Electronic Supplementary Material (ESI) for Chemical Communications

Supporting Information for: Expanding the accessible chemical space by solid phase synthesis of bicyclic homodetic peptides

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e-mail: jean-louis.reymond@ioc.unibe.ch;

Contents

Table S1. Homodetic polycyclic peptides from the literature.

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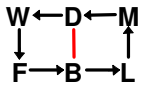
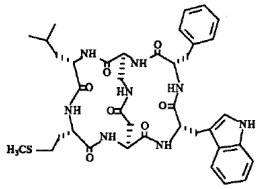
bicyclo[2.2.0]hexane</td><td>  </td><td>  </td></tr>
<tr><td colspan="3">(a) C. A. Maggi, M. Astolfi, S. Giuliani, C. Goso, S. Manzini, S. Meini, R. Patacchini, V. Pavone, C. Pedone, L. Quartara, A. R. Renzetti, A. Giachetti, J. Pharmacol. Exp. Ther., 1994, 271, 1489; (b) L. Quartara, V. Pavone, C. Pedone, A. Lombardi, A. R. Renzetti, C. A. Maggi, Regul. Pept., 1996, 65, 55; (c) A. Lombardi, G. D'Auria, O. Maglio, F. Nastri, L. Quartara, C. Pedone, V. Pavone, J. Am. Chem. Soc., 1998, 120, 5879; (d) M. Teixido, M. Altamura, L. Quartara, A. Giolitti, C. A. Maggi, E. Giralt, F. Albericio, J. Comb. Chem., 2003, 5, 760.</td></tr>
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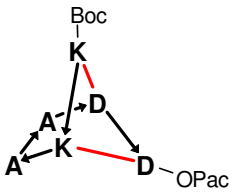
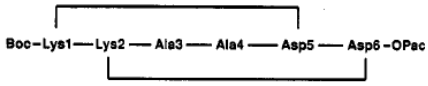
bicyclo[2.1.1]hexane</td><td>  </td><td>  </td></tr>
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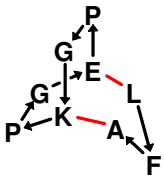
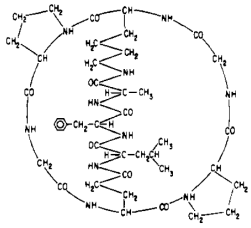
bicyclo[3.2.2]nonane</td><td>  </td><td>  </td></tr>
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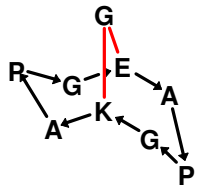
bicyclo[3.3.1]nonane</td><td>  </td><td>cyclo-(Glu¹–Ala²–Pro³–Gly⁴–Lys⁵–Ala⁶–Pro⁷–Gly⁸)-cyclo-(1γ → 5ε) Gly^c</td></tr>
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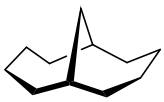
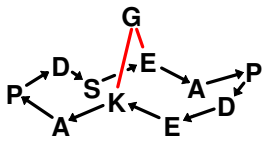
<tr><td> 
bicyclo[4.4.1]undecane</td><td>  </td><td>cyclo-(Ala¹-Pro²-Asp³-Glu⁴-Lys⁵-Ala⁶-Pro⁷
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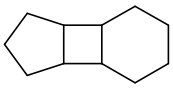
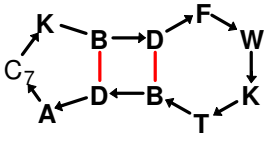
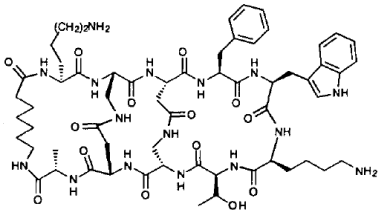
<tr><td> 
tricyclo[5.4.0.0^{2,6}]undecane</td><td> 
C_7 = 7-aminoheptanoic acid</td><td>  </td></tr>
<tr><td colspan="3">(a) R. Hirschmann, W. Yao, B. Arison, L. Maechler, A. Rosegay, P. A. Sprengler, A. B. Smith III, Tetrahedron Lett., 1996, 37, 5637; (b) R. Hirschmann, W. Yao, B. Arison, L. Maechler, A. Rosegay, P. A. Sprengler, A. B. Smith III, Tetrahedron, 1998, 54, 7179.</td></tr>
</table>

General Information

All reagents were purchased in the highest quality available either from Sigma Aldrich, Bachem, Acros Organics, Iris Biotech, Fluorochem UK. PyBOP, amino acids and their derivatives were purchased from Advanced ChemTech or Novabiochem. For SPPS amino acids were used as the following derivatives: Fmoc-Ala-OH, Fmoc-Dap(Boc)-OH, Fmoc-Glu(OAll)-OH, Fmoc-Glu-OAll, Fmoc-Gly-OH, Fmoc-Leu-OH, Fmoc-Lys(Boc)-OH, Fmoc-D-Lys(Boc)-OH, Fmoc-Phe-OH, Fmoc-Pro-OH, Fmoc-Val-OH. Fmoc-Ala-TentaGel S AC resin was purchased from Rapp Polymere, Fmoc-Gly-Wang resin was purchased from Fluorochem UK, Fmoc-Phe-Wang and Fmoc-Val-Wang resins from Novabiochem. Cyclic peptide syntheses were performed manually in polypropylene syringes fitted with a polyethylene frit, a Teflon stopcock and stopper. All solvents used in reactions on solid phase and in solution were bought in p.a. quality and distilled prior to use. Analytical RP-HPLC was performed with an Ultimate 3000 Rapid Separation LC System (DAD-3000RS diode array detector) using an Acclaim RSLC 120 C18 column (2.2 µm, 120 Å, 3×50 mm, flow 1.2 mL/min) from Dionex. Data recording and processing was done with Dionex Chromeleon Management System Version 6.80 (analytical RP-HPLC). Preparative RP-HPLC was performed with a Waters Prep LC Controller System using a Dr. Maisch GmbH Reprospher column (C18-DE, 100×30 mm, particle size 5 µm, pore size 100 Å, flow rate 60 mL/min). Compounds were detected by UV absorption at 214 nm using a Waters 486 Tunable Absorbance Detector. The following elution solutions were used for all RP-HPLC: **A** Milli-Q deionized H_2O with 0.1% TFA; **D** Milli-Q deionized H_2O/HPLC-grade acetonitrile N (40:60) with 0.1% TFA. MS spectra, recorded on a Thermo Scientific LTQ OrbitrapXL, were provided by the MS analytical service of the Department of Chemistry and Biochemistry at the University of Bern (group PD Dr. Stefan Schürch).

NMR data were acquired at a temperature of 298 K using a Bruker AvanceII 500 MHz NMR spectrometer equipped with an inverse dual channel (^{1}H, X) *z*-gradient probehead (BBI), or on a Bruker AvanceII 400 MHz NMR spectrometer equipped with an inverse dual channel (^{1}H, X) *z*-gradient probehead (BBI). All spectra were recorded in 90% H_2O/D_2O, with the exception of **6b** which was analyzed in 50% H_2O/DMSO-d_6. Proton resonances were assigned using TOCSY and HSQC data. All NMR data were processed using Topspin (versions 2.1 or 3.0, Bruker Switzerland).

Half-lives $t_{1/2}$ of H/D exchange in D_2O were measured by fitting residual peak intensity to an exponential decay function (f = fast, $t_{1/2}$ < 5 min) and were extracted from 1D ^{1}H-NMR data obtained on a Bruker AvanceI 300 MHz NMR spectrometer equipped with an inverse dual channel (^{1}H, X) (BBI). 1D ^{1}H-NMR data were acquired with 16 to 64 transients into 32K data points over a ppm width of 12 ppm using a W5 sequence[1] to eliminate the water resonance. A relaxation delay of 6 s was applied between transients. 2D ^{1}H-TOCSY and ROESY NMR data were acquired over a frequency width of 12 ppm in both F_2 and F_1 into 2K complex data points in F_2 using 128 to 256 t_1 increments depending on the sample. A relaxation delay of 2 s between transients was used for all experiments. ^{1}H-TOCSY data were recorded using 8 or 32 transients, ^{1}H-ROESY using 32 to 96 transients, depending on the sample. Water suppression was achieved using a WATERGATE routine[2] after the final read pulse. The 2D TOCSY NMR data were acquired with a spin-lock time of 70 ms. 2D ROESY NMR (Tr-ROESY scheme)[3] data were acquired with a mixing time of 150 ms. Data were processed using standard apodizing functions prior to Fourier transformation.

2D ^{1}H-^{13}C HSQC NMR data were acquired, with ^{13}C decoupling during the acquisition period, over an F2 frequency width of 12 ppm into 2K complex data points. 16 to 32 transients were

[1] M. Liu, X. Mao, C. He, H. Huang, J.K. Nicholson, J.C. Lindon, *J. Magn. Reson.* 1998, **132**, 125.
[2] (*a*) M. Piotto, V. Saudek, V. Sklenar, *J. Biomol. NMR* 1992, **2**, 661; (*b*) V. Sklenar, M. Piotto, R. Leppik, V. Saudek, *J. Magn. Reson.* 1993, **102**, 241.
[3] T.-L. Hwang, A.J. Shaka, *J. Am. Chem. Soc.* 1992, **114**, 3157.

accumulated for each of 128 *t*1 increments over an F1 frequency width of 180 ppm centered at 90 ppm. Phase-sensitive data were acquired in a sensitivity-improved manner using an echo-antiecho acquisition mode. 2D ^{1}H-^{13}C HMBC NMR data were acquired over an F2 frequency width of 12 ppm into 2K complex data points. 64 to 96 transients were accumulated for each of 128 *t*1 increments over an F1 frequency width of 200 ppm centered at 100 ppm. Phase-sensitive data were acquired in a sensitivity-improved manner using an echo-antiecho acquisition mode.

Monocyclic Peptides

Monocyclic peptides were synthesized by adding 300-400 mg of resin (Fmoc-Ala-TentaGel S AC resin for **1a**, Fmoc-Val-Wang resin for **2a**, Fmoc-Phe-Wang resin for **3a**, Fmoc-Gly-Wang resin for **4a**, **5a**, *epi*-**5a** and **6a**) in a 10 mL polypropylene syringe fitted with a polyethylene frit, a Teflon stopcock and stopper. The resin was swollen in DCM for 15 min. After removal of DCM the Fmoc protecting group was removed. Stirring of the reaction mixture at any given step described below was performed by attaching the closed syringe to a rotating axis. The following conditions were used:

Removal of the Fmoc protecting group – At each step the Fmoc protecting group was removed with 6 mL of DMF/piperidine (4:1) for 10 min. After filtration the procedure was repeated and finally the resin was washed (2× each) with NMP, MeOH and DCM.

Coupling of the Fmoc-protected amino acids – 3 eq of Fmoc-protected amino acid, 3 eq of PyBOP in 6 mL of NMP were added to the resin. 6 eq of DIEA were added and the reaction was stirred for 60 min (reaction times were prolonged to 120 min for couplings of Fmoc-Glu(OAll)-OH, Fmoc-Glu-OAll and Fmoc-Dap(Boc)-OH, which were performed with 2 eq of both Fmoc-protected amino acid and HATU as coupling agent). The resin was then washed (2× each) with NMP, MeOH and DCM. The effectiveness of the coupling was monitored by TNBS or chloranil test.

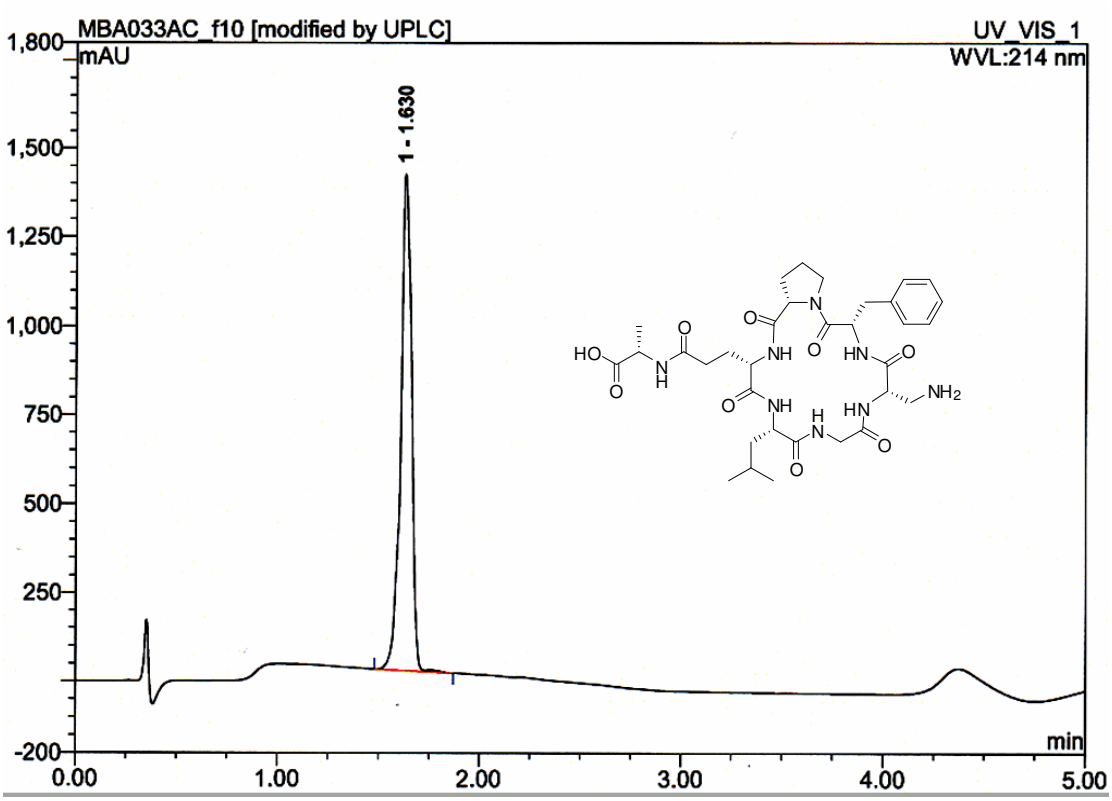
Allyl and N-terminal Fmoc deprotection – After the last coupling, the polypropylene syringe was equipped with a septum and dried under vacuum for one hour. It was then swollen in dry DCM for 15 min under argon. After removal of the solvent, $Pd(PPh_3)_4$ (0.25 eq) was diluted in 4 mL of dry DCM and added to the resin under argon. Phenylsilane (25 eq) was also diluted in 4 mL of dry DCM and added to the resin. The reaction was stirred under argon bubbling for 20 min. The reagents were then removed by filtration and the resin washed with dry DCM. The procedure was repeated twice. Finally, the resin was washed with dry DCM (10 mL, 2×15 min) and the last Fmoc protecting group was removed as previously described.

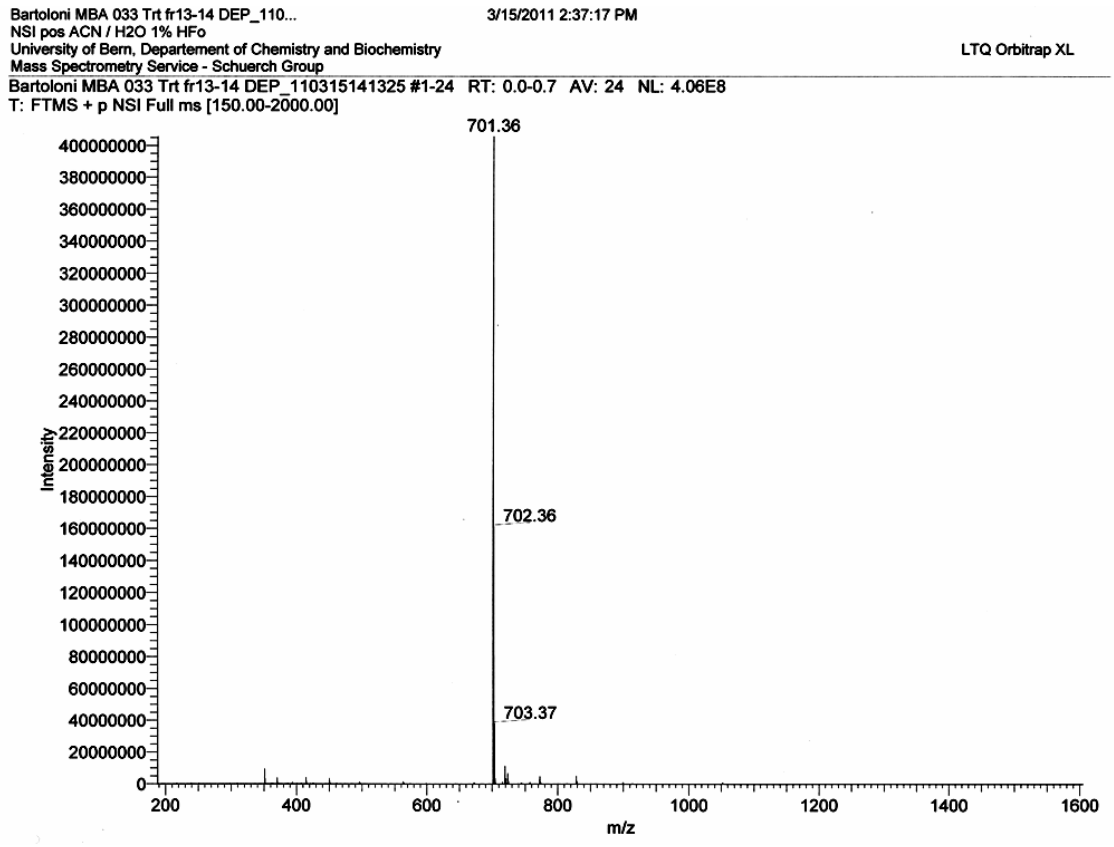
On-resin cyclization – HATU (3 eq) and DIPEA (6 eq) were added to the deprotected peptidyl-resin in 6 mL of NMP/DMSO (4:1)[4] and the mixture was stirred at room temperature overnight for 16 h. The reagents were removed by filtration and the resin washed (2× each) with NMP, MeOH and DCM. The TNBS test was used to check the effectiveness of the cyclization. In case of partially positive test, the cyclization step was repeated once more.

TFA cleavage and purification – The cleavage was carried out using a TFA/TIS/H_2O (95:4:1) solution for 2 h. The peptide solutions were separated from the resin by filtration, evaporated and dried under high vacuum and finally lyophilized from a freshly-prepared water/acetonitrile solution. The lyophilized crude was then dissolved in a water/acetonitrile mixture, purified by preparative RP-HPLC and lyophilized again. Yields were calculated for the TFA salts of the products.

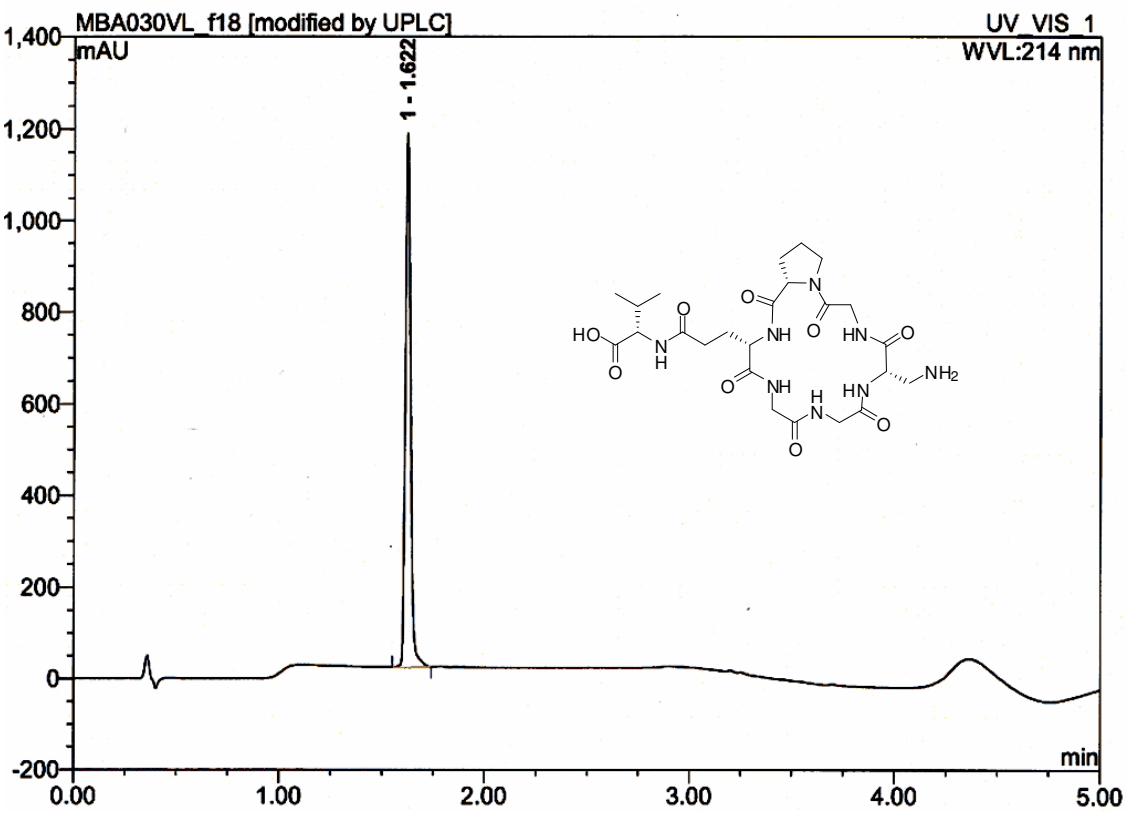
[4] W. Zhang, J. W. Taylor, *Tetrahedron Lett.* 1996, **37**, 2173.

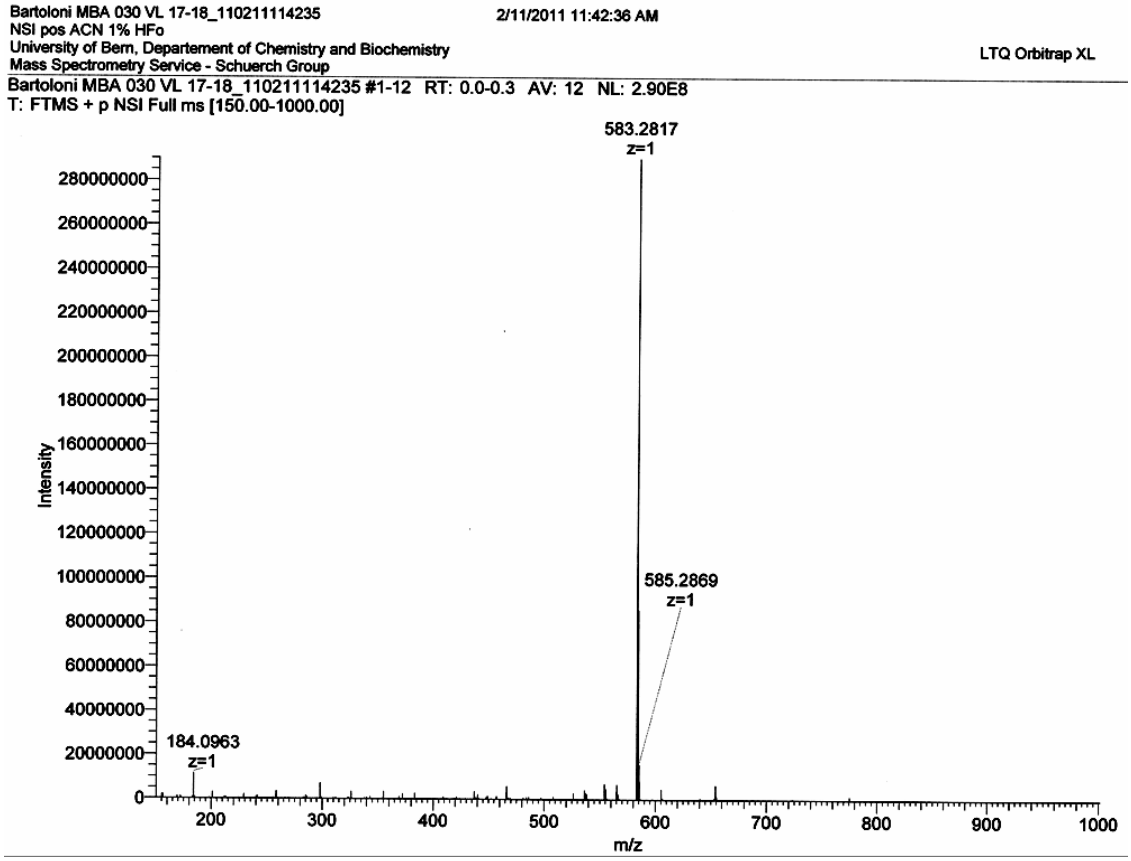
cyclo(Leu-Gly-Dap-Phe-Pro-Glu(Ala-OH)) (1a, L[1]GBFPE[1]γA). From Fmoc-Ala-TentaGel S AC resin (1000 mg, 0.21 mmol/g, bead size 90 µm), **1a** was obtained as foamy white solid after preparative RP-HPLC (45.2 mg, 55.5 µmol, 26%). Analytical RP-HPLC: t_R = 1.630 min (A/D 100:0 to 0:100 in 2.2 min, λ = 214 nm). MS (ESI+): $C_{33}H_{48}N_8O_9$ calc./obs. 701.36/701.36 $[M+H]^+$.



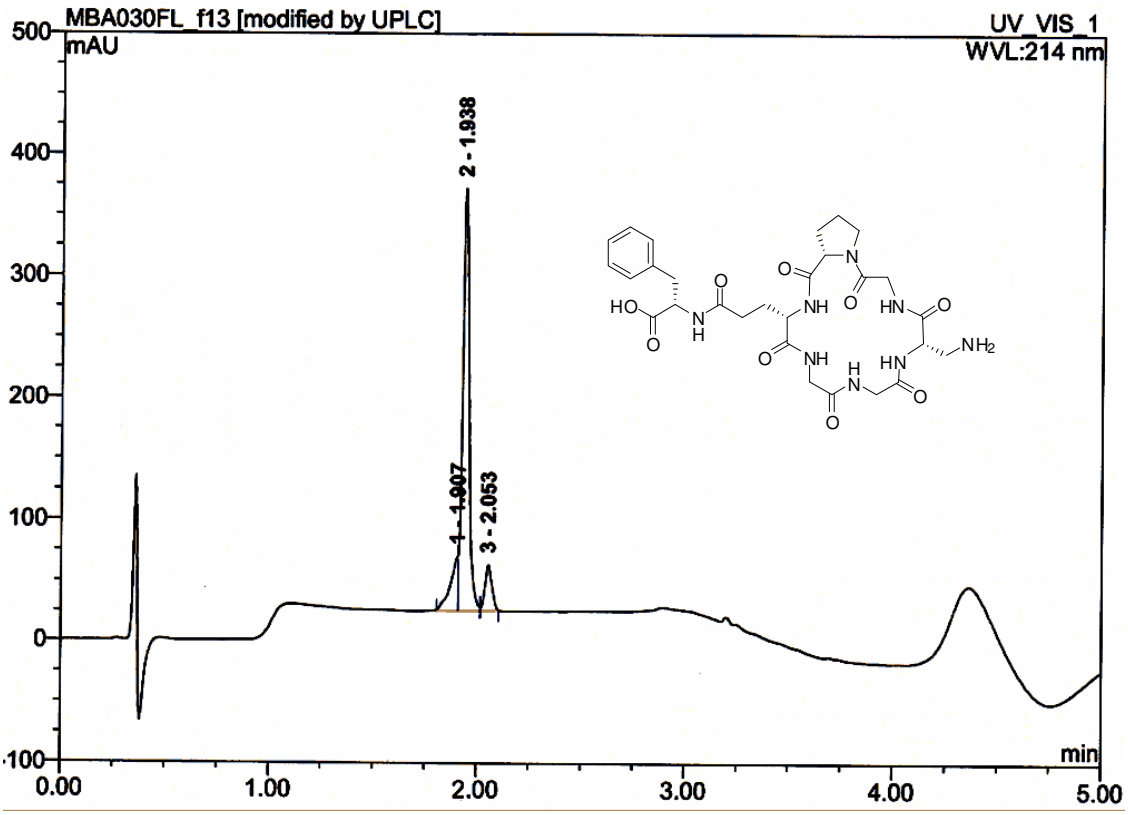


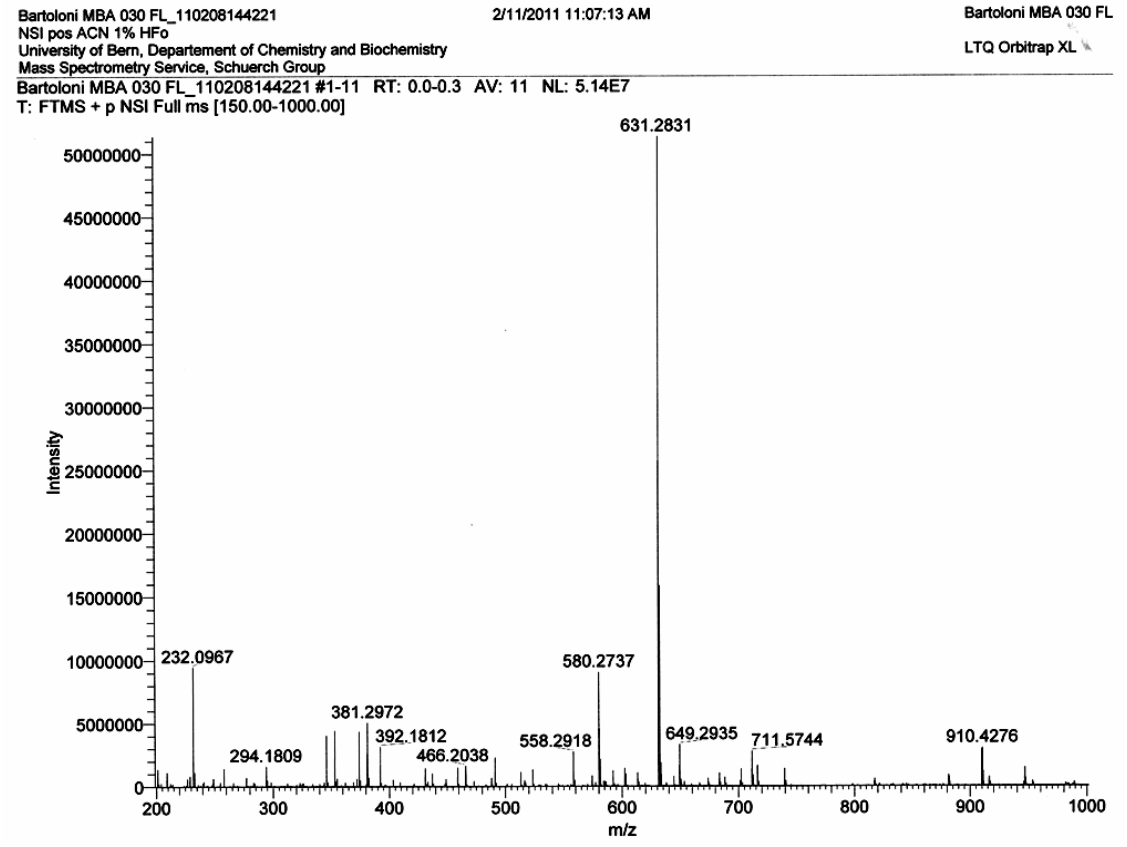
cyclo(Gly-Gly-Dap-Gly-Pro-Glu(Val-OH)) (2a, $G^1GBGPE^1\gamma V$). From Fmoc-Val-Wang resin (183 mg, 0.61 mmol/g, 100-200 mesh), **2a** was obtained as foamy white solid after preparative RP-HPLC (9.0 mg, 12.9 µmol, 12%). Analytical RP-HPLC: t_R = 1.622 min (A/D 100:0 to 50:50 in 2.2 min, λ = 214 nm). MS (ESI+): $C_{24}H_{38}N_8O_9$ calc./obs. 583.28/583.28 $[M+H]^+$.



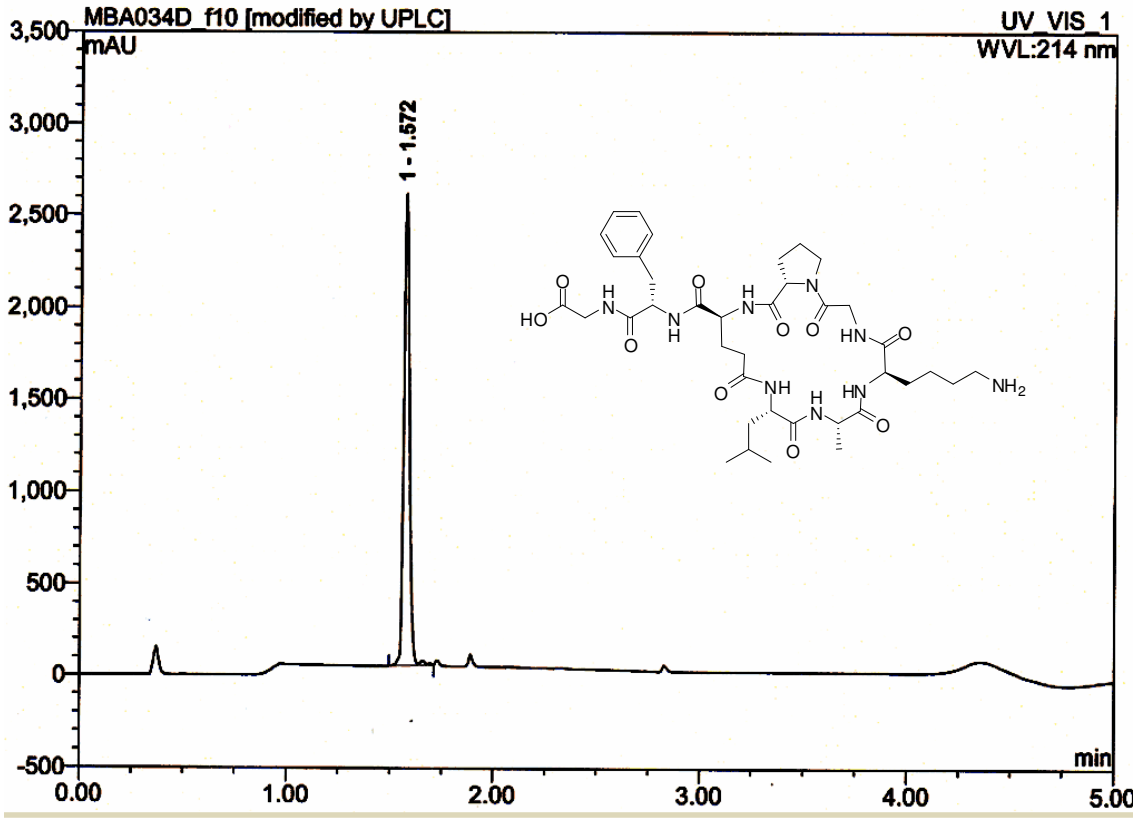


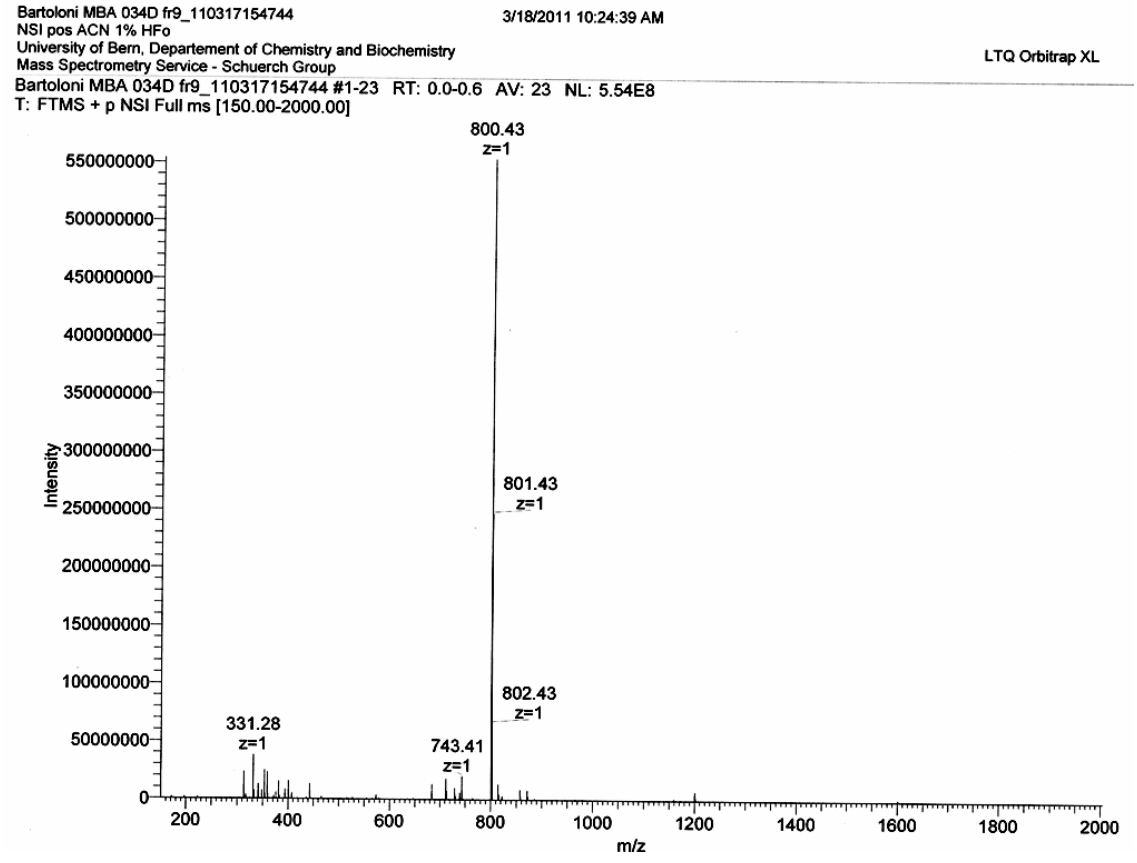
cyclo(Gly-Gly-Dap-Gly-Pro-Glu(Phe-OH)) (3a, $G^1GBGPE^1\gamma F$). From Fmoc-Phe-Wang resin (61 mg, 1.0 mmol/g, 100-200 mesh), **3a** was obtained as foamy white solid after preparative RP-HPLC (3.8 mg, 5.1 µmol, 8%). Analytical RP-HPLC: t_R = 1.938 min (A/D 100:0 to 50:50 in 2.2 min, λ = 214 nm). MS (ESI+): $C_{28}H_{38}N_8O_9$ calc./obs. 631.28/631.28 $[M+H]^+$.



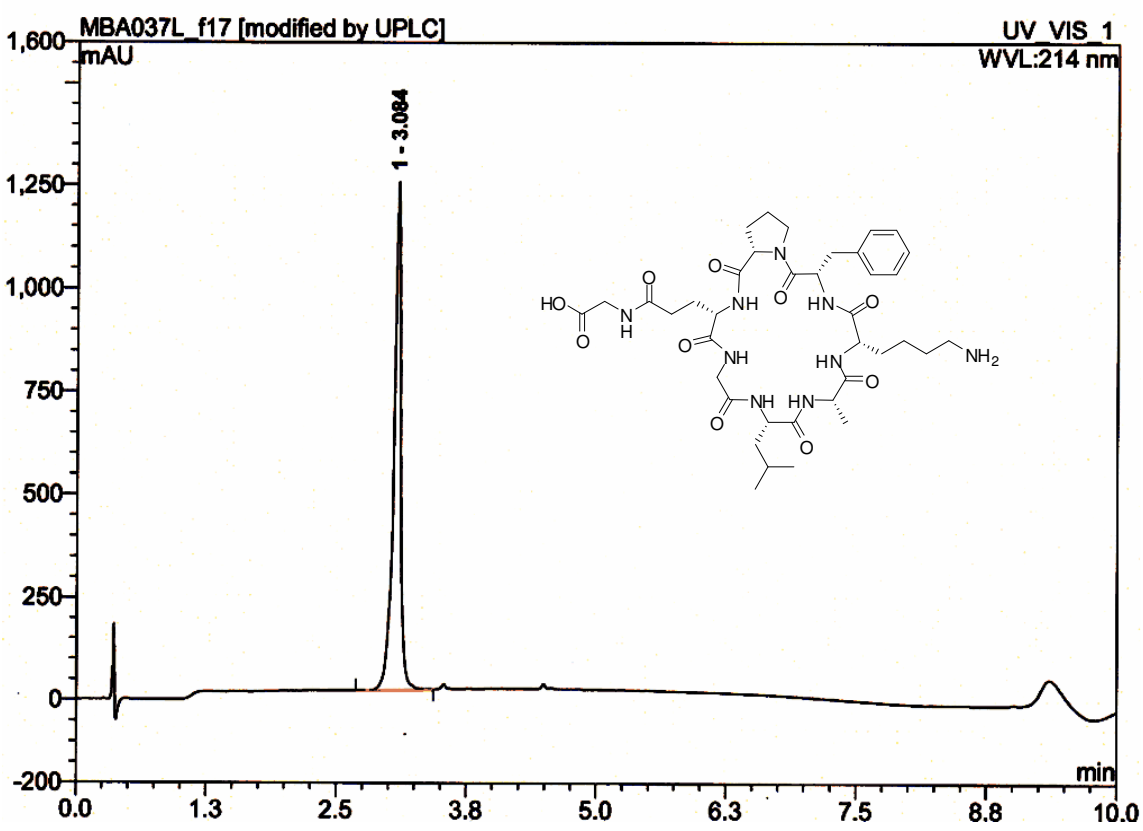


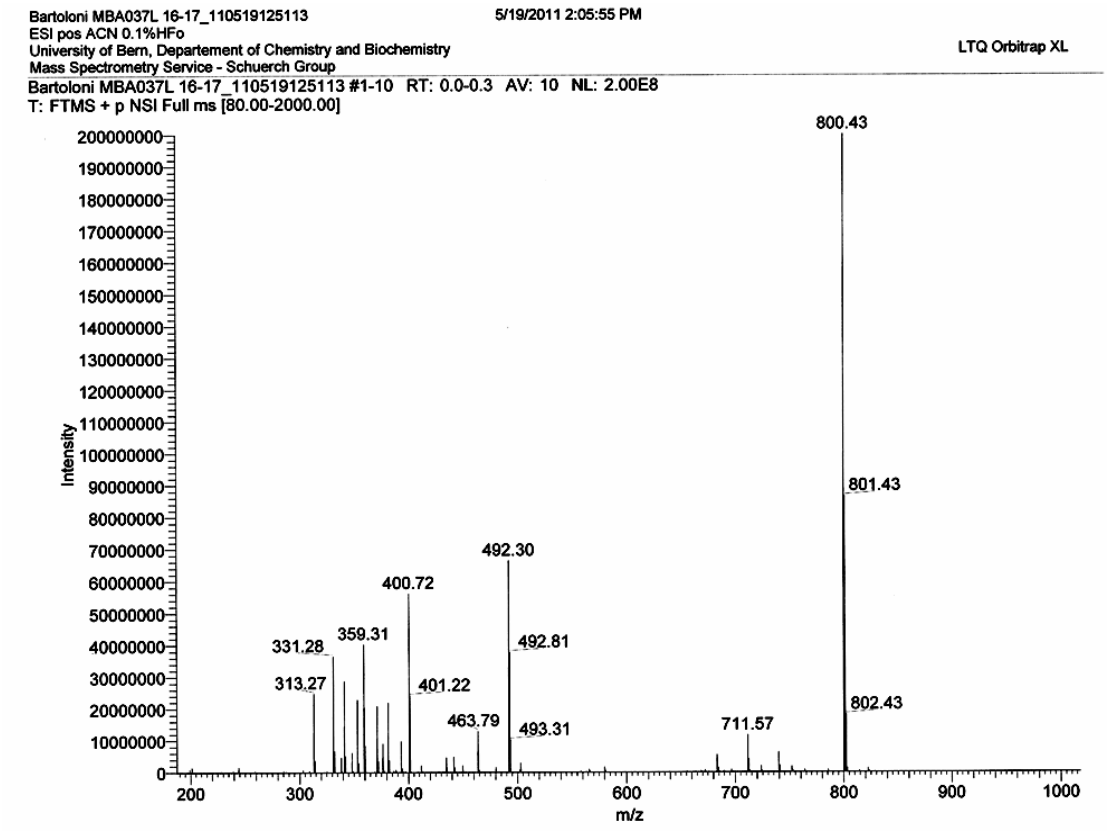
cyclo(Glu(Leu-Ala-lys-Gly-Pro))-Phe-Gly-OH (4a, L[1]AkGPE[1]FG). From Fmoc-Gly-Wang resin (300 mg, 0.56 mmol/g), **4a** was obtained as foamy white solid after preparative RP-HPLC (48.1 mg, 52.6 µmol, 31%). Analytical RP-HPLC: t_R = 1.572 min (A/D 100:0 to 0:100 in 2.2 min, λ = 214 nm). MS (ESI+): $C_{38}H_{57}N_9O_{10}$ calc./obs. 800.43/800.43 $[M+H]^+$.



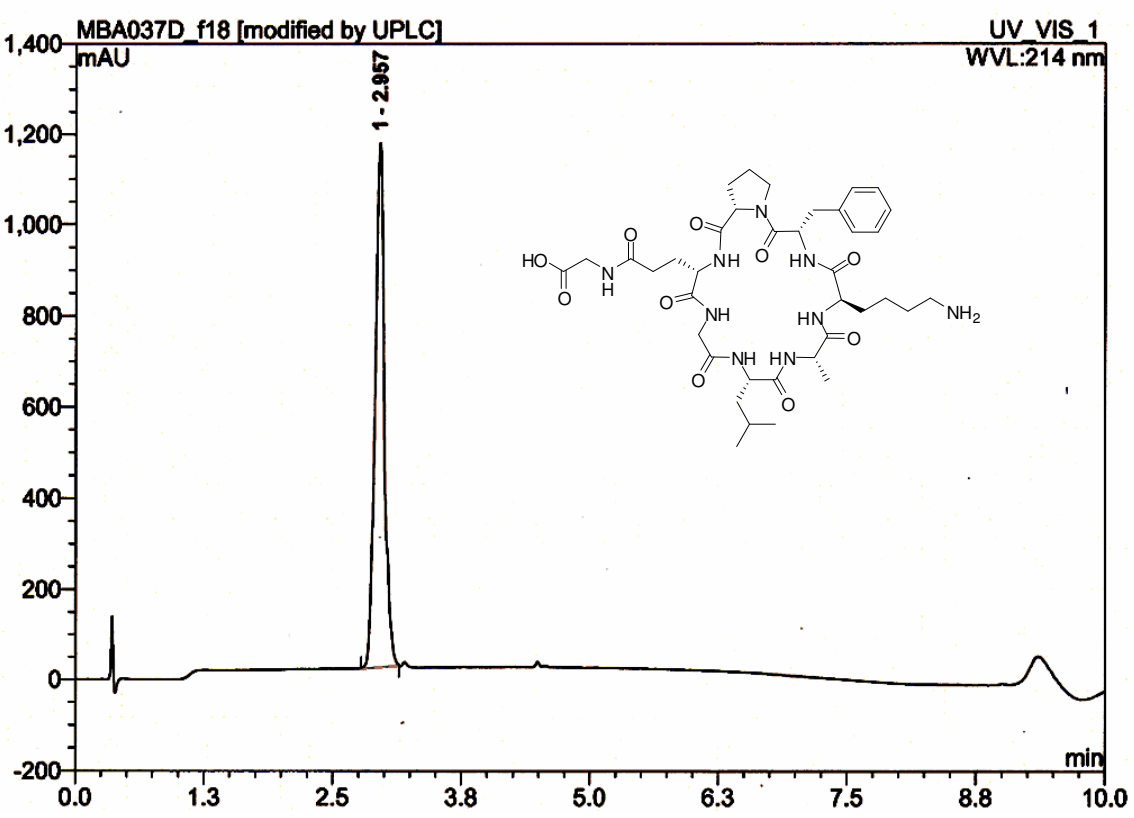


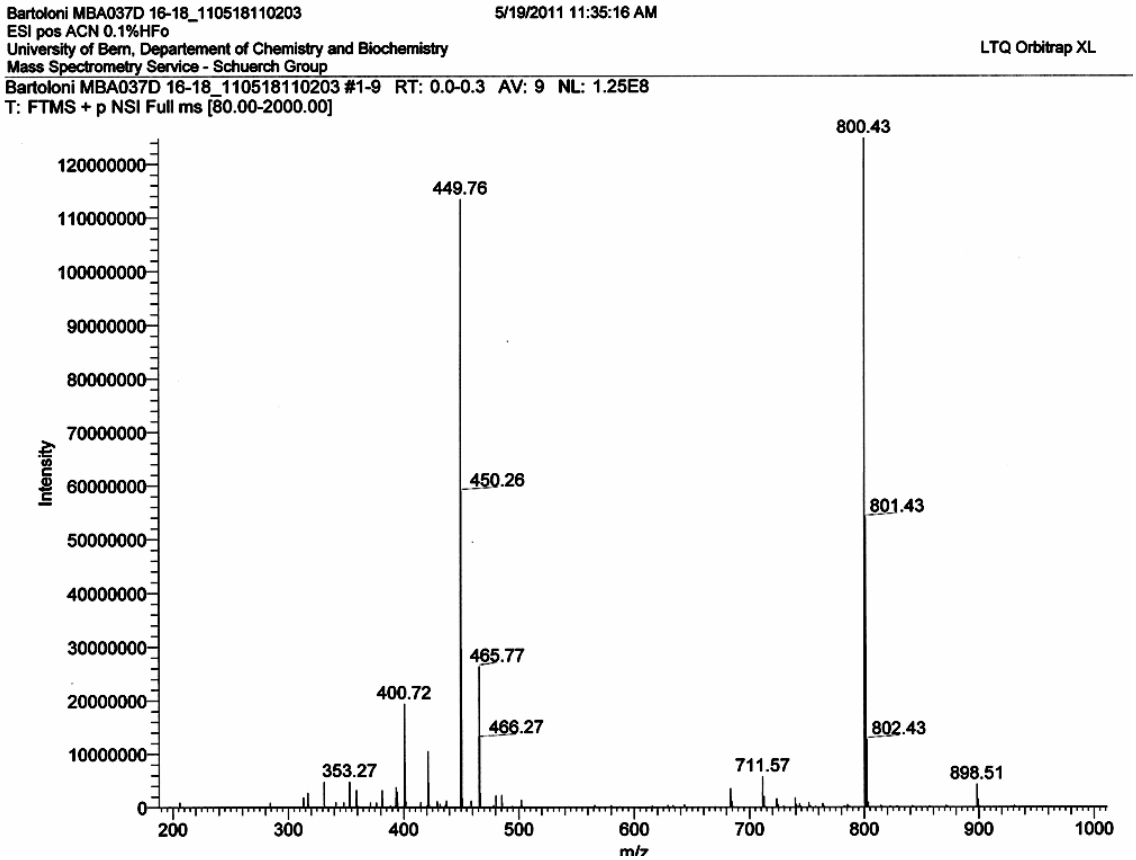
cyclo(Gly-Leu-Ala-Lys-Phe-Pro-Glu(Gly-OH)) (5a, G[1]LAKFPE[1]γG). From Fmoc-Gly-Wang resin (400 mg, 0.56 mmol/g), **5a** was obtained as foamy white solid after preparative RP-HPLC (24.0 mg, 26.3 µmol, 12%). Analytical RP-HPLC: t_R = 3.084 min (A/D 100:0 to 0:100 in 7.5 min, λ = 214 nm). MS (ESI+): $C_{38}H_{57}N_9O_{10}$ calc./obs. 800.43/800.43 $[M+H]^+$.



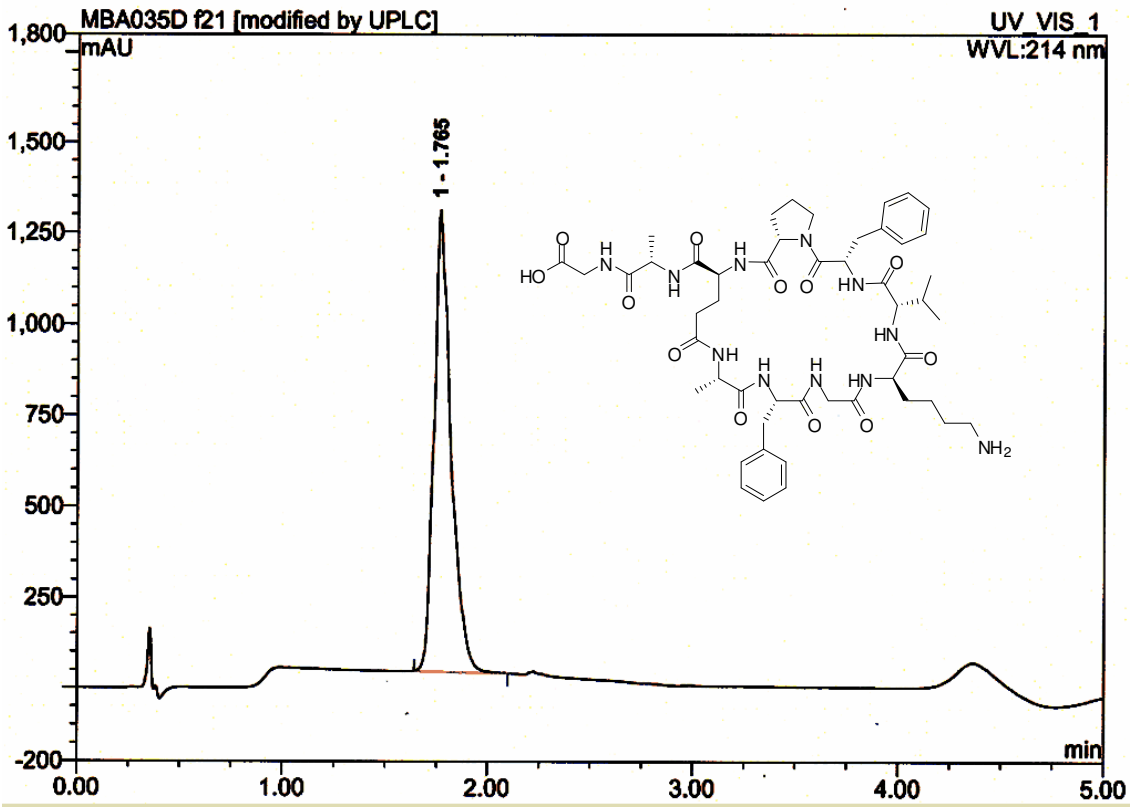


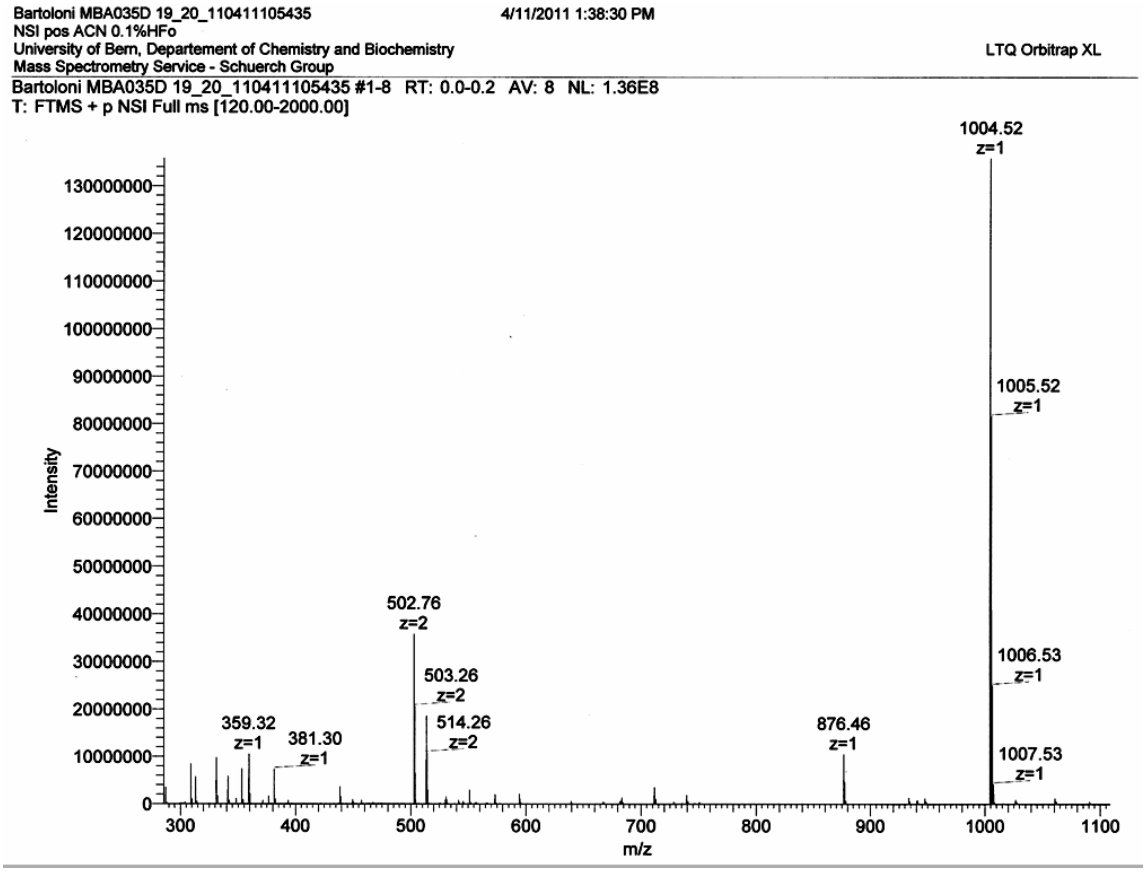
cyclo(Gly-Leu-Ala-lys-Phe-Pro-Glu(Gly-OH)) (*epi*-**5a**, **G^{1}LAkFPE^{1}γG).** From Fmoc-Gly-Wang resin (400 mg, 0.56 mmol/g), *epi*-**5a** was obtained as foamy white solid after preparative RP-HPLC (43.5 mg, 47.6 µmol, 21%). Analytical RP-HPLC: t_R = 2.957 min (A/D 100:0 to 0:100 in 7.5 min, λ = 214 nm). MS (ESI+): $C_{38}H_{57}N_9O_{10}$ calc./obs. 800.43/800.43 $[M+H]^+$.





cyclo(Glu(Ala-Phe-Gly-lys-Val-Phe-Pro))-Ala-Gly-OH (6a, A[1]FGkVFPE[1]AG). From Fmoc-Gly-Wang resin (350 mg, 0.56 mmol/g), **6a** was obtained as foamy white solid after preparative RP-HPLC (67.5 mg, 60.4 µmol, 27%). Analytical RP-HPLC: t_R = 1.765 min (A/D 100:0 to 0:100 in 2.2 min, λ = 214 nm). MS (ESI+): $C_{49}H_{69}N_{11}O_{12}$ calc./obs. 1004.52/1004.52 $[M+H]^+$, 502.76/502.76 $[M+2H]^{2+}$.





Bicyclic peptides

Bicyclic peptides were synthesized with an analogue procedure by subjecting monocyclic peptides to amide bond formation conditions in high dilution (1 mM)[5]. To a solution of phosphonium coupling agent (3 eq PyAOP[6] for **1**-**4b**, PyBOP for **5b**, *epi*-**5b** and **6b**) and DIEA (6 eq) in DCM/DMF (10:1), a 10 mM solution of monocyclic peptide in DMF was slowly added dropwise at 0 °C under vigorous stirring. The reaction was left stirring overnight for 16 h at r.t. and monitored via RP-HPLC, until complete disappearance of the starting material. The reaction was then quenched by addition of TFA (6 eq) and the solvent removed by evaporation. The crude was then dissolved in a water/acetonitrile mixture, purified by preparative RP-HPLC and lyophilized. Yields were calculated by considering TFA salts of monocyclic peptides as starting material.

Note: Some MS spectra of bicyclic peptides show a poor S/N ratio due to scarce ionization in experimental conditions. Common noise peaks at 313.27, 331.28, 359.32, 381.30, 683.54, 711.57, 739.60 m/z.

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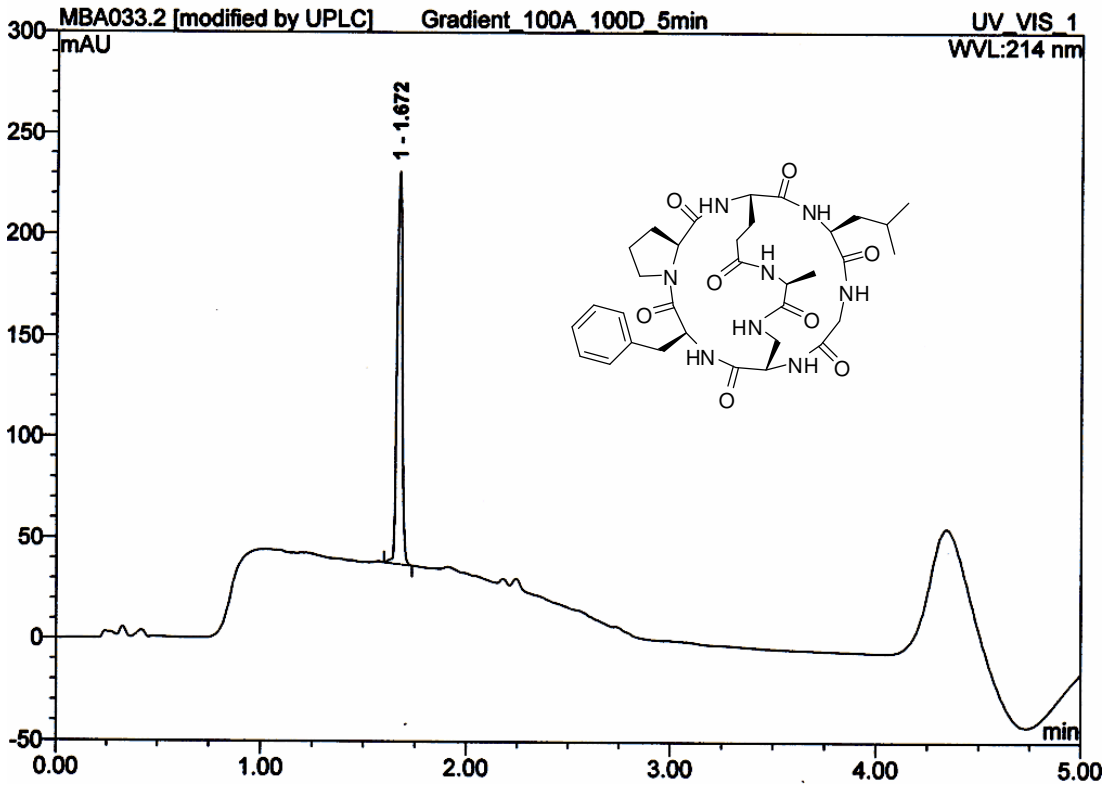
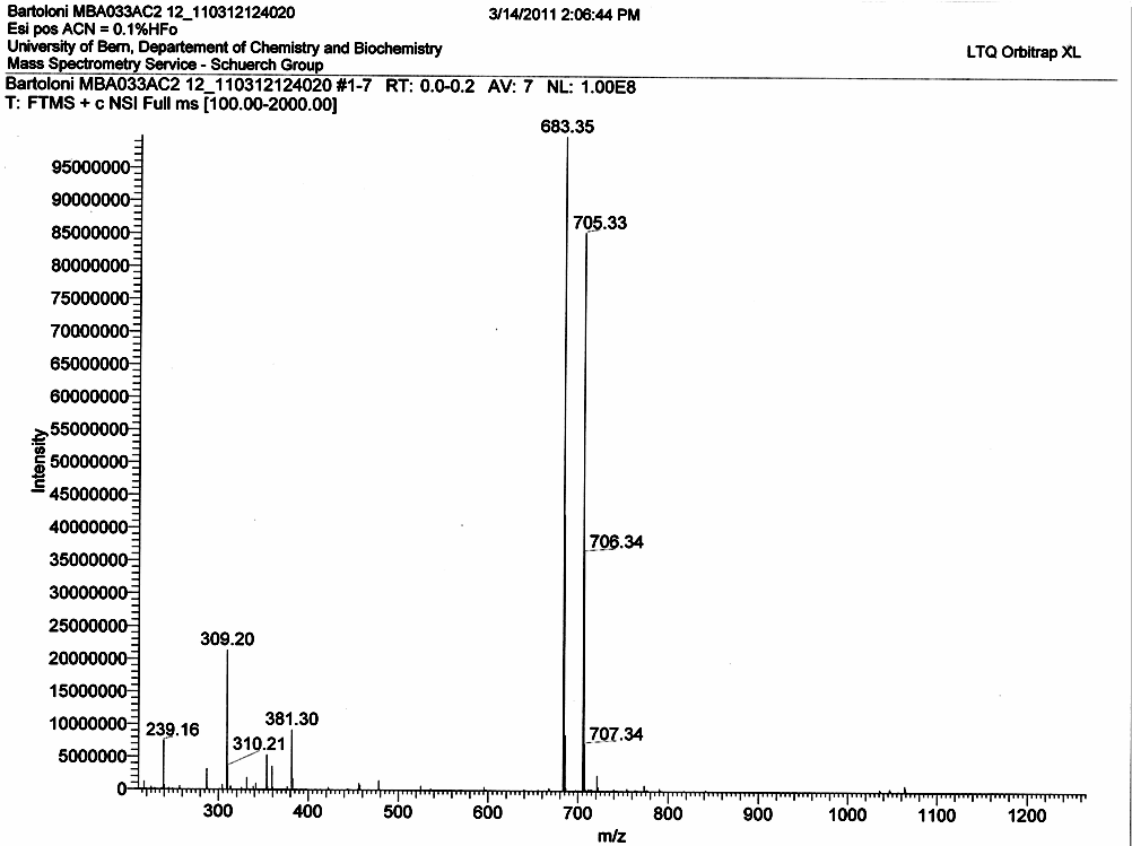
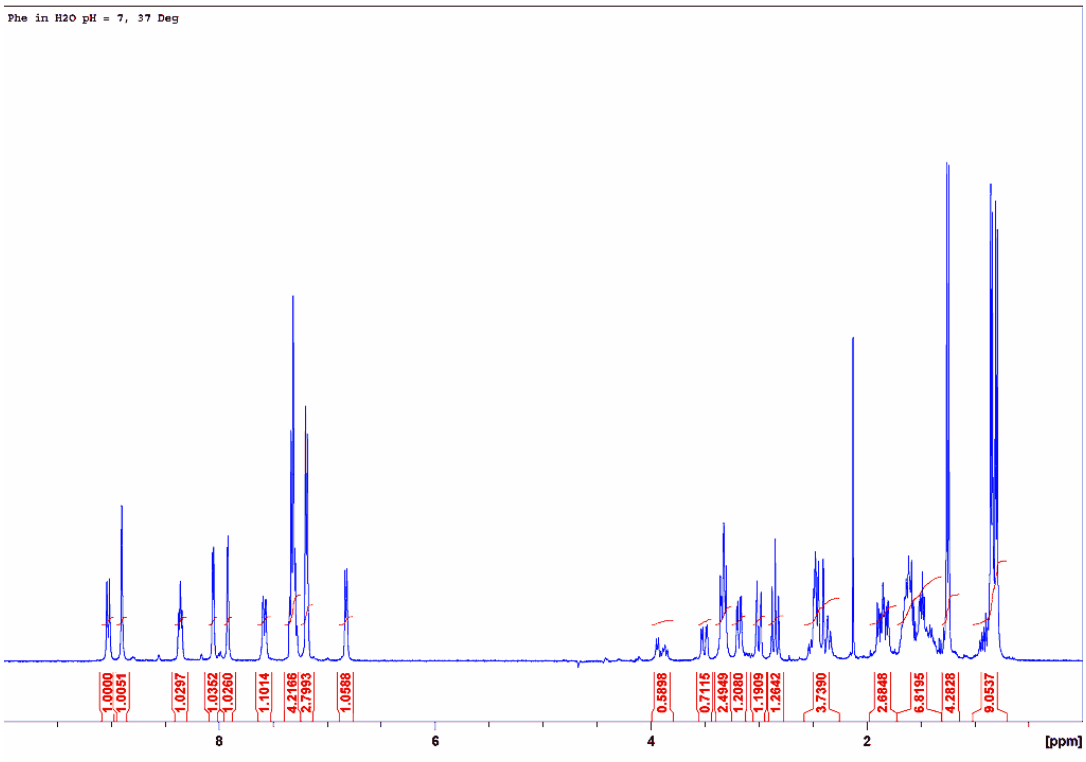
[5] V. Caciagli, F. Cardinali, A. Hänsicke, G. Tuchalski, P. Lombardi, *J. Pept. Sci.* 1997, **3**, 224.
[6] F. Albericio, M. Cases, J. Alsina, S. A. Triolo, L. A. Carpino, S. A. Kates, *Tetrahedron Lett.* 1997, **38**, 4853.

cyclo(Leu^{1}Gly^{2}Dap^{3}Phe^{4}Pro^{5}Glu^6)cyclo(6γ→3β)Ala^7 (1b, L^{1}G*B*^{2}FP*E*^{1}γA^2). Starting from **1a** (44.6 mg, 54.7 µmol), **1b** was obtained as foamy white solid after preparative RP-HPLC (14.0 mg, 20.5 µmol, 37%). Analytical RP-HPLC: t_R = 1.672 min (A/D 100:0 to 0:100 in 2.2 min, λ = 214 nm). MS (ESI+): $C_{33}H_{46}N_8O_8$ calc./obs. 683.35/683.35 $[M+H]^+$, 705.33/705.33 $[M+Na]^+$.

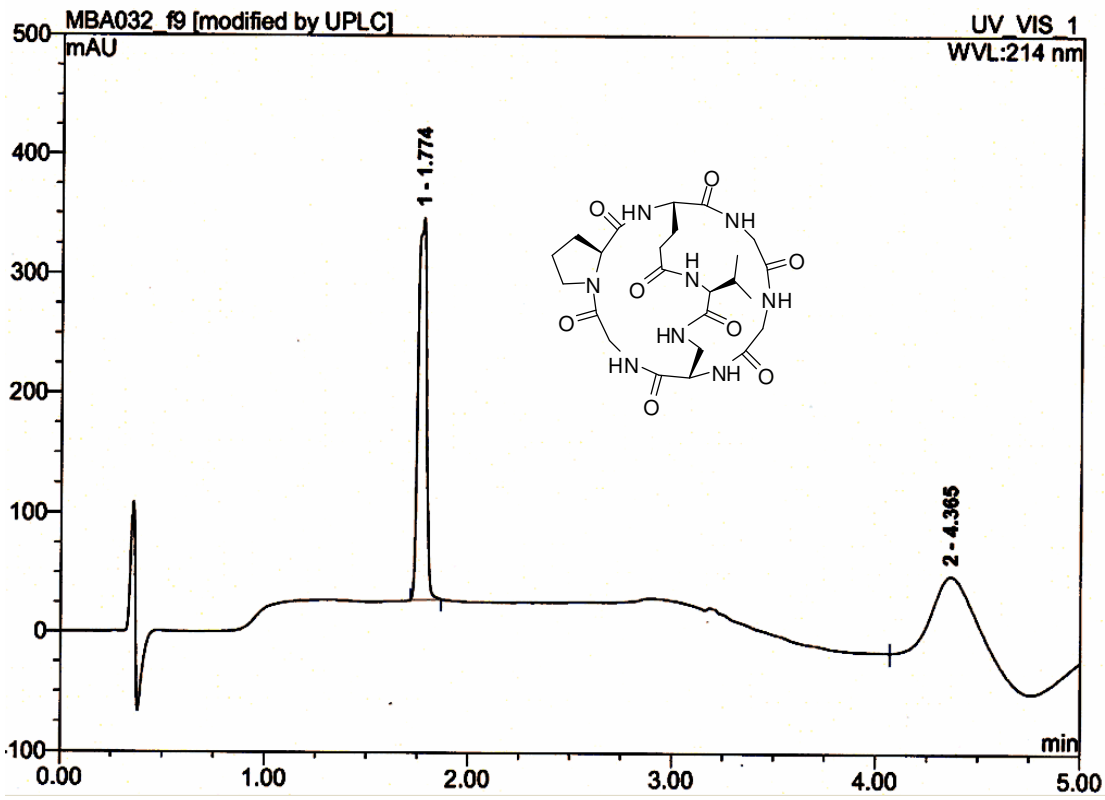


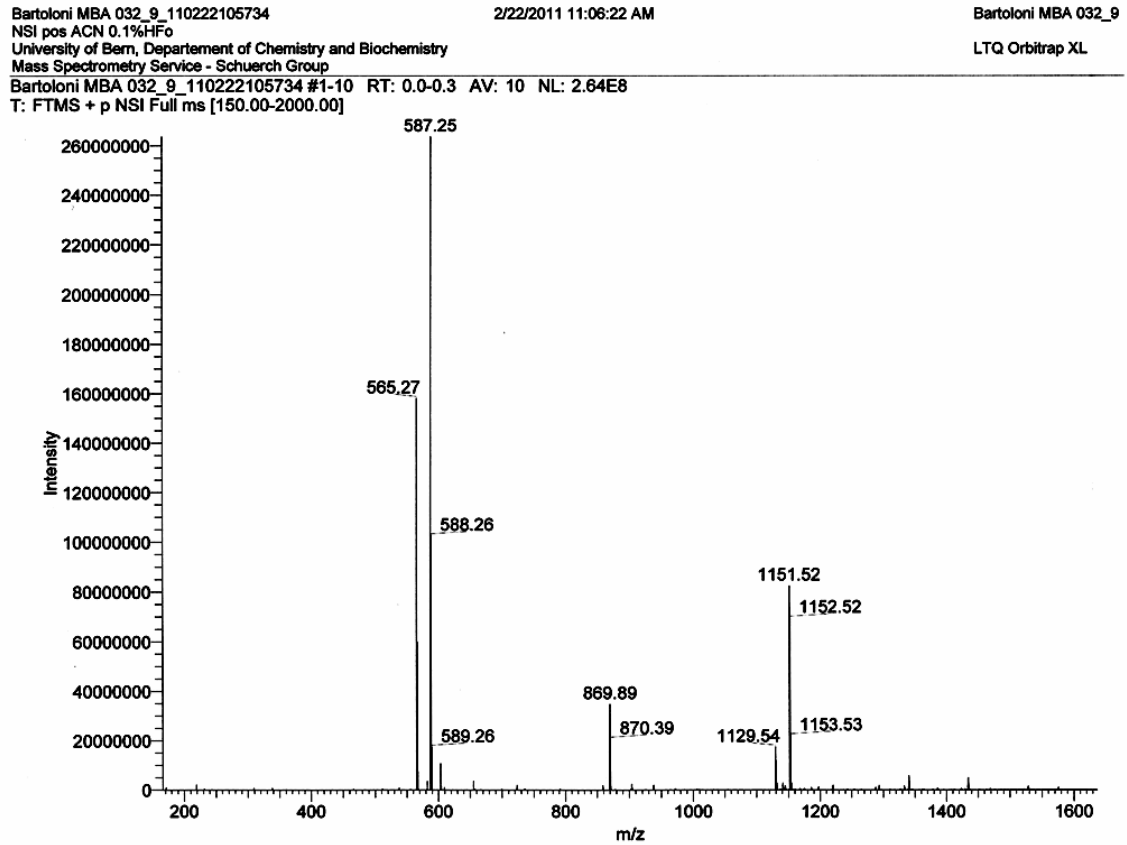


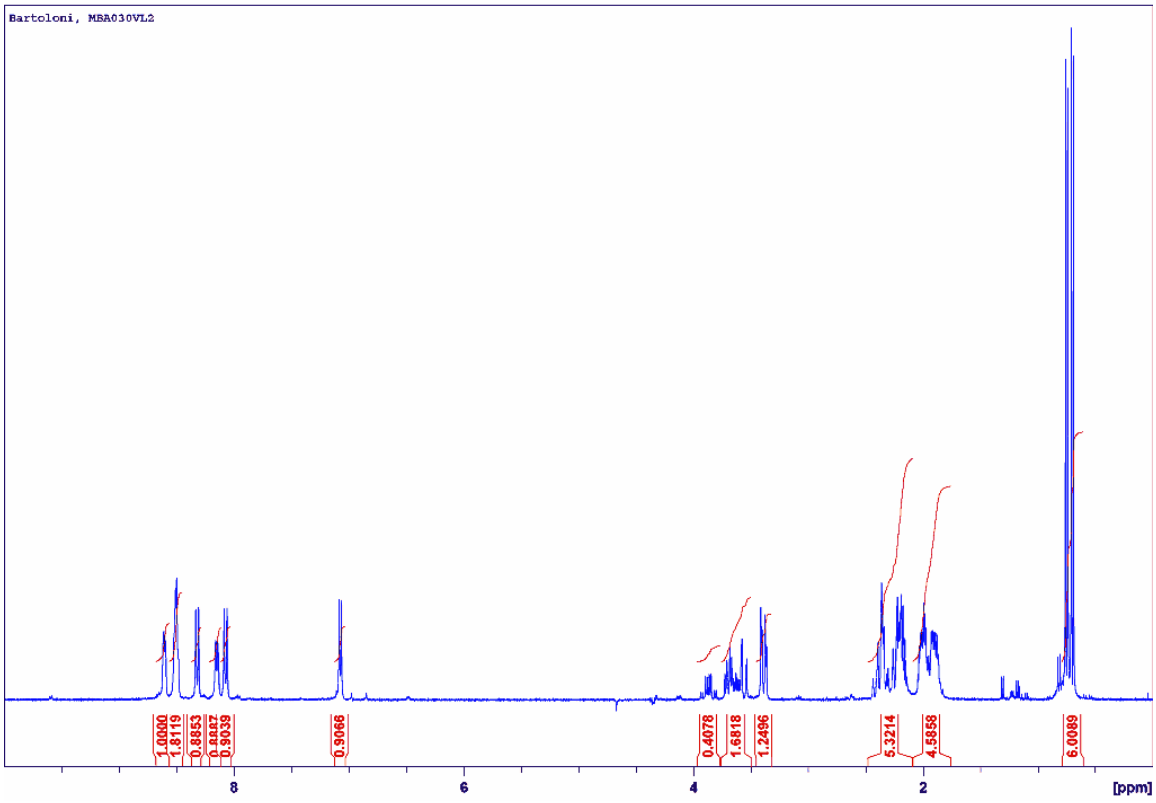


residue	*proton*	*δ (ppm)*	*J (Hz)*
Leu[1]	NH	7.92	^3J(NH–αCH) = 2.69
	αCH	3.91	^3J(δCH–γCH) = 6.34
	β'CH	1.65	
	β"CH	1.53	
	γCH	1.64	
	δ'CH_3	0.89	
	δ"CH_3	0.85	
Gly[2]	NH	8.36	^3J(NH–α'CH) = 7.77
	α'CH	3.96	^3J(NH–α"CH) = 5.47
	α"CH	3.55	^2J(α'CH–α"CH) = 16.97
Dap[3]	αNH	6.83	^3J(αNH–αCH) = 6.71
	αCH	4.34	^3J(βNH–β'CH) = 10.21
	β'CH	4.18	^3J(βNH–β"CH) = 2.28
	β"CH	3.05	^2J(β'CH–β"CH) = 14.31
	βNH	7.58	
Phe[4]	αNH	8.90	^3J(β'CH–αCH) = 4.66
	αCH	4.46	^3J(β"CH–αCH) = 12.23
	β'CH	3.23	^2J(β"CH–β'CH) = 12.23
	β"CH	2.89	
	2,6H	7.24	
	3,4,5H	7.36	
Pro[5]	αCH	3.36	
	β'CH	1.88	
	β"CH	0.95	
	γ'CH	1.69	
	γ"CH	1.46	
	δCH_2	3.38	
Glu[6]	NH	9.03	^3J(NH–αCH) = 9.65
	αCH	4.84	
	β'CH	2.41	
	β"CH	1.92	
	γCH_2	2.51	
Ala[7]	NH	8.06	^3J(NH–αCH) = 4.83
	αCH	3.99	^3J(βCH–αCH) = 7.38
	βCH_3	1.30	

cyclo(Gly^{1}Gly^{2}Dap^{3}Gly^{4}Pro^{5}Glu^6)cyclo(6γ→3β)Val^7 (2b, G^{1}G*B*^{2}GP*E*^{1}γV^2). Starting from **2a** (9.0 mg, 12.9 µmol), **2b** was obtained as foamy white solid after preparative RP-HPLC (3.3 mg, 5.8 µmol, 45%). Analytical RP-HPLC: t_R = 1.774 min (A/D 100:0 to 50:50 in 2.2 min, λ = 214 nm). MS (ESI+): $C_{24}H_{36}N_8O_8$ calc./obs. 565.27/565.27 $[M+H]^+$, 587.25/587.25 $[M+Na]^+$, 1151.52/1151.52 $[M+M+Na]^+$.

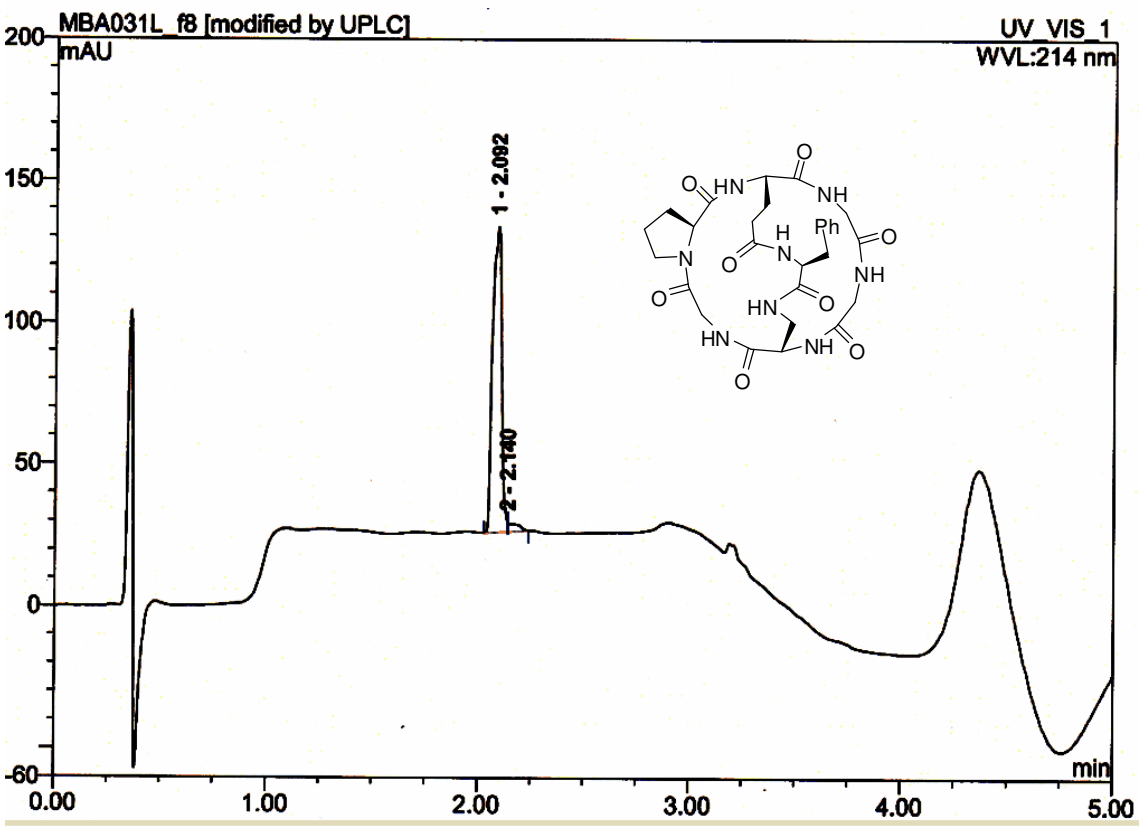


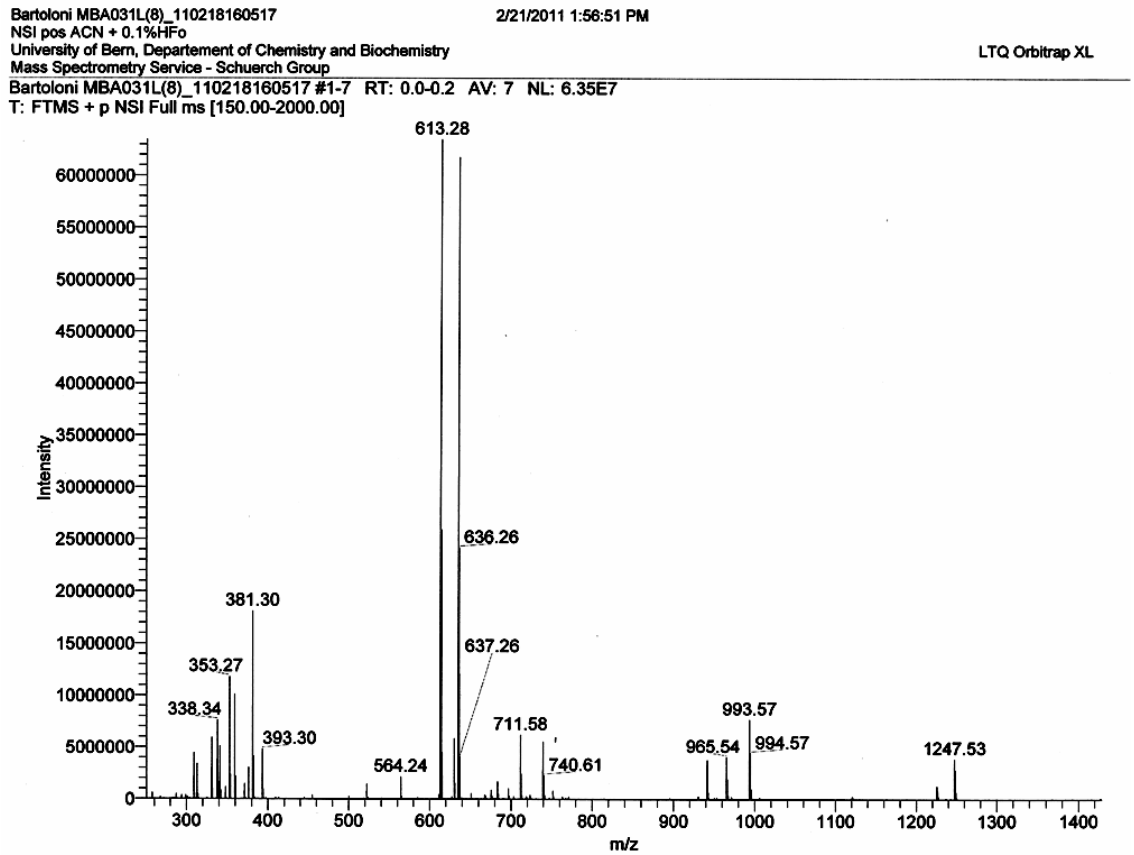


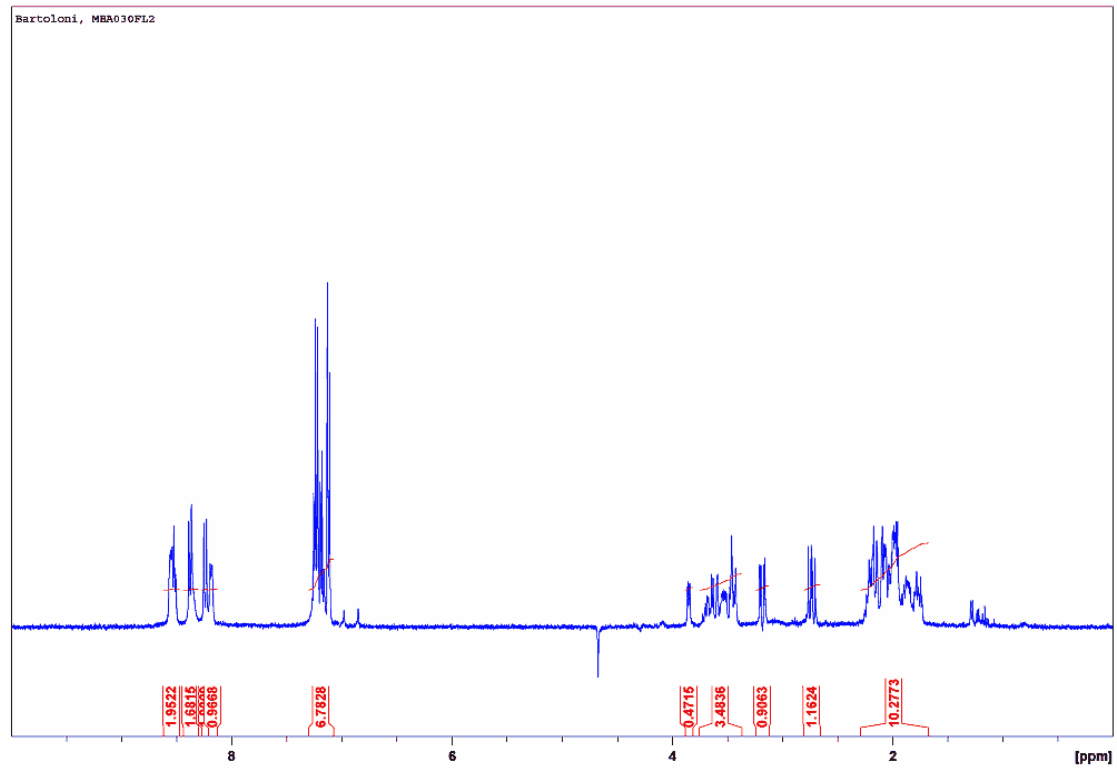


residue	*proton*	*δ (ppm)*	*J (Hz)*
Gly[1]	NH	8.50	^{3}J(α'CH–NH) = 6.73
	α'CH	3.79	^{3}J(α"CH–NH) = 2.66
	α"CH	3.66	^{2}J(α'CH–α"CH) = 16.52
Gly[2]	NH	8.61	^{3}J(α'CH–NH) = 6.85
	α'CH	4.01	^{3}J(α"CH–NH) = 6.25
	α"CH	3.93	^{2}J(α'CH–α"CH) = 17.54
Dap[3]	αNH	8.32	^{3}J(αCH–NH) = 9.79
	αCH	4.95	
	β'CH	3.73	
	β"CH	3.49	
	βNH	8.15	
Gly[4]	NH	8.51	
	α'CH	4.46	
	α"CH	3.49	
Pro[5]	αCH	4.45	
	β'CH	2.33	
	β"CH	2.11	
	γ'CH	2.08	
	γ"CH	2.00	
	δ'CH_2	4.21	
	δ"CH_2	3.80	
Glu[6]	NH	7.08	^{3}J(αCH–NH) = 7.53
	αCH	4.72	
	β'CH	2.33	
	β"CH	2.00	
	γCH_2	2.45	
Val[7]	αNH	8.08	^{3}J(αCH–NH) = 9.84
	αCH	4.40	^{3}J(γCH–βCH) = 7.03
	βCH	2.26	
	γ'CH_3	0.85	
	γ"CH_3	0.80	

cyclo(Gly^{1}Gly^{2}Dap^{3}Gly^{4}Pro^{5}Glu^6)cyclo(6γ→3β)Phe^7 (3b, G^{1}G*B*^{2}GP*E*^{1}γF^2). Starting from **3a** (3.7 mg, 5.0 µmol), **3b** was obtained as foamy white solid after preparative RP-HPLC (1.2 mg, 2.0 µmol, 39%). Analytical RP-HPLC: t_R = 2.092 min (A/D 100:0 to 50:50 in 2.2 min, λ = 214 nm). MS (ESI+): $C_{28}H_{36}N_8O_8$ calc./obs. 613.27/613.28 $[M+H]^+$, 635.25/635.36 $[M+Na]^+$.

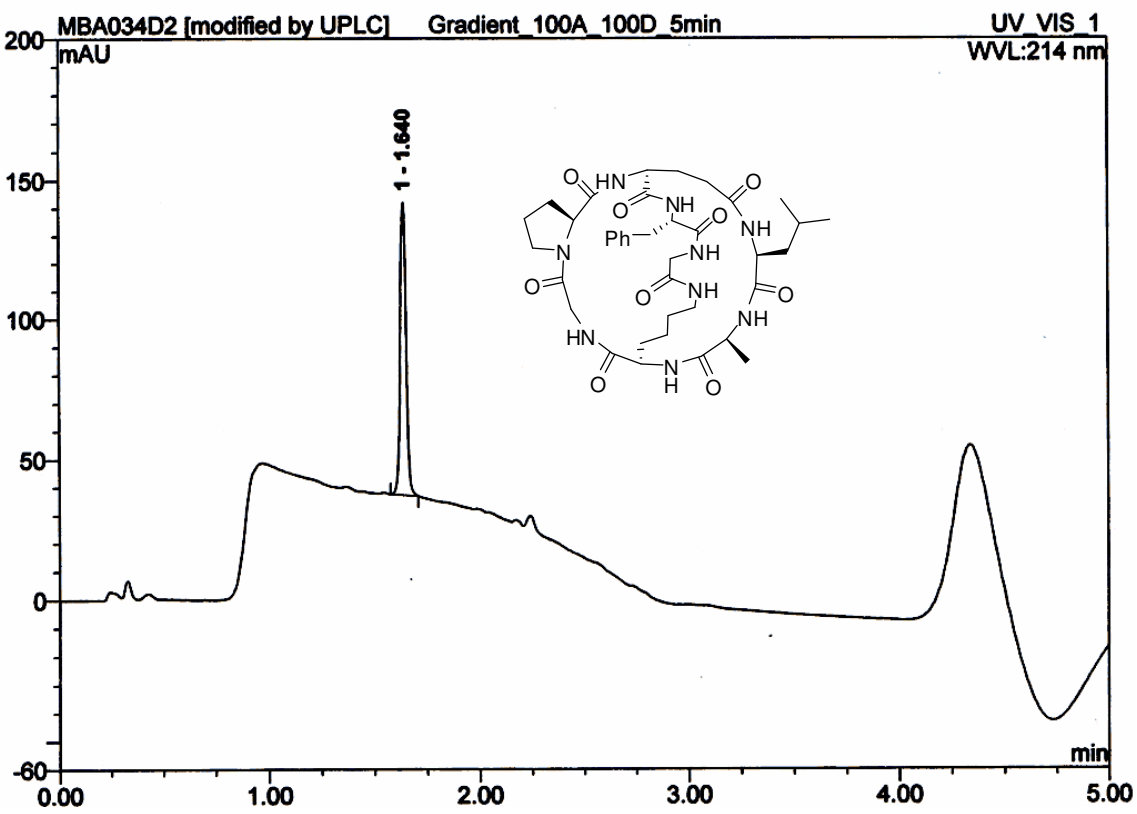


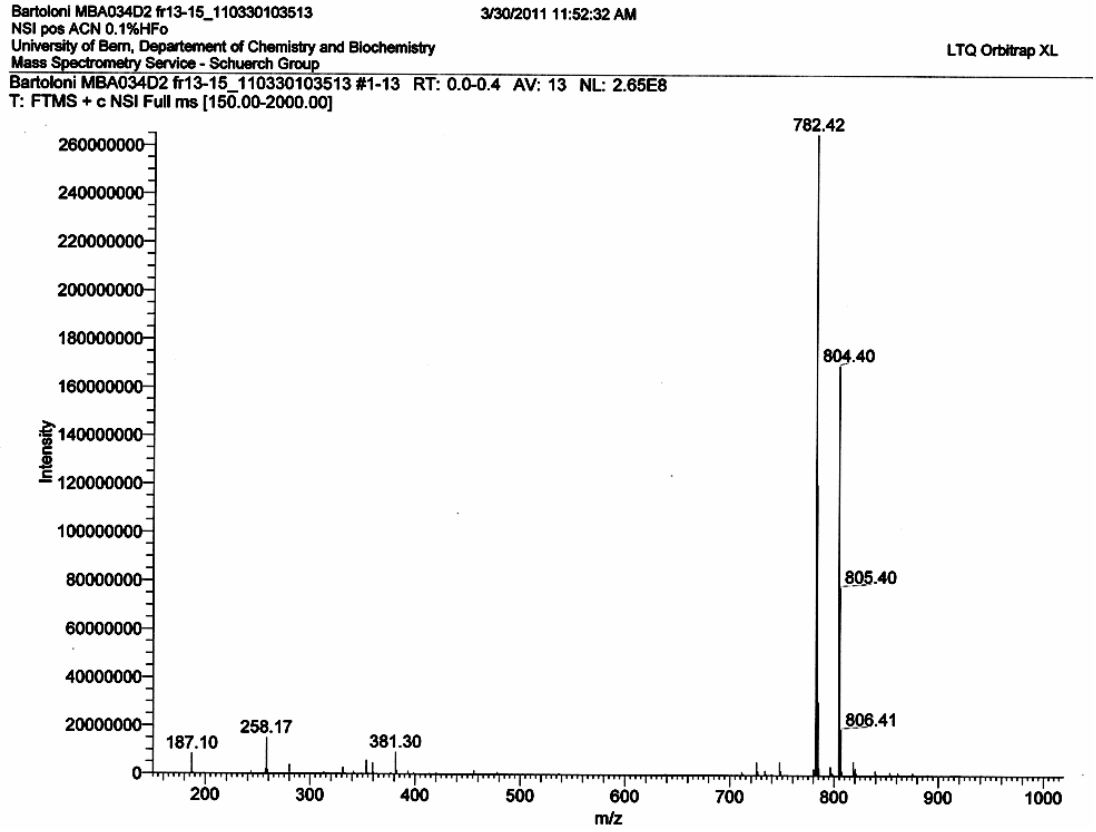


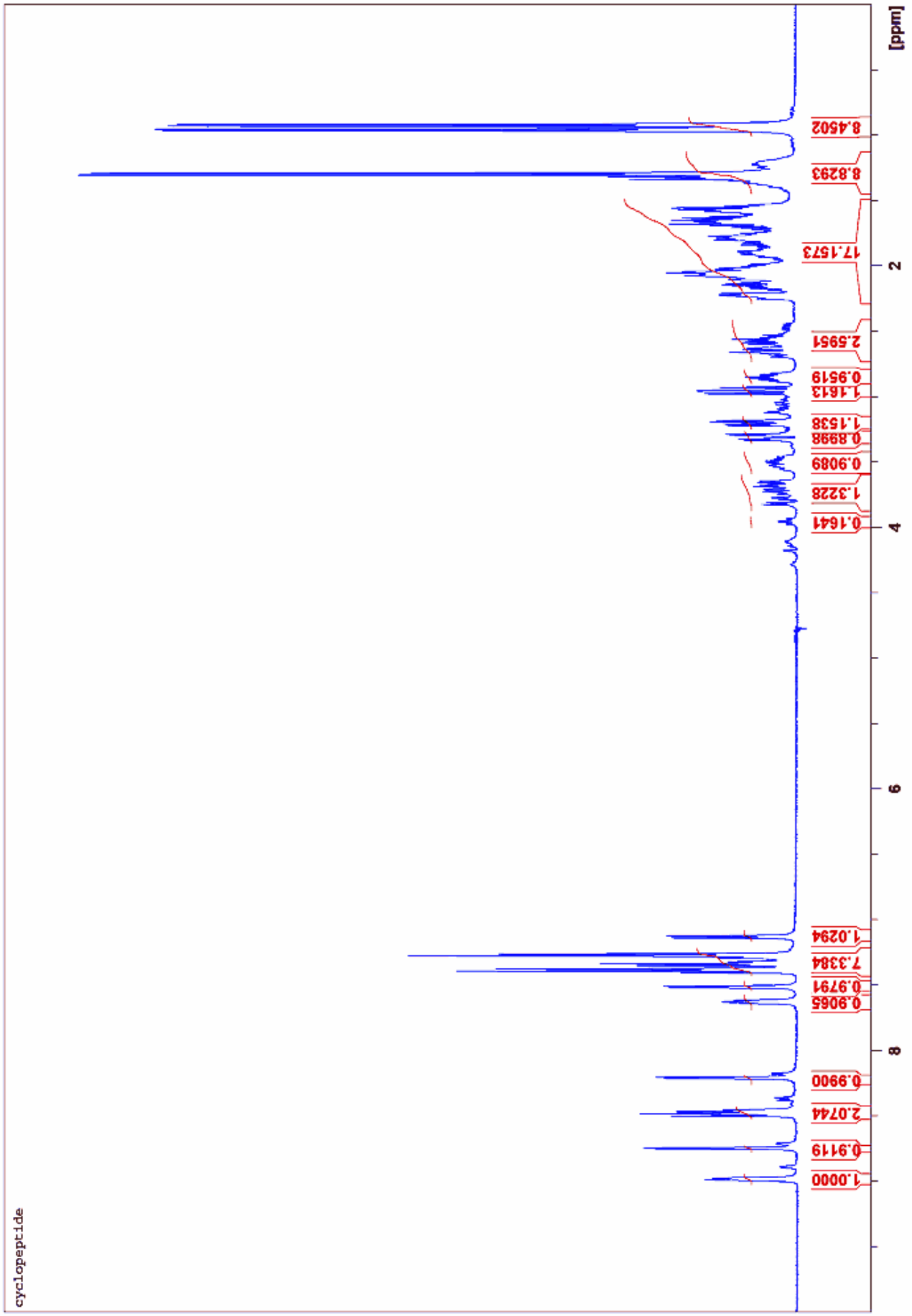


residue	*proton*	*δ (ppm)*	*J (Hz)*
Gly[1]	NH	8.55	
	α'CH	3.74	
	α''CH	3.67	
Gly[2]	NH	8.52	
	αCH_2	3.94	
Dap[3]	αNH	8.38	^3J(αNH–αCH) = 9.49
	αCH	4.91	
	β'CH	3.62	
	β''CH	3.54	
	βNH	8.19	
Gly[4]	NH	8.36	
	α'CH	4.39	
	α''CH	3.54	
Pro[5]	αCH	4.42	
	β'CH	2.20	
	β''CH	1.98	
	γ'CH	1.99	
	γ''CH	1.87	
	$\delta'CH_2$	4.09	
	$\delta''CH_2$	3.70	
Glu[6]	NH	7.24	
	αCH	4.64	
	β'CH	2.15	
	β''CH	1.85	
	γ'CH	2.17	
	γ''CH	2.07	
Phe[7]	αNH	8.24	^3J(NH–αCH) = 9.52
	αCH	4.65	^3J(β'CH–αCH) = 4.57
	β'CH	3.27	^3J(β''CH–αCH) = 10.29
	β''CH	2.83	^2J(β'CH– β''CH) = 14.17
	2,6H	7.21	
	3,5H	7.33	
	4H	7.27	

cyclo(Leu^{1}Ala^{2}lys^{3}Gly^{4}Pro^{5}Glu^6)cyclo(6α→3ε)Phe^{7}Gly^8 (4b, L^{1}A*k*^{2}GP*E*^{1}FG^2). Starting from **4a** (46.4 mg, 50.8 µmol), **4b** was obtained as foamy white solid after preparative RP-HPLC (24.6 mg, 31.5 µmol, 62%). Analytical RP-HPLC: t_R = 1.640 min (A/D 100:0 to 0:100 in 2.2 min, λ = 214 nm). MS (ESI+): $C_{38}H_{55}N_9O_9$ calc./obs. 782.42/782.42 $[M+H]^+$, 804.40/804.40 $[M+Na]^+$.



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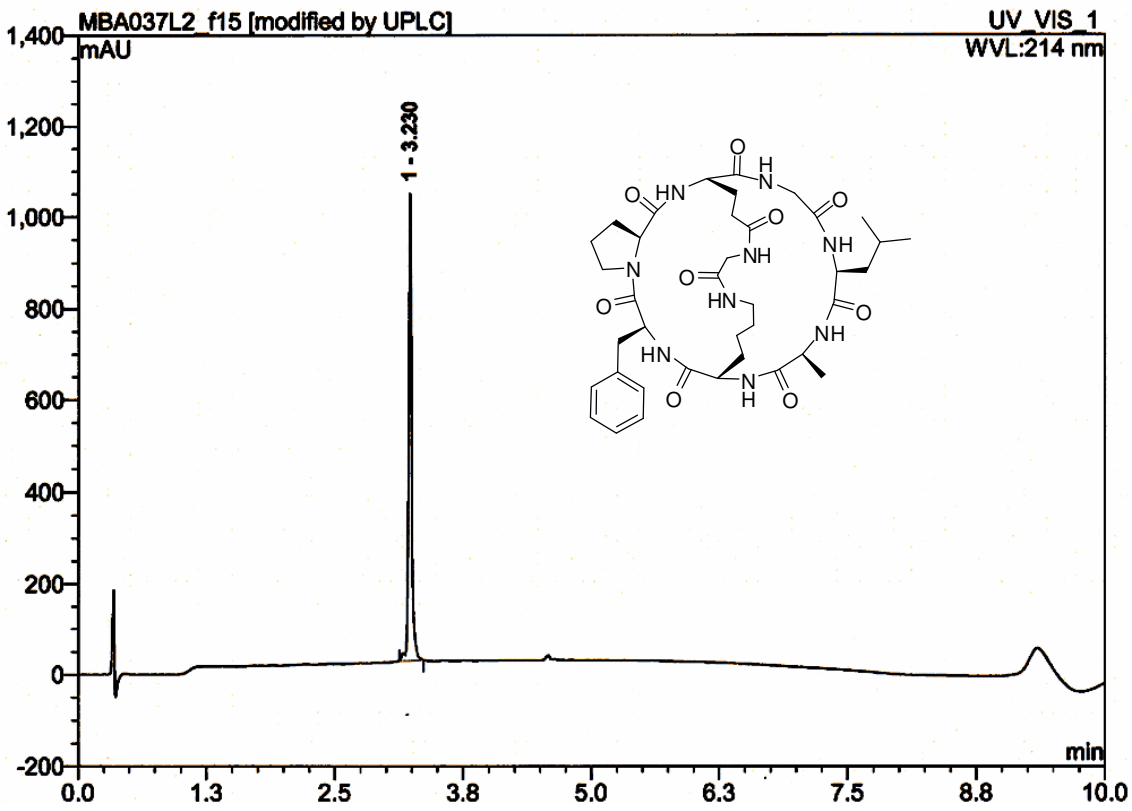
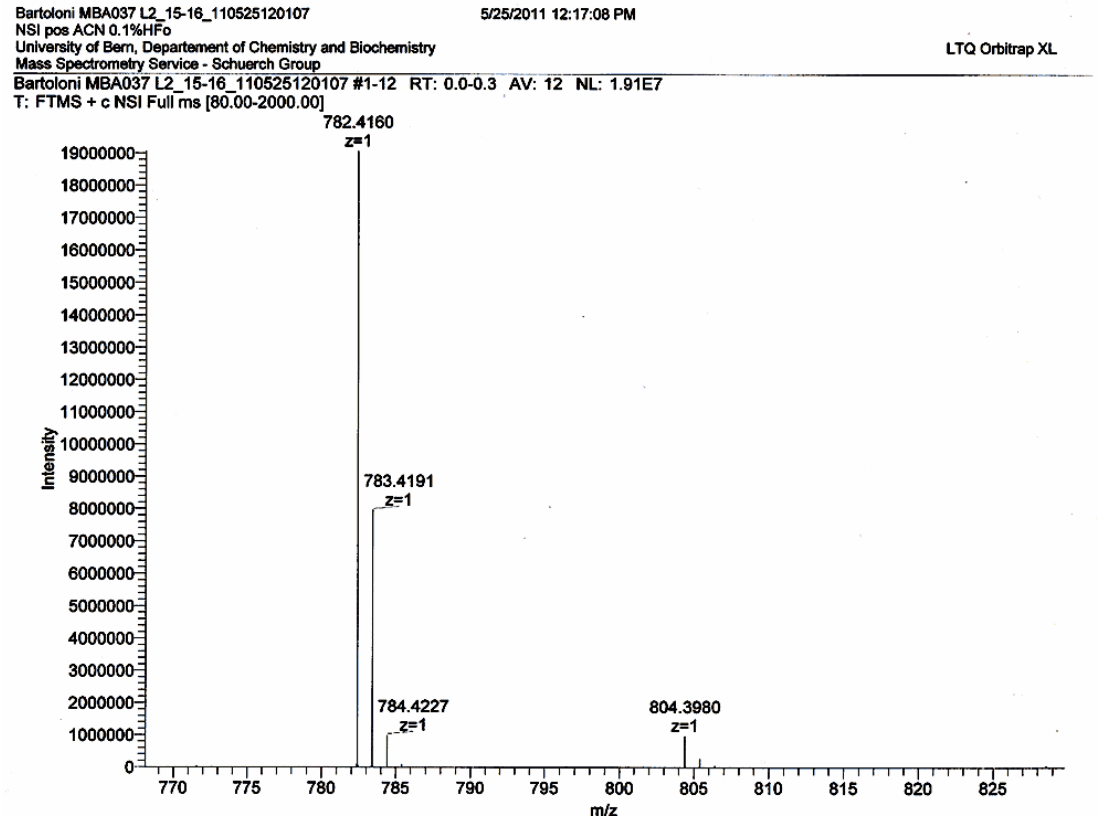
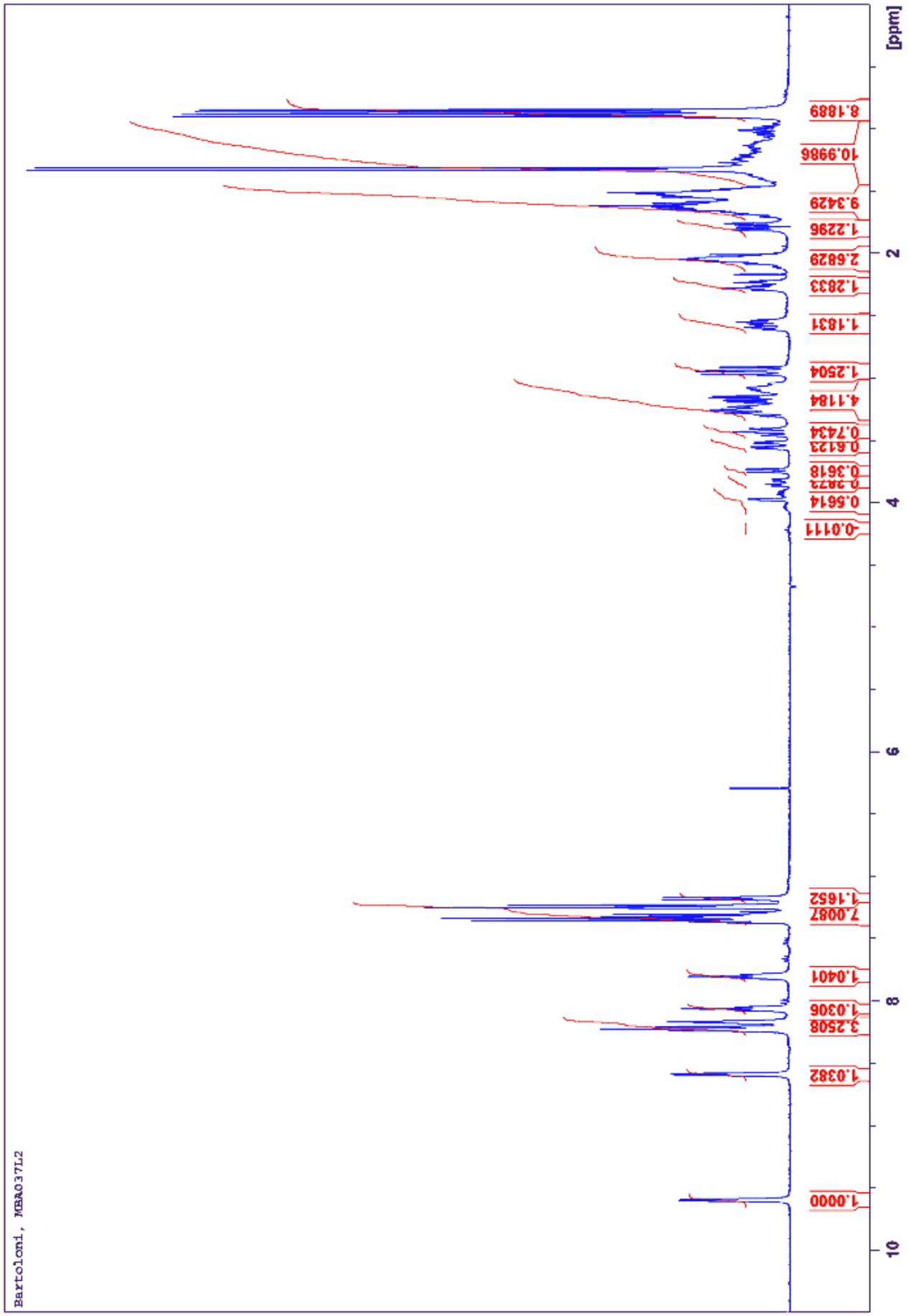
1.0000
2
4
6
8

residue	*proton*	*δ (ppm)*	*J (Hz)*
Leu[1]	NH	8.21	^3J(NH–αCH) = 3.93
	αCH	4.14	^3J(δCH–γCH) = 6.62
	β'CH	1.67	
	β''CH	1.60	
	γCH	1.71	
	δ'CH_3	0.99	
	δ''CH_3	0.95	
Ala[2]	NH	8.49	^3J(NH–αCH) = 9.06
	αCH	4.54	^3J(βCH–αCH) = 7.18
	βCH_3	1.32	
Lys[3]	αNH	7.13	^3J(αNH–αCH) = 7.41
	αCH	4.33	^3J(εNH–εCH) = 7.54, 4.78
	βCH_2	1.81	
	γCH_2	1.34	
	δ'CH	1.63	
	δ''CH	1.25	
	ε'CH	3.52	
	ε''CH	2.89	
	εNH	7.27	
Gly[4]	NH	8.98	^3J(NH–α'CH) = 1.74
	α'CH	4.19	^3J(α''CH–NH) = 7.02
	α''CH	3.70	^2J(α'CH–α''CH) = 15.07
Pro[5]	αCH	4.68	
	β'CH	2.25	
	β''CH	2.18	
	γ'CH	2.10	
	γ''CH	1.92	
	δ'CH_2	3.98	
	δ''CH_2	3.74	
Glu[6]	NH	7.52	^3J(NH–αCH) = 5.27
	αCH	4.17	
	βCH_2	2.06	
	γ'CH	2.67	
	γ''CH	2.59	
Phe[7]	αNH	8.75	^3J(NH–αCH) = 2.50
	αCH	4.31	^3J(β'CH–αCH) = 6.06
	β'CH	3.22	^3J(β''CH–αCH) = 10.61
	β''CH	2.99	^2J(β'CH– β''CH) = 13.32
	2,6H	7.31	
	3,5H	7.42	
	4H	7.37	
Gly[8]	NH	8.47	^3J(NH–α'CH) = 8.57
	α'CH	3.84	^3J(α''CH–NH) = 5.26
	α''CH	3.35	^2J(α'CH–α''CH) = 17.79

cyclo(Gly^{1}Leu^{2}Ala^{3}Lys^{4}Phe^{5}Pro^{6}Glu^7)cyclo(7γ→4ε)Gly^8 (5b, G^{1}LA*K*^{2}FP*E*^{1}γG^2). Starting from **5a** (24.0 mg, 26.3 µmol), **5b** was obtained as foamy white solid after preparative RP-HPLC (11.2 mg, 14.3 µmol, 55%). Analytical RP-HPLC: t_R = 3.230 min (A/D 100:0 to 0:100 in 7.5 min, λ = 214 nm). MS (ESI+): $C_{38}H_{55}N_9O_9$ calc./obs. 782.42/782.42 $[M+H]^+$.



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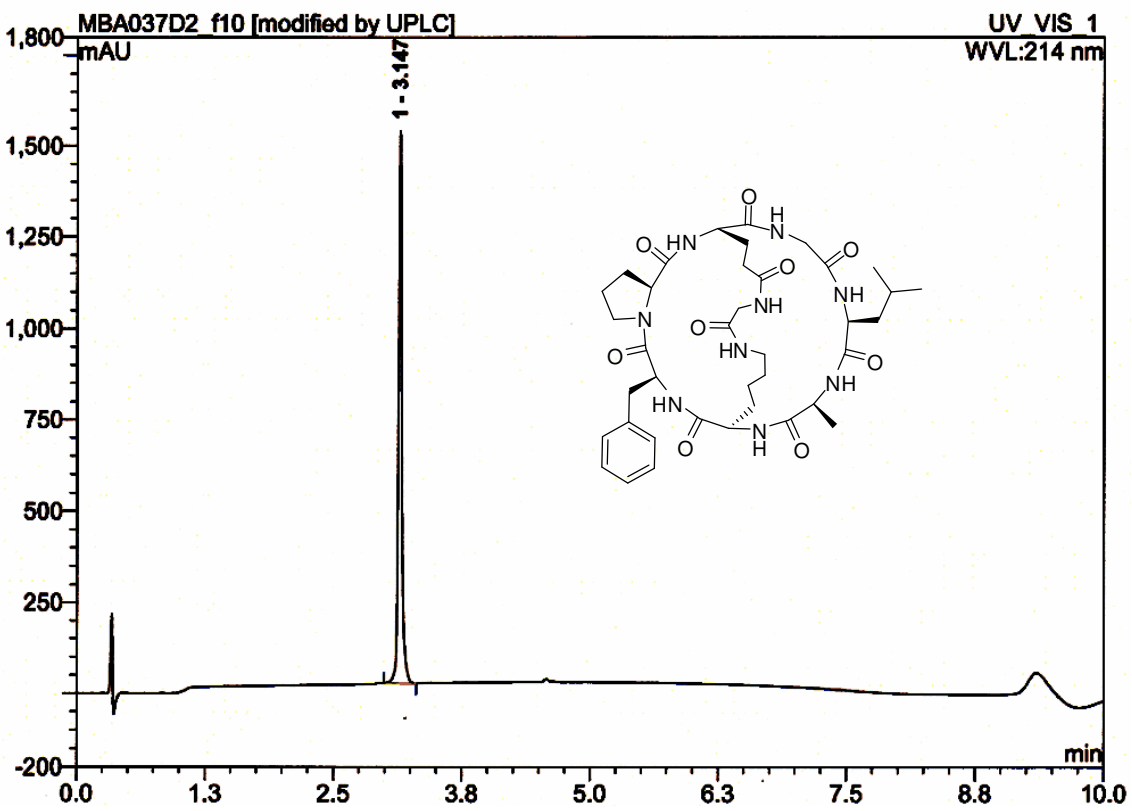
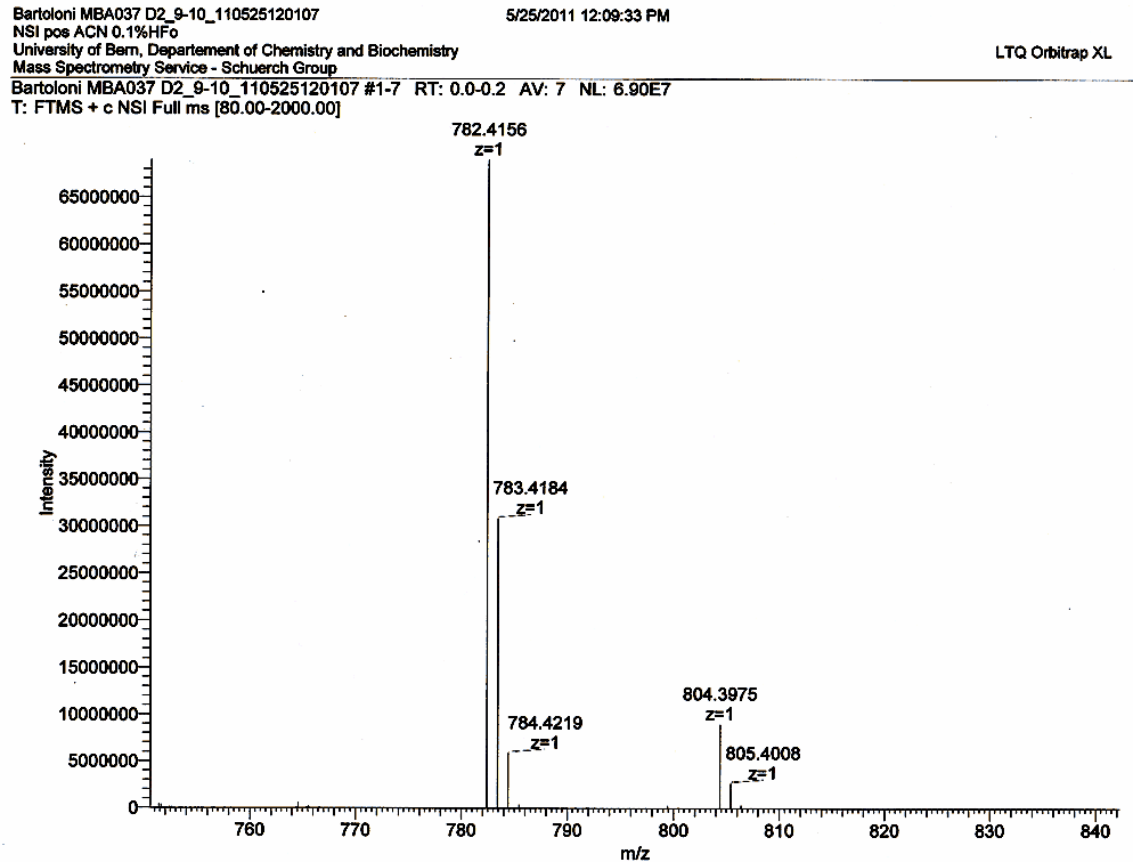
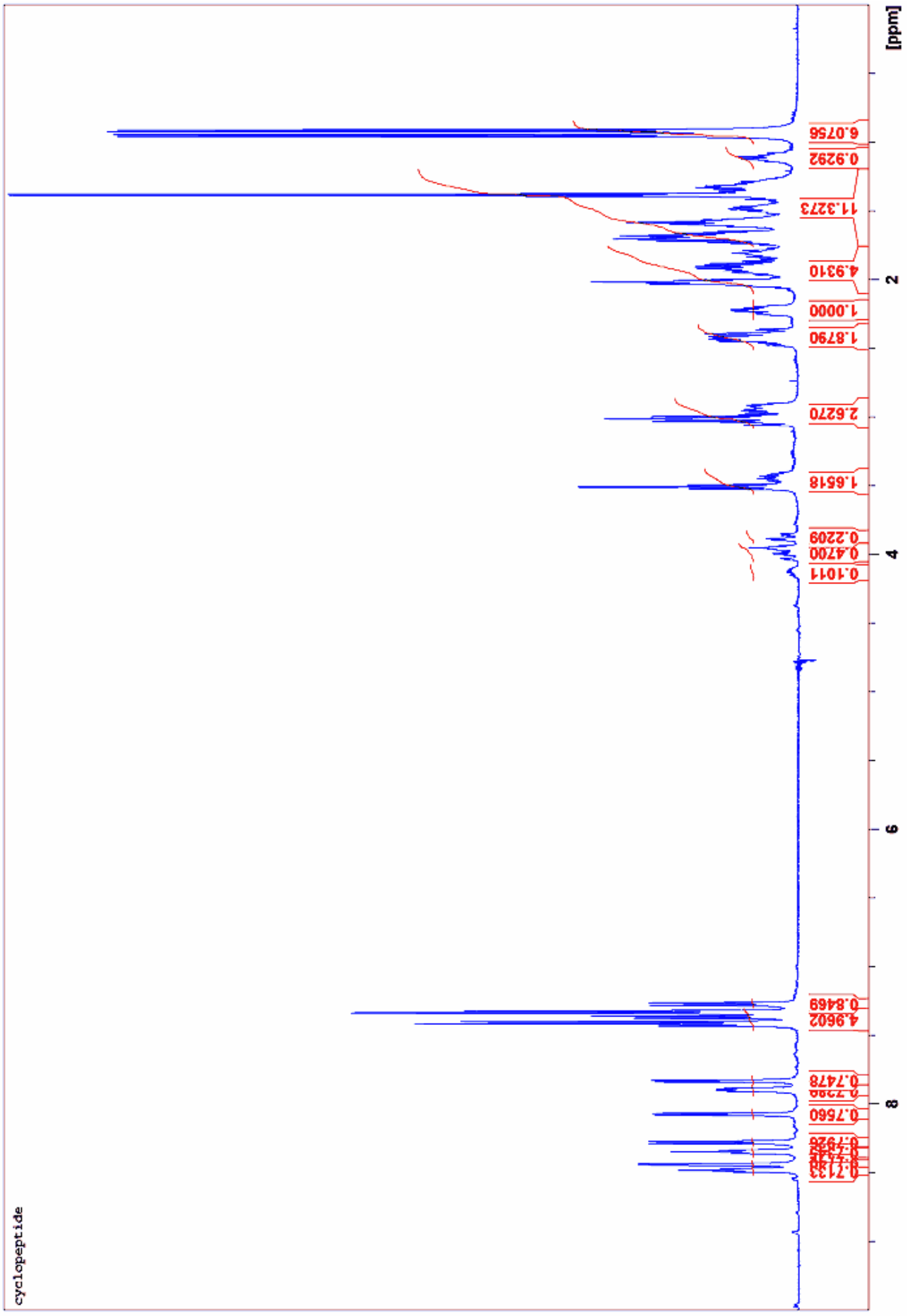
residue	*proton*	*δ (ppm)*	*J (Hz)*
Gly[1]	NH	8.16	^3J(NH–αCH) = 1.73, 5.88
	αCH_2	3.99	
Leu[2]	NH	8.23	^3J(NH–αCH) = 3.81
	αCH	4.05	^3J(δCH–γCH) = 6.38
	β'CH	1.62	
	β''CH	1.53	
	γCH	1.64	
	$\delta'CH_3$	0.91	
	$\delta''CH_3$	0.87	
Ala[3]	NH	8.22	^3J(NH–αCH) = 7.33
	αCH	4.23	^3J(βCH–αCH) = 7.38
	βCH_3	1.34	
Lys[4]	αNH	7.18	^3J(αNH–αCH) = 7.21
	αCH	4.30	^3J(εNH–εCH) = 4.73, 7.21
	β'CH	1.62	
	β''CH	1.53	
	γ'CH	1.23	
	γ''CH	1.15	
	δ'CH	1.55	
	δ''CH	1.32	
	ε'CH	3.25	
	ε''CH	3.10	
	εNH	7.81	
Phe[5]	αNH	8.59	^3J(NH–αCH) = 5.31
	αCH	4.66	^3J(β'CH–αCH) = 6.13
	β'CH	3.19	^3J(β''CH–αCH) = 10.30
	β''CH	2.96	^2J(β'CH– β''CH) = 13.22
	2,6H	7.26	
	3,5H	7.36	
	4H	7.32	
Pro[6]	αCH	3.76	^3J(αCH–β'CH) = 8.34
	β'CH	1.80	
	β''CH	1.03	
	γ'CH	1.69	
	γ''CH	1.39	
	δ'CH	3.45	
	δ''CH	3.29	
Glu[7]	NH	9.60	^3J(NH–αCH) = 4.88
	αCH	3.94	
	βCH_2	2.06	
	γ'CH	2.60	
	γ''CH	2.27	
Gly[8]	NH	8.07	^3J(α'CH–NH) = 5.50
	α'CH	3.85	^3J(α''CH–NH) = 5.84
	α''CH	3.56	^2J(α'CH–α''CH) = 15.85

cyclo(Gly^{1}Leu^{2}Ala^{3}lys^{4}Phe^{5}Pro^{6}Glu^7)cyclo(7γ→4ε)Gly^8 (*epi*-**5b**, **G^{1}LA*k*^{2}FP*E*^{1}γG^2).** Starting from *epi*-**5a** (43.5 mg, 47.6 µmol), *epi*-**5b** was obtained as foamy white solid after preparative RP-HPLC (5.1 mg, 6.5 µmol, 14%). Analytical RP-HPLC: t_R = 3.147 min (A/D 100:0 to 0:100 in 7.5 min, λ = 214 nm). MS (ESI+): $C_{38}H_{55}N_9O_9$ calc./obs. 782.42/782.42 $[M+H]^+$.



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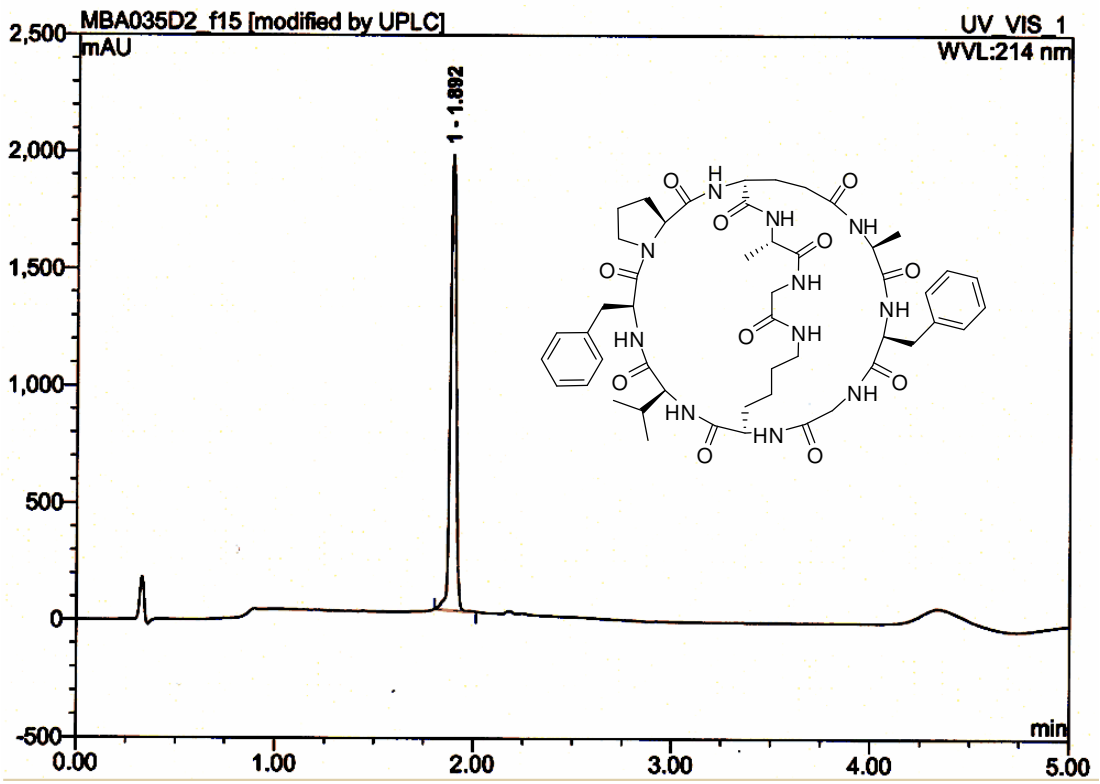
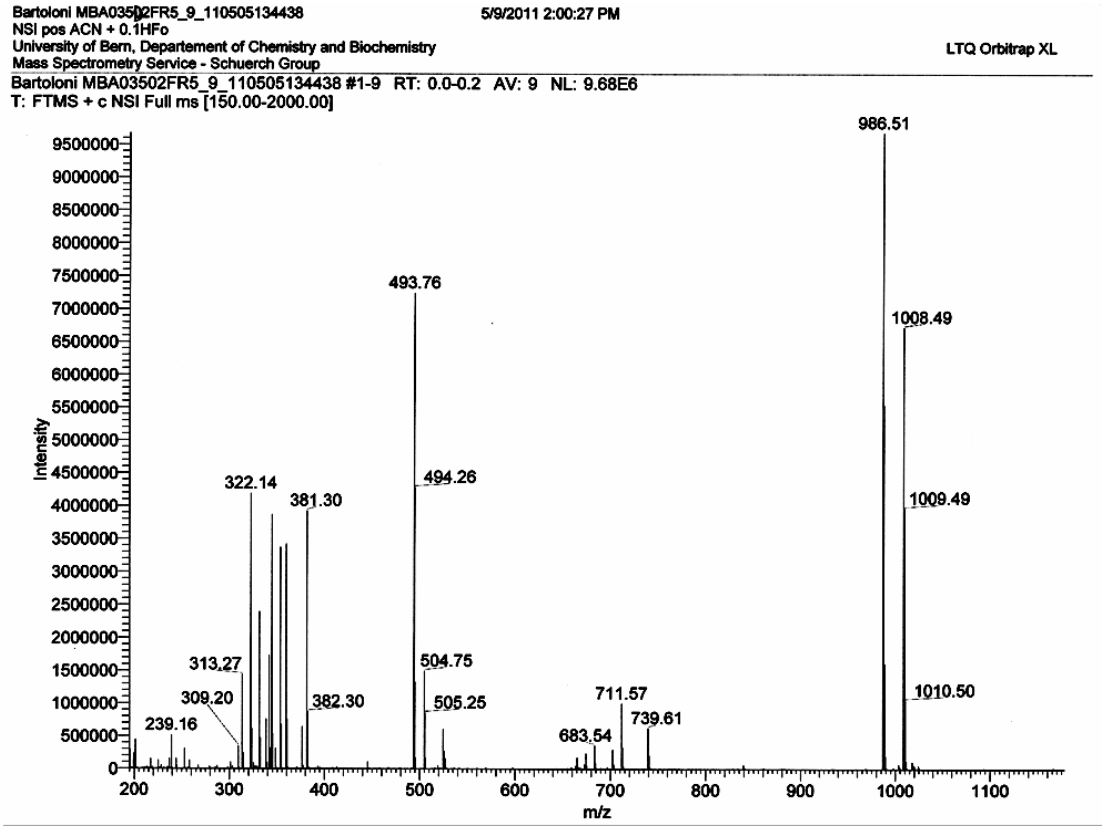
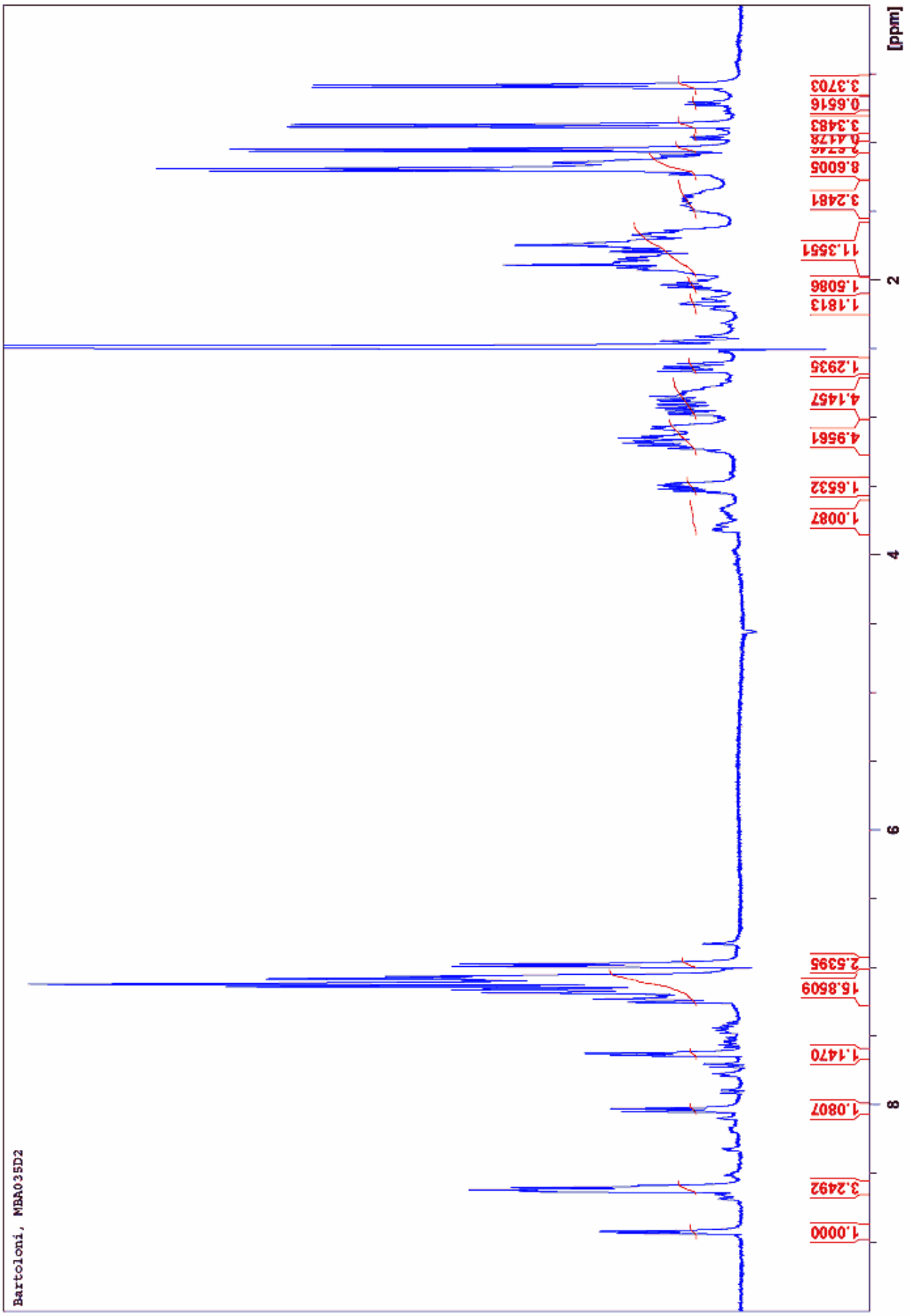
6
8

residue	*proton*	*δ (ppm)*	*J (Hz)*
Gly[1]	NH	8.50	^3J(NH–α'CH) = 7.14
	α'CH	4.35	^3J(α"CH–NH) = 4.62
	α"CH	4.00	^2J(α"CH–α'CH) = 17.71
Leu[2]	NH	8.46	^3J(NH–αCH) = 4.06
	αCH	4.10	^3J(δCH–γCH) = 6.51
	β'CH	1.67	
	β"CH	1.58	
	γCH	1.69	
	δ'CH_3	0.94	
	δ"CH_3	0.91	
Ala[3]	NH	8.29	^3J(NH–αCH) = 7.50
	αCH	4.40	^3J(βCH–αCH) = 7.34
	βCH_3	1.38	
D-Lys[4]	αNH	7.28	^3J(αNH–αCH) = 8.32
	αCH	4.33	^3J(εNH–εCH) = 3.65, 7.50
	β'CH	1.80	
	β"CH	4.57	
	γ'CH	1.30	
	γ"CH	1.11	
	δ'CH	1.47	
	δ"CH	1.33	
	ε'CH	3.43	
	ε"CH	2.93	
	εNH	7.91	
Phe[5]	αNH	8.09	^3J(NH–αCH) = 5.64
	αCH	4.54	
	βCH_2	3.01	
	2,6H	7.33	
	3,5H	7.41	
	4H	7.36	
Pro[6]	αCH	3.96	^3J(δCH–γCH) = 6.54, 7.90
	βCH_2	2.01	
	γ'CH	1.88	
	γ"CH	1.69	
	δCH_2	3.50	
Glu[7]	NH	7.84	^3J(NH–αCH) = 5.23
	αCH	4.14	
	βCH	2.22	
	β"CH	1.93	
	γCH_2	2.42	
Gly[8]	NH	8.36	^3J(NH–α'CH) = 4.68
	α'CH	3.96	^3J(α"CH–NH) = 6.21
	α"CH	3.86	^2J(α'CH–α"CH) = 17.07

cyclo(Ala[1]Phe[2]Gly[3]lys[4]Val[5]Phe[6]Pro[7]Glu[8])cyclo(8α→4ε)Ala[9]Gly[10] (6b, A[1]FG*k*[2]VFP*E*[1]AG[2]). Starting from **6a** (67.5 mg, 60.4 µmol), **6b** was obtained as foamy white solid after preparative RP-HPLC (22.8 mg, 23.1 µmol, 38%). Analytical RP-HPLC: t_R = 1.892 min (A/D 100:0 to 0:100 in 2.2 min, λ = 214 nm). MS (ESI+): $C_{49}H_{67}N_{11}O_{11}$ calc./obs. 986.51/986.51 $[M+H]^+$, 1008.49/1008.49 $[M+Na]^+$, 493.76/493.76 $[M+2H]^{2+}$.



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11.3551
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3.3483
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residue	*proton*	*δ (ppm)*	*J (Hz)*
Ala[1]	NH	8.03	^3J(NH–αCH) = 8.17
	αCH	4.43	^3J(βCH–αCH) = 7.16
	βCH_3	1.04	
Phe[2]	αNH	8.92	^3J(NH–αCH) = 5.96
	αCH	4.07	^3J(β"CH–αCH) = 9.38
	β'CH	3.15	^2J(β"CH– β'CH) = 14.10
	β"CH	2.87	
	2,6H	6.98	
	3,5H	7.12	
	4H	7.10	
Gly[3]	NH	8.60	
	α'CH	3.50	
	α"CH	3.79	
Lys[4]	αNH	7.17	
	αCH	4.09	
	βCH_2		
	γCH_2		
	δ'CH	1.90	
	δ"CH	1.13	
	ε'CH	3.16	
	ε"CH	2.81	
	εNH	7.17	
Val[5]	αNH	7.05	^3J(γ'CH–βCH) = 6.80
	αCH	3.49	^3J(γ"CH–βCH) = 7.07
	βCH	1.89	
	$\gamma' CH_3$	0.87	
	$\gamma'' CH_3$	0.58	
Phe[6]	αNH	7.23	^3J(NH–αCH) = 9.40
	αCH	4.90	^3J(β'CH–αCH) = 5.81
	β'CH	2.94	^3J(β"CH–αCH) = 8.69
	β"CH	2.62	^2J(β'CH– β"CH) = 14.10
	2,6H	6.98	
	3,5H	7.12	
	4H	7.10	
Pro[7]	αCH	4.14	
	β'CH	2.03	
	β"CH	1.63	
	γ'CH	1.84	
	γ"CH	1.77	
	$\delta' CH_2$	3.65	
	$\delta'' CH_2$	3.22	
Glu[8]	NH	7.62	^3J(NH–αCH) = 6.26
	αCH	4.41	
	β'CH	2.43	
	β"CH	1.67	
	γ'CH	2.17	
	γ"CH	1.88	
Ala[9]	NH	8.62	^3J(NH–αCH) = 2.33
	αCH	3.97	^3J(βCH–αCH) = 7.02
	βCH_3	1.18	
Gly[10]	NH	8.60	
	α'CH	3.50	
	α"CH	3.79	

NMR Structure determination

Tables S2-S6 report the used restraints in the following format: *[residue#1 number] [residue#1 name] [atom#1 label] [residue#2 number] [residue#2 name] [atom#2 label] [distance]*. Integration of rOe cross-peaks was performed using Topspin, followed by peak sorting and calculation via Microsoft Excel. rOe cross peaks of geminal proton couples were used as standard values for the calculation. Diastereotopic protons were not stereospecifically assigned. All protons were labelled according to *http://archive.ambermd.org/201009/att-0820/map-DG_modified.AMBER*. Distances given in Å.

Bicyclic peptide structure building – The starting structures for all cyclic peptides in the present study were built with L- and D-amino acids ordered as extended conformation using the peptide building dictionary interface in Maestro (version 8.5) in Desmond suite.[7] Parameters for bridgehead lysine and diaminopropanoic acid were obtained from natural amino acid building blocks based on transferability of AMBER force field ff99SB. The final modified topology, coordinates and parameters for refinement were built using the Xleap module of AmberTools 10.0. [8]

rOe distance restraint refinement protocol – All minimization and refinement calculations were performed using SANDER module of AMBER 10.0. The 500 steps of steepest descent energy minimization were performed using the generalized-Born implicit solvent model (GB)[9] on starting built structures to remove bad contacts associated to residues. This was followed by distance restraint Molecular Dynamics simulated annealing using GB model. rMDSA was performed for a period of 20 ps using input rOe distance restraints (Tables S1-S5). The protocol involved: 0-5000 steps heating the system from 0 K to 600 K, 5001-18000 steps cooling the system gradually from 600 K to 100 K with long TAUTP, 18001-20000 steps cooling to 0 K, 0-5000 steps tight coupling for heating and equilibration (TAUTP = 0.4), 5001-18000 steps of slow cooling (TAUTP = 4.0-1.0), 18001-19000 steps of faster cooling (TAUTP = 1.0) and 19001-20000 steps of fast cooling, like a minimization (TAUTP = 1.0-0.1). The weight of the restraints gradually increased the weight from 0.1 to 1 between step 0 and step 3000 and for the rest of the run (step 3001 to 20000) we kept the weight of the restraints at 1.

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[7] J. B. Kevin, C. Edmond, X. Huafeng, O. D. Ron, P. E. Michael, A. G. Brent, L. K. John, K. Istvan, A. M. Mark, D. S. Federico, K. S. John, S. Yibing, E. S. David, *Proceedings of the 2006 ACM/IEEE conference on Supercomputing*, ACM Tampa, Florida, 2006.

[8] *a*) D.A. Pearlman, D.A. Case, J.W. Caldwell, W.S. Ross, T.E. Cheatham, III, S. DeBolt, D. Ferguson, G. Seibel, P. Kollman, *Comp. Phys. Commun.* 1995, **91**, 1; *b*) D.A. Case, T. Cheatham, T. Darden, H. Gohlke, R. Luo, K.M. Merz, Jr., A. Onufriev, C. Simmerling, B. Wang, R. Woods, *J. Comput. Chem*. 2005, **26**, 1668.

[9] *a*) A. Onufriev, D. Bashford, D. A. Case, *J. Phys. Chem. B* 2000, **104**, 3712; *b*) B. Xia, V. Tsui, D. A. Case, H. J. Dyson, P. E. Wright, *J. Biomol. NMR* 2002, **22**, 317.

Table S2 Proton distance restraints for bicyclic peptide **1b**.

1 LEU NH	1 LEU αCH	2.7
1 LEU NH	2 GLY NH	3.0
1 LEU NH	3 DAP NH	4.0
2 GLY NH	2 GLY α"CH	2.4
2 GLY NH	2 GLY α'CH	2.6
2 GLY NH	3 DAP NH	2.4
2 GLY α"CH	3 DAP NH	3.1
3 DAP NH	3 DAP αCH	2.6
3 DAP NH	3 DAP εNH	3.5
3 DAP εNH	3 DAP β"CH	2.5
3 DAP εNH	3 DAP β'CH	2.8
3 DAP NH	4 PHE NH	4.2
3 DAP αCH	4 PHE NH	2.2
3 DAP β"CH	4 PHE NH	3.3
3 DAP εNH	6 GLU NH	4.0
3 DAP εNH	6 GLU γCH_2	3.5
3 DAP NH	6 GLU NH	3.8
3 DAP NH	7 ALA NH	4.1
3 DAP NH	7 ALA αCH	3.3
3 DAP εNH	7 ALA NH	2.7
3 DAP εNH	7 ALA αCH	3.3
4 PHE NH	4 PHE αCH	2.7
4 PHE NH	4 PHE β"CH	2.7
4 PHE NH	4 PHE β'CH	2.5
4 PHE αCH	6 GLU NH	2.6
5 PRO αCH	6 GLU NH	2.8
6 GLU NH	6 GLU αCH	2.6
6 GLU NH	6 GLU β"CH	2.9
7 ALA NH	7 ALA αCH	2.7

Table S3 Proton distance restraints for bicyclic peptide **4b**.

1 LEU NH	1 LEU αCH	2.7
1 LEU αCH	2 ALA NH	2.6
2 ALA NH	2 ALA αCH	2.5
2 ALA NH	3 LYS αNH	2.6
2 ALA αCH	3 LYS αNH	2.7
3 LYS αNH	3 LYS αCH	2.8
3 LYS εNH	3 LYS δ'CH	3.0
3 LYS εNH	3 LYS ε"CH	3.0
3 LYS εNH	3 LYS ε'CH	3.1
3 LYS αNH	4 GLY NH	3.8
3 LYS αCH	4 GLY NH	2.2
3 LYS εNH	8 GLY NH	2.5
3 LYS ε"CH	8 GLY NH	3.2
3 LYS εNH	8 GLY α"CH	3.3
3 LYS εNH	8 GLY α'CH	3.1
4 GLY NH	4 GLY α"CH	2.3
5 PRO αCH	6 GLU NH	3.0
5 PRO δ"CH	6 GLU NH	3.0
6 GLU NH	6 GLU αCH	2.7
6 GLU αCH	7 PHE NH	2.1
7 PHE NH	7 PHE αCH	2.6
7 PHE NH	7 PHE β"CH	2.6
7 PHE NH	7 PHE β'CH	2.4
7 PHE αCH	8 GLY NH	2.1
8 GLY NH	8 GLY α"CH	2.3
8 GLY NH	8 GLY α'CH	2.9

Table S4 Proton distance restraints for bicyclic peptide **5b**.

1 GLY NH	4 LYS αCH	3.5
1 GLY NH	5 PHE αCH	2.7
1 GLY NH	7 GLU NH	2.8
1 GLY NH	7 GLU βCH_2	3.0
2 LEU NH	2 LEU αCH	2.3
3 ALA NH	3 ALA αCH	2.8
3 ALA NH	3 ALA βCH_3	2.5
3 ALA NH	4 LYS αNH	2.6
3 ALA βCH_3	4 LYS αNH	3.1
4 LYS εNH	4 LYS ε"CH	2.8
4 LYS εNH	4 LYS ε'CH	2.9
4 LYS αNH	5 PHE NH	3.6
4 LYS αCH	5 PHE NH	2.2
4 LYS εNH	7 GLU γ"CH	4.0
4 LYS εNH	8 GLY NH	3.2
4 LYS εNH	8 GLY α"CH	3.6
4 LYS εNH	8 GLY α'CH	2.7
5 PHE NH	5 PHE αCH	2.5
5 PHE NH	5 PHE β"CH	2.6
5 PHE NH	5 PHE β'CH	2.6
5 PHE β"CH	6 PRO αCH	3.0
5 PHE αCH	7 GLU NH	2.8
6 PRO αCH	7 GLU NH	3.3
6 PRO γ"CH	7 GLU NH	3.3
6 PRO δ"CH	7 GLU NH	3.0
6 PRO δ"CH	7 GLU γ"CH	3.5
6 PRO β"CH	7 GLU NH	4.6
7 GLU NH	7 GLU αCH	2.9
7 GLU NH	7 GLU βCH_2	2.7
7 GLU NH	7 GLU γ"CH	3.0
7 GLU NH	7 GLU γ'CH	4.1
7 GLU βCH_2	8 GLY NH	3.8
7 GLU γ"CH	8 GLY NH	2.8
7 GLU γ'CH	8 GLY NH	2.6
8 GLY NH	8 GLY α"CH	2.5
8 GLY NH	8 GLY α'CH	2.8

Table S5 Proton distance restraints for bicyclic peptide *epi*-**5b**.

1 GLY NH	1 GLY α"CH	2.4
1 GLY α"CH	2 LEU NH	2.2
1 GLY NH	7 GLU αCH	2.1
1 GLY NH	7 GLU β'CH	2.8
2 LEU β"CH	3 ALA NH	2.8
2 LEU β'CH	3 ALA NH	2.7
3 ALA NH	3 ALA αCH	2.6
3 ALA NH	3 ALA βCH_3	2.3
3 ALA NH	4 LYS NH	2.4
4 LYS NH	4 LYS αCH	2.5
4 LYS NH	4 LYS β'CH	2.4
4 LYS NH	4 LYS β"CH	2.6
4 LYS εNH	4 LYS ε"CH	2.5
4 LYS εNH	4 LYS δ'CH	2.8
4 LYS εNH	4 LYS ε'CH	2.6
4 LYS εNH	7 GLU αCH	2.5
4 LYS εNH	8 GLY α"CH	2.7
4 LYS εNH	8 GLY α'CH	2.6
5 PHE NH	5 PHE β'CH	2.2
6 PRO αCH	7 GLU NH	2.1
6 PRO βCH_2	7 GLU NH	2.4

7 GLU NH	7 GLU αCH	2.6
7 GLU NH	7 GLU β''CH	2.4
7 GLU αCH	8 GLY NH	2.6
7 GLU γ'CH	8 GLY NH	2.2
8 GLY NH	8 GLY α''CH	2.3

Table S6 Proton distance restraints for bicyclic peptide **6b**

1 ALA NH	1 ALA αCH	2.6
1 ALA NH	1 ALA βCH_3	2.5
1 ALA αCH	2 PHE NH	2.2
1 ALA βCH_3	2 PHE NH	2.4
1 ALA NH	8 GLU β'CH	2.2
2 PHE NH	2 PHE αCH	2.1
5 VAL NH	6 PHE β''CH	2.5
5 VAL NH	6 PHE β'CH	2.4
7 PRO αCH	8 GLU NH	1.9
8 GLU NH	8 GLU αCH	2.7
8 GLU αCH	9 ALA NH	2.1
9 ALA NH	9 ALA βCH_3	2.1

Estimation of Combinatorial Possibilities

By taking into account the structural restrictions of our synthetic approach, we were able to calculate the number of unique sequences that can undergo cyclization to form bicyclic peptides and thus estimate the number of unique peptides that can be constructed.
To calculate the amount of possible bicyclic peptide structures, mathematical graphs were generated with the program GenG[10], which can be interpreted as peptides scaffolds, where nodes correspond to amino acids and edges correspond to peptide bonds. The maximal connectivity of a node was restricted to three, and the minimal ring size set to four nodes. Only graphs containing exactly two rings were considered (Fig. S3). For each number of amino acids (5-15 nodes) all possible scaffolds were generated, and for each scaffold, the number of possible sequences was calculated according to the formula

$$nSeq/Scf = dAA^{(SCL-2)} * dBH_1 * dBH_2 * ScfF$$

where nSeq/Scf is the number of possible unique sequences per scaffold, dAA is the number of different amino acids (e.g. 20), SCL is the total number of amino acids in the sequence (e.g. the chain length), dBH_1 and dBH_2 are the number of different bridge-head amino acids and ScfF a factor that takes into account the inequality of bridges in bicyclic peptides (e.g. [2.2.1] ≠ [2.1.2] ≠ [1.2.2]; ScfF = 1 if all bridges have the same length, ScfF = 3 if two bridges have same length, ScfF = 6 if none of the bridges have same length). If a scaffold contained a four ring, the total number of possibilities for this scaffold was divided by two as a means to account for sequences that are unlikely to undergo the second cyclization due to increased ring strain (e.g. in peptides where bridge-head residues point in opposite directions with respect to the first cycle).

Library Generation – Libraries of 1 M random sequences were generated for each length (5-10 AA) of amino acids for the linear and monocyclic peptides, and for each scaffold for the bicyclic peptides. The amino acids were grouped according to their chemical properties into the following groups: small and polar (Gly, Ala, Ser, Thr, Asn, Gln), hydrophobic (Val, Leu, Ile, Met), aromatic (Phe, Tyr, Trp), positively charged (His, Lys, Arg) and negatively charged (Asp, Glu). At each variable position in the sequence, each group had the same probability of occurance, while within each group each amino acid had the same probability of occurance. Only natural L-amino acids were allowed. At the bridge-head positions, L- and D-Dap, L- and D-Lys and L- and D-Glu were allowed. The same scaffolds restrictions as before were applied. Starting from SMILES, the three-dimensional structures of the bicyclic peptides were generated using CORINA[11]. Principal component analysis was used on the atomic coordinates to give the variance of atom positions in each dimension.

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[10] B. McKay, Practical Graph Isomorphism. *Congressus Numerantium* 1981, **30**, 45-87.
[11] J. Sadowski, J. Gasteiger, G. Klebe, *J. Chem. Inf. Comput. Sci.* 1994, **34**, 1000-1008.

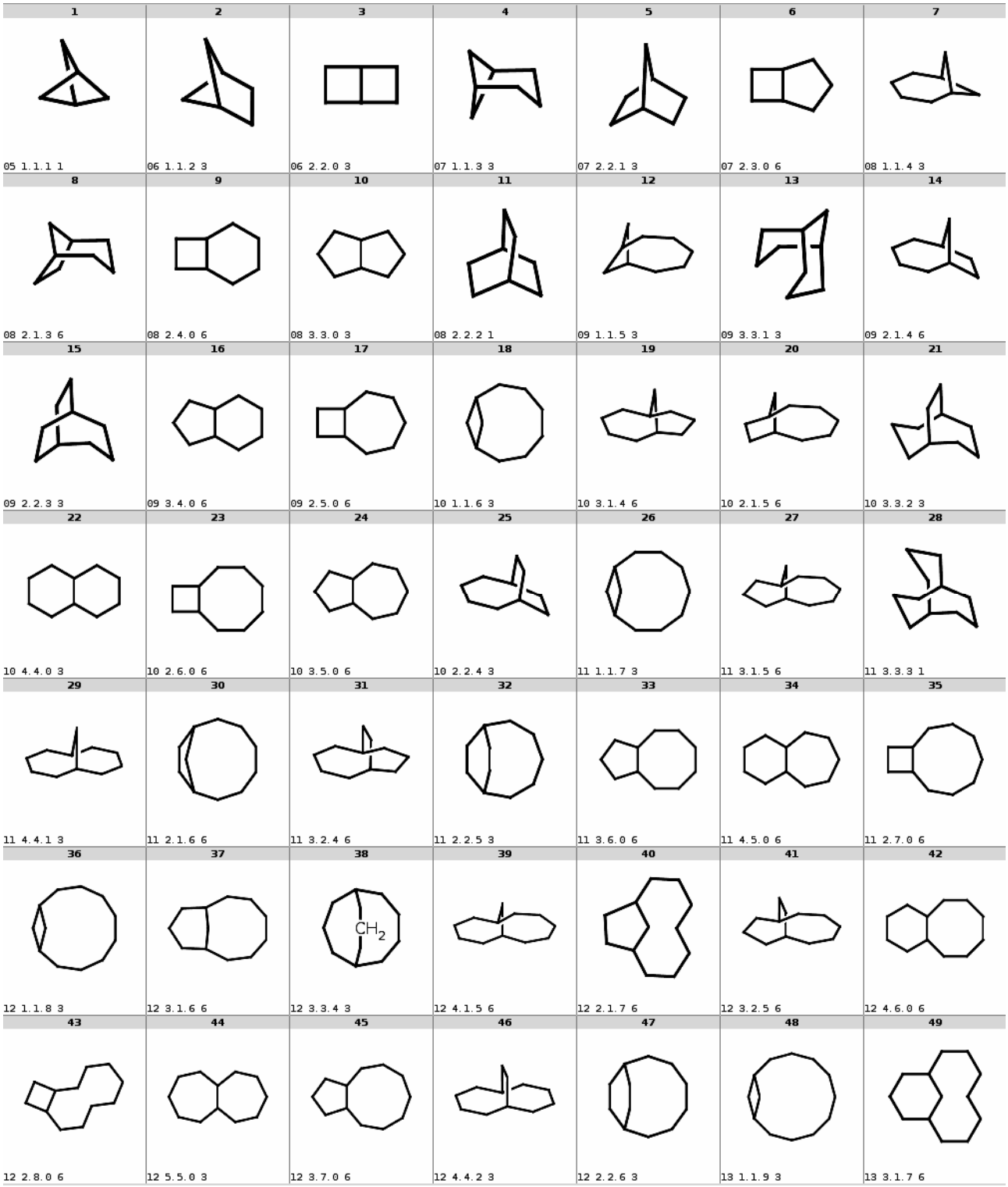

Figure S1. A. no. 1-49 of all possible scaffolds for bicyclic peptides shown as alkanes. The first number is the chain length of the sequence, the second number is the number of amino acids in each bridge, and the third number is the scaffold factor (ScfF).

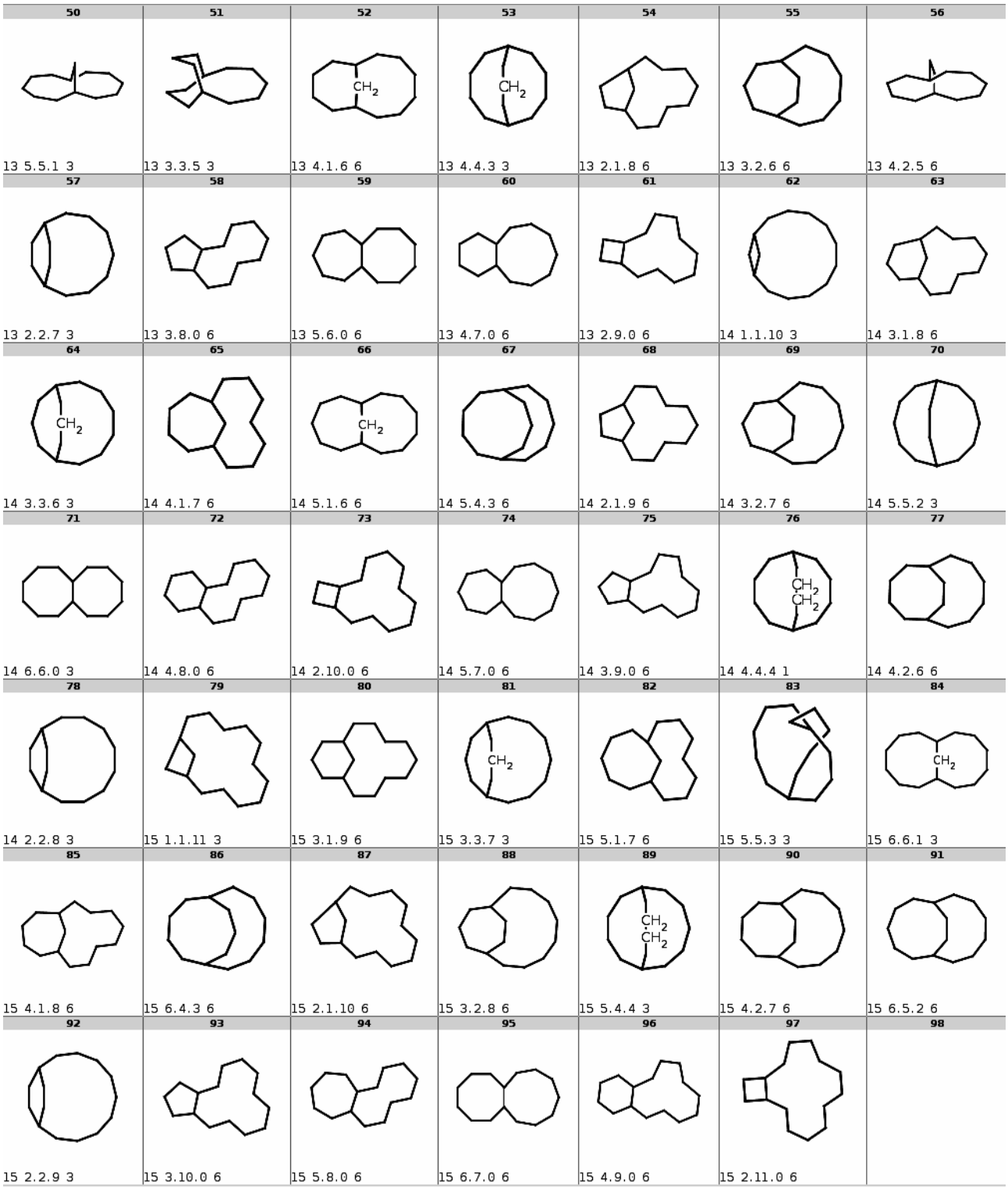

Figure S1. B. No. 50-97 of all possible scaffolds for bicyclic peptides shown as alkanes. The first number is the chain length of the sequence, the second number is the number of amino acids in each bridge, and the third number is the scaffold factor (ScfF).

Analysis of Molecular Dimensions

Table S7. Molecular dimensions of polycyclic peptides.[a)]

# of AA	PC1	PC2	PC3
SI-Example 1	63.23	31.32	5.44
SI-Example 3	58.42	32.56	9.02
SI-Example 4	44.99	29.69	25.32
SI-Example 5	47.75	39.01	13.24
SI-Example 6	60.30	23.27	16.43
1b	42.30	33.48	24.21
4b	51.60	27.74	20.66
5b	52.54	29.95	17.51
epi-**5b**	43.82	35.26	20.92
6b	49.74	30.35	19.91

[a)] the structure generated by CORINA was used for the SI examples 1-6 (from Table S1), and the experimentally determined NMR structures were used for **1b-6b.** There was no significant difference is PCs between CORINA-generated and experimentally determined structures for **1b-6b**.

Table S8. Molecular dimensions of virtually generated peptides and peptide segments from proteins.

Set Name	Number of Structures	PC1	PC2	PC3
Linear Peptides 5-10AA	8'202'571	86.7 ± 5.5	9.2 ± 4.1	4.0 ± 1.9
Monocyclic Peptides 5-10AA	8'199'968	54.1 ± 4.9	37.7 ± 4.5	8.2 ± 2.8
Bicyclic Peptides 5-10AA, containing no 0 Bridges	16'967'279	55.6 ± 7.5	28.6 ± 5.5	15.8 ± 4.5
Bicyclic Peptides 5-10AA, containing 0 Bridges	12'291'492	63.4 ± 7.9	26.6 ± 6.0	10.0 ± 4.4
α-Helical Segments 5-10AA extracted from 2RH1	1'309	55.6 ± 7.3	28.3 ± 5.4	16.1 ± 3.9
β-Sheet Segments 5-10AA extracted from 1GZT	58	84.1 ± 5.0	12.8 ± 4.2	3.1 ± 1.4