

## Supporting information

### 1.0 General experimental

#### 1.1 Instrumentation

Melting points (m.p.) were determined using a Reichert hot-stage melting point apparatus and are uncorrected.

Infrared spectra (IR) spectra were recorded on a Perkin-Elmer 1600 series Fourier Transform infrared spectrophotometer as thin films of liquid (neat) between sodium chloride plates. IR absorptions ( $\nu_{\max}$ ) are reported in wavenumbers ( $\text{cm}^{-1}$ ) with the relative intensities expressed as s (strong), m (medium), w (weak) or prefixed b (broad).

Proton nuclear magnetic resonance ( $^1\text{H}$  n.m.r.) spectra were recorded on a Bruker DRX400 spectrometer operating at 400 MHz, as solutions in deuterated solvents as specified. Each resonance was assigned according to the following convention: chemical shift (rotamers); multiplicity; number of protons; observed coupling constants ( $J$  Hz) and proton assignment. Chemical shifts ( $\delta$ ), measured in parts per million (ppm), are reported relative to the residual proton peak in the solvent used as specified. Multiplicities are denoted as singlet (s), doublet (d), triplet (t), quartet (q), pentet (p), multiplet (m) or prefixed broad (b), or a combination where necessary.

Carbon-13 nuclear magnetic resonance ( $^{13}\text{C}$  n.m.r.) spectra were recorded on a Bruker DRX400 spectrometer operating at 100 MHz, as solutions in deuterated solvents as specified. Chemical shifts ( $\delta$ ), measured in parts per million (ppm), are reported relative to the residual proton peak in the deuterated solvent (as specified).

Assignments were determined from *J*-Modulated Spin Echo experiments showing quaternary and methylene signals in the opposite phase to those of methine and methyl resonances.

Correlation spectroscopy (COSY) was used to correlate chemical shifts of protons coupled to one another. Heteronuclear Multiple Quantum Correlation (HMQC) spectroscopy was used to correlate directly bonded  $^{13}\text{C}$ - $^1\text{H}$  nuclei. Heteronuclear Multiple Bond Correlation (HMBC) spectroscopy was used to determine long range  $^{13}\text{C}$ - $^1\text{H}$  connectivity. All two-dimensional (2D)-n.m.r. experiments were recorded on a Bruker DRX400 spectrometer.

Low resolution electrospray ionisation (ESI) mass spectra were recorded on a Micromass Platform Electrospray mass spectrometer (QMS-quadrupole mass electrospectroscopy) as solutions in specified solvents. Spectra were recorded in the positive mode (ESI<sup>+</sup>). High resolution electrospray mass spectra (HRMS) were recorded on a Bruker BioApex 47e Fourier Transform mass spectrometer (4.7 Tesla magnet) fitted with an analytical electrospray source. The mass spectrometer was calibrated with an internal standard solution of sodium iodide in MeOH. Matrix assisted laser desorption ionisation time of flight (MALDI-TOF) mass spectra were recorded on a Bruker AUTOFLEX II instrument in either linear or reflector mode at 19.5 kV, with a 20 kV gradient reflector. Samples were crystallised within a 2,5-dihydroxybenzoic acid matrix.

Automated microwave-assisted solid phase peptide synthesis (SPPS) was carried out using a CEM Liberty-Discover<sup>TM</sup> synthesiser. This involved the flow of dissolved reagents from external nitrogen pressurised bottles to a resin-containing microwave reactor vessel fitted with a porous filter. Coupling and deprotection reactions were

carried out within this vessel and were aided by microwave energy. Each reagent delivery, wash and evacuation step was carried out according to automated protocols of the instrument controlled by PepDriver software.

Microwave ring closing metathesis (RCM) reactions were carried out on a CEM Discover<sup>TM</sup> system fitted with the Benchmate<sup>TM</sup> option. The instrument produces a continuous focussed beam of microwave radiation at a maximum power delivery selected by the user, which reaches and maintains a selected temperature. Reactions were performed in 10 mL high pressure glass microwave vessels fitted with self-sealing Teflon septa as a pressure relief device. The vessels employed magnetic stirrer beads and the temperature of each reaction was monitored continuously with a non-contact infrared sensor located below the microwave cavity floor. Reaction times were measured from the time the microwave reached its maximum temperature until the reaction period had lapsed (cooling periods not inclusive).

Analytical reverse phase high performance liquid chromatography (RP-HPLC) was performed on an Agilent 1200 series instrument equipped with photodiode array (PDA) detection (controlled by ChemStation software) and an automated injector (100  $\mu$ L loop volume). Analytical separations were performed on a Vydac C18 (4.6 x 250 mm, 5  $\mu$ m) analytical column at flow rates of 1.5 mL min<sup>-1</sup>. Preparative RP-HPLC was performed on an Agilent 1200 series instrument equipped with multivariable wavelength (MVW) detection (controlled by ChemStation software) and an Agilent unit injector (2 mL loop volume). Preparative separations were performed on a Vydac C18 (22 x 250 mm, 10  $\mu$ m) preparative column at flow rates of 10 mL min<sup>-1</sup>. The solvent system used throughout this study was buffer A: 0.1%

aqueous TFA; buffer B: 0.1% TFA in MeCN. Linear gradients of 0.1% TFA in MeCN (buffer B) were employed as specified.

## 1.2 Solvents and reagents

Acetonitrile (MeCN), ethyl acetate (EtOAc), dichloromethane (DCM), diethyl ether (Et<sub>2</sub>O), light petroleum and methanol (MeOH) were used as supplied by Merck. *N,N'*-Dimethylformamide (DMF) was supplied by Auspep and stored over 4Å molecular sieves. Piperidine and trifluoroacetic acid (TFA) were used as supplied by Auspep. Acetic anhydride, anisole, diisopropylethylamine (DIPEA), dimethyl sulfoxide (DMSO), *N*-methylmorpholine (NMM), thioanisole, triisopropyl silane (TIPS), tricyclohexylphosphine[1,3-bis(2,4,6-trimethylphenyl)-4,5-dihydroimidazol-2-ylidene] (benzylidene)ruthenium(II) dichloride (2<sup>nd</sup> generation Grubbs' catalyst), tris(triphenylphosphine)rhodium(I) chloride (Rh[(PPh<sub>3</sub>)<sub>3</sub>Cl], Wilkinson's catalyst) and *cis*-2-butene (99%) were used as supplied by Aldrich. *N*-Methyl-2-pyrrolidone (NMP) was used as supplied by Fluka. (2*S*)-Amino-pent-4-enoic acid (L-allylglycine, Agl) was used as supplied by Peptech. *N*-Fluorenylmethoxycarbonylamino succinimide (Fmoc-OSu), *O*-(benzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate (HBTU), *N*-hydroxybenzotriazol (HOBt), Fmoc-Arg(Pbf)-Wang resin (0.30 mmol g<sup>-1</sup>) and sidechain-protected Fmoc-amino acids were used as supplied by GL Biochem. High purity (<10 ppm oxygen) argon and hydrogen were supplied by BOC gases and additional purification was achieved by passage of the gases through water, oxygen and hydrocarbon traps. The DCM, MeOH and solution of lithium chloride in DMF (0.4 M LiCl/DMF) used in all metal-catalysed metathesis and hydrogenation reactions were degassed with high purity argon prior to use. (2*S*,7*S*)-2,7-Diaminooctanedioic acid (2,7-diaminosuberic

acid, Das), (2*S*,7*S*)-2,7-diaminooct-4-enedioic acid (2,7-diaminodehydrosuberic acid,  $\Delta^4$ Das), (2*S*,7*S*)-2-amino-5-methylhex-4-enoic acid (Pre) and (2*S*,7*S*)-2-aminohex-4-enoic acid (Crt) are generated under the catalysis conditions.

### 1.3 Peptide synthesis procedure

Automated microwave-assisted solid phase peptide synthesis (SPPS) was carried out using a CEM Liberty-Discover<sup>TM</sup> system as described in Section 1.1. In a 50 mL centrifuge tube, Fmoc-Arg(Pbf)-Wang resin (0.30 mmol g<sup>-1</sup>) was swollen with DMF (10 mL, 1 x 60 min.) and connected to the Liberty<sup>TM</sup> resin manifold. The Fmoc-amino acids (0.2 M in DMF), activators (0.5 M HBTU : HOBT in DMF), activator base (2 M DIPEA in NMP) and deprotection agent (20% v/v piperidine in DMF) were measured out and solubilised in an appropriate volume of specified solvent as calculated by the PepDriver software program. The default microwave conditions used in the synthesis of each linear peptide included: Initial deprotection (36 W, 37°C, 2 min.), deprotection (45 W, 75°C, 10 min.), preactivation (0 W, 25°C, 2 min.) and coupling (25 W, 75°C, 10 min.). Each arginine residue underwent a double coupling involving the filtration and delivery of fresh reagents and a second preactivation (0 W, 25°C, 2 min.) and coupling (25 W, 75°C, 10 min.) step. Upon synthesis completion, the resin-bound peptides were automatically returned to the Liberty<sup>TM</sup> resin manifold as a suspension in DMF : DCM (1 : 1) and filtered through polypropylene Terumo syringes (10 mL) fitted with a polyethylene porous (20  $\mu$ m) filter prior to acid-mediated cleavage (Section 1.4). Resin wash and filtering steps were aided by the use of a Visprep<sup>TM</sup> SPE DL 24-port model vacuum manifold as supplied by Supelco.

#### 1.4 TFA cleavage

A small aliquot of resin-bound peptide (approx. 3 mg) was suspended in a cleavage solution (1 mL; 95 : 2 : 2 : 1; TFA : TIPS : water : anisol) and shaken gently using a KS125 basic KA elliptical shaker supplied by Labortechnik, at 400 motions min.<sup>-1</sup>, for 2 h. The mixture was filtered through a fritted syringe and the beads rinsed with TFA (1 x 0.2 mL). The filtrate was concentrated under a constant stream of air and the resultant oil was induced to precipitate in ice-cold diethyl ether (1 mL). Cleaved peptides were collected by centrifugation (3 x 5 min.) at a speed of 4500 cycles min.<sup>-1</sup> on a Hermle Z200A centrifuge supplied by Medos. The supernatant liquid was decanted and the pellet was re-suspended in diethyl ether (1 mL). This procedure was repeated three times before the peptide was collected and dried for analysis by analytical RP-HPLC and mass spectrometry.

For full scale resin cleavages, 10 mL of cleavage solution was used and after 4 h, the resin then rinsed with TFA (3 x 3 mL). The filtrate was concentrated under a constant stream of air and the resultant oil was induced to precipitate in ice-cold diethyl ether (35 mL). Collection by centrifugation proceeded over 5 x 6 min. spin times.

#### 1.5 Microwave-accelerated ring closing metathesis (RCM)

Microwave RCM reactions were carried out on a CEM Discover<sup>TM</sup> system as described in Section 1.1. A microwave reactor vessel was loaded with substrate (170-679 mg), deoxygenated solvent (4.75 mL), deoxygenated chaotropic salt solution (0.25 mL) and catalyst (20 mol%) in an inert (nitrogen) environment. The system was sealed and the reaction mixture irradiated with microwave energy whilst being stirred at 100°C for 2 h. After cooling to room temperature, the resin-bound peptide was filtered through a fritted syringe and washed with DCM (5 mL, 3 x 1 min.), DMF (5

mL, 3 x 1 min.) then MeOH (5 mL, 3 x 1 min.) and dried *in vacuo* for 30 min.. The resin was then subjected to acid-mediated cleavage (Section 1.4) and the resultant isolated solid analysed by RP-HPLC and mass spectrometry. Metathesis experiments are described using the following format: Substrate (mmol), solvent (mL), additive (mL), catalyst (mol%), microwave power (W), reaction temperature (°C) and reaction time (h).

## 1.6 Hydrogenation procedure

Fischer-Porter shielded aerosol pressure reactors (100 mL) fitted with pressure gauge heads and stirrer beads were loaded with substrate (270-679 mg), deoxygenated solvents (5 mL) and Wilkinson's catalyst (1 mol%) under an inert (nitrogen) environment. The system was sealed and the pressure vessel then connected to a hydrogenation manifold and purged three times using vacuum and argon flushing cycles, before being charged with hydrogen gas to 80 psi. The reaction mixture was stirred at room temperature for a reported time period and the reaction terminated by venting the hydrogen gas. The resin-bound peptide was filtered through a fritted syringe and washed with DCM (7 mL, 3 x 1 min.), DMF (10 mL, 3 x 1 min.) then MeOH (10 mL, 3 x 1 min.) and left to dry *in vacuo* for 1 h prior to resin cleavage (Section 1.4) and subsequent analysis by RP-HPLC and mass spectrometry. Hydrogenation experiments are described by the following format: Substrate (mmol), solvent (mL), catalyst (mol%), hydrogen pressure (psi), reaction temperature (°C) and reaction time (h).

## 1.7 Cross metathesis with *cis*-2-butene

Fischer-Porter shielded aerosol pressure reactors (100 mL), fitted with pressure gauge heads and stirrer beads, were loaded with substrate (270-679 mg), deoxygenated solvent (4.75 - 9.5 mL), deoxygenated chaotropic salt solution (0.25-0.50 mL) and catalyst (20 mol%). The system was sealed and the pressure vessel then connected to a vacuum manifold and purged three times using vacuum and argon flushing cycles, before being charged to 12 psi with *cis*-2-butene. The reaction mixture was stirred at reflux for 72 h, cooled to room temperature and filtered through a fritted syringe. The resin-bound peptide was washed with DCM (5 mL, 3 x 1 min.), DMF (5 mL, 3 x 1 min.) and MeOH (5 mL, 3 x 1 min.) and dried *in vacuo* for 30 min.. The resin was then subjected to acid-mediated cleavage (Section 1.4) and the resultant solid analysed by RP-HPLC and mass spectrometry. Metathesis experiments are described using the following format: Substrate (mmol), solvent (mL), catalyst (mol%), microwave power (W), reaction temperature (°C) and reaction time (h).

## 2.0 Synthetic procedures

### 2.1 (2*S*)-2*N*-Fluorenylmethoxycarbonylamino-5-methylhex-4-enoic acid (Fmoc-L-Pre-OH) **7**

Fmoc-protected allylglycine **8** was subjected to the conventional cross metathesis procedure with 2-methyl-2-butene (Section 1.7) under the following conditions: (2*S*)-2*N*-Fluorenylmethoxycarbonylamino-5-methylhex-4-enoic acid **8** (600 mg, 1.79 mmol), DCM (10 mL), 2<sup>nd</sup> generation Grubbs' catalyst (75.8 mg, 89.3 μmol), 2-methyl-2-butene (2 mL), 50°C, 12 h, 100% conversion. The reaction mixture was concentrated

under reduced pressure to yield a brown oil (660 mg). The crude product was purified *via* column chromatography (SiO<sub>2</sub>; 1 : 1 : 0.1; light petroleum : EtOAc : MeOH) to give the required prenylglycine derivative **7** (0.59 g, 1.62 mmol) as yellow solid in 91% yield (> 90% purity).  $\nu_{\max}$  (neat): 3426w, 3324w, 3066w, 2932m, 1716s, 1514m, 1478w, 1450m, 1378w, 1338m, 1265m, 1220w, 1106w, 1057m, 910m, 855w, 759w, 738s, 704w, 648w, 621w cm<sup>-1</sup>. <sup>1</sup>H n.m.r. (400 MHz, CDCl<sub>3</sub>):  $\delta$  1.63 (s, 3H), 1.73 (s, 3H), 2.49-2.65 (m, 2H), 4.23 (t, *J* 6.7 Hz, 1H), 4.40 (d, *J* 6.7 Hz, 2H), 5.11 (m, 1H), 5.41 (bd, *J* 7.5 Hz, 1H), 7.31 (t, *J* 7.3 Hz, 2H), 7.40 (t, *J* 7.3 Hz, 2H), 7.58-7.66 (m, 2H), 7.76 (d, *J* 7.4 Hz, 2H), 9.22 (bs, 1H). <sup>13</sup>C n.m.r. (100 MHz, CDCl<sub>3</sub>):  $\delta$  18.1, 26.0, 30.8, 47.3, 53.8, 67.2, 117.5, 120.1, 125.2, 127.2, 127.8, 136.9, 141.4, 143.9, 156.1, 176.2. HRMS (ESI<sup>+</sup>, MeOH): Found: *m/z* 388.1522 (M+Na)<sup>+</sup>, C<sub>22</sub>H<sub>23</sub>NNaO<sub>4</sub> requires 388.1525. Further purification was carried out by trituration from Et<sub>2</sub>O : light petroleum to give Fmoc-prenylglycine **7** (0.34 g, 0.93 mmol) as a colourless solid, m.p. 109-111°C in 53% yield (> 99% purity). In most cases, Fmoc-prenylglycine **7** was used in SPPS without purification.

## 2.2 Linear [2,8]-Agl-[3,12]-Pre-dicarba conotoxin RgIA:

### Fmoc-Gly-Agl-Pre-Ser-Asp-Pro-Arg-Agl-Arg-Tyr-Arg-Pre-Arg **5**

The automated microwave-accelerated procedure outlined in Section 1.3 was used in the synthesis of peptide **5** onto Fmoc-Arg(Pbf)-Wang resin (833 mg, 0.25 mmol). Quantities of HBTU, HOBt, DIPEA, piperidine and each Fmoc-amino acid were used as determined by the automated protocols of the instrument and remained constant throughout this synthesis. The total amount of each coupling reagent and successive Fmoc-amino acid required is summarised in Table 1.

**Table 1: Quantities of reagents and amino acids used in the synthesis of peptide 5**

<i>Reagent (in DMF unless specified)</i>	<i>Total volume (mL)</i>	<i>Mass (g) or Volume (mL)</i>
0.5 M HBTU : HOBt	67.5	12.80 : 4.55 g
2.0 M DIPEA in NMP	35.0	12.30 mL
Fmoc-L-Arg(Pbf)-OH	40.0	5.20 g
Fmoc-L-Asp(O <sup>t</sup> Bu)-OH	15.0	1.23 g
Fmoc-L-Gly-OH	15.0	0.90 g
Fmoc-L-Agl-OH	27.5	1.85 g
Fmoc-L-Pre-OH	27.5	2.0 g
Fmoc-L-Pro-OH	15.0	1.0 g
Fmoc-L-Ser( <sup>t</sup> Bu)-OH	15.0	1.15 g
Fmoc-L-Tyr( <sup>t</sup> Bu)-OH	15.0	1.38 g

After sequence completion, the resin-bound peptide was transferred into a fritted syringe and treated with a capping solution (94 : 5 : 1; DMF : acetic anhydride : NMM) for 2 h. The resin was then washed with DMF (3 x 1 min.), DCM (3 x 1 min.) and MeOH (3 x 1 min.) and left to dry *in vacuo* for 30 min.. A small aliquot of the resin-bound peptide was subjected to acid-mediated cleavage (Section 1.4) and mass spectral analysis of the resultant solid confirmed formation of the required linear peptide **5**. Mass spectrum (ESI<sup>+</sup>, MeCN : H<sub>2</sub>O : HCOOH):  $m/z$  610.5 [ $\frac{1}{3}(M+3H)$ ]<sup>+</sup>,  $\frac{1}{3}(C_{86}H_{128}N_{25}O_{20})$  requires 610.3; 616.5 [ $\frac{1}{3}(M+H_2O+3H)$ ]<sup>+</sup>,  $\frac{1}{3}(C_{86}H_{130}N_{25}O_{21})$  requires 616.3.

### 2.3 Linear [2,8]-Pre-[3,12]-Agl-Dicarba conotoxin RgIA:

#### Fmoc-Gly-Pre-Agl-Ser-Asp-Pro-Arg-Pre-Arg-Tyr-Arg-Agl-Arg 6

The automated microwave-accelerated procedure outlined in Section 1.3 was used in the synthesis of peptide **6** onto Fmoc-Arg(Pbf)-Wang resin (333 mg, 0.10 mmol). Quantities of HBTU, HOBt, DIPEA, piperidine and each Fmoc-amino acid were used as determined by the automated protocols of the instrument and remained constant throughout this synthesis. The total amount of each coupling reagent and successive Fmoc-amino acid required is summarised in Table 2.

**Table 2: Quantities of reagents and amino acids used in the synthesis of peptide 6**

<i>Reagent (in DMF unless specified)</i>	<i>Total volume (mL)</i>	<i>Mass (g) or Volume (mL)</i>
0.5 M HBTU : HOBt	27.0	5.12 : 1.82 g
2.0 M DIPEA in NMP	14.0	4.90 mL
Fmoc-L-Arg(Pbf)-OH	16.0	2.08 g
Fmoc-L-Asp(O <sup>t</sup> Bu)-OH	6.0	0.49 g
Fmoc-L-Gly-OH	6.0	0.36 g
Fmoc-L-Agl-OH	11.0	0.74 g
Fmoc-L-Pre-OH	11.0	0.80 g
Fmoc-L-Pro-OH	6.0	0.40 g
Fmoc-L-Ser( <sup>t</sup> Bu)-OH	6.0	0.46 g
Fmoc-L-Tyr( <sup>t</sup> Bu)-OH	6.0	0.55 g

After sequence completion, the resin-bound peptide was transferred into a fritted syringe and treated with a capping solution (94 : 5 : 1, DMF : acetic anhydride :

NMM) for 2 h. The resin was then washed with DMF (3 x 1 min.), DCM (3 x 1 min.) and MeOH (3 x 1 min.) and left to dry *in vacuo* for 30 min.. A small aliquot of the resin-bound peptide was subjected to acid-mediated cleavage (Section 1.4) and mass spectral analysis of the resultant solid supported formation of the desired linear peptide **6**. Mass spectrum (ESI<sup>+</sup>, MeCN : H<sub>2</sub>O : HCOOH): *m/z* 610.6 [ $\frac{1}{3}(M+3H)$ ]<sup>+</sup>,  $\frac{1}{3}(C_{86}H_{128}N_{25}O_{20})$  requires 610.3; 616.5 [ $\frac{1}{3}(M+H_2O+3H)$ ]<sup>+</sup>,  $\frac{1}{3}(C_{86}H_{130}N_{25}O_{21})$  requires 616.3; 915.0 [ $\frac{1}{2}(M+2H)$ ]<sup>+</sup>,  $\frac{1}{2}(C_{86}H_{127}N_{25}O_{20})$  requires 915.0, 924.0 [ $\frac{1}{2}(M+H_2O+2H)$ ]<sup>+</sup>,  $\frac{1}{2}(C_{86}H_{129}N_{25}O_{21})$  requires 924.0.

#### 2.4 *c*[ $\Delta^4$ 2,8]-[3,12]-Pre-dicarba conotoxin RgIA:

##### **Fmoc-Gly-*c*[ $\Delta^4$ Das-Pre-Ser-Asp-Pro-Arg]-Arg-Tyr-Arg-Pre-Arg **10****

Resin-bound peptide **5** was subjected to the general microwave-accelerated RCM procedure outlined in Section 1.5 under the following conditions: Resin-peptide **10** (390 mg, 87.0  $\mu$ mol), DCM (4.75 mL), 0.4 M LiCl/DMF (0.25 mL), 2<sup>nd</sup> generation Grubbs' catalyst (14 mg, 17.1  $\mu$ mol), 80 W, 100°C, 2 h. Post metathesis, a small aliquot of peptidyl-resin was subjected to the acid-mediated cleavage procedure outlined in Section 1.4. Mass spectral analysis of the isolated solid supported the formation of cyclic peptide **10**. Mass spectrum (ESI<sup>+</sup>, MeCN : H<sub>2</sub>O : HCOOH): *m/z* 601.3 [ $\frac{1}{3}(M+3H)$ ]<sup>+</sup>,  $\frac{1}{3}(C_{84}H_{124}N_{25}O_{20})$  requires 601.0; 607.2 [ $\frac{1}{3}(M+H_2O+3H)$ ]<sup>+</sup>,  $\frac{1}{3}(C_{84}H_{126}N_{25}O_{21})$  requires 607.0; 900.9 [ $\frac{1}{2}(M+2H)$ ]<sup>+</sup>,  $\frac{1}{2}(C_{84}H_{123}N_{25}O_{20})$  requires 901.0; 910.0 [ $\frac{1}{2}(M+H_2O+2H)$ ]<sup>+</sup>,  $\frac{1}{2}(C_{84}H_{125}N_{25}O_{21})$  requires 910.0.

#### 2.5 [2,8]-Pre-*c*[ $\Delta^4$ 3,12]-dicarba conotoxin RgIA:

##### **Fmoc-Gly-Pre-*c*[ $\Delta^4$ Das-Ser-Asp-Pro-Arg-Pre-Arg-Tyr-Arg]-Arg **11****

Resin-bound peptide **6** was subjected to the general microwave-accelerated RCM procedure outlined in Section 1.5 under the following conditions: Resin-peptide **6** (679 mg, 0.10 mmol), DCM (4.75 mL), 0.4 M LiCl/DMF (0.25 mL), 2<sup>nd</sup> generation Grubbs' catalyst (17 mg, 20  $\mu$ mol), 80 W, 100°C, 2 h. Post metathesis, a small aliquot of peptidyl-resin was subjected to the acid-mediated cleavage procedure outlined in Section 1.4. Mass spectral analysis of the isolated solid supported the formation of the desired cyclic peptide **11**. Mass spectrum (ESI<sup>+</sup>, MeCN : H<sub>2</sub>O : HCOOH):  $m/z$  601.2 [ $\frac{1}{3}(M+3H)$ ]<sup>+</sup>,  $\frac{1}{3}(C_{84}H_{124}N_{25}O_{20})$  requires 601.0; 607.1 [ $\frac{1}{3}(M+H_2O+3H)$ ]<sup>+</sup>,  $\frac{1}{3}(C_{84}H_{126}N_{25}O_{21})$  requires 607.0; 900.8 [ $\frac{1}{2}(M+2H)$ ]<sup>+</sup>,  $\frac{1}{2}(C_{84}H_{123}N_{25}O_{20})$  requires 901.0; 909.9 [ $\frac{1}{2}(M+H_2O+2H)$ ]<sup>+</sup>,  $\frac{1}{2}(C_{84}H_{125}N_{25}O_{21})$  requires 910.0.

## 2.6 *c*[2,8]-[3,12]-Pre-dicarba conotoxin RgIA:

### Fmoc-Gly-*c*[Das-Pre-Ser-Asp-Pro-Arg]-Arg-Tyr-Arg-Pre-Arg **12**

Resin-bound peptide **10** was subjected to the general Wilkinson's hydrogenation procedure outlined in Section 1.6 under the following conditions: Resin-peptide **10** (343 mg, 77.0  $\mu$ mol), DCM (4.5 mL), MeOH (0.5 mL), Wilkinson's catalyst, 80 psi of H<sub>2</sub>, 20°C, 22 h. At the end of the reaction period, a small aliquot of peptidyl-resin was subjected to acid-mediated cleavage (Section 1.4) and mass spectral analysis of the isolated solid supported formation of saturated cyclic peptide **12**. Mass spectrum (ESI<sup>+</sup>, MeCN : H<sub>2</sub>O : HCOOH):  $m/z$  601.9 [ $\frac{1}{3}(M+3H)$ ]<sup>+</sup>,  $\frac{1}{3}(C_{84}H_{126}N_{25}O_{20})$  requires 601.7; 607.5 [ $\frac{1}{3}(M+H_2O+3H)$ ]<sup>+</sup>,  $\frac{1}{3}(C_{84}H_{128}N_{25}O_{21})$  requires 608.0; 902.0 [ $\frac{1}{2}(M+2H)$ ]<sup>+</sup>,  $\frac{1}{2}(C_{84}H_{125}N_{25}O_{20})$  requires 902.0; 910.9 [ $\frac{1}{2}(M+H_2O+2H)$ ]<sup>+</sup>,  $\frac{1}{2}(C_{84}H_{127}N_{25}O_{21})$  requires 911.0.

## 2.7 [2,8]-Pre-*c*[3,12]-dicarba conotoxin RgIA:

### Fmoc-Gly-Pre-*c*[Das-Ser-Asp-Pro-Arg-Pre-Arg-Tyr-Arg]-Arg 13

Resin-bound peptide **11** was subjected to the general Wilkinson's hydrogenation procedure outlined in Section 1.6 under the following conditions: Resin-peptide **11** (679 mg, 0.10 mmol), DCM (4.5 mL), MeOH (0.5 mL), Wilkinson's catalyst, 80 psi of H<sub>2</sub>, 20°C, 22 h. Following hydrogenation, a small aliquot of the resin-bound peptide was subjected to acid-mediated cleavage (Section 1.4) and mass spectral analysis of the resultant solid supported formation of saturated cyclic peptide **13**. Mass spectrum (ESI<sup>+</sup>, MeCN : H<sub>2</sub>O : HCOOH): *m/z* 601.5 [ $\frac{1}{3}(M+3H)$ ]<sup>+</sup>,  $\frac{1}{3}(C_{84}H_{126}N_{25}O_{20})$  requires 601.7; 607.5 [ $\frac{1}{3}(M+H_2O+3H)$ ]<sup>+</sup>,  $\frac{1}{3}(C_{84}H_{128}N_{25}O_{21})$  requires 608.0; 902.0 [ $\frac{1}{2}(M+2H)$ ]<sup>+</sup>,  $\frac{1}{2}(C_{84}H_{125}N_{25}O_{20})$  requires 902.0; 910.9 [ $\frac{1}{2}(M+H_2O+2H)$ ]<sup>+</sup>,  $\frac{1}{2}(C_{84}H_{127}N_{25}O_{21})$  requires 911.0.

## 2.8 *c*[2,8]-[3,12]-Crt-dicarba conotoxin RgIA:

### Fmoc-Gly-*c*[Das-Crt-Ser-Asp-Pro-Arg]-Arg-Tyr-Arg-Crt-Arg 14

Resin-bound peptide **12** was subjected to the general cross metathesis procedure outlined in Section 1.7 under the following conditions: Resin-peptide **12** (270 mg, 60 μmol), DCM (4.75 mL), 0.4 M LiCl/DMF (0.25 mL), 2<sup>nd</sup> generation Grubbs' catalyst (10.8 mg, 12.5 μmol), 12 psi of *cis*-2-butene, 50°C, 72 h. Post metathesis, a small aliquot of peptidyl-resin was subjected to the acid-mediated cleavage procedure outlined in Section 1.4. Mass spectral analysis of the isolated solid indicated the presence of the desired product **14** and a partially metathesised peptide (mono-Crt containing). Mass spectrum (ESI<sup>+</sup>, MeCN : H<sub>2</sub>O : HCOOH): *m/z* 592.4 [ $\frac{1}{3}(M_{14}+3H)$ ]<sup>+</sup>,  $\frac{1}{3}(C_{82}H_{122}N_{25}O_{20})$  requires 592.3; 597.3 [ $\frac{1}{3}(M_{12}+3H)$ ]<sup>+</sup> (mono-Crt),  $\frac{1}{3}(C_{83}H_{124}N_{25}O_{20})$  requires 597.0; 602.3 [ $\frac{1}{3}(M_{12}+H_2O+3H)$ ]<sup>+</sup> (mono-Crt),

$\frac{1}{3}(\text{C}_{83}\text{H}_{126}\text{N}_{25}\text{O}_{21})$  requires 603.0; 607.5  $[\frac{1}{3}(\text{M}_{12}+\text{H}_2\text{O}+3\text{H})]^+$ ,  $\frac{1}{3}(\text{C}_{84}\text{H}_{128}\text{N}_{25}\text{O}_{21})$  requires 608.0; 888.5  $[\frac{1}{2}(\text{M}_{14}+2\text{H})]^+$ ,  $\frac{1}{2}(\text{C}_{82}\text{H}_{121}\text{N}_{25}\text{O}_{20})$  requires 888.0; 895.5  $[\frac{1}{2}(\text{M}+2\text{H})]^+$  (mono-Crt),  $\frac{1}{2}(\text{C}_{83}\text{H}_{123}\text{N}_{25}\text{O}_{20})$  requires 895.0; 902.1  $[\frac{1}{2}(\text{M}_{12}+2\text{H})]^+$ ,  $\frac{1}{2}(\text{C}_{84}\text{H}_{125}\text{N}_{25}\text{O}_{20})$  requires 902.0; 910.5  $[\frac{1}{2}(\text{M}_{12}+\text{H}_2\text{O}+2\text{H})]^+$ ,  $\frac{1}{2}(\text{C}_{84}\text{H}_{127}\text{N}_{25}\text{O}_{21})$  requires 911.0.

## 2.9 [2,8]-Crt-c[3,12]-dicarba conotoxin RgIA:

### Fmoc-Gly-Crt-c[Das-Ser-Asp-Pro-Arg-Crt-Arg-Tyr-Arg]-Arg 15

Resin-bound peptide **13** was subjected to the general cross metathesis procedure outlined in Section 1.7 under the following conditions: Resin-peptide **13** (679 mg, 0.10 mmol), DCM (9.5 mL), 0.4 M LiCl/DMF (0.5 mL), 2<sup>nd</sup> generation Grubbs' catalyst (17 mg, 20  $\mu\text{mol}$ ), 12 psi of *cis*-2-butene, 50°C, 72 h. Post metathesis, a small aliquot of resin-bound peptide was subjected to the acid-mediated cleavage procedure outlined in Section 1.4. Mass spectral analysis of the resultant solid confirmed the formation of **15**. Starting material **13** was also observed. Mass spectrum (ESI<sup>+</sup>, MeCN : H<sub>2</sub>O : HCOOH): *m/z* 888.3  $[\frac{1}{2}(\text{M}_{15}+2\text{H})]^+$ ,  $\frac{1}{2}(\text{C}_{82}\text{H}_{121}\text{N}_{25}\text{O}_{20})$  requires 888.0; 895.5  $[\frac{1}{2}(\text{M}_{13}+2\text{H})]^+$  (mono-Crt),  $\frac{1}{2}(\text{C}_{83}\text{H}_{123}\text{N}_{25}\text{O}_{20})$  requires 895.0; 902.0  $[\frac{1}{2}(\text{M}_{13}+2\text{H})]^+$ ,  $\frac{1}{2}(\text{C}_{84}\text{H}_{125}\text{N}_{25}\text{O}_{20})$  requires 902.0; 910.9  $[\frac{1}{2}(\text{M}_{13}+\text{H}_2\text{O}+2\text{H})]^+$ ,  $\frac{1}{2}(\text{C}_{84}\text{H}_{127}\text{N}_{25}\text{O}_{21})$  requires 911.0.

## 2.10 c[2,8]-c[ $\Delta^4$ 3,12]-dicarba conotoxin RgIA:

### Gly-c[Das-c( $\Delta^4$ Das-Ser-Asp-Pro-Arg)-Arg-Tyr-Arg]-Arg 16

Resin-bound peptide **14** was subjected to the general microwave-accelerated RCM procedure outlined in Section 1.5 under the following conditions: Resin-peptide **14** (170 mg, 38.0  $\mu\text{mol}$ ), DCM (4.75 mL), 0.4 M LiCl/DMF (0.25 mL), 2<sup>nd</sup> generation

Grubbs' catalyst (6.5 mg, 7.6  $\mu\text{mol}$ ), 80 W, 100°C, 2 h. Post metathesis, a small aliquot of peptidyl-resin was subjected to acid-mediated cleavage (Section 1.4) and mass spectral analysis of the isolated solid supported formation of bicyclic peptide **16**. Mono-cyclic peptides **12** (mono-Crt) and **14** were also observed. Mass spectrum (ESI<sup>+</sup>, MeCN : H<sub>2</sub>O : HCOOH):  $m/z$  596.4 [ $\frac{1}{3}(\text{M}_{12}+3\text{H})$ ]<sup>+</sup> (mono-Crt),  $\frac{1}{3}(\text{C}_{83}\text{H}_{124}\text{N}_{25}\text{O}_{20})$  requires 597.0; 602.3 [ $\frac{1}{3}(\text{M}_{12}+\text{H}_2\text{O}+3\text{H})$ ]<sup>+</sup> (mono-Crt),  $\frac{1}{3}(\text{C}_{83}\text{H}_{126}\text{N}_{25}\text{O}_{21})$  requires 603.0; 859.5 [ $\frac{1}{2}(\text{M}_{16}+2\text{H})$ ]<sup>+</sup>,  $\frac{1}{2}(\text{C}_{78}\text{H}_{113}\text{N}_{25}\text{O}_{20})$  requires 859.9; 881.5 [ $\frac{1}{2}(\text{M}_{16}+2\text{Na})$ ]<sup>+</sup>,  $\frac{1}{2}(\text{C}_{78}\text{H}_{111}\text{N}_{25}\text{Na}_2\text{O}_{20})$  requires 881.9; 902.0 [ $\frac{1}{2}(\text{M}_{12}+2\text{H})$ ]<sup>+</sup> (mono-Crt),  $\frac{1}{2}(\text{C}_{84}\text{H}_{125}\text{N}_{25}\text{O}_{20})$  requires 902.0.

The remaining resin-bound peptide **16** was treated with a solution of DMSO : DMF (5 mL, 1 : 1) for 72 h and then subjected to Fmoc-deprotected in the presence of 20% v/v piperidine in DMF (1 x 1 min., 2 x 20 min.). After filtration, the resin was washed with DMF (5 x 1 min.), DCM (3 x 1 min.) and MeOH (3 x 1 min.) and dried *in vacuo* for 1 h. The Fmoc-deprotected peptidyl-resin was then subjected to acid-mediated cleavage (Section 1.4) and mass spectral analysis of the isolated solid (80 mg) confirmed formation of cyclic peptide **2**. Mass spectrum (ESI<sup>+</sup>, MeCN : H<sub>2</sub>O : HCOOH):  $m/z$  727.4 [ $\frac{1}{3}(\text{M}_{12}+3\text{H})$ ]<sup>+</sup>,  $\frac{1}{3}(\text{C}_{69}\text{H}_{113}\text{N}_{25}\text{O}_{18})$  requires 727.6; 750.0 [ $\frac{1}{2}(\text{M}_{16}+2\text{H})$ ]<sup>+</sup>,  $\frac{1}{2}(\text{C}_{63}\text{H}_{103}\text{N}_{25}\text{O}_{18})$  requires 748.9; 771.4 [ $\frac{1}{2}(\text{M}_{16}+2\text{Na})$ ]<sup>+</sup>,  $\frac{1}{2}(\text{C}_{63}\text{H}_{101}\text{N}_{25}\text{Na}_2\text{O}_{18})$  requires 770.8; 800.3 [ $\frac{1}{2}(\text{M}_{12}+\text{H}_2\text{O}+2\text{H})$ ]<sup>+</sup>,  $\frac{1}{2}(\text{C}_{69}\text{H}_{117}\text{N}_{25}\text{O}_{19})$  requires 799.9. The crude product was purified by preparative RP-HPLC (Agilent: Vydac C18 preparative column, 0 → 25% buffer B over 30 min.,  $t_{\text{R}}$  20.98 min.) as described in Section 1.1. Selected fractions were combined and lyophilised to yield target peptide **2** (760  $\mu\text{g}$ , 1% yield, > 90% purity) as a colourless oil. Mass spectrum (MALDI-TOF, MeCN : H<sub>2</sub>O):  $m/z$  1497.1 (M+H)<sup>+</sup>,  $\text{C}_{63}\text{H}_{102}\text{N}_{25}\text{O}_{18}$  requires 1496.8.

RP-HPLC (Agilent: Vydac C18 analytical column, 0 → 30% buffer B over 30 min.,  $t_R$  13.59 min.).

## 2.11 $c[\Delta^4,8]-c[3,12]$ -dicarba conotoxin RgIA:

### Gly- $c[\Delta^4\text{Das}-c(\text{Das-Ser-Asp-Pro-Arg})-\text{Ala-Trp-Arg})-\text{Arg}$ **17**

Resin-bound peptide **15** was subjected to the general microwave-accelerated RCM procedure outlined in Section 1.5 under the following conditions: Resin-peptide **15** (679 mg, 0.10 mmol), DCM (4.75 mL), 0.4 M LiCl/DMF (0.25 mL), 2<sup>nd</sup> generation Grubbs' catalyst (17 mg, 20  $\mu\text{mol}$ ), 100°C, 80 W, 2 h. Post metathesis, a small aliquot of resin-bound peptide was subjected to the acid-mediated cleavage procedure outlined in Section 1.4. Mass spectral analysis of the resultant solid supported the formation of cyclic peptide **17**. Mono-cyclic peptides **13**, **13** (mono-Crt) and **15** were also observed. Mass spectrum (ESI<sup>+</sup>, MeCN : H<sub>2</sub>O : HCOOH):  $m/z$  601.2 [ $\frac{1}{3}(\text{M}_{13}+3\text{H})$ ]<sup>+</sup>,  $\frac{1}{3}(\text{C}_{84}\text{H}_{126}\text{N}_{25}\text{O}_{20})$  requires 601.6; 607.3 [ $\frac{1}{3}(\text{M}_{13}+\text{H}_2\text{O}+3\text{H})$ ]<sup>+</sup>,  $\frac{1}{3}(\text{C}_{84}\text{H}_{128}\text{N}_{25}\text{O}_{21})$  requires 607.6; 859.5 [ $\frac{1}{2}(\text{M}_{17}+2\text{H})$ ]<sup>+</sup>,  $\frac{1}{2}(\text{C}_{78}\text{H}_{113}\text{N}_{25}\text{O}_{20})$  requires 859.9; 888.3 [ $\frac{1}{2}(\text{M}_{15}+2\text{H})$ ]<sup>+</sup>,  $\frac{1}{2}(\text{C}_{82}\text{H}_{121}\text{N}_{25}\text{O}_{20})$  requires 887.9; 902.0 [ $\frac{1}{2}(\text{M}_{13}+2\text{H})$ ]<sup>+</sup> (mono-Crt),  $\frac{1}{2}(\text{C}_{84}\text{H}_{125}\text{N}_{25}\text{O}_{20})$  requires 902.0; 911.1 [ $\frac{1}{2}(\text{M}_{13}+\text{H}_2\text{O}+2\text{H})$ ]<sup>+</sup>,  $\frac{1}{2}(\text{C}_{84}\text{H}_{127}\text{N}_{25}\text{O}_{20})$  requires 911.0.

The remaining resin bound peptide **17** was treated with a solution of DMSO : DMF (5 mL; 1 : 1) for 72 h and then subjected to Fmoc-deprotection in the presence of 20% v/v piperidine in DMF (1 x 1 min., 2 x 20 min.). After filtration, the resin was washed with DMF (5 x 1 min.), DCM (3 x 1 min.) and MeOH (3 x 1 min.) and dried *in vacuo* for 1 h. The Fmoc-deprotected peptidyl-resin was then subjected to acid-mediated cleavage (Section 1.4) and mass spectral analysis of the isolated residue (151 mg)

confirmed formation of cyclic peptide **3**. The crude product was purified by preparative RP-HPLC (Agilent: Vydac C18 preparative column, 0 → 30% buffer B over 30 min.,  $t_R$  17.55 min.) as described in Section 1.1. Selected fractions were combined and lyophilised to yield the target peptide **3** (460  $\mu$ g, 0.6% yield, > 90% purity) as a colourless solid. Mass spectrum (MALDI-TOF, MeCN : H<sub>2</sub>O):  $m/z$  1497.2 (M+H)<sup>+</sup>, C<sub>63</sub>H<sub>102</sub>N<sub>25</sub>O<sub>18</sub>) requires 1496.8. RP-HPLC (Agilent: Vydac C18 analytical column, 0 → 30% buffer B over 30 min.,  $t_R$  14.79 min.).

## 2.12 *c*[2,8]-*c*[3,12]-dicarba conotoxin RgIA:

### *c*[Das-*c*(Das-Ser-Asp-Pro-Arg)-Arg-Tyr-Arg)-Arg **4**

Resin-bound peptide **16** was subjected to the general Wilkinson's hydrogenation procedure outlined in Section 1.6 under the following conditions: Resin-peptide **16** (270 mg, 60  $\mu$ mol), DCM (4.5 mL), MeOH (0.5 mL), Wilkinson's catalyst, 80 psi of H<sub>2</sub>, 22°C, 24 h. Following hydrogenation, the resin bound peptide **14** was agitated in a solution of DMSO : DMF (5 mL; 1 : 1) for 72 h and then subjected to Fmoc-deprotection in the presence of 20% v/v piperidine in DMF (1 x 1 min., 2 x 20 min.). After filtration, the resin was washed with DMF (5 x 1 min.), DCM (3 x 1 min.) and MeOH (3 x 1 min.) and dried *in vacuo* for 1 h. The Fmoc-deprotected peptidyl-resin was then subjected to acid-mediated cleavage (Section 1.4) and mass spectral analysis of the isolated solid (80 mg) confirmed formation of saturated bicyclic peptide **4**. The fully reduced monocyclic peptide **12** was also observed. Mass spectrum (ESI<sup>+</sup>, MeCN : H<sub>2</sub>O : HCOOH):  $m/z$  750.0 [ $\frac{1}{2}$ (M<sub>4</sub>+2H)]<sup>+</sup>,  $\frac{1}{2}$ (C<sub>63</sub>H<sub>105</sub>N<sub>25</sub>O<sub>18</sub>) requires 749.9; 772.0 [ $\frac{1}{2}$ (M<sub>4</sub>+2Na)]<sup>+</sup>,  $\frac{1}{2}$ (C<sub>63</sub>H<sub>103</sub>N<sub>25</sub>Na<sub>2</sub>O<sub>18</sub>) requires 771.8; 792.9 [ $\frac{1}{2}$ (M<sub>12</sub>+2H)]<sup>+</sup> (fully reduced),  $\frac{1}{2}$ (C<sub>69</sub>H<sub>117</sub>N<sub>25</sub>O<sub>18</sub>) requires 793.0 The crude product was purified by preparative RP-HPLC (Agilent: Vydac C18 preparative column, 0 → 25% buffer B

over 30 min.,  $t_R$  21.53 min.) as described in Section 1.1. Selected fractions were combined and lyophilised to yield target peptide **4** (670  $\mu\text{g}$ , 0.8% yield, > 90% purity) as a colourless oil. Mass spectrum (MALDI-TOF, MeCN : H<sub>2</sub>O):  $m/z$  1499.0 (M+H)<sup>+</sup>, C<sub>63</sub>H<sub>104</sub>N<sub>25</sub>O<sub>18</sub> requires 1498.8. RP-HPLC (Vydac C18 analytical column, 0 → 30% buffer B over 30 min.,  $t_R$  14.16 min.).