Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Synthesis and *in vitro* bone cell activity of analogues of the cyclohexapeptide Dianthin G

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Supporting information

General methods

Materials

All reagents were acquired as reagent grade and used without further purification. Solvents for RP-HPLC were purchased as HPLC grade and used without further purification. 6-Chloro-1-hydroxybenzotriazole (6-Cl-HOBt) was purchased from Aapptec (Louisville, Kentucky). *O*-(Benzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate (HBTU), 2-(7-aza-1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU), 2-chlorotrityl chloride resin, 4-(hydroxmethyl)phenoxyacetic acid (HMP linker), di-*tert*-butyl dicarbonate (Boc_2O), Fmoc-allylglycine-OH (Fmoc-Agl), Fmoc-Pro-OH, Fmoc-Leu-OH, Fmoc-Thr(tBu)-OH, Fmoc-Phe-OH, and Fmoc-Gly-OH were purchased from GL Biochem (Shanghai, China). *N,N*-Dimethylformamide (DMF) (AR grade), and acetonitrile (CH_3CN) [high-performance liquid chromatography (HPLC) grade] were purchased from Scharlau (Barcelona, Spain). *N,N'*-Diisopropylethylamine (iPr_2EtN), *N,N'*-diisopropylcarbodiimide (DIC), 2-mercaptoethanol, 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), 2,4,6-trimethylpyridine (*sym*-collidine), Hoveyda-Grubbs' II catalyst, 2,2,2-trifluoroethanol (TFE), dimethylsulfate (DMS), 2-nitrobenzenesulfonyl chloride (*o*-NBS-Cl), formic acid, 1-methyl-2-pyrrolidinone (NMP), and piperidine were purchased from Sigma–Aldrich (Sydney, Australia). Dichloromethane (CH_2Cl_2) was purchased from ECP Limited (Auckland, New Zealand). Triisopropylsilane (iPr_3SiH) was purchased from Alfa Aesar (Lankashire, U.K). Dimethyl sulfoxide-d_6 (DMSO-d_6) was purchased from Cambridge Isotope Laboratories (Massachusetts, USA) Trifluoroacetic acid (TFA) was purchased from Halocarbon (New Jersey, USA). Dimethyl sulfoxide (DMSO) was purchased from Romil Ltd (Cambridge, UK). Fmoc-Gly-O-CH_2-Phi-OCH_2-CH_2-COOH (Fmoc-Gly-HMPP) was purchased from PolyPeptide Laboratories Group (Strasbourg, France). Aminomethyl polystyrene resin was purchased from Rapp Polymere (Tuebingen, Germany).

HPLC, MS and NMR

Analytical RP-HPLC spectra were performed on a Dionex (California, USA) Ultimate 3000 System equipped with a two-channel UV detector using an analytical column

(XTerra® MS C_{18} column, 4.6 mm x 150 mm, 5 µm) and a linear gradient of 5% to 75%B over 35 mins (*ca.* 2%B per minute) at a flow rate of 1 mL min^{-1}. The solvent system used was A (0.1% trifluoroacetic acid in H_2O) and B (0.1% trifluoroacetic acid in CH_3CN). Peptide masses were confirmed by a Bruker micrOTOF-Q II mass spectrometer (Bremen, Germany) or a Hewlett Packard (HP) 1100 series mass spectrometer (California, USA) using direct flow injection at 0.3 mL min^{-1} into an ESI source in the positive mode.

Peptides were purified using a Waters (Massachusetts, USA) S600E system using a semi-preparative column (Waters XTerra® C_{18}, 300 mm x 19 mm, 10 µm) at a flow rate of 10 mL min^{-1} and eluted using a one-step slow gradient protocol with detection at 210 nm.[1] Fractions were collected, analysed by analytical RP-HPLC or ESI-MS, pooled and lyophilised three times from 10 mM aq HCl.[2]

Nuclear magnetic resonance spectra were recorded on a Bruker AVANCE 600 spectrometer (Bremen, Germany) using DMSO-d6 as a solvent. Assignments were made with the aid of HSQC, TOCSY, and NOESY experiments.

Peptide synthesis

Loading of the *C*-terminal amino acid to the resin

For peptides **1**-**5**:

A solution of Fmoc-Gly-O-CH_2-phi-OCH_2-CH_2-COOH (190.2 mg, 0.4 mmol) and DIC (62 µL, 0.4 mmol) in CH_2Cl_2/DMF (*v/v*; 2:1, 3 mL) was added to pre-swollen (CH_2Cl_2, 3 mL, 20 min) aminomethyl polystyrene resin (220.0 mg, 0.2 mmol) and the mixture gently agitated for 5 h, at room temperature, filtered and washed with DMF (4 x 3 mL). A negative Kaiser test[3] confirmed the coupling.

For peptide **6**:

A solution of Fmoc-Gly-OH (119.0 mg, 0.4 mmol) and *i*Pr_2EtN (70 µL, 0.4 mmoL) in CH_2Cl_2/DMF (*v/v*; 2:1, 3 mL) was added to pre-swollen (CH_2Cl_2, 3 mL, 20 min) 2-ClTrtCl resin (150.0 mg, 0.2 mmol) and the mixture was shaken for 1 h at room temperature, filtered, and washed with DMF (4 x 3 mL).

For peptides **7**, **8**, **9**, and **10**:

A solution of HMP linker (109.3 mg, 0.6 mmol) and DIC (93 µL, 0.6 mmol) in CH_2Cl_2/DMF (*v/v*; 2:1, 3 mL) was added to pre-swollen (CH_2Cl_2, 3 mL, 20 min)

aminomethyl polystyrene resin (220.0 mg, 0.2 mmol) and the mixture was shaken for 5 h at room temperature, filtered, and washed with DMF (4 x 3 mL). A negative Kaiser test[3] confirmed the coupling.

For peptides **7**, **8**, and **9,** a mixture of Fmoc-allylglycine (202.4 mg, 0.6 mmol), DIC (93 µL, 0.6 mmol) and DMAP (2.44 mg, 0.02 mmol) in DMF (3 mL) was then added to the resin and the reaction mixture was shaken for 1 h at room temperature. This procedure was repeated once with fresh reagents. For peptide **10**, a mixture of the Fmoc-Gly-OH (178.4 mg, 0.6 mmol), DIC (93 µL, 0.6 mmol) and DMAP (2.44 mg, 0.02 mmol) in DMF (3 mL) was then added to the resin and the reaction mixture was shaken for 1 h at room temperature (repeated once).

Elongation of the peptide sequence

Extension of the *C*-terminal amino acid on the resin was performed at room temperature using manual Fmoc-SPPS. Deprotection of the Fmoc group was accomplished using 20% *v/v* piperidine/DMF (3 mL) for 5 min twice with consecutive DMF washes after each addition. A solution of Fmoc-amino acid (0.8 mmol), HATU (281.4 mg, 0.74 mmol), *i*Pr_2EtN (278.7 µL, 1.6 mmol) in DMF (3 mL) was then added to peptidyl-resin and the mixture was shaken at room temperature for 45 min, then filtered and washed with DMF (4 x 3 mL).

Following on-resin elonagation of the linear precursor of RCM peptides (peptidyl-resin **18**, **19**, and **22**), Boc_2O (436.5 mg, 2.0 mmol) in DMF (3 mL) was added to the peptidyl-resin and the mixture was shaken at room temperature for 2 h, filtered, and washed with DMF (4 X 3 mL), CH_2Cl_2 (4 x 3 mL), and dried under vacuum.

Peptide cleavage and isolation

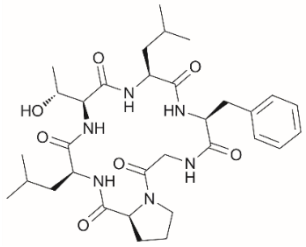
The resulting peptides were released from the resin with concomitant removal of the threonine side chain protecting group by treatment with TFA/*i*Pr_3SiH/H_2O (*v/v/v*; 95:2.5:2.5, 5 mL) at room temperature for 3 h. The resin was removed by filtration, washed with TFA (2 x 3 mL) and the combined filtrates were concentrated, re-suspended with H_2O/CH_3CN (*v/v*; 1:1) and lyophilised.

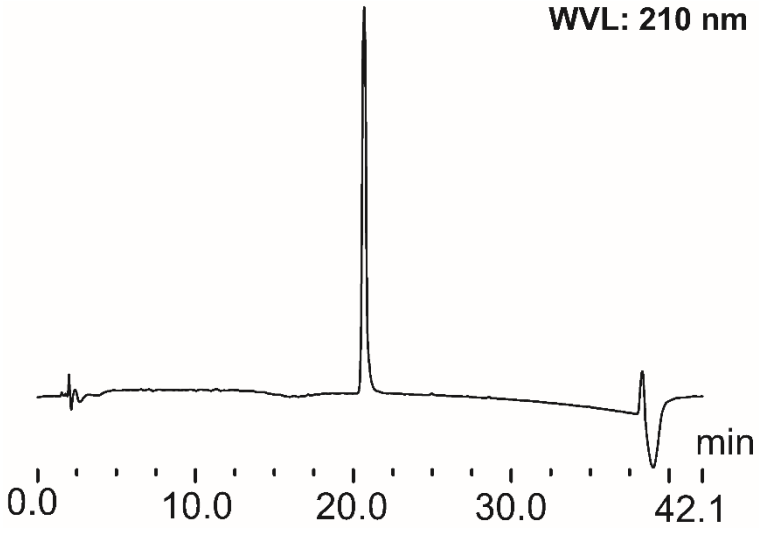
Peptide cyclisation

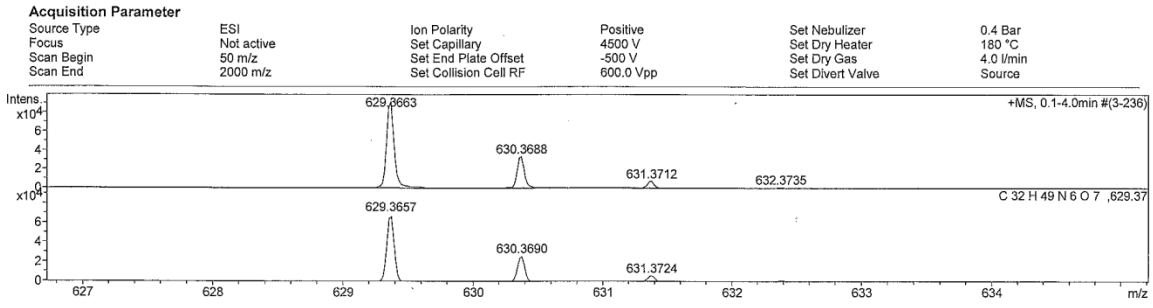
Cyclisation was carried out in solution using the pseudo-high dilution conditions described by Brimble *et al.*[4] Peptide **2** is used here as an example of the synthetic procedure followed for macrolactamisation. To a stirring solution of *i*Pr_2EtN (139.4 µL, 0.8 mmol) in CH_2Cl_2 (150 mL) was added a mixture of the linear peptide (105.0 mg, 0.16 mmol, 0.8 mM), HBTU (182.0 mg, 0.48 mmol), and 6-Cl-HOBt (81.40 mg, 0.48 mmol) in CH_2Cl_2/DMF (*v/v*; 9:1, 50 mL) dropwise at a rate of 2.0 mL h^{-1}. After complete addition of the reagents, the reaction mixture was concentrated under reduced pressure, diluted with H_2O (15 mL) and lyophilised. The crude peptide was purified by RP-HPLC to yield peptide **2** as a white fluffy solid (49.0 mg, 38% overall yield, 98% purity).

A. Dianthin G (1)

Dianthin G (**1**) was synthesised using manual Fmoc-SPPS on an aminomethyl polystyrene resin (0.2 mmol), which following the cleavage conditions in the general procedure afforded the crude linear precursor as a white solid (110 mg, 85% yield). The crude linear precursor (110 mg, 0.17 mmol) was cyclised using solution-phase macrolactamisation as outlined in the general methods section. RP-HPLC purification afforded peptide **1** as a white fluffy solid (65.4 mg, 52% overall yield, 99% purity); R_t 20.40 min; *m/z* (HR-MS) 629.3663 ($[M+H]^+$ requires for $C_{32}H_{49}N_6O_7$: 629.3584).





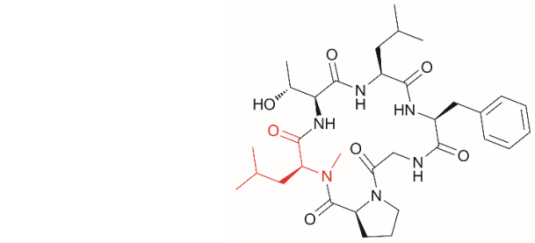


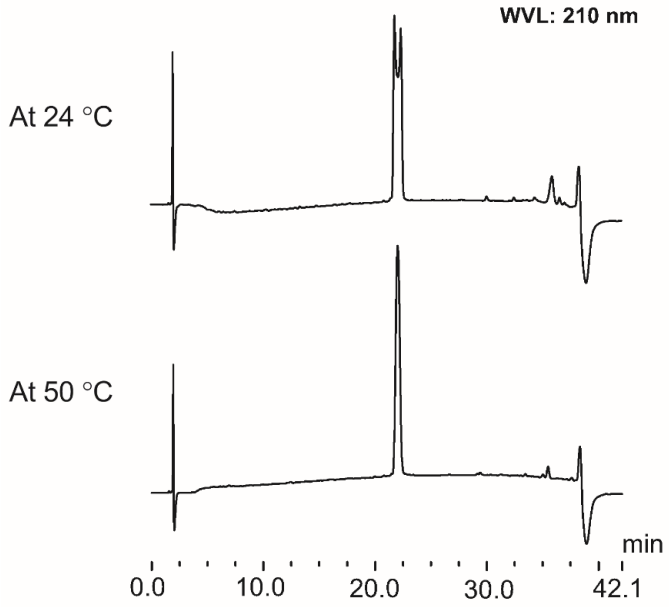
Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	e⁻ Conf	N-Rule
629.3663	1	C 32 H 49 N 6 O 7	100.00	629.3657	-0.6	-1.0	2.8	11.5	even	ok
	2	C 34 H 51 N 3 O 8	85.07	629.3671	0.7	1.1	9.0	11.0	odd	ok
	3	C 33 H 45 N 10 O 3	75.98	629.3671	0.7	1.1	15.0	16.5	even	ok
	4	C 36 H 53 O 9	31.71	629.3684	2.1	3.3	15.3	10.5	even	ok
	5	C 35 H 47 N 7 O 4	28.28	629.3684	2.1	3.3	21.0	16.0	odd	ok
	6	C 44 H 45 N 4	5.48	629.3639	-2.5	-3.9	66.6	24.5	even	ok
	7	C 46 H 47 N O	11.62	629.3652	-1.1	-1.8	72.6	24.0	odd	ok

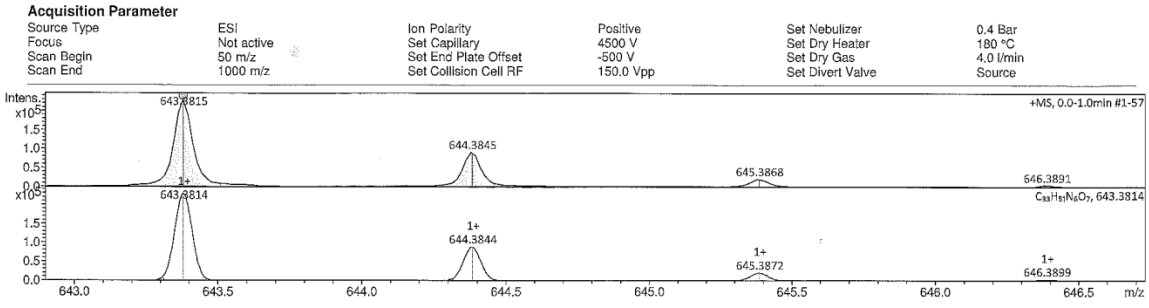
Figure S1. Analytical RP-HPLC and HR-MS data of dianthin G (**1**).

B. Peptide 2

Peptide **2** was synthesised using manual Fmoc-SPPS on an aminomethyl polystyrene resin (0.2 mmol), which following the cleavage conditions in the general procedure afforded the crude linear precursor (105 mg, 79% yield). The crude linear precursor (105 mg, 0.16 mmol) was cyclised using solution-phase macrolactamisation as outlined in the general methods section. RP-HPLC purification afforded peptide **2** as a white fluffy solid (49.0 mg, 38% overall yield, 98% purity); R_t 21.56 min; *m/z* (HR-MS) 643.3815 ($[M+H]^+$ requires for $C_{33}H_{51}N_6O_7$: 643.3741).





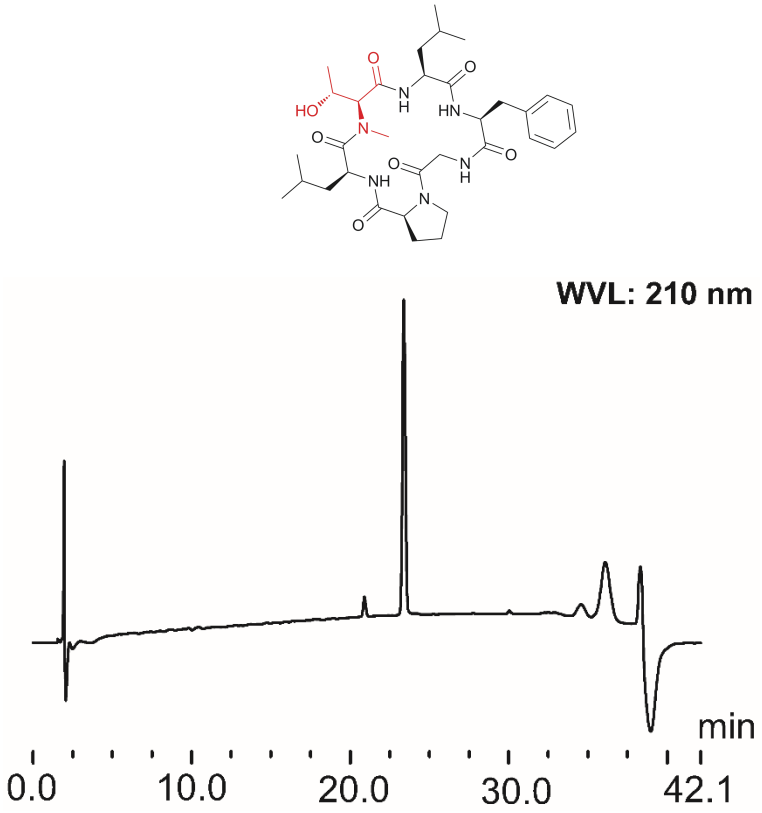


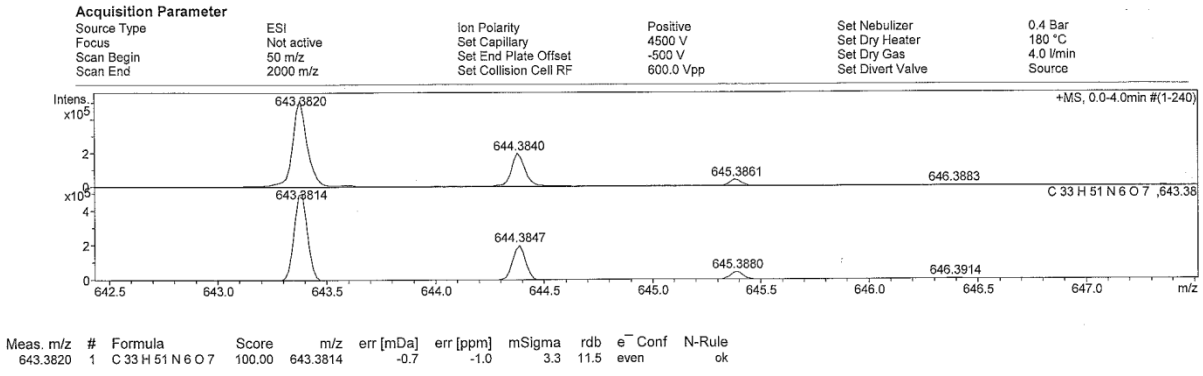
Meas. m/z	#	Ion Formula	m/z	err [ppm]	mSigma	# Sigma	Score	rdb	e⁻ Conf	N-Rule
643.3815	1	C33H51N6O7	643.3814	0.3	4.8	1	100.00	11.5	even	ok
	1	C33H51N6O7	643.3814	0.3	4.8	1	100.00	11.5	even	ok
	1	C33H54N3NaO8	643.3803	1.9	10.0	1	100.00	8.0	odd	ok

Figure S2. Analytical RP-HPLC and HR-MS data of peptide **2**.

C. Peptide 3

Peptide **3** was synthesised using manual Fmoc-SPPS on an aminomethyl polystyrene resin (0.2 mmol), which following the cleavage conditions in the general procedure afforded the crude linear precursor (67.4 mg, 51% yield). The crude linear precursor (67.4 mg, 0.1 mmol) was cyclised using solution-phase macrolactamisation as outlined in the general methods section. RP-HPLC purification afforded peptide **3** as a white fluffy solid (28.3 mg, 22% overall yield, 97% purity); R_t 23.17 min; *m/z* (HR-MS) 643.3820 ($[M+H]^+$ requires for $C_{33}H_{51}N_6O_7$: 643.3741).



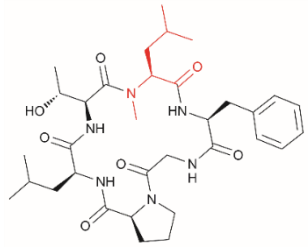


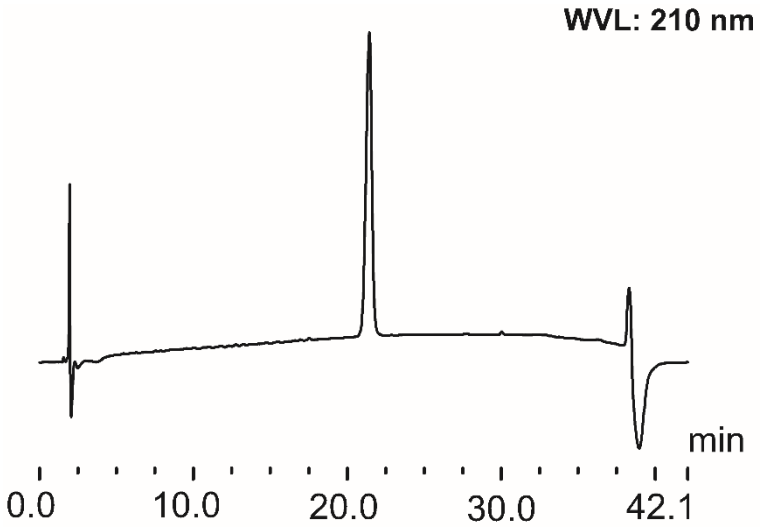
Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	e⁻ Conf	N-Rule
643.3820	1	C 33 H 51 N 6 O 7	100.00	643.3814	-0.7	-1.0	3.3	11.5	even	ok

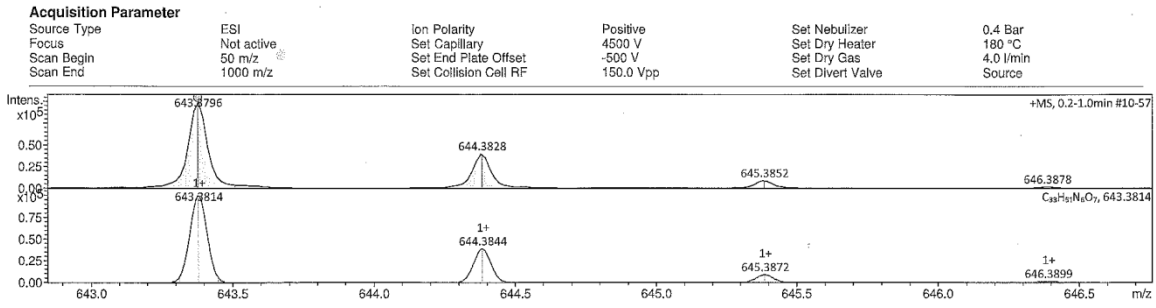
Figure S3. Analytical RP-HPLC and HR-MS data of peptide **3**.

D. Peptide 4

Peptide **4** was synthesised using manual Fmoc-SPPS on an aminomethyl polystyrene resin (0.2 mmol), which following the cleavage conditions in the general procedure afforded the crude linear precursor (95.2 mg, 72% yield). The crude linear precursor (95.2 mg, 0.14 mmol) was cyclised using solution-phase macrolactamisation as outlined in the general methods section. RP-HPLC purification afforded peptide **4** as a white fluffy solid (65.6 mg, 51% overall yield, 99% purity); R_t 20.78 min; *m/z* (HR-MS) 643.3796 ([M+H]$^+$ requires for $C_{33}H_{51}N_6O_7$: 643.3741).





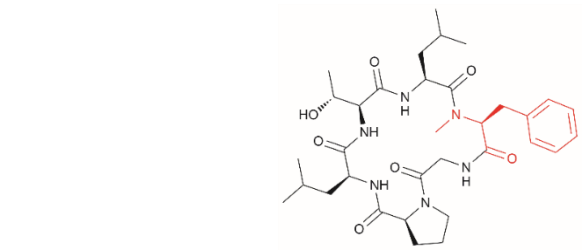


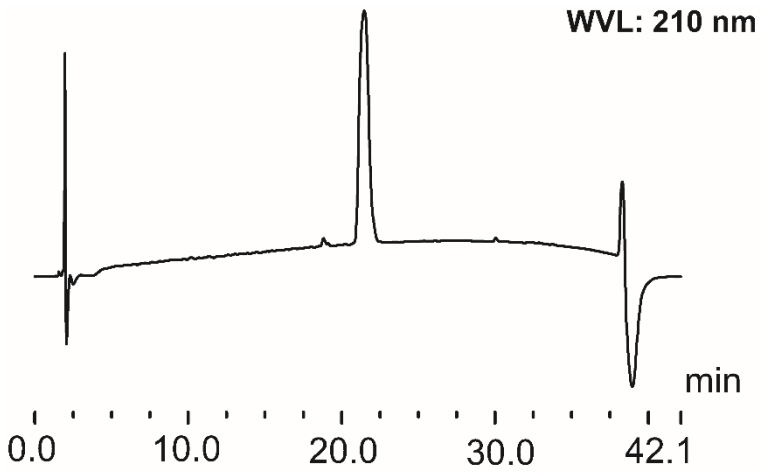
Meas. m/z	#	Ion Formula	m/z	err [ppm]	mSigma	# Sigma	Score	rdb	e⁻ Conf	N-Rule
643.3796	1	C33H51N6O7	643.3814	2.8	6.4	1	100.00	11.5	even	ok
	1	C33H51N6O7	643.3814	2.8	6.4	1	100.00	11.5	even	ok
	1	C33H54N3NaO8	643.3803	-1.2	11.6	1	100.00	8.0	odd	ok

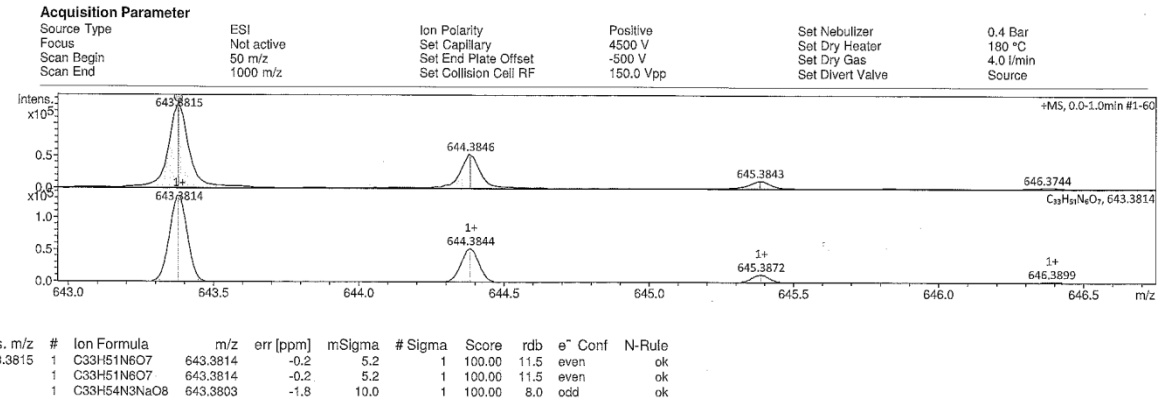
Figure S4. Analytical RP-HPLC and HR-MS data of peptide **4**.

E. Peptide 5

Peptide **5** was synthesised using manual Fmoc-SPPS on an aminomethyl polystyrene resin (0.2 mmol), which following the cleavage conditions in the general procedure afforded the crude linear precursor (84.6 mg, 64% yield). The crud linear precursor (84.6 mg, 0.13 mmol) was cyclised using solution-phase macrolactamisation as outlined in the general methods section. RP-HPLC purification afforded peptide **5** as a white fluffy solid (55.3 mg, 43% overall yield, 98% purity); R_t 20.80 min; *m/z* (HR-MS) 643.3815 ([M+H]$^+$ requires for $C_{33}H_{51}N_6O_7$: 643.3741).







Meas. m/z	#	Ion Formula	m/z	err [ppm]	mSigma	# Sigma	Score	rdb	e⁻ Conf	N-Rule
643.3815	1	C33H51N6O7	643.3814	-0.2	5.2	1	100.00	11.5	even	ok
	1	C33H51N6O7	643.3814	-0.2	5.2	1	100.00	11.5	even	ok
	1	C33H54N3NaO8	643.3803	-1.8	10.0	1	100.00	8.0	odd	ok

Figure S5. Analytical RP-HPLC and HR-MS data of peptide **5**.

F. Peptide 6

Peptide **6** was synthesised using manual Fmoc-SPPS on a 2-chlorotrityl chloride resin (0.2 mmol), which following the cleavage conditions in the general procedure afforded the crude linear precursor (80.6 mg, 61% yield). The crude linear precursor (80.6 mg, 0.12 mmol) was cyclised using solution-phase macrolactamisation as outlined in the general methods section. RP-HPLC purification afforded peptide **6** as a white fluffy solid (50.1 mg, 39% overall yield, 98% purity); R_t 22.09 min; *m/z* (HR-MS) 643.3803 ($[M+H]^+$ requires for $C_{33}H_{51}N_6O_7$: 643.3741).

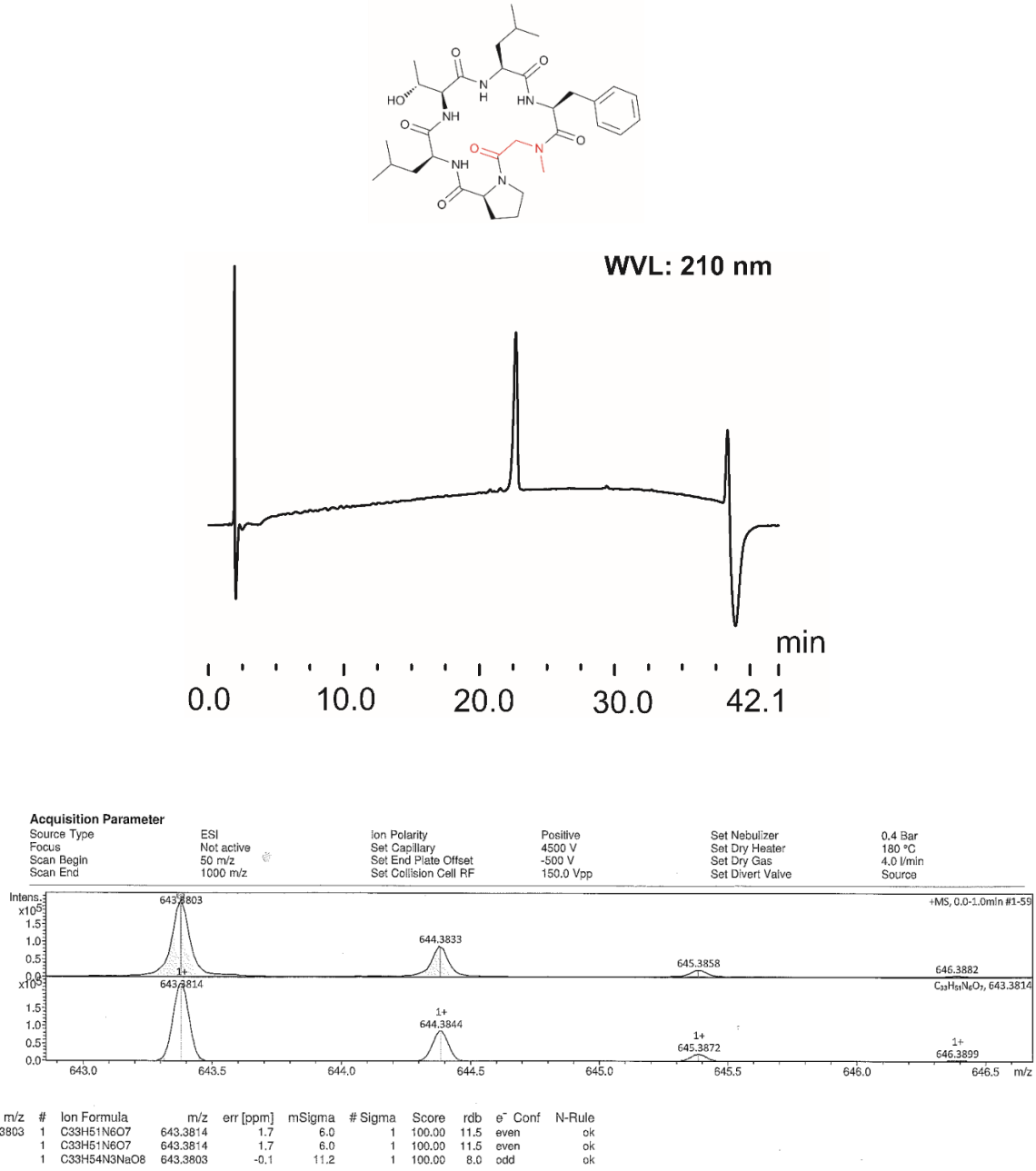
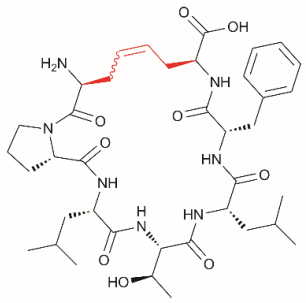
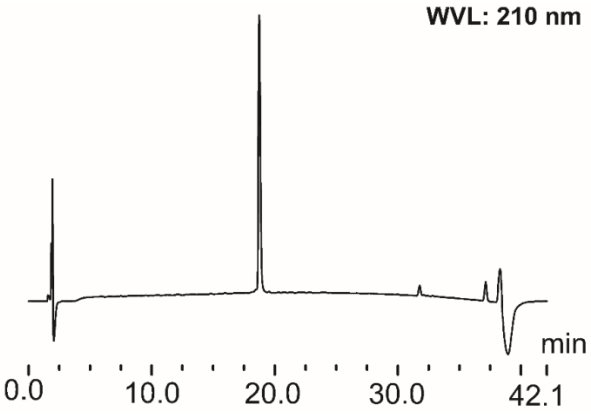


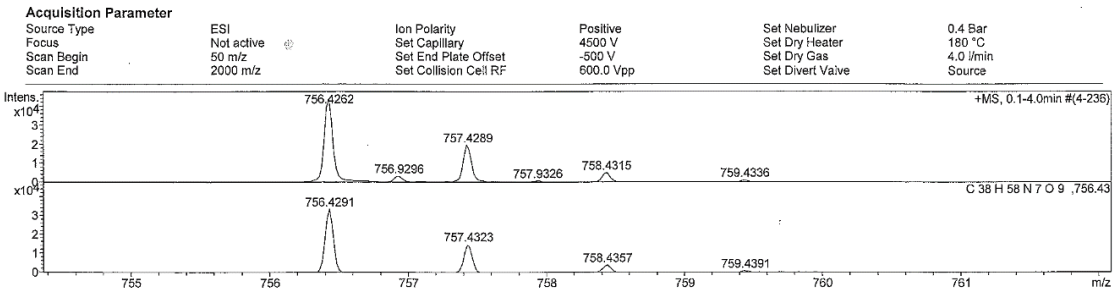
Figure S6. Analytical RP-HPLC and HR-MS data of peptide **6**.

G. Peptide 7

The peptidyl-resin **18** was synthesised using manual Fmoc-SPPS on an aminomethyl polystyrene resin (0.2 mmol) and the peptidyl-resin (67.2 mg, 32.5 x 10^{-3} mmol) was subjected to microwave-assisted ring closing metathesis as outlined in the general methods section. Following cleavage from the resin, RP-HPLC purification afforded peptide **7** as a white fluffy solid (4.84 mg, 16% yield from resin-bound peptide **18**, 99% purity); R_t 18.75 min; *m/z* (HR-MS) 756.4262 ($[M+H]^+$ requires for $C_{38}H_{58}N_7O_9$: 756.4218).





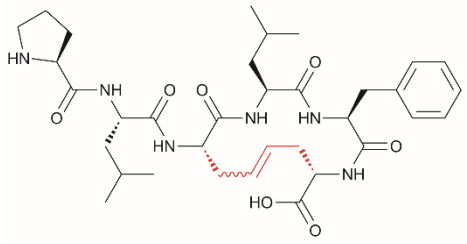


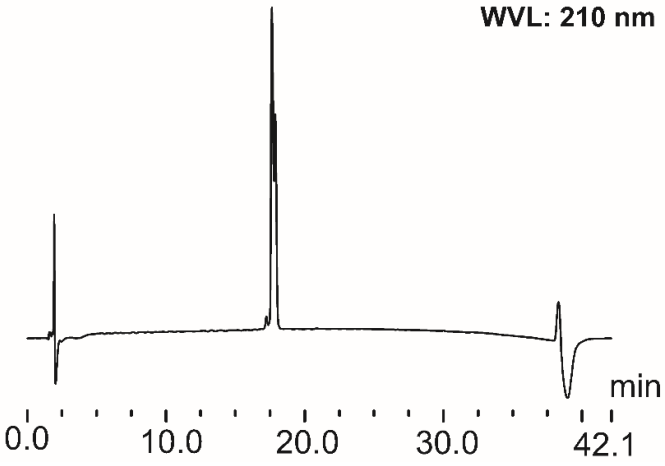
Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	e⁻ Conf	N-Rule
756.4262	1	C 38 H 58 N 7 O 9	55.86	756.4291	2.8	3.7	3.5	13.5	even	ok
	2	C 39 H 64 O 14	51.04	756.4291	2.8	3.7	8.3	8.0	odd	ok
	3	C 47 H 56 N 4 O 5	55.69	756.4245	-1.7	-2.3	43.5	22.0	odd	ok
	4	C 44 H 63 K N O 7	25.14	756.4236	-2.6	-3.5	45.9	13.5	even	ok
	5	C 43 H 57 K N 8 O 2	24.26	756.4236	-2.6	-3.5	47.0	19.0	odd	ok
	6	C 46 H 50 N 11	46.35	756.4245	-1.7	-2.3	49.6	27.5	even	ok
	7	C 49 H 58 N O 6	100.00	756.4259	-0.4	-0.5	50.1	21.5	even	ok
	8	C 45 H 59 K N 5 O 3	56.31	756.4249	-1.3	-1.7	52.6	18.5	even	ok
	9	C 48 H 52 N 8 O	83.52	756.4259	-0.4	-0.5	55.7	27.0	odd	ok
	10	C 47 H 61 K N 2 O 4	88.14	756.4263	0.1	0.1	58.4	18.0	odd	ok
	11	C 52 H 56 N 2 O 3	19.85	756.4285	2.3	3.0	62.0	26.0	odd	ok
	12	C 50 H 54 N 5 O 2	49.09	756.4272	1.0	1.3	62.7	26.5	even	ok
	13	C 48 H 57 K N 6	32.53	756.4276	1.4	1.8	67.0	23.0	odd	ok

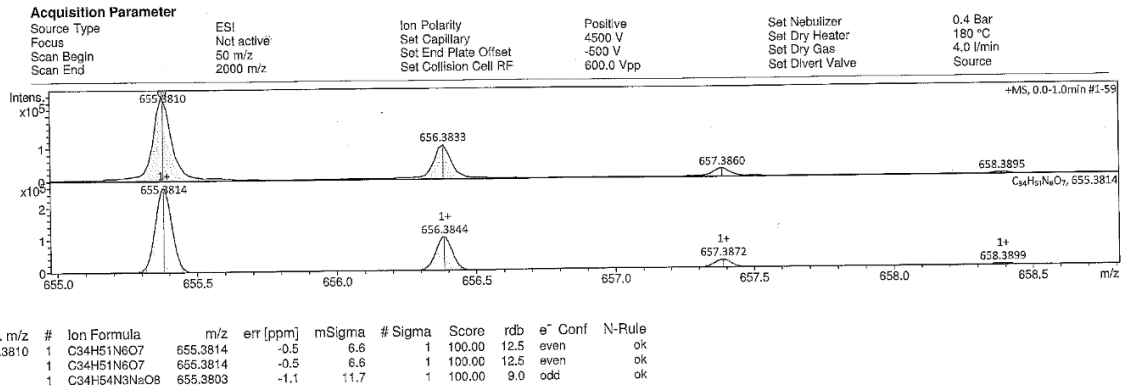
Figure S7. Analytical RP-HPLC and HR-MS data of peptide **7**.

H. Peptide 8

The peptidyl-resin **19** was synthesised using manual Fmoc-SPPS on an aminomethyl polystyrene resin (0.2 mmol) and the peptidyl-resin (66.5 mg, 32.5 x 10^{-3} mmol) was subjected to microwave-assisted ring closing metathesis as outlined in the general methods section. Following cleavage from the resin, RP-HPLC purification afforded peptide **8** as a white fluffy solid (6.55 mg, 25% yield from resin-bound peptide **19**, 98% purity); R_t 17.6 min; *m/z* (HR-MS) 655.3810 ($[M+H]^+$ requires for $C_{34}H_{51}N_6O_7$: 655.4218).





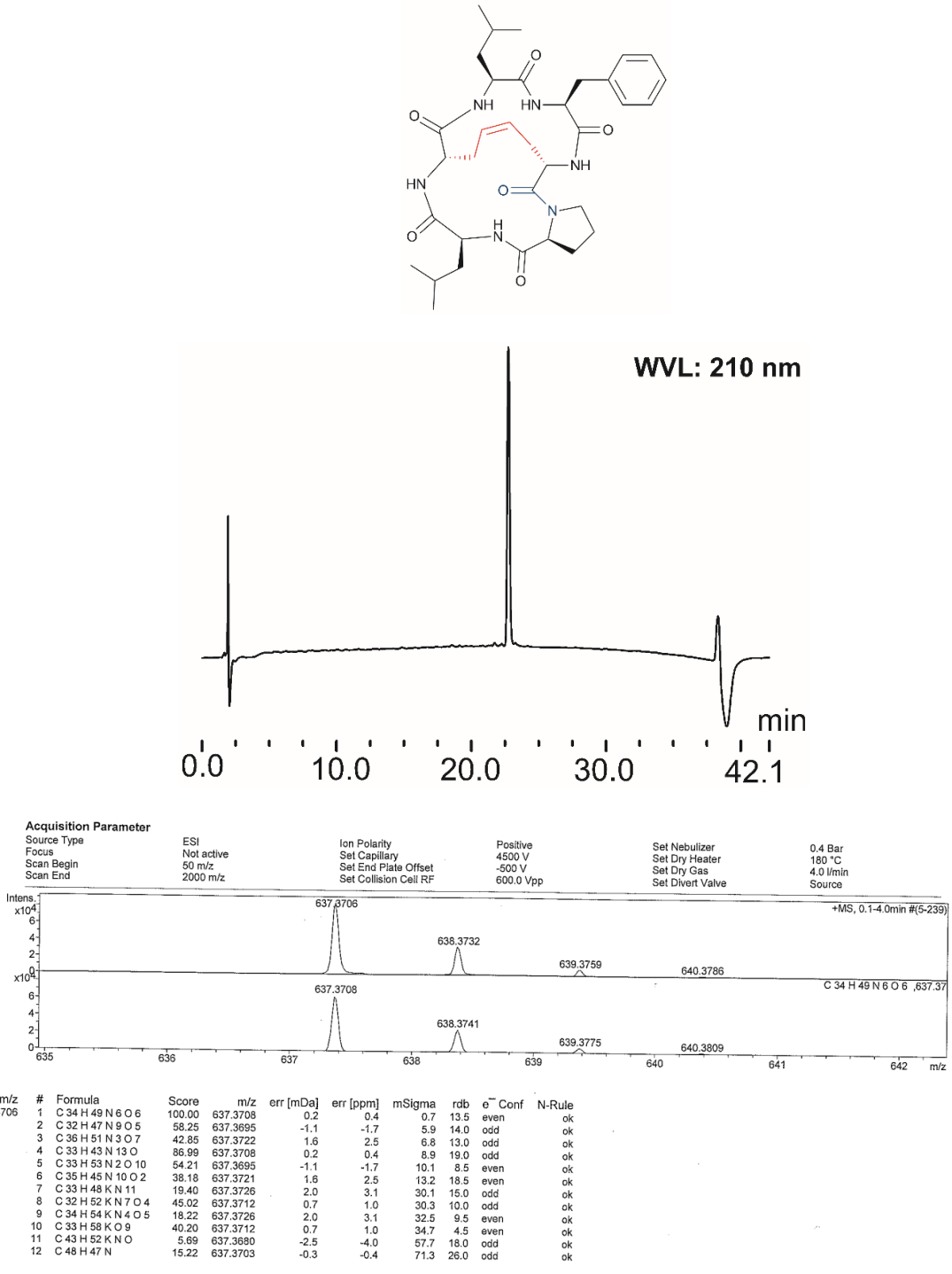


Meas. m/z	#	Ion Formula	m/z	err [ppm]	mSigma	# Sigma	Score	rdb	e⁻ Conf	N-Rule
655.3810	1	C34H51N6O7	655.3814	-0.5	6.6	1	100.00	12.5	even	ok
	1	C34H51N6O7	655.3814	-0.5	6.6	1	100.00	12.5	even	ok
	1	C34H54N3NaO8	655.3803	-1.1	11.7	1	100.00	9.0	odd	ok

Figure S8. Analytical RP-HPLC and HR-MS data of peptide **8**.

I. Peptide 9

Peptide **8** (6.0 mg, 9.2 µmol) was cyclised in solution, as outlined in the general methods, to yield the crude peptide **9**. RP-HPLC purification afforded peptide **9** as a white fluffy solid (4.07 mg, 68% yield from peptide **8** and 16% overall yield, 99% purity); R_t 21.80 min; *m/z* (HR-MS) 637.3706 ($[M+H]^+$ requires for $C_{34}H_{49}N_6O_6$: 637.3605).

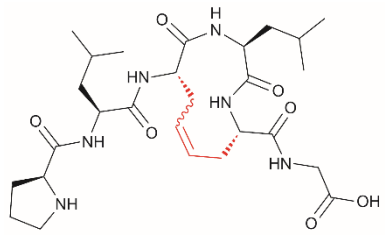


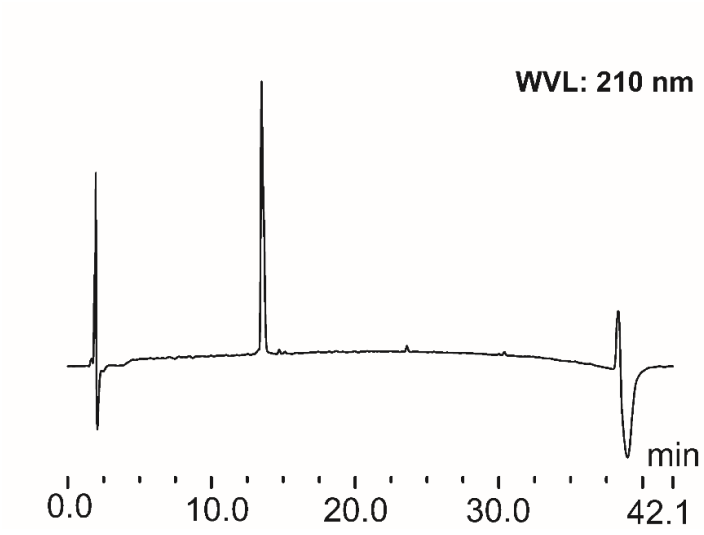
Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	e⁻ Conf	N-Rule
637.3706	1	C 34 H 49 N 6 O 6	100.00	637.3708	0.2	0.4	0.7	13.5	even	ok
	2	C 32 H 47 N 9 O 5	58.25	637.3695	-1.1	-1.7	5.9	14.0	odd	ok
	3	C 36 H 51 N 3 O 7	42.85	637.3722	1.6	2.5	6.8	13.0	odd	ok
	4	C 33 H 43 N 13 O	86.99	637.3708	0.2	0.4	8.9	19.0	odd	ok
	5	C 33 H 53 N 2 O 10	54.21	637.3695	-1.1	-1.7	10.1	8.5	even	ok
	6	C 35 H 45 N 10 O 2	38.18	637.3721	1.6	2.5	13.2	18.5	even	ok
	7	C 33 H 48 K N 11	19.40	637.3726	2.0	3.1	30.1	15.0	odd	ok
	8	C 32 H 52 K N 7 O 4	45.02	637.3712	0.7	1.0	30.3	10.0	odd	ok
	9	C 34 H 54 K N 4 O 5	18.22	637.3726	2.0	3.1	32.5	9.5	even	ok
	10	C 33 H 58 K O 9	40.20	637.3712	0.7	1.0	34.7	4.5	even	ok
	11	C 43 H 52 K N O	5.69	637.3680	-2.5	-4.0	57.7	18.0	odd	ok
	12	C 48 H 47 N	15.22	637.3703	-0.3	-0.4	71.3	26.0	odd	ok

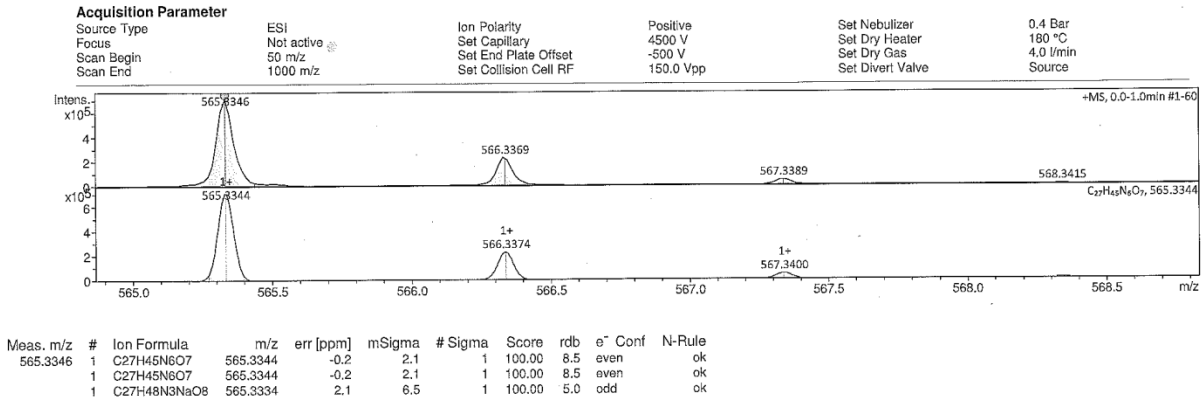
Figure S9. Analytical RP-HPLC and HR-MS data of peptide **9**.

J. Peptide 10

The peptidyl-resin **22** was synthesised using manual Fmoc-SPPS on an aminomethyl polystyrene resin (0.2 mmol) and the peptidyl-resin (56.2 mg, 32.5 x 10^{-3} mmol) was subjected to microwave-assisted ring closing metathesis as outlined in the general methods section. Following cleavage from the resin, RP-HPLC purification afforded peptide **10** as a white fluffy solid (4.52 mg, 20% yield from resin-bound peptide **22**, 99% purity); R_t 13.00 min; *m/z* (HR-MS) 565.3346 ($[M+H]^+$ requires for $C_{27}H_{45}N_6O_7$: 565.3271).





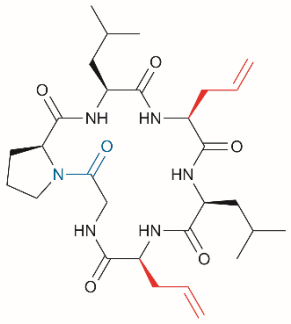


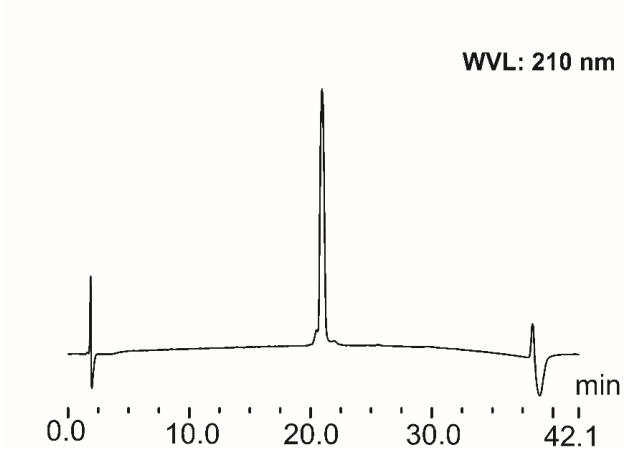
Meas. m/z	#	Ion Formula	m/z	err [ppm]	mSigma	# Sigma	Score	rdb	e⁻ Conf	N-Rule
565.3346	1	C27H45N6O7	565.3344	-0.2	2.1	1	100.00	8.5	even	ok
	1	C27H45N6O7	565.3344	-0.2	2.1	1	100.00	8.5	even	ok
	1	C27H48N3NaO8	565.3334	2.1	6.5	1	100.00	5.0	odd	ok

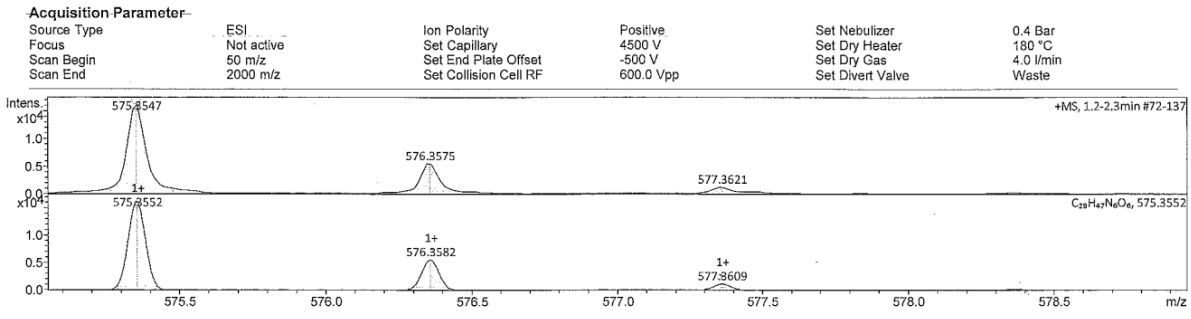
Figure S10. Analytical RP-HPLC and HR-MS data of peptide **10**.

K. Peptide 23

Peptide **23** was synthesised using manual Fmoc-SPPS on an aminomethyl polystyrene resin (0.2 mmol), which following the cleavage conditions in the general procedure afforded the crude linear precursor as a white solid (77.0 mg, 65% yield). The crude linear precursor (77.0 mg, 0.13 mmol) was cyclised using solution-phase macrolactamisation as outlined in the general methods section. RP-HPLC purification afforded peptide **23** as a white fluffy solid (45.98 mg, 40% overall yield, 96% purity); R_t 21.20 min; *m/z* (HR-MS) 575.3547 ([M+H]$^+$ requires for $C_{29}H_{47}N_6O_6$: 575.3479).







Meas. m/z	#	Ion Formula	m/z	err [ppm]	mSigma	# Sigma	Score	rdb	e⁻ Conf	N-Rule
575.3547	1	C29H47N6O6	575.3552	0.8	7.4	1	100.00	9.5	even	ok
	2	C28H41N13O	575.3552	0.8	11.8	2	92.46	15.0	odd	ok
	3	C28H51N2O10	575.3538	-1.5	12.5	3	73.93	4.5	even	ok
	4	C30H43N10O2	575.3565	-3.1	15.0	4	39.01	14.5	even	ok
	1	C29H50N3NaO7	575.3541	-1.0	8.5	1	100.00	6.0	odd	ok
	2	C28H44N10NaO2	575.3541	-1.0	10.0	2	97.03	11.5	even	ok
	3	C30H46N7NaO3	575.3554	-1.3	11.1	3	88.52	11.0	odd	ok

Figure S11. Analytical RP-HPLC and HR-MS data of peptide **23**.

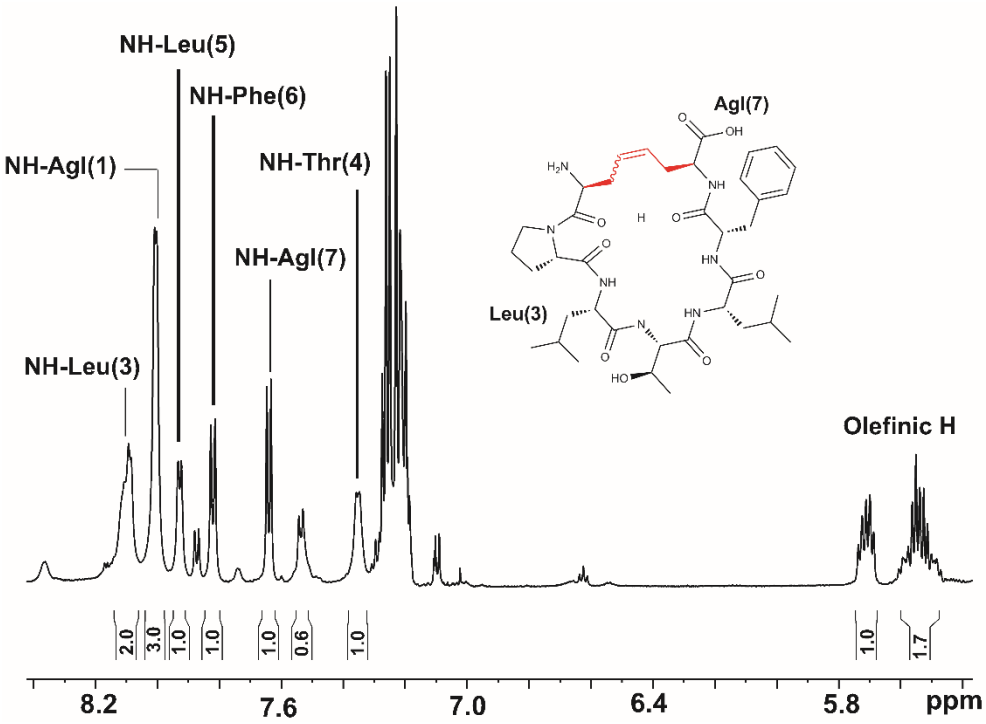


Figure S12. Partial ^{1}H NMR spectrum of **7** in DMSO-d6 at 298K.

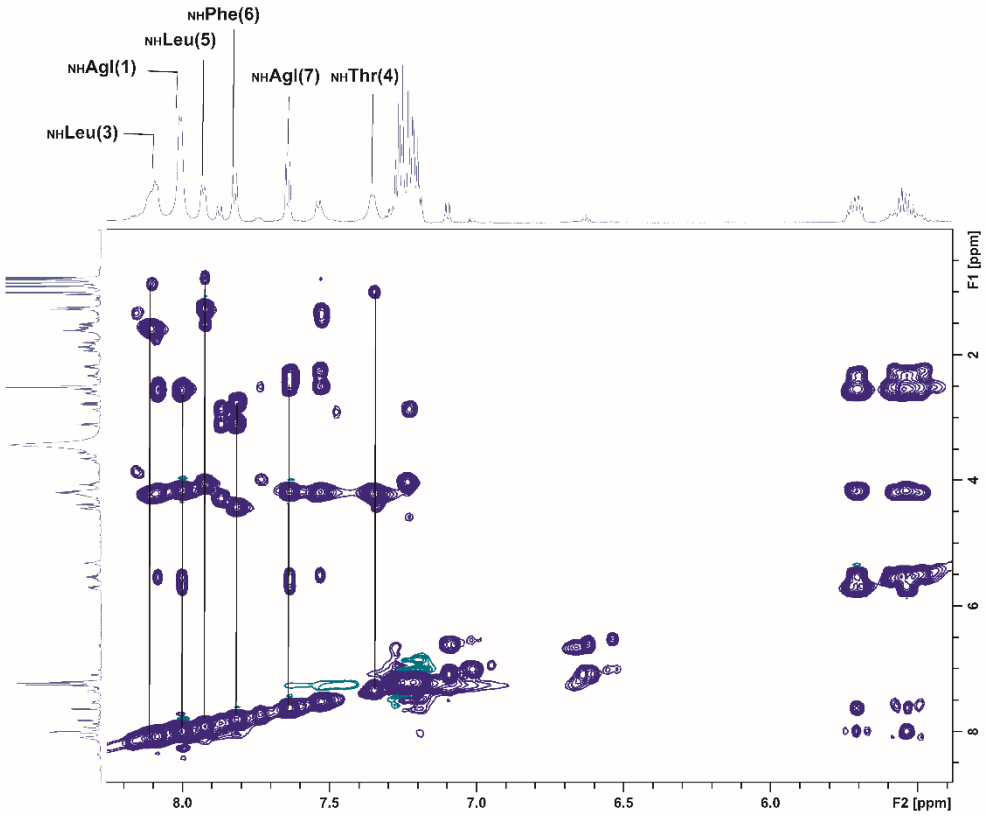


Figure S13. Partial 600 MHz TOCSY spectrum of **7** in DMSO-d6 at 298K.

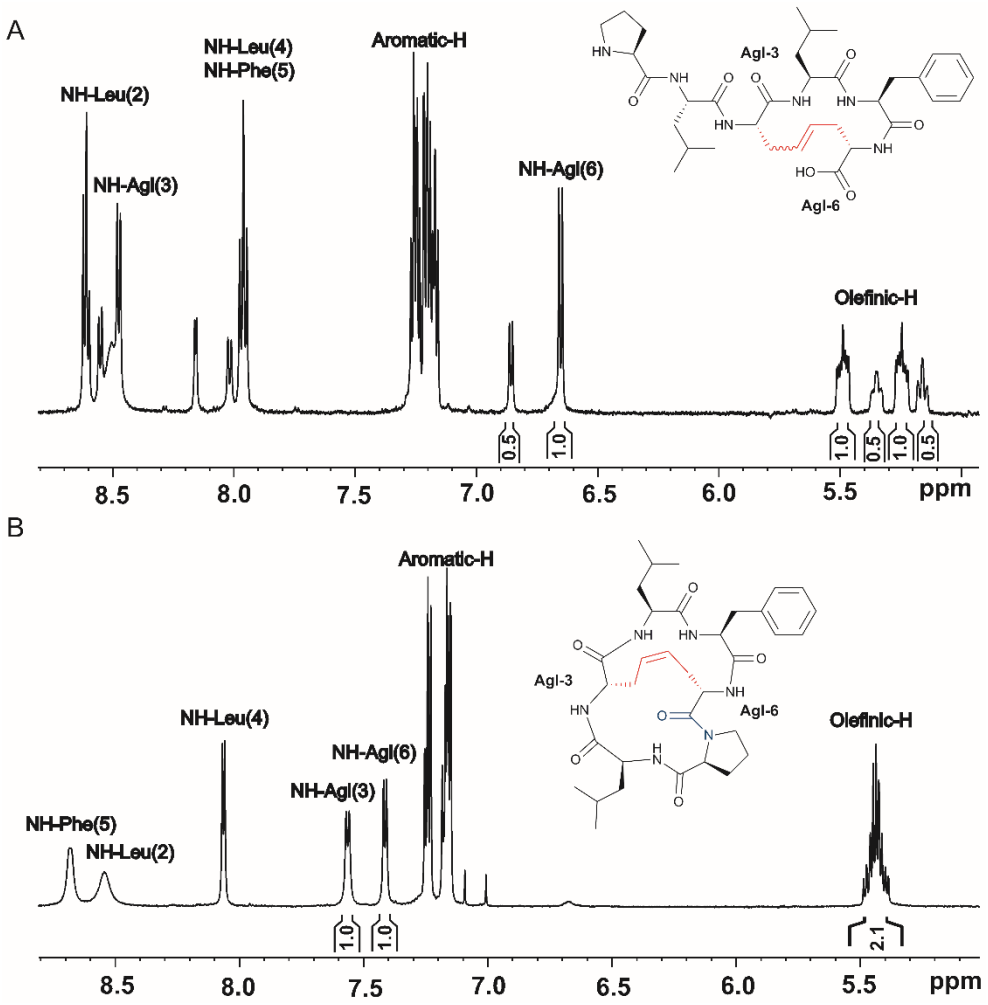


Figure S14. Amide and alkene regions of the ^{1}H NMR spectra of (A) dicarba analogue **8** at 298 K with a 2:1 ratio of major:minor signals for the olefinic hydrogens, (B) bicyclic analogue **9** at 298 K showing the presence of only one diastereomer.

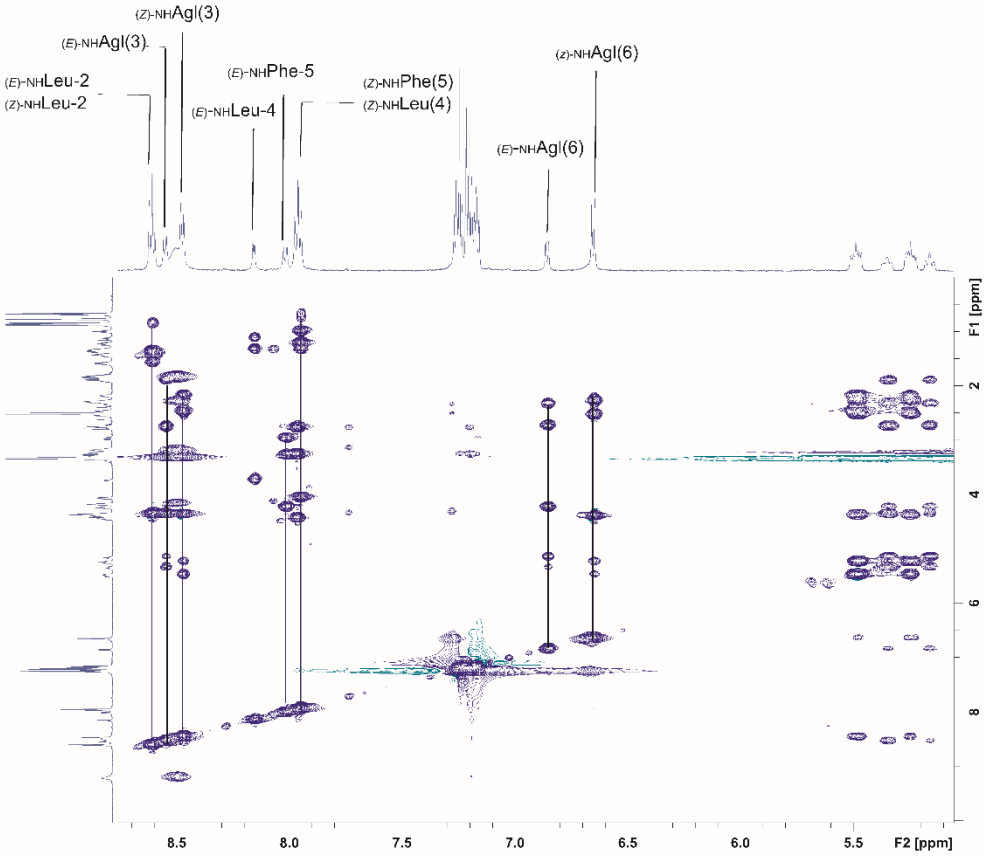


Figure S15. Partial 600 MHz TOCSY spectrum of **8** in DMSO-d6 at 298K.

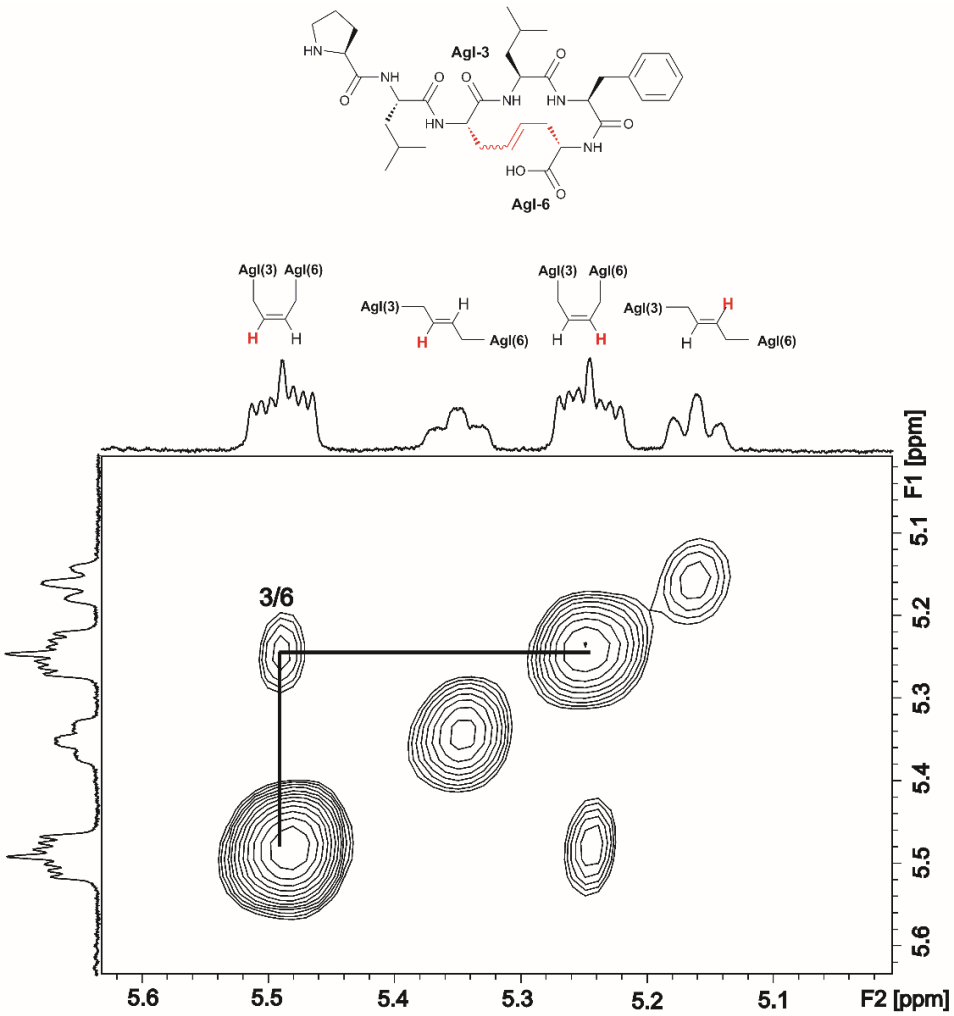


Figure S16. Selected 600 MHz NOESY spectra of the dicarba analogue **8** showing the olefinic-H connectivities with the *cis*-isomer isomer exhibiting strong nOe.

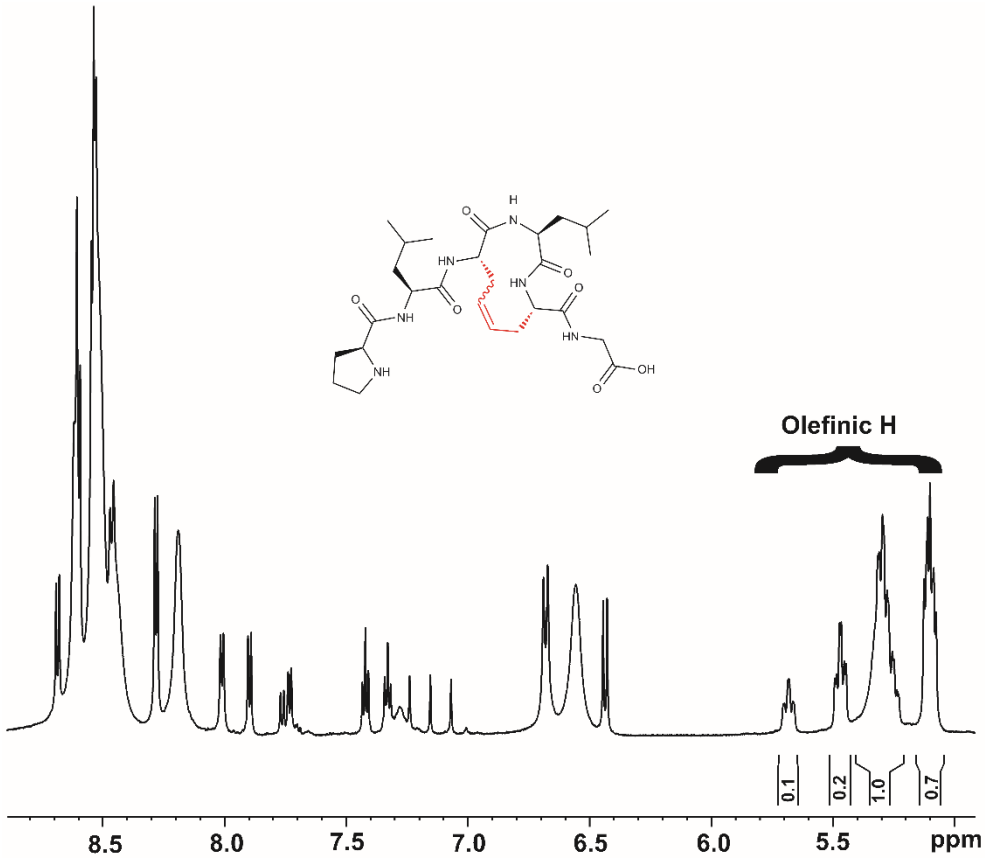


Figure S17. Partial ^{1}H NMR spectrum of **10** in DMSO-d6 at 298K.

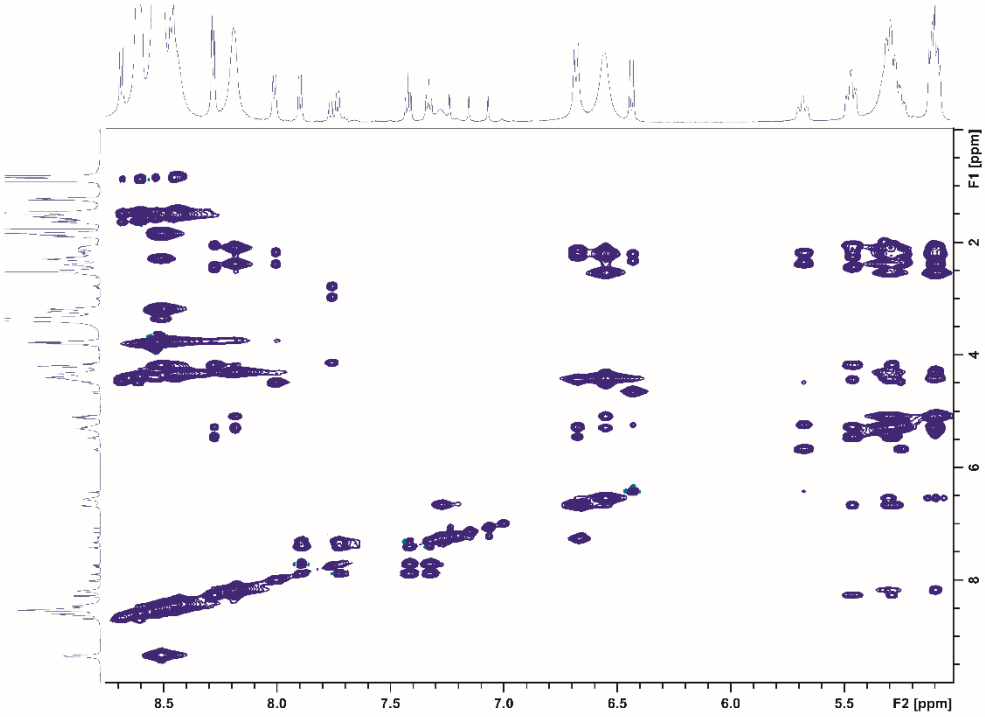


Figure S18. Partial 600 MHz TOCSY spectrum of **10** in DMSO-d6 at 298K.

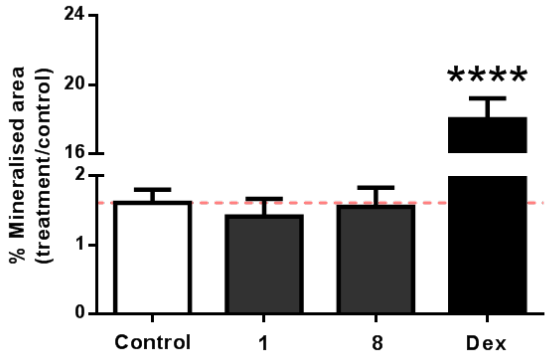


Figure S19. Effects of dianthin G **(1)** (10^{-8} M) and the dicarba analogue **8** (10^{-8} M) in primary human osteoblasts on cell differentiation, assessed by percentage of mineralisation. Data are expressed as a ratio of treatment to control, mean ± SEM from a representative experiment. Dexamethasone [Dex] (10^{-8} M) is included as a positive control. ****significantly different from control ($P < 0.0001$).

Circular dichroism spectroscopy

All CD spectra were recorded using a Pi Star-180 (Applied Photophysics, Surrey, UK) spectrometer at 20 ^{o}C with a cell of 0.1 cm path length in the range from 190 nm to 300 nm at 0.5 nm intervals with a 5 s response time. Each CD spectrum measurement represents the average of four scans obtained with a 2 nm optical bandwidth. Baseline spectrum was collected with the solvent alone (30% TFE in water) and then subtracted from the raw peptide spectra. The measurements were performed at peptide concentrations of 86 µM in 30% TFE in water, in 1 mm quartz cuvettes (Hellma Analytics, Mullheim, Germany). Data are expressed as mean residue ellipticities [Θ] in (deg cm^2 dmol^{-1}), calculated as follows:

$$\Theta = S / (10 \times c \times L \times n)$$

Where S is the raw CD signal in millidegrees, c is the peptide concentration (M), L is the cuvette path length (cm), and n is the number of peptide bonds.

References

1. (a) P. W. R. Harris, D. J. Lee and M. A. Brimble, *J. Pept. Sci.,* 2012, **18**, 549-555; (b) Y. Chen, C. T. Mant and R. S. Hodges, *J. Chromatogr. A.,* 2007, **1140**, 112-120.

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4. H. Kaur, A. M. Heapy, R. Kowalczyk, Z. Amso, M. Watson, J. Cornish and M. A. Brimble, *Tetrahedron,* 2014, **70**, 7788-7794.