Electronic Supplementary Material (ESI) for ChemComm.

Fmoc Solid-Phase Synthesis of Protected C-terminal Modified Peptides by Formation of Backbone Cyclic Urethane Moiety

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Supporting Information

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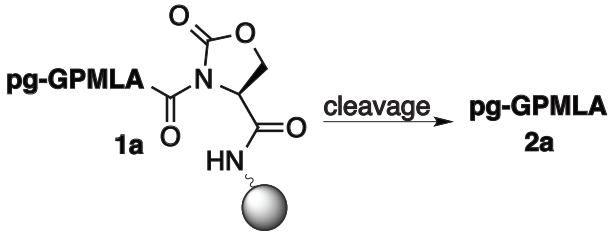
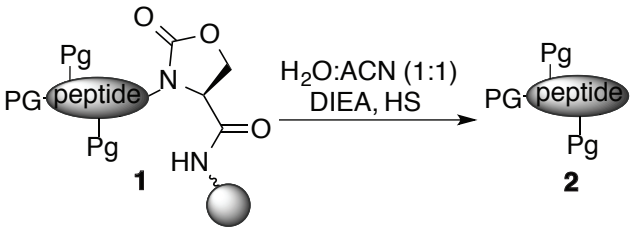
Table S1: Optimization of reaction conditions for synthesis of protected peptide acids, **2a**.[a]



Entry	pg	Solvent	Temp. (°C)	Time (h)	Conversion[b] (%)
1	Ac	Water	RT	15	30
2	Ac	Water:DMF (4:1)	RT	15	56
3	Ac	Water	50	15	45
4	Ac	Water: DMF (4:1)	50	15	77
5	Ac	Water: DMF (1:1)	50	15	99
6	Ac	Water: DMF (1:1)	65	4	59
7	Ac	Water: ACN (1:1)	65	4	99
8	**Ac**	**Water: ACN (1:1)**	**65**	**2**	**99**
9	NH_2	Water: ACN (1:1)	65	2	99
10[c]	Fmoc	Water: ACN (1:1)	65	2	99

Reaction conditions: Peptide (25 mg, 0.70 mm/g) on solid support was reacted with DSC (100mg), DIEA (70 μL) and a crystal of DMAP in DMF at room temperature for 16 h followed by hydrolysis. [b]Conversion to **2a** was calculated from the absorbance at 220 nm using HPLC. The entry in bold represents the optimized reaction conditions for hydrolysis. [c]Mixture of completely protected peptide and Fmoc deprotected peptide was observed. DIEA = Diisopylethylamine, DMAP = 4-(*N*,*N*-dimethylamino)-pyridine.

Table S2. Substrate scope of CUT in the synthesis of peptide acids.



Entry	Substrate	Peptide	PG	Time (h)	Conversion (%)
1	**1b**	LFK(Boc)N(Trt)A	Ac	2	95
2	**1c**	LFK(Boc)N(Trt)A	NH_2	2	95
3[c]	**1d**	LFK(Boc)N(Trt)A	Fmoc	2	80
4	**1e**	FE(tBu)S(tBu)Q(Trt)I	NH_2	2	90
5	**1f**	R(Pbf)D(tBu)PMLG	Ac	2 x 2	95
6	**1g**	R(Pbf)D(tBu)PMLG	NH_2	2	90
7	**1h**	Y(tBu)LFK(Boc)N(Trt)A	Ac	2	95
8	**1i**	Y(tBu)LFK(Boc)N(Trt)A	NH_2	2	90
9	**1j**	VWR(Pbf)A	Ac	2 x 2	90
10	**1k**	T(tBu)C(tBu)D(tBu)V	Ac	2 x 2	90
11[c]	**1l**	Y(tBu)LFK(Boc)N(Trt)A	Fmoc	2	80
12	**1m**	VWH(Trt)A	Ac	2 x 2	95
13[d]	**1n**	T(Trt)C(Trt)GGH(Trt)A	Ac	2 x 2	80
14[c d]	**1o**	T(Trt)C(Trt)GGH(Trt)A	Fmoc	2	80

Reaction conditions: Peptide (25 mg, 0.7 mm/g) on solid support was reacted with DSC (15 equiv), DIEA (15 equiv) and a crystal of DMAP in DMF at room temperature followed by hydrolysis. [b]Conversion to **2** was calculated from the absorbance at 220 nm using HPLC. [c]Mixture of completely protected peptide and Fmoc deprotected peptide was observed. [d]Trt protecting group of cysteine was deprotected under the reaction conditions, more labile groups like Trt on cysteine underwent deprotection under hydrolysis conditions. HS = heated shaker

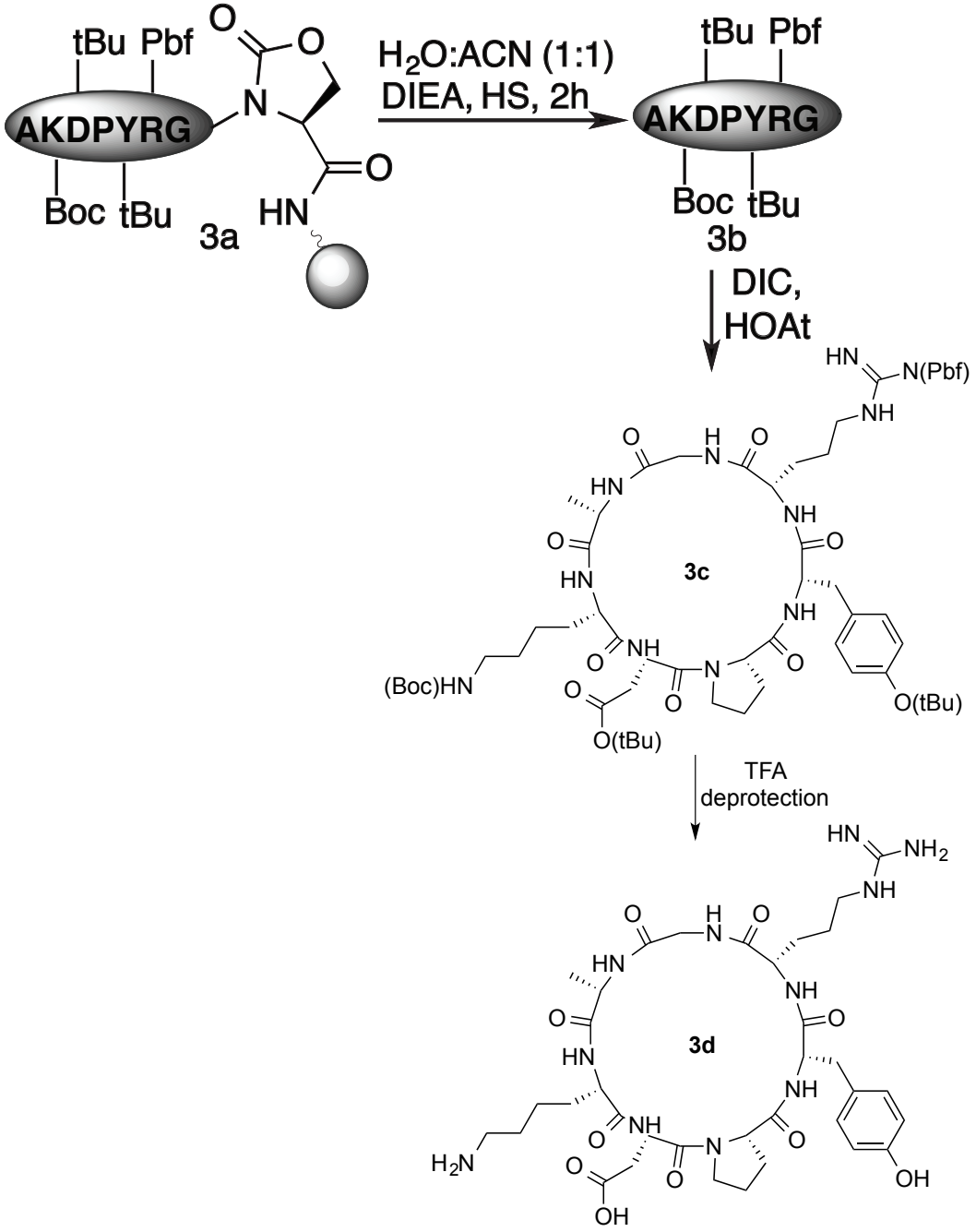
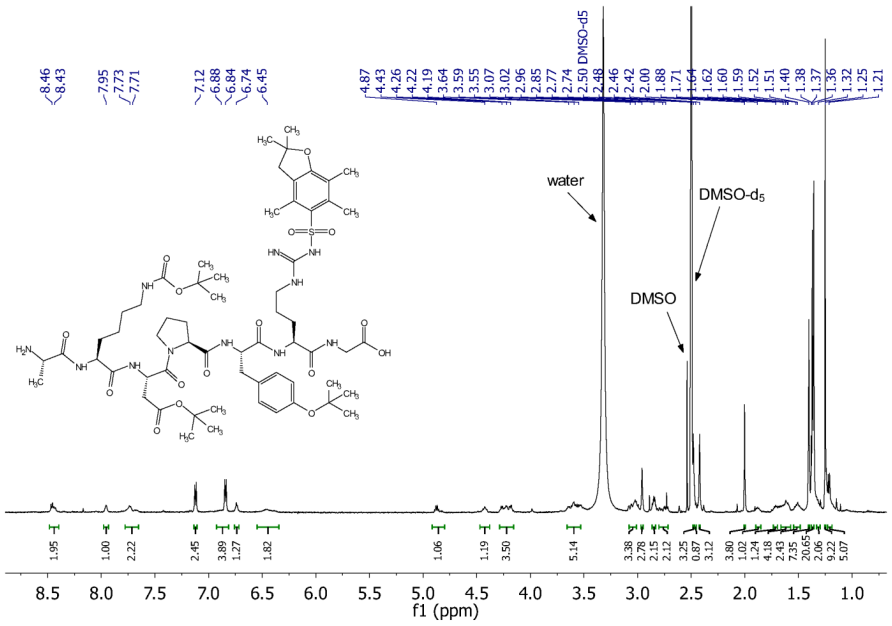


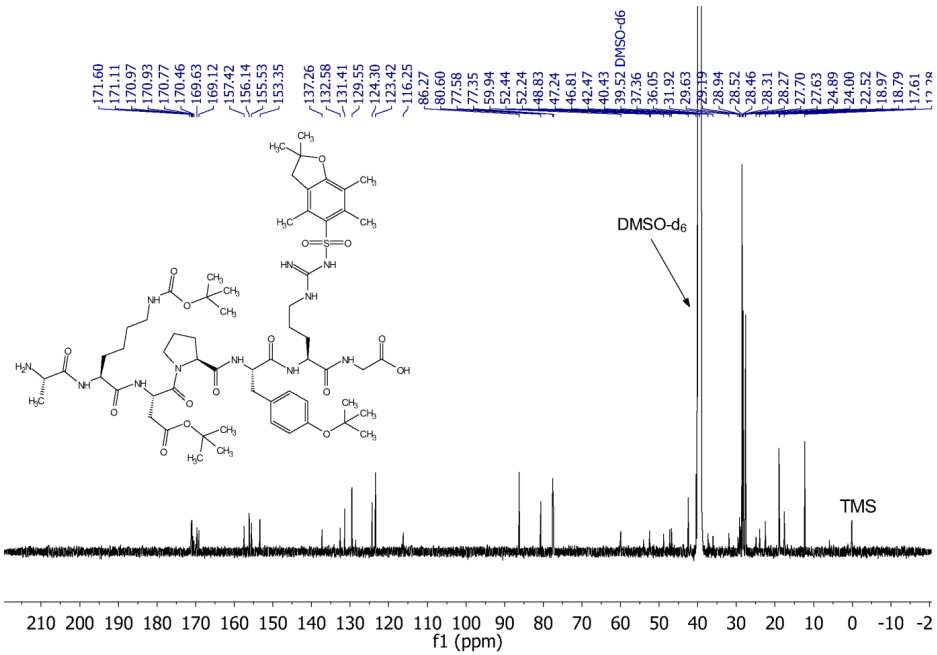
Figure S1. Application of CUT in the synthesis of macrocyclic peptide **3d**. AKDPYRGS-Rink AM was synthesized on the solid support, followed by serine selective deprotection and activation to generate **3a** which is followed by hydrolysis with water to obtain protected linear peptide acid AK(Boc)D(tBu)PY(tBu)R(Pbf)G-OH **3b**. Next, the protected peptide acid AK(Boc)D(tBu)PY(tBu)R(Pbf)G-OH **3b** was subjected to coupling reagents (DIC and HOAt) to obtain head to tail macrocyclized protected peptide cyc(AK(Boc)D(tBu)PY(tBu)R(Pbf)G) **3c**.

This was followed by the removal of side-chain protecting group of macrocyclic peptide using TFA cocktail to generate deprotected monocyclic peptide cyc(AKDPYRG) **3d**.

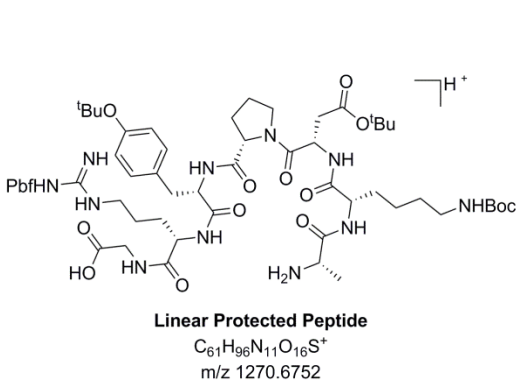
^{1}H NMR spectrum for linear protected peptide **3b**, AK(Boc)D(tBu)PY(tBu)R(Pbf)G-OH, recorded at 600 MHz

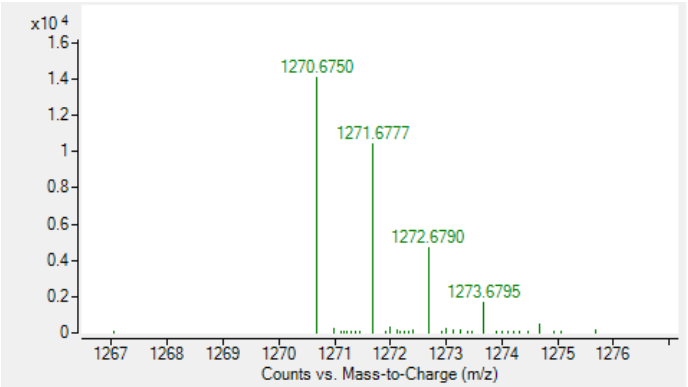


^{13}C NMR spectrum for linear protected peptide **3b**, AK(Boc)D(tBu)PY(tBu)R(Pbf)G-OH, recorded at 151 MHz

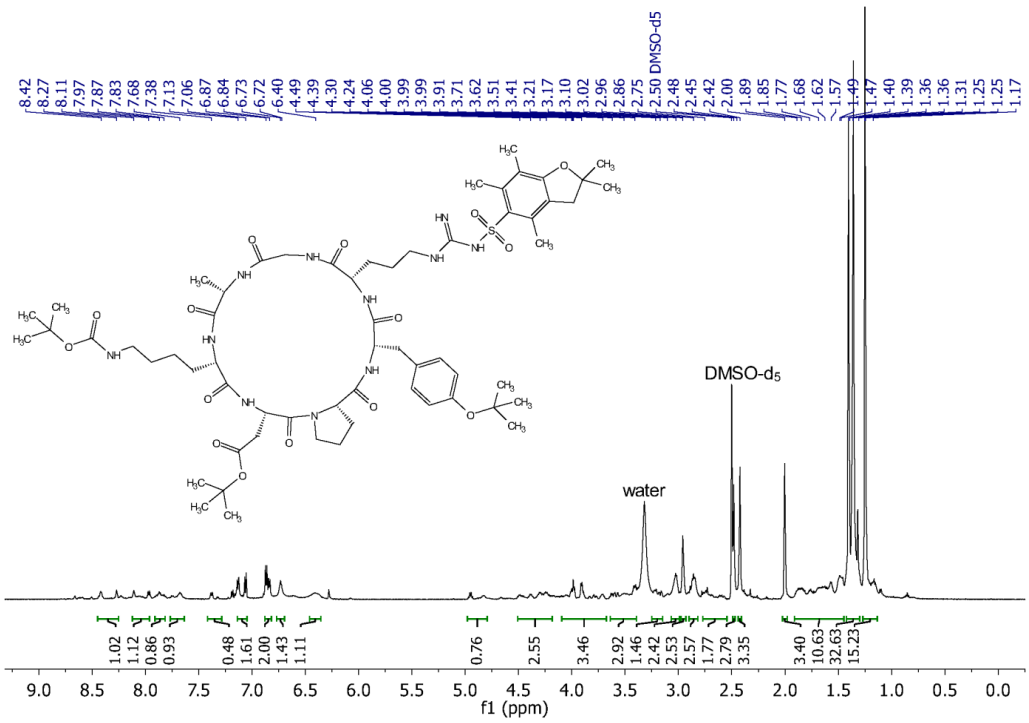


HRMS (+ESI) data for linear protected peptide **3b**, AK(Boc)D(tBu)PY(tBu)R(Pbf)G-OH

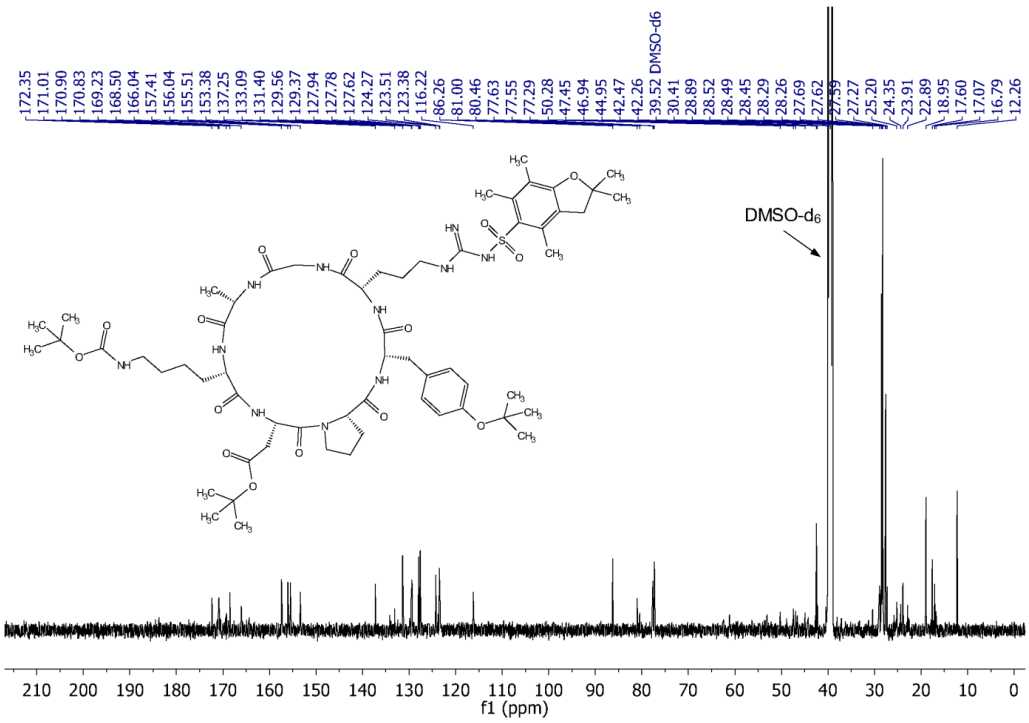




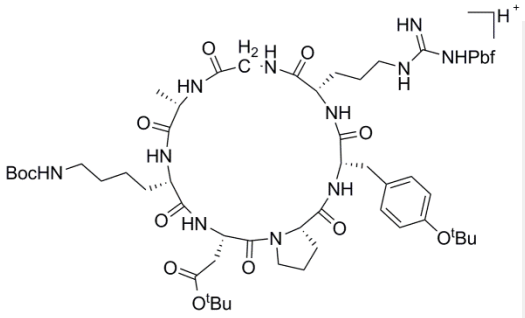
^{1}H NMR spectrum for cyclic protected peptide **3c**, cyc(AK(Boc)D(tBu)PY(tBu)R(Pbf)G), recorded at 600 MHz

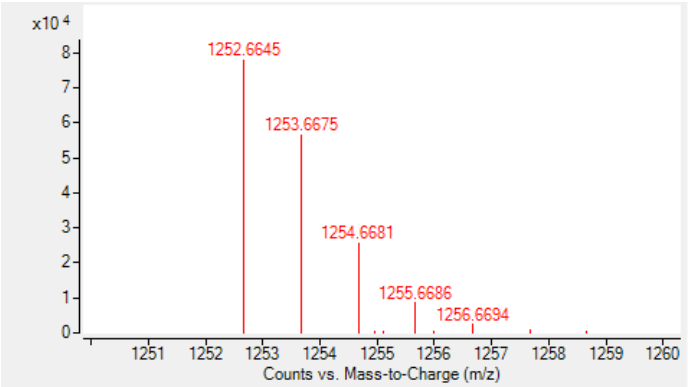


^{13}C NMR spectrum for cyclized protected peptide **3c**, cyc(AK(Boc)D(tBu)PY(tBu)R(Pbf)G), recorded at 151 MHz

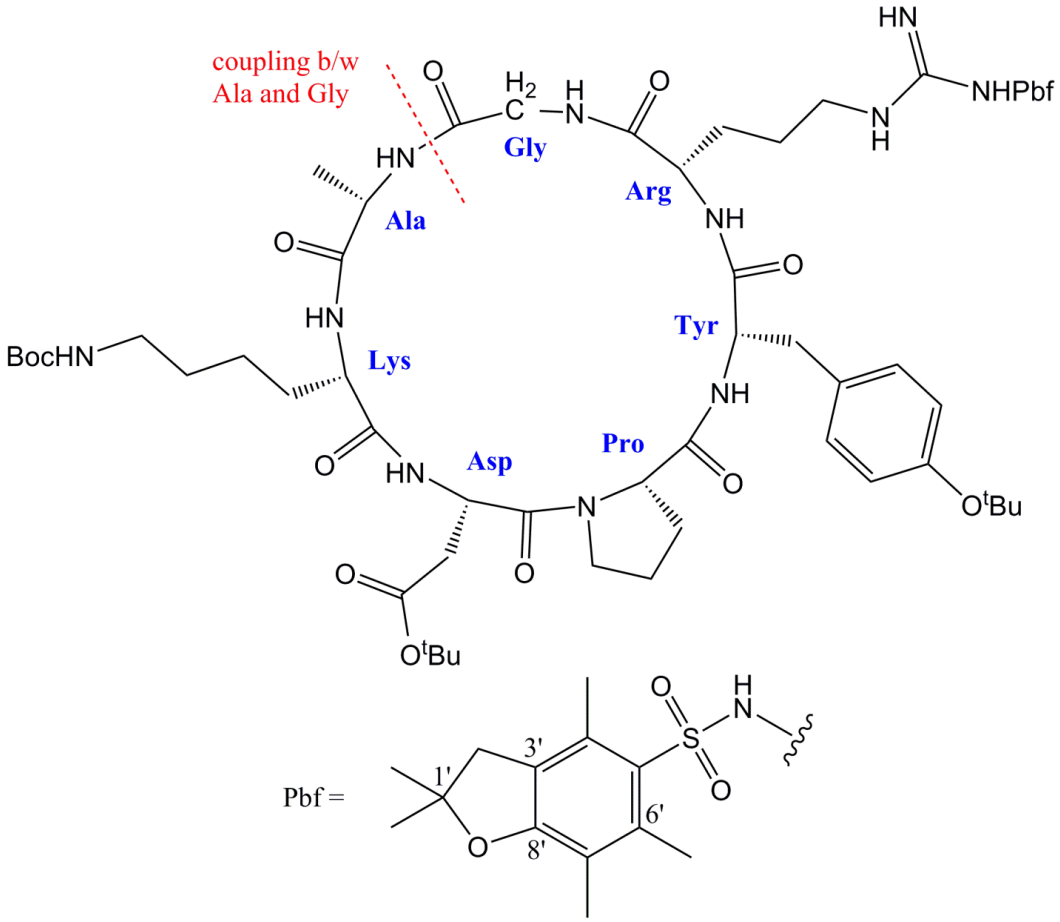


HRMS (+ESI) data for cyclized protected peptide **3c**, cyc(AK(Boc)D(tBu)PY(tBu)R(Pbf)G)





Structure and Pbf numbering convention for NMR data

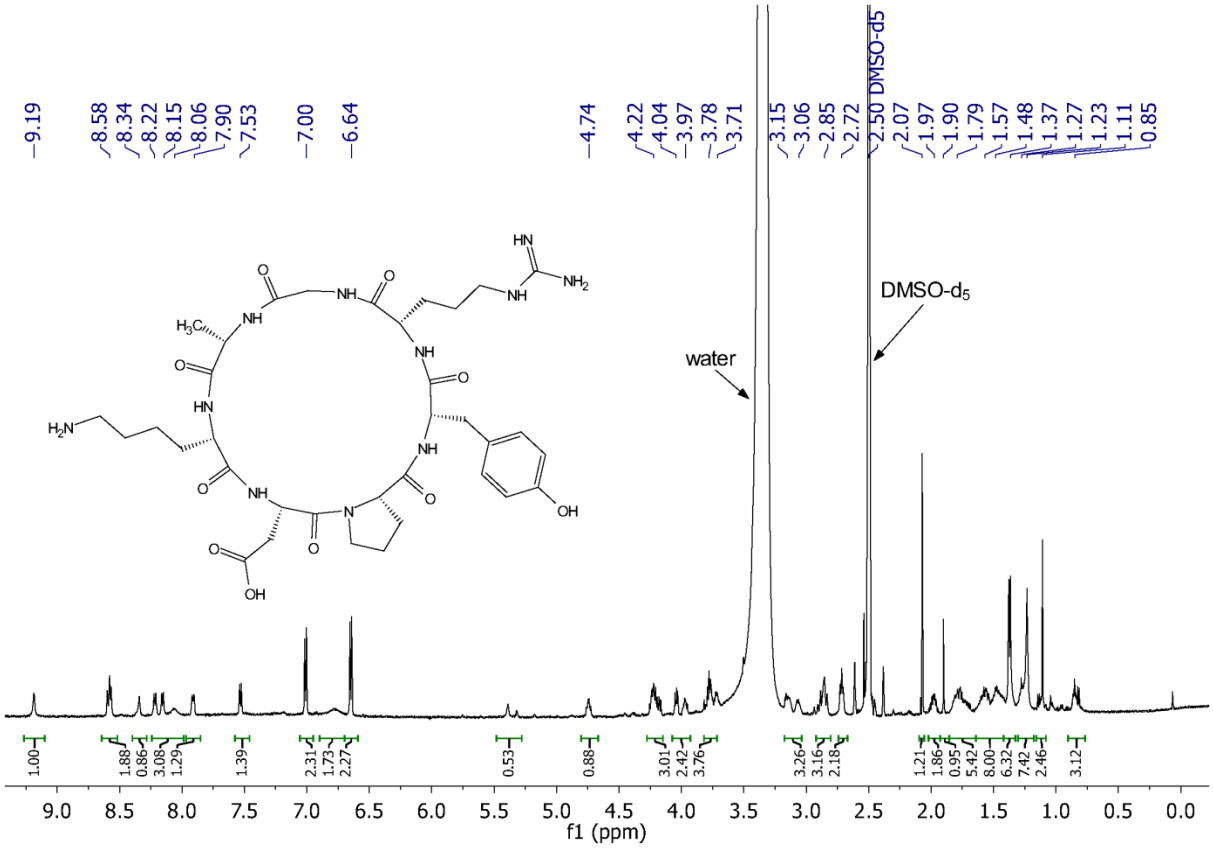


^{1}H chemical shifts (ppm), coupling constants (Hz), and multiplicities					
	Compound:	Linear, unprotected	Linear, protected	Cyclized, protected	Cyclized, protected
Residue	Atom			conformer 1	conformer 2
Ala	NH	8.11, d (5.3 Hz)	6.55 - 6.35, br s	8.42, d (5.7 Hz)	7.85 - 7.91, m [a]
	Hα	3.92 - 3.83, m	3.64, d (6.5 Hz)	3.92 - 3.89, m [a]	4.23 - 4.19, m [a]
	Hβ	1.32, d (7.1 Hz)	1.22, d (7.0 Hz)	1.28 - 1.22, m [a]	1.29 - 1.22, m [a]
Lys	NH	8.49, d (7.9 Hz)	8.43, d (7.8 Hz)	7.97, d (8.4 Hz)	7.84 - 7.79, m [a]
	Hα	4.29 - 4.22, m [a]	4.28 - 4.20, m [a]	4.01 - 3.96, m [a]	4.21 - 4.14, m [a]
	Hβ	1.64 - 1.57, m [a] 1.55 - 1.44, m [a]	1.66 - 1.58, m [a] 1.55 - 1.48, m [a]	1.80 - 1.74, m [a] 1.64 - 1.59, m [a]	1.60 - 1.54, m [a] 1.49 - 1.44, m [a]
	Hγ	1.35 - 1.24, m [a]	1.23 - 1.16, m [a]	1.24 - 1.12, m [a]	1.19 - 1.15, m [a]
	Hδ	1.56 - 1.44, m [a]	1.36 - 1.30, m [a]	1.35 - 1.28, m [a]	1.34 - 1.31, m [a]
	Hε	2.79 - 2.69, m [a]	2.85, q (6.8 Hz)	2.93 - 2.79, m [a]	2.91 - 2.81, m [a]
	$NH_{terminal}$	7.79, t (5.1 Hz)	6.74, t (5.6 Hz)	6.73, t (5.6 Hz)	6.73, t (5.6 Hz)
	Boc	N/A	1.36, s	1.37 - 1.34, m [a]	1.37 - 1.34, m [a]
Asp	NH	8.42, d (7.4 Hz)	8.46, d (8.5 Hz)	7.38, d (8.4 Hz)	8.30 - 8.21, m [a]
	Hα	4.78, q (7.0 Hz)	4.87, q (7.4 Hz)	4.95, q (7.2 Hz)	4.85 - 4.80, br s
	Hβ	2.77, dd (17.0, 6.7 Hz) 2.48, dd (17.0, 6.9 Hz)	2.82 - 2.70, m [a] 2.52 - 2.42, m [a]	2.77 - 2.72, m [a] 2.47 - 2.42, m [a]	2.70, dd (16.6, 6.8 Hz) 2.42 - 2.38, m [a]
	tBu	N/A	1.37, s	1.37 - 1.34, m [a]	1.37 - 1.34, m [a]
Pro	Hα	4.24, dd (7.6, 3.2 Hz)	4.18, dd (7.7, 3.8 Hz)	4.00 - 3.97, m [a]	4.26 - 4.22, m [a]
	Hβ	1.93 - 1.83, m 1.71 - 1.65, m [a]	1.88, t (7.1 Hz) 1.66 - 1.58, m [a]	1.92 - 1.84, m [a] 1.50 - 1.44, m [a]	1.88 - 1.82, m [a] 1.65 - 1.61, m [a]
	Hγ	1.79 - 1.70, m [a] 1.57 - 1.63, m [a]	1.75 - 1.66, m [a] 1.67 - 1.62, m [a]	1.88 - 1.78, m [a]	1.74 - 1.66, m [a] 1.61 - 1.53, m [a]
	Hδ	3.64 - 3.54, m	3.57 - 3.50, m	3.74 - 3.68, m 3.65 - 3.60, m	3.59 - 3.43, m
Tyr	NH	7.58, d (8.0 Hz)	7.79 - 7.63, m	8.11 - 8.00, m [a]	7.71 - 7.67, m [a]
	Hα	4.37, dt (8.5, 4.7)	4.43, dt (8.5, 4.5 Hz)	4.05 - 3.98, m [a]	4.51 - 4.57, m
	Hβ	2.93, dd (14.0, 4.5 Hz) 2.73 - 2.68, m [a]	3.07, dd (14.1, 4.4 Hz) 2.78 - 2.71, m [a]	3.17, dd (14.0, 4.6 Hz) 3.12 - 3.07, m [a]	3.06 - 3.01, m [a] 2.76 - 2.71, m [a]
	Hδ (Ar.)	7.04, d (8.5 Hz)	7.12, d (8.4 Hz)	7.06, d (8.5 Hz)	7.13, d (8.4 Hz)
	Hε (Ar.)	6.64, d (8.4 Hz)	6.84, d (8.4 Hz)	6.87, d (8.4 Hz)	6.84, d (8.4 Hz)
	tBu	N/A	1.25, s	1.25, s	1.25, s
Arg	NH	7.85, d (8.2 Hz)	7.79 - 7.63, m [a]	7.70 - 7.64, m [a]	7.85 - 7.80, m [a]
	Hα	4.33 - 4.27, m [a]	4.27, q (6.4 Hz)	4.38, q (7.2 Hz)	4.32 - 4.26, m [a]
	Hβ	1.77 - 1.65, m [a] 1.55 - 1.45, m [a]	1.62 - 1.57, m [a] 1.55 - 1.47, m [a]	1.71 - 1.65, m [a] 1.51 - 1.46, m [a]	1.71 - 1.65, m [a] 1.43 - 1.38, m [a]
	Hγ	1.55 - 1.45, m [a]	1.43 - 1.35, m [a]	1.44 - 1.38, m [a]	1.52 - 1.45, m [a]
	Hδ	3.09, q (6.5 Hz)	3.04 - 2.98, m	3.06 - 3.00, m [a]	3.08 - 2.99, m [a]
	$NH_{terminal}$	7.64, t (5.7 Hz)	6.93 - 6.80, br s	6.77 - 6.67, br s	6.76 - 6.69, br s
	Pbf_C1'Me	N/A	1.40, s	1.41, s	1.40, s
	Pbf_C2'	N/A	2.96, s	2.96, s	2.95, s
	Pbf_C4'Me	N/A	2.42, s	2.42, s	2.43, s
	Pbf_C6'Me	N/A	2.48, s	2.48, s	2.48, s
	Pbf_C7'Me	N/A	2.00, s	2.00, s	2.00, s
Gly	NH	8.08, t (5.9 Hz)	7.98 - 7.93, br s	8.11, t (5.3 Hz)	7.88 - 7.85, m [a]
	Hα	3.77, dd (17.5, 5.8 Hz) 3.72, dd (17.6, 5.9 Hz)	3.63 - 3.55, m [a]	4.02 - 3.97, m [a] 3.58, dd (17.2, 3.9 Hz)	3.94 - 3.88, m [a]

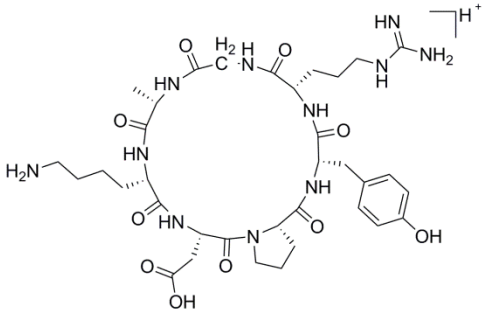
[a:] overlapped signals

^{13}C chemical shifts (ppm)					
Compound:		Linear, unprotected	Linear, protected	Cyclized, protected	Cyclized, protected
Residue	Atom			conformer 1	conformer 2
Ala	C=O	169.4	169.2	172.4	172.3
	Cα	48.1	48.8	50.3	49.8
	Cβ	17.2	18.8	17.1	16.8
Lys	C=O	170.8	170.8	171.0	171.3
	Cα	52.3	52.4	53.4	52.4
	Cβ	31.5	31.9	30.4	31.6
	Cγ	22.2	22.5	22.9	22.6
	Cδ	26.6	27.7	27.7	27.7
	Cε	38.7	39.7	39.4	39.4
	Boc ($C\underline{C}H_3$)	N/A	28.3	28.3	28.3
	Boc ($\underline{C}CH_3$)	N/A	77.4	77.6	77.6
	Boc (C=O)	N/A	155.5	155.5	155.5
Asp	C=O	169.6	169.6	169.2	168.9
	Cα	47.5	47.2	46.9	47.4
	Cβ	35.9	37.4	38.1	37.1
	COO	172.2	170.5	170.5	169.7
	tBu ($C\underline{C}H_3$)	N/A	27.6	27.6	27.6
	tBu ($\underline{C}CH_3$)	N/A	80.6	81.0	80.5
Pro	C=O	171.1	171.1	170.9	171.0
	Cα	59.7	59.4	61.2	59.7
	Cβ	29.0	28.9	28.9	28.8
	Cγ	23.9	24.0	24.4	23.9
	Cδ	46.8	46.8	47.5	46.6
Tyr	C=O	170.9	170.9	171.0	169.4
	Cα	54.4	54.0	56.4	53.7
	Cβ	36.2	36.1	34.7	36.4
	Cγ (Ar.)	127.8	132.6	133.1	132.4
	Cδ (Ar.)	130.1	129.6	129.4	129.6
	Cε (Ar.)	114.9	123.4	123.5	123.4
	Cζ (Ar.)	155.8	153.4	153.4	153.4
	tBu ($C\underline{C}H_3$)	N/A	28.5	28.5	28.5
	tBu ($\underline{C}CH_3$)	N/A	77.6	77.3	77.3
Arg	C=O	171.4	171.6	170.8	171.0
	Cα	51.7	52.2	53.1	52.0
	Cβ	29.3	29.6	28.9	29.1
	Cγ	24.8	24.9	25.2	24.3
	Cδ	40.4	40.4	39.7	39.7
	C=NH	156.8	156.1	156.0	156.0
	Pbf_C1’	N/A	86.3	86.3	86.3
	Pbf_C1’Me	N/A	28.3	28.3	28.3
	Pbf_C2’	N/A	42.5	42.5	42.5
	Pbf_C3’	N/A	124.3	124.3	124.3
	Pbf_C4’	N/A	131.4	131.4	131.4
	Pbf_C4’Me	N/A	19.0	19.0	19.0
	Pbf_C5’	N/A	134.2	135.1	134.1
	Pbf_C6’	N/A	137.3	137.3	137.3
	Pbf_C6’Me	N/A	17.6	17.6	17.6
	Pbf_C7’	N/A	116.2	116.2	116.2
	Pbf_C7’Me	N/A	12.3	12.3	12.3
	Pbf_C8’	N/A	157.4	157.4	157.4
Gly	C=O	171.0	171.0	168.5	166.0
	Cα	40.7	40.4	42.7	40.5

^{1}H NMR spectrum for cyclic unprotected peptide **3d**, cyc(AKDPYRG), recorded at 600 MHz



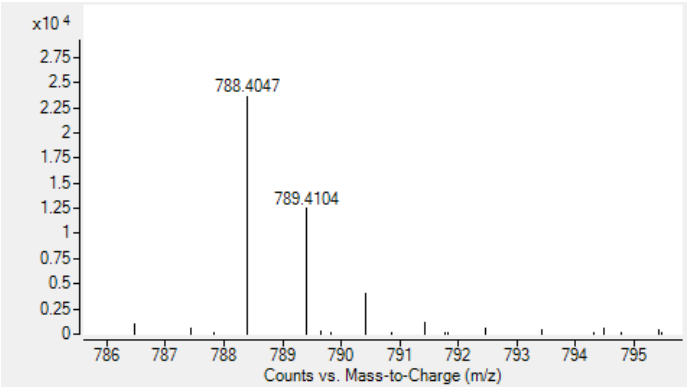
HRMS (+ESI) data for cyclized unprotected peptide **3d**, cyc(AKDPYRG)

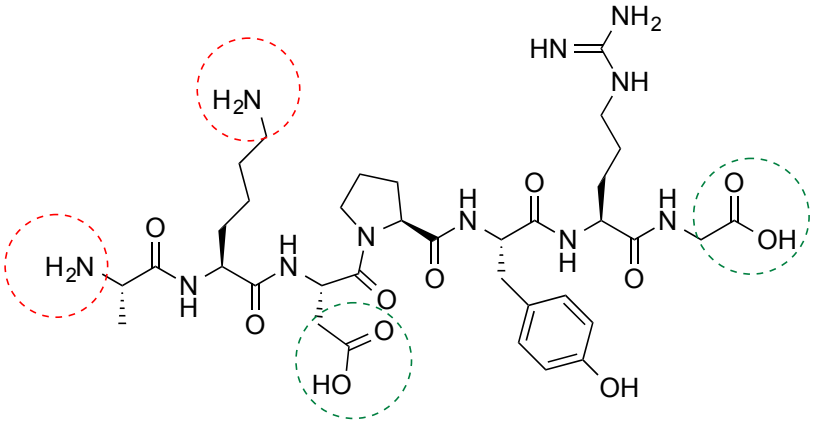


Cyclic Unprotected Peptide

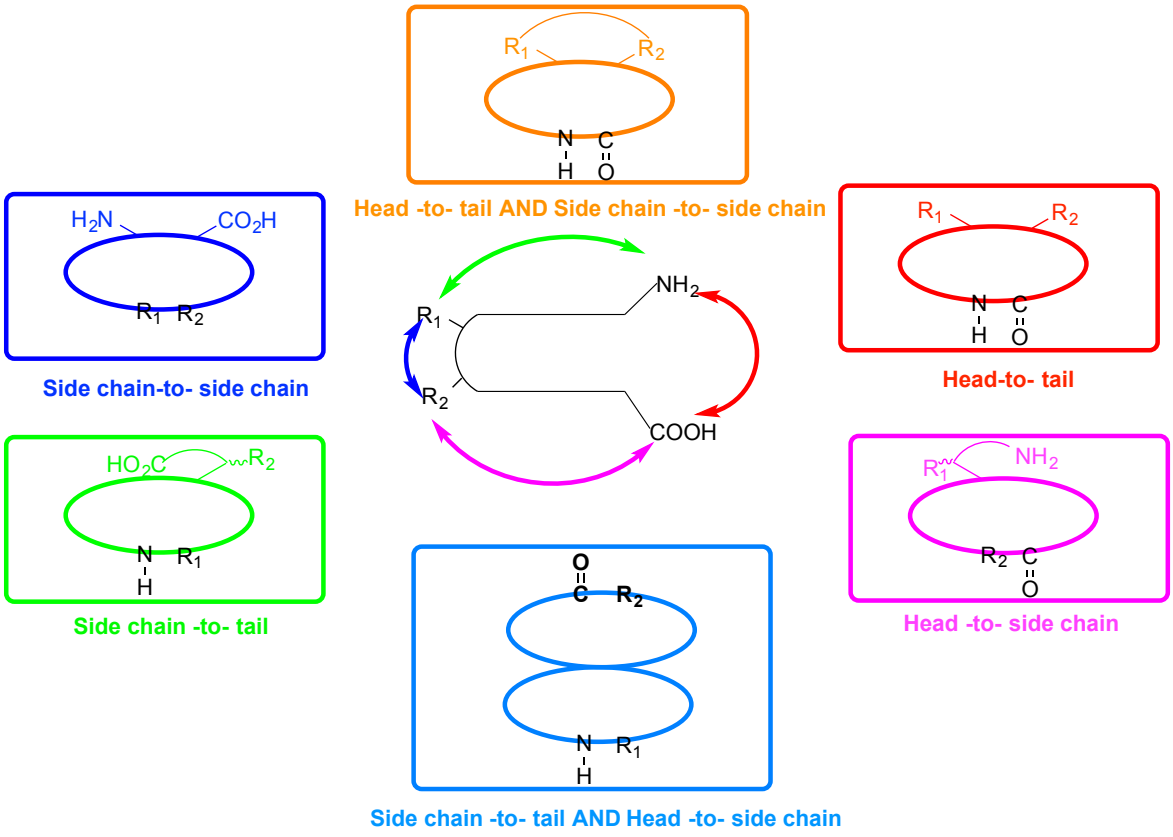
$C_{35}H_{54}N_{11}O_{10}^{+}$

m/z 788.4050





Unprotected Peptide



Various Possibilities of cyclization by using unprotected peptide

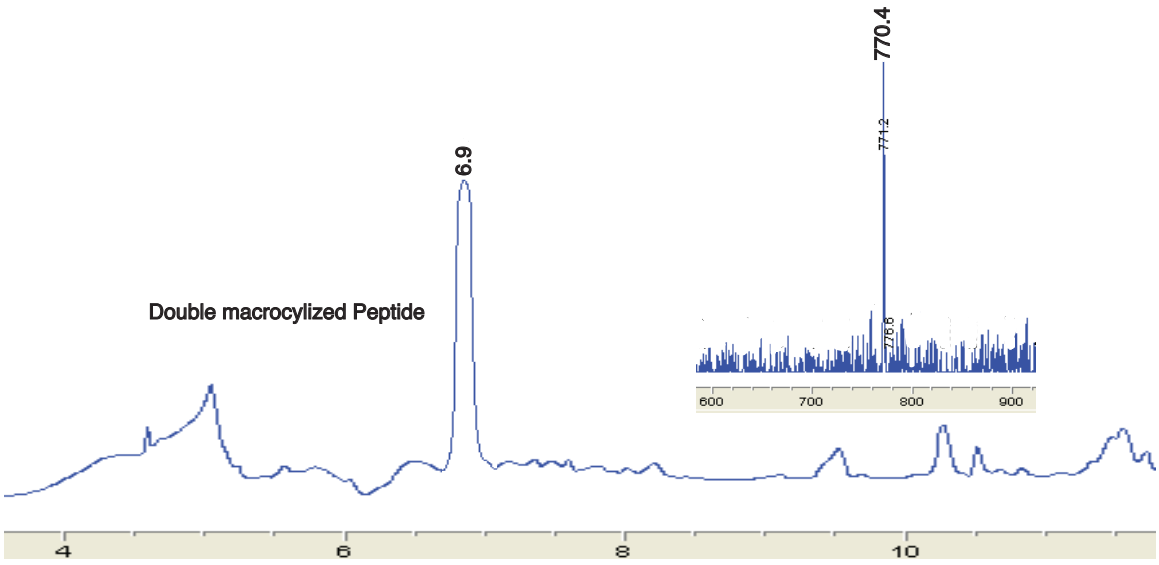
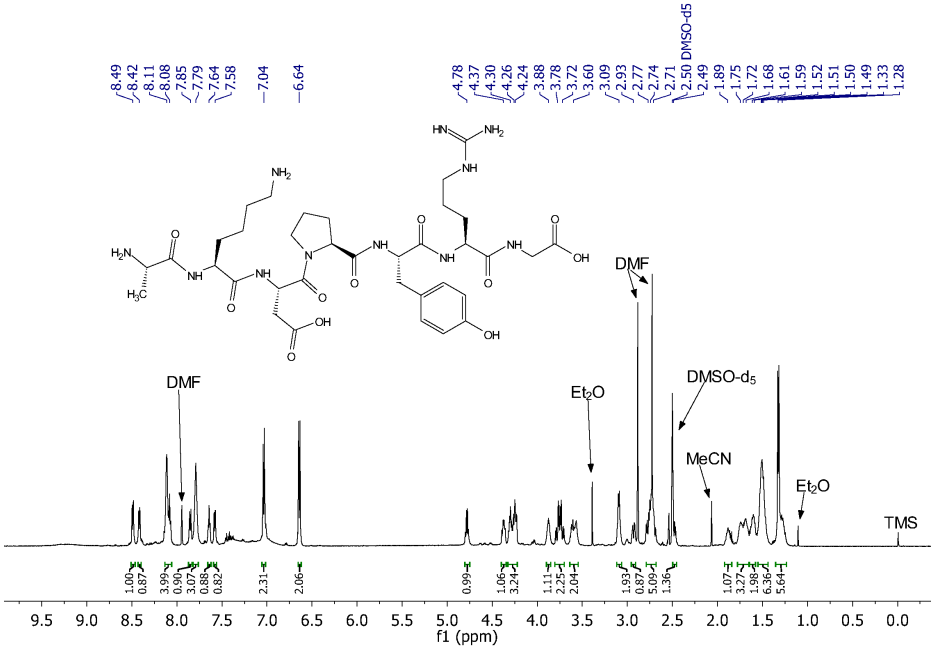
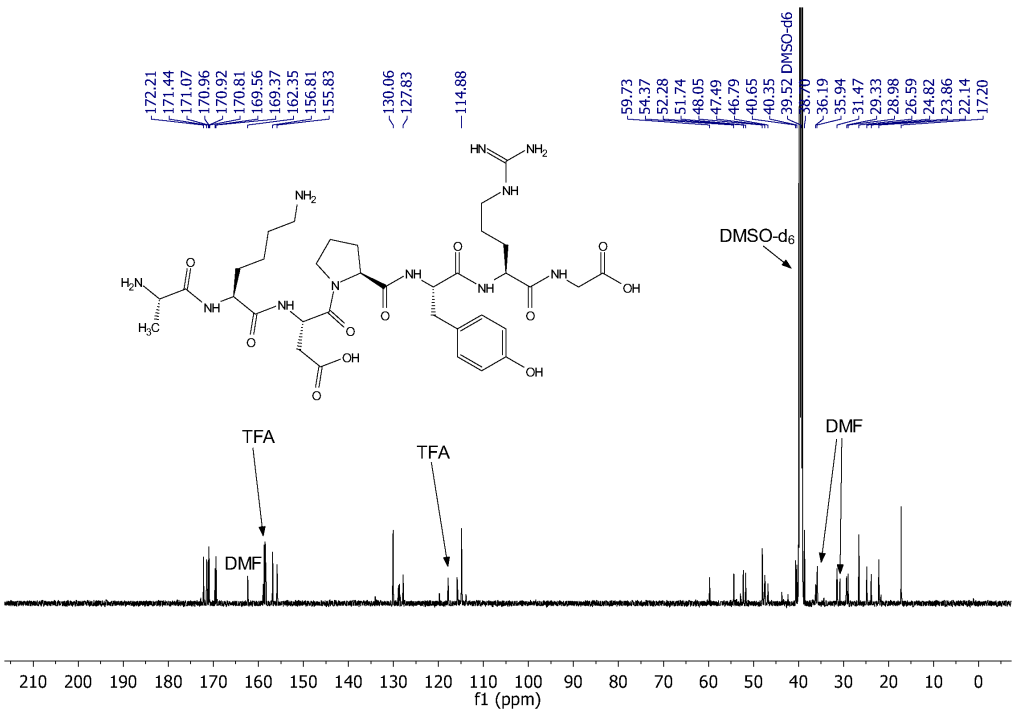


Figure S2. Synthesis of macrocyclic peptide from unprotected linear peptide acid AKDPYRG obtained from Wang resin. Various possibilities of the cyclization of linear unprotected peptide AKDPYRG. Double cyclization of unprotected peptide cyc_2(AKDPYRG) was observed as shown by LCMS.

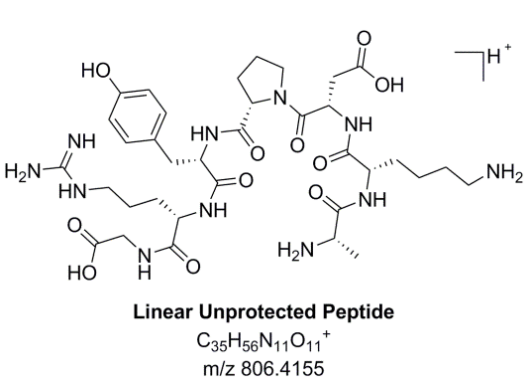
^{1}H NMR spectrum for linear unprotected peptide, AKDPYRG, recorded at 600 MHz

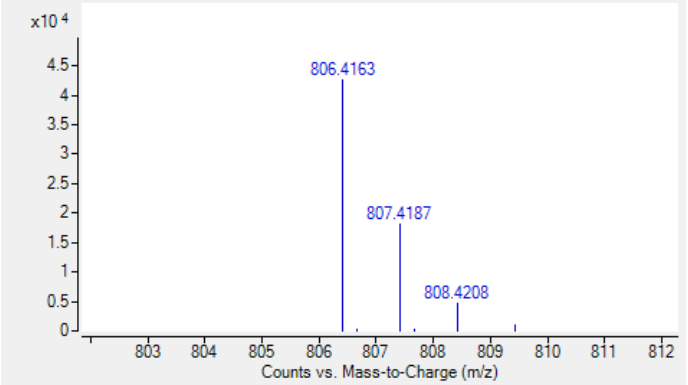


^{13}C NMR spectrum for linear unprotected peptide, AKDPYRG, recorded at 151 MHz

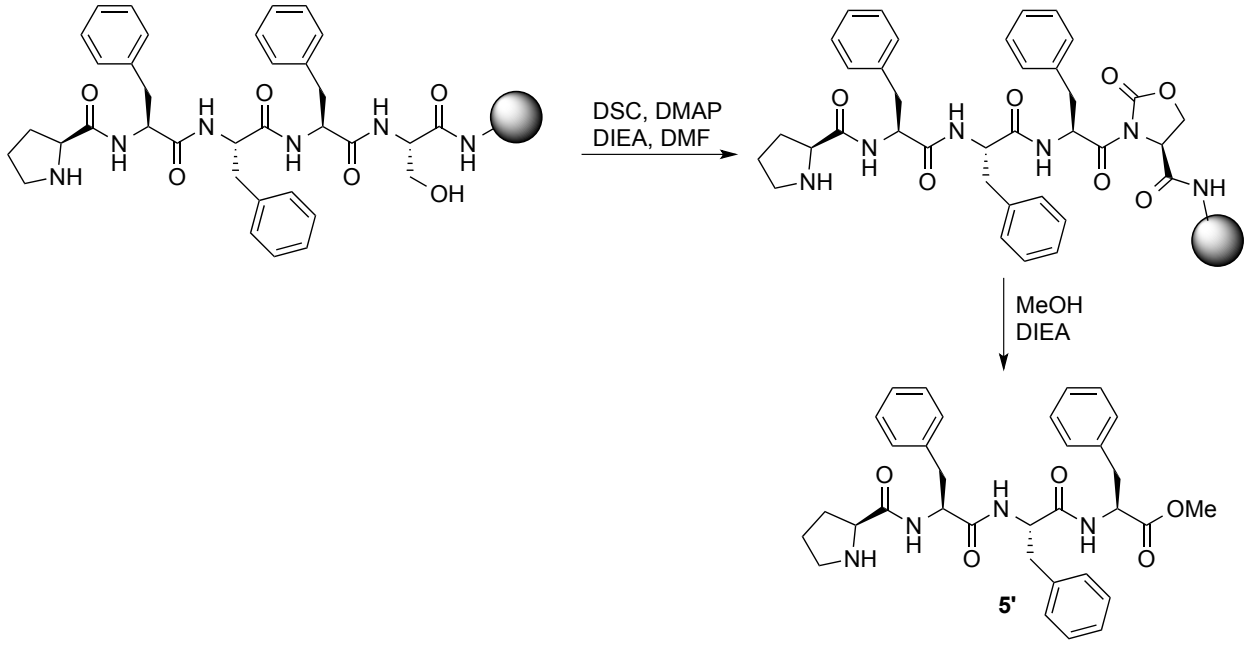


HRMS (+ESI) data for linear unprotected peptide, AKDPYRG





(a)



(b)

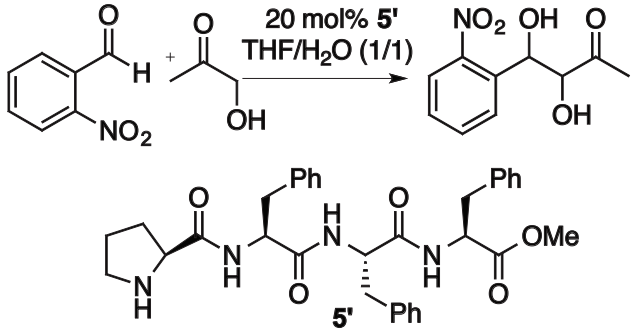
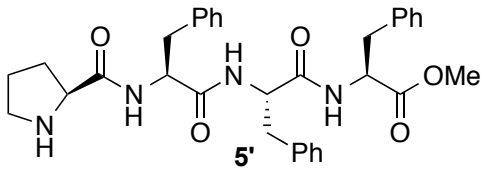


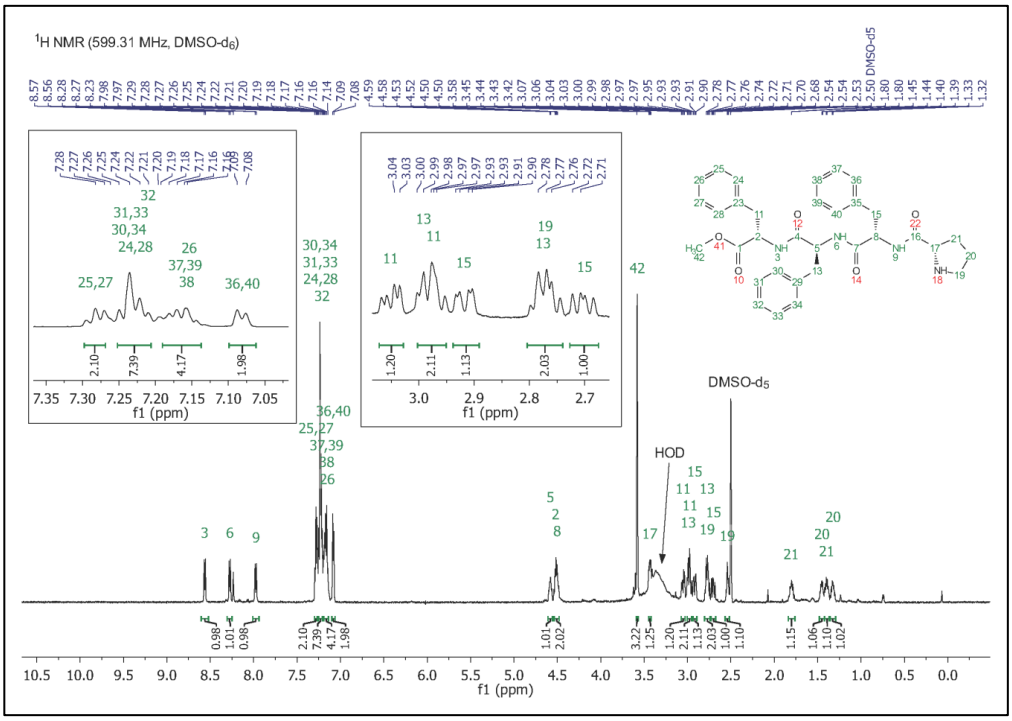
Figure S3. (a) Application of CUT in the synthesis of a catalyst PFFFOMe **5'** (b) Use of PFFFOMe **5'** for catalyzing asymmetric aldol reaction.

Catalyst 5':

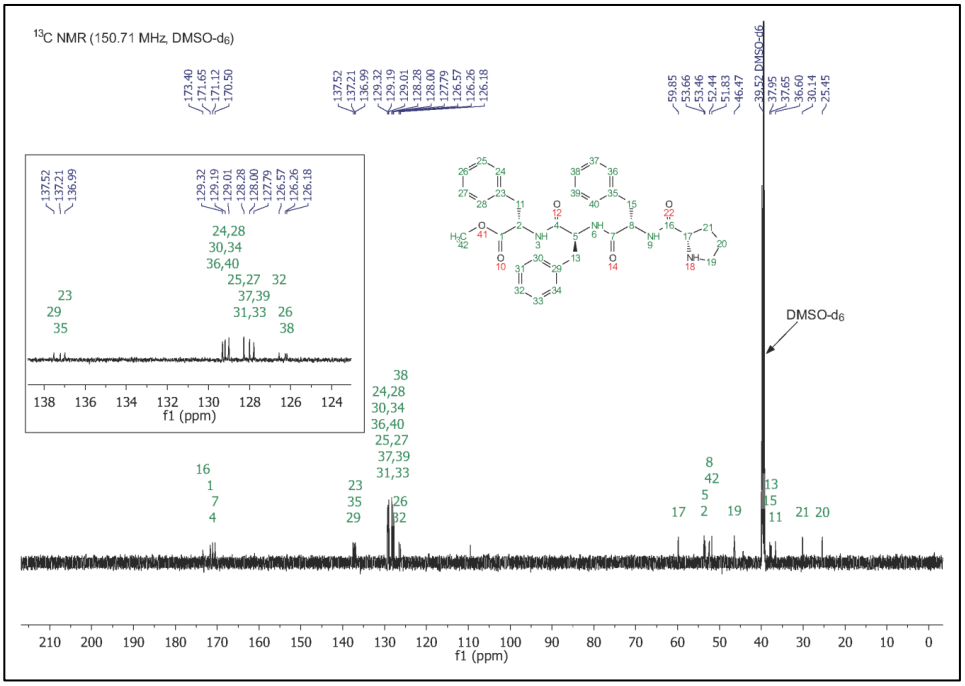


Methyl L-prolyl-L-phenylalanyl-L-phenylalanyl-L-phenylalaninate (5'): ^{1}H NMR (599 MHz, DMSO-d_6) d 8.56 (d, *J* = 7.4 Hz, 1H), 8.28 (d, *J* = 8.4 Hz, 1H), 7.98 (d, *J* = 8.8 Hz, 1H), 7.28 (t, *J* = 7.30 Hz, 2H), 7.26 – 7.20 (om, 7H), 7.20 – 7.12 (om, 4H), 7.08 (d, *J* = 7.2 Hz, 2H), 4.58 (ddd, *J* = 8.40, 8.35, 5.06 Hz, 1H), 4.55 – 4.46 (om, 2H), 3.58 (s, 3H), 3.43 (dd, *J* = 8.4, 4.8 Hz, 1H), 3.05 (dd, *J* = 14.0, 6.0 Hz, 1H), 3.01 – 2.94 (om, 2H), 2.92 (dd, *J* = 13.9, 4.4 Hz, 1H), 2.81 – 2.74 (om, 2H), 2.70 (dd, *J* = 13.8, 8.9 Hz, 1H), 2.57 – 2.51 (m, 1H), 1.86 – 1.74 (m, 1H), 1.49 – 1.36 (om, 2H), 1.36 – 1.29 (m, 1H). ^{13}C NMR (151 MHz, DMSO-d_6) d 173.4, 171.7, 171.1, 170.5, 137.5, 137.2, 137.0, 129.3, 129.2, 129.0, 128.3, 128.0, 127.8, 126.6, 126.3, 126.2, 59.9, 53.7, 53.5, 52.4, 51.8, 46.5, 38.0, 37.7, 36.6, 30.1, 25.5. HRMS (+ESI) calc. for $C_{33}H_{39}N_4O_5^+$: 571.2915, found 571.2925.

^{1}H NMR spectrum for catalyst PFFFOMe 5', recorded at 600 MHz

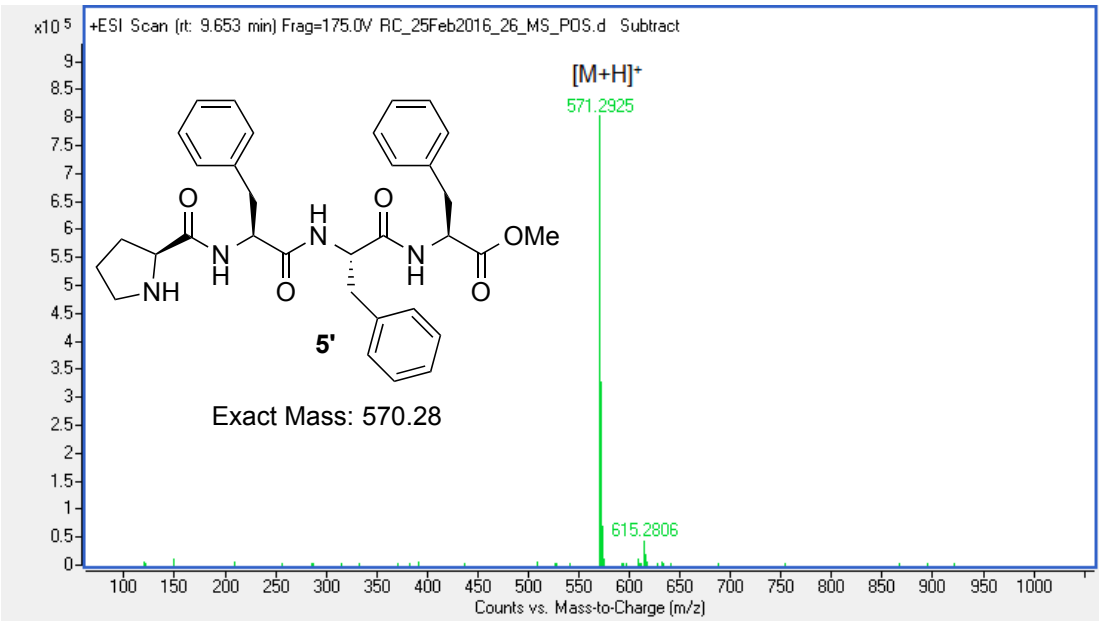


^{13}C NMR spectrum for catalyst PFFFOMe 5', recorded at 151 MHz

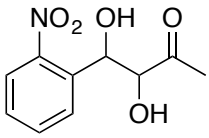


HRMS (+ESI) data for catalyst PFFFOMe 5'

HRMS:



Aldol Product:

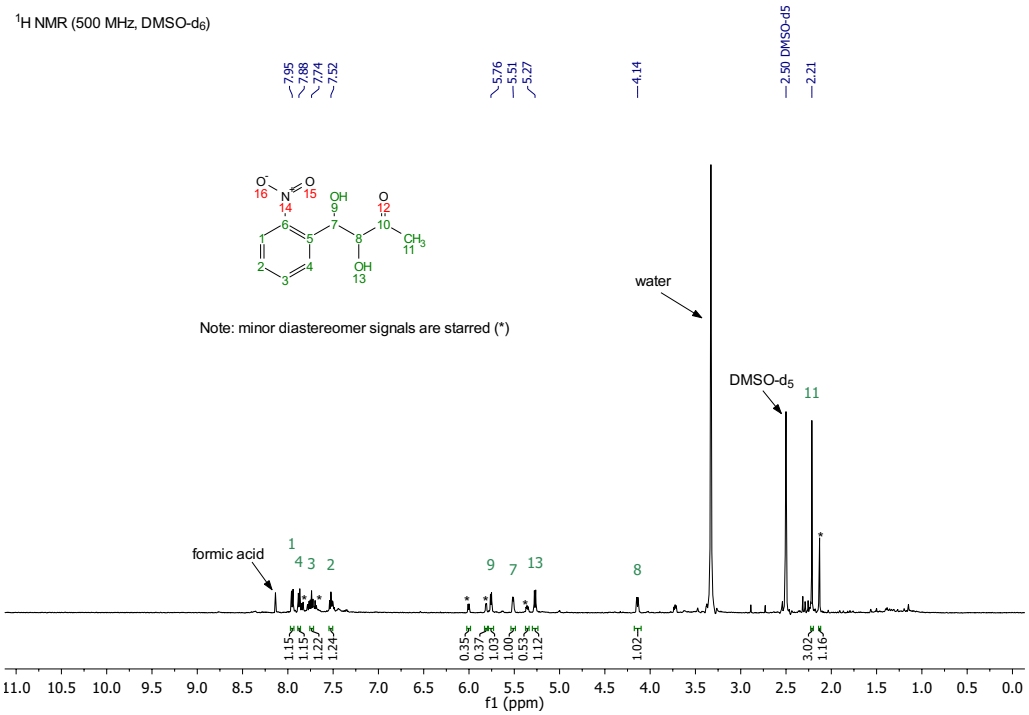


The molar ratio of major to minor diastereomers is 1.00 : 0.36.

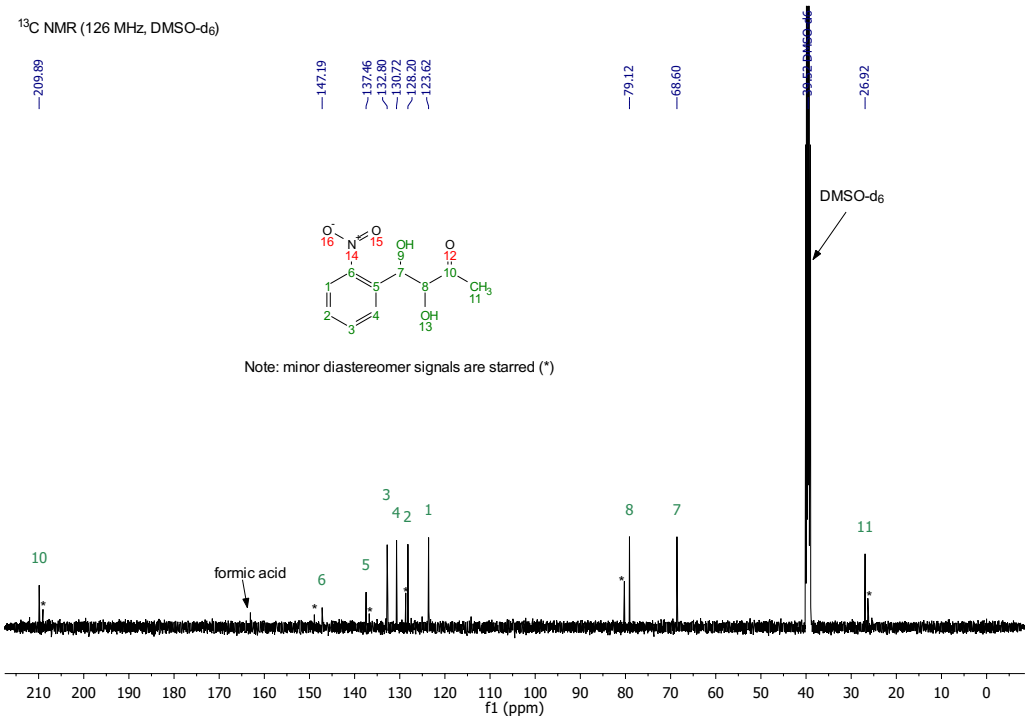
3,4-dihydroxy-4-(2-nitrophenyl)butan-2-one (major): ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.84 (dd, J = 8.1, 1.2 Hz, 1H), 7.77 (dd, J = 8.0, 1.4 Hz, 1H), 7.70 (dt, J = 7.7, 1.2 Hz, 1H), 7.50 (dt, J = 7.7, 1.5 Hz, 1H), 6.00 (d, J = 5.4 Hz, 1H), 5.80 (d, J = 5.5, 1H), 5.36 (dd, J = 7.3, 5.3 Hz, 1H), 3.73 (dd, J = 7.5, 5.5 Hz, 1H), 2.13 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 209.0, 148.9, 136.8, 132.6, 128.7, 128.2, 123.6, 80.2, 68.5, 26.3. HRMS (+ESI) calc. for $C_{10}H_{12}NO_5^+$: 226.0710, found 226.0715.

3,4-dihydroxy-4-(2-nitrophenyl)butan-2-one (minor): ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.95 (dd, J = 8.1, 1.1 Hz, 1H), 7.88 (dd, J = 7.9, 1.3 Hz, 1H), 7.74 (dt, J = 7.7, 0.9 Hz, 1H), 7.52 (dt, J = 7.9, 1.3 Hz, 1H), 5.76 (d, J = 5.8 Hz, 1H), 5.51 (dd, J = 5.6, 2.3 Hz, 1H), 5.27 (d, J = 7.3 Hz, 1H), 4.14 (dd, J = 7.3, 2.3 Hz, 1H), 2.21 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 209.9, 147.2, 137.5, 132.8, 130.7, 128.2, 123.6, 79.1, 68.6, 26.9. HRMS (+ESI) calc. for $C_{10}H_{12}NO_5^+$: 226.0710, found 226.0697.

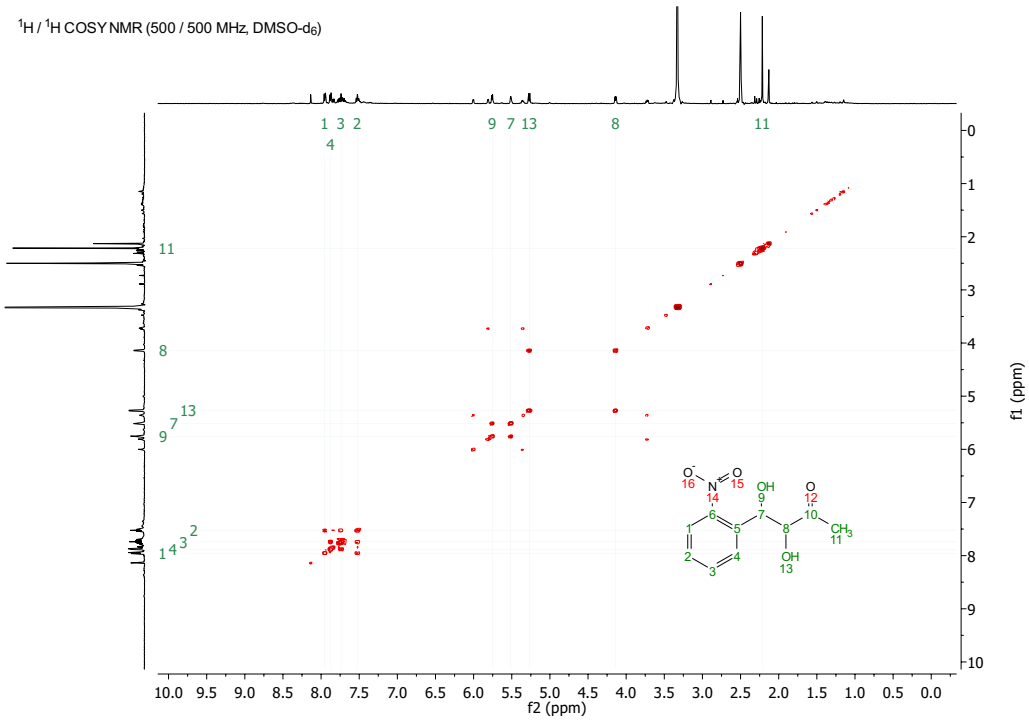
^{1}H NMR spectrum for Aldol product, recorded at 500 MHz



^{13}C NMR spectrum for Aldol product, recorded at 126 MHz



^{1}H/^{1}H COSY NMR spectrum for Aldol product, recorded at 500 MHz



(a)

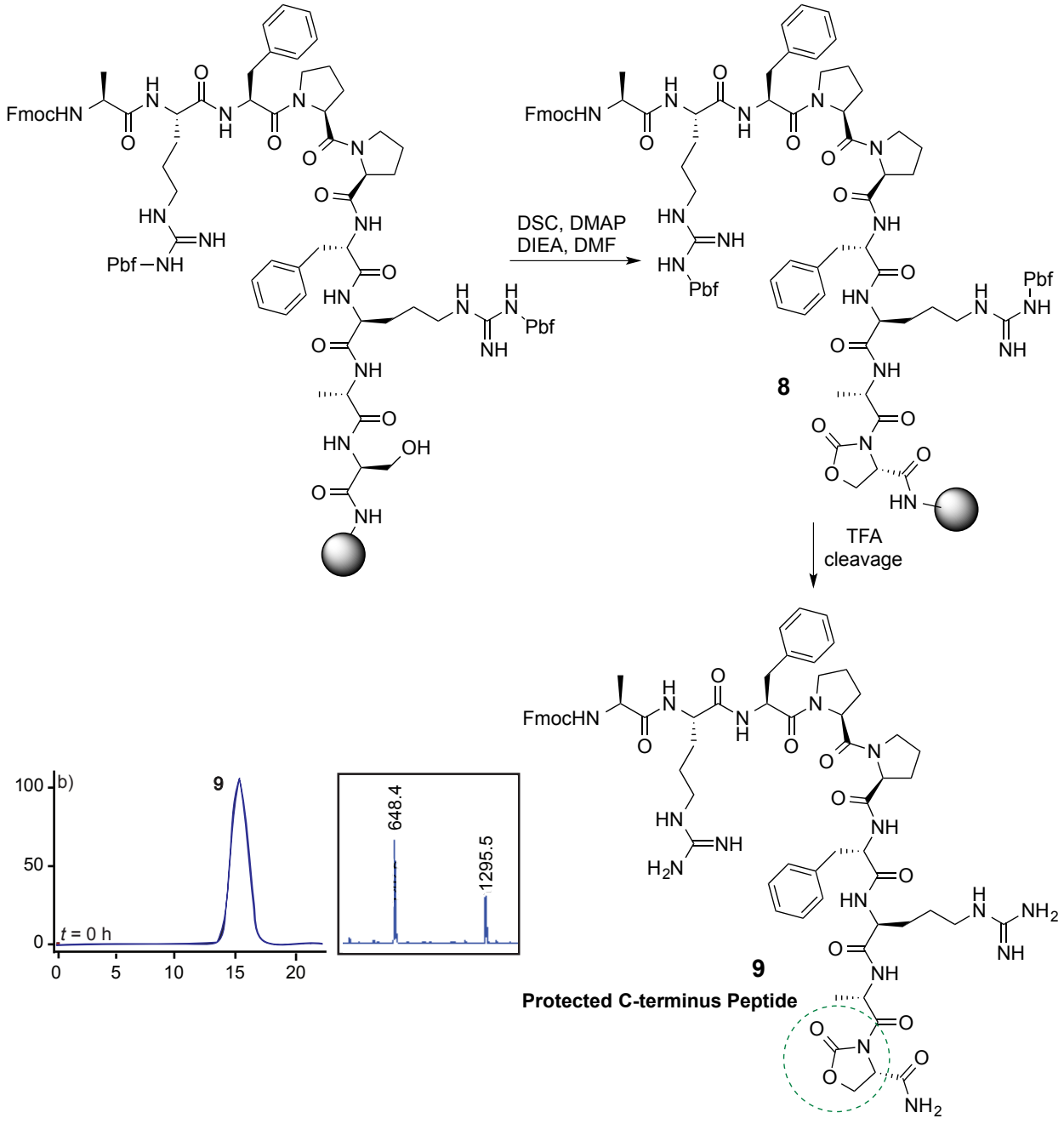
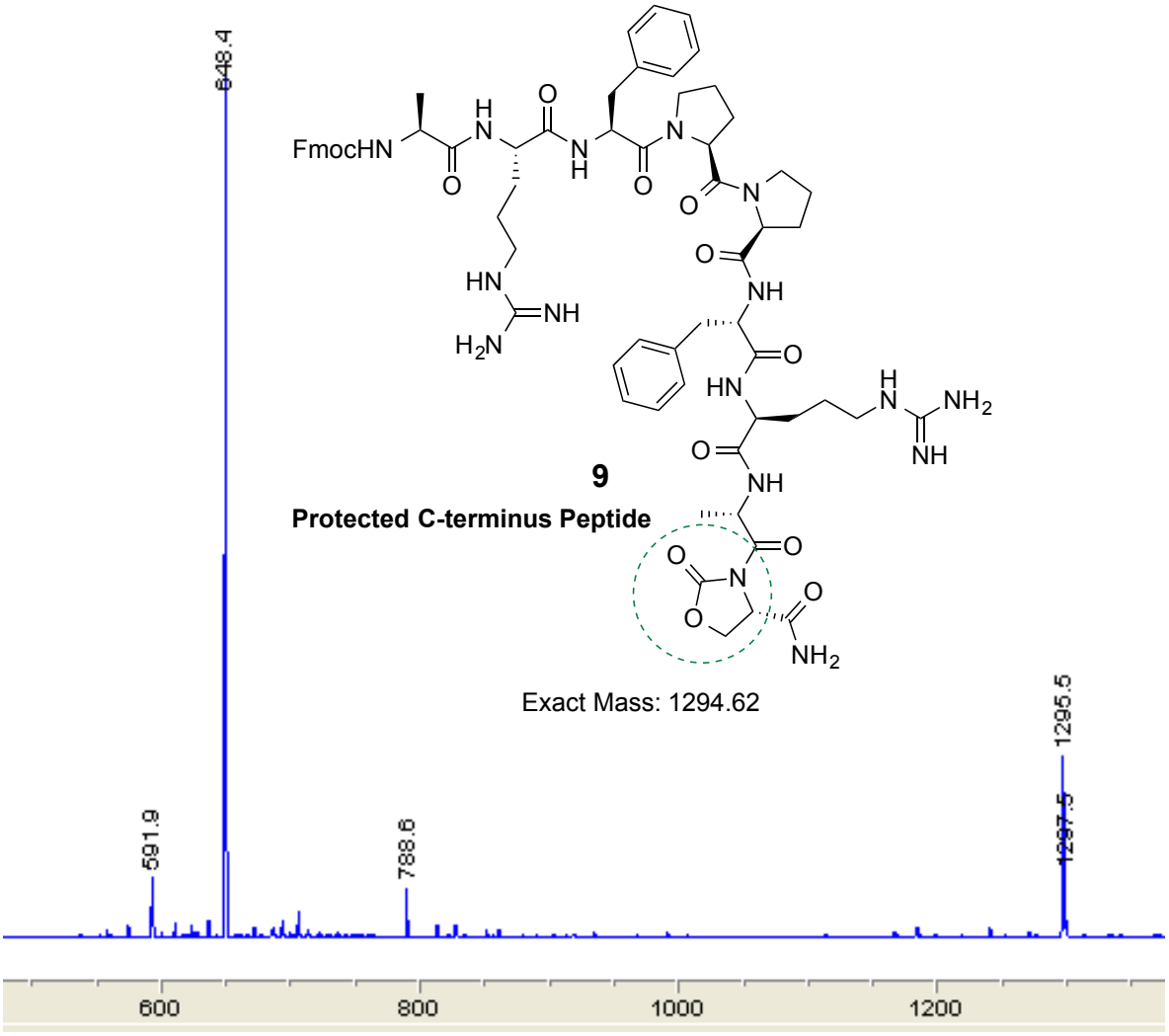
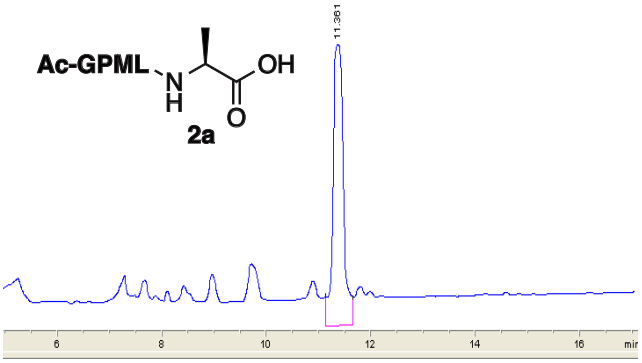


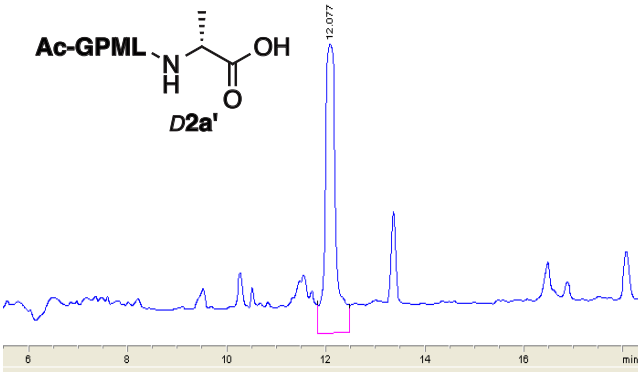
Figure S4. (a) Synthesis of peptide Fmoc-ARFPPFRAOxd, **9** with semi-permanent protecting group on the C-terminus using cyclic urethane technique (CUT). (b) HPLC and MS chromatogram of C-terminal protected peptide Fmoc ARFPPFRA-Oxd, **9.**

MS data for protected C-terminal peptide Fmoc-ARFPPFRA-Oxd, **9**.



HPLC traces of (a) crude peptide acid 2a Ac-GPMLA-(L)-OH (b) crude diastereoisomer $_D$2a' Ac-GPML-A-(D)-OH





Methods

I. General. All commercial materials (Aldrich, Fluka, Nova) were used without further purification. All solvents were reagent grade or HPLC grade (Fisher). Anhydrous THF, diethyl ether, CH_2Cl_2, and DMF were obtained from a dry solvent system (passed through column of alumina) and used without further drying. All reactions were performed under air in round bottom flask. Yields refer to chromatographically pure compounds; % yield were obtained by comparison of HPLC peak areas of products and starting material. HPLC was used to monitor the reaction progress.

Materials. Fmoc-amino acids were obtained from Nova Biochem is under (EMD Millipore Corporation)(Billerica, Massachusetts) and CreoSalus (Louisville, Kentucky). Rink amide, Wang, Chem Matrix and TentaGel resins were obtained from ChemPep Inc (Wellington, Florida). N,N,N′,N′-Tetramethyl-O-(1H-benzotriazol-1-yl)uronium hexafluorophosphate (HBTU) was obtained from CreoSalus (Louisville, Kentucky). N,N′-Disuccinimidyl carbonate (DSC) was obtained from Nova Biochem, under (EMD Millipore Corporation) (Billerica, Massachusetts). 4-Dimethylaminopyridine (DMAP): Merck KGaA (Darmstadt, Germany). N,N-Dimethylformamide (DMF): Macron Fine Chemicals (Center Valley, Pennsylvania). Dichloroethane (DCE), acetonitrile, N,N- Diisopropylethylamine (DIEA), N,N'-diisopropylcarbodiimide (DIC), 1-Hydroxy-7-azabenzotriazole (HOAt), were purchased from (EMD Millipore Corporation)(Billerica, Massachusetts). Piperidine was purchased from Alfa Aesar (Ward Hill, Massachusetts). Trifluoroacetic acid (TFA) was purchased from VWR 100 Matsonford RoadRadnor, PA. Diethyl Ether: Sigma Aldrich (St. Louis, Missouri). Water was purified using a Millipore MilliQ water purification system.

NMR:

Proton NMR spectra were acquired at 25 °C in DMSO-d_6 using an Agilent DD2 (600 MHz) spectrometer and carbon NMR spectra on a 151 MHz equipped with a 3-mm He HCN cryoprobe. All NMR chemical shifts (δ) are referenced in ppm relative to residual solvent or internal tetramethylsilane. ^{1}H NMR chemical shifts referenced to residual DMSO-d_5 at 2.50 ppm, and ^{13}C NMR chemical shifts referenced to DMSO-d_6 at 39.52 ppm. Carbon NMR spectra are proton decoupled. NMR spectral data are reported as chemical shift (multiplicity, coupling constants (*J*), integration). Multiplicity is reported as follows: singlet (s), broad singlet (bs), doublet (d), doublet of doubles (dd), doublet of triplet (td), triplet (t) and multiplet (m). Coupling constant (*J*) in Hertz (Hz).

HPLC

Semi-Preparative HPLC:

Preparative HPLC chromatography (HPLC) was performed on Beckman Coulter equipped with System Gold 168 detector and 125P solvent module HPLC with a 10 mm C-18 reversed-phase column. All separations involved a mobile phase of 0.1% FA (v/v) in water (solvent A) and 0.1% FA (v/v) in acetonitrile (solvent B). Semi-preparative HPLC method using a linear gradient of 0–80% acetonitrile in 0.1% aqueous FA over 30 min at room temperature with a flow rate of 3.0

mL min^{-1}. The eluent was monitored by absorbance at 220 nm and 254 nm unless otherwise noted.

Analytical HPLC:

Analytical HPLC chromatography (HPLC) was performed on an Agilent 1100 series HPLC equipped with a 4.6 mm C-18 reversed-phase column. All separations involved mobile phase of 0.1% FA (v/v) in water (solvent A) and 0.1% FA (v/v) in acetonitrile (solvent B). Peptide compositions were evaluated by analytical reverse phase HPLC using a gradient of 0.1% FA in acetonitrile versus 0.1% FA in water. Analytical HPLC method using a linear gradient of 0–80% 0.1% FA (v/v) acetonitrile in 0.1% aqueous FA over 30 min at room temperature with a flow rate of 1.0 mL min^{-1}. The eluent was monitored by absorbance at 220 nm unless otherwise noted.

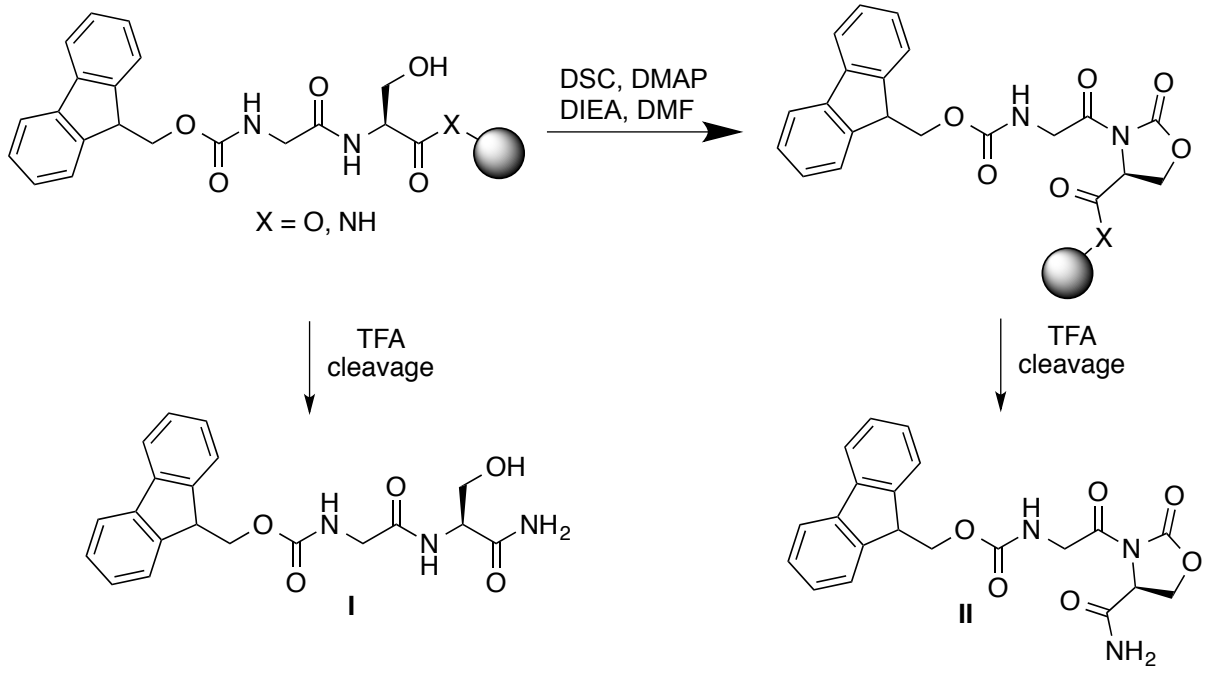
LCMS:
Mass spectrometry was performed using ultra high performance liquid chromatography-mass spectrometry using the Agilent 1100 Series LCMSD VL MS Spectrometer.

HRMS:

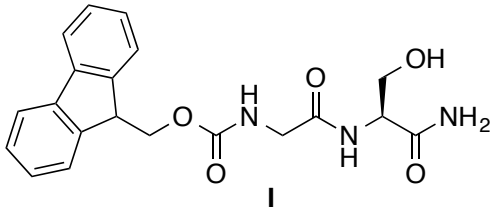
HRMS data were acquired on a Q-ToF mass spectrometer using positive polarity electrospray ionization (+ESI). Tandem MS experiments were performed using collision induced dissociation (CID) with N_2 as the collision gas.

Fmoc Solid-Phase Peptide Synthesis.[1] Peptides were synthesized manually on a 0.25 mm scale using Rink amide, Wang, Chem Matrix and TentaGel resins. Fmoc–group was deprotected using 20% piperidine–DMF for 20 min to obtain a deprotected peptide-resin. Fmoc-protected amino acids (1.25 mm) were sequentially coupled on the resin using a HBTU (1.25 mm) and DIEA (1.25 mm) for 2 h at room temperature. Peptides were synthesized using standard protocols.[1] The peptide was cleaved from the resin using a cocktail of 95:2.5:2.5, trifluoroacetic acid:triisopropyl silane:water for 2 h. The resin was removed by filtration and the resulting solution was concentrated. The oily residue was triturated with diethyl ether to obtain a white suspension. The resulting solid was purified by HPLC.

(II) Serine Cyclization and NMR Spectra:

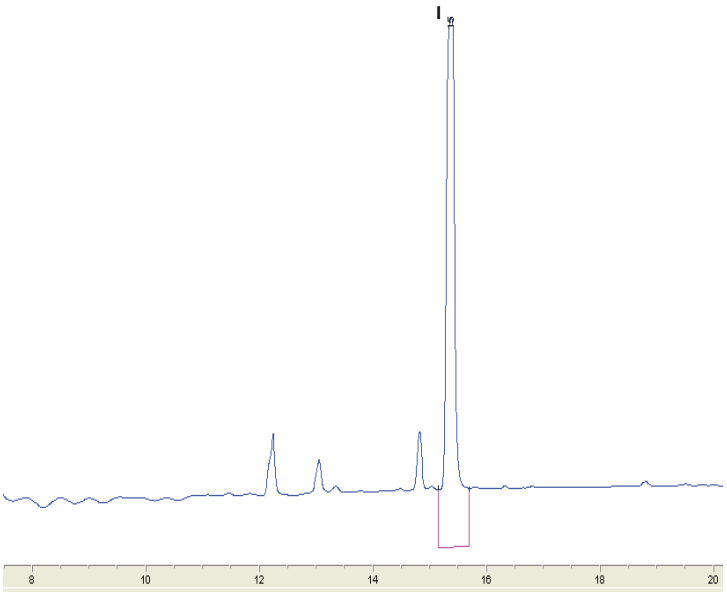


General procedure for the activation of serine to cyclic urethane moiety on solid support: To a peptide on the solid support (25-100 mg (0.25-0.69 mm/g) was added a solution of DSC (15 equiv.), DIEA (15 equiv.) and catalytic amount of DMAP in dimethylformamide (DMF). The resin was left on shaker for 17 h. The solution was drained and resin was washed with DMF followed by cleavage using a cocktail of 95:2.5:2.5, trifluoroacetic acid:triisopropyl silane:water for 2 h. The resin was removed by filtration and the resulting solution was concentrated. The oily residue was triturated with diethyl ether to obtain a white suspension. The resulting solid was purified by HPLC for analysis by MS and NMR.



I

HPLC Trace: Retention time = 15.37, Purity: >95% (HPLC analysis at 254 nm)



HRMS: *m/z* 384.155, (calcd $[M+H]^+$ = 384.148)

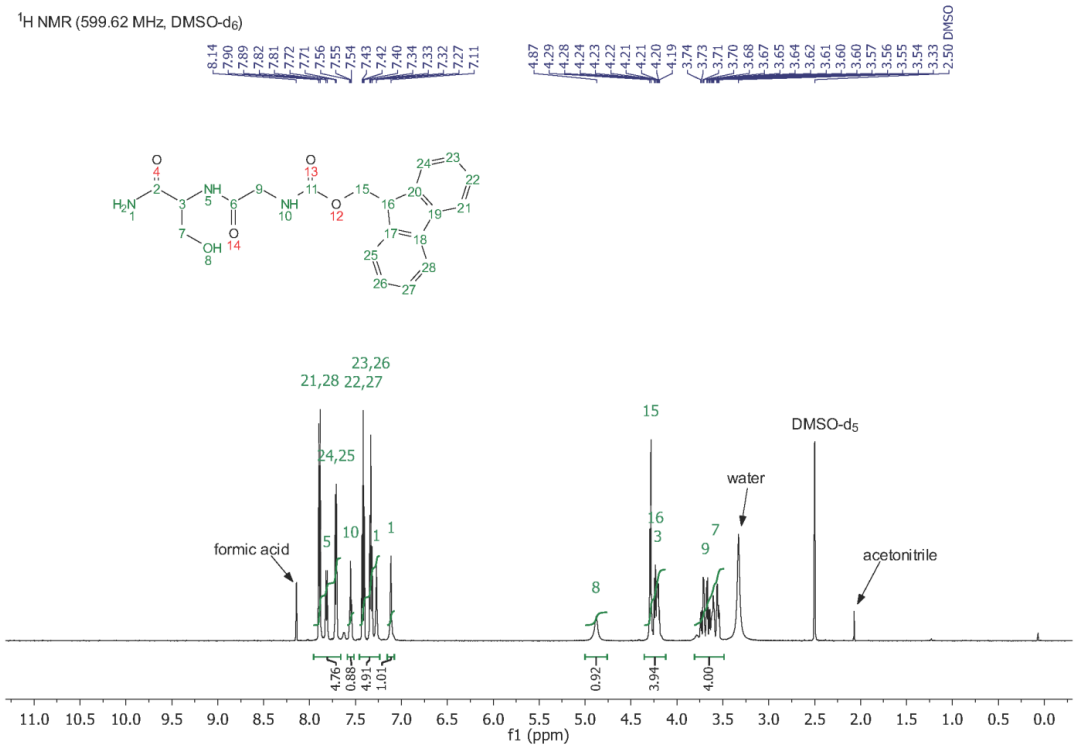
NMR

^{1}H NMR chemical shifts referenced to residual DMSO-d_6 at 2.50 ppm, and ^{13}C NMR chemical shifts referenced to DMSO-d_6 at 39.52 ppm.

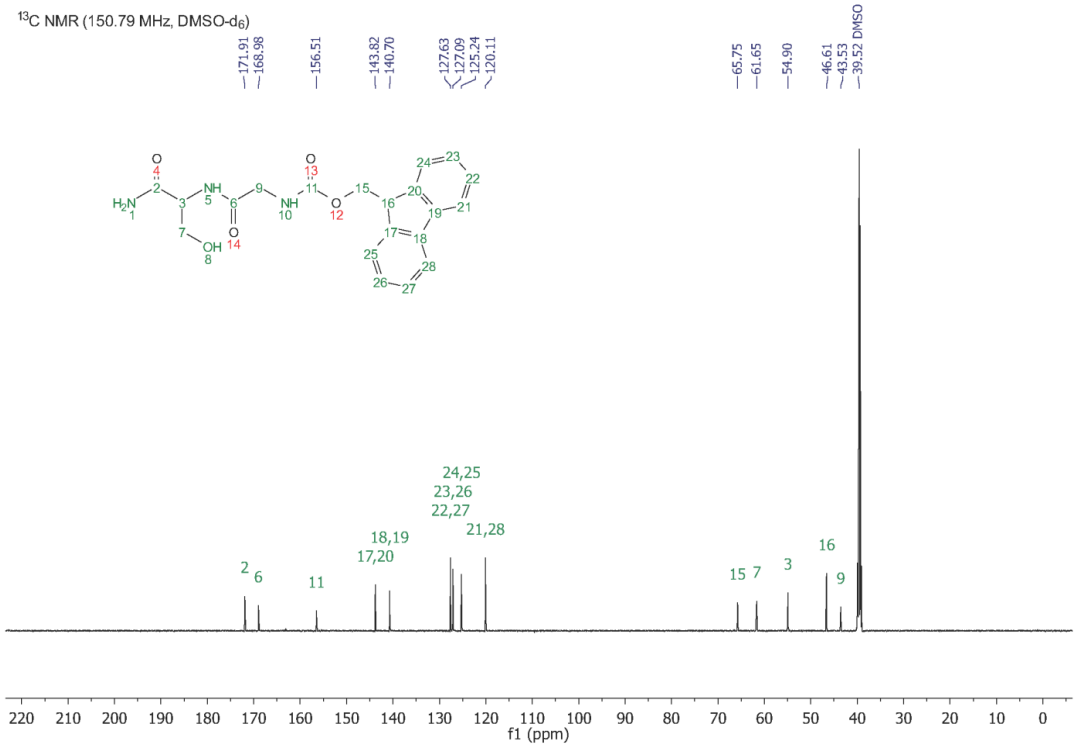
^{1}H NMR: (600 MHz, DMSO-d_6) δ 7.89 (d, J = 7.5 Hz, 2H), 7.81 (d, J = 8.0 Hz, 1H), 7.71 (d, J = 7.5 Hz, 2H), 7.55 (t, J = 6.0 Hz, 1H), 7.42 (t, J = 7.4 Hz, 2H), 7.33 (t, J = 7.4 Hz, 2H), 7.27 (s, 1H), 7.11 (s, 1H), 4.87 (br s, 1H), 4.29 (d, J = 6.8 Hz, 2H), 4.26 – 4.17 (om, 2H), 3.72 (dd, J = 16.8, 6.0 Hz, 1H), 3.66 (dd, J = 16.8, 6.0 Hz, 1H), 3.61 (dd, J = 10.5, 5.4 Hz, 1H), 3.55 (dd, J = 10.5, 4.8 Hz, 1H).

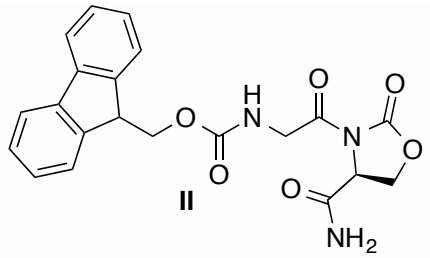
^{13}C NMR: (151 MHz, DMSO-d_6) δ 171.9, 169.0, 156.5, 143.8, 140.7, 127.6, 127.1, 125.2, 120.1, 65.8, 61.7, 54.9, 46.6, 43.5.

^{1}H NMR (599.62 MHz, DMSO-d$_6$)

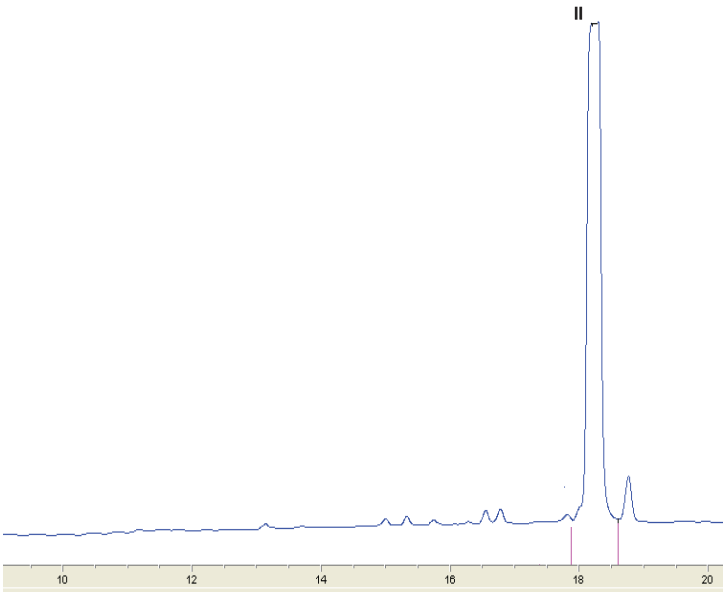


^{13}C NMR (150.79 MHz, DMSO-d$_6$)





HPLC Trace: Retention time = 18.3 min, Purity: >95% (HPLC analysis at 254 nm)



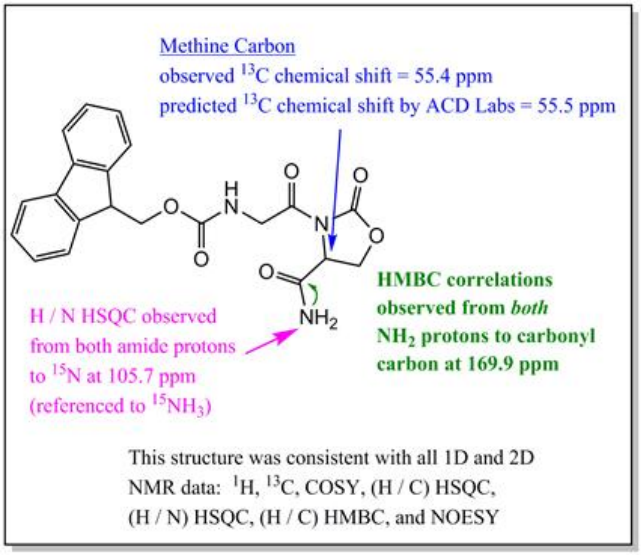
HRMS: *m/z* 432.116, (calcd $[M+Na]^+$ = 432.121)

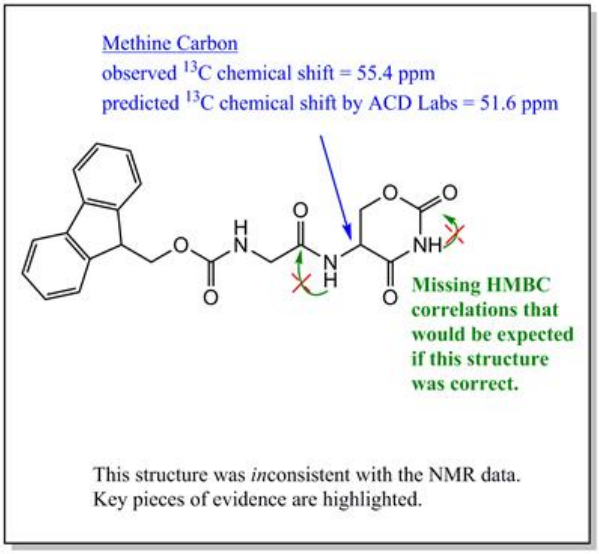
NMR

^{1}H NMR chemical shifts referenced to residual DMSO-d_6 at 2.50 ppm, and ^{13}C NMR chemical shifts referenced to DMSO-d_6 at 39.52 ppm.

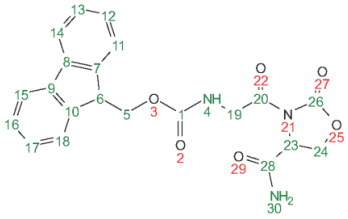
^{1}H NMR (600 MHz, DMSO-d_6) δ 7.90 (d, J = 7.5 Hz, 2H), 7.75 (s, 1H), 7.72 (d, J = 7.4 Hz, 2H), 7.64 (t, J = 6.0 Hz, 1H), 7.42 (t, J = 7.5 Hz, 2H), 7.39 (s, 1H), 7.33 (t, J = 7.4 Hz, 2H), 4.73 (dd, J = 9.1, 3.1 Hz, 1H), 4.60 (t, J = 9.0 Hz, 1H), 4.36 – 4.15 (m, 6H).

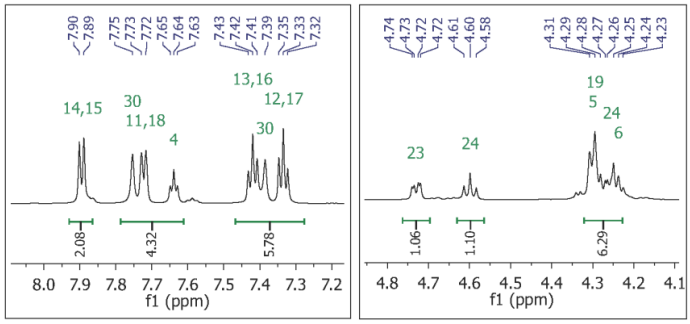
^{13}C NMR (151 MHz, DMSO-d_6) δ 169.9, 169.1, 156.4, 153.5, 143.8, 140.7, 127.6, 127.1, 125.2, 120.1, 66.8, 65.8, 55.4, 46.6, 44.1.

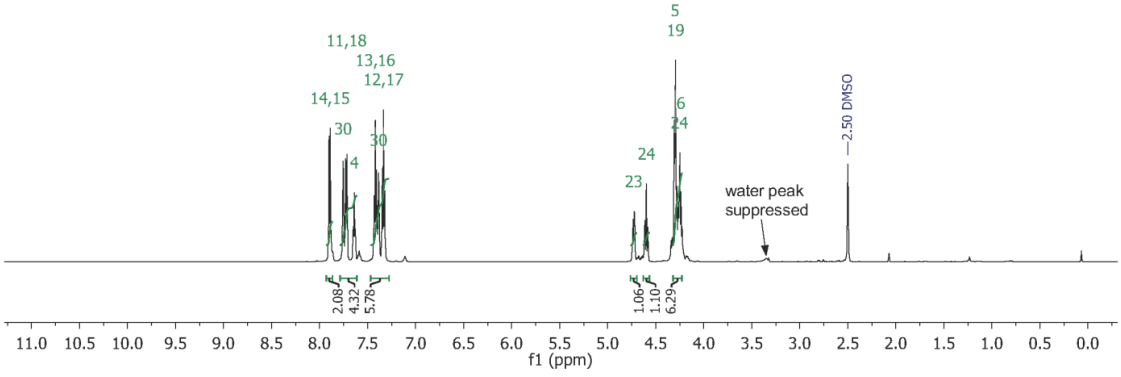


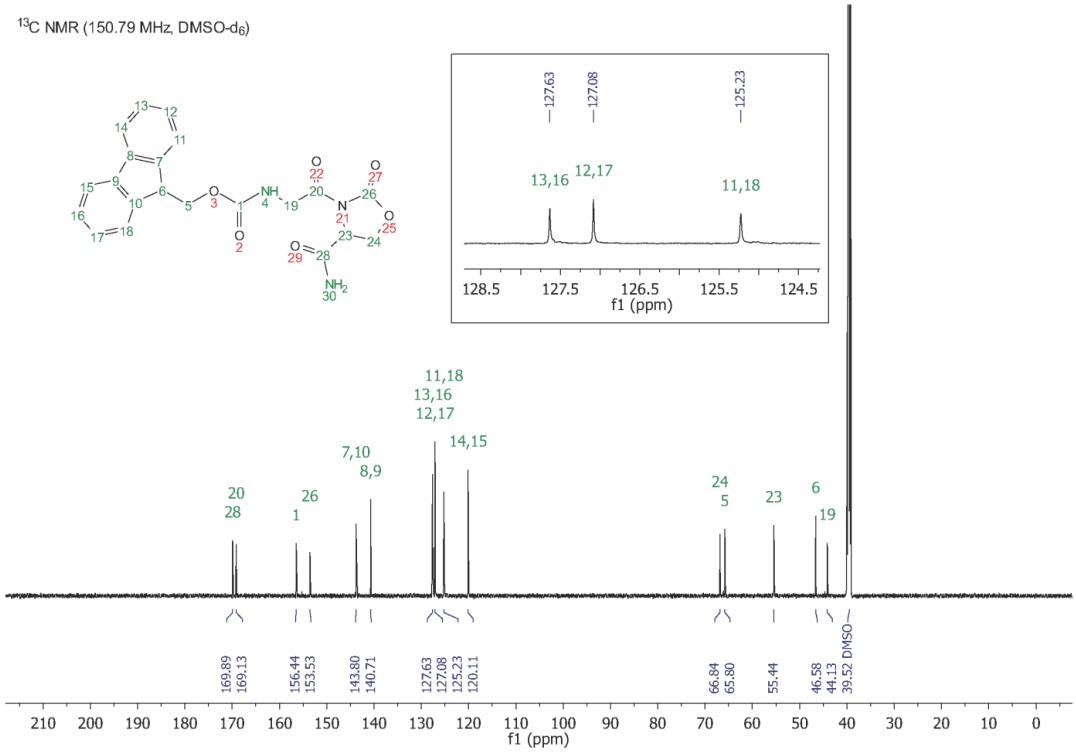


^{1}H NMR (599.62 MHz, DMSO-d_6)
presaturation used for water suppression

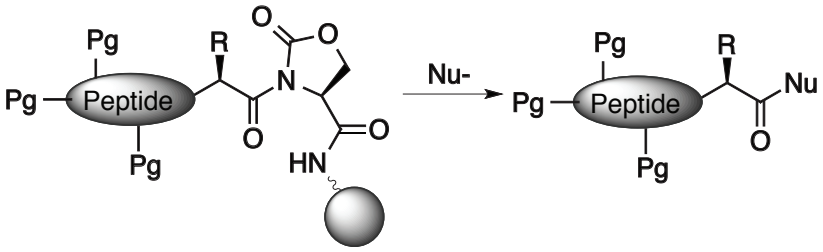







13C NMR (150.79 MHz, DMSO-d6)
127.63
127.08
125.23
13,16
12,17
11,18
128.5
127.5
126.5
125.5
124.5
f1 (ppm)
11,18
13,16
12,17
14,15
7,10
8,9
20
28
26
1
24
5
23
6
19
169.89
169.13
156.44
153.53
143.80
140.71
127.63
127.08
125.23
120.11
66.84
65.80
55.44
46.58
44.13
39.52 DMSO
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

III. Synthesis of C-terminally modified peptides from cyclic urethane technique (CUT) on the solid support.



General procedure for the synthesis of peptide acids from solid support: To an activated peptide as cyclic urethane moiety on the solid support, 1 mL H_2O:ACN (1:1) and 20 μL of DIEA was added and resin was left on the heated shaker at 65 °C for 2 h. The resin was filtered which is followed by the removal of solvent under high vacuum. The resulting peptide acid was analyzed by MS and purified by HPLC to obtain white solid. HPLC: 0.1% FA (v/v) in water (solvent A): 0.1% FA (v/v) acetonitrile (solvent B); gradient 0-80 %, 0.1% FA (v/v) acetonitrile in 25 min, flow rate = 1.0 mL/min, detection wavelength 220 nm.

General procedure for the synthesis of peptide esters from solid support: To an activated peptide as cyclic urethane moiety on the solid support, 1 mL MeOH and 100 μL of DIEA was added and resin was left on the heated shaker at 65 °C for 4 h. The resin was filtered which is followed by the removal of solvent under high vacuum. The resulting peptide ester was analyzed by MS and purified by HPLC to obtain white solid. HPLC: 0.1% FA (v/v) in water (solvent A): 0.1% FA (v/v) acetonitrile (solvent B); gradient 0-80 %, 0.1% FA (v/v) acetonitrile in 25 min, flow rate = 1.0 mL/min, detection wavelength 220 nm.

General procedure for the synthesis of peptide N-aryl amide from solid support: To an activated peptide as cyclic urethane moiety on the solid support, 1 mL ACN and 100 μL of benzyl amine was added and resin was left on the heated shaker at 65 °C for 4 h. The resin was filtered which is followed by the removal of solvent under high vacuum. The resulting peptide N-aryl amide was analyzed by MS and purified by HPLC to obtain white solid. HPLC: 0.1% FA (v/v) in water (solvent A): 0.1% FA (v/v) acetonitrile (solvent B); gradient 0-80 %, 0.1% FA (v/v) acetonitrile in 25 min, flow rate = 1.0 mL/min, detection wavelength 220 nm.

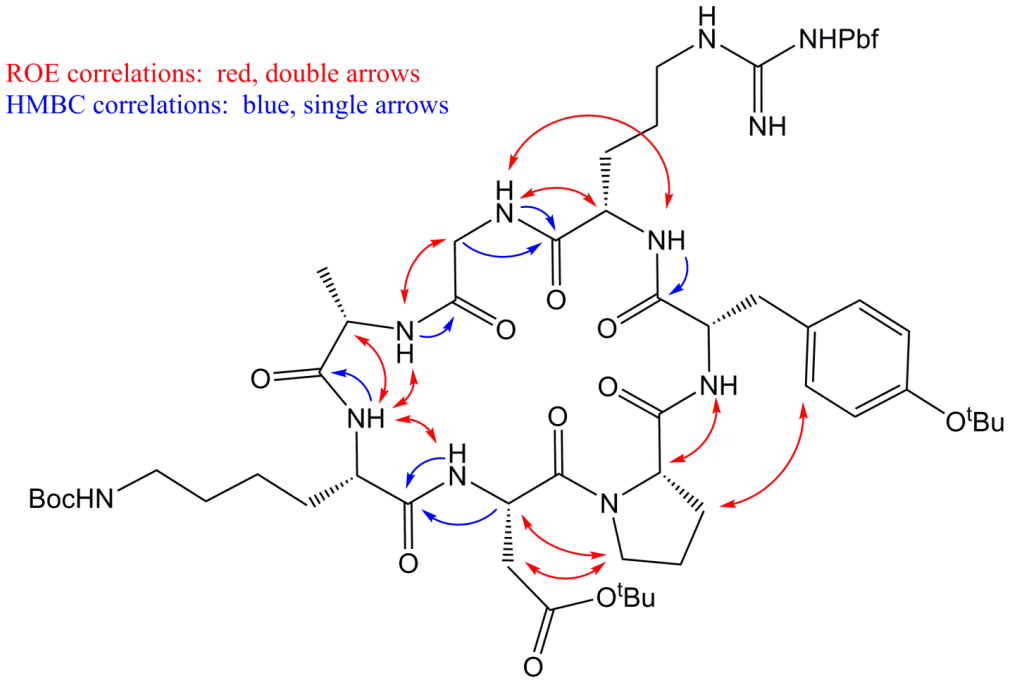
General procedure for the synthesis of peptide alcohol from solid support: To an activated peptide as cyclic urethane moiety on the solid support, 100 mg sodium borohydride in 1 mL THF was added and resin was left on the shaker overnight at room temperature. The resin was filtered which is followed by the removal of solvent under high vacuum. The resulting peptide alcohol was analyzed by MS and purified by HPLC to obtain white solid. HPLC: 0.1% FA (v/v) in water (solvent A): 0.1% FA (v/v) acetonitrile (solvent B); gradient 0-80 %, 0.1% FA (v/v) acetonitrile in 25 min, flow rate = 1.0 mL/min, detection wavelength 220 nm.

IV. Deprotection of protecting groups from C-terminal modified peptides: C-terminally modified peptides were deprotected by using a cocktail of 95:2.5:2.5, trifluoroacetic acid:triisopropyl silane:water for 2 h in solution and the resulting solution was concentrated. The oily residue was triturated with diethyl ether to obtain a white suspension. The resulting solid was purified by HPLC and analyzed by MS.

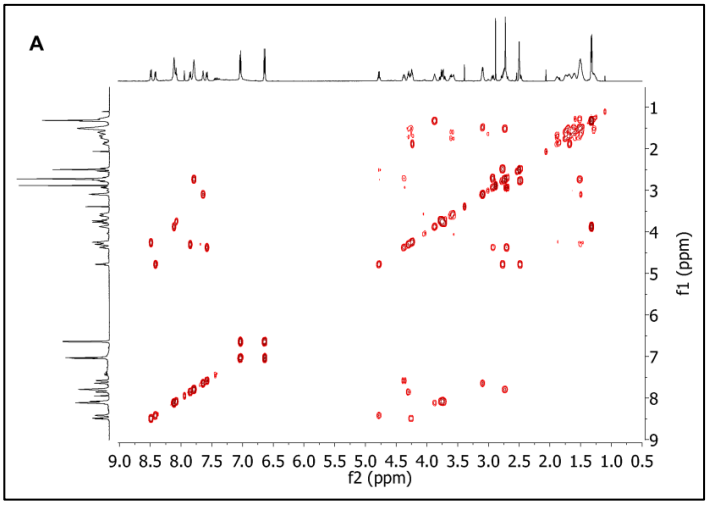
V. General procedure for the synthesis of protected C-terminal peptides: To an activated peptide as cyclic urethane moiety on the solid support, cleavage cocktail of 95:2.5:2.5, trifluoroacetic acid:triisopropyl silane:water was added and left on shaker for 2 h. The resin was removed by filtration and the resulting solution was concentrated. The oily residue was triturated with diethyl ether to obtain a white suspension. The resulting protected C-terminal peptide was purified by HPLC and analyzed by MS. HPLC: 0.1% FA (v/v) in water (solvent A): 0.1% FA (v/v) acetonitrile (solvent B); gradient 0-80 %, 0.1% FA (v/v) acetonitrile in 25 min, flow rate = 1.0 mL/min, detection wavelength 220 nm.

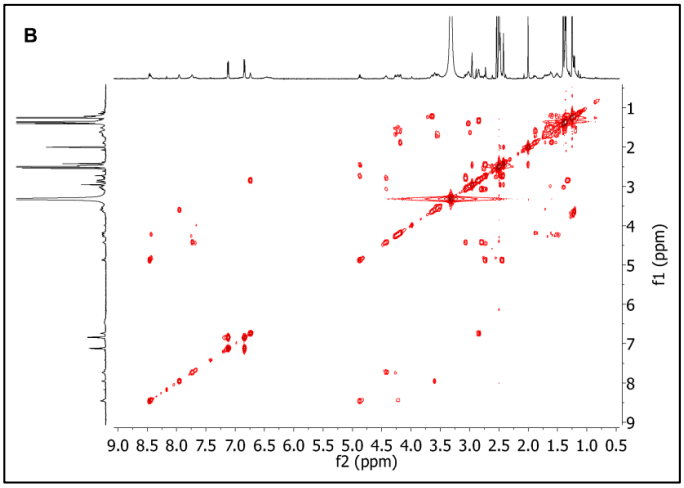
VI. NMR and MS data for Linear and Cyclized Peptide, AKDPYRG

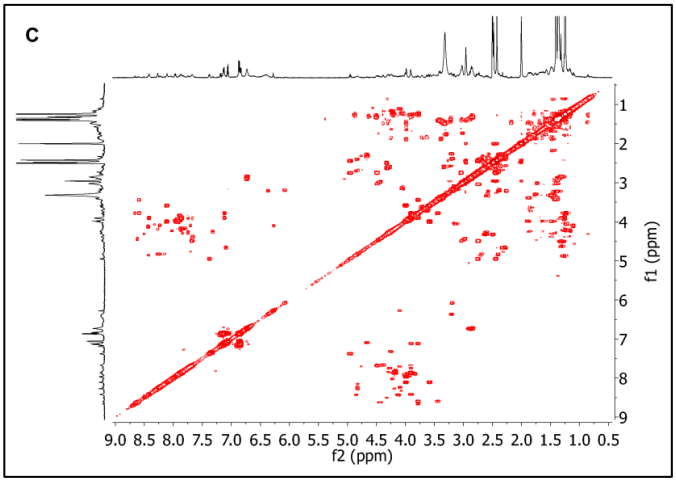
Observed key HMBC and ROE correlations for conformer 1 of cyclized protected peptide cyc(AK(Boc)D(tBu)PY(tBu)R(Pbf)G) **3c**.



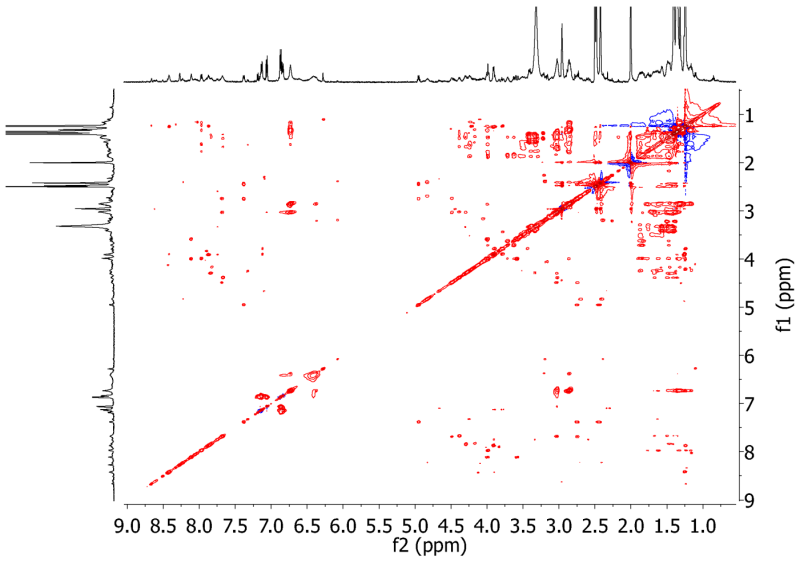
COSY NMR spectra for linear unprotected peptide (A) AKDPYRG, linear protected peptide **3b** (B) AK(Boc)D(tBu)PY(tBu)R(Pbf)G, and cyclized protected peptide **3c** (C), cyc(AK(Boc)D(tBu)PY(tBu)R(Pbf)G), recorded at 600 MHz / 600 MHz (^{1}H / ^{1}H)



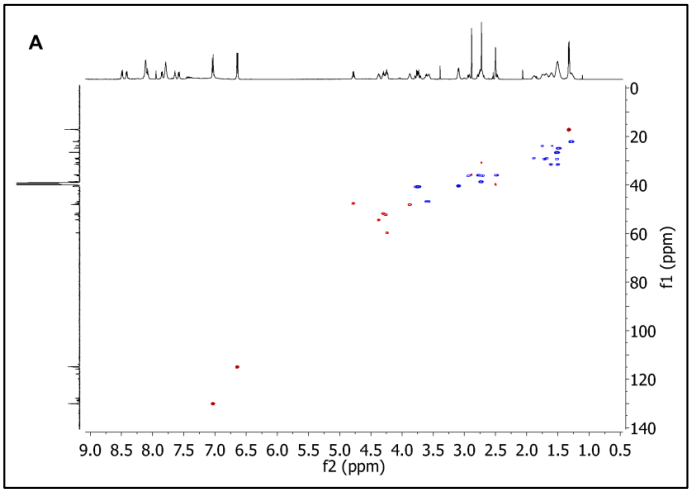


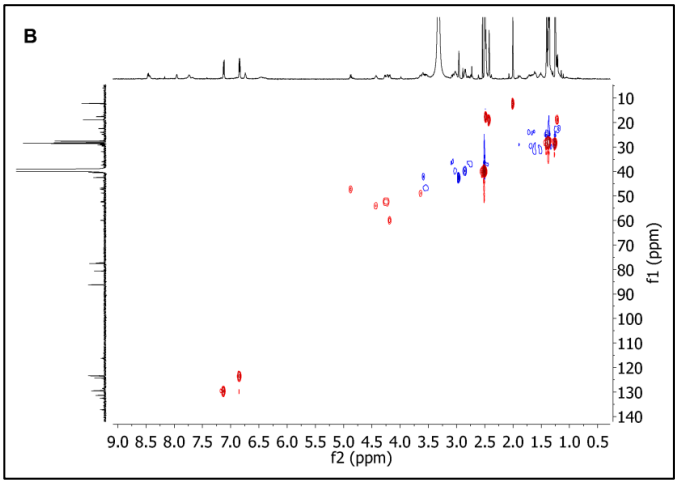


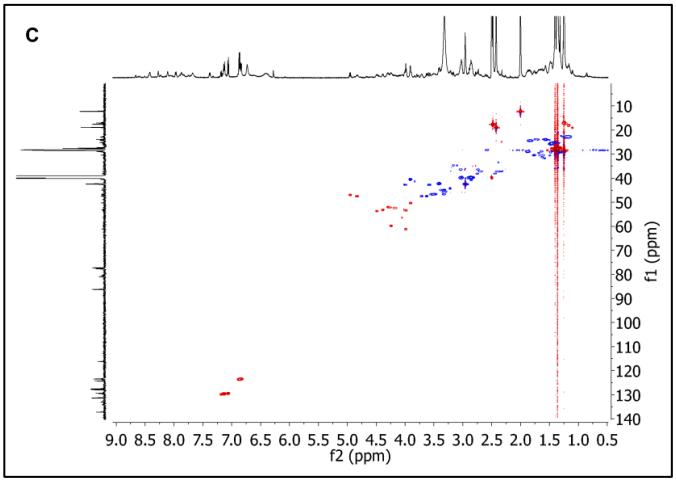
TOCSY NMR spectra for cyclized protected peptide **3c**, cyc(AK(Boc)D(tBu)PY(tBu)R(Pbf)G), recorded at 600 MHz / 600 MHz (^{1}H / ^{1}H) using an 80 ms mixing time



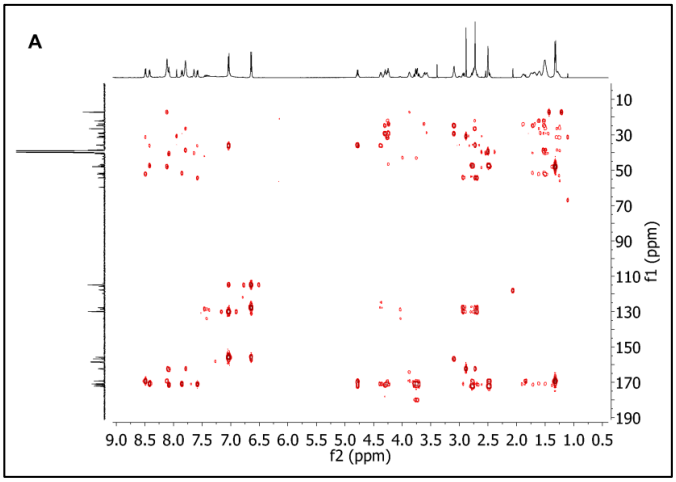
Multiplicity-edited HSQC NMR spectra for linear unprotected peptide (A), linear protected peptide **3b** (B), and cyclized protected peptides **3c** (C), AKDPYRG, recorded at 600 MHz / 151 MHz (1H / 13C) with $J_{H,C}$ optimized to 145 Hz

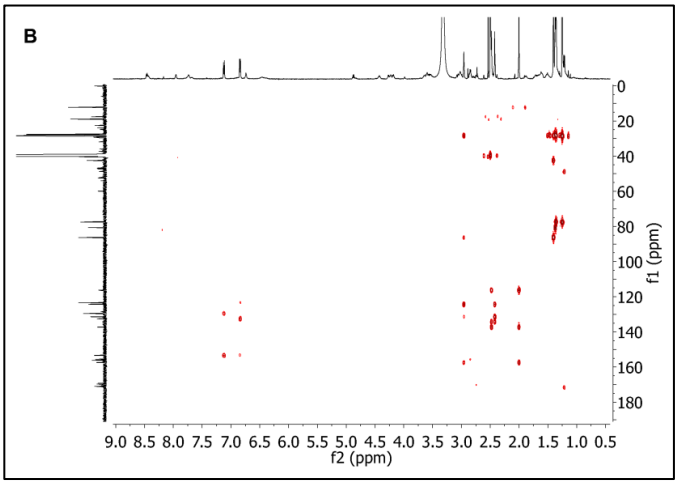


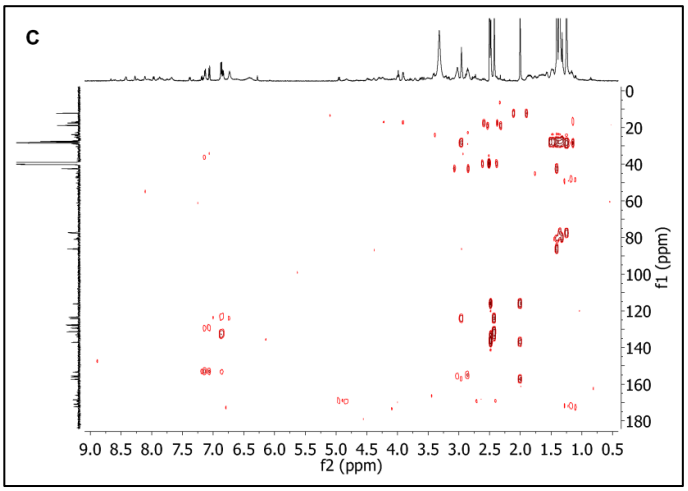




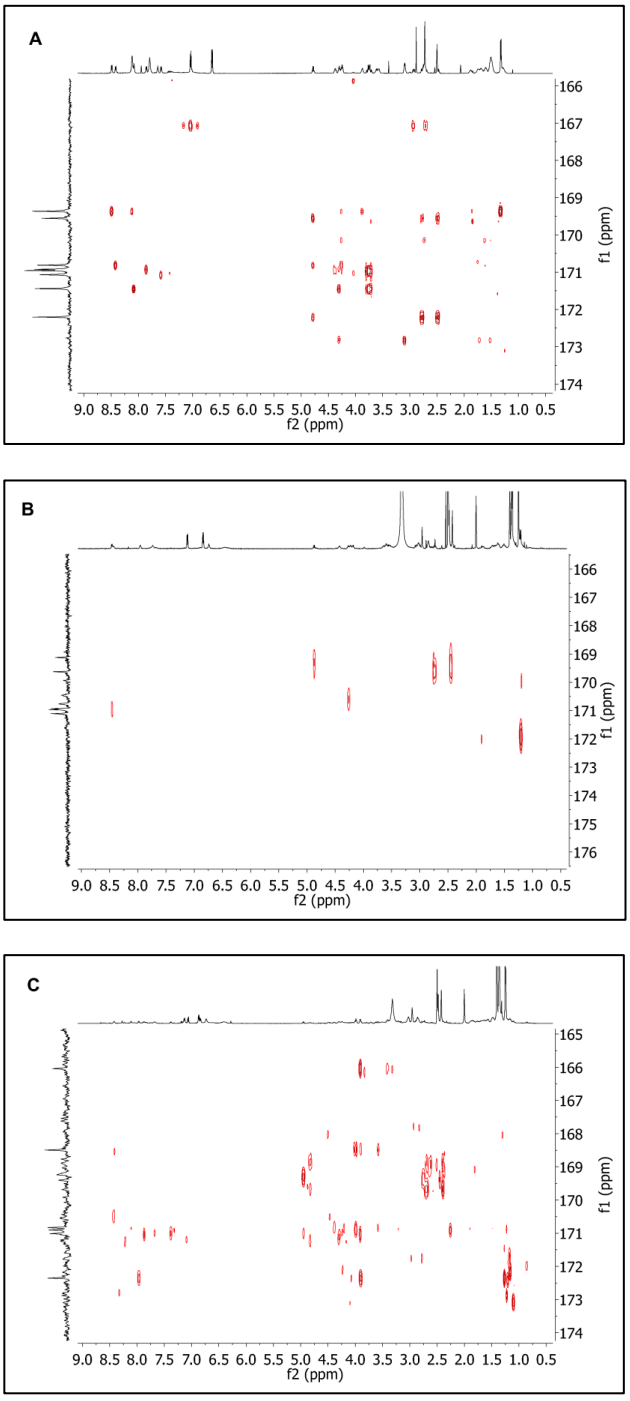
HMBC NMR spectra for linear unprotected peptide (A), linear protected peptide **3b** (B), and cyclized protected peptide **3c** (C), AKDPYRG, recorded at 600 MHz / 151 MHz (1H / 13C) with $J_{H,C}$ optimized to 8 Hz and one bond suppression set to 165 Hz





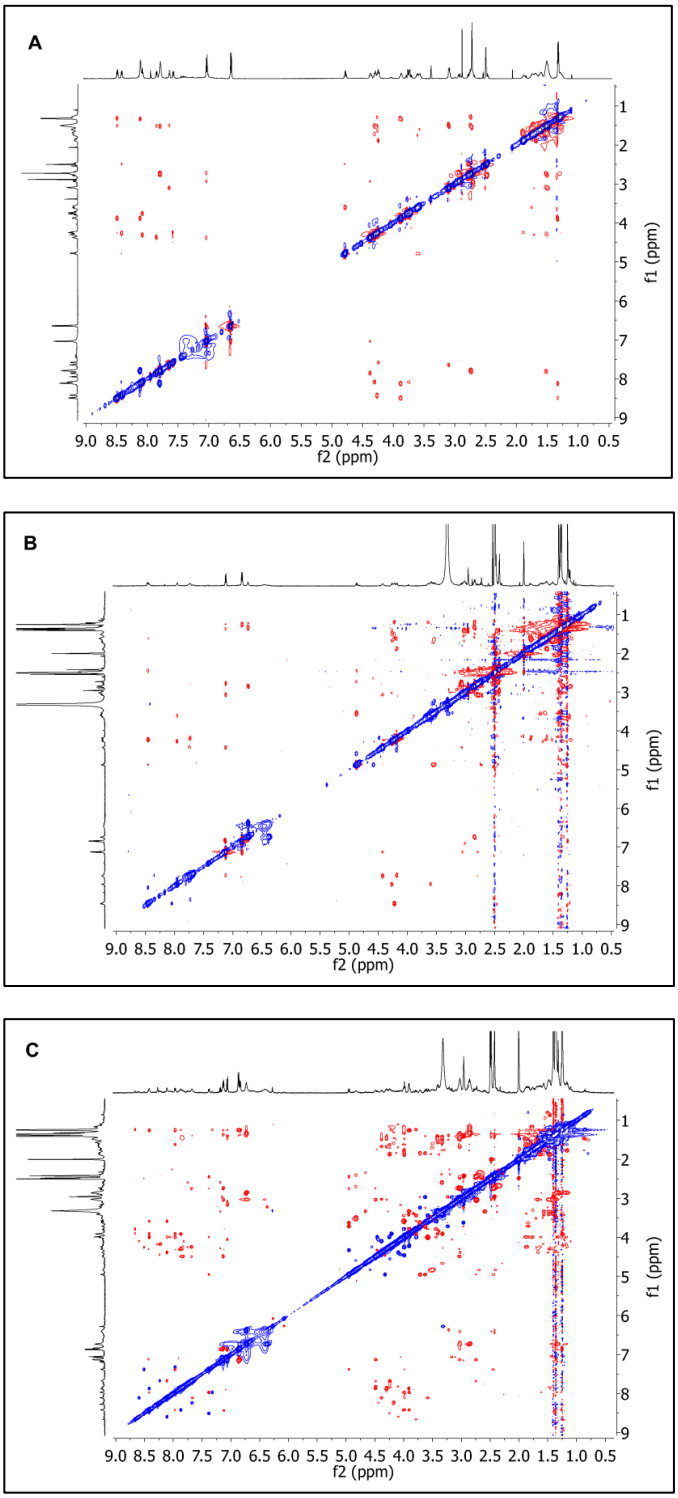


HMBC NMR spectra of carbonyl region for linear unprotected peptide (A), AKDPYRG, linear protected peptide **3b** (B) AK(Boc)D(tBu)PY(tBu)R(Pbf)G, and cyclized protected peptide **3c** (C), cyc(AK(Boc)D(tBu)PY(tBu)R(Pbf)G), recorded at 600 MHz / 151 MHz (1H / 13C) with $J_{H,C}$ optimized to 10 Hz and one bond suppression set to 165 Hz

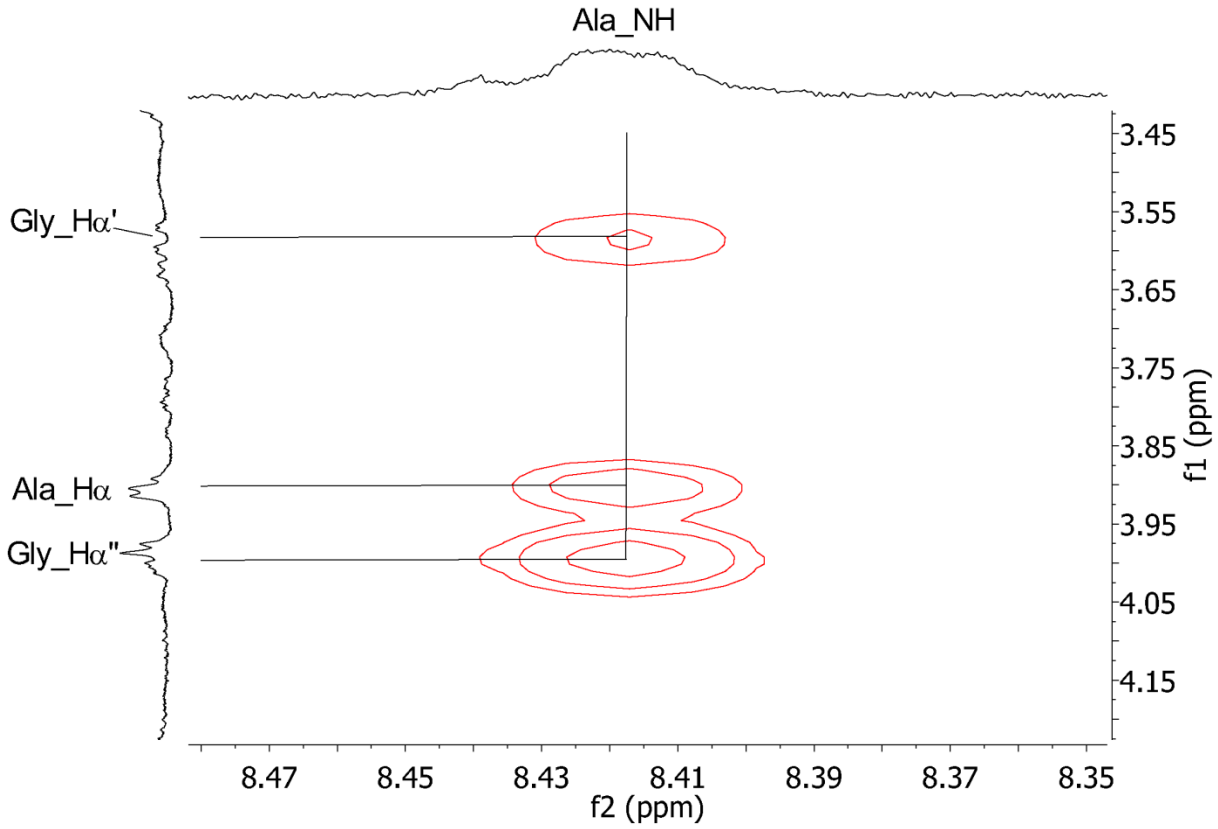


To improve resolution, a spectral width of 73 to 177 ppm was chosen with 220 indirect increments. This resulted in aliphatic resonances being intentionally folded to a non-interfering region.

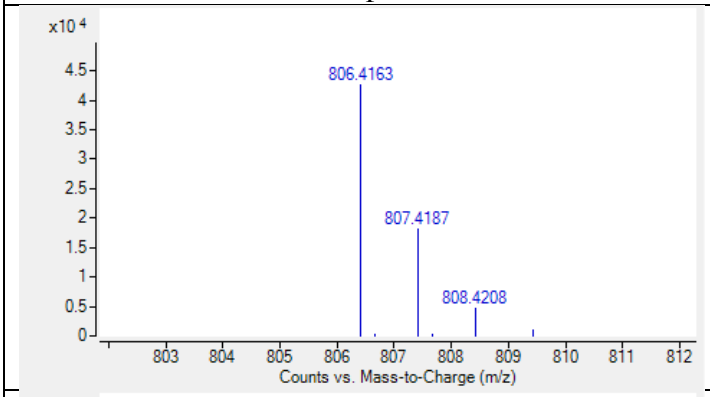
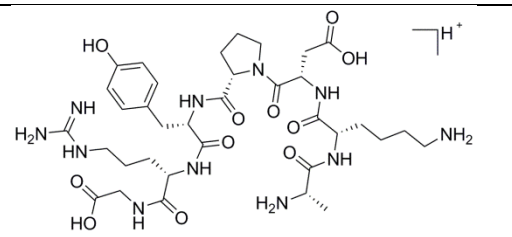
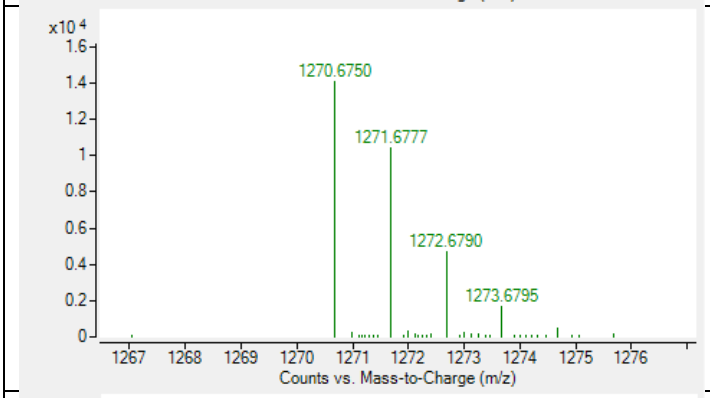
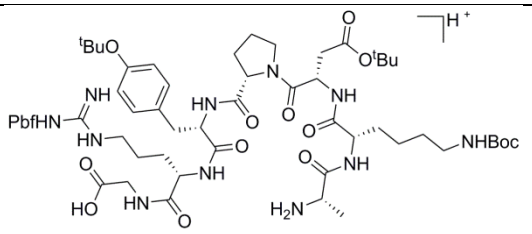
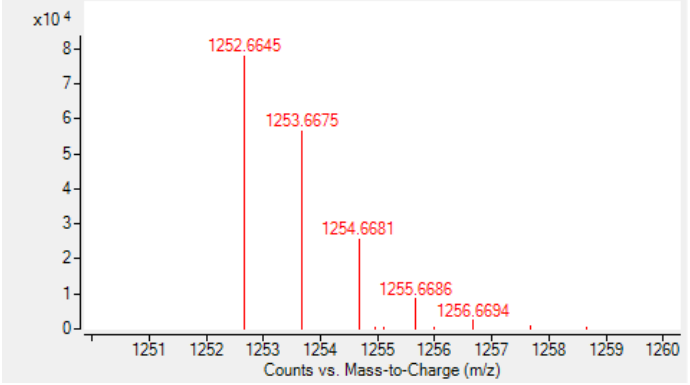
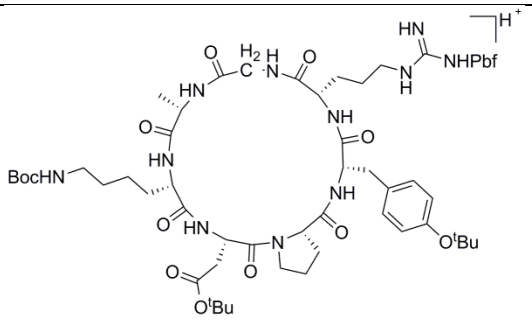
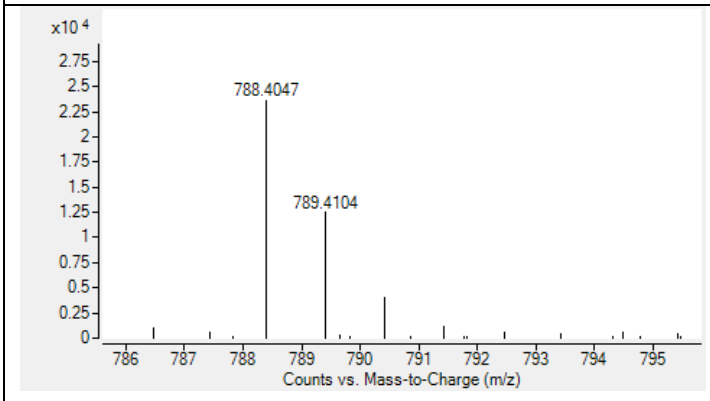
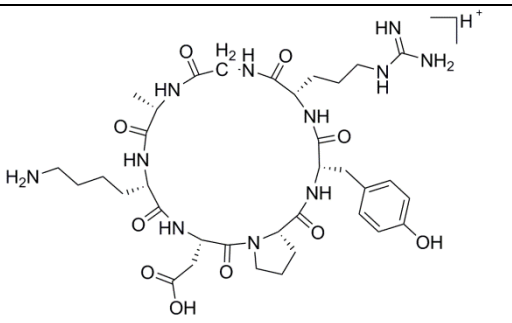
ROESY NMR spectra for linear unprotected peptide (A), AKDPYRG, linear protected peptide **3b** (B) AK(Boc)D(tBu)PY(tBu)R(Pbf)G, and cyclized protected peptide **3c** (C), cyc(AK(Boc)D(tBu)PY(tBu)R(Pbf)G), recorded at 600 MHz / 600 MHz (^{1}H / ^{1}H) using a 200 ms mixing time



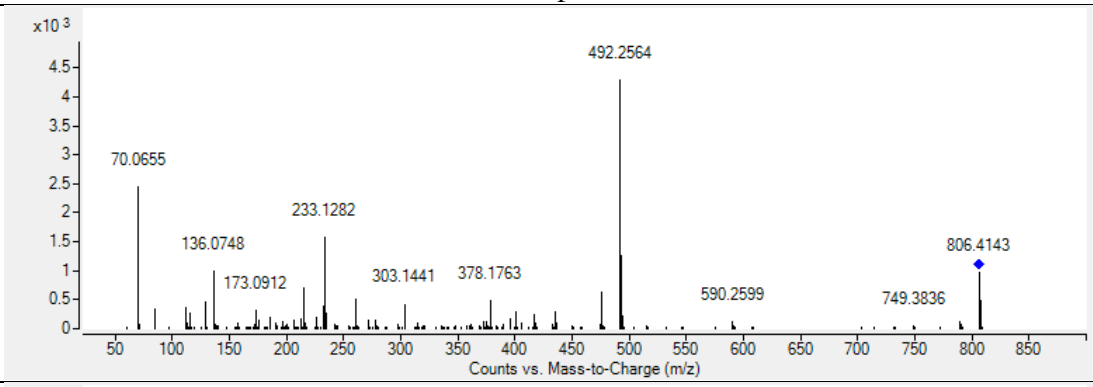
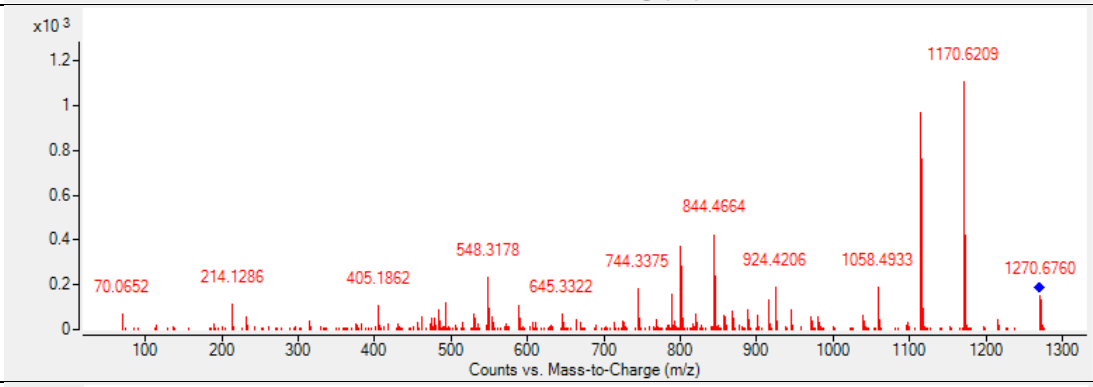
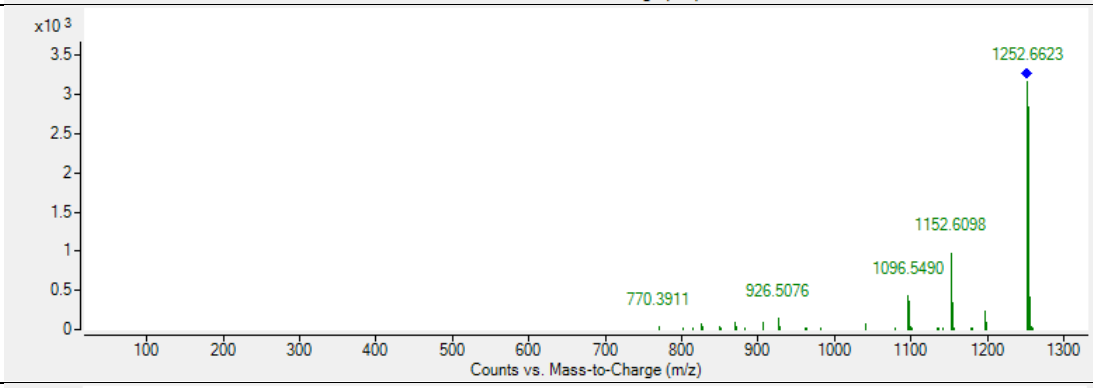
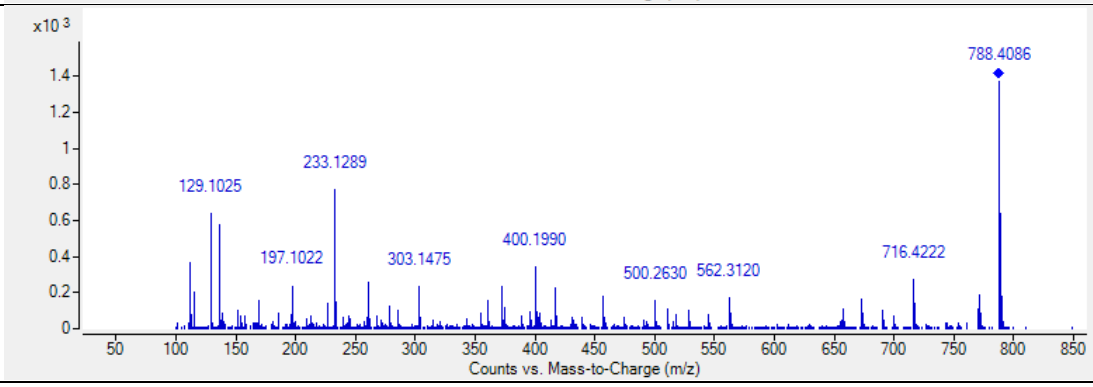
ROESY spectrum of protected cyclized peptide **3c** cyc(AK(Boc)D(tBu)PY(tBu)R(Pbf)G), highlighting key ROE cross peaks between Ala and Gly residues



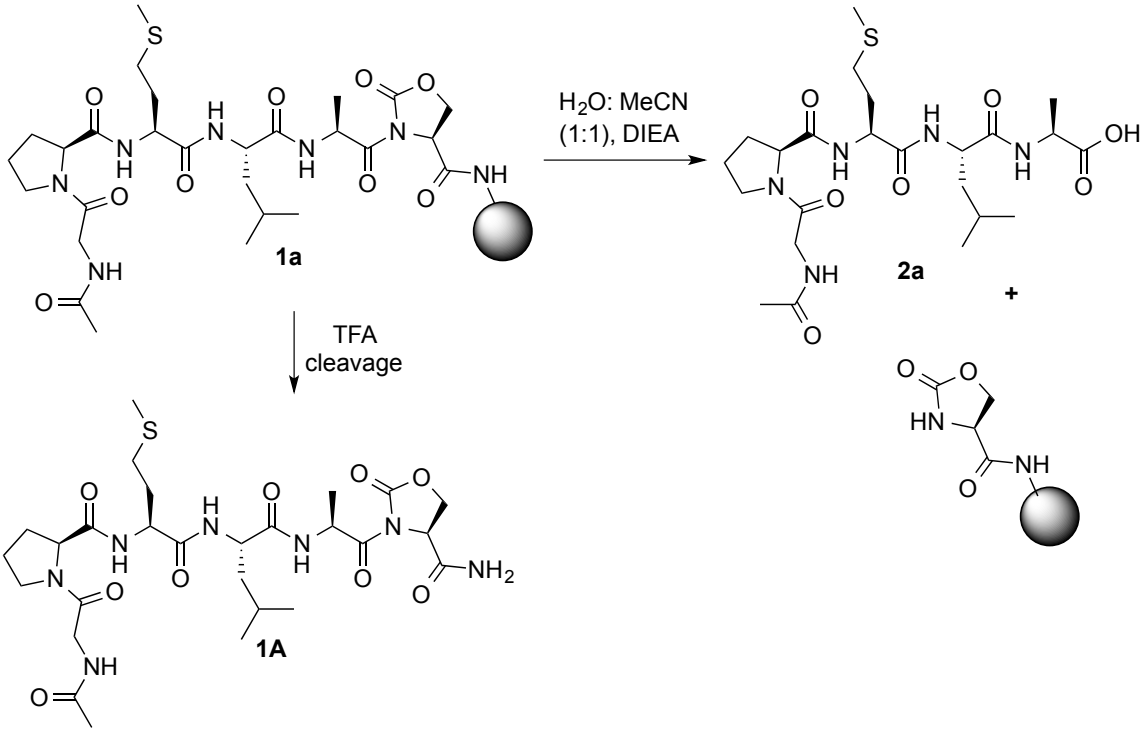
HRMS (+ESI) data for linear unprotected peptide (A), linear protected peptide **3b** (B), cyclized protected peptide **3c** (C), and cyclized unprotected peptide **3d** (D), AKDPYRG

HRMS spectrum	Structure and Calculated m/z
	 **Linear Unprotected Peptide** $C_{35}H_{56}N_{11}O_{11}^+$ m/z 806.4155
	 **Linear Protected Peptide** $C_{61}H_{96}N_{11}O_{16}S^+$ m/z 1270.6752
	 **Cyclic Protected Peptide** $C_{61}H_{94}N_{11}O_{15}S^+$ m/z 1252.6646
	 **Cyclic Unprotected Peptide** $C_{35}H_{54}N_{11}O_{10}^+$ m/z 788.4050

MS/MS (+ESI) data for linear unprotected peptide (A), linear protected peptide **3b** (B), cyclized protected peptide **3c** (C), and cyclized unprotected peptide **3d** peptide (D), AKDPYRG

Molecule	MS/MS spectrum
Linear Unprotected	
Linear Protected (3b)	
Cyclic Protected (3c)	
Cyclic Unprotected (3d)	

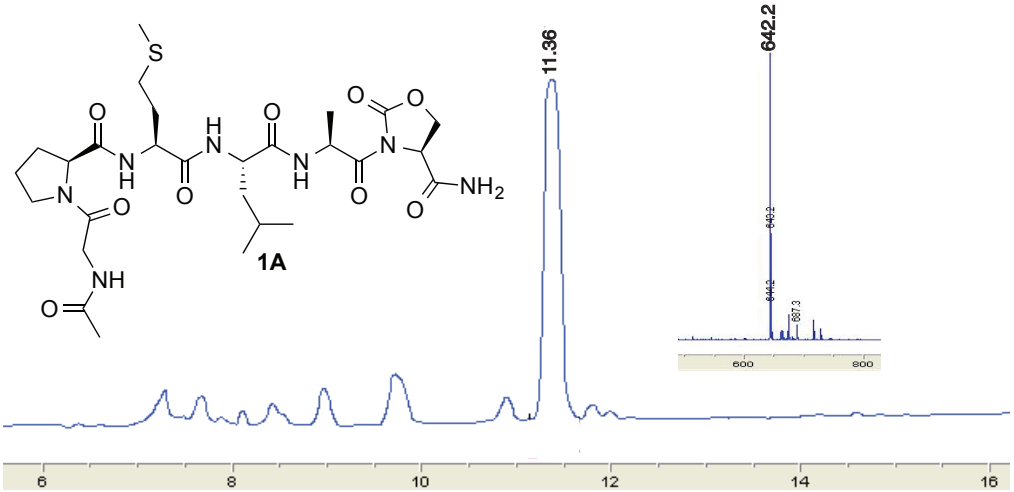
VII. Peptide Characterization and HPLC Traces

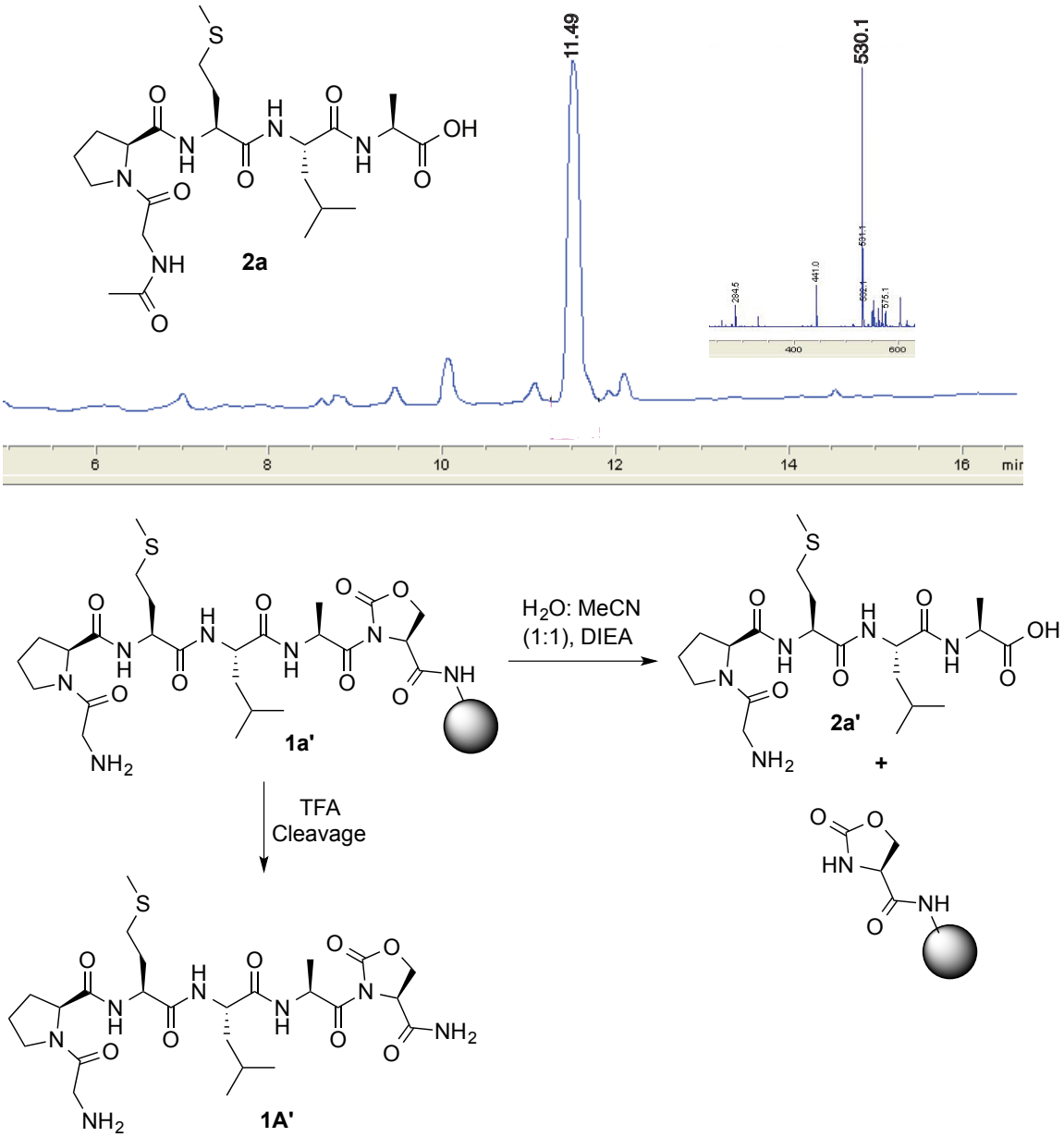


Ac-Gly-Pro-Met-Leu-Ala-Oxd (1A). LCMS: *m/z* 642.20 (calcd $[M+H]^+$ = 642.28), Purity: >95% (HPLC analysis at 220 nm). Retention time: 11.36

Ac-Gly-Pro-Met-Leu-Ala (2a). LCMS: *m/z* 530.1 (calcd $[M+H]^+$ = 530.26), Purity: >95% (HPLC analysis at 220 nm). Retention time: 11.49 min

HPLC spectra of the cyclized starting material and the crude reaction mixture after hydrolysis; the product peak is labeled.

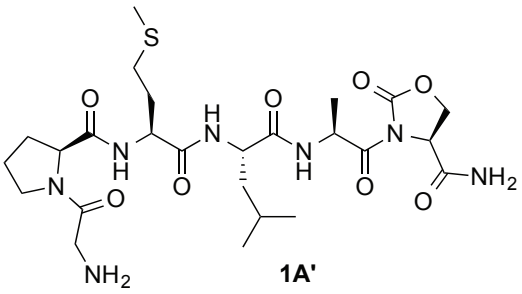




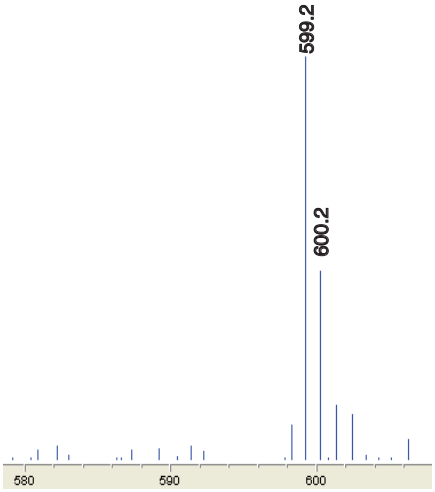
Gly-Pro-Met-Leu-Ala-Oxd (1A'). LCMS: *m/z* 600.2 (calcd $[M+H]^+$ = 600.27), Purity: >95% (HPLC analysis at 220 nm). Retention time: 8.11 min

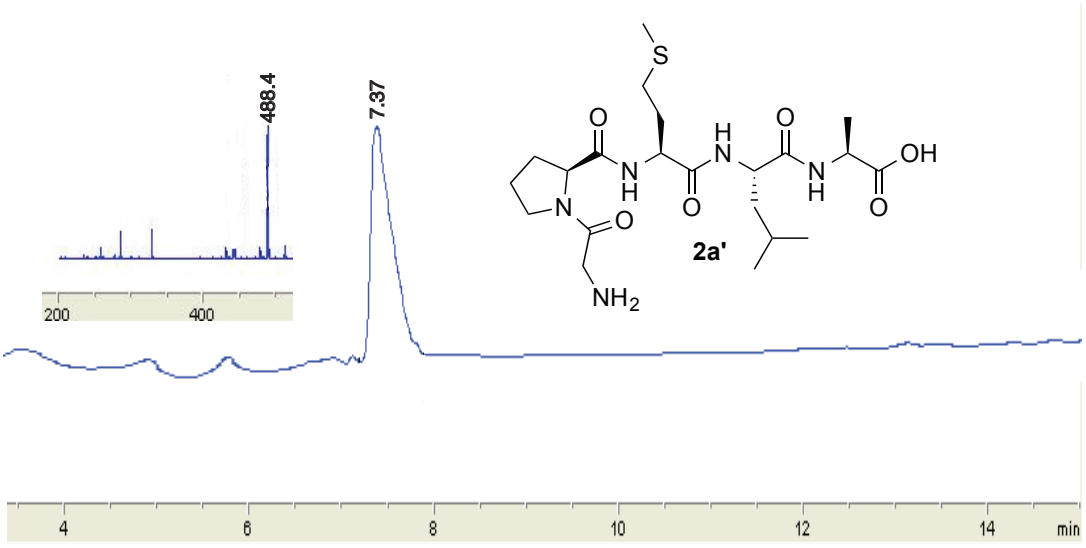
Gly-Pro-Met-Leu-Ala (2a'). LCMS: *m/z* 488.40 (calcd $[M+H]^+$ = 488.25), Purity: >95% (HPLC analysis at 220 nm). Retention time: 7.37 min

HPLC spectra of the cyclized starting material and the crude reaction mixture after hydrolysis; the product peak is labeled.

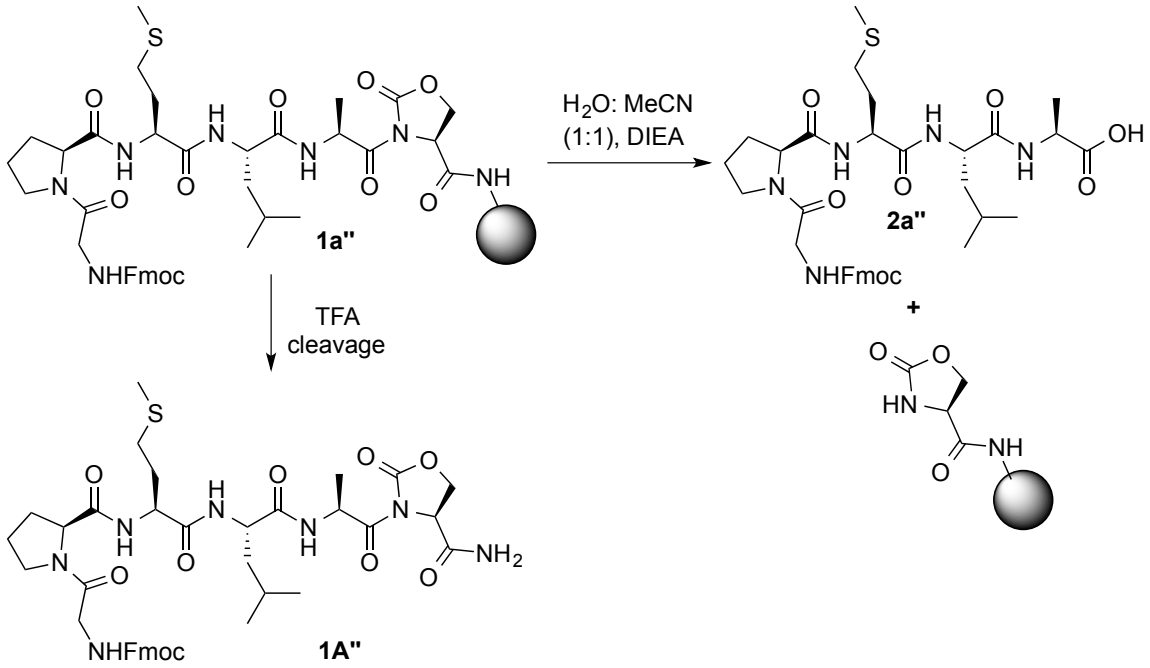

S
O
N
H
O
O
N
H
O
O
N
O
O
NH2
O
NH2

1A'


599.2
600.2
580
590
600


488.4
7.37
200
400
S
O
N
H
O
O
N
H
O
O
N
H
OH
O
NH2
4
6
8
10
12
14
min

2a'

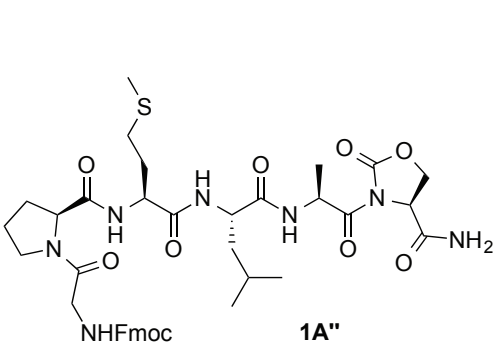


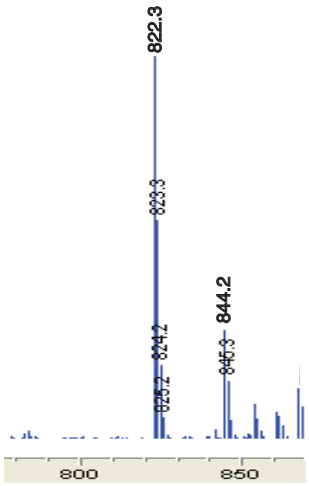
Fmoc-Gly-Pro-Met-Leu-Ala-Oxd (1A''). LCMS: *m/z* 822.3 (calcd $[M+H]^+$ = 822.34), 844.2 (calcd $[M+Na]^+$ = 844.34); Purity: >95% (HPLC analysis at 220 nm). Retention time: 18.2 min.

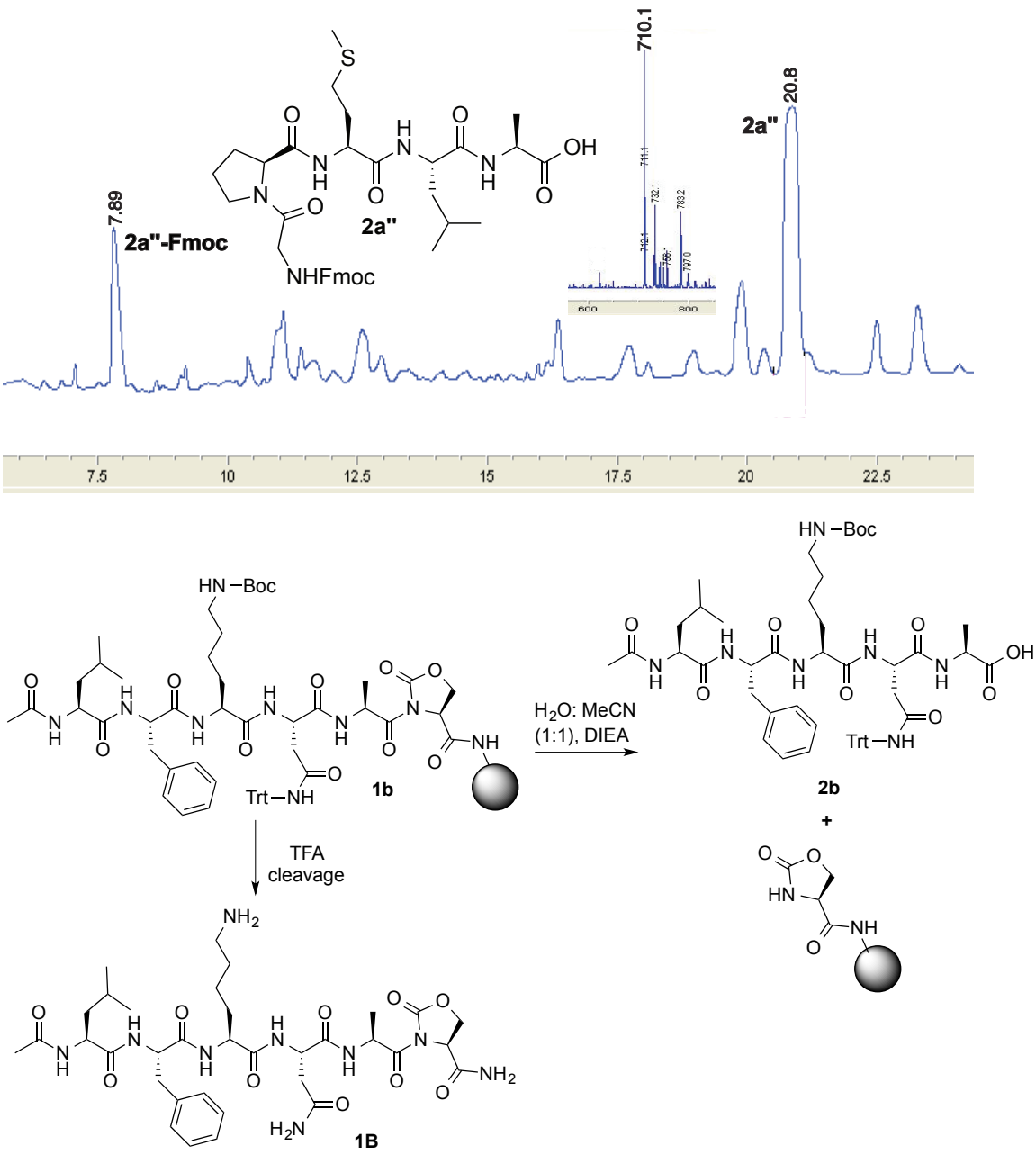
Fmoc-Gly-Pro-Met-Leu-Ala (2a''). LCMS: *m/z* 710.1 (calcd $[M+H]^+$ = 710.31), (HPLC analysis at 220 nm). Retention time: 20.8 min.

Gly-Pro-Met-Leu-Ala (2a''-Fmoc or 2a'). LCMS: *m/z* 488.4 (calcd $[M+H]^+$ = 488.25), (HPLC analysis at 220 nm). Retention time: 7.89 min.

HPLC spectra of the cyclized starting material and the crude reaction mixture after hydrolysis; the product peak is labeled.



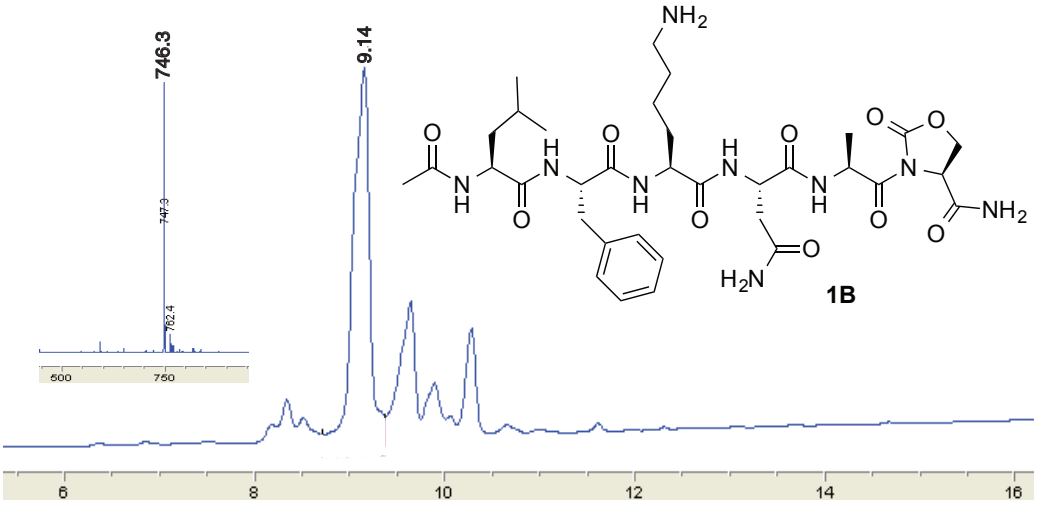


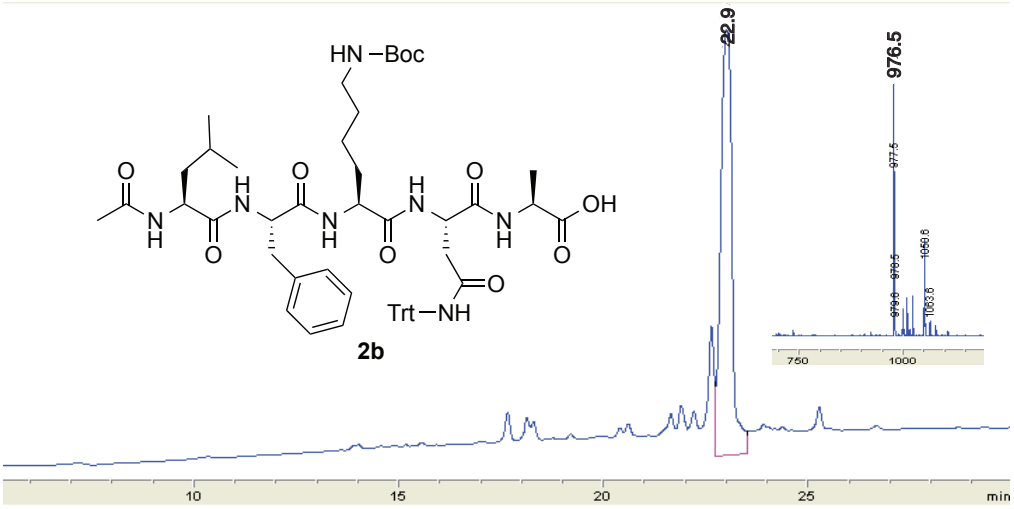


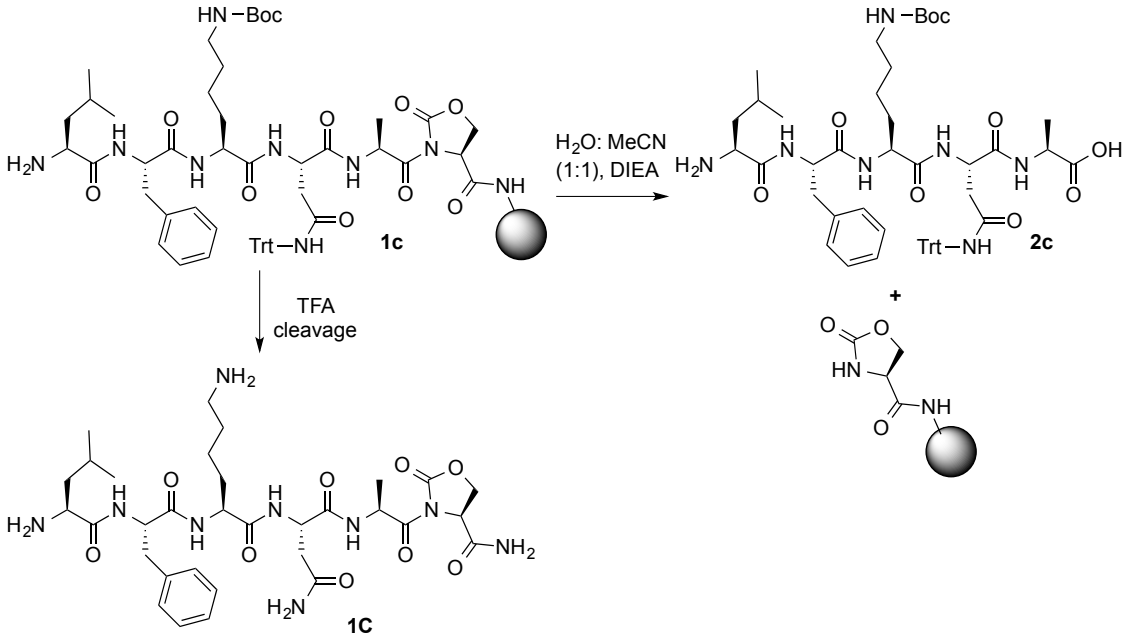
Ac-Leu-Phe-Lys-Asn-Ala-Oxd (1B). LCMS: *m/z* 746.3 (calcd $[M+H]^+$ = 746.38), Purity: >95% (HPLC analysis at 220 nm). Retention time: 9.14 min.

Ac-Leu-Phe-Lys(Boc)-Asn(Trt)-Ala (2b). LCMS: *m/z* 976.5 (calcd $[M+H]^+$ = 976.51), Purity: >95% (HPLC analysis at 220 nm). Retention time: 22.97 min.

HPLC spectra of the cyclized starting material and the crude reaction mixture after hydrolysis; the product peak is labeled.


746.3
9.14
1B
6
8
10
12
14
16

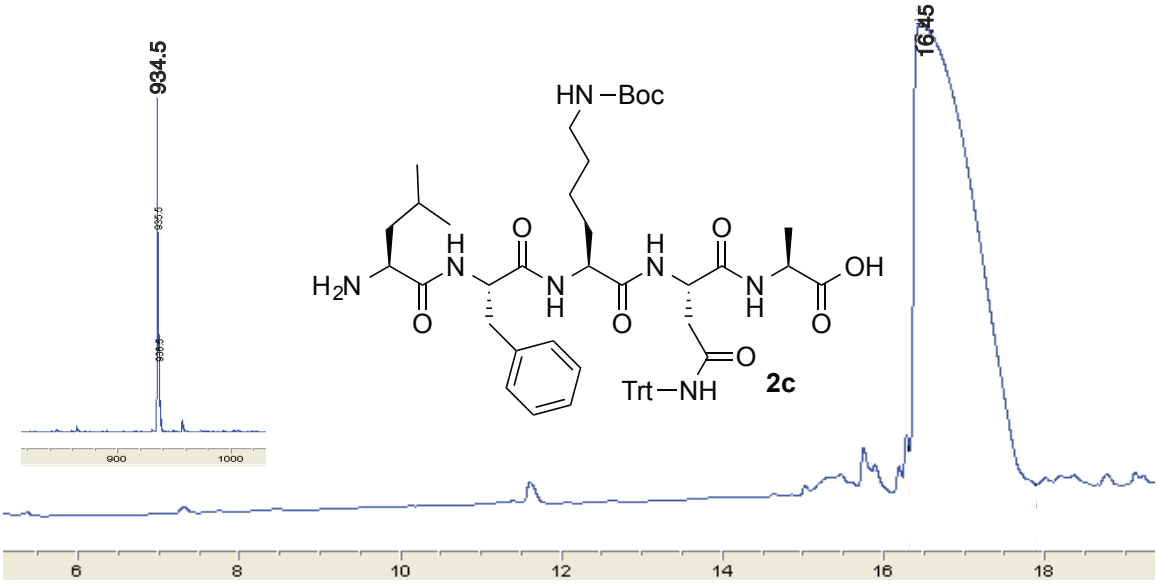

HN –Boc
Trt–NH
2b
22.9
976.5
10
15
20
25
min

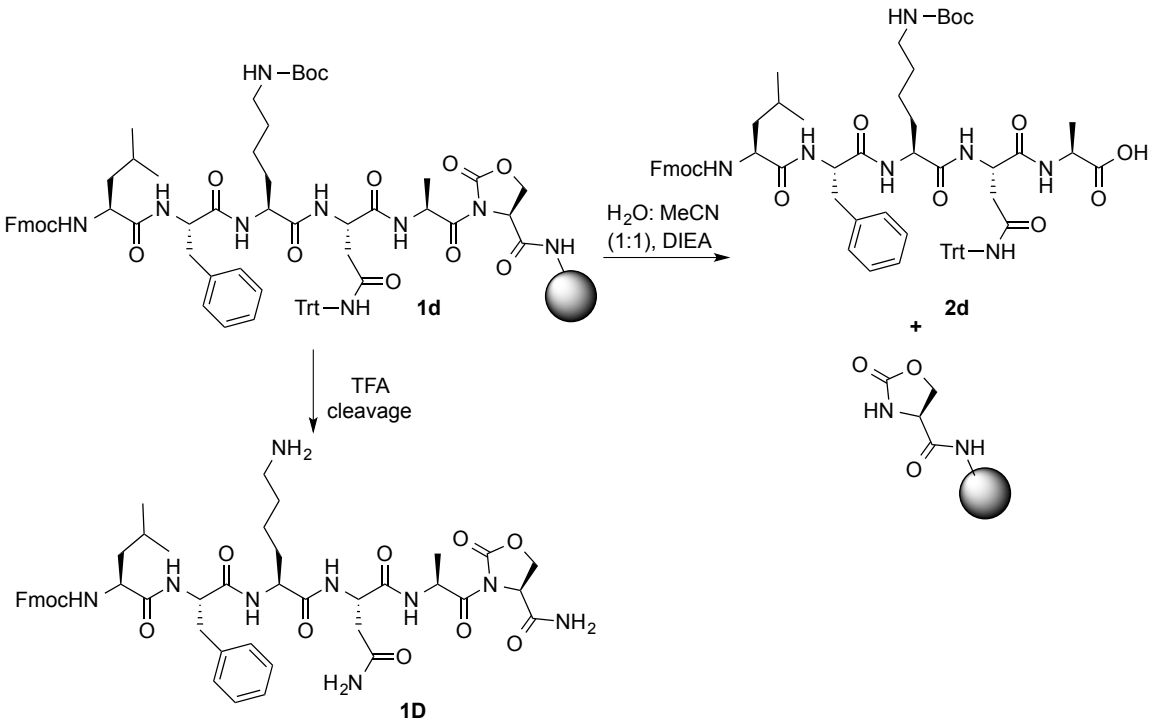

HN –Boc
Trt–NH
1c
H2O: MeCN
(1:1), DIEA
2c
TFA
cleavage
1C

Leu-Phe-Lys-Asn-Ala-Oxd (1C). LCMS: *m/z* 704.3 (calcd $[M+H]^+$ = 704.37), Purity: >95% (HPLC analysis at 220 nm).

Leu-Phe-Lys(Boc)-Asn(Trt)-Ala (2c). LCMS: *m/z* 934.5 (calcd $[M+H]^+$ = 934.57), Purity: >95% (HPLC analysis at 220 nm). Retention time: 16.45 min.

HPLC spectra of the cyclized starting material and the crude reaction mixture after hydrolysis; the product peak is labeled.



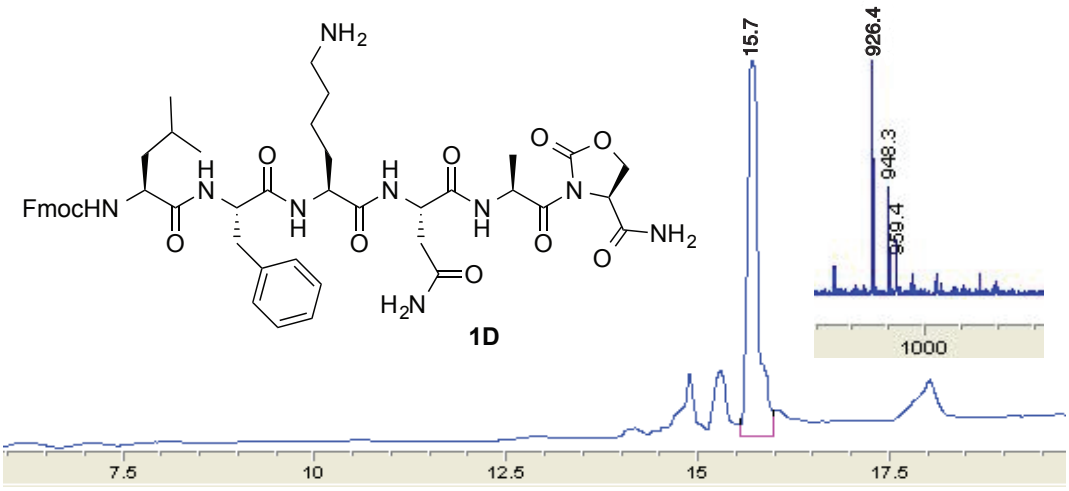


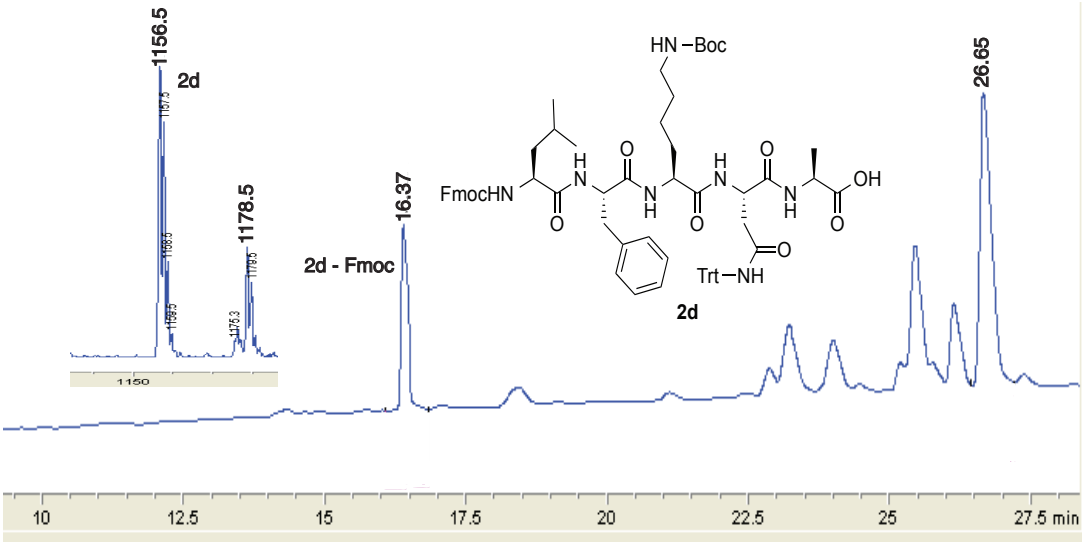
Fmoc-Leu-Phe-Lys-Asn-Ala-Oxd (1D). LCMS: *m/z* 926.4 (calcd $[M+H]^+$ = 926.43), 948.3 (calcd $[M+Na]^+$ = 948.43), Purity: >95% (HPLC analysis at 220 nm). Retention time: 15.7 min.

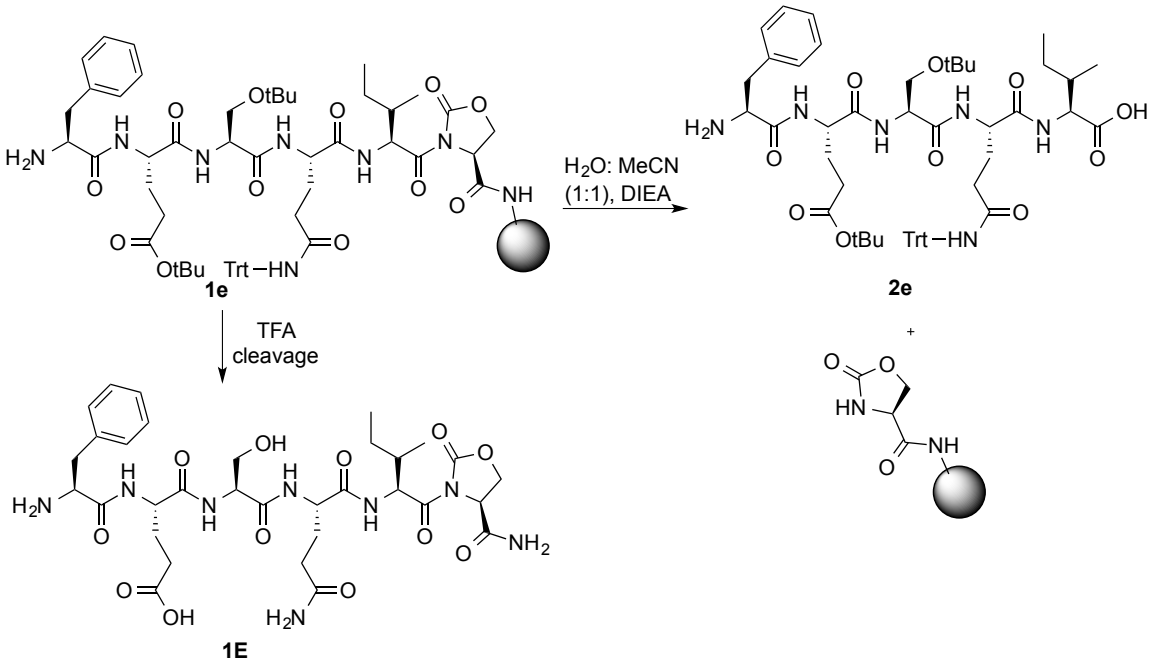
Fmoc-Leu-Phe-Lys(Boc)-Asn(Trt)-Ala (2d). LCMS: *m/z* 1156.5 (calcd $[M+H]^+$ = 1156.57), 1178.5 (calcd $[M+Na]^+$ = 1178.57), Purity: >95% (HPLC analysis at 220 nm). Retention time: 26.65 min.

Leu-Phe-Lys(Boc)-Asn(Trt)-Ala (2d-Fmoc or 2c). LCMS: *m/z* 934.5 (calcd $[M+H]^+$ = 934.57), Purity: >95% (HPLC analysis at 220 nm). Retention time: 16.37 min.

HPLC spectra of the cyclized starting material and the crude reaction mixture after hydrolysis; the product peak is labeled.


FmocHN
NH2
H2N
NH2
1D
15.7
926.4
948.3
959.4
1000
7.5
10
12.5
15
17.5

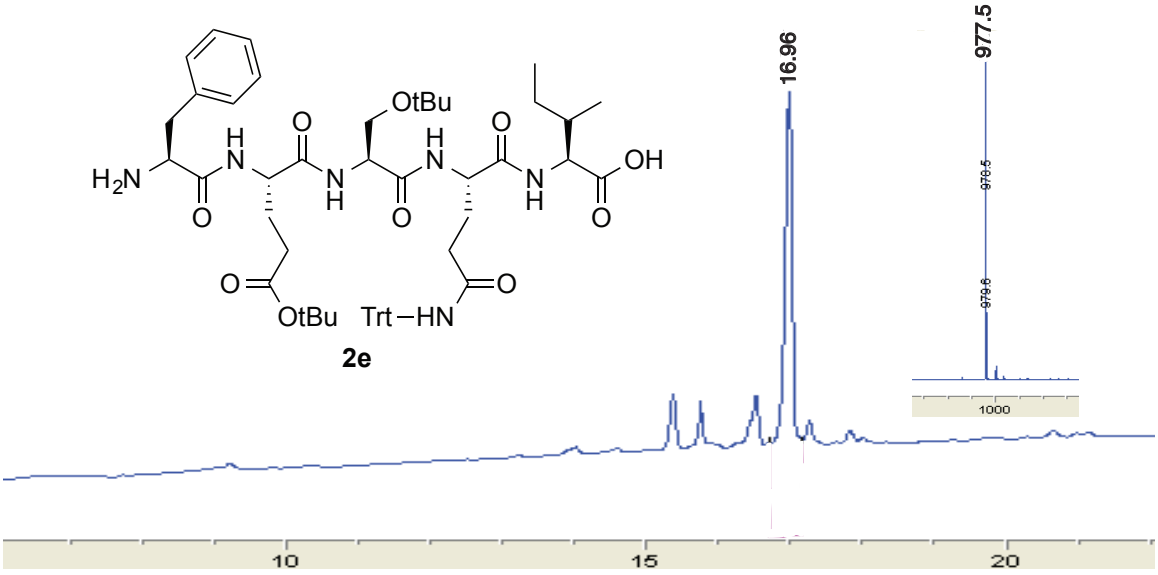

1156.5
2d
1178.5
2d - Fmoc
16.37
HN–Boc
FmocHN
OH
Trt–NH
2d
26.65
10
12.5
15
17.5
20
22.5
25
27.5 min

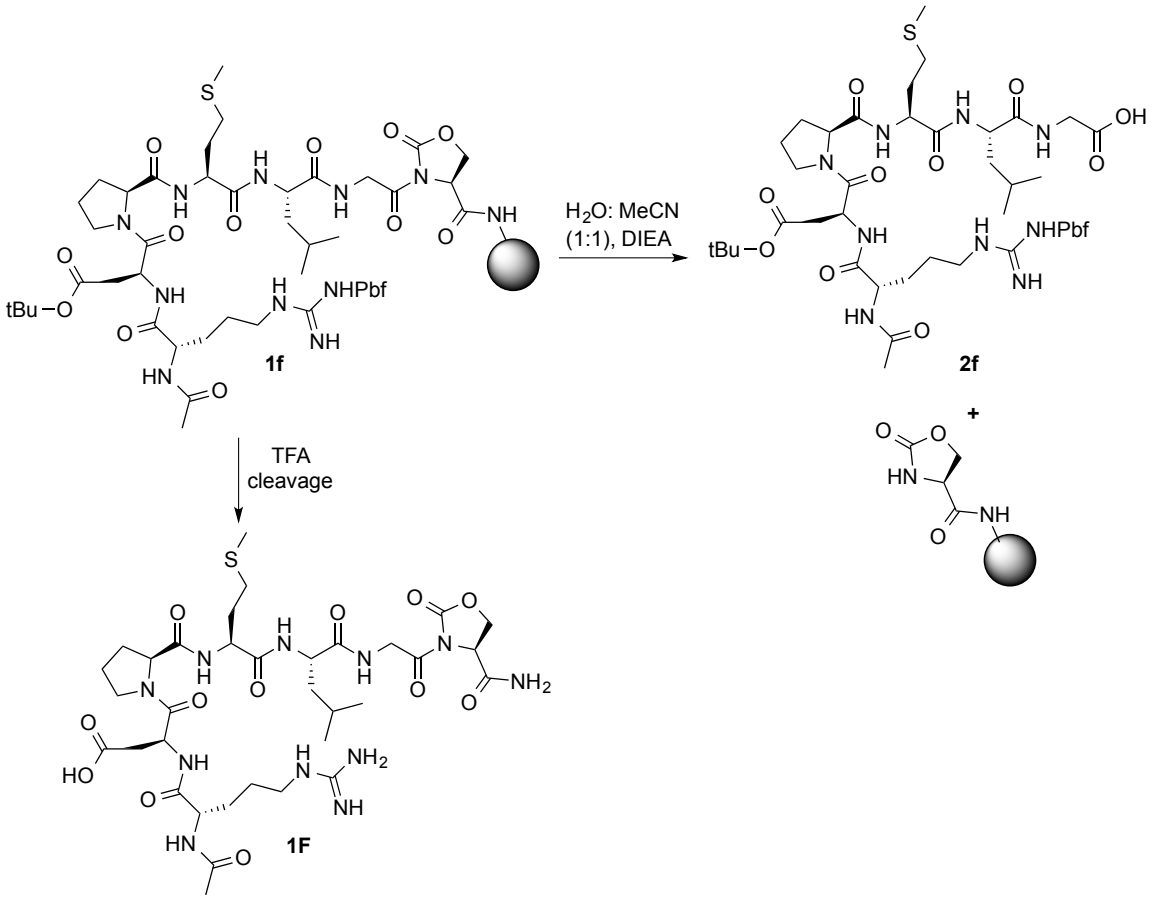


Phe-Glu-Ser-Gln-Ile-Oxd (1E). LCMS: *m/z* 735.3 (calcd $[M+H]^+$ = 735.32), Purity: >95% (HPLC analysis at 220 nm).

Phe-Glu(tBu)-Ser(tBu)-Gln(Trt)-Ile (2e). LCMS: *m/z* 977.5 (calcd $[M+H]^+$ = 977.53), Purity: >95% (HPLC analysis at 220 nm). Retention time: 16.96 min.

HPLC spectra of the cyclized starting material and the crude reaction mixture after hydrolysis; the product peak is labeled.

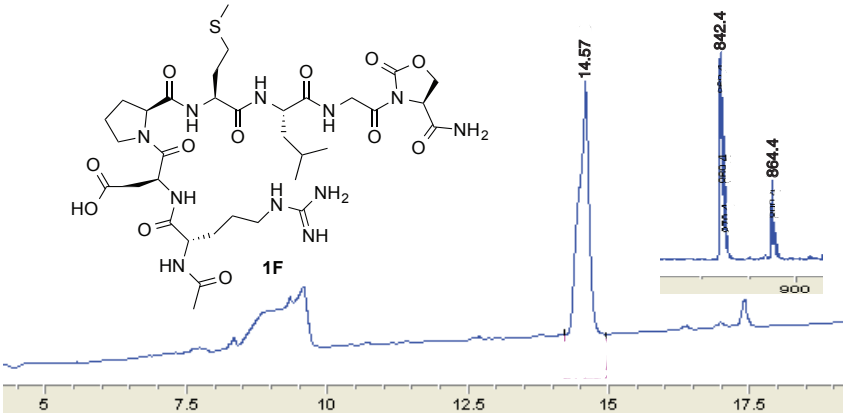


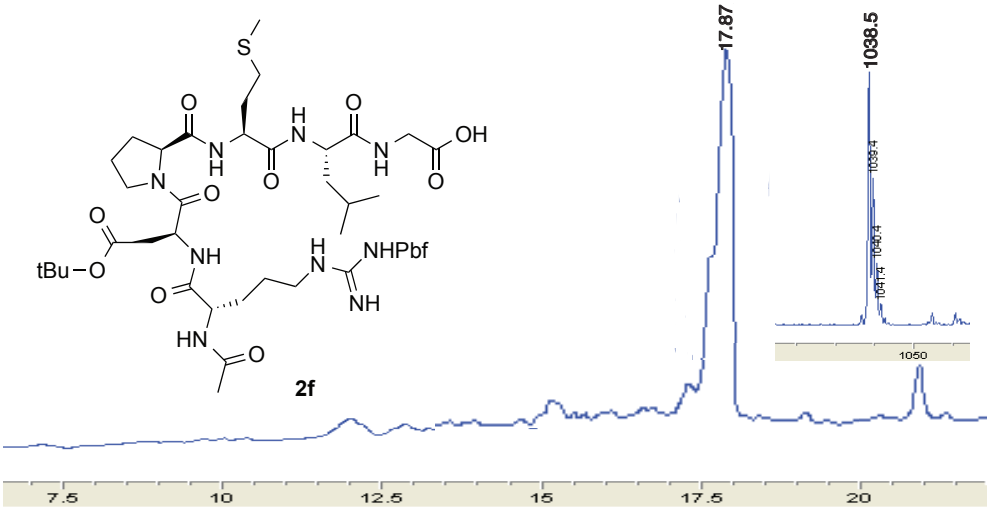


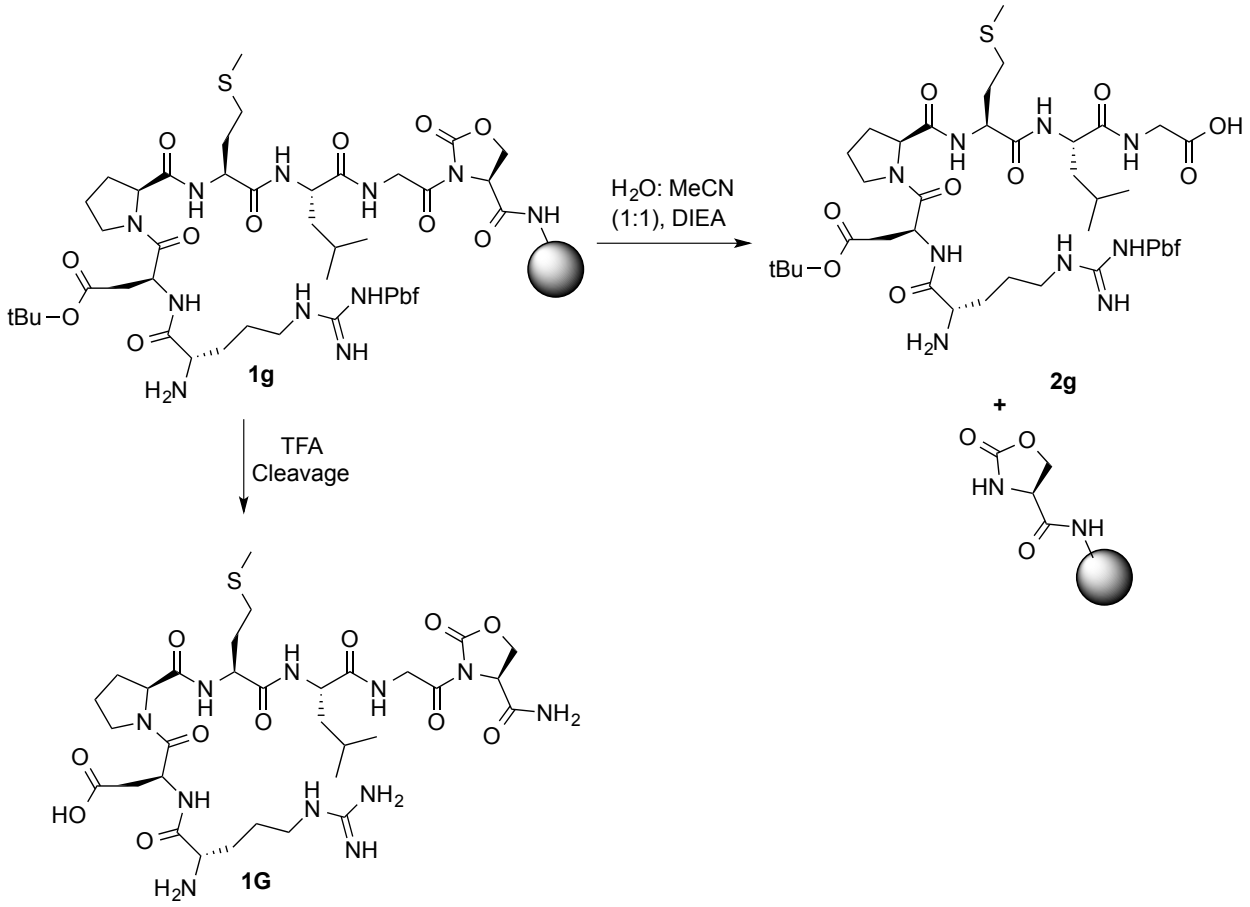
Ac-Arg-Asp-Pro-Met-Leu-Gly-Oxd (1F). LCMS: *m/z* 842.4 (calcd $[M+H]^+$ = 842.38), 864.4 (calcd $[M+Na]^+$ = 864.38), Purity: >95% (HPLC analysis at 220 nm). Retention time: 14.57 min.

Ac-Arg(Pbf)-Asp(tBu)-Pro-Met-Leu-Gly (2f). LCMS: *m/z* 1038.5 (calcd $[M+H]^+$ = 1038.49), Purity: >95% (HPLC analysis at 220 nm). Retention time: 17.87 min.

HPLC spectra of the cyclized starting material and the crude reaction mixture after hydrolysis; the product peak is labeled.





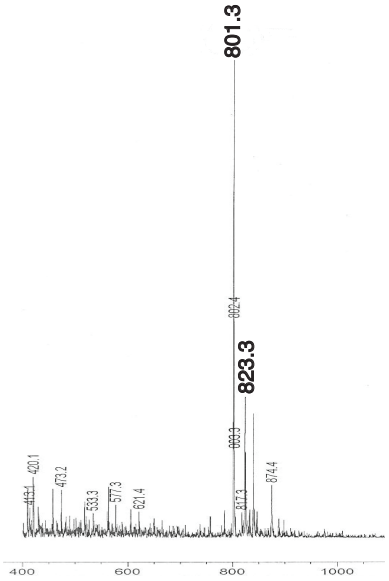


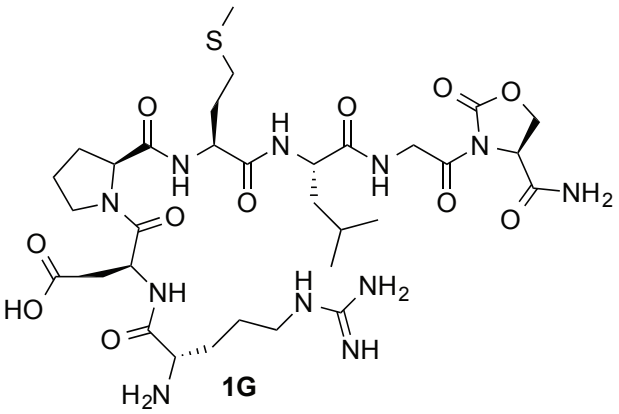
Arg-Asp-Pro-Met-Leu-Gly-Oxd (1G). LCMS: *m/z* 801.3 (calcd $[M+H]^+$ = 801.36), 823.3 (calcd $[M+Na]^+$ = 823.36) Purity: >95% (HPLC analysis at 220 nm).

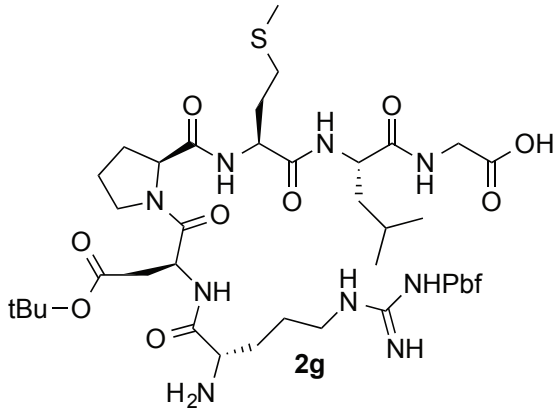
Arg(Pbf)-Asp(tBu)-Pro-Met-Leu-Gly (2g). LCMS: *m/z* 996.4 (calcd $[M+H]^+$ = 996.48), 1018.5 (calcd $[M+Na]^+$ = 1018.48), Purity: >95% (HPLC analysis at 220 nm). Retention time:

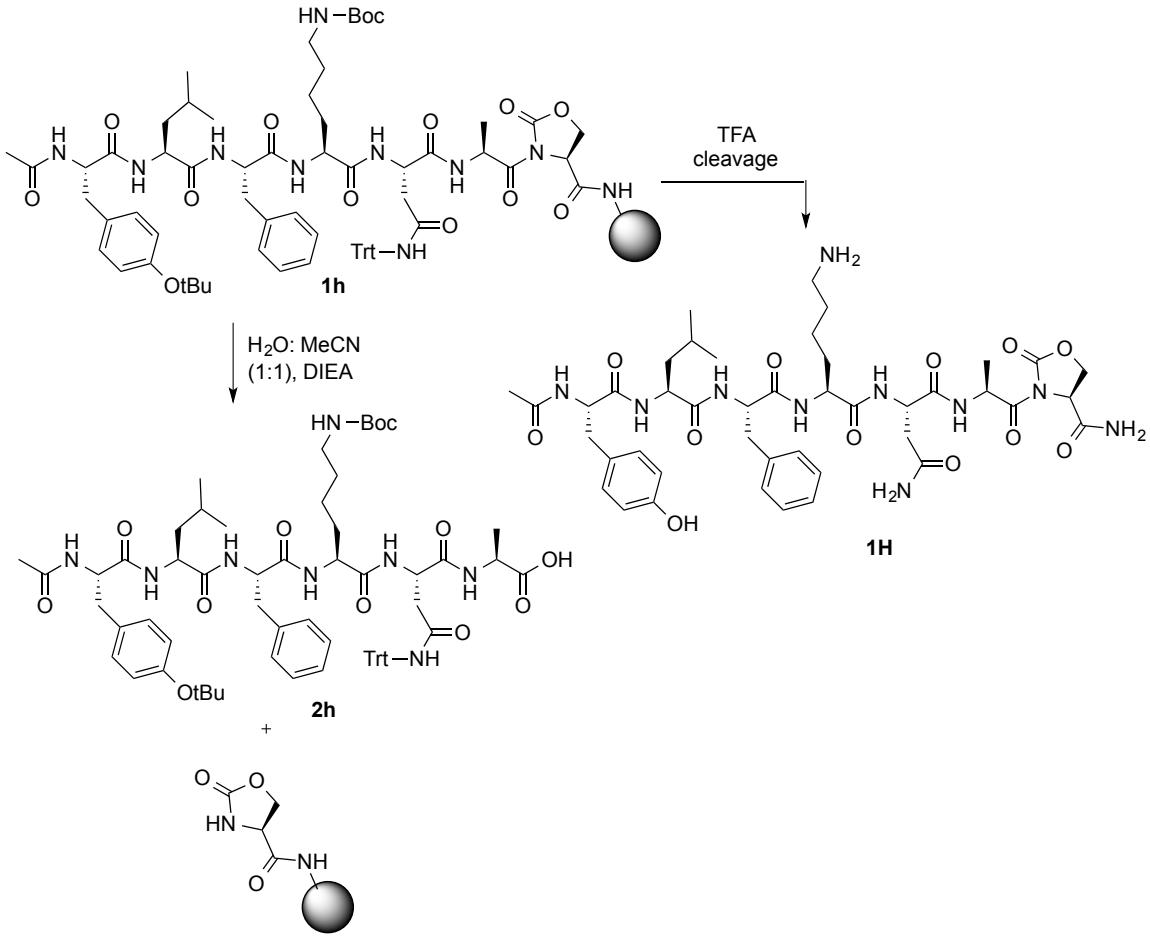
15.09 min.

HPLC spectra of the cyclized starting material and the crude reaction mixture after hydrolysis; the product peak is labeled.





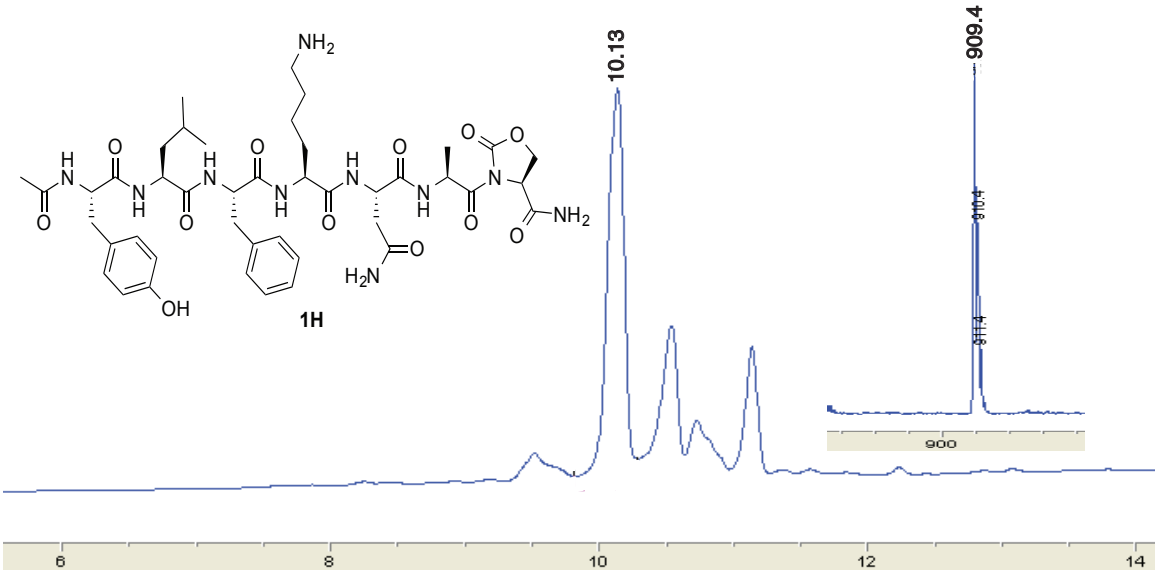




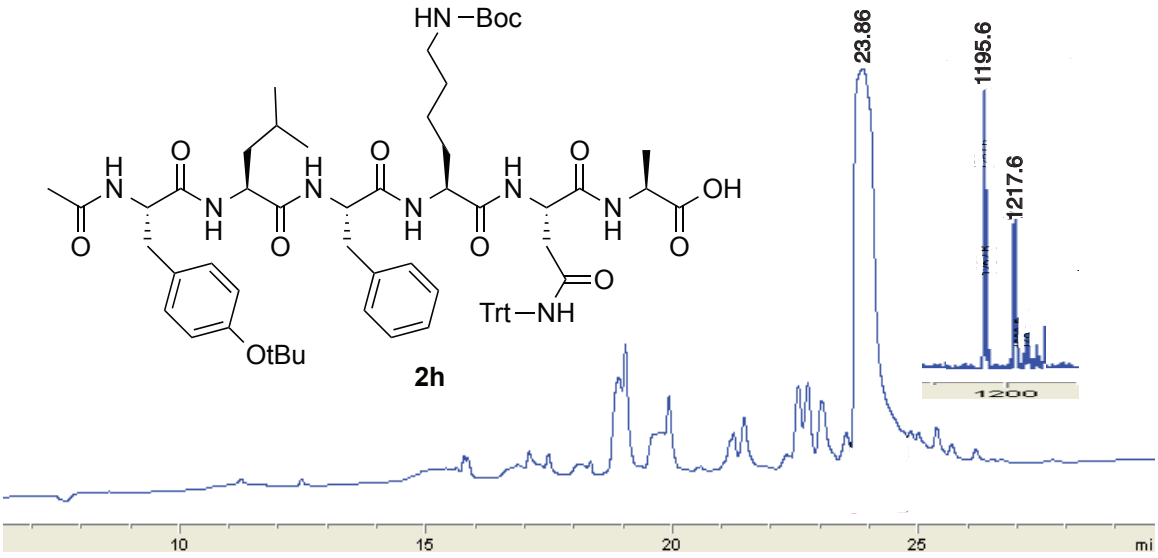
Ac-Tyr-Leu-Phe-Lys-Asn-Ala-Oxd (1H). LCMS: *m/z* 909.4 (calcd $[M+H]^+$ = 909.44) Purity: >95% (HPLC analysis at 220 nm). Retention time: 10.13 min.

Ac-Tyr(tBu)-Leu-Phe-Lys(Boc)-Asn(Trt)-Ala (2h). LCMS: *m/z* 1195.6 (calcd $[M+H]^+$ = 1195.64), 1217.6 (calcd $[M+Na]^+$ = 1217.64) Purity: >95% (HPLC analysis at 220 nm). Retention time: 23.86 min.

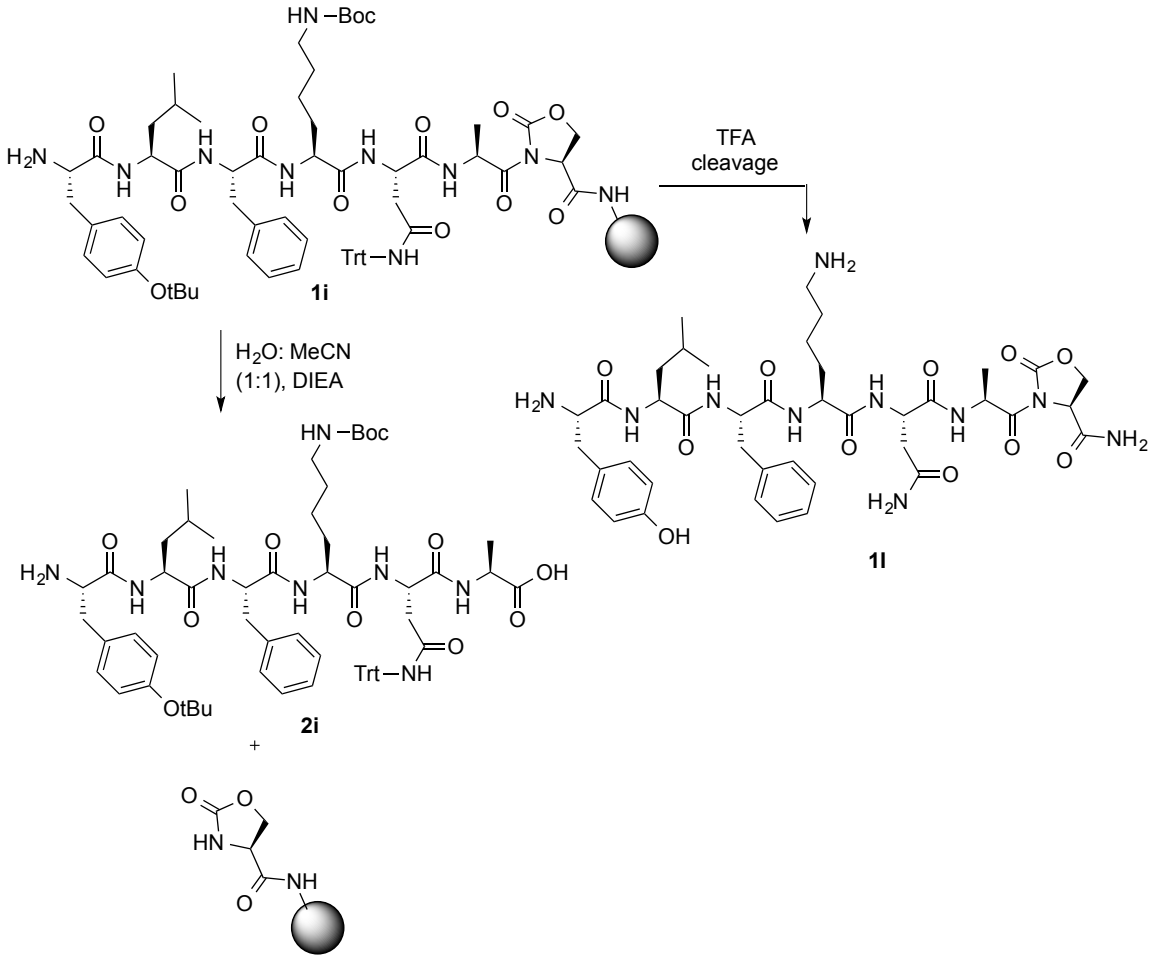
HPLC spectra of the cyclized starting material and the crude reaction mixture after hydrolysis; the product peak is labeled.


10.13
909.4
910.4
911.4
900
6
8
10
12
14

1H


HN–Boc
OtBu
Trt–NH
OH
23.86
1195.6
1217.6
1200
10
15
20
25
mi

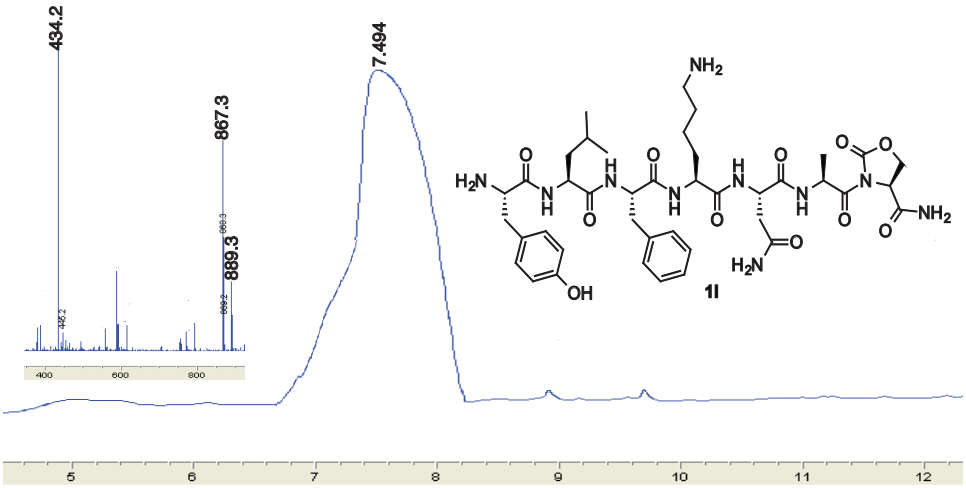
2h

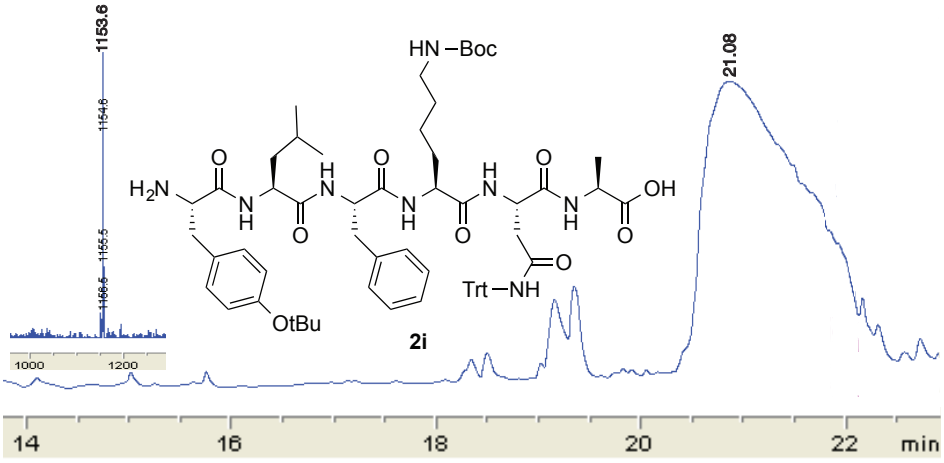


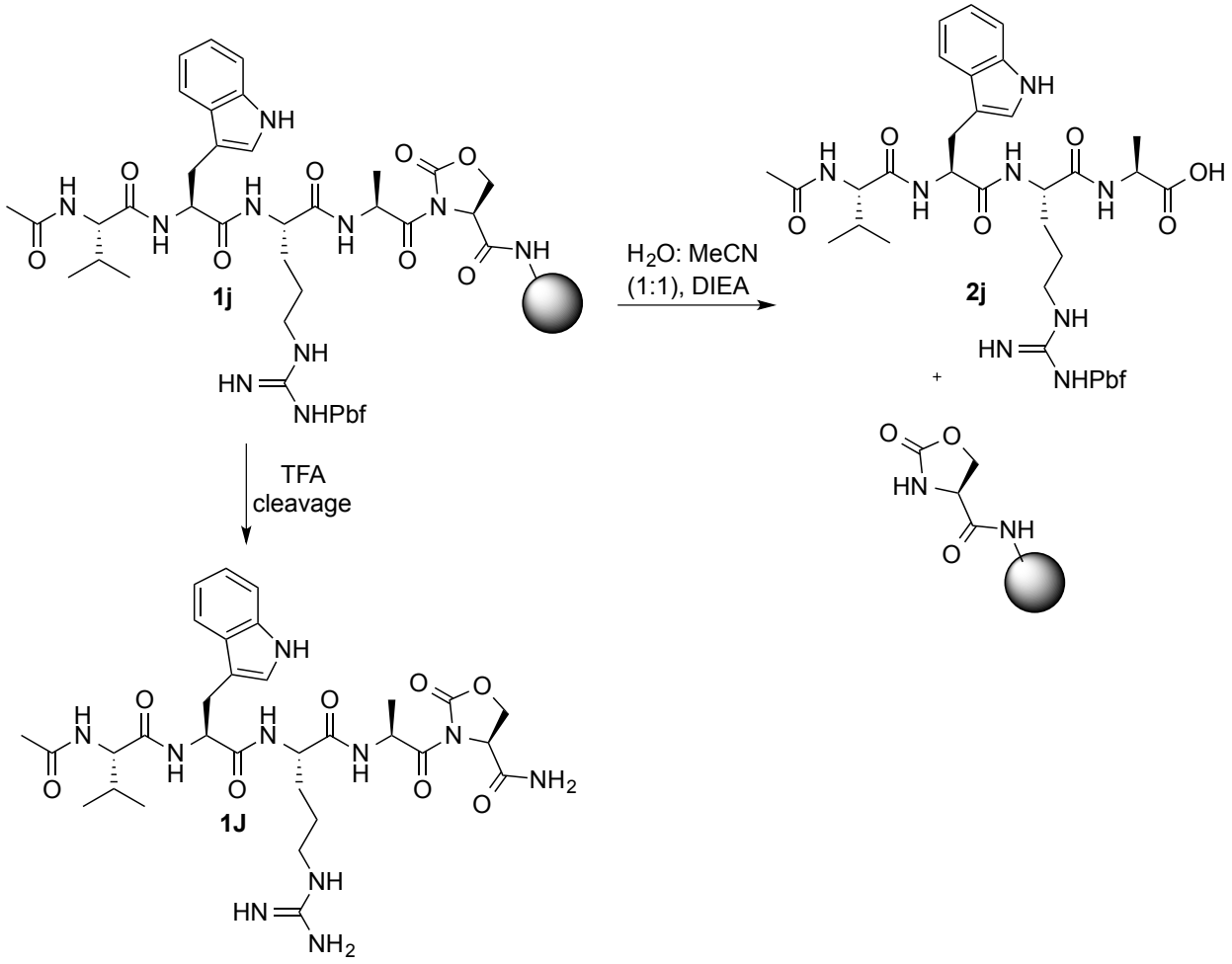
Tyr-Leu-Phe-Lys-Asn-Ala-Oxd (1I). LCMS: *m/z* 867.3 (calcd $[M+H]^+$ = 867.43), 434.2 (calcd $[M+2H]^{2+}$ = 434.21), 889.3 (calcd $[M+Na]^+$ = 889.43), Purity: >95% (HPLC analysis at 220 nm). Retention time: 7.49 min.

Tyr(tBu)-Leu-Phe-Lys(Boc)-Asn(Trt)-Ala (2i). LCMS: *m/z* 1153.6 (calcd $[M+H]^+$ = 1153.63), Purity: >95% (HPLC analysis at 220 nm). Retention time: 21.08 min.

HPLC spectra of the cyclized starting material and the crude reaction mixture after hydrolysis; the product peak is labeled.


434.2
867.3
889.3
7.494
1l
5
6
7
8
9
10
11
12

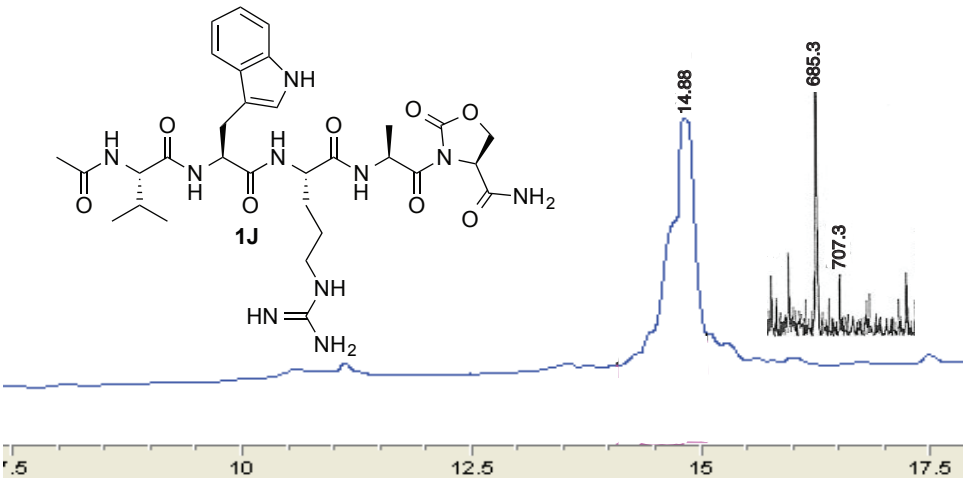

1153.6
HN–Boc
21.08
OtBu
Trt–NH
2i
14
16
18
20
22
min

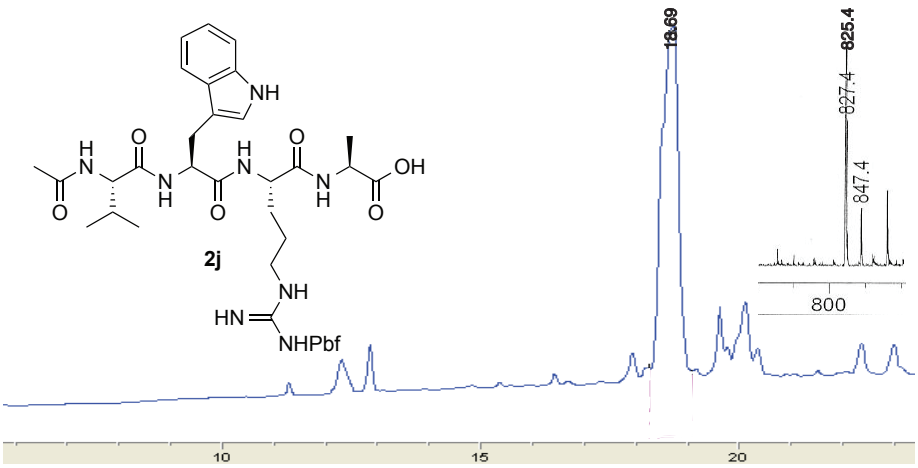


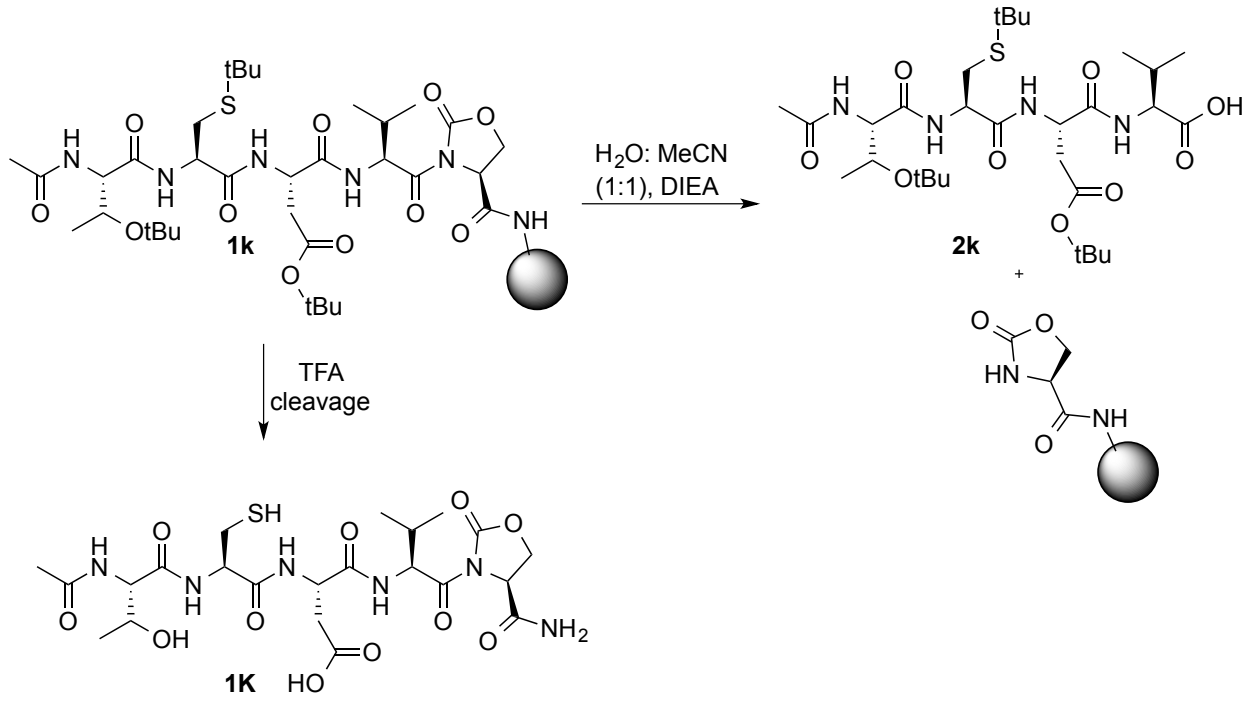
Ac-Val-Trp-Arg-Ala-Oxd (1J). LCMS: *m/z* 685.3 (calcd $[M+H]^+$ = 685.76), 707.4 (calcd $[M+Na]^+$ = 707.76), Purity: >95% (HPLC analysis at 220 nm). Retention time: 14.88 min.

Ac-Val-Trp-Arg(Pbf)-Ala (2j). LCMS: *m/z* 825.4 (calcd $[M+H]^+$ = 825.63), 847.4 (calcd $[M+Na]^+$ = 847.63), Purity: >95% (HPLC analysis at 220 nm). Retention time: 18.69 min.

HPLC spectra of the cyclized starting material and the crude reaction mixture after hydrolysis; the product peak is labeled.


1J
14.88
685.3
707.3
10
12.5
15
17.5

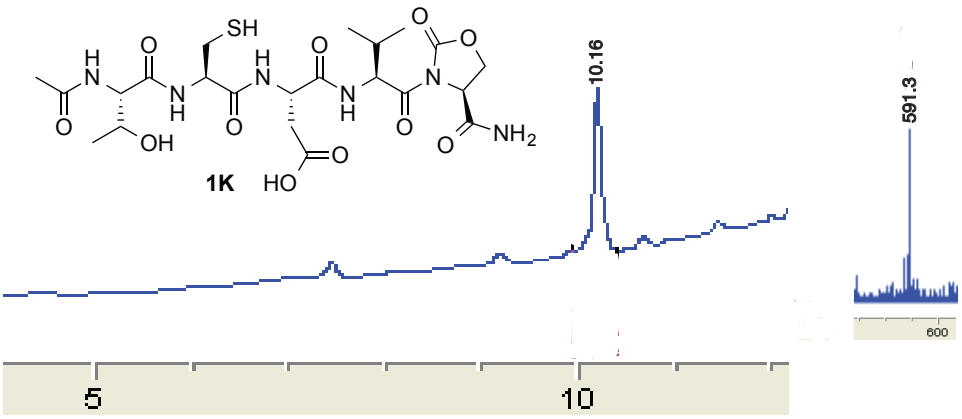

2j
18.69
825.4
827.4
847.4
800
10
15
20

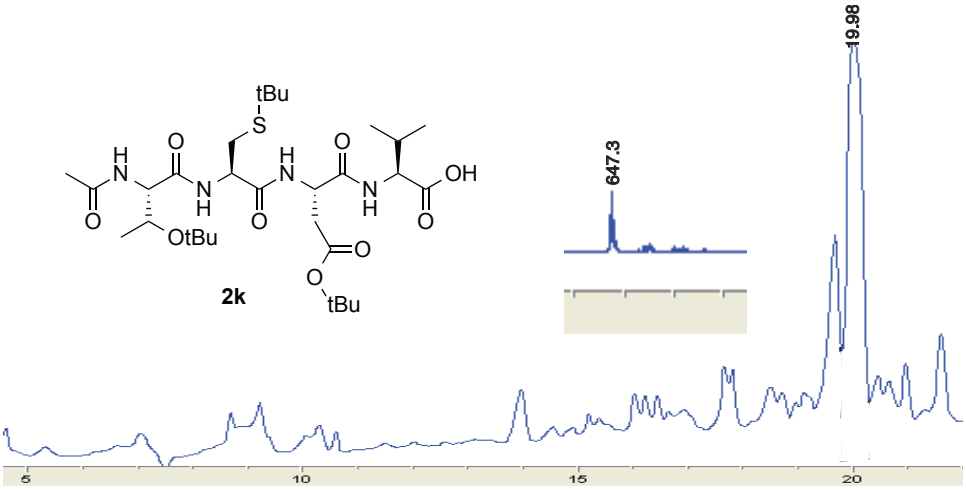

1k
H2O: MeCN
(1:1), DIEA
2k
TFA
cleavage
1K

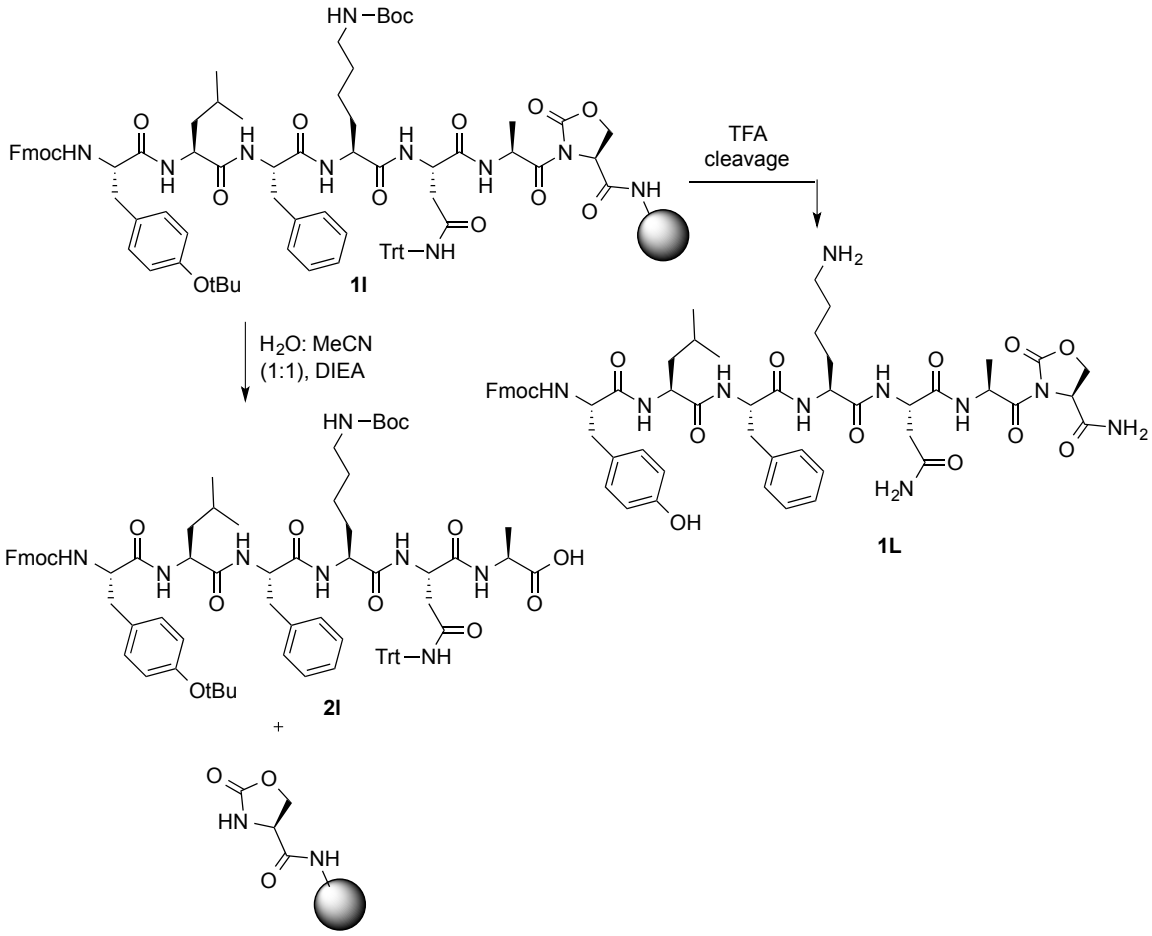
Ac-Thr-Cys-Asp-Val-Oxd (1K). LCMS: *m/z* 591.3 (calcd $[M+H]^+$ = 591.5), Purity: >95% (HPLC analysis at 220 nm). Retention time: 10.16 min.

Ac-Thr(tBu)-Cys(tBu)-Asp(tBu)-Val (2k). LCMS: *m/z* 647.3 (calcd $[M+H]^+$ = 647.36), Purity: >95% (HPLC analysis at 220 nm). Retention time: 19.98 min.

HPLC spectra of the cyclized starting material and the crude reaction mixture after hydrolysis; the product peak is labeled.





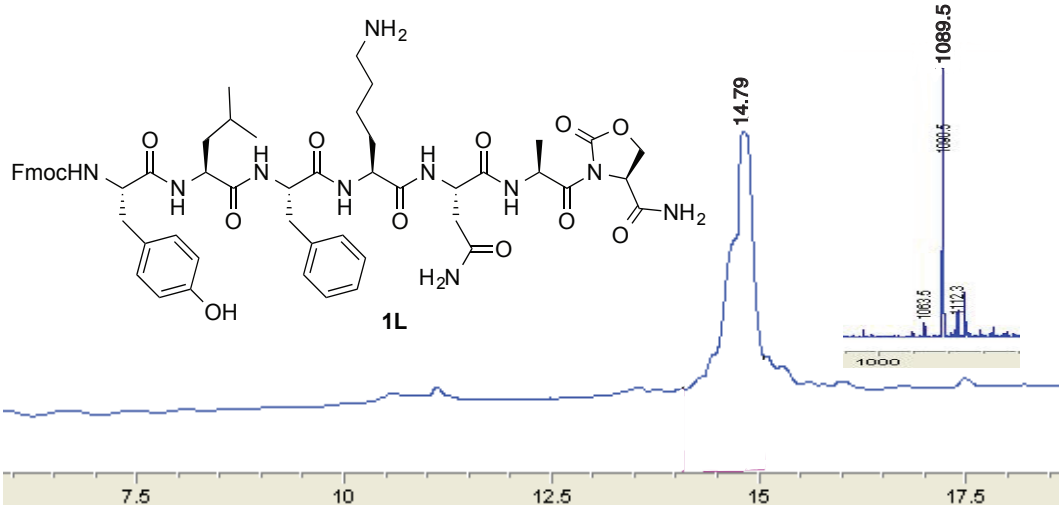


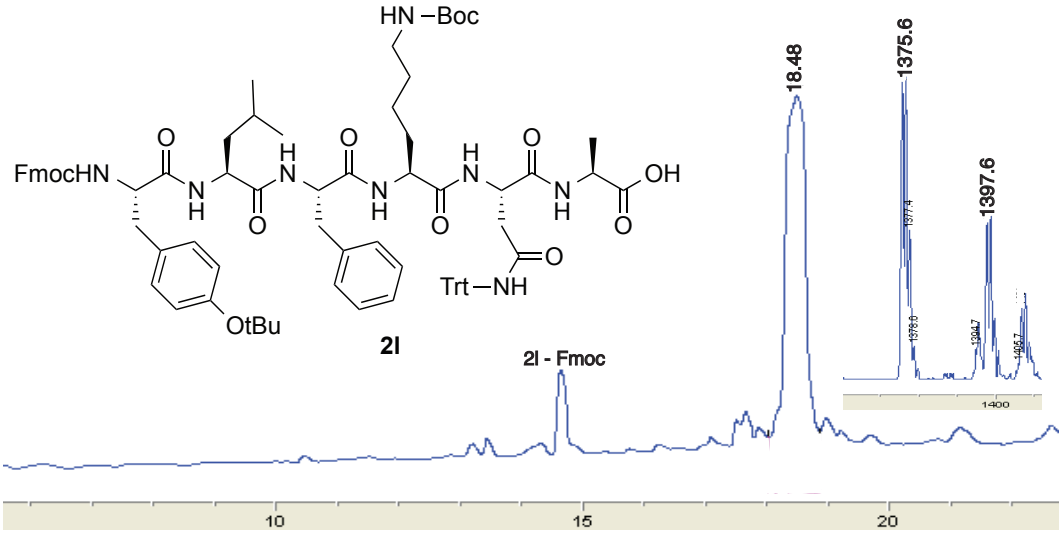
Fmoc-Tyr-Leu-Phe-Lys-Asn-Ala-Oxd (1L). LCMS: *m/z* 1089.5 (calcd $[M+H]^+$ = 1089.50), Purity: >95% (HPLC analysis at 220 nm). Retention time: 14.79 min.

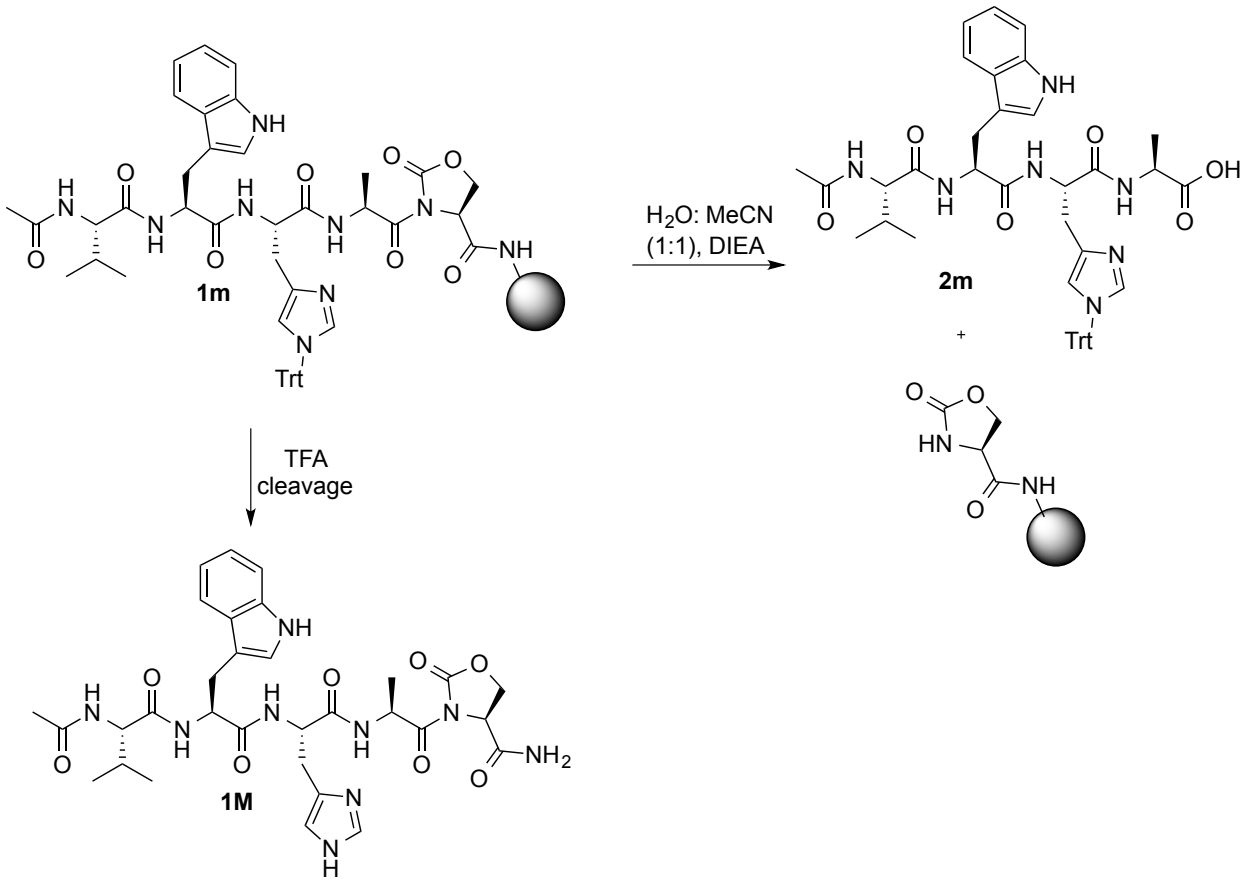
Fmoc-Tyr(tBu)-Leu-Phe-Lys(Boc)-Asn(Trt)-Ala (2l). LCMS: *m/z* 1375.6 (calcd $[M+H]^+$ = 1375.69), 1397.6 (calcd $[M+Na]^+$ = 1397.69) Purity: >95% (HPLC analysis at 220 nm). Retention time: 18.48 min

Tyr(tBu)-Leu-Phe-Lys(Boc)-Asn(Trt)-Ala (2l-Fmoc). LCMS: *m/z* 1153.6 (calcd $[M+H]^+$ = 1153.69), Purity: >95% (HPLC analysis at 220 nm). Retention time: 14.9 min

HPLC spectra of the cyclized starting material and the crude reaction mixture after hydrolysis; the product peak is labeled.


FmocHN
NH2
OH
NH2
H2N
1L
14.79
1089.5
1000
7.5
10
12.5
15
17.5

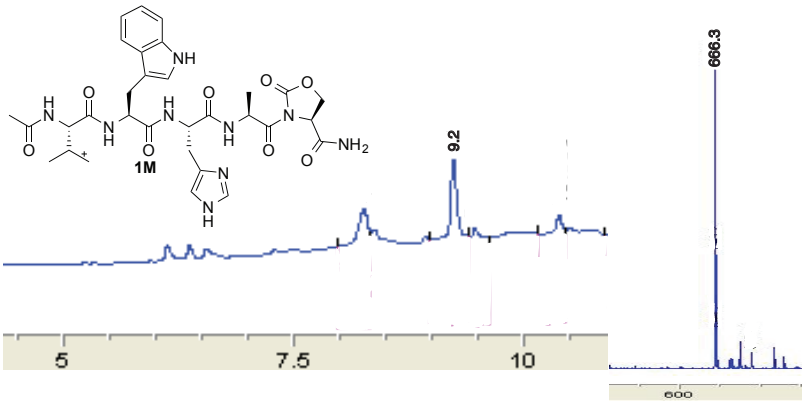

HN –Boc
FmocHN
OH
OtBu
Trt–NH
2l
2l - Fmoc
18.48
1375.6
1397.6
1400
10
15
20

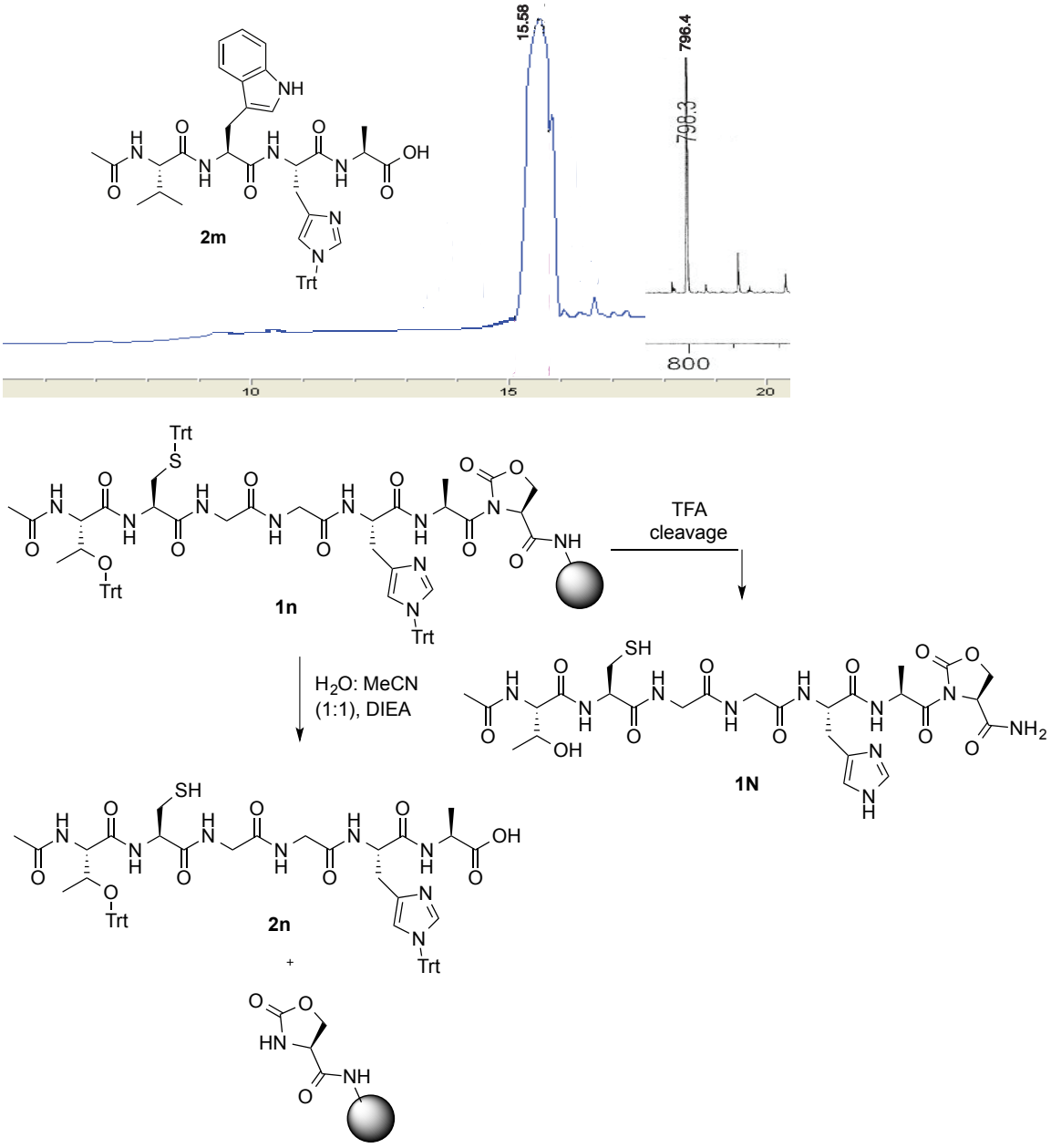


Ac-Val-Trp-His-Ala-Oxd (1M). LCMS: *m/z* 666.3 (calcd $[M+H]^+$ = 666.6), Purity: >95% (HPLC analysis at 220 nm). Retention time: 9.2 min

Ac-Val-Trp-His(Trt)-Ala (2m). LCMS: *m/z* 796.4 (calcd $[M+H]^+$ = 796.5), Purity: >95% (HPLC analysis at 220 nm). Retention time: 15.58 min

HPLC spectra of the cyclized starting material and the crude reaction mixture after hydrolysis; the product peak is labeled.

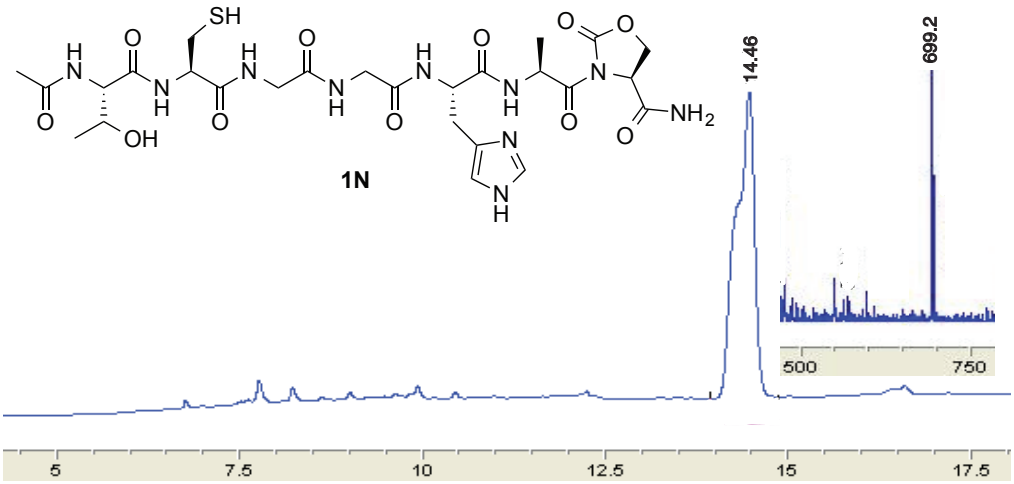


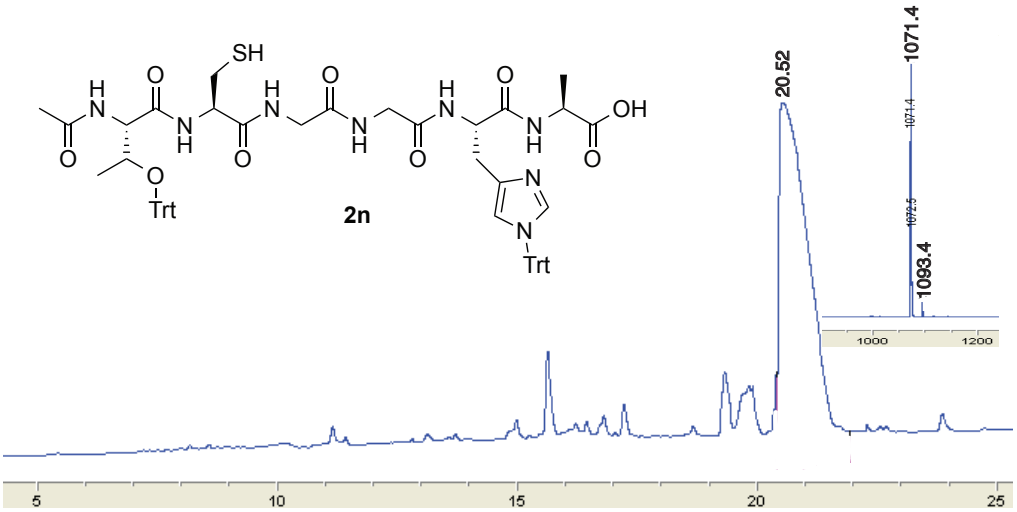


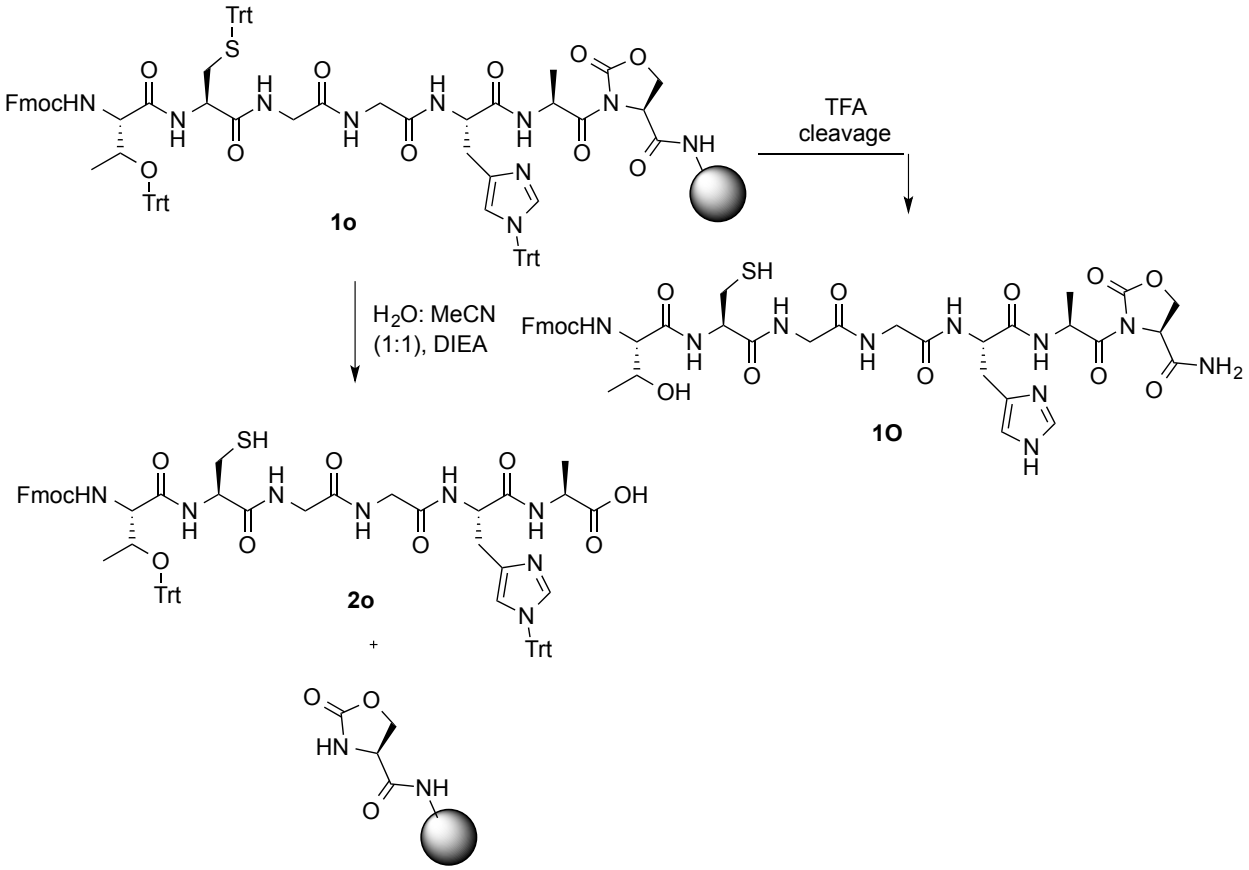
Ac-Thr-Cys-Gly-Gly-His-Ala-Oxd (1N). LCMS: *m/z* 699.2 (calcd $[M+H]^+$ = 699.66), Purity: >95% (HPLC analysis at 220 nm). Retention time: 14.46 min

Ac-Thr(Trt)-Cys-Gly-Gly-His(Trt)-Ala (2n). LCMS: *m/z* 1071.4 (calcd $[M+H]^+$ = 1071.5), 1093.4 (calcd $[M+Na]^+$ = 1093.5) Purity: >95% (HPLC analysis at 220 nm). Retention time: 20.52 min

HPLC spectra of the cyclized starting material and the crude reaction mixture after hydrolysis; the product peak is labeled.


SH
OH
NH2
1N
14.46
699.2
500
750
5
7.5
10
12.5
15
17.5


SH
OH
Trt
2n
Trt
20.52
1071.4
1093.4
1000
1200
5
10
15
20
25

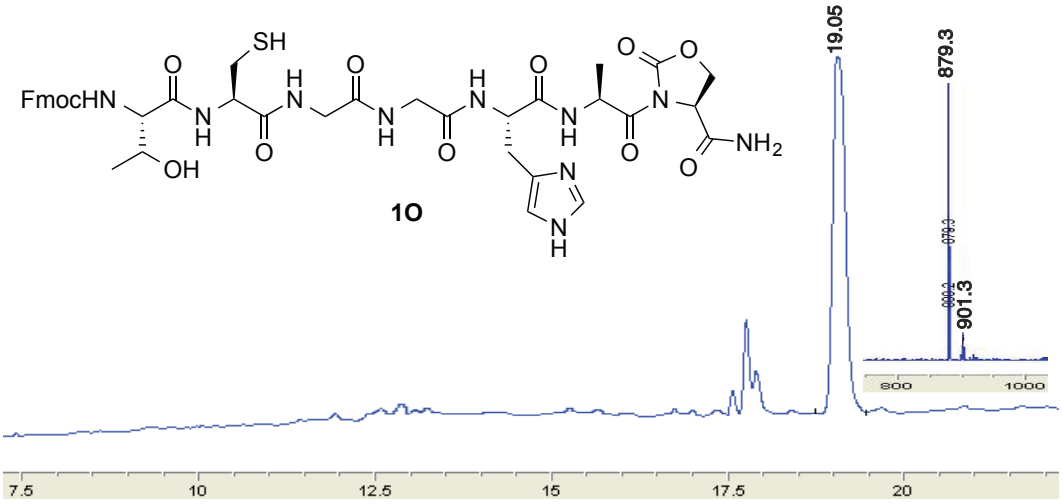


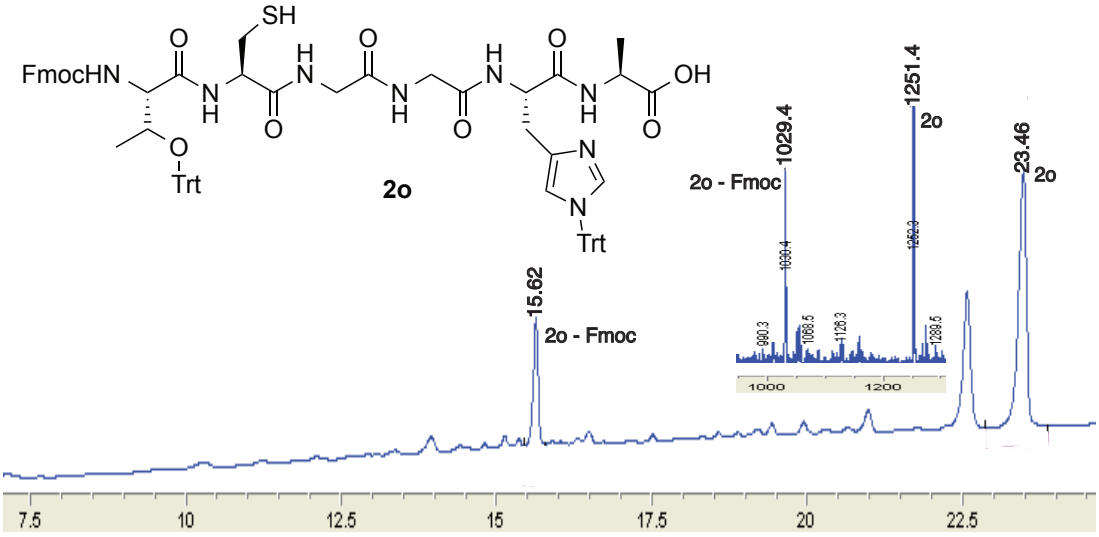
Fmoc-Thr-Cys-Gly-Gly-His-Ala-Oxd (1O). LCMS: *m/z* 879.3 (calcd $[M+H]^+$ = 879.66), 901.3 (calcd $[M+Na]^+$ = 900.66), Purity: >95% (HPLC analysis at 220 nm). Retention time: 19.05 min

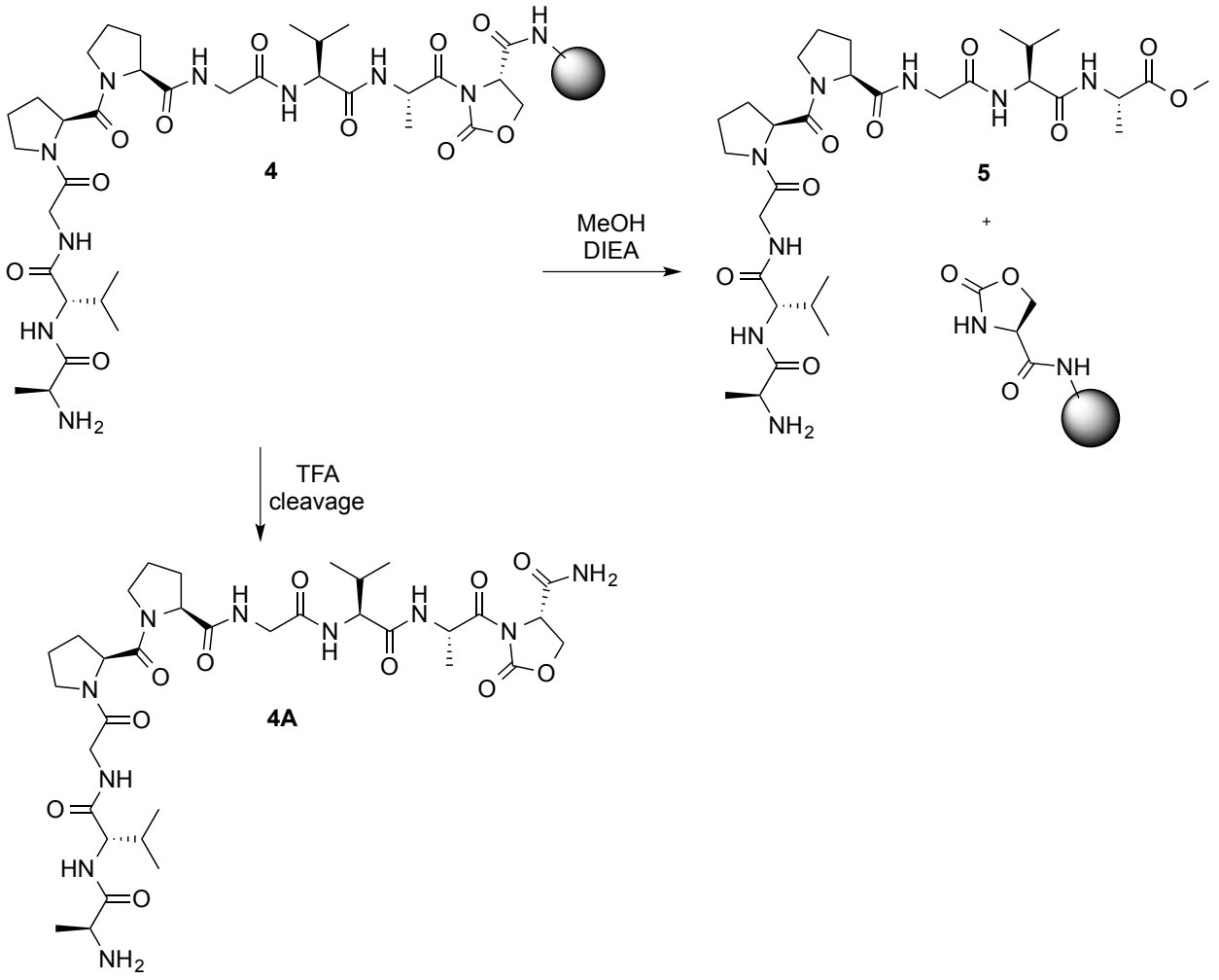
Fmoc-Thr(Trt)-Cys-Gly-Gly-His(Trt)-Ala (2o). LCMS: *m/z* 1251.4 (calcd $[M+H]^+$ = 1251.57, Purity: >95% (HPLC analysis at 220 nm). Retention time: 23.46 min

Thr(Trt)-Cys-Gly-Gly-His(Trt)-Ala (2o-Fmoc). LCMS: *m/z* 1029.4 (calcd $[M+H]^+$ = 1029.57), Purity: >95% (HPLC analysis at 220 nm). Retention time: 15.62 min

HPLC spectra of the cyclized starting material and the crude reaction mixture after hydrolysis; the product peak is labeled.


FmocHN
SH
OH
NH2
1O
19.05
879.3
901.3
800
1000
7.5
10
12.5
15
17.5
20

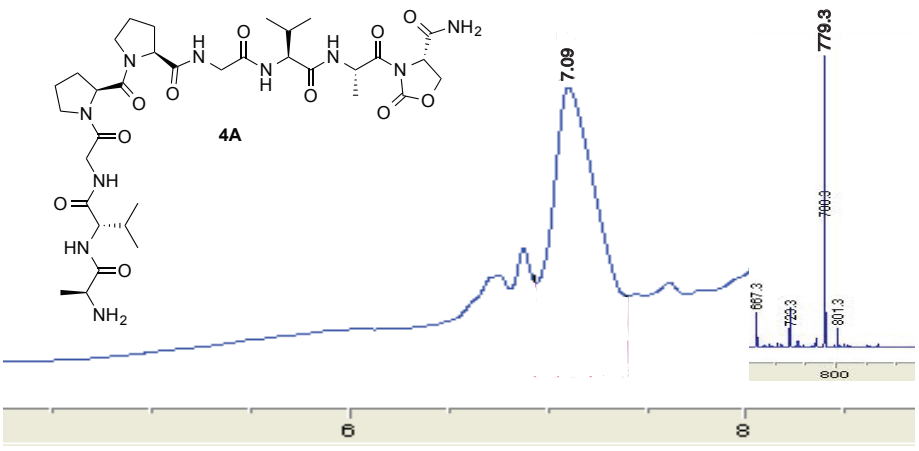

FmocHN
SH
OH
Trt
Trt
2o
15.62
2o - Fmoc
1029.4
2o - Fmoc
1251.4
2o
23.46
2o
1000
1200
7.5
10
12.5
15
17.5
20
22.5

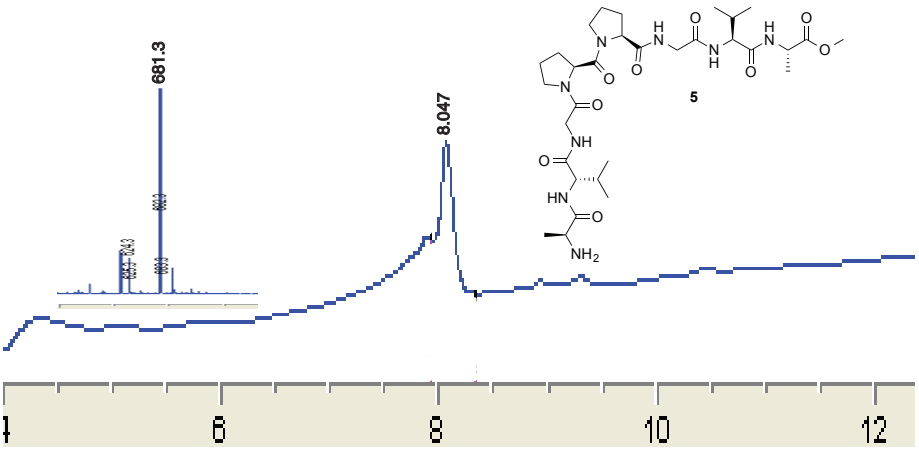


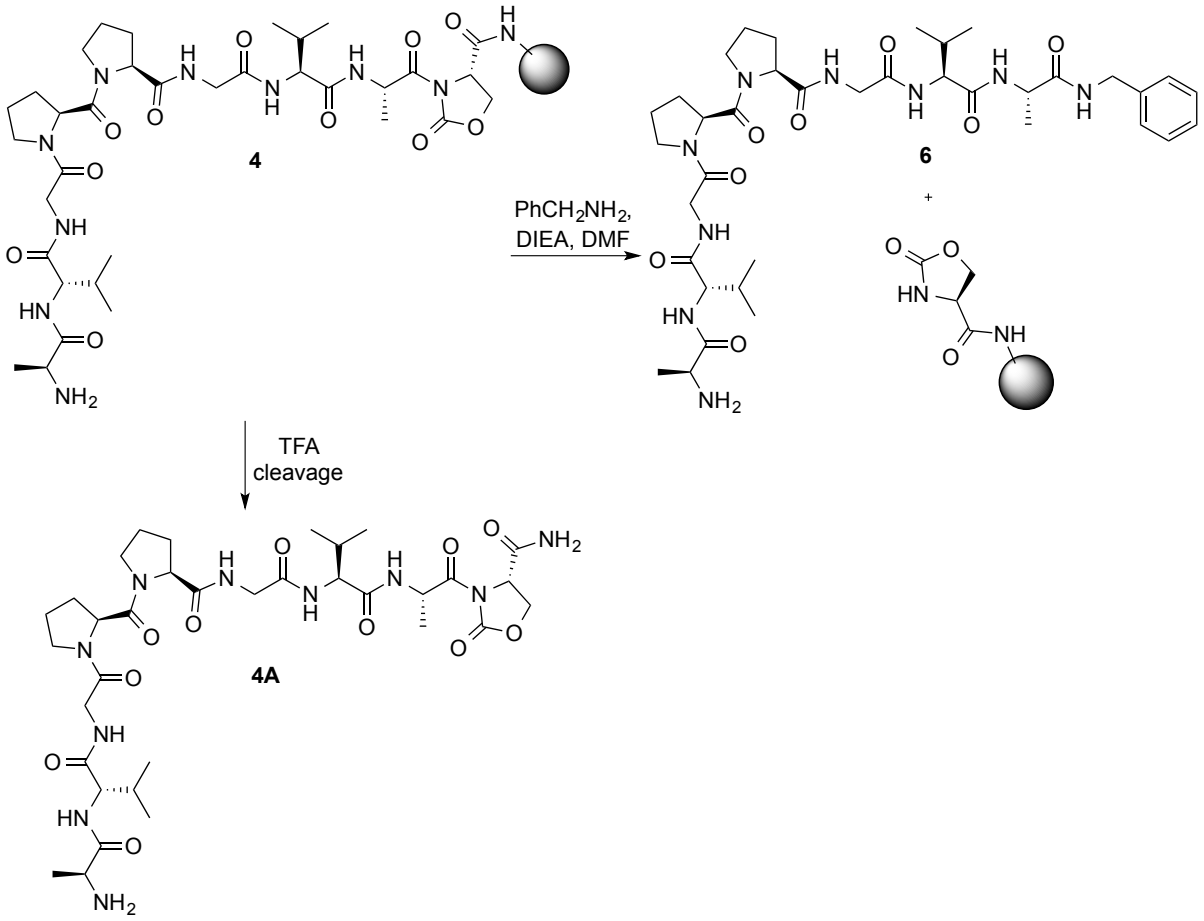
Ala-Val-Gly-Pro-Pro-Gly-Val-Ala-Oxd (4A). LCMS: *m/z* 779.3 (calcd $[M+H]^+$ = 779.37), Purity: >95% (HPLC analysis at 220 nm). Retention time: 7.09 min

Ala-Val-Gly-Pro-Pro-Gly-Val-Ala-OMe (5). LCMS: *m/z* 681.3 (calcd $[M+H]^+$ = 681.46, Purity: >95% (HPLC analysis at 220 nm). Retention time: 8.047 min

HPLC spectra of the cyclized starting material and the crude reaction mixture after incubation in methanol solution; the product peak is labeled.


4A
7.09
779.3
788.3
667.3
729.3
801.3
800
6
8

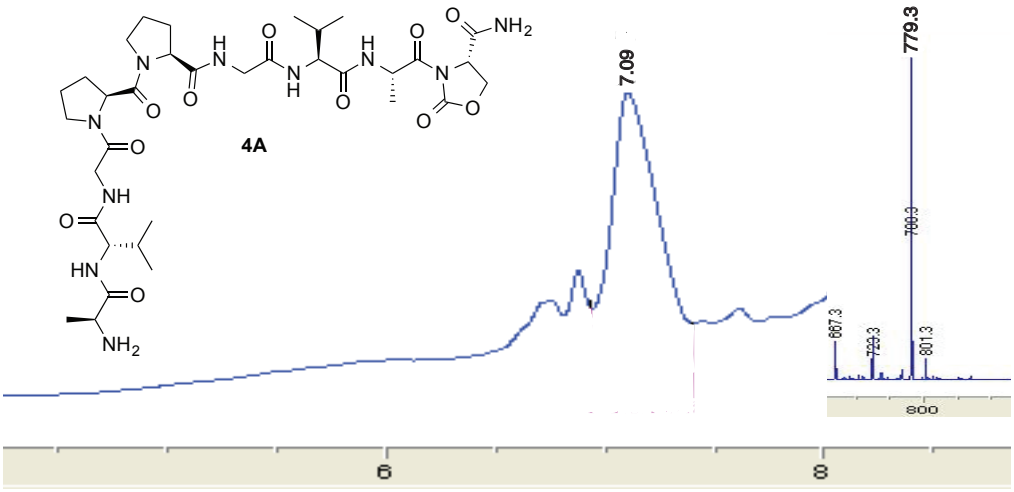

681.3
8.047
5
4
6
8
10
12

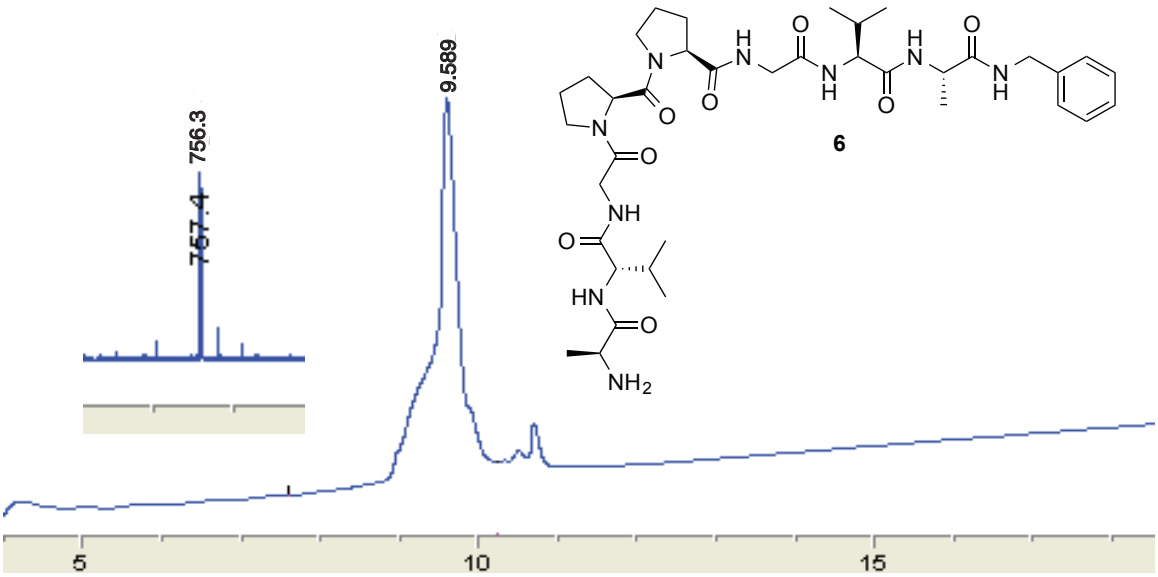


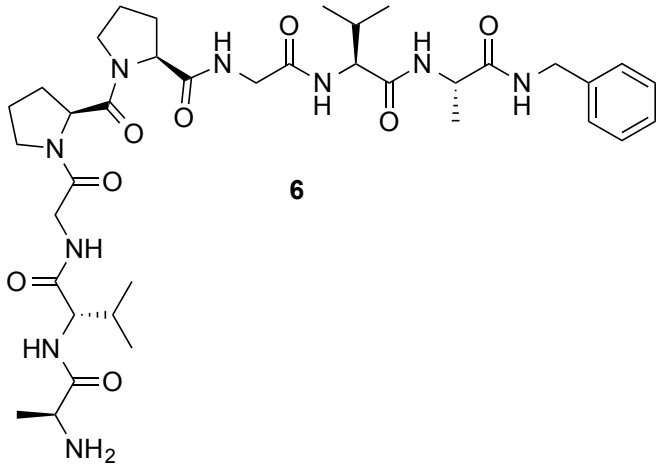
Ala-Val-Gly-Pro-Pro-Gly-Val-Ala-Oxd (4A). LCMS: *m/z* 779.3 (calcd $[M+H]^+$ = 779.37), Purity: >95% (HPLC analysis at 220 nm). Retention time: 7.09 min

Ala-Val-Gly-Pro-Pro-Gly-Val-Ala-NHBz (6). LCMS: *m/z* 756.3 (calcd $[M+H]^+$ = 756.57, Purity: >95% (HPLC analysis at 220 nm). Retention time: 9.58 min

HPLC spectra of the cyclized starting material and the crude reaction mixture after incubation in solution of benzyl amine in DMF; the product peak is labeled.

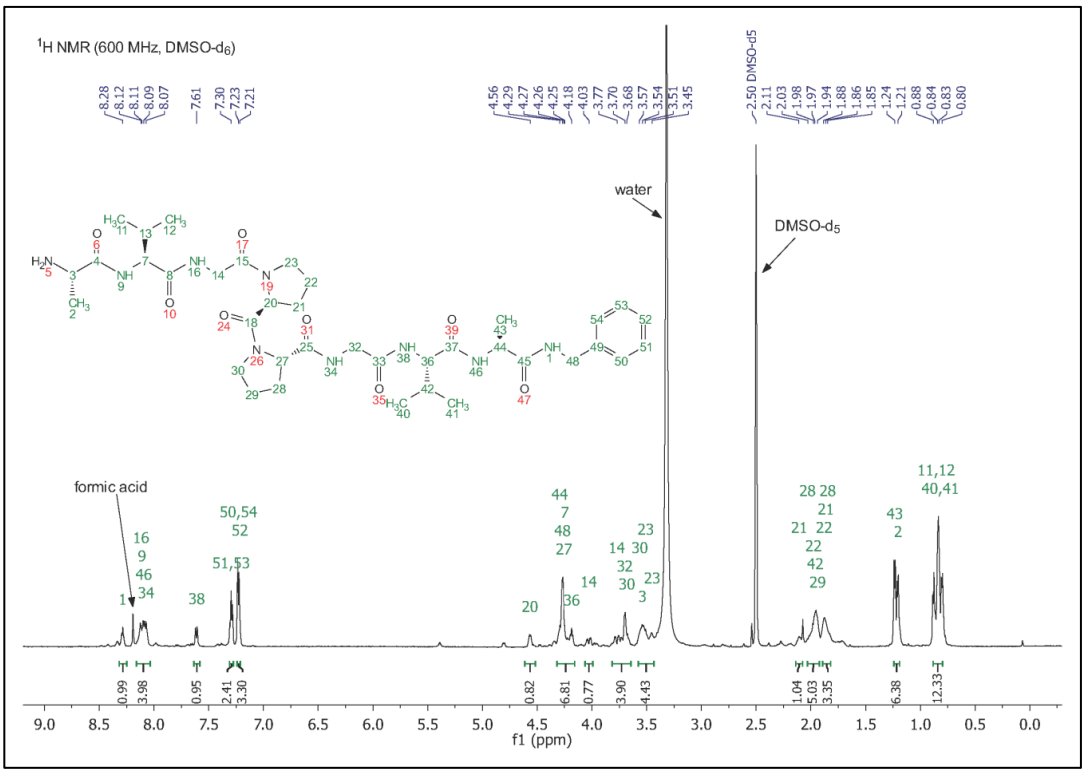

4A
7.09
779.3
780.3
667.3
729.3
801.3
800
6
8


9.589
756.3
757.4
6
5
10
15

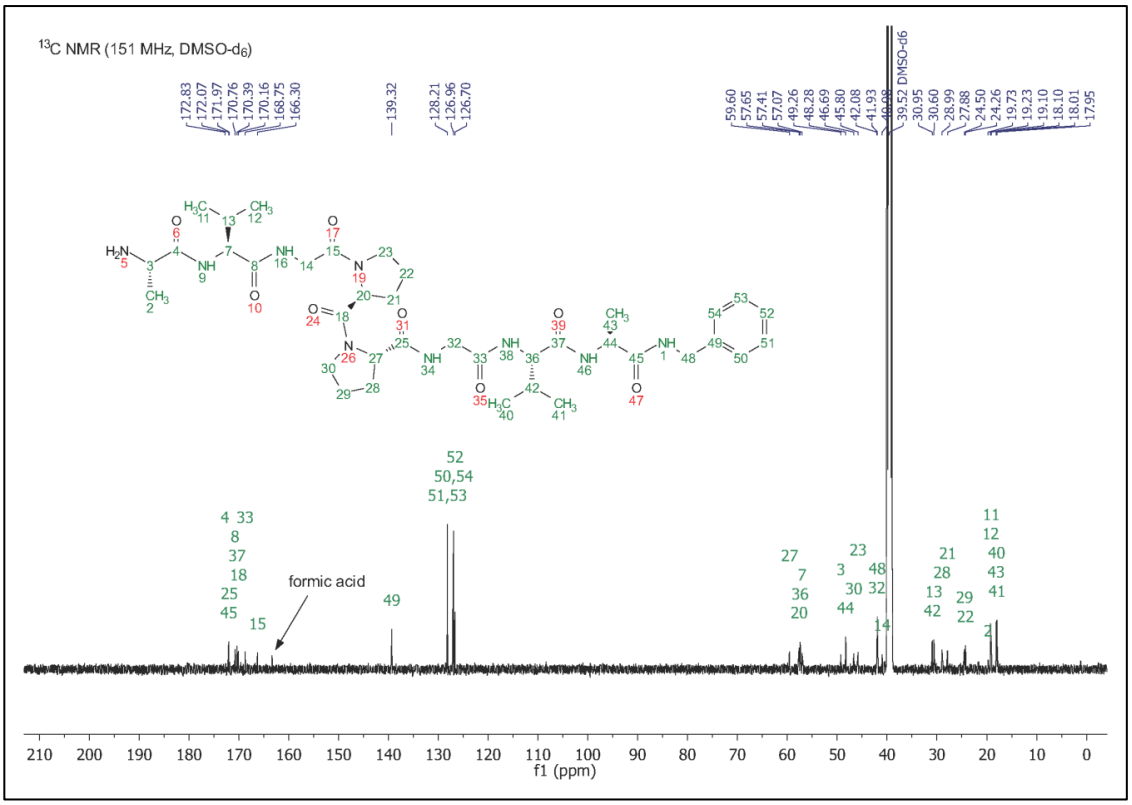

6

Ala-Val-Gly-Pro-Pro-Gly-Val-Ala-NHBz (6): ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.28 (t, *J* = 6.2 Hz, 1H), 8.14 – 8.05 (om, 4H), 7.61 (d, *J* = 8.6 Hz, 1H), 7.29 (t, *J* = 7.3 Hz, 2H), 7.25 – 7.20 (om, 3H), 4.56 (d, *J* = 8.3 Hz, 1H), 4.32 – 4.23 (om, 5H), 4.18 (t, *J* = 7.6 Hz, 1H), 4.03 (dd, *J* = 17.3, 5.6 Hz, 1H), 3.77 (dd, *J* = 17.3, 4.6 Hz, 1H), 3.72 – 3.65 (om, 3H), 3.59 – 3.42 (om, 4H), 2.14 – 2.07 (m, 1H), 2.04 – 1.80 (om, 9H), 1.24 (d, *J* = 6.5 Hz, 3H), 1.21 (d, *J* = 7.1 Hz, 3H), 0.90 – 0.78 (om, 12H), note: N-terminal protons were not detected. ^{13}C NMR (151 MHz, DMSO-d_6) δ 172.8,172.1, 172.0, 170.8, 170.4, 170.2, 168.8, 166.3, 139.3, 128.2, 127.0, 126.7, 59.6, 57.7, 57.4, 57.1, 49.3, 48.3, 46.7, 45.8, 42.1, 41.9, 41.0, 31.0, 30.6, 29.0, 27.9, 24.5, 24.3, 19.7, 19.2, 19.1, 18.1, 18.0, 17.9. HRMS (+ESI) calc. for $C_{37}H_{58}N_9O_8^+$: 756.4403, found 756.4403.

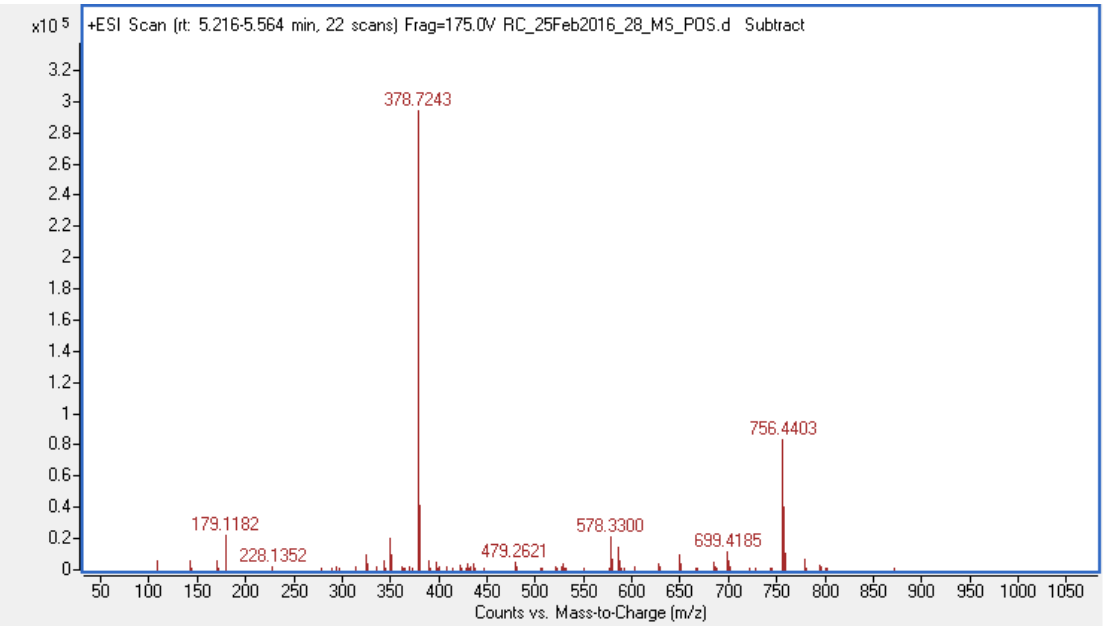
^{1}H NMR spectrum for Ala-Val-Gly-Pro-Pro-Gly-Val-Ala-NHBz 6, recorded at 600 MHz

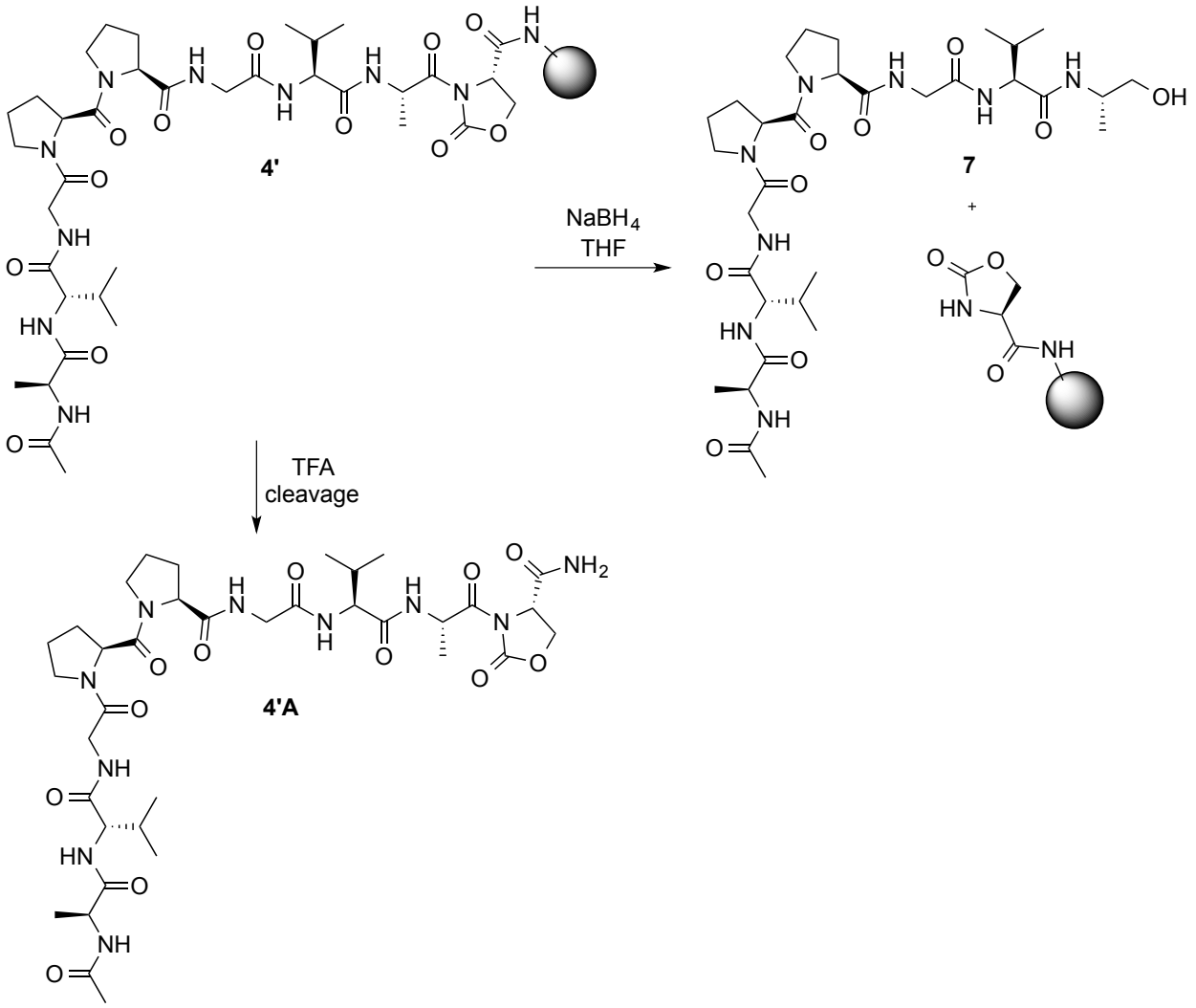


^{13}C NMR spectrum for **Ala-Val-Gly-Pro-Pro-Gly-Val-Ala-NHBz 6**, recorded at 151 MHz



HRMS (+ESI) data for **Ala-Val-Gly-Pro-Pro-Gly-Val-Ala-NHBz 6**

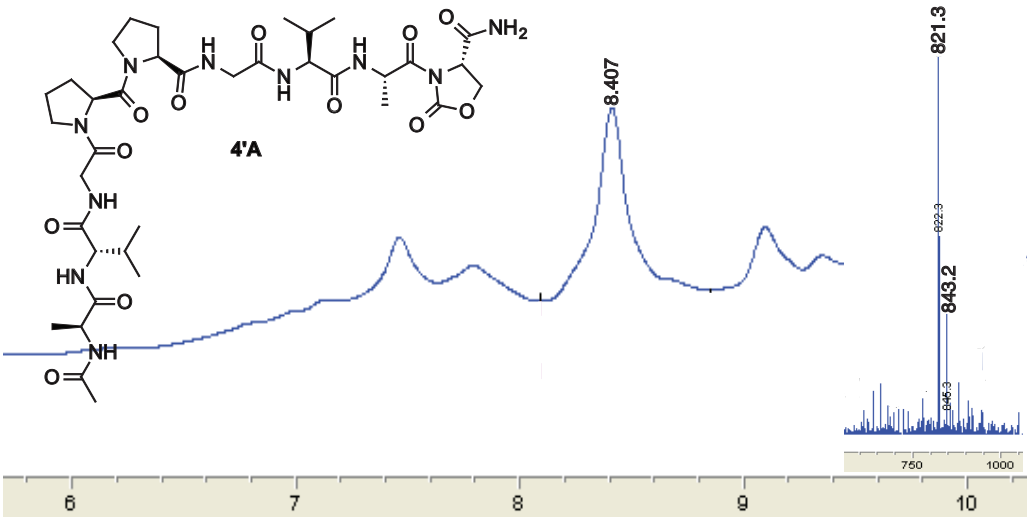


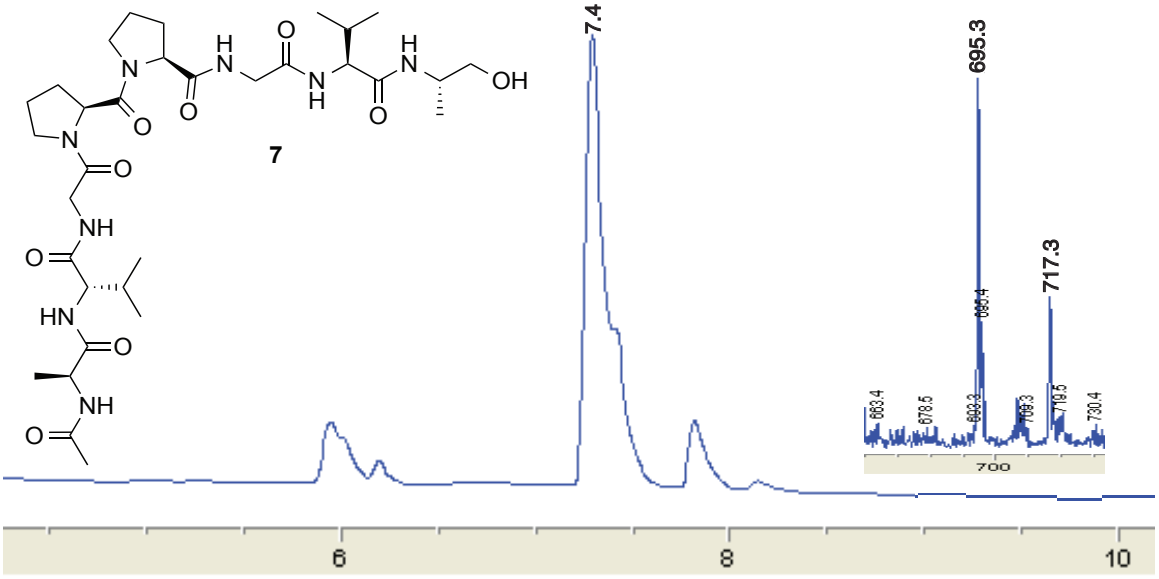


Ac-Ala-Val-Gly-Pro-Pro-Gly-Val-Ala-Oxd (4'A). LCMS: *m/z* 821.3 (calcd $[M+H]^+$ = 821.66), *m/z* 843.2 (calcd $[M+Na]^+$ = 843.66), Purity: >95% (HPLC analysis at 220 nm). Retention time: 8.407 min

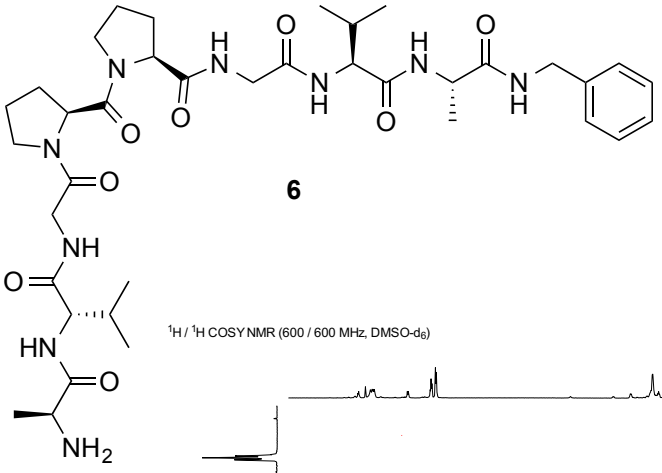
Ac-Ala-Val-Gly-Pro-Pro-Gly-Val-Ala-CH_2OH (7). LCMS: *m/z* 695.3 (calcd $[M+H]^+$ = 695.37); 717.3 (calcd $[M+Na]^+$ = 717.37) Purity: >95% (HPLC analysis at 220 nm). Retention time: 7.4 min

HPLC spectra of the cyclized starting material and the crude reaction mixture after incubation in sodium borohydride solution in THF; the product peak is labeled.

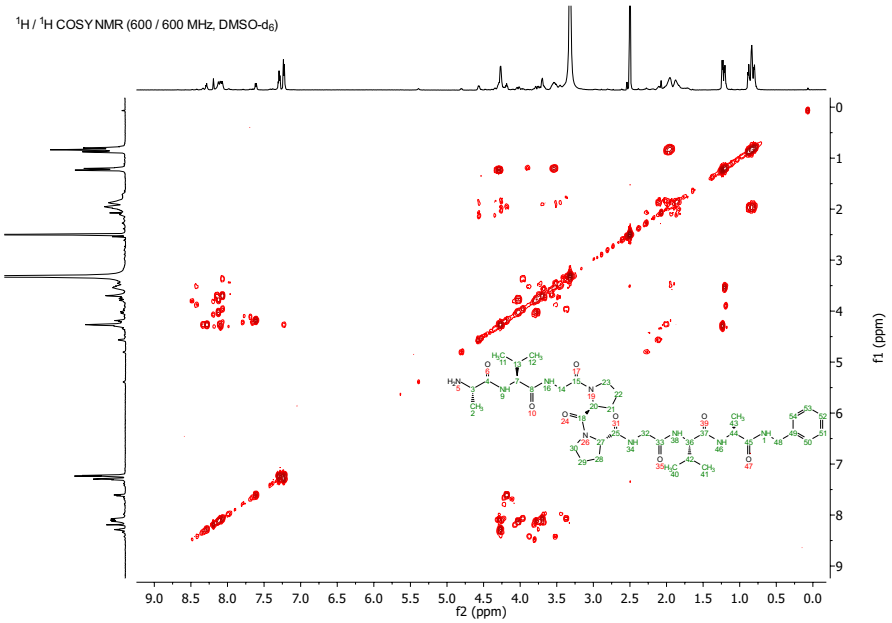

4'A
8.407
821.3
843.2
6
7
8
9
10
750
1000

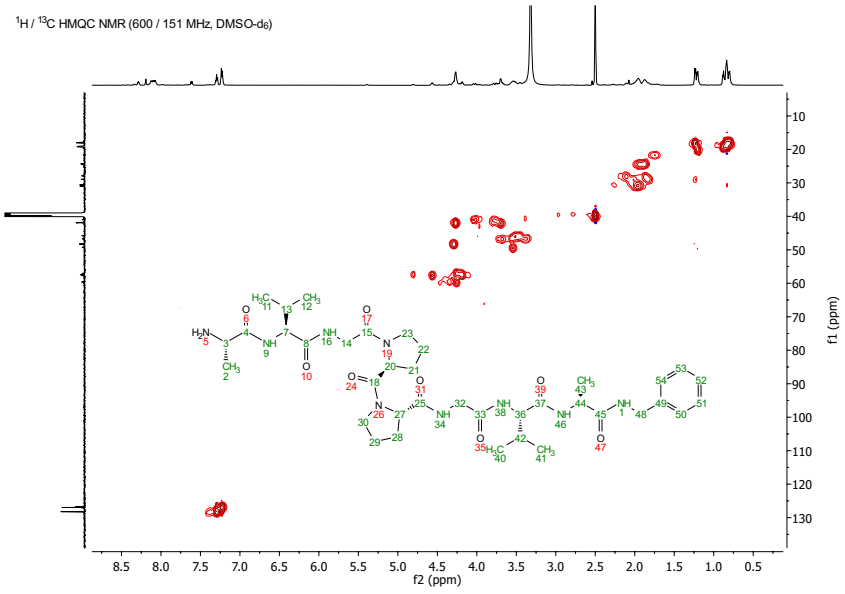

7
7.4
695.3
717.3
6
8
10
700

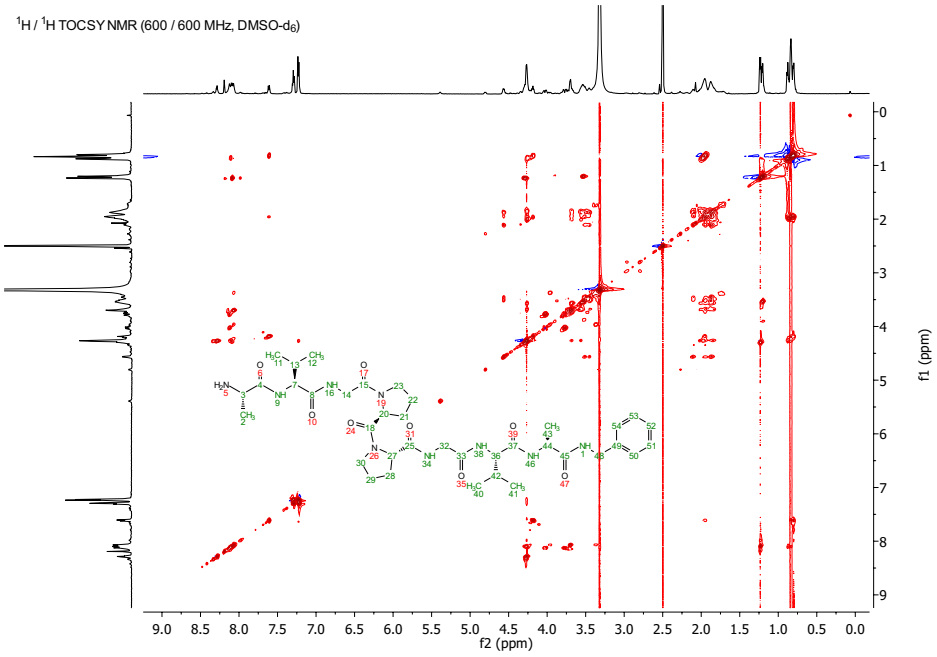
2 D NMR Data for Ala-Val-Gly-Pro-Pro-Gly-Val-Ala-NHBz, 6

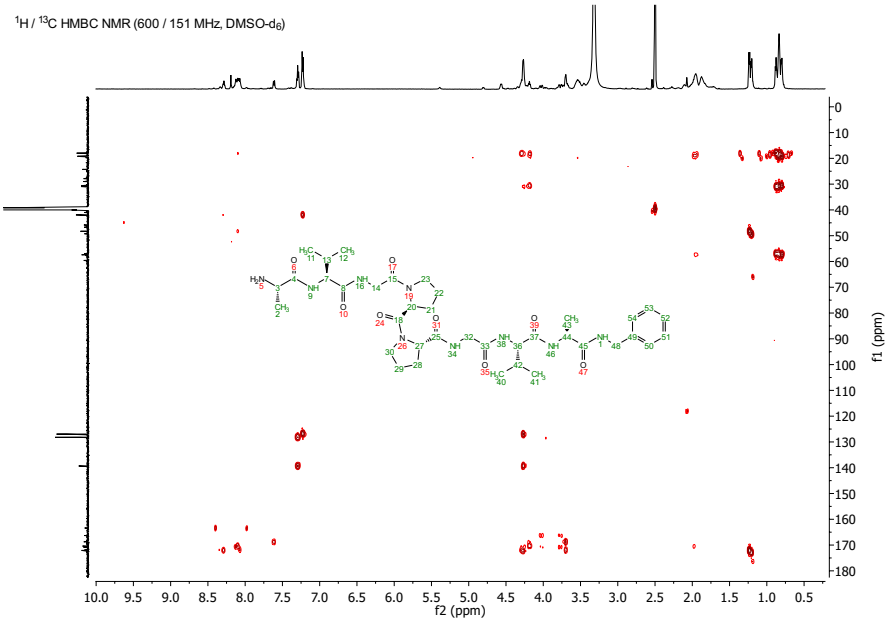


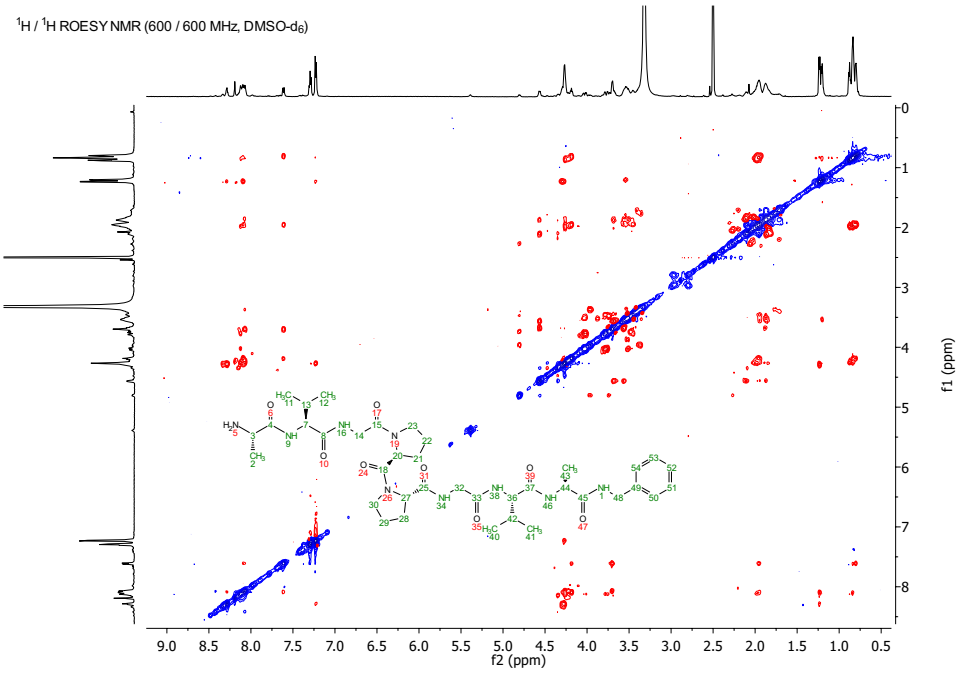
6






^{1}H / ^{1}H TOCSY NMR (600 / 600 MHz, DMSO-d$_6$)
f2 (ppm)
f1 (ppm)


^{1}H / ^{13}C HMBC NMR (600 / 151 MHz, DMSO-d$_6$)
f2 (ppm)
f1 (ppm)



References:

(1) Chan, W. C.; White, P. D. *Fmoc solid phase peptide synthesis : a practical approach*; Oxford University Press: New York, 2000.