Supporting information for

Oxidative α,ω-diyne coupling as an approach towards novel peptidic macrocycles

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1. Model study

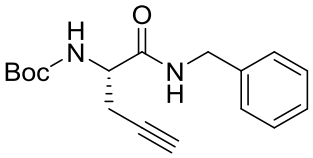
A model study to evaluate the optimal reaction conditions for the intermolecular dimerisation of alkynylated amino acids **1a** and **1b** was performed (Table 1). The general reaction procedure consisted of adding the solvent (0.5ml) to a mixture of **1a** (0.08mmol) or **1b** (0.08mmol) with a Cu-catalyst (0.5 equiv.) and/or Ni-catalyst (0.5 equiv.). The reaction mixture was stirred at rt and the base (3 equiv.), ligand (5 equiv.) and oxidant were added. In case of O_2 as oxidant, the reaction mixture was stirred under O_2-atmosphere using a O_2-balloon. In case of MnO_2, pyridine *N*-oxide or H_2O_2 (35 wt-% in H_2O) as oxidants, these reagents were added in excess (5 equiv.) to the reaction mixture and stirred open to the atmosphere, but using a plug of cotton wool to prevent excessive loss of solvents in case of long reaction times. Also in case of air as oxidant, the reaction mixture was stirred open to the atmosphere as described above. The reaction temperature was increased to 60°C when no or slow conversion towards the corresponding diyne was observed. Conversions were determined via HPLC by direct sampling of the reaction mixture.

AA	Cu-cat.	Ni-cat.	Base	Ligand	Solvent	oxidant	t (h)	T	Conv. (%)
1b	$Cu(OAc)_2.H_2O$	/	Et_3N	pyridine	EtOH	O_2	5	rt	>99
1b	$Cu(OAc)_2.H_2O$	$NiCl_2$	Et_3N	pyridine	EtOH	O_2	4	rt	>99
1a	$Cu(OAc)_2.H_2O$	/	Et_3N	pyridine	EtOH	O_2	120	rt	50
1a	$Cu(OAc)_2.H_2O$	$NiCl_2$	Et_3N	pyridine	EtOH	O_2	120	rt-60°C	60
1b	**$Cu(OAc)_2.H_2O$**	/	Et_3N	pyridine	EtOH	O_2	5	rt	>99
1b	**$CuSO_4.5H_2O$**	/	Et_3N	pyridine	EtOH	O_2	96	rt	90
1b	**CuI**	/	Et_3N	pyridine	EtOH	O_2	144	rt	93
1b	**$Cu(CH_3CN)_4PF_6$**	/	Et_3N	pyridine	EtOH	O_2	24	rt	>99
1b	$Cu(OAc)_2.H_2O$	**$NiCl_2.6H_2O$**	Et_3N	pyridine	EtOH	O_2	168	rt	95
1b	$Cu(OAc)_2.H_2O$	**$NiCl_2.(acac)_2$**	Et_3N	pyridine	EtOH	O_2	168	rt	93
1b	$Cu(OAc)_2.H_2O$	**$NiCl_2.(PPh_3)_2$**	Et_3N	pyridine	EtOH	O_2	168	rt	5
1b	$Cu(OAc)_2.H_2O$	$NiCl_2$	**Et_2NH**	pyridine	EtOH	O_2	24	rt	95
1b	$Cu(OAc)_2.H_2O$	$NiCl_2$	**DIPEA**	pyridine	EtOH	O_2	24	rt	97
1b	$Cu(OAc)_2.H_2O$	$NiCl_2$	**TMEDA**	pyridine	EtOH	O_2	168	rt	87
1b	$Cu(OAc)_2.H_2O$	$NiCl_2$	**DBU**	pyridine	EtOH	O_2	24	rt	>99
1b	$Cu(OAc)_2.H_2O$	$NiCl_2$	Et_3N	**4-Me piperidine**	EtOH	O_2	4	rt	90
1b	$Cu(OAc)_2.H_2O$	$NiCl_2$	Et_3N	**pyrrolidine**	EtOH	O_2	4	rt	50
1b	$Cu(OAc)_2.H_2O$	$NiCl_2$	Et_3N	pyridine	**DMF**	O_2	48	rt	40
1b	$Cu(OAc)_2.H_2O$	$NiCl_2$	Et_3N	pyridine	**H_2O**	O_2	192	rt-60°C	0
1b	$Cu(OAc)_2.H_2O$	$NiCl_2$	Et_3N	pyridine	**H_2O/ Dioxane (1/1)**	O_2	216	rt-60°C	90
1b	$Cu(OAc)_2.H_2O$	$NiCl_2$	Et_3N	pyridine	**H_2O/ CH_3CN (1/1)**	O_2	216	rt-60°C	0
1b	$Cu(OAc)_2.H_2O$	$NiCl_2$	Et_3N	pyridine	**DMSO**	O_2	48	rt	93
1b	$Cu(OAc)_2.H_2O$	$NiCl_2$	Et_3N	pyridine	EtOH	**MnO_2**	72	rt	>99
1b	$Cu(OAc)_2.H_2O$	$NiCl_2$	Et_3N	pyridine	EtOH	**H_2O_2**	72	rt	50
1b	$Cu(OAc)_2.H_2O$	$NiCl_2$	Et_3N	pyridine	EtOH	**air**	120	rt	92
1b	$Cu(OAc)_2.H_2O$	$NiCl_2$	Et_3N	pyridine	EtOH	**Pyridine *N*-oxide**	24	rt	>99

Table 1: Model study

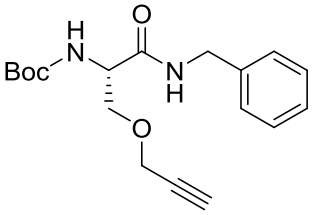
Synthesis of starting materials 1a and 1b

tert-Butyl (S)-(1-(benzylamino)-1-oxopent-4-yn-2-yl)carbamate **1a**



(*S*)-2-((*tert*-Butoxycarbonyl)amino)pent-4-ynoic acid (1.41 mmol), 1-hydroxy-7-azabenzotriazole (HOAt) (4.22 mmol) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC.HCl) (1.41 mmol) were dissolved in CH_2Cl_2 (40 ml). DIPEA (*N*,*N*-diisopropylethylamine) (4.22 mmol) was added dropwise and the mixture was stirred at rt for 5min. The reaction was cooled to 0 °C and benzylamine (1.41 mmol) in CH_2Cl_2 (5 ml) was added dropwise. The reaction mixture was stirred at rt for 26 h protected from atmospheric moisture via a $CaCl_2$-tube, concentrated *in vacuo* and the resulting residue was triturated in EtOAc (15 ml) and filtered. The filtrate was washed with a saturated aqueous citric acid solution (15 ml), a saturated aqueous $KHCO_3$ solution (2x15 ml) and brine (2x15 ml). The organic phase was dried over $MgSO_4$, filtered and concentrated *in vacuo* to afford *tert*-butyl (*S*)-(1-(benzylamino)-1-oxopent-4-yn-2-yl)carbamate as a white solid in 71% yield (0.31 g); mp: 89.5 – 91.1 °C; IR (neat): 3230, 2074, 1736, 1675 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$): δ 7.22 – 7.38 (m, 5 H), 6.62 (br. s, 1 H), 5.28 (br. s, 1 H), 4.44 – 4.53 (m, 2 H), 4.25 – 4.39 (m, 1 H), 2.79 – 2.91 (m, 1 H), 2.56 – 2.69 (m, 1 H), 2.06 (t, *J* = 2.7 Hz, 1 H), 1.43 (s, 9 H); ^{13}C NMR (163 MHz, $CDCl_3$) δ 170.5, 155.6, 137.8, 128.9, 127.9, 127.8, 81.0, 79.6, 72.0, 53.3, 43.9, 28.4, 22.6; HRMS Calcd for [$C_{17}H_{22}N_2O_3$ – Boc + H^+]: 203.1179. Found 203.1183.

t*ert*-Butyl (*S*)-(1-(benzylamino)-1-oxo-3-(prop-2-yn-1-yloxy)propan-2-yl)carbamate **1b**

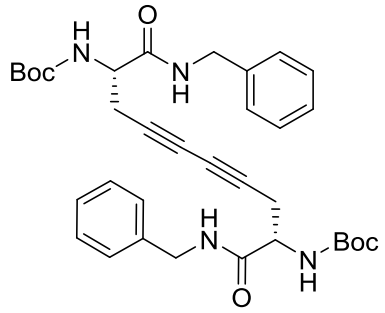


N-(*tert*-Butoxycarbonyl)-*O*-(prop-2-yn-1-yl)-L-serine (21.46 mmol), HOAt (64.40 mmol) and EDC.HCl (64.40 mmol) were dissolved in CH_2Cl_2 (150 ml). DIPEA (64.40 mmol) was added dropwise and the mixture was stirred at rt for 5 min. The reaction mixture was cooled to 0 °C and benzylamine (64.40 mmol) in CH_2Cl_2 (5 ml) was added dropwise. After stirring for 36h at rt protected from atmospheric moisture via a $CaCl_2$-tube, the reaction mixture was concentrated *in vacuo*. The resulting residue was triturated with EtOAc (50 ml) and filtered. The filtrate was washed with an aqueous saturated citric acid solution (50 ml), an aqueous saturated $KHCO_3$ solution (2x50 ml) and brine (2x50 ml). The organic phase was dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residue was purified via column silica gel chromatography (EtOAc/hexane 1/1) to afford *tert*-butyl (*S*)-(1-(benzylamino)-1-oxo-3-(prop-2-yn-1-yloxy)propan-2-yl)carbamate as a white solid in 45% yield (3.18 g); mp: 100.0 – 102.0 °C; IR (neat): 3300, 2178, 1742, 1095 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$): δ 7.20 – 7.36 (m, 5 H), 6.81 (t, *J* = 5.4 Hz, 1 H), 5.36 – 5.55 (m, 1 H), 4.41 – 4.51 (m, 2 H), 4.33 (br. s., 1 H), 4.15 (m, 2 H), 3.94 (dd, *J* = 4.4 and 3.8 Hz, 1 H), 3.69 (dd, *J* = 6.3 and 4.4 Hz, 1 H), 2.45 (t, *J* = 2.4 Hz, 1 H), 1.42 (s, 9 H); ^{13}C NMR (63 MHz, $CDCl_3$): δ 170.0, 155.5, 137.9, 128.6,

127.5, 127.4, 80.4, 78.9, 75.2, 69.6, 58.6, 54.1, 43.4, 28.3; HRMS Calcd for [$C_{18}H_{24}N_2O_4$ – Boc + H^+]: 233.1284. Found 233.1270. The obtained spectroscopic data were in accordance with literature data.[1]

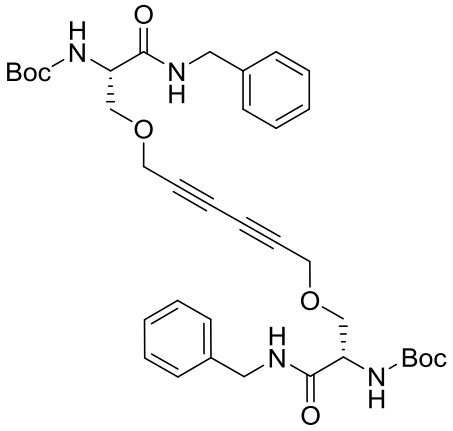
Synthesis of diynes 2a and 2b

Di-*tert*-butyl ((2*S*,9*S*)-1,10-bis(benzylamino)-1,10-dioxodeca-4,6-diyne-2,9-diyl)dicarbamate **2a**



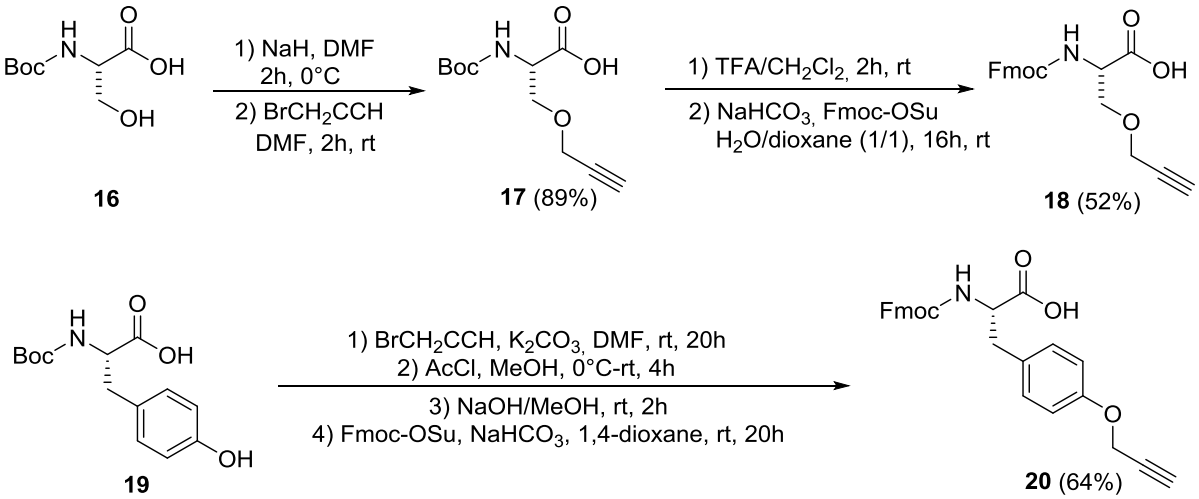
t*ert*-Butyl (*S*)-(1-(benzylamino)-1-oxo-3-(prop-2-yn-1-yloxy)propan-2-yl)carbamate (**1a**) (0.083 mmol), $Cu(OAc)_2.H_2O$ (0.04 mmol) and $NiCl_2$ (0.04 mmol) were added to EtOH (0.5 ml). Subsequently, Et_3N (0.25 mmol) and pyridine (0.41 mmol) were added dropwise to the mixture. The reaction mixture was stirred under O_2-atmosphere at rt for 4d. Afterwards, the reaction mixture was concentrated *in vacuo*. EtOAc (2 ml) was added and the mixture was acidified to pH 5 with an aqueous saturated citric acid solution and extracted with EtOAc (2x5 ml). The combined organic phases were washed with a saturated NH_4Cl solution (5 ml), brine (2x5 ml), dried with $MgSO_4$, filtered and concentrated *in vacuo*. The resulting mixture was purified via column silica gel chromatography (EtOAc/hexane 1/1) to afford di-*tert*-butyl (2*S*,9*S*)-1,10-bis(benzylamino)-1,10-dioxodeca-4,6-diyne-2,9-diyl)dicarbamate as a yellow solid in 36% yield (18 mg); mp: 82.0-82.9 °C; IR (neat): 2217, 2143, 1729, 1681 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$): δ 7.15 – 7.41 (m, 10 H), 6.78 – 6.88 (m, 2 H), 5.45 (d, *J* = 7.8 Hz, 2 H), 4.26 – 4.50 (m, 6 H), 2.77 (ddd, *J* = 19.6, 6.2, 6.2 Hz, 4 H), 1.41 (s, 18 H); ^{13}C NMR (63 MHz, $CDCl_3$) δ 170.0, 155.3, 137.7, 128.7, 127.6, 127.5, 80.7, 73.3, 67.9, 53.0, 43.5, 28.2, 23.4; HRMS Calcd for [$C_{34}H_{42}N_4O_6$ + Na^+]: 625.2997. Found 625.3010.

Di-*tert*-butyl ((4*S*,15*S*)-3,16-dioxo-1,18-diphenyl-6,13-dioxa-2,17-diazaoctadeca-8,10-diyne-4,15-diyl)dicarbamate **2b**



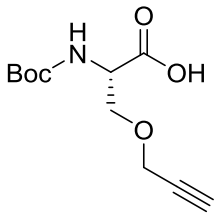
t*ert*-Butyl (*S*)-(1-(benzylamino)-1-oxo-3-(prop-2-yn-1-yloxy)propan-2-yl)carbamate (**1b**) (6.72 mmol) and $Cu(OAc)_2.H_2O$ (3.36 mmol) were added to EtOH (50 ml). Triethylamine (20.15 mmol) and pyridine (33.6 mmol) were added dropwise to the reaction mixture and the reaction mixture was stirred for 22h under O_2-atmosphere at rt. The reaction mixture was concentrated in vacuo and EtOAc (30 ml) was added to the residue. The mixture was acidified to pH 5 with an aqueous saturated citric acid solution and extracted with EtOAc (2x30 ml). The combined organic phases were washed with an aqueous saturated NH_4Cl solution (10 ml), brine (2x20 ml), dried over $MgSO_4$, filtered and concentrated *in vacuo*. The resulting residue was purified via column silica gel chromatography (hexane/EtOAc 1/2) to afford di-*tert*-butyl ((4*S*,15*S*)-3,16-dioxo-1,18-diphenyl-6,13-dioxa-2,17-diazaoctadeca-8,10-diyne-4,15-diyl)dicarbamate as yellow solid in 61% yield (2.69 g); mp: 64.1 – 66.2 °C; IR (neat): 2236, 2156, 1753, 1666, 1070 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$): δ 7.19 – 7.40 (m, 10H), 6.68 (t, *J* = 5.2 Hz, 2H), 5.37 (br. S., 2H), 4.48 (d, *J* = 5.9 Hz, 4H), 4.32 (br. S., 2H), 4.25 (d, *J* = 4.8 Hz, 4H), 3.95 (dd, *J* = 4.4 and 3.8 Hz, 2H), 3.69 (dd, *J* = 6.3 and 4.4 Hz, 2H), 1.43 (s, 18H); ^{13}C NMR (63 MHz, $CDCl_3$) δ 169.8, 155.4, 137.9, 128.7, 127.5, 80.5, 75.0, 70.9, 69.8, 59.1, 54.1, 43.5, 28.3; HRMS Calcd for [$C_{36}H_{46}N_4O_8$ + H^+]: 663.3399. Found: 663.3389.

2. Synthesis of Fmoc-AA(*O*-propargyl)-OH 18 and 20



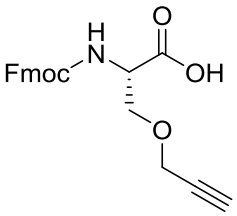
Scheme 1: Synthesis of Fmoc protected amino acids **18** and **20**.

N-(*tert*-butoxycarbonyl)-O-(prop-2-yn-1-yl)-L-serine **17**:



N-(*tert*-Butoxycarbonyl)-O-(prop-2-yn-1-yl)-L-serine **17** was synthesised according to literature.[2] (t*ert*-Butoxycarbonyl)-L-serine (24.37 mmol) was dissolved in DMF (150 ml). The solution was cooled to 0 °C and NaH (58.50 mmol, 60 wt-% suspension in mineral oil) was added in portions. After stirring for 2h protected from atmospheric moisture with a $CaCl_2$-tube, propargylbromide (34.10 mol, 80 wt-% in toluene) was added dropwise. After stirring for 2h at rt, the excess NaH was destroyed by addition of H_2O (50 ml), where the first 2 ml were carefully dropped. The resulting reaction mixture was concentrated *in vacuo*, dissolved in H_2O (150 ml), washed with Et_2O (50 ml) and acidified with 1M $KHSO_4$ to pH 1-2. The acidic aqueous phase was extracted with EtOAc (2x50 ml) and the combined organic phases were washed with brine (5x20 ml). The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo* to afford *N*-(*tert*-butoxycarbonyl)-*O*-(prop-2-yn-1-yl)-L-serine as a orange oil in 89% yield (6.80 g). IR (neat): 3336, 2078, 1736, 1559, 1123 cm^{-1}; ^1H NMR ($CDCl_3$, 250 MHz): δ 5.39 (d, *J* = 8.2 Hz, 1H), 4.49 (d, *J* = 8.2 Hz, 1H), 4.16 – 4.23 (m, 2H), 4.00 (dd, *J* = 9.3 and 3.0 Hz, 1H), 3.81 (dd, *J* = 9.4 and 3.5 Hz, 1H), 2.47 (t, *J* = 2.4 Hz, 1H), 1.47 (s, 9 H); ^{13}C NMR ($CDCl_3$, 63 MHz): δ 175.2, 156.0, 80.7, 78.9, 75.5, 69.7, 58.9, 53.9, 28.5; HRMS Calcd for [$C_{11}H_{17}NO_5$ + Na^+]: 266.0999. Found: 266.0992. The obtained spectroscopic data were in accordance with literature data. [2]

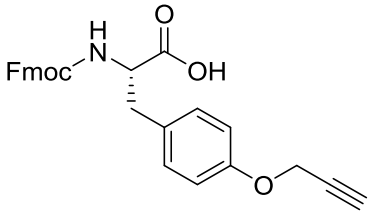
N-(((9H-fluoren-9-yl)methoxy)carbonyl)-*O*-(prop-2-yn-1-yl)-L-serine **18**:



t*ert*-Butyl (*S*)-(1-(benzylamino)-1-oxo-3-(prop-2-yn-1-yloxy)propan-2-yl)carbamate (21.71 mmol) was dissolved in CH_2Cl_2 (50 ml), followed by the addition of an excess of TFA (40 ml). The reaction mixture was stirred at rt for 2h and concentrated *in vacuo*. The obtained oil was dissolved in H_2O (50 ml) and the pH of the solution was adjusted to pH 10 with an aqueous saturated $NaHCO_3$ solution (30 ml). This basic aqueous solution was added to a solution of Fmoc-OSu **(***N*-(9-fluorenylmethoxycarbonyloxy)succinimide) (23.88 mmol) in 1,4-dioxane (75 ml). Afterwards, the reaction mixture was stirred at rt for 16h. The reaction mixture was concentrated *in vacuo* and the residual oil was dissolved in EtOAc (140 ml). The organic phase was extracted with an aqueous 1% $NaHCO_3$ solution (2x100 ml). The pH of the combined aqueous layers were adjusted to pH 1 with an aqueous 6M HCl, followed by extraction with EtOAc (4x10 0ml). The combined organic phases were washed with brine (2x50 ml), dried over $MgSO_4$, filtered and concentrated *in vacuo*. The resulting residue was triturated with Et_2O (50 ml), filtered and and the obtained solids were dried (0.05 mmHg) to

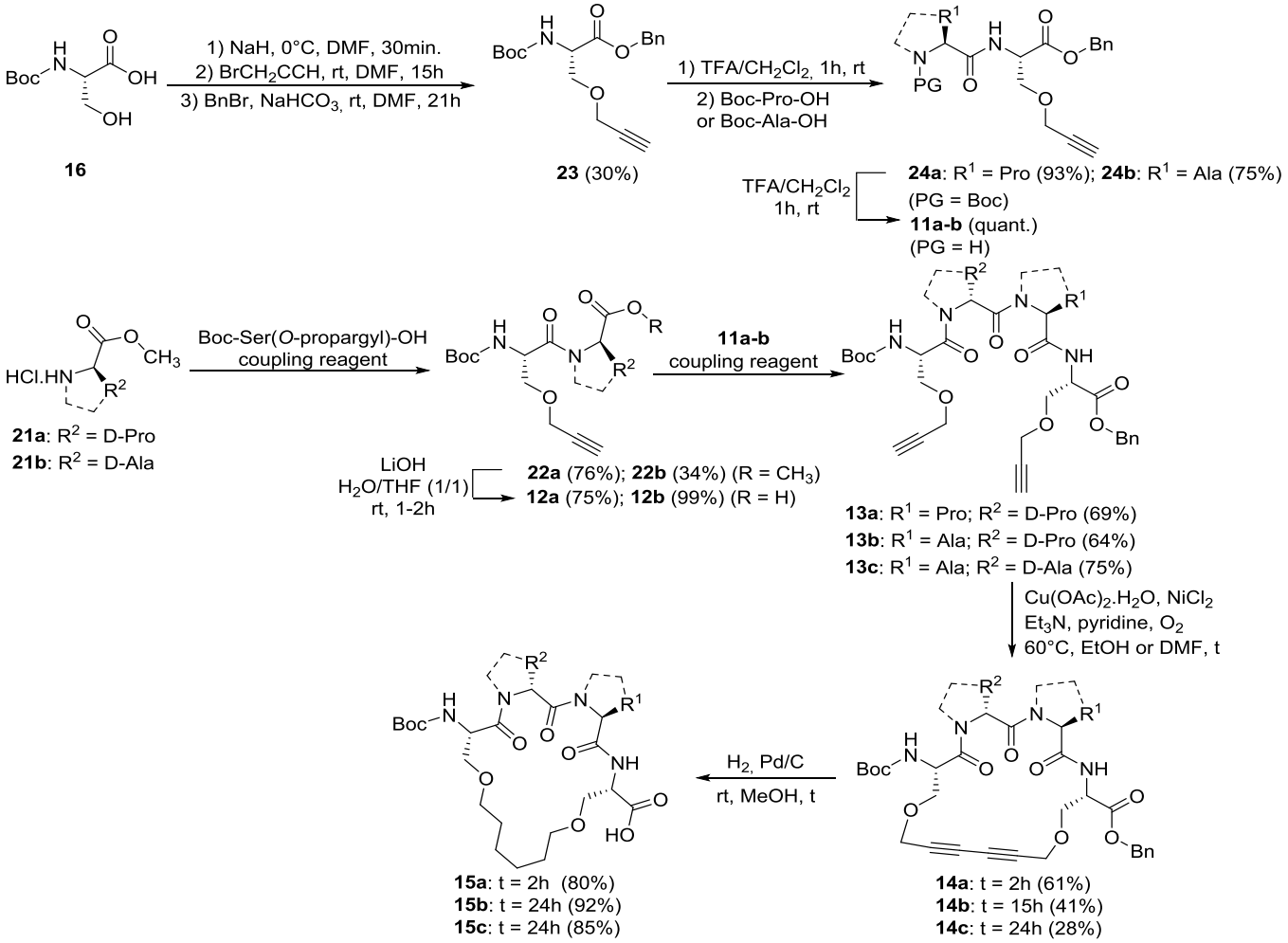
afford *N*-(((9H-fluoren-9-yl)methoxy)carbonyl)-*O*-(prop-2-yn-1-yl)-L-serine as a white solid in 52% yield (4.10 g); mp: 156.1 – 157.9 °C; IR (neat): 3300, 2166, 1753, 1427, 1070 cm^{-1}; ^{1}H NMR (250 MHz, DMSO-d_6): δ 12.81 (br. S, H), 7.88 (d, *J* = 7.3 Hz, 2H), 7.74 (d, *J* = 7.1 Hz, 2H), 7.67 (d, *J* = 8.2 Hz, 1H), 7.36 (dtd, *J*= 23.5 and 7.8 and 1.4 Hz, 4H), 4.17 – 4.30 (m, 4H), 4.15 (d, *J* = 2.3 Hz, 2H), 3.70 (d, *J* = 5.6 Hz, 2H), 3.46 (t, *J* = 2.3 Hz, 1H); ^{13}C NMR (63 MHz, DMSO-d_6) δ 171.4, 156.0, 143.8, 140.7, 127.6, 127.0, 125.3, 120.1, 79.8, 77.5, 68.6, 65.8, 57.7, 53.9, 46.6; HRMS Calcd for [$C_{21}H_{19}NO_5$ + H^+]: 366.1348. Found: 366.1336.

(*S*)-2-((((9H-Fluoren-9-yl)methoxy)carbonyl)amino)-3-(4-(prop-2-yn-1-yloxy)phenyl)propanoic acid **20**



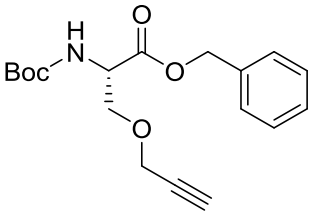
(*S*)-2-((((9H-Fluoren-9-yl)methoxy)carbonyl)amino)-3-(4-(prop-2-yn-1-yloxy)phenyl)propanoic acid was synthesised according to literature.[3] K_2CO_3 (64.0 mmol) was added to a solution of (t*ert*-butoxycarbonyl)-L-tyrosine (21.33 mmol) in DMF (30 ml). Propargylbromide (64.0 mmol, 80 wt-% in toluene) was added dropwise to the reaction mixture and stirred at rt for 20h closed from the atmospheric moisture with a $CaCl_2$-tube. The reaction mixture was diluted with H_2O (150 ml) and extracted with EtOAc (2x100 ml). The combined organic phases were dried over $MgSO_4$, filtered and concentrated *in vacuo*. The resulting residue was added to a solution of acetyl chloride (21 ml) in MeOH (180 ml) at 0 °C and stirred for 4h. The reaction mixture was concentrated *in vacuo* and the obtained residue was added to a mixture of 2M NaOH (42 ml) and MeOH (30 ml) and stirred at rt for 2h. The pH of the solution was adjusted to pH 7 with concentrated HCl and stirred overnight at 0 °C. The formed precipitate was filtered, washed with cold H_2O and dried to obtain a white powder. The obtained solid was dissolved in a 1,4-dioxane/H_2O mixture (1/1) (80 ml), followed by the addition of $NaHCO_3$ (5.50 mmol). After stirring for 30min at rt, Fmoc-OSu (5.50 mmol) was added and the resulting reaction mixture was stirred for 20h at rt. The reaction mixture was concentrated *in vacuo* and the obtained residue was purified via column silica gel chromatography (CH_2Cl_2/MeOH/AcOH 100/10/1) to afford (*S*)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-(4-(prop-2-yn-1-yloxy)phenyl)propanoic acid as a white solid in 64% yield (2.38 g); mp: 132.4 – 137.2 °C; IR (neat): 3283, 3039, 2134, 1740 cm^{-1}; ^{1}H NMR (250 MHz, DMSO-d_6): δ 12.68 (br. S, 1H), 7.87 (d, *J* = 7.3 Hz, 2H), 7.66 (m, 3H), 7.40 (t, *J* = 7.4 Hz, 2H), 7.29 (q, *J* =7.5 Hz, 2H), 7.19 (d, *J* = 8.6 Hz, 2H), 6.87 (d, *J* = 8.6 Hz, 2H), 4.72 (d, *J* = 2.5 Hz, 2H), 4.03 – 4.24 (m, 4H), 3.52 (t, *J* = 2.6 Hz, 1H), 3.01 (dd, *J* = 6.9 and 3.8 Hz, 1 H), 2.80 (dd, *J* = 10.0 and 7.5 Hz, 1H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 174.0, 156.6, 156.5, 144.4, 141.4, 131.3, 130.8, 128.3, 127.7, 126.0, 120.8, 115.2, 80.1, 78.7, 86.3, 56.4, 56.0, 47.3, 36.3; HRMS Calcd for [$C_{27}H_{23}NO_5$ + Na^+]: 464.1469. Found 464.1466.

3. Solution phase synthesis of peptides **13a-c**, **14a-c** and **15a-c**



Scheme 2: Synthesis of dipeptides **11a-b** and **12a-b** and tetrapeptides **13a-c**, **14a-c** and **15a-c**

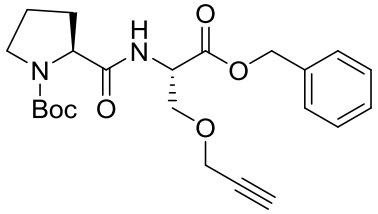
Synthesis of dipeptides 12a-b and 24a-b:
Benzyl *N*-(*tert*-butoxycarbonyl)-*O*-(prop-2-yn-1-yl)-L-serinate **23**



Benzyl *N*-(*tert*-butoxycarbonyl)-*O*-(prop-2-yn-1-yl)-L-serinate **23** was synthesised according to literature.[4] (t*ert*-Butoxycarbonyl)-L-serine (21.10 mmol) was dissolved in DMF (150 ml). NaH (42.20 mmol, 60 wt-% suspension in mineral oil) was added slowly. After stirring for 30min, propargylbromide (29.50 mmol, 80 wt-% in toluene) was added. The reaction mixture was stirred at rt for 15h closed for atmospheric moisture with a $CaCl_2$-tube. The reaction mixture was diluted with brine (200 ml) and extracted with EtOAc (2x100 ml). The aqueous layer was acidified to pH 1-2 with 1M $KHSO_4$ and extracted with EtOAc (3x150 ml). The

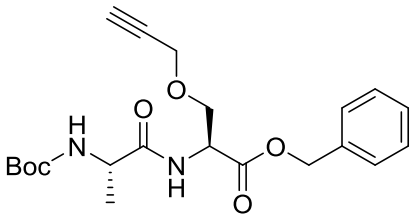
combined organic layers were dried over $MgSO_4$, filtered, concentrated *in vacuo* and the resulting residue was dissolved in DMF (150 ml). $NaHCO_3$ (42.20 mmol) and benzyl bromide (52.80 mmol) were added dropwise. The reaction mixture was stirred at rt for 21h, diluted with brine (150 ml) and extracted with EtOAc (3x100 ml). The combined organic layers were washed with brine (2x100 ml), dried over $MgSO_4$, filtered and concentrated *in vacuo*. The obtained mixture was purified via column silica gel chromatography (hexane/EtOAc 1/1) to afford benzyl *N*-(*tert*-butoxycarbonyl)-*O*-(prop-2-yn-1-yl)-L-serinate as a colourless liquid in 30% yield (2.07 g). IR (neat): 3289, 2134, 1746 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$): δ 7.29 – 7.44 (m, 5 H), 5.41 (d, *J* = 8.8 Hz, 1 H), 5.23 (dd, *J* = 12.5 Hz, 2 H), 4.46 – 4.57 (m, 1 H), 4.12 (d, *J* = 2.5 Hz, 2 H), 4.01 (dd, *J* = 5.0, 2.5 Hz, 1 H), 3.78 (dd, *J* = 5.0, 2.5 Hz, 1 H), 2.42 (t, *J* = 2.3 Hz, 1 H), 1.46 (s, 9 H); ^{13}C NMR ($CDCl_3$, 126 MHz,): δ= 170.6, 155.7, 135.7, 128.8, 128.5, 128.3, 80.3, 79.0, 75.3, 70.0, 67.4, 58.8, 54.2, 28.5; HRMS Calcd for [$C_{18}H_{23}NO_5$ + Na^+]: 356.1469. Found 356.1475. The obtained spectroscopic data were in accordance with literature data.[4]

t*ert*-Butyl (*S*)-2-(((*S*)-1-(benzyloxy)-1-oxo-3-(prop-2-yn-1-yloxy)propan-2-yl)carbamoyl)pyrrolidine-1-carboxylate **24a**



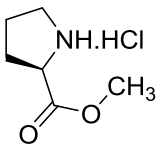
Benzyl *N*-(*tert*-butoxycarbonyl)-*O*-(prop-2-yn-1-yl)-L-serinate (3.0 mmol) was dissolved in TFA (5 ml) and CH_2Cl_2 (5 ml) and stirred for 1h at rt protected from atmospheric moisture with a $CaCl_2$-tube. The reaction mixture was concentrated *in vacuo* and dissolved in CH_2Cl_2 (20 ml). The reaction mixture was cooled to 0 °C and *N*-Boc-Proline (3.0 mmol) and HOAt (6.0 mmol) were added. EDC.HCl (6.0 mmol) and DIPEA (6.0 mmol) were added next and the reaction mixture was stirred for 1h at rt protected from atmospheric moisture with a $CaCl_2$-tube. The reaction mixture was diluted with EtOAc (20 ml) and washed with H_2O (3x20 ml) and brine (3x20 ml). The organic layer was dried over $MgSO_4$, filtered, concentrated *in vacuo* and purified via column silica gel chromatography (petroleumether/EtOAc 5/1) to afford *tert*-butyl (*S*)-2-(((*S*)-1-(benzyloxy)-1-oxo-3-(prop-2-yn-1-yloxy)propan-2-yl)carbamoyl)pyrrolidine-1-carboxylate as a yellow oil in 80% yield (1.03 g). IR (neat): 3288, 2114, 1744, 1674 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$): δ = 7.32 – 7.43 (m, 5 H), 6.88 (br. S, 1 H), 5.22 (dd, *J* = 12.5 Hz and 12.5 Hz, 2 H), 4.81 (br. S., 1 H), 4.31 (br. S., 1 H), 4.11 (d, *J* = 2.5 Hz, 2 H), 4.00 (dd, *J* = 2.5 Hz and 2.5 Hz, 1 H), 3.79 (dd, *J* = 2.5 Hz and 2.5 Hz, 1 H), 3.34 – 3.58 (m, 2 H), 2.40 (t, *J* = 2.4 Hz, 1 H), 2.17 (br. S., 2 H), 1.83 – 1.90 (m, 4 H), 1.47 (s, 9 H) ; ^{13}C NMR (126 MHz, $CDCl_3$): δ 172.8, 169.8, 149.9, 135.6, 128.8, 128.6, 128.4, 79.0, 78.1, 75.3, 69.6, 67.5, 61.2, 58.7, 52.7, 47.2, 31.0, 28.6, 23.9; HRMS Calcd for [$C_{23}H_{30}N_2O_6$ + Na^+]: 453.1996. Found 453.1991.

Benzyl *N*-((*tert*-butoxycarbonyl)-L-alanyl)-*O*-(prop-2-yn-1-yl)-L-serinate **24b**



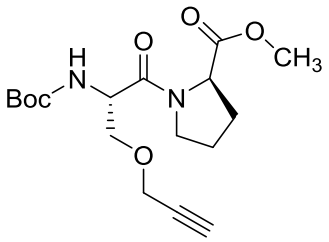
Benzyl *N*-(*tert*-butoxycarbonyl)-*O*-(prop-2-yn-1-yl)-L-serinate (12.6 mmol) was dissolved in TFA (40 ml) and CH_2Cl_2 (40 ml) and stirred for 1h at rt protected from atmospheric moisture with a $CaCl_2$-tube. The reaction mixture was concentrated *in vacuo* and dissolved in DMF (100 ml), followed by the addition of DIPEA (12.6 mmol). This mixture was added to a mixture of *N*-Boc-Alanine (11.5 mmol), HATU (13.7 mmol) and DIPEA (12.6 mmol), dissolved in DMF (100 ml) and stirred for 30min at 0 °C. The resulting mixture was stirred for 15h protected from atmospheric moisture with a $CaCl_2$-tube at rt. The reaction mixture was concentrated *in vacuo* and the obtained residu was dissolved in EtOAc (100 ml) and washed with an aqueous 1M $KHSO_4$ solution (100 ml), an aqueous 1M NaOH solution (100 ml) and brine (3x10 0ml). The organic layer was dried over $MgSO_4$, filtered, concentrated *in vacuo* and purified via column silica gel chromatography (petroleumether/EtOAc 3/1) to afford benzyl *N*-((*tert*-butoxycarbonyl)-L-alanyl)-*O*-(prop-2-yn-1-yl)-L-serinate as a yellow liquid in 75% yield (3.5 g). IR (neat) 3351, 2173, 1723, 1699 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$): δ = 7.30 - 7.38 (m, 5 H), 6.78 (d, *J* = 8.3 Hz, 1 H), 5.20 (dd, *J* = 7.5 and 5.0 Hz, 2 H), 5.05 (br. s, 1 H), 4.78 – 4.81 (m, 1 H), 4.21 (br. s, 1 H), 4.09 (d, *J* = 2.4 Hz, 2 H), 4.01 (dd, *J* = 9.4 and 3.2 Hz, 1 H), 3.76 (dd, *J* = 9.4 and 3.2 Hz, 1 H), 2.40 (t, *J* = 2.4 Hz, 1 H), 1.44 (s, 9 H), 1.36 (d, *J* = 7.1 Hz, 3 H); ^{13}C NMR (126 MHz, $CDCl_3$): δ = 172.8, 169.8, 155.5, 135.5, 128.8, 128.6, 128.4, 80.3, 79.0, 75.4, 69.5, 67.6, 58.8, 52.8, 50.3, 28.5, 18.9; HRMS Calcd for [$C_{21}H_{28}N_2O_6$ + Na^+]: 427.1840. Found 427.1836.

Methyl D-prolinate hydrochloride **21b**



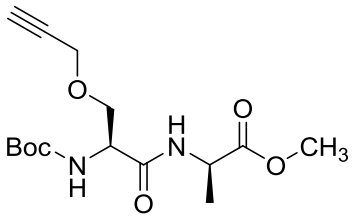
Methyl D-prolinate hydrochloride **21b** was synthesised according to literature.[5] To a cooled solution of *D*-proline (4.34 mmol) in MeOH (5 ml) was added $SOCl_2$ (5.65 mmol) dropwise over 1.5h. The reaction mixture was stirred for 15h at rt protected from atmospheric moisture with a $CaCl_2$-tube. The solution was concentrated *in vacuo* to obtain a yellow oil. After high vacuum (0.05 mmHg) drying methyl D-prolinate hydrochloride was obtained as a gray oil in quantitative yield (0.72 g). IR (neat): 3391, 1739, 1232 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$): δ 10.90 (br. s, 1 H), 9.13 (br. s, 1 H), 4.50 (br. s, 1 H), 3.86 (s, 3 H), 3.42 – 3.74 (m, 2 H), 2.35 – 2.56 (m, 1 H), 1.99 – 2.31 (m, 3 H) ; ^{13}C NMR (126 MHz, $CDCl_3$): δ 169.2, 59.4, 53.9, 46.3, 29.0, 23.8; HRMS Calcd for [$C_6H_{11}NO_2$ + H^+]: 130.0863. Found 130.0868. The obtained spectroscopic data were in accordance with literature data.[5]

Methyl *N*-(*tert*-butoxycarbonyl)-*O*-(prop-2-yn-1-yl)-L-seryl-D-prolinate **22a**



A solution of *N*-(*tert*-butoxycarbonyl)-O-(prop-2-yn-1-yl)-L-serine (0.82mmol) in CH_2Cl_2 (10 ml) was cooled to 0°C and treated with (1-[bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate) (HATU) (1.15 mmol). Methyl D-prolinate hydrochloride (0.99 mmol) was added, followed by the addition of *N*-methylmorpholine (NMM) (3.29 mmol). The reaction mixture was stirred for 15h at 0 °C closed from atmospheric moisture with a $CaCl_2$-tube. The reaction mixture was concentrated *in vacuo* and the obtained residue was dissolved in EtOAc (10 ml). The organic phase was washed with H_2O (2x10 ml), 1M HCl (10 ml), saturated aqueous $NaHCO_3$ solution (10 ml) and brine (10 ml). The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo*. The obtained residue was purified via column silica gel chromatography (petroleum ether/EtOAc 2/1) to afford methyl *N*-(*tert*-butoxycarbonyl)-*O*-(prop-2-yn-1-yl)-L-seryl-D-prolinate as a yellow oil in 76% yield (0.22 g). IR (neat): 3276, 2976, 2114, 1744, 1709, 1647; ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.38 (d, *J* = 8.5 Hz, 1 H), 4.68 – 4.75 (m, 1 H), 4.48 (dd, *J* = 2.5 Hz and 2.5 Hz, 1 H), 4.09 – 4.20 (m, 2 H), 3.60 – 3.80 (m, 7 H), 2.42 (t, *J* = 2.5 Hz, 1 H), 2.17 – 2.28 (m, 1 H), 1.89 – 2.13 (m, 3 H), 1.45 (s, 9 H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 172.4, 169.1, 155.1, 79.9, 79.2, 74.8, 70.3, 59.1, 58.6, 52.2, 51.5, 47.2, 29.2, 28.3, 24.7; HRMS Calcd for [$C_{17}H_{26}N_2O_6$ + H^+]: 355.1864. Found 355.1859.

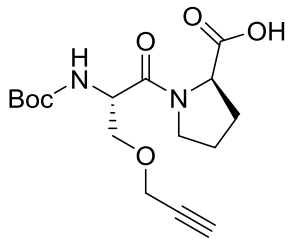
Methyl *N*-(*tert*-butoxycarbonyl)-*O*-(prop-2-yn-1-yl)-L-seryl-D-alaninate **22b**



A solution of *N*-(*tert*-butoxycarbonyl)-*O*-(prop-2-yn-1-yl)-L-serine (20.6 mmol) in DMF (125 ml) was cooled to 0 °C and treated with benzotriazol-1-ol (HOBt) (24.7 mmol), EDC.HCl (24.7 mmol), DIPEA (22.6 mmol) and a solution of methyl D-alaninate hydrocholoride (22.6 mmol) and DIPEA (22.6 mmol) in DMF (125 ml). The reaction mixture was stirred for 15h at 0 °C closed from atmospheric moisture with a $CaCl_2$-tube. The reaction mixture was concentrated *in vacuo* and the obtained residue was dissolved in EtOAc (100 ml). The organic phase was washed with an aqueous 1M $KHSO_4$ solution (100 ml), an aqueous 1M NaOH solution (100 ml) and brine (3x100 ml). The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo*. The obtained residue was purified via column silica gel chromatography (petroleum ether/EtOAc 2/1) to afford methyl *N*-(*tert*-butoxycarbonyl)-*O*-(prop-2-yn-1-yl)-L-seryl-D-alaninate as a yellow oil in 34% yield (2.32 g). IR (neat): 3294, 2156, 1735, 1654; ^{1}H NMR (500 MHz, $CDCl_3$): δ 6.86 (br. s, 1 H), 5.35 (br. s, 1 H), 4.56

(quin, *J* = 7.2 Hz, 1 H), 4.24 - 4.32 (m, 1 H), 4.09 - 4.21 (m, 2 H), 3.89 (br. s, 1 H), 3.72 (s, 3 H), 3.66 (dd, *J* = 9.4, 6.0 Hz, 1 H), 2.43 (t, *J* = 1.0 Hz, 1 H), 1.43 (s, 9 H), 1.39 ppm (d, *J* = 6.8 Hz, 3 H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 173.3, 169.7, 155.8, 80.6, 79.2, 75.4, 69.4, 58.8, 54.1, 52.7, 48.4, 28.5, 18.5 ppm; HRMS Calcd for [$C_{15}H_{24}N_2O_6$ + H^+]: 329.1701. Found 329.1709.

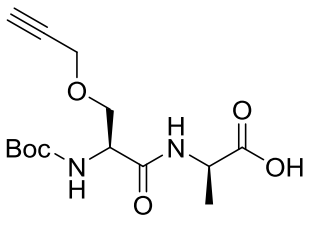
N-(*tert*-Butoxycarbonyl)-*O*-(prop-2-yn-1-yl)-L-seryl-D-proline **12a**



To a solution of *N*-(*tert*-butoxycarbonyl)-*O*-(prop-2-yn-1-yl)-L-seryl-D-prolinate (0.71 mmol) in a mixture of H_2O (10 ml) and THF (10 ml) was LiOH.H_2O (1.06 mmol) added. The reaction mixture was stirred for 15h at rt protected from atmospheric moisture with a $CaCl_2$-tube. The reaction mixture was diluted with H_2O (10 ml) and EtOAc (10 ml). The aqueous phase was separated and the pH was adjusted to pH 1-2 with a 1M HCl solution. The resulting mixture was extracted with EtOAc (3x20 ml). The organic phase was washed with brine (3x20 ml), dried over $MgSO_4$, filtered and concentrated *in vacuo*. After high vacuum (0.05mm Hg) drying *N*-(*tert*-butoxycarbonyl)-*O*-(prop-2-yn-1-yl)-L-seryl-D-proline was obtained as a yellow solid in 61% yield (0.18 g). mp: 49.2 – 52.3 °C; IR (neat): 3275, 2115, 1704, 1635, 1633 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.36 (s, 1 H), 6.87 and 7.29 (d, *J* = 8.2 Hz, 1 H)*, 4.45 – 4.54 and 4.77 – 4.83 (m, 1 H)*, 4.16 – 4.21 and 4.45 – 4.54 (m, 1 H)*, 4.07 – 4.08 and 4.13 – 4.15 (m, 2 H)*, 3.46 – 3.52 and 3.53 – 3.70 (m, 2 H)*, 3.40 – 3.41 and 3.44 – 3.47 (t, *J* = 2.0 Hz, 1 H)*, 3.33 – 3.37 and 3.53 – 3.70 (m, 2 H)*, 2.07 – 2.19 (m, 1 H), 1.65 – 1.74 and 1.82 – 1.95 (m, 3 H)*, 1.39 and 1.37 (2×s, 9 H)*; ^{13}C NMR (126 MHz, DMSO-d_6): δ ^{13}C NMR (DMSO-d_6 ,126MHz): δ 173.6 + 173.0*, 168.7 + 168.1*, 155.4 + 154.9*, 80.0, 78.3, 77.3 + 77.1*, 68.9 + 68.6*, 58.7, 57.7, 51.8 + 51.3*, 46.4 + 46.1*, 30.6 + 28.6*, 28.1 + 27.7*, 24.3; HRMS Calcd for [$C_{16}H_{24}N_2O_6$ –Boc + H^+]: 241.1183. Found 241.1178.

*Double signals due to rotamerism across the C-N bond of the amide as well as the carbamate.

N-(*tert*-Butoxycarbonyl)-*O*-(prop-2-yn-1-yl)-L-seryl-D-alanine **12b**



To a solution of methyl *N*-(*tert*-butoxycarbonyl)-*O*-(prop-2-yn-1-yl)-L-seryl-D-alaninate (1.5 mmol) in a mixture of H_2O (10 ml) and THF (10 ml) was LiOH (3.8 mmol) added. The reaction mixture was stirred for 2h at rt protected from atmospheric moisture with a $CaCl_2$-tube. The reaction mixture was diluted with H_2O (20 ml) and EtOAc (20 ml). The aqueous phase was separated and the pH was adjusted to pH 1-2 with an aqueous 1M HCl solution. The resulting mixture was extracted with EtOAc (3x25 ml). The organic phase was washed with brine (3x25 ml), dried over $MgSO_4$, filtered and concentrated *in vacuo* to afford *N*-(*tert*-butoxycarbonyl)-*O*-(prop-2-yn-1-yl)-L-seryl-D-alanine as a yellow solid in 99% yield (0.48 g). mp: 95.2 - 97.3 °C; IR (neat): 3281, 2135, 1714, 1655 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 7.02 (br. s, 1 H), 5.45 - 5.56 (m, 1 H), 4.58 (br. s, 1 H), 4.47 (br. s, 1 H), 4.09 - 4.22 (m, 2 H), 3.86 (dd, *J* = 9.2, 4.3 Hz, 1 H), 3.68 (br. s, 1 H), 2.44 (t, *J* = 2.4 Hz, 1 H), 1.43 ppm (s, 12 H)); ^{13}C NMR (126 MHz, $CDCl_3$): δ 175.5, 170.3, 156.2, 81.0, 79.1, 75.5, 69.6, 58.8, 54.0, 54.0, 48.4, 28.5, 18.3 ppm; HRMS Calcd for [$C_{14}H_{22}N_2O_6 + H^+$]: 315.1551. Found 315.1556.

Difficult *D*-Pro Pro coupling:

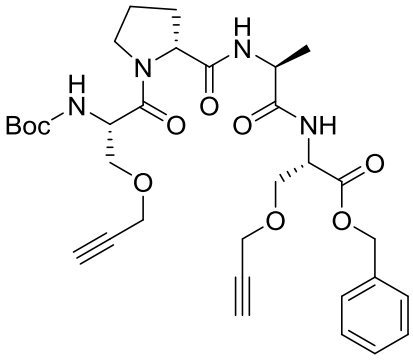
The coupling of Boc-Pro-OH with dipeptide **11**, after Boc deprotection of **24**, appeared to be problematic due to a difficult D-Pro Pro coupling. Several coupling reagents were tried and the conversions were rather poor (Table 2). Therefore, instead of a linear synthesis, a convergent synthesis was chosen towards tetrapeptide **13**, where HATU as coupling reagent in DMF afforded the highest conversion (>80%).

Entry	Coupling reagent	solvent	t (h)	Conv. (%)
1	HOBt/EDC	CH_2Cl_2	48	40
2	HOAt/EDC	CH_2Cl_2	48	40
3	HATU	CH_2Cl_2	48	10
4	TBTU	DMF	24	13
5	T3P	EtOAc	72	30
6	BOP-Cl	CH_2Cl_2	24	0
7[a]	PyBOP	CH_3CN	72	30

Table 2: Evalution of the *D*-Pro Pro coupling with several coupling reagentia ([a] a complex reaction mixture was obtained)

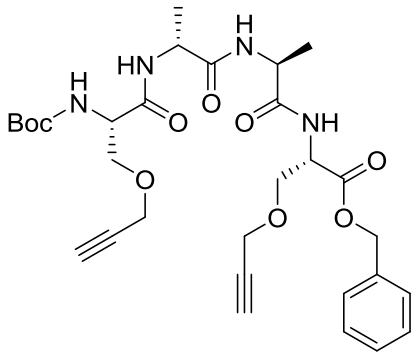
Synthesis of tetrapeptides 13b-c, 14b-c and 15b-c:

Benzyl *N*-*N*-(*tert*-butoxycarbonyl)-*O*-(prop-2-yn-1-yl)-L-seryl-D-prolyl-L-alanyl-*O*-(prop-2-yn-1-yl)-L-serinate **13b**



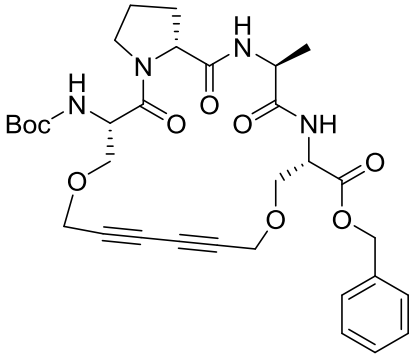
Benzyl *N*-((*tert*-butoxycarbonyl)-L-alanyl)-*O*-(prop-2-yn-1-yl)-L-serinate (2.7 mmol) was dissolved in a mixture of TFA (5 ml) and CH_2Cl_2 (5 ml). The reaction mixture was stirred for 1h closed from atmopsheric moisture with a $CaCl_2$-tube and concentrated *in vacuo* to obtain **11b** as a TFA salt. To a cooled solution of *N*-(*tert*-butoxycarbonyl)-*O*-(prop-2-yn-1-yl)-L-seryl-D-proline (2.6 mmol) in CH_2Cl_2 (10 ml) was added HOBt (3.1 mmol), EDC.HCl (3.1 mmol) and DIPEA (5.7 mmol) and the reaction mixture was stirred for 30min at 0 °C. The TFA salt (**11b**), dissolved in CH_2Cl_2 (100 ml), was added to the reaction mixture, followed by the addition of DIPEA (2.9 mmol). The reaction mixture was stirred for 15h at rt protected from atmospheric moisture with a $CaCl_2$-tube. The reaction mixture was concentrated *in vacuo* and the obtained residue was purified via column silica gel chromatography ($CHCl_3$/iPrOH 30/1) to afford benzyl *N*-*N*-(tert-butoxycarbonyl)-*O*-(prop-2-yn-1-yl)-L-seryl-D-prolyl-L-alanyl-*O*-(prop-2-yn-1-yl)-L-serinate as a yellow oil in 64% yield (1.08 g). HRMS Calcd for [$C_{32}H_{42}N_4O_9 + Na^+$]: 649.2844. Found 649.2865.

Benzyl *N*-*N*-(*tert*-butoxycarbonyl)-*O*-(prop-2-yn-1-yl)-L-seryl-D-alanyl-L-alanyl-*O*-(prop-2-yn-1-yl)-L-serinate **13c**



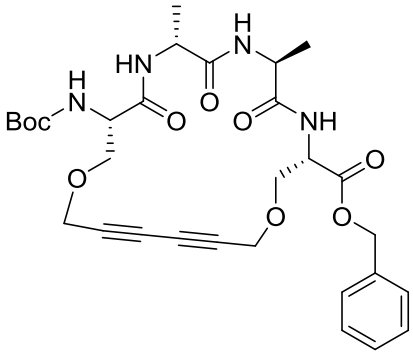
Benzyl *N*-((*tert*-butoxycarbonyl)-L-alanyl)-*O*-(prop-2-yn-1-yl)-L-serinate (8.80 mmol) was dissolved in a mixture of TFA (100 ml) and CH_2Cl_2 (100 ml) and stirred for 2h. The reaction mixture was evaporated *in vacuo*, dissolved in DMF (150 ml), followed by the addition of DIPEA (8.80 mmol). This mixture was added to a mixture of *N*-(tert-butoxycarbonyl)-*O*-(prop-2-yn-1-yl)-L-seryl-D-alanine (7.98 mmol), HOAt (9.57 mmol), EDC.HCl (9.57 mmol) and DIPEA (8.80 mmol), dissolved in DMF (150 ml) and stirred for 30min at 0 °C. The resulting reaction mixture was stirred for 15h at rt, evaporated *in vacuo* and the resulting crude mixture was purified via column silica gel chromatography ($CHCl_3$/MeOH 99/1) to afford benzyl *N*-*N*-(*tert*-butoxycarbonyl)-*O*-(prop-2-yn-1-yl)-L-seryl-D-alanyl-L-alanyl-*O*-(prop-2-yn-1-yl)-L-serinate as a yellow solid in 75% yield (3.60 g). HRMS Calcd for [$C_{30}H_{40}N_4O_9 + Na^+$]: 623.2687. Found 623.2681.

Benzyl (3S,6S,9R,12S)-12-((*tert*-butoxycarbonyl)amino)-tetrahydropyrrolo-[2,1-i]-6-methyl-5,8,11-trioxo-1,14-dioxa-4,7,10-triazacycloicosa-16,18-diyne-3-carboxylate **14b**



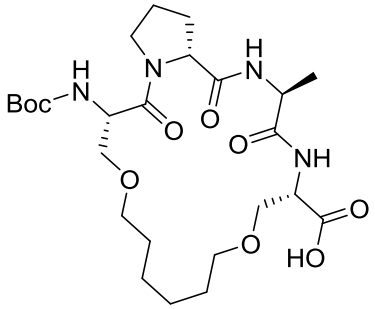
Benzyl *N*-*N*-(*tert*-butoxycarbonyl)-*O*-(prop-2-yn-1-yl)-L-seryl-D-prolyl-L-alanyl-*O*-(prop-2-yn-1-yl)-L-serinate (1.5 mmol), $Cu(OAc)_2.H_2O$ (1.5 mmol) and $NiCl_2$ (1.5 mmol) were added to EtOH (750 ml). Et_3N (4.5 mmol) and pyridine (7.5 mmol) were added and the reaction mixture was stirred at 60 °C under O_2-atmosphere for 15h. The reaction mixture was concentrated *in vacuo* and purified via column silica gel chromatography (CH_2Cl_2/iPrOH 97/3) to afford benzyl (3S,6S,9R,12S)-12-((tert-butoxycarbonyl)amino)-10-pyrolo-6-dimethyl-5,8,11-trioxo-1,14-dioxa-4,7,10-triazacycloicosa-16,18-diyne-3-carboxylate as a red solid in 41% yield (0.38 g). HRMS Calcd for [$C_{32}H_{40}N_4O_9$ + Na^+]: 647.2687. Found 647.2694.

Benzyl (3*S*,6*S*,9*R*,12*S*)-12-((*tert*-butoxycarbonyl)amino)-6,9-dimethyl-5,8,11-trioxo-1,14-dioxa-4,7,10-triazacycloicosa-16,18-diyne-3-carboxylate **14c**



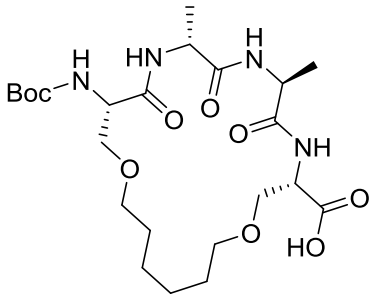
Benzyl *N*-*N*-(*tert*-butoxycarbonyl)-*O*-(prop-2-yn-1-yl)-L-seryl-D-alanyl-L-alanyl-*O*-(prop-2-yn-1-yl)-L-serinate (2.50 mmol), $Cu(OAc)_2.H_2O$ (2.50 mmol) and $NiCl_2$ (2.50 mmol) were added to DMF (1200 ml). Et_3N (7.49 mmol) and pyridine (12.49 mmol) were added and the reaction mixture was stirred at 60 °C under O_2-atmosphere for 24h. The reaction mixture was concentrated *in vacuo* and purified via column silica gel chromatography ($CHCl_3$/MeOH 99/1) to afford benzyl (3*S*,6*S*,9*R*,12*S*)-12-((tert-butoxycarbonyl)amino)-6,9-dimethyl-5,8,11-trioxo-1,14-dioxa-4,7,10-triazacycloicosa-16,18-diyne-3-carboxylate as a white solid in 28% yield (410 mg). HRMS Calcd for [$C_{30}H_{38}N_4O_9$ + H^+]: 599.2712. Found 599.2718.

(3*S*,6*S*,17*S*,22*aR*)-17-((*tert*-Butoxycarbonyl)amino)-3-methyl-1,4,18-trioxooctadecahydro-1H,16H-pyrrolo[2,1-i][1,14]dioxa[4,7,10]triazacycloicosine-6-carboxylic acid **15b**



To a mixture of benzyl (3S,6S,9R,12S)-12-((*tert*-butoxycarbonyl)amino)-10-pyrolo-6-dimethyl-5,8,11-trioxo-1,14-dioxa-4,7,10-triazacycloicosa-16,18-diyne-3-carboxylate 0.032 mmol) in MeOH (1 ml) was added palladium on carbon (10 wt-% Pd on C, 10 mg). The reaction mixture was stirred under H_2 (1 atm) at rt for 24h, filtered over Celite and concentrated *in vacuo* to afford (3*S*,6*S*,17*S*,22*aR*)-17-((tert-butoxycarbonyl)amino)-3-methyl-1,4,18-trioxooctadecahydro-1H,16H-pyrrolo[2,1-i][1,14]dioxa[4,7,10]triazacycloicosine-6-carboxylic acid as a white solid in 92% yield (16.0 mg). HRMS Calcd for [$C_{32}H_{48}N_4O_9$ + Na^+]: 565.2844. Found 565.2830.

(3*S*,6*S*,9*R*,12*S*)-12-((tert-butoxycarbonyl)amino)-6,9-dimethyl-5,8,11-trioxo-1,14-dioxa-4,7,10-triazacycloicosane-3-carboxylic acid **15c**



To a mixture of benzyl (3*S*,6*S*,9*R*,12*S*)-12-((*tert*-butoxycarbonyl)amino)-6,9-dimethyl-5,8,11-trioxo-1,14-dioxa-4,7,10-triazacycloicosa-16,18-diyne-3-carboxylate (0.017 mmol) in MeOH (1 ml) was added palladium on carbon (10 wt-% Pd on C, 5 mg). The reaction mixture was stirred under H_2 (1 atm) at rt for 24h, filtered over Celite and concentrated *in vacuo* to afford (3*S*,6*S*,17*S*,22*aR*)-17-((tert-butoxycarbonyl)amino)-3-methyl-1,4,18-trioxooctadecahydro-1H(3*S*,6*S*,9*R*,12*S*)-12-((tert-butoxycarbonyl)amino)-6,9-dimethyl-5,8,11-trioxo-1,14-dioxa-4,7,10-triazacycloicosane-3-carboxylic acid as a white solid in 85% yield (7.5 mg). HRMS Calcd for [$C_{23}H_{40}N_4O_9$ + Na^+]: 539.2687. Found 539.2693.

4. Solid Phase Peptide Synthesis of 5a-k, 7 and 9

5a: 39% yield; HPLC (standard gradient): t_{ret} = 10.27 min.; HRMS Calcd for [$C_{24}H_{33}N_5O_7$ + H^+]: 504.2453. Found 504.2444.

5b: 43% yield; HPLC (standard gradient): t_{ret} = 8.62 min.; HRMS Calcd for [$C_{21}H_{29}N_5O_7$ + H^+]: 464.2140. Found 464.2132.

5c: 12% yield; HPLC (standard gradient): t_{ret} = 9.15 min.; HRMS Calcd for [$C_{22}H_{31}N_5O_7$ + H^+]:478.2296 . Found 478.2296.

5d: 27% yield; HPLC (standard gradient): t_{ret} = 8.95 min.; HRMS Calcd for [$C_{22}H_{31}N_5O_7$ + H^+]: 478.2296. Found 478.2273.

5e: 41% yield; HPLC (standard gradient): t_{ret} = 8.96 min.; HRMS Calcd for [$C_{21}H_{31}N_5O_7$ + H^+]: 466.2296. Found 466.2290.

5f: 4 % yield: HPLC (standard gradient): t_{ret} = 9.65 min.; HMRS Calcd for [$C_{23}H_{33}N_5O_7$ + H^+]: 492.2453. Found 492.2540.

5g: 23% yield; HPLC (standard gradient): t_{ret} = 8.25 min.; HRMS Calcd for [$C_{20}H_{29}N_5O_7$ + Na^+]: 474.1959. Found 474.1957.

5h: 34% yield; HPLC (standard gradient): t_{ret} = 8.15 min.; HRMS Calcd for [$C_{20}H_{29}N_5O_7$ + H^+]: 452.2140. Found 452.2122.

5i: 29% yield; HPLC (standard gradient): t_{ret} = 7.10 min.; HRMS Calcd for [$C_{20}H_{28}N_6O_8$ + H^+]: 481.2041. Found 481.2030.

5j: 23% yield; HPLC (standard gradient): t_{ret} = 12.64 min.; HRMS Calcd for [$C_{28}H_{35}N_5O_9$+ H^+]: 586.2507. Found 586.2514.

5k: 15 % yield; HPLC (standard gradient): t_{ret} = 12.82 min.; HRMS Calcd for [$C_{31}H_{42}N_6O_9$ + H^+]: 643.3086. Found 643.3085.

7: 5% yield; HPLC (standard gradient): t_{ret} = 12.63 min.; HRMS Calcd for $C_{30}H_{37}N_5O_7$ + Na^+]: 602.2585. Found 602.2583.

9: 9% yield; HPLC (standard gradient): t_{ret} = 14.63 min.; HRMS Calcd for [$C_{36}H_{41}N_5O_7$ + H^+]: 656.3079. Found 656.3076.

5. Cyclization and purification of peptides 5a-k, 7 and 9

a. Purification using RP preparative HPLC

The obtained ethanolic reaction mixtures containing the diyne, pyridine, triethylamine and catalysts were evaporated *in vacuo* and directly loaded on the RP HPLC column to give analytically pure samples as white solids after lyophilization.

6a 9% yield (3.5 mg); HPLC (standard gradient): t_{ret} = 9.46 min.; HRMS Calcd for [$C_{24}H_{31}N_5O_7$ + H^+]: 502.2267. Found 502.2296.

6b: 39% yield; HPLC (standard gradient): t_{ret} = 8.74 min.; HRMS Calcd for [$C_{21}H_{27}N_5O_7$ + H^+]: 462.1983. Found 462.1982.

6c: 14% yield; HPLC (standard gradient): t_{ret} = 9.02 min.; HRMS Calcd for [$C_{22}H_{29}N_5O_7$ + H^+]: 476.2140. Found 476.2122.

6d: 40% yield; HPLC (standard gradient): t_{ret} = 9.11 min.; HRMS Calcd for [$C_{22}H_{29}N_5O_7$ + H^+]: 476.2140. Found 476.2136.

6e: 4% yield; HPLC (standard gradient): t_{ret} = 8.68 min.; HRMS Calcd for [$C_{21}H_{29}N_5O_7$ + H^+]: 464.2140. Found 464.2145.

6f: 4 % yield: HPLC (standard gradient): t_{ret} = 9.16 min.; HMRS Calcd for [$C_{23}H_{31}N_5O_7$ + H^+]: 490.2296. Found 490.2292.

6g: 10% yield; HPLC (standard gradient): t_{ret} = 8.21 min.; HRMS Calcd for [$C_{20}H_{27}N_5O_7$ + H^+]: 450.1983. Found 450.1985.

6h: 25% yield; HPLC (standard gradient): t_{ret} = 7.46 min.; HRMS Calcd for [$C_{20}H_{27}N_5O_7$ + H^+]: 450.1983. Found 450.1980.

6i: 4% yield; HPLC (standard gradient): t_{ret} = 7.32 min.; HRMS Calcd for [$C_{20}H_{26}N_6O_8$ + H^+]: 479.1885. Found: 479.1862.

6j: 5% yield; HPLC (standard gradient): t_{ret} = 11.55 min.; HRMS Calcd for [$C_{28}H_{33}N_5O_9$ + H^+]: 584.2351. Found: 584.2299.

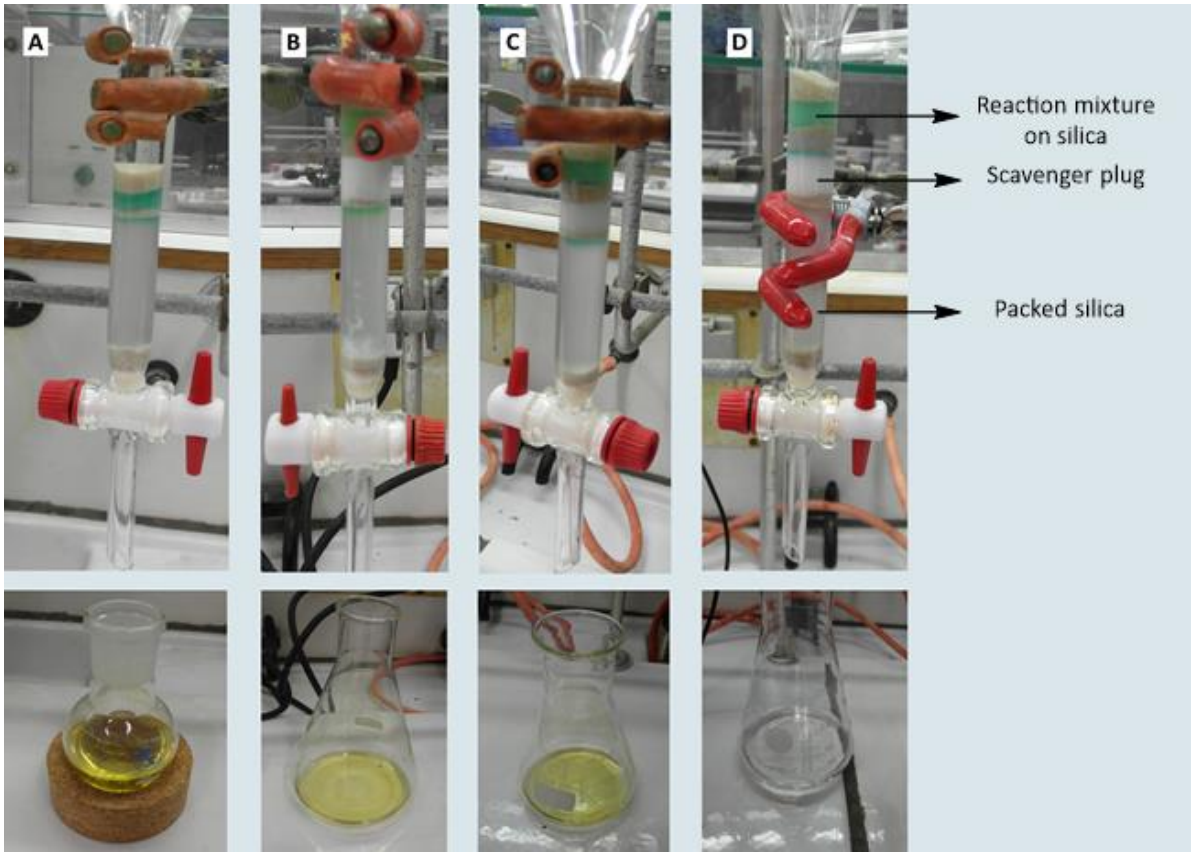
6k: 17 % yield; HPLC (standard gradient): t_{ret} = 12.00 min.; HRMS Calcd for [$C_{31}H_{40}N_6O_9$ + H^+]: 641.2930. Found: 641.2910.

8: 5% yield; HPLC (standard gradient): t_{ret} = 12.18 min.; HRMS Calcd for [$C_{30}H_{35}N_5O_7$ + H^+]: 578.2609. Found 578.2607.

10: 4% yield; HPLC (standard gradient): t_{ret} = 14.45 min. (90% pure); HRMS Calcd for [$C_{36}H_{39}N_5O_7$ + Na^+]: 676.2742. Found 676.2738.

b. Purification using silica gel column chromatography

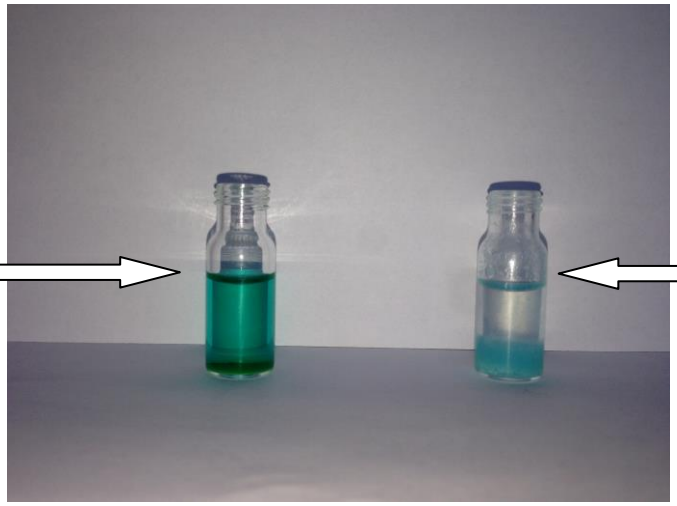
As stated in the manuscript, the silica gel column chromatography of poorly soluble peptides (in CH_2Cl_2) resulted in green coloured compounds because of the presence of co-eluted copper salts. Therefore, different chelators were tested as plugs over a silica column to verify whether these salts can be retained during chromatography. Conditions were evaluated where the reaction mixture was treated (a) with a blank (no scavenger), (b) 5 equiv Na_4EDTA, (c) 5 equiv Na_3-citrate and (4) 5 equiv Na_3PO_4. The mixture was stirred for a few minutes and silica was added before the solvent was evaporated. This resulted in blue-green coated silica which was afterwards brought onto the purification column. As can be seen from the figure below, only the use of Na_3PO_4 as scavenger gave good results.



Scheme 0.1. Different scavengers tested to complex the Cu- and Ni-salts; A = blank, B = Na_4EDTA, C = Na_3-citrate and D = Na_3PO_4.

In a control experiment, the effect of Na_3PO_4 on complexing the copper salts was demonstrated. In the figure below (left) the reaction mixture containing the Cu- and Ni-salts is compared to the same solution but treated with 5 equiv of Na_3PO_4. It can be seen that the Na_3PO_4 is responsible for clearing the supernatant solution from the excess of coloured salts.

(a) Untreated reaction mixture of diyne, copper and Ni-salts, pyridine and triethyl amine in EtOH



(b) Identical reaction mixture as (a), after treatment with 5 aquiv of aq. Sat. Na_3PO_4

The use of silica gel column chromatography as purification method was demonstrated for peptide derivatives **6a**, **6c**, **6e**, **6g**, **6i**, **8** and **10** which gave low yield via RP preparative HPLC. The mixture was treated with 5 equiv of Na_3PO_4 (as aq. sat. solution) during 15 minutes. Afterwards the mixture was concentrated, loaded on a plug of silica gel (2cm, diameter 0.5cm) and eluted using 20% MeOH in CH_2Cl_2. After evaporation in vacuo, 5mL of hexane

was added and the mixture was left without stirring to remove traces of pyridine. After decanting the hexane, the remaining solids were further evaporated at high vacuum (0,1 mbar) resulting in cyclic peptides as white solids.

6a 68% yield; TLC (silica, 20% MeOH in CH_2Cl_2): R_f = 0.29. HPLC (standard gradient): t_{ret} = 9.46 min.

6c 66% yield; TLC (silica, 20% MeOH in CH_2Cl_2): R_f = 0.22. HPLC (standard gradient): t_{ret} = 9.02 min.

6e 48% yield; TLC (silica, 20% MeOH in CH_2Cl_2): R_f = 0.15. HPLC (standard gradient): t_{ret} = 8.68 min.

6g 45% yield; TLC (silica, 20% MeOH in CH_2Cl_2): R_f = 0.12. HPLC (standard gradient): t_{ret} = 8.21 min.

6i 61% yield; TLC (silica, 20% MeOH in CH_2Cl_2): R_f = 0.05. HPLC (standard gradient): t_{ret} = 7.32 min.

8 54% yield; TLC (silica, 20% MeOH in CH_2Cl_2): R_f = 0.29. HPLC (standard gradient): t_{ret} = 12.18 min.

10 76% yield; TLC (silica, 20% MeOH in CH_2Cl_2): R_f = 0.31. HPLC (standard gradient): t_{ret} = 14.45 min.

c. Solid phase cyclisation of peptide 5a

Compound **5a** was synthesised on solid phase via the general procedure for SPPS. However, instead of acetylating the amine terminus, cleaving the peptide from the resin and puryfing the acyclic peptide via preparative HPLC, the solid supported *N*-Fmoc protected peptide was directly cyclised on solid phase using a BIOTAGE Initiator^{+} SP Microwave Synthesizer. $Cu(OAc)_2.H_2O$ (1 equiv.), $NiCl_2$ (1 equiv.), Et_3N (3 equiv.) and pyridine (5 equiv.) were dissolved in $CHCl_3$ (1 ml) and added to the resin (50 mg). After reaction for 30min at 50 °C in the microwave, 90% conversion towards the cyclic peptide is observed according to HPLC and LC-MS. As a sideproduct, dimer **6a'** is formed in 10%, according to HPLC and LC-MS.

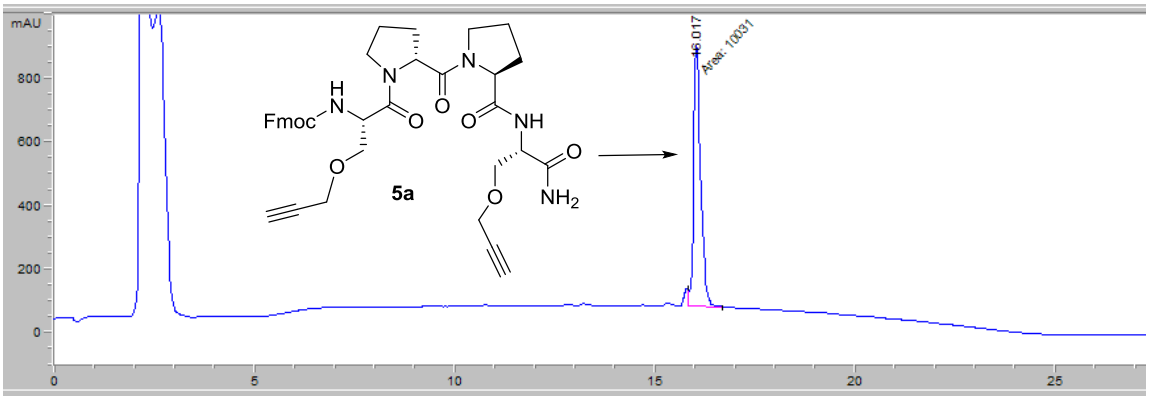


Figure 1a: HPLC chromatogram of crude starting material **5a**

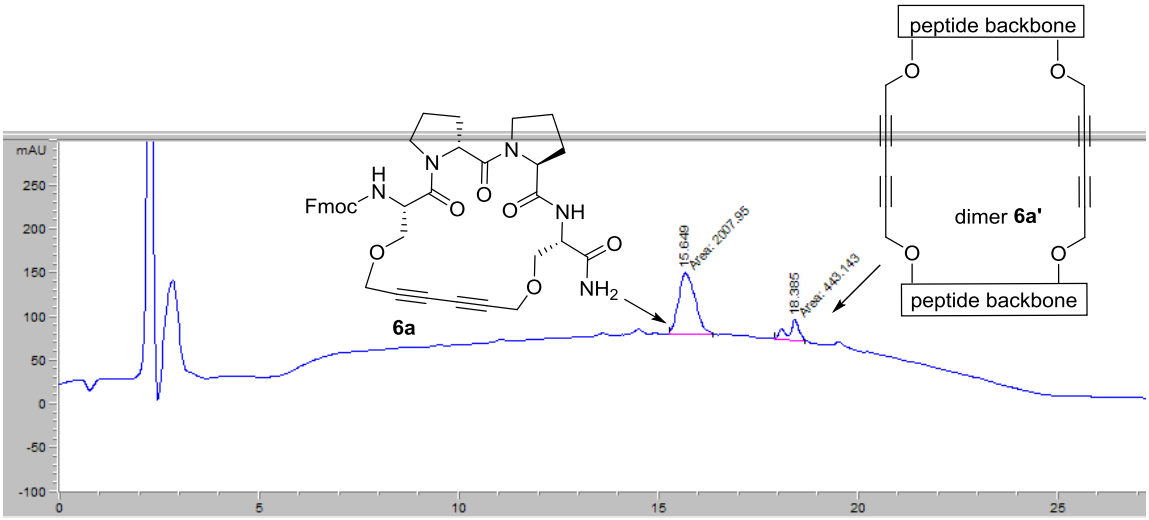


Figure 1b: HPLC chromatogram of the crude reaction mixture of the solid phase cyclisation for peptide **5a**

6. Copies of ^{1}H and ^{13}C NMR of compounds 1, 2, 12, 13a, 14a, 15a, 17, 18, 20-24 and ROESY NMR of compounds 13a, 14a and 15a

1a

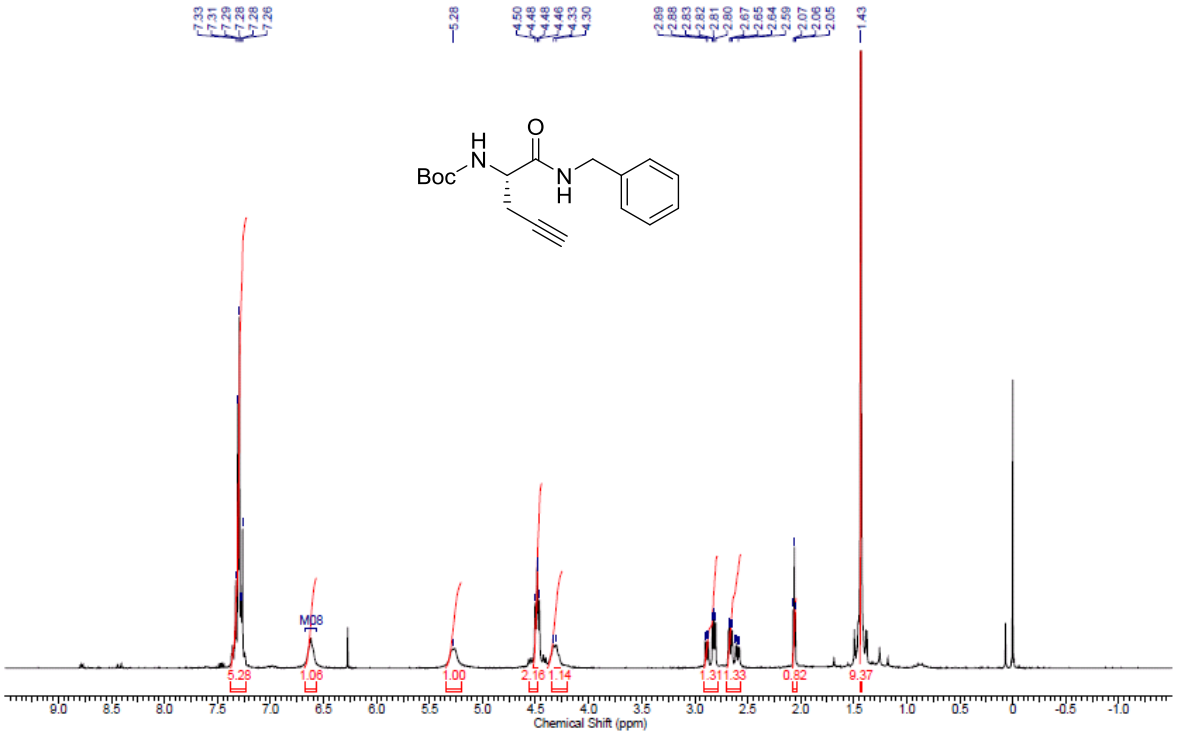


Figure 2: ^{1}H NMR spectrum of **1a** (250 MHz, $CDCl_3$)

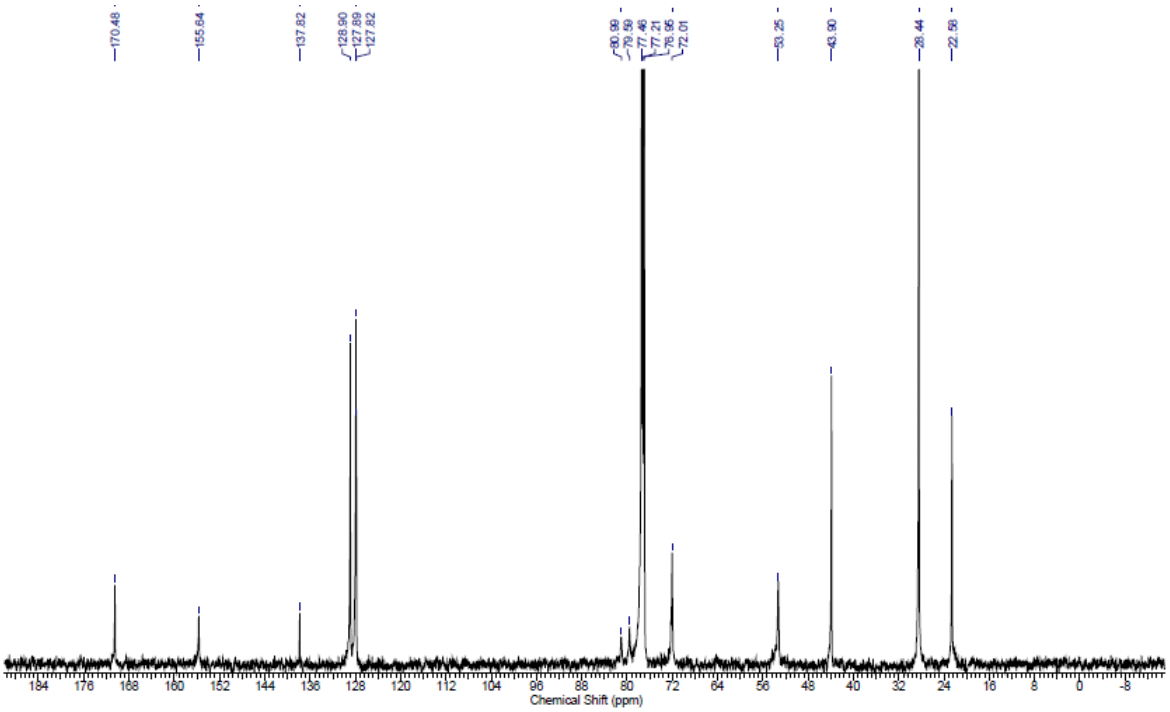


Figure 3: ^{13}C NMR spectrum of **1a** (126 MHz, $CDCl_3$)

1b

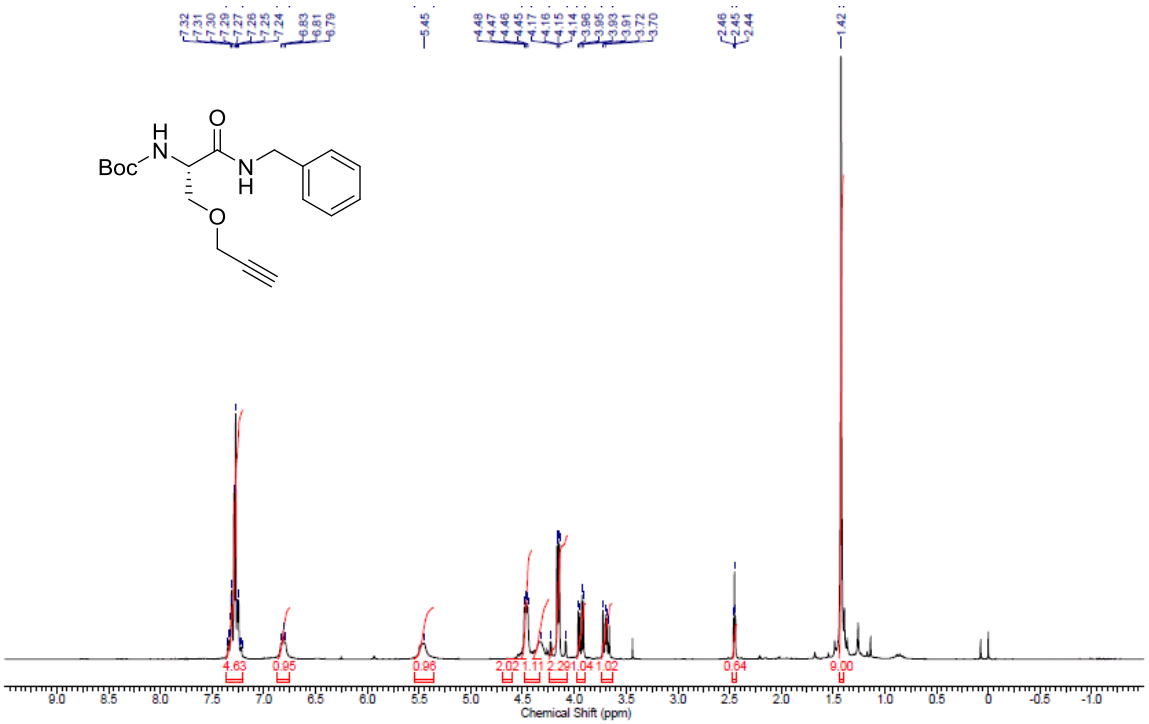


Figure 4: ^{1}H NMR spectrum of **1b** (250 MHz, $CDCl_3$)

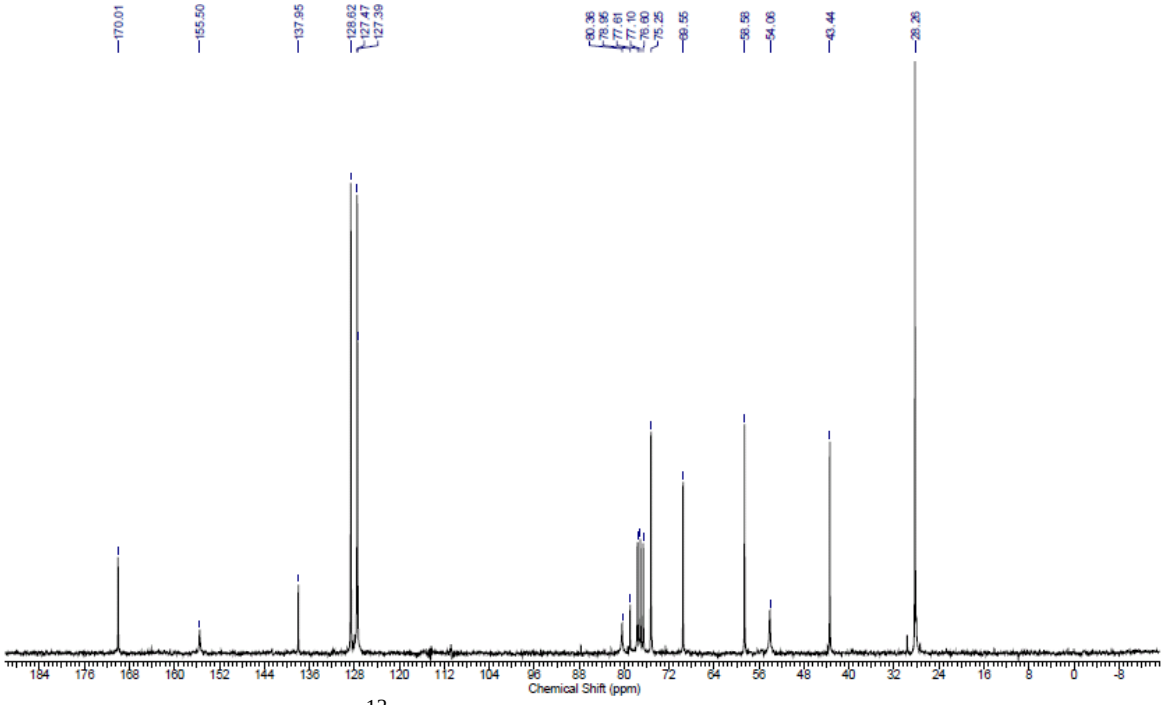


Figure 5: ^{13}C NMR spectrum of **1b** (63 MHz, $CDCl_3$)

2a

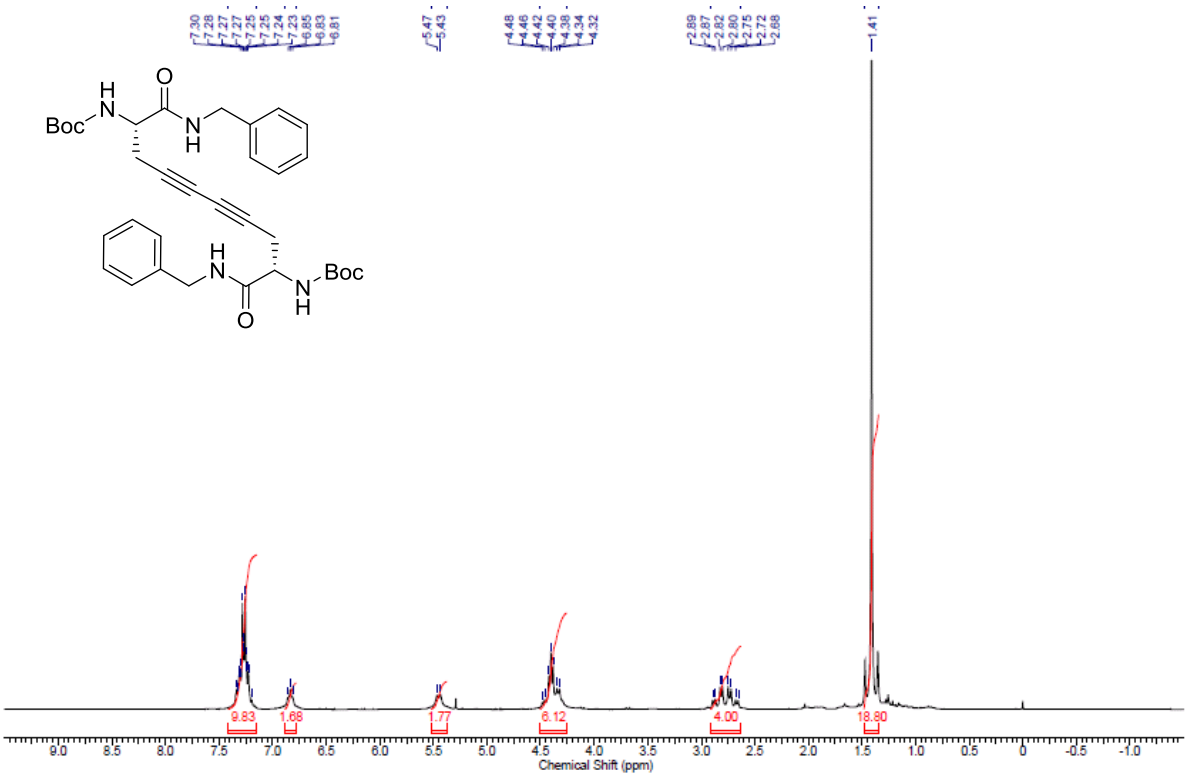


Figure 6: ^{1}H NMR spectrum of **2a** (250 MHz, $CDCl_3$)

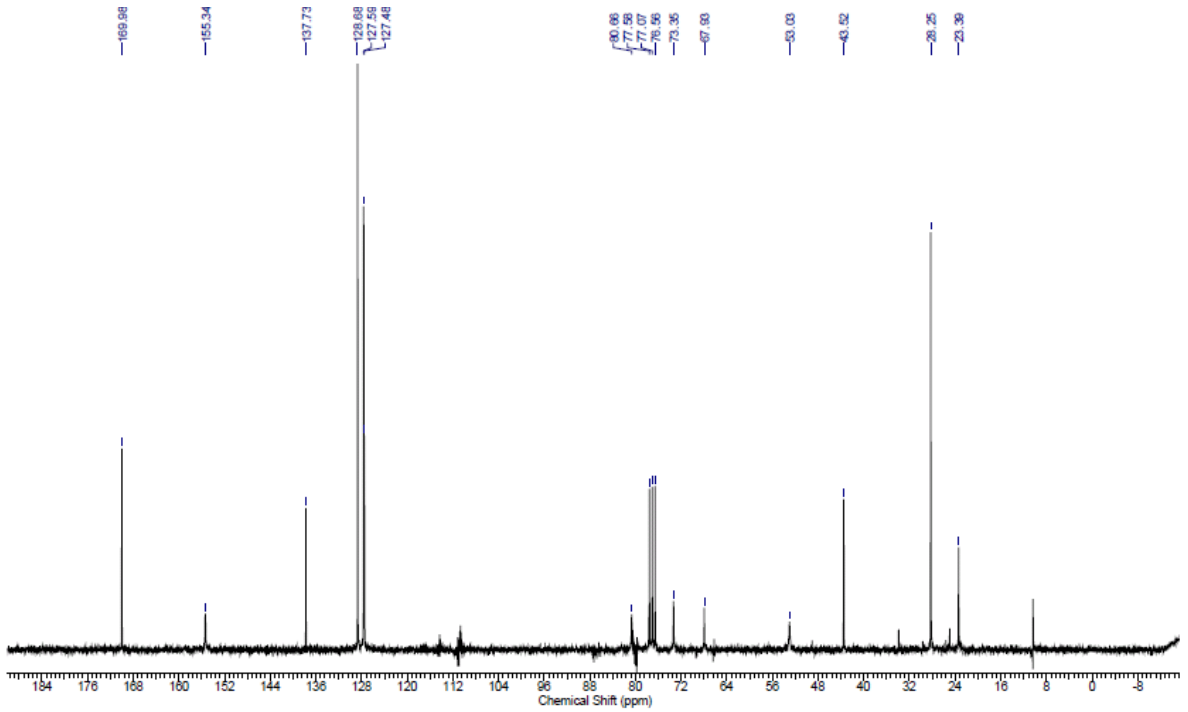


Figure 7: ^{13}C NMR spectrum of **2a** (63 MHz, $CDCl_3$)

2b

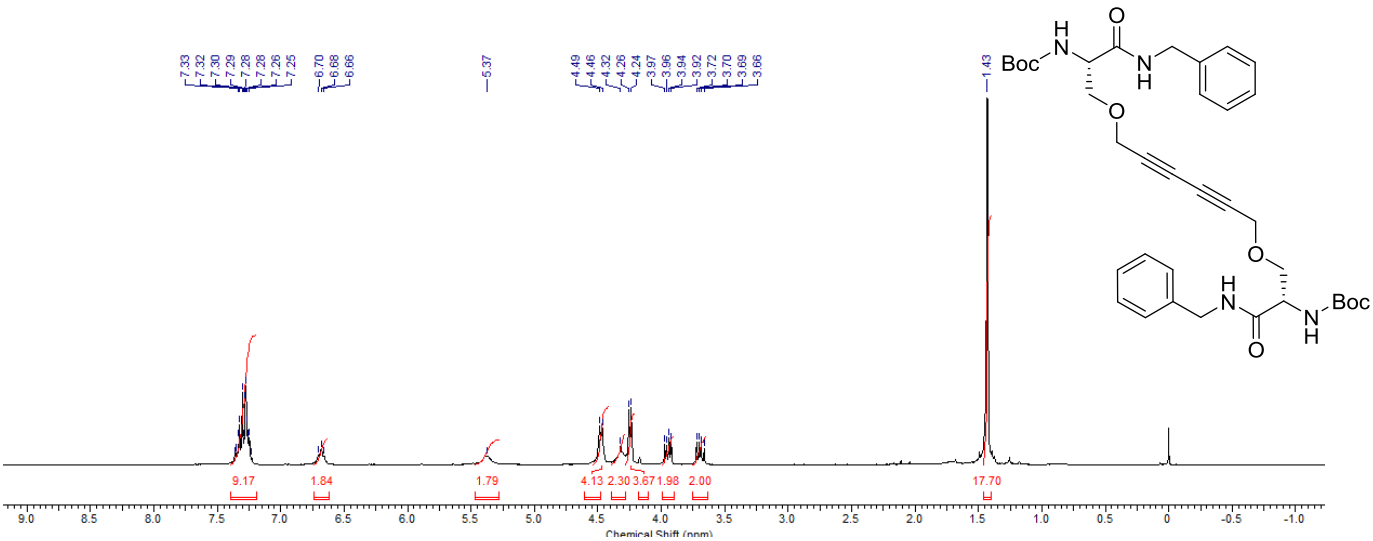


Figure 8: ^{1}H NMR spectrum of **2b** (250 MHz, $CDCl_3$)

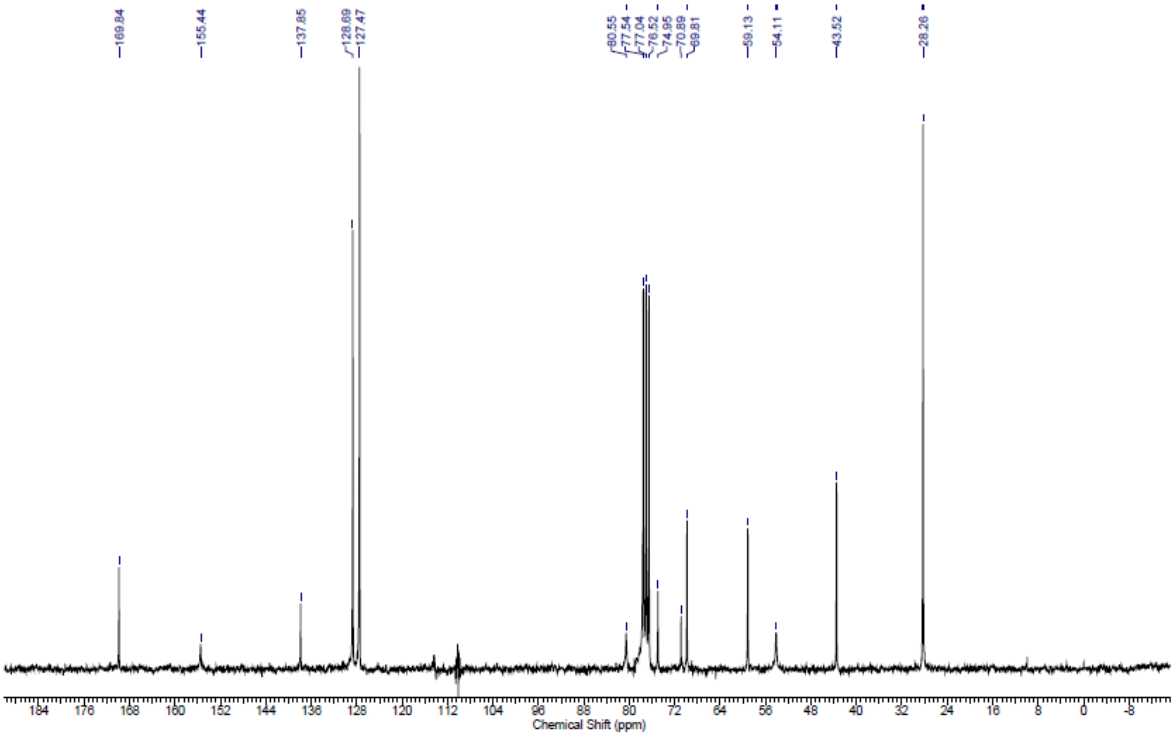


Figure 9: ^{13}C NMR spectrum of **2b** (63 MHz, $CDCl_3$)

17

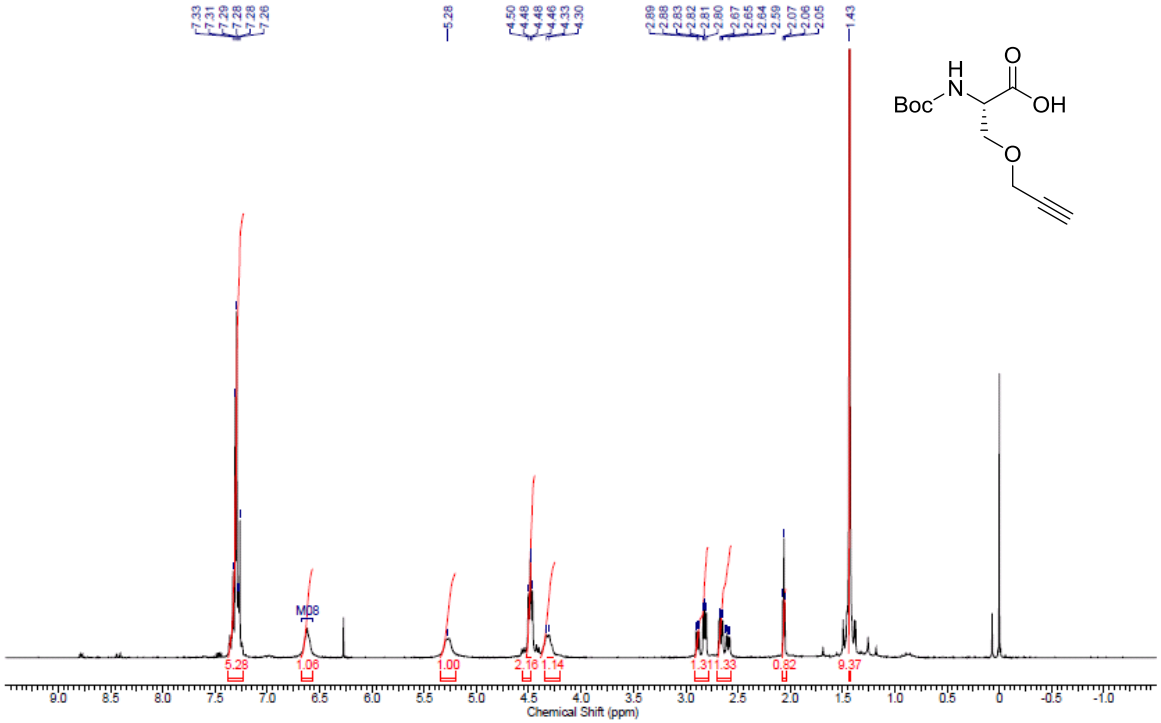


Figure 10: ^{1}H NMR spectrum of **17** (250 MHz, $CDCl_3$)

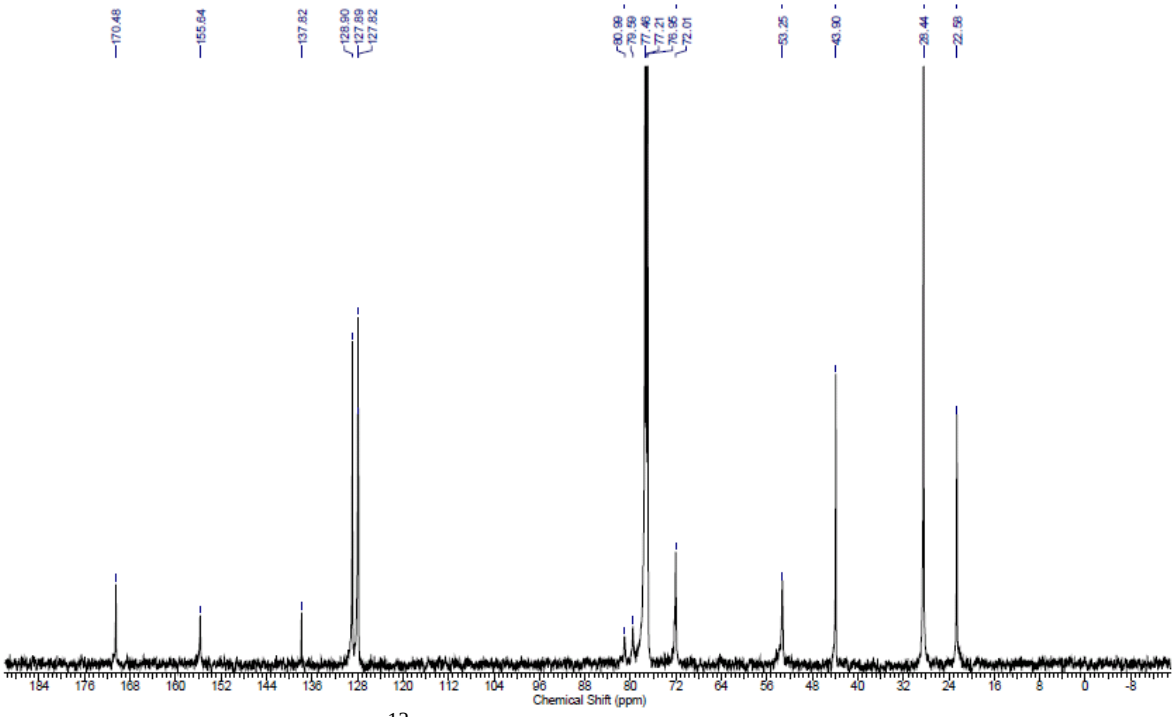


Figure 11: ^{13}C NMR spectrum of **17** (63 MHz, $CDCl_3$)

18

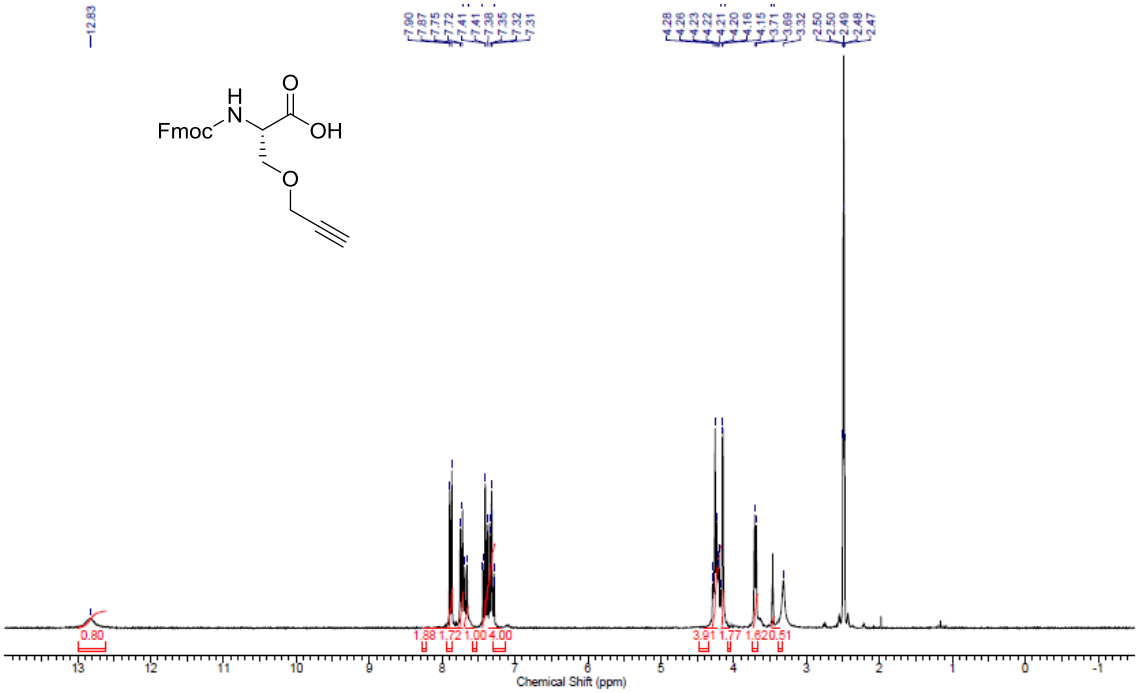


Figure 12: ^{1}H NMR spectrum of **18** (250 MHz, DMSO-d_6)

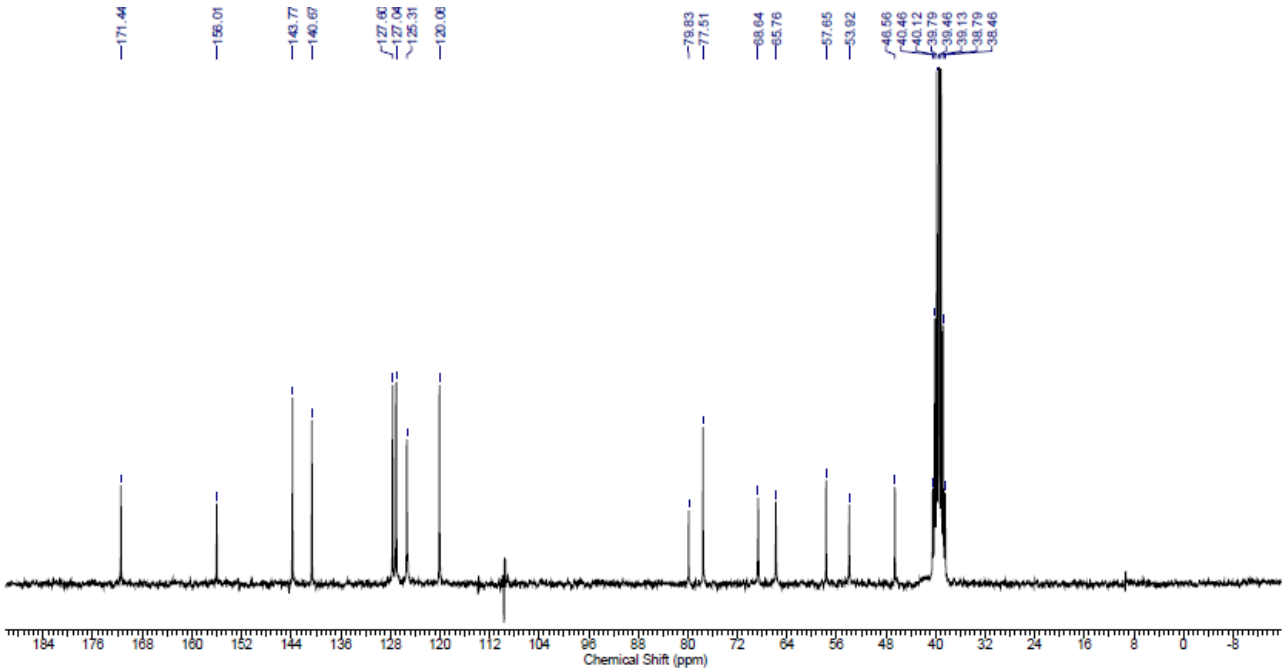


Figure 13: ^{13}C NMR spectrum of **18** (63 MHz, DMSO-d_6)

20

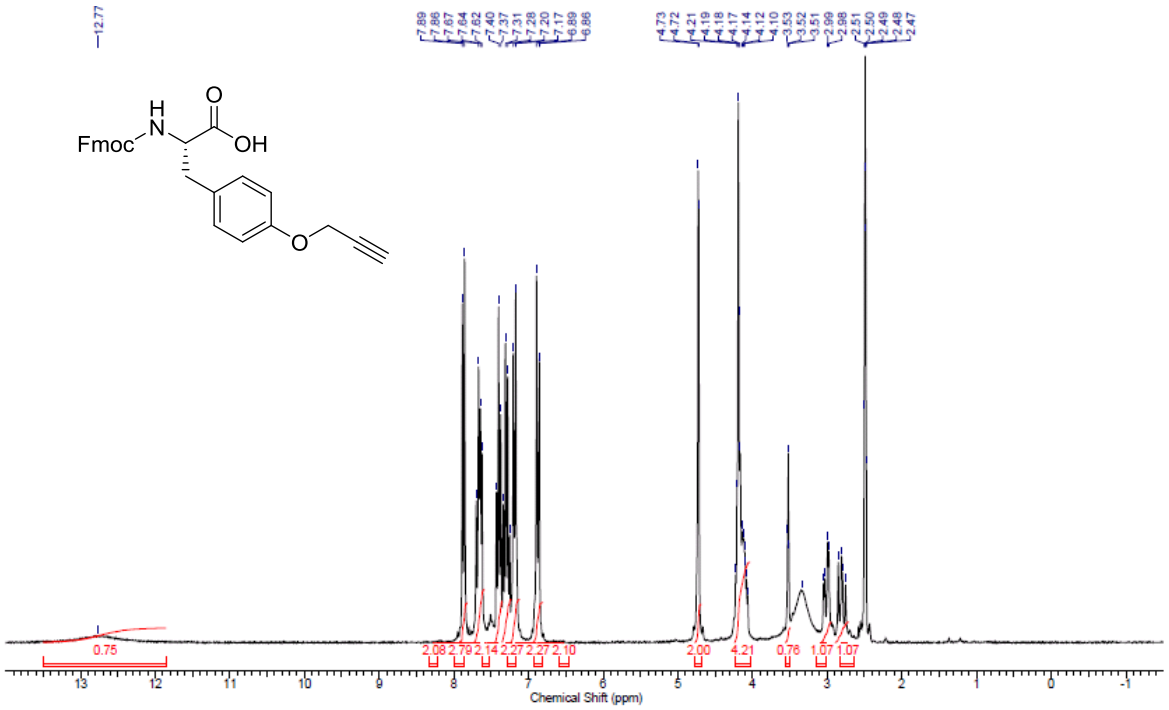


Figure 14: ^{1}H NMR spectrum of **20** (250 MHz, DMSO-d_6)

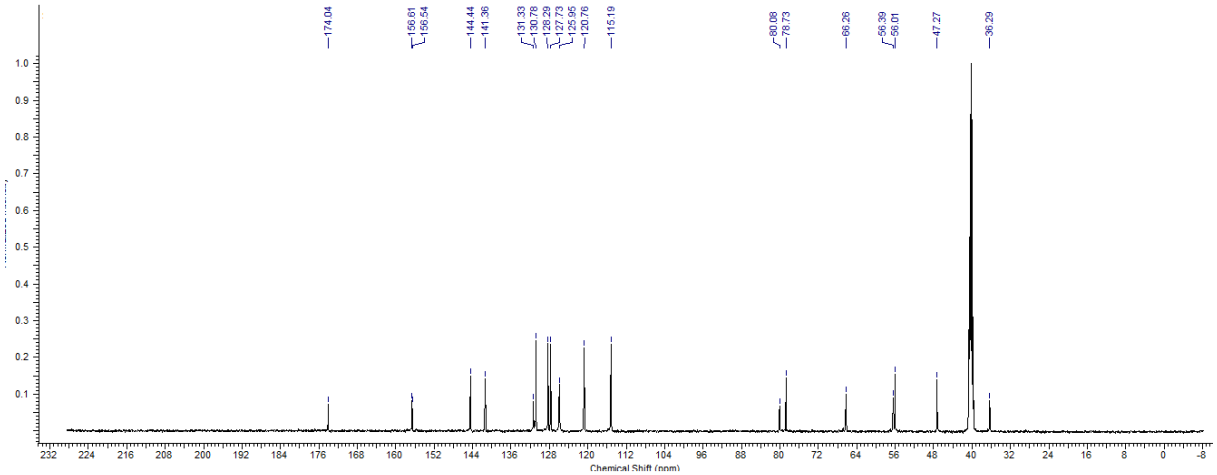


Figure 15: ^{13}C NMR spectrum of **20** (126 MHz, DMSO-d_6)

23

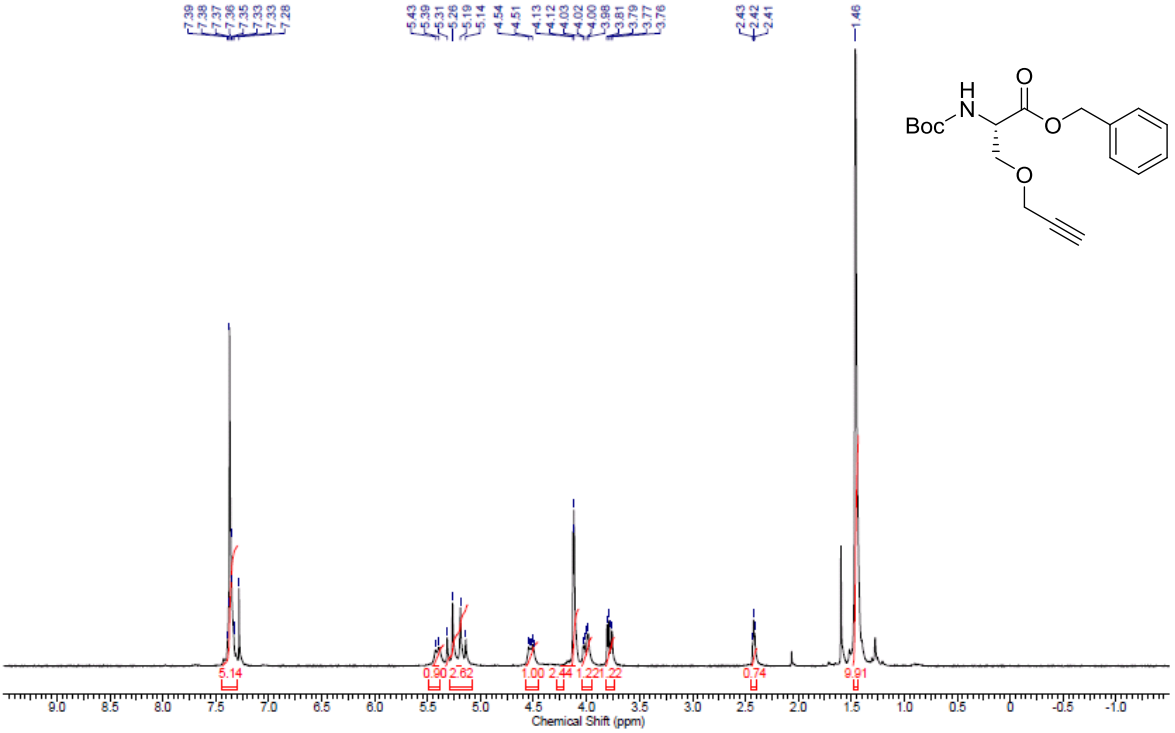


Figure 16: ^{1}H NMR spectrum of **23** (250 MHz, $CDCl_3$)

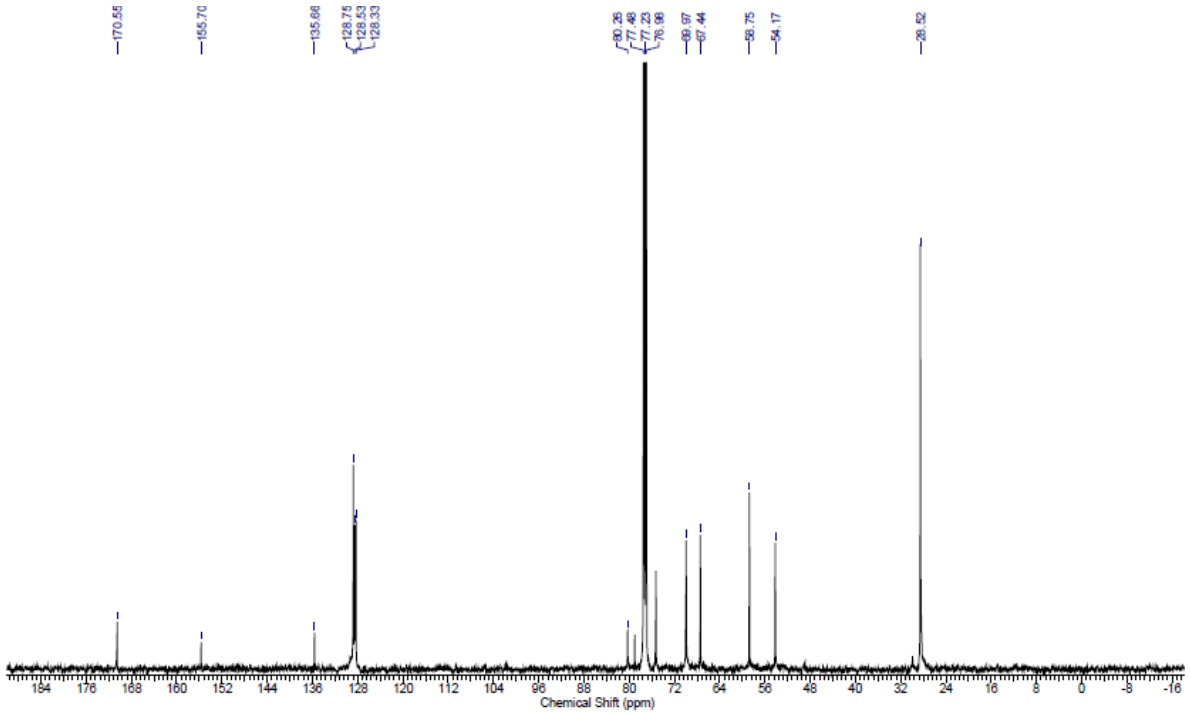


Figure 17: ^{13}C NMR spectrum of **23** (126 MHz, $CDCl_3$)

24a

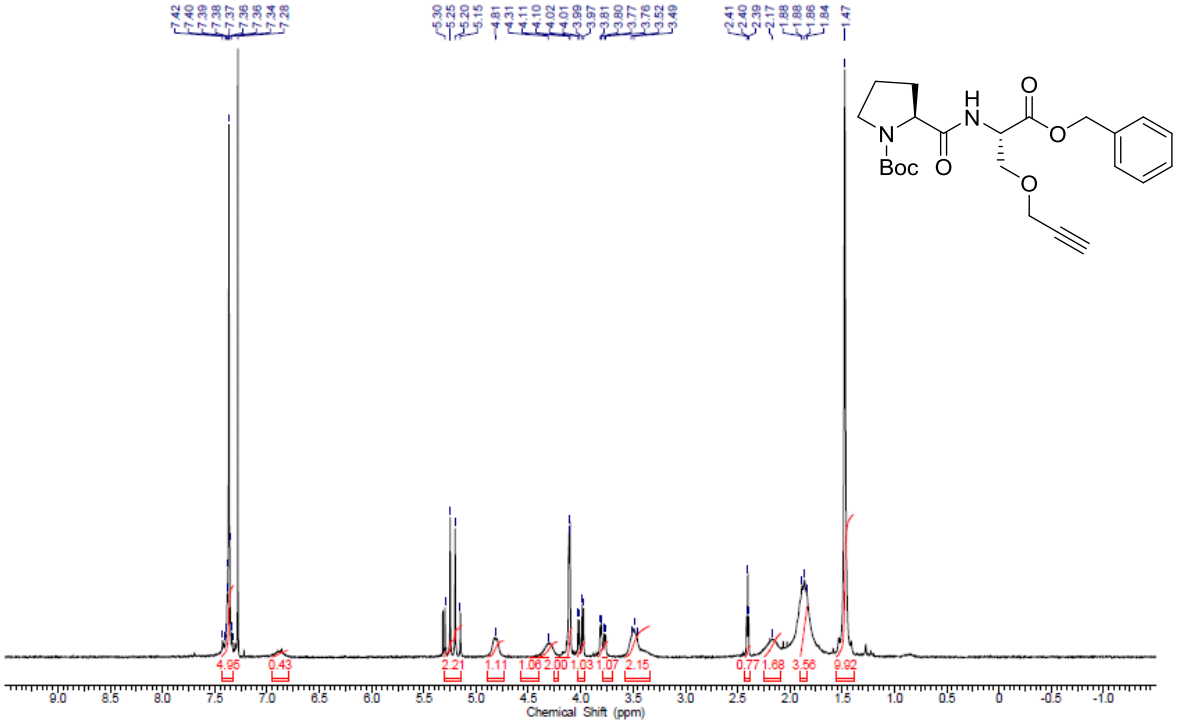


Figure 18: ^{1}H NMR spectrum of **24a** (250 MHz, $CDCl_3$)

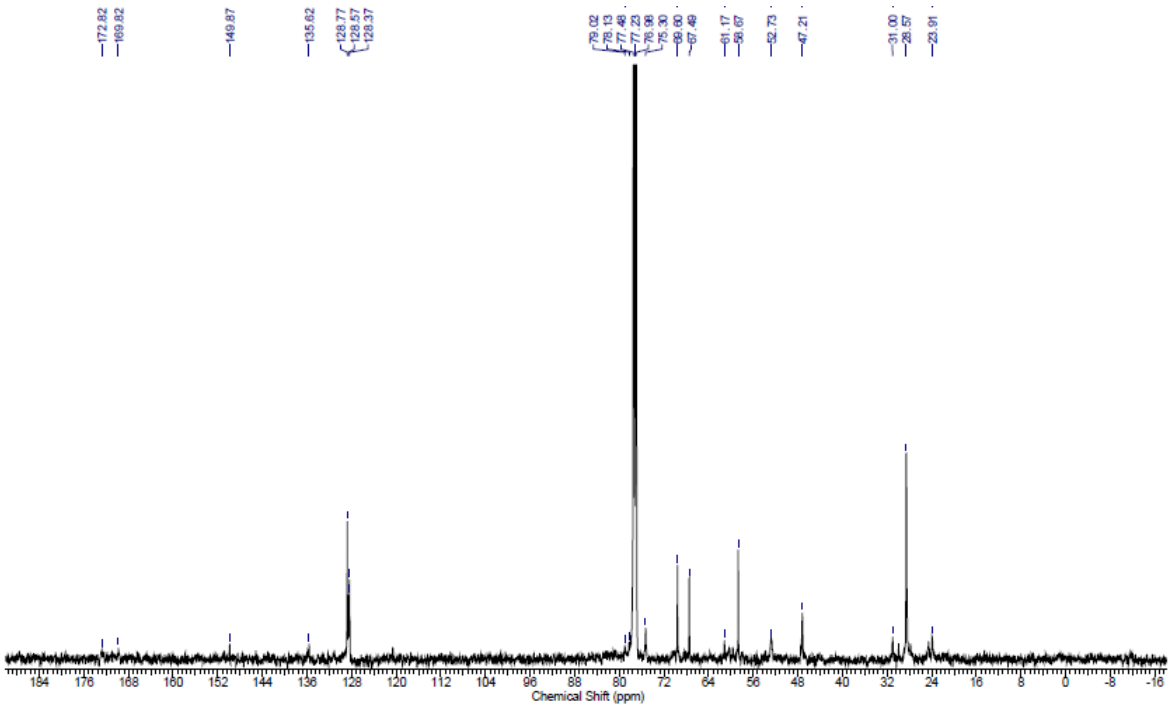


Figure 19: ^{13}C NMR spectrum of **24a** (126 MHz, $CDCl_3$)

24b

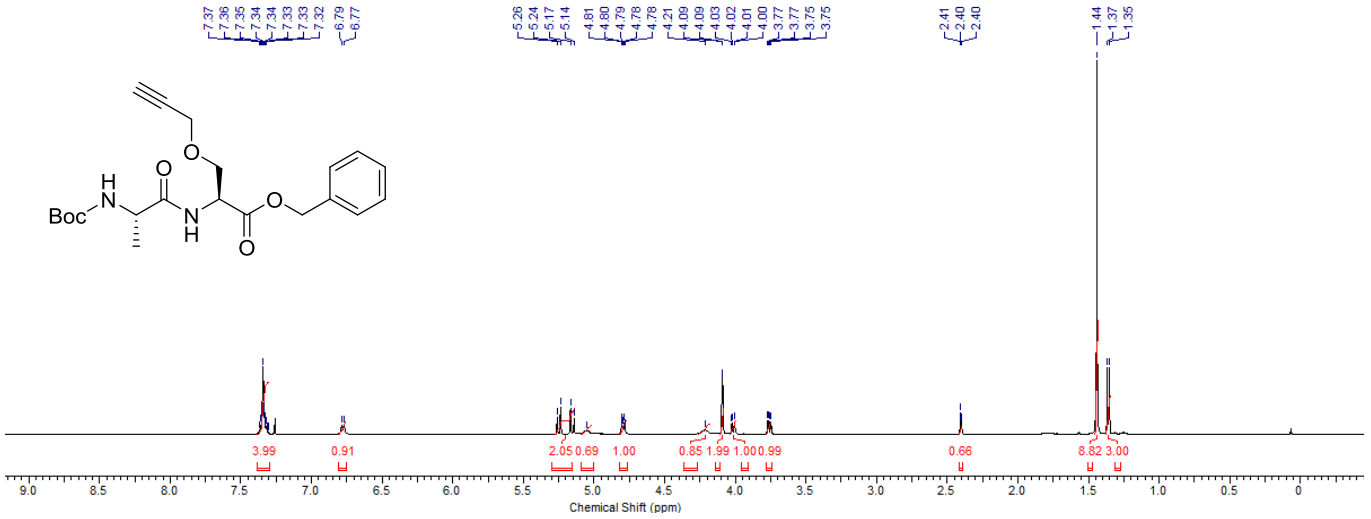


Figure 20: ^{1}H NMR spectrum of **24b** (250 MHz, $CDCl_3$)

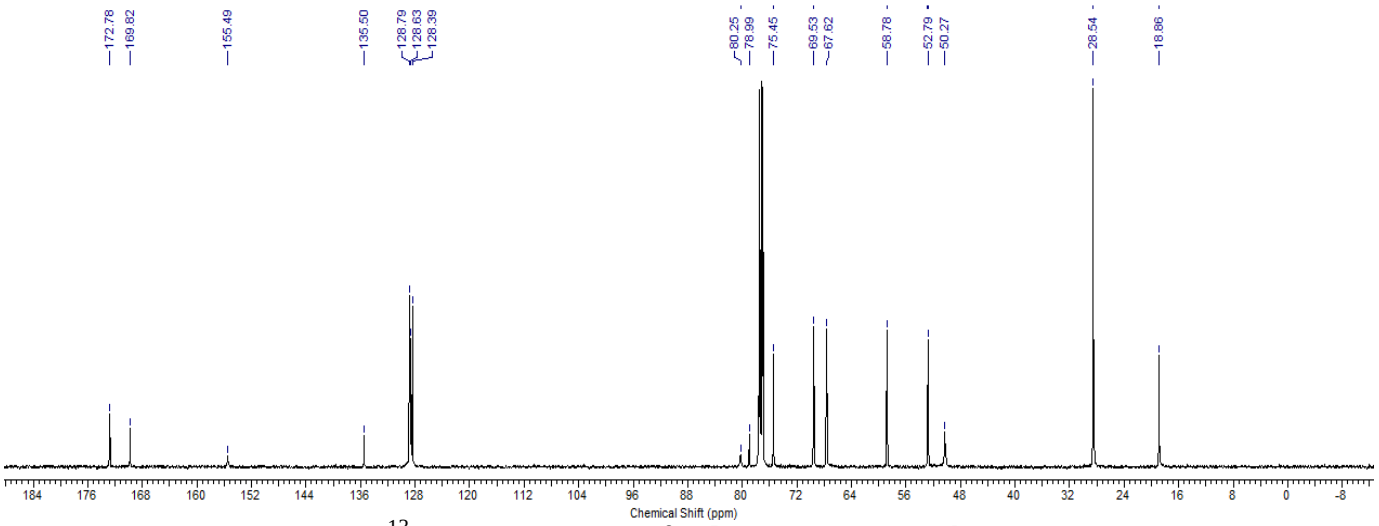


Figure 21: ^{13}C NMR spectrum of **24b** (126 MHz, $CDCl_3$)

21

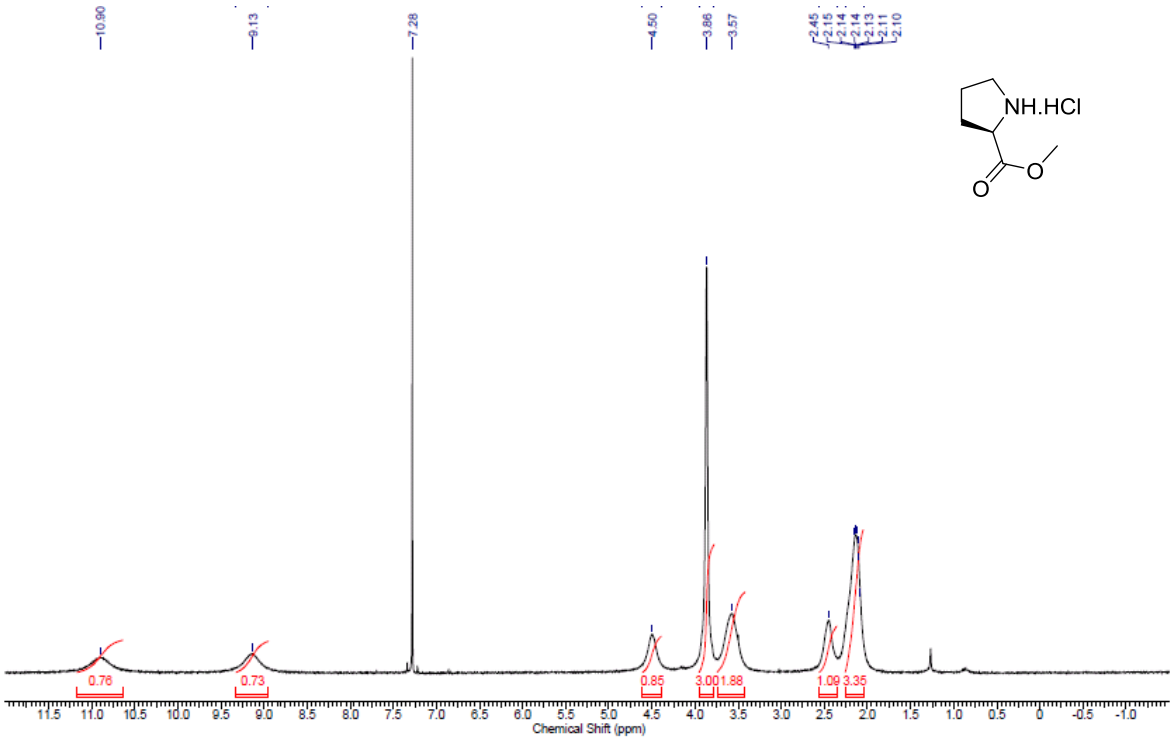


Figure 22: ^{1}H NMR spectrum of **21** (250 MHz, $CDCl_3$)

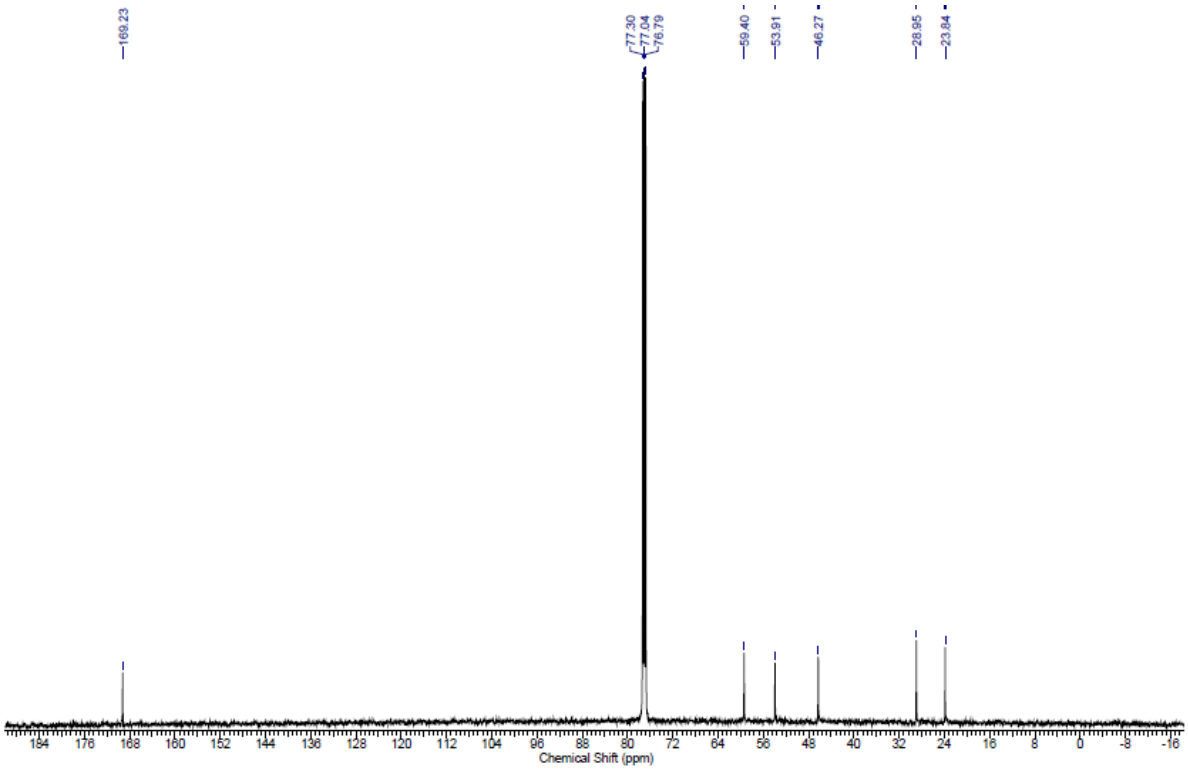


Figure 23: ^{13}C NMR spectrum of **21** (63 MHz, $CDCl_3$)

22a

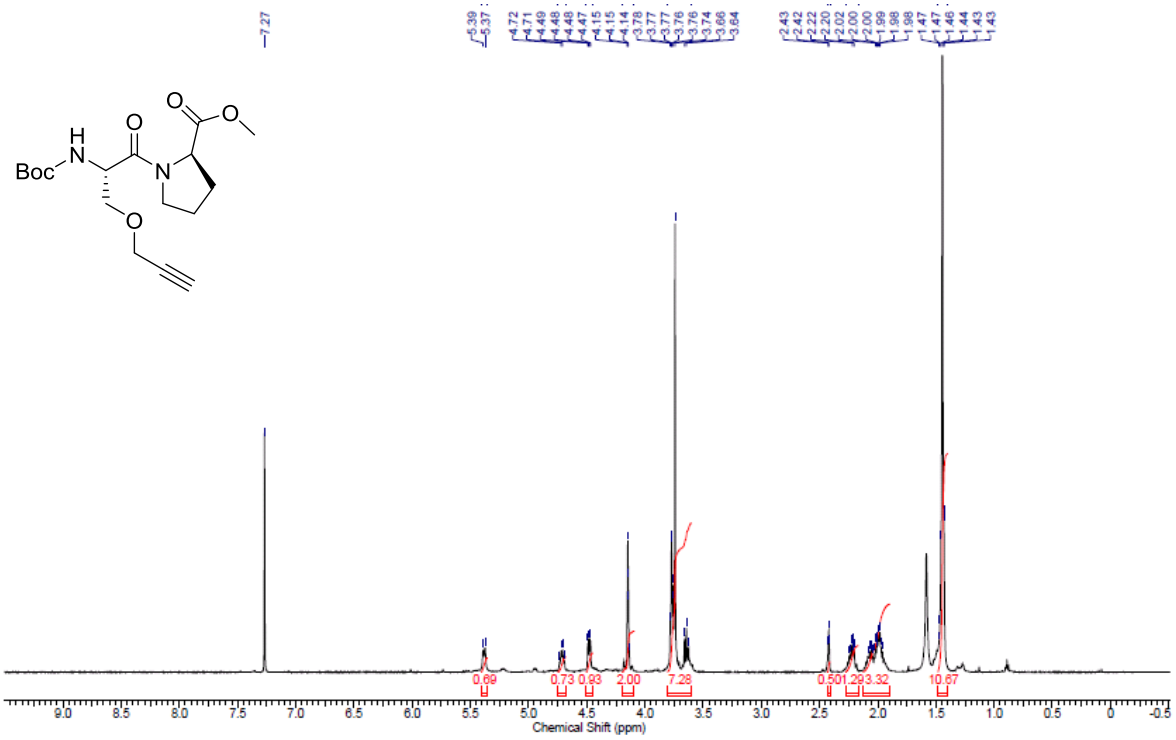


Figure 24: ^{1}H NMR spectrum of **22a** (500 MHz, $CDCl_3$)

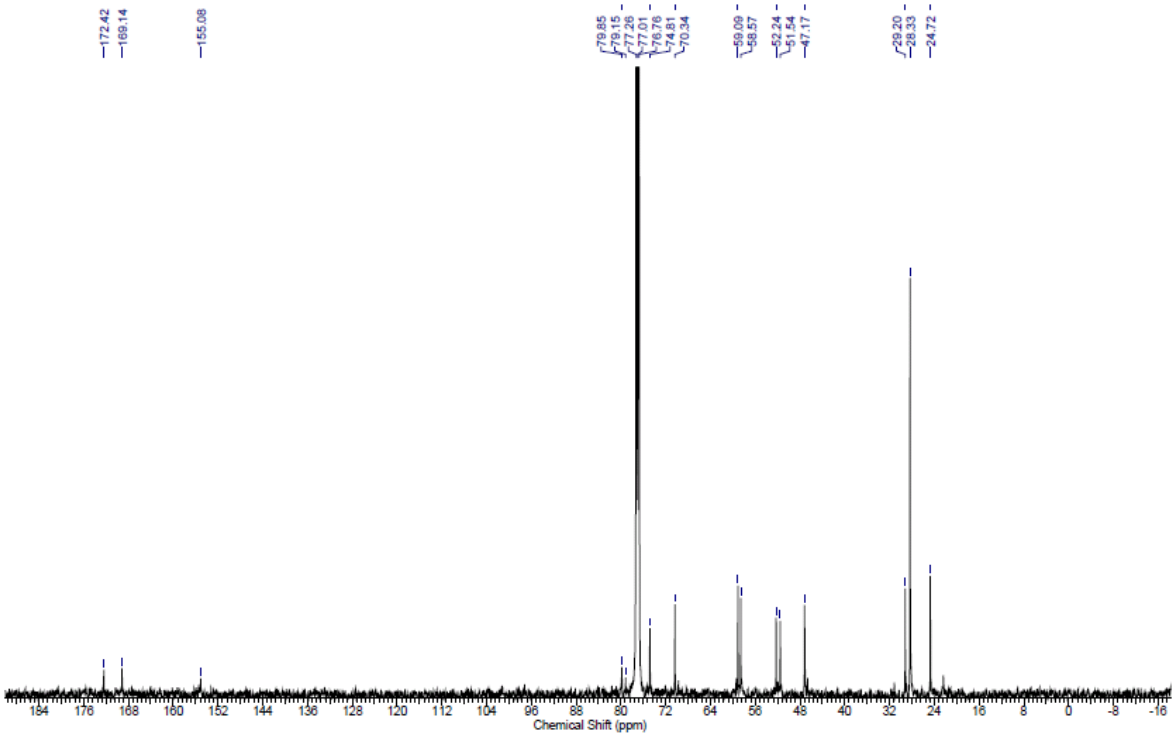


Figure 25: ^{13}C NMR spectrum of **22a** (126 MHz, $CDCl_3$)

22b

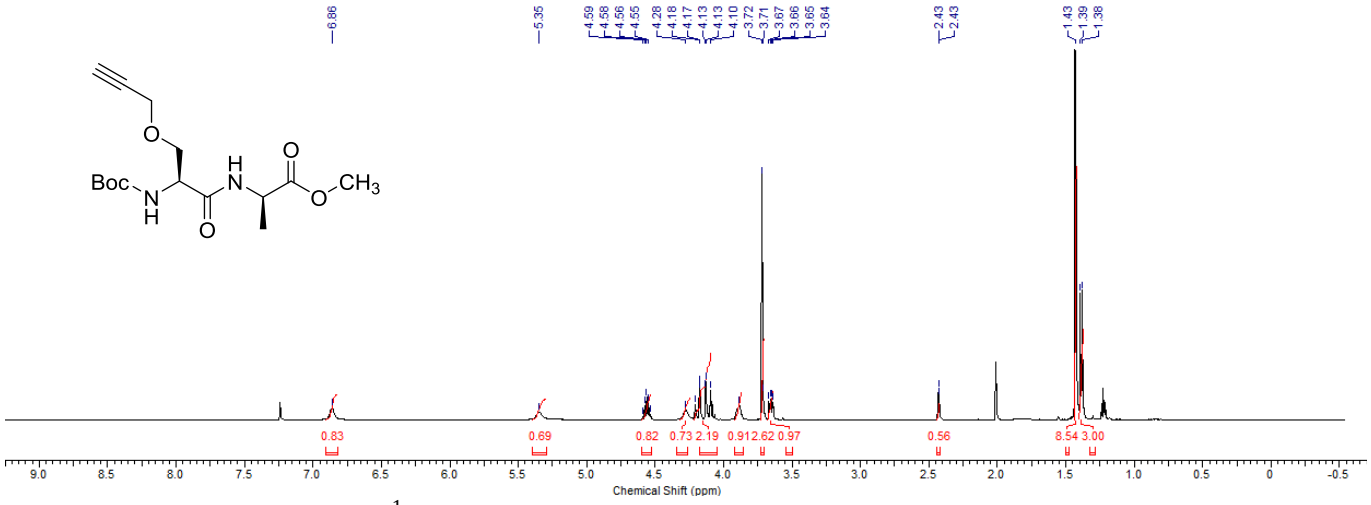


Figure 26: ^{1}H NMR spectrum of **22b** (500 MHz, $CDCl_3$)

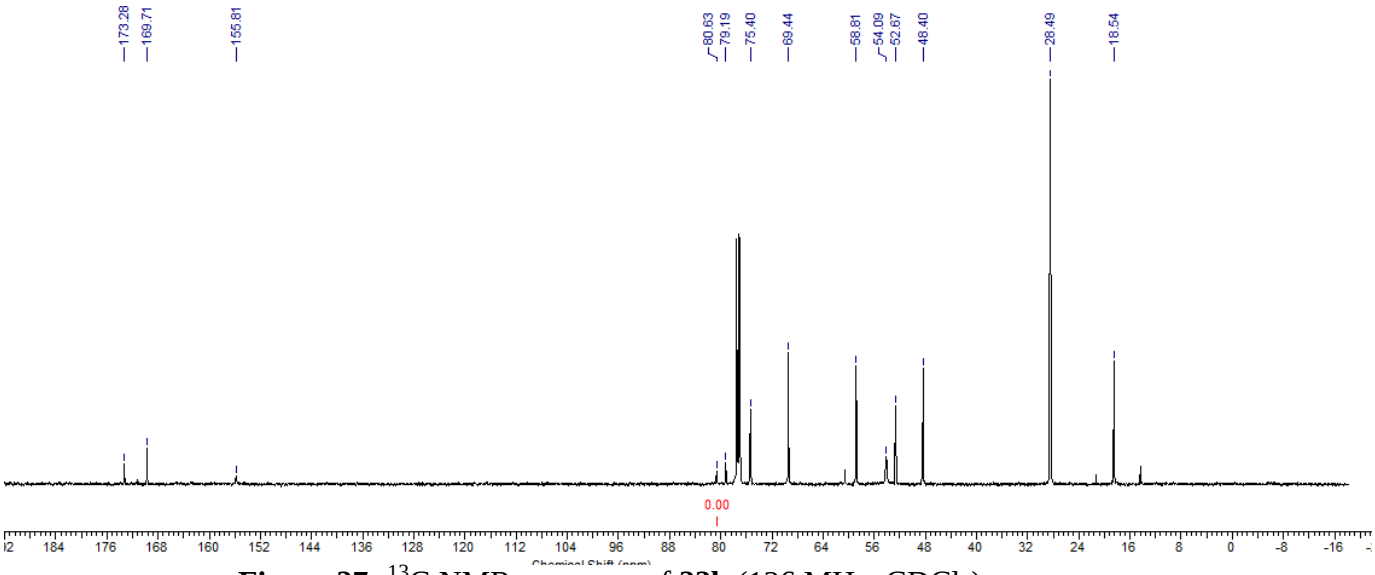


Figure 27: ^{13}C NMR spectrum of **22b** (126 MHz, $CDCl_3$)

12a

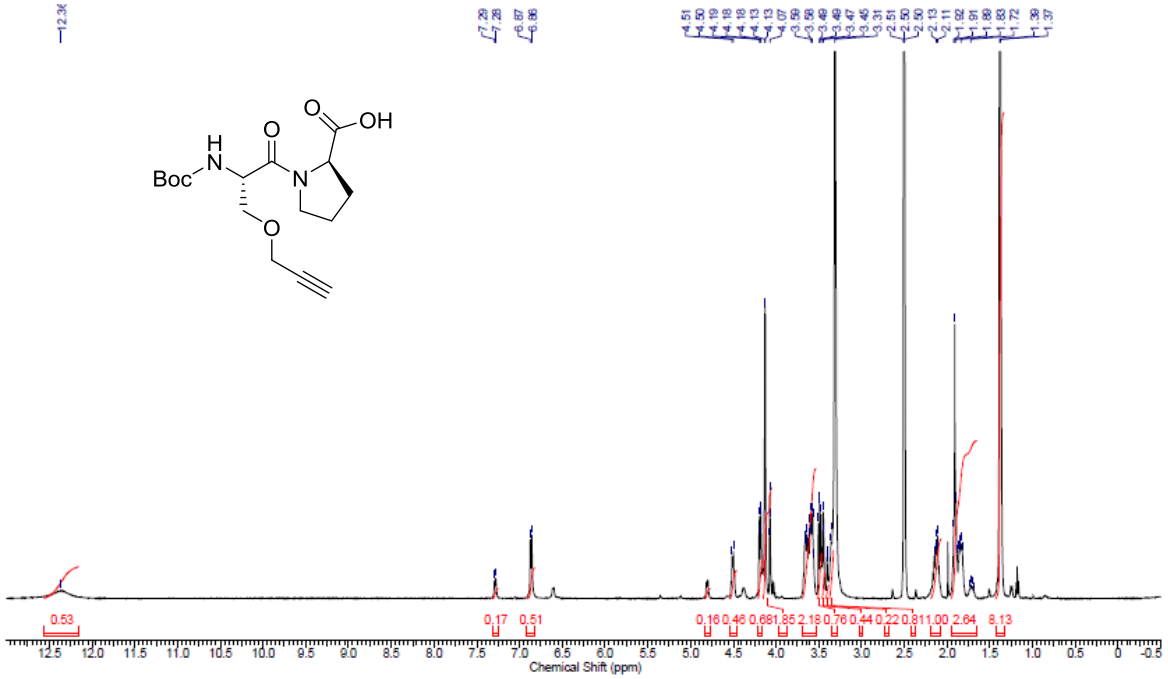


Figure 28: ^{1}H NMR spectrum of **12a** (500 MHz, DMSO-d_6)

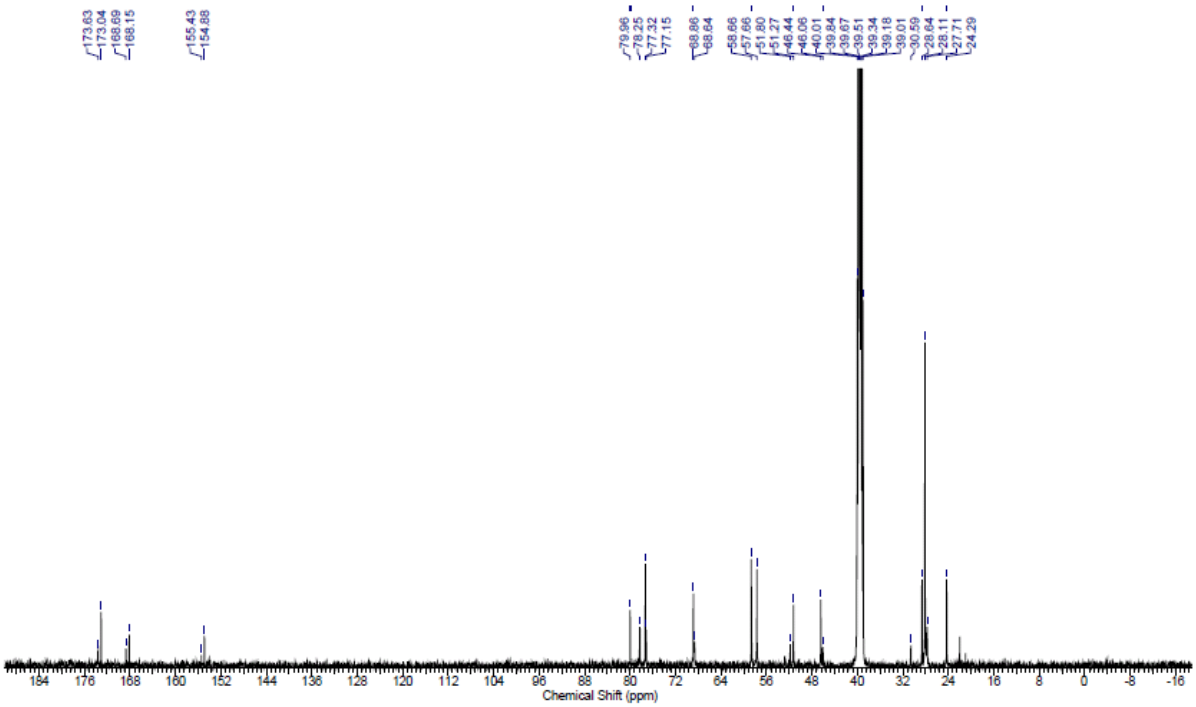


Figure 29: ^{13}C NMR spectrum of **12a** (126 MHz, DMSO-d_6)

12b

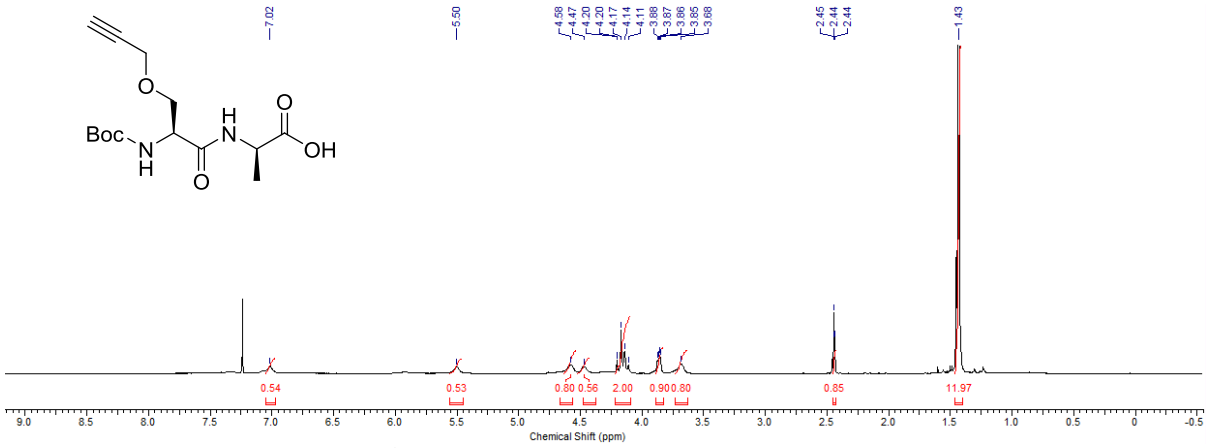


Figure 30: ^{1}H NMR spectrum of **12a** (500 MHz, $CDCl_3$)

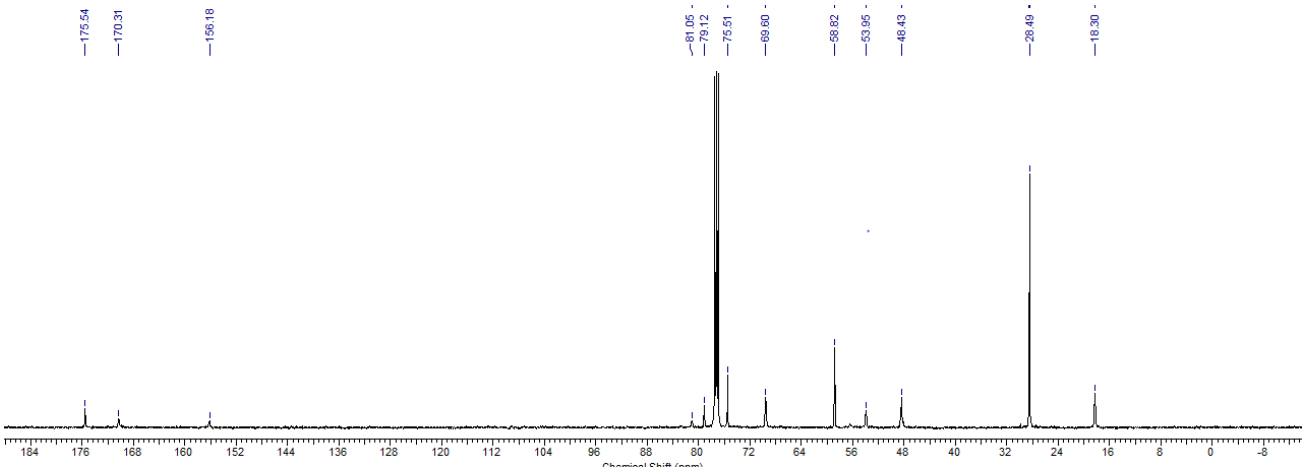


Figure 31: ^{13}C NMR spectrum of **12a** (126 MHz, $CDCl_3$)

13a

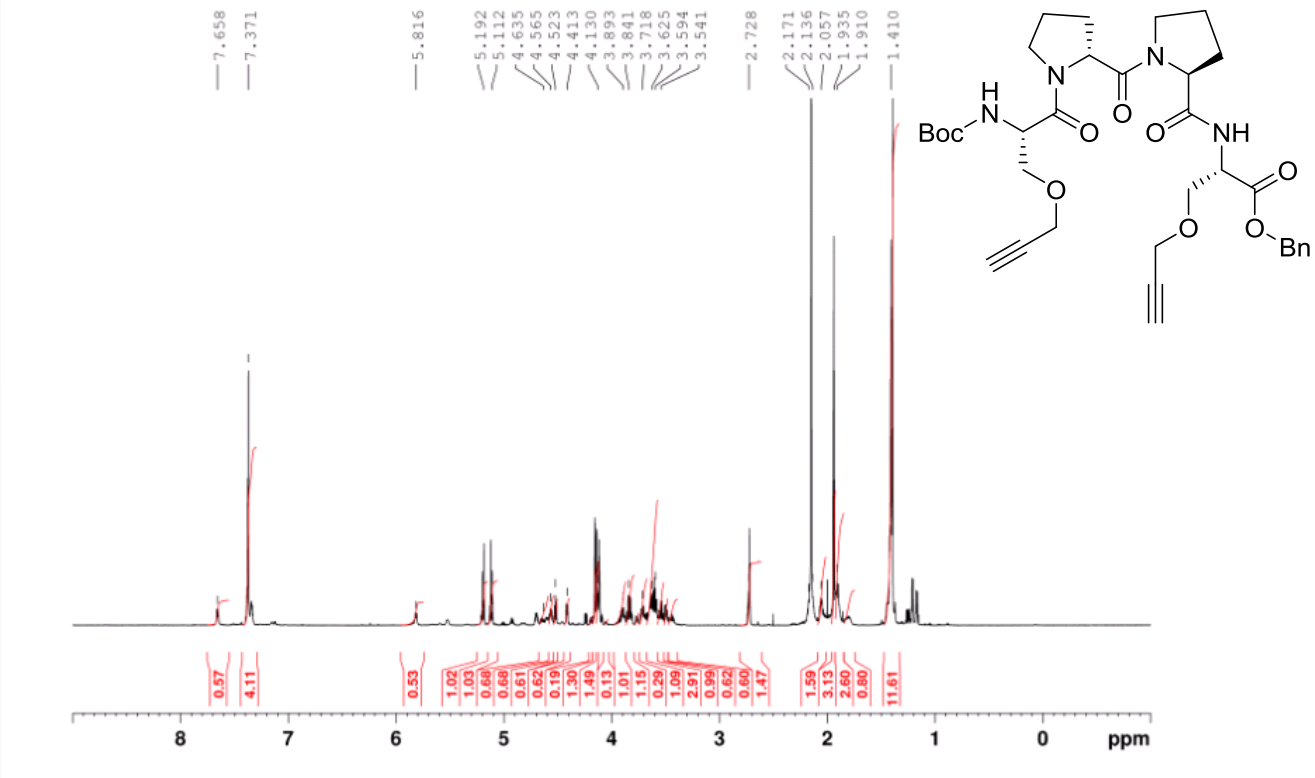


Figure 32: ^{1}H spectrum of **13a** (700 MHz, CD_3CN)

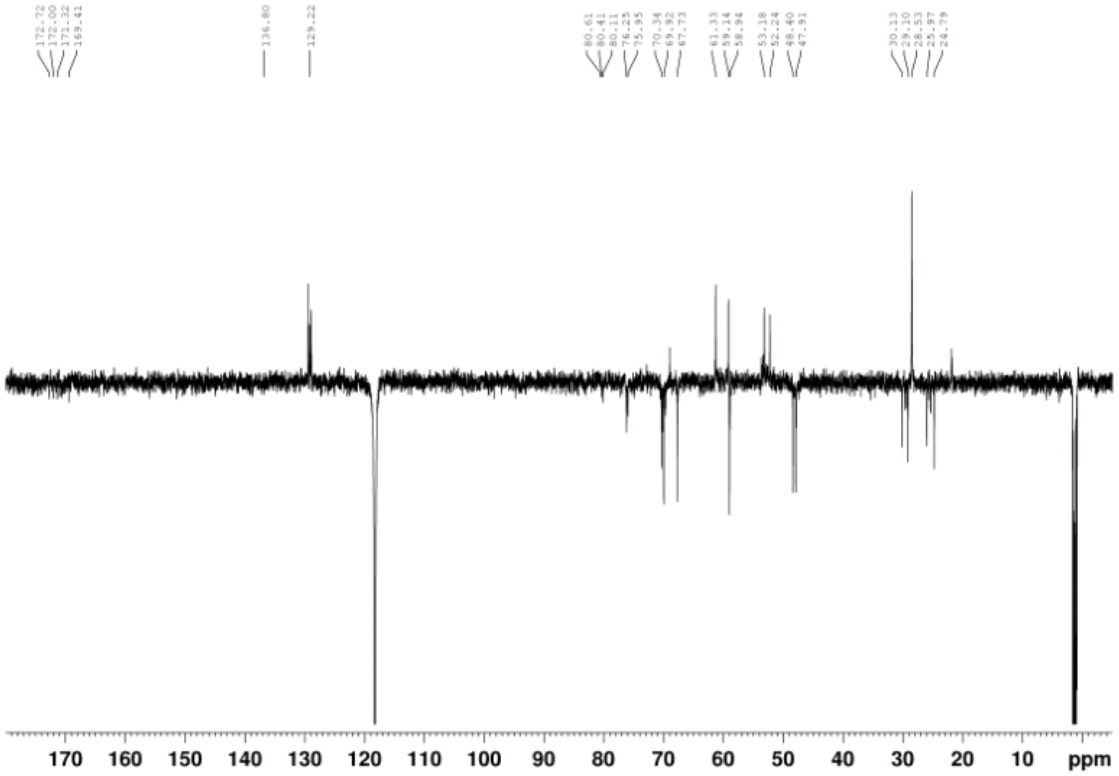


Figure 33: ^{13}C DEPT 135 spectrum of **13a** (176 MHz, CD_3CN)

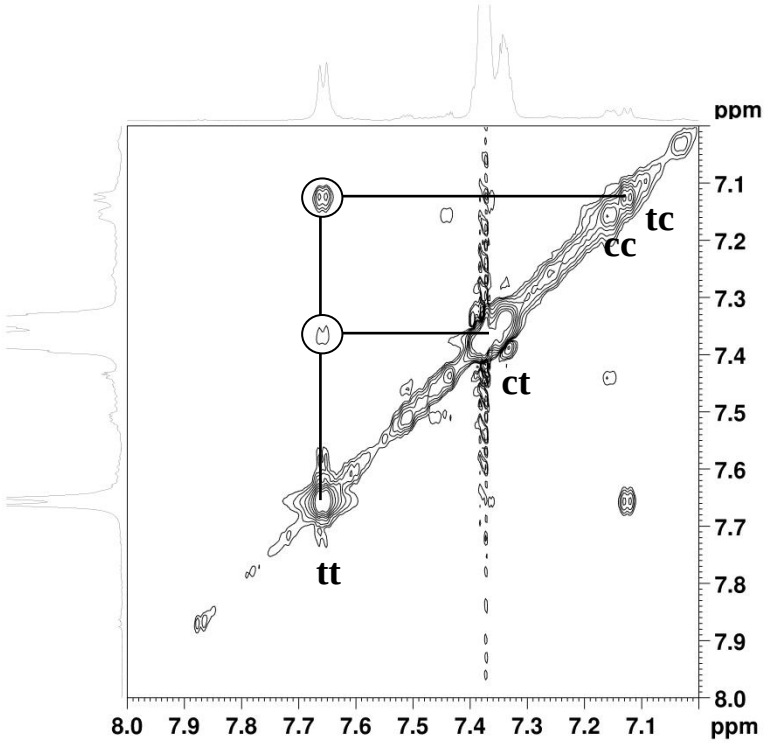


Figure 33: ^{1}H-^{1}H ROESY spectrum of **13a** (700 MHz, 250 ms mixing time, CD_3CN)

The main isomer (*trans-trans*) of **13a** is exchanging slowly on the NMR time scale, as evidenced by the exchange cross peaks in the ^{1}H-^{1}H ROESY spectrum. A cross peak between e.g. the *trans-trans* isomer and the *cis-cis* isomer is not witnessed due to the low probability of two peptide bonds changing geometry at the same time.

14a

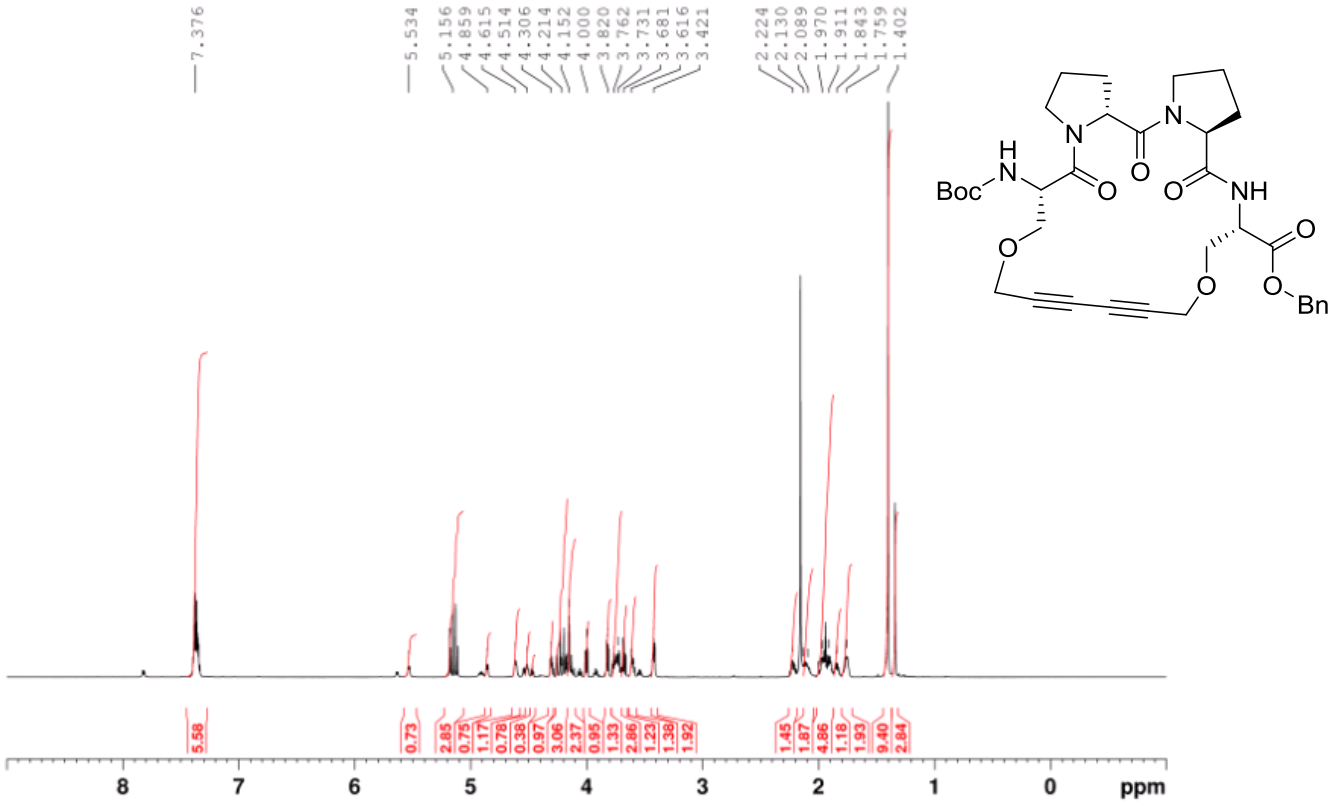


Figure 34: ^{1}H spectrum of **14a** (700 MHz, CD_3CN)

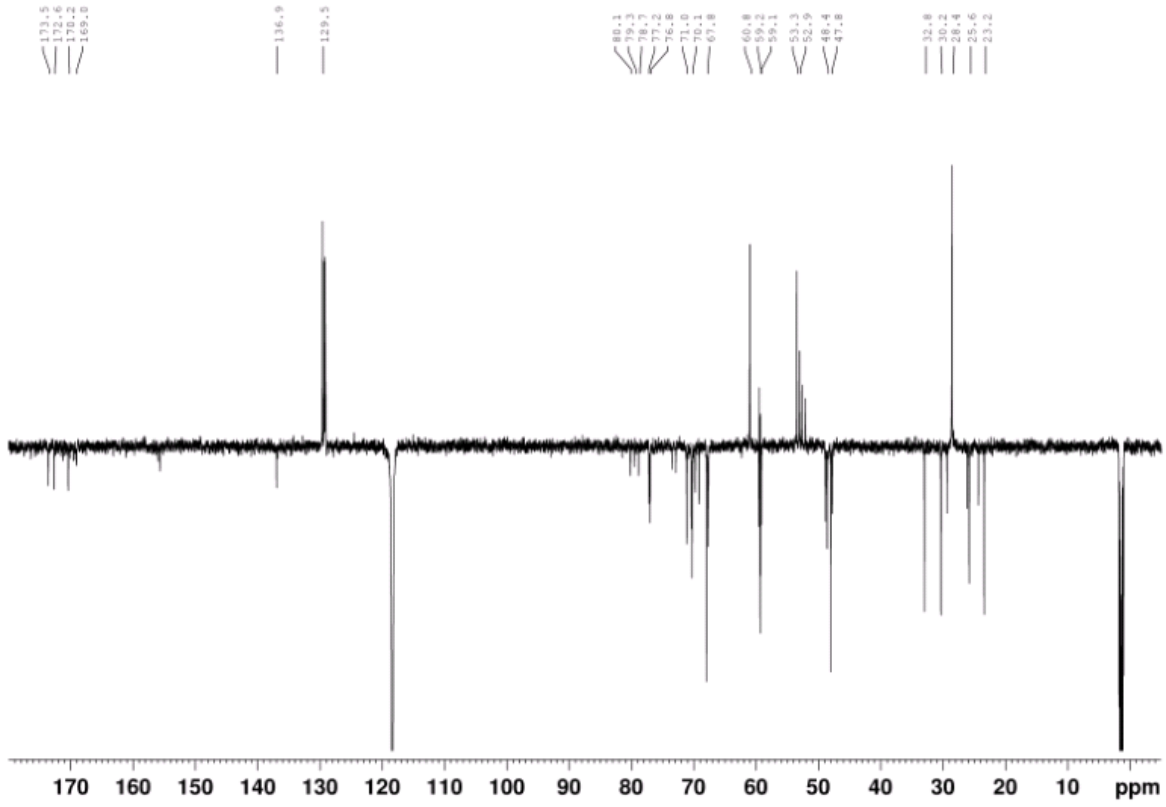


Figure 35: ^{13}C DEPT 135 spectrum of **14a** (176 MHz, CD_3CN)

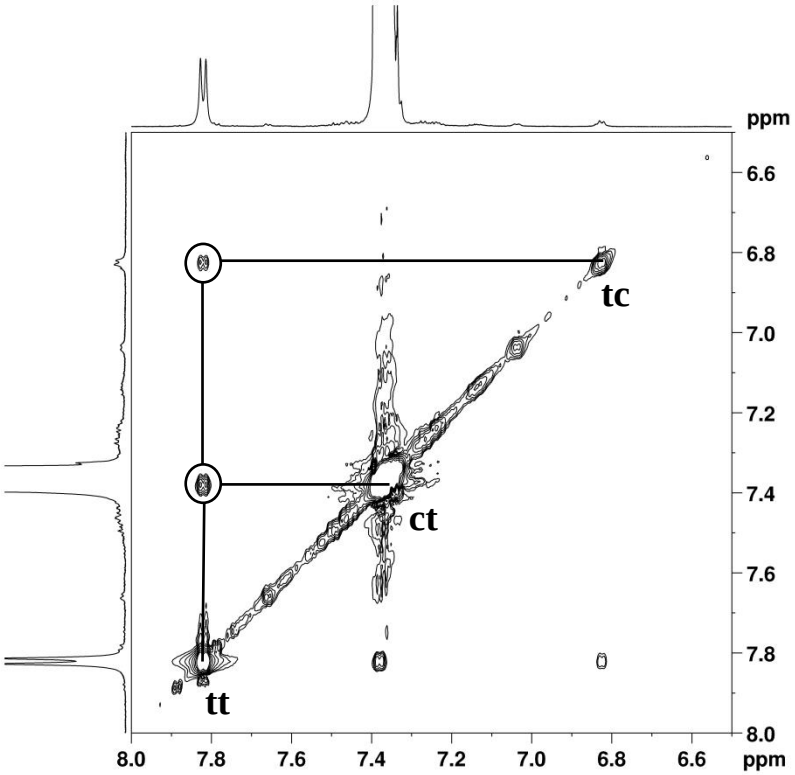


Figure 36: ^{1}H-^{1}H ROESY spectrum of **14a** (700 MHz, 250 ms mixing time, CD_3CN)

15a

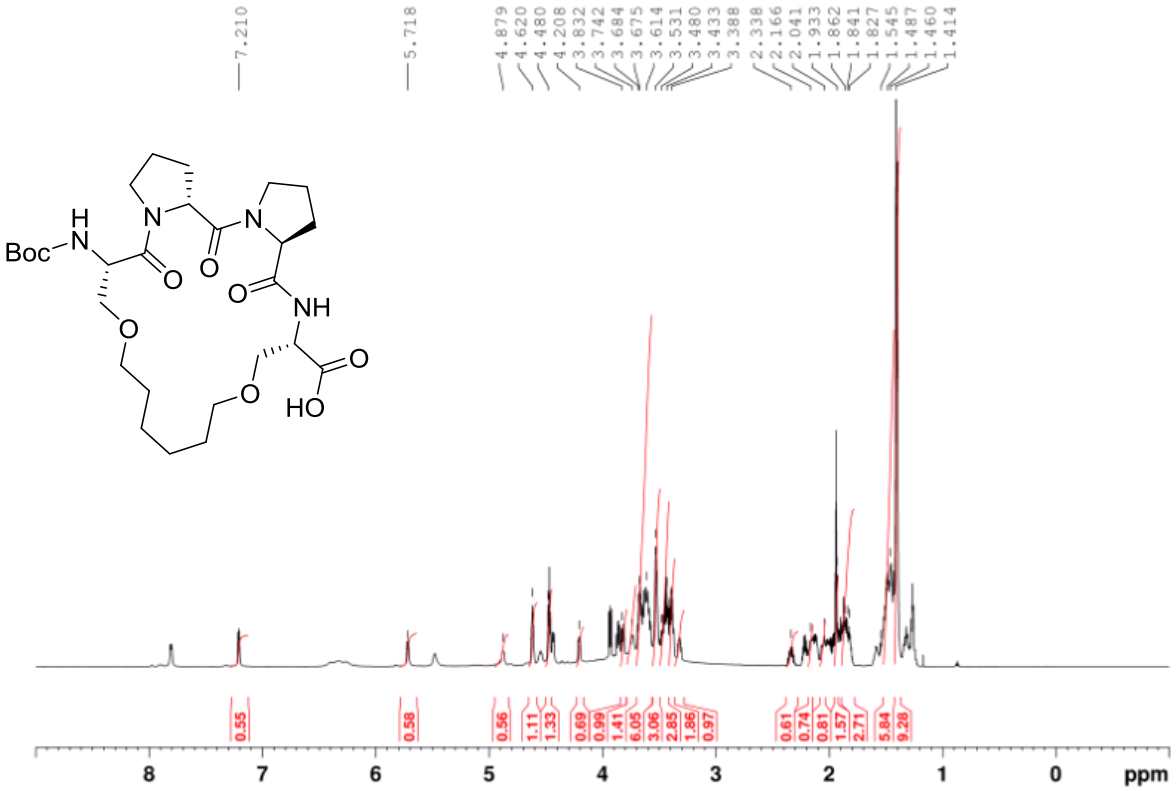


Figure 37: ^{1}H spectrum of **15a** (700 MHz, CD_3CN)

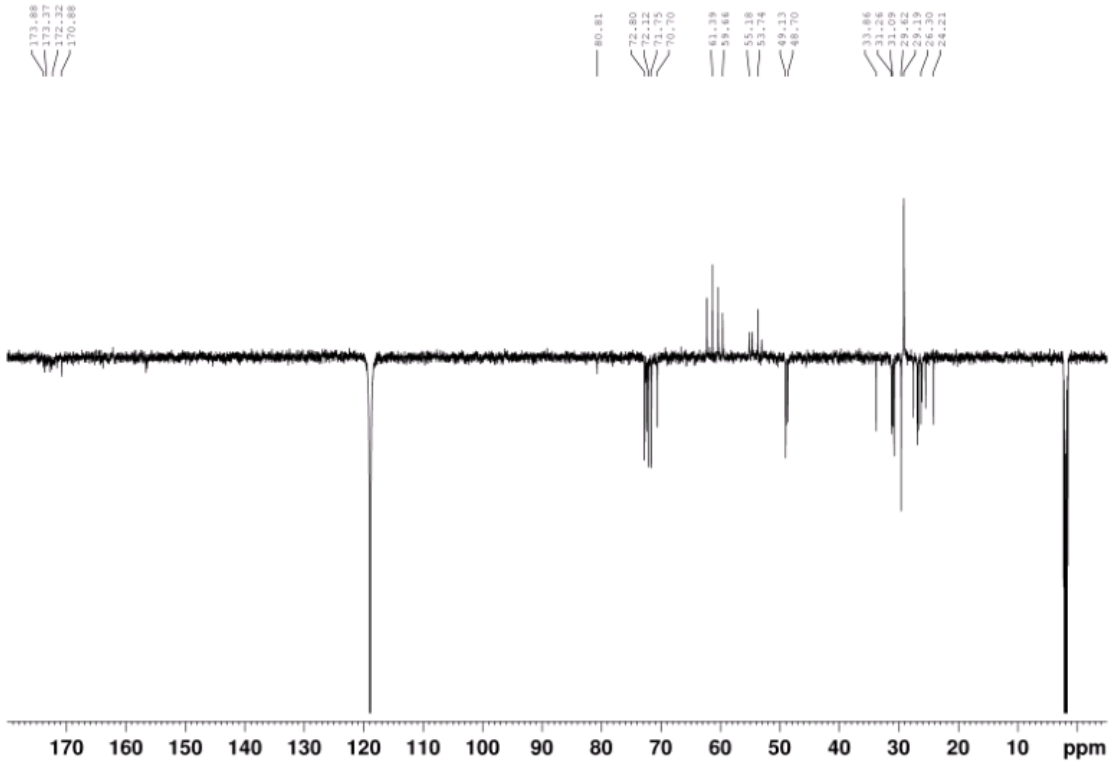


Figure 38: ^{13}C DEPT 135 spectrum of **15a** (176 MHz, CD_3CN)

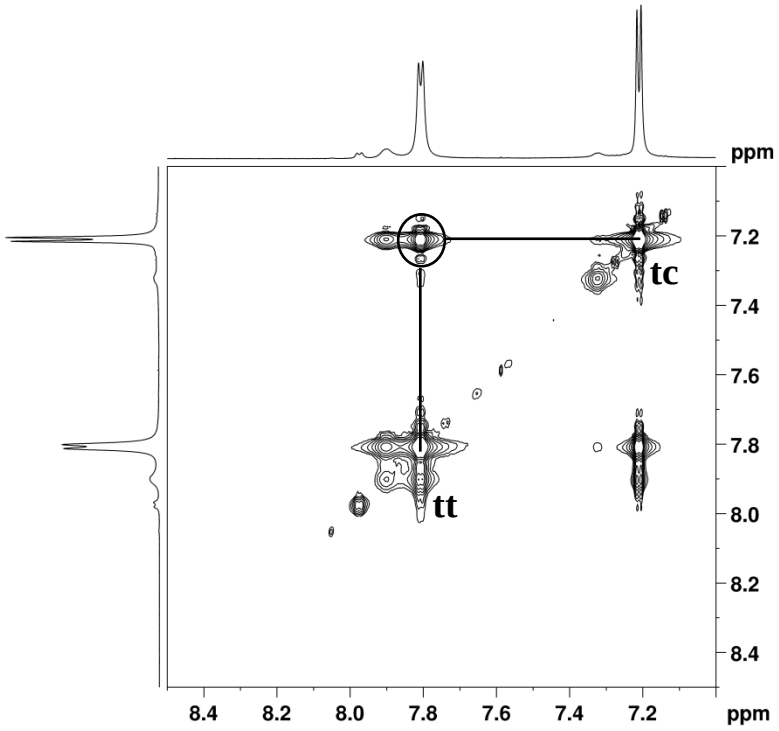
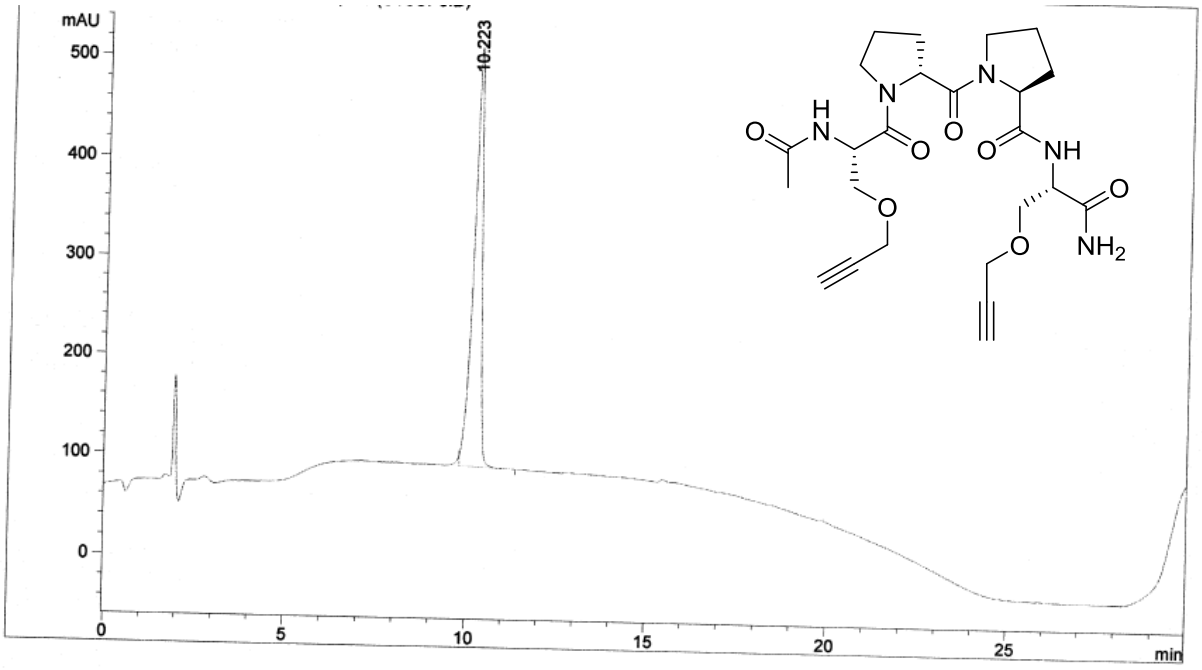


Figure 39: ^{1}H-^{1}H ROESY spectrum of **15a** (700 MHz, 250 ms mixing time, CD_3CN)

7. Copies of HPLC chromatograms of purified peptides 5a-k, 7 and 9 and crude reaction mixtures of peptides 6a-k, 8 and 10



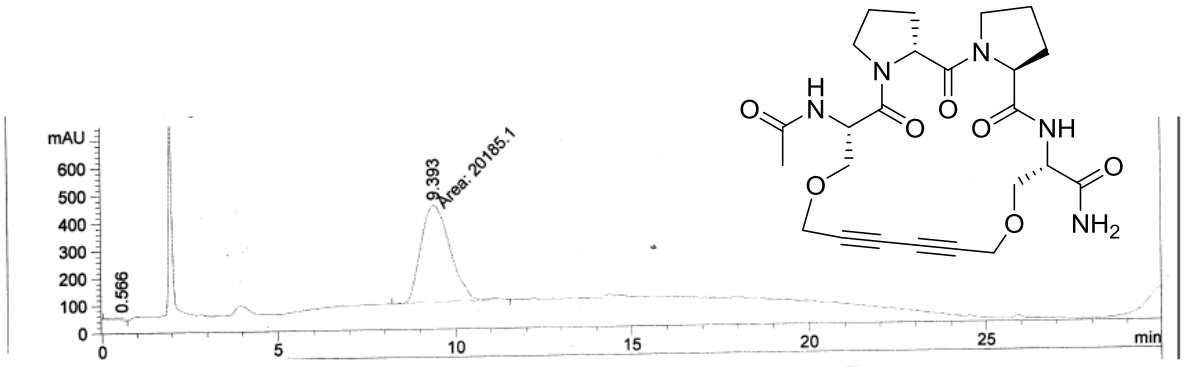
Area Percent Report

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000

Signal 1: DAD1 A, Sig=215,4 Ref=400,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	10.223	BP	0.2426	6888.23926	421.70276	100.0000

Figure 40: HPLC chromatogram of **5a** purified via preparative RP-HPLC.



Area Percent Report

Sorted By : Retention Time
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Dilution : 1.0000

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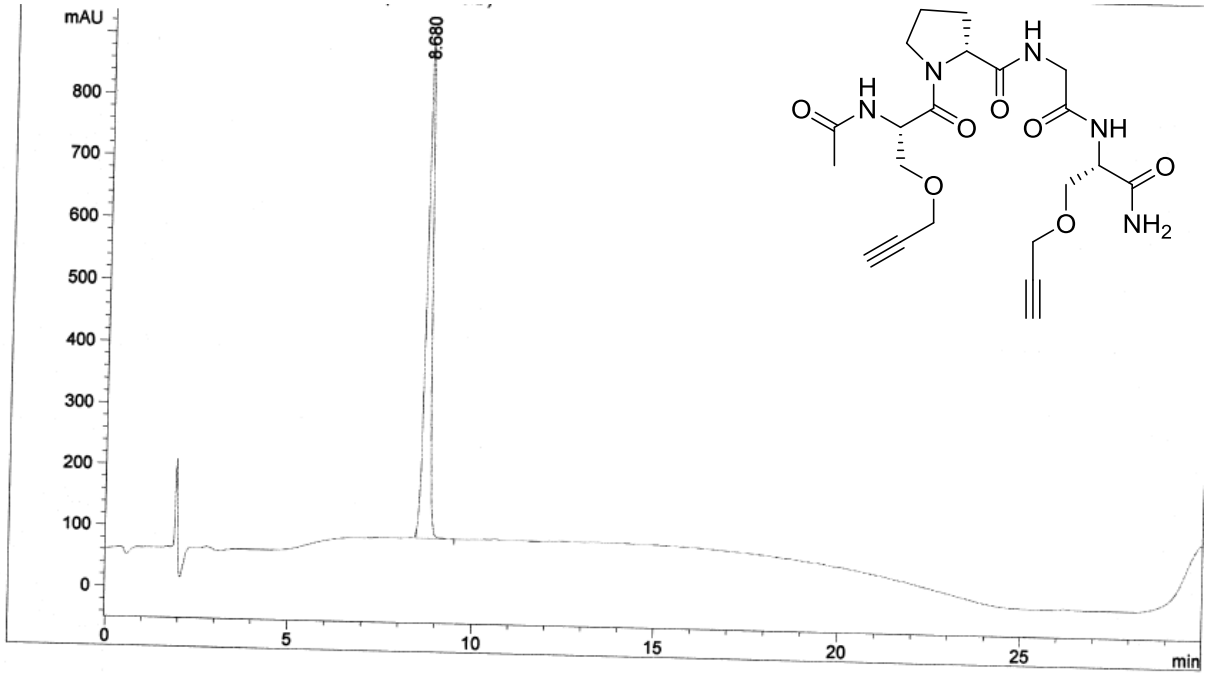
Signal 2: DAD1 B, Sig=254,16 Ref=360,100

Peak #	RetTime [min]	Sig	Type	Area [mAU*s]	Height [mAU]
1	0.566	1	BP	222.41255	9.67116
2	1.956	2	BB	1.31282e4	1676.87720

Figure 41: HPLC chromatogram of the crude reaction mixture of **6a**.



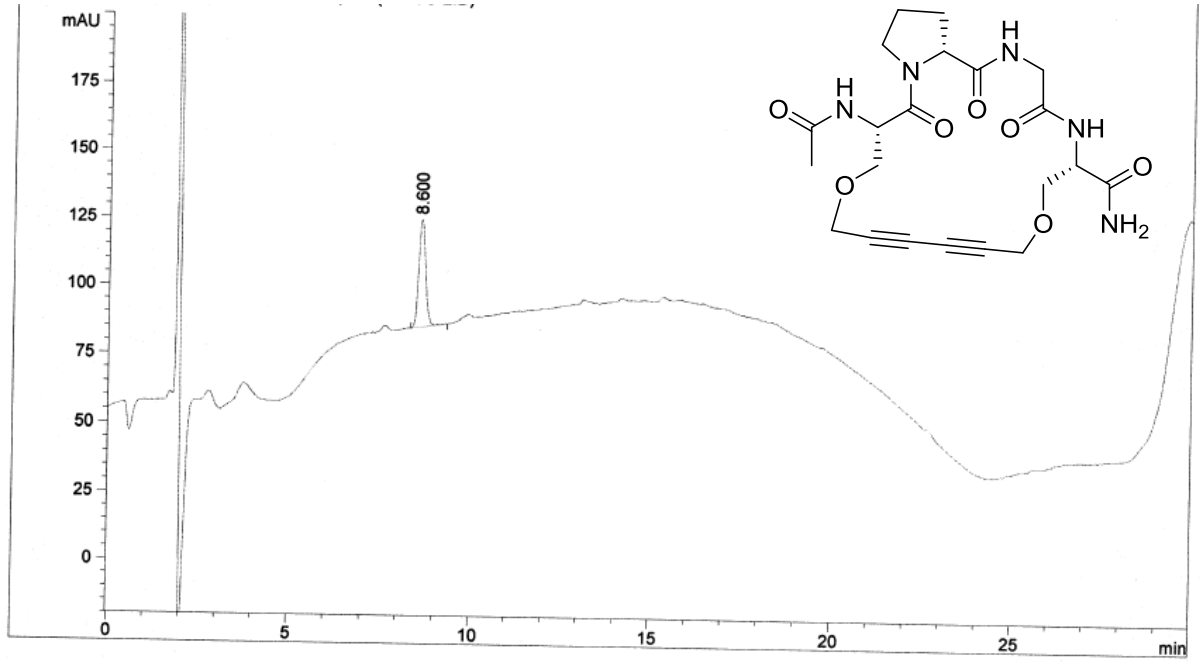
Area Percent Report

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000

Signal 1: DAD1 A, Sig=215,4 Ref=400,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	8.680	BB	0.1629	8413.03027	798.90167	100.0000

Figure 42: HPLC chromatogram of **5b** purified via preparative RP-HPLC.



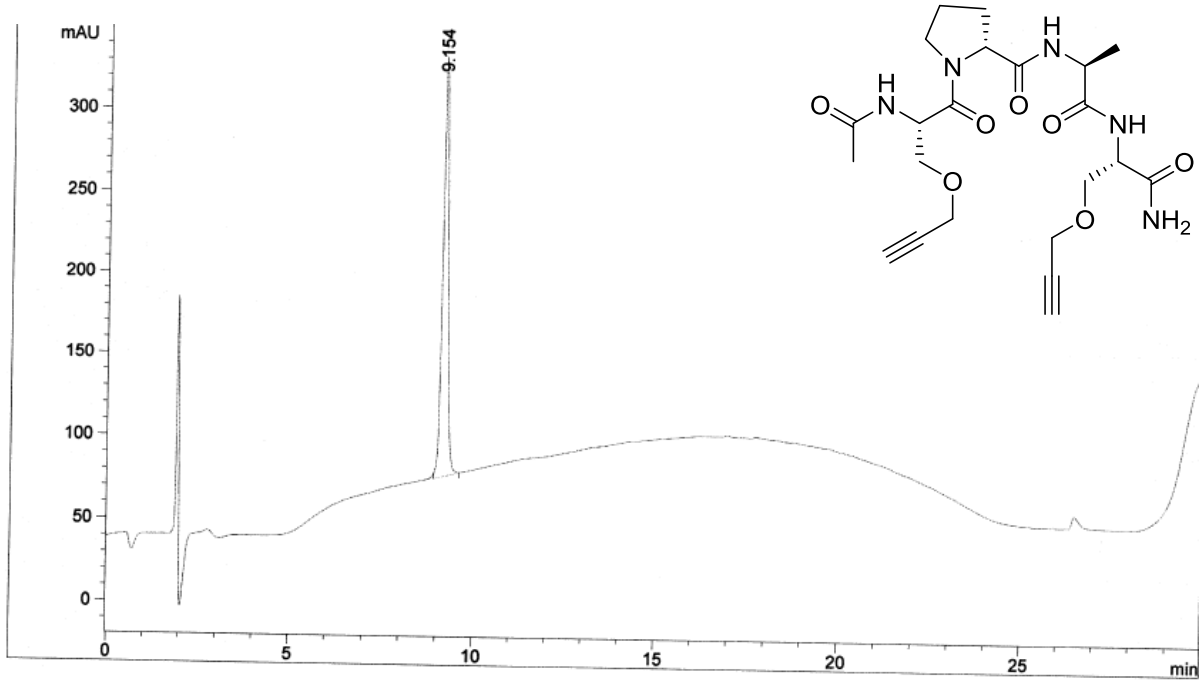
Area Percent Report

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000

Signal 1: DAD1 A, Sig=215,4 Ref=400,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	8.600	BP	0.2101	525.68933	39.80061	100.0000

Figure 43: HPLC chromatogram of the crude reaction mixture of **6b**.



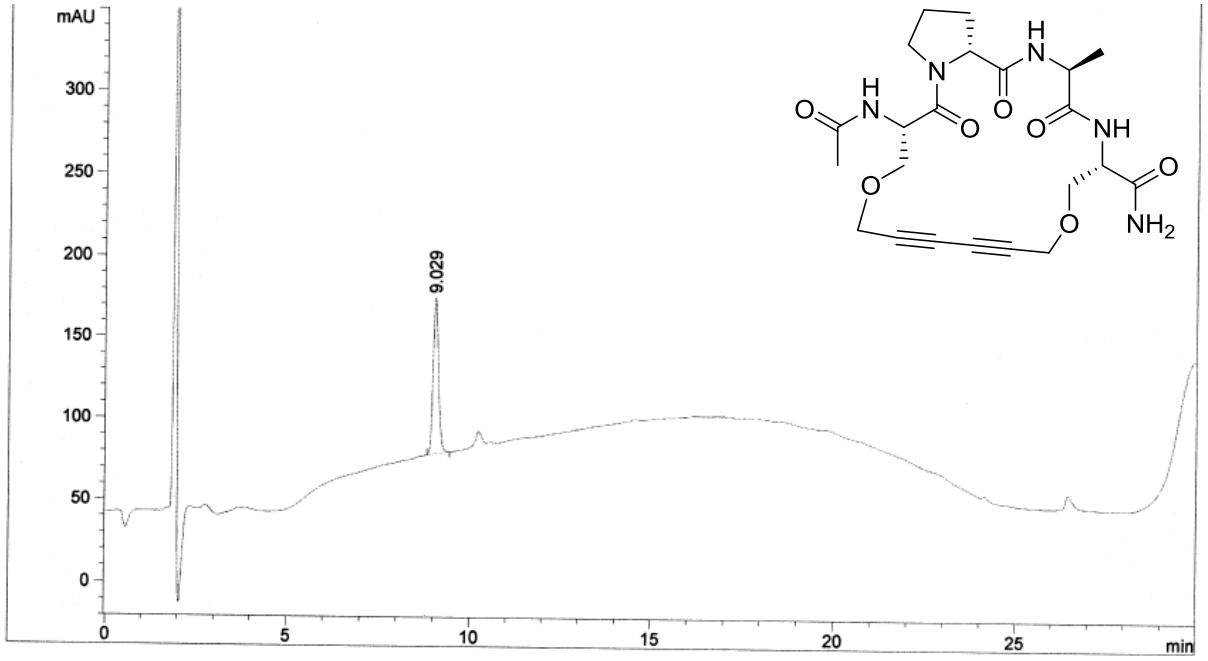
Area Percent Report

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000

Signal 1: DAD1 A, Sig=215,4 Ref=400,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	9.154	PB	0.1564	2638.44238	255.59734	100.0000

Figure 44: HPLC chromatogram of **5c** purified via preparative RP-HPLC.



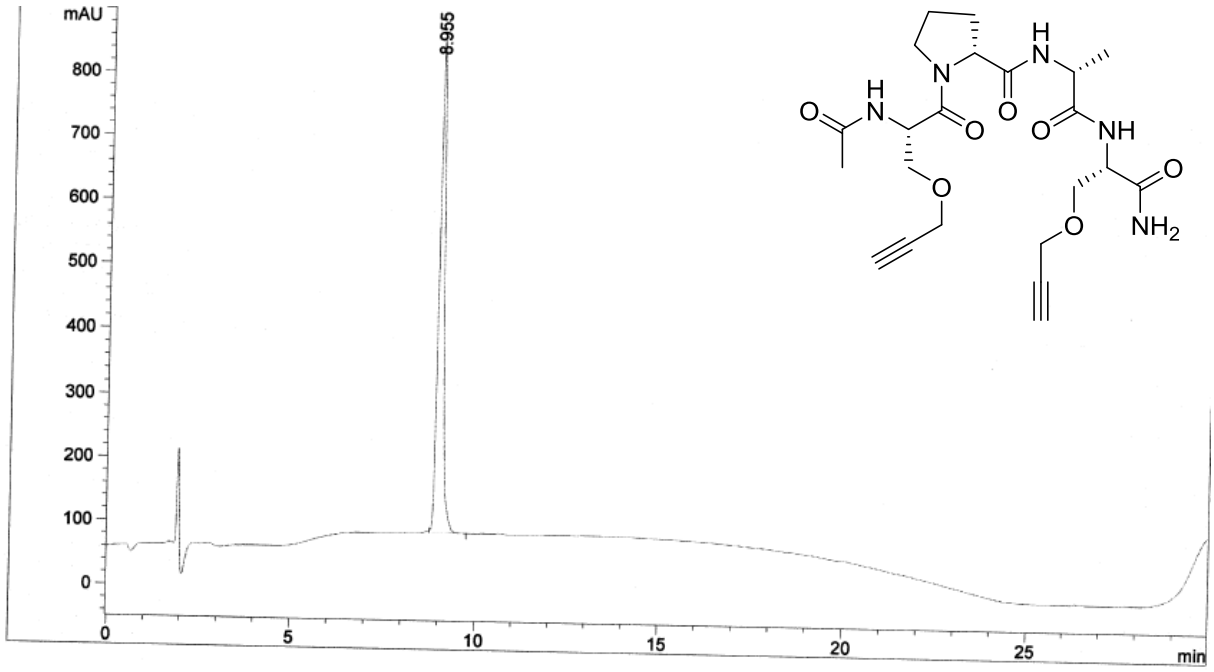
Area Percent Report

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000

Signal 1: DAD1 A, Sig=215,4 Ref=400,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	9.029	BP	0.1738	1106.80615	96.47875	100.0000

Figure 45: HPLC chromatogram of the crude reaction mixture of **6c**.

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================================================================================
                            Area Percent Report
================================================================================

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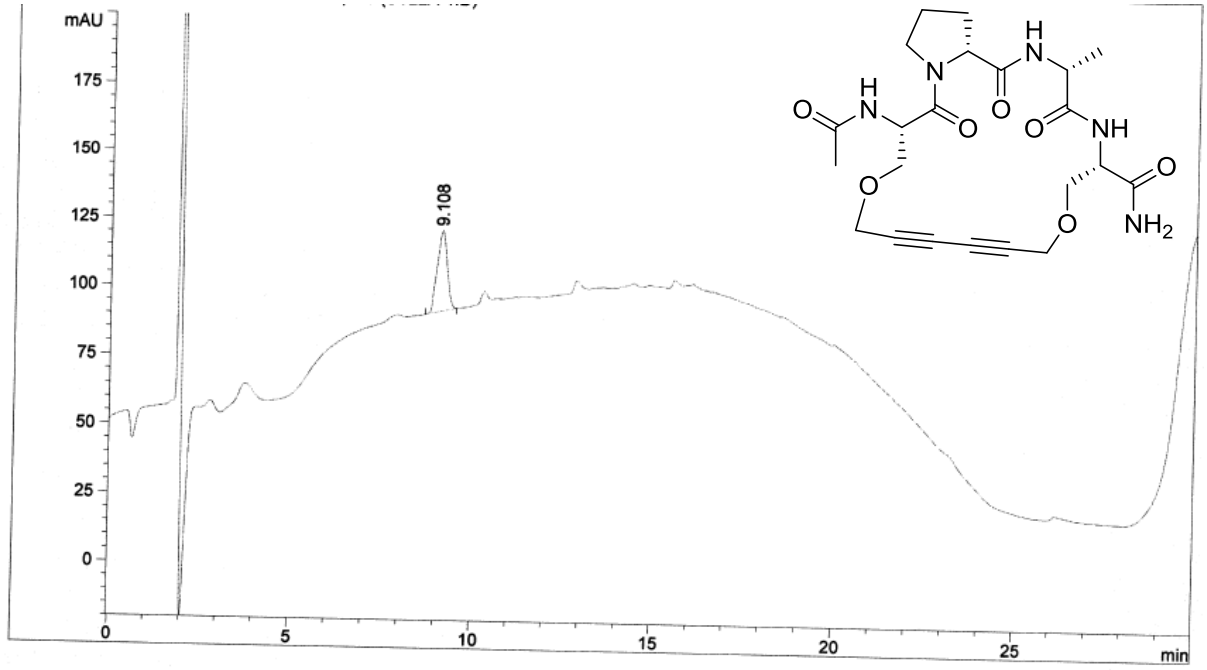
Multiplier            :      1.0000
Dilution              :      1.0000

Signal 1: DAD1 A, Sig=215,4 Ref=400,100

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]    [mAU*s]    [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   8.955 BP    0.1587 7848.36719  771.18408 100.0000
```

Figure 46: HPLC chromatogram of **5d** purified via preparative RP-HPLC.



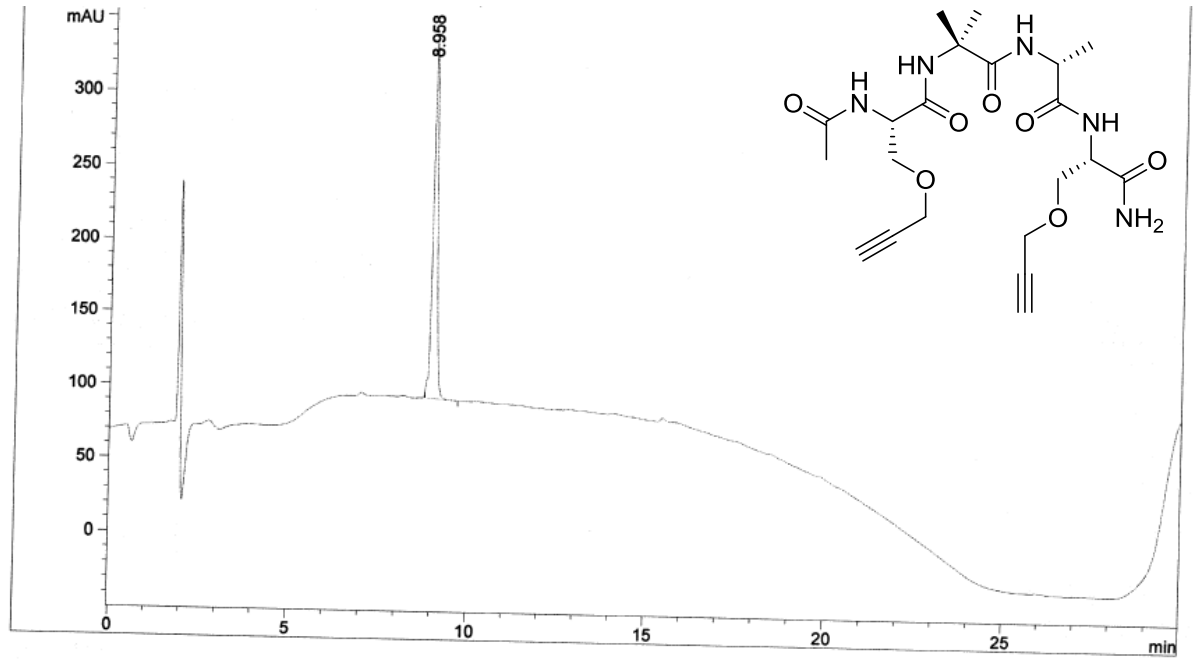
Area Percent Report

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000

Signal 1: DAD1 A, Sig=215,4 Ref=400,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	9.108	PP	0.2881	556.07678	29.47807	100.0000

Figure 47: HPLC chromatogram of the crude reaction mixture of **6d**.



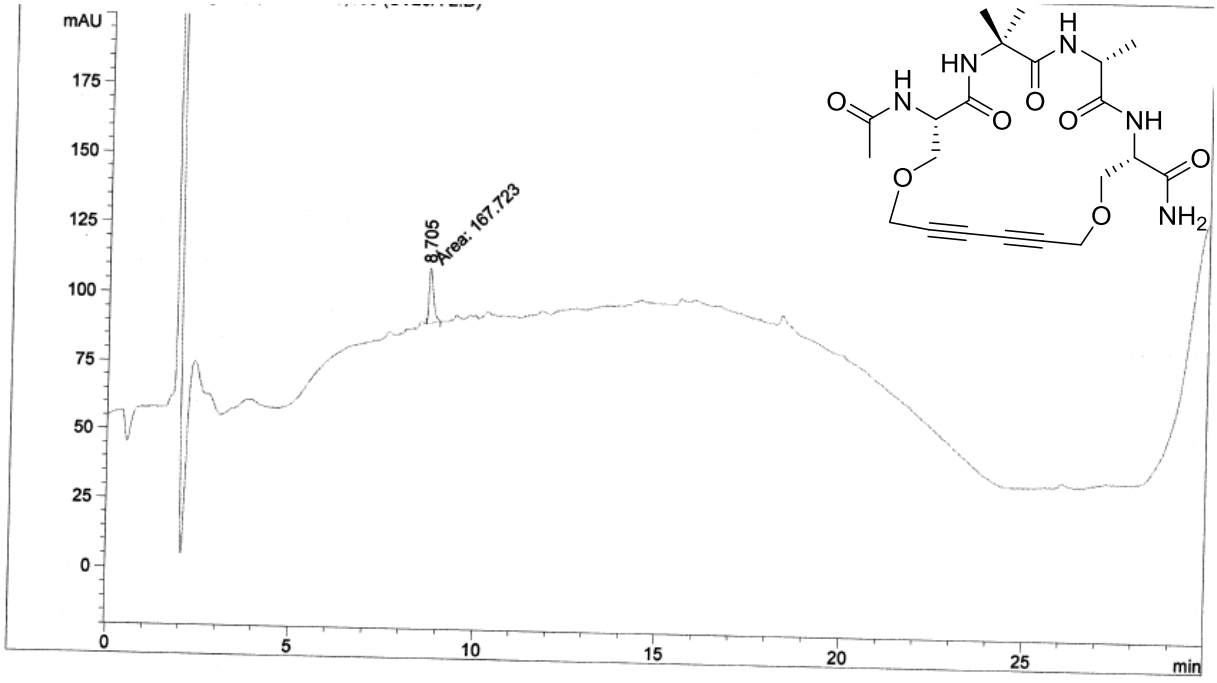
Area Percent Report

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000

Signal 1: DAD1 A, Sig=215,4 Ref=400,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	8.958	BP	0.1400	2209.20557	243.20943	100.0000

Figure 48: HPLC chromatogram of **5e** purified via preparative RP-HPLC.



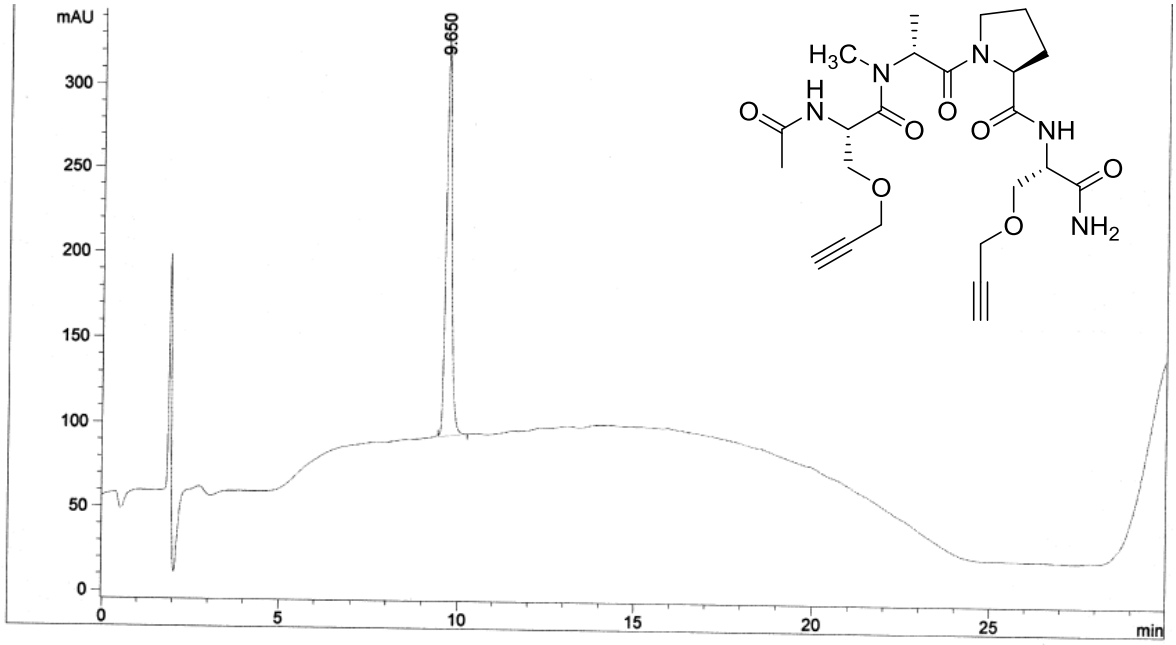
Area Percent Report

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000

Signal 1: DAD1 A, Sig=215,4 Ref=400,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	8.705	MM	0.1418	167.72296	19.71524	100.0000

Figure 49: HPLC chromatogram of the crude reaction mixture of **6e**.



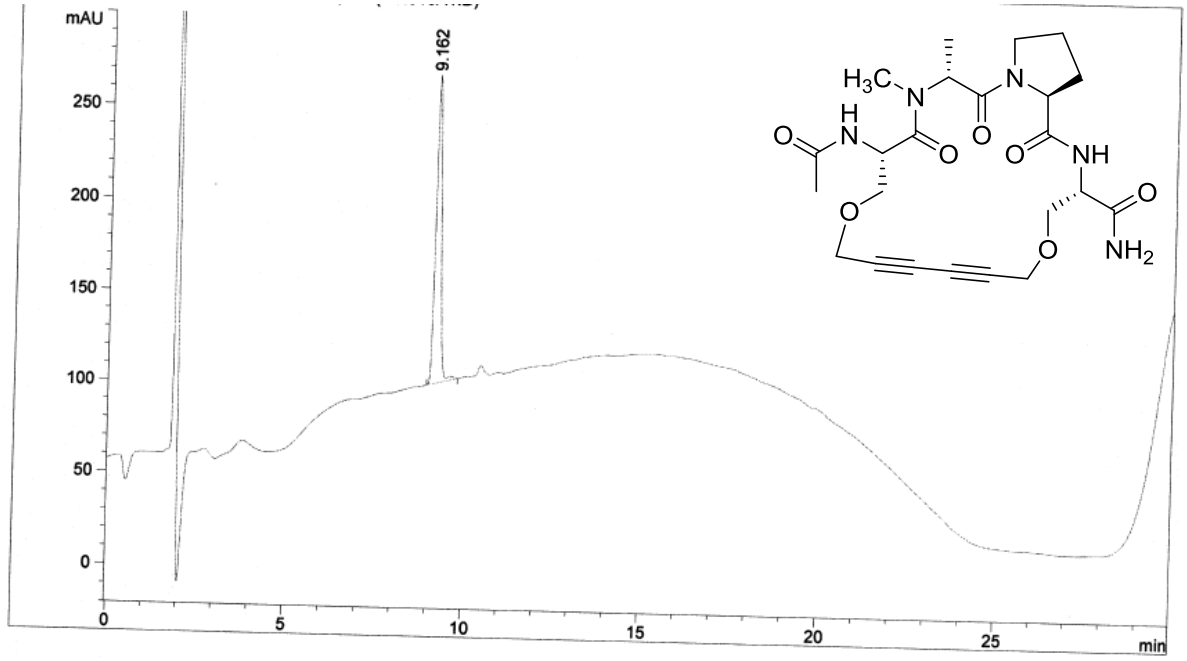
Area Percent Report

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000

Signal 1: DAD1 A, Sig=215,4 Ref=400,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	9.650	BB	0.1656	2518.04663	233.90530	100.0000

Figure 50: HPLC chromatogram of **5f** purified via preparative RP-HPLC.



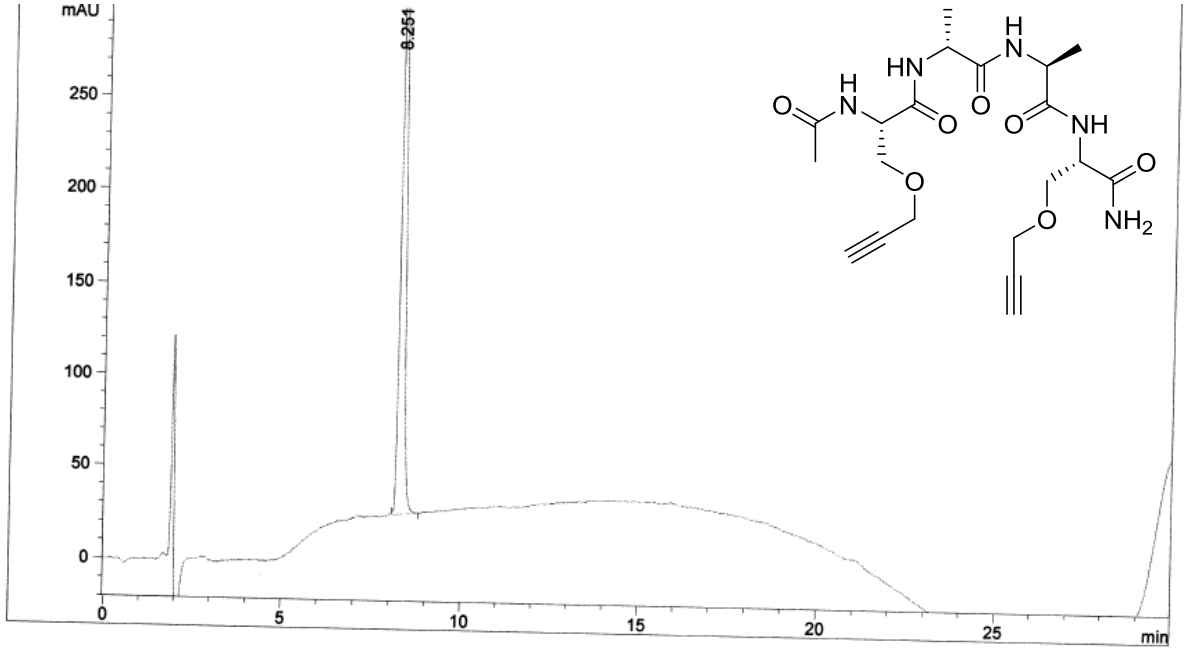
Area Percent Report

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000

Signal 1: DAD1 A, Sig=215,4 Ref=400,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	9.162	BP	0.1654	1796.84961	167.15628	100.0000

Figure 51: HPLC chromatogram of the crude reaction mixture of **6f**.



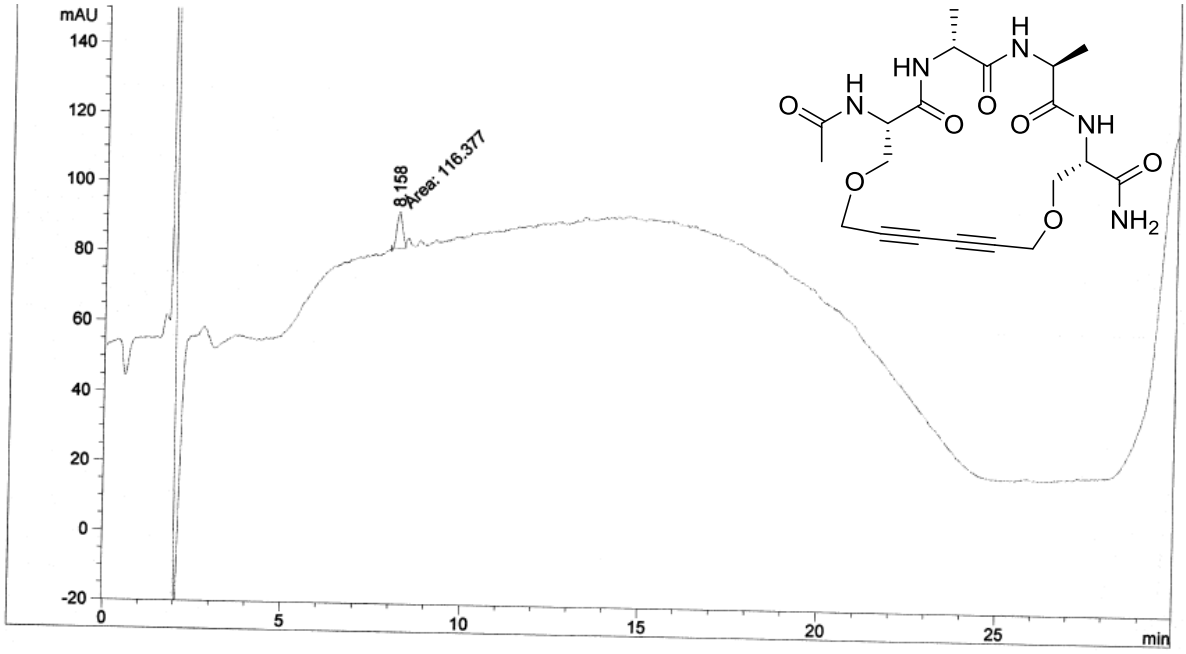
Area Percent Report

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000

Signal 1: DAD1 A, Sig=215,4 Ref=400,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	8.251	BP	0.1478	3263.34644	346.68845	100.0000

Figure 52: HPLC chromatogram of **5g** purified via preparative RP-HPLC.



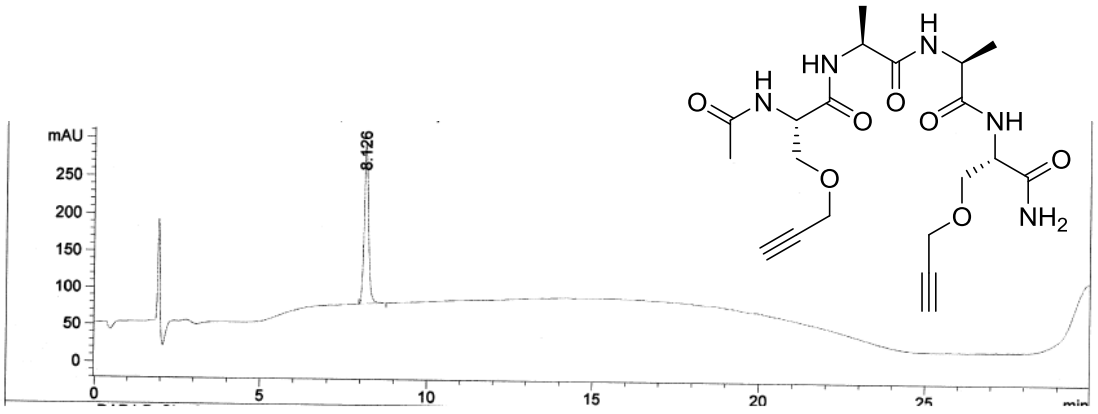
Area Percent Report

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000

Signal 1: DAD1 A, Sig=215,4 Ref=400,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	8.116	Fsho	0.0000	0.00000	8.82814	0.0000
2	8.158	MM	0.1843	116.37746	10.52448	100.0000

Figure 53: HPLC chromatogram of the crude reaction mixture of **6g**.



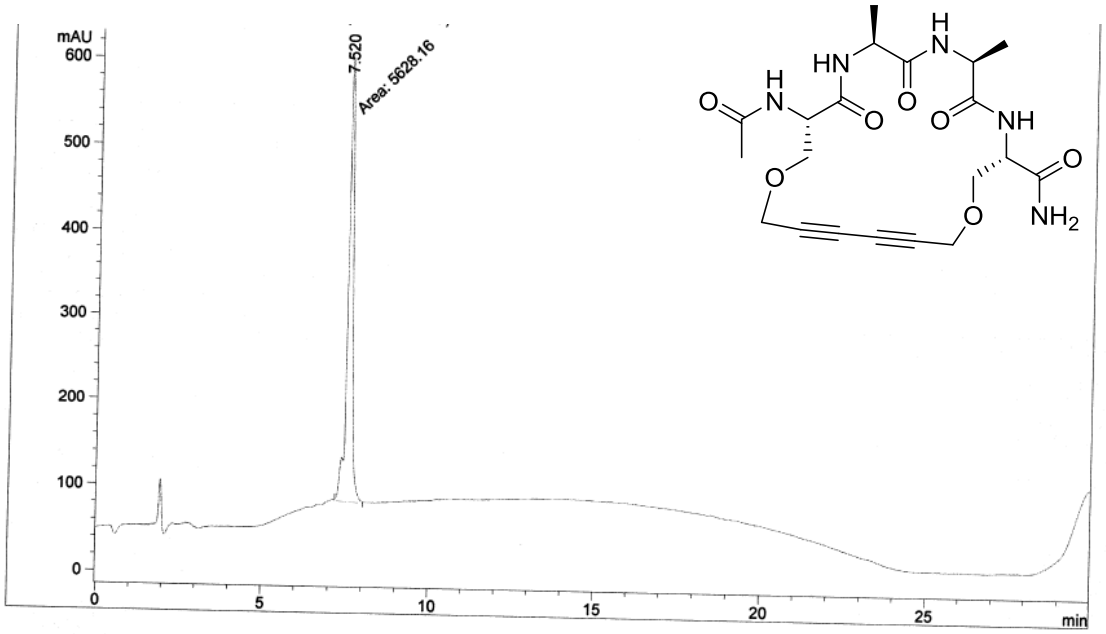
Area Percent Report

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000

Signal 1: DAD1 A, Sig=215,4 Ref=400,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	8.126	BB	0.1495	2100.66089	215.99884	100.0000

Figure 54: HPLC chromatogram of **5h** purified via preparative RP-HPLC.

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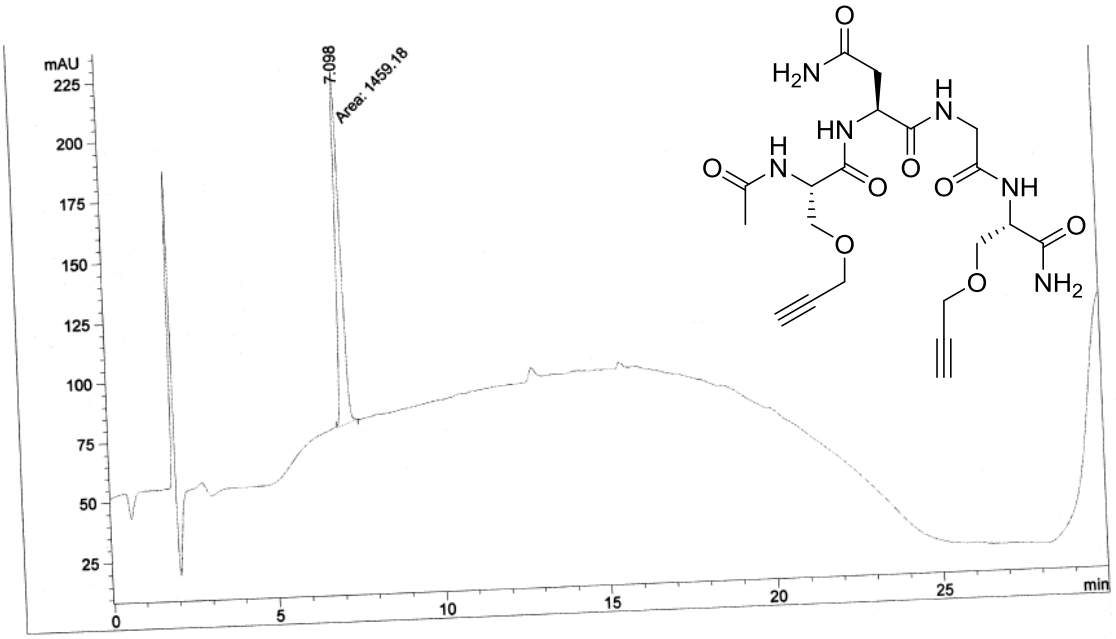
================================================================================
                              Area Percent Report
================================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000

Signal 1: DAD1 A, Sig=215,4 Ref=400,100

Peak RetTime Type  Width     Area      Height      Area
  #   [min]        [min]   [mAU*s]     [mAU]        %
----|-------|----|-------|----------|----------|--------|
   1   7.520 MM    0.1810 5628.16309  518.11969 100.0000
   2   7.953 Rsho  0.0000    0.00000    4.87684   0.0000
```

Figure 55: HPLC chromatogram of the crude reaction mixture of **6h**.



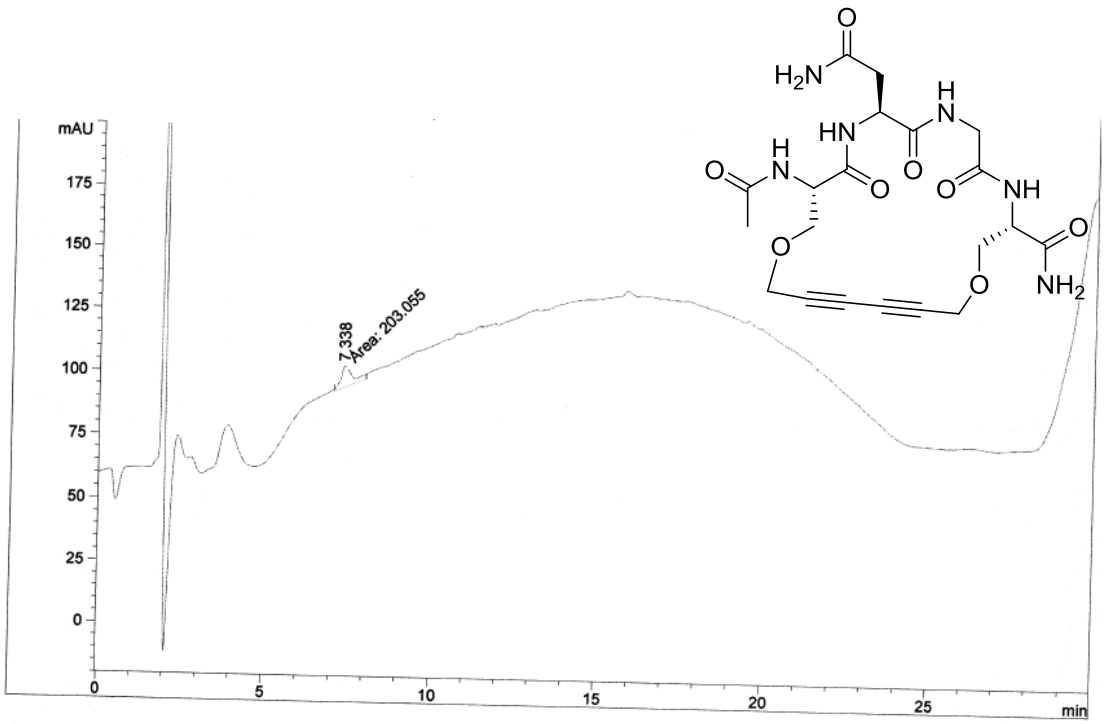
Area Percent Report

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000

Signal 1: DAD1 A, Sig=215,4 Ref=400,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	7.098	MM	0.1645	1459.18115	147.87390	100.0000
2	7.465	Rsho	0.0000	0.00000	2.73038	0.0000

Figure 56: HPLC chromatogram of **5i** purified via preparative RP-HPLC.

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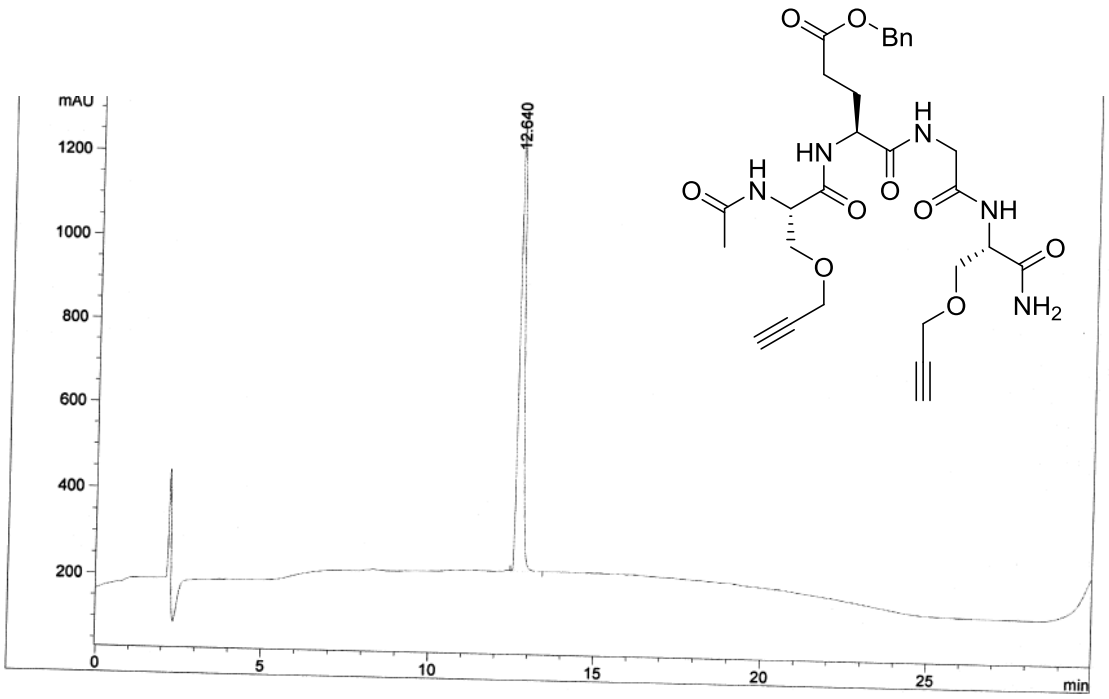
================================================================================
                            Area Percent Report
================================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000

Signal 1: DAD1 A, Sig=215,4 Ref=400,100

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   7.161 Fsho 0.0000    0.00000    2.04270   0.0000
   2   7.338 MM   0.3952  203.05481    8.56279 100.0000
```

Figure 57: HPLC chromatogram of the crude reaction mixture of **6i**.



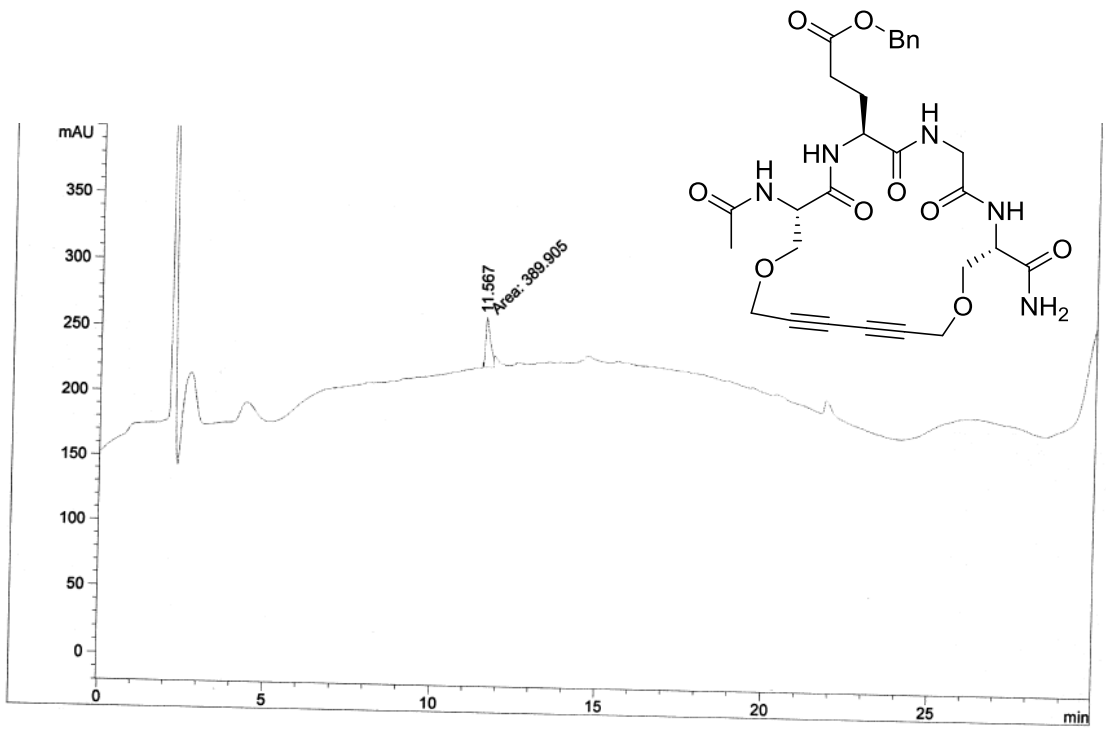
Area Percent Report

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000

Signal 1: DAD1 A, Sig=215,4 Ref=400,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	12.640	BP	0.1680	1.10882e4	1043.47583	100.0000

Figure 58: HPLC chromatogram of **5j** purified via preparative RP-HPLC.

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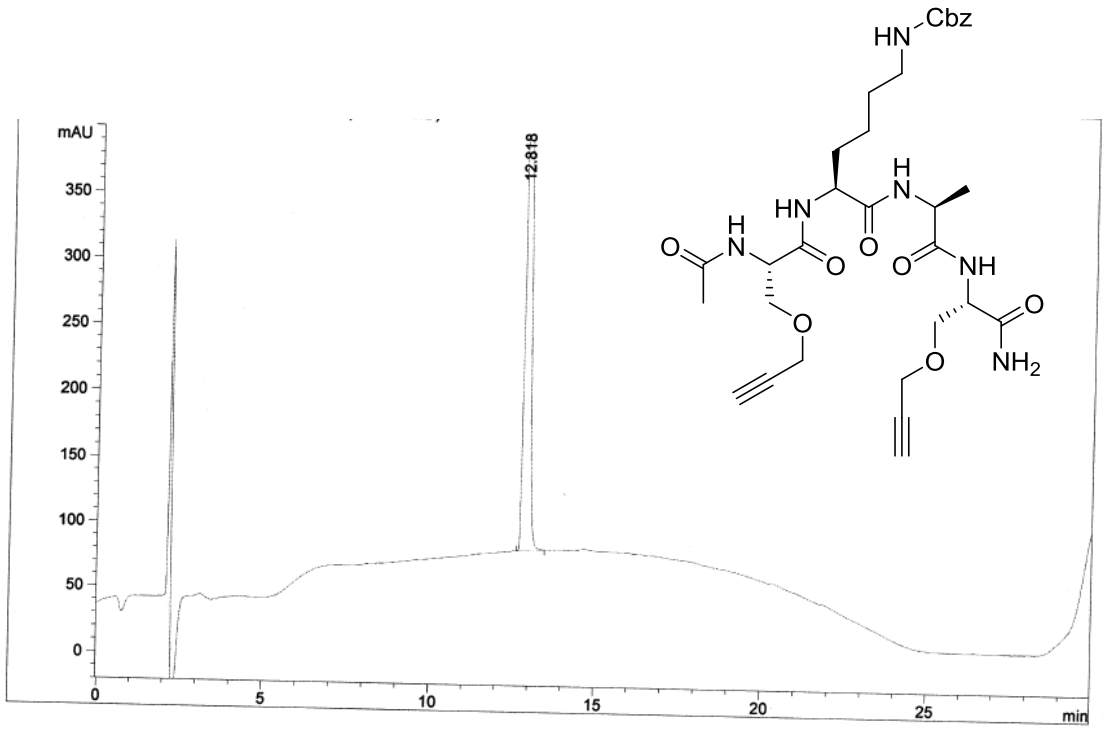
================================================================================
                            Area Percent Report
================================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000

Signal 1: DAD1 A, Sig=215,4 Ref=400,100

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1  11.567 MM    0.1710  389.90521   38.01034 100.0000
   2  11.716 Rsho  0.0000    0.00000   14.19488   0.0000
```

Figure 59: HPLC chromatogram of the crude reaction mixture of **6j**.



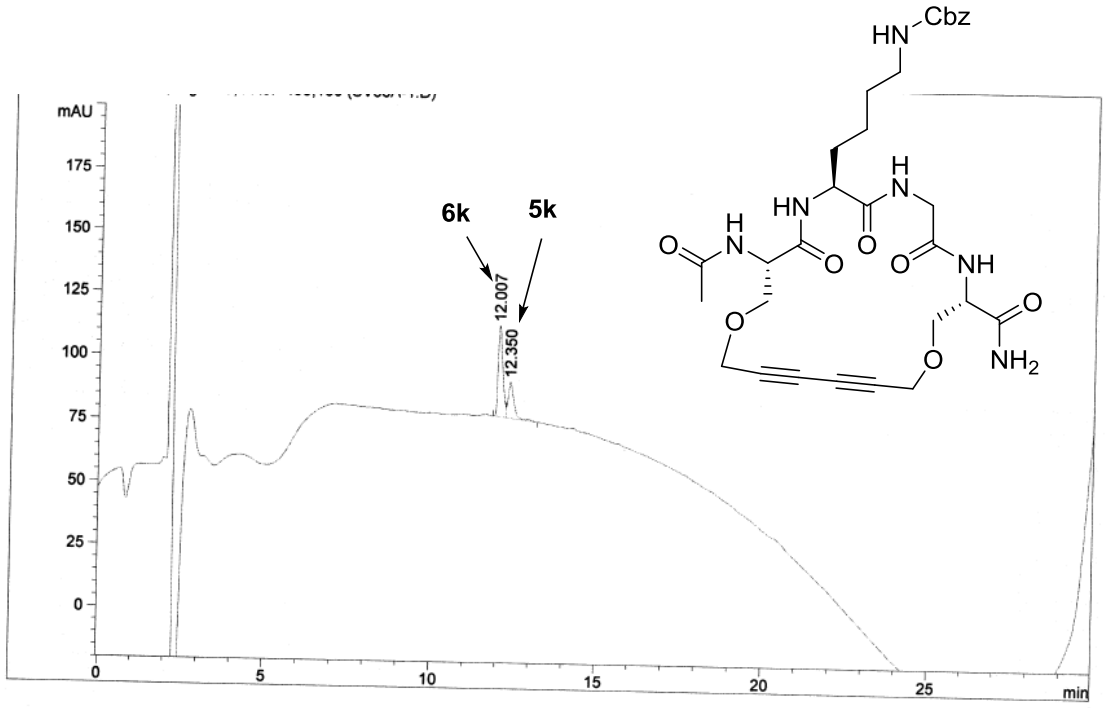
Area Percent Report

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000

Signal 1: DAD1 A, Sig=215,4 Ref=400,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	12.818	BB	0.1627	5832.69434	554.40863	100.0000

Figure 60: HPLC chromatogram of **5k** purified via preparative RP-HPLC.

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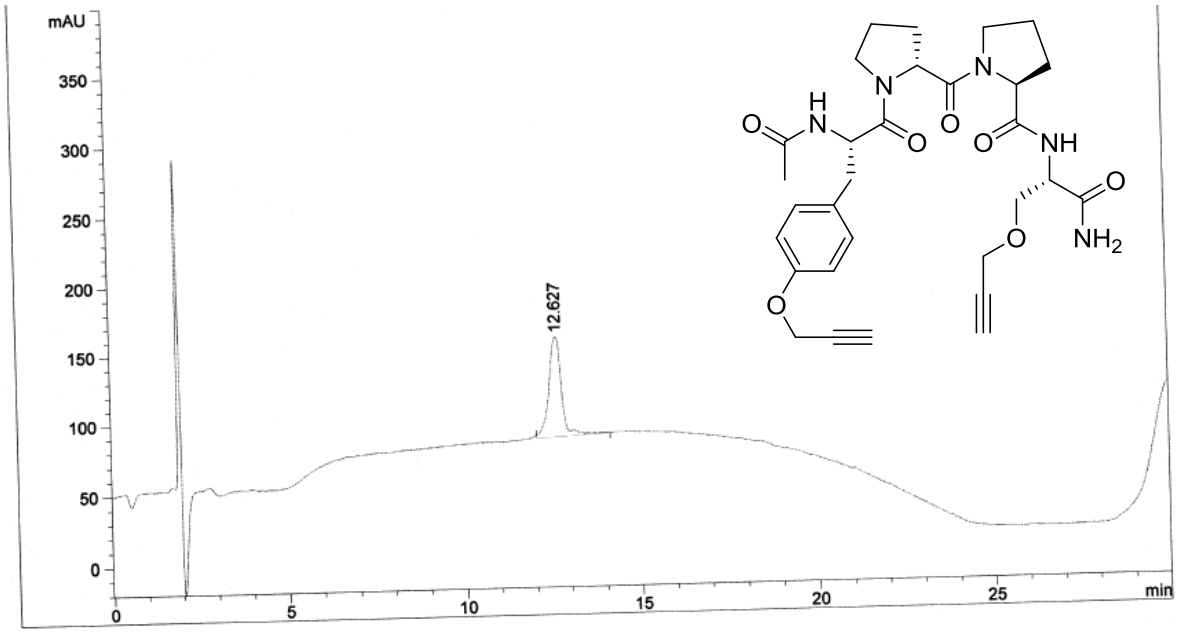
================================================================================
                            Area Percent Report
================================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000

Signal 1: DAD1 A, Sig=215,4 Ref=400,100

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]    [mAU*s]    [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1  12.007 PV    0.1554  351.28323   35.51619  66.9451
   2  12.350 VB    0.1868  173.44995   14.16775  33.0549
```

Figure 61: HPLC chromatogram of the crude reaction mixture of **6k**.



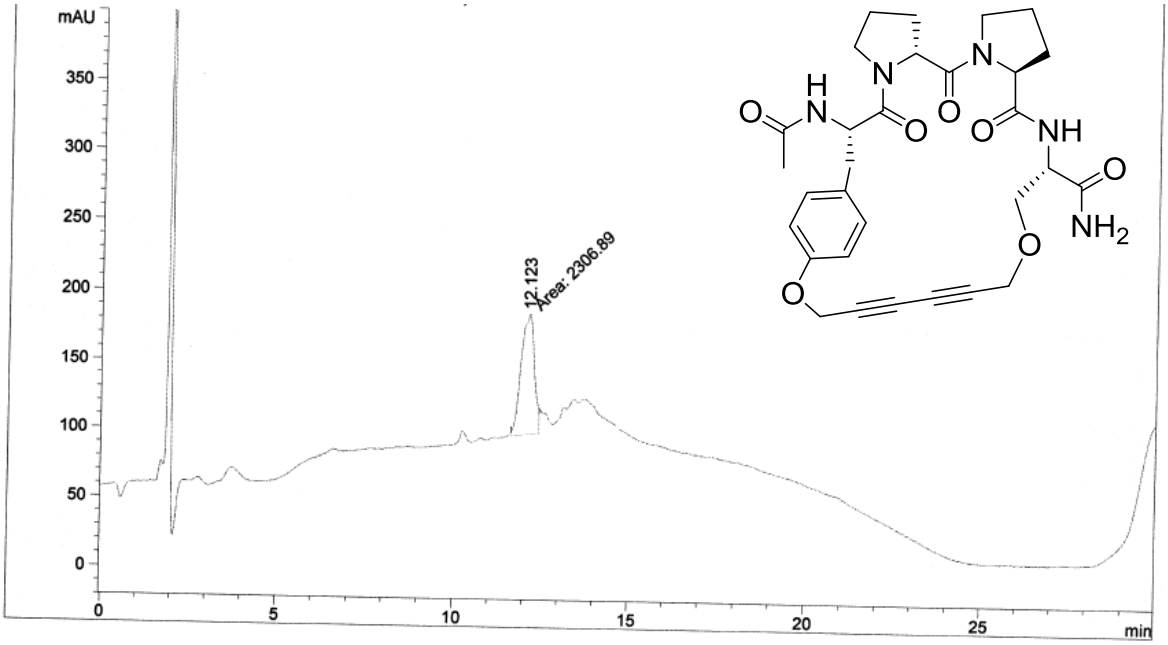
Area Percent Report

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000

Signal 1: DAD1 A, Sig=215,4 Ref=400,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	12.627	BP	0.3803	1808.35681	72.16608	100.0000

Figure 64: HPLC chromatogram of **7** purified via preparative RP-HPLC.



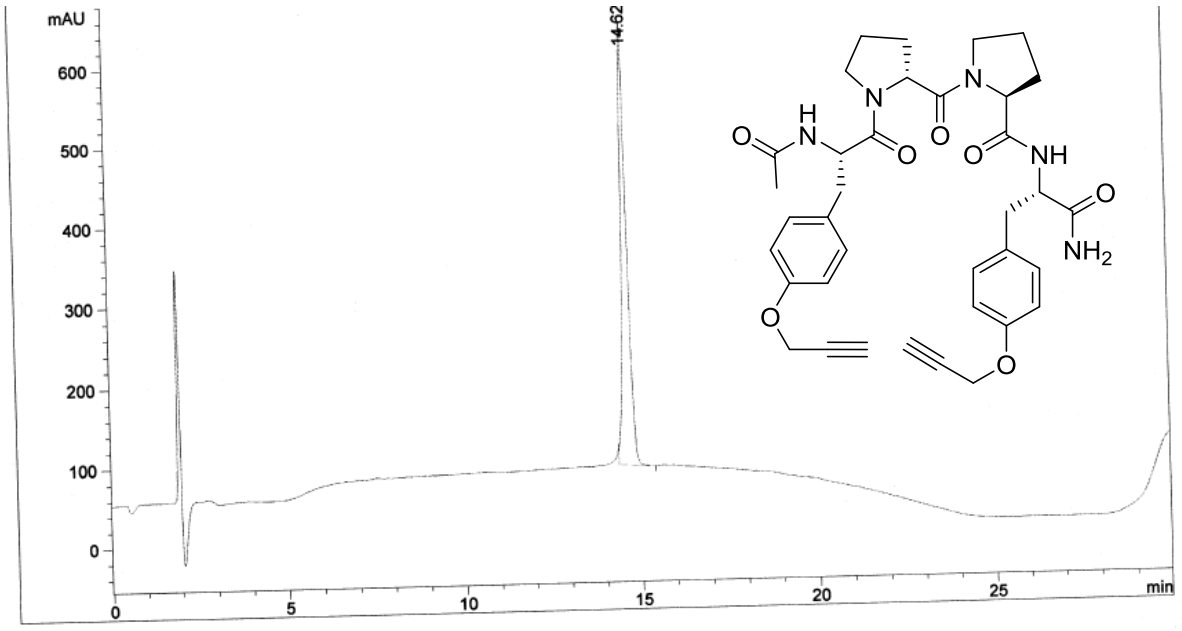

```
================================================================================
                            Area Percent Report
================================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000

Signal 1: DAD1 A, Sig=215,4 Ref=400,100

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1  11.901 Fsho  0.0000    0.00000   59.58928   0.0000
   2  12.123 MM    0.4385 2306.89209   87.67314 100.0000
```

Figure 65: HPLC chromatogram of the crude reaction mixture of **8**.



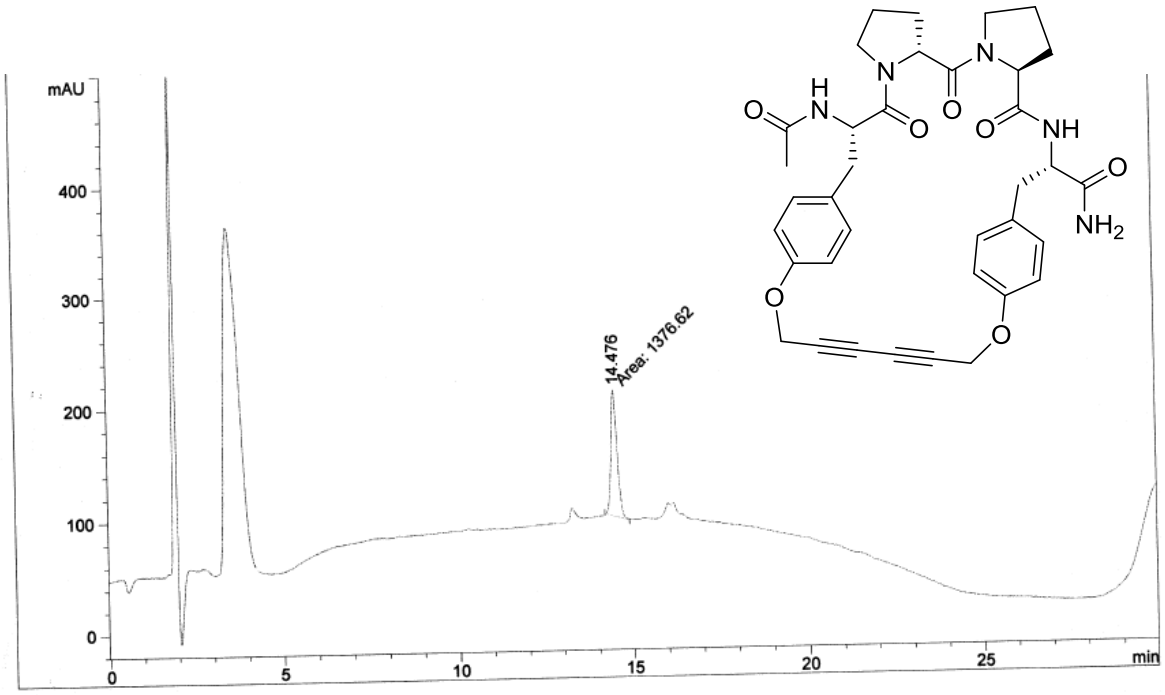
Area Percent Report

Sorted By	:	Signal
Multiplier	:	1.0000
Dilution	:	1.0000

Signal 1: DAD1 A, Sig=215,4 Ref=400,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	14.629	BP	0.1724	6683.43848	555.22015	100.0000

Figure 62: HPLC chromatogram of **9** purified via preparative RP-HPLC.



Area Percent Report

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000

Signal 1: DAD1 A, Sig=215,4 Ref=400,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	14.476	MM	0.2046	1376.61584	112.15251	100.0000
2	14.774	Rsho	0.0000	0.00000	6.88563	0.0000

Figure 63: HPLC chromatogram of the crude reaction mixture of **10**.

8. References

[1] A. M. King, C. Salomé, J. Dinsmore, E. Salomé-Grosjean, M. De Ryck, R. Kaminiski, A. Valade, H. Kohn, *J. Med. Chem.*, 2011, **54**, 4815.
[2] H. T. ten Brink, D. T. S. Rijkers, R. M. J. Liskamp, *J. Org. Chem.* 2006, **71**, 1817.
[3] S. Reitz, M. Cebi, P. Reiβ, G. Studnik, U. Linne, U. Koert, L.-O. Essen, *Angew. Chem. Int. Ed.*, 2009, **48**, 4853.
[4] C. Li, J. Tang, J. Xie, *Tetrahedron*, 2009, **65**, 7935.
[5] C. Reuter, P. Huy, J.-M. Neudörfl, R. Kühne, H.-G. Schmalz, *Chem. Eur. J.*, 2011, **17**, 12037.