Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry

Supporting Information

N-Activated β-Lactams as Versatile Reagents for Acyl Carrier Protein Labeling

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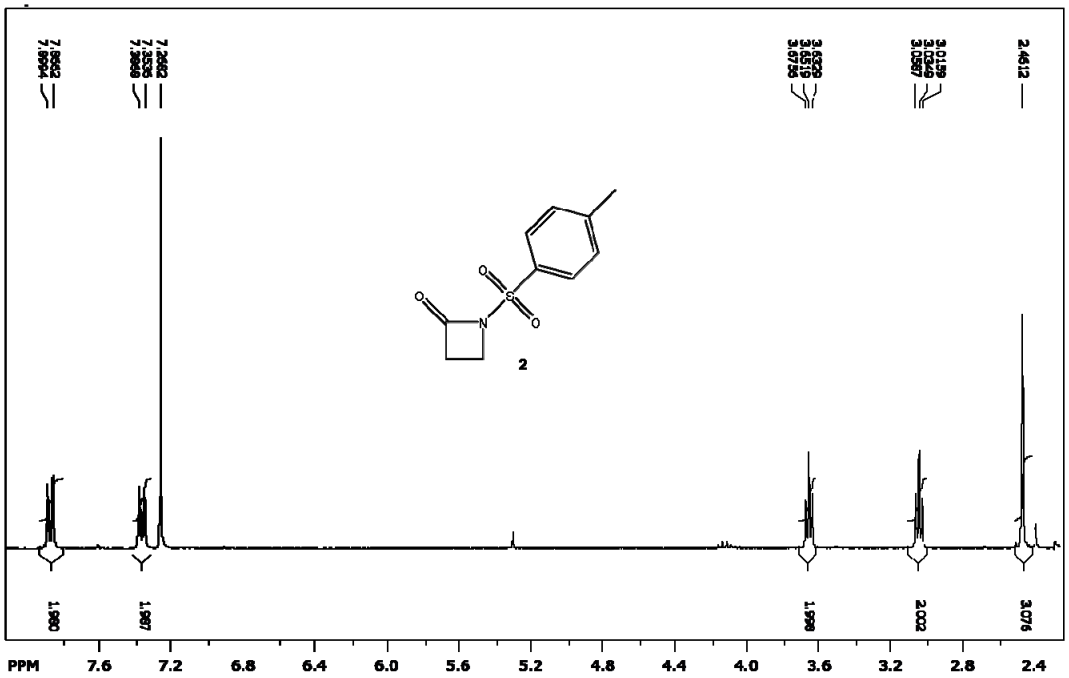
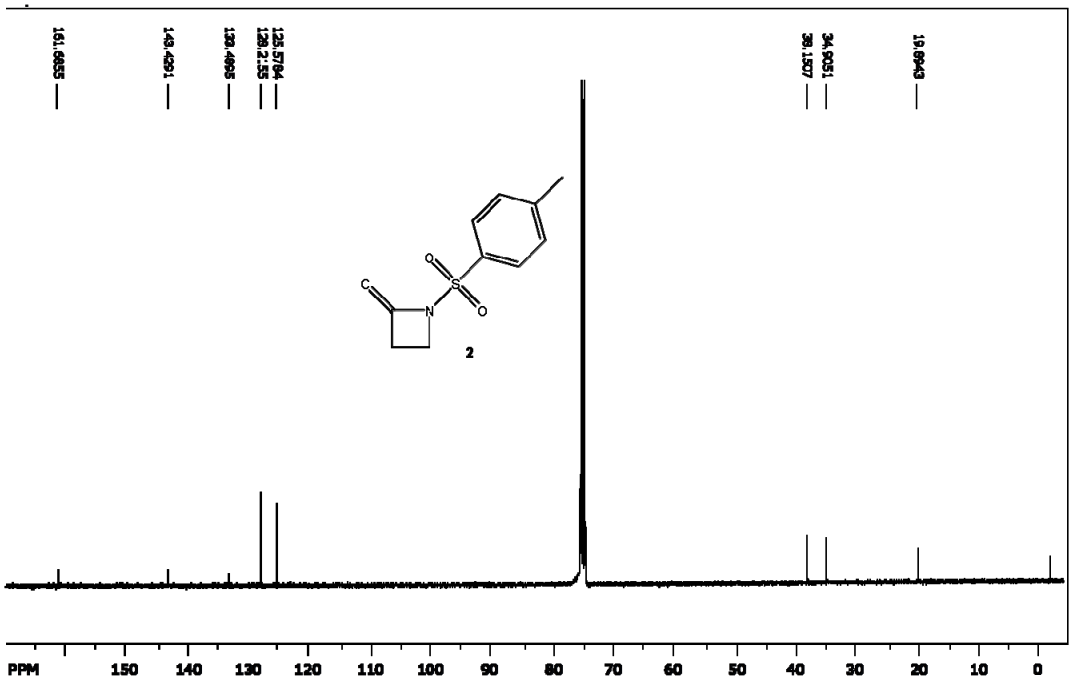
Spectra and Chromatograms

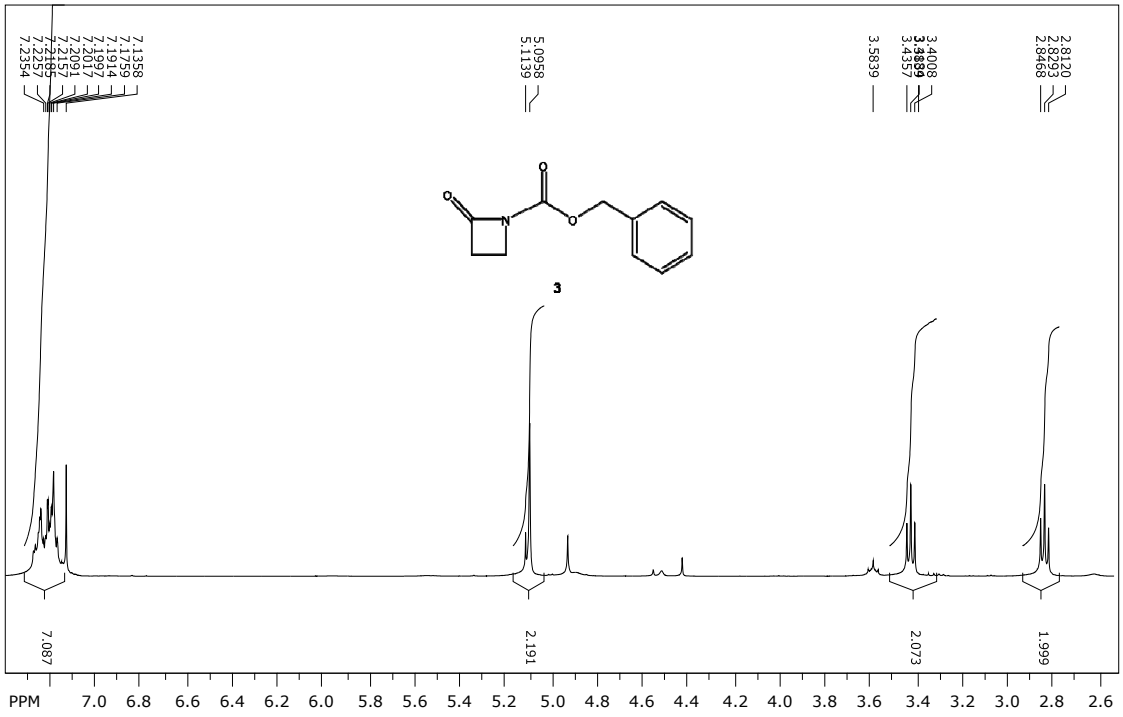
^{1}H NMR of 2



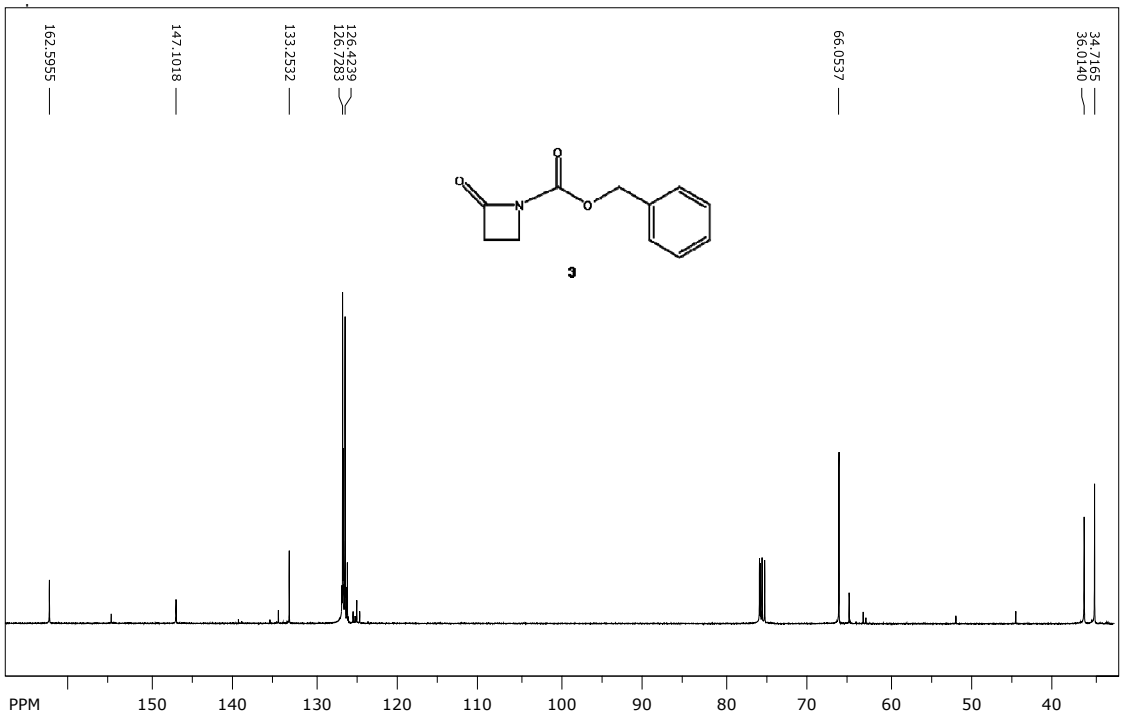
^{13}C NMR of 2



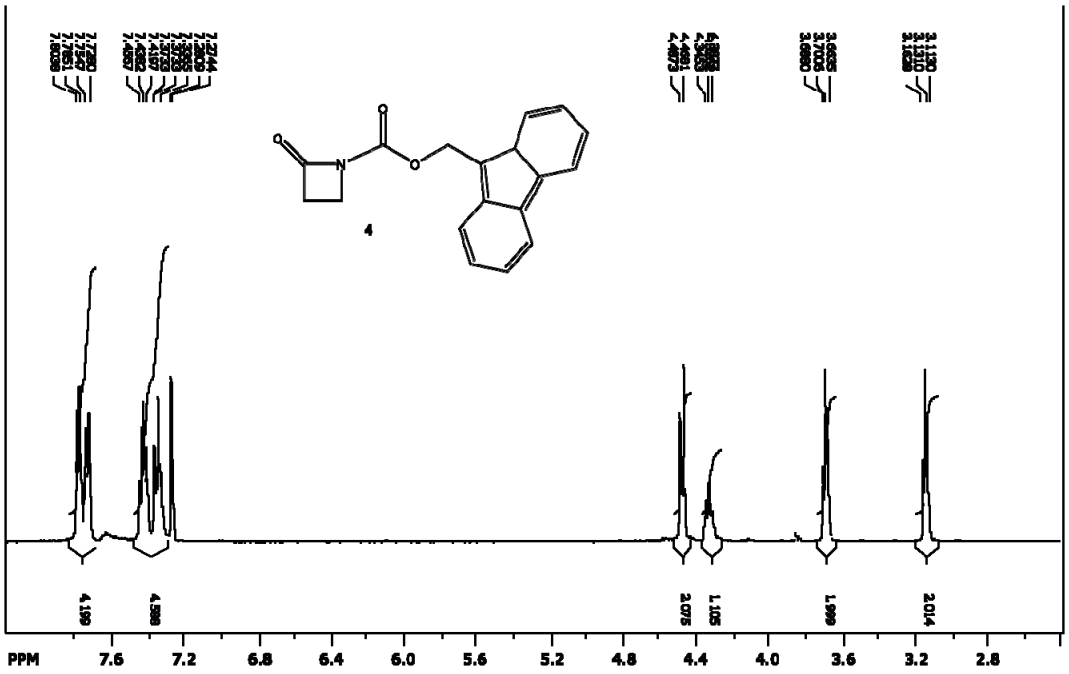
^{1}H NMR of 3



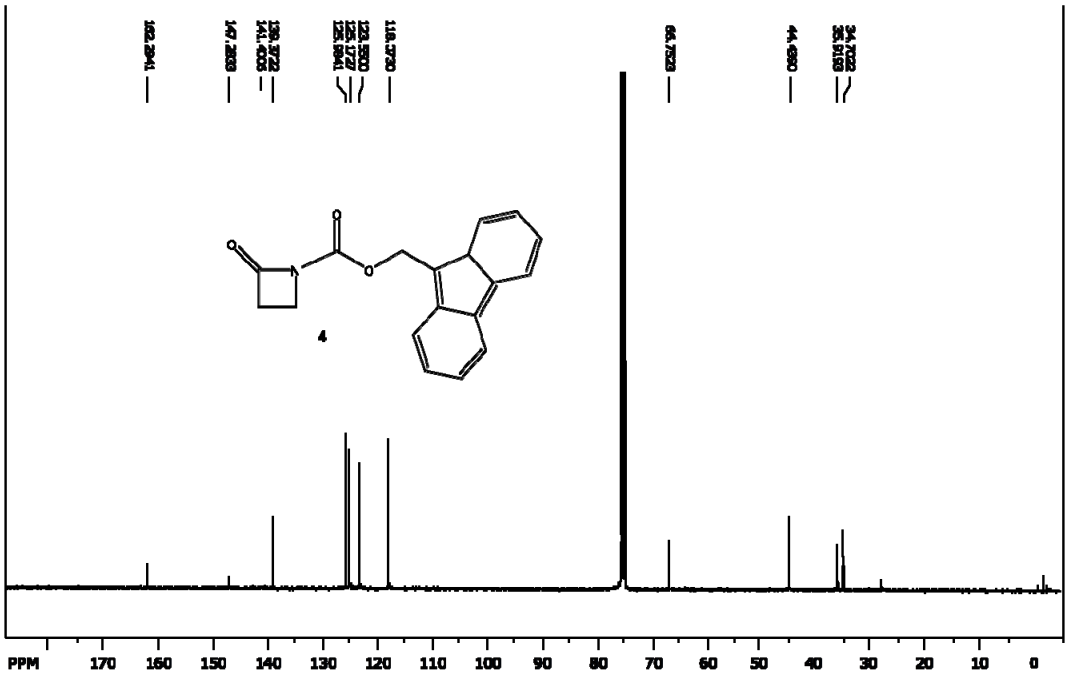
^{13}C NMR of 3



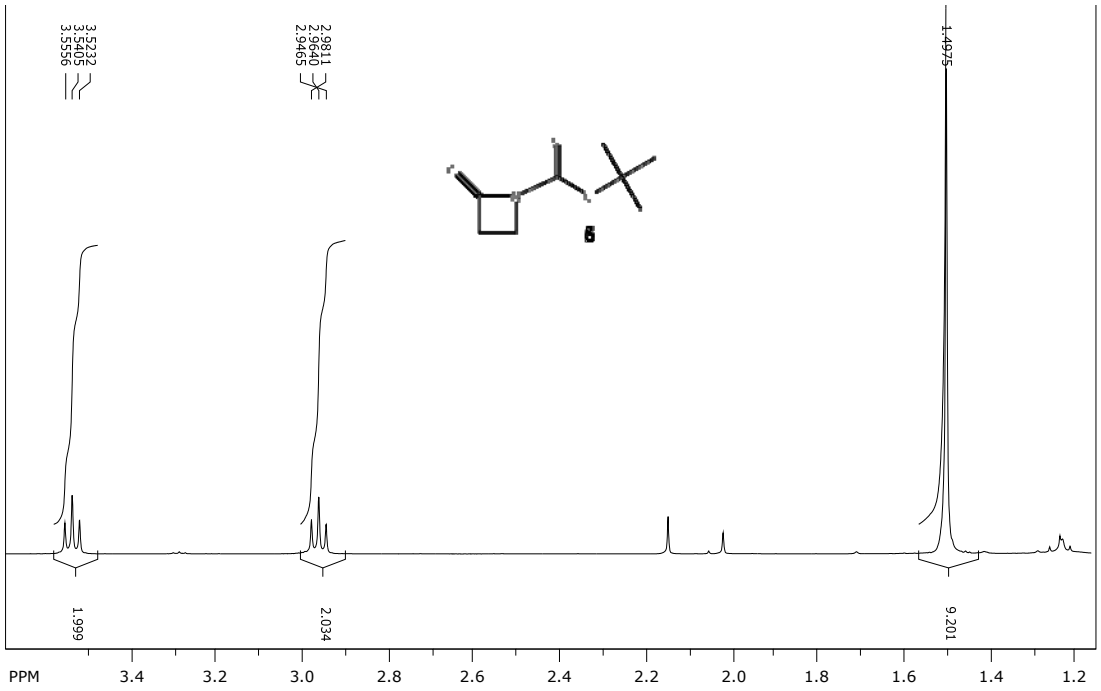
^{1}H NMR of 4



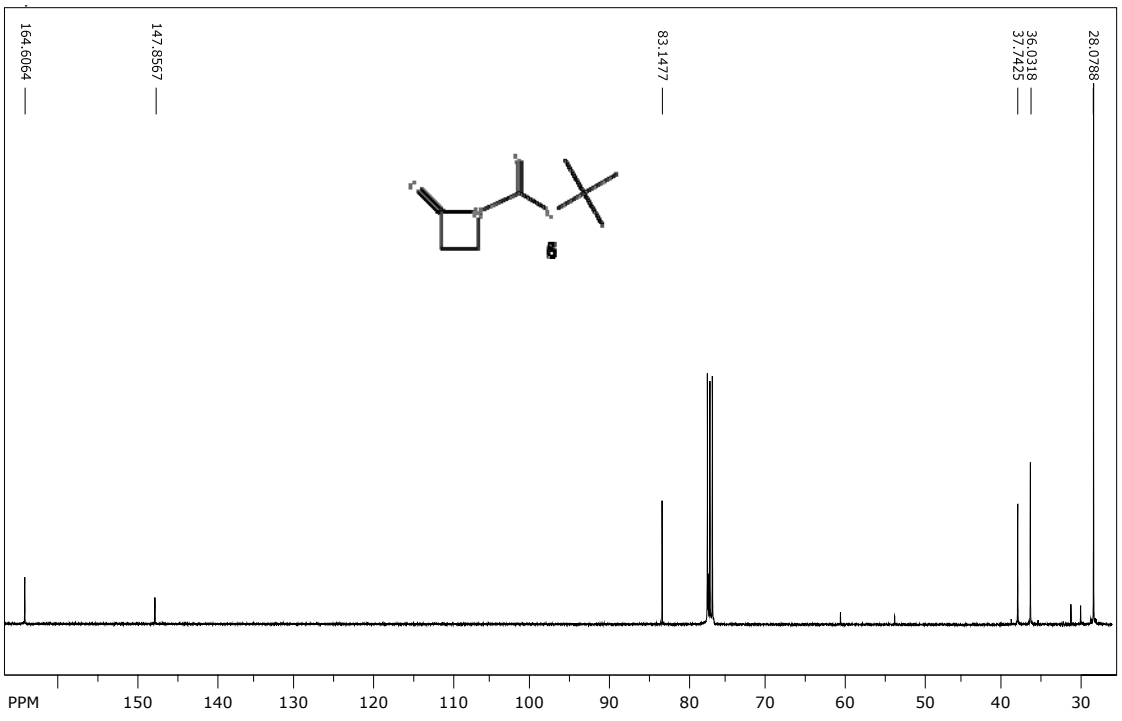
^{13}C NMR of 4



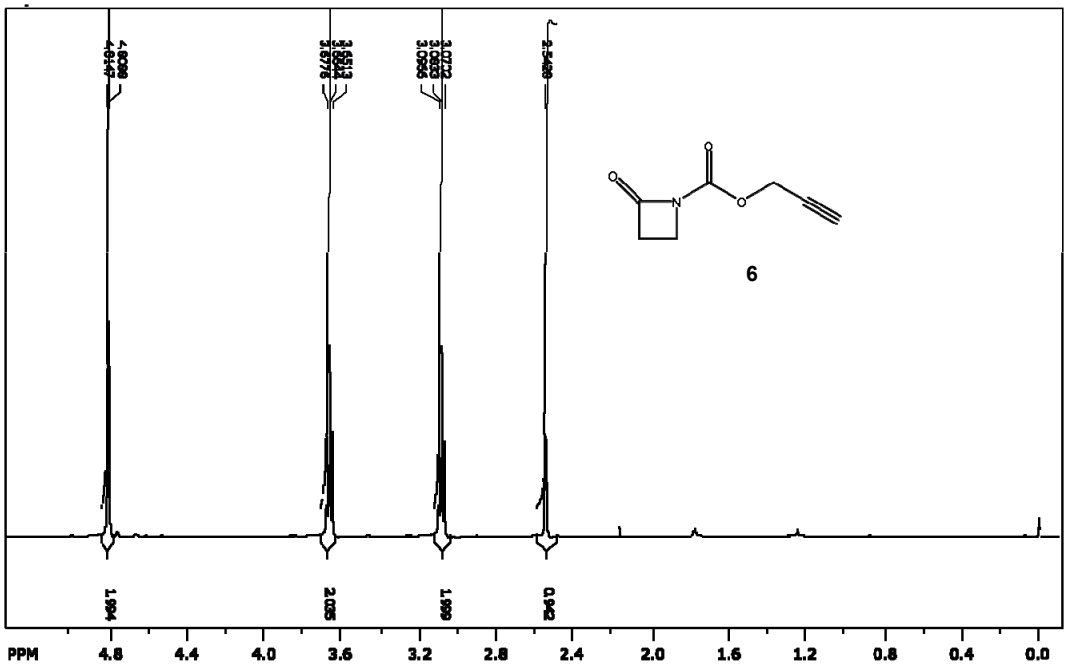
^{1}H NMR of 5



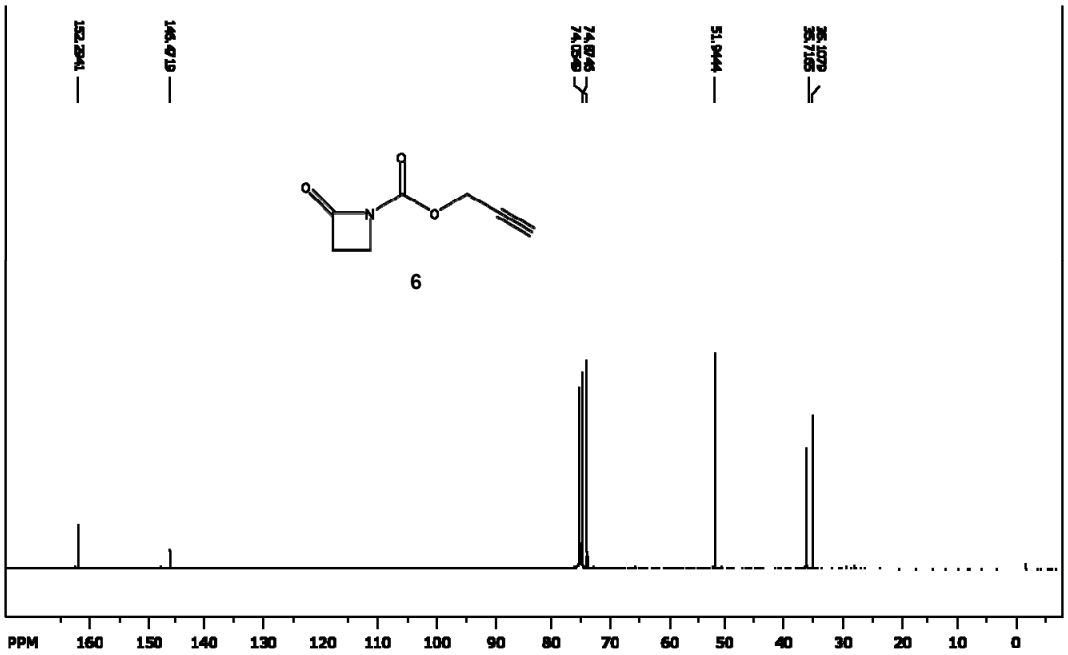
^{13}C NMR of 5



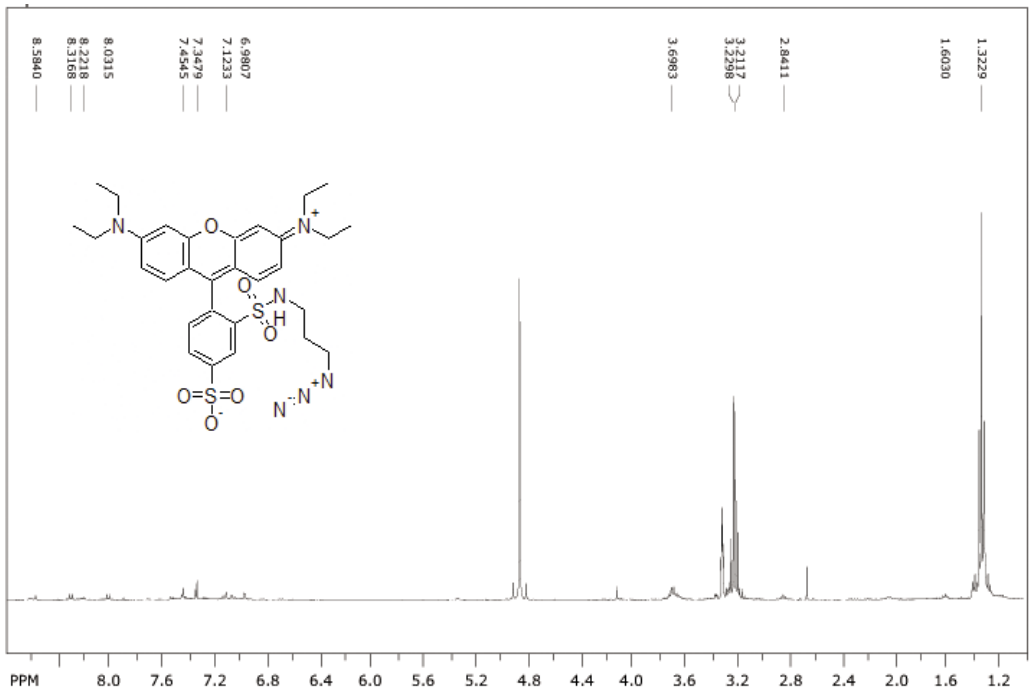
^{1}H NMR of 6



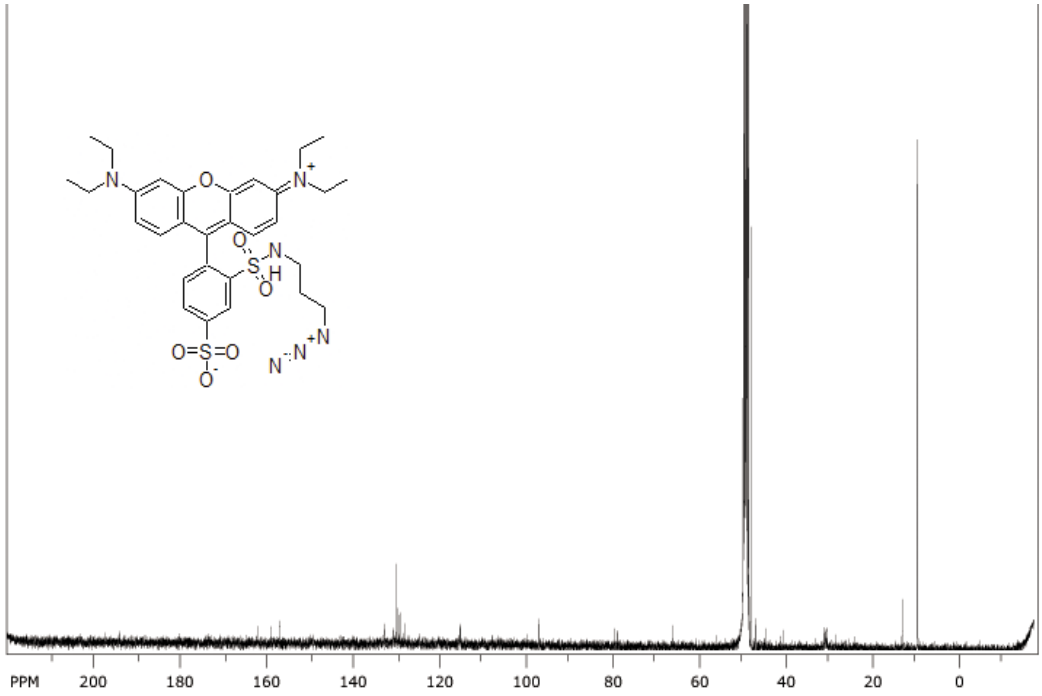
^{13}C NMR of 6



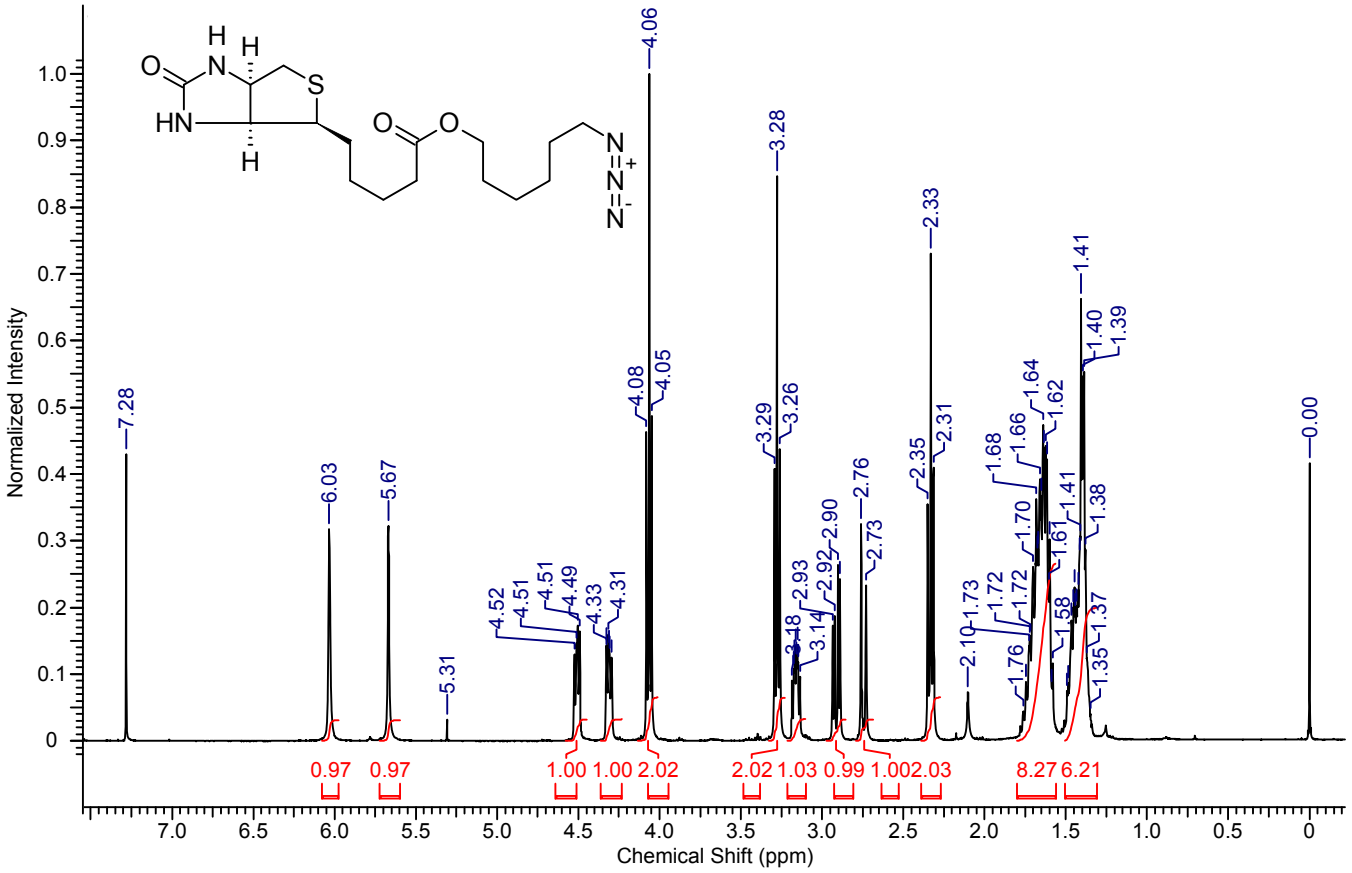
^{1}H NMR of S1



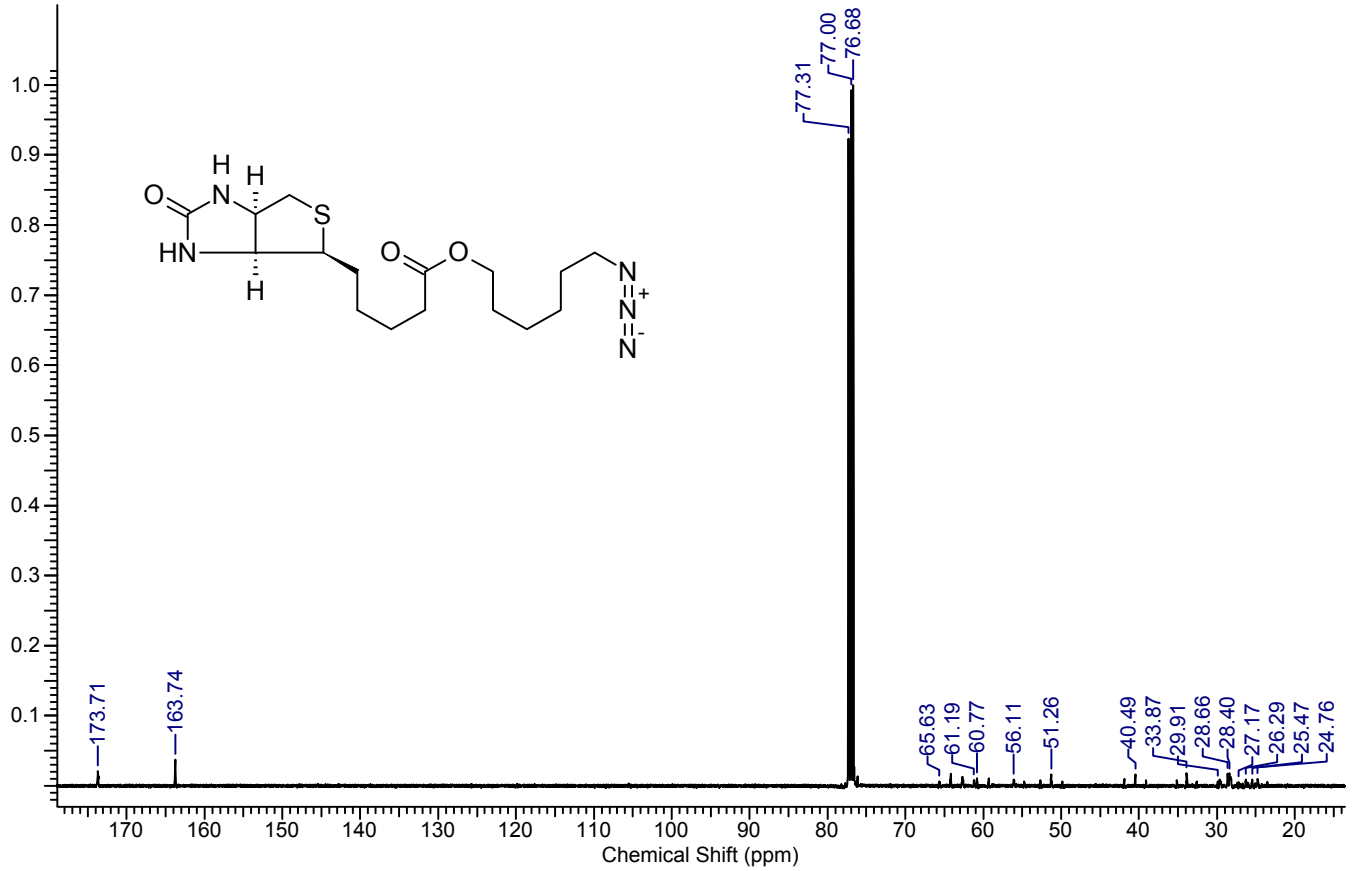
^{13}C NMR of S1



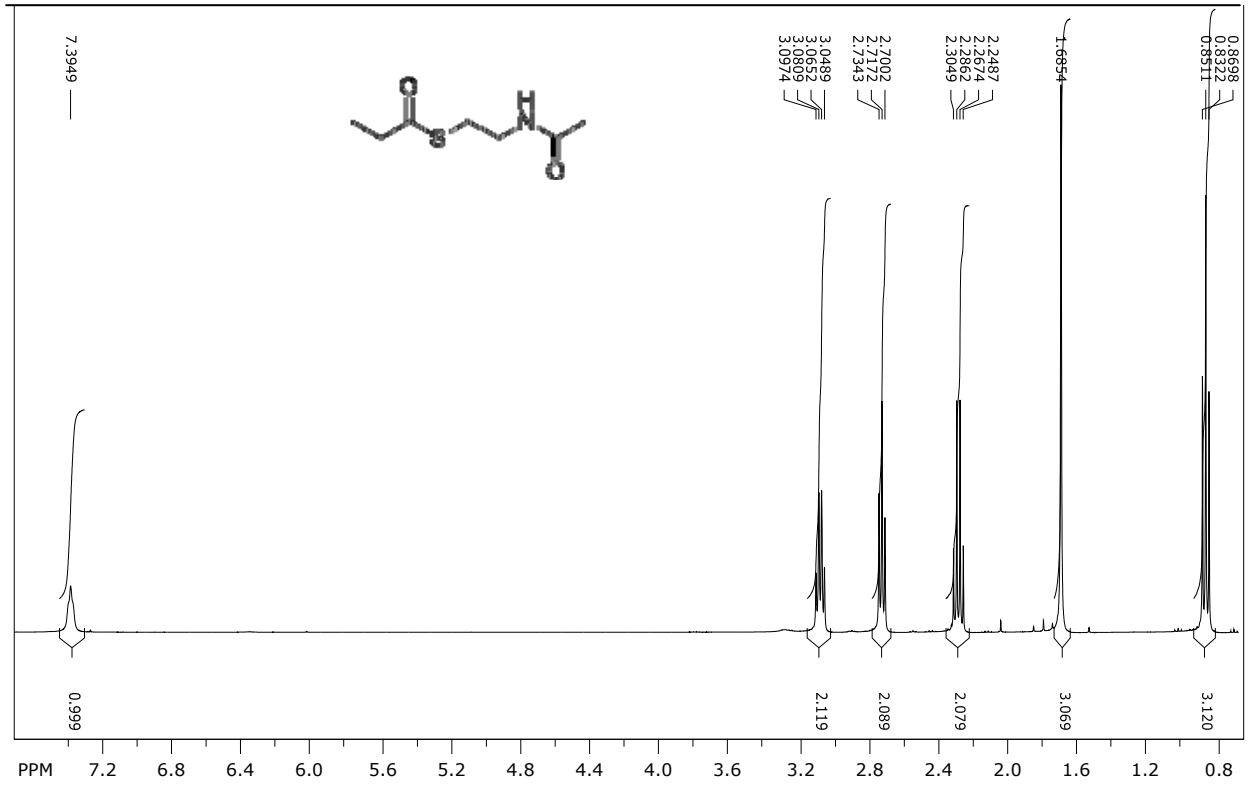
^{1}H NMR of S2


Normalized Intensity
Chemical Shift (ppm)
7.28
6.03
5.67
5.31
4.52
4.51
4.49
4.33
4.31
4.08
4.06
4.05
3.29
3.28
3.26
2.93
2.92
2.90
2.76
2.73
2.48
3.14
2.35
2.33
2.31
2.10
1.73
1.72
1.72
1.70
1.68
1.66
1.64
1.62
1.61
1.58
1.41
1.41
1.40
1.39
1.38
1.37
1.35
1.76
0.00
0.97
0.97
1.00
1.00
2.02
2.02
1.03
0.99
1.00
2.03
8.27
6.21

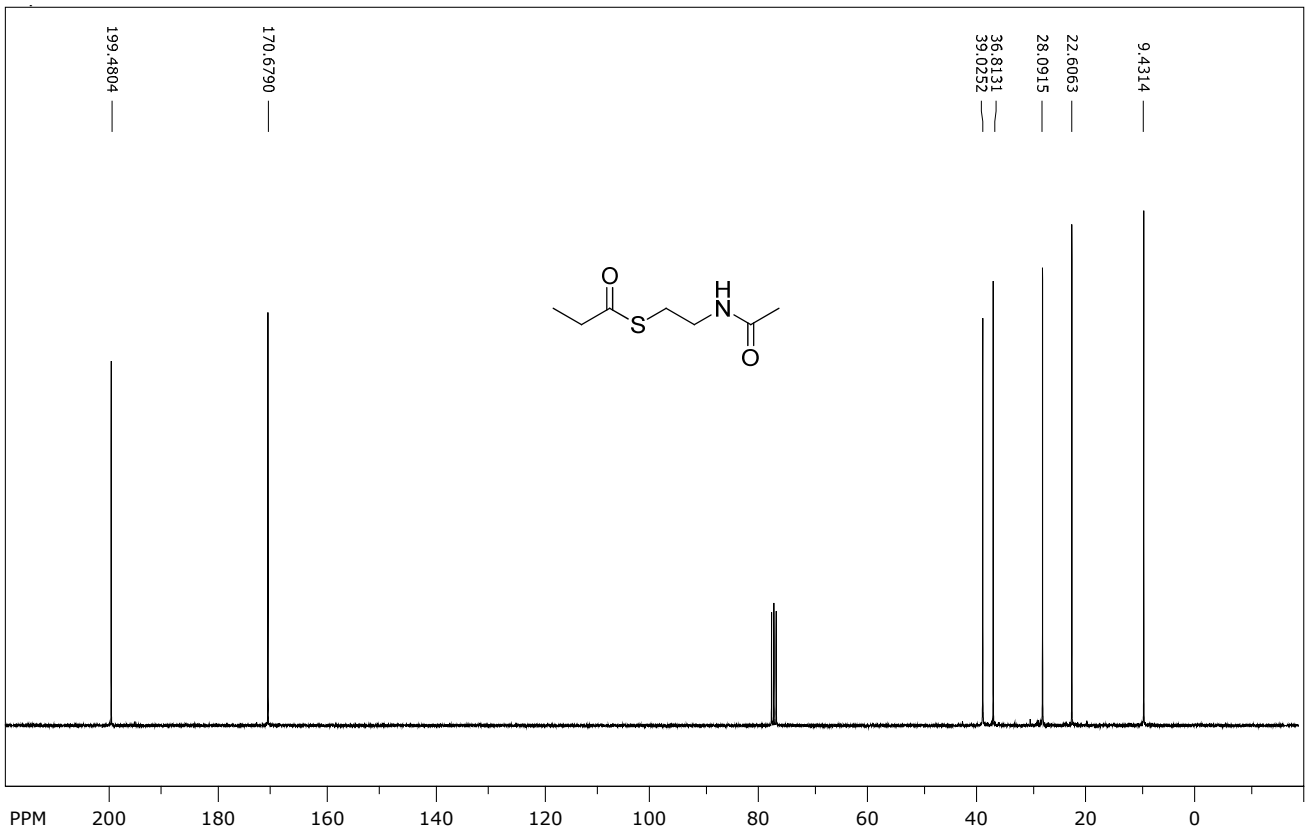
^{13}C NMR of S2



^{1}H NMR of Propionyl-SNAc



^{13}C NMR of Propionyl-SNAc



Representative data for determination of lactam loading:

Tryptic digests and the exact masses of the resultant peptides were calculated using the Expasy Peptide Mass tool < http://ca.expasy.org/tools/peptide-mass.html>. The masses of adducts were calculated by adding the exact mass of the peptide to the exact mass of the lactam probe.

To determine the extent of lactam loading, the calculated mass of the modified and unmodified peptides were extracted from the TIC, then the mass of the modified and unmodified peptides were were compared using the formula:

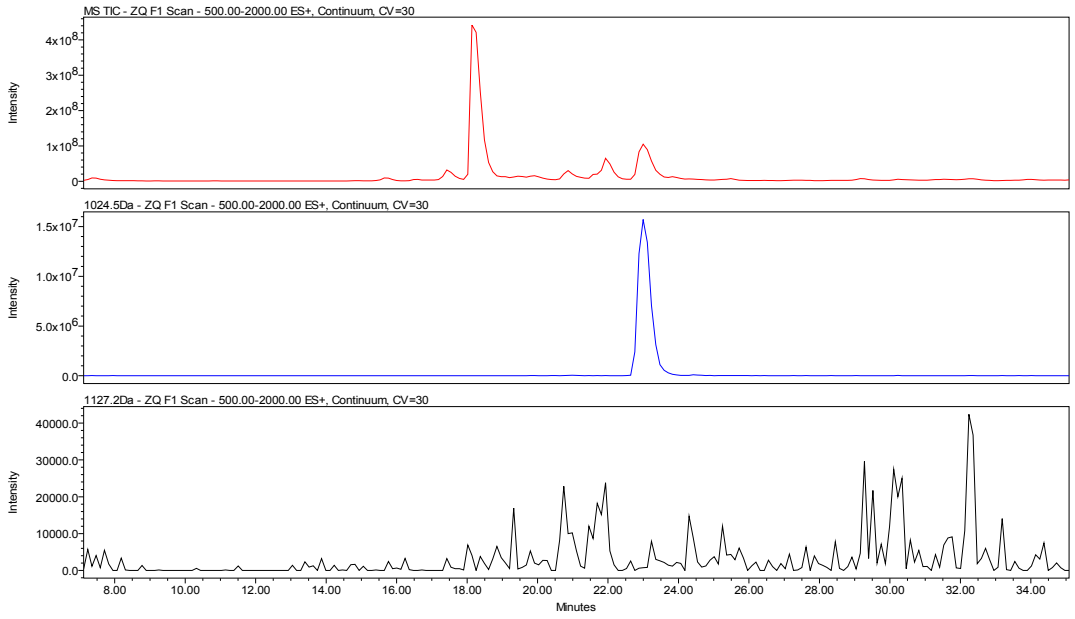
% loading = $(I_m / (I_m + I_u)) \times 100$

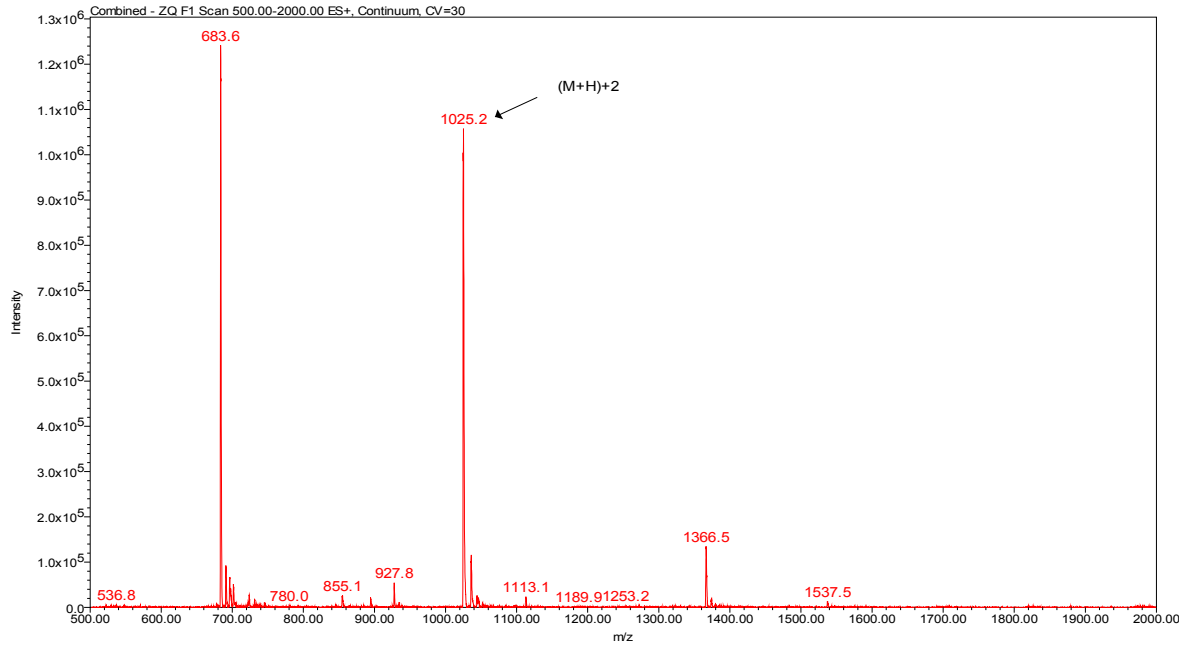
I_m = intensity of the modified peptide

I_u = intensity of unmodified peptide

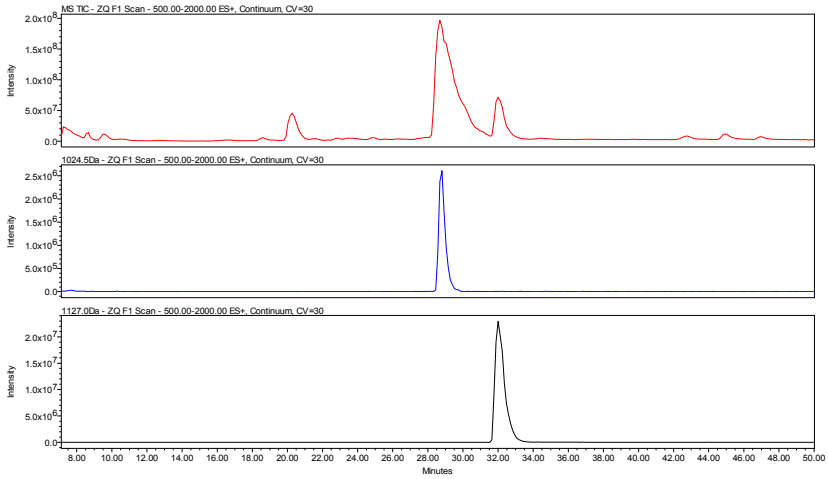
LC-MS spectra:

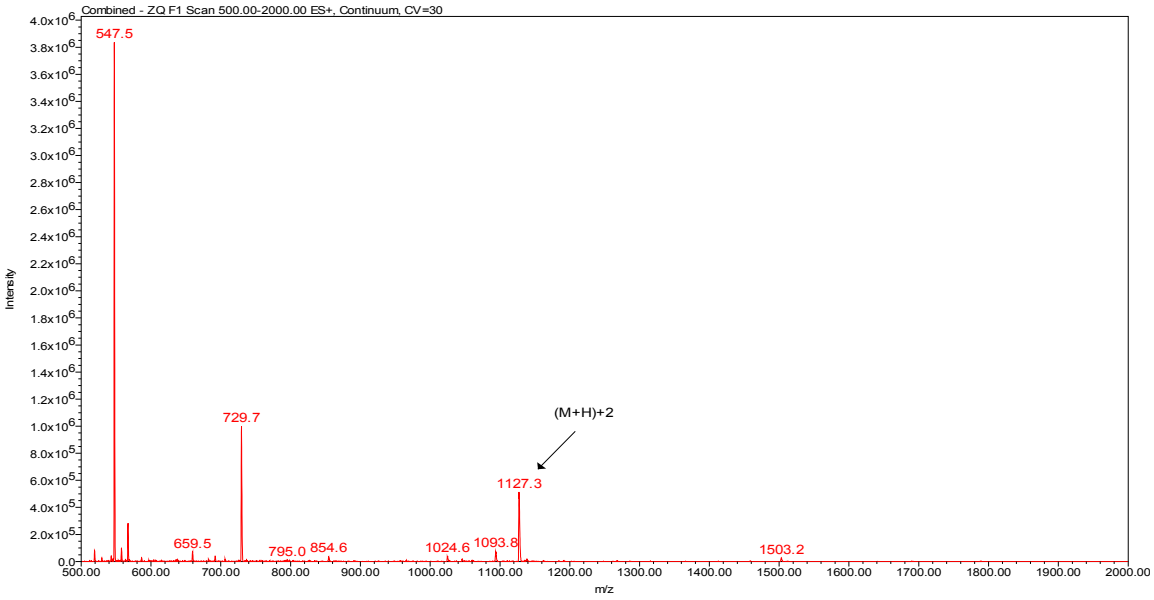
1. LC-MS data for trypsinized DEBS ACP3 in the absence of β-lactam (unmodified). The spectrum of the Ppant containing peptide fragment is shown. m/z value of +2 is labeled.





2. LC-MS data for trypsinized DEBS ACP3 + 10x N-Cbz-lactam (3). The spectrum of the Ppant containing peptide fragment is shown. m/z value of +2 is labeled





Representative % loading calculation for saturation curves: ACP3+ 10x N-Cbz-β-lactam 3

	m/z	Intensity
unmodified	1024.5	2745654.387
adduct	1127.3	23146385.076

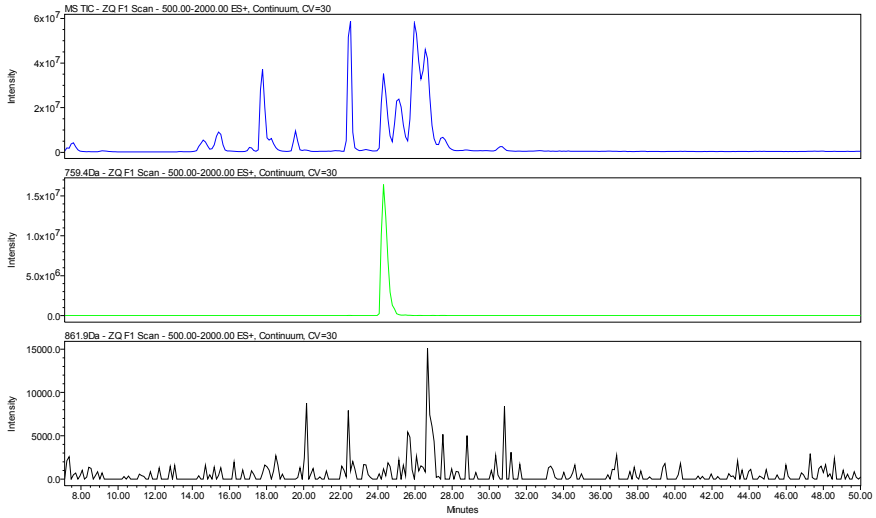
$$\% \text{ loading} = (I_m / (I_m + Iu)) \times 100$$

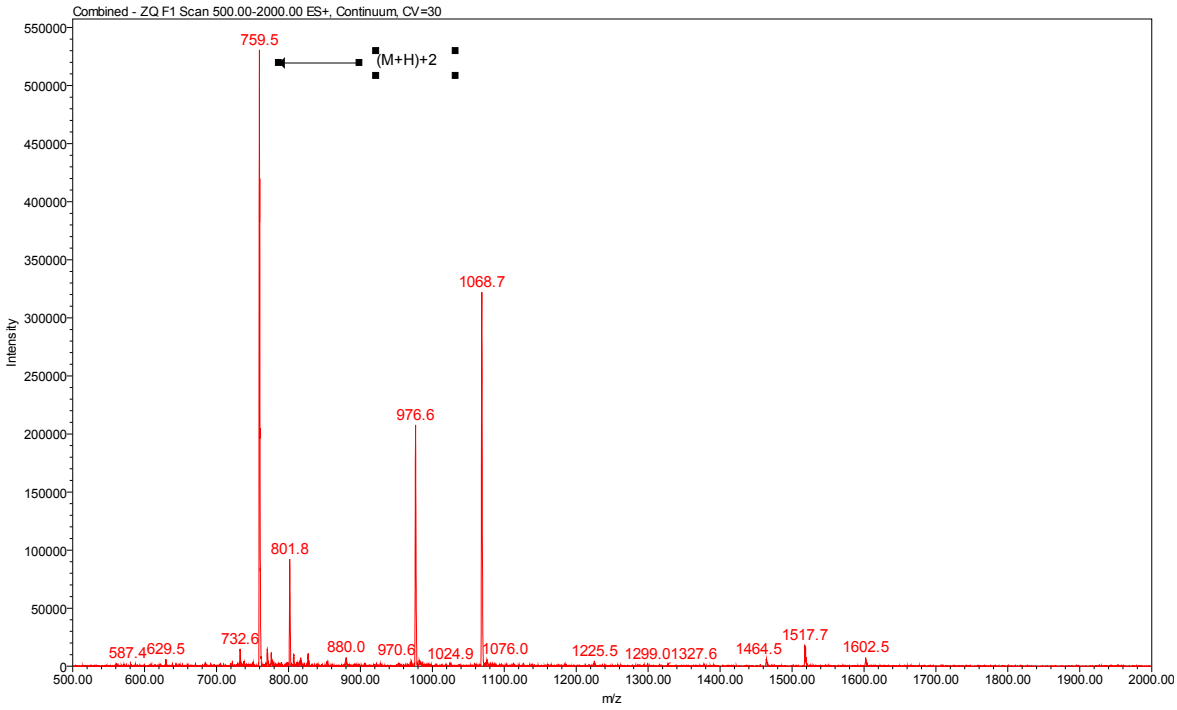
I_m = intensity of the modified peptide

I_u = intensity of unmodified peptide

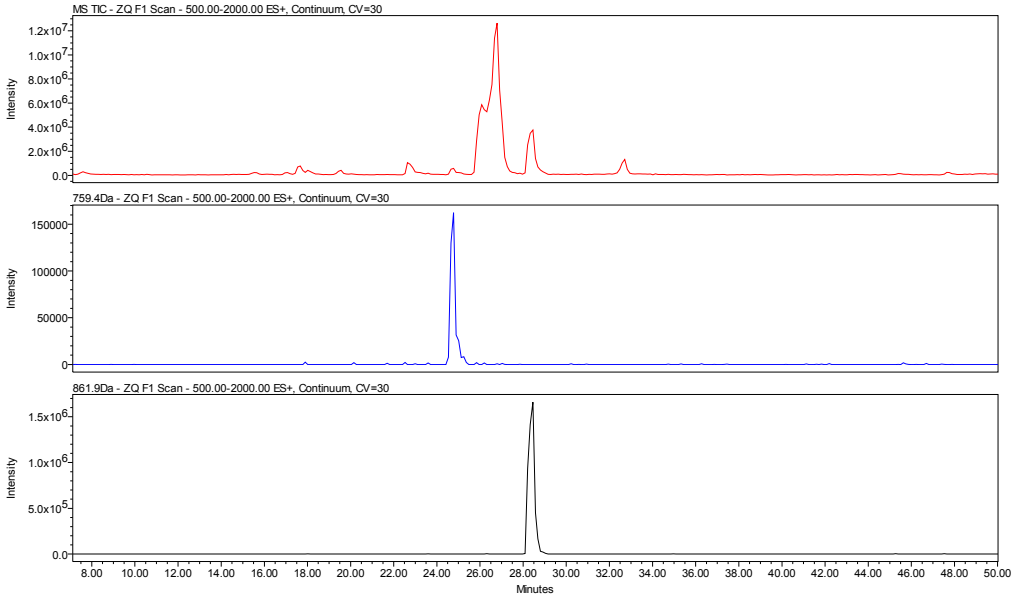
% loading: ((23146385.076/ 2745654.387+23146385.076)) * 100 = 83.2%

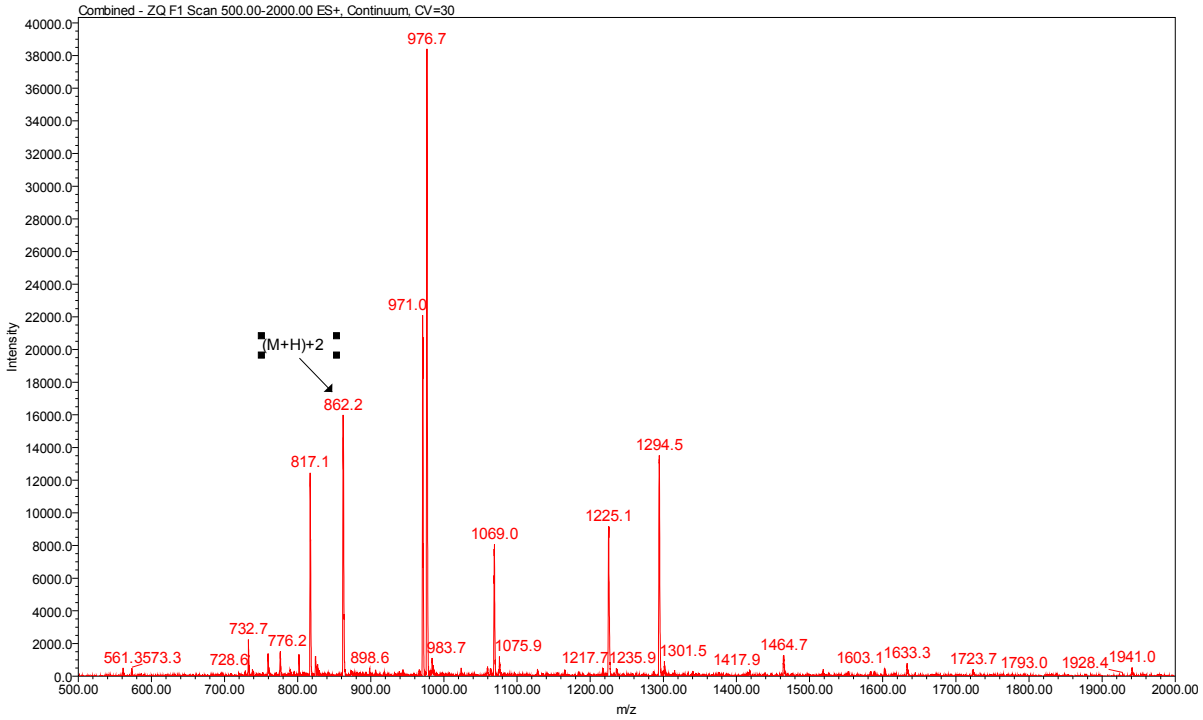
3. LC-MS data for trypsinized DEBS ACP2 in the absence of β-lactam(unmodified). The spectrum of the Ppant containing peptide fragment is shown. m/z value of +2 is labeled



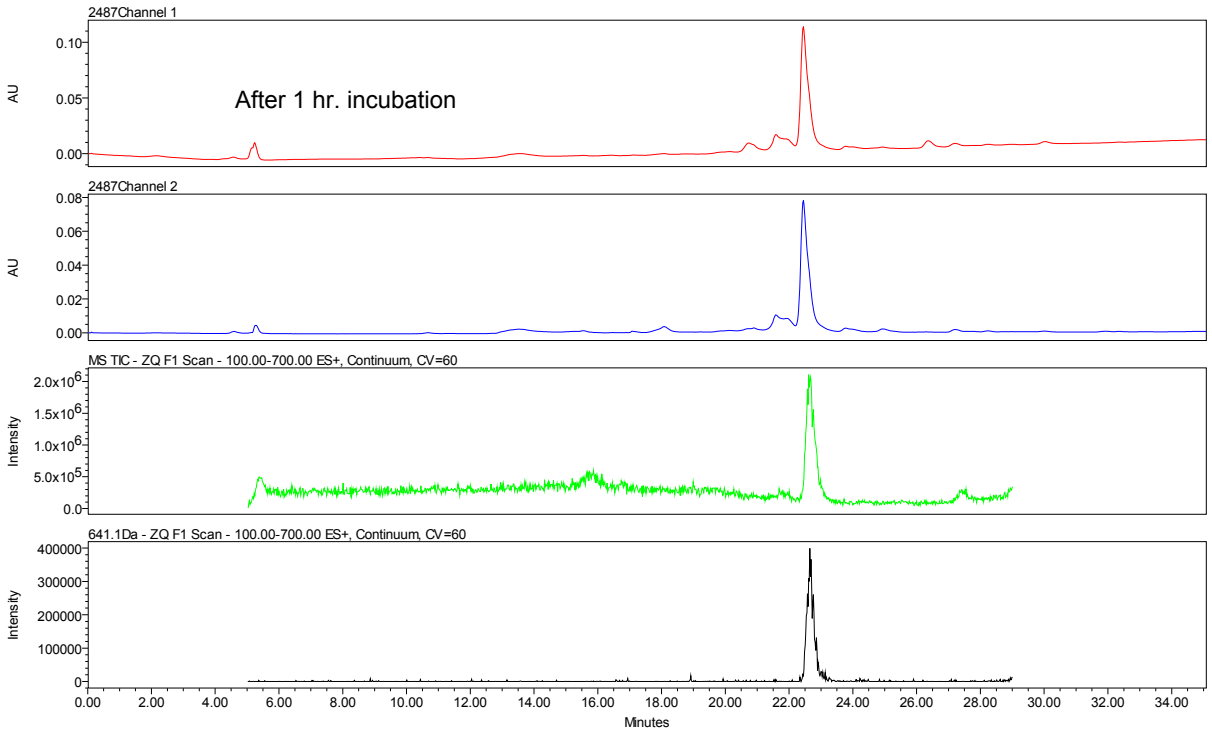


4. LC-MS data for trypsinized DEBS ACP2 + 10x N-Cbz-lactam (3). The spectrum of the Ppant containing peptide fragment is shown. m/z value of +2 is labeled

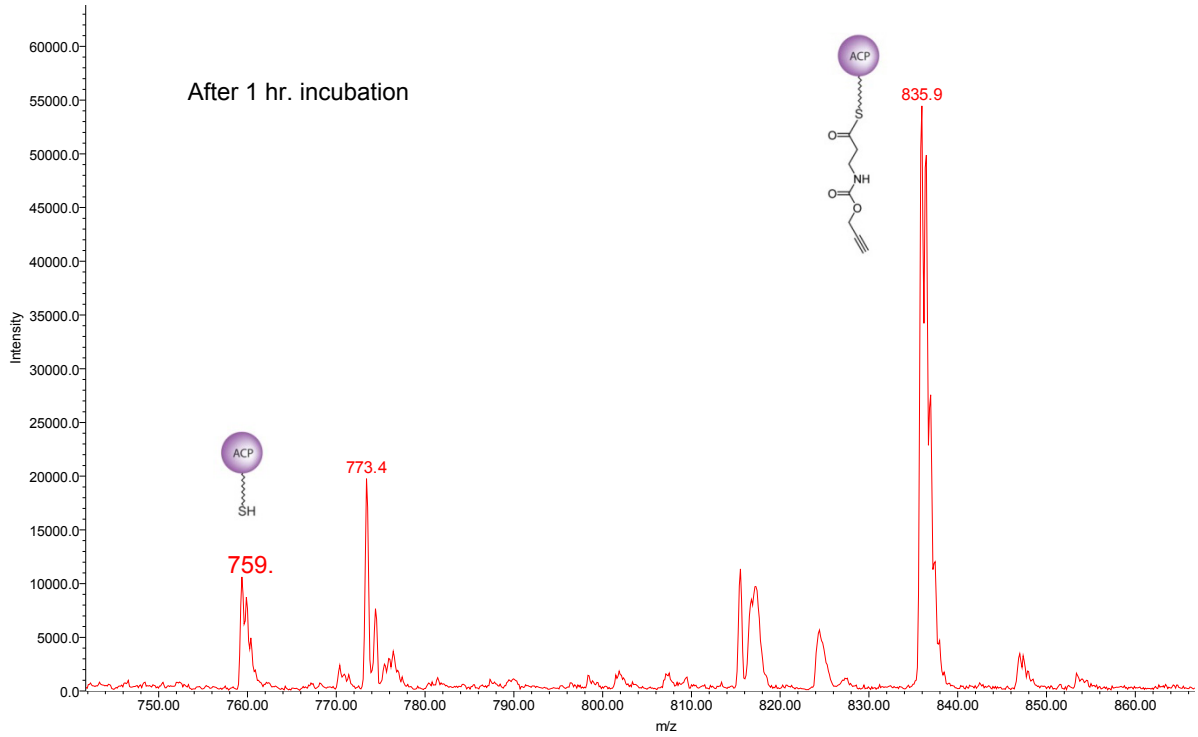


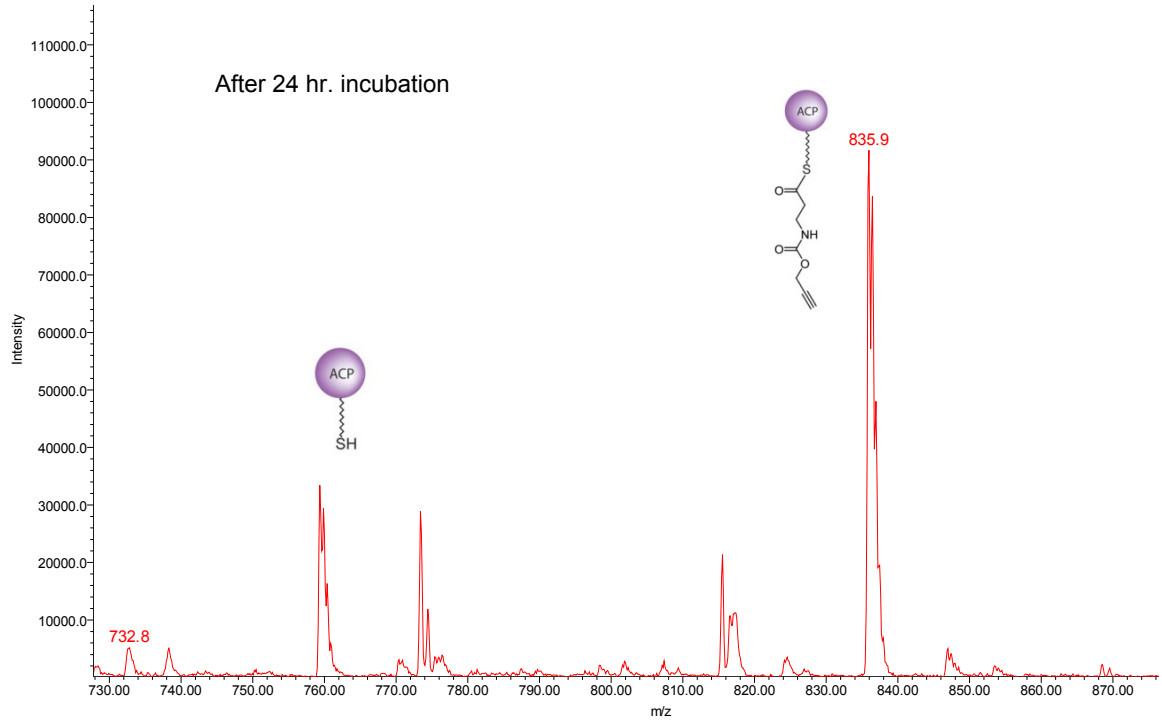


S1: LC-MS data for Sulforhadamine-B Azide; a = 254 nm, b = 280 nm, c = TIC, d = TIC extracted at m/z = 641.1 $[m+H]^+$



Stability data for ACP-thioester: ACP 2 was incubated with 20 equivalents of compound 6 in same conditions as used in other labelling experiments. The samples were incubated for 1hour and another for 24 hours before trypsin digestion. The extent of loading was observed to decrease by only 10%.





Data for Fig.3: Percentage loading determined by LC-MS for saturation curves

Table 1: ACP2

	Compound 2					Compound 3					Compound 4				
Equivalents				St. Dev	Avg.				St. Dev	Avg.				St. Dev	Avg.
0.5	12.3	15.0	18.9	2.7	15.3	9.3	12.0	13.9	1.9	11.6	27.0	29.0	23.0	2.5	26.4
1.0	50.2	54.2	56.1	2.4	53.3	21.1	23.7	24.3	1.4	22.8	47.2	51.8	44.0	2.5	47.8
5.0	78.5	79.9	85.6	2.9	81.6	82.3	85.4	89.0	2.7	85.0	73.5	68.0	76.9	3.2	72.8
10.0	80.5	83.4	89.6	3.8	84.5	84.4	92.0	93.0	3.8	90.0	82.0	85.4	78.0	3.0	81.9
20.0	84.5	92.4	93.0	3.9	89.9	90.4	95.4	96.4	2.7	94.0	88.1	90.0	94.7	2.7	91.0
50.0	92.0	93.6	96.5	1.9	94.0	94.5	98.7	99.5	2.2	97.5	92.0	98.4	98.0	2.9	96.1

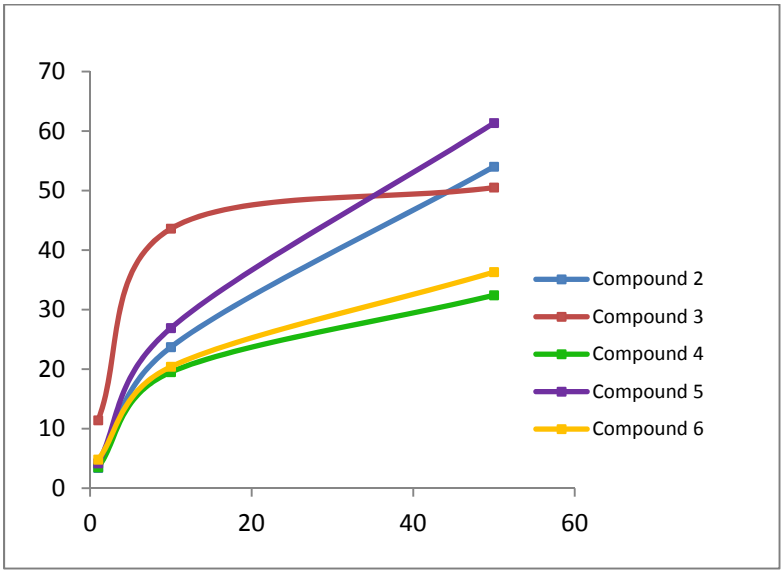
	Compound 5					Compound 6				
Equivalents				St. Dev	Avg.				St. Dev	Avg.
0.5	2.1	2.9	4.8	1.1	3.3	10.4	12.0	13.4	1.2	11.9
1.0	7.5	8.9	11.1	1.4	9.2	26.0	29.0	30.6	1.9	28.5
5.0	9.8	15.3	15.8	2.7	13.7	65.2	69.0	72.9	3.1	69.0
10.0	36.7	39.8	45.9	3.9	40.8	91.1	94.9	96.0	2.1	94.0
20.0	68.7	70.4	76.3	3.2	71.7	96.2	98.9	99.5	1.5	98.3
50.0	98.4	97.8	93.1	2.8	96.6	96.8	98.9	99.8	2.4	98.6

Table2: ACP3

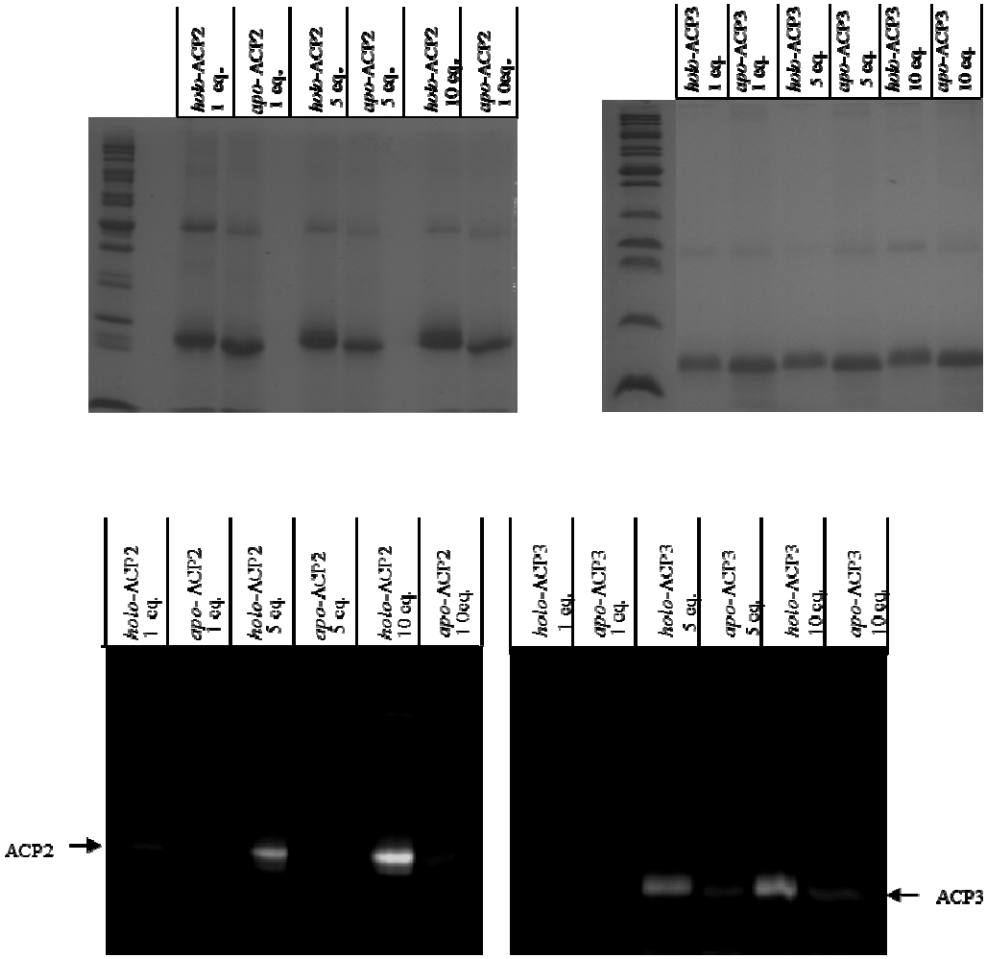
	Compound 2					Compound 3					Compound 4				
Equivalents				St. Dev	Avg.				St. Dev	Avg.				St. Dev	Avg.
0.5	41.6	39.8	42.9	1.2	41.9	18.6	21.0	22.5	1.6	20.7	18.0	16.5	13.0	2.1	15.8
1.0	58.0	59.9	56.0	1.6	57.9	42.1	43.7	44.4	1.0	43.3	38.0	41.0	36.0	2.1	38.2
5.0	62.5	64.6	66.2	1.5	64.4	52.5	55.8	56.9	1.9	55.1	46.0	44.0	45.8	0.9	45.2
10.0	73.1	77.4	84.3	4.6	78.3	78.0	85.8	86.2	3.8	83.2	76.0	71.0	80.0	3.7	75.6
20.0	80.2	81.9	83.7	1.4	82.0	89.9	89.7	95.7	2.8	91.8	85.0	79.0	89.0	4.1	84.4
50.0	81.4	85.8	88.3	2.9	85.0	94.8	98.9	99.4	2.4	98.1	27.0	29.0	23.0	3.7	94.7

	Compound 5					Compound 6				
Equivalents				St. Dev	Avg.				St. Dev	Avg.
0.5	5.2	6.0	7.2	0.8	6.0	11.4	12.1	14.2	1.2	12.5
1.0	14.0	13.4	16.0	1.1	14.5	28.6	32.0	33.1	1.9	31.1
5.0	24.5	28.0	30.2	2.3	27.6	75.6	81.2	83.1	3.1	79.4
10.0	55.0	61.0	68.4	5.5	61.4	83.1	86.9	87.9	2.1	85.8
20.0	67.5	72.9	74.1	2.9	71.4	89.4	92.0	93.2	1.5	91.6
50.0	89.4	94.3	98.0	3.5	93.9	90	94	95.6	2.4	93.4

Saturation curve for acylation for KS-AT6.

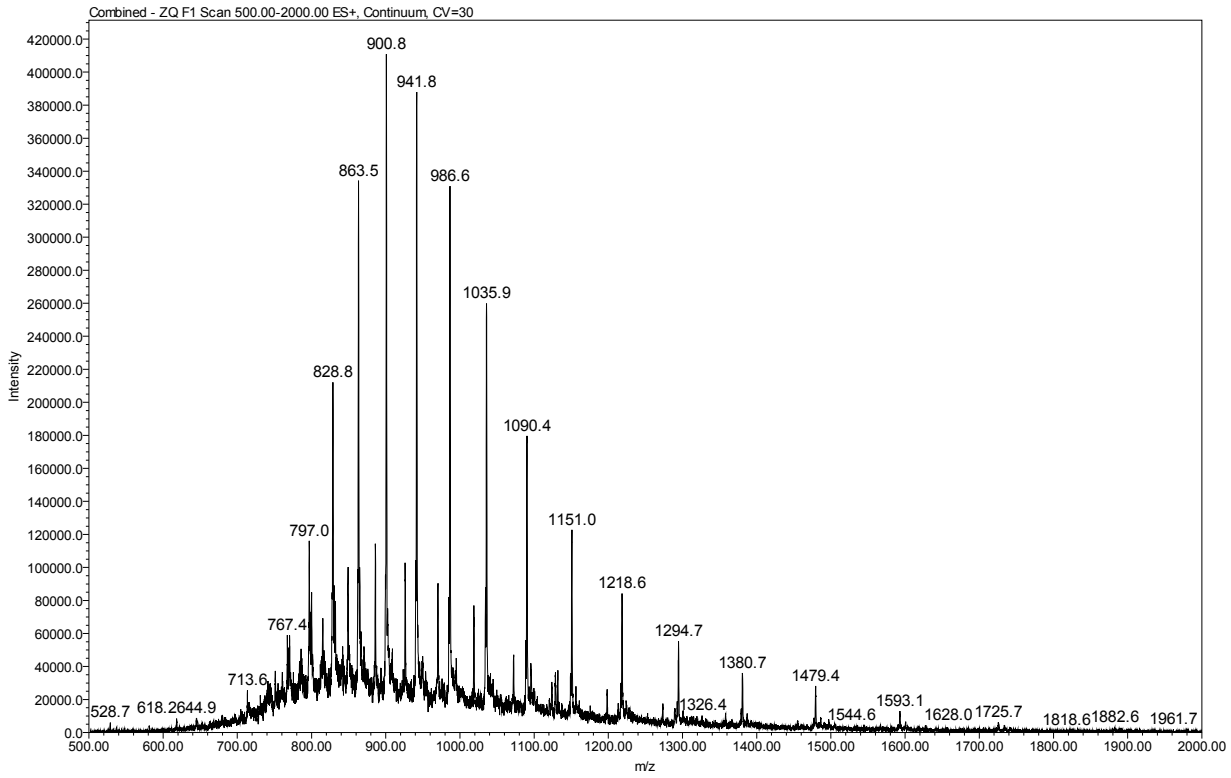


Same gel as Fig. 5 in text stained with Fisher GelCode Blue. Far left lane contains Fisher EZ-Run *Rec* protein ladder. An unrelated experiment has been cropped out.

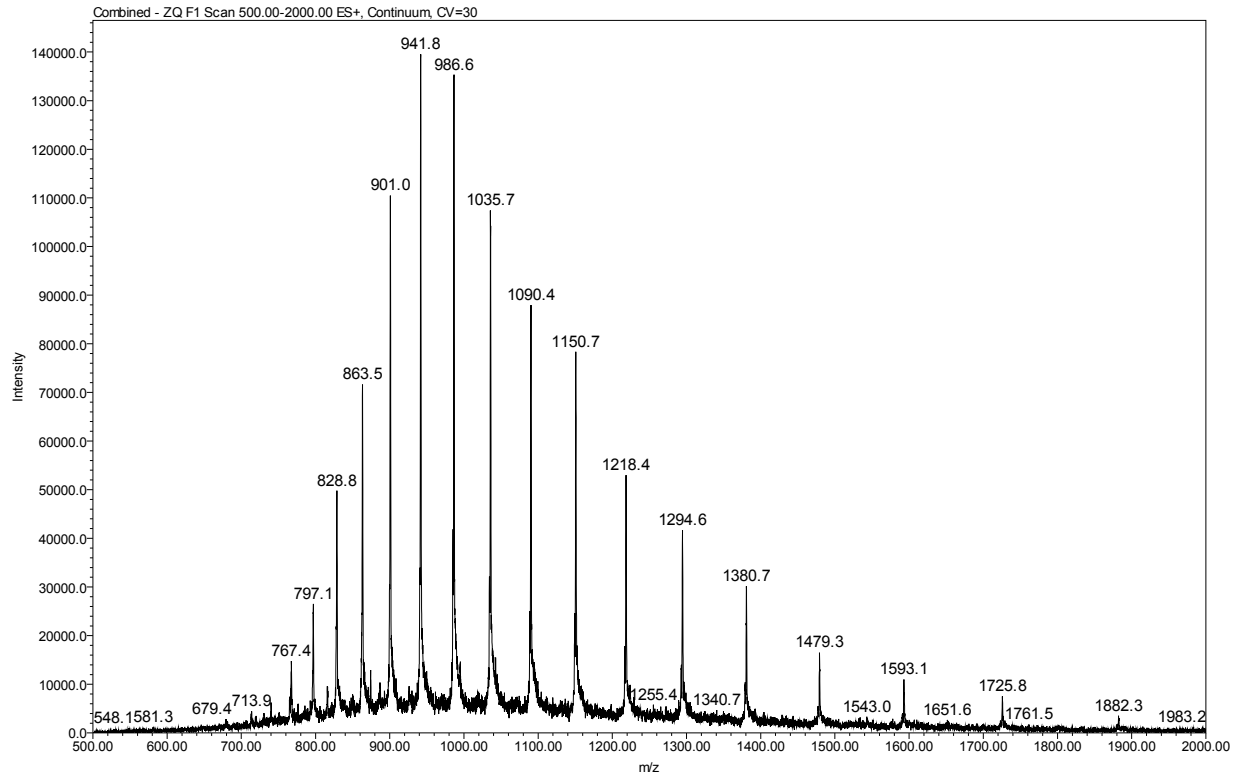


Full spectrum for Fig. 6

Initial 80:20 *holo*:*apo* protein; pre-purification, full spectrum

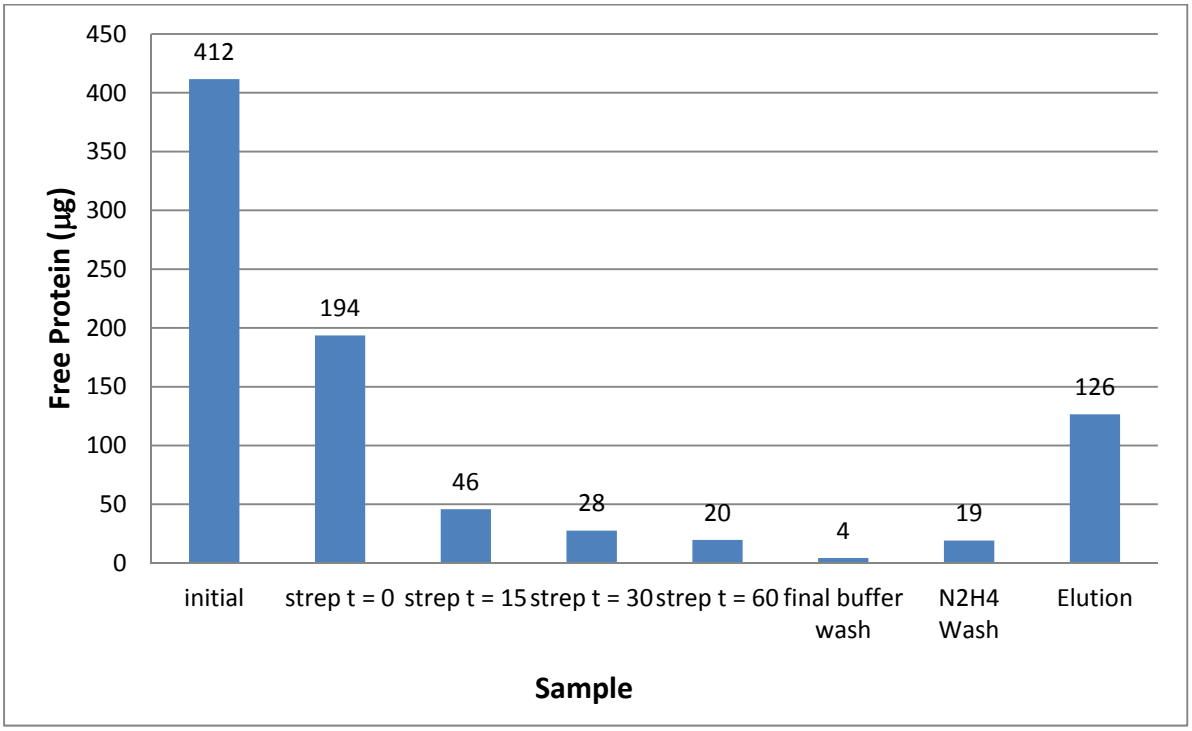


Initial 80:20 *holo*:*apo* protein post-purification, full spectrum

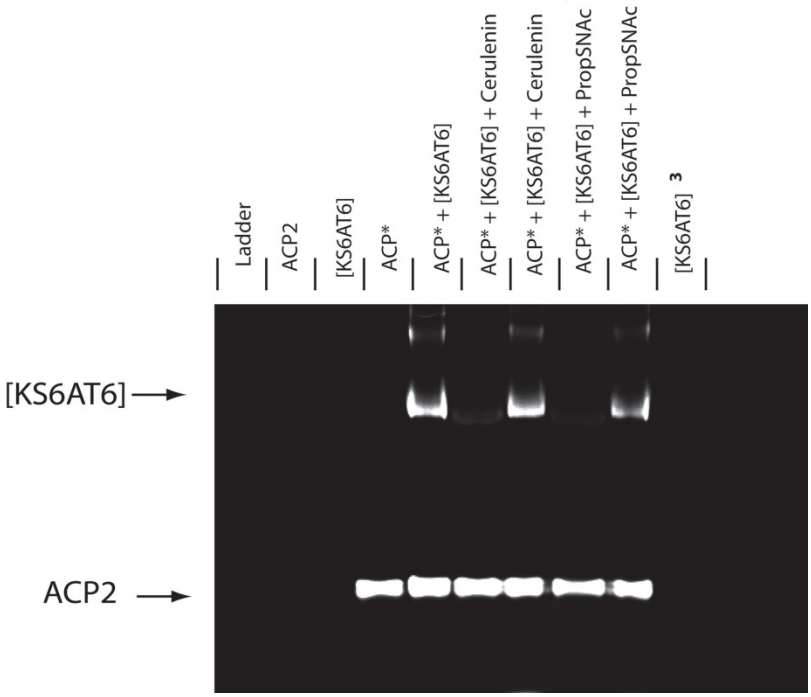


Timecourse Bradford assay for protein quantitation

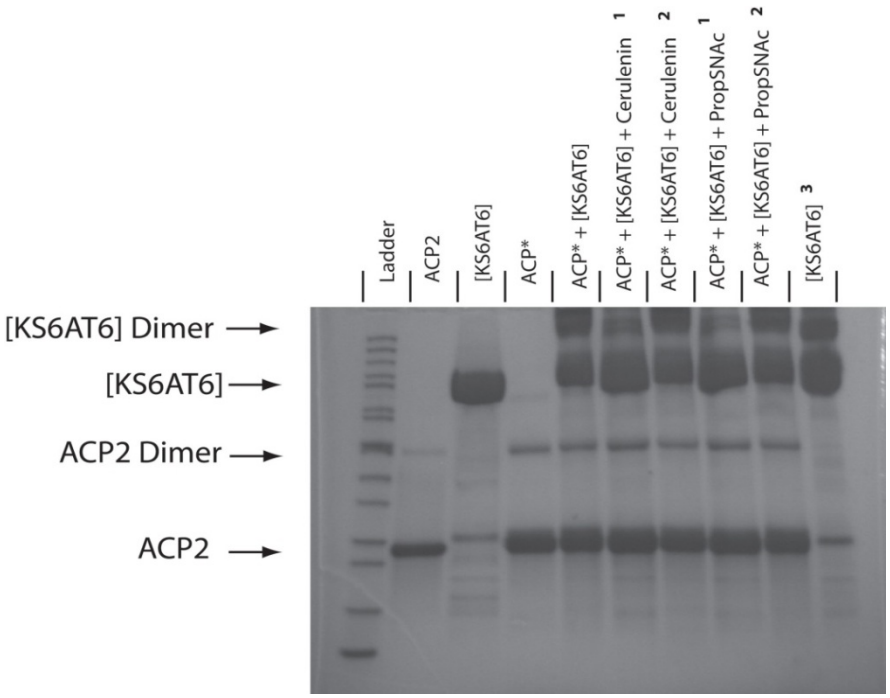
100% *holo* ACP2 was used to quantitate free protein at various points in the procedure. Aliquots were removed at specified points and subjected to Bradford assays to determine the free protein content.



Uncropped gel of Fig. 9



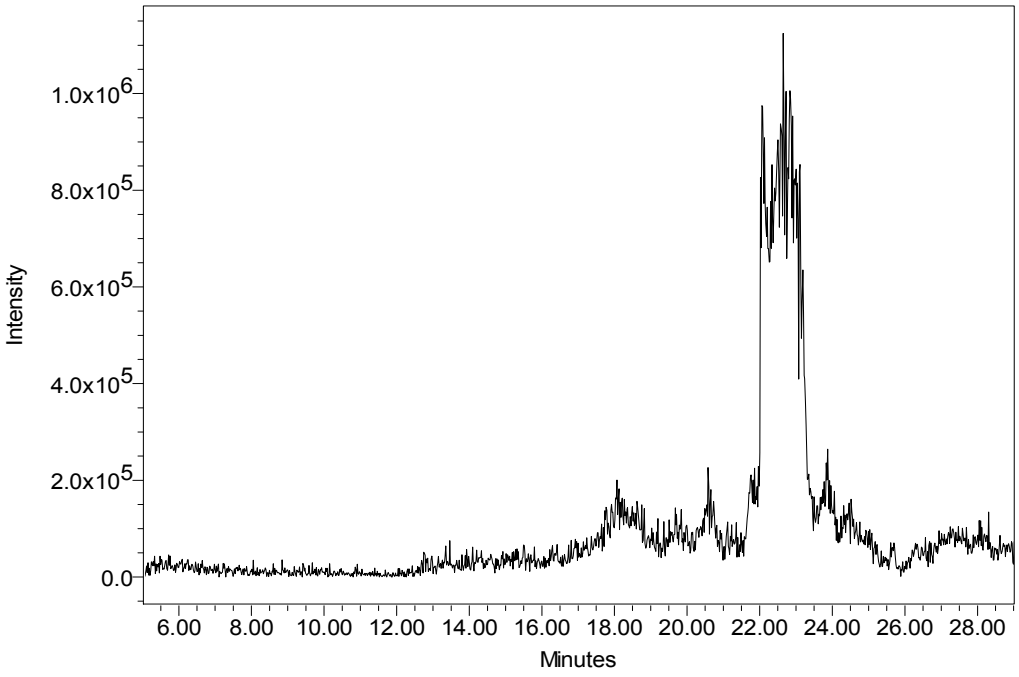
Fluorescent competition assay for ACP to KS acyl transfer. Fluorescent ACP2 (ACP2*) is prepared via click coupling of rhodamine azide and ACP2, preacylated with compound 6. ACP2* is then incubated with [KS6At6] under various conditions. Lanes are marked with the corresponding components for each reaction. 1[KS6AT6] was mixed with competitor (cerulenin or propionyl-SNAc) for 1 hour prior to addition of ACP2*. 2ACP2* and competitor were introduced to [KS6AT6] simultaneously. 3[KS6AT6] was mixed with 10 eq of the click product in the absence of ACP2. SNAc = N-acetylcysteamine.



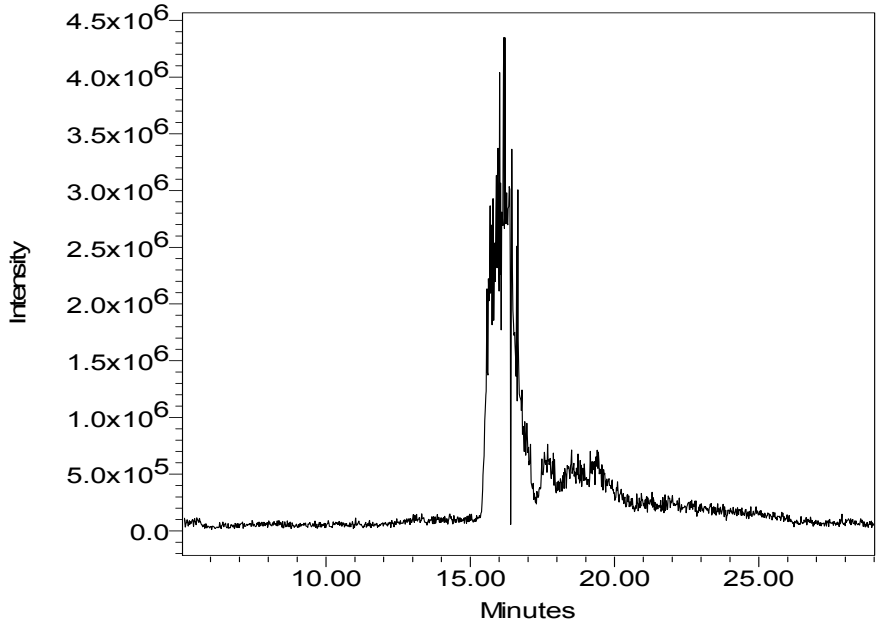
Gel stained with Fisher GelCode Blue. Far left lane contains Fisher EZ-Run *Rec* protein ladder. Lane 1&2 in the stained gel are standards for buffered ACP and KS without any treatment. The KSAT in lane 5-10 undergoes substantial dimerization which is Cu-dependent.

HPLC data:

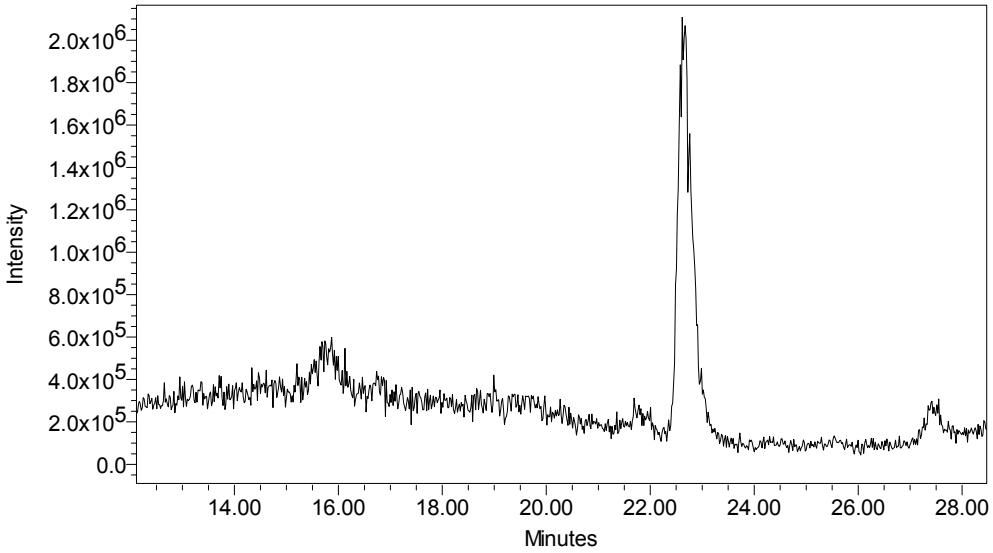
(9*H*-Fluoren-9-yl)methyl 2-oxoazetidin-1-carboxylate.



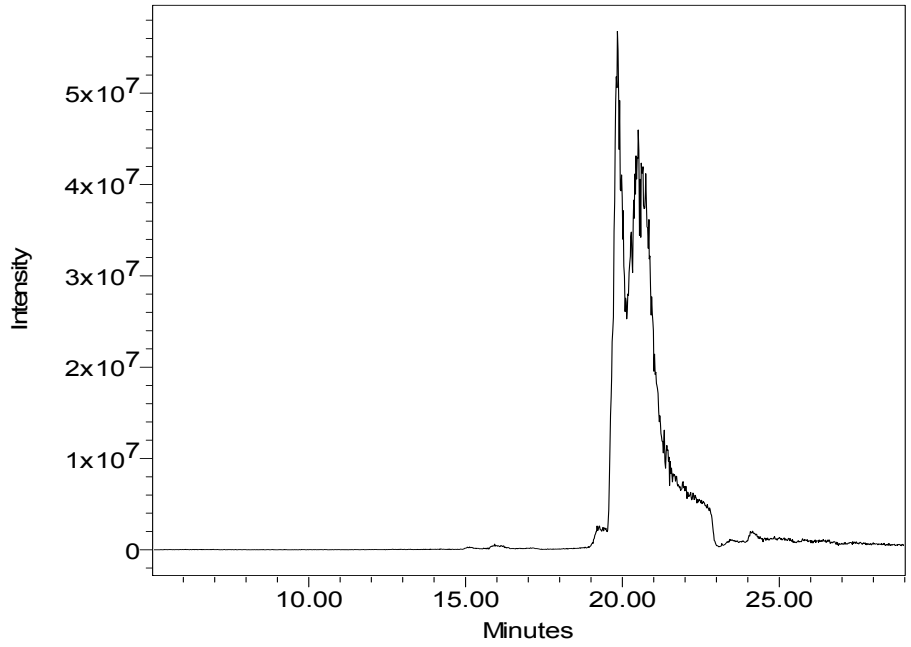
Prop-2-yn-1-yl 2-oxoazetidine-1-carboxylate



Sulforhodamine-B Azide



Biotin Azide



Propionyl-*N*-acetylcysteamine (Propionyl-SNAc)