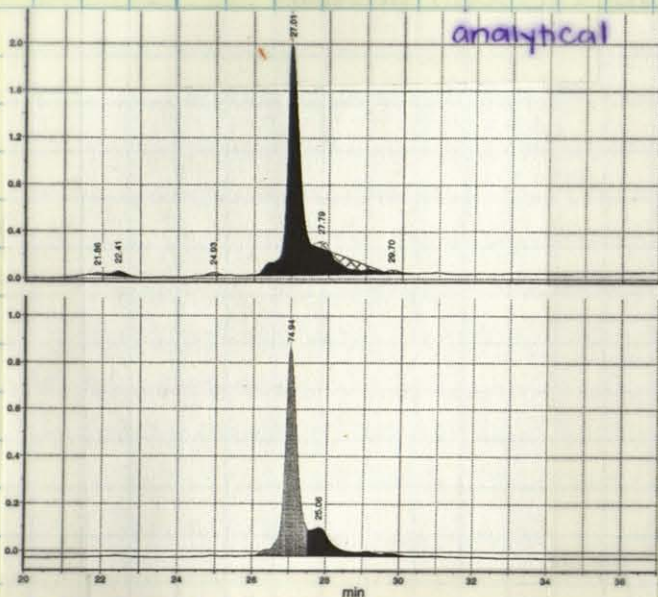


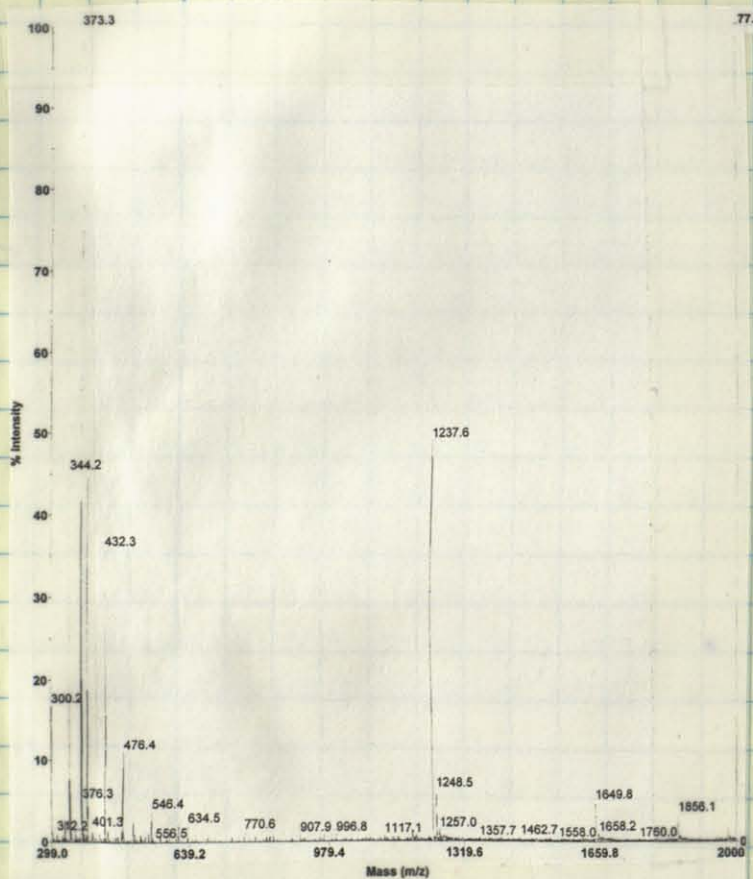
ACT run (to precursors to pax(1-36) peptide)

well 1 Fmoc-ISKRPVFLSEETP(pY)SYPT [G-TGT resin]
 and well 2
 well 13 Fmoc-Fmoc-SYPT (Gly-loaded TGT resin)



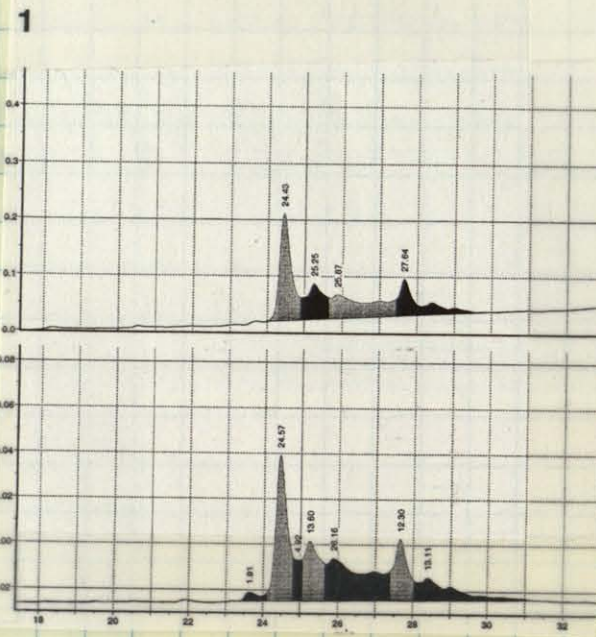
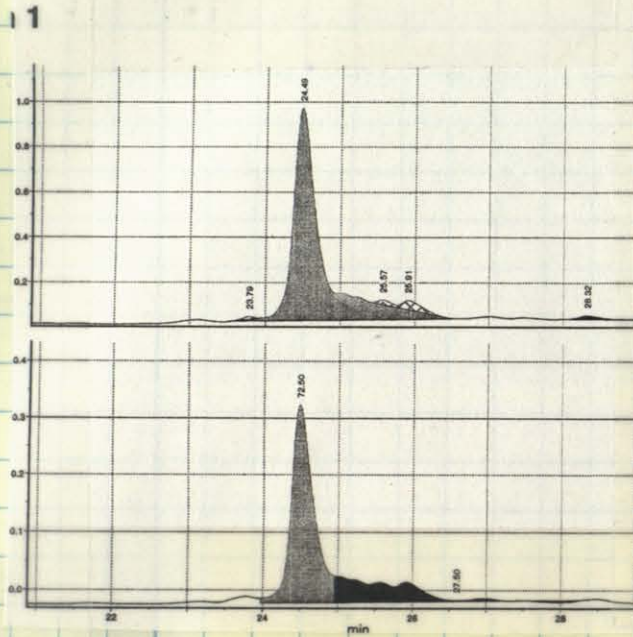
pepcalc. quant. for:
 Fmoc-ISK... PTG-COOH
 $C_{115}H_{161}O_{33}N_{23}$ 2393.69
 - 1 (H)
 + 80 $(PO_3)^{2-}$
 2472.69
 $(M+2H)^{2+}$: 1237.35
 found: 1237.6

93/7 water/acetonitrile to
 0/100% " over 35 minutes



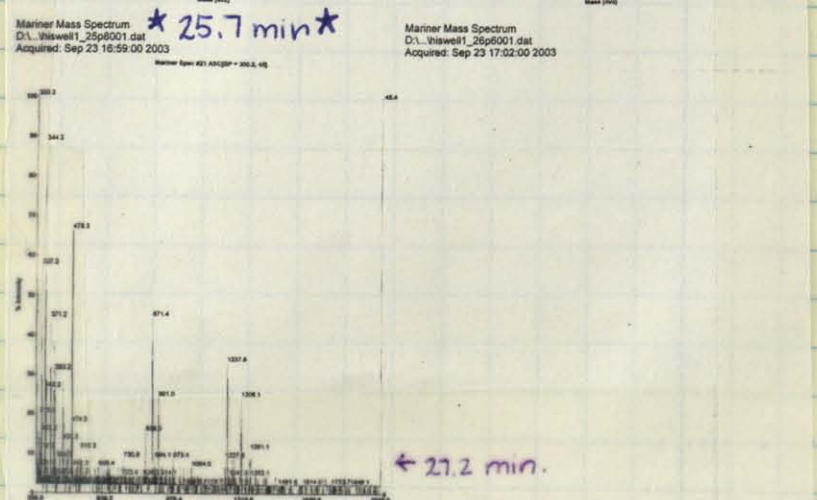
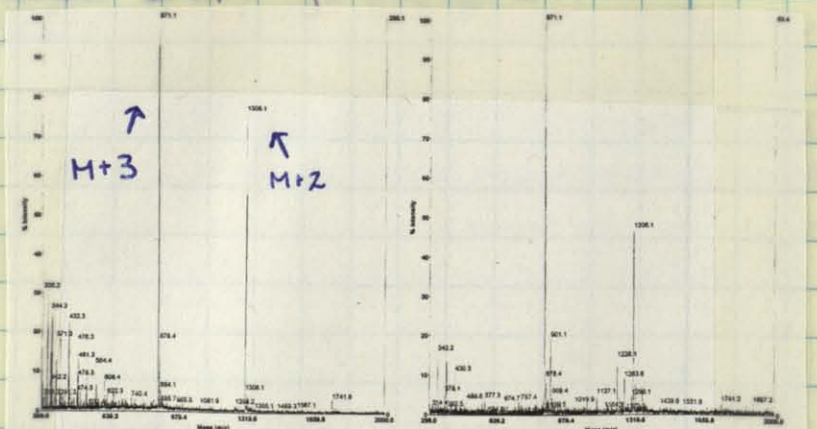
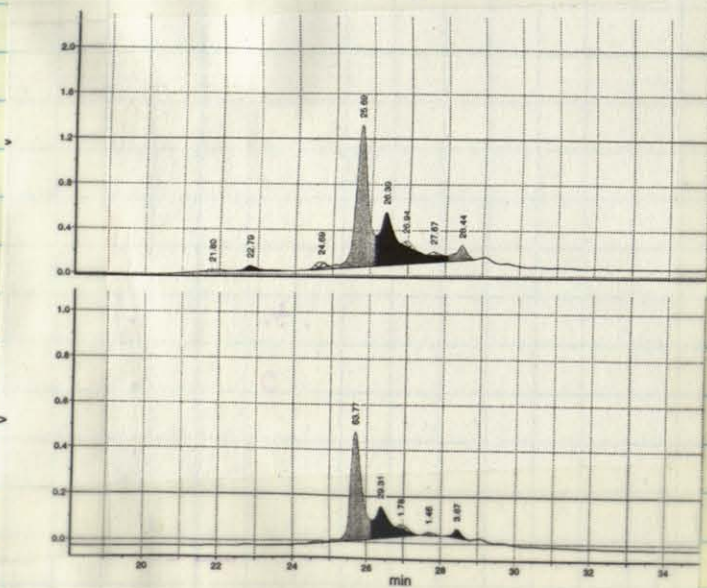
HIS addition to Fmoc-ISKRPVFLSEETP(pY)SYPT(G-TGT)

ACT well 2: Fmoc-His(Trt)-OH added with ² eq. of HATU and 2 eq. of DIPEA by hand. TNBS test positive before coupling and negative afterwards.



ACT Well 1: Fmoc-His(Trt)-OH coupled w/ PyBOP/DIPEA by hand. TNBS test was negative after coupling. More His was added (for a double coupling) w/ HATU/DIEA.

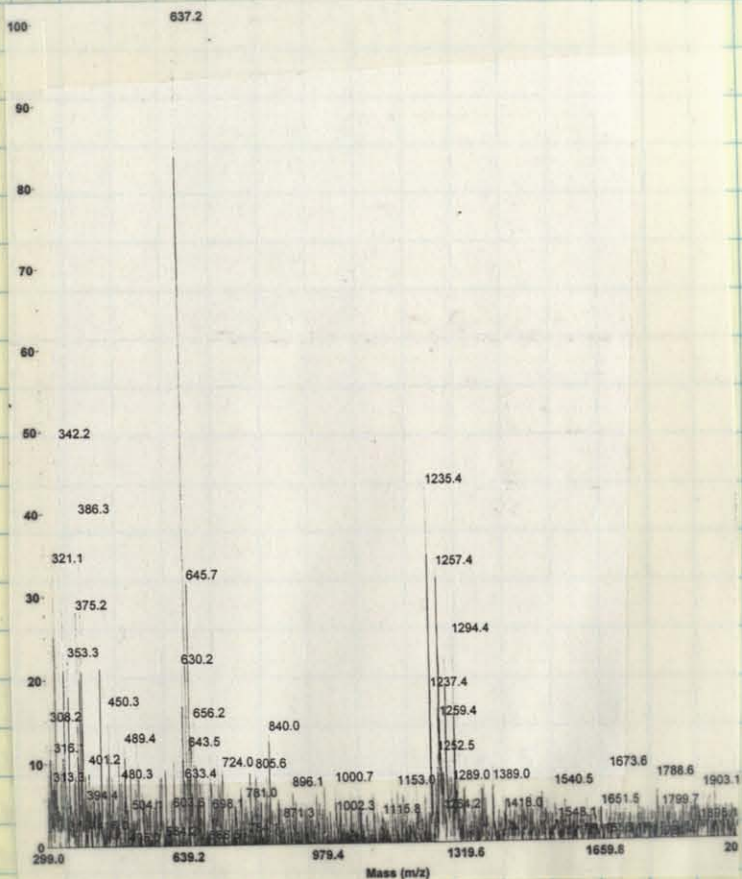
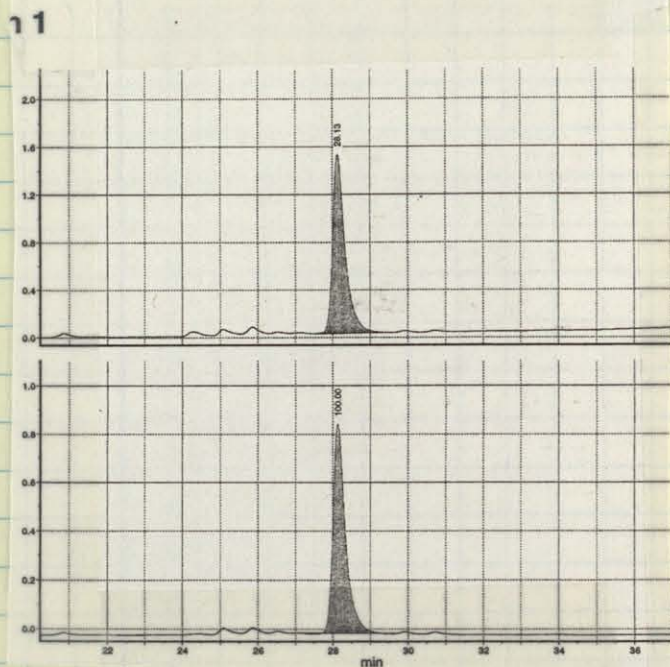
Run 1 After PyBOP coupling only



pepcalc (w/out phospho)
2530.83
+phospho - H :

Fmoc-P (caged phospho Y) SYPT-G (TGT resin)

- cpTyr-OH and Fmoc-Pro-OH were added by hand with the couplings checked after deprotection with a positive TNBS test and after coupling with a negative TNBS test.



pepcalc for Fmoc-PYSYPTG-OH:
 1006.08
 caged phospho: 229.
 1235.08

ACT run:

well 1

Fmoc-ISKPPVFLSEET-
 (to PopYSYPTG[TGT])

well 2

Fmoc-DDLDALLADLESTTS-
 (to HISK...G[TGT])

w/ pTYR31

Rack name	Ves	Reagent	MolWt	Dens	Molar	Vol(mi)	Grams
none	---	1-DMF	---	---	---	1713.37	---
none	---	4-DMF	---	---	---	469.77	---
AA32	16	Ser	383.4	0.00	0.50	4.60	0.88
AA32	17	Thr	397.5	0.00	0.50	3.45	0.69
AA32	6	Glu	425.5	0.00	0.50	3.45	0.73
AA32	11	Leu	353.4	0.00	0.50	5.75	1.02
AA32	14	Phe	387.4	0.00	0.50	2.34	0.44
AA32	4	Asp	411.5	0.00	0.50	4.60	0.95
AA32	20	Val	339.4	0.00	0.50	2.30	0.40
AA32	1	Ala	311.3	0.00	0.50	2.30	0.36
AA32	15	Pro	337.4	0.00	0.50	2.30	0.38
AA32	2	Arg	648.8	0.00	0.50	2.30	0.74
AA32	12	Lys	468.6	0.00	0.50	2.30	0.54
AA32	10	Ile	353.4	0.00	0.50	2.30	0.40
RG4	1	PIP	85.2	1.45	0.50	37.20	1.58
RG5	1	DIP	129.3	0.74	0.50	31.05	2.01
RG2	1	HBT	379.3	0.00	0.50	31.05	5.89
RG3	1	Cap	0.0	0.00	0.50	62.10	0.00

NOTE: Volume and Grams have been increased by 15% to support normal losses. System fluids are exempt from this increase.