

## Supplementary Material

### *Instrumental Details, Abbreviations, General Methods and Procedures*

Mass spectra (ESI<sup>+</sup>) were measured on a Bruker BioApex 47 e<sup>-</sup> FTMS (Fourier Transform Mass Spectrometer) with a 4.7 Tesla magnet and an Analytica electrospray source. Liquid chromatography-mass spectrometry (LC-MS) was conducted on a Gilson instrument equipped with a Gilson 215/819 injector module, 306 gradient pumps and an Agilent 1100 diode array detector. Peptides were visualised with UV irradiation between 200-300 nm and a Micromass ZMD mass spectrometer in positive electrospray ionisation (ESI<sup>+</sup>) mode. The solvent system used throughout this study (except where specified) was buffer A: 0.1% aqueous formic acid; buffer B: 0.1% formic acid in MeOH. Analytical experiments were performed on a Phenomenex Luna RP-C8 analytical column (3 x 50 mm, 5 μm) at a flow rate of 1.1 ml/min. The instrument was equipped with a Gilson FC 405 fraction collector that was triggered by mass detection in the ESI Source. The flow to the detectors was reduced with an LC packing post column splitter.

NMR spectra were recorded on a 500 MHz Varian Mercury 500 spectrometer. The solvent <sup>1</sup>H signal, δ<sub>H</sub> 1.94 for d<sub>6</sub>-DMSO, was used as an internal reference. Reverse phase HPLC (RP-HPLC) was performed using an automated gradient controlled Waters apparatus (2 x 6000A) with a tunable absorbance detector. The solvent system used was buffer A: 0.1% aqueous TFA; buffer B: 0.1% TFA in MeCN. Preparative RP-HPLC was performed using a μBONDAPAK C18 column (10 μm, 7.8 mm ID, 300 mm) with a flow rate of 2.0 ml/min. The separations were carried out using an isocratic gradient 5%→40%→100% B unless otherwise specified. Microwave reactions were carried out on a CEM Discover System<sup>TM</sup> fitted with a benchmate option. The instrument produces a continuous focused beam of microwave irradiation at a power delivery of 60W, which reaches and maintains a temperature of 100°C. Reactions were performed in 10 ml high pressure quartz microwave vessels fitted with self-sealing Teflon septa. The vessels contained 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 elapsed (cooling periods and ramping periods are not included).

Abbreviations: 2-Aminohex-4-enoic acid (Δ<sup>4</sup>Ahx), 2-Amino-5-phenylpent-4-enoic acid (Δ<sup>4</sup>App), 2-Amino-5-phenylpentanoic acid (App), 2-Aminopent-4-enoic acid (allylglycine, Agl), 2,7-Diaminooctanedioic acid (2,7-diaminosuberic acid, Das), 2,7-Diaminooct-4-enedioic acid (2,7-diaminodehydrosuberic acid, Δ<sup>4</sup>Das), *N,N*-Dimethylformamide (DMF), Diisopropylethylamine (DIPEA), *N*-Methylmorpholine (NMM), *O*-(Benzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate (HBTU), *O*-(7-Azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate (HATU), Pentafluorophenyl diphenylphosphinate (FDDP), *N*-(9-Fluorenyl-methoxycarbonyl)-L-glycine (Fmoc-L-Gly-OH), *N*-(9-Fluorenylmethoxycarbonyl)-L-phenylalanine (Fmoc-L-Phe-OH), *N*-(9-Fluorenylmethoxycarbonyl)-*O*-*tert*-butyl-L-threonine (Fmoc-L-

Thr(<sup>t</sup>Bu)-OH), Tricyclohexylphosphine[1,3-bis(2,4,6-trimethylphenyl)-4,5-dihydroimidazol-2-ylidene]-(benzylidene)-ruthenium(II) dichloride (2<sup>nd</sup> generation Grubbs' catalyst).

### ***Automated Microwave Assisted Solid Phase Peptide Synthesis***

Automated microwave-accelerated SPPS was carried out on a CEM Liberty-Discover<sup>TM</sup> synthesiser, involving the flow of liquid solvent and reagents from external nitrogen pressurised bottles to a 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 v2.3.0.

In a 50 ml centrifuge tube, the Wang-Gly resin (or other cited resin) (0.25 mmol) was allowed to swell in DMF (10 ml, 1 x 60 min) and connected to an empty compartment on the CEM Liberty<sup>TM</sup> resin manifold. The Fmoc-amino acids (0.2 M Fmoc-Xaa(PG)-OH in DMF), activator (0.5 M HBTU : HOBt in DMF), activator base (2 M DIPEA in NMP) and deprotection agent (20% piperidine in DMF) were measured out and solubilised in the appropriate volume of specified solvent as calculated by the PepDriver software program provided. The concentration of each reagent delivered to the reaction chamber during linear peptide synthesis remained constant. The volume of reagents delivered also remained constant throughout each synthesis but varied upon alteration of the resin scale.

Each synthesis commenced with the delivery of resin to the microwave reactor vessel in a stream of DMF : DCM (14 ml; 1 : 1) with washing and draining in triplicate to ensure all traces of resin were transferred. After an initial swelling period (1 x 10 min), each subsequent amino acid coupling was then performed according to the default PepDriver method. Upon synthesis completion, the resin was automatically returned to the Liberty<sup>TM</sup> resin manifold as a suspension in DMF : DCM (1 : 1) in readiness for further workup.

Peptides were isolated using polypropylene Terumo syringes (10 ml or 5 ml) fitted with a polyethylene porous (20 µm) frits (0.3 cm thick). Terumo syringes fitted with teflon taps were placed on a Visprep vacuum manifold (Visprep<sup>TM</sup> SPE DL 24-port model vacuum manifold supplied by Supelco). All washings were performed with a known volume of solution and resin immersion time and each wash was evacuated *via* a water aspirator before the next wash or coupling step. Cleavage mixtures were agitated on a KS125 basic KA elliptical shaker supplied by Labortechnik at 400 motions per minute unless otherwise stated. Centrifugation of cleaved peptides was performed at 4000 rpm on a Hermle Z200A centrifuge supplied by Medos.

### ***General Capping Procedure***

To prevent free amines from inactivating 2<sup>nd</sup> generation Grubbs' catalyst, the peptidyl-resin was capped with an anhydride solution (5% acetic anhydride, 94% DMF, 1% NMM) and shaken for 2 hrs before being filtered, washed with DMF (7 ml, 3 x 1 min), DCM (7 ml, 3 x 1 min), MeOH (7 ml, 3 x 1 min) and left to dry *in vacuo* for 30 mins..

### ***General TFA Cleavage Procedure***

A small aliquot of the resin-tethered peptide was suspended in a cleavage solution (10 mL): 95% TFA : 5% TIPS) and gently stirred for 4 h.. After this period of time, the mixture was filtered and the resin beads were rinsed with TFA (2 x 0.5 ml). The filtrate was concentrated by a constant stream of air to yield crude product as an oil. The peptide was then precipitated with ice-cold Et<sub>2</sub>O (4 - 40 ml) and collected by centrifugation (2 x 10 min). The supernatant liquid was decanted and the solid was collected for mass spectrometric analysis.

### ***Synthesis of the Linear Mahafacyclin B Analogues on Wang-Gly resin***

#### **Synthesis of Fmoc-Thr-Agl-Phe-Gly-Phe-Agl-Gly-OH (2)**

The linear heptapeptide **2** was synthesised on Wang-Gly resin (0.25 mmol) using the automated peptide synthesis described above. The resin-tethered heptapeptide was isolated and capped (method described above) before a small aliquot was subjected to TFA cleavage (procedure outlined above) for mass spectrometric analysis. Mass spectrum (ESI<sup>+</sup>, MeCN/H<sub>2</sub>O): *m/z* 966.4 (M + Na)<sup>+</sup>, C<sub>51</sub>H<sub>57</sub>N<sub>7</sub>NaO<sub>11</sub>.

#### **Synthesis of H<sub>2</sub>N-Thr-Agl-Phe-Gly-Phe-Agl-Gly-OH**

The linear sequence was prepared as per the Fmoc-Thr-Agl-Phe-Gly-Phe-Agl-Gly-OH **2** synthesis described above. The peptidyl-resin was subjected to Fmoc-deprotection using 20% piperidine in DMF and cleaved from the resin to afford the desired peptide as a colourless solid (176 mg, 98%). Mass spectrum (ESI<sup>+</sup>, MeCN/H<sub>2</sub>O): *m/z* 722.2 (M + H)<sup>+</sup>, C<sub>36</sub>H<sub>48</sub>N<sub>7</sub>O<sub>9</sub>.

#### **Synthesis of Fmoc-Phe-Agl-Thr-Gly-Agl-Phe-Gly-OH (4)**

The linear sequence was prepared as per the Fmoc-Thr-Agl-Phe-Gly-Phe-Agl-Gly-OH **2** synthesis described above. Mass spectrum (ESI<sup>+</sup>, MeCN/H<sub>2</sub>O): *m/z* 966.1 (M + Na)<sup>+</sup>, C<sub>51</sub>H<sub>57</sub>N<sub>7</sub>NaO<sub>11</sub>.

#### **Synthesis of Fmoc-Gly-Agl-Phe-Gly-Phe-Agl-Gly-OH (5)**

The linear sequence was prepared as per the Fmoc-Thr-Agl-Phe-Gly-Phe-Agl-Gly-OH **2** synthesis described above. Mass spectrum (ESI<sup>+</sup>, MeCN/H<sub>2</sub>O): *m/z* 922.4 (M + Na)<sup>+</sup>, C<sub>49</sub>H<sub>53</sub>N<sub>7</sub>NaO<sub>10</sub>.

### ***Ring Closing Metathesis of Mahafacyclin B Analogues on Wang-Gly resin***

The reactions were carried out under conventional conditions and/or microwave conditions (as specified):

#### **General Conventional Metathesis Conditions:**

The vessel was charged with substrate (50 - 500 mg), deoxygenated solvent (4 - 5 ml) and catalyst (20 mol%) in an inert nitrogen or argon atmosphere. The reaction mixture was heated to the desired temperature for a specific period of time and the metathesis reactions were terminated upon exposure to oxygen. In solution

phase experiments the solvent was removed under reduced pressure. Reactions carried out on the solid phase were subjected to the same metathesis conditions, however at the end of the reaction period, the reaction mixture containing the resin-bound peptide was filtered through a fritted syringe. The resin was then washed with DCM (7 ml, 3 x 1 min), DMF (7 ml, 3 x 1 min) and MeOH (7 ml, 3 x 1 min) and then left to dry *in vacuo* for 1 h before being subjected to TFA resin cleavage. The peptides were analysed by LC-MS to determine the % conversion to product.

#### **General Microwave Metathesis Conditions:**

A microwave reactor vessel was charged with substrate (50 - 749 mg), deoxygenated solvent (4 - 5 ml) and catalyst (20 mol%) in an inert (nitrogen or argon) environment. The reaction mixture was irradiated with microwave energy and stirred at 100°C for 1 h, cooled to ambient temperature and then terminated upon exposure to oxygen. The reaction mixtures were analysed as for conventional metathesis reactions and the % conversion to product was determined by LC-MS.

#### ***Synthesis of tethered peptides:***

##### **Synthesis of H<sub>2</sub>N-Thr-*c*[Δ<sup>4</sup>Das-Phe-Gly-Phe]-Gly-OH (3)**

The resin-bound peptide **2** was subjected to the microwave RCM procedure outlined above. Peptidyl-resin (95 mg, 0.068 mmol), DCM (3 ml), 2<sup>nd</sup> generation Grubbs' catalyst (12 mg, 0.014 mmol), LiCl/DMF (0.4M, 0.2 ml), 100°C, 1 h.. LC-MS was performed to assess reaction completion (LC-MS: *m/z* 938.4 C<sub>49</sub>H<sub>53</sub>N<sub>7</sub>NaO<sub>11</sub> (t<sub>R</sub> 9.67 min)) and was considered complete after 1 h.. The peptidyl-resin was then subjected to Fmoc-deprotection using 20% piperidine in DMF and cleaved from the resin to afford the desired peptide as an off-white solid (47 mg, 100%). Mass Spectrum (ESI<sup>+</sup>, MeCN/H<sub>2</sub>O): *m/z* 694.2 (M + H)<sup>+</sup>, C<sub>34</sub>H<sub>44</sub>N<sub>7</sub>O<sub>9</sub>.

##### **Synthesis of Fmoc-Thr-*c*[Δ<sup>4</sup>Das-Phe-Gly-Phe]-Gly-OH**

The resin-bound peptide **2** was subjected to the microwave RCM procedure outlined above. Peptidyl-resin (592 mg, 0.42 mmol), DCM (3 ml), 2<sup>nd</sup> generation Grubbs' catalyst (71 mg, 0.084 mmol), LiCl/DMF (0.2 ml), 100°C, 1 h.. The peptidyl-resin was then cleaved from the resin to afford the desired peptide as an off-white solid (372 mg, 97%). Mass Spectrum (ESI<sup>+</sup>, MeCN/H<sub>2</sub>O): *m/z* 938.1 (M + Na)<sup>+</sup>, C<sub>49</sub>H<sub>53</sub>N<sub>7</sub>NaO<sub>11</sub>.

##### **Synthesis of H<sub>2</sub>N-Phe-*c*[Δ<sup>4</sup>Das-Thr-Gly]-Phe-Gly-OH (4)**

The metathesis was performed as per the synthesis of H<sub>2</sub>N-Thr-*c*[Δ<sup>4</sup>Das-Phe-Gly-Phe]-Gly-OH **3** using linear peptide Fmoc-Phe-Agl-Thr-Gly-Agl-Phe-Gly-OH **4** (615 mg, 0.25 mmol) and 2<sup>nd</sup> generation Grubbs' catalyst (42 mg, 0.049 mmol). The peptidyl-resin was then subjected to Fmoc-deprotection using 20%

piperidine in DMF and cleaved from the resin to afford a light brown peptide (172 mg, 99%). Mass Spectrum (ESI<sup>+</sup>, MeCN/H<sub>2</sub>O): *m/z* 694.3 (M + H)<sup>+</sup>, C<sub>34</sub>H<sub>44</sub>N<sub>7</sub>O<sub>9</sub>.

#### **Synthesis of H<sub>2</sub>N-Gly-*c*[Δ<sup>4</sup>Das-Phe-Gly-Phe]-Gly-OH (5)**

The metathesis was performed as per the synthesis of H<sub>2</sub>N-Thr-*c*[Δ<sup>4</sup>Das-Phe-Gly-Phe]-Gly-OH **3** using linear peptide Fmoc-Gly-Agl-Phe-Gly-Phe-Agl-Gly-OH **5** (644 mg, 0.25 mmol) and 2<sup>nd</sup> generation Grubbs' catalyst (42 mg, 0.049 mmol). The peptidyl-resin was then subjected to Fmoc-deprotection using 20% piperidine in DMF and cleaved from the resin to afford a light brown peptide (145 mg, 89%). Mass Spectrum (ESI<sup>+</sup>, MeCN/H<sub>2</sub>O): *m/z* 650.2 (M + H)<sup>+</sup>, C<sub>32</sub>H<sub>40</sub>N<sub>7</sub>O<sub>8</sub>.

#### ***Macrolactamisation of the Tethered Peptides:***

##### **Synthesis of *Cyclo*-[Thr-*c*[Δ<sup>4</sup>Das-Phe-Gly-Phe]-Gly] (6)**

Macrolactamisation was carried out following a modified literature procedure.<sup>5</sup> A stirred solution of Fmoc-deprotected peptide **3** (20 mg, 0.029 mmol) in DMF (6 ml) under an atmosphere of nitrogen was cooled to 0°C and treated with pentafluorophenyl diphenylphosphinate (FDDP) (18 mg, 0.046 mmol) and DIPEA (16 μl, 0.092 mmol). The mixture was then warmed to ambient temperature and stirring was continued for 3 hrs.. The solvent was removed under reduced pressure and the residue partitioned between sat. aq. NaHCO<sub>3</sub> (20 ml) and CHCl<sub>3</sub>/*i*-PrOH (3:1 v/v; 30 ml). The aqueous phase was extracted with CHCl<sub>3</sub>/*i*-PrOH (3:1 v/v; 2 x 30 ml) and the combined organic phase was washed with saturated brine, dried with (MgSO<sub>4</sub>) and concentrated under reduced pressure to afford the target peptide as a light brown solid (20 mg). The residue was purified by preparative reverse phase HPLC (*t<sub>R</sub>* 28.9 min). The residue was then lyophilised for 18 h to give the desired peptide **6** as a colourless solid (10.7 mg, 55%). Mass spectrum (ESI<sup>+</sup>, MeCN/H<sub>2</sub>O): *m/z* 698.2 (M + Na)<sup>+</sup>, C<sub>34</sub>H<sub>41</sub>N<sub>7</sub>NaO<sub>8</sub>.

##### **Synthesis of *Cyclo*-[Thr-*c*[Δ<sup>4</sup>Das-Phe-Gly-Phe]-Gly] (6)**

The lactamisation reaction was carried out as per the above synthesis of *cyclo*-[Thr-*c*[Δ<sup>4</sup>Das-Phe-Gly-Phe]-Gly] (**6**) using H<sub>2</sub>N-Phe-*c*[Δ<sup>4</sup>Das-Thr-Gly]-Phe-Gly-OH (10 mg, 0.014 mmol), FDDP (9 mg, 0.023 mmol) and DIPEA (8 μl, 0.046 mmol). The desired peptide **6** was isolated as a colourless solid (8.8 mg, 91%). The residue was purified by preparative reverse phase HPLC (*t<sub>R</sub>* 28.9 min). The residue was then lyophilised for 18 h to give the desired peptide **6** (4.5 mg, 46%). Mass spectrum (ESI<sup>+</sup>, MeCN/H<sub>2</sub>O): *m/z* 698.1 (M + Na)<sup>+</sup>, C<sub>34</sub>H<sub>41</sub>N<sub>7</sub>NaO<sub>8</sub>.

### Synthesis of *Cyclo*-[Fmoc-Thr-*c*[ $\Delta^4$ Das-Phe-Gly-Phe]-Gly]

The reaction was carried out as per the above synthesis of *cyclo*-[Thr-*c*[ $\Delta^4$ Das-Phe-Gly-Phe]-Gly] (**6**) using Fmoc-Thr-*c*[ $\Delta^4$ Das-Phe-Gly-Phe]-Gly-OH (40 mg, 0.044 mmol), FDDP (26 mg, 0.068 mmol) and DIPEA (24  $\mu$ l, 0.139 mmol). The desired bicyclic ester (precursor to (**8**, R=CH<sub>3</sub>)) was isolated as a colourless solid (37 mg, 94%). Mass spectrum (ESI<sup>+</sup>, MeCN/H<sub>2</sub>O): *m/z* 920.0 (M + Na)<sup>+</sup>, C<sub>49</sub>H<sub>51</sub>N<sub>7</sub>NaO<sub>10</sub>.

### Synthesis of *Cyclo*-[Thr-Agl-Phe-Gly-Phe-Agl-Gly] (**7**, R=H)

The macrocyclisation reaction was carried out as per the above synthesis of *cyclo*-[Thr-*c*[ $\Delta^4$ Das-Phe-Gly-Phe]-Gly] (**6**) using the linear peptide H<sub>2</sub>N-Thr-Agl-Phe-Gly-Phe-Agl-Gly-OH (84 mg, 0.116 mmol), FDDP (71 mg, 0.185 mmol) and DIPEA (64  $\mu$ l, 0.367 mmol). Crude peptide was isolated as a colourless solid (41 mg, 50%). The residue was purified by preparative reverse phase HPLC. The chromatogram showed two unresolvable peaks of identical and correct *m/z* for the isomeric lactam (**7**, R=H) and lactone (**8**, R=H) (*t<sub>R</sub>* 35.8 min and *t<sub>R</sub>* 34.3 min). The residue was lyophilised for 18 h to give a mixture of the cyclic peptides as a colourless solid (5 mg, 6%). Mass spectrum (ESI<sup>+</sup>, MeCN/H<sub>2</sub>O): *m/z* 726.1 (M + Na)<sup>+</sup>, C<sub>36</sub>H<sub>45</sub>N<sub>7</sub>NaO<sub>8</sub>.

### Synthesis of *Cyclo*-[Gly-*c*[ $\Delta^4$ Das-Phe-Gly-Phe]-Gly]

The macrocyclisation reaction was carried out as per the above synthesis of *cyclo*-[Thr-*c*[ $\Delta^4$ Das-Phe-Gly-Phe]-Gly] (**6**) using H<sub>2</sub>N-Gly-*c*[ $\Delta^4$ Das-Phe-Gly-Phe]-Gly-OH (134 mg, 0.206 mmol), FDDP (127 mg, 0.330 mmol) and DIPEA (0.11 ml, 0.66 mmol). The desired peptide was isolated as a light brown solid (83 mg, 64%). Mass Spectrum (ESI<sup>+</sup>, MeCN/H<sub>2</sub>O): *m/z* 654.2 (M + Na)<sup>+</sup>, C<sub>32</sub>H<sub>37</sub>N<sub>7</sub>NaO<sub>7</sub>.

### Ring Opening of bicyclic peptides

#### Synthesis of *Cyclo*-[Phe- $\Delta^4$ Ahx-Thr-Gly- $\Delta^4$ Ahx-Phe-Gly] (**7**, R=Me)

A Fischer-Porter tube was loaded with substrate **6** (14 mg, 0.020 mmol), 2<sup>nd</sup> generation Grubbs' catalyst (4 mg, 0.004 mmol), DCM (~5 mL) and *cis*-2-butene (15 psi). The reaction mixture was stirred under an inert atmosphere at 50°C for 48 h. The reaction mixture was then concentrated under reduced pressure to obtain a brown solid (15 mg). The residue was purified by preparative reverse phase HPLC (*t<sub>R</sub>* 33.4 min). The residue was then lyophilised for 18 h to give the desired peptide **7** (R=Me) as a colourless solid (6.1 mg, 42%). <sup>1</sup>H NMR (DMSO/500MHz):  $\delta$  1.06, (d, *J* 6.0 Hz, 3H); 1.21, (bs, 1H); 1.55 (m, 4H); 2.08, (s, 6H); 2.90-3.19 (m, 6H), 3.77, (dd, 6.1, 16.2 Hz, 2H); 3.88-4.28, (m, 5H); 4.42-4.52 (m, 2H); 5.12-5.22 (m, 2H); 5.32-5.42 (m, 2H); 5.44-5.58 (m, 2H); 7.14-7.38 (m, 8H); 7.44 (d, *J* 6.1 Hz, 1H); 7.61 (d, 8.9 Hz, 1H); 8.12-8.22 (m, 4H); 8.35 (s, 1H); 8.45, (d, *J* 5.7 Hz, 1H). Mass spectrum (ESI<sup>+</sup>, MeCN/H<sub>2</sub>O): *m/z* 732.3 (M + H)<sup>+</sup>, C<sub>38</sub>H<sub>50</sub>N<sub>7</sub>O<sub>8</sub>.

### Synthesis of *Cyclo*-[H<sub>2</sub>N-Thr- $\Delta^4$ Ahx-Phe-Gly-Phe- $\Delta^4$ Ahx-Gly] (**8**, R=Me)

The reaction was carried out as above using *cyclo*-[Fmoc-Thr-*c*[ $\Delta^4$ Das-Phe-Gly-Phe]-Gly] (37 mg, 0.041 mmol), 2<sup>nd</sup> generation Grubbs' catalyst (7 mg, 0.008 mmol), DCM (~5 ml) and *cis*-2-butene (15 psi). The reaction mixture was then stirred under an inert atmosphere at 50°C for 48 h days before being concentrated under reduced pressure to give a brown solid (39 mg, 99%). The reaction mixture was stirred overnight with 20% piperidine (10 ml). The solvent was removed under reduced pressure to afford a brown solid (20 mg). The residue was purified by preparative reverse phase HPLC (*t<sub>R</sub>* 35.8 min). The residue was then lyophilised for 18 h to give the desired peptide (**8**, R=Me) as a colourless solid (8.5 mg, 28%). Mass spectrum (ESI<sup>+</sup>, MeCN/H<sub>2</sub>O): *m/z* 754.0 (M + Na)<sup>+</sup>, C<sub>38</sub>H<sub>49</sub>N<sub>7</sub>NaO<sub>8</sub>.

### Synthesis of *Cyclo*-[Phe- $\Delta^4$ App-Thr-Gly- $\Delta^4$ App-Phe-Gly] (**9**)

#### Method A

A Fischer-Porter tube was loaded with substrate **6** (20 mg, 0.03 mmol), 2<sup>nd</sup> generation Grubbs' catalyst (5 mg, 0.006 mmol), solvents (~3 ml DCM) and styrene (32 mg, 0.3mmol). The reaction mixture was stirred under an inert atmosphere at 50°C for 6 days. The reaction mixture was then concentrated under reduced pressure to obtain a brown solid (25 mg). The solid was dissolved in a minimum amount of DCM (~ 2 ml) and precipitated using diethyl ether (~30 ml). The isolated peptide was subjected to mass spectrometric analysis and showed only the presence of starting material **6**.

#### Method B

The crude peptide isolated from the synthesis of *cyclo*-[Phe- $\Delta^4$ Ahx-Thr-Gly- $\Delta^4$ Ahx-Phe-Gly] (**7**, R=Me) was subjected to cross metathesis with styrene. A Fischer-Porter tube was loaded with the reaction mixture **7** (30 mg, 0.041 mmol), 2<sup>nd</sup> generation Grubbs' catalyst (7 mg, 0.008 mmol), solvent (~3 ml DCM) and freshly distilled styrene (47  $\mu$ l, 0.41 mmol). The reaction mixture was stirred under an inert atmosphere at 50°C for 72 h. before being concentrated under reduced pressure to give a brown solid (27 mg). Mass spectral analysis showed the presence of the desired product **9** together with the bicyclic peptide **6** (1:3 ratio respectively). The residue was purified by preparative reverse phase HPLC (*t<sub>R</sub>* 42 min for **9**) and then lyophilised for 18 h to give the desired peptide **9** as a colourless solid. Mass spectrum (ESI<sup>+</sup>, MeCN/H<sub>2</sub>O): *m/z* 856.2 (M + H)<sup>+</sup>, C<sub>48</sub>H<sub>54</sub>NO<sub>8</sub>.

### *Hydrogenation of the cross metathesised peptide*

#### *General hydrogenation procedure*

A Fischer-Porter tube was charged with substrate (100 - 650 mg), dry deoxygenated solvent (5 - 10 ml) and Wilkinson's catalyst (~1 - 5 mg) in an inert (argon) environment. The pressure vessel was connected to a

hydrogenation manifold and purged three times using vacuum and argon flushing cycles, before being charged with hydrogen gas to the reported pressure. The reaction was stirred at room temperature for a reported period of time and the reaction was then terminated by venting the hydrogen gas and exposure to air.

#### **Synthesis of *Cyclo*-[Phe-App-Thr-Gly-App-Phe-Gly] (10)**

Crude *cyclo*-[Phe- $\Delta^4$ App-Thr-Gly- $\Delta^4$ App-Phe-Gly] **9** was hydrogenated without prior purification. The brown solid (100 mg, 0.116 mmol) was loaded into a Fischer-Porter tube with Wilkinson's catalyst (5 mg, 0.006 mmol), methanol (~3 ml) and hydrogen (90 psi). The reaction mixture was stirred at room temperature for 18 h. The reaction mixture was then concentrated under reduced pressure to obtain a brown solid (20 mg). The residue was purified by preparative reverse phase HPLC (gradient: 30%→40%→100% B:  $t_R$  30.7 min). The residue was then lyophilised for 18 h to give the desired peptide as a colourless solid. Mass spectrum (ESI<sup>+</sup>, MeCN/H<sub>2</sub>O):  $m/z$  860.2 (M + H)<sup>+</sup>, C<sub>48</sub>H<sub>58</sub>N<sub>7</sub>O<sub>8</sub>.





















