SUPPORTING INFORMATION

Structural basis of the inhibition of GH1 β-glucosidases by multivalent pyrrolidine iminosugars

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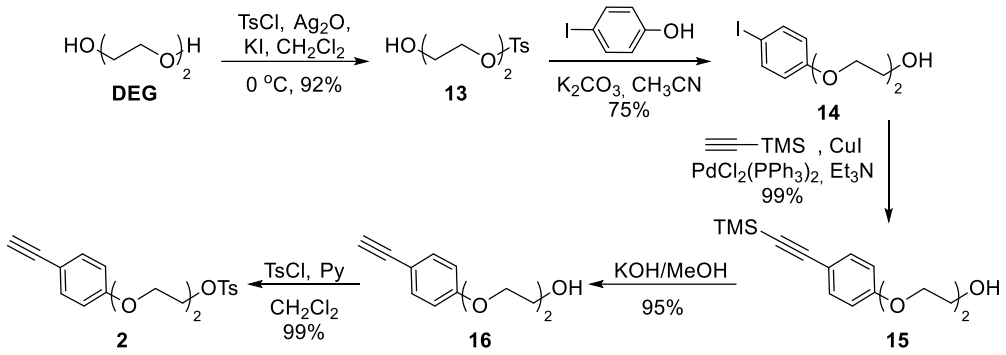
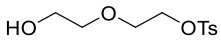
1. Synthesis of **2**, **5**, **7**, **8**.

1.1. *Synthesis of **2***

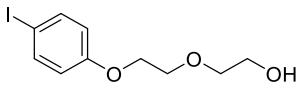


Monotosylated diethylene glycol[1] (13)



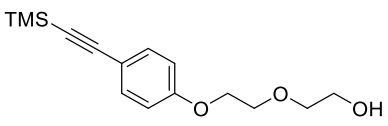
To a solution of diethylene glycol (500 μL, 5.22 mmol) in anhydrous CH_2Cl_2 (25 mL) at 0 °C, Ag_2O (928 mg, 3.96 mmol), TsCl (509 mg, 2.64 mmol) and KI (88 mg, 0.53 mmol) were added. After stirring at r.t. for 30 min, the mixture was filtered through celite and the solvent was removed under vacuum. The crude product was purified by chromatography column on silica gel (EtOAc:cyclohexane 2:1) to give **13** (633 mg, 2.43 mmol, 92%) as a colourless oil.

2-(2-(4-Iodophenoxy)ethoxy)ethan-1-ol[2] (14)



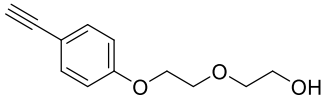
To a solution of **13** (345 mg, 1.33 mmol) in CH_3CN (3.3 mL), 4-iodophenol (310 mg, 1.40 mmol) and K_2CO_3 (222 mg, 1.59 mmol) were added and the mixture was refluxed for 4 h. The mixture was diluted with EtOAc and washed with H_2O and brine. The organic layer was dried over Na_2SO_4, filtered and evaporated. The resulting residue was purified by chromatography column on silica gel (EtOAc:cyclohexane 1:1) to give **14** (308 mg, 1.00 mmol, 75%) as a colourless oil.

2-(2-(4-(Trimethylsilylethynyl)phenoxy)ethoxy)ethan-1-ol[3] (15)



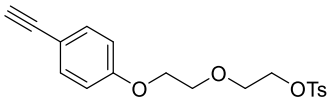
To a mixture of **14** (297 mg, 0.964 mmol), CuI (2 mg, 0.01 mmol) and $PdCl_2(PPh_3)_2$ (14 mg, 0.020 mmol), a solution of trimethylsilylacetylene (200 μL, 1.39 mmol) in Et_3N (4 mL) was added and the mixture was stirred at r.t. overnight. The mixture was filtered through celite and the solvent was removed under vacuum. The crude product was dissolved in CH_2Cl_2 and washed with HCl (1M) and H_2O. The organic layer was dried over Na_2SO_4, filtered and evaporated. The resulting residue was purified by chromatography column on silica gel (EtOAc:cyclohexane 1:1) to give **15** (265 mg, 0.952 mmol, 99%) as a yellow oil.

2-(2-(4-Ethynylphenoxy)ethoxy)ethan-1-ol[3] (16)



A mixture of **15** (252 mg, 0.905 mmol) and KOH/MeOH (5%, 3.6 mL) was stirred at r.t. for 1 h. After this time, HCl (1M) was added and the mixture was extracted with CH_2Cl_2. The organic layer was dried over Na_2SO_4, filtered and evaporated. The resulting residue was purified by chromatography column on silica gel (EtOAc:cyclohexane 2:1) to give **16** (178 mg, 0.863 mmol, 95%) as a pale yellow solid.

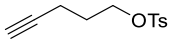
2-(2-(4-Ethynylphenoxy)ethoxy)ethyl-4-methylbenzenesulfonate[3] (2)



To a solution of **16** (2.79 g, 13.5 mmol) in a mixture CH_2Cl_2:pyridine 4:1 (anhydrous, 30 mL) at 0 °C, TsCl (4.69 g, 24.4 mmol) was added. After stirring at r.t. for 6.5 h, the mixture was washed with HCl (1M) and H_2O. The organic layer was dried over Na_2SO_4, filtered and evaporated. The resulting residue was purified by chromatography column on silica gel (EtOAc:cyclohexane 1:4) to give **2** (4.84 g, 13.4 mmol, 99%) as a purple oil.

*1.2. Synthesis of **5***

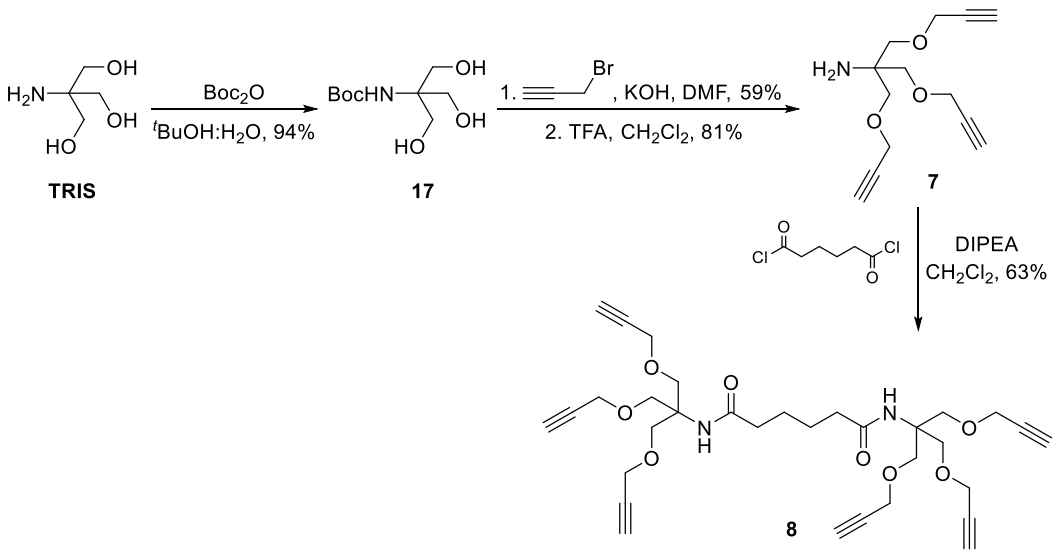
Pent-4-yn-1-yl 4-methylbenzenesulfonate[4] (5)



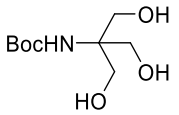
To a solution of pent-4-yn-1-ol (1.5 mL, 15 mmol) in anhydrous pyridine (40 mL) at 0 °C, TsCl (8.91 g, 46.3 mmol) was added. After stirring at r.t. for 5 h, water was added and the solvent was removed under vacuum. The residue was dissolved in CH_2Cl_2, washed with HCl (1M), sat. aq. soln. of $NaHCO_3$ and brine. The organic layer was dried over Na_2SO_4, filtered and evaporated.

The crude product was purified by chromatography column on silica gel (EtOAc:cyclohexane 1:10) to give **5** (3.34 g, 14.0 mmol, 91%) as a colourless oil.

*1.3. Synthesis of **7** and **8***

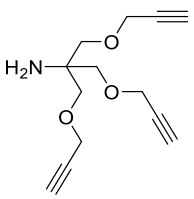


_N_-(_tert_-Butoxycarbonyl)tris(hydroxymethyl)aminomethane[5] (17)



A solution of Boc_2O (2.39 g, 10.7 mmol) in ^{t}BuOH (10 mL) was added to a suspension of TRIS (1.01 g, 8.30 mmol) in ^{t}BuOH:H_2O (1:1, 15 mL) and the reaction mixture was stirred at r.t. for 1 d. The solvent was removed under vacuum and the product was purified by precipitation with cold EtOAc. Compound **17** (1.72 g, 7.77 mmol, 94%) was obtained as a white solid.

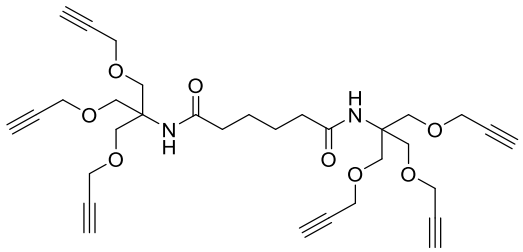
Tris[(propargyloxy)methyl]aminomethane (7)



To a solution of **17** (1.63 g, 7.37 mmol) in anhydrous DMF (20 mL) at 0 ^{o}C, propargyl bromide (4.8 mL, 45 mmol) and KOH (2.92 g, 44.2 mmol) were added (addition of KOH in portions during 15 min). The reaction mixture was stirred at 35 ^{o}C for 1 d. After this time, the mixture was diluted with EtOAc and washed with H_2O (three times). The organic layer was dried over Na_2SO_4, filtered

and evaporated. The resulting residue was purified by chromatography column on silica gel (EtOAc:cyclohexane 1:7→EtOAc) to give the corresponding tripropargylated derivative (1.45 g, 4.32 mmol, 59%) as a yellow solid. To a solution of this compound (1.43 g, 4.26 mmol) in anhydrous CH_2Cl_2 (17 mL) at 0 ºC, TFA (7.0 mL, 94 mmol) was added slowly and the reaction mixture was stirred at r.t. for 2 h. Evaporation of the solvent and chromatographic purification on Dowex 50WX8 eluting with MeOH, H_2O and NH_4OH 17%, afforded **7** (809 mg, 3.44 mmol, 81%) as a yellow solid. IR (ν cm^{-1}) 3366, 3282, 3250 (≡C-H, NH), 2923, 2103 (C≡C), 1590, 1440, 1359, 1265, 1090, 911, 727. ^{1}H-NMR (300 MHz, $CDCl_3$, δ ppm, *J* Hz) δ 4.15 (d, 6H, $^4J_{H,H}$ = 2.4, -OC*H*$_2$CC≡H), 3.47 (s, 6H, H_2NCC*H*$_2$O), 2.42 (t, 3H, -OCH$_2$CC≡*H*), 1.58 (br. s, 2H, -NH_2). ^{13}C-NMR (75.4 MHz, $CDCl_3$, δ ppm) δ 79.9 (-OCH$_2$*C*C≡H), 74.5 (-OCH$_2$C*C*≡H), 72.2 (H_2NC*C*H$_2$O), 58.8 (-O*C*H$_2$CC≡H), 55.7 (H_2N*C*CH$_2$O). HRESIMS *m/z* found 236.1277, calc. for $C_{13}H_{18}NO_3$ $[M+H]^+$: 236.1281.

Hexakis[(propargyloxy)methyl]-*N,N'*-dimethyladipamide (8)



A suspension of adipic acid (58 mg, 0.40 mmol) in $SOCl_2$ (1 mL) was refluxed for 3 h under nitrogen atmosphere. After cooling to r.t., the solvent was evaporated and the crude adipoyl chloride was used directly for the next reaction without further purification. To a solution of **7** (242 mg, 1.03 mmol) in anhydrous CH_2Cl_2 (2.5 mL), DIPEA (420 μL, 2.40 mmol) was added. After cooling at 0 ºC, a solution of adipoyl chloride (73 mg, 0.40 mmol) in anhydrous CH_2Cl_2 was added and the reaction was stirred at r.t. overnight. The mixture was washed with HCl (0.5M) and water (three times). The organic phase was dried over Na_2SO_4, filtered and evaporated. The resulting residue was purified by chromatography column on silica gel (EtOAc:cyclohexane 1:1→2:1) to give **8**[6] (147 mg, 0.253 mmol, 63%) as a white solid. IR (ν cm^{-1}) 3314, 3270 (≡C-H, NH), 2952, 2117 (C≡C), 1667, 1642 (C=O), 1556, 1438, 1358, 1288, 1088, 958, 804. ^{1}H-NMR (300 MHz, $CDCl_3$, δ ppm, *J* Hz) δ 5.73 (br. s, 2H, NH), 4.13 (d, 12H, $^4J_{H,H}$ = 2.4, -OC*H*$_2$CC≡H), 3.82 (s, 12H, -NHCC*H*$_2$O), 2.44 (t, 6H, -OCH$_2$CC≡*H*), 2.17-2.13 (m, 4H, -CH$_2$C*H*$_2$C=O), 1.64-1.60 (m, 4H, -C*H*$_2$CH$_2$C=O). ^{13}C-NMR (75.4 MHz, $CDCl_3$, δ ppm) δ 173.1 (C=O), 79.7 (-OCH$_2$*C*C≡H), 74.8 (-OCH$_2$C*C*≡H), 68.7 (-NHC*C*H$_2$O), 59.3 (-NH*C*CH$_2$O), 58.8 (-O*C*H$_2$CC≡H), 37.0 (-CH$_2$*C*H$_2$C=O), 25.0 (-*C*H$_2$CH$_2$C=O). HRESIMS *m/z* found 603.2667, calc. for $C_{32}H_{40}N_2O_8Na$ $[M+Na]^+$: 603.2677.

2. Dixon and Cornish-Bowden plots[7] for the determination of the type of inhibition and K_i.

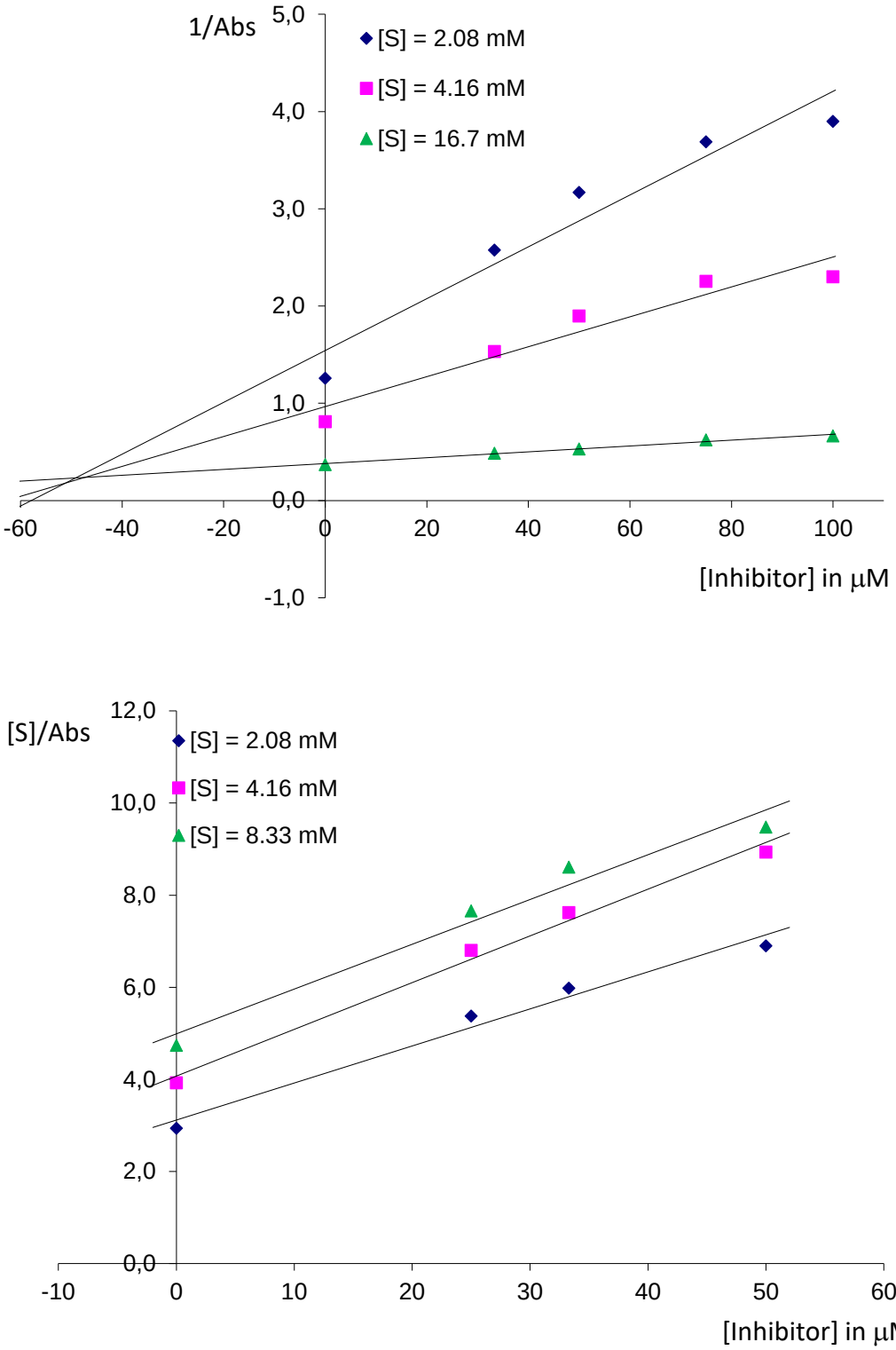
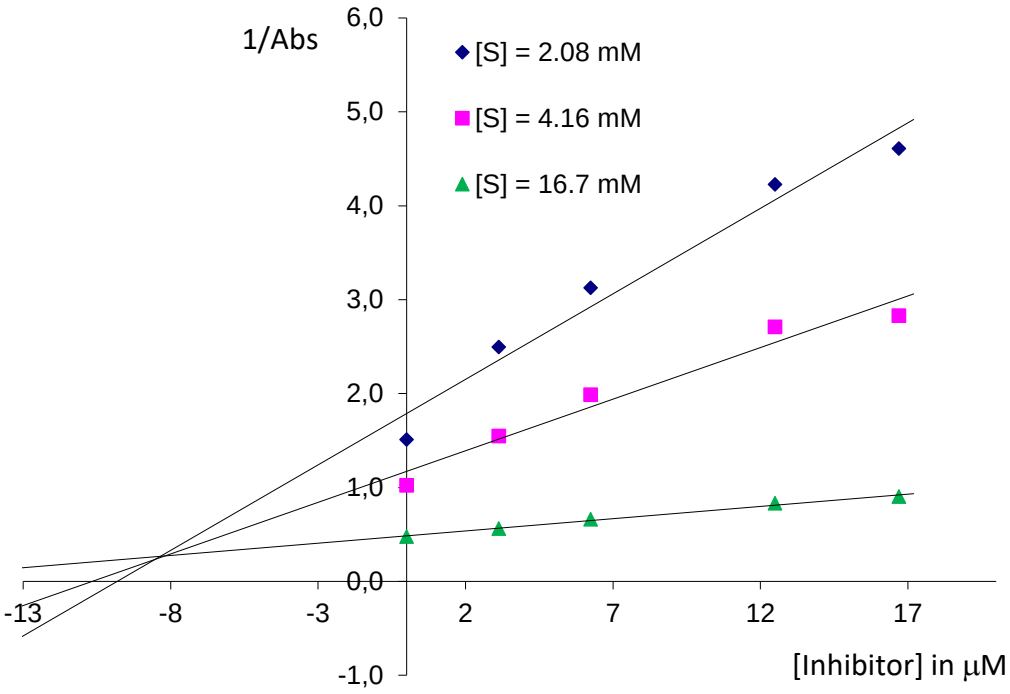


Figure S1. Dixon and Cornish-Bowden plots for compound **4**.



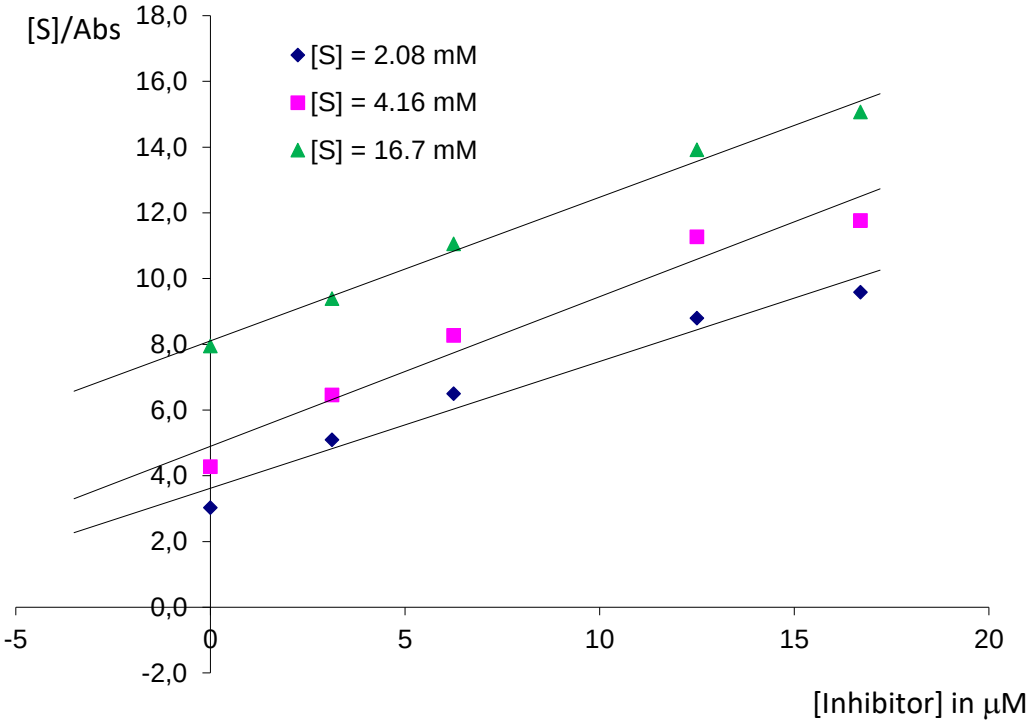
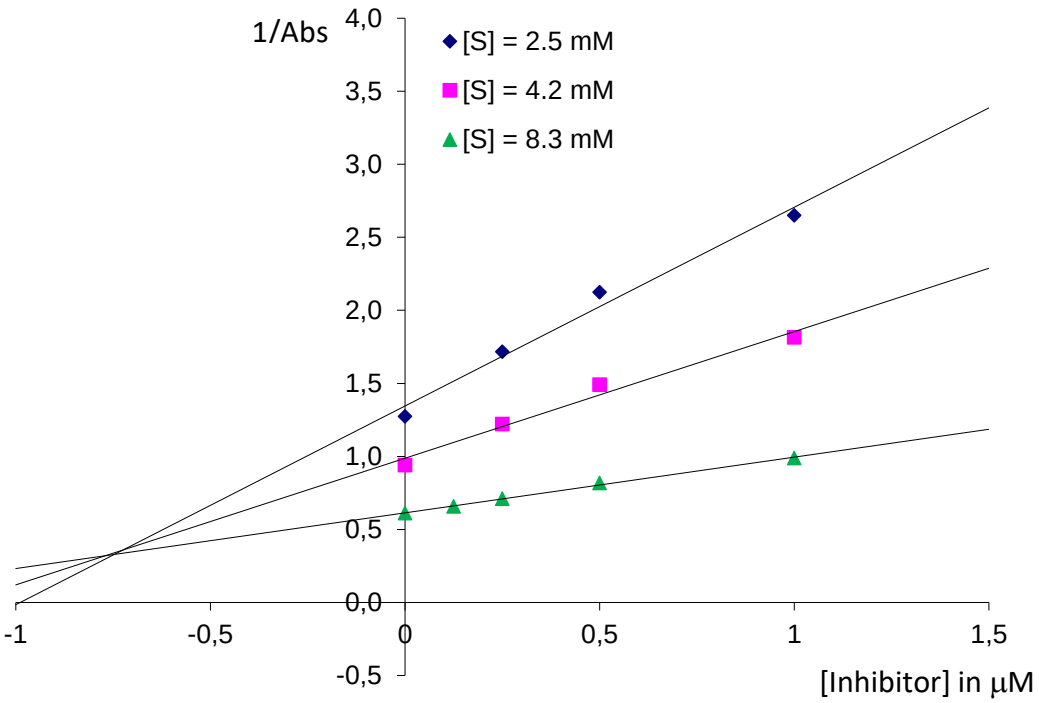


Figure S2. Dixon and Cornish-Bowden plots for compound **10**.



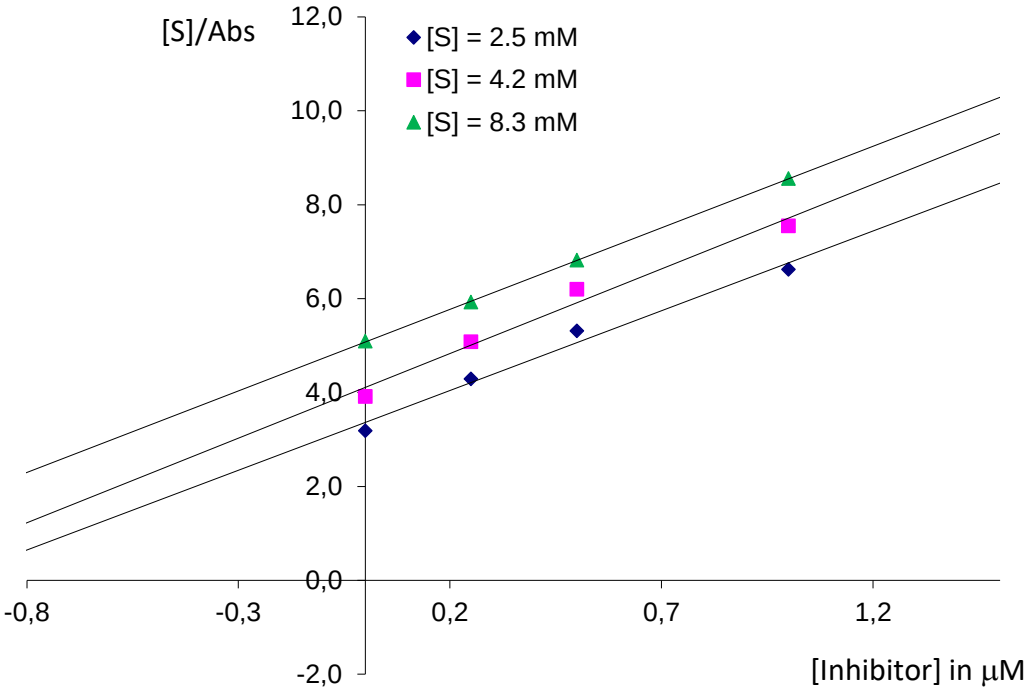


Figure S3. Dixon and Cornish-Bowden plots for compound **12**.

3. Crystallographic statistics for the BglA:**4** and BglA:**10** complex crystal structures

Crystallographic statistics
(Values in parentheses are for the high resolution shell)

Crystal data	BglA/ compound **4**	BglA/ compound **10**
Space group	P 4 2_1 2	P 4 2_1 2
Unit cell parameters		
a (Å)	146.73	146.10
b (Å)	146.73	146.10
c (Å)	140.05	140.35
Data collection		
Beamline	XALOC (ALBA)	XALOC (ALBA)
Temperature (K)	100	100
Wavelength (Å)	0.97926	0.97926
Resolution (Å)	48.91-2.13 (2.17-2.13)	48.70-2.85 (3.00-2.85)
Data processing		
Total reflections	572842 (27706)	225830 (33371)
Unique reflections	85545 (4461)	32518(4772)
Multiplicity	6.7 (6.2)	6.9 (7.0)
Completeness (%)	99.9 (99.6)	91.1 (93.0)
Mean I/σ (I)	11.6 (2.2)	9.2 (2.5)
R_{merge}[†] (%)	10.4 (70.1)	14.1 (69.1)
R_{pim}[††] (%)	4.3 (29.9)	7. 0 (34.1)
Molecules per ASU	2	2
Refinement		
R_{work} / R_{free}[†††] (%)	21.59/24.13	18.53/23.25
N° of atoms/average B (Å^2)		
Protein	7294/34.35	7294/48.53
Other molecules	56/45.04	50/60.35
Water Molecules	458/46.44	150/33.31
All atoms	7808/35.14	7494/48.30
Ramachandran plot (%)		
Favoured	96.41	96.08
Outliers	0.11	0.67
RMS deviations		
Bonds (Å)	0.01	0.005
Angles (°)	1.2	1.39
PDB accession codes	6R4K	6QWI

[†]$R_{merge} = \Sigma_{hkl} \Sigma_i | I_i(hkl) - [I(hkl)]| / \Sigma_{hkl} \Sigma_i I_i(hkl)$, where $I_i(hkl)$ is the ith measurement of reflection hkl and [I(hkl)] is the weighted mean of all measurements.

[††]$R_{pim} = \Sigma_{hkl} [1/(N - 1)] 1/2 \Sigma_i | I_i(hkl) - [I(hkl)]| / \Sigma_{hkl} \Sigma_i I_i(hkl)$, where N is the redundancy for the hkl reflection.

[†††]R_{work} / $R_{free} = \Sigma_{hkl} | Fo - Fc | / \Sigma_{hkl} | Fo |$, where Fc is the calculated and Fo is the observed structure factor amplitude of reflection hkl for the working / free (5%) set, respectively.

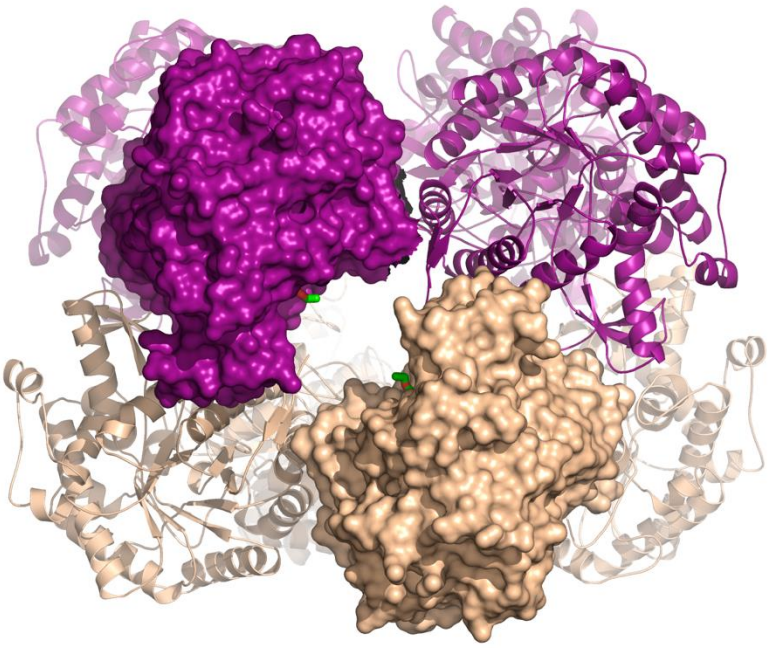
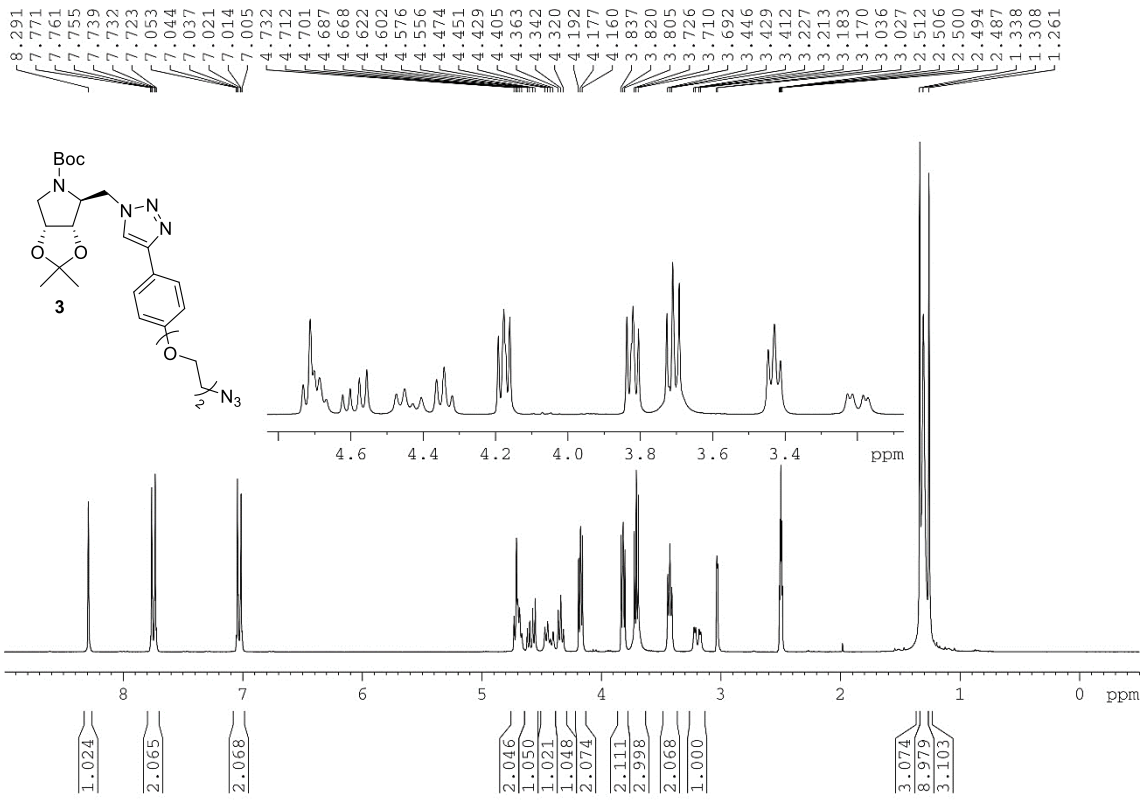
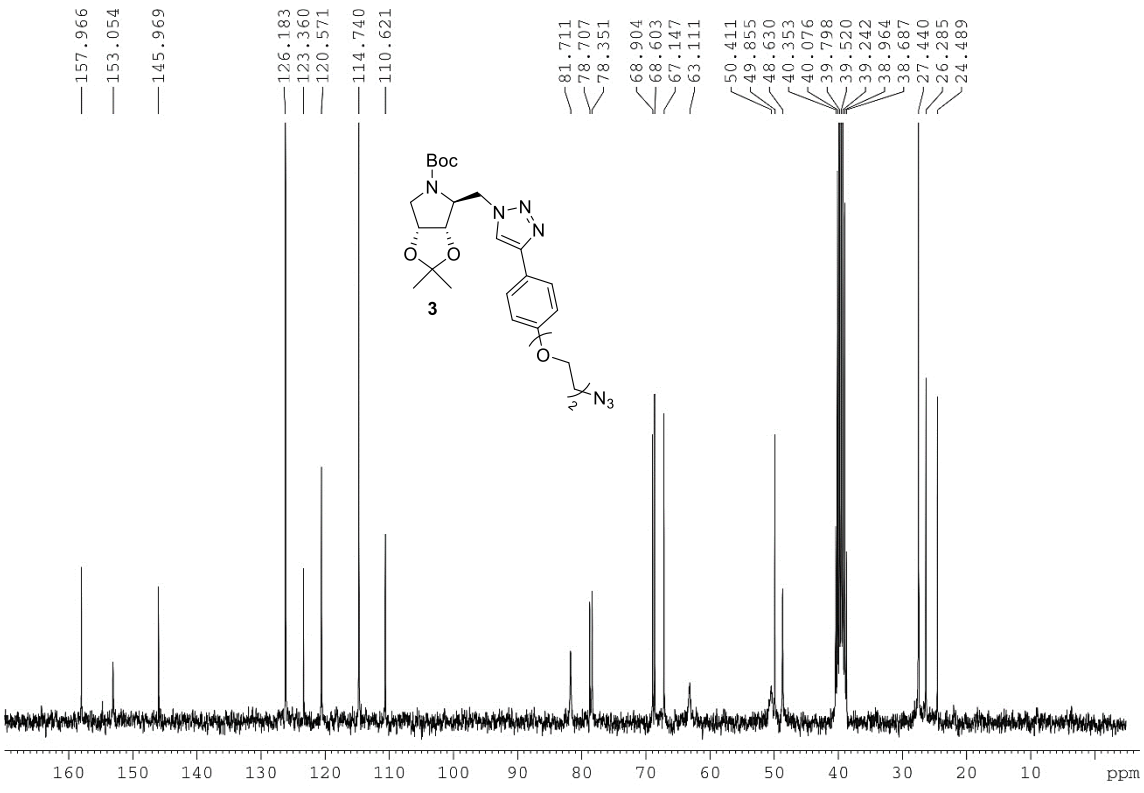


Figure S4. A view of the BglA octamer complexed with compound **10**, highlighting the observed part of the inhibitor bound in two of the subunits

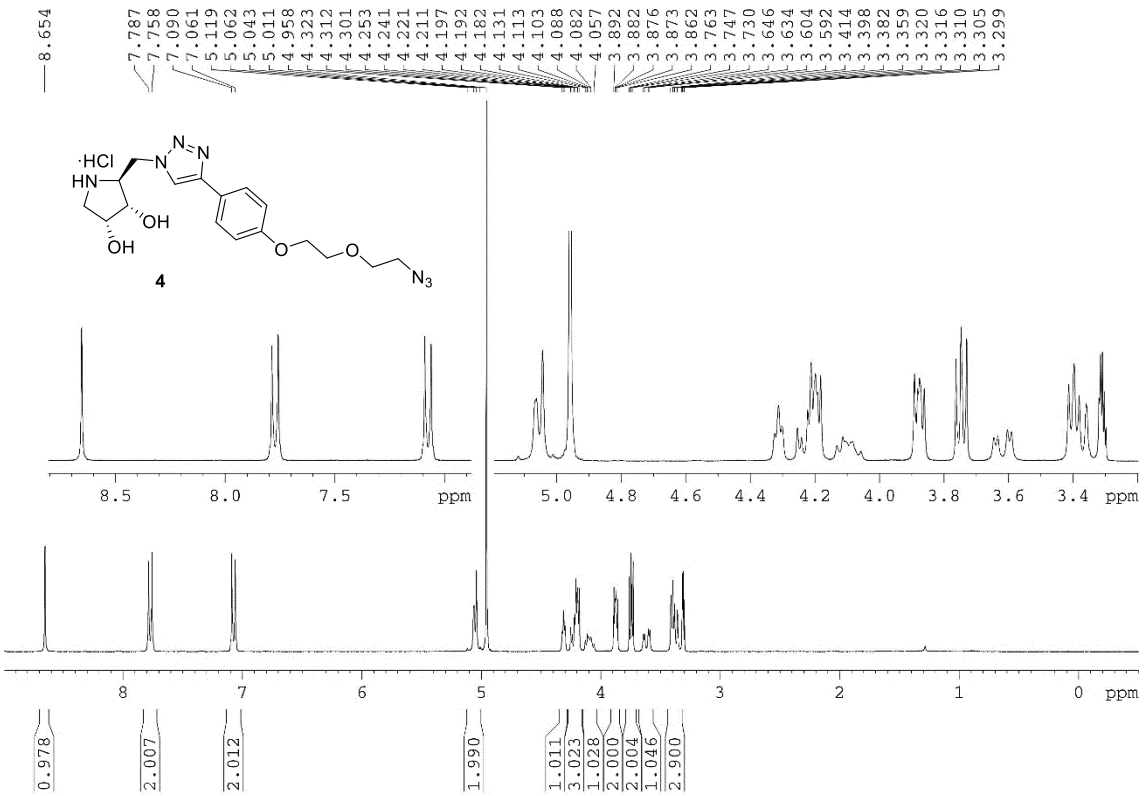
4. ^{1}H- and ^{13}C-NMR spectra for new compounds.



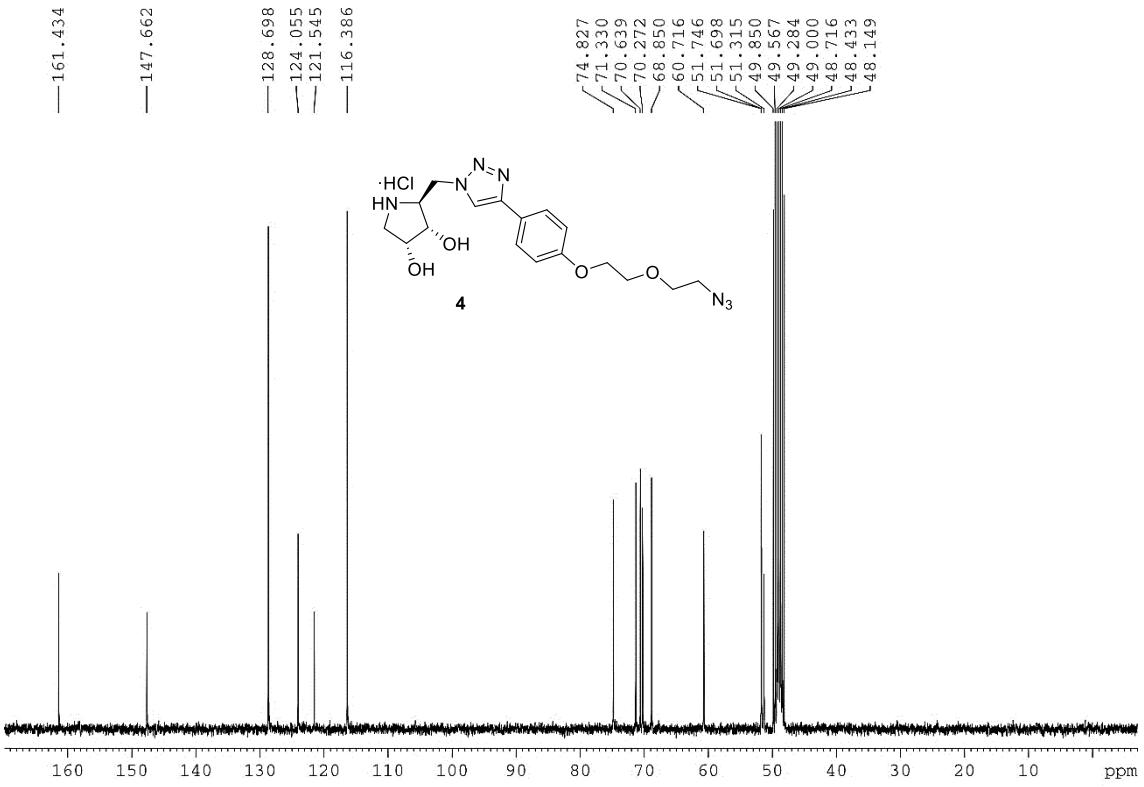
^{1}H-NMR (300 MHz, DMSO-d_6, 363 K)



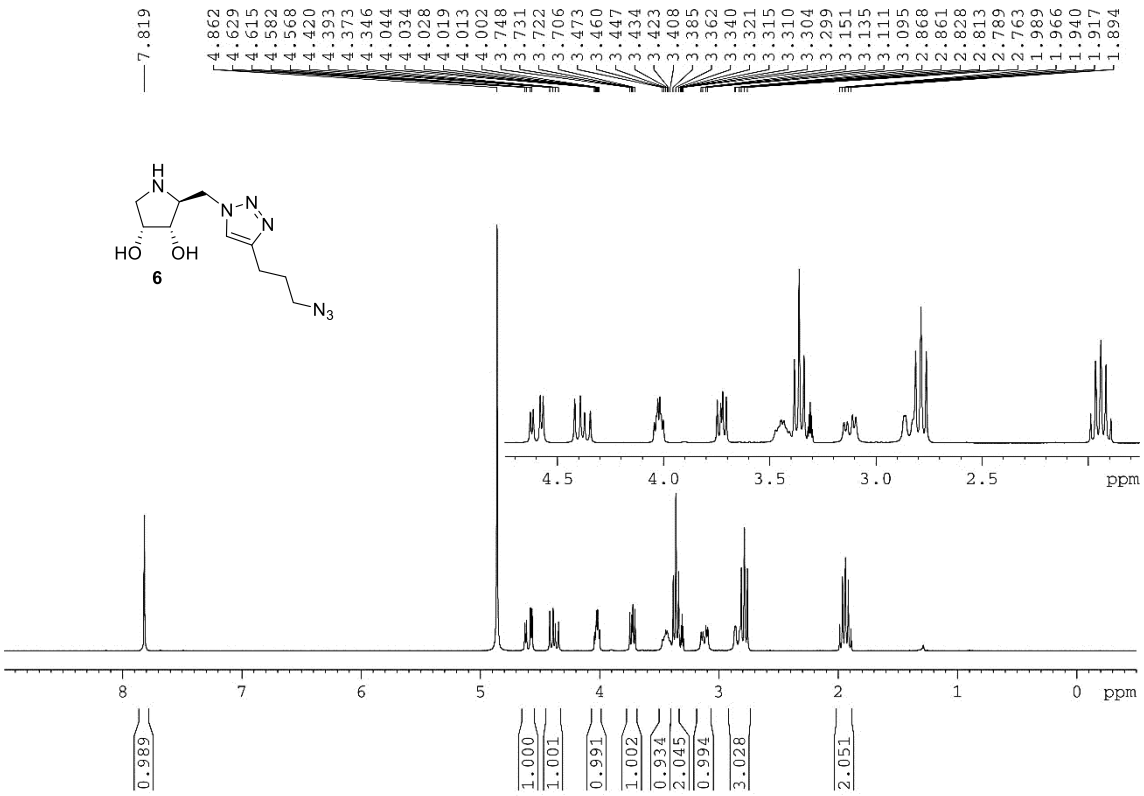
^{13}C-NMR (75.4 MHz, DMSO-d_6, 363 K)



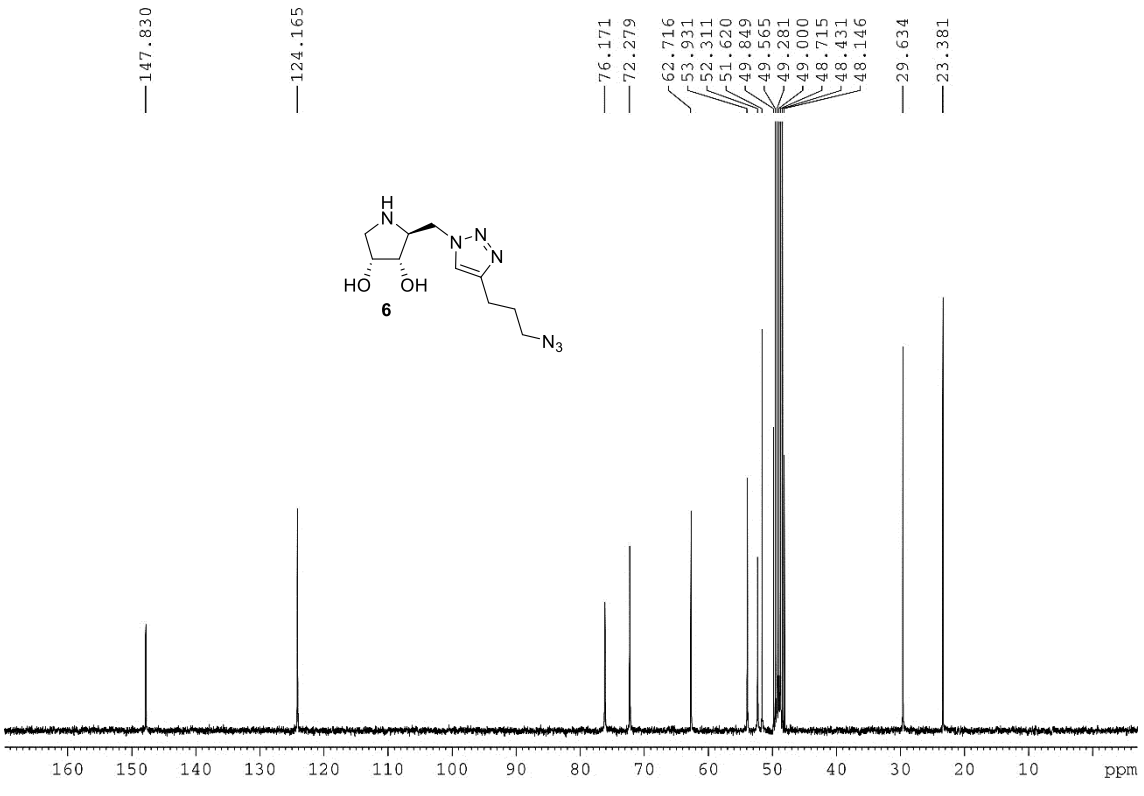
^{1}H-NMR (300 MHz, CD_3OD)



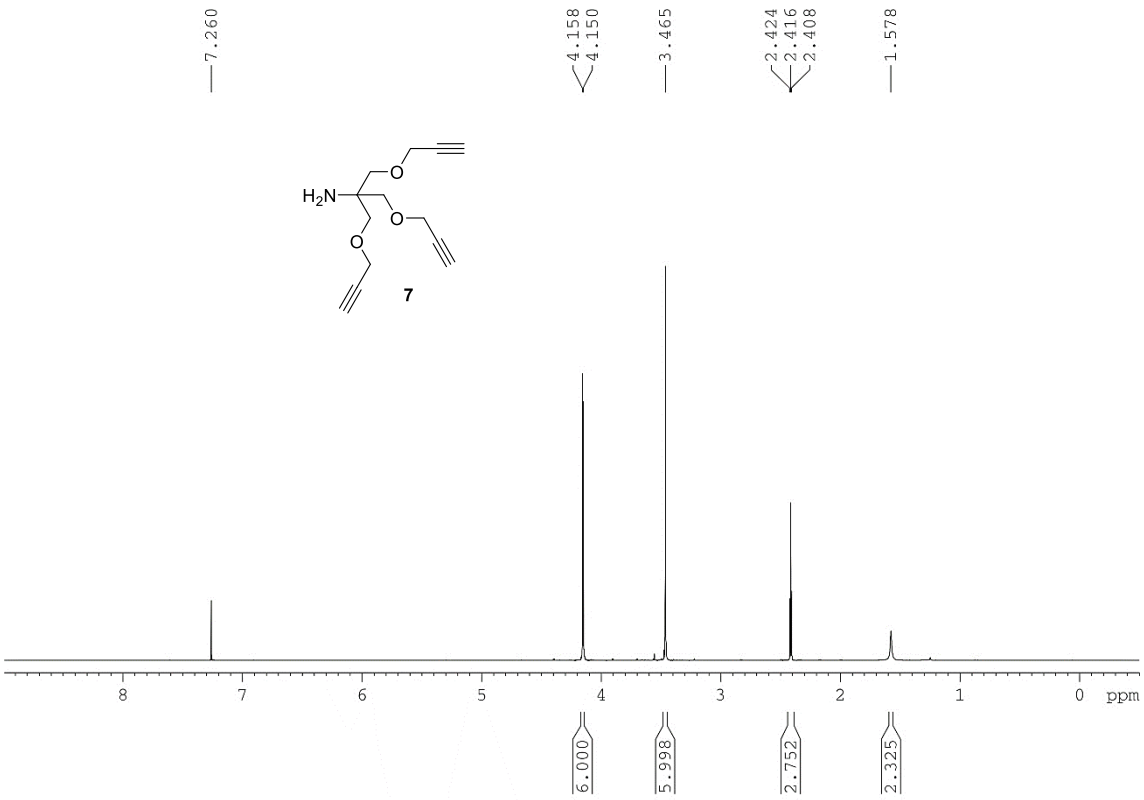
^{13}C-NMR (75.4 MHz, CD_3OD)



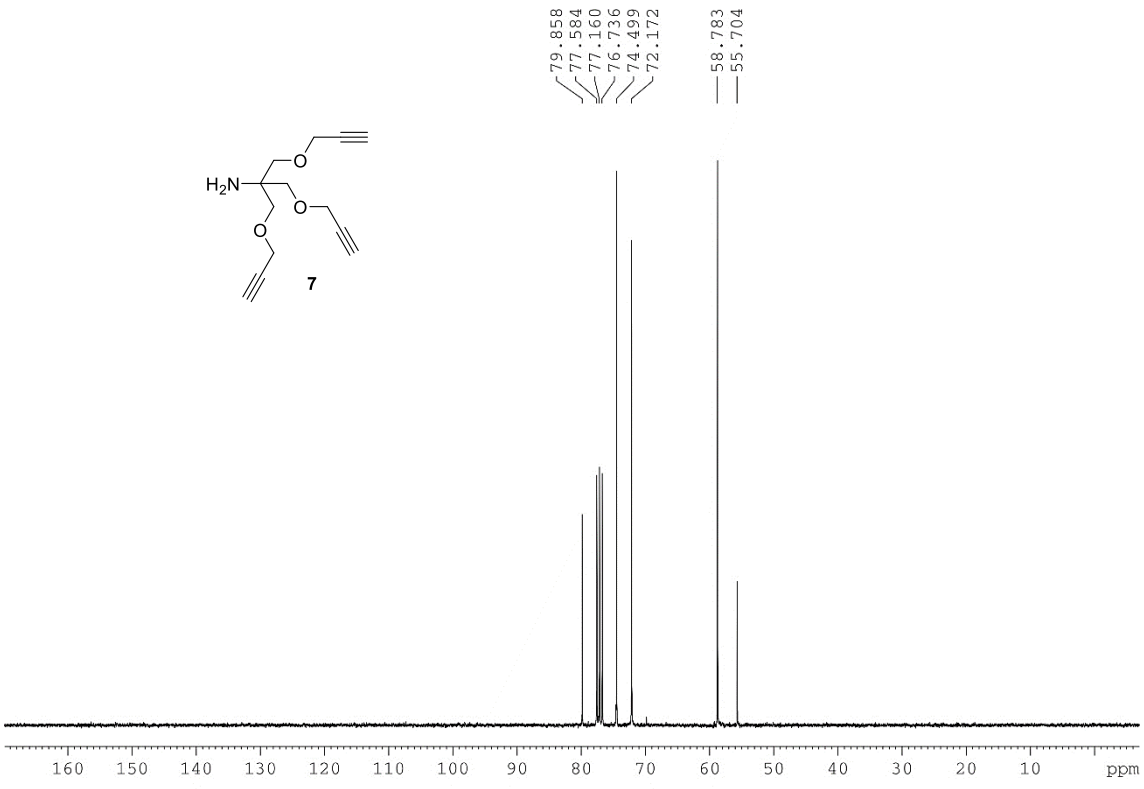
^{1}H-NMR (300 MHz, CD_3OD)



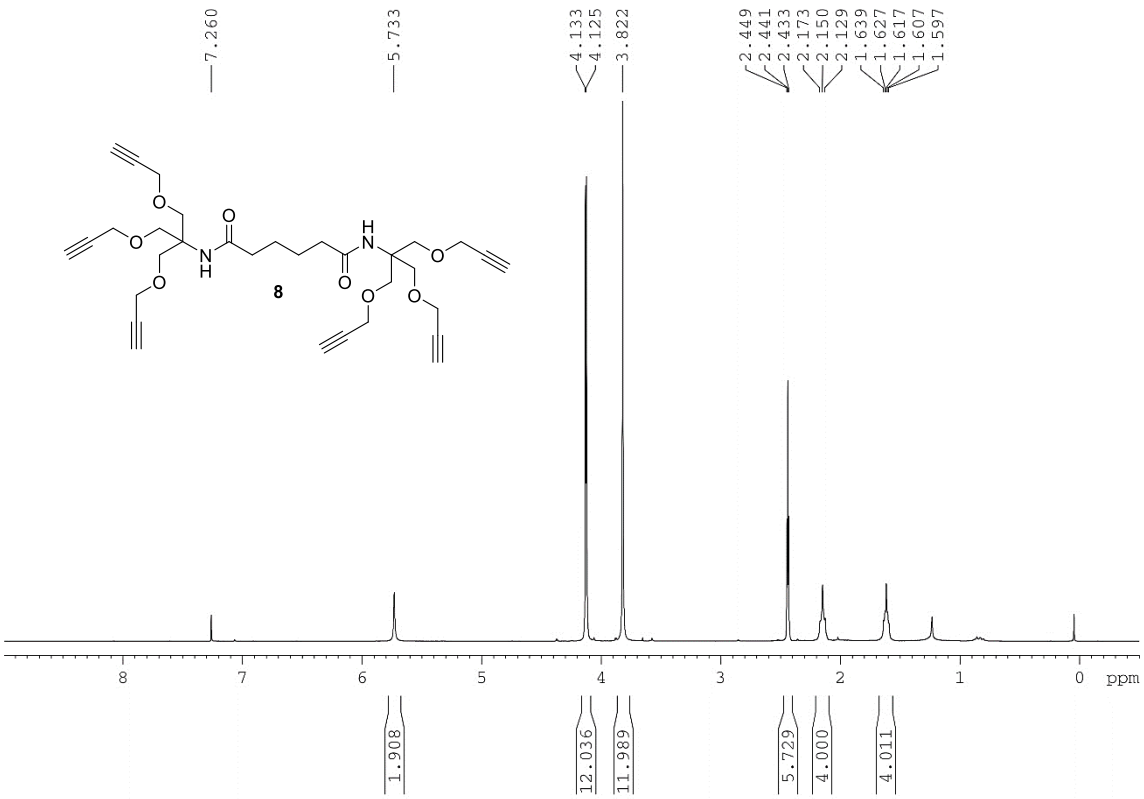
^{13}C-NMR (75.4 MHz, CD_3OD)



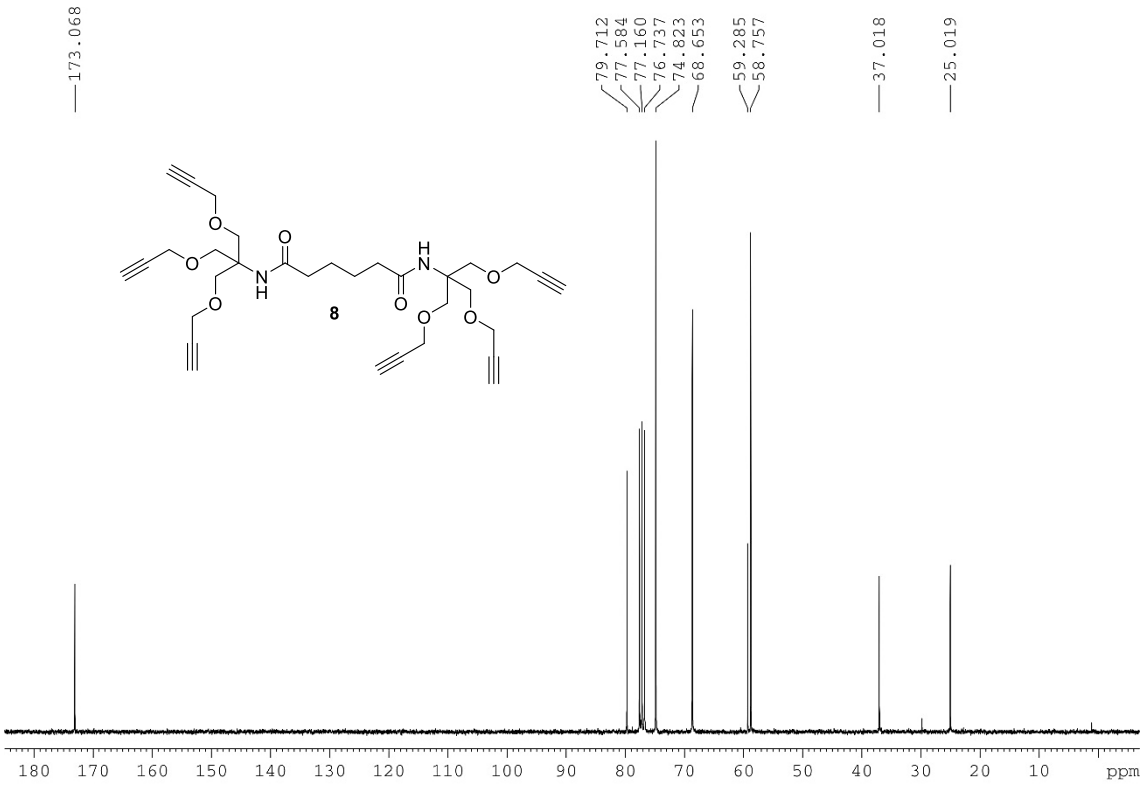
^{1}H-NMR (300 MHz, $CDCl_3$)



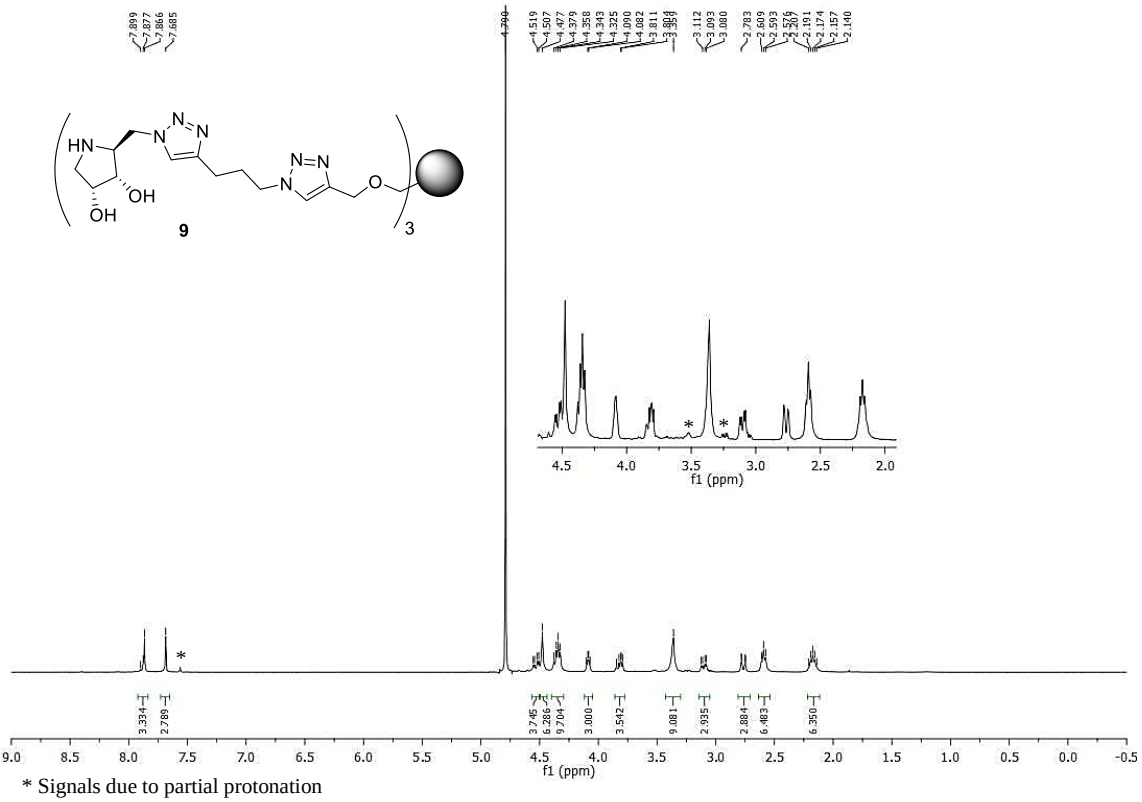
^{13}C-NMR (75.4 MHz, $CDCl_3$)



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

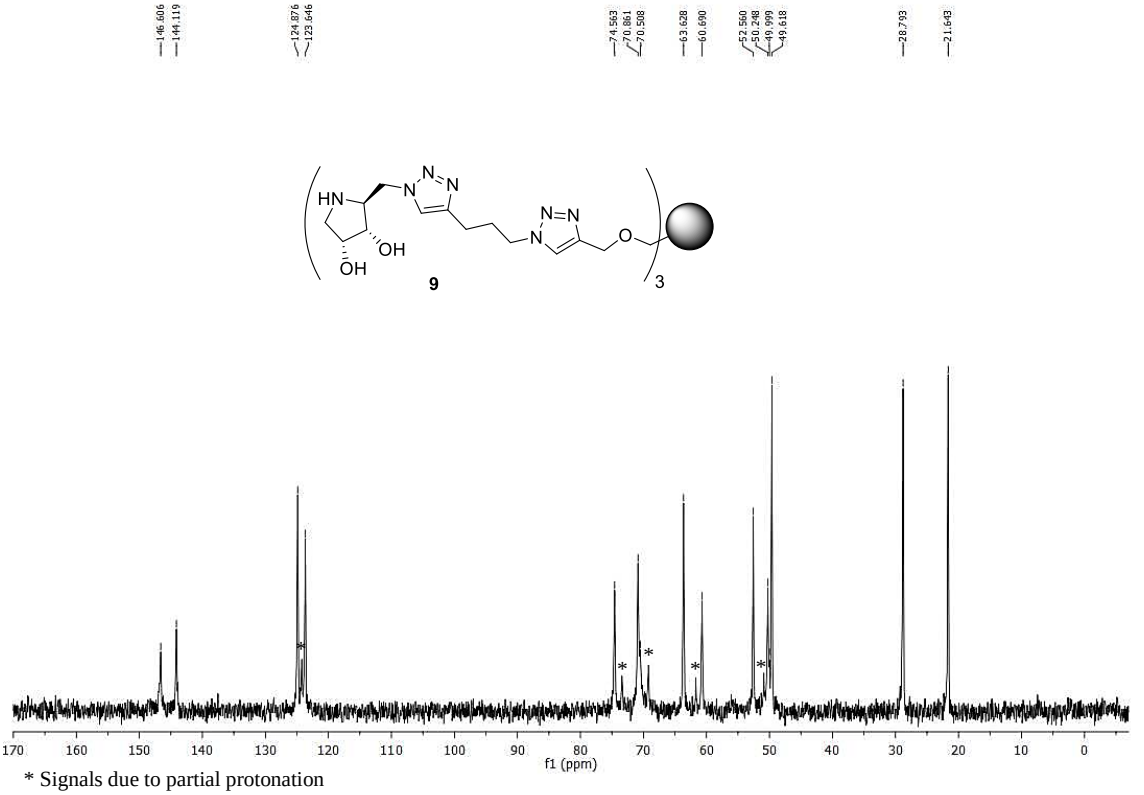


^{13}C-NMR (75.4 MHz, $CDCl_3$)



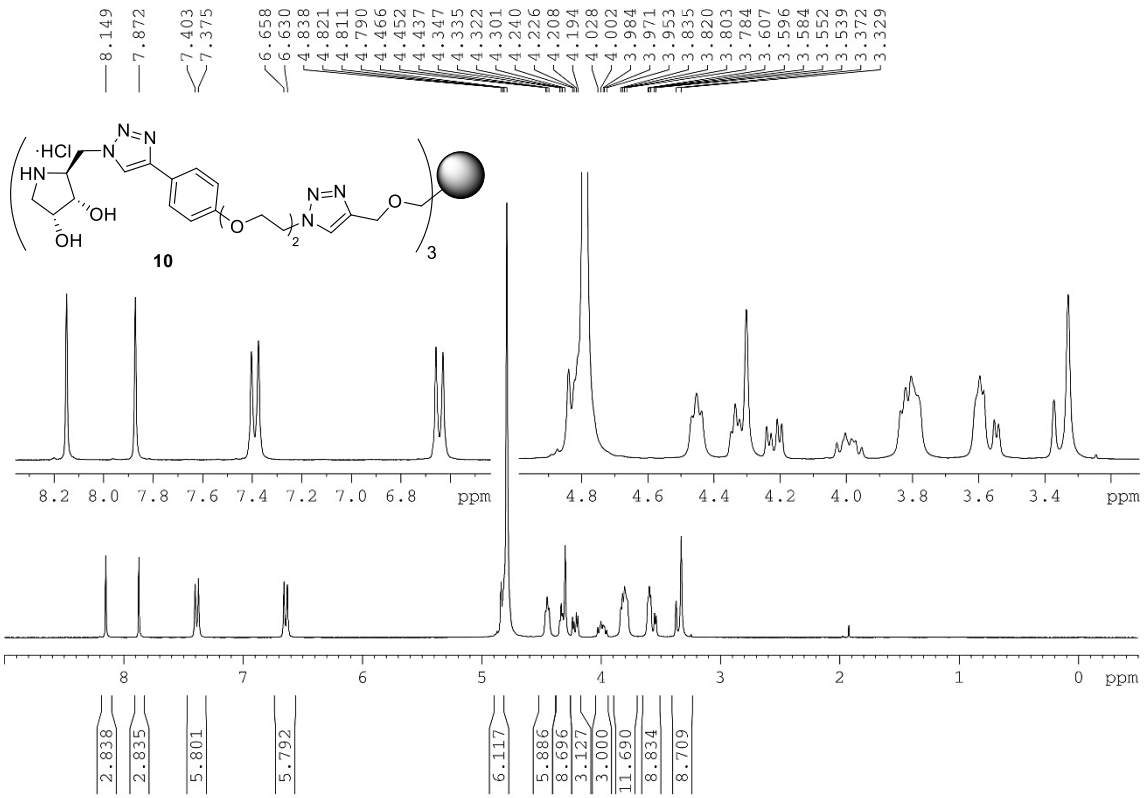
* Signals due to partial protonation

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

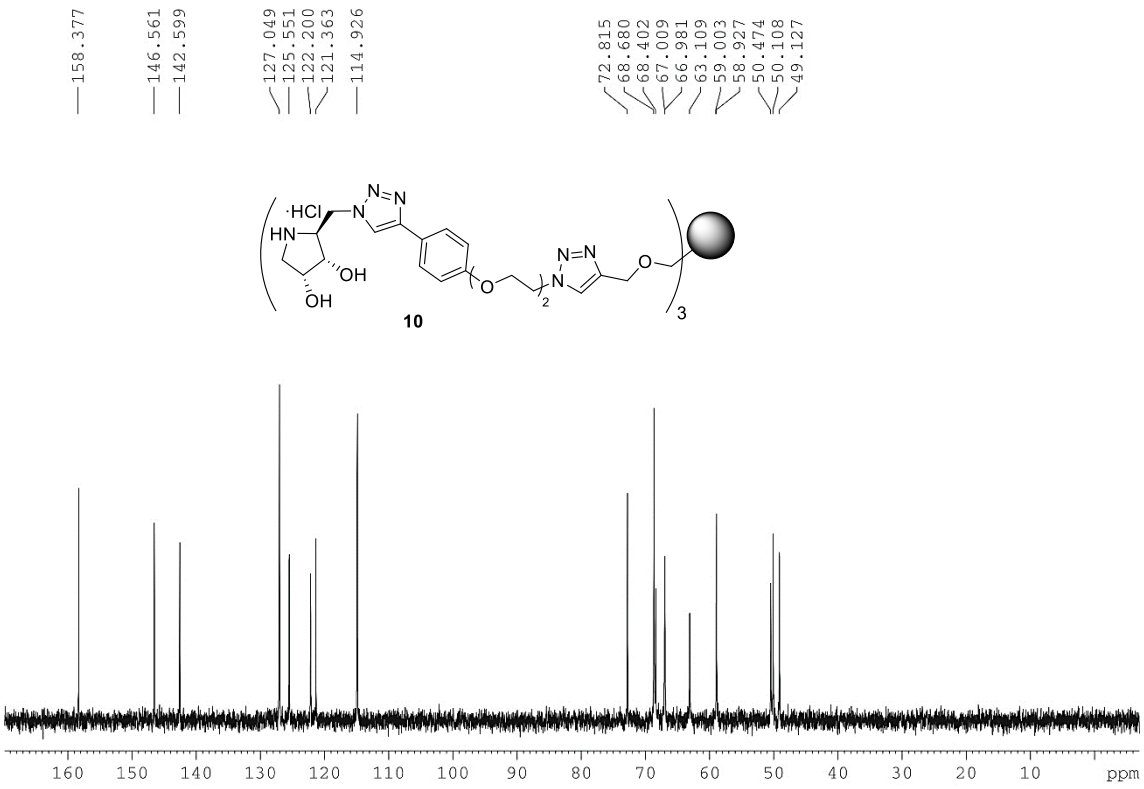


* Signals due to partial protonation

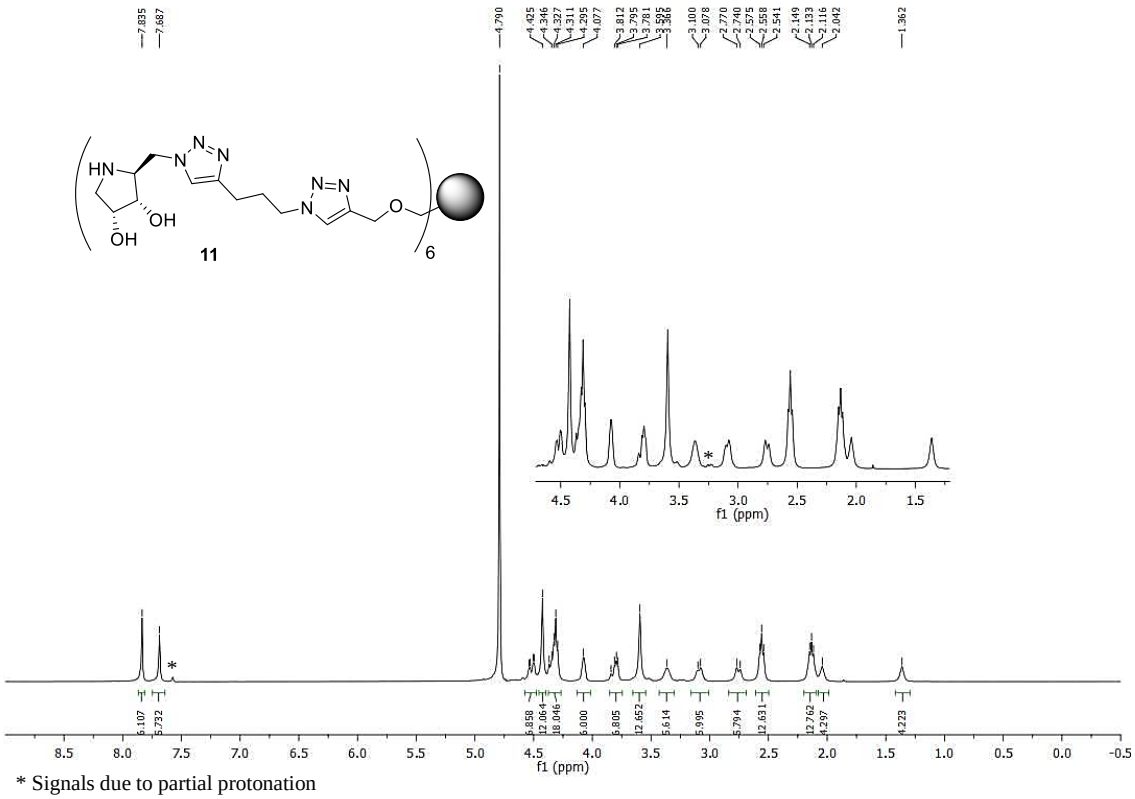
^{13}C-NMR (50 MHz, D_2O)



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

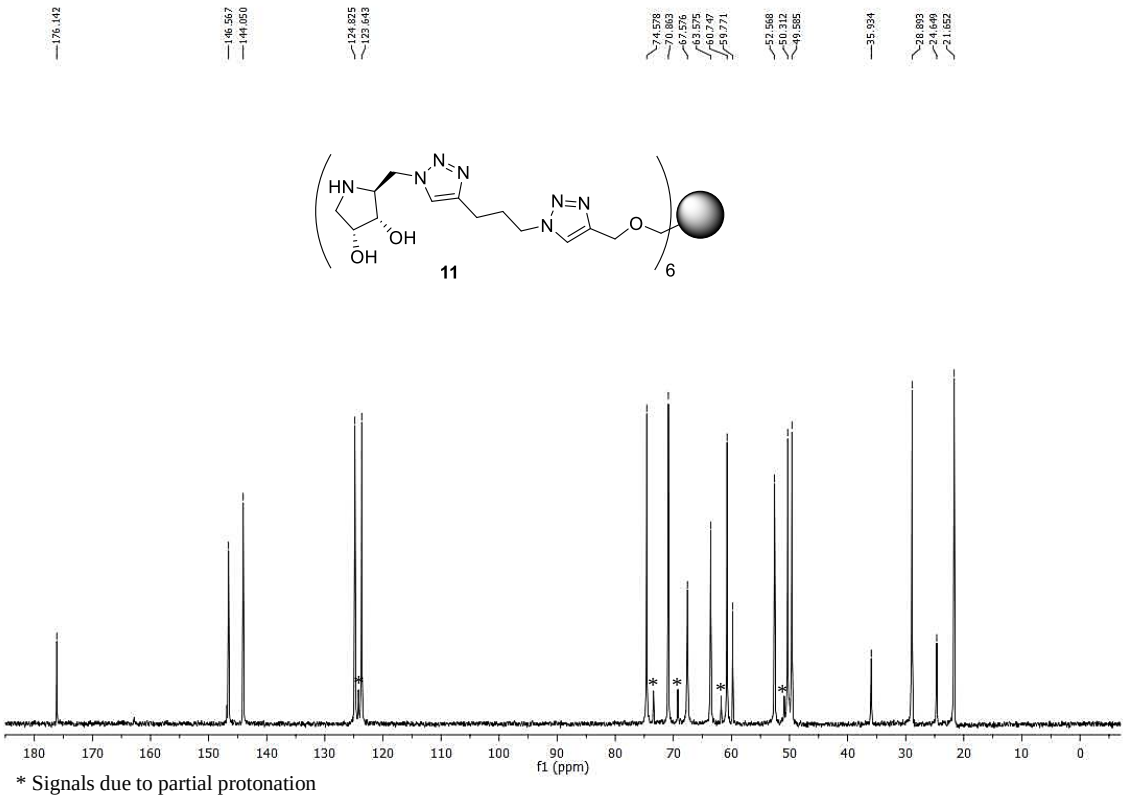


^{13}C-NMR (75.4 MHz, D_2O)



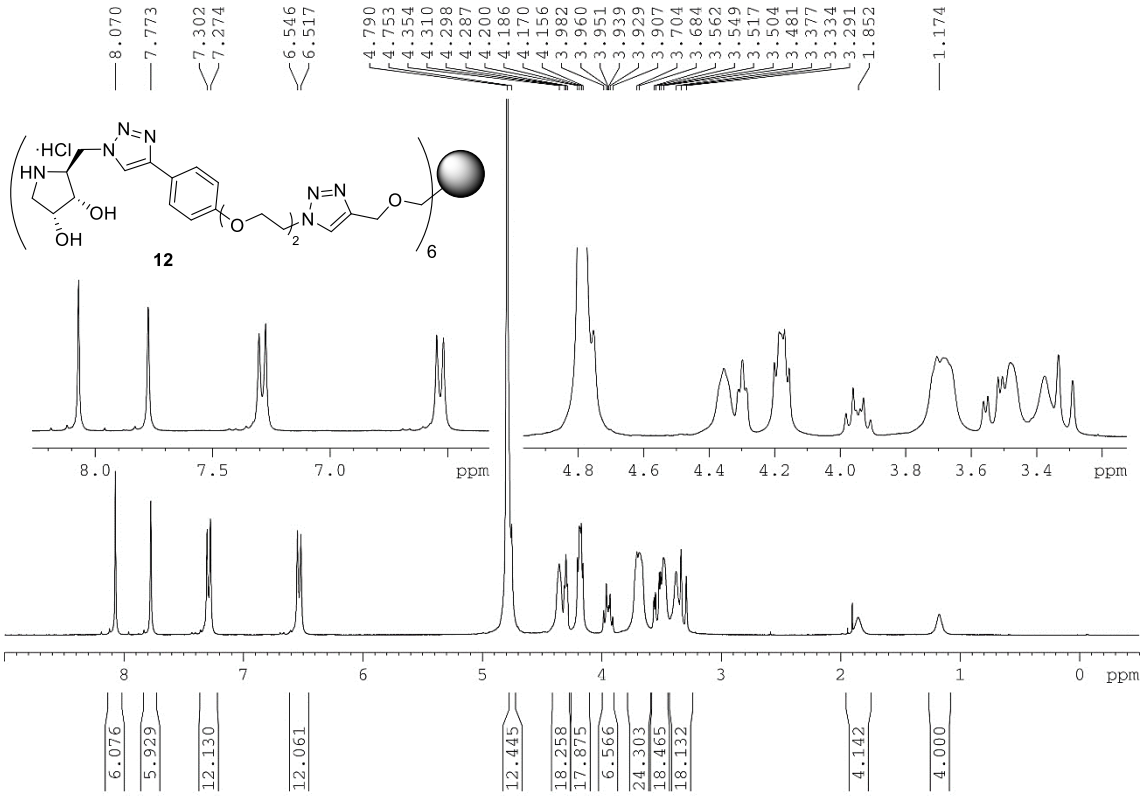
* Signals due to partial protonation

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

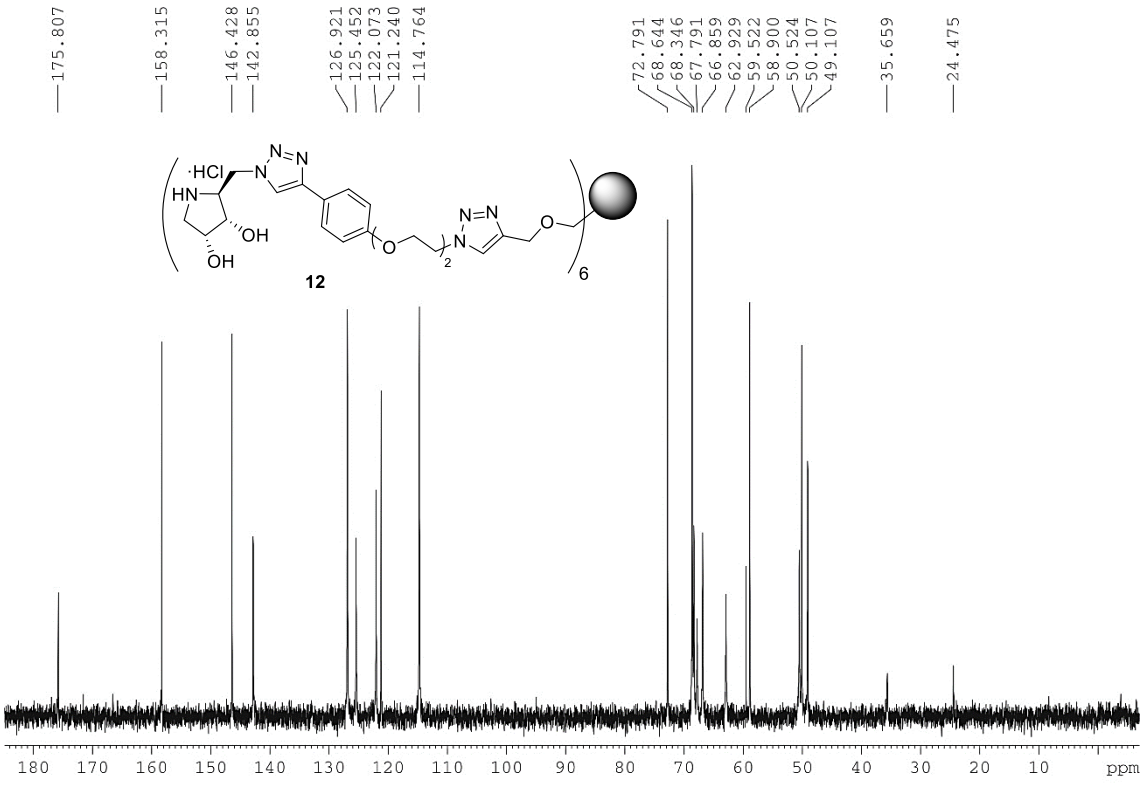


* Signals due to partial protonation

^{13}C-NMR (50 MHz, D_2O)



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



^{13}C-NMR (75.4 MHz, D_2O)

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