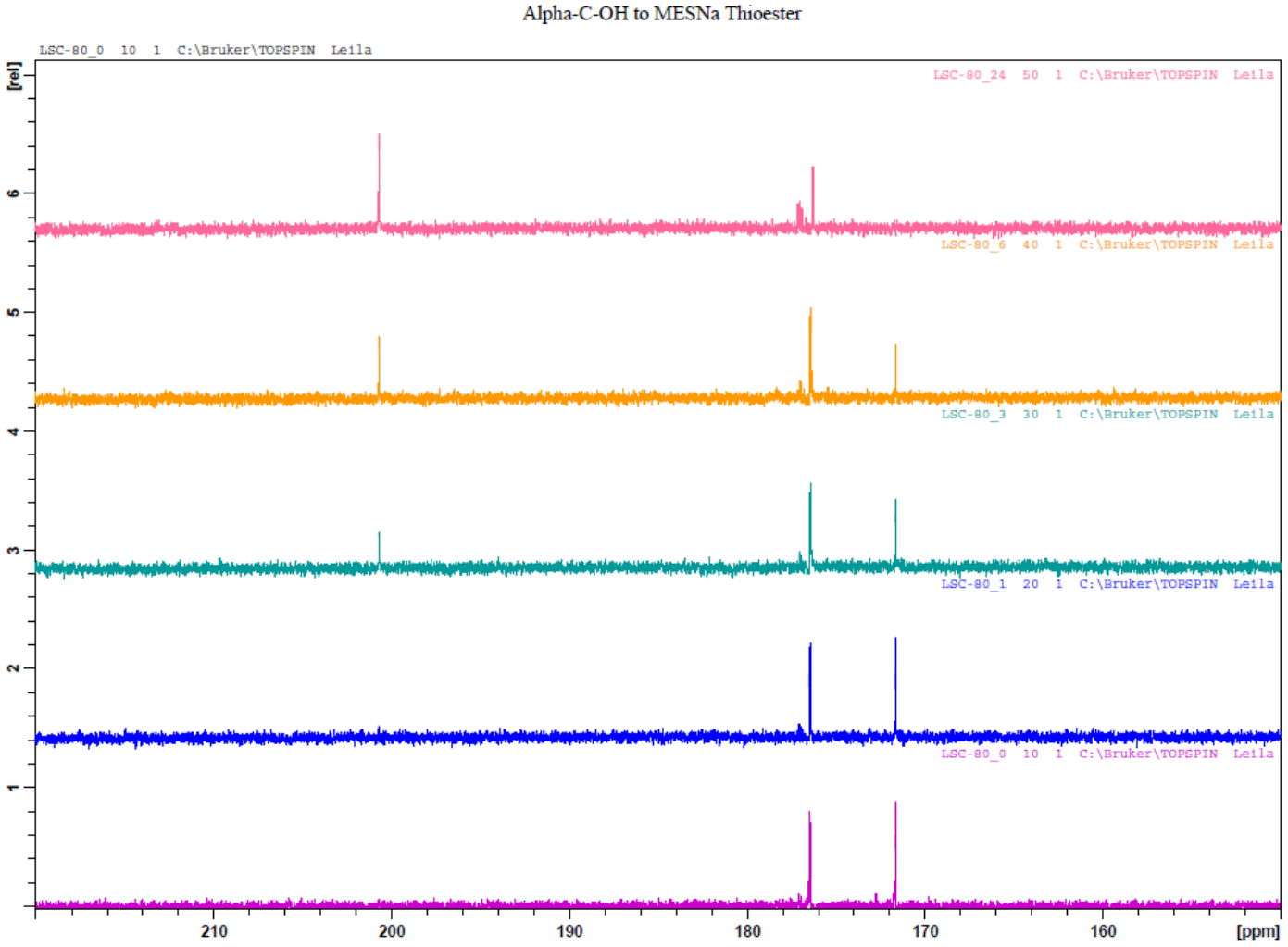
Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Supporting information

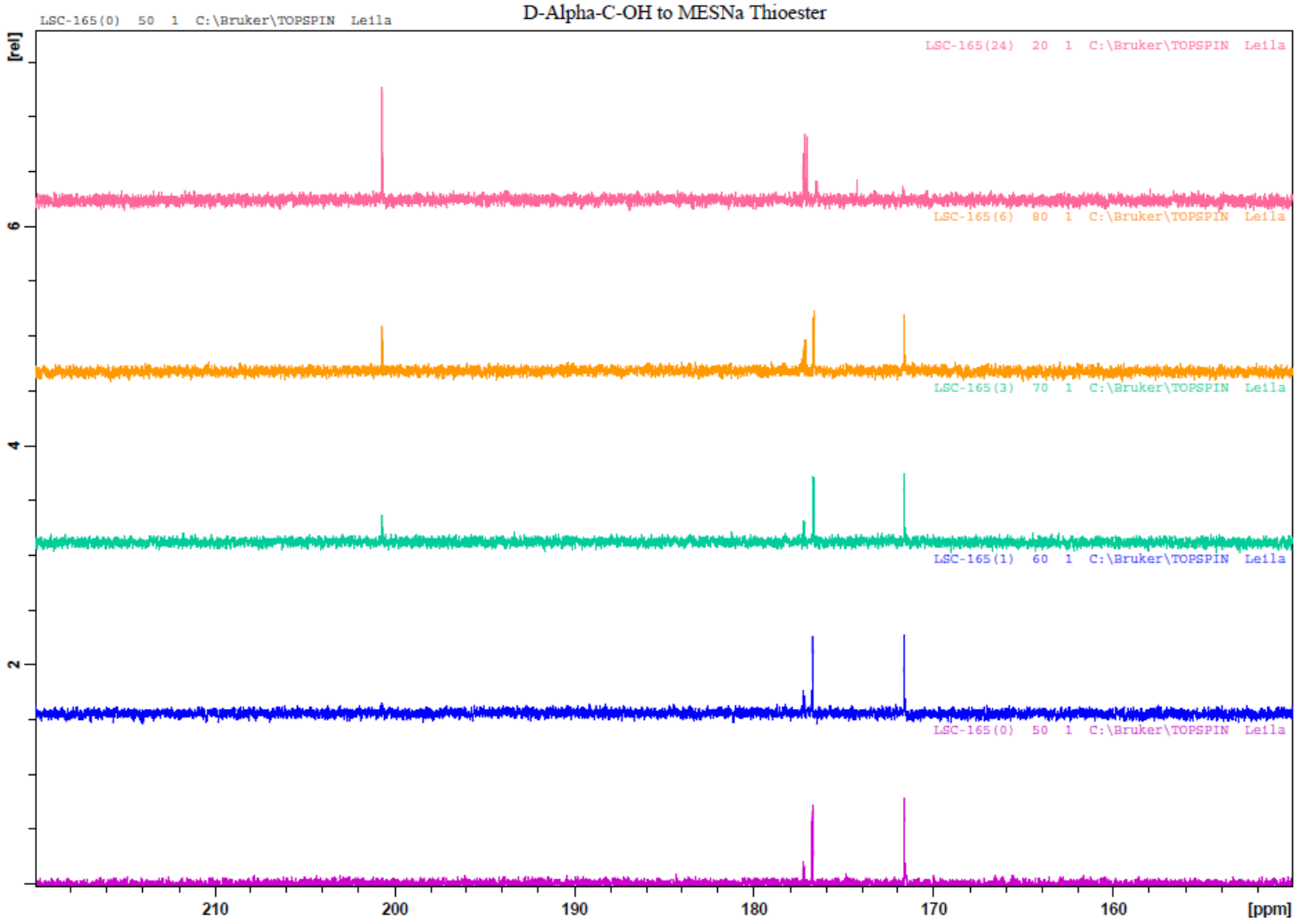
Expanding the scope of N→S acyl transfer in native peptide sequences

Ben Cowper, Leila Shariff, Wenjie Chen, Samantha M. Gibson, Wei-Li Di and Derek Macmillan

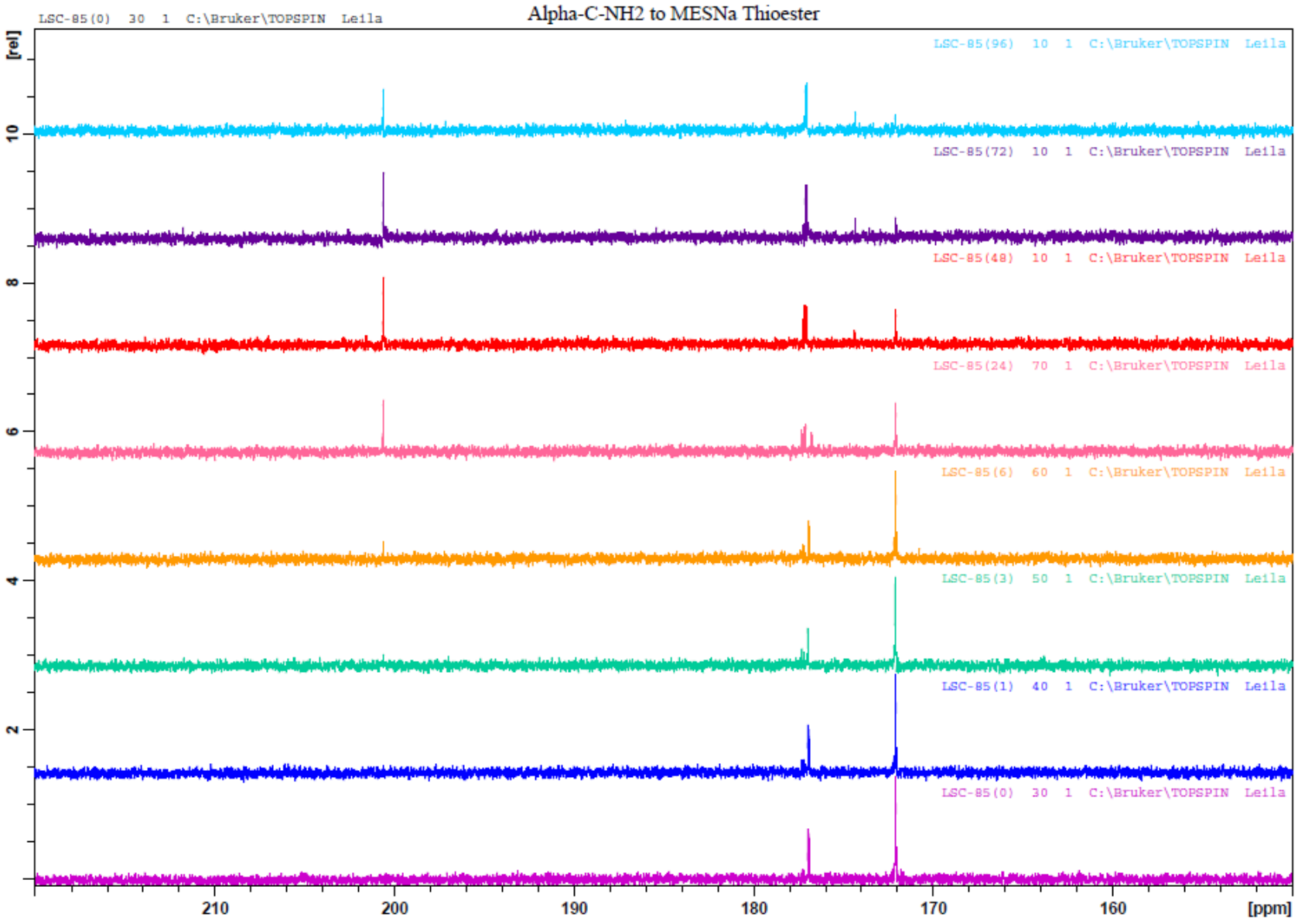
^{13}C analysis data for thioester formation (Gly-Cys-OH)



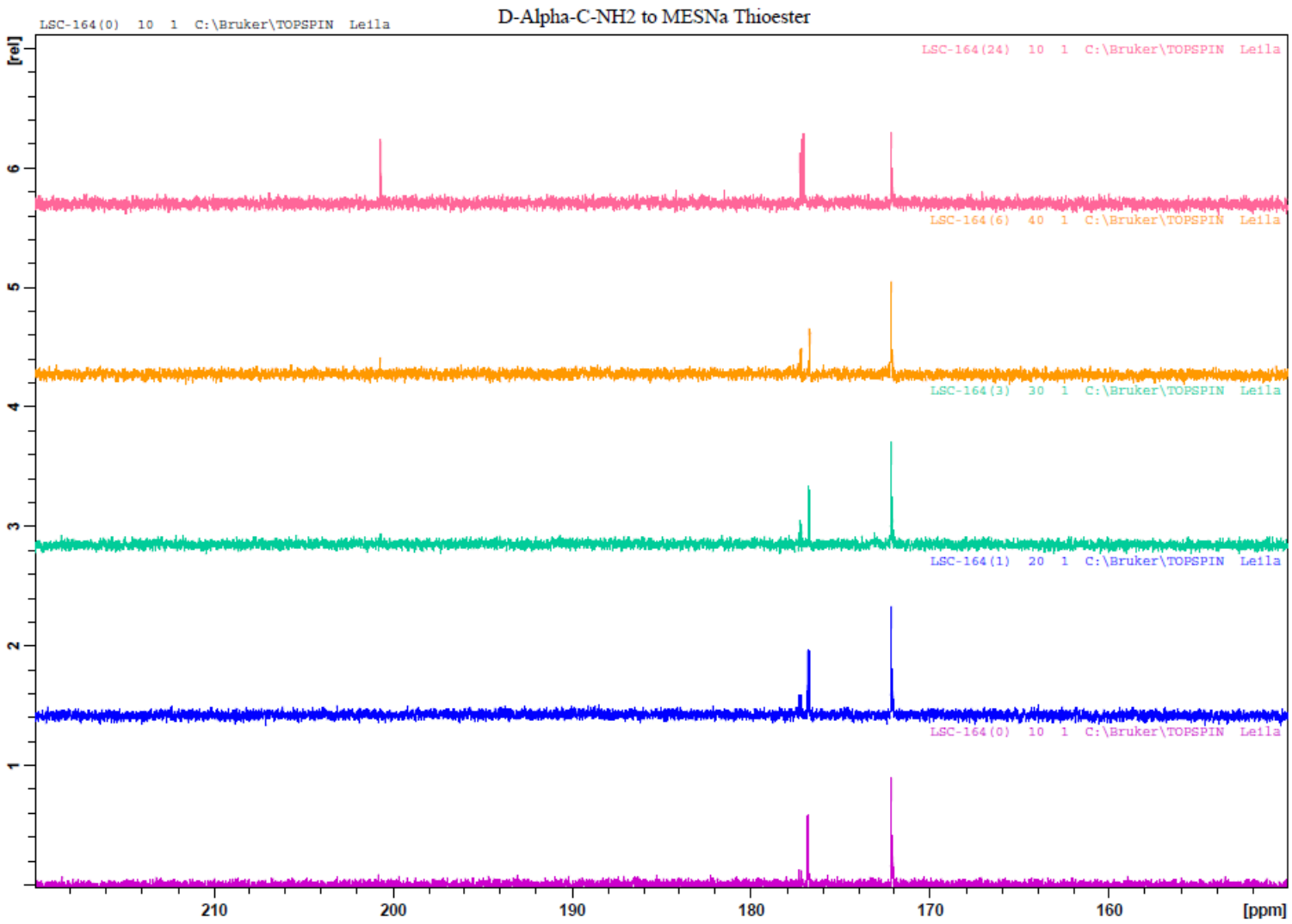
^{13}C analysis data for thioester formation (Xaa-**D-Cys**-OH)



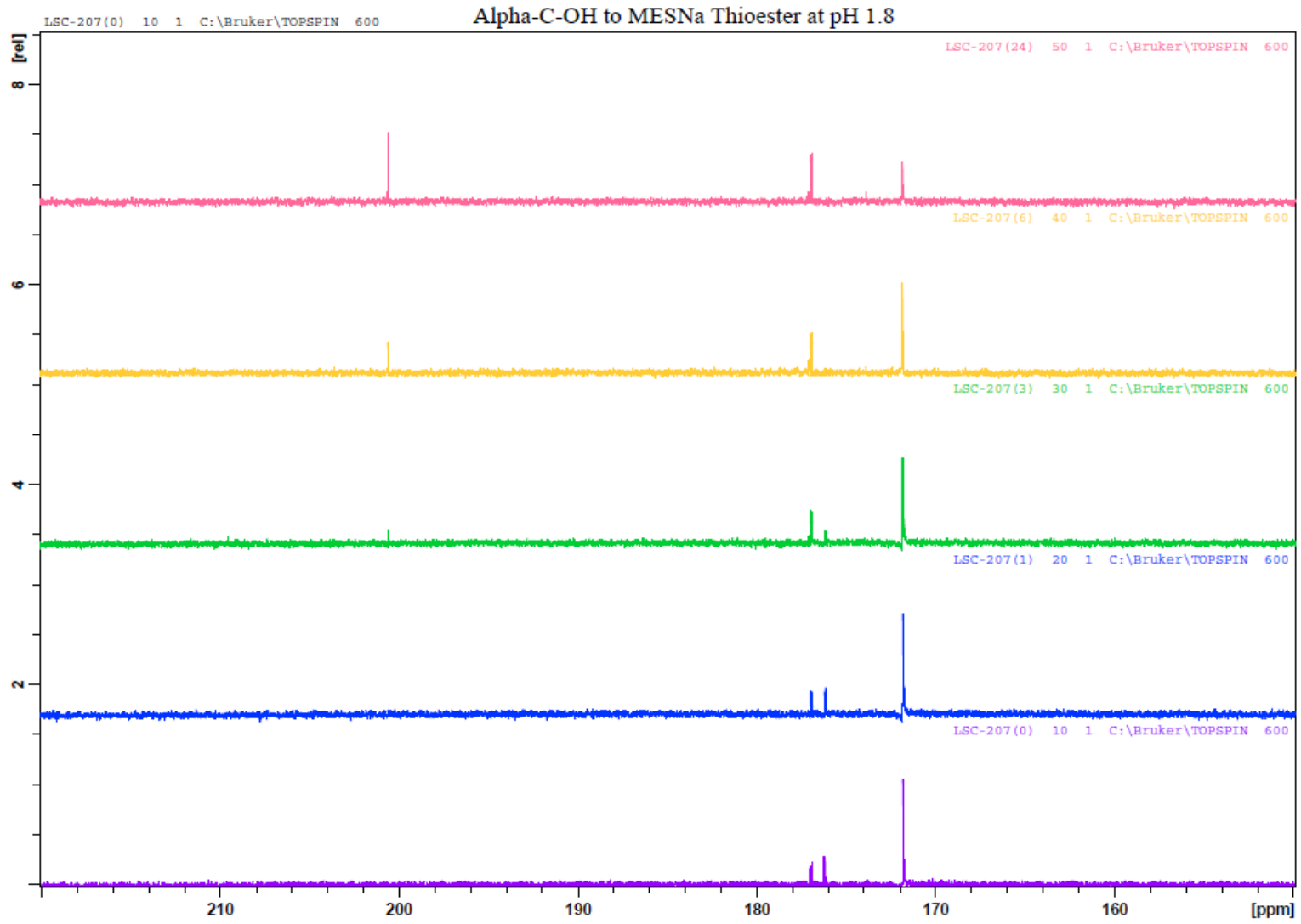
^{13}C analysis data for thioester formation (Xaa-Cys-NH_2)



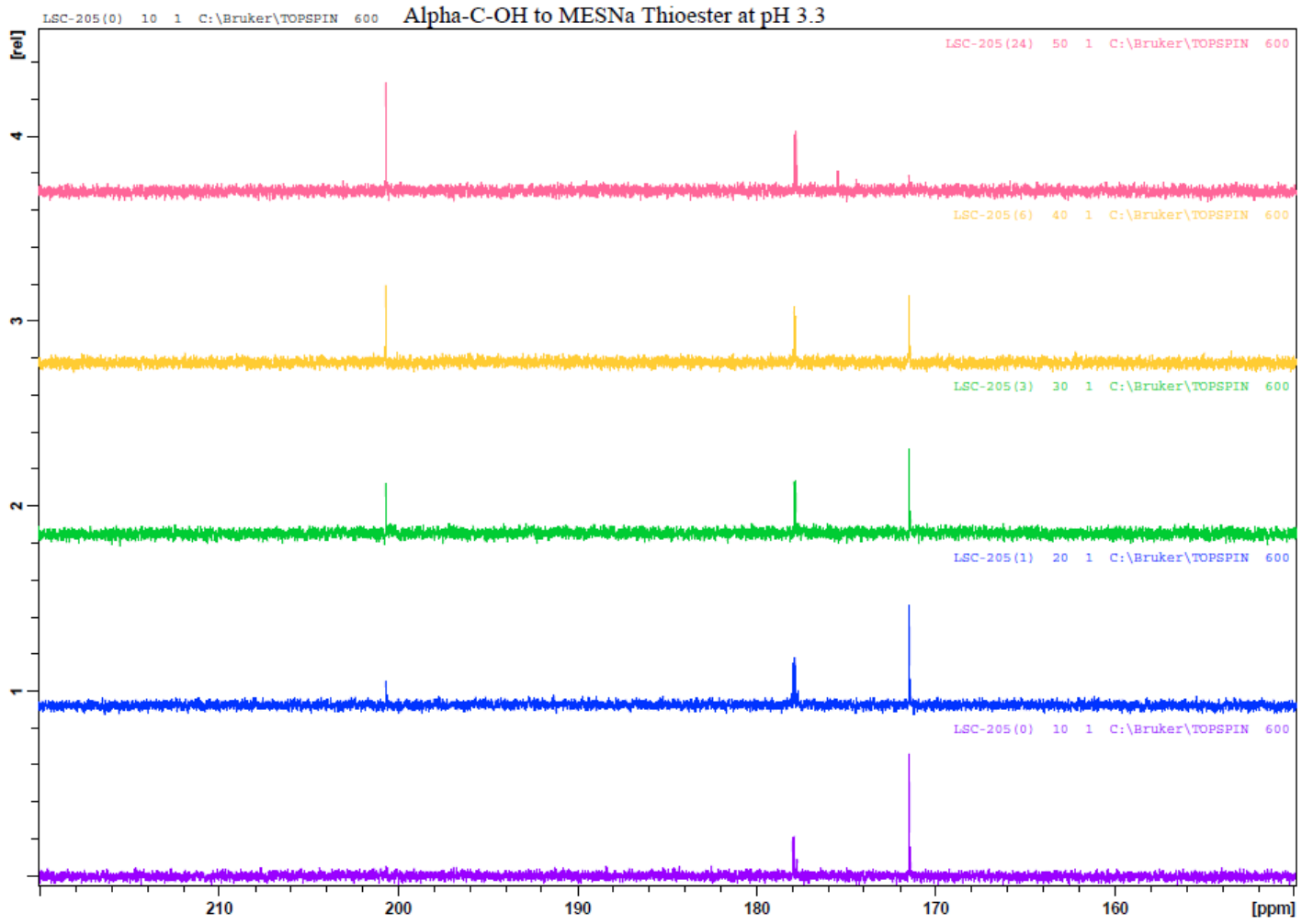
^{13}C analysis data for thioester formation (Xaa-**D-Cys**-NH_2)



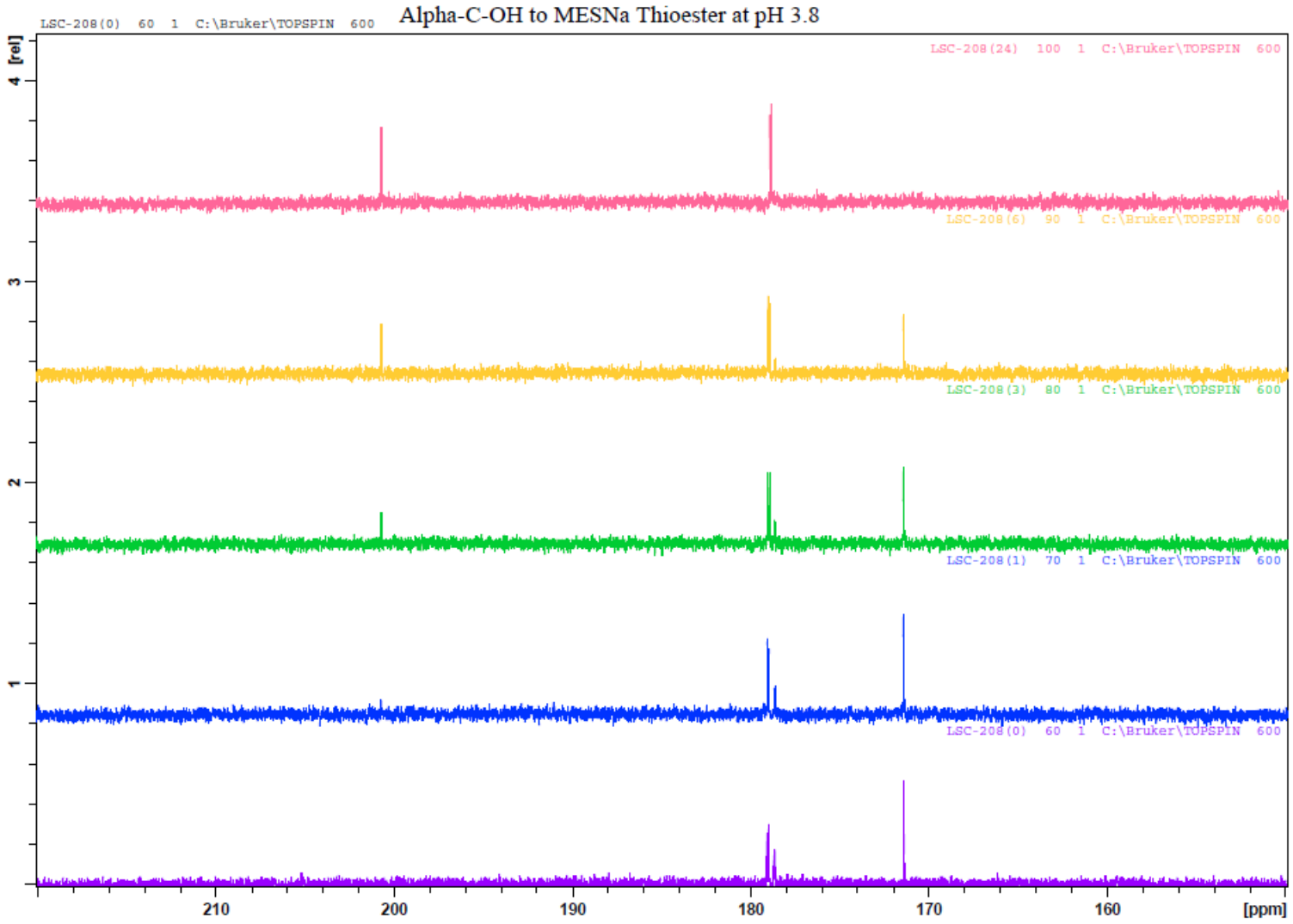
^{13}C analysis data for thioester formation at pH 1.8 (Xaa-Cys-OH)



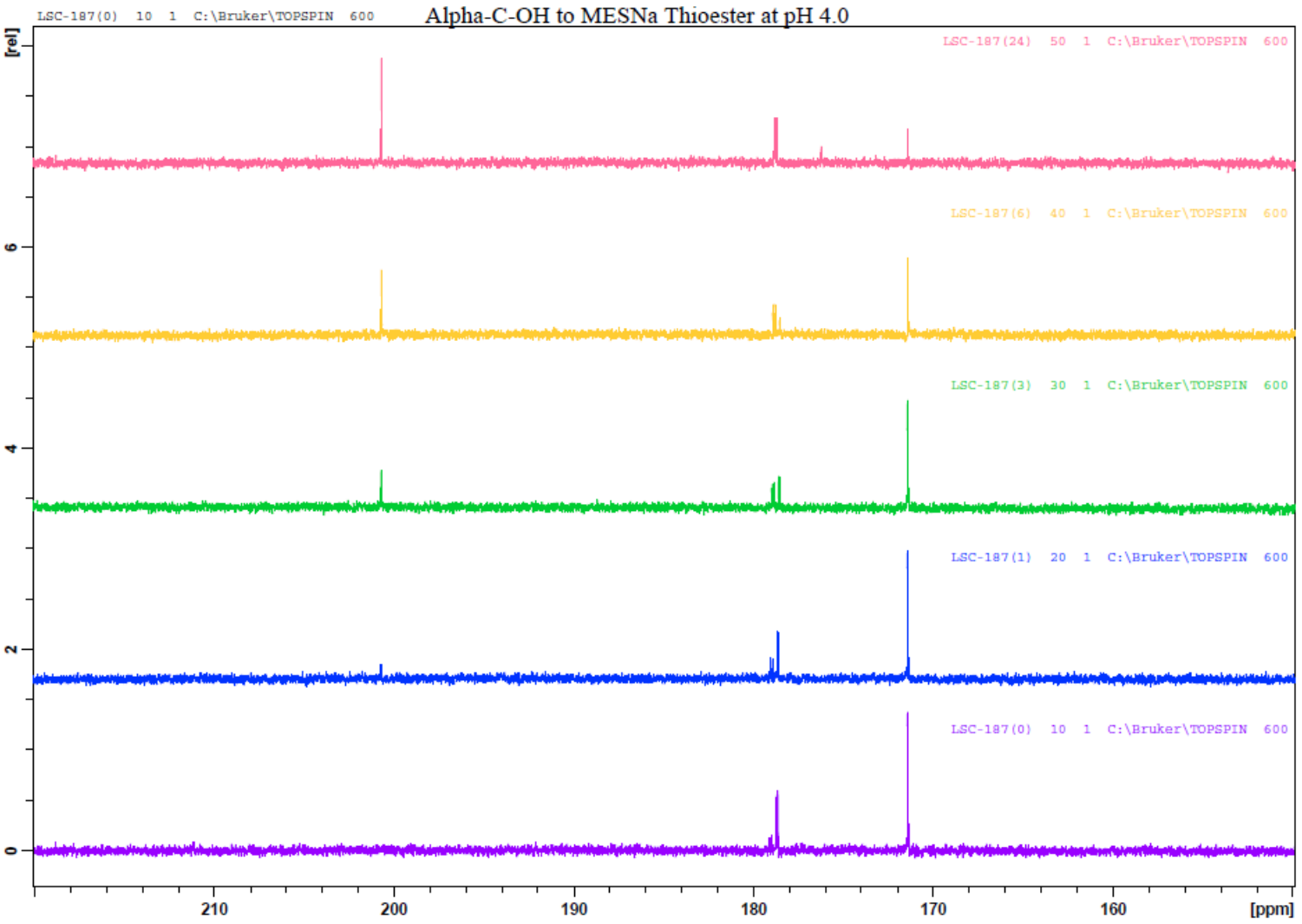
^{13}C analysis data for thioester formation at pH 3.3 (Xaa-Cys-OH)



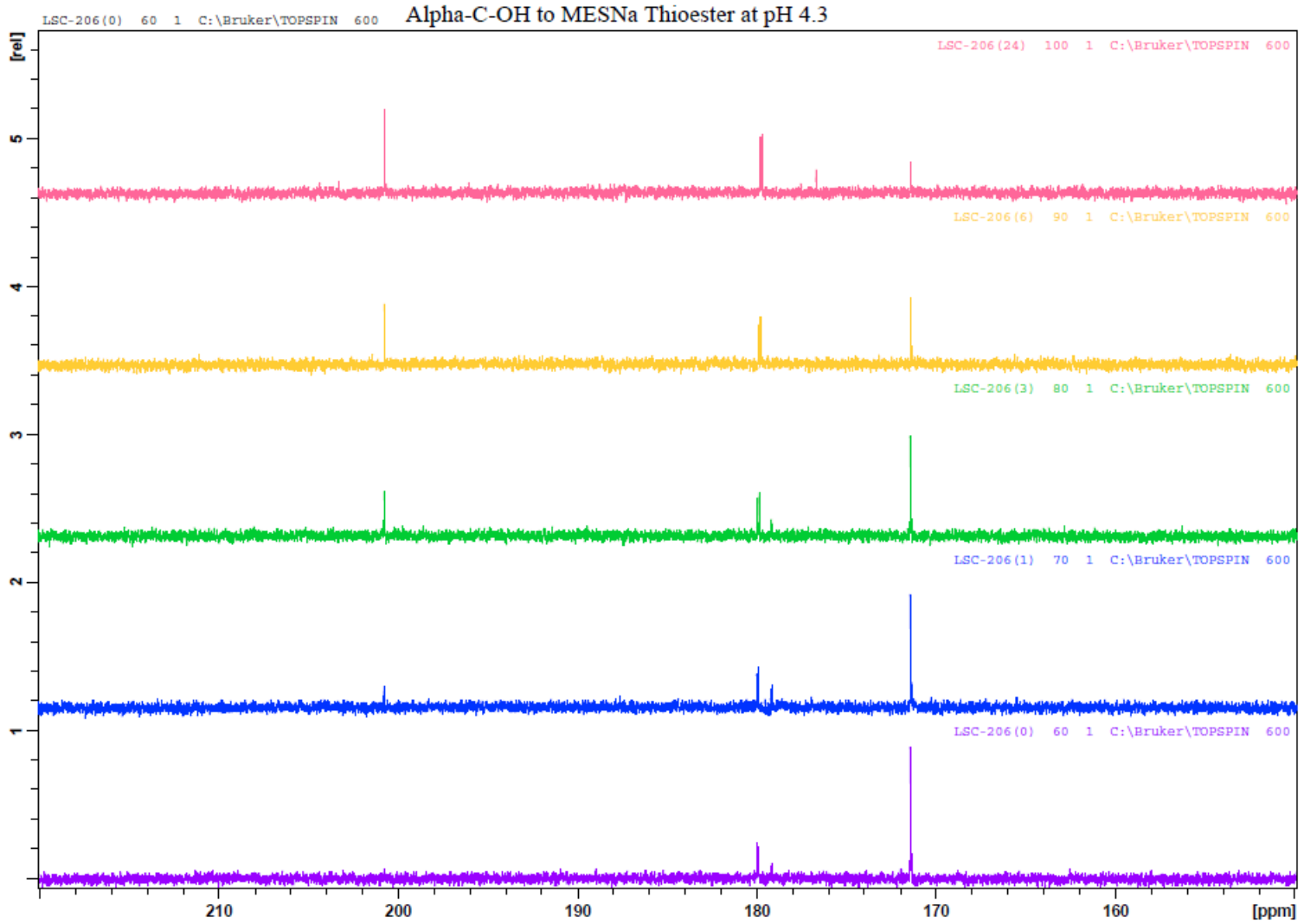
^{13}C analysis data for thioester formation at pH 3.8 (Xaa-Cys-OH)



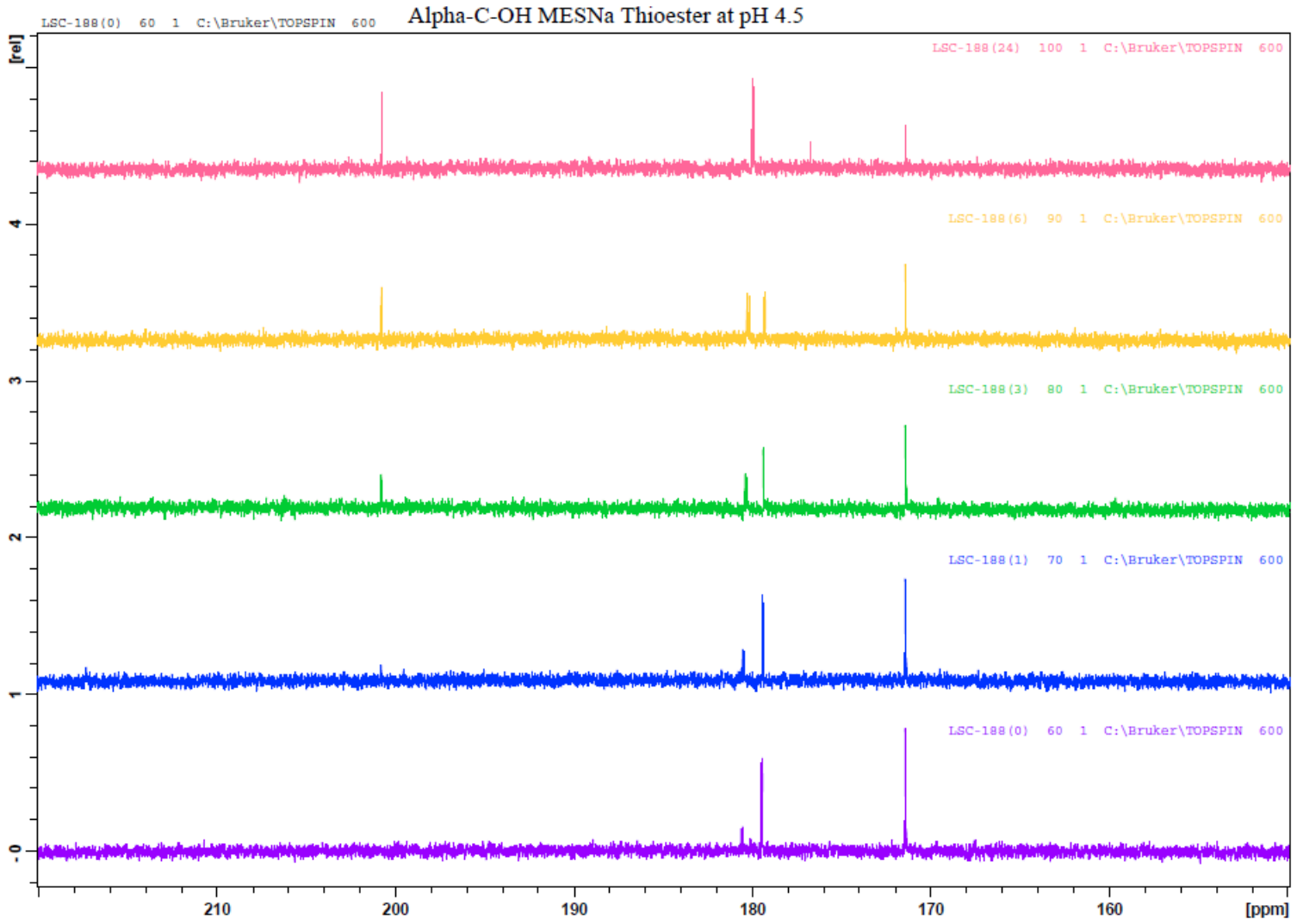
^{13}C analysis data for thioester formation at pH 4.0 (Xaa-Cys-OH)



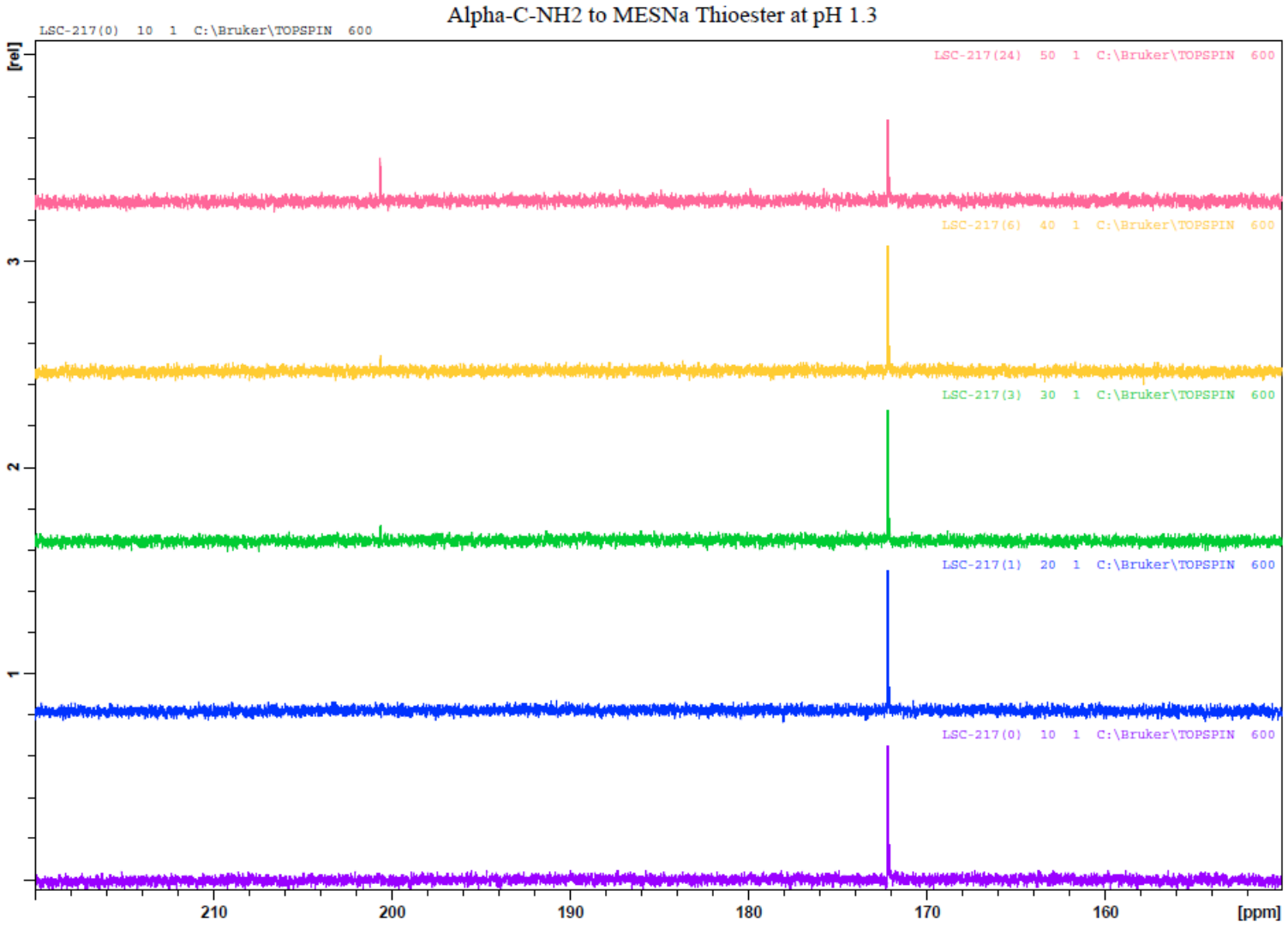
^{13}C analysis data for thioester formation at pH 4.3 (Xaa-Cys-OH)



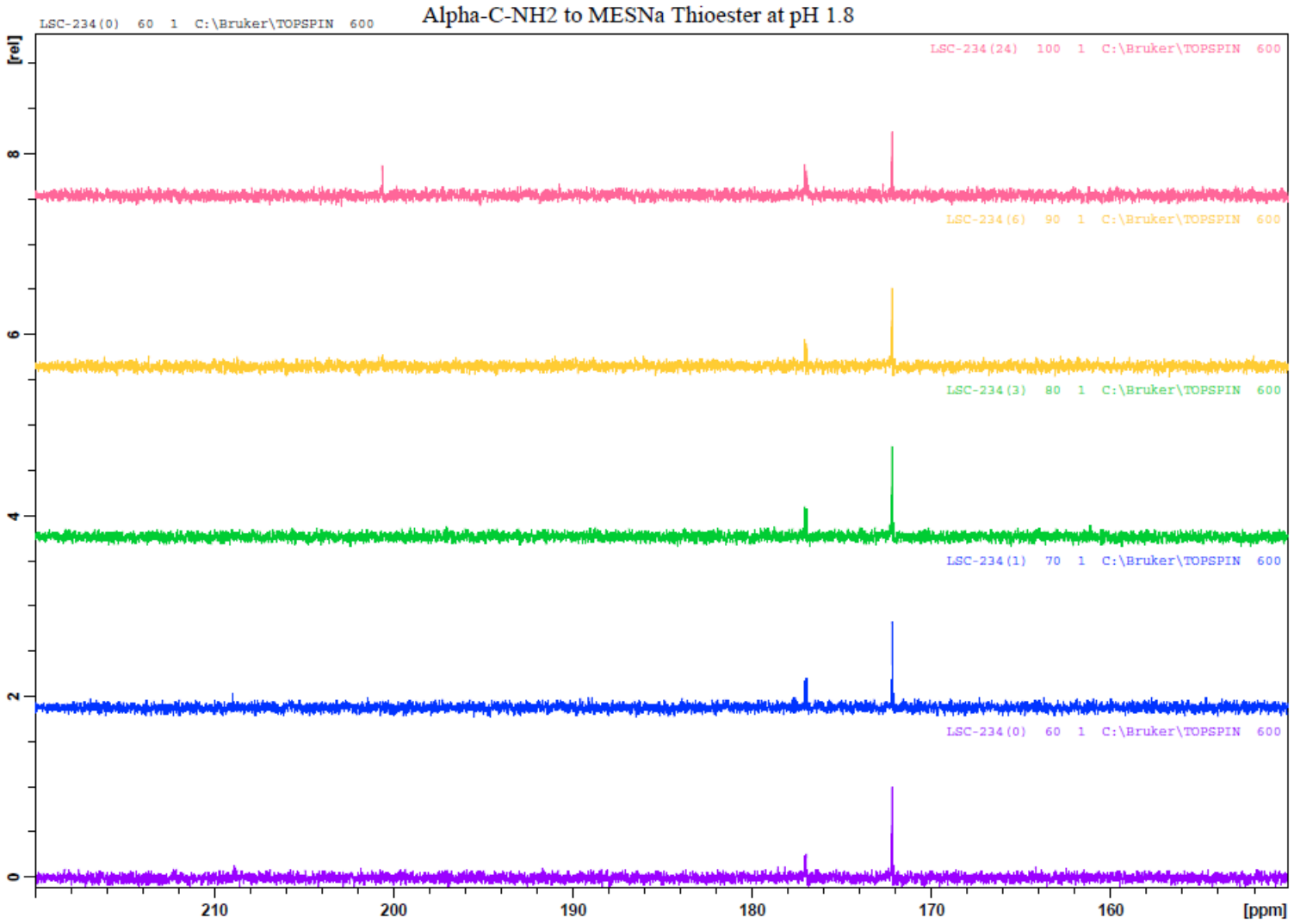
^{13}C analysis data for thioester formation at pH 4.5 (Xaa-Cys-OH)



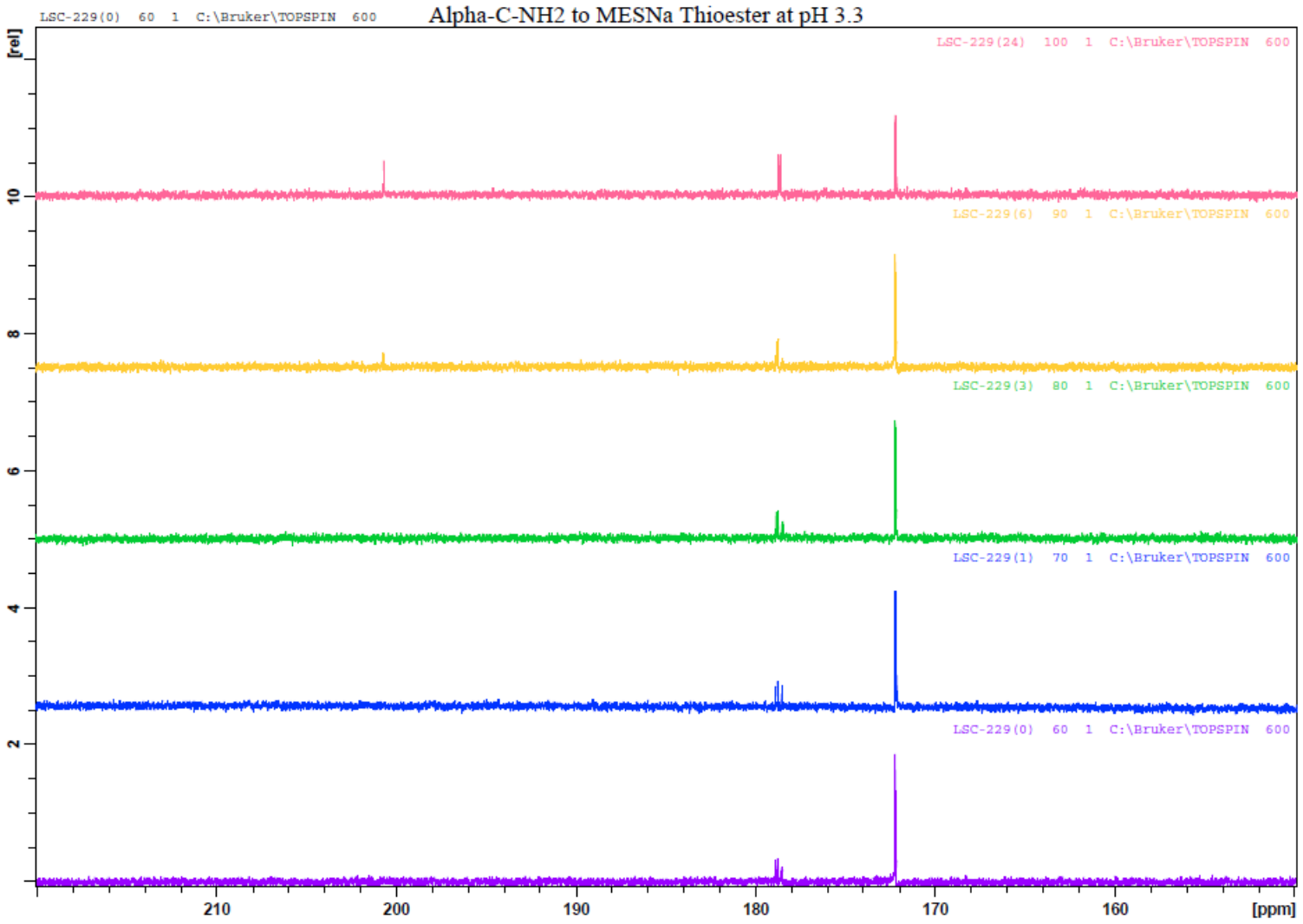
^{13}C analysis data for thioester formation at pH 1.3 (Xaa-Cys-NH_2)



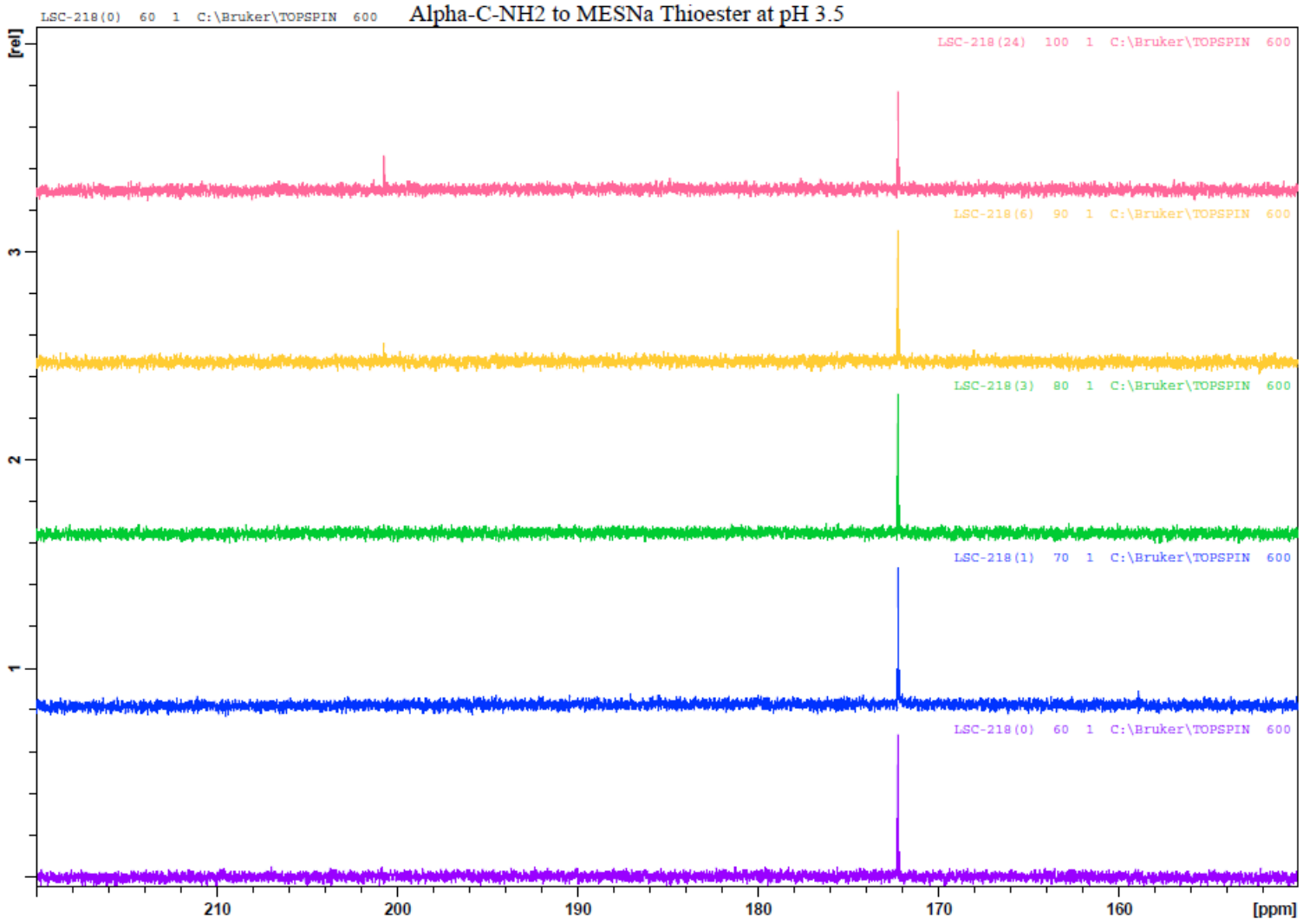
^{13}C analysis data for thioester formation at pH 1.8 (Xaa-Cys-NH_2)



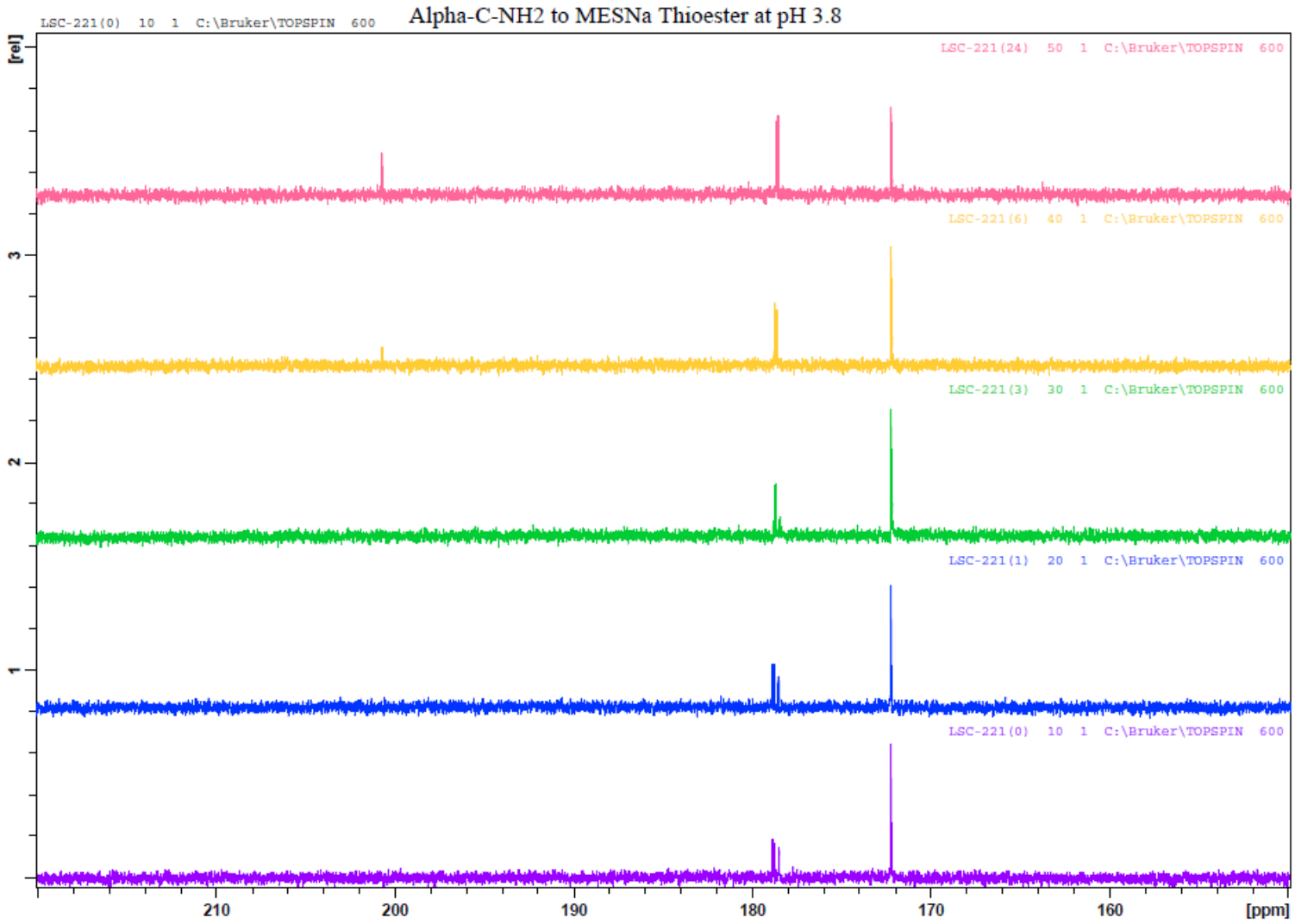
^{13}C analysis data for thioester formation at pH 3.3 (Xaa-Cys-NH_2)



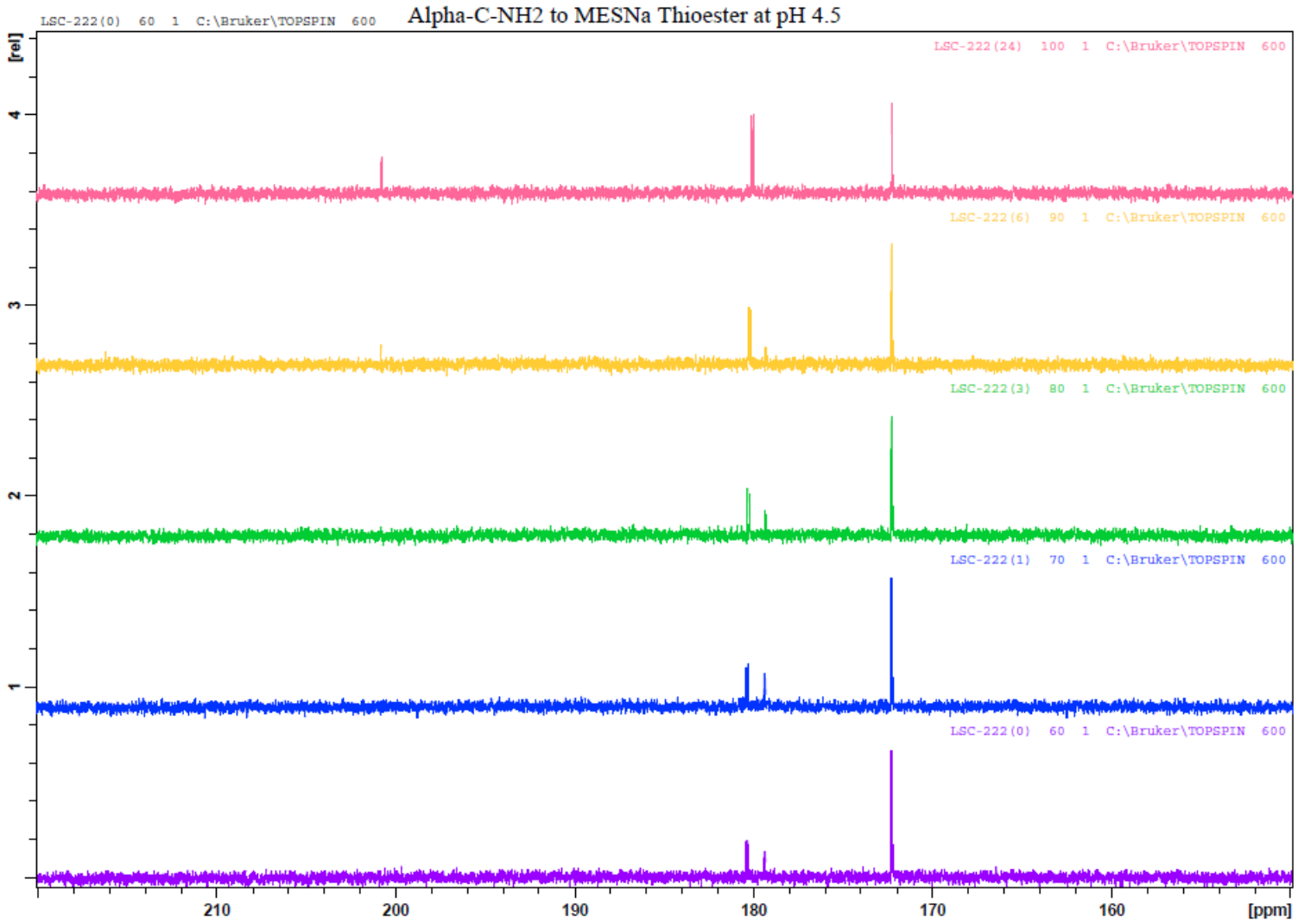
^{13}C analysis data for thioester formation at pH 3.5 (Xaa-Cys-NH_2)



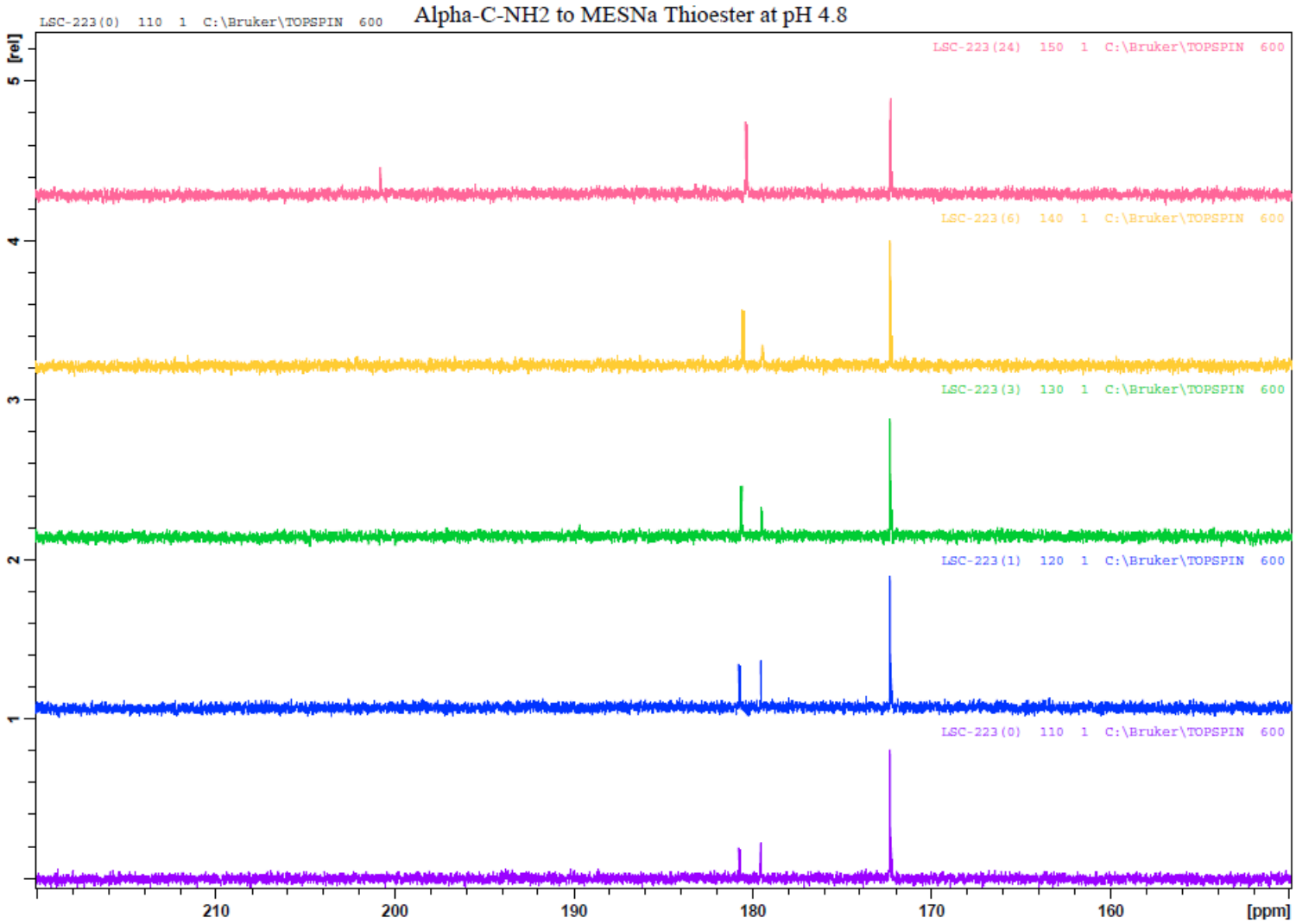
^{13}C analysis data for thioester formation at pH 3.8 (Xaa-Cys-NH_2)



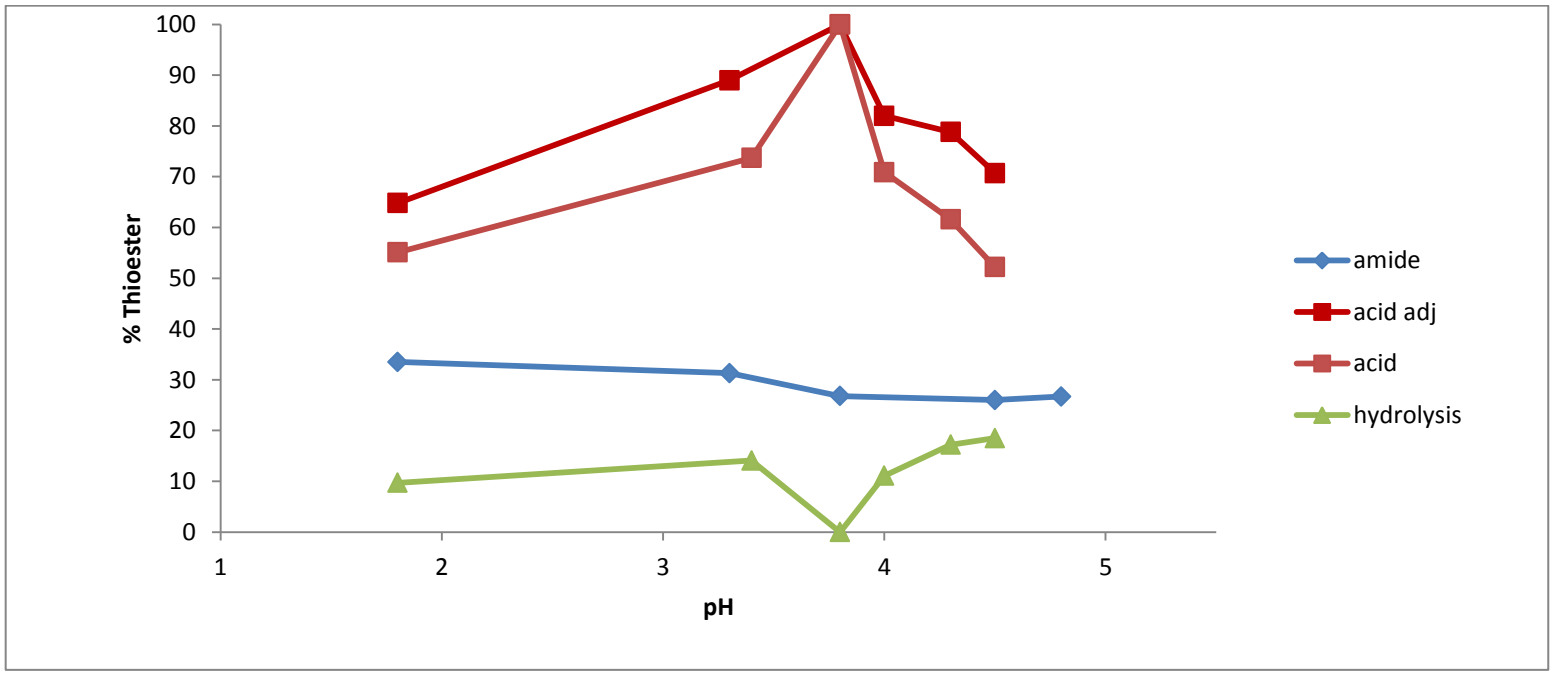
^{13}C analysis data for thioester formation at pH 4.5 (Xaa-Cys-NH_2)



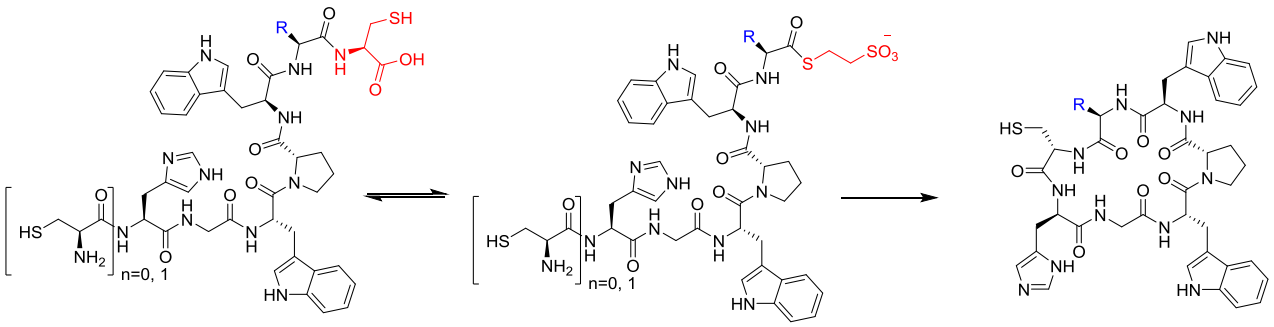
^{13}C analysis data for thioester formation at pH 4.8 (Xaa-Cys-NH_2)



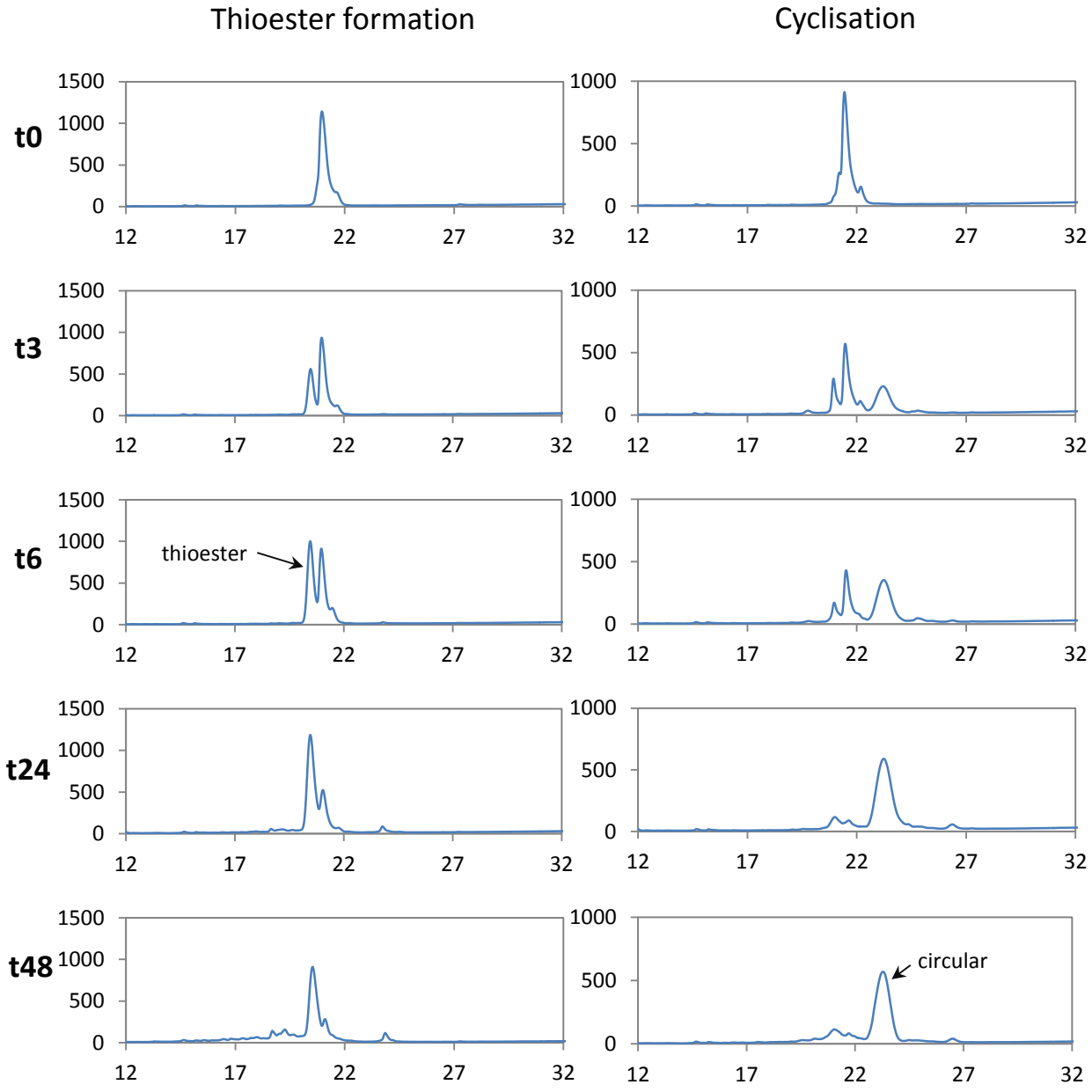
Summary of pH data:

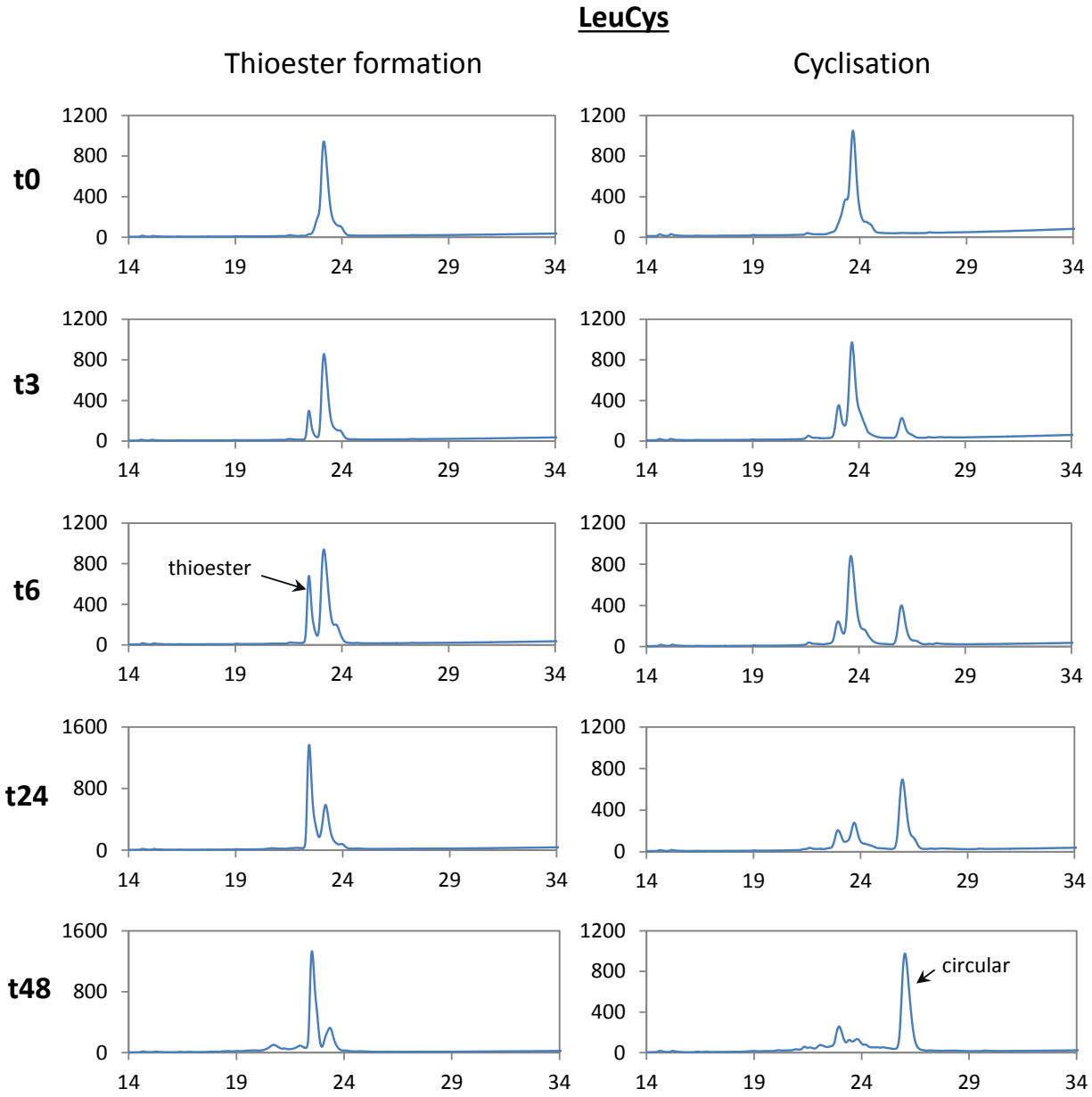


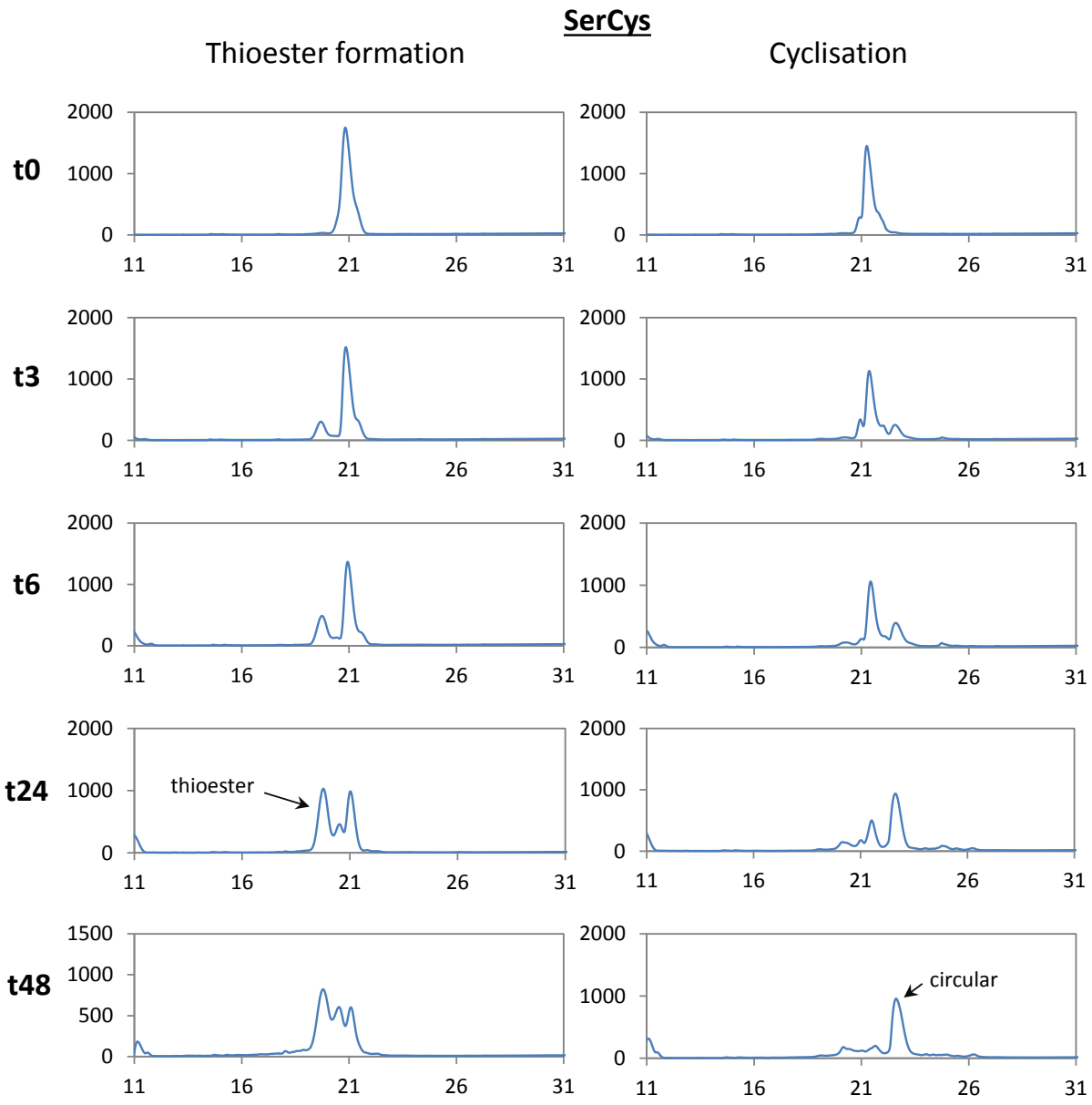
HPLC Analysis of Thioester formation and cyclisation of Agardhipeptin analogues:

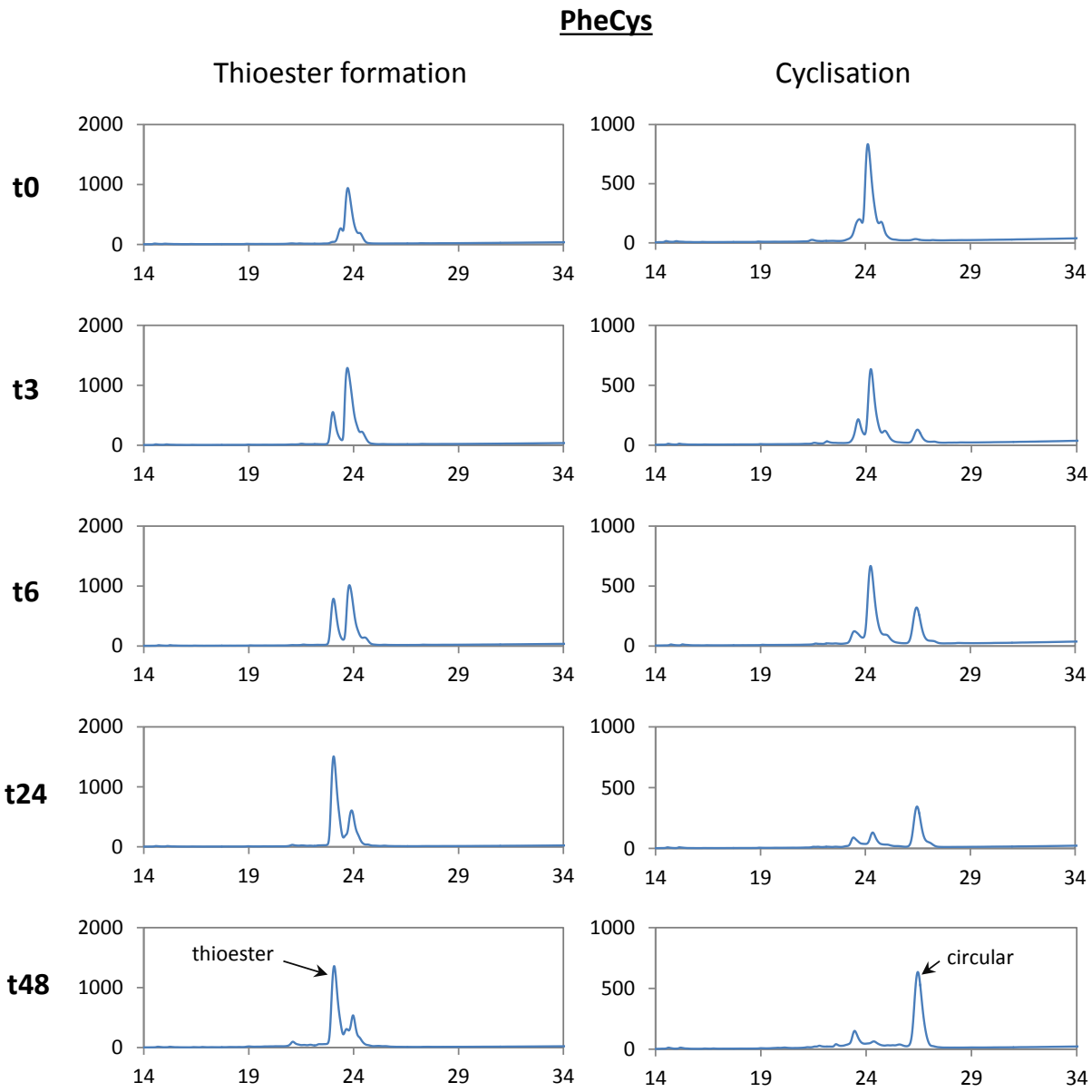


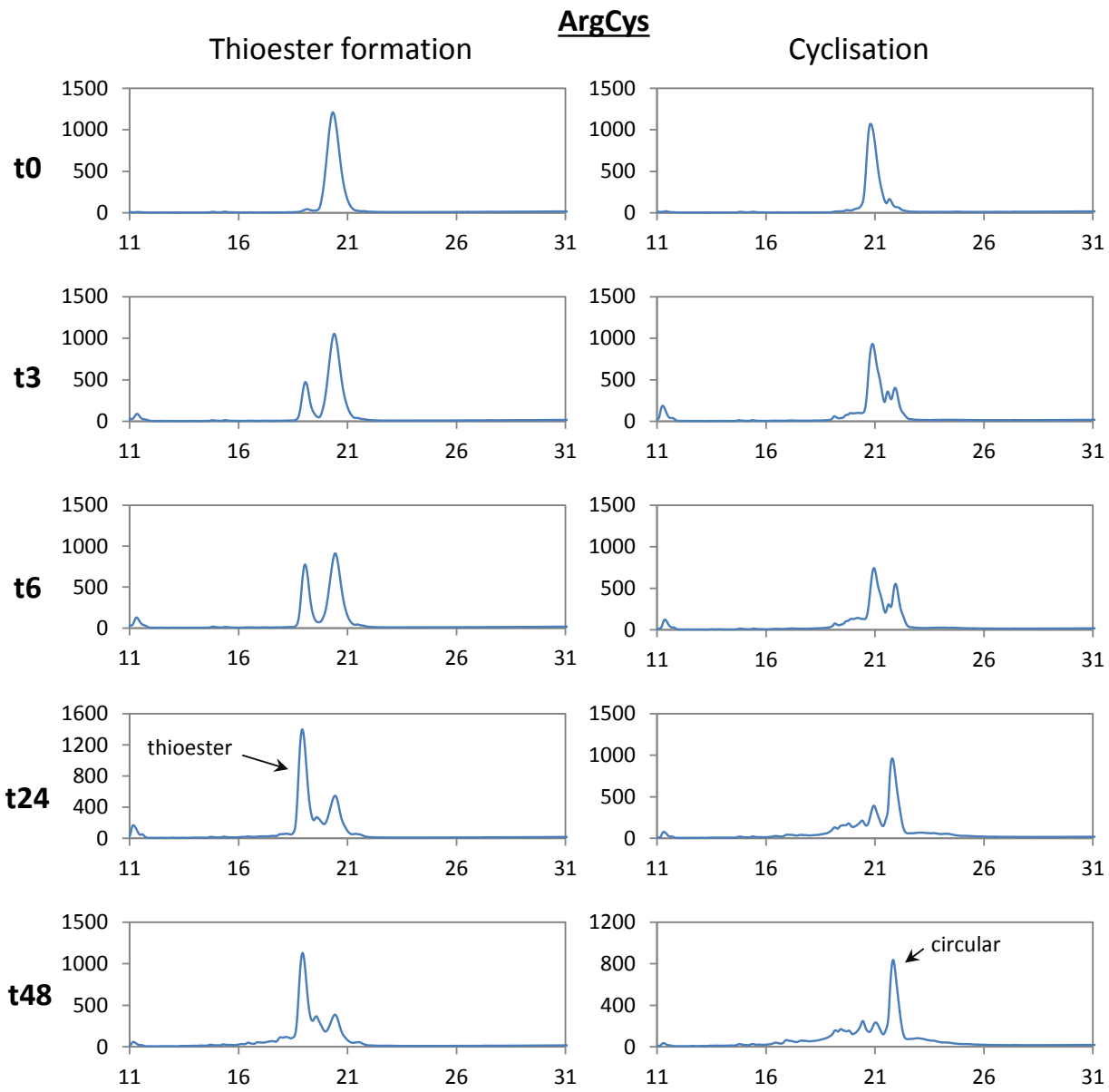
GlyCys

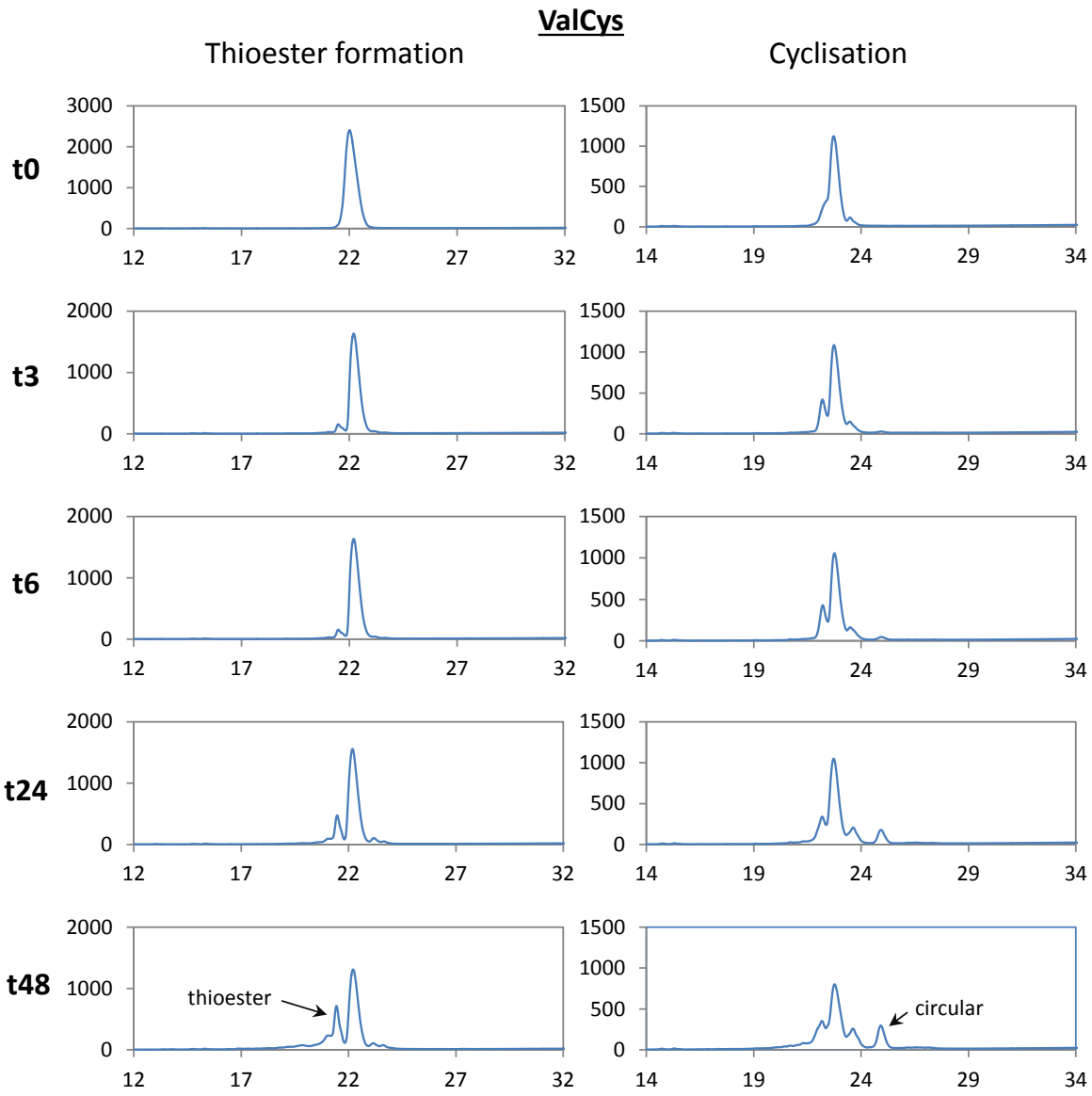



LeuCys
Thioester formation
Cyclisation
t0
t3
t6
t24
t48
thioester
circular
1200
800
400
0
1600
14
19
24
29
34


SerCys
Thioester formation
Cyclisation
t0
t3
t6
t24
t48
thioester
circular
2000
1000
0
1500
500
11
16
21
26
31


PheCys
Thioester formation
Cyclisation
t0
t3
t6
t24
t48
2000
1000
0
1000
500
0
14
19
24
29
34
thioester
circular

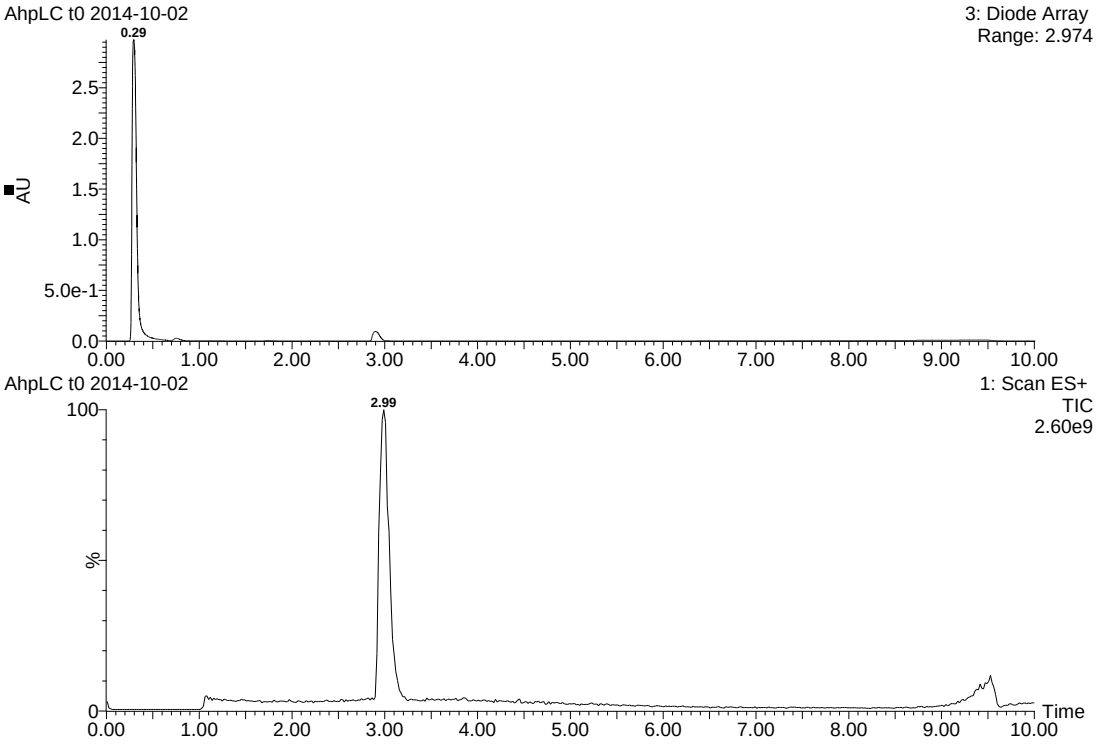

ArgCys
Thioester formation
Cyclisation
t0
t3
t6
t24
t48
thioester
circular
11
16
21
26
31


ValCys
Thioester formation
Cyclisation
t0
t3
t6
t24
t48
thioester
circular

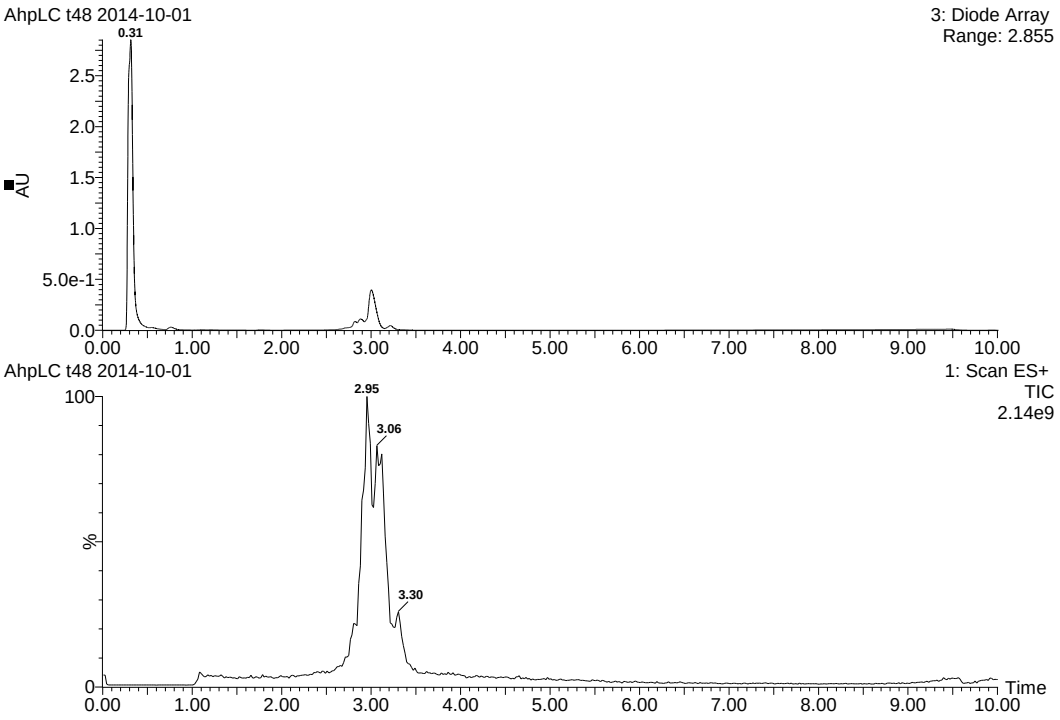
Selected LC-MS data for Agardhipeptin thioester formation/Cyclisation studies

LeuCys: THIOESTER FORMATION

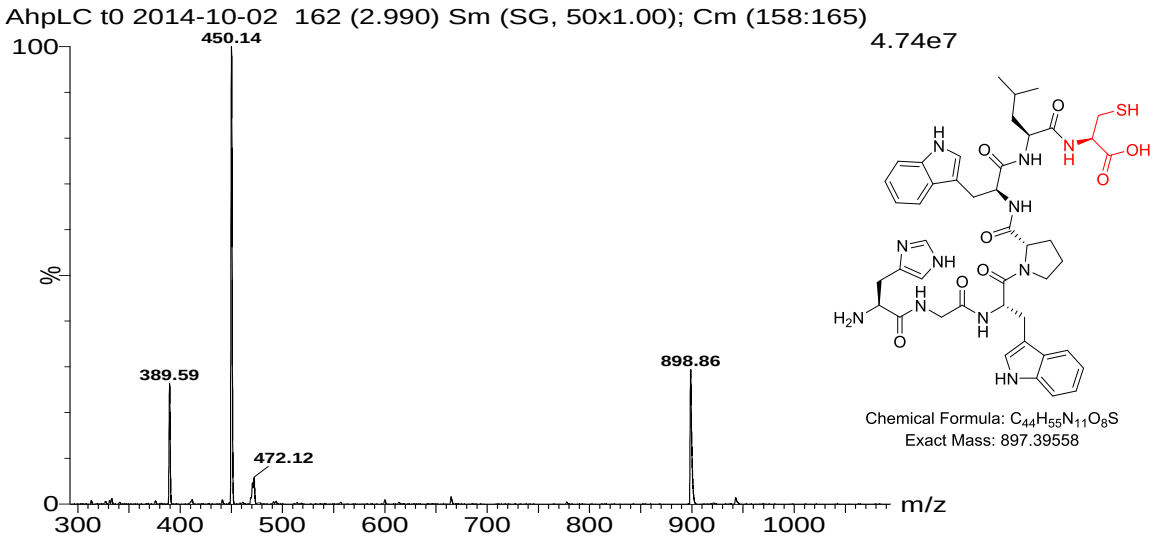
t0



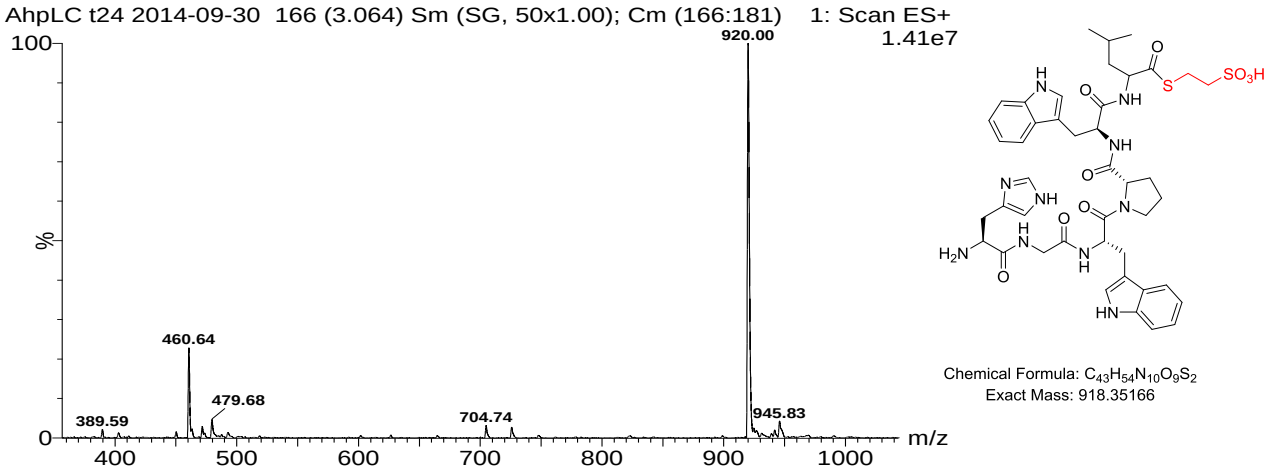
t48



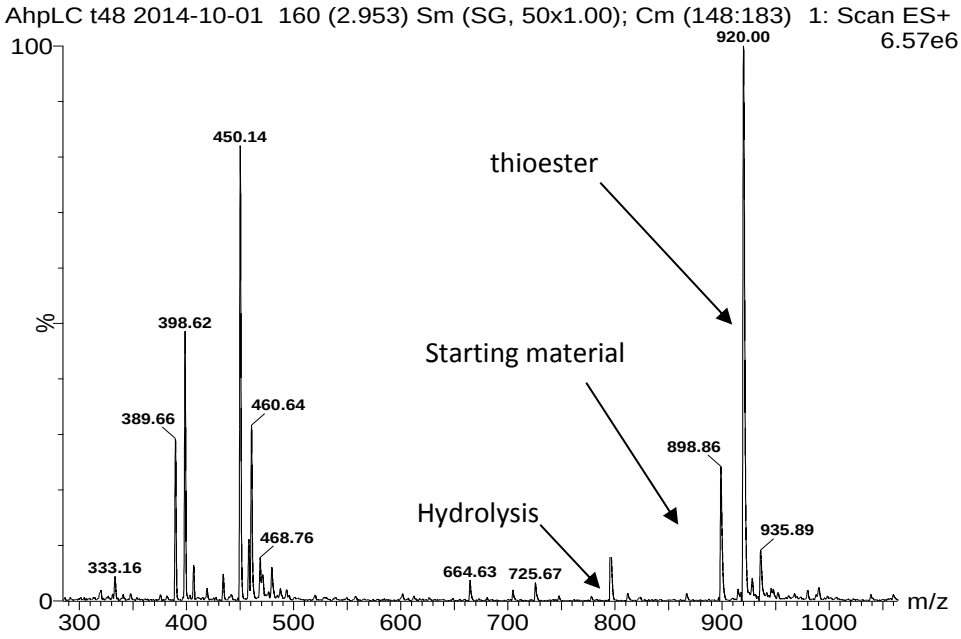
starting material **8b**



Product **10b** (after 24h)

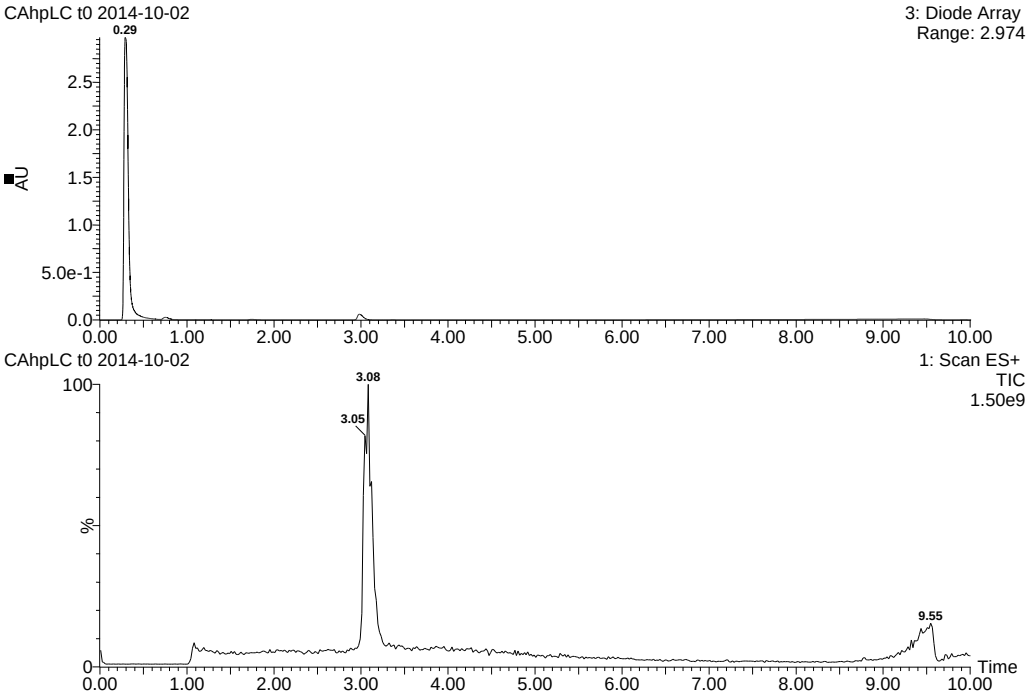


whole TIC @ t48

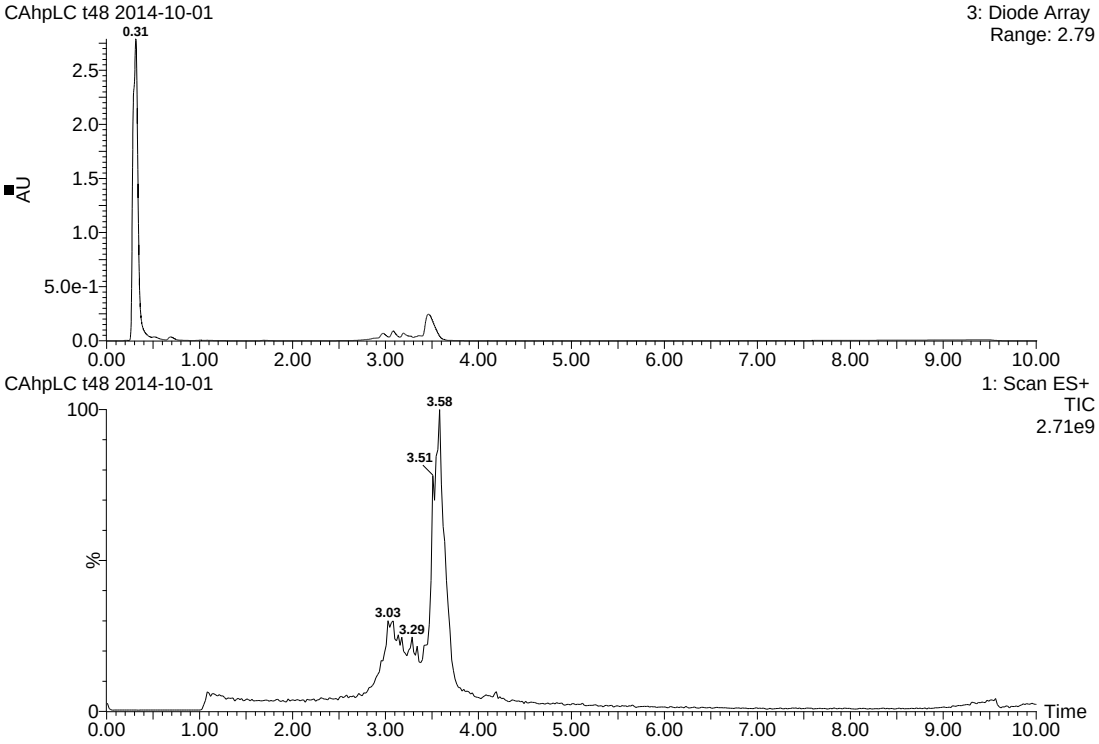


Leu-Cys: CYCLISATION

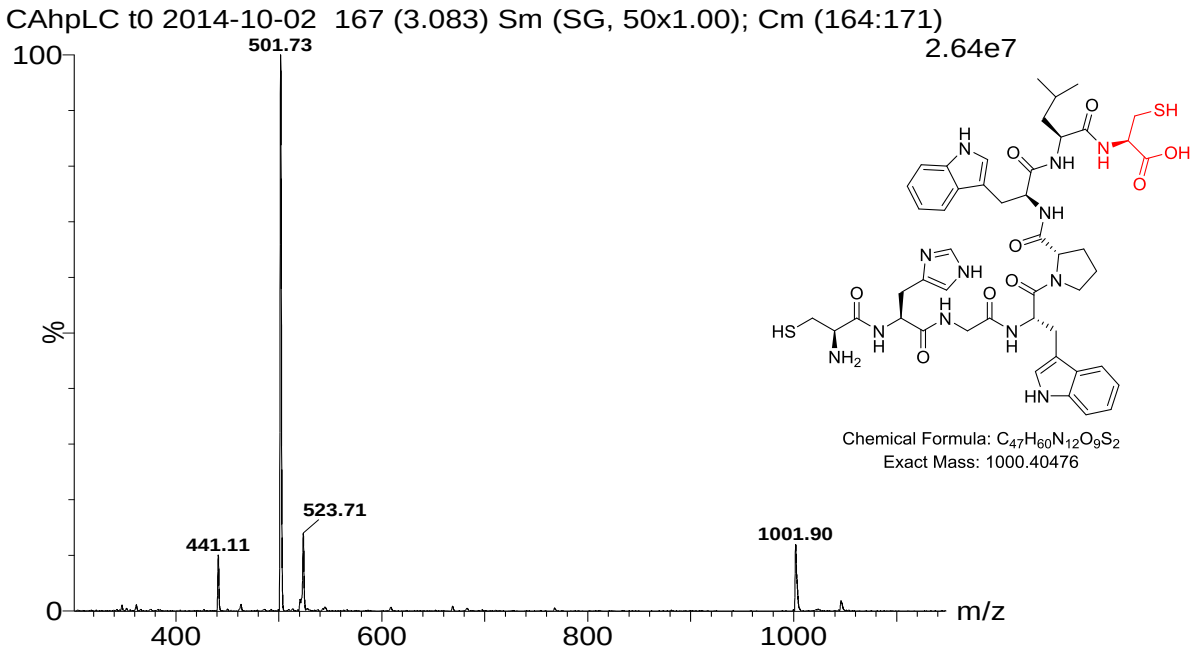
t0



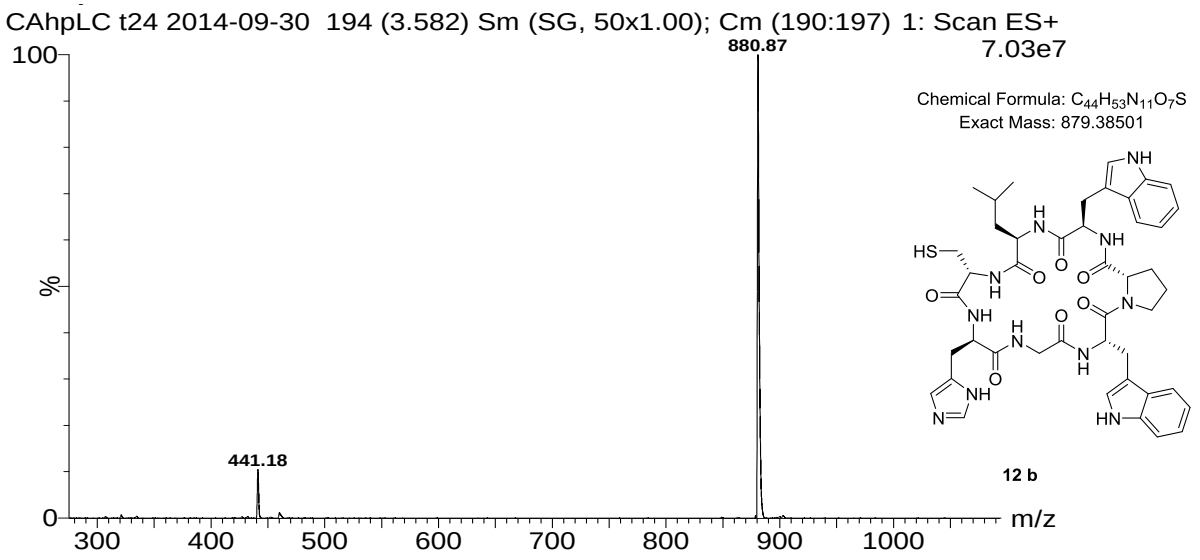
t48



starting material **9b**



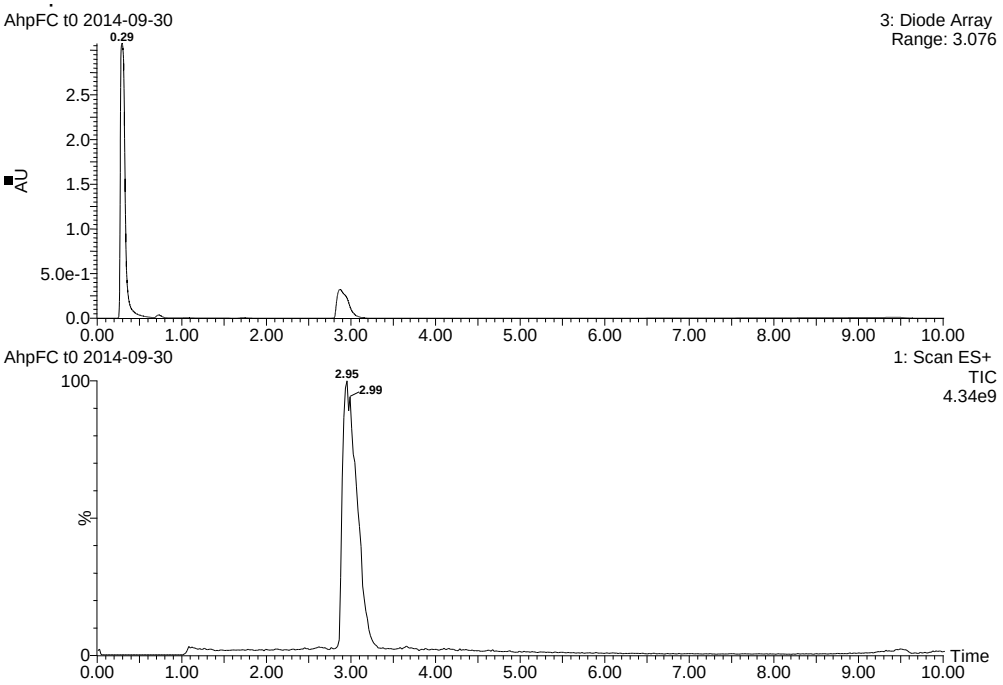
Product **12 b**



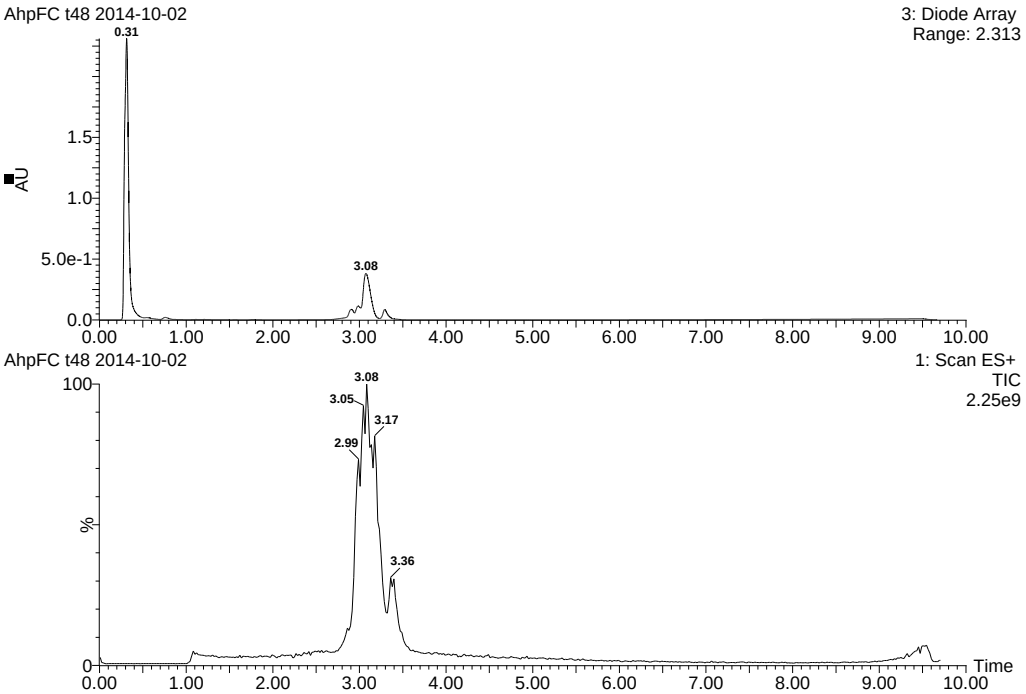
PheCys

THIOESTER FORMATION

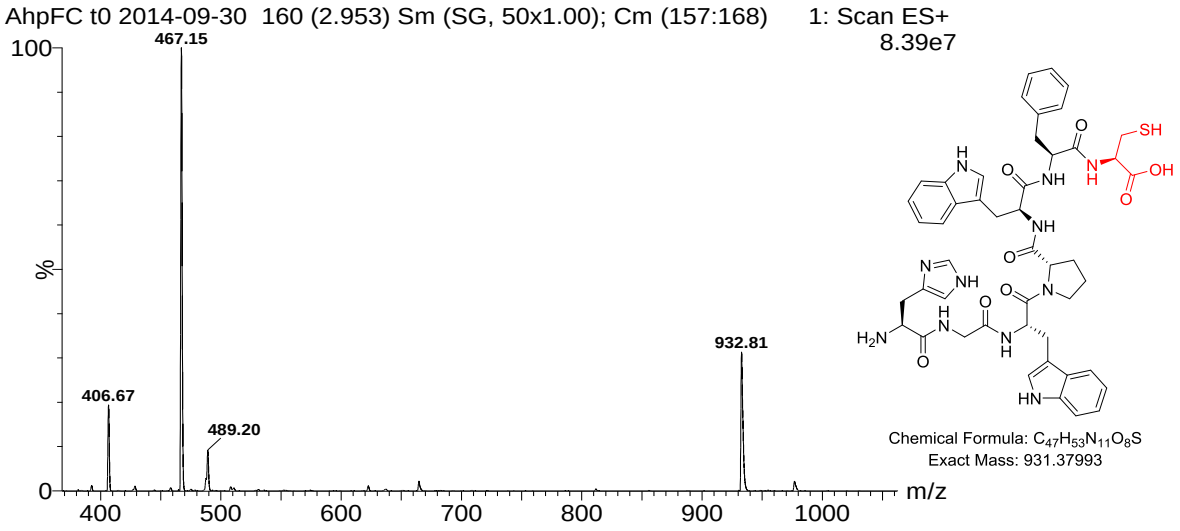
t0



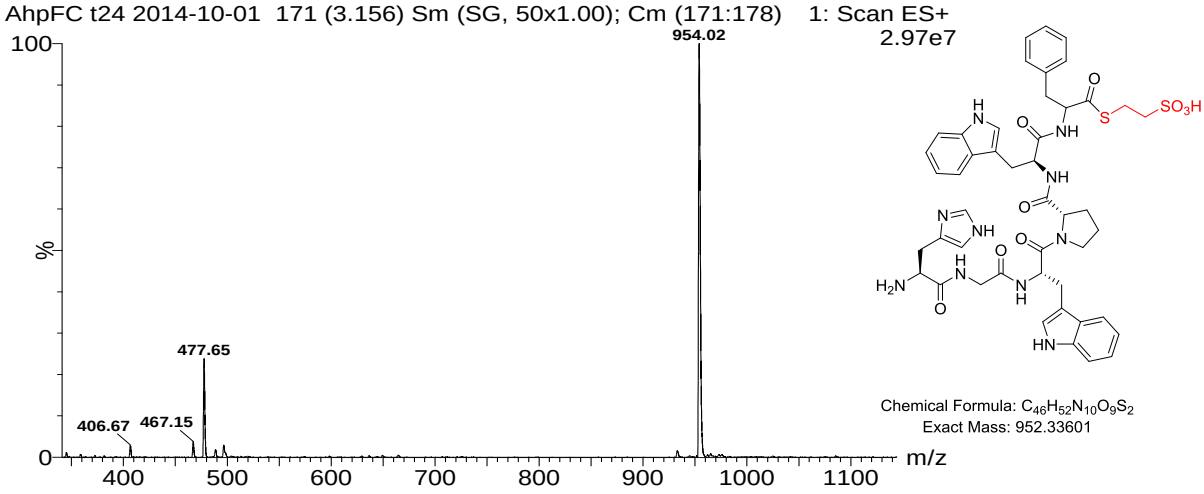
t48



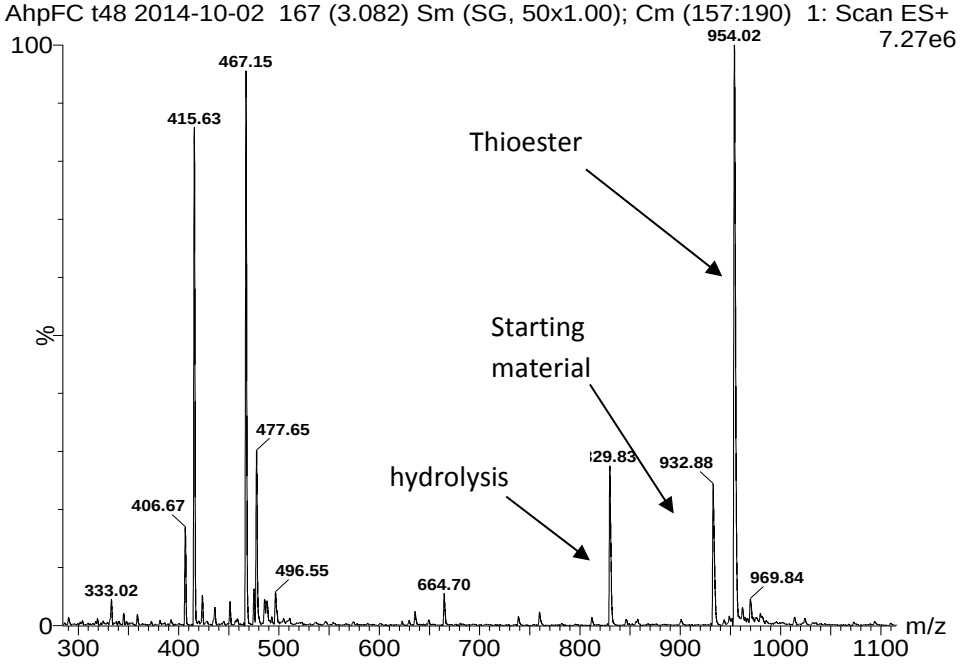
starting material **8e**



Product **10e**

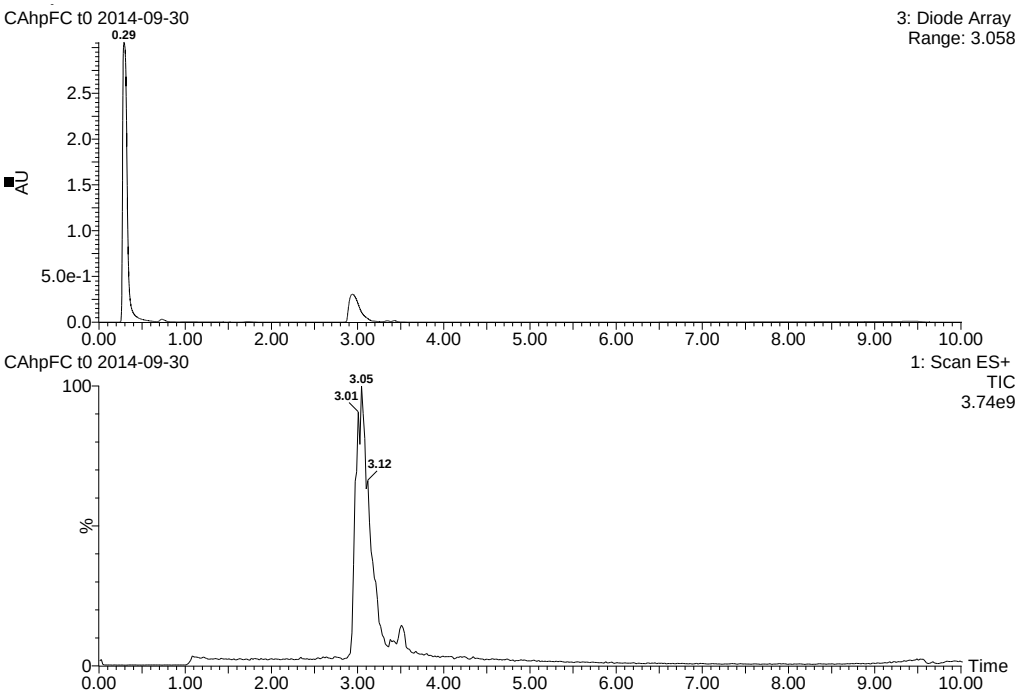


combined TIC @ t48

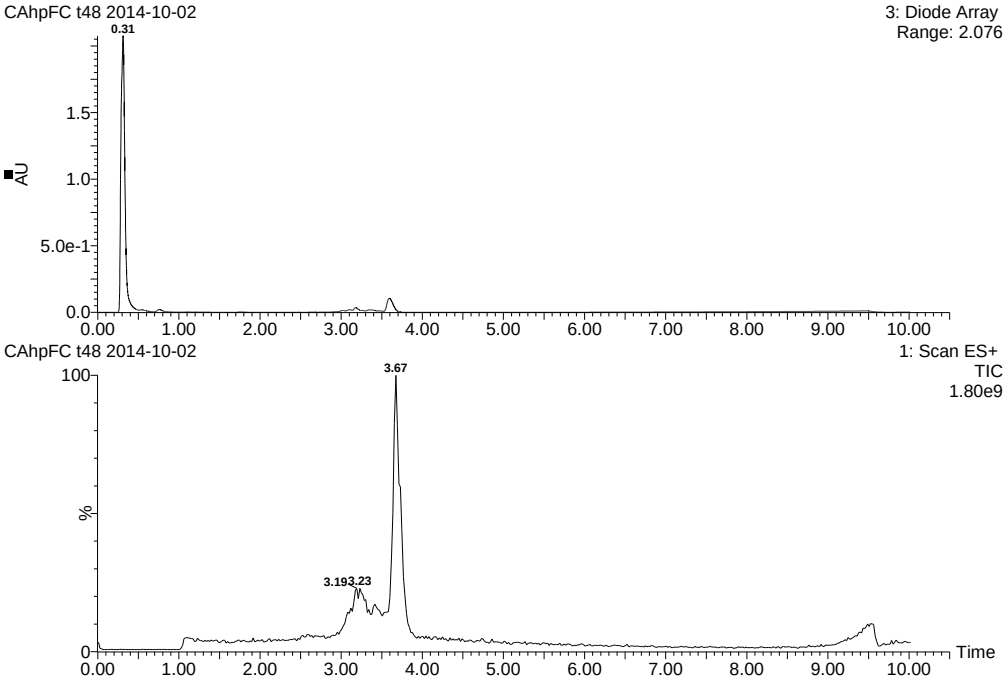


CYCLISATION

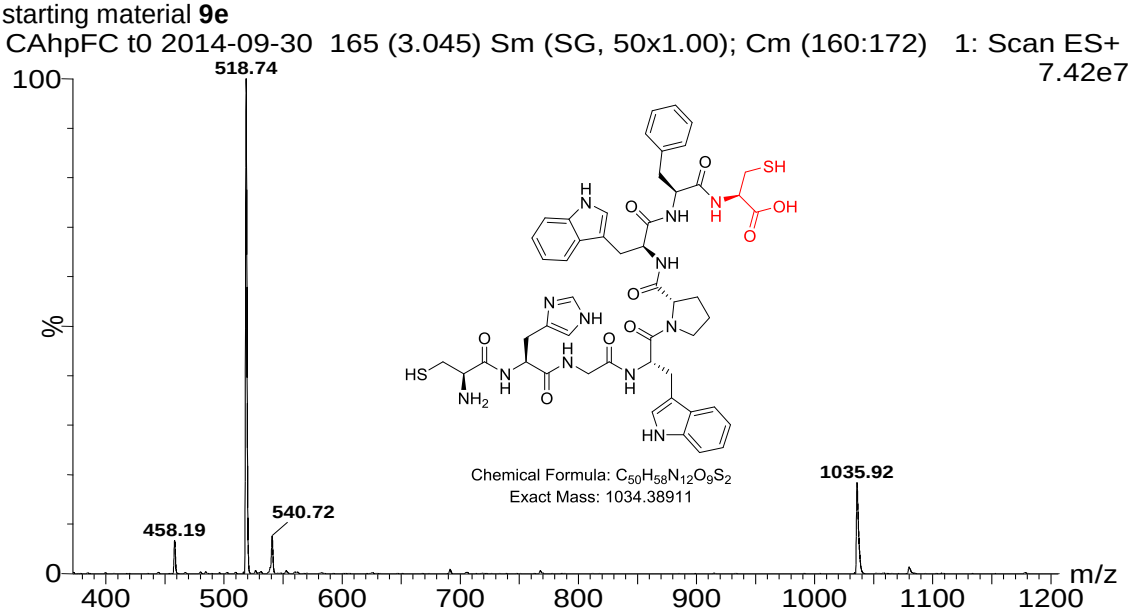
t0



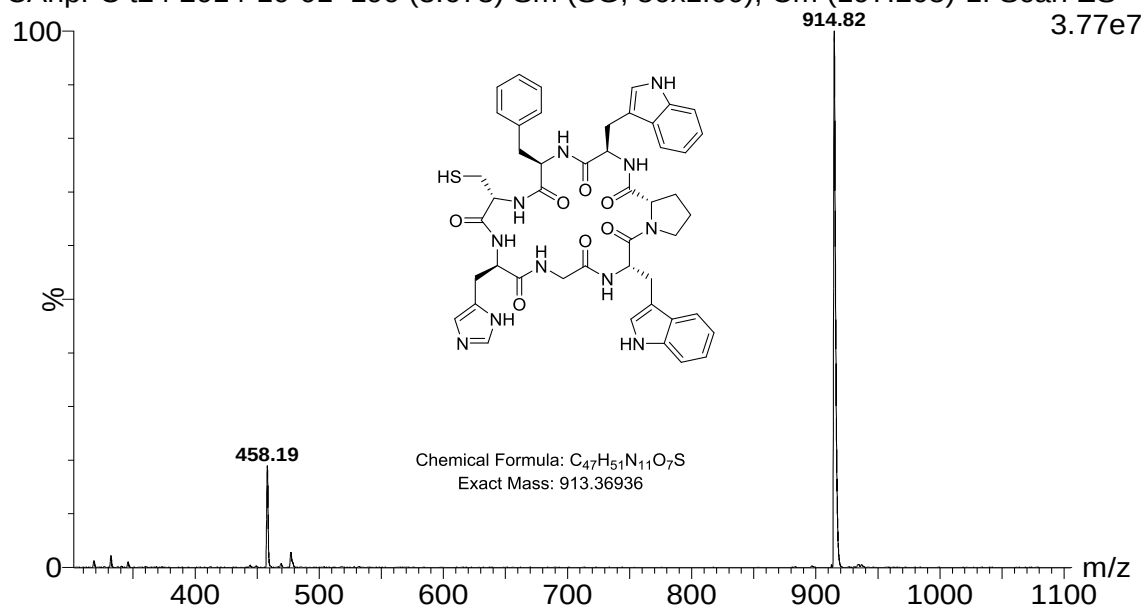
t48



starting material **9e**

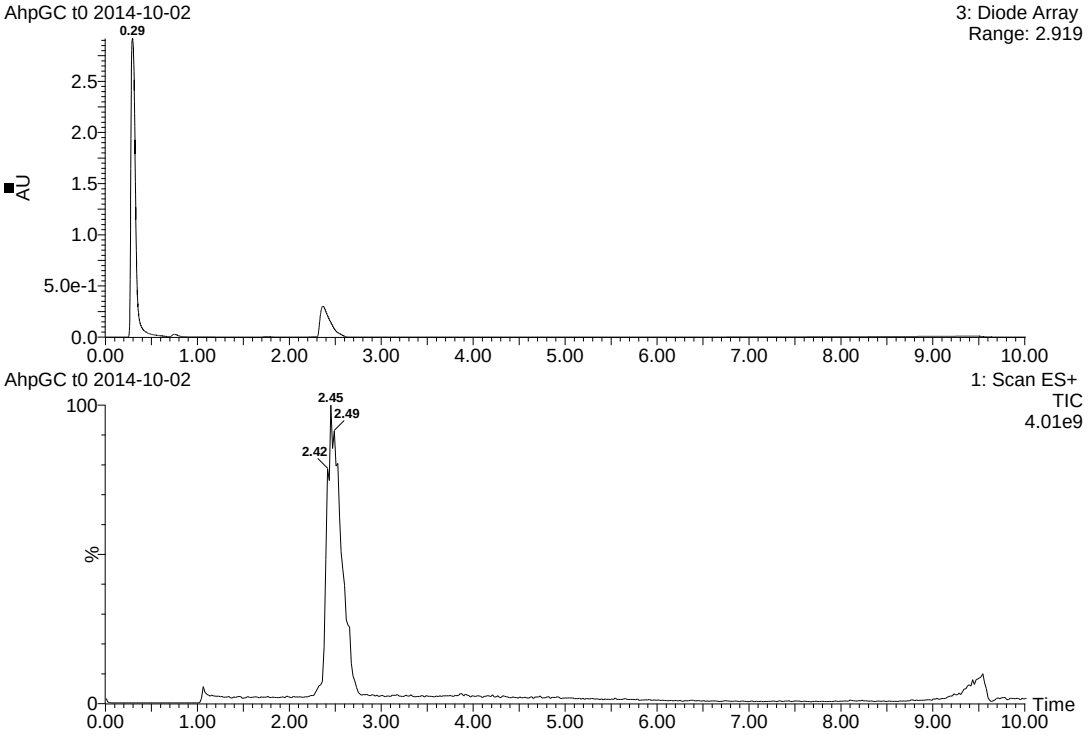


Product **12e**

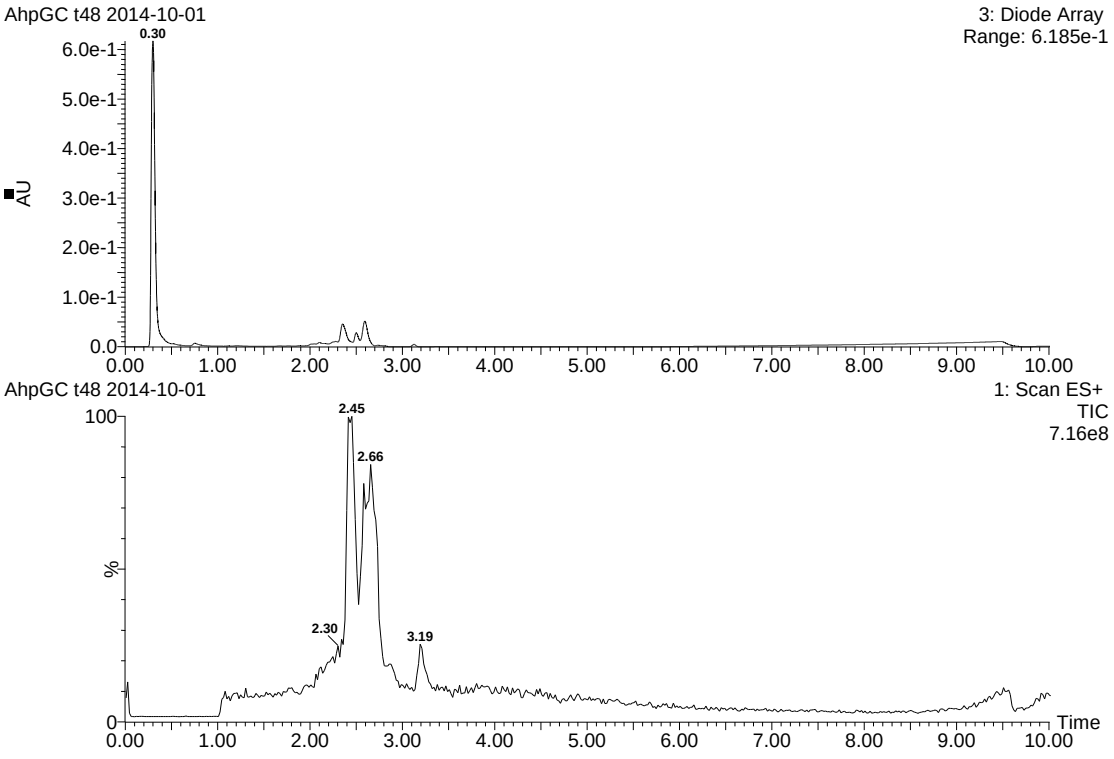


GlyCys: THIOESTER FORMATION

t0

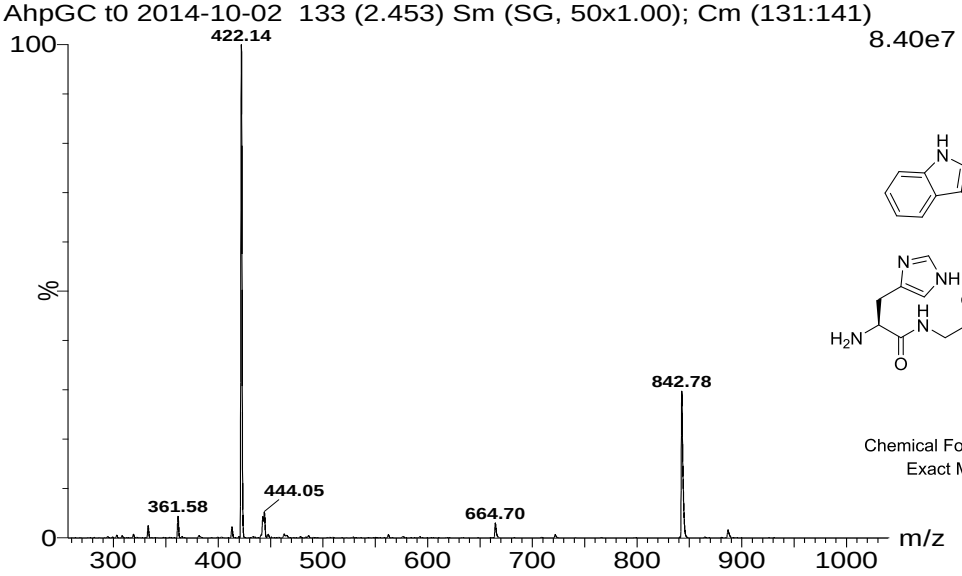


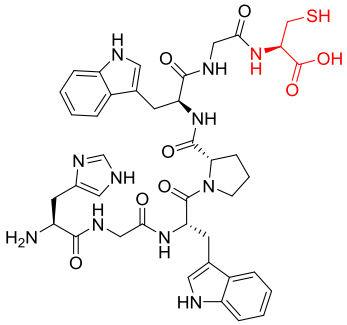
t48



starting material **8a**

AhpGC t0 2014-10-02 133 (2.453) Sm (SG, 50x1.00); Cm (131:141)

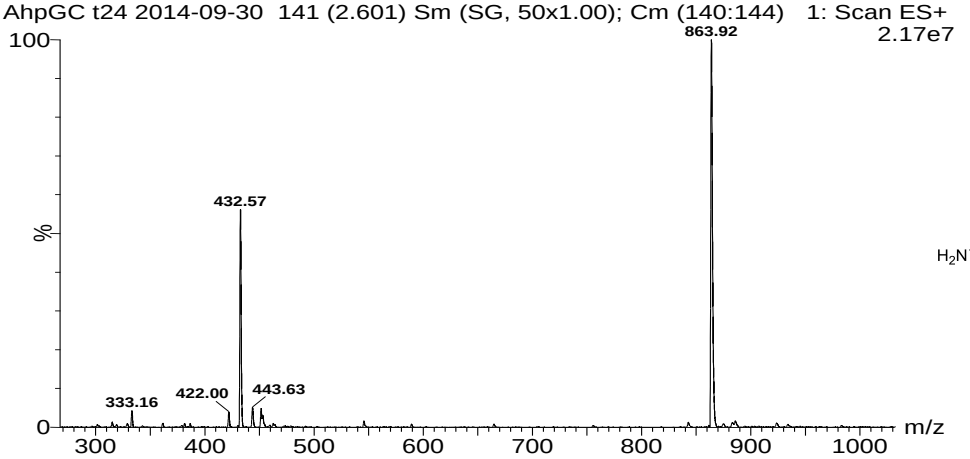


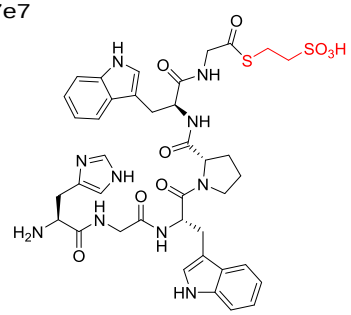


Chemical Formula: $C_{40}H_{47}N_{11}O_8S$
Exact Mass: 841.33298

Product **10a**

AhpGC t24 2014-09-30 141 (2.601) Sm (SG, 50x1.00); Cm (140:144) 1: Scan ES+

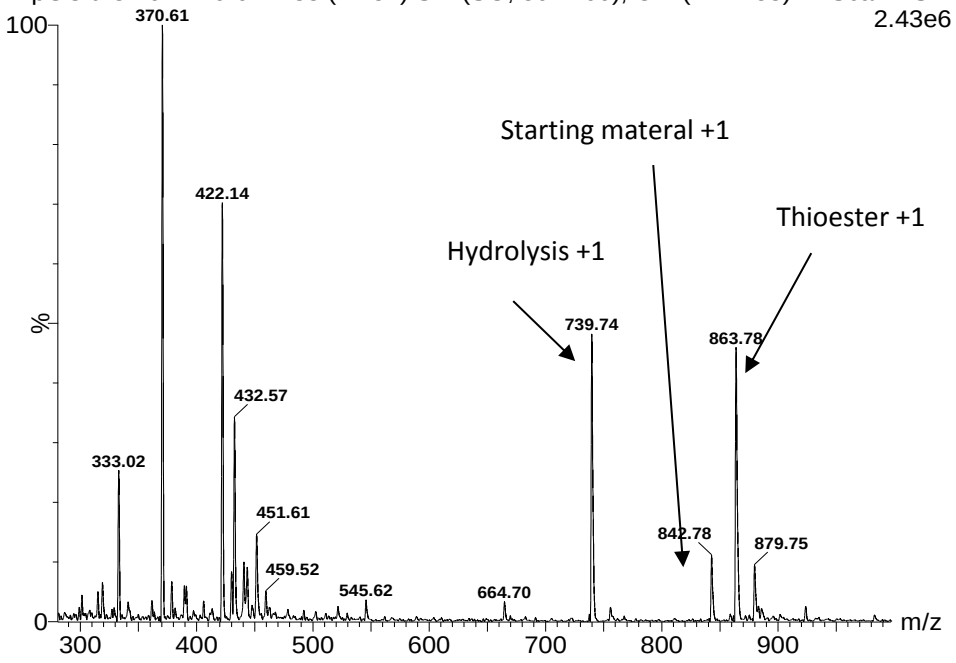




Chemical Formula: $C_{39}H_{46}N_{10}O_9S_2$
Exact Mass: 862.28906

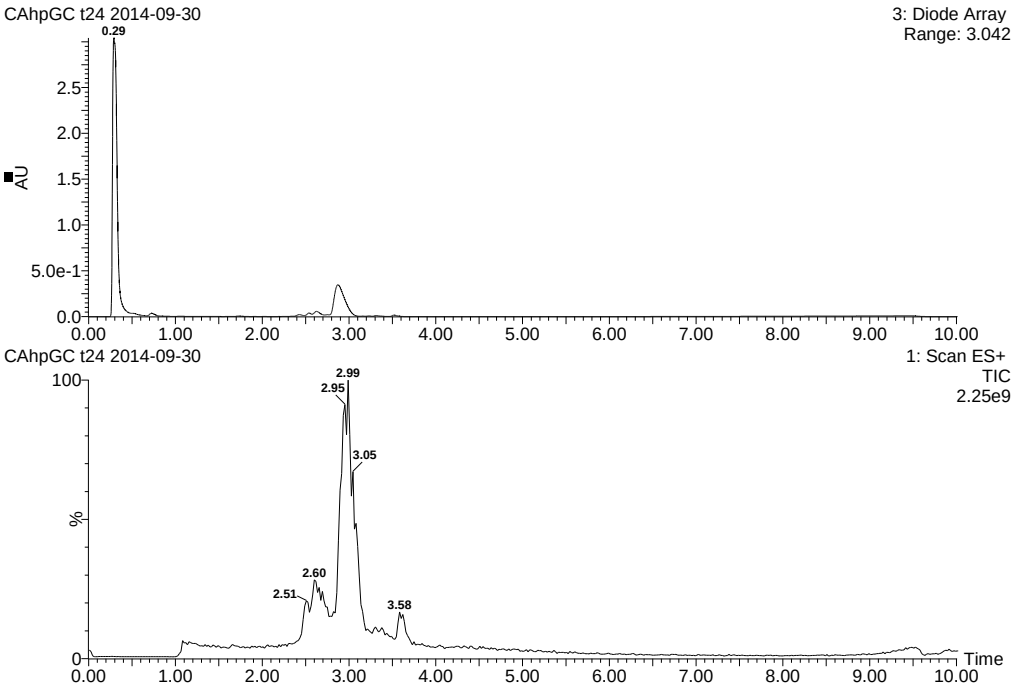
combined TIC @ t48

AhpGC t48 2014-10-01 133 (2.452) Sm (SG, 50x1.00); Cm (121:153) 1: Scan ES+

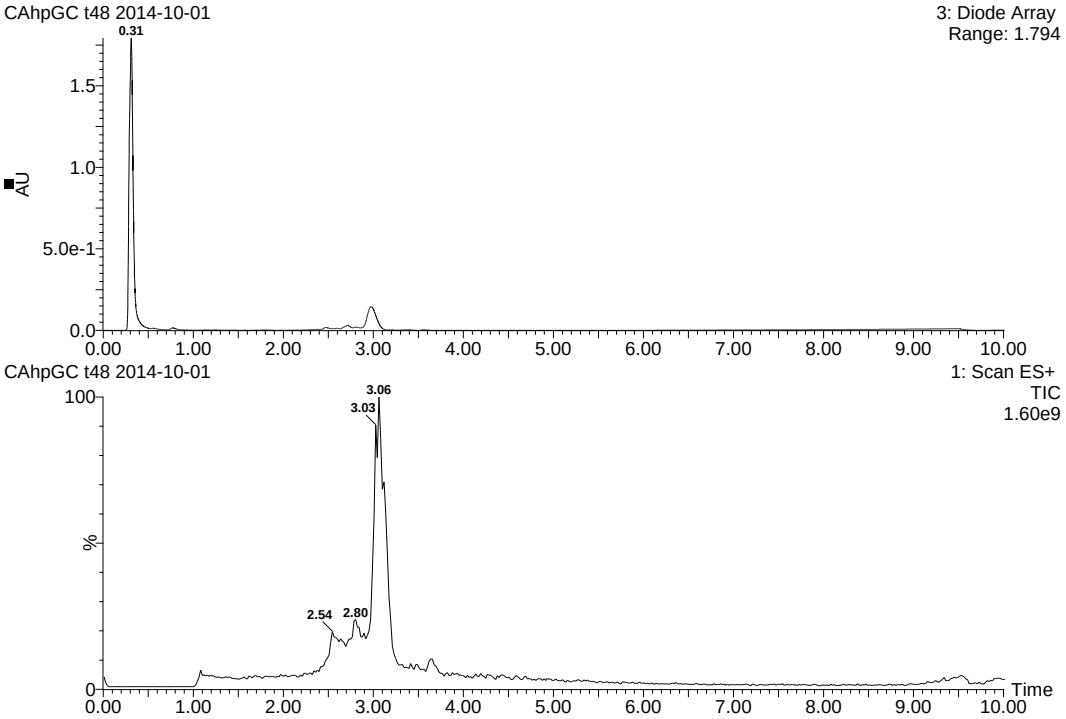


CYCLISATION

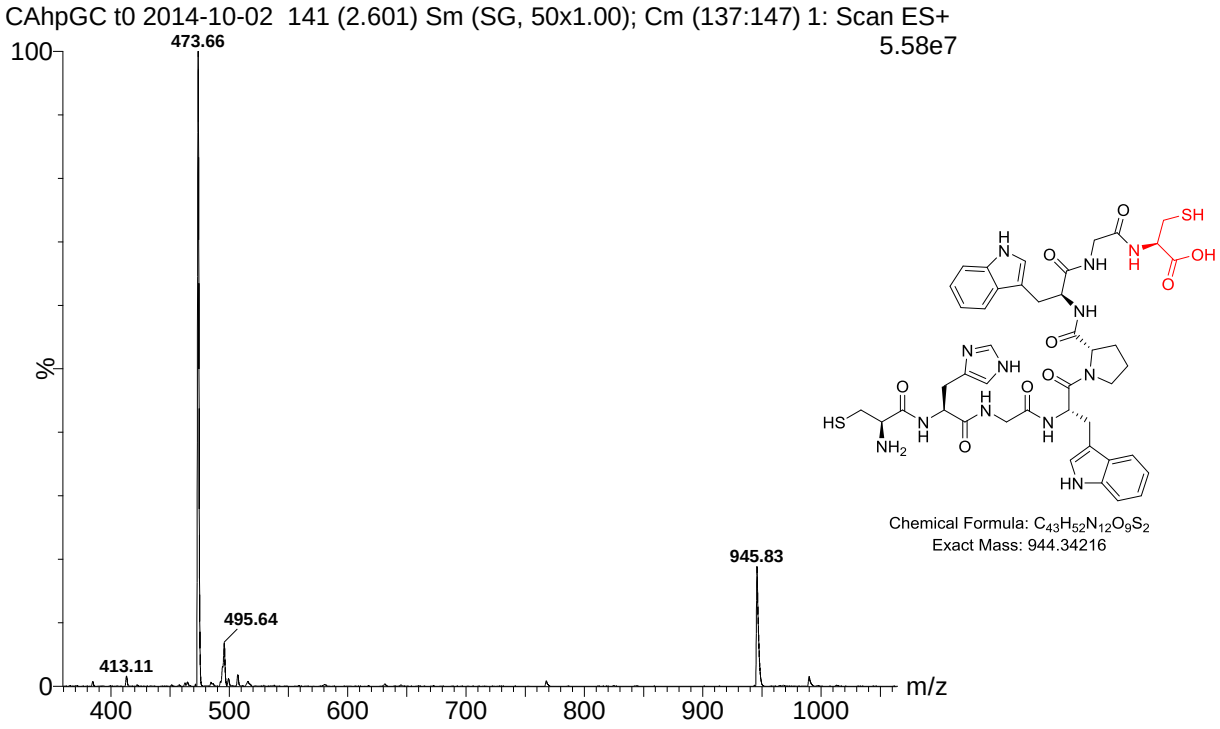
t24



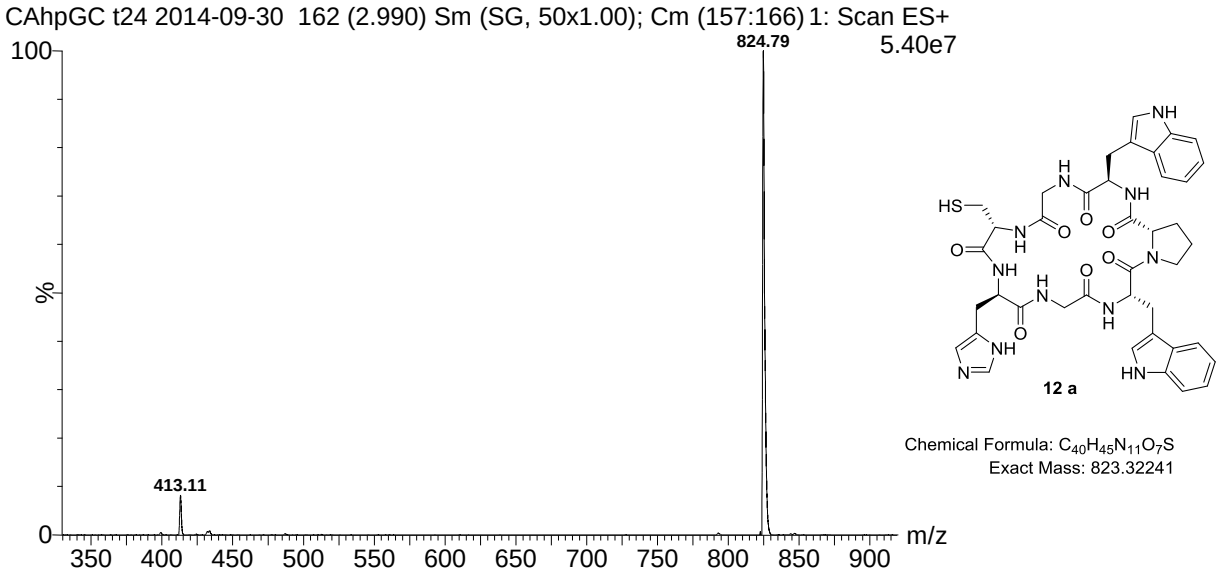
t48



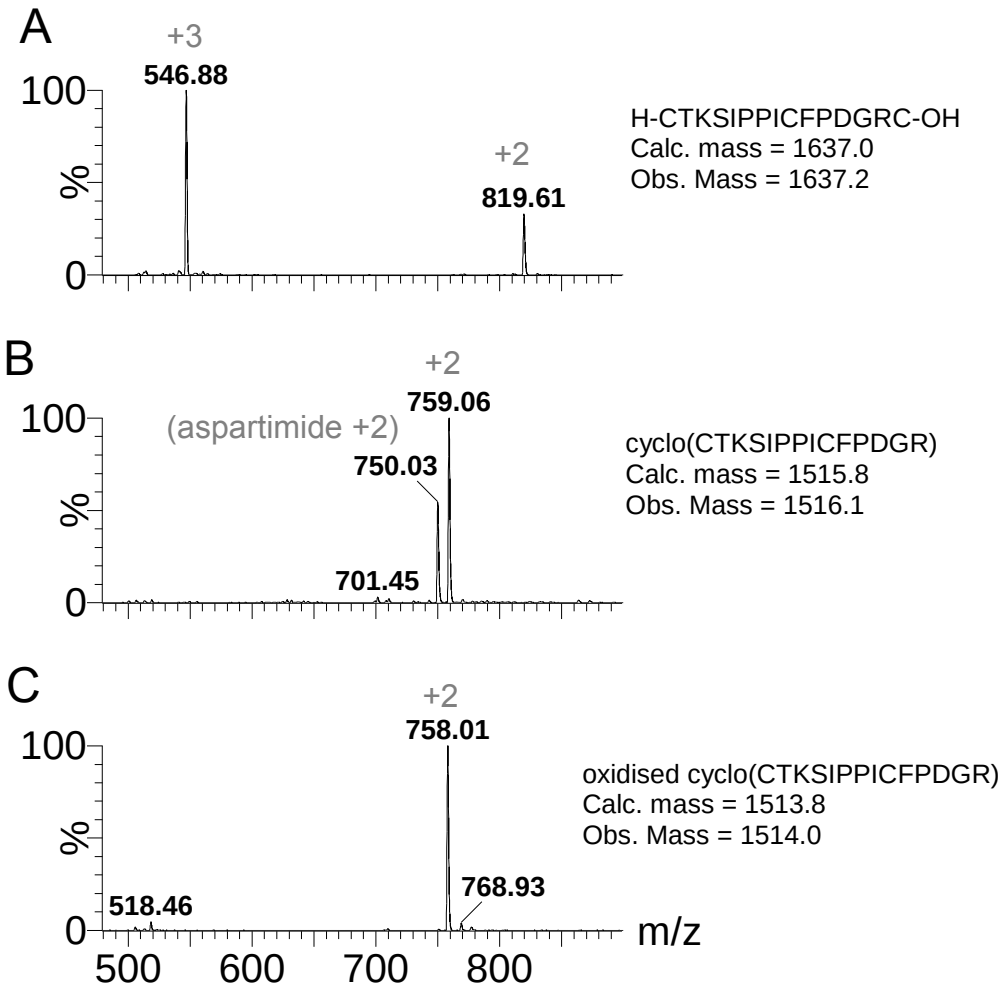
starting material **9a**



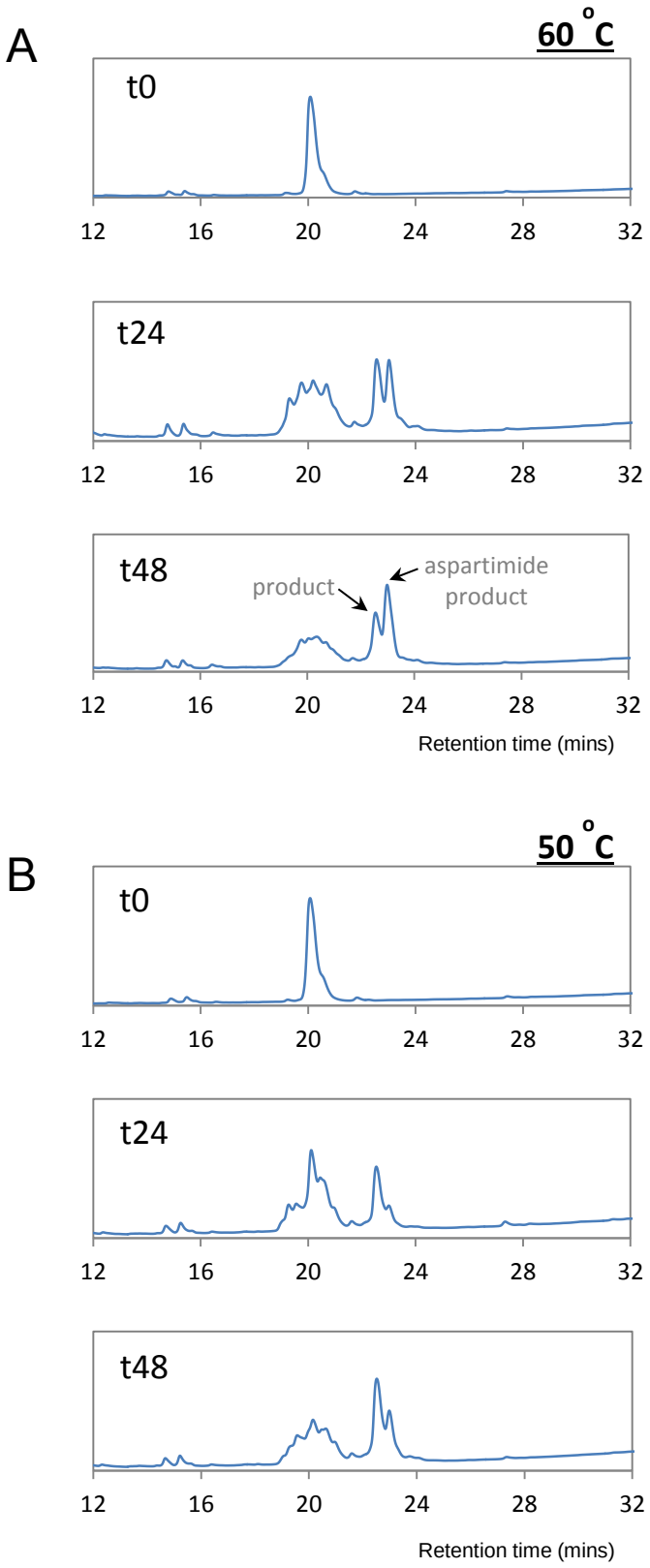
Product **12a**



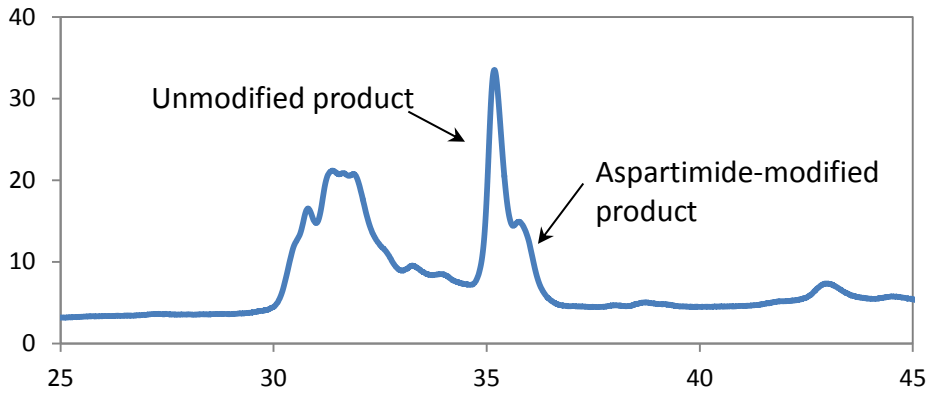
SFTI-1 synthesis



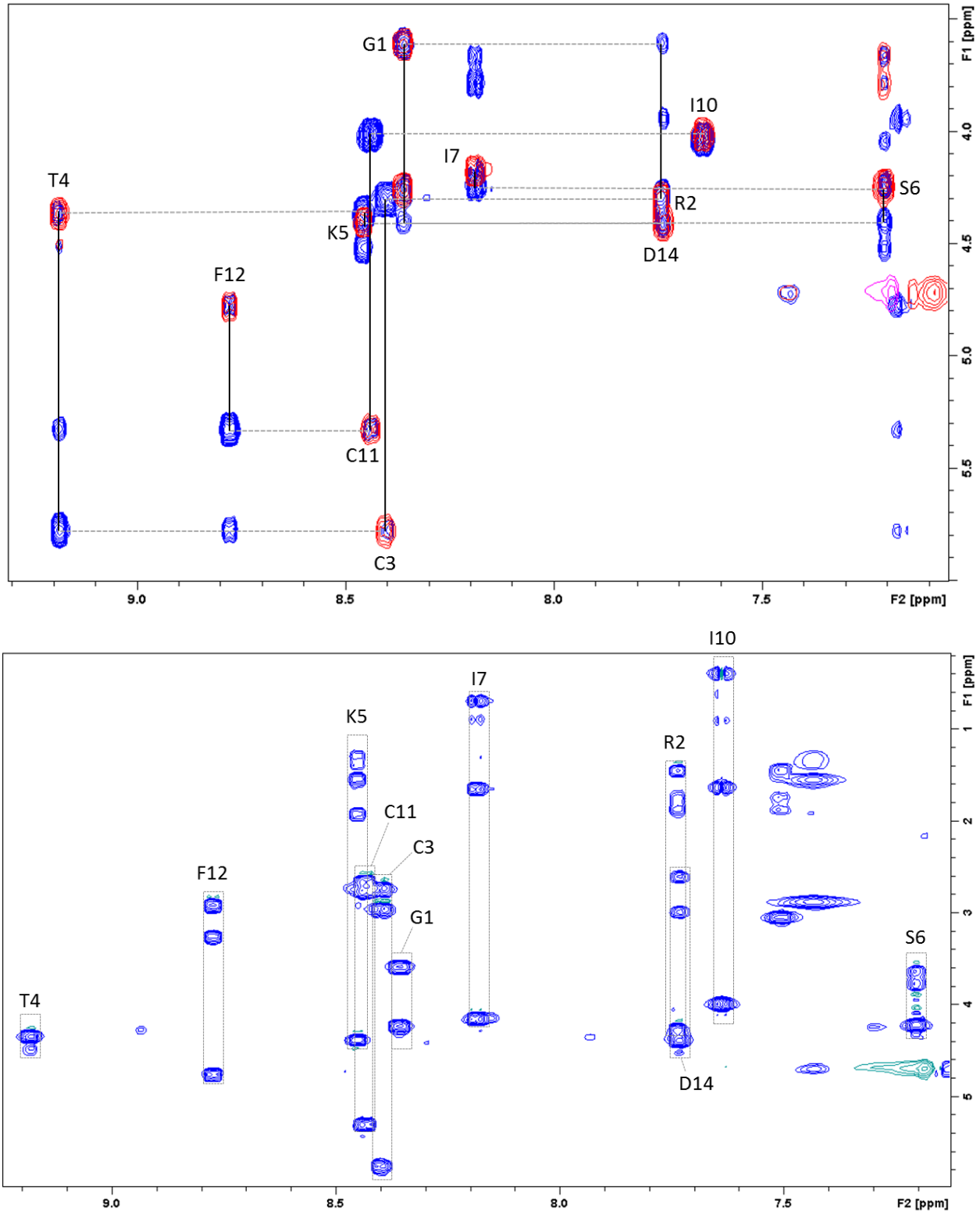
mass spectra of linear SFTI-1 precursor (**A**), cyclised (**B**) and cyclised/ oxidised (**C**) SFTI-1.



Analytical HPLC of cyclisation reactions performed at 60 °C (**A**) and 50 °C (**B**).



Separation of unmodified and aspartimide-modified cyclic SFTI-1 using preparative RP-HPLC.



A) overlaid ^{1}H-^{1}H NOESY and ^{1}H-^{1}H TOCSY spectra of wild-type SFTI-1 showing sequential assignment of backbone amide chemical shifts. **B)** Annotated ^{1}H-^{1}H TOCSY spectrum of wild-type SFTI-1 showing complete side-chain resonances for each amino acid.