Supporting Information

carba-Nucleopeptides (*c*NPs): A Novel Biopharmaceutical Modality Through Aqueous Rhodamine B Photoredox Catalysis

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General Information

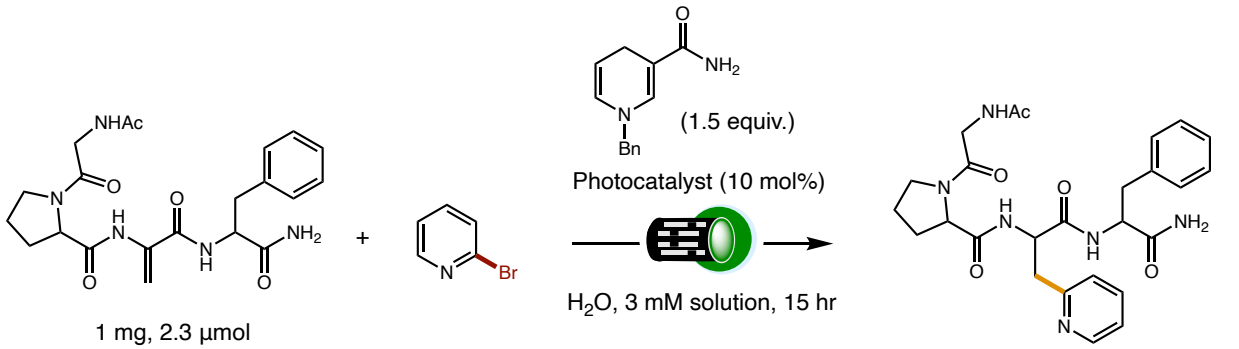
Unless stated otherwise, all reactions were performed under an atmosphere of N_2 with magnetic stirring. Materials received from commercial suppliers were used directly without further purification. Bromides were purchased from Sigma-Aldrich and Combi-Blocks. Fmoc-protected amino acids were purchased from Chem-Impex. The organic solvents (reagent grade) used for reaction optimization and purification were purchased from Sigma Aldrich, Fisher Scientific, and Oakwood Chemicals. Thin-layer chromatography (TLC) was performed on silica gel coated aluminum TLC plates (Merck, TLC Silica gel 60 F_{254}) and visualized using a UV lamp (254 nm) in combination with KMnO4 stains. All reactions were performed with Blue LED lights (PR160L, 440 nm, 40 W) or Green LED lights (PR160L, 525 nm, 40 W) from Kessil while stirring on a Corning PC-620D stir plate at 1150 rpm. The products were purified by flash column chromatography on an automated Buchi Pure C-815 Flash instrument or on a Isolera 1 Biotage® instrument. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Ascend™ 400 NMR spectrometer. ^{1}H NMR and ^{13}C NMR spectra were referenced to $CDCl_3$ (7.26 ppm and 77.16 ppm), CD_3CN (1.94 ppm), or CD_3COD (3.31 ppm). Peak multiplicities are designated by the following abbreviations: s, singlet; d, doublet; dd, doublet of doublets; ddd, doublet of doublets of doublets; dddd, doublet of doublets of doublets of doublets; ddt, doublet of doublet of triplets; dtd, doublet of triplet of doublets; t, triplet; and m, multiplet. NMR Spectra were recorded at 298 K. The small molecule mass characterization was acquired on a LCT Premier (Waters Corp.) time of flight mass spectrometer (HRMS) with an electrospray ion source. LC chromatograms and mass characterizations for peptides were performed on a Waters Acquity UPLC H-Class with a Waters QDa Mass Detector. MS/MS data was acquired on a Quatro Ultima (Waters Corp.) triple quadrupole mass analyzer with an electrospray ion source. All LC chromatograms were recorded at 214 nm unless otherwise specified.

Reaction Optimization

Effect of photocatalyst identity on the reaction

Procedure: To a 1 mL clear glass shell vial (purchased from Analytical Sales & Services Inc.) charged with a small stir bar, Ac-Gly-Pro-Dha-Phe-NH_2 (1 mg, 2.3 μmol, 1 equiv.), 2-bromopyridine (1 μL, 11.7 μmol, 5 equiv.), photocatalyst (0.23 μmol, 0.1 equiv.), and ddH_2O (577 μL, N_2 sparged for 5 minutes) were added. 1-benzyl-3-carbamoylpyridinium bromide was dissolved in 200 μL of ddH_2O, and sodium cyanoborohydride was added until the solution turned yellow. The reduced nicotinamide was then added to the vial. The vial was purged under N_2 for 3 minutes, parafilmed, and placed 7 cm away from two Blue or Green LED lights with stirring at 1150 rpm on the Corning PC-620D stir plate (under fan cooling) for fifteen hours. After that time, the sample was filtered and subjected to UPLC/MS analysis.

Table S1. Effect of photocatalyst identity on the reaction



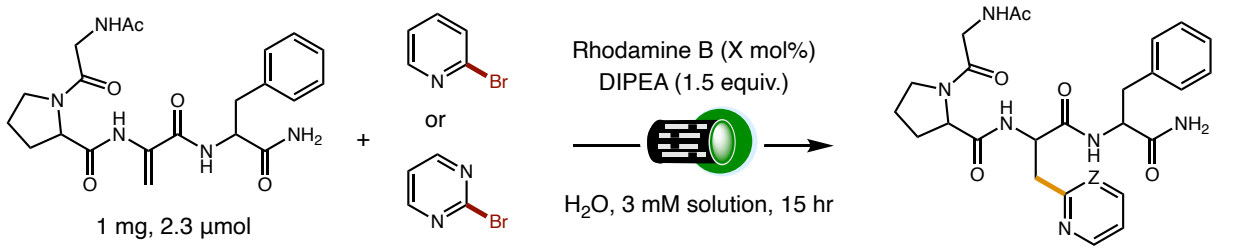
Entry:	Photocatalyst Identity:	Light Source:	Conversion[a]
1	$Ru(dtbby)_3$	Blue 440 nm	0%
2	$Ru(bpz)_3PF_6$	Blue 440 nm	<1%
3	Fluorescein	Blue 440 nm	4%
4	Fluorescein	Green 525 nm	0%
5	Rose Bengel	Green 525 nm	<1%
6	Eosin Y	Green 525 nm	0%
7	Eosin B	Green 525 nm	0%
8	$Cu(dap)_2Cl$	Green 525 nm	0%
9	Erythrosine	Green 525 nm	<1%
10	Methylene Blue	Green 525 nm	0%
11	Carminic acid	Green 525 nm	0%
12	**Rhodamine B**	**Green 525 nm**	**21%**
13	Rhodamine 6G	Green 525 nm	0%
14	**Rhodamine B + DIPEA (no Nicotinamide)**	**Green 525 nm**	**30%**

[a] % Conversion determined by integrating the % area of product peaks vs. Dha peptide peaks using LC.

Effect of catalyst loading on the reaction

Procedure: To a 1 mL clear glass shell vial (purchased from Analytical Sales & Services Inc.) charged with a small stir bar, Ac-Gly-Pro-Dha-Phe-NH_2 (1 mg, 2.3 µmol, 1 equiv.), 2-bromopyridine (1 µL, 11.7 µmol, 5 equiv.) or 2-bromopyrimidine (2 mg, 11.7 µmol, 5 equiv.), rhodamine B, diisopropylethylamine (0.6 µL, 3.5 µmol, 1.5 equiv.), and ddH_2O (777 µL total, N_2 sparged for 5 minutes) were added. The vial was purged under N_2 for 3 minutes, parafilmed, and placed 7 cm away from two Green LED lights with stirring at 1150 rpm on the Corning PC-620D stir plate (under fan cooling) for fifteen hours. After that time, the sample was filtered and subjected to UPLC/MS analysis.

Table S2. Effect of catalyst loading on the reaction



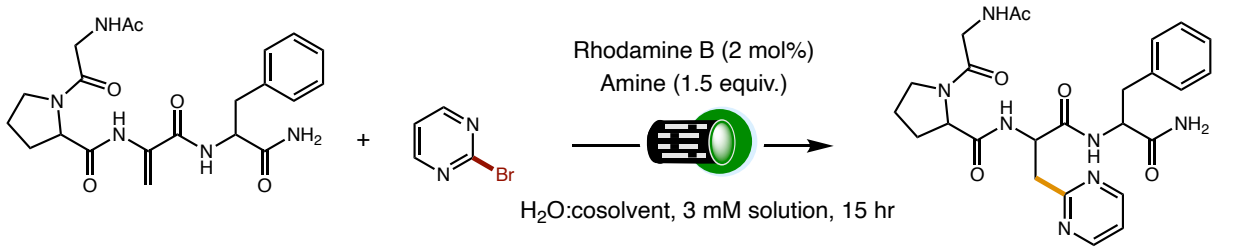
Entry:	mol% Pcat:	Bromoheterocycle:	Conversion[a]
1	10	2-bromopyridine	30%
2	5	2-bromopyridine	23%
3	3	2-bromopyridine	31%
4	**2**	**2-bromopyridine**	**44%**
5	1	2-bromopyridine	20%
6	0.5	2-bromopyridine	<17%
7	**2**	**2-bromopyrimidine**	**52%**
8	0.5	2-bromopyrimidine	46%

[a] % Conversion determined by integrating the % area of product peaks vs. Dha peptide peaks using LC.

Effect of amine identity on the reaction

Procedure: To a 1 mL clear glass shell vial (purchased from Analytical Sales & Services Inc.) charged with a small stir bar, Ac-Gly-Pro-Dha-Phe-NH_2 (1 mg, 2.3 µmol, 1 equiv.), 2-bromopyrimidine (2 mg, 11.7 µmol, 5 equiv.), 40 µL of rhodamine B from a 1.16 mM stock solution in ddH_2O (22 µg, 0.047 µmol, 2 mol%), amine (3.5 µmol, 1.5 equiv.), and ddH_2O (777 µL total, N_2 sparged for 5 minutes) were added. The vial was purged under N_2 for 3 minutes, parafilmed, and placed 7 cm away from two Green LED lights with stirring at 1150 rpm on the Corning PC-620D stir plate (under fan cooling) for fifteen hours. After that time, the sample was filtered and subjected to UPLC/MS analysis.

Table S3. Effect of amine identity on the reaction



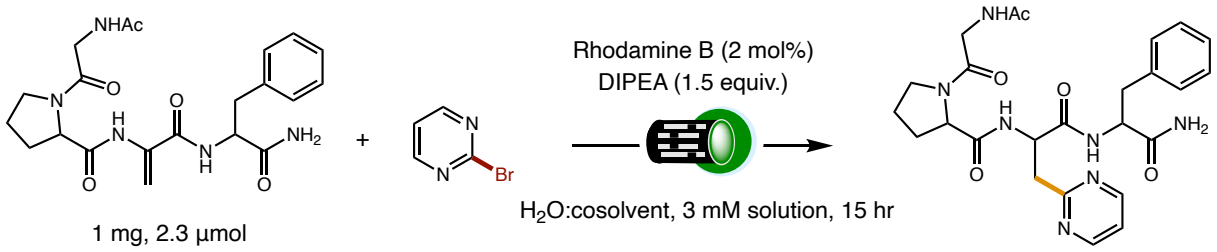
Entry:	Amine Identity:	Conversion[a]
1	Tributylamine	<1%
2	Triisobutylamine	22%
3	Triphenylamine	<1%
4	Piperidine	5%
5	4-Methylmorpholine	11%
6	N,N-Dimethylglycine	2%
7	4-Dimethylaminopyridine	<1%
8	1,4-Diazabicyclo[2.2.2]octane	2%
9	N,N,N',N'-Tetramethylenediamine	33%
10	**Diisopropylethylamine**	**52%**

[a] % Conversion determined by integrating the % area of product peaks vs. Dha peptide peaks using LC.

Effect of cosolvent identity and ratio on the reaction

Procedure: To a 1 mL clear glass shell vial (purchased from Analytical Sales & Services Inc.) charged with a small stir bar, Ac-Gly-Pro-Dha-Phe-NH_2 (1 mg, 2.3 μmol, 1 equiv.), 2-bromopyrimidine (2 mg, 11.7 μmol, 5 equiv.), 40 μL of rhodamine B from a 1.16 mM stock solution in ddH_2O (22 μg, 0.047 μmol, 2 mol%), diisopropylethylamine (0.6 μL, 3.5 μmol, 1.5 equiv.), cosolvent, and ddH_2O (N_2 sparged for 5 minutes) were added. The vial was purged under N_2 for 3 minutes, parafilmed, and placed 7 cm away from two Green LED lights with stirring at 1150 rpm on the Corning PC-620D stir plate (under fan cooling) for fifteen hours. After that time, the sample was filtered and subjected to UPLC/MS analysis.

Table S4. Effect of cosolvent identity and ratio on the reaction



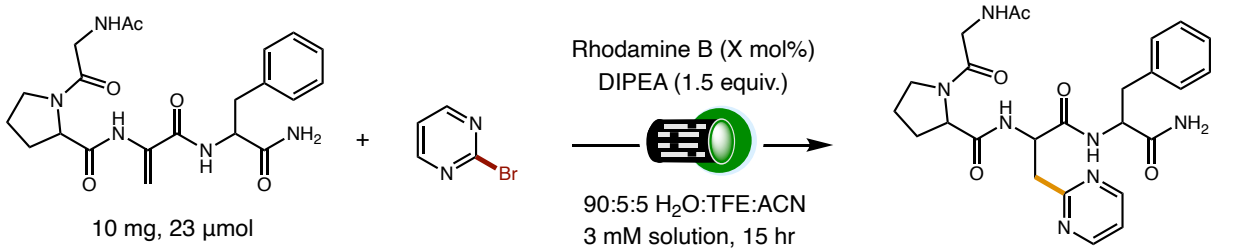
Entry:	Cosolvent Identity:	Ratio of H_2O:Cosolvent:	Conversion[a]
1	Dimethyl sulfoxide	95:5	28%
2	N,N-Dimethylformamide	95:5	40%
3	Methanol	95:5	40%
4	Ethanol	95:5	31%
5	Isopropanol	95:5	22%
6	tert-Butanol	95:5	47%
7	Methyl Acetate	95:5	5%
8	Glycerol	95:5	7%
9	1,1,1,3,3,3-Hexafluoro-2-propanol	95:5	2%
10	Acetonitrile	95:5	38%
11	2,2,2-Trifluoroethanol	95:5	80%
12	**2,2,2-Trifluoroethanol/ Acetonitrile**	**90:5:5**	**87%**

[a] % Conversion determined by integrating the % area of product peaks vs. Dha peptide peaks using LC.

Effect of scaling the reaction

Procedure: To a 2-dram vial charged with a stir bar, Ac-Gly-Pro-Dha-Phe-NH_2 (10 mg, 23.3 µmol, 1 equiv.), 2-bromopyrimidine (19 mg, 117 µmol, 5 equiv.), 400 µL or 100 µL of rhodamine B from a 1.16 mM stock solution in ddH_2O (0.22 mg, 0.47 µmol, 2 mol%) or (56 µg, 0.12 µmol, 0.5 mol%), diisopropylethylamine (6 µL, 35 µmol, 1.5 equiv.), 2,2,2-trifluoroethanol (389 µL), acetonitrile (389 µL), and ddH_2O (7 mL total, N_2 sparged for 5 minutes) were added. The vial was purged under N_2 for 5 minutes, parafilmed, and placed 1 cm away from two Green LED lights with stirring at 1150 rpm on the Corning PC-620D stir plate (under fan cooling) for fifteen hours. After that time, the sample was washed with dichloromethane twice and subjected to UPLC/MS analysis to determine % conversion. The products were then concentrated using a rotavapor, redissolved in minimal water, and purified by reverse phase flash chromatography (acetonitrile:H_2O) using a Biotage® Sfär C18 Duo column.

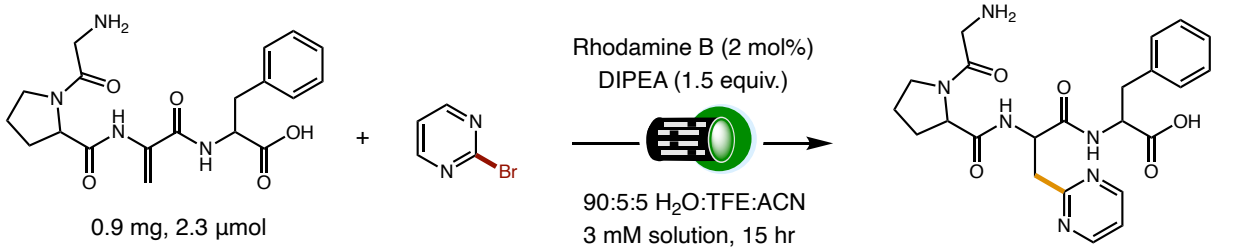
Table S5. Effect of scaling the reaction



Entry:	mol% Pcat:	Conversion[a]	Isolated Yield:
1	2	97%	67%
2	**0.5**	**>99%**	**77%**

[a] % Conversion determined by integrating the % area of product peaks vs. Dha peptide peaks using LC.

Effect of using NH_2-Gly-Pro-Dha-Phe-CO_2H as peptide

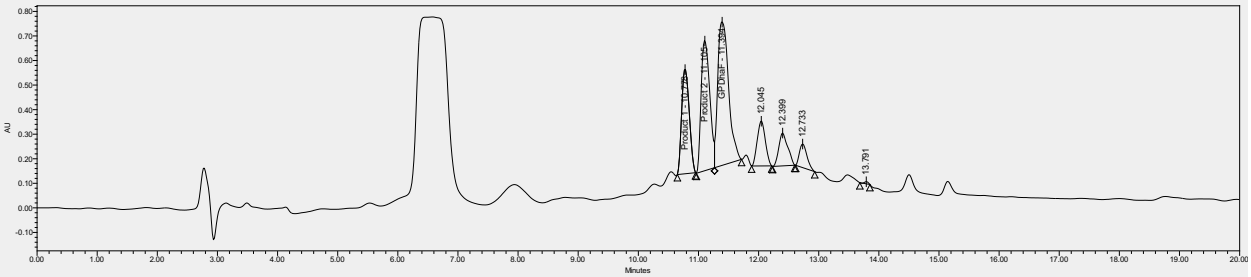


Procedure: To a 1 mL clear glass shell vial (purchased from Analytical Sales & Services Inc.) charged with a small stir bar, NH_2-Gly-Pro-Dha-Phe-CO_2H (0.9 mg, 2.3 µmol, 1 equiv.), 2-bromopyrimidine (2 mg, 11.7 µmol, 5 equiv.), 40 µL of rhodamine B from a 1.16 mM stock solution in ddH_2O (22 µg, 0.047 µmol, 2 mol%), diisopropylethylamine (0.6 µL, 3.5 µmol, 1.5 equiv.), 2,2,2-trifluoroethanol (39 µL), acetonitrile (39 µL), and ddH_2O (700 µL total, N_2 sparged for 5 minutes) were added. The vial was purged under N_2 for 3 minutes, parafilmed, and placed 7 cm away from two Green LED lights with stirring at 1150 rpm on the Corning PC-620D stir plate (under fan cooling) for fifteen hours. After that time, the sample was filtered and subjected to UPLC/MS analysis Supelco Discovery Bio Wide Pore C8 (3.0 µ, 4.6 mm x 10 cm) column using the method below (44% conversion).

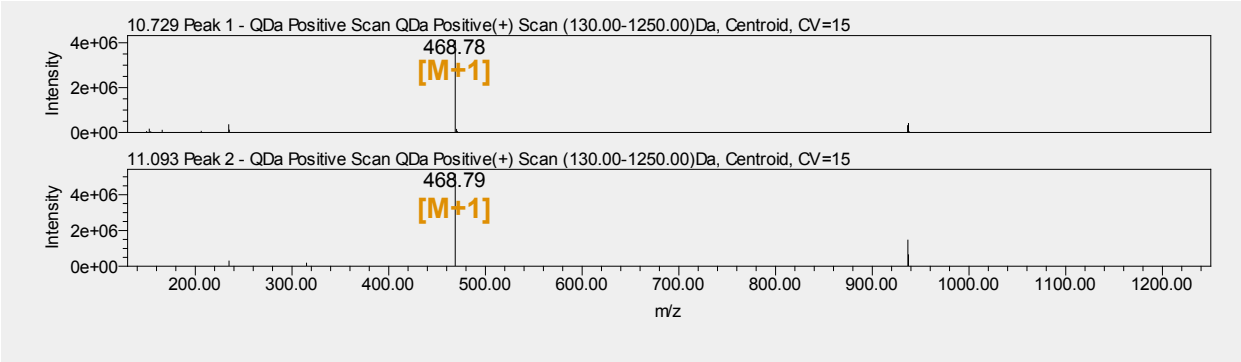
A = Acetonitrile with 1% formic acid modifier, B = ddH_2O with 1% formic acid modifier.

Time:	%A:	%B:
0.0	5.0	95.0
2.0	5.0	95.0
3.0	10.0	90.0
9.0	30.0	70.0
17.0	50.0	50.0

17.5	95.0	5.0
19.0	95.0	5.0
19.5	5.0	95.0
20.0	5.0	95.0



	Name	Retention Time	Area	% Area
1	Product 1	10.778	3747727	18.41
2	Product 2	11.105	5288231	25.98
3	GPDhaF	11.394	7352501	36.13
4		12.045	1702657	8.37
5		12.399	1375380	6.76
6		12.733	845225	4.15
7		13.791	40034	0.20



Experimental Procedures

Picture of reaction setups

A) B) C)

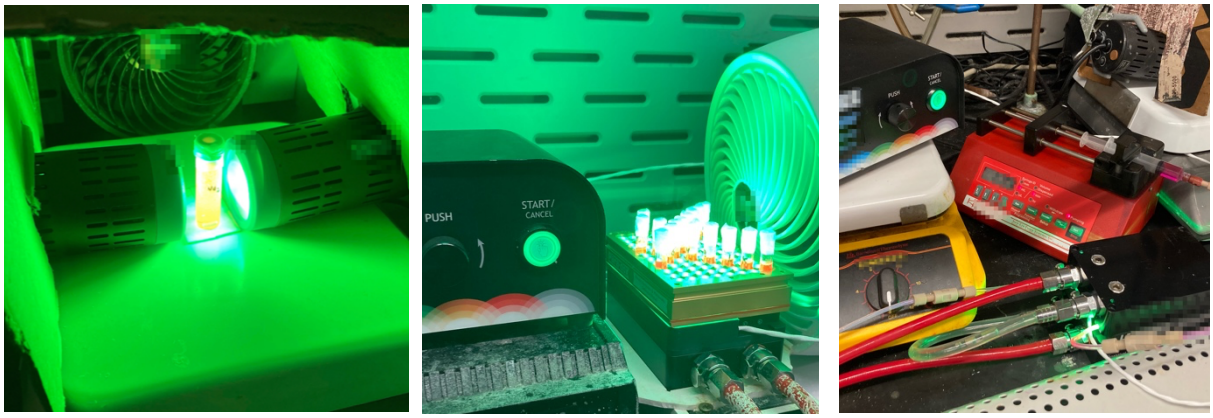


Figure S1. Reaction setup photographs. A) Batch synthesis. B) Parallel Synthesis. C) Flow Synthesis.

General procedure for the synthesis of pyrimidine sidechains

To a 2-dram vial charged with a stir bar, Ac-Gly-Pro-Dha-Phe-NH_2 (10 mg, 23.3 μmol, 1 equiv.), bromopyrimidine (117 μmol, 5 equiv.), 100 μL of rhodamine B from a 1.16 mM stock solution in ddH_2O (56 μg, 0.12 μmol, 0.5 mol%), diisopropylethylamine (6 μL, 35 μmol, 1.5 equiv.), 2,2,2-trifluoroethanol (389 μL), acetonitrile (389 μL), and ddH_2O (7 mL total, N_2 sparged for 5 minutes) were added. The vial was purged under N_2 for 5 minutes, parafilmed, and placed 1 cm away from two Green LED lights with stirring at 1150 rpm on the Corning PC-620D stir plate (under fan cooling) for fifteen hours. After that time, the sample was washed with dichloromethane twice and subjected to UPLC/MS analysis to determine % conversion (defined as the % area of the product peaks at 214 nm as compared to all other peptide peaks in the LC chromatogram). The products were then concentrated using a rotavapor, redissolved in minimal water, and purified by reverse phase flash chromatography (acetonitrile:H_2O) using a Biotage® Sfär C18 Duo column.

General procedure for the synthesis of purine sidechains

To a 2-dram vial charged with a stir bar, bromopurine (117 μmol, 5 equiv.), acetonitrile (389 μL), and up to 4 mL of ddH_2O were added. The bromopurine was then dissolved in the solution as much as possible using heat and sonication as necessary (Note: this pre-dissolving step is essential to the success of these reactions). After the vial has cooled, Ac-Gly-Pro-Dha-Phe-NH_2 (10 mg, 23.3 μmol, 1 equiv.), 100 μL of rhodamine B from a 1.16 mM stock solution in ddH_2O (56 μg, 0.12 μmol, 0.5 mol%), diisopropylethylamine (6 μL, 35 μmol, 1.5 equiv.), 2,2,2-trifluoroethanol (389 μL), and the rest of the ddH_2O (7 mL total, N_2 sparged for 5 minutes) were added. The vial was purged under N_2 for 5 minutes, parafilmed, and placed 1 cm away from two Green LED lights with stirring at 1150 rpm on the Corning PC-620D stir plate (under fan cooling) for fifteen hours. After that time, the sample was washed with dichloromethane twice and subjected to UPLC/MS analysis

to determine % conversion (defined as the % area of the product peaks at 214 nm as compared to all other peptide peaks in the LC chromatogram). The products were then concentrated using a rotavapor, redissolved in minimal water, and purified by reverse phase flash chromatography (acetonitrile:H_2O) using a Biotage® Sfär C18 Duo column.

Deviation 1: In some cases, the products partition into the organic layer. These reactions were washed with diethyl ether instead.

Deviation 2: In some cases, the Dha-peptide eluted with a similar retention time to the products (as indicated). These reactions were transferred to a 1-dram vial containing 20 mg of 2-mercaptoethylamine, polymer-bound resin and 10 mg of TCEP.[1] The vial was heated to 40 °C for two hours to remove the remaining Dha-peptide. The solution was then filtered and purified by reverse phase flash chromatography (acetonitrile:H_2O).

Synthesis of cysteine-containing peptides

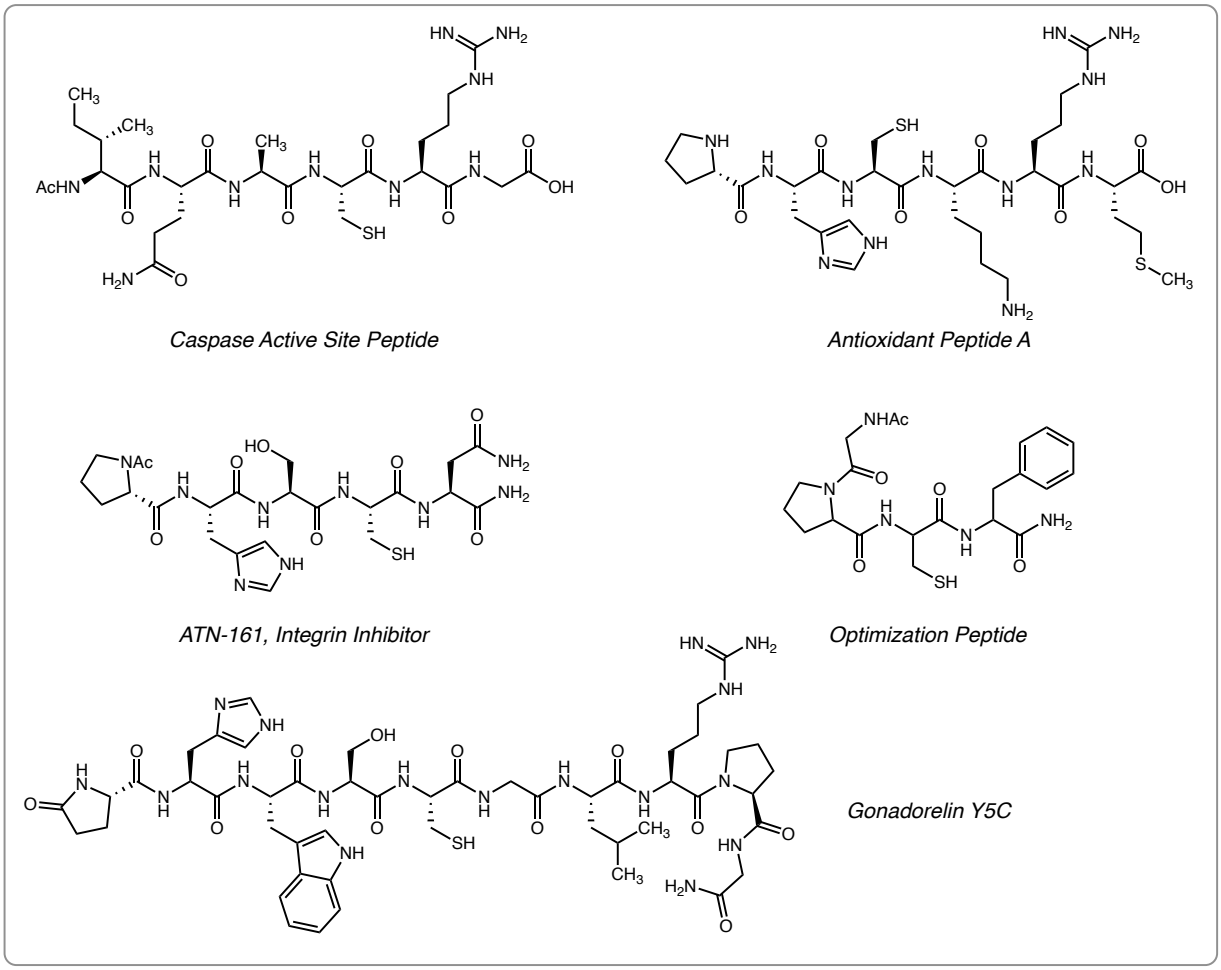


Figure S2. Cysteine-containing peptides synthesized.

All cysteine-containing peptides were synthesized by solid phase peptide synthesis using Fmoc-protected amino acids on rink amide resin or 2-chlorotrityl chloride resin, cleaved with 95:2.5:2.5 TFA:TIPS:H_2O solution, and purified by reverse phase flash chromatography. All peptide products were analyzed on a Supelco Discovery Bio Wide Pore C8 (3.0 μ, 4.6 mm x 10 cm) column using one of the two methods below.

A = Acetonitrile with 1% formic acid modifier, B = ddH_2O with 1% formic acid modifier.

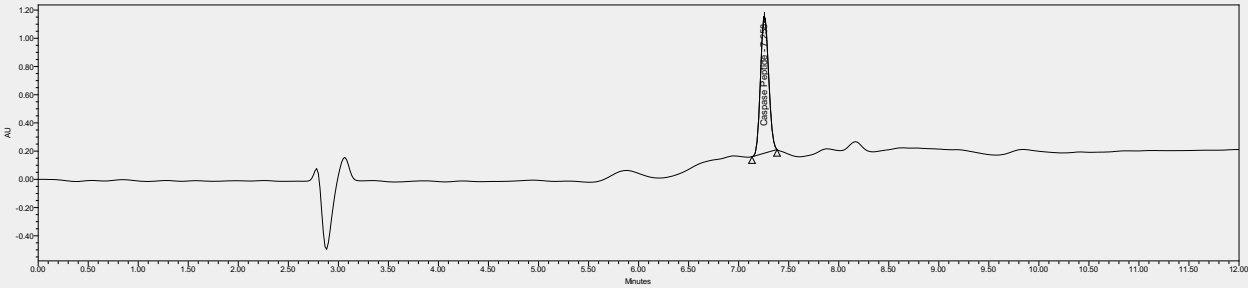
ATN-161 and Antioxidant Peptide A:
0.4 mL/min flow rate

Time:	%A:	%B:
0.0	0.0	100.0
1.0	0.0	100.0
2.0	5.0	95.0
10.5	95.0	5.0
12.0	95.0	5.0

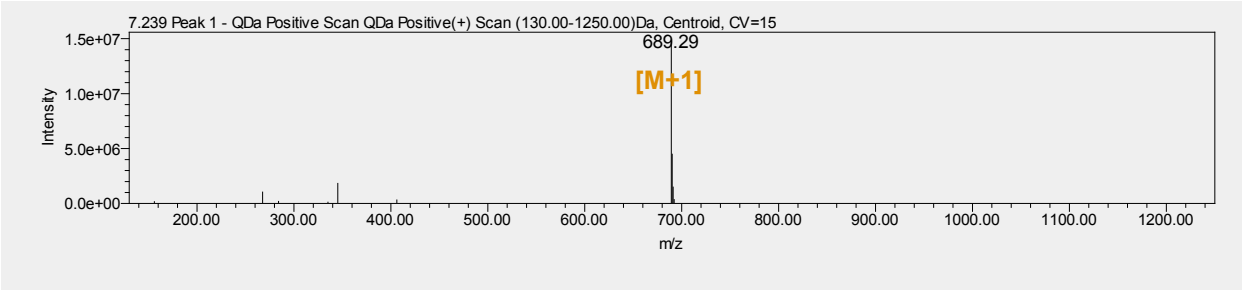
All other peptides:
0.5 mL/min flow rate

Time:	%A:	%B:
0.0	5.0	95.0
2.0	5.0	95.0
10.5	95.0	5.0
12.0	95.0	5.0

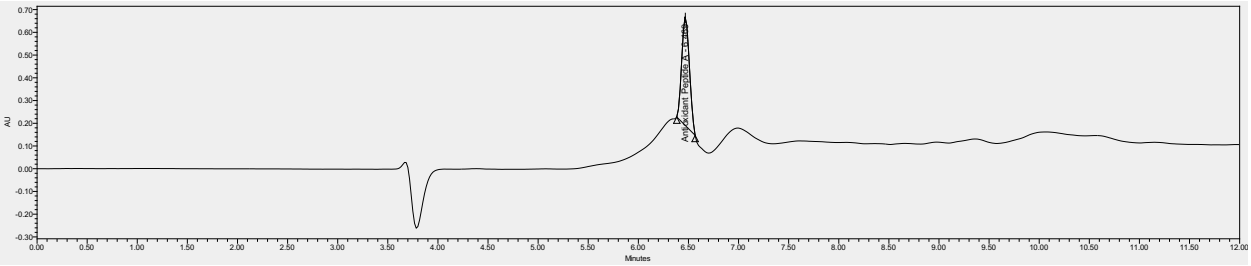
Caspase Active Site Peptide:



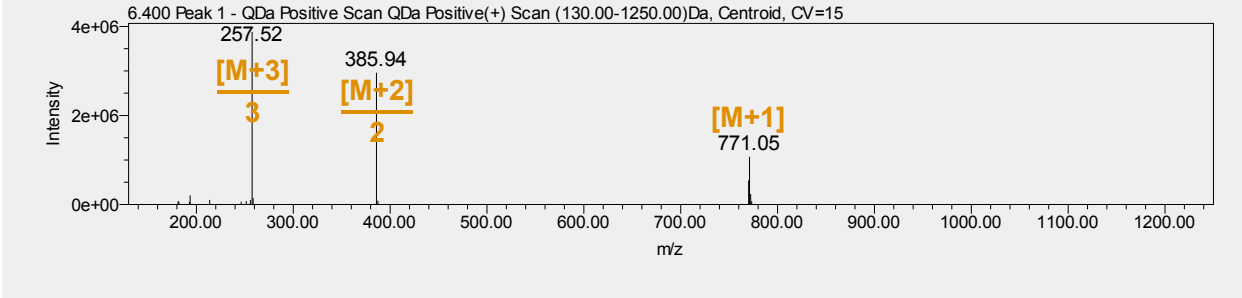
	Name	Retention Time	Area	% Area
1	Caspase Peptide	7.258	5486993	100.00



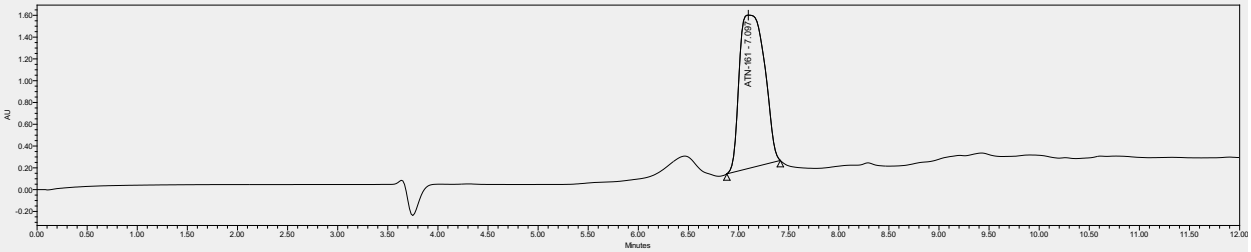
Antioxidant Peptide A:



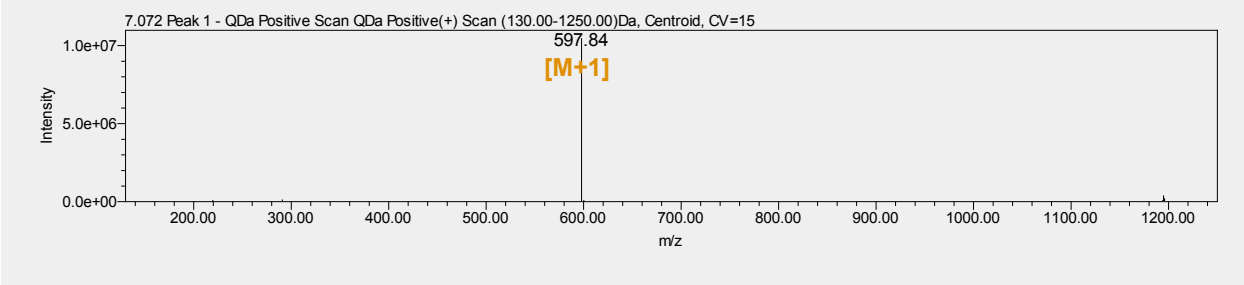
	Name	Retention Time	Area	% Area
1	Antioxidant Peptide A	6.469	2527061	100.00



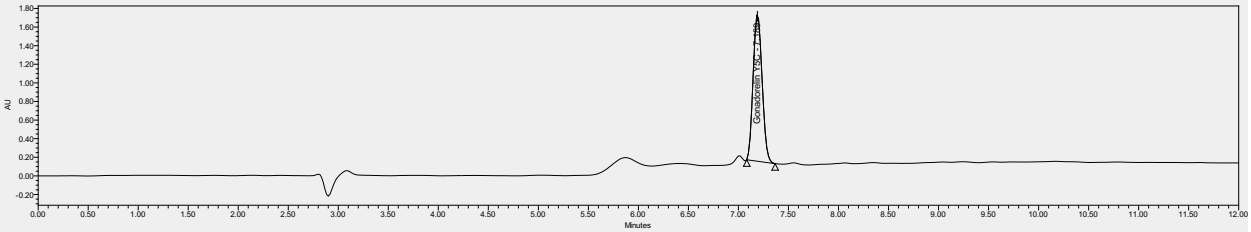
ATN-161, Integrin Inhibitor:



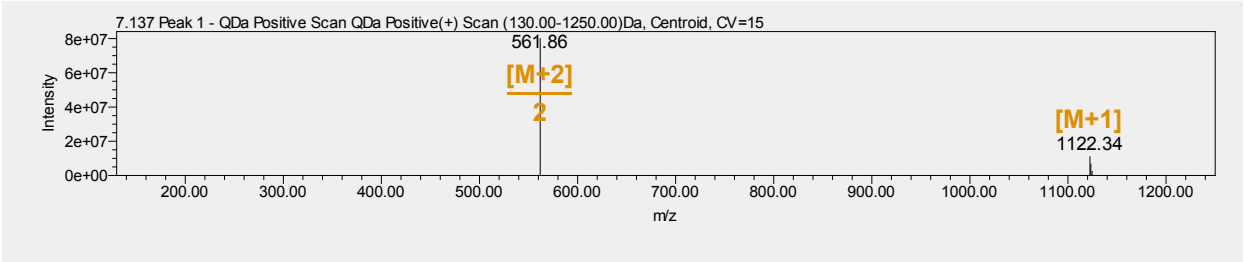
	Name	Retention Time	Area	% Area
1	ATN-161	7.097	24467853	100.00



Gonadorelin Y5C:

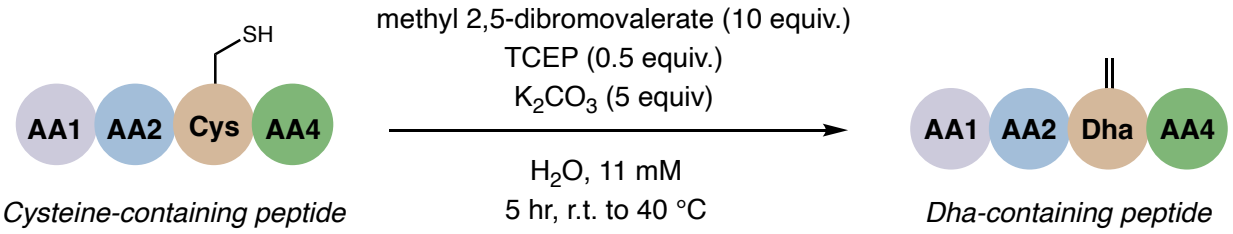


	Name	Retention Time	Area	% Area
1	Gonadorelin Y5C	7.189	9810992	100.00



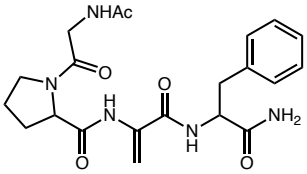
Synthesis of Dha-containing peptides

General Procedure:

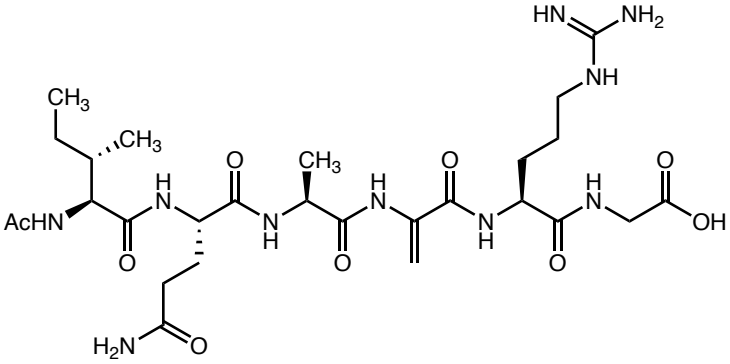


Cysteine-containing peptide *Dha-containing peptide*

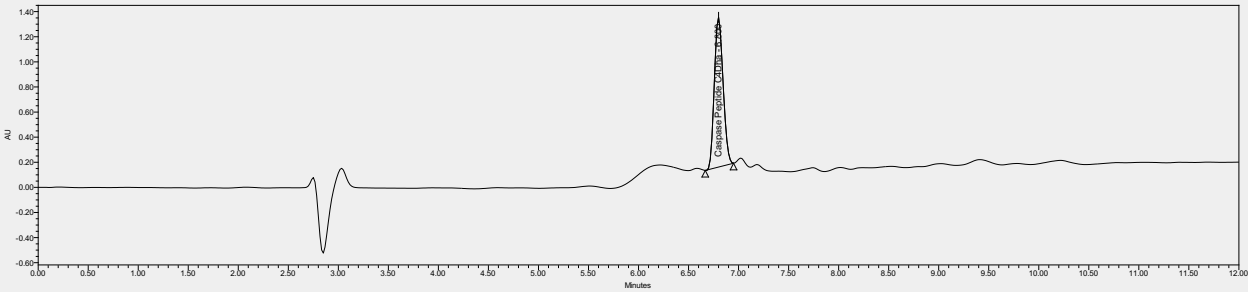
To a 50 mL flask or a 20 mL scintillation vial charged with a stir bar, peptide, TCEP, and water were added and stirred at room temperature for 1 hour. Potassium carbonate and methyl 2,5-dibromovalerate were then added, and the solution was stirred for another hour at room temperature. The reaction was then heated to 40 °C for 3 hours while monitoring for full conversion by UPLC/MS. Upon completion, the reaction was washed with ethyl acetate, concentrated, and purified by reverse phase flash chromatography (acetonitrile:H_2O).



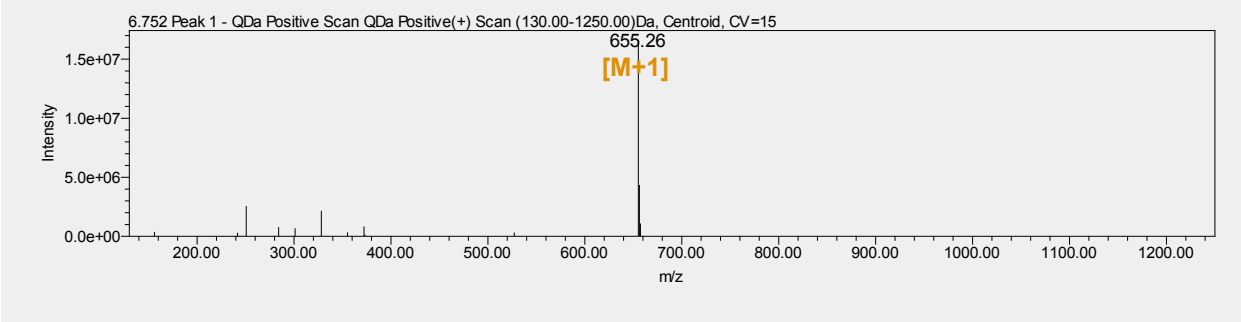
Ac-Gly-Pro-Dha-Phe-NH_2 was synthesized according to the reported procedure.[1]

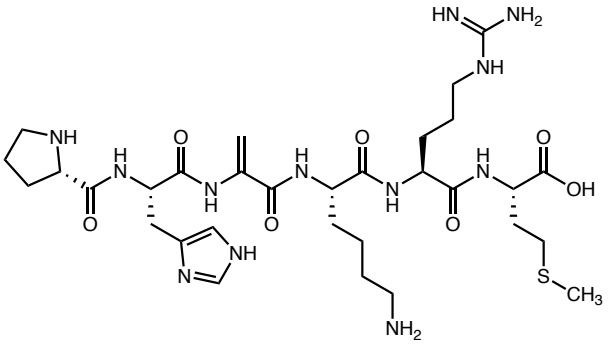


Caspase Active Site Peptide C4Dha was synthesized on a 60 μmol scale according to the general procedure (with the exception of not washing with ethyl acetate upon completion of the reaction) to yield 14.8 mg of pure peptide (38% yield).

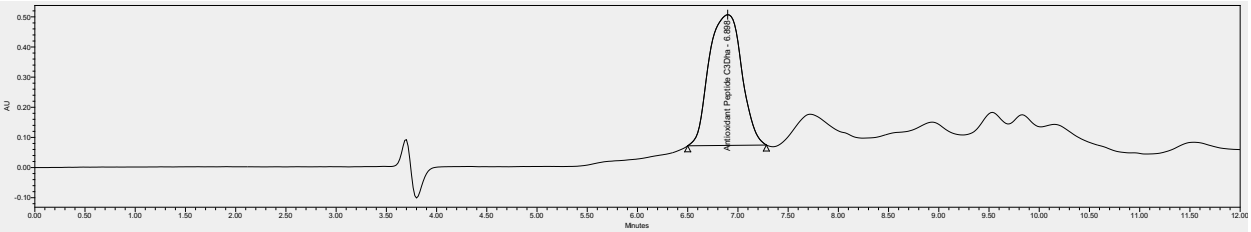


	Name	Retention Time	Area	% Area
1	Caspase Peptide C4Dha	6.800	7251089	100.00

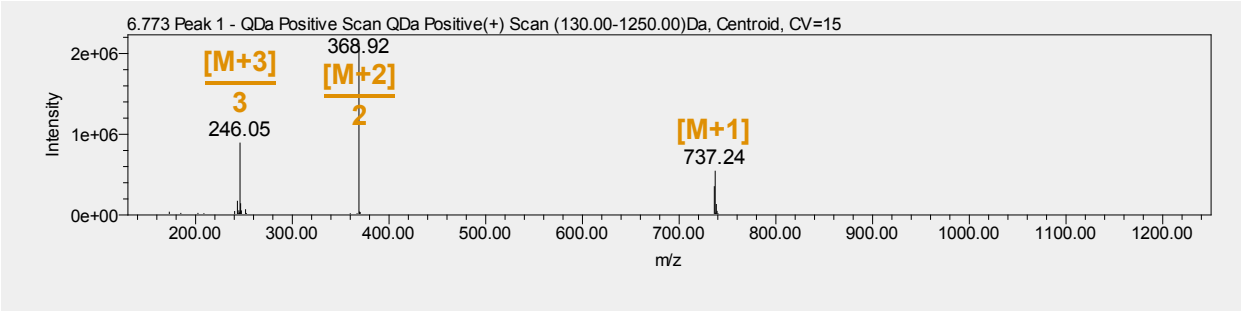


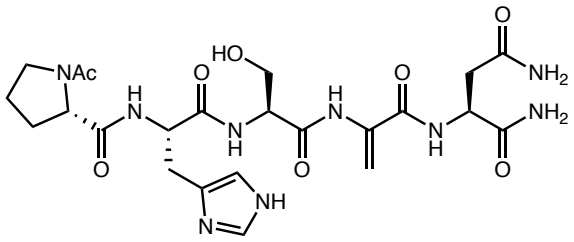


Antioxidant Peptide A C3Dha was synthesized on a 65 µmol scale according to the general procedure to yield 7.0 mg of pure peptide (15% yield).

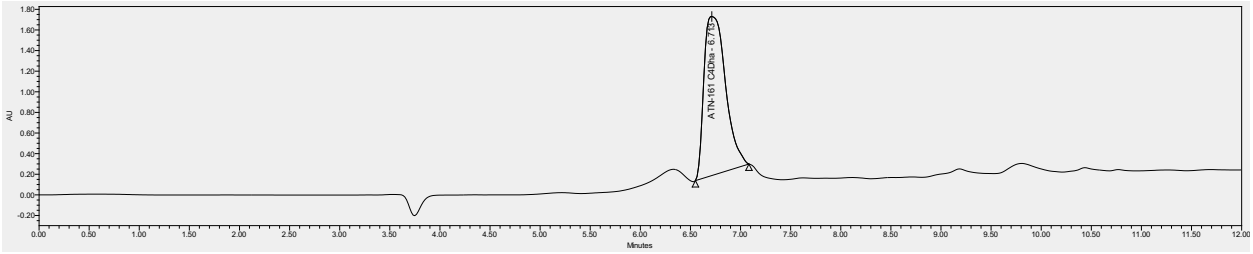


	Name	Retention Time	Area	% Area
1	Antioxidant Peptide C3Dha	6.898	9817482	100.00

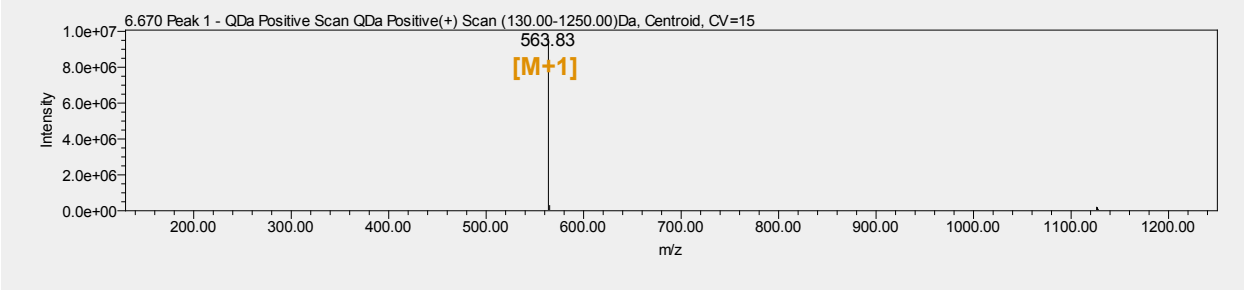


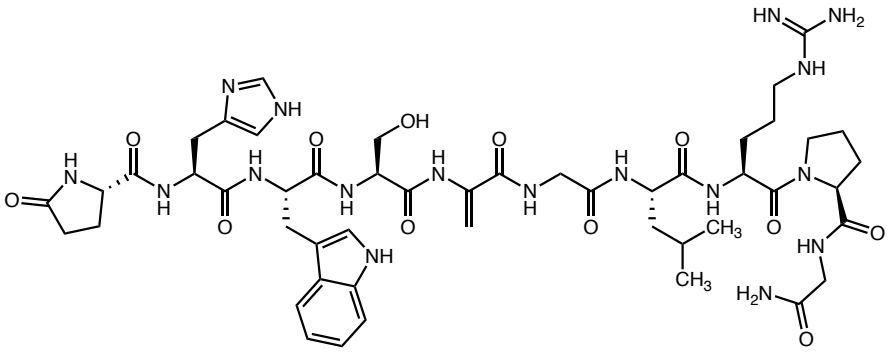


ATN-161 C4Dha was synthesized on an 84 μmol scale according to the general procedure to yield 16.9 mg of pure peptide (36% yield).

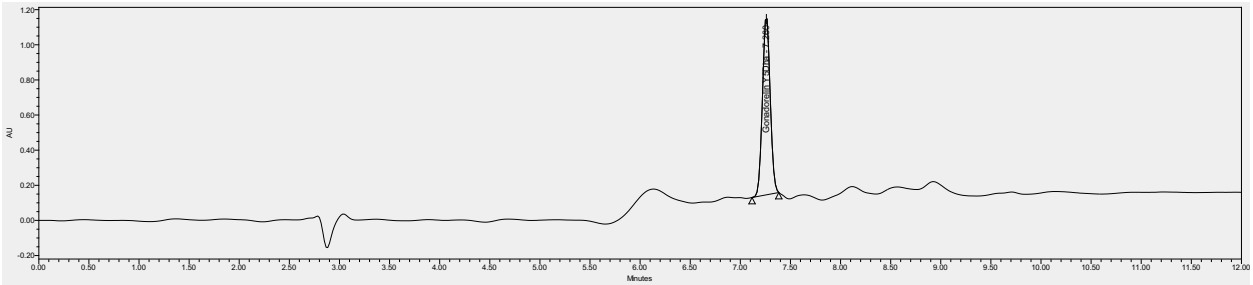


	Name	Retention Time	Area	% Area
1	ATN-161 C4Dha	6.713	23078837	100.00

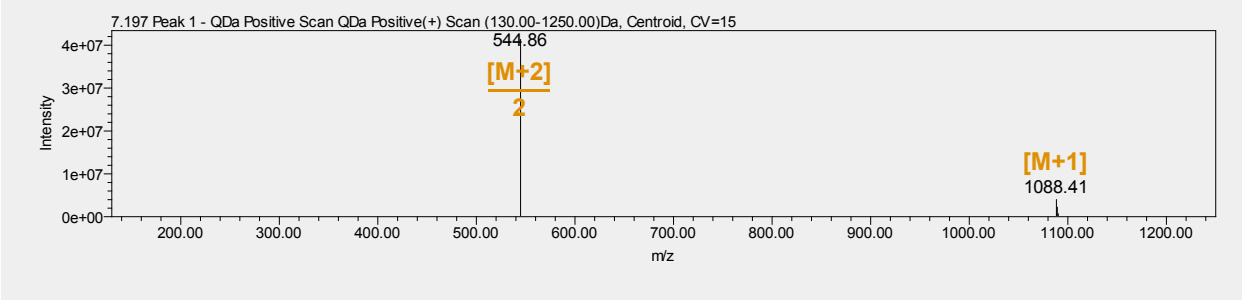




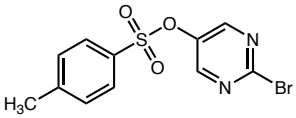
Gonadorelin Y5Dha was synthesized on a 31 µmol scale according to the general procedure to yield 11 mg of pure peptide (33% yield).



	Name	Retention Time	Area	% Area
1	Gonadorelin Y5Dha	7.260	5459586	100.00

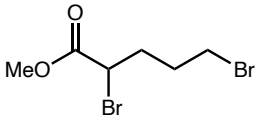


Synthesis of small molecules



2-bromopyrimidin-5-yl 4-methylbenzenesulfonate: To a 2-dram vial charged with a stir bar, 2-bromopyrimidin-5-ol (200 mg, 1.1 mmol, 1 equiv.) was dissolved in 4.4 mL of dichloromethane. The solution was cooled to 0 °C using an ice bath followed by the addition of triethylamine (159 µL, 1.1 mmol, 1 equiv.). p-Toluenesulfonyl chloride (218 mg, 1.1 mmol, 1 equiv.) was then added while stirring. The reaction was allowed to warm to room temperature and was stirred for 1 hour, after which, the reaction appeared to be complete by TLC (ethyl acetate/hexanes). The solution was then diluted with dichloromethane and washed twice with ddH_2O and once with brine. The

organic layer was dried with $MgSO_4$, filtered, and concentrated under reduced pressure. The crude product was purified using normal phase flash chromatography (ethyl acetate/hexanes) to give 220 mg of pure product (61% yield).



Methyl 2,5-dibromovalerate was synthesized according to the reported procedure.[2]

Parallel Synthesis

Procedure for parallel synthesis

To sixteen 1 mL shell vials (Thermo Fisher Scientific) each charged with a stir bar, sixteen distinct bromides (11.7 μmol, 5 equiv.) were added. Diisopropylethylamine (0.6 μL, 3.5 μmol, 1.5 equiv.), 2,2,2-trifluoroethanol (39 μL), acetonitrile (39 μL), Ac-Gly-Pro-Dha-Phe-NH_2 (1 mg, 2.3 μmol, 1 equiv.), rhodamine B (22 μg, 0.047 μmol, 2.0 mol%), and ddH_2O (700 μL total, N_2 sparged for 5 minutes) were added to each vial. Ac-Gly-Pro-Dha-Phe-NH_2 and rhodamine B were added by first creating a stock solution in ddH_2O and distributing the appropriate amounts among the vials. Each vial was individually capped and purged under N_2 for 3 minutes, parafilmed, and placed on the Lumidox® 96-well LED array (527 nm). The reactions were illuminated under water recirculation and fan cooling while stirring at ~350 rpm using a VP 710-C5 from V&P Scientific Inc. for 15 hours. Upon completion of the reaction, each cap was removed and ~10 equiv. of TCEP and 10 equiv. of 2-mercaptoethylamine, polymer-bound resin was added to each vial. The vials were stirred in a water bath at 40 °C for 5 hours. After this, the vials were filtered using a 96-well filter plate, removing excess Dha peptide. Next, diethyl ether extraction was performed by adding ~300 μL of diethyl ether to each well and extracting the aqueous phase of each well with a glass pipette to remove the bromide. Finally, Waters Oasis HLB 96-well Plate (60 mg sorbent per well) was used to remove rhodamine B. The sorbent was first washed and filtered with 1 mL of methanol and 1 mL of water. The sample solutions were then loaded onto the sorbent and filtered. The sorbent was washed by filtering 1 mL of a 5% NaOH solution through each well, and the products were eluted with 1 mL of a 50% trifluoroethanol solution in water. The products were then submitted to a UPLC for analysis of purity and yield using a Waters Zorbax StableBondC18 3.5 μ, 2.1 x 100 mm column with a 0.5 mL/min flow rate using the method below. (Note: In our run, some NaOH solution eluted with our products allowing the samples to be readily buffered and stored if desired. If no buffer is preferred, each sorbent well should be washed with 1 mL of water after the wash step with 5% NaOH and prior to the trifluoroethanol elution step.)

A = Acetonitrile with 1% formic acid modifier, B = ddH_2O with 1% formic acid modifier.

Time:	%A:	%B:
0.0	5.0	95.0
2.0	5.0	95.0
3.0	10.0	90.0
10.0	20.0	80.0
12.0	30.0	70.0
16.5	95.0	5.0
18.0	95.0	5.0

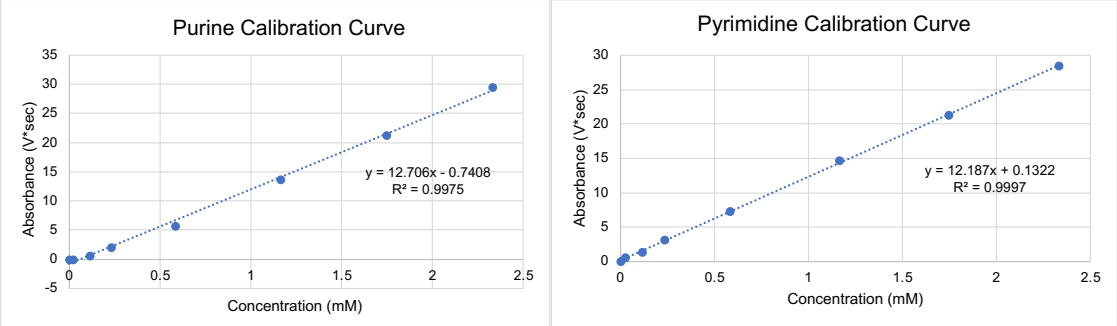


Figure S3. Calibration curves for the pyrimidine (compound **1**) and purine (compound **11**) *c*NPs

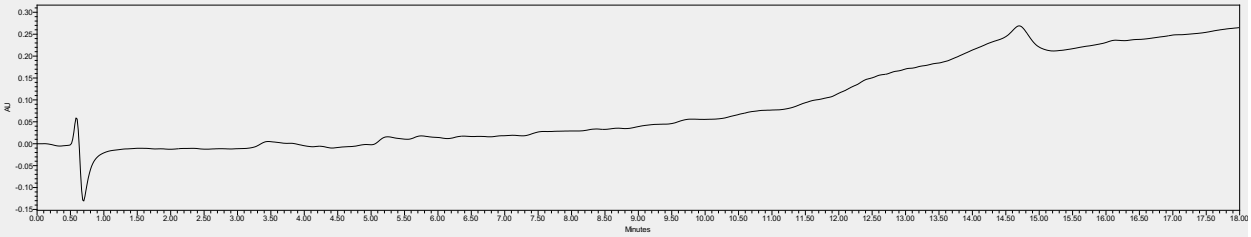
Results from parallel synthesis

Purity, amount, and yield were all determined from the UPLC spectrum. Amount and yield were determined by building a calibration curve for the pyrimidine nucleobases using the simple pyrimidine heterocycle *c*NP (compound **1**) and for the purine nucleobases using the simple purine heterocycle *c*NP (compound **11**). For the two aryl products (compounds **19** and **20**), the amounts were calculated from a variant of the Beer-Lambert's Law as described previously.[1]

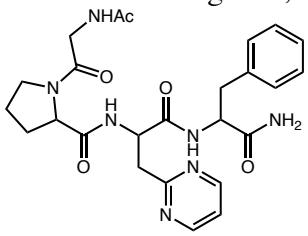
Table S6. Results from parallel synthesis

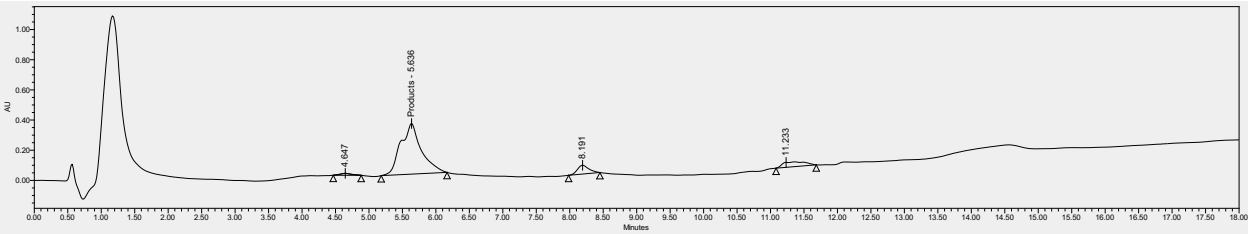
*c*NP Product:	MW:	Purity:	Yield of Epimers:	Amount: (mg)
1	509.6	81.80%	24.9%	0.30
2	509.6	0%	0%	0.0
3	527.6	91.3%	2.8%	0.03
4	544.0	90.0%	35.7%	0.45
5	588.5	79.2%	16.6%	0.23
6	523.6	15.5%	1.2%	0.01
7	524.6	0%	0%	0.0
8	549.6	0%	0%	0.0
9	525.6	0%	0%	0.0
11	549.6	0%	0%	0.0
12	564.6	0%	0%	0.0
13	548.6	81.5%	71.2%	0.91
14	548.6	66.8%	19.9%	0.25
15	548.6	90.0%	17.3%	0.22
19	549.6	92.0%	4.4%	0.06
20	535.6	8.1%	1.0%	0.01

Background spectrum:

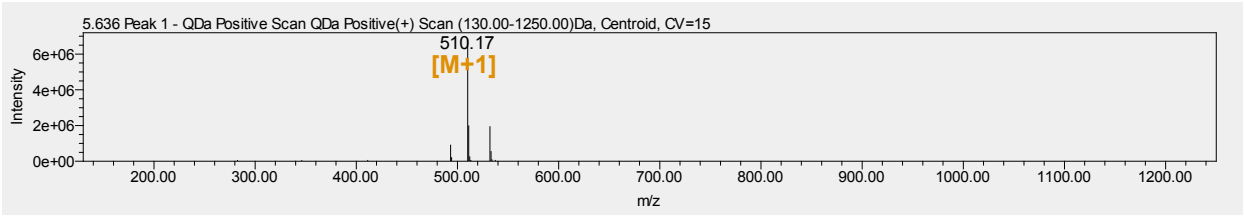


1: MW = 509.6 g/mol, Purity = 81.8%, Yield = 24.9% [0.30 mg]

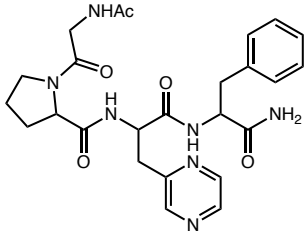


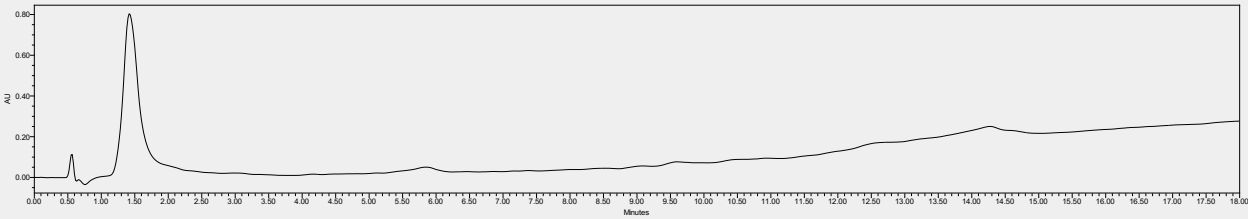


	Name	Retention Time	Area	% Area
1		4.647	151630	1.72
2	Products	5.636	7196903	81.87
3		8.191	698166	7.94
4		11.233	744171	8.47

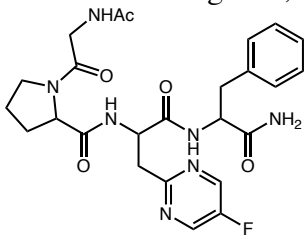


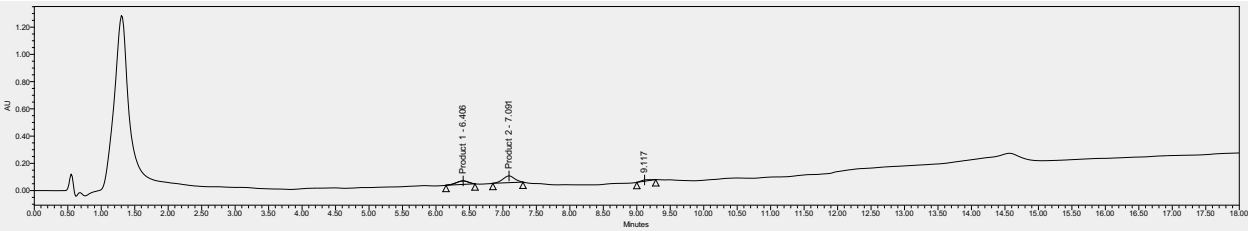
2: MW = 509.6 g/mol, Purity = 0%, Yield = 0% [0.0 mg]



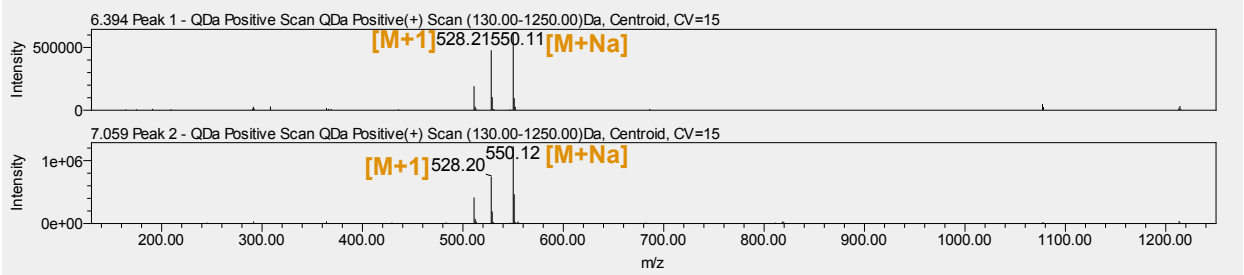


3: MW = 527.6 g/mol, Purity = 91.3%, Yield = 2.8% [0.03 mg]

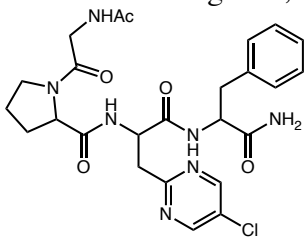


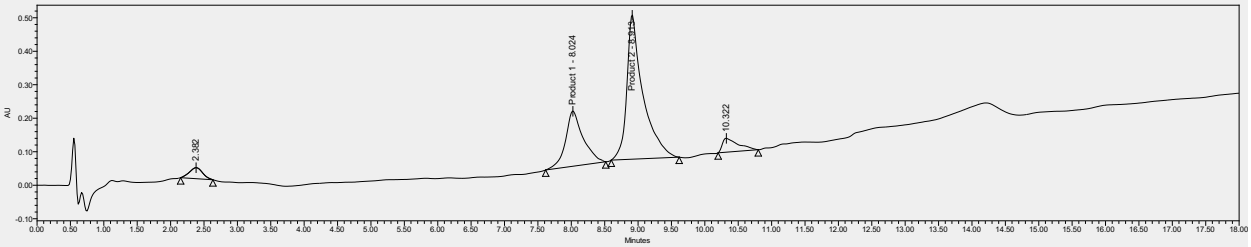


	Name	Retention Time	Area	% Area
1	Product 1	6.406	327722	32.13
2	Product 2	7.091	603078	59.13
3		9.117	89054	8.73

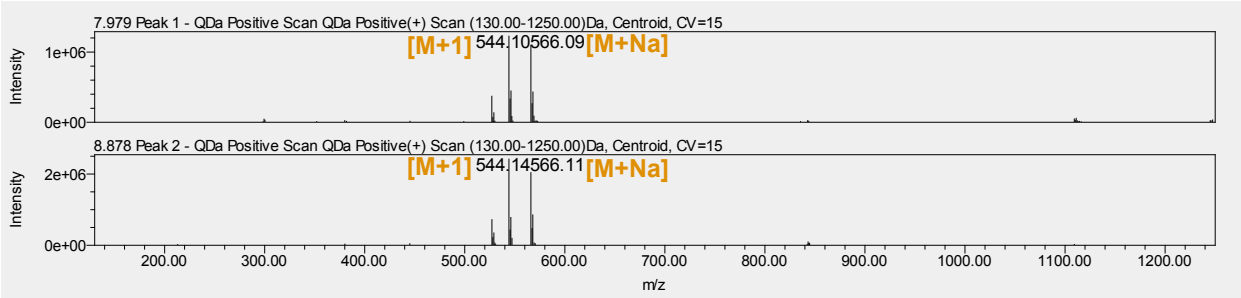


4: MW = 544.0 g/mol, Purity = 90.0%, Yield = 35.7% [0.45 mg]

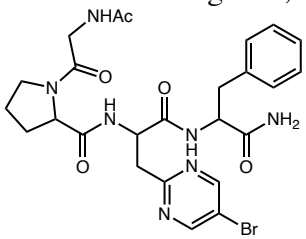


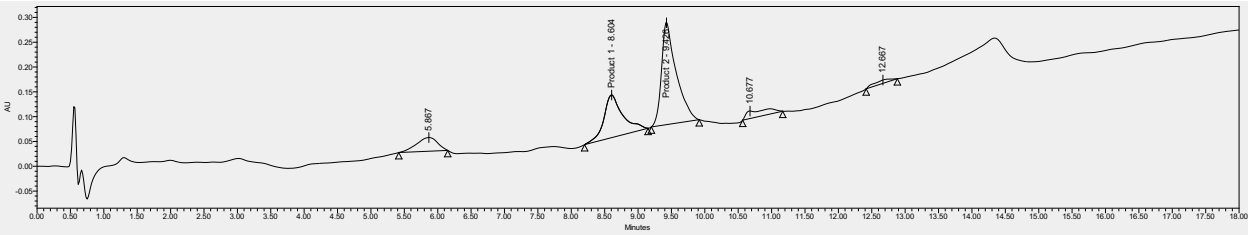


	Name	Retention Time	Area	% Area
1		2.382	448889	3.94
2	Product 1	8.024	3023418	26.54
3	Product 2	8.913	7237362	63.53
4		10.322	681693	5.98

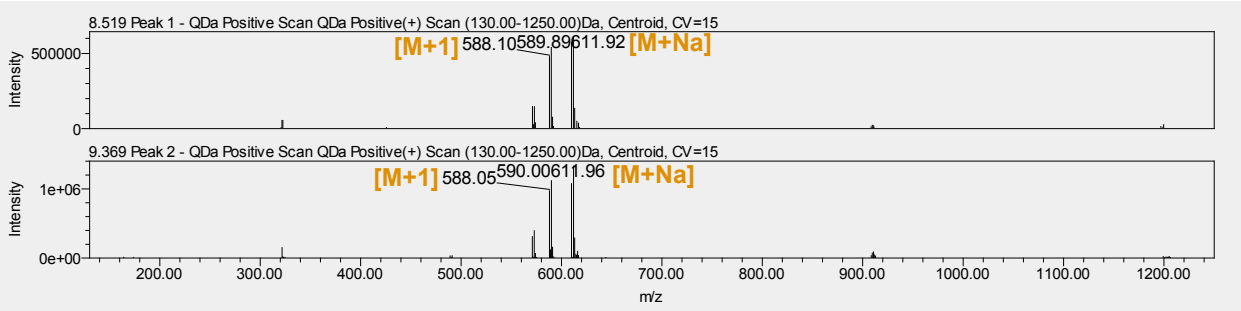


5: MW = 588.5 g/mol, Purity = 79.2%, Yield = 16.6% [0.23 mg]

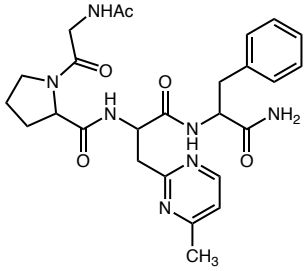


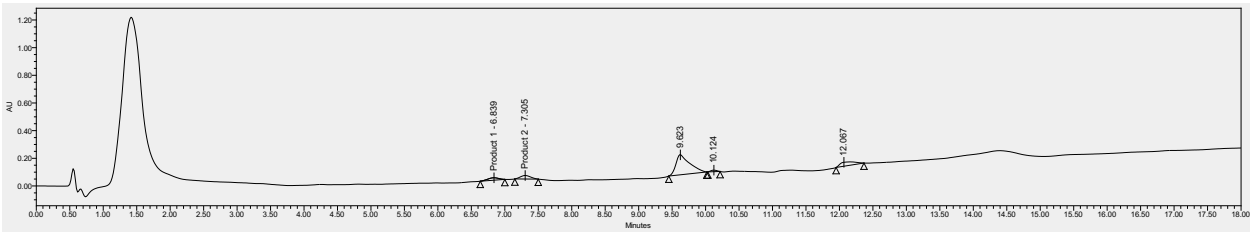


	Name	Retention Time	Area	% Area
1		5.867	606740	10.24
2	Product 1	8.604	1693447	28.57
3	Product 2	9.426	3164475	53.38
4		10.677	337422	5.69
5		12.667	125994	2.13

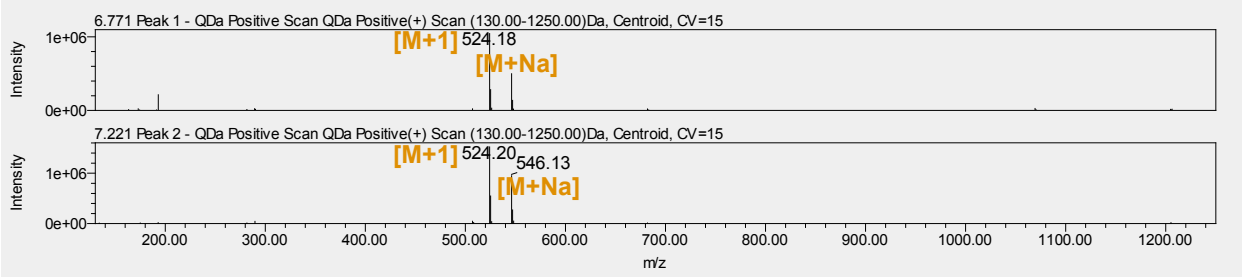


6: MW = 523.6 g/mol, Purity = 15.5%, Yield = 1.2% [0.01 mg]

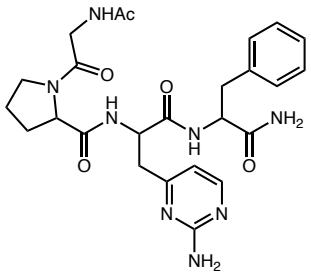


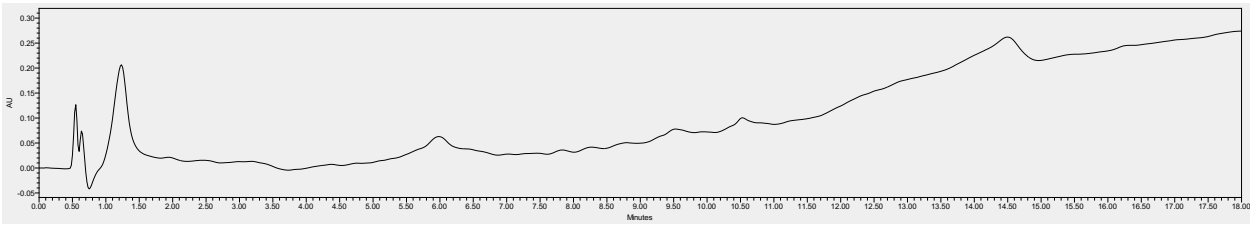


	Name	Retention Time	Area	% Area
1	Product 1	6.839	201422	6.62
2	Product 2	7.305	271461	8.92
3		9.623	2072836	68.14
4		10.124	71236	2.34
5		12.067	425052	13.97

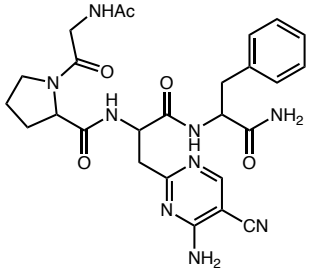


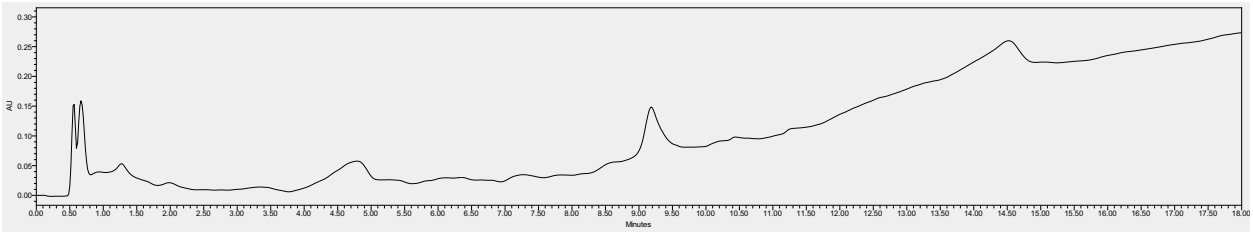
7: MW = 524.6 g/mol, Purity = 0%, Yield = 0% [0.0 mg]



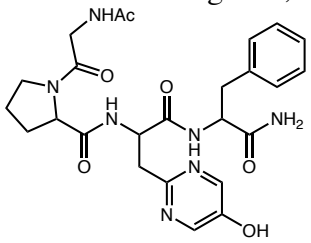


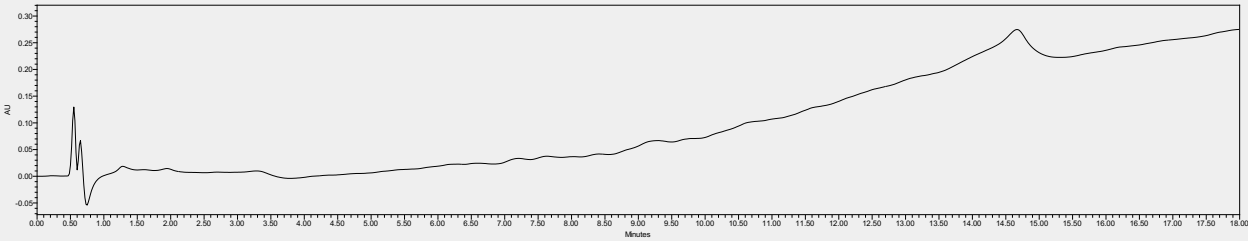
8: MW = 549.6 g/mol, Purity = 0%, Yield = 0% [0.0 mg]



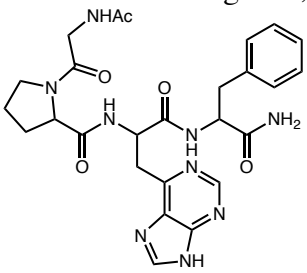


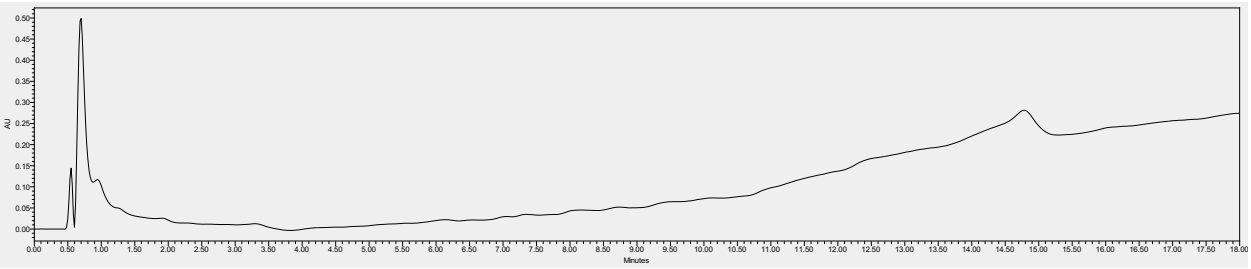
9: MW = 525.6 g/mol, Purity = 0%, Yield = 0% [0.0 mg]



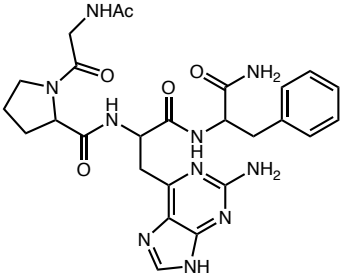


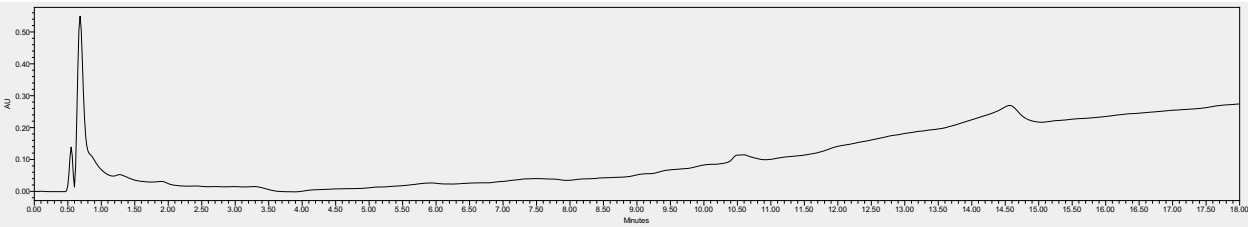
11: MW = 549.6 g/mol, Purity = 0%, Yield = 0% [0.0 mg]



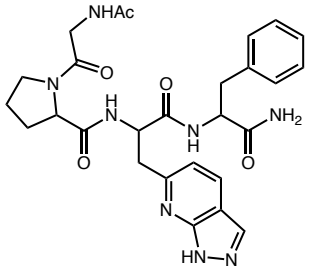


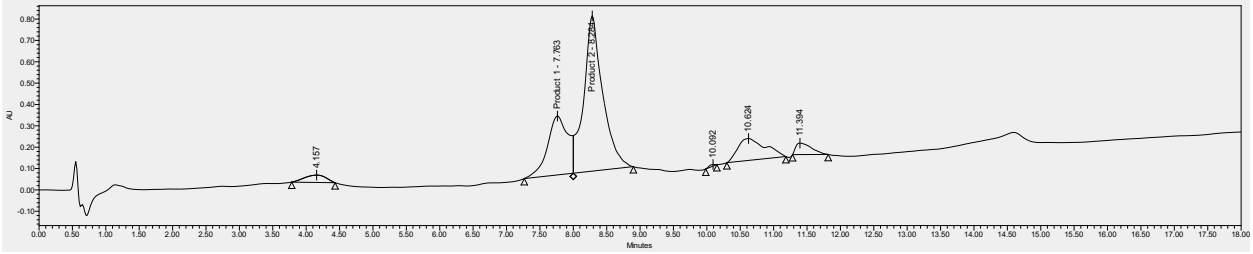
12: MW = 564.6 g/mol, Purity = 0%, Yield = 0% [0.0 mg]



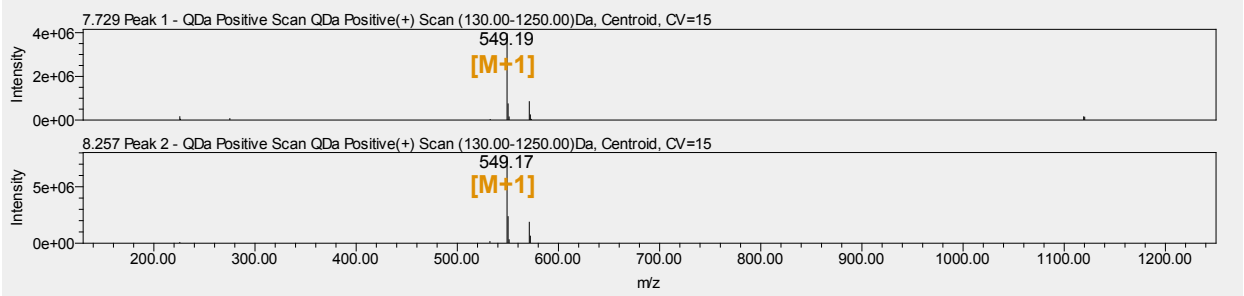


13: MW = 548.6 g/mol, Purity = 81.5%, Yield = 71.2% [0.91 mg]

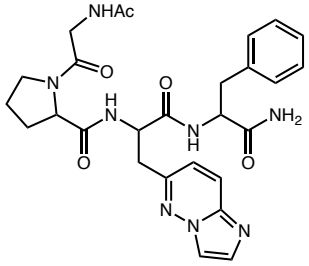


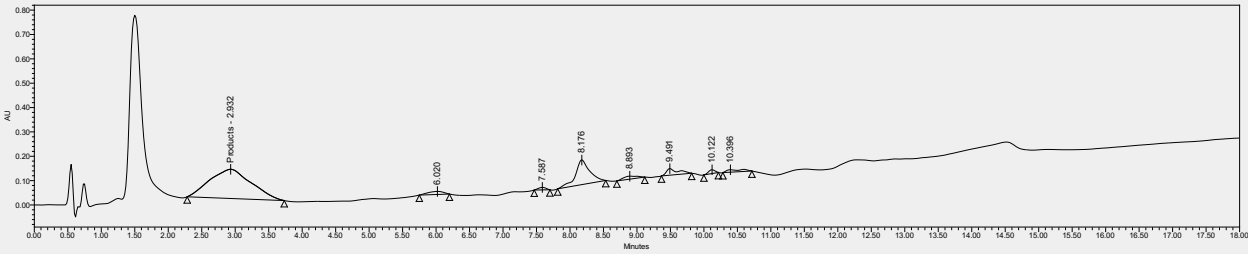


	Name	Retention Time	Area	% Area
1		4.157	770710	3.09
2	Product 1	7.763	5908602	23.66
3	Product 2	8.284	14441511	57.82
4		10.092	53465	0.21
5		10.624	2919691	11.69
6		11.394	881515	3.53

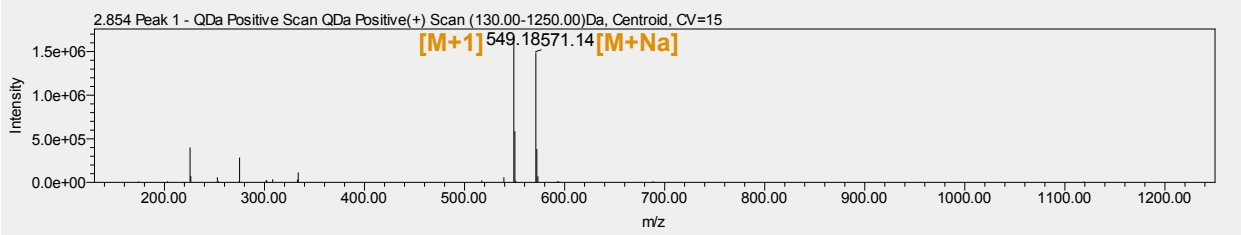


14: MW = 548.6 g/mol, Purity = 66.8%, Yield = 19.9% [0.25 mg]

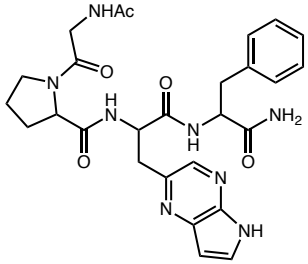


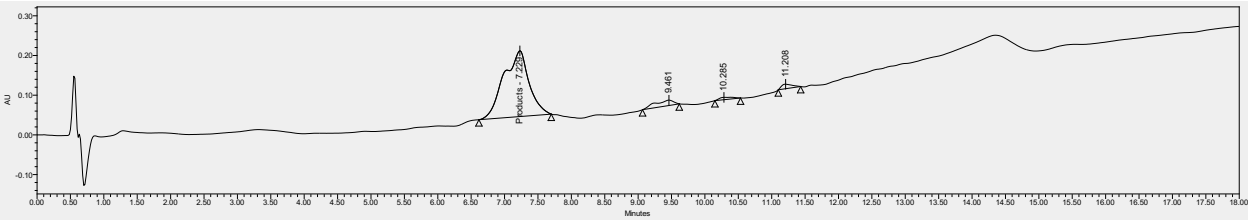


	Name	Retention Time	Area	% Area
1	Products	2.932	5163237	66.79
2		6.020	204566	2.65
3		7.587	82485	1.07
4		8.176	1501962	19.43
5		8.893	177214	2.29
6		9.491	330069	4.27
7		10.122	104324	1.35
8		10.396	167118	2.16

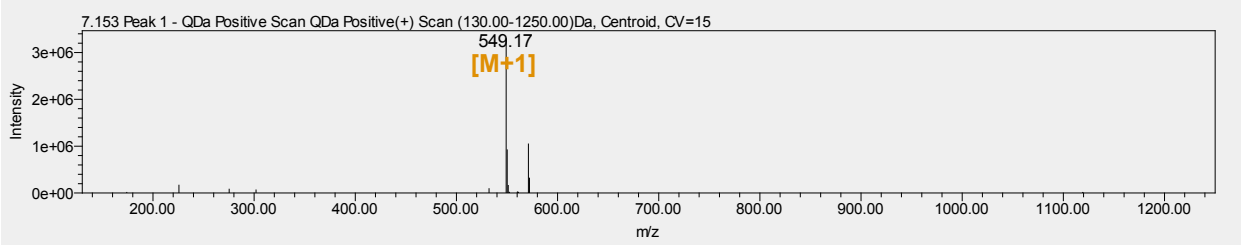


15: MW = 548.6 g/mol, Purity = 90.0%, Yield = 17.3% [0.22 mg]

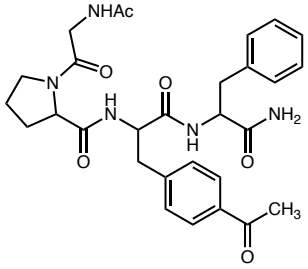


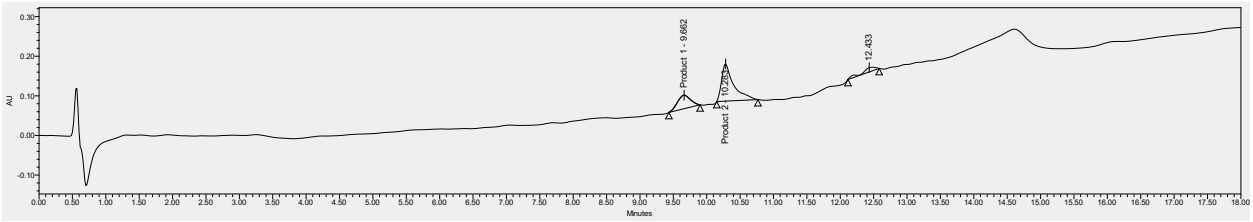


	Name	Retention Time	Area	% Area
1	Products	7.229	4366749	89.97
2		9.461	280842	5.79
3		10.285	79754	1.64
4		11.208	126384	2.60

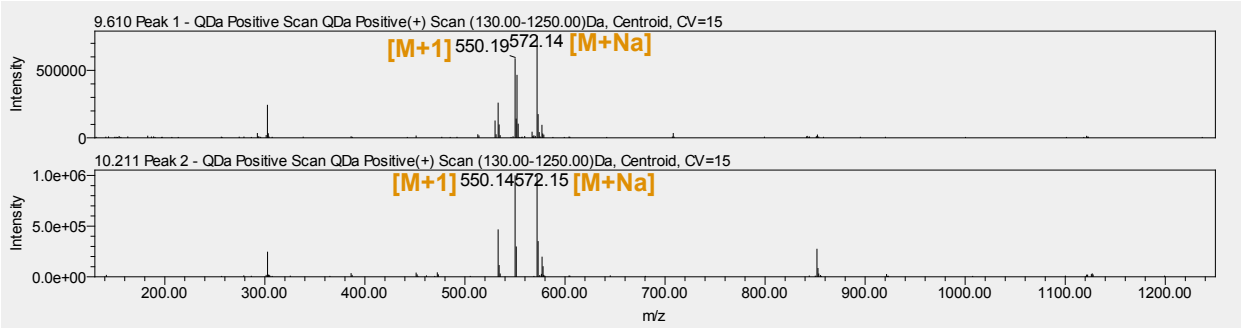


19: MW = 549.6 g/mol, Purity = 92.0%, Yield = 4.4% [0.6 mg]

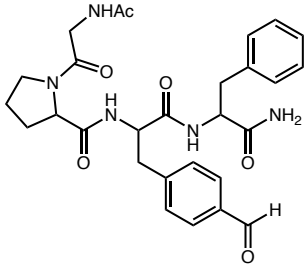


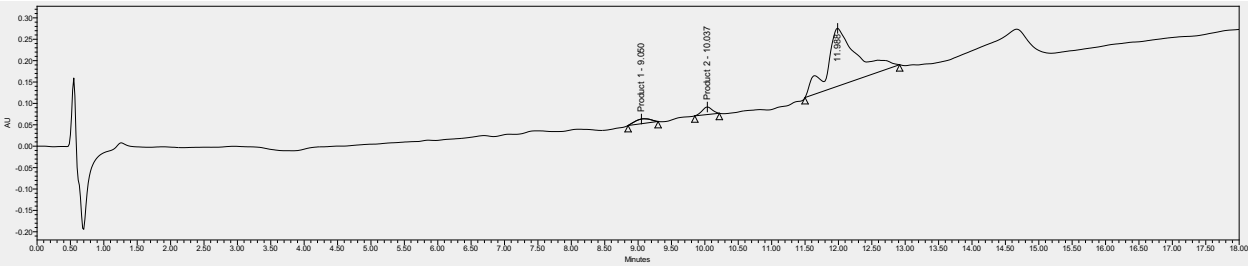


	Name	Retention Time	Area	% Area
1	Product 1	9.662	465141	26.06
2	Product 2	10.283	1176728	65.92
3		12.433	143144	8.02

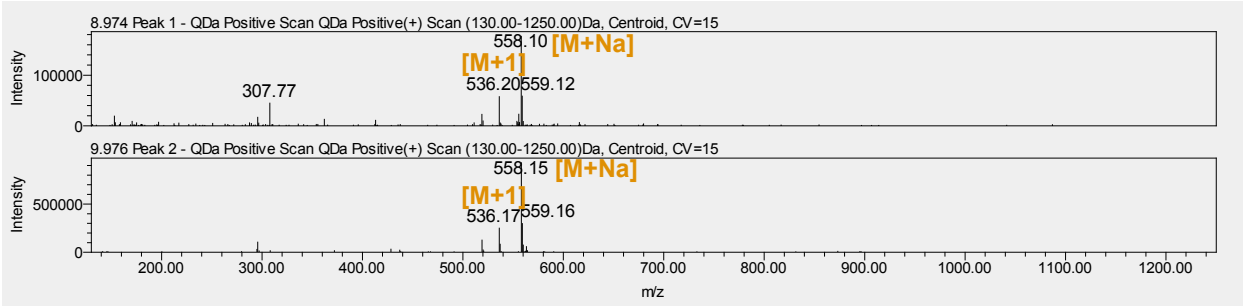


20: MW = 535.6 g/mol, Purity = 8.1%, Yield = 1.0% [0.01 mg]





	Name	Retention Time	Area	% Area
1	Product 1	9.050	177589	3.94
2	Product 2	10.037	185148	4.11
3		11.988	4139146	91.94



Flow Synthesis

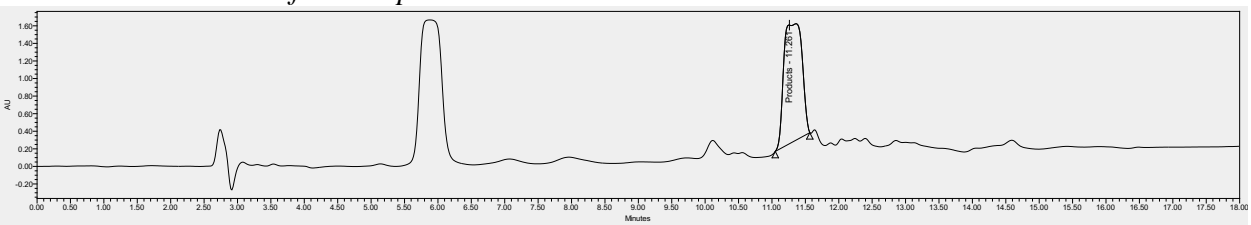
Procedure for flow synthesis and results

To a 1-dram vial, Ac-Gly-Pro-Dha-Phe-NH_2 (25 mg, 58 μmol, 1 equiv.), 2-bromopyrimidine (14 mg, 87 μmol, 1.5 equiv.), rhodamine B (140 μg, 0.29 μmol, 0.5 mol%), diisopropylethylamine (15 μL, 87 μmol, 1.5 equiv.), 2,2,2-trifluoroethanol (29 μL), acetonitrile (29 μL), and ddH_2O (500 μL total, overall concentration 0.1 M, N_2 sparged for 5 minutes) were added. ddH_2O was purged with N_2 for 5 minutes and was used to rinse the tubing. The vial was then purged under N_2 for 5 minutes and transferred to the tubing using a syringe. The syringe was then refilled with N_2 purged ddH_2O and hooked up to a syringe pump (New Era Pump Systems Inc.) with a flow rate set to 14 μL/min. The solution was irradiated with the Lumidox® 96-well LED array (527 nm) over 2 hours and 36 minutes (1 hour and 55-minute residence time) and was collected into a 1-dram vial. (Note: Air was left in between the ddH_2O prior to the reaction solution and the ddH_2O after the reaction solution. A little extra ddH_2O was then collected with the reaction solution after completion of the reaction due to some loss of solution on the tubing walls during flow.) The sample was then submitted for UPLC/MS analysis using a Supelco Discovery Bio Wide Pore C8 (3.0 μ, 4.6 mm x 10 cm) column with a 0.5 mL/min flow rate using the method below. (45% conversion). The product was then purified by reverse phase flash chromatography (acetonitrile:H_2O) to yield 11.1 mg of compound **1** (38% yield).

A = Acetonitrile with 1% formic acid modifier, B = ddH_2O with 1% formic acid modifier.

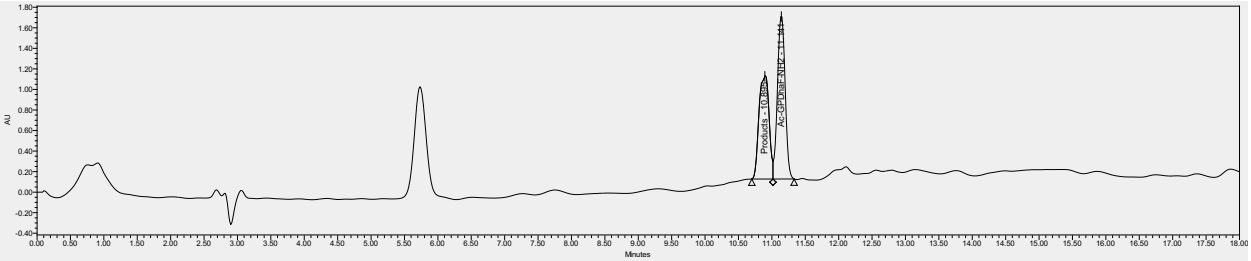
Time:	%A:	%B:
0.0	5.0	95.0
2.0	5.0	95.0
3.0	10.0	90.0
9.0	30.0	70.0
17.0	50.0	50.0
17.5	95.0	5.0
18.0	95.0	5.0

*Crude Batch Reaction for Compound **1**:*



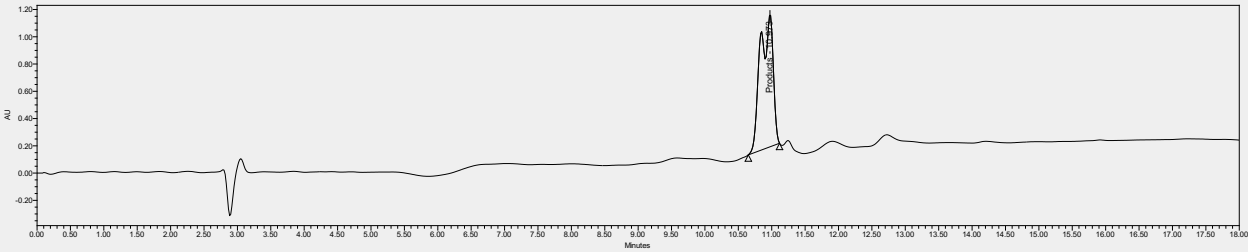
	Name	Retention Time	Area	% Area
1	Products	11.261	25518563	100.00

*Crude Flow Reaction for Compound **1**:*

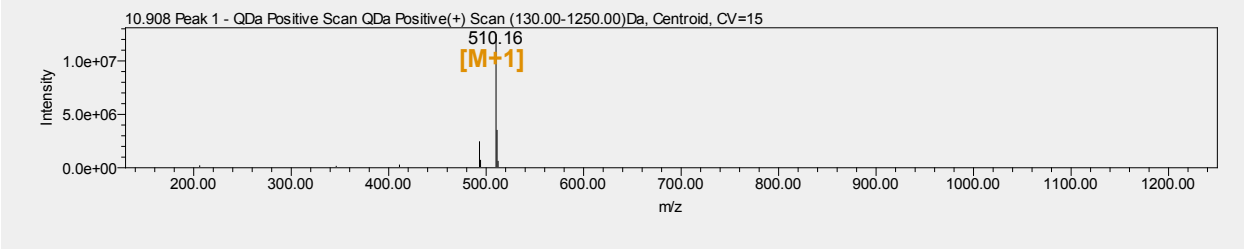


	Name	Retention Time	Area	% Area
1	Products	10.895	10517566	45.28
2	Ac-GPDhaF-NH2	11.141	12708612	54.72

Isolated Epimers:

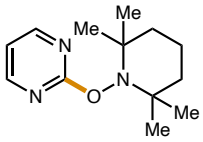


	Name	Retention Time	Area	% Area
1	Products	10.973	13102767	100.00

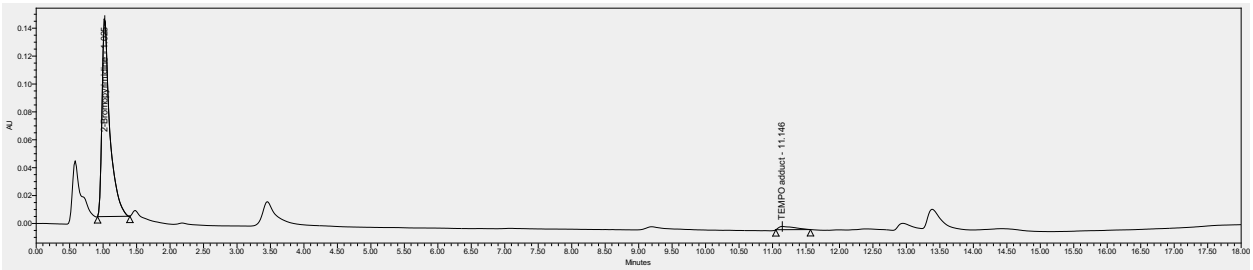


Mechanistic Studies

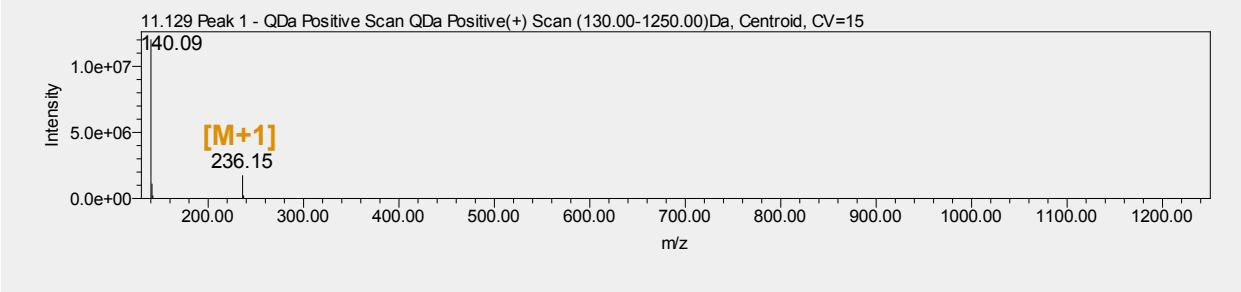
TEMPO radical trapping



To a 1-dram vial charged with a stir bar, 2-bromopyrimidine (9 mg, 58 μmol, 1 equiv.), TEMPO (9 mg, 58 μmol, 1 equiv.), 200 μL of rhodamine B from a 1.16 mM stock solution in ddH_2O (111 μg, 0.23 μmol, 0.4 mol%), diisopropylethylamine (3 μL, 17 μmol, 0.3 equiv.), 2,2,2-trifluoroethanol (194 μL), acetonitrile (194 μL), and ddH_2O (3.5 mL total, N_2 sparged for 5 minutes) were added. The vial was purged under N_2 for 5 minutes, parafilmed, and placed 1 cm away from two Green LED lights with stirring at 1150 rpm on the Corning PC-620D stir plate (under fan cooling) for fifteen hours. After that time, the sample was filtered and subjected to UPLC/MS analysis to determine % conversion (defined as the % area of the product peak compared to the 2-bromopyrimidine peak at 280 nm). (3% conversion).



	Name	Retention Time	Area	% Area
1	2-Bromopyrimidine	1.025	1148340	96.73
2	TEMPO adduct	11.146	38795	3.27



Isodesmic calculations

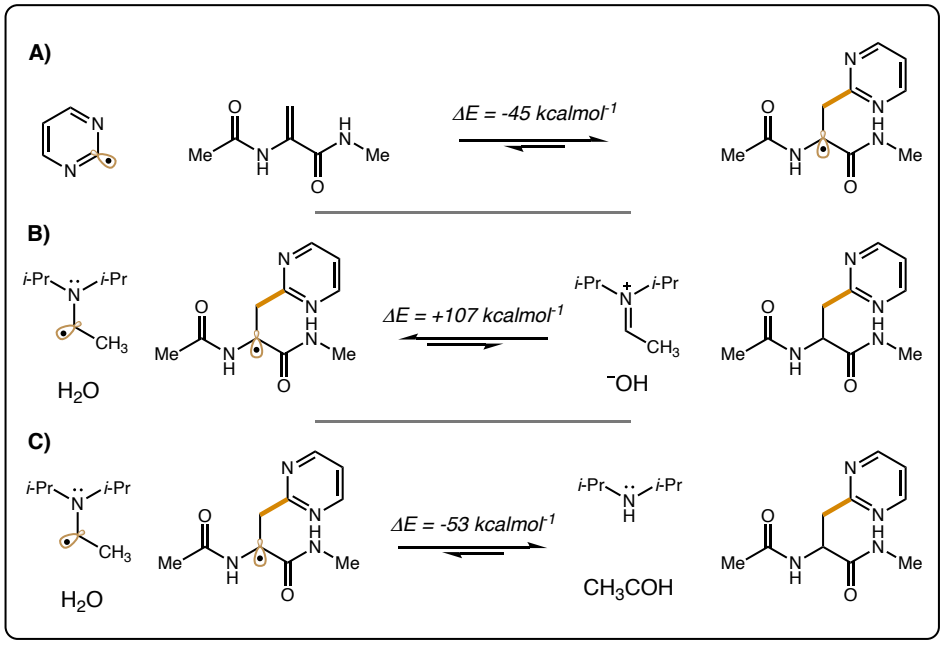
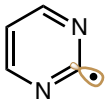


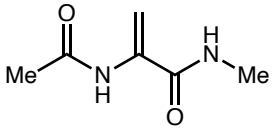
Figure S4. Isodesmic calculations for the A) radical addition to Dha, B) the unfavorable intermediate reduction by DIPEA, and C) the overall favorable process to form the *c*NP product.



Job type: Single point.
Method: UB3LYP
Basis set: 6-311+G**
Number of basis functions: 150
Number of electrons: 41 (1 unpaired)
Parallel Job: 4 threads

SCF model:
An unrestricted hybrid HF-DFT SCF calculation will be performed using Pulay DIIS + Geometric Direct Minimization

SCF total energy: -263.7110795 hartrees

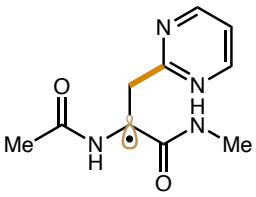


Job type: Single point.
Method: RB3LYP
Basis set: 6-311+G**
Number of basis functions: 280

Number of electrons: 76
Parallel Job: 4 threads

SCF model:
A restricted hybrid HF-DFT SCF calculation will be performed using Pulay DIIS + Geometric Direct Minimization

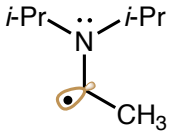
SCF total energy: -494.7808416 hartrees



Job type: Single point.
Method: UB3LYP
Basis set: 6-311+G**
Number of basis functions: 430
Number of electrons: 117 (1 unpaired)
Parallel Job: 4 threads

SCF model:
An unrestricted hybrid HF-DFT SCF calculation will be performed using Pulay DIIS + Geometric Direct Minimization

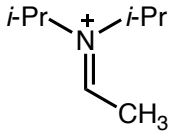
SCF total energy: -758.5640844 hartrees



Job type: Single point.
Method: UB3LYP
Basis set: 6-311+G**
Number of basis functions: 306
Number of electrons: 73 (1 unpaired)
Parallel Job: 4 threads

SCF model:
An unrestricted hybrid HF-DFT SCF calculation will be performed using Pulay DIIS + Geometric Direct Minimization

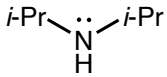
SCF total energy: -370.4927896 hartrees



Job type: Single point.
Method: RB3LYP
Basis set: 6-311+G**
Number of basis functions: 306
Charge: +1
Number of electrons: 72
Parallel Job: 2 threads

SCF model:
A restricted hybrid HF-DFT SCF calculation will be performed using Pulay DIIS + Geometric Direct Minimization

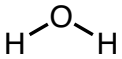
SCF total energy: -370.3192909 hartrees



Job type: Single point.
Method: RB3LYP
Basis set: 6-311+G**
Number of basis functions: 244
Number of electrons: 58
Parallel Job: 4 threads

SCF model:
A restricted hybrid HF-DFT SCF calculation will be performed using Pulay DIIS + Geometric Direct Minimization

SCF total energy: -292.5129296 hartrees



Job type: Single point.
Method: RB3LYP
Basis set: 6-311+G**
Number of basis functions: 34
Number of electrons: 10
Parallel Job: 4 threads

SCF model:
A restricted hybrid HF-DFT SCF calculation will be performed using Pulay DIIS + Geometric Direct Minimization

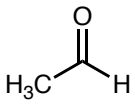
SCF total energy: -76.4584607 hartrees

^{-}OH

Job type: Single point.
Method: RB3LYP
Basis set: 6-311+G**
Number of basis functions: 28
Charge: -1
Number of electrons: 10
Parallel Job: 2 threads

SCF model:
A restricted hybrid HF-DFT SCF calculation will be performed using Pulay DIIS + Geometric Direct Minimization

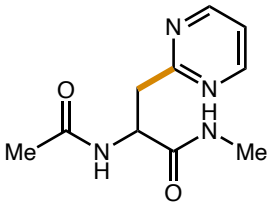
SCF total energy: -75.8273302 hartrees



Job type: Single point.
Method: RB3LYP
Basis set: 6-311+G**
Number of basis functions: 90
Number of electrons: 24
Parallel Job: 4 threads

SCF model:
A restricted hybrid HF-DFT SCF calculation will be performed using Pulay DIIS + Geometric Direct Minimization

SCF total energy: -153.8821290 hartrees



Job type: Single point.
Method: RB3LYP
Basis set: 6-311+G**
Number of basis functions: 436
Number of electrons: 118
Parallel Job: 4 threads

SCF model:

A restricted hybrid HF-DFT SCF calculation will be performed using Pulay DIIS + Geometric Direct Minimization

SCF total energy: -759.2045780 hartrees

HRMS of crude reaction

Protonated DIPEA Base calculated $[C_8H_{20}N]^+ = 130.16$
Protonated Diisopropylamine calculated $[C_6H_{16}N]^+ = 102.13$

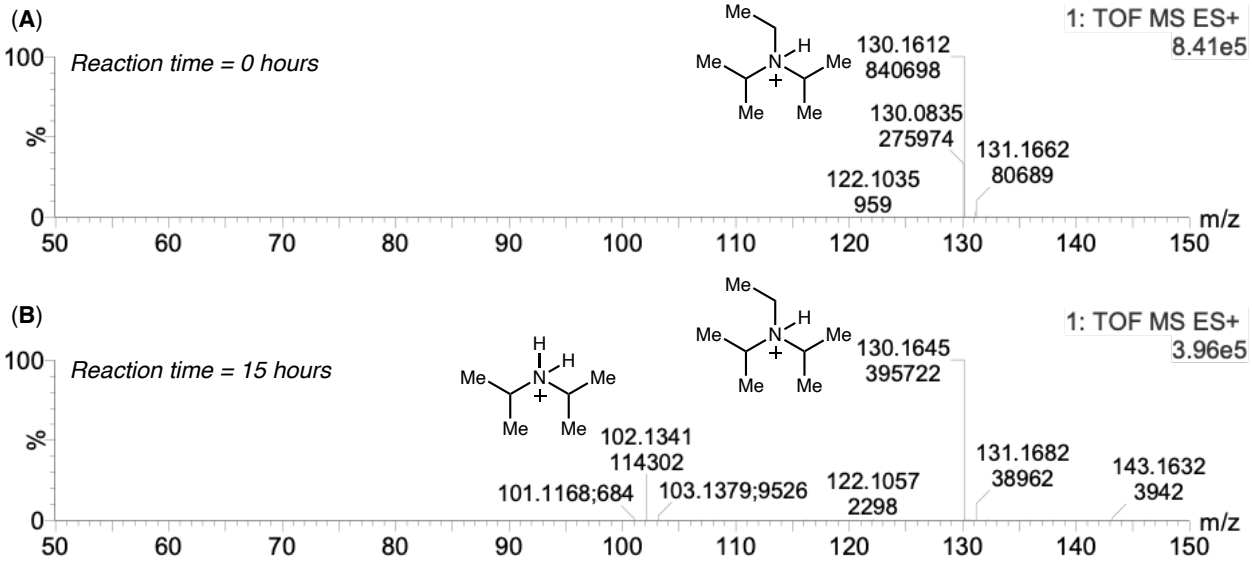
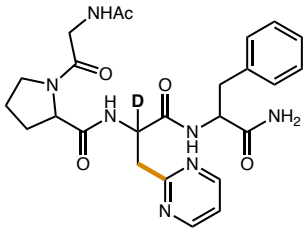


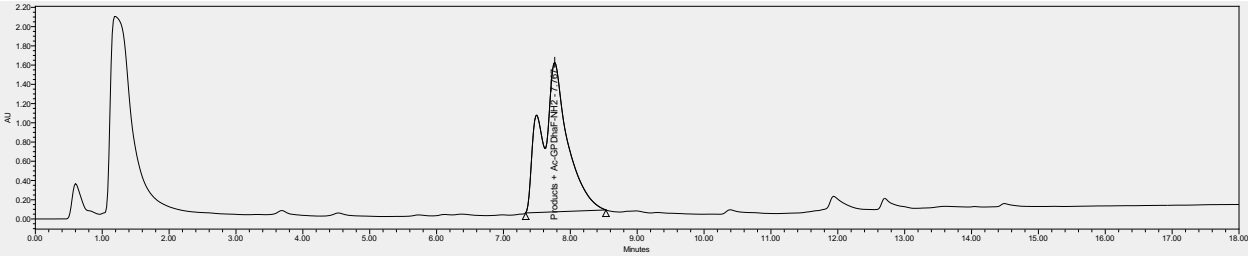
Figure S5. A) HRMS of the reaction with 2-bromopyrimidine before Green LED irradiation. B) HRMS of the reaction with 2-bromopyrimidine after 15 hours of Green LED irradiation.

Deuterium labeling experiment

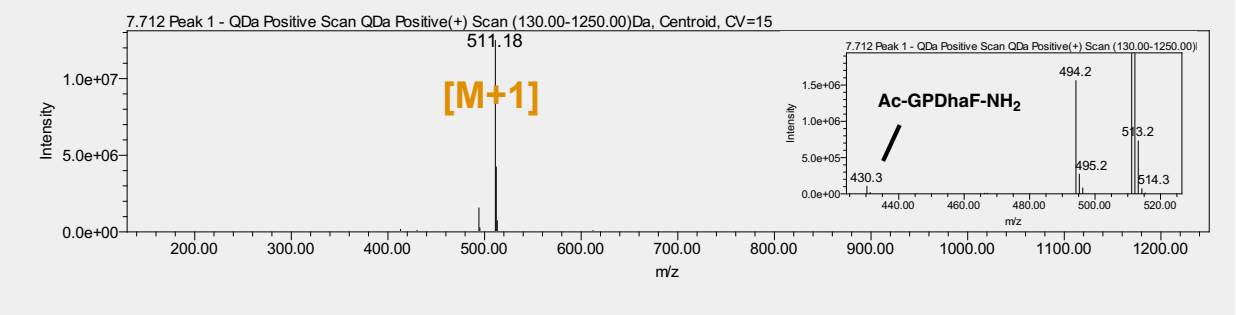


The reaction was set up according to the general procedure for pyrimidine sidechains with the exception of exchanging all ddH_2O for D_2O. After 15 hours, the crude reaction was then submitted to UPLC/MS analysis. >95% deuterium incorporation was observed.

Crude

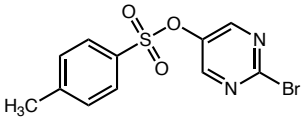


	Name	Retention Time	Area	% Area
1	Products + Ac-GPDhaF-NH2	7.767	42320533	100.00

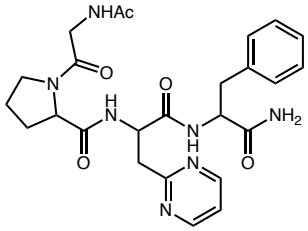


Analytical Data of Products

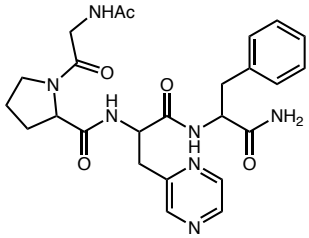
NMR shifts



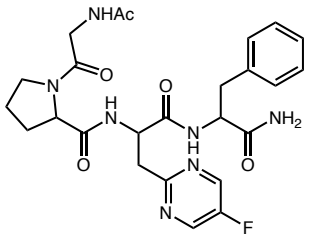
2-bromopyrimidin-5-yl 4-methylbenzenesulfonate
Isolated Yield 61% (220 mg). **^{1}H NMR** (400 MHz, Chloroform-*d*) δ 8.24 (s, 2H), 7.80 – 7.62 (m, 2H), 7.45 – 7.32 (m, 2H), 2.48 (s, 3H). **^{13}C NMR** (101 MHz, Chloroform-*d*) δ 153.55, 149.94, 147.14, 144.79, 130.58, 128.74, 21.96. **HRMS (ESI-ToF)** m/z calculated for $C_{11}H_{10}BrN_2O_3S$ $[MH]^+$: 328.9596. Found 328.9593.



Compound 1
^{1}H NMR (400 MHz, CD_3CN) δ 8.62 (d, *J* = 4.9 Hz, 2H), 7.96 – 7.31 (m, 2H), 7.28 – 7.10 (m, 6H), 6.99 – 6.68 (m, 2H), 6.00 (m, 1H), 4.77 – 4.58 (m, 1H), 4.51 – 4.30 (m, 1H), 4.20 (m, 1H), 4.05 – 3.82 (m, 2H), 3.67 – 3.42 (m, 2H), 3.44 – 3.29 (m, 1H), 3.28 – 3.09 (m, 2H), 3.03 – 2.78 (m, 1H), 2.19 – 1.65 (m, 4H), 1.84 – 1.81 (-NHCO**CH_3** two s, 3H).

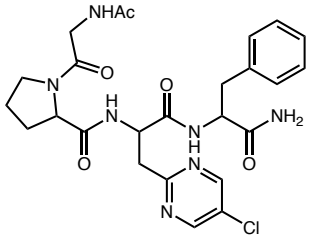


Compound 2
^{1}H NMR (400 MHz, CD_3CN) δ 8.51 – 8.32 (m, 3H), 7.69 – 7.38 (m, 2H), 7.36 – 7.12 (m, 5H), 7.02 – 6.63 (m, 2H), 5.87 (m, 1H), 4.61 – 4.46 (m, 1H), 4.41 (ddt, *J* = 9.6, 8.5, 4.8 Hz, 1H), 4.23 – 4.09 (m, 1H), 4.06 – 3.78 (m, 2H), 3.66 – 3.41 (m, 2H), 3.33 – 3.00 (m, 3H), 2.89 (m, 1H), 2.14 – 1.63 (m, 4H), 1.86 – 1.81 (-NHCO**CH_3** two s, 3H).



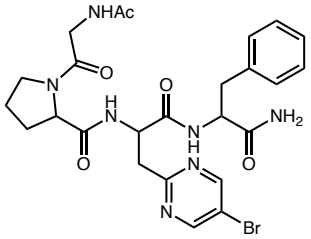
Compound 3

^{1}H NMR (400 MHz, CD_3CN) δ 8.55 (dd, *J* = 5.1, 0.9 Hz, 2H), 7.31 – 7.14 (m, 5H), 4.68 (ddd, *J* = 7.7, 6.7, 5.1 Hz, 1H), 4.41 (ddd, *J* = 9.6, 4.9, 2.5 Hz, 1H), 4.30 – 4.11 (m, 1H), 4.10 – 3.77 (m, 2H), 3.68 – 3.41 (m, 2H), 3.32 (m, 1H), 3.24 – 3.13 (m, 2H), 2.89 (ddd, *J* = 14.2, 9.8, 4.5 Hz, 1H), 2.16 – 1.68 (m, 4H), 1.86 – 1.81 (-NHCO**CH_3** two s, 3H). **^{19}F NMR** (376 MHz, CD_3CN) δ -142.02, -142.55.



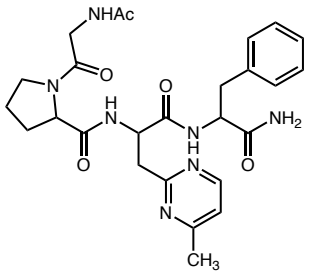
Compound 4

^{1}H NMR (400 MHz, CD_3CN) δ 8.63 (d, *J* = 8.1 Hz, 2H), 7.21 (dtd, *J* = 15.7, 6.7, 2.1 Hz, 5H), 4.74 – 4.67 (m, 1H), 4.46 – 4.39 (m, 1H), 4.24 – 4.14 (m, 1H), 4.06 – 3.80 (m, 2H), 3.62 – 3.44 (m, 2H), 3.37 – 3.12 (m, 4H), 2.14 – 1.69 (m, 4H), 1.86 – 1.82 (-NHCO**CH_3** two s, 3H).



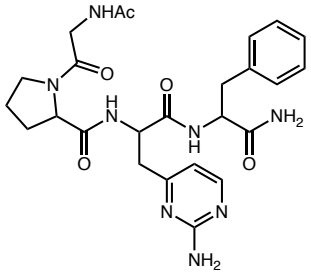
Compound 5

^{1}H NMR (400 MHz, CD_3CN) δ 8.71 (d, *J* = 8.3 Hz, 2H), 7.31 – 7.13 (m, 5H), 4.74 – 4.68 (m, 1H), 4.45 – 4.39 (m, 1H), 4.19 (ddd, *J* = 14.1, 12.4, 5.5 Hz, 1H), 4.05 – 3.80 (m, 2H), 3.63 – 3.43 (m, 2H), 3.33 – 3.10 (m, 3H), 2.85 (m, 1H), 2.14 – 1.68 (m, 4H), 1.87 – 1.82 (-NHCO**CH_3** two s, 3H).



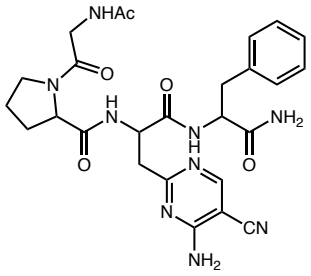
Compound 6

^{1}H NMR (400 MHz, CD_3CN) δ 8.45 (dd, *J* = 10.0, 5.2 Hz, 1H), 7.91 – 7.44 (m, 2H), 7.30 – 7.15 (m, 5H), 7.11 (dd, *J* = 7.9, 5.2 Hz, 1H), 7.07 – 6.74 (m, 2H), 6.04 (d, *J* = 23.2 Hz, 1H), 4.66 (dddd, *J* = 8.6, 7.3, 5.0, 2.7 Hz, 1H), 4.41 (dddd, *J* = 9.6, 8.5, 7.5, 4.8 Hz, 1H), 4.26 – 4.16 (m, 1H), 4.06 – 3.81 (m, 2H), 3.67 – 3.46 (m, 2H), 3.31 – 3.05 (m, 3H), 2.89 (ddd, *J* = 14.0, 9.7, 4.3 Hz, 1H), 2.42 – 2.41 (s, 3H), 2.16 – 1.68 (m, 4H), 1.85 – 1.81 (-NHCO**CH_3** two s, 3H).



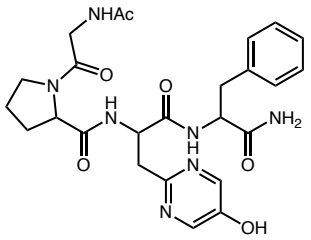
Compound 7

^{1}H NMR (400 MHz, CD_3CN) δ 8.09 (t, *J* = 4.4 Hz, 1H), 7.77 (dd, *J* = 121.5, 8.4 Hz, 1H), 7.47 (dd, *J* = 21.4, 7.8 Hz, 1H), 7.30 – 7.14 (m, 6H), 6.97 – 6.63 (m, 2H), 6.42 (t, *J* = 5.4 Hz, 1H), 5.73 (dd, *J* = 133.9, 36.3 Hz, 2H), 4.56 – 4.35 (m, 2H), 4.30 – 4.12 (m, 1H), 3.97 – 3.73 (m, 2H), 3.67 – 3.42 (m, 2H), 3.29 – 2.72 (m, 4H), 2.06 – 1.71 (m, 4H), 1.86 – 1.83 (-NHCO**CH_3** two s, 3H).



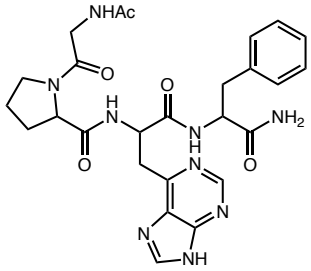
Compound 8

^{1}H NMR (400 MHz, CD_3CN) δ 8.42 – 8.29 (m, 1H), 7.30 – 7.10 (m, 5H), 4.74 – 4.62 (m, 1H), 4.49 – 4.39 (m, 1H), 4.28 – 4.20 (m, 1H), 4.17 – 3.79 (m, 2H), 3.63 – 3.39 (m, 2H), 3.10 – 3.06 (m, 2H), 2.98 – 2.80 (m, 2H), 2.13 – 1.68 (m, 4H), 1.85 (-NHCO**CH_3** two s, 3H).



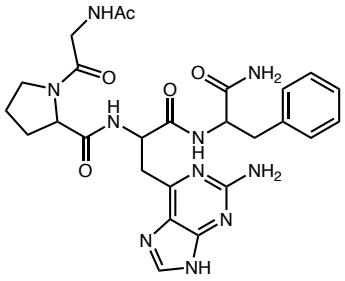
Compound 9

^{1}H NMR (400 MHz, CD_3CN) δ 8.17 (s, 2H), 7.32 – 7.11 (m, 5H), 4.63 – 4.57 (m, 1H), 4.46 – 4.35 (m, 1H), 4.29 – 4.17 (m, 1H), 4.04 – 3.82 (m, 2H), 3.67 – 3.42 (m, 2H), 3.21 – 3.06 (m, 4H), 2.15 – 1.69 (m, 4H), 1.87 – 1.82 (-NHCO**CH_3** two s, 3H).



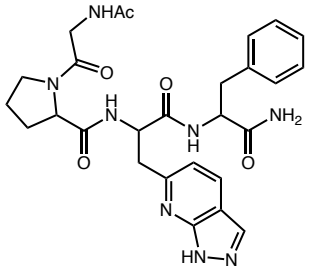
Compound 11

^{1}H NMR (400 MHz, CD_3CN) δ 8.74 (t, *J* = 6.4 Hz, 1H), 8.32 (d, *J* = 15.2 Hz, 1H), 7.25 – 7.11 (m, 5H), 4.79 (t, *J* = 4.5 Hz, 1H), 4.46 – 4.37 (m, 1H), 4.21 – 4.13 (m, 1H), 4.03 – 3.79 (m, 2H), 3.65 – 3.40 (m, 4H), 3.20 – 3.11 (m, 1H), 2.92 – 2.82 (m, 1H), 2.13 – 1.54 (m, 4H), 1.87 – 1.81 (-NHCO**CH_3** two s, 3H).



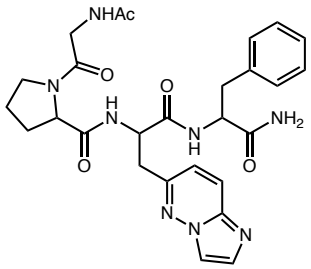
Compound 12

^{1}H NMR (400 MHz, CD_3CN) δ 7.93 (d, *J* = 19.7 Hz, 1H), 7.27 – 7.11 (m, 5H), 4.77 – 4.68 (m, 1H), 4.44 (ddd, *J* = 14.9, 9.2, 5.4 Hz, 1H), 4.21 (ddt, *J* = 13.0, 8.8, 4.9 Hz, 1H), 4.00 – 3.81 (m, 2H), 3.62 – 3.41 (m, 2H), 3.36 – 3.09 (m, 4H), 2.07 – 1.52 (m, 4H), 1.87 – 1.82 (-NHCO**CH_3** two s, 3H).



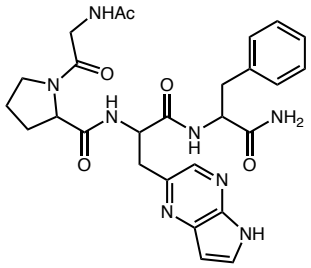
Compound 13

^{1}H NMR (400 MHz, CD_3CN) δ 8.08 – 7.95 (m, 2H), 7.80 – 7.58 (m, 1H), 7.52 – 7.31 (m, 1H), 7.30 – 7.15 (m, 5H), 7.15 – 6.93 (m, 2H), 6.84 – 6.65 (m, 2H), 5.97 – 5.66 (m, 1H), 4.71 – 4.49 (m, 1H), 4.48 – 4.29 (m, 1H), 4.25 – 4.15 (m, 1H), 4.05 – 3.76 (m, 2H), 3.66 – 3.42 (m, 2H), 3.41 – 3.11 (m, 2H), 3.10 – 2.81 (m, 2H), 2.02 – 1.66 (m, 4H), 1.89 – 1.81 (-NHCO**CH_3** two s, 3H).



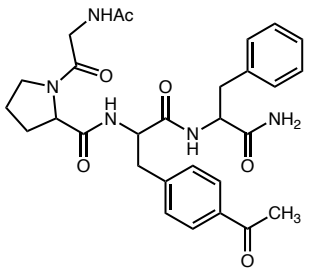
Compound 14

^{1}H NMR (400 MHz, CD_3CN) δ 7.94 (m, 1H), 7.81 (d, *J* = 9.4 Hz, 1H), 7.68 – 7.57 (m, 3H), 7.48 (dd, *J* = 26.9, 8.0 Hz, 1H), 7.30 – 7.16 (m, 5H), 6.98 (dd, *J* = 9.4, 2.5 Hz, 1H), 6.88 – 6.76 (m, 1H), 5.86 (d, *J* = 24.7 Hz, 1H), 4.50 – 4.39 (m, 2H), 4.22 – 4.15 (m, 1H), 4.15 – 3.78 (m, 2H), 3.65 – 3.41 (m, 2H), 3.37 – 3.29 (m, 1H), 3.25 – 3.04 (m, 2H), 2.98 – 2.84 (m, 1H), 2.09 – 1.60 (m, 4H), 1.84 (-NHCO**CH_3** two s, 3H).



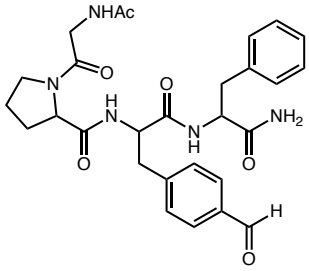
Compound 15

^{1}H NMR (400 MHz, CD_3CN) δ 8.01 (d, *J* = 3.3 Hz, 1H), 7.68 (dd, *J* = 7.6, 3.7 Hz, 1H), 7.24 – 7.14 (m, 5H), 6.55 (dd, *J* = 6.4, 3.6 Hz, 1H), 4.52 (ddd, *J* = 16.8, 8.7, 4.8 Hz, 1H), 4.41 (ddd, *J* = 9.8, 4.7, 2.2 Hz, 1H), 4.23 – 4.14 (m, 1H), 4.12 – 3.81 (m, 2H), 3.68 – 3.40 (m, 2H), 3.34 – 3.05 (m, 3H), 2.88 (ddd, *J* = 13.5, 9.7, 2.7 Hz, 1H), 2.12 – 1.60 (m, 4H), 1.85 – 1.81 (-NHCO**CH_3** two s, 3H).



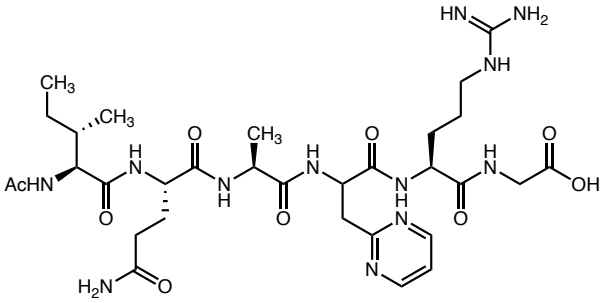
Compound 19

^{1}H NMR (400 MHz, CD_3CN) δ 7.83 (m, 2H), 7.34 – 7.16 (m, 7H), 4.47 – 4.41 (m, 1H), 4.37 (dd, *J* = 10.1, 4.9 Hz, 1H), 4.22 – 4.11 (m, 1H), 4.03 – 3.75 (m, 2H), 3.63 – 3.38 (m, 2H), 3.28 – 3.03 (m, 2H), 2.94 – 2.85 (m, 2H), 2.53 (s, 3H), 2.06 – 1.74 (m, 4H), 1.89 – 1.83 (-NHCO**CH_3** two s, 3H).



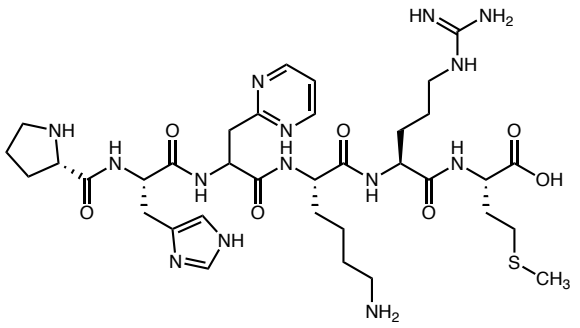
Compound 20

^{1}H NMR (400 MHz, CD_3CN) δ 9.95 (s, 1H), 7.78 (d, *J* = 7.9 Hz, 2H), 7.56 – 7.40 (m, 2H), 7.39 – 7.17 (m, 7H), 7.06 – 6.57 (m, 2H), 5.82 (d, *J* = 31.6 Hz, 1H), 4.46 – 4.36 (m, 1H), 4.25 (ddd, *J* = 10.3, 8.1, 4.7 Hz, 1H), 4.14 (dd, *J* = 8.2, 5.0 Hz, 1H), 4.05 – 3.78 (m, 2H), 3.65 – 3.39 (m, 2H), 3.31 – 3.07 (m, 2H), 3.06 – 2.84 (m, 2H), 2.05 – 1.63 (m, 4H), 1.88 – 1.81 (-NHCO**CH_3** two s, 3H).



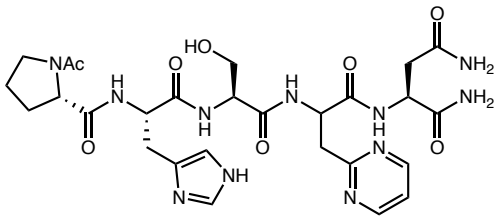
Compound 21

^{1}H NMR (500 MHz, MeOD) δ 8.74 (dd, *J* = 5.1, 2.8 Hz, 2H), 7.46 – 7.42 (m, 1H), 4.99 (dd, *J* = 8.5, 5.4 Hz, 1H), 4.94 – 4.89 (m, 1H), 4.44 – 4.35 (m, 1H), 4.31 (dt, *J* = 9.3, 5.0 Hz, 1H), 4.24 (dt, *J* = 11.3, 7.1 Hz, 1H), 4.19 – 4.08 (m, 2H), 3.75 (d, *J* = 10.5 Hz, 2H), 3.57 – 3.33 (m, 2H), 3.23 – 3.15 (m, 2H), 2.33 (q, *J* = 7.3 Hz, 2H), 2.02 (d, *J* = 2.9 Hz, 3H), 2.14 – 1.87 (m, 2H), 1.85 – 1.69 (m, 2H), 1.68 – 1.44 (m, 2H), 1.40 – 1.15 (m, 4H), 0.89 (dt, *J* = 12.2, 7.3 Hz, 6H).



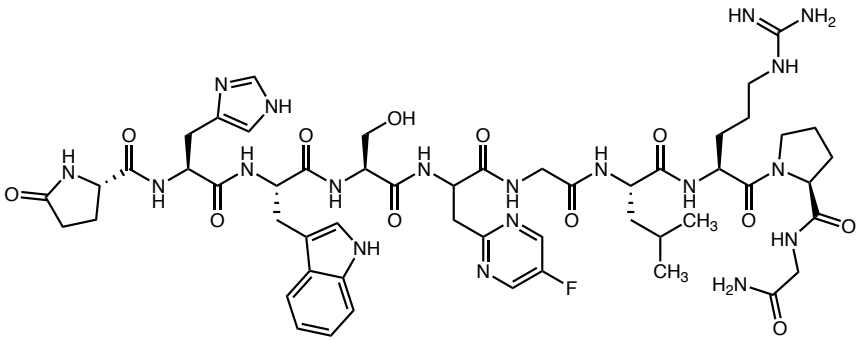
Compound 22

^{1}H NMR (500 MHz, MeOD) δ 8.74 (m, 2H), 7.92 – 7.77 (m, 1H), 7.43 (t, *J* = 5.0 Hz, 1H), 7.06 – 6.92 (m, 1H), 4.97 (dd, *J* = 8.0, 5.7 Hz, 1H), 4.91 (dd, *J* = 8.3, 5.6 Hz, 1H), 4.59 (dd, *J* = 8.4, 5.5 Hz, 1H), 4.53 (dd, *J* = 8.2, 6.6 Hz, 1H), 4.40 – 4.31 (m, 1H), 4.30 – 4.21 (m, 1H), 3.50 – 3.41 (m, 2H), 3.40 – 3.33 (m, 2H), 3.23 – 3.16 (m, 2H), 3.14 – 3.01 (m, 2H), 3.00 – 2.93 (m, 2H), 2.53 – 2.41 (m, 6H), 2.15 – 2.06 (m, 4H), 2.08 (d, *J* = 2.8 Hz, 3H), 2.00 – 1.85 (m, 4H), 1.73 – 1.64 (m, 4H).



Compound 23

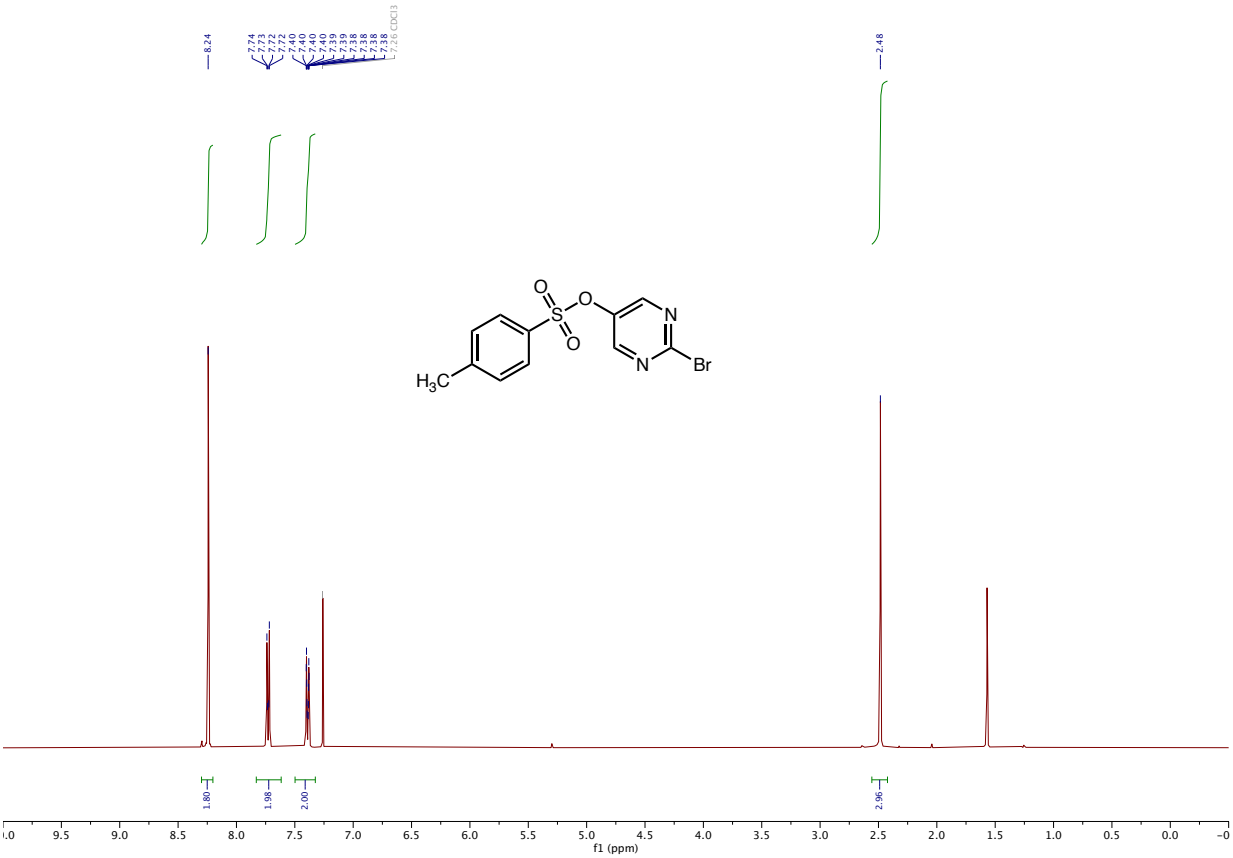
^{1}H NMR (500 MHz, MeOD) δ 8.75 – 8.69 (m, 2H), 7.61 (m, 1H), 7.36 (m, 1H), 6.91 (m, 1H), 4.99 – 4.96 (m, 1H), 4.70 – 4.66 (m, 1H), 4.57 – 4.51 (m, 1H), 4.36 – 4.31 (m, 1H), 4.30 – 4.25 (m, 1H), 3.86 – 3.77 (m, 1H), 3.76 – 3.64 (m, 2H), 3.63 – 3.55 (m, 1H), 3.54 – 3.39 (m, 2H), 3.19 – 2.98 (m, 2H), 2.80 – 2.63 (m, 2H), 2.23 – 2.15 (m, 1H), 2.15 – 2.10 (-NCO**CH$_3$** two s, 3H), 2.00 – 1.83 (m, 3H).

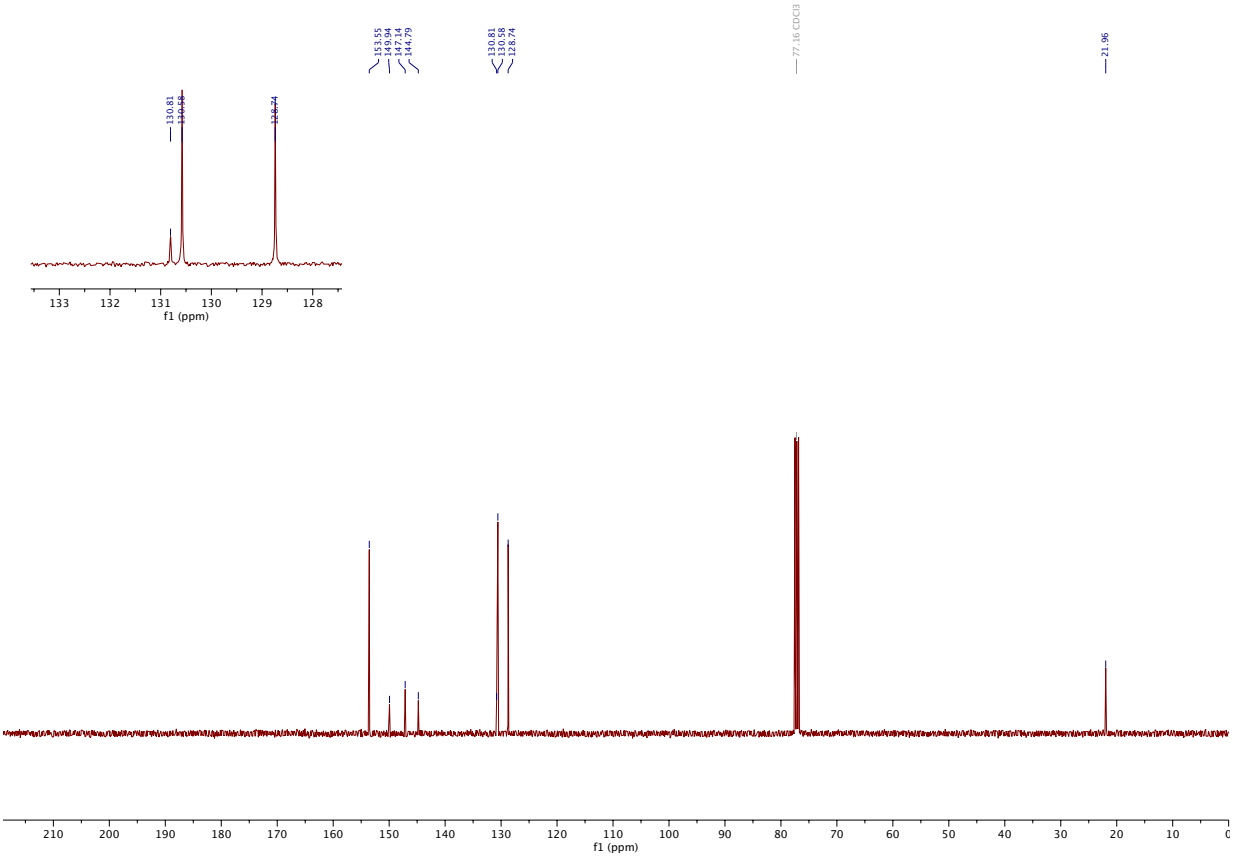


Compound 24

^{1}H NMR (500 MHz, MeOD) δ 8.64 (d, *J* = 2.4 Hz, 2H), 7.53 – 7.33 (m, 4H), 7.13 (m, 1H), 7.07 – 6.99 (m, 1H), 6.90 (d, *J* = 26.8 Hz, 1H), 4.92 – 4.87 (m, 2H), 4.59 – 4.55 (m, 1H), 4.54 – 4.48 (m, 1H), 4.41 – 4.32 (m, 2H), 4.28 – 4.13 (m, 2H), 3.98 – 3.82 (m, 4H), 3.81 – 3.71 (m, 3H), 3.70 – 3.37 (m, 5H), 3.25 – 3.11 (m, 4H), 2.38 – 2.17 (m, 3H), 2.20 (s, 6H), 1.94 – 1.88 (m, 2H), 1.68 – 1.58 (m, 4H), 0.95 – 0.83 (m, 6H). **^{19}F NMR** (376 MHz, MeOD) δ -140.64, -140.71.

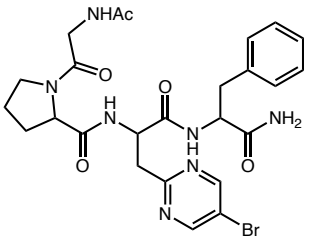
NMR Small Molecule Spectrum





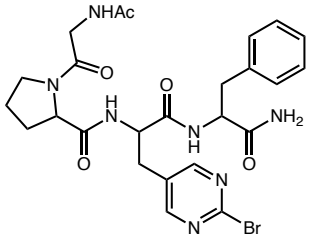
Determining the chemoselectivity of compound 5

5A



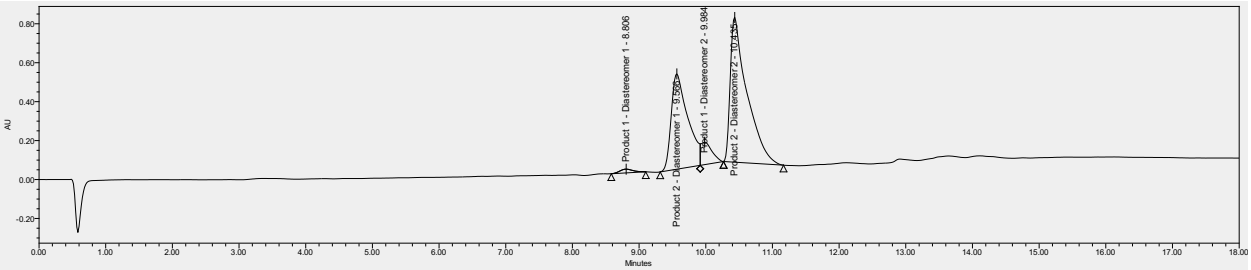
or

5B

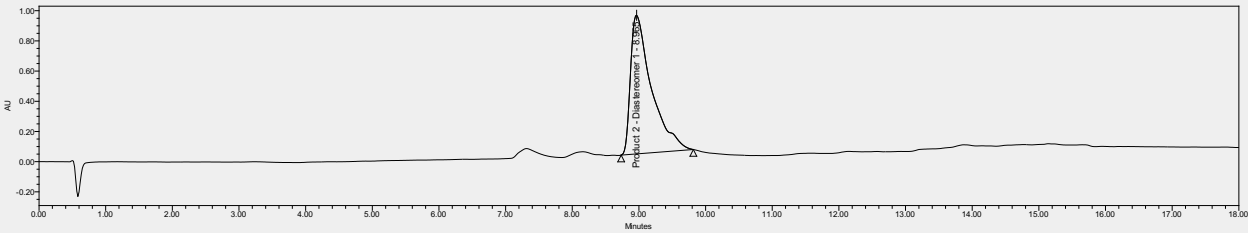


To assess whether the diastereomers of *c*NP **5A** or **5B** were the major products in this reaction, the purified mixture of **5** was dissolved in CD_3CN and subjected to ^1H NMR analysis. Two sets of diastereomeric protons were observed in the 8 ppm to 9 ppm region that seemed to correlate with the pyrimidine C-Hs. The major products appeared to belong to **5A** due to the greater downfield shift expected in **5A** than **5B** because of the ortho bromine atom present. To validate that the peaks at 8.7 ppm belong to **5A**, the purified mixture was subjected to High Performance Liquid Chromatography (HPLC) purification using a Alltech Econosil C18 (10 μ, 4.6 x 250 mm) HPLC column with acetonitrile:H_2O. The two major diastereomers were isolated and subjected to UPLC/MS analysis to determine purity and ^1H NMR analysis to determine the correct identity. ^1H NMR confirmed that the 8.7 ppm peaks belonged to **5A** making **5A** the major product and **5B** the minor product in this reaction.

Purified Product Mixture:

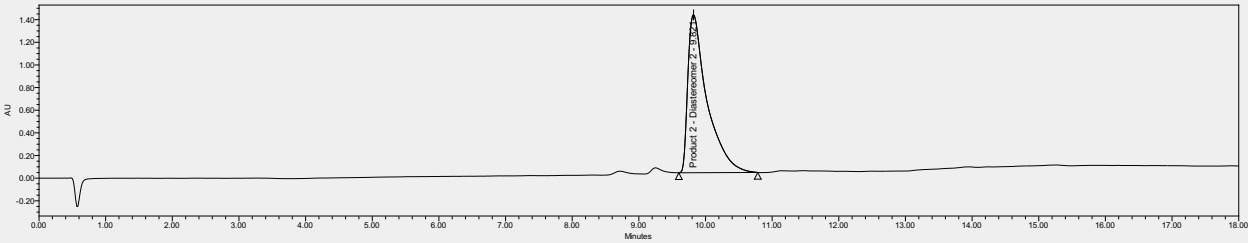


Major Product - Diastereomer 1:



	Name	Retention Time	Area	% Area
1	Product 2 - Diastereomer 1	8.965	20005346	100.00

Major Product - Diastereomer 2:



	Name	Retention Time	Area	% Area
1	Product 2 - Diastereomer 2	9.821	27807872	100.00

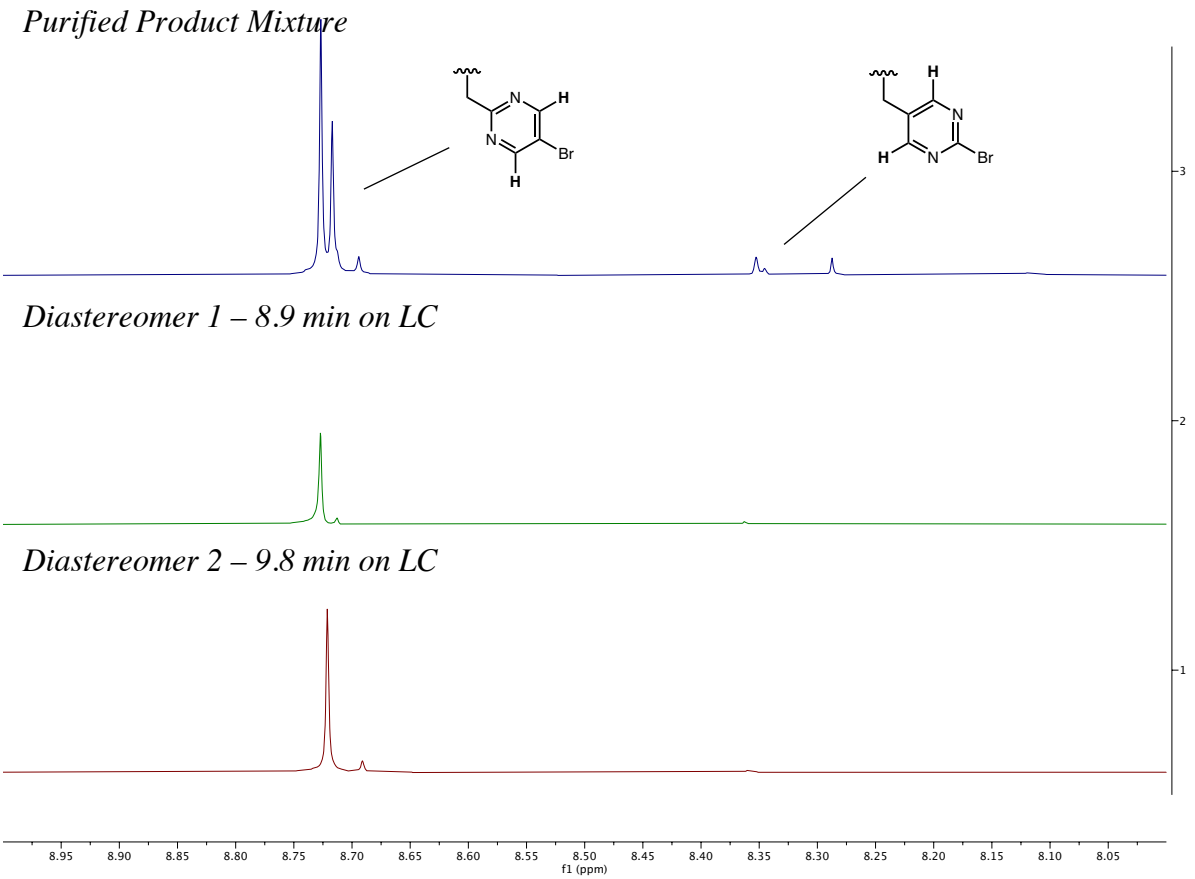


Figure S6. Determination of the chemoselectivity of product **5** by ^{1}H NMR with CD_3CN as solvent.

Peptide products from batch reactions

UPLC Column Methods:

A = Acetonitrile with 1% formic acid modifier, B = ddH_2O with 1% formic acid modifier.

For compounds **1-16**, **18-20**: Waters Zorbax StableBondC18 3.5 μ, 2.1 x 100 mm column (0.5 mL/min flow rate).

Time:	%A:	%B:
0.0	5.0	95.0
2.0	5.0	95.0
3.0	10.0	90.0
10.0	20.0	80.0
12.0	30.0	70.0
16.5	95.0	5.0
18.0	95.0	5.0

For compound **17**: Waters XBridge BEH C18 (2.5 μ, 4.6 x 150 mm) column (1.0 mL/min flow rate).

Time:	%A:	%B:
0.0	5.0	95.0
2.0	5.0	95.0
8.0	95.0	5.0
10.0	95.0	5.0
11.5	5.0	95.0
12.0	5.0	95.0

For compound **21-24**: Supelco Discovery Bio Wide Pore C8 (3.0 μ, 4.6 mm x 10 cm) column).

*Compounds **22** and **23**:*
0.4 mL/min flow rate

Time:	%A:	%B:
0.0	0.0	100.0
1.0	0.0	100.0
2.0	5.0	95.0
10.5	95.0	5.0
12.0	95.0	5.0

*Compounds **21** and **24**:*
0.5 mL/min flow rate

Time:	%A:	%B:
0.0	5.0	95.0
2.0	5.0	95.0
10.5	95.0	5.0
12.0	95.0	5.0

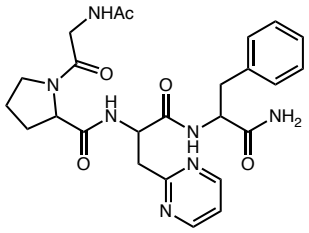
*Compounds **2** and **8** (for determining % conversions)*
0.5 mL/min flow rate

Time:	%A:	%B:
0.0	5.0	95.0
2.0	5.0	95.0
3.0	10.0	90.0
9.0	30.0	70.0
17.0	50.0	50.0
17.5	95.0	5.0
19.0	95.0	5.0
19.5	5.0	95.0
20.0	5.0	95.0

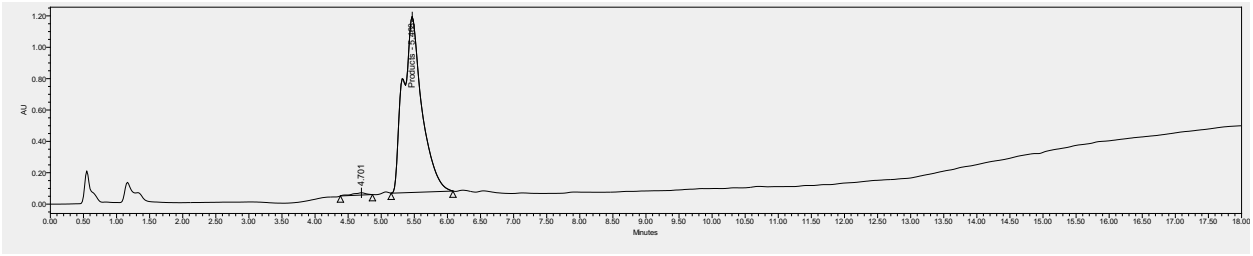
Note: ^{1}H NMR spectra were primarily taken in CD_3CN or CD_3OD to improve resolution, but a small amount of D_2O was also required for many peptides to achieve sufficient solubility.

1: Synthesized by the general procedure for pyrimidine sidechains

MW = 509.6, % Conversion = >99%, Isolated yield = 77% [9.1 mg]

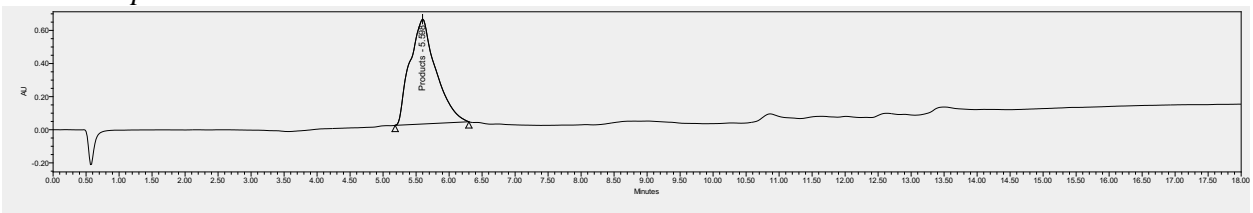


Crude:

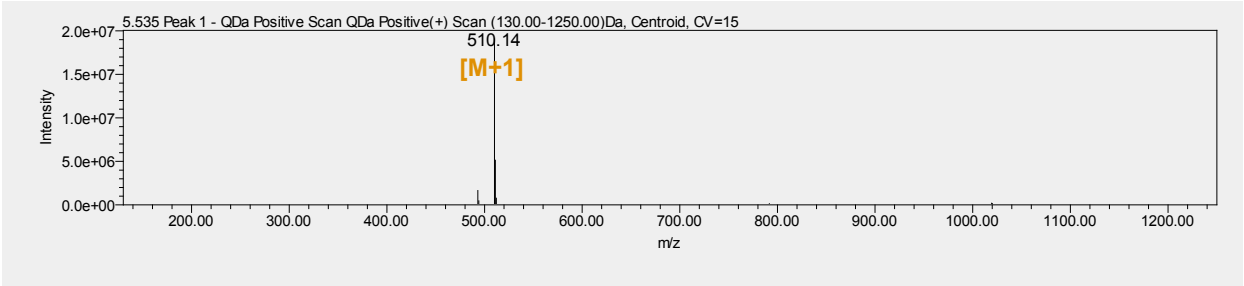


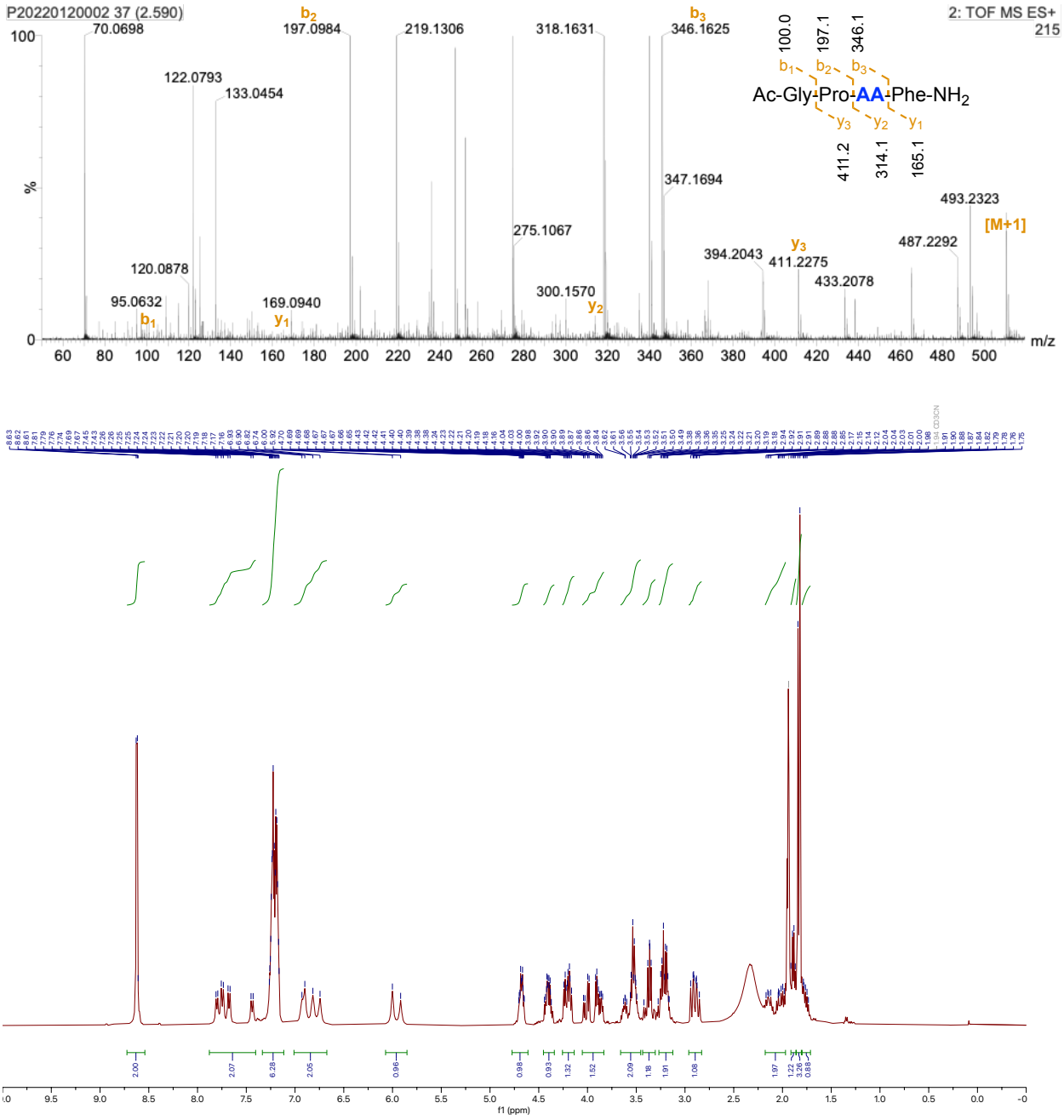
	Name	Retention Time	Area	% Area
1		4.701	208937	0.93
2	Products	5.468	22161585	99.07

Isolated Epimers:



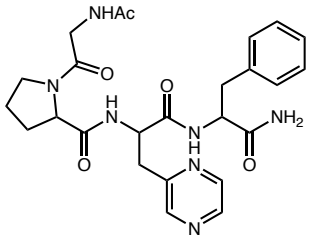
	Name	Retention Time	Area	% Area
1	Products	5.598	17300170	100.00



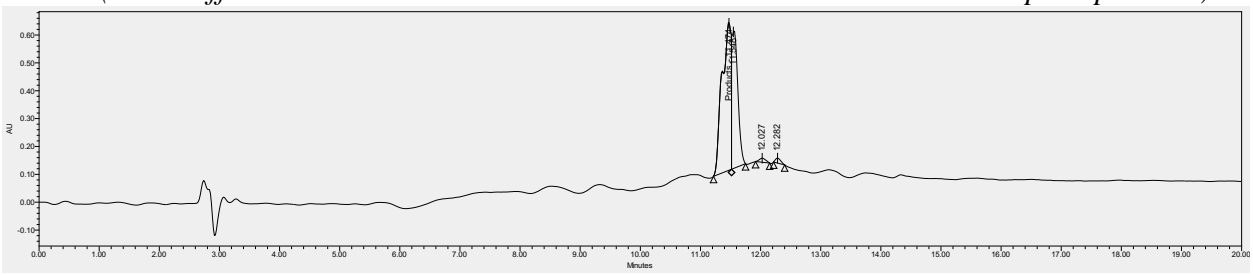

P20220120002 37 (2.590)
2: TOF MS ES+
215
100
%
0
70.0698
122.0793
133.0454
120.0878
95.0632
b1
169.0940
y1
b2
197.0984
219.1306
275.1067
300.1570
y2
318.1631
b3
346.1625
347.1694
394.2043
y3
411.2275
433.2078
487.2292
493.2323
[M+1]
100.0
197.1
346.1
b1 b2 b3
Ac-Gly-Pro-AA-Phe-NH2
y3 y2 y1
411.2
314.1
165.1
m/z
60 80 100 120 140 160 180 200 220 240 260 280 300 320 340 360 380 400 420 440 460 480 500
f1 (ppm)

2: Synthesized by the general procedure for pyrimidine sidechains with **deviation 2**

MW = 509.6, % Conversion = 60%, Isolated yield = 44% [5.2 mg]

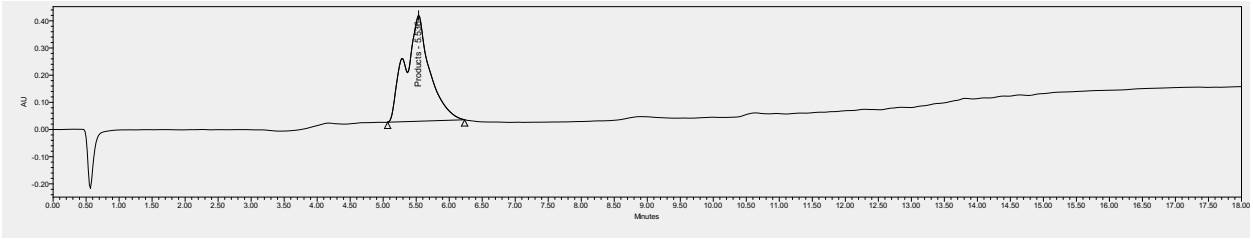


Crude: (Used different column method to determine % conversion due to overlap in spectrum)

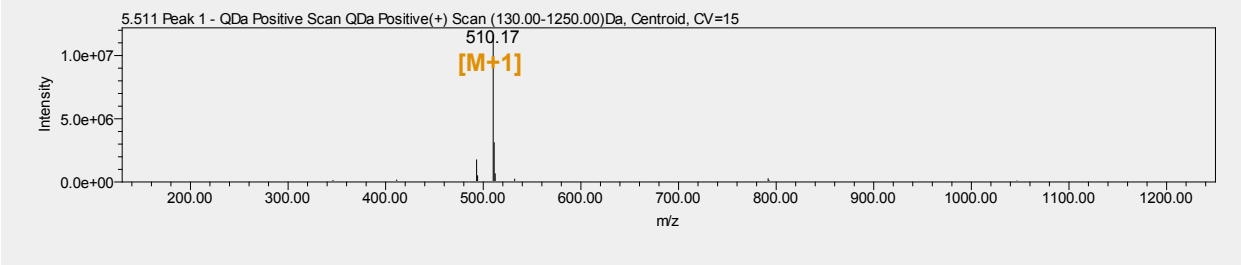


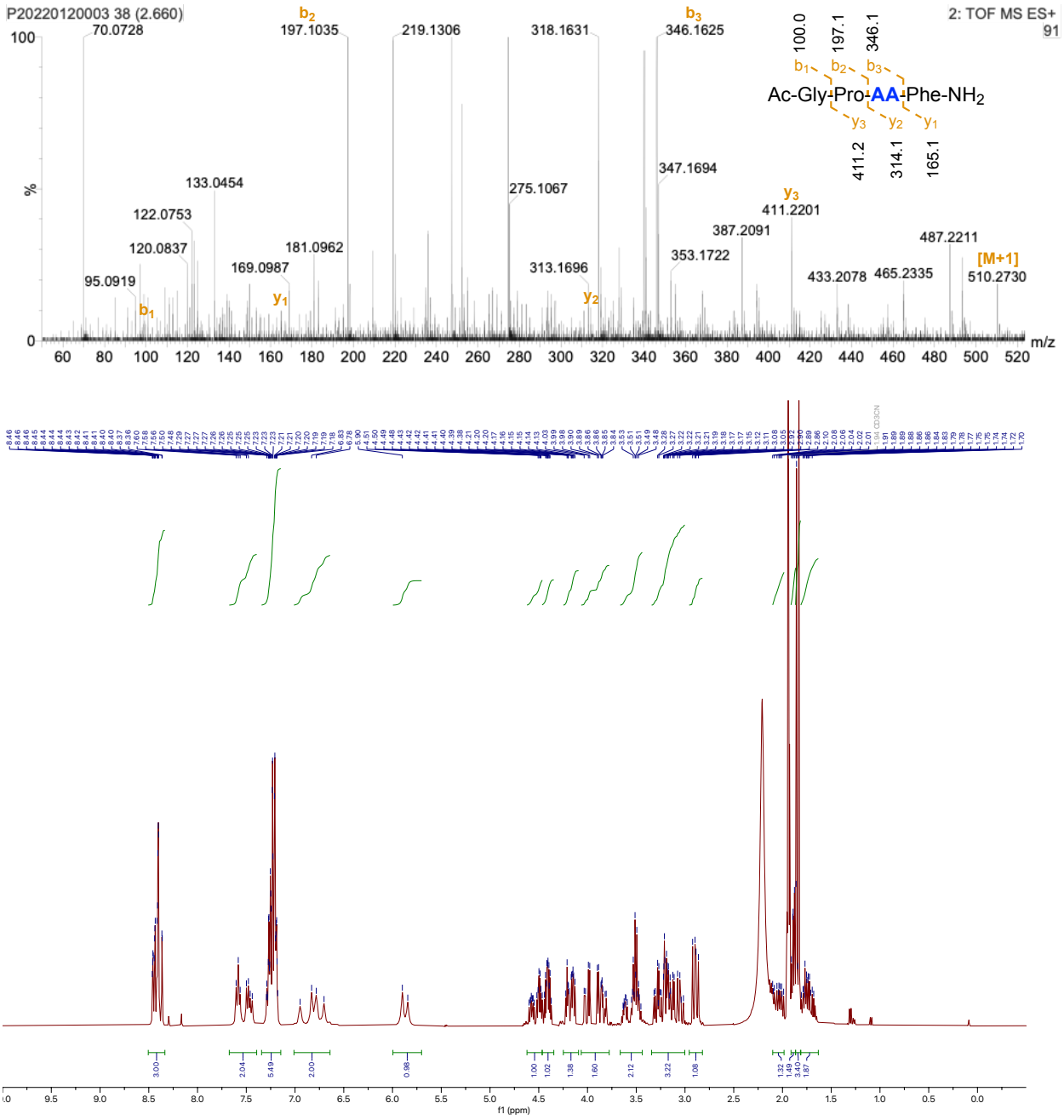
	Name	Retention Time	Area	% Area
1	Products	11.474	5491389	59.93
2		11.548	3452401	37.68
3		12.027	110226	1.20
4		12.282	109286	1.19

Isolated Epimers:



	Name	Retention Time	Area	% Area
1	Products	5.536	10028917	100.00




P20220120003 38 (2.660)
2: TOF MS ES+
91
100
%
0
m/z
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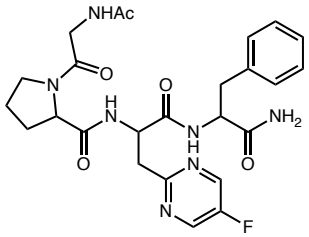
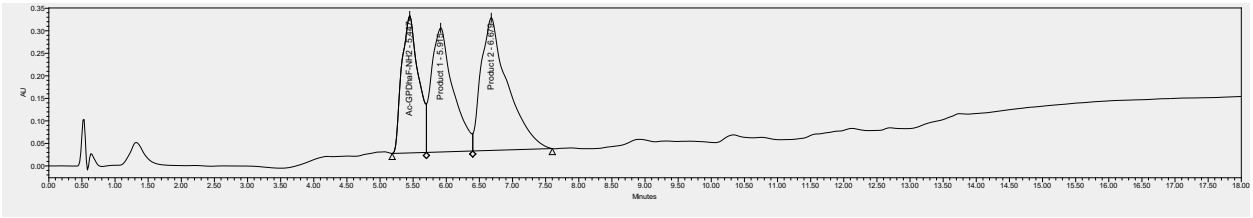
b2
197.1035
219.1306
318.1631
b3
346.1625
347.1694
133.0454
275.1067
y3
411.2201
122.0753
387.2091
487.2211
120.0837
181.0962
353.1722
[M+1]
169.0987
313.1696
433.2078
465.2335
510.2730
95.0919
y1
y2
b1
100.0
197.1
346.1
b1 b2 b3
Ac-Gly-Pro-AA-Phe-NH2
y3 y2 y1
411.2
314.1
165.1
f1 (ppm)

3: Synthesized by the general procedure for pyrimidine sidechains with **deviation 2**

MW = 527.6, % Conversion = 73%, Isolated yield = 47% [5.8 mg]

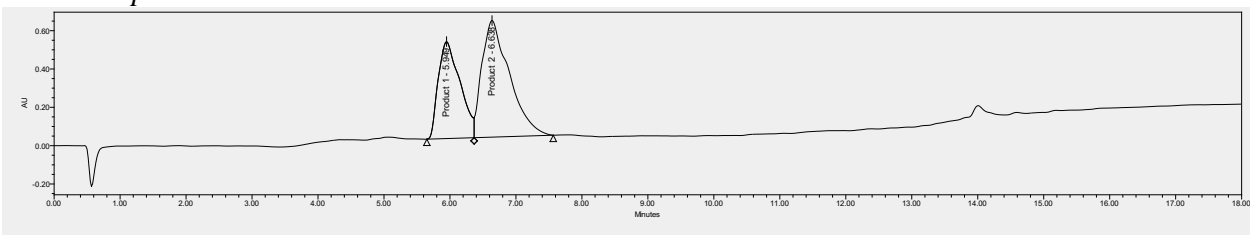


Crude:

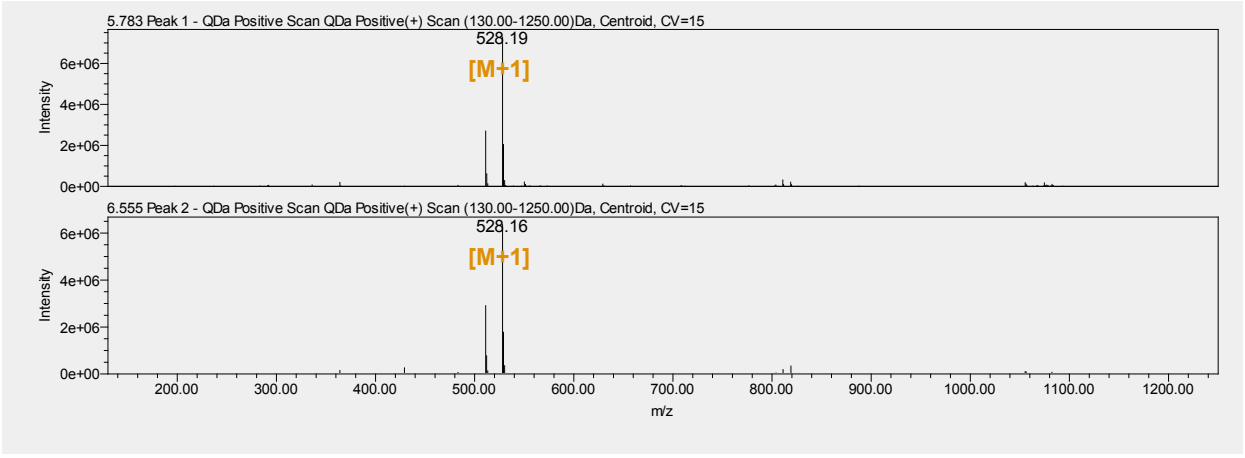


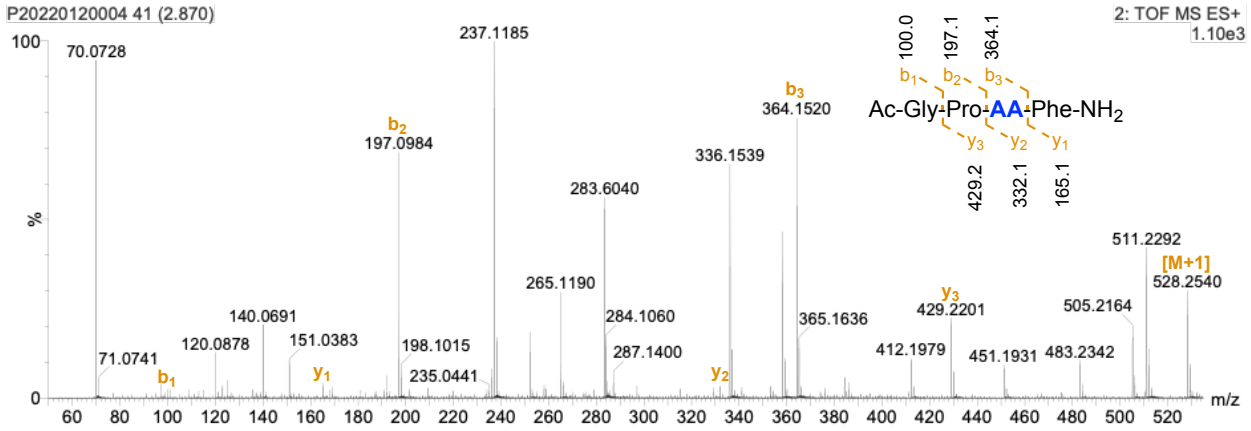
	Name	Retention Time	Area	% Area
1	Ac-GPDhaF-NH2	5.447	5301248	26.75
2	Product 1	5.915	6312401	31.85
3	Product 2	6.679	8205988	41.40

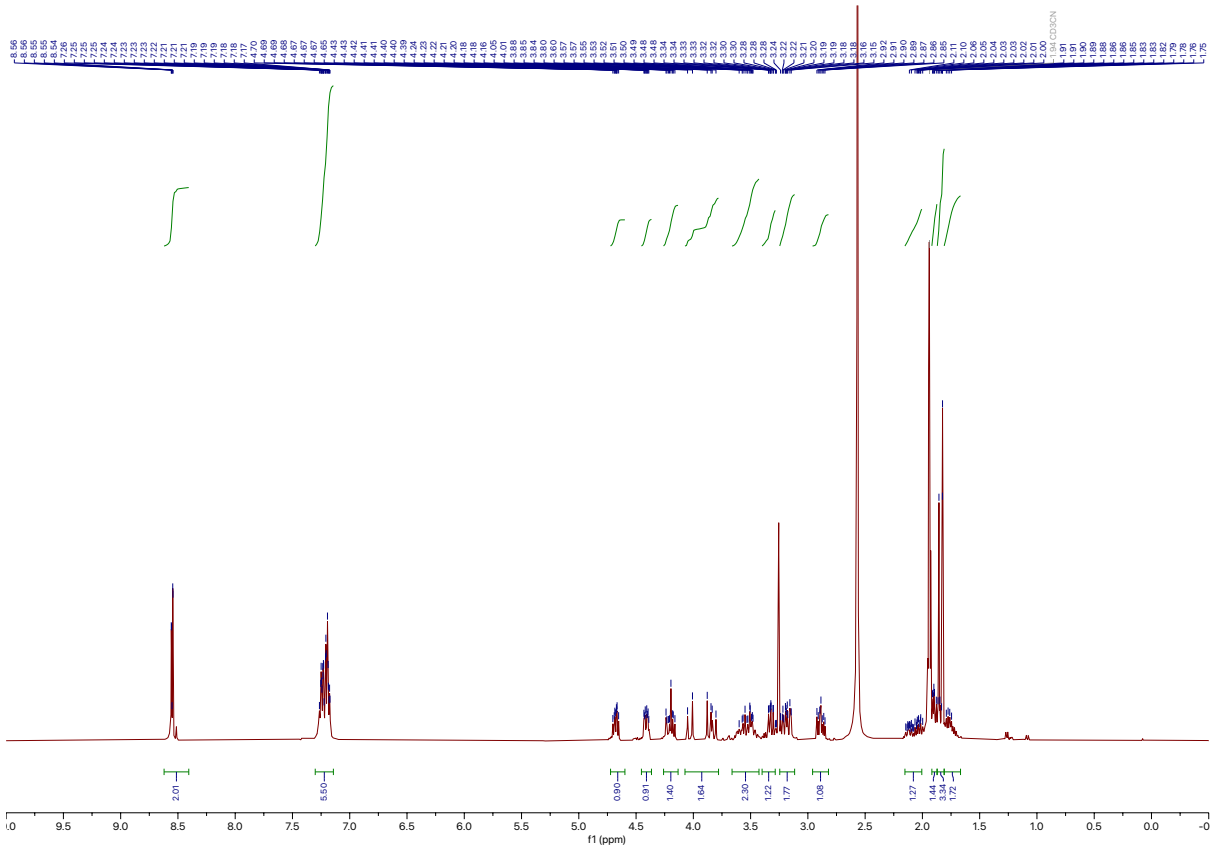
Isolated Epimers:

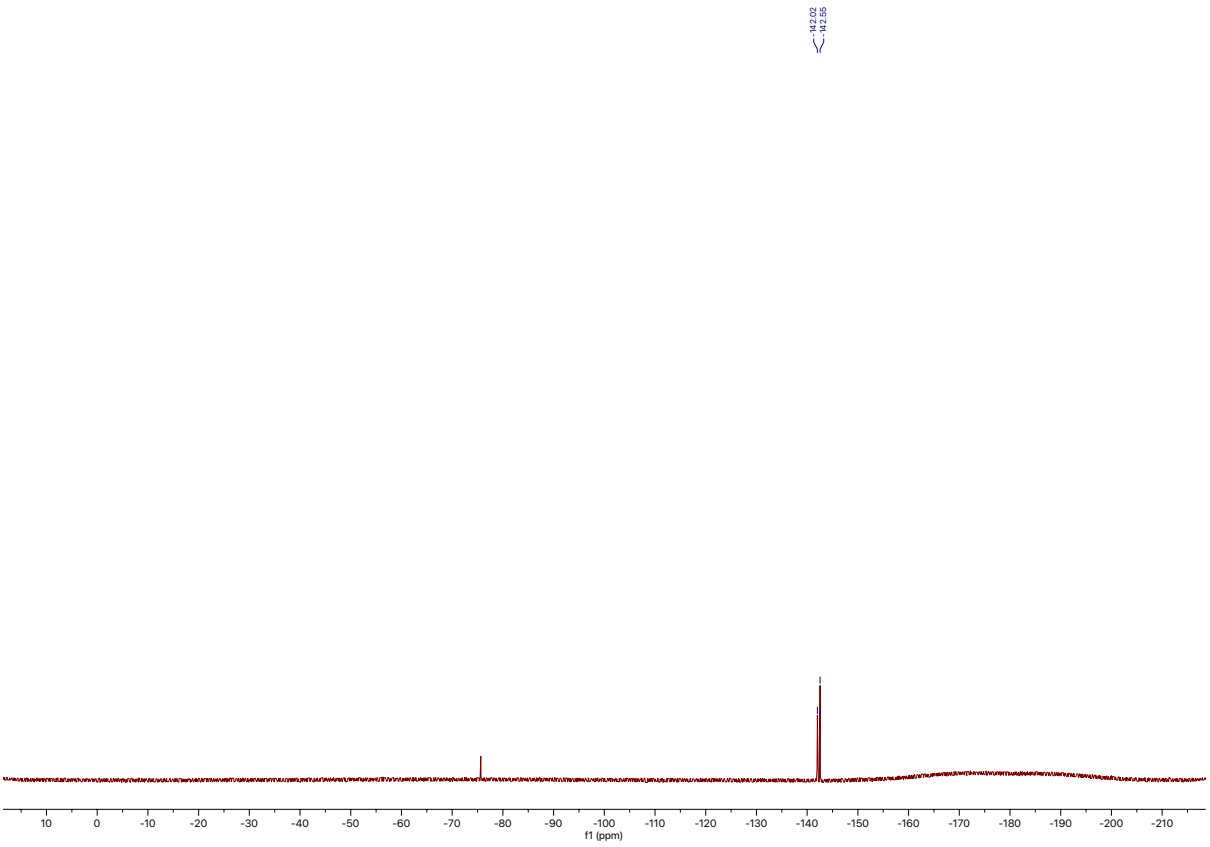


	Name	Retention Time	Area	% Area
1	Product 1	5.949	11585118	39.57
2	Product 2	6.638	17691956	60.43



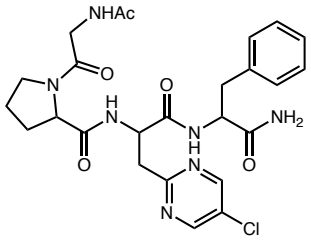

P20220120004 41 (2.870)
2: TOF MS ES+
1.10e3
100
%
0
70.0728
237.1185
197.0984
364.1520
336.1539
283.6040
265.1190
140.0691
120.0878
151.0383
71.0741
198.1015
235.0441
284.1060
287.1400
365.1636
412.1979
429.2201
451.1931
483.2342
505.2164
511.2292
528.2540
[M+1]
100.0
197.1
364.1
429.2
332.1
165.1
Ac-Gly-Pro-AA-Phe-NH2
m/z


f1 (ppm)

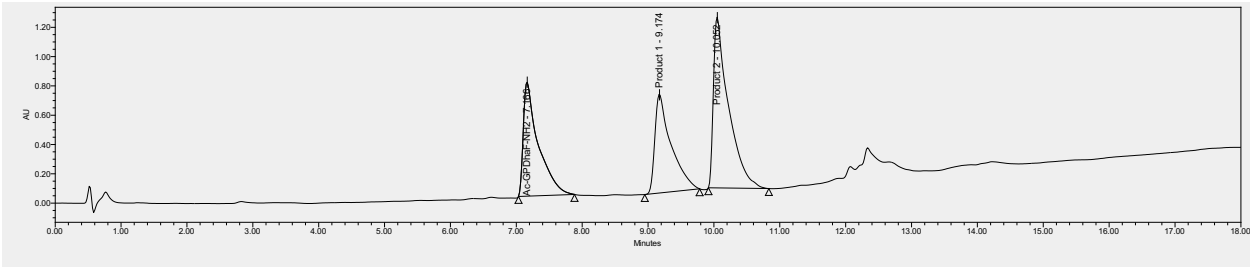

-142.02
-142.55
10
0
-10
-20
-30
-40
-50
-60
-70
-80
-90
-100
-110
-120
-130
-140
-150
-160
-170
-180
-190
-200
-210
f1 (ppm)

4: Synthesized by the general procedure for pyrimidine sidechains with **deviations 1 and 2**

MW = 544.0, % Conversion = 71%, Isolated yield = 51% [6.5 mg]

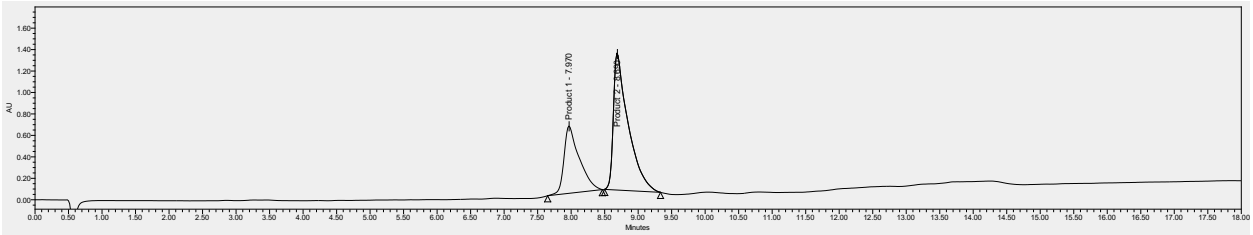


Crude:

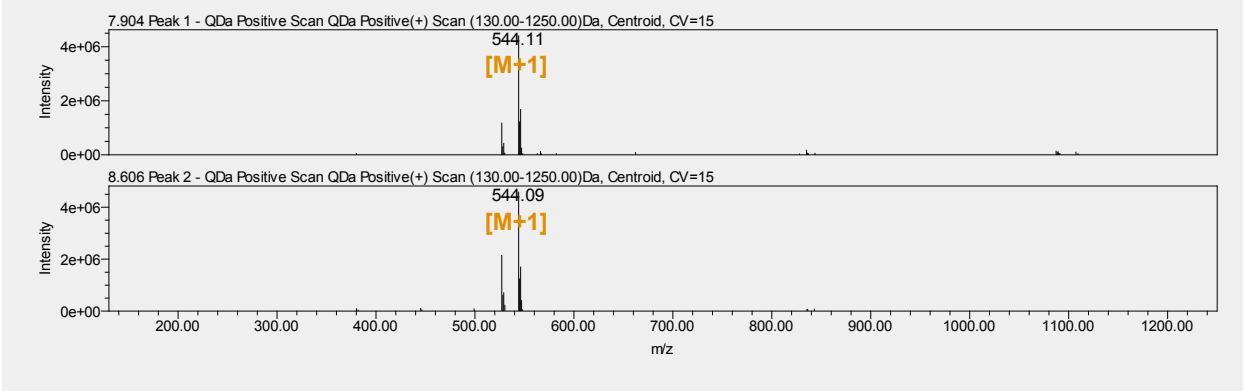


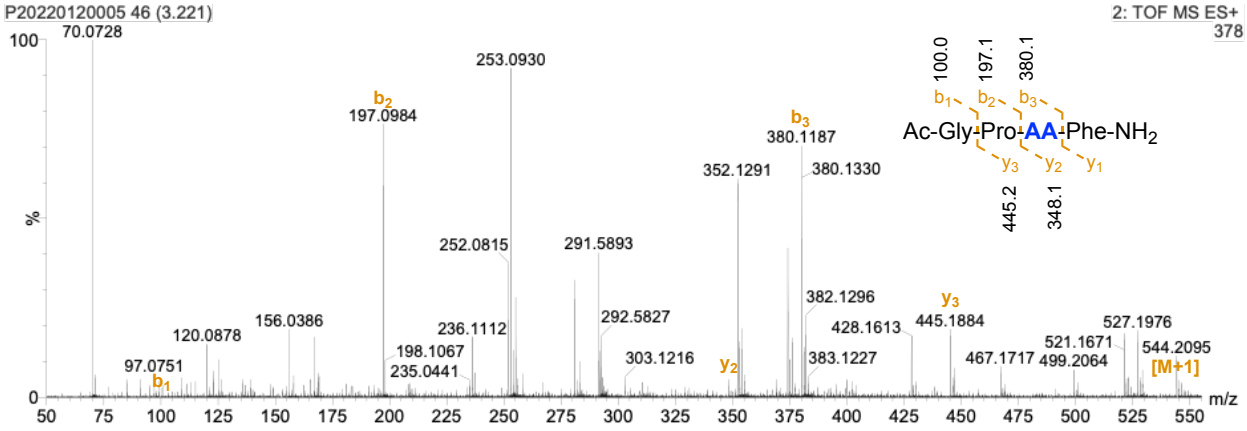
	Name	Retention Time	Area	% Area
1	Ac-GPDhaF-NH2	7.166	12271100	28.99
2	Product 1	9.174	11520827	27.22
3	Product 2	10.052	18539017	43.80

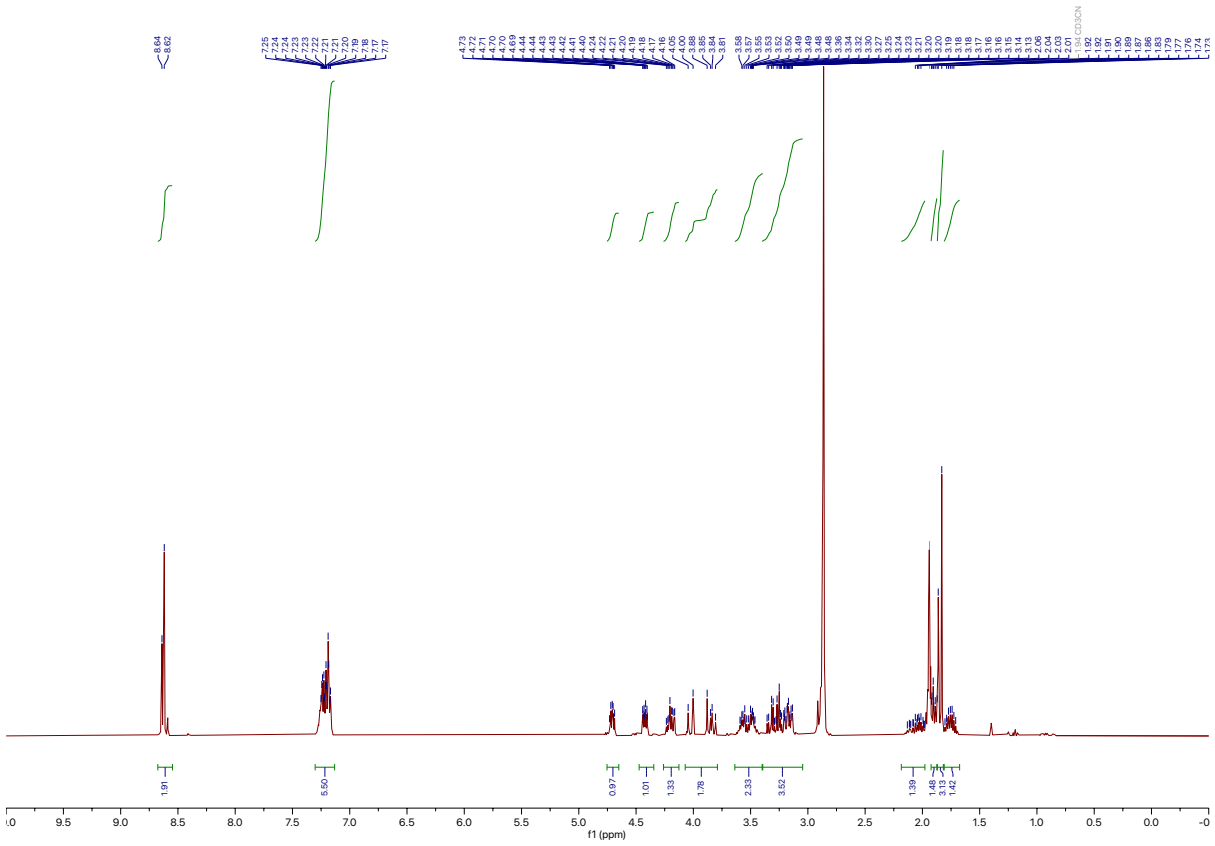
Isolated Epimers:



	Name	Retention Time	Area	% Area
1	Product 1	7.970	9937794	33.69
2	Product 2	8.690	19556276	66.31

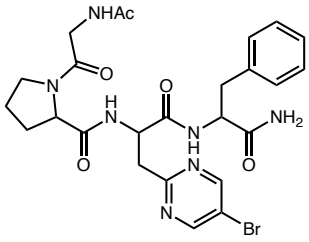



P20220120005 46 (3.221)
2: TOF MS ES+
378
100
%
0
70.0728
253.0930
b2
197.0984
b3
380.1187
352.1291
380.1330
252.0815
291.5893
382.1296
y3
445.1884
428.1613
292.5827
236.1112
156.0386
120.0878
198.1067
235.0441
303.1216
y2
383.1227
467.1717
521.1671
499.2064
527.1976
544.2095
[M+1]
97.0751
b1
m/z
50 75 100 125 150 175 200 225 250 275 300 325 350 375 400 425 450 475 500 525 550
100.0
197.1
380.1
b1 b2 b3
Ac-Gly-Pro-AA-Phe-NH2
y3 y2 y1
445.2
348.1

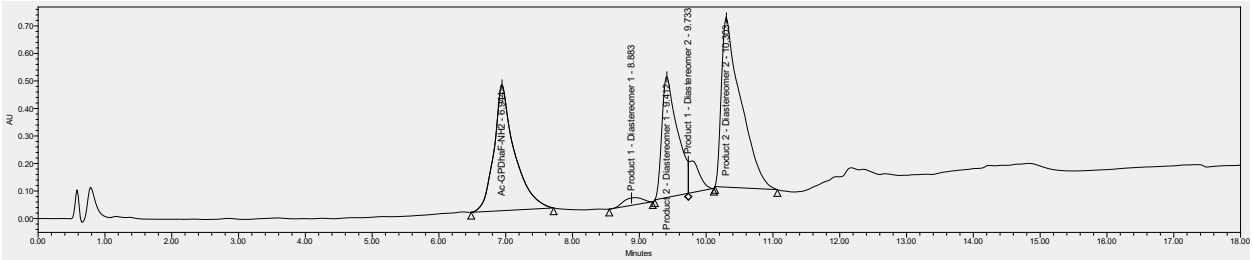

1.91
5.50
0.97
1.01
1.33
1.78
2.33
3.52
1.39
1.48
3.13
1.42
10 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0
f1 (ppm)

5: Synthesized by the general procedure for pyrimidine sidechains with **deviations 1 and 2**

MW = 588.5, % Conversion = 69%, Combined isolated yield = 46% [6.3 mg]

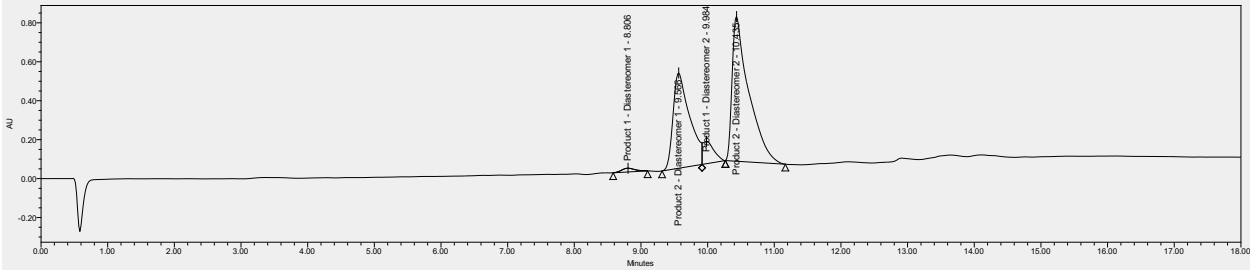


Crude:

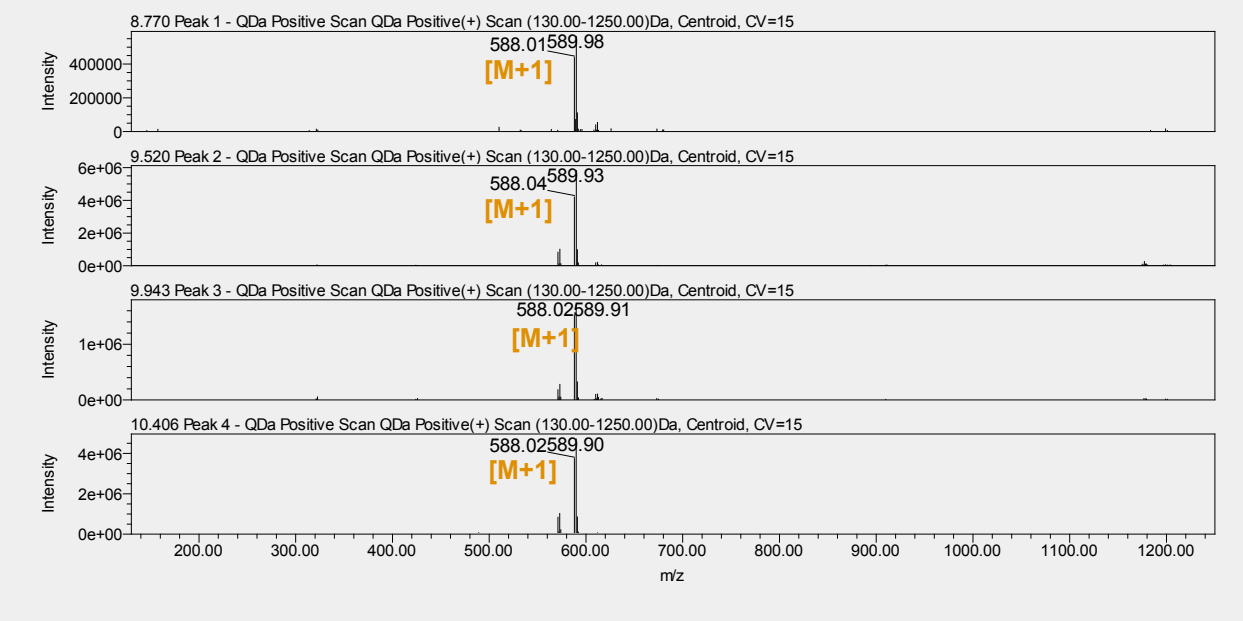


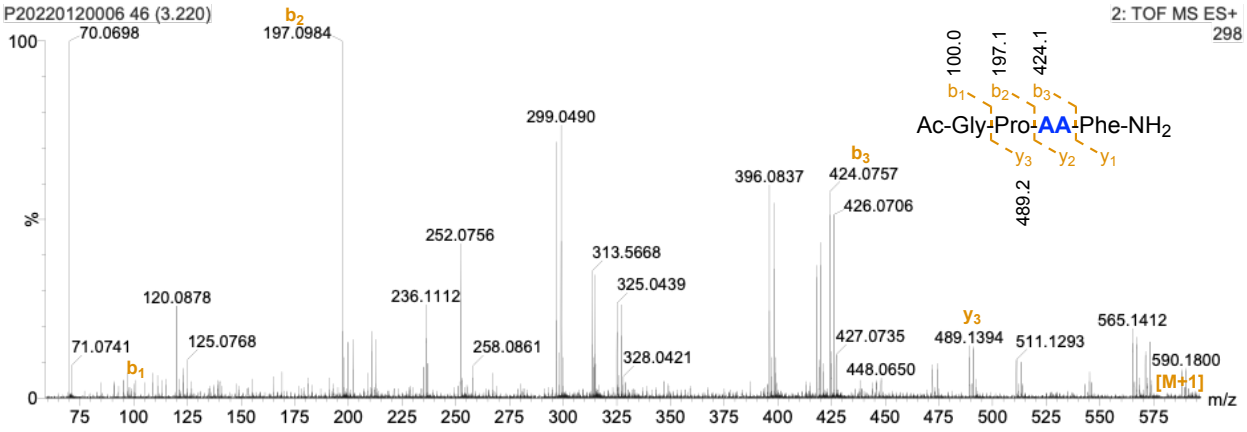
	Name	Retention Time	Area	% Area
1	Ac-GPDhaF-NH2	6.944	9759544	31.36
2	Product 1 - Diastereomer 1	8.883	533502	1.71
3	Product 2 - Diastereomer 1	9.412	7092923	22.79
4	Product 1 - Diastereomer 2	9.733	1291671	4.15
5	Product 2 - Diastereomer 2	10.303	12442885	39.98

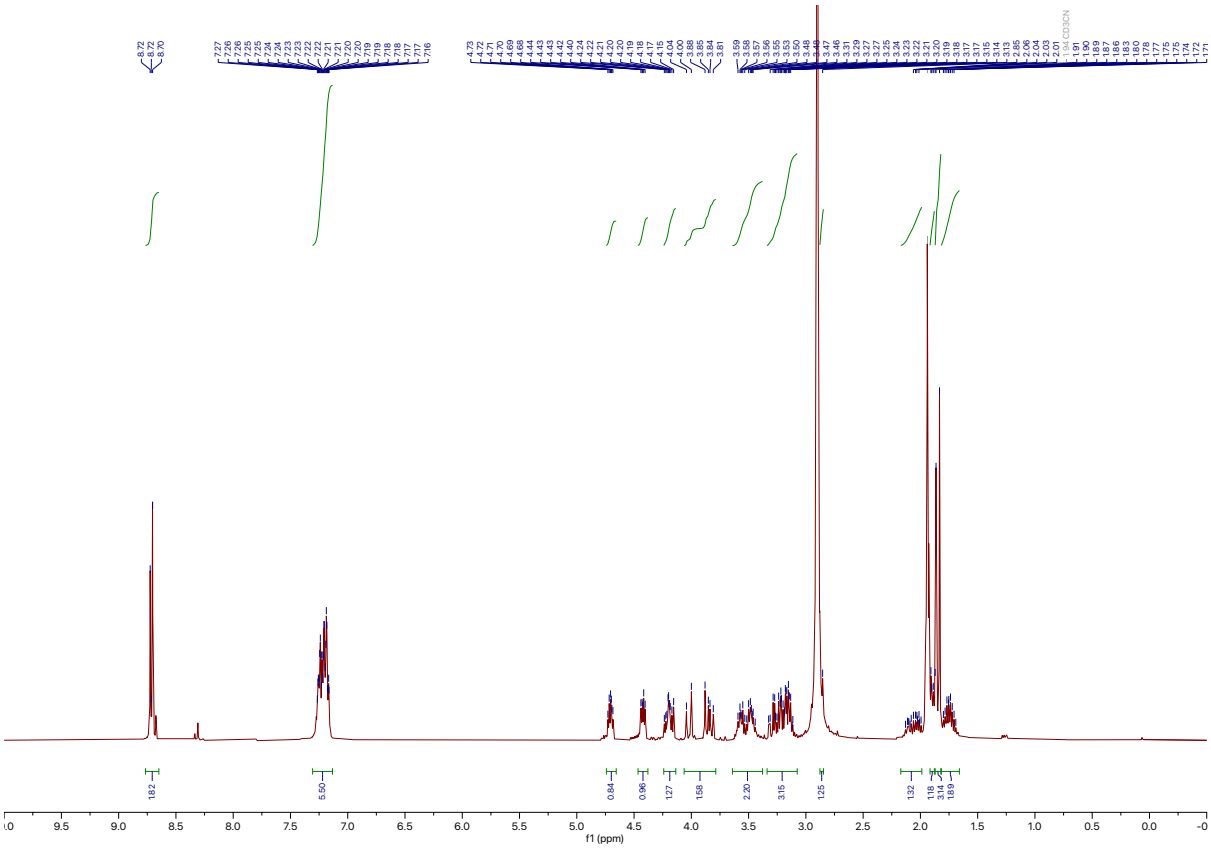
Isolated Epimers:



	Name	Retention Time	Area	% Area
1	Product 1 - Diastereomer 1	8.806	268653	1.17
2	Product 2 - Diastereomer 1	9.566	8422663	36.73
3	Product 1 - Diastereomer 2	9.984	1323721	5.77
4	Product 2 - Diastereomer 2	10.435	12913701	56.32

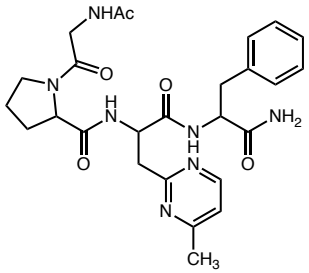

8.770 Peak 1 - QDa Positive Scan QDa Positive(+) Scan (130.00-1250.00)Da, Centroid, CV=15
588.01
589.98
[M+1]
9.520 Peak 2 - QDa Positive Scan QDa Positive(+) Scan (130.00-1250.00)Da, Centroid, CV=15
588.04
589.93
[M+1]
9.943 Peak 3 - QDa Positive Scan QDa Positive(+) Scan (130.00-1250.00)Da, Centroid, CV=15
588.02
589.91
[M+1]
10.406 Peak 4 - QDa Positive Scan QDa Positive(+) Scan (130.00-1250.00)Da, Centroid, CV=15
588.02
589.90
[M+1]
Intensity
m/z


P20220120006 46 (3.220)
2: TOF MS ES+
298
Ac-Gly-Pro-AA-Phe-NH2
b1
b2
b3
y3
y2
y1
100.0
197.1
424.1
489.2
70.0698
197.0984
299.0490
396.0837
424.0757
426.0706
252.0756
313.5668
325.0439
120.0878
236.1112
125.0768
258.0861
328.0421
71.0741
427.0735
448.0650
489.1394
511.1293
565.1412
590.1800
[M+1]
%
m/z

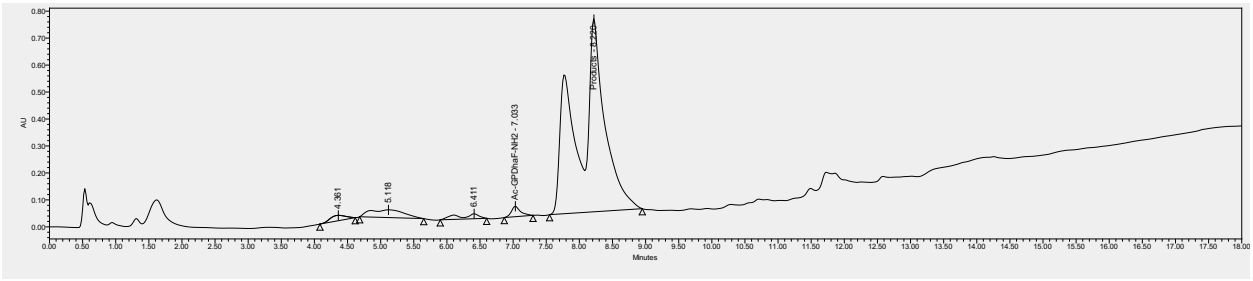

f1 (ppm)

6: Synthesized by the general procedure for pyrimidine sidechains with **deviation 1**

MW = 523.6, % Conversion = 90%, Isolated yield = 62% [7.6 mg]

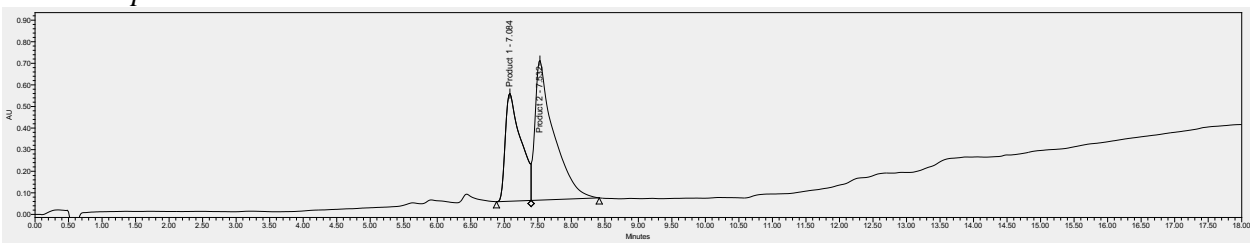


Crude:

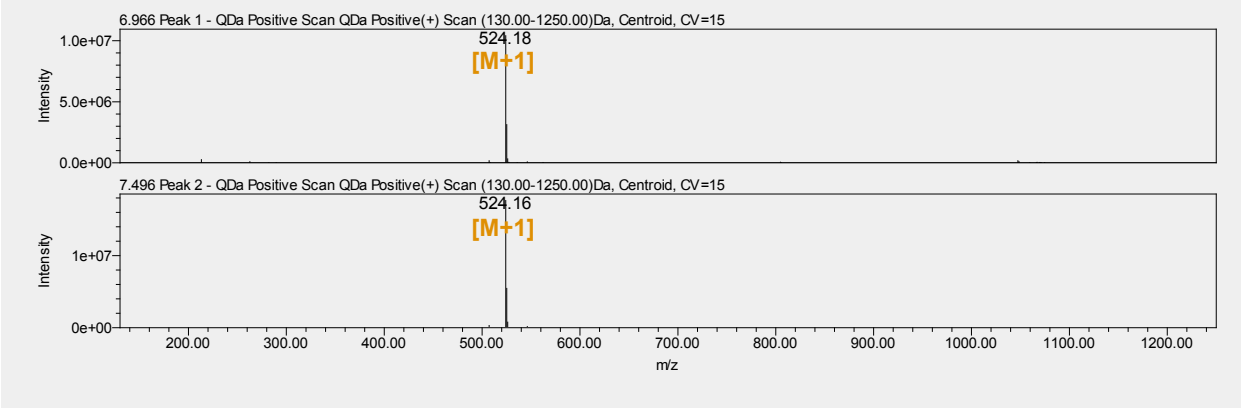


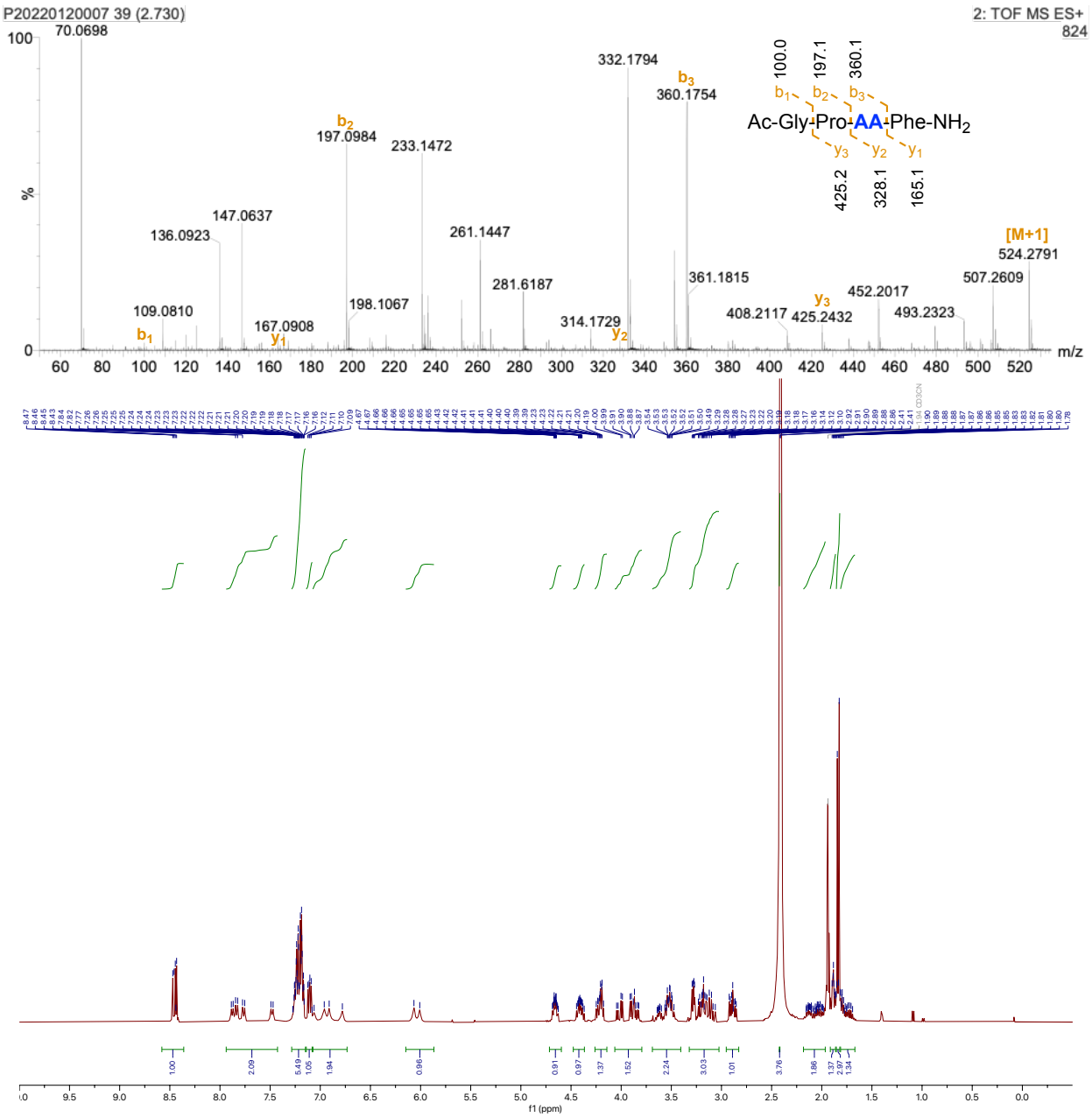
	Name	Retention Time	Area	% Area
1		4.361	331694	1.51
2		5.118	1040142	4.74
3		6.411	392906	1.79
4	Ac-GPDhaF-NH2	7.033	369767	1.69
5	Products	8.220	19799802	90.27

Isolated Epimers:



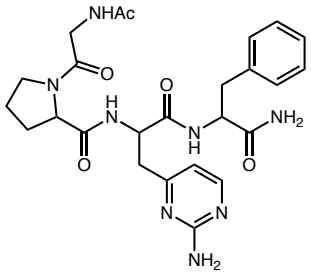
	Name	Retention Time	Area	% Area
1	Product 1	7.084	8119399	39.12
2	Product 2	7.532	12638016	60.88


6.966 Peak 1 - QDa Positive Scan QDa Positive(+) Scan (130.00-1250.00)Da, Centroid, CV=15
524.18
[M+1]
Intensity
7.496 Peak 2 - QDa Positive Scan QDa Positive(+) Scan (130.00-1250.00)Da, Centroid, CV=15
524.16
[M+1]
m/z

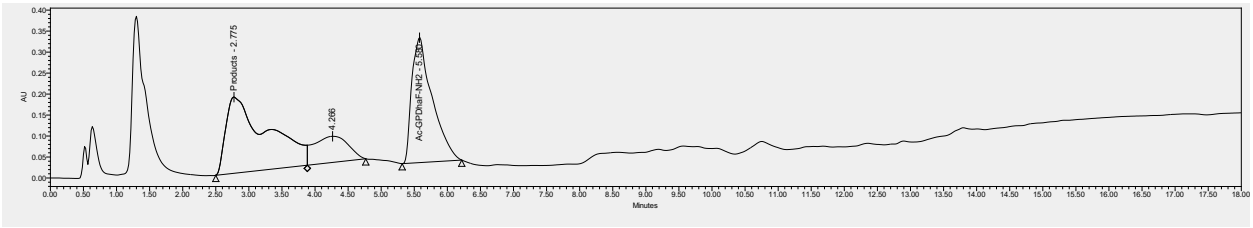

P20220120007 39 (2.730)
2: TOF MS ES+
824
70.0698
332.1794
360.1754
197.0984
233.1472
147.0637
136.0923
261.1447
281.6187
361.1815
109.0810
198.1067
167.0908
314.1729
408.2117
425.2432
452.2017
493.2323
507.2609
524.2791
Ac-Gly-Pro-AA-Phe-NH2
%
m/z
f1 (ppm)

7: Synthesized by the general procedure for pyrimidine sidechains with **deviation 2**

MW = 524.6, % Conversion = 48%, Isolated yield = 29% [3.5 mg]

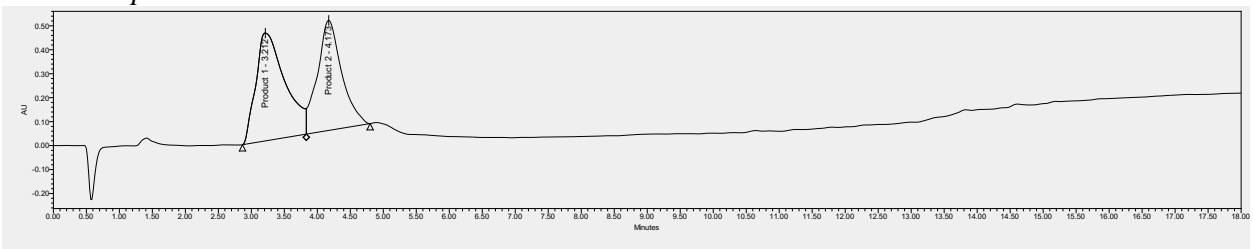


Crude:

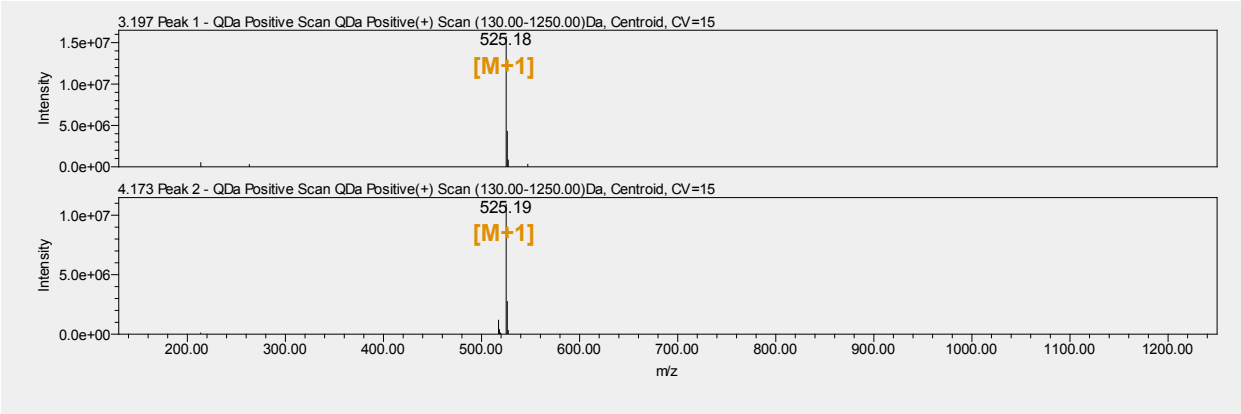


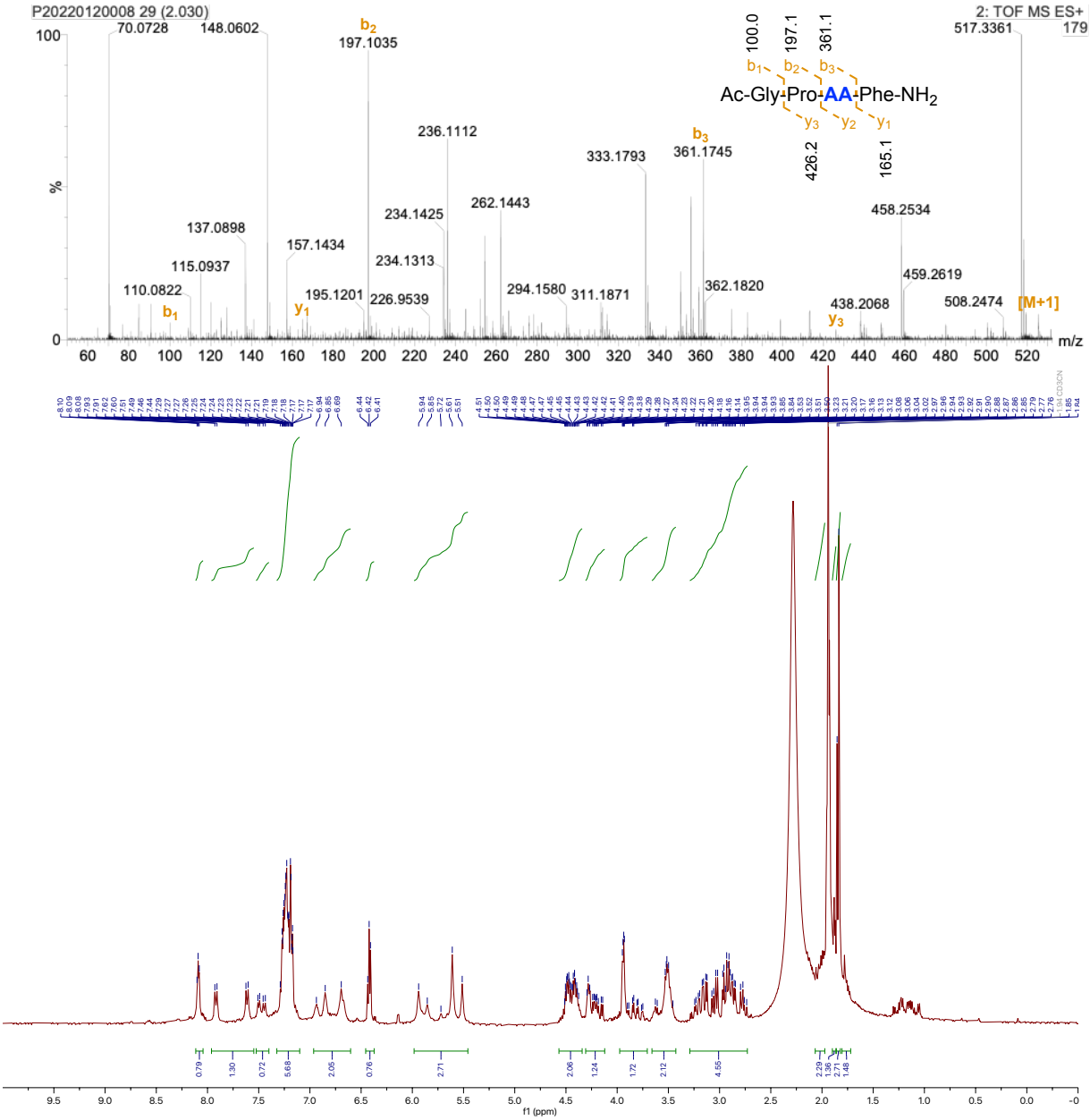
	Name	Retention Time	Area	% Area
1	Products	2.775	7901374	47.70
2		4.266	2273859	13.73
3	Ac-GPDhaF-NH2	5.580	6389207	38.57

Isolated Epimers:



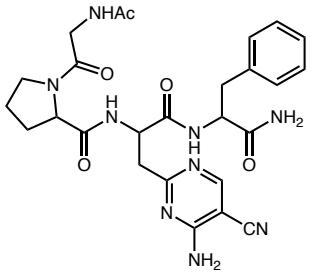
	Name	Retention Time	Area	% Area
1	Product 1	3.212	13855801	54.05
2	Product 2	4.173	11781005	45.95


3.197 Peak 1 - QDa Positive Scan QDa Positive(+) Scan (130.00-1250.00)Da, Centroid, CV=15
525.18
[M+1]
4.173 Peak 2 - QDa Positive Scan QDa Positive(+) Scan (130.00-1250.00)Da, Centroid, CV=15
525.19
[M+1]
Intensity
m/z

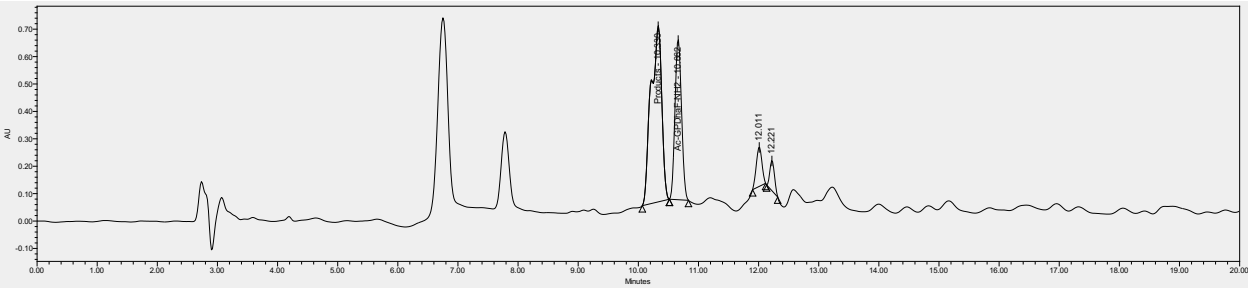

P20220120008 29 (2.030)
2: TOF MS ES+
517.3361
179
Ac-Gly-Pro-AA-Phe-NH2
100.0
197.1
361.1
426.2
165.1
70.0728
148.0602
197.1035
236.1112
333.1793
361.1745
262.1443
234.1425
137.0898
157.1434
115.0937
234.1313
110.0822
195.1201
226.9539
294.1580
311.1871
362.1820
458.2534
459.2619
438.2068
508.2474
[M+1]
m/z
f1 (ppm)

8: Synthesized by the general procedure for pyrimidine sidechains with **deviation 2**

MW = 549.6, % Conversion = 57%, Isolated yield = 52% [6.7 mg]

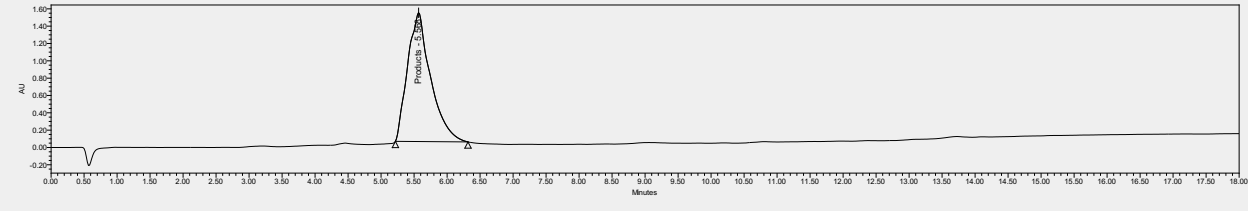


Crude: (Used different column method to determine % conversion due to overlap in spectrum)

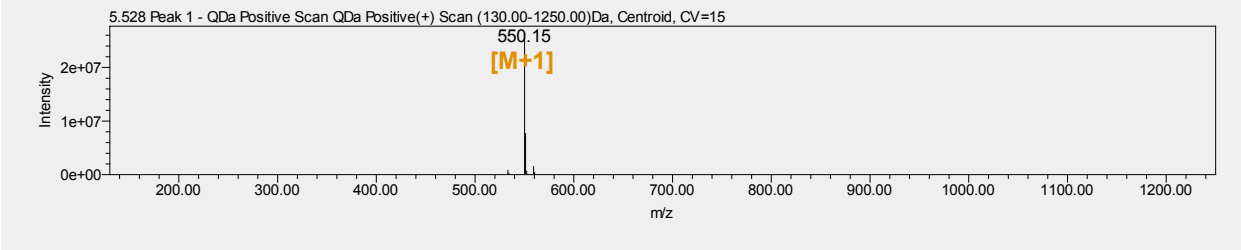


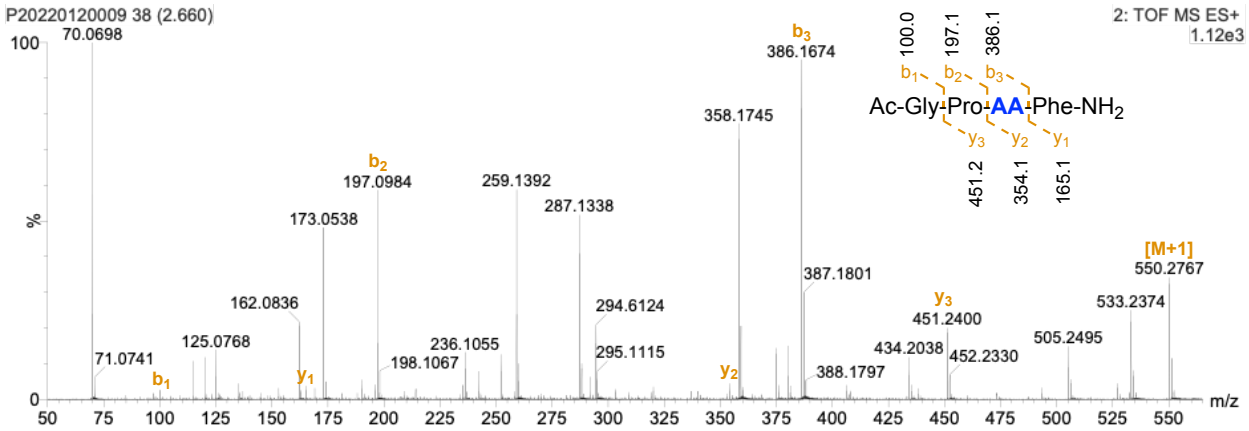
	Name	Retention Time	Area	% Area
1	Products	10.330	7971446	57.22
2	Ac-GPDhaF-NH2	10.662	4427018	31.78
3		12.011	938731	6.74
4		12.221	593434	4.26

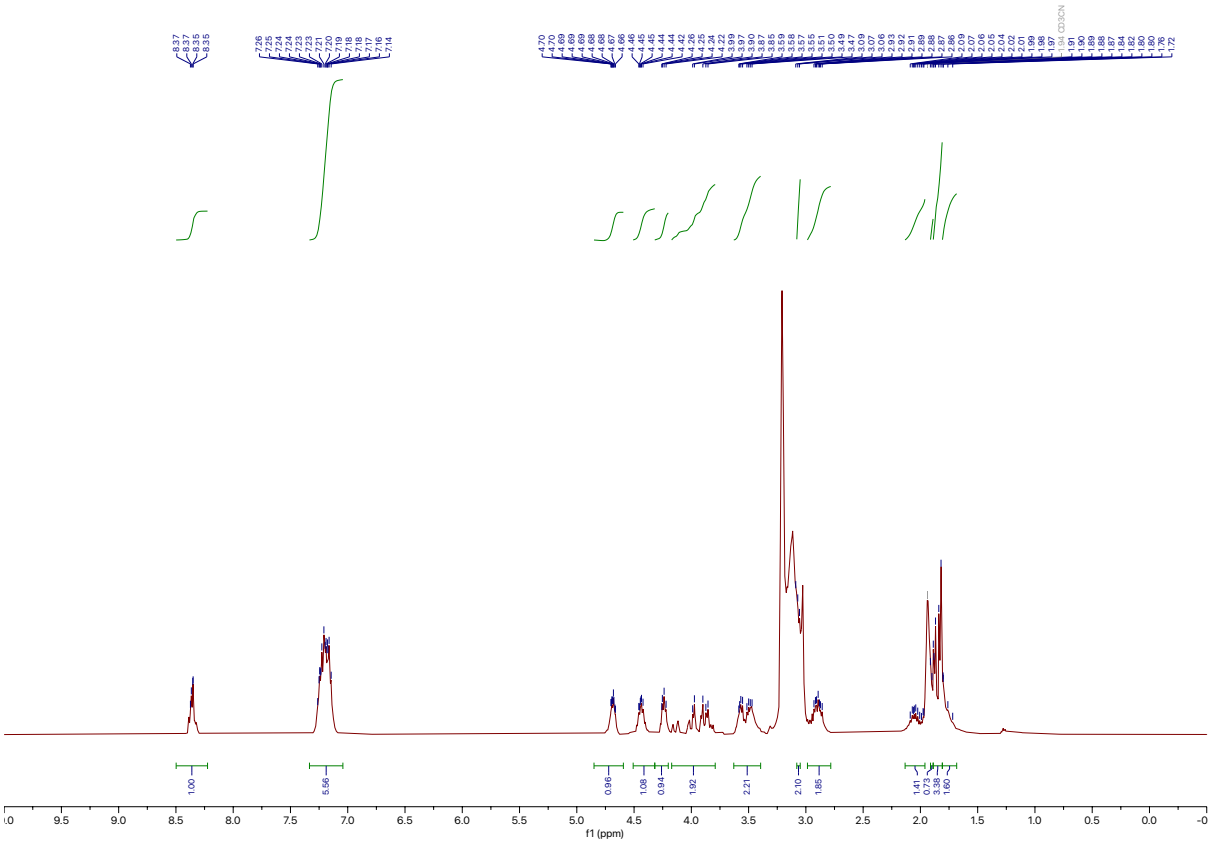
Isolated Epimers:



	Name	Retention Time	Area	% Area
1	Products	5.568	35999453	100.00

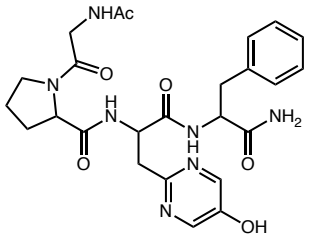



P20220120009 38 (2.660)
2: TOF MS ES+
1.12e3
100
%
0
m/z
70.0698
386.1674
358.1745
197.0984
259.1392
287.1338
173.0538
387.1801
162.0836
294.6124
451.2400
533.2374
[M+1]
550.2767
125.0768
236.1055
295.1115
434.2038
452.2330
505.2495
71.0741
198.1067
388.1797
b1
y1
y2
y3
100.0
197.1
386.1
451.2
354.1
165.1
Ac-Gly-Pro-AA-Phe-NH2

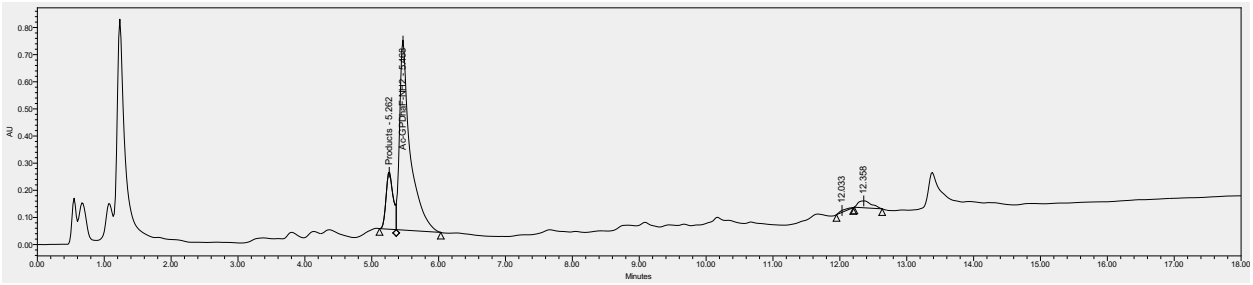

f1 (ppm)

9: Synthesized by the general procedure for pyrimidine sidechains

MW = 525.6, % Conversion = 16%, Isolated yield = 10% [1.2 mg]

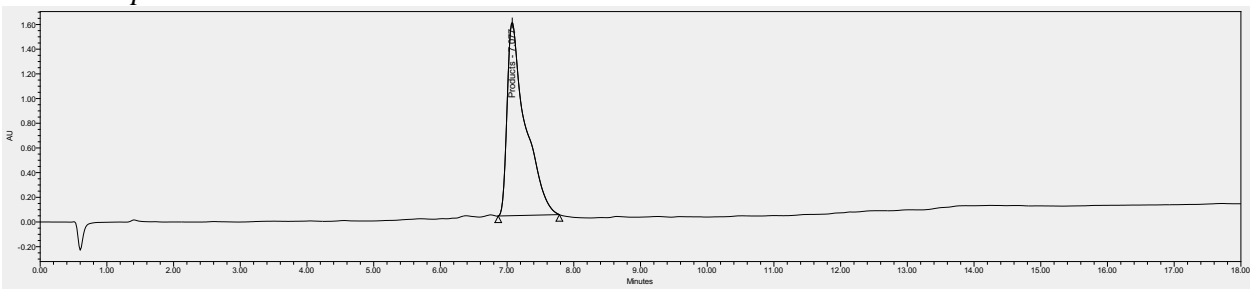


Crude:

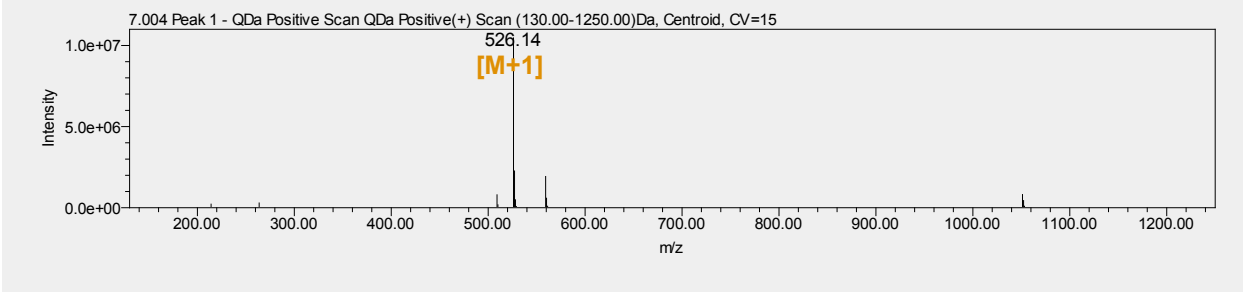


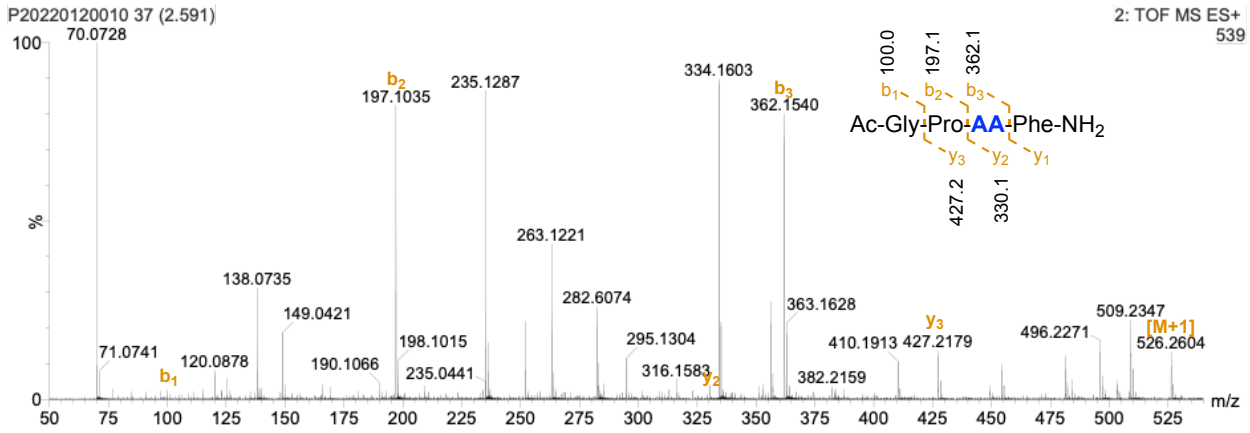
	Name	Retention Time	Area	% Area
1	Products	5.262	1561868	16.11
2	Ac-GPDhaF-NH2	5.468	7703626	79.48
3		12.033	67653	0.70
4		12.358	359055	3.70

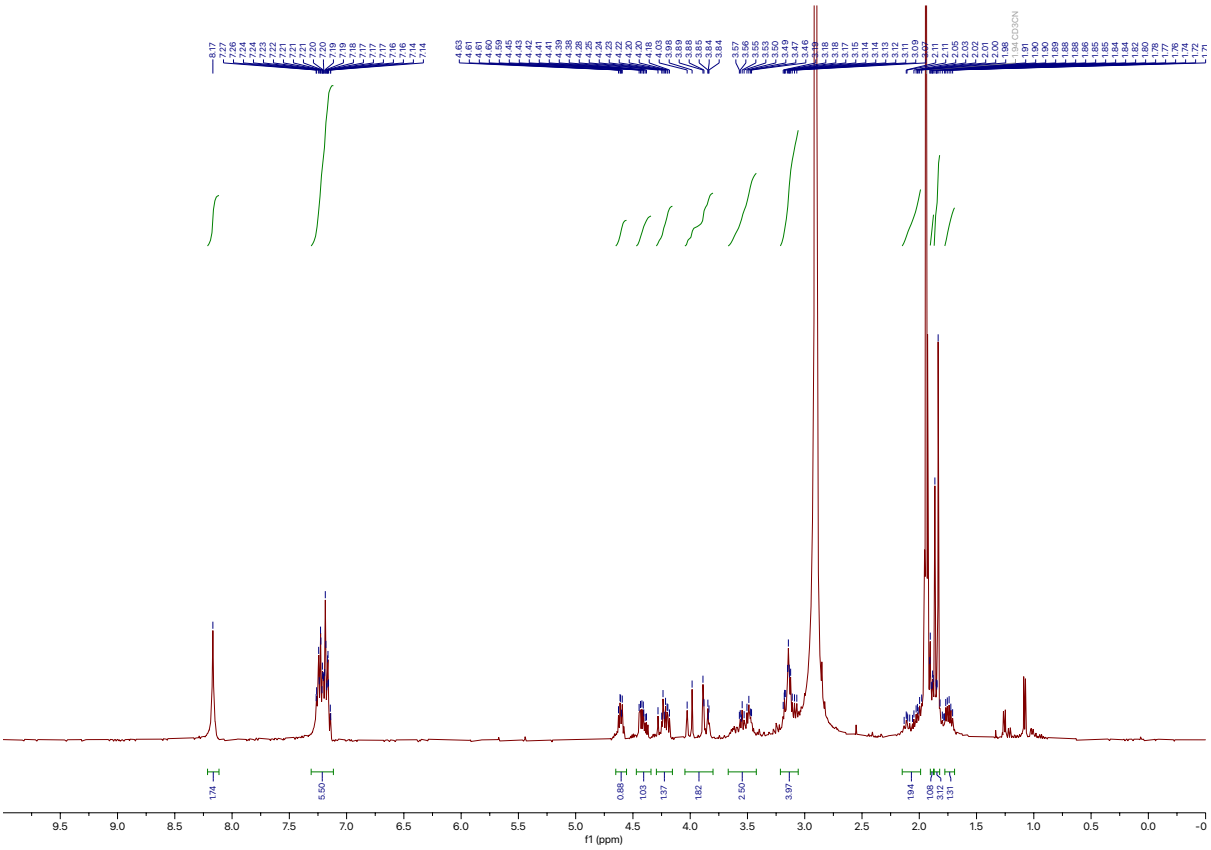
Isolated Epimers:



	Name	Retention Time	Area	% Area	Base Peak	Base Peak (Combined)
1	Products	7.077	29639278	100.00	526.18	526.14

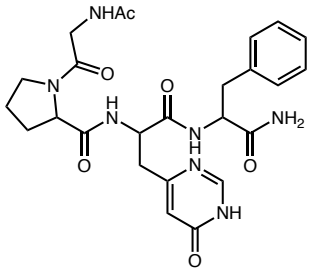



P20220120010 37 (2.591)
2: TOF MS ES+
539
100
%
0
70.0728
b2
197.1035
235.1287
334.1603
b3
362.1540
263.1221
138.0735
282.6074
149.0421
363.1628
y3
509.2347
[M+1]
71.0741
198.1015
295.1304
410.1913
427.2179
496.2271
526.2604
b1
120.0878
190.1066
235.0441
316.1583
y2
382.2159
m/z
50 75 100 125 150 175 200 225 250 275 300 325 350 375 400 425 450 475 500 525
100.0
197.1
362.1
b1 b2 b3
Ac-Gly-Pro-AA-Phe-NH2
y3 y2 y1
427.2
330.1

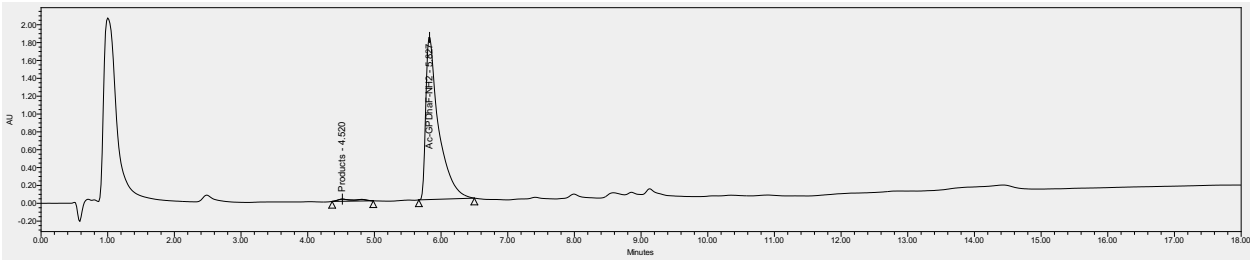

f1 (ppm)
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0

10: Synthesized by the general procedure for pyrimidine sidechains with **deviation 1**

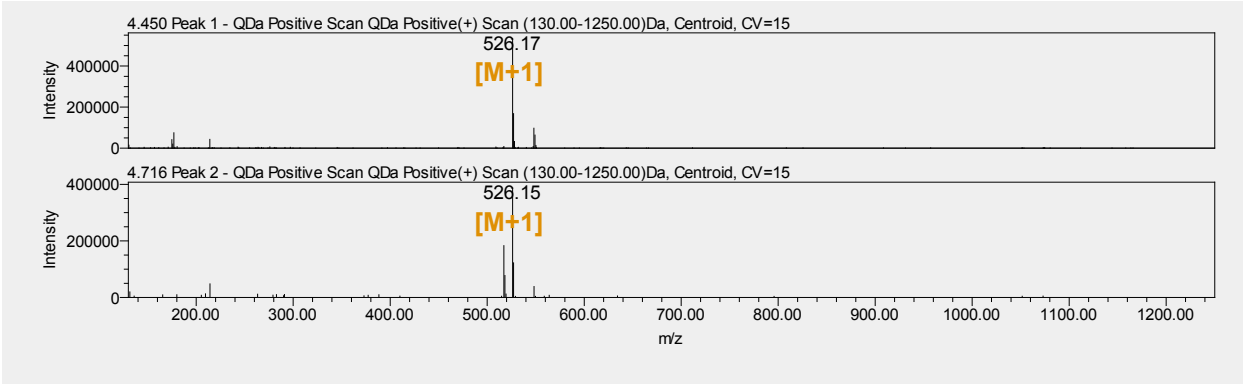
MW = 525.6, % Conversion = 2%, Isolated yield = Not isolated.



Crude:

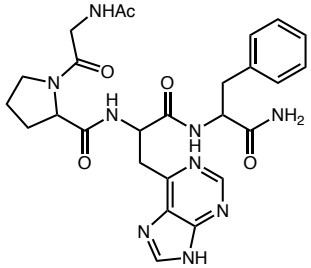


	Name	Retention Time	Area	% Area
1	Products	4.520	476153	1.92
2	Ac-GPDhaF-NH2	5.827	24348484	98.08

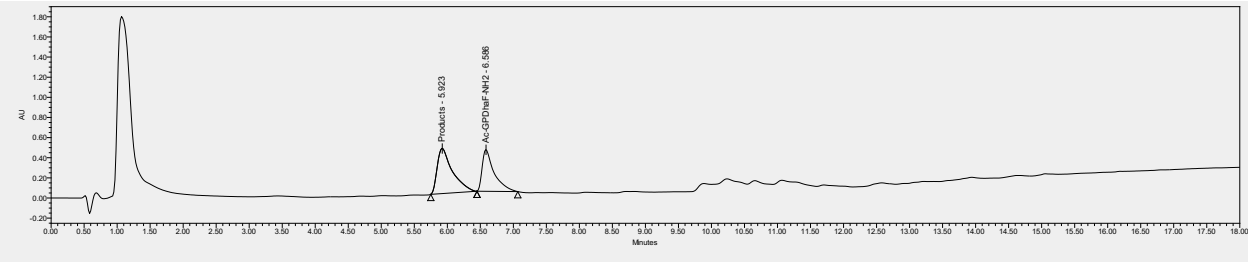


11: Synthesized by the general procedure for purine sidechains with **deviation 2**

MW = 549.6, % Conversion = 58%, Isolated yield = 25% [3.2 mg]

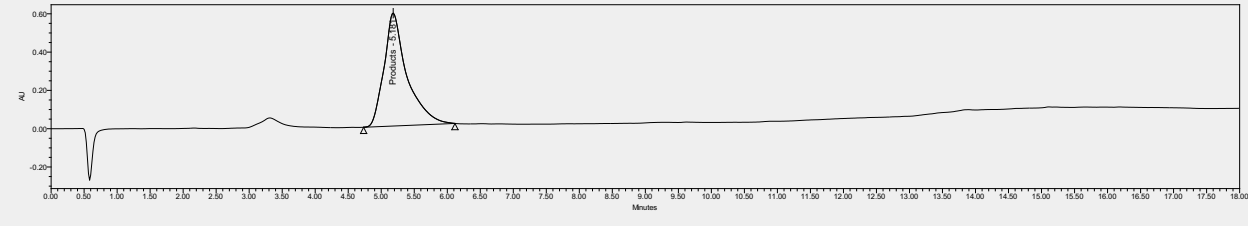


Crude:

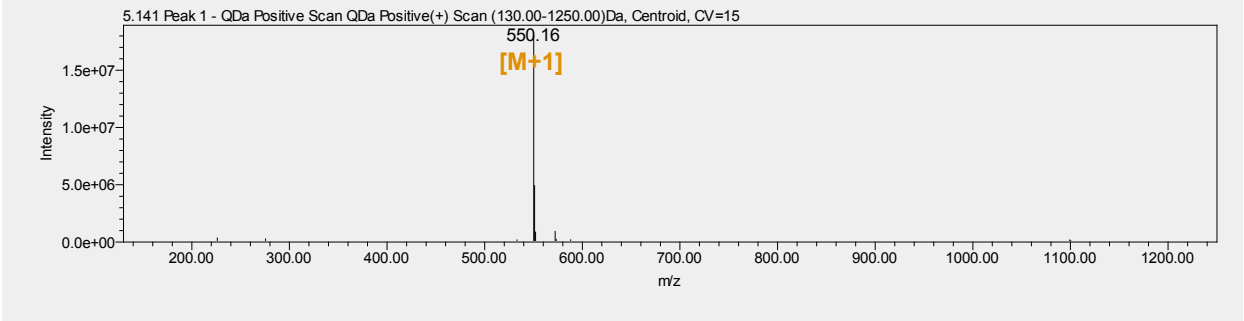


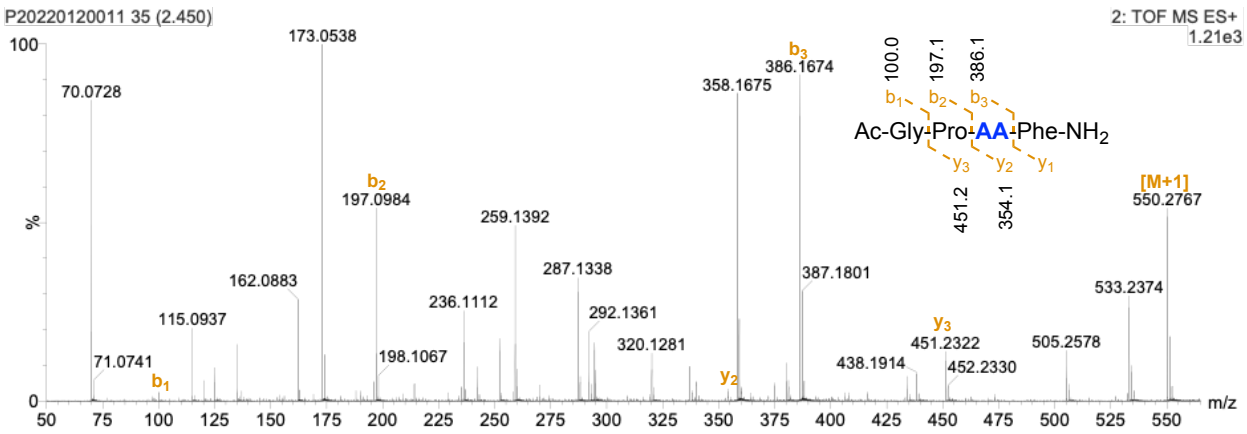
	Name	Retention Time	Area	% Area
1	Products	5.923	7193294	58.15
2	Ac-GPDhaF-NH2	6.586	5177177	41.85

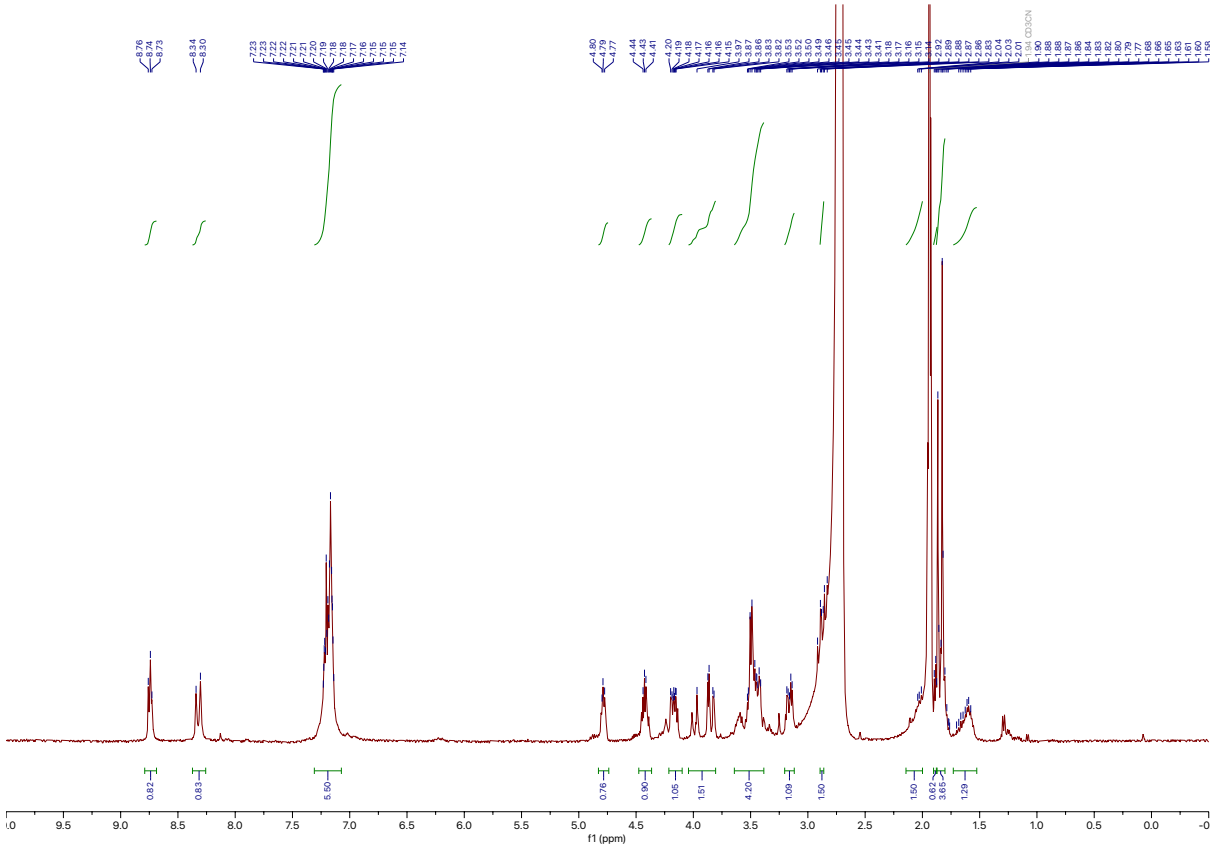
Isolated Epimers:



	Name	Retention Time	Area	% Area
1	Products	5.181	14181453	100.00

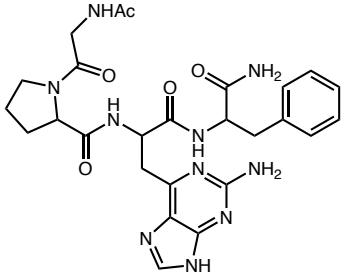



P20220120011 35 (2.450)
2: TOF MS ES+
1.21e3
100
%
0
173.0538
70.0728
b2
197.0984
259.1392
162.0883
115.0937
236.1112
287.1338
292.1361
320.1281
71.0741
b1
198.1067
y2
358.1675
b3
386.1674
387.1801
y3
451.2322
438.1914
452.2330
505.2578
533.2374
[M+1]
550.2767
m/z
50 75 100 125 150 175 200 225 250 275 300 325 350 375 400 425 450 475 500 525 550
100.0
197.1
386.1
b1 b2 b3
Ac-Gly-Pro-AA-Phe-NH2
y3 y2 y1
451.2
354.1

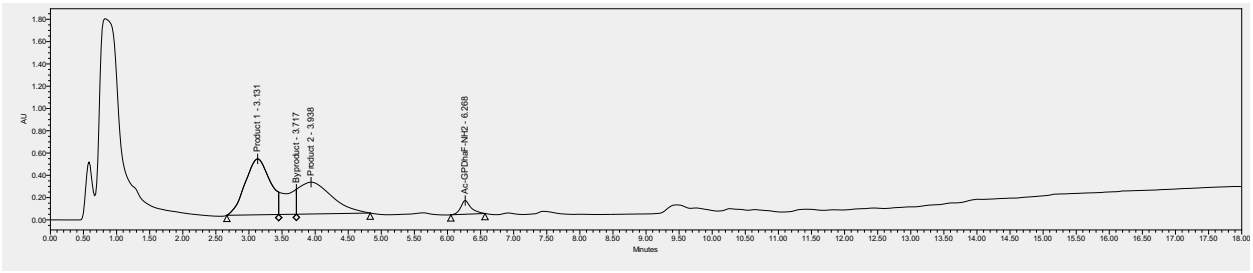

f1 (ppm)

12: Synthesized by the general procedure for purine sidechains

MW = 564.6, % Conversion = 84%, Isolated yield = 45% [5.9 mg]

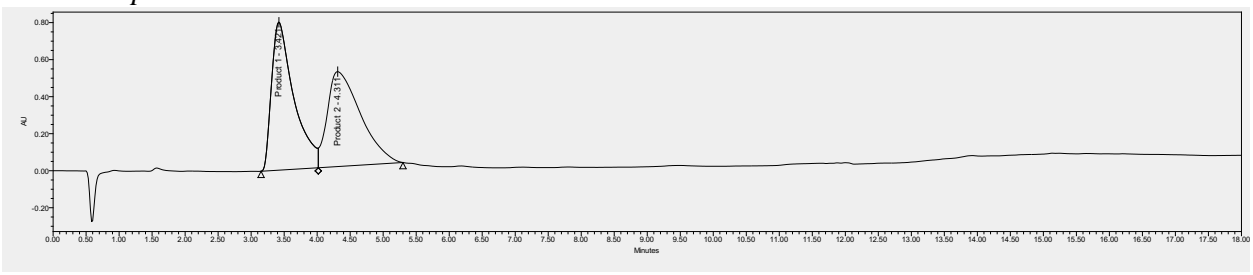


Crude:

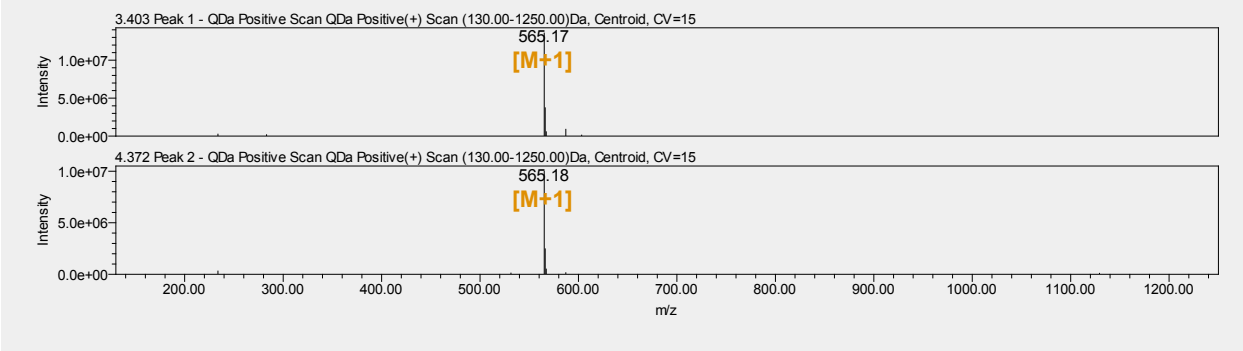


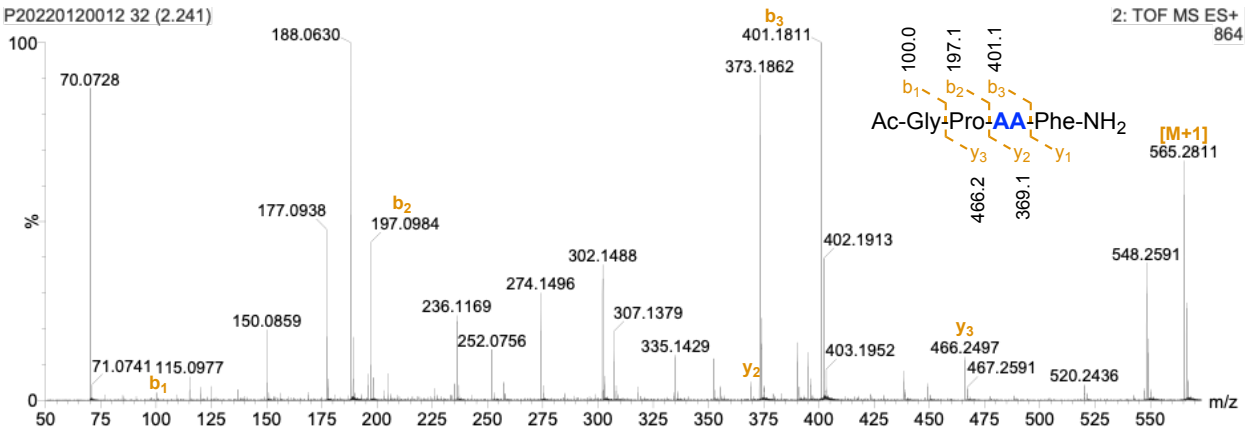
	Name	Retention Time	Area	% Area
1	Product 1	3.131	13122382	47.84
2	Byproduct	3.717	3126040	11.40
3	Product 2	3.938	9847165	35.90
4	Ac-GPDhaF-NH2	6.268	1333837	4.86

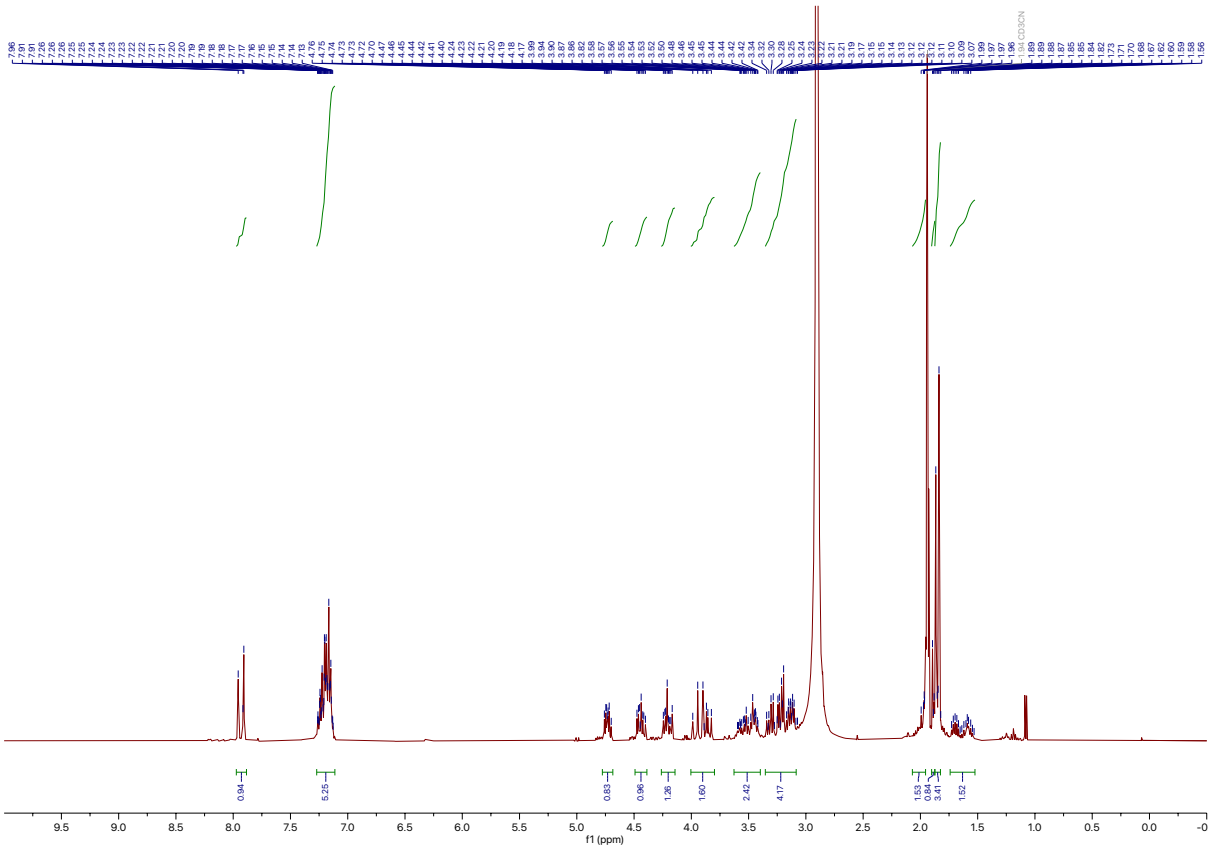
Isolated Epimers:



	Name	Retention Time	Area	% Area
1	Product 1	3.421	18736309	51.07
2	Product 2	4.311	17953852	48.93

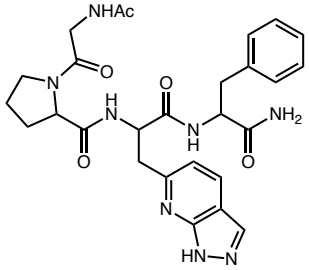

3.403 Peak 1 - QDa Positive Scan QDa Positive(+) Scan (130.00-1250.00)Da, Centroid, CV=15
565.17
[M+1]
Intensity
1.0e+07
5.0e+06
0.0e+00
4.372 Peak 2 - QDa Positive Scan QDa Positive(+) Scan (130.00-1250.00)Da, Centroid, CV=15
565.18
[M+1]
200.00
300.00
400.00
500.00
600.00
700.00
800.00
900.00
1000.00
1100.00
1200.00
m/z


P20220120012 32 (2.241)
2: TOF MS ES+
864
100
%
0
70.0728
188.0630
177.0938
150.0859
71.0741
115.0977
b1
b2
197.0984
236.1169
252.0756
274.1496
302.1488
307.1379
335.1429
b3
401.1811
373.1862
y2
402.1913
403.1952
y3
466.2497
467.2591
520.2436
548.2591
[M+1]
565.2811
100.0
197.1
401.1
b1 b2 b3
Ac-Gly-Pro-AA-Phe-NH2
y3 y2 y1
466.2
369.1
50
75
100
125
150
175
200
225
250
275
300
325
350
375
400
425
450
475
500
525
550
m/z

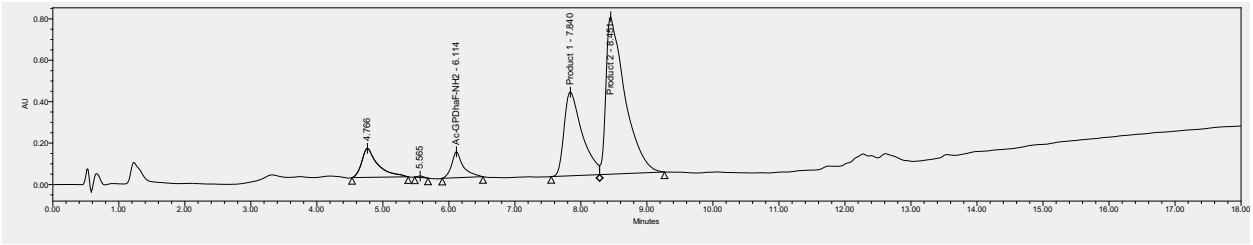

0.94
5.25
0.83
0.96
1.26
1.60
2.42
4.17
1.53
0.84
3.41
1.52
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0
f1 (ppm)

13: Synthesized by the general procedure for purine sidechains

MW = 548.6, % Conversion = 85%, Isolated yield = 48% [6.1 mg]

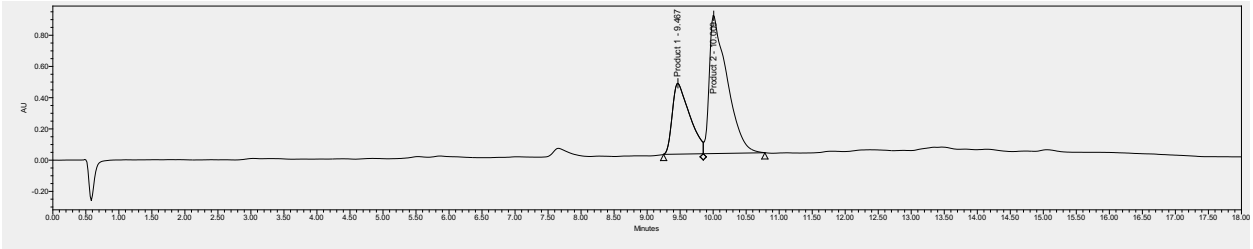


Crude:

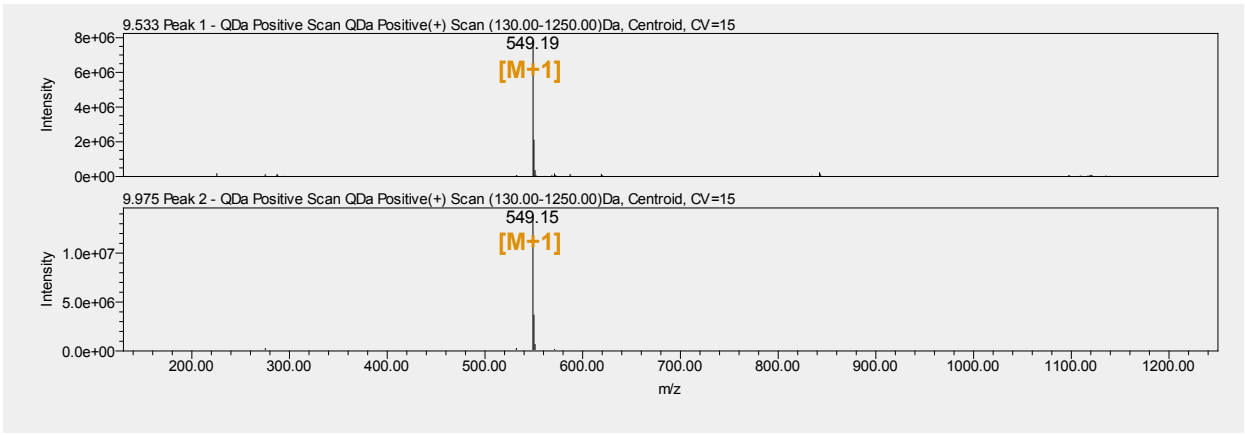


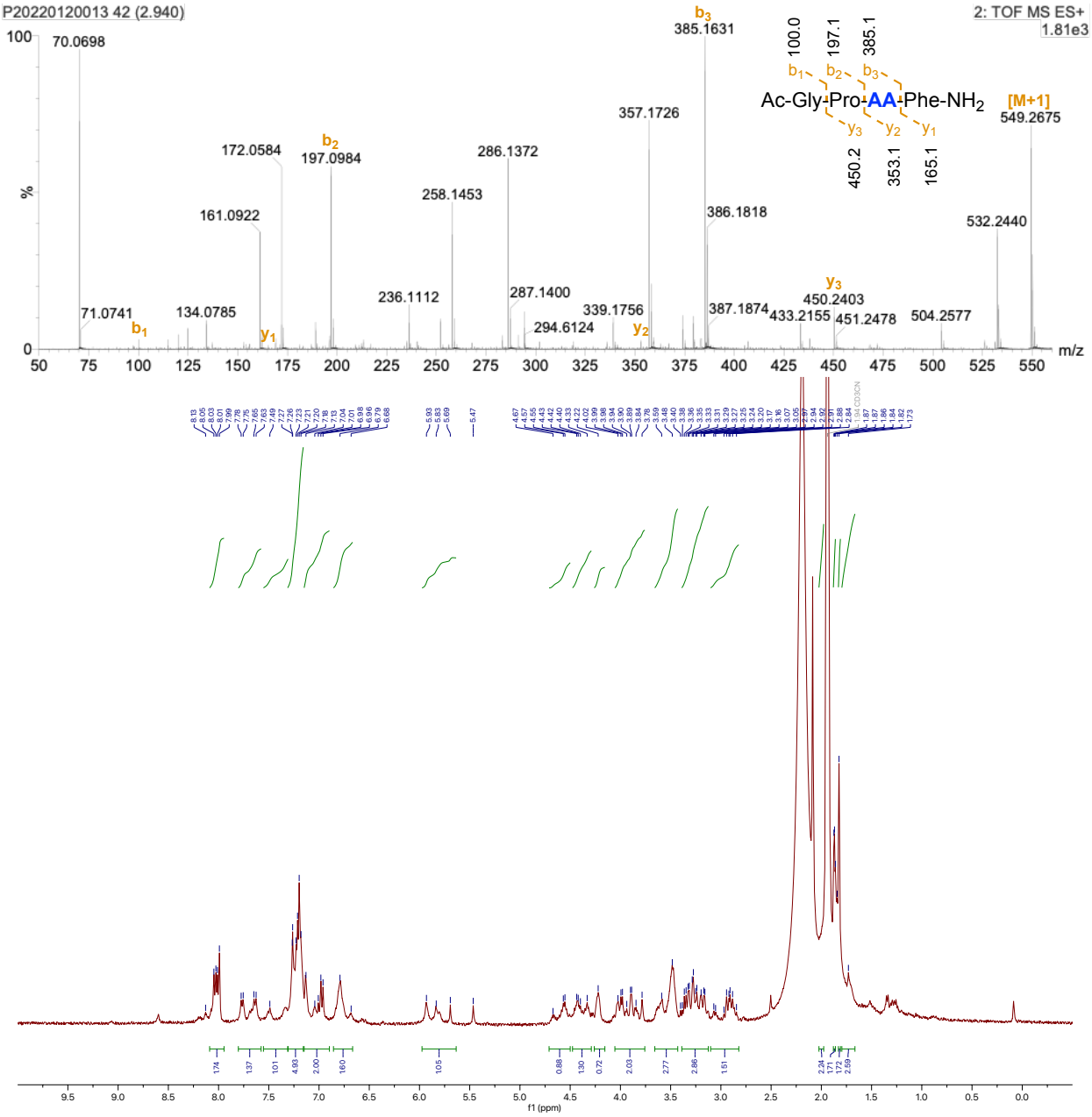
	Name	Retention Time	Area	% Area
1		4.766	2388288	8.94
2		5.565	44793	0.17
3	Ac-GPDhaF-NH2	6.114	1528740	5.72
4	Product 1	7.840	7641600	28.61
5	Product 2	8.451	15108236	56.56

Isolated Epimers:



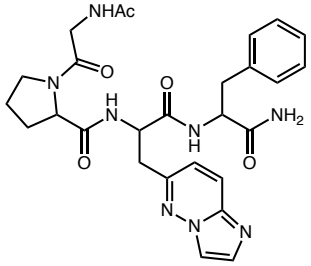
	Name	Retention Time	Area	% Area
1	Product 1	9.467	8271847	33.26
2	Product 2	10.009	16601806	66.74


9.533 Peak 1 - QDa Positive Scan QDa Positive(+) Scan (130.00-1250.00)Da, Centroid, CV=15
549.19
[M+1]
Intensity
9.975 Peak 2 - QDa Positive Scan QDa Positive(+) Scan (130.00-1250.00)Da, Centroid, CV=15
549.15
[M+1]
Intensity
m/z

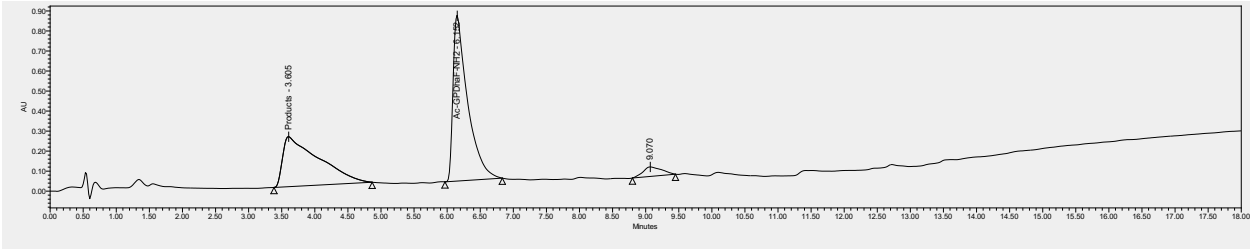

P20220120013 42 (2.940)
2: TOF MS ES+
1.81e3
70.0698
b3
385.1631
100.0
197.1
385.1
b1 b2 b3
Ac-Gly-Pro-AA-Phe-NH2
[M+1]
549.2675
y3 y2 y1
450.2
353.1
165.1
357.1726
172.0584
b2
197.0984
286.1372
258.1453
161.0922
386.1818
532.2440
y3
450.2403
236.1112
287.1400
339.1756
387.1874
433.2155
451.2478
504.2577
71.0741
134.0785
b1
y1
294.6124
y2
%
m/z

14: Synthesized by the general procedure for purine sidechains with **deviation 2**

MW = 548.6, % Conversion = 41%, Isolated yield = 27% [3.5 mg]

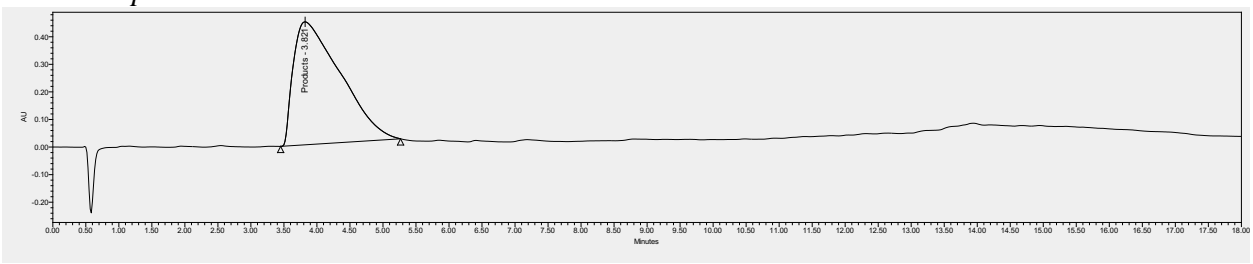


Crude:

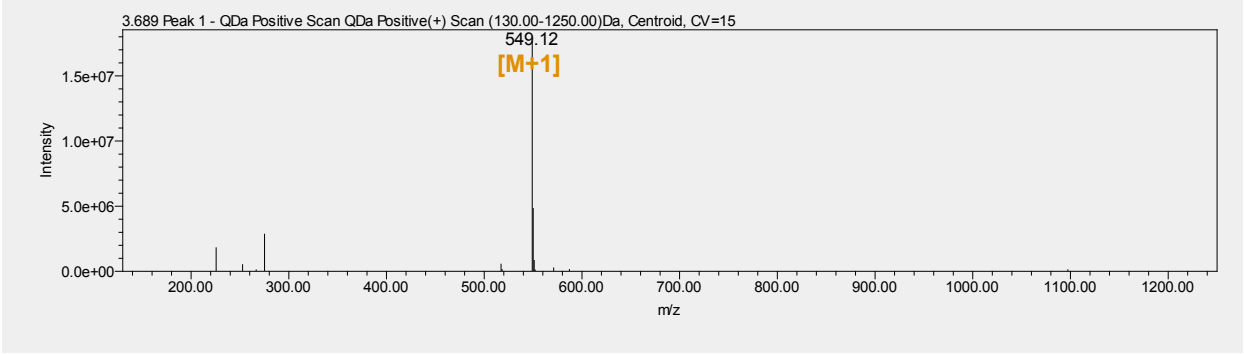


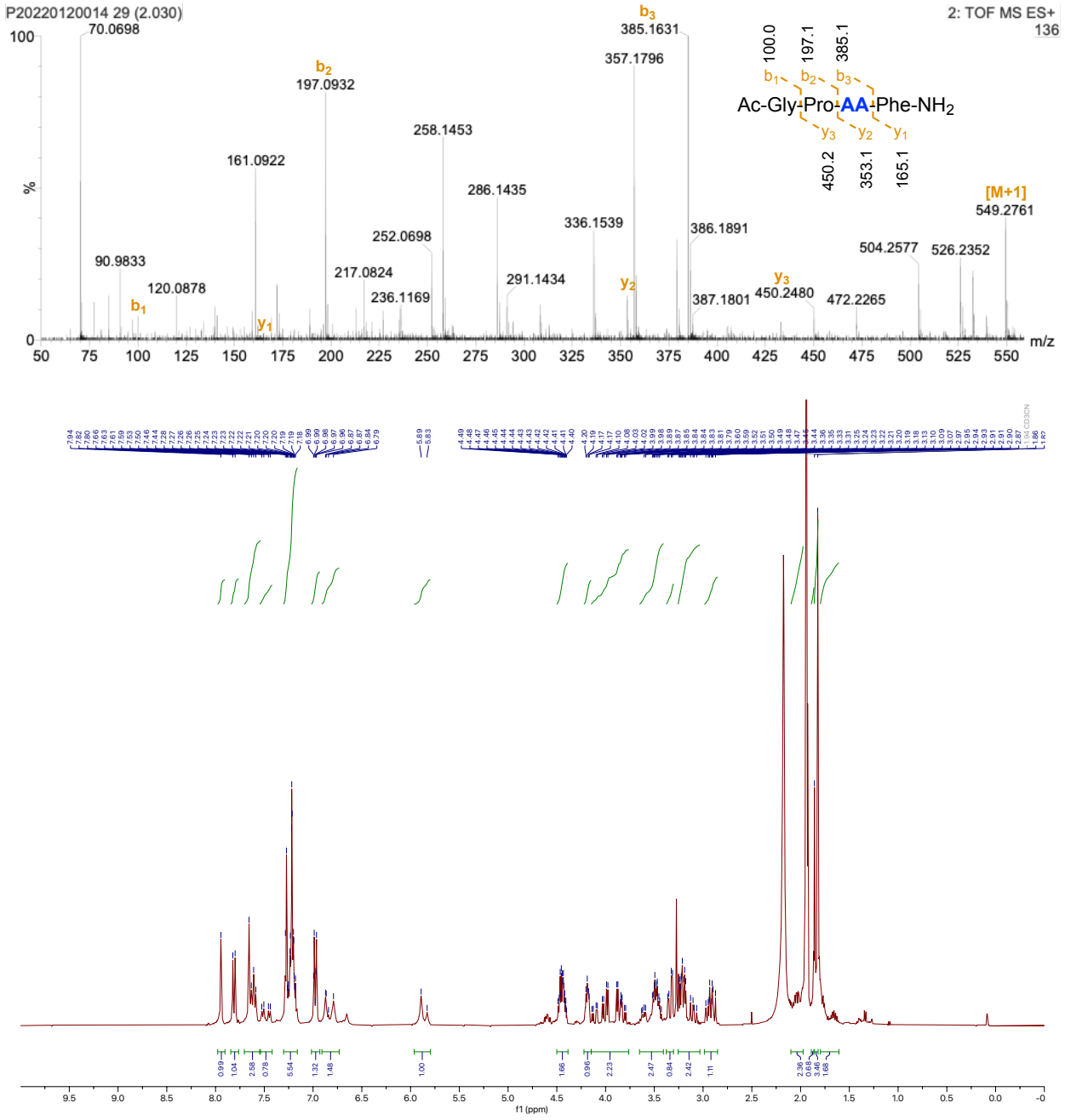
	Name	Retention Time	Area	% Area
1	Products	3.605	9379878	40.85
2	Ac-GPDhaF-NH2	6.152	12653953	55.11
3		9.070	927843	4.04

Isolated Epimers:



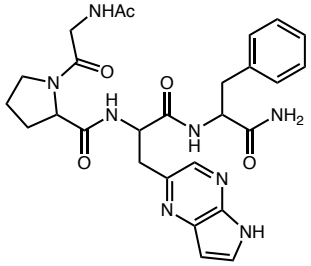
	Name	Retention Time	Area	% Area
1	Products	3.821	22070976	100.00



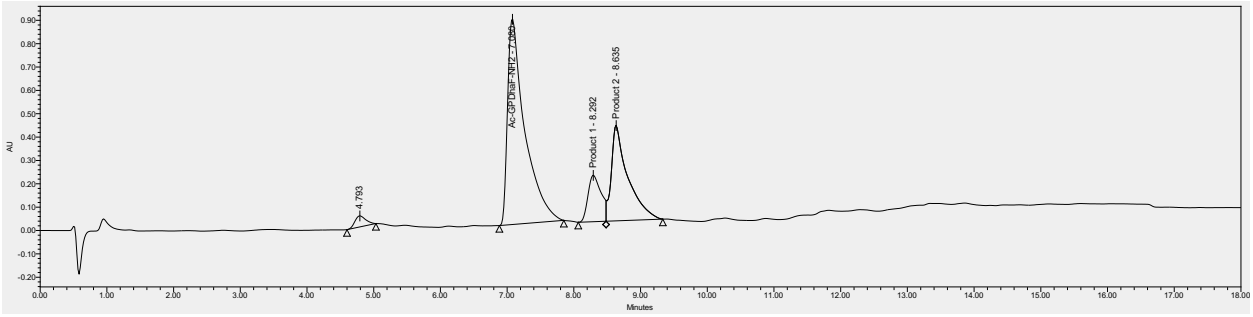

P20220120014 29 (2.030)
2: TOF MS ES+
136
100
%
0
70.0698
b2
197.0932
b3
385.1631
357.1796
258.1453
161.0922
286.1435
336.1539
252.0698
386.1891
90.9833
217.0824
291.1434
y2
y3
450.2480
504.2577
526.2352
[M+1]
549.2761
120.0878
b1
y1
236.1169
387.1801
472.2265
m/z
100.0
197.1
385.1
b1 b2 b3
Ac-Gly-Pro-AA-Phe-NH2
y3 y2 y1
450.2
353.1
165.1
f1 (ppm)

15: Synthesized by the general procedure for purine sidechains with **deviation 2**

MW = 548.6, % Conversion = 37%, Isolated yield = 24% [3.1 mg]

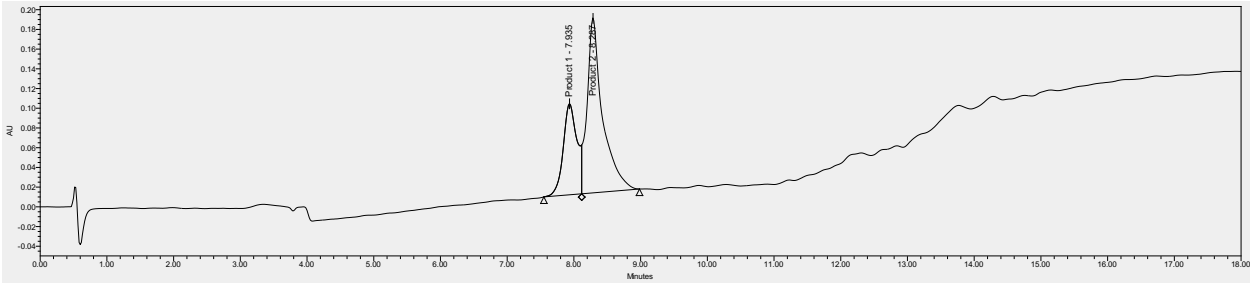


Crude:

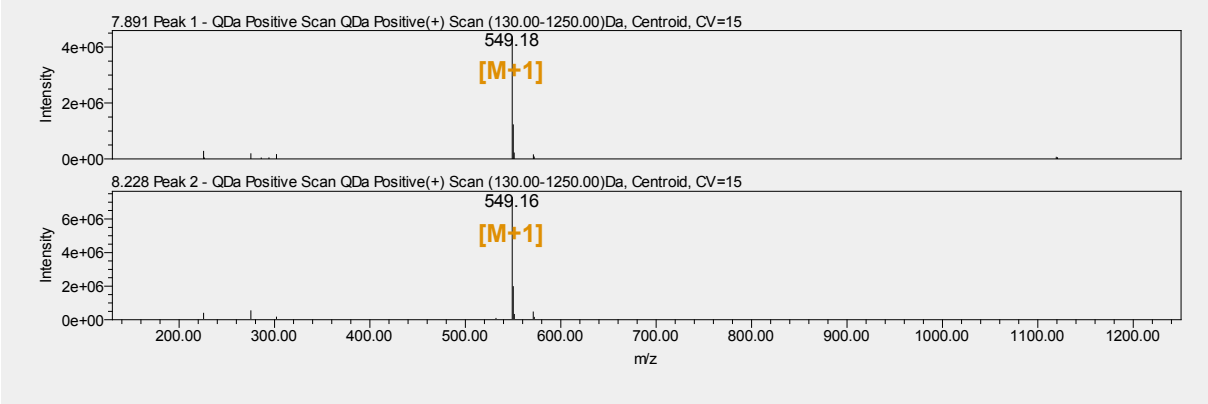


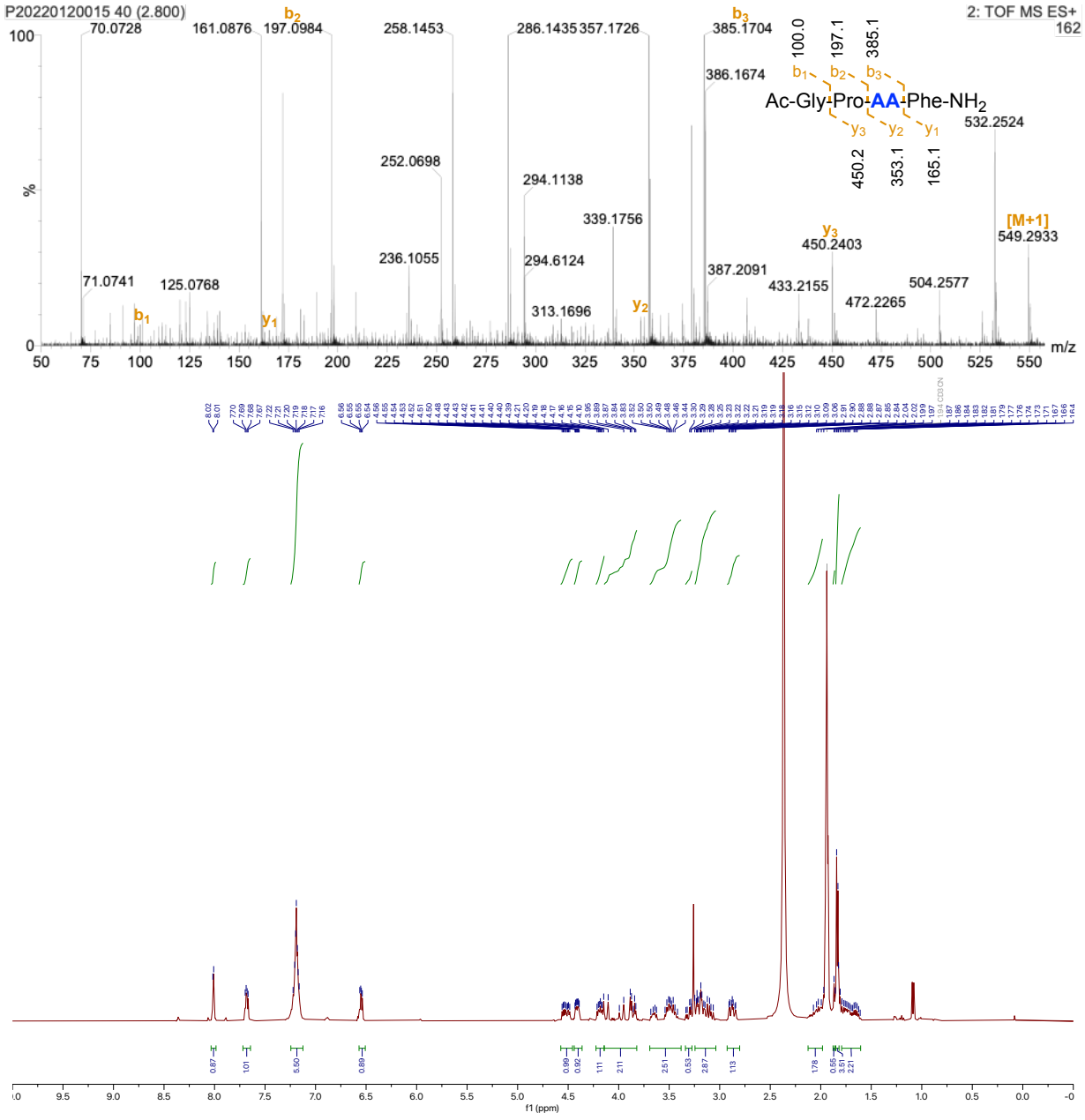
	Name	Retention Time	Area	% Area
1		4.793	539210	2.07
2	Ac-GPDhaF-NH2	7.080	15750030	60.54
3	Product 1	8.292	2705191	10.40
4	Product 2	8.635	7020994	26.99

Isolated Epimers:



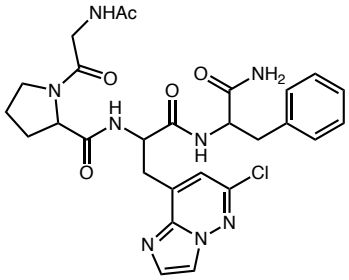
	Name	Retention Time	Area	% Area
1	Product 1	7.935	1323194	31.67
2	Product 2	8.287	2854790	68.33


7.891 Peak 1 - QDa Positive Scan QDa Positive(+) Scan (130.00-1250.00)Da, Centroid, CV=15
549.18
[M+1]
Intensity
8.228 Peak 2 - QDa Positive Scan QDa Positive(+) Scan (130.00-1250.00)Da, Centroid, CV=15
549.16
[M+1]
m/z

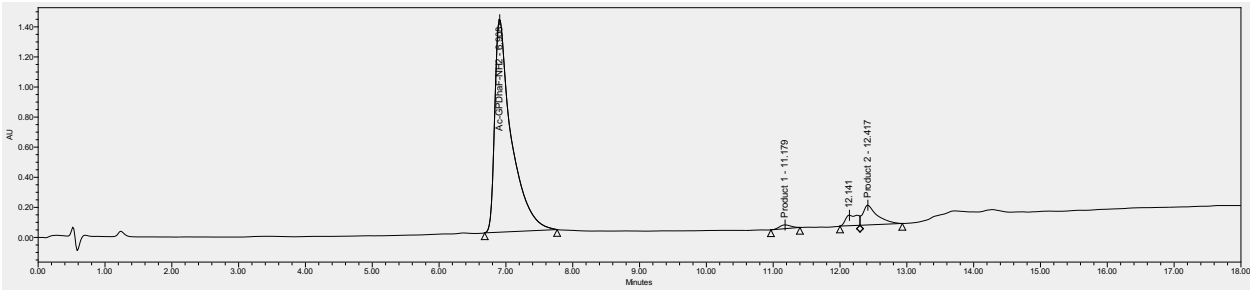

P20220120015 40 (2.800)
2: TOF MS ES+
Ac-Gly-Pro-AA-Phe-NH2
[M+1]
549.2933
m/z
f1 (ppm)

16: Synthesized by the general procedure for purine sidechains (**with 4 mol% Rhodamine B**)

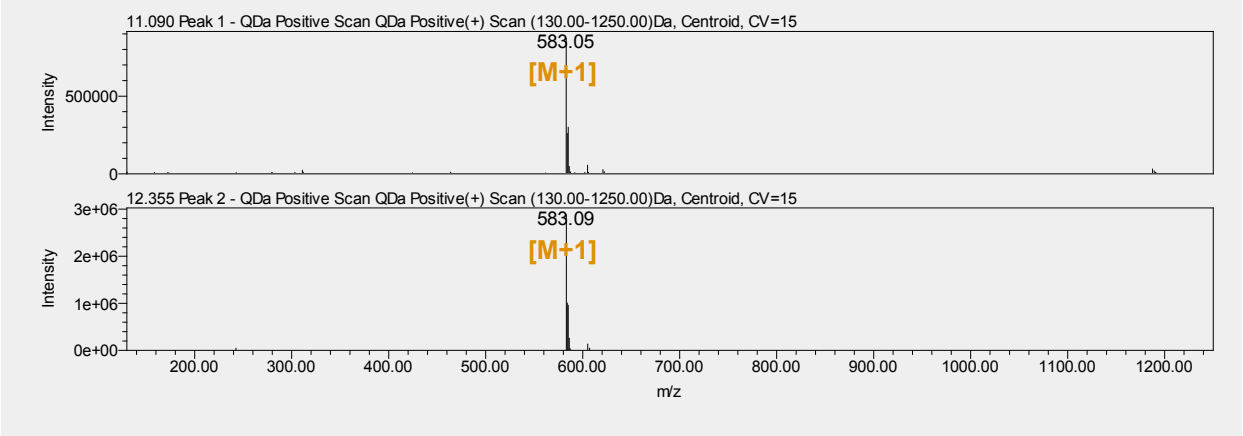
MW = 583.0, % Conversion = 8%, Isolated yield = Not isolated.



Crude:

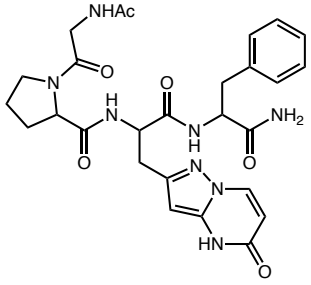


	Name	Retention Time	Area	% Area
1	Ac-GPDhaF-NH2	6.908	24238288	88.46
2	Product 1	11.179	297686	1.09
3		12.141	907696	3.31
4	Product 2	12.417	1956828	7.14

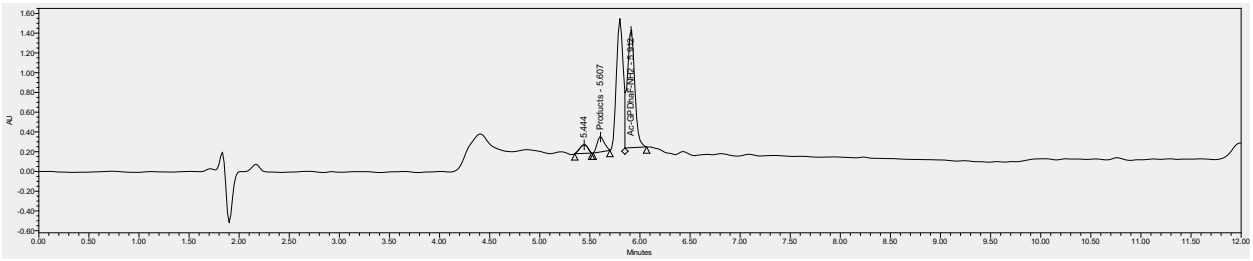


17: Synthesized by the general procedure for purine sidechains with **deviation 1**

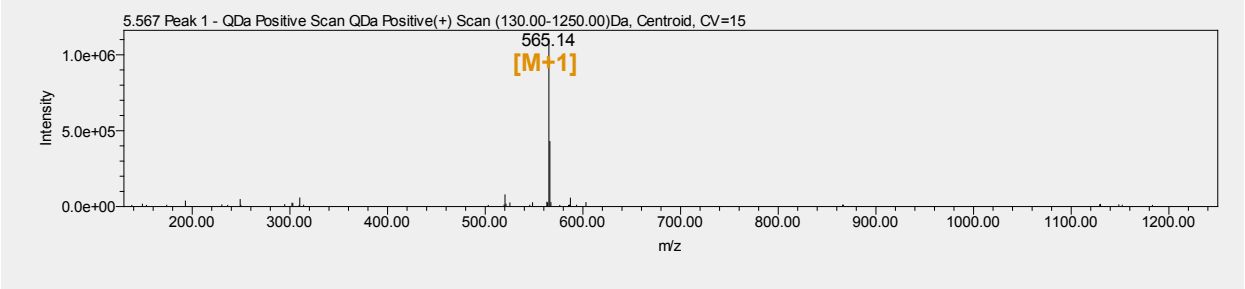
MW = 564.6, % Conversion = 10%, Isolated yield = Not isolated.



Crude:

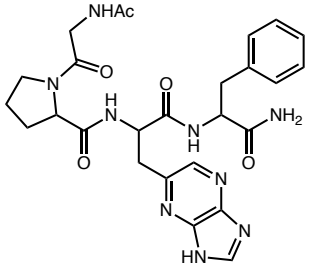


	Name	Retention Time	Area	% Area
1		5.444	486082	6.49
2	Products	5.607	772545	10.32
3	Ac-GPDhaF-NH2	5.912	6226989	83.19

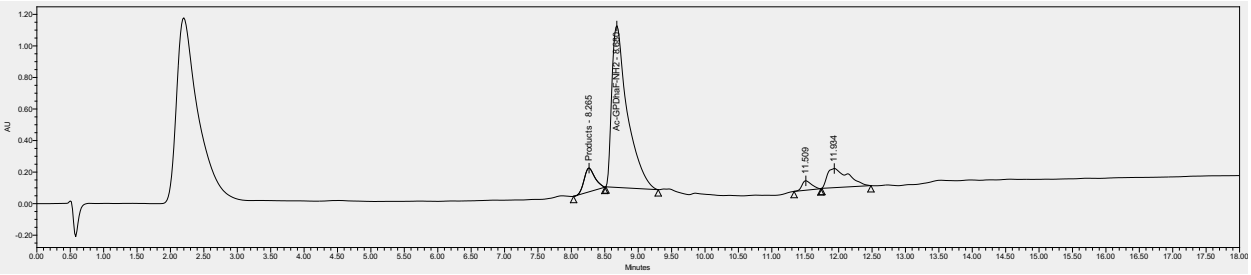


18: Synthesized by the general procedure for purine sidechains with **deviation 1**

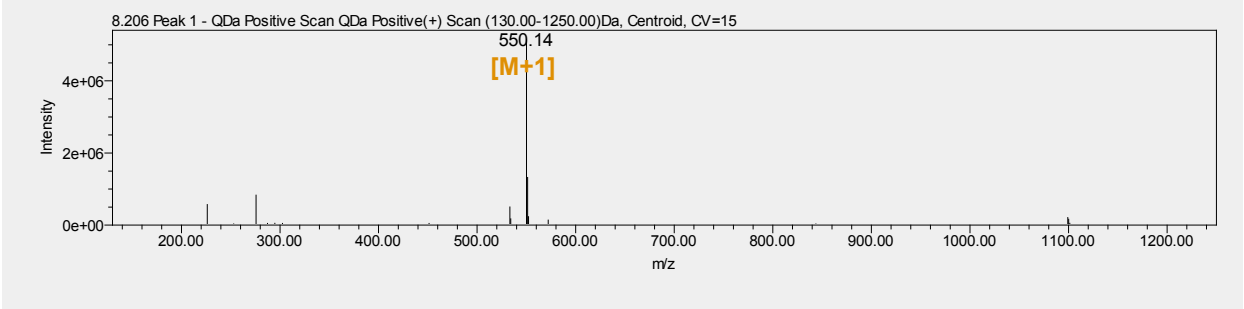
MW = 549.6, % Conversion = 8%, Isolated yield = Not isolated.



Crude:

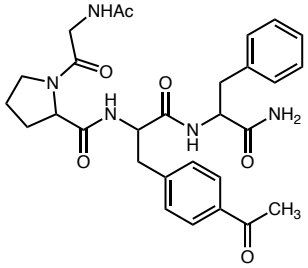


	Name	Retention Time	Area	% Area
1	Products	8.265	1698284	7.90
2	Ac-GPDhaF-NH2	8.680	16555388	77.03
3		11.509	596003	2.77
4		11.934	2641084	12.29

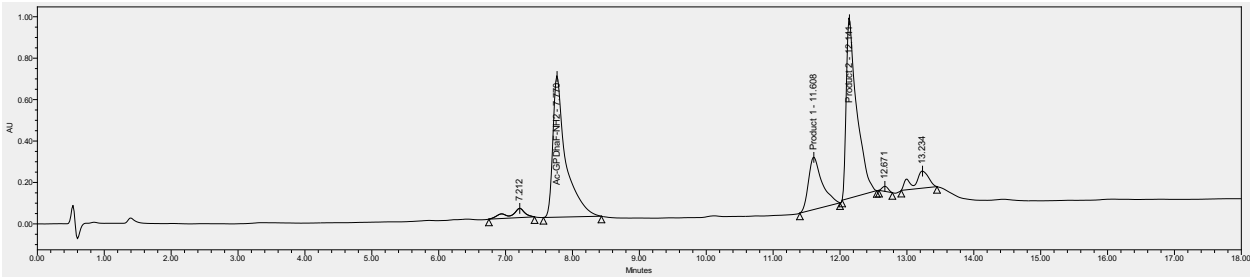


19: Synthesized by the general procedure for pyrimidine sidechains with **deviation 1**

MW = 549.6, % Conversion = 54%, Isolated yield = 40% [5.1 mg]

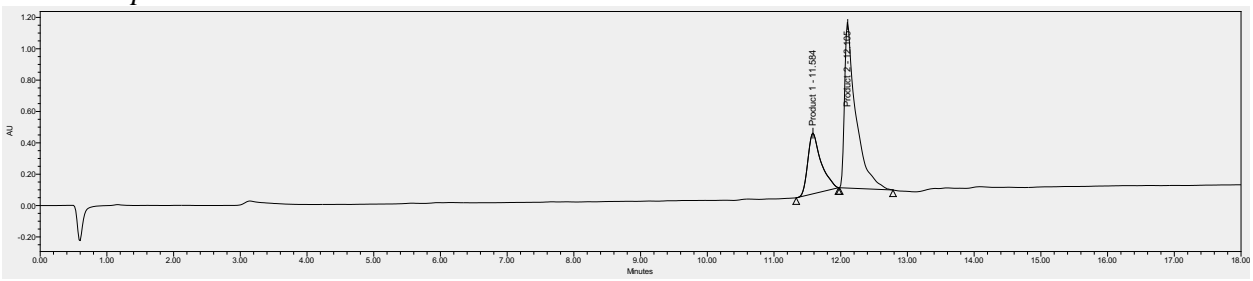


Crude:

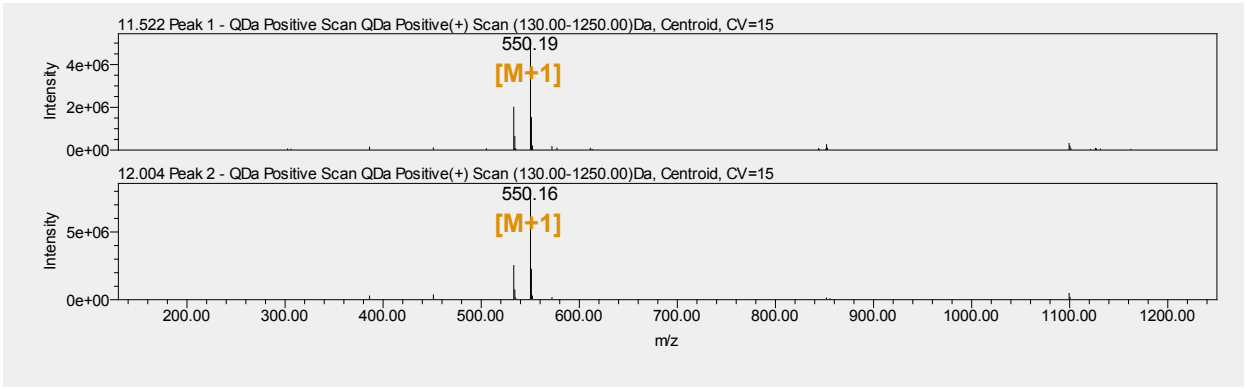


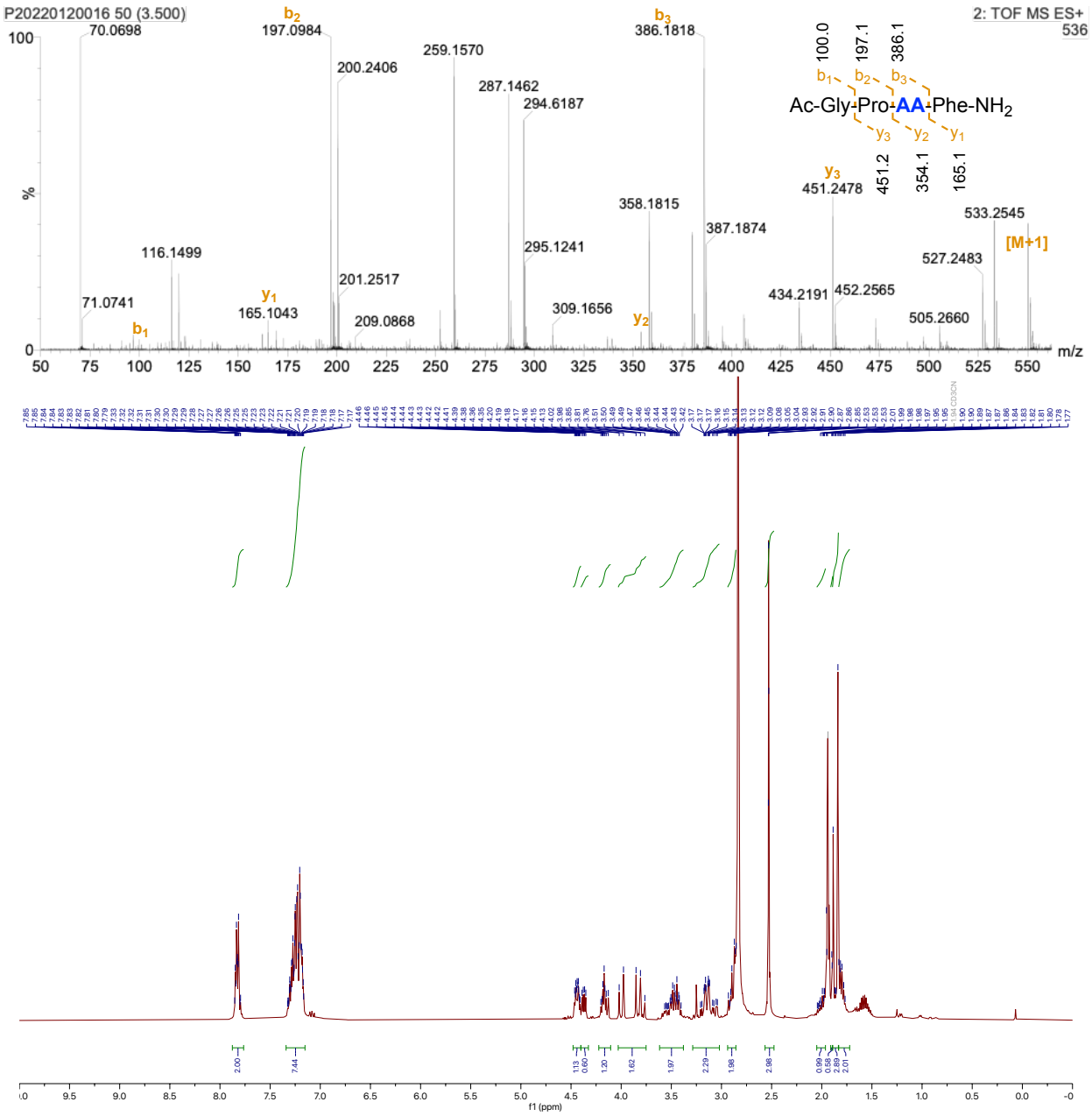
	Name	Retention Time	Area	% Area
1		7.212	706465	2.91
2	Ac-GPDhaF-NH2	7.770	9102381	37.50
3	Product 1	11.608	3555460	14.65
4	Product 2	12.141	9505369	39.16
5		12.671	152757	0.63
6		13.234	1251006	5.15

Isolated Epimers:



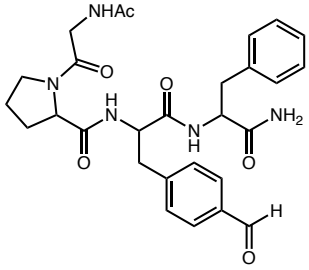
	Name	Retention Time	Area	% Area
1	Product 1	11.584	5354157	30.12
2	Product 2	12.105	12423517	69.88


11.522 Peak 1 - QDa Positive Scan QDa Positive(+) Scan (130.00-1250.00)Da, Centroid, CV=15
550.19
[M+1]
Intensity
4e+06
2e+06
0e+00
12.004 Peak 2 - QDa Positive Scan QDa Positive(+) Scan (130.00-1250.00)Da, Centroid, CV=15
550.16
[M+1]
Intensity
5e+06
0e+00
200.00
300.00
400.00
500.00
600.00
700.00
800.00
900.00
1000.00
1100.00
1200.00
m/z

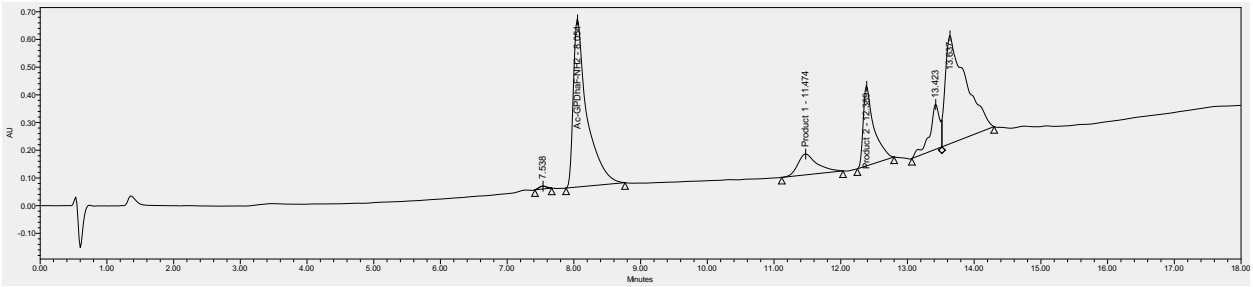

P20220120016 50 (3.500)
2: TOF MS ES+
536
100
%
0
70.0698
b2
197.0984
200.2406
259.1570
287.1462
294.6187
b3
386.1818
358.1815
387.1874
295.1241
116.1499
71.0741
b1
y1
165.1043
201.2517
209.0868
309.1656
y2
y3
451.2478
434.2191
452.2565
505.2660
527.2483
533.2545
[M+1]
100.0
197.1
386.1
b1
b2
b3
Ac-Gly-Pro-AA-Phe-NH2
y3
y2
y1
451.2
354.1
165.1
50
75
100
125
150
175
200
225
250
275
300
325
350
375
400
425
450
475
500
525
550
m/z
f1 (ppm)

20: Synthesized by the general procedure for pyrimidine sidechains with **deviation 1**

MW = 535.6, % Conversion = 20%, Isolated yield = 15% [1.9 mg]

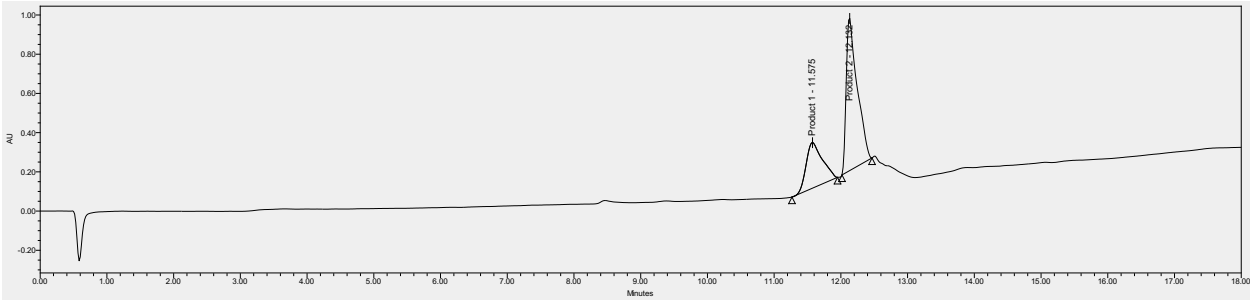


Crude:

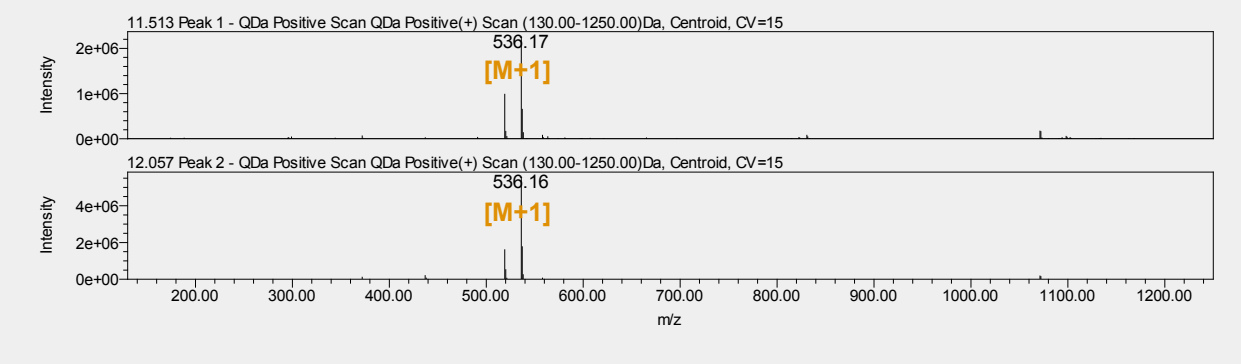


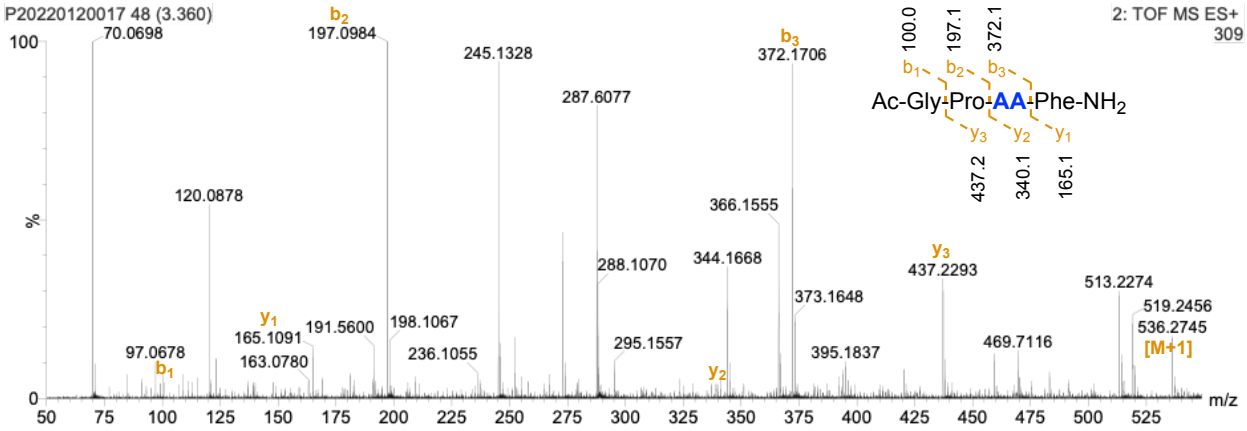
	Name	Retention Time	Area	% Area
1		7.538	83138	0.35
2	Ac-GPDhaF-NH2	8.054	8609978	36.66
3	Product 1	11.474	1517470	6.46
4	Product 2	12.389	3224650	13.73
5		13.423	1709371	7.28
6		13.637	8343618	35.52

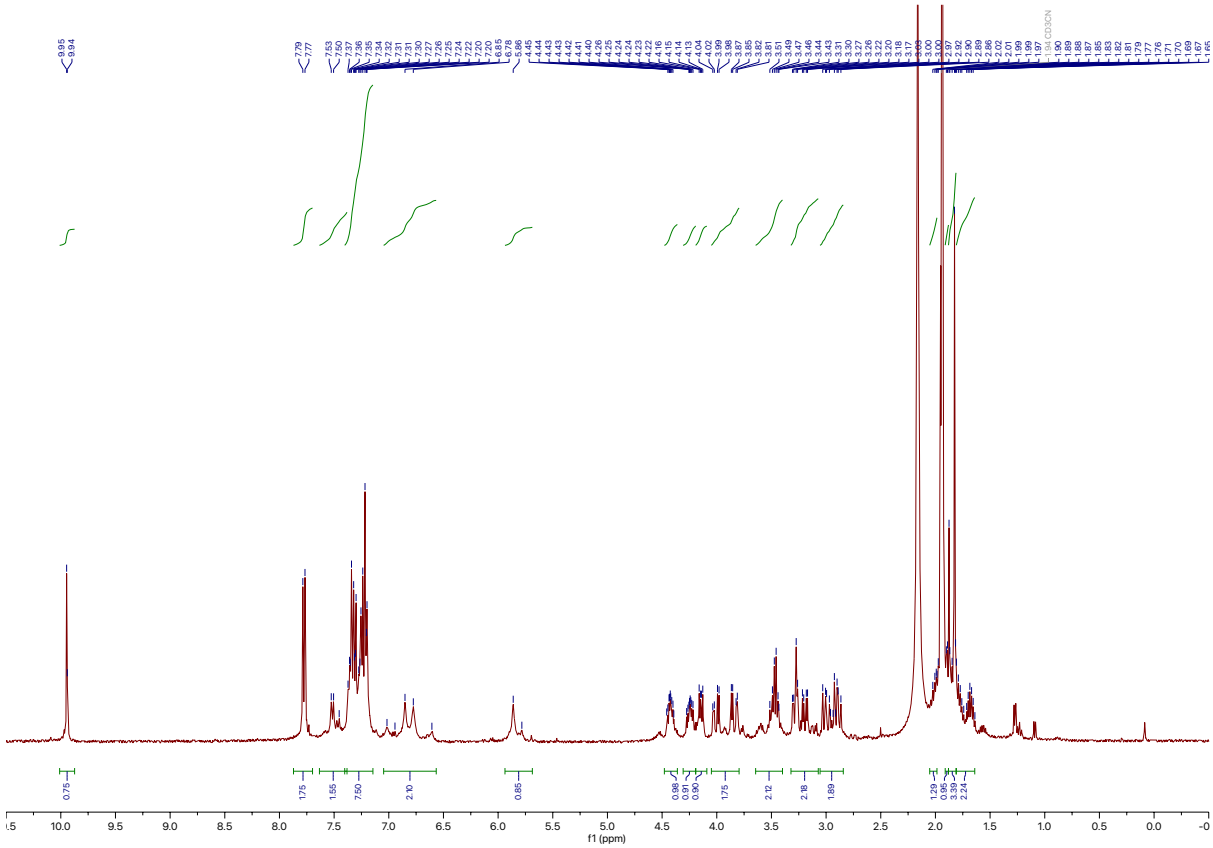
Isolated Epimers:



	Name	Retention Time	Area	% Area
1	Product 1	11.575	3916782	30.62
2	Product 2	12.132	8874456	69.38


11.513 Peak 1 - QDa Positive Scan QDa Positive(+) Scan (130.00-1250.00)Da, Centroid, CV=15
536.17
[M+1]
Intensity
12.057 Peak 2 - QDa Positive Scan QDa Positive(+) Scan (130.00-1250.00)Da, Centroid, CV=15
536.16
[M+1]
m/z

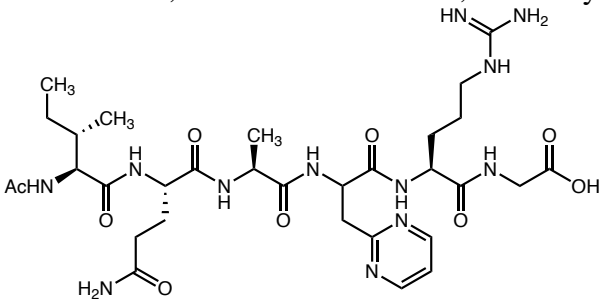

P20220120017 48 (3.360)
2: TOF MS ES+
309
Ac-Gly-Pro-AA-Phe-NH2
70.0698
197.0984
245.1328
372.1706
287.6077
120.0878
366.1555
344.1668
288.1070
373.1648
437.2293
513.2274
519.2456
536.2745
[M+1]
165.1091
191.5600
198.1067
97.0678
163.0780
236.1055
295.1557
395.1837
469.7116
437.2
340.1
165.1
m/z


f1 (ppm)

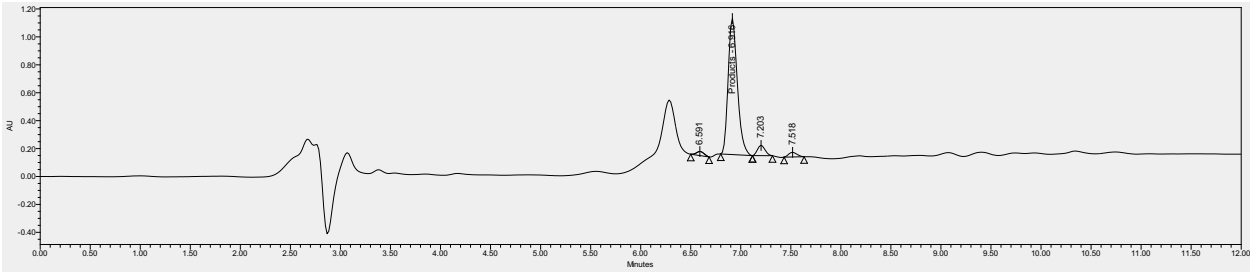
21: *Caspase Active Site Peptide*

Synthesized by the general procedure for pyrimidine sidechains with **deviation 1**

MW = 734.8, % Conversion = 90%, Isolated yield = 56% [9.6 mg]

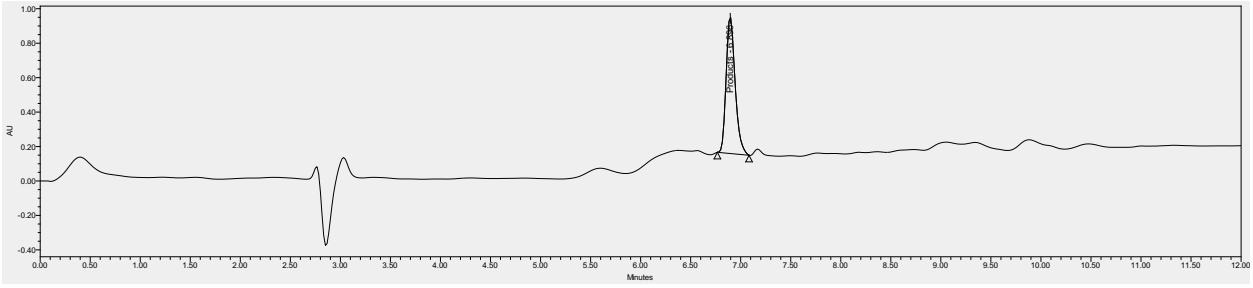


Crude:

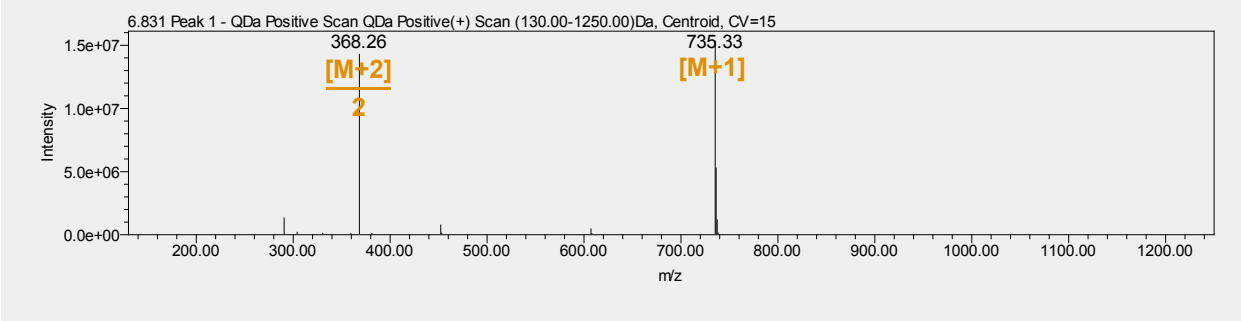


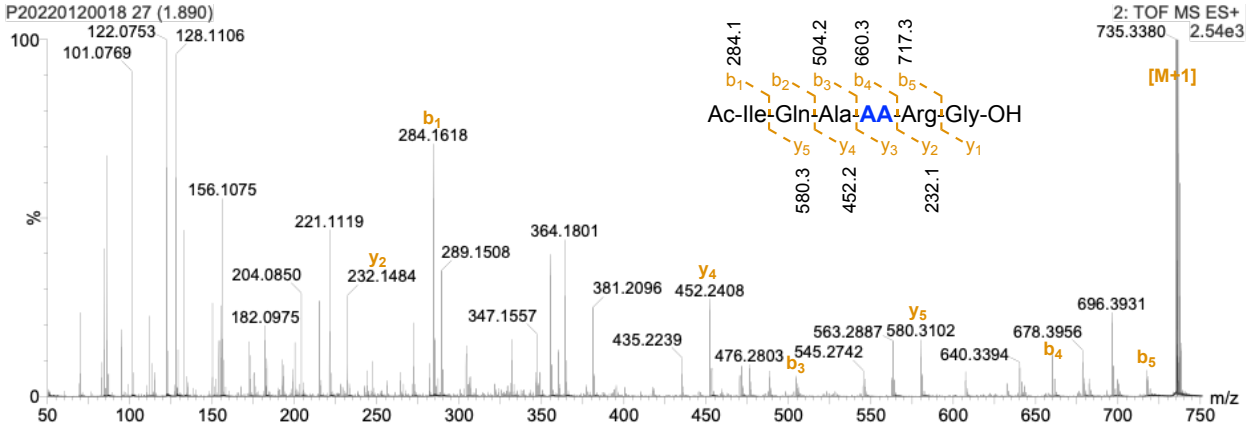
	Name	Retention Time	Area	% Area
1		6.591	154462	2.21
2	Products	6.916	6239620	89.46
3		7.203	389447	5.58
4		7.518	191448	2.74

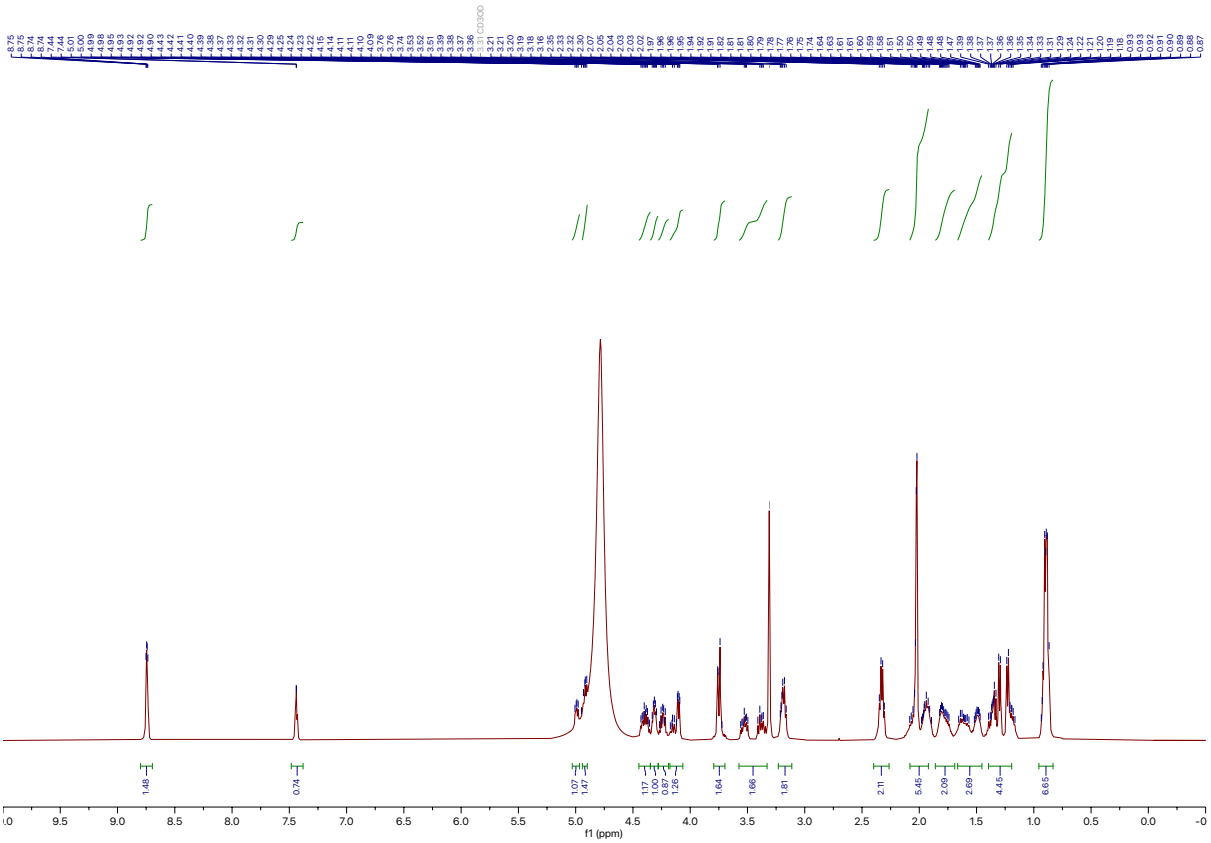
Isolated Epimers:



	Name	Retention Time	Area	% Area
1	Products	6.896	4948360	100.00


6.831 Peak 1 - QDa Positive Scan QDa Positive(+) Scan (130.00-1250.00)Da, Centroid, CV=15
368.26
[M+2]
2
735.33
[M+1]
Intensity
m/z

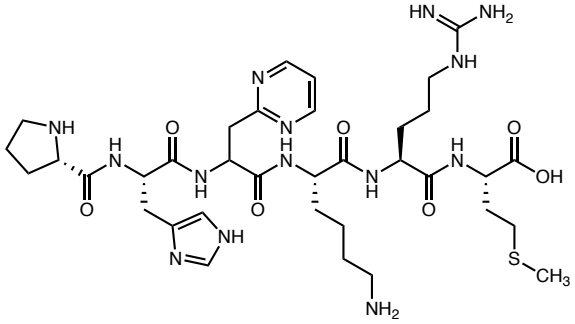

P20220120018 27 (1.890)
2: TOF MS ES+
735.3380
2.54e3
[M+1]
Ac-Ile-Gln-Ala-AA-Arg-Gly-OH
284.1
504.2
660.3
717.3
580.3
452.2
232.1
122.0753
128.1106
101.0769
284.1618
156.1075
221.1119
364.1801
289.1508
204.0850
232.1484
381.2096
452.2408
182.0975
347.1557
435.2239
476.2803
545.2742
563.2887
580.3102
640.3394
678.3956
696.3931
%
m/z


f1 (ppm)

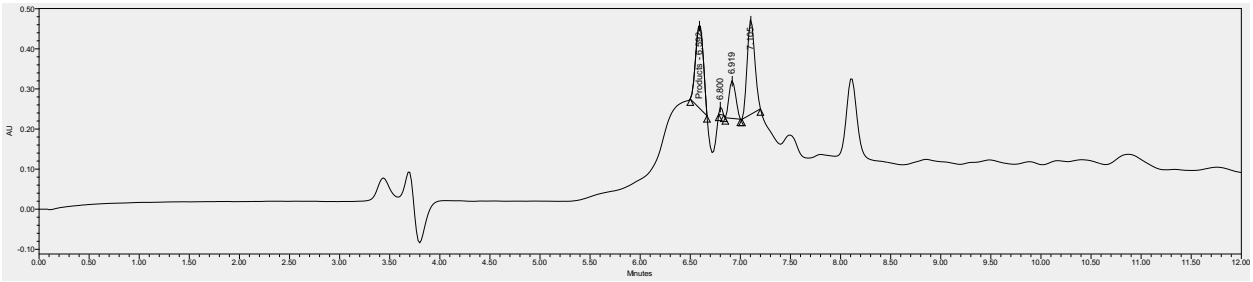
22: *Antioxidant Peptide A*

Synthesized by the general procedure for pyrimidine sidechains with **deviations 1 and 2**

MW = 817.0, % Conversion = 39%, Isolated yield = 37% [7.1 mg]

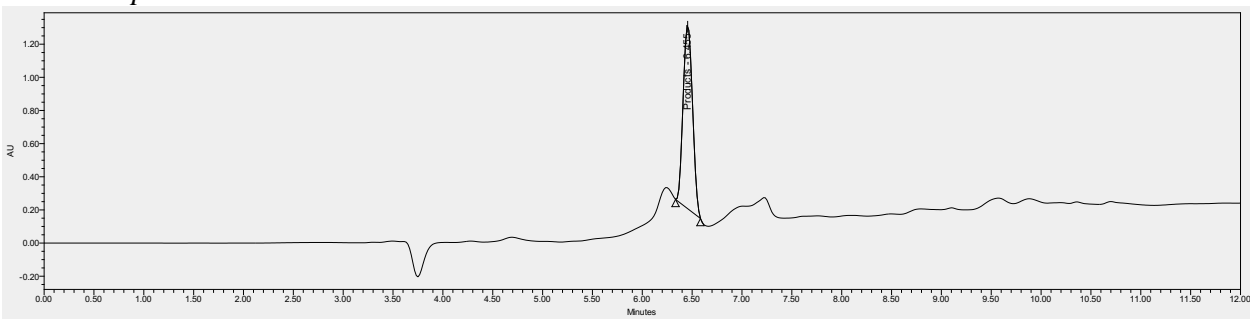


Crude:

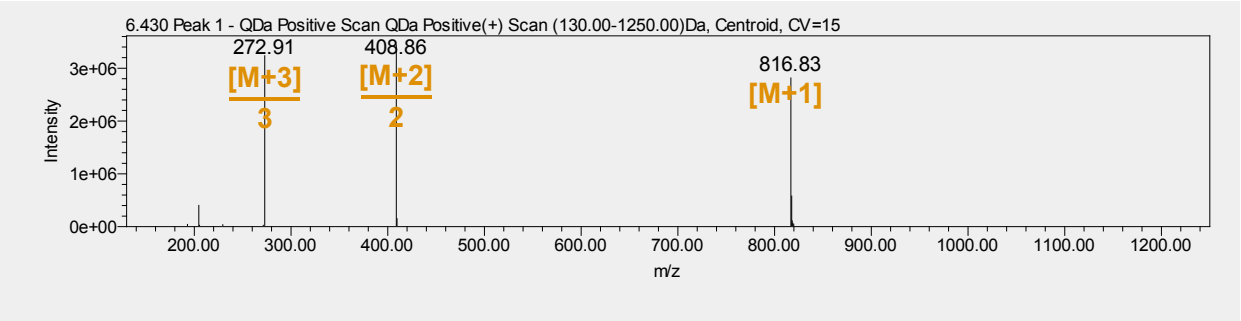


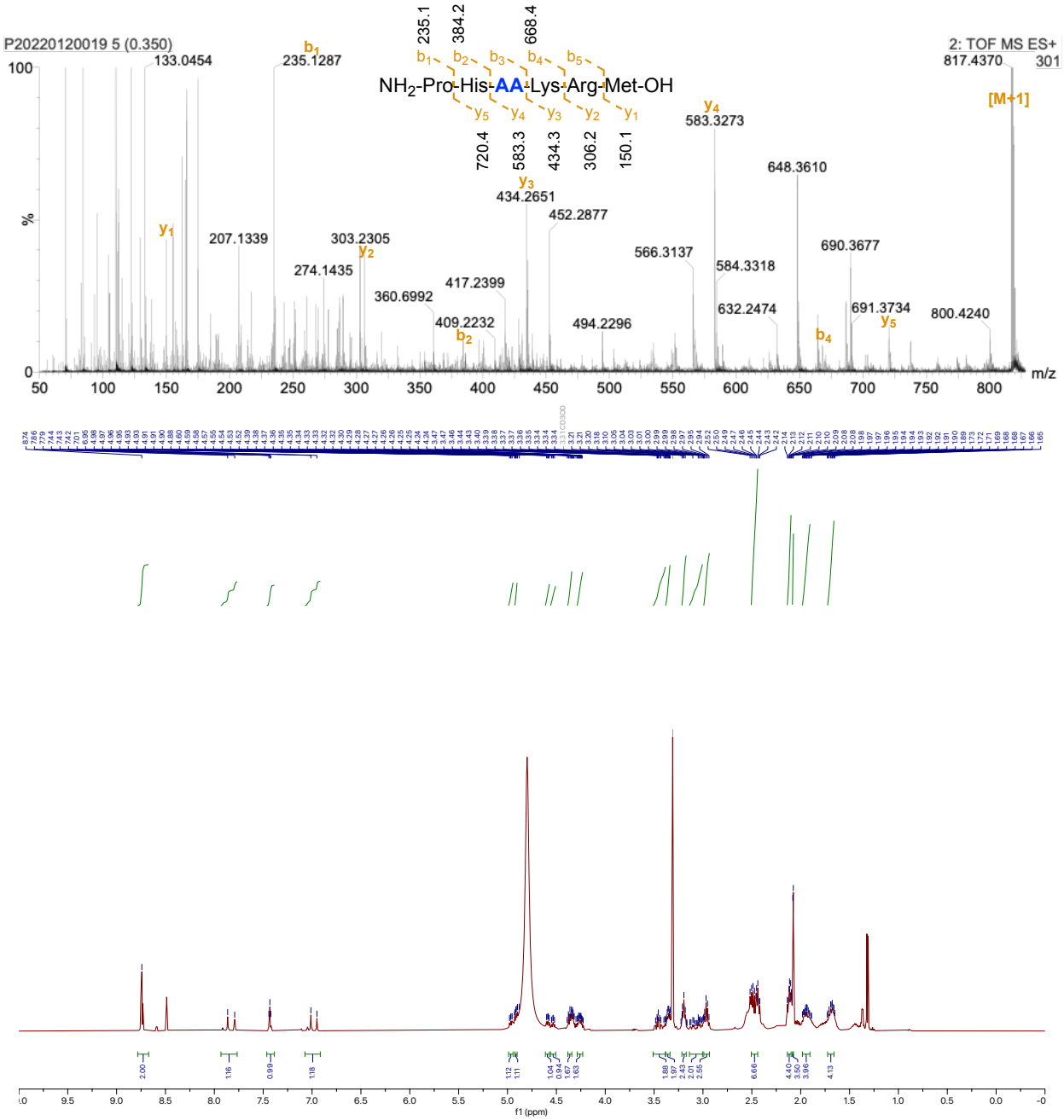
	Name	Retention Time	Area	% Area
1	Products	6.592	1102319	39.26
2		6.800	33669	1.20
3		6.919	435090	15.50
4		7.105	1236581	44.04

Isolated Epimers:



	Name	Retention Time	Area	% Area
1	Products	6.455	7253178	100.00

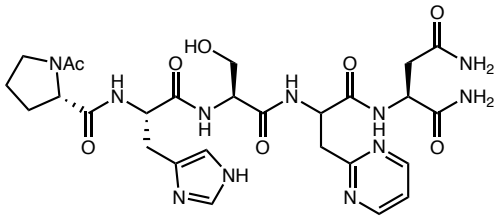

6.430 Peak 1 - QDa Positive Scan QDa Positive(+) Scan (130.00-1250.00)Da, Centroid, CV=15
272.91
[M+3]
3
408.86
[M+2]
2
816.83
[M+1]
Intensity
m/z


P20220120019 5 (0.350)
2: TOF MS ES+
817.4370
301
[M+1]
NH2-Pro-His-AA-Lys-Arg-Met-OH
b1 235.1287
133.0454
y4 583.3273
648.3610
y3 434.2651
452.2877
y1 207.1339
303.2305
y2
274.1435
566.3137
584.3318
690.3677
417.2399
360.6992
632.2474
691.3734
y5
800.4240
409.2232
b2
494.2296
b4
%
m/z
f1 (ppm)

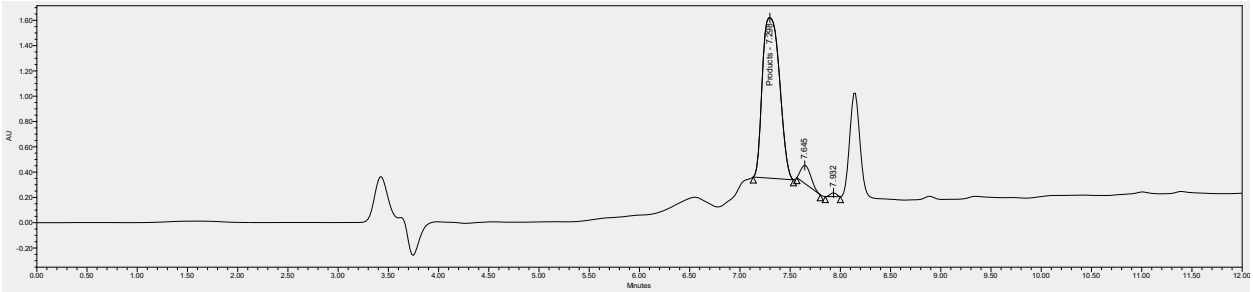
23: *ATN-161, Integrin Inhibitor*

Synthesized by the general procedure for pyrimidine sidechains with **deviation 1**

MW = 643.7, % Conversion = 93%, Isolated yield = 59% [8.8 mg]

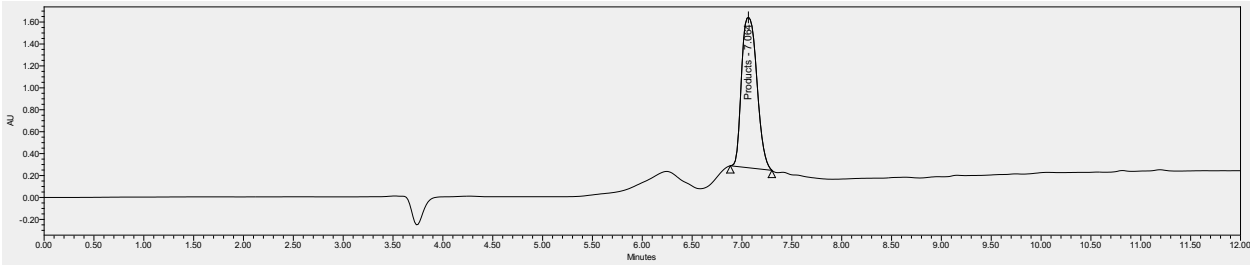


Crude:

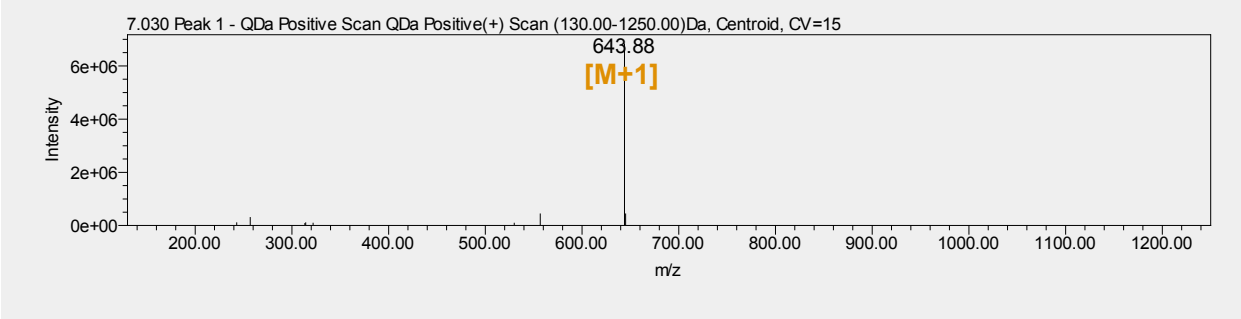


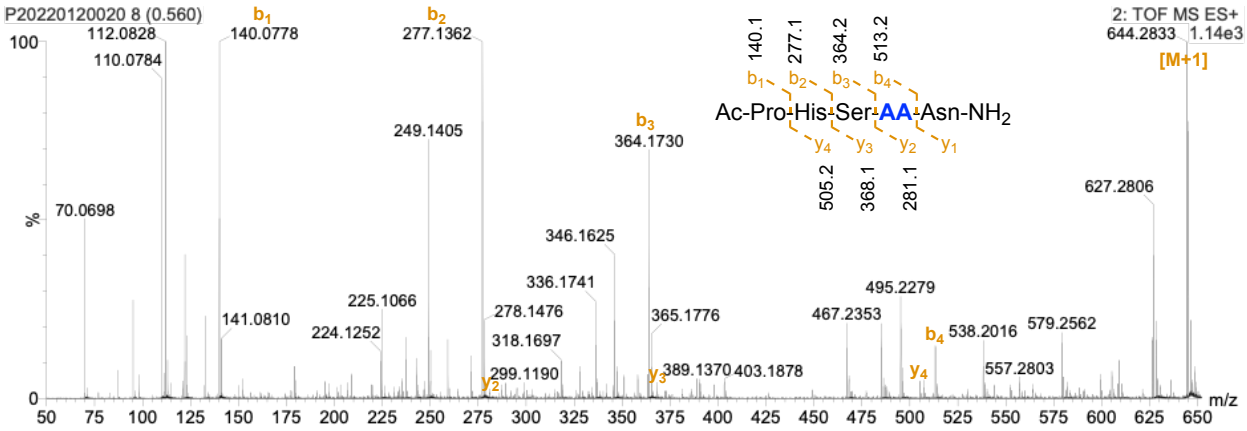
	Name	Retention Time	Area	% Area
1	Products	7.298	15091815	92.96
2		7.645	1010426	6.22
3		7.932	132746	0.82

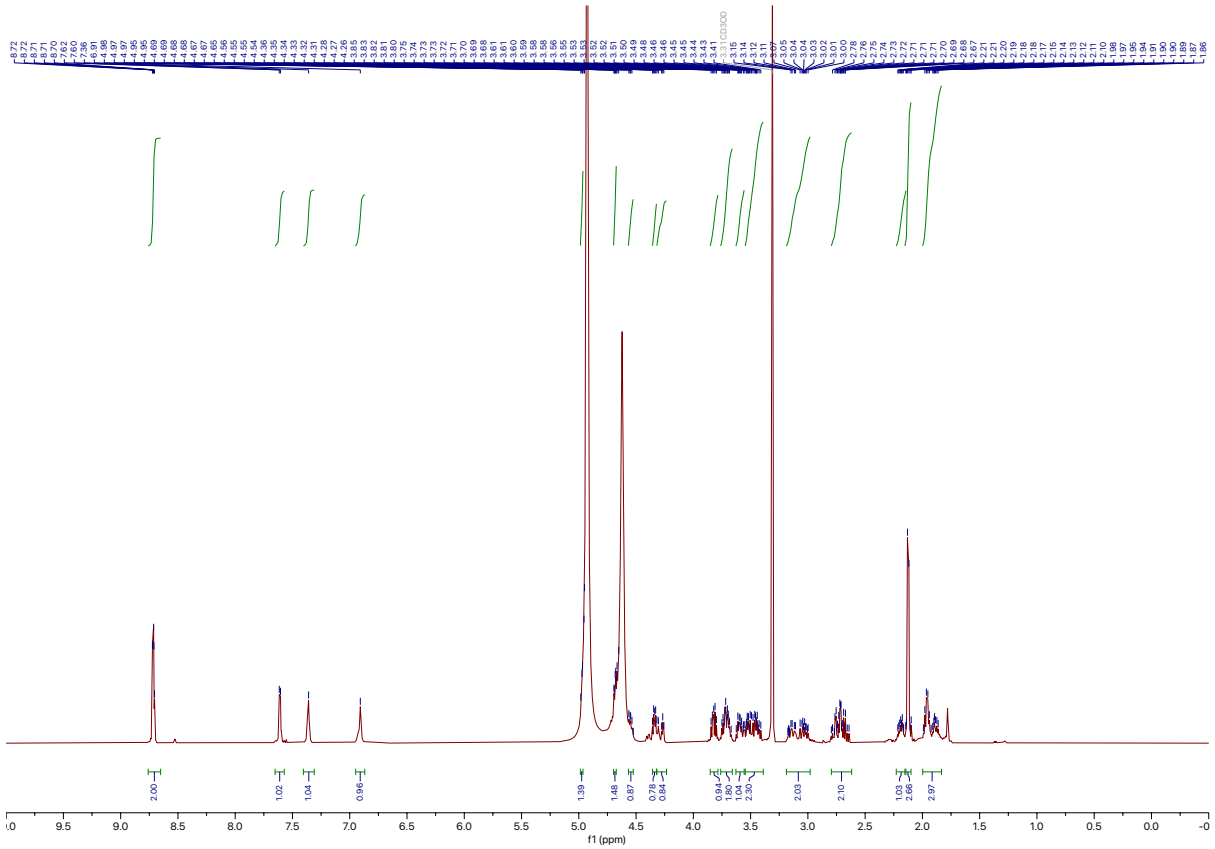
Isolated Epimers:



	Name	Retention Time	Area	% Area
1	Products	7.064	14688336	100.00


7.030 Peak 1 - QDa Positive Scan QDa Positive(+) Scan (130.00-1250.00)Da, Centroid, CV=15
643.88
[M+1]
Intensity
6e+06
4e+06
2e+06
0e+00
200.00
300.00
400.00
500.00
600.00
700.00
800.00
900.00
1000.00
1100.00
1200.00
m/z

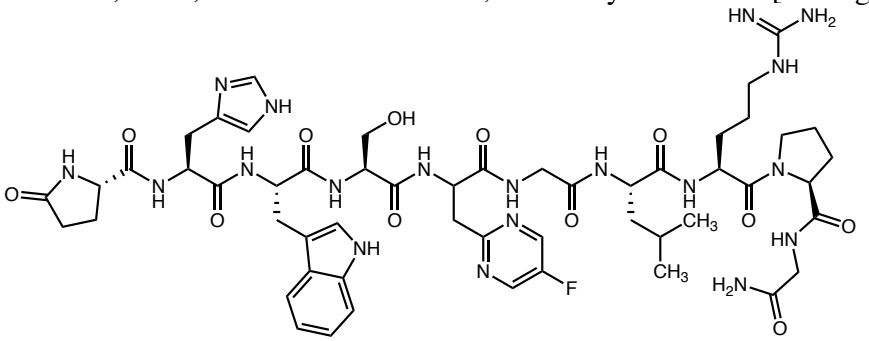

P20220120020 8 (0.560)
2: TOF MS ES+
644.2833 1.14e3
[M+1]
b1 140.0778
b2 277.1362
b3 364.1730
y2
y3
y4
112.0828
110.0784
249.1405
70.0698
346.1625
336.1741
225.1066
278.1476
141.0810
224.1252
318.1697
365.1776
299.1190
389.1370
403.1878
467.2353
495.2279
538.2016
579.2562
557.2803
627.2806
140.1
277.1
364.2
513.2
b1 b2 b3 b4
Ac-Pro-His-Ser-AA-Asn-NH2
y4 y3 y2 y1
505.2
368.1
281.1
%
100
0
50 75 100 125 150 175 200 225 250 275 300 325 350 375 400 425 450 475 500 525 550 575 600 625 650
m/z


f1 (ppm)

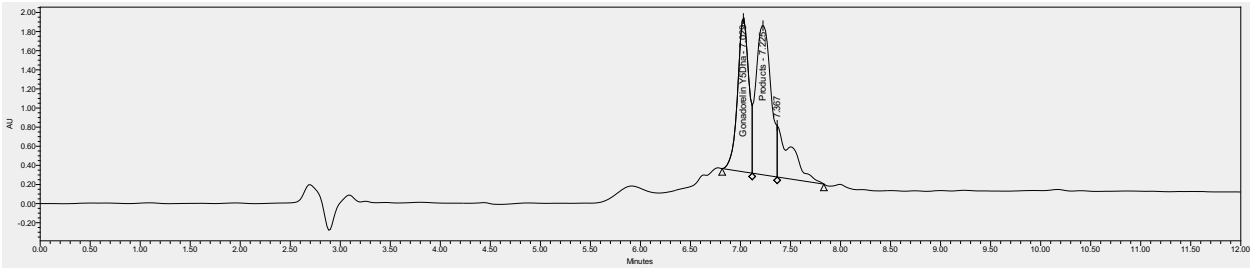
24: *Gonadorelin, Ovulating Agent*

Synthesized by the general procedure for pyrimidine sidechains on a **10 μmol scale**

MW = 1,187.3, % Conversion = 48%, Isolated yield = 18% [2.1 mg]

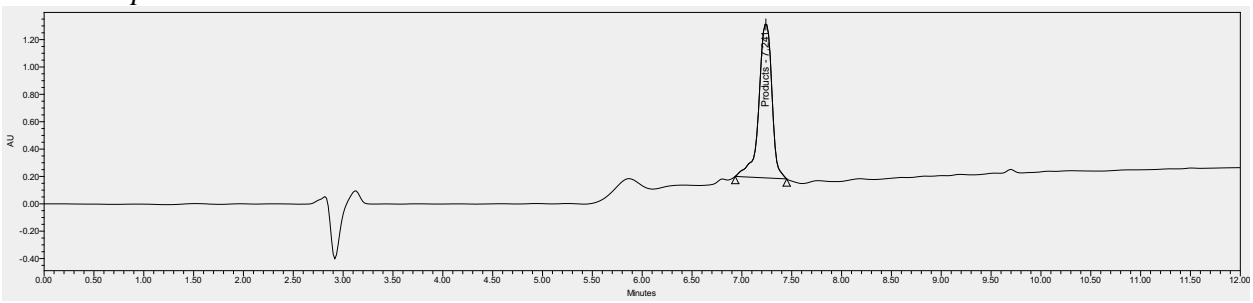


Crude:

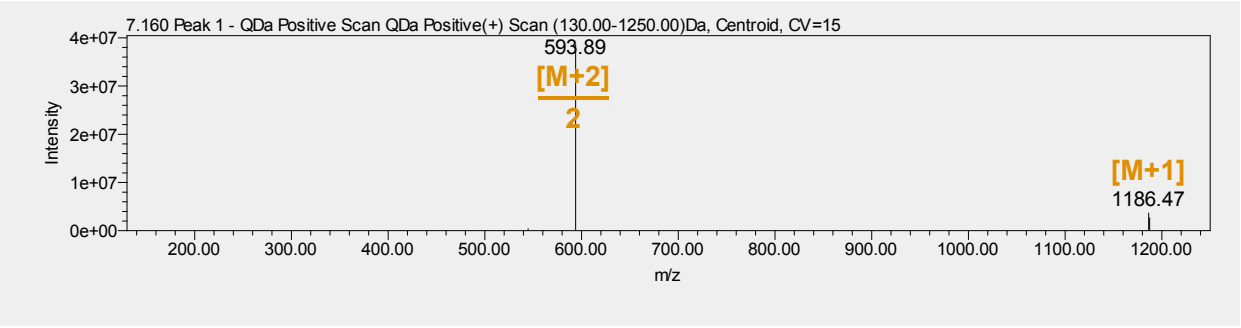


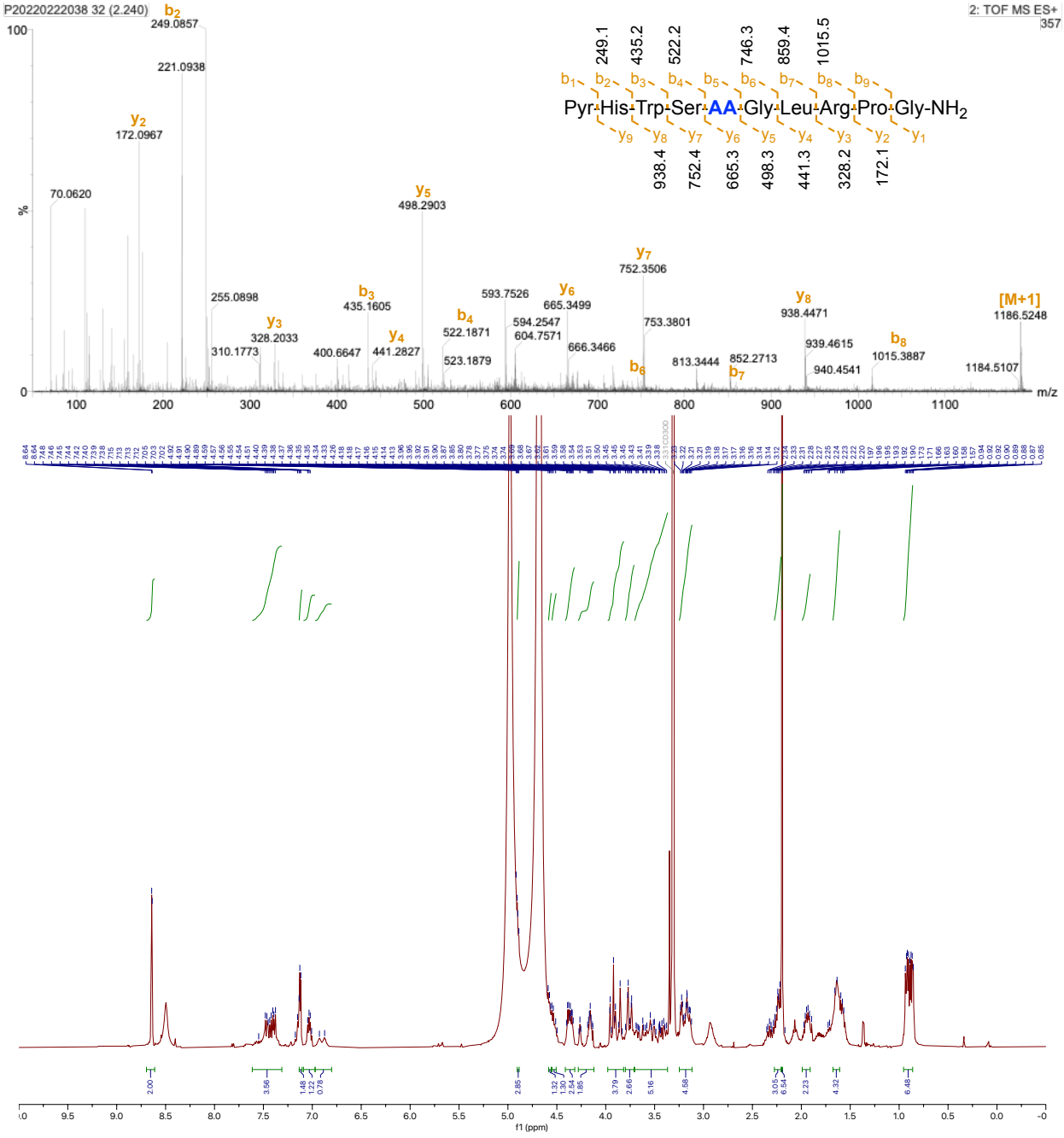
	Name	Retention Time	Area	% Area
1	Gonadorelin Y5Dha	7.029	12492255	36.42
2	Products	7.225	16399254	47.81
3		7.367	5406777	15.76

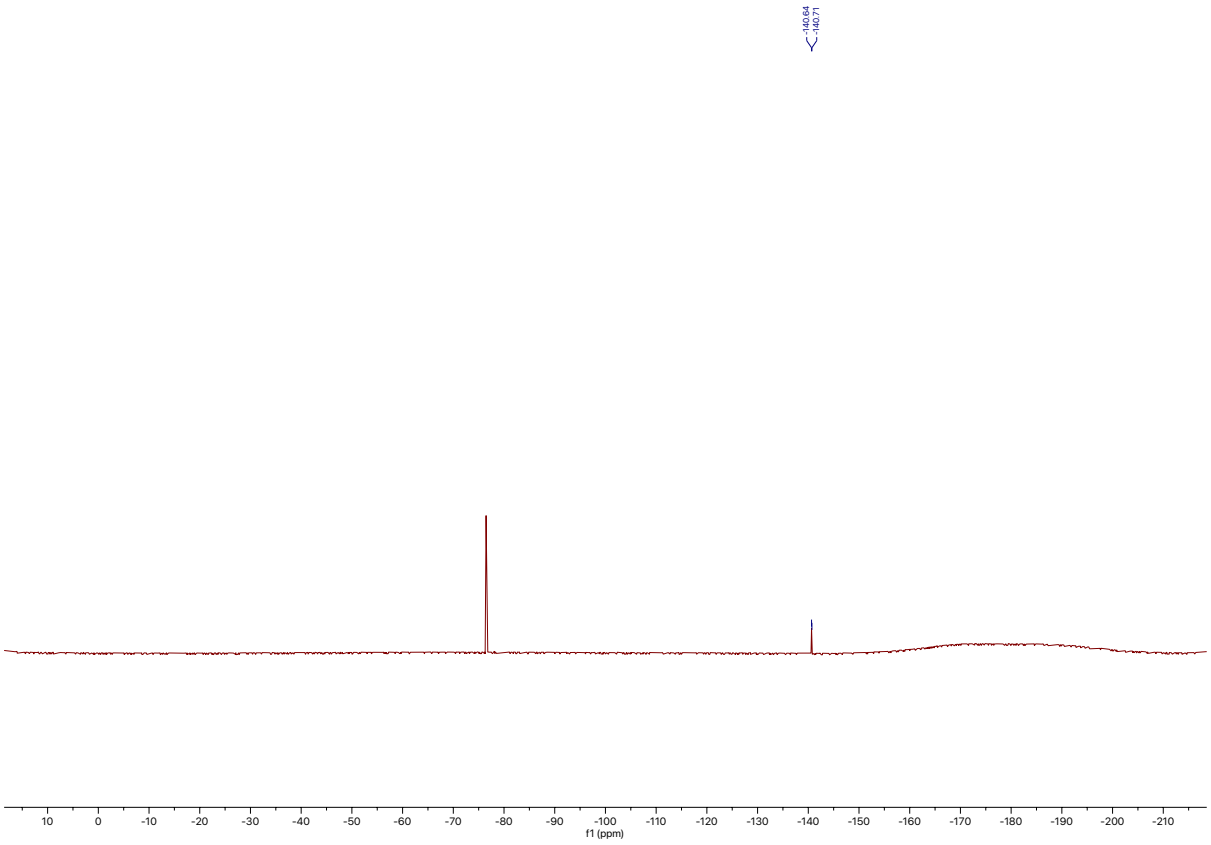
Isolated Epimers:



	Name	Retention Time	Area	% Area
1	Products	7.241	10296248	100.00


7.160 Peak 1 - QDa Positive Scan QDa Positive(+) Scan (130.00-1250.00)Da, Centroid, CV=15
Intensity
4e+07
3e+07
2e+07
1e+07
0e+00
593.89
[M+2]
2
[M+1]
1186.47
200.00
300.00
400.00
500.00
600.00
700.00
800.00
900.00
1000.00
1100.00
1200.00
m/z


P20220222038 32 (2.240)
2: TOF MS ES+
357
Pyr-His-Trp-Ser-AA-Gly-Leu-Arg-Pro-Gly-NH2
b2 249.0857
221.0938
y2 172.0967
70.0620
y5 498.2903
y7 752.3506
b3 435.1605
y6 665.3499
593.7526
255.0898
y3 328.2033
b4 522.1871
y4 441.2827
310.1773
400.6647
523.1879
594.2547
604.7571
666.3466
753.3801
y8 938.4471
[M+1] 1186.5248
b8 1015.3887
939.4615
940.4541
1184.5107
b6
b7
813.3444
852.2713
m/z
f1 (ppm)


-140.64
-140.71
10
0
-10
-20
-30
-40
-50
-60
-70
-80
-90
-100
-110
-120
-130
-140
-150
-160
-170
-180
-190
-200
-210
f1 (ppm)

References

[1] J. R. Immel, M. Chilamari, S. Bloom, *Chem. Sci.* **2021**, *12*, 10083–10091.
[2] P. M. Morrison, P. J. Foley, S. L. Warriner, M. E. Webb, *Chem. Commun.* **2015**, *51*, 13470–13473.