Supplementary Material for:

Orthogonal Optical Control of a G Protein-Coupled Receptor with a SNAP-Tethered Photochromic Ligand

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Author Contributions [§]J.B., A.D., and J.L.: equal contribution.

Contents

1 Experimental

1.1 General

Solvents for chromatography and reactions were purchased HPLC grade (except DMF was purchased from Acros, 99.8 %, extra dry over molecular sieves) or distilled over an appropriate drying reagent prior to use. If necessary, solvents were degassed either by freeze-pump-thaw or by bubbling N_2 through the vigorously stirred solution for several minutes. Unless otherwise stated, all other reagents were used without further purification from commercial sources.

Flash column chromatography was carried out on silica gel 60 (0.040–0.063 mm) purchased from Merck. Reactions and chromatography fractions were monitored by thin layer chromatography (TLC) on Merck silica gel 60 F254 glass plates. The plates were visualized under UV light at 254 nm.

NMR spectra were recorded in deuterated solvents on a BRUKER Avance III HD 400 (equipped with a CryoProbe™) instruments and calibrated to residual solvent peaks ($^{1}H/^{13}C$ in ppm): DMSO-d_6 (2.50/39.52), Me_3OD-d_4 (3.31/49.00). Multiplicities are abbreviated as follows: s = singlet, d = doublet, t = triplet, q = quartet, br = broad, m = multiplet. Spectra are reported based on appearance, not on theoretical multiplicities derived from structural information.

High-resolution electrospray (ESI) mass spectra were obtained on a Varian MAT 711 MS instrument operating in either positive or negative ionization modes.

LC-MS was performed on an Agilent 1260 Infinity HPLC System, MS-Agilent 1100 Series, Type: 1946D, Model: SL, equipped with a Agilent Zorbax Eclipse Plus C18 (100 x 4.6 mm, particle size 3.5 micron) RP column with a constant flow-rate of 2 mL/min, if not stated otherwise. Retention times (t_R) are given in minutes (min).

HPLC was performed on a Varian Prep Star HPLC System, Model SD-1 equipped with Varian Dynamax columns (RP-Analytical: Microsorb 60 C18, 250 x 4.6 mm, particle size 8 µm; RP-SemiPrep: Microsorb 60 C18, 250 x 21.4 mm, particle size 8 µm; RP-Prep: Microsorb 60 C18, 250 x 41.4 mm, particle size 8 µm). Prior to injection, samples were filtered through a syringe filter (Chromafil Xtra GF100/25, pore size 1 µm).

1.2 Synthesis of BGAGs

A. General procedure for attachement of the Azide-functionalized PEG-linker

In a schlenk flask, **8** or *N*-Boc-*D*-redAG0 **10 (**1.1 equiv.), Azido-$(PEG)_n$-NHS-ester (Baseclick, n = 4 (**11**, #BCL-001), n = 8 (**12**, #BCL-032), n = 12 (**13**, #BCL-033); 1.0 equiv.) and DIPEA (2.0 equiv.) were added to degassed, anhydrous DMF (0.5 mL) under N_2-atmosphere. The reaction mixture was stirred at r.t. while reaction progress was monitored by LCMS. Upon completion, the crude reaction mixture was purified by C18 reverse phase (RP) flash column chromatography (100/0 → 60/40 = 1 mM HCl/MeCN) or by RP-HPLC (MeCN/H_2O/formic acid = 10/90/0.1 → 80/20/0.1 over 42 min). The product containing fractions were combined, concentrated *in vacuo* and freeze-dried to obtain the desired product.

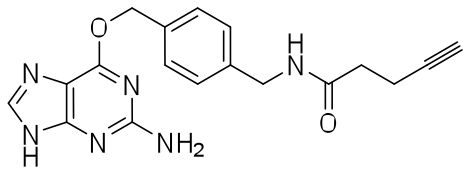
B. General procedure for click reaction

In a schlenk flask, the reaction mixture was prepared with the corresponding azide (1.0 equiv.) and BG-alkyne **(SI2.1)** (1.1 eq) in degassed DMSO (2−4 mL) under N_2-atmosphere. Stock solutions of sodium ascorbate (NaAsc, 131 mM) and copper-(II)-sulfate pentahydrate (105 mM) were prepared separately in degassed water. 50 µL of each stock solution were mixed under N_2 to preform the catalytically active Cu^I species and quickly transferred to the reaction mixture. The resulting reaction mixture was heated to 90 °C and reaction progress was monitored by LCMS. Upon completion, the crude product was purified by RP flash column chromatography (100/0 → 60/40 = 1 mM HCl/MeCN) or RP-HPLC (MeCN/H_2O/formic acid = 10/90/0.1 → 80/20/0.1 over 42 min). The product containing fractions were combined, concentrated *in vacuo* and freeze-dried to obtain the desired product.

C. General procedure for Boc-deprotection:

In a falcon tube, neat TFA (250 µL) was added to the Boc-protected molecule and stirred at r.t. for 10 min. Diethylether (50 mL) was added and the resulting suspension was centrifuged (4000 rpm, 20 min, 4 °C). The supernatant was discarded, the solid was washed again with diethylether and finally dried under high vacuum to obtain the desired product.

1.2.1 *N*-(4-(((2-Amino-9*H*-purin-6-yl)oxy)methyl)benzyl)pent-4-ynamide (3)



6-((4-(Aminomethyl)benzyl)oxy)-9*H*-purin-2-amine[1] (**BG**) (382 mg, 1.41 mmol, 1.2 equiv.), 4-pentynoic acid (**2**) (115 mg, 1.17 mmol, 1.0 equiv.), HBTU (489 mg, 1.29 mmol, 1.1 equiv.) and DIPEA (302 mg, 2.34 mmol, 409 µL, 2.0 equiv.) were dissolved in DMF (5 mL). Reaction progress was monitored by LCMS and after completion, the crude reaction mixture was subjected to RP-HPLC (MeCN/H_2O/FA = 10/90/0.1 → 80/20/0.1 over 40 min). The product containing fractions were combined, concentrated *in vacuo* and dried under high vacuum to obtain the product BG-alkyne (**3**) (178 mg, 0.508 mmol) as a white powder in 43% yield.

NMR spectroscopy revealed two rotamers, proven by heating the NMR sample to 50 °C and merging of the spectroscopic signals (data not shown). Peaks are reported for the major rotamer.

^{1}H NMR (400 MHz, DMSO-d_6): δ [ppm] = 8.45–8.34 (m, 1H), 8.13 (s, 1H), 8.10 (s, 1H), 7.47 (d, *J* = 8.4 Hz, 2H), 7.29 (d, *J* = 8.4 Hz, 2H), 6.59 (br s, 2H), 5.48 (s, 2H), 4.32–4.21 (m, 2H), 2.78 (m, 1H), 2.43–2.28 (m, 4H).

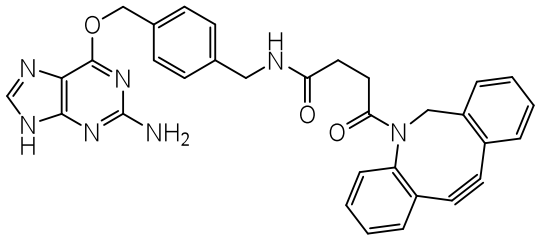
^{13}C NMR (101 MHz, DMSO-d_6): δ [ppm] = 170.3, 159.2, 159.1, 155.0, 141.0, 139.5, 134.7, 128.7, 127.3, 126.4, 83.8, 71.4, 67.2, 41.9, 34.2, 14.3.

HRMS (ESI): calc. for $C_{18}H_{19}N_6O_2^+$ $(M+H)^+$: 351.1564, found: 351.1562.

UV/Vis (LCMS): λ_{max1} = 196 nm, λ_{max2} = 212 nm, λ_{max3} = 287 nm.

t_R (LCMS; MeCN/H_2O/formic acid = 10/90/0.1 → 90/10/0.1 over 7 min) = 1.955 min.

1.2.2 *N*-(4-(((2-Amino-9*H*-purin-6-yl)oxy)methyl)benzyl)-4-(11,12-dehydrodibenzo[b,f]azocin-5(6*H*)-yl)-4-oxobutanamide (5)



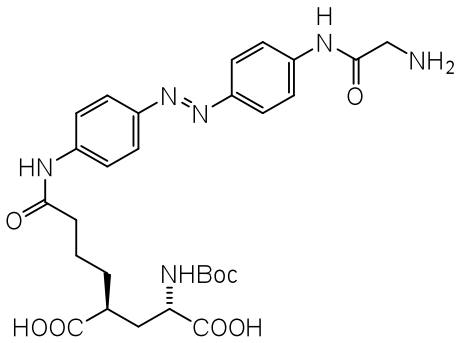
6-((4-(Aminomethyl)benzyl)oxy)-9*H*-purin-2-amine[1] (**BG**) (6.7 mg, 24.9 µmol, 1.0 equiv.), DBCO-NHS-ester (Jena Bioscience, CLK-A133-25, **4**) (10.0 mg, 24.9 µmol, 1.0 equiv.) and DIPEA (6.4 mg, 49.8 µmol, 8.7 µL, 2.0 equiv.) were dissolved in DMSO (0.5 mL). Reaction progress was monitored by LCMS and after completion the crude reaction mixture was subjected to RP-HPLC (MeCN/H_2O/FA = 5/95/0.1 → 80/20/0.1 over 40 min). The product containing fractions were combined, concentrated *in vacuo* and dried under high vacuum to obtain **5** (1.0 mg, 1.79 µmol) in 7% yield. The low yield can be attributed to residual water in the used DMSO as the corresponding DBCO-acid was obtained as the main product.

HRMS (ESI): calc. for $C_{32}H_{28}N_7O_3^+$ $[M+H]^+$: 558.2248, found: 558.2254.

UV/Vis (LCMS): λ_{max} = 290 nm.

t_R (LCMS; MeCN/H_2O/formic acid = 10/90/0.1 → 90/10/0.1 over 7 min) = 3.118 min.

1.2.3 (2*S*,4*S*)-2-(4-((4-((*E*)-(4-(2-Aminoacetamido)phenyl)diazenyl)phenyl)amino)-4-oxobutyl)-4-((*tert*-butoxycarbonyl)amino)pentanedioic acid (6)



6 was prepared according to a literature procedure and analytical data matched the one reported.[2]

^{1}H-NMR (400 MHz, DMSO-d_6) δ [ppm] = 10.29 (s, 1H), 7.93–7.75 (m, 9H), 6.60 (d, *J* = 7.8 Hz, 1H), 3.90 (q, *J* = 8.1 Hz, 1H), 3.78 (s, 2H), 2.44–2.25 (m, 3H), 1.88–1.42 (m, 6H), 1.36 (s, 9H).

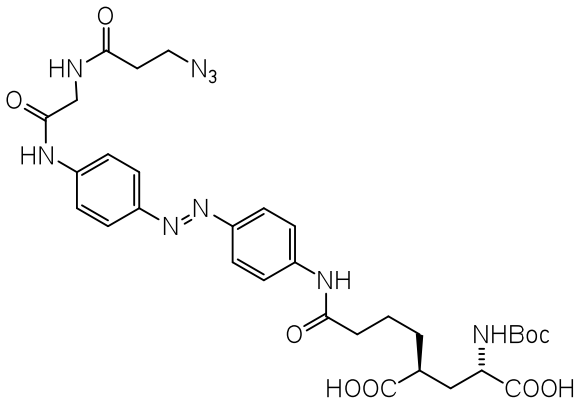
^{13}C NMR (101 MHz, DMSO-d_6) δ [ppm] = 176.3, 174.0, 171.5, 165.9, 155.1, 148.0, 147.4, 142.2, 140.9, 123.5, 123.4, 119.4, 119.2, 77.9, 55.0, 52.0, 41.6, 40.1, 36.5, 30.9, 28.2, 23.0.

HRMS (ESI): calc. for $C_{28}H_{37}N_6O_8^+$ $[M+H]^+$: 585.2667, found: 585.2673.

UV/VIS (LCMS): λ_{max} ($\pi \rightarrow \pi^*$) = 368 nm.

t_R (LCMS, MeCN/H_2O/formic acid = 90/10/0.1 → 10/90/0.1 over 7 min) = 2.418 min.

1.2.4 (2*S*,4*S*)-2-(4-((4-((*E*)-(4-(2-(3-azidopropanamido)acetamido)phenyl)diazenyl)phenyl)-amino)-4-oxobutyl)-4-((*tert*-butoxycarbonyl)amino)pentanedioic acid (14)



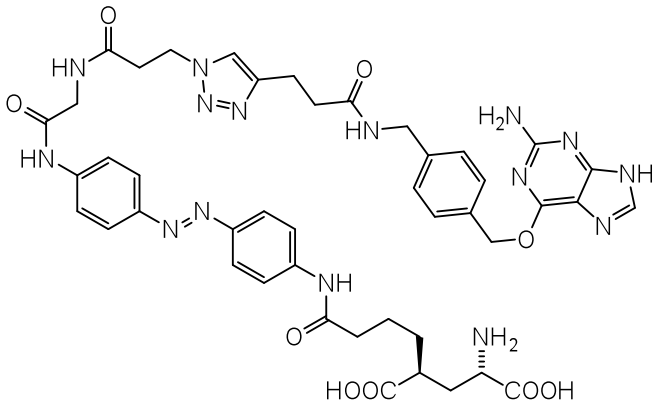
A schlenk flask was charged with **8** (10 mg, 17 µmol, 1.0 equiv.), HBTU (7.2 mg, 19 µmol, 1.1 equiv.) and 3-azidopropionic acid[3] (3.0 mg, 16 µmol, 1.5 equiv.) under a N_2-atmosphere. Degassed, anhydrous DMF (1 mL) was added and the reaction mixture was cooled to 0 °C before DIPEA (6.0 µL, 34 µmol, 2.0 equiv.) was added dropwise. The reaction mixture was allowed to warm to r.t. and reaction progress was monitored by LCMS. Upon completion, the crude reaction mixture was purified by RP-HPLC (10/90/0.1 →80/20/0.1 = MeCN/H_2O/formic acid over 45 min). The product containing fractions were combined, concentrated *in vacuo* and freeze-dried to obtain 10 mg (15 µmol) of **14** as a yellow solid in 88% yield.

^{1}H NMR (400 MHz, DMSO-d_6) δ [ppm] = 10.34 (s, 1H), 10.25 (s, 1H), 8.43 (t, *J* = 5.8 Hz, 1H), 7.98–7.71 (m, 8H), 3.96 (d, *J* = 5.7 Hz, 2H), 3.89 (t, *J* = 7.3 Hz, 1H), 3.53 (t, *J* = 6.4 Hz, 2H), 2.67 (s, 1H), 2.43–2.23 (m, 4H), 1.86–1.42 (m, 6H), 1.36 (s, 9H).

HRMS (ESI): calc. for $C_{31}H_{38}N_9O_9^-$ $[M-H]^-$: 680.2798, found: 680.2798.

t_R (RP-HPLC, MeCN/H_2O/formic acid = 10/90/0.1 → 80/20/0.1 over 42 min)= 26.2 min (*trans*)

1.2.5 (2*S*,4*S*)-2-Amino-4-(4-((4-((*E*)-(4-(2-(3-(4-(3-((4-(((2-amino-9*H*-purin-6-yl)oxy)methyl) benzyl)amino)-3-oxopropyl)-1*H*-1,2,3-triazol-1-yl)propanamido)acetamido)-phenyl)diazenyl)phenyl)amino)-4-oxobutyl)pentanedioic acid, $BGAG_0$



$BGAG_0$ was prepared according to general procedures B and C.

Amounts:

3 (5.8 mg, 17 µmol, 1.1 equiv.)

14 (10 mg, 15 µmol, 1.0 equiv.)

yield: 3.3 mg (3.5 µmol, 23% over two steps), yellow solid.

^{1}H NMR (400 MHz, DMSO-d_6) δ 10.52 (s, 1H), 10.33 (s, 1H), 10.27 (s, 1H), 8.51–8.24 (m, 3H), 7.96–7.59 (m, 8H), 7.52 (dd, *J* = 8.9, 3.1 Hz, 1H), 7.41 (m, 2H), 7.23 (t, *J* = 9.0 Hz, 3H), 7.18–7.06 (m, 1H), 6.98 (s, 1H), 6.82 (t, *J* = 7.4 Hz, 1H), 6.29 (br s, 2H), 5.42 (br s, 2H), 4.52 (t, *J* = 7.0 Hz, 2H), 4.27 (t, *J* = 4.9 Hz, 2H), 3.94 (d, *J* = 5.7 Hz, 2H), 3.60 (s, 1H), 3.16 (d, *J* = 4.0 Hz, 1H), 2.90–2.83 (m, 2H), 2.79 (t, *J* = 6.8 Hz, 2H), 2.67 (p, *J* = 1.7 Hz, 1H), 2.41–2.31 (m, 3H), 1.88–1.33 (m, 6H).

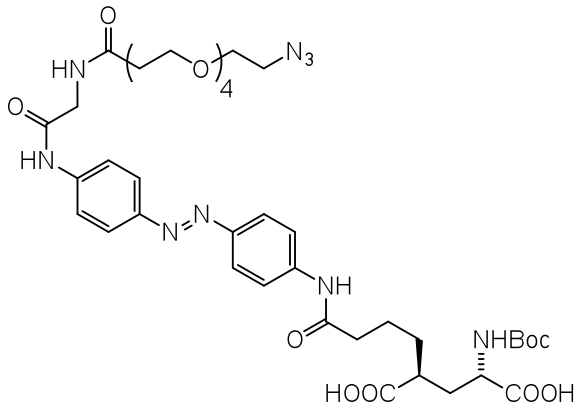
HRMS (ESI): calc. for $C_{44}H_{48}N_{15}O_9^-$ $[M-H]^-$: 930.3765, found: 930.3770.

t_R (RP-HPLC, MeCN/H_2O/formic acid = 10/90/0.1 → 80/20/0.1 over 42 min) = 18.4 min (*trans*, before Boc-deprotection).

UV/VIS (LCMS): λ_{max} ($\pi \rightarrow \pi^*$) = 368 nm.

t_R (LCMS, MeCN/H_2O/formic acid = 90/10/0.1 → 10/90/0.1 over 10 min, flow: 1 ml/min) = 3.960 min.

1.2.6 (2*S*,4*S*)-2-(4-((4-((*E*)-(4-((1-Azido-15-oxo-3,6,9,12-tetraoxa-16-azaoctadecan-18-yl)amino)phenyl)diazenyl)phenyl)amino)-4-oxobutyl)-4-((*tert*-butoxycarbonyl)-amino)pentanedioic acid (15)



15 was prepared according to general procedure A.

Amounts:

N_3-PEG_4-NHS-ester (#BCL-001, 8.6 mg, 22 µmol, 1.1 equiv.)

8 (14.3 mg, 24 µmol, 1.0 equiv.)

DIPEA (7.7 µL, 44 µmol, 2.0 equiv.)

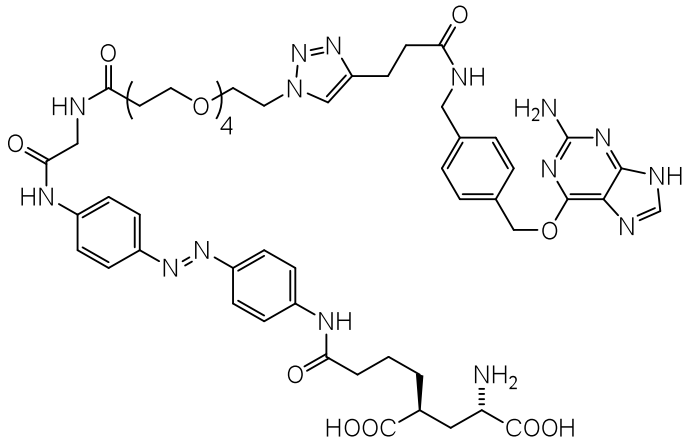
yield: 18.0 mg (21 µmol, 86%), yellow solid.

HRMS (ESI): calc. for $C_{39}H_{54}N_9O_{13}^-$ $[M-H]^-$: 856.3847, found: 856.3844.

UV/VIS (LCMS): λ_{max} ($\pi \rightarrow \pi^*$) = 368 nm.

t_R (LCMS, MeCN/H_2O/formic acid = 90/10/0.1 → 10/90/0.1 over 10 min) = 5.130 min.

1.2.7 (2*S*,4*S*)-2-Amino-4-(4-((4-((*E*)-(4-(1-(4-(3-((4-(((2-amino-9*H*-purin-6-yl)oxy)methyl)-benzyl)amino)-3-oxopropyl)-1*H*-1,2,3-triazol-1-yl)-15-oxo-3,6,9,12-tetraoxa-16-aza-octadecan-18-amido)phenyl)diazenyl)phenyl)amino)-4-oxobutyl)-pentanedioic acid, $BGAG_4$



$BGAG_4$ was prepared according to general procedures B and C.

Amounts:

3 (7.8 mg, 23 µmol, 1.1 equiv.)

15 (18 mg, 21 µmol, 1.0 equiv)

yield: 5.1 mg (5 µmol, 24% over two steps).

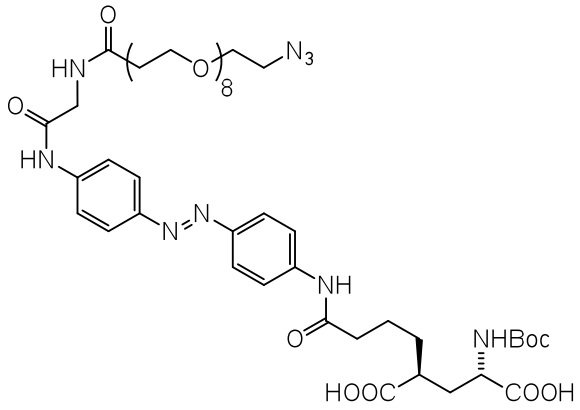
^{1}H NMR (400 MHz, DMSO-d_6) δ [ppm] =10.58 (s, 1H), 10.33 (s, 1H), 10.30 (s, 1H), 8.37 (t, *J* = 6.2 Hz, 2H), 8.28 (t, *J* = 5.8 Hz, 2H), 7.89–7.69 (m, 12H), 7.23 (d, *J* = 7.9 Hz, 2H), 7.14 (d, *J* = 7.9 Hz, 2H), 6.28 (s, 2H), 4.44 (t, *J* = 5.1 Hz, 4H), 4.23 (d, *J* = 5.9 Hz, 2H), 3.93 (d, *J* = 5.7 Hz, 2H), 3.77 (t, *J* = 5.3 Hz, 3H), 3.62 (t, *J* = 6.5 Hz, 2H), 3.54–3.44 (m, 12H), 2.86 (t, *J* = 7.6 Hz, 2H), 2.45–2.30 (m, 5H), 1.97–1.72 (m, 3H), 1.70–1.39 (m, 6H).

HRMS (ESI): calc. for $C_{52}H_{66}N_{15}O_{13}^+$ $[M+H]^+$: 1108.4959,found: 1108.4943.

UV/VIS (LCMS): λ_{max} ($\pi \rightarrow \pi^*$) = 368 nm.

t_R (LCMS, MeCN/H_2O/formic acid = 90/10/0.1 → 10/90/0.1 over 10 min) = 2.927 min.

1.2.8 (2*S*,4*S*)-2-(4-((4-((*E*)-(4-((1-Azido-27-oxo-3,6,9,12,15,18,21,24-octaoxa-28-azatriacontan-30-yl)amino)phenyl)diazenyl)-phenyl)amino)4-oxobutyl)-4-((*tert*-butoxycarbonyl)-amino)pentanedioic acid (16)



16 was prepared according to general procedure A.

Amounts:

N_3-PEG_8-NHS-ester (#BCL-032, 10.0 mg, 17.7 µmol, 1.0 equiv.)

8 (11.4 mg, 19.5 µmol, 1.1 equiv.)

DIPEA (6.2 µL, 35.4 µmol, 2.0 equiv.)

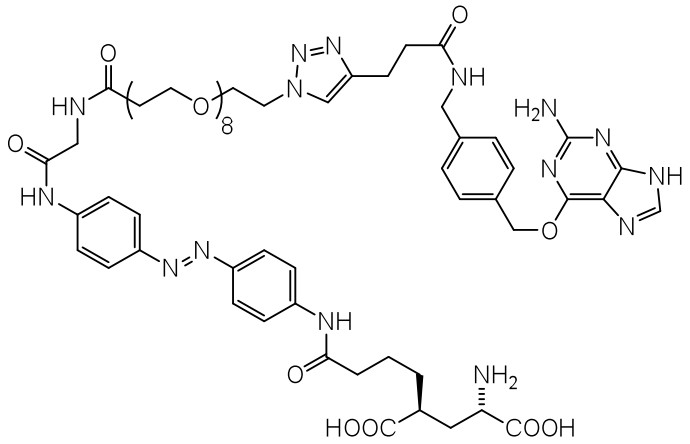
yield: 18.0 mg (17 µmol, 89%), yellow solid.

HRMS (ESI): calc. for $C_{47}H_{69}N_9O_{17}^{2-}$ $[M\text{-}2H]^{2-}$: 515.7411, found: 515.7407.

UV/VIS (LCMS): λ_{max} ($\pi \rightarrow \pi^*$) = 368 nm.

t_R (LCMS, MeCN/H_2O/formic acid = 90/10/0.1 → 10/90/0.1 over 10 min) = 4.998 min.

1.2.9 (2*S*,4*S*)-2-Amino-4-(4-((4-((*E*)-(4-(1-(4-(3-((4-(((2-amino-9*H*-purin-6-yl)oxy)methyl)-benzyl)amino)-3-oxopropyl)-1*H*-1,2,3-triazol-1-yl)-27-oxo-3,6,9,12,15,18,21,24-octa-oxa-28-azatriacontan-30-amido)phenyl)diazenyl)phenyl)amino)-4-oxobutyl)pentane-dioic acid, $BGAG_8$



$BGAG_8$ was prepared according to general procedures B and C.

Amounts:

3 (7.1 mg, 21 µmol, 1.2 equiv.)

16 (18 mg, 17 µmol, 1.0 equiv.)

yield: 9.6 mg (7.5 µmol, 44% over two steps), yellow solid.

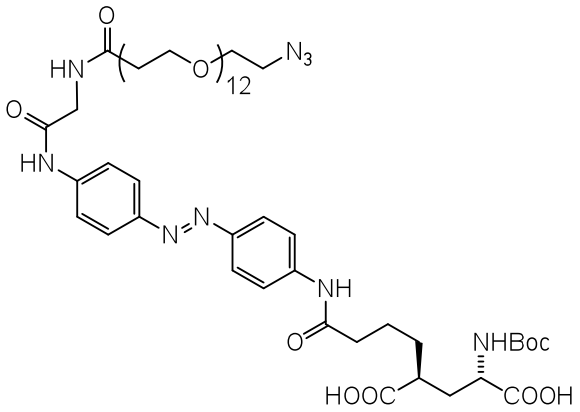
^{1}H NMR (400 MHz, DMSO-d_6) δ 10.91 (s, 1H), 10.32 (s, 1H), 10.29 (s, 1H), 8.43–8.20 (m, 5H), 8.09 (s, 1H), 7.89–7.72 (m, 10H), 7.23 (d, *J* = 7.7 Hz, 2H), 7.14 (d, *J* = 7.7 Hz, 2H), 6.55 (s, 2H), 4.73 (s, 1H), 4.50–4.39 (m, 4H), 4.23 (d, *J* = 6.3 Hz, 2H), 3.93 (d, *J* = 5.8 Hz, 2H), 3.77 (t, *J* = 5.3 Hz, 2H), 3.62 (t, *J* = 6.6 Hz, 2H), 3.49 (d, *J* = 4.6 Hz, 28H), 2.86 (t, *J* = 7.7 Hz, 2H), 2.46–2.31 (m, 6H), 2.10–1.96 (m, 1H), 1.85 (dt, *J* = 14.2, 7.0 Hz, 1H), 1.58 (d, *J* = 6.3 Hz, 5H).

HRMS (ESI): calc. for $C_{60}H_{82}N_{15}O_{17}^+$ $[M+H]^+$: 1284.6008, found: 1284.6004.

UV/VIS (LCMS): λ_{max} ($\pi \rightarrow \pi^*$)= 368 nm.

t_R (LCMS, MeCN/H_2O/formic acid = 90/10/0.1 → 10/90/0.1 over 10 min) = 3.059 min.

1.2.10 (2*S*,4*S*)-2-(4-((4-((*E*)-(4-(1-Azido-39-oxo-3,6,9,12,15,18,21,24,27,30,33,36-dodecaoxa-40-azadotetracontan-42-amido)phenyl)diazenyl)phenyl)amino)-4-oxobutyl)-4-((*tert*-butoxycarbonyl)amino)pentanedioic acid (17)



17 was prepared according to general procedure A.

Amounts:

N_3-PEG_{12}-NHS-Ester (Baseclick #BCL-033, 10.0 mg, 13.5 µmol, 1.0 equiv.)

8 (8.7 mg, 14.8 µmol, 1.1 equiv.)

DIPEA (3.5 mg, 27.0 µmol, 4.7 µL, 2.0 equiv.)

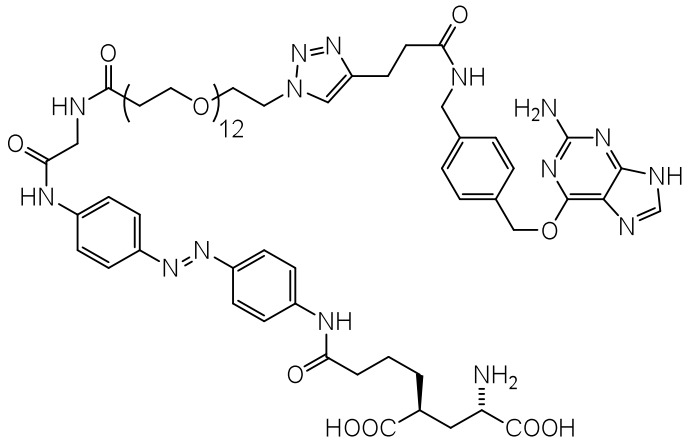
yield: 13.0 mg (10.8 µmol, 80%), yellow solid.

HRMS (ESI): calc. for $C_{55}H_{85}N_9O_{21}^{2-}$ $[M\text{-}2H]^{2-}$: 603.7936, found: 603.7937.

UV/Vis (LCMS): λ_{max} ($\pi \rightarrow \pi^*$) = 368 nm.

t_R (LCMS; MeCN/H_2O/formic acid = 10/90/0.1 → 90/10/0.1 over 7 min) = 3.232 min.

1.2.11 (2*S*,4*S*)-2-Amino-4-(4-((4-((*E*)-(4-(1-(4-(3-((4-(((2-amino-9*H*-purin-6-yl)oxy)methyl)benzyl)amino)-3-oxopropyl)-1*H*-1,2,3-triazol-1-yl)-39-oxo-3,6,9,12,15,18,21,24,27,30,33,36-dodecaoxa-40-azadotetracontan-42-amido)phenyl)diazenyl)phenyl)amino)-4-oxobutyl)pentanedioic acid, $BGAG_{12}$



$BGAG_{12}$ was prepared according to general procedures B and C.

Amounts:

3 (4.2 mg, 11.9 µmol, 1.2 equiv.)

17 (12.0 mg, 9.9 µmol, 1.0 equiv.)

yield: 6.8 mg (7.06 µmol, 65% over two steps), orange solid.

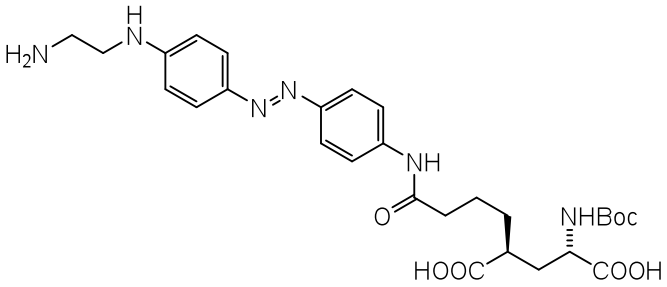
^{1}H NMR (400 MHz, DMSO-d_6): δ [ppm] =10.30 (s, 1H), 10.26 (s, 1H), 8.47 (br s, 1H), 8.81 (t, *J* = 6.0 Hz, 1H), 8.36–8.16 (m, 3H), 7.93–7.68 (m, 8H), 7.48 (d, *J* = 6.0 Hz, 2H), 7.24 (d, *J* = 6.0 Hz, 2H), 6.88–6.74 (m, 1H), 5.53 (br s, 2H), 4.44 (t, *J* = 5.2 Hz, 2H), 4.27 (d, *J* = 6.0 Hz, 2H), 3.94 (d, *J* = 5.6 Hz, 2H), 3.87–3.78 (m, 1H), 3.76 (t, *J* = 5.2 Hz, 2H), 3.63 (t, *J* = 6.8 Hz, 2H), 3.56–3.41 (m, 44H), 2.86 (t, *J* = 8.0 Hz, 2H), 2.62–2.55 (m, 2H), 2.46–2.28 (m, 7H), 2.16–1.91 (m, 2H), 1.90–1.74 (m, 1H), 1.66–1.51 (m, 4H), 1.48–1.28 (m, 2H).

HRMS (ESI): calc. for $C_{68}H_{99}N_{15}O_{21}^{2+}[M+2H]^{2+}$: 730.8565, found: 730.8564.

UV/Vis (LCMS): λ_{max} ($\pi \rightarrow \pi^*$) = 369 nm.

t_R (LCMS; MeCN/H_2O/formic acid = 10/90/0.1 → 90/10/0.1 over 7 min) = 2.463 min.

1.2.12 (2*S*,4*S*)-2-(4-((4-((*E*)-(4-((2-aminoethyl)amino)phenyl)diazenyl)phenyl)amino)-4-oxobutyl)-4-((*tert*-butoxycarbonyl)amino)pentanedioic acid (10)



Dimethyl (2S,4S)-2-(4-((4-((E)-(4-aminophenyl)diazenyl)phenyl)amino)-4-oxobutyl)-4-((tert-butoxycarbonyl)amino)pentanedioate[2] (200 mg, 0.360 mmol, 1.0 equiv.) and Fmoc-2-aminoacetaldehyde[4] (101 mg, 0.360 mmol, 1.0 equiv.) were dissolved in DCE (10 mL) and one drop of acetic acid was added. Sodium triacetoxyborohydride (270 mg, 1.28 mmol, 3.6 equiv.) was added portionwise and the reaction mixture was stirred at r.t. for 4.5 h. The reaction mixture was quenched and subsequently washed with sat. aq. $NaHCO_3$ (3x) before the organic layer was concentrated *in vacuo*. The residue was redissolved in DMF (47.5 mL), piperidine (2.5 mL) was added and the deprotecting reaction was stirred overnight. The reaction mixture was concentrated *in vacuo*, the residue was redissolved in EtOAc, washed with sat. aq. $NaHCO_3$ (2x) and brine (sat.) before the organic layer was dried over $MgSO_4$. The solvent was removed *in vacuo* before purification by flash column chromatography (90/10/1 = DCM/MeOH/TEA) followed by RP column chromatography (100/0 → 60/40 = 1mM HCl/MeCN). The product containing fractions were combined, concentrated *in vacuo* and freeze-dried to obtain the intermediate bis-methylester dimethyl (2*S*,4*S*)-2-(4-((4-((*E*)-(4-((2-aminoethyl)amino)phenyl)diazenyl)phenyl)amino)-4-oxobutyl)-4-((*tert*-butoxycarbonyl)amino) pentanedioate as its red-orange HCl salt (84 mg, 0.13 mmol) in 36% yield over 2 steps.

^{1}H-NMR (400 MHz, MeOD-d_4) δ [ppm] = 7.82–7.66 (m, 6H), 6.80 (d, *J* = 8.9 Hz, 2H), 4.16 (dd, *J* = 8.4, 6.2 Hz, 1H), 3.71–3.65 (m, 6H), 3.55 (t, *J* = 6.1 Hz, 2H), 3.18 (t, *J* = 6.8 Hz, 2H), 2.52 (p, *J* = 6.9 Hz, 1H), 2.44–2.35 (m, 2H), 2.01–1.90 (m, 2H), 1.73–1.57 (m, 3H), 1.42 (s, 9H), 1.29 (q, *J* = 6.6, 5.9 Hz, 1H).

^{13}C-NMR (101 MHz, MeOH-d_4) δ [ppm] = 177.3, 174.3, 174.0, 157.9, 152.8, 149.7, 145.6, 141.5, 126.5, 123.7, 121.2, 113.7, 80.7, 53.4, 52.7, 52.4, 43.5, 41.7, 39.8, 37.6, 34.8, 32.6, 28.7, 24.2.

HRMS (ESI): calc. for $C_{30}H_{43}O_7N_6^+$ $[M+H]^+$: 599.3188, found: 599.3191.

UV/VIS (LCMS): λ_{max} = 412 nm.

t_R (LCMS, MeCN/H_2O/formic acid = 90/10/0.1 → 10/90/0.1 over 7 min) = 2.915 min.

In a round bottom flask, dimethyl (2*S*,4*S*)-2-(4-((4-((*E*)-(4-((2-aminoethyl)amino)phenyl)-diazenyl)phenyl)amino)-4-oxobutyl)-4-((*tert*-butoxycarbonyl)amino) pentanedioate (84 mg, 0.13 mmol, 1.0 equiv.) was dissolved in a mixture of H_2O (3.7 mL) and THF (7.3 mL) before lithium hydroxide (78 mg, 3.3 mmol, 25 equiv.) was added as a solid at 0 °C in one portion. The reaction was stirred for 2 h at 0 °C ,

before it was neutralized by addition of formic acid (0.12 mL, 3.3 mmol, 25 equiv.). The THF was removed *in vacuo* before the aqueous phase was subjected to RP column chromatography (100/0 → 75/25 = 1 mM HCl/MeCN). The product containing fractions were combined, concentrated i*n vacuo* and freeze-dried to obtain **10** as its red HCl salt (33.5 mg, 55 µmol) in 43% yield.

^{1}H-NMR (400 MHz, DMSO-d_6) δ [ppm] 10.18 (s, 1H), 7.80–7.64 (m, 6H), 6.82 (s, 1H), 6.75 (d, *J* = 8.6 Hz, 2H), 6.51 (d, *J* = 7.7 Hz, 1H), 3.91 (q, *J* = 8.2 Hz, 1H), 3.46–3.36 (m, 2H), 3.02 (t, *J* = 6.3 Hz, 2H), 2.47–2.37 (m, 1H), 2.32 (t, *J* = 7.2 Hz, 2H), 1.72–1.44 (m, 5H), 1.37 (s, 9H), 1.34 (s, 1H).

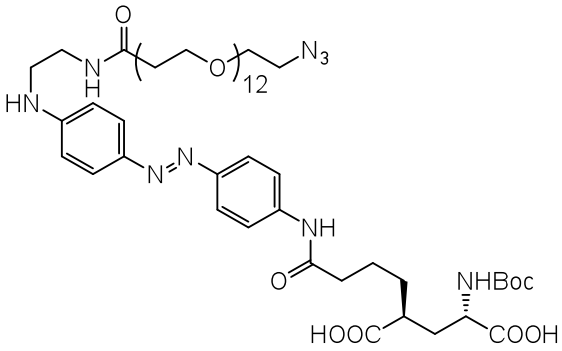
^{13}C-NMR (101 MHz, DMSO-d_6) δ [ppm] = 176.9, 174.7, 171.8, 155.3, 151.5, 148.2, 143.8, 141.3, 125.2, 123.0, 119.7, 112.4, 78.2, 52.9, 42.4, 40.7, 38.3, 37.0, 35.8, 31.6, 28.7, 23.6.

HRMS (ESI): calc. for $C_{28}H_{39}O_7N_6^+$ $[M+H]^+$: 571.2875, found: 571.2877.

UV/VIS (LCMS): λ_{max} = 412 nm.

t_R (LCMS, MeCN/H_2O/formic acid = 90/10/0.1 → 10/90/0.1 over 7 min) = 2.438 min.

1.2.13 (2*S*,4*S*)-2-(4-((4-((*E*)-(4-((1-azido-39-oxo-3,6,9,12,15,18,21,24,27,30,33,36-dodecaoxa-40-azadotetracontan-42-yl)amino)phenyl)diazenyl)phenyl)amino)-4-oxobutyl)-4-((*tert*-butoxycarbonyl)amino)pentanedioic acid, (18)



18 was prepared according to general procedure A.

Amounts:

N_3-PEG_{12}-NHS-Ester (Baseclick #BCL-033, 10.0 mg, 13.5 µmol, 1.0 equiv.)

10 (8.5 mg, 14.9 µmol, 1.1 equiv.)

DIPEA (3.5 mg, 27.0 µmol, 4.7 µL, 2.0 equiv.)

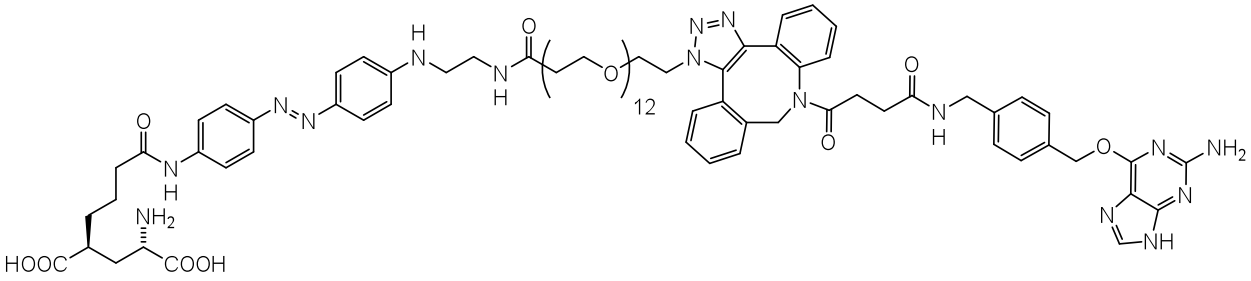
yield: 14.0 mg (11.7 µmol, 87%), red-orange solid.

HRMS (ESI): calc. for $C_{55}H_{87}N_9O_{20}^{2-}$ $[M-2H]^{2-}$: 596.8039, found: 596.8035.

UV/Vis (LCMS): λ_{max} = 412 nm.

t_R (LCMS; MeCN/H_2O/formic acid = 10/90/0.1 → 90/10/0.1 over 10 min) = 5.117 min.

1.2.14 (2*S*,4*S*)-2-amino-4-(4-((4-((*E*)-(4-((1-(8-(4-((4-(((2-amino-9H-purin-6-yl)oxy)methyl)-benzyl)amino)-4-oxobutanoyl)-8,9-dihydro-1*H*-dibenzo[b,f][1,2,3]triazolo[4,5-d]azo-cin-1-yl)-39-oxo-3,6,9,12,15,18,21,24,27,30,33,36-dodecaoxa-40-azadotetra-contan-42-yl)amino)phenyl)diazenyl)phenyl)amino)-4-oxobutyl)pentanedioic acid, $BGAG_{12,460}$



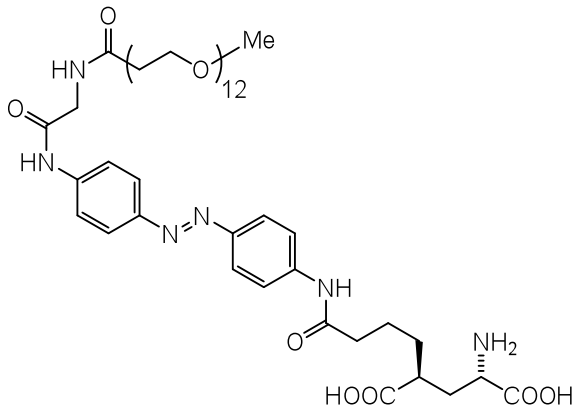
In a round bottom flask, **5** (1.0 mg, 1.79 µmol) and **18** (2.1 mg, 1.79 µmol) were combined and dissolved in MeOH. After stirring at r.t. for 30 min all starting material was consumed according to LCMS and all volatiles were removed *in vacuo* to obtain the crude protected triazole. The solid was treated with 0.35 mL neat TFA for 10 min at r.t. before Et_2O was added and the suspension was subjected to sedimentation (4,000 rpm, r.t., 20 min) to collect a deep-red solid, which was washed again with Et_2O and dried under HV to obtain 1.2 mg (1.03 µmol) $\mathbf{BGAG_{12}}_{(460)}$ in 58% yield (over 2 steps).

HRMS (ESI): calc. for $C_{82}H_{110}N_{16}O_{21}^{2+}$ $(M+2H)^{2+}$: 827.4010, found: 827.4015.

UV/Vis (LCMS): λ_{max} = 413 nm.

t_R (LCMS; MeCN/H_2O/formic acid = 10/90/0.1 → 90/10/0.1 over 7 min) = 2.601 min.

1.2.15 (2*S*,4*S*)-2-Amino-4-(4-oxo-4-((4-((*E*)-(4-(38-oxo-2,5,8,11,14,17,20,23,26,29,32,35-dodecaoxa-39-azahentetracontan-41-amido)phenyl)diazenyl)phenyl)amino)butyl)-pentanedioic acid, *D*-AG$_{12}$



***D*-AG12** was prepared according to general procedures A and C.

Amounts:

Methyl-PEG$_{12}$-NHS-ester (Thermo Scientific #22685, 9.7 mg, 14 µmol, 1.0 equiv.)

8 (9.1 mg, 16 µmol, 1.1 equiv.)

DIPEA (4.9 µL, 28 µmol, 2 equiv.)

yield: 8.0 mg (7.6 µmol, 54% over two steps), orange solid.

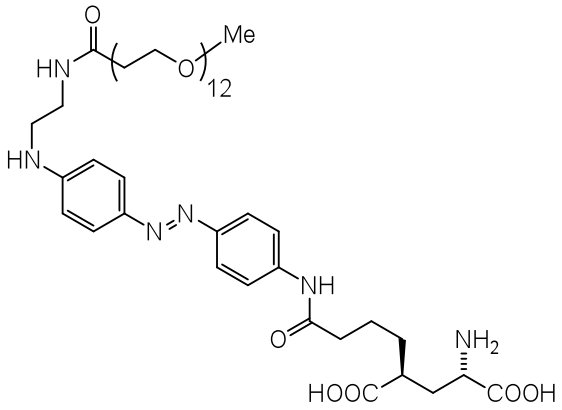
1H-NMR (400 MHz, DMSO-d$_6$) δ [ppm] = 10.31 (s, 1H), 10.27 (s, 1H), 8.27 (t, *J* = 5.8 Hz, 1H), 7.88–7.76 (m, 8H), 3.93 (d, *J* = 5.7 Hz, 2H), 3.63 (t, *J* = 6.5 Hz, 5H), 3.50 (s, 45H), 3.23 (s, 4H), 2.61 (s, 1H), 2.43 (t, *J* = 6.5 Hz, 5H), 2.36 (t, *J* = 7.0 Hz, 2H), 1.83 (s, 1H), 1.60 (p, *J* = 11.4, 10.7 Hz, 3H), 1.48–1.39 (m, 1H).

HRMS (ESI): calc. for $C_{49}H_{79}N_6NaO_{19}^{2+}$ $[M+Na+H]^{2+}$: 539.2643, found: 539.2639.

UV/VIS (LCMS): λ_{max} ($\pi \rightarrow \pi^*$) = 368 nm.

t_R (LCMS, MeCN/H_2O/formic acid = 90/10/0.1 → 10/90/0.1 over 10 min) = 3.346 min.

1.2.16 (2*S*,4*S*)-2-Amino-4-(4-oxo-4-((4-((*E*)-(4-((38-oxo-2,5,8,11,14,17,20,23,26,29,32,35-dodecaoxa-39-azahentetracontan-41-yl)amino)phenyl)diazenyl)phenyl)amino)-butyl)pentanedioic acid, *D*-AG$_{12(445)}$



***D*-AG$_{12(445)}$** was prepared according to general procedures A and C.

Amounts:

Methyl-PEG12-NHS-ester (Thermo Scientific, #22685, 9.8 mg, 14 µmol, 1.0 equiv.)

18 (9.0 mg, 16 µmol, 1.1 equiv.)

yield: 12 mg, (12 µmol, 73% over two steps), red solid.

^{1}H NMR (400 MHz, DMSO-d_6) δ [ppm] = 10.17 (s, 1H), 8.03 (t, *J* = 5.5 Hz, 1H), 7.71 (dt, *J* = 12.6, 9.1 Hz, 6H), 6.70 (d, *J* = 8.8 Hz, 2H), 6.62 (s, 1H), 3.62 (dt, *J* = 18.7, 7.1 Hz, 4H), 3.49 (d, *J* = 3.0 Hz, 50H), 2.60 (q, *J* = 7.3 Hz, 1H), 2.34 (q, *J* = 6.6 Hz, 5H), 1.95–1.74 (m, 2H), 1.68–1.37 (m, 5H).

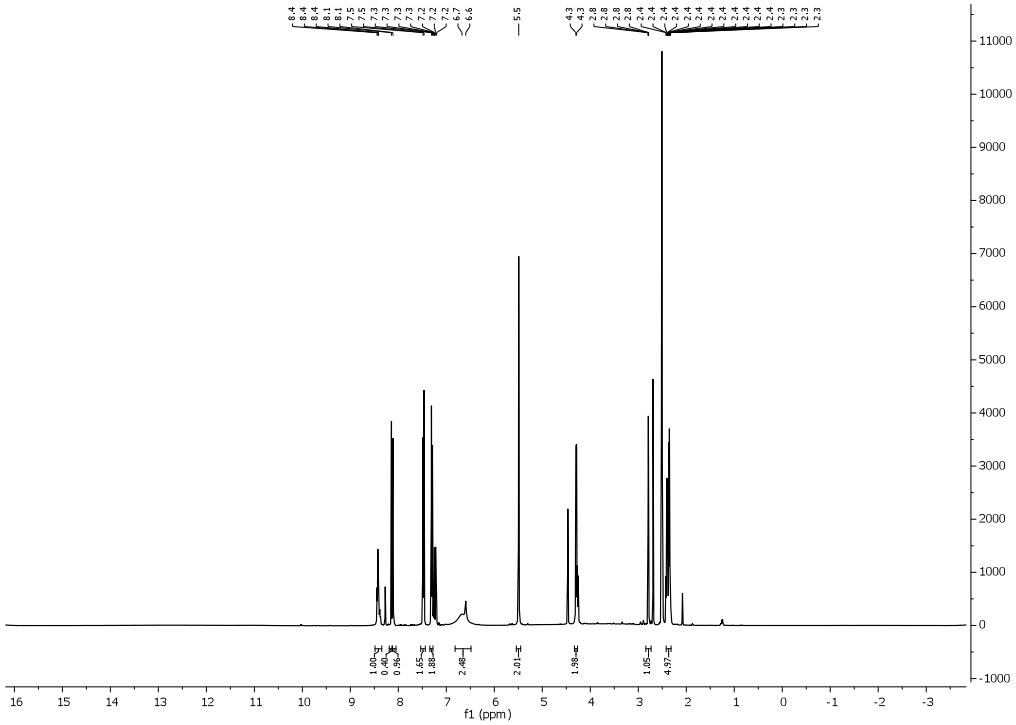
HRMS (ESI): calc. for $C_{49}H_{81}N_6NaO_{18}^{2+}$ $[M+Na+H]^+$: 532.2747, found: 532.2743.

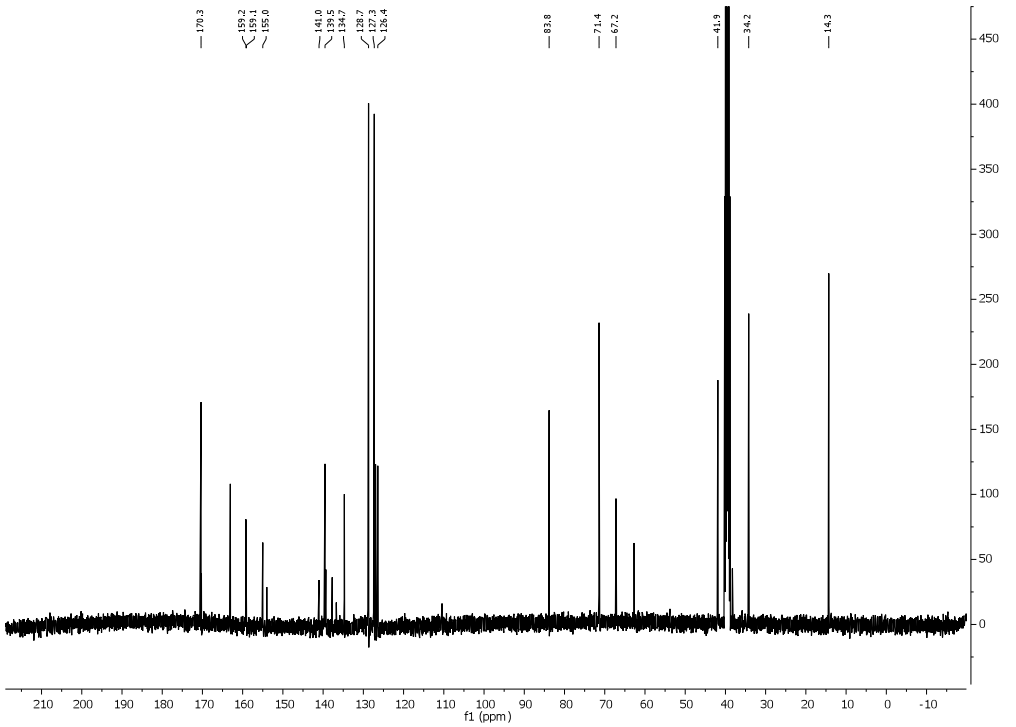
UV/VIS (LCMS): λ_{max} = 412 nm.

***t*$_R$** (LCMS, MeCN/H_2O/formic acid = 90/10/0.1 → 10/90/0.1 over 10 min) = 3.397 min.

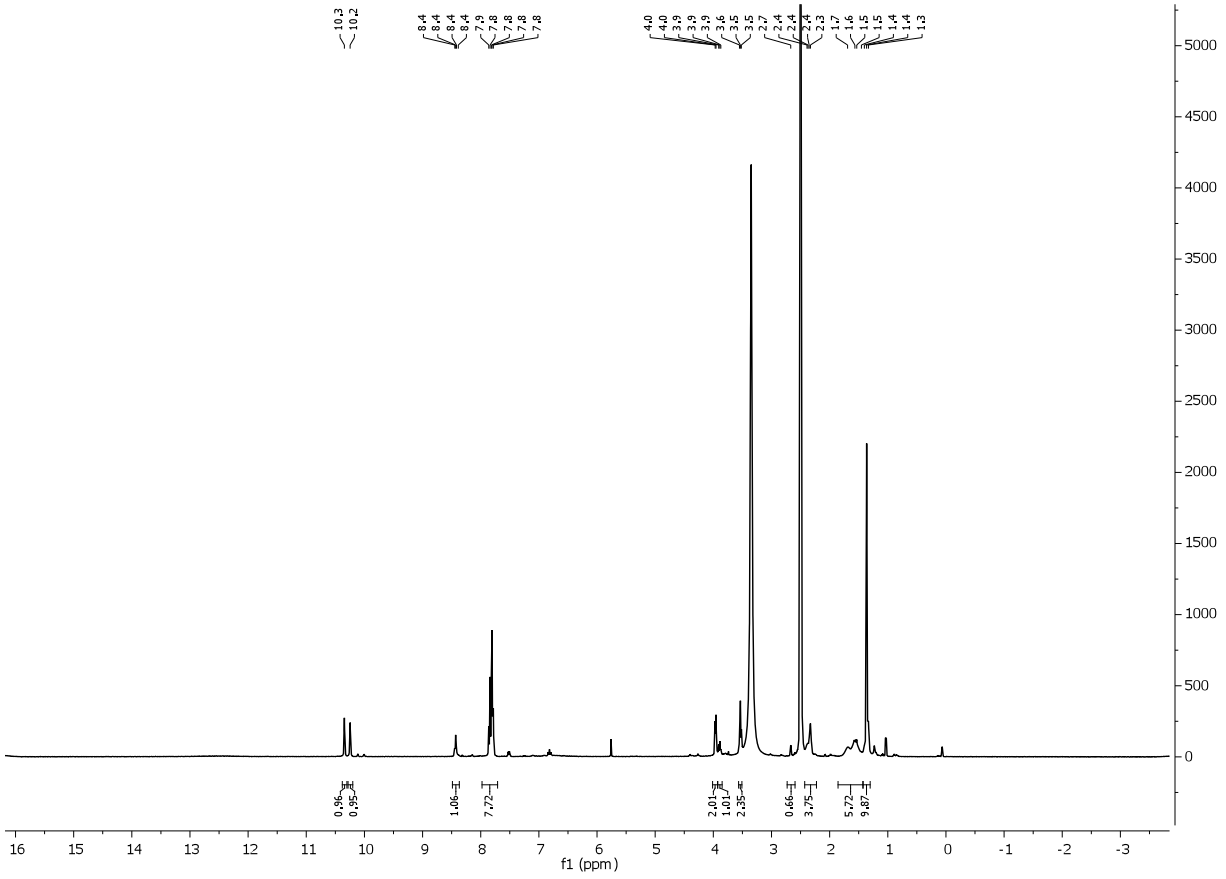
2 Spectral data

2.1 *N*-(4-(((2-Amino-9*H*-purin-6-yl)oxy)methyl)benzyl)pent-4-ynamide (3)

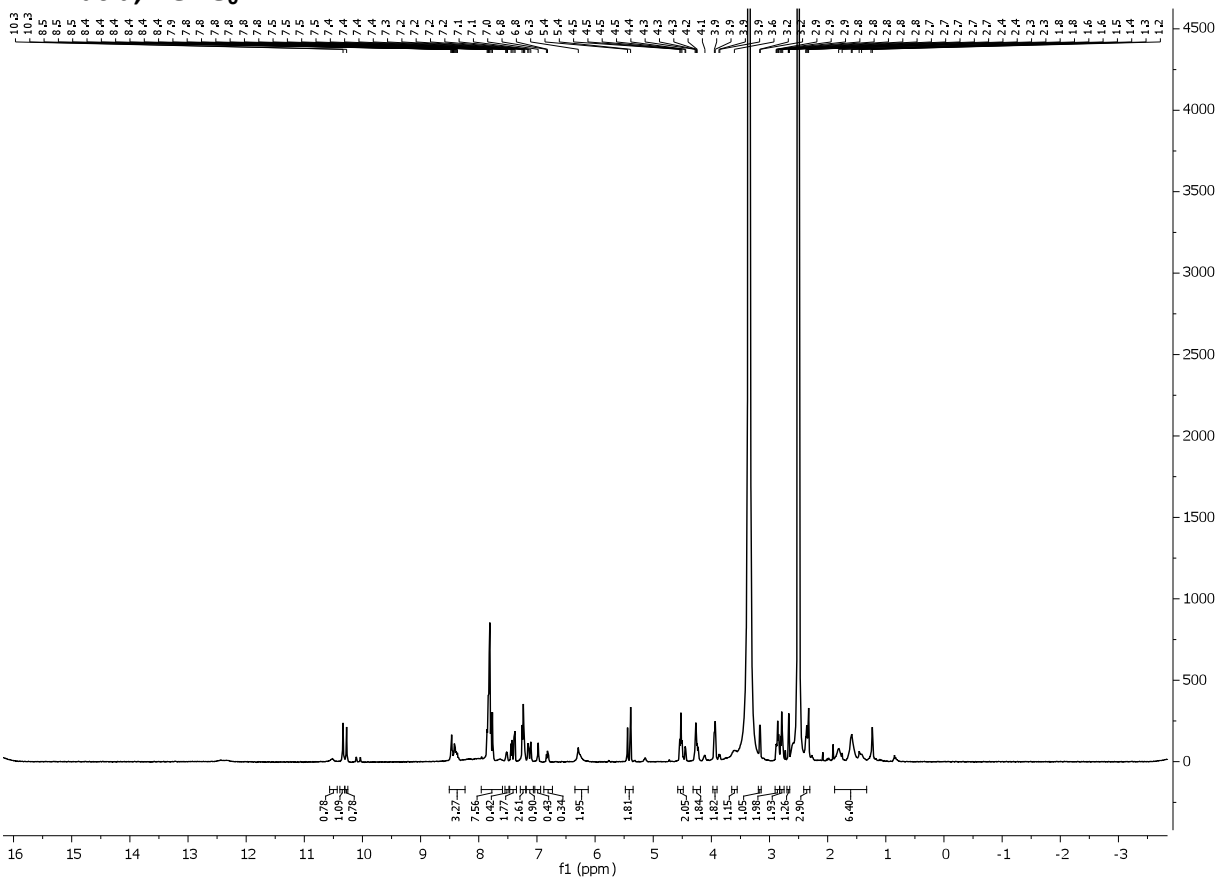




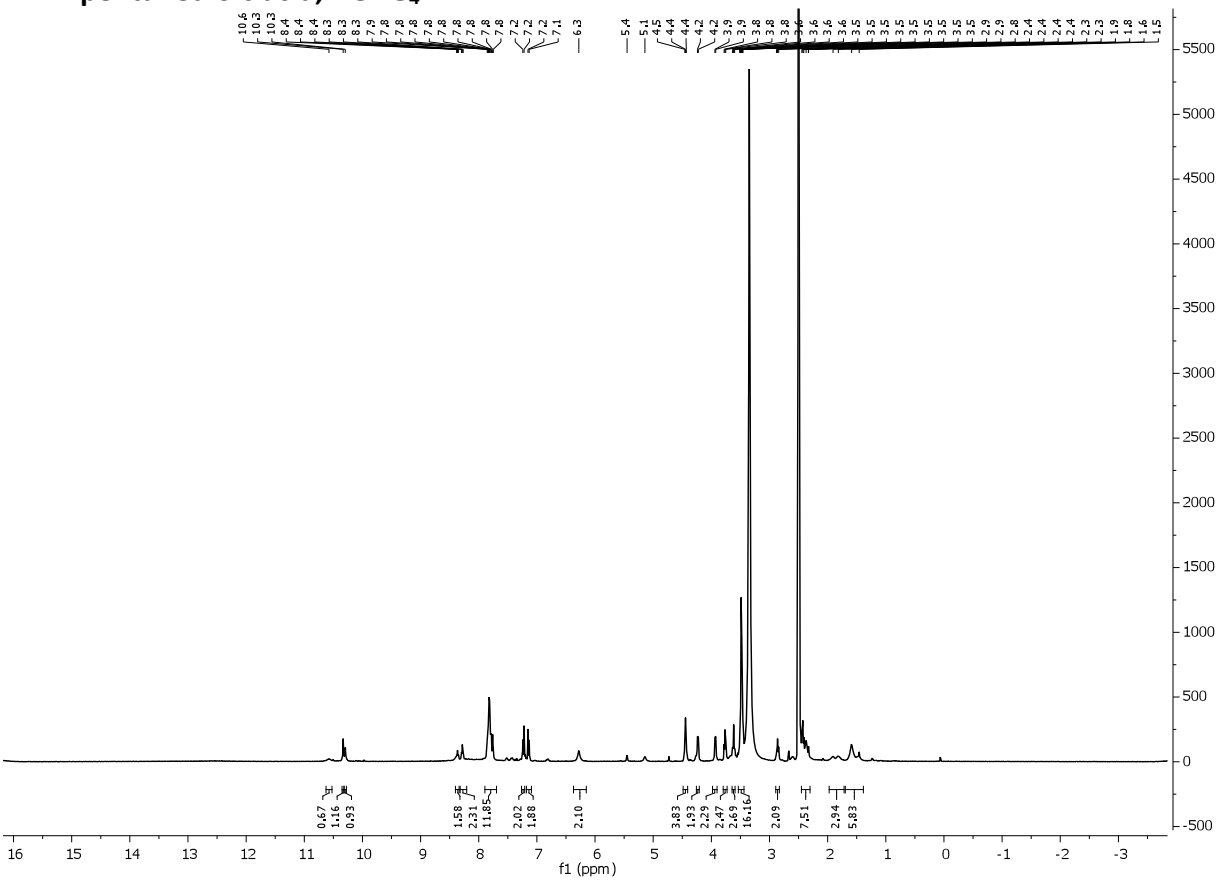
2.2 (2*S*,4*S*)-2-(4-((4-((*E*)-(4-(2-(3-azidopropanamido)acetamido)phenyl)diazenyl)phenyl)-amino)-4-oxobutyl)-4-((*tert*-butoxycarbonyl)amino)pentanedioic acid (14)



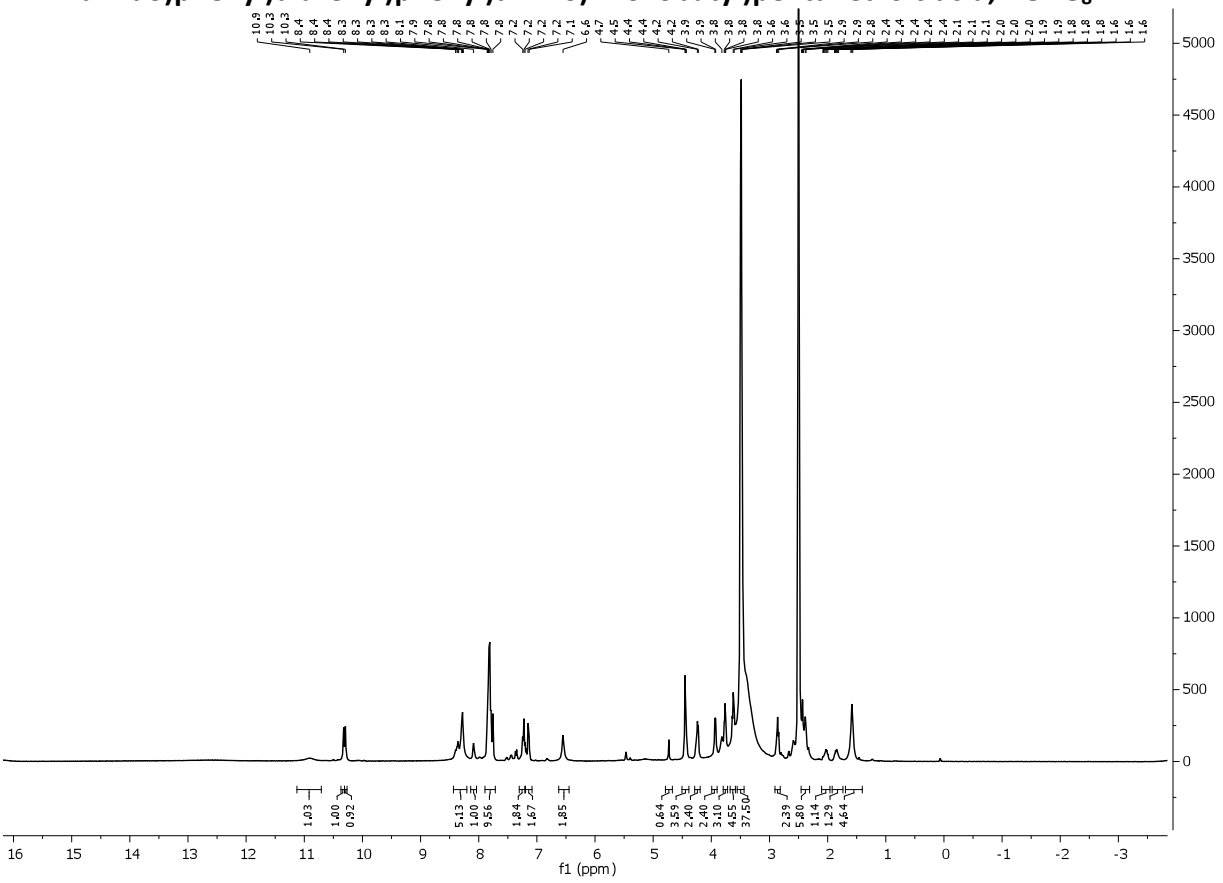
2.3 (2*S*,4*S*)-2-amino-4-(4-((4-((*E*)-(4-(2-(3-(4-(3-(((4-(((2-amino-9*H*-purin-6-yl)oxy)methyl) benzyl)amino)-3-oxopropyl)-1*H*-1,2,3-triazol-1-yl)propanamido)acetamido)phenyl)diazenyl) phenyl)amino)-4-oxobutyl)pentanedioic acid, $BGAG_0$



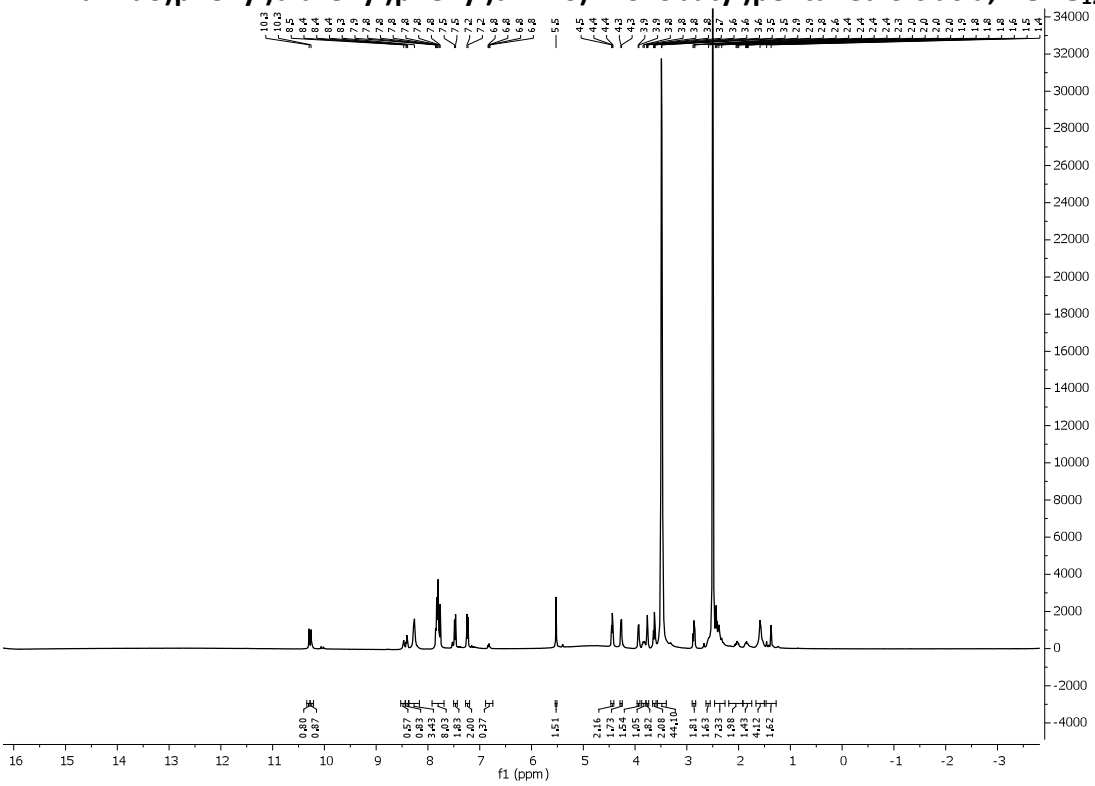
2.4 (2*S*,4*S*)-2-Amino-4-(4-((4-((*E*)-(4-(1-(4-(3-((4-(((2-amino-9*H*-purin-6-yl)oxy)methyl)benzyl)amino)-3-oxopropyl)-1*H*-1,2,3-triazol-1-yl)-15-oxo-3,6,9,12-tetraoxa-16-azaoctadecan-18-amido)phenyl)diazenyl)phenyl)amino)-4-oxobutyl)-pentanedioic acid, $BGAG_4$

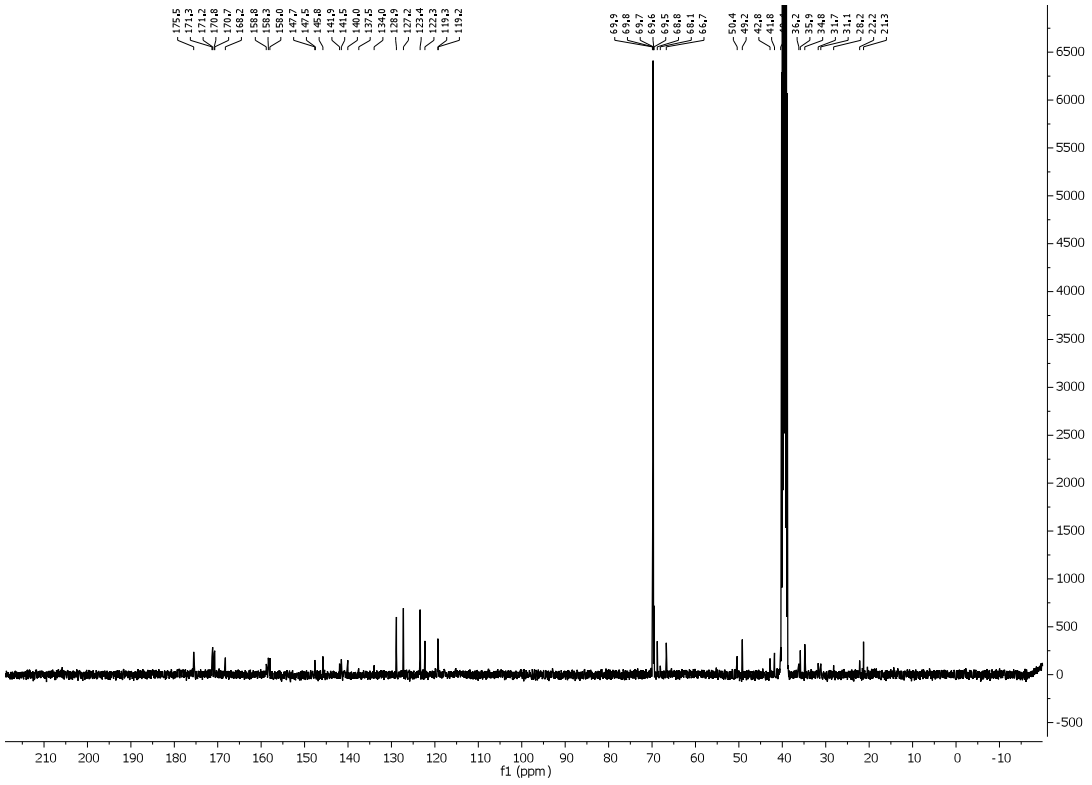


2.5 (2*S*,4*S*)-2-Amino-4-(4-((4-((*E*)-(4-(1-(4-(3-((4-(((2-amino-9*H*-purin-6-yl)oxy)methyl)benzyl)amino)-3-oxopropyl)-1*H*-1,2,3-triazol-1-yl)-27-oxo-3,6,9,12,15,18,21,24-octaoxa-28-azatriacontan-30-amido)phenyl)diazenyl)phenyl)amino)-4-oxobutyl)pentanedioic acid, $BGAG_8$

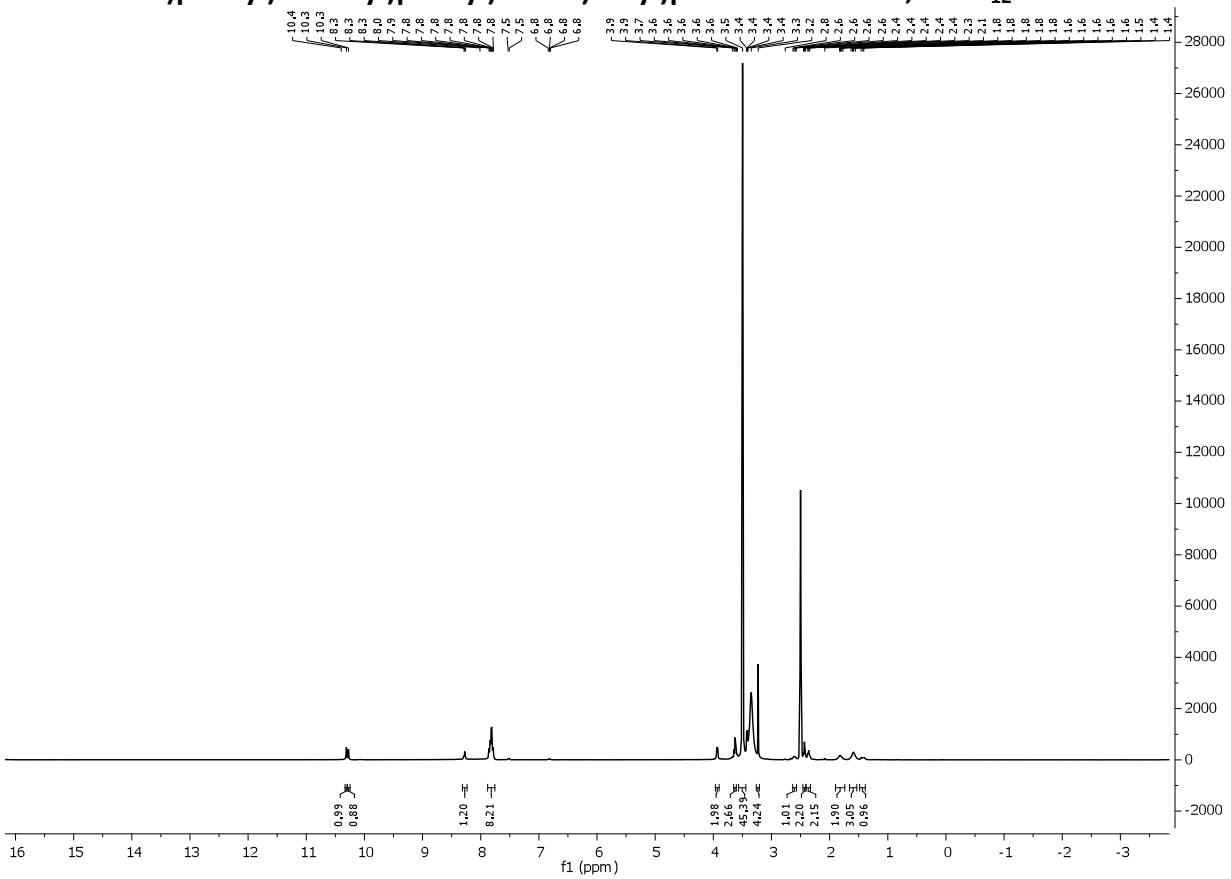


2.6 (2*S*,4*S*)-2-Amino-4-(4-((4-((*E*)-(4-(1-(4-(3-((4-(((2-amino-9*H*-purin-6-yl)oxy)methyl)benzyl)amino)-3-oxopropyl)-1*H*-1,2,3-triazol-1-yl)-39-oxo-3,6,9,12,15,18,21,24,27,30,33,36-dodecaoxa-40-azadotetracontan-42-amido)phenyl)diazenyl)phenyl)amino)-4-oxobutyl)pentanedioic acid, $BGAG_{12}$

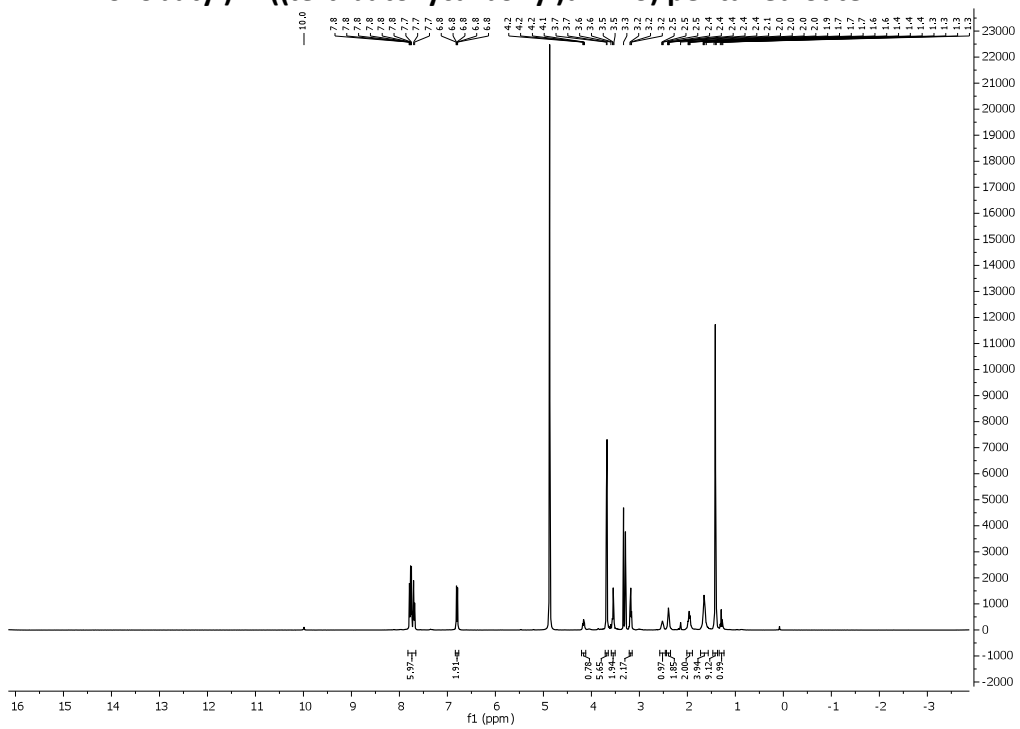


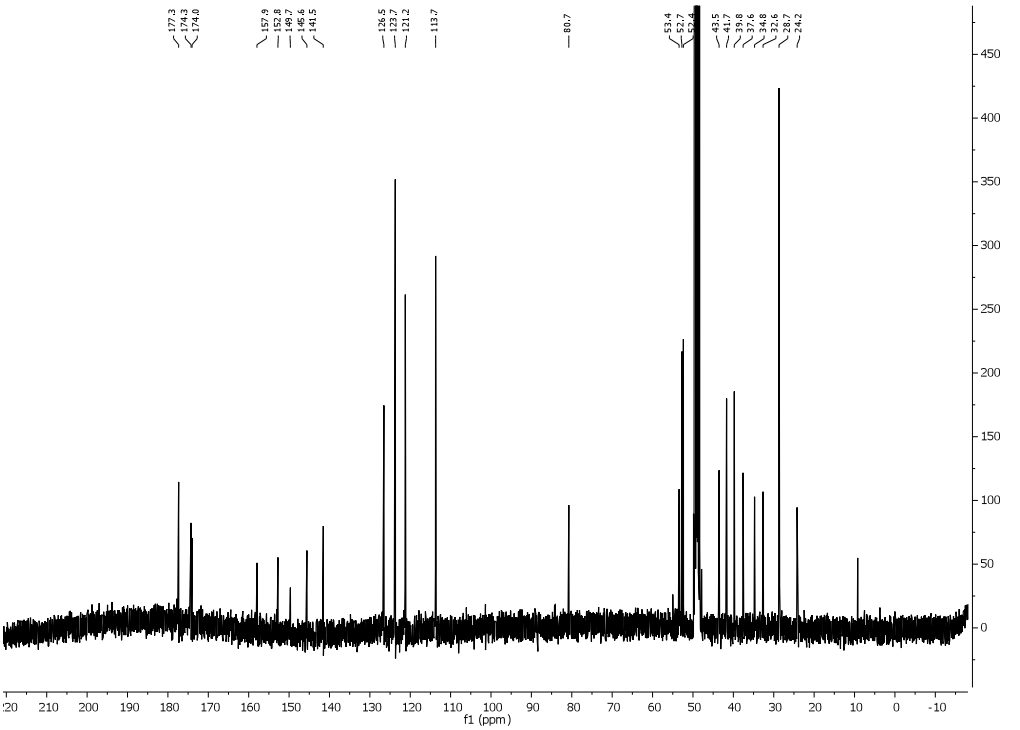

175.5
171.3
171.2
170.8
170.7
168.2
158.8
158.3
158.0
147.7
147.5
146.8
141.3
141.1
140.0
137.5
134.0
128.9
127.2
123.4
122.3
119.3
119.2
69.9
69.8
69.7
69.6
69.5
68.8
68.1
66.7
50.4
49.2
42.8
41.8
36.2
35.9
34.8
31.7
31.1
28.2
22.2
21.3
6500
6000
5500
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
-500
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)

2.7 (2*S*,4*S*)-2-amino-4-(4-oxo-4-((4-((*E*)-(4-(38-oxo-2,5,8,11,14,17,20,23,26,29,32,35-dodecaoxa-39-azahentetracontan-41-amido)phenyl)diazenyl)phenyl)amino)butyl)pentanedioic acid, *D*-AG$_{12}$

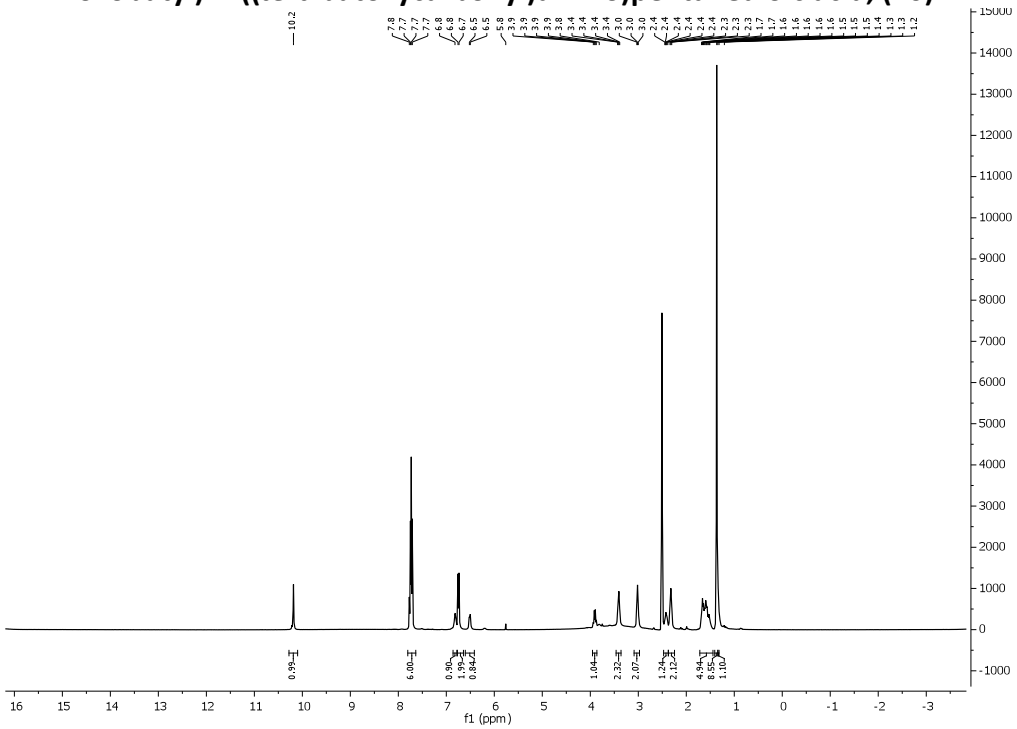


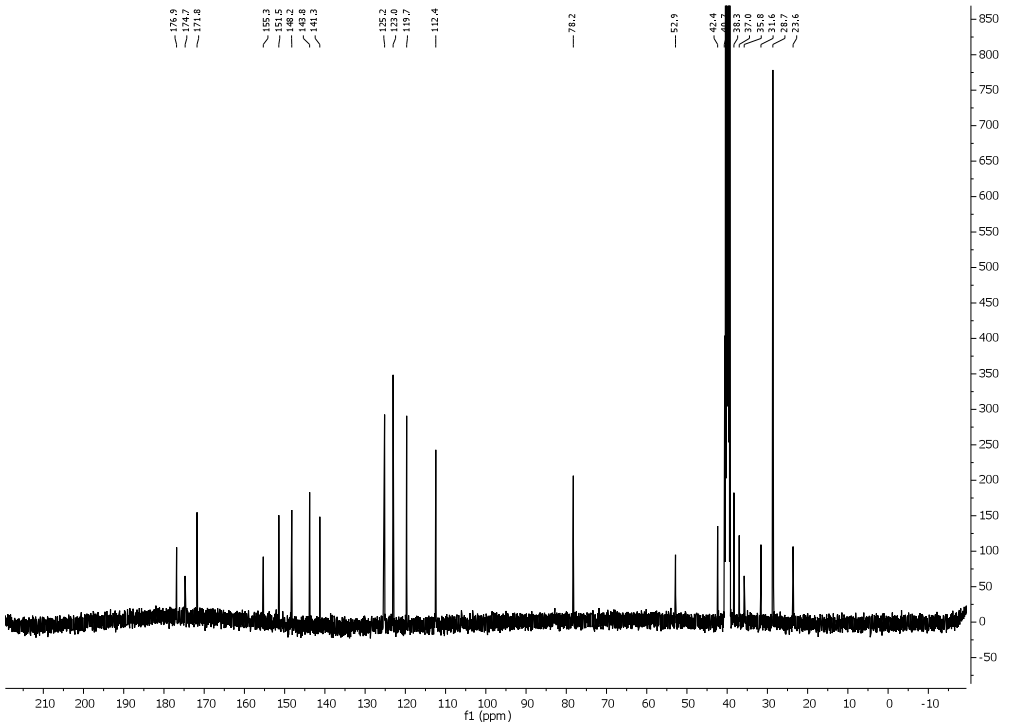
2.8 Dimethyl (2*S*,4*S*)-2-(4-((4-((*E*)-(4-((2-aminoethyl)amino)phenyl)diazenyl) phenyl)amino)-4-oxobutyl)-4-((*tert*-butoxycarbonyl)amino) pentanedioate



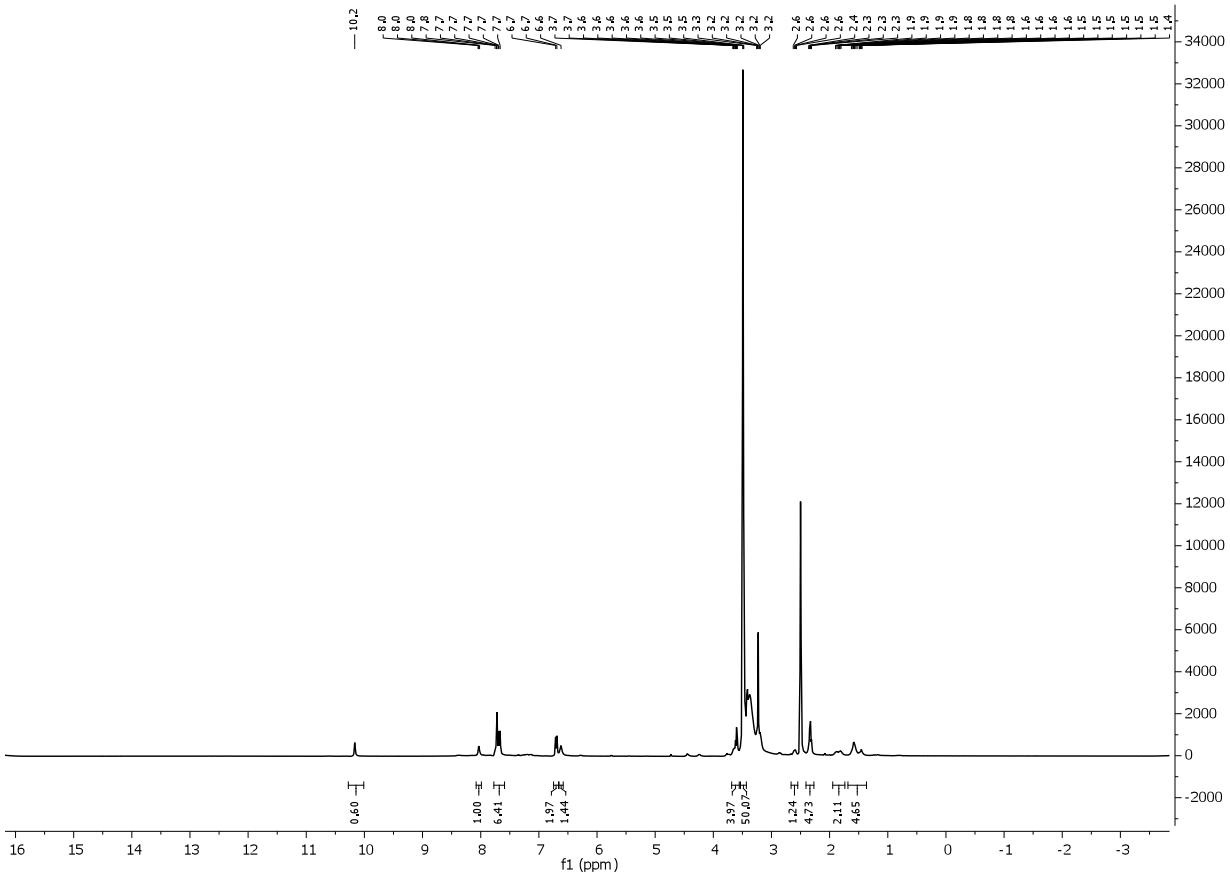


2.9 (2*S*,4*S*)-2-(4-((4-((*E*)-(4-((2-aminoethyl)amino)phenyl)diazenyl)phenyl)amino)-4-oxobutyl)-4-((*tert*-butoxycarbonyl)amino)pentanedioic acid, (10)





2.10 (2*S*,4*S*)-2-Amino-4-(4-oxo-4-((4-((*E*)-(4-((38-oxo-2,5,8,11,14,17,20,23,26,29,32,35-dodecaoxa-39-azahentetracontan-41-yl)amino)phenyl)diazenyl)phenyl)-amino)butyl)pentanedioic acid, *D*-AG$_{12,445}$



3 Supporting Figures

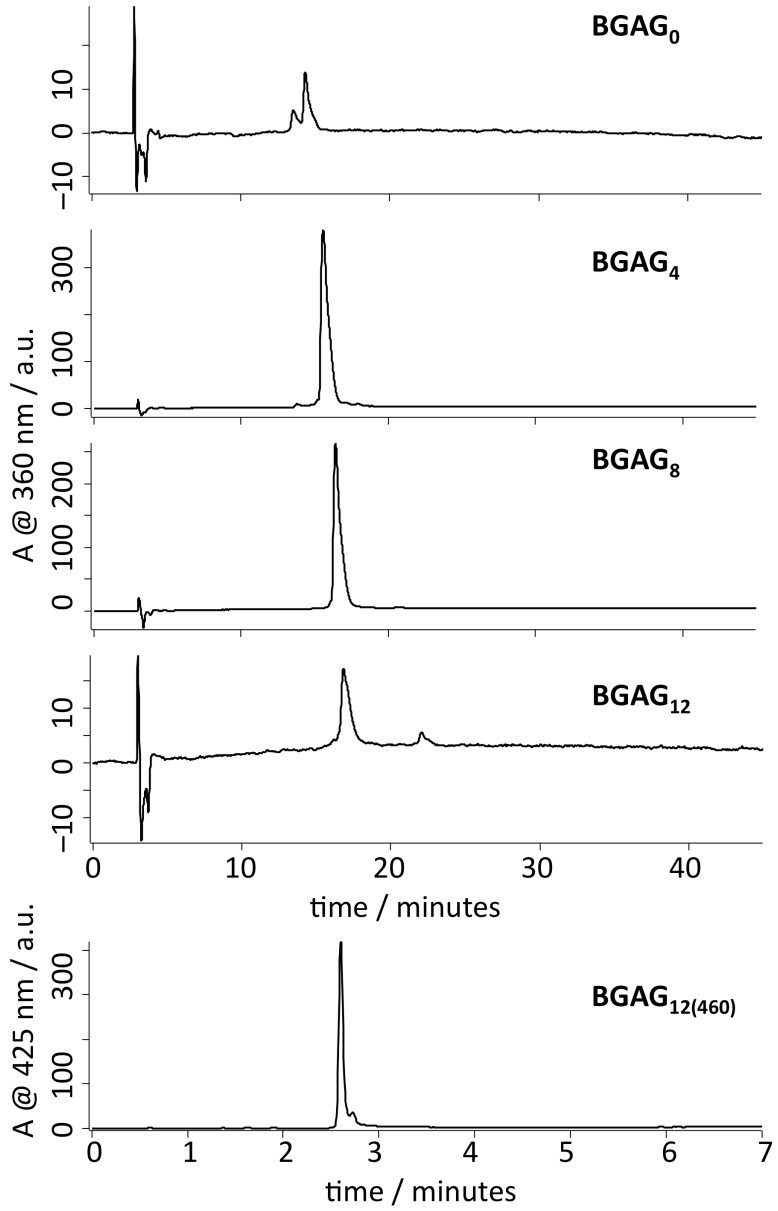


Figure S1: HPLC traces of the **BGAG** library demonstrating its purity.

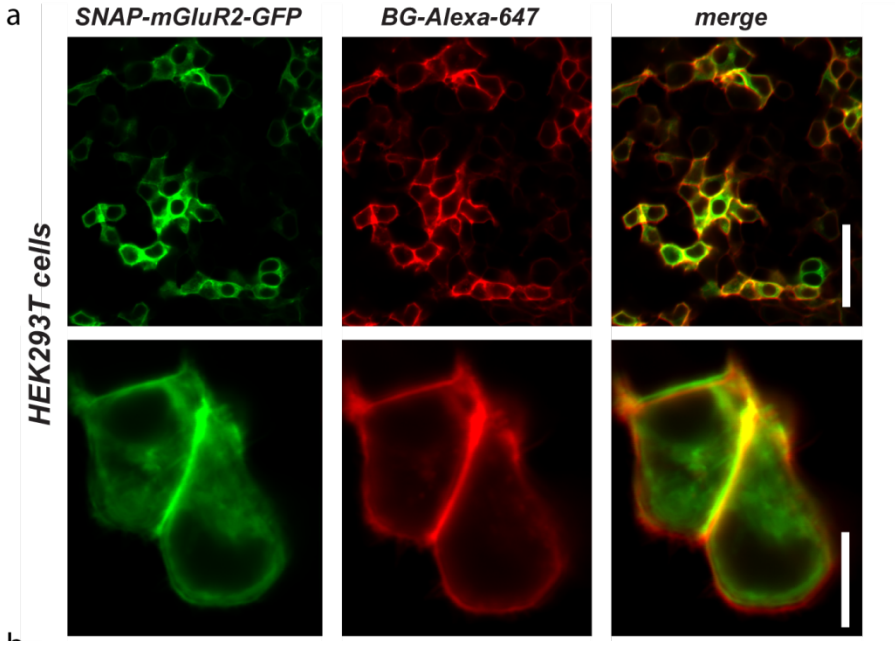


Figure S2: Expression and fluorophore labeling with benzylguanine-Alexa-647 (BG-Alexa-647) of SNAP-mGluR2-GFP in HEK293T cells. First column: GFP fluorescence. Second column: BG-Alexa-647 fluorescence. Third column: merge. Scale bars represents 50 µM (top) and 10 µM (bottom) .

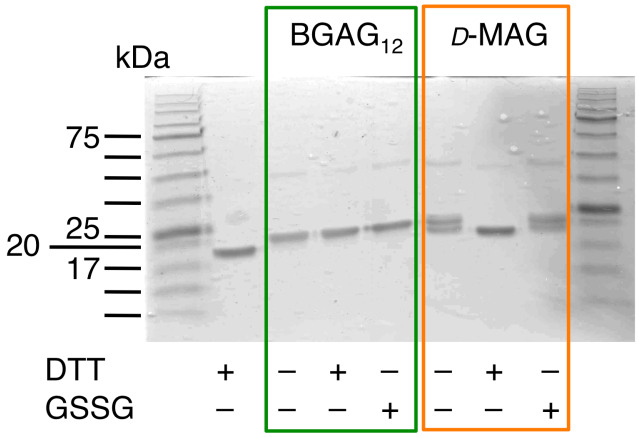


Figure S3: SDS-PAGE after *in vitro* SNAP-tag labeling (New England Biolabs, #P9312S) with **BGAG$_{12}$** and *D*-MAG. Reductive (dithiothreitol, DTT), oxidative (oxidized glutathione, GSSG) or neutral conditions (no additive) were employed according to the manufacturer's instructions. **BGAG$_{12}$** reacts with the SNAP-tag under each condition, while *D*-MAG reacts only under oxidative and neutral conditions (although slower as indicated by the unlabelled SNAP-tag band) and is unreactive towards the SNAP-tag under reductive conditions.

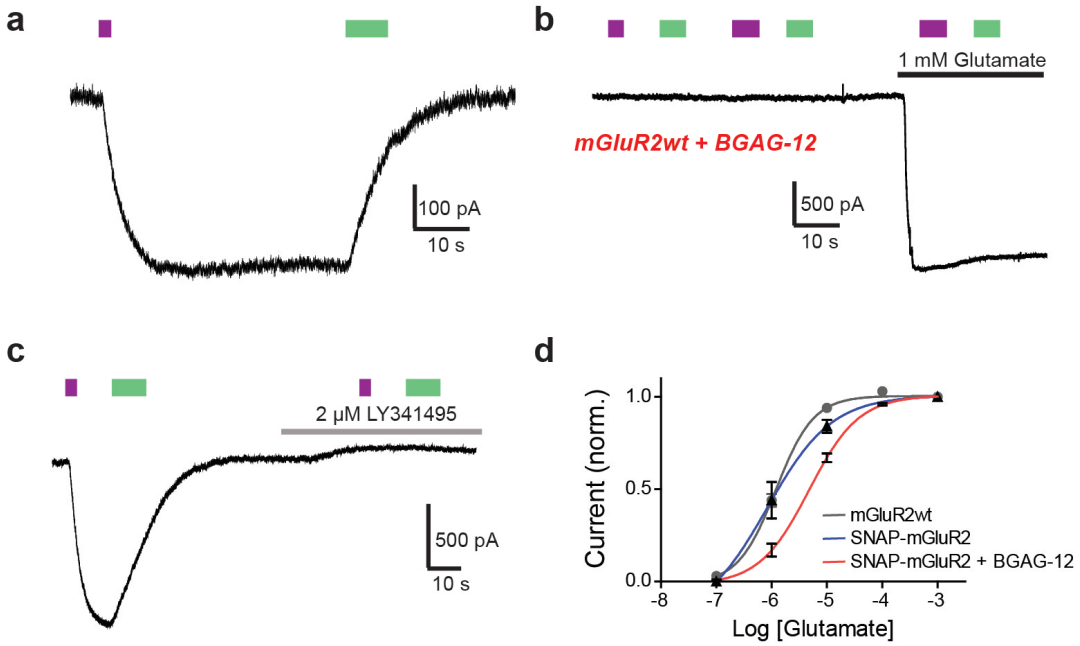


Figure S4: Further characterization of SNAG-mGluR2. **a**) Representative patch-clamp recording demonstrates reversible, bistable optical control of SNAP-mGluR2 with **BGAG$_{12}$** (*i.e.* SNAG-mGluR2). SNAG-mGluR2 is activated by a brief pulse of UV light (λ = 380 nm, gray) and deactivated with a brief pulse of green light (λ = 500 nm, green). Black bar represents no light. **b**) When wild type mGluR2 (mGluR2wt) is incubated with **BGAG$_{12}$**, no photoresponse is seen after wash-out but application of 1 mM glutamate still produces a large inward current. **c**) Photoactivation of SNAG-mGluR2 is fully blocked by the competitive mGluR2 antagonist LY341495. **d**) Glutamate concentration-response curves for mGluR2wt and SNAP-mGluR2 with or without **BGAG$_{12}$** labeling. Glutamate titration was performed in the dark for all conditions.

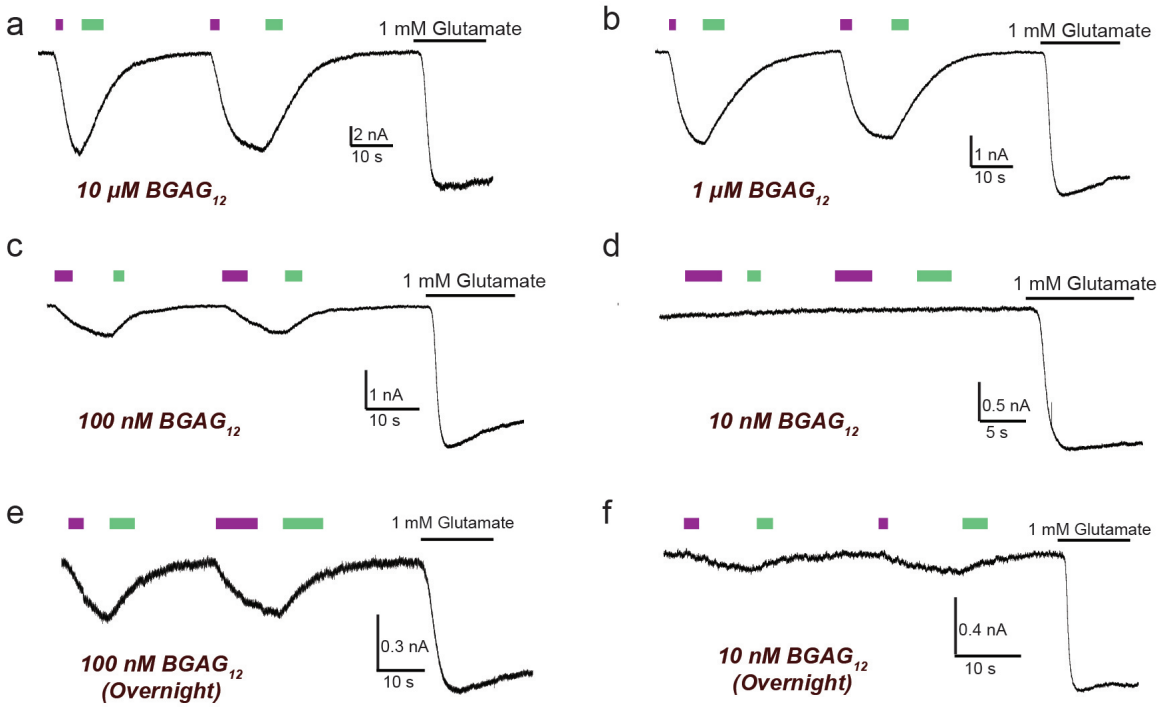


Figure S5: Summary of labeling conditions for **$BGAG_{12}$** on SNAP-mGluR2 in HEK293T cells. **a-d**) Representative traces showing photoactivation of SNAG-mGluR2 following incubation with $BGAG_{12}$ for 45 minutes in standard extracellular solution at various concentrations. Purple bars indicate 380 nm light and green bars indicate 500 nm light. **e-f**) Representative trace showing photoactivation of SNAG-mGluR2 following overnight labeling with 100 nM (**e**) or 10 nM (**f**) **$BGAG_{12}$**.

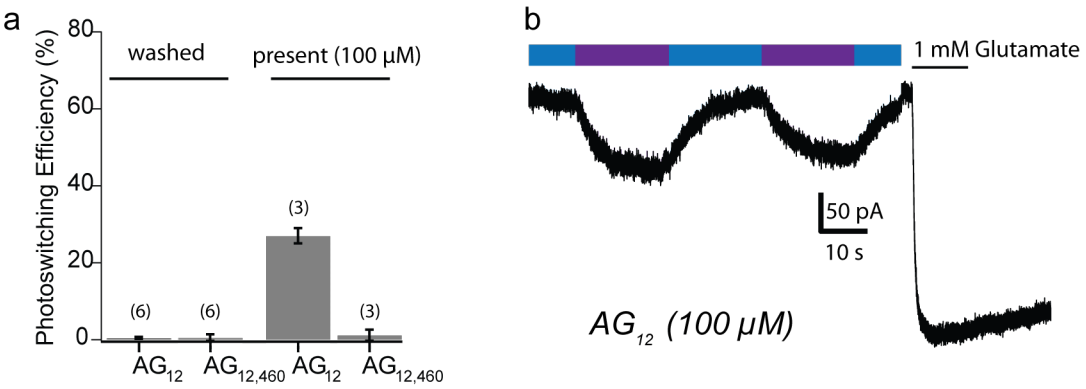


Figure S6: Optical control of SNAG-mGluR2 with AGs in HEK 293T cells. **a**) Summary of photoswitch efficiency relative to 1 mM glutamate for AG_{12} and $AG_{12,460}$ either treated as **$BGAG_{12}$** (incubated for 1 h (10µM) then washed) or with the photoswitch present (washed in (100 µM)). Error bars represent SEM; the numbers of cells tested are in parentheses. **b**) Representative patch-clamp recording demonstrates the reversible optical control of SNAG-mGluR2 with AG_{12}. SNAG-mGluR2 is activated with a UV light (λ = 380 nm, violet) and deactivated with blue light (λ = 440 nm, blue). Application of saturating 1 mM glutamate gives full activation and prevents further photoactivation in all cases.

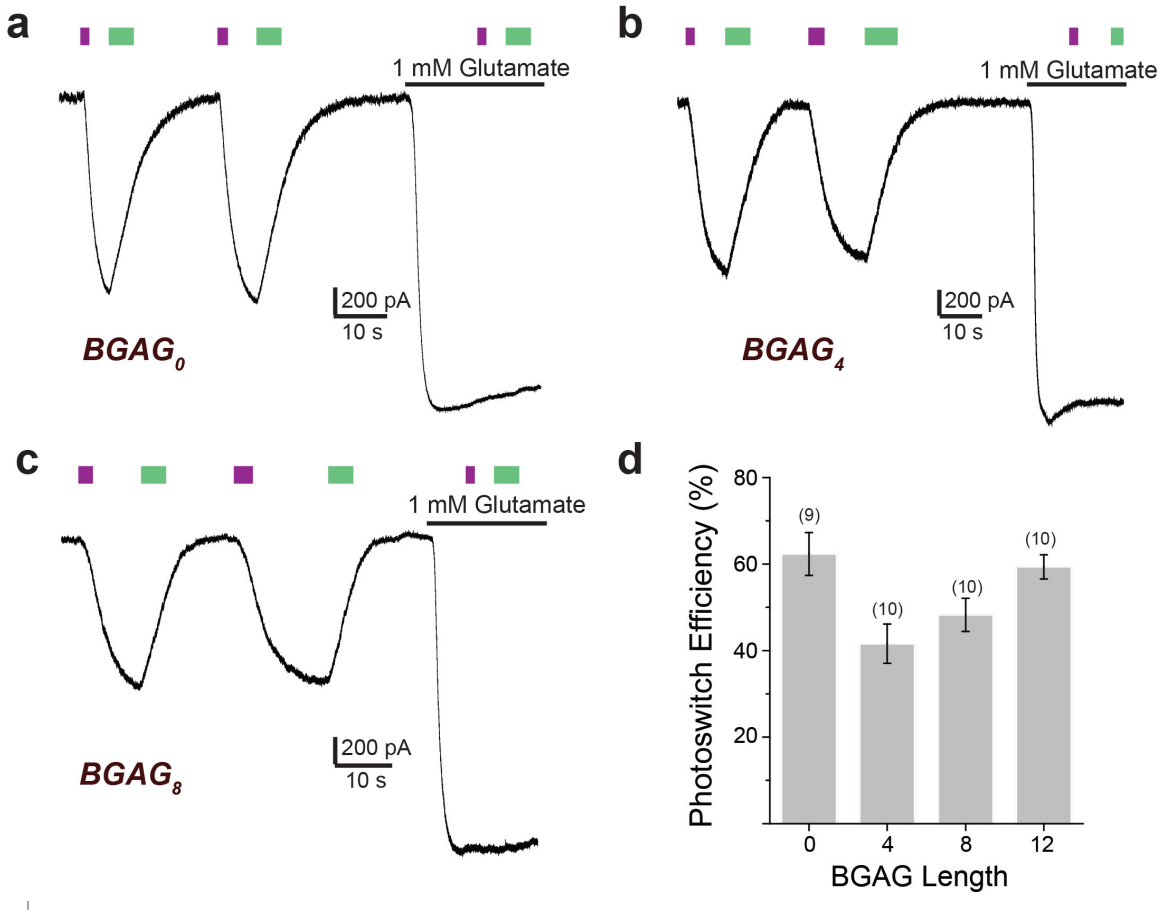


Figure S7: Optical control of SNAG-mGluR2 with variable length BGAGs in HEK 293T cells. **a-c**) Representative patch-clamp recording demonstrates the reversible optical control of SNAG-mGluR2 with either **$BGAG_0$** (**a**), **$BGAG_4$** (**b**), or **$BGAG_8$** (**c**). SNAG-mGluR2 is activated with a brief pulse of UV light (λ = 390 nm, gray) and deactivated with a brief pulse of green light (λ = 500 nm, green). Application of saturating 1 mM glutamate gives full activation and prevents further photoactivation in all cases. **d**) Summary of photoswitch efficiency relative to 1 mM glutamate for all BGAG variants. Error bars represent SEM; the numbers of cells tested are in parentheses.

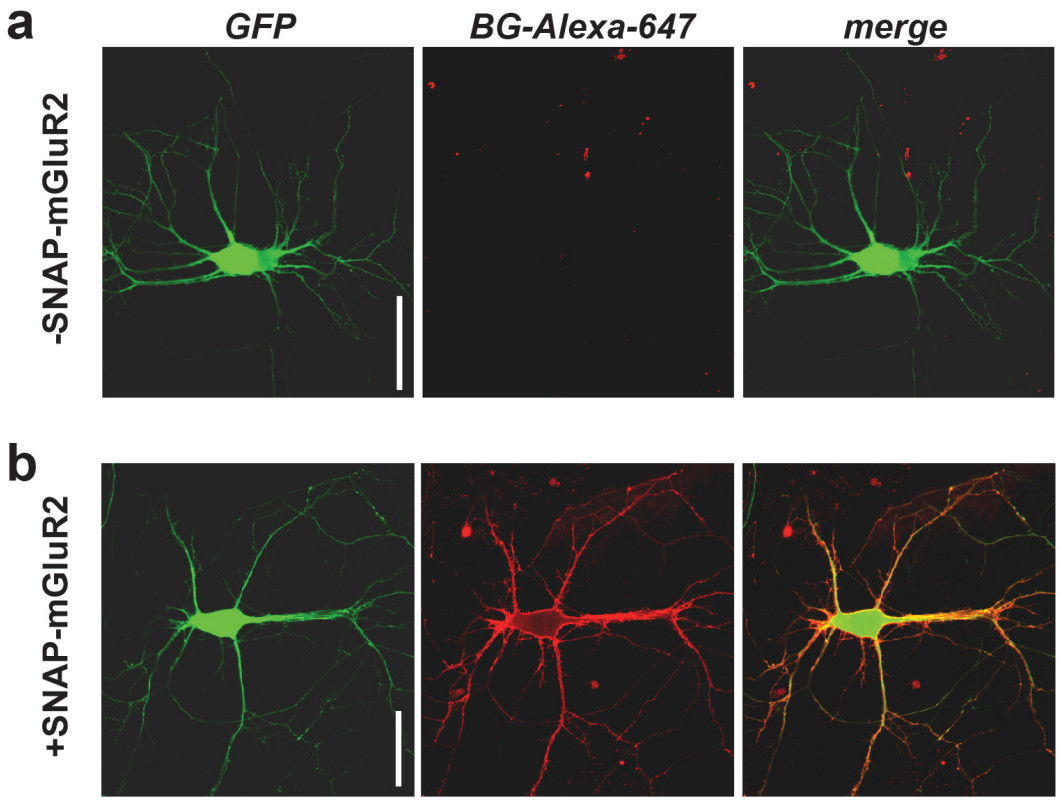


Figure S8: BG-Alexa-647 labeling controls for hippocampal neurons. **a-b**) Representative images showing BG-Alexa-647 labeling of GFP-expressing neurons in the absence (**a**) or presence (**b**) of SNAP-mGluR2 coexpression.

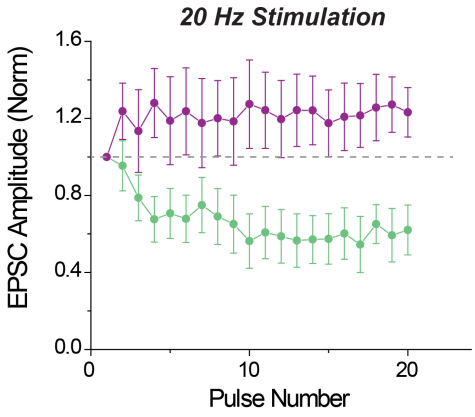


Figure S9: SNAG-mGluR2 mediated optical modulation of short term plasticity. Summary of response to high frequency (20 Hz) stimulation of a SNAG-mGluR2-expressing neuron in the presence of 380 nm (violet) or 500 nm (green) illumination. EPSC amplitude is normalized to the amplitude of the first pulse within the train.

4 References

1. Keppler, A. *et al.* A general method for the covalent labeling of fusion proteins with small molecules in vivo. *Nat. Biotechnol.* **21,** 86–89 (2003).

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