CHEMISTRY
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Supporting Information

Intra-annular Savige-Fontana reaction: One step conversion of one class of monocyclic peptides into another class of bicyclic peptides.

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Contents

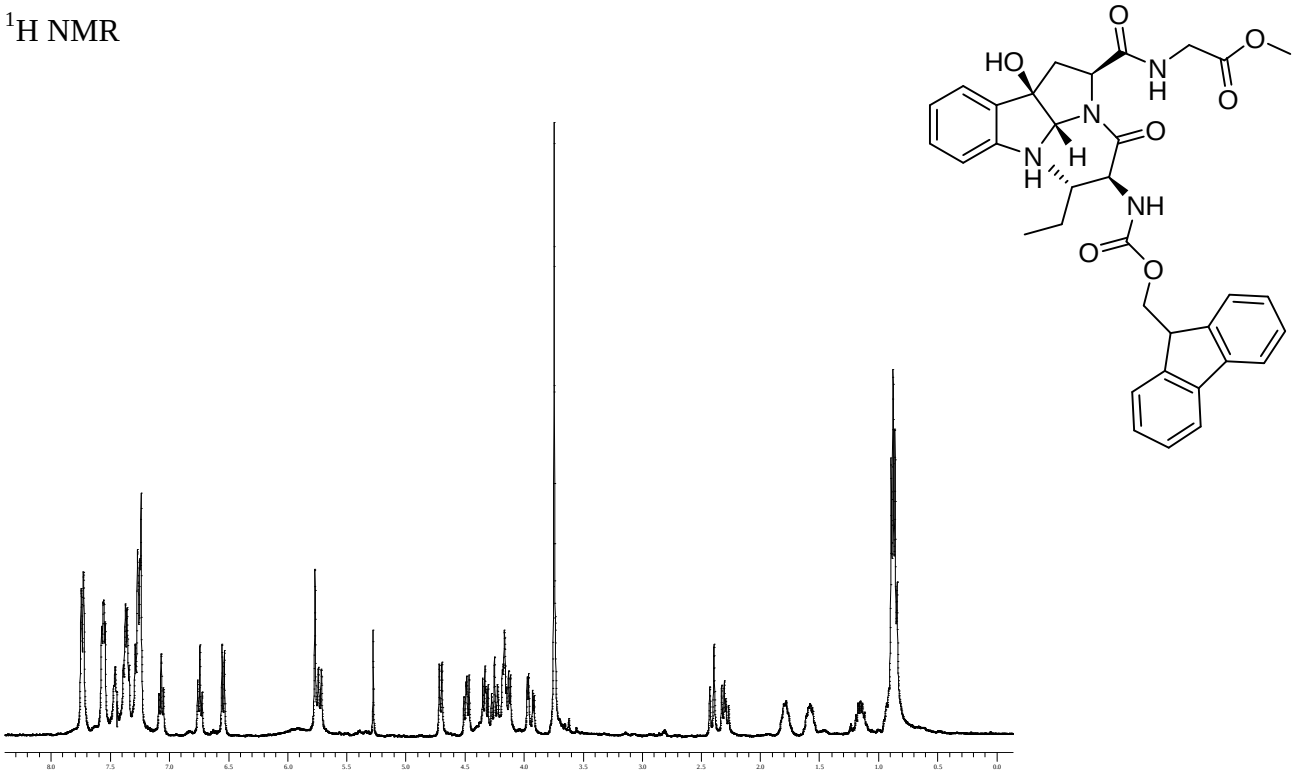
General experimental details

^{1}H-NMR and ^{13}C-NMR were performed at 300/400/600 MHz and 75/100 MHz respectively. Chemical shifts for all spectra were reported in parts per million and referenced to the solvent peak.
Mass spectrometry data was acquired using positive or negative ionization mode in MeOH or MeCN.
UV spectra were recorded on a spectrophotometer in 1 mL quartz cuvettes.
HPLC was performed using a reverse-phase C18 column (8.5 x 15 x 1.5 mm) with gradients combining buffer A and B. Buffer A = (H_2O + 0.1 % TFA); Buffer B = (MeCN + 0.05% TFA).
Thin layer chromatography was performed on Merck silica plates.
All amino-acids are L-amino-acids unless otherwise stated.
Preparations and full characterisation of both diastereomers of H-Hpi-Gly-OMe have been described in our previous paper.[1]

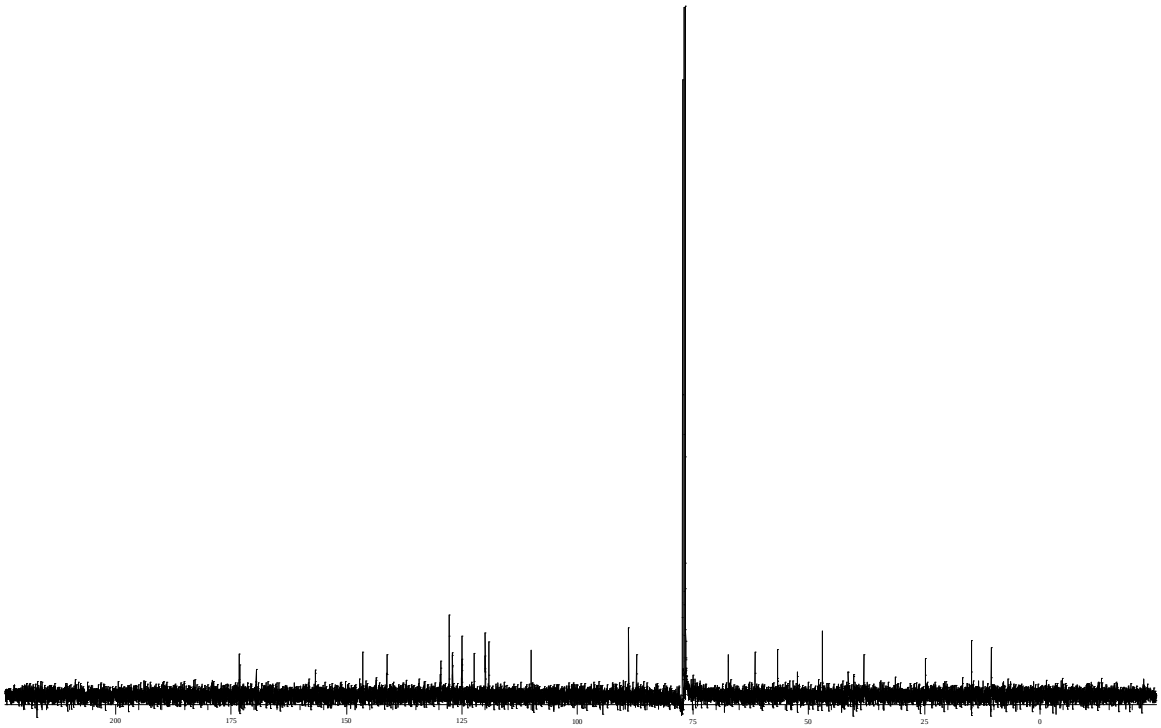
[1] May, J.P.; Fournier, P.; Pellicelli, J.; Patrick, B. O.; Perrin, D. M.; *J. Org. Chem.* **2005**, *70*, 8424-8430.

Compound **4**: [*syn-cis*]-Fmoc-Ile-Hpi-Gly-OMe

^{1}H NMR

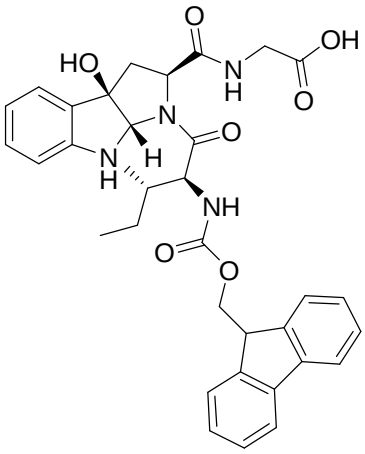


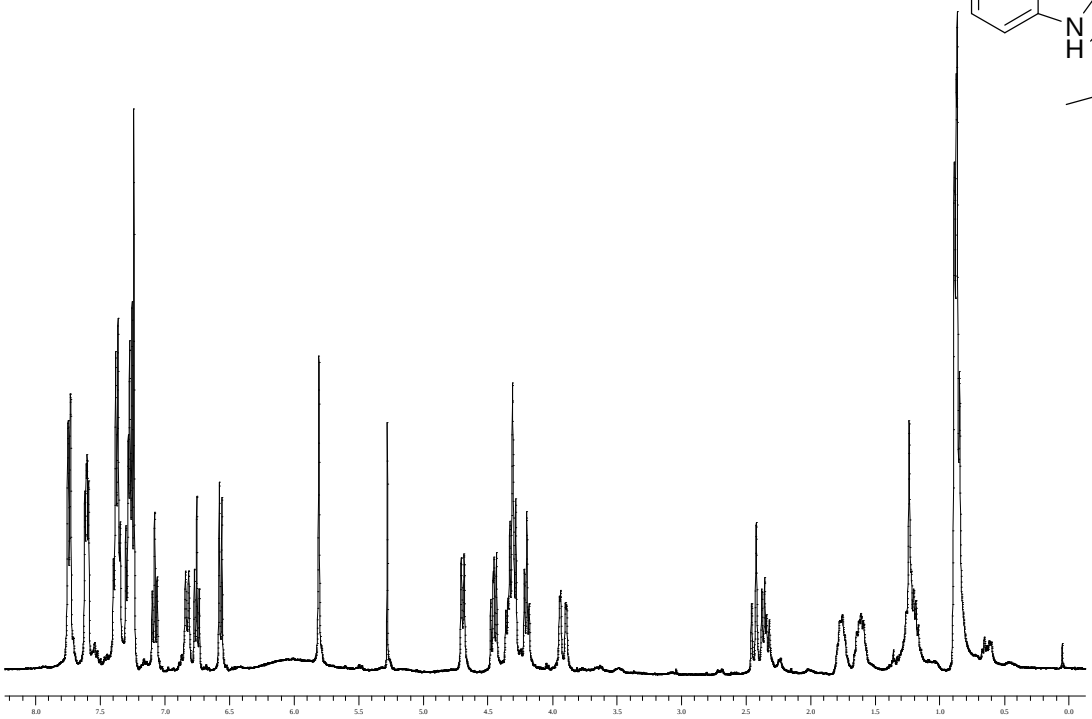
^{13}C NMR



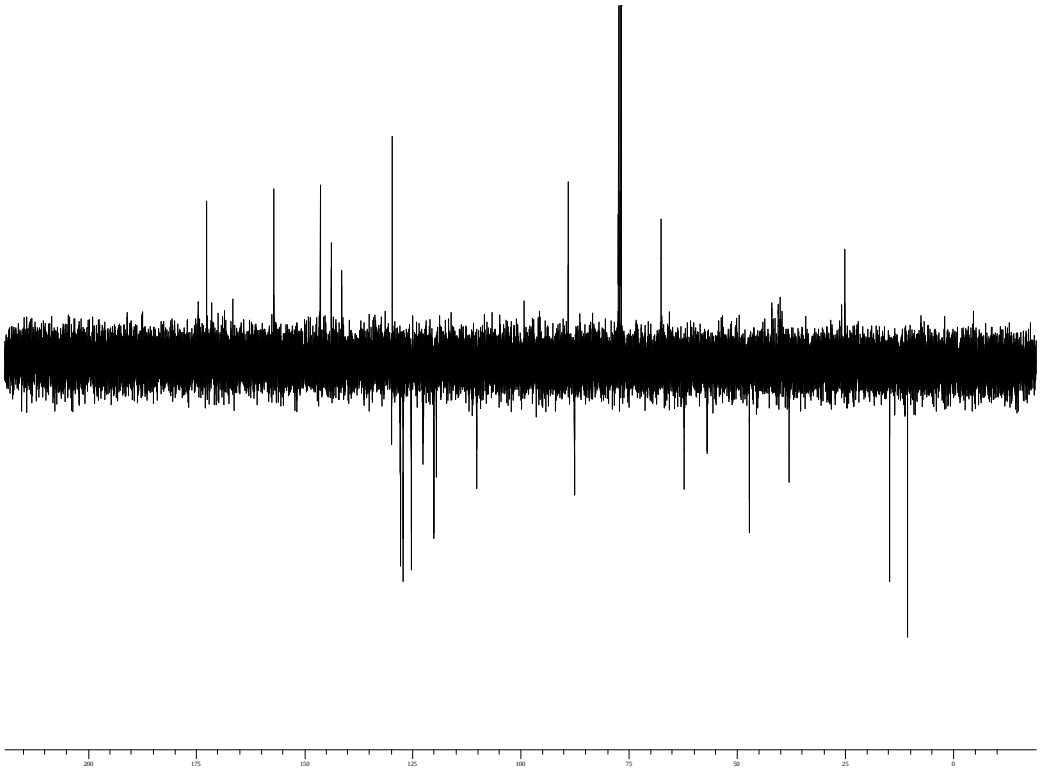
Compound **5**: [*syn-cis*]-Fmoc-Ile-Hpi-Gly-OH

^{1}H NMR

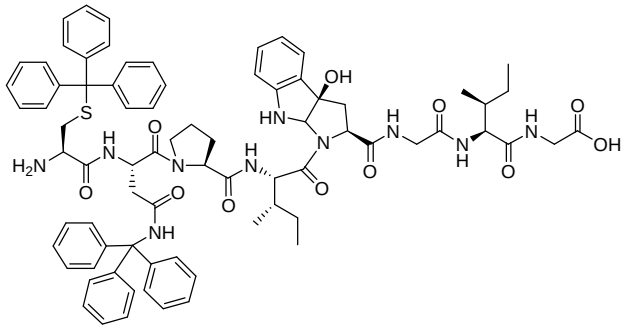




^{13}C NMR

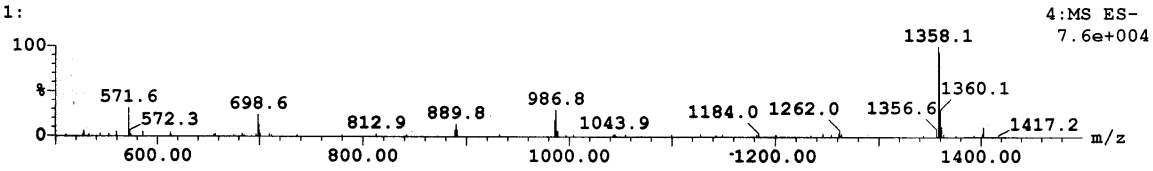


Compound **8**: H-Cys(Tr)-Asn(Tr)-Pro-Ile-Hpi-Gly-Ile-Gly-OH

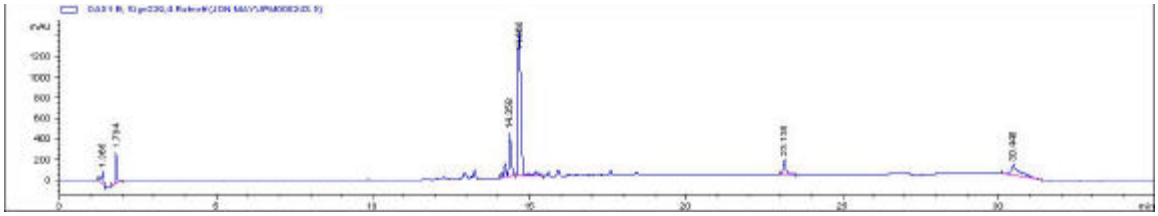


Chemical Formula: $C_{77}H_{86}N_{10}O_{11}S$
Exact Mass: 1358.6198
Molecular Weight: 1359.6321

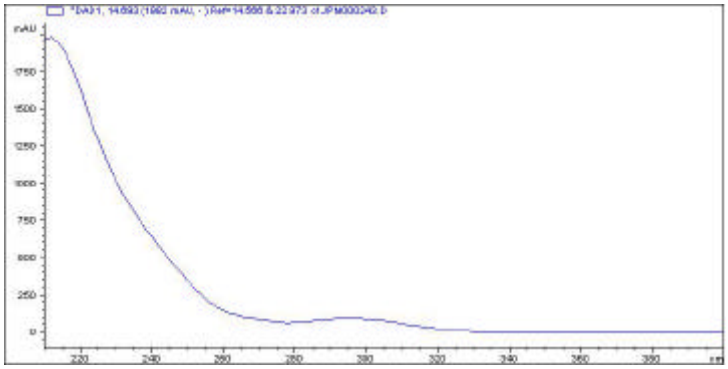
MS/ES –ve



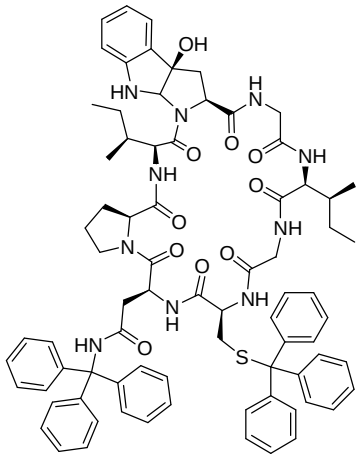
crude HPLC trace



UV of major peak

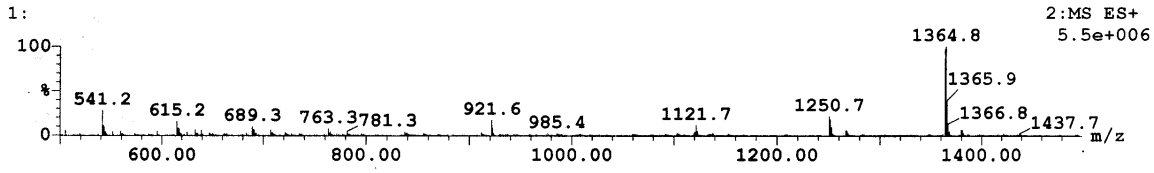


Compound **9**: [*syn-cis*]-Cyclo(Cys(Tr)-Asn(Tr)-Pro-Ile-Hpi-Gly-Ile-Gly)

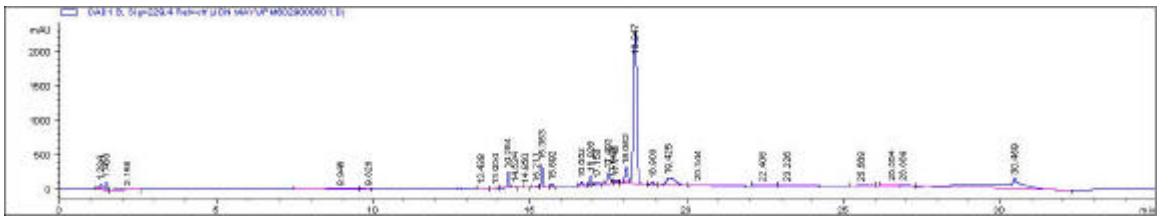


Chemical Formula: $C_{77}H_{84}N_{10}O_{10}S$
Exact Mass: 1340.6093
Molecular Weight: 1341.6169

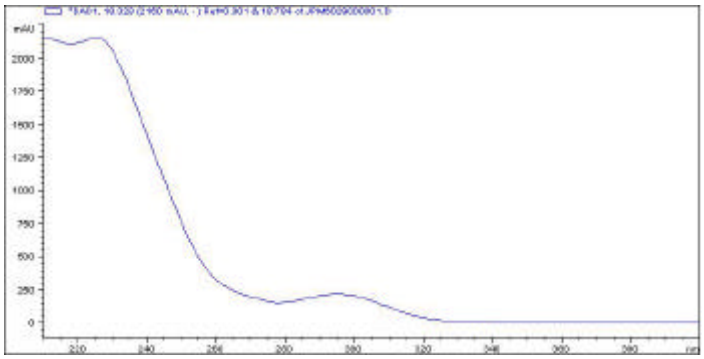
MS/ES +ve



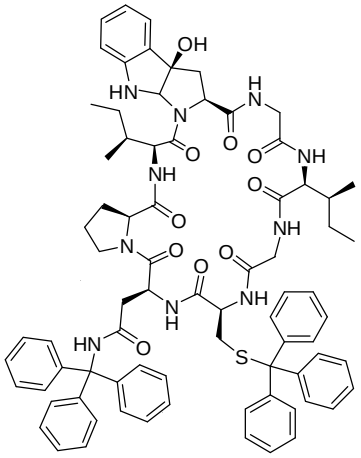
HPLC trace

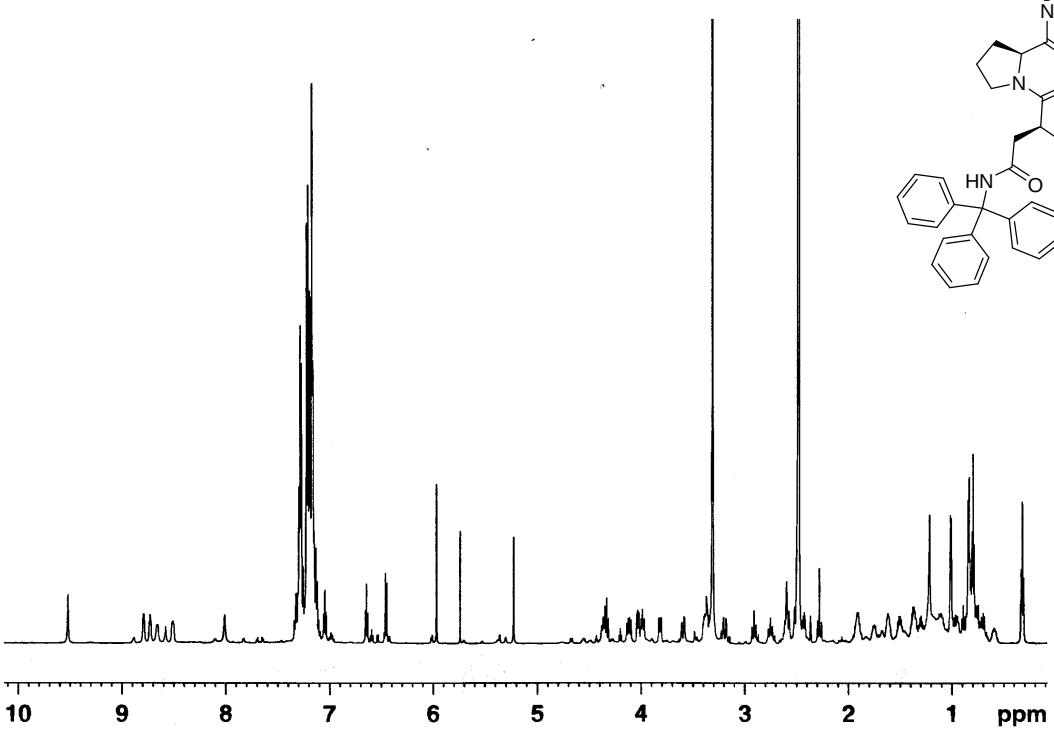


UV of major peak from HPLC

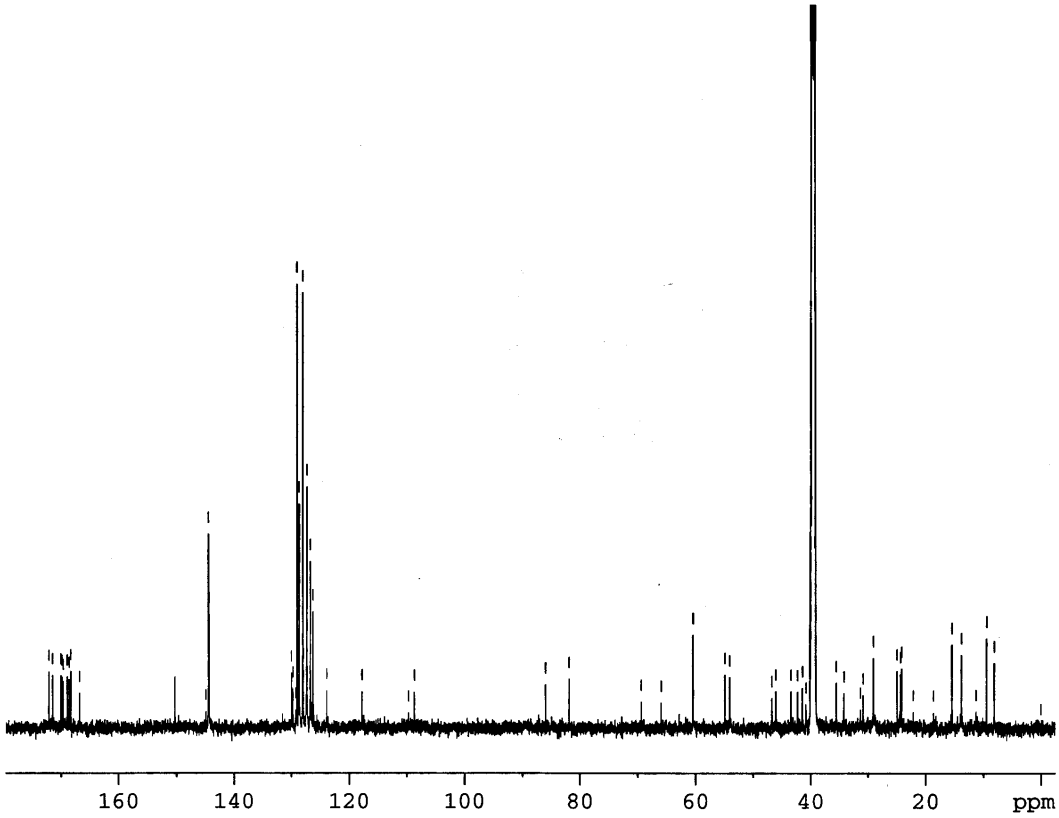


^{1}H NMR

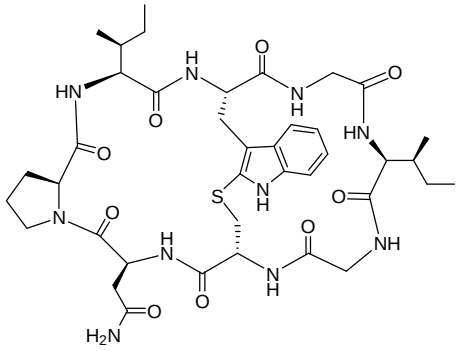




^{13}C NMR

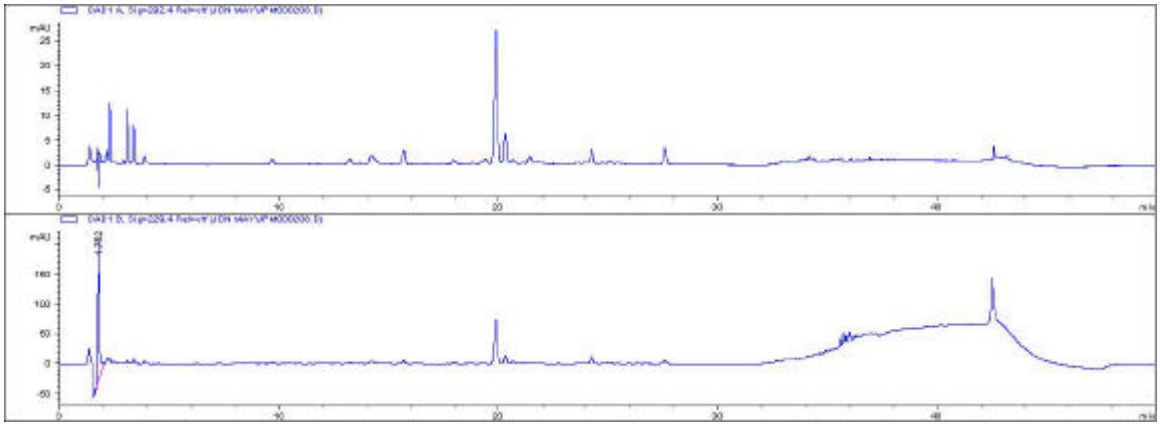


Compound **10**: Pro[2]-Ile[3]-*S*-deoxo-amaninamide

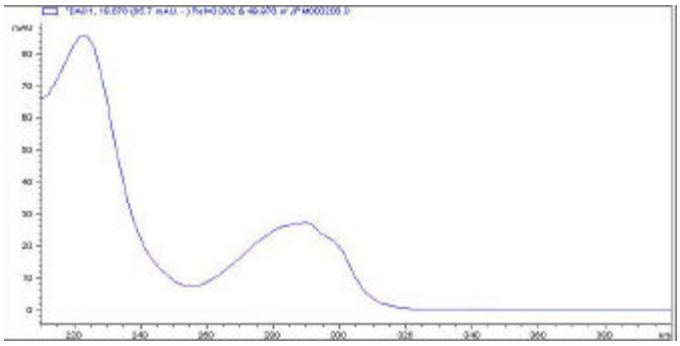


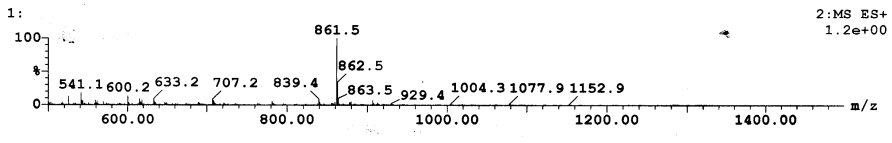
Chemical Formula: $C_{39}H_{54}N_{10}O_9S$
Exact Mass: 838.3796
Molecular Weight: 838.9727

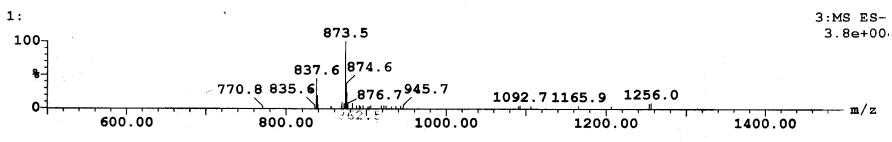
Crude HPLC



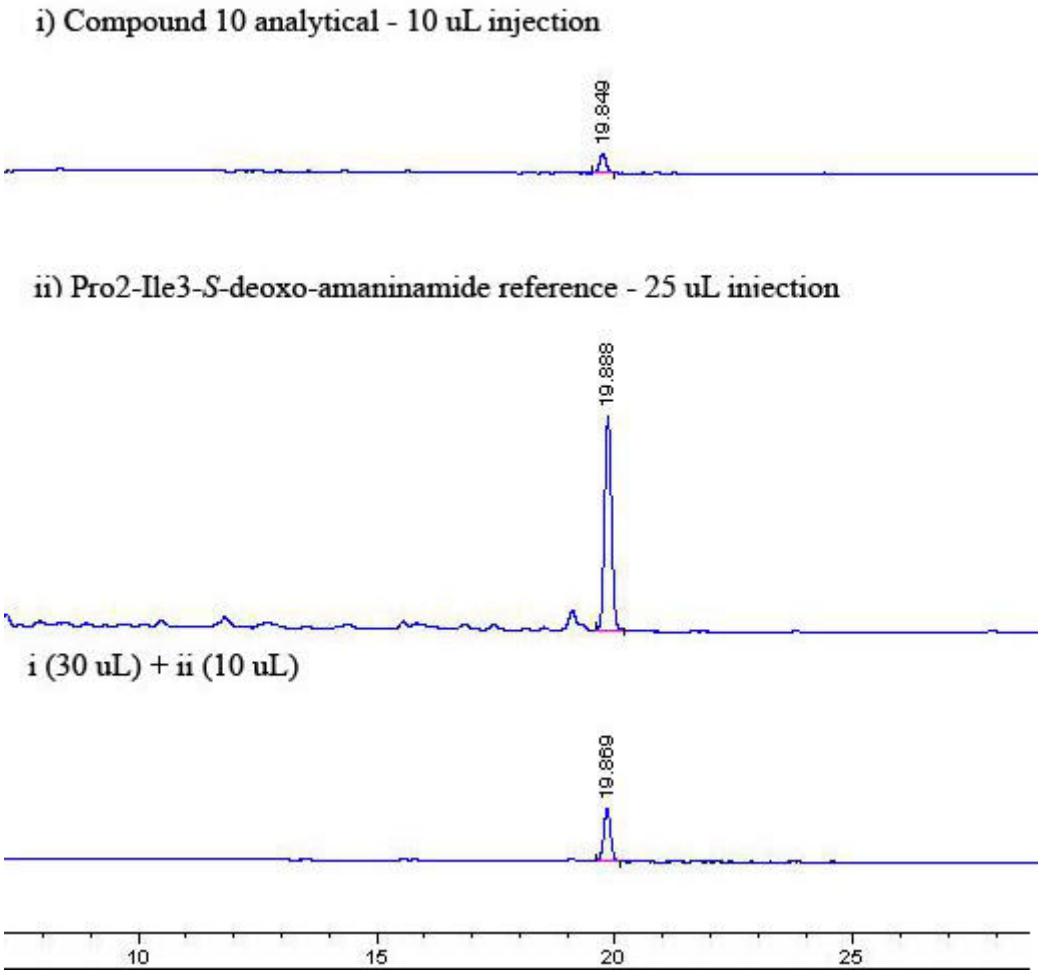
UV of major peak from HPLC







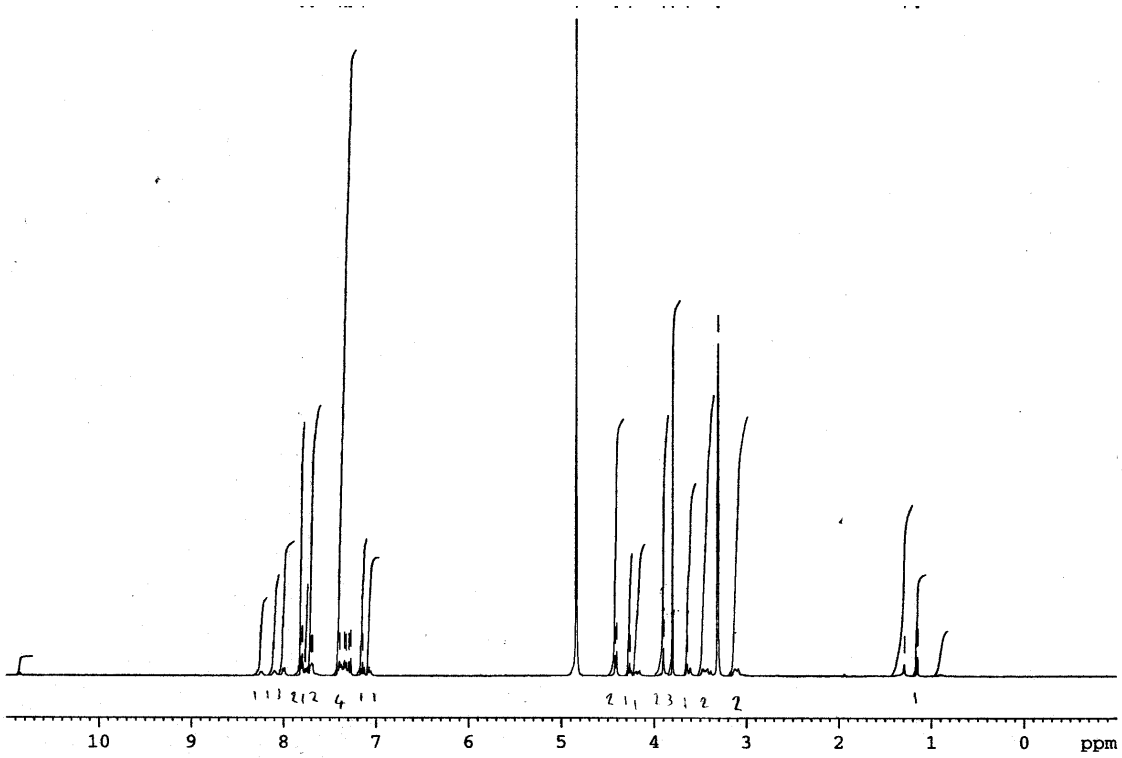
HPLC analysis of compound **10** relative to reference Pro^2-Ile^3-*S*-deoxo-amaninamide[2]



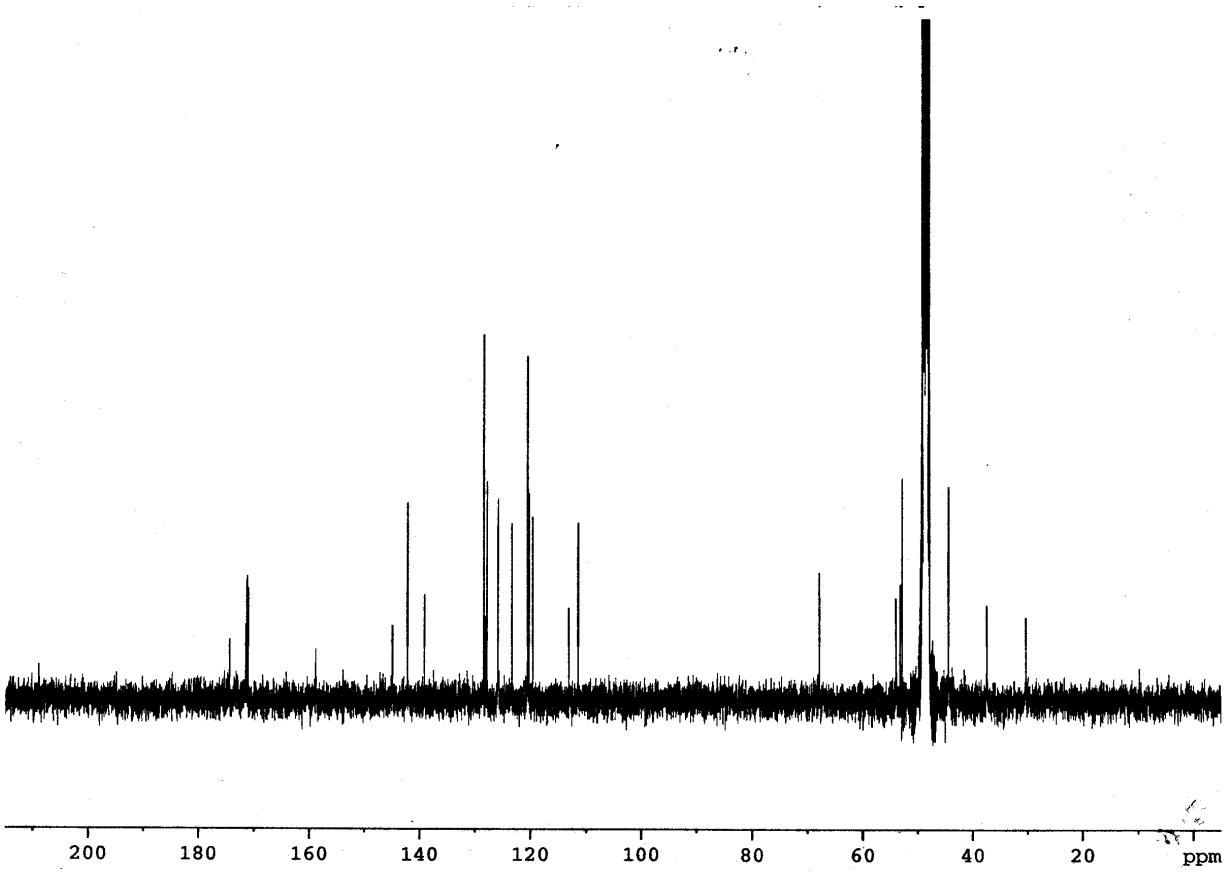
[2] J. P. May, P. Fournier, B. O. Patrick, D. M. Perrin, *Chem. Eur. J. in press* **2007**.

Compound **13**: cyclic tryptathionine - Fmoc-Gly-Trp-Gly-Cys-OMe

^{1}H NMR

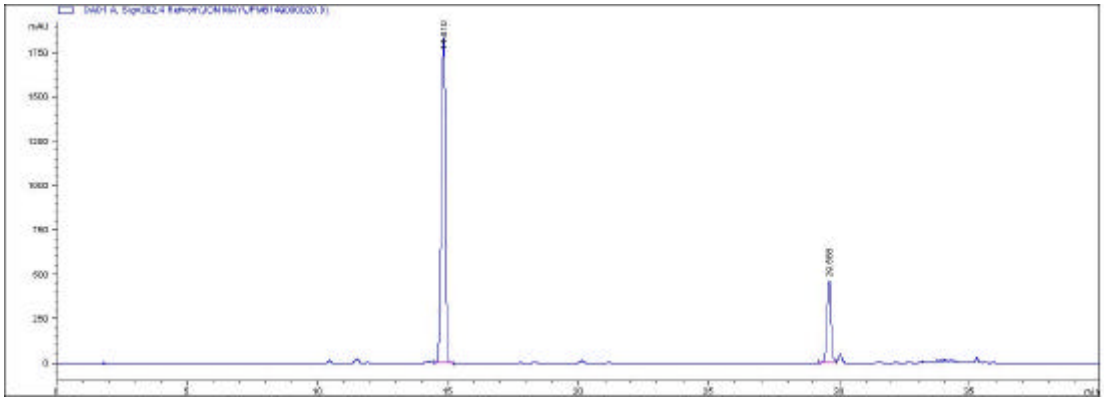


^{13}C NMR

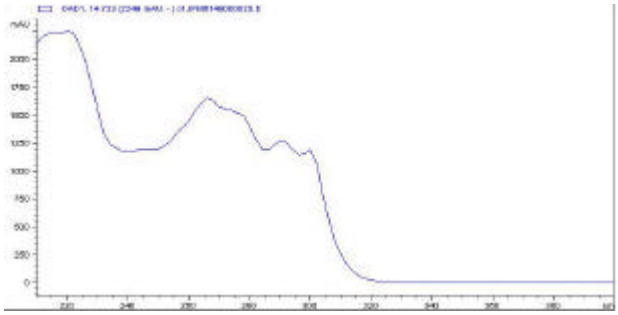


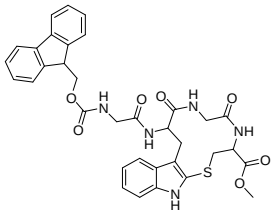
HPLC study on cyclic compound 13:

HPLC trace of crude **13** showed two peaks. The first peak (rt = 14.8) was the desired product.

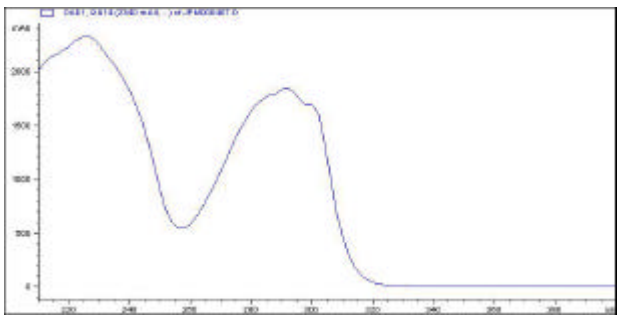


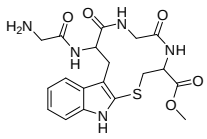
Peak 1 (rt = 14.8) was collected and characterized (NMR, MS, UV), but UV didn't show the characteristic absorption of a tryptathionine, because it was partially masked by the Fmoc moiety.





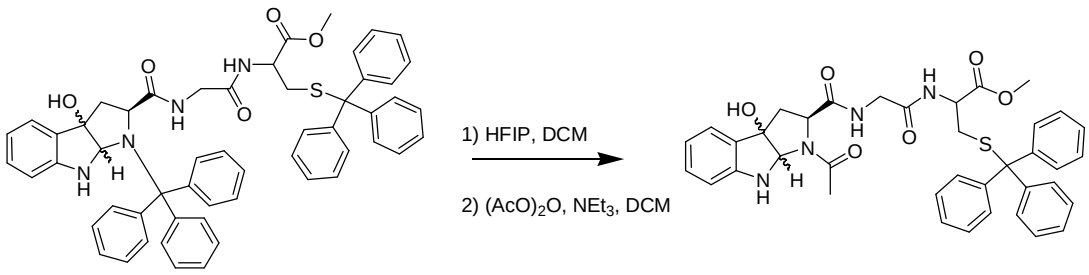
Hence, a small sample of **13** was treated with piperidine, evaporated to dryness and redissolved in MeOH/H_2O for analysis by HPLC. Three peaks were seen. Peak 1 had a characteristic UV spectra and MS of desired Fmoc-deprotected tryptathionine compound (see below). The other two peaks corresponded to starting material and Fmoc-piperidine byproduct.





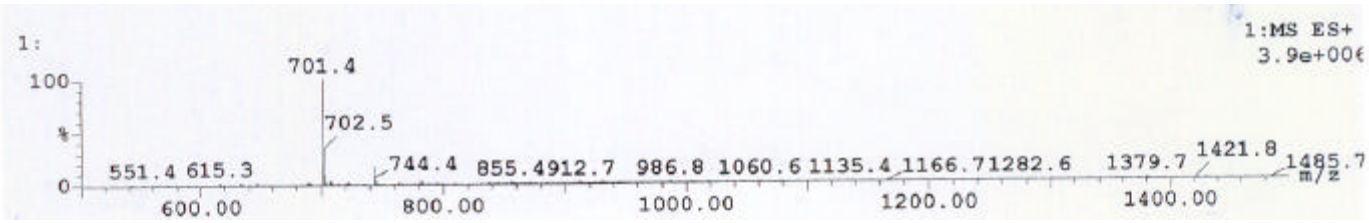
Study on Compound 14 (Ac-Hpi-Gly-Cys(Tr)-OMe)

The tripeptide Tr-Hpi-Gly-Cys(Tr)-OMe (May *et al. J. Org. Chem.* **2005**, *70*, 8424) was treated with HFIP/CH_2Cl_2 (1:4) for 10 mins, then the solvent was evaporated to dryness. The residue was redissolved in CH_2Cl_2 and acetic anhydride (1 eq.) and triethylamine (excess) were added. After 1hr the reaction was diluted with CH_2Cl_2 and washed with citric acid, sodium bicarbonate and brine. The organic phase was dried over sodium sulphate and purified with a silica column (CH_2Cl_2/MeOH 0-10%). The desired product was found by mass spec and a single peptide product was observed by HPLC.

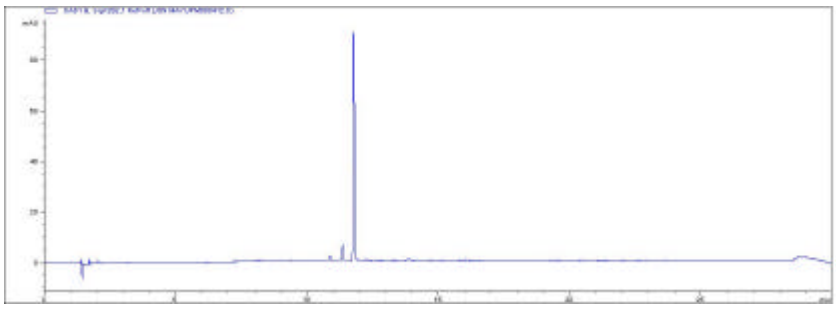


Chemical Formula: $C_{38}H_{38}N_4O_6S$
Exact Mass: 678.2512

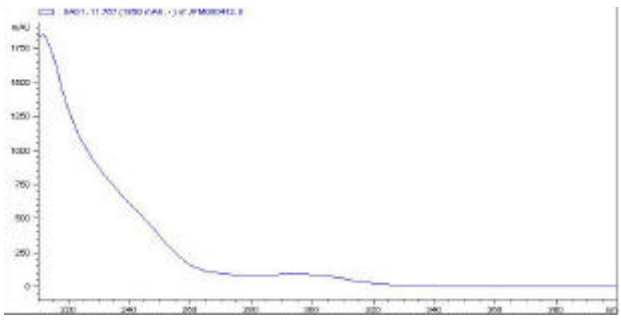
MS of product



Analytical HPLC of product

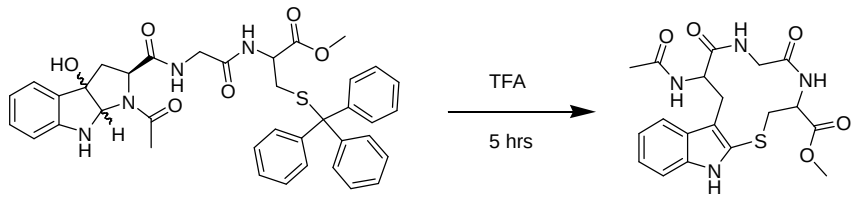


UV absorption of product

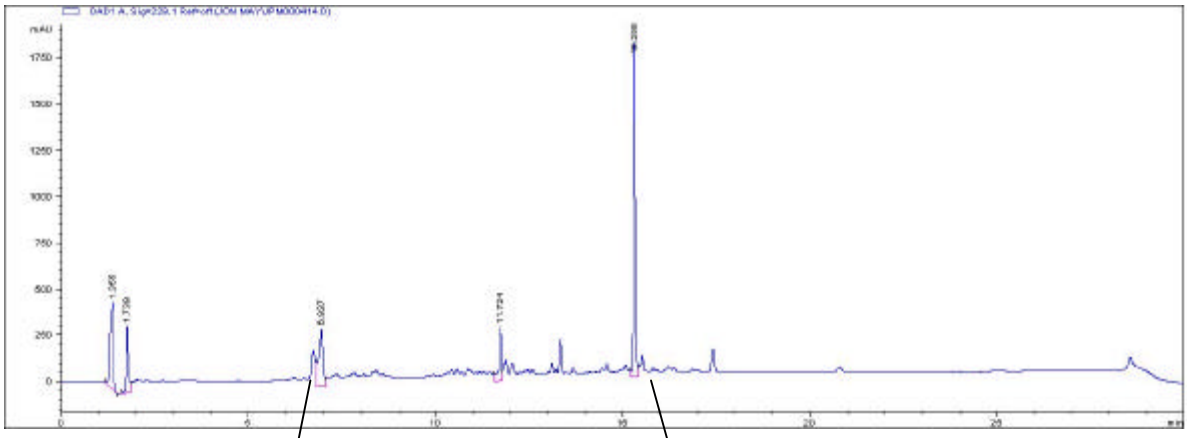


N.B. No significant UV absorption shown in 250-350 region: no tryptathionine.

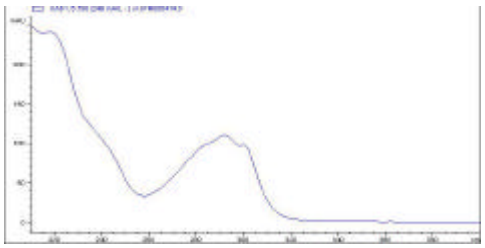
Compound **14** was then treated with neat TFA for 5 hrs.



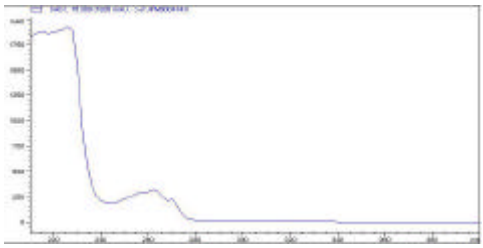
Chemical Formula: $C_{19}H_{22}N_4O_5S$
Exact Mass: 418.1311



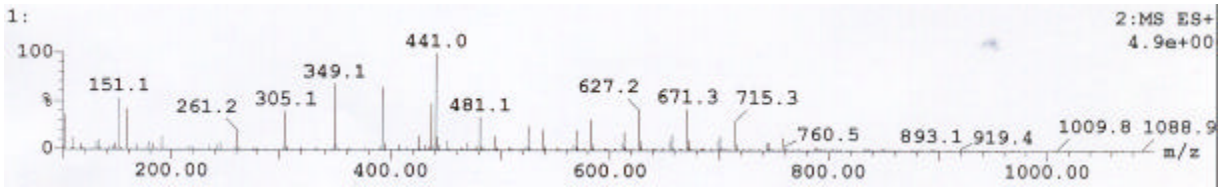
Peak 1



Peak 2



Peaks corresponding to tryptathionine (peak 1) and oxindole (peak 2) were observed. The desired mass of the tryptathionine compound was seen in mass spectrometry of the crude reaction mixture ($(M+Na)^+$ = 441).



[*anti-cis*]-Fmoc-Ile-Hpi-Gly-Cys(Tr)-OMe: Method was identical to that used for compound 1. White foam 0.16 g (50%). R_f = 0.5 (CH_2Cl_2/MeOH 9:1); ^{1}H NMR (400 MHz, DMSO-d6) δ= 8.01-7.75 (m, 4H, NHCO, ArH^{Fmoc}), 7.71-7.65 (m, 2H, ArH^{Fmoc}), 7.46-7.37 (m, 2H, ArH^{Fmoc}), 7.36-7.22 (m, 2H, ArH^{Fmoc}), 7.11 (d, 1H, *J* = 7.5 Hz, ArH^{indole}), 7.03 (t, 1H, *J* = 7.5 Hz, ArH^{indole}), 6.75 (d, 1H, *J* = 4.0 Hz, NH^{indole}), 6.63 (t, 1H, *J* = 7.5 Hz, ArH^{indole}), 6.54 (d, 1H, *J* = 7.5 Hz, ArH^{indole}), 5.98 (s 1H, OH), 5.82 (d, 1H, *J* = 4.1 Hz, CH^{Hpi8a}), 4.60 (t, 1H, *J* = 8.1 Hz, CH^{Ilea}), 4.30-4.12 (m, 4H, $CH_2^{Fmoc,}$ CH^{Fmoc} CH^{Hpia}), 3.75 (dd, 1H, *J* = 11.6, 6.0 Hz, CH^{glya}), 3.56-3.41 (m, 4H, CH_3^{OMe}, CH_2^{Glya}), 2.58-2.39 (m, 1H, $CH^{Hpiß}$), 2.01 (dd, *J* = 8.2, 4.9 Hz, CH ^{Hpiß}), 1.84-1.78 (m, 1H, $CH^{Ileß}$), 1.69-1.49 (m, 1H, $CH^{Ile?}$), 1.25-1.08 (m, 1H, $CH^{Ile?}$), 0.99-0.75 (m, 6H, $CH^{Ile?',Iled}$); ^{13}C NMR (100 MHz, DMSO-d6) δ= 173.9 (C^{COOH}), 172.3 (C^{CONH}), 171.4 (C^{CONH}), 157.6 (C^{CONH}), 149.8 (C^{Hpi}), 145.4, 145.1 (C^{Fmoc}), 142.2, 142.1 (C^{Fmoc}), 133.5 (C^{3b}), 130.4 (CH^{6}), 129.1 (CH^{Fmoc}), 128.5 (CH^{Fmoc}), 126.9, 126.8 (CH^{Fmoc}), 124.0 (CH^{4}), 121.5 (CH^{Fmoc}), 119.6 (CH^{5}), 111.6 (CH^{7}), 88.8 (C^{3a}), 87.1 (C^{8a}), 67.3 (CH^{Fmoc}), 62.3 (CH^{Ilea}), 58.2 (CH^{Fmoc}), 53.1 (CH^{OMe}), 48.1 (CH^{Hpia}), 43.7 ($CH^{Hpiß}$), 42.1 (CH^{Glya}), 38.2 ($CH^{Ileß}$), 25.8 ($CH_2^{Ile?}$), 16.4 ($CH^{Ile?'}$), 12.7 (CH^{Iled}); ES^+/MS: 649.2 $(M+Na)^+$.

[*anti-cis*]-Fmoc-Ile-Hpi-Gly-OMe (in DMSO)