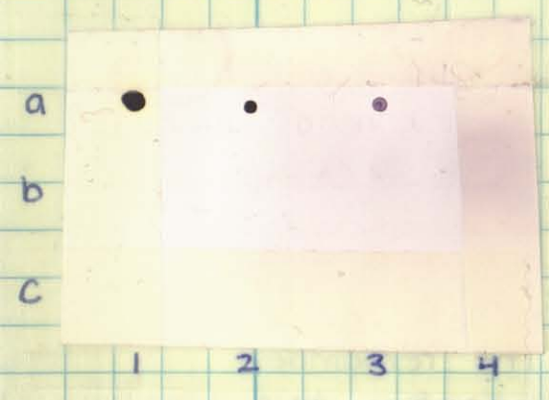
Dot Blots / Attempted visualization with anti-His antibodies

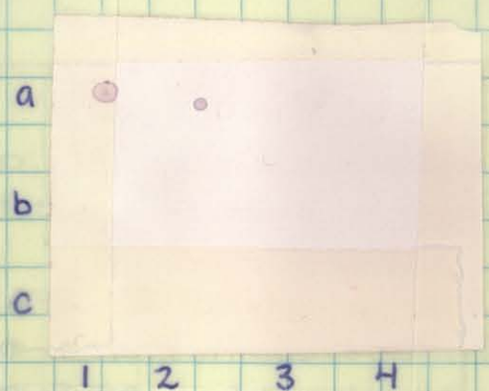
Ac-HHHHGKW-NH_2 (979.07 g/mol)
0.005 g in 200 µL of water [on balance 0.0000]
(25 µg/µL or 25.5 nmol/µL)

His6-ENLYFQC-Pax(38-557)-FLAG
concentration (biorad assay) 98 µg/mL or 1.3 nmol/mL
(further concentrated to 0.753 µg/µL or 0.01 nmol/µL)

anti-hexahistidine (mouse) 1°
(5 µL in 15 mL TBST)
goat anti-mouse alkaline phos. 2°
(10 µL in 10 mL TBST)



Quiagen anti-tetraHis (mouse) 1°
goat anti-mouse alk. phos 2° antibody
(10 µL in 10 mL TBST)

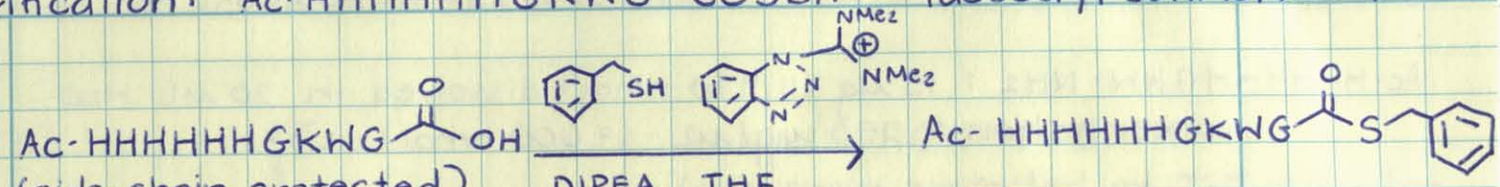


a1 3 µL of 0.01 nmol/µL His-Pax-FL (2.3 µg, 0.03 nmol)
a2 1 µL of 0.01 nmol/µL His-Pax-FL (0.75 µg) (0.01 nmol)
a3 1 µL of 0.001 nmol/µL His-Pax-FL (75 ng, 0.001 nmol)
a4 1 µL of 0.0001 nmol/µL His-Pax-FL (7.5 ng, 0.0001 nmol)

b1 3 µL AcHHHHHHGKW-NH_2
b2 1 µL AcHHHHHHGKW-NH_2
b3 1 µL AcHHHHHHGKW-NH_2
b4 1 µL AcHHHHHHGKW-NH_2
} estimated to be the same concentrations as for AcHHHHGKW-NH_2 below

c1 3 µL of 25 nmol/µL Ac-HHHHGKW-NH_2 (75 µg, 75 nmol)
c2 1 µL of 25 nmol/µL Ac-HHHHGKW-NH_2 (25 µg, 25 nmol)
c3 1 µL of 2.5 nmol/µL Ac-HHHHGKW-NH_2 (2.5 µg, 2.5 nmol)
c4 1 µL of 0.25 nmol/µL Ac-HHHHGKW-NH_2 (250 ng, 0.25 nmol)

Thioesterification: Ac-HHHHHHGKWG-COSBn Tuesday, Jan. 13, 2004



	mw	eq.	d.	moles	amount
3-37	~~795~~	1.0		≈ 70 μmol	
benzylmercaptan	124.21	4.0	1.058	280 μmol	32 μL
HBTU		4.0		280 μmol	0.105 g
DIPEA	129.25	8.0	0.742	560 μmol	
dry THF					30 ~~mmol~~ mL

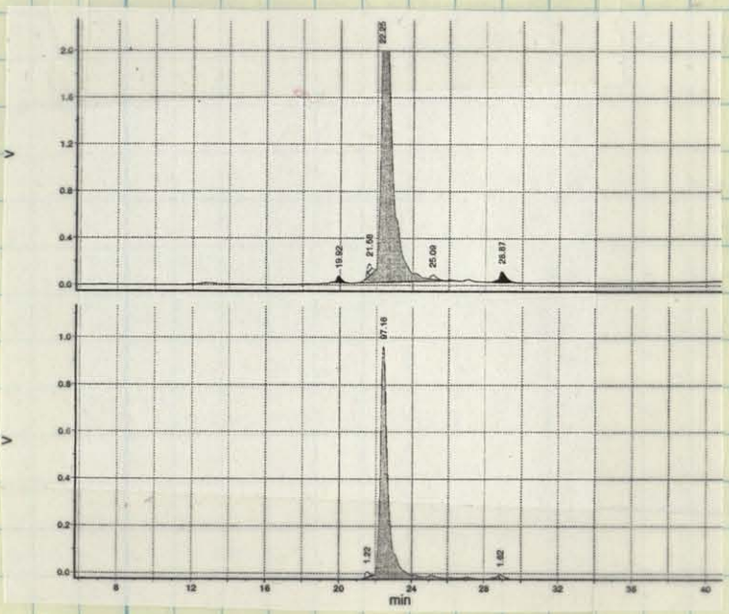
The peptide was cleaved from the TGT resin with 0.5% TFA in DCM for about 1.5 hours. The resin was rinsed with DCM into a 200-mL rb flask and rinsed well. 100 mL of hexanes were added and the solvent was removed in vacuo, leaving a thick light yellow liquid. The peptide was transferred to a 100-mL flask with some DCM, which was then removed in vacuo.

The flask was flushed with nitrogen. 30 mL of dry THF were added. To the stirring solution was then added HBTU, DIPEA, and benzylmercaptan. The resulting mixture was stirred overnight at room temp. under argon.

The solvent was removed on the rotovap (cleaning the trap w/ bleach afterwards to get rid of the thiol smell) and then further dried on the vaccuum pump for ≈ 1 hour. A second trap was set up in the hood to avoid thiols going through the manifold etc. A white powder was present after solvent removal.

10 to 15 mL of 95% TFA, 2.5% TIS, 2.5% water was added to the rb flask and then transferred to a 50-mL conical tube. After 2 h. of shaking The solvent was evaporated with a stream of N_2, then triturated with 2 × 30 mL of ethyl ether. The peptide was dissolved in 100% water for its purification (p. 109)

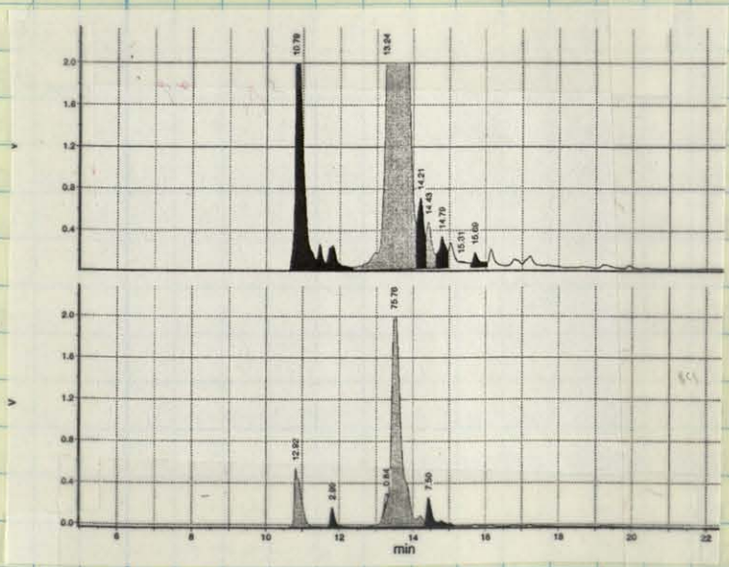
Purification of Ac-HHHHHHGKWG-COSBn Thursday, January 15, 2004



Ac-HHHHHHGKWG-COOH
(side-chain protected on TGT resin for thioesterification. For test cleavage side chains deprotected)

$C_{59}H_{74}O_{12}N_{24}$
pepcalc: 1311.39 - 656.7 (M+2), 438.1 (M+3)

ES/MS: two major peaks for 22.3 min.
656.2 (M+2)
437.8 (M+3)



Ac-HHHHHHGKWG-COSBn
$C_{66}H_{80}O_{11}N_{24}S$
pepcalc w/ thioester calc:
≈ 1417 / 1416.5 - 709.5/3 (M+2), 473.8 (M+3)

ES/MS for major peak (13.24 min)
709.2 (M+2)
473.3 (M+3)

8 mg of the purified thioester (5.6 μmol dissolved in 112 μL of PBS)
⇒ 50 mM solution

HPLC table for prep:
70 30% A (water) to ~~70~~ 30% water
over 30 min. (total run time: 41 min)