# **Supporting Information**

# **Ring-Closing Metathesis of Peptides in Water**

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Synthesis of Fmoc-L-Sac-OH



*S*-allyl-L-cysteine (Sac) (2.00 g, 12.4 mmol) and NaHCO<sub>3</sub> (3.13 g, 37.2 mmol) were dissolved in water (60 mL) and stirred at room temperature. A solution of FmocOSu (4.59 g, 13.6 mmol) in THF (60 mL) was then added to the aqueous solution and stirring was continued for an additional 2 hours at room temperature. The reaction mixture was acidified to pH 1 with 6 M HCl and extracted with EtOAc (4 x 30 mL). The organic phase was dried over anhydrous sodium sulfate, filtered, concentrated *in vacuo* and purified by flash chromatography (silica gel, 97:2:1 CH<sub>2</sub>Cl<sub>2</sub> : *i*-PrOH : AcOH), yielding Fmoc-L-Sac-OH as a white solid (1.30 g, 27%). [ $\alpha$ ]<sub>D</sub> -12.2° (*c* 1.00, CH<sub>2</sub>Cl<sub>2</sub>); IR (CHCl<sub>3</sub> cast) 3325, 3065, 1685, 1532 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 600 MHz):  $\delta$  7.77 (d, 2H, *J* = 7.6 Hz, Fmoc-H), 7.62-7.60 (m, 2H, Fmoc-H), 7.40 (t, 2H, *J* = 7.4 Hz, Fmoc-H), 7.31 (t, 2H,

J = 7.5 Hz, Fmoc-H), 5.76-5.73 (m, 1H, C<u>H</u>=CH<sub>2</sub>), 5.64 (d, 1H, J = 7.8 Hz, NH), 5.13 (m, 2H, CH=C<u>H<sub>2</sub></u>), 4.60-4.66 (m, 1H, Cys-H<sub>a</sub>), 4.44-4.46 (m, 2H, Fmoc CHC<u>H<sub>2</sub></u>), 4.24 (1H, t, J = 7.1 Hz, Fmoc C<u>H</u>CH<sub>2</sub>), 3.15 (2H, d, J = 7.1 Hz, C<u>H<sub>2</sub></u>CH=CH<sub>2</sub>), 3.04 (dd, 1H, J = 14.2, 4.5, Cys-H<sub>a</sub>), 2.94 (dd, 1H, J = 14.0, 4.5 Hz, Cys-H<sub>a</sub>); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 125 MHz):  $\delta$  175.4, 155.9, 143.6 (d, rotamers), 141.3, 133.5, 127.8, 127.1, 125.0, 120.0, 118.2, 67.4, 53.4, 47.1, 35.3, 32.5; HRMS (ES) Calcd for C<sub>21</sub>H<sub>21</sub>NO<sub>4</sub>SNa[M+Na]<sup>+</sup> 406.1083, found 406.1079.

#### Elucidation of the structure of the +14 Da side product:

Comparison of the LC MS/MS spectra of the isolated +14 Da side product and a 1-Pip-6-Pgl-oxytocin standard (synthesized by SPPS) reveals they are identical. The extracted mass chromatograms of the +14 Da side product (37.09 mins) and the synthetic standard (36.80 mins) are also very similar. These results led us to propose that 1-Pip-6-Pgl-oxytocin **3k** is the +14 Da side product:



#### **Extracted Mass Chromatograms:**



#### LC MS/MS spectra:

0

100

200





#### **Summary of RCM experiments:**

Table 1. RCM of peptide analogues on-resin and in aqueous solution.

	System								
	Resin			30 % t-BuOH			Micelles		
Peptide	HGII	Time	Yield	HGII	Time	Yield	HGII	Time	Yield
	(equiv)	( <b>h</b> )	(%) <sup>a</sup>	(equiv)	(h)	(%) <sup>b</sup>	(equiv)	( <b>h</b> )	(%) <sup>b</sup>
3a	0.2	24	50	50	24	0	50	24	0
3b	0.2	24	100	50	24	< 1	20	24	0
3c	0.2	24	100	50	24	< 1	20	24	0
3d	0.2	24	100	0.5	3	78	50	24	0
3e	0.2	24	0	10	24	0	20	24	0
4a	0.2	24	0	50	24	0	50	24	0
4b	0.2	24	0	50	24	< 1	50	24	0
4c	0.2	24	0	50	24	< 1	20	24	0
<b>4d</b>	0.2	24	0	0.5	24	63	20	24	0
4e	0.2	24	0	10	24	0	20	24	0

All aqueous reactions were conducted at 37 °C with 5000 equiv MgCl<sub>2</sub>. A concentration of 0.1 mM peptide or less was necessary for the large excess of salt to dissolve. (a) Yields of the on-resin RCM reactions were calculated from the ratio of cyclic products to linear starting material by HPLC integration; (b) isolated yields.

# **HPLC Traces:**

#### 1,6-Agl-oxytocin (3a)



#### 1,6-Pgl-oxytocin (3b)





## 1,6-Oas-oxytocin (3c)

#### 1,6-Sac-oxytocin (3d)





## Fmoc-1,6-Sac-oxytocin (3d')

#### 1,6-X-oxytocin (3e)





## 7,14-Agl-crotalphine (4a)

#### 7,14-Pgl-crotalphine (4b)



#### 7,14-Oas-crotalphine (4c)



#### 7,14-Sac-crotalphine (4d)



<sup>171</sup> Channel 1 🚺 171 Channel 2

# 7,14-X-crotalphine (4e)





#### Cyclized cis-1,6-Agl-Oxytocin (3f)



# Cyclized trans-1,6-Agl-Oxytocin (3g)





Cyclized 1,6-Pgl-oxytocin (3h)





#### Cyclized 1,6-Sac-oxytocin (3j)



S13

## Cyclized Fmoc-1,6-Sac-oxytocin (3j')



#### 1-Pip-6-Pgl-oxytocin (3k)





## Cyclized 7,14-Oas-crotalphine (4g)

Cyclized 7,14-Pgl-crotalphine (4h)



S15



## Cyclized 7,14-Sac-crotalphine (4i)

S16

## **MALDI-TOF Spectra:**

## 1,6-Agl-oxytocin (3a)

Voyager Spec #1=>AdvBC(32,0.5,0.1)[BP = 1019.5, 22490]



L:\...\run1\_0004.dat Acquired: 09:33:00, June 13, 2011

S18

## 1,6-Pgl-oxytocin (3b)



L:\...\pureHPLCfraction\_0001.dat Acquired: 02:08:00, November 17, 2011

## 1,6-Oas-oxytocin (3c)



L:\...\HPLCfraction\_0001.dat Acquired: 01:54:00, November 14, 2011

## 1,6-Sac-oxytocin (3d)



.:\...\pureHPLCfraction\_0001.dat \cquired: 00:24:00, November 15, 2011

## Fmoc-1,6-Sac-oxytocin (3d')





L1...\F6\_0002.dat Acquired: 16:58:00, March 14, 2012

# 1,6-X-oxytocin (3e)



#### S23

## 7,14-Agl-crotalphine (4a)



Cro-ana-with-allylGly L1...\Cro-ana-with-allylGly\_0001.dat Acquired: 18:01:00, June 09, 2011

# [M-H]<sup>-</sup>

S24

# 7,14-Pgl-crotalphine (4b)



L1...\BishomoGly-cro-run-f-3\_0001.dat Acquired: 05:56:00, December 30, 2011

# [M-H]<sup>-</sup>

## 7,14-Oas-crotalphine (4c)



L1...\Ser-allyi-Cro-f-1\_0001.dat Acquired: 06:43:00, December 26, 2011

#### Voyager Spec #1=>BC=>AdvBC(32,0.5,0.1)[BP = 1582.6, 44648]

S25

 $[M+Na]^+$ 

# 7,14-Sac-crotalphine (4d)



L1...\Allyl-Cys-cro-f-2-pos\_0001.dat Acquired: 02:25:00, November 29, 2011

[M-H]<sup>-</sup>



# 7,14-X-crotalphine (4e)



L1...\Novel-Cys-Cro-July-12\_0001.dat Acquired: 11:38:00, July 12, 2012

 $[M+H]^+$ 

S28

## Cyclized cis- and trans-1,6-Agl-oxytocin (3f, 3g)



 $[M+H]^+$ 

S29

# Cyclized 1,6-Pgl-oxytocin (3h)



L1...\F2'\_0001.dat Acquired: 03:22:00, January 18, 2012

 $[M+H]^+$ 

S30

# Cyclized 196-Oas-oxytocin (3i)



L1...\F4\_0001.dat Acquired: 11:20:00, April 03, 2012

 $[M+H]^+$ 

S31

# Cyclized 1,6-Sac-oxytocin (3j)

#### Voyager Spec #1=>AdvBC(32,0.5,0.1)=>BC[BP = 1061.1, 16622]



.1...\F10\_0001.dat Acquired: 09:14:00, June 11, 2012  $[M+Na]^+$ 

S32

# Cyclized Fmoc-1,6-Sac-oxytocin (3j')





L1...\HPLCfraction26mins\_0001.dat Acquired: 19:35:00, February 29, 2012

S33  $[M+H]^+$ 1-Pip-6-Pgl-oxytocin (3k) [M+Na] Voyager Spec #1[BP = 1039.4, 24387] \_2.4E+4 1039.4 061 70 <sup>Alsea</sup>u¶₽¶K] 40 30 20 10 تهاد بالمسارب بعالته الثار البارانا المالا والمعتبة والكاوم ألؤالة أأطأم لتتبأ أومن بألهما الالامام يقار Pula 0-3360.6 5001.0 1720.2 2540.4 4180.8 Mass (m/z)

L1...\pureHPLCfraction\_0001.dat Acquired: 00:05:00, February 15, 2012

#### [M-H]<sup>-</sup>

S34

# Cyclized 7,14-Pgl-crotalphine (4h)



L1...\15-good\_0001.dat Acquired: 15:16:00, April 02, 2012

 $[M+H]^+$ 

S35

# Cyclized 7,14-Oas-crotalphine (4i)



L1...\cyclic-AllylSer-Cro-pos-good\_0001.dat Acquired: 18:59:00, April 05, 2012 [M-H]<sup>-</sup>

S36

# Cyclized 7,14-Sac-crotalphine (4j)



L1...\150312-ZeduSallyinxnneg\_0001.dat Acquired: 11:32:00, March 16, 2012



S37

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S38

FmocHN





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S40





S41























9

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6

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<u>}</u>.5

S51











S57



