ONLINE SUPPLEMENTAL MATERIALS

Investigation of Unanticipated Alkylation at the N(π) Position of a Histidyl Residue Under Mitsunobu Conditions and Synthesis of New Orthogonally Protected Histidine Analogues

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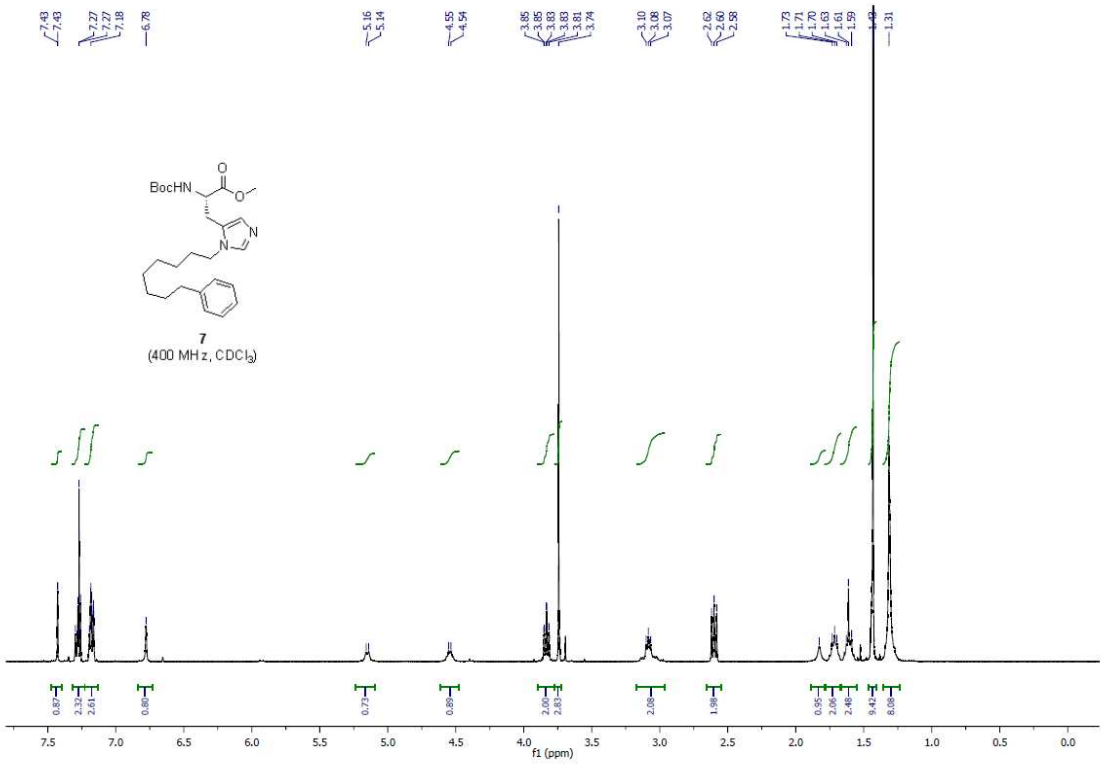
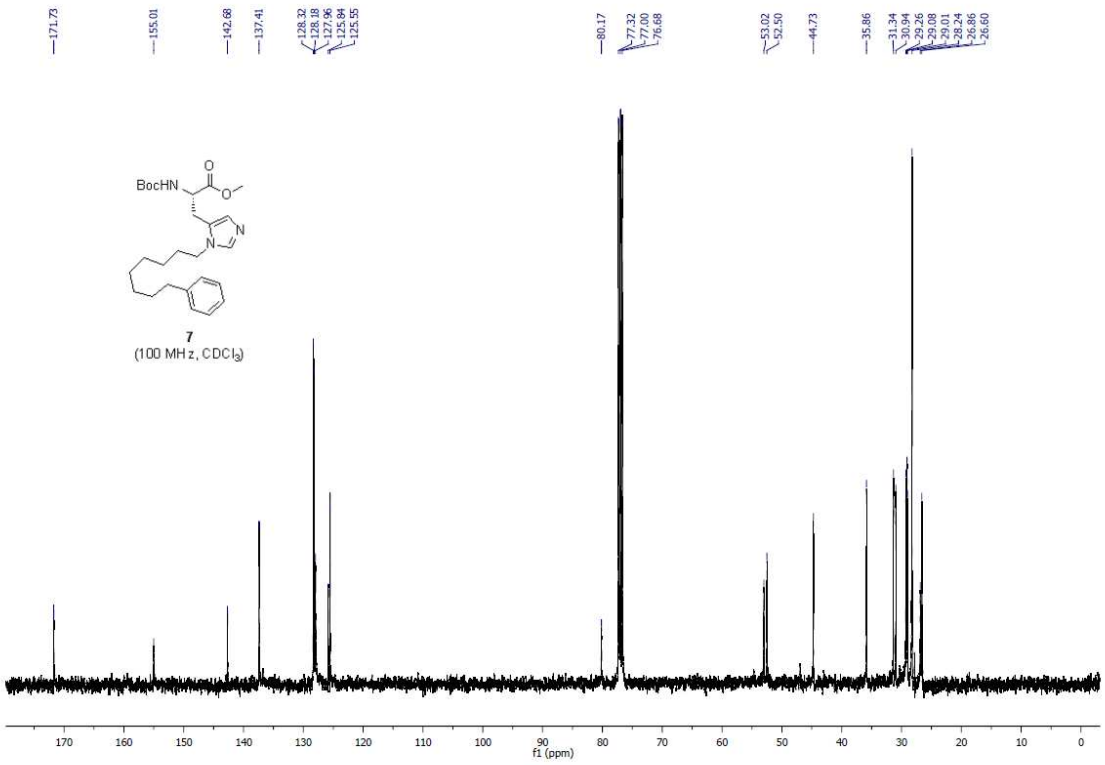
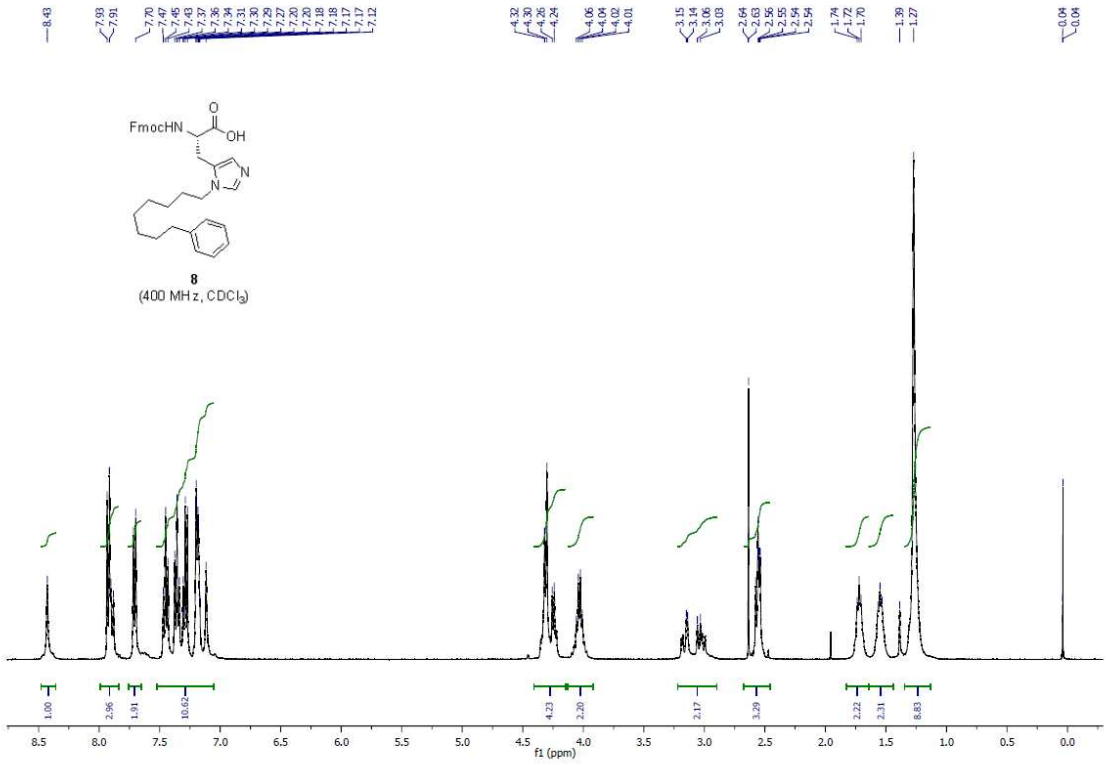
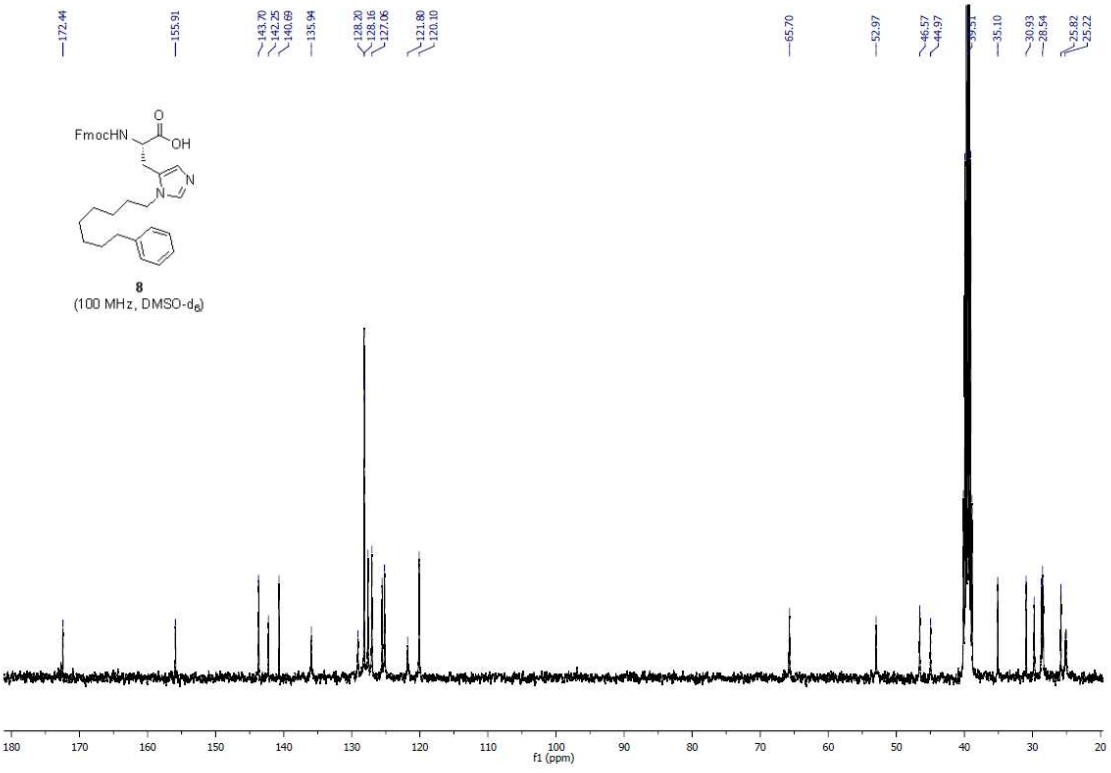
^{1}H and ^{13}C NMR spectra of 7





^{1}H and ^{13}C NMR spectra of 8





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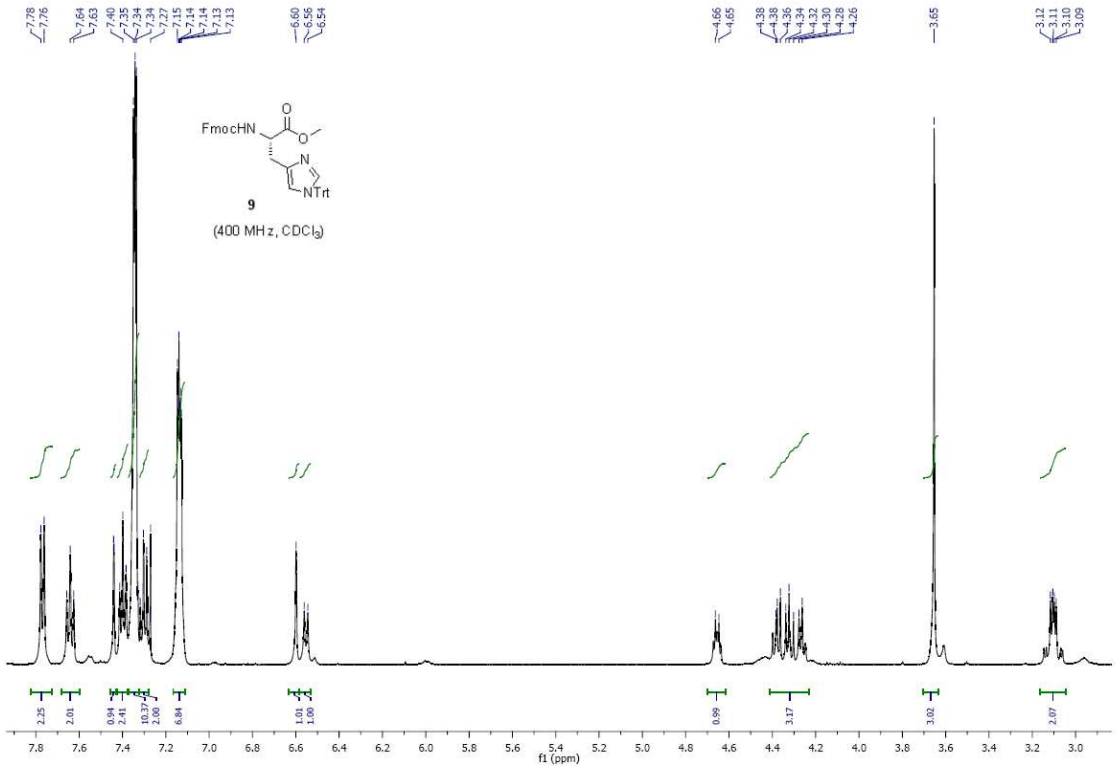
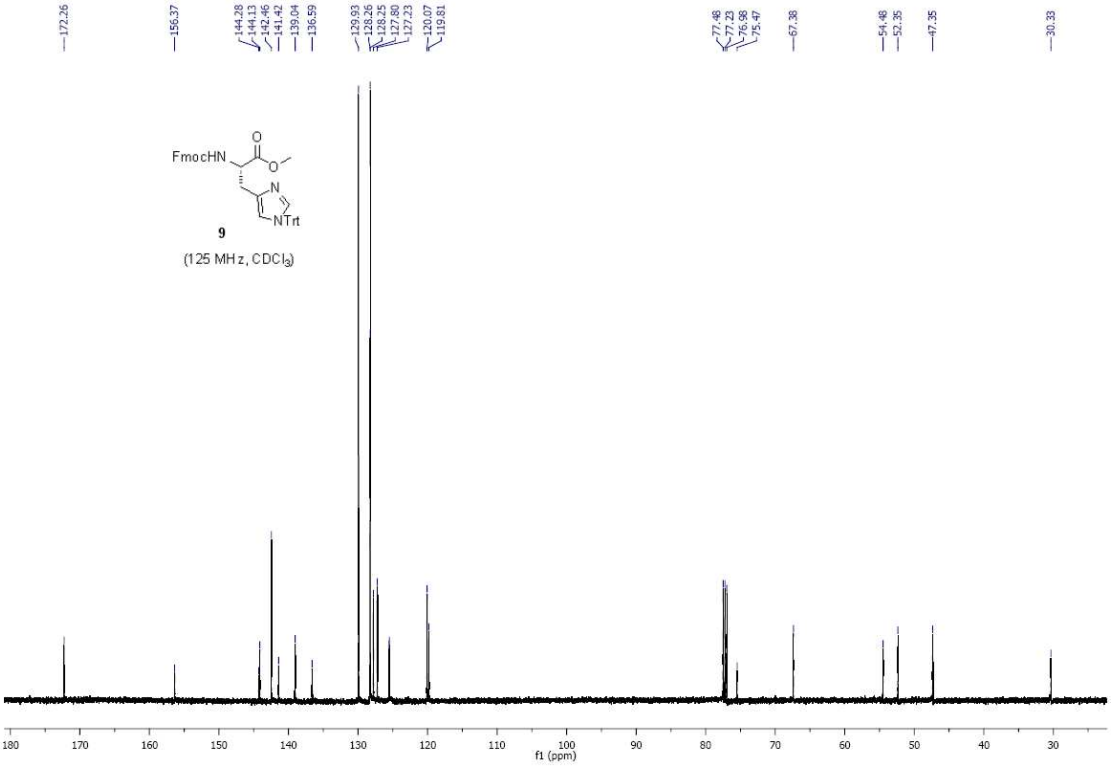
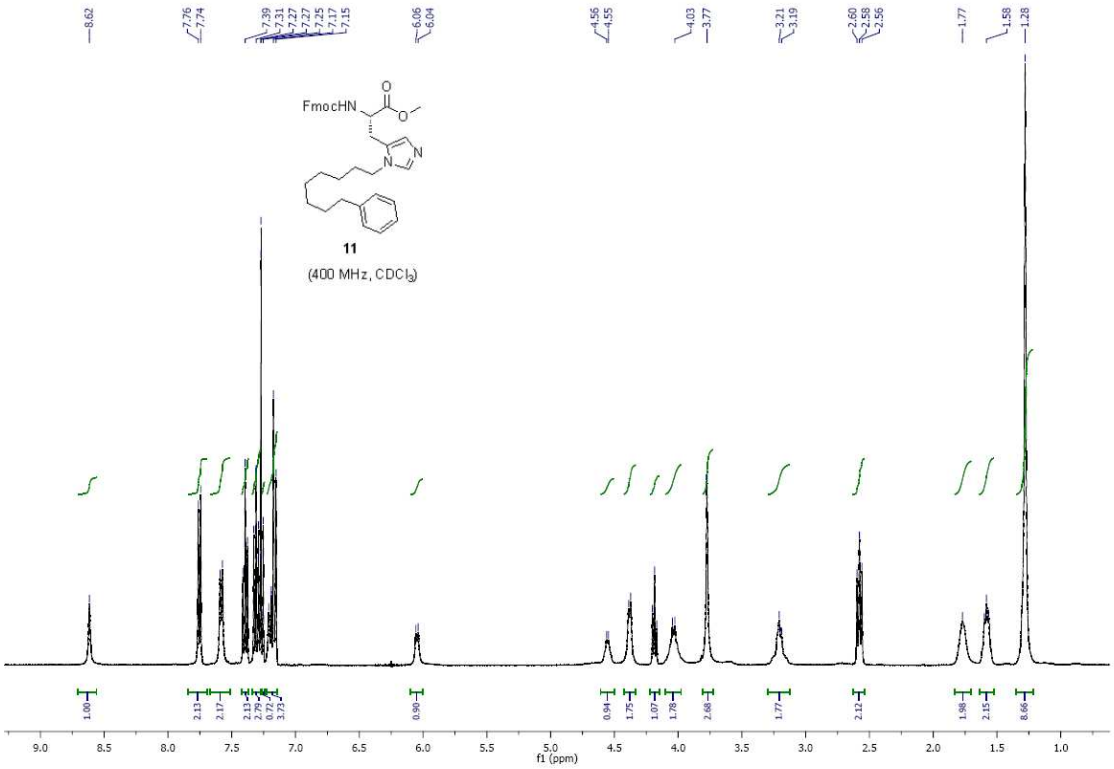
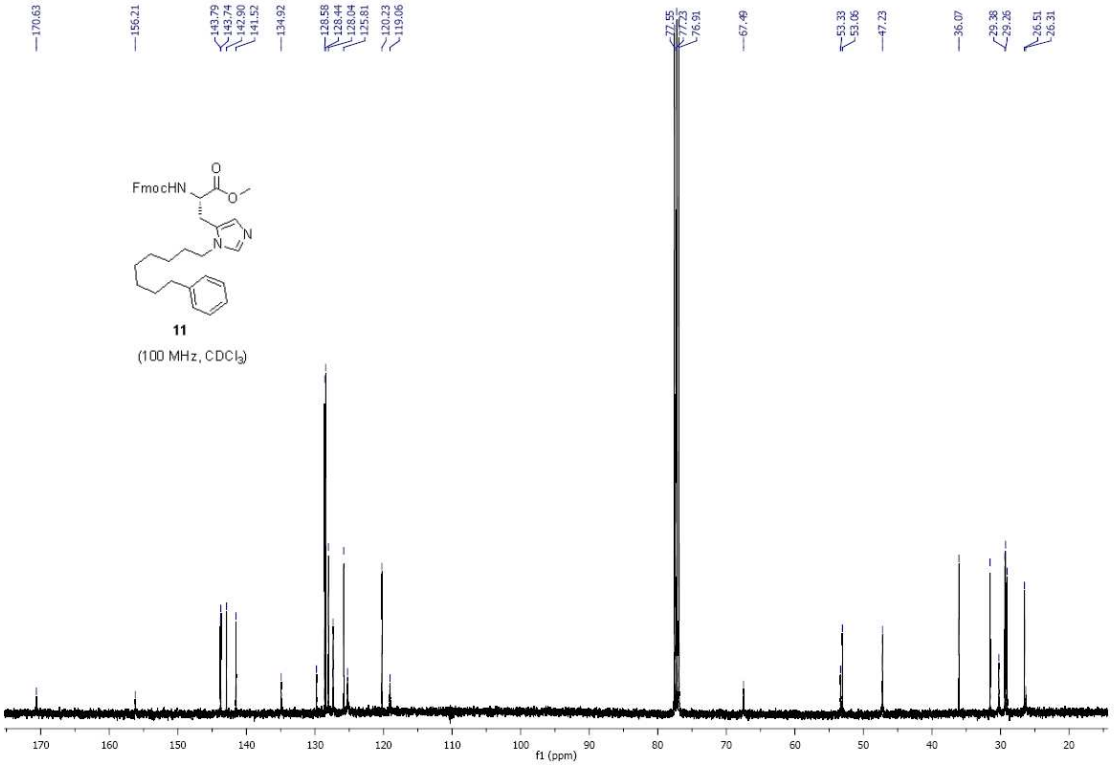


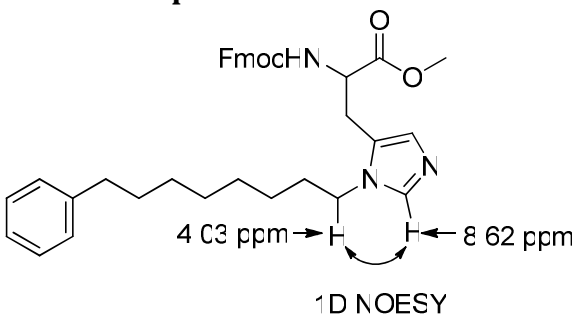


^{1}H and ^{13}C NMR spectra of 11



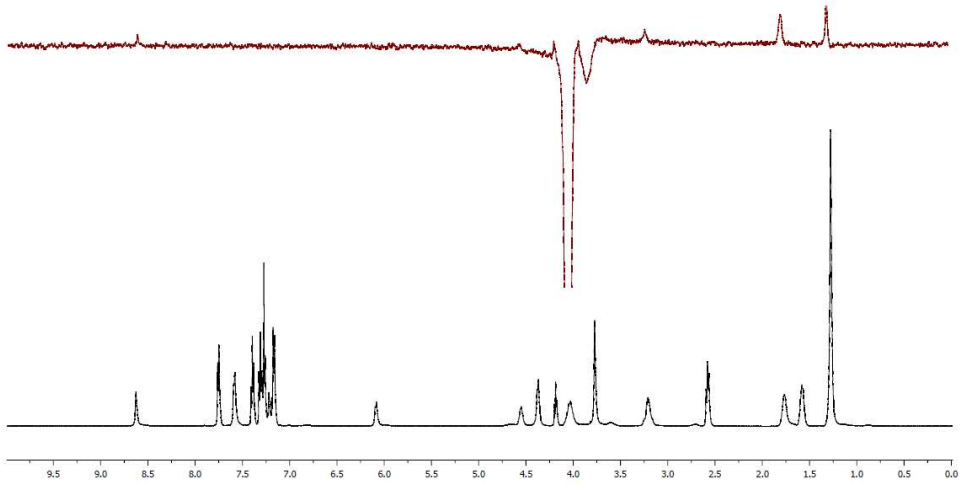


NOE of Compound 11

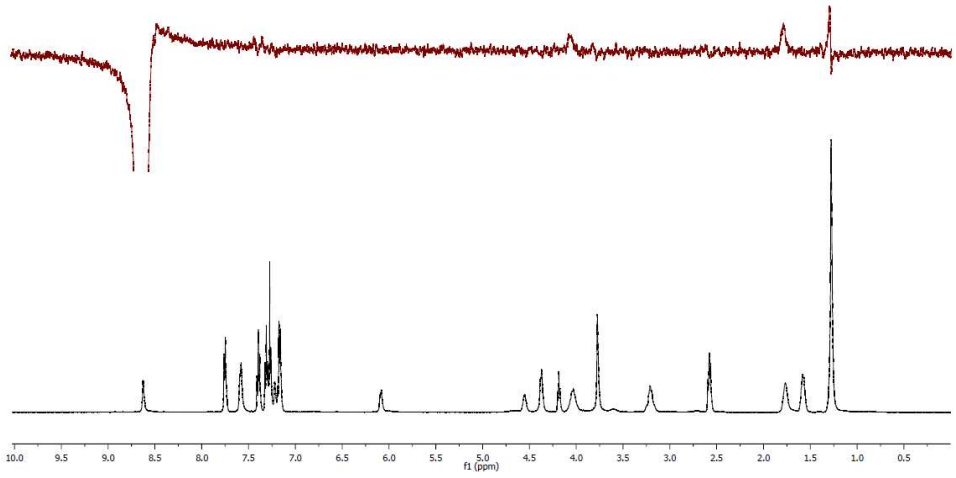


1D NOESY

500 MHz, relax. Delay 1.0 sec, mixing time 500 ms
Selective band center: 4.03 ppm



Selective band center: 8.62 ppm



HPLC Charts for Peptide 2, 3, 4, 5, 13

Table 1. MS and HPLC retention time

No	**Expect $(M + H)^+$**	**Observed $(M + H)^+$**	**Retention time/ min**	**HPLC method[a]**
2	863.4	863.4	25.2	II
3	863.4	863.4	24.3	II
4	1051.6	1051.5	29.8	I
5	1037.6	1037.4	29.2	I
13	861.5	861.4	23.3	I

[a] HPLC Method:

λ= 220 nm, flow rate: 10 mL/min

Solvent A: H_2O, 0.1% TFA; Solvent B: MeCN, 0.1% TFA

I:

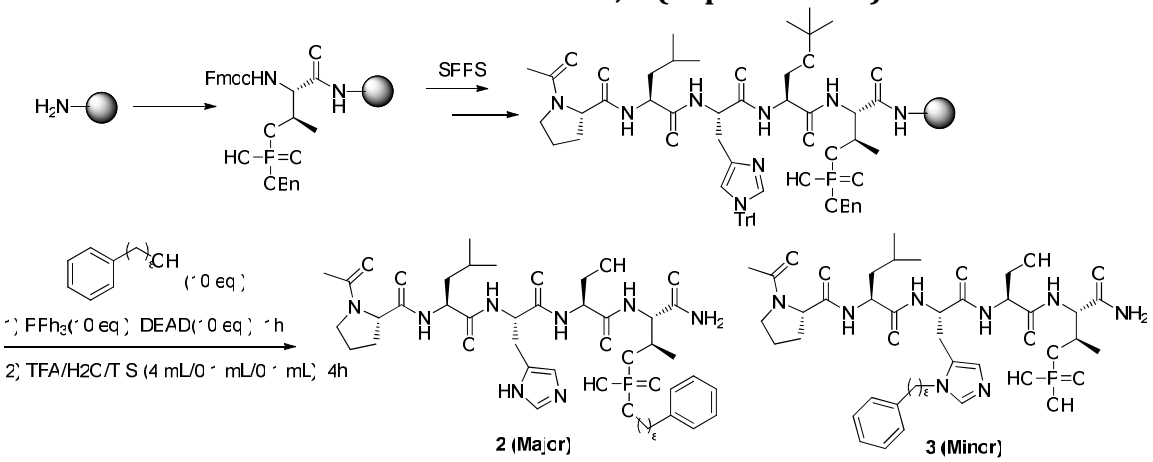
Time	0	30	30.1	35
B%	0	100	100	100

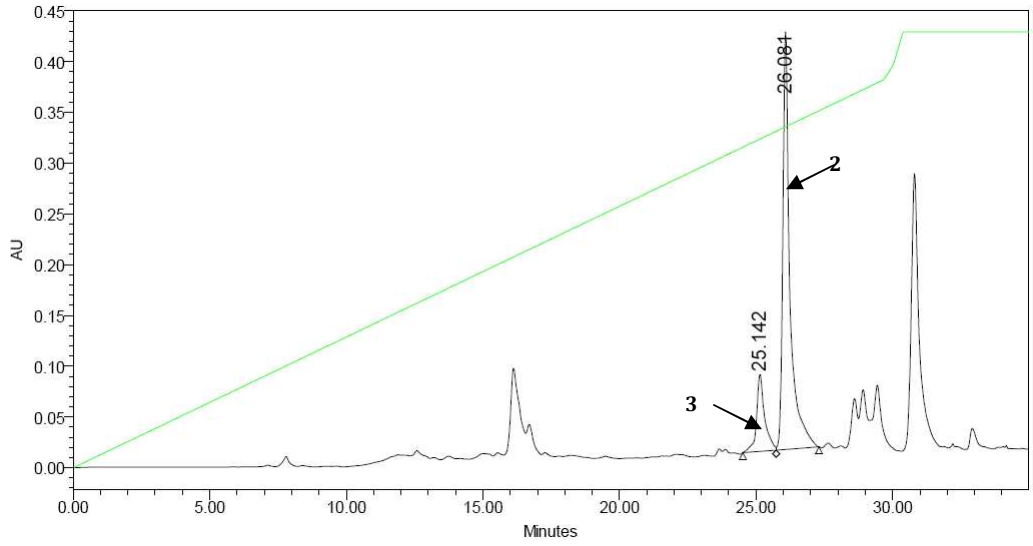
II:

Time	0	30	30.1	35
B%	0	90	100	100

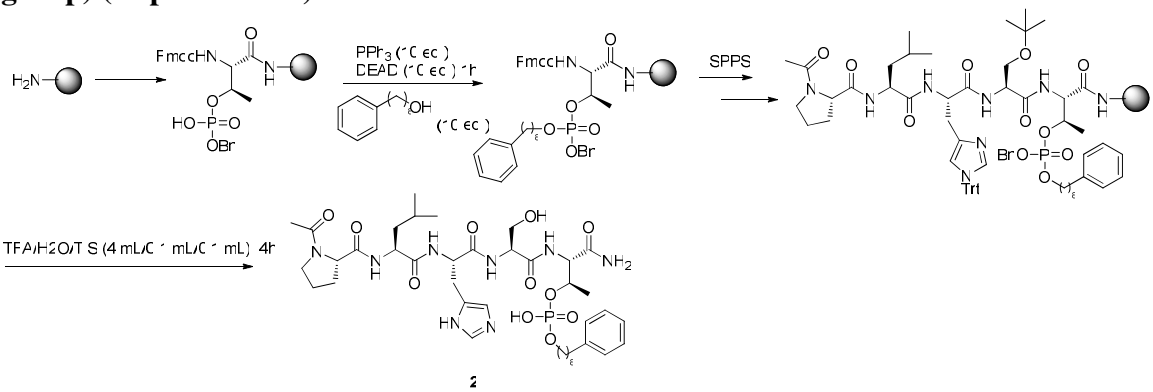
HPLC spectra:

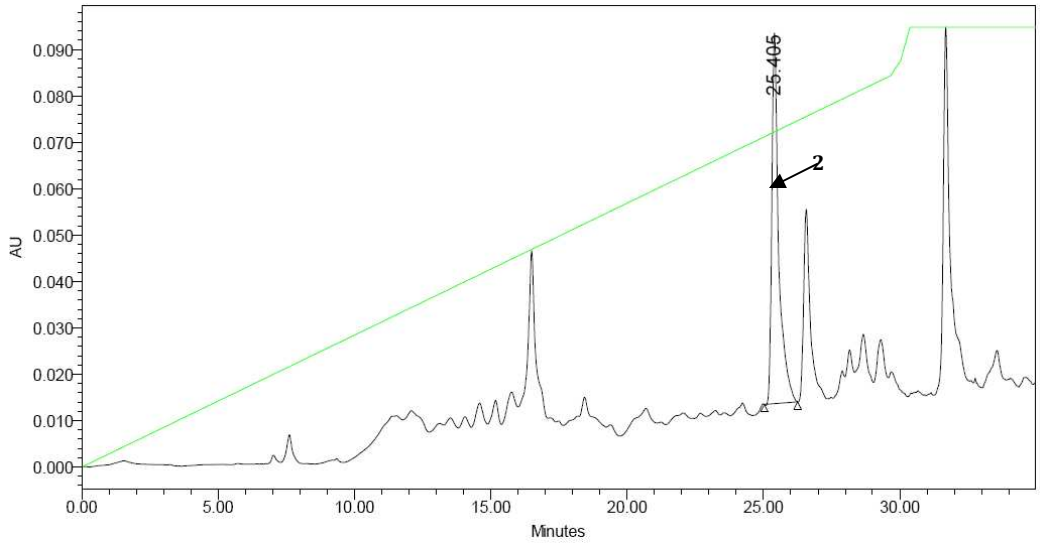
1. on-resin mistunobu reaction to afford 2, 3 (Experiment A)



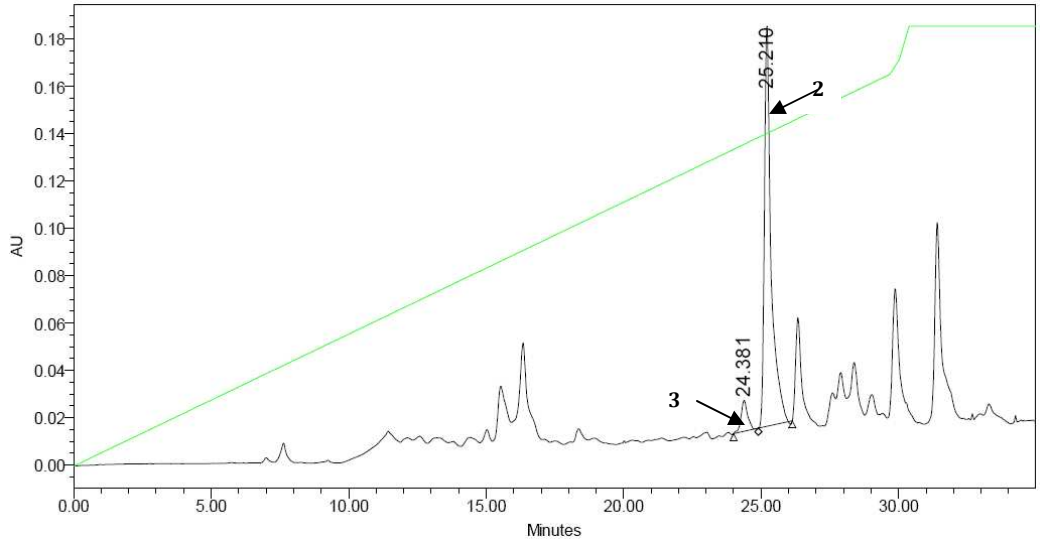


2. Test of Mechanism I (Intramolecular alkyl transfer of the phosphoryl ester group) (Experiment B)



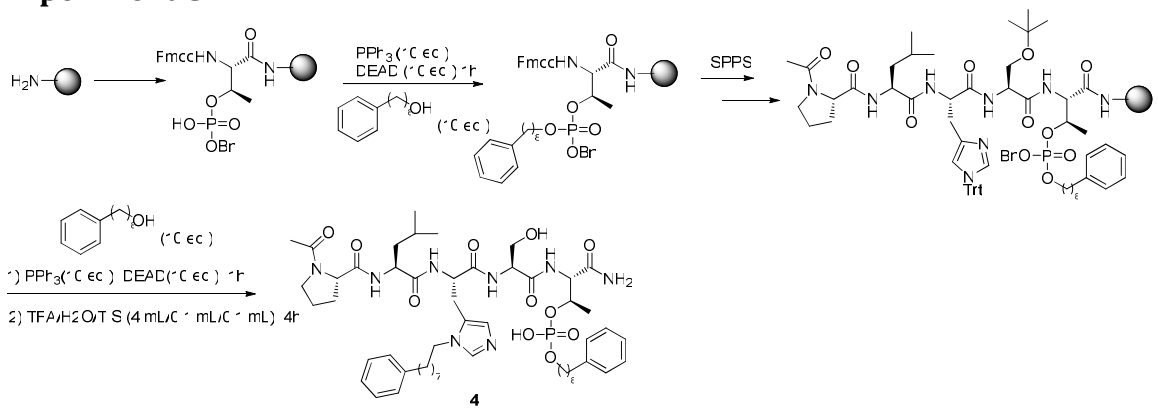


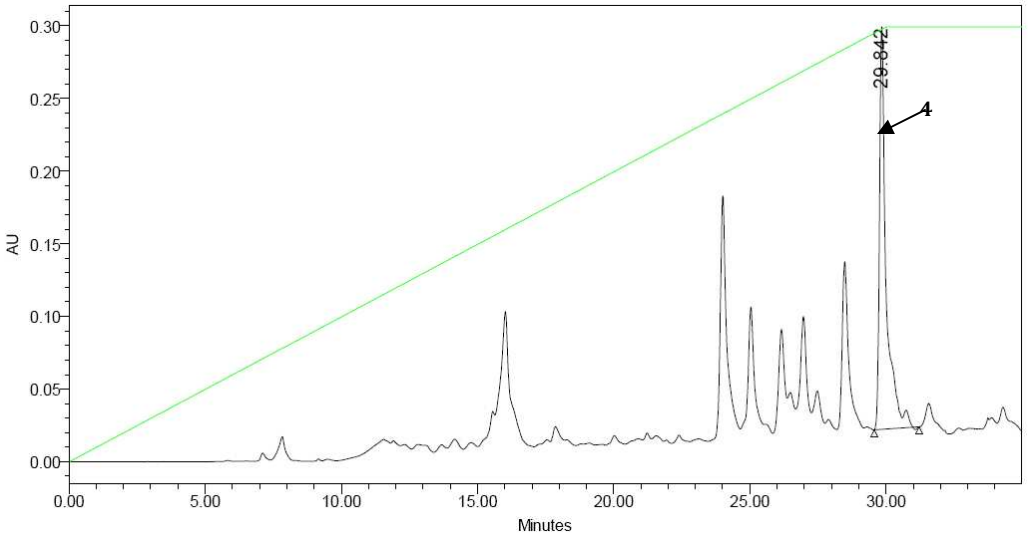
3. Mixture Test (Experiment A mixed with Experiment B)



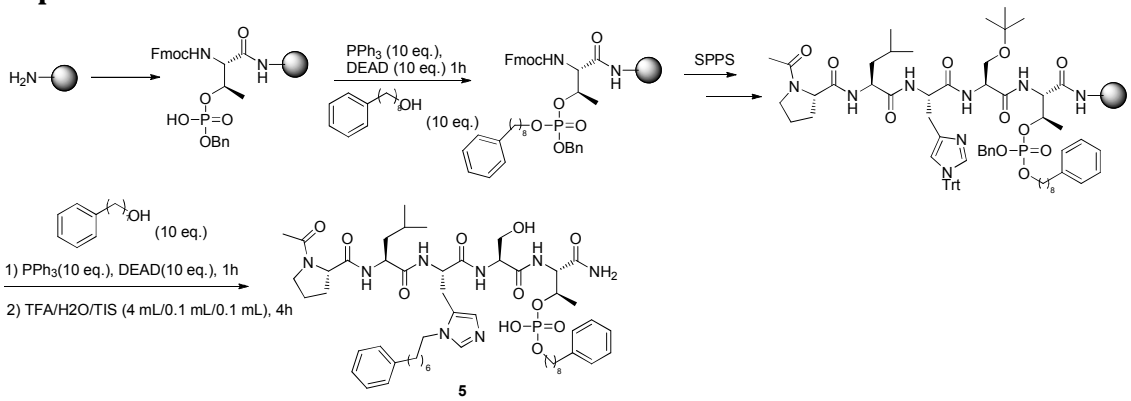
4. Test of Mechanism II (Experiment C and D)

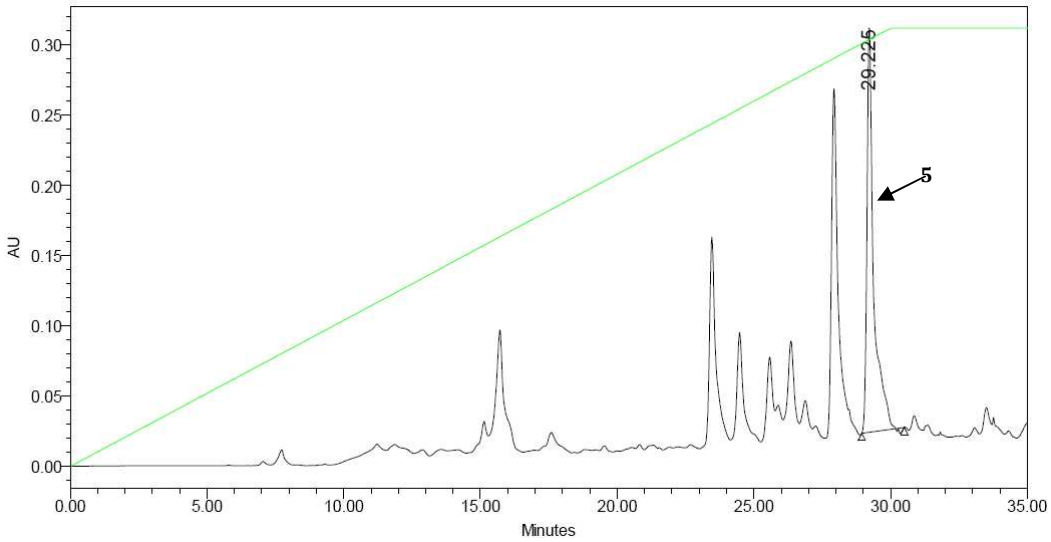
Experiment C:



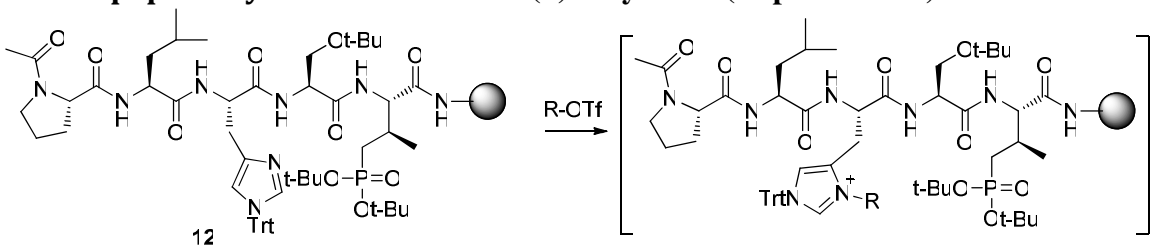


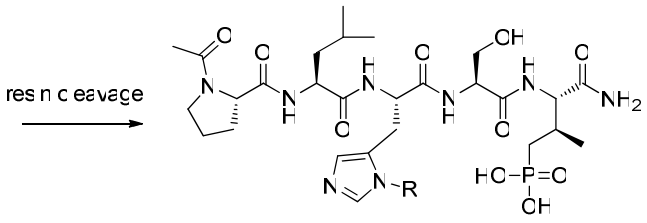
Experiment D:



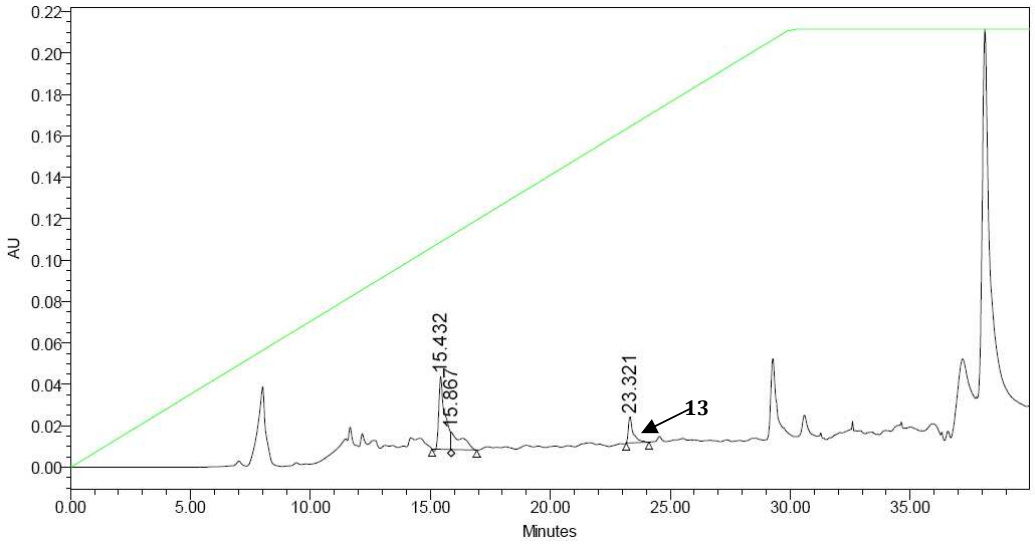


5. Post-peptide synthesis on-resin N(π)-alkylation (Experiment E)





13 R = $Ph(CH_2)_8$ -



Purity of Peptide 2, 3, 4, 5, 13

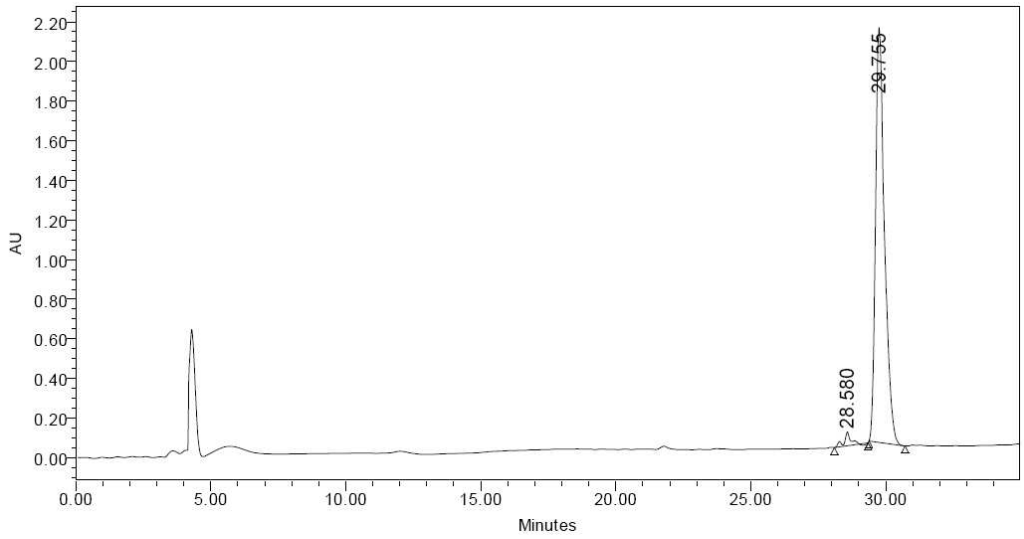
Table 2. Purity of Peptides

No	2	3	4	5	13
Purity	**97%**	**96%**	**98%**	**98%**	**100%**

HPLC Method:
λ= 220 nm, flow rate: 1.0 mL/min
Solvent A: H_2O, 0.1% TFA; Solvent B: MeCN, 0.1% TFA

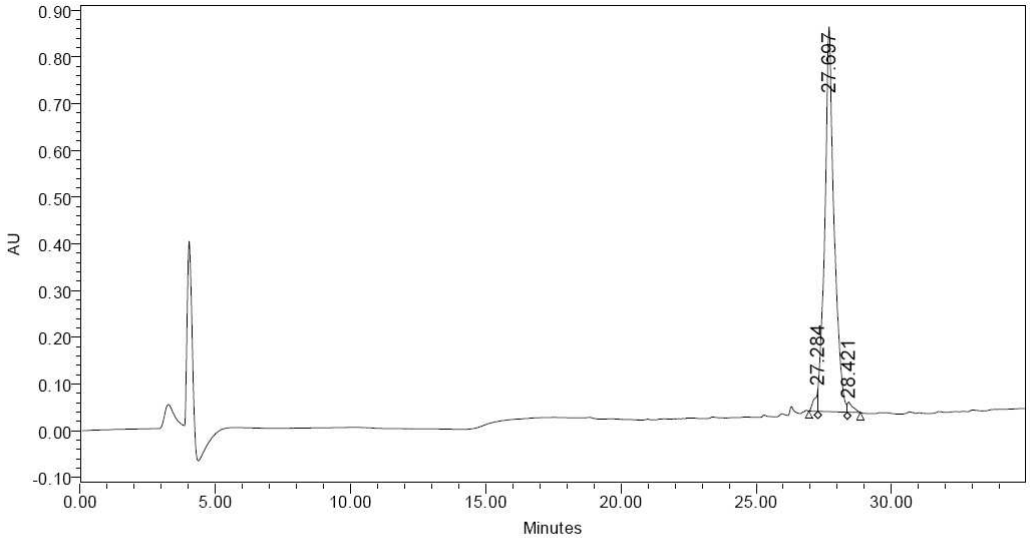
Time	0	30	30.1	35
B%	0	100	100	100

Peptide 2:



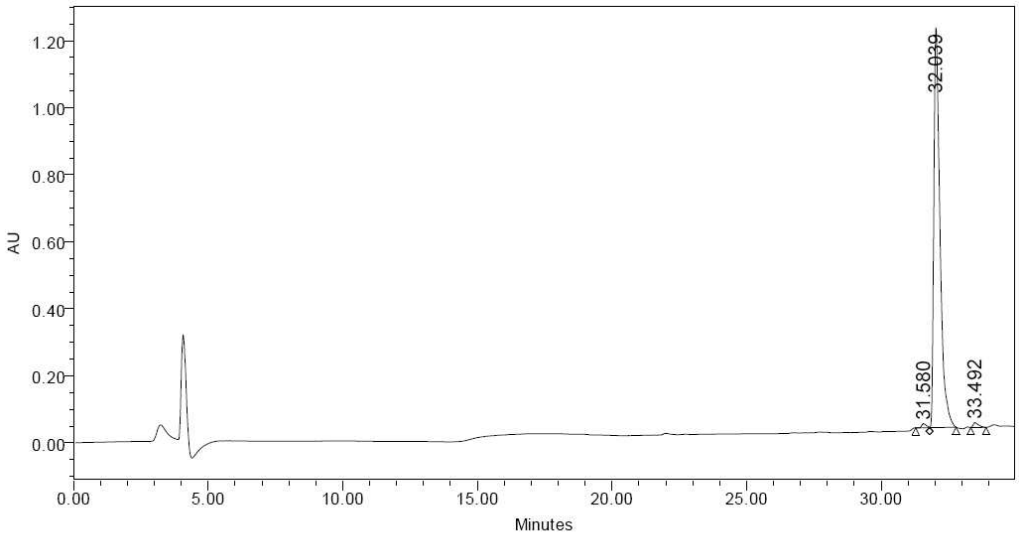
	RT	Area	% Area	Height
1	28.580	1298495	2.62	69683
2	29.755	48329081	97.38	2088105

Peptide 3



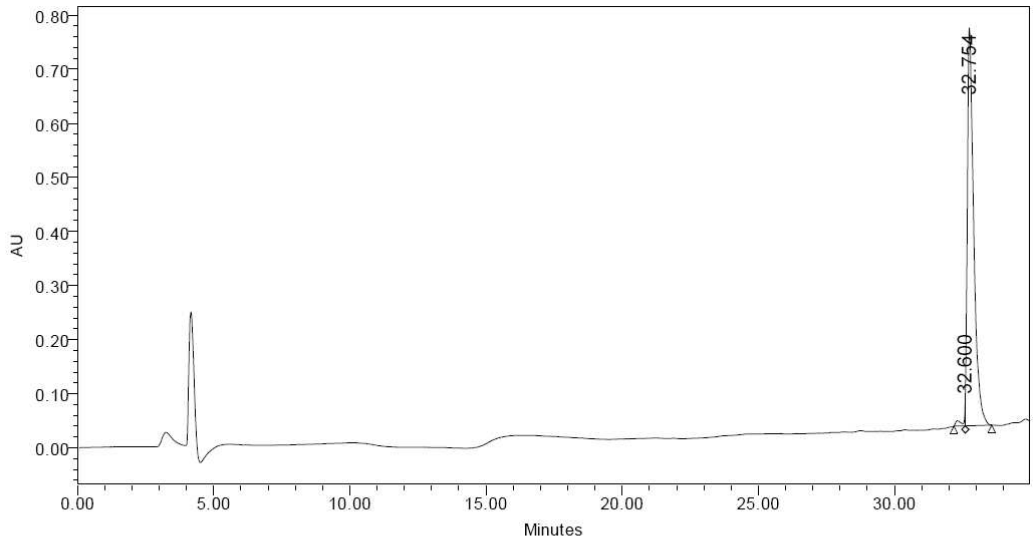
	RT	Area	% Area	Height
1	27.284	403588	1.98	47621
2	27.697	19721340	96.58	823302
3	28.421	293918	1.44	21107

Peptide 4



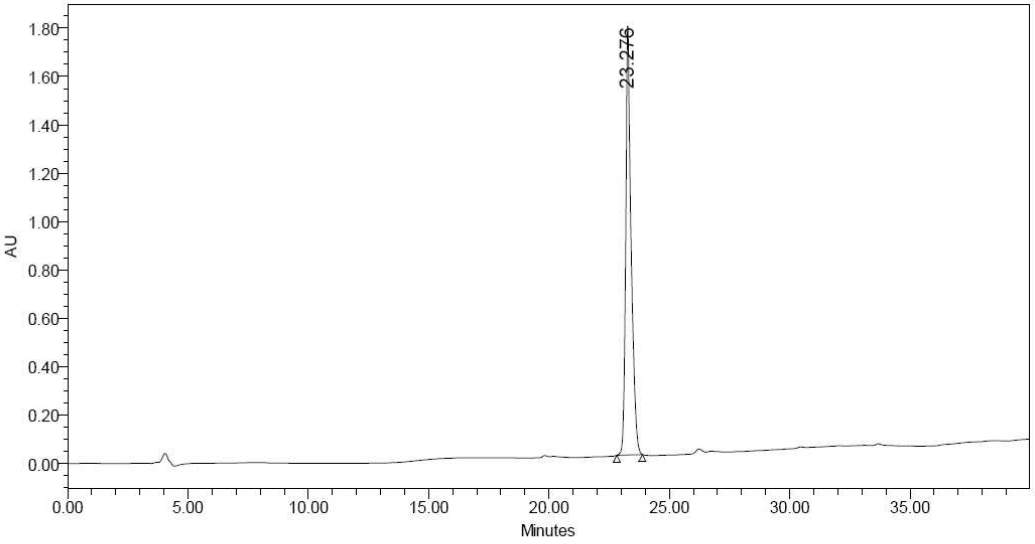
	RT	Area	% Area	Height
1	31.580	149603	0.78	11971
2	32.039	18959805	98.22	1195612
3	33.492	193813	1.00	14291

Peptide 5



	RT	Area	% Area	Height
1	32.600	201622	1.66	51715
2	32.754	11917745	98.34	736127

Peptide 13



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