Supporting Information

Ring-Closing Metathesis of Peptides in Water

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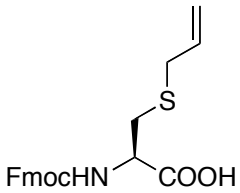
Synthesis of Fmoc-L-Sac-OH

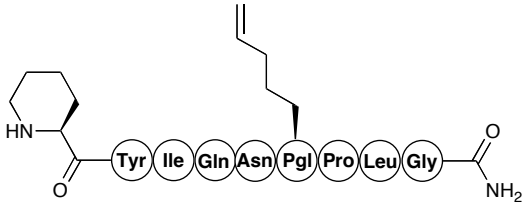


S-allyl-L-cysteine (Sac) (2.00 g, 12.4 mmol) and $NaHCO_3$ (3.13 g, 37.2 mmol) were dissolved in water (60 mL) and stirred at room temperature. A solution of FmocOSu (4.59 g, 13.6 mmol) in THF (60 mL) was then added to the aqueous solution and stirring was continued for an additional 2 hours at room 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_2Cl_2 : *i*-PrOH : AcOH), yielding Fmoc-L-Sac-OH as a white solid (1.30 g, 27%). $[\alpha]_D$ -12.2° (*c* 1.00, CH_2Cl_2); IR ($CHCl_3$ cast) 3325, 3065, 1685, 1532 cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz): δ 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$_2$), 5.64 (d, 1H, *J* = 7.8 Hz, NH), 5.13 (m, 2H, CH=C<u>H$_2$</u>), 4.60-4.66 (m, 1H, Cys-H$_\alpha$), 4.44-4.46 (m, 2H, Fmoc CHC<u>H$_2$</u>), 4.24 (1H, t, *J* = 7.1 Hz, Fmoc C<u>H</u>CH$_2$), 3.15 (2H, d, *J* = 7.1 Hz, C<u>H$_2$</u>CH=CH$_2$), 3.04 (dd, 1H, *J* = 14.2, 4.5, Cys-H$_\beta$), 2.94 (dd, 1H, *J* = 14.0, 4.5 Hz, Cys-H$_\beta$); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 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 $C_{21}H_{21}NO_4SNa[M+Na]^+$ 406.1083, found 406.1079.

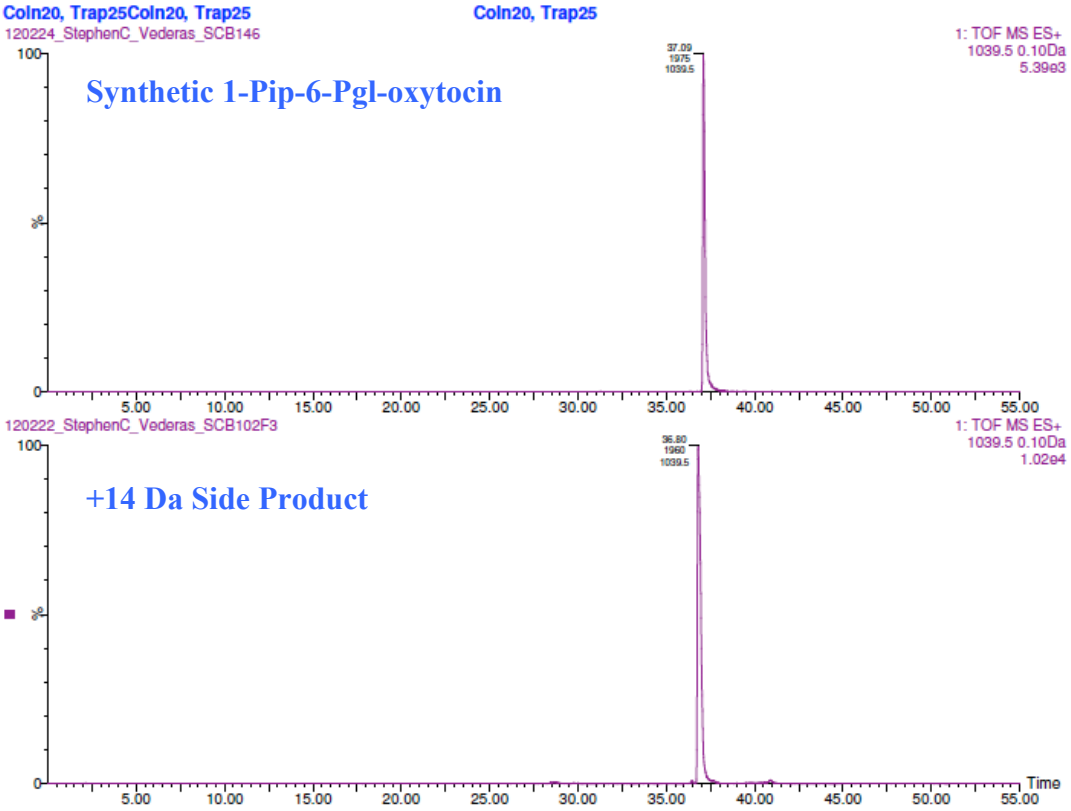
Elucidation of the structure of the +14 Da side product:

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:

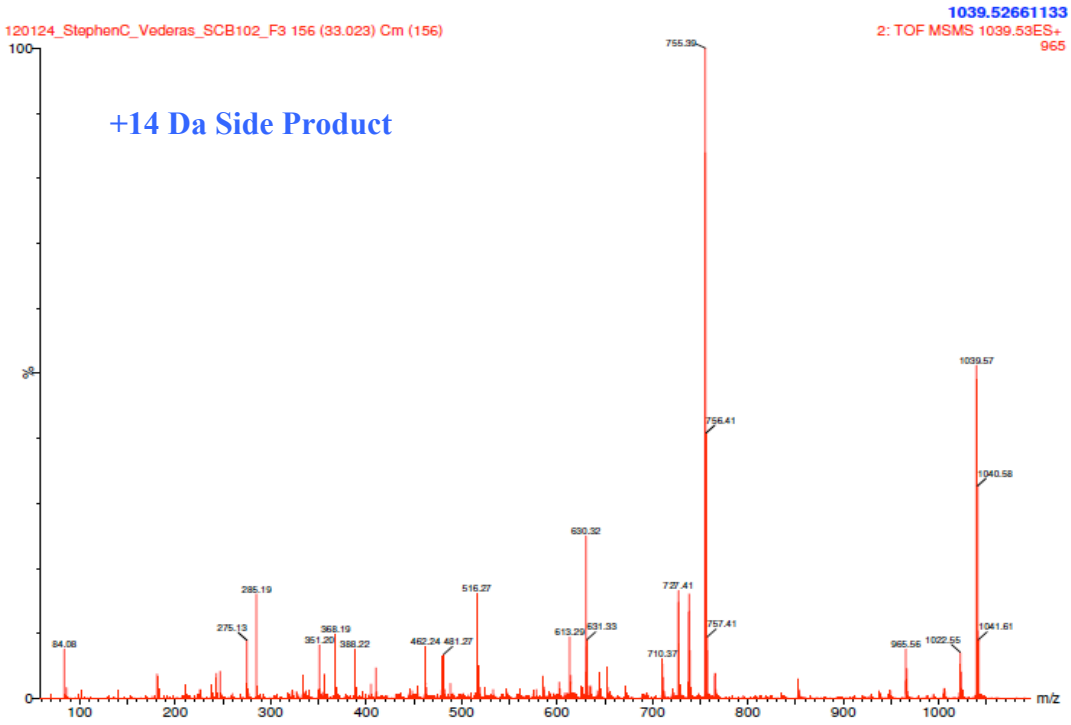


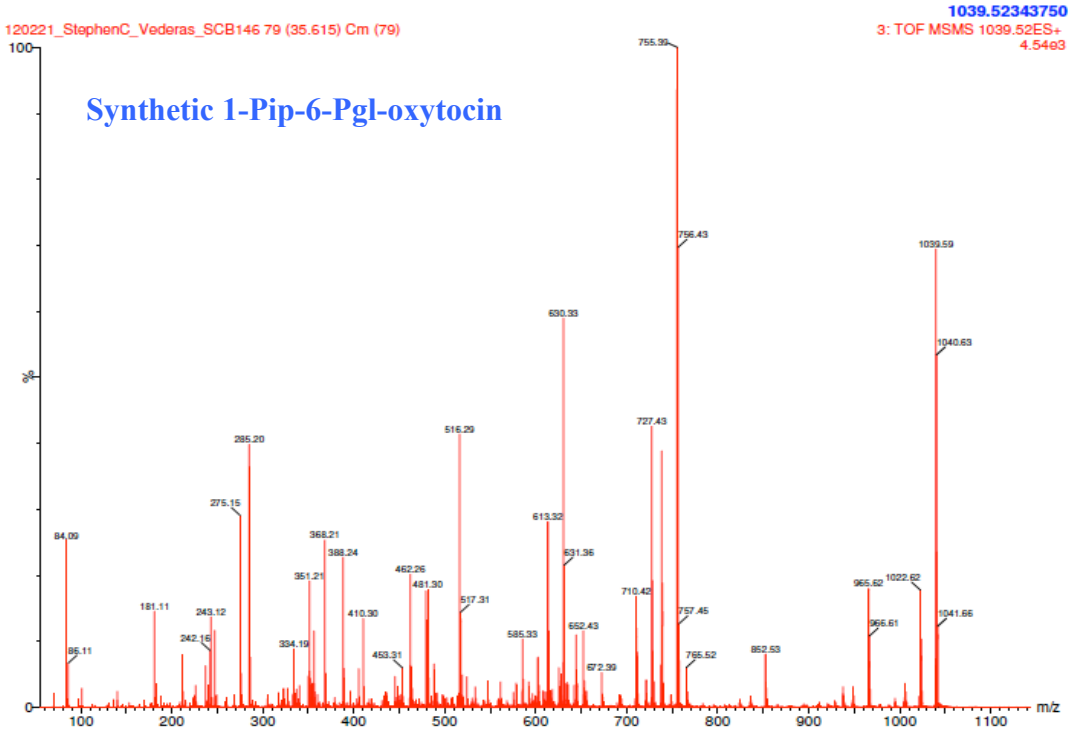
3k

Extracted Mass Chromatograms:



LC MS/MS spectra:





Summary of RCM experiments:

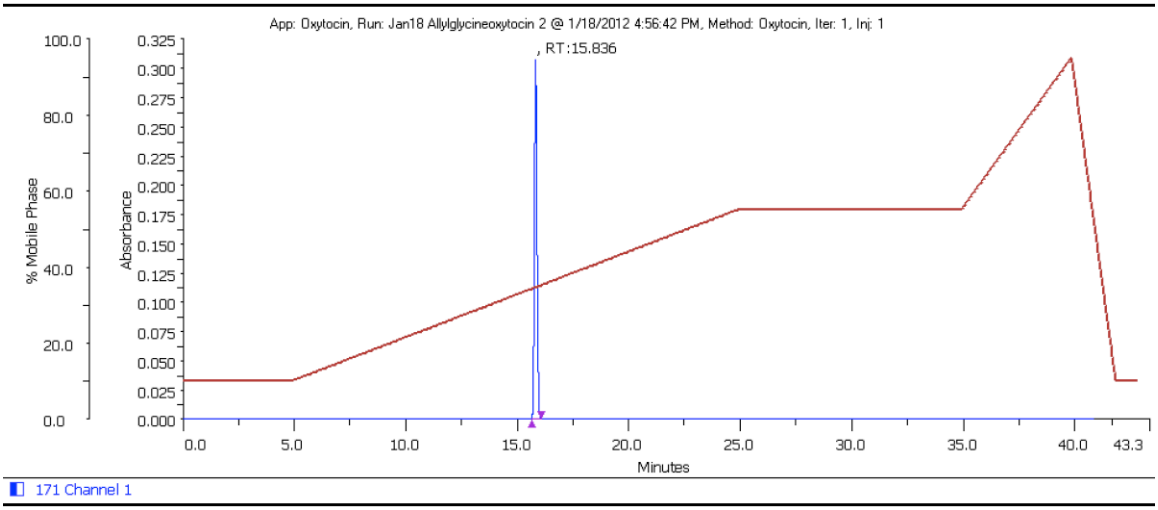
Table 1. RCM of peptide analogues on-resin and in aqueous solution.

	System								
	Resin			30 % *t*-BuOH			Micelles		
Peptide	**HGII (equiv)**	**Time (h)**	**Yield (%)[a]**	**HGII (equiv)**	**Time (h)**	**Yield (%)[b]**	**HGII (equiv)**	**Time (h)**	**Yield (%)[b]**
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
4d	0.2	24	0	0.5	24	63	20	24	0
4e	0.2	24	0	10	24	0	20	24	0

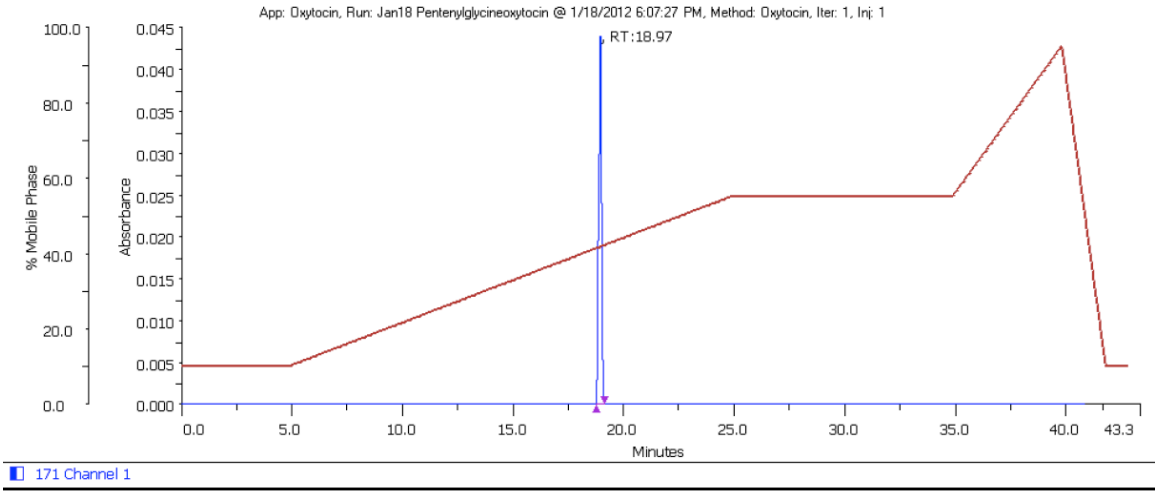
All aqueous reactions were conducted at 37 °C with 5000 equiv $MgCl_2$. A concentration of 0.1 mM peptide or less was necessary for the large excess of salt to dissolve. (a) Yields of the on-resin RCM reactions were calculated from the ratio of cyclic products to linear starting material by HPLC integration; (b) isolated yields.

HPLC Traces:

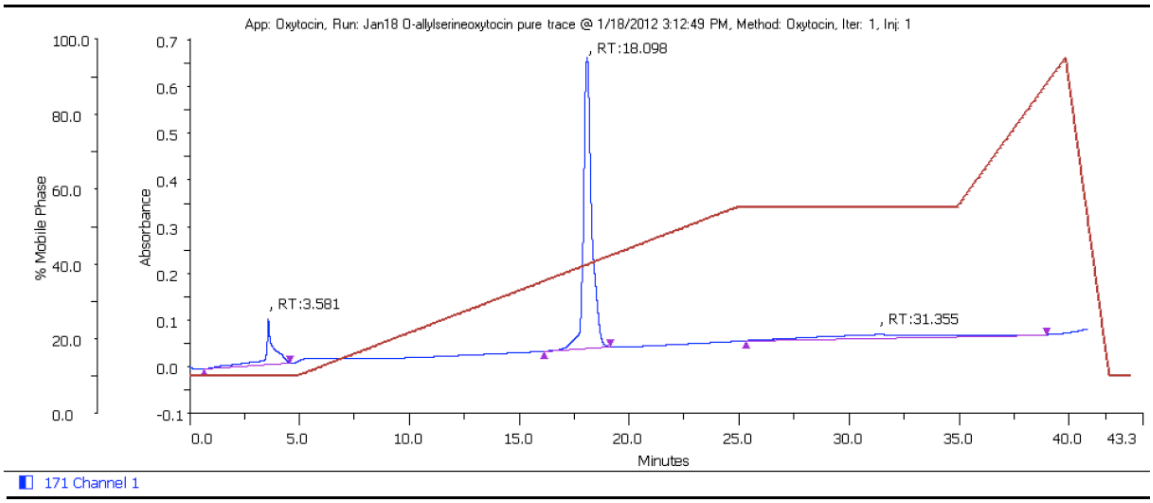
1,6-Agl-oxytocin (3a)



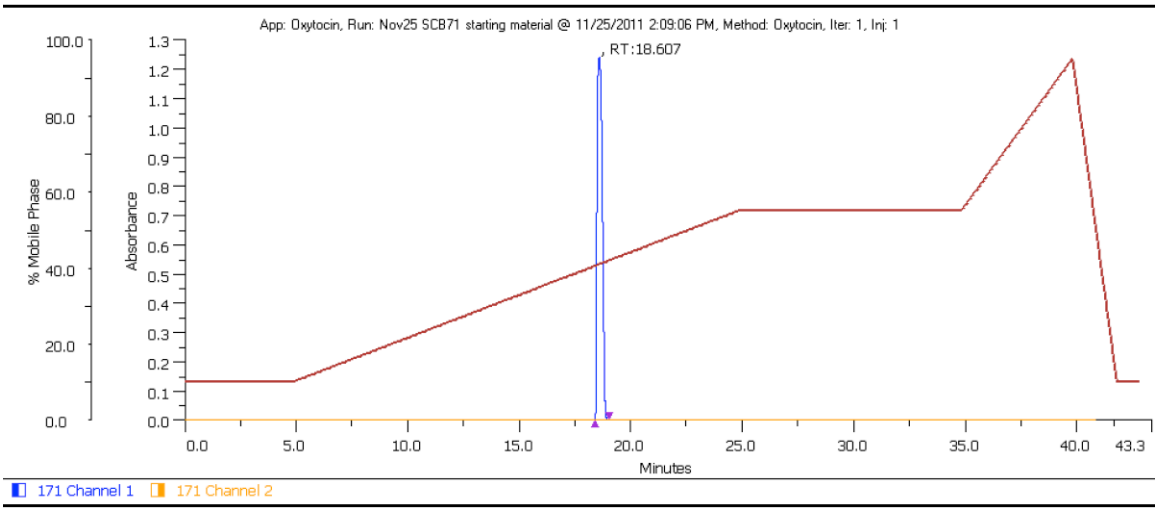
1,6-Pgl-oxytocin (3b)



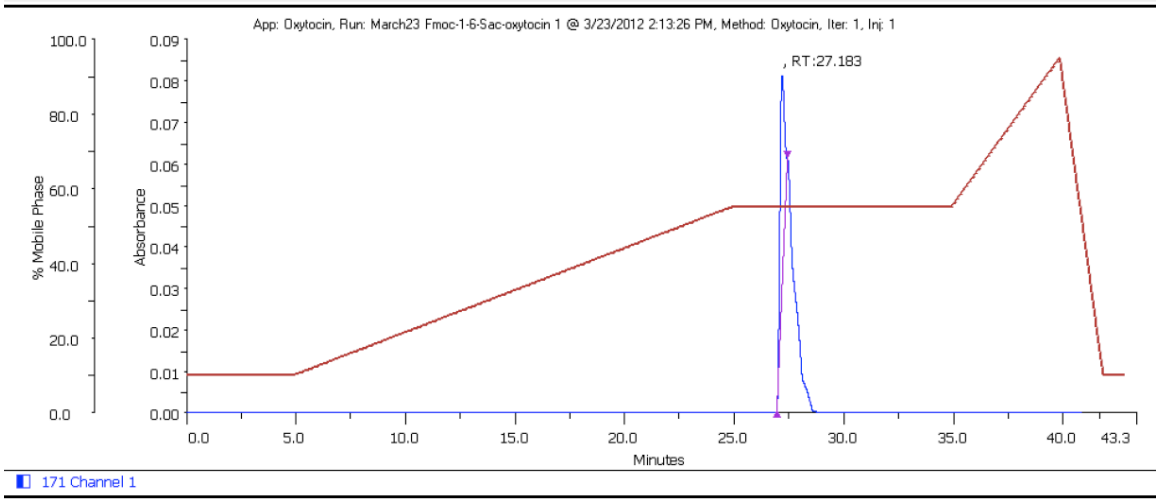
1,6-Oas-oxytocin (3c)



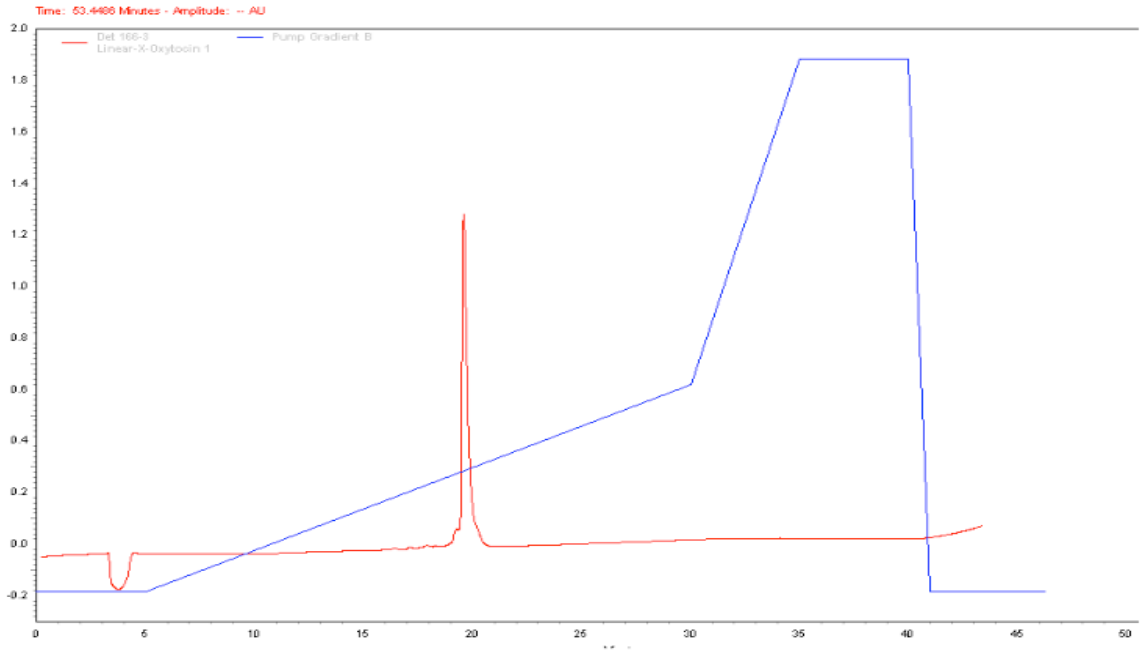
1,6-Sac-oxytocin (3d)



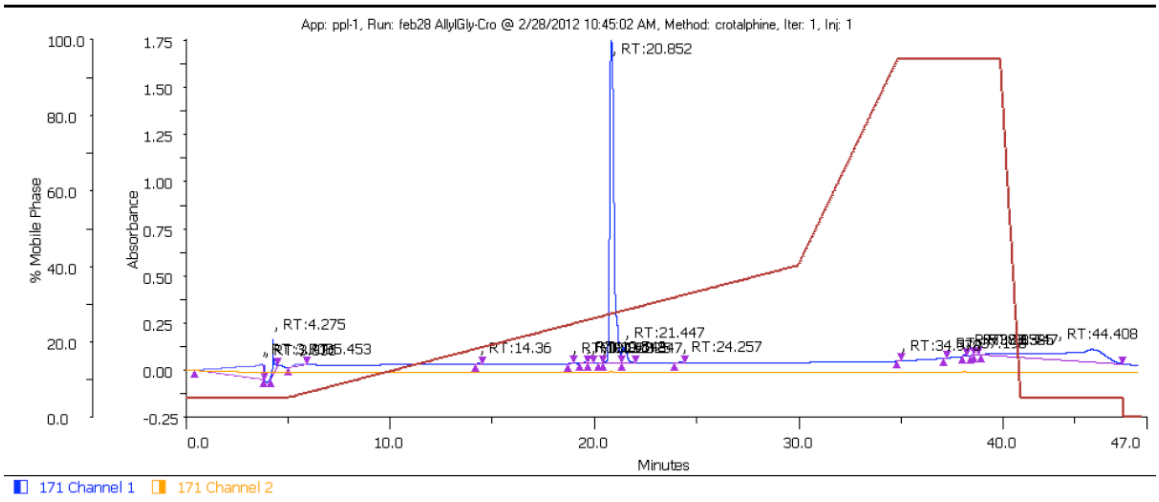
Fmoc-1,6-Sac-oxytocin (3d')



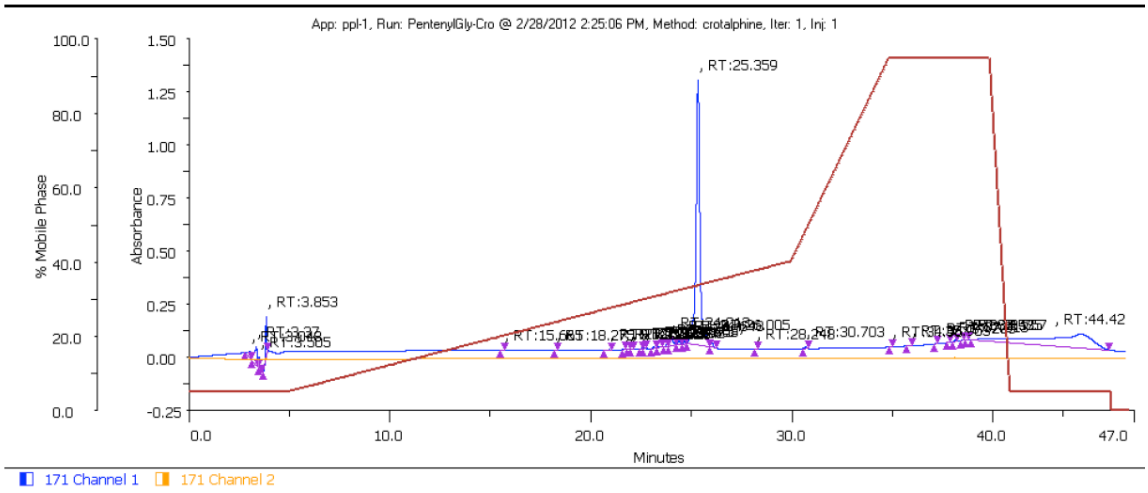
1,6-X-oxytocin (3e)



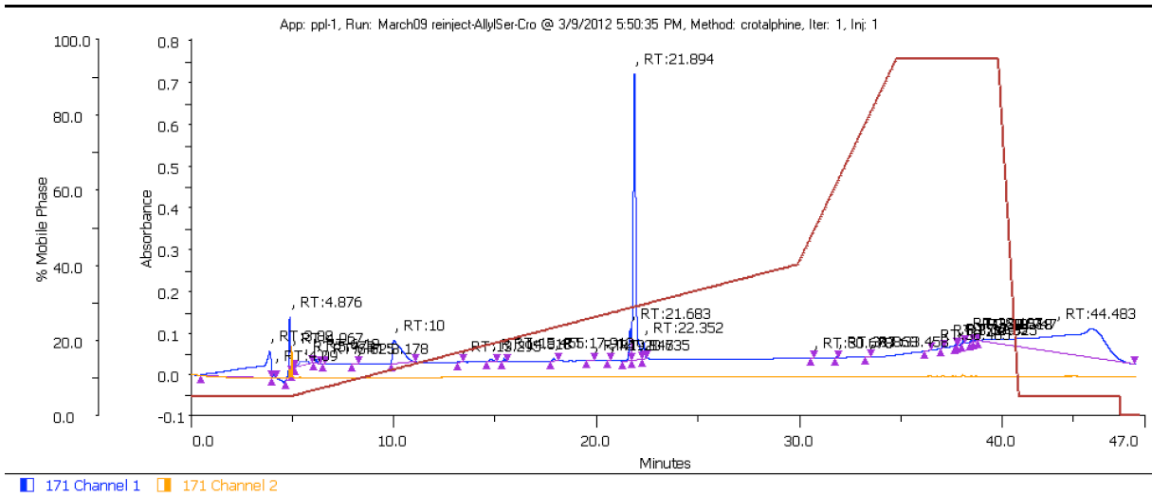
7,14-Agl-crotalphine (4a)



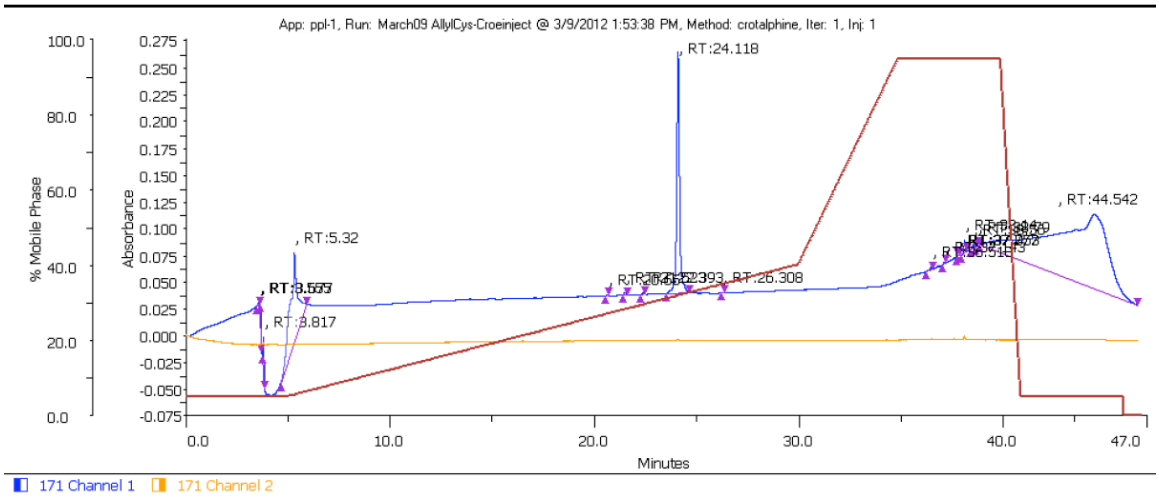
7,14-Pgl-crotalphine (4b)



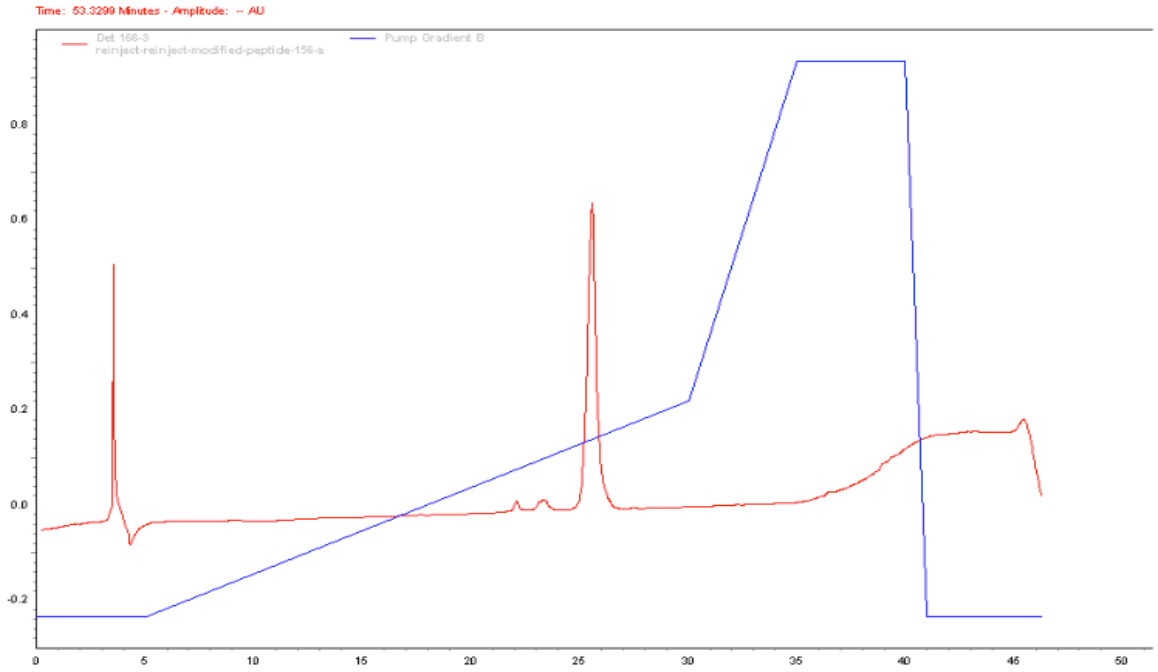
7,14-Oas-crotalphine (4c)



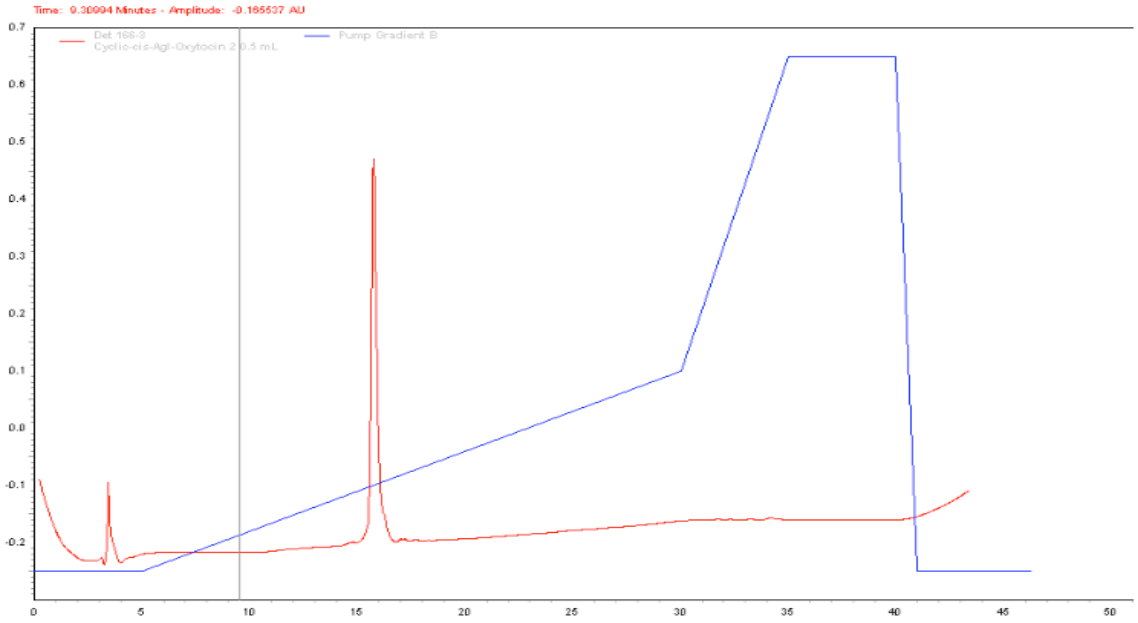
7,14-Sac-crotalphine (4d)



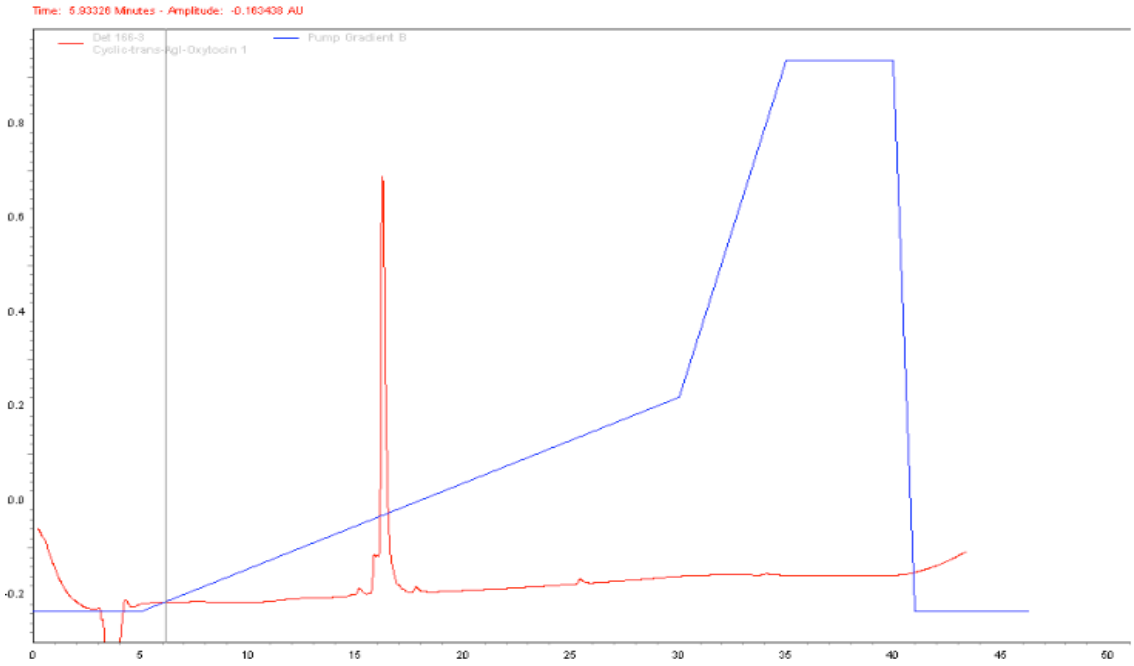
7,14-X-crotalphine (4e)



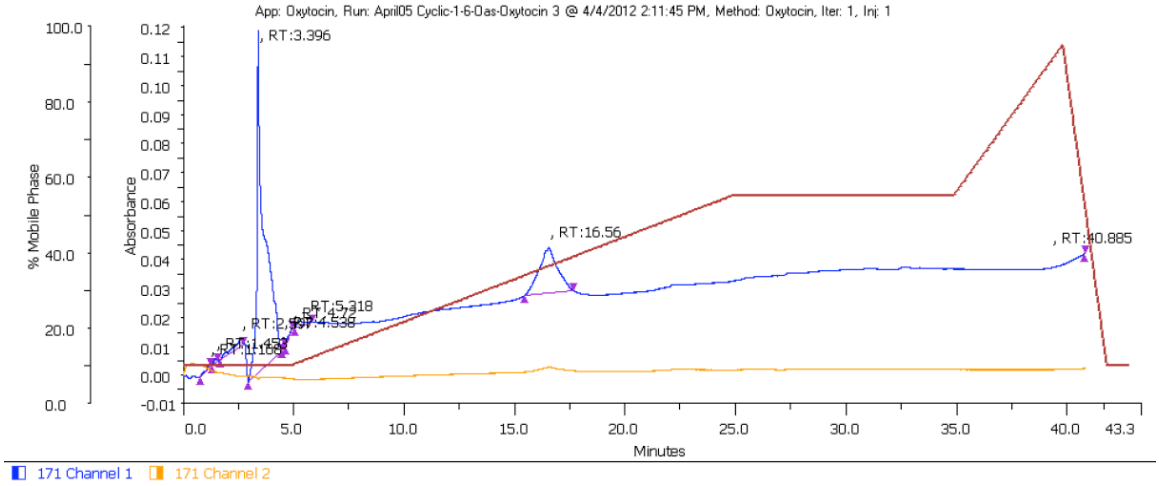
Cyclized *cis*-1,6-Agl-Oxytocin (3f)



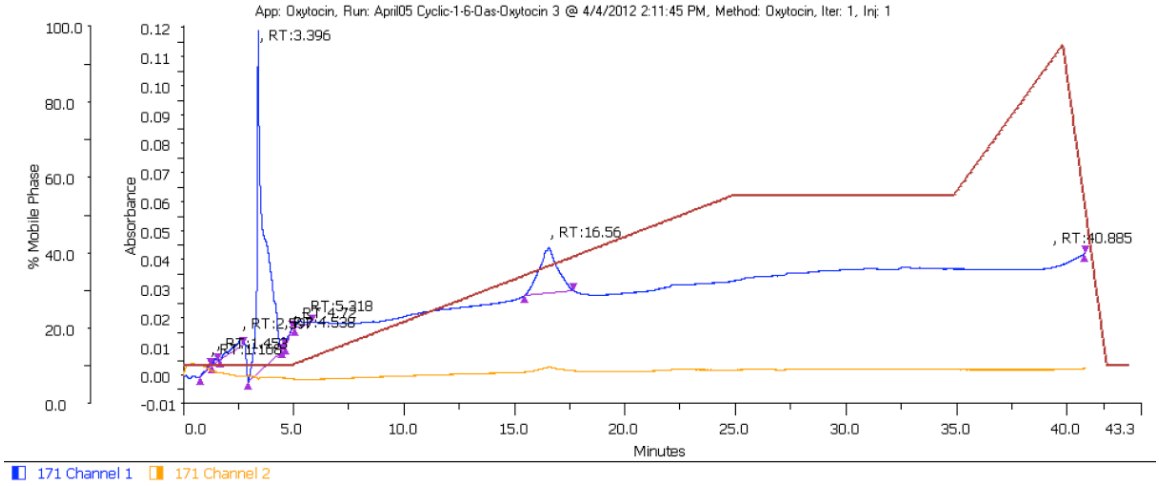
Cyclized *trans*-1,6-Agl-Oxytocin (3g)



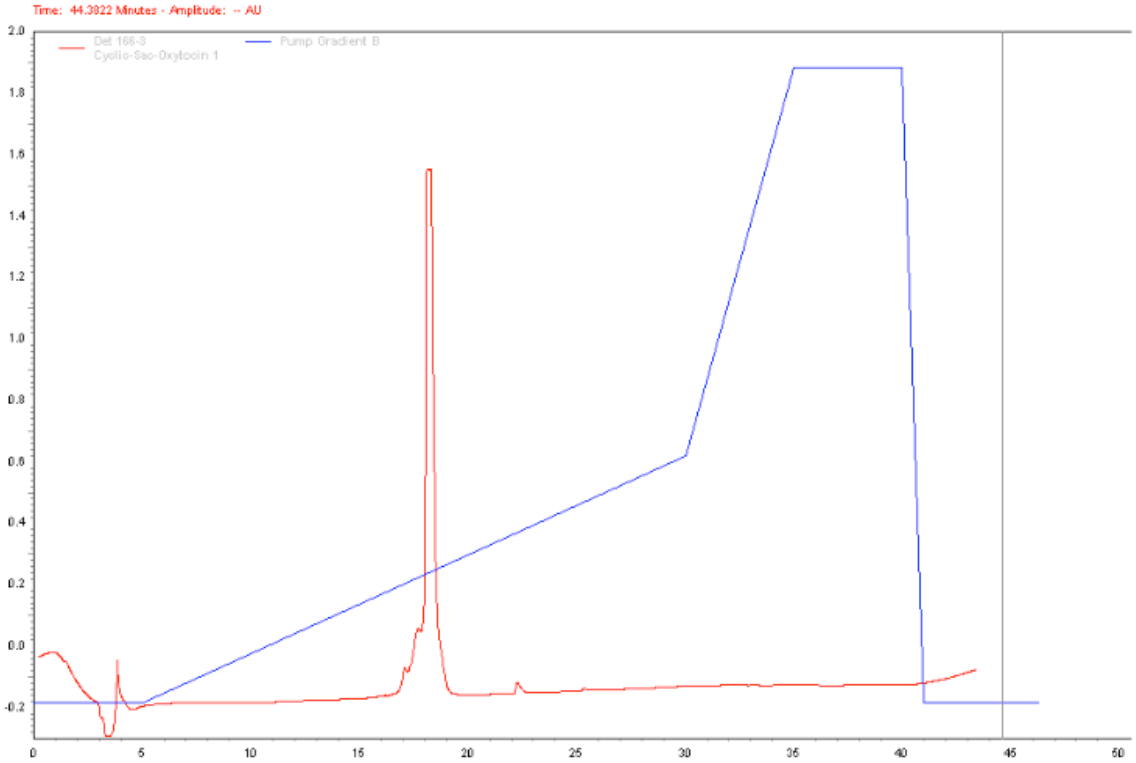
Cyclized 1,6-Pgl-oxytocin (3h)



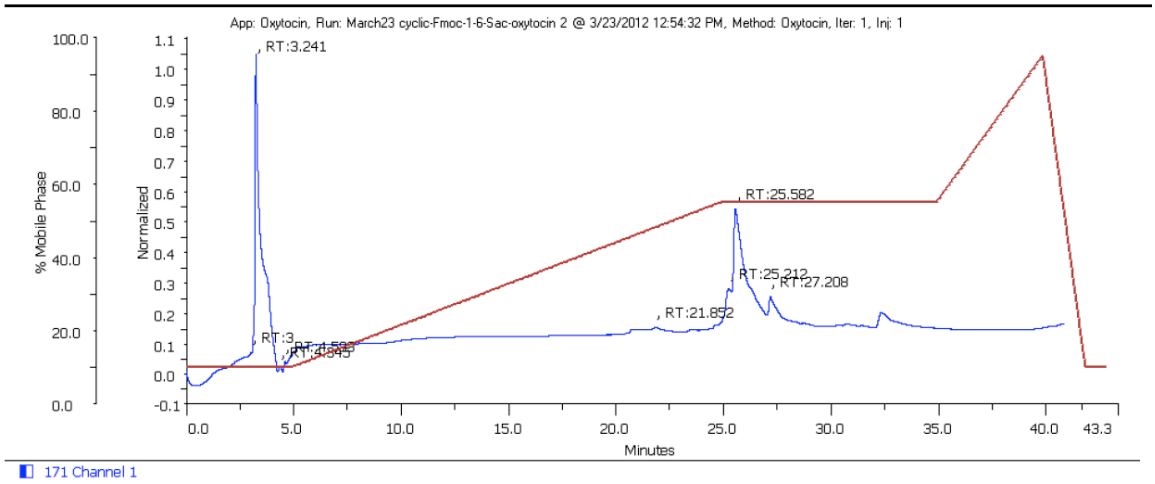
Cyclized 1,6-Oas-Oxytocin (3i)



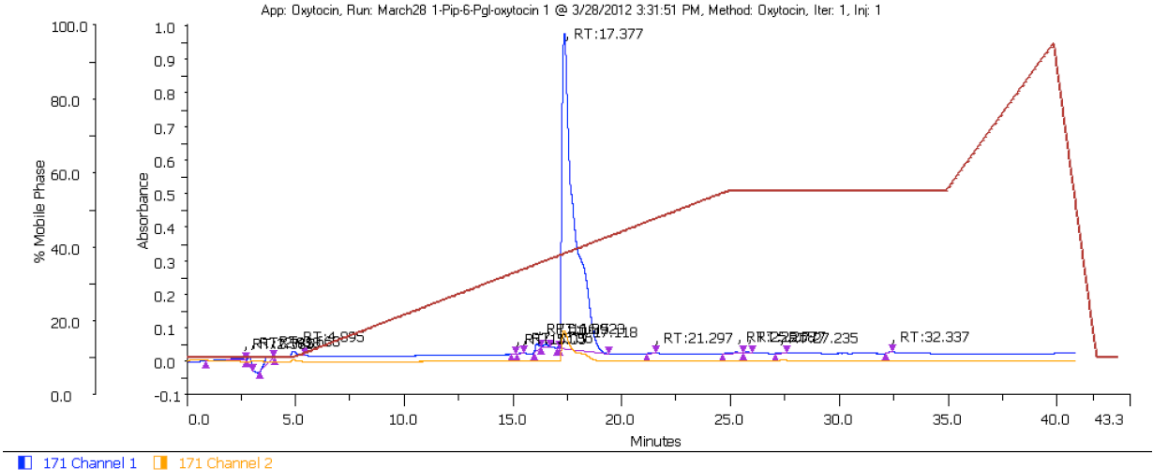
Cyclized 1,6-Sac-oxytocin (3j)



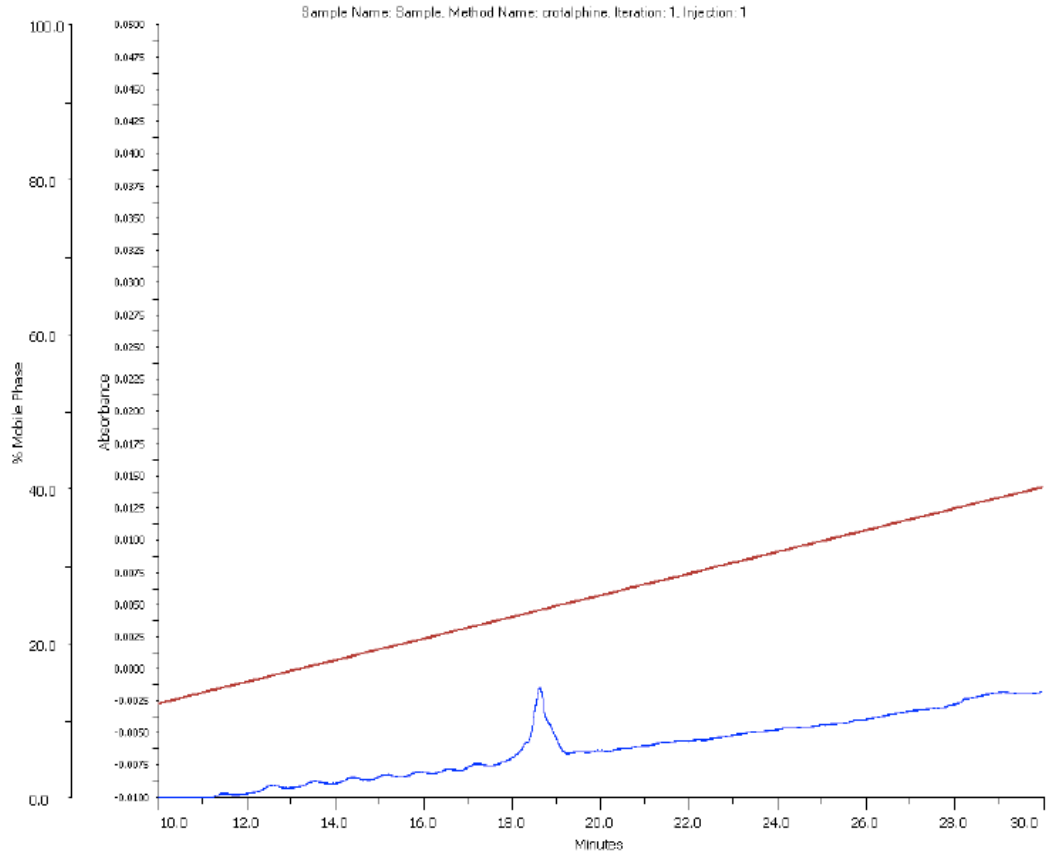
Cyclized Fmoc-1,6-Sac-oxytocin (3j')



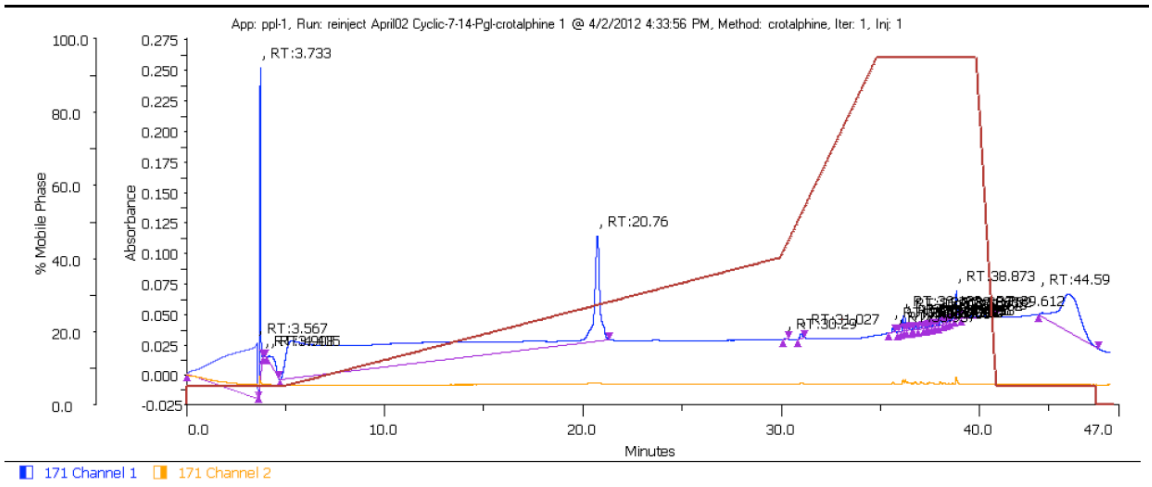
1-Pip-6-Pgl-oxytocin (3k)



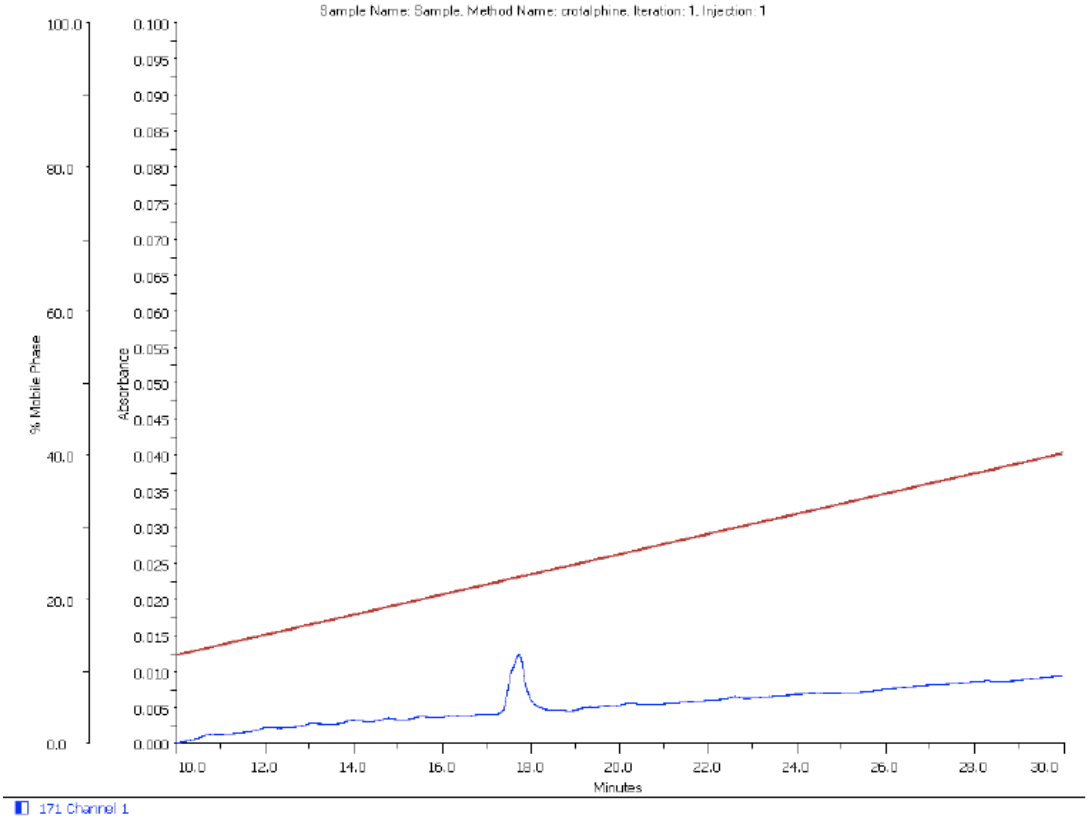
Cyclized 7,14-Oas-crotalphine (4g)



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

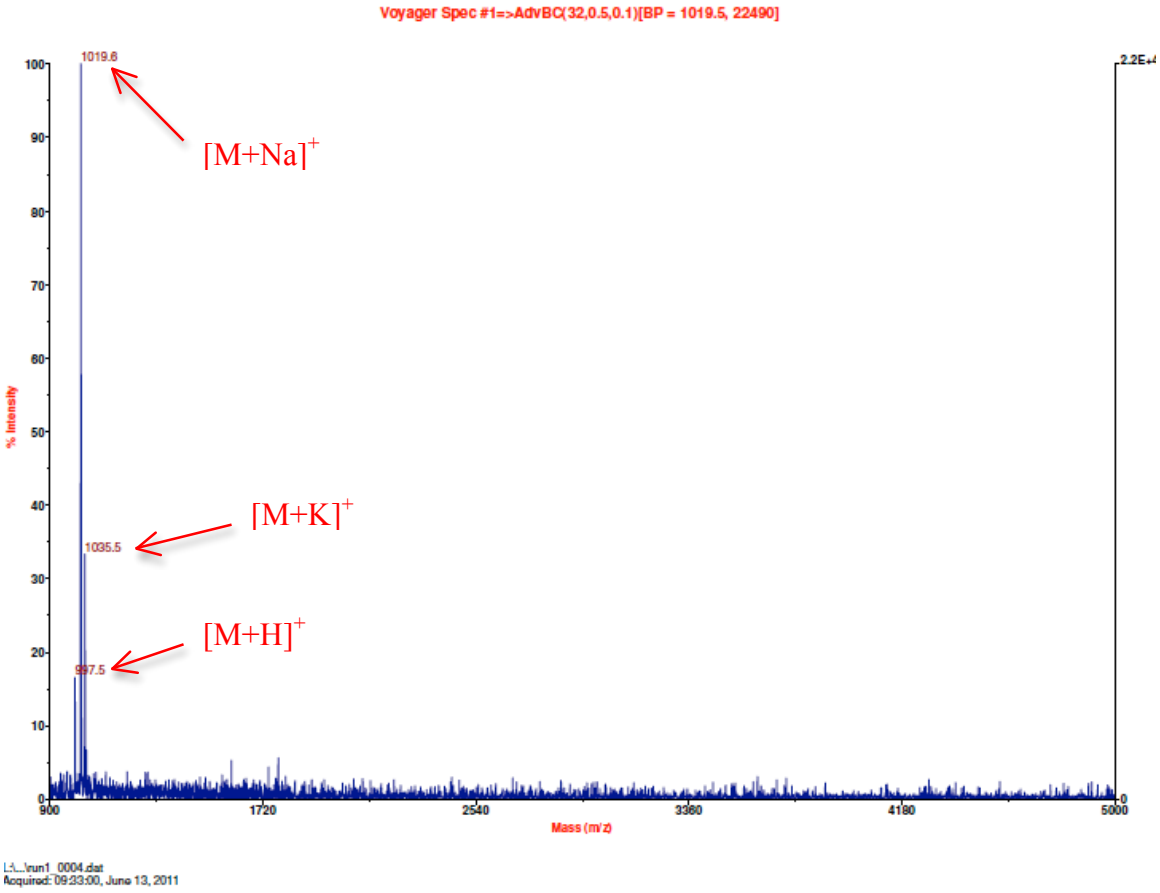


Cyclized 7,14-Sac-crotalphine (4i)

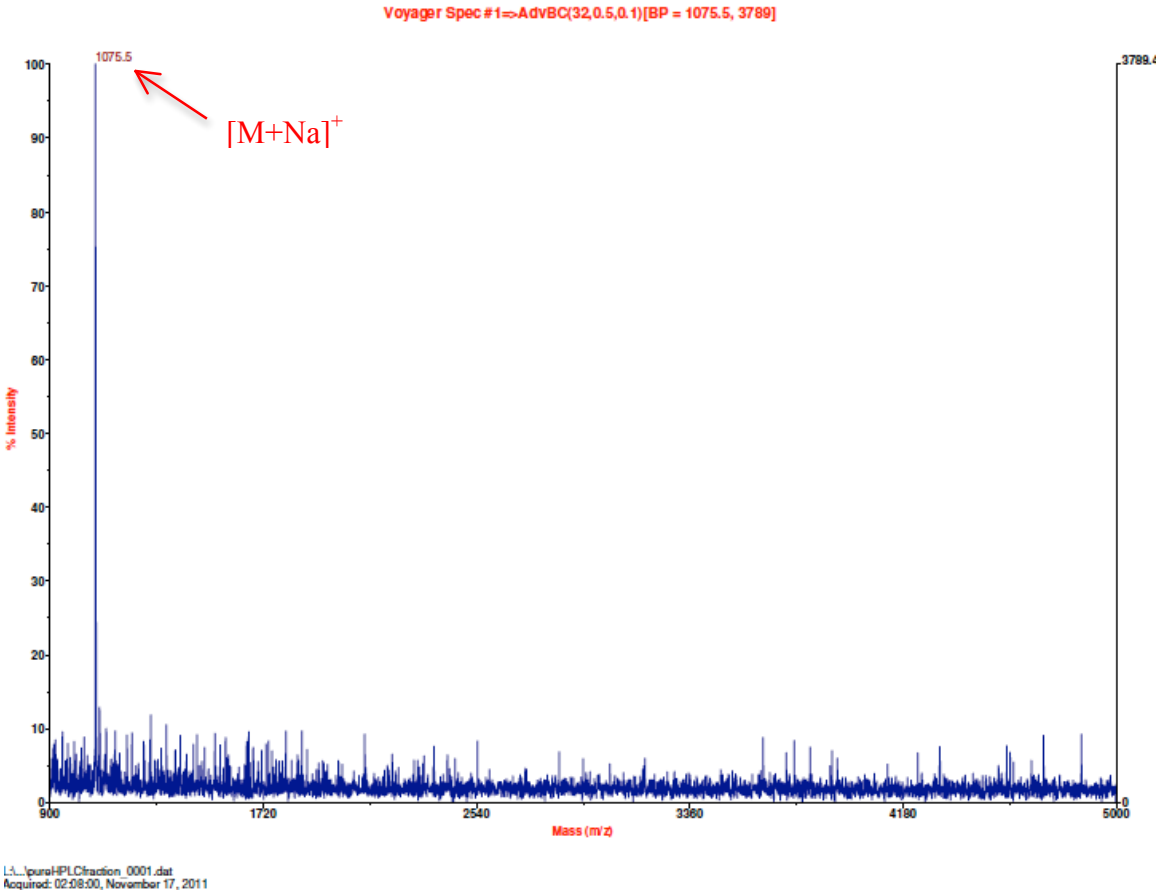


MALDI-TOF Spectra:

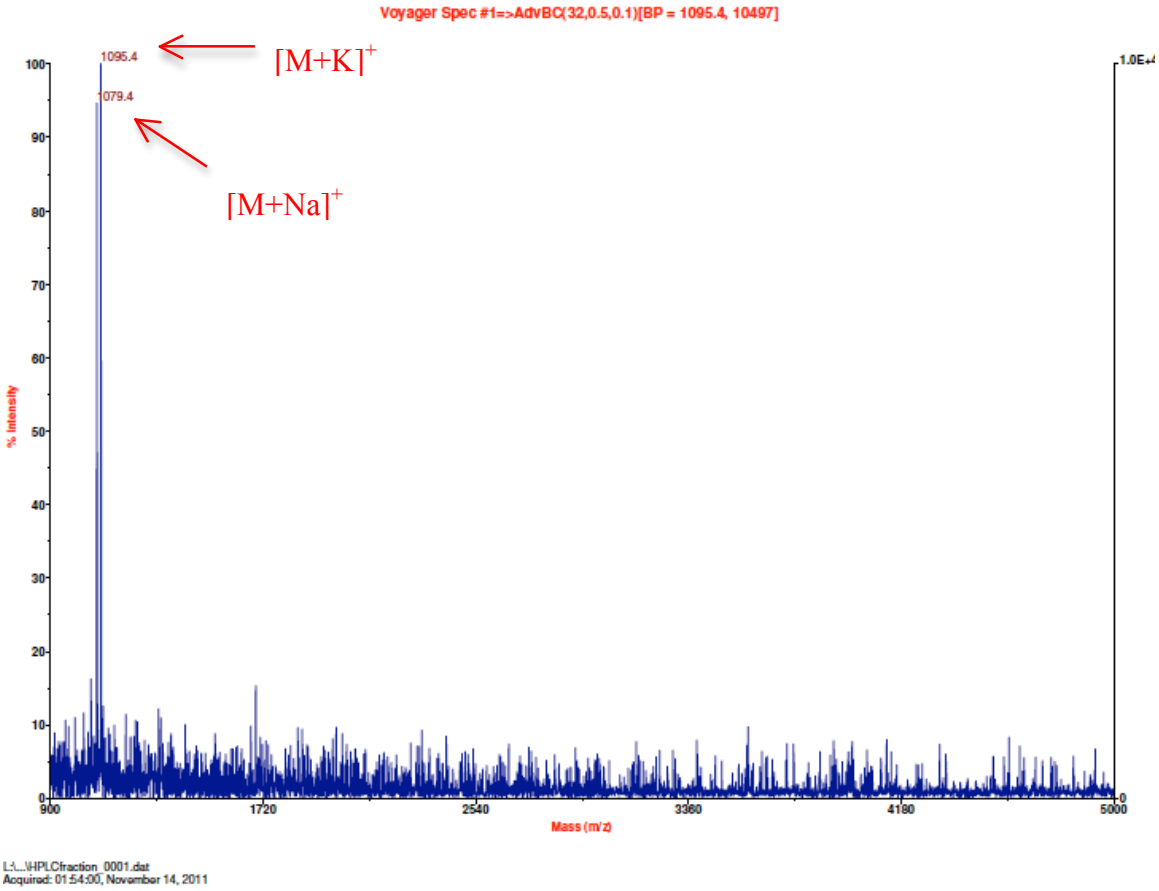
1,6-Agl-oxytocin (3a)



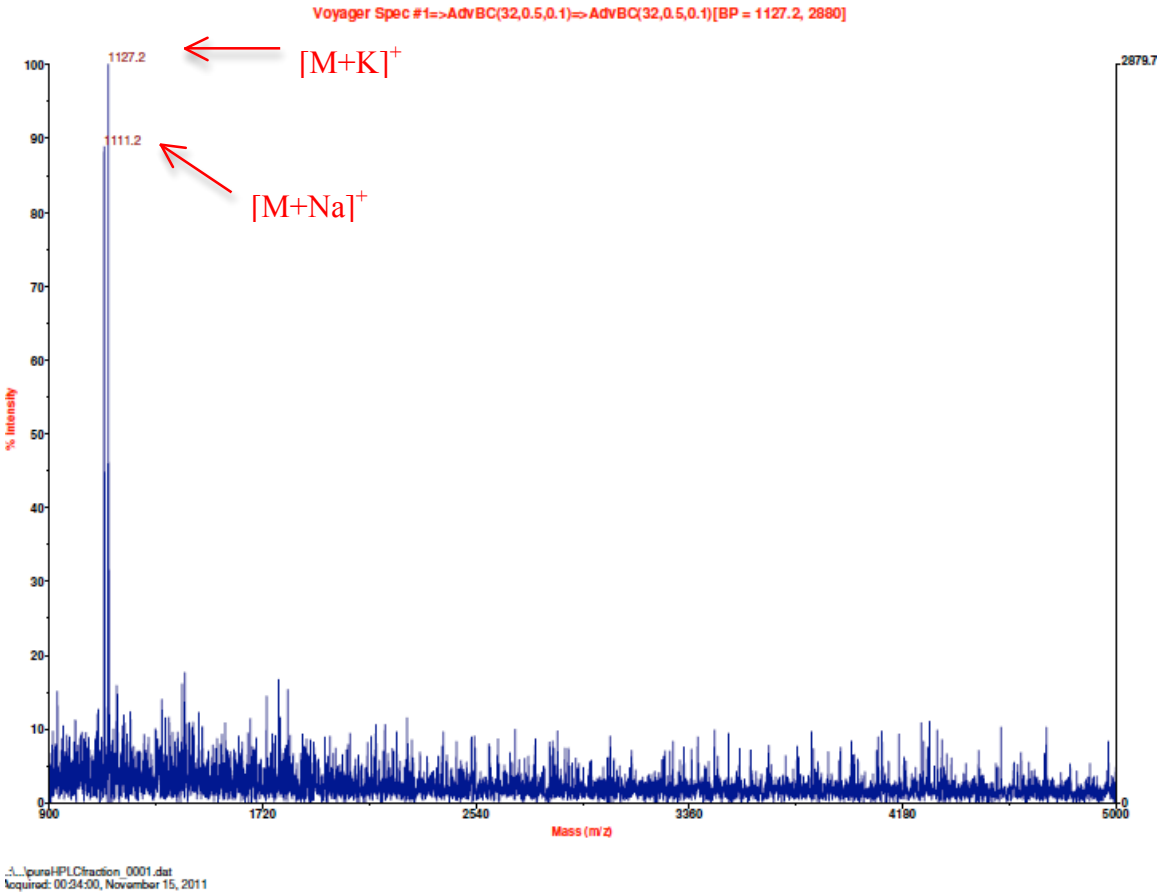
1,6-Pgl-oxytocin (3b)



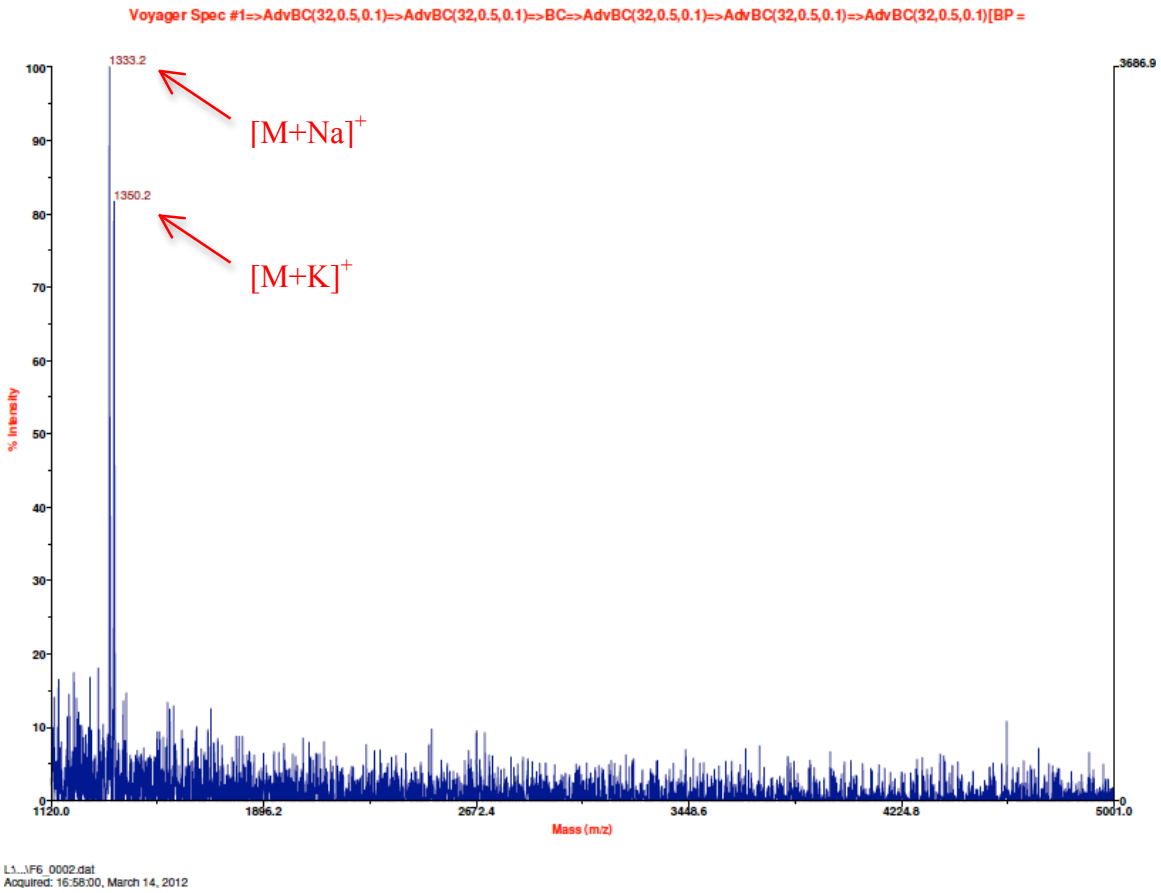
1,6-Oas-oxytocin (3c)



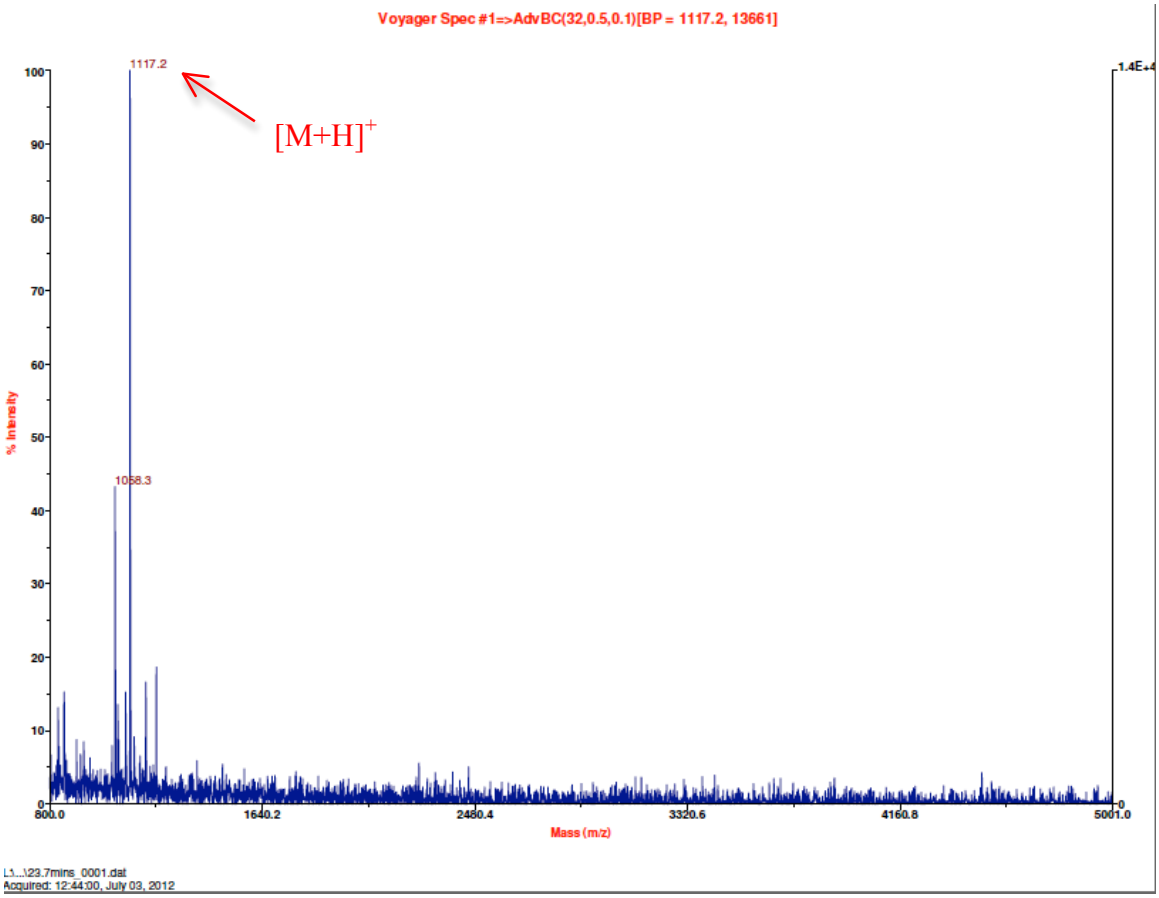
1,6-Sac-oxytocin (3d)



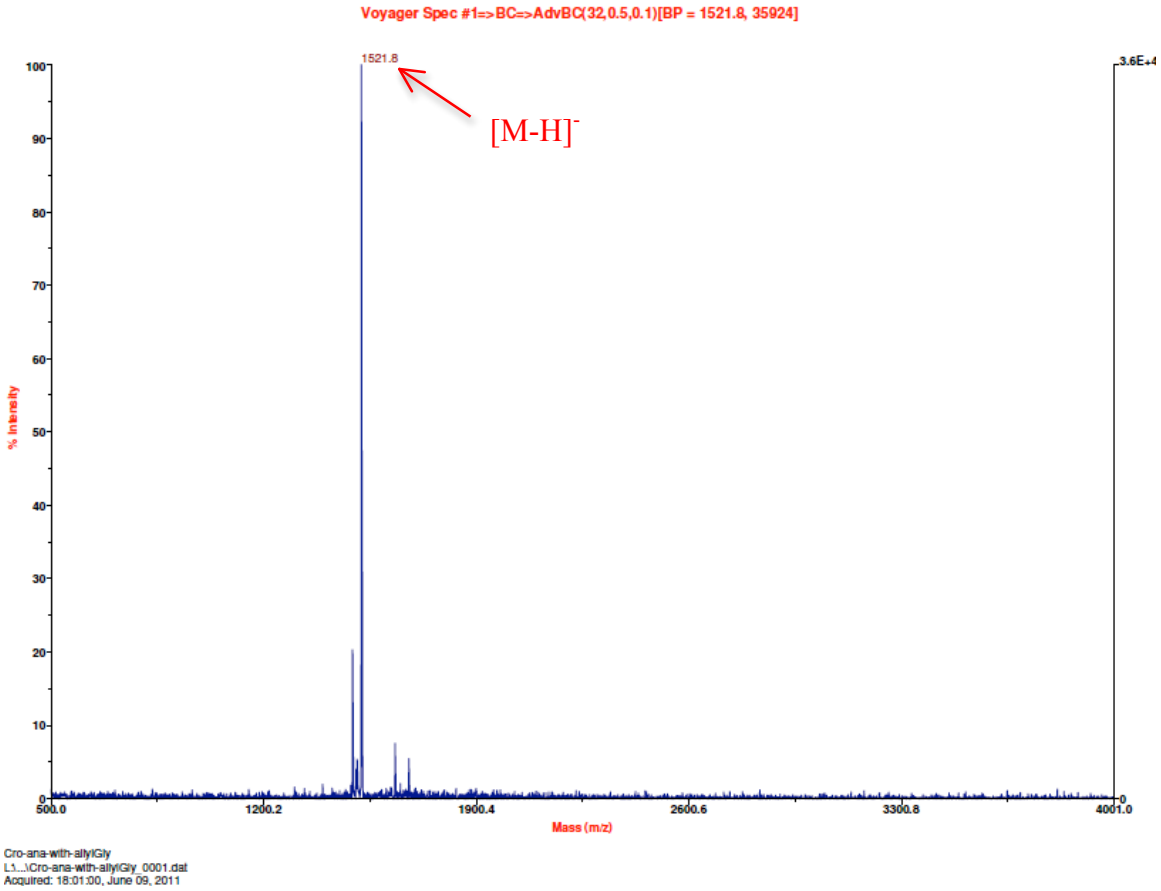
Fmoc-1,6-Sac-oxytocin (3d')



1,6-X-oxytocin (3e)

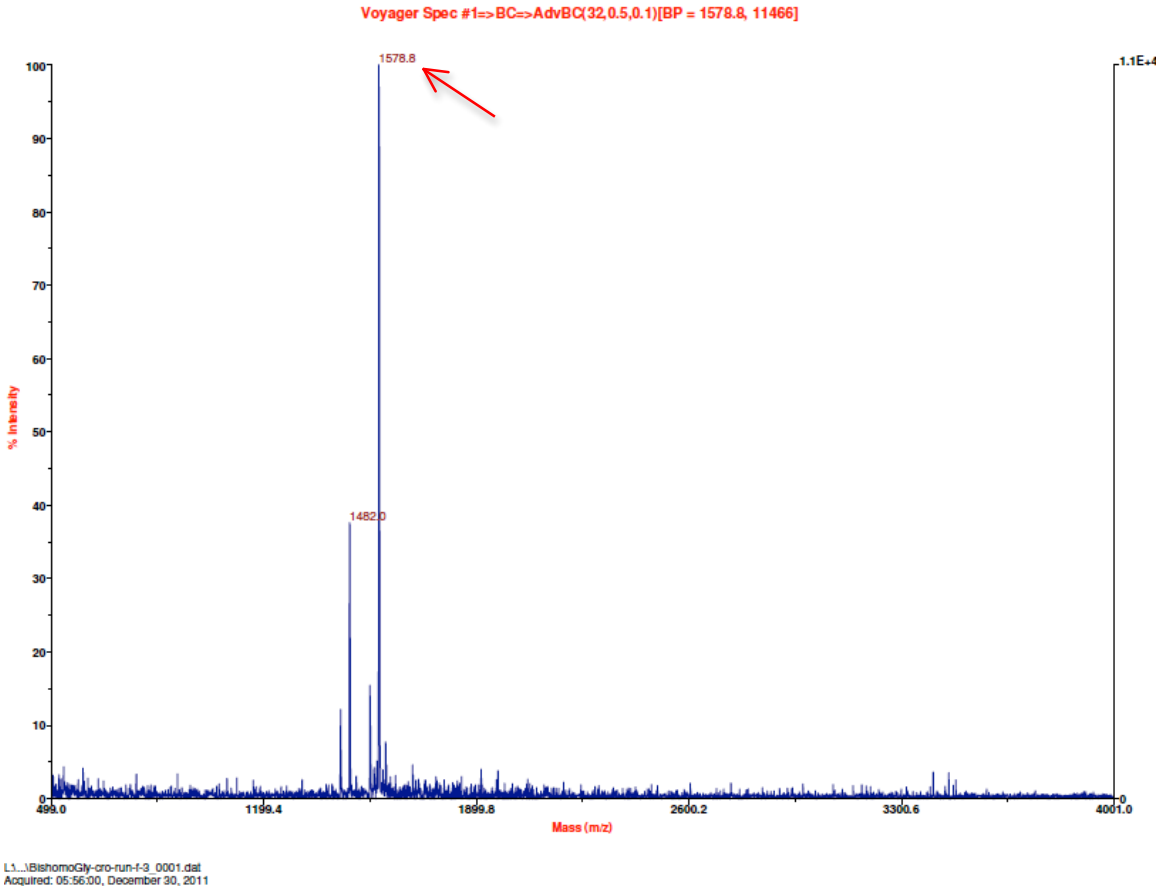


7,14-Agl-crotalphine (4a)



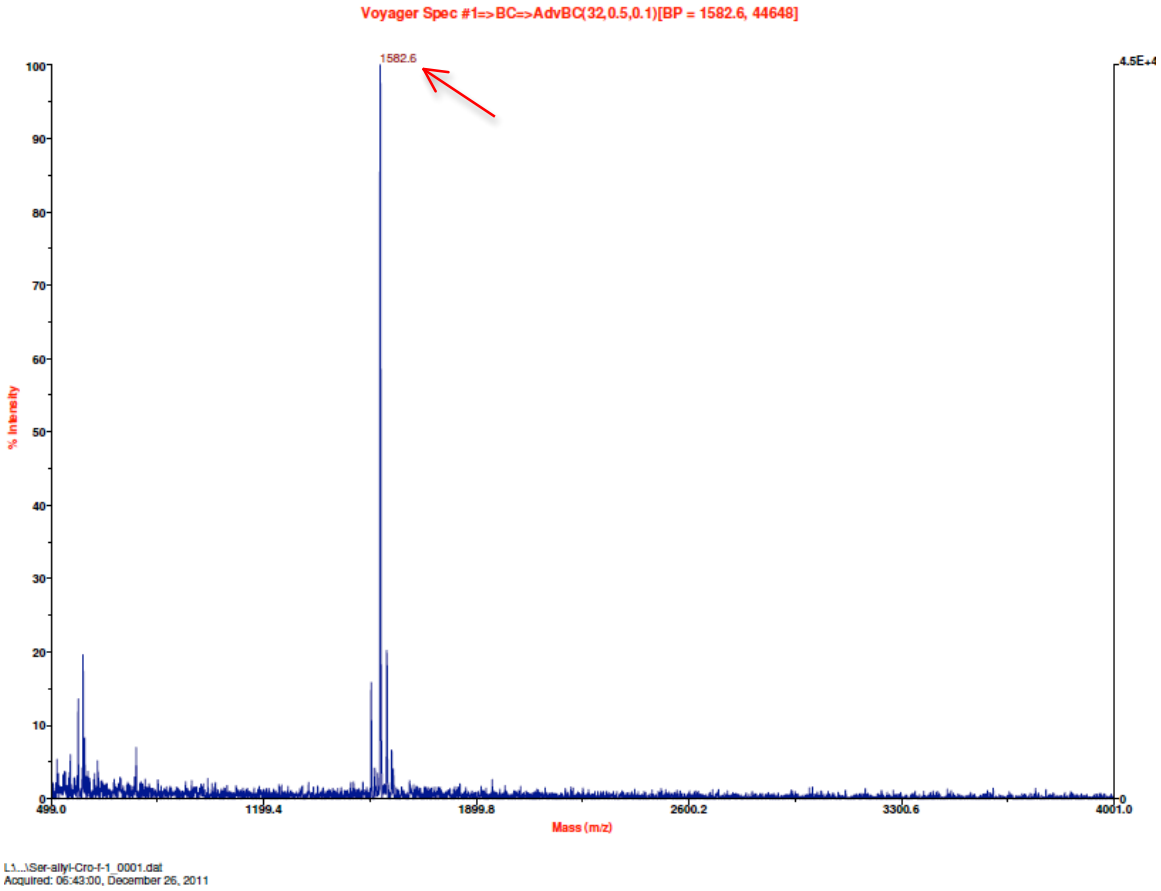
$[M-H]^-$

7,14-Pgl-crotalphine (4b)



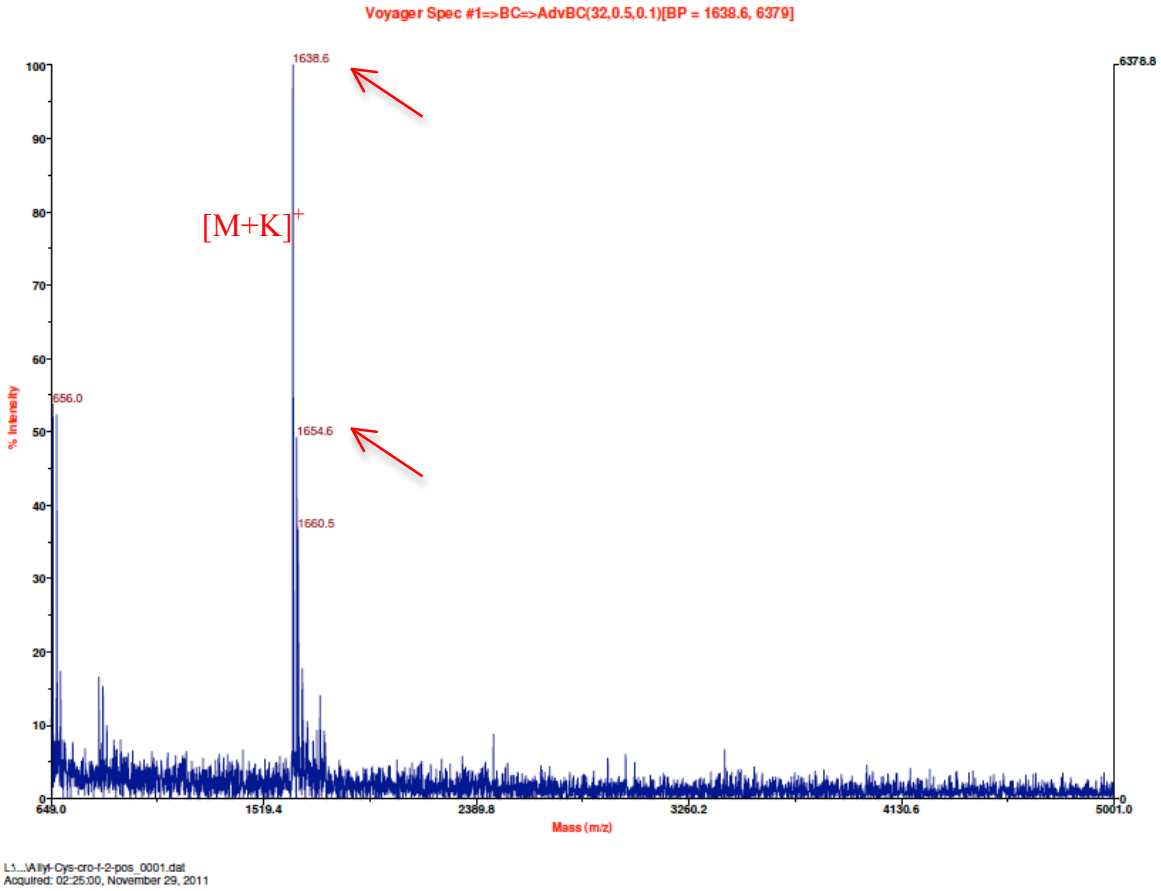
$[M-H]^-$

7,14-Oas-crotalphine (4c)



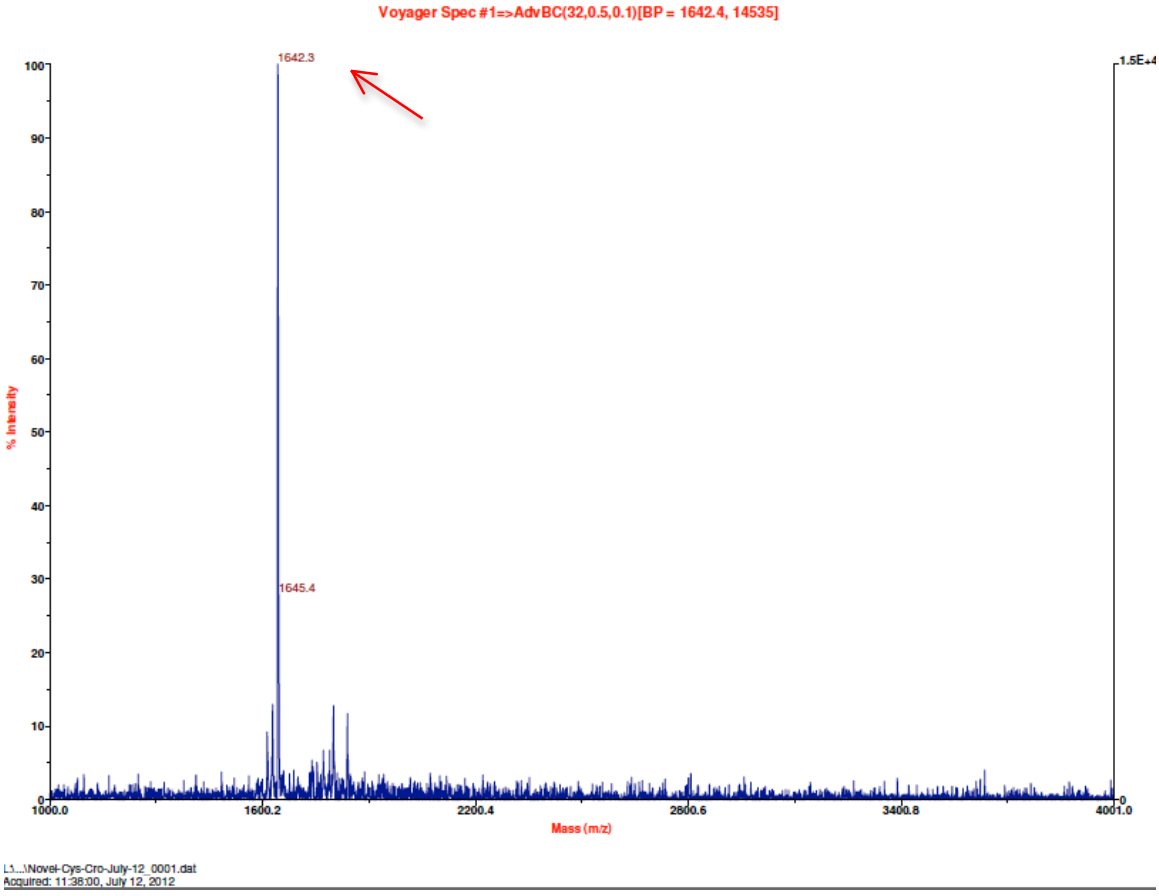
$[M+Na]^+$

7,14-Sac-crotalphine (4d)



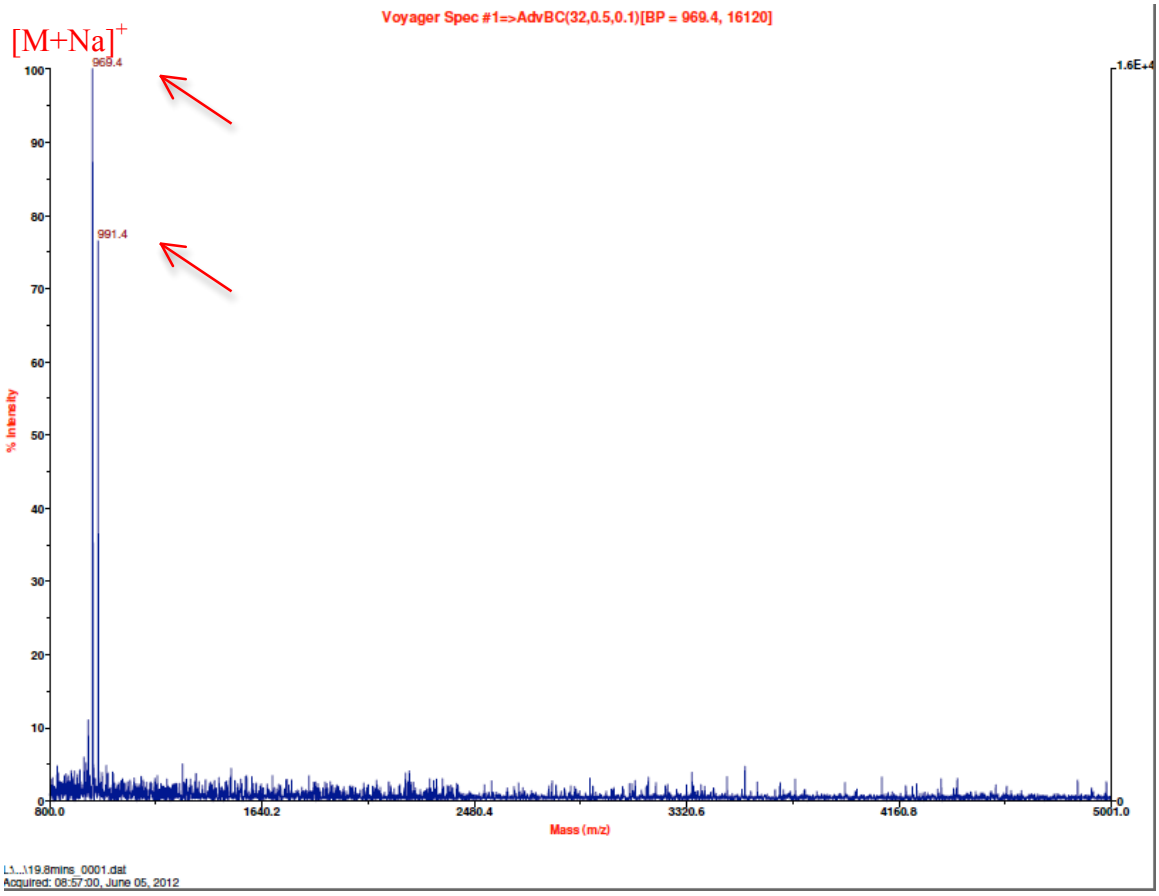
$[M-H]^-$

7,14-X-crotalphine (4e)



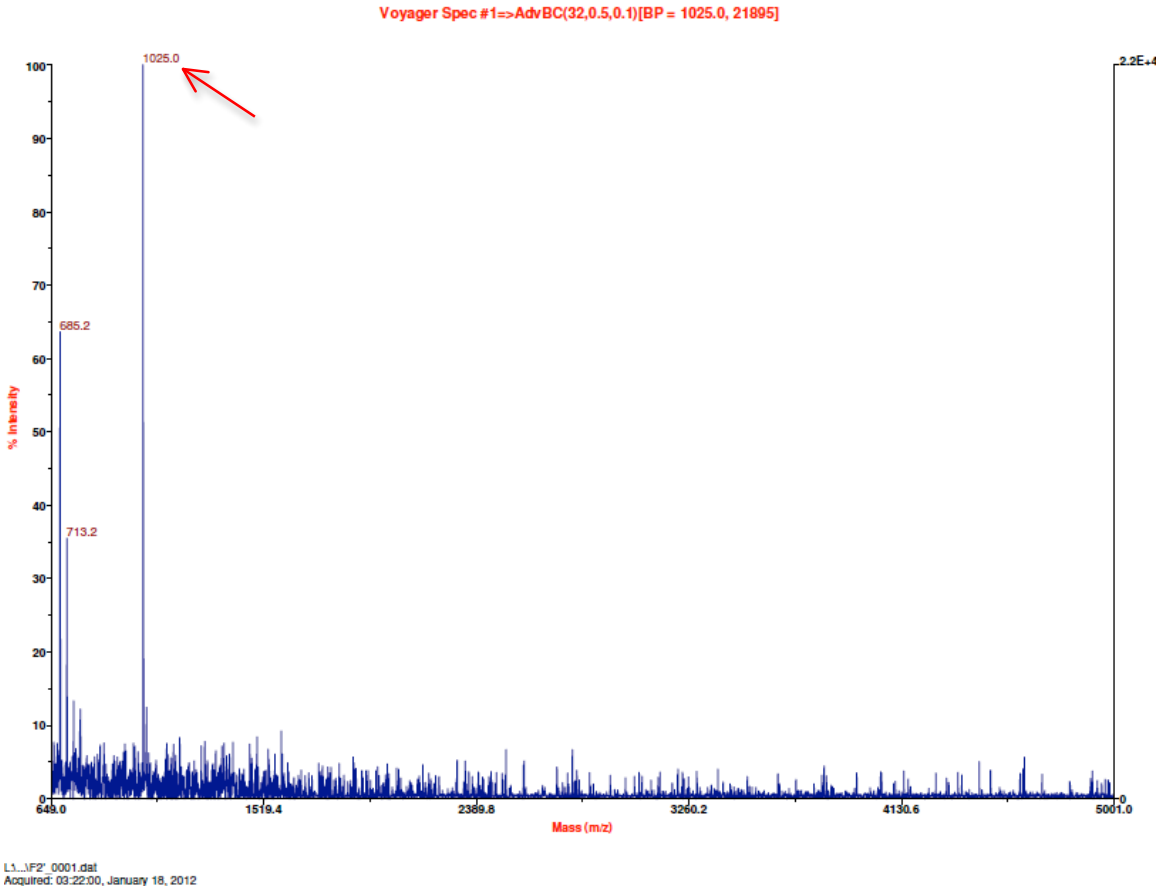
$[M+H]^+$

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

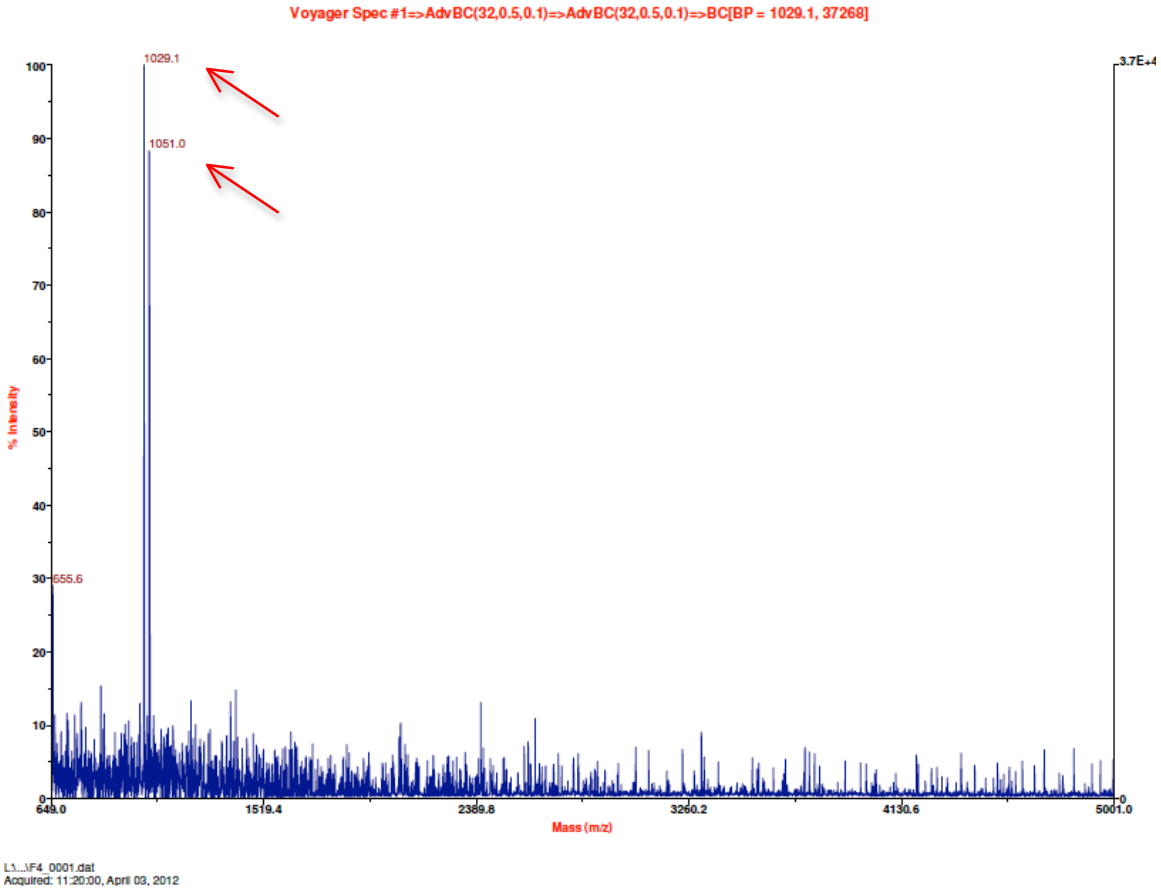


$[M+H]^+$

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

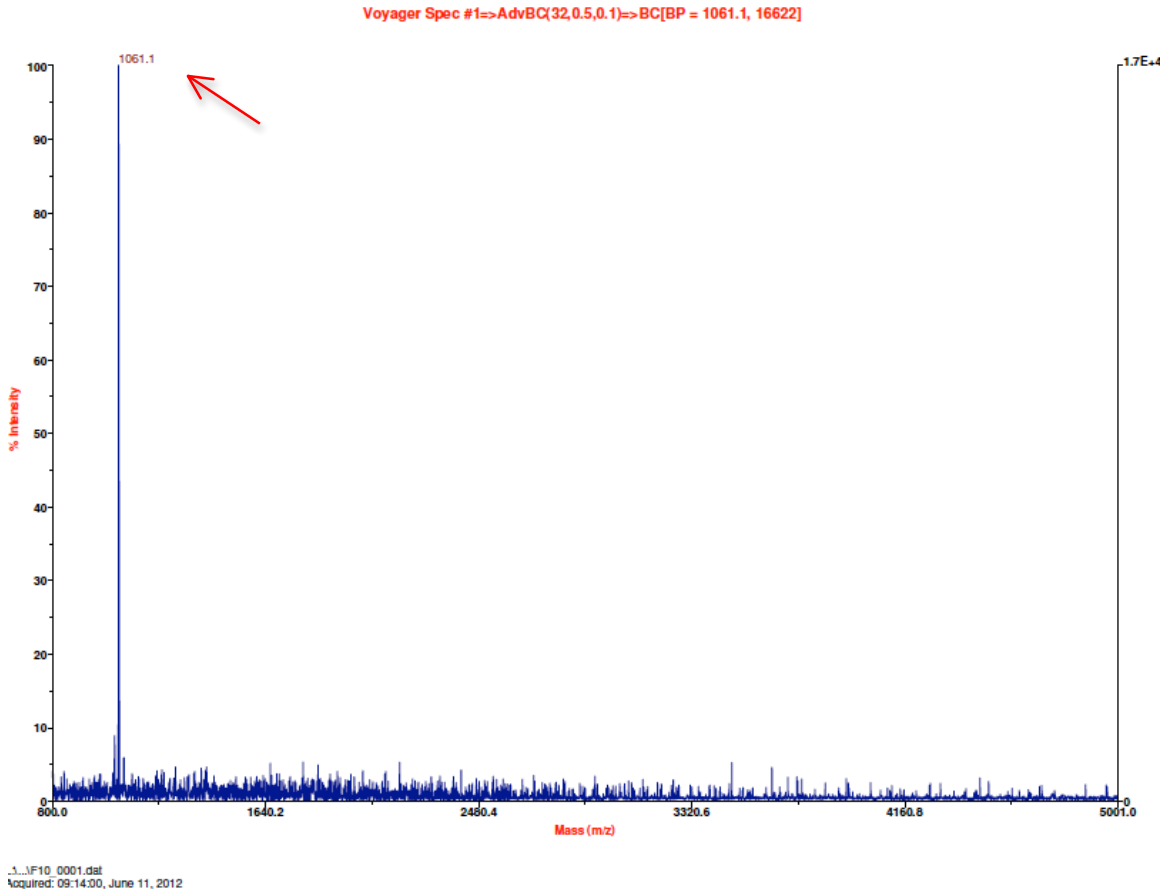


Cyclized 1,6-Oas-oxytocin (3i)



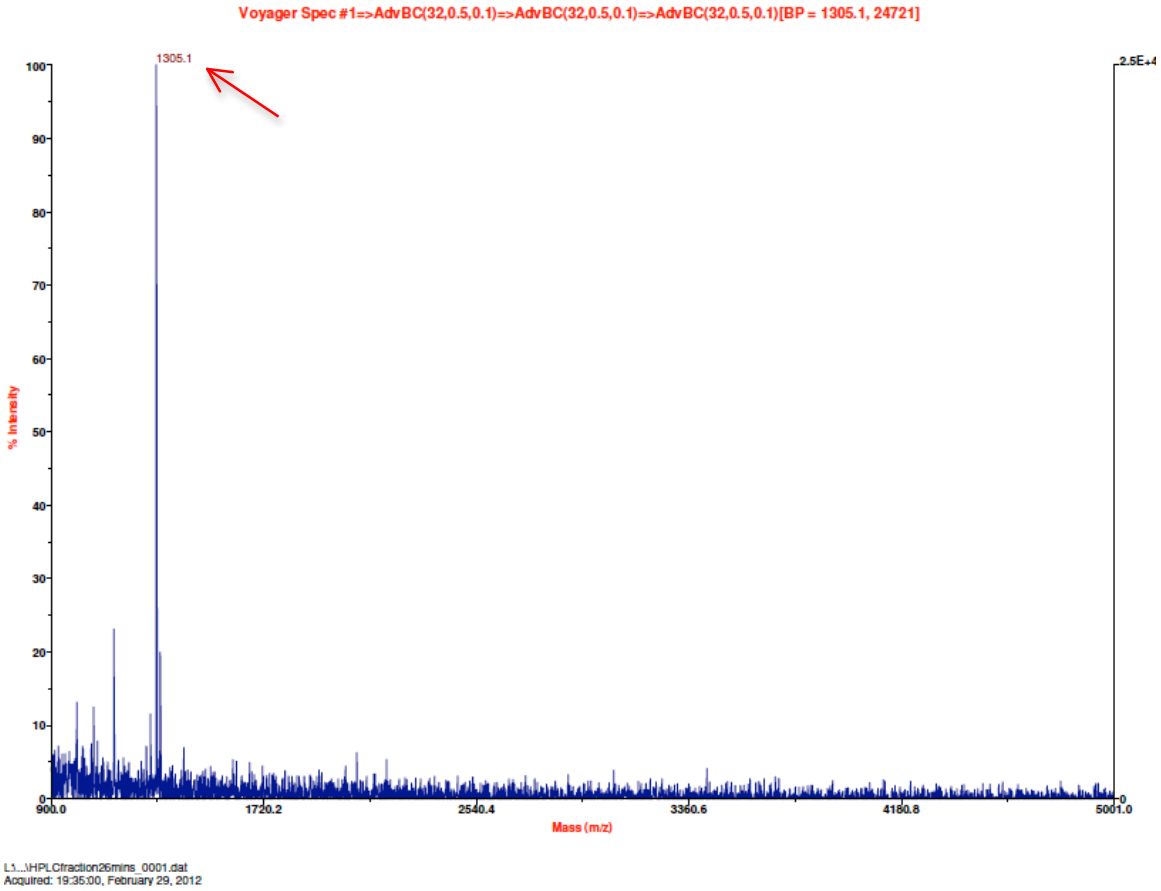
$[M+H]^+$

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



$[M+Na]^+$

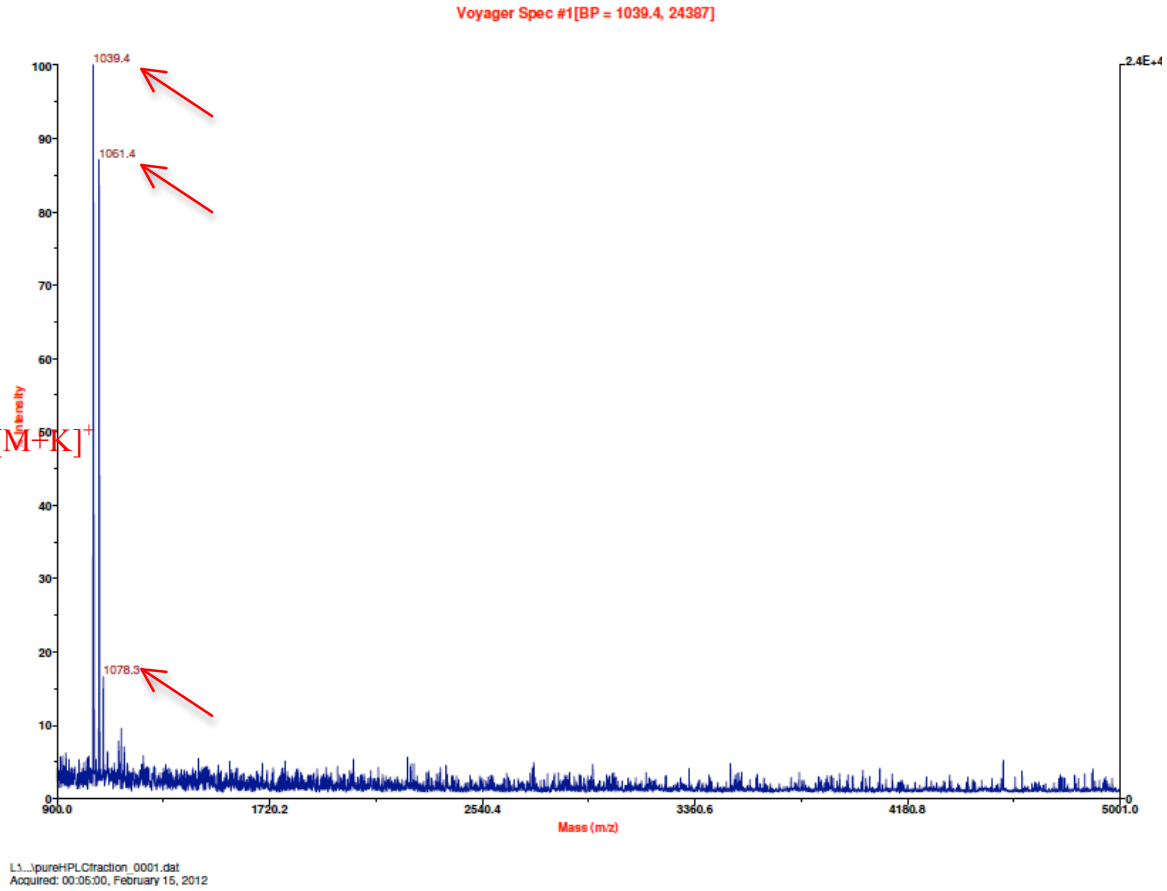
Cyclized Fmoc-1,6-Sac-oxytocin (3j')



[M+H]$^+$

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

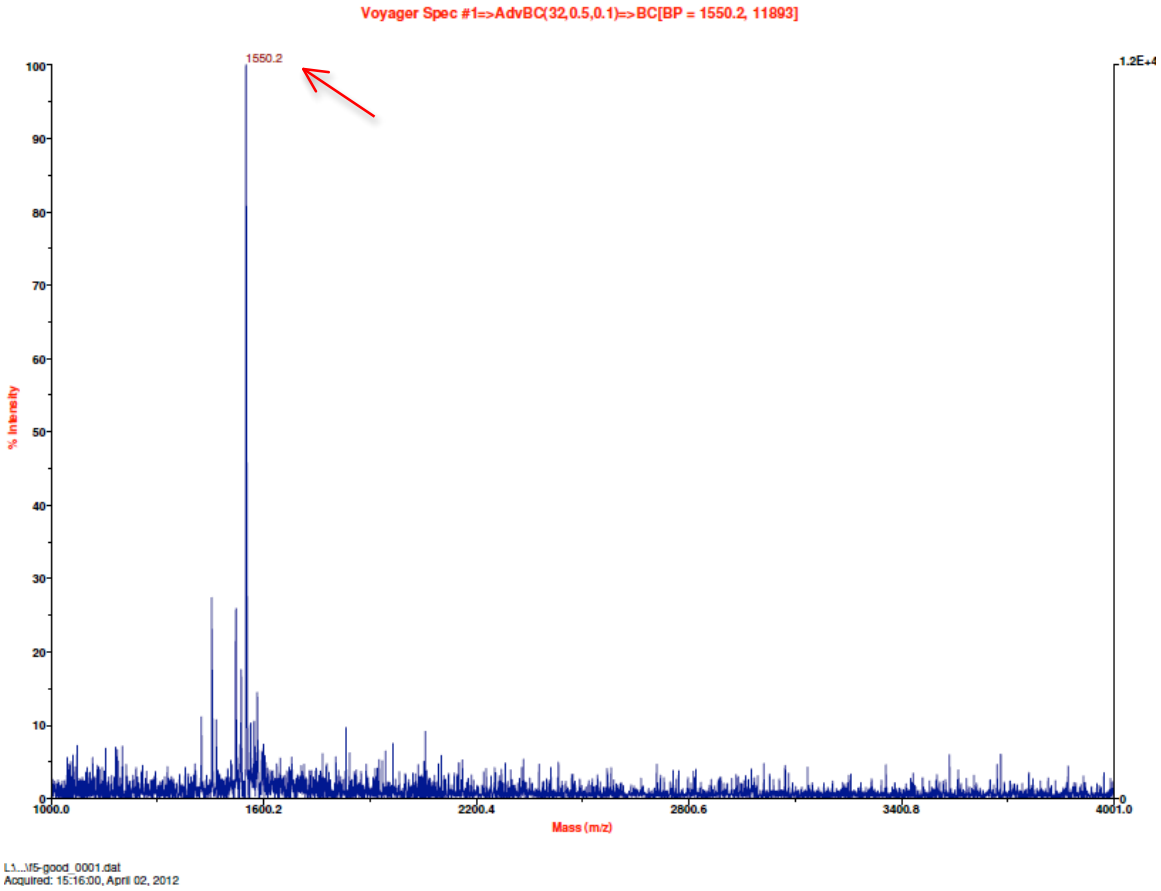
[M+Na]$^+$



[M+K]$^+$

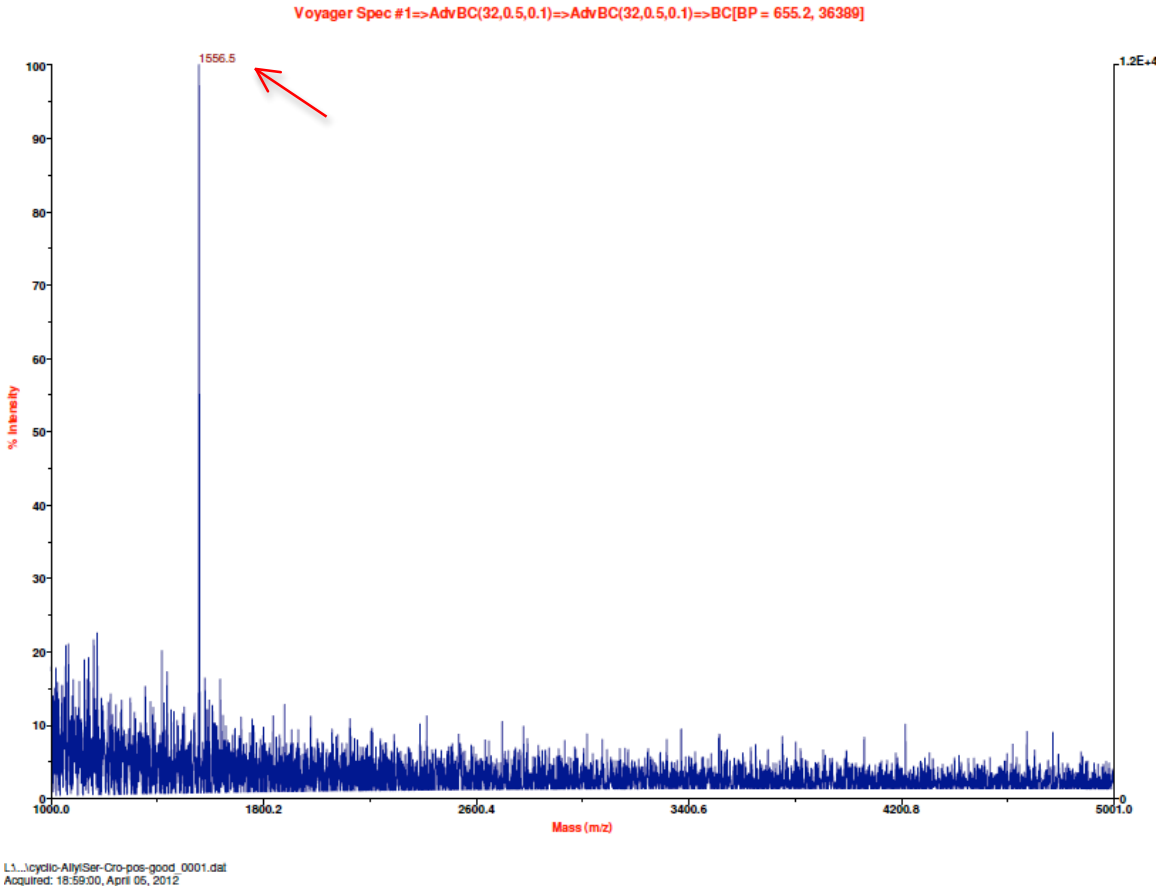
$[M-H]^-$

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



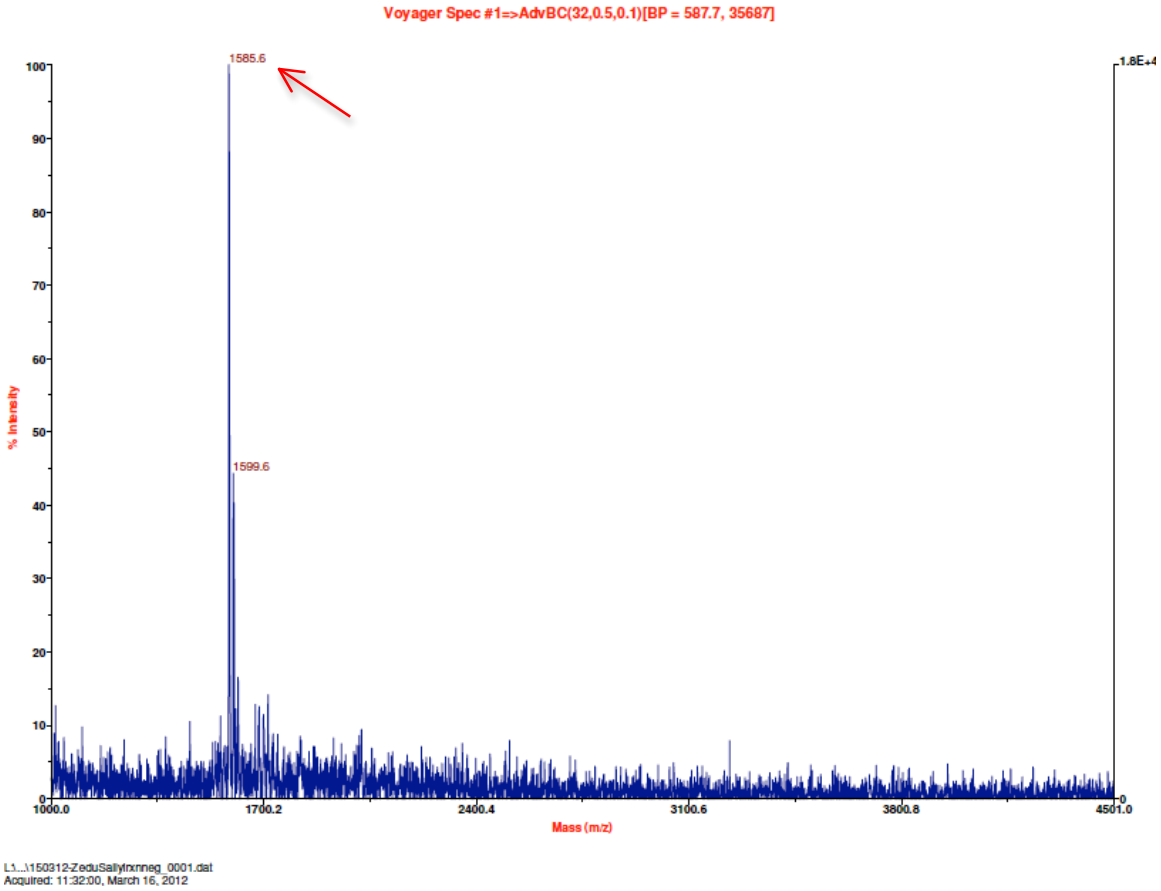
$[M+H]^+$

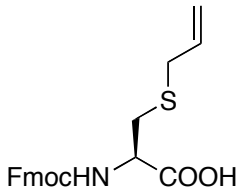
Cyclized 7,14-Oas-crotalphine (4i)

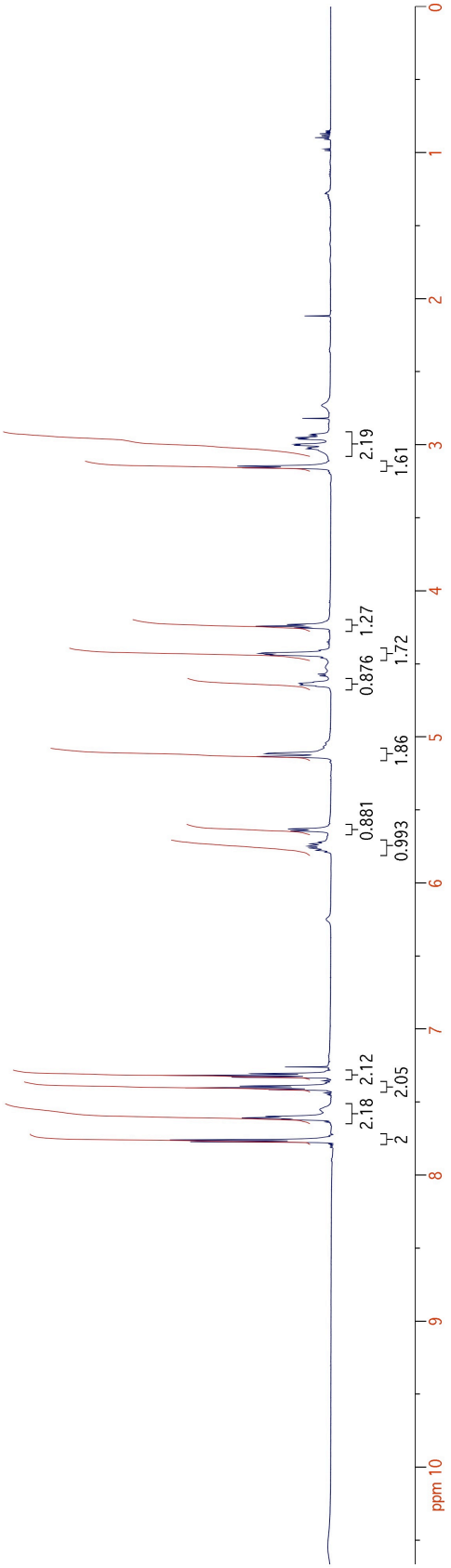


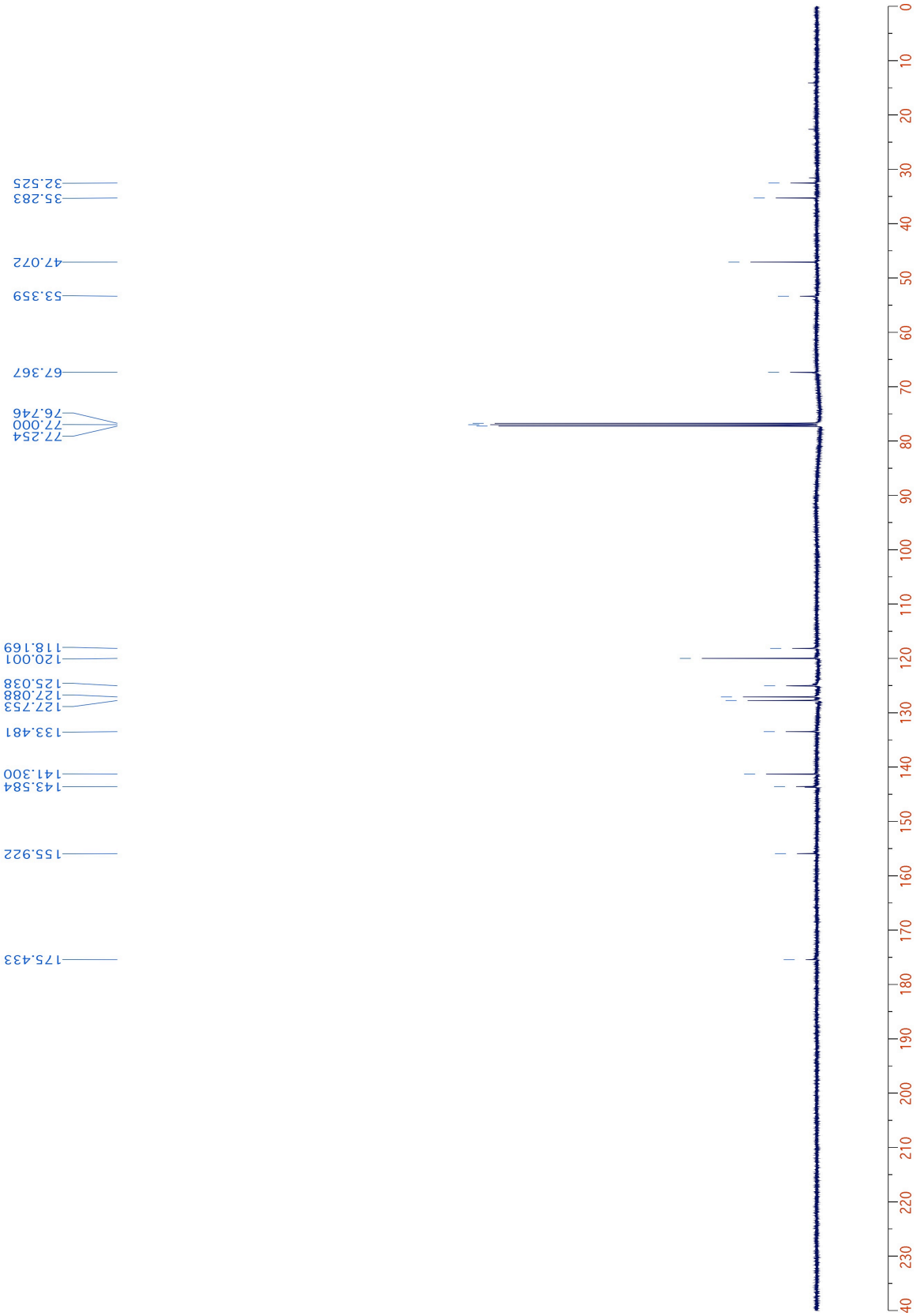
$[M-H]^-$

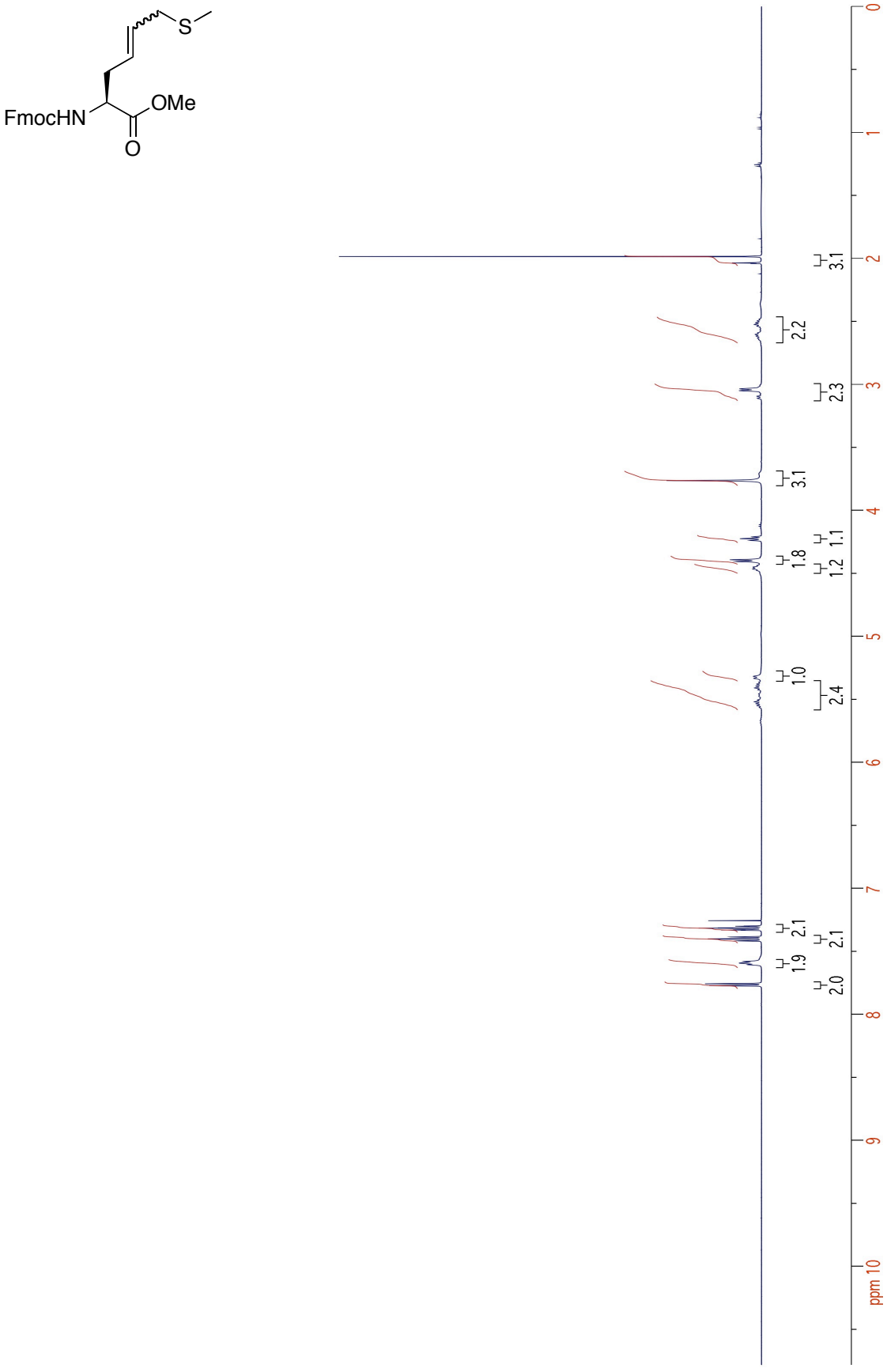
Cyclized 7,14-Sac-crotalphine (4j)

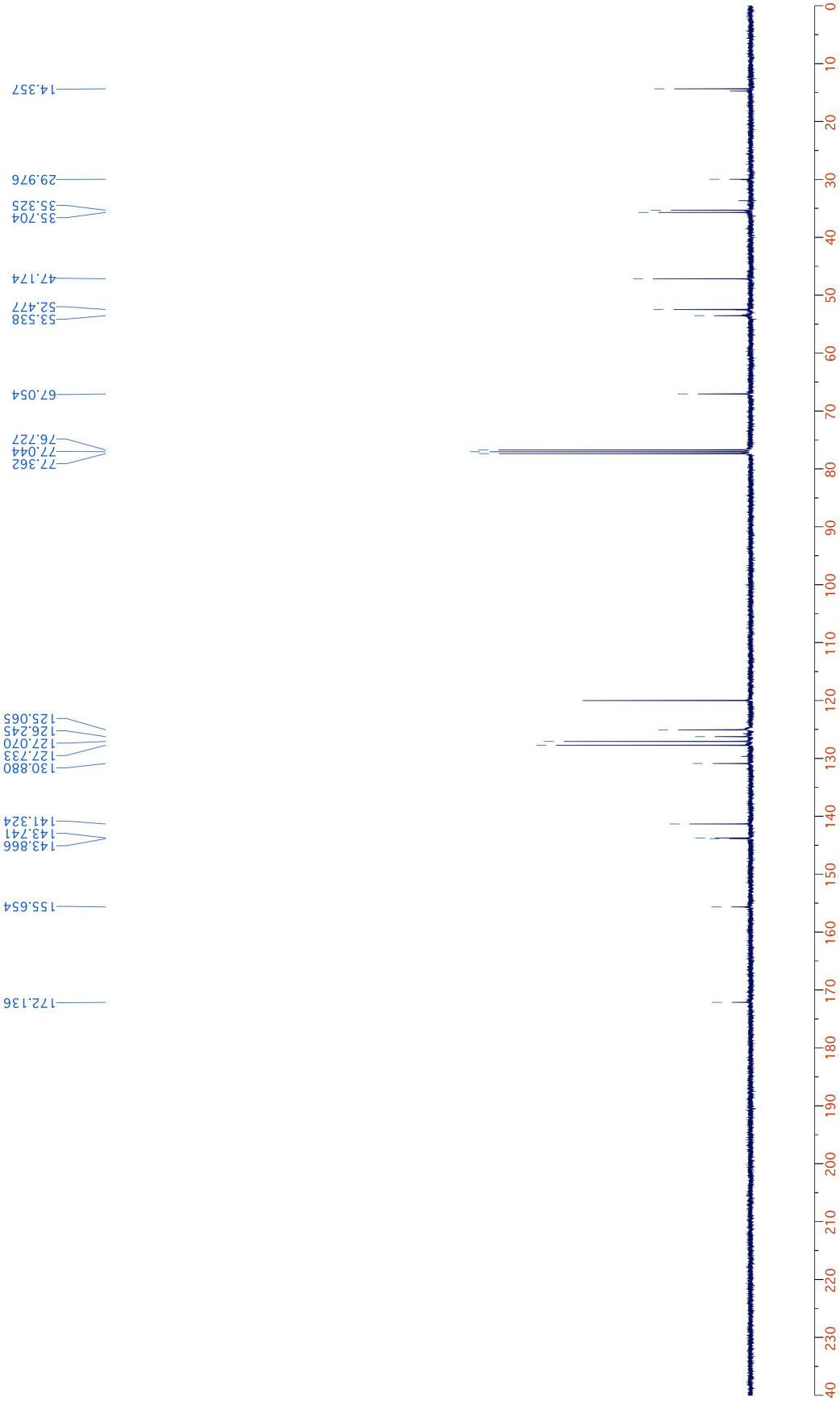








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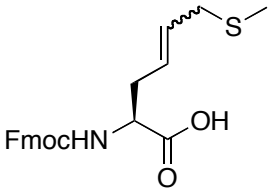

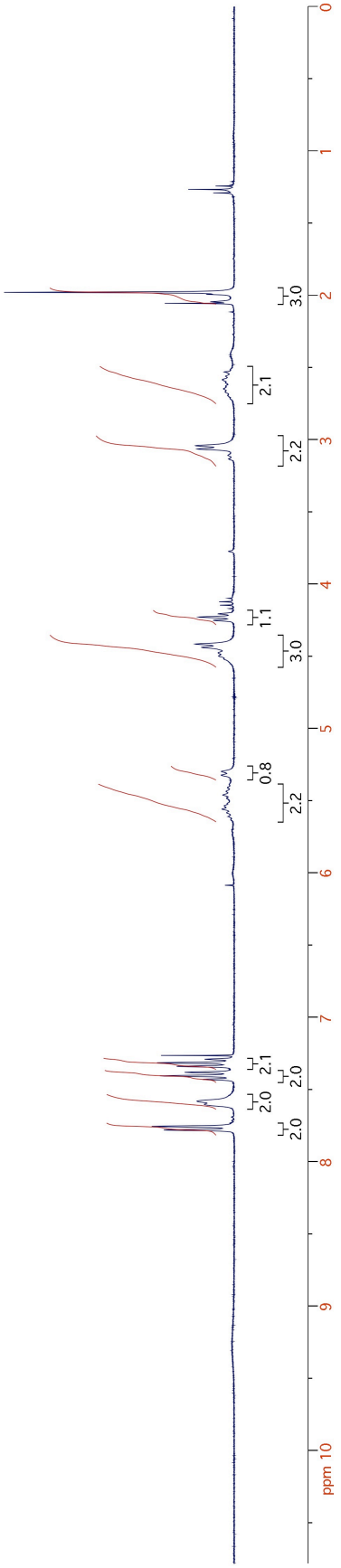

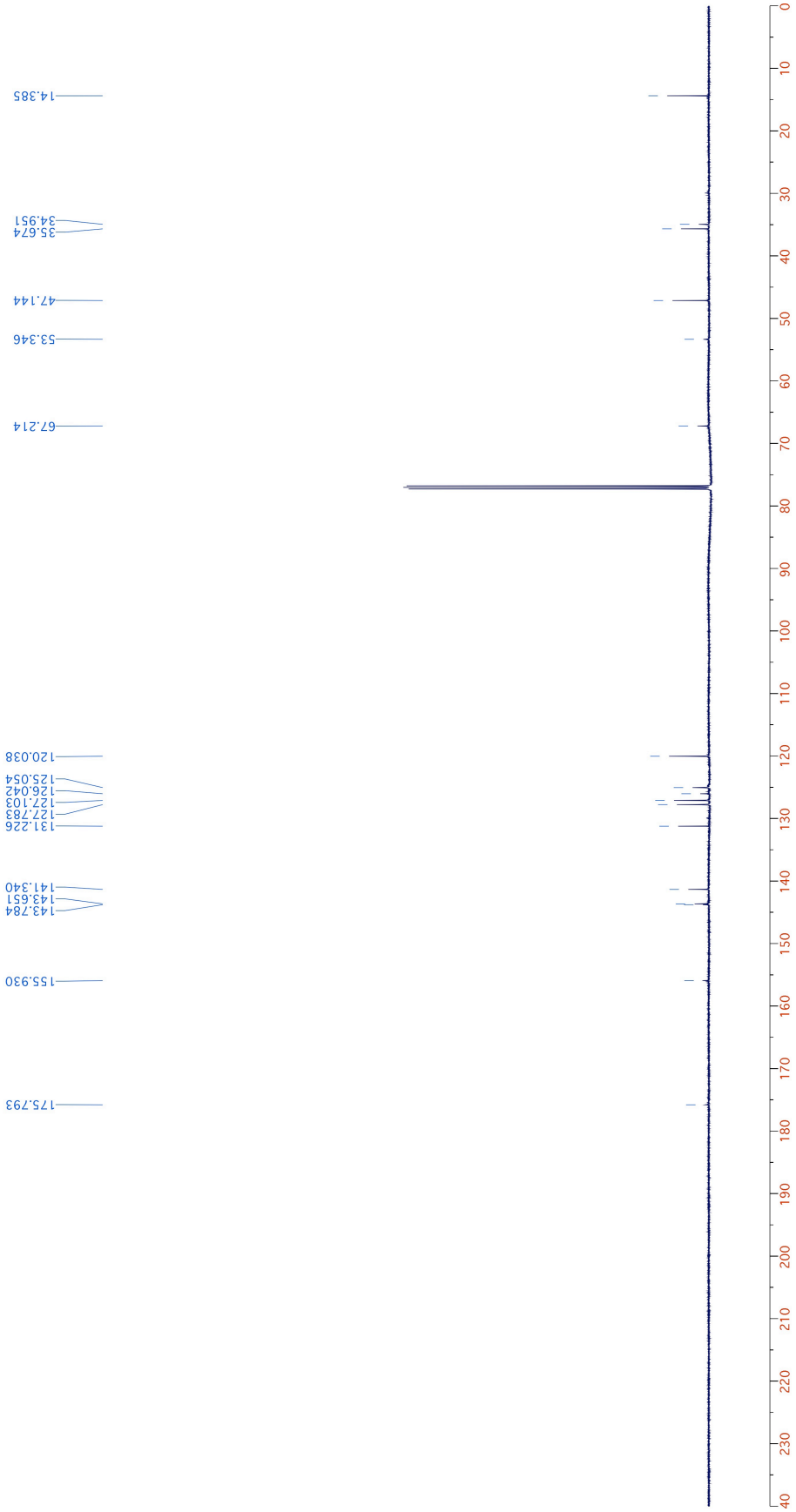
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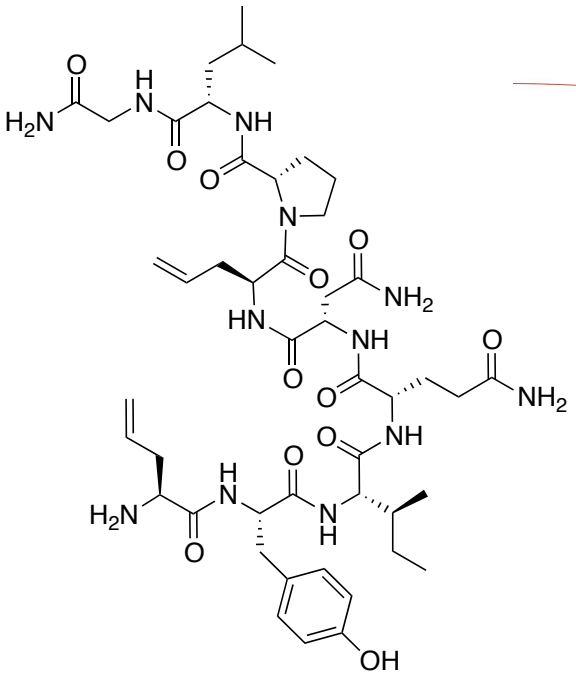

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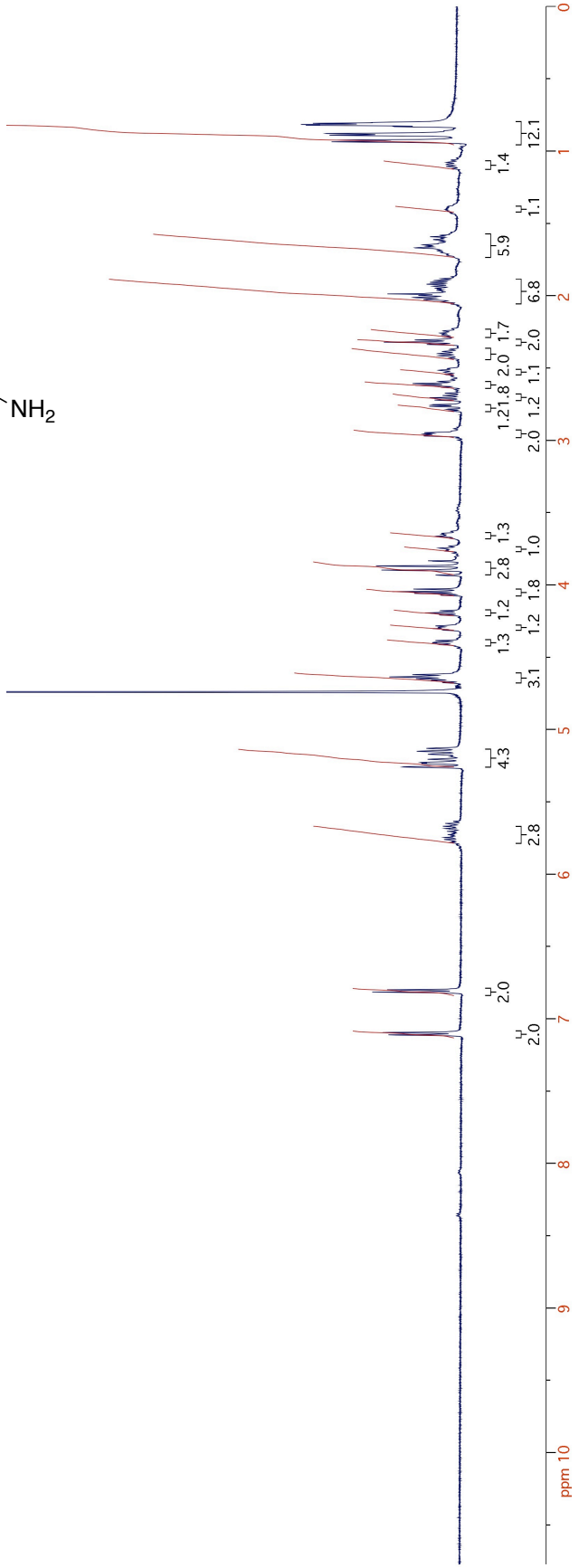

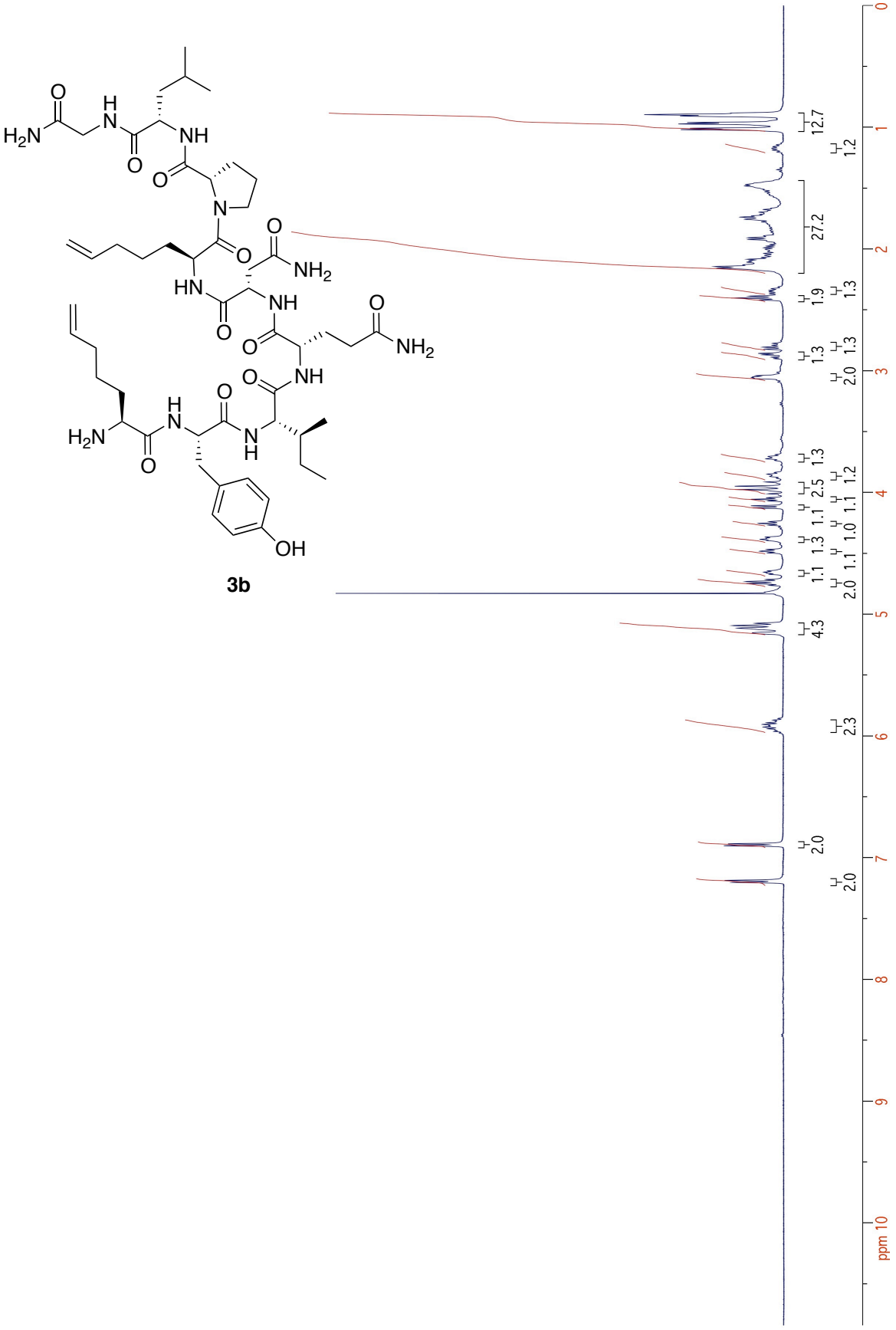

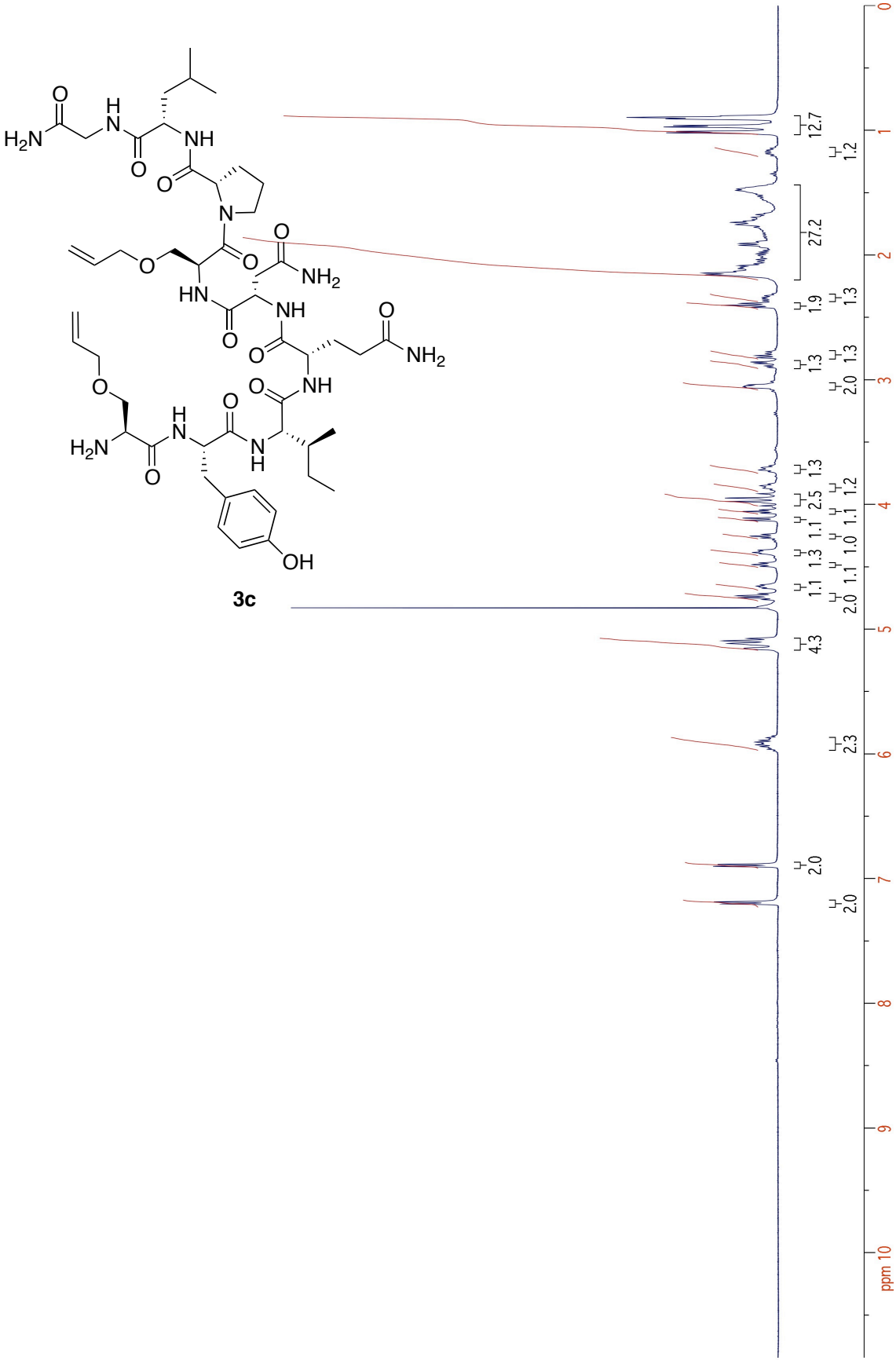

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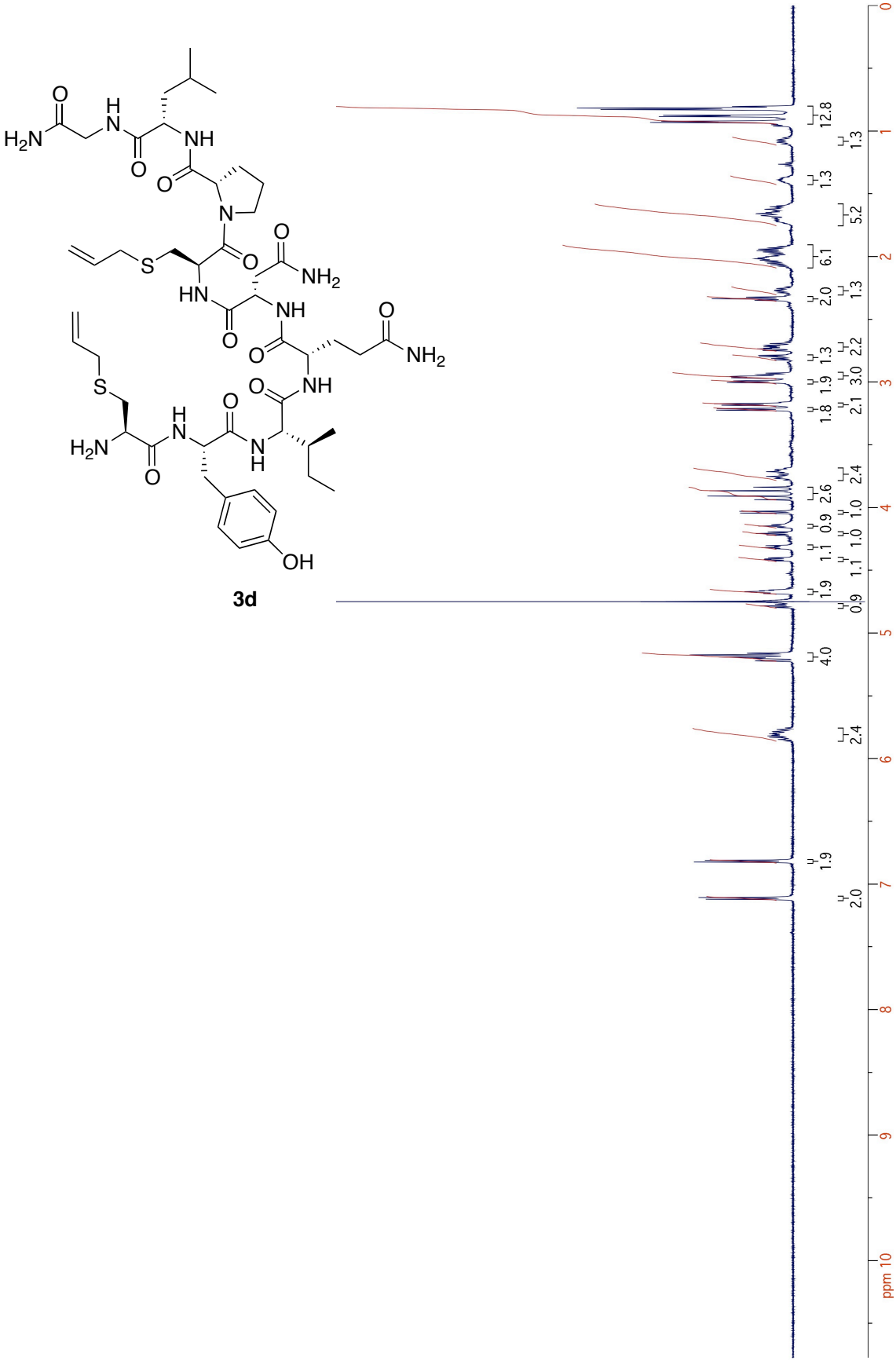

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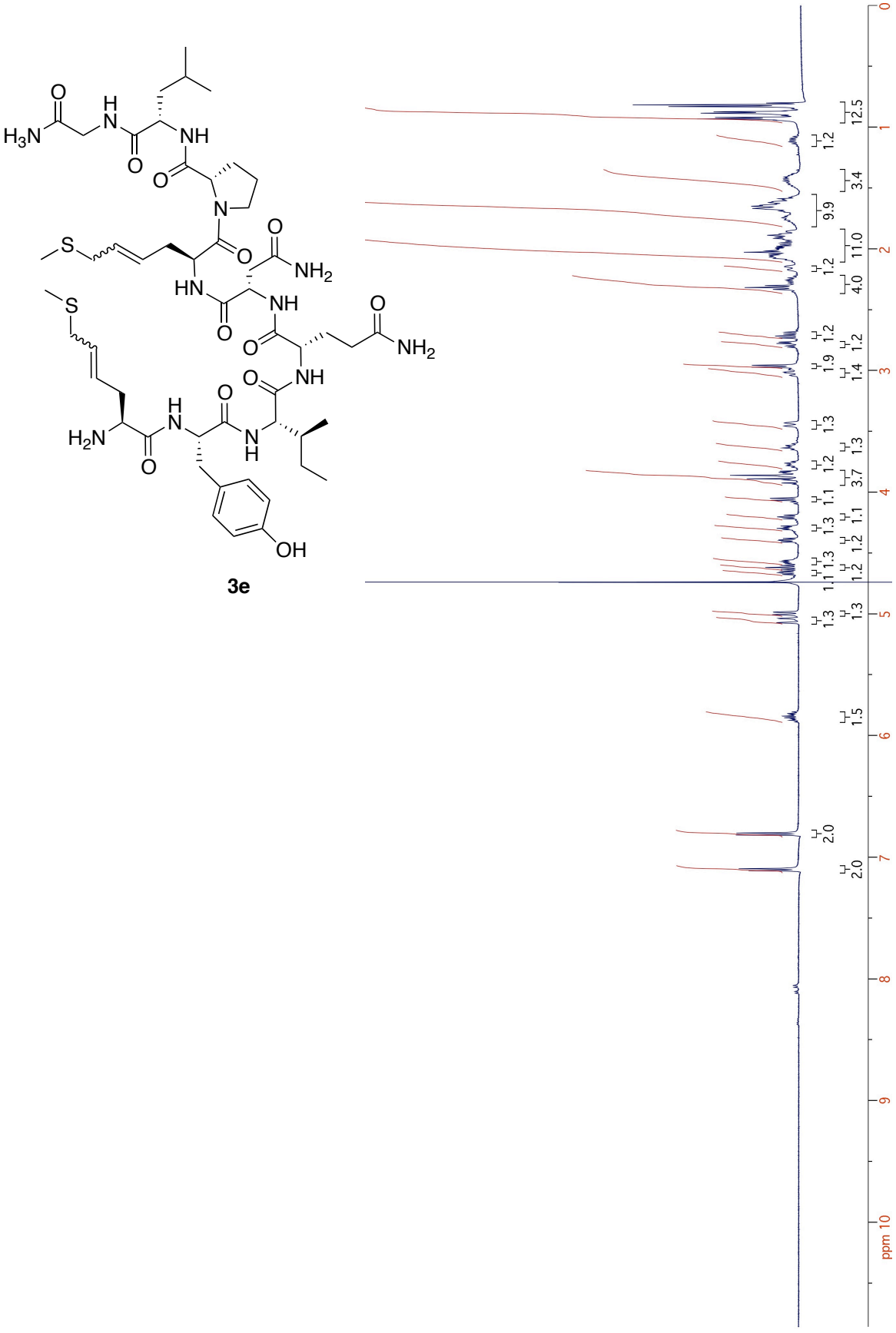

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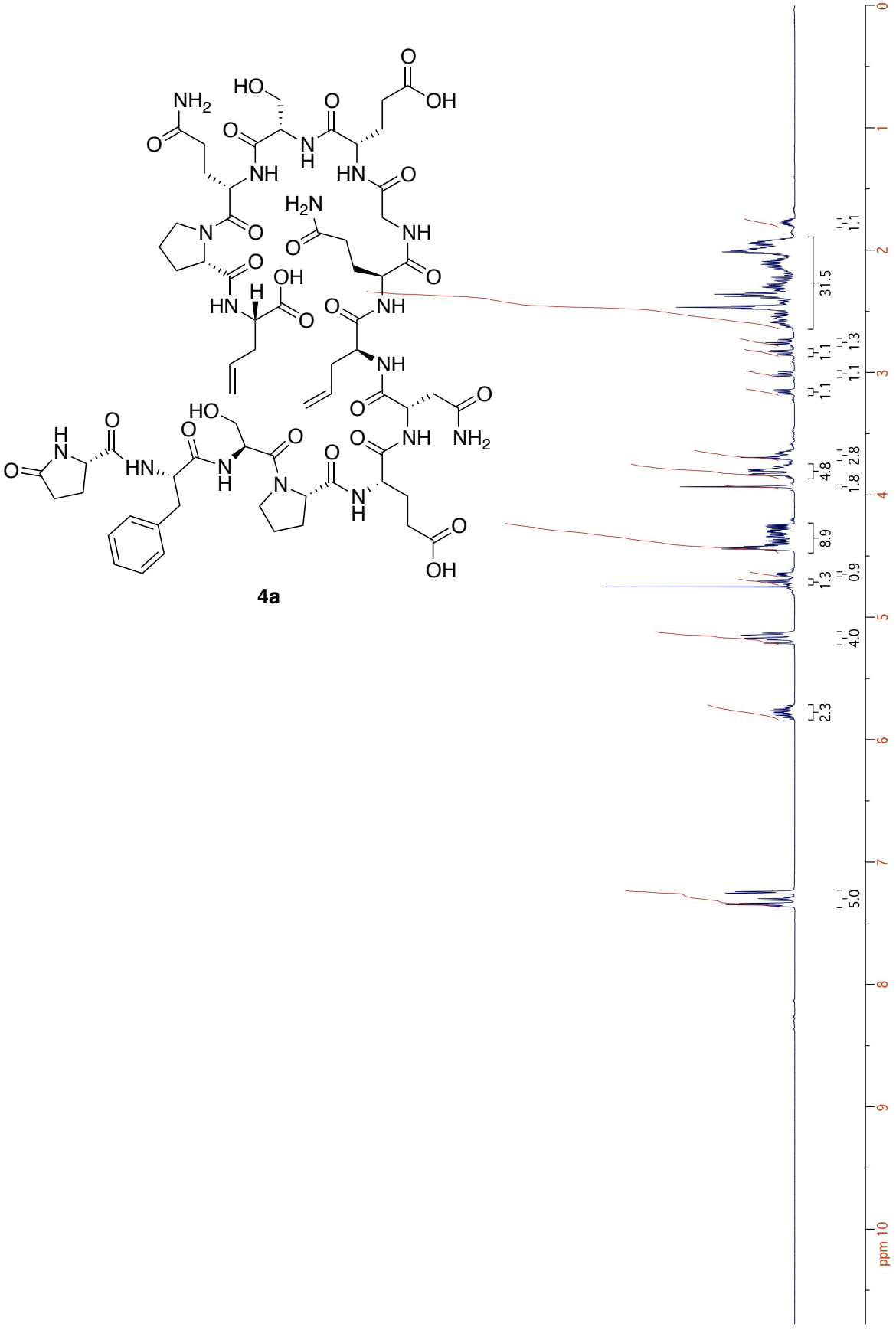

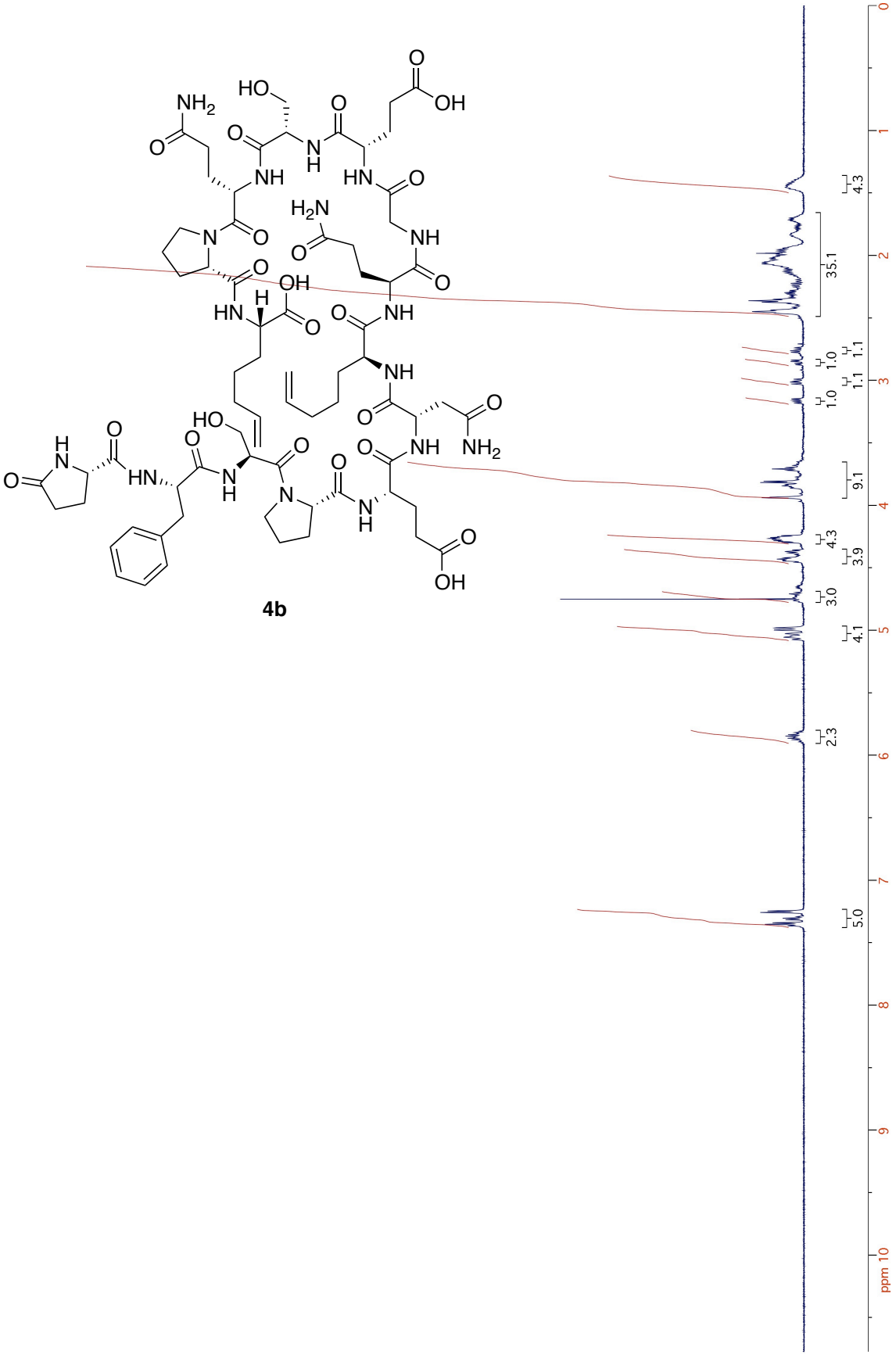

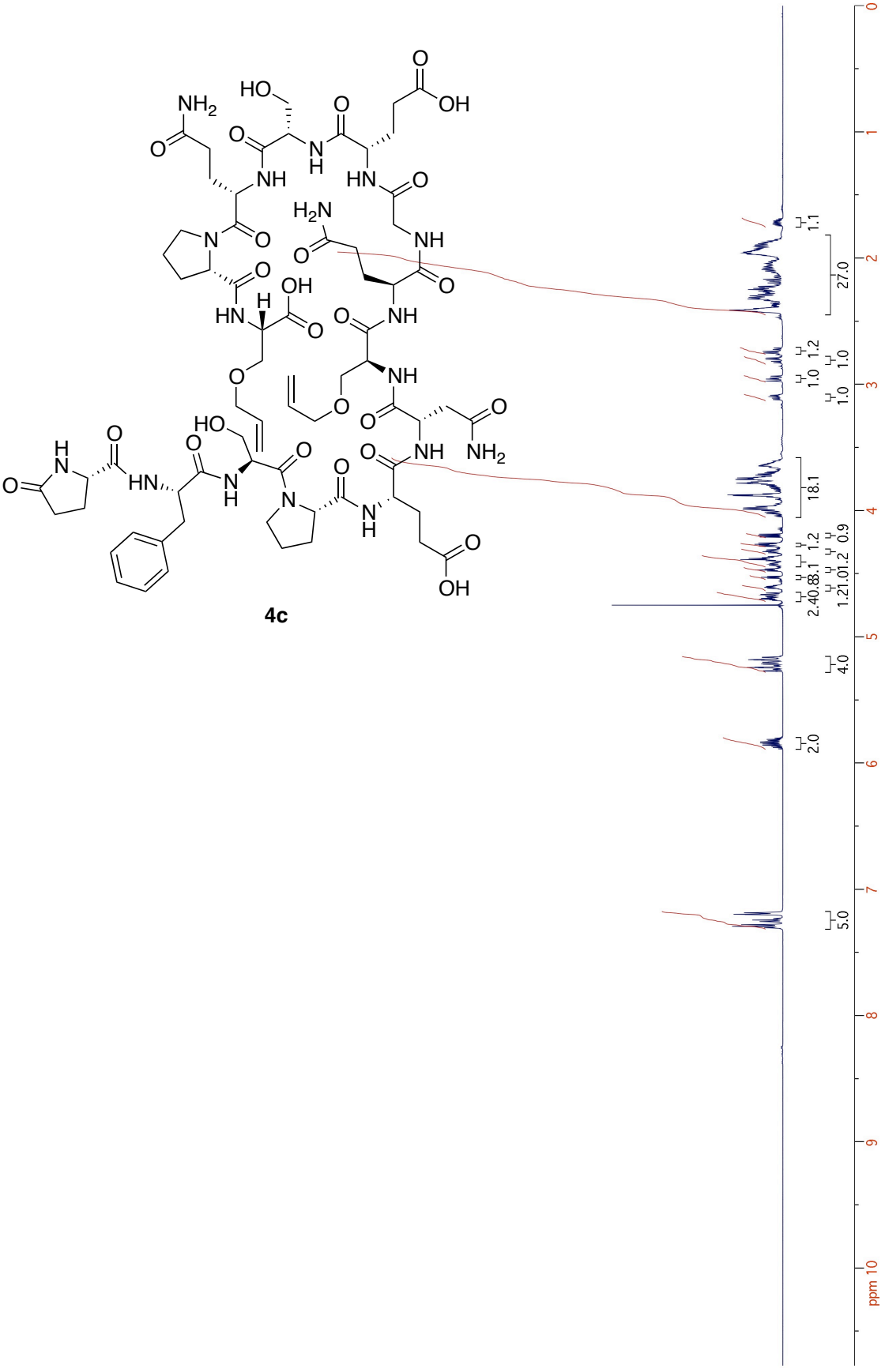

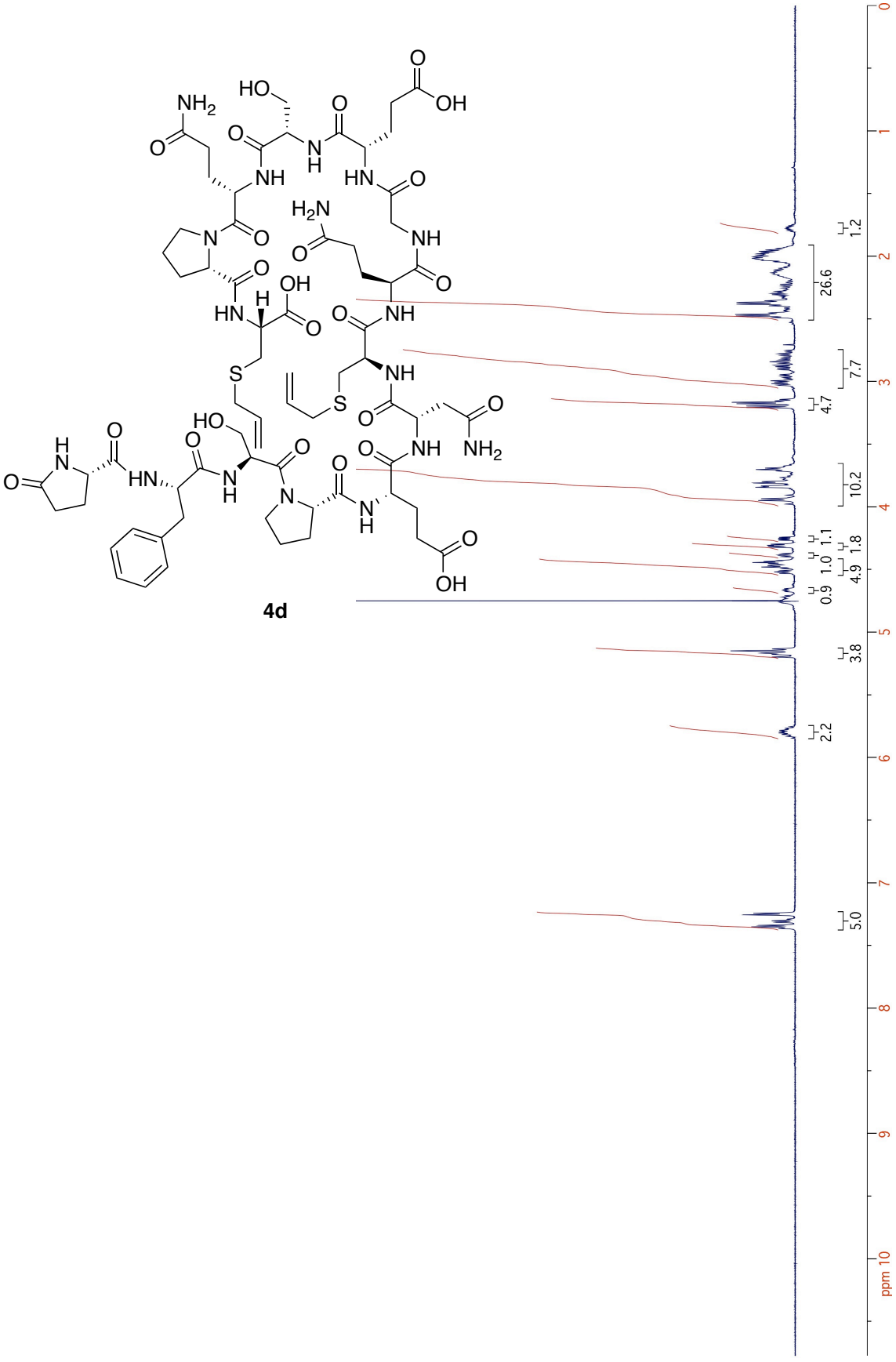

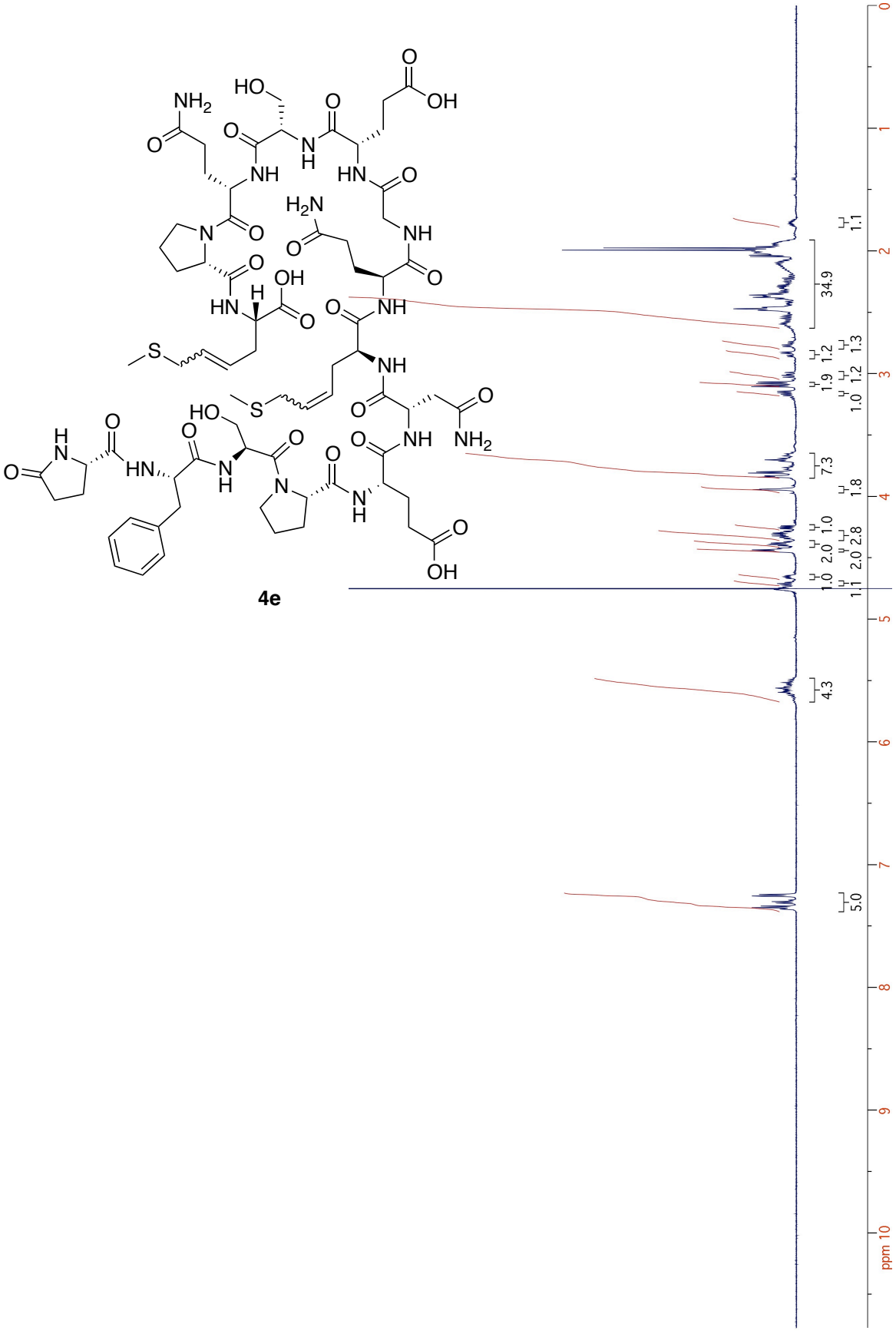

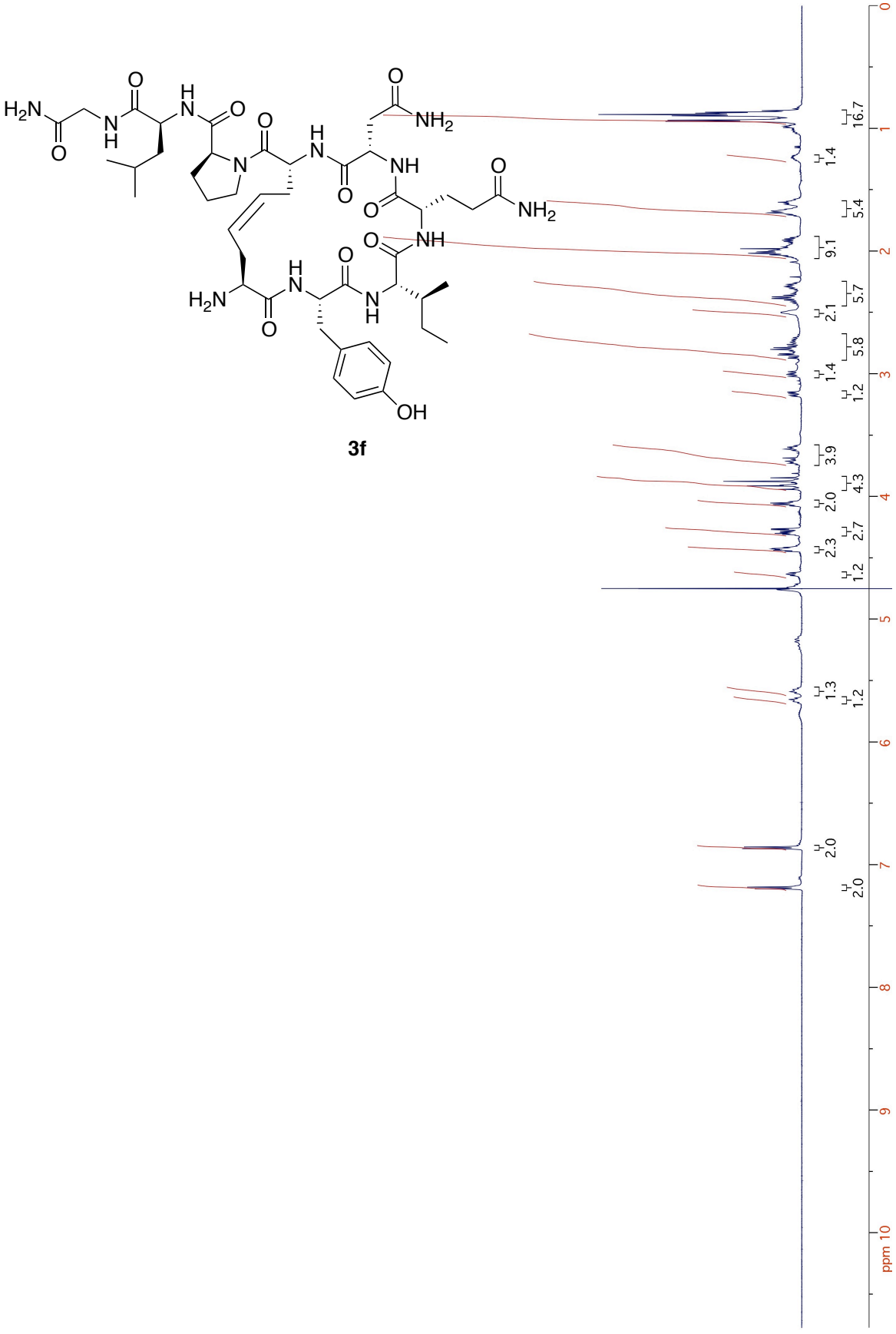

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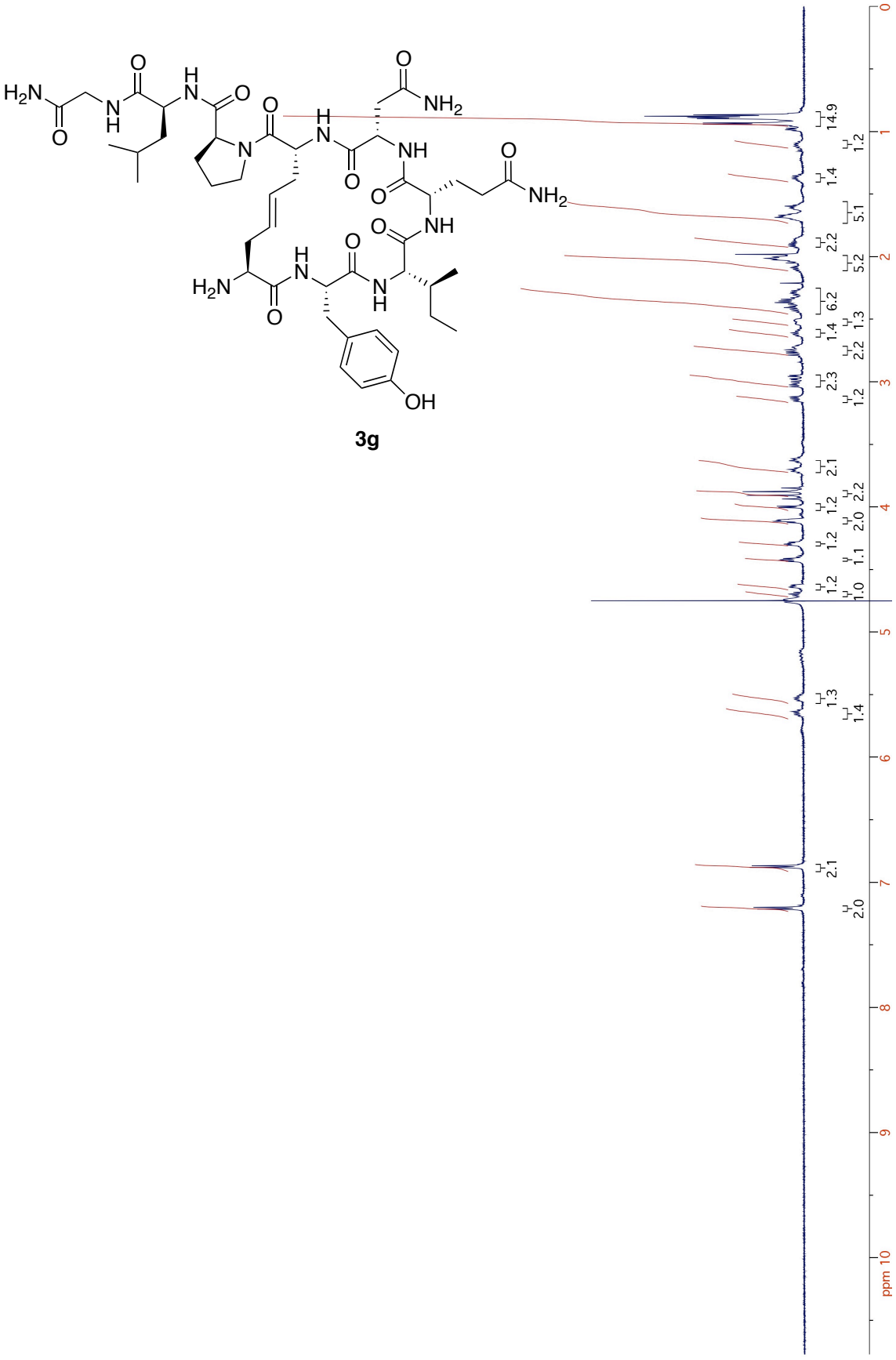

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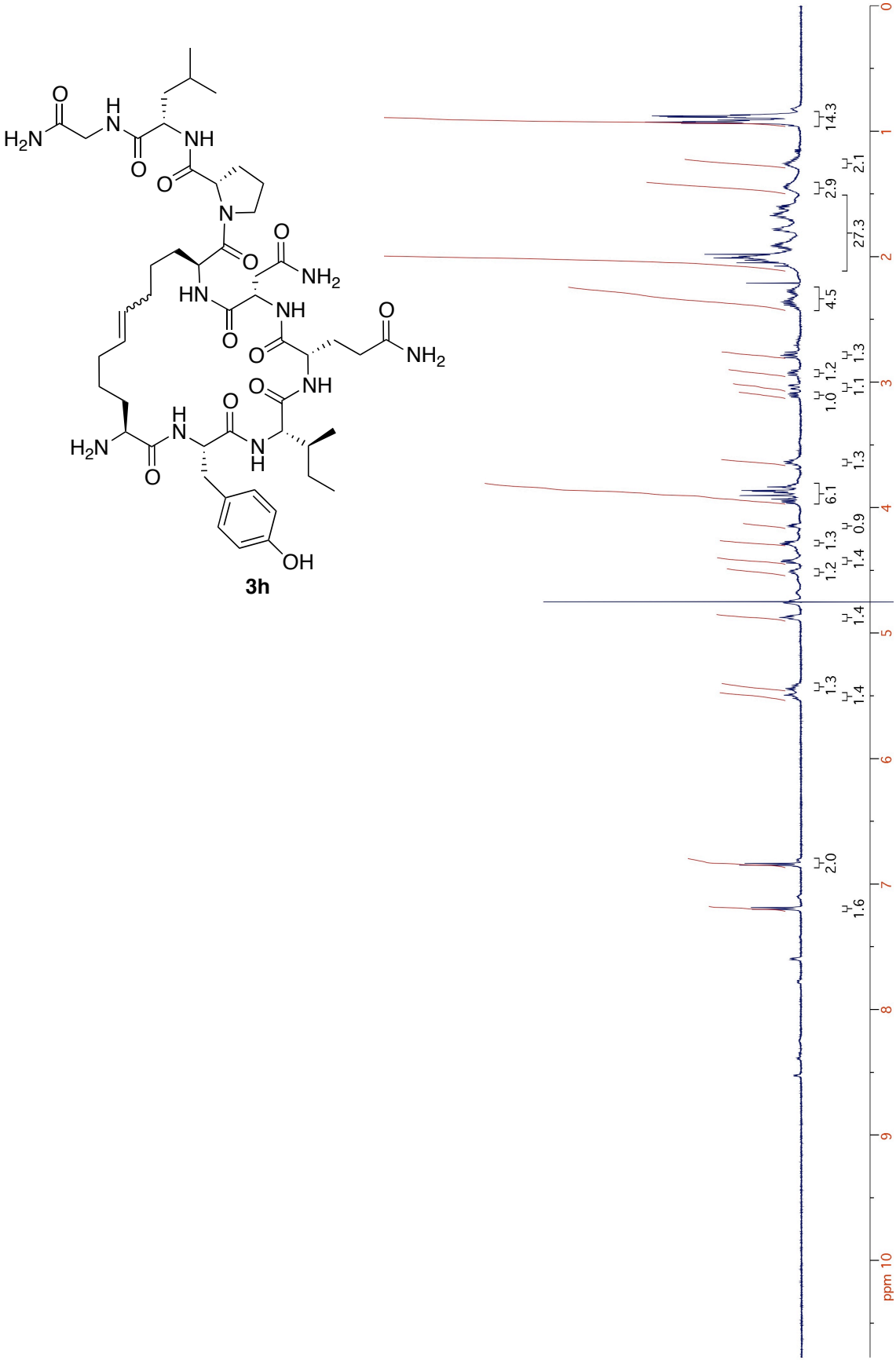

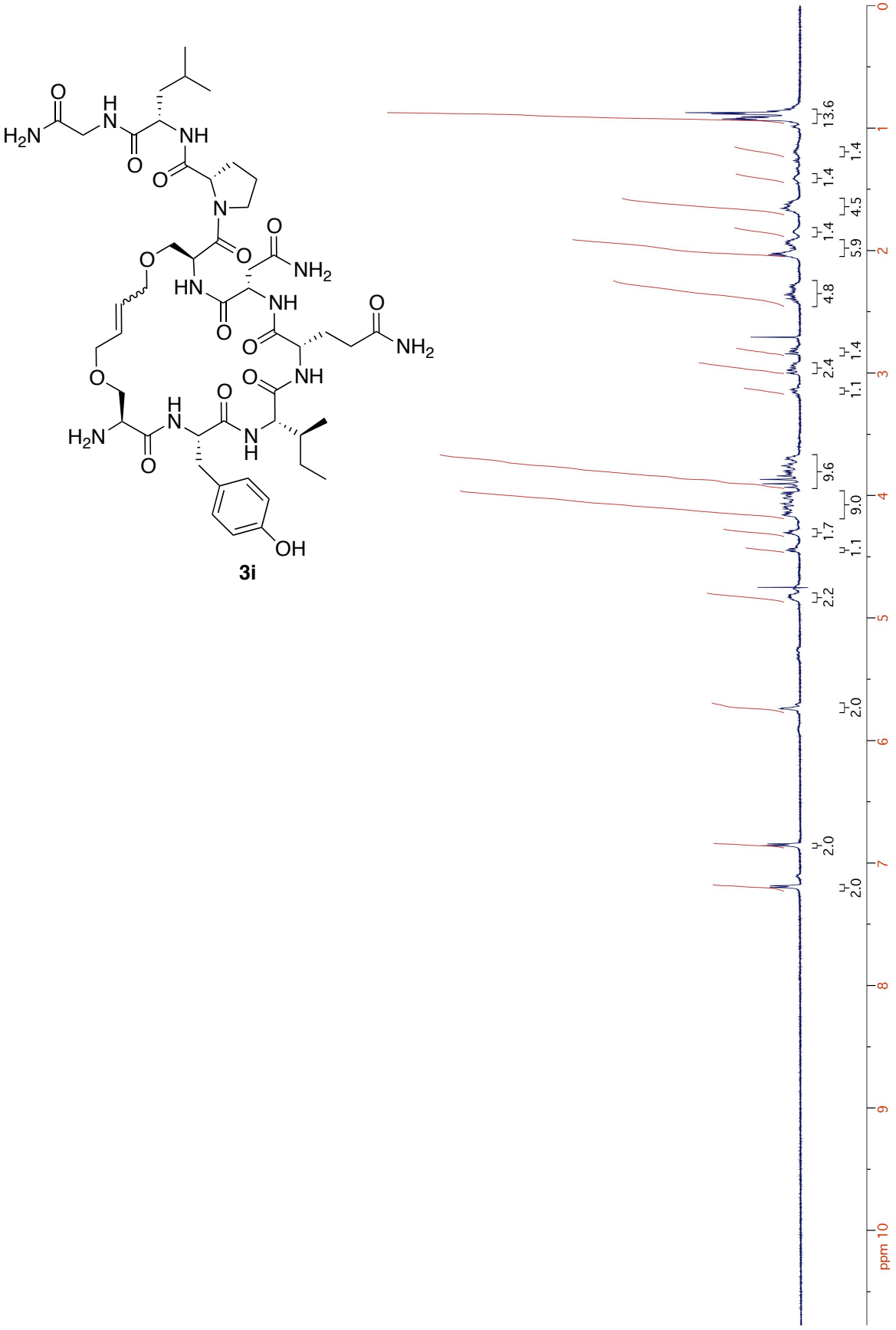

3i