Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.

Supporting Information: "Can the absolute configuration of cyclic peptides be determined with vibrational circular dichroism?"

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Additional IR and VCD spectra of 7a, 7b and 8

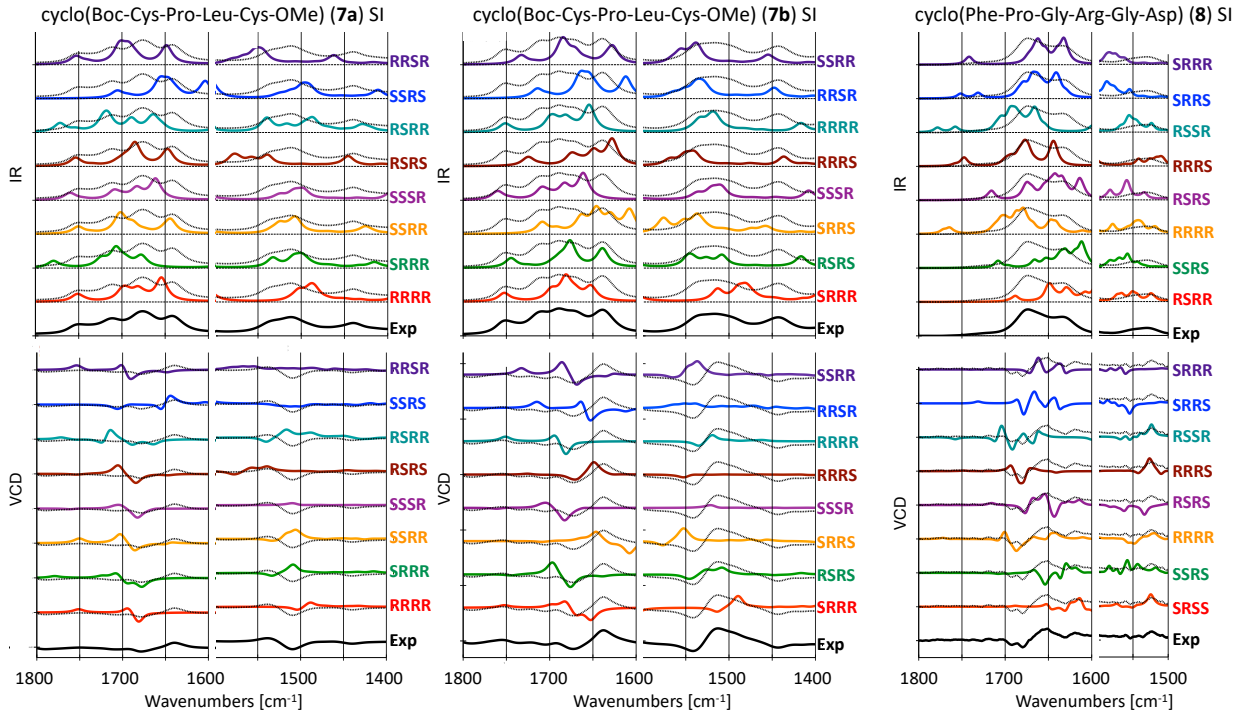

Figure S1: Experimental and computed (B3LYP/6-31+G*/CPCM) IR (top) and VCD spectra (bottom) of **7a** in ACN-d_3,[S19] **7b** in ACN-d_3[S19] and **8** in TFE.[S27] Two different scaling factors are used for the amide I and amide II region, see Table S3. The experimental spectrum is overlaid with the computed spectra as a dotted black line.

Scaling Factors

Table S1: Scaling factors used for molecules with two chiral centers: cyclo(Ala-β-Ala-Ala-β-Ala) (**1**), cyclo(Gly-Gly-Ser-Gly-Gly-Ala) (**2**) and cyclo(Gly-Gly-Ser-Gly-Gly-Val) (**3**).

	1		**2**		**3**	
	Amide I	Amide II	Amide I	Amide II	Amide I	Amide II
SS	0.990	0.970	0.980	0.985	0.980	0.990
RR	0.980	0.995	0.995	0.960	0.97	0.97
SR	0.995	0.985	0.975	0.970	0.975	0.965
RS	0.985	1.000	0.985	0.985	0.985	0.990

Table S2: Scaling factors used for molecules with three chiral centers: cyclo(Boc-Cys-Pro-Gly-Cys-OMe) (**4**), cyclo(Gly-Ala-Gly-Ser-Gly-Val) (**5**) and cyclo(Gly-Ala-Gly-Ser-Gly-Leu) (**6**).

	4		**5**		**6**	
	Amide I	Amide II	Amide I	Amide II	Amide I	Amide II
SRR	0.970	0.975	0.995	1.000	0.995	0.995
RSS	0.975	1.000[a]	0.980	0.975	0.980	0.975
RSR	0.985	0.975	0.990	1.000	0.990	1.000
SRS	0.960	1.000[a]	0.980	0.980	0.975	0.985
SSR	1.000	1.000[a]	0.980	0.980	0.990	0.980
RRS	0.970	0.970	0.990	1.000	0.975	1.000
SSS	0.960	0.965	0.985	0.975	0.985	0.970
RRR	0.990	0.975	0.970	0.995	0.995	0.985

[a] No positive overlap integral for this region. Scling factor 1.000 is used.

Table S3: Scaling factors used for molecules with four chiral centers: cyclo(Boc-Cys-Pro-S-Leu-Cys-OMe) (**7a**), cyclo(Boc-Cys-Pro-S-Leu-Cys-OMe) (**7b**) and Cyclo(Phe-Pro-Gly-Arg-Gly-Asp) (**8**).

	7a		**7b**		**8**	
	Amide I	Amide II	Amide I	Amide II	Amide I	Amide II
SRRR	1.000	0.975	0.985	0.960	0.980	1.000[a]
RSSS	0.975	0.995	0.965	0.990	1.000	0.980
RSRR	1.000	1.000[a]	1.000	1.000	0.960	0.990
SRSS	0.980	0.975	0.975	0.975	1.000	0.965
SSRR	0.990	0.975	0.980	0.995	0.965	0.985
RRSS	0.970	1.000	0.960	0.975	0.985	0.965
SSRS	0.965	0.965	0.990	0.965	0.965	1.000[a]
RRSR	0.992	1.000[a]	0.970	0.990	0.985	0.985
SRSR	0.970	0.970	0.965	0.995	0.985	0.000
RSRS	0.990	0.995	0.985	0.975	0.975	1.000[a]
RSSR	0.985	0.975	0.985	0.970	1.000	0.980
SRRS	0.960	0.995	0.965	1.000	0.985	1.000[a]
SSSR	0.995	0.970	0.995	0.975	0.975	1.000[a]
RRRS	0.970	0.990	0.975	0.995	0.990	0.970
SSSS	0.970	0.980	0.965	0.960	0.975	0.975
RRRR	0.990	0.965	0.990	0.985	0.995	0.990

[a] No positive overlap integral for this region. Scling factor 1.000 is used.

Peptide synthesis

Resin loading and first amino acid coupling: 2-Chlorotrityl chloride resin (4 g) was swelled in DCM (dichloromethane) for one hour. The resin was then added thionyl chloride (1.2 equiv.) and dry DCM and stirred with gentle agitation under argon for two hours. The resin was drained, rinsed thoroughly with dry DCM, and treated with Fmoc-Gly-OH (3 equiv.) in dry DCM and DIPEA (diisopropylethylamine, 6 equiv.). The mixture was stirred with gentle agitation under argon, overnight at room temperature. The solution was flushed out and the unreacted sites was end-capped with a solution of DCM, MeOH and DIPEA (80:15:5, 10 ml) for 30 minutes. The mixture was drained, and the resin was thoroughly rinsed with DCM and dried under nitrogen flow.

Linear peptide synthesis and resin cleavage: The linear peptide precursors were prepared by an automated solid phase peptide synthesiser (Biotage Initiator+ Alstra) in a 0.200 mmol scale. The preloaded resin was swelled in DMF (dimethylformamide) for 20 min at 70 °C. The Fmoc group was then removed by 20 % piperidine in DMF (two times with 4,5 ml for 7 minutes), and the amino acids (3.5 equiv.) was coupled using HBTU (2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate, 3.4 equiv.), HOBt (1-hydroxybenzotriazole hydrate, 3.5 equiv.), and DIPEA (7 equiv.) for 5 minutes at 75 °C. After each coupling the Fmoc group was removed by 20 % piperidine in DMF and the resin was washed with DMF. After the final amino acid coupling the resin was washed with DCM. The linear peptide was cleaved from the resin using 20 % hexafluoroisopropanol in DCM (two times with 5 ml, for 1 h) with slow stirring. The filtrates were combined and concentrated by reduced pressure.

Peptide cyclisation and side-chain protection cleavage: A solution of linear peptide and DIPEA (6 equiv., 0.21 ml) in DMF (10 ml) was added dropwise to a solution of Py-BOP (benzotriazole-1-yl-oxy-tris-pyrrolidino-phosphonium hexafluorophosphate, 3 equiv., 312 mg) and HOBt (3 equiv., 103 mg) in DMF (200 ml) under vigorous stirring, and the reaction was stirred at room temperature overnight. The solvents was removed under re-

duced pressure and treated with a solution of trifluoroacetic acid/triisopropylsilane)/water (95:2.5:2.5, 10 ml) for 3 hours. The mixture was concentrated under reduced pressure followed by precipitation using cold diethyl ether. The precipitate was washed with diethyl ether three times before it was dried and purified.

Purification and analysis: The crude cyclic peptides were purified by preparative reverse phase-HPLC using a Teledyne ISCO CombiFlash EZ prep system using a YMC-Actus Triart C18 column (150 x 20.0 mm, 5 µm) and gradients of 0 to 40 % buffer B (buffer A: water, buffer B: acetonitrile, both containing 0.1 % TFA) over 30 minutes with an 11 ml/min. flow rate. Peptide fractions was collected and lyophilized. NMR spectra was recorded on a 400 MHz Bruker Advance III HD spectrometer equipped with a 5 mm SmartProbe BB/1H (BB = 19F, 31P-15N) at 20 °C. The raw data was analysed with MestReNova (Version 14.2.2-28739). High resolution mass spectra were recorded on a Thermo Scientific Orbitrap Exploris 120 in either positive or negative electrospray ionization (ESI) mode. The data was analysed using Thermo Scientific Freestyle 1.8SP2 software.

Cyclo(Ala-Gly-Gly-Ser-Gly-Gly) (**2**) was obtained as a white powder (21 mg, 26 % yield). ^{1}**H NMR** (400 MHz, DMSO-*d*6) δ 8.40 (m, 4H), 7.55 (q, J = 5.3 Hz, 2H), 4.08 (p, J = 6.9 Hz, 1H), 4.02 (q, J = 6.0 Hz, 1H), 3.95 – 3.51 (m, 11H), 1.21 (d, J = 7.1 Hz, 3H). ^{13}**C NMR** (101 MHz, DMSO-*d*6) δ 172.26, 170.32, 169.63, 169.10, 169.08, 169.01, 60.30, 56.61, 49.20, 42.40, 42.29, 41.98, 41.81, 16.55. **HRMS** (ESI): calculated for: $C_{14}H_{23}N_6O_7^+$ $[M+H]^+$ 387.1623; found 387.1622.

Cyclo(Val-Gly-Gly-Ser-Gly-Gly) (**3**) was isolated as white fluffy powder (34 mg, 41 % yield) containing minor impurities. ^{1}**H NMR** (400 MHz, DMSO-*d*6) δ 8.57 (t, J = 6.0 Hz, 1H), 8.45 (dt, J = 6.1, 3.0 Hz, 2H), 8.21 (d, J = 6.7 Hz, 1H), 7.50 (t, J = 5.0 Hz, 1H), 7.45 (t, J = 4.8 Hz, 1H), 4.04 – 3.91 (m, 2H), 3.89 – 3.73 (m, 6H), 3.73 – 3.50 (m, 5H), 2.02 – 1.89 (m, 1H), 0.88 (dd, J = 13.2, 6.7 Hz, 6H). ^{13}**C NMR** (101 MHz, DMSO-*d*6) δ 171.49, 170.33, 169.32, 169.13, 168.99, 168.93, 60.23, 59.70, 56.50, 42.38 (2C), 41.98 (2C), 28.78, 19.19, 18.77. **HRMS** (ESI): calculated for: $C_{16}H_{25}N_6O_7^+$ $[M+H]^+$ 415.1936; found

415.1934.

Cyclo(Val-Gly-Ser-Gly-Ala-Gly) (**5**) was isolated as a white powder (22 mg, 26 %) containing minor impurities. ^{1}**H NMR** (400 MHz, DMSO-*d*6) δ 8.45 (t, J = 5.8 Hz, 1H), 8.38 (t, J = 5.7 Hz, 1H), 8.07 – 7.99 (m, 2H), 7.90 (d, J = 7.8 Hz, 1H), 7.79 (d, J = 7.2 Hz, 1H), 4.22 (m, 2H), 3.99 (t, J = 7.3 Hz, 1H), 3.82 (m, 2H), 3.77 – 3.53 (m, 7H), 2.12 – 1.99 (m, 1H), 1.22 (d, J = 7.1 Hz, 3H), 0.85 (dd, J = 9.7, 6.8 Hz, 6H). ^{13}**C NMR** (101 MHz, DMSO-*d*6) δ 172.10, 171.43, 170.32, 168.92, 168.84, 168.48, 61.16, 58.56, 55.10, 48.19, 42.82, 42.69, 42.46, 29.14, 19.11, 18.26, 17.37. **HRMS** (ESI): calculated for: $C_{17}H_{29}N_6O_7^+$ $[M+H]^+$ 429.2092; found 429.2091.

Cyclo(Leu-Gly-Ser-Gly-Ala-Gly) (**6**) was isolated as a white fluffy powder (19 mg, 22 % yield). ^{1}**H NMR** (400 MHz, DMSO-*d*6) δ 8.38 - 8.30 (m, 2H), 8,20 (d, J = 7.4 Hz, 1H), 8.02 - 7.94 (m, 2H), 7.78 (d, J = 7.2 Hz, 1H), 4.28 - 4.19 (m, 2H), 4.18 – 4.09 (m, 1H), 3.92 – 3.69 (m, 4H), 3.69 – 3.54 (m, 4H), 1.53 (m, 3H), 1.22 (d, J = 7.1 Hz, 3H), 0.86 (dd, J = 18.9, 5.4 Hz, 6H). ^{13}**C NMR** (101 MHz, DMSO-d6) δ 172.05, 172.00, 170.42, 169.06, 168.92, 168.59, 61.36, 55.17, 51.56, 48.17, 42.88, 42.59, 42.43, 39.43, 24.23, 22.88, 21.64, 17.49. **HRMS** (ESI): calculated for: $C_{18}H_{31}N_6O_7^+$ $[M+H]^+$ 443.2249; found 443.2246.

NMR spectra for synthesised cyclic peptides

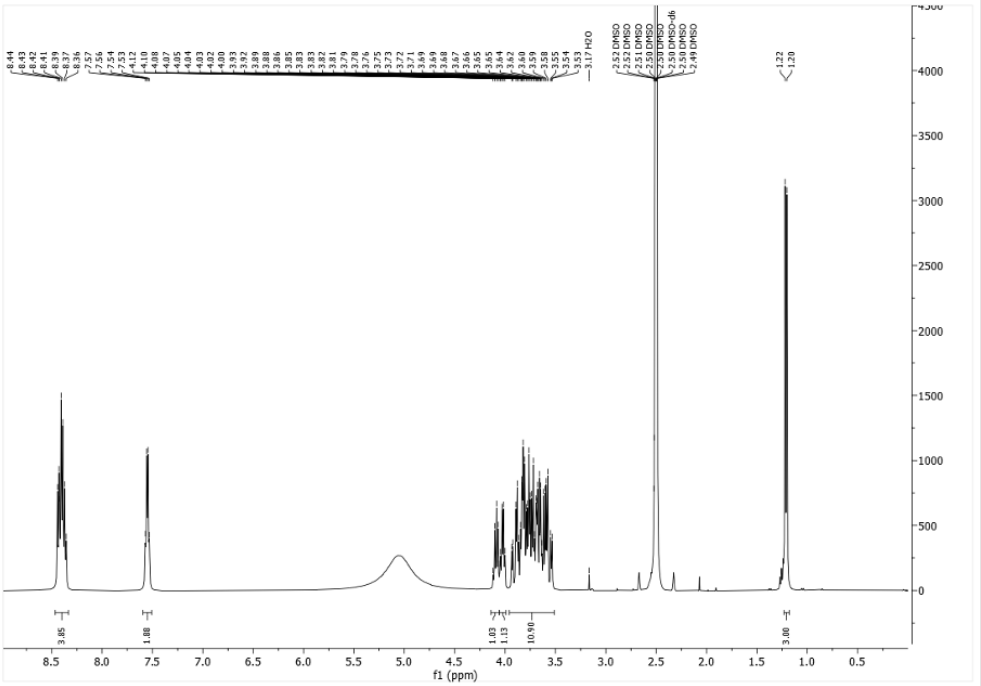

Figure S2: ^{1}**H NMR** spectrum of cyclo(Ala-Gly-Gly-Ser-Gly-Gly) (**2**).

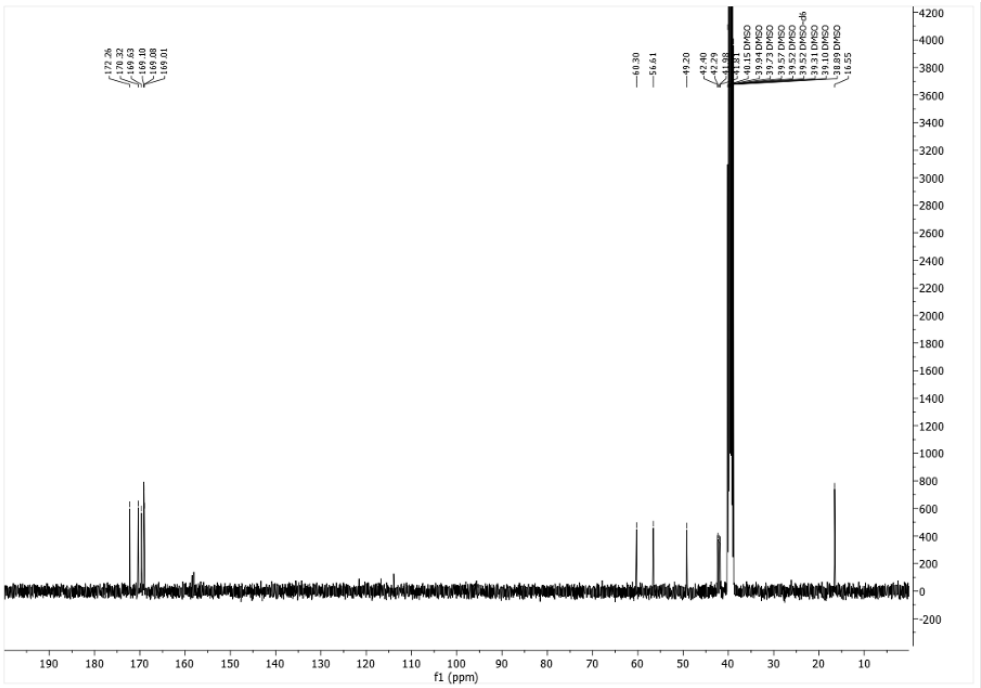

Figure S3: ^{13}**C NMR** spectrum of cyclo(Ala-Gly-Gly-Ser-Gly-Gly) (**2**).

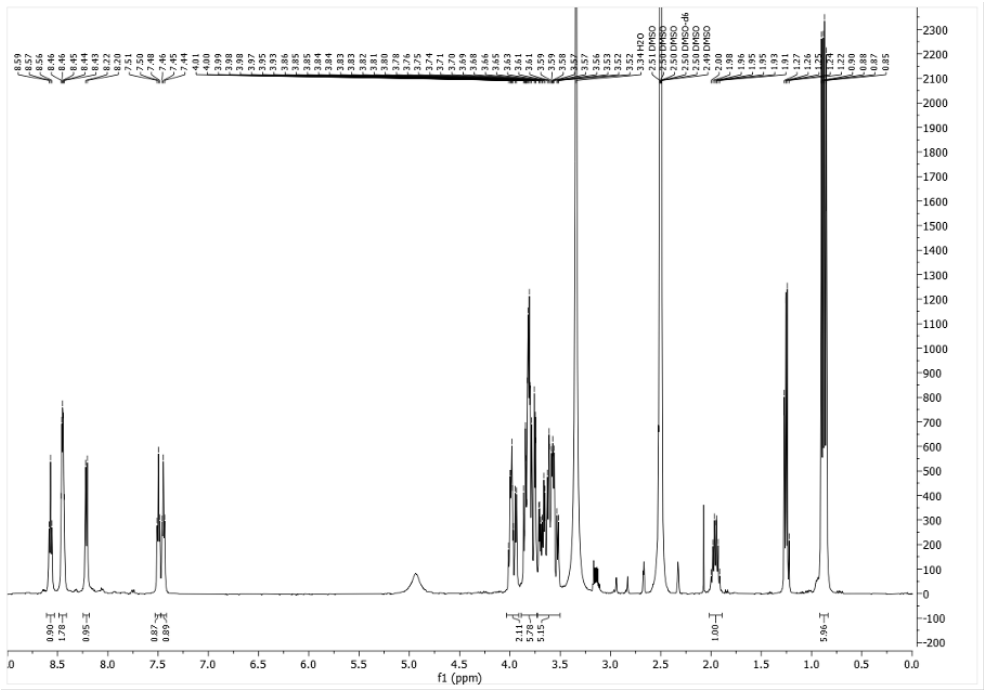

Figure S4: ^{1}**H NMR** spectrum of cyclo(Val-Gly-Gly-Ser-Gly-Gly) (**3**).

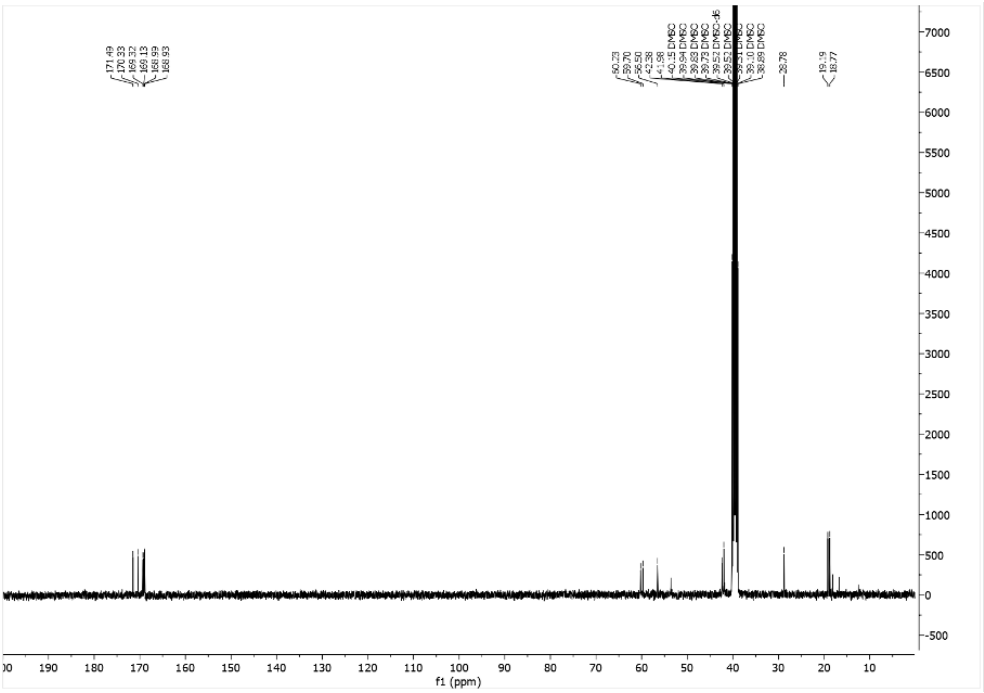

Figure S5: ^{13}**C NMR** spectrum of cyclo(Val-Gly-Gly-Ser-Gly-Gly) (**3**).

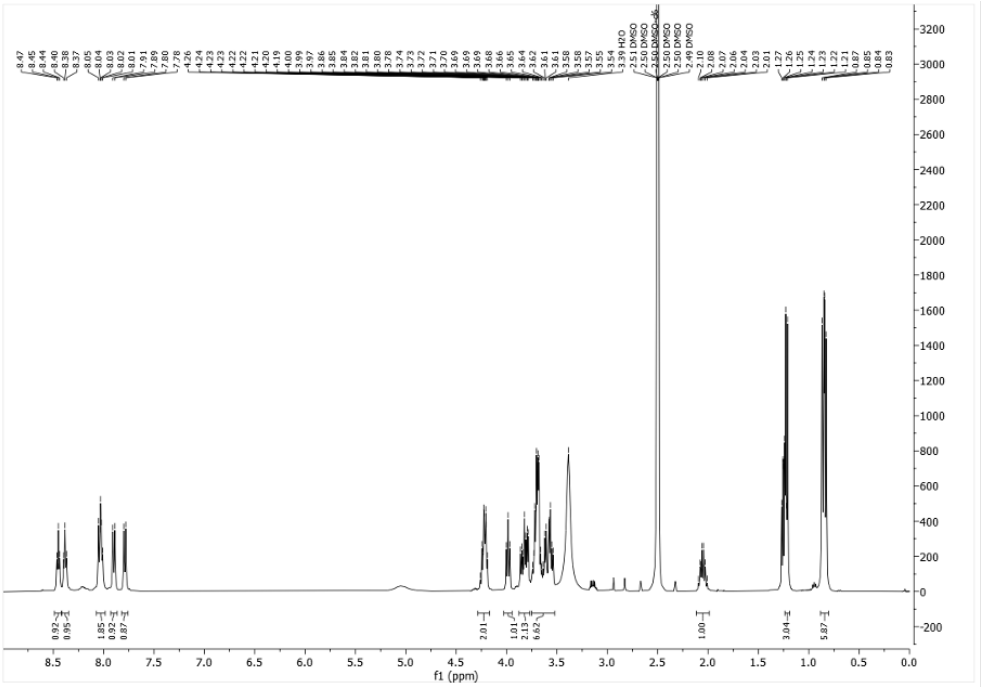

Figure S6: ^{1}**H NMR** spectrum of cyclo(Val-Gly-Ser-Gly-Ala-Gly) (**5**).

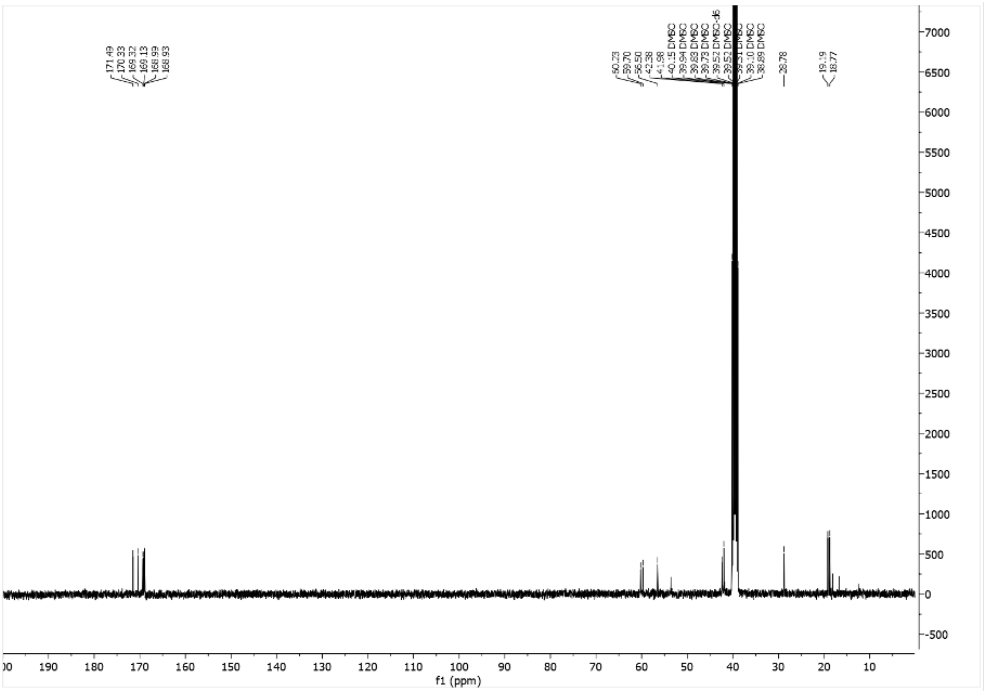

Figure S7: ^{13}**C NMR** spectrum of cyclo(Val-Gly-Ser-Gly-Ala-Gly) (**5**).

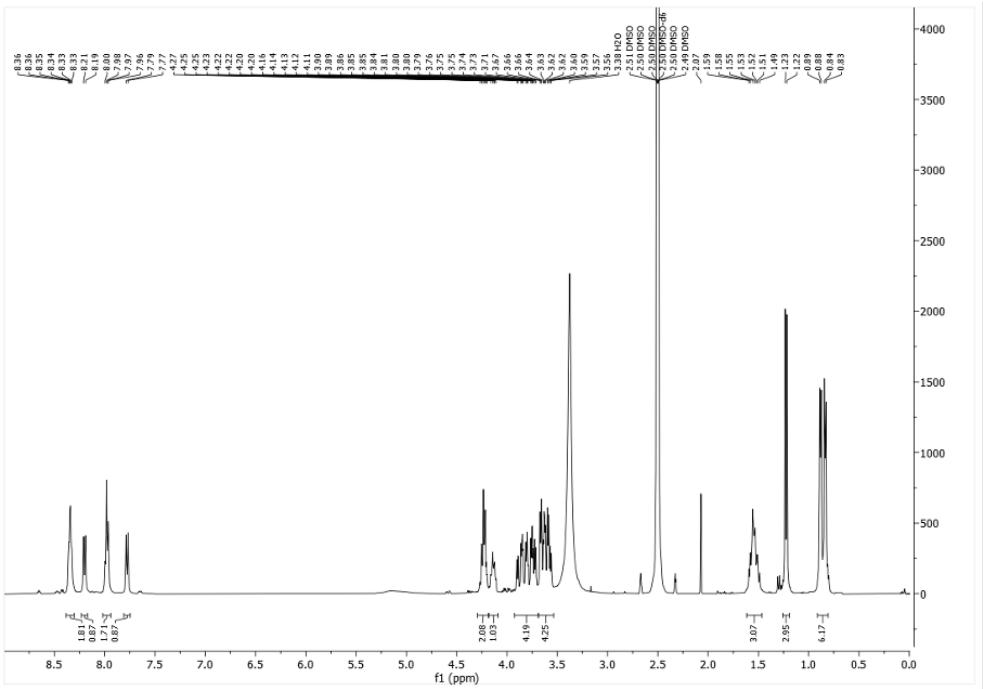

Figure S8: ^{1}**H NMR** spectrum of cyclo(Leu-Gly-Ser-Gly-Ala-Gly) (**6**).

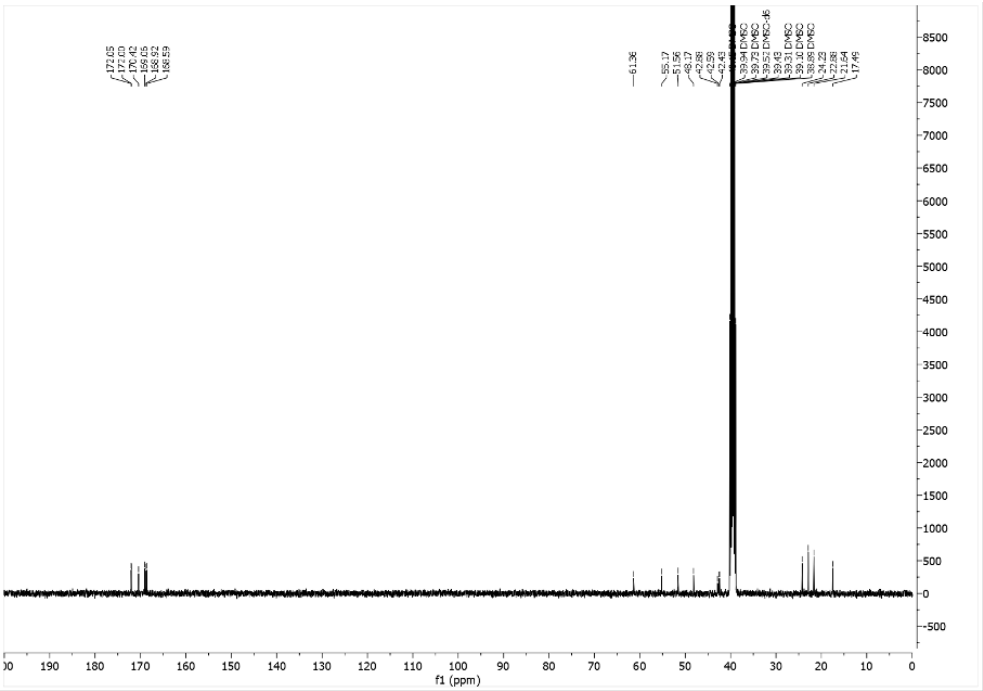

Figure S9: ^{13}**C NMR** spectrum of cyclo(Leu-Gly-Ser-Gly-Ala-Gly) (**6**).