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Supporting Information

Multivalency Increases the Binding Strength of RGD Peptidomimetic-Paclitaxel Conjugates to Integrin $\alpha_V\beta_3$

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Materials and methods

All manipulations requiring anhydrous conditions were carried out in flame-dried glassware, with magnetic stirring and under a nitrogen atmosphere. All commercially available reagents were used as received. Anhydrous solvents were purchased from commercial sources and withdrawn from the container by syringe, under a slight positive pressure of nitrogen. The reactions were monitored by analytical thin-layer chromatography (TLC) using silica gel 60 F_{254} pre-coated glass plates (0.25 mm thickness). Visualization was accomplished by irradiation with a UV lamp and/or staining with a potassium permanganate alkaline solution, ninhydrin or ceric ammonium molibdate solution. Flash column chromatography was performed according to the method of Still and co-workers[S1] using Chromagel 60 ACC (40-63 μm) silica gel. Automated chromatography was performed with Grace Reveleris instrument. Proton NMR spectra were recorded on a spectrometer operating at 400.16 MHz. Proton chemical shifts are reported in ppm (δ) with the solvent reference relative to tetramethylsilane (TMS) employed as the internal standard ($CDCl_3$ δ = 7.26 ppm; CD_2Cl_2, δ = 5.32 ppm; $[D]_6$DMSO, δ = 2.50 ppm; CD_3OD, δ = 3.33 ppm). The following abbreviations are used to describe spin multiplicity: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, bs = broad signal, dd = doublet of doublet, ddd = doublet of doublet of doublet, ddt = doublet of doublet of triplet. Carbon NMR spectra were recorded on a spectrometer operating at 100.63 MHz, with complete proton decoupling. Carbon chemical shifts are reported in ppm (*δ*) relative to TMS with the respective solvent resonance as the internal standard ($CDCl_3$, δ = 77.16 ppm; CD_2Cl_2, δ = 54.00 ppm; $[D]_6$DMSO, δ = 39.51 ppm; CD_3OD, δ = 49.05 ppm).

ESI-MS spectra were recorded on the ion trap mass spectrometer Finnigan LCQ Advantage or Micro Waters Q-Tof (ESI source). The MALDI-TOF-MS spectra were recorded on the instrument Bruker Microflex™ LT, supporting the sample on the 2,5-dihydroxybenzoic acid (DHB), α-cyano-4-hydroxycinnamic acid (HCCA) and sinapinic acid (SIN) matrices. The peptide calibration standard (300-3000 Da range), which consisted of Angiotensin II, Angiotensin I, Substance P, Bombesin; ACTH clip 1-17, ACTH clip 18-39, Somatostatin 28, was purchased from Bruker Daltonics® and used to calibrate the MALDI-TOF-MS instrument. The sample was mixed in equal volumes with the matrix solution: a small amount (1 μL) of this mixture was spotted on the target surface. The target matrix was dried at room temperature and then analyzed.

High-resolution mass spectra (HRMS) were performed with a Fourier Transform Ion Cyclotron Resonance (FT-ICR) Mass Spectrometer APEX II & Xmass software (Bruker Daltonics) – 4.7 T Magnet (Magnex) equipped with ESI source, available at CIGA (Centro Interdipartimentale Grandi Apparecchiature) c/o Università degli Studi di Milano.

HPLC purifications and HPLC traces of final products were performed on Dionex Ultimate 3000 equipped with Dionex RS Variable Wavelenght Detector (column: Atlantis Prep T3 OBD™ 5 μm 19 × 100 mm; flow 15 mL/min unless stated otherwise). The crude reaction mixture was dissolved in H_2O or, if the compound

was insoluble in water, adding first DMF, then diluting slowly with H_2O until reaching a 1:1 mixture DMF/H_2O (ultrasonic sonicator was used to assist the dissolution). The solution so obtained was filtered (polypropylene, 0.45 μm, 13 mm ø, PK/100) and injected in the HPLC, affording purified products. Purity analyses were carried on a Dionex Ultimate 3000 instrument equipped with a Dionex RS Variable Wavelenght detector (column: Atlantis® Prep T3 OBDTM 5 μm 19 × 100 mm). 1 mg of analyte was dissolved in 1 mL of H_2O and was injected using the same gradient used in the purification step. The analysis of the integrals and the relative percentage of purity was performed with the software Cromeleon 6.80 SR11 Build 3161.

Freeze-drying: The product was dissolved in water and frozen with dry ice: the freeze-drying was carried out at least for 48 h at -50 °C using the instrument 5Pascal Lio5P DGT.

BIOLOGICAL ASSAYS

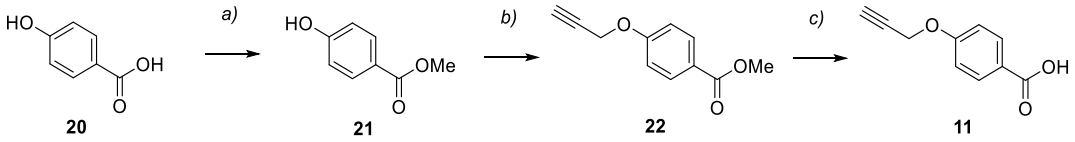
Solid Phase Receptor Binding Assays

Human integrin $\alpha_v\beta_3$ receptor (R&D Systems, Minneapolis, MN, USA) was diluted to 0.5 μg mL^{-1} in coating buffer containing 20 mmol L^{-1} tris(hydroxymethyl) amino methane–HCl (Tris-HCl; pH 7.4), 150 mmol L^{-1} NaCl, 1 mmol L^{-1} $MnCl_2$, 2 mmol L^{-1} $CaCl_2$, and 1 mmol L^{-1} $MgCl_2$. An aliquot of diluted receptor (100 μL $well^{-1}$) was added to 96-well microtiter plates (NUNC MW 96F MAXISORP STRAIGHT) and incubated overnight at 4 ºC. The plates were then incubated with blocking solution (coating buffer plus 1% bovine serum albumin) for an additional 2 h at room temperature to block nonspecific binding; this was followed by a 3 h incubation shaking the plate at room temperature with various concentrations (10^{-12} -10^{-5} M) of test compounds in the presence of 1 μg mL^{-1} vitronectin biotinylated by using an EZ-Link Sulfo-NHS-Biotinylation kit (Pierce, Rockford, IL). After being washed, the plates were incubated shaking for 1 h at room temperature with streptavidin biotinylated peroxidase complex (Amersham Biosciences, Uppsala, Sweden). Then the plates were washed again and finally incubated for 30 min with Substrate Reagent Solution (100 μL; R&D Systems, Minneapolis, MN), before the reaction was stopped by addition of 2 N H_2SO_4 (50 μL). The absorbance at 415 nm was read in a Synergy HT Multi-Detection Microplate Reader (BioTek Instruments, Inc.). Each data point is the result of the average of triplicate wells and was analyzed by nonlinear regression analysis with the GraphPad Prism program. Each experiment was repeated in triplicate. For each data set, normalization was performed by attributing 100% of vitronectin binding to the highest value of absorbance and 0% of vitronectin binding to the lowest value of absorbance. The concentration required for 50% inhibition of vitronectin binding was determined with the GraphPad Prism program.

Synthesis of *cyclo*[DKP-RGD]$_n$-Val-Ala-PTX conjugates (*n* = 1-4)

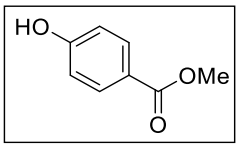
Synthesis of Mono- and polyalkyne scaffolds (11-14)

Synthesis of aromatic mono-alkyne scaffold (11)



Scheme S1. Synthesis of monomeric aromatic scaffold (**11**). REAGENTS AND CONDITIONS: *a)* H_2SO_4, MeOH, reflux, 4 h, Y.: quantitative; *b)* propargyl bromide, K_2CO_3, acetone, r.t., 24 h, Y.: 99%; *c)* NaOH, MeOH/H_2O (3:1), r.t., 5 h, Y.: 98%.

Methyl 4-hydroxybenzoate (**21**)[S2]

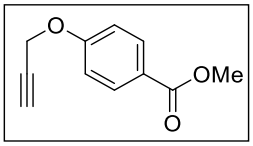


Chemical Formula: $C_8H_8O_3$
Molecular Weight: 152,15

Commercial 4-hydroxybenzoic acid **20** (100 mg, 0.724 mmol, 1 equiv) was dissolved in MeOH (1.3 mL), and H_2SO_4 (19 µL, 0.362 mmol, 0.5 equiv) was added thereto, and stirred for 4 hours under reflux. The reaction mixture was concentrated, diluted in water, and extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure. Then, the residue was purified by flash chromatography on silica gel (1:1 Et_2O/*n*-Hexane) affording the desired product **21** as a white solid (110 mg, yield: quantitative).

R_f = 0.48 (1:1, Et_2O/*n*-Hexane); ^{1}H NMR (400 MHz, CD_2Cl_2-d_2) δ 7.98 (d, *J* = 8.8 Hz, 2H), 6.92 (d, *J* = 8.7 Hz, 2H), 5.90 (s, 1H), 3.90 (s, 3H); MS (ESI+) *m/z* calcd for $[C_8H_8O_3]^+$: 153.03 $[M + H]^+$; found: 153.08.

Methyl 4-(prop-2-yn-1-yloxy)benzoate (**22**)[S2]

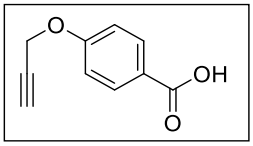


Chemical Formula: $C_{11}H_{10}O_3$
Molecular Weight: 190,20

Compound **21** (110 mg, 0.723 mmol, 1 equiv) was dissolved in dry acetone (7 mL) under nitrogen atmosphere. The solution was cooled in an ice bath. Propargyl bromide (250 µL, 2.9 mmol, 4 equiv) and K_2CO_3 (400 mg, 2.9 mmol, 4 equiv) were added, and the mixture was stirred at room temperature 72 h at 30 ºC. The mixture was concentrated, then the crude was dissolved in AcOEt (70 mL) and washed with water (3 × 10 mL). The organic phase was dried over Na_2SO_4 and concentrated, affording the desired product **22** as an orange solide (135 mg, yield: 99%).

R_f = 0.77 (1:1, *n*-Hexane/EtOAc); ^{1}H NMR (400 MHz, CD_2Cl_2-d_2) δ 7.99 (d, *J* = 9.0 Hz, 2H), 7.01 (d, *J* = 8.9 Hz, 2H), 4.76 (d, *J* = 2.4 Hz, 2H), 3.86 (s, 3H), 2.61 (t, *J* = 2.4 Hz, 1H); MS (ESI+) *m/z* calcd for $[C_{11}H_{10}O_3]^+$: 190.06 $[M + H]^+$; found: 191.08.

4-(Prop-2-yn-1-yloxy)benzoic acid (**11**)[S2]

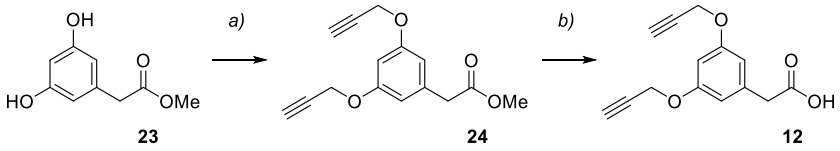


Chemical Formula: $C_{10}H_8O_3$
Molecular Weight: 176,17

Compound **22** (135 mg, 0.710 mmol, 1 equiv) was dissolved in MeOH (6 mL) under a nitrogen atmosphere. The solution was cooled to 0 °C, then NaOH (142 mg, 3.6 mmol, 5 equiv) in H_2O (2 mL) was added. The mixture was stirred 5 h at r.t.. The mixture evaporated until the dryness under reduced pressure and later acidified to ca. pH = 1 with a 1 M $KHSO_4$ aqueous solution and extracted with EtOAc (4 × 20 mL). The organic phase was dried over Na_2SO_4 and concentrated, affording **11** as an orange solid (120 mg, yield: 98%).

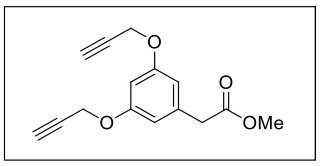
R_f = 0.15 (3:2, *n*-Hexane/EtOAc); ^{1}H NMR (400 MHz, CD_3OD) δ 7.98 (d, *J* = 8.9 Hz, 2H), 7.05 (d, *J* = 8.9 Hz, 2H), 4.81 (d, *J* = 2.4 Hz, 2H), 2.99 (t, *J* = 2.4 Hz, 1H). MS (ESI+) *m/z* calcd for $[C_{10}H_8O_3]^+$: 176.05 [*M* + H]$^+$; found: 177.06; *m/z* calcd $[C_{10}H_8NaO_3]^+$: 199.39 [*M* + Na]$^+$; found: 199.45.

Synthesis of bis-alkyne scaffold (12)



Scheme S2. Synthesis of dimeric scaffold (**12**). REAGENTS AND CONDITIONS: *a)* propargyl bromide, K_2CO_3, acetone, r.t., 24 h, Y.:58; *b)* LiOH·H_2O, THF/H_2O (2:1), 0 °C, 1.5 h Y.: quantitative.

Methyl 3,5-bis(propynyloxy)phenyl acetate (**24**)

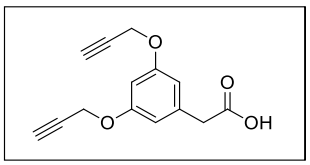


Chemical Formula: $C_{15}H_{14}O_4$
Molecular Weight: 258,27

Commercial methyl 3,5-hydroxyphenyl acetate **23** (200 mg, 1.09 mmol, 1 equiv) was dissolved in dry acetone (11 mL) under nitrogen atmosphere. The solution was cooled in an ice bath. Propargyl bromide (760 µL, 8.8 mmol, 8 equiv) and K_2CO_3 (1.2 g, 8.8 mmol, 8 equiv) were added, and the mixture was stirred at room temperature 72 h. The mixture was concentrated, then the crude was dissolved in AcOEt (70 mL) and washed with water (3 × 10 mL). The organic phase was dried over Na_2SO_4 and concentrated. The crude residue was purified by a Grace Reveleris system (column: Reveleris Silica 12 g; dry load; flow rate: 30 $mLmin^{-1}$; ramp: from 100% hexane to 100% AcOEt in 18 min) to afford **24** as a white solid (167 mg, yield: quantitative).

R_f = 0.58 (1:1, *n*-Hexane/AcOEt,); ^{1}H NMR (400 MHz, $CDCl_3$) *δ* 6.53 (bs, 3H), 4.65 (d, *J* = 2.4 Hz, 4H), 3.68 (s, 3H), 3.56 (s, 2H), 2.53 (t, *J* = 2.4 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) *δ* 171.6, 158.7, 136.2, 109.1, 101.0, 78.4, 75.8, 55.9, 52.2, 41.4.

3,5-bis(Propynyloxy)phenyl acetic acid (**12**)

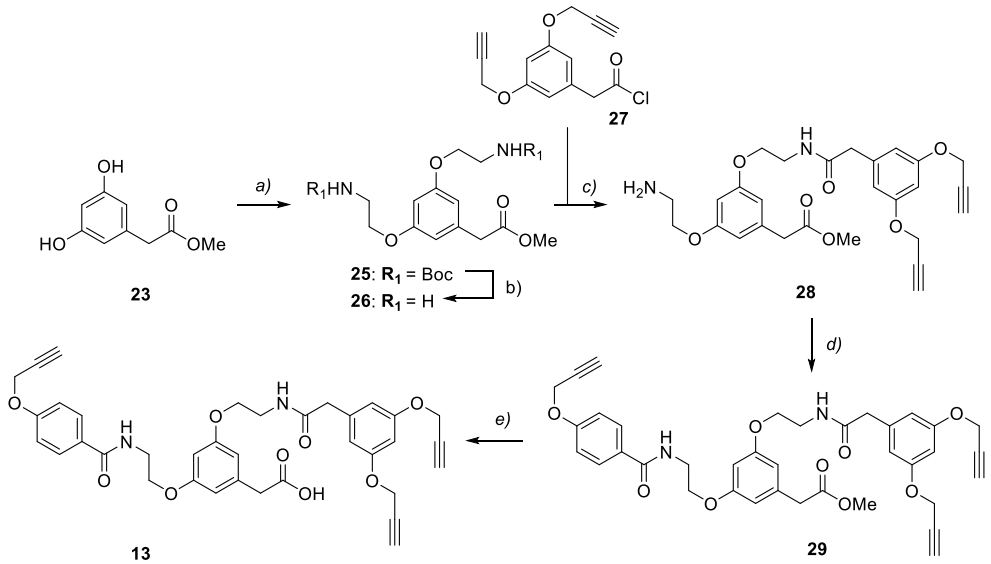


Chemical Formula: $C_{14}H_{12}O_4$
Molecular Weight: 244,24

Compound **24** (165 mg, 0.64 mmol, 1 equiv) was dissolved in THF (20 mL) under a nitrogen atmosphere. The solution was cooled to 0 °C, then a solution of $LiOH{\cdot}H_2O$ (67 mg, 1.6 mmol, 2.5 equiv) in H_2O (10 mL) was added. The mixture was stirred 1.5 h at 0 °C. The mixture was acidified to ca. pH = 2 with a 1 M $KHSO_4$ aqueous solution and extracted with CH_2Cl_2 (4 × 20 mL). The organic phase was dried over Na_2SO_4 and concentrated, affording **12** as a white solid (158 mg, quantitative yield).

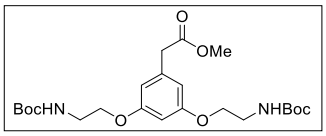
R_f = 0.36 (1:1, *n*-Hexane/AcOEt,); ^{1}H NMR (400 MHz, CD_3OD) δ 6.59-6.51 (m, 3H), 4.70 (d, J = 2.4 Hz, 4H), 3.54 (s, 2H), 2.92 (t, J = 2.4 Hz, 2H).

Synthesis of tris-alkyne scaffold (13)



Scheme S3. Synthesis of trimeric scaffold (**13**). REAGENTS AND CONDITIONS: *a)* 2-(Boc-amino)ethyl bromide, K_2CO_3, acetone, reflux, overnight, Y.: 87%; *b)* TFA/CH_2Cl_2 (1:2), r.t., 45min; *c)* **27**, Et_3N, DMF, r.t., overnight; *d)* **11**, HATU, HOAT, *i*Pr_2NEt, DMF, overnight, Y.: 30% (over three steps); *e)* $LiOH{\cdot}H_2O$, THF/H_2O (2:1), 0 °C, 1.5 h, Y.: 99%.

Methyl 2-(3,5-bis(2-((tert-butoxycarbonyl)amino)ethoxy)phenyl)acetate (**25**)

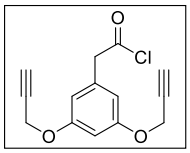


Chemical Formula: $C_{23}H_{36}N_2O_8$
Molecular Weight: 468,55

Methyl 3,5-hydroxyphenyl acetate **23** (500 mg, 2.75 mmol, 1 equiv) was dissolved in dry acetone (20 mL) under nitrogen atmosphere. 2-(Boc-amino)ethyl bromide (3.7 g, 16.5 mmol, 6 equiv) in dry acetone (7 mL) was added to the starting material at 0 ºC together with K_2CO_3 (2.3 g, 16.5 mmol, 6 equiv), and the mixture was stirred under reflux, overnight. The mixture was concentrated, and later diluted in AcOEt (70 mL). The organic layer was washed with H_2O (2 × 30 mL) and brine (1 × 20 mL). The organic phase was dried over Na_2SO_4 and concentrated, then the crude was purified by flash chromatography [eluent: 9:1 CH_2Cl_2/AcOEt] to afford protected diamine **25** as a transparent oil (1.12 g, 87% yield).

R_f = 0.38 (9:1, CH_2Cl_2/AcOEt); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.42 (d, *J* = 2.2 Hz, 2H), 6.34 (t, *J* = 2.2 Hz, 1H), 4.99 (s, 2H), 3.97 (t, *J* = 5.1 Hz, 4H), 3.68 (s, 3H), 3.53 (s, 2H), 3.51-3.46 (m, 4H), 1.44 (s, 18H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.62, 159.82, 155.86, 136.23, 108.31, 100.14, 67.26, 52.06, 41.30, 40.09, 28.39, 27.34.

2-(3,5-bis(Prop-2-yn-1-yloxy)phenyl)acetyl chloride (**27**)

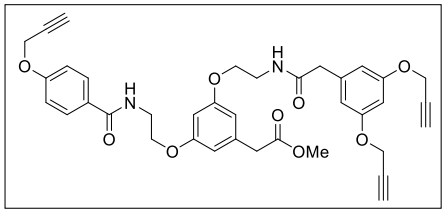


Chemical Formula: $C_{14}H_{11}ClO_3$
Molecular Weight: 262,69

Thionyl chloride (90 µL, 1.23 mmol, 3 equiv) and DMF (16 µL, 1.3 mmol, 0.5 equiv) were added to a solution of acid **23** (100 mg, 0.410 mmol, 1 equiv) in dry CH_2Cl_2 (1.3 mL). After the mixture was stirred at room temperature for 1 h, the solvent was removed in vacuum to obtain the corresponding acetyl chloride **27** (110 mg, quantitative yield) as yellow oil, which was used directly in the next step.

^{1}H NMR (400 MHz, CD_2Cl_2-d_2) δ 6.59 (t, *J* = 2.3 Hz, 1H), 6.53 (d, *J* = 2.2 Hz, 2H), 4.69 (d, *J* = 2.5 Hz, 4H), 4.12 (s, 2H), 2.60 (t, *J* = 2.4 Hz, 2H).

Methyl 2-(3-(2-(2-(3,5-bis(prop-2-yn-1-yloxy)phenyl)acetamido)ethoxy)-5-(2-(4-(prop-2-yn-1-yloxy)benzamido)ethoxy)phenyl)acetate (**29**)



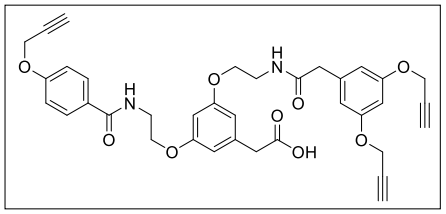
Chemical Formula: $C_{37}H_{36}N_2O_9$
Molecular Weight: 652,70

A solution of Boc-protected compound **25** (550 mg, 2.05 mmol, 1 equiv) in dry CH_2Cl_2 (67 mL) was cooled to 0 °C under a nitrogen atmosphere and TFA (33 mL) was added. The mixture was then allowed to reach room temperature and stirred for 45 min. The solvent was removed affording the corresponding

trifluoroacetate salt **26**, without further purifications. The obtained solid (550 mg, 2.05 mmol, 5 equiv) was dissolved in a pre-flame dry flask with DMF (14 mL) and Et_3N (57 µL, 0.41 mmol, 1 equiv) and stirred about 20 min at r.t. Then, the acetyl chloride **27** (110 mg, 0.410 mmol, 1 equiv) dissolved in DMF (5 mL) was slowly added (dropwise) to the TFA salt **26** at 0 ºC and the reaction mixture was stirred overnight at r.t. AcOEt (100 mL) was added and the solution was washed with $NaHCO_3$ (2 × 30 mL) and brine (1 × 15 mL). The organic phase was dried over Na_2SO_4 and concentrated, giving a yellow crude as amine **28** (MS (ESI+): *m/z* calcd for $[C_{27}H_{31}N_2O_7]^+$: 495.21, $[M + H]^+$; found 495.25). Without being purified, the amine **28** was used directly at the further coupling. A solution of acid **11** (109 mg, 0.62 mmol, 1.5 equiv) in dry DMF (15 mL) was cooled to 0 °C under a nitrogen atmosphere. HATU (265 mg, 0.70 mmol, 1.7 equiv), HOAT (95 mg, 0.70 mmol, 1.7 equiv) and *i*Pr_2NEt (286 µL, 1.64 mmol, 4 equiv) were added and the mixture was stirred for 20 min at 0 °C. A solution of **28** (203 mg, 0.410 mmol, 1 equiv) in dry DMF (5 mL) was added to the stirred mixture. The reaction was allowed to slowly reach room temperature and stirred overnight. The mixture was diluted with AcOEt (200 mL) and washed with aqueous solution of $NaHCO_3$ sat. (2 × 30 mL), a saturated aqueous solution of K_2CO_3 (1 × 30 mL) and brine (2 × 20 mL). The organic phase was dried over Na_2SO_4 and concentrated. The crude was purified by flash chromatography [gradient: from 3:2 AcOEt/Petroleum Ether to 4:1 AcOEt/Petroleum Ether] to afford carbamate **29** as a light orange solid (80 mg, 30% yield over three steps).

R_f = 0.29 (25:75, Petroleum Ether/AcOEt); ^{1}H NMR (600 MHz, CD_2Cl_2-d_2) δ 7.77 (d, *J* = 8.8 Hz, 2H), 6.98 (d, *J* = 8.8 Hz, 2H), 6.87 (t, *J* = 5.5 Hz, 1H), 6.51 (d, *J* = 3.5 Hz, 3H), 6.43 (t, *J* = 1.8 Hz, 1H), 6.35 (dt, *J* = 13.6, 2.1 Hz, 2H), 6.28 (t, *J* = 5.3 Hz, 1H), 4.73 (d, *J* = 2.4 Hz, 2H), 4.62 (d, *J* = 2.5 Hz, 4H), 4.08 (t, *J* = 5.3 Hz, 2H), 3.95 (t, *J* = 5.3 Hz, 2H), 3.78 (q, *J* = 5.4 Hz, 2H), 3.66 (s, 3H), 3.55 (q, *J* = 5.6 Hz, 2H), 3.52 (s, 2H), 3.48 (s, 2H), 2.62 (t, *J* = 2.4 Hz, 1H), 2.58 (t, *J* = 2.5 Hz, 2H). ^{13}C NMR (151 MHz, CD_2Cl_2-d_2) δ 172.19, 171.03, 167.36, 160.54, 160.36, 160.27, 159.40, 138.15, 137.02, 129.34, 128.12, 115.07, 109.51, 109.01, 108.82, 101.42, 100.45, 78.91, 78.59, 76.34, 76.14, 67.36, 67.21, 56.40, 52.49, 44.15, 41.58, 39.92, 39.51, 38.92; MS (MALDI): *m/z* calcd for $[C_{37}H_{36}N_2O_9]^+$: 652.24 $[M + H]^+$; found: 653.27 (DHB matrix); *m/z* calcd for $[C_{37}H_{36}NaN_2O_9]^+$: 675.24 $[M + Na]^+$; found: 675.56 (DHB matrix).

2-(3-(2-(2-(3,5-bis(Prop-2-yn-1-yloxy)phenyl)acetamido)ethoxy)-5-(2-(4-(prop-2-yn-1-yloxy)benzamido)ethoxy)phenyl)acetic acid (**13**)

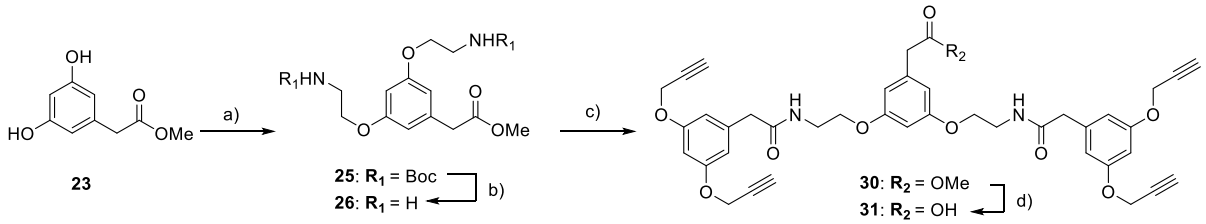


Compound **29** (71 mg, 0.11 mmol, 1 equiv) was dissolved in THF (4 mL) under a nitrogen atmosphere. The solution was cooled to 0 °C, then a solution of $LiOH·H_2O$ (12 mg, 0.27 mmol, 2.5 equiv) in H_2O (2 mL) was added. The mixture was stirred 1.5 h at 0 °C. The mixture was acidified to ca. pH = 2 with a 1 M $KHSO_4$ aqueous solution and extracted with CH_2Cl_2 (4 × 20 mL). The organic phase was dried over Na_2SO_4 and concentrated, affording **13** as a white solid (158 mg, quantitative yield).

R_f = 0.39 (9:1, CH_2Cl_2/MeOH); ^{1}H NMR (500 MHz, CD_3OD) δ 7.80 (d, *J* = 8.8 Hz, 2H), 7.01 (d, *J* = 8.9 Hz, 2H), 6.53 (d, *J* = 2.3 Hz, 2H), 6.50-6.46 (m, 2H), 6.44 (s, 1H), 6.41 (t, *J* = 2.3 Hz, 1H), 4.76 (d, *J* = 2.4 Hz,

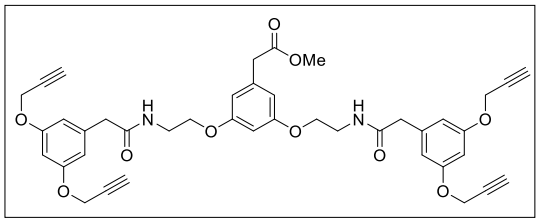
2H), 4.61 (d, *J* = 2.5 Hz, 4H), 4.10 (t, *J* = 5.7 Hz, 2H), 3.97 (t, *J* = 5.2 Hz, 2H), 3.72 (t, *J* = 5.6 Hz, 2H), 3.52 (t, *J* = 6.1 Hz, 2H), 3.50 (s, 2H), 3.45 (s, 2H), 2.97 (t, *J* = 2.4 Hz, 1H), 2.90 (t, *J* = 2.4 Hz, 2H); ^{13}C-DEPT135 NMR (126 MHz, CD_3OD) δ 128.77, 114.29, 108.37, 108.23, 108.06, 100.52, 99.74, 78.34, 77.95, 75.87, 75.54, 66.13, 66.10, 55.32, 42.56, 40.93, 39.27, 38.88. MS (MALDI): *m/z* calcd for $[C_{36}H_{35}N_2O_9]^+$: 638.24 $[M + H]^+$; found: 639.27 (DHB matrix); *m/z* calcd for $[C_{36}H_{34}NaN_2O_9]^+$: 661.24 $[M + Na]^+$; found: 661.27 (DHB matrix); *m/z* calcd for $[C_{36}H_{34}KN_2O_9]^+$: 677.24 $[M + K]^+$; found: 677.27 (DHB matrix).

Synthesis of tetra-alkyne scaffold (14)



Scheme S4. Synthesis of tetrameric scaffold (**14**). REAGENTS AND CONDITIONS: *a)* 2-(Boc-amino)ethyl bromide, K_2CO_3, acetone, reflux, overnight, Y.: 87%; *b)* TFA/CH_2Cl_2 (1:2), r.t., 45 min; *c)* **12**, HATU, HOAT, *i*Pr_2NEt, DMF, overnight, Y.: 77% (over two steps); *d)* LiOH·H_2O, THF/H_2O (2:1), 0 °C, 1.5 h, Y.: 85%.

Methyl 2-(3,5-bis(2-(2-(3,5-bis(prop-2-yn-1-yloxy)phenyl)acetamido)ethoxy)phenyl)acetate (**30**)



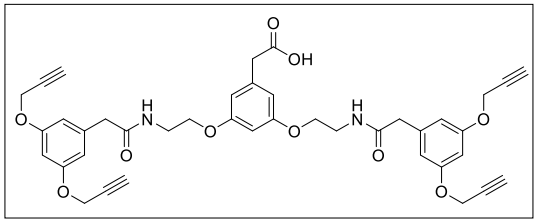
Chemical Formula: $C_{41}H_{40}N_2O_{10}$
Molecular Weight: 720,78

A solution of Boc-protected compound **25** (200 mg, 0.43 mmol, 1 equiv) in dry CH_2Cl_2 (14 mL) was cooled to 0 °C under a nitrogen atmosphere and TFA (7 mL) was added. The mixture was then allowed to reach room temperature and stirred for 45 min. The solvent was removed affording the corresponding trifluoroacetate salt **26**, without further purification. Then, the acid **12** (315 mg, 1.29 mmol, 3 equiv) was solubilized in dry DMF (15 mL) and cooled to 0 °C under a nitrogen atmosphere. HATU (556 mg, 1.46 mmol, 3.6 equiv), HOAT (199 mg, 1.46 mmol, 3.6 equiv) and *i*Pr_2NEt (449 µL, 2.58 mmol, 6 equiv) were added and the mixture was stirred for 20 min at 0 °C. Later, the salt **26** (200 mg, 0.43 mmol, 1 equiv) was dissolved in dry DMF (9 mL) and slowly added (dropwise) the reaction mixture that was stirred overnight at r.t. The mixture was diluted with AcOEt (100 mL) and washed with 1 M aqueous solution of $KHSO_4$ (2 × 15 mL), a saturated aqueous solution of $NaHCO_3$ (1 × 15 mL) and brine (1 × 20 mL). The organic phase was dried over Na_2SO_4 and concentrated. The crude was purified by flash chromatography [eluent: CH_2Cl_2/MeOH, 9:1] to afford diamide **30** as a light orange solid (238 mg, 77% yield over two steps).

R_f = 0.28 (99:1, CH_2Cl_2/MeOH); ^{1}H NMR (400 MHz, CD_2Cl_2-d_2) δ 6.50 (s, 6H), 6.37-6.34 (m, 2H), 6.27 (s, 1H), 6.02 (t, *J* = 5.7 Hz, 2H), 4.63 (d, *J* = 2.3 Hz, 8H), 3.96 (t, *J* = 5.2 Hz, 4H), 3.66 (s, 3H), 3.56 (q, *J* = 5.5 Hz, 4H), 3.52 (s, 2H), 3.48 (s, 4H), 2.56 (t, *J* = 2.5 Hz, 4H). ^{13}C NMR (101 MHz, CD_2Cl_2-d_2) δ 172.12,

170.86, 160.29, 159.47, 138.10, 137.11, 109.56, 108.93, 101.54, 100.53, 78.91, 76.11, 67.30, 56.46, 52.50, 44.28, 41.62, 39.54. MS (ESI+): *m/z* calcd for $[C_{41}H_{40}NaN_2O_{10}]^+$: 743.11, $[M + Na]^+$; found 743.10.

2-(3,5-bis(2-(2-(3,5-bis(Prop-2-yn-1-yloxy)phenyl)acetamido)ethoxy)phenyl)acetic acid (**31**)

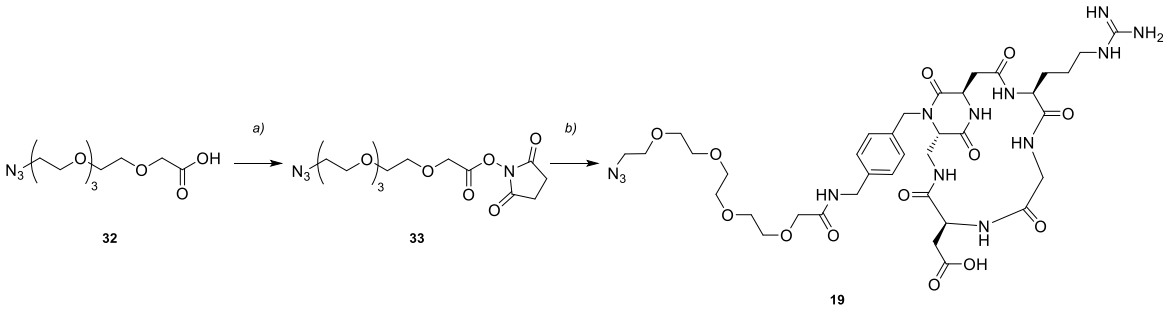


Chemical Formula: $C_{40}H_{38}N_2O_{10}$
Molecular Weight: 706,75

Compound **32** (137 mg, 0.2 mmol, 1 eq) was dissolved in THF (6.6 mL) under a nitrogen atmosphere. The solution was cooled to 0 °C, then a solution of $LiOH{\cdot}H_2O$ (21 mg, 0.5 mmol, 2.5 eq) in H_2O (3.3 mL) was added. The mixture was stirred 1.5 h at 0 °C. The mixture was acidified to ca. pH = 2 with a 1 M $KHSO_4$ aqueous solution and extracted with CH_2Cl_2 (4 × 20 mL). The organic phase was dried over Na_2SO_4 and concentrated. The crude was purified by flash chromatography [eluent: 9:1:0.1 CH_2Cl_2/MeOH/AcOH] affording **33** as a white solid (120 mg, 85% yield).

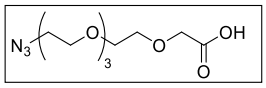
R_f = 0.2 (95:5, CH_2Cl_2/MeOH); ^{1}H NMR (400 MHz, CD_3OD) δ 6.56 (d, *J* = 2.3 Hz, 4H), 6.51 (t, *J* = 2.3 Hz, 2H), 6.47 (d, *J* = 2.2 Hz, 2H), 6.37 (d, *J* = 2.2 Hz, 1H), 4.64 (d, *J* = 2.4 Hz, 8H), 4.01 (t, *J* = 5.4 Hz, 4H), 3.56 (t, *J* = 5.3 Hz, 4H), 3.52 (s, 2H), 3.48 (s, 4H), 2.91 (t, *J* = 2.4 Hz, 4H). ^{13}C NMR (101 MHz, CD_3OD) δ 173.97, 161.21, 160.27, 139.04, 138.69, 109.77, 109.55, 108.51, 101.94, 101.07, 79.71, 76.87, 67.50, 56.72, 43.95, 42.75, 40.26. MS (ESI+): *m/z* calcd for $[C_{40}H_{38}NaN_2O_{10}]^+$: 729.42, $[M + Na]^+$; found 729.45.

Synthesis of ligand *cyclo*[DKP-RGD]-PEG$_4$-Azide (19)



Scheme S5. Synthesis of *cyclo*[DKP-RGD]-PEG$_4$-Azide (**19**). REAGENTS AND CONDITIONS: *a)* EDC·HCl, *N*-Hydroxysuccinimide, CH_2Cl_2, overnight, Y.: 64%; *b) cyclo*[DKP- RGD]-CH_2NH_2, MeCN/PBS pH 7.5 (1:1), overnight; Y.: 77%.

14-Azido-3,6,9,12-tetraoxatetradecanoic acid (**32**)[S3]

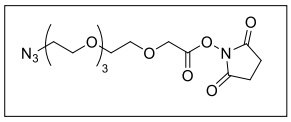


Chemical Formula: $C_{10}H_{19}N_3O_6$
Molecular Weight: 277,28

The commercially available tetraethylene glycol (4.58 g, 23.6 mmol, 3 equiv) was dissolved in dry CH_2Cl_2 (80 mL) under inert atmosphere; then DMAP (0.19 g, 1.58 mmol, 0.2 equiv) and Et_3N (3.3 mL, 23.6 mmol, 3 equiv) were added. The mixture was cooled at 0 °C and a solution of TsCl (1.5 g, 7.9 mmol, 1 equiv) in dry CH_2Cl_2 (40 mL) was added dropwise. The reaction was stirred at r.t. for 2 h. Hence, 100 mL of CH_2Cl_2 were added and the mixture was washed with a solution of HCl 1 M (2 × 40 mL) and brine (1 × 50 mL). The organic phase was dried over Na_2SO_4 and concentrated. Then the crude was dissolved in dry DMF (15 mL) and NaN_3 (1.03 g, 15.8 mmol, 2 equiv) was added under inert atmosphere. The suspension was stirred at 80 °C overnight. The solvent was removed and the crude was purified by flash chromatography on silica gel (gradient from 1% MeOH to 5% MeOH in AcOEt) affording the corresponding azide. Later, to a suspension of NaH (0.6 g, 25 mmol, 5 equiv) in dry THF (25 mL), azido-tetraehtylene glycol (1.09 g, 5 mmol, 1 equiv) dissolved in dry THF (25 mL) was added dropwise over a period of 30 min under inert atmosphere. The reaction was stirred for 1 h more at room temperature. Then, a solution of bromoacetic acid (1.387 g, 10 mmol, 2 equiv) in dry THF (21 mL) was added dropwise over a period of 1 h and then the reaction was stirred overnight at r.t. After addition of cold water, THF was removed by vacuum and a solution of HCl 1M was added until pH = 1. The mixture was extracted with AcOEt (5 × 80 mL) and the collected organic phases were washed with brine (1 × 150 mL). The organic phase was dried over Na_2SO_4 and concentrated, then the crude was purified by flash chromatography on silica gel (eluent: CH_2Cl_2/MeOH, 9:1 + 0.1% CH_3COOH) affording the corresponding carboxylic acid (**37**) as a pale yellow oil (1.09 g, 79% yield).

R_f = 0.25 (9:1, CH_2Cl_2/MeOH + 0,1% CH_3COOH); ^{1}H NMR (400 MHz, CD_2Cl_2-d_2) δ 3.96 (s, 2H), 3.70-3.61 (m, 14H), 3.39 (t, J = 5.0 Hz, 2H).

Azido-tetraethylene glycol-N-hydroxysuccinimidyl ester (**33**)[S3]

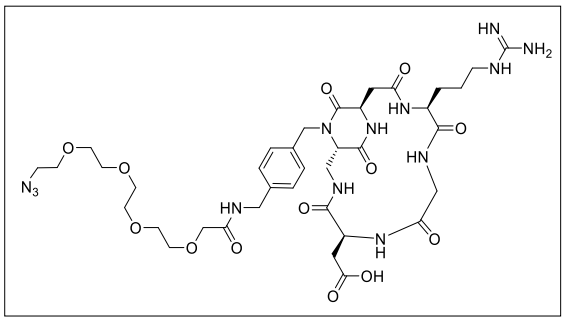


Chemical Formula: $C_{14}H_{22}N_4O_8$
Molecular Weight: 374,35

Carboxylic acid **37** (77 mg, 0.28 mmol, 1 equiv) was dissolved in dry THF and cooled to 0 °C under a nitrogen atmosphere. EDC·HCl (69 mg, 0.36 mmol, 1.3 equiv) and *N*-hydroxysuccinimide (41 mg, 0.36 mmol, 1.3 equiv) were added and the mixture was allowed to reach r.t. and stirred overnight. The solvent was removed and the crude was purified over a pad of silica [eluent: AcOEt/hexane, 8:2] affording ester **33** as a colorless oil (67 mg, 64% yield).

R_f = 0.43 (9:1, CH_2Cl_2/MeOH); ^{1}H NMR (400 MHz, CD_2Cl_2-d_2) δ 4.50 (s, 2H), 3.78-3.73 (m, 2H), 3.68-3.59 (m, 12H), 3.37 (t, J = 5.1 Hz, 2H), 2.83 (s, 4H); MS (ESI+): *m/z* calcd for $[C_{14}H_{22}N_4NaO_8]^+$: 397.13, [M + Na]$^+$; found 397.19.

cyclo[DKP-RGD]-Tetraethylene glycol-azide (**19**)

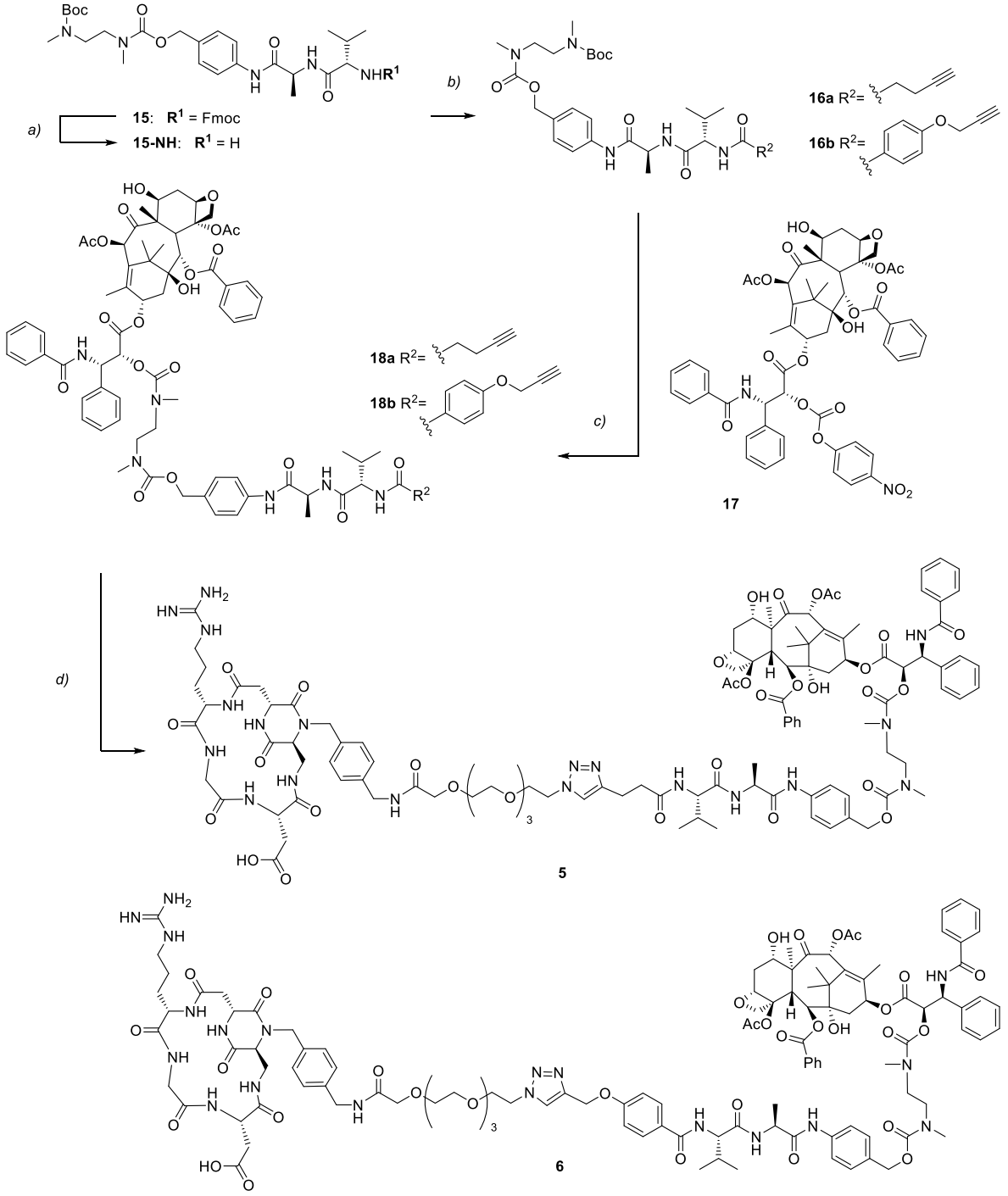


$C_{37}H_{55}N_{13}O_{13}$
MW: 889,91 g/mol

To a solution of **33** (17 mg, 0.045 mmol, 1.3 equiv) in MeCN (2 mL) under a nitrogen atmosphere, *cyclo*[DKP-RGD]-CH_2NH_2 (30 mg, 0.035 mmol, 1 equiv) dissolved in pH 7.5 phosphate buffer solution (1.5 mL) was added at 0 °C under a nitrogen atmosphere. The reaction mixture was allowed to reach r.t. and stirred overnight. During the first 3 h the pH value was kept near 7.3-7.5 adding 0.2 M aqueous NaOH aq. when necessary. The crude was then purified by semipreparative-HPLC [Waters Atlantis 21 mm × 10 cm column, flow: 9 mL/min, gradient: 90% (H_2O + 0.1% CF_3COOH) / 10% (CH_3CN + 0.1% CF_3COOH) to 55% (H_2O + 0.1% CF_3COOH) / 45% (CH_3CN + 0.1% CF_3COOH) in 10 min; t_R (product): 8.3 min]. The purified product was then freeze dried to give the desired **19** as a white solid (27 mg, 77% yield).

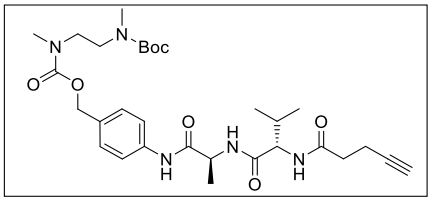
^{1}H NMR (400 MHz, D_2O) δ 7.36 (d, J = 8.1 Hz, 2H), 7.32 (d, J = 8.2 Hz, 2H), 5.12 (d, J = 15.4 Hz, 1H), 4.90 (t, J = 7.1 Hz, 1H), 4.59 (dd, J = 7.9, 5.4 Hz, 1H), 4.48 (s, 2H), 4.34 (d, J = 17.1 Hz, 1H), 4.22 (dd, J = 9.6, 5.2 Hz, 1H), 4.19-4.11 (m, 4H), 4.01 (d, J = 14.6 Hz, 1H), 3.80-3.75 (m, 3H), 3.75-3.69 (m, 3H), 3.69-3.59 (m, 10H), 3.47-3.43 (m, 2H), 3.25 (t, J = 6.8 Hz, 2H), 3.01-2.88 (m, 2H), 2.81 (dd, J = 16.9, 7.1 Hz, 1H), 2.67 (dd, J = 14.0, 5.4 Hz, 1H), 2.08-1.97 (m, 1H), 1.90-1.78 (m, 1H), 1.77-1.60 (m, 2H); ^{13}C NMR (101 MHz, D_2O) δ 174.1, 173.9, 173.1, 172.8, 172.7, 170.9, 170.1, 168.6, 156.8, 137.8, 134.1, 128.1, 127.8, 70.5, 69.5, 69.2, 59.3, 54.0, 52.1, 50.1, 49.4, 47.6, 42.5, 42.2, 40.6, 39.2, 38.0, 34.6, 25.8, 24.7; MS (ESI+) *m/z* calcd for $[C_{37}H_{56}N_{13}O_{13}]^+$: 890.41 $[M + H]^+$; found: 890.47; *m/z* calcd $[C_{37}H_{55}N_{13}NaO_{13}]^+$: 912.39 $[M + Na]^+$; found: 912.45.

Synthesis of *cyclo*[DKP-RGD]-Val-Ala-PTX conjugates (5-6)



Scheme S6. Synthesis of monomeric conjugates bearing an aliphatic (**5**) and aromatic (**6**) scaffold. REAGENTS AND CONDITIONS: *a)* piperidine, DMF, r.t., 2 h *b)* 4-pentynoic acid, HATU, HOAt, *i*Pr$_2$NEt, DMF, r.t., overnight, Y.: 88% (**16a**) (over two steps); **11**, HATU, HOAt, *i*Pr$_2$NEt, DMF, r.t., overnight, Y.: 86% (**16b**) (over two steps); *c)* 1) TFA/CH_2Cl_2 (1:2), 45 min; 2) **17**, *i*Pr$_2$NEt, DMF, r.t., overnight, Y.: 66% (**18a**) (over two steps), Y.: 93% (**18b**) (over two steps); *d)* $CuSO_4 \cdot 5H_2O$, sodium ascorbate, DMF/H_2O (1:1), 30 ºC, overnight, Y.: 81% (**5**), Y.: 70% (**6**).

tert-Butyl (4-((S)-2-((S)-3-methyl-2-(pent-4-ynamido)butanamido)propanamido)benzyl) ethane-1,2-diylbis(methylcarbamate) (**16a**)

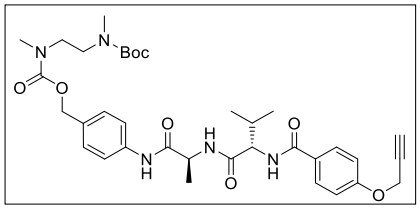


Chemical Formula: $C_{30}H_{45}N_5O_7$
Molecular Weight: 587,72

N-Fmoc-protected compound **15** (150 mg, 0.206 mmol, 1 equiv) was dissolved in DMF under a nitrogen atmosphere. The solution was cooled to 0 °C and piperidine (102 µL, 1.1 mmol, 5 equiv) was added. The reaction was stirred at room temperature for 2 h. The mixture was diluted with AcOEt (20 × volume of DMF) and washed twice with a saturated aqueous solution of $NaHCO_3$. The organic phase was dried over Na_2SO_4 and concentrated at rotavapor. CH_2Cl_2 was added to the residue and evaporated to afford a white solid **15-NH** (105 mg, 0.206 mmol) which was used directly at the next step. A solution commercial 4-pentynoic acid (31 mg, 0.31 mmol, 1.5 equiv) in dry DMF (7 mL) was cooled to 0 °C under a nitrogen atmosphere. HATU (134 mg, 0.35 mmol, 1.7 equiv), HOAT (48 mg, 0.35 mmol, 1.7 equiv) and *i*Pr_2NEt (145 µL, 0.83 mmol, 4 equiv) were added and the mixture was stirred for 20 min at 0 °C. A solution of **15-NH** (105 mg, 0.206 mmol, 1 equiv) in dry DMF (3 mL) was added to the stirred mixture. The reaction was allowed to slowly reach room temperature and stirred overnight. The mixture was diluted with an AcOEt/CH_2Cl_2, 4:1 mixture (100 mL) and washed with 1 M aqueous solution of $KHSO_4$ (2 × 15 mL), a saturated aqueous solution of $NaHCO_3$ (1 × 15 mL) and brine (1 × 20 mL). The organic phase was dried over Na_2SO_4 and concentrated. The solid was suspended in Et_2O. The product was collected by centrifugation and purified by flash chromatography [gradient: from 99:1 CH_2Cl_2/MeOH to 97:3 CH_2Cl_2/MeOH] to afford amide **16a** as a white solid (106 mg, 88% yield over two steps).

R_f = 0.39 (9:1, CH_2Cl_2/MeOH); ^{1}H NMR (500 MHz, [D]$_6$DMSO) δ 9.71 (s, 1H), 7.93 (d, *J* = 7.2 Hz, 1H), 7.75 (d, *J* = 8.5 Hz, 1H), 7.58 (d, *J* = 8.5 Hz, 2H), 7.29 (d, *J* = 8.2 Hz, 2H), 5.00 (s, 2H), 4.44 (p, *J* = 7.0 Hz, 1H), 4.21 (dd, *J* = 8.4, 6.5 Hz, 1H), 3.38-3.29 (m, 4H), 2.87 (s, 3H), 2.75 (s, 3H), 2.62 (t, *J* = 2.4 Hz, 1H), 2.46-2.32 (m, 4H), 2.09-1.99 (m, 1H), 1.39 (s, 9H), 1.34 (d, *J* = 7.1 Hz, 3H), 0.90 (dd, *J* = 11.3, 6.8 Hz, 6H); ^{13}C NMR (126 MHz, [D]$_6$DMSO) δ 171.41, 171.23, 171.14, 155.93, 155.28, 139.02, 132.35, 128.64, 121.72, 120.36, 119.73, 84.25, 78.98, 71.33, 66.47, 58.45, 49.56, 34.73, 34.48, 30.77, 19.58, 18.53, 18.36, 14.79. MS (ESI+) *m/z* calcd for $[C_{30}H_{45}N_5O_7]^+$: 587.75 $[M + H]^+$; found: 588.77; *m/z* calcd $[C_{30}H_{45}NaN_5O_7]^+$: 610.72 $[M + Na]^+$; found: 610.70.

tert-Butyl (4-((S)-2-((S)-3-methyl-2-(4-(prop-2-yn-1-yloxy)benzamido)butanamido)propanamido)benzyl) ethane-1,2-diylbis(methylcarbamate) (**16b**)



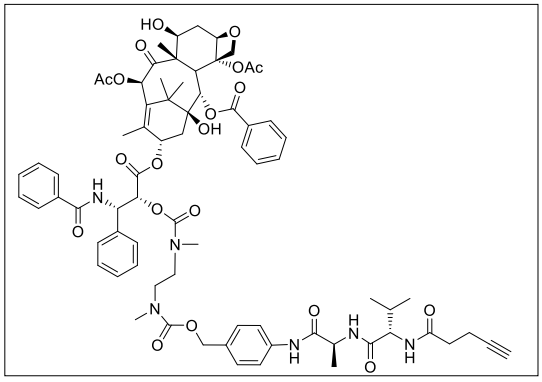
Chemical Formula: $C_{35}H_{47}N_5O_8$
Molecular Weight: 665,79

N-Fmoc-protected compound **15** (70 mg, 0.096 mmol, 1 equiv) was dissolved in DMF under a nitrogen atmosphere. The solution was cooled to 0 °C and piperidine (50 µL, 0.48 mmol, 5 equiv) was added. The

reaction was stirred at room temperature for 2 h. The mixture was diluted with AcOEt (20 × volume of DMF) and washed twice with a saturated aqueous solution of $NaHCO_3$. The organic phase was dried over Na_2SO_4 and concentrated at rotavapor. CH_2Cl_2 was added to the residue and evaporated to afford a white solid **15-NH** (49 mg, 0.096 mmol) which was used directly at the next step. A solution acid **11** (26 mg, 0.15 mmol, 1.5 equiv) in dry DMF (4 mL) was cooled to 0 °C under a nitrogen atmosphere. HATU (93 mg, 0.25 mmol, 1.7 equiv), HOAT (34 mg, 0.25 mmol, 1.7 equiv) and *i*Pr_2NEt (67 µL, 0.39 mmol, 4 equiv) were added and the mixture was stirred for 20 min at 0 °C. A solution of **15-NH** (49 mg, 0.096 mmol, 1 equiv) in dry DMF (2 mL) was added to the stirred mixture. The reaction was allowed to slowly reach room temperature and stirred overnight. The mixture was diluted with an AcOEt/CH_2Cl_2, 4:1 mixture (100 mL) and washed with 1 M aqueous solution of $KHSO_4$ (2 × 15 mL), a saturated aqueous solution of $NaHCO_3$ (1 × 15 mL) and brine (1 × 20 mL). The organic phase was dried over Na_2SO_4 and concentrated. The solid was solubilized in DCM/MeOH (95:5) and purified by flash chromatography [gradient: from 100 CH_2Cl_2 to 95:5 CH_2Cl_2/MeOH] to afford amide **16b** as a white solid (55 mg, 86% yield over two steps).

R_f = 0.35 (95:5, CH_2Cl_2/MeOH); ^{1}H NMR (400 MHz, CD_2Cl_2-d_2) δ 8.73 (s, 1H), 7.82 (d, *J* = 8.8 Hz, 2H), 7.58 (d, *J* = 8.1 Hz, 2H), 7.29 (d, *J* = 8.1 Hz, 2H), 7.13 (t, *J* = 6.4 Hz, 1H), 6.99 (d, *J* = 8.8 Hz, 2H), 6.90 (d, *J* = 7.7 Hz, 1H), 5.04 (s, 2H), 4.74 (d, *J* = 2.4 Hz, 2H), 4.66 (p, *J* = 7.1 Hz, 1H), 4.50 (t, *J* = 7.2 Hz, 1H), 3.38-3.27 (m, 4H), 2.91 (s, 3H), 2.76 (s, 3H), 2.61 (t, *J* = 2.4 Hz, 1H), 2.24 (q, *J* = 6.8 Hz, 1H), 1.43 (d, *J* = 11.8 Hz, 12H), 1.01 (dd, *J* = 6.8, 4.0 Hz, 6H). ^{13}C NMR (101 MHz, CD_2Cl_2-d_2) δ 171.86, 170.91, 167.23, 160.23, 138.02, 132.73, 129.23, 128.66, 128.44, 127.08, 119.82, 114.50, 79.11, 77.96, 75.83, 66.59, 66.47, 59.13, 55.83, 49.66, 46.55, 38.37, 29.67, 28.08, 19.10, 18.50, 18.02. MS (ESI+) *m/z* calcd for $[C_{35}H_{47}N_5O_8]^+$: 665.34 $[M + H]^+$; found: 666.71; *m/z* calcd $[C_{35}H_{47}NaN_5O_8]^+$: 688.35 $[M + Na]^+$; found: 711.23

Aliphatic alkyne-Val-Ala-PTX (**18a**)

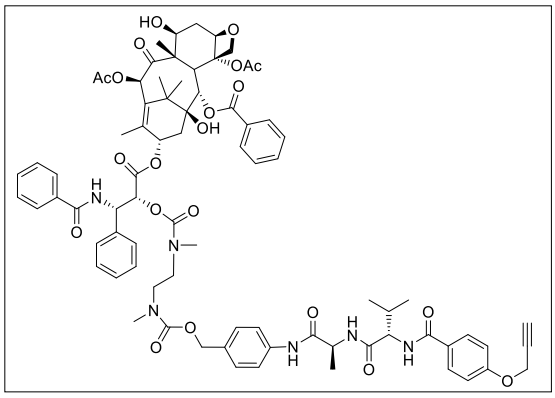


Chemical Formula: $C_{73}H_{86}N_6O_{20}$
Molecular Weight: 1367,51

A solution of Boc-protected compound **16a** (20 mg, 0.034 mmol, 1 equiv) in dry CH_2Cl_2 (1.3 mL) was cooled to 0 °C under a nitrogen atmosphere and TFA (567 µL) was added. The mixture was then allowed to reach room temperature and stirred for 45 min. The solvent was removed affording the corresponding trifluoroacetate salt, without further purifications. The solid was dissolved in dry DMF (500 µL) and *i*Pr_2NEt (25 µL, 0.136 mmol, 4 equiv). The resulting solution was added at 0 °C to a stirred solution of **17** (52 mg, 0.051 mmol, 1.5 equiv) in dry DMF (500 µL), under a nitrogen atmosphere. The reaction was then allowed to reach room temperature and stirred overnight. AcOEt (100 mL) was added and the solution was washed with a 1 M aqueous solution of $KHSO_4$ (2 × 10 mL) and brine (1 × 15 mL). The organic phase was dried over Na_2SO_4 and concentrated, then the crude was purified by flash chromatography [gradient: from 9:1 CH_2Cl_2/AcOEt to 7:3 CH_2Cl_2/AcOEt] to afford carbamate **18a** as a white solid (30 mg, 66% yield).

R_f = 0.33 (CH_2Cl_2/AcOEt, 9:1); MS (MALDI-TOF): *m/z* calcd for $[C_{73}H_{86}NaN_6O_{20}]^+$: 1389.98 [*M* + Na]$^+$; found: 1398.70 (HCCA matrix), 1390.08 (SA matrix); HRMS (ESI+): *m/z* calcd for $[C_{73}H_{86}NaN_6O_{20}]^+$: 1389.98, [*M* + Na]$^+$; found 1389.57; *m/z* calcd for $[C_{73}H_{86}Na_2N_6O_{20}]^{2+}$ 706.14, [*M* + 2Na]$^{2+}$ found 706.28.

Aromatic alkyne-Val-Ala-PTX (**18b**)

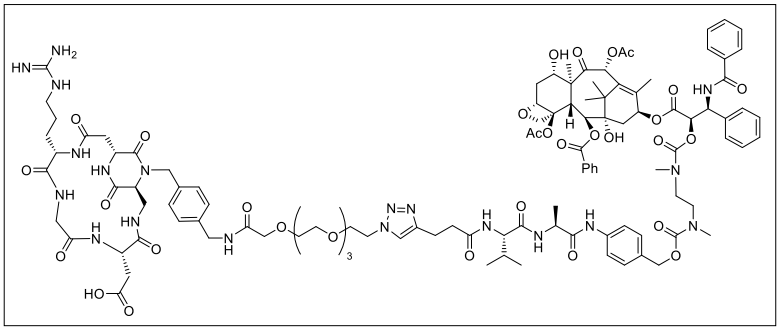


Chemical Formula: $C_{78}H_{88}N_6O_{21}$
Molecular Weight: 1445,58

A solution of Boc-protected compound **16b** (20 mg, 0.03 mmol, 1 equiv) in dry CH_2Cl_2 (1 mL) was cooled to 0 °C under a nitrogen atmosphere and TFA (500 µL) was added. The mixture was then allowed to reach room temperature and stirred for 45 min. The solvent was removed affording the corresponding trifluoroacetate salt, without further purifications. The solid was dissolved in dry DMF (500 µL) and *i*Pr_2NEt (21 µL, 0.12 mmol, 4 equiv). The resulting solution was added at 0 °C to a stirred solution of **17** (46 mg, 0.045 mmol, 1.5 equiv) in dry DMF (500 µL), under a nitrogen atmosphere. The reaction was then allowed to reach room temperature and stirred overnight. AcOEt (100 mL) was added and the solution was washed with a 1 M aqueous solution of $KHSO_4$ (2 × 10 mL) and brine (1 × 15 mL). The organic phase was dried over Na_2SO_4 and concentrated, then the crude was purified by flash chromatography [eluent: 9:1 CH_2Cl_2/AcOEt] to afford carbamate **18b** as a white solid (39 mg, 93% yield).

R_f = 0.30 (9:1, CH_2Cl_2/AcOEt); MS (MALDI-TOF): *m/z* calcd for $[C_{78}H_{88}NaN_6O_{21}]^+$: 1467.99 [*M* + Na]$^+$; found: 1469.02 (HCCA matrix), 1469.09 (SA matrix); HRMS (ESI+): *m/z* calcd for $[C_{78}H_{88}NaN_6O_{21}]^+$: 1467.99, [*M* + Na]$^+$; found 1457.58; *m/z* calcd for $[C_{78}H_{88}Na_2N_6O_{21}]^{2+}$ 745.25, [*M* + 2Na]$^{2+}$ found 745.28.

cyclo[DKP-RGD]-Val-Ala-PTX aliphatic scaffold conjugate **(5)**

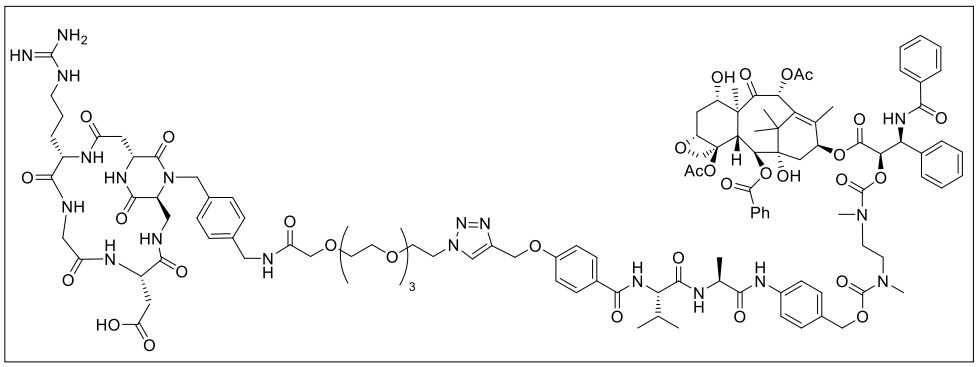


Chemical Formula: $C_{110}H_{141}N_{19}O_{33}$
Molecular Weight: 2257,40

Alkyne **18a** (10 mg, 0.0075 mmol, 1.5 equiv) and azide **19** (5 mg, 0.005 mmol, 1 equiv) were dissolved in a degased 1:1 mixture of H_2O/DMF (500 μL) under a nitrogen atmosphere. Degased aqueous solutions of $CuSO_4 \cdot 5H_2O$ (0.74 mg, 0.5 equiv) and sodium ascorbate (0.6 mg, 0.6 equiv) were added at room temperature and the mixture was stirred overnight at 30 ºC. The solvent was removed under vacuum, and the crude residue was purified by semipreparative HPLC [Waters Atlantis 21 mm x 10 cm column; gradient: 90% (H_2O+0.1% CF_3COOH)/10% (CH_3CN+0.1% CF_3COOH) to 100% (CH_3CN+0.1% CF_3COOH) in 20 min; t_R (product)=11.9 min]. The purified product was then freeze-dried to give the desired compound **5** as a white solid (9 mg, 81% yield).

MS (MALDI-TOF): *m/z* calcd for $[C_{110}H_{141}N_{19}O_{33}]^+$: 2257,40 [*M* + H]$^+$; found: 2258.30 (HCCA matrix), 2258.30 (SA matrix); HRMS (ESI+): *m/z* calcd for $[C_{110}H_{140}Na_3N_{19}O_{33}]^{2+}$: 1161.90, [*M* + 3Na]$^{2+}$; found 1161.97; *m/z* calcd for $[C_{110}H_{141}Na_2N_{19}O_{33}]^{2+}$: 1150.90, [*M* + 2Na]$^{2+}$ found 1150.98.

Cyclo[DKP-RGD]-Val-Ala-PTX aromatic scaffold conjugate **(6)**

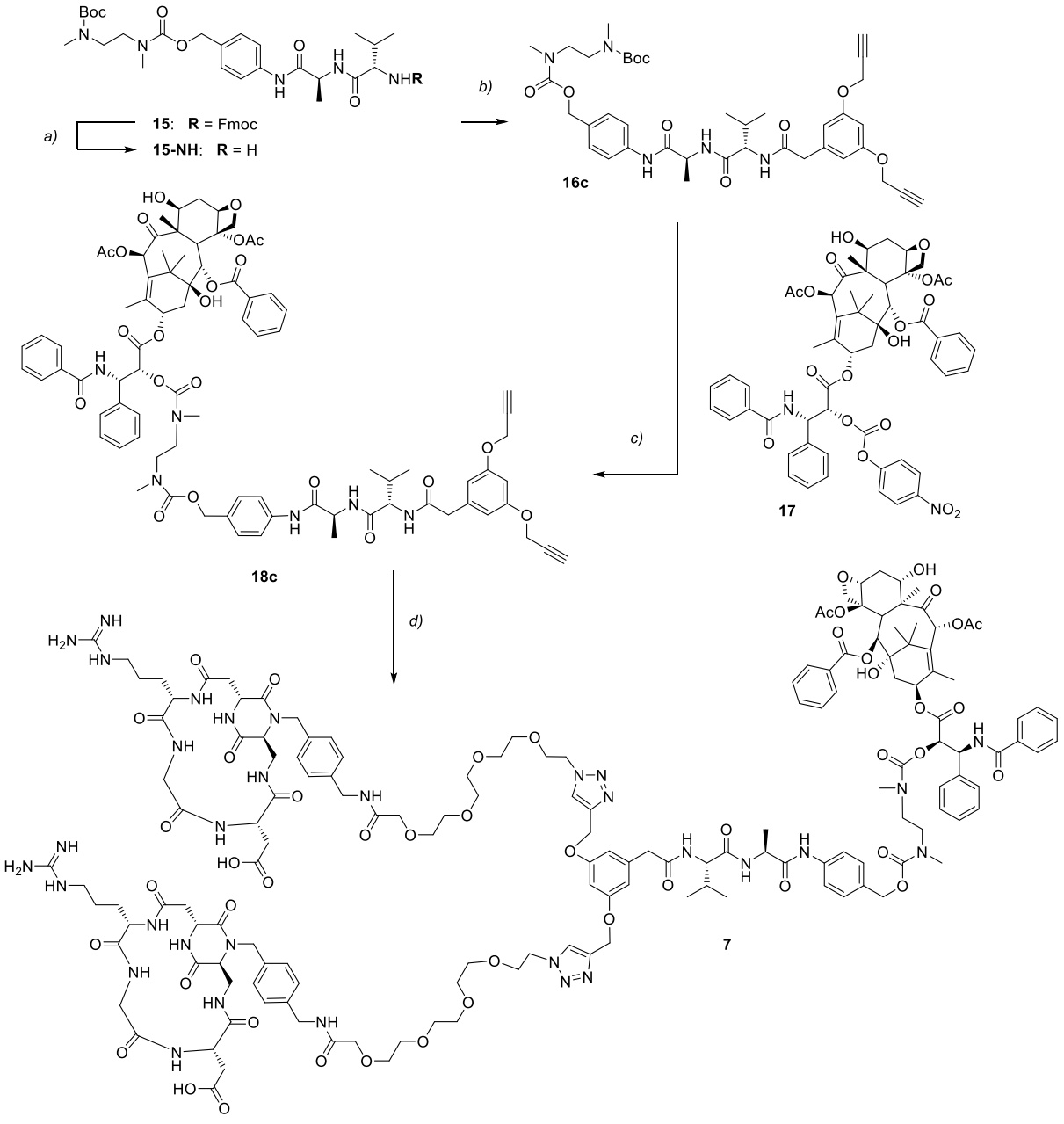


Chemical Formula: $C_{115}H_{143}N_{19}O_{34}$
Molecular Weight: 2335,51

Alkyne **18b** (6.5 mg, 0.005 mmol, 1.5 equiv) and azide **19** (3 mg, 0.003 mmol, 1 equiv) were dissolved in a degased 1:1 mixture of H_2O/DMF (360 μL) under a nitrogen atmosphere. Degased aqueous solutions of $CuSO_4 \cdot 5H_2O$ (0.44 mg, 0.5 equiv) and sodium ascorbate (0.36 mg, 0.6 equiv) were added at room temperature and the mixture was stirred overnight at 30 ºC. The solvent was removed under vacuum, and the crude residue was purified by semipreparative HPLC [Waters Atlantis 21 mm x 10 cm column; gradient: 90% (H_2O+0.1% CF_3COOH)/10% (CH_3CN+0.1% CF_3COOH) to 100% (CH_3CN+0.1% CF_3COOH) in 20 min; t_R (product)=12.9 min]. The purified product was then freeze-dried to give the desired compound **5** as a white solid (5 mg, 70% yield).

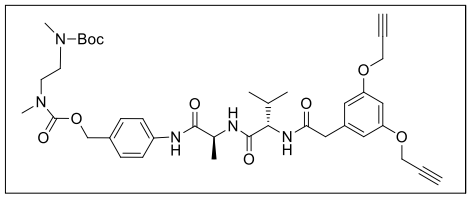
MS (MALDI-TOF): *m/z* calcd for $[C_{115}H_{144}N_{19}O_{34}]^+$: 2335,47 $[M + H]^+$; found: 2236 (HCCA matrix), 2236 (SA matrix); HRMS (ESI+): *m/z* calcd for $[C_{115}H_{144}NaN_{19}O_{34}]^{2+}$: 1178.98, $[M + H + Na]^{2+}$; found 1179.00; *m/z* calcd for $[C_{115}H_{143}Na_2N_{19}O_{34}]^{2+}$ 1189.96, $[M + 2Na]^{2+}$ found 1189.99; *m/z* calcd for $[C_{115}H_{142}Na_3N_{19}O_{34}]^{2+}$ 1200.86, $[M + 2Na - H]^{2+}$ found 1200.98.

Synthesis of (*cyclo*[DKP-RGD]$_2$)-Val-Ala-PTX conjugate (7)



Scheme S7. Synthesis of (*cyclo*[DKP-RGD]$_2$)-Val-Ala-PTX conjugate (**7**). REAGENTS AND CONDITIONS: *a)* piperidine, DMF, r.t., 2 h *b)* **12**, HATU, HOAt, *i*Pr$_2$NEt, DMF, r.t., overnight, Y.: 71% (over two steps); *c)* 1) TFA/CH_2Cl_2 (1:2), 45 min; 2) **17**, *i*Pr$_2$NEt, DMF, r.t., overnight, Y.: 77% (over two steps); *d)* $CuSO_4 \cdot 5H_2O$, sodium ascorbate, DMF/H_2O (1:1), 30 ºC, overnight, Y.: quantitative.

[3,5-bis(Propynyloxy)phenylacetyl]-Val–Ala-N-[4-[[[(N-(Boc)-N,N'-dimethylethylenediamine) carbonyl]oxy]methyl]phenyl] **(16c)**

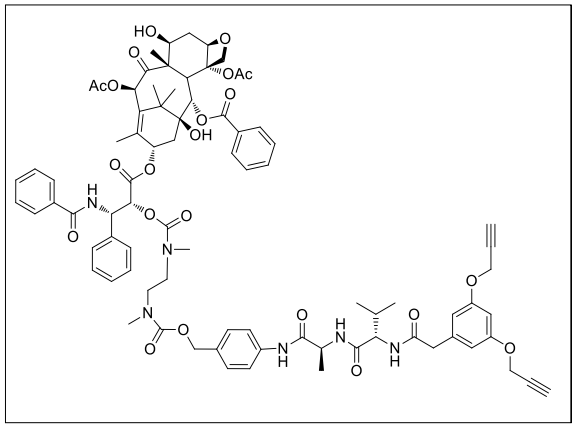


Chemical Formula: $C_{39}H_{51}N_5O_9$
Molecular Weight: 733,85

N-Fmoc-protected compound **15** (160 mg, 0.219 mmol, 1 equiv) was dissolved in DMF under a nitrogen atmosphere. The solution was cooled to 0 °C and piperidine (108 µL, 1.1 mmol, 5 equiv) was added. The reaction was stirred at room temperature for 2 h. The mixture was diluted with AcOEt (20 × volume of DMF) and washed twice with a saturated aqueous solution of $NaHCO_3$. The organic phase was dried over Na_2SO_4 and concentrated at rotavapor. CH_2Cl_2 was added to the residue and evaporated to afford a yellow solid **15-NH** (90 mg, 81% yield). A solution of acid **12** (68 mg, 0.28 mmol, 1.5 equiv) in dry DMF (2.3 mL) was cooled to 0 °C under a nitrogen atmosphere. HATU (114 mg, 0.3 mmol, 1.6 equiv), HOAT (41 mg, 0.3 mmol, 1.6 equiv) and *i*Pr_2NEt (100 µL, 0.57 mmol, 3 equiv) were added and the mixture was stirred for 20 min at 0 °C. A solution of **15-NH** (96 mg, 0.19 mmol, 1 equiv) in dry DMF (2.3 mL) was added to the stirred mixture. The reaction was allowed to slowly reach room temperature and stirred overnight. The mixture was diluted with a AcOEt/CH_2Cl_2, 4:1 mixture (100 mL) and washed with 1 M aqueous solution of $KHSO_4$ (2 × 15 mL), a saturated aqueous solution of $NaHCO_3$ (1 × 15 mL) and brine (1 × 20 mL). The organic phase was dried over Na_2SO_4 and concentrated. The solid was suspended in Et_2O. The product was collected by centrifugation and purified by flash chromatography [gradient: from 99:1 CH_2Cl_2/MeOH to 97:3 CH_2Cl_2/MeOH] to afford amide **16c** as a white solid (101 mg, 72% yield).

R_f = 0.3 (100 %, AcOEt); ^{1}H NMR (400 MHz, CD_3OD + $[D]_6$DMSO) δ 7.57 (m, 2H), 7.32 (m, 2H), 6.60 (d, *J* = 2.2 Hz, 2H), 6.53 (t, *J* = 2.2 Hz, 1H), 5.06 (s, 2H), 4.70 (d, *J* = 2.4 Hz, 4H), 4.46 (q, *J* = 7.1 Hz, 1H), 4.20 (d, *J* = 7.2 Hz, 1H), 3.56 (s, 1H), 3.41 (m, 4H), 3.03 (t, *J* = 2.4 Hz, 2H), 2.95 (m, rotamer A+B, 3H), 2.85 (bs, rotamer A, 3H), 2.75 (bs, rotamer B, 3H), 2.11 (m, 1H), 1.42 (m, 12H), 0.99 (d, *J* = 6.9 Hz, 3H), 0.97 (d, *J* = 6.9 Hz, 3H); ^{13}C NMR (101 MHz, CD_3OD + $[D]_6$DMSO) δ 173.6, 173.3, 172.9, 160.2, 139.3, 130.0, 129.7, 121.0, 110.0, 101.8, 79.9, 77.2, 68.1, 67.9, 60.4, 56.7, 51.0, 43.6, 35.6, 35.3, 34.7, 31.9, 28.8, 19.8, 18.7, 18.1.

[3,5-bis(Propynyloxy)phenylacetyl]-Val–Ala-PTX (**18c**)

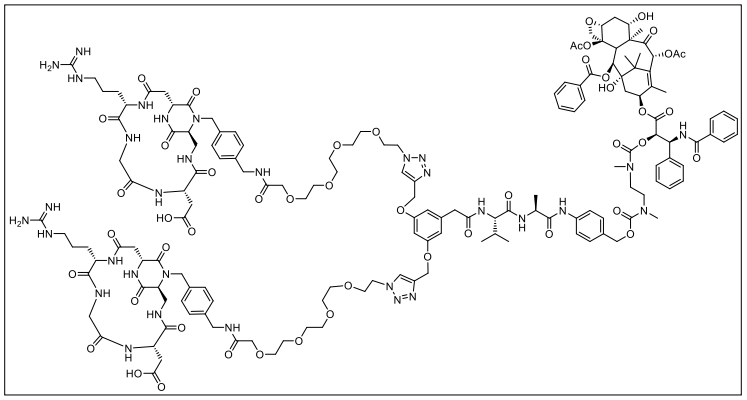


$C_{82}H_{92}N_6O_{22}$
MW: 1513,63 g/mol

A solution of Boc-protected compound **16c** (75 mg, 0.1 mmol, 1 equiv) in dry CH_2Cl_2 (5 mL) was cooled to 0 °C under a nitrogen atmosphere and TFA (2.5 mL) was added. The mixture was then allowed to reach room temperature and stirred for 45 min. The solvent was removed affording the corresponding trifluoroacetate salt, without further purifications. The solid was dissolved in dry DMF (2 mL) and *i*Pr_2NEt (52 µL, 0.3 mmol, 3 equiv). The resulting solution was added at 0 °C to a stirred solution of **17** (204 mg, 0.2 mmol, 2 equiv) in dry DMF (1 mL), under a nitrogen atmosphere. The reaction was then allowed to reach room temperature and stirred overnight. AcOEt (100 mL) was added and the solution was washed with a 1 M aqueous solution of $KHSO_4$ (2 × 10 mL) and brine (1 × 15 mL). The organic phase was dried over Na_2SO_4 and concentrated, then the crude was purified by a Grace Reveleris system (column: Reveleris Silica HP 12 g, dry load, flow rate: 25 mL/min., ramp from 0% to 15% of MeOH in CH_2Cl_2 in 15 min) to afford carbamate **18c** as a white solid (116 mg, 77% yield).

R_f = 0.22 (100%, AcOEt); MS (ESI+) *m/z* calcd for $[C_{82}H_{92}N_6NaO_{22}]^+$: 1535.62 [*M* + Na]$^+$; found: 1535.89. MS (MALDI-TOF): *m/z* calcd for $[C_{82}H_{92}N_6NaO_{22}]^+$: 1535.62 [*M* + Na]$^+$; found: 1536 (HCCA matrix), 1536 (SA matrix); HRMS (ESI+): *m/z* calcd for $[C_{82}H_{92}N_6NaO_{22}]^+$: 1535.62, [*M* + Na]$^+$; found 1535.68; *m/z* calcd for $[C_{82}H_{92}N_6Na_2O_{22}]^{2+}$ 779.01, [*M* + 2Na]$^{2+}$ found 779.02.

(*cyclo*[DKP-RGD]$_2$)-Val-Ala-PTX (7)



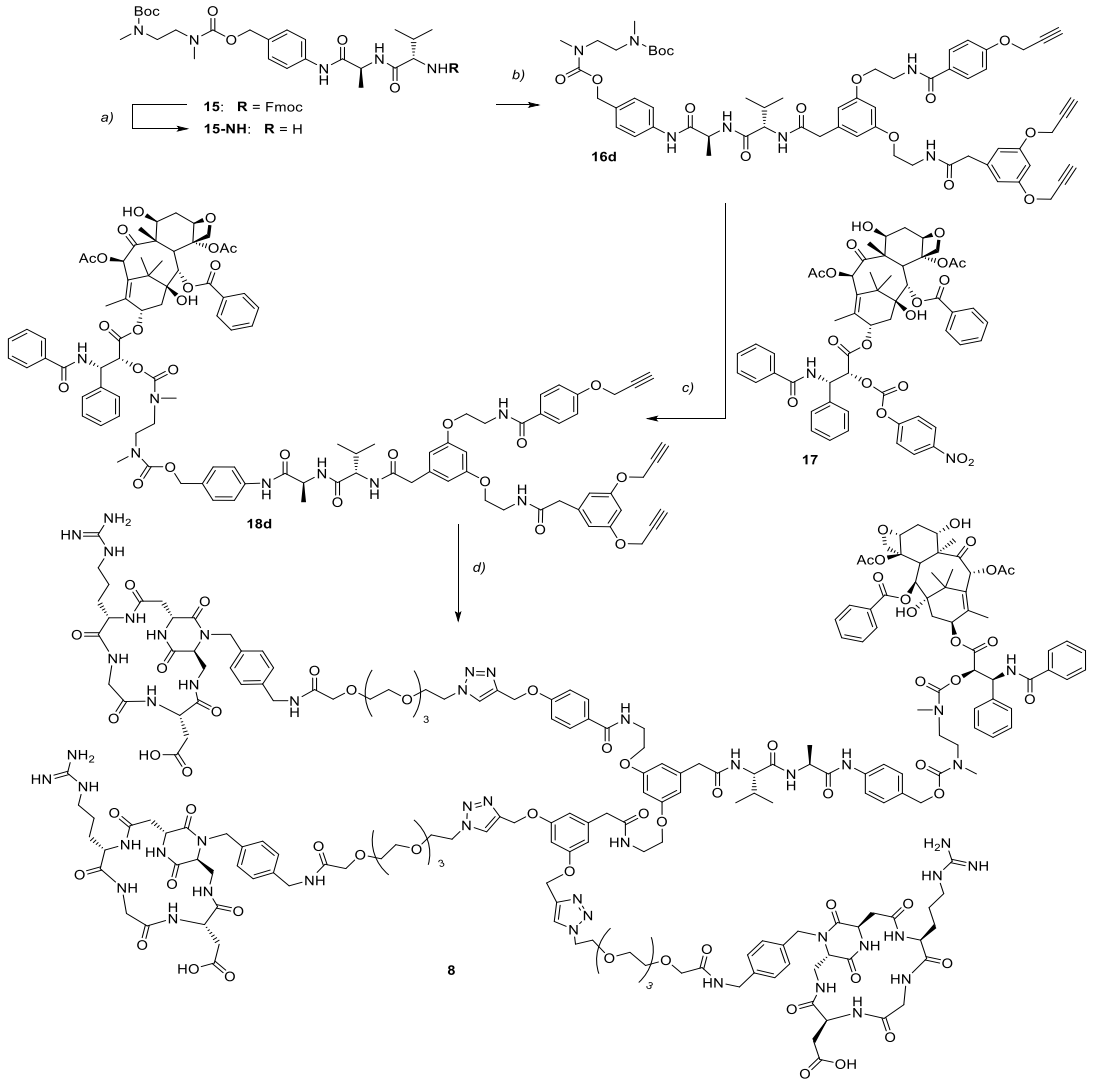
Chemical Formula: $C_{156}H_{202}N_{32}O_{48}$
Molecular Weight: 3293,51

The bis-alkyne **18c** (5 mg, 33.2×10^{-2} mmol, 1 equiv) and azide **19** (10 mg, 99.6×10^{-2} mmol, 3 equiv) were dissolved in a degased 1:1 mixture of H_2O/DMF (400 µL) under a nitrogen atmosphere. Degased aqueous solutions of $CuSO_4\cdot5H_2O$ (0.017 M, 96 µL, 1 equiv) and sodium ascorbate (0.03 M, 96 µL, 1.2 equiv) were

added in the darkness at room temperature and the mixture was stirred overnight. The solvent was removed under vacuum, and the crude residue was purified by semipreparative HPLC [Waters Atlantis 21 mm x 10 cm column; gradient: 90% (H_2O+0.1% CF_3COOH)/10% (CH_3CN+0.1% CF_3COOH) to 100% (CH_3CN+0.1% CF_3COOH) in 26 min; t_R (product) = 14.9 min]. The purified product was then freeze-dried to give the desired compound **7** as a white solid (11 mg, quantitative yield).

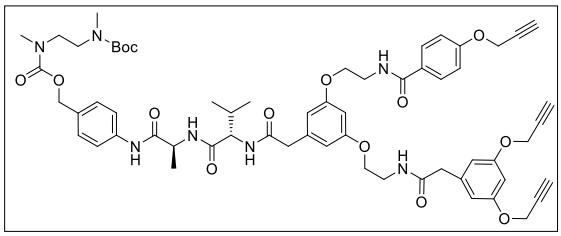
MS (ESI+): *m/z* calcd for $[C_{156}H_{204}N_{32}O_{48}]^{2+}$: 1646.73, $[M + 2H]^{2+}$; found 1647.02; MS (ESI+): *m/z* calcd for $[C_{156}H_{203}N_{32}NaO_{48}]^{2+}$: 1657.72, $[M + H + Na]^{2+}$; found 1658.01; MS (MALDI): *m/z* calcd for $[C_{156}H_{203}N_{32}O_{48}]^{+}$: 3294,47 $[M + H]^{+}$; found: 3291 (HCCA matrix), 3294 (SA matrix); HRMS (ESI+): *m/z* calcd for $[C_{156}H_{204}N_{32}O_{48}]^{2+}$: 1646.7248, $[M + 2H]^{2+}$; found 1646.7260; *m/z* calcd for $[C_{156}H_{205}N_{32}O_{48}]^{3+}$ 1098.1523, $[M + 3H]^{3+}$ found 1098.1475.

Synthesis of (*cyclo*[DKP-RGD]$_3$)-Val-Ala-PTX conjugate (8)



Scheme S8. Synthesis of (*cyclo*[DKP-RGD]$_3$)-Val-Ala-PTX conjugate (**8**). REAGENTS AND CONDITIONS: *a)* piperidine, DMF, r.t., 2 h *b)* **13**, HATU, HOAt, *i*Pr2NEt, DMF, r.t., overnight, Y.: 80% (over two steps); *c)* 1) TFA/CH_2Cl_2 (1:2), r.t., 45 min; 1) **17**, *i*Pr_2NEt, DMF, r.t., overnight, Y.: 69% (over two steps); *d)* $CuSO_4 \cdot 5H_2O$, sodium ascorbate, DMF/H_2O (1:1), 30 ºC, overnight, Y.: 62%.

Tri-alkyne-Val-Ala-diamine-Boc **(16d)**

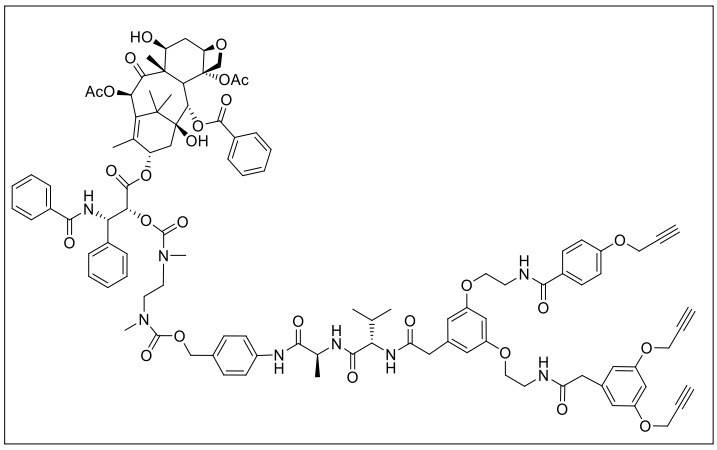


Chemical Formula: $C_{61}H_{73}N_7O_{14}$
Molecular Weight: 1128,29

N-Fmoc-protected compound **15** (65 mg, 0.091 mmol, 1 equiv) was dissolved in DMF under a nitrogen atmosphere. The solution was cooled to 0 °C and piperidine (45 µL, 0.455 mmol, 5 equiv) was added. The reaction was stirred at room temperature for 2 h. The mixture was diluted with AcOEt (20 × volume of DMF) and washed twice with a saturated aqueous solution of $NaHCO_3$. The organic phase was dried over Na_2SO_4 and concentrated at rotavapor. CH_2Cl_2 was added to the residue and evaporated to afford a yellow solid **15-NH** (45 mg, quantitative yield). A solution of acid **13** (70 mg, 0.11 mmol, 1.2 equiv) in dry DMF (2 mL) was cooled to 0 °C under a nitrogen atmosphere. HATU (59 mg, 0.16 mmol, 1.7 equiv), HOAT (22 mg, 0.16 mmol, 1.7 equiv) and *i*Pr_2NEt (64 µL, 0.364 mmol, 4 equiv) were added and the mixture was stirred for 20 min at 0 °C. A solution of **15-NH** (45 mg, 0.091 mmol, 1 equiv) in dry DMF (3 mL) was added to the stirred mixture. The reaction was allowed to slowly reach room temperature and stirred overnight. The mixture was diluted with AcOEt (100 mL) and washed with 1 M aqueous solution of $KHSO_4$ (2 × 15 mL), a saturated aqueous solution of $NaHCO_3$ (1 × 15 mL) and brine (1 × 20 mL). The organic phase was dried over Na_2SO_4 and concentrated. The crude was purified by flash chromatography [eluent: CH_2Cl_2/MeOH, 9:1] to afford amide **16d** as a light yellow solid (73 mg, 73% yield over two steps).

R_f = 0.41 (9:1, CH_2Cl_2/MeOH); ^{1}H NMR (400 MHz, [D]$_6$DMSO) δ 9.77 (s, 1H), 8.39 (t, *J* = 5.5 Hz, 1H), 8.10-8.00 (m, 2H), 7.89-7.81 (m, 3H), 7.55 (d, *J* = 8.5 Hz, 2H), 7.27 (d, *J* = 8.3 Hz, 2H), 7.03 (d, *J* = 8.9 Hz, 2H), 6.53 (d, *J* = 2.2 Hz, 2H), 6.50 (d, *J* = 2.2 Hz, 2H), 6.48 (s, 1H), 6.40 (t, *J* = 2.3 Hz, 1H), 4.98 (s, 2H), 4.85 (d, *J* = 2.4 Hz, 2H), 4.72 (d, *J* = 2.4 Hz, 4H), 4.41 (p, *J* = 7.0 Hz, 1H), 4.20 (dd, *J* = 8.7, 6.6 Hz, 1H), 4.08 (t, *J* = 6.0 Hz, 2H), 3.97 (t, *J* = 5.8 Hz, 2H), 3.60 (q, *J* = 5.8 Hz, 2H), 3.51-3.45 (m, 2H), 3.44-3.37 (m, 7H), 3.32 (dd, *J* = 12.3, 4.7 Hz, 4H), 2.85 (s, 3H), 2.74 (s, 3H), 2.00 (h, *J* = 6.8 Hz, 1H), 1.37 (s, 9H), 1.30 (d, *J* = 7.1 Hz, 3H), 0.85 (dd, *J* = 9.5, 6.8 Hz, 6H); ^{13}C NMR (101 MHz, [D]$_6$DMSO) δ 170.73, 170.53, 169.74, 165.80, 159.27, 159.20, 159.12, 157.98, 155.18, 154.54, 138.44, 138.33, 138.26, 131.52, 128.68, 127.93, 127.23, 118.93, 114.18, 108.69, 107.95, 100.04, 99.41, 78.91, 78.64, 78.24, 77.95, 77.68, 66.19, 66.03, 65.75, 57.52, 55.44, 48.89, 45.92, 42.19, 38.66, 38.24, 33.73, 30.28, 28.67, 27.78, 18.86, 17.81, 17.60. MS (MALDI): *m/z* calcd for $[C_{61}H_{73}N_7O_{14}]^+$: 1127.52 [*M* + H]$^+$; found: 1128.52 (DHB matrix); *m/z* calcd for $[C_{61}H_{73}NaN_7O_{14}]^+$: 1150.54 [*M* + Na]$^+$; found: 1150.55 (DHB matrix); *m/z* calcd for $[C_{61}H_{73}KN_7O_{14}]^+$: 1166.24 [*M* + K]$^+$; found: 1166.27 (DHB matrix).

Tri-alkyne-Val–Ala-PTX (**18d**)

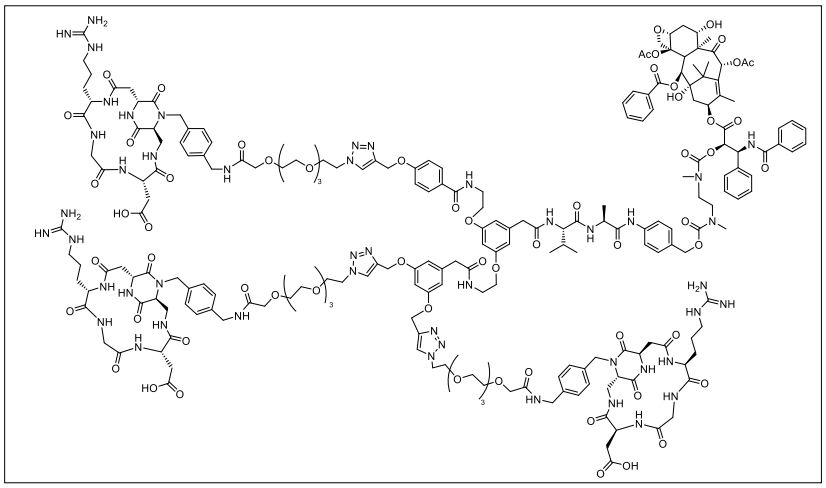


Chemical Formula: $C_{104}H_{114}N_8O_{27}$
Molecular Weight: 1908,09

A solution of Boc-protected compound **16d** (40 mg, 0.035 mmol, 1 equiv) in dry CH_2Cl_2 (1.2 mL) was cooled to 0 °C under a nitrogen atmosphere and TFA (600 µL) was added. The mixture was then allowed to reach room temperature and stirred for 45 min. The solvent was removed affording the corresponding trifluoroacetate salt, without further purifications. The solid was dissolved in dry DMF (500 µL) and *i*Pr_2NEt (25 µL, 0.14 mmol, 4 equiv). The resulting solution was added at 0 °C to a stirred solution of **17** (54 mg, 0.052 mmol, 1.5 equiv) in dry DMF (700 µL), under a nitrogen atmosphere. The reaction was then allowed to reach room temperature and stirred overnight. AcOEt (60 mL) was added and the solution was washed with a 1 M aqueous solution of $KHSO_4$ (2 × 10 mL) and brine (1 × 15 mL). The organic phase was dried over Na_2SO_4 and concentrated, then the crude was purified by a flash chromatography (eluent: CH_2Cl_2/MeOH from 100% to 95% of CH_2Cl_2) to afford carbamate **18d** as a white solid (44 mg, 69% yield over two steps).

R_f = 0.43 (9:1, CH_2Cl_2/MeOH); MS (ESI+): *m/z* calcd for $[C_{104}H_{114}NaN_8O_{27}]^+$: 1930.03, $[M + Na]^+$; found 1930.04; MS (MALDI-TOF): *m/z* calcd for $[C_{104}H_{114}NaN_8O_{27}]^+$: 1930.03 $[M + Na]^+$; found: 1930.03 (DHB matrix); *m/z* calcd for $[C_{104}H_{114}KN_8O_{27}]^+$: 1946.07 $[M + K]^+$; found: 1946.07 (DHB matrix); HRMS (ESI+): *m/z* calcd for $[C_{104}H_{114}NaN_8O_{27}]^+$: 1930.03, $[M + Na]^+$; found 1930.75; *m/z* calcd for $[C_{104}H_{114}Na_2N_8O_{27}]^{2+}$ 976.39, $[M + 2Na]^{2+}$ found 976.38.

***[cyclo(DKP-RGD)$_3$]-Val-Ala-PTX* (8)**

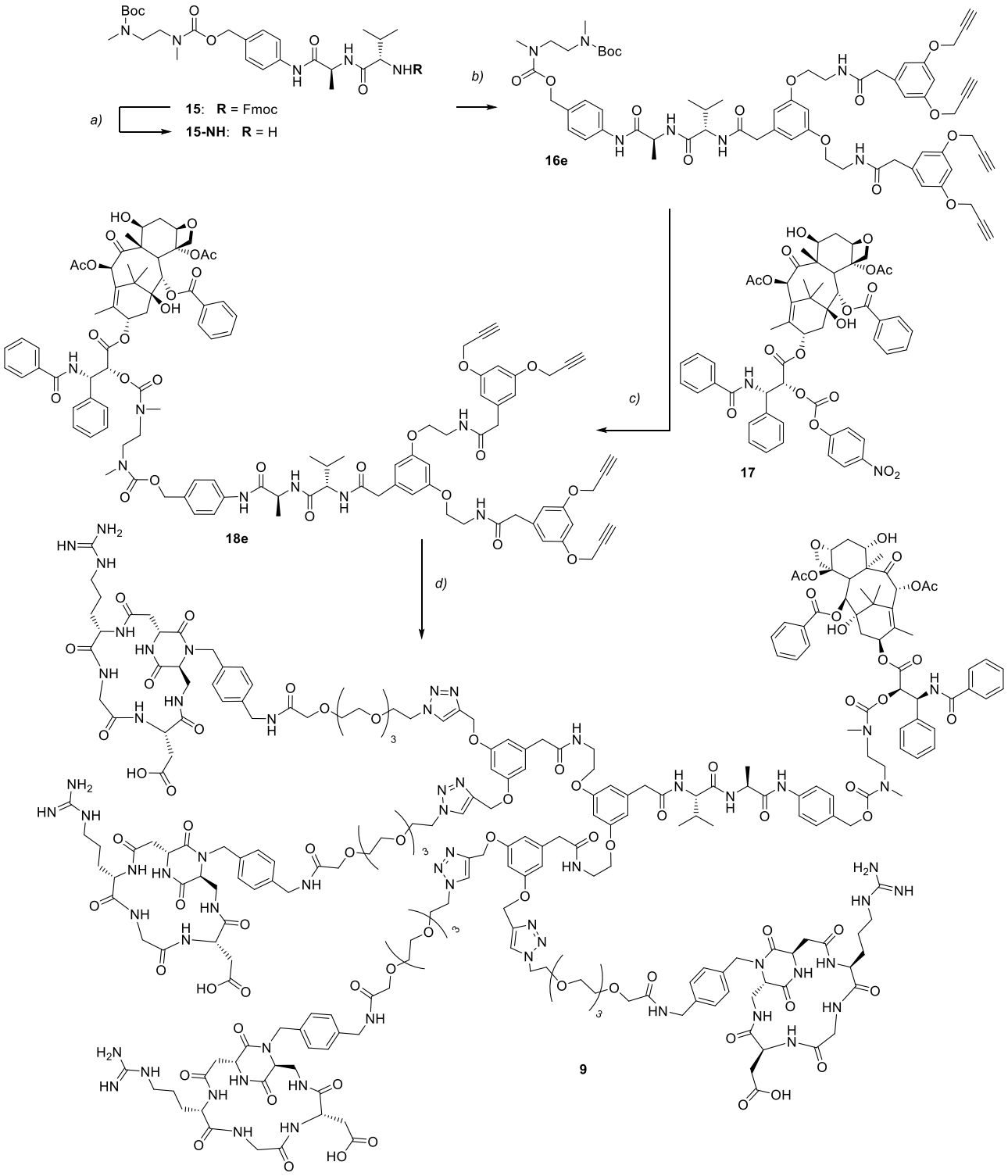


Chemical Formula: $C_{215}H_{279}N_{47}O_{66}$
Molecular Weight: 4577.86

Tri-alkyne **18d** (5 mg, 0.0026 mmol, 1 equiv) and azide **19** (9.5 mg, 0.0094 mmol, 3.6 equiv) were dissolved in a degased 1:1 mixture of H_2O/DMF (260 μL) under a nitrogen atmosphere. Degased aqueous solutions of $CuSO_4 \cdot 5H_2O$ (1.15 mg, 0.0039 mmol, 1.5 equiv) and sodium ascorbate (0.47 mg, 0.0047 mmol, 1.8 equiv) were added in the darkness at room temperature and the mixture was stirred overnight. The solvent was removed under vacuum, and the crude residue was purified by semipreparative HPLC [Waters Atlantis 21 mm x 10 cm column; gradient: 90% (H_2O+0.1% CF_3COOH)/10% (CH_3CN+0.1% CF_3COOH) to 100% (CH_3CN+0.1% CF_3COOH) in 20 min; t_R (product)=11.5 min]. The purified product was then freeze-dried to give the desired compound **8** as a white solid (6.9 mg, 62% yield).

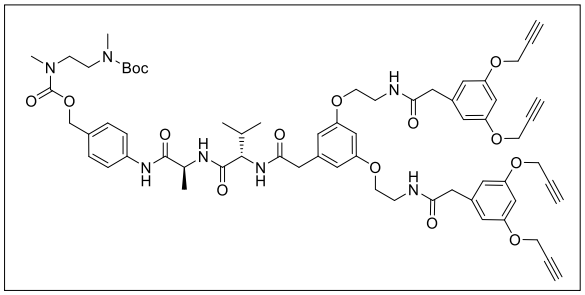
MS (ESI+): *m/z* calcd for $[C_{215}H_{281}N_{47}O_{66}]^{2+}$: 2289.85, $[M + 2H]^{2+}$; found 2289.89; *m/z* calcd for $[C_{215}H_{282}N_{47}O_{66}]^{3+}$: 1526.86, $[M + 3H]^{3+}$; found 1527.00; *m/z* calcd for $[C_{215}H_{283}N_{47}O_{66}]^{4+}$: 1145.86, $[M + 4H]^{4+}$; found 1145.51; *m/z* calcd for $[C_{215}H_{284}N_{47}O_{66}]^{5+}$: 916.23, $[M + 5H]^{5+}$; found 916.61; MS (MALDI-TOF): *m/z* calcd for $[C_{215}H_{280}N_{47}O_{66}]^{+}$: 4577 $[M + H]^{+}$; found: 4582 (DHB matrix); HRMS (ESI+): *m/z* calcd for $[C_{215}H_{279}Na_3N_{47}O_{66}]^{3+}$: 1548.99, $[M + 3Na]^{3+}$; found 1548.92; *m/z* calcd for $[C_{215}H_{279}Na_4N_{47}O_{66}]^{4+}$ 1167.43, $[M + 4Na]^{4+}$ found 1167.24.

Synthesis of (*cyclo*[DKP-RGD]$_4$)-Val-Ala-PTX conjugate (9)



Scheme S9. Synthesis of (*cyclo*[DKP-RGD]$_4$)-Val-Ala-PTX conjugate (**9**). REAGENTS AND CONDITIONS: *a)* piperidine, DMF, r.t., 2 h *b)* **14**, HATU, HOAt, *i*Pr$_2$NEt, DMF, r.t., overnight, Y.: 92% (over two steps); *c)* 1) 1:2 TFA/CH_2Cl_2, r.t., 45 min; 2) **17**, *i*Pr$_2$NEt, DMF, r.t., overnight, Y.: 75% (over two steps); *d)* $CuSO_4 \cdot 5H_2O$, sodium ascorbate, 1:1 DMF/H_2O, 30 ºC, overnight, Y.: 73%.

Tetra-alkyne-Val-Ala-diamine-Boc (**16e**)

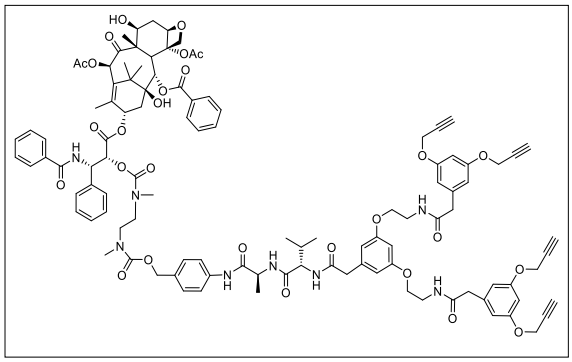


Chemical Formula: $C_{65}H_{77}N_7O_{15}$
Molecular Weight: 1196,37

N-Fmoc-protected compound **15** (35 mg, 0.047 mmol, 1 equiv) was dissolved in DMF under a nitrogen atmosphere. The solution was cooled to 0 °C and piperidine (24 µL, 0.235 mmol, 5 equiv) was added. The reaction was stirred at room temperature for 2 h. The mixture was diluted with AcOEt (20 × volume of DMF) and washed twice with a saturated aqueous solution of $NaHCO_3$. The organic phase was dried over Na_2SO_4 and concentrated at rotavapor. CH_2Cl_2 was added to the residue and evaporated to afford a yellow solid **15-NH** (24 mg, quantitative yield). A solution of acid **14** (50 mg, 0.071 mmol, 1.5 equiv) in dry DMF (1 mL) was cooled to 0 °C under a nitrogen atmosphere. HATU (31 mg, 0.08 mmol, 1.7 equiv), HOAT (11 mg, 0.08 mmol, 1.7 equiv) and *i*Pr_2NEt (33 µL, 0.189 mmol, 4 equiv) were added and the mixture was stirred for 20 min at 0 °C. A solution of **15-NH** (24 mg, 0.047 mmol, 1 equiv) in dry DMF (1.6 mL) was added to the stirred mixture. The reaction was allowed to slowly reach room temperature and stirred overnight. The mixture was diluted with AcOEt (100 mL) and washed with 1 M aqueous solution of $KHSO_4$ (2 × 15 mL), a saturated aqueous solution of $NaHCO_3$ (1 × 15 mL) and brine (1 × 20 mL). The organic phase was dried over Na_2SO_4 and concentrated. The crude was purified by flash chromatography [eluent: CH_2Cl_2/MeO, 95:5 H] to afford amide **16e** as a light yellow solid (54 mg, 92% yield over two steps).

R_f = 0.27 (95:5, CH_2Cl_2/MeOH); ^{1}H NMR (400 MHz, [D]$_6$DMSO) δ 9.82 (s, 1H), 8.18-8.07 (m, 3H), 7.91 (d, *J* = 8.6 Hz, 1H), 7.56 (d, *J* = 8.5 Hz, 2H), 7.28 (d, *J* = 8.2 Hz, 2H), 6.54 (d, *J* = 2.3 Hz, 2H), 6.51 (t, *J* = 2.2 Hz, 5H), 6.49 (d, *J* = 2.3 Hz, 2H), 4.99 (s, 2H), 4.73 (d, *J* = 2.4 Hz, 8H), 4.41 (p, *J* = 6.9 Hz, 1H), 4.21 (dd, *J* = 8.7, 6.6 Hz, 1H), 3.97 (t, *J* = 5.9 Hz, 4H), 3.50-3.38 (m, 14H), 3.33 (dd, *J* = 12.1, 4.6 Hz, 4H), 2.86 (s, 3H), 2.74 (s, 3H), 2.01 (h, *J* = 6.7 Hz, 1H), 1.38 (s, 9H), 1.31 (d, *J* = 7.1 Hz, 3H), 0.86 (dd, *J* = 10.3, 6.8 Hz, 6H). ^{13}C NMR (101 MHz, [D]$_6$DMSO) δ 170.82, 170.61, 169.78, 159.29, 159.13, 158.00, 141.44, 138.52, 138.38, 138.31, 131.54, 128.03, 108.67, 108.25, 107.94, 107.69, 107.44, 100.00, 99.65, 99.36, 78.96, 78.28, 77.81, 66.21, 65.80, 57.45, 55.44, 48.91, 42.24, 40.42, 38.28, 30.41, 27.83, 18.93, 17.88, 17.68; MS (MALDI-TOF): *m/z* calcd for $[C_{65}H_{77}NaN_7O_{15}]^+$: 1218.03 $[M + Na]^+$; found: 1218.2 (HCCA matrix).

Tetra-alkyne-Val–Ala-PTX (**18e**)

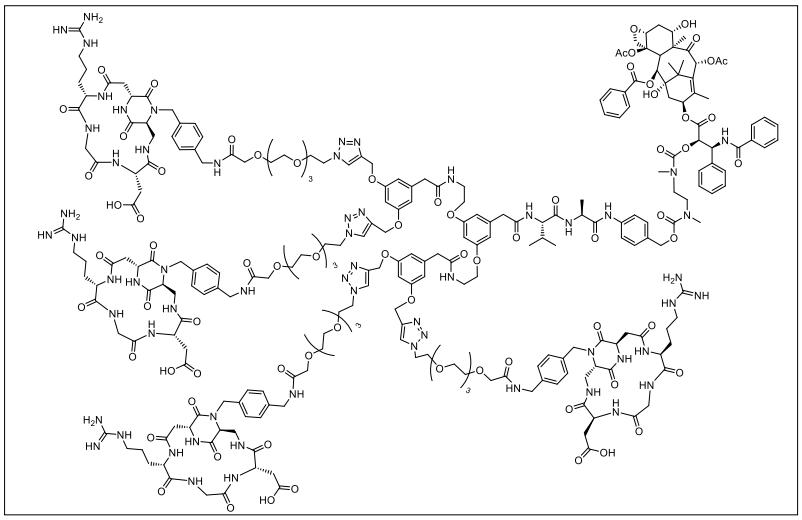


Chemical Formula:
$C_{108}H_{118}N_8O_{28}$
Molecular Weight: 1976,16

A solution of Boc-protected compound **16e** (40 mg, 0.033 mmol, 1 equiv) in dry CH_2Cl_2 (1.1 mL) was cooled to 0 °C under a nitrogen atmosphere and TFA (550 μL) was added. The mixture was then allowed to reach room temperature and stirred for 45 min. The solvent was removed affording the corresponding trifluoroacetate salt, without further purifications. The solid was dissolved in dry DMF (500 μL) and *i*Pr_2NEt (24 μL, 0.13 mmol, 4 equiv). The resulting solution was added at 0 °C to a stirred solution of **17** (51 mg, 0.05 mmol, 1.5 equiv) in dry DMF (500 μL), under a nitrogen atmosphere. The reaction was then allowed to reach room temperature and stirred overnight. AcOEt (60 mL) was added and the solution was washed with a 1 M aqueous solution of $KHSO_4$ (2 × 10 mL) and brine (1 × 15 mL). The organic phase was dried over Na_2SO_4 and concentrated, then the crude was purified by a flash chromatography (eluent: AcOEt/MeOH from 100% to 95% of AcOEt) to afford carbamate **18e** as a white solid (49 mg, 75% yield over two steps).

R_f = 0.25 (95:5, CH_2Cl_2/MeOH); MS (MALDI-TOF): *m/z* calcd for $[C_{108}H_{118}N_8O_{28}]^+$: 1975.01 $[M + H]^+$; found: 1976.2 (HCCA matrix); *m/z* calcd for $[C_{108}H_{118}NaN_8O_{28}]^+$: 1997.9 $[M + Na]^+$; found: 1998.02 (HCCA matrix); HRMS (ESI+): *m/z* calcd for $[C_{108}H_{118}NaN_8O_{28}]^+$: 1997.99, $[M + Na]^+$; found 1997.79; *m/z* calcd for $[C_{108}H_{118}Na_2N_8O_{28}]^{2+}$: 1010.31, $[M + 2Na]^{2+}$: found 1010.39.

***(cyclo[DKP-RGD]$_4$)-Val-Ala-PTX* (9)**



Chemical Formula: $C_{256}H_{338}N_{60}O_{80}$
Molecular Weight: 5535.86

Tetra-alkyne **18e** (4 mg, 0.002 mmol, 1 equiv) and azide **19** (9.8 mg, 0.0097 mmol, 4.8 equiv) were dissolved in a degased 1:1 mixture of H_2O/DMF (200 μL) under a nitrogen atmosphere. Degased aqueous solutions of $CuSO_4 \cdot 5H_2O$ (1.2 mg, 0.004 mmol, 2 equiv) and sodium ascorbate (0.95 mg, 0.0048 mmol, 2.4 equiv) were added in the darkness at room temperature and the mixture was stirred overnight. The solvent was removed under vacuum, and the crude residue was purified by semipreparative HPLC [Waters Atlantis 21 mm x 10 cm column; gradient: 90% (H_2O + 0.1% CF_3COOH)/10% (CH_3CN + 0.1% CF_3COOH) to 100% (CH_3CN + 0.1% CF_3COOH) in 20 min; t_R (product)=10.9 min]. The purified product was then freeze-dried to give the desired compound **9** as a white solid (8 mg, 73% yield).

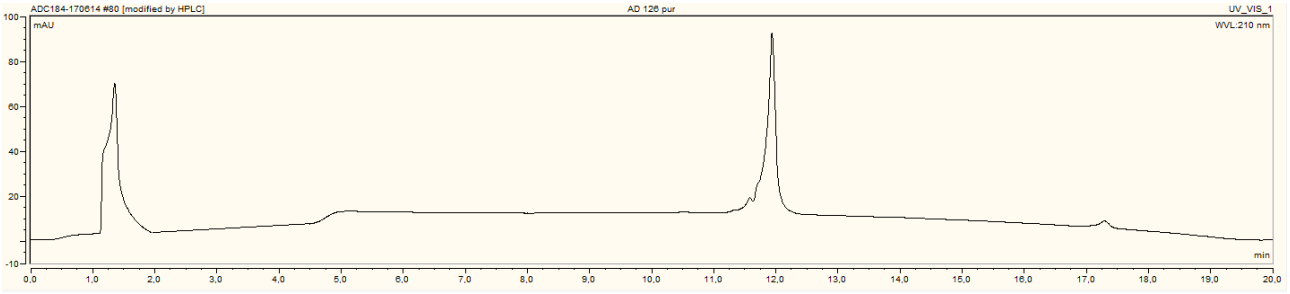
MS (MALDI-TOF): *m/z* calcd for $[C_{256}H_{338}N_{60}O_{80}]^+$: 5532.78 $[M + H]^+$; found: 5532.90 (HCCA matrix); HRMS (ESI+): *m/z* calcd for $[C_{256}H_{338}Na_4N_{60}O_{80}]^{4+}$: 1406.93, $[M + 4Na]^{4+}$; found 1406.84; *m/z* calcd for $[C_{256}H_{338}Na_5N_{60}O_{80}]^{5+}$: 1130.14, $[M + 5Na]^{5+}$: found 1130.07.

HPLC traces of the final products

cyclo[DKP-RGD]-Val-Ala-PTX aliphatic scaffold conjugate **(5)**

Waters Atlantis 21 mm × 10 cm column, gradient from 90% (H_2O + 0.1% CF_3COOH) / 10% (CH_3CN + 0.1% CF_3COOH) to 100% (CH_3CN + 0.1% CF_3COOH) in 20 min.

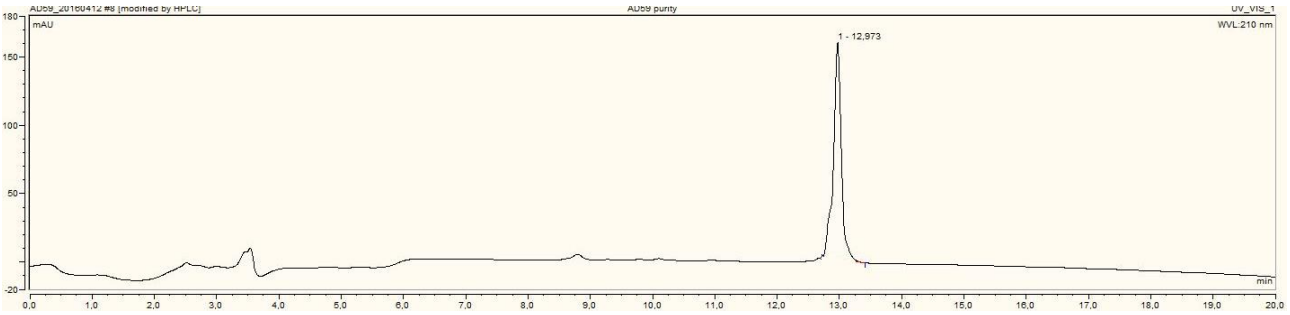
Purity: 98%



cyclo[DKP-RGD]-Val-Ala-PTX aromatic scaffold conjugate **(6)**

Waters Atlantis 21 mm × 10 cm column, gradient from 90% (H_2O + 0.1% CF_3COOH) / 10% (CH_3CN + 0.1% CF_3COOH) to 100% (CH_3CN + 0.1% CF_3COOH) in 20 min.

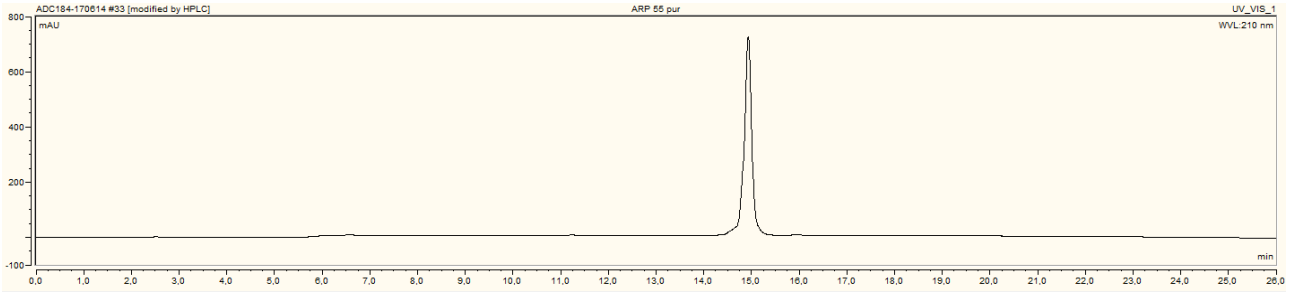
Purity: 98%



*(cyclo[DKP-RGD])$_2$-Val-Ala-PTX (**7**)*

Waters Atlantis 21 mm × 10 cm column, gradient from 90% (H_2O + 0.1% CF_3COOH) / 10% (CH_3CN + 0.1% CF_3COOH) to 100% (CH_3CN + 0.1% CF_3COOH) in 26 min.

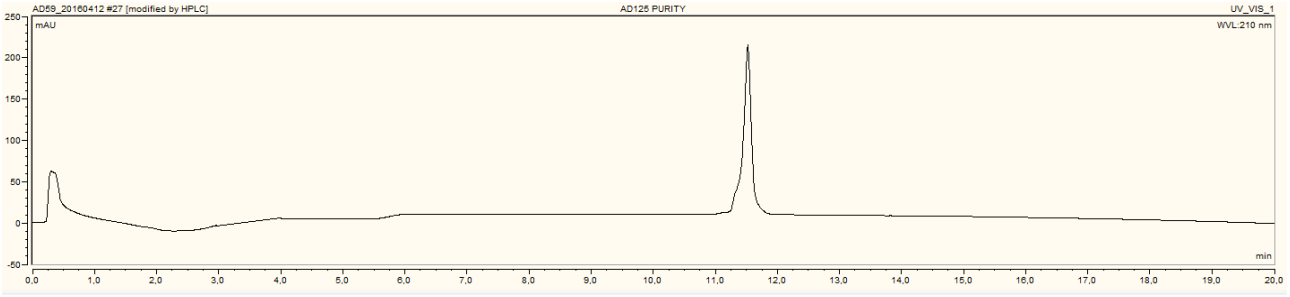
Purity: >99%



*(cyclo[DKP-RGD])$_3$-Val-Ala-PTX (**8**)*

Waters Atlantis 21 mm × 10 cm column, gradient from 90% (H_2O + 0.1% CF_3COOH) / 10% (CH_3CN + 0.1% CF_3COOH) to 100% (CH_3CN + 0.1% CF_3COOH) in 20 min.

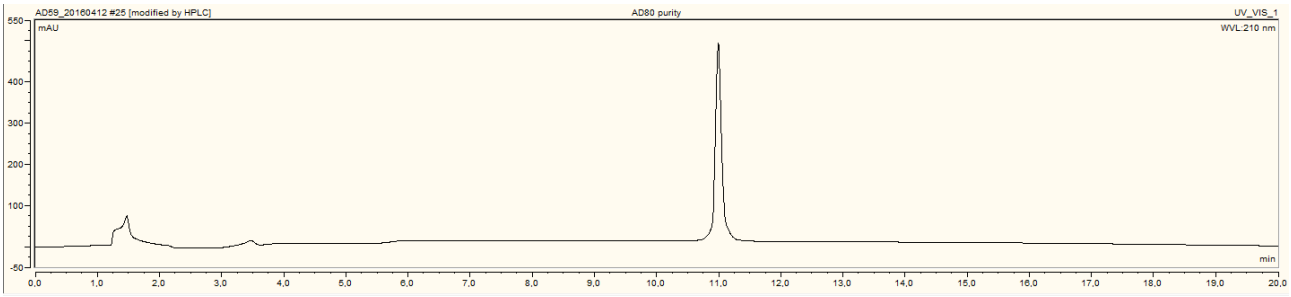
Purity: >99%



*(cyclo[DKP-RGD])$_4$-Val-Ala-PTX (**9**)*

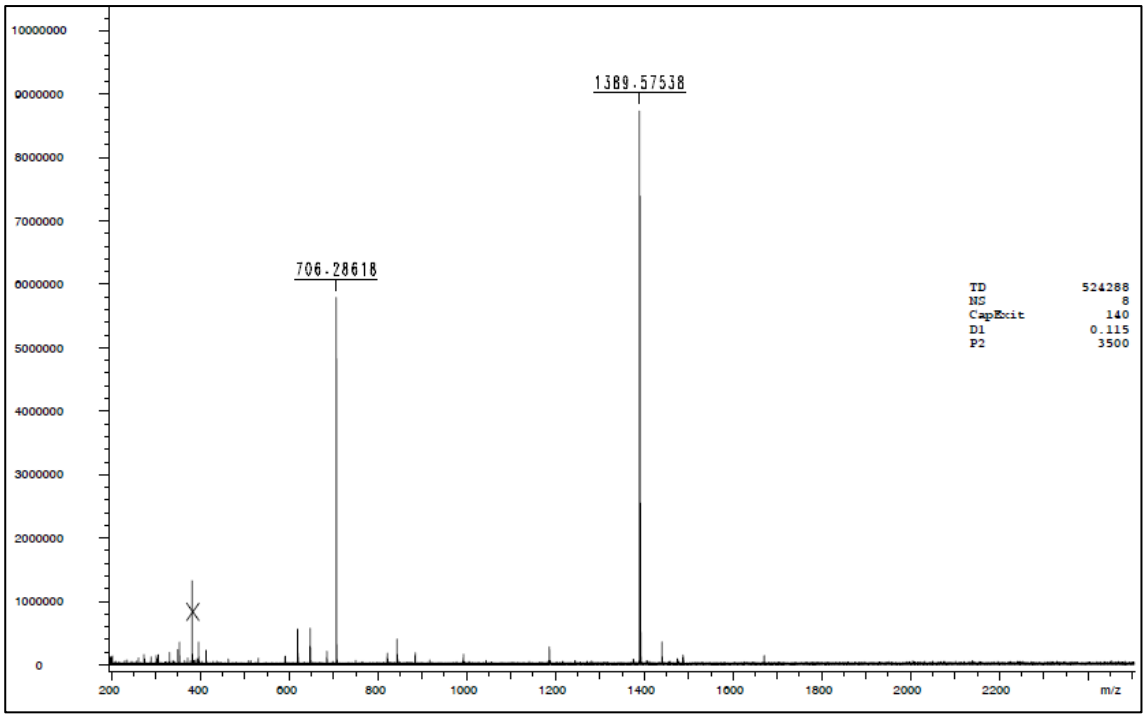
Waters Atlantis 21 mm × 10 cm column, gradient from 90% (H_2O + 0.1% CF_3COOH) / 10% (CH_3CN + 0.1% CF_3COOH) to 100% (CH_3CN + 0.1% CF_3COOH) in 20 min.

Purity: >99%

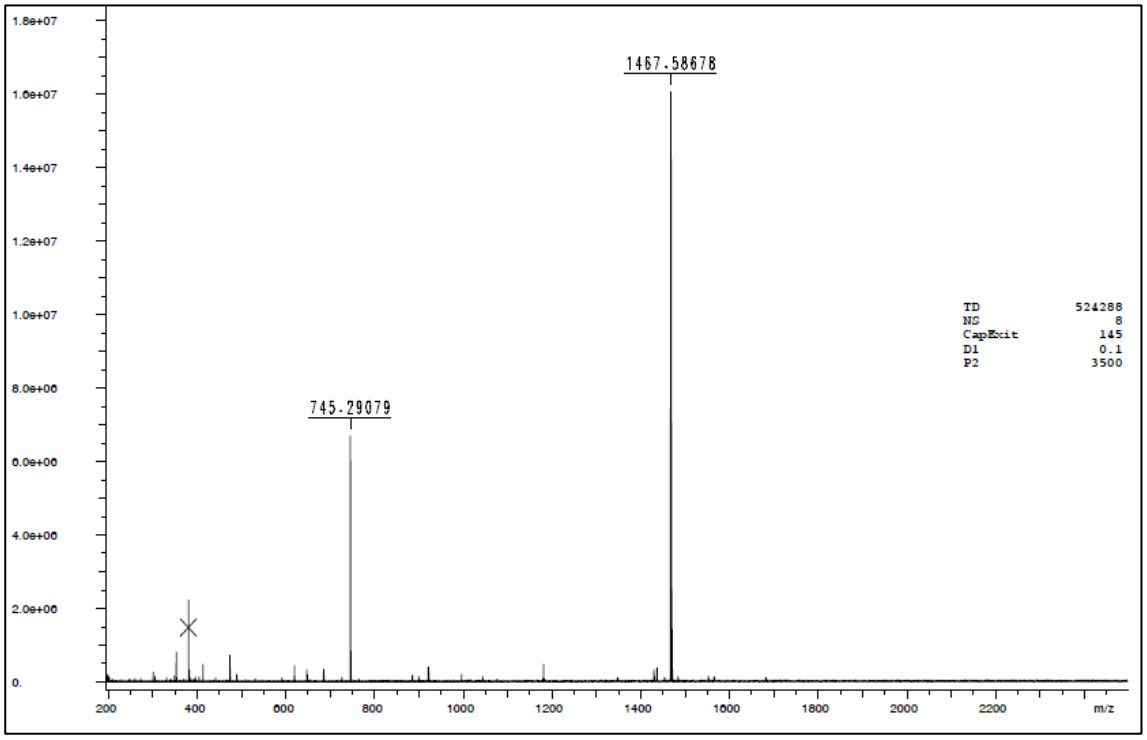


HRMS Spectra

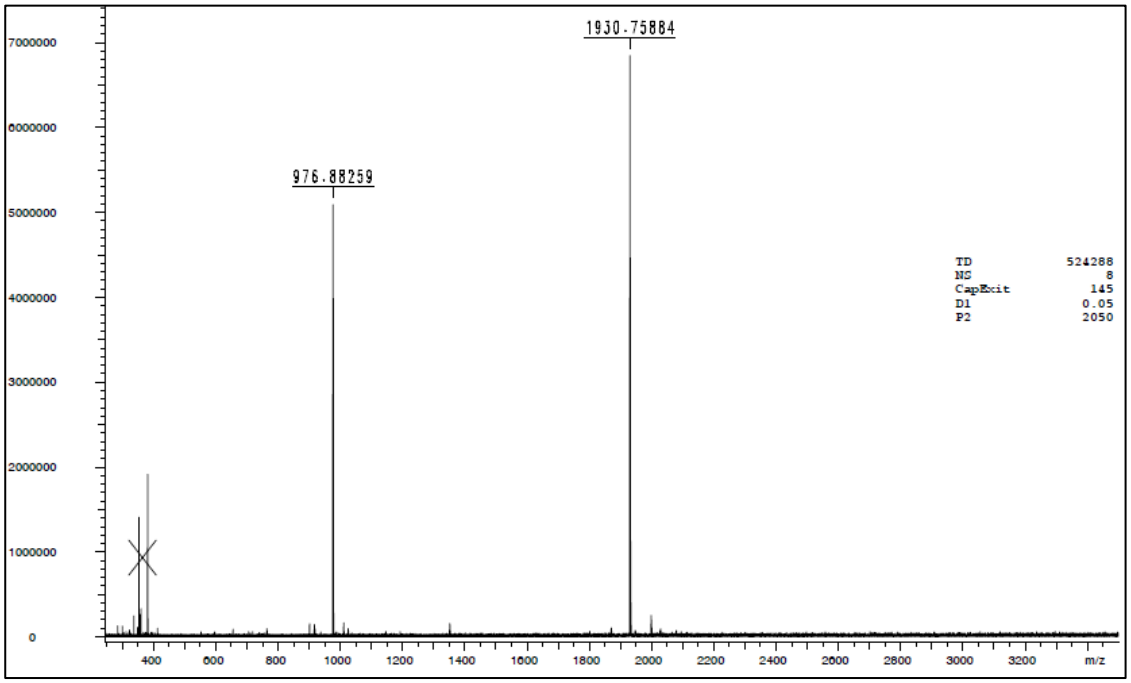
Aliphatic alkyne-Val-Ala-PTX (**18a**)



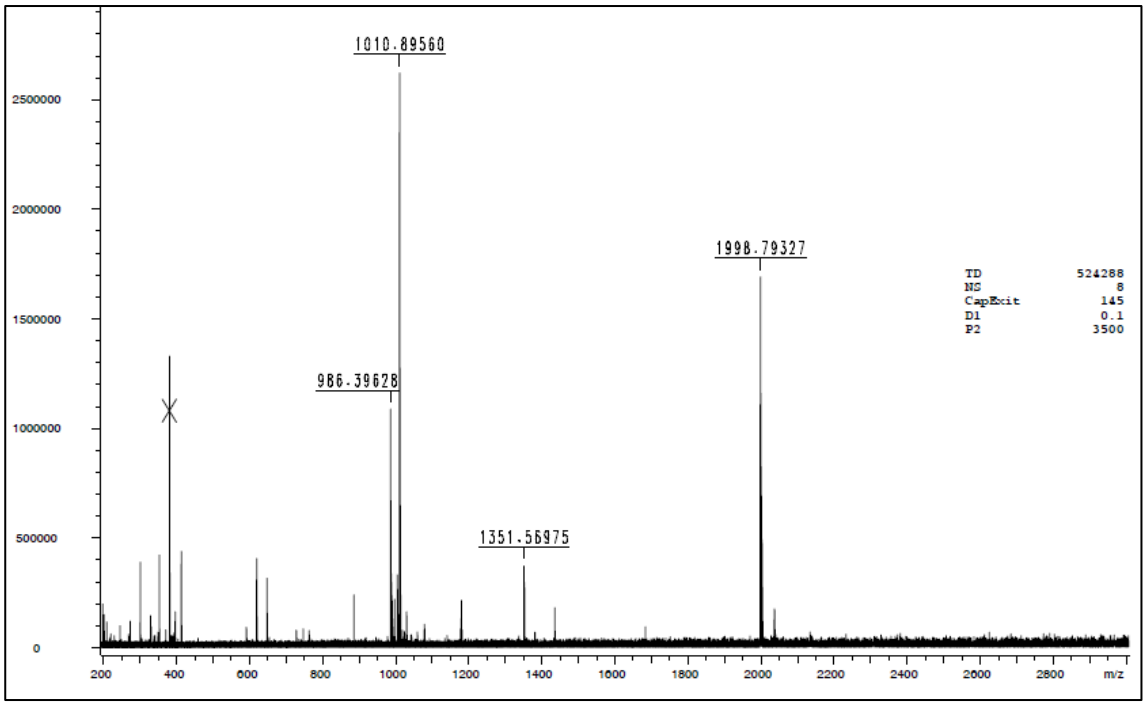
Aromatic alkyne-Val-Ala-PTX (**18b**)



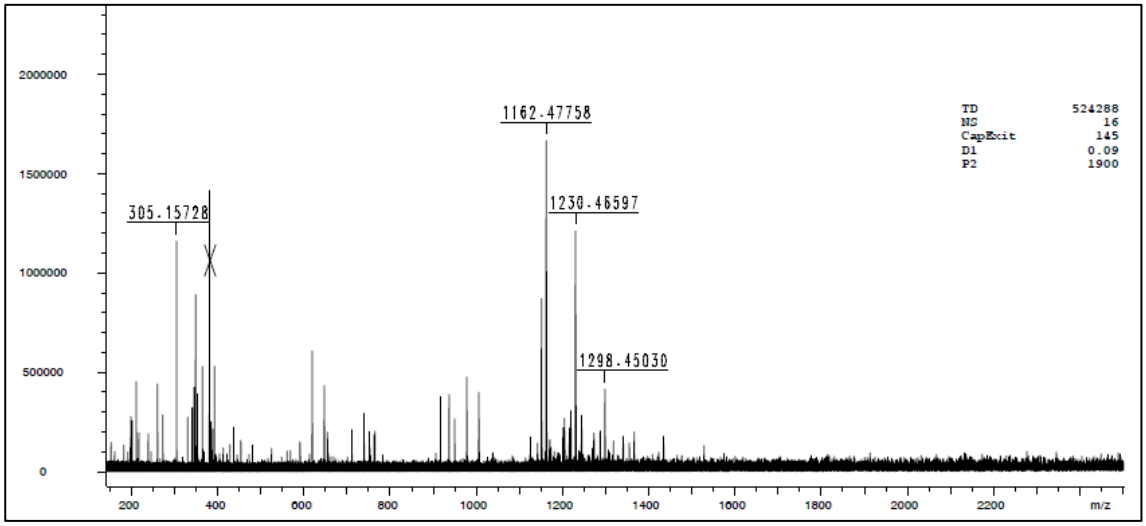
Tri-alkyne-Val–Ala-PTX (**18d**)



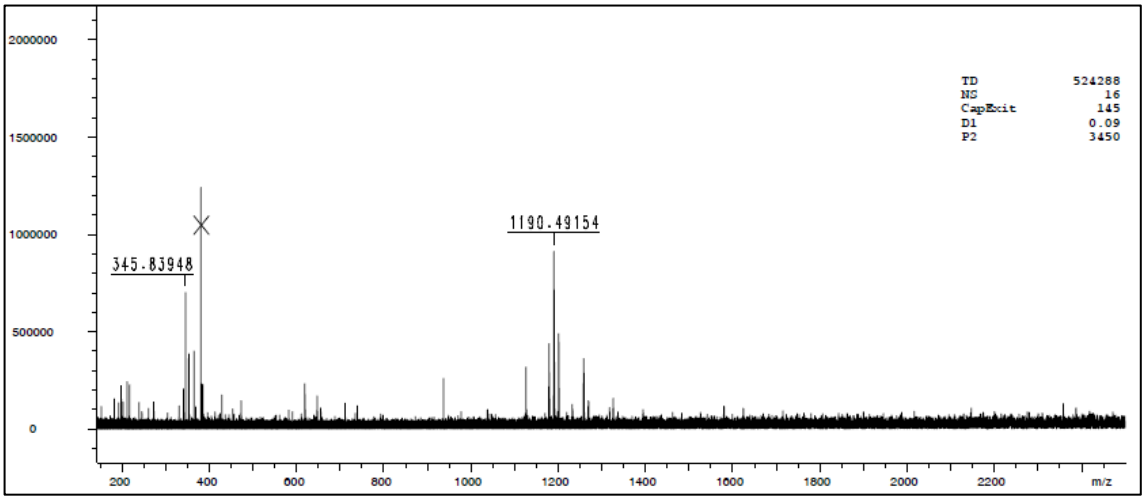
Tetra-alkyne-Val–Ala-PTX (**18e**)



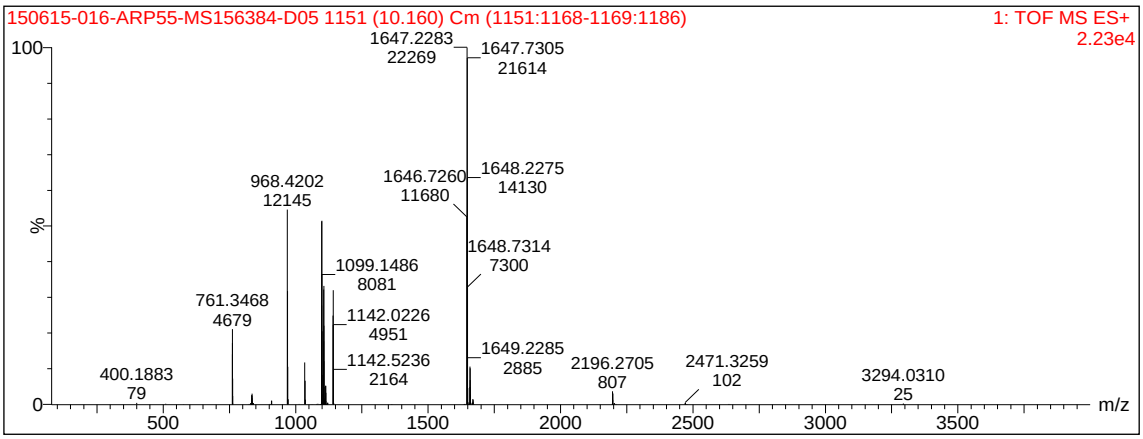
cyclo[DKP-RGD]-Val-Ala-PTX aliphatic scaffold conjugate **(5)**



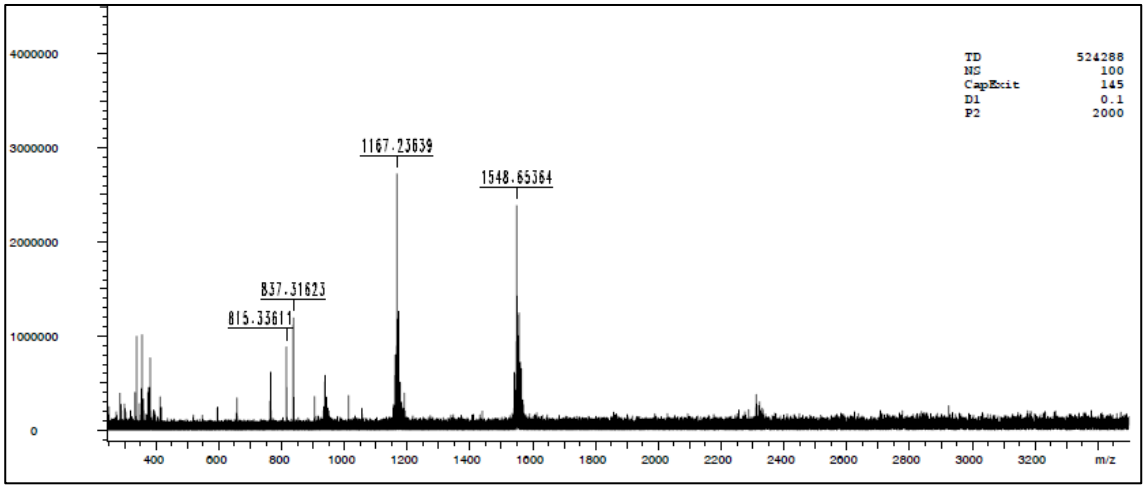
cyclo[DKP-RGD]-Val-Ala-PTX aromatic scaffold conjugate **(6)**



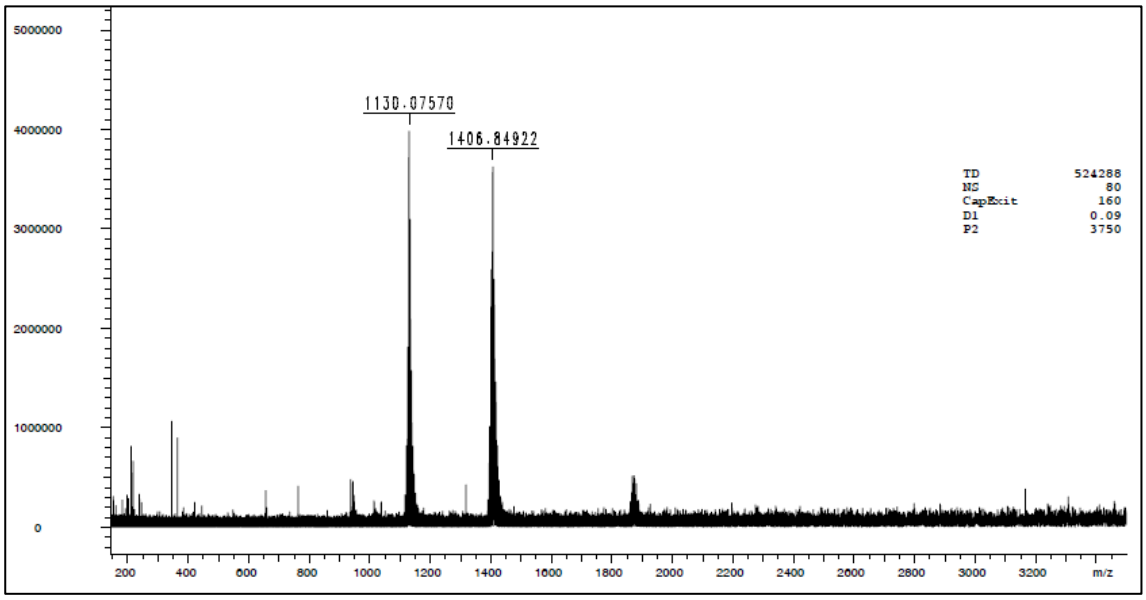
cyclo[DKP-RGD]$_2$-Val-Ala-PTX **(7)**



cyclo[DKP-RGD]$_3$-Val-Ala-PTX **(8)**



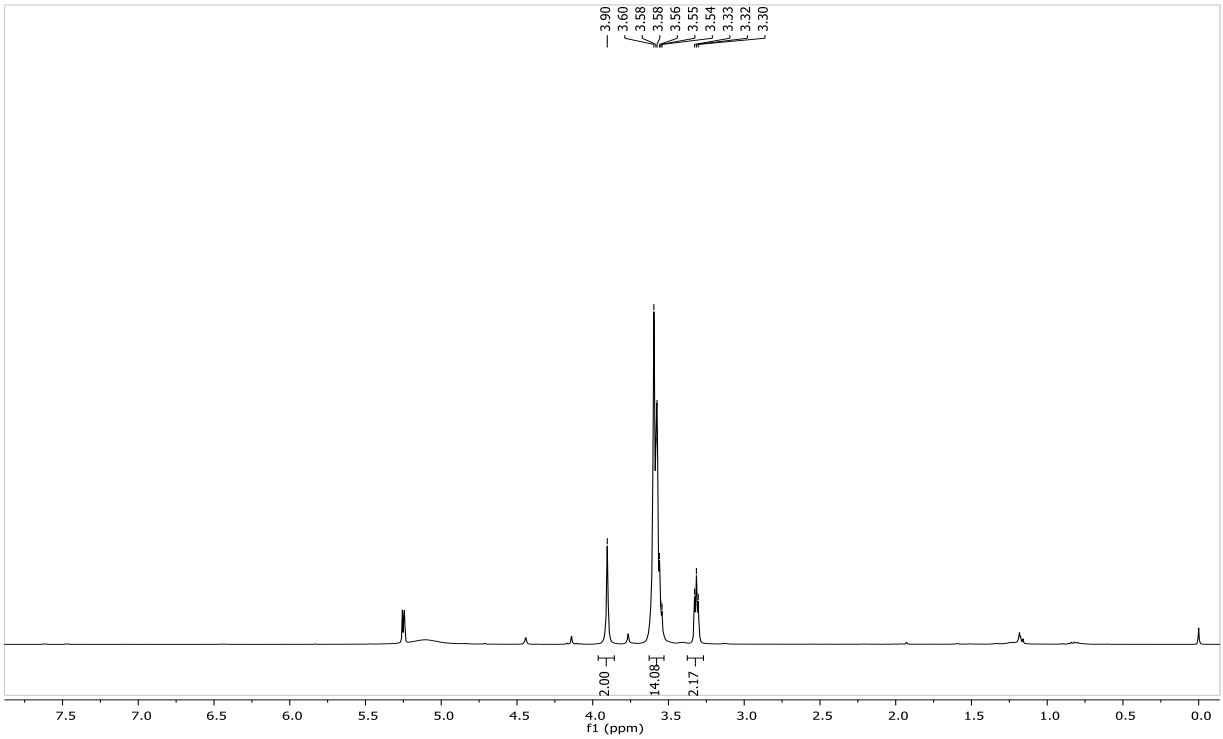
cyclo[DKP-RGD]$_4$-Val-Ala-PTX **(9)**



^{1}H-NMR and ^{13}C-NMR spectra

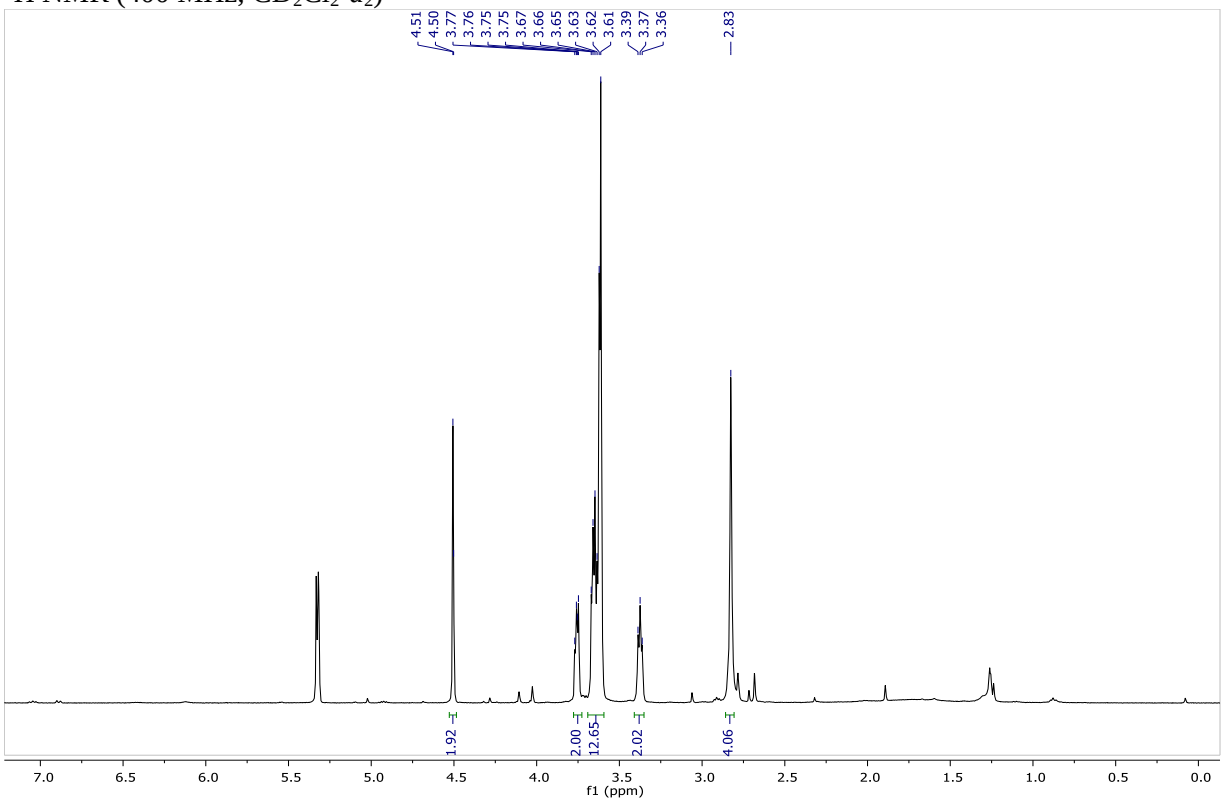
14-azido-3,6,9,12-tetraoxatetradecanoic acid (**32**)

^{1}H NMR (400 MHz, CD_2Cl_2-d_2)



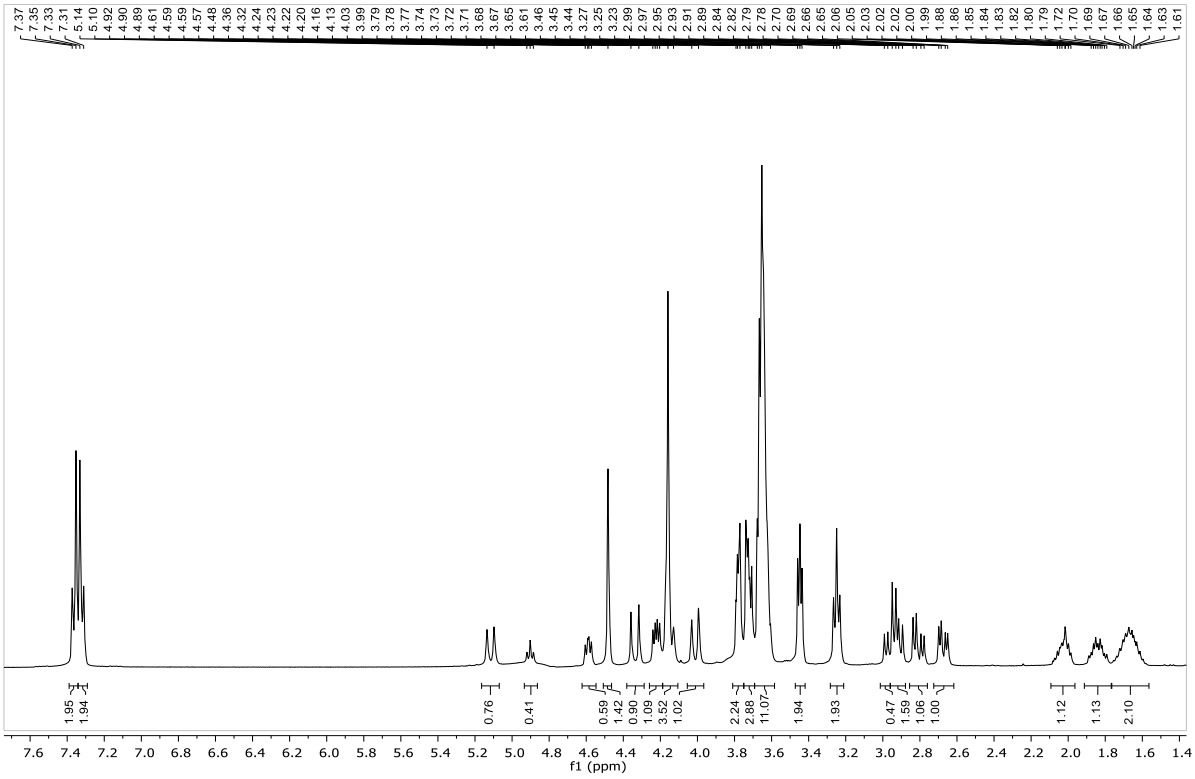
Azido-tetraethylene glycol-N-hydroxysuccinimidyl ester (**33**)

^{1}H NMR (400 MHz, CD_2Cl_2-d_2)

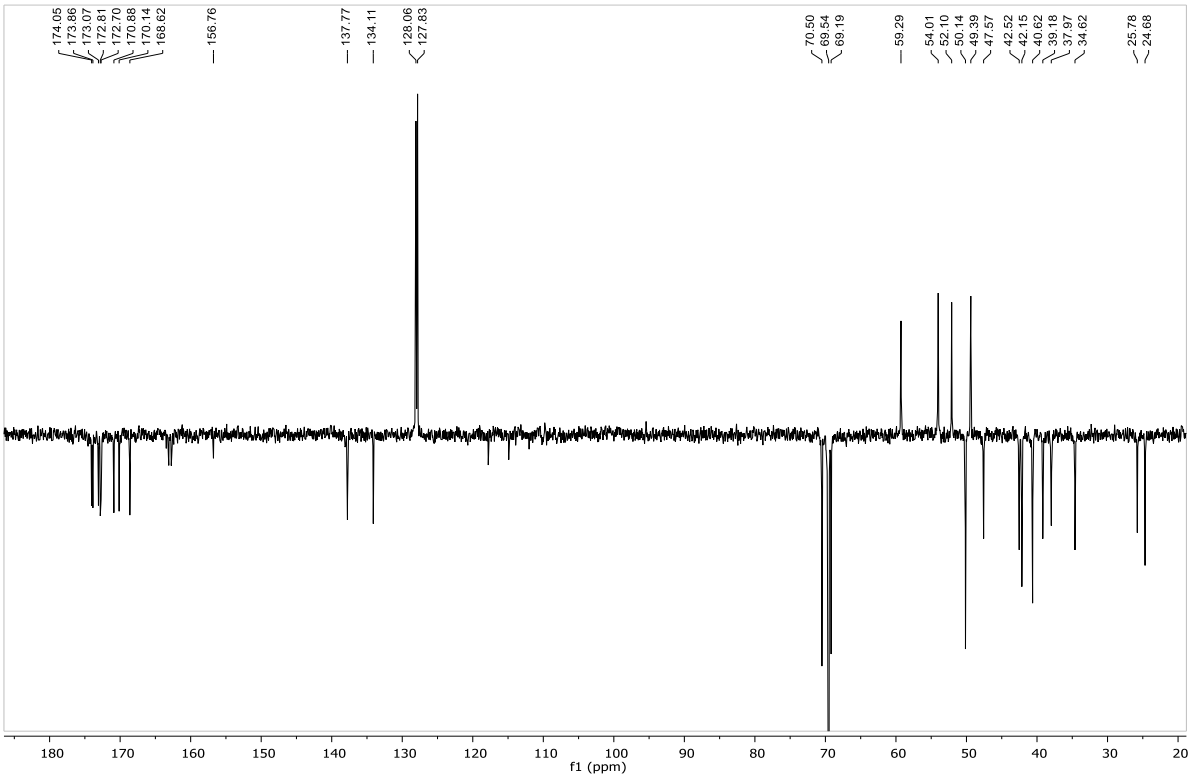


cyclo[DKP-RGD]-tetraethylene glycol-azide (**19**)

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

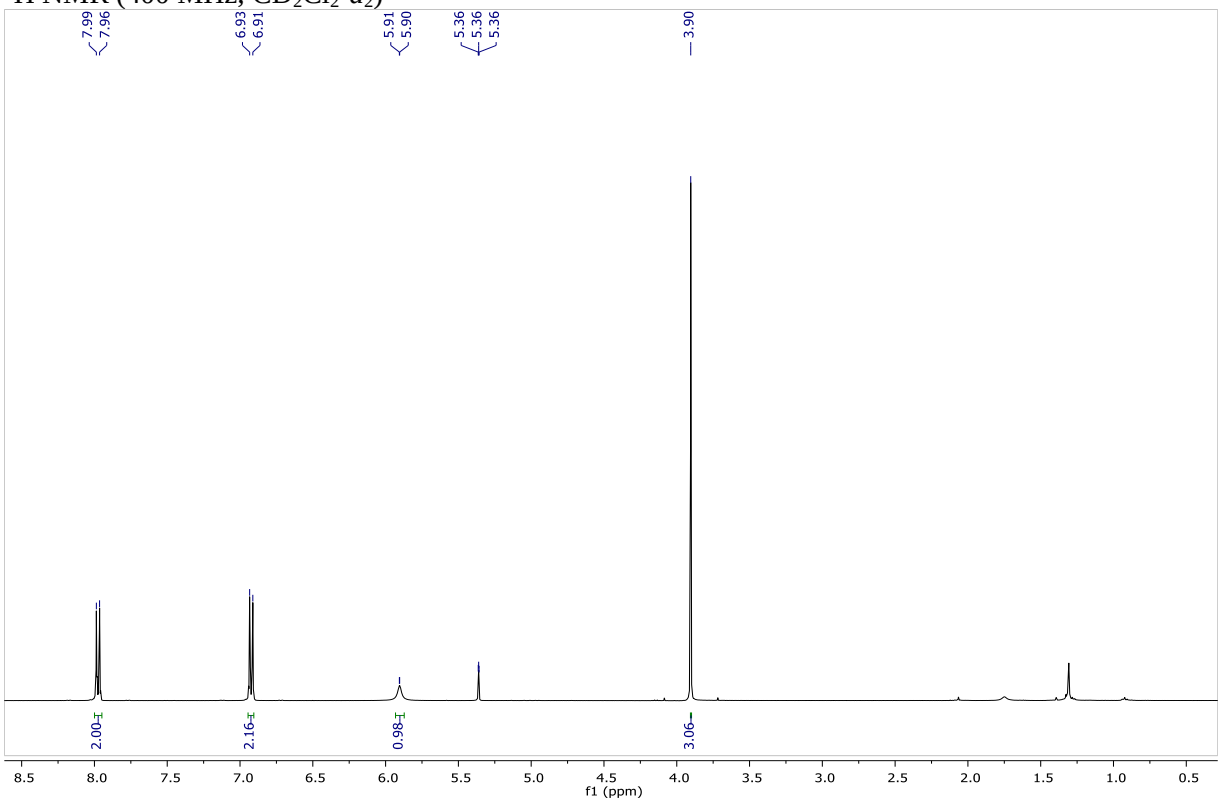


^{13}C NMR (101 MHz, D_2O)



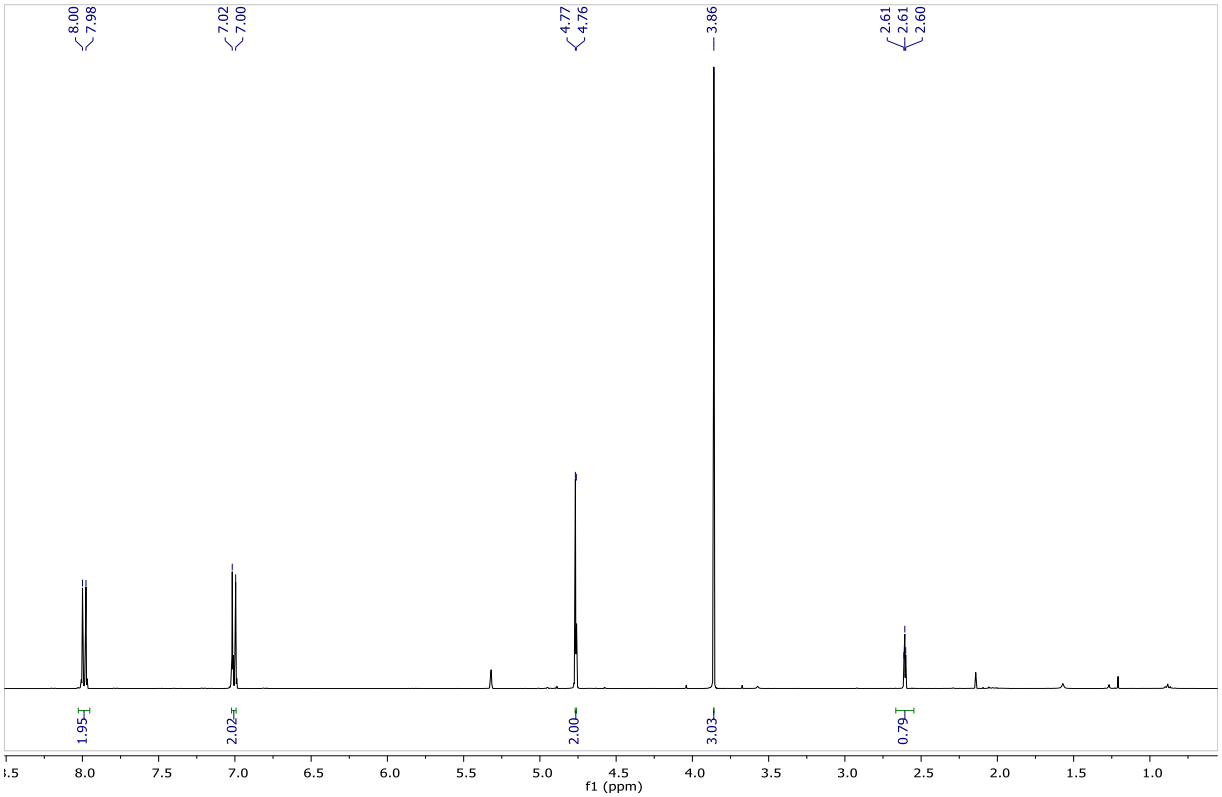
methyl 4-hydroxybenzoate (**21**)

^{1}H NMR (400 MHz, CD_2Cl_2-d_2)



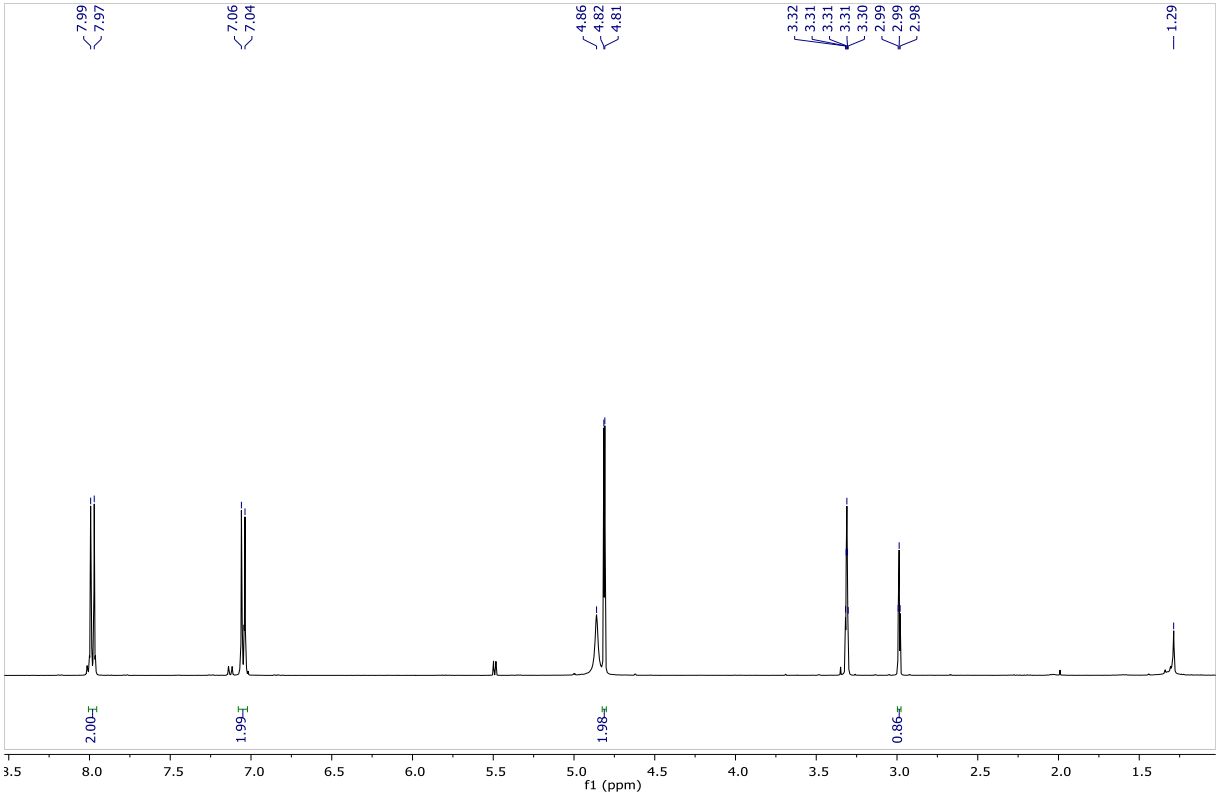
methyl 4-(prop-2-yn-1-yloxy)benzoate (**22**)

^{1}H NMR (400 MHz, CD_2Cl_2-d_2)



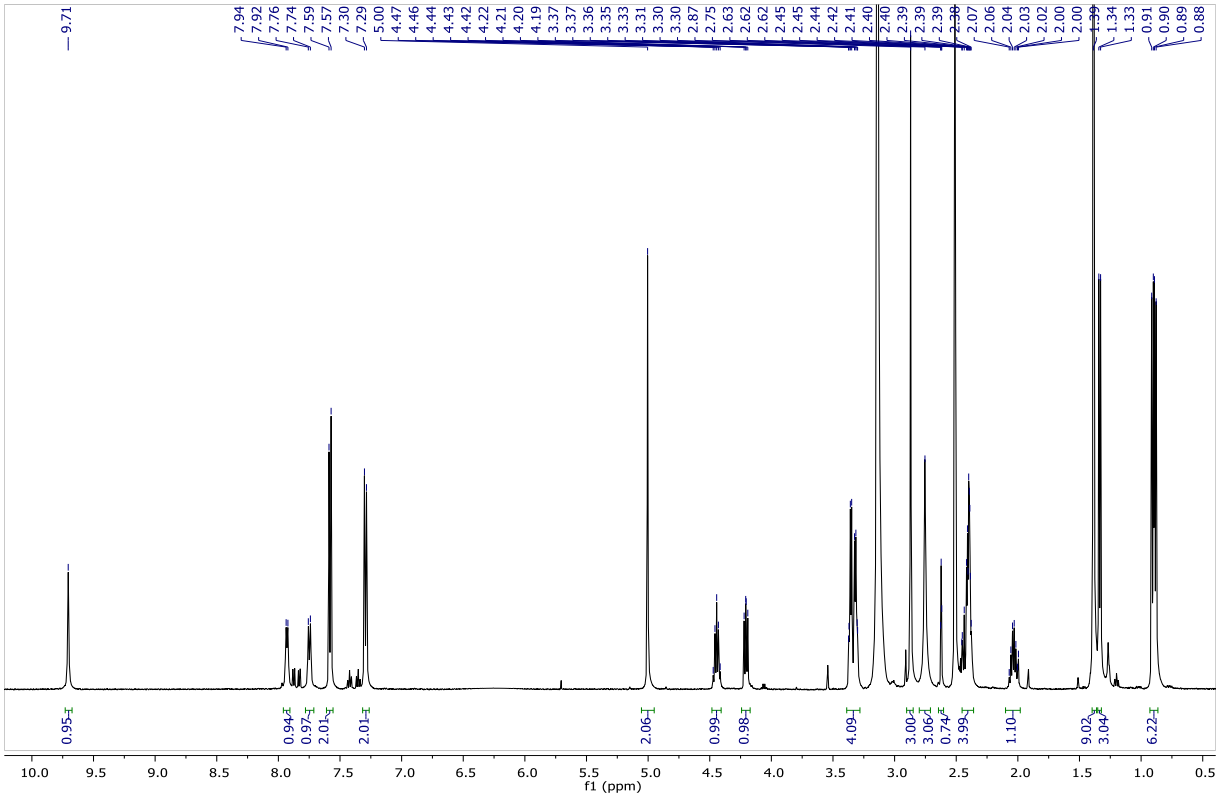
4-(prop-2-yn-1-yloxy)benzoic acid (**11**)

^{1}H NMR (400 MHz, CD_3OD)

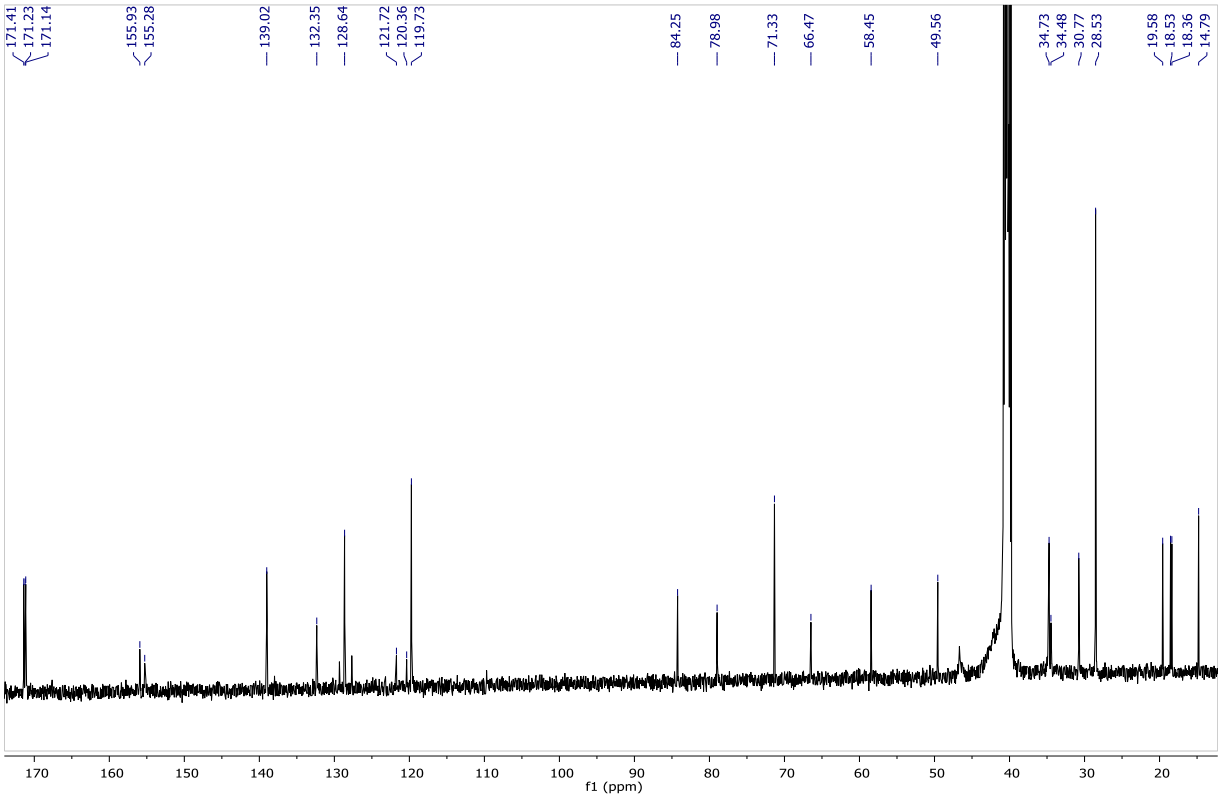


tert-butyl (4-((S)-2-((S)-3-methyl-2-(pent-4-ynamido)butanamido)propanamido)benzyl) ethane-1,2-diylbis(methylcarbamate) (**16a**)

^{1}H NMR (500 MHz, $[D]_6DMSO$), T= 70° C

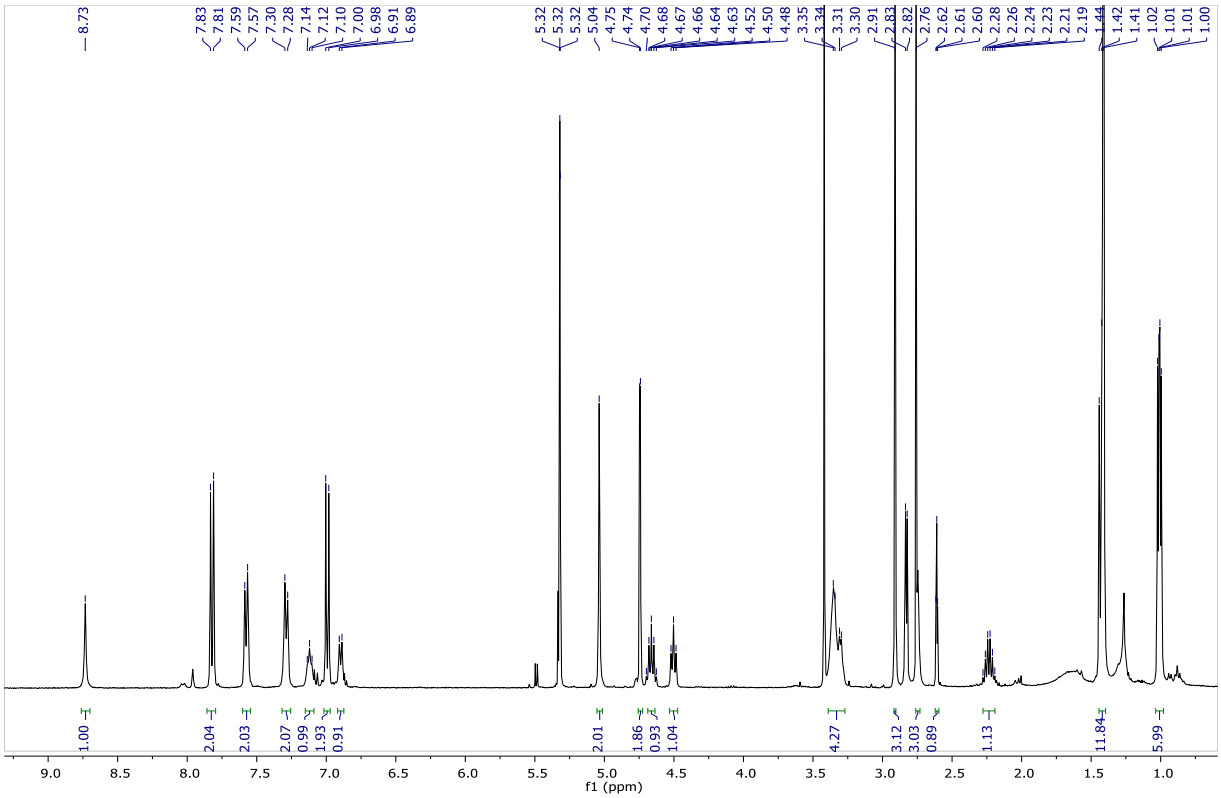


^{13}C NMR (126 MHz, [D]$_6$DMSO), T = 70 ℃

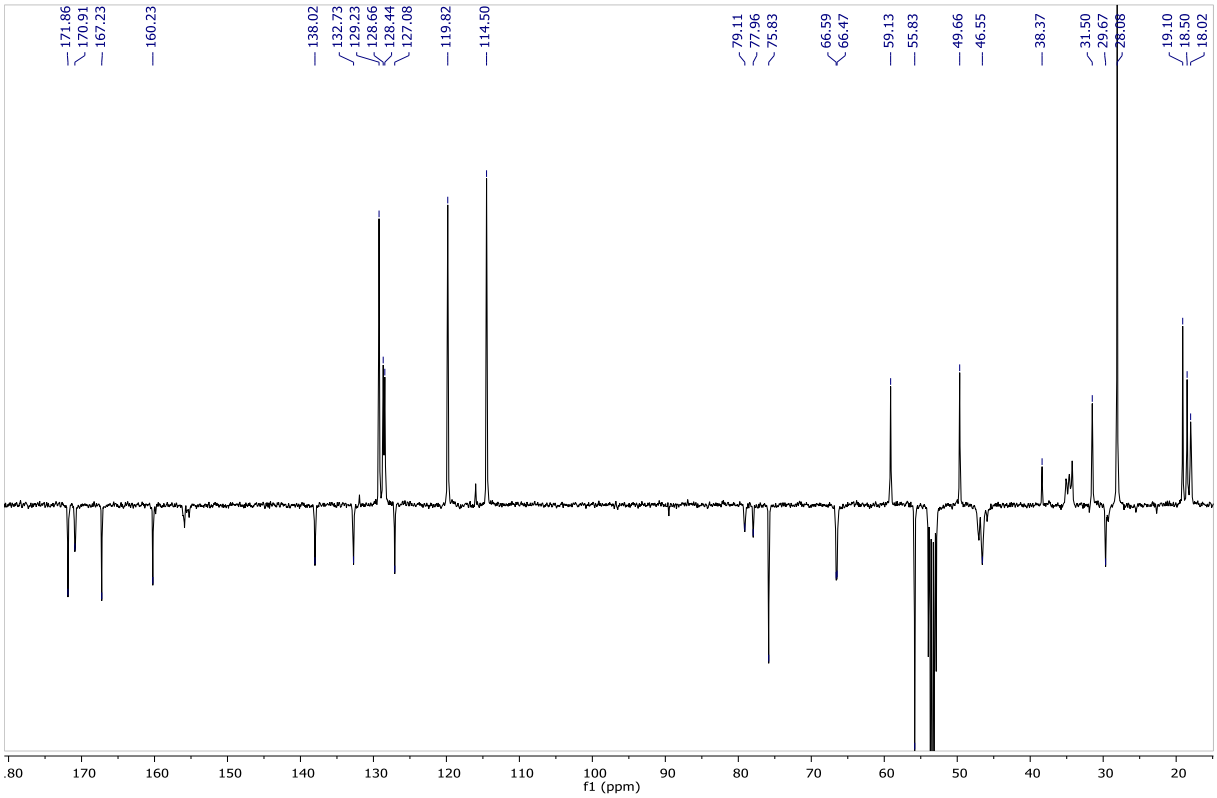


tert-Butyl (4-((S)-2-((S)-3-methyl-2-(4-(prop-2-yn-1-yloxy)benzamido)butanamido)propanamido)benzyl) ethane-1,2-diylbis(methylcarbamate) (**16b**)

^{1}H NMR (400 MHz, CD_2Cl_2-d_2)

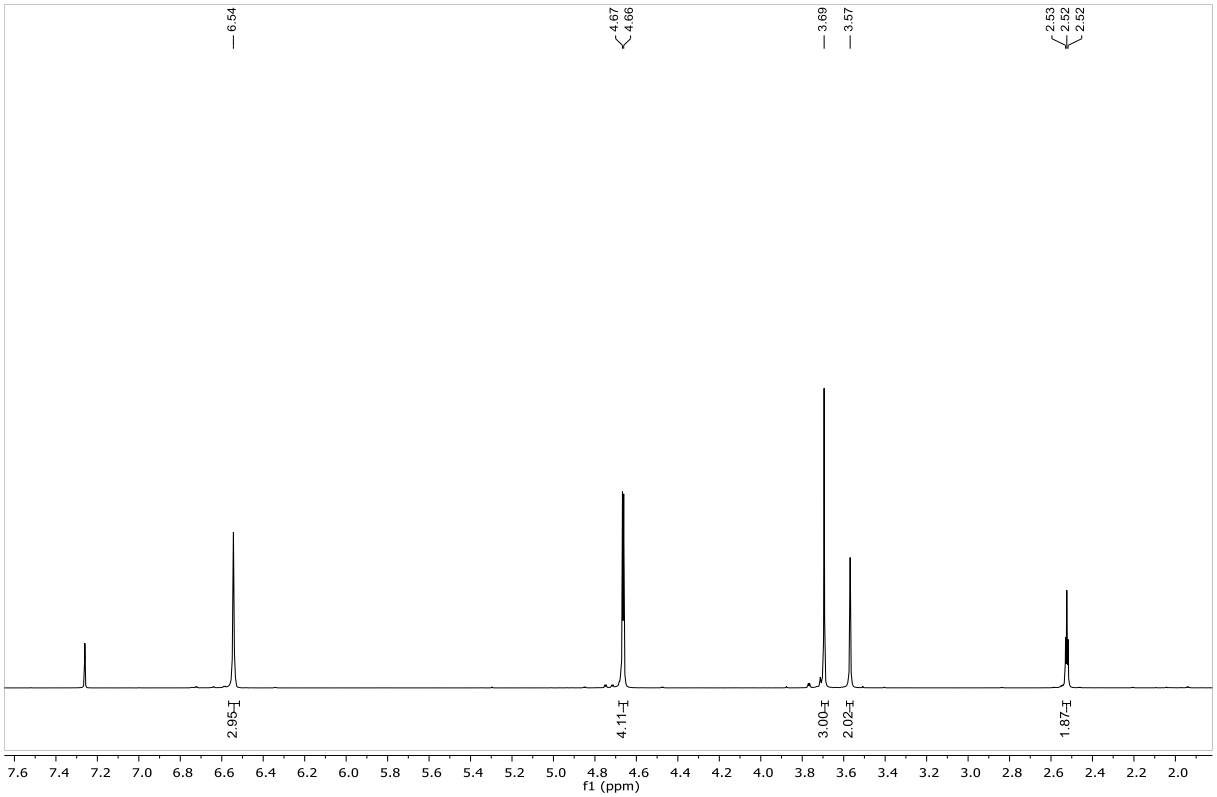


^{13}C NMR (101 MHz, CD_2Cl_2-d_2)

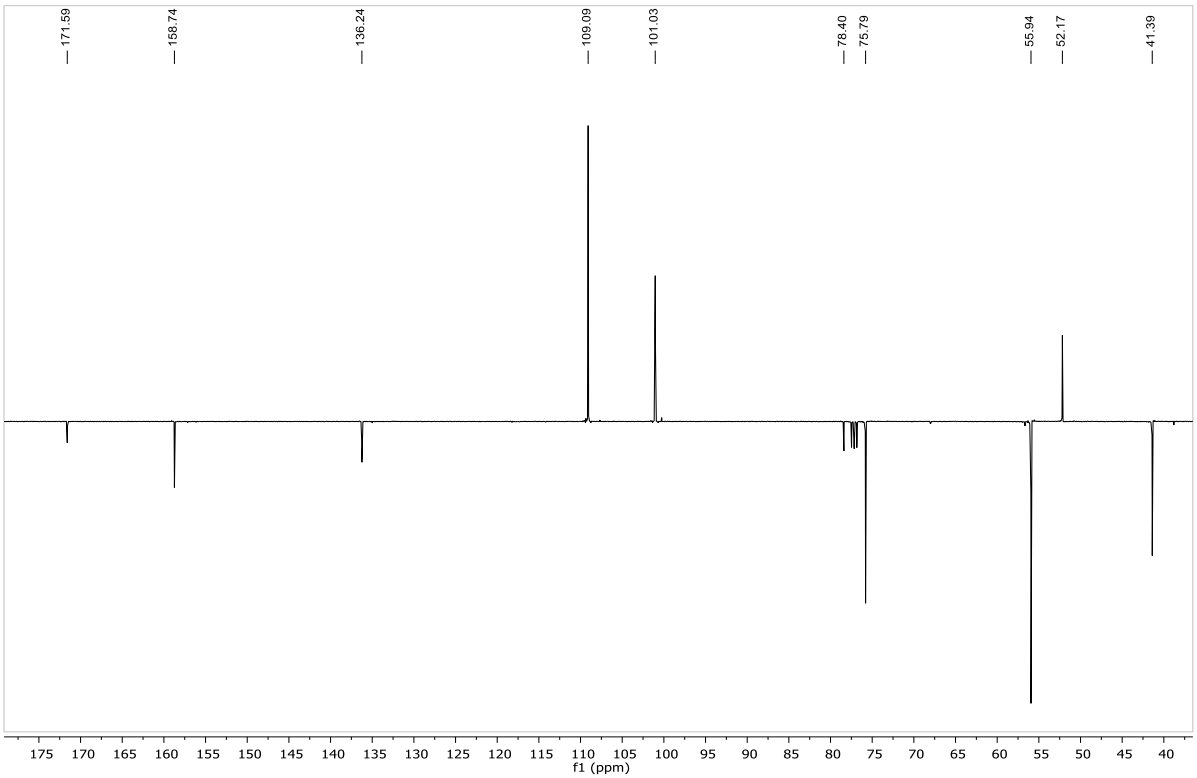


Methyl 3,5-bis(propynyloxy)phenyl acetate (**24**)

^{1}H NMR (400 MHz, $CDCl_3$)

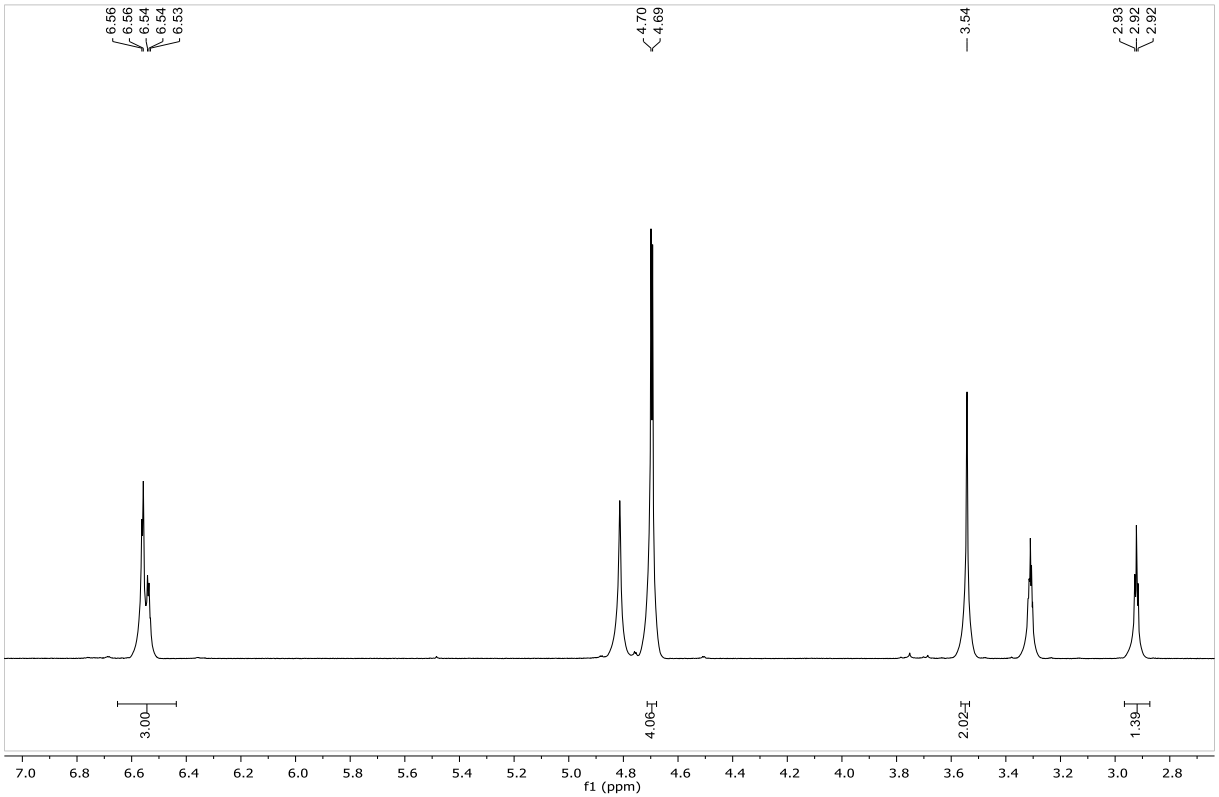


^{13}C NMR (101 MHz, $CDCl_3$)



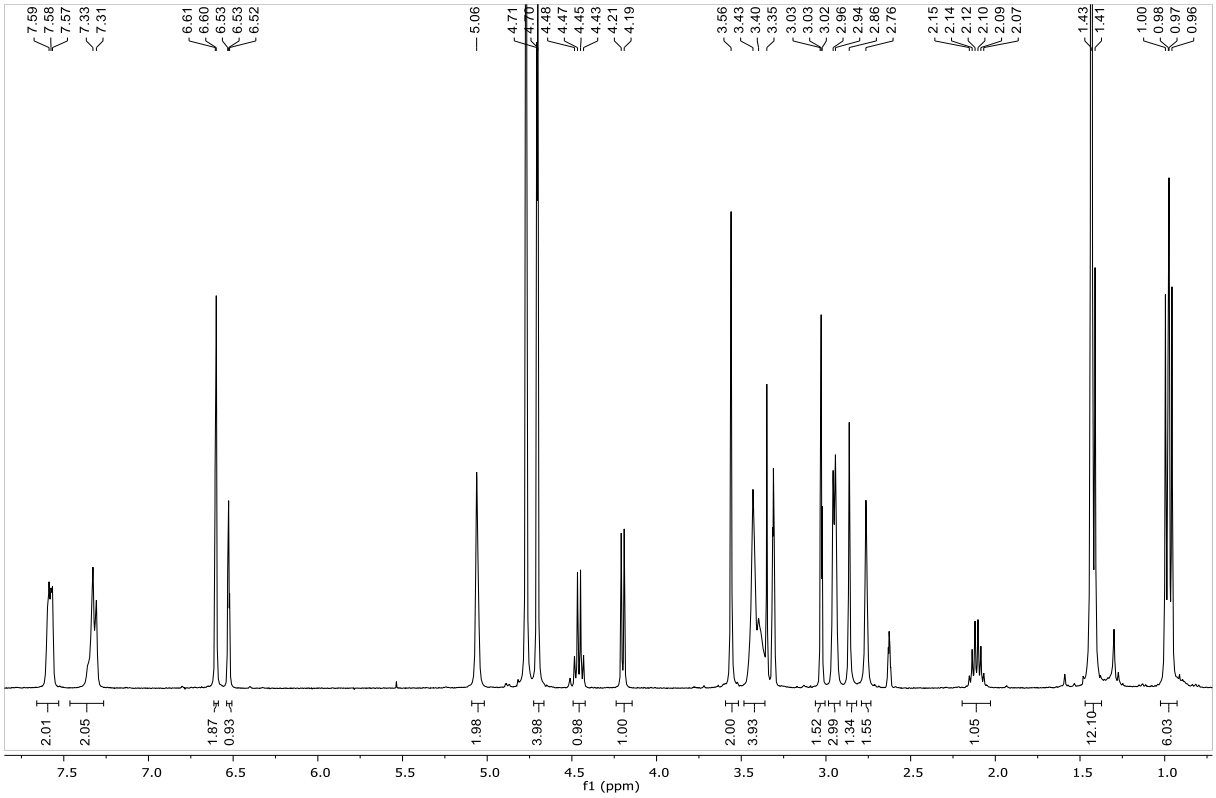
3,5-bis(Propynyloxy)phenyl acetic acid (**12**)

^{1}H NMR (400 MHz, CD_3OD)

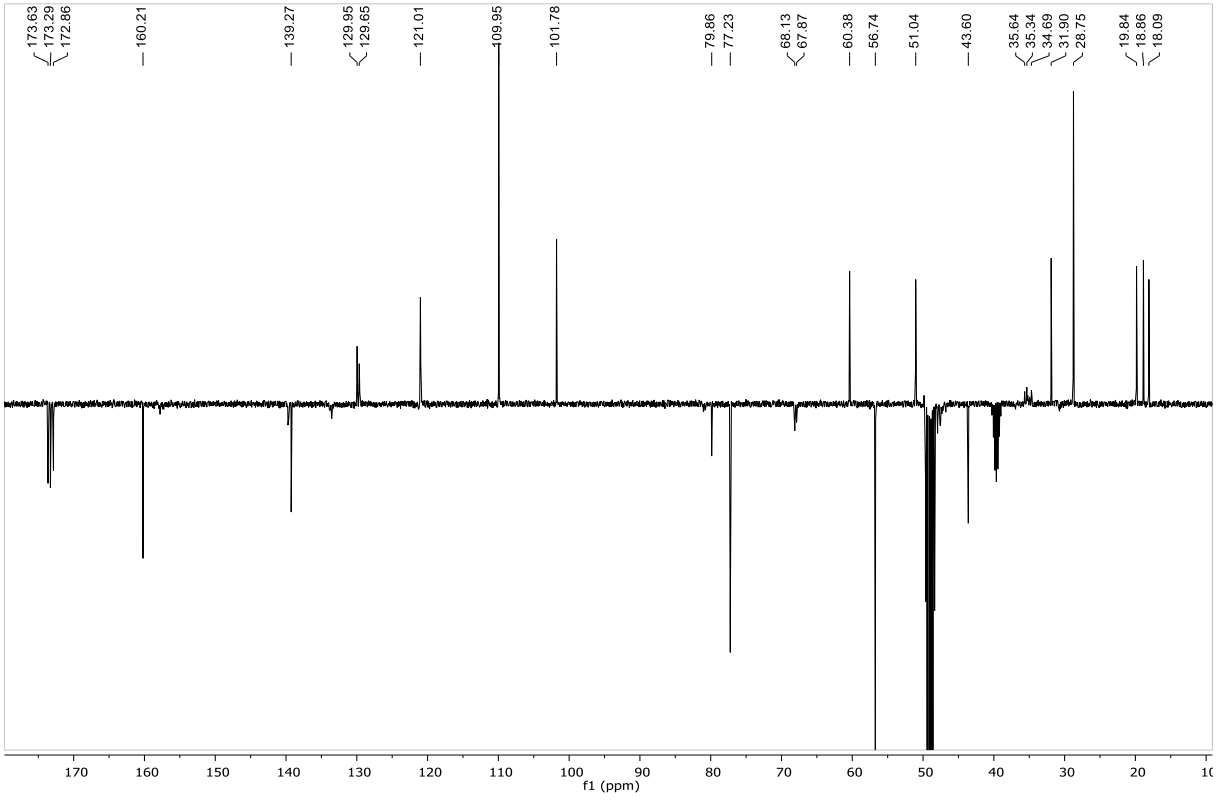


[3,5-bis(Propynyloxy)phenylacetyl]-Val–Ala-N-[4-[[[(N-(Boc)-N,N'-dimethylethylenediamine) carbonyl]oxy]methyl]phenyl] **(16c)**

^{1}H NMR (400 MHz, CD_3OD + $[D]_6DMSO$)

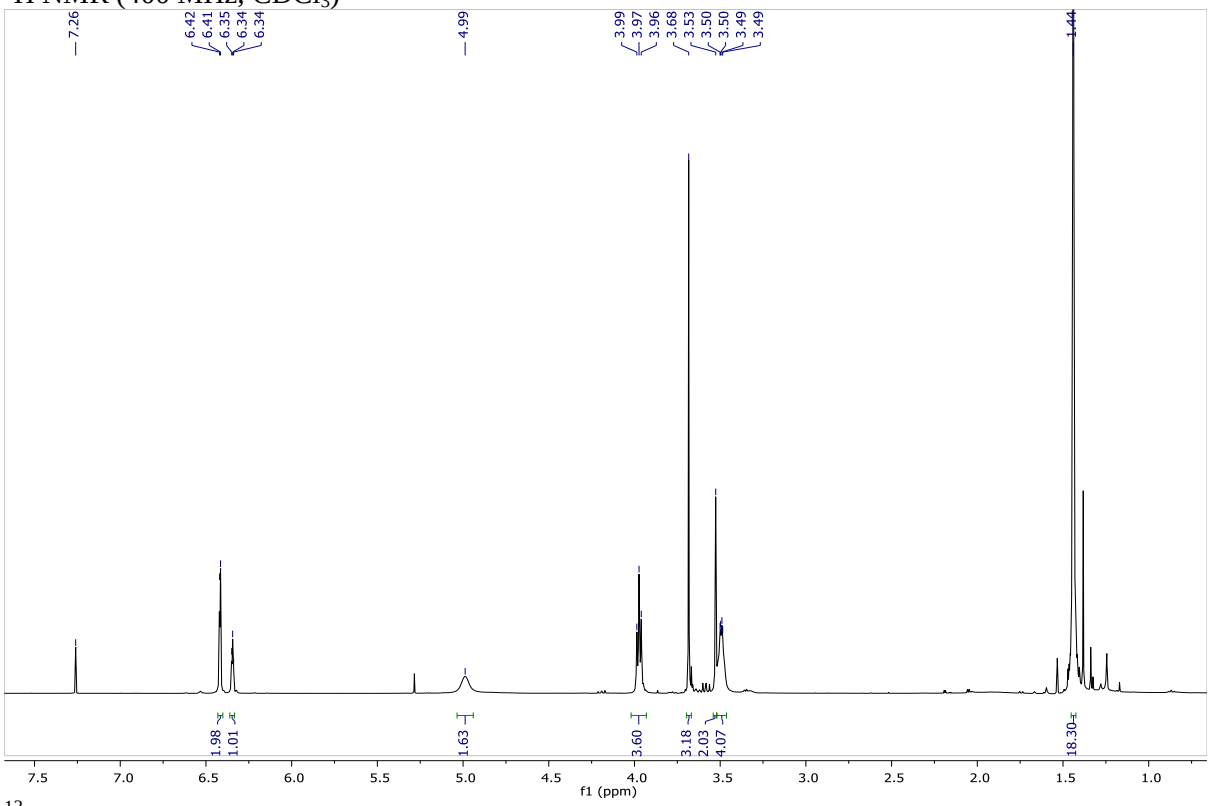


^{13}C NMR (101 MHz, CD_3OD + $[D]_6DMSO$)

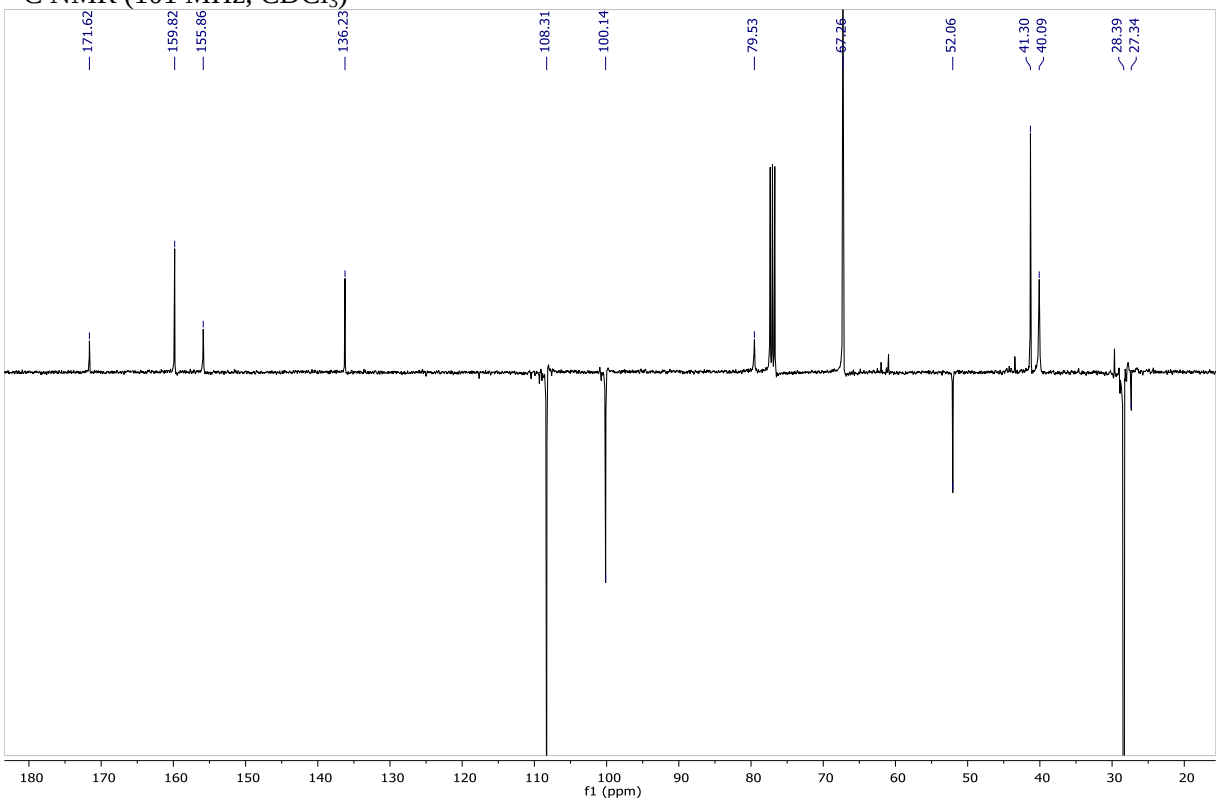


Methyl 2-(3,5-bis(2-((tert-butoxycarbonyl)amino)ethoxy)phenyl)acetate **(25)**

^{1}H NMR (400 MHz, $CDCl_3$)

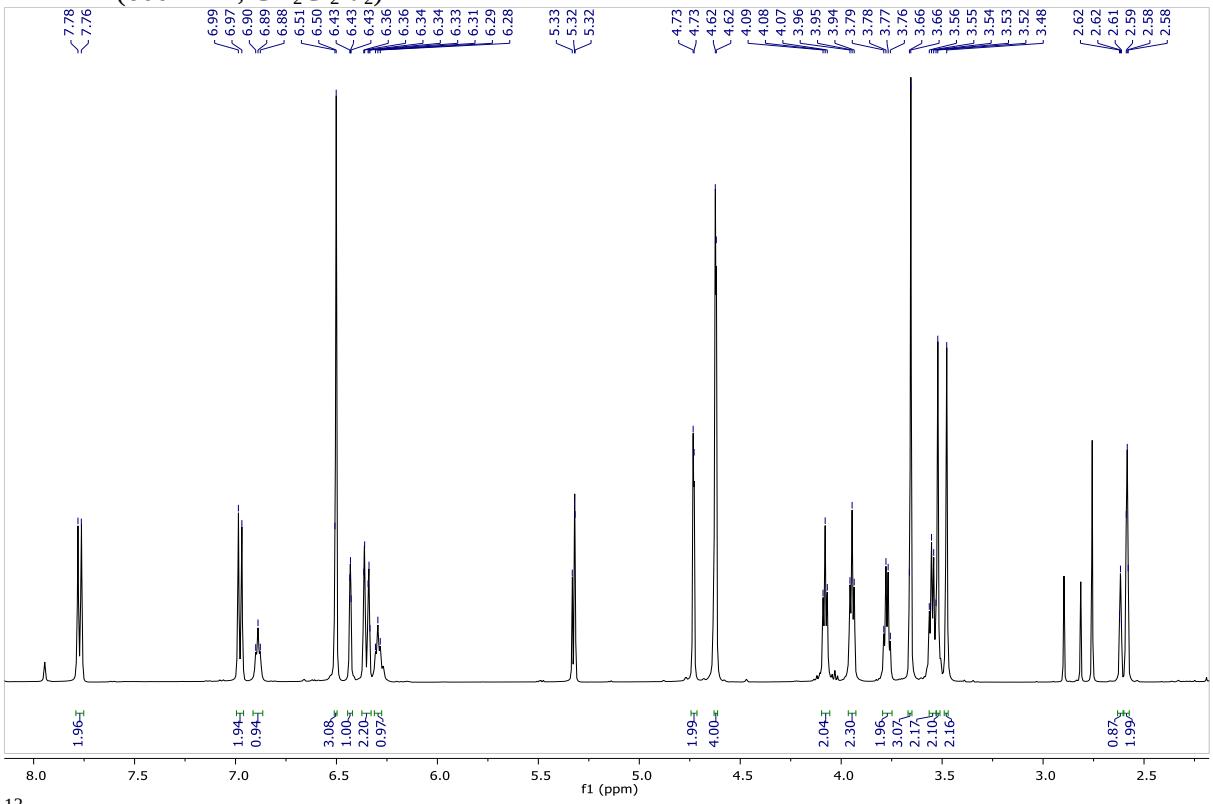


^{13}C NMR (101 MHz, $CDCl_3$)

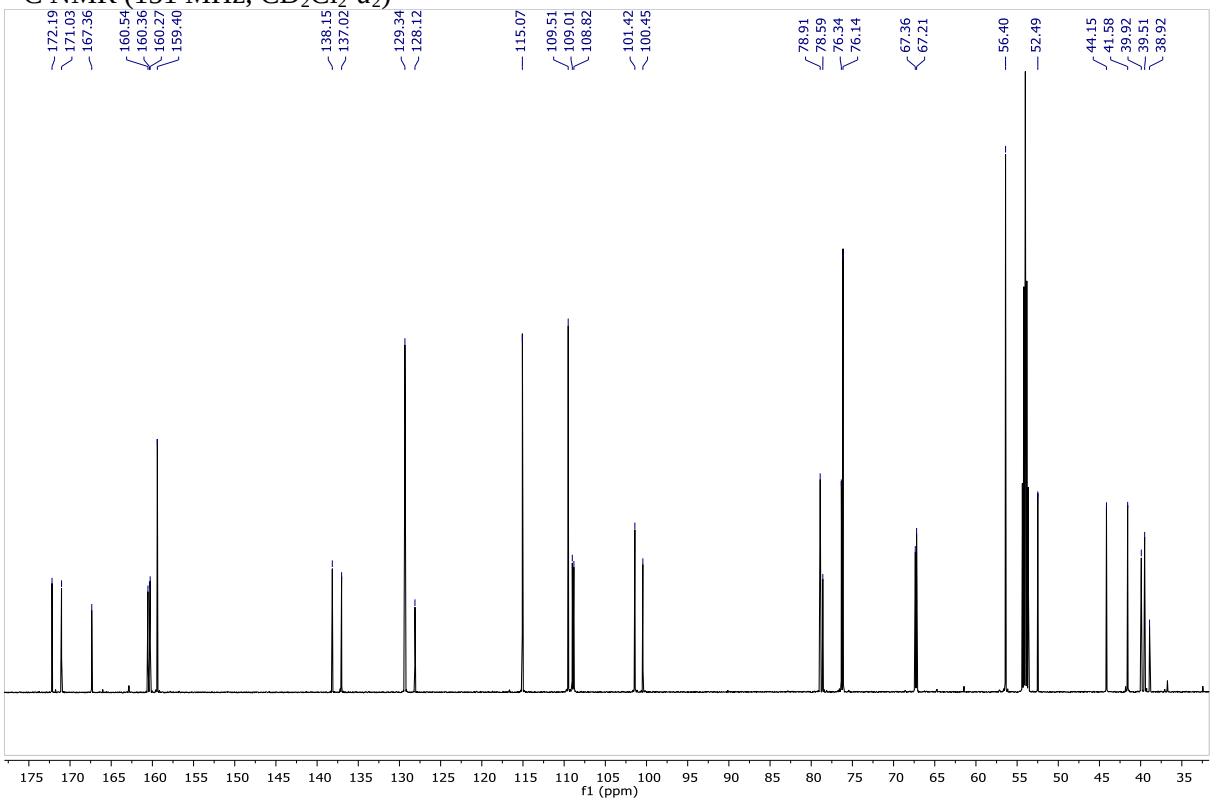


Methyl 2-(3-(2-(2-(3,5-bis(prop-2-yn-1-yloxy)phenyl)acetamido)ethoxy)-5-(2-(4-(prop-2-yn-1-yloxy)benzamido)ethoxy)phenyl)acetate (**29**)

^{1}H NMR (600 MHz, CD_2Cl_2-d_2)

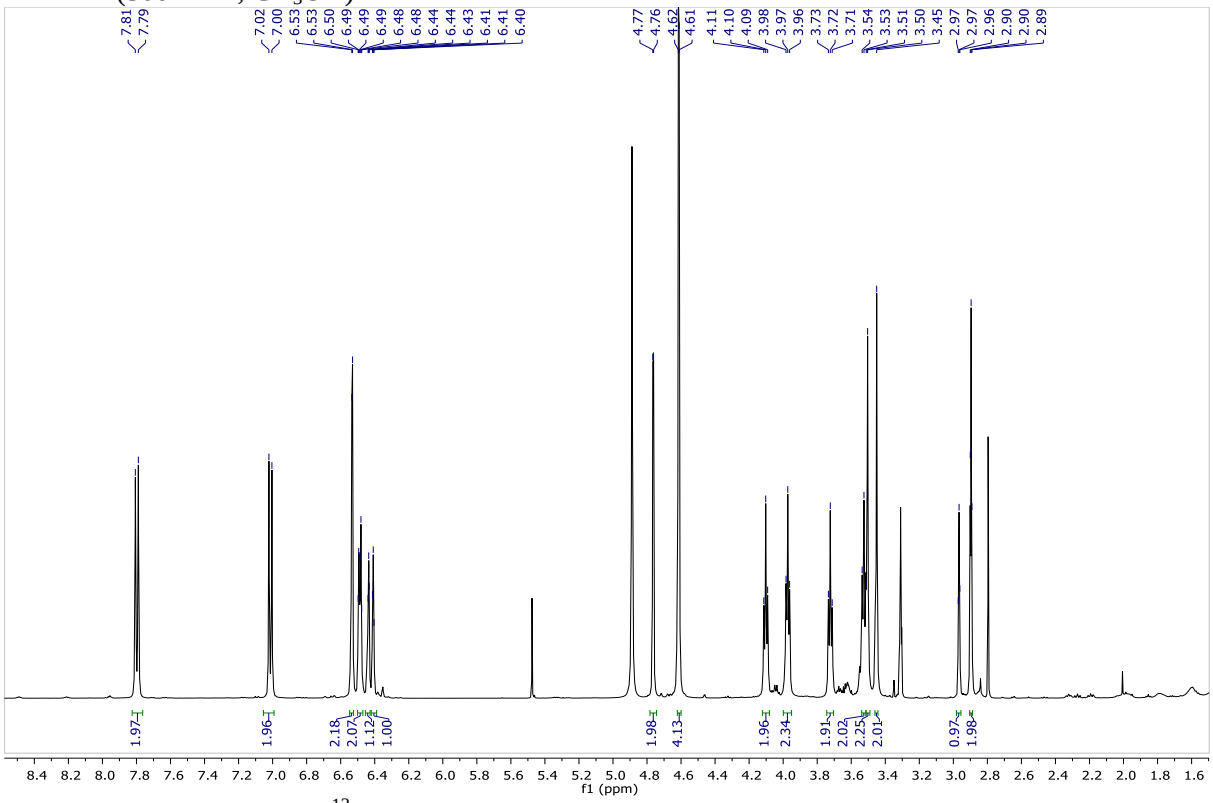


^{13}C NMR (151 MHz, CD_2Cl_2-d_2)

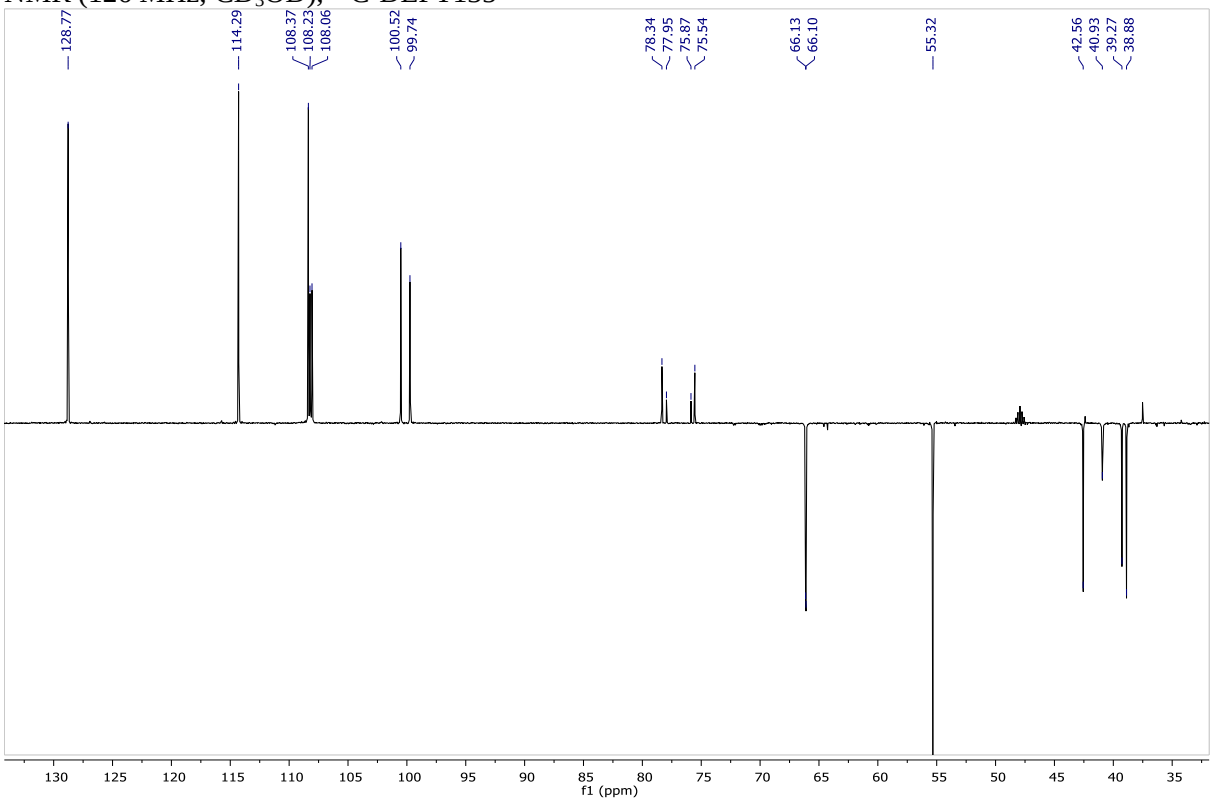


2-(3-(2-(2-(3,5-bis(Prop-2-yn-1-yloxy)phenyl)acetamido)ethoxy)-5-(2-(4-(prop-2-yn-1-yloxy)benzamido)ethoxy)phenyl)acetic acid (**13**)

^{1}H NMR (500 MHz, CD_3OD)

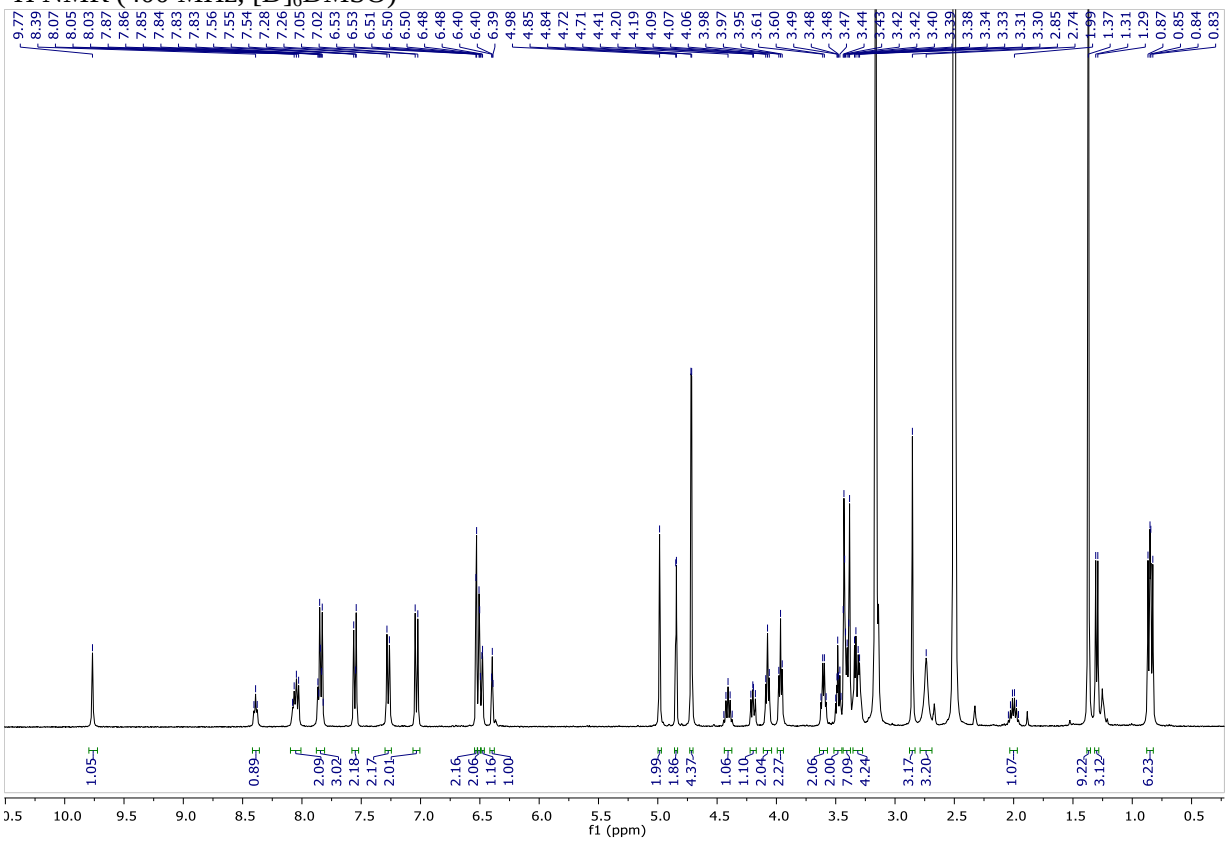


NMR (126 MHz, CD_3OD), ^{13}C-DEPT135

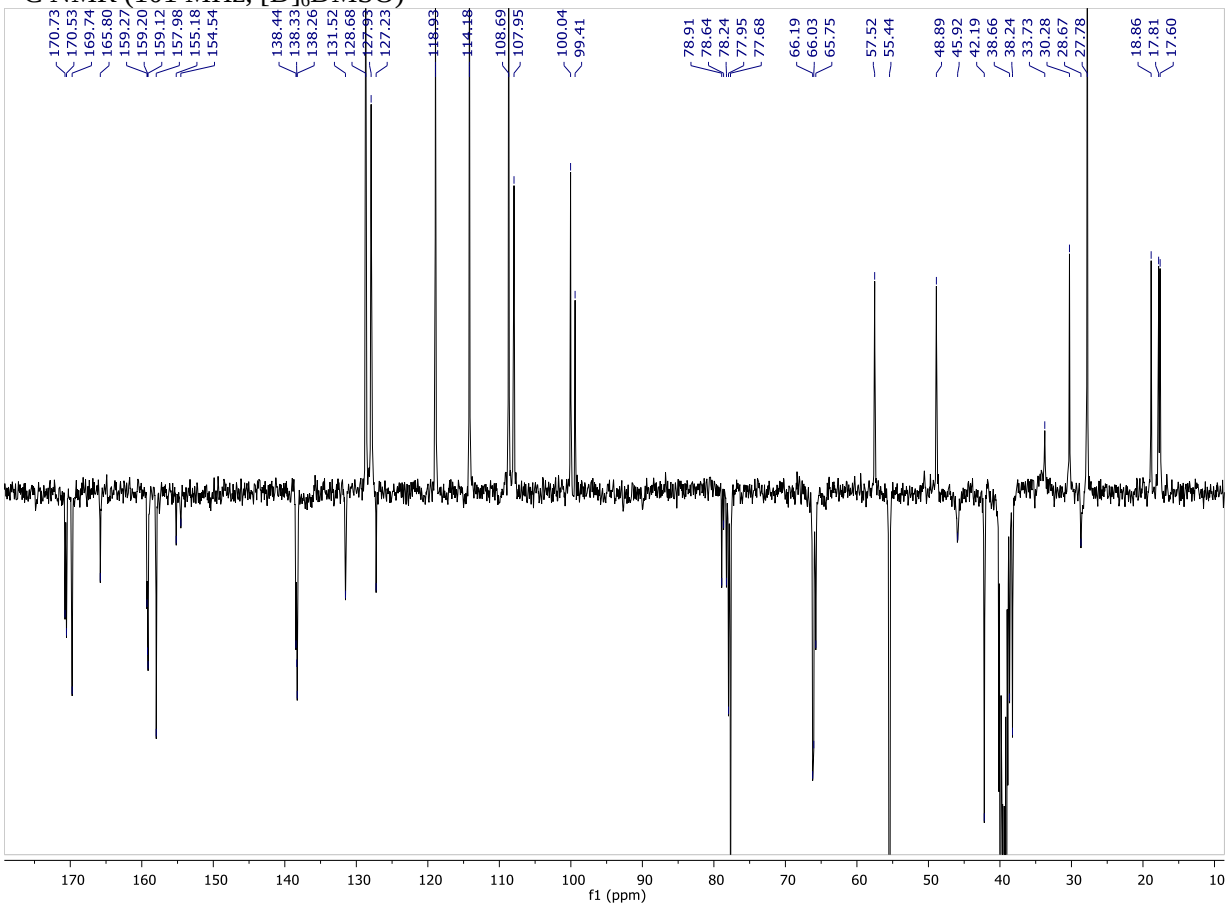


Tris-alkyne-Val-Ala-diamine-Boc (**16d**)

^{1}H NMR (400 MHz, [D]$_6$DMSO)

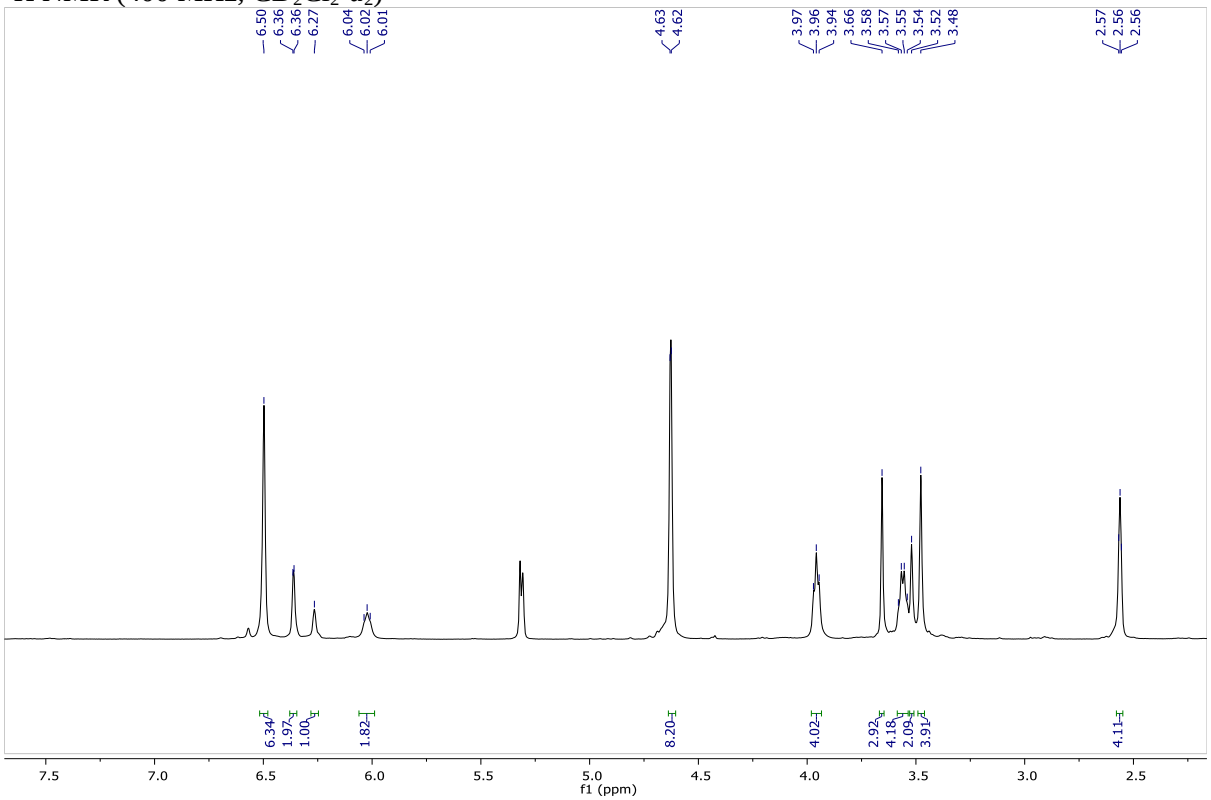


^{13}C NMR (101 MHz, [D]$_6$DMSO)

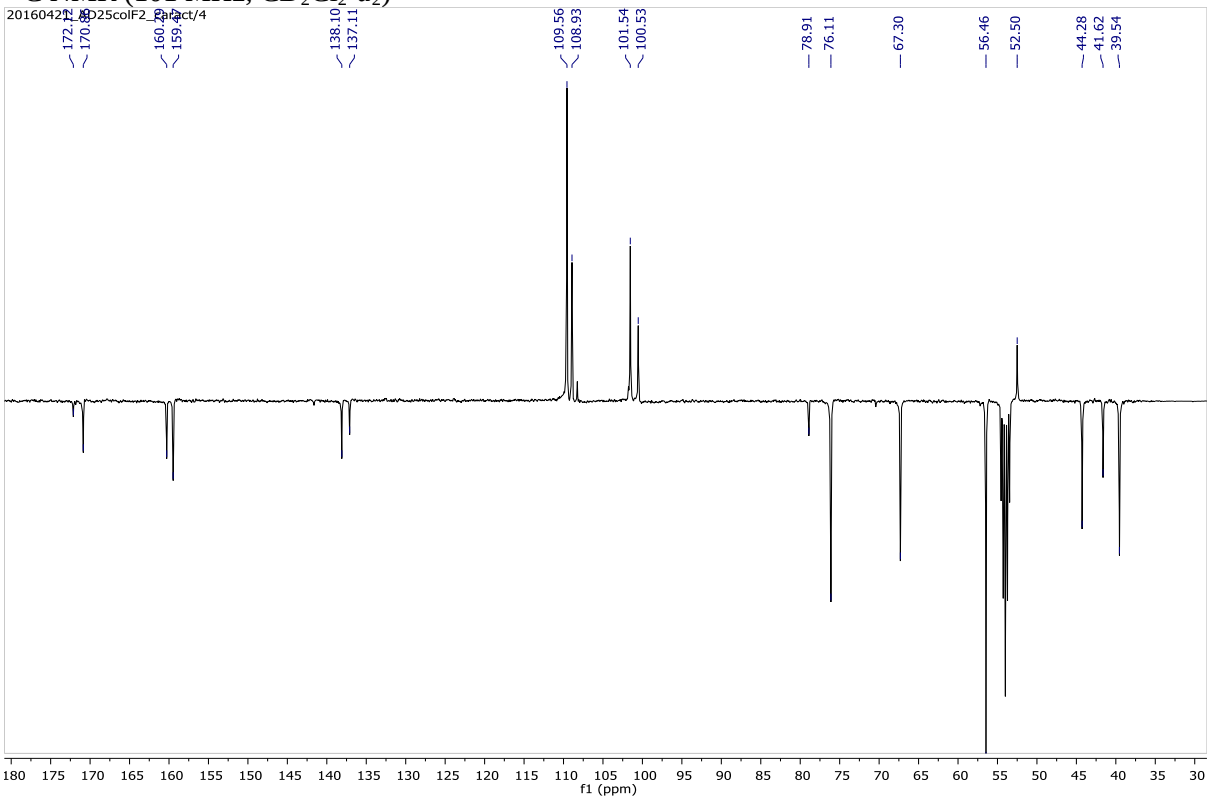


Methyl 2-(3,5-bis(2-(2-(3,5-bis(prop-2-yn-1-yloxy)phenyl)acetamido)ethoxy)phenyl)acetate (**30**)

^{1}H NMR (400 MHz, CD_2Cl_2-d_2)

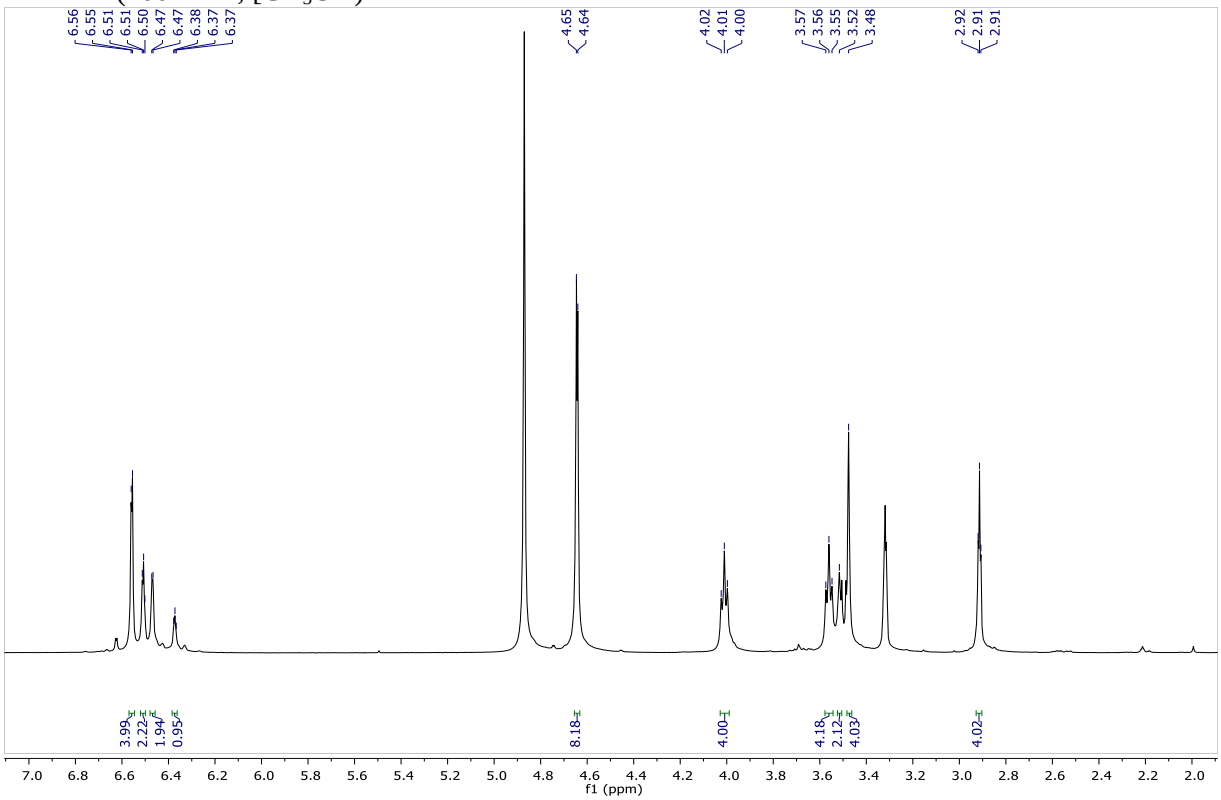


^{13}C NMR (101 MHz, CD_2Cl_2-d_2)

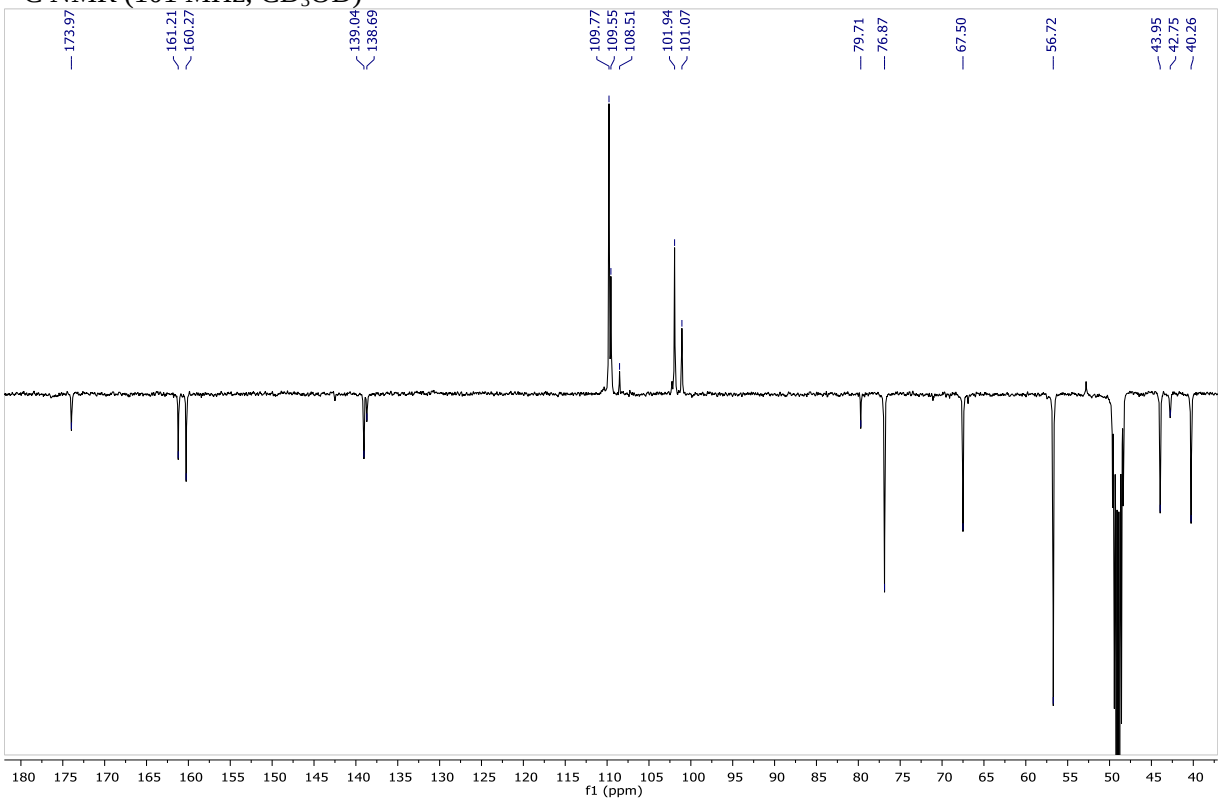


2-(3,5-bis(2-(2-(3,5-bis(Prop-2-yn-1-yloxy)phenyl)acetamido)ethoxy)phenyl)acetic acid (**31**)

^{1}H NMR (400 MHz, [CD_3OD)

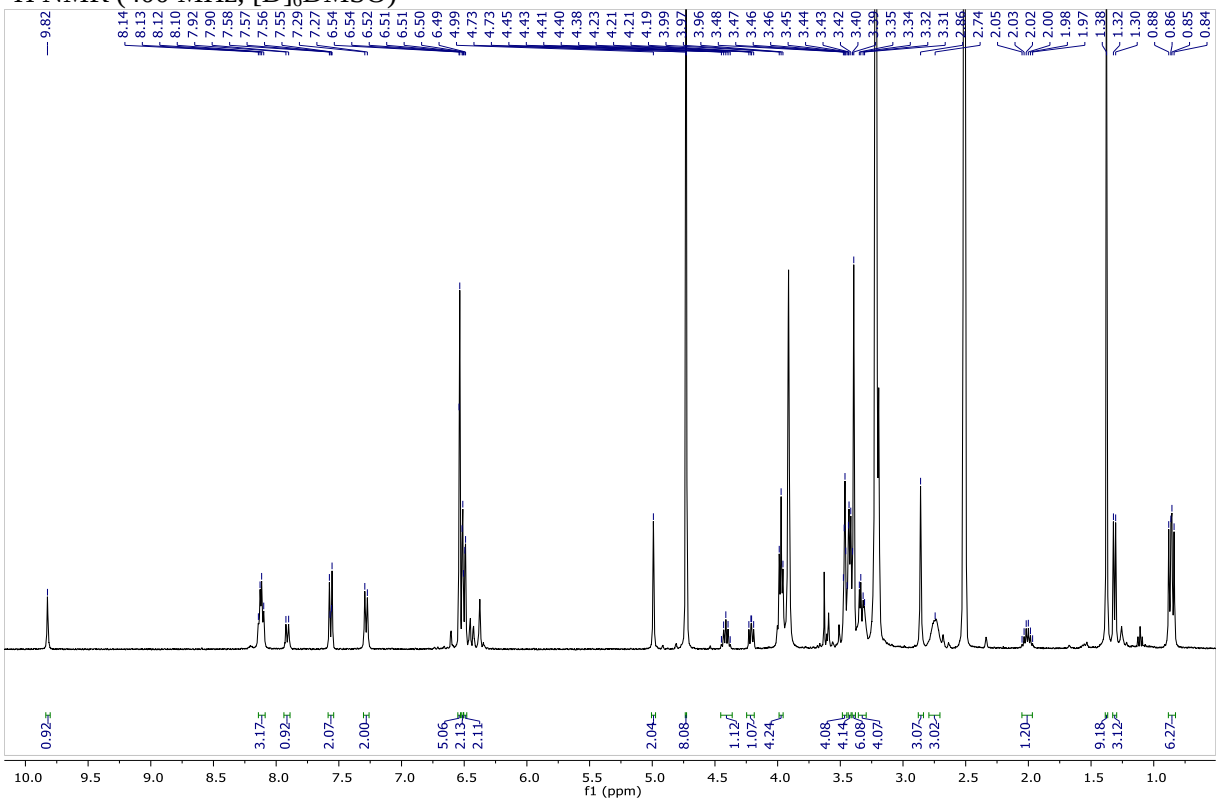


^{13}C NMR (101 MHz, CD_3OD)

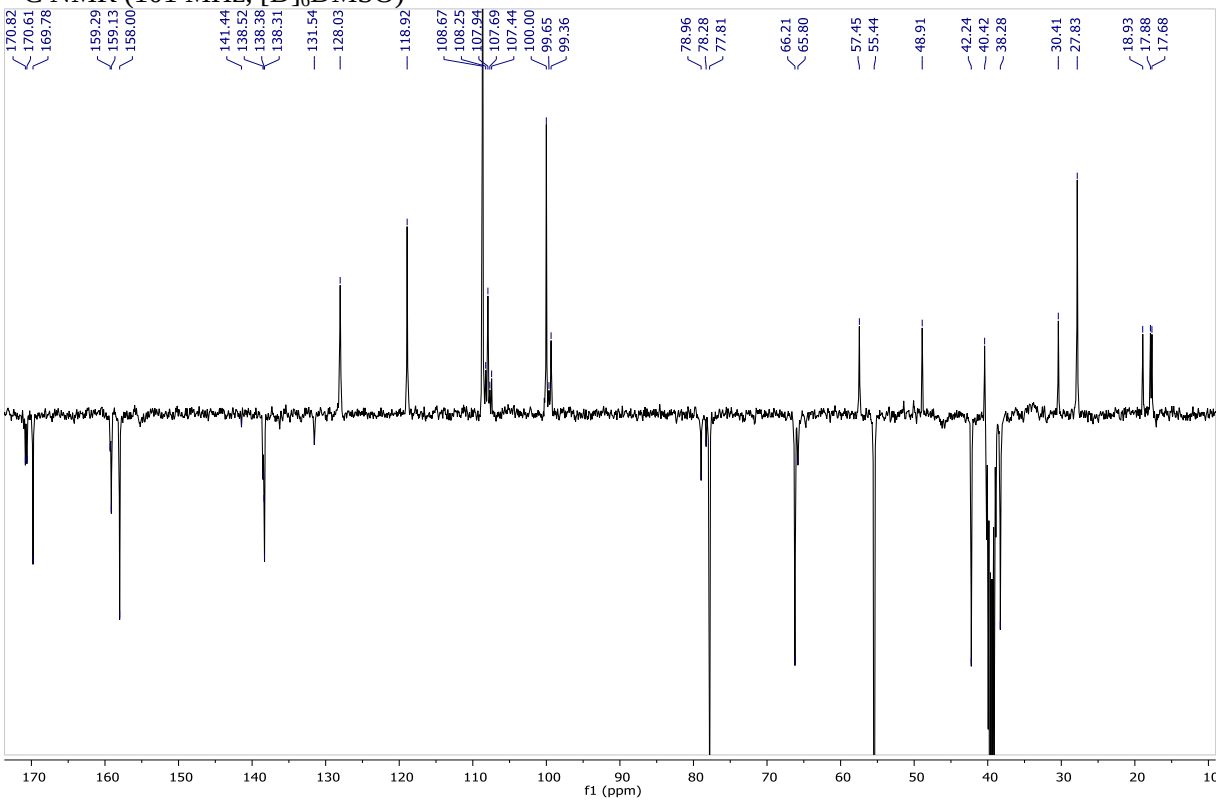


Tetra-alkyne-Val-Ala-PABA-diamine-Boc (**16e**)

^{1}H NMR (400 MHz, [D]$_6$DMSO)



^{13}C NMR (101 MHz, [D]$_6$DMSO)



[S1] W. C. Still, M. Kahn, A. Mitra, *J. Org. Chem.* **1978**, *43*, 2923-2925.

[S2] J. I. Gavrilyuk, U. Wuellner, S. Salahuddin, R. K. Goswami, S. C. Sinha, C. F. Barbas, *Bioorg. Med. Chem. Lett.* **2009**, *19*, 3716-3720.

[S3] Z.-X. Jiang, Y. Feng, Y. B. Yu, *Chem. Commun.* **2011**, *47*, 7233-7235; M. Assali, J. J. Cid, M. Pernía-Leal, M. Muñoz-Bravo, I. Fernández, R. E. Wellinger, N. Khiar, *ACS Nano* **2013**, *7*, 2145-2153.