SUPPORTING INFORMATION

Click chemistry-generated Auristatin F-linker-benzylguanine for a SNAP-tag based recombinant antibody-drug conjugate demonstrating selective cytotoxicity towards EGFR-overexpressing tumour cells

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MATERIALS, METHODS, AND SPECTROMETERS

All precursor chemicals were obtained commercially from Sigma Aldrich and used without further purification. All reaction solvents were freshly distilled under nitrogen before use and the removal of water was afforded by distilling over sodium wire for tetrahydrofuran (THF, with benzophenone as an indicator), P_2O_5 for dichloromethane (DCM) and CaH_2 for acetonitrile (ACN), *N,N*-dimethylformamide (DMF) and pyridine.

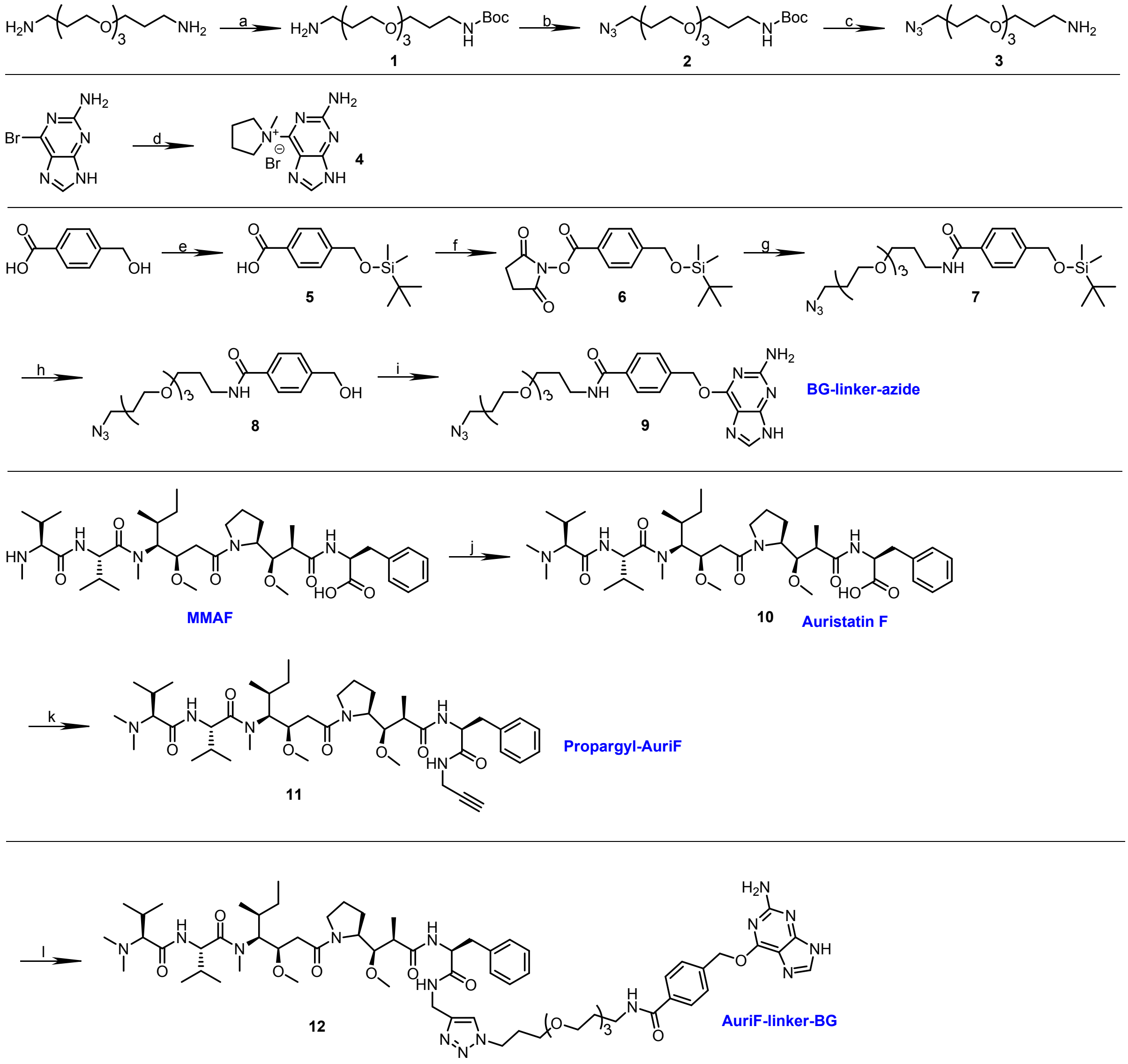
TLC was performed using Merck aluminium-backed silica 60 F_{254} plates, with visualisation provided by short-wave (254 nm) or long-wave (365 nm) UV-light or by using stains with subsequent heating. Anisaldehyde stain was prepared as a 2.5% solution of anisaldehyde in sulfuric acid and ethanol (1:10 v/v); ninhydrin stain was prepared as a 0.3% solution of ninhydrin in absolute ethanol and glacial acetic acid (97:3 v/v) and the $KMnO_4$ stain was prepared by dissolving 1.5 g of $KMnO_4$, 10 g K_2CO_3, and 1.25 mL 10% NaOH in 200 mL water.

Purification by column chromatography was carried out using silica gel 60 from Sigma Aldrich, with the mobile phase comprising mixtures of methanol and DCM or ethyl acetate (EtOAc) and hexane. Purity data was afforded by high-performance liquid chromatography (HPLC) with a UV detector, using an Agilent 1220 unit equipped with an Agilent ZORBAX Eclipse Plus C-18 column.

The characterisation of isolated compounds by nuclear magnetic resonance (NMR) was performed on Varian Mercury 300 MHz, Bruker 400 MHz and Bruker 600 MHz spectrometers. Chemical shifts were recorded relative to residual methanol in CD_3OD (^{1}H NMR: δ 3.31 ppm, ^{13}C NMR: δ 49.00 ppm), residual chloroform in $CDCl_3$ (^{1}H NMR: δ 7.26 ppm, ^{13}C NMR: δ 77.16 ppm) or residual DMSO in DMSO-d_6 (^{1}H NMR: δ 2.50 ppm, ^{13}C NMR: δ 39.52 ppm). Infrared spectroscopy was performed on a Perkin-Elmer Spectrum Two FT-IR. Mass spectrometry was performed either by LC-MS or HRMS, the former using an Agilent 6120 Quadrupole system with positive ESI and the latter at the Central Analytical Facility at Stellenbosch University, using a Waters Synapt G2 system with an ESI probe in positive mode.

Compounds were named according to the IUPAC nomenclature system, generated automatically by Perkin-Elmer ChemDraw Professional 17.1.

SCHEME FOR SYNTHESIS



Scheme S1: Synthesis of an AuriF-linker-BG for SNAP-tag conjugation. a. Boc_2O, DCM, 94%; b. NaN_3, Tf_2O, H_2O, toluene; $NaHCO_3$; $CuSO_4.5H_2O$, $NaHCO_3$, toluene, **1**, H_2O; MeOH, 95%; c. TFA, DCM, 88%; d. *N*-methylpyrrolidine, DMF, 83%; e. imidazole, TBSCl, THF; K_2CO_3, H_2O; $KHSO_4$, H_2O, 96%; f. NHS, DCC, DMAP, ACN, 95%; g. **3**, EtN(*i*-Pr)$_2$, ACN, 92%; h. HCl, THF; K_2CO_3, 90%; i. *t*-BuOK, **4**, very dry DMF, 69%; j. paraformaldehyde, AcOH, Pd/C, H_2, MeOH, 98%; k. propargylamine, EDC, HOBt, DMF, 57%; l. **9**, $CuSO_4.5H_2O$, THPTA, sodium ascorbate, *t*-BuOH, H_2O, 31%.

EXPERIMENTAL SECTION

tert-Butyl (3-{2-[2-(3-aminopropoxy)ethoxy]ethoxy}propyl)carbamate[1] (1)

4,7,10-Trioxa-1,13-tridecanediamine (7.75 mL, 35.4 mmol) was taken up in anhydrous DCM (25 mL) and stirred under an argon atmosphere at rt. Di-*tert*-butyl dicarbonate (0.772 g, 3.54 mmol) dissolved in anhydrous DCM (10 mL) was added in a dropwise fashion and the resulting reaction mixture stirred for 3 hours at rt. The reaction mixture was subsequently reduced *in vacuo* and the residue purified by column chromatography (8% MeOH/DCM, 1% NEt_3) to yield **1** as an amber oil (1.065 g, 94%). ^1H NMR δ (300 MHz, $CDCl_3$) 1.35 (s, 9H), 1.45 (br s, 2H), 1.59 – 1.71 (m, 4H), 2.71 (t, 2H, *J* = 6.7 Hz), 3.12 (app. q, 2H, *J* = 6.0 Hz), 3.42 - 3.58 (m), 5.16 (br s, 1H); ^{13}C NMR δ (100.6 MHz, $CDCl_3$) 28.4, 29.7, 33.3, 38.5, 39.6, 69.4, 69.4, 70.2, 70.2, 70.6, 70.6, 78.7, 156.1; IR 1692, 1102 cm^{-1}. The spectroscopic data of **1** matched that presented in literature.[1]

tert-Butyl (3-{2-[2-(3-azidopropoxy)ethoxy]ethoxy}propyl)carbamate[1] (2)

NaN_3 (0.930 g, 14.3 mmol) was taken up in H_2O (2.3 mL) to which toluene (2.3 mL) was added and the biphasic solution cooled to 0 °C. With vigorous stirring, triflic anhydride (1.55 mL, 9.21 mmol) was introduced in a dropwise manner and the reaction kept at 0 °C for 30 min. The temperature of the reaction was then allowed to rise to 10 °C and the stirring continued for a further 2 hours, after which a saturated, aqueous $NaHCO_3$ solution (2 mL) was added dropwise until the evolution of CO_2 halted. The toluene was separated, and the resulting solution extracted twice with toluene (2.3 mL). Compound **1** (1.00 g, 3.12 mmol), $NaHCO_3$ (1.061 g, 12.63 mmol) and Cu(II)$SO_4 \cdot 5H_2O$ (0.030 g, 0.12 mmol) were then taken up in H_2O (4 mL), to which the triflic azide toluene extracts (~7.0 mL) were added. This was followed by the addition of MeOH (27.0 mL) to afford a homogenous solution, which was stirred vigorously for 6 hours at rt. The organic solvents were removed in vacuo and the resulting aqueous solution extracted with DCM (3 x 10 mL) before drying with anhydrous $MgSO_4$, followed by filtration. The filtrate was subsequently adsorbed onto silica by rotary evaporation and the residue subjected to column chromatography (30% EtOAc/Hexane) to yield product **2** as a colourless oil (1.030 g, 95%). ^1H NMR δ (300 MHz, $CDCl_3$) 1.41 (s, 9H), 1.68 – 1.77 (m, 2H), 1.77 – 1.87 (m, 2H), 3.13 – 3.24 (m, 2H), 3.32 – 3.40 (m, 2H), 3.48 - 3.65 (m, 12H), 4.97 (br s, 1H); ^{13}C NMR δ (100.6 MHz, $CDCl_3$) 28.6, 29.3, 29.8, 38.7, 48.6, 68.0, 69.7, 70.4, 70.5, 70.7, 70.7, 79.0, 156.2; IR 2095, 1701, 1109 cm^{-1}. The spectroscopic data of **2** matched that presented in literature.[1]

3-{2-[2-(3-Azidopropoxy)ethoxy]ethoxy}propan-1-amine[1] (3)

Compound **2** (1.00 g, 2.89 mmol) was taken up in anhydrous DCM (10 mL) and stirred under an argon atmosphere. To the resulting solution was added TFA (1.80 mL, 23.5 mmol) in a dropwise fashion, and the reaction was stirred for 5 hours at rt. Thereafter, the reaction mixture was reduced in vacuo and purified by flash chromatography (8% MeOH/DCM, 1% NEt_3) to yield **3** as an amber oil (0.625 g, 88%). ^1H NMR δ (300 MHz, $CDCl_3$) 1.61 – 1.82 (m, 4H), 2.70 – 2.80 (m, 2H), 3.14 (br s, 2H), 3.30 (t, 2H, *J* = 6.7 Hz), 3.42 - 3.58 (m, 12H); ^{13}C NMR δ (100.6 MHz, $CDCl_3$) 29.0, 32.1, 39.2, 48.4, 67.8, 69.3, 70.1, 70.2, 70.4, 70.5; IR 2094, 1103 cm^{-1}. The spectroscopic data of **3** matched that presented in literature.[1]

1-(2-Amino-9*H*-purin-6-yl)-1-methylpyrrolidin-1-ium bromide[2] (4)

2-Amino-6-bromopurine (0.300 g, 1.40 mmol) was dissolved in anhydrous DMF (2 mL) and allowed to stir, under an argon atmosphere, for 10 min before the addition of *N*-methylpyrrolidine (0.40 mL, 3.9 mmol). The resulting reaction mixture was stirred for 48 hours, at rt, after which acetone was added until the evolution of a precipitate halted. The heterogenous mixture was then filtered by gravity and the solid product washed with acetone before drying to yield **4** as a colourless crystalline material (0.350 g, 83%). ^1H NMR δ (400 MHz, DMSO-d_6) 2.00–2.11 (m, 2H), 2.20–

2.31 (m, 2H), 3.65 (s, 3H), 3.93-4.02 (m, 2H), 4.57 – 4.64 (m, 2H), 7.07 (s, 2H), 8.33 (s, 1H), 13.26 (br s, 1H); ^{13}C NMR δ (100.6 MHz, DMSO-d$_6$) 21.4, 51.6, 64.1, 116.0, 142.9, 151.6, 158.5, 159.0. The spectroscopic data of **4** matched that presented in literature.[2]

4-{[(*tert*-Butyldimethylsilyl)oxy]methyl}benzoic acid[3] (5)

4-(Hydroxymethyl)benzoic acid (0.350 g, 2.30 mmol) and imidazole (0.314 g, 4.61 mmol) were taken up in anhydrous THF (6 mL) and stirred for 15 min under an argon atmosphere. TBSCl (0.694 g, 4.61 mmol) was added, upon which a white precipitate was formed, and the reaction stirred for 16 hours at rt. This reaction mixture was filtered, and the filtrate concentrated, resulting in a pale-yellow oil. THF:H_2O (6 mL, 1:1) was then added, followed by K_2CO_3 (0.180 g, 1.30 mmol), and the solution stirred for 5 hours. This was followed by the addition of 0.5 M $KHSO_4$, until the evolution of precipitate halted, after which the organic acid was extracted into DCM (3 x 20 mL) and the extracts washed with brine (50 mL) and water (50 mL). The organic phase was dried over $MgSO_4$, which was followed by filtration and concentrated by rotary evaporation to yield **5** as a colourless crystalline material (0.587 g, 96%). ^{1}H NMR δ (300 MHz, $CDCl_3$) 0.12 (s, 6H), 0.96 (s, 9H), 4.82 (s, 2H), 7.43 (d, 2H, *J* = 8.3 Hz), 8.09 (d, 2H, *J* = 8.3 Hz); ^{13}C NMR δ (100.6 MHz, $CDCl_3$) -5.1, 18.6, 26.1, 64.7, 126.0, 128.1, 130.4, 148.0, 172.0. The spectroscopic data of **5** matched that presented in literature.[3]

2,5-Dioxopyrrolidin-1-yl 4-{[(*tert*-butyldimethylsilyl)oxy]methyl}benzoate (6)

N-Hydroxysuccinimide (0.285 g, 2.48 mmol) and *N,N'*-dicyclohexylcarbodiimide (0.511 g, 2.48 mmol) were taken up in anhydrous ACN (15 mL) at 0 °C under an argon atmosphere and stirred for roughly 10 min until dissolved. Compound **5** (0.550 g, 2.06 mmol) as well as DMAP (0.050 g, 0.41 mmol) were then added, along with anhydrous ACN (5 mL), and the reaction allowed to warm to rt and stir for 12 hours. The resulting reaction mixture was placed at -20 °C for 3 hours, the precipitate filtered, and the filtrate concentrated to a residue that was purified by column chromatography (30% EtOAc/Hexane). The pure product **6** was achieved as a pearly, colourless crystalline material (0.713 g, 95%). ^{1}H NMR δ (400 MHz, $CDCl_3$) 0.11 (s, 6H), 0.95 (s, 9H), 2.90 (s, 4H), 4.82 (s, 2H), 7.46 (d, 2H, *J* = 8.6 Hz), 8.10 (d, 2H, *J* = 8.6 Hz); ^{13}C NMR δ (100.6 MHz, $CDCl_3$) -5.2, 18.5, 25.9, 26.0, 64.6, 123.8, 126.2, 130.8, 149.4, 162.0, 169.3; IR 2941, 1741, 1214, 1072 cm^{-1}.

N-(3-{2-[2-(3-Azidopropoxy)ethoxy]ethoxy}propyl)-4-{[(*tert*-butyldimethylsilyl)oxy]methyl}benzamide (7)

Compound **6** (1.00 g, 2.75 mmol) and **3** (0.680 g, 2.76 mmol) were taken up in anhydrous ACN (50 mL), under an argon atmosphere, and stirred for roughly 10 min at rt. DIPEA (1.30 mL, 7.46 mmol) was then added, the reaction warmed to 55 °C and stirred under reflux for 12 hours. The reaction mixture was adsorbed onto silica *in vacuo*, and the residue subjected to column chromatography (40-60% EtOAc/Hexane, elution gradient), yielding the product **7** as an oil (1.250 g, 92%). ^{1}H NMR δ (300 MHz, $CDCl_3$) 0.10 (s, 6H), 0.94 (s, 9H), 1.80 (p, 2H, *J* = 6.5 Hz), 1.89 (p, 2H, *J* = 5.8 Hz), 3.34 (t, 2H, *J* = 6.5 Hz), 3.45-3.54 (m, 4H), 3.55-3.69 (m, 10H), 4.76 (s, 2H), 7.04 (br s, 1H), 7.36 (d, 2H, *J* = 8.4 Hz), 7.76 (d, 2H, *J* = 8.4 Hz); ^{13}C NMR δ (100.6 MHz, $CDCl_3$) -5.1, 18.5, 26.1, 29.1, 29.3, 39.1, 48.6, 64.7, 68.0, 70.5, 70.7, 70.7, 70.9, 70.9, 126.0, 127.1, 133.7, 145.0, 167.2. LC/MS (ES) *m/z*: $[M + H]^+$ Calcd. for $C_{24}H_{43}N_4O_5Si$ 495.3; Found 495.2. IR 2096, 1640, 1092 cm^{-1}.

N-(3-{2-[2-(3-Azidopropoxy)ethoxy]ethoxy}propyl)-4-(hydroxymethyl)benzamide (8)

Compound **7** (0.860 g, 1.74 mmol) was dissolved in THF (20 mL) and stirred at 0 °C. To this solution was added 32% HCl (0.20 mL, 2.0 mmol) in a dropwise fashion, and the solution was stirred for 45 min. The reaction was then allowed to warm to rt and stirred for an additional 2 hours. Thereafter, K_2CO_3 (1.00 g, 7.24 mmol) was added, and the neutralisation allowed to proceed for 30 min before filtering. The filtrate was subsequently adsorbed onto silica in vacuo and the residue purified by column chromatography (100% EtOAc) to yield **8** as an oil (0.595 g, 90%). ^{1}H NMR δ (300 MHz, $CDCl_3$) 1.79 (app. p, 2H, *J* = 6.5 Hz), 1.85 – 1.93 (m, 2H), 2.44 (br s, 1H), 3.33 (t, 2H, *J* = 6.5 Hz), 3.44-3.50 (m, 4H), 3.54-3.67 (m, 10H), 4.71 (d, 2H, *J* = 4.1 Hz), 7.14 (br s, 1H), 7.37 (d, 2H, *J* = 8.4 Hz), 7.74 (d, 2H, *J* = 8.4 Hz); ^{13}C NMR δ (100.6 MHz, $CDCl_3$) 29.0, 29.2, 39.2, 48.6, 64.8, 68.0, 70.3, 70.5, 70.6, 70.6, 71.0, 127.0, 127.3, 134.1, 144.5, 167.2. LC/MS (ES) *m/z*: [M + H]$^+$ Calcd. for $C_{18}H_{29}N_4O_5$ 381.2; Found 381.1. IR 3350, 2096, 1631, 1097 cm^{-1}.

4-{[(2-Amino-9*H*-purin-6-yl)oxy]methyl}-*N*-(3-{2-[2-(3-azidopropoxy)ethoxy]ethoxy}propyl)benzamide (9)

Compound **8** (0.300 g, 0.789 mmol) was taken up in freshly distilled, anhydrous DMF (4 mL) under an argon atmosphere, to which *t*-BuOK (0.450 g, 4.01 mmol) was added and the solution stirred for 30 min or until the solution had changed to a dark orange colour. Compound **4** (0.300 g, 1.00 mmol) was then added, and the resulting reaction mixture allowed to stir for 4 hours at 27 °C. The DMF was then evaporated by use of a vacuum pump, the resulting concentrate adsorbed onto silica *in vacuo* and the residue subjected to column chromatography (5% MeOH/DCM) to furnish **9** as a waxy, colourless solid (0.280 g, 69%). ^{1}H NMR δ (300 MHz, $CDCl_3$) 1.77 (p, 2H, *J* = 6.6 Hz), 1.88 (p, 2H, *J* = 5.9 Hz), 3.31 (t, 2H, *J* = 6.6 Hz), 3.45 (t, 2H, *J* = 6.1 Hz), 3.48 - 3.64 (m, 12H), 5.16 (s, 2H), 5.48 (s, 2H), 7.30 (t, 1H, *J* = 4.9 Hz), 7.39 (d, 2H, *J* = 8.1 Hz), 7.70 (d, 2H, *J* = 8.1 Hz), 7.70 (s, 1H); ^{13}C NMR δ (100.6 MHz, $CDCl_3$) 29.0, 29.2, 38.9, 48.6, 67.5, 68.0, 70.4, 70.4, 70.5, 70.6, 70.6, 127.3, 128.0, 134.7, 139.7, 159.5, 167.5 (4 quaternary BG resonances not observed due to relaxation). LC/MS (ES) *m/z*: [M + H]$^+$ Calcd. for $C_{23}H_{32}N_9O_5$ 514.3; Found 514.1. IR 2096, 1628, 1582, 1096 cm^{-1}.

{(2*R*,3*R*)-3-[(*S*)-1-((3*R*,4*S*,5*S*)-4-{(*S*)-2-[(*S*)-2-(Dimethylamino)-3-methylbutanamido]-*N*,3-dimethylbutanamido}-3-methoxy-5-methylheptanoyl)pyrrolidin-2-yl]-3-methoxy-2-methylpropanoyl}-*L*-phenylalanine[4] (Auristatin F) (10)

MMAF (0.050 g, 0.068 mmol) was dissolved in anhydrous MeOH (4 mL) and the solution degassed with argon before the addition of AcOH (0.20 mL, 3.5 mmol), paraformaldehyde (0.120 g, 4.00 mmol) and 10% Pd/C (0.040 g, 0.038 mmol). The resulting heterogeneous solution was enriched with H_2, by use of a balloon attached to the reaction vessel, and the reaction was allowed to stir for 60 hours at rt. The reaction mixture was subsequently filtered through Celite, which was rinsed with MeOH (10 mL), and the resulting solution reduced in vacuo to yield Auristatin F as a colourless solid (0.050 g, 98%). ^{1}H NMR δ (600 MHz, MeOD) 0.85 - 0.89 (m, 5H), 0.98 - 1.02 (m, 10H), 1.03 - 1.06 (m, 8H), 1.08 - 1.10 (m, 6H), 1.13 (d, 3H, *J* = 6.4 Hz), 1.15 (d, 2H, *J* = 6.7 Hz), 1.21 (d, 3H, *J* = 6.5 Hz), 1.26 - 1.33 (m, 1H), 1.39 - 1.46 (m, 2.34H), 1.52 - 1.58 (m, 1H), 1.61 - 1.66 (m, 0.67H), 1.75 - 1.81 (m, 2.34H), 1.85 - 1.91 (m, 2.67H), 2.06 - 2.12 (m, 0.67H), 2.16 - 2.21 (m, 1H), 2.23 - 2.28 (m, 1H), 2.31 - 2.34 (m, 0.67H), 2.39 - 2.49 (m, 4H), 2.90 - 2.91 (m, 5H), 2.92 - 2.93 (m, 6.67H), 3.15 (s, 2H), 3.18 - 3.21 (m, 1H), 3.24 (s, 3H), 3.28 - 3.30 (m, 3H), 3.30 - 3.31 (m, 2H), 3.34 - 3.35 (m, 7H), 3.42 - 3.43 (m, 1H), 3.64 - 3.74 (m, 3.34H), 3.85 - 3.86 (m, 0.67H), 4.04 - 4.09 (m, 0.67H), 4.12 - 4.20 (m, 1H), 4.69 - 4.80 (m, 5H), 7.16 - 7.20 (m, 1.67H), 7.23 - 7.29 (m, 6.67H); ^{13}C NMR δ (151 MHz, MeOD) 10.9, 10.9, 14.9, 15.6, 15.9, 16.3, 19.1, 19.2, 19.2, 19.8, 19.9, 19.9, 20.3, 20.8, 24.5, 25.5, 25.8, 26.5, 27.1, 27.2, 28.6, 31.7, 31.8, 32.9, 33.0, 33.6, 33.8, 36.8, 38.0, 38.2, 42.5, 45.3, 48.0, 53.9, 54.7, 56.8, 56.9, 58.0, 58.3, 58.5, 60.6, 60.7, 61.4, 62.0, 68.4, 68.4, 79.2, 79.7, 83.4, 86.9, 127.7, 127.8, 129.5, 129.6, 129.9, 130.2, 138.8, 138.8, 166.8, 166.8, 171.7, 172.1, 174.5, 174.7, 174.9, 175.2, 176.5, 176.8. The spectroscopic data of **5** matched that presented in literature.[5]

(*S*)-2-[(*S*)-2-(Dimethylamino)-3-methylbutanamido]-*N*-{(3*R*,4*S*,5*S*)-3-methoxy-1-[(*S*)-2-((1*R*,2*R*)-1-methoxy-2-methyl-3-oxo-3-{[(*S*)-1-oxo-3-phenyl-1-(prop-2-yn-1-ylamino)propan-2-yl]amino}propyl)pyrrolidin-1-yl]-5-methyl-1-oxoheptan-4-yl}-*N*,3-dimethylbutanamide (11)

To a solution of Auristatin F (0.030 g, 0.040 mmol) and EDC (0.010 g, 0.052 mmol) in anhydrous DMF (0.5 mL), at 0 °C, was added propargylamine (0.008 mL, 0.1 mmol) and HOBt (0.001 g, 0.007 mmol) and the resulting solution warmed to 30 °C. The reaction was allowed to stir for 36 hours after which point the DMF was removed in vacuo. The residue was adsorbed on silica and purified by column chromatography (2-4% MeOH/DCM, elution gradient) to yield **11** as a colourless solid (0.018 g, 57%). HRMS (ES) *m/z*: $[M + H]^+$ Calcd. for $C_{43}H_{71}N_6O_7$ 783.5384; Found 783.5384.

4-{[(2-Amino-9*H*-purin-6-yl)oxy]methyl}-*N*-(3-{2-[2-(3-{4-[((*S*)-2-{(2*R*,3*R*)-3-[(*S*)-1-((3*R*,4*S*,5*S*)-4-{(*S*)-2-[(*S*)-2-(dimethylamino)-3-methylbutanamido]-*N*,3-dimethylbutanamido}-3-methoxy-5-methylheptanoyl)pyrrolidin-2-yl]-3-methoxy-2-methylpropanamido}-3-phenylpropanamido)methyl]-1*H*-1,2,3-triazol-1-yl}propoxy)ethoxy]ethoxy}propyl)benzamide (12)

Compound **11** (0.018 g, 0.023 mmol) and compound **9** (0.011 g, 0.021 mmol) (this had an Rf close to the product and was used as the limiting reagent) were dissolved in *t*-BuOH (0.5 mL) and allowed to stir. An aqueous solution (0.5 mL) of $Cu_2SO_4 \cdot 5H_2O$ (0.0015 g, 0.0060 mmol) and the click copper ligand, THPTA (0.003 g, 0.006 mmol), was prepared, which were allowed to complex for 5 minutes before adding to the *t*-BuOH solution. This was followed by the addition of sodium ascorbate (0.005 g, 0.03 mmol) and the resulting reaction allowed to stir for 60 hours before the solution was freeze-dried and the residue purified directly by column chromatography (1-5% MeOH/DCM, elution gradient) to yield **12** (Aurif-linker-BG) as a colourless solid (0.0087 g, 31%). See below for the HRMS data.

^{1}H NMR and ^{13}C NMR SPECTRA; MASS SPECTRA

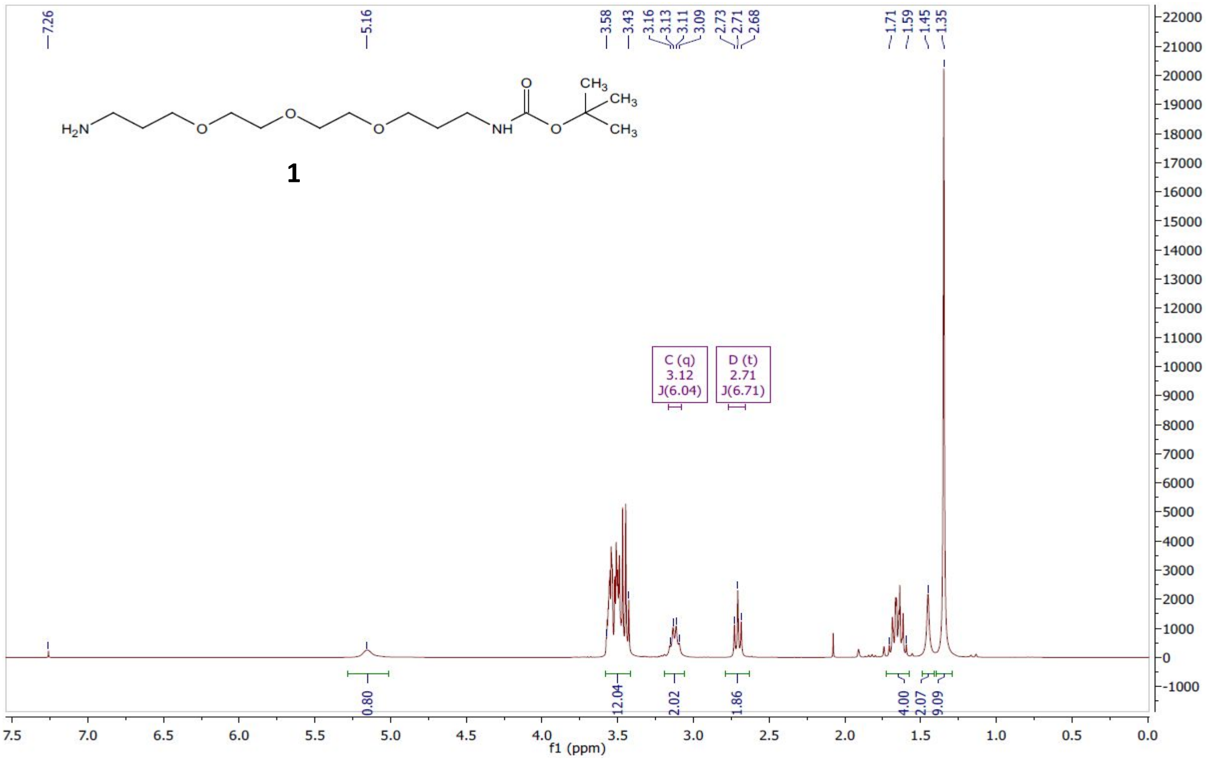


Figure S1: ^{1}H NMR Spectrum of 1.

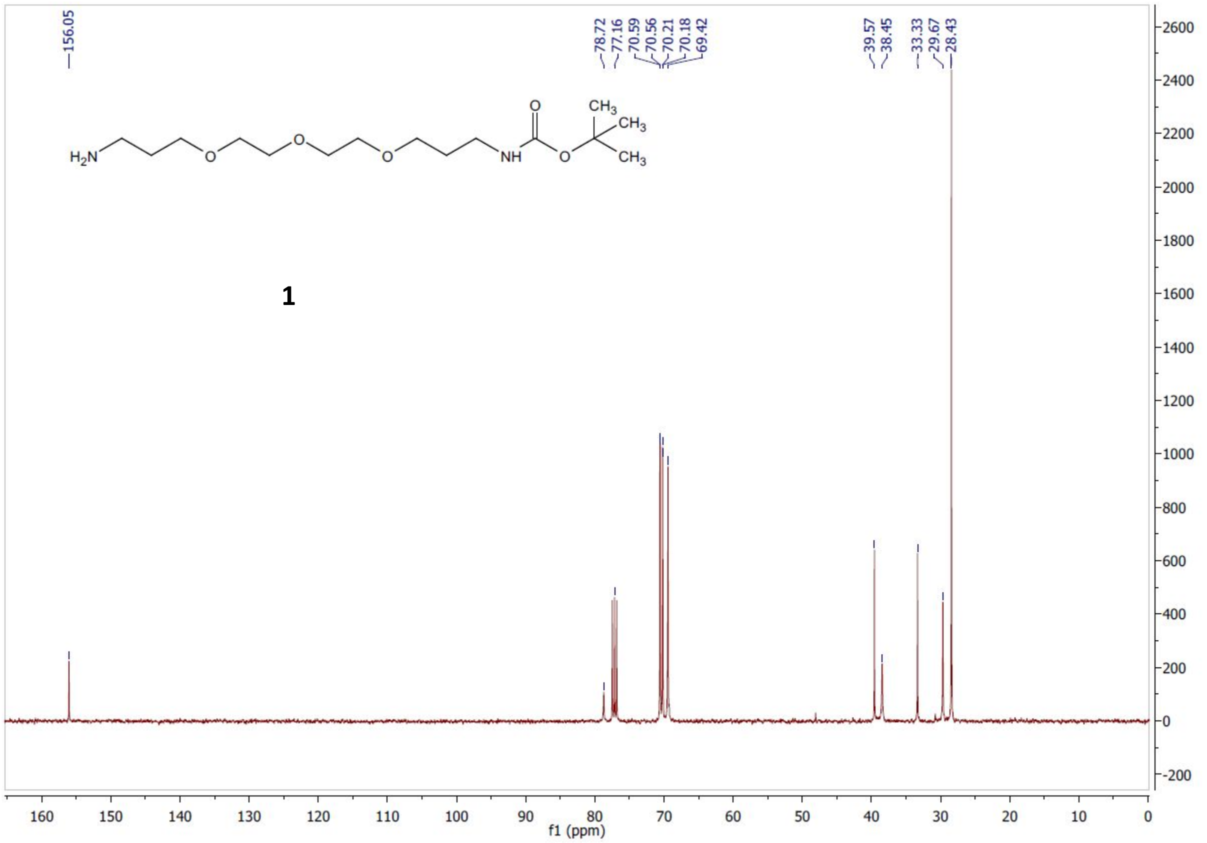


Figure S2: ^{13}C NMR Spectrum of 1.

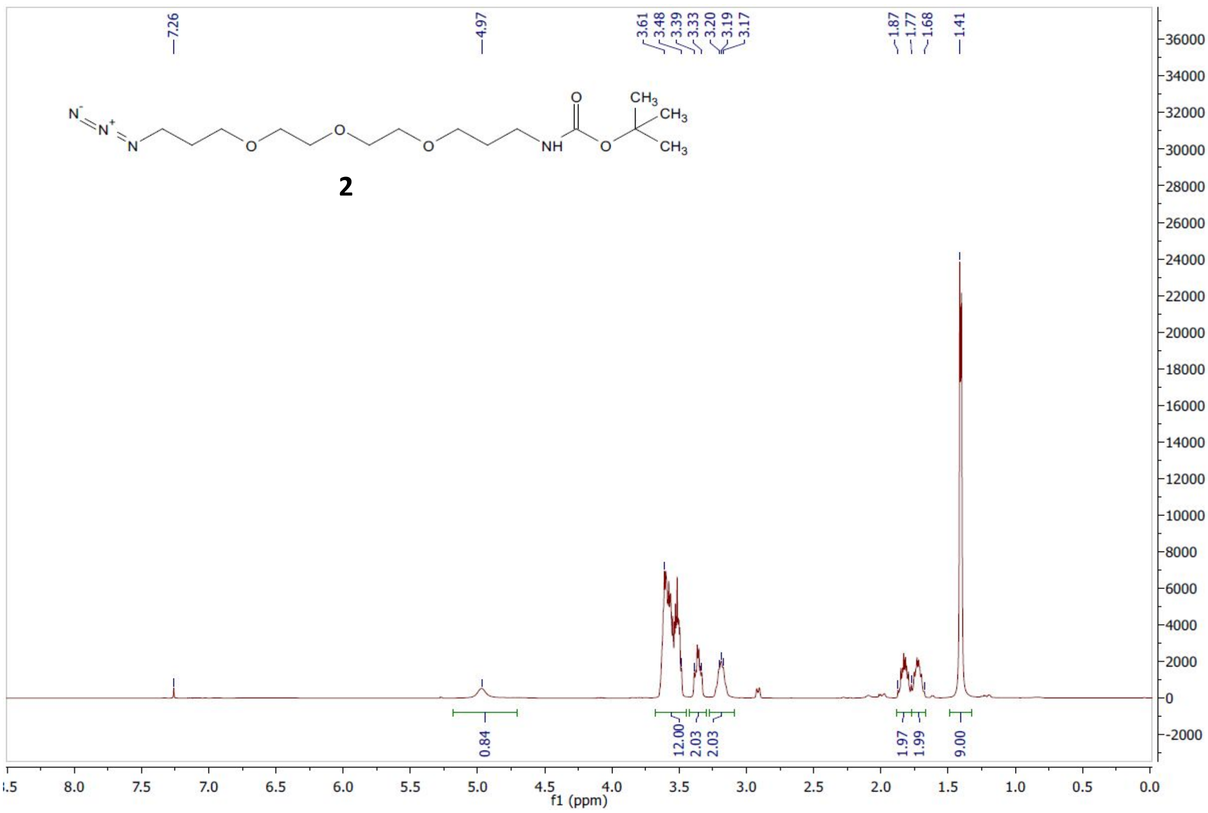


Figure S3: ^{1}H NMR Spectrum of 2.

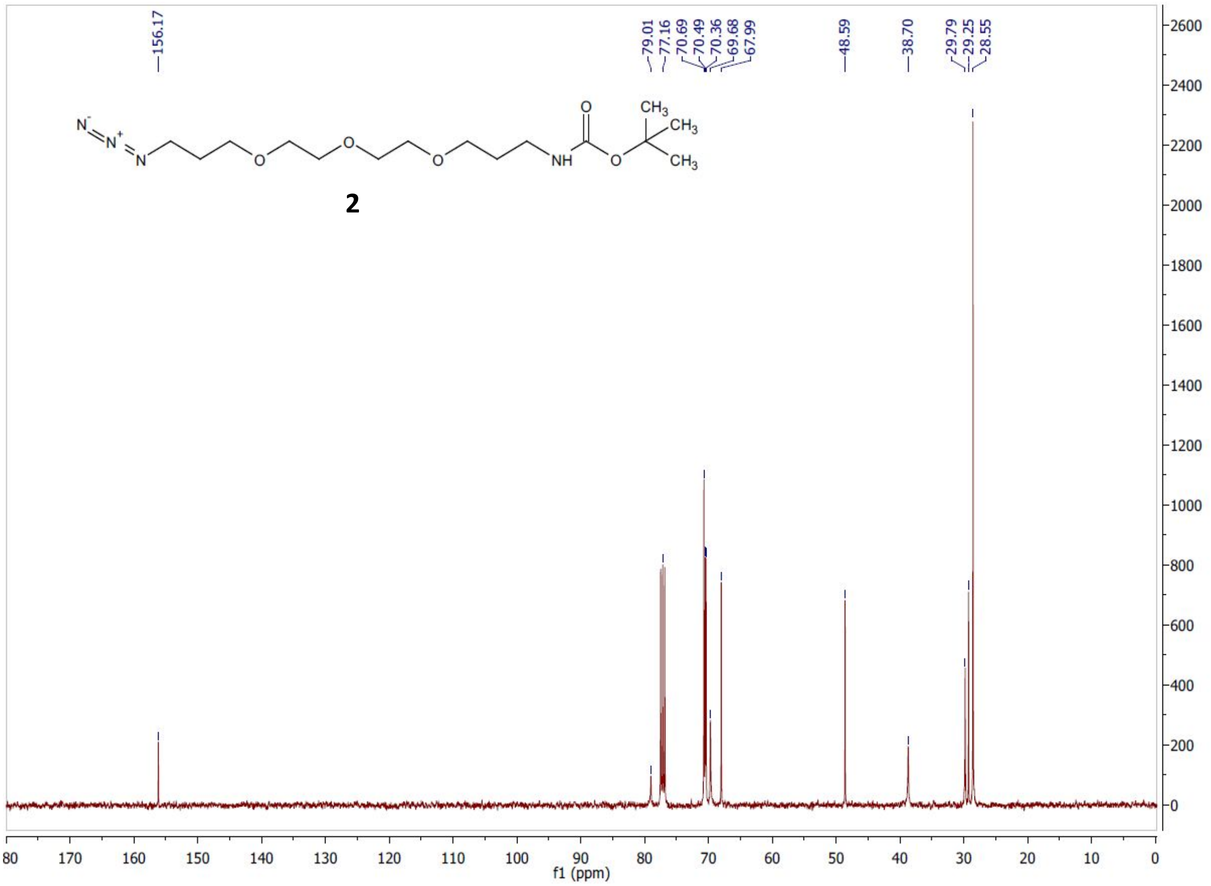


Figure S4: ^{13}C NMR Spectrum of 2.

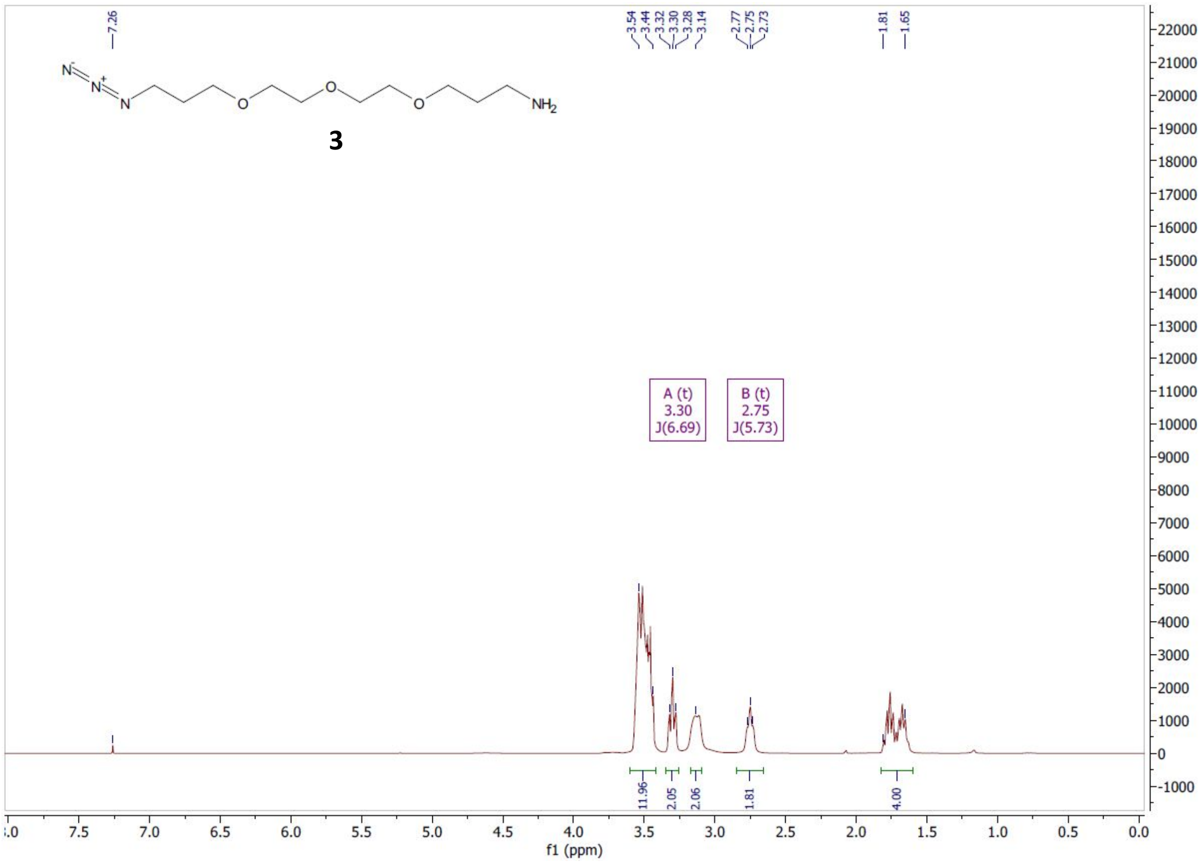


Figure S5: ^{1}H NMR Spectrum of 3.

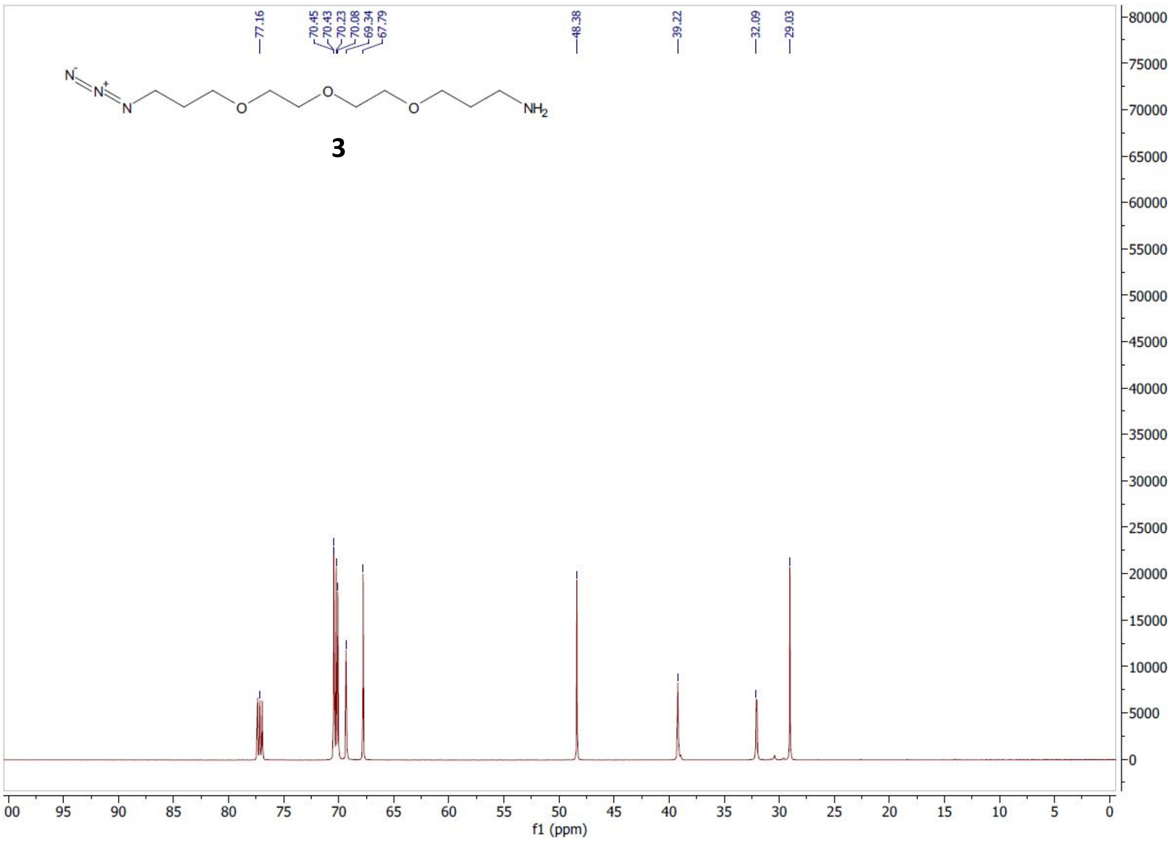


Figure S6: ^{13}C NMR Spectrum of 3.

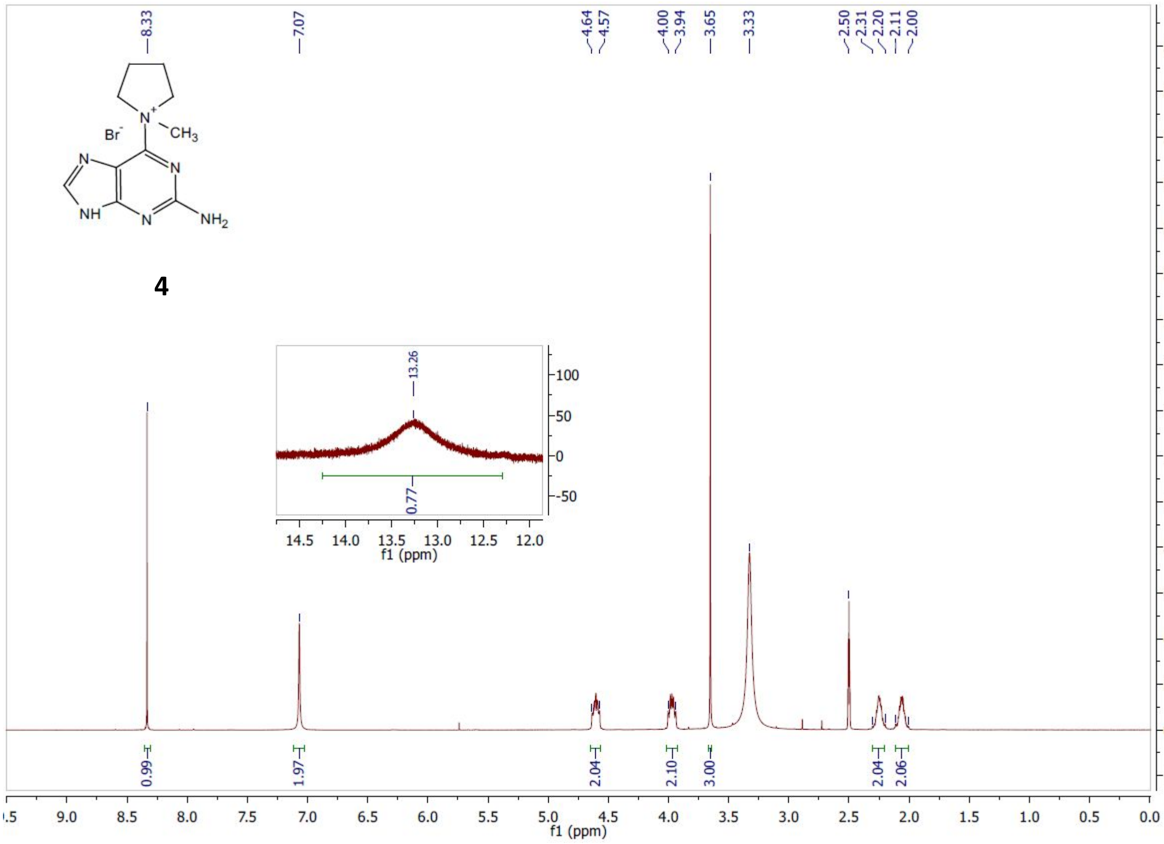


Figure S7: ^{1}H NMR Spectrum of 4.

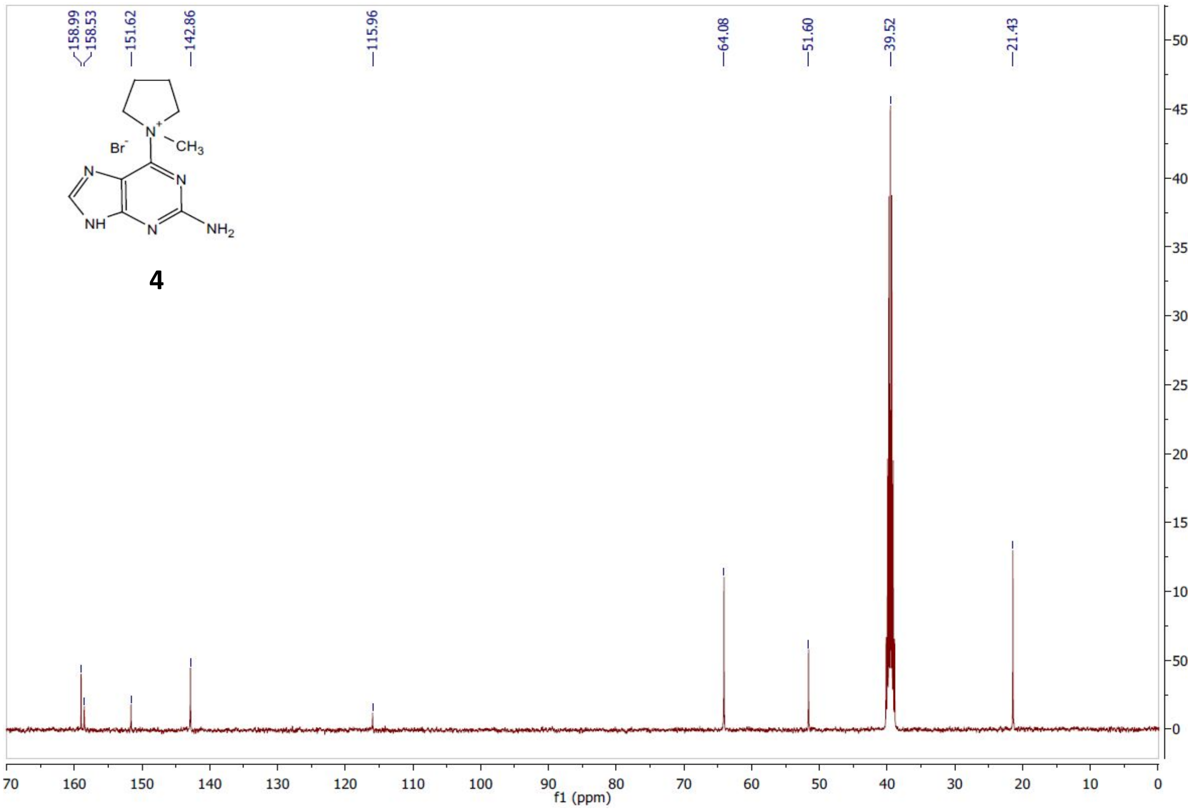


Figure S8: ^{13}C NMR Spectrum of 4.

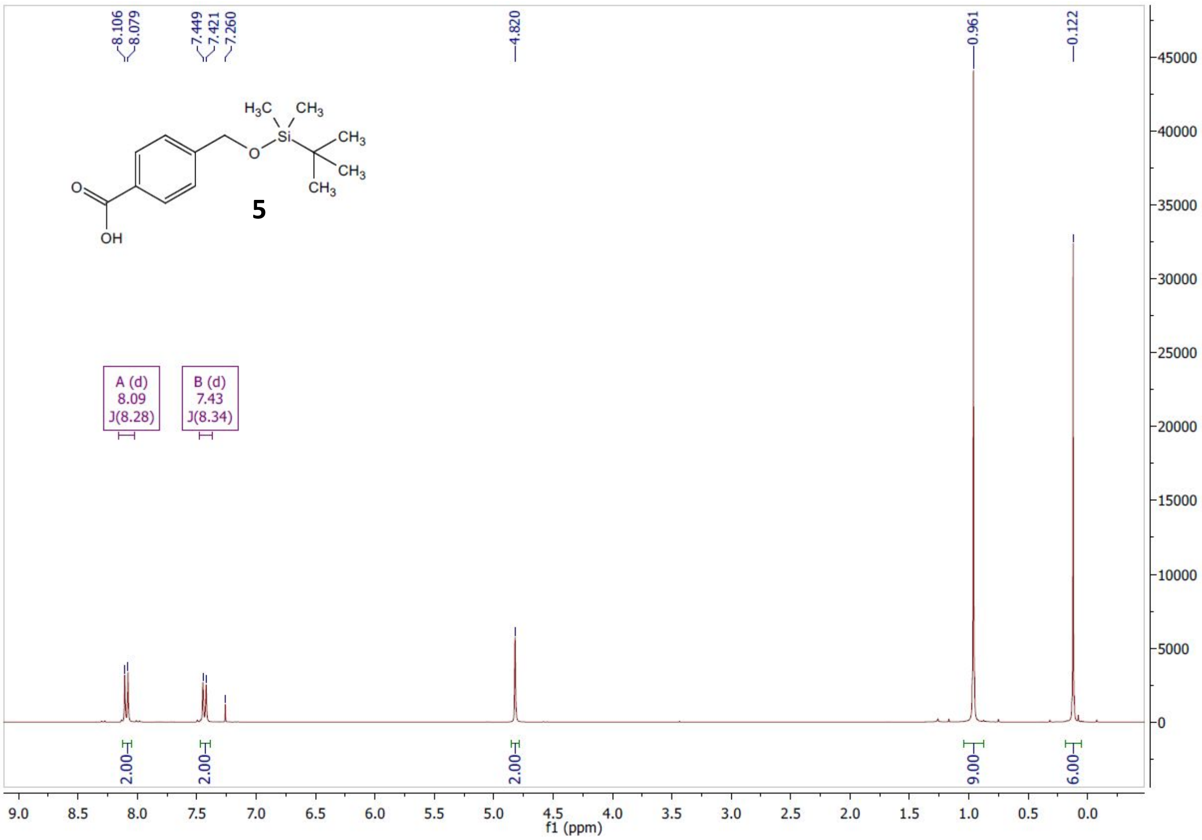


Figure S9: ^{1}H NMR Spectrum of 5.

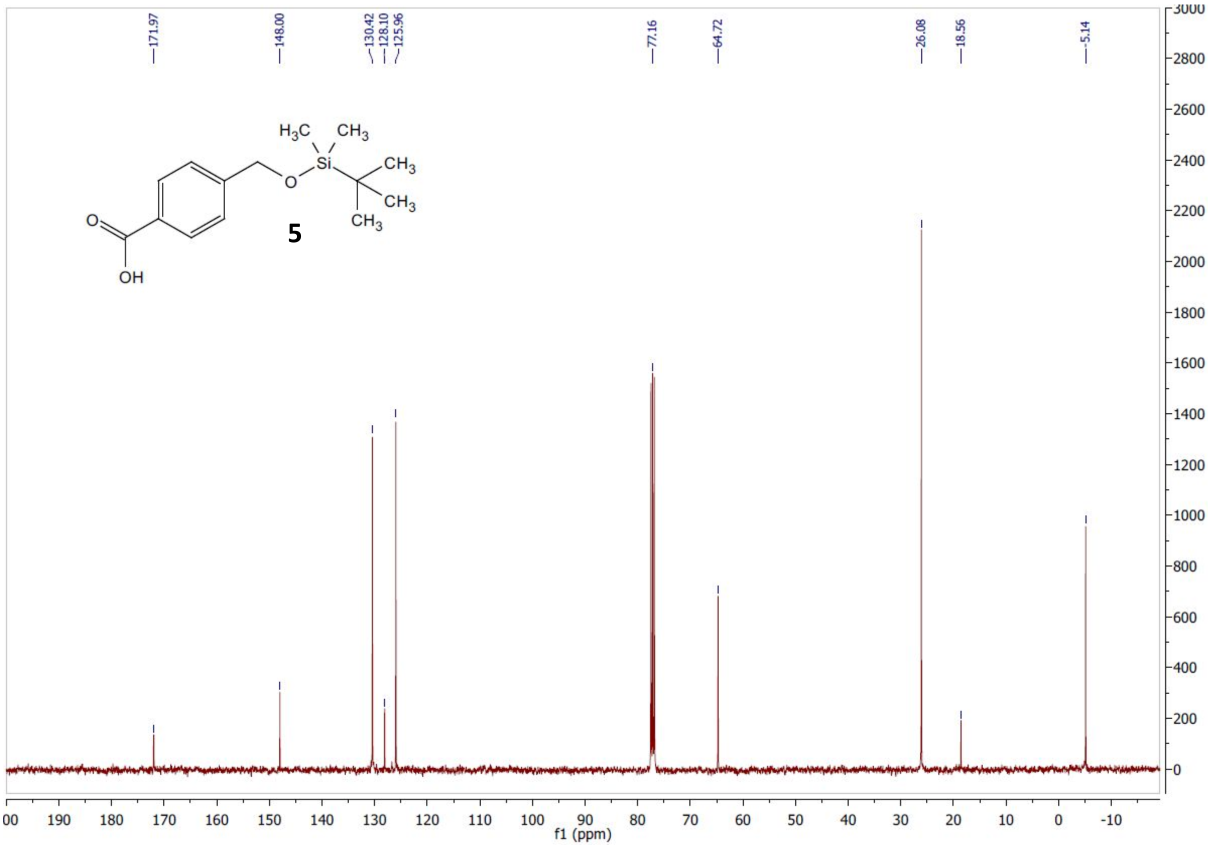


Figure S10: ^{13}C NMR Spectrum of 5.

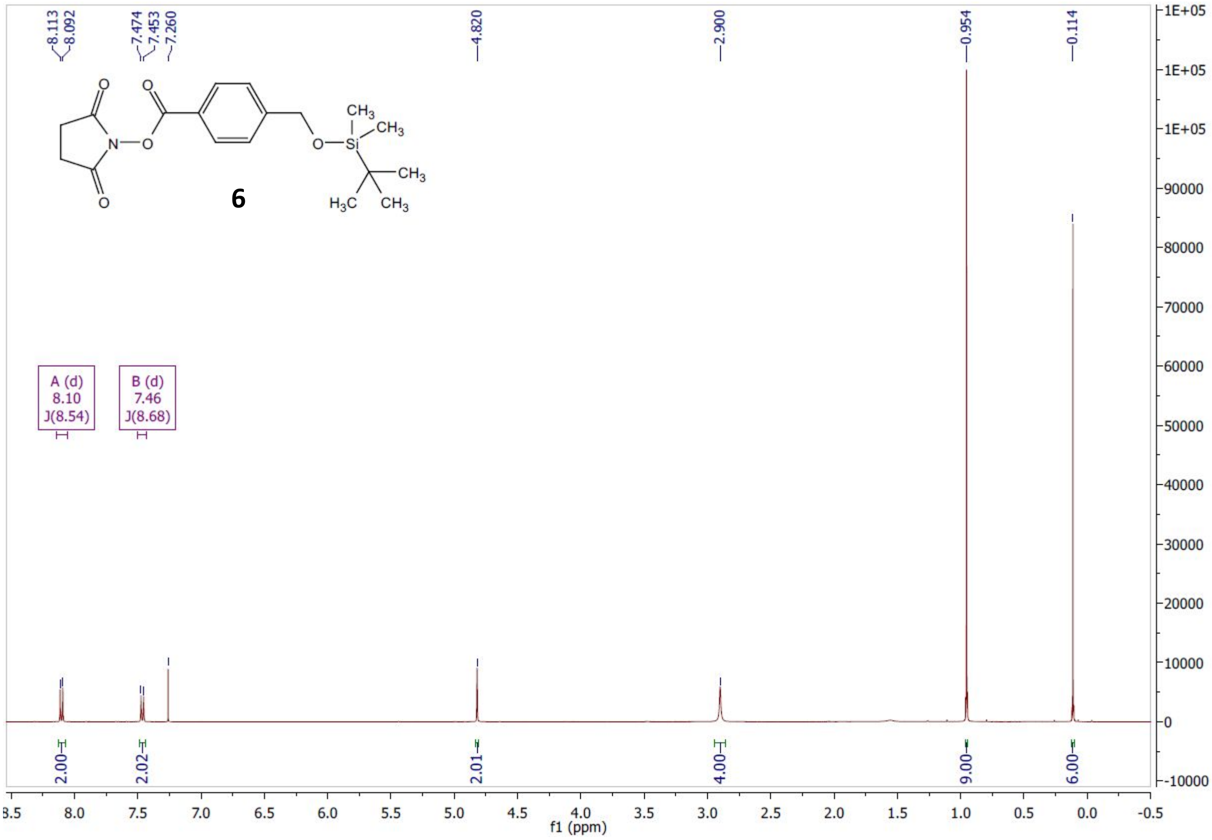


Figure S11: ^{1}H NMR Spectrum of 6.

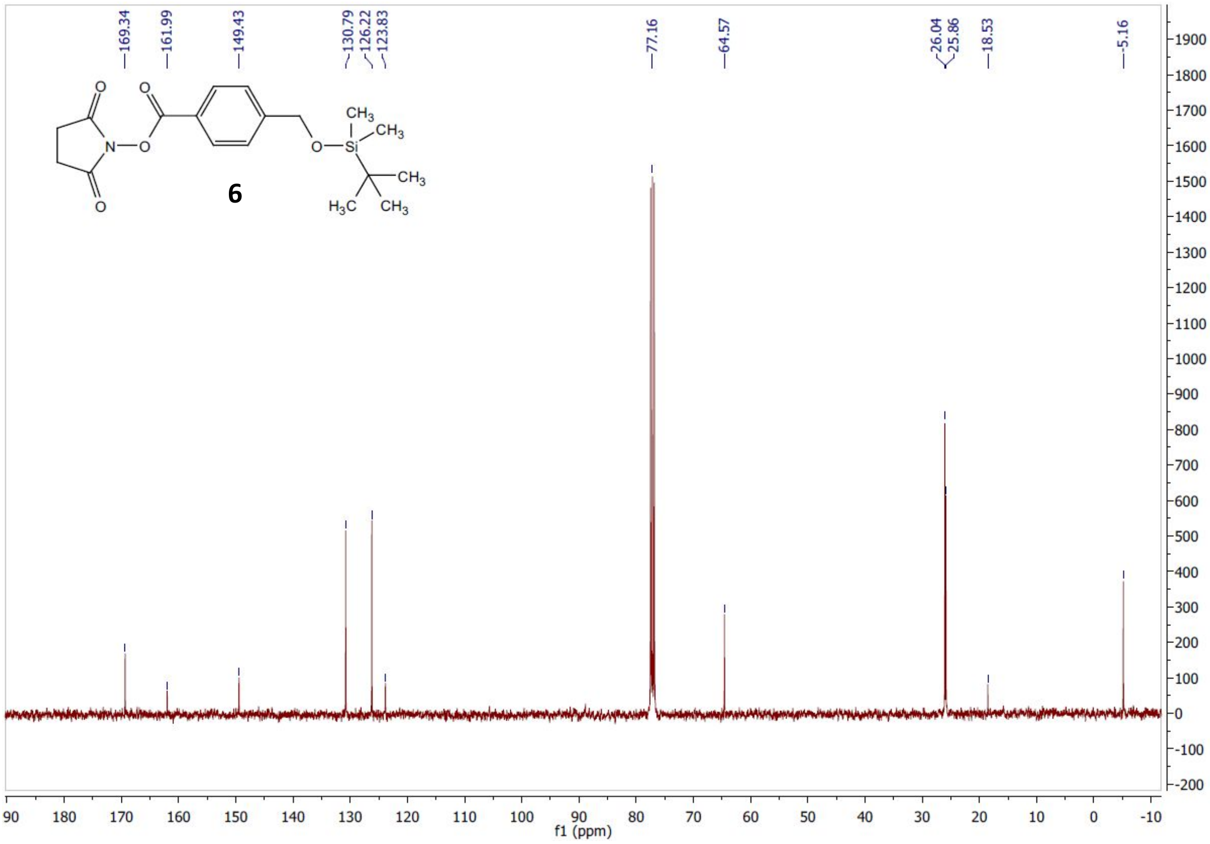


Figure S12: ^{13}C NMR Spectrum of 6.

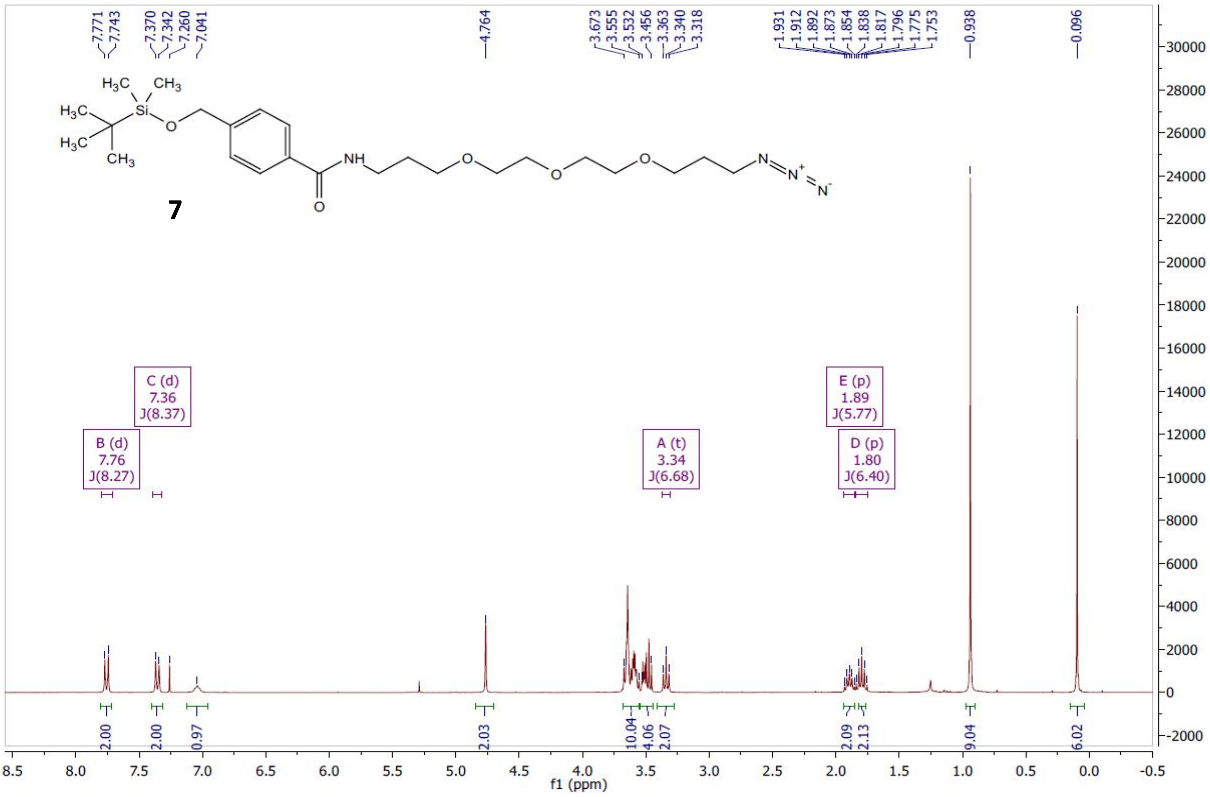


Figure S13: ^{1}H NMR Spectrum of 7.

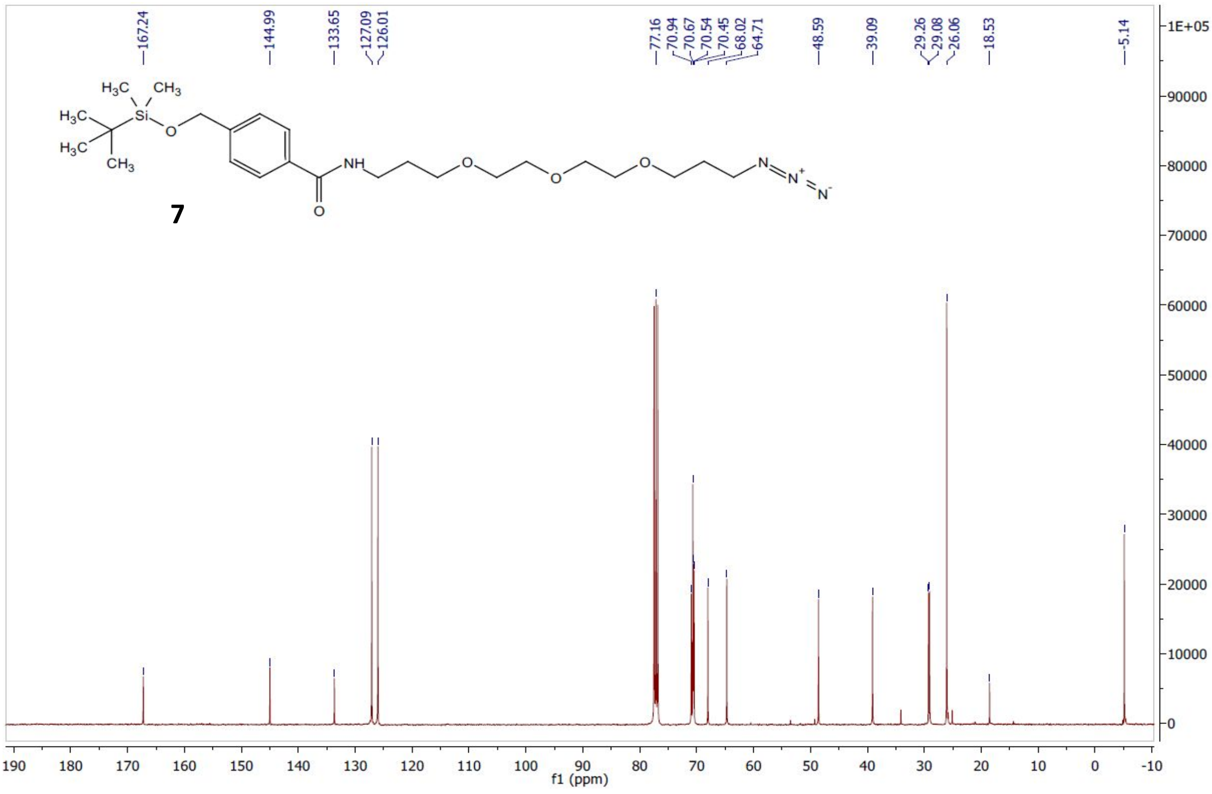


Figure S14: ^{13}C NMR Spectrum of 7.

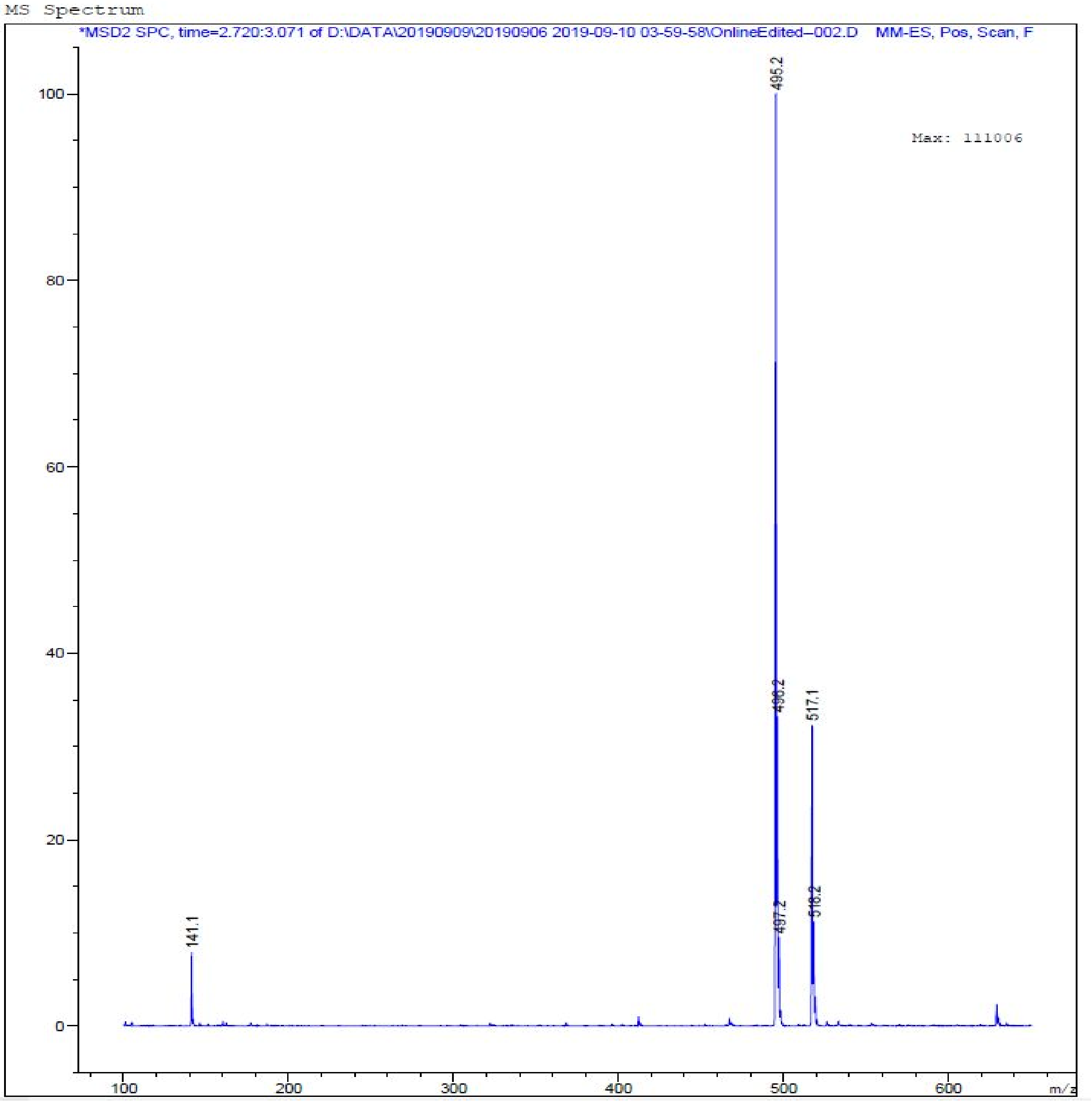


Figure S15: Low resolution *m/z* of 7.

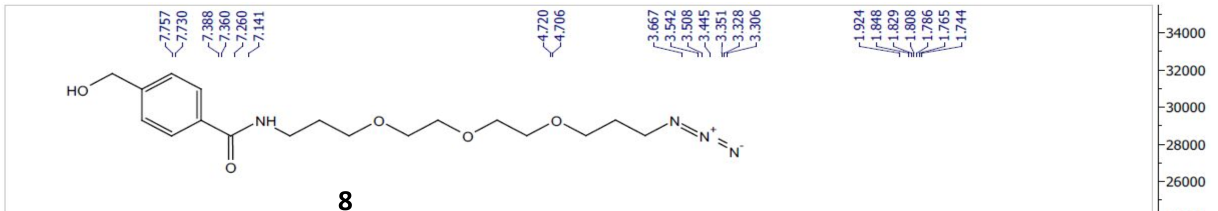


Figure S16: ^{1}H NMR Spectrum of 8.

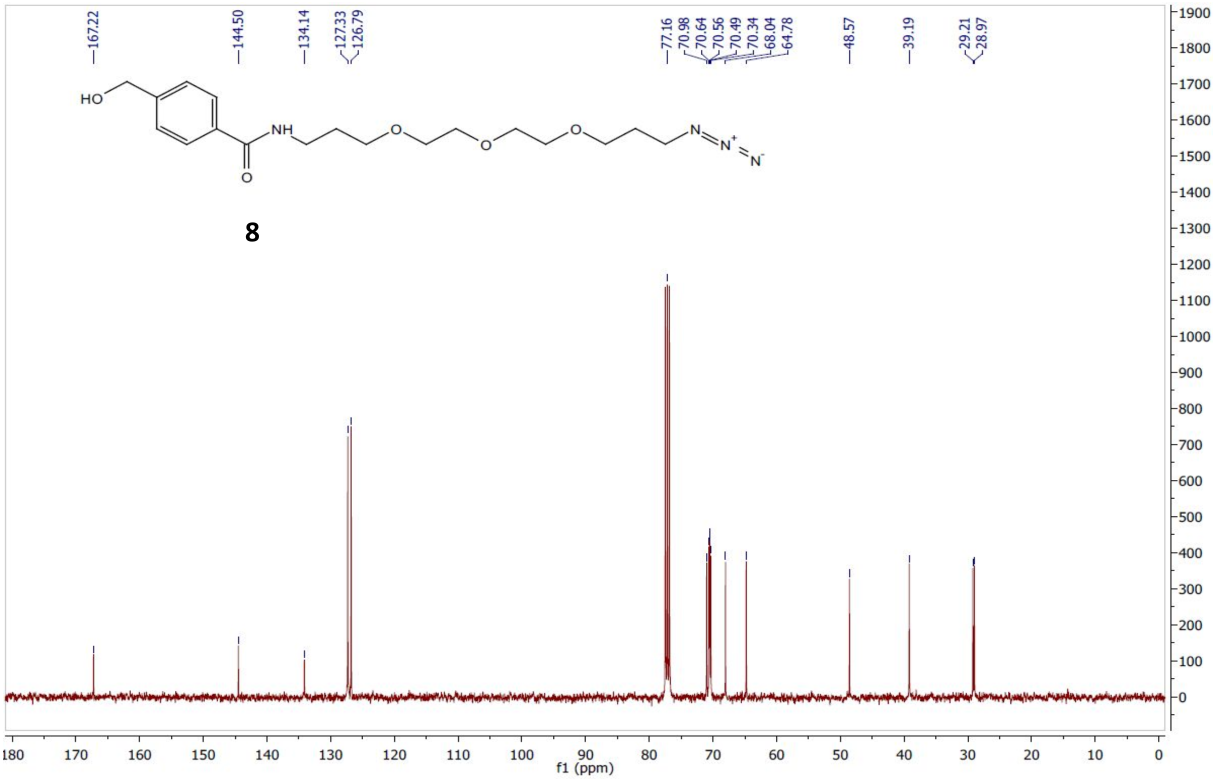


Figure S17: ^{13}C NMR Spectrum of 8.

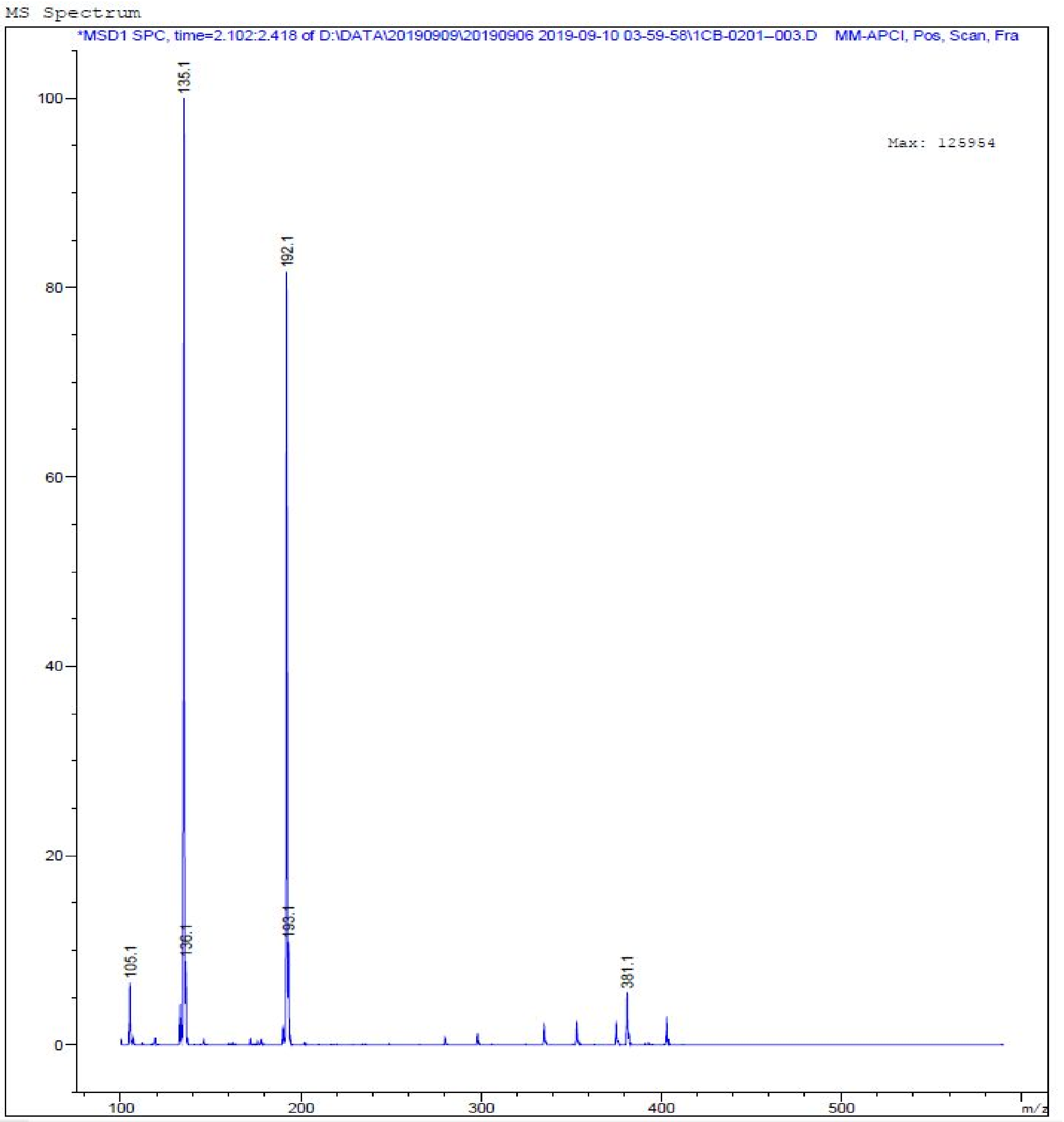


Figure S18: Low resolution *m/z* of 8.

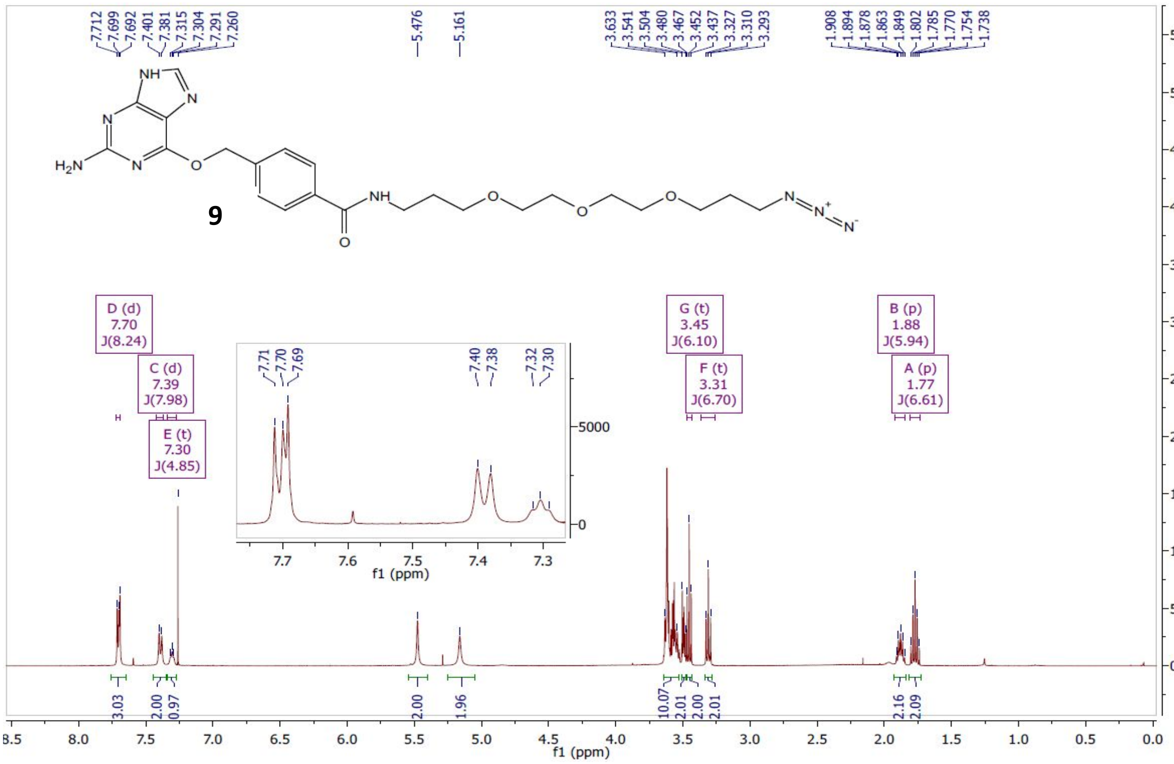


Figure S19: ^{1}H NMR Spectrum of 9.

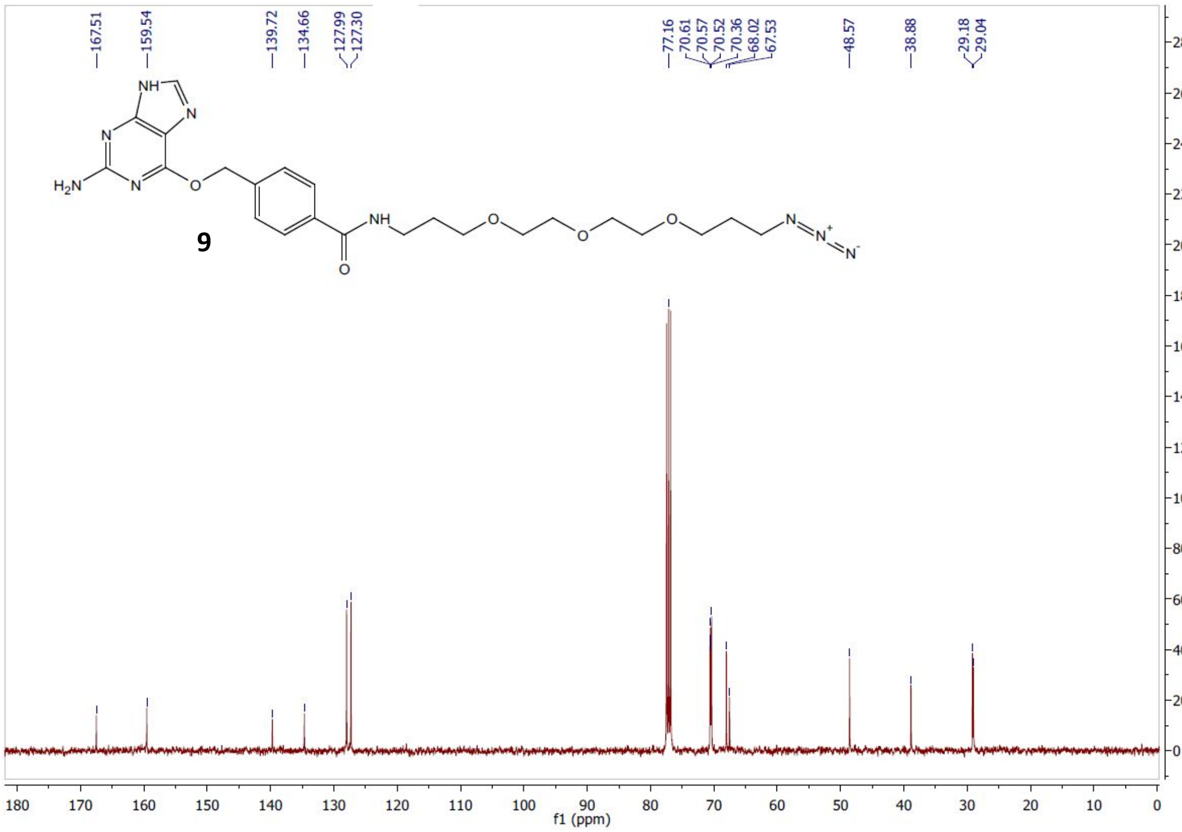


Figure S20: ^{13}C NMR Spectrum of 9.

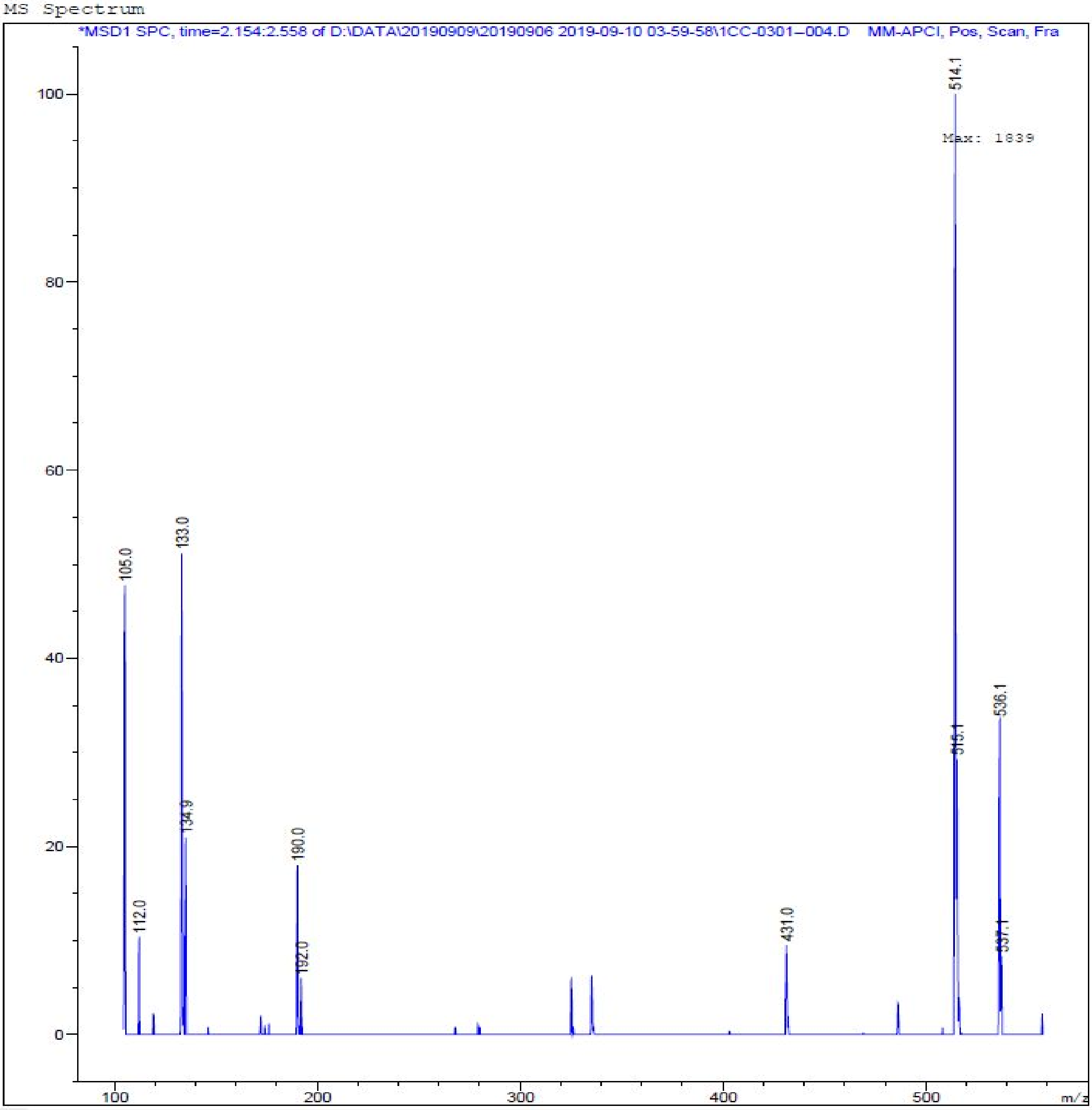


Figure S21: Low resolution *m/z* of 9.

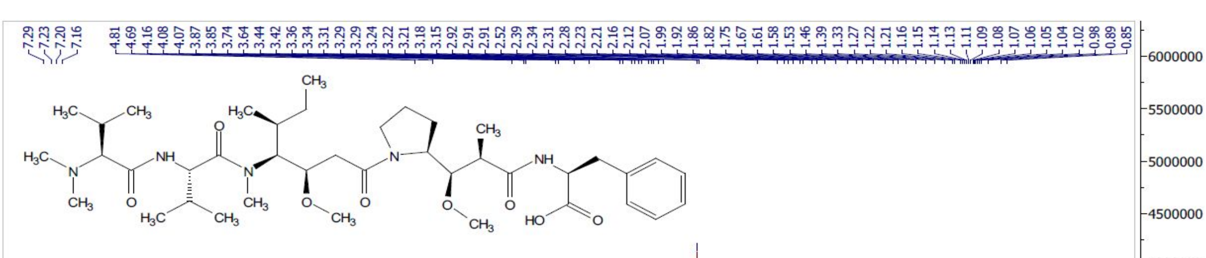


Figure S22: ^{1}H NMR Spectrum of 10.

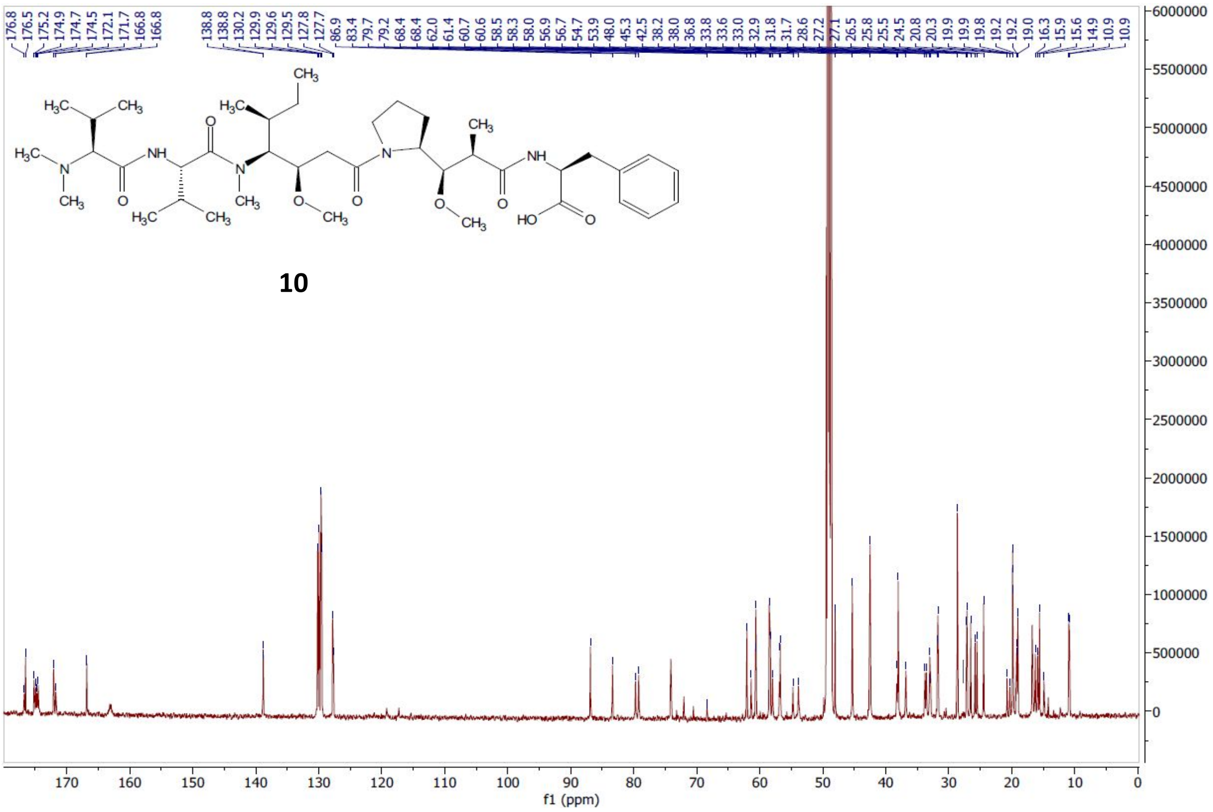


Figure S23: ^{13}C NMR Spectrum of 10.

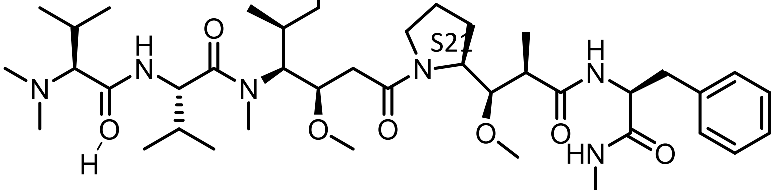


Figure S24: Molecular structure of the $[M + H]^+$ ion of 11.

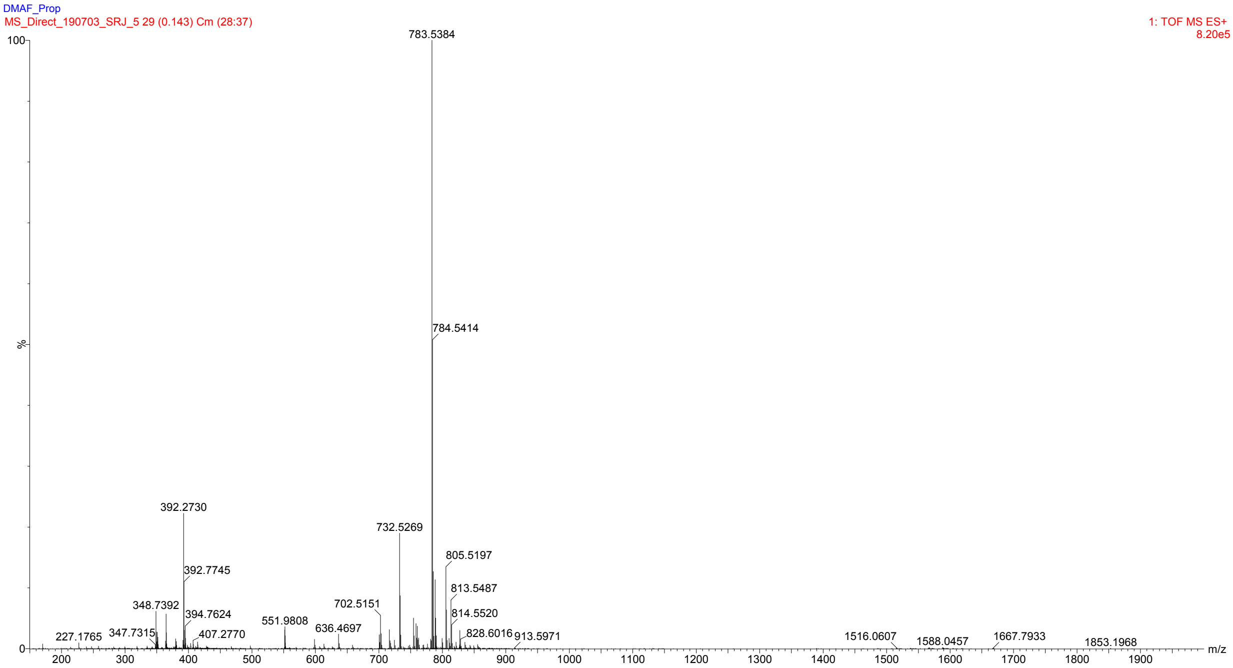


Figure S25: High resolution *m/z* of 11.

Product 12 as AURIF-tether-BG

Product **12** was only faintly visible on TLC under the UV (254 nm) light (presumably due to quenching of the aromatic chromophores) but responded well to a $KMnO_4$ spray. A picture showing a single spot for the product chromatography fractions is given below against the less polar reference, propargyl-auristatin F (compound **11**).

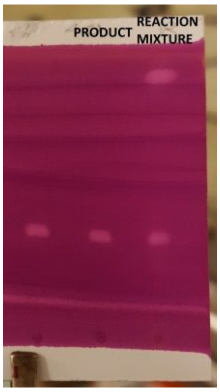
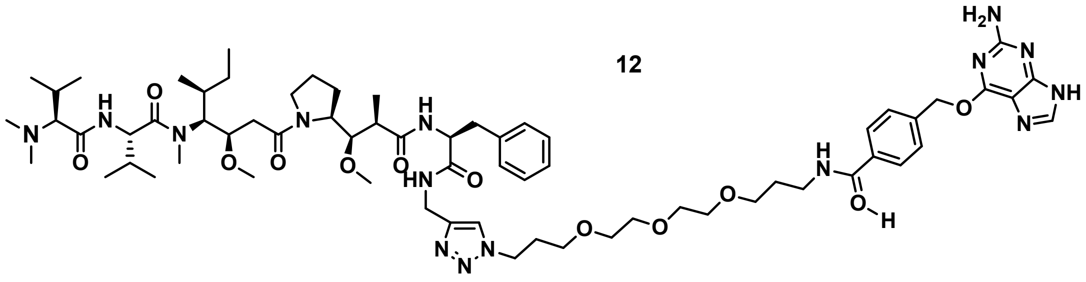


Figure S26: TLC showing the product 12 as a single polar spot with the less polar 11 at the top (on the right in a mixed spot).

The ^{1}H and ^{13}C NMR spectra of **12** were extremely complex due to rotamers,[4] but an important singlet at 5.61 ppm in an uncluttered spectral region for 2H in the ^{1}H NMR spectrum could be discerned for the benzylic methylene group attached to the guanine group (essential for SNAP-tag).

HRMS data for **12** was excellent, though:
HRMS (ES) *m/z*: $[M + H]^+$ Calcd for $C_{66}H_{102}N_{15}O_{12}$ 1296.7832; Found 1296.7878.
HRMS (ES) *m/z*: $[M + Na]^+$ Calcd for $C_{66}H_{101}N_{15}NaO_{12}$ 1318.7652; Found 1318.7592.

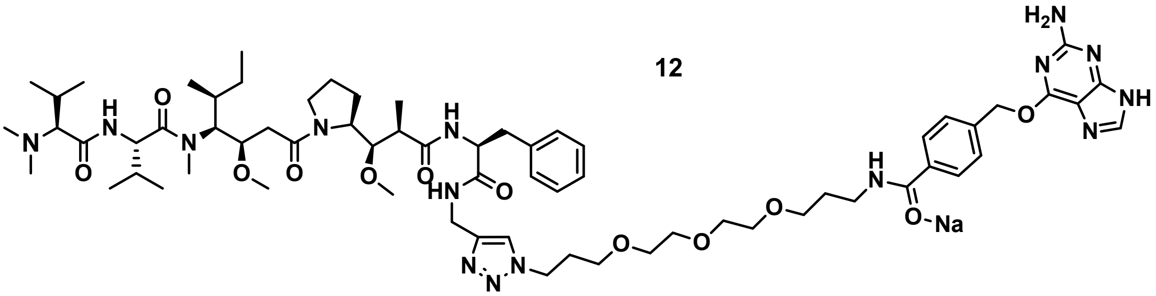
12



Chemical Formula: $C_{66}H_{102}N_{15}O_{12}$
Exact Mass: 1296.7832
Molecular Weight: 1297.6094
m/z: 1296.7832 (100.0%), 1297.7866 (71.4%), 1298.7899 (25.1%), 1299.7933 (5.8%), 1297.7803 (5.5%), 1298.7836 (4.0%), 1298.7875 (2.5%), 1299.7908 (1.8%), 1299.7870 (1.4%), 1297.7895 (1.2%)
Elemental Analysis: C, 61.09; H, 7.92; N, 16.19; O, 14.80

Figure S27: Molecular structure of the $[M + H]^+$ ion of 12.

12



Chemical Formula: $C_{66}H_{101}N_{15}NaO_{12}$
Exact Mass: 1318.7652
Molecular Weight: 1319.5912
m/z: 1318.7652 (100.0%), 1319.7685 (71.4%), 1320.7719 (25.1%), 1321.7752 (5.8%), 1319.7622 (5.5%), 1320.7656 (4.0%), 1320.7694 (2.5%), 1321.7728 (1.8%), 1321.7689 (1.4%), 1319.7715 (1.2%)
Elemental Analysis: C, 60.07; H, 7.71; N, 15.92; Na, 1.74; O, 14.55

Figure S28: Molecular structure of the [M + Na]$^+$ ion of 12.

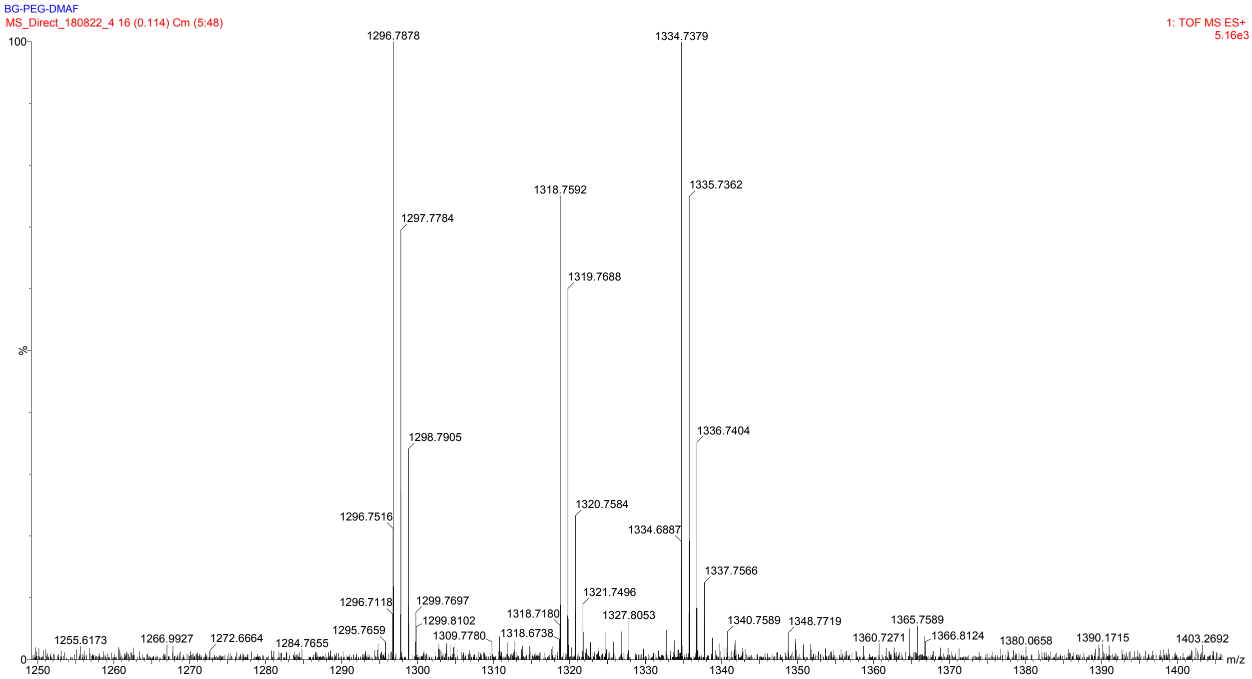


Figure S29: High resolution *m/z* of 12.

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