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Supporting Information

Neutrophil Elastase Promotes Linker Cleavage and Paclitaxel Release from an Integrin-Targeted Conjugate

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Supplementary Figures

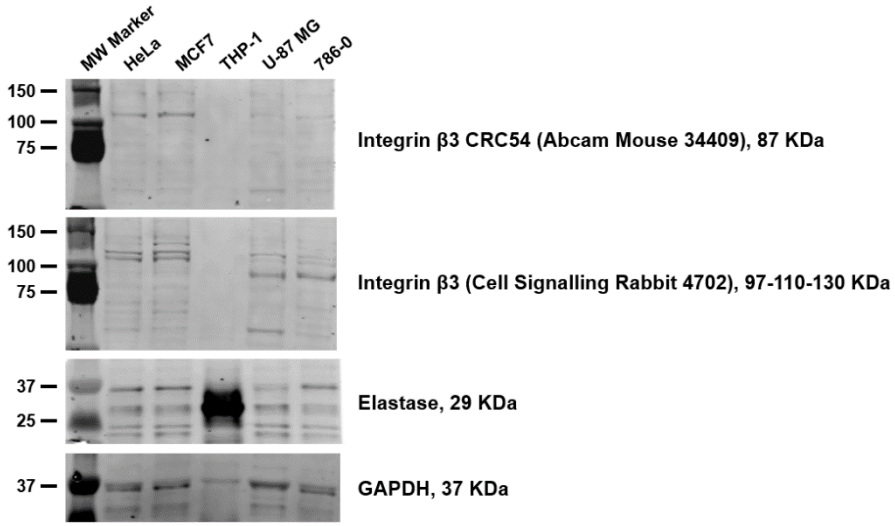


Figure S1. Western Blot analysis for the detection of β_3-integrin subunit, elastase and glyceraldehyde 3-phosphate dehydrogenase (GADPH) in HeLa, MCF7 (breast adenocarcinoma), THP-1 (acute monocytic leukemia), U87-MG (glioblastoma) and 786-O (renal cell adenocarcinoma) cells.

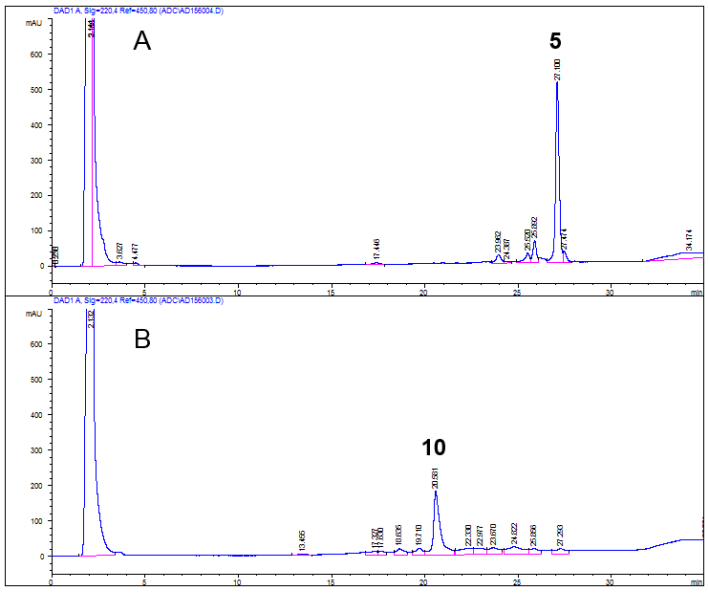
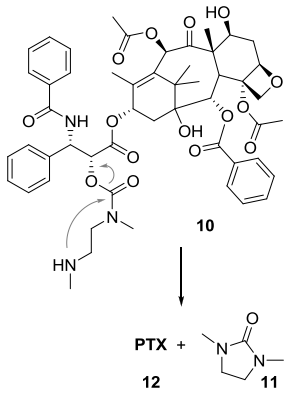
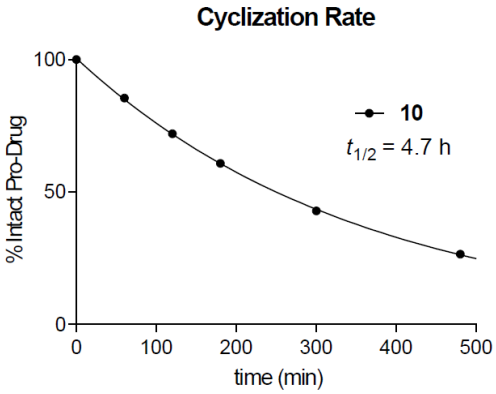


Figure S2. HPLC-MS chromatograms from cleavage experiments of RGD-PTX conjugate **5**. **A**) Analysis of **5** upon 2 h treatment with TFA-inactivated elastase; **B**) Analysis of **5** upon 2 h treatment with activated elastase. *m/z* 968.4 signal was attributed to compound **10**.

A





B

	IC_{50} [nM]
Paclitaxel prodrug (**10**)	64.2
Paclitaxel (PTX) (**12**)	5.0

Figure S3. **A)** Cyclization of the ethylenediamine self-immolative spacer in compound **10**, leading to release of cyclic urea **11** and Paclitaxel (**12**). The graph represents the decay of compound **10** in a DMSO:PBS mixture at pH 7.5 and 37 °C; half-life of 4.7 h was measured. **B)** Antiproliferative activity of Paclitaxel (**12**) and prodrug **10** in $\alpha_v\beta_3$-expressing human renal cell carcinoma 786-O cells after 110 h treatment. IC_{50} values were calculated as the concentration of compound required for 50% inhibition of cell viability.

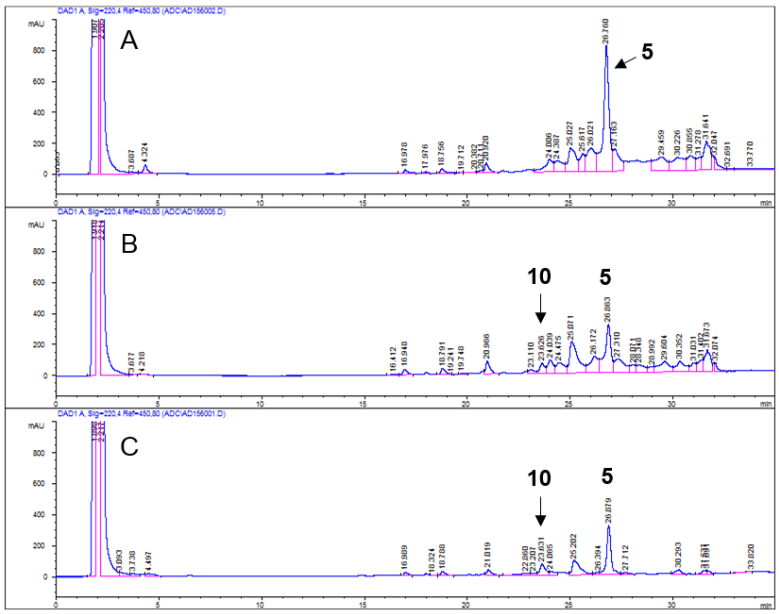


Figure S4. HPLC-MS profiles of RGD-PTX conjugate **5** upon treatment with lysosomal extract for 2 h. **A**) Analysis of **5** in the presence of inactivated lysosomal extract; **B**) lysosomal extract digestion of **5** in the presence of cysteine proteases inhibitor E-64; **C**) Analysis of **5** in the presence of lysosomal extract.

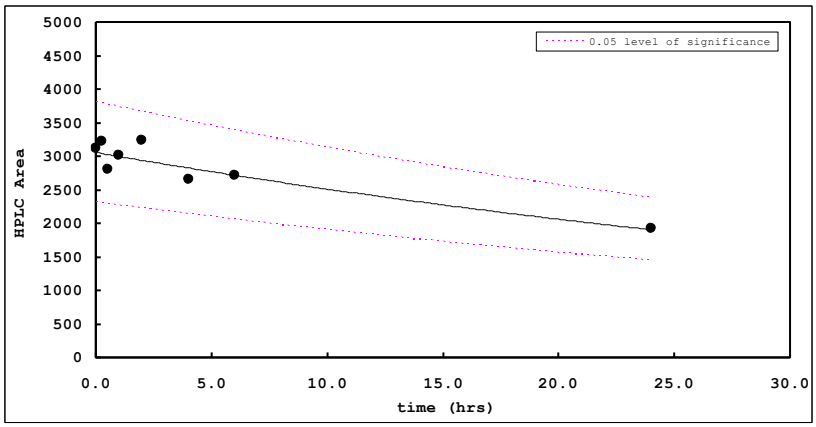


Figure S5. Plasma stability of conjugate **5**: half-life of 35.27 h was calculated.

Materials and Methods

Paclitaxel prodrug **10** was synthesized according to a procedure published by Scheeren and co-workers.[S1] 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[S2] 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; $[D]_6DMSO$, 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; $[D]_6DMSO$, 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: Waters Atlantis 21 mm × 10 cm column; flow 15 mL/min unless stated otherwise). The crude reaction mixture was dissolved in H_2O or, if the compound was insoluble in water,

[S1] F. M. H. de Groot, W. J. Loos, R. Koekkoek, L. W. A. van Berkom, G. F. Busscher, A. E. Seelen, C. Albrecht, P. de Bruijn, H. W. Scheeren, *J. Org. Chem.* **2001**, *66*, 8815-8830.
[S2] W. C. Still, M. Kahn, A. Mitra, *J. Org. Chem.* **1978**, *43*, 2923-2925.

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: Waters Atlantis 21 mm × 10 cm column). 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.

Protocols for Biological Evaluations

Solid Phase Receptor Binding Assays

Recombinant 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 in the dark, 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 Prism GraphPad Prism software. Each experiment was repeated in duplicate.

Confocal Microscopy Analysis

786-O renal cell adenocarcinoma cells (NCI) were cultured in RPMI 1640 medium in the presence of 1% HEPES and 10% Fetal Calf Serum (FCS). The cells were then harvested at 50-70% confluence by trypsinization (trypsin/EDTA solution, Sigma Aldrich T4049) for 5 min at 37 °C. Cell count was performed with Vi-CELL (Beckman Coulter) and the viability was assessed. Cells were seeded in cover glass chambers (200,000 cells/chamber in 1 ml, Sarstedt 94.6190.402) and incubated overnight at 37 °C, 5% CO_2. The cell medium was replaced with fresh medium containing the tested compounds (1 μM concentration) and the

cells were incubated for 2 h. Confocal microscopy analysis of live cells was performed using confocal laser scanning microscope SP2, Leica equipped with a 633 objective (oil immersion).

Enzymatic Cleavage Assays

100 µM solution of conjugate **5** was digested in PBS/10% MeCN at 37 °C for 2 h with 25 mUnits of Elastase from human leucocytes (ELANE, E-8140, Sigma-Aldrich). Samples were analyzed by ESI-LC/MS on a PLRP-S column (Agilent Technologies; 2.1 × 150 mm, 8 µm, 1000 Å) with an Agilent 1100 HPLC system equipped with a diode array detector with an electrospray ion source. Mobile phase A was composed of 0.05% TFA in water, and mobile phase B was 0.05% TFA in MeCN. Samples (45 µM) were eluted at 0.25 ml/min by using a gradient from 20 to 50% B in 30 min, raised to 80% B and held at 80% B for 5 min; the UV signal was recorded at 220 and 280 nm, and MS detection was set in full-scan mode from 300-2000 amu. Data are shown in Figure S2.

0.2 mM solutions of conjugate **5** were digested in 200 mM sodium acetate (pH 5.5)/1 mM EDTA containing 1 mM cysteine at 37 °C for 2 h with 0.5 mg/ml of lysosomal enriched extract (prepared from rat liver) in the presence or absence of 20 µM E-64 protease inhibitors (Enzo Life Sciences). Samples were analyzed by ESI-LC/MS on a PLRP-S column (Agilent Technologies; 2.1 × 150 mm, 8 µm, 1000 Å) with an Agilent 1100 HPLC system equipped with a diode array detector with an electrospray ion source. Mobile phase A was composed of 0.05% TFA in water, and mobile phase B was 0.05% TFA in MeCN. Samples (45 µM) were eluted at 0.25 ml/min by using a gradient from 20 to 50% B in 30 min, raised to 80% B and held at 80% B for 5 min; the UV signal was recorded at 220 and 280 nm, and MS detection was set in full-scan mode from 300-2000 amu. Data are shown in Figure S4.

Kinetic Analysis of PTX Release

200 µl of a 2 mM solution of compound **10** were diluted with 100 µl DMSO, 200 µl H_2O and 200 µl of a 0.1 M phosphate buffer solution pH = 7.5 (final concentration **10** = 0.6 mM; buffer: 28.6 mM). The mixture was stirred at 37 °C and aliquots (110 µl) were taken at different time points (0, 1, 2, 3, 5, 8 h), diluted with a 30% MeCN mixture in H_2O + 0,2% TFA (110 µl) and analyzed by HPLC (2 Waters 515 HPLC pumps equipped with 996 photodiode array detector and Waters Atlantis® T3 - 5 µm - 4.6 × 100 mm column; eluent A: H_2O + 0,1% TFA, eluent B: MeCN + 0,1% TFA; gradient from 20% B to 80% B in 20 min; flow rate: 1 ml/min). LC spectrum was registered at 254 nm and the integrated peak of 10 was compared to the peak of free PTX. Data were plotted and the exponential decay of **10** was fitted using GraphPad Prism software (Figure S3).

Plasma Stability Assays

A 1 mM solution of conjugate **5** in DMSO was diluted to 5 µM with blank mouse plasma and incubated at 37 °C under mixing for 24 h. Aliquots of 20 µl were double collected at the time points 0, 0.25, 0.50, 1, 2, 4, 6 and 24 h and frozen up to analysis. Sample plasma proteins were precipitated by adding 180 ml of MeCN/MeOH (9:1) to the unfrozen aliquots. After mixing for 15 minutes, aliquots were centrifuged for 5

minutes at 12000 rpm; the supernatants were collected and transferred into 96-well plate; the latter was further centrifuged for 5 minutes at 4000 rpm. The samples were analyzed against standard appropriately diluted for calibration line in MeCN /MeOH (9:1). All samples were analyzed by LC-MS/MS method on a UPLC-MS Acquity TQD System Waters equipped with an electrospray ion source, with a BEH C18 column; 2.1 x 50 mm, 1.7 µm Acquity Waters, mobile phase A was composed of 5% MeCN in 5 mM Ammonium Formate pH 3.5 and mobile phase B was 5% 5 mM Ammonium Formate pH 3.5 in MeCN. Data were analyzed with Mass Lynx 4.1 SCN919 software. Half-life degradation ($t_{1/2}$) was evaluated with a pseudo first order product decay and the calculated value was 35.27 hours (Figure S5).

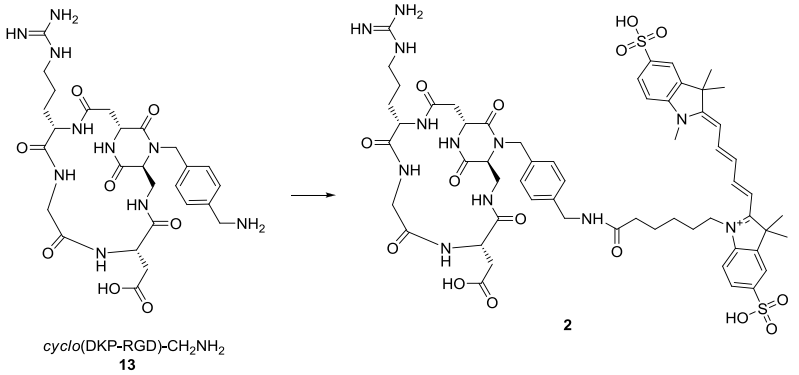
Cell Antiproliferative Assays

786-O renal cell adenocarcinoma cells (NCI) were cultured in RPMI 1640 medium supplemented with 10% fetal calf serum (FCS) at 37 °C and 5% CO_2. Cells were seeded at a density of 2000 cells/well (200 µl/well) in 96-well plates and, 24 hours after seeding. Cells were treated in duplicate with serial dilutions of SMDC products **5-7** in the range 5.0 µM - 9.8 nM and with serial dilutions of PTX in the range 0.1 µM – 0.2 nM, in the presence or absence of Elastase from human leucocytes (ELANE, 324681, Millipore). In the case of presence of elastase, the latter was added immediately after compound treatment at a final concentration of 50 nM in half of replicates, then the plates were incubated for 96 hours at 37 °C.

Cell viability was assessed with the CellTiter-Glo luciferase-based ATP detection assay (Promega) and Envision (PerkinElmer) microplate reader. Growth inhibitory activity was evaluated at the end of incubation by using GraphPad Prism software. Experimental data were normalized versus untreated control samples and interpolated by linear regression analysis with GraphPad Prism software to generate dose-response curves.

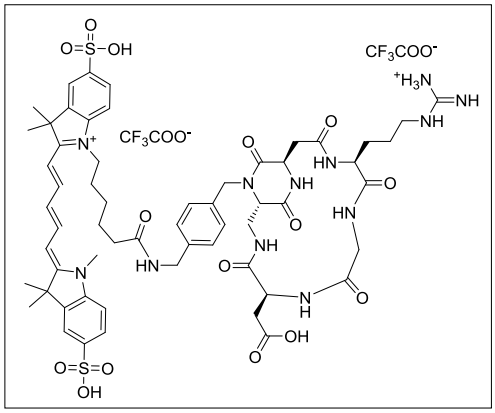
Synthetic Procedures

Synthesis of *cyclo*(DKP-RGD)-sCy5 (2).



Scheme S1: Synthesis of *cyclo*(DKP-RGD)-sCy5 **2.** REAGENTS AND CONDITIONS: SulfoCy5-NHS, *i*Pr_2NEt, DMF, r.t., 4 h, Y: 40%.

cyclo(DKP-RGD)-sCy5 (**2**)



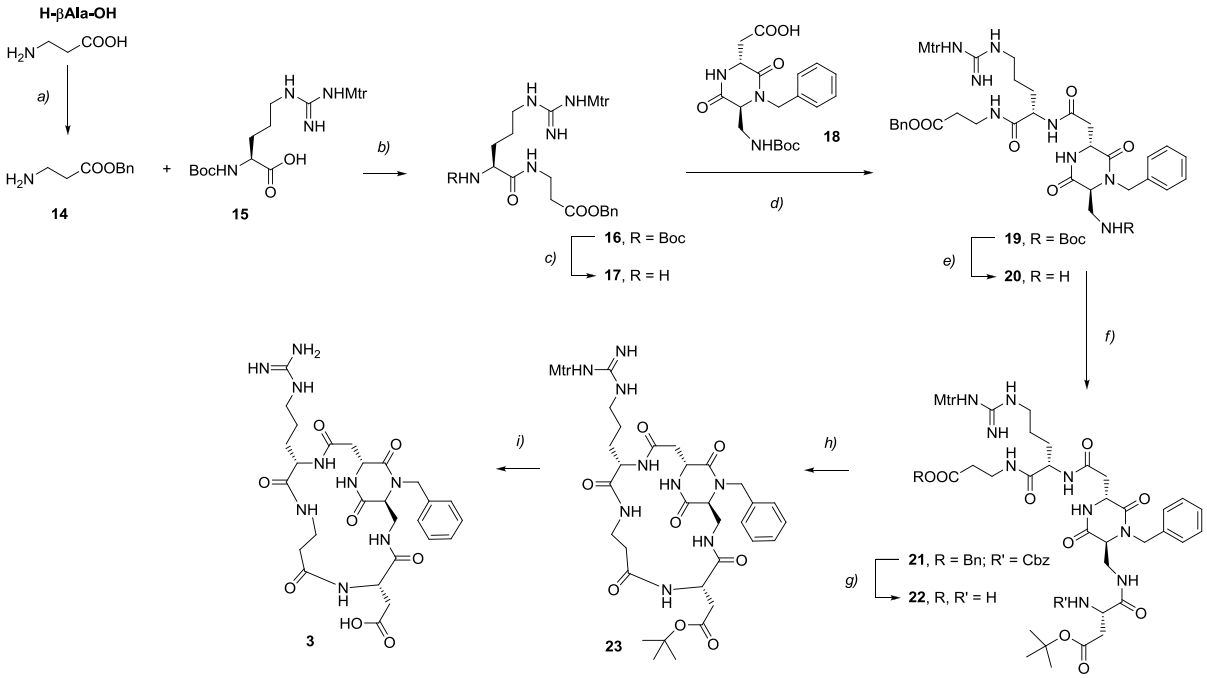
$C_{59}H_{76}N_{12}O_{15}S_2^{2+}$
MW: 1257,4449 + TFA

To a solution of *cyclo*(DKP-RGD)-CH_2NH_2 **13**[S3] (2.4 mg, 0.0028 mmol, 1 eq) in DMF (400 µl), *i*Pr_2NEt (2 µl, 0.0112 mmol, 4 eq) was added adjusting the pH at 8-9; then SulfoCy5 (3 mg, 0.0039 mmol, 1.5 eq) was added and the reaction mixture was stirred for 4 h at room temperature in the darkness. Then the mixture was directly purified by semipreparative-HPLC [gradient: 90% (H_2O + 0.1% CF_3COOH) / 10% (MeCN + 0.1% CF_3COOH) to 40% (H_2O + 0.1% CF_3COOH) / 60% (MeCN + 0.1% CF_3COOH) in 9 min; t_R (product): 7 min]. The purified product was then freeze-dried to give compound **2** as a blue solid (1.5 mg, 40% yield).

HRMS (ESI+): *m/z* calcd for $[C_{59}H_{75}N_{12}O_{15}S_2]^+$: 1255.4911 $[M]^+$; found: 1255.4932 - *m/z* calcd for $[C_{59}H_{75}N_{12}O_{15}S_2Na]^{2+}$: 639.2404 $[M+Na]^{2+}$; found: 639.2391.

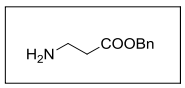
[S3] R. Colombo, M. Mingozzi, L. Belvisi, D. Arosio, U. Piarulli, N. Carenini, P. Perego, N. Zaffaroni, M. De Cesare, V. Castiglioni, E. Scanziani, C. Gennari, *J. Med. Chem.* **2012**, *55*, 10460-10474.

Synthesis of *cyclo*(DKP-RβAD) (3).



Scheme S2: Synthesis of *cyclo*(DKP-RβAD) **3**; REAGENTS AND CONDITIONS: *a)* benzyl alcohol, trimethylsilyl chloride, 100 °C, 4 h, Y.: 30%; *b)* HATU, HOAt, *i*Pr_2NEt, DMF, r.t., 16 h, Y.: 67%; *c)* TFA/ CH_2Cl_2 (1:2), r.t., 2.5 h, Y.: quantitative; *d)* HATU, HOAt, *i*Pr_2NEt, DMF, r.t., 16 h, Y.: 56%; *e)* TFA/ CH_2Cl_2 (1:2), r.t., 2 h, Y.: quantitative; *f)* Cbz-Asp(OtBu)-OH, HATU, HOAt, *i*Pr_2NEt, DMF, r.t., 16 h, Y.: 69%; *g)* H_2, 10% Pd/C, THF/H_2O (1:1), r.t., 16 h, Y.: 91%; *h)* HATU, HOAt, *i*Pr_2NEt, DMF, r.t., 16 h, Y.: 50%; *i)* TFA /thioanisol/EDT/anisole (90:5:3:2), 3.5 h, r.t., Y.: 45%.

H-βAla-OBn (**14**)

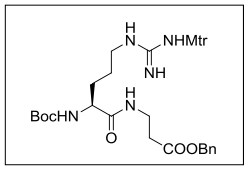


$C_{10}H_{13}NO_2$
MW: 179.22

To a solution of H-βAla-OH (200 mg, 2.25 mmol, 1 eq) in benzyl alcohol (1.2 ml, 11.2 mmol, 5 eq) under N_2 atmosphere, chlorotrimethylsilane (0.46 ml, 3.59 mmol, 1.6 eq) was added and the reaction was stirred at 100 °C for 4 h. The reaction mixture was cooled to room temperature and washed 3 times with cold Et_2O, and centrifuged. The resulting precipitate was isolated and dried under vacuum, affording the benzyl protected β-alanine (120 mg, 30% yield).

^{1}H NMR (400 MHz, MeOD) δ 7.38 (m, 1H), 5.20 (s, 1H), 3.22 (t, J = 6.5 Hz, 1H), 2.79 (t, J = 6.5 Hz, 1H).

Boc-Arg(Mtr)-βAla-OBn (**16**)

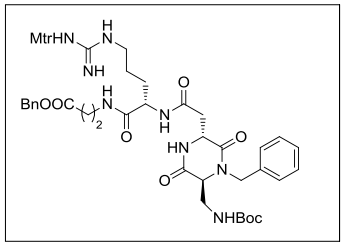


$C_{31}H_{44}N_5O_8S$
MW: 647.78

To a solution of Boc-Arg(Mtr)-OH (**15**, 297 mg, 0.61 mmol, 1 eq) in dry DMF (10 ml) at 0 °C under N_2 atmosphere, HATU (232 mg, 0.61 mmol, 1 eq), HOAt (83 mg, 0.61 mmol, 1 eq) and *i*Pr_2NEt (0.3 ml, 3.054 mmol, 3 eq) were added. After stirring the mixture for 1 h at 0 °C, a solution of **14** in dry DMF (4 ml) was added followed by *i*Pr_2NEt (0.2 ml, 3.05 mmol, 2 eq). The reaction mixture was then warmed to room temperature and stirred overnight. The mixture was diluted with AcOEt (50 ml) and consecutively washed with 1 M aqueous solution of $KHSO_4$ (4 × 30 ml), a saturated aqueous solution of $NaHCO_3$ (4 × 30 ml) and brine (1 × 75 ml), dried over Na_2SO_4 and the solvent removed under reduced pressure to afford the crude product. The residue was purified with flash chromatography on silica gel (AcOEt: Hex 8:2, solid load) to afford the desired product **16** (290 mg, 67% yield).

R_f = 0.32 (AcOEt: Hex 9:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33 (m, 5H), 6.53 (s, 1H), 5.10 (s, 2H), 4.12 (d, J = 7.1 Hz, 1H), 3.82 (s, 3H), 3.54 (m, 2H), 3.26 (m, 2H), 2.68 (s, 3H), 2.61 (s, 3H), 2.12 (s, 3H), 1.93 - 1.67 (m, 4H), 1.41 ppm (s, 9H). MS (ESI+): *m/z* calcd for $[C_{31}H_{43}N_5O_8S]^-$: 645.28 $[M\text{-}H]^-$; found: 646.12; *m/z* calcd for $[C_{31}H_{44}N_5O_8SNa]^+$: 670.28 $[M+Na]^+$; found: 670.65.

Boc-DKP-Arg(Mtr)-βAla-OBn (**19**)



$C_{45}H_{60}N_8O_{11}S$
MW: 921.07

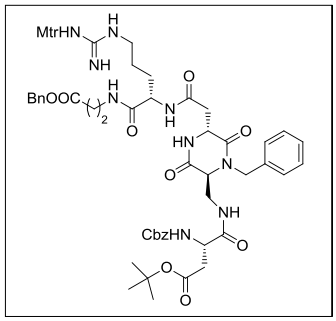
To a solution of deprotected DKP **18**[S4] (64 mg, 0.16 mmol, 1 eq) in dry DMF (1.2 ml) at 0 °C under N_2 atmosphere, HATU (73 mg, 0.19 mmol, 1.2 eq), HOAt (26 mg, 0.19 mmol, 1.2 eq) and *i*Pr_2NEt (0.11 ml, 0.64 mmol, 4 eq) were added. After stirring the mixture for 30 min at 0 °C, the trifluoroacetate salt of deprotected dipeptide Boc-Arg(Mtr)-βAla-OBn **17** (140 mg, 0.18 mmol, 1.5 eq) in dry DMF (0.6 ml) was added. The reaction mixture was stirred at 0 °C for 1 h and room temperature overnight. The solvent was removed and the mixture was diluted with AcOEt (50 ml) and consecutively washed with 1 M aqueous solution of $KHSO_4$ (3 × 25 ml), a saturated aqueous solution of $NaHCO_3$ (3 × 25 ml) and brine (1 × 30 ml), dried over Na_2SO_4 and the solvent removed under reduced pressure to afford the crude product. The residue

[S4] M. Marchini, M. Mingozzi, R. Colombo, I. Guzzetti, L. Belvisi, F. Vasile, D. Potenza, U. Piarulli, D. Arosio, C. Gennari *Chem. Eur. J.* **2012**, *18*, 6195-6207.

was purified with flash chromatography on silica gel (CH_2Cl_2/MeOH 96:4, solid load) to afford the desired product **19** as a yellow foam (83 mg, 56% yield).

R_f=0.45 (CH_2Cl_2: MeOH 9:1); ^{1}H NMR (400 MHz, MeOD) δ 7.44 – 7.13 (m, 10H), 6.63 (s, 1H), 5.38 (d, J = 15.4 Hz, 1H), 5.08 (d, J = 9.0 Hz, 2H), 4.53 (t, J = 5.4 Hz, 1H), 4.29 (dd, J = 8.4, 5.0 Hz, 1H), 4.04 (d, J = 15.5 Hz, 1H), 3.92 – 3.70 (m, 5H), 3.50 – 3.33 (m, 3H), 3.22 – 3.03 (m, 2H), 2.86 (ddd, J = 21.8, 15.6, 5.5 Hz, 2H), 2.67 (s, 3H), 2.60 (s, 3H), 2.54 (t, J = 6.8 Hz, 2H), 2.09 (s, 3H), 1.76 (m, 1H), 1.67 – 1.47 (m, 3H), 1.41 ppm (s, 9H); ^{13}C NMR (101 MHz, MeOD) δ 174.2, 173.0, 172.0, 168.5, 159.9, 158.1, 139.5, 137.9, 137.4, 137.1, 129.9, 129.5, 129.2, 128.9, 128.8, 125.7, 112.8, 80.7, 67.4, 61.4, 56.0, 54.3, 52.5, 41.5, 38.7, 36.4, 34.7, 30.1, 28.8, 24.4, 18.8, 12.1 ppm. MS (ESI-): *m/z* calcd for $[C_{45}H_{59}N_8O_{11}S]^-$: 919.40 [*M-H*]$^-$; found: 919.52.

Cbz-Asp(OtBu)-DKP-Arg(Mtr)-βAla-OBn (**21**)

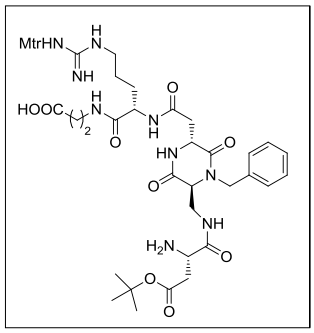


$C_{56}H_{71}N_9O_{14}S$
MW: 1126.28

To a solution of Cbz-L-Asp(O*t*Bu)-OH (48 mg, 0.149 mmol, 1.5 eq) in dry DMF (1.6 ml) at 0 °C under N_2 atmosphere, HATU (60 mg, 0.158 mmol, 1.6 eq), HOAt (21.5 mg, 0.158 mmol, 1.6 eq) and *i*Pr_2NEt (0.07 ml, 0.396 mmol, 4 eq) were added. After stirring the mixture for 30 min at 0°C, the trifluoroacetate salt of DKP-Arg(Mtr)-βAla-OBn **20** (92.56 mg, 0.099 mmol, 1 eq) in dry DMF (0.8 ml) was added. The reaction mixture was stirred at 0 °C for 1 h and then room temperature overnight. The mixture was diluted with AcOEt (50 ml) and consecutively washed with 1 M aqueous solution of $KHSO_4$ (3 × 30 ml), a saturated aqueous solution of $NaHCO_3$ (2 × 25 ml) and brine (1 × 30 ml), dried over Na_2SO_4 and the solvent evaporated under reduced pressure to afford the crude product. The residue was purified with flash chromatography on silica gel (CH_2Cl_2/MeOH 95:5, solid load) to afford the desired product **21** as yellow foam (77 mg, 69% yield).

R_f= 0.43 (CH_2Cl_2: MeOH 95:5); ^{1}H NMR (400 MHz, MeOD) δ 7.43 – 7.14 (m, 15H), 6.61 (s, 1H), 5.33 (d, J = 15.4 Hz, 1H), 5.20 – 4.92 (m, 4H), 4.63 (m, 1H), 4.50 (m, 1H), 4.30 (m, 1H), 4.11 (d, J = 15.5 Hz, 1H), 3.85 – 3.78 (m, 5H), 3.63 (m, 1H), 3.31 (s, 2H), 3.20 - 3.10 (m, 2H), 2.96 – 2.71 (m, 3H), 2.66 (s, 3H), 2.58 (s, 3H), 2.56 - 2.50 (m, 3H), 2.07 (s, 3H), 1.78 (m, 1H), 1.64 – 1.46 (m, 3H), 1.37 ppm (s, 9H); ^{13}C NMR (101 MHz, MeOD) δ 174.1, 174.0, 172.9, 171.9, 171.3, 168.8, 168.5, 159.7, 158.2, 158.0, 139.4, 137.8, 137.7, 137.3, 137.0, 134.7, 129.8, 129.4, 129.4, 129.1, 129.1, 129.0, 128.9, 128.8, 128.7, 125.6, 112.7, 82.3, 67.9, 67.3, 60.7, 55.9, 54.1, 53.2, 52.2, 40.4, 38.3, 38.2, 36.3, 34.6, 30.0, 28.2, 24.3, 18.8, 12.1 ppm. MS (ESI-): *m/z* calcd for $[C_{56}H_{70}N_9O_{14}S]^-$: 1125.27 [*M-H*]$^-$; found: 1125.54.

H-Asp(OtBu)-DKP-Arg(Mtr)-βAla-OH (**22**)

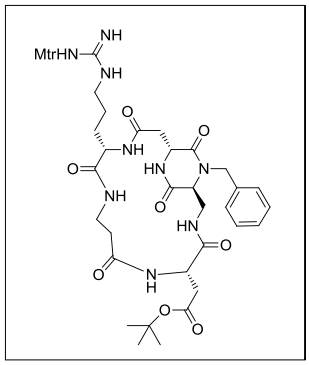


$C_{41}H_{59}N_9O_{12}S$
MW: 902.03

A solution of Cbz-Asp(O*t*Bu)-DKP-Arg(Mtr)-βAla-OBn **21** (77 mg, 0.068 mmol, 1 eq) in THF/H_2O 1:1 (6.8 ml) was treated with 10% Pd/C (1.45 mg, 0.014 mmol, 0.2 eq) and the flask was purged three times with vacuum/H_2. The mixture was stirred at room temperature overnight under H_2 atmosphere, then filtered through a pad of Celite and then was washed thoroughly with MeOH. The solvents were removed under vacuum to give the crude product **22** as white solid (56 mg, 91% yield), which was used without further purification.

R_f= 0.05 (9:1CH_2Cl_2/MeOH).

cyclo[DKP-Arg(Mtr)-βAla-Asp(OtBu)] (**23**)



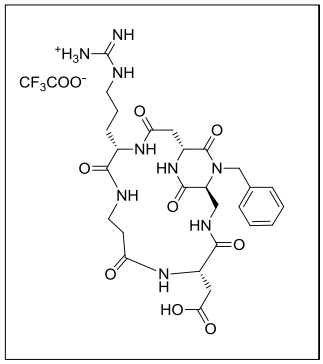
$C_{41}H_{57}N_9O_{11}S$
MW: 884.01

To a solution of H-Asp(O*t*Bu)-DKP-Arg(Mtr)-βAla-OH **22** (56 mg, 0.062 mmol, 1 eq) in dry DMF (44 ml, 0.0014 M), under nitrogen atmosphere and at 0 °C, HATU (94.3 mg, 0.248 mmol, 4 eq), HOAt (33.8 mg, 0.248 mmol, 4 eq) and *i*Pr_2NEt (0.065 ml, 0.372 mmol, 6 eq) were added. The reaction was warmed up to room temperature and stirred overnight. DMF was then removed under reduced pressure. The residue was dissolved in AcOEt (70 ml) and consecutively washed with 1 M aqueous solution of $KHSO_4$ (3 × 25 ml), brine (1 × 30 ml), dried over Na_2SO_4 and the solvent removed under reduced pressure to afford the crude product. The residue was purified with flash chromatography on silica gel (CH_2Cl_2/MeOH 95:5, solid load) to afford the desired product **23** as a pale yellow solid (27 mg, 50% yield).

R_f= 0.40 (9:1 CH_2Cl_2/MeOH); ^{1}H NMR (400 MHz, MeOD) δ 7.47 – 7.07 (m, 5H), 6.65 (s, 1H), 5.52 (d, J = 15.5 Hz, 1H), 4.93 (t, J = 7.4 Hz, 1H), 4.70 (dd, J = 9.0, 5.2 Hz, 1H), 4.07 - 3.98 (m, 4H), 3.88 (t, J = 5.6 Hz, 1H), 3.82 (s, 3H), 3.73 (dt, J = 13.4, 3.6 Hz, 1H), 3.53 (dd, J = 14.4, 5.6 Hz, 1H), 3.21 - 3.16 (m, 3H), 2.89

(m, 1H), 2.67 – 2.40 (m, 10H), 2.28 (dd, *J* = 14.0, 3.1 Hz, 1H), 2.12 (s, 3H), 1.99 (m, 1H), 1.85 (m, 1H), 1.68 – 1.50 (m, 2H), 1.44 ppm (s, 9H); ^{13}C NMR (101 MHz, MeOD) δ 174.7, 174.7, 173.6, 172.7, 170.9, 170.1, 169.3, 159.9, 139.4, 137.9, 137.4, 134.9, 129.9, 129.0, 128.9, 125.7, 112.8, 82.7, 61.0, 56.0, 55.3, 52.3, 51.7, 39.5, 38.4, 37.8, 36.9, 35.5, 28.3, 27.9, 24.3, 18.8, 12.1 ppm; MS (ESI+): *m/z* calcd for $[C_{41}H_{56}N_9O_{11}S]^-$: 882.38 $[M\text{-}H]^-$; found: 882.86.

cyclo(DKP-RβAD) (**3**)

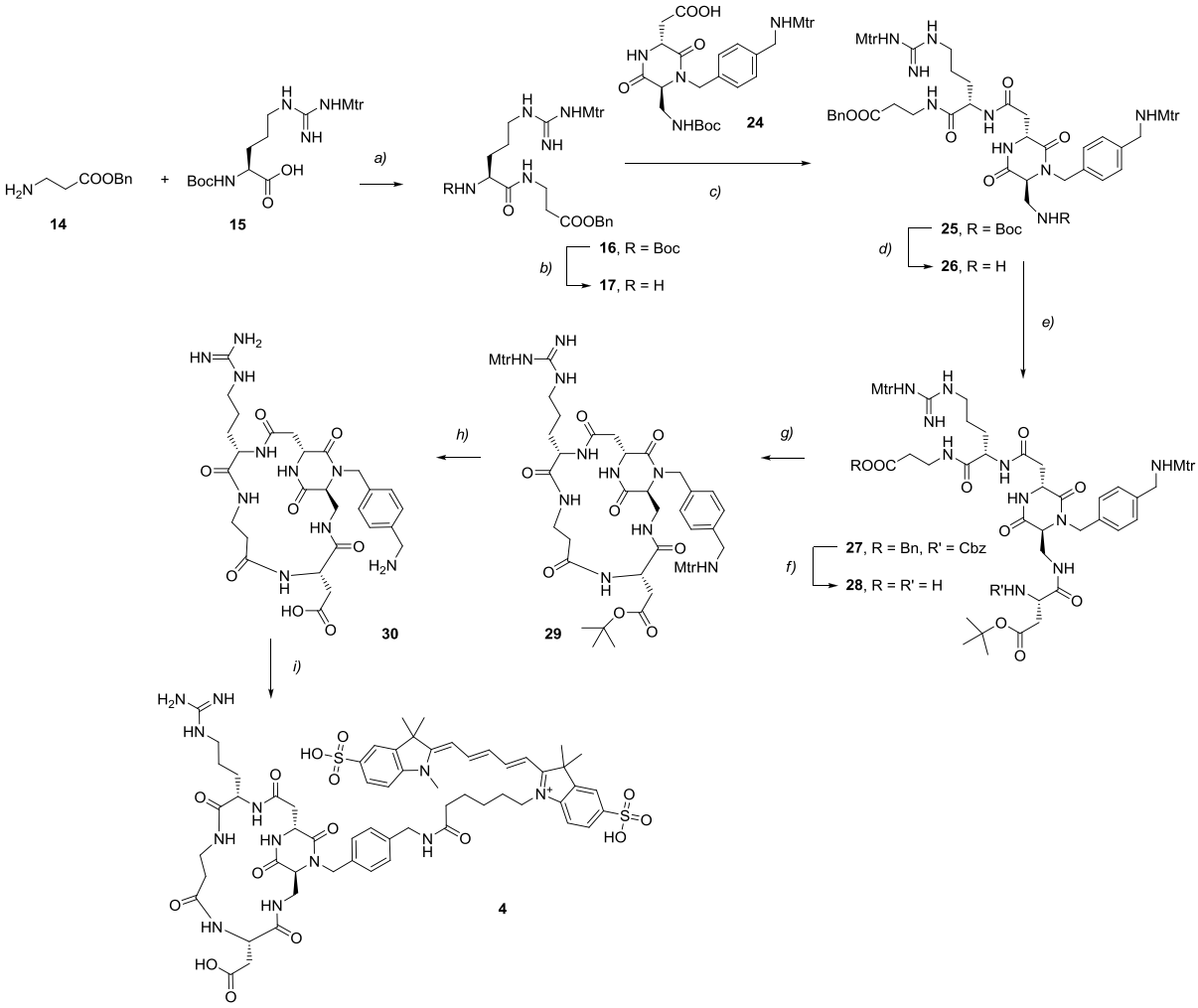


$C_{27}H_{37}N_9O_8$·TFA
MW: 615.64

To a solution of *cyclo*[DKP- Arg(Mtr)- βAla-Asp(O*t*Bu)] **23** (27 mg, 0.0306 mmol, 1 eq), a cleavage cocktail of thioanisole (0.15 ml), ethanedithiol (0.09 ml) and anisole (0.06 ml) in TFA (3 ml) was added at 0 °C under N_2 atmosphere, the flask was opened and the mixture warmed up to room temperature and stirred for 2 h. All volatiles were then evaporated and the crude was dissolved in a mixture of iPr$_2$O/H$_2$O (1:1, 10 ml).The aqueous phase was washed with iPr$_2$O (3 × 5 ml) and then concentrated under reduced pressure to give the crude compound, which was purified by semipreparative-HPLC [Water's Atlantis 21 mm × 10 cm column; gradient 90% (H_2O + 0.1% CF_3COOH)/(10% MeCN + 0.1% CF_3COOH) to 72% (H_2O + 0.1% CF_3COOH)/28% (MeCN + 0.1% CF_3COOH) in 12 min; t_R (product): 8 min] to give the desired compound **3** as a trifluoroacetate salt (10 mg, 45% yield), after freeze-drying.

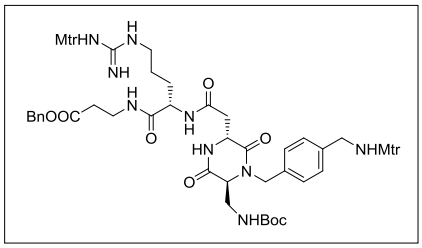
^{1}H NMR (400 MHz, D_2O) δ 7.49 – 7.30 (m, 5H), 5.34 (d, *J* = 15.5 Hz, 1H), 4.80 - 4.76 (m, 2H), 4.20 – 3.99 (m, 5H), 3.75 – 3.66 (m, 2H), 3.25 - 3.20 (m, 3H), 3.02 (dd, *J* = 15.4, 6.5 Hz, 1H), 2.93 – 2.66 (m, 3H), 2.50 – 2.46 (m, 2H), 2.03 (m, 1H), 1.87 (m, 1H), 1.70 – 1.65 ppm (m, 2H); ^{13}C NMR (101 MHz, D_2O) δ 174.3, 173.9, 173.8, 173.0, 172.2, 169.2, 168.0, 163.1, 162.8, 135.1, 129.1, 128.1, 127.6, 127.6, 117.8, 114.9, 59.6, 53.9, 51.3, 50.2, 48.5, 40.6, 38.9, 36.9, 35.8, 35.3, 33.9, 25.9, 24.7; MS (ESI+): *m/z* calcd for $[C_{27}H_{38}N_9O_8]^+$: 616.28 $[M+H]^+$; found: 616.21.

Synthesis of *cyclo*(DKP-RβAD)-sCy5 (4).



Scheme S3: Synthesis of *cyclo*(DKP-RβAD)-sCy5 **4**. REAGENTS AND CONDITIONS: *a)* HATU, HOAt, *i*Pr_2NEt, DMF, r.t., 16 h, Y.: 55%; *b)* TFA/CH_2Cl_2 (1:2), r.t., 2.5 h, Y.: quantitative; *c)* HATU, HOAt, *i*Pr_2NEt, DMF, r.t., 16 h, Y.: 65%; *d)* TFA/CH_2Cl_2 (1:2), r.t., 2 h, Y.: quantitative; *e)* Cbz-Asp(O*t*Bu)-OH, HATU, HOAt, *i*Pr_2NEt, DMF, r.t., 16 h, Y.: 74%; *f)* H_2, 10% Pd/C, THF/H_2O (1:1), r.t., 16 h, Y.: 85%-quantitative; *g)* HATU, HOAt, *i*Pr_2NEt, DMF, r.t., 16 h, Y.: 60%; *h)* TFA/TMSBr/thioanisol/EDT/phenol (70:14:10:5:1), 2 h, r.t., Y.: 66%; *i)* SulfoCy5-NHS, *i*Pr_2NEt, DMF, r.t., 4 h, Y: 50%.

Boc-DKP-Arg(Mtr)-βAla-OBn (**25**)

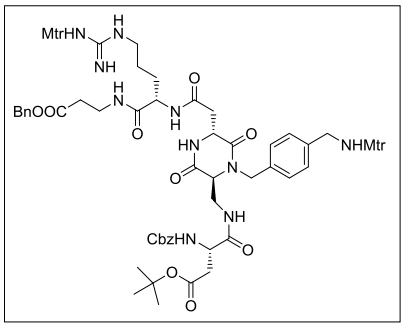


$C_{56}H_{77}N_9O_{14}S_2$
MW: 1161.49

To a solution of functionalized DKP **24**[S3] (95 mg, 0.15 mmol, 1 eq) in dry DMF (1 ml) at 0 °C under N_2 atmosphere, HATU (76 mg, 0.2 mmol, 1.3 eq), HOAt (27 mg, 0.2 mmol, 1.3 eq) and *i*Pr_2NEt (0.104 ml, 0.6 mmol, 4 eq) were added. After stirring the mixture for 30 min at 0 °C, the trifluoroacetate salt of deprotected dipeptide Boc-Arg(Mtr)-βAla-OBn (200 mg, 0.3 mmol, 2 eq) in dry DMF (0.7 ml) was added. The reaction mixture was stirred at 0 °C for 1 h, then warmed to room temperature and stirred overnight. The solvent was removed and the mixture was diluted with AcOEt (50 ml) and washed with 1 M aqueous solution of $KHSO_4$ (3 × 25 ml), a saturated aqueous solution of $NaHCO_3$ (3 × 25 ml) and brine (1 × 30 ml), dried over Na_2SO_4 and the solvent removed under reduced pressure to afford the crude product. The residue was purified with flash chromatography on silica gel (CH_2Cl_2/MeOH, starting at 96:4 and finishing at 92:8, dry load) to afford the desired product **25** as pale yellow foam (114 mg, 65% yield).

R_f= 0.35 (CH_2Cl_2: MeOH 96:4); ^{1}H NMR (400 MHz, MeOD) δ 7.36 – 7.25 (m, 5H), 7.10 (dd, J = 19.5, 8.2 Hz, 4H), 6.69 (s, 1H), 6.65 (s, 1H), 5.30 (d, J = 15.5 Hz, 1H), 5.07 (s, 3H), 4.50 (m, 1H), 4.28 (m, 1H), 4.00 – 3.94 (m, 3H), 3.84 - 3.71 (m, 9H), 3.55 – 3.35 (m, 8H), 3.15 (m, 2H), 2.85 (m, 2H), 2.66 (s, 3H), 2.60 (s, 6H), 2.53 (t, J = 6.8 Hz, 2H), 2.48 (s, 3H), 2.08 (s, 3H), 2.06 (s, 3H), 1.77 (m, 1H), 1.62 – 1.49 (m, 3H), 1.41 ppm (s, 9H); ^{13}C NMR (101 MHz, MeOD) δ 174.2, 173.1, 172.0, 168.5, 160.7, 159.8, 139.9, 139.5, 138.5, 137.9, 137.5, 136.2, 134.8, 130.9, 129.6, 129.6, 129.4, 129.3, 129.3, 129.2, 128.8, 126.1, 125.7, 113.2, 112.9, 112.8, 67.4, 61.4, 59.0, 56.2, 56.0, 54.3, 52.5, 46.8, 36.4, 34.7, 30.9, 30.1, 28.8, 24.5, 24.4, 18.9, 18.2, 12.1, 12.1, 12.1 ppm; MS (ESI): *m/z* calcd for $[C_{56}H_{76}N_9O_{14}S_2]^-$: 1161.49 [*M-H*]$^-$; found: 1160.77.

Cbz-Asp(OtBu)-DKP-Arg(Mtr)-βAla-OBn (**27**)



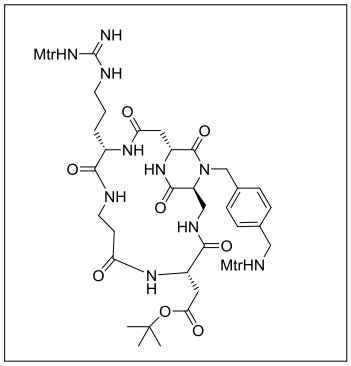
$C_{67}H_{88}N_{10}O_{17}S_2$
MW: 1366.55

To a solution of Cbz-L-Asp(O*t*Bu)-OH (48 mg, 0.147 mmol, 1.5 eq) in dry DMF (1.4 ml) at 0 °C under N_2 atmosphere, HATU (60 mg, 0.157 mmol, 1.6 eq), HOAt (21 mg, 0.157 mmol, 1.6 eq) and *i*Pr_2NEt (0.07 ml, 0.396 mmol, 4 eq) were added. After stirring the mixture for 30 min at 0 °C, the trifluoroacetate salt of Boc-

free DKP-Arg(Mtr)-βAla-OBn (**26**, 115 mg, 0.098 mmol, 1 eq) in dry DMF (0. 6ml) was added. The reaction mixture was stirred at 0 °C for 1 h, then warmed to room temperature and stirred overnight. The mixture was diluted with AcOEt (50 ml) and washed with 1 M aqueous solution of $KHSO_4$ (3 × 30 ml), a saturated aqueous solution of $NaHCO_3$ (2 × 25 ml) and brine (1 × 30 ml), dried over Na_2SO_4 and the solvent removed under reduced pressure to afford the crude product. The residue was purified with flash chromatography on silica gel (CH_2Cl_2/MeOH starting at 97:3 and finishing at 95:5, solid load) to afford the desired product **27** as a yellow foam (99 mg, 74% yield).

R_f = 0.26 (CH_2Cl_2: MeOH 96:4); ^{1}H NMR (400 MHz, MeOD) δ 7.38 – 7.21 (m, 10H), 7.11 (dd, J = 25.3, 8.1 Hz, 2H), 6.71 (s, 1H), 6.59 (s, 1H), 5.26 (d, J = 15.4 Hz, 1H), 5.19 – 4.99 (m, 4H), 4.60 (t, J = 5.3 Hz, 1H), 4.48 (dd, J = 8.6, 5.1 Hz, 1H), 4.29 (m,, 1H), 4.05 (d, J = 15.4 Hz, 1H), 3.97 (s, 2H), 3.89 – 3.71 (m, 8H), 3.61 (m, 1H), 3.36 (m, 2H), 3.23 – 3.02 (m, 2H), 2.94 – 2.70 (m, 4H), 2.66 (s, 3H), 2.62 (s, 3H), 2.58 (s, 3H), 2.53 (t, J = 7.6 Hz, 2H), 2.48 (s, 3H), 2.17 (m, 6H), 1.84 – 1.44 (m, 4H), 1.40 ppm (s, 9H); ^{13}C NMR (101 MHz, MeOD) δ 174.4, 173.3, 172.2, 169.0, 160.9, 160.1, 140.1, 139.7, 138.7, 138.1, 136.4, 129.8, 129.7, 129.6, 129.5, 129.4, 129.3, 129.2, 129.1, 128.8, 128.2, 126.3, 113.4, 113.0, 82.6, 68.2, 67.7, 60.9, 56.4, 56.2, 54.4, 53.5, 52.5, 40.7, 38.6, 36.6, 34.9, 31.0, 30.3, 28.6, 28.5, 24.7, 24.6, 19.1, 18.4, 12.3 ppm; MS (ESI): *m/z* calcd for $[C_{67}H_{87}N_{10}O_{17}S_2]^-$: 1365.55 [*M-H*]$^-$; found: 1365.91.

cyclo[DKP-Arg(Mtr)-βAla-Asp(OtBu)] (**29**)



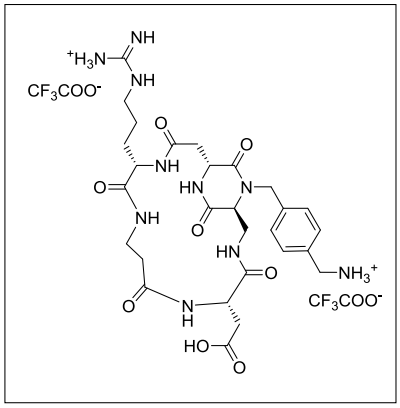
$C_{52}H_{72}N_{10}O_{14}S_2$
MW: 1125.47

To a solution of **28** (82 mg, 0.072 mmol, 1 eq) in dry DMF (51.4 ml), under nitrogen atmosphere and at 0 °C, HATU (110 mg, 0.288 mmol, 4 eq), HOAt (39 mg, 0.288 mmol, 4 eq) and *i*Pr$_2$Net (0.075 ml, 0.432 mmol, 6 eq) were added. The reaction stirred at 0 °C for 1.5 h and then at room temperature overnight. The solvent was then removed under reduced pressure. The residue was dissolved in AcOEt (100 ml) and washed with 1 M aqueous solution of $KHSO_4$ (3 × 50 ml), and brine (1 × 60 ml), dried over Na_2SO_4 and the solvent evaporated under reduced pressure to afford the crude product. The residue was purified with flash chromatography on silica gel (CH_2Cl_2/MeOH starting at 95:5 and finishing at 93:7, solid load) to afford the desired product **29** as pale yellow solid (48.8 mg, 60% yield).

R_f= 0.4 (9:1 CH_2Cl_2/MeOH); ^{1}H NMR (400 MHz, MeOD) δ 7.09 (s, 4H), 6.68 (s, 1H), 6.65 (s, 1H), 5.43 (d, J = 15.5 Hz, 1H), 4.89 (t, J = 7.4 Hz, 1H), 4.69 (dd, J = 9.0, 5.2 Hz, 1H), 4.10 – 3.94 (m, 4H), 3.92 (d, J = 15.5 Hz, 1H), 3.84 (s, 3H), 3.83 – 3.79 (m, 4H), 3.73 (m, 1H), 3.51 (dd, J = 14.4, 5.6 Hz, 1H), 3.23 – 3.12

(m, 3H), 2.89 (dd, *J* = 15.3, 7.6 Hz, 1H), 2.72 – 2.53 (m, 12H), 2.50 – 2.39 (m, 4H), 2.28 (m, 1H), 2.11 (s, 3H), 2.08 (s, 3H), 2.01 (m, 1H), 1.86 (m, 1H), 1.59 (m, 2H), 1.44 ppm (s, 9H); ^{13}C NMR (101 MHz, MeOD) δ 174.7, 174.7, 173.6, 172.7, 170.9, 170.0, 169.2, 160.7, 159.9, 139.9, 139.5, 138.6, 137.9, 136.5, 129.5, 129.5, 129.5, 129.4, 129.4, 129.4, 128.9, 128.8, 126.1, 125.7, 113.2, 112.8, 82.6, 61.0, 56.2, 56.2, 56.1, 56.0, 56.0, 55.3, 52.3, 51.7, 39.5, 38.4, 37.8, 36.8, 35.5, 30.7, 28.6, 28.3, 28.3, 27.8, 27.3, 24.4, 24.3, 18.8, 18.2, 18.2, 12.1, 12.1, 12.1 ppm; MS (ESI): *m/z* calcd for $[C_{52}H_{71}N_{10}O_{14}S_2]^-$: 1124.47 $[M\text{-}H]^-$; found: 1123.83.

cyclo(DKP-RβAD)-CH$_2$NH$_2$ (**30**)

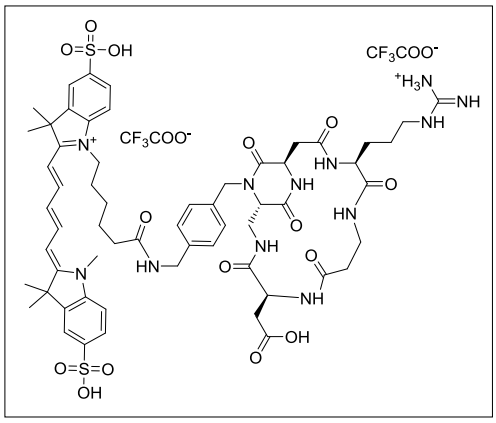


$C_{28}H_{42}N_{10}O_8^{2+}$
646,7049 + TFA

To a solution of **29** (49 mg, 0.044 mmol, 1 eq), a cleavage cocktail of phenol (74 mg), thioanisole (0.734 ml) and ethanedithiol (0.368 ml) in TFA (4.9 ml) was added. Trimethylsilylbromide (0.98 ml) was added at 0 °C under N_2 atmosphere, the flask was opened and the mixture warmed up to room temperature and stirred for 2 h. Volatiles were then evaporated and the crude was dissolved in a mixture of H_2O/*i*PrO_2 (1:1, 60 ml). The aqueous phase was washed with *i*PrO_2 (3 × 30 ml) and then concentrated under reduced pressure to give the crude compound, which was purified by semipreparative-HPLC [Water's Atlantis 21 mm × 10 cm column; gradient 95% (H_2O + 0.1% CF_3COOH)/(5% MeCN + 0.1% CF_3COOH) to 78% (H_2O + 0.1% CF_3COOH)/22% (MeCN + 0.1% CF_3COOH) in 10 min; t_R (product): 6.5 min] to give the desired compound as trifluoroacetate salt as a white solid (**30**, 25 mg, 66% yield), after freeze-drying from water.

^{1}H NMR (400 MHz, D_2O) δ 7.34 (m, 4H), 5.18 (m, 1H), 4.73 (dd, *J* = 11.0, 4.0 Hz, 1H), 4.64 (dd, *J* = 8.5, 5.5 Hz, 1H), 4.23 (m, 1H), 4.14 – 3.92 (m, 5H), 3.70 – 3.54 (m, 2H), 3.17 (m, 2H), 2.99 – 2.61 (m, 4H), 2.39 (dd, *J* = 8.3, 3.7 Hz, 2H), 1.94 (m, 1H), 1.77 (m, 1H), 1.64 – 1.52 ppm (m, 2H); ^{13}C NMR (101 MHz, D_2O) δ 174.2, 173.9, 173.8, 173.0, 172.2, 169.3, 168.2, 136.1, 132.3, 129.4, 128.3, 128.1, 59.8, 58.2, 53.8, 52.5, 51.8, 51.2, 50.3, 49.9, 48.4, 42.7, 40.5, 40.4, 38.8, 36.8, 35.8, 35.4, 34.0, 26.1, 24.7, 24.5 ppm; MS (ESI): *m/z* calcd for $[C_{28}H_{39}N_{10}O_8]^-$: 643.30 $[M\text{-}H]^-$; found: 643.76; *m/z* calcd for $[C_{28}H_{41}N_{10}O_8]^+$: 645.31 $[M+H]^-$; found: 645.66.

Cyclo(DKP-RGD)-sCy5 (**4**)

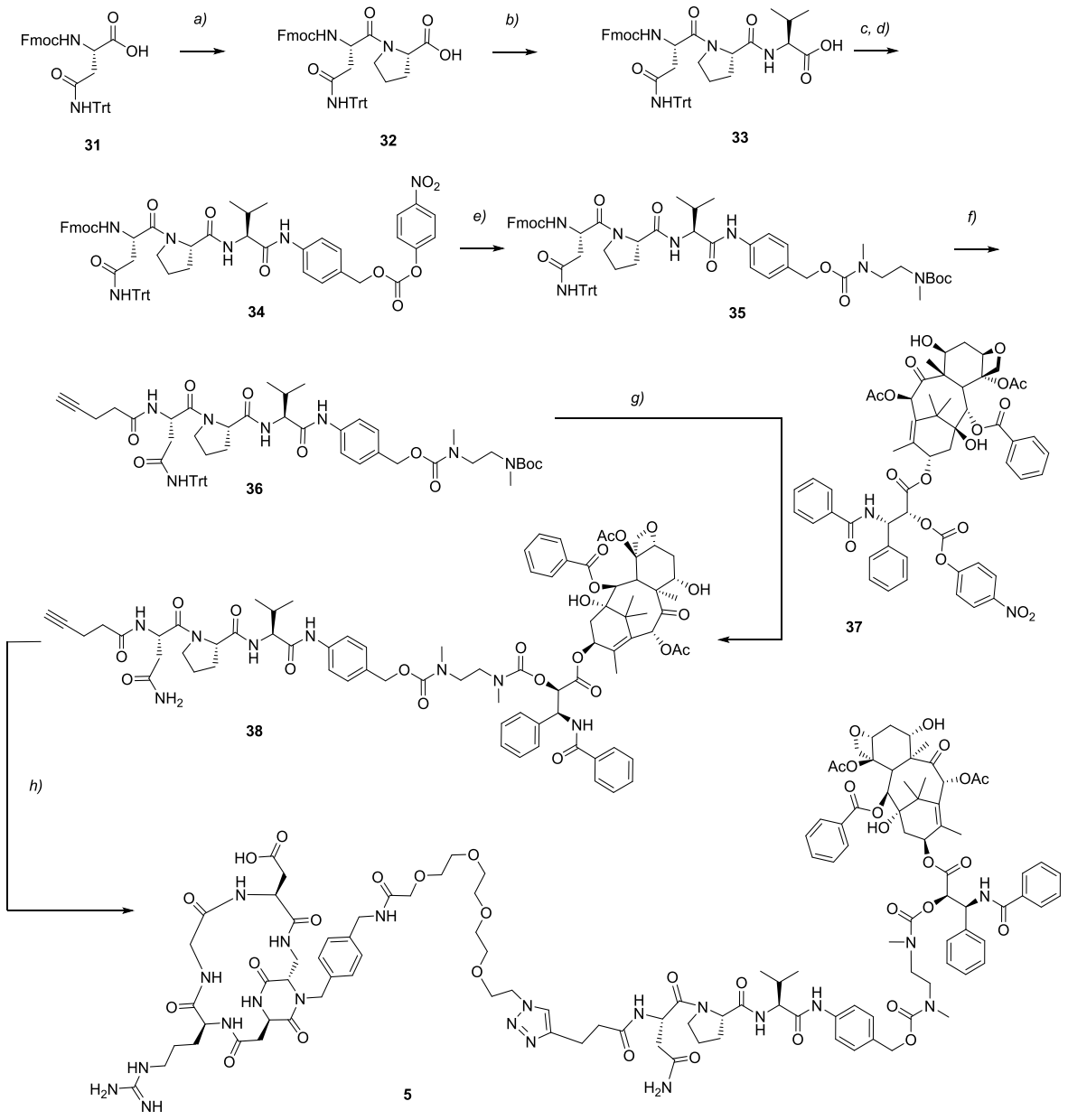


$C_{60}H_{78}N_{12}O_{15}S_2^{2+}$
MW: 1271,4719 + TFA

To a solution of compound **30** (4 mg, 0.0046 mmol, 1 eq) in DMF (600 µl), *i*Pr_2NEt (3 µl, 0.0184 mmol, 4 eq) was added adjusting the pH at 8-9; then SulfoCy5 (5.3 mg, 0.0069 mmol, 1.5 eq) was added and the reaction mixture was stirred for 4 h at room temperature in the darkness. Then the mixture was directly purify by semipreparative-HPLC [gradient: 90% (H_2O + 0.1% CF_3COOH) / 10% (MeCN + 0.1% CF_3COOH) to 40% (H_2O + 0.1% CF_3COOH) / 60% (MeCN + 0.1% CF_3COOH) in 9 min; t_R (product): 7 min]. The purified product was then freeze-dried to give compound **4** as a blue solid (3 mg, 50% yield).

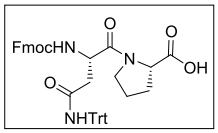
HRMS (ESI-): *m/z* calcd for $[C_{60}H_{75}N_{12}O_{15}S_2]^-$: 1267.4922 $[M-2H]^-$; found: 1267.4928 - *m/z* calcd for $[C_{60}H_{74}N_{12}O_{15}S_2]^{2-}$: 633.2424 $[M-3H]^{2-}$; found: 633.2426.

Synthesis of *cyclo*(DKP-RGD)-Asn-Pro-Val-PTX conjugate (5)



Scheme S4. Synthesis of *cyclo*(DKP-RGD)-Asn-Pro-Val-PTX conjugate (**5**). REAGENTS AND CONDITIONS: *a)* 1) NHS, EDC·HCl, CH_2Cl_2, RT, overnight; 2) L-Proline, $NaHCO_3$, THF/H_2O (1:1), RT, overnight, Y.: 52% (over two steps); *b)* NHS, EDC·HCl, CH_2Cl_2, RT, overnight; 2) L-Valine, $NaHCO_3$, THF/H_2O (1:1), RT, overnight, Y.: 82% (over two steps); *c)* 4-aminobenzyl alcohol, EEDQ, CH_2Cl_2/MeOH (2:1), RT, overnight; *d)* 4-nitrophenyl chloroformate, pyridine, RT, THF, 2 h, Y.: 54% (over two steps); *e)* *N*-(Boc)-*N,N'*-dimethylethylenediamine, *i*Pr_2EtN, RT, THF, overnight, Y.: 80%; *f)* 1) piperidine, DMF, RT, 2 h; 2) 4-pentynoic acid, HATU, HOAt, *i*Pr_2EtN, DMF, RT, overnight, Y.: 90% (over two steps); *g)* 1) TFA/CH_2Cl_2 (1:2), 15 min; 2) **37**, *i*Pr_2EtN, DMF, RT, overnight, Y.: 71% (over two steps); *h) cyclo*[DKP-RGD]-PEG4-N_3, $CuSO_4 \cdot 5H_2O$, sodium ascorbate, DMF/H_2O (1:1), 30 ºC, overnight, Y.: 94%.

Fmoc-Asn(Trt)-Pro-OH (**32**)

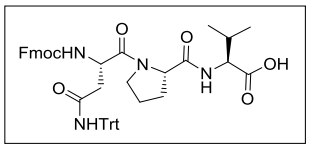


$C_{43}H_{39}N_3O_6$
MW: 693,79

Fmoc-Asn(Trt)-OH **31** (2 g, 3.35 mmol, 1 equiv) and EDC·HCl (1.9 g, 10.05 mmol, 3 equiv) were dissolved in dry CH_2Cl_2 (33 mL) under nitrogen atmosphere. *N*-hydroxysuccinimide (771 mg, 6.70 mmol, 2 equiv) was added and the mixture was stirred overnight at room temperature. The reaction mixture was diluted with CH_2Cl_2 (30 mL) and washed with HCl aq. 1 M (1 × 25 mL) and brine. The organic layer was washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure. Then, the residue was purified by filtration over a pad of silica gel (eluent: 2:8 AcOEt/CH_2Cl_2) affording the desired activated Fmoc-Asn(Trt)-OSu as a white solid. Proline (464 mg, 4.02 mmol, 1.2 equiv) was dissolved in H_2O (20 mL) and $NaHCO_3$ (563 mg, 6.70 mmol, 2 equiv) was added. The previously obtained Fmoc-Asn(Trt)-OSu was dissolved in THF (20 mL) and added to the stirred solution of H-Pro-OH and $NaHCO_3$. The mixture was stirred overnight at room temperature. The solvent was concentrated, followed by addition of a 1 M aqueous solution of $KHSO_4$ (40 mL). The suspension was extracted with CH_2Cl_2 (4 × 20 mL), then the collected organic phases were dried and concentrated. The crude was purified by flash chromatography [eluent: 2:8 AcOEt/CH_2Cl_2 + 0,1% AcOH] affording Fmoc-Asn(Trt)-Pro-OH **32** (1.2 g, 52% yield).

R_f = 0.15 (8:2, CH_2Cl_2/AcOEt + 0.1% AcOH); ^{1}H NMR (500 MHz, CD_2Cl_2-d_2) δ 7.80 (d, *J* = 7.6 Hz, 2H), 7.60 (t, *J* = 8.7 Hz, 2H), 7.42 (t, *J* = 7.7 Hz, 2H), 7.34 – 7.18 (m, 18H), 6.08 (s, 1H), 4.83 – 4.74 (m, 1H), 4.37 (d, *J* = 7.0 Hz, 3H), 4.19 (t, *J* = 6.8 Hz, 1H), 3.64 – 3.53 (m, 1H), 3.43 (s, 1H), 2.84 (dd, *J* = 15.4, 7.3 Hz, 1H), 2.72 (dd, *J* = 15.4, 5.8 Hz, 1H), 2.19 – 2.04 (m, 2H), 1.95 – 1.82 (m, 2H); MS (MALDI-TOF): *m/z* calcd for $[C_{43}H_{40}N_3O_6]^+$: 694.79 [*M* + H]$^+$; found: 694.81; *m/z* calcd $[C_{43}H_{39}NaN_3O_6]^+$: 716.79 [*M* + Na]$^+$; found: 716.80 (DHB matrix).

Fmoc-Asn(Trt)-Pro-Val-OH (**33**)



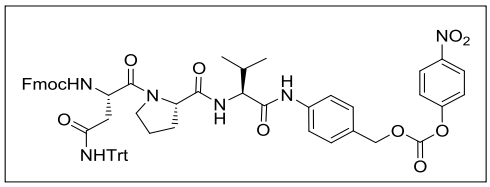
$C_{48}H_{48}N_4O_7$
MW: 792,92

Fmoc-Asn(Trt)-Pro-OH **32** (1.18 g, 1.70 mmol, 1 equiv) and EDC·HCl (977 mg, 5.10 mmol, 3 equiv) were dissolved in dry CH_2Cl_2 (17 mL) under nitrogen atmosphere. *N*-hydroxysuccinimide (400 mg, 3.40 mmol, 2 equiv) was added and the mixture was stirred overnight at room temperature. The reaction mixture was diluted with CH_2Cl_2 (20 mL) and washed with HCl aq. 1 M (1 × 15 mL) and brine, dried over Na_2SO_4 and concentrated under reduced pressure affording the desired activated Fmoc-Asn(Trt)-Pro-OSu as a white solid. Valine (240 mg, 2.04 mmol, 1.2 equiv) was dissolved in H_2O (11 mL) and $NaHCO_3$ (290 mg, 3.40 mmol, 2 equiv) was added. The previously obtained Fmoc-Asn(Trt)-Pro-OSu was dissolved in THF (11 mL)

and added to the stirred solution of H-Val-OH and $NaHCO_3$. The mixture was stirred overnight at room temperature. The solvent was concentrated, followed by addition of a 1 M aqueous solution of $KHSO_4$ (20 mL). The suspension was extracted with CH_2Cl_2 (4 × 15 mL), then the collected organic phases were dried and concentrated. The crude was purified by flash chromatography [eluent: 95:5 CH_2Cl_2/MeOH + 0,1% AcOH] affording Fmoc-Asn(Trt)-Pro-Val-OH **33** (1.10 g, 82% yield).

R_f = 0.21 (95:5, CH_2Cl_2/MeOH + 0.1% AcOH); ^{1}H NMR (500 MHz, CD_2Cl_2-d_2) δ 7.85 (d, *J* = 8.2 Hz, 1H), 7.66 (d, *J* = 6.9 Hz, 2H), 7.51 – 7.41 (m, 3H), 7.27 (td, *J* = 7.3, 3.5 Hz, 2H), 7.20 - 7.05 (m, 17H), 6.60 (d, *J* = 8.4 Hz, 1H), 4.44 (td, *J* = 9.5, 3.6 Hz, 1H), 4.21 – 4.08 (m, 3H), 4.04 (t, *J* = 7.3 Hz, 1H), 3.85 (t, *J* = 8.3 Hz, 1H), 3.27 (q, *J* = 8.6 Hz, 1H), 2.95 – 2.82 (m, 1H), 2.71 – 2.64 (m, 1H), 2.61 (dd, *J* = 14.0, 3.7 Hz, 1H), 1.92 – 1.76 (m, 2H), 1.70 – 1.58 (m, 2H), 1.47 (p, *J* = 10.7, 9.8 Hz, 1H), 0.67 (d, *J* = 6.7 Hz, 3H), 0.44 (d, *J* = 6.6 Hz, 3H); ^{13}C-DEPT45 NMR (126 MHz, CD_2Cl_2-d_2) δ 128.87, 128.81, 127.99, 127.86, 127.80, 127.18, 127.05, 125.34, 125.33, 120.02, 67.24, 61.55, 59.26, 49.72, 47.60, 47.15, 40.58, 29.81, 29.56, 29.15, 24.70, 19.36, 18.87. MS (MALDI-TOF): *m/z* calcd for $[C_{48}H_{49}N_4O_7]^+$: 793.92 $[M + H]^+$; found: 793.99; *m/z* calcd $[C_{48}H_{48}NaN_4O_7]^+$: 815.92 $[M + Na]^+$; found: 816.01 (DHB matrix).

Fmoc-Asn(Trt)-Pro-Val-PABC-PNP (**34**)

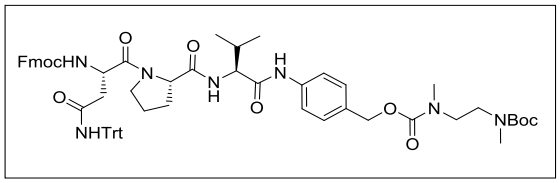


$C_{62}H_{58}N_6O_{11}$
MWt: 1063,16

Compound **33** (880 mg, 1.11 mmol, 1 equiv) was dissolved in dry CH_2Cl_2/MeOH (2:1, 11 mL) under a nitrogen atmosphere. EEDQ (686 mg, 2.77 mmol, 2.5 equiv) and 4-aminobenzyl alcohol (273 mg, 2.22 mmol, 2 equiv) were added at 0 °C, under a nitrogen atmosphere. Then, the mixture was allowed to reach room temperature and stirred overnight under nitrogen atmosphere. The solvent was removed under reduced pressure affording a yellow solid. The crude was diluted in Et_2O (3 × 30 mL) and filtered in a fritz. The solid was purified by flash chromatography [gradient: from 0% MeOH / 100% CH_2Cl_2 to 5% MeOH / 95% CH_2Cl_2] to afford the Fmoc-Asn(Trt)-Pro-Val-PABA as a white solid (MS (MALDI-TOF): *m/z* calcd for $[C_{55}H_{55}NaN_5O_7]^+$: 921.05, $[M + Na]^+$; found: 921.23). A solution of Fmoc-Asn(Trt)-Pro-Val-PABA in a mixture of dry THF (65 mL) under nitrogen atmosphere was cooled to 0 °C. Pyridine (224 μL, 2.77 mmol, 2.5 equiv) and 4-nitrophenylchloroformate (448 mg, 2.22 mmol, 2 equiv) were added, then the mixture could reach room temperature and stirred for 2 h. The reaction mixture was concentrated under reduced pressure and AcOEt (150 mL) was added and the solution was washed with a 1 M aqueous solution of $KHSO_4$ (3 × 20 mL) and brine (20 mL). The organic phase was dried and concentrated, then the crude was purified by flash chromatography [eluent: 4:6 *n*-Hexane/EtOAc] affording compound **34** (640 mg, 54% yield) as a white solid.

R_f = 0.28 (4:6, *n*-Hexane/EtOAc); ^{1}H NMR (500 MHz, CD_2Cl_2-d_2) δ 8.57 (s, 1H), 8.28 (d, *J* = 8.9 Hz, 2H), 7.89 – 7.80 (m, 4H), 7.77 (d, *J* = 8.6 Hz, 1H), 7.62 (dd, *J* = 13.4, 7.5 Hz, 2H), 7.47 – 7.40 (m, 6H), 7.37 – 7.17 (m, 18H), 5.86 (d, *J* = 8.4 Hz, 1H), 5.31 (s, 2H), 4.48 (t, *J* = 10.1 Hz, 1H), 4.43 – 4.35 (m, 3H), 4.22 (t, *J* = 6.9 Hz, 1H), 4.10 (t, *J* = 8.2 Hz, 1H), 3.41 (q, *J* = 8.5 Hz, 1H), 3.00 (t, *J* = 12.6 Hz, 1H), 2.73 – 2.66 (m, 1H), 2.62 (dd, *J* = 13.8, 3.0 Hz, 1H), 2.22 – 2.13 (m, 1H), 2.00 – 1.92 (m, 1H), 1.90 – 1.75 (m, 2H), 1.62 – 1.53 (m, 1H), 0.80 (d, *J* = 6.7 Hz, 3H), 0.44 (d, *J* = 6.6 Hz, 3H). ^{13}C-APT NMR (151 MHz, CD_2Cl_2-d_2) δ 171.61, 171.25, 169.93, 168.88, 155.64, 155.36, 152.49, 145.38, 143.83, 143.78, 143.69, 141.28, 141.24, 139.61, 129.48, 128.59, 127.95, 127.73, 127.07, 127.02, 125.18, 124.97, 121.90, 120.04, 119.98, 70.83, 70.77, 66.99, 61.85, 60.01, 49.41, 49.31, 47.62, 47.11, 40.68, 29.85, 29.67, 28.45, 28.11, 24.62, 19.56, 19.05. MS (MALDI-TOF): *m/z* calcd for $[C_{62}H_{58}NaN_6O_{11}]^+$: 1086.92 [*M* + Na]$^+$; found: 1087.03 (DHB matrix).

Fmoc-Asn(Trt)-Pro-Val-PABC-N-(Boc)-N,N′-dimethylethylenediamine (**35**)



$C_{65}H_{73}N_7O_{10}$
MWt: 1112,32

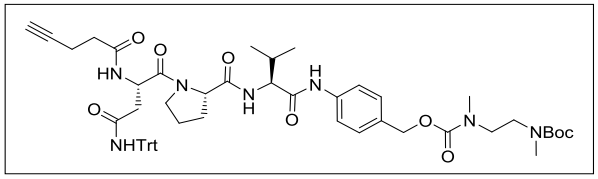
A solution of *N*-(Boc)-*N*,*N*′-dimethylethylenediamine[S5] (208 μL, 1.03 mmol, 2 equiv) in dry THF (7 mL) and *i*Pr_2NEt (250 mg, 1.38 mmol, 2.5 equiv) were added under nitrogen to a solution of **34** (587 mg, 0.552 mmol, 1 equiv) in dry THF (15 mL) kept at 0 ºC. The mixture was stirred overnight at room temperature, then the solvent was removed under vacuum. AcOEt (100 mL) was added and the solution was washed with 1 M aqueous solution of $KHSO_4$ (3 × 20 mL), a saturated aqueous solution of $NaHCO_3$ (2 × 20 mL) and brine (20 mL). The organic phase was dried and concentrated. The crude was purified by flash chromatography (gradient: 3:2 AcOEt/*n*-Hexane to 4:1 AcOEt/*n*-Hexane), affording **35** (490 mg, 80% yield) as white solid.

R_f = 0.19 (1:4, *n*-Hexane/EtOAc); ^{1}H NMR (500 MHz, CD_2Cl_2-d_2) δ 8.47 (d, *J* = 11.0 Hz, 1H), 7.86 – 7.76 (m, 4H), 7.72 (d, *J* = 7.0 Hz, 1H), 7.65 – 7.58 (m, 2H), 7.43 (s, 2H), 7.38 – 7.06 (m, 21H), 5.11 (s, 2H), 4.48 (t, *J* = 10.2 Hz, 1H), 4.41 – 4.34 (m, 3H), 4.25 – 4.21 (m, 1H), 4.16 – 4.06 (m, 1H), 3.51 – 3.25 (m, 5H), 3.06 – 2.93 (m, 4H), 2.86 (s, 2H), 2.75 (s, 1H), 2.70 – 2.62 (m, 2H), 2.19 – 2.12 (m, 1H), 1.97 – 1.93 (m, 1H), 1.91 – 1.76 (m, 2H), 1.58 – 1.55 (m, 1H), 1.46 (s, 9H), 0.77 (d, *J* = 6.8 Hz, 3H), 0.41 (d, *J* = 6.8 Hz, 3H). ^{13}C NMR (151 MHz, CD_2Cl_2-d_2) δ 171.53, 171.33, 171.24, 169.72, 168.94, 156.21, 155.97, 155.41, 143.80, 143.74, 141.27, 141.24, 138.71, 138.58, 132.31, 132.19, 128.60, 127.93, 127.72, 127.04, 125.01, 119.96, 119.77, 79.31, 70.74, 67.01, 66.72, 66.53, 61.85, 59.90, 59.68, 49.40, 47.57, 47.10, 46.98, 46.68, 46.42, 40.70, 35.00, 34.46, 34.22, 29.87, 29.67, 28.59, 28.13, 24.61, 19.59, 18.98, 18.90. MS (MALDI-TOF): *m/z*

[S5] A. Dal Corso, M. Caruso, L. Belvisi, D. Arosio, U. Piarulli, C. Albanese, F. Gasparri, A. Marsiglio, F. Sola, S. Troiani, B. Valsasina, L. Pignataro, D. Donati, C. Gennari, *Chem. Eur. J.* **2015**, *21*, 6921-6929.

calcd for $[C_{65}H_{74}N_7O_{10}]^+$: 1113.32 [*M* + H]$^+$; found: 1113.87; calcd for $[C_{65}H_{73}NaN_7O_{10}]^+$: 1135.32 [*M* + H]$^+$; found: 1135.75 (DHB matrix).

4-pentynamido-Asn(Trt)-Pro-Val-PABC-N-(Boc)-N,N′-dimethylethylenediamine (**36**)

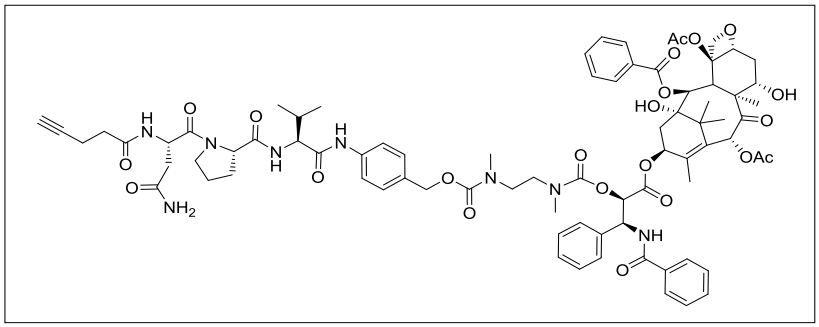


$C_{55}H_{67}N_7O_9$
MW: 970,16

N-Fmoc-protected compound **35** (90 mg, 0.081 mmol, 1 equiv) was dissolved in DMF (1.1 mL) under a nitrogen atmosphere. The solution was cooled to 0 °C and piperidine (40 µL, 0.405 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 $NaHCO_3$ (10 mL). The organic phase was dried over Na_2SO_4 and concentrated under vacuum. Methylene chloride was added to the residue and evaporated to afford a white solid which was used directly at the next step. A solution of commercially available 4-pentynoic acid (12 mg, 0.122 mmol, 1.5 equiv) in dry DMF (1.5 mL) was cooled to 0 °C under a nitrogen atmosphere. HATU (51 mg, 0.134 mmol, 1.7 equiv), HOAT (18 mg, 0.134 mmol, 1.7 equiv) and *i*Pr_2NEt (56 µL, 0.324 mmol, 4 equiv) were added and the mixture was stirred for 20 min at 0 °C. A solution of the above mentioned white solid 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 purified by flash chromatography [gradient: from CH_2Cl_2 100% to 99:1 CH_2Cl_2/MeOH] to afford amide **36** as a white solid (70 mg, 90% yield over two steps).

R_f = 0.30 (95:5, CH_2Cl_2/MeOH); ^{1}H NMR (400 MHz, CD_2Cl_2-d_2) δ 8.46 (s, 1H), 7.84 – 7.69 (m, 3H), 7.57 (s, 1H), 7.37 – 7.14 (m, 17H), 6.84 (s, 1H), 5.10 (s, 2H), 4.74 (t, *J* = 9.7 Hz, 1H), 4.33 (dd, *J* = 9.0, 3.8 Hz, 1H), 4.09 (t, *J* = 8.8 Hz, 1H), 3.52 – 3.27 (m, 5H), 3.09 – 2.92 (m, 4H), 2.81 – 2.66 (m, 5H), 2.56 – 2.35 (m, 4H), 2.23 – 2.09 (m, 1H), 2.03 (t, *J* = 2.7 Hz, 1H), 1.94 (p, *J* = 6.0, 5.4 Hz, 1H), 1.86 – 1.75 (m, 2H), 1.65 – 1.53 (m, 1H), 1.47 (s, 9H), 0.76 (d, *J* = 6.7 Hz, 3H), 0.43 (d, *J* = 6.5 Hz, 3H); ^{13}C NMR (101 MHz, CD_2Cl_2-d_2) δ 171.57, 171.33, 170.32, 169.76, 169.21, 143.85, 138.67, 138.53, 132.26, 132.17, 128.63, 128.07, 127.88, 127.77, 126.99, 119.74, 82.86, 79.33, 79.08, 70.69, 68.98, 66.77, 66.63, 61.87, 60.09, 59.87, 47.75, 47.57, 47.04, 46.68, 46.44, 40.33, 38.36, 34.98, 34.66, 34.46, 34.29, 29.91, 29.68, 28.68, 28.15, 24.63, 19.59, 19.08, 14.47. MS (MALDI-TOF): *m/z* calcd for $[C_{55}H_{67}NaN_7O_9]^+$: 993.16 [*M* + Na]$^+$; found: 993.47.

4-pentynamido-Asn-Pro-Val-PTX (**38**)

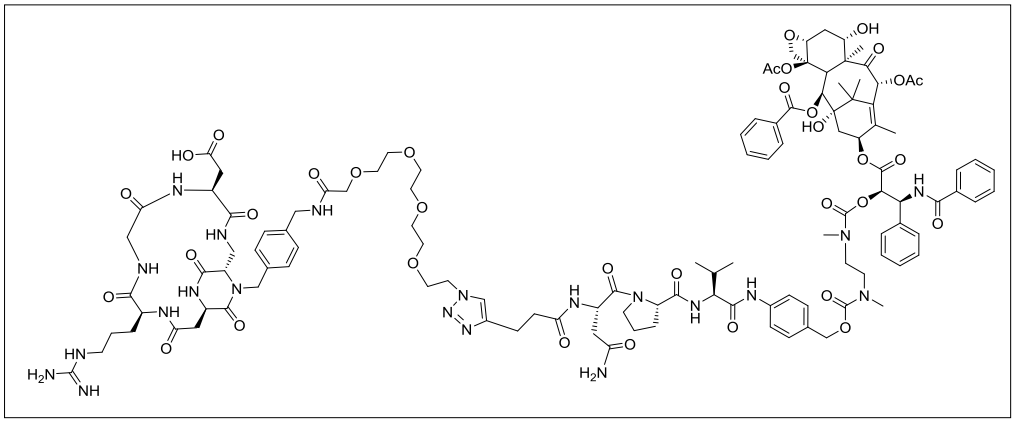


$C_{79}H_{94}N_8O_{22}$
MW: 1507,63

A solution of Boc-protected compound **36** (58 mg, 0.060 mmol, 1 equiv) in dry CH_2Cl_2 (3 mL) was cooled to 0 °C under a nitrogen atmosphere and TFA (1.5 mL) was added dropwise. The mixture was then allowed to reach room temperature and stirred for 30 min. The solvent was removed affording the corresponding trifluoroacetate salt, without further purifications. The solid was dissolved in dry DMF (900 µL) and *i*Pr_2NEt (42 µL, 0.240 mmol, 4 equiv). The resulting solution was added at 0 °C to a stirred solution of **37**[S5] (68 mg, 0.066 mmol, 1.1 equiv) in dry DMF (900 µL), under a nitrogen atmosphere. The reaction was then allowed to reach room temperature and stirred overnight. AcOEt (50 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 99:1 CH_2Cl_2/MeOH to 95:5 CH_2Cl_2/MeOH] to afford carbamate **38** as a white solid (63 mg, 71% yield over two steps).

R_f = 0.28 (CH_2Cl_2/MeOH, 9:1); MS (MALDI-TOF): *m/z* calcd for $[C_{79}H_{94}NaN_8O_{22}]^+$: 1530.63 [*M* + Na]$^+$; found: 1530.03 (HCCA matrix), 1532.08 (SA matrix); HRMS (ESI+): *m/z* calcd for $[C_{79}H_{94}NaN_8O_{22}]^+$: 1529.64, [*M* + Na]$^+$; found 1529.63; *m/z* calcd for $[C_{79}H_{94}Na_2N_8O_{22}]^{2+}$: 1529.63, [*M* + 2Na]$^{2+}$ found 776.31.

cyclo(DKP-RGD)-Asn-Pro-Val-PTX conjugate (**5**)



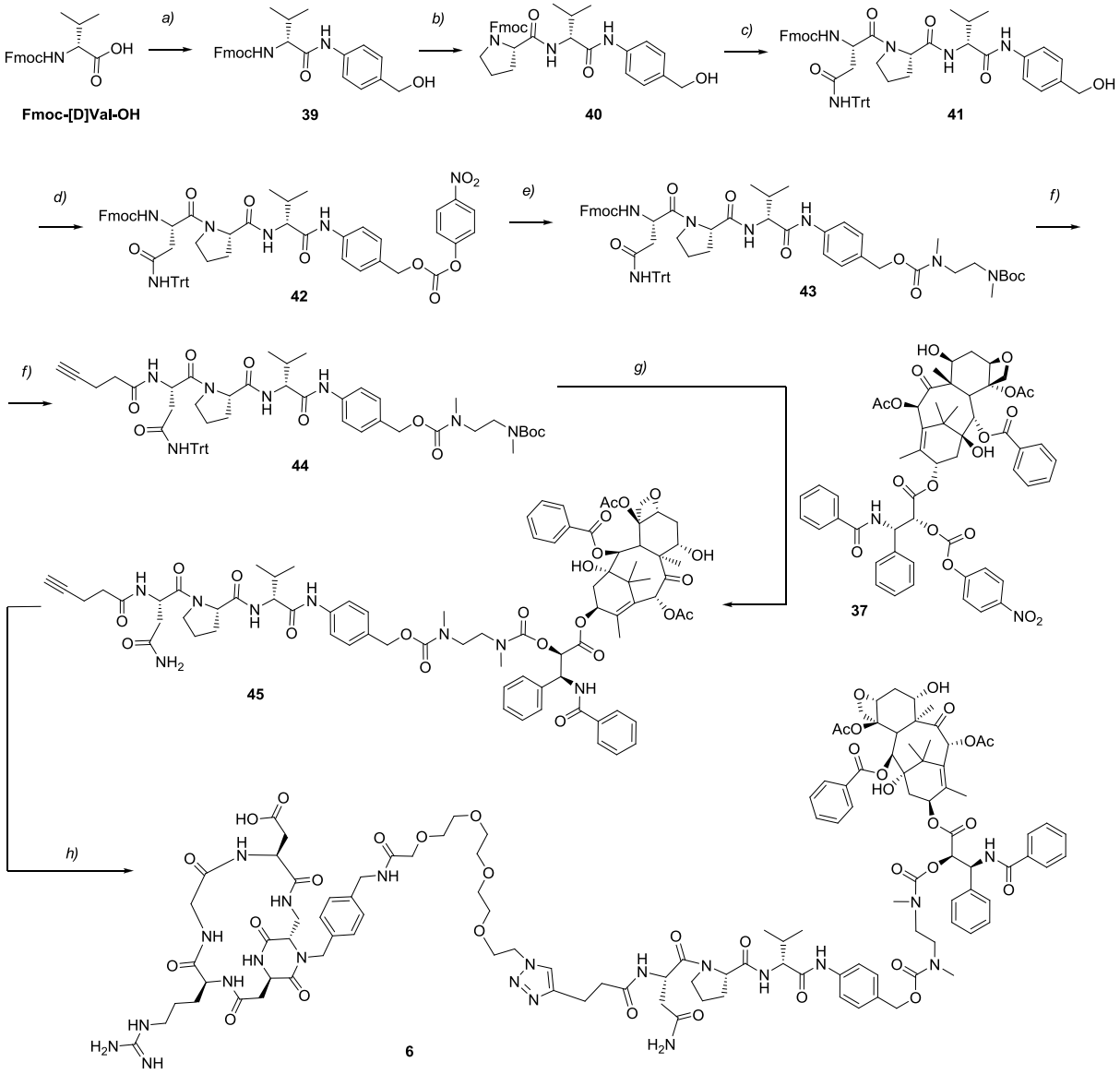
$C_{116}H_{149}N_{21}O_{35}$
MW: 2397,54 + TFA

Alkyne **38** (7.8 mg, 0.0052 mmol, 1.3 equiv) and *cyclo*(DKP-RGD)-PEG4-N_3[S6] (4 mg, 0.004 mmol, 1 equiv) were dissolved in a degased 1:1 mixture of H_2O/DMF (400 μL) under a nitrogen atmosphere. Degased aqueous solutions of $CuSO_4 \cdot 5H_2O$ (0.59 mg, 0.5 equiv) and sodium ascorbate (0.48 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)=12.5 min]. The purified product was then freeze-dried to give the desired compound **5** as a white solid (9 mg, 94% yield).

MS (MALDI-TOF): *m/z* calcd for $[C_{116}H_{150}N_{21}O_{35}]^+$: 2398,54 [*M* + H]$^+$; found: 2398.99 (HCCA matrix), 2399.52 (SA matrix); HRMS (ESI+): *m/z* calcd for $[C_{116}H_{149}Na_2N_{21}O_{35}]^{2+}$: 1221.01, [*M* + 2Na]$^{2+}$; found 1221.51.

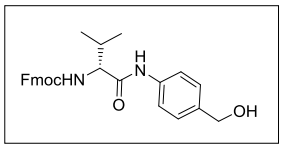
[S6] A. Raposo Moreira Dias, A. Pina, A. Dal Corso, D. Arosio, L. Belvisi, L. Pignataro, M. Caruso, C. Gennari, *Chem. Eur. J.* **2017**, *23*, 14410-14415.

Synthesis of *cyclo*(DKP-RGD)-Asn-Pro-val-PTX conjugate (6)



Scheme S5. Synthesis of *cyclo*(DKP-RGD)-Asn-Pro-val-PTX conjugate (**6**). REAGENTS AND CONDITIONS: *a)* 4-aminobenzyl alcohol, EEDQ, CH_2Cl_2/MeOH (2:1), RT, overnight, Y.: 84%; *b)* [1] piperidine, DMF, RT, 2 h; [2] Fmoc-Pro-OH, HATU, HOAt, *i*Pr_2NEt, DMF, RT, overnight; *c)* [1] piperidine, DMF, RT, 2 h; [2] Fmoc-Asn(Trt)-OH, HATU, HOAt, *i*Pr_2NEt, DMF, RT, overnight; *d)* 4-nitrophenyl chloroformate, pyridine, RT, THF, 2 h, Y.: 47% (over five steps); *e) N*-(Boc)-*N,N'*-dimethylethylenediamine, *i*Pr_2NEt, RT, THF, overnight, Y.: 87%; *f)* [1] piperidine, DMF, RT, 2 h; [2] 4-pentynoic acid, HATU, HOAt, *i*Pr_2NEt, DMF, RT, overnight, Y.: 94% (over two steps); *g)* [1] TFA/CH_2Cl_2 (1:2), 15 min; [2] **37**, *i*Pr_2NEt, DMF, RT, overnight, Y.: 54% (over two steps); *h) cyclo*(DKP-RGD)-PEG4-N_3, $CuSO_4 \cdot 5H_2O$, sodium ascorbate, DMF/H_2O (1:1), 30 °C, overnight, Y.: 70%.

Fmoc-val-PABOH (**39**)

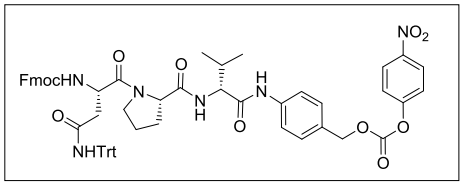


$C_{27}H_{28}N_2O_4$
MW: 444,52

Commercial Fmoc-D-Valine-OH (150 mg, 0.442 mmol, 1 equiv) was dissolved in dry CH_2Cl_2/MeOH (2:1, 6 mL) under a nitrogen atmosphere. EEDQ and 4-aminobenzyl alcohol were added at 0 °C, under a nitrogen atmosphere. Then, the mixture was allowed to reach room temperature and stirred overnight under nitrogen atmosphere. The solvent was removed under reduced pressure affording a yellow solid. The crude was diluted in Et_2O (3 × 25 mL) and filtered in a frit affording compound **39** as white solid (165 mg, 84% yield).

R_f = 0.18 (98:2, CH_2Cl_2/MeOH + 0.1% AcOH); ^{1}H NMR (400 MHz, MeOD-d_4) δ 7.79 (d, *J* = 7.6 Hz, 2H), 7.67 (t, *J* = 7.4 Hz, 2H), 7.54 (d, *J* = 8.4 Hz, 2H), 7.37 (t, *J* = 7.5 Hz, 2H), 7.34 – 7.26 (m, 4H), 4.56 (s, 2H), 4.45 – 4.33 (m, 2H), 4.23 (t, *J* = 6.8 Hz, 1H), 4.03 (d, *J* = 7.6 Hz, 1H), 2.10 (dq, *J* = 13.9, 6.9 Hz, 1H), 1.00 (d, *J* = 6.8 Hz, 3H); MS (ESI+) *m/z* calcd for $[C_{27}H_{29}N_2O_4]^+$: 445.20 [*M* + H]$^+$; found: 445.34; *m/z* calcd for $[C_{27}H_{28}NaN_2O_4]^+$: 467.52 [*M* + Na]$^+$; found: 467.71.

Fmoc-Asn(Trt)-Pro-val-PABC-PNP (**42**)



$C_{62}H_{58}N_6O_{11}$
MW:: 1063,16

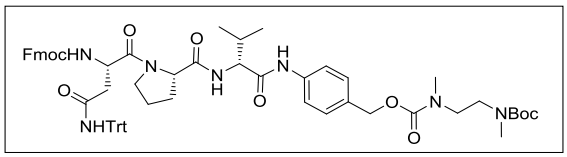
N-Fmoc-protected compound **39** (163 mg, 0.367 mmol, 1 equiv) was dissolved in DMF under a nitrogen atmosphere. The solution was cooled to 0 °C and piperidine (181 μL, 1.835 mmol, 5 equiv) was added. The reaction was stirred at room temperature, and after 2 h was concentrated under vacuum affording the desired free amine. The crude was used directly in the next step. A solution of Fmoc-L-Proline-OH (186 mg, 0.550 mmol, 1.5 equiv) in dry DMF (5 mL) was cooled to 0 °C under a nitrogen atmosphere. HATU (238 mg, 0.624 mmol, 1.7 equiv), HOAT (85 mg, 0.624 mmol, 1.7 equiv) and *i*Pr_2NEt (256 μL, 1.47 mmol, 4 equiv) were added and the mixture was stirred for 20 min at 0 °C. A solution of the above mentioned crude (82 mg, 0.367 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 solvent was removed and the crude was purified by flash chromatography [gradient: from CH_2Cl_2 99:1 to 99:3 CH_2Cl_2/MeOH] to afford intermediate **40**. The latter was subjected to the same protocol described above (Fmoc deprotection and coupling with Fmoc-Asn(Trt)-OH) to give intermediate **41**.

Fmoc-Asn(Trt)-Pro-val-PABA **41** was dissolved in dry THF (21 mL) under nitrogen atmosphere and cooled to 0 °C. Pyridine (74 μL, 0.918 mmol, 2.5 equiv) and 4-nitrophenylchloroformate (148 mg, 0.734 mmol, 2 equiv) were added, then the mixture could reach room temperature and stirred for 2 h. AcOEt (200 mL) was added and the solution was washed with a 1 M aqueous solution of $KHSO_4$ (3 × 20 mL) and brine (20 mL).

The organic phase was dried and concentrated, then the crude was purified by flash chromatography [gradient: from AcOEt/Hexane 1:1 to 7:3] affording compound **42** (185 mg, 47% yield, after five steps).

R_f = 0.36 (2:8, *n*-Hexane/EtOAc); ^{1}H NMR (400 MHz, CD_2Cl_2-d_2) δ 8.40 (s, 1H), 8.24 (d, *J* = 9.2 Hz, 2H), 7.77 (d, *J* = 7.6 Hz, 2H), 7.56 (d, *J* = 7.4 Hz, 2H), 7.47 – 7.12 (m, 27H), 5.93 (s, 1H), 5.22 (s, 2H), 4.65 (s, 1H), 4.50 – 4.28 (m, 3H), 4.19 (t, *J* = 6.7 Hz, 1H), 3.89 (d, *J* = 8.3 Hz, 1H), 3.49 (d, *J* = 8.9 Hz, 1H), 3.13 (s, 1H), 2.97 (t, *J* = 11.3 Hz, 1H), 2.63 (d, *J* = 14.0 Hz, 1H), 2.14 – 2.01 (m, 2H), 1.91 – 1.70 (m, 3H), 0.80 (dd, *J* = 15.9, 6.6 Hz, 6H). ^{13}C NMR (101 MHz, CD_2Cl_2-d_2) δ 171.67, 170.38, 169.87, 168.97, 155.61, 152.45, 145.41, 143.93, 143.72, 141.27, 138.92, 129.80, 129.39, 128.59, 127.94, 127.73, 127.09, 125.20, 125.06, 121.86, 120.11, 119.96, 70.82, 70.68, 67.14, 61.00, 60.24, 50.08, 47.39, 47.10, 40.21, 29.67, 28.91, 24.75, 19.35, 18.65. MS (MALDI-TOF): *m/z* calcd for $[C_{62}H_{59}N_6O_{11}]^+$: 1064.16 [*M* + H]$^+$; found: 1064.22 (HCCA matrix), 1065.25 (SA matrix).

Fmoc-Asn(Trt)-Pro-val-PABC-N-(Boc)-N,N′-dimethylethylenediamine (**43**)

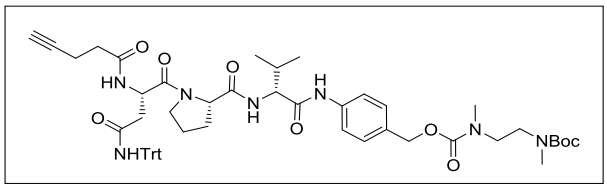


$C_{65}H_{73}N_7O_{10}$
MW: 1112,32

A solution of *N*-(Boc)-*N*,*N*′-dimethylethylenediamine (64 µL, 0.339 mmol, 2 equiv) in dry THF (2 mL) and *i*Pr_2NEt (74 µL, 0.423 mmol, 2.5 equiv) were added under nitrogen to a solution of **42** (180 mg, 0.169 mmol, 1 equiv) in dry THF (5 mL) kept at 0 ºC. The mixture was stirred overnight at room temperature, then the solvent was removed under vacuum. AcOEt (50 mL) was added and the solution was washed with 1 M aqueous solution of $KHSO_4$ (3 × 20 mL), a saturated aqueous solution of $NaHCO_3$ (2 × 20 mL) and brine (20 mL). The organic phase was dried and concentrated. The crude was purified by flash chromatography (8:2 AcOEt/*n*-Hexane), affording **43** (140 mg, 87% yield) as a white solid.

R_f = 0.19 (8:2 AcOEt/*n*-Hexane); ^{1}H NMR (400 MHz, MeOD-d_4) δ 7.80 (d, *J* = 7.6 Hz, 2H), 7.65 (t, *J* = 7.2 Hz, 4H), 7.39 (t, *J* = 7.6 Hz, 2H), 7.33 – 7.16 (m, 19H), 5.05 (s, 2H), 4.61 (t, *J* = 7.0 Hz, 1H), 4.51 – 4.32 (m, 3H), 4.24 – 4.15 (m, 2H), 3.63 (q, *J* = 6.9, 6.1 Hz, 1H), 3.42 – 3.35 (m, 4H), 3.29 – 3.18 (m, 1H), 2.91 (s, 3H), 2.85 (s, 2H), 2.77 – 2.71 (m, 3H), 2.18 – 2.04 (m, 2H), 2.01 – 1.85 (m, 3H), 1.42 (s, 9H), 0.91 (d, *J* = 5.9 Hz, 6H). ^{13}C NMR (101 MHz, MeOD-d_4) δ 173.23, 171.26, 170.46, 169.59, 156.66, 156.03, 144.33, 143.75, 141.19, 138.15, 138.03, 132.42, 132.20, 128.64, 128.27, 127.42, 127.34, 126.82, 126.38, 124.77, 124.74, 120.08, 119.56, 79.79, 79.50, 70.32, 66.88, 66.67, 60.68, 59.66, 50.09, 46.56, 46.15, 38.08, 34.18, 33.87, 33.23, 29.43, 29.34, 29.14, 27.32, 24.54, 18.44, 17.34. MS (MALDI-TOF): *m/z* calcd for $[C_{65}H_{73}N_7O_{10}]^+$: 1112.32 [*M* + H]$^+$; found: 1112.54 (HCCA matrix), 1112.55 (SA matrix).

4-pentynamido-Asn(Trt)-Pro-val-PABC-N-(Boc)-N,N'-dimethylethylenediamine (**44**)

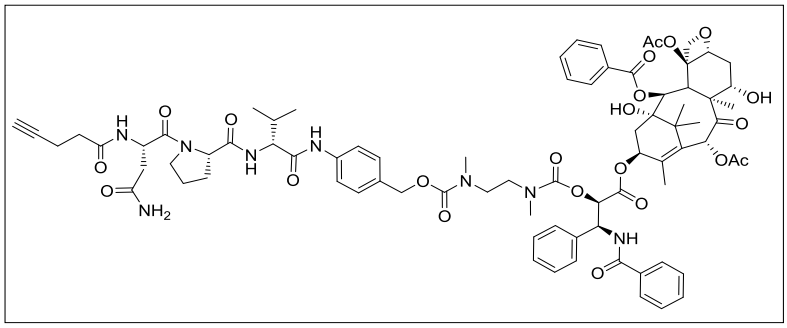


$C_{55}H_{67}N_7O_9$
MW: 970,16

N-Fmoc-protected compound **43** (153 mg, 0.138 mmol, 1 equiv) was dissolved in DMF under a nitrogen atmosphere. The solution was cooled to 0 °C and piperidine (70 µL, 0.688 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 under vacuum. CH_2Cl_2 was added to the residue and evaporated to afford a white solid (123 mg, 0.138 mmol) which was used directly in the next step. HATU (80 mg, 0.207 mmol, 1.7 equiv), HOAt (30 mg, 0.207 mmol, 1.7 equiv) and *i*Pr_2NEt (97 µL, 0.552 mmol, 4 equiv) were added to a solution of commercially available 4-pentynoic acid (25 mg, 0.235 mmol, 1.5 equiv) in dry DMF (2 mL) at 0 °C under a nitrogen atmosphere. The mixture was stirred for 20 min at 0 °C and a solution of the above mentioned crude (123 mg, 0.138 mmol, 1 equiv) in dry DMF (5 mL) was added. The reaction was allowed to slowly reach room temperature and stirred overnight. The mixture was diluted with a 4:1 AcOEt/CH_2Cl_2 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 crude compound was purified by flash chromatography [gradient: from CH_2Cl_2 100% to 97:3 CH_2Cl_2/MeOH] to afford amide **44** as fade white solid (125 mg, 94% yield over two steps).

R_f = 0.37 (95:5, CH_2Cl_2/MeOH); ^{1}H NMR (400 MHz, CD_2Cl_2-d_2) δ 8.26 – 8.16 (m, 1H), 7.62 – 7.46 (m, 1H), 7.35 (s, 2H), 7.28 – 7.15 (m, 18H), 6.72 (s, 1H), 5.03 (s, 2H), 4.83 (td, *J* = 7.8, 3.7 Hz, 1H), 4.46 (dd, *J* = 8.4, 3.4 Hz, 1H), 3.83 (s, 1H), 3.45 (s, 1H), 3.39 – 3.26 (m, 4H), 3.06 (s, 1H), 2.91 (s, 4H), 2.83 (s, 2H), 2.75 (s, 1H), 2.70 (dd, *J* = 14.1, 3.7 Hz, 1H), 2.46 (td, *J* = 7.6, 6.6, 2.4 Hz, 2H), 2.40 – 2.30 (m, 2H), 2.17 – 2.06 (m, 1H), 2.05 – 1.98 (m, 1H), 1.97 (t, *J* = 2.6 Hz, 1H), 1.88 – 1.76 (m, 2H), 1.71 (s, 1H), 1.42 (s, 9H), 0.79 (dd, *J* = 16.8, 6.6 Hz, 6H); ^{13}C NMR (101 MHz, CD_2Cl_2-d_2) δ 171.59, 170.42, 170.30, 169.58, 169.01, 144.01, 138.08, 132.47, 128.62, 128.37, 127.88, 127.01, 120.01, 70.70, 69.00, 66.68, 66.55, 60.98, 48.42, 47.32, 40.10, 34.84, 28.95, 28.57, 28.11, 24.66, 19.49, 18.72, 14.44. MS (MALDI-TOF): *m/z* calcd for $[C_{55}H_{67}NaN_7O_9]^+$: 993.16 [*M* + Na]$^+$; found: 993.20 (HCCA matrix), 993.22 (SA matrix).

4-pentynamido-Asn-Pro-val-PTX (**45**)

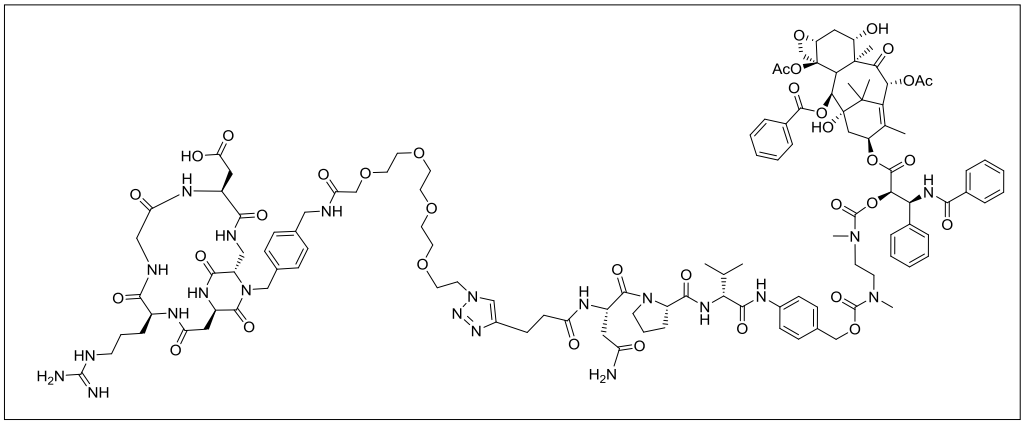


$C_{79}H_{94}N_8O_{22}$
MW: 1507,63

A solution of Boc-protected compound **44** (50 mg, 0.052 mmol, 1 equiv) in dry CH_2Cl_2 (1.8 mL) was cooled to 0 °C under a nitrogen atmosphere and TFA (900 µL) was added. The mixture was then allowed to reach room temperature and stirred for 15 min. The solvent was removed affording the corresponding trifluoroacetate salt, without further purifications. The solid was dissolved in dry DMF (800 µL) and *i*Pr_2NEt (37 µL, 0.208 mmol, 4 equiv). The resulting solution was added at 0 °C to a stirred solution of **37** (55 mg, 0.057 mmol, 1.1 equiv) in dry DMF (800 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 CH_2Cl_2 100% to 95:5 CH_2Cl_2/MeOH] to afford carbamate **45** as a light-yellow solid (41 mg, 54% yield over two steps).

R_f = 0.19 (95:5, CH_2Cl_2/MeOH); MS (MALDI-TOF): *m/z* calcd for $[C_{79}H_{94}NaN_8O_{22}]^+$: 1530.65 [*M* + Na]$^+$; found: 1530.08 (HCCA matrix), 1530.52 (SA matrix); HRMS (ESI+): *m/z* calcd for $[C_{79}H_{94}NaN_8O_{22}]^+$: 1529.64, [*M* + Na]$^+$: found 1529.63.

cyclo(DKP-RGD)-Asn-Pro-val-PTX conjugate (**6**)

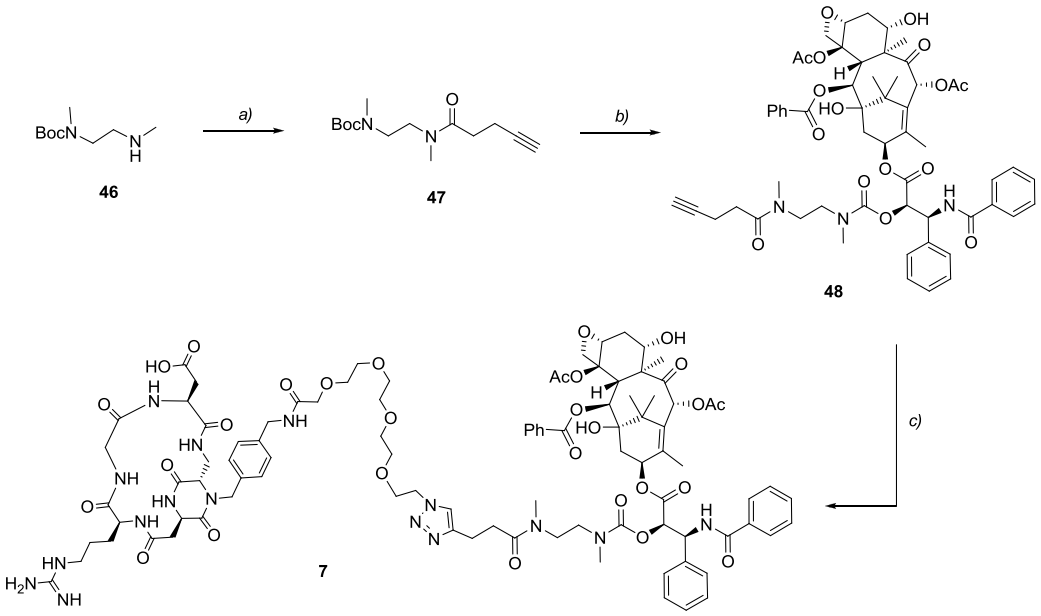


$C_{116}H_{149}N_{21}O_{35}$
MW: 2397,54 + TFA

Alkyne **45** (5.6 mg, 0.0036 mmol, 1.2 equiv) and *cyclo*(DKP-RGD)-PEG4-N_3[S6] (3 mg, 0.003 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 $CuSO_4 \cdot 5H_2O$ (0.45 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) = 12.5 min]. The purified product was then freeze-dried to give the desired compound **6** as a white solid (5 mg, 70% yield).

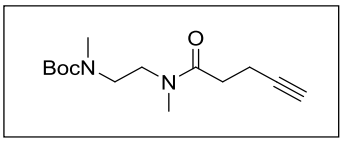
MS (MALDI-TOF): *m/z* calcd for $[C_{116}H_{150}N_{21}O_{35}]^+$: 2398.52 $[M + H]^+$; found: 2398.29 (HCCA matrix), 2399.52 (SA matrix); *m/z* calcd for $[C_{116}H_{149}NaN_{21}O_{35}]^+$: 2420.22 $[M + Na]^+$; found: 2420.32 (HCCA matrix), 2420.52 (SA matrix); HRMS (ESI+): *m/z* calcd for $[C_{116}H_{150}NaN_{21}O_{35}]^{2+}$: 1210.02, $[M + Na]^{2+}$; found 1210.03; *m/z* calcd for $[C_{116}H_{150}Na_2N_{21}O_{35}]^{3+}$: 814.34, $[M + 2Na]^{3+}$ found 814.35.

Synthesis of *cyclo*(DKP-RGD)-Uncleavable-PTX conjugate (7)



Scheme S6. Synthesis of *cyclo*(DKP-RGD)-uncleavable-PTX conjugate (**7**). REAGENTS AND CONDITIONS: *a)* 4-pentynoic acid, HATU, HOAt, *i*Pr_2NEt, DMF, RT, overnight, Y.: 98%; *b)* [1] TFA/CH_2Cl_2 (1:2), 45 min; [2] **37**, *i*Pr_2NEt, DMF, RT, overnight, Y.: 84% (over two steps); *c) cyclo*(DKP-RGD)-PEG4-N_3, $CuSO_4 \cdot 5H_2O$, sodium ascorbate, DMF/H_2O (1:1), 30 ºC, overnight, Y.: quant.

tert-butyl methyl(2-(N-methylpent-4-ynamido)ethyl)carbamate (**47**)



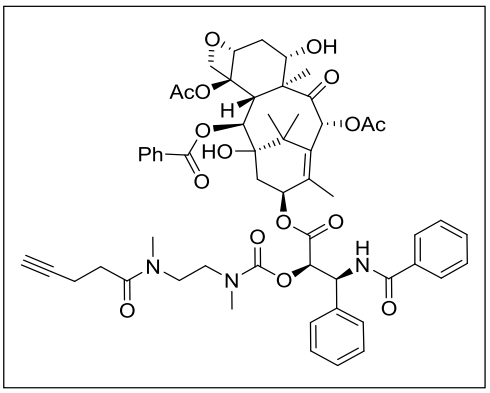
$C_{14}H_{24}N_2O_3$
MW: 268,35

A solution of commercial 4-pentynoic acid (78 mg, 0.796 mmol, 1.5 equiv) in dry DMF (4 mL) was cooled to 0 °C under a nitrogen atmosphere. HATU (343 mg, 0.902 mmol, 1.7 equiv), HOAT (123 mg, 0.902 mmol, 1.7 equiv) and *i*Pr_2NEt (370 µL, 2.124 mmol, 4 equiv) were added and the mixture was stirred for 20 min at 0 °C. A solution of *N*-(Boc)-*N*,*N'*-dimethylethylenediamine **46** (100 mg, 0.531 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 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 purified by flash chromatography [eluent: CH_2Cl_2 100%] to afford amide **47** as dark yellow oil (140 mg, 98%).

R_f = 0.46 (95:5, CH_2Cl_2/MeOH); ^{1}H NMR (400 MHz, CD_2Cl_2-d_2) δ 3.43 – 3.31 (m, 2H), 3.24 (t, *J* = 6.2 Hz, 2H), 2.91 (s, rotamer A, 3H), 2.83 (s, rotamer B, 3H), 2.75 (s, rotamer A + B, 3H), 2.49 – 2.36 (m, 4H), 1.90 (q, *J* = 2.2 Hz, 1H), 1.35 (s, rotamer A + B, 9H); ^{13}C NMR (101 MHz, CD_2Cl_2-d_2) δ 171.14, 156.27, 84.35,

79.57, 68.74, 48.30 (rotamer A), 48.09 (rotamer B), 47.72, 46.95 (rotamer A), 46.60 (rotamer B), 46.22 (rotamer A), 45.72 (rotamer B), 36.35 (rotamer A), 35.05, 34.24 (rotamer B), 33.08 (rotamer A), 32.20 (rotamer B), 28.71, 15.04 (rotamer A), 14.76 (rotamer B). MS (ESI +): *m/z* calcd for $[C_{14}H_{25}N_2O_3]^+$: 269.10 $[M + H]^+$; found: 269.09.

4-pentynamido-PTX (**48**)

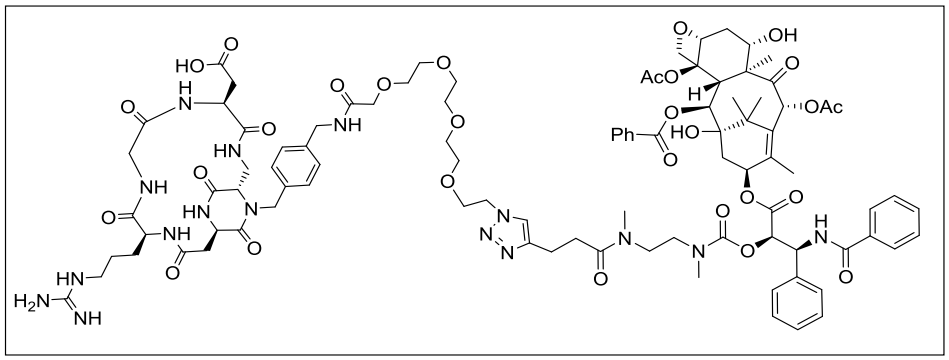


$C_{57}H_{65}N_3O_{16}$
MW: 1048,14

A solution of Boc-protected compound **47** (67 mg, 0.249 mmol, 1 equiv) in dry CH_2Cl_2 (8 mL) was cooled to 0 °C under a nitrogen atmosphere and TFA (4 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 dark yellow oil was dissolved in dry DMF (0.5 mL) and *i*Pr_2NEt (87 µL, 0.5 mmol, 10 equiv). The resulting solution was added at 0 °C to a stirred solution of **37** (50 mg, 0.05 mmol, 1 equiv) in dry DMF (1 mL), under a nitrogen atmosphere. The reaction was then allowed to reach room temperature and stirred overnight. AcOEt (50 mL) was added and the solution was washed with a 1 M aqueous solution of $KHSO_4$ (2 × 5 mL) and brine (1 × 5 mL). The organic phase was dried over Na_2SO_4 and concentrated, then the crude was purified by flash chromatography [gradient: from AcOEt/*n*-Hexane 1:1 to 4:1] to afford carbamate **48** as a white solid (41 mg, 54% yield over two steps).

R_f = 0.31 (4:1, AcOEt/*n*-Hexane 1:1); ^{1}H NMR (400 MHz, $CDCl_3$-*d*) δ 8.71 (d, *J* = 9.8 Hz, 1H), 8.21 – 8.15 (m, 2H), 7.87 – 7.79 (m, 2H), 7.61 – 7.55 (m, 1H), 7.54 – 7.46 (m, 5H), 7.44 – 7.34 (m, 5H), 7.33 – 7.27 (m, 2H), 6.35 – 6.28 (m, 2H), 6.19 (dd, *J* = 9.8, 3.0 Hz, 1H), 5.69 (d, *J* = 7.3 Hz, 1H), 5.45 (d, *J* = 3.0 Hz, 1H), 5.00 (dd, *J* = 10.0, 2.3 Hz, 1H), 4.47 (td, *J* = 6.8, 3.3 Hz, 1H), 4.35 – 4.29 (m, 1H), 4.24 (dd, *J* = 8.4, 1.0 Hz, 1H), 4.00 – 3.89 (m, 1H), 3.84 (d, *J* = 7.1 Hz, 1H), 3.64 – 3.54 (m, 1H), 3.12 – 3.06 (m, 1H), 3.05 – 2.98 (m, 4H), 2.98 – 2.94 (m, 2H), 2.90 (s, 3H), 2.63 – 2.52 (m, 5H), 2.51 – 2.46 (m, 3H), 2.24 – 2.21 (m, 3H), 1.99 (d, *J* = 1.4 Hz, 3H), 1.93 (t, *J* = 2.6 Hz, 2H), 1.69 (s, 3H), 1.23 (s, 3H), 1.13 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$-*d*) δ 133.56, 131.28, 130.31, 128.75, 128.67, 127.97, 127.95, 127.75, 126.81, 84.51, 83.14, 77.22, 76.48, 75.82, 75.71, 75.24, 72.14, 71.35, 52.73, 46.76, 45.80, 45.57, 36.78, 35.81, 35.55, 35.52, 32.34, 30.32, 29.69, 26.78, 22.79, 22.69, 22.35, 20.82, 14.88, 14.25, 14.11, 9.66. MS (MALDI-TOF): *m/z* calcd for $[C_{57}H_{66}N_3O_{16}]^+$: 1049.14 $[M + H]^+$; found: 1049.22 (HCCA matrix); *m/z* calcd for $[C_{57}H_{65}NaN_3O_{16}]^+$: 1071.14 $[M + Na]^+$; found: 1071.26.

cyclo(DKP-RGD)-Uncleavable-PTX (**7**)



$C_{94}H_{120}N_{16}O_{29}$
MW: 1938,05 + TFA

Alkyne **48** (7 mg, 0.0065 mmol, 1.3 equiv) and *cyclo*(DKP-RGD)-PEG4-N_3[S6] (5 mg, 0.005 mmol, 1 equiv) were dissolved in a degased 1:1 mixture of H_2O/DMF (400 μL) under a nitrogen atmosphere. Degased aqueous solutions of $CuSO_4 \cdot 5H_2O$ (0.74 mg, 0.5 equiv) and sodium ascorbate (0.60 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 min]. The purified product was then freeze-dried to give the desired compound **7** as a white solid (9.7 mg, 100% yield).

MS (MALDI-TOF): *m/z* calcd for $[C_{94}H_{121}N_{16}O_{29}]^+$: 1939.05 [*M* + H]$^+$; found: 1939.70 (HCCA matrix); HRMS (ESI+): *m/z* calcd for $[C_{94}H_{121}N_{16}O_{29}]^+$: 1937.85, [*M* + H]$^+$; found 1937.85; *m/z* calcd for $[C_{94}H_{120}NaN_{16}O_{29}]^+$: 1959.83, [*M* + Na]$^+$; found 1959.83; *m/z* calcd for $[C_{94}H_{120}Na_2N_{16}O_{29}]^{2+}$: 991.41, [*M* + 2Na]$^{2+}$ found 991.41; *m/z* calcd for $[C_{94}H_{120}NaN_{16}O_{29}]^{2+}$: 980.41, [*M* + Na]$^{2+}$; found 980.42; *m/z* calcd for $[C_{94}H_{120}Na_3N_{16}O_{29}]^{2+}$: 1002.40, [*M* + 3Na]$^{2+}$; found 1002.41.

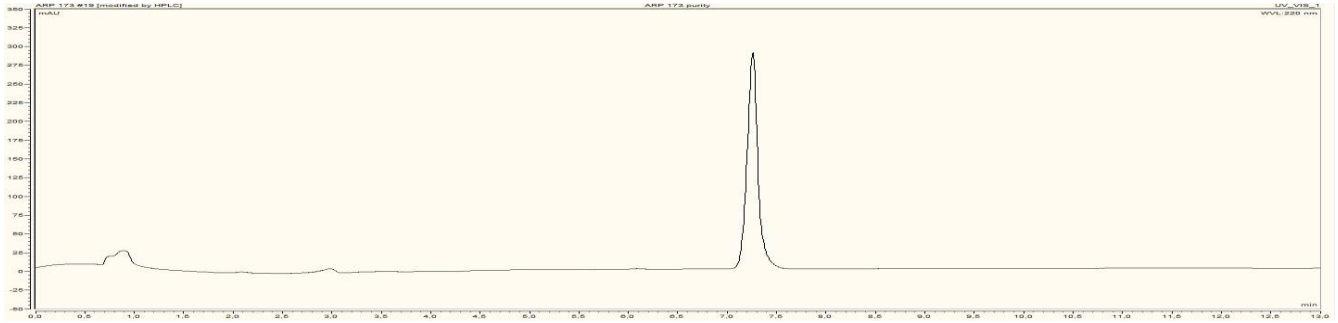
GENERAL PROCEDURE FOR Boc DEPROTECTION REACTIONS

To a 0.03 M CH_2Cl_2 solution of the *N*-Boc-protected compound (**16**, **19**, **25**) half volume of TFA was added, and the reaction was stirred at r.t. for 1 h. The solvent was evaporated and then CH_2Cl_2 was added for five times to the residue followed by evaporation under vacuum, to afford the amine TFA salt as a crude compound which was used in the next step without further purification.

HPLC Traces of Final Products

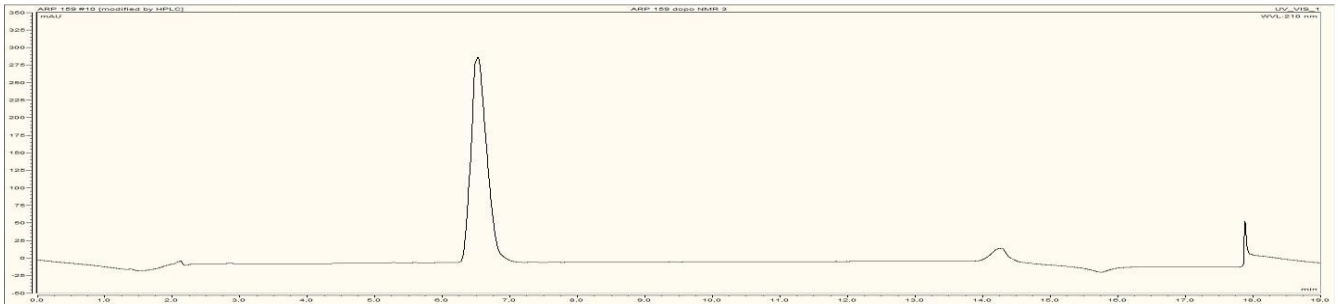
cyclo(DKP-RGD)-sCy5 conjugate (**2**)

Waters Atlantis 21 mm × 10 cm column, gradient: 90% (H_2O + 0.1% CF_3COOH) / 10% (MeCN + 0.1% CF_3COOH) to 40% (H_2O + 0.1% CF_3COOH) / 60% (MeCN + 0.1% CF_3COOH) in 9 min.



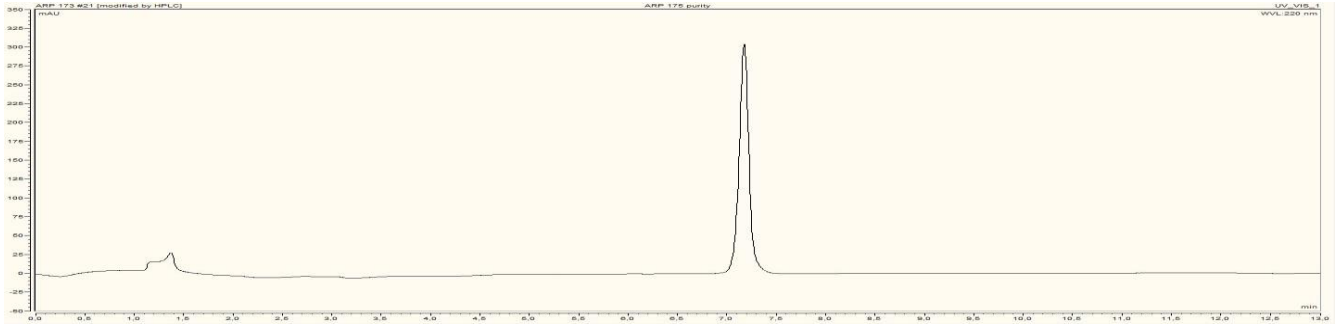
cyclo(DKP-RβAD) (**3**)

Waters Atlantis 21 mm × 10 cm column, gradient: 95% (H_2O + 0.1% CF_3COOH) / 5% (MeCN + 0.1% CF_3COOH) to 78% (H_2O + 0.1% CF_3COOH) / 22% (MeCN + 0.1% CF_3COOH) in 10 min.



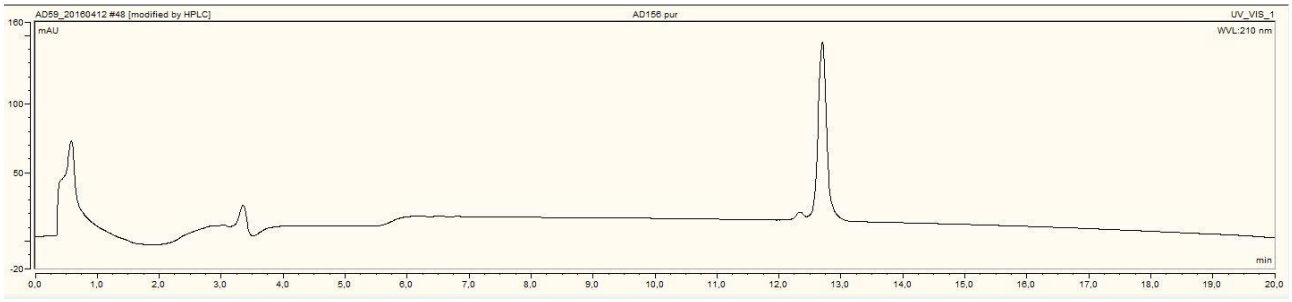
cyclo(DKP- RβAD)-sCy5 conjugate (**4**)

Waters Atlantis 21 mm × 10 cm column, gradient: 90% (H_2O + 0.1% CF_3COOH) / 10% (MeCN + 0.1% CF_3COOH) to 40% (H_2O + 0.1% CF_3COOH) / 60% (MeCN + 0.1% CF_3COOH) in 9 min.



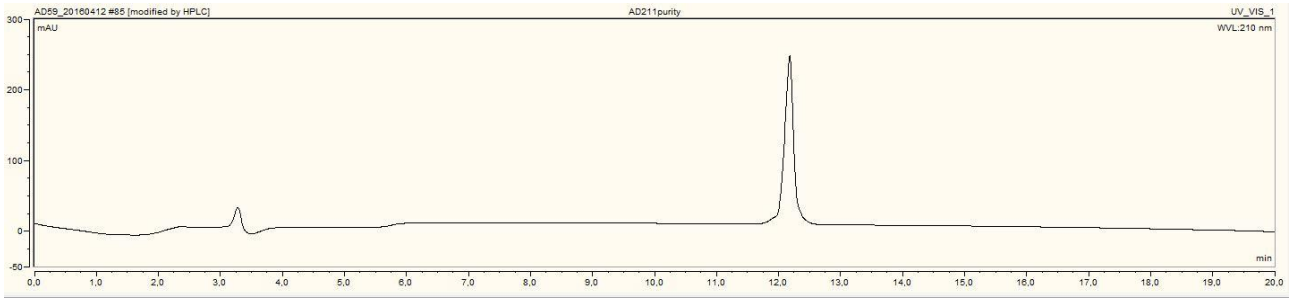
cyclo(DKP-RGD)-Asn-Pro-Val-PTX conjugate (**5**)

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.



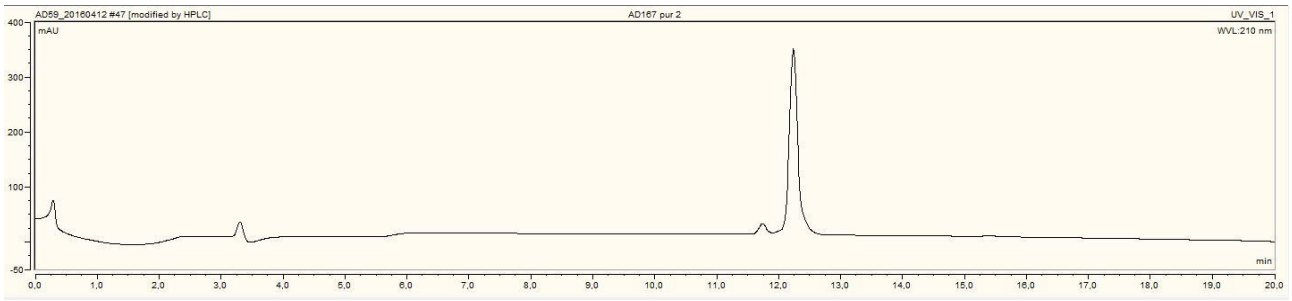
cyclo(DKP-RGD)-Asn-Pro-[D]Val-PTX conjugate (**6**)

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.



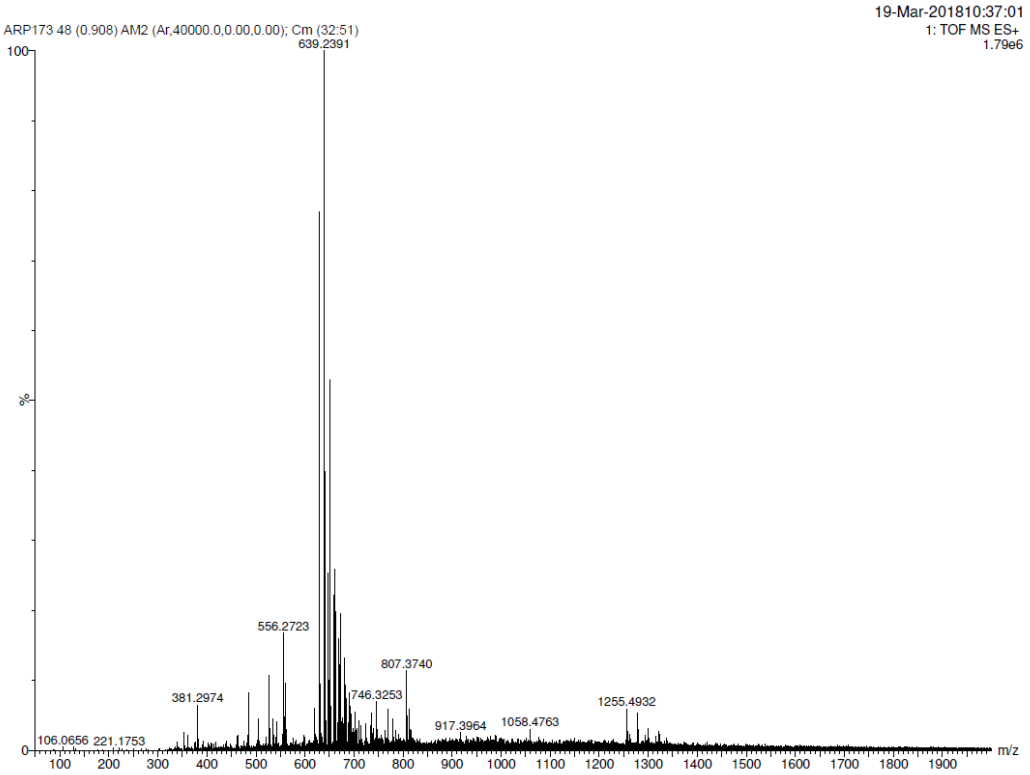
cyclo(DKP-RGD)-unc-PTX conjugate (**7**)

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 20 min.

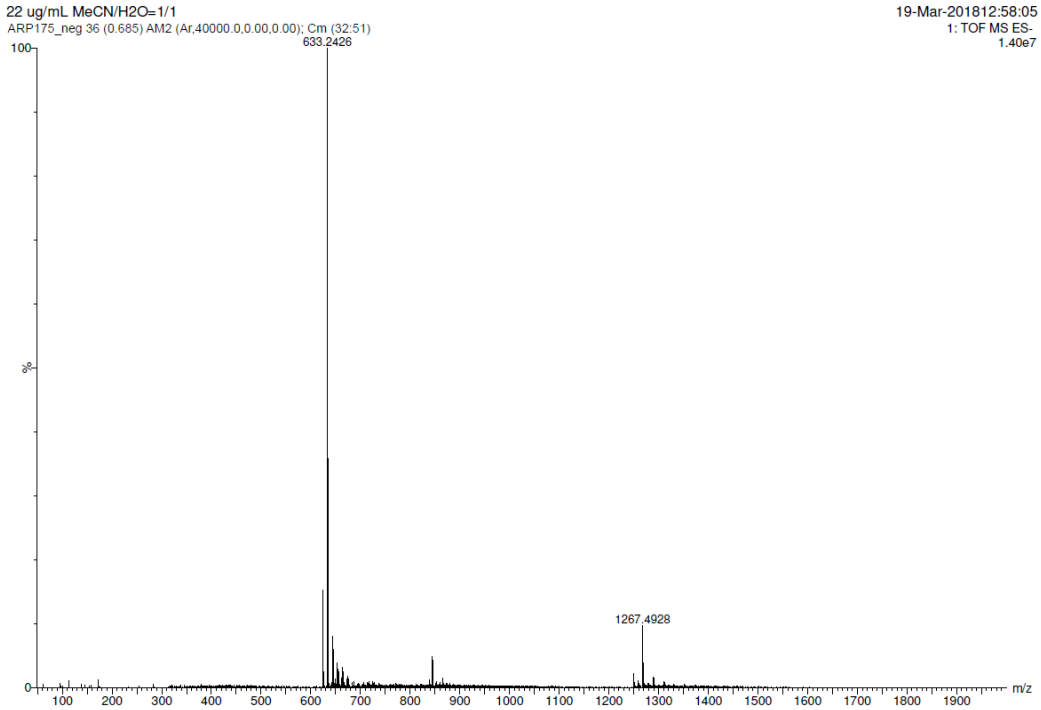


High-Resolution Mass Spectrometry Data

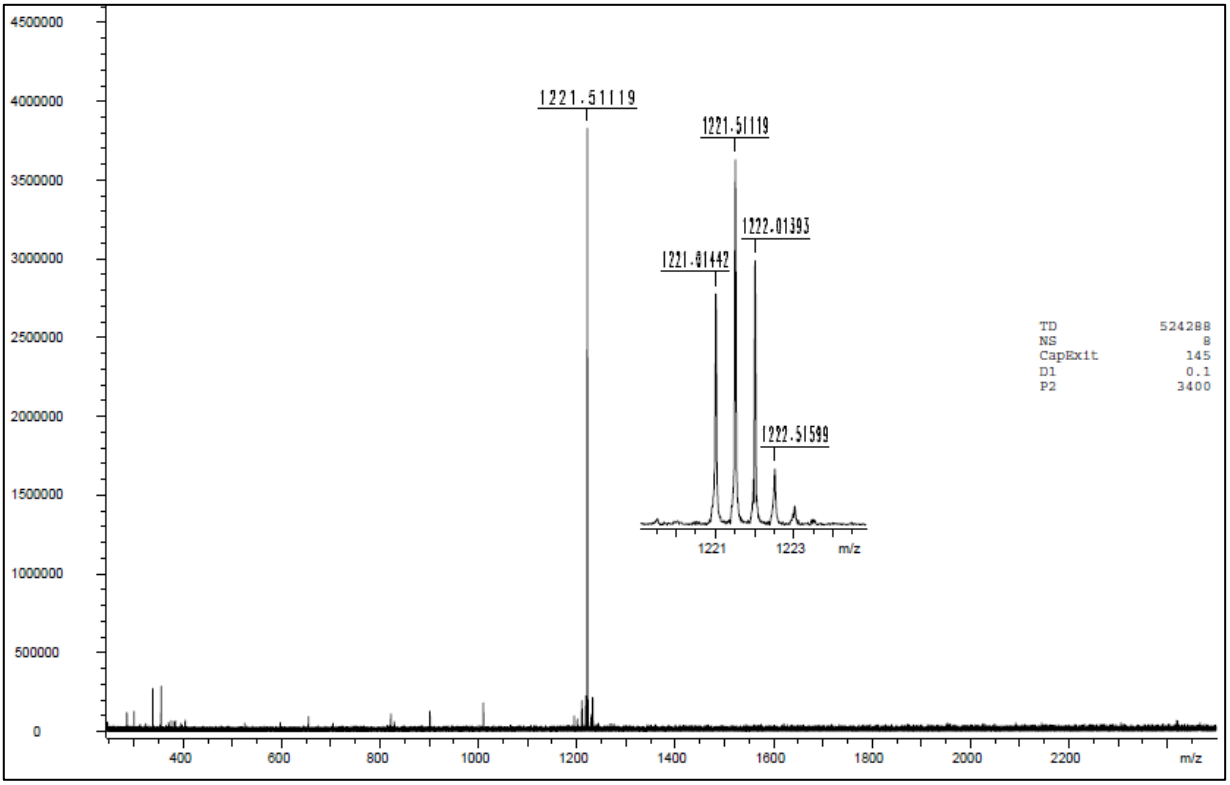
cyclo(DKP-RGD)-sCy5 conjugate (**2**)



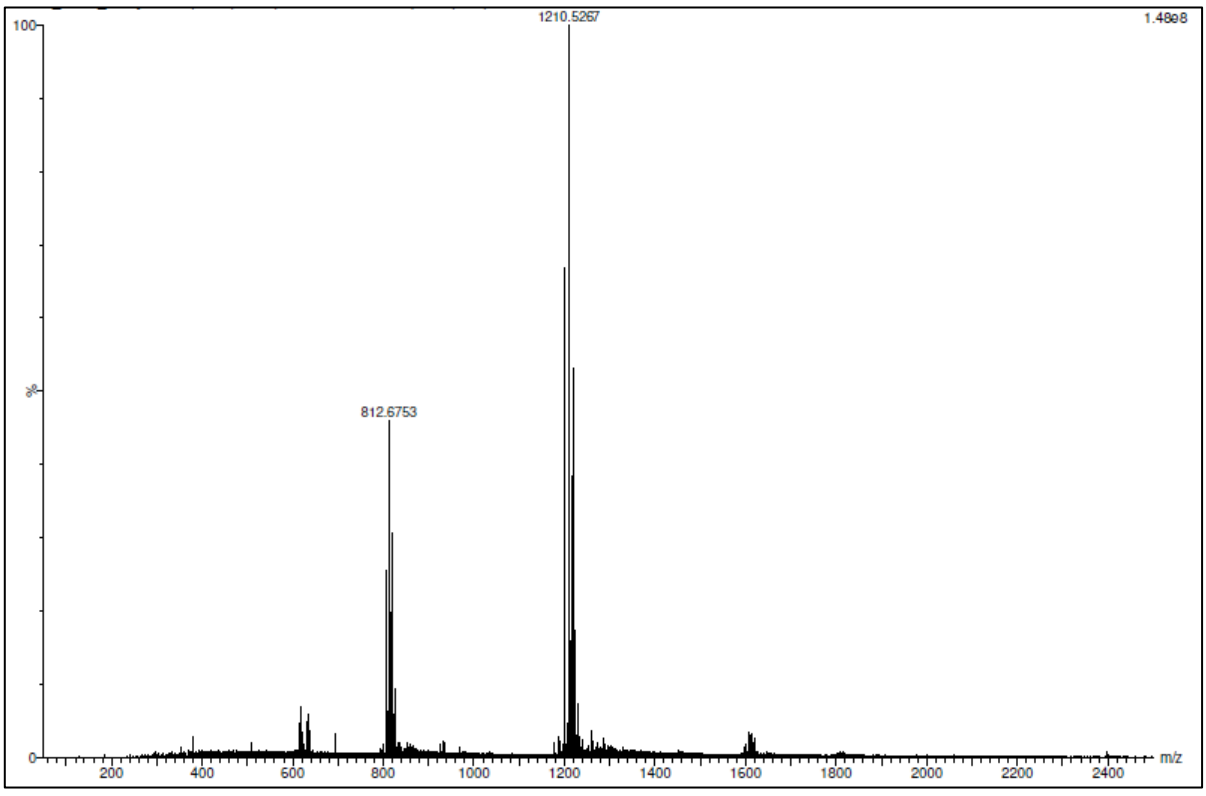
cyclo(DKP- RβAD)-sCy5 conjugate (**4**)



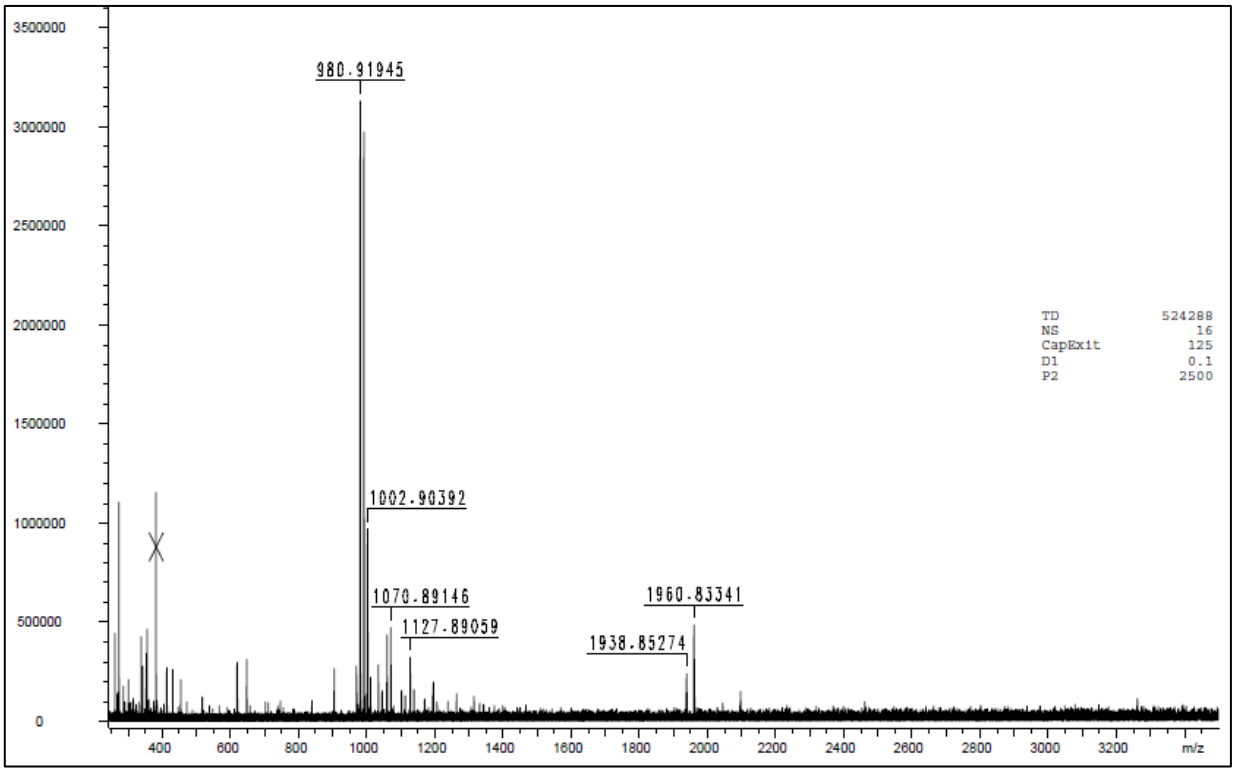
cyclo(DKP-RGD)-Asn-Pro-Val-PTX conjugate (**5**)



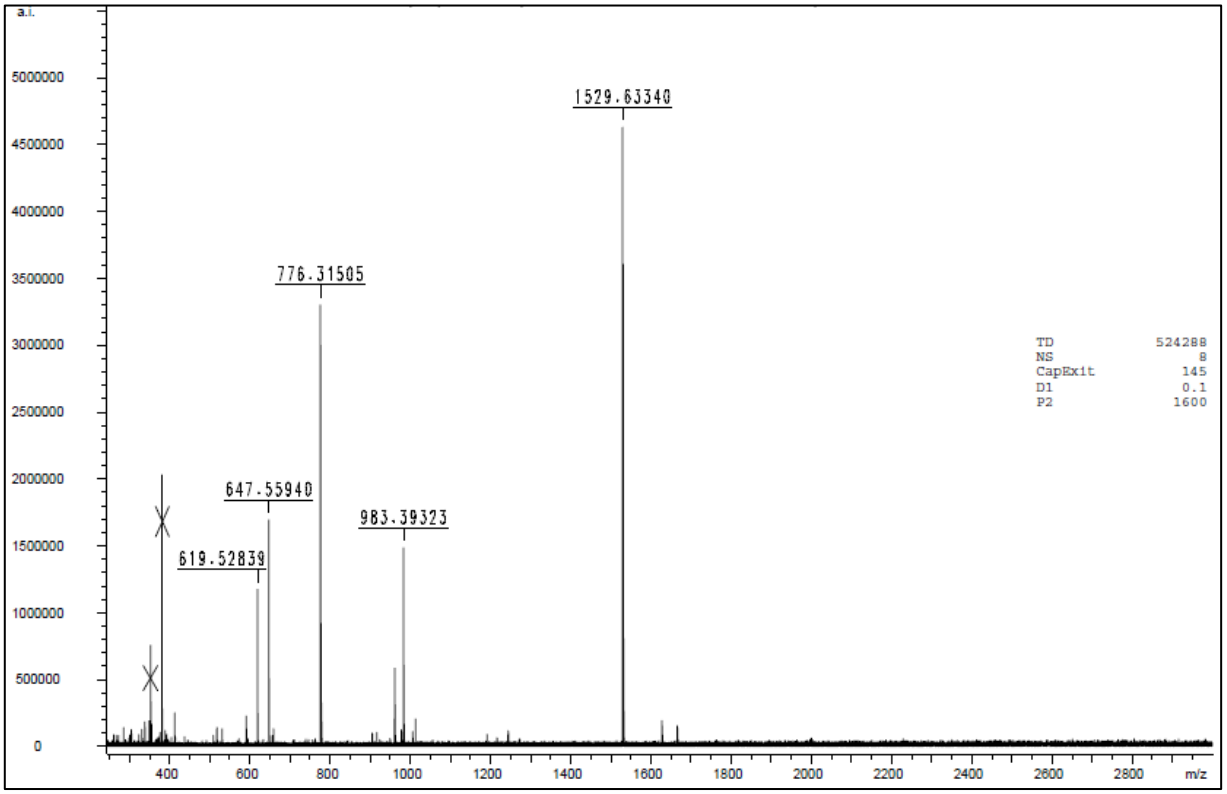
cyclo(DKP-RGD)-Asn-Pro-[D]Val-PTX conjugate (**6**)



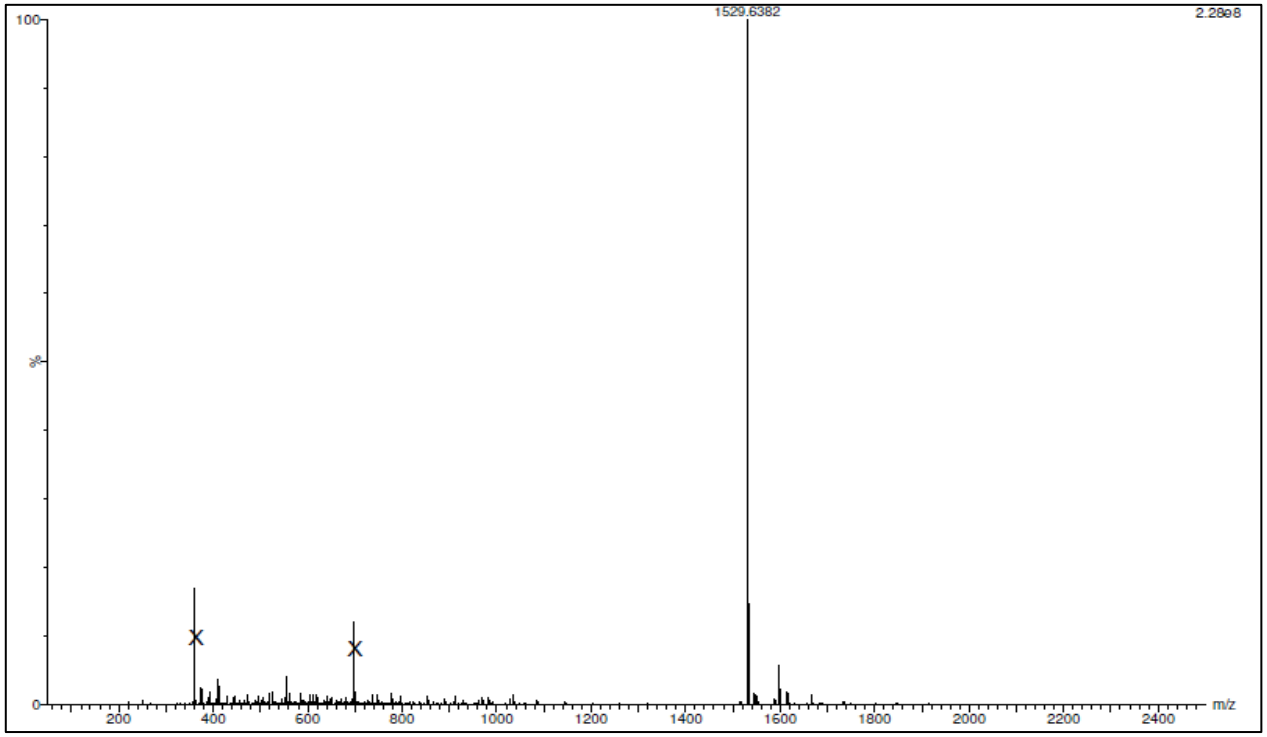
cyclo(DKP-RGD)-unc-PTX conjugate (**7**)



4-pentynamido-Asn-Pro-Val-PTX (**38**)



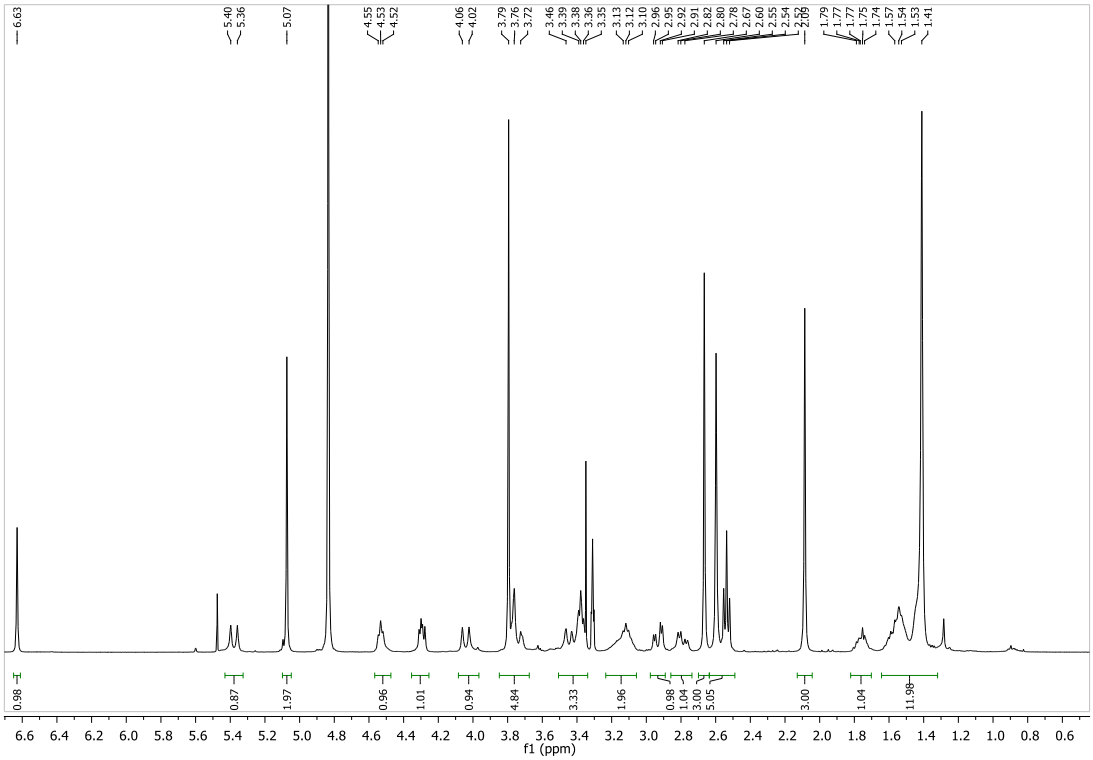
4-pentynamido-Asn-Pro-val-PTX (**45**)



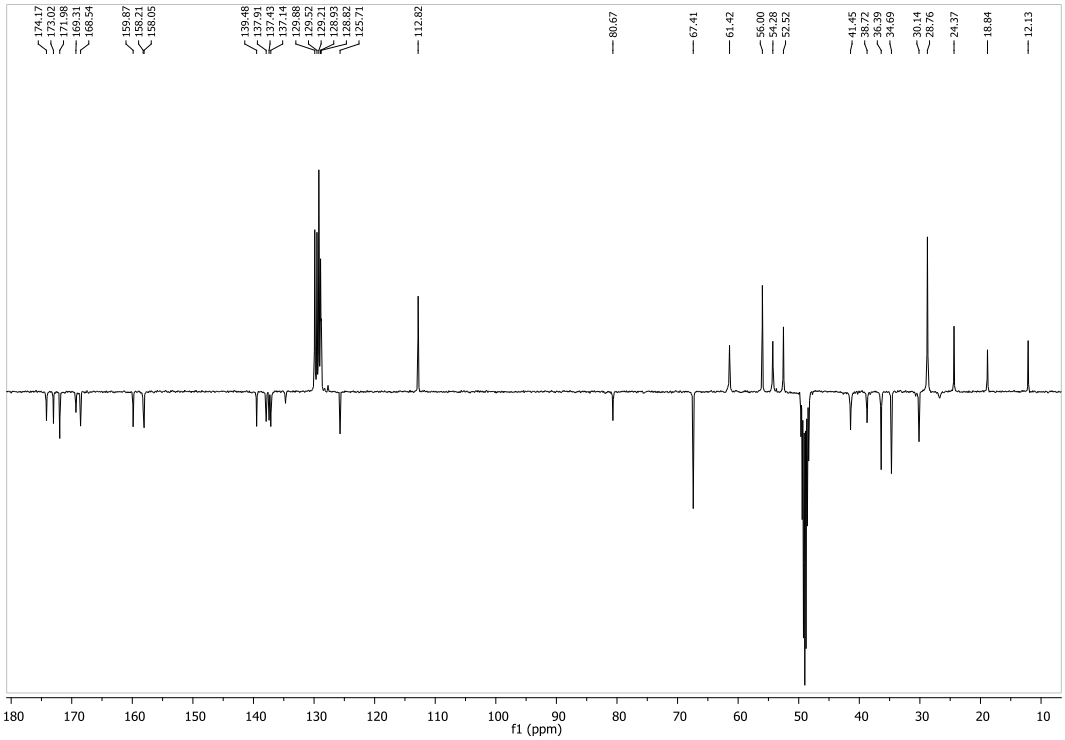
^{1}H-NMR and ^{13}C-NMR Spectra

Boc-DKP-Arg(Mtr)-βAla-OBn (**19**)

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

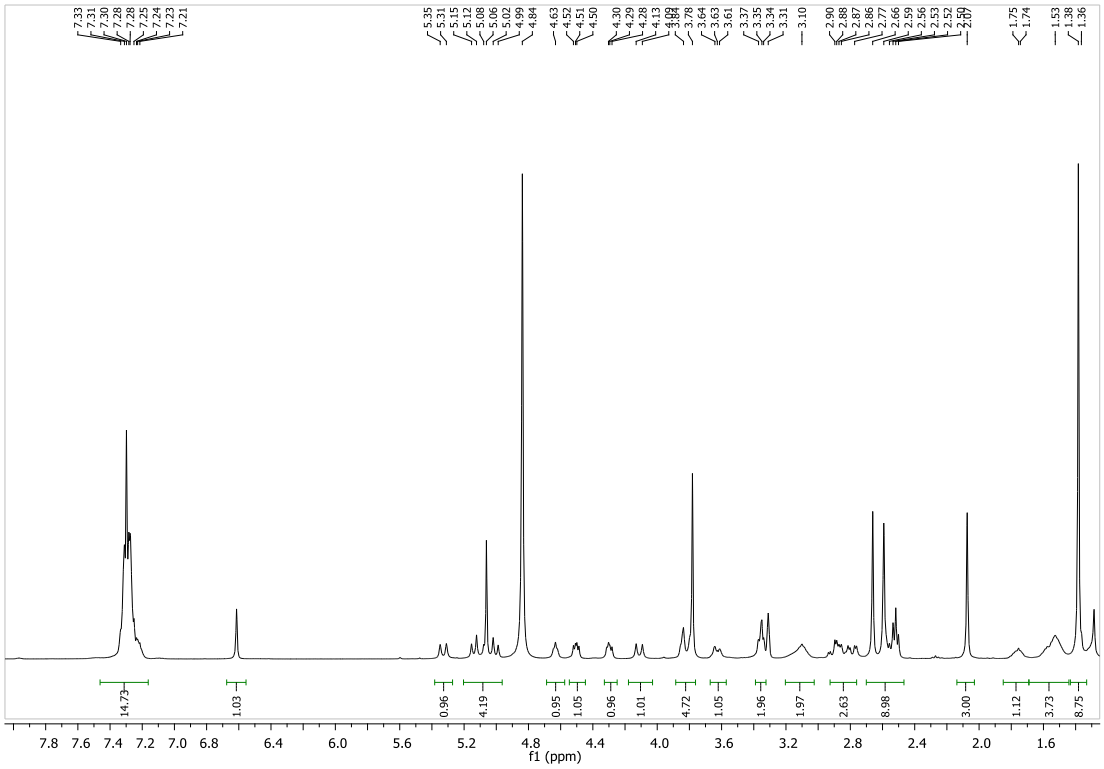


^{13}C NMR (101 MHz, MeOD)

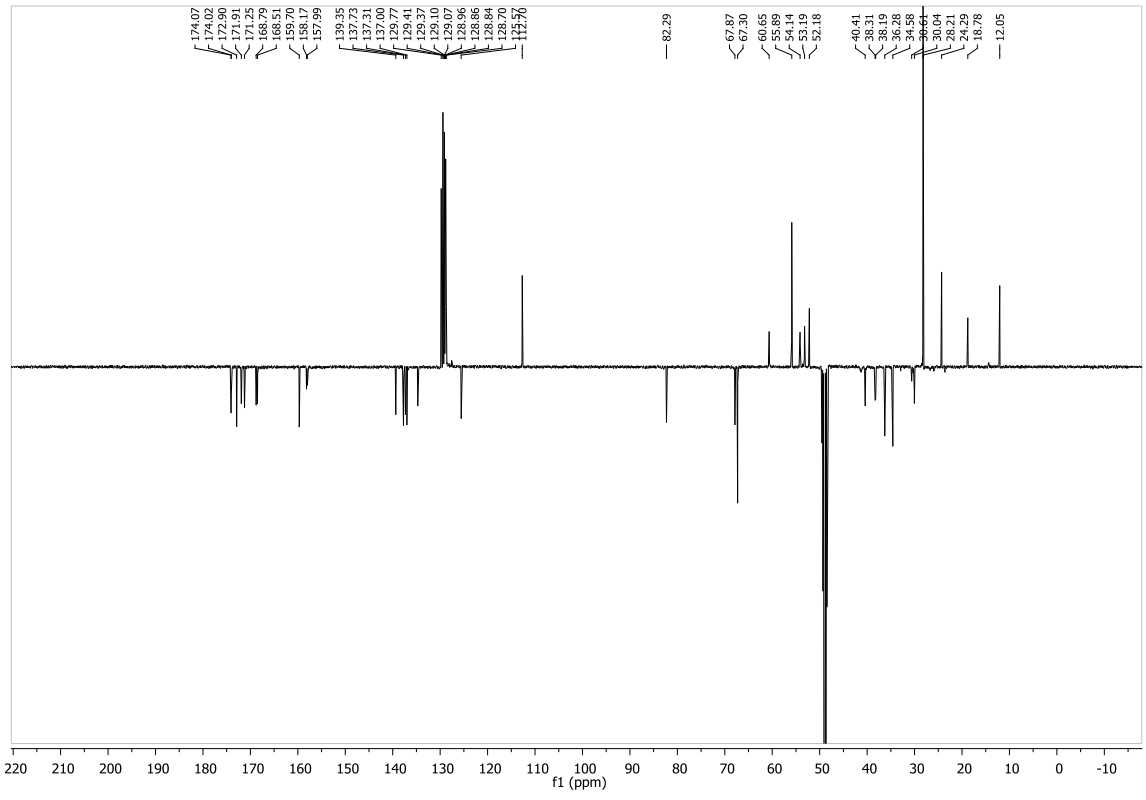


Cbz-Asp(OtBu)-DKP-Arg(Mtr)-βAla-OBn (**21**)

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

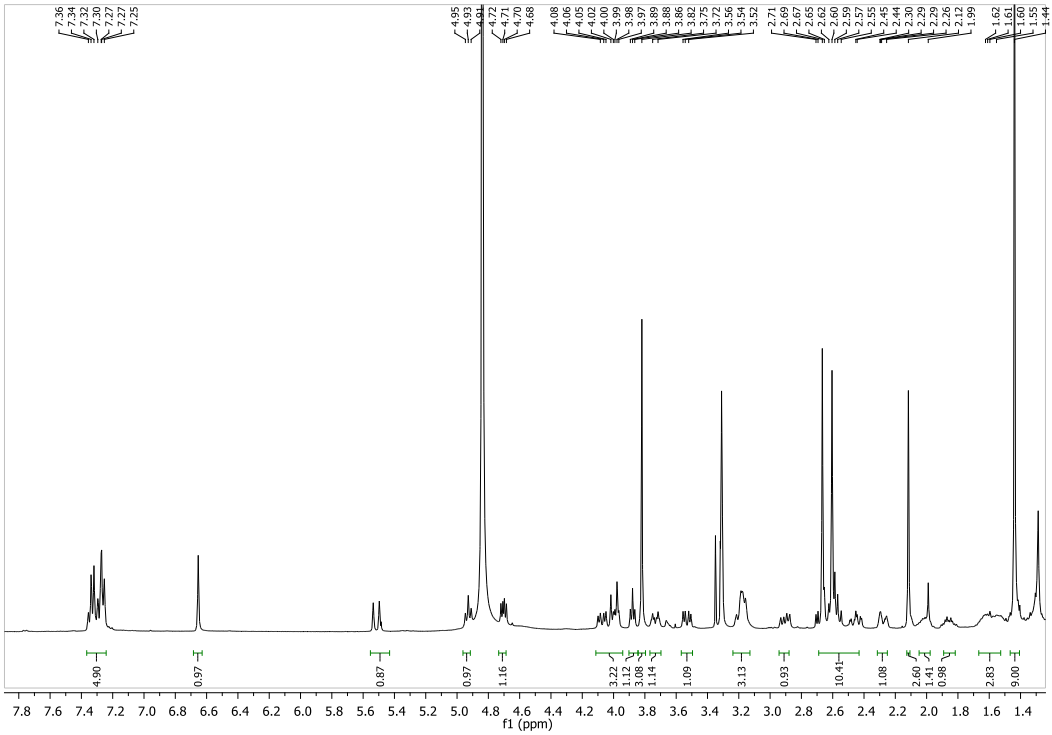


^{13}C NMR (101 MHz, MeOD)

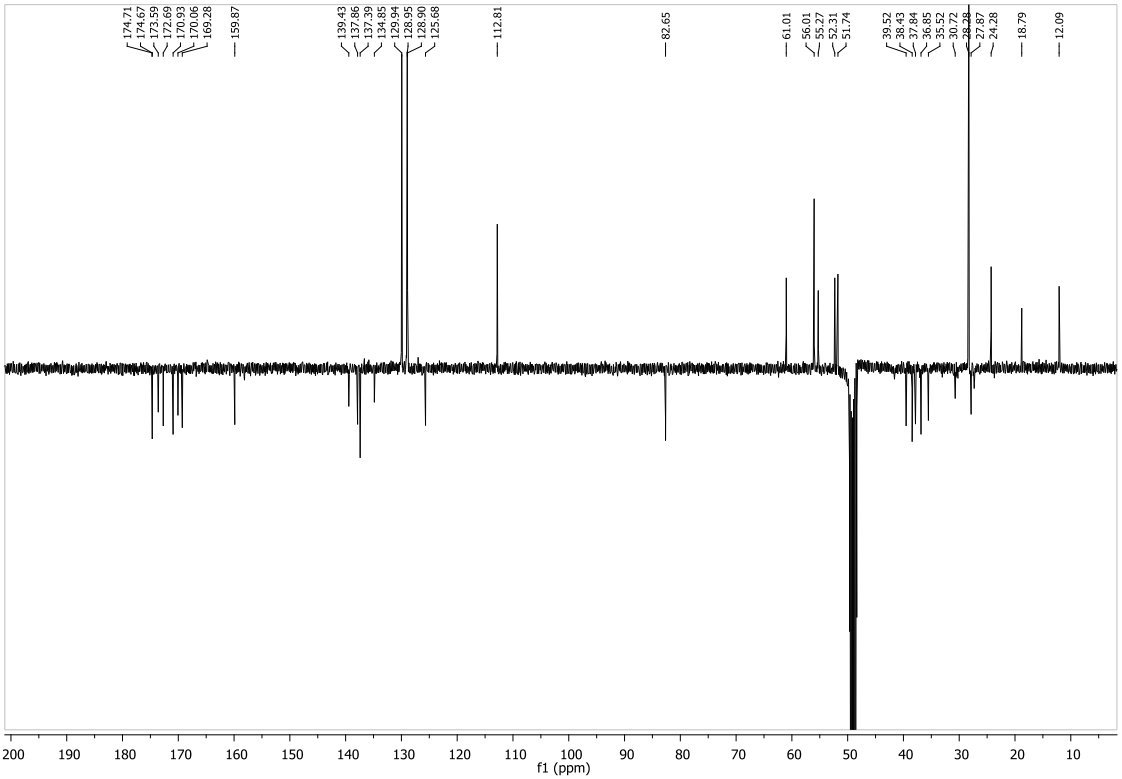


cyclo[DKP-Arg(Mtr)-βAla-Asp(OtBu)] (**23**)

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

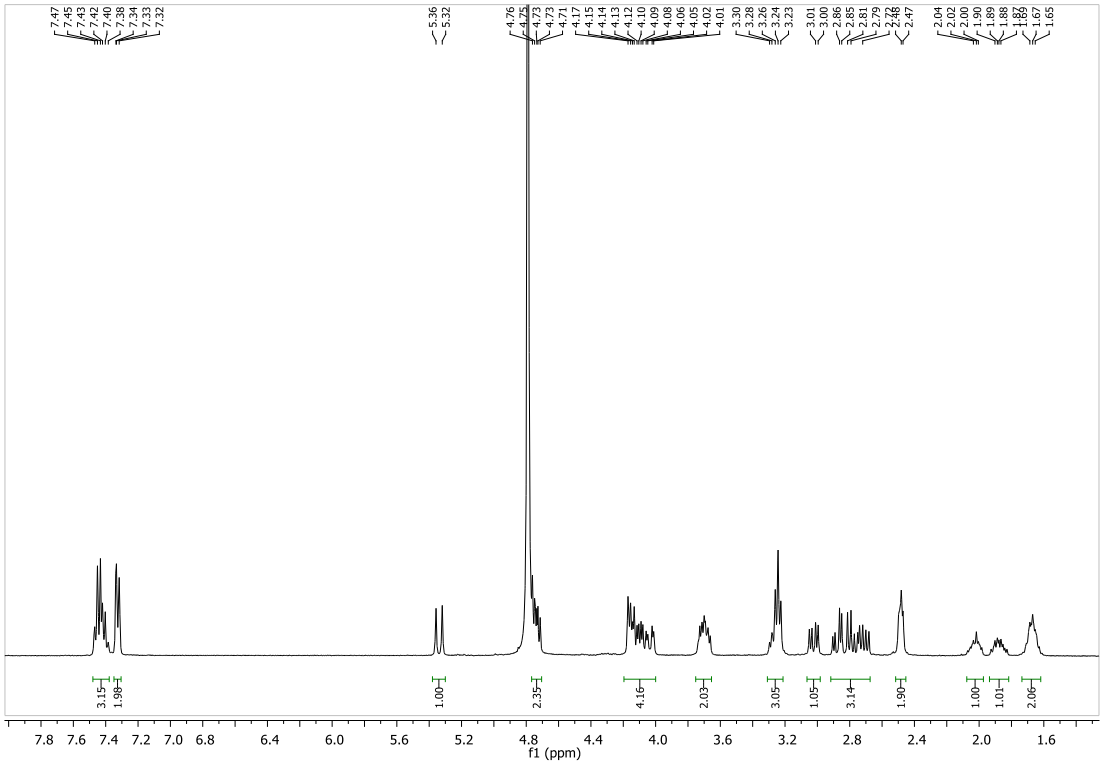


^{13}C NMR (101 MHz, MeOD)

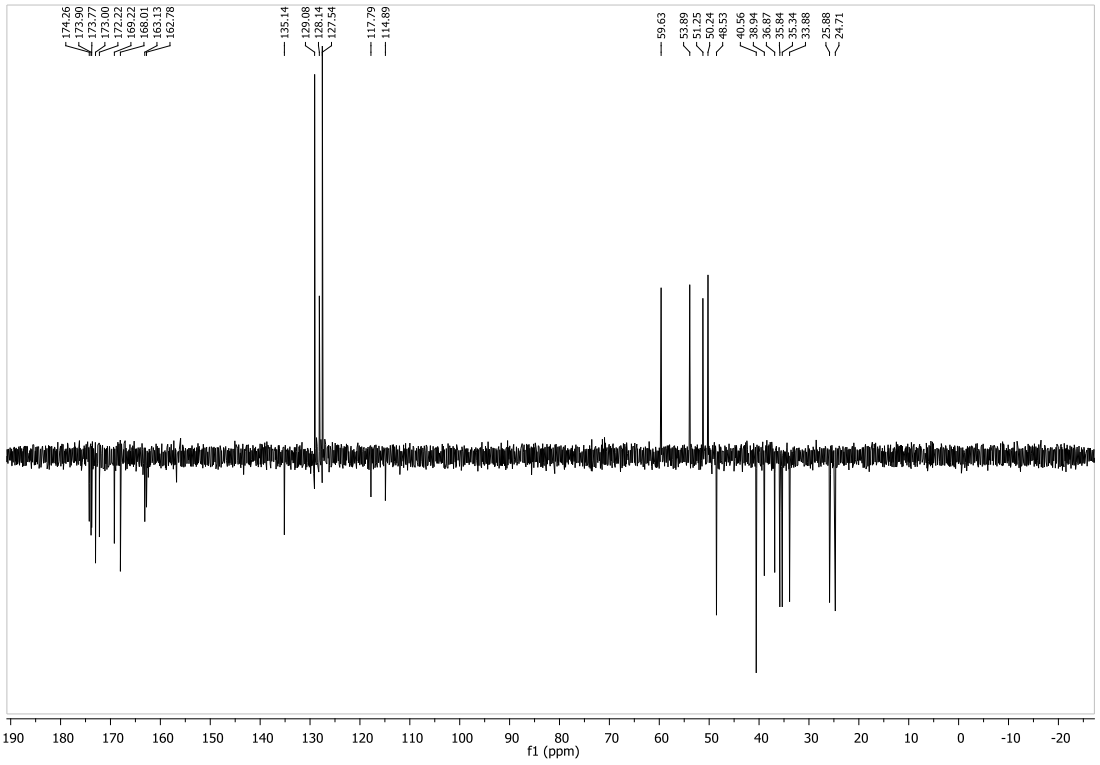


cyclo(DKP-RβAD) (**3**)

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

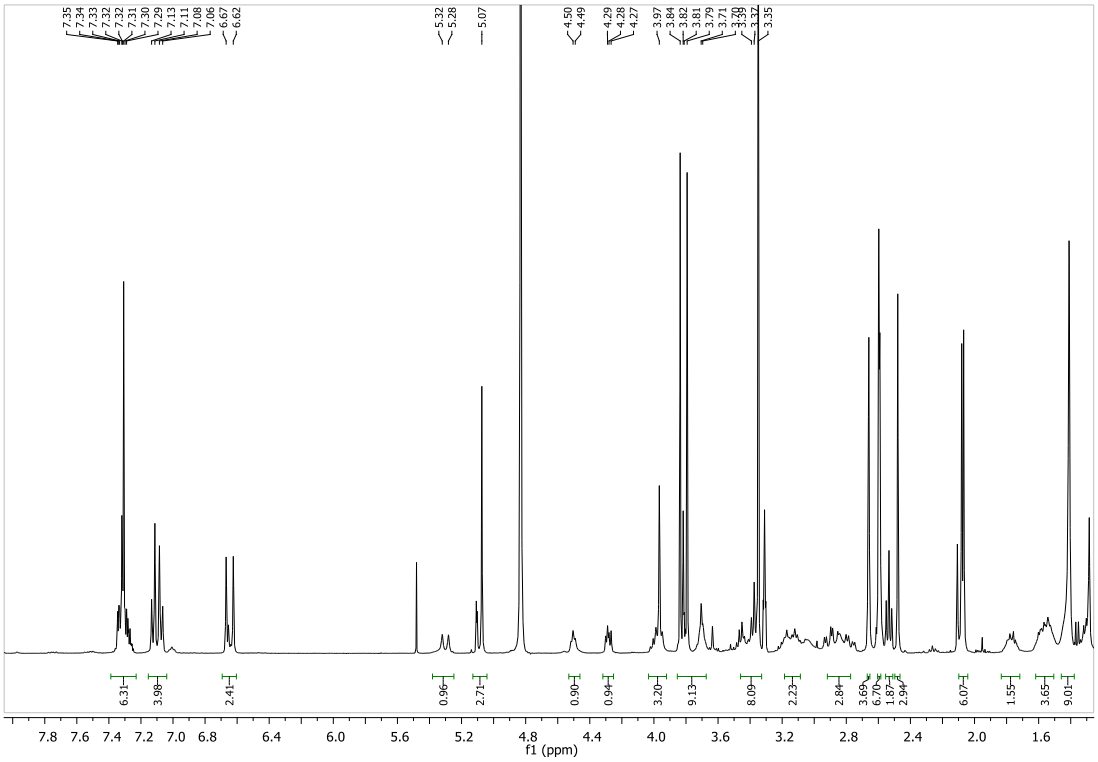


^{13}C NMR (101 MHz, D_2O)

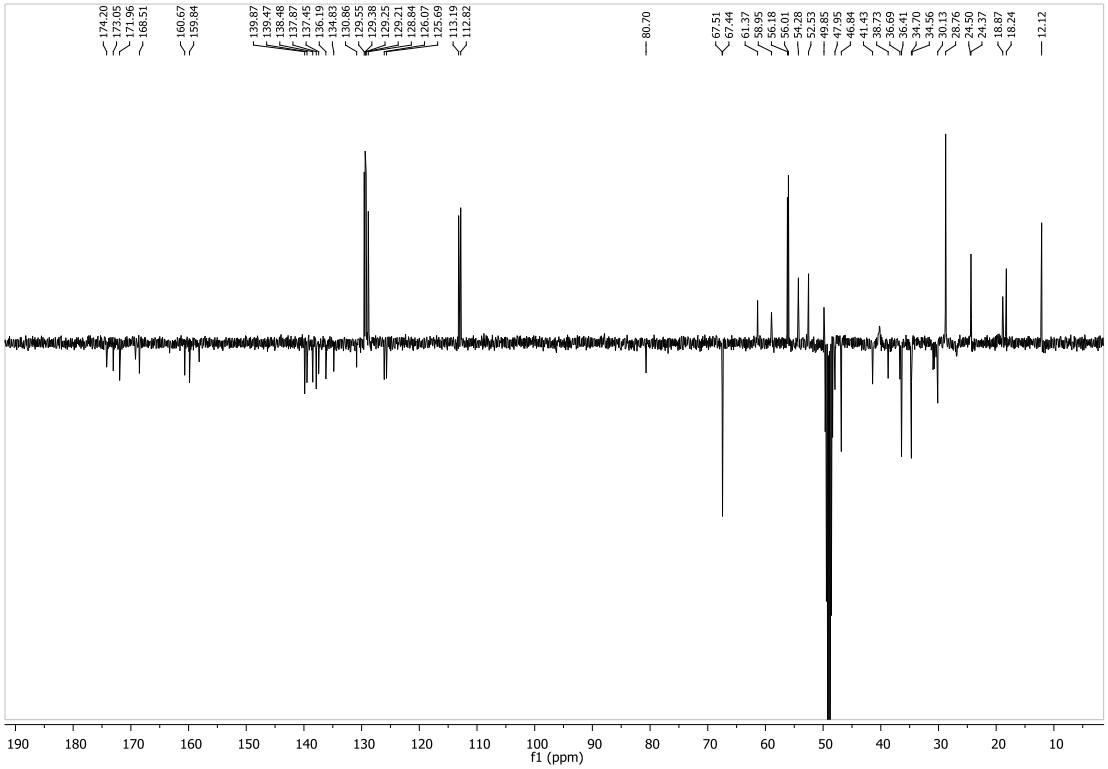


Boc-DKP-Arg(Mtr)-βAla-OBn (**25**)

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

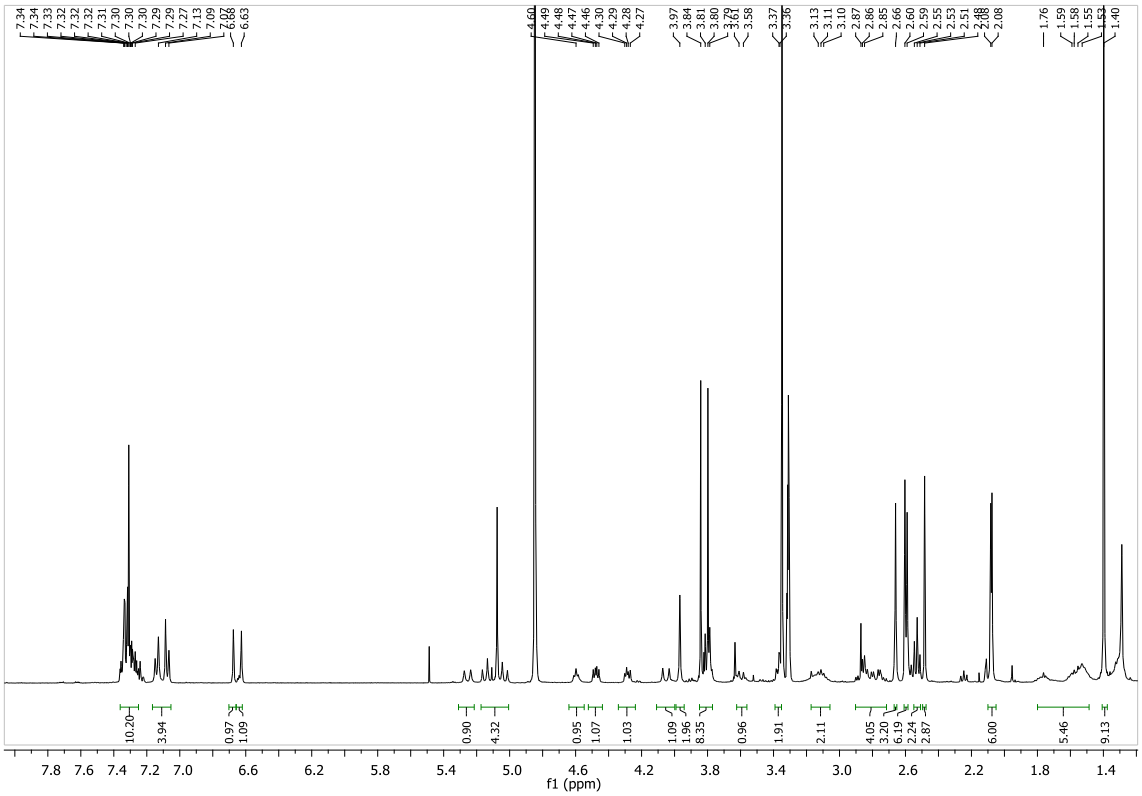


^{13}C NMR (101 MHz, MeOD)

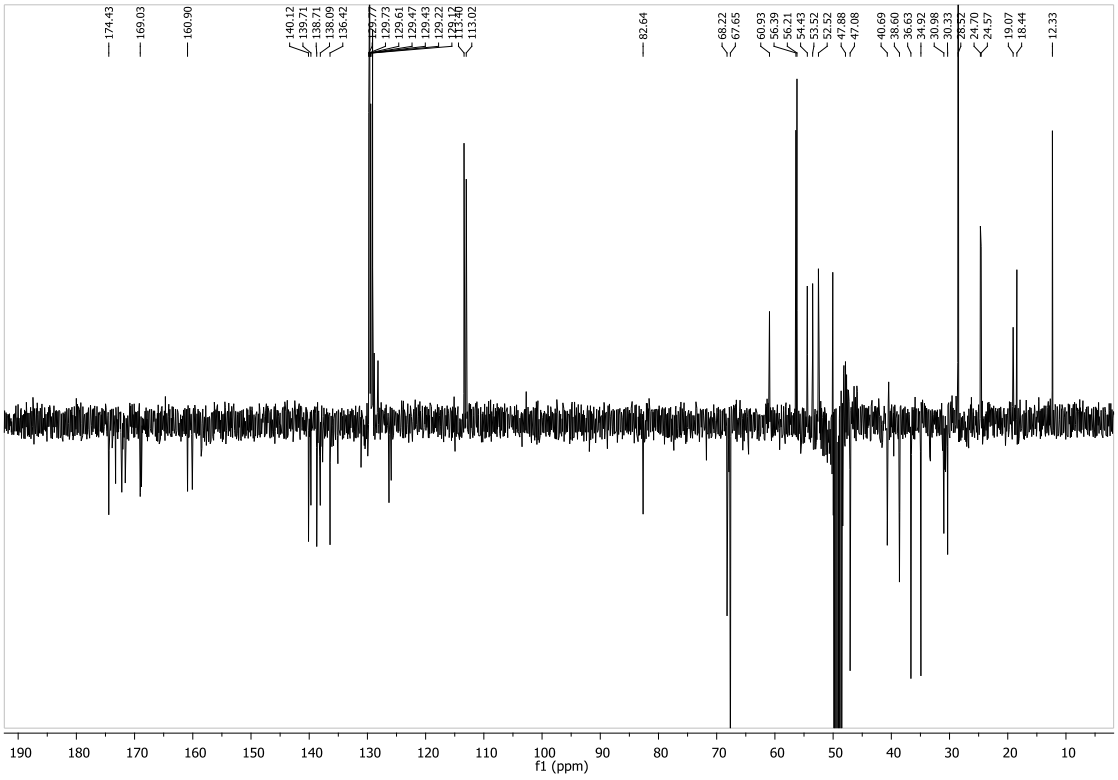


Cbz-Asp(OtBu)-DKP-Arg(Mtr)-βAla-OBn (**27**)

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

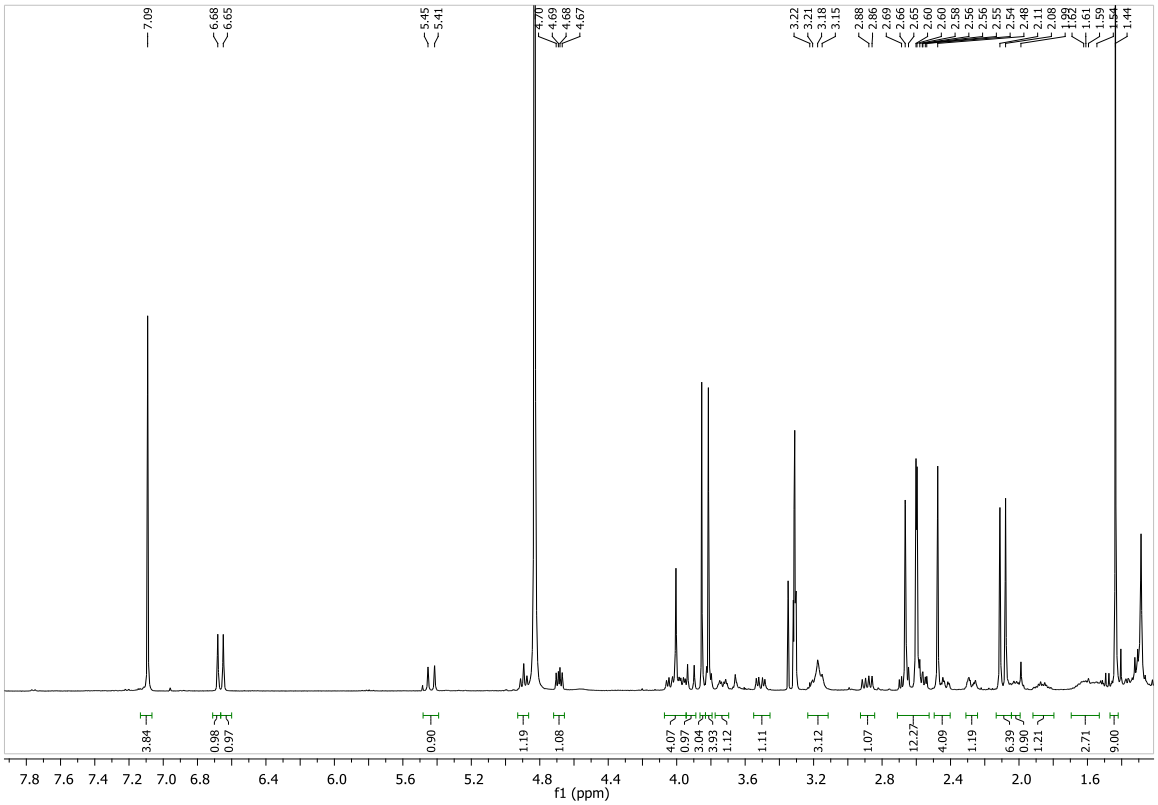


^{13}C NMR (101 MHz, MeOD)

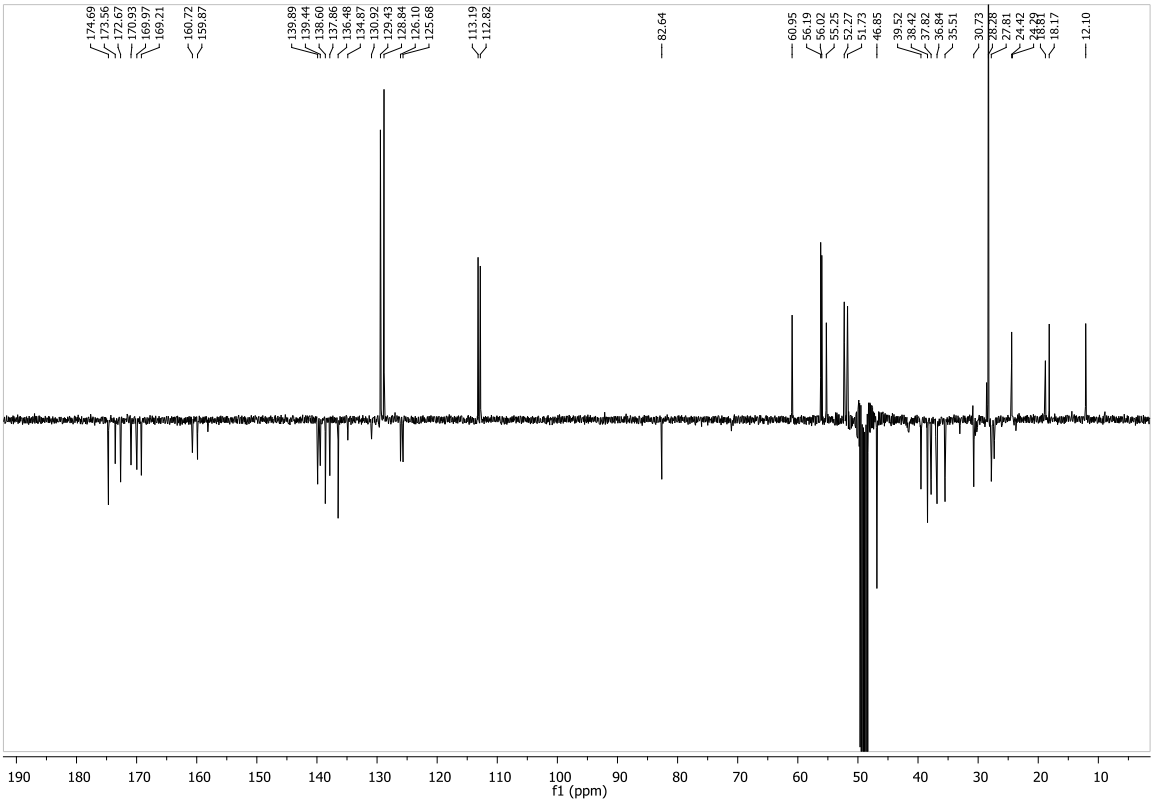


cyclo[DKP-Arg(Mtr)-βAla-Asp(OtBu)] (**29**)

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

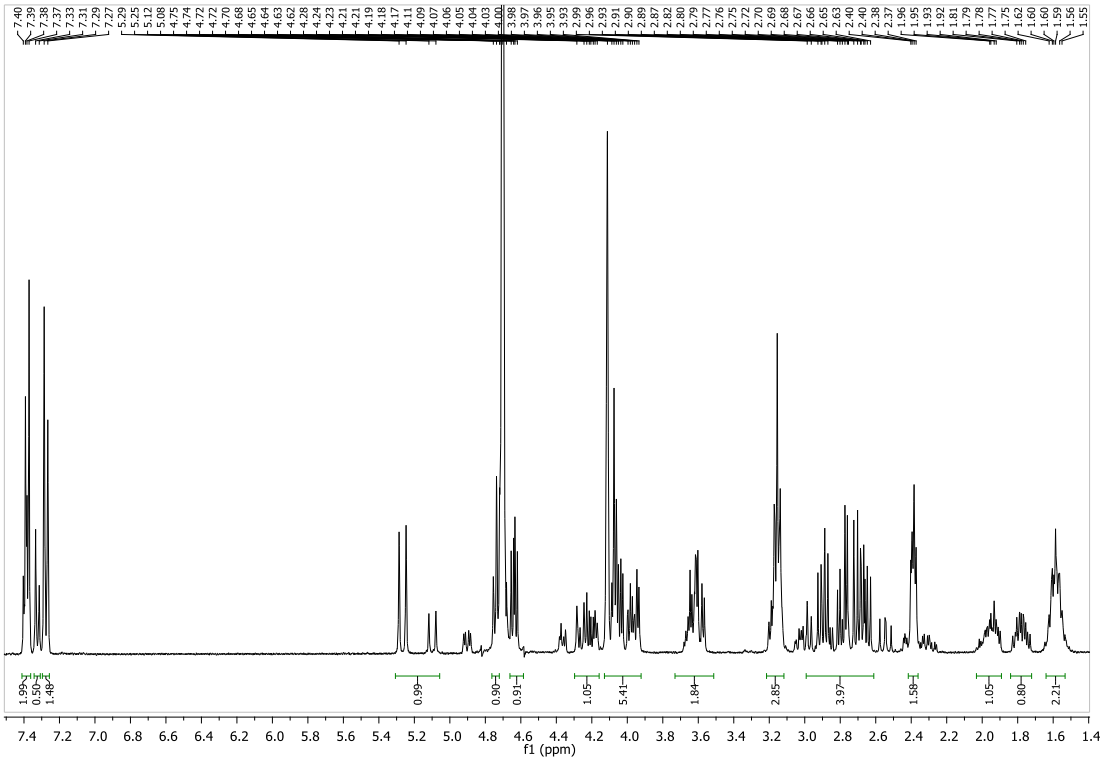


^{13}C NMR (101 MHz, MeOD)

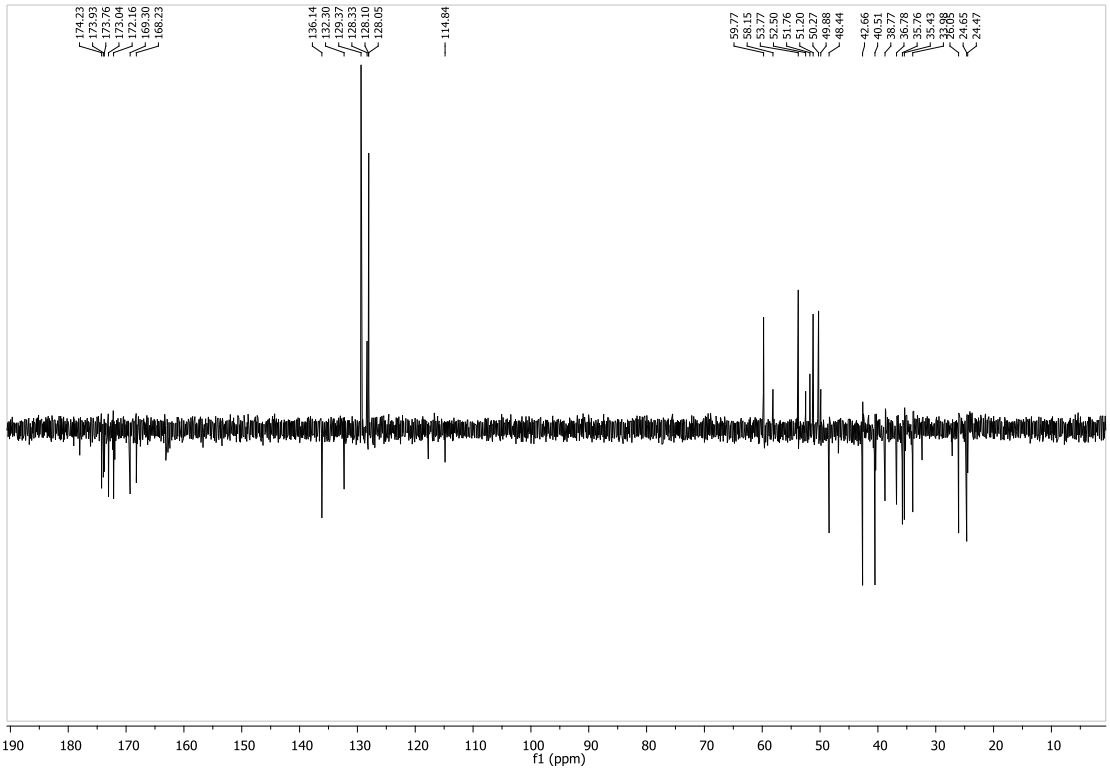


cyclo(DKP-RβAD)-CH_2NH_2 (**30**)

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

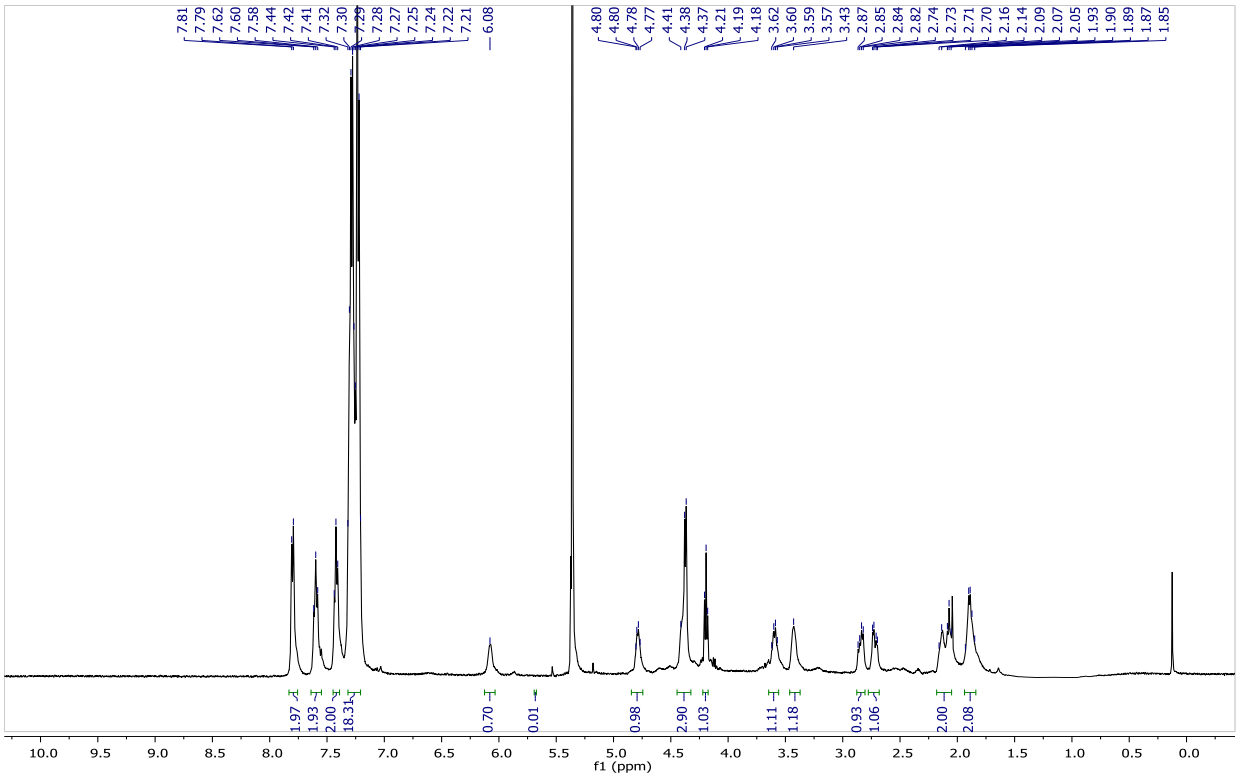


^{13}C NMR (101 MHz, D_2O)



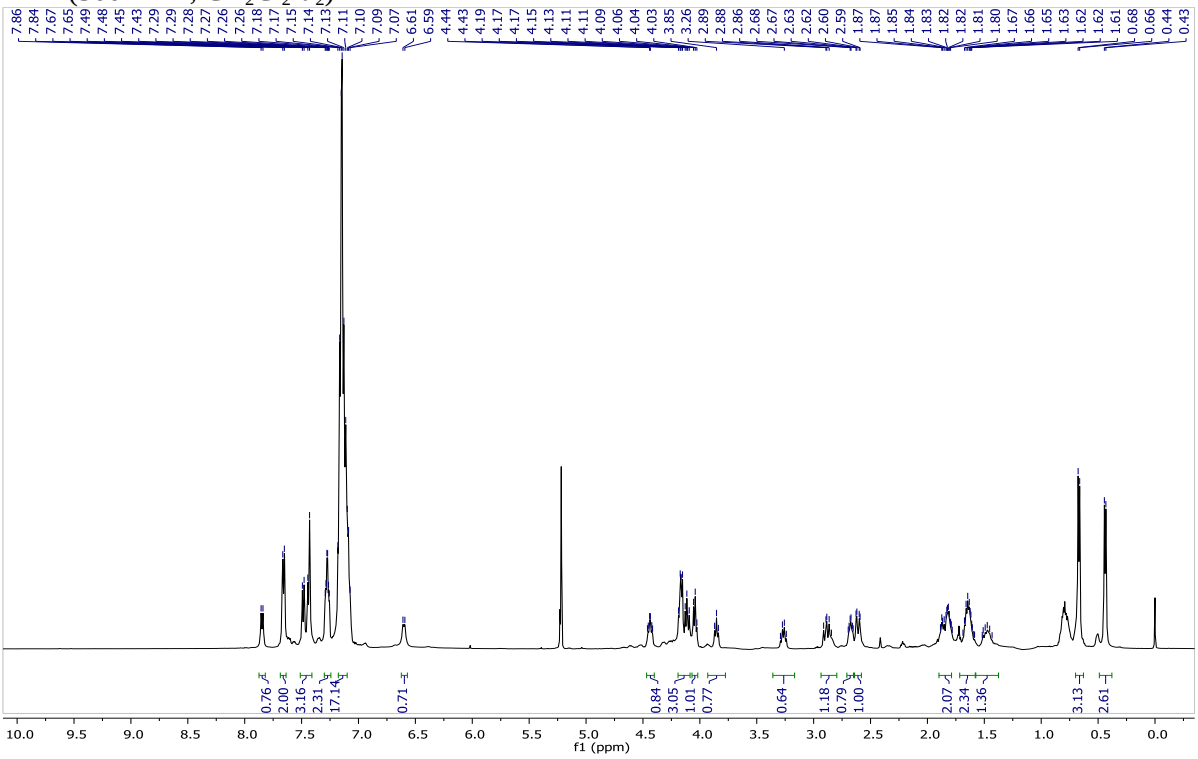
Fmoc-Asn(Trt)-Pro-OH (**32**)

^{1}H NMR (500 MHz, CD_2Cl_2-d_2)

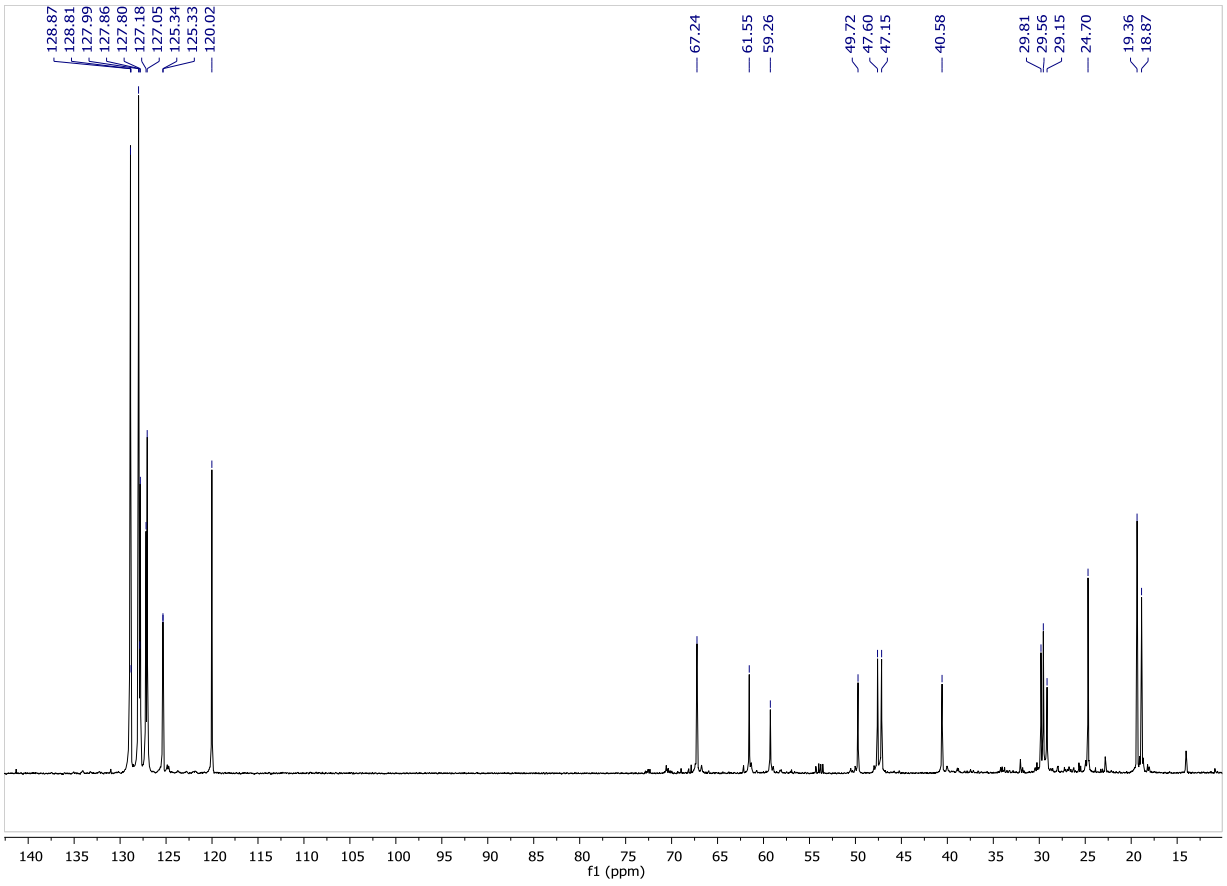


Fmoc-Asn(Trt)-Pro-Val-OH (**33**)

^{1}H NMR (500 MHz, CD_2Cl_2-d_2)

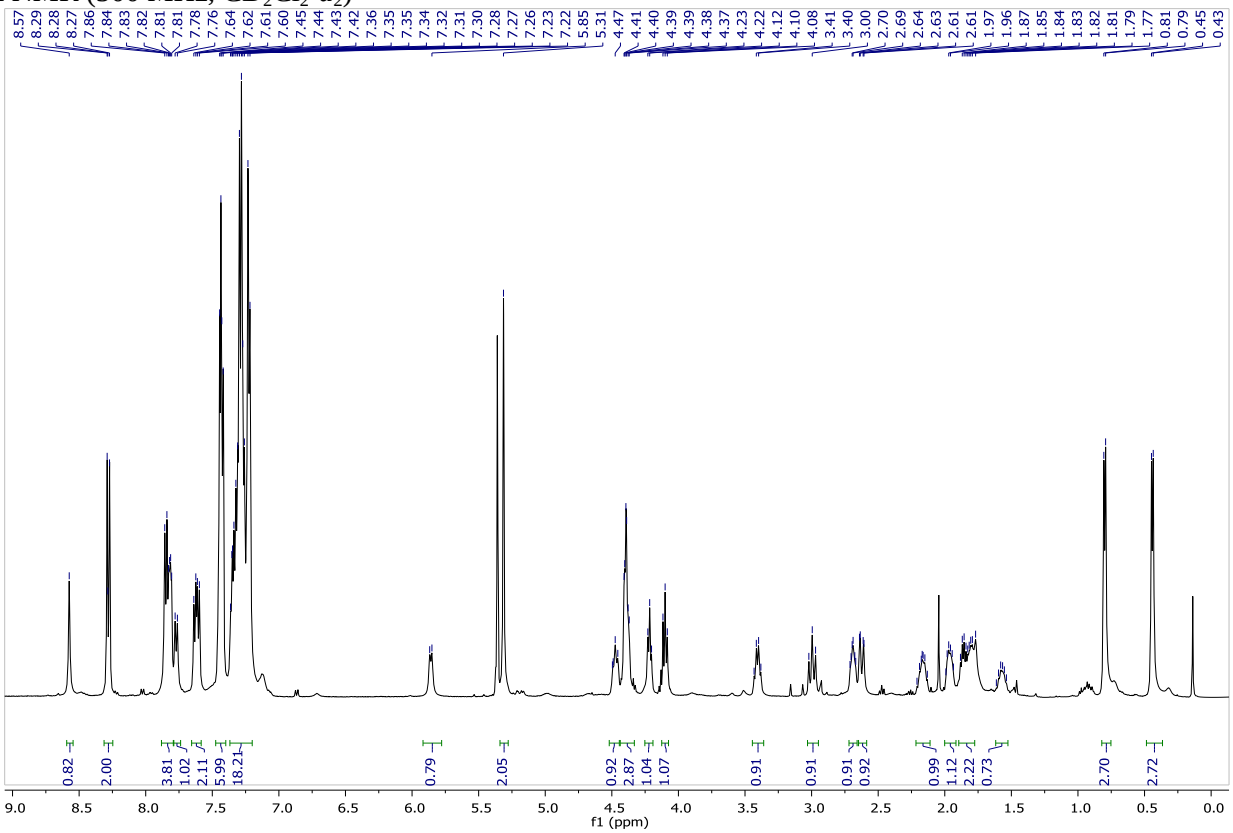


^{13}C-DEPT45 NMR (126 MHz, CD_2Cl_2-d_2)

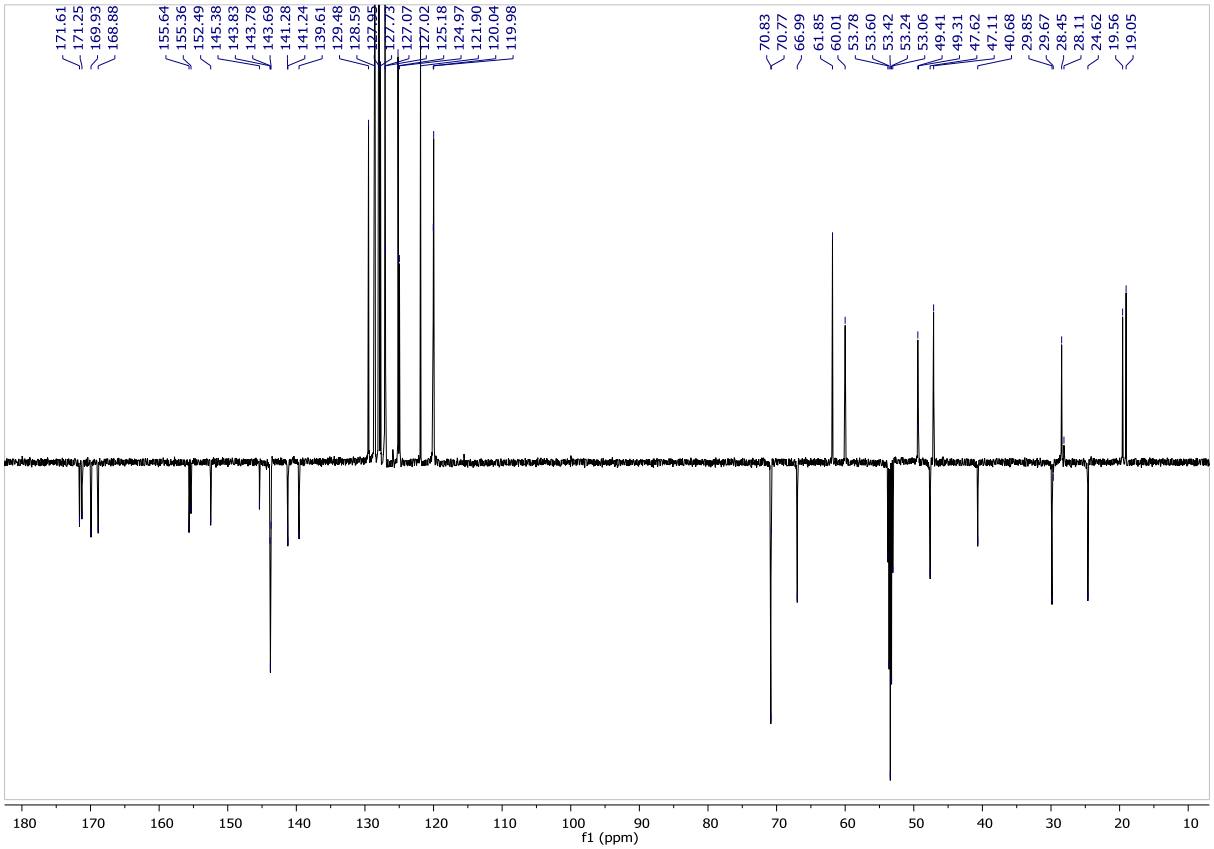


Fmoc-Asn(Trt)-Pro-Val-PABC-PNP (**34**)

^{1}H NMR (500 MHz, CD_2Cl_2-d_2)

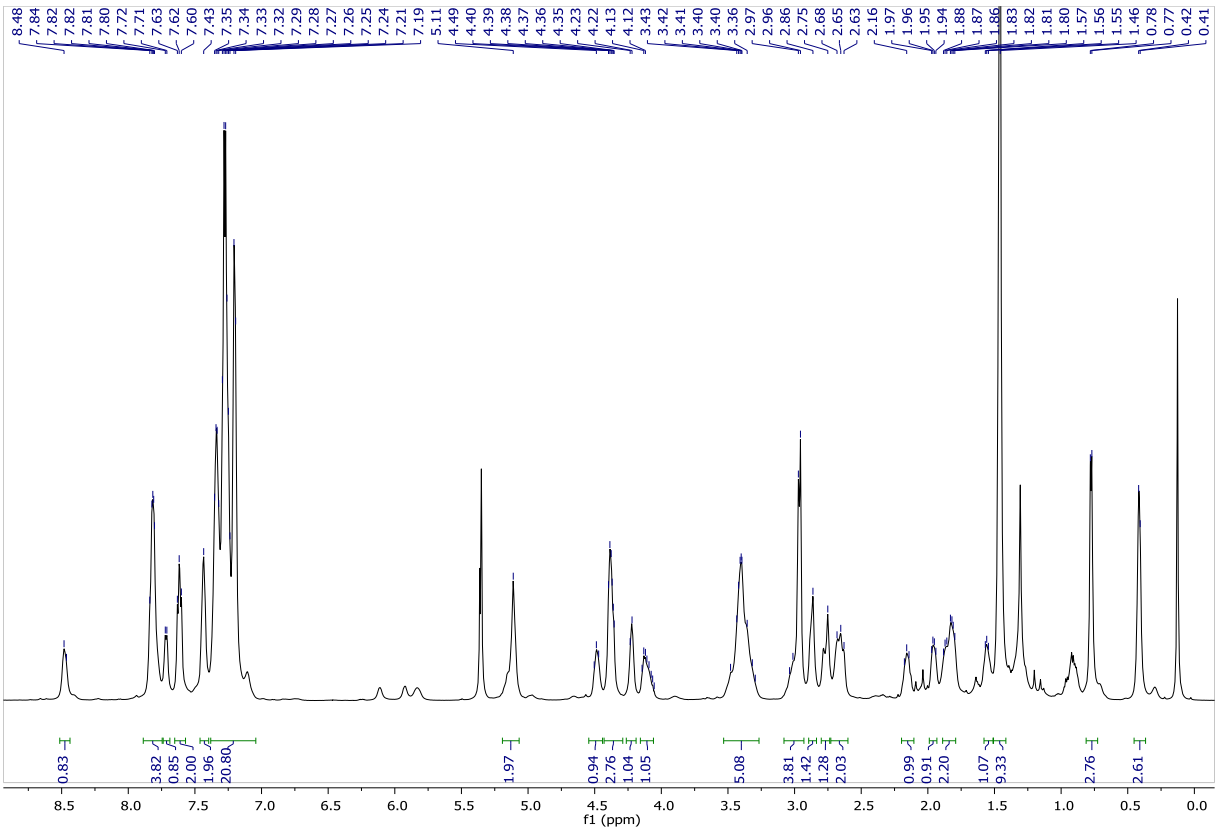


^{13}C-APT NMR (126 MHz, CD_2Cl_2-d_2)

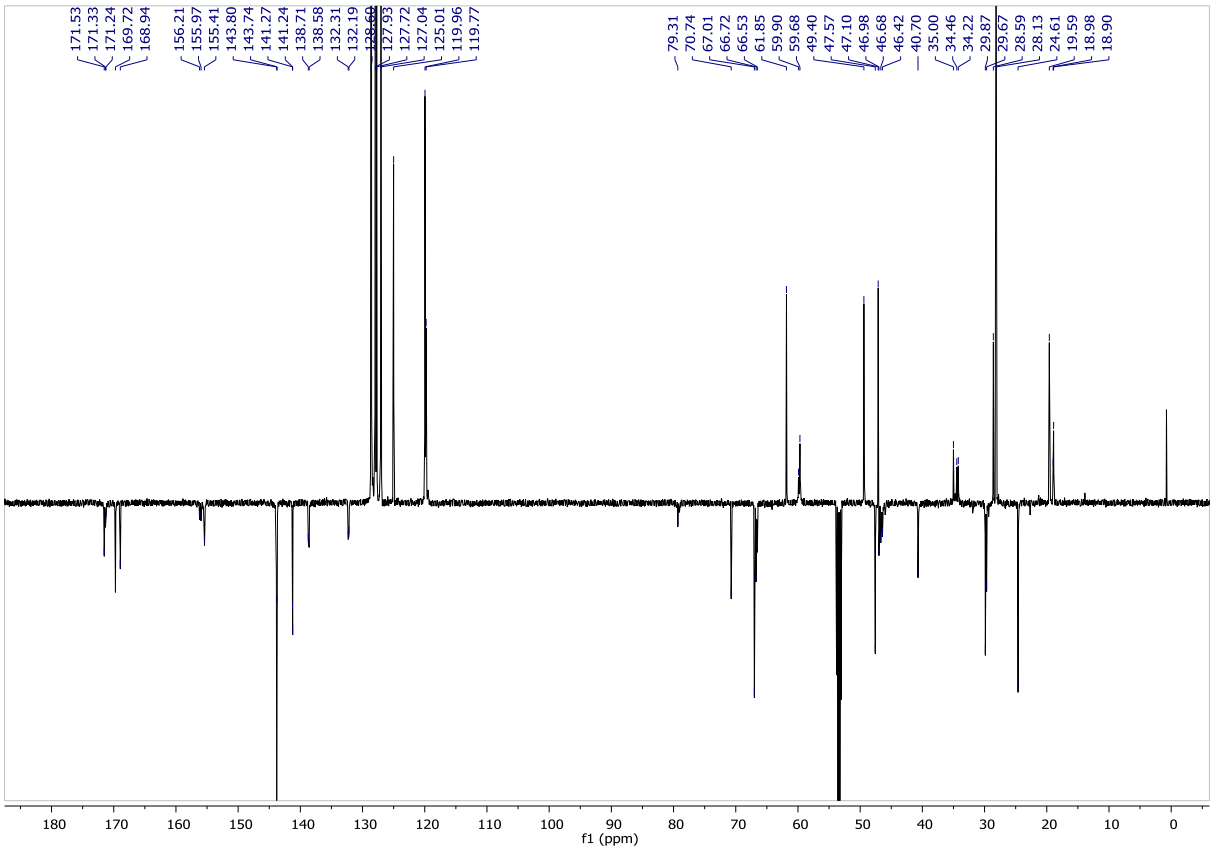


Fmoc-Asn(Trt)-Pro-Val-PABC-N-(Boc)-N,N'-dimethylethylenediamine (**35**)

^{1}H NMR (500 MHz, CD_2Cl_2-d_2)

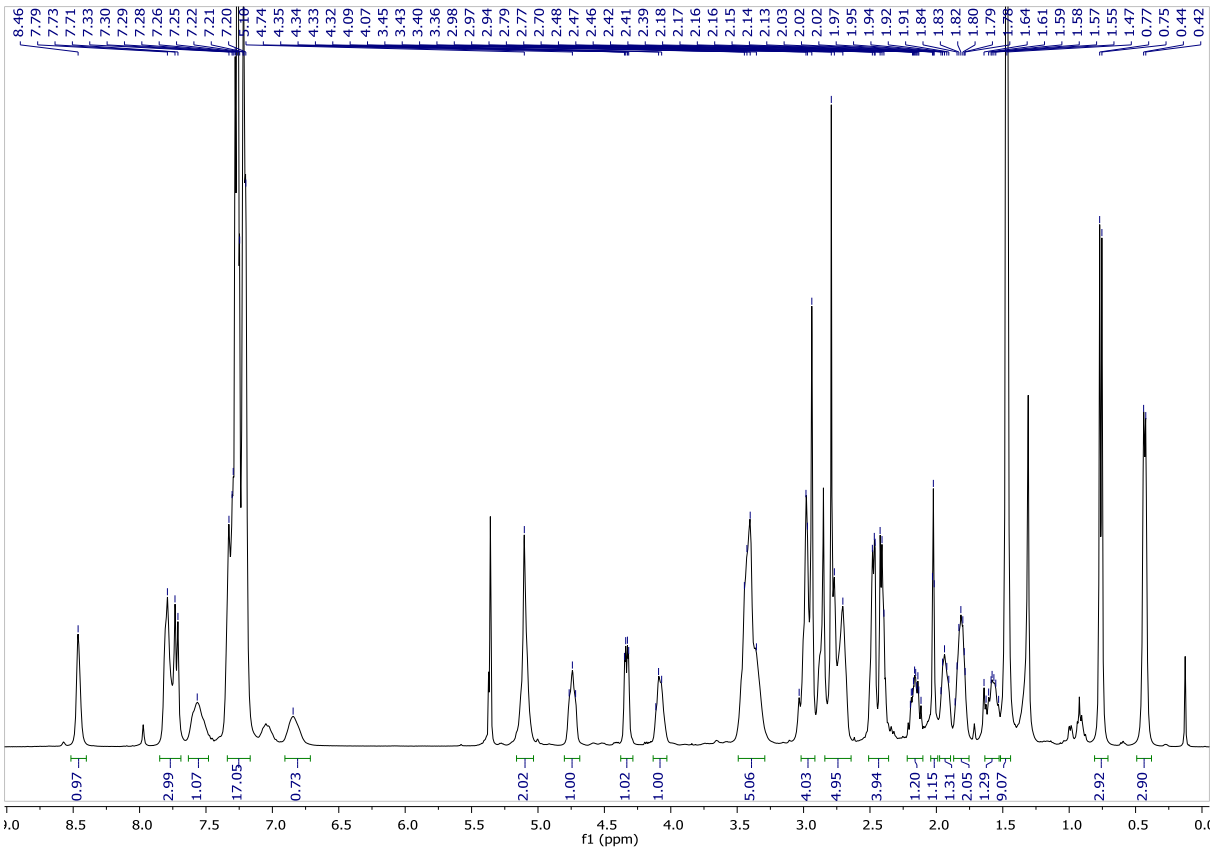


^{13}C-APT NMR (126 MHz, CD_2Cl_2-d_2)

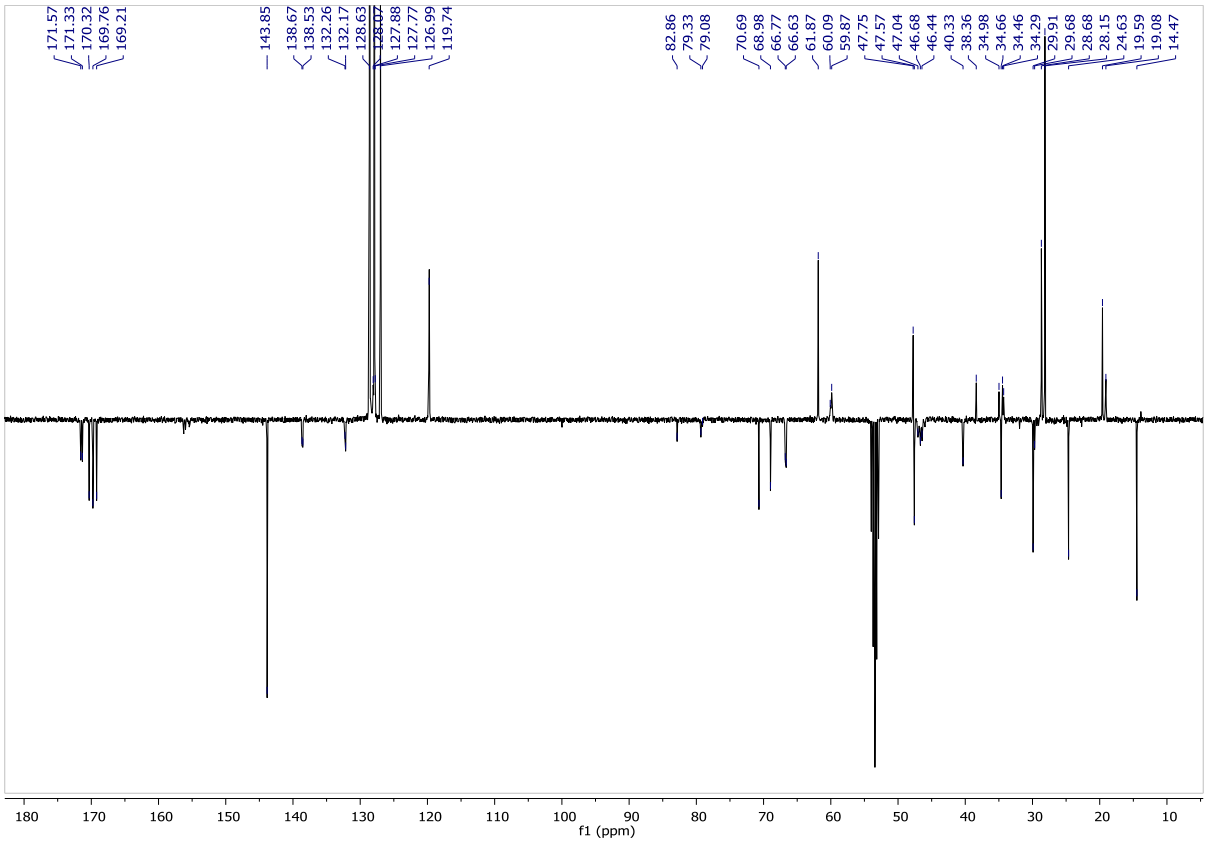


4-pentynamido-Asn(Trt)-Pro-Val-PABC-N-(Boc)-N,N'-dimethylethylenediamine (**36**)

^{1}H NMR (500 MHz, CD_2Cl_2-d_2)

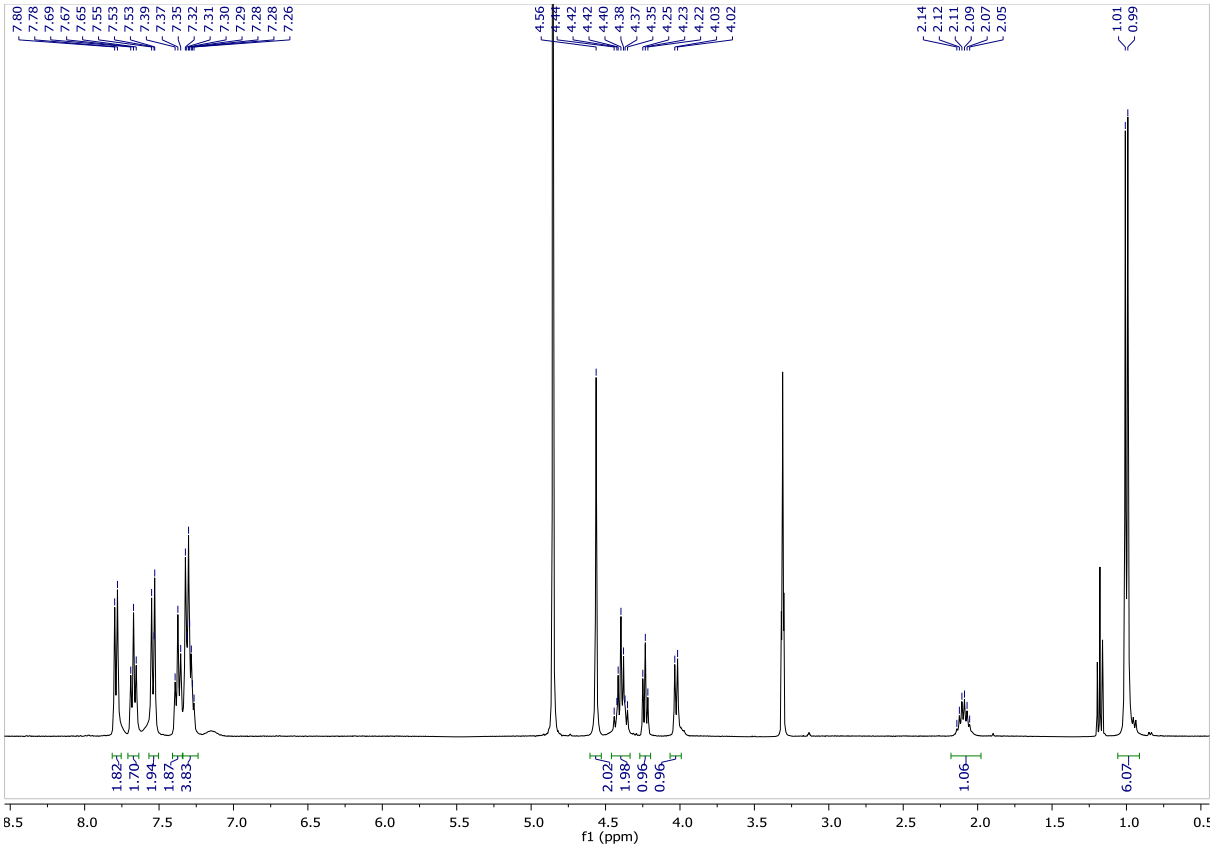


^{13}C-APT NMR (126 MHz, CD_2Cl_2-d_2)



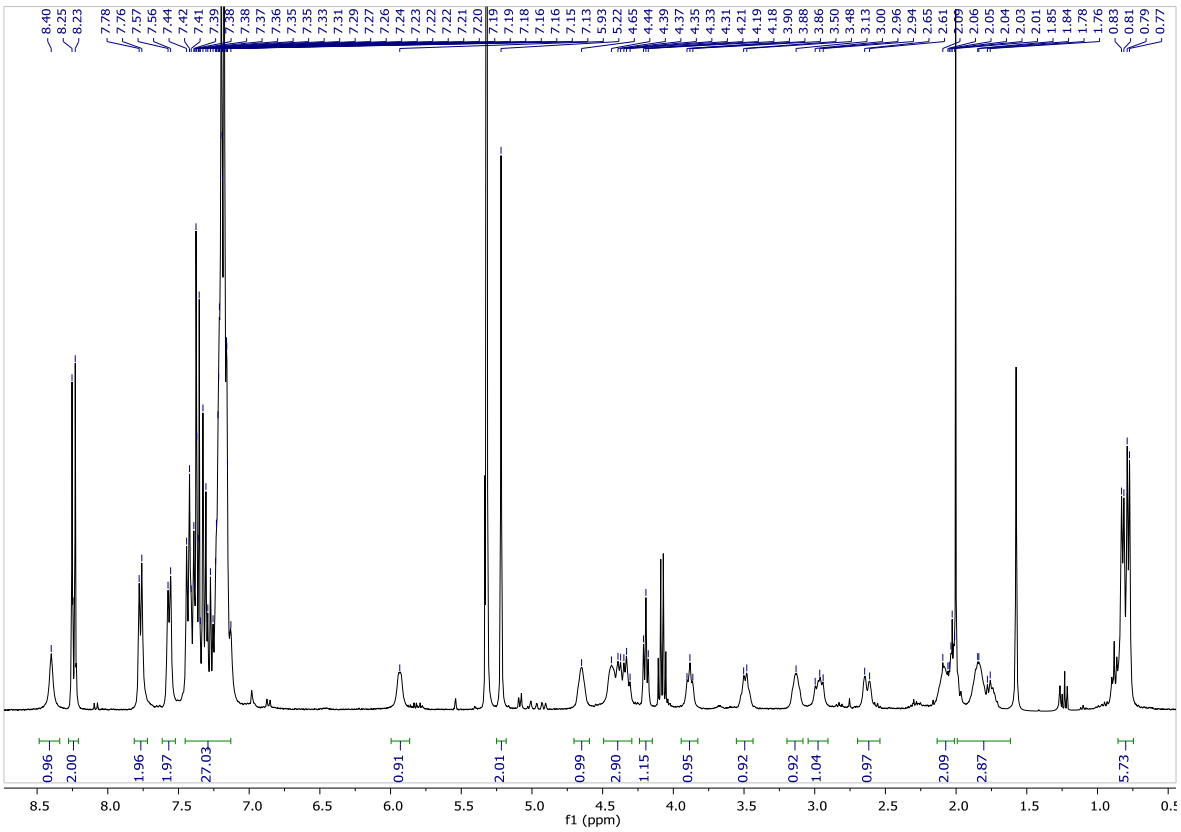
Fmoc-val-PABOH (**39**)

^{1}H NMR (400 MHz, MeOD-d_4)

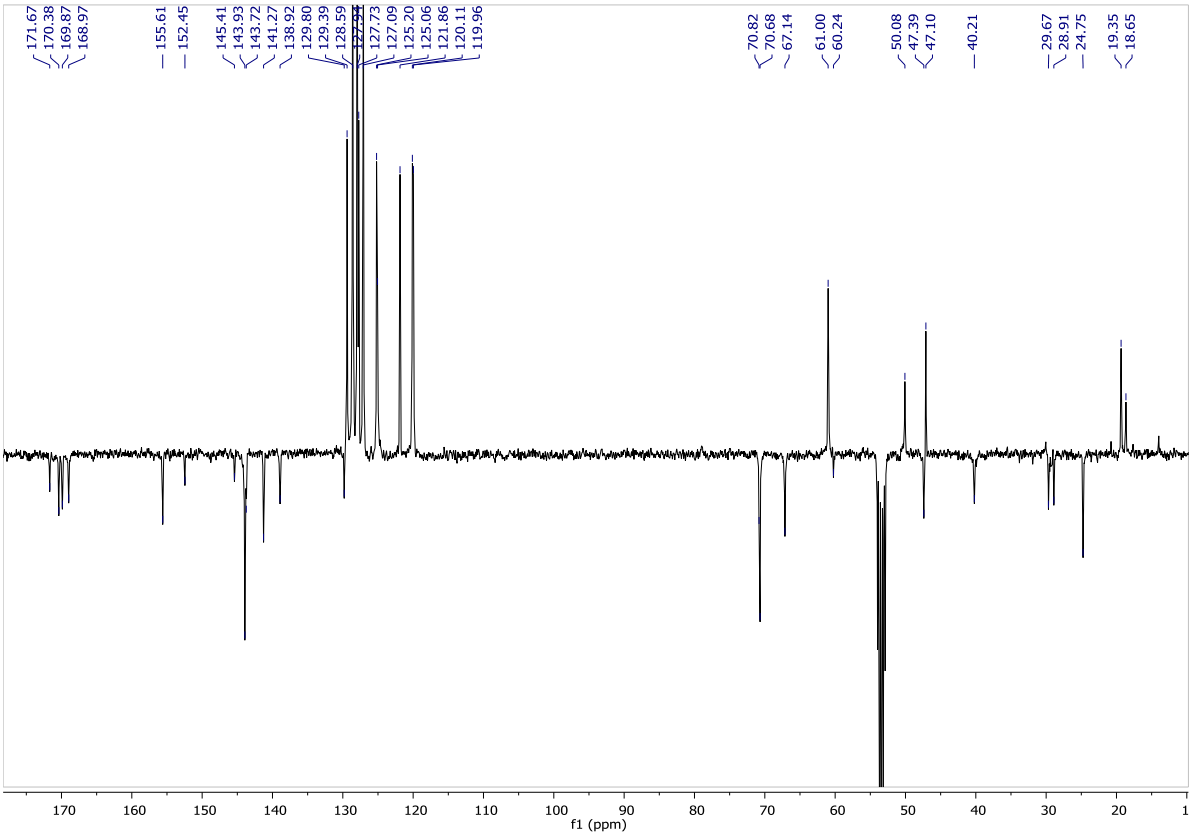


Fmoc-Asn(Trt)-Pro-val-PABC-PNP (**42**)

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

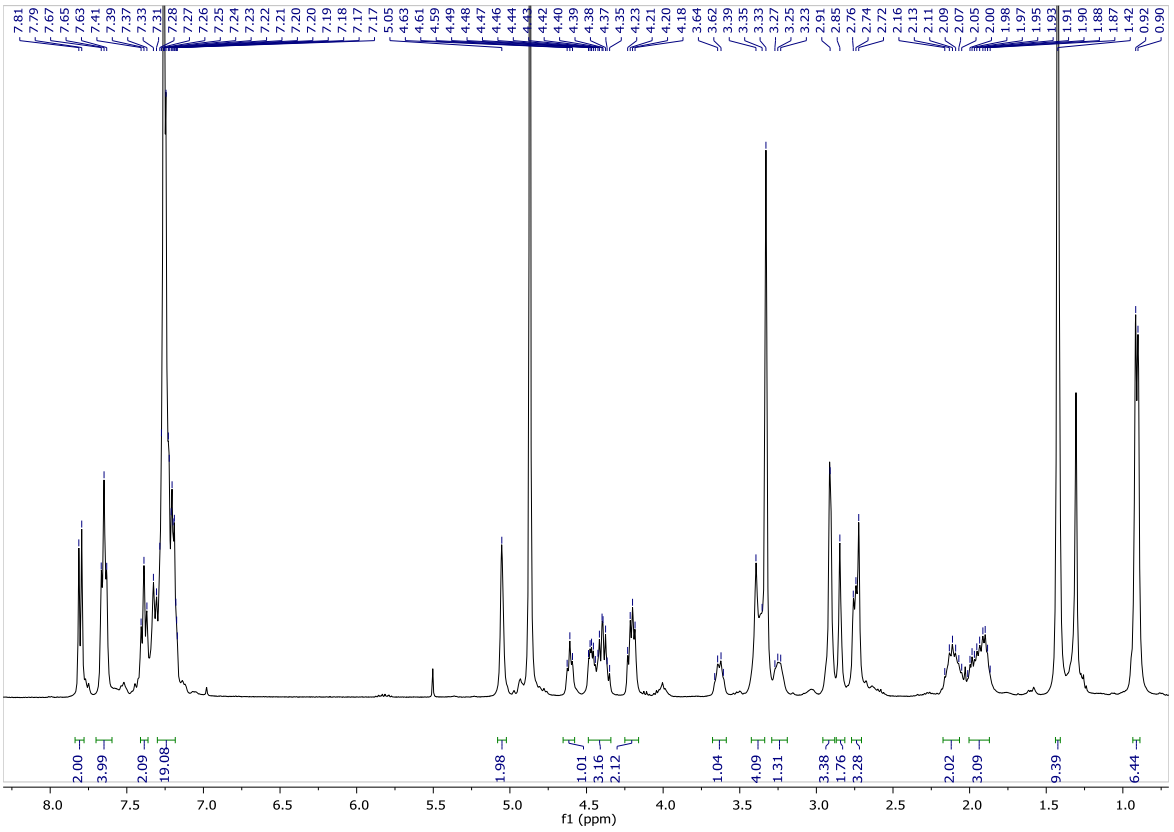


^{13}C-APT NMR (101 MHz, CD_2Cl_2-d_2)

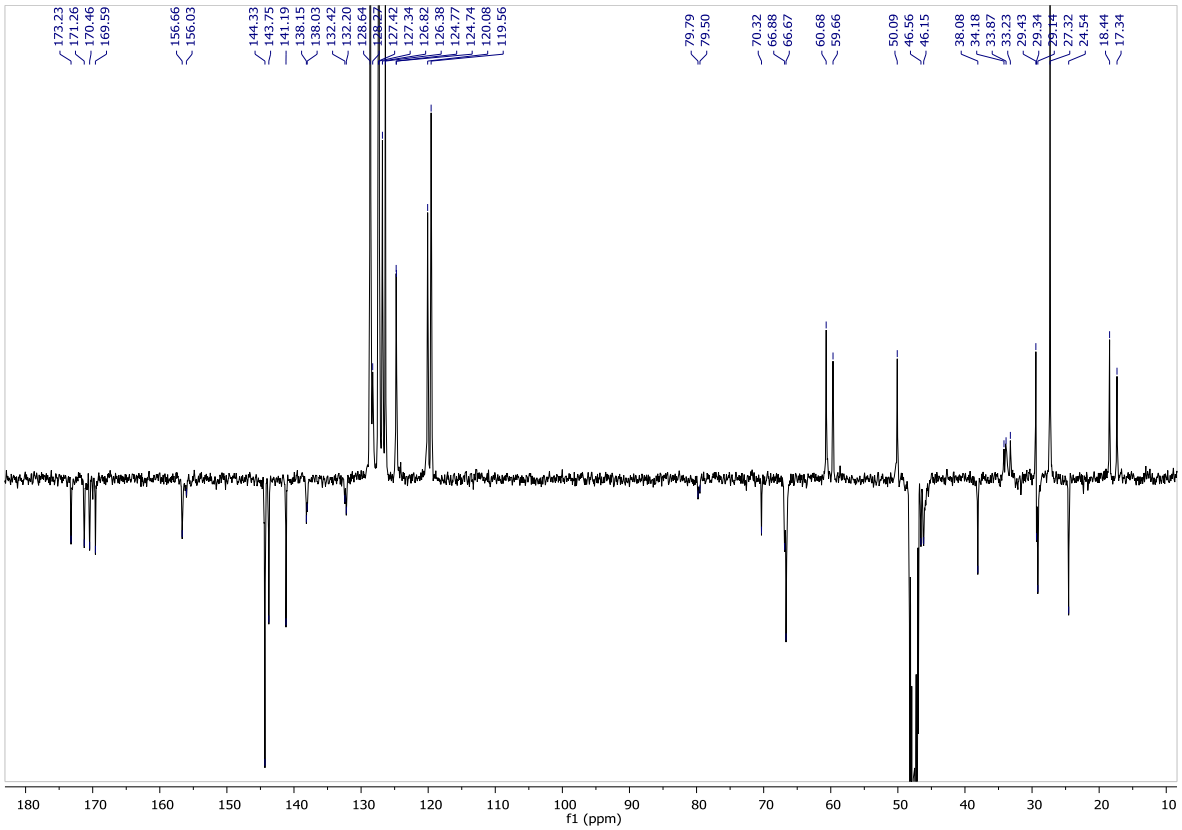


Fmoc-Asn(Trt)-Pro-val-PABC-N-(Boc)-N,N'-dimethylethylenediamine (**43**)

^{1}H NMR (400 MHz, MeOD-d_4)

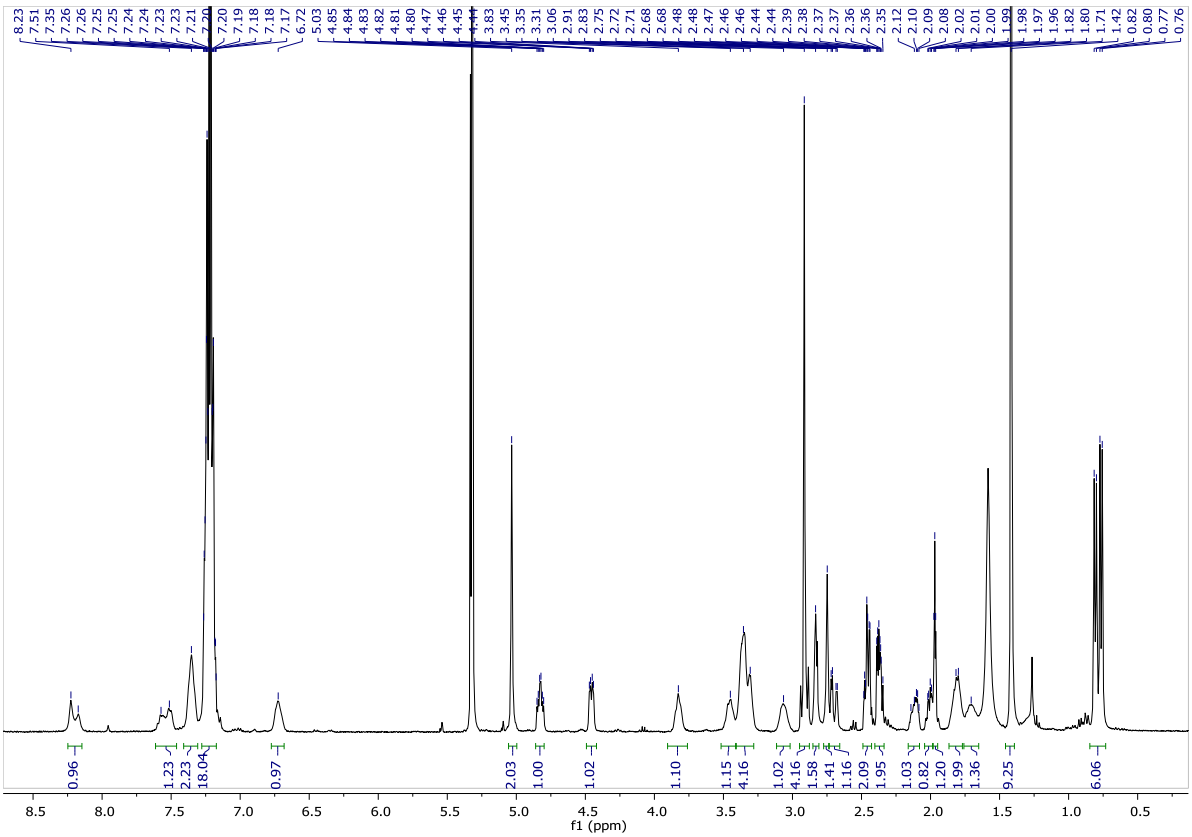


^{13}C-APT NMR (101 MHz, MeOD-d_4)

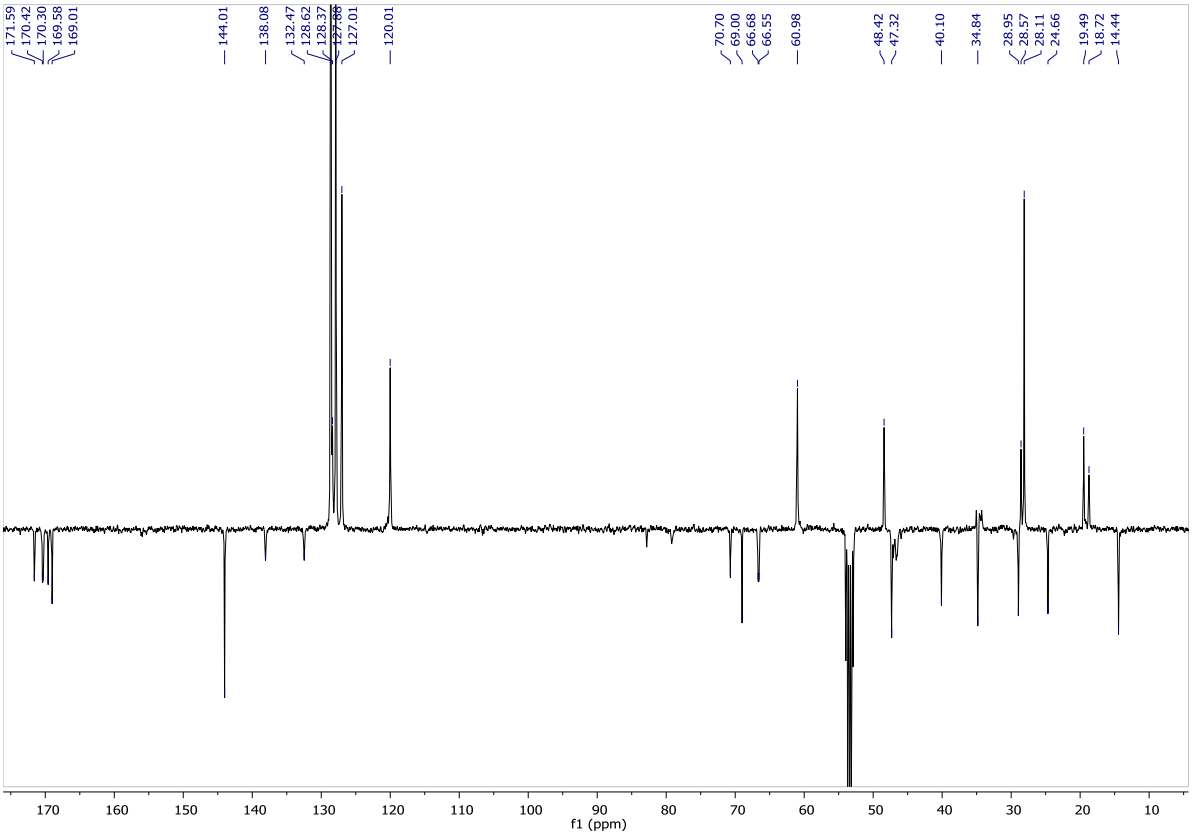


4-pentynamido-Asn(Trt)-Pro-val-PABC-N-(Boc)-N,N'-dimethylethylenediamine (**44**)

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

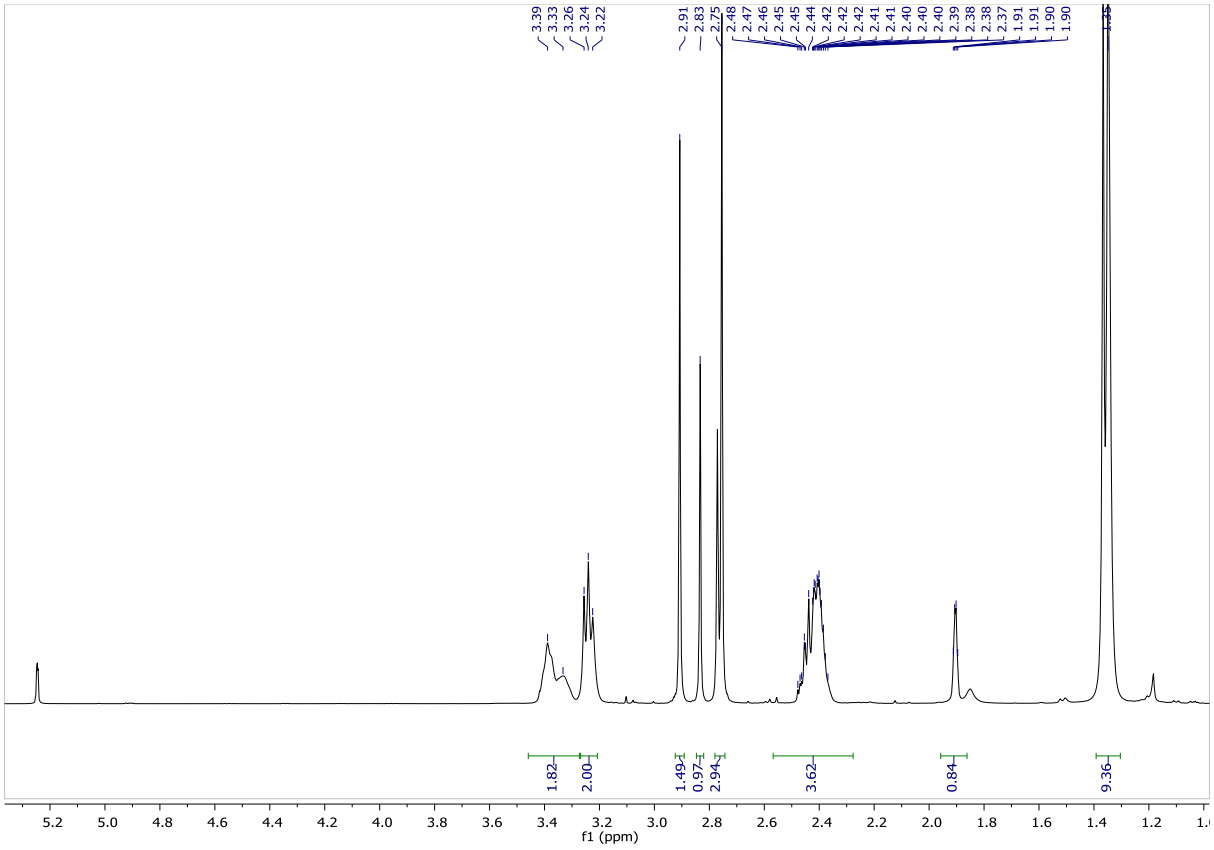


^{13}C-APT NMR (101 MHz, CD_2Cl_2-d_2)

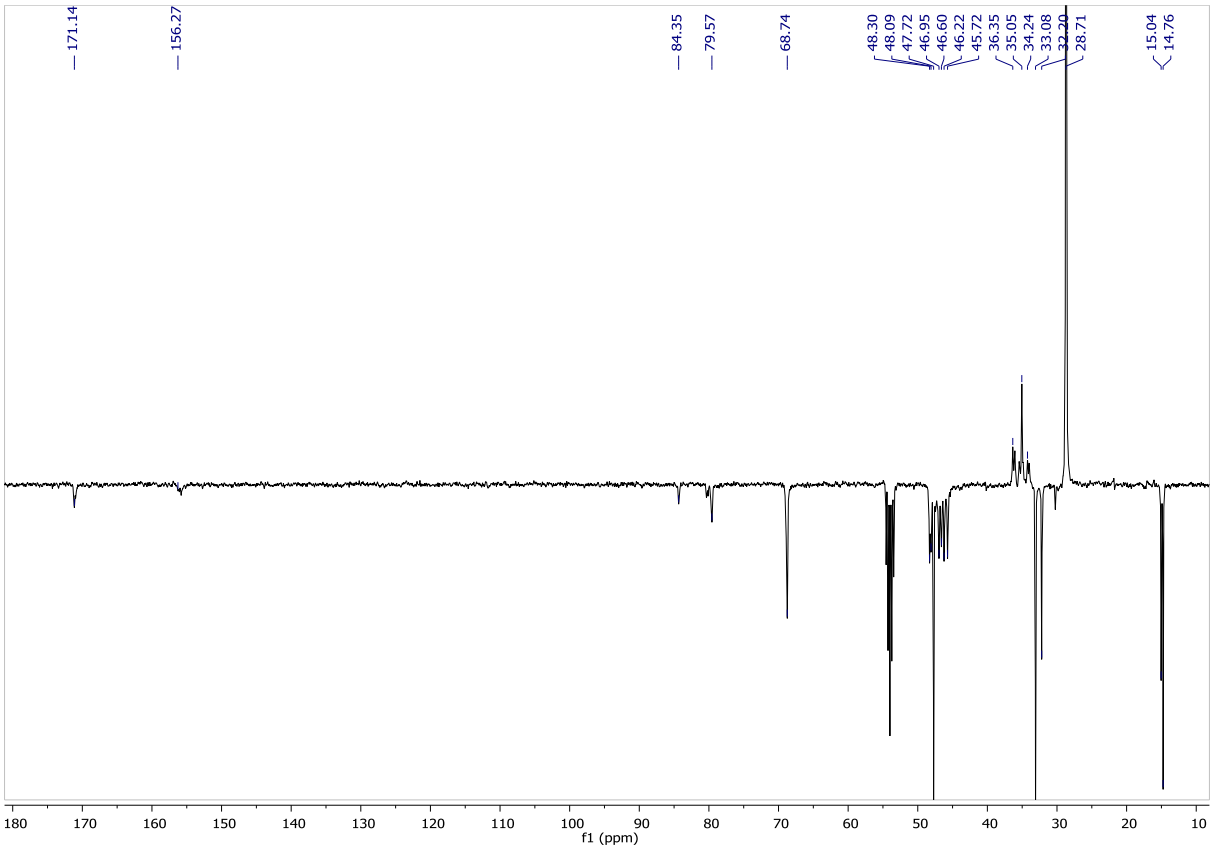


tert-butyl methyl(2-(N-methylpent-4-ynamido)ethyl)carbamate (**47**)

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

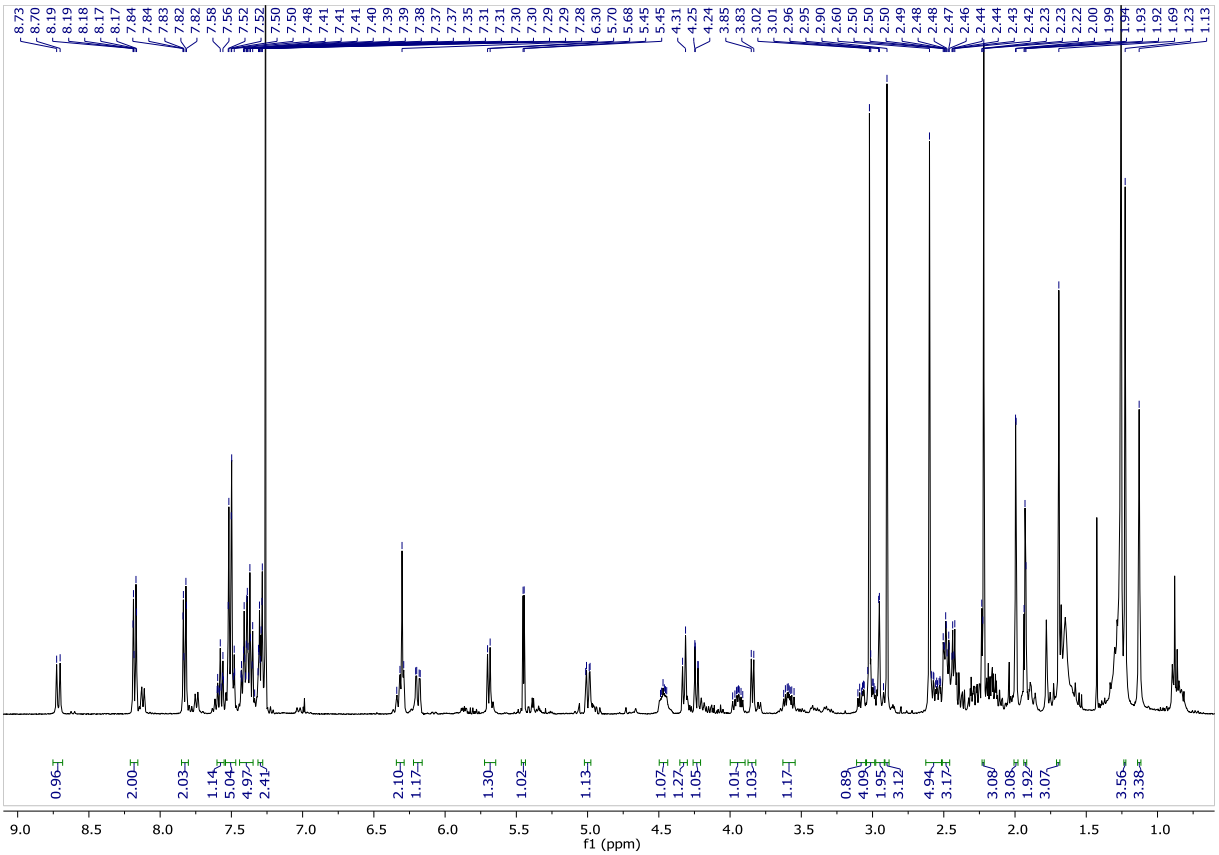


^{13}C-APT NMR (101 MHz, CD_2Cl_2-d_2)



Compound (**48**)

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



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