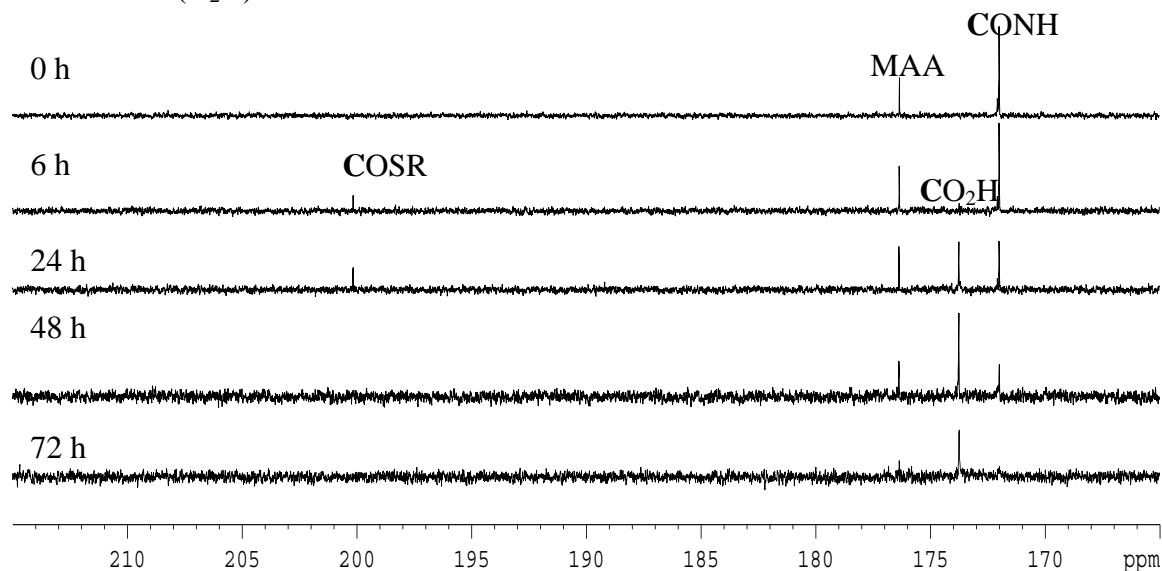


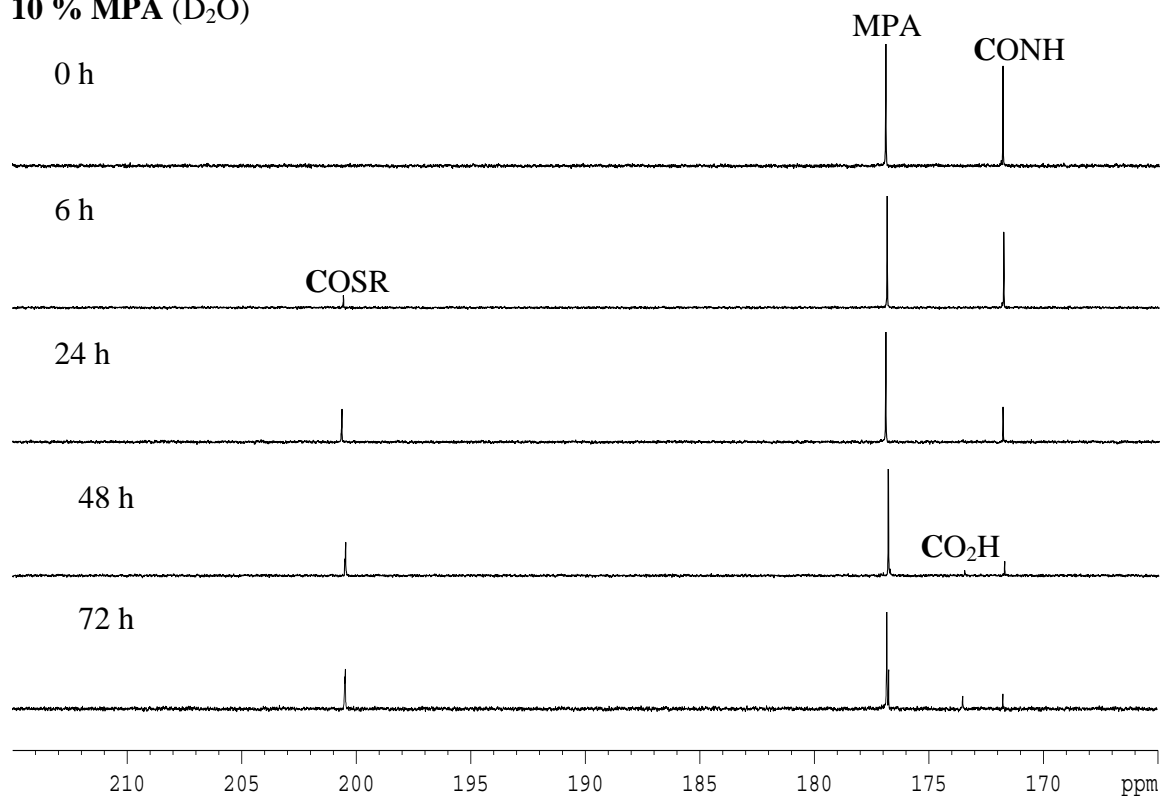
**Supporting Information for Peptide thioester synthesis through N-S acyl-transfer:
Application to the synthesis of a beta-Defensin.**

Jaskiranjit Kang, Natalie L. Reynolds, Christine Tyrrell, Julia Dorin, and Derek Macmillan*

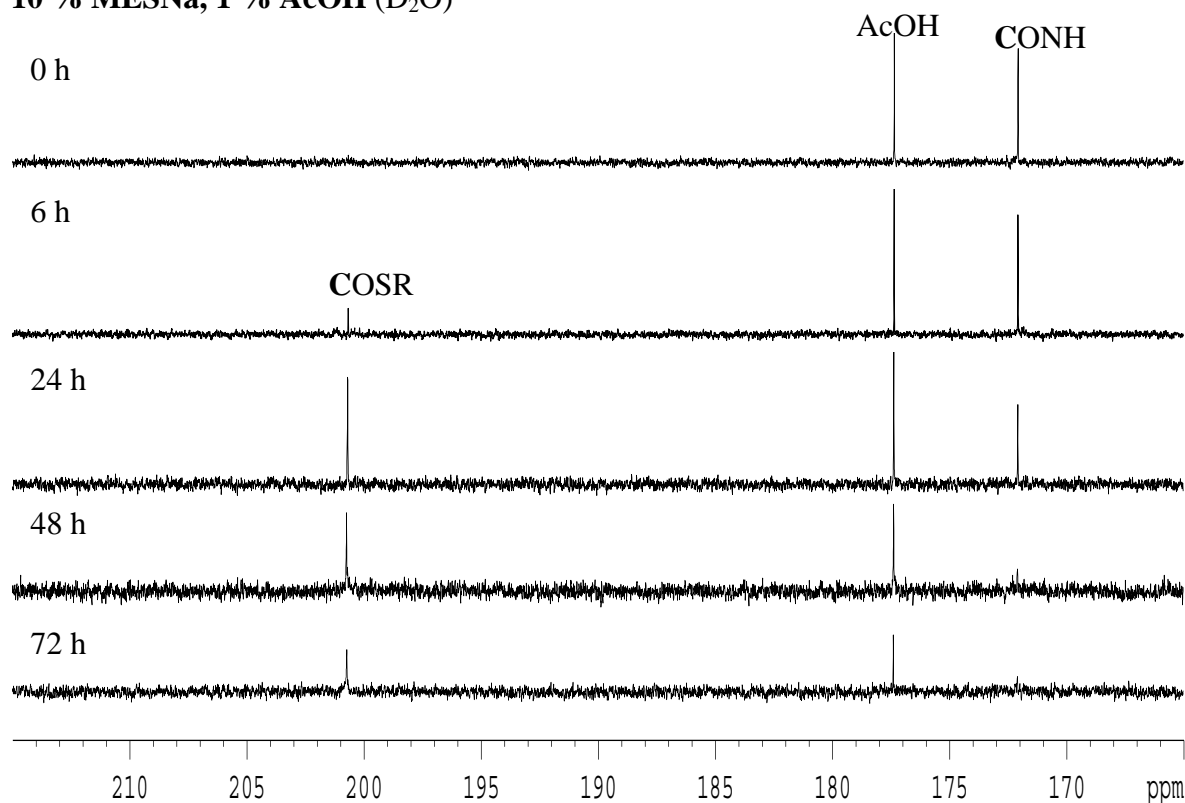
10 % MAA (D₂O)



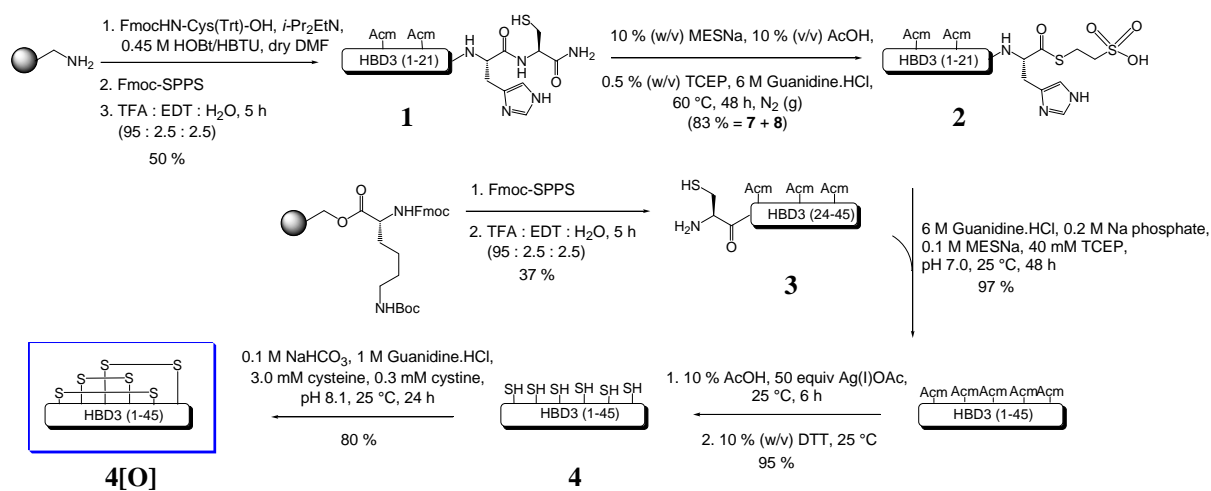
10 % MPA (D₂O)



10 % MESNa, 1 % AcOH (D₂O)

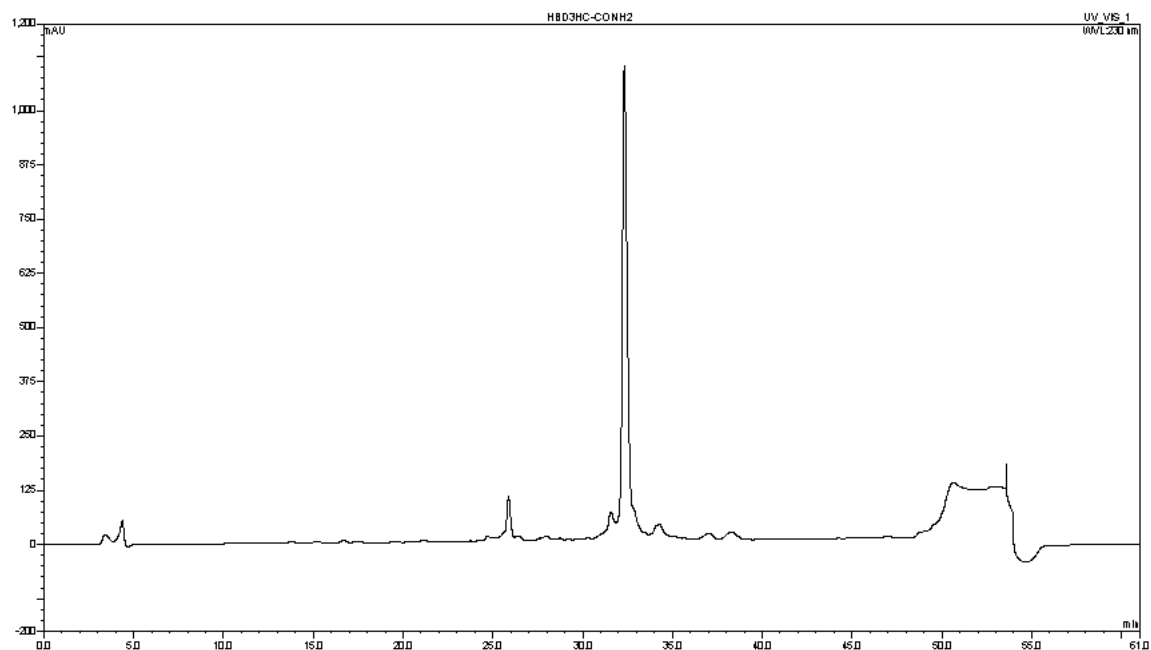


Biological application of novel peptide thioesterification method: synthesis of human β -defensin 3 (HBD3) with a Ser22His mutation

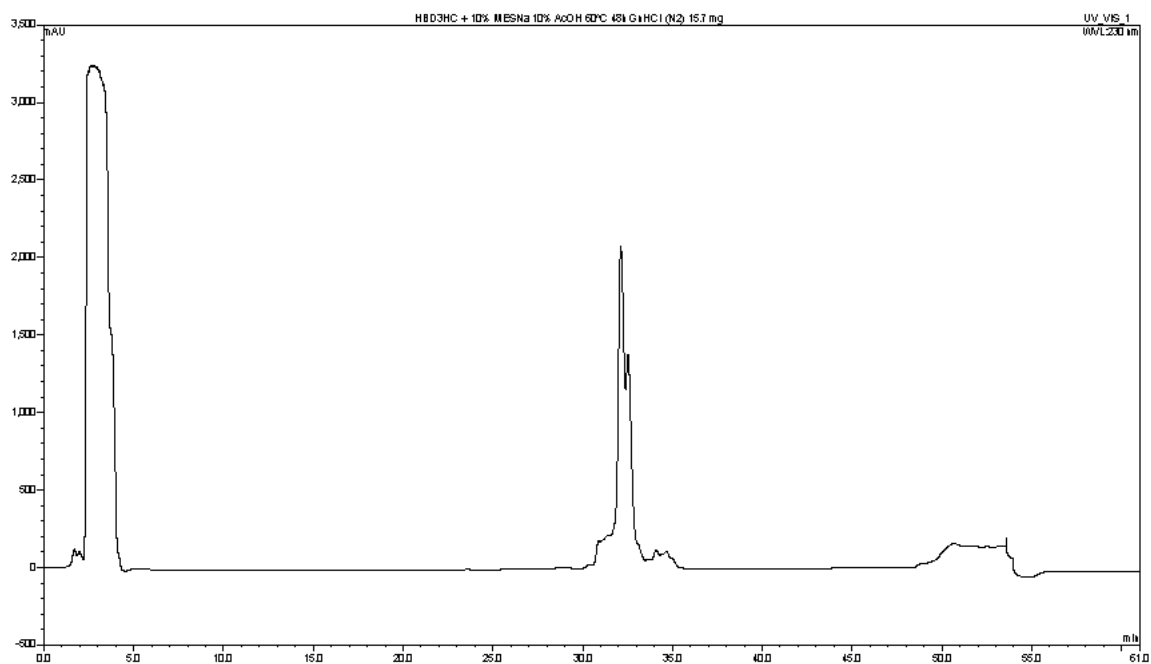


Scheme 2. Synthesis of full-length oxidised HBD3 13

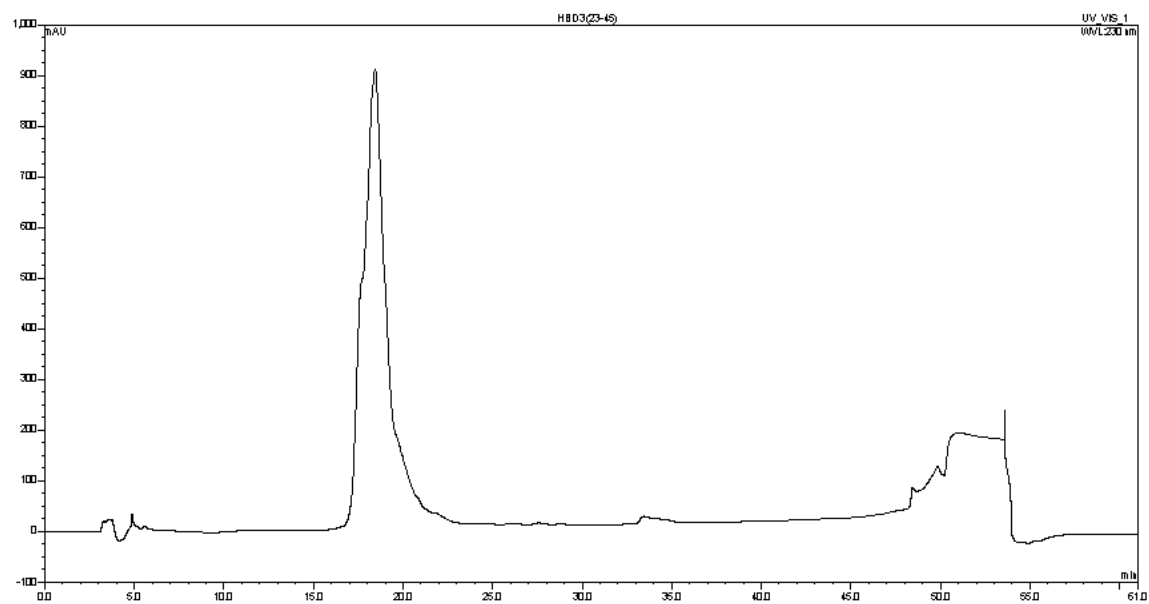
HPLC analysis of key transformations in the synthesis of folded HBD3 using the optimised peptide thioesterification procedure, with a linear gradient of 5-50 % MeCN over 45 min in 0.1 % aqueous TFA at a flow rate of 4.0 mLmin⁻¹, absorption detected at 230 nm.



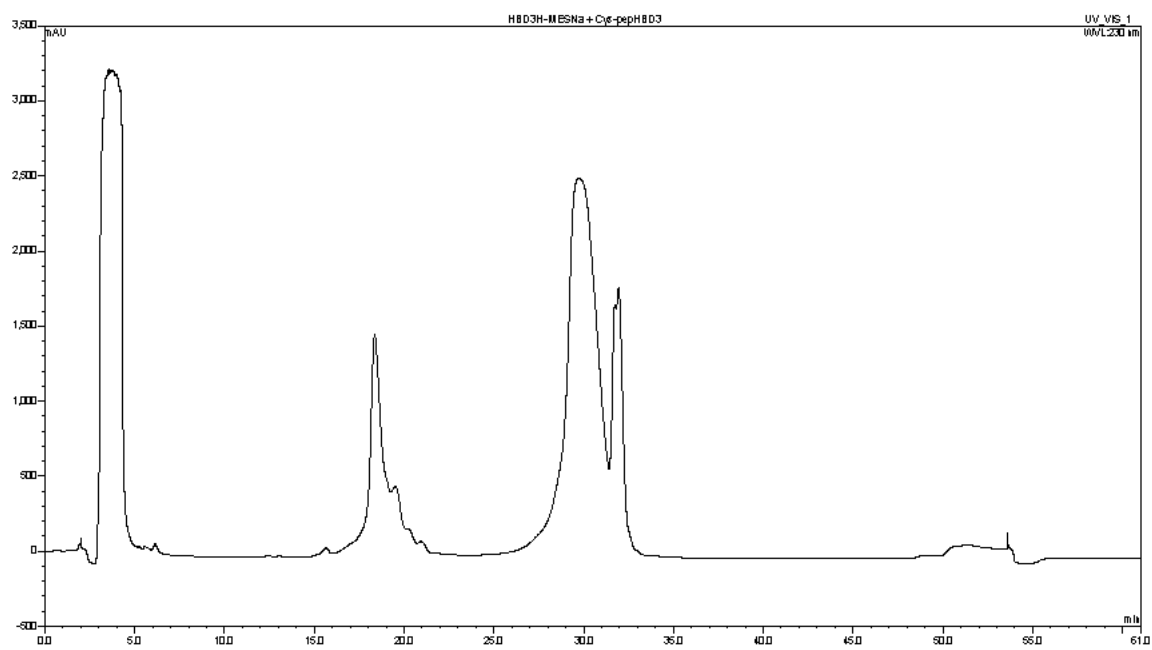
Starting material (1): HBD3(1-21)HC-NH₂: 32.3 min.



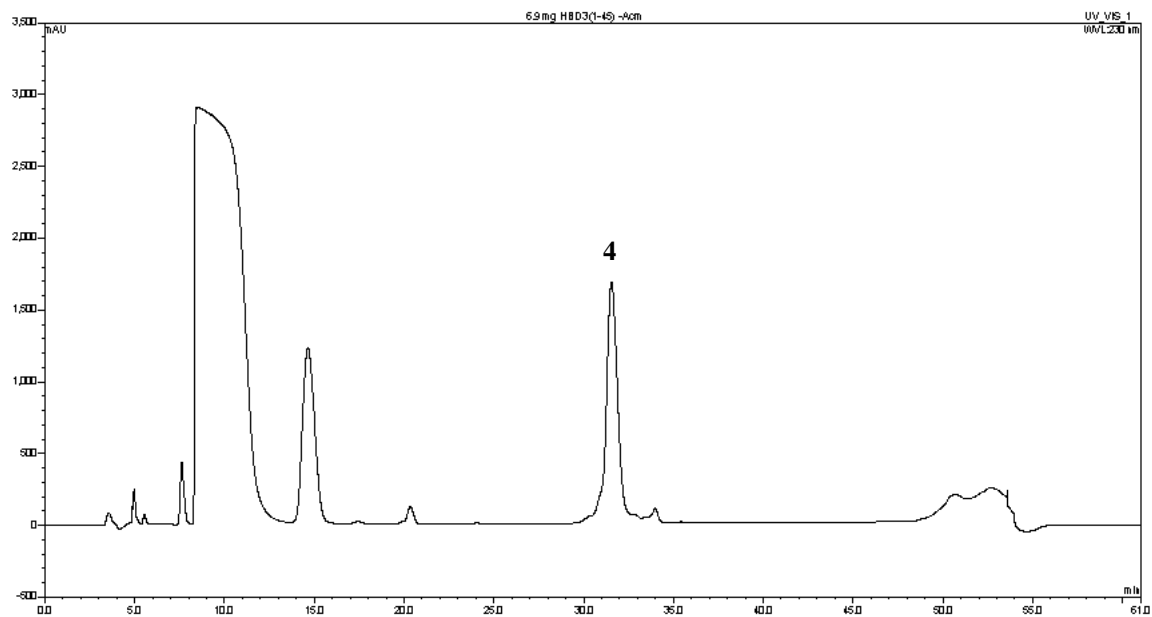
Peptide thioester: co-eluted HBD3(1-21)HC-NH₂ (1) + HBD3(1-21)H-SCH₂CH₂SO₃H (2):
32.1 and 32.5 min.



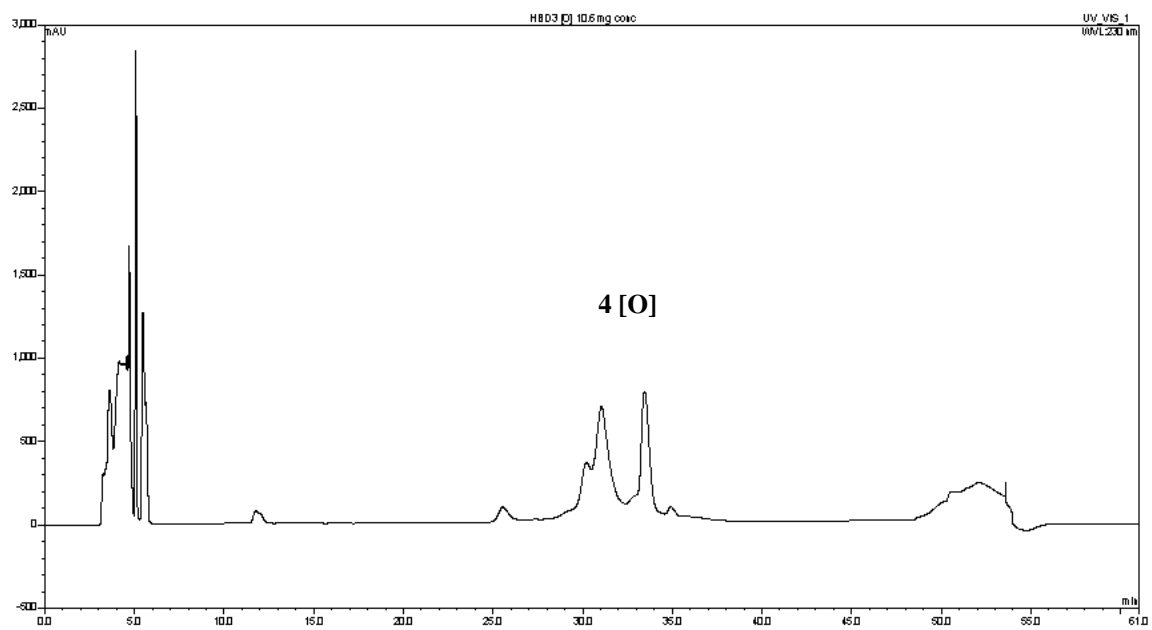
Cysteiny peptide: HBD3(23-45) **3**: 18.4 min.



Native chemical ligation: Cys-peptide: 18.4 min, HBD3(1-45) + Ac_m₅: 29.7 min, unreacted SM: 32.0 min.



Deprotected full-length HBD3(1-45) – Ac_m₅: 31.6 min.



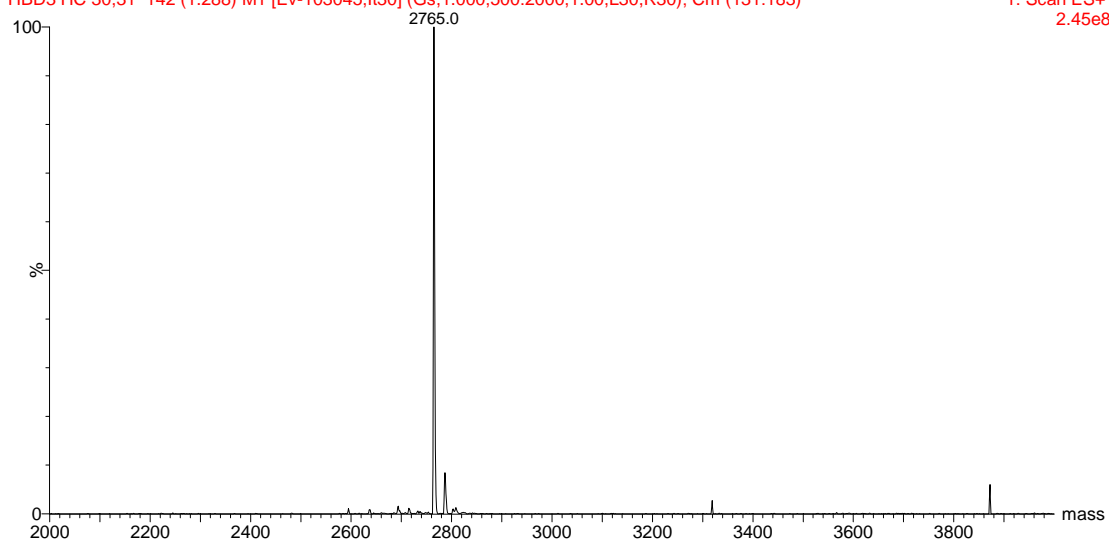
Oxidised full-length HBD3(1-45): 30.2, 31.1, and 33.5 min.

Mass spectra of key transformation in the synthesis of HBD3(1-45).

HBD3(1-21)HC-NH₂ (1)

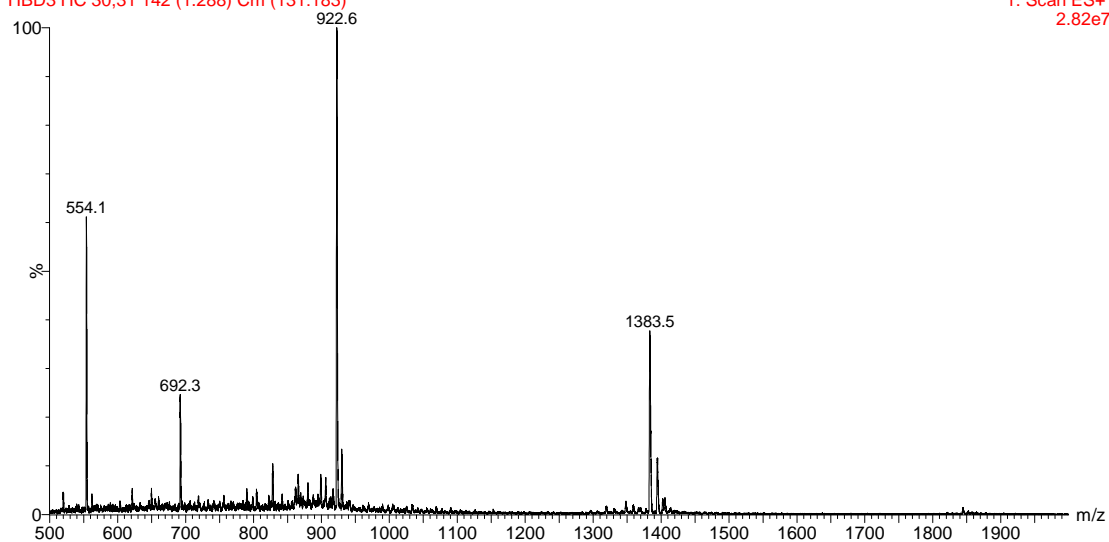
HBD3 HC 30,31 142 (1.288) M1 [Ev-103045,It30] (Gs,1,000,500:2000,1.00,L30,R30); Cm (131:183)

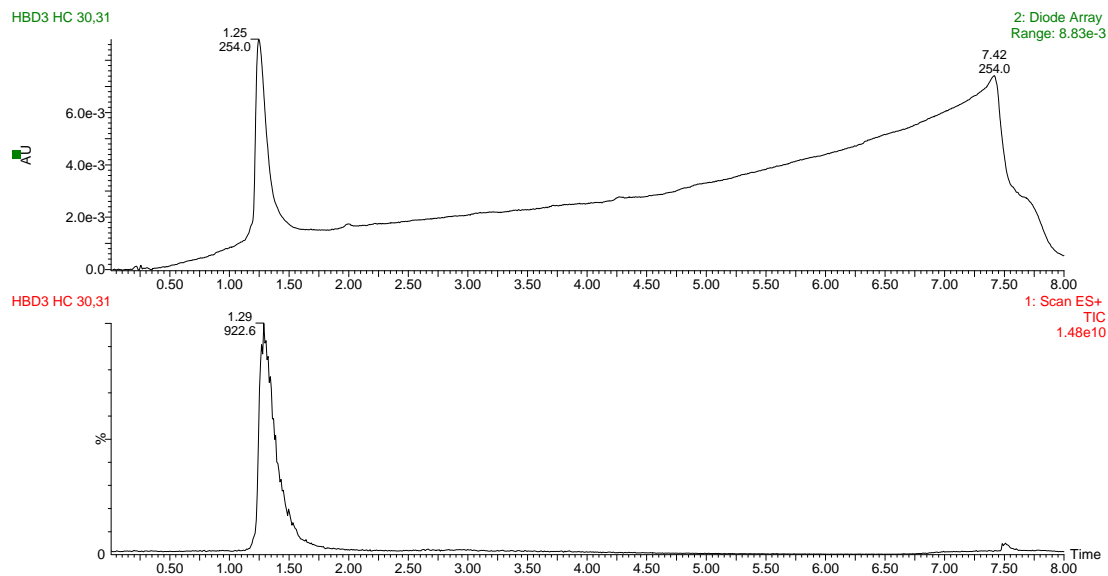
1: Scan ES+
2.45e8



HBD3 HC 30,31 142 (1.288) Cm (131:183)

1: Scan ES+
2.82e7

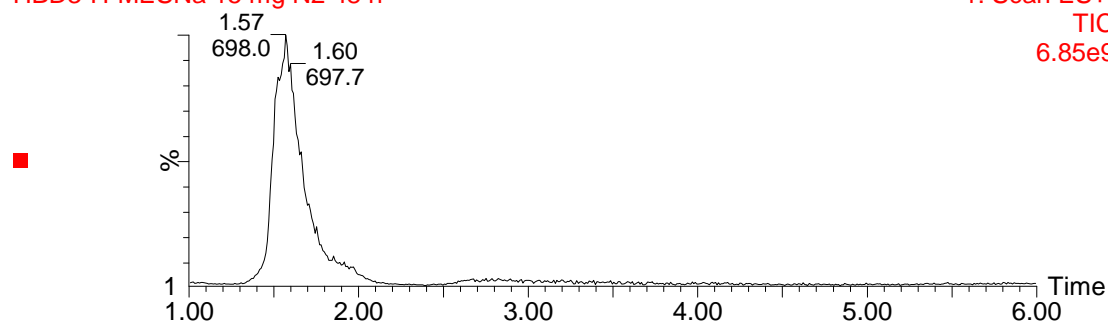




HBD3(1-21)HC-NH₂ + HBD3(1-21)H-SCH₂CH₂SO₃H (1 + 2)

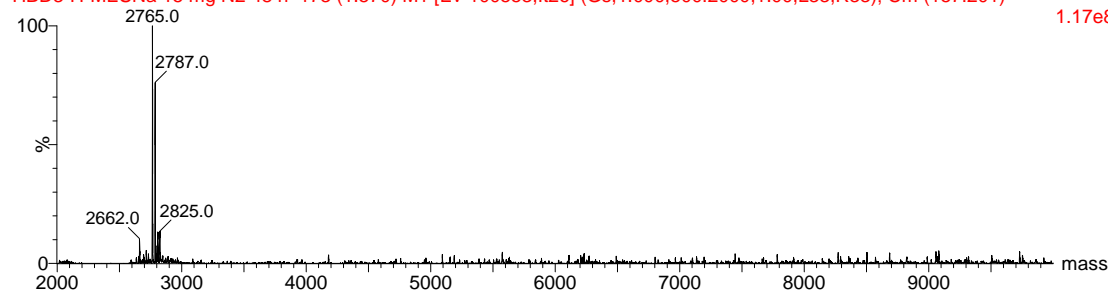
HBD3-H-MESNa 15 mg N2 48 h

1: Scan ES+
TIC
6.85e9



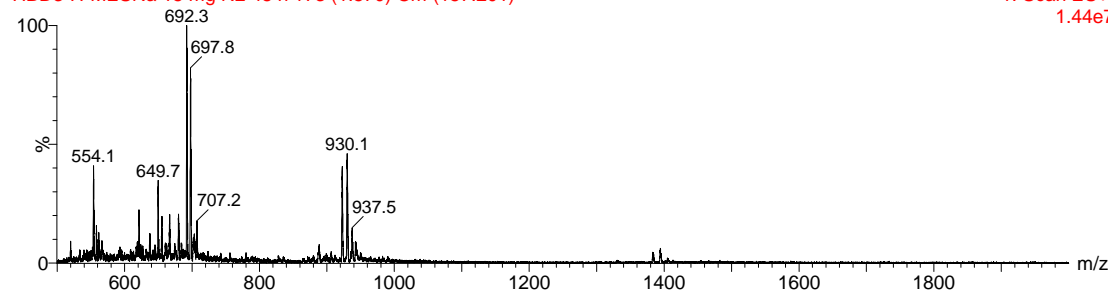
HBD3-H-MESNa 15 mg N2 48 h 173 (1.570) M1 [Ev-100553,It26] (Gs,1,000,500:2000,1,00,L33,R33); Cm (157:201)

1.17e8

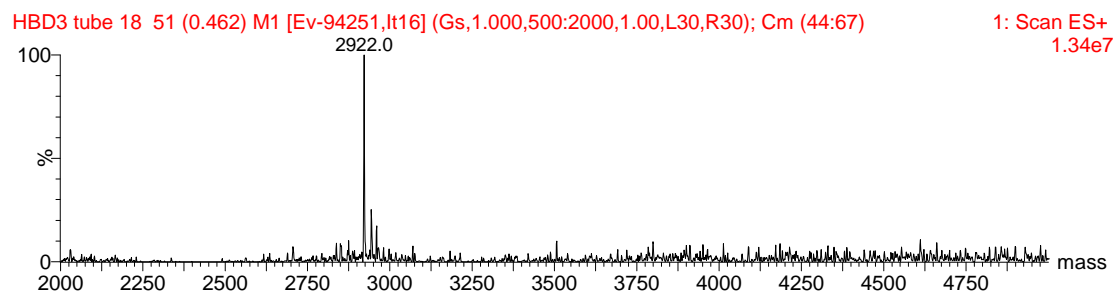
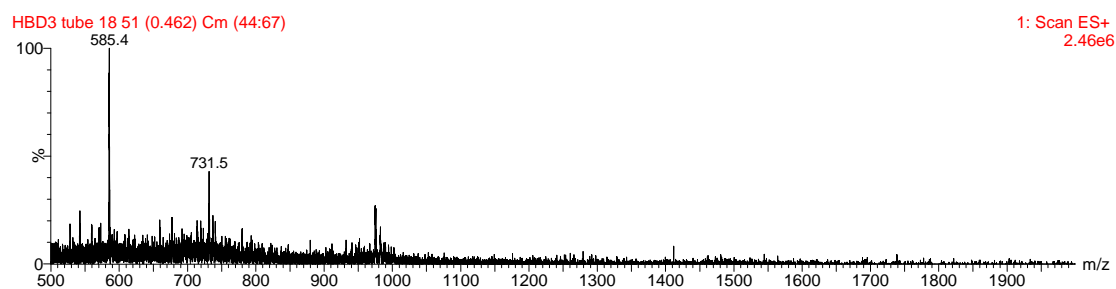
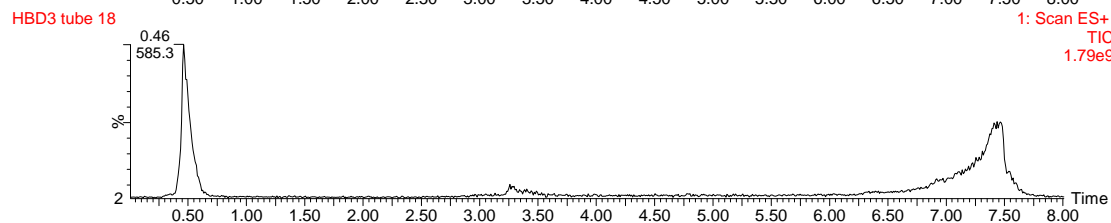
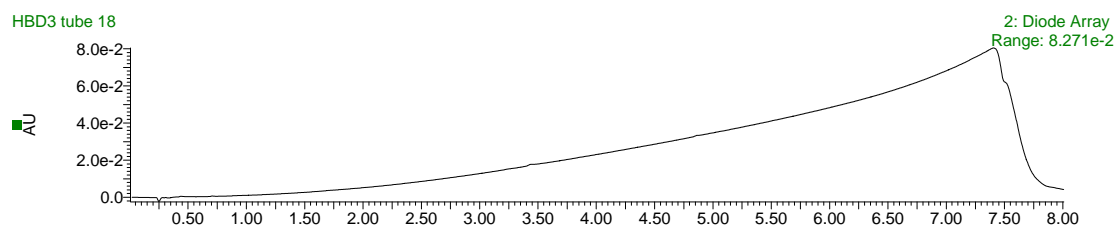


HBD3-H-MESNa 15 mg N2 48 h 173 (1.570) Cm (157:201)

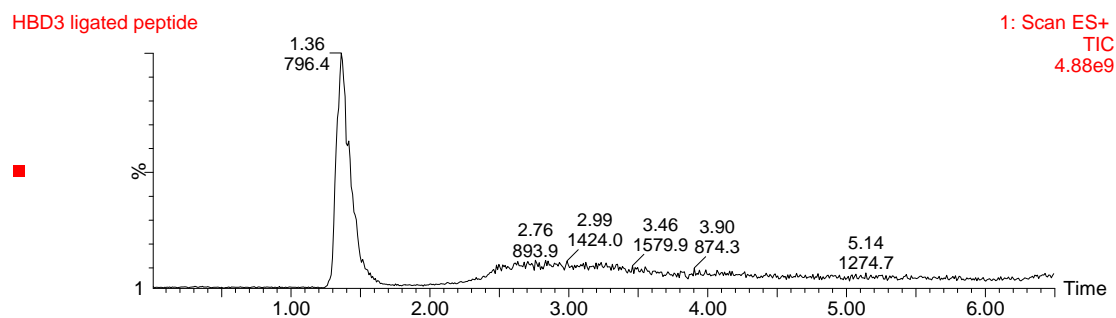
1: Scan ES+
1.44e7

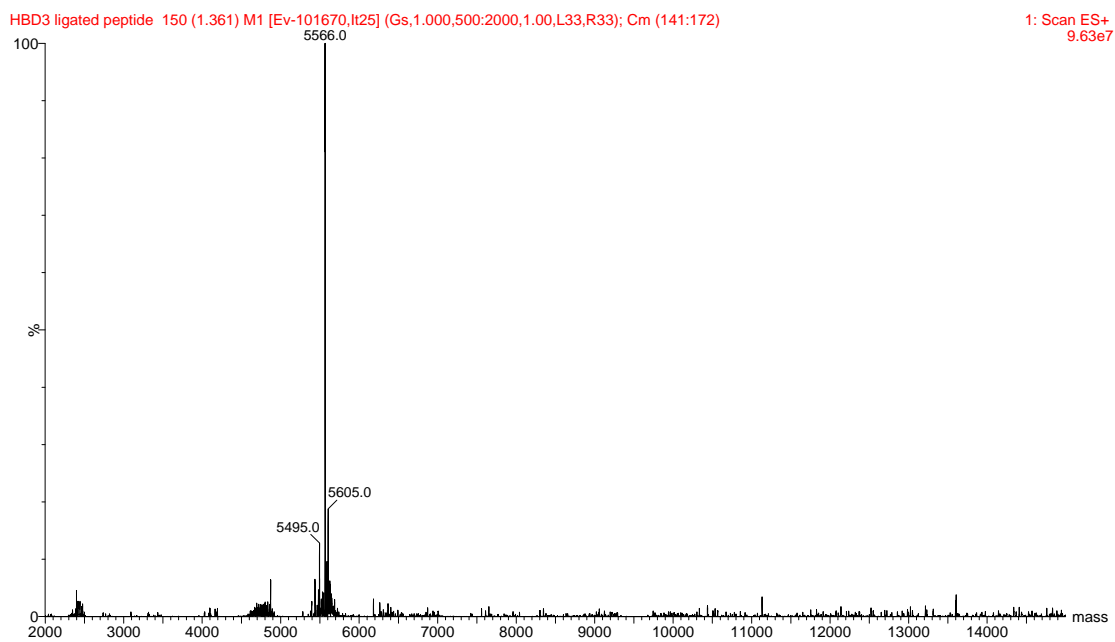
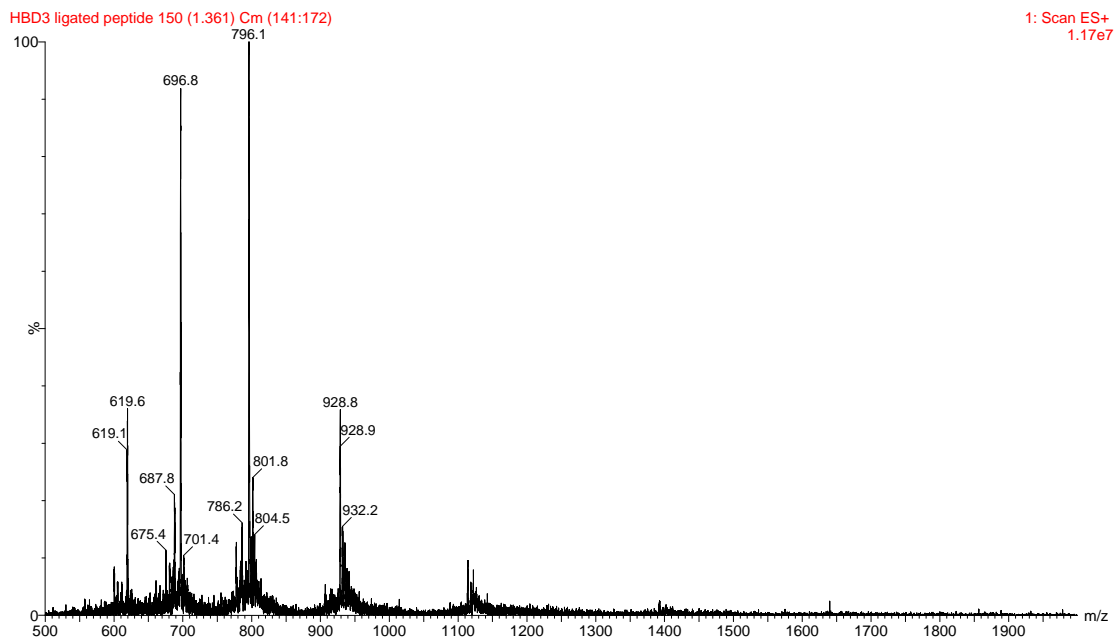


HBD3(23-45): Cysteinyl peptide (3)

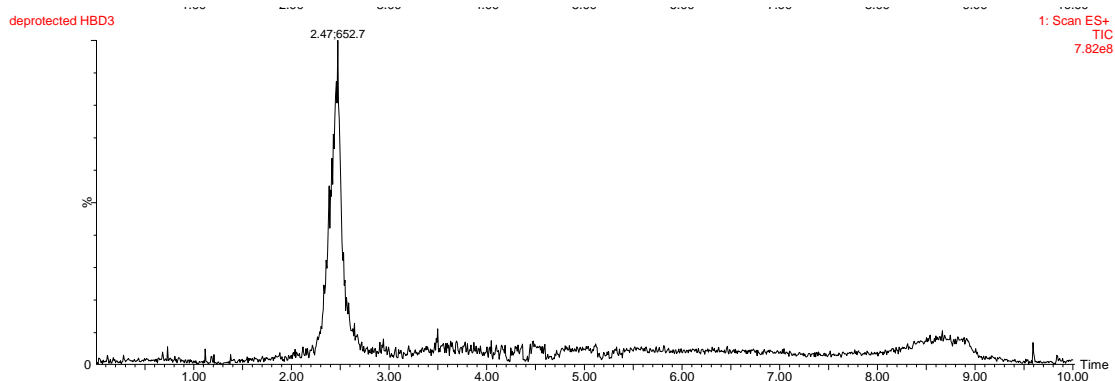


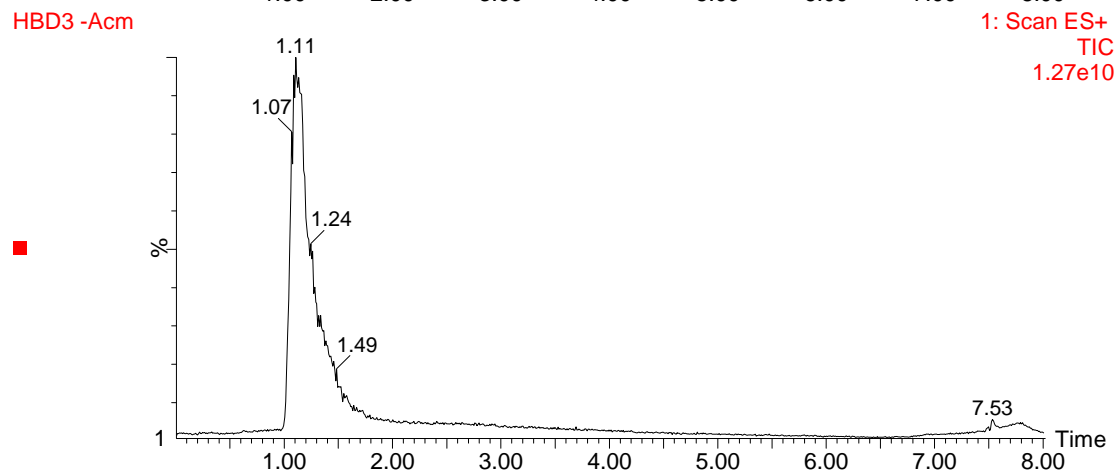
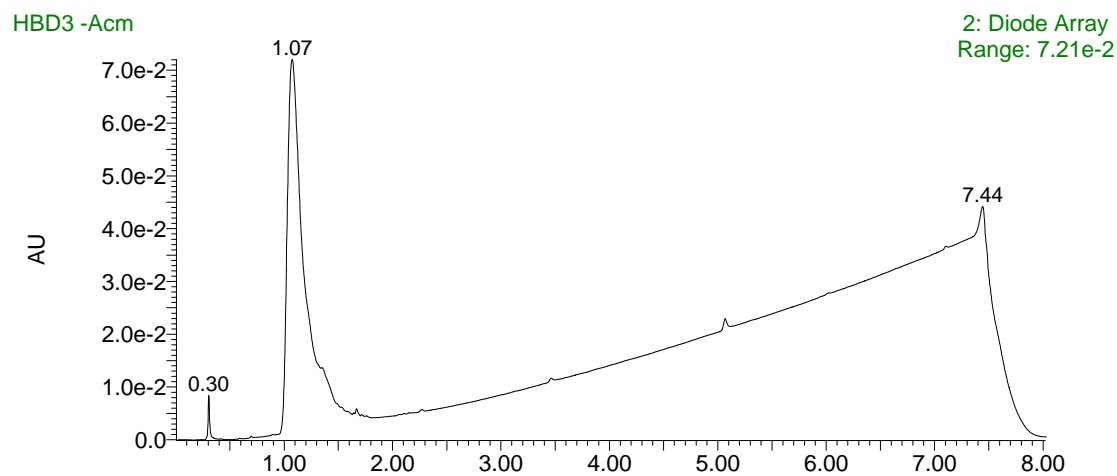
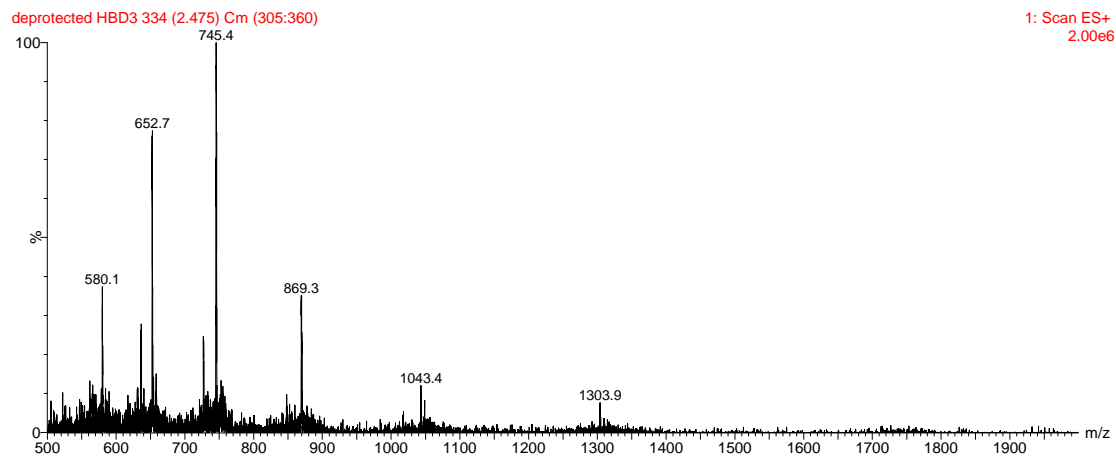
HBD3(1-45) + Acm₅ ligated product

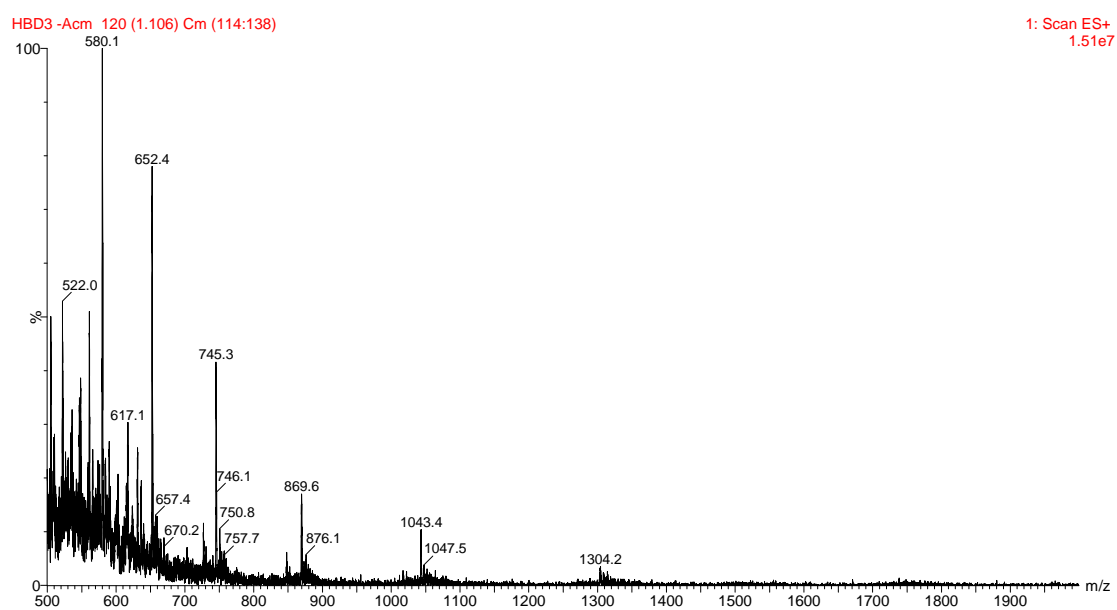
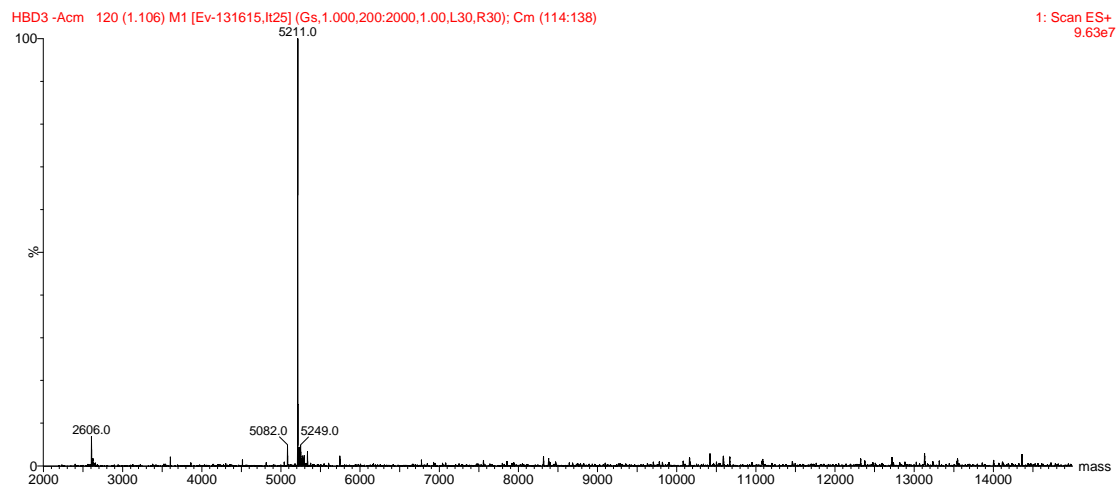




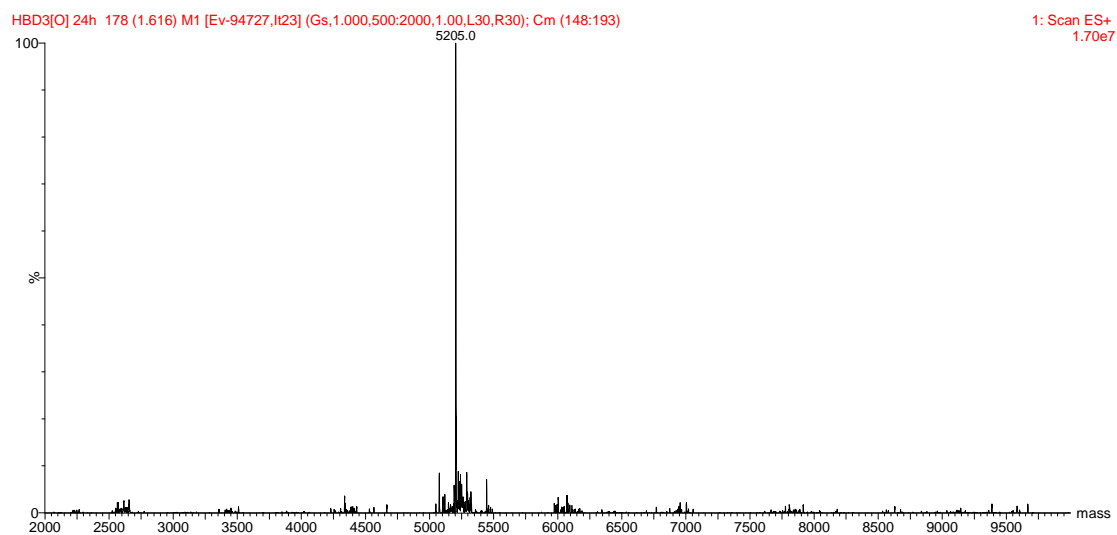
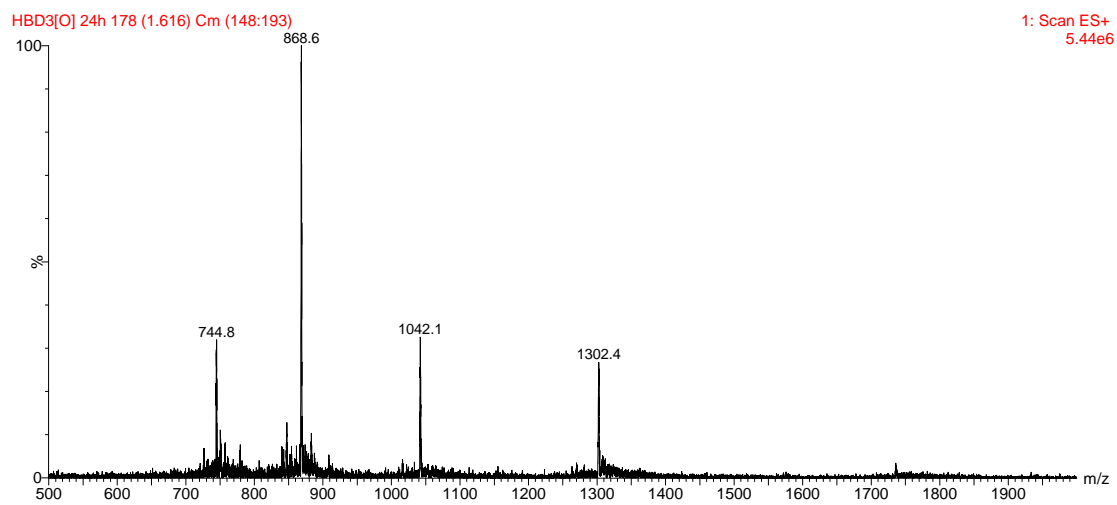
Deprotected HBD3(1-45) – Acm₅ (4)



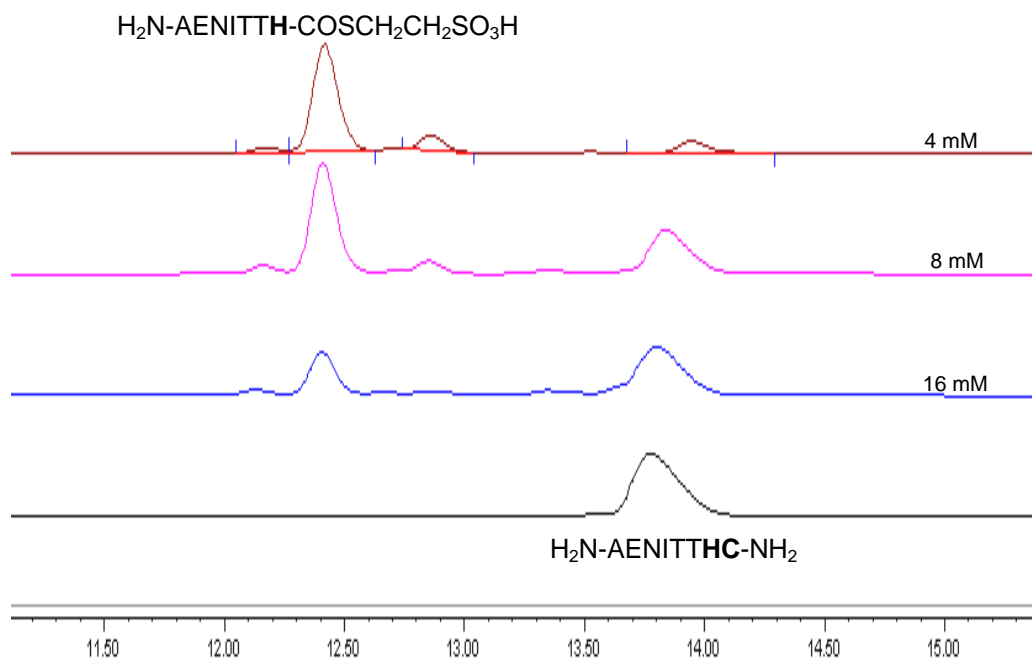




Folded HBD3(1-45)



Peptide concentration dependence in H-AENITTGHC-NH₂ thioesterification



Reactions all performed at 60 °C in 10 % w/v MESNa containing 1% v/v AcOH for 24 h.