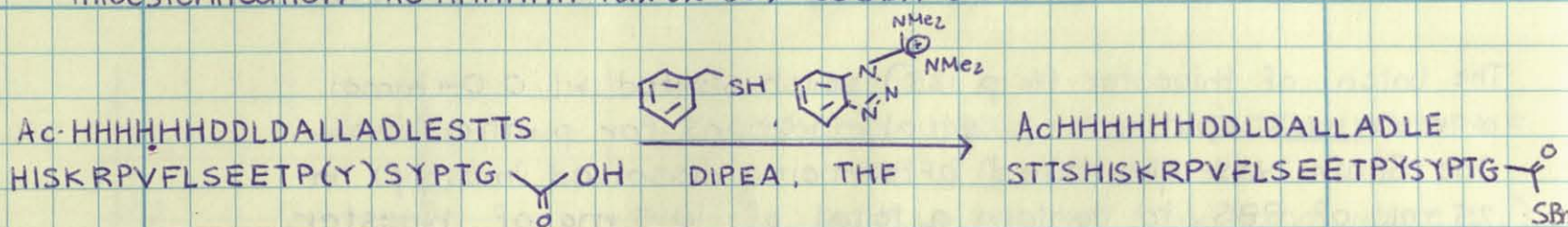
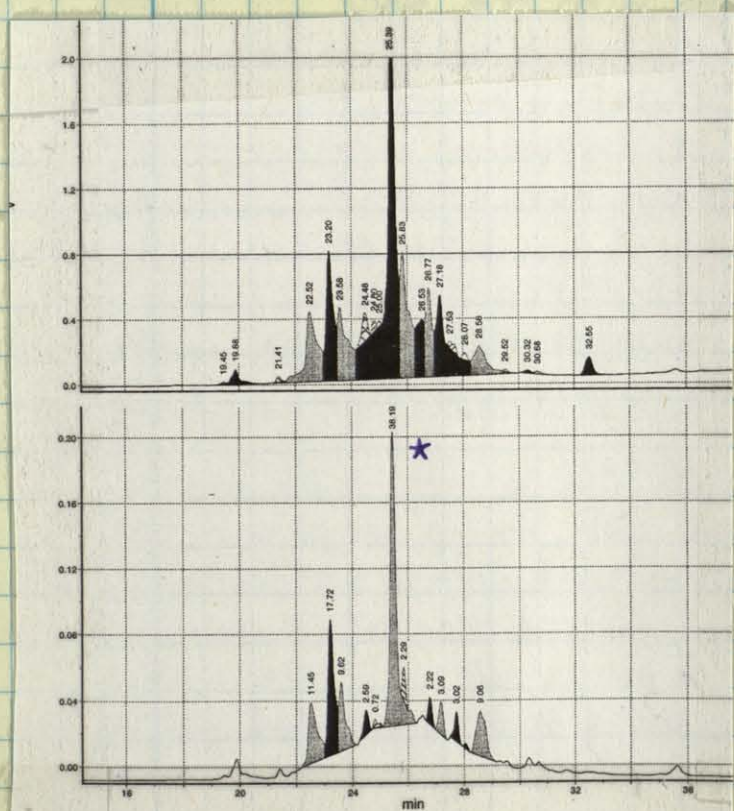


Thioesterification: Ac-HHHHHH-Pax(2-36)-COSBn (Y31)



The thioesterification was carried out on 0.04 mmol resin as described on p 4-119. The last 5 residues of the hexahistidine tag were coupled using 4 equiv. of Fmoc-His(Trt)-OH and PyBOP.



2/27/04 Ach6-Pax(Y31) HisTrt

Page

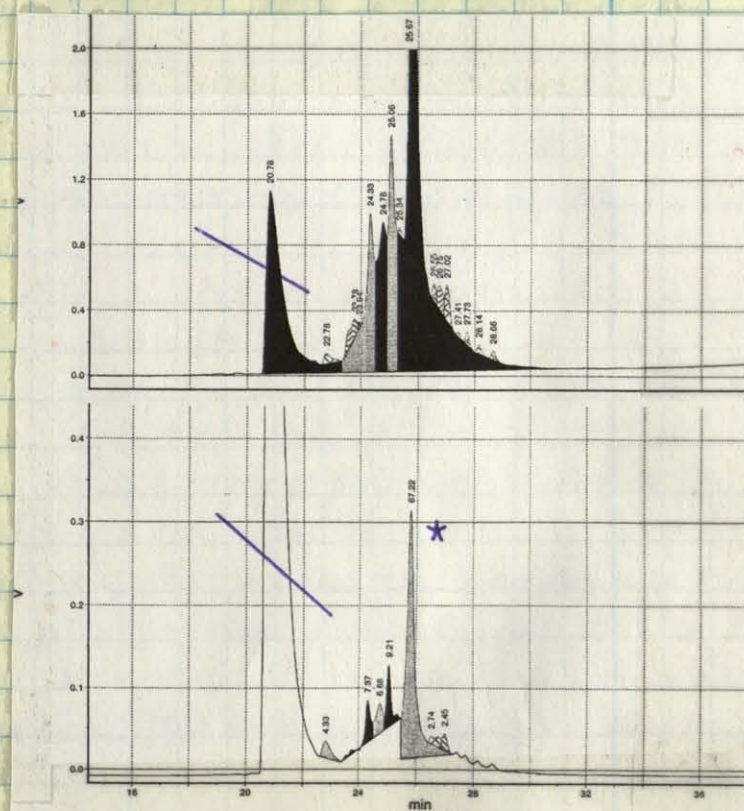
Ac-His6Pax(2-36)(Y31)-COOH
 calc. mass: C₂₀₉H₃₀₇O₆₈N₅₉
 mw: 4734.11 (M+5: 947.8, M+6: 790.01)

ESI/MS peak at:

22.7 min: 952.7 M+2

23.2 min: 1107.4 + Ac-ISKRPV...G-COOH

* 25.4 min: 947.5, 789.8 **



3/01/04 Ach6D...P(Y)SYPTG-SBn

Page

Ac-His6Pax(2-36)(Y31)-COSBn
 calculated mass: C₂₁₆H₃₁₃O₆₇N₅₉S
 4839.22 (M+5: 968.9)

ESI/MS peak at:

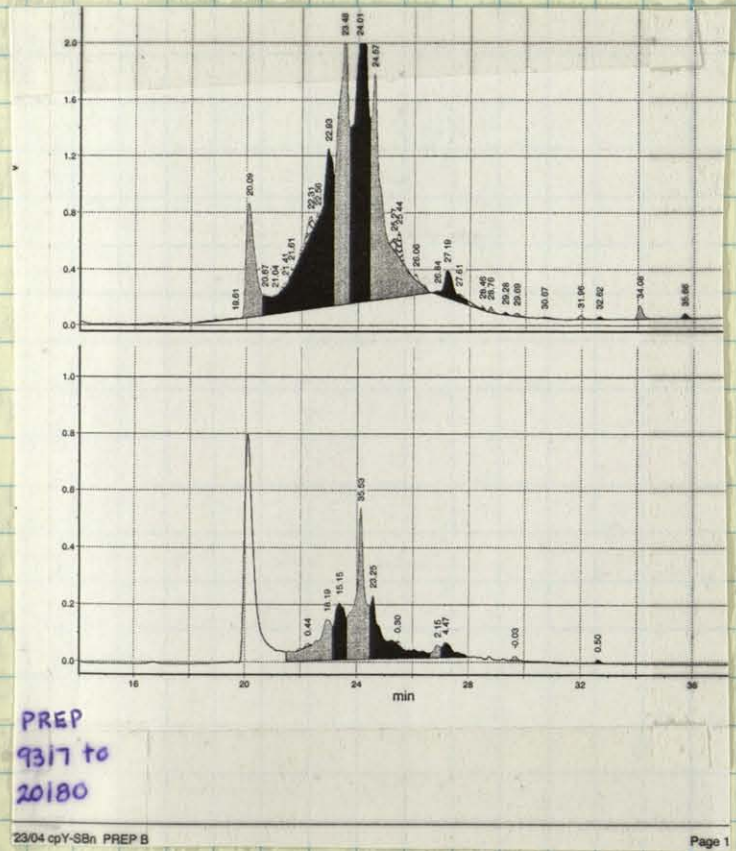
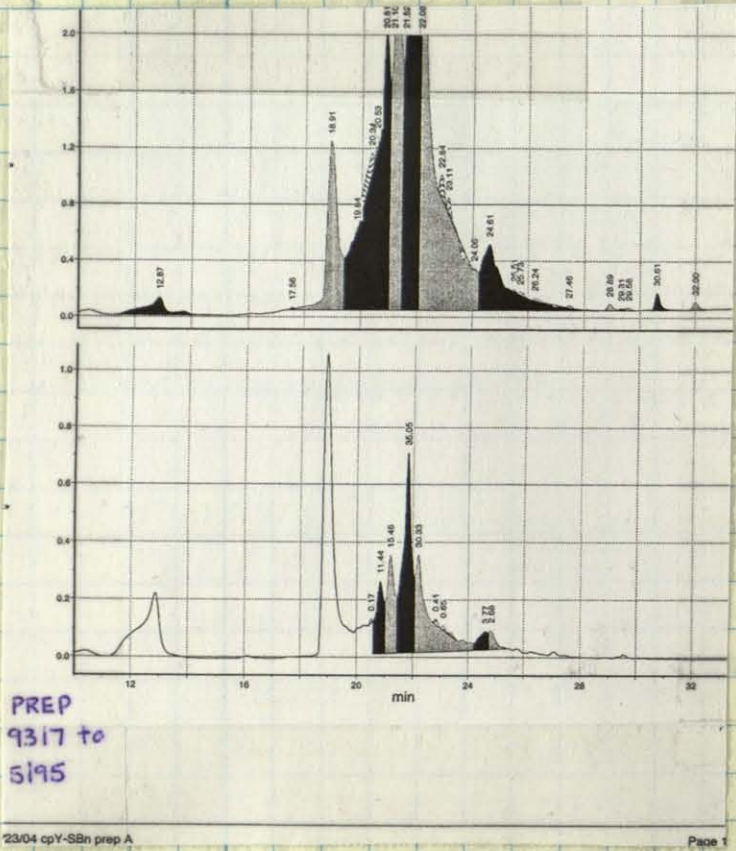
25.1 min: 1160.4

25.7 min: 968.7 ***

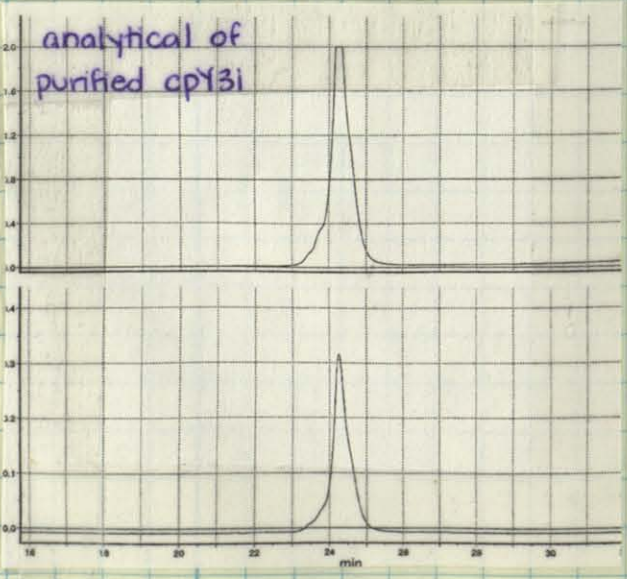
After cleavage for 2 h w/ 95% TFA, 2.5% TIS and 2.5% water, the 20 mL TFA solution was split into 2 batches to blow off the solvent and twice triturate w/ diethyl ether. One batch was to be purified by prep HPLC and the other by a Ni-NTA agarose hexahistidine affinity column.

Thioesterification / Purification of AcHis6Pax(2-36,cpY)SBn (cpY31)

The remaining 0.04 mmol of Ac-His6Pax(2-36,cpY)-COOH was converted to the thioester (as on p.4-119) and purified by reverse phase HPLC.

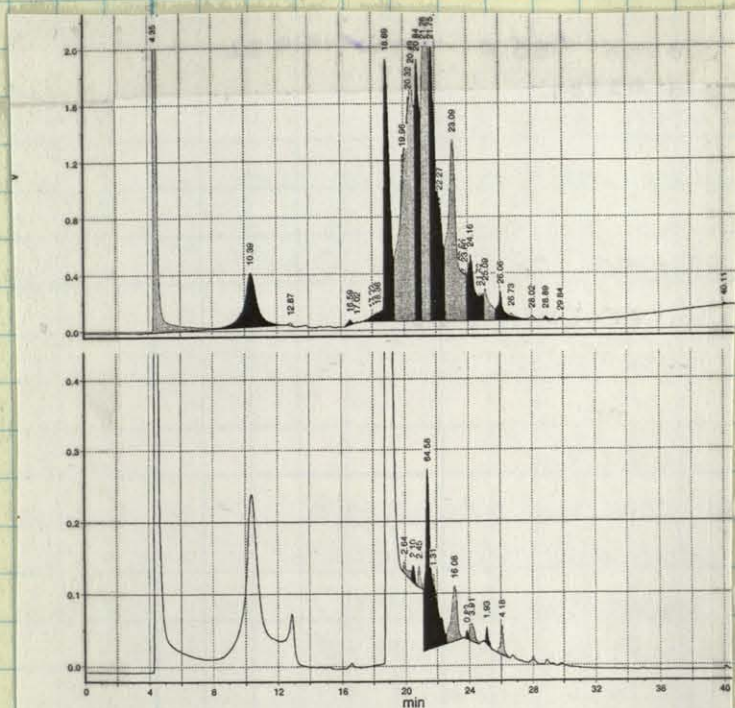


The purified peptide was lyophilized and then dissolved in 2 mL of water for quantification w/ a BSA assay. A 1/1000 dilution determined the concentration to be 3.4 mg/mL. 1 mL (est. at 3.4 mg) was lyophilized to remove the water, then redissolved



Thioesterification / Purification of ACH6Pax (2-36, pY31)-SBn (pY31)

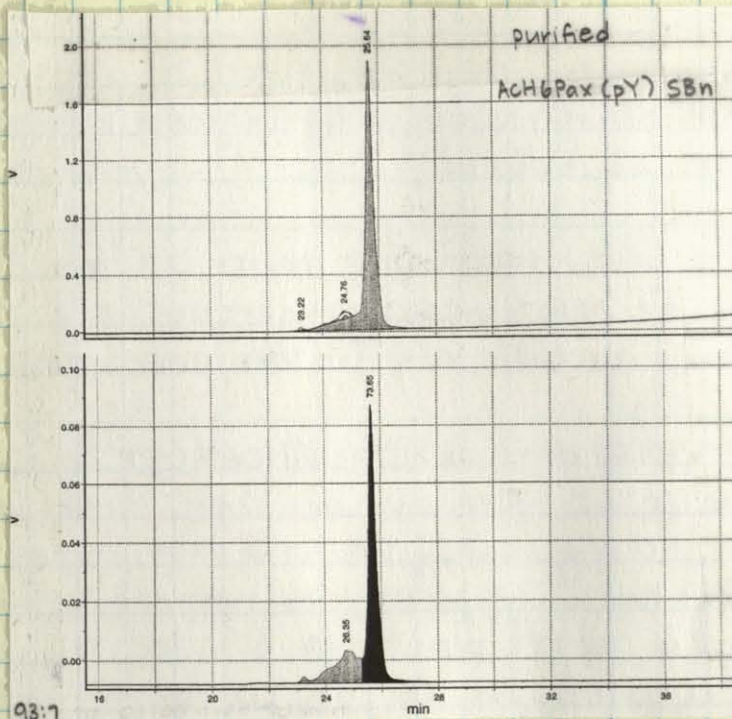
0.08 mmol of the free acid was derivatized to the thioester and purified by HPLC.



93:7 to 5:95 over 30 min

v22/04 (pY31)SBn prep A

Page



← m/z for ESIMS
peak at 21.3 (and purified peptide)
found*