

Investigation of the ring-closing metathesis of peptides in water†

Cite this: *Org. Biomol. Chem.*, 2013, **11**, 630

Stephen A. Cochrane,‡ Zedu Huang‡ and John C. Vederas*

Received 2nd October 2012,
Accepted 21st November 2012

DOI: 10.1039/c2ob26938d

www.rsc.org/obc

A systematic study of the ring-closing metathesis (RCM) of unprotected oxytocin and crotalphine peptide analogues in water is reported. The replacement of cysteine with *S*-allyl cysteine enables RCM to proceed readily in water containing excess MgCl_2 with 30% *t*-BuOH as a co-solvent. The presence of the sulfur atom is vital for efficient aqueous RCM to occur, with non-sulfur containing analogues undergoing RCM in low yields.

Introduction

Disulfide bonds are common in naturally occurring peptides and often play an important role in their correct folding, stability and biological activity.^{1,2} Two examples are the mammalian hormone oxytocin (1), which controls mammary and uterine smooth muscle contraction,³ and the orally active analgesic crotalphine (2), isolated from the venom of the South American rattlesnake *Crotalus durissus terrificus* (Fig. 1).⁴ As disulfide bonds are susceptible to reduction to free thiols, which can result in scrambling of bridges and loss of activity, there is considerable interest in replacement of these moieties in peptides with more stable linkages.⁵ Ring-closing metathesis (RCM) in peptides^{6,7} allows for the replacement of disulfide bridges with a dicarba bridge. The resulting stapled peptides are more stable than the natural analogues and many

examples have been shown to retain their biological activity.⁷ Solution-phase RCM of peptides has been mostly limited to hydrophobic peptides in organic solvents.⁸ The RCM of resin bound peptides is an alternative to the solution phase process,^{7,9} but aggregation of the peptide chain with the resin or peptide complexation to the catalyst can hinder the reaction.¹⁰ The RCM of unprotected peptides in an aqueous environment is an attractive strategy. Peptides generally have high solubility in water, and performing these reactions in aqueous media could allow cyclisations to be performed that are not possible on-resin. A major drawback with this approach is the insolubility of commercially available ruthenium-based metathesis catalysts and the competing catalyst decomposition pathways in water.¹¹ It is possible to chemically modify ruthenium catalysts to solubilize them in water, however this requires a series of chemical steps to make appropriate ligands.¹²

Lipshutz and co-workers recently reported the use of aqueous micelles as media to achieve cross-metathesis (CM) and RCM on small organic molecules.¹³ Several common detergents including sodium dodecyl sulfate (SDS) and Triton X-100 were used at critical micelle concentrations to perform CM reactions using 2% Grubbs 2nd generation catalyst in moderate yields.^{13a} Davis and co-workers have also reported a novel system for aqueous CM.¹⁴ A mutant of the serine protease subtilisin *Bacillus lentus* containing *S*-allyl cysteine (Sac) at position 156 successfully underwent CM in high yields with a number of substrates using 200 equivalents Hoveyda–Grubbs 2nd generation catalyst (HGII) and 10 000 equivalents MgCl_2 in water with 30% *t*-BuOH as a co-solvent. The authors found that Mg^{2+} is required for success of this reaction, presumably to disrupt non-productive chelation between the catalyst and protein. They proposed that the sulfur atom in the allyl sulfide coordinates to the ruthenium centre of the catalyst and brings the reactive alkene moieties of the protein and substrate into close proximity, thereby enhancing the rate of reaction and

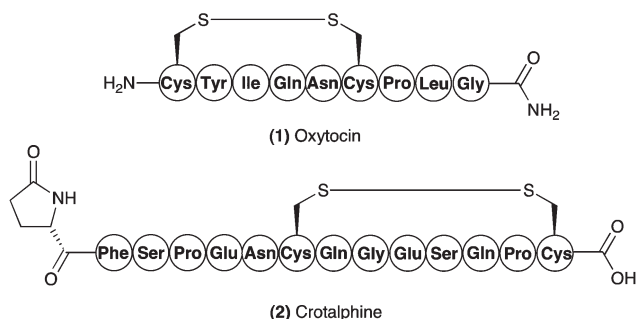


Fig. 1 Structures of oxytocin (1) and crotalphine (2).

Department of Chemistry, University of Alberta, Edmonton, Alberta, Canada
T6G 2G2. E-mail: john.vederas@ualberta.ca

†Electronic supplementary information (ESI) available: ¹H- and ¹³C-NMR spectra, MALDI spectra and HPLC traces. See DOI: 10.1039/c2ob26938d

‡Stephen A. Cochrane and Zedu Huang contributed equally to this project.

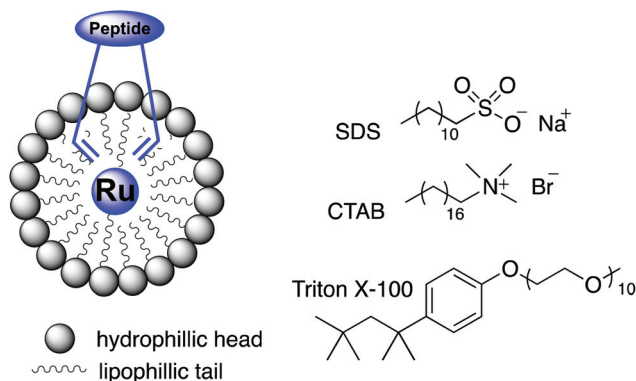


Fig. 2 Proposed RCM of peptide in a micellar system.

competing with non-productive chelation to the protein. This method was also used by Schultz and co-workers to perform RCM on a large protein bearing adjacent genetically encoded *O*-crotylserine residues.¹⁵

We now report our studies on the RCM of oxytocin **3a–e** and crotalphine **4a–e** analogues in water, using micelles or *t*-BuOH as an additive to solubilise the catalyst. The micellar systems can potentially protect the insoluble catalyst from decomposition by water by sequestration inside the micelle (Fig. 2). The alkene chains could then enter the lipophilic environment for RCM. Given that oxytocin (**1**) is positively charged and crotalphine (**2**) is negatively charged at neutral pH, polar head groups on the micelle could affect the RCM of these peptides. Three commercially available detergents were therefore selected for study at their critical micelle concentrations; (1) anionic SDS, (2) neutral Triton X-100 and (3) cationic cetyl trimethylammonium bromide (CTAB). In addition, 30% *t*-BuOH was used in a non-micellar solvent system. The Hoveyda–Grubbs 2nd generation catalyst was chosen for these reactions as it displays superior air and moisture stability compared to other commercially available catalysts. All peptides were synthesised using standard Fmoc solid-phase peptide synthesis (SPPS). Several variants were selected to test the influence of chain length and of heteroatoms in the alkene-bearing side chain (Fig. 3). The replacement of cysteines in oxytocin and crotalphine with allylglycine (Agl) affords analogues that have sulfur replaced directly with CH upon cyclization.^{7a,b} *S*-Allylcysteine (Sac) and *O*-allylserine (Oas) containing peptides allow catalyst coordination effects to be probed.^{14,15} Pentenylglycine (Pgl) incorporation can probe the effect of chain length.

Results and discussion

The synthesis of Fmoc-protected alkene containing amino acids was required for the SPPS of the oxytocin and crotalphine analogues. Asymmetric synthesis of Fmoc-Pgl-OH was accomplished using the chiral Ni(II) Schiff-base method reported by Belokon *et al.*,¹⁶ Fmoc-Oas-OH was synthesised from commercially available Boc-Ser-OH using a previously reported literature protocol.¹⁷ Fmoc-Sac-OH was obtained by

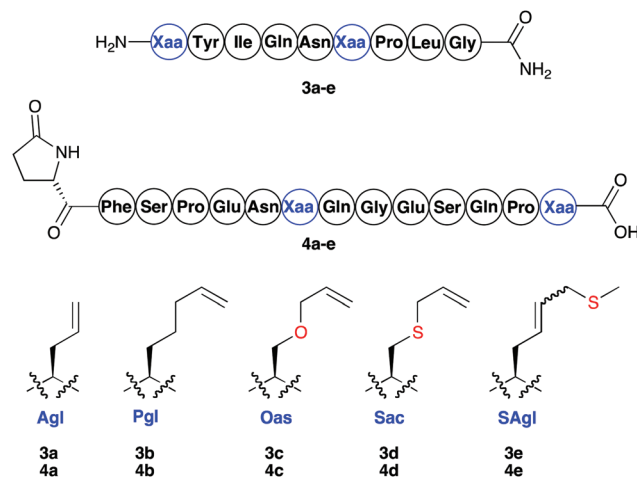


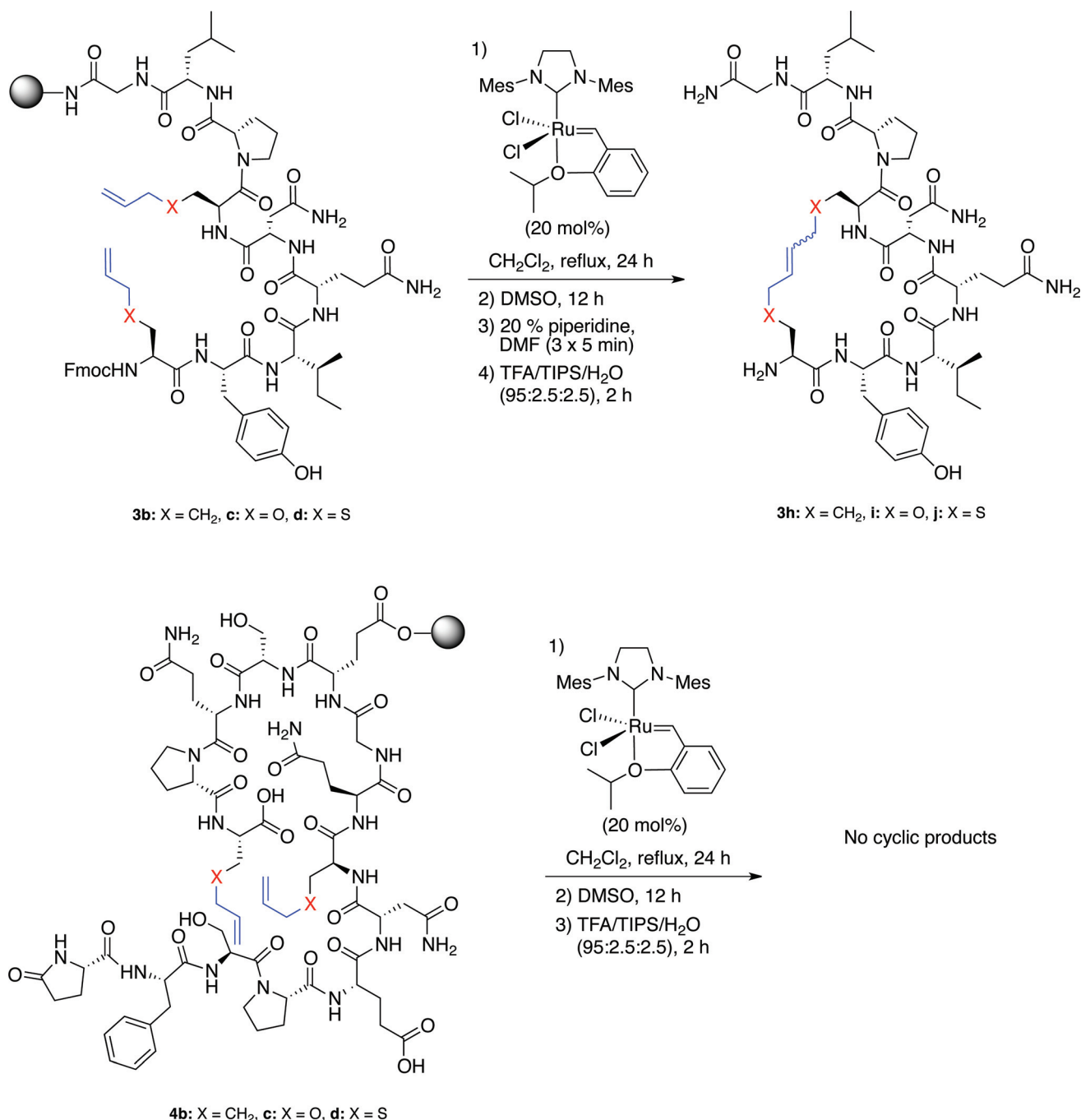
Fig. 3 Linear oxytocin analogues **3a–e** and crotalphine analogues **4a–e**.

protection of commercially available *S*-allylcysteine using a standard Fmoc-protection procedure.¹⁸

The RCM of oxytocin and crotalphine analogues were first performed on-resin for comparison with the latter aqueous reactions (Scheme 1). The resin bound Fmoc-protected peptides were refluxed in CH₂Cl₂ with 20 mol% HGII for 24 hours, followed by the addition of DMSO (50 equiv. relative to the catalyst loading) and stirred for a further 12 hours at room temperature.^{7b} Deprotection of the Fmoc-carbamate, acidic cleavage from resin and RP-HPLC purification were then performed. The RCM of resin bound Agl oxytocin analogue **3a** proceeded in 50% yield. The 1:1 mixture of *cis*- and *trans*-isomers were separable by RP-HPLC. The resin bound Pgl, Oas and Sac oxytocin analogues **3b–d** underwent complete conversion to the desired cyclic products as an inseparable mixture of *cis*-*trans* isomers. Attempts to cyclize the crotalphine analogues on-resin were unsuccessful, presumably due to complexation between the catalyst and amide backbone and/or hydrophobic protecting groups of the peptides.¹⁰

Our efforts were then directed towards the RCM of fully unprotected oxytocin and crotalphine analogues in water. RCM reactions failed to proceed for any analogues when CTAB or Triton X-100 were used as additives. MALDI analysis showed some product formation in the 8.2 mM SDS system for the Pgl, Oas and Sac analogues, however product was not successfully isolated from this system. We therefore directed our attention to the use of 30% *t*-BuOH as a co-solvent.

We first focused on the RCM of the Agl oxytocin analogue **3a** and Agl crotalphine analogue **4a** as the resulting olefin bridge is an isostere of a disulfide-bond. Initial attempts to perform RCM on these analogues using stoichiometric amounts of HGII at 37 °C were unsuccessful. Performing the reaction at higher temperatures (up to 80 °C) had no effect on the reaction. The loading of HGII was then increased to 50 equiv. but MALDI analysis after 24 hours revealed only the formation of ruthenium-peptide adducts. Davis and co-workers observed a similar problem in their cross-metathesis reactions on proteins, which was circumvented by adding a



Scheme 1 Method for the on-resin RCM of peptide analogues.

large excess of MgCl_2 (10 000 equiv.) to disrupt non-productive chelation.¹⁴ Even with the addition of 10 000 equiv. MgCl_2 no reaction occurred in our case.

To determine if the failure of AgI analogues to cyclize in aqueous media is due to side chain length, the Pgl oxytocin analogue **3b** and Pgl crotalphine analogue **4b** were examined. Cyclic products were detected by MALDI when the reactions were performed in the *t*-BuOH system using 50 equiv. HGII however the isolated yields were typically <1%. Lower loadings of HGII gave no product and higher loadings did not increase

the yield. The major products were ruthenium-peptide adducts and addition of excess MgCl_2 had little effect on disrupting the non-productive chelation. For the Pgl oxytocin analogue **3b**, trace amounts of a side-product 14 Da higher in mass than the RCM product were detected. Comparison of the LC MS/MS spectra of the isolated +14 Da side-product with a synthetic standard (**3k**) showed that a piperidine ring had been formed at the N-terminus, possibly by a hydroamination reaction between the N-terminal amine and a ruthenium carbene on residue 1 (Fig. 4).

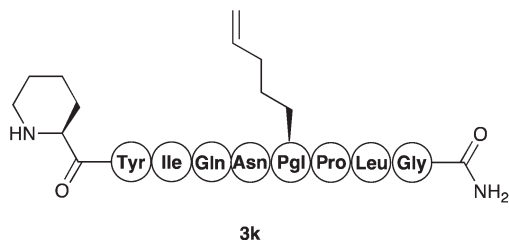


Fig. 4 1-Pip-6-Pgl-oxytocin (**3k**).

The role of a heteroatom within the alkene side-chain was studied by examining the Oas and Sac analogues. The Oas oxytocin analogue **3c** and Oas crotalphine analogue **4c** displayed similar reactivity to the Pgl analogues. A +14 Da side product was also detected in the RCM of Oas oxytocin analogue **3c**, presumably analogous to Pgl oxytocin side-product **3k**. The peptide analogues containing *S*-allylcysteine underwent remarkably faster and cleaner reactions than all the other analogues (Scheme 2). The Sac crotalphine analogue **4d** underwent RCM with 50 mol% HGII in 63% isolated yield. It is important to note that this reaction failed on-resin. Initial attempts to cyclize Sac oxytocin analogue **3d** failed because it decomposed rapidly upon exposure to HGII. When the N-terminus was protected as the Fmoc-carbamate, Sac oxytocin underwent complete conversion (78% isolated yield) to the desired cyclic product in 3 hours using 50 mol% HGII. The enhanced reactivity of *S*-allyl containing peptides is in full agreement with the results of Davis and co-workers. The ability to perform these cyclisations using sub-stoichiometric amounts of HGII allows the cost-effective synthesis of valuable biologically relevant peptide analogues in milligram quantities.

The enhanced reactivity of the sulfur containing analogues led us to synthesize linear peptides **3e** and **4e**. Fmoc-SAgI-OH (**7**) was synthesised from commercially available Fmoc-L-Agl-OH in 3 steps (Scheme 3). Protection as a methyl ester was necessary for effective purification in the subsequent step. Cross-metathesis between **5** and allyl methyl sulfide was performed, followed by an ester hydrolysis using Pascal's conditions,¹⁹ yielding Fmoc-SAgI-OH (**7**). The unnatural amino acid **7** is analogous to *S*-allyl cysteine with the positions of the sulfur and alkene switched. Performing RCM on these analogues would yield the same cyclic products as the RCM of **3a** and **4a** respectively. We hoped the sacrificial allyl sulfide within **7** would facilitate aqueous RCM through coordination between the ruthenium in the catalyst and sulfur on the side chain of **7**. Attempts to perform the RCM of resin bound SAgI oxytocin analogue **3e** and SAgI crotalphine analogue **4e** failed with only the linear starting materials recovered. The aqueous reactions were also unsuccessful. Analysis of the MALDI spectra after 24 hours revealed only ruthenium-peptide adducts, suggesting that the chain length of the alkene side chain is also an important factor in aqueous RCM. This is highlighted by comparison of the reactivity of the AgI, Pgl and Oas analogues. AgI analogues **3a** and **4a** also formed

ruthenium-peptide adducts but failed to cyclize, whereas both the Pgl and Oas analogues underwent RCM, albeit in low yields, in the *t*-BuOH system.

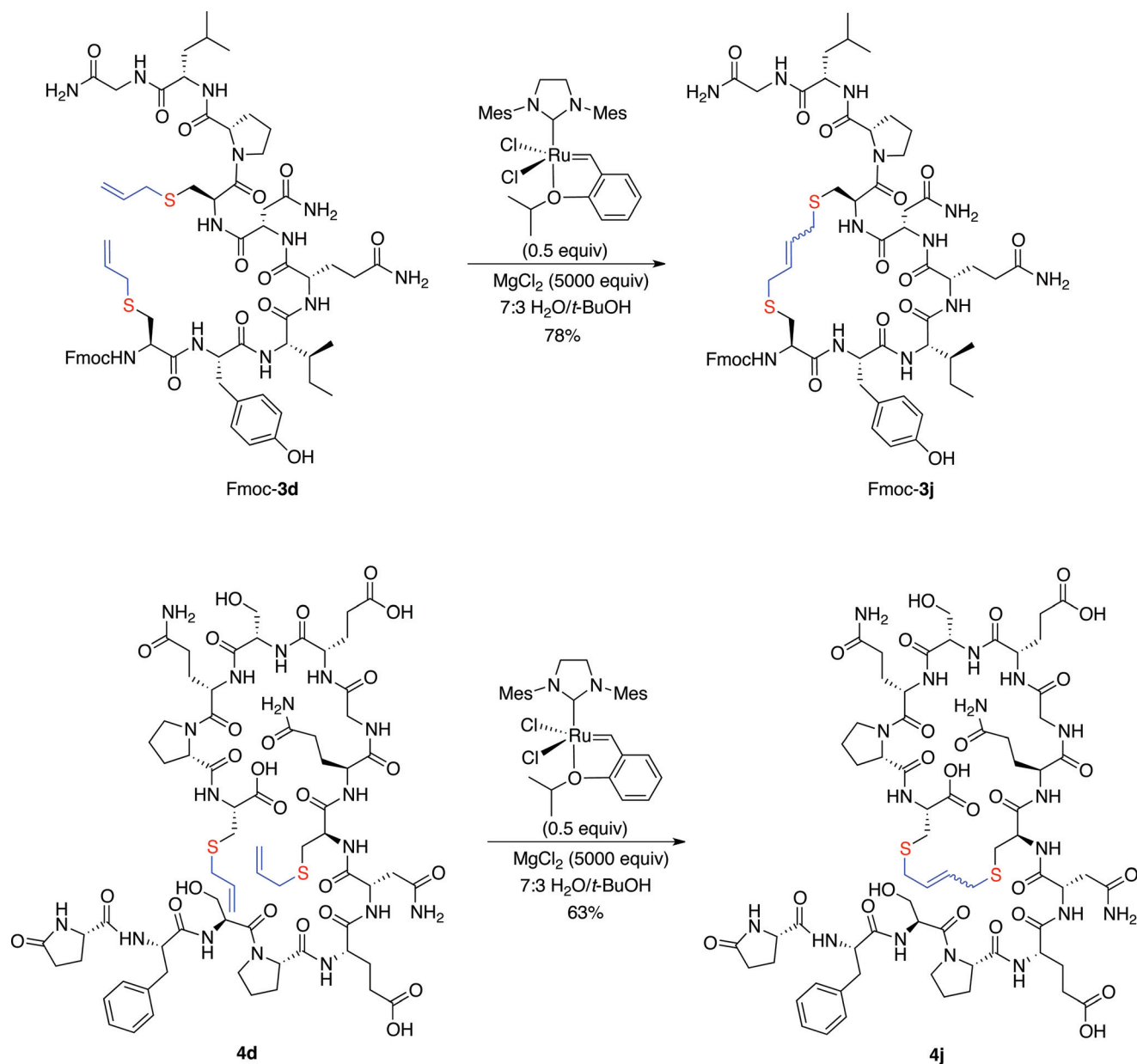
Conclusions

The RCM of oxytocin analogues containing allylglycine, pentenylglycine, *O*-allylserine or *S*-allylcysteine can be achieved in good to excellent yields on-resin. None of the crotalphine analogues could be cyclized by this method. *S*-Allylcysteine analogues of oxytocin and crotalphine also undergo efficient RCM in water containing excess MgCl₂ and 30% *t*-BuOH as a co-solvent. These solution-phase RCM reactions took place on peptides containing unprotected hydroxyl, carboxyl and primary amide moieties. The addition of MgCl₂ proved vital to disrupt non-productive chelation between the resulting ruthenium-carbene and these coordinating heteroatoms. The sulfur atom within *S*-allylcysteine drastically improved the efficiency of RCM compared to pentenylglycine and *O*-allylserine analogues. This methodology provides an alternative to the on-resin cyclization of *S*-allylcysteine, which is particularly attractive in instances where on-resin cyclization is not possible.

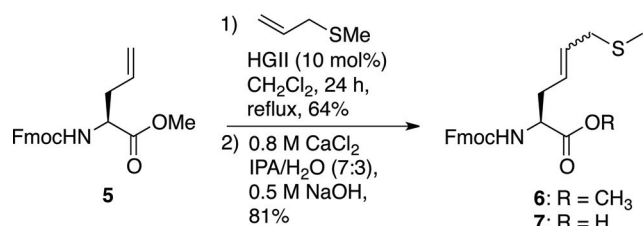
Experimental section

General information

NMR spectra were recorded on a Varian Inova 600, Inova 500, Inova 400, or Unity 500 spectrometer. For ¹H NMR spectra, δ values were referenced to CDCl₃ (7.26 ppm), D₂O (4.79 ppm), (CD₃)₂SO (2.50 ppm), and for ¹³C NMR spectra, δ values were referenced to CDCl₃ (77.0 ppm), (CD₃)₂SO (39.5 ppm) as the solvents. Infrared spectra (IR) were recorded on a Nicolet Magna 750 FT-IR or Nic-Plan FT-IR Microscope spectrometers. Cast refers to the evaporation of a solution on a NaCl plate. Mass spectra (MS) were recorded on an Agilent Technologies 6220 oaTOF (HR-ES) or on a Perspective Biosystems VoyagerTM Elite MALDI-TOF (LR-MALDI), or on Bruker 9.4T Apex-Qe FTICR (HR-MALDI), or on Waters (Micromass) Q-TOF Premier (LC-MS/MS). 4-Hydroxy- α -cyanocinnamic acid (HCCA) was used as matrix. Optical rotations were measured on a Perkin Elmer 241 polarimeter with a microcell (10 cm, 1 mL) at ambient temperature. All commercially available reagents and protected amino acids were purchased and used without further purification. All the solvents used for reactions were distilled over appropriate drying reagents prior to use. Commercially available ACS grade solvents (>99.0% purity) were used for column chromatography without any further purification. All reactions and fractions from column chromatography were monitored by thin layer chromatography (TLC) using glass plates with a UV fluorescent indicator (normal SiO₂, Merck 60 F₂₅₄). Following method was used for visualization: UV absorption by fluorescence quenching, staining with phosphomolybdic acid in ethanol (10 g/100 mL) or staining with Ninhydrin (Ninhydrin:acetic acid:*n*-butanol/0.6 g:6



Scheme 2 Aqueous RCM of Fmoc-1,6-Sac-oxytocin (Fmoc-3d) and 7,14-Sac-crotalphine (4d) in the *t*-BuOH system.



Scheme 3 Synthesis of Fmoc-SAgl-OH (7).

mL:200 mL) spray. Flash chromatography was performed using Merck type 60, 230–400 mesh silica gel. High Performance Liquid Chromatography (HPLC) was performed on a Gilson Preparative HPLC system equipped with a model 322

HPLC pump, GX-271 liquid handler, 156 UV/vis detector and a 10 mL sample loop, or a Gilson Analytical HPLC system equipped with a 322 HPLC pump, FC 203B fraction collector, 171 diode array detector and a 100 to 1000 μ L sample loop, or a Beckman System Gold chromatograph equipped with a model 166 variable wavelength UV detector and a Rheodyne 7225i injector fitted with a 100 to 1000 μ L sample loop. The columns used were Vydac C₁₈ (5 μ m, 4.6 \times 250 mm), Vydac C₈ (5 μ m, 4.6 \times 250 mm), Vydac C₁₈ (5 μ m, 10 \times 250 mm), Phenomenex C₁₈ (5 μ m, 21.2 \times 250 mm) and Zorbax C₈ (21.2 \times 250 mm). All HPLC solvents were filtered with a Millipore filtration system under vacuum before use.

(*S*)-Methyl-2-(((9*H*-fluoren-9-yl)methoxy)carbonylamino)-6-(methylthio)hex-4-enoate (6). Fmoc-L-Agl-OMe (5) (1.00 g,

2.85 mmol) was added to a flame-dried flask under a blanket of argon and dissolved in dry CH_2Cl_2 (12 mL, 0.25 M). Allyl methyl sulfide (0.62 mL, 5.70 mmol) was added, followed by the addition of Hoveyda–Grubbs 2nd generation catalyst (182 mg, 10 mol%) in parts to avoid excessive effervescence. The resulting green solution was refluxed under argon for 24 hours, concentrated *in vacuo* and purified by column chromatography (silica gel, 7:1 hexanes–EtOAc) to yield methyl ester **6** as a yellow oil and mixture of *E/Z* isomers (750 mg, 64%). $[\alpha]_{\text{D}}^{+21.97^\circ}$ (*c* 0.83, CH_2Cl_2); IR (KBr disc) 3335, 3065, 2951, 1722, 1520 cm^{-1} ; ^1H NMR (CDCl_3 , 500 MHz): δ 7.77 (d, 2H, *J* = 7.5, Fmoc-H), 7.6 (m, 2H, Fmoc-H), 7.40 (app. t, 2H, *J* = 7.5, Fmoc-H), 7.32 (app. t, 2H, *J* = 7.5, Fmoc-H), 5.55–5.36 (m, 2H, $\text{RCH}=\text{CHR}'$), 5.32 (d, 1H, *J* = 8.0 Hz, NH), 4.48–4.44 (m, 1H, Fmoc- CHCH_2), 4.40 (d, 2H, *J* = 7.1 Hz, Fmoc- CHCH), 4.23 (t, *J* = 7.0 Hz, 1H, H_α), 3.76–3.74 (m, 3H, OCH_3), 3.11–3.02 (m, 2H, H_β), 2.64–2.49 (m, 2H, H_β), 2.04–2.00 (m, 3H, SCH_3); ^{13}C NMR (CDCl_3 , 125 MHz): δ 172.2, 155.7, 143.9, 143.8, 141.3, 130.9, 127.7, 127.1, 126.3, 125.1, 120.0, 67.1, 53.5, 52.5, 47.2, 35.7, 35.3, 30.0, 14.4; HRMS (ES) Calcd for $\text{C}_{23}\text{H}_{25}\text{NO}_4\text{SNa}$ [$\text{M} + \text{Na}$] $^+$ 434.1385, found 434.1385.

(S)-2-(((9H-Fluoren-9-yl)methoxy)carbonylamino)-6-(methylthio)hex-4-enoic acid (Fmoc-X-OH) (7). Methyl ester **6** (680 mg, 1.65 mmol) was dissolved in CH_2Cl_2 (2 mL), *i*-PrOH (22.5 mL) and water (10.5 mL). $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ (4.11 mg, 28.0 mmol) was then added and the reaction mixture stirred until all solids had dissolved. 0.5 M NaOH (6.8 mL) was added and the resulting suspension stirred for 75 min at room temperature. The reaction mixture was concentrated to remove *i*-PrOH, acidified to pH 1 with 6 M HCl (~25 drops) and washed with EtOAc (2 \times 40 mL). The EtOAc extracts were combined, dried over anhydrous Na_2SO_4 , filtered and concentrated *in vacuo* to yield the Fmoc amino acid **7** as a white solid (530 mg, 81%). $[\alpha]_{\text{D}}^{+13.31^\circ}$ (*c* 0.40, CH_2Cl_2); IR (KBr disc) 3313, 3065, 2916, 1721, 1523 cm^{-1} ; ^1H NMR (CDCl_3 , 400 MHz): δ 7.77 (d, 2H, *J* = 7.5, Fmoc-H), 7.6 (m, 2H, Fmoc-H), 7.40 (app. t, 2H, *J* = 7.3, Fmoc-H), 7.32 (app. t, 2H, *J* = 7.4, Fmoc-H), 5.62–5.40 (m, 2H, $\text{RCH}=\text{CHR}'$), 5.30 (d, 1H, *J* = 8.0 Hz, NH), 4.57–4.40 (m, 3H, Fmoc- CHCH_2 , Fmoc- CHCH_2), 4.23 (t, *J* = 6.6 Hz, 1H, H_α), 3.13–3.04 (m, 2H, H_β), 2.62–2.53 (m, 2H, H_β), 2.04–1.98 (m, 3H, SCH_3); ^{13}C NMR (CDCl_3 , 125 MHz): δ 175.8, 155.9, 143.8, 143.7, 141.3, 131.2, 127.8, 127.1, 126.0, 125.1, 120.0, 67.2, 53.3, 47.1, 35.7, 35.0, 14.4; HRMS (ES) Calcd for $\text{C}_{22}\text{H}_{23}\text{NO}_4\text{SNa}$ [$\text{M} + \text{Na}$] $^+$ 420.1240, found 420.1233.

Peptide syntheses

All peptides were synthesized on a CEM Liberty 1 Microwave Peptide Synthesizer. Solid phase synthesis was carried out on a 0.1 mmol scale using Fmoc chemistry on Rink Amide NovaGelTM resin (0.63 mmol g^{-1} loading) for oxytocin analogues, 2-chlorotrityl resin (0.80 mmol g^{-1} loading) for 7,14-Sac-crotalphine and 7,14-SAgI-crotalphine, and Wang resin (0.65 mmol g^{-1} loading) for the remaining crotalphine analogues. Commercially available asymmetrically protected amino acids and synthesized amino acids were loaded on the peptide synthesizer as 0.2 M DMF solutions. All amino acid

subunits were coupled using *O*-benzotriazole-*N,N,N',N'*-tetramethyl-uronium-hexafluoro-phosphate (HBTU) as the activating agent and heated at 70 $^\circ\text{C}$ (50 $^\circ\text{C}$ for cysteines) for a 5 min coupling time. Fmoc residues were deprotected using a 20% solution of piperidine in DMF and the dibenzofulvene adduct absorption monitored at 301 nm using a UV monitor.

General procedure for cleavage and purification of peptides

To simultaneously cleave the peptide from resin and remove side chain protecting groups a solution of 95 : 2.5 : 2.5 trifluoroacetic acid (TFA) : triisopropylsilane (TIPS) : H_2O was added to the resin-bound peptide for 2 hours. The resin beads were removed *via* filtration through glass wool and the filtrate was concentrated *in vacuo*. The crude peptide was obtained by precipitation with cold Et_2O . The crude peptide was then dissolved in 10 mL water and purified by HPLC. The following HPLC methods were used for purification. Oxytocin analogues prep-scale (Method A): Zorbax C_8 , flow rate 10 mL min^{-1} , detected at 220 nm. Gradient: starting from 10% MeCN (0.1% TFA) and 90% water (0.1% TFA) for 5 min, ramping up to 55% MeCN over 20 min, staying at 55% MeCN for 10 min, ramping up to 95% MeCN over 4 min, staying at 95% MeCN for 1 min, ramping down to 10% MeCN over 2 min and staying at 10% MeCN for 3 min. Crotalphine analogues prep-scale (Method B): Phenomenex C_{18} column, flow rate 10 mL min^{-1} , detected at 220 nm. Gradient: starting from 5% MeCN (0.1% TFA) and 95% water (0.1% TFA) for 5 min, ramping up to 40% MeCN over 25 min, then ramping up to 95% MeCN over 5 min, staying at 95% MeCN for 5 min, ramping down to 5% MeCN over 1 min, then staying at 5% MeCN for 5 min. Oxytocin analogues analytical-scale (Method C): Analytical C_{18} column, flow rate 1.0 mL min^{-1} , detected at 220 nm. Gradient: starting from 10% MeCN (0.1% TFA) and 90% water (0.1% TFA) for 5 min, ramping up to 55% MeCN over 20 min, staying at 55% MeCN for 10 min, ramping up to 95% MeCN over 4 min, ramping down to 10% MeCN over 2 min and staying at 10% MeCN for 1 min. Crotalphine analogues prep-scale (Method D): Vydac C_{18} column, flow rate 1.0 mL min^{-1} , detected at 220 nm. Gradient: starting from 5% MeCN (0.1% TFA) and 95% water (0.1% TFA) for 5 min, ramping up to 40% MeCN over 25 min, then ramping up to 95% MeCN over 5 min, staying at 95% MeCN for 5 min, ramping down to 5% MeCN over 1 min, then staying at 5% MeCN for 5 min. Semi-Prep method (Method E): Vydac C_{18} (5 μm , 10 \times 250 mm) 5.0 mL min^{-1} , detected at 220 nm. Gradient: starting from 10% MeCN (0.1% TFA) and 90% water (0.1% TFA) for 5 min, ramping up to 40% MeCN over 20 min, ramping up to 95% MeCN over 4 min, staying at 95% MeCN for 1 min, ramping down to 10% MeCN over 2 min and staying at 10% MeCN for 3 min. The peptide was collected, concentrated *in vacuo* to remove acetonitrile and lyophilized to give the final product.

Procedure for loading amino acids onto 2-chlorotrityl resin

In a manual SPPS vessel 2-chlorotrityl resin (190 mg, 0.15 mmol) was bubbled with argon in CH_2Cl_2 for 15 min. The resin was then filtered and transferred to a screw-top vial.

Diisopropylethyl amine (DIPEA) (0.21 mL, 1.2 mmol) and Fmoc-L-Sac-OH (206 mg, 0.6 mmol) were dissolved in CH_2Cl_2 (3 mL) and added to the resin. The resulting slurry was shaken for 2.5 hours, filtered and washed with CH_2Cl_2 (3×10 mL). The resin was then capped by bubbling with argon in MeOH-DIPEA- CH_2Cl_2 (10:5:85) for 15 min. The solution was removed by filtration and the resin then washed with DMF (3×10 mL) and CH_2Cl_2 (3×10 mL) and dried under argon.

Procedure for loading amino acids onto Wang resin

A 3-necked round bottom flask equipped with a stirring bar was flame dried and cooled in a desiccator under argon. The amino acid (10.0 equiv.) was dissolved in dry CH_2Cl_2 and cooled to 0 °C. Diisopropylcarbodiimide (DIC) (5.0 equiv.) was added and the reaction was stirred at 0 °C for 20 min. The reaction mixture was then concentrated *in vacuo* and re-dissolved in DMF (10 mL). Wang resin (1.0 equiv.) was added to a manual SPPS vessel and washed with CH_2Cl_2 (2×5 mL) and DMF (5 mL). The resin was pre-swollen by bubbling with argon in DMF (5 mL) for 1 hour and filtered. The activated anhydride solution was added to the resin followed by 4-dimethylaminopyridine (DMAP) (0.1 equiv.) and bubbled with argon for 2 hours. The solvent was removed by filtration and the resin washed with DMF (3×5 mL). The resin was then capped by bubbling with argon in 20% acetic anhydride in DMF (10 mL) for 15 min and filtered. The resin was washed with DMF (3×5 mL) and CH_2Cl_2 (3×5 mL) and dried under argon.

1,6-Agl-oxytocin (3a). Peptide was isolated as a single peak using C_8 RP-HPLC Method A (16 mg, 16%). Retention time (Method C) = 15.8 min. ^1H NMR (D_2O , 500 MHz): δ 7.10 (d, 2H, J = 8.0 Hz, Tyr Ar-H), 6.81 (d, 2H, J = 8.0 Hz, Tyr Ar-H), 5.84–5.62 (m, 2H, $\text{CH}=\text{CH}_2$), 5.28–5.12 (m, 4H, $\text{CH}=\text{CH}_2$), 4.70–4.60 (m, 3H), 4.44–4.38 (m, 1H), 4.33–4.26 (m, 1H), 4.21–4.19 (m, 1H), 4.08–4.02 (m, 2H), 3.88 (app. q, 2H, J = 14.4 Hz), 3.80–3.72 (m, 1H), 3.70–3.62 (m, 1H), 3.00–2.90 (m, 2H), 2.78 (dd, 1H, J = 15.7, 5.2 Hz), 2.70 (dd, 1H, J = 15.7, 8.1 Hz), 2.64–2.60 (m, 2H), 2.55–2.50 (m, 1H), 2.43–2.36 (m, 2H), 2.32 (t, 1H, J = 7.7 Hz), 2.28–2.23 (m, 2H), 2.08–1.86 (m, 7H), 1.76–1.56 (m, 6H), 1.46–1.36 (m, 1H), 1.16–1.14 (m, 1H), 0.92–0.80 (m, 12H). MW calculated for $\text{C}_{47}\text{H}_{73}\text{N}_{12}\text{O}_{12}$ 997.5465, found *high resolution* (FTICR-ESI-MS) 997.5461 ($\text{M} + \text{H}$) $^+$.

1,6-Pgl-oxytocin (3b). Peptide was isolated as a single peak using C_8 RP-HPLC Method A (16 mg, 16%). Retention time (Method C) = 19.0 min. ^1H NMR (D_2O , 500 MHz): δ 7.20 (d, 2H, J = 8.1 Hz, Tyr Ar-H), 6.90 (d, 2H, J = 8.2 Hz, Tyr Ar-H), 6.00–5.82 (m, 2H, $\text{CH}=\text{CH}_2$), 5.14–5.06 (m, 4H, $\text{CH}=\text{CH}_2$), 4.77–4.71 (m, 2H), 4.68–4.62 (m, 1H), 4.48 (t, 1H, J = 7.0 Hz), 4.42–4.35 (m, 1H), 4.25 (t, 1H, J = 7.3 Hz), 4.12 (d, 1H, J = 8.7 Hz), 4.06 (t, 1H, J = 6.4 Hz), 3.96 (app. q, 2H, J = 15.6 Hz), 3.90–3.82 (m, 1H), 3.75–3.68 (m, 1H), 3.10–2.00 (m, 2H), 2.88 (dd, 1H, J = 15.5, 5.7 Hz), 2.80 (dd, 1H, J = 15.5, 8.2 Hz), 2.40 (t, 2H, J = 7.8 Hz), 2.37–2.31 (m, 1H), 2.28–1.40 (m, 27 H), 1.22–1.14 (m, 1H), 1.16–0.87 (m, 12H). MW calculated for $\text{C}_{51}\text{H}_{81}\text{N}_{12}\text{O}_{12}$ 1053.6078, found *high resolution* (FTICR-ESI-MS) 1053.6086 ($\text{M} + \text{H}$) $^+$.

1,6-Oas-oxytocin (3c). Peptide was isolated as a single peak using C_8 RP-HPLC Method A (15 mg, 15%). Retention time (Method C) = 18.1 min. ^1H NMR (D_2O , 500 MHz): δ 7.10 (d, 2H, J = 8.2 Hz, Tyr Ar-H), 6.81 (d, 2H, J = 8.3 Hz, Tyr Ar-H), 5.94–5.82 (m, 2H, $\text{CH}=\text{CH}_2$), 5.35–5.22 (m, 4H, $\text{CH}=\text{CH}_2$), 4.84 (app. t, 1H, J = 5.7 Hz), 4.70 (dd, 1H, J = 7.9, 5.8 Hz), 4.66 (app. t, 1H, J = 7.8 Hz), 4.41 (dd, 1H, J = 8.4, 6.0 Hz), 4.34–4.29 (m, 1H), 4.24–4.39 (m, 2H), 4.08–4.00 (m, 5H), 3.94–3.75 (m, 7H), 3.72–3.66 (m, 2H), 2.98 (d, 1H, J = 7.6 Hz), 2.80 (dd, 1H, J = 15.5, 5.5 Hz), 2.72 (dd, 2H, J = 15.4, 8.0 Hz), 2.33 (app. t, 2H, J = 7.8 Hz), 2.30–2.24 (m, 1H), 2.08–1.90 (m, 6H), 1.78–1.58 (m, 5H), 1.44–1.36 (m, 1H), 1.14–1.04 (m, 1H), 0.92–0.80 (m, 12H). MW calculated for $\text{C}_{49}\text{H}_{77}\text{N}_{12}\text{O}_{14}$ 1057.5677, found *high resolution* (FTICR-ESI-MS) 1057.5675 ($\text{M} + \text{H}$) $^+$.

1,6-Sac-oxytocin (3d). Peptide was isolated as a single peak using C_8 RP-HPLC Method A (15 mg, 14%). Retention time (Method C) = 18.6 min. ^1H NMR (D_2O , 500 MHz): δ 7.02 (d, 2H, J = 8.4 Hz, Tyr Ar-H), 6.82 (d, 2H, J = 8.2 Hz, Tyr Ar-H), 5.76–5.86 (m, 2H, $\text{CH}=\text{CH}_2$), 5.14–5.24 (m, 4H, $\text{CH}=\text{CH}_2$), 4.78 (t, 1H, J = 7.2 Hz), 4.70–4.64 (m, 2H), 4.41 (dd, 1H, J = 8.3, 5.5 Hz), 4.34–4.29 (m, 1H), 4.21 (dd, 1H, J = 9.2, 5.4 Hz), 4.15 (t, 1H, J = 6.4 Hz), 4.04 (d, 1H, J = 8.6 Hz), 3.89 (app. q, 2H, J = 21.3 Hz), 3.80–3.72 (m, 2H), 3.22 (d, 2H, J = 7.1 Hz), 3.18 (d, 2H, J = 7.1 Hz), 3.00 (d, 2H, J = 7.7 Hz), 2.97–2.92 (m, 3H), 2.83–2.77 (m, 1H), 2.78–2.66 (m, 2H), 2.34 (t, 2H, J = 7.6 Hz), 2.31–2.34 (m, 1H), 2.10–1.92 (m, 6H), 1.78–1.58 (m, 5H), 1.42–1.36 (m, 1H), 1.14–1.04 (m, 1H), 0.98–0.80 (m, 12H). MW calculated for $\text{C}_{49}\text{H}_{77}\text{N}_{12}\text{O}_{12}\text{S}_2$ 1089.5220, found *high resolution* (FTICR-ESI-MS) 1089.5217 ($\text{M} + \text{H}$) $^+$.

Fmoc-1,6-Sac-oxytocin (3d'). Peptide was isolated as a single peak using C_8 RP-HPLC Method A (4 mg, 3%). Retention time (Method C) = 27.2 min. MW calculated for $\text{C}_{64}\text{H}_{87}\text{N}_{12}\text{O}_{14}\text{S}_2$ 1311.5906, found *high resolution* (FTICR-ESI-MS) 1311.5918 ($\text{M} + \text{H}$) $^+$.

Fmoc-1,6-Sagl-oxytocin (3e). Peptide was isolated as a single peak using C_{18} RP-HPLC Method E (4.5 mg, 0.03 mmol scale, 13%). Retention time (Method C) = 19.4 min. ^1H NMR (D_2O , 600 MHz): δ 7.10 (d, 2H, J = 8.7 Hz, Tyr Ar-H), 6.81 (d, 2H, J = 8.5 Hz, Tyr Ar-H), 5.14–5.24 (m, 4H, $\text{CRH}=\text{CR'H}$), 4.68–4.60 (m, 2H), 4.41–4.39 (m, 1H), 4.32–4.29 (m, 1H), 4.23–4.20 (m, 1H), 4.07–4.05 (m, 2H), 3.89 (app. q, 2H, J = 15.8 Hz), 3.77–3.62 (m, 2H), 3.13–3.08 (m, 2H), 2.97 (d, 2H, J = 7.7 Hz), 2.82–2.50 (m, 6H), 2.43–2.40 (m, 1H), 2.35–2.32 (m, 2H), 2.28–2.23 (m, 1H), 2.06–1.89 (m, 7H), 1.73–1.59 (m, 4H), 1.45–1.41 (m, 1H), 1.14–1.08 (m, 1H), 0.98–0.80 (m, 12H). MW calculated for $\text{C}_{51}\text{H}_{80}\text{N}_{12}\text{O}_{12}\text{S}_2$ 1117.5538, found *high resolution* (HRMS-ESI-MS) 1117.5533 ($\text{M} + \text{H}$) $^+$.

7,14-Agl-crotalphine (4a). Peptide was isolated as a single peak using C_{18} RP-HPLC Method B (20 mg, 13%). Retention time (Method D) = 20.9 min. ^1H NMR (D_2O , 600 MHz): δ 7.37–7.23 (m, 5H, Phe-ArH), 5.84–5.71 (m, 2H, $\text{CH}=\text{CH}_2$), 5.22–5.10 (m, 4H, $\text{CH}=\text{CH}_2$), 4.73–4.68 (m, 1H), 4.65 (dd, 1H, J = 9.1, 4.9 Hz), 4.46–4.24 (m, 9H), 3.94 (s, 2H), 3.86–3.72 (m, 5H), 3.72–3.62 (m, 3H), 3.16 (dd, 1H, J = 13.8, 6.6 Hz), 3.01 (dd, 1H, J = 13.8, 9.0 Hz), 2.84 (dd, 1H, J = 16.2, 7.8 Hz), 2.75 (dd, 1H, J = 15.6, 7.2 Hz), 2.64–1.72 (m, 33H). MW calculated for

$C_{66}H_{94}N_{17}O_{25}$ 1524.6601, found *high resolution* (FTICR-ESI-MS) 1524.6591 ($M + H$)⁺.

7,14-Pgl-crotalphine (4b). Peptide was isolated as a single peak using C_{18} RP-HPLC Method B (15 mg, 10%). Retention time (Method D) = 25.3 min. 1H NMR (D_2O , 600 MHz): δ 7.37–7.23 (m, 5H, Phe-ArH), 5.90–5.80 (m, 2H, $CH=CH_2$), 5.08–4.95 (m, 4H, $CH=CH_2$), 4.78–4.60 (m, 3H), 4.45–4.37 (m, 4H), 4.30–4.23 (m, 4H), 3.96–3.64 (m, 9H), 3.17 (dd, 1H, J = 13.8, 6.6 Hz), 3.01 (dd, 1H, J = 13.8, 9.0 Hz), 2.86 (dd, 1H, J = 15.6, 6.6 Hz), 2.76 (dd, 1H, J = 15.6, 7.2 Hz), 2.48–1.66 (m, 35H), 1.50–1.36 (m, 4H). MW calculated for $C_{70}H_{102}N_{17}O_{25}$ 1580.7227, found *high resolution* (FTICR-ESI-MS) 1580.7229 ($M + H$)⁺.

7,14-Oas-crotalphine (4c). Peptide was isolated as a single peak using C_{18} RP-HPLC Method B (21 mg, 13%). Retention time (Method D) = 21.9 min. 1H NMR (D_2O , 600 MHz): δ 7.32–7.18 (m, 5H, Phe-ArH), 5.90–5.80 (m, 2H, $CH=CH_2$), 5.28–5.14 (m, 4H, $CH=CH_2$), 4.72–4.64 (m, 2H), 4.62–4.58 (m, 1H), 4.53 (t, 1H, J = 4.2 Hz), 4.41 (t, 1H, J = 5.4 Hz), 4.46–4.36 (m, 3H), 4.36–4.30 (m, 1H), 4.28–4.26 (m, 1H), 4.20 (dd, 1H, J = 9.0, 4.2 Hz), 4.06–3.60 (m, 18H), 3.01 (dd, 1H, J = 13.8, 6.6 Hz), 2.95 (dd, 1H, J = 13.8, 9.0 Hz), 2.81 (dd, 1H, J = 16.2, 6.6 Hz), 2.73 (dd, 1H, J = 16.2, 7.2 Hz), 2.49–1.70 (m, 27H), 1.67–1.75 (m, 1H). MW calculated for $C_{68}H_{98}N_{17}O_{27}$ 1584.6813, found *high resolution* (FTICR-ESI-MS) 1584.6831 ($M + H$)⁺.

7,14-Sac-crotalphine (4d). Peptide was isolated as a single peak using C_{18} RP-HPLC Method B (20 mg, 12%). Retention time (Method D) = 24.1 min. 1H NMR (D_2O , 600 MHz): δ 7.38–7.24 (m, 5H, Phe-ArH), 5.85–5.75 (m, 2H, $CH=CH_2$), 5.21–5.13 (m, 4H, $CH=CH_2$), 4.68–4.66 (m, 1H), 4.54–4.20 (m, 5H), 4.39 (t, 1H, J = 6.0 Hz), 4.31 (dd, 2H, J = 4.8, 3.6 Hz), 4.25 (dd, 1H, J = 9.6, 4.8 Hz), 3.98–3.66 (m, 10H), 3.19 (dd, 4H, J = 18.0, 7.2 Hz), 3.16–3.70 (m, 8H), 2.52–1.74 (m, 27H), 1.80–1.75 (m, 1H). MW calculated for $C_{68}H_{98}N_{17}O_{25}S_2$ 1616.6356, found *high resolution* (FTICR-ESI-MS) 1616.6342 ($M + H$)⁺.

7,14-Sagl-crotalphine (4e). Peptide was isolated as a single peak using C_{18} RP-HPLC Method B (8 mg, 0.03 mmol scale, 12%). Retention time (Method D) = 25.3 min. 1H NMR (D_2O , 600 MHz): δ 7.36–7.24 (m, 5H, Phe-ArH), 5.63–5.49 (m, 4H, $RCH=CHR'$), 4.71 (t, 1H, J = 6.7 Hz), 4.66 (dd, 1H, J = 9.0, 4.8 Hz), 4.45–4.42 (m, 2H), 4.41–4.37 (m, 2H), 4.34–4.28 (m, 3H), 4.25 (dd, 1H, J = 9.3, 4.7 Hz), 3.92–3.96 (m, 2H), 3.85–3.64 (m, 7H), 3.16 (dd, 1H, J = 13.8, 7.0 Hz), 3.09 (dd, 2H, J = 15.3, 6.9 Hz), 3.01 (dd, 1H, J = 13.8, 9.0 Hz), 2.84 (dd, 1H, J = 15.8, 6.1 Hz), 2.76 (dd, 1H, J = 15.8, 7.6 Hz), 2.60–1.90 (m, 35H), 1.79–1.75 (m, 1H). MW calculated for $C_{70}H_{99}N_{17}O_{25}S_2$ 820.8225, found *high resolution* (HRMS-ESI-MS) 820.8222 ($M - 2H$)^{−2}.

General procedure for RCM on-resin

To a flame-dried 10 mL round-bottomed flask equipped with a magnetic stirring bar and flushed with argon was added linear Fmoc-1,6-Sac-oxytocin on rink amide resin (110 mg, 0.03 mmol). The resin was suspended in CH_2Cl_2 (5 mL) and Hoveyda–Grubbs 2nd generation catalyst (4.4 mg, 20 mol%) was added. The resulting suspension was refluxed with gentle stirring under argon for 24 hours and cooled to room

temperature. DMSO (0.02 mL) was added and the reaction stirred for a further 12 hours. The resin was then filtered through a manual SPPS vessel and washed successively with methanol and CH_2Cl_2 . A small amount of resin was cleaved using TFA–TIPS– H_2O (95 : 2.5 : 2.5) to confirm cyclization had occurred. Fmoc-deprotection was performed by bubbling the resin in a solution of 20% piperidine in DMF (3×10 mL \times 5 min) with argon. The resin was washed with DMF (3×10 mL), DCM (3×10 mL) and dried under argon. The peptide was then cleaved from resin using TFA–TIPS– H_2O (95 : 2.5 : 2.5), filtered, concentrated *in vacuo* and precipitated with cold ether. The crude peptide was dissolved in water (4 mL) and acetonitrile (2 mL) and purified by RP-HPLC (Method E).

General procedure for RCM in the *t*-BuOH system

Fmoc-1,6-Sac-oxytocin **3d'** (1.0 mg, 0.8 μ mol) and $MgCl_2 \cdot 6H_2O$ (813 mg, 4.0 mmol) were dissolved in Milli-Q H_2O (7.0 mL) and stirred at room temperature. A solution of Hoveyda–Grubbs 2nd Generation Catalyst (0.25 mg, 0.4 μ mol) in *t*-BuOH (3.0 mL) was then added and the resulting solution stirred at 37 °C. The reaction progress was monitored by MALDI and after 3 hours the starting material had been completely consumed. The product was then purified by prep-scale C_8 RP-HPLC using Method A. The product containing fractions were pooled, concentrated and lyophilized to yield cyclized Fmoc-1,6-Sac-oxytocin as a fluffy white powder (0.8 mg, 78%).

General procedure for RCM in the micellar system

Hoveyda–Grubbs 2nd Generation Catalyst (12.5 mg, 20.0 μ mol) was suspended in 8.2 mM SDS (10.0 mL) and stirred for 10 min. 1,6-Oas-oxytocin (1.0 mg, 1.0 μ mol) and $MgCl_2 \cdot 6H_2O$ (1.0 g, 5.0 mmol) were then added and the resulting green micellar solution stirred at 37 °C for 24 hours. The reaction mixture was washed with CH_2Cl_2 (3×10 mL) and the resulting colourless solution was diluted with Milli-Q water (10.0 mL). The reaction mixture was passed through a prep-scale C_8 RP-HPLC using Method A.

Cyclized *cis*-1,6-Agl-oxytocin (3f). The stereochemistry was determined to be *cis* by comparison of the alkene splitting pattern with previously published spectra.^{7a} Peptide was isolated as a single peak using C_{18} RP-HPLC Method E (0.7 mg, 0.03 mmol scale, 2.4% overall yield). Retention time (Method C) = 15.5 min. 1H NMR (D_2O , 600 MHz): δ 7.20 (d, 2H, J = 8.4 Hz, Tyr Ar-H), 6.9 (d, 2H, J = 8.4 Hz, Tyr Ar-H), 5.68–5.64 (m, 1H, $RCH=CHR'$), 5.60–5.56 (m, 1H, $RCH=CHR'$), 4.64–4.62 (m, 1H), 4.44–4.42 (m, 2H), 4.31–4.26 (m, 3H), 4.08–4.04 (m, 2H), 3.90 (m, 2H), 3.73–3.60 (m, 2H), 3.17 (dd, 1H, J = 14.4, 6.1 Hz), 3.00 (m, dd, 1H, J = 14.3, 8.6 Hz), 2.87–2.71 (m, 6H), 2.53–2.47 (m, 2H), 2.43–2.26 (m, 6H), 2.09–1.89 (m, 9H), 1.70–1.60 (m, 5H), 1.27–1.20 (m, 1H), 0.94–0.87 (m, 12H). MW calculated for $C_{45}H_{69}N_{12}O_{12}$ 969.5152, found *high resolution* (FTICR-ESI-MS) 969.5144 ($M + H$)⁺.

Cyclized *trans*-1,6-Agl-oxytocin (3g). The stereochemistry was determined to be *trans* by comparison of the alkene splitting pattern with previously published spectra.^{7a} Peptide was

isolated as a single peak using C_{18} RP-HPLC Method E (0.8 mg, 0.03 mmol scale, 2.8% overall yield). Retention time (Method C) = 16.0 min. ^1H NMR (D_2O , 600 MHz): δ 7.21 (d, 2H, J = 8.5 Hz, Tyr Ar-H), 6.9 (d, 2H, J = 8.4 Hz, Tyr Ar-H), 5.65 (dt, 1H, J = 15.1, 7.4 Hz, $\text{RCH}=\text{CHR}'$), 5.53 (dt, 1H, J = 14.7, 7.4 Hz, $\text{RCH}=\text{CHR}'$), 4.71–4.68 (m, 1H), 4.64–4.62 (m, 1H), 4.44–4.41 (m, 1H), 4.31–4.28 (m, 1H), 4.13–4.10 (m, 2H), 4.00 (d, 1H, J = 6.2 Hz), 3.90 (app. q, 2H, J = 17.6 Hz), 3.73–3.60 (m, 2H), 3.14 (dd, 1H, J = 14.4, 7.1 Hz), 3.04–2.95 (m, 2H), 2.79–2.71 (m, 2H), 2.64–2.59 (m, 1H), 2.55–2.50 (m, 1H), 2.45–2.27 (m, 6H), 2.11–1.98 (m, 5H), 1.92–1.85 (m, 2H), 1.70–1.60 (m, 5H), 1.39–1.34 (m, 1H), 1.16–1.13 (m, 1H), 0.94–0.87 (m, 12H). MW calculated for $\text{C}_{45}\text{H}_{69}\text{N}_{12}\text{O}_{12}$ 969.5153, found *high resolution* (FTICR-ESI-MS) 969.5144 ($\text{M} + \text{H}$) $^+$.

Cyclized 1,6-Pgl-oxytocin (3h). Peptide was isolated as a mixture of isomers using C_8 RP-HPLC Method A (1.5 mg, 0.03 mmol scale, 5% overall yield). Retention time (Method C) = 16.9 min. ^1H NMR (D_2O , 600 MHz): δ 7.20 (d, 2H, J = 8.6 Hz, Tyr Ar-H), 6.84 (d, 2H, J = 8.6 Hz, Tyr Ar-H), 5.53–5.49 (m, 1H, $\text{RCH}=\text{CHR}'$), 5.45–5.40 (m, 1H, $\text{RCH}=\text{CHR}'$), 4.89–5.87 (m, 1H), 4.52–4.50 (m, 1H), 4.44–4.42 (m, 1H), 4.29–4.27 (m, 1H), 4.15–4.13 (m, 1H), 3.97–3.81 (m, 6H), 3.67–3.62 (m, 1H), 3.10 (dd, 1H, J = 14.3, 6.3 Hz), 3.04 (dd, 1H, J = 16.8, 4.1 Hz), 2.92 (dd, 1H, J = 14.2, 9.0 Hz), 2.78 (dd, 1H, J = 16.2, 10.2 Hz), 2.42–2.24 (m, 4H), 2.08–1.53 (m, 27 H), 1.50–1.40 (m, 2H), 1.29–1.21 (m, 2H), 0.93–0.87 (m, 12H). MW calculated for $\text{C}_{49}\text{H}_{77}\text{N}_{12}\text{O}_{12}$ 1025.5778, found *high resolution* (FTICR-ESI-MS) 1025.5781 ($\text{M} + \text{H}$) $^+$.

Cyclized 1,6-Oas-oxytocin (3i). Peptide was isolated as a mixture of isomers using C_8 RP-HPLC Method A (2.5 mg, 0.03 mmol scale, 8% overall yield). Retention time (Method C) = 16.6 min. ^1H NMR (D_2O , 600 MHz): δ 7.20 (d, 2H, J = 8.5 Hz, Tyr Ar-H), 6.85 (d, 2H, J = 8.4 Hz, Tyr Ar-H), 5.77–5.70 (m, 2H, $\text{RCH}=\text{CHR}'$), 4.85–4.80 (m, 2H), 4.46–4.43 (m, 1H), 4.31–4.28 (m, 2H), 4.16–3.97 (m, 9H), 3.93–3.69 (m, 9H), 3.15 (dd, 1H, J = 14.5, 5.6 Hz), 3.00–2.92 (m, 2H), 2.82 (dd, 1H, J = 16.0, 10.0 Hz), 2.43–2.27 (m, 5H), 2.06–1.91 (m, 6H), 1.68–1.59 (m, 5H), 1.42–1.39 (m, 1H), 1.22–1.15 (m, 1H), 0.94–0.82 (m, 12H). MW calculated for $\text{C}_{47}\text{H}_{73}\text{N}_{12}\text{O}_{14}$ 1029.5364, found *high resolution* (FTICR-ESI-MS) 1029.5360 ($\text{M} + \text{H}$) $^+$.

Cyclized 1,6-Sac-oxytocin (3j). Peptide was isolated as a mixture of isomers using C_8 RP-HPLC Method A (2 mg, 0.03 mmol scale, 6%). Retention time (Method C) = 18.0 min. ^1H NMR (D_2O , 600 MHz): δ 7.21 (d, 2H, J = 8.5 Hz, Tyr Ar-H), 6.86 (d, 2H, J = 8.6 Hz, Tyr Ar-H), 5.66–5.60 (m, 2H, $\text{RCH}=\text{CHR}'$), 4.84–4.80 (m, 1H), 4.46 (dd, 1H, J = 8.6, 5.2 Hz), 4.32 (dd, 1H, J = 10.0, 4.5 Hz), 4.16 (t, 1H, J = 6.1 Hz), 4.12 (dd, 1H, J = 8.5, 5.4 Hz), 4.03 (d, 1H, J = 6.0 Hz), 3.90 (app. q, 2H, J = 21.5 Hz), 3.82–3.72 (m, 2H), 3.26–3.14 (m, 4H), 3.12–3.08 (m, 1H), 3.01–2.92 (m, 5H), 2.83–2.76 (m, 2H), 2.41–2.26 (m, 4H), 2.07–1.89 (m, 8H), 1.87–1.81 (m, 1H), 1.71–1.59 (m, 4H), 1.42–1.35 (m, 1H), 1.21–1.15 (m, 1H), 0.95–0.86 (m, 12H). MW calculated for $\text{C}_{47}\text{H}_{73}\text{N}_{12}\text{O}_{12}\text{S}_2$ 1061.4907, found *high resolution* (FTICR-ESI-MS) 1061.4905 ($\text{M} + \text{H}$) $^+$.

Cyclized Fmoc-1,6-Sac-oxytocin (3j'). Peptide was isolated as mixture of isomers C_8 RP-HPLC Method A. Retention time

(Method C) = 25.6 min. MW calculated for $\text{C}_{62}\text{H}_{83}\text{N}_{12}\text{O}_{14}\text{S}_2$ 1283.5588, found *high resolution* (FTICR-ESI-MS) 1283.5593 ($\text{M} + \text{H}$) $^+$.

1-Pip-6-Pgl-oxytocin (3k). Peptide was isolated as a single peak using C_8 RP-HPLC Method A (4 mg, 4%). This was used as a standard to confirm the identity of the hydroamination side-product by LC MS/MS comparison. Retention time (Method C) = 17.4 min. ^1H NMR (D_2O , 500 MHz): δ 7.10 (d, 2H, J = 8.3 Hz, Tyr Ar-H), 6.80 (d, 2H, J = 8.3 Hz, Tyr Ar-H), 5.85 (ddt, 1H, J = 17.1, 10.4, 6.6 Hz, $\text{CH}=\text{CH}_2$), 5.06 (d, 1H, J = 17.3, $\text{CH}=\text{CHH}$), 5.00 (d, 1H, J = 10.3, $\text{CH}=\text{CHH}$), 4.66 (dd, 1H, J = 7.8, 6.0 Hz), 4.62 (t, 1H, J = 7.8 Hz), 4.58 (dd, 1H, J = 8.9, 4.9 Hz), 4.40 (dd, 1H, J = 8.1, 5.9 Hz), 4.29 (dd, 1H, J = 9.5, 4.9 Hz), 4.20 (t, 1H, J = 7.3 Hz), 4.06 (d, 1H, J = 8.6 Hz), 3.94–3.82 (m, 4H), 3.80–3.75 (m, 1H), 3.66–3.60 (m, 1H), 3.44 (d, 1H, J = 12.7 Hz), 3.02 (td, 1H, J = 12.4, 2.1 Hz), 2.97 (d, 2H, 7.8 Hz), 2.79 (dd, 1H, J = 15.5, 5.8 Hz), 2.71 (dd, 1H, J = 15.5, 8.0 Hz), 2.37–2.22 (m, 4H), 2.19–2.13 (m, 1H), 2.11–1.84 (m, 11 H), 1.80–1.56 (m, 10H), 1.51–1.37 (m, 3H), 1.16–1.08 (m, 1H), 0.96–0.79 (m, 12H). MW calculated for $\text{C}_{50}\text{H}_{79}\text{N}_{12}\text{O}_{12}$ 1039.5935, found *high resolution* (FTICR-ESI-MS) 1039.5931 ($\text{M} + \text{H}$) $^+$.

Cyclized 7,14-Pgl-crotalphine (4h). Peptide was isolated as a mixture of isomers peak using C_{18} RP-HPLC Method B. Retention time (Method D) = 20.8 min. MW calculated for $\text{C}_{68}\text{H}_{98}\text{N}_{17}\text{O}_{25}$ 1552.6901, found *high resolution* (FTICR-ESI-MS) 1552.6916 ($\text{M} + \text{H}$) $^+$.

Cyclized 7,14-Oas-crotalphine (4i). Peptide was isolated as a mixture of isomers using C_{18} RP-HPLC Method B. Retention time (Method D) = 18.6 min. MW calculated for $\text{C}_{66}\text{H}_{94}\text{N}_{17}\text{O}_{27}$ 1556.6500, found *high resolution* (FTICR-ESI-MS) 1556.6479 ($\text{M} + \text{H}$) $^+$.

Cyclized 7,14-Sac-crotalphine (4j). Peptide was isolated as a mixture of isomers using C_{18} RP-HPLC Method B. Retention time (Method D) = 17.7 min. ^1H NMR (D_2O , 500 MHz): δ 7.37–7.22 (m, 5H, Phe-ArH), 5.63–5.54 (m, 2H, $\text{RCH}=\text{CHR}'$), 4.55–4.38 (m, 6H), 4.33–4.27 (m, 1H), 4.25 (dd, 1H, J = 9.3, 4.7 Hz), 4.05–4.00 (m, 1H), 3.98–3.66 (m, 9H), 3.16 (dd, 2H, J = 14.1, 6.5 Hz), 3.05–2.75 (m, 7H), 2.50–1.90 (m, 25H), 1.81–1.75 (m, 1H). MW calculated for $\text{C}_{66}\text{H}_{94}\text{N}_{17}\text{O}_{25}\text{S}_2$ 1588.6043, found *high resolution* (FTICR-ESI-MS) 1588.6053 ($\text{M} + \text{H}$) $^+$.

Acknowledgements

We thank Dr Randy Whittal, Jing Zheng and Bela Reiz for their assistance with LC MS/MS and FTICR-ESI-MS experiments. These investigations were supported by the Natural Sciences and Engineering Research Council of Canada (NSERC) and the Canada Research Chair in Bioorganic and Medicinal Chemistry (J. C. V.).

Notes and references

- (a) K. J. Woycechowsky and R. T. Raines, *Curr. Opin. Chem. Biol.*, 2000, **4**, 533–539; (b) N. L. Daly, K. J. Rosengren and

- D. J. Craik, *Adv. Drug Delivery Rev.*, 2009, **61**, 918–930; (c) A. Walewska, J. J. Skalicky, D. R. Davis, M. M. Zhang, E. Lopez-Vera, M. Watkins, T. S. Han, D. Yoshikami, B. M. Olivera and G. Bulaj, *J. Am. Chem. Soc.*, 2008, **130**, 14280–14286; (d) A. M. Almeida, R. Li and S. H. Gellman, *J. Am. Chem. Soc.*, 2012, **134**, 75–78.
- 2 For an example see: H. Liu, M. A. Boudreau, J. Zheng, R. M. Whittall, P. Austin, C. D. Roskelley, M. Roberge, R. J. Andersen and J. C. Vederas, *J. Am. Chem. Soc.*, 2010, **132**, 1486–1487.
- 3 (a) A. L. Lehninger, D. L. Nelson and M. Cox, *Principles of Biochemistry*, Worth Publishers, Inc., New York, 2nd edn, 1993, pp. 750–752; (b) S. C. Sweetman, *Martindale: The Complete Drug Reference*, Pharmaceutical Press, London, 34th edn, 2004.
- 4 (a) V. P. Gutierrez, V. O. Zambelli, G. Picolo, M. Chacur, S. C. Sampaio, P. Brigatte, K. Konno and Y. Cury, *Behav. Pharmacol.*, 2012, **23**, 14–24; (b) K. Konno, G. Picolo, V. P. Gutierrez, P. Brigatte, V. O. Zambelli, A. C. M. Camargo and Y. Cury, *Peptides*, 2008, **29**, 1293–1304; (c) Z. Huang, D. J. Derksen and J. C. Vederas, *Org. Lett.*, 2010, **12**, 2282–2285.
- 5 (a) P. J. Knerr, A. Tzekou, D. Ricklin, H. Qu, H. Chen, W. A. van der Donk and J. D. Lambris, *ACS Chem. Biol.*, 2011, **6**, 753–760 and references therein (b) J. B. Binder and R. T. Raines, *Curr. Opin. Chem. Biol.*, 2008, **12**, 767–773; (c) R. M. J. Liskamp, D. T. S. Rijkers, J. A. W. Kruijtzter and J. Kemmink, *ChemBioChem*, 2011, **12**, 1626–1653; (d) K. Holland-Nell and M. Meldal, *Angew. Chem., Int. Ed.*, 2011, **50**, 5204–5206; (e) J.-D. Malcor, N. Payrot, M. David, A. Faucon, K. Abouzid, G. Jacquot, N. Floquet, F. Debarbieux, G. Rougon, J. Martinez, M. Khrestchatsky, P. Vlieghe and V. Lisowski, *J. Med. Chem.*, 2012, **55**, 2227–2241; (f) A. D. de Araujo, M. Mobli, G. F. King and P. F. Alewood, *Angew. Chem., Int. Ed.*, 2012, **51**, 10298–10302; (g) Z. Dekan, I. Vetter, N. L. Daly, D. J. Craik, R. J. Lewis and P. F. Alewood, *J. Am. Chem. Soc.*, 2011, **133**, 15866–15869.
- 6 For some early reports, see: (a) S. J. Miller, H. E. Blackwell and R. H. Grubbs, *J. Am. Chem. Soc.*, 1996, **118**, 9606–9614; (b) R. M. Williams and J. Liu, *J. Org. Chem.*, 1998, **63**, 2130–2132; (c) Y. Gao, P. Lane-Bell and J. C. Vederas, *J. Org. Chem.*, 1998, **63**, 2133–2143.
- 7 (a) J. L. Stymiest, B. F. Mitchell, S. Wong and J. C. Vederas, *Org. Lett.*, 2003, **5**, 47–49; (b) J. L. Stymiest, B. F. Mitchell, S. Wong and J. C. Vederas, *J. Org. Chem.*, 2005, **70**, 7799–7809; (c) M. A. Hossain, K. J. Rosengren, S. Zhang, R. A. D. Bathgate, G. W. Tregear, B. J. van Lierop, A. J. Robinson and J. D. Wade, *Org. Biomol. Chem.*, 2009, **7**, 1547–1553; (d) H. Andersson, H. Demaegdt, A. Johnsson, G. Vauquelin, G. Lindeberg, M. Hallberg, M. Érdélyi, A. Karlén and A. Hallberg, *J. Med. Chem.*, 2011, **54**, 3779–3792; (e) M. P. C. Mulder, J. A. W. Kruijtzter, E. J. Breukink, J. Kemmink, R. J. Pieters and R. M. J. Liskamp, *Bioorg. Med. Chem.*, 2011, **19**, 6505–6517; (f) M. I. García-Aranda, P. Marrero, B. Gautier, M. Martín-Martínez, N. Inguimbert, M. Vidal, M. T. García-López, M. A. Jiménez, R. González-Muñiz and M. Pérez de Vega, *J. Bioorg. Med. Chem.*, 2011, **19**, 1978–1986; (g) M. J. Pérez de Vega, M. I. García-Aranda and R. González-Muñiz, *Med. Res. Rev.*, 2011, **31**, 677–715.
- 8 (a) L. K. Henchey, A. L. Jochim and P. S. Arora, *Curr. Opin. Chem. Biol.*, 2008, **12**, 692–697; (b) J. F. Reichwein, C. Versluis and R. M. J. Liskamp, *J. Org. Chem.*, 2000, **65**, 6187–6195.
- 9 (a) C. E. Schafmeister, J. Po and G. L. Verdine, *J. Am. Chem. Soc.*, 2000, **122**, 5891–5892; (b) N. Schmiedeberg and H. Kessler, *Org. Lett.*, 2002, **4**, 59–62; (c) E. R. Jarvo, G. T. Copeland, N. Papaioannou, P. J. Bonitatebus Jr. and S. J. Miller, *J. Am. Chem. Soc.*, 1999, **121**, 11638–11643; (d) A. Baron, P. Verdić, J. Martinez and F. J. Lamaty, *Org. Chem.*, 2011, **76**, 766–772; (e) A. M. Heapy, G. M. Williams, J. D. Fraser and M. A. Brimble, *Org. Lett.*, 2012, **14**, 878–881.
- 10 D. J. Derksen, J. L. Stymiest and J. C. Vederas, *J. Am. Chem. Soc.*, 2006, **128**, 14252–14253.
- 11 (a) M. B. Dinger and J. C. Mol, *Organometallics*, 2003, **22**, 1089–1095; (b) M. B. Dinger and J. C. Mol, *Eur. J. Inorg. Chem.*, 2003, 2827–2823.
- 12 For some examples of water-soluble ruthenium catalysts: (a) D. M. Lynn, B. Mohr, R. H. Grubbs, L. M. Henling and M. W. Day, *J. Am. Chem. Soc.*, 2000, **122**, 6601–6609; (b) S. H. Hong and R. H. Grubbs, *J. Am. Chem. Soc.*, 2006, **128**, 3508–3509; (c) J. P. Jordan and R. H. Grubbs, *Angew. Chem., Int. Ed.*, 2007, **46**, 5152–5155.
- 13 (a) B. H. Lipshutz, G. T. Aguinaldo, S. Ghorai and K. Voigtritter, *Org. Lett.*, 2008, **10**, 1325–1328; (b) B. H. Lipshutz, S. Ghorai, W. W. Y. Leong and B. R. Taft, *J. Org. Chem.*, 2011, **76**, 5061–5073.
- 14 (a) Y. A. Lin, J. M. Chalker, N. Floyd, G. J. L. Bernardes and B. G. Davis, *J. Am. Chem. Soc.*, 2008, **130**, 9642–9643; (b) Y. A. Lin, J. M. Chalker and B. G. Davis, *ChemBioChem*, 2009, **10**, 959–969; (c) Y. A. Lin, J. M. Chalker and B. G. Davis, *J. Am. Chem. Soc.*, 2010, **132**, 16805–16811.
- 15 H. Ai, W. Shen, E. Brustad and P. G. Schultz, *Angew. Chem., Int. Ed.*, 2010, **49**, 935–937.
- 16 Y. N. Belokon, V. I. Tararov, V. I. Maleev, T. F. Savel'eva and M. G. Ryzhov, *Tetrahedron: Asymmetry*, 1998, **9**, 4249–5252.
- 17 A. K. Boal, I. Guryanov, A. Moretto, M. Crisma, E. L. Lanni, C. Toniolo, R. H. Grubbs and D. J. O'Leary, *J. Am. Chem. Soc.*, 2007, **129**, 6986–6987.
- 18 See ESI†
- 19 R. Pascal and R. Sola, *Tetrahedron Lett.*, 1998, **39**, 5031–5034.