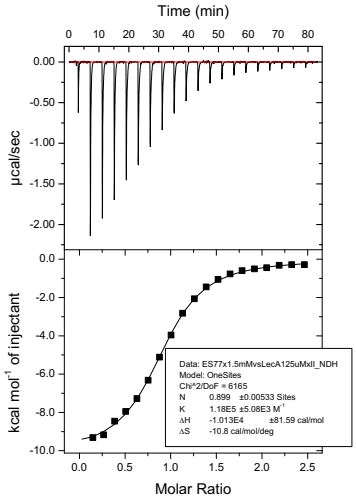
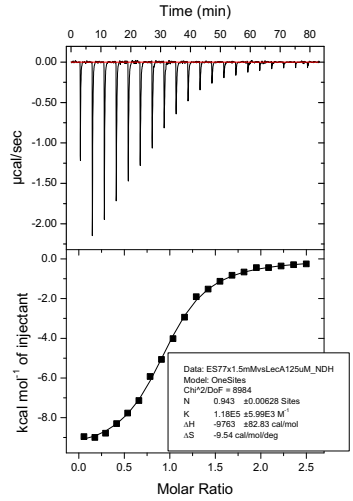
PDB ID	Number of cavities	Nature	Volume (Å^3)
1l7l	0		
1oko	1	Central pocket	236
1uoj	0		
2vxj	1	Central pocket	297
2wyf	1	Central pocket	307
2wyf	2	Central pocket	297
3zyf	0		
3zyh	1	Additional cavity	263
3zyh	2	Central pocket	243
4a6s	0		
4al9	1	Central pocket	280
4cp9	1	Central pocket	303
4cp9	2	Central pocket	286
4cpb	1	Central pocket	253
4ljh	1	Central pocket	314
4ljh	2	Central pocket	273
4lk6	1	Central pocket	344
4lk6	2	Central pocket	297
4lk7	1	Central pocket	294
4lk7	2	Central pocket	260
4lkd	1	Central pocket	284
4lke	1	Central pocket	341

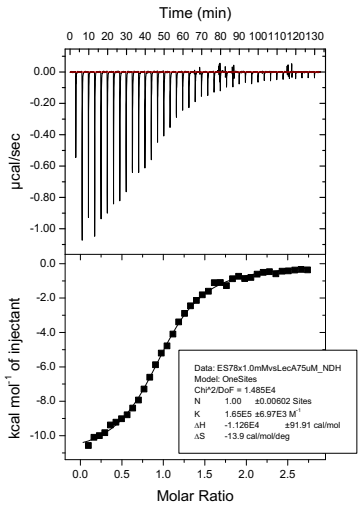
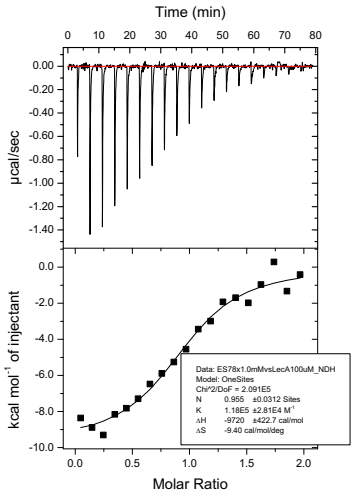
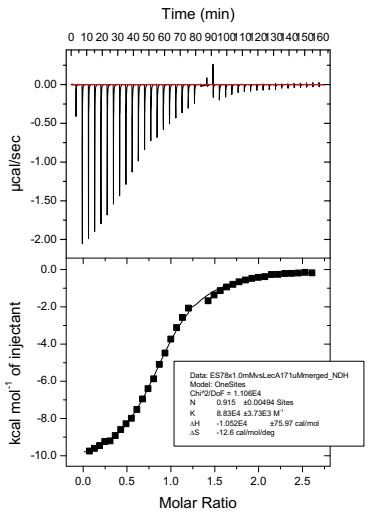
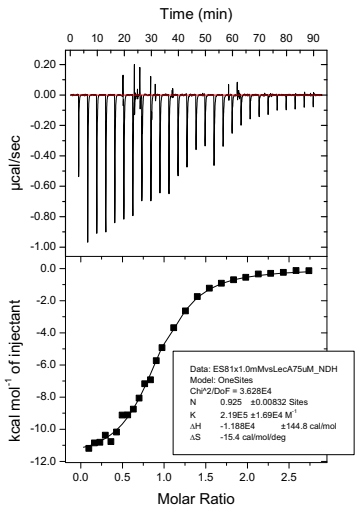
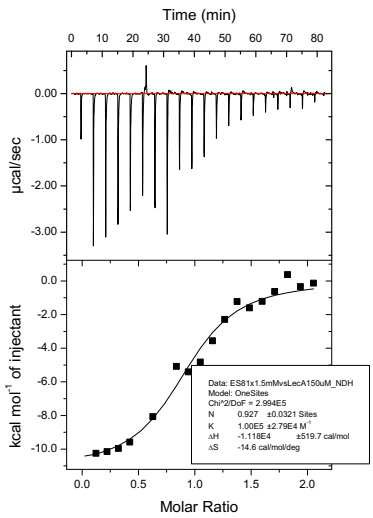
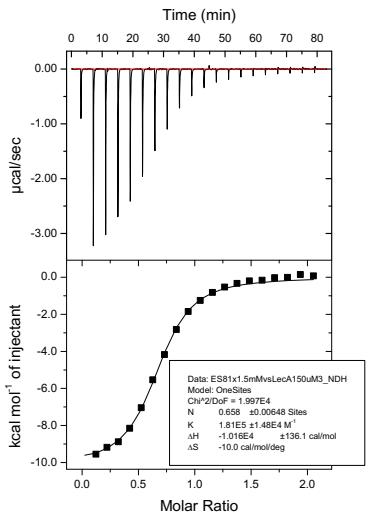
4lke	2	Central pocket	314
4lkf	1	Central pocket	297
4lkf	2	Central pocket	287
4yw6	0		
4yw7	1	Central pocket	300
4ywa	1	Central pocket	182
5d21	0		
5mih	1	Central pocket	277
5mih	2	Central pocket	263

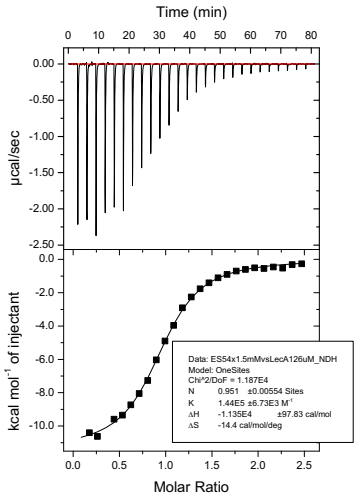
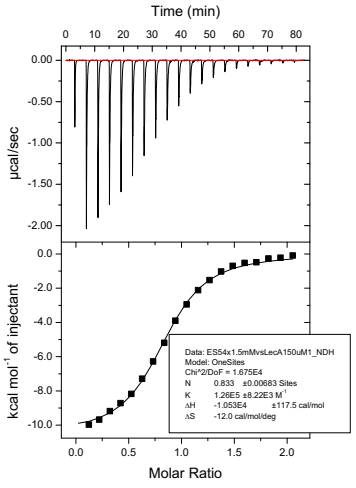
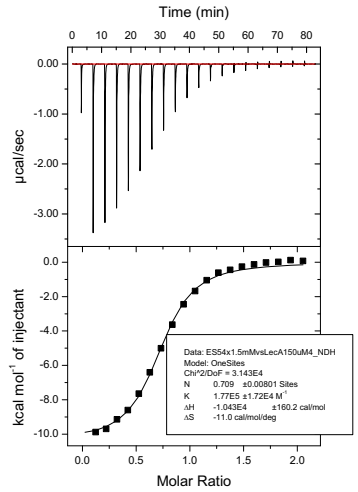
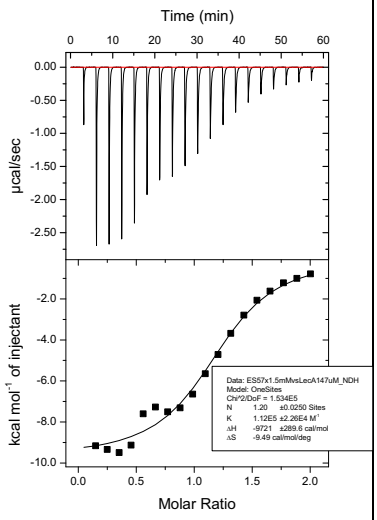
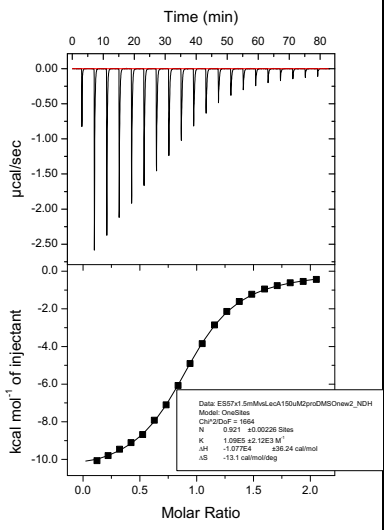
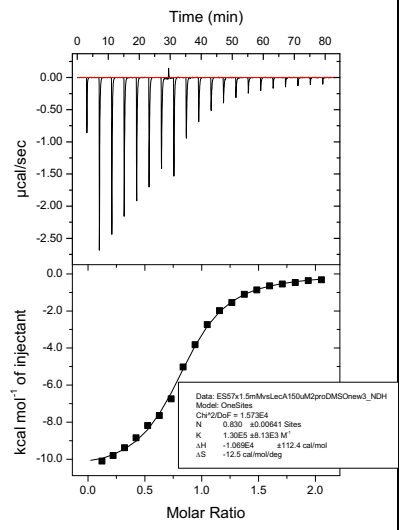
527

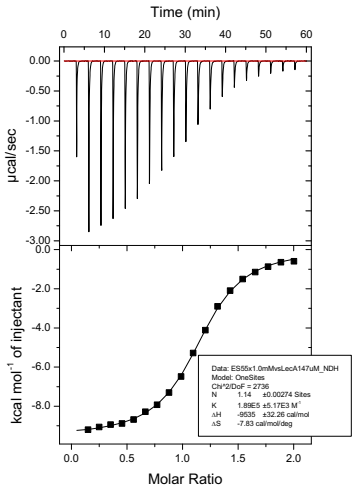
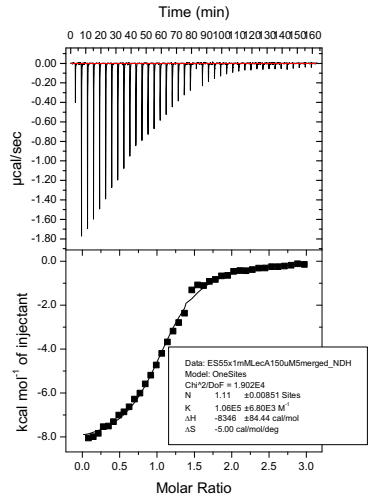
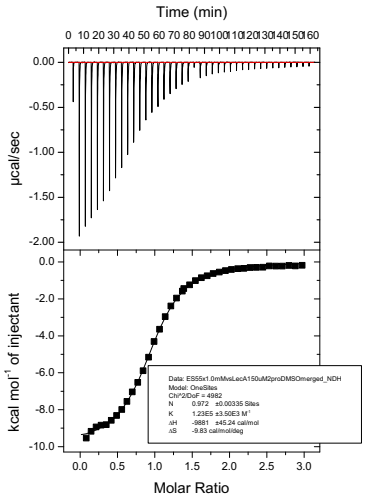
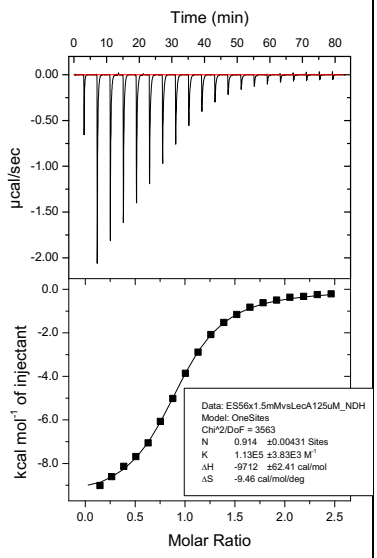
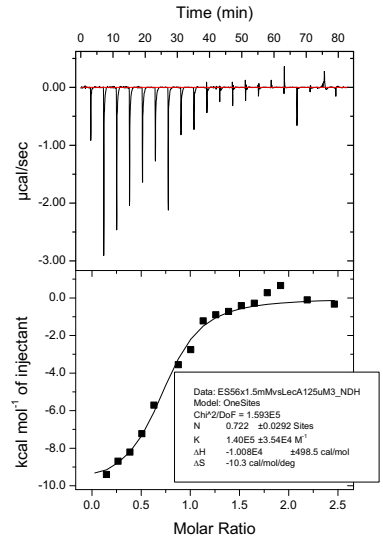
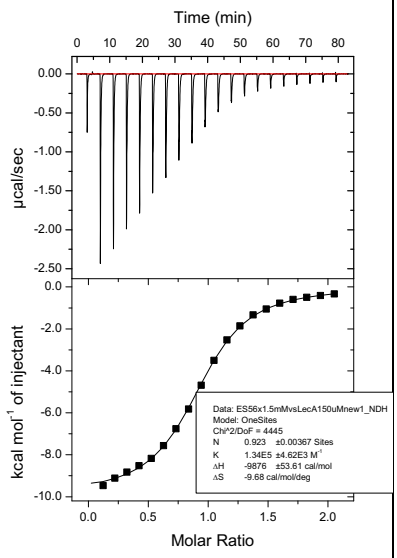
Isothermal Titration Calorimetry (ITC)

		
Compound **S6** = 1.5 mM LecA = 126 µM	Compound **S6** = 1.5 mM LecA = 150 µM	Compound **S6** = 1.5 mM LecA = 150 µM
		
Compound **1** = 1.0 mM LecA = 134 µM	Compound **1** = 1.0 mM LecA = 144 µM	Compound **1** = 1.5 mM LecA = 121 µM

		
Compound **2** = 1.0 mM LecA = 147 µM	Compound **2** = 1.0 mM LecA = 232 µM	Compound **2** = 1.0 mM LecA = 100 µM
		
Compound **3** = 1.5 mM LecA = 150 µM	Compound **3** = 1.5 mM LecA = 125 µM	Compound **3** = 1.5 mM LecA = 125 µM

		
Compound **4** = 1.0 mM LecA = 75 µM	Compound **4** = 1.0 mM LecA = 100 µM	Compound **4** = 1.0 mM LecA = 171 µM
		
Compound **5** = 1.0 mM LecA = 75 µM	Compound **5** = 1.5 mM LecA = 150 µM	Compound **5** = 1.5 mM LecA = 150 µM

		
Compound **6** = 1.5 mM LecA = 126 µM	Compound **6** = 1.5 mM LecA = 150 µM	Compound **6** = 1.5 mM LecA = 150 µM
		
Compound **7** = 1.5 mM LecA = 147 µM	Compound **7** = 1.5 mM LecA = 150 µM 2% DMSO in buffer	Compound **7** = 1.5 mM LecA = 150 µM 2% DMSO in buffer

		
Compound **8** = 1.0 mM LecA = 147 µM	Compound **8** = 1.0 mM LecA = 150 µM	Compound **8** = 1.0 mM LecA = 150 µM 2%DMSO in buffer
		
Compound **9** = 1.5 mM LecA = 125 µM	Compound **9** = 1.5 mM LecA = 125 µM	Compound **9** = 1.5 mM LecA = 150 µM

529 **Figure S1**: ITC titration experiments with LecA.

530

19F PrOF NMR

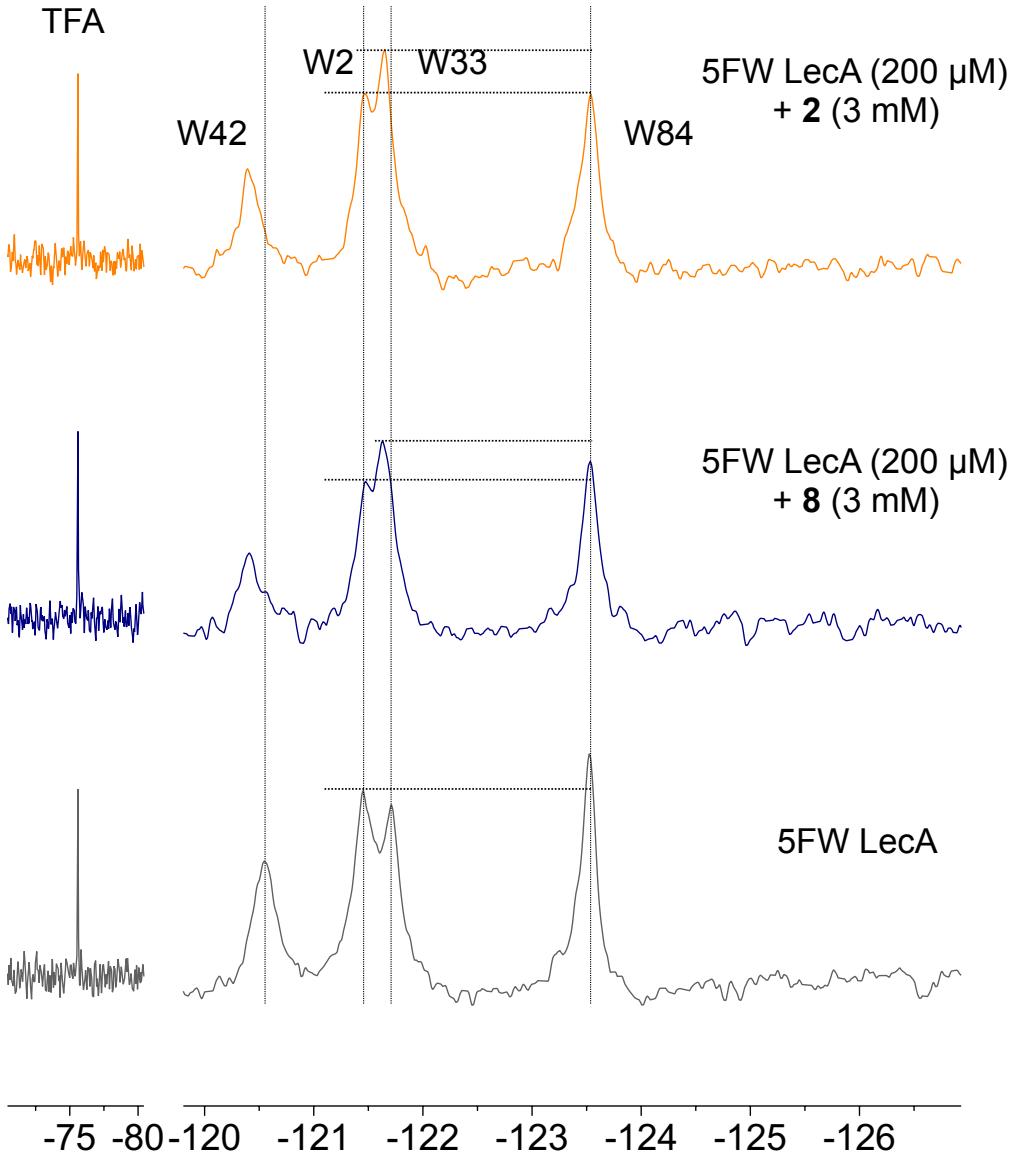
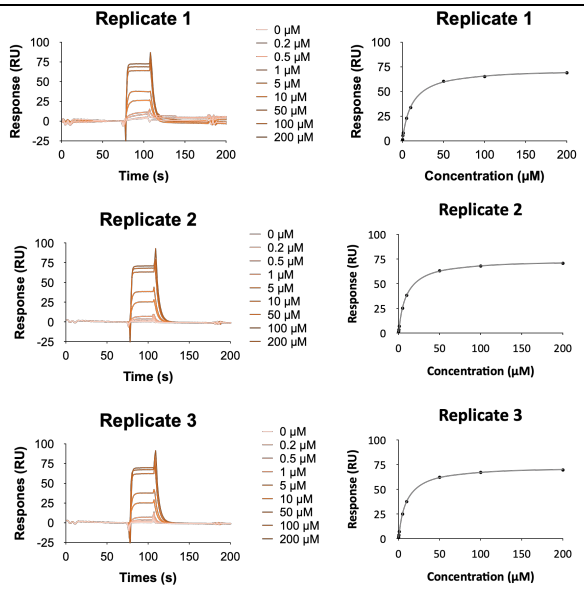
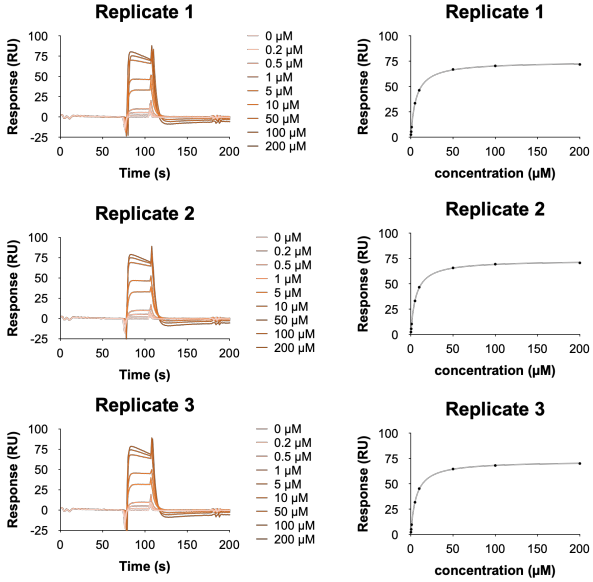
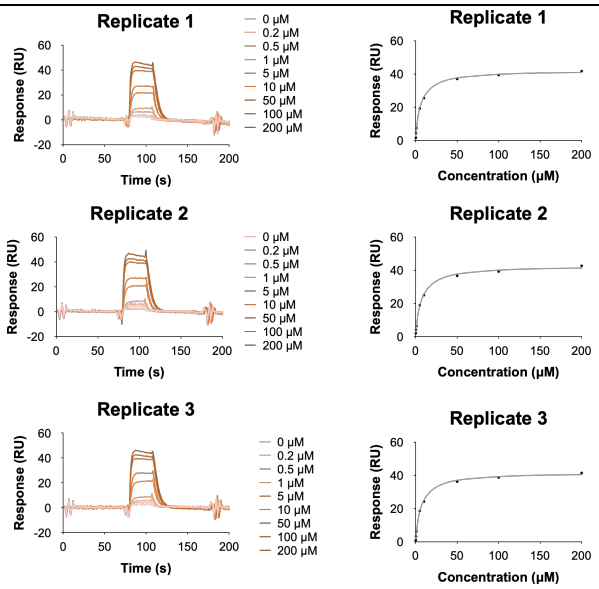
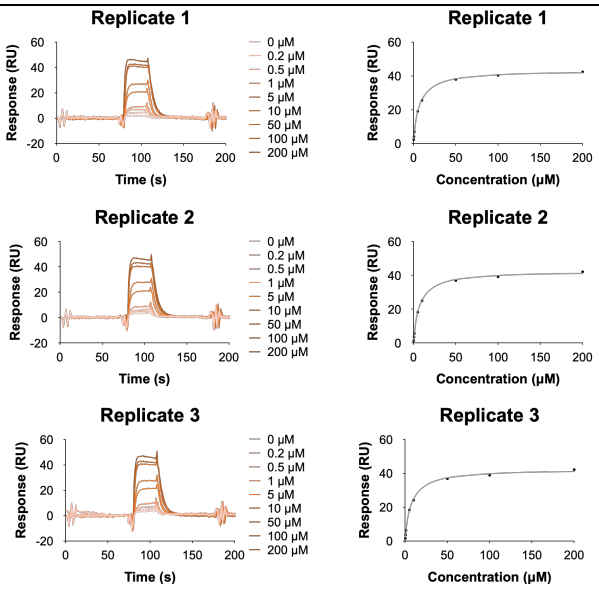
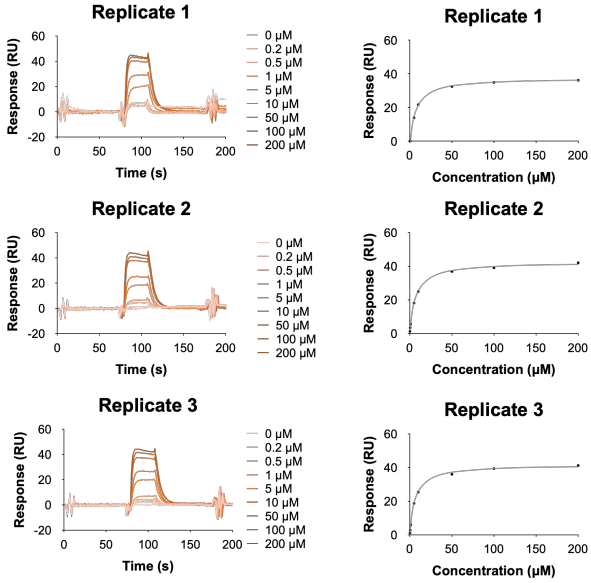
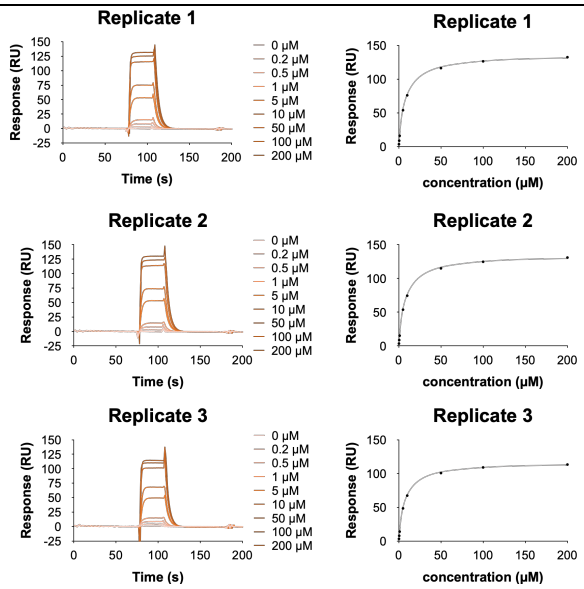


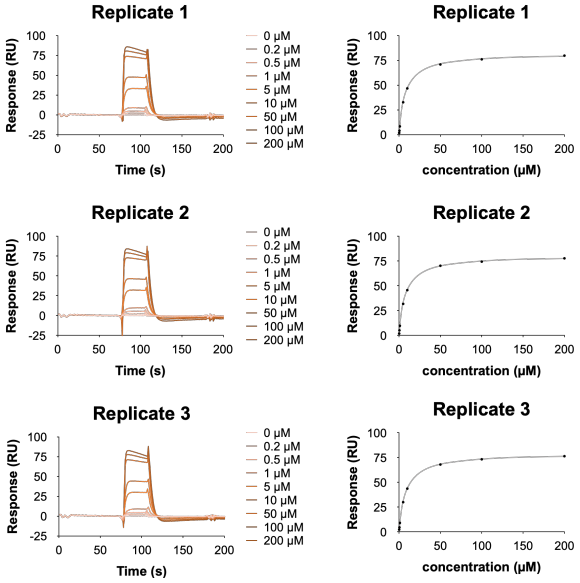
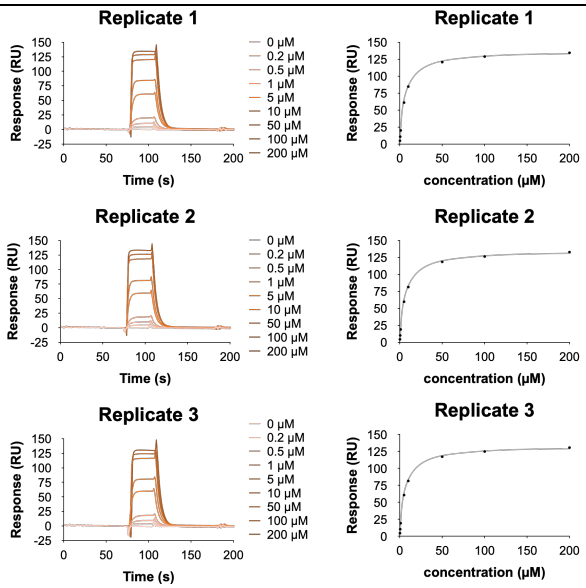
Figure S2: 19F PrOF NMR of 5FW LecA: without ligand (gray), with the benzyl ether **2** (orange) and the napththyl compound **8** (blue). A chemical shift perturbation of Trp42 and Trp33 has been observed in presence of both ligands indicating that ligands target the carbohydrate and the central pockets, respectively.

Surface Plasmon Resonance (SPR)

1	
2	

3	 Replicate 1 Response (RU) Time (s) 0 µM 0.2 µM 0.5 µM 1 µM 5 µM 10 µM 50 µM 100 µM 200 µM Replicate 1 Response (RU) Concentration (µM) Replicate 2 Response (RU) Time (s) Replicate 2 Response (RU) Concentration (µM) Replicate 3 Response (RU) Time (s) Replicate 3 Response (RU) Concentration (µM)
4	 Replicate 1 Response (RU) Time (s) 0 µM 0.2 µM 0.5 µM 1 µM 5 µM 10 µM 50 µM 100 µM 200 µM Replicate 1 Response (RU) Concentration (µM) Replicate 2 Response (RU) Time (s) Replicate 2 Response (RU) Concentration (µM) Replicate 3 Response (RU) Time (s) Replicate 3 Response (RU) Concentration (µM)

5	 Replicate 1 Response (RU) Time (s) 0 µM 0.2 µM 0.5 µM 1 µM 5 µM 10 µM 50 µM 100 µM 200 µM Replicate 1 Response (RU) Concentration (µM) Replicate 2 Response (RU) Time (s) Replicate 2 Response (RU) Concentration (µM) Replicate 3 Response (RU) Time (s) Replicate 3 Response (RU) Concentration (µM)
6	 Replicate 1 Response (RU) Time (s) 0 µM 0.2 µM 0.5 µM 1 µM 5 µM 10 µM 50 µM 100 µM 200 µM Replicate 1 Response (RU) concentration (µM) Replicate 2 Response (RU) Time (s) Replicate 2 Response (RU) concentration (µM) Replicate 3 Response (RU) Time (s) Replicate 3 Response (RU) concentration (µM)

7	 Replicate 1 Response (RU) Time (s) 0 µM 0.2 µM 0.5 µM 1 µM 5 µM 10 µM 50 µM 100 µM 200 µM Replicate 1 Response (RU) concentration (µM) Replicate 2 Response (RU) Time (s) Replicate 2 Response (RU) concentration (µM) Replicate 3 Response (RU) Time (s) Replicate 3 Response (RU) concentration (µM)
8	 Replicate 1 Response (RU) Time (s) 0 µM 0.2 µM 0.5 µM 1 µM 5 µM 10 µM 50 µM 100 µM 200 µM Replicate 1 Response (RU) concentration (µM) Replicate 2 Response (RU) Time (s) Replicate 2 Response (RU) concentration (µM) Replicate 3 Response (RU) Time (s) Replicate 3 Response (RU) concentration (µM)

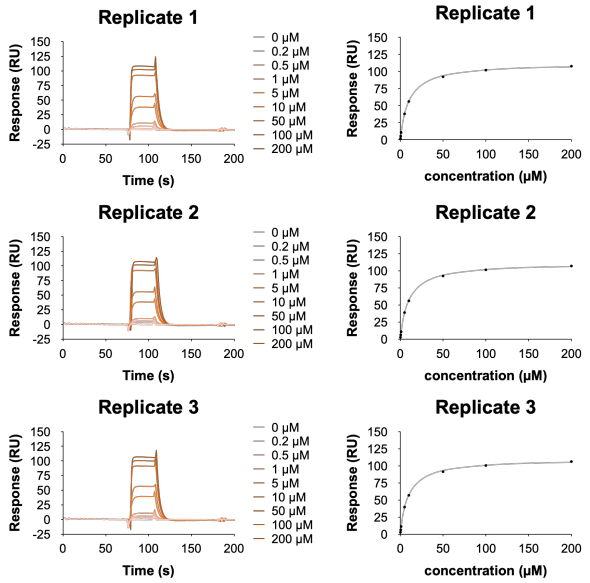
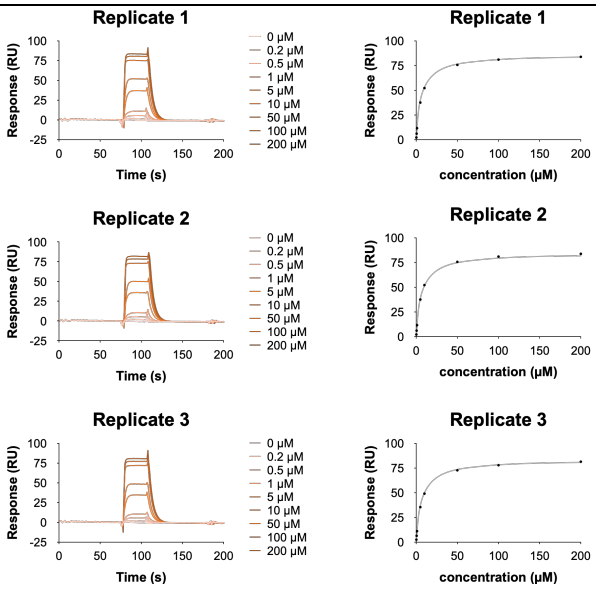
9	
S6	

Figure S3: Individual sensorgrams and fits of of all replicates for binding to LecA.

X-Ray Crystallography

Table S3. X-ray data collection and processing of LecA in complex with **1**

PDB code	7FIO
Data Collection	
Beamline	PROXIMA1 (SOLEIL)
Wavelength (Å)	0.9786
Detector	EigerX-16M
Resolution (Å)[a]	47.29-1.50 (1.53-1.50)
Space Group	$P2_12_12_1$
a, b, c (Å)	49.60 52.68 156.97
α, β, γ (°)	90.0,90.0 ,90.0
Total observations	690,166
Unique reflections	66,700
Multiplicity[a]	10.3 (9.3)
Mean $I/\sigma(I)$[a]	13.5 (1.7)
Completeness (%)[a]	99.8 (99.1)
R_{merge}[a,b]	0.089 (0.999)
$CC_{½}$[a,c]	1.0 (0.6)
Refinement	
Reflections: working/free[d]	66,623/3,419
R_{work}/ R_{free}[e]	0.177/0.212
Ramachandran plot: allowed/favoured/outliers (%)	3/97/0
R.m.s. bond deviations (Å)	0.0162
R.m.s. angle deviations (°)	1.896
Mean *B*-factors: protein/ligand[f]**/ /water (Å²)**	20/41/29

[a] Values for the outer resolution shell are given in parentheses.
[b] $R_{merge} = \sum_{hkl} \sum_i |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl} \sum_i I_i(hkl)$.
[c] $CC_{½}$ is the correlation coefficient between symmetry-related intensities taken from random halves of the dataset.
[d] The data set was split into "working" and "free" sets consisting of 95 and 5% of the data, respectively. The free set was not used for refinement.
[e] The R-factors R_{work} and R_{free} are calculated as follows: $R = \sum(|F_{obs} - F_{calc}|)/\sum|F_{obs}|$, where F_{obs} and F_{calc} are the observed and calculated structure factor amplitudes, respectively
[f] refers to ligands bound in the active site and potential surface binding sites

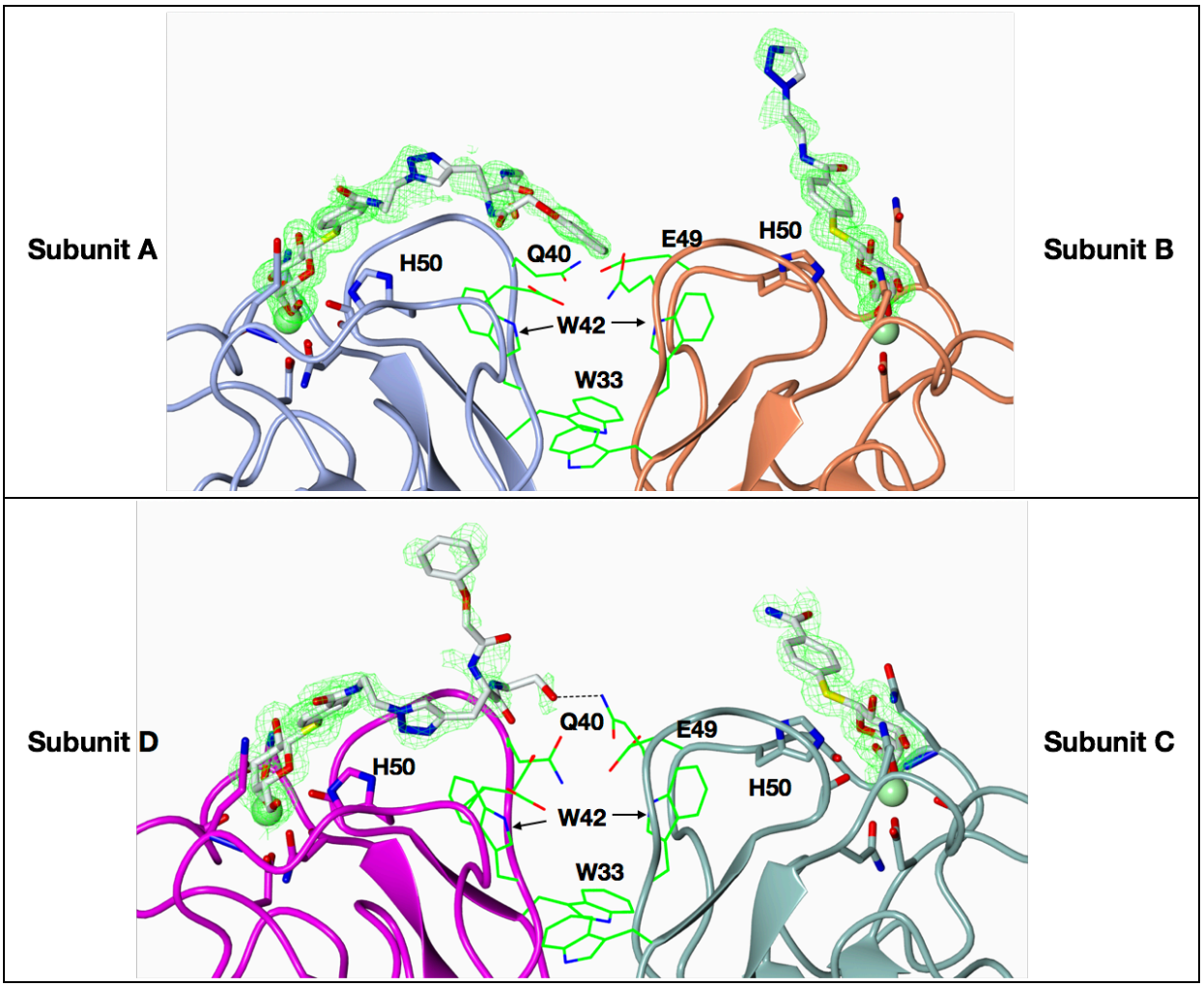


Figure S4: Co-crystal structure of compound **1** and tetrameric LecA. All carbohydrate binding sites of the four monomers are occupied by one ligand by coordination to the calcium ion with the galactose moiety. Subunit A shows the ligand with its side chain at the entrance of the central pocket. Subunit B and C are not completely resolved. Subunit D shows a hydrogen bonding between the ethanol amine function of **1** with Q40 of the neighboring subunit C.

References

[1] G. R. Fulmer, A. J. M. Miller, N. H. Sherden, H. E. Gottlieb, A. Nudelman, B. M. Stoltz, J. E. Bercaw, K. I. Goldberg, *Organometallics* **2010**, *29*, 2176–2179.

[2] A. Novoa, T. Eierhoff, J. Topin, A. Varrot, S. Barluenga, A. Imberty, W. Römer, N. Winssinger, *Angew. Chemie Int. Ed.* **2014**, *53*, 8885–8889.

[3] S. Kuhaudomlarp, E. Siebs, E. Shanina, J. Topin, I. Joachim, P. da Silva Figueiredo Celestino Gomes, A. Varrot, D. Rognan, C. Rademacher, A. Imberty, A. Titz, *Angew. Chemie Int. Ed.* **2021**, *60*, 8104-8114.

[4] K. Anbarasu, K. K. Ilavenil, *Asian J. Chem.* **2018**, *30*, 2238–2240.

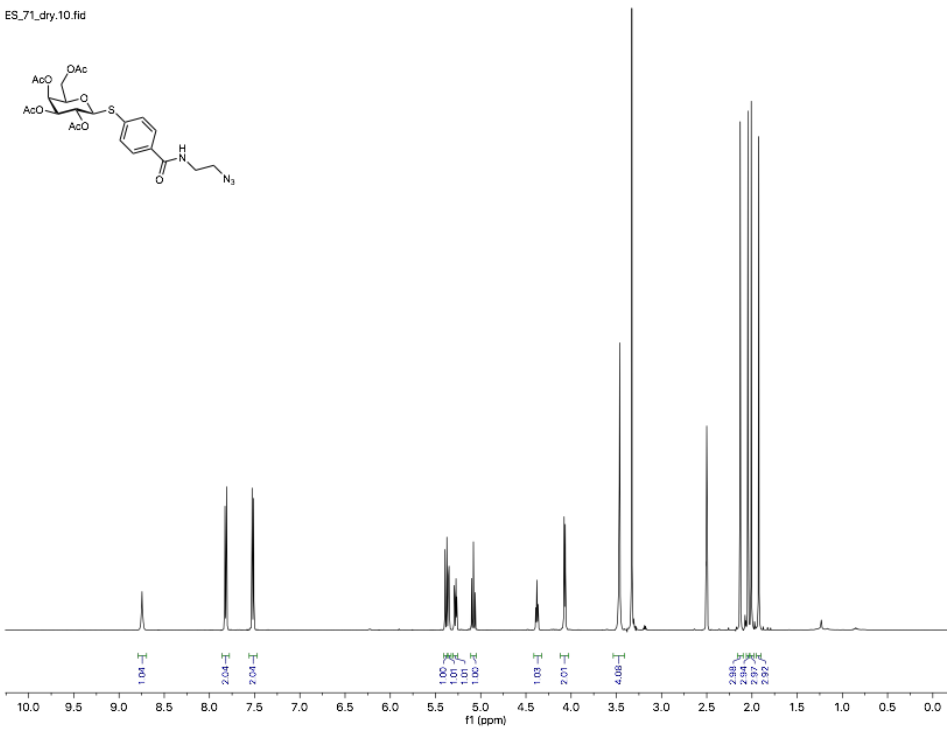
[5] D. Li, S. Xiong, T. Guo, D. Shu, H. Xiao, G. Li, D. Guo, *Dye. Pigment.* **2018**, *158*, 28–35.

[6] P. J. Machín, D. N. Hurst, R. M. Bradshaw, L. C. Blaber, D. T. Burden, A. D. Fryer, R. A. Melarange, C. Shivdasani, *J. Med. Chem.* **1983**, *26*, 1570–1576.

[7] T. E. Kristensen, K. Vestli, M. G. Jakobsen, F. K. Hansen, T. Hansen, *J. Org. Chem.* **2010**, *75*, 1620–1629.

[8] J. C. Sheehan, G. L. Boshart, P. A. Cruickshank, *J. Org. Chem.* **1961**, *26*, 2525–2528.

Spectra



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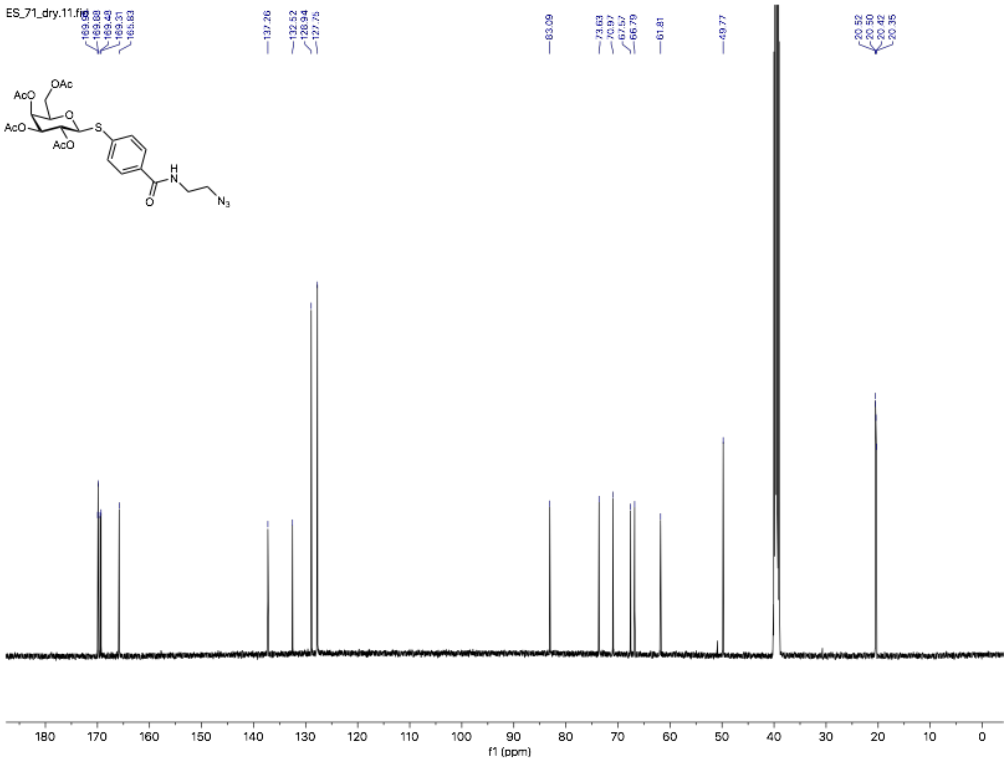

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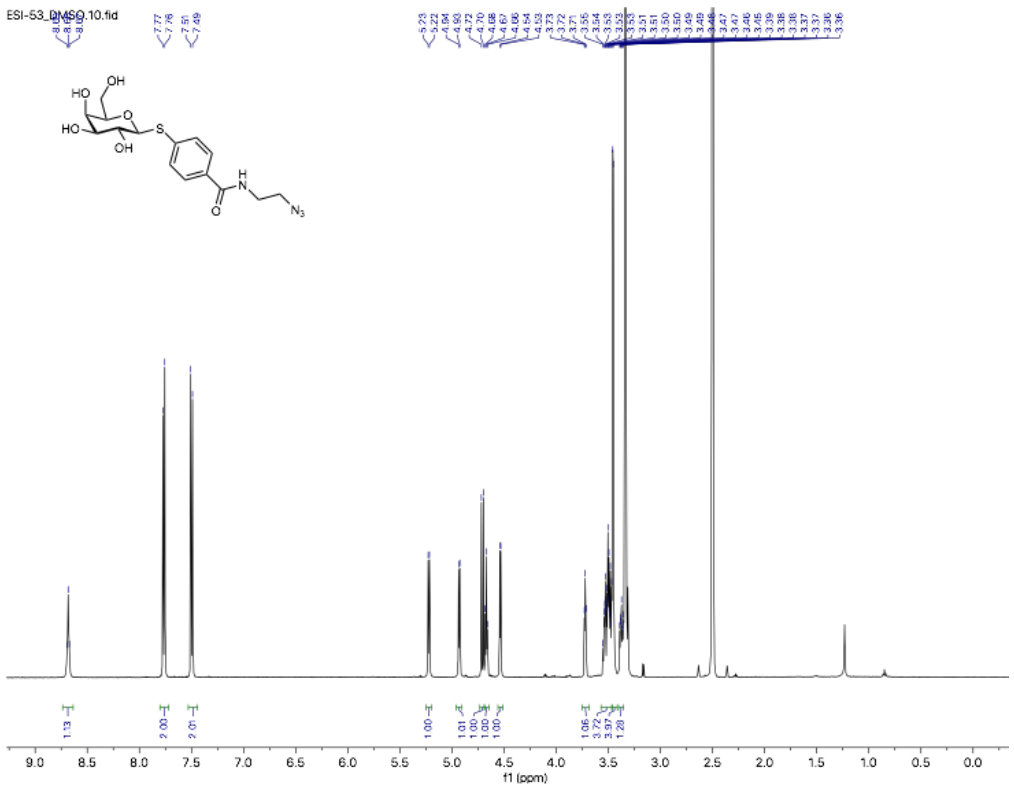

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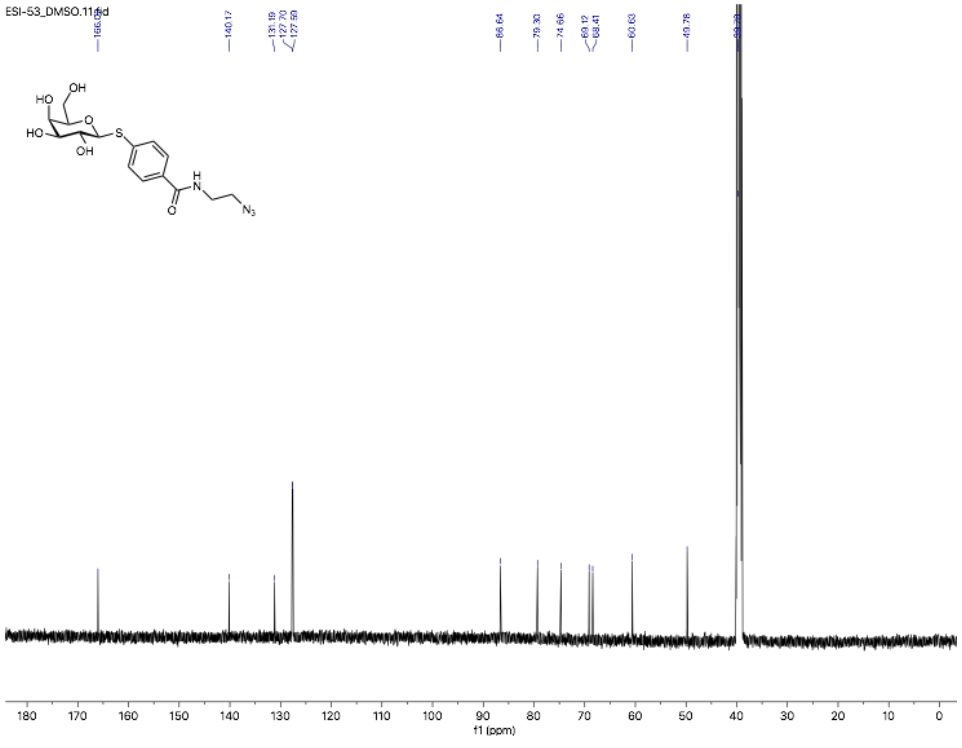

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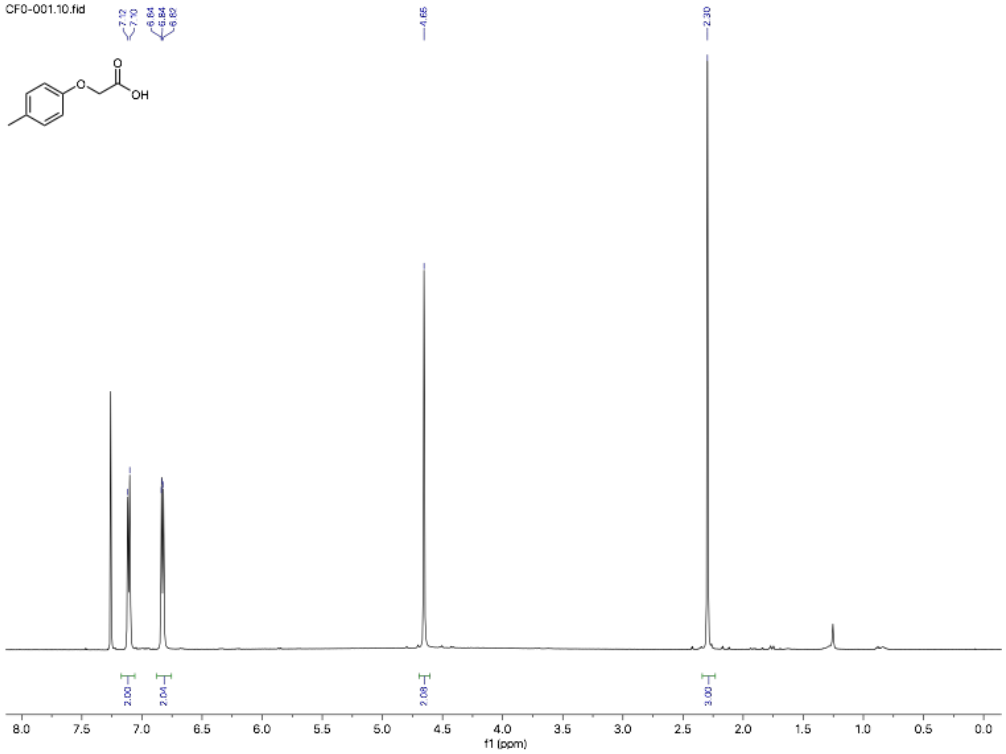
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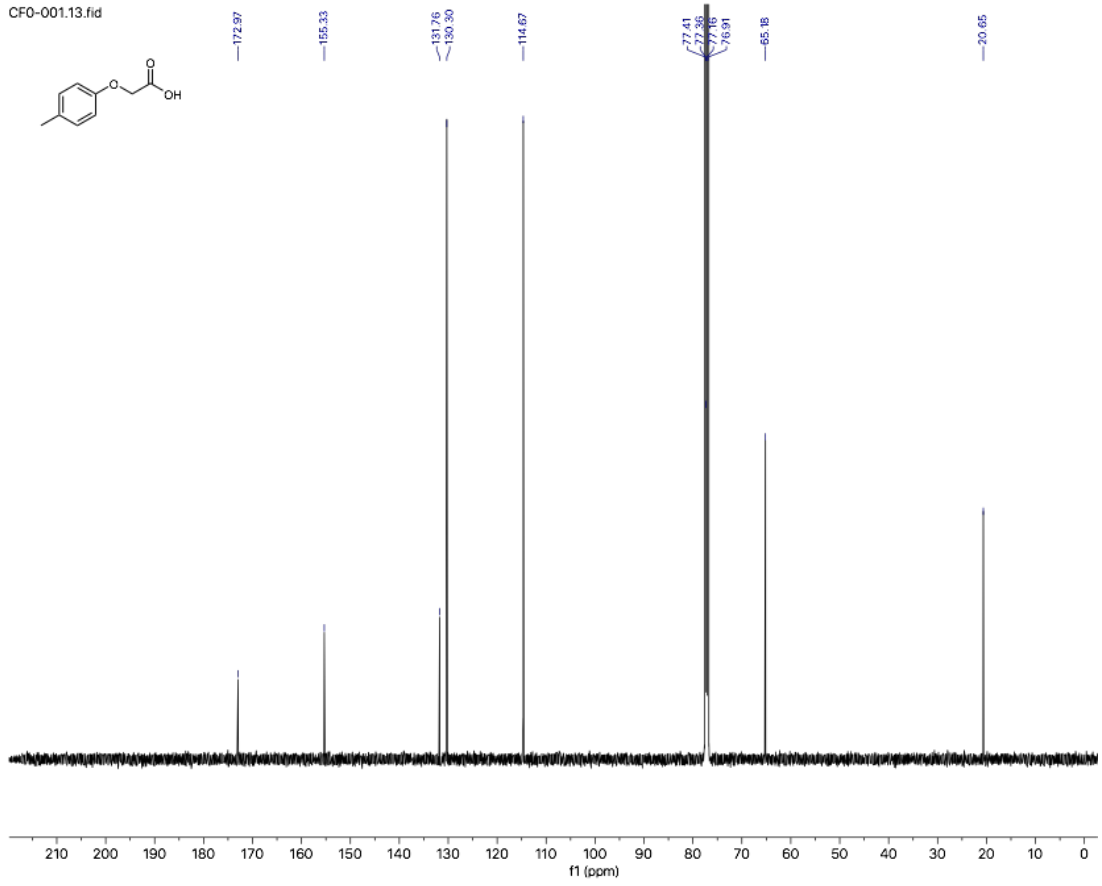
7.10
6.84
6.82
6.69
4.65
2.30
2.00
2.04
2.08
3.00
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)
O
OH

583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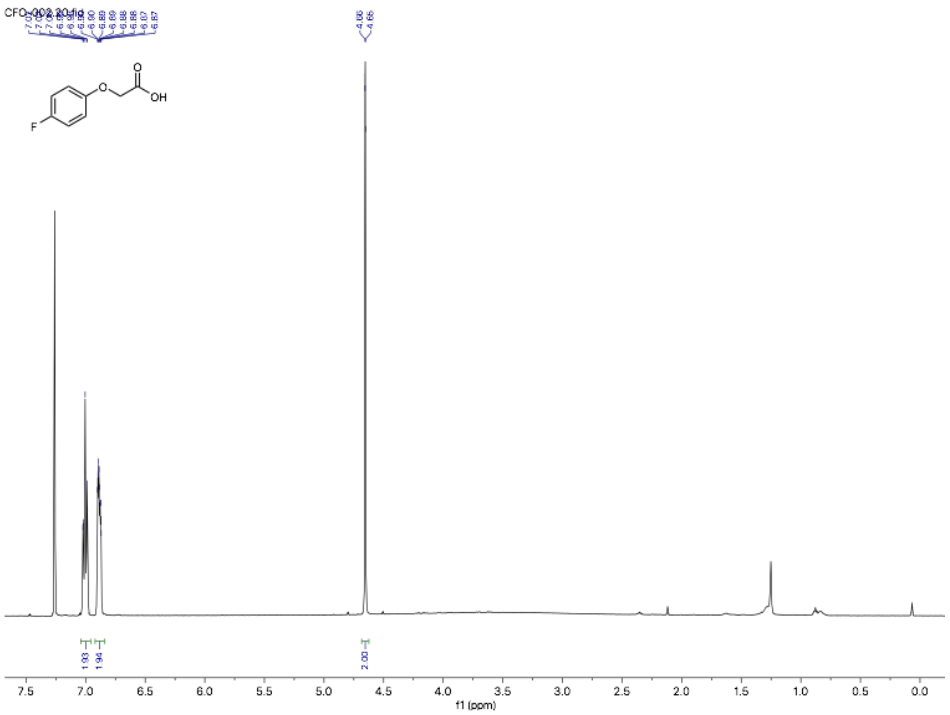
155.33
131.76
130.30
114.67
77.41
77.16
76.91
65.18
20.65
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)
O
OH

584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4.65
O
OH
F
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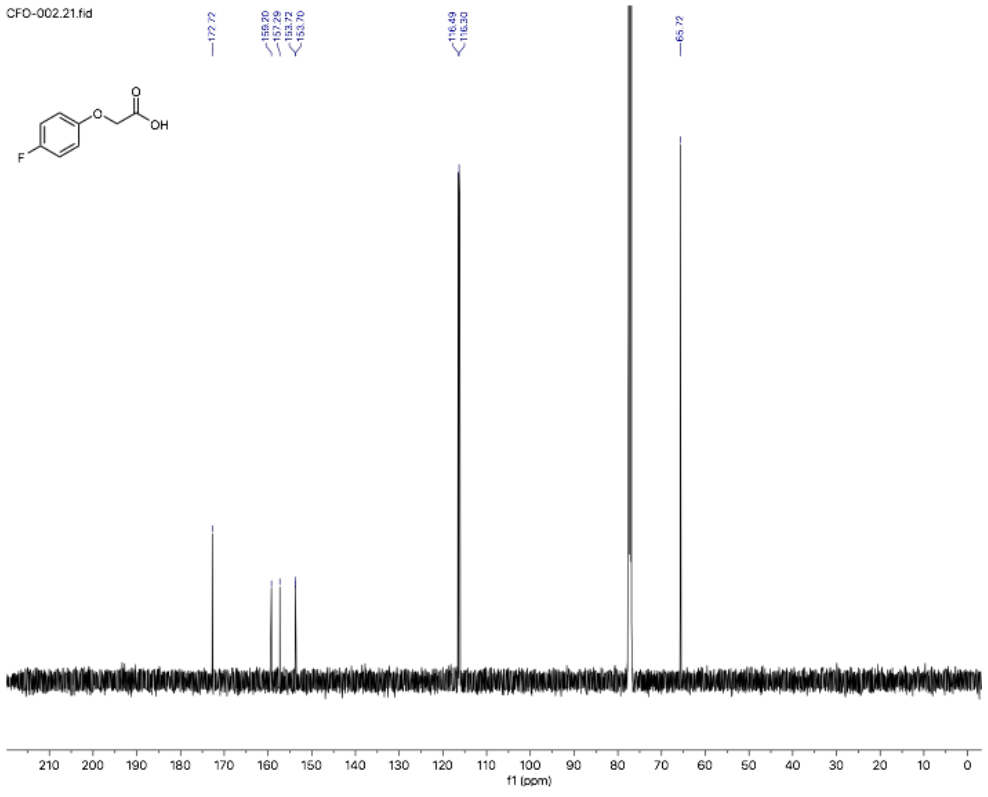
1.94
2.00
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

585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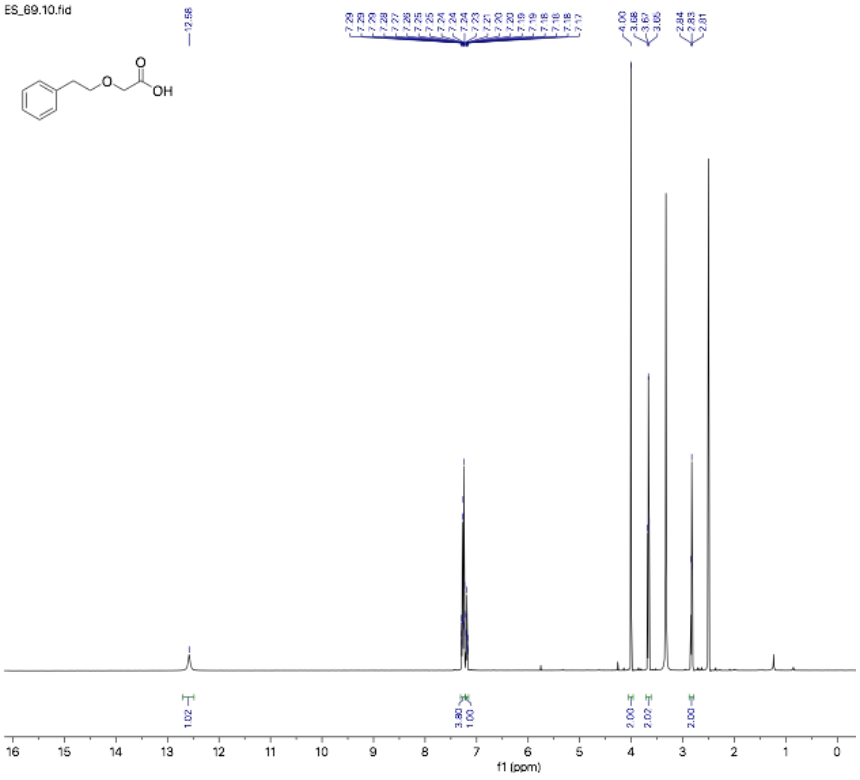
158.20
157.29
153.72
153.70
116.49
116.30
65.72
O
OH
F
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

586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7.29
7.28
7.27
7.26
7.25
7.25
7.24
7.24
7.24
7.23
7.21
7.20
7.20
7.19
7.19
7.18
7.16
7.16
7.15
7.17
4.00
3.88
3.87
3.85
2.84
2.83
2.81
O
O
OH
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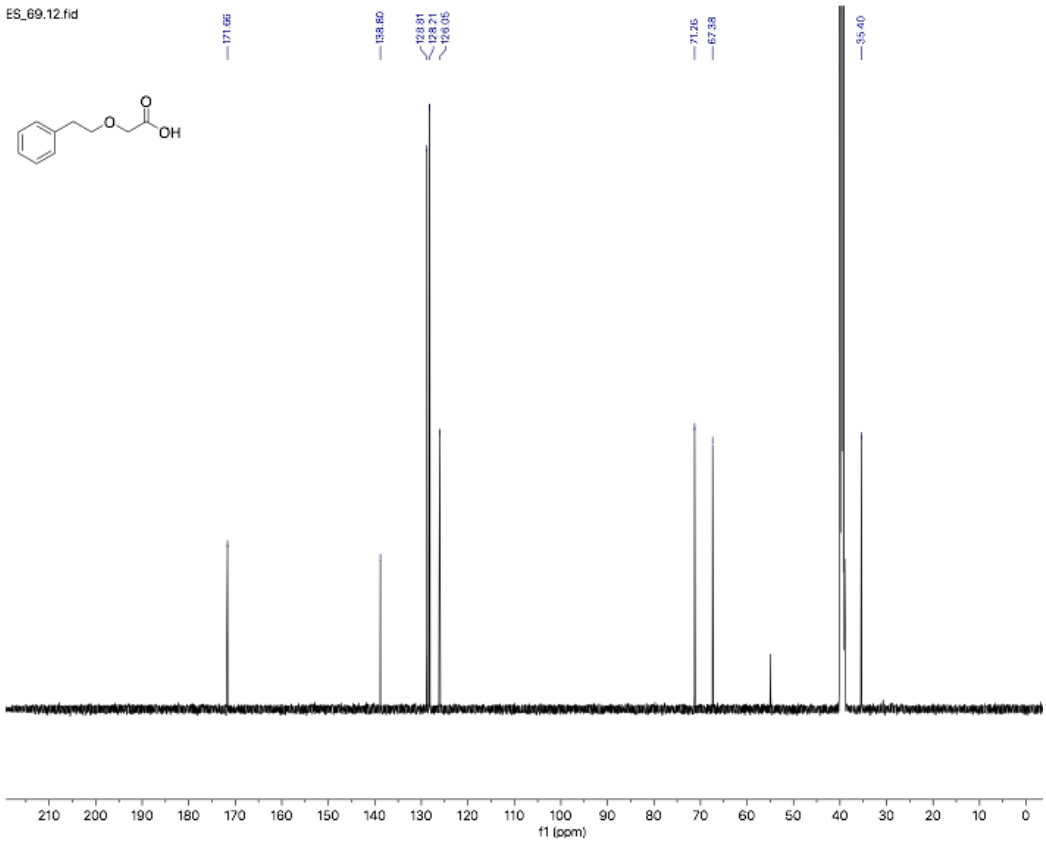
1.00
2.00
2.02
2.00
16 15 14 13 12 11 10 9 8 7 6 5 4 3 2 1 0
f1 (ppm)

587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129.01
128.21
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71.26
67.38
35.40
O
O
OH
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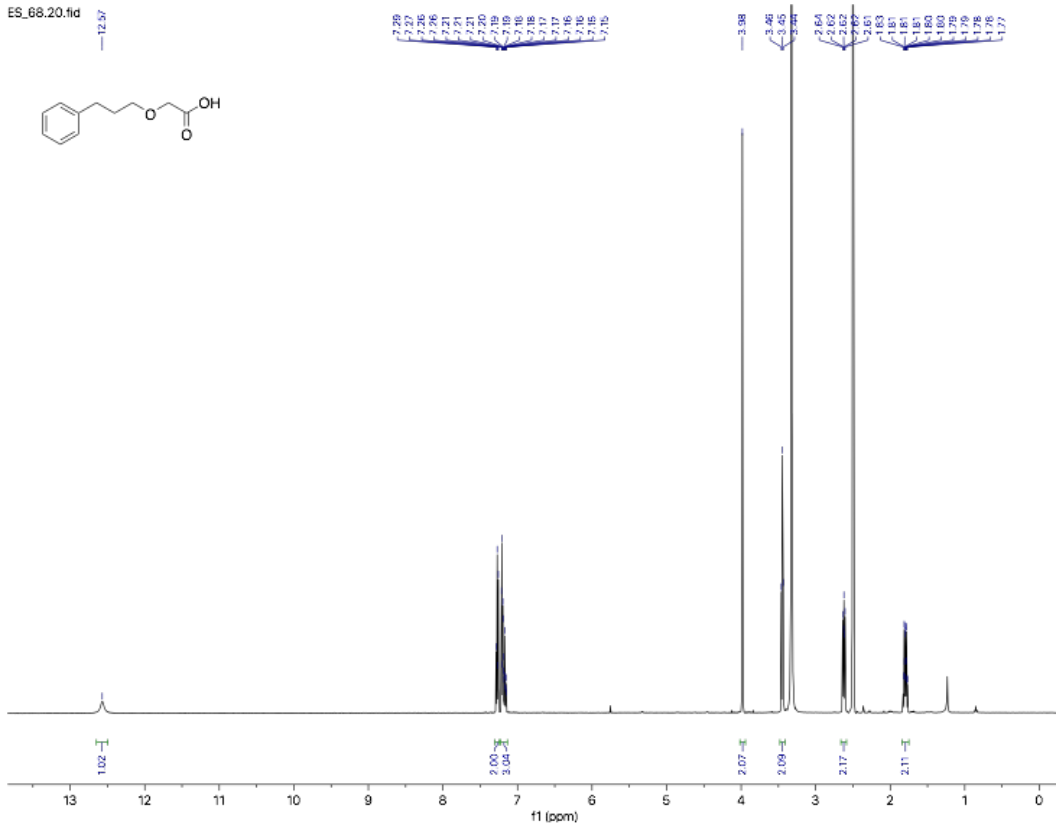
f1 (ppm)

588



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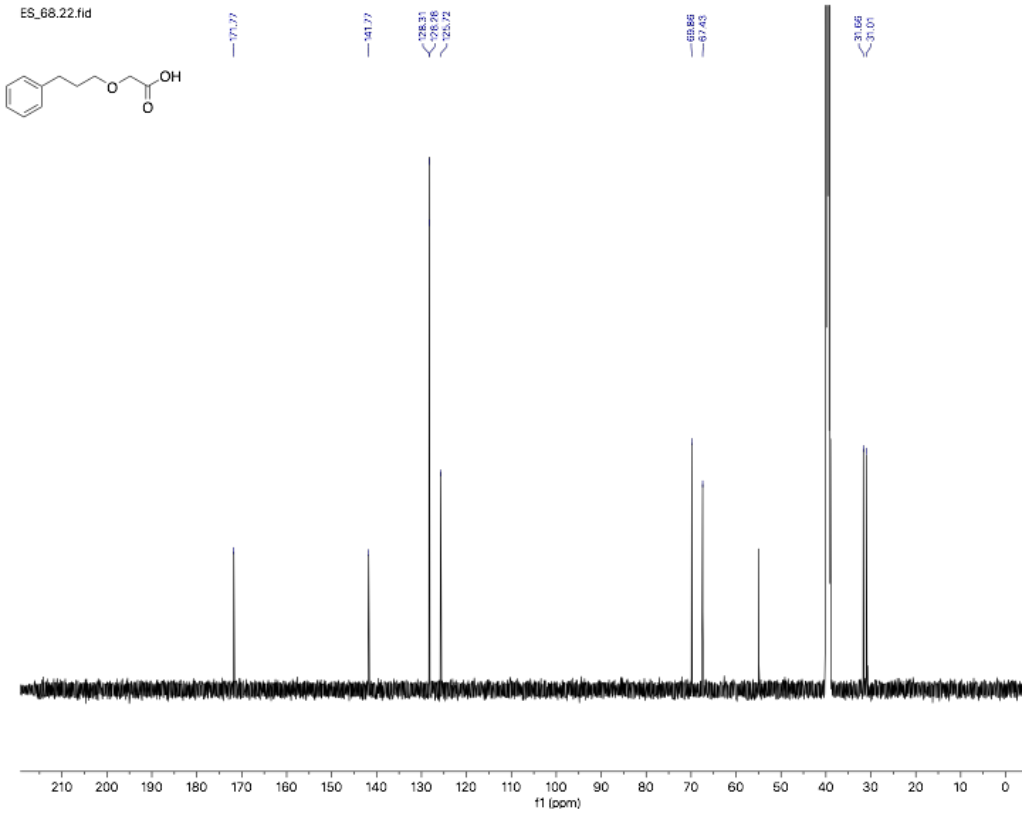
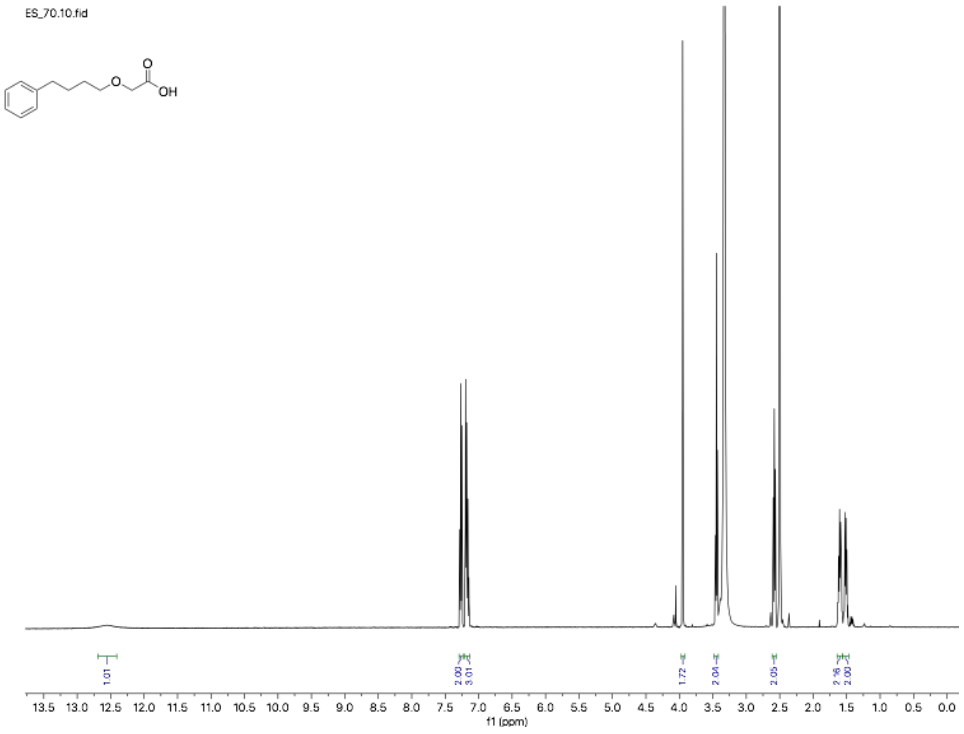


590

591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O
OH
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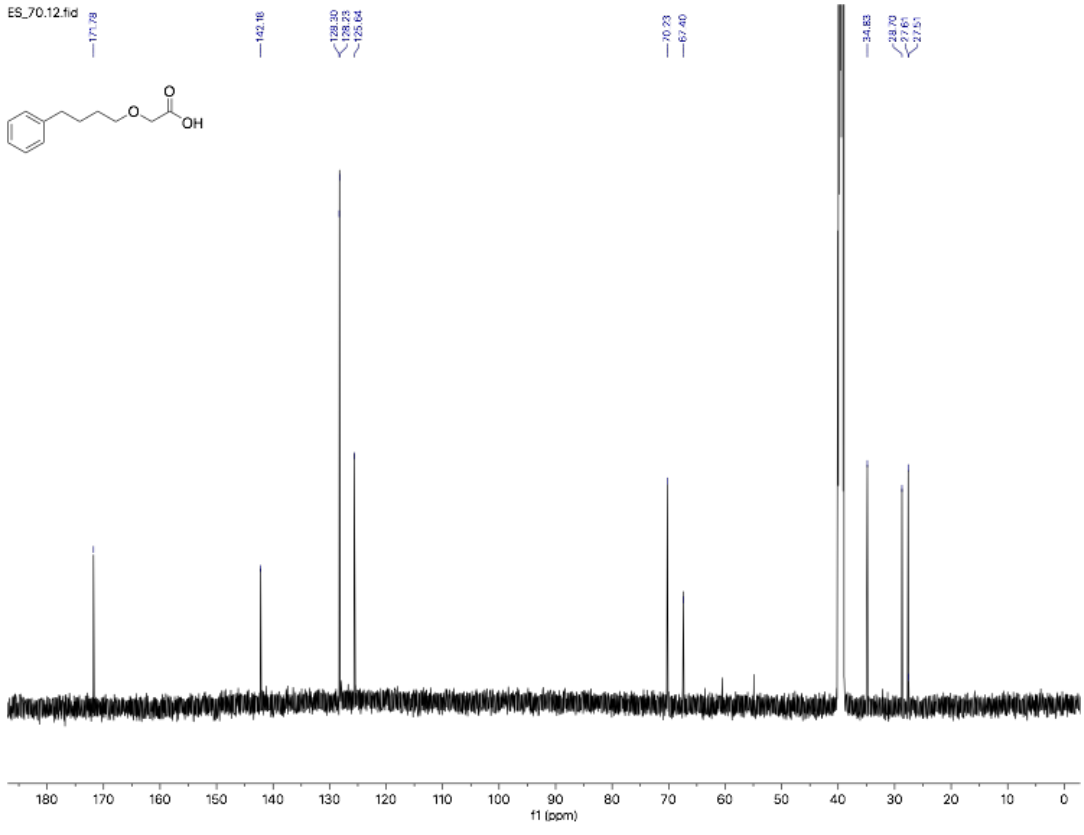
2.00
3.01
1.72
2.04
2.05
2.16
2.00
13.5 13.0 12.5 12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

592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O
O
OH
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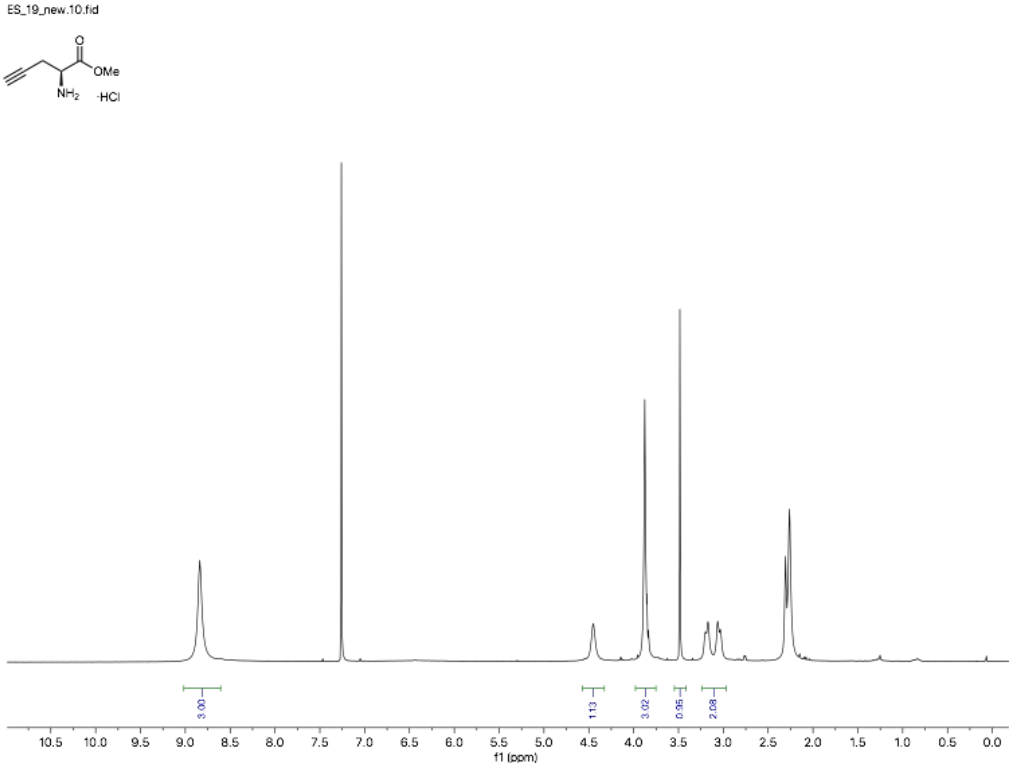
f1 (ppm)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OMe
NH2 ·HCl
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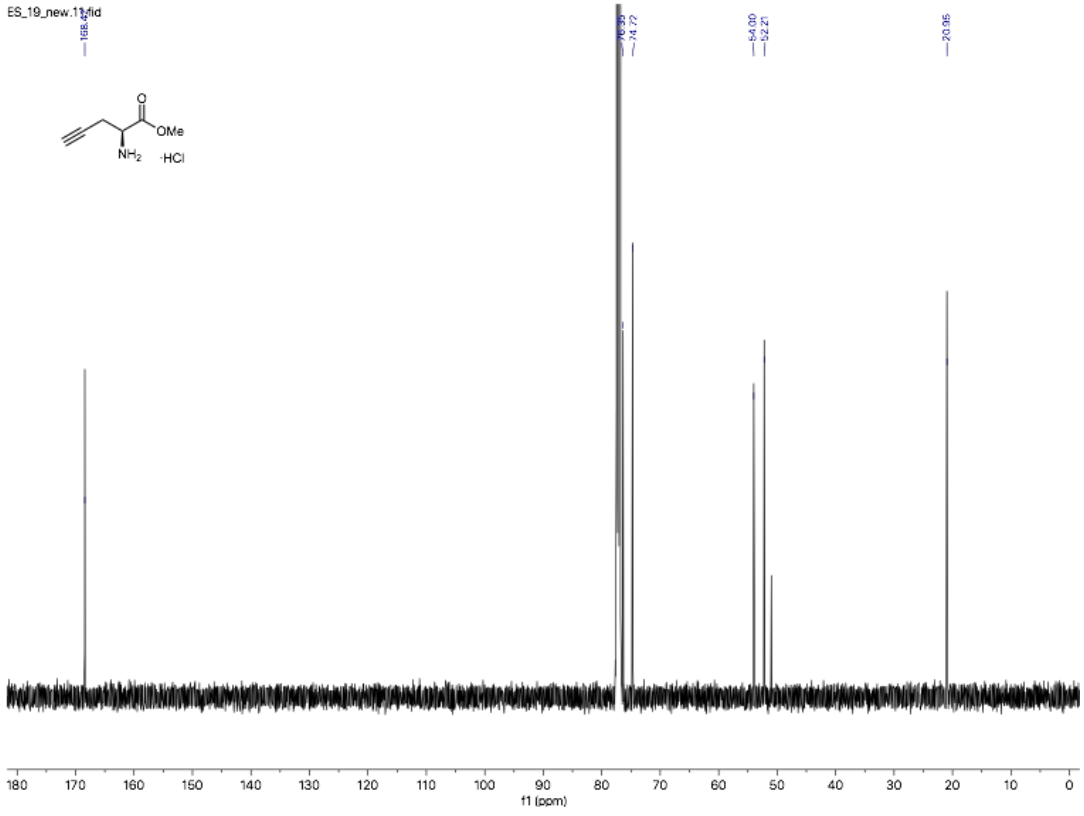
0.95
2.08
f1 (ppm)

593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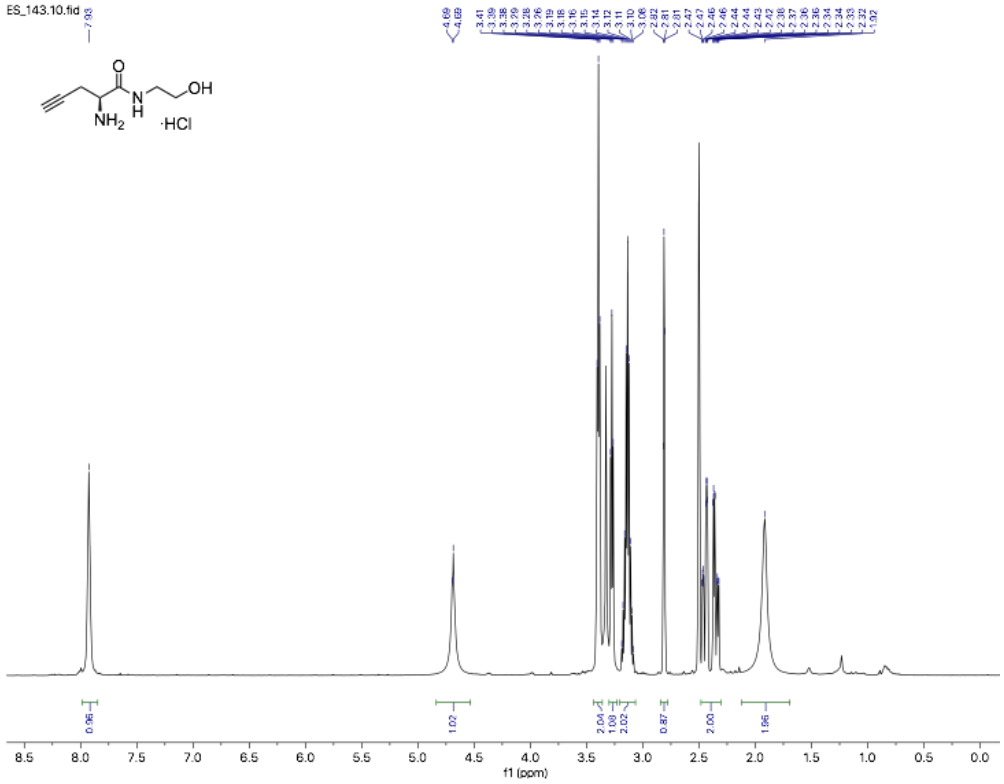
OMe
NH2 ·HCl
f1 (ppm)

594



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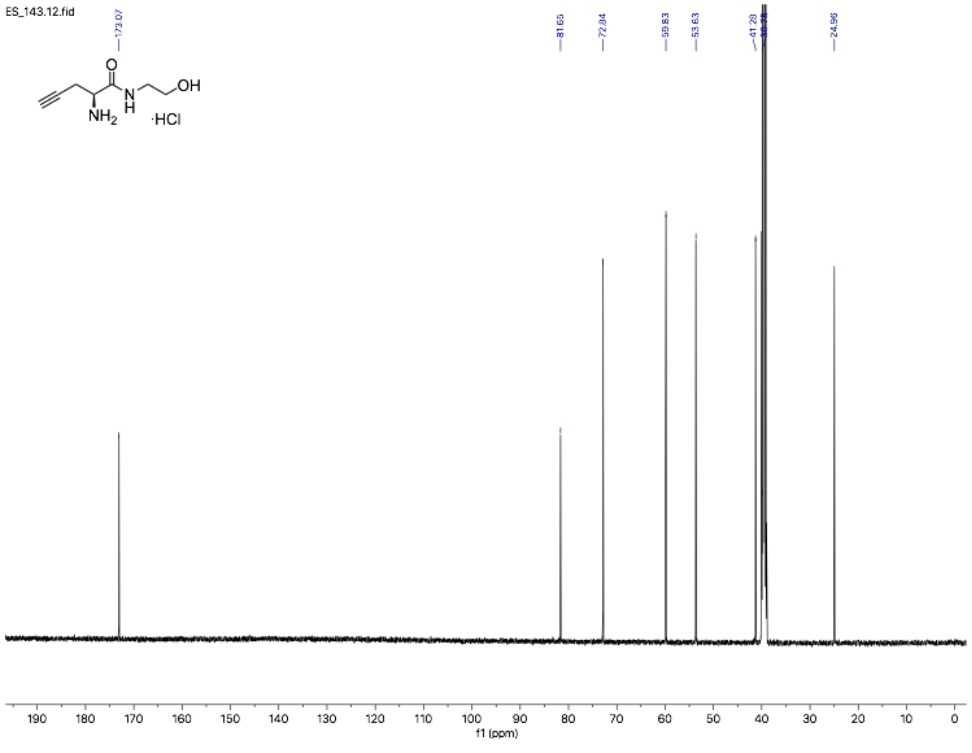

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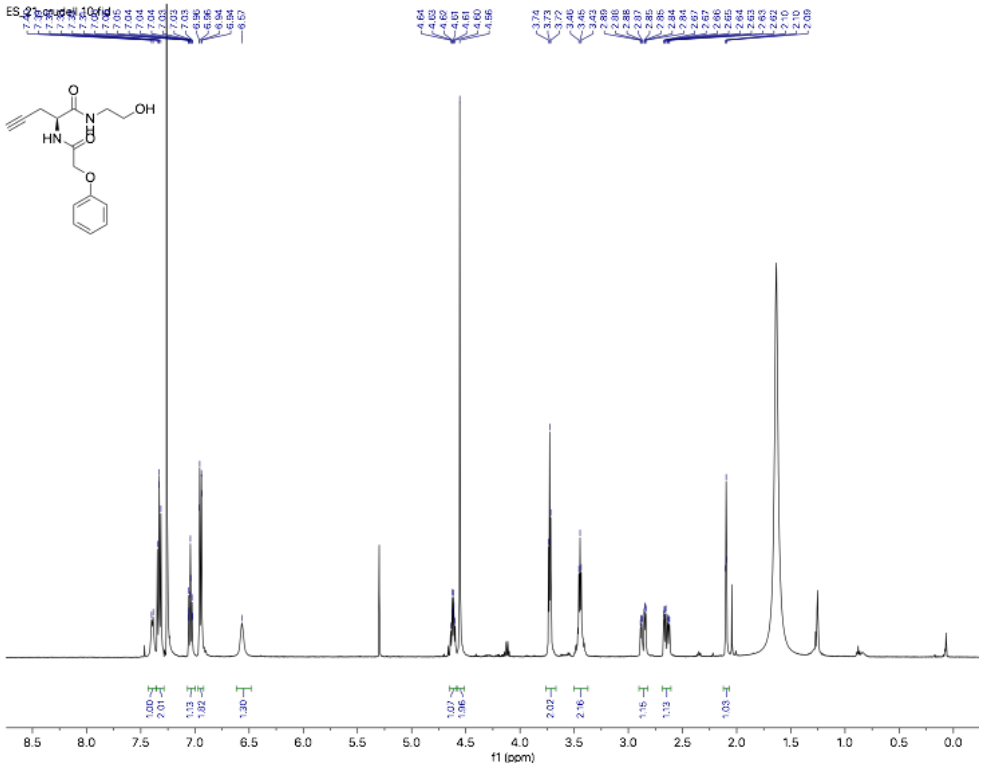
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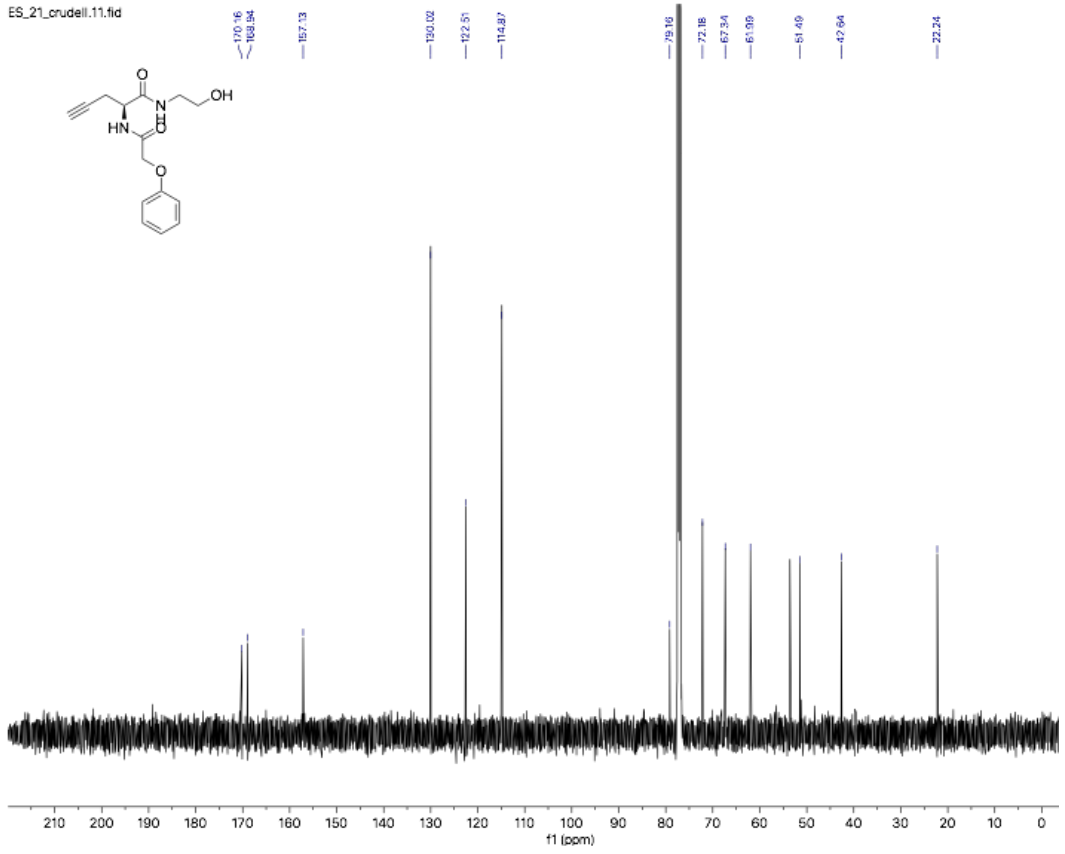
OH
HN
O
N
f1 (ppm)

597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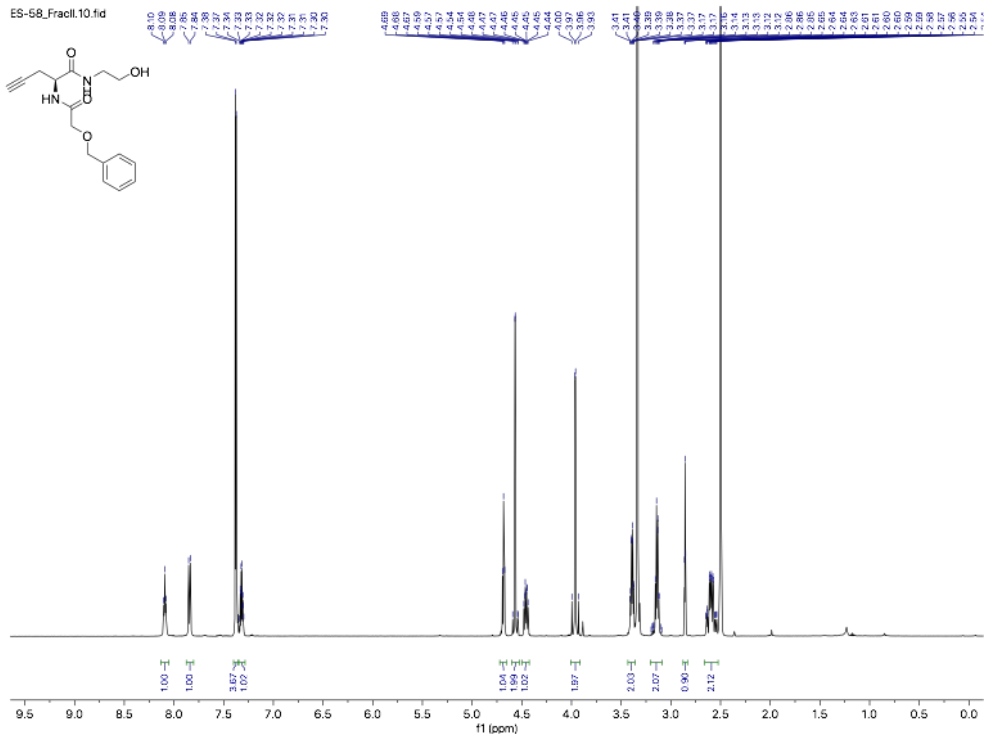
OH
HN
O
N
f1 (ppm)

598



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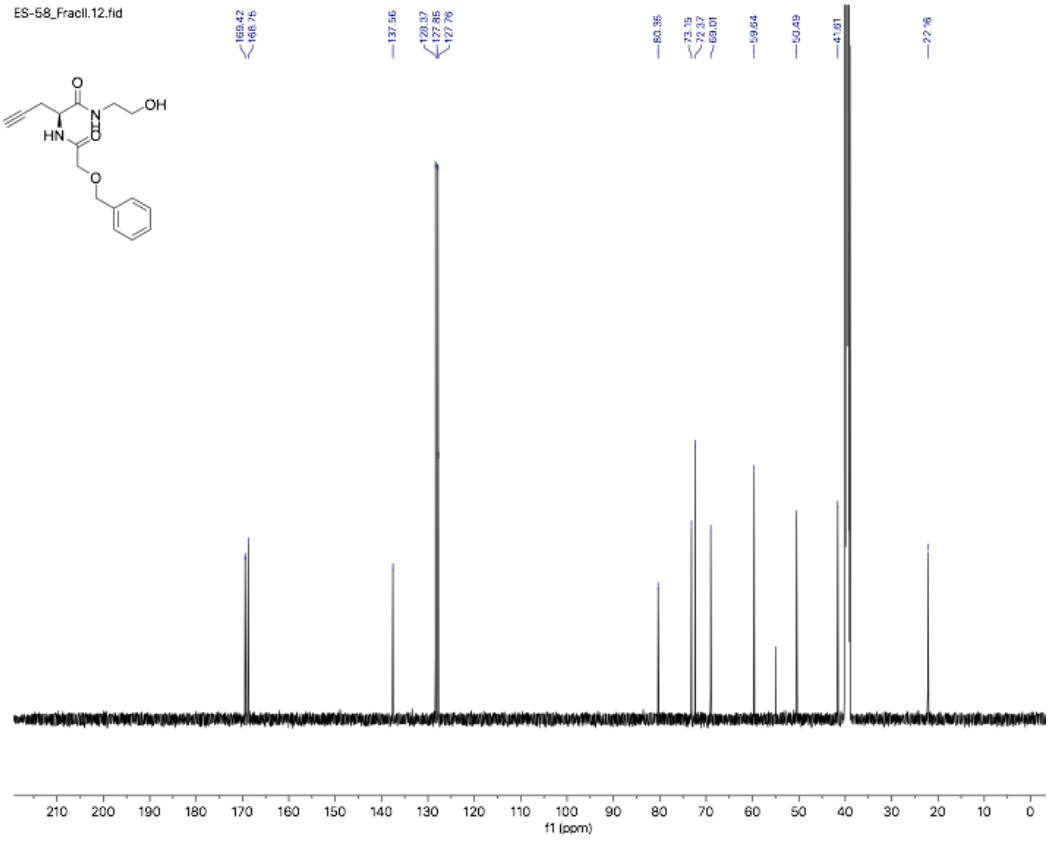

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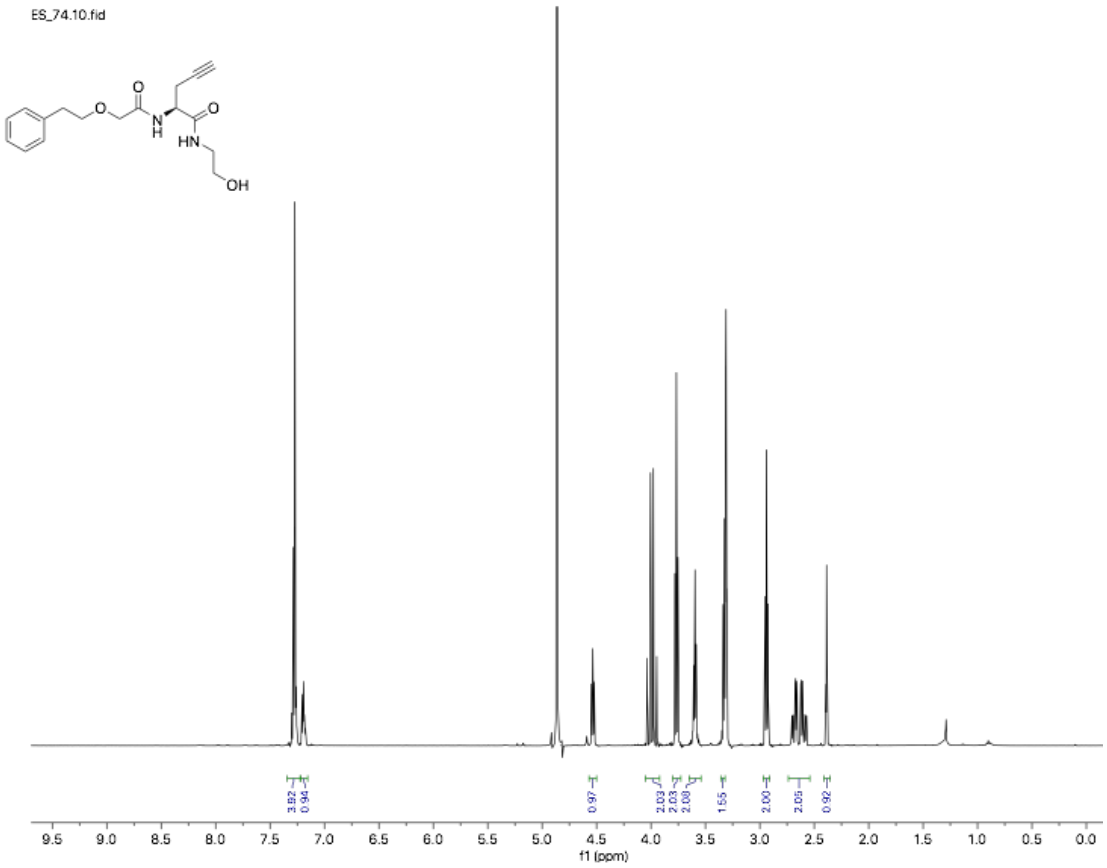
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O
N
H
O
HN
OH
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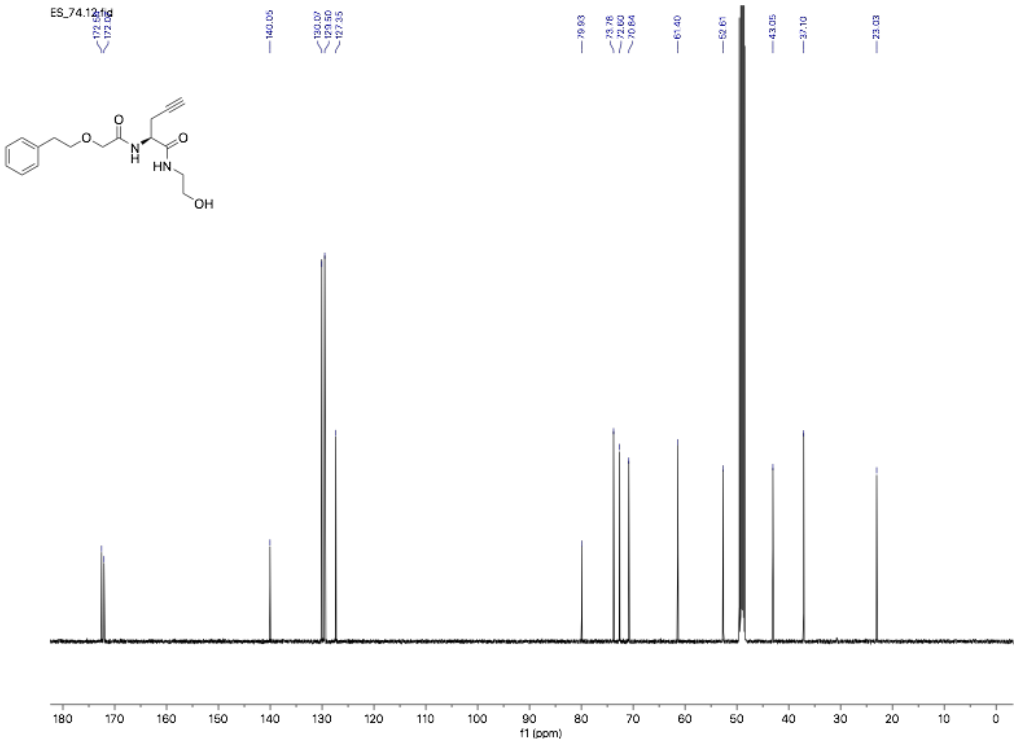
0.94
0.97
2.03
2.03
2.08
1.55
2.00
2.05
0.92
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

601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73.16
72.83
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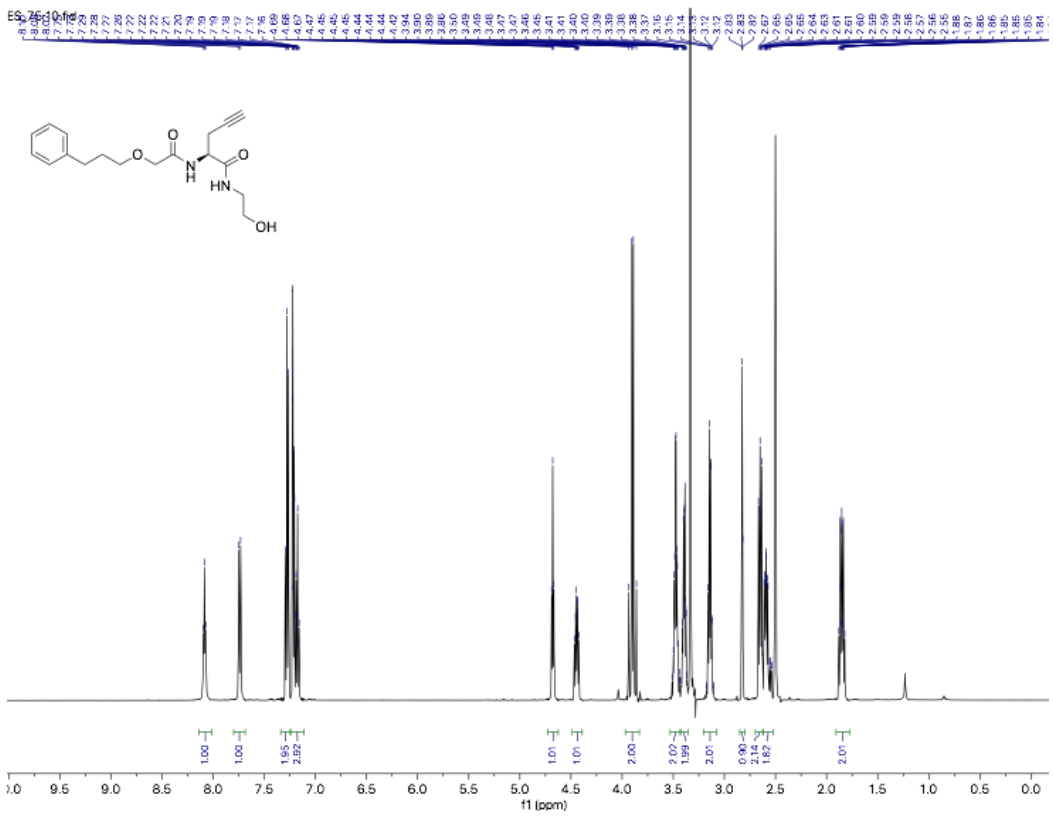
61.40
52.61
43.05
37.10
23.03
O
O
N
H
O
HN
OH
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

602



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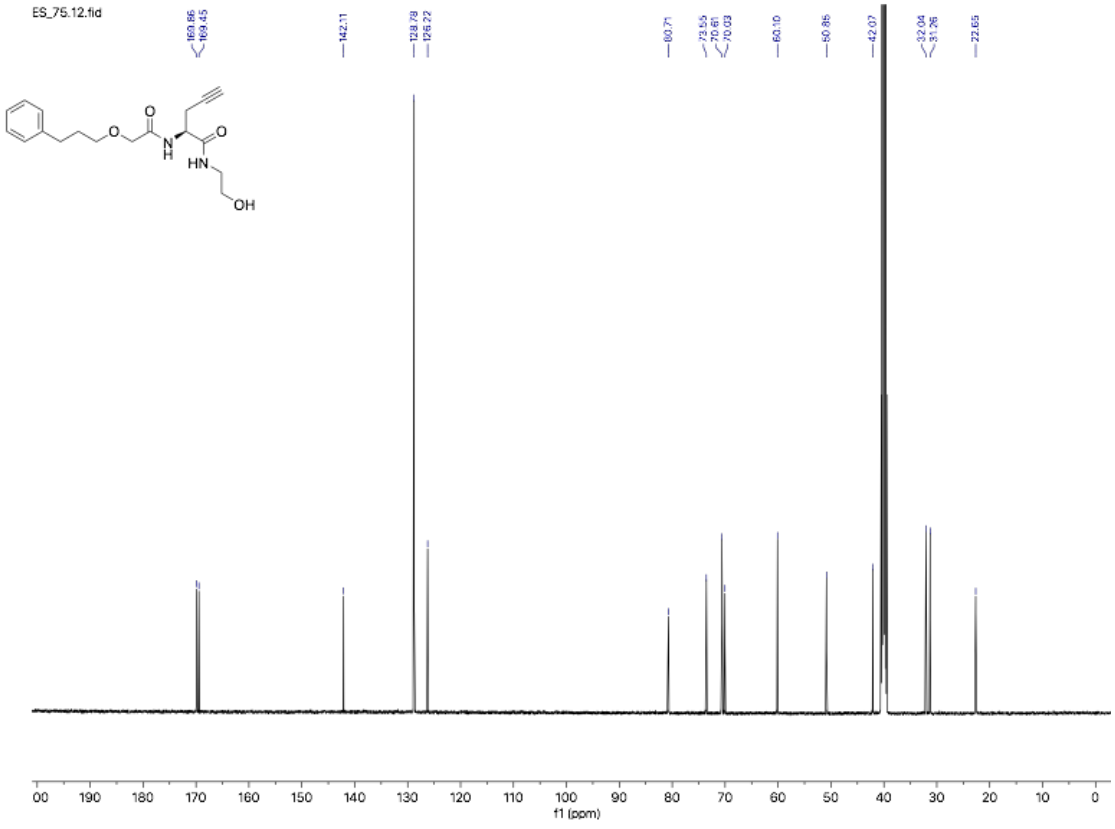

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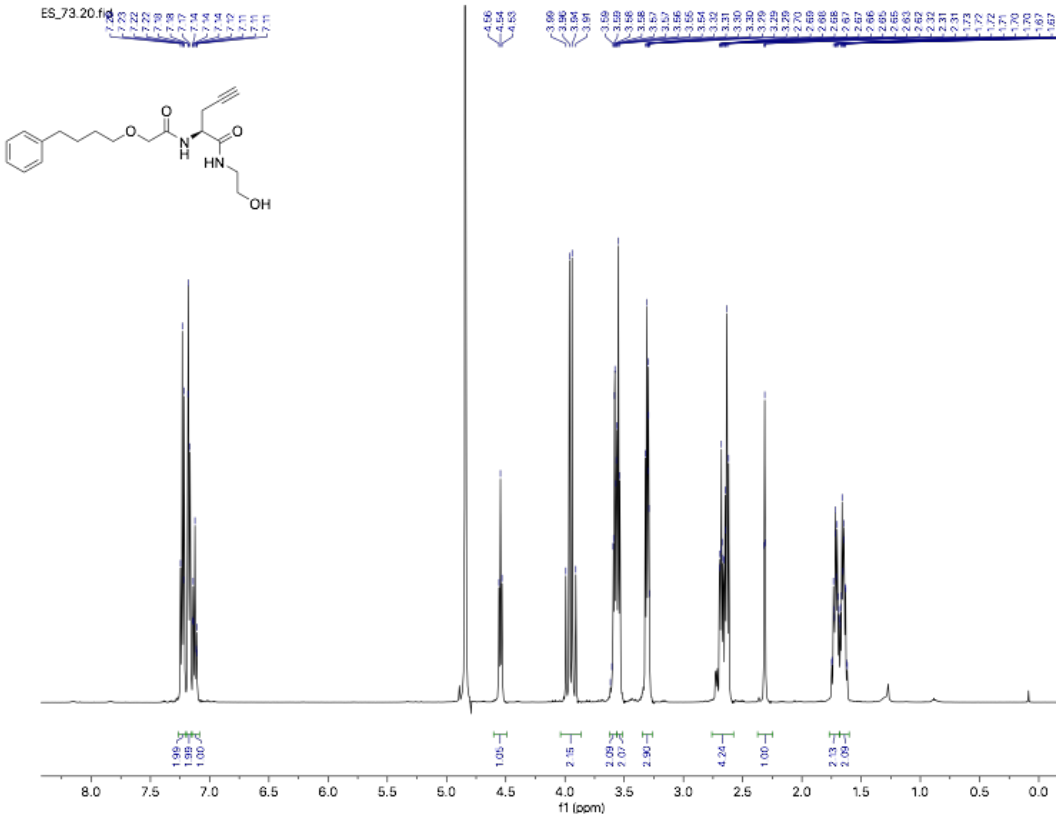
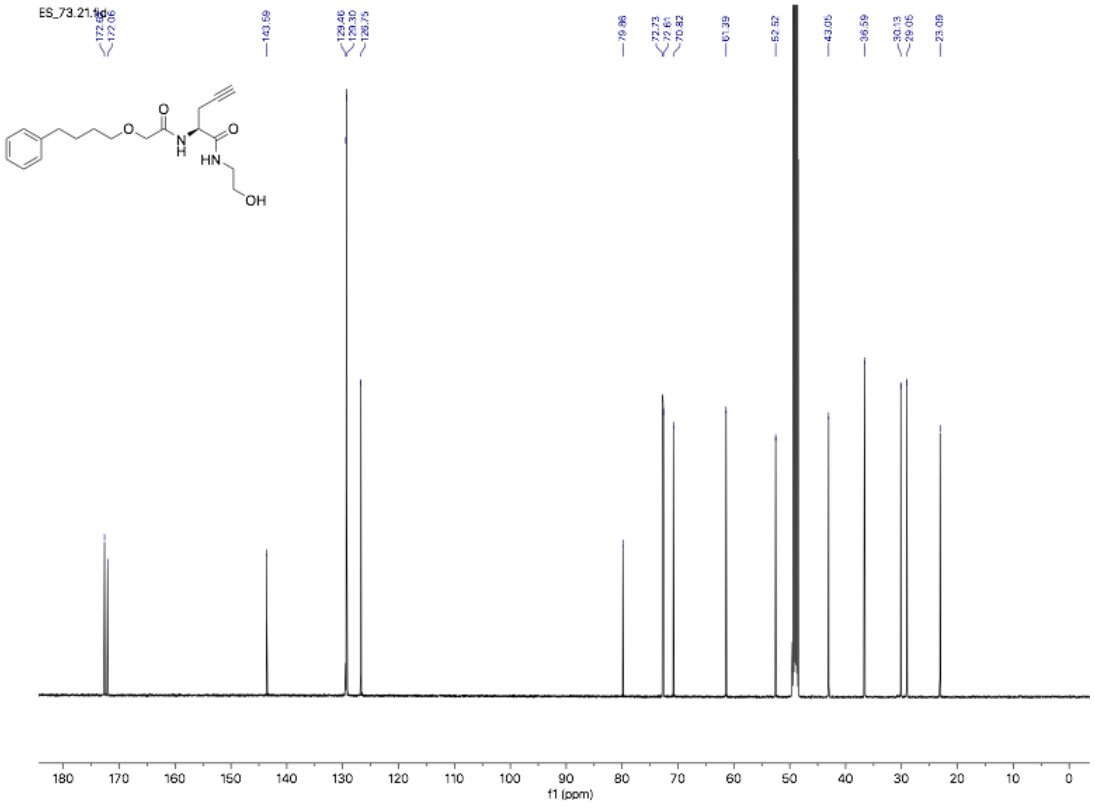
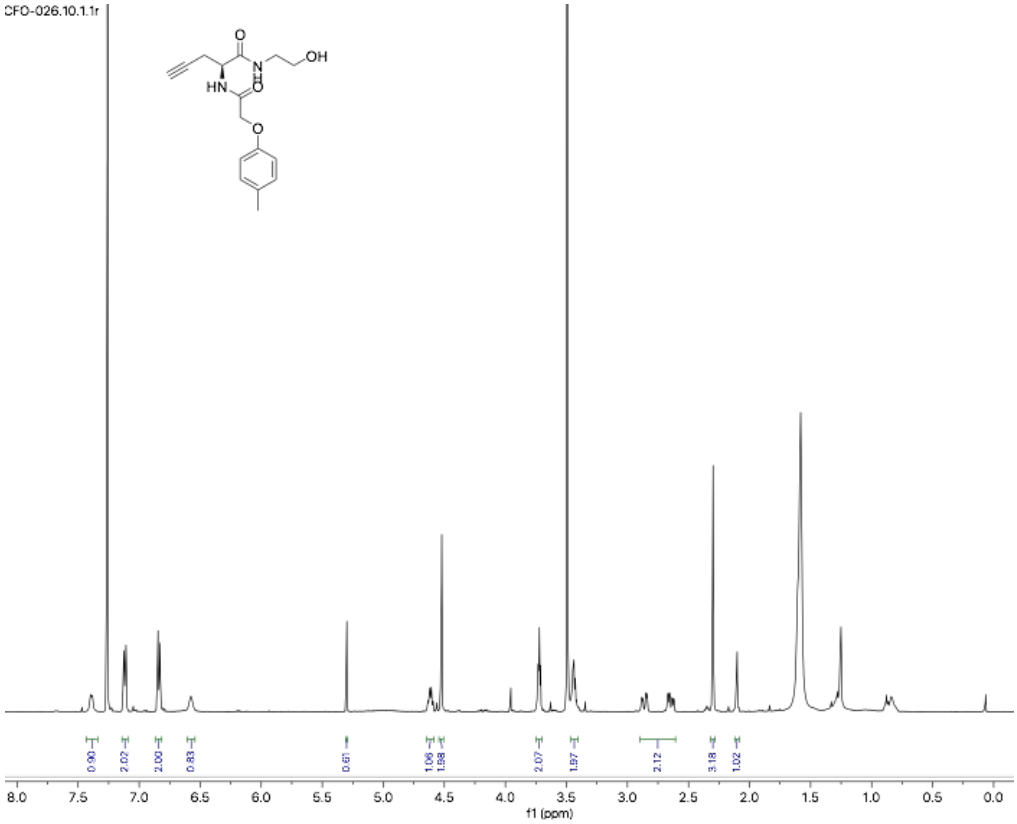


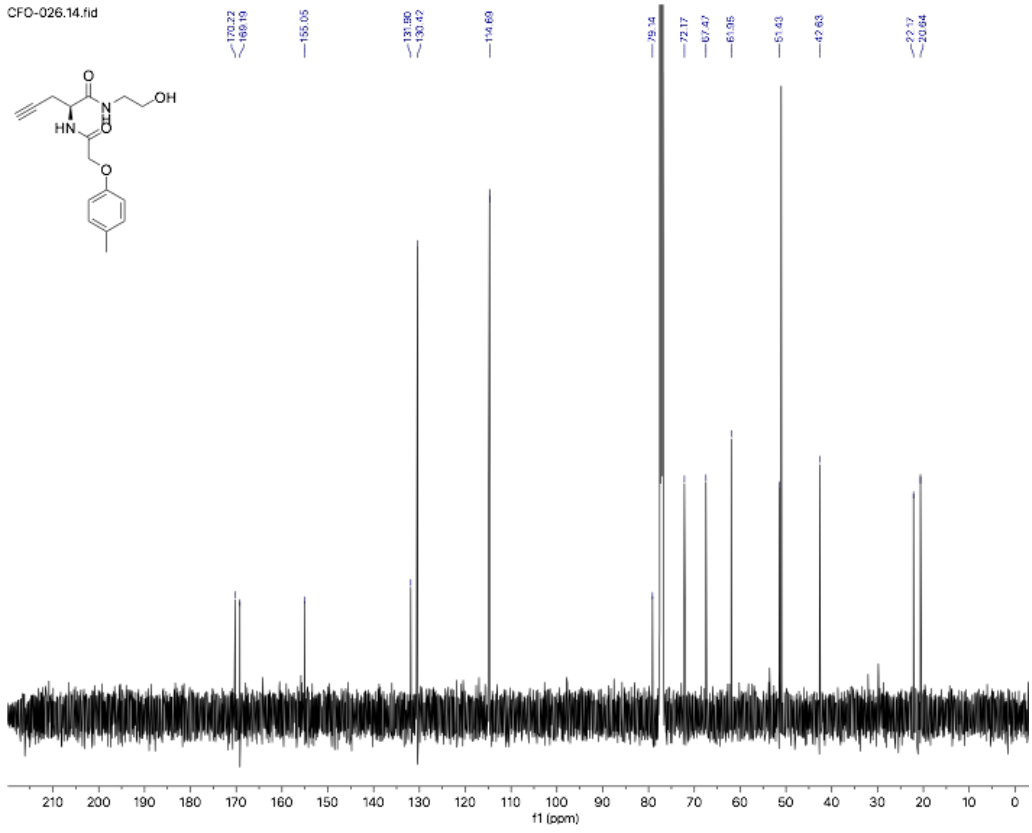
605



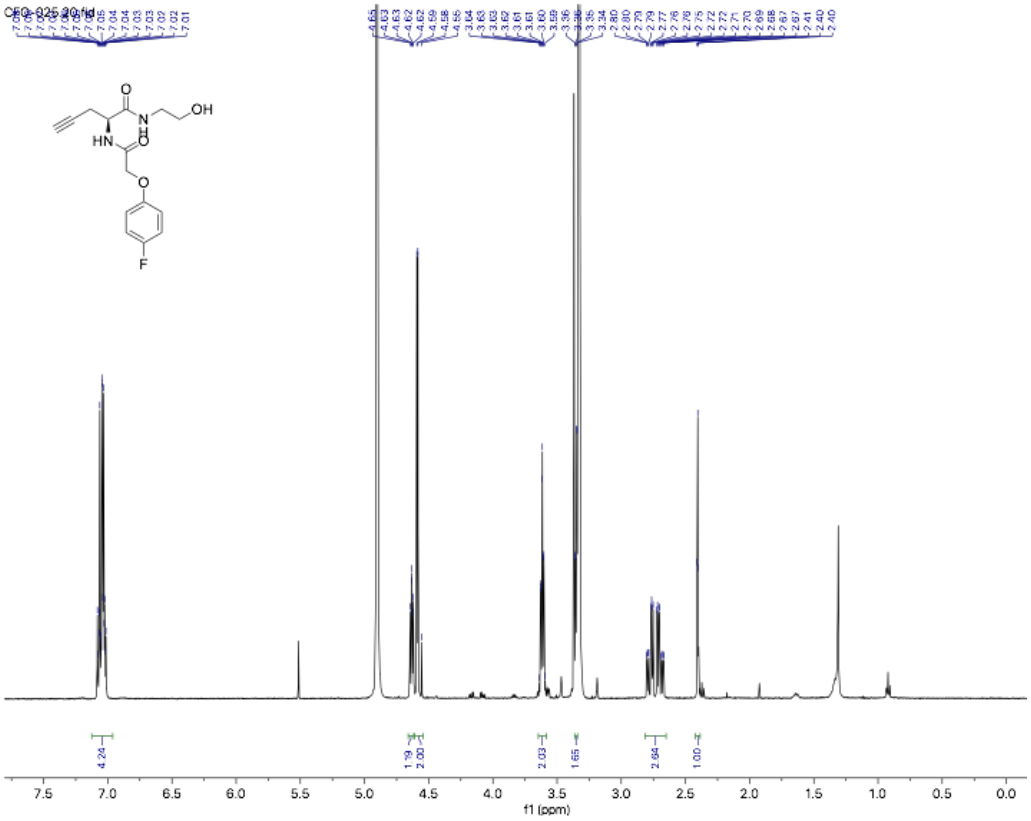
606



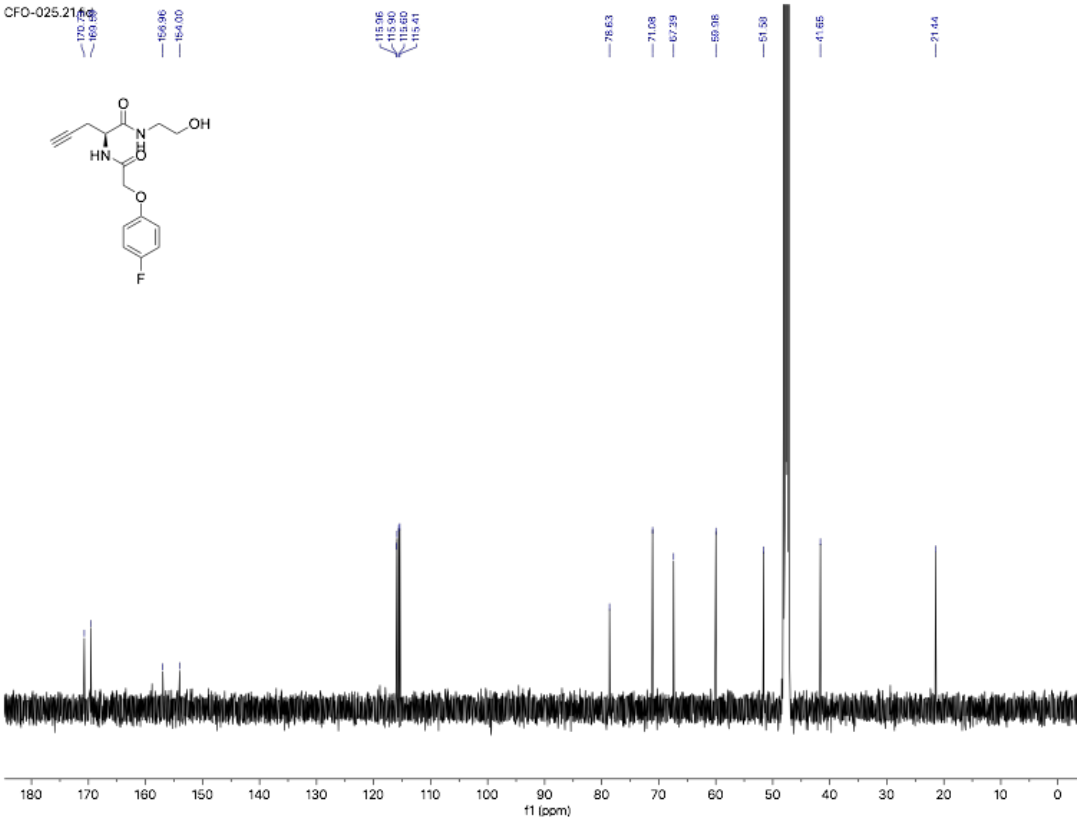
607



608

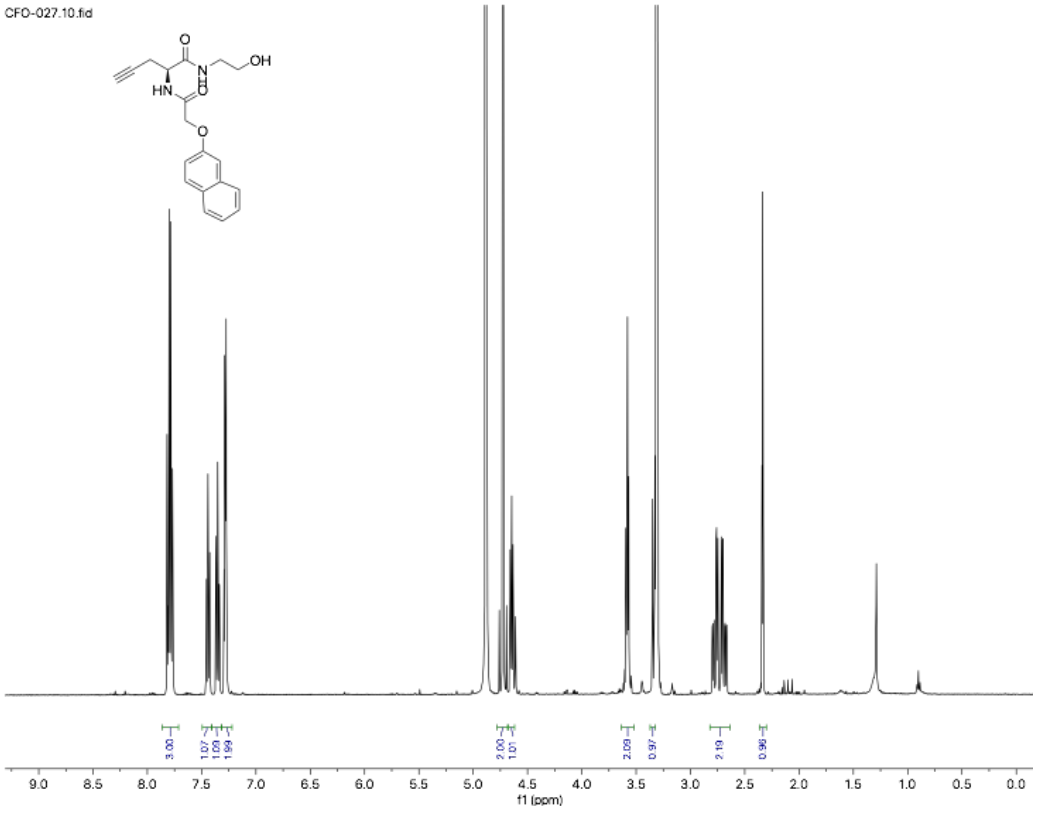

CFO-025.20.fid
OH
HN
O
F
4.24
1.19
2.00
2.03
1.65
2.84
1.00
f1 (ppm)

609

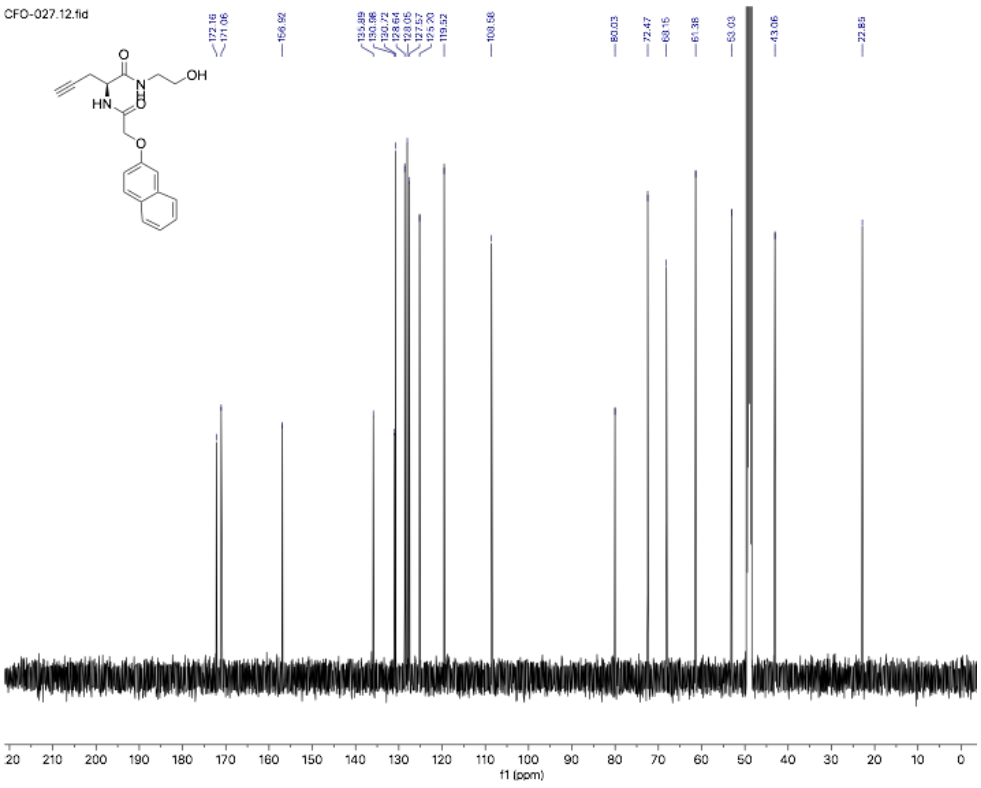

CFO-025.21.fid
170.73
169.98
159.96
154.00
115.96
115.90
115.60
115.41
78.63
71.08
67.39
59.98
51.59
41.65
21.44
OH
HN
O
F
f1 (ppm)

610

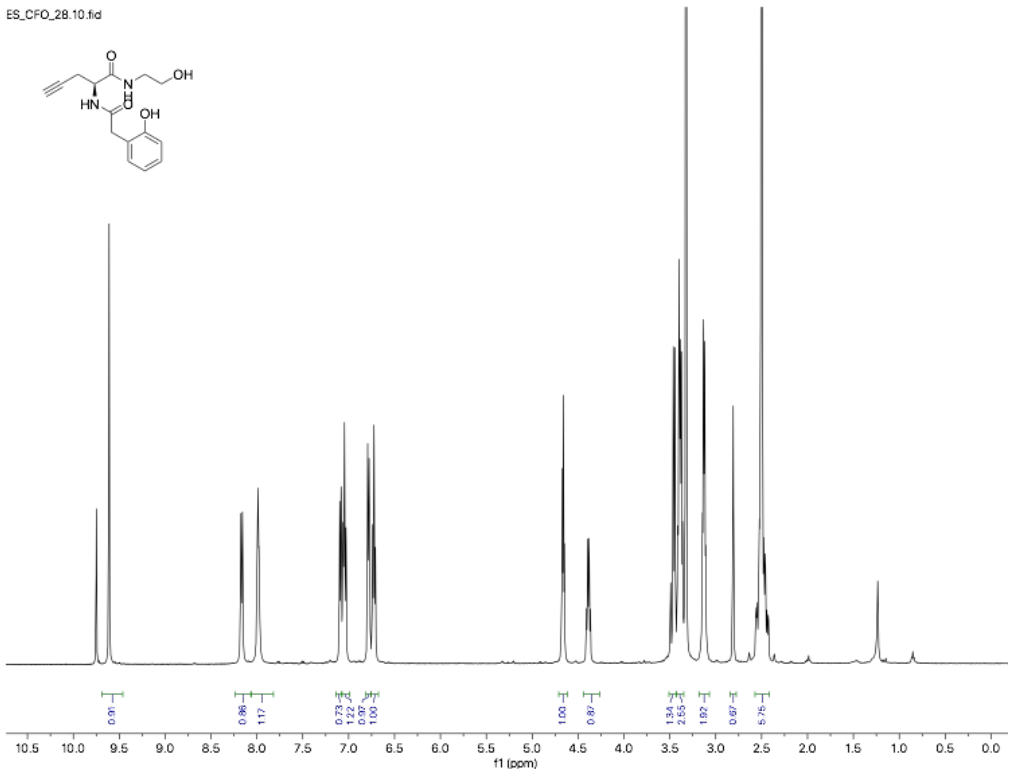
611



612



613


ES_CFO_28.10.fid
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N
OH
HN
O
OH
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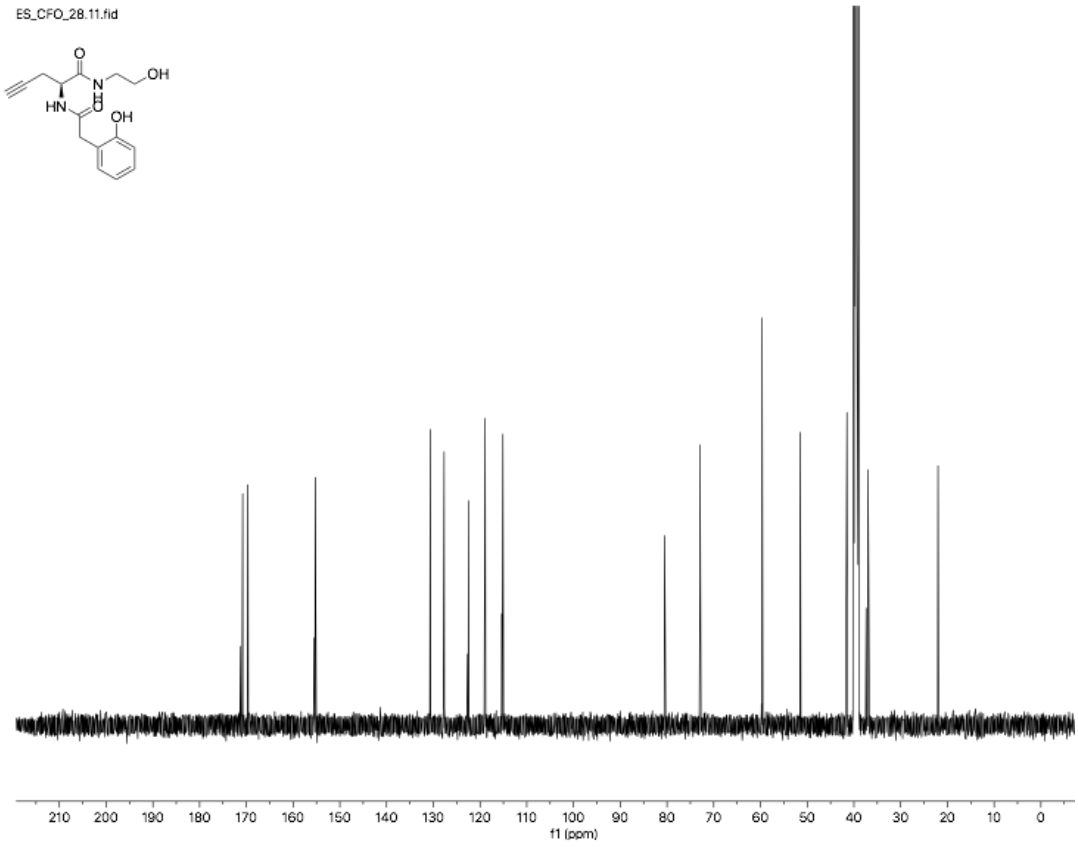
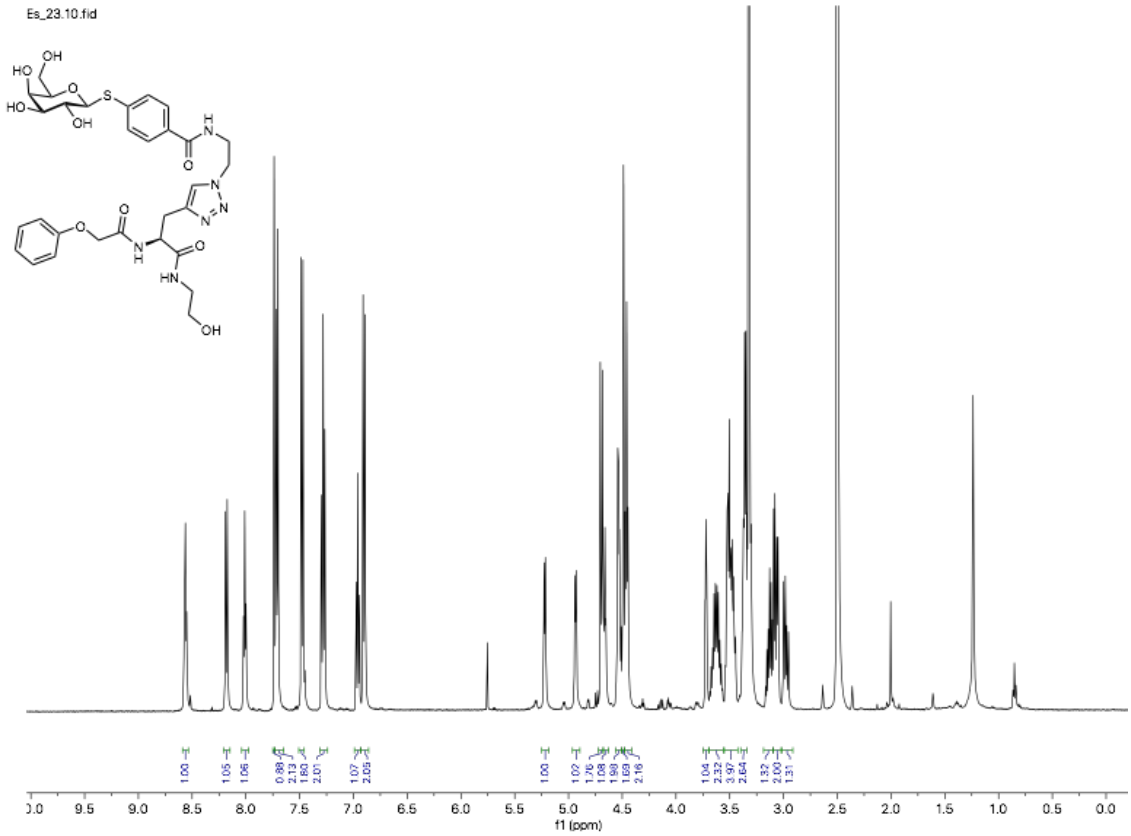
0.86
1.17
0.73
1.22
0.97
1.00
1.00
0.87
1.34
2.55
1.92
0.67
5.75
10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

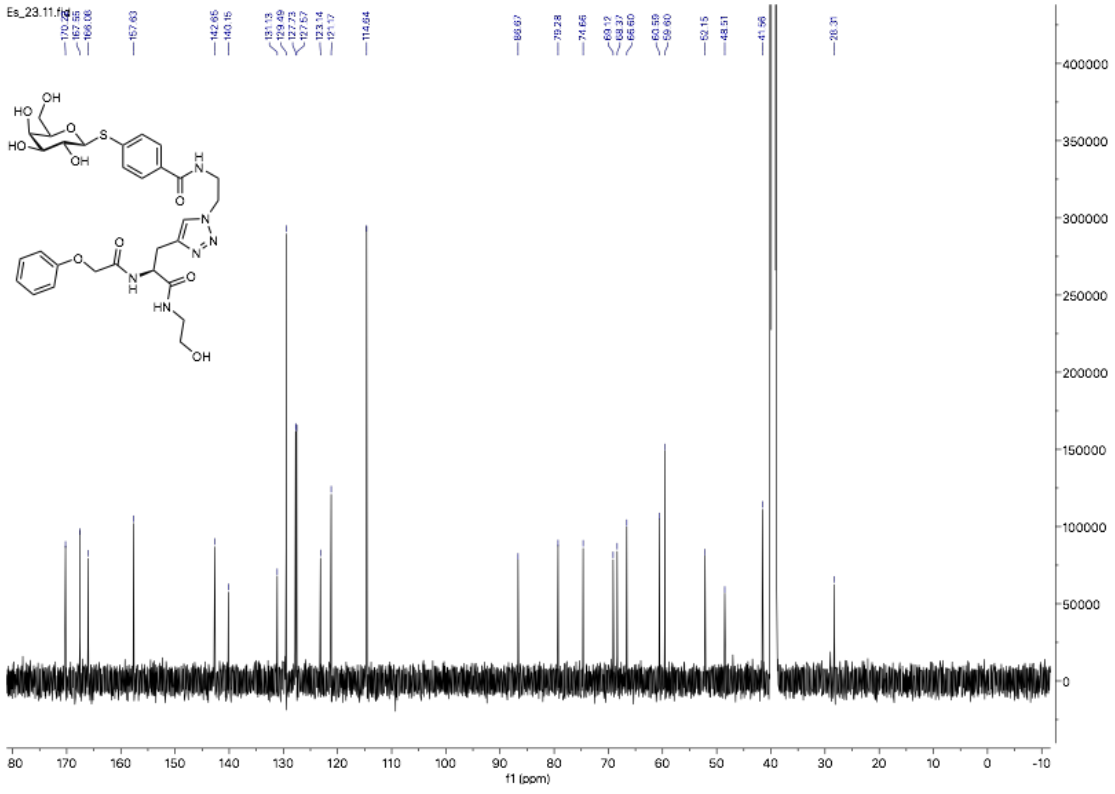
614


ES_CFO_28.11.fid
O
N
OH
HN
O
OH
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

615

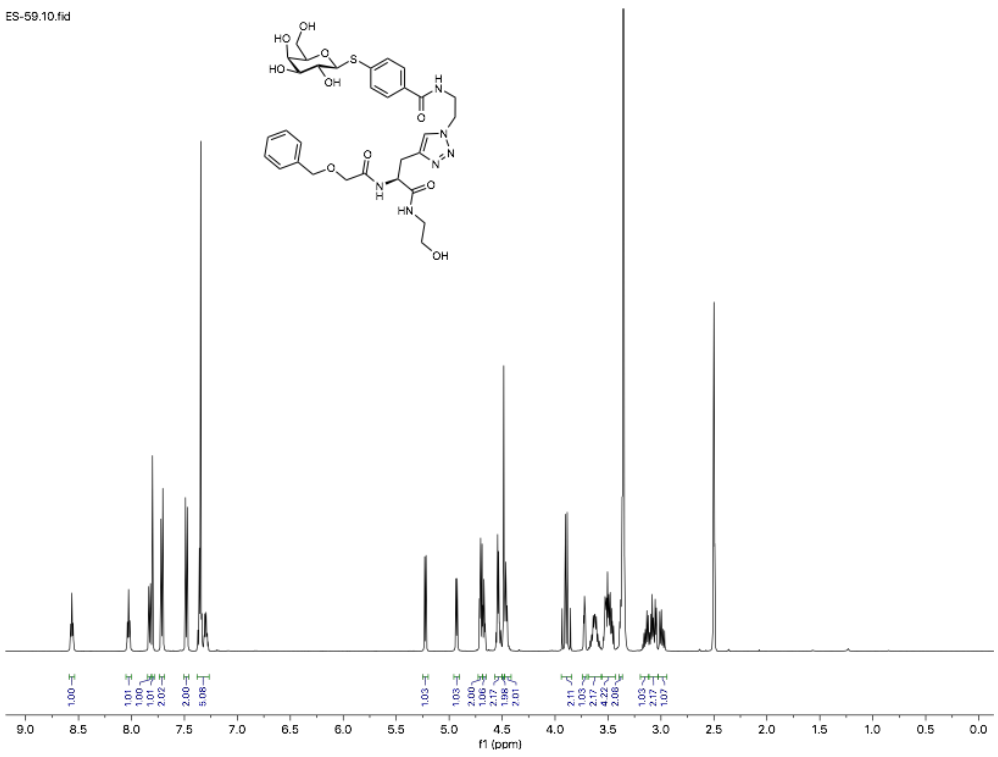


616

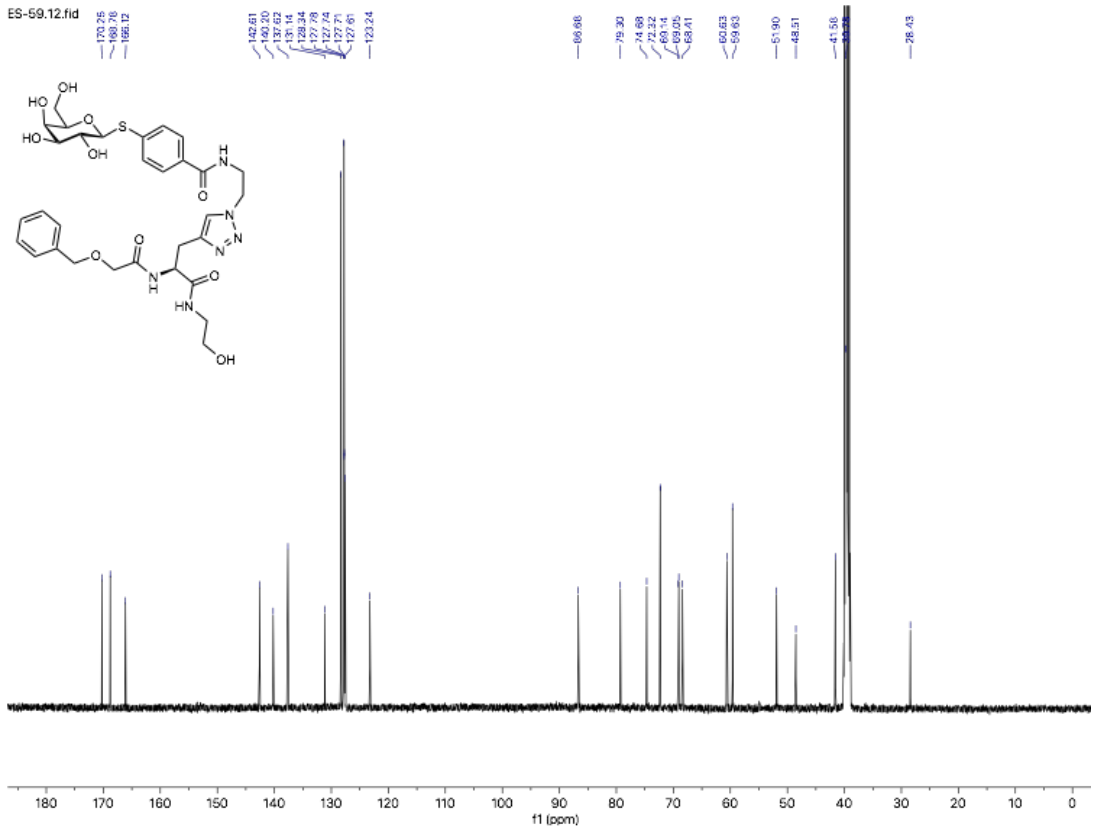


617

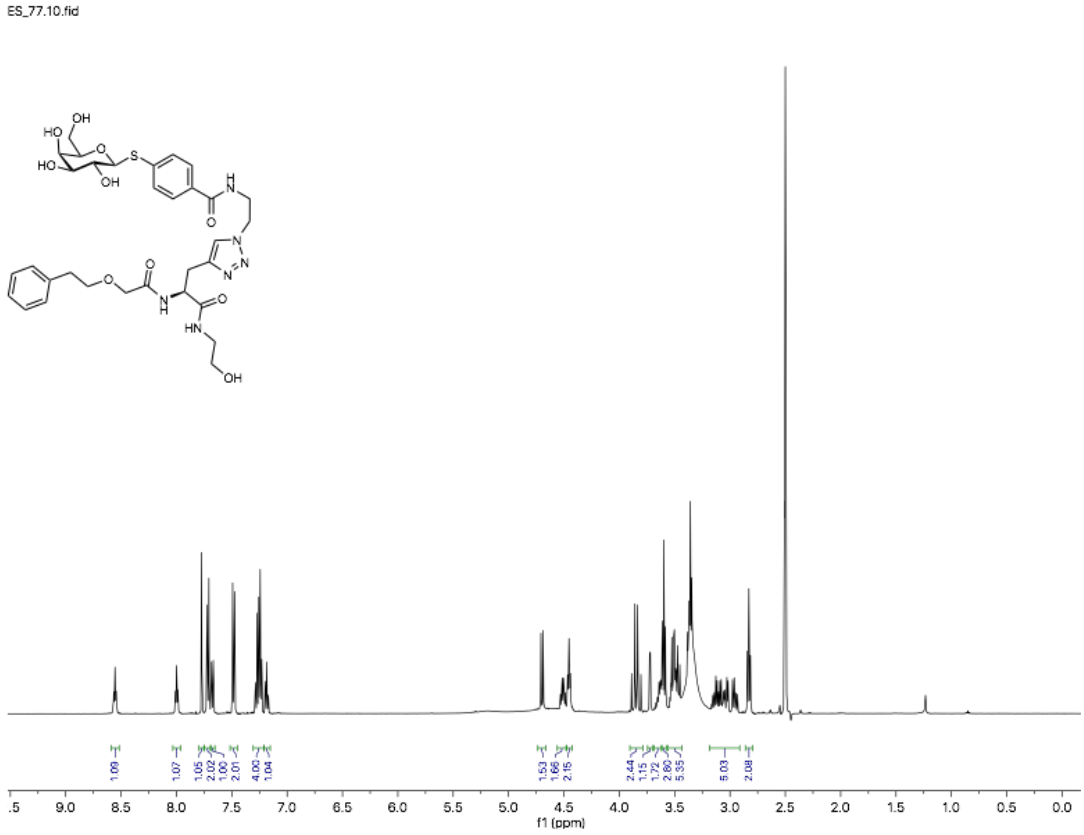
618


ES-59.10.fid
f1 (ppm)

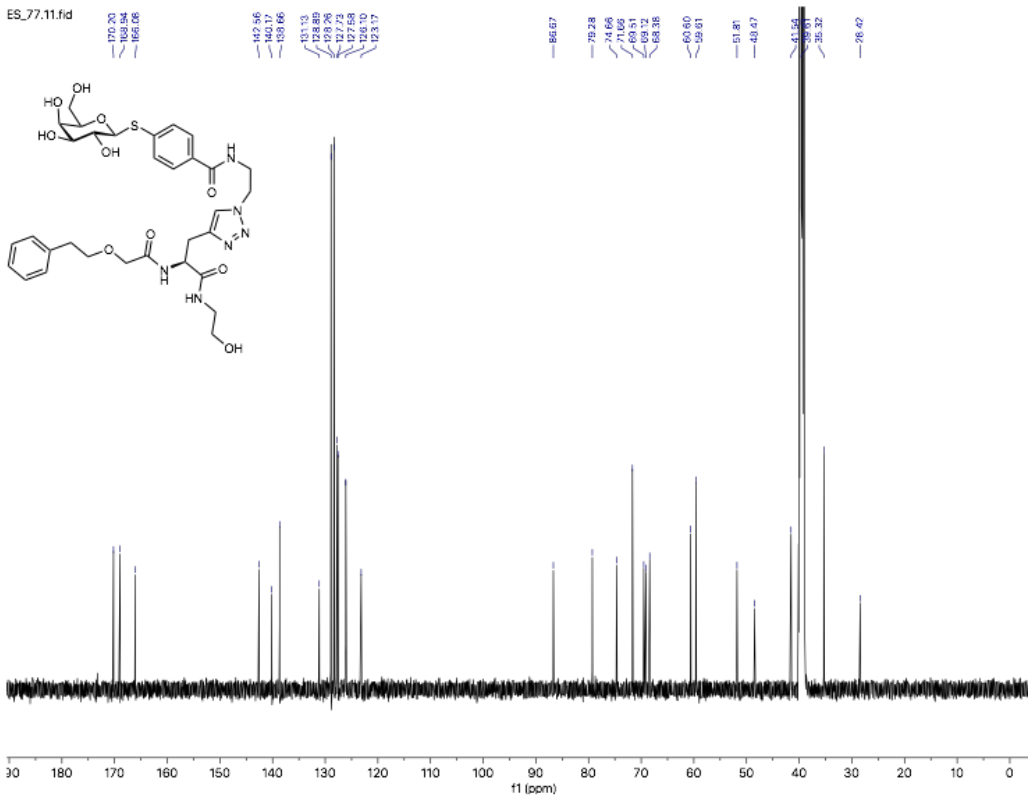
619


ES-59.12.fid
170.25
169.78
166.12
142.61
140.20
137.62
131.14
129.34
127.78
127.74
127.71
127.61
123.24
66.66
79.30
74.69
72.32
69.14
68.75
63.41
60.63
59.63
51.90
48.51
41.56
28.43
f1 (ppm)

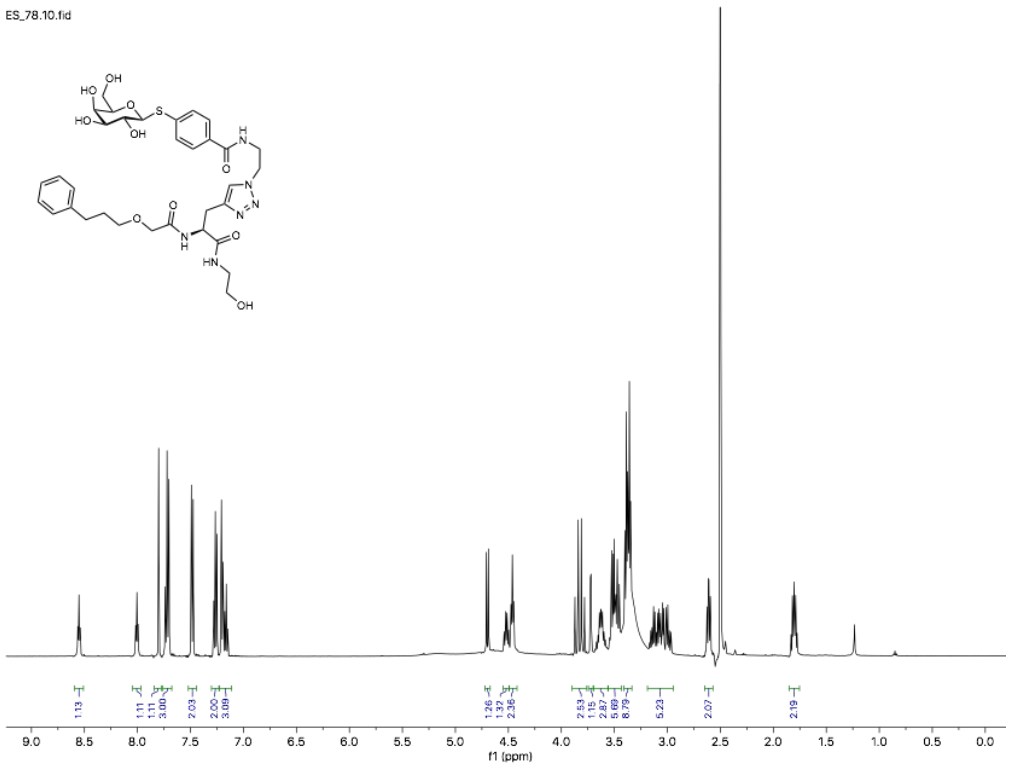
620



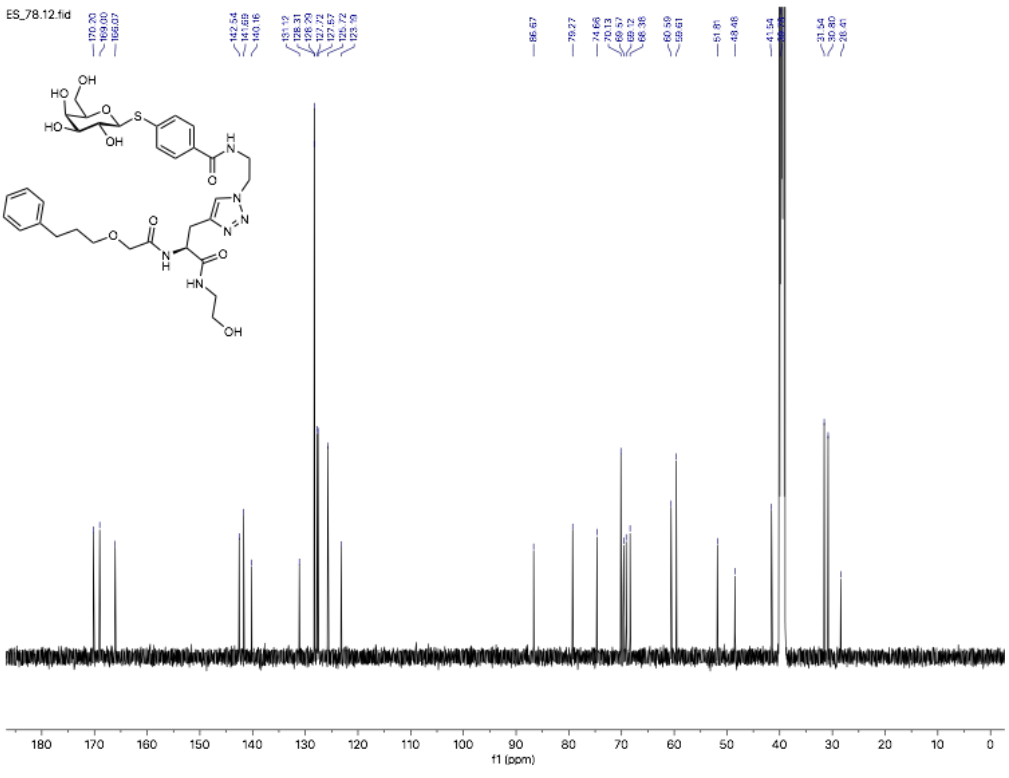
621



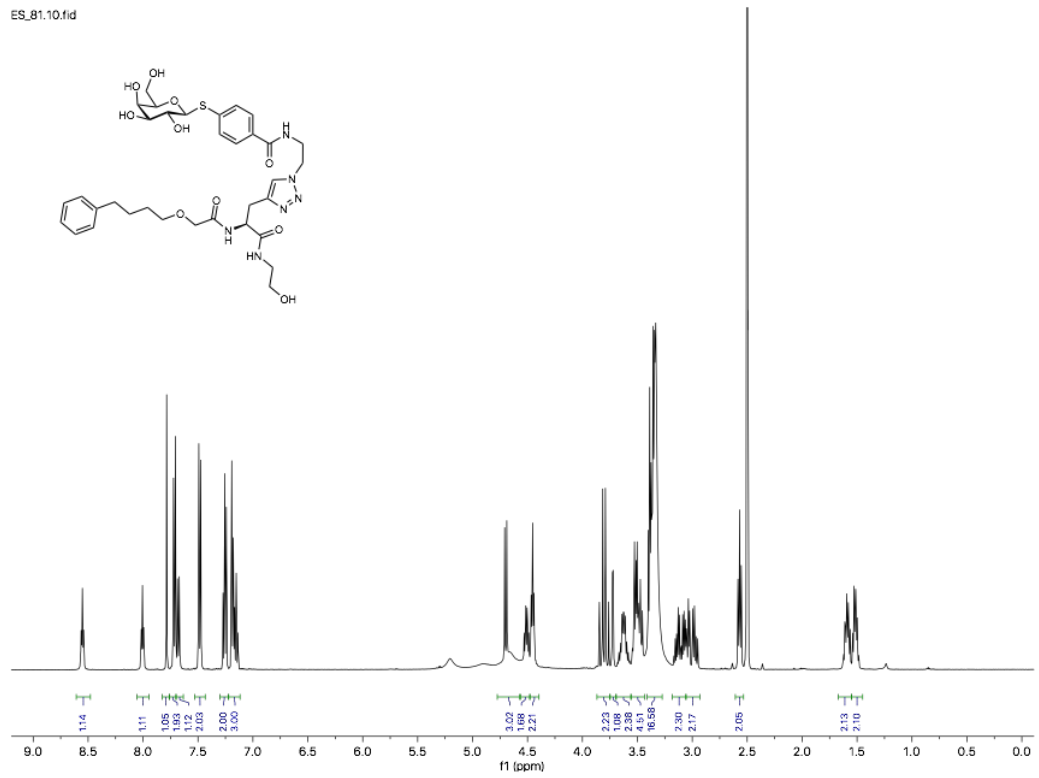
622



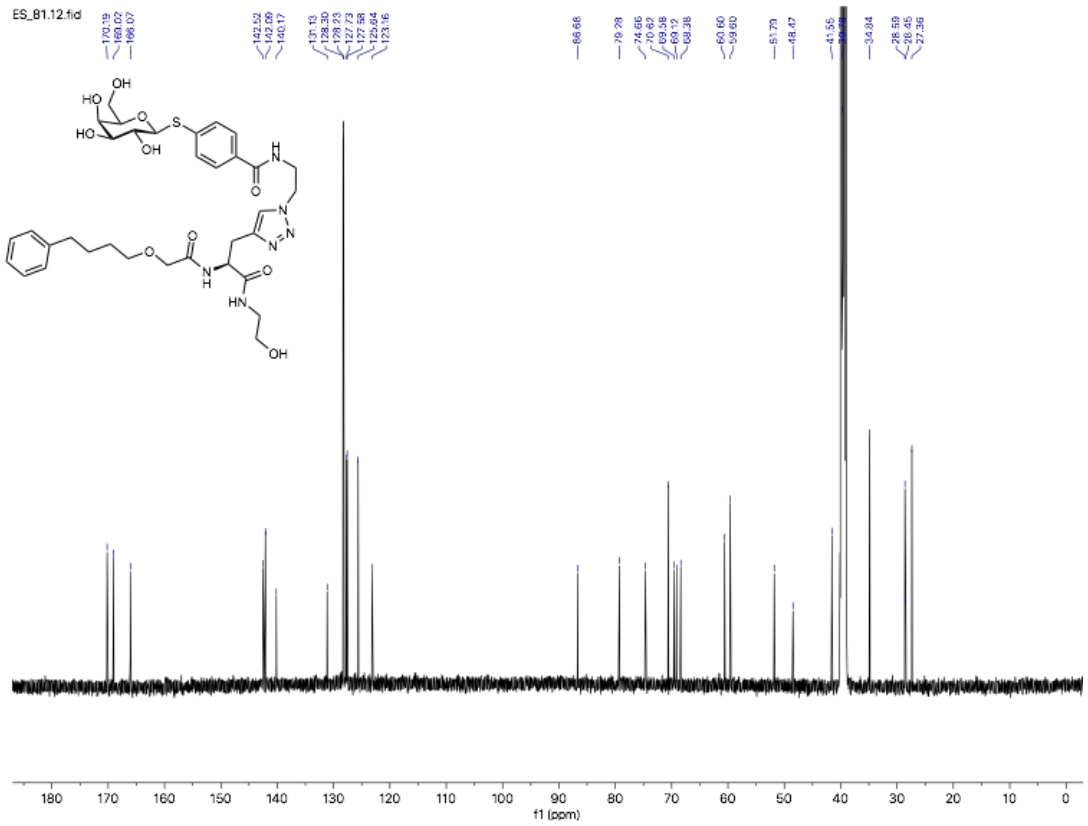
623



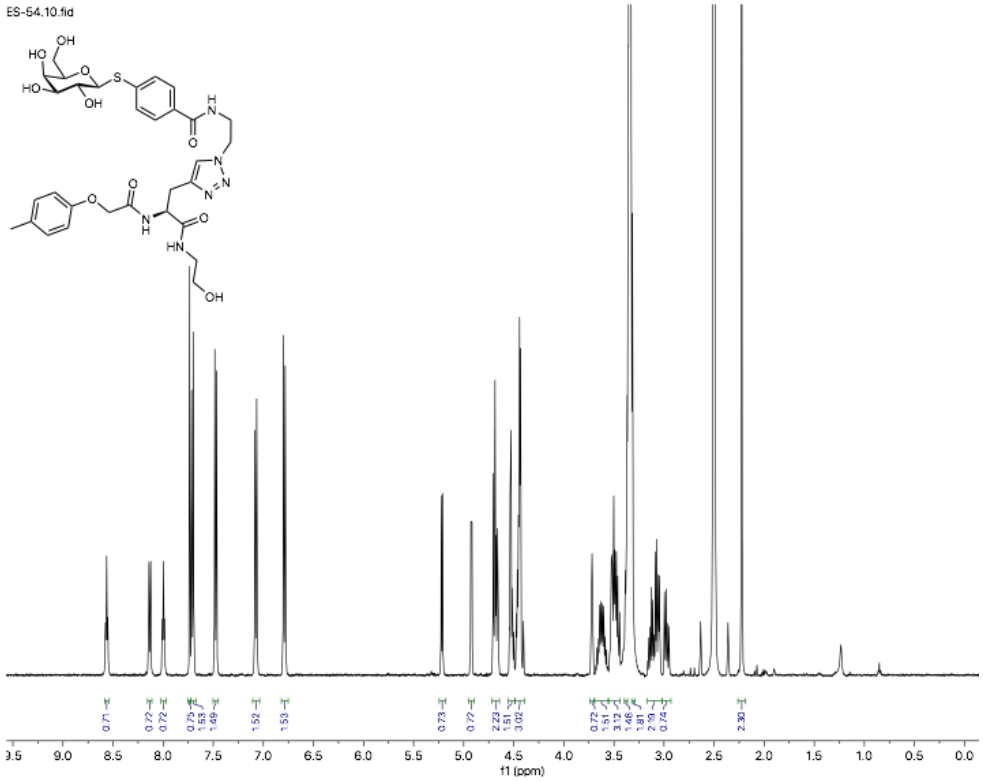
624



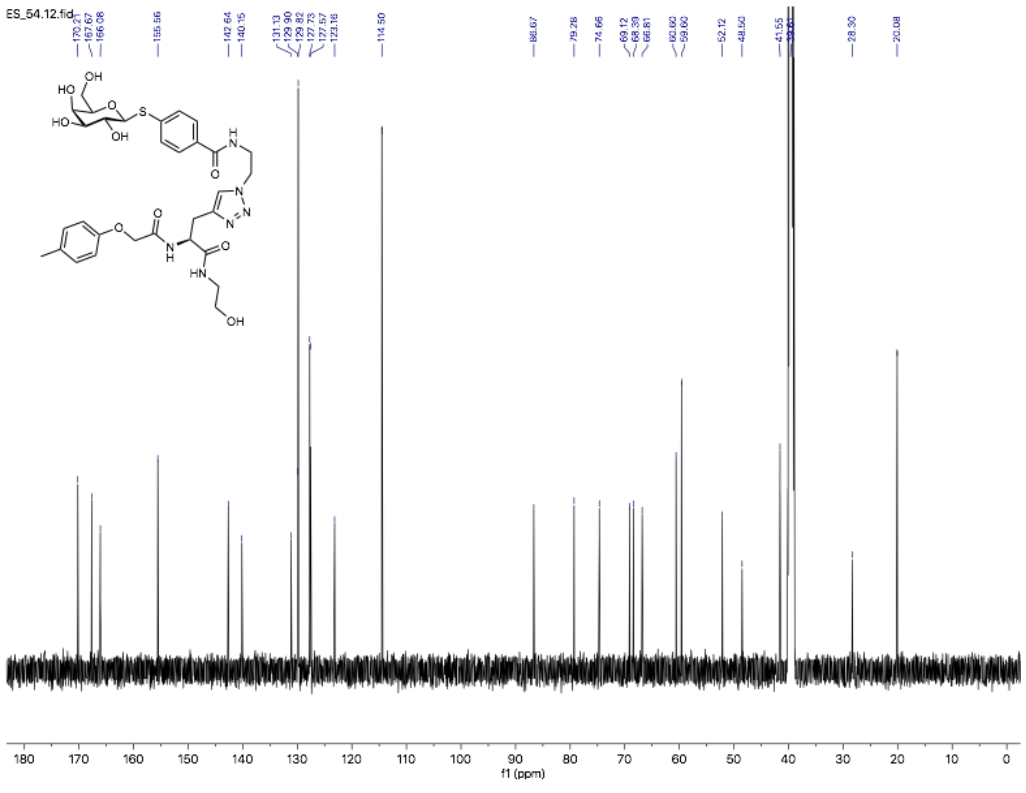
625



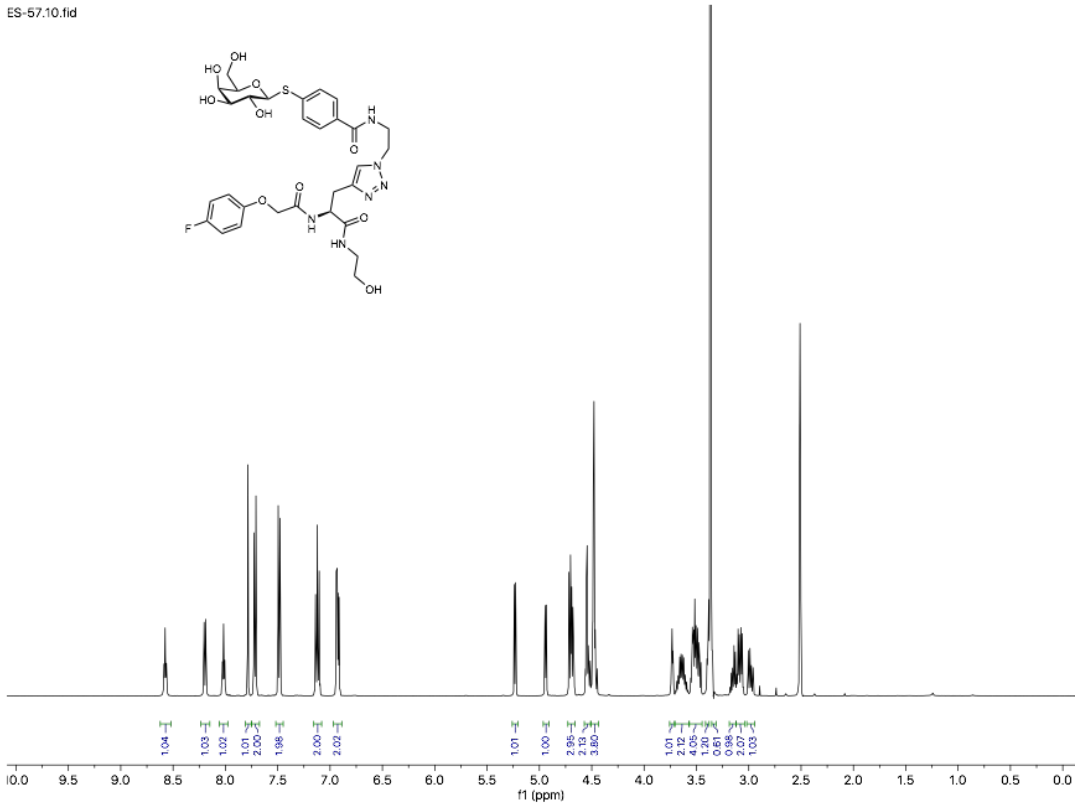
626



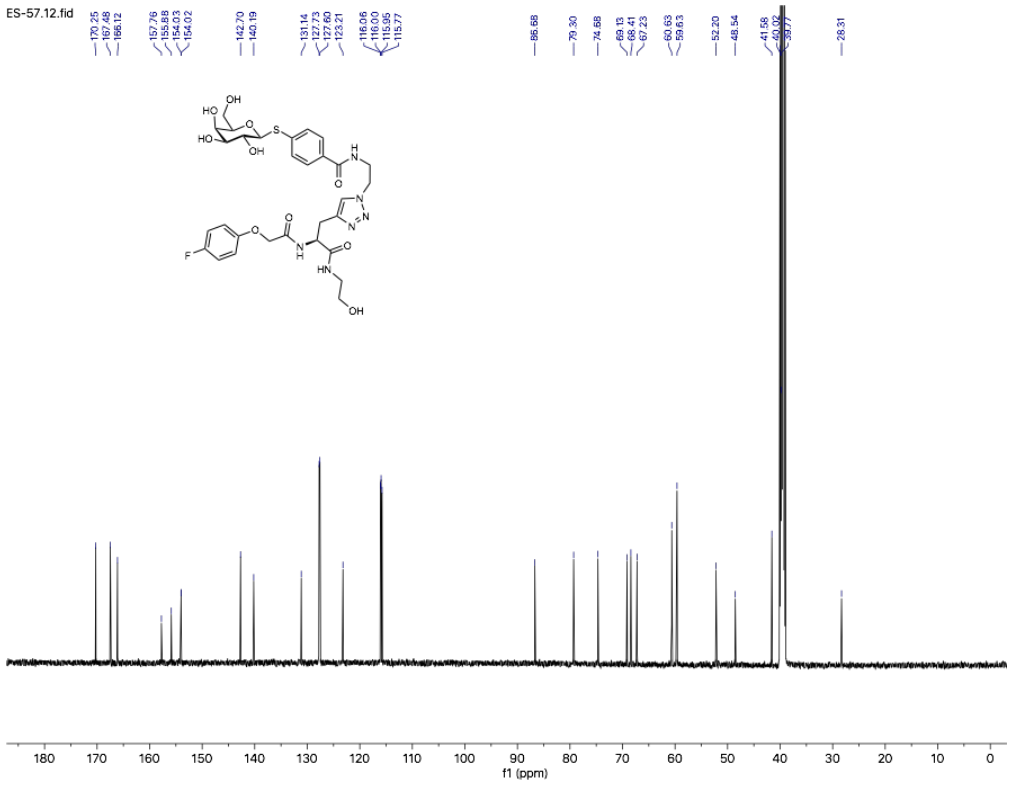
627



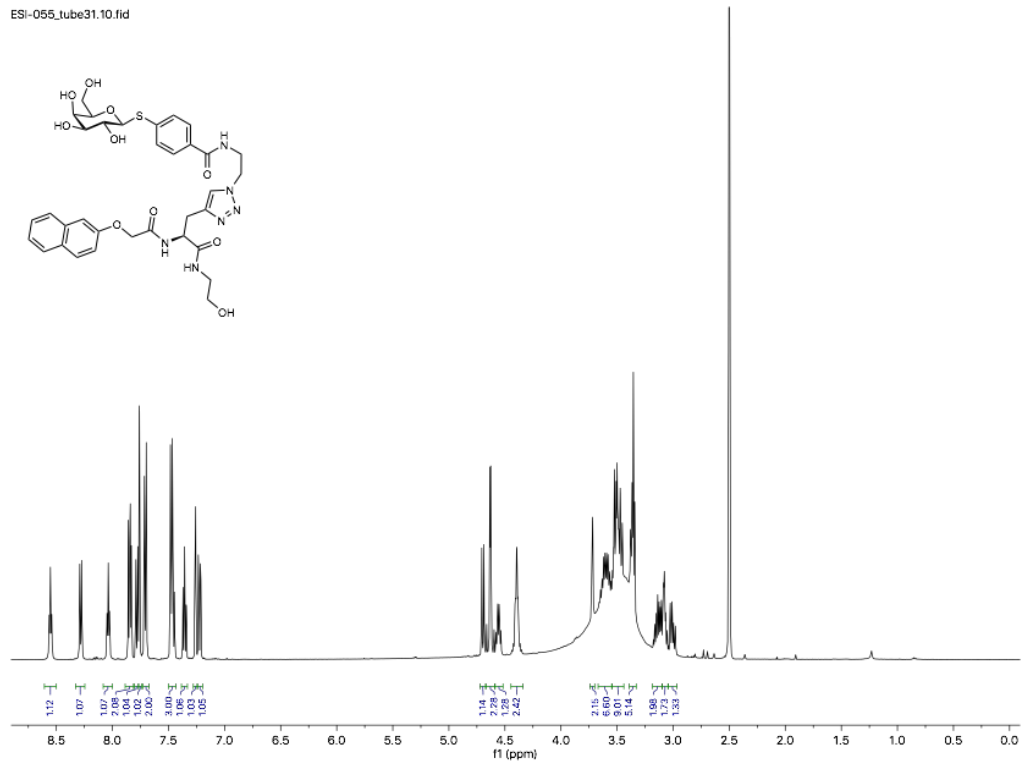
628



629



630



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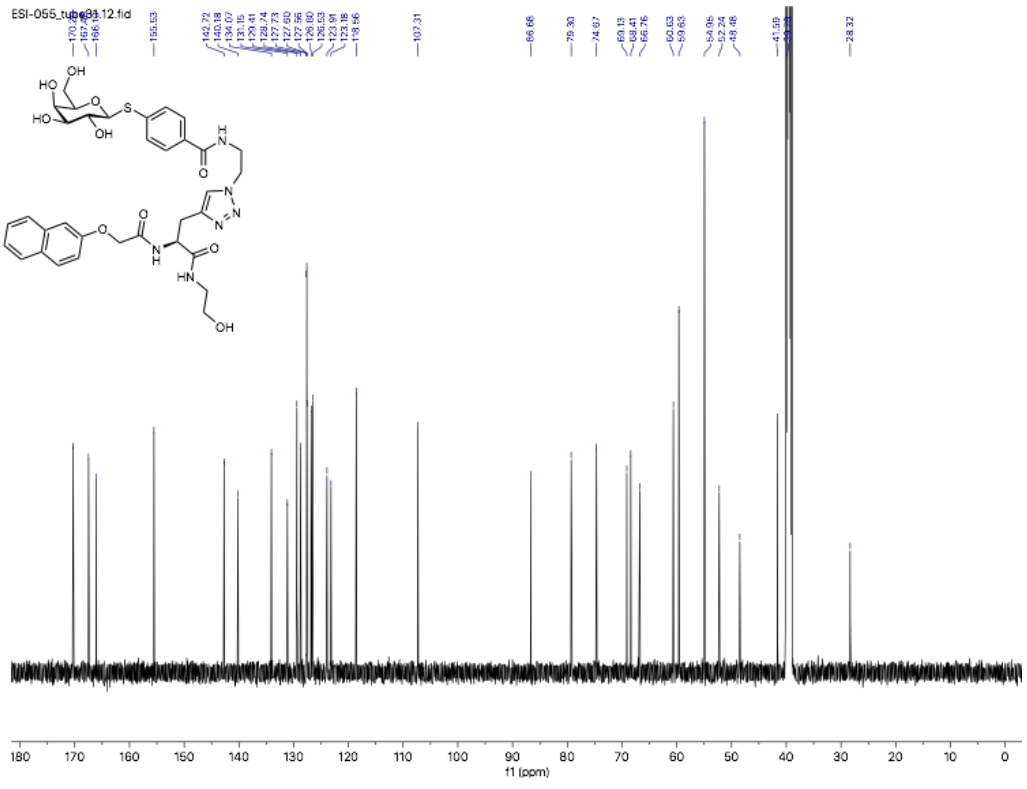

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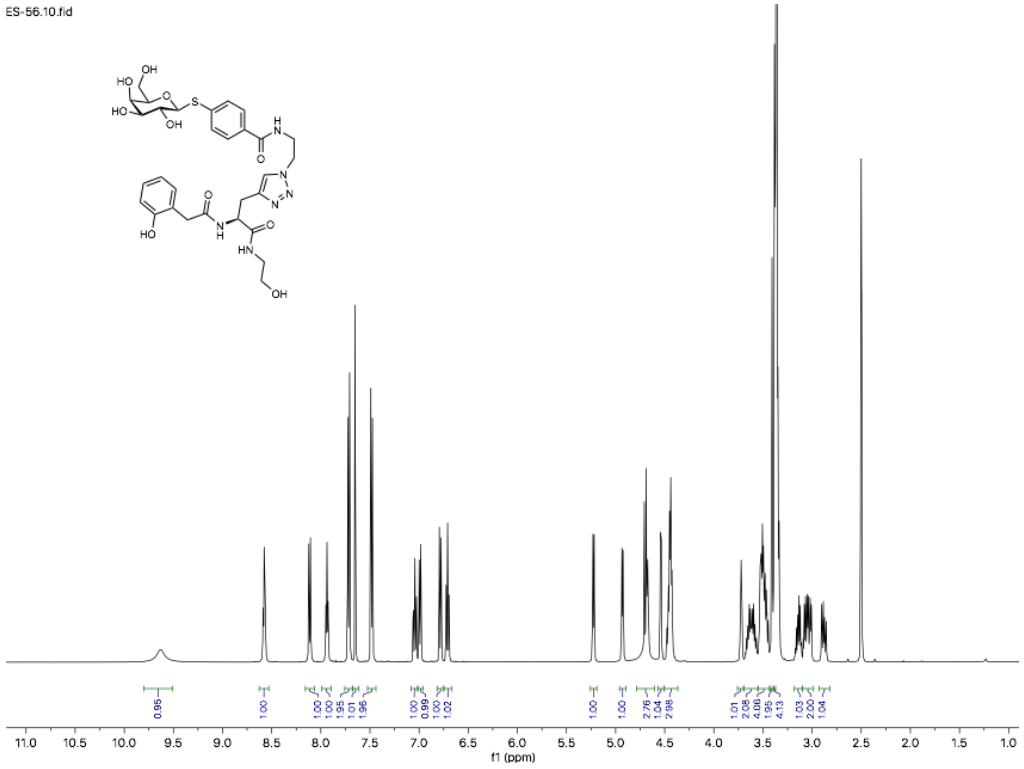
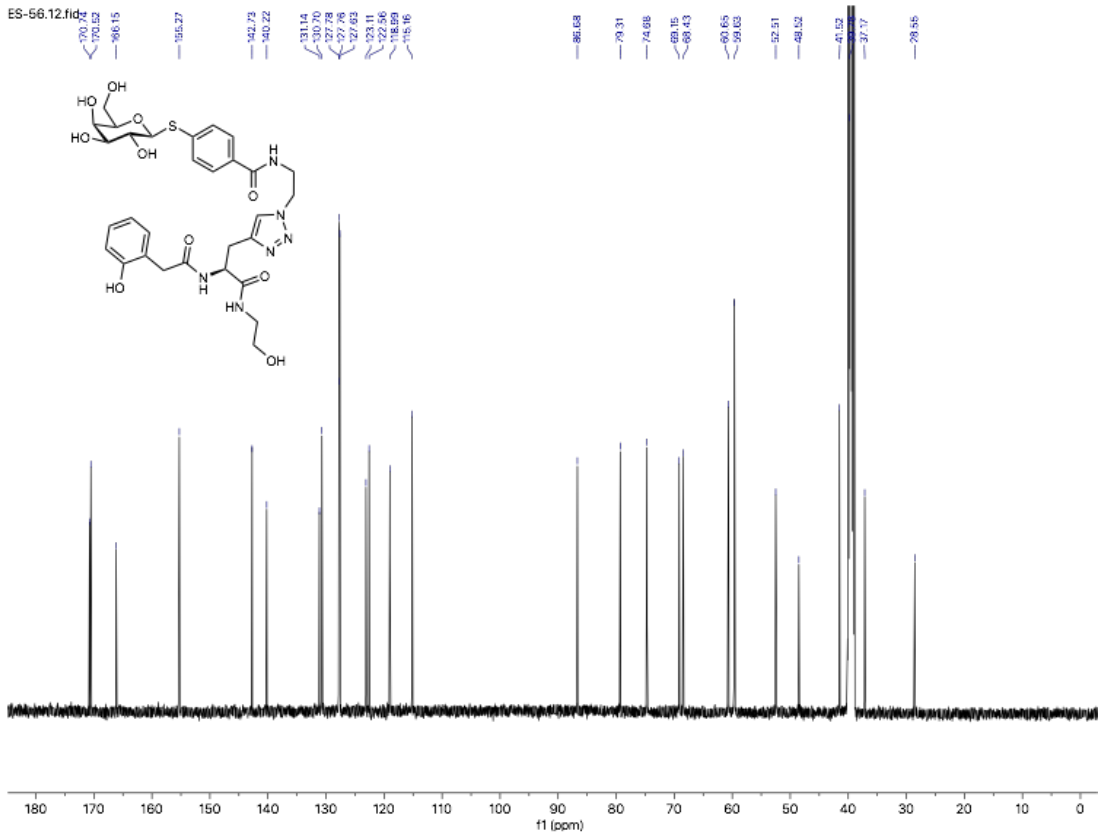


633



634