Supporting Information

Late-Stage Installation and Functionalization of Alkyl Pyridiniums: A General HTE Amenable Strategy to Access Diverse Aryl Alanine Containing Macrocyclic Peptides

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1. General Comments

All materials, reagents and solvents were obtained from commercial suppliers and used without further purification. Reactions were performed in oven-dried vials with Teflon-lined caps or oven-dried round bottomed flasks unless otherwise noted. Flasks were fitted with rubber septa, and reactions were conducted under a positive pressure of N_2. Manual and automated solid phase peptide synthesis (SPPS) were performed on peptide synthesizer Symphony X. Proton nuclear magnetic spectra (^{1}H NMR), carbon nuclear magnetic spectra (^{13}C NMR) were recorded on 500 MHz spectrometer. Chemical shifts are given in parts per million (p.p.m., δ units) relative to tetramethyl silane or NMR solvent (DMSO-d_6 = δ 2.50) peak and coupling constants (*J*) are reported in Hz. The following NMR abbreviations are used: s= singlet, d = doublet, q = quartet, m = multiplet, dd = doublet of doublets, ddd = doublet of doublet of doublet, ddt = doublet of doublet of triplet, td = triplet of doublet, br s = broad singlet.

Macrocyclic peptide (MP) characterization: LC and HRMS

Instrument :Waters Acquity UPLC	
LC Conditions	
Column :	Aquity UPLC BEH C18 1.7µm
Flow rate:	0.3mL/min
Mobile phase :	A : 0.1% FA in Water
	B : 0.1% FA in Acetonitrile
Gradient :	5% B 0.0 min
	5% B 2.0 min
	30% B 4.0 min
	95% B 9.0 min
	95% B 13.0 min
	5% B 13.5 min
	5% B 15 min

Instrument :Waters Xevo XS Tof	
MS Conditions:	
Ionization mode:	ESI+
Source Temp.:	120 °C
Scan range:	m/z 400-4000

LCMS Method used in HTE.

Instrument :Waters Acquity UPLC	
LC Conditions	
Column :	Aquity UPLC BEH C18 1.7µm
Flow rate:	0.35mL/min
Mobile phase :	A : 0.1% TFA in Water
	B : 0.1% TFA in Acetonitrile
Gradient :	5% B 0.0 min
	100% B 4.4 min
	100% B 4.8 min
	5% B 4.82 min

Instrument : Waters Single Quadrupole Mass Detector	
MS Conditions:	
Ionization mode:	ESI+
Source Temp.:	400 °C
Scan range:	m/z 300-2000

Purification methods:

Method A: Macrocyclic peptides were purified by preparative HPLC on a Gilson GX-281 prep system, equipped with kromasil 100-10 C8 column (Dimensions: 50 x 250mm), using mobile phase of Acetonitrile and H_2O with both containing 0.05% TFA and a linear gradient 30-100% Acetonitrile over 30 minutes.

Method B: Macrocyclic peptides were purified by Waters Dual-Pump Mass-Directed AutoPure preparative instrument, equipped with two XSelect CSH C-18 columns (Dimensions: 19 x 100 mm), using mobile phase of Acetonitrile and H_2O with both containing 0.16% TFA using a linear gradient 46-61% Acetonitrile over 6 minutes.

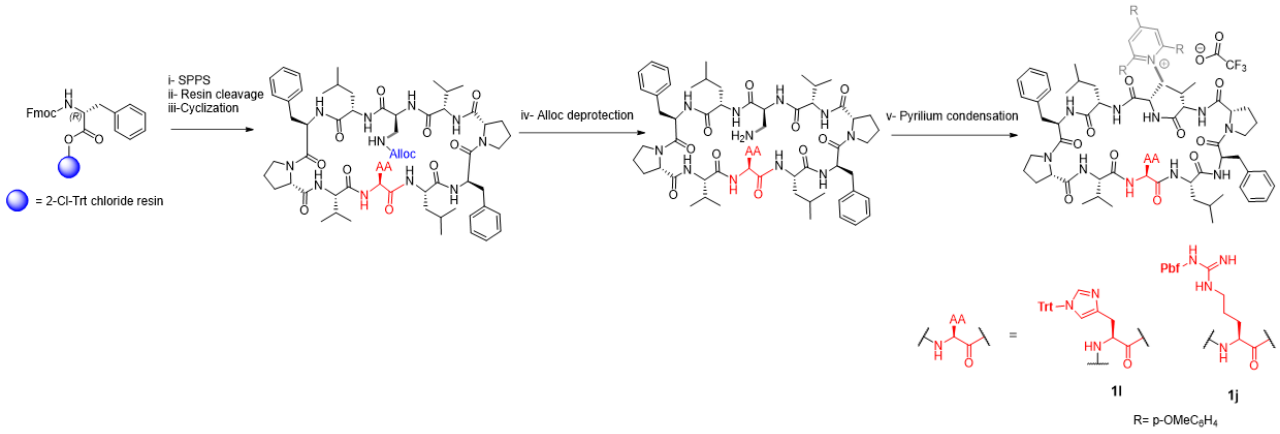
2. Macrocyclic peptide synthesis and characterization.

2.1 Synthesis of MP-Pyridinium salts **1a-1l** and **3**.

Synthesis strategy of **1a-1h**, **1k**,**1l** and **3** were depicted in manuscript scheme 2-A and detailed procedure for the synthesis of these peptides is described below.

A modified procedure was used for the synthesis of **1i** (His contain MP-pyridinium) and **1j** (Arg contain MP-pyridinium) (Figure S1). As shown below, these two peptides were synthesized with the protecting group intact (Trityl (Trt) for **1i** and and 2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl (Pbf) for **1j**). In the presence of free imidazole and guanidinium, pyrylium condensation reactions were not clean.

Figure S1: Synthesis strategy of **1i** and **1j**:



2.1.1 General loading protocol exemplified by Fmoc-D-Phe-OH loading on 2Cl-trt chloride resin.

2-Chlorotrityl chloride resin (1.56 mmol/g, 1g, 1.56 mmol) was swelled in DCM (10 mL) for 5 minutes in a fritted syringe then drained. A solution of Fmoc-D-Phe-OH (0.7 eq) and DIPEA (3 eq) was prepared and transferred to the resin-containing syringe. The reaction was shaken at rt for 3 h. After draining the crude solution, the resin was treated with 10 mL of DCM/MeOH/DIPEA (17:2:1) for 15 minutes and drained. This capping step with MeOH was repeated twice. The resin bound Fmoc-D-Phe was washed with DCM (3x10 mL), DMF (3x10 mL), and DCM(3x10 mL), then dried under vacuum overnight.

The loading rate was determined by gain in weight, and batch to batch varied between 0.7 to 0.85 mmol/g.

2.1.2 General procedure for linear Chain Assembly by SPPS and resin cleavage

Linear peptide precursors were synthesized via solid-phase peptide synthesis on an automated Gyros Protein Symphony X peptide synthesizer at 0.3 mmol scale. Fmoc deprotection was performed with 12 mL of 20% (v/v) pyrrolidine in DMF at rt for 4 minutes, repeated twice. Each amino acid monomer was coupled by addition of 4 eq HATU (0.4M in DMF), 4 eq of amino acid monomer (0.2 M in DMF) and 8 eq NMM (0.8 M in DMF) at room temperature for 5 minutes, repeated once. Upon completion of peptide synthesis, the linear peptide precursor was cleaved from resin by treatment with 10 mL of 30% (v/v) HFIP in DCM for 5 min, repeated twice. The peptide solution was concentrated under reduced pressure to yield the crude linear peptide, as confirmed by LC/MS.

2.1.3 General procedure for the cyclization

Crude linear peptide (assumed quantitative yield, 0.3 mmol) was dissolved in 70 mL THF. To the solution was added 4 eq DIPEA and 0.95 eq HATU (0.4M in ACN) sequentially. The reaction was allowed to stir at rt for 15 min. Upon completion monitored by LC/MS, the reaction mixture was concentrated under reduced pressure, then dried further under vacuum, and used as crude in the following step.

2.1.4 General procedures for the deprotection of Dap (functional handle) as well as global deprotection:

Protocol A: Boc deprotection to obtain deprotected cyclized precursors of **1a**-**1c**, **1k**,**1l**, and **3**.

Cyclized peptide residue (0.3mmol) was dissolved in 10 mL of 20% (v/v) TFA in DCM and allowed to stir at rt for 30 minutes, after which the solution was concentrated via rotary evaporation. The residue was redissolved in 15 mL ACN/water (3:1) and lyophilized. Dissolution in ACN/water and lyophilization was repeated twice more to remove any remaining TFA.

Protocol B: Global deprotection to obtain deprotected cyclized precursors of **1d**, **1e**, **1f**, **1g**, **1h**, and **1m**.

Cyclized peptide residue (0.3 mmol) was dissolved in 10 mL of cleavage cocktail (TFA/water/TIPS (95:2.5:2.5) and allowed to stir at rt for 30 minutes, after which the solution was concentrated via rotary evaporation. The residue was redissolved in 15 mL ACN/water (3:1) and lyophilized. Dissolution in ACN/water and lyophilization was repeated twice more to remove any remaining TFA.

Protocol C: Alloc Deprotection to obtain Dap deprotected cyclized precursors of **1i**, and **1j**.

Cyclized peptide residue (0.3 mmol) was dissolved in 6 mL of anhydrous DCM under N_2 atmosphere. 1.5 mL tetrakis(triphenylphosphine)palladium (0) solution (0.015 M in DCM, 0.07 eq) and phenylsilane (0.185 mL, 1.5 mmol, 5 eq) were added, and the resulting mixture was allowed to stir under N_2 at room temperature for 1h. After confirming reaction completion by LC/MS, the reaction mix was concentrated under reduced pressure and used in the next step without any purification.

2.1.5 General procedures for the Pyrylium condensation

0.150 mmol of cyclic deprotected peptide (half of crude lyophilized powder) was dissolved in 15 mL of EtOH in a Biotage® microwave reaction vial. To this solution was added DIPEA (4 eq) and the mixture was stirred at rt for 5 min. Pyrylium tetrafluoroborate salt (1 eq) was added to the mixture. The reaction vial was sealed and stirred at 90 °C using a Biotage® Initiator+ microwave. Reactions were monitored via LC/MS, with reaction times differing based on substrate as indicated below. An additional 0.3 eq of pyrylium was added to reactions that were less than half complete at the 1 h mark. After confirming reaction completion by LC/MS, the reaction mixture was concentrated under reduced pressure. Crude mixture was dissolved in DMSO and directly purified on reverse phase HPLC using C8 column, eluting with 20-100% ACN (containing 0.5%TFA) in H_2O (containing 0.5% TFA) on a 30-minute linear gradient to obtain desired product as light yellow or orange solid. The purities listed below in Table S1 are based on LCMS analysis of the purified products.

Table S1: Pyrylium condensation reaction times, isolated yield, and purities.

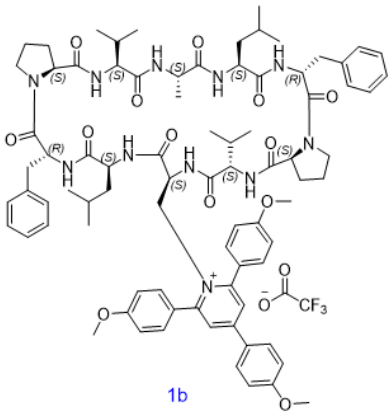
MP#	Pyrylium	Reaction time (h)	Isolated yield (%)	Isolated Purity (%)
1a	Pyrylium-H	5	35	94
1b	Pyrylium-OMe	5	44	95
1c	Pyrylium-Me	5	40	95
1d	Pyrylium-OMe	1	52	98
1e	Pyrylium-OMe	6	35	98
1f	Pyrylium-OMe	1	21	98
1g	Pyrylium-OMe	9	23	98
1h	Pyrylium-OMe	10	27	92
1i	Pyrylium-OMe	1*	12	74
1j	Pyrylium-OMe	9	14	88
1k	Pyrylium-OMe	1	49	99
1l	Pyrylium-OMe	1	19	97
3	Pyrylium-OMe	1	49	96

*Reaction did not reach completion but was stopped to prevent formation of byproducts.

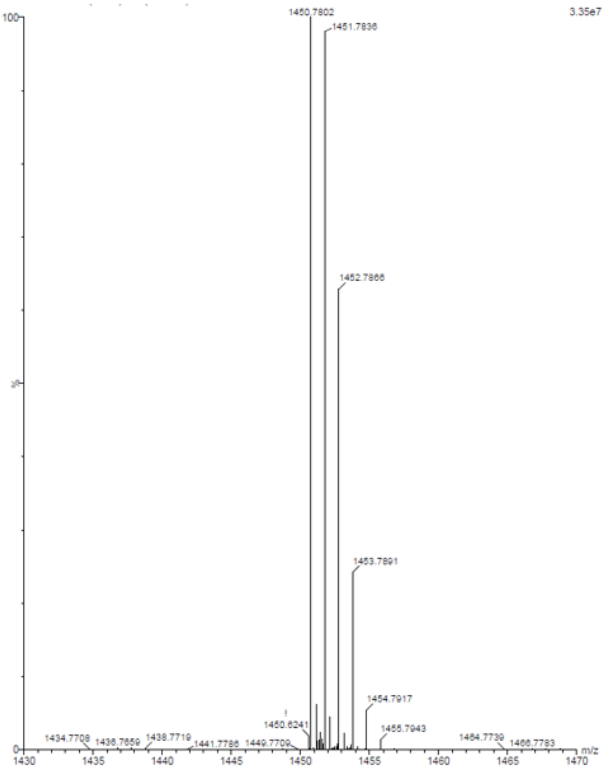
Above reported isolated yields in Table S1 are for the overall synthesis of MP-pyridiniums.

2.1.6 Characterization of MP-Pyridinium salts **1a**-**1l** and **3**.

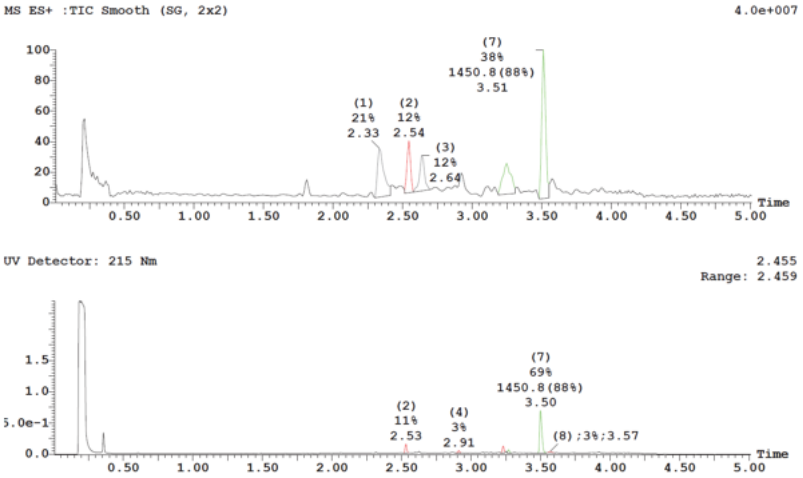
Note the green peaks in analytical HPLC traces below are desired product peaks.



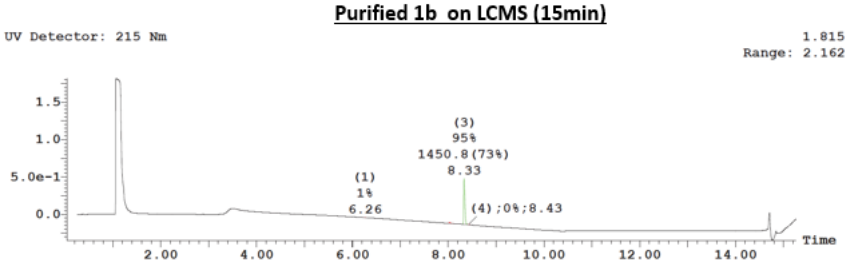
HRMS_SUMMARY			
Exact Mass	Observed Mass	Δm Error [ppm]	Formula
1450.7815	1450.7802	**-0.9**	C82H104N11O13

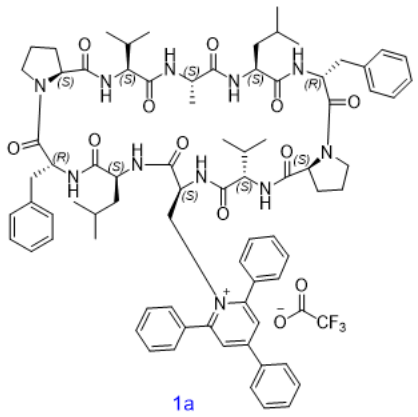


Crude Profile of 1b on LCMS



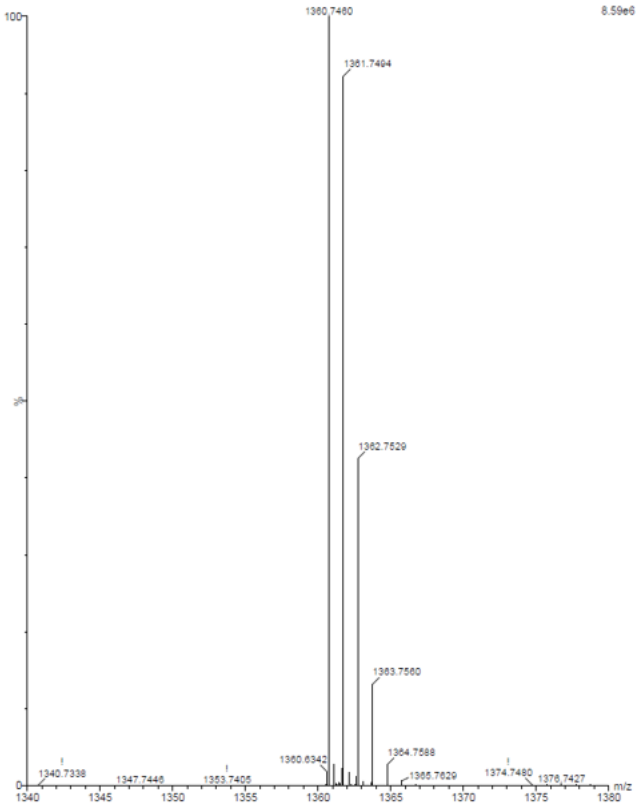
Purified 1b on LCMS (15min)



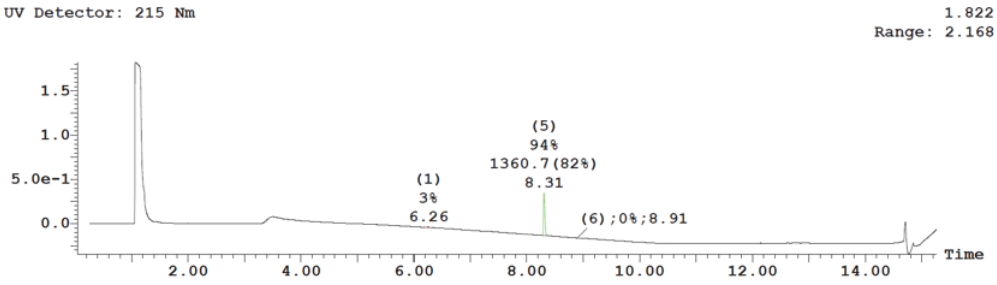


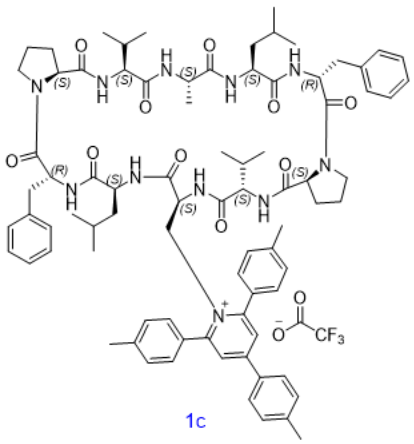
1a

HRMS_SUMMARY			
Exact Mass	Observed Mass	Δm Error [ppm]	Formula
1360.7498	1360.746	-2.8	C79H98N11O10



Purified 1a on LCMS (15min)





1c

HRMS_SUMMARY			
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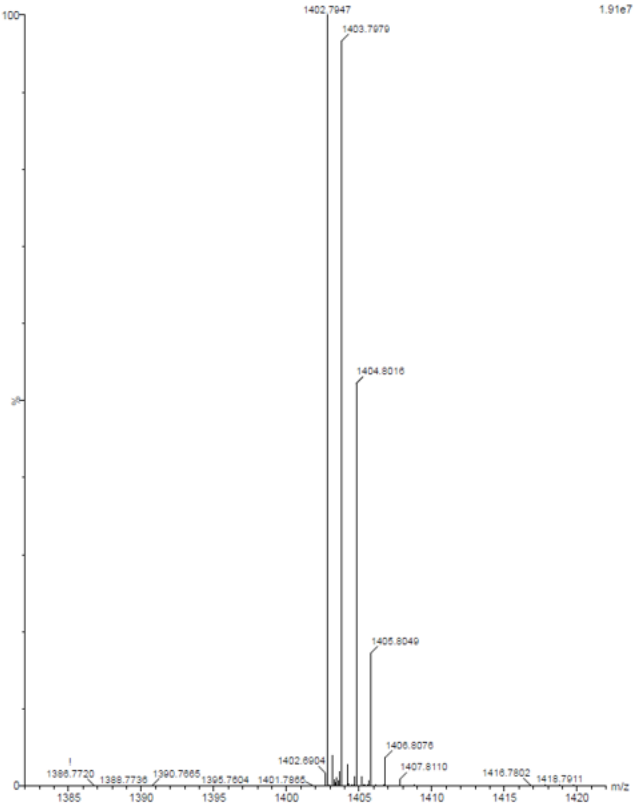
1402.7968	1402.7947	-1.5	C82H104N11O10



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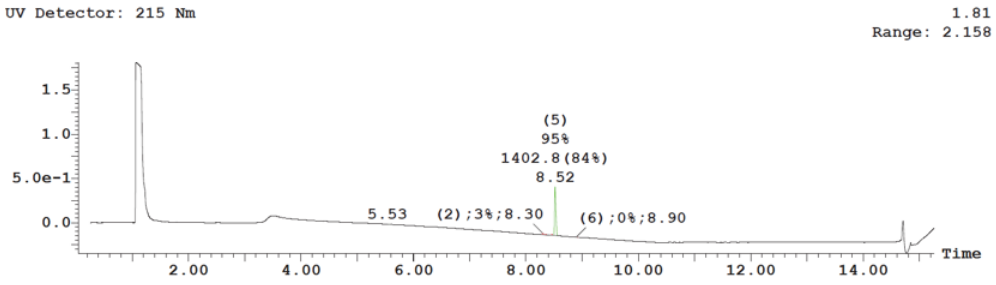

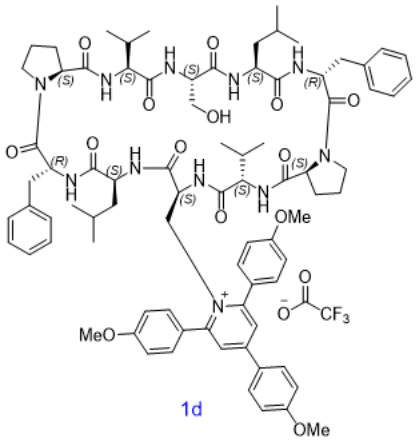

HRMS_SUMMARY			
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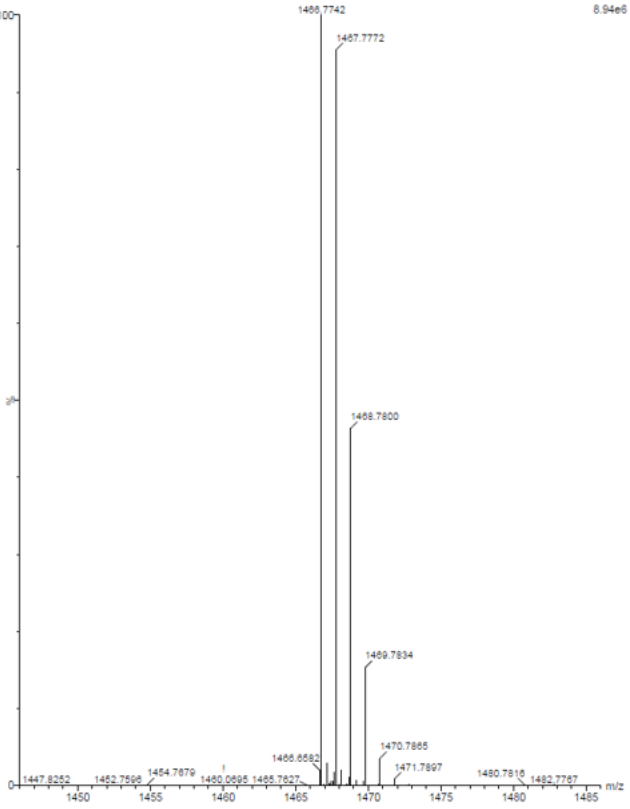
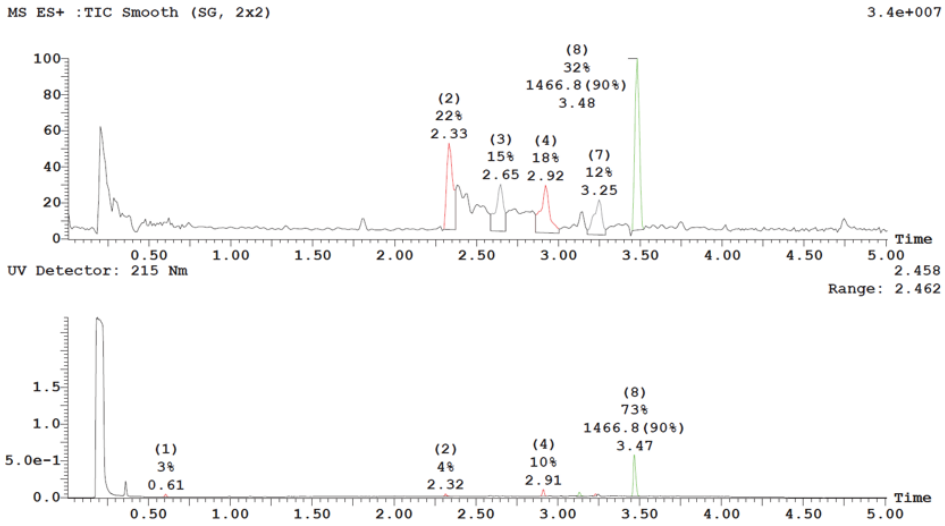
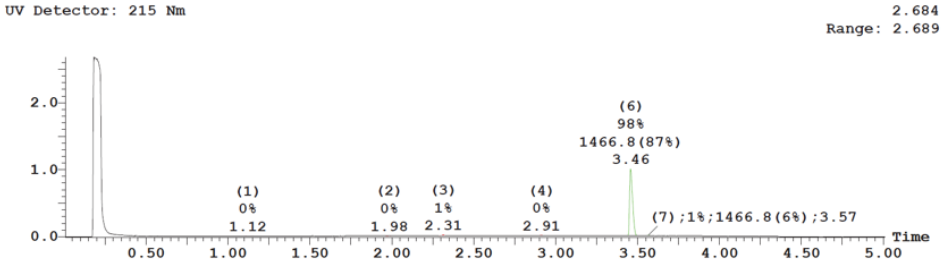
1466.7764	1466.7742	-1.5	C82H104N11O14

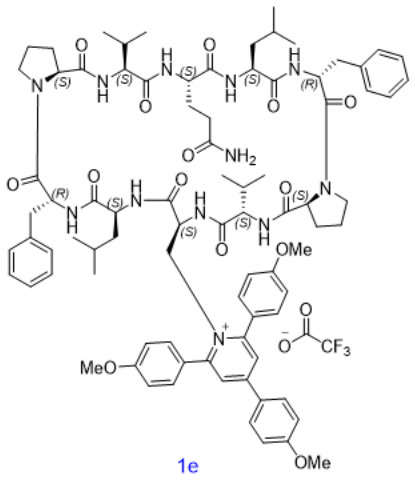


Crude Profile of 1d on LCMS



Purified 1d on LCMS





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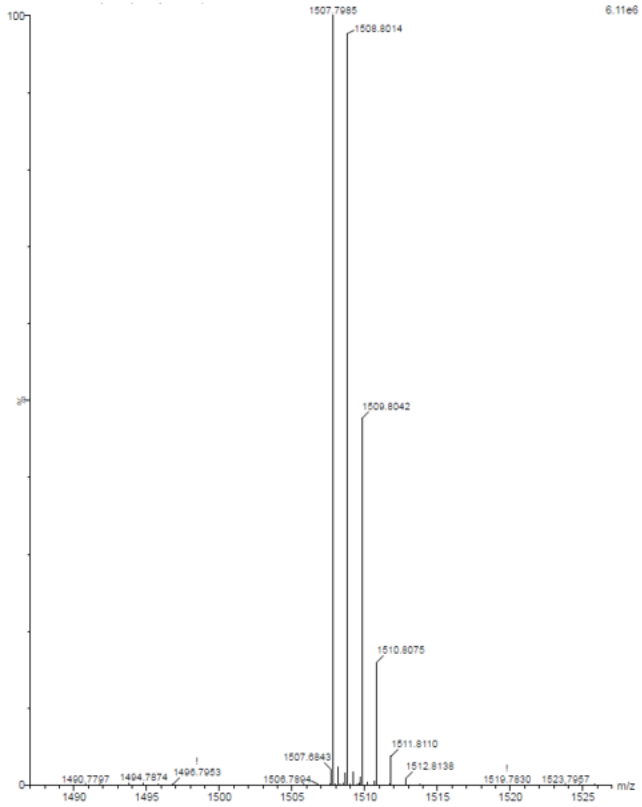
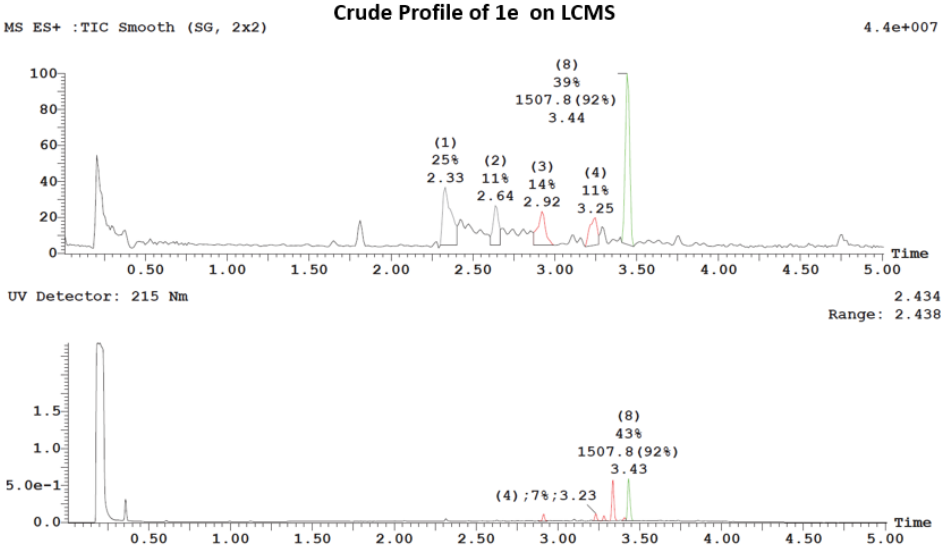
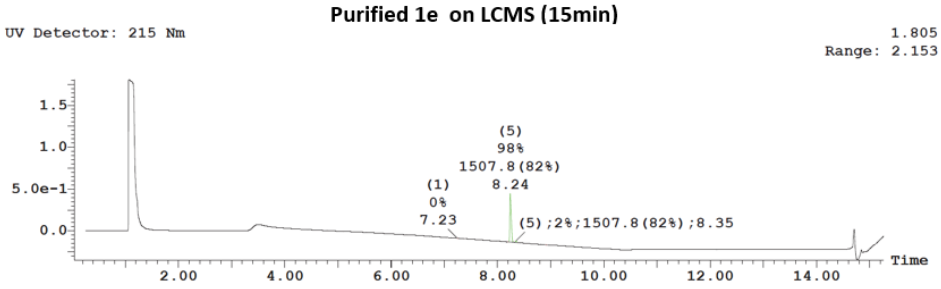
1507.803	1507.7985	-3	C84H107N12O14

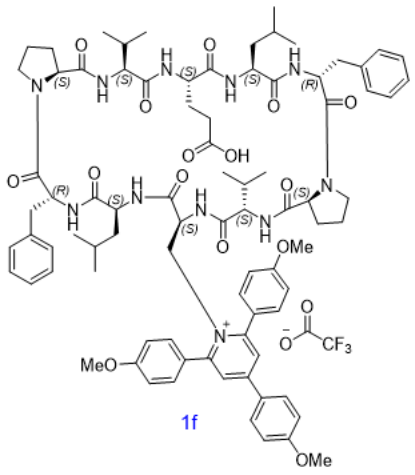


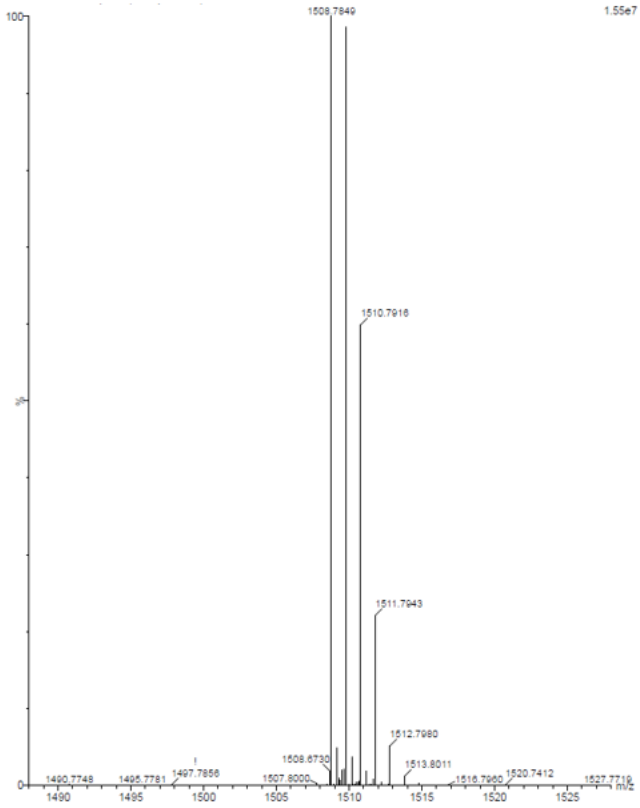
Crude Profile of 1e on LCMS



Purified 1e on LCMS (15min)







HRMS_SUMMARY			
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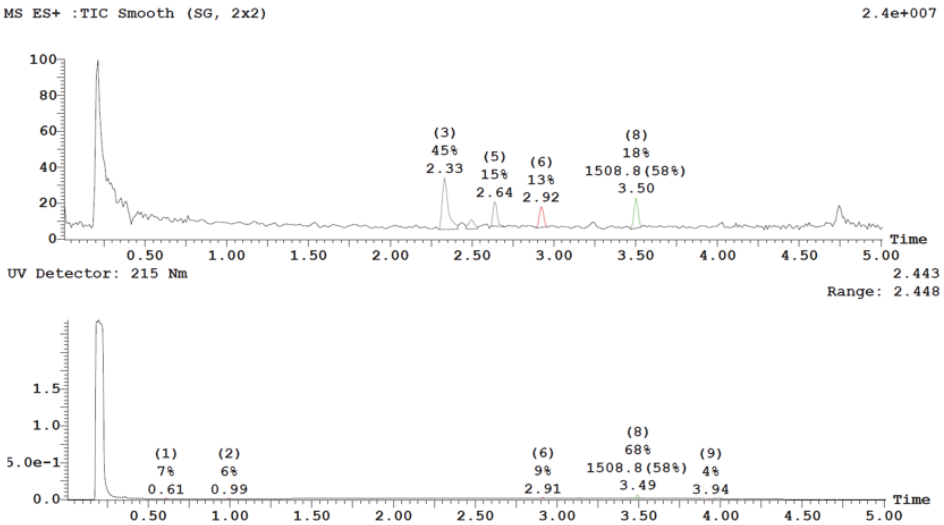
1508.787	1508.7849	-1.4	C84H106N11O15

Crude Profile of 1f on LCMS



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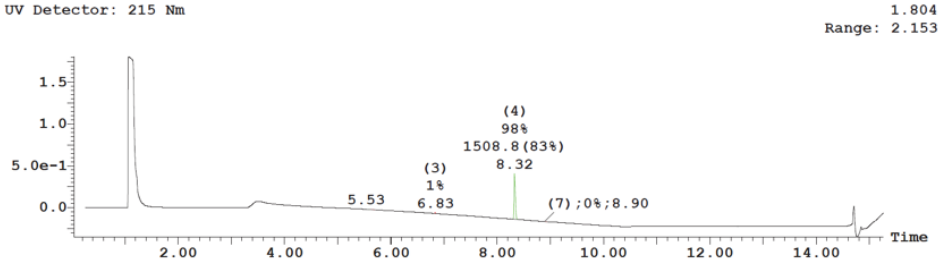

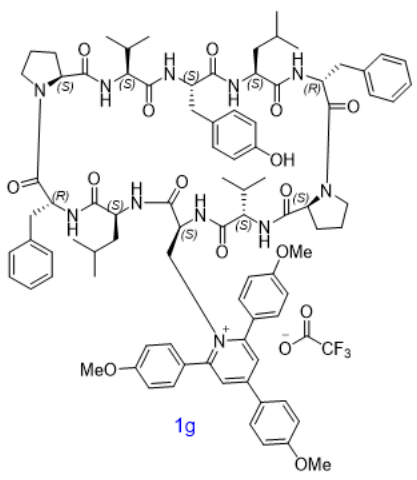

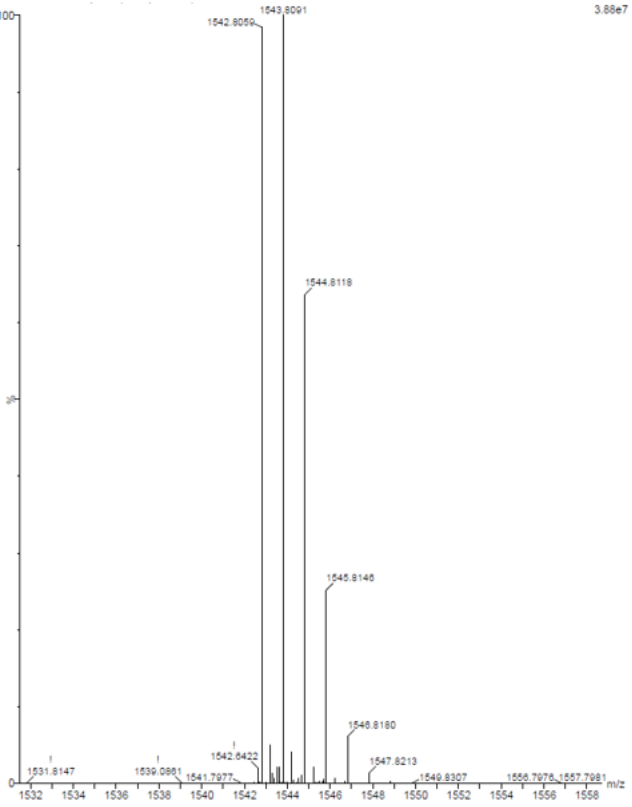

HRMS_SUMMARY			
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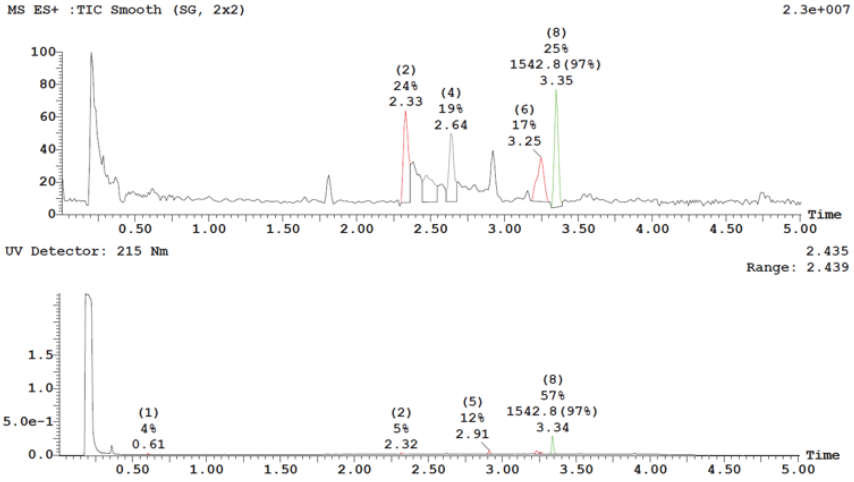
1542.8077	1542.8059	-1.2	C88H108N11O14

Crude Profile of 1g on LCMS



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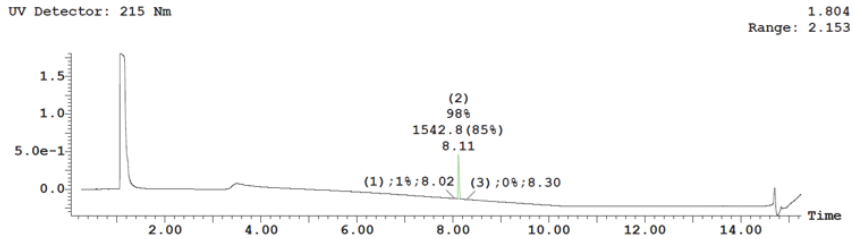

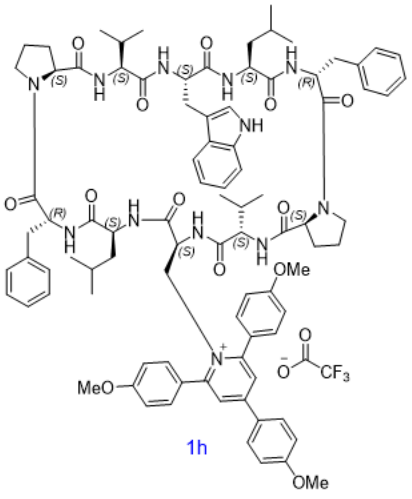

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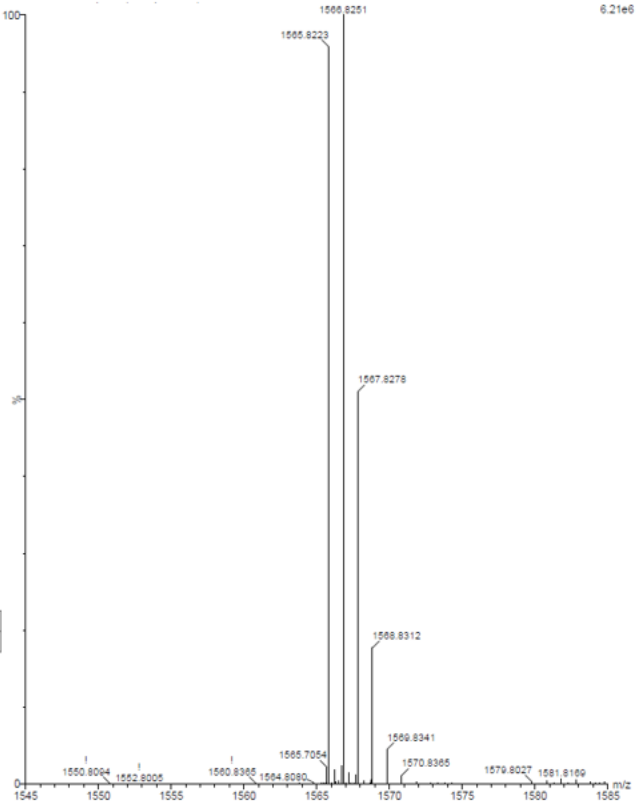
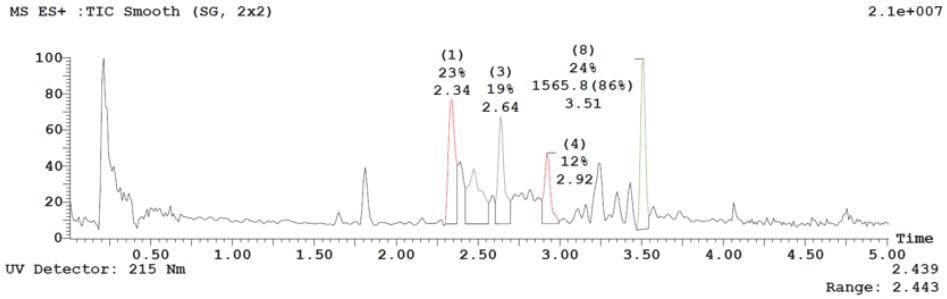
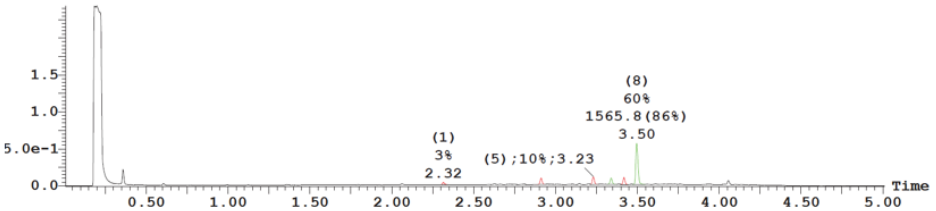
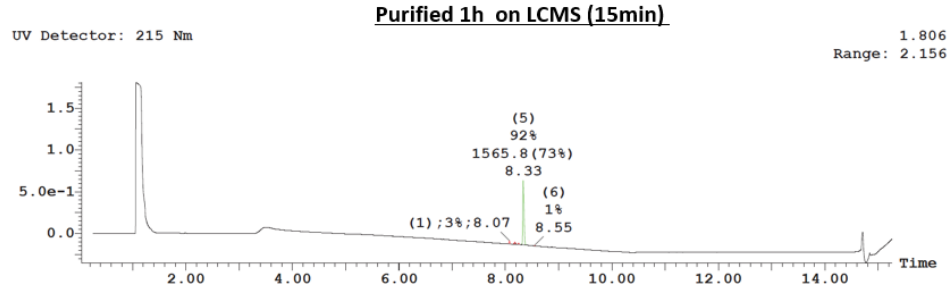


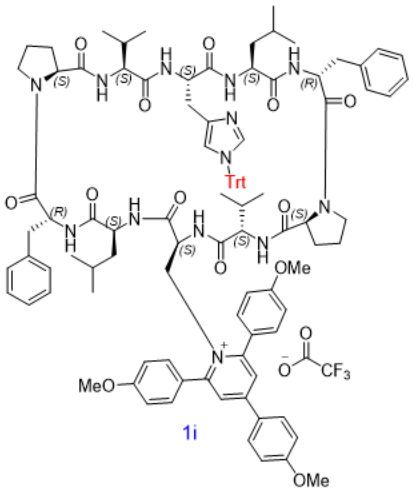
Crude Profile of 1h on LCMS

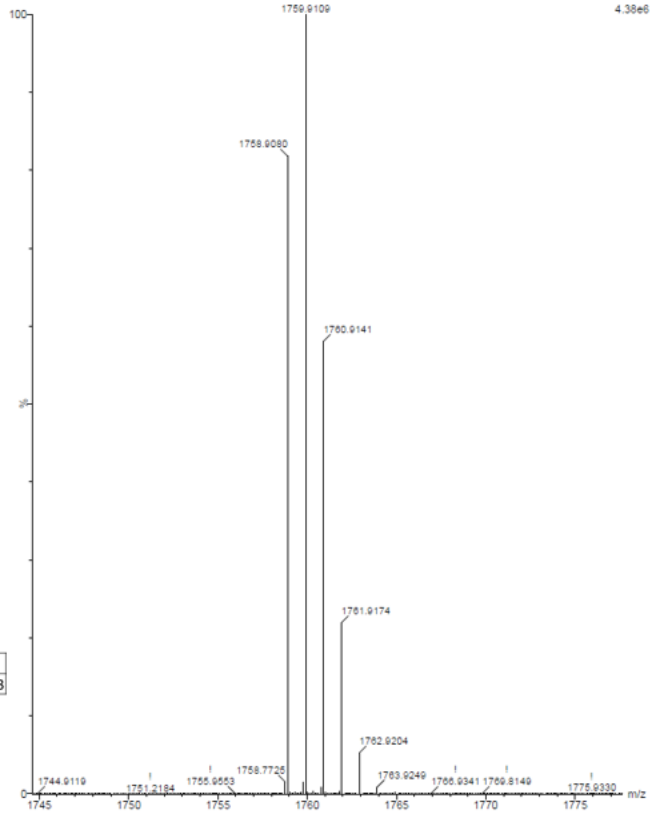




Purified 1h on LCMS (15min)







HRMS_SUMMARY			
Exact Mass	Observed Mass	Δm Error [ppm]	Formula
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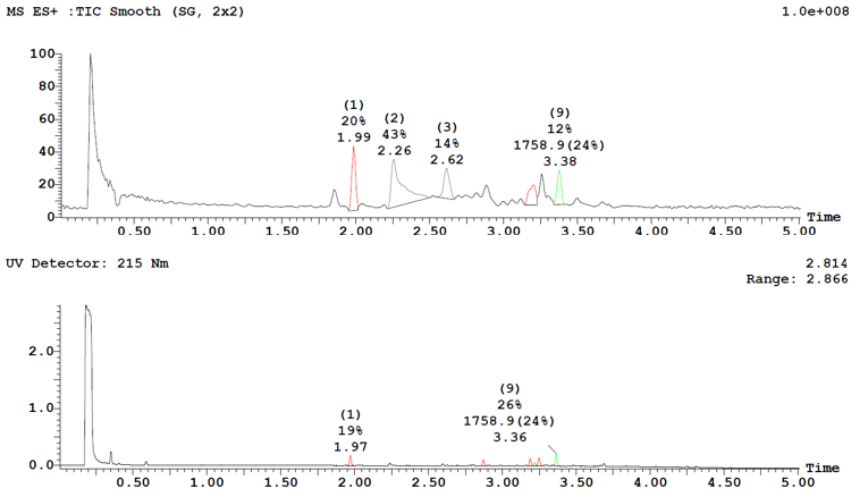
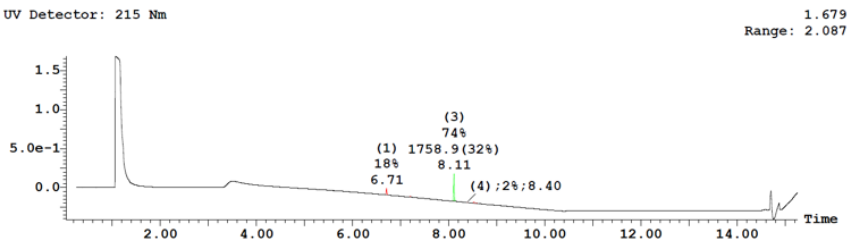
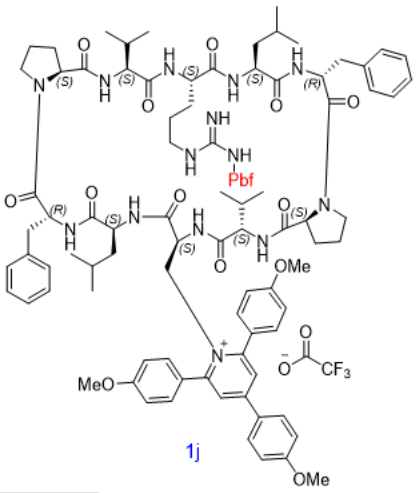
Crude Profile of 1i on LCMS

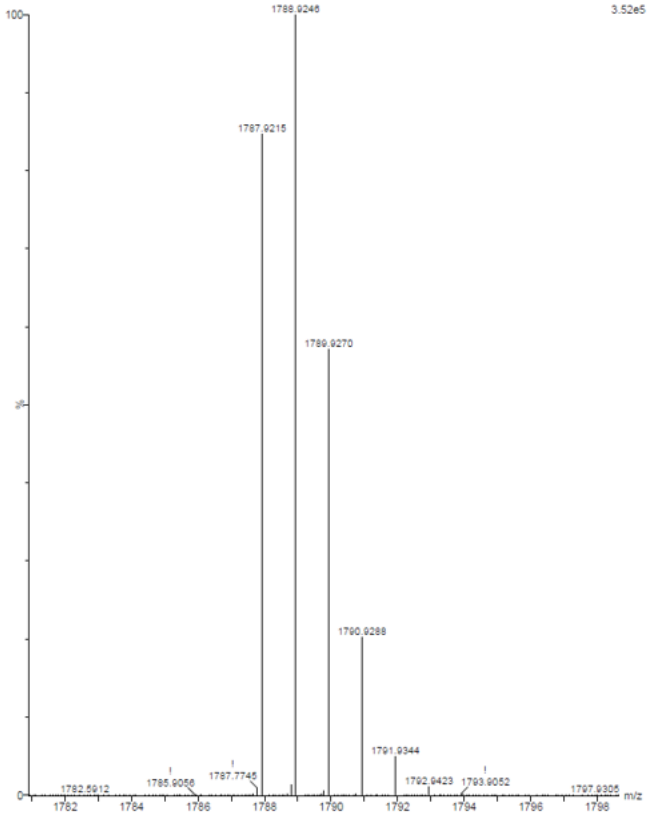


Purified 1i on LCMS (15min)

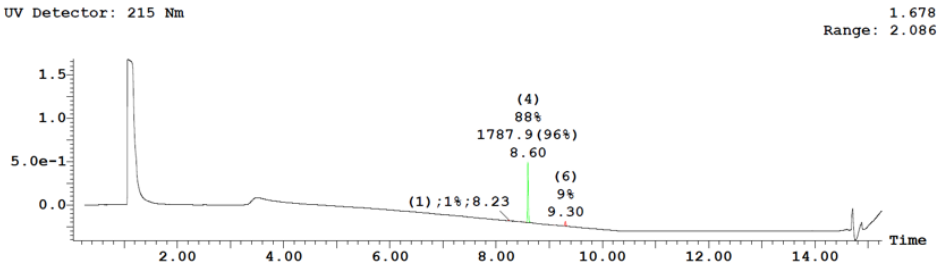


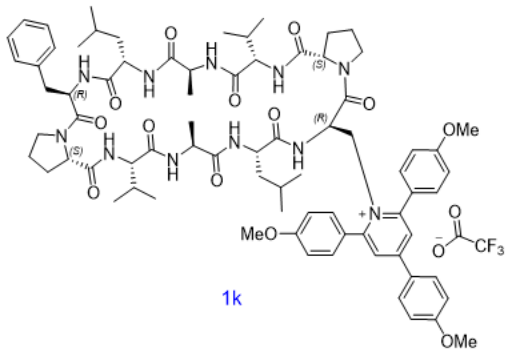


HRMS_SUMMARY			
Exact Mass	Observed Mass	Δm Error [ppm]	Formula
1787.9275	1787.9215	-3.3	C98H127N14O16S



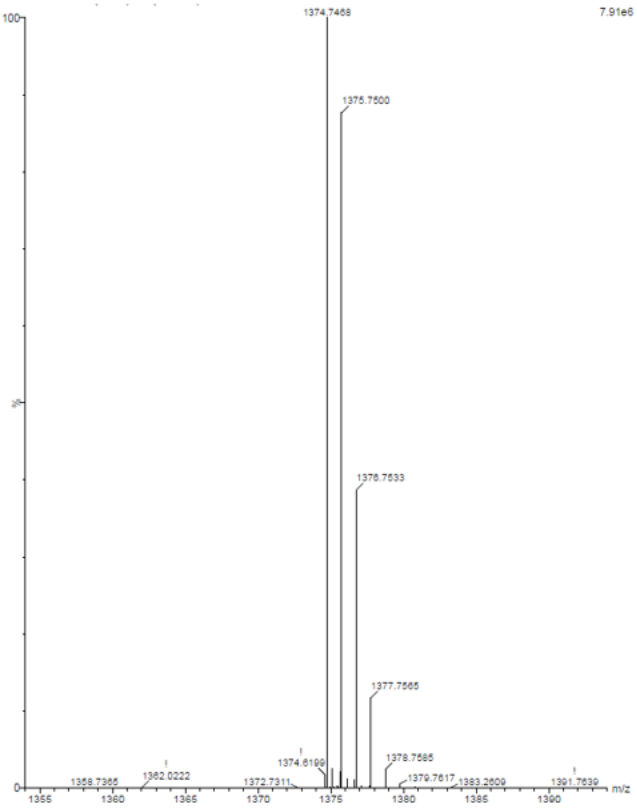
Purified 1j on LCMS (15min)



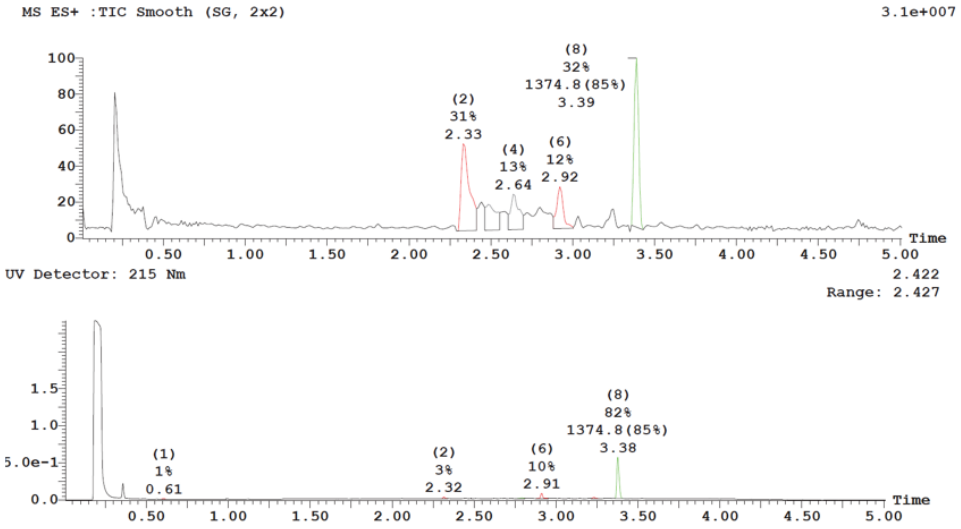


1k

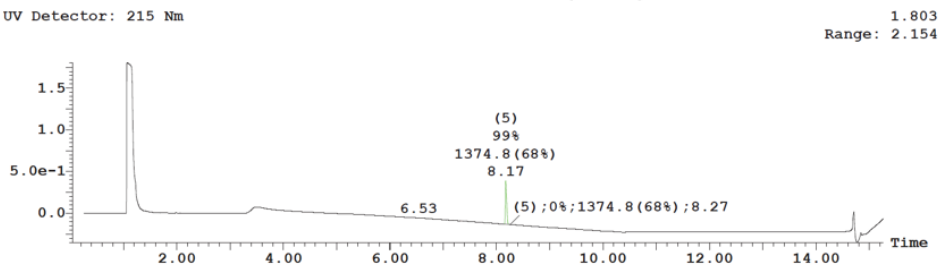
HRMS_SUMMARY			
Exact Mass	Observed Mass	Δm Error [ppm]	Formula
1374.7502	1374.7468	-2.5	C76H100N11O13

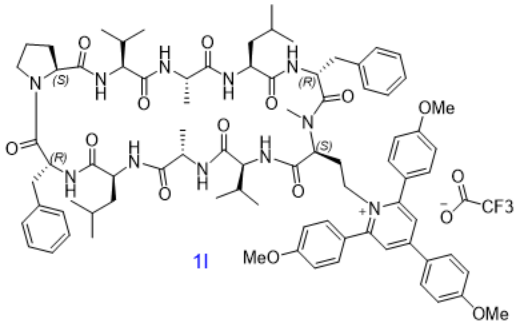


Crude Profile of 1k on LCMS

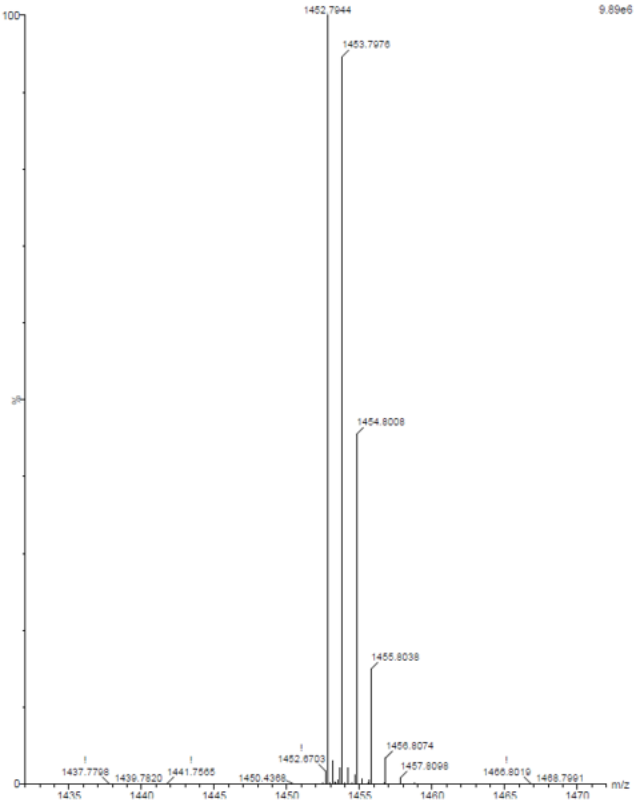


Purified 1k on LCMS (15min)

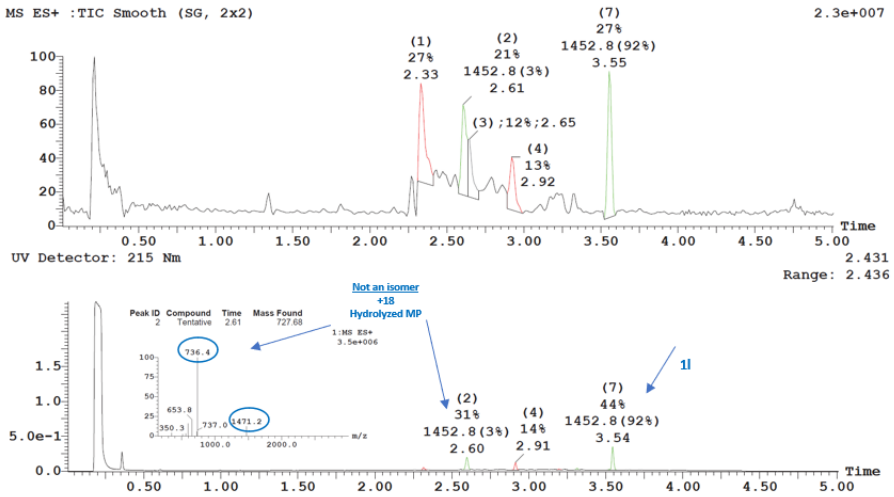




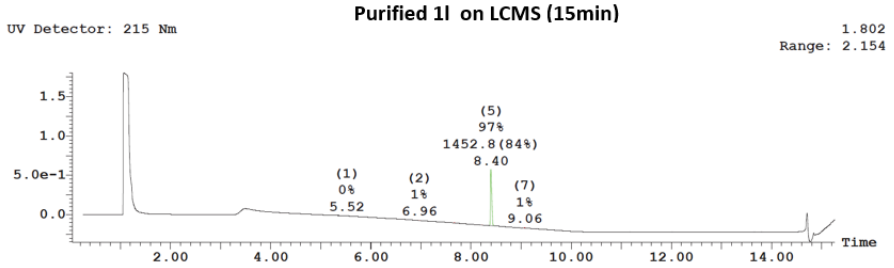
HRMS_SUMMARY			
Exact Mass	Observed Mass	Δm Error [ppm]	Formula
1452.7972	1452.7944	-1.9	C82H106N11O13



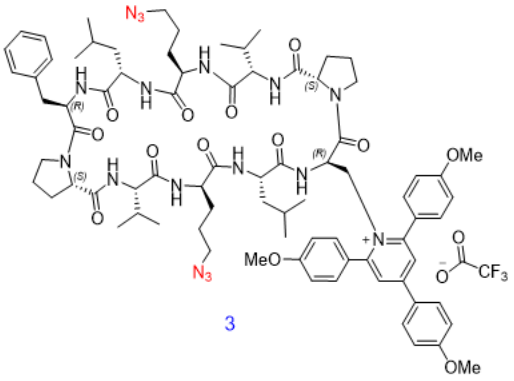
Crude Profile of 1l on LCMS



Purified 1l on LCMS (15min)

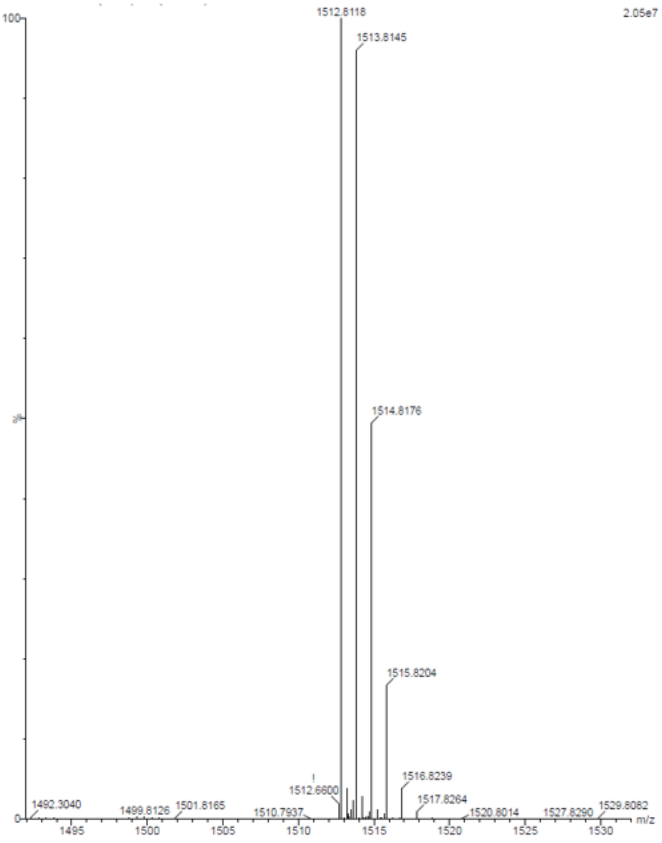


Under pyrylium condensation condition, hydrolysis of **1l** leads to the corresponding pyridinium containing linear precursor and the ratio of hydrolyzed product to the desired product is ~3:4 based on the LCMS chromatogram.

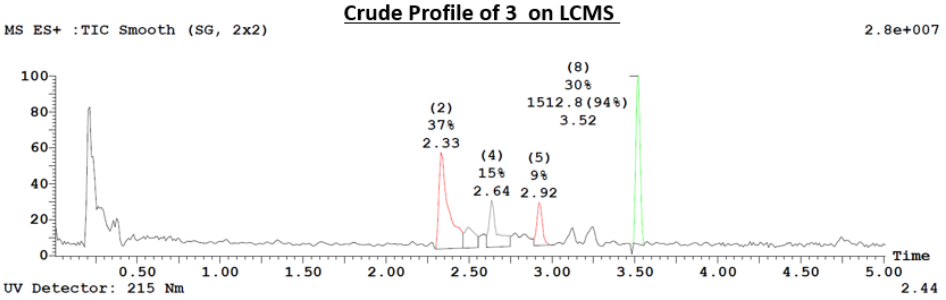


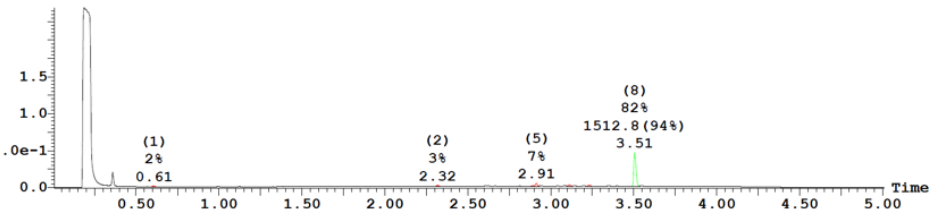
3

HRMS_SUMMARY			
Exact Mass	Observed Mass	Δm Error [ppm]	Formula
1512.8156	1512.8118	-2.5	C80H106N17O13

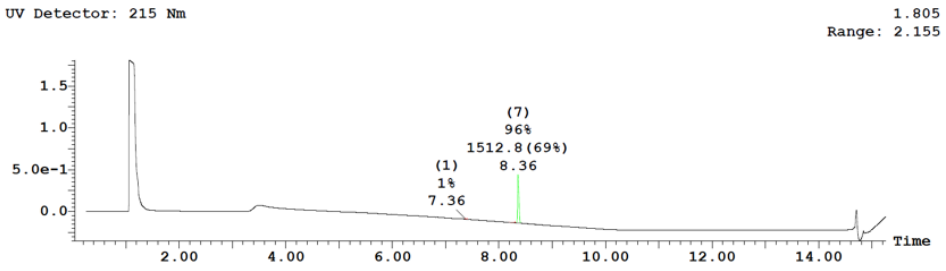


Crude Profile of 3 on LCMS



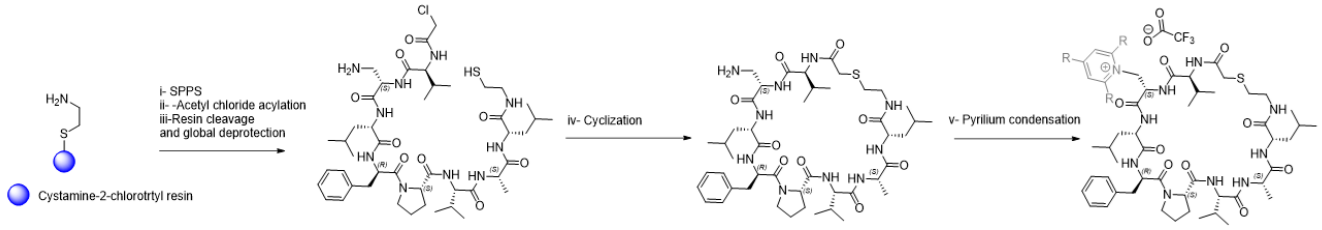


Purified 3 on LCMS (15min)



2.2 Synthesis of MP-Pyridinium salt,**1m**:

Figure S2: Synthesis strategy.



Linear chain assembly and global deprotection

Linear chain assembly was done according to general condition described in section 2.1.2 using commercially available Cystamine-2-chlorotrtyl resin (1.73mmol/g). At the end of the assembly, resin bound linear precursor (0.25 mmol) was allowed to react with 5 mL of chloroacetic anhydride (0.8 M in DMF, 16 eq) for 5 minutes. This capping was repeated once more. The resin bound linear intermediate was washed with DCM (3x10 mL), DMF (3x10 mL), and DCM(3 x10 mL), then dried under vacuum.

Protocol B (Global deprotection), described in section 2.1.4 was then applied to cleave resin as well as for the deprotection of the Dap.

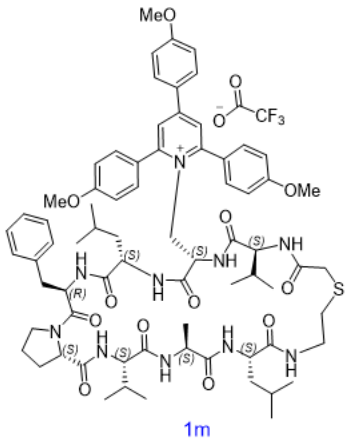
Cyclization:

Crude linear peptide intermediate (0.25 mmol) was dissolved in 22 mL of DMSO and treated with 0.44 mL of DIPEA (10 eq). The resulting mixture was stirred at rt for overnight. After confirming completion by LC/MS, directly purified on reverse phase HPLC, Method-A to obtain desired product as white solid (123.6 mg, 0.131 mmol, 52.4% yield). MS (ESI) m/z (M+1) Calcd for $C_{46}H_{74}N_{10}O_9S^+$ 943.54, observed 944.0.

Pyrylium condensation

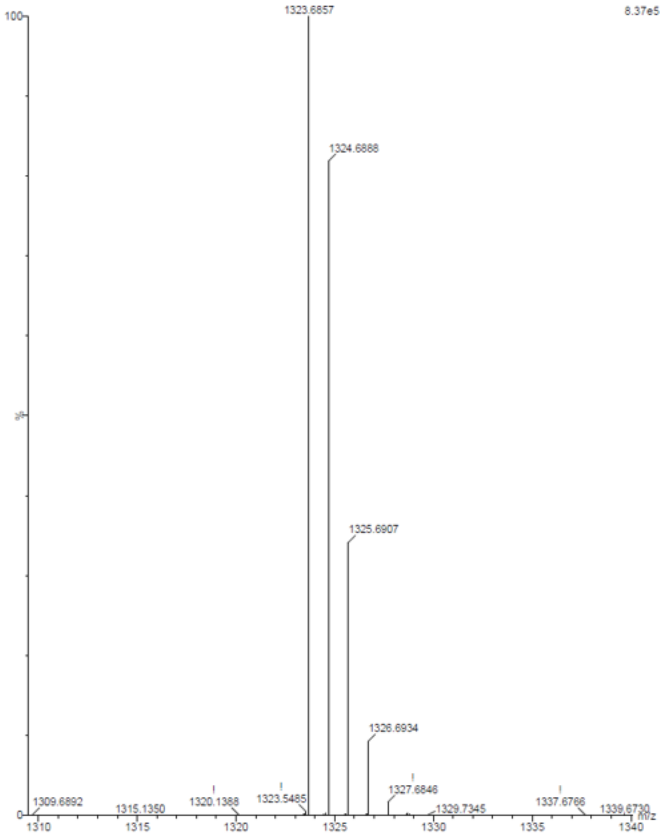
Cyclized peptide (0.131 mmol) was dissolved in 12 mL of EtOH in a Biotage® microwave reaction vial. To this solution was added 92 µL of DIPEA (4 eq) and the mixture was stirred at rt for 5 min. 127 mg of 4,6-tris(4-methoxyphenyl) pyrylium tetrafluoroborate salt (2 eq) was added to the mixture. The reaction vial was sealed and stirred at 90 °C using a Biotage® Initiator+ microwave for 10 h. After confirming completion by LC/MS, the reaction mixture was concentrated under reduced pressure. Crude mixture was dissolved in DMSO and directly purified on reverse phase HPLC using Method-A to obtain desired product, **MP-1m** as light yellow solid (120 mg, 0.091 mmol, 69.2% yield). HRMS: calculated for $C_{72}H_{95}N_{10}O_{12}S^+$:1323.6852, observed 1323.6857.

Characterization of MP-Pyridinium salt, **1m**

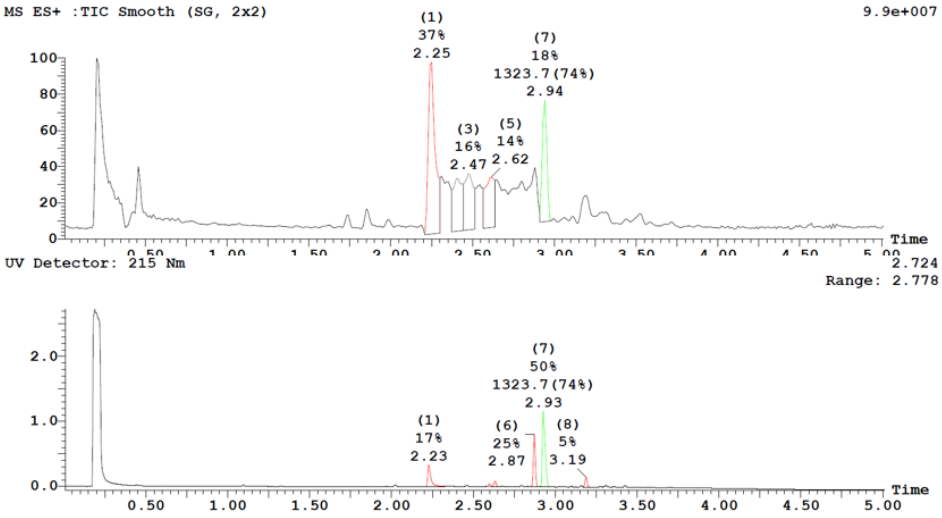


1m

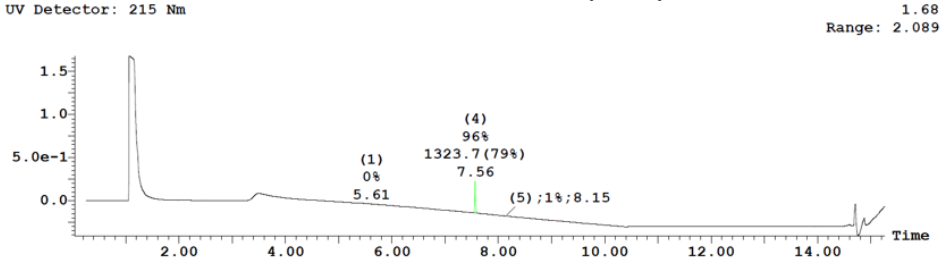
HRMS_SUMMARY			
Exact Mass	Observed Mass	Δm Error [ppm]	Formula
1323.6852	1323.6857	0.4	C72H95N10O12S



Crude Profile of 1m on LCMS

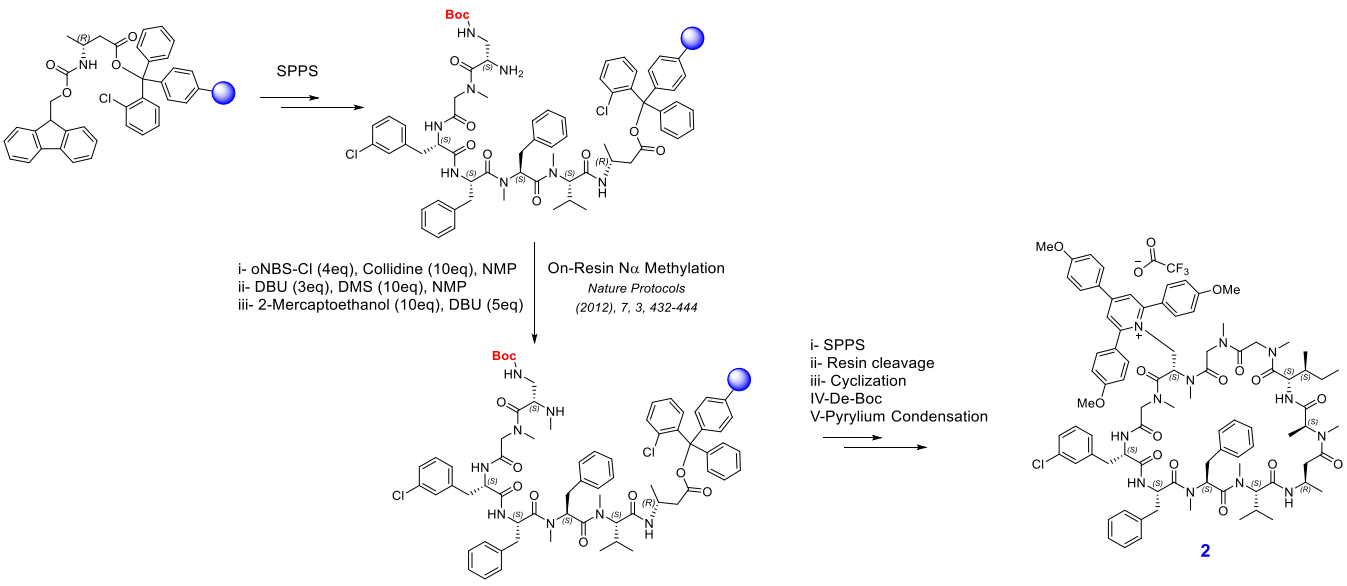


Purified 1m on LCMS (15min)



2.3. Synthesis of MP-Pyridinium salt, **2**:

Figure S3: Synthesis strategy.



Linear chain assembly, and on-resin Nα methylation of the Dap residue.

Linear precursor of compound **2** was assembled according to literature procedure[1] with some modifications which are described here.

Manual operation mode of the Gyros Protein Symphony X peptide synthesizer was used for the assembly.

Step 1: Loading of the first residue was done according to procedure described in section 2.1.1.

Step 2: Fmoc-D-3-Abu-O-trt(2-Cl)-resin (365 mg, 0.3 mmol) was swelled in DCM (4 mL x 3 x 1min) and washed with DMF (4 mL x 3 x 30sec).

Step 3: The resin was reacted with solution of 2% (v/v) DBU in DMF (4.2 mL) to remove Fmoc group. (Room temperature, 30 min).

Step 4: The resin washed with DMF (4 mL x 6 x 1min).

Step 5: 3.6 eq of amino acid, and 2.3 eq of HOAt were weighted in a 4 mL vial and dissolved in NMP (1.8 mL). A solution of DIC (2 mL, 0.71M in DMF, 5.2 eq) was combined with the AA and HOAt and added the reaction vessel containing resin bound intermediate. Allowed to react at room temp under N_2 mixing. Please see the chart below for the reaction time of each individual monomer.

Step 6: The resin washed with DMF (4 mL x 6 x 1min).

Step 7: Step 3-6 were repeated sequentially to assemble the full linear peptide.

On-resin *N*-Methylation step: according to publish procedure [2].

Table S2: Coupling time for each residue.

Position	Residue	Coupling Time	Notes
11	NMeA	Single_RT_2h	
10	Ile	Single_RT_2h	
9	Sar	Single_RT_2h	
8	Sar	Single_RT_2h	
7	Dap*(Boc)	Single_RT_2h	*Nα-Methylated
6	Sar	Single_RT_2h	
5	Phe3Cl	Single_RT_2h	
4	Phe	Single_RT_2h	
3	NMePhe	Dou-ble_RT_2h+16h	
2	NMeVal	Single_RT_1h	
1	D-3-Abu	Preloaded	(0.823mmole/g)

Resin cleavage:

Upon completion of SPPS, the linear peptide precursor was cleaved from resin by treatment with 10 mL of 30% (v/v) HFIP in DCM for 5 min, repeated twice. The peptide solution was concentrated under reduced pressure to yield the crude linear peptide, as confirmed by LC/MS.

Cyclization:

Crude linear peptide (0.3 mmol) was dissolved in DMF (91 mL) and reacted with DIPEA (0.21 mL, 1.2 mmol) and the solution of HATU (0.72 mL, 0.4 M in DMF, 0.95 eq). The resulting mixture was allowed to stir at room temperature for 2 h. After confirming completion by LC/MS, crude mixture was directly concentrated down using a genevac (high boiling method) to obtain crude cyclized product.

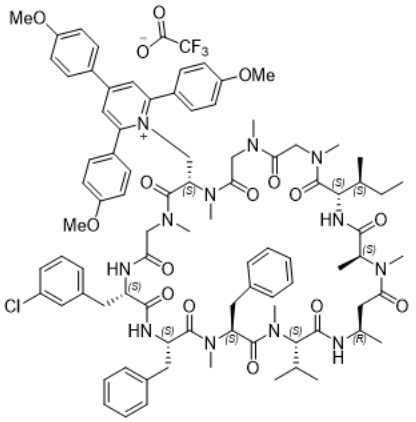
Boc deprotection to free Dap functional handle:

Cyclized peptide residue (0.3 mmol) was dissolved in 48 mL of 20% (v/v) TFA in DCM at 0°C and allowed to stir at same temperature for 20 minutes, after which the solution was concentrated via rotary evaporation. The residue was redissolved in 15 mL ACN/water (3:1) and lyophilized. Dissolution in ACN/water and lyophilization was repeated twice more to remove any remaining TFA. Used in the next step without any purification.

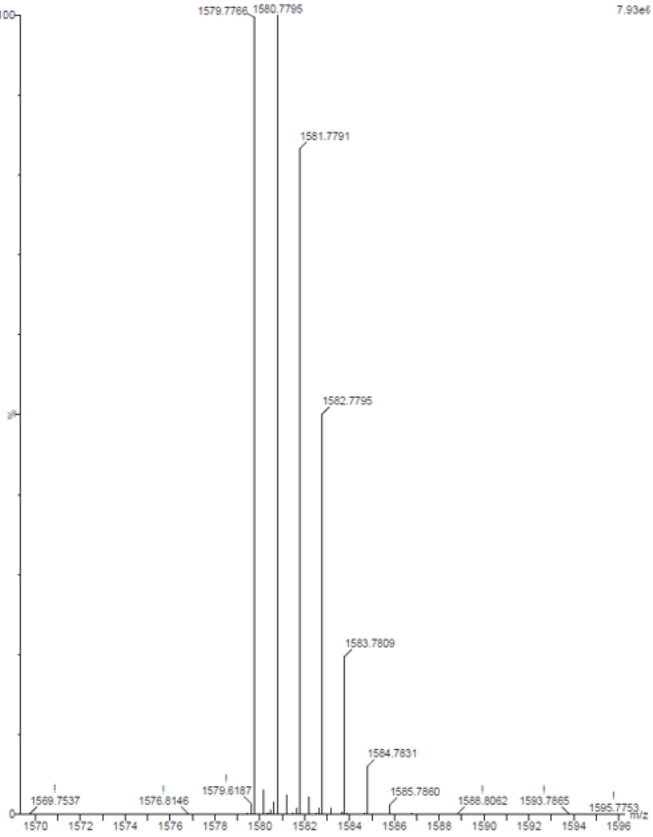
Pyrylium condensation:

Cyclized and Dap free peptide (0.15 mmol, half of crude lyophilized powder) was dissolved in 15 mL of EtOH in a Biotage® microwave reaction vial. To this solution was added 105 µL of DIPEA (4 eq) and the mixture was stirred at rt for 5 min. 88 mg of 4,6-tris(4-methoxyphenyl) pyrylium tetrafluoroborate salt (1.2 eq) was added to the mixture. The reaction vial was sealed and stirred at 90 °C using a Biotage® Initiator+ microwave for 2h. After confirming completion by LC/MS, the reaction mixture was concentrated under reduced pressure. Crude mixture was dissolved in DMSO and directly purified on reverse phase HPLC using Method-A to obtain MP **2** as light yellow solid (58.4 mg, 0.037 mmol, 21.4% yield). HRMS: calculated for $C_{87}H_{108}ClN_{12}O_{14}^{+}$:1579.7796, observed 1579.7766.

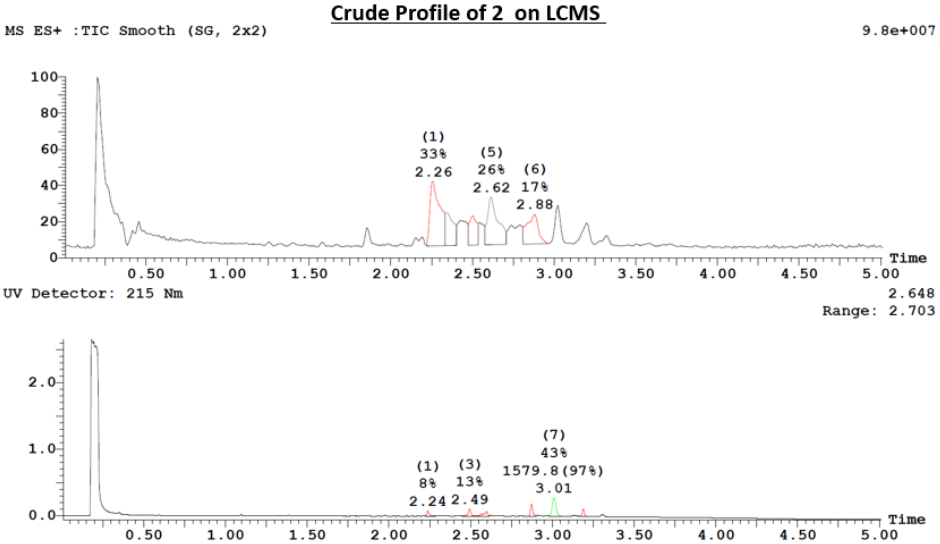
Characterization of MP-Pyridinium salt **2**:



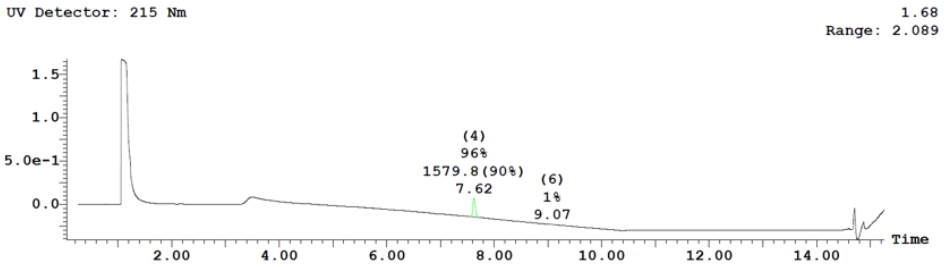
HRMS_SUMMARY			
Exact Mass	Observed Mass	Δm Error [ppm]	Formula
1579.7796	1579.7766	-1.8	C87H108ClN12O1



Crude Profile of 2 on LCMS



Purified 2 on LCMS (15min)



3. Late-stage reductive couplings of MP-pyridiniums with aryl bromides,

3.1 High-throughput experimentation (HTE), 2 μmol scale procedures.

Microscale procedures for MP-pyridinium salt and reductant screen (Scheme-2B)

In a nitrogen-filled glovebox, to 1-mL vials containing stir bars, MP-pyridiniums, (preloaded, 2 μmol, 1 eq), and secured in a 24 well aluminum block was added 3-bromoanisole (25 μL, 0.12 M solution in DMA, 3 μmol, 1.5 eq), precomplexed $NiBr_2$.glyme/2-pyridylcarboxamidine ligand mixture* (4 μL, 0.1M mixture in DMA, 0.4 μmol, 0.2 eq.) and Zn or Mn (13.3 μL, 0.6 M slurry in DMA, 8 μmol, 4eq.) sequentially. The reaction block was sealed, placed on a tumble stirrer preheated to 60°C for 24 h. The reaction block was cooled to room temperature and taken out of the glovebox. An analytical plate was prepared by diluting 2 μL of the crude reaction mixtures with 198 μL of DMSO. The reactions were analyzed using UPLC-UV/MS analysis to obtain the product LCAPs.

*Preparation of 0.1M stock solution of $NiBr_2$.glyme/2-pyridylcarboxamidine ligand mixture: In a nitrogen-filled glovebox, a 4-mL vial (equipped with stir bar) was charged with $NiBr_2$.glyme (0.049mmol) and pyridine-2,6-bis(carboximidamide)dihydrochloride (0.058 mmol) in 472.2 μL of DMA. The mixture was stirred for ~30 minutes (resulting in a green or blue slurry) before being dosed to the reaction plate. Catalyst, Zn and Mn stock slurries were dosed while stirring at 800 rpm.

Microscale procedure for aryl halide scope screen (Manuscript Schemes 3 and 4)

In a nitrogen-filled glovebox, to 1-mL vials containing stir bars, MP-pyridinium, **1b** (preloaded, 2 μmol, 1 eq), and secured in a 24 well aluminum block was added aryl bromide (25 μL, 0.12M solution in DMA, 3 μmol, 1.5 eq), precomplexed $NiBr_2$.glyme/2-pyridylcarboxamidine ligand mixture* (4 μL, 0.1M mixture in DMA, 0.4μmol, 0.2 eq.) and Zn (13.3 μL, 0.6 M slurry in DMA, 8μmol, 4eq.) sequentially. The reaction block was sealed, placed on a tumble stirrer preheated to 60°C for 24 h. The reaction block was cooled to room temperature and taken out of the glovebox. An analytical plate was prepared by diluting 2 μL of the crude reaction mixtures with 198 μL of DMSO. The reactions were analyzed using UPLC-UV/MS analysis to obtain the product LCAPs.

*Preparation of 0.1M stock solution of $NiBr_2$.glyme/2-pyridylcarboxamidine ligand mixture: In a nitrogen-filled glovebox, a 4-mL vial (equipped with stir bar) was charged with $NiBr_2$.glyme (0.049 mmol) and pyridine-2,6-bis(carboximidamide)dihydrochloride (0.058 mmol) in 472.2 μL of DMA. The mixture was stirred for ~30 minutes (resulting in a green or blue slurry) before being dosed to the reaction plate. Catalyst, and Zn stock slurries were dosed while stirring at 800 rpm.

Microscale procedures for the coupling of diverse MP-pyridiniums with 12 aryl bromides (Manuscipt Figure 2)

In a nitrogen-filled glovebox, to 1-mL vials containing stir bars, MP-pyridinium, (preloaded, 2 μmol, 1 eq), and secured in a 24 well aluminum block was added aryl bromide (25 μL, 0.12M solution in DMA, 3 μmol, 1.5 eq), pre-complexed $NiBr_2$.glyme/2-pyridylcarboxamidine ligand mixture* (4μL, 0.1M mixture in DMA, 0.4μmol, 0.2 eq.) and Zn (13.3 μL, 0.6 M slurry in DMA, 8 μmol, 4 eq.) sequentially. The reaction block was sealed, placed on a tumble stirrer preheated to 60°C for 24 h. The reaction block was cooled to room temperature and taken out of the glovebox. An analytical plate was prepared by diluting 2 μL of the crude reaction mixtures with 198 μL of DMSO. The reactions were analyzed using UPLC-UV/MS analysis to obtain the product LCAPs.

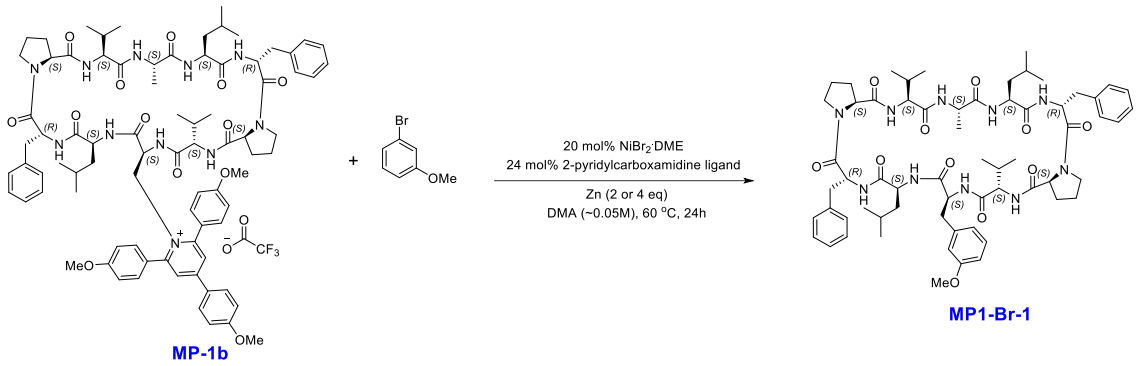
*Preparation of 0.1 M stock solution of $NiBr_2$.glyme/2-pyridylcarboxamidine ligand mixture: In a nitrogen-filled glovebox, a 4-mL vial (equipped with stir bar) was charged with $NiBr_2$.glyme (0.049 mmol) and pyridine-2,6-bis(carboximidamide)dihydrochloride (0.058 mmol) in 472.2 μL of DMA. The mixture was stirred for ~30 minutes (resulting in a green or blue slurry) before being dosed to the reaction plate. Catalyst, and Zn stock slurries were dosed while stirring at 800 rpm.

3.2 Reproducibility study of 2 μmol-scale experiments.

In a nitrogen-filled glovebox, to 1-mL vials containing stir bars, MP-pyridiniums, **1a** or **1b** (preloaded, 2 μmol, 1 eq), and secured in a 24 well aluminum block was added 3-bromoanisole (25 μL, 0.12 M solution in DMA, 3 μmol, 1.5 eq), DMA (6.65 μL or 0 μL), pre-complexed $NiBr_2$.glyme/2-pyridylcarboxamidine ligand mixture* (4 μL, 0.1 M mixture in DMA, 0.4 μmol, 0.2 eq.) and Zn (6.65 μL or 13.3 μL, 0.6 M slurry in DMA, 4 μmol or 8 μmol, 2 eq or 4 eq.) sequentially. The reaction block was sealed, placed on a tumble stirrer preheated to 60°C for 24 h. The reaction block was cooled to room temperature and taken out of the glovebox. An analytical plate was prepared by diluting 2 μL of the crude reaction mixtures with 198 μL of DMSO. The reactions were analyzed using UPLC-UV/MS analysis to obtain the product LCAPs.

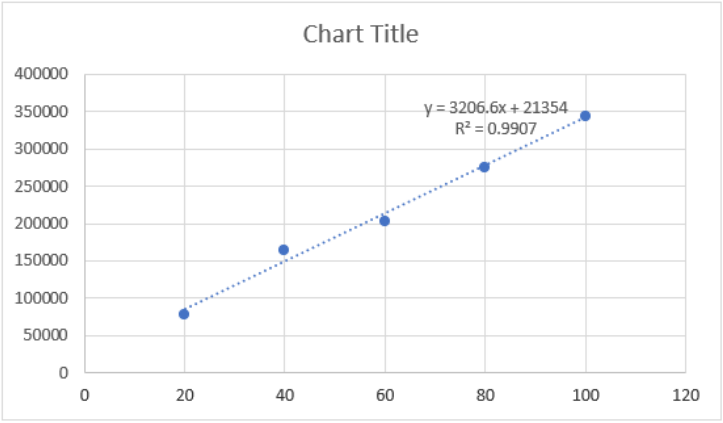
*Preparation of 0.1 M stock solution of $NiBr_2$.glyme/2-pyridylcarboxamidine ligand mixture: In a nitrogen-filled glovebox, a 4-mL vial (equipped with stir bar) was charged with $NiBr_2$.glyme (0.049 mmol) and pyridine-2,6-bis(carboximidamide)dihydrochloride (0.058 mmol) in 472.2 μL of DMA. The mixture was stirred for ~30 minutes (resulting in a green or blue slurry) before being dosed to the reaction plate. Catalyst, Zn stock slurries were dosed while stirring at 800 rpm.

Scheme S1: Experimental product LCAPs and Assay yields of replicate experiments.



A- Known concentration of product, **MP1-Br-1** vs UV210-AreaAbs

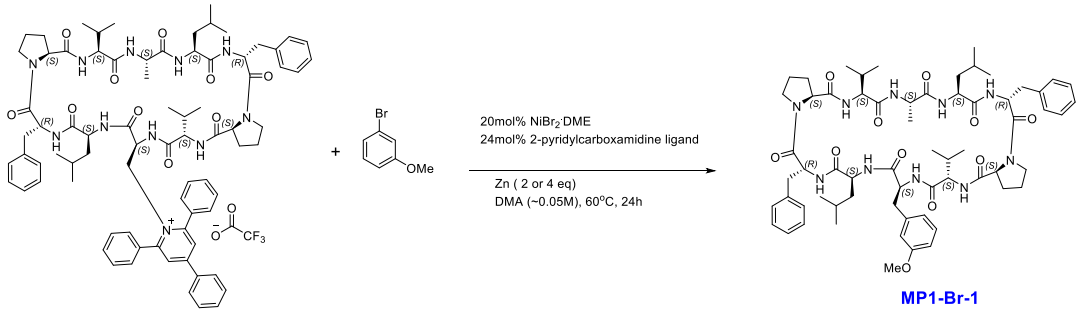
Known (% yield)	UV210_Prod AreaAbs
20	79191.56068
40	165182.099
60	204243.3664
80	275383.6586
100	344752.2125



B- Experimental product LCAPs and Assay yields of replicate experiments for MP-Pyridinium **1b** coupling.

Replicates	eq of Zn	LCAP	Avarage LCAP	Standard deviation	UV210_Prod AreaAbs	AY(%)	Avarage AY (%)	Standard deviation
1	2eq	48	69	10.5	191757.6699	53	54	8.3
2		82			183628.9759	51		
3		60			166268.3558	45		
4		75			194648.937	54		
5		73			170404.079	46		
6		70			249324.8766	71		
7		73			213171.7972	60		
8		73			195852.1375	55		
1	4eq	88	87	1.8	236115.517	67	69	3.2
2		87			241514.1235	69		
3		86			249754.9779	71		
4		84			225820.8269	64		
5		89			244594.9424	70		
6		86			255586.8534	73		

Reported LCAPs are the UV210_Prod Area%.

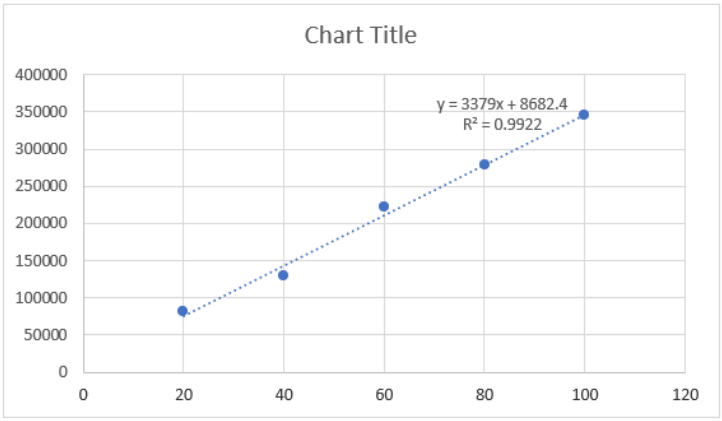


MP-1a

MP1-Br-1

C- Known concentration of product, **MP1-Br-1** vs UV210-AreaAbs

Known (% yield)	UV210_Prod AreaAbs
20	82459.53382
40	129159.251
60	221597.0497
80	277905.5598
100	345984.2864



D- Experimental product LCAPs and Assay yields of replicate experiments for MP-Pyridinium **1a** coupling.

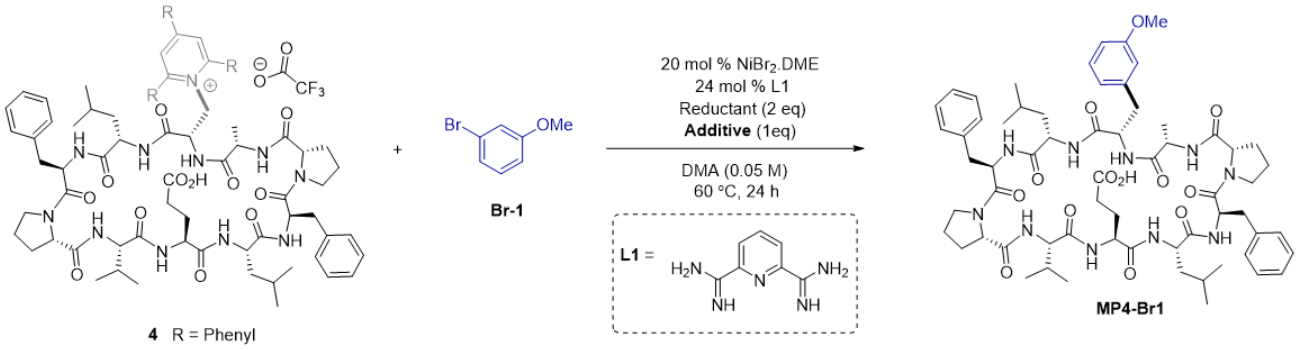
Replicates	eq of Zn	LCAP	Avarage LCAP	Standard deviation	UV210_Prod AreaAbs	AY(%)	Avarage AY (%)	Standard deviation
1	2eq	53	54	2.2	133544.8976	37	42	4.3
2		51			159121.8648	45		
3		56			166216.225	47		
4		55			136110.5869	38		
5		56			146959.9875	41		
1	4eq	48	47	3.2	128334.8542	35	34	2
2		50			127718.31010	35		
3		45			116996.599	32		
4		50			129519.1862	36		
5		45			118885.8303	33		
6		42			114625.982	31		

Reported LCAPs are the UV210_Prod Area%.

3.3 Additive Screening:

No improvement observed in the presence of additive.

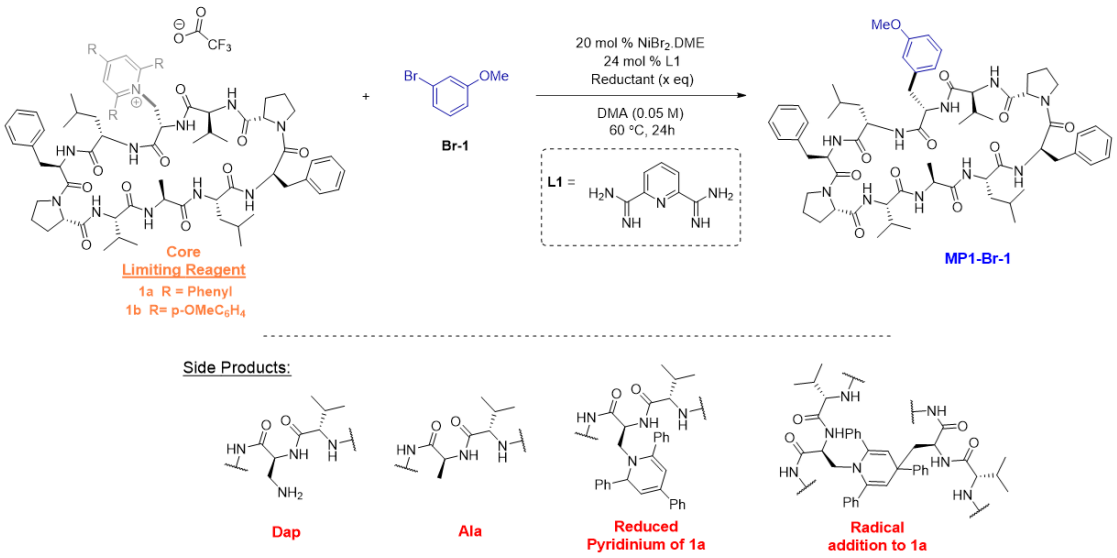
Starting material, 4 was prepared according to procedure described in section 2.1, and it was isolated via precipitation in cold ether to obtain desired product as white solid (148.9 mg, 0.099 mmol, 77% yield). MS (ESI) m/z (M+1) Calcd for $C_{76}H_{96}N_{11}O_{12}^{+}$ 1391.7, observed 1392.1.

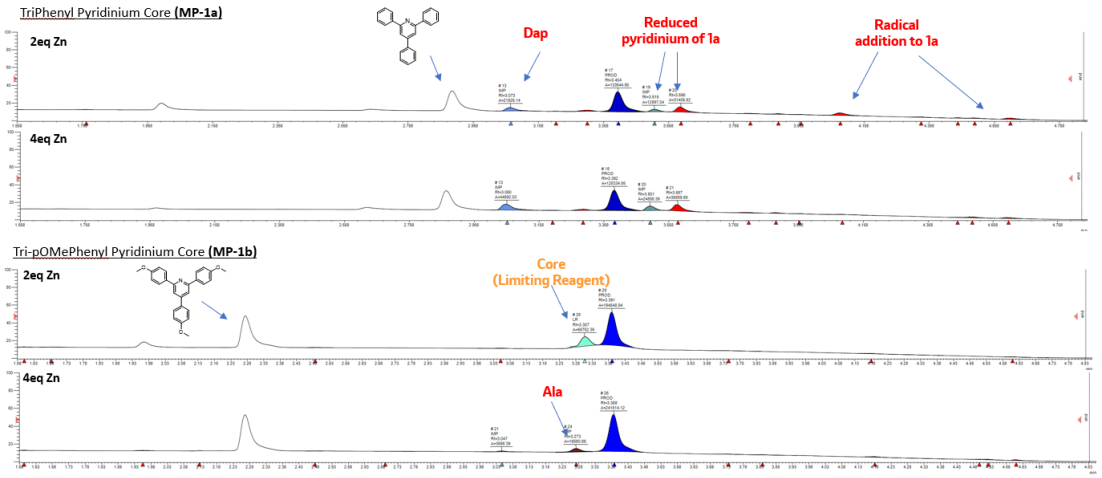


Entry	Reductant	Additive	LCAP
1	Zn	TBAI	36 %
2	Mn		14 %
3	Zn	LiCl	36%
4	Mn		31%
5	Zn	$MgCl_2$	29%
6	Mn		20%
7	Zn	None	42%
8	Mn		28%

Reported LCAPs are the UV210_Prod Area%.

3.4 Analysis and comparison of the UPLC chromatograms with respect to side product formation.



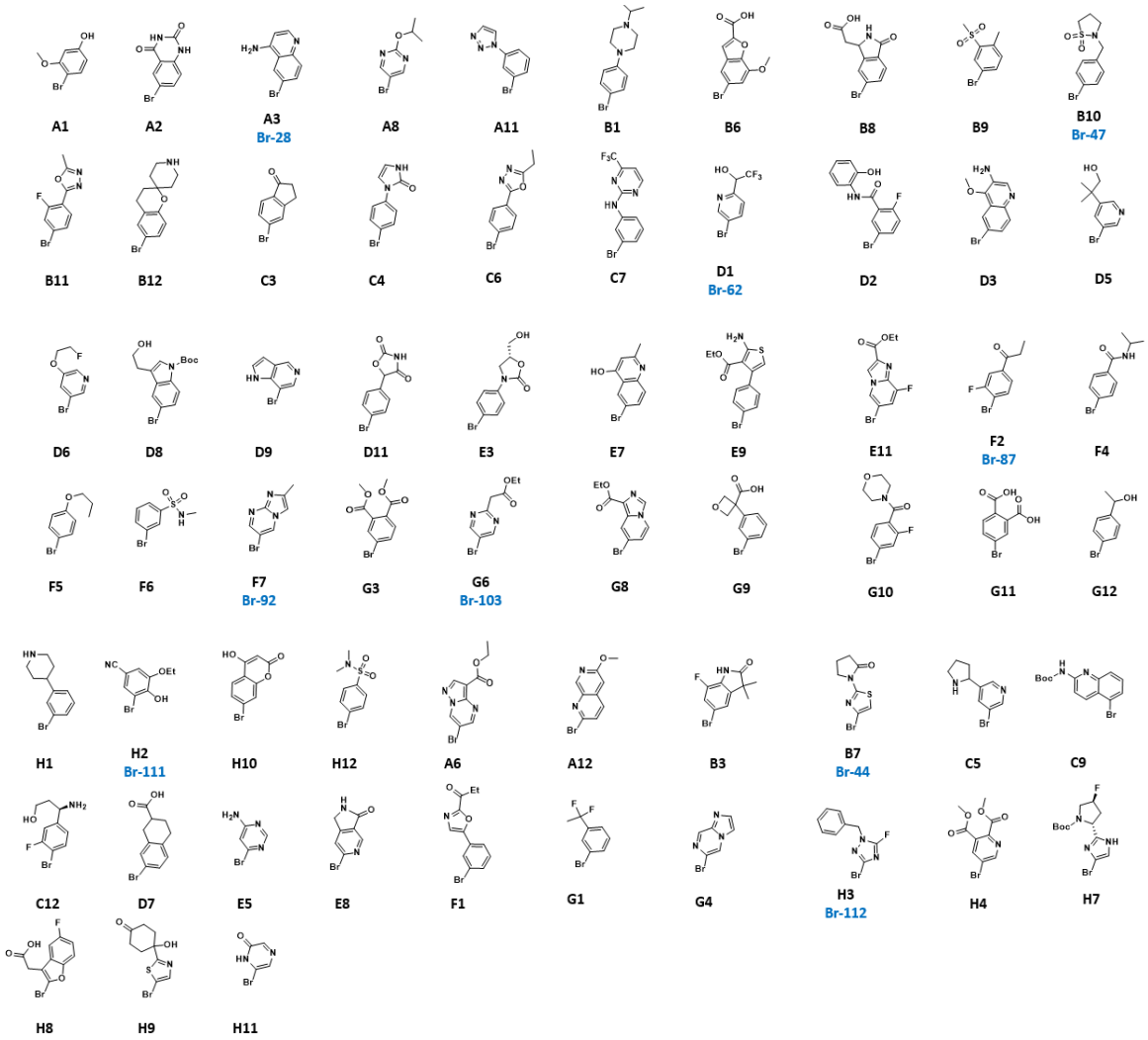


3.5 Selection process for ArBr and 96 selected Aryl bromides in manuscript Scheme 4

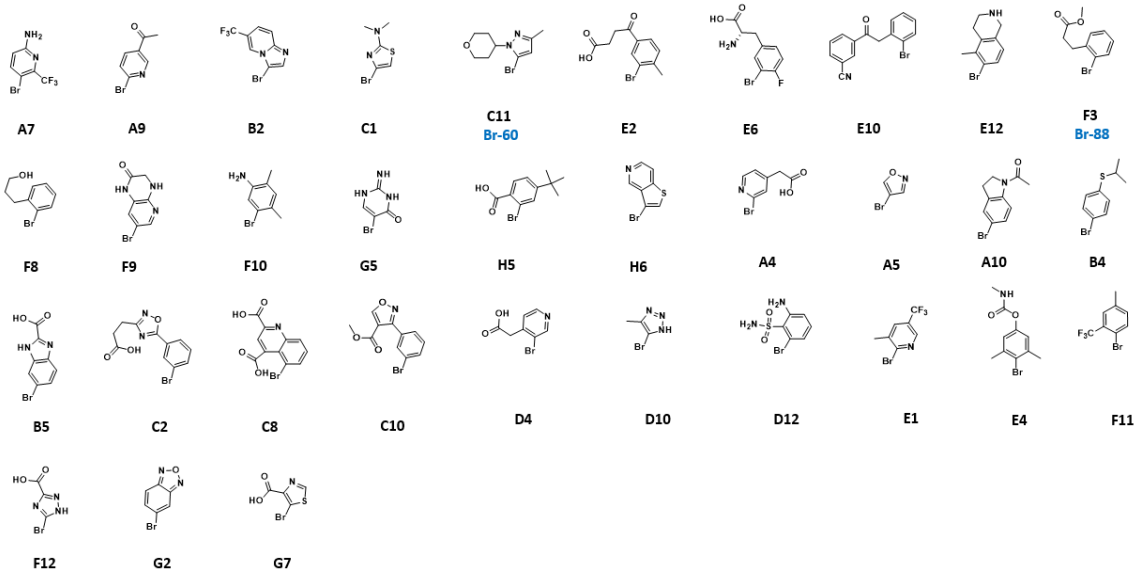
Venn Diagram: The ArBr and ArI in the Venn Diagram represent those that are available from MBBC. The 5968 MBBC ArBr in the Venn diagram are those that only have one C_{Ar}-Br bond, zero C_{Ar}-I and zero C_{Ar}-Cl bonds. The 760 MBBC ArI in the Venn diagram are those that only have one C_{Ar}-I bond, zero C_{Ar}-Br and zero C_{Ar}-Cl bonds. The 799 commercial Ar are the aryl groups in the aryl alanine side chain of the commercially available (SciFinder search combined with WuxiTIDE Amino acid collection) Fmoc protected aryl alanines.

ArBr selection for the HTE library in Scheme 4 of the manuscript: 5378 aryl bromides in the Venn diagram that lead to aryl alanines that are unavailable from commercial Fmoc protected amino acids or Aryl iodides were subjected to functional group filtration: MW up to 350; calculated rotatable bond count up to 6; calculated HBD count up to 4; calculated number of aromatic rings up to 2; and removing functional groups (alkene, alkyne, alkyl chloride, cyclopropyl, enamine, enol ether, nitro, *N*-oxide and primay anilines with only one C_{Ar}-NH_2). On the resulting 3991 ArBr after applying these filters, statistical unsupervised learning modeling were carried out in python using the scikit-learn[3] library. 208 molecular descriptors and 2048 molecular fingerprint bits were calculated with the open-source cheminformatics toolkit RDKit.[4] To reduce the dimensionality of the reconstructed data to 2-dimensional chemical space visualization, t-distributed stochastic neighbor embedding (t-SNE) with default hyperparameter settings was used. K-Means clustering was done on the reduced feature space to generate 96 clusters and 96 ArBr were selected to span the chemical space. The aryl bromides closest to the cluster center were chosen. In cases where the aryl bromide closest to the cluster center was unavailable the next closest ArBr was chosen from the same cluster.

ArBr `s with LCAP > 20



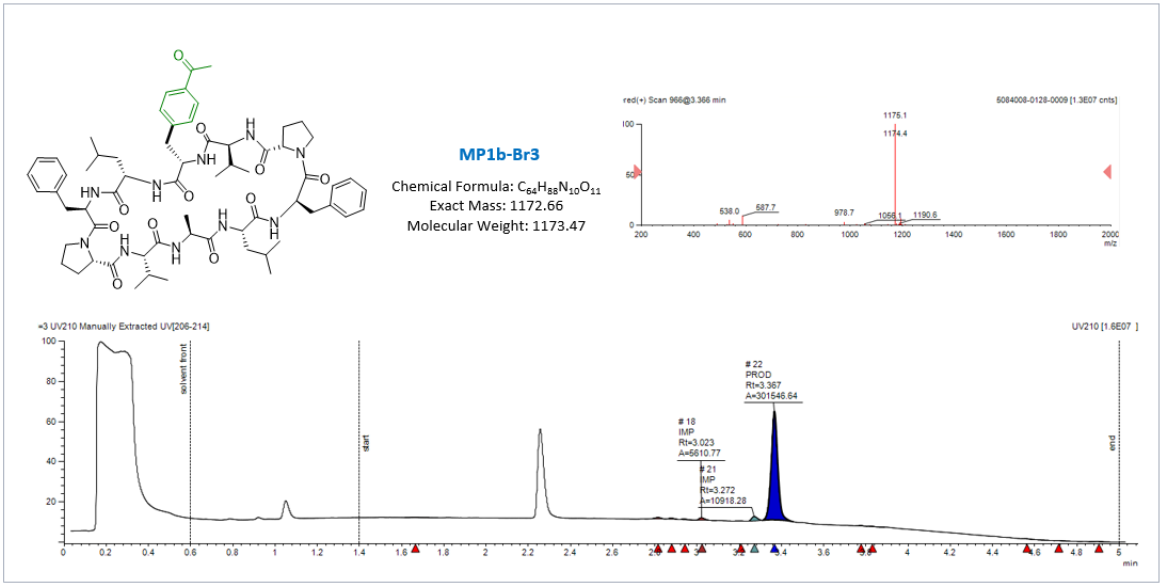
Limitations: ArBr `s with LCAP < 20

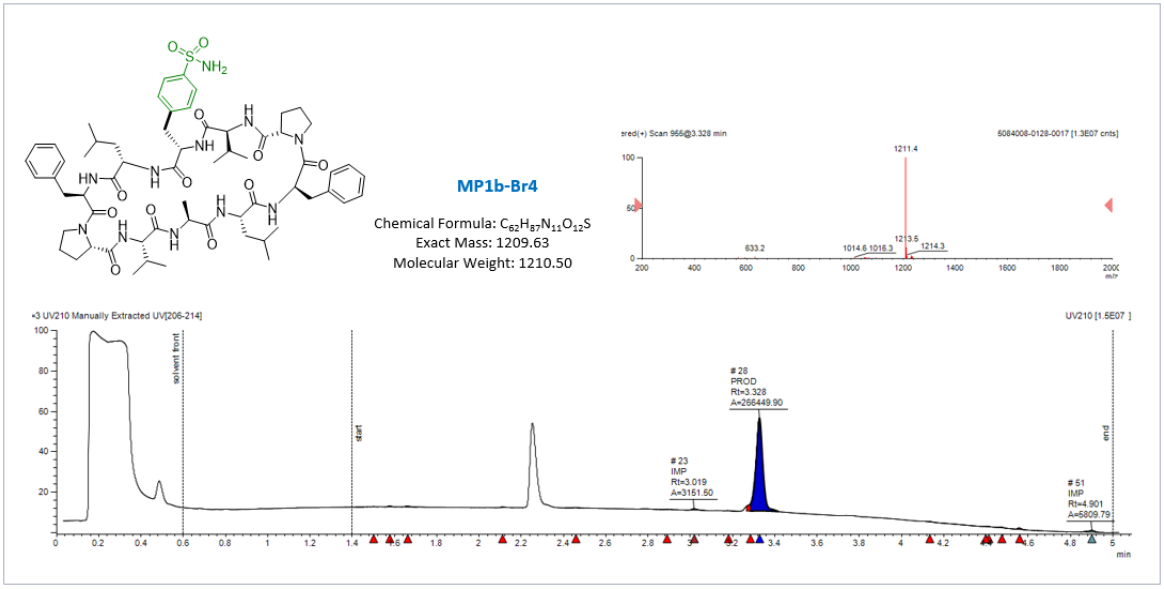


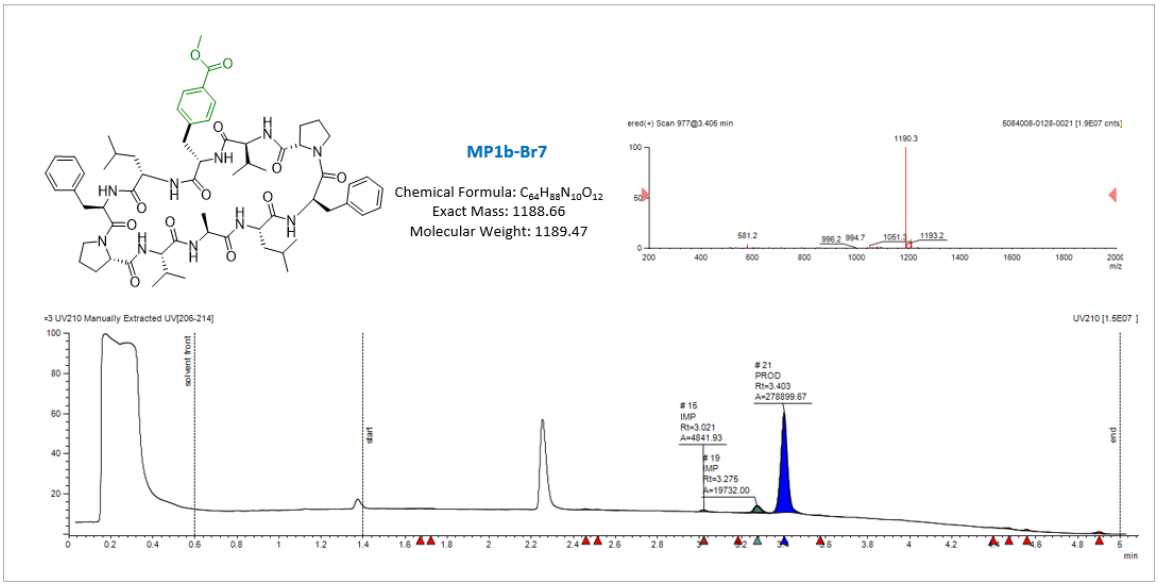
3.6 UPLC/MS chromatograms of the crude reaction mixtures: The representative examples from manuscript Scheme 3 & 4 and Figure 2.

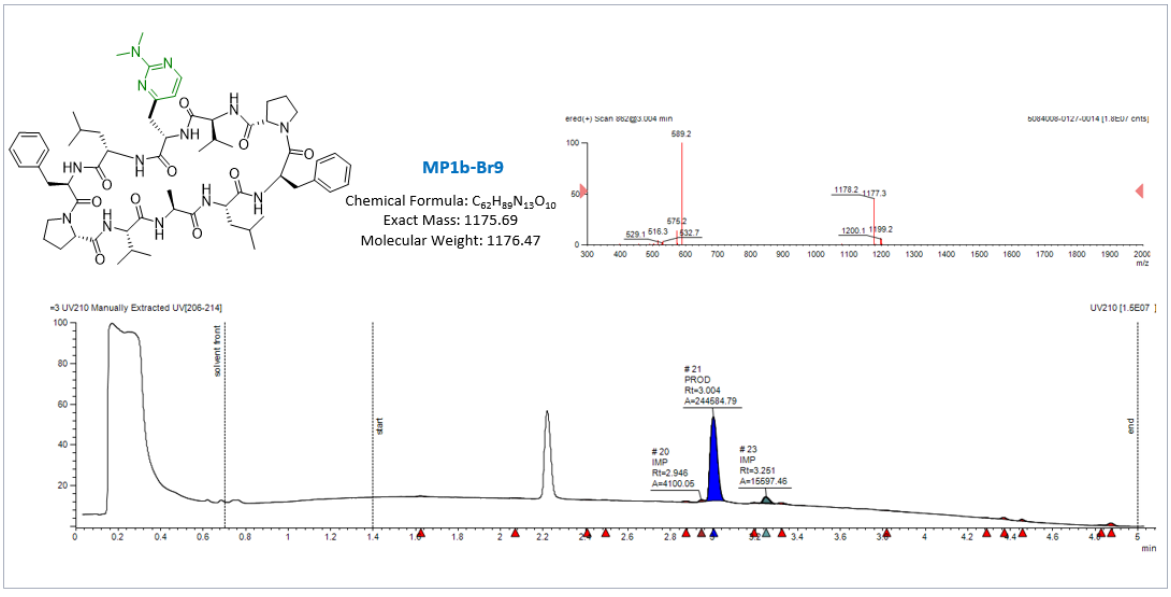
The peak areas of the non-peptide containing species such as 2,4,6-tris(4-methoxyphenyl) pyridine (retention time ~2.2-2.4 min), aryl bromide and des-bromo aryl were excluded from the integration. The peak areas for all relevant peptide containing species on the chromatogram (retention time 1.4 to 5 minutes) were integrated using virscidian v10.7 software. The product peaks are highlighted in blue in the LC chromatograms.

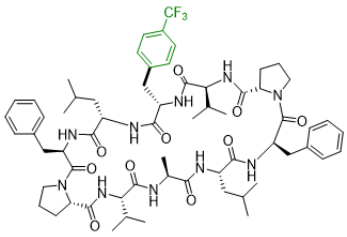
Manuscript Scheme 3 representative chromatograms




MP1b-Br4
Chemical Formula: C62H87N11O12S
Exact Mass: 1209.63
Molecular Weight: 1210.50
UV210 [1.5E07]
28
PROD
Rt=3.328
A=266449.90
# 23
IMP
Rt=3.019
A=3151.50
# 51
IMP
Rt=4.901
A=5809.79
solvent front
start
end
min

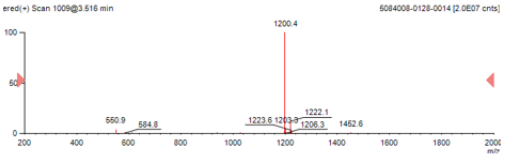

MP1b-Br7
Chemical Formula: C64H88N10O12
Exact Mass: 1188.66
Molecular Weight: 1189.47
UV210 [1.5E07]
21
PROD
Rt=3.403
A=278899.67
# 15
IMP
Rt=3.021
A=4841.93
# 19
IMP
Rt=3.275
A=19732.00
solvent front
start
end
min

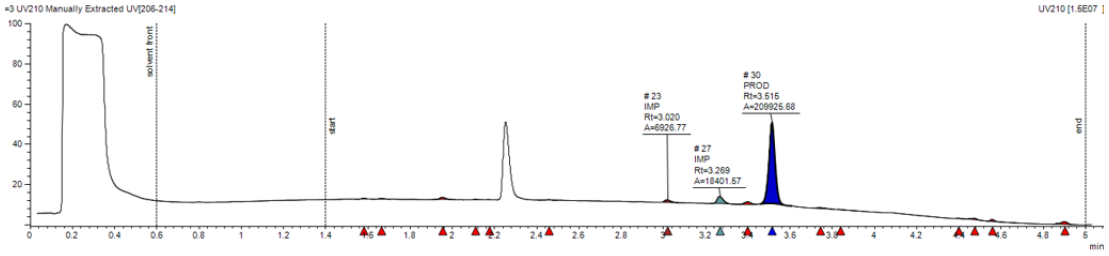

MP1b-Br9
Chemical Formula: C62H89N13O10
Exact Mass: 1175.69
Molecular Weight: 1176.47
UV210 [1.5E07]
21
PROD
Rt=3.004
A=244584.79
# 20
IMP
Rt=2.946
A=4100.06
# 23
IMP
Rt=3.251
A=15597.46
solvent front
start
end
min

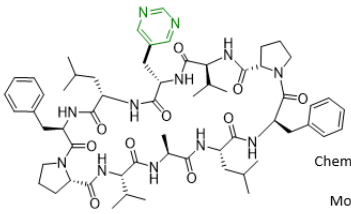


MP1b-Br12

Chemical Formula: $C_{63}H_{85}F_3N_{10}O_{10}$
Exact Mass: 1198.64
Molecular Weight: 1199.43

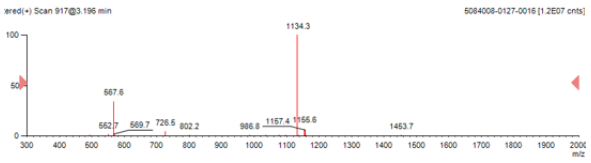


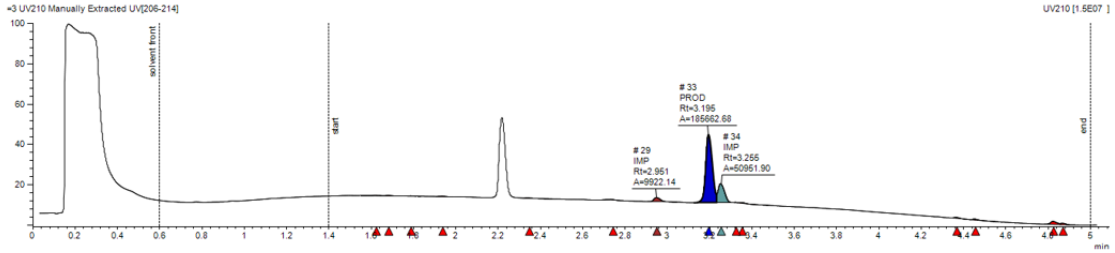


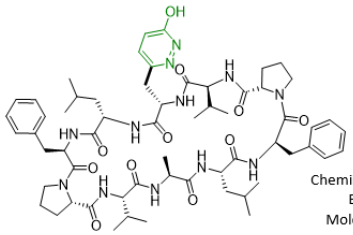


MP1b-Br16

Chemical Formula: $C_{60}H_{84}N_{12}O_{10}$
Exact Mass: 1132.64
Molecular Weight: 1133.41

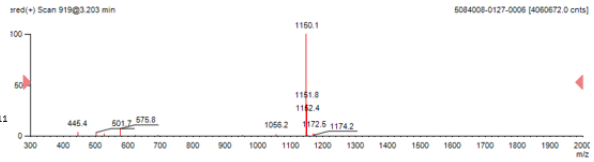


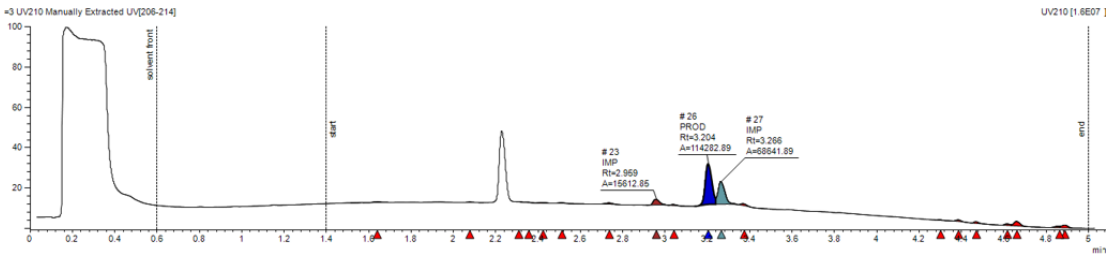


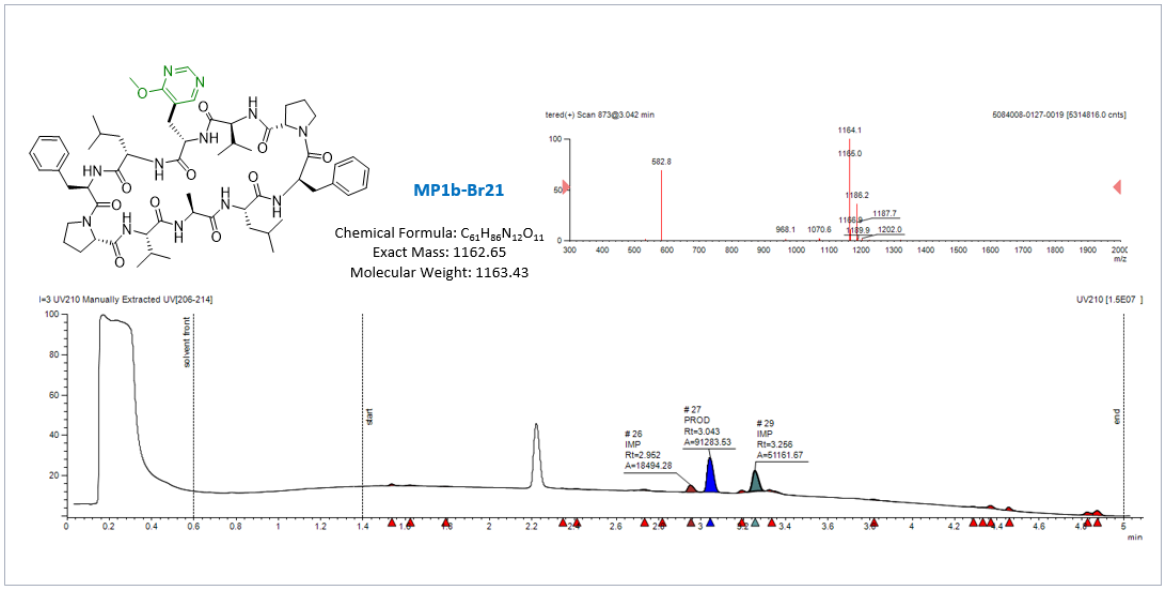


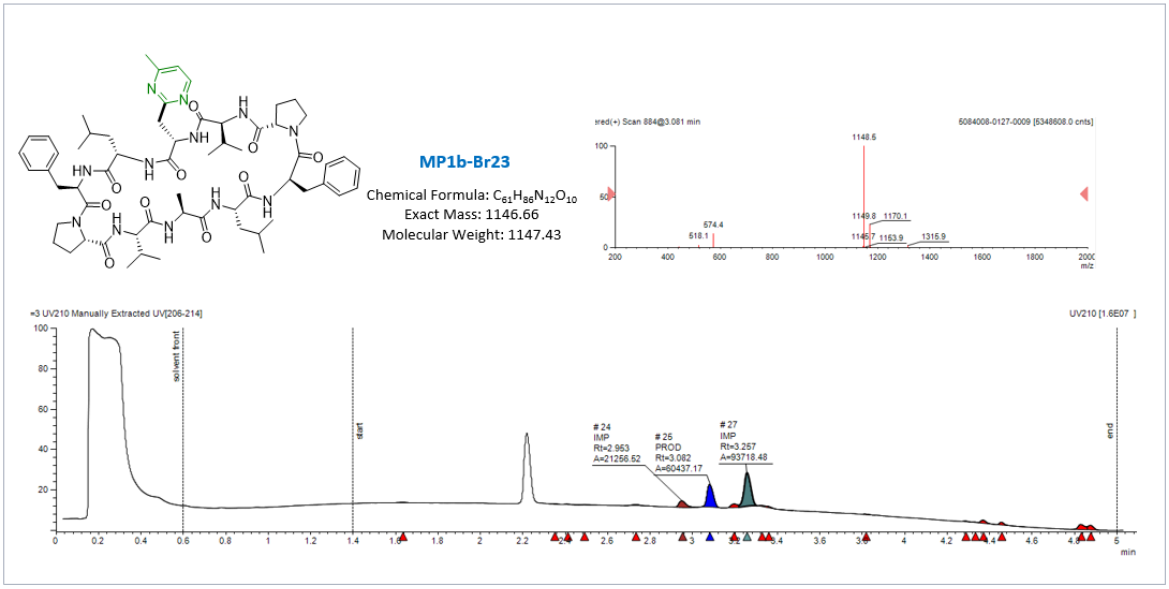
MP1b-Br19

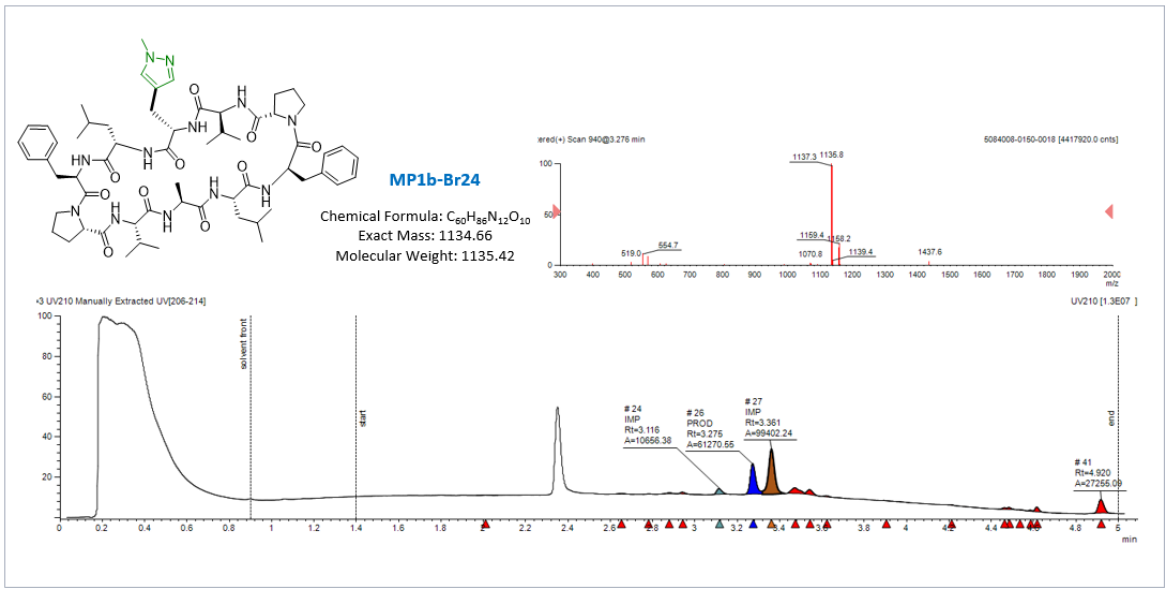
Chemical Formula: $C_{60}H_{84}N_{12}O_{11}$
Exact Mass: 1148.64
Molecular Weight: 1149.40

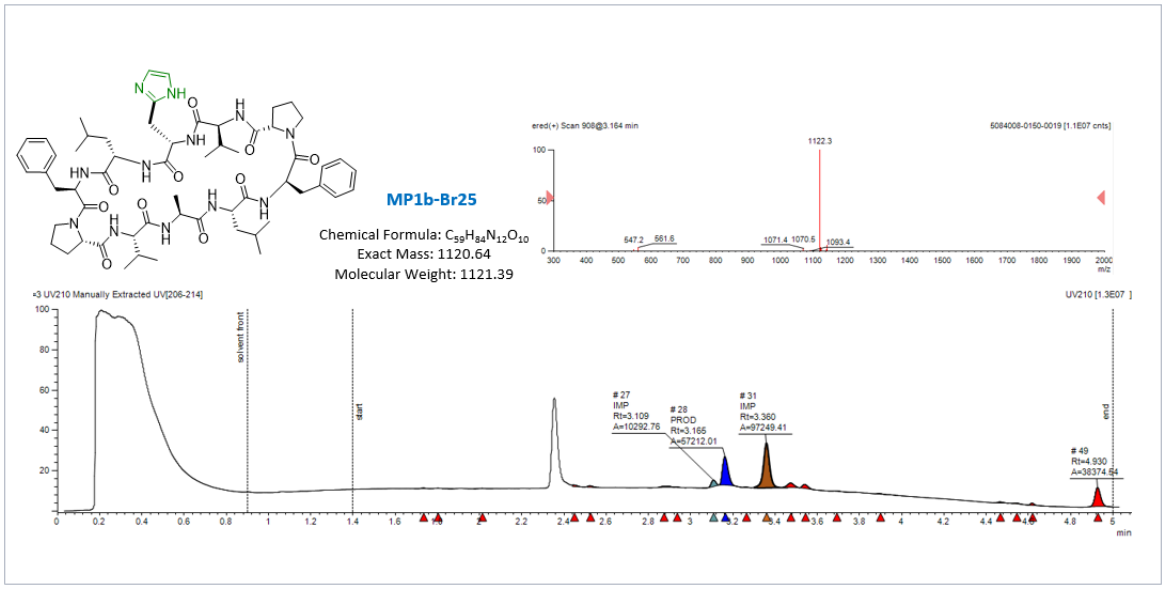




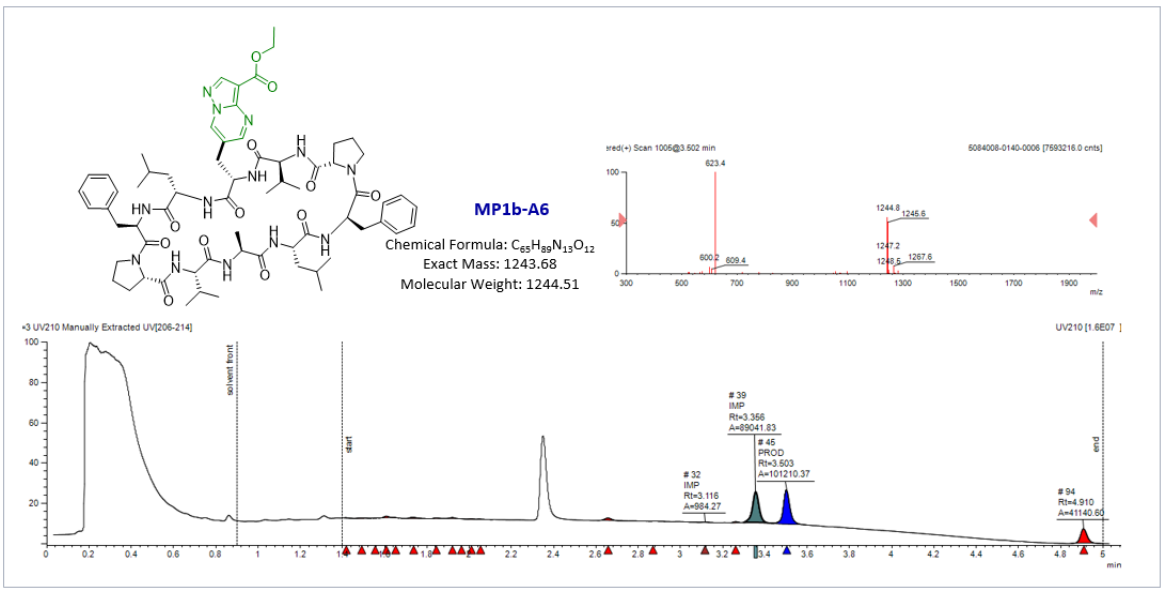

MP1b-Br21
Chemical Formula: $C_{61}H_{86}N_{12}O_{11}$
Exact Mass: 1162.65
Molecular Weight: 1163.43
UV210 [1.5E07]
solvent front
start
end
26
IMP
Rt=2.952
A=18494.28
# 27
PROD
Rt=3.043
A=91283.53
# 29
IMP
Rt=3.256
A=51161.67
min

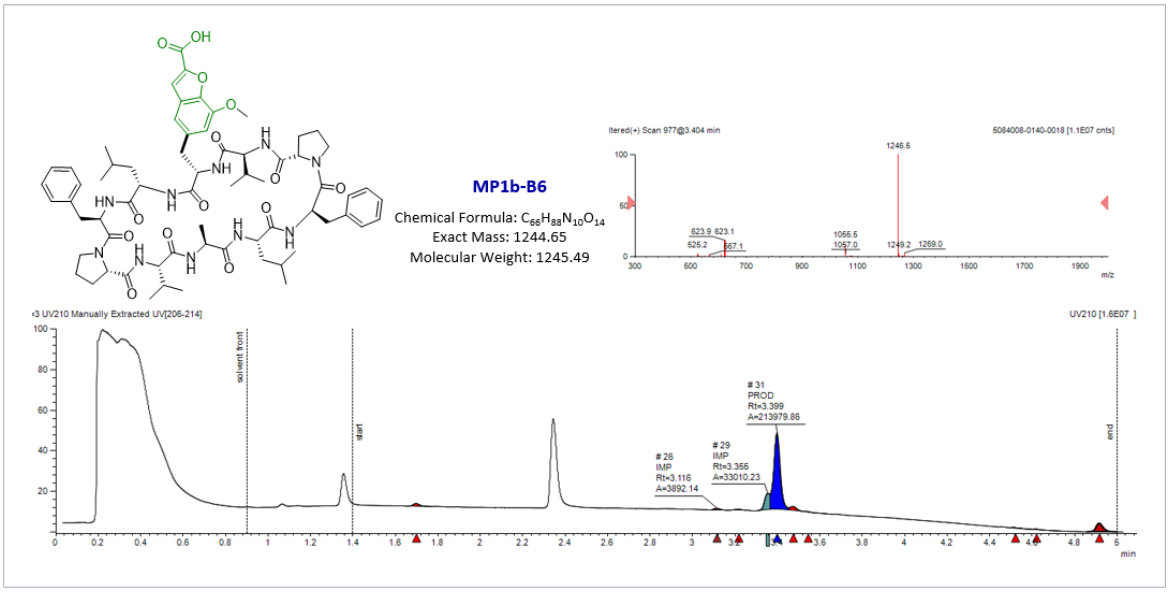

MP1b-Br23
Chemical Formula: $C_{61}H_{86}N_{12}O_{10}$
Exact Mass: 1146.66
Molecular Weight: 1147.43
UV210 [1.6E07]
solvent front
start
end
24
IMP
Rt=2.963
A=21266.62
# 25
PROD
Rt=3.082
A=60437.17
# 27
IMP
Rt=3.257
A=93718.48
min





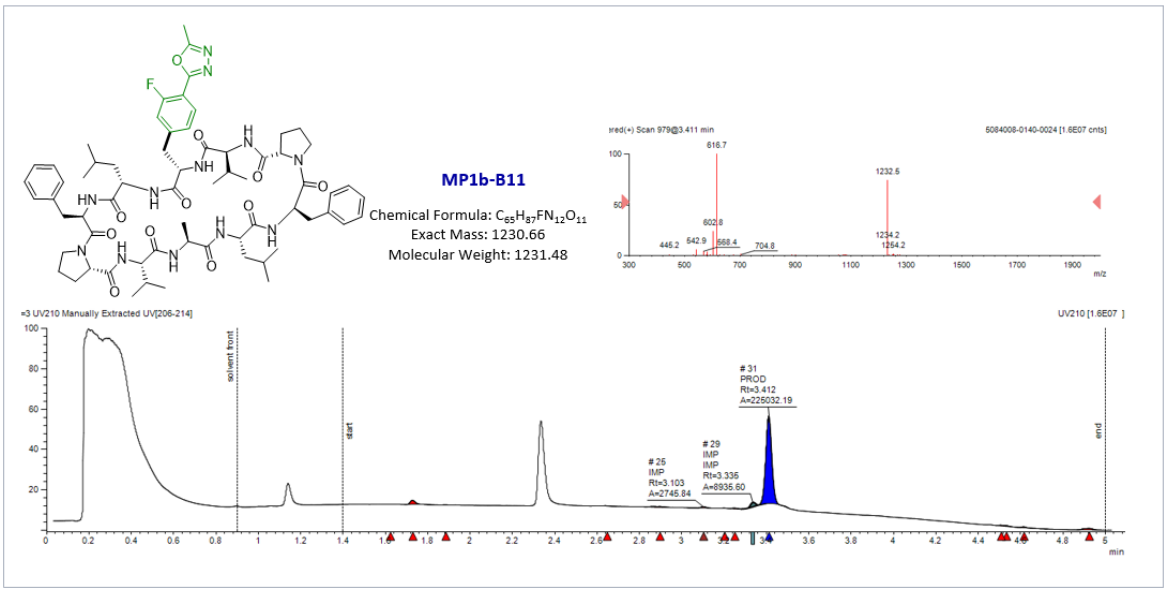
Manuscript Scheme 4 representative chromatograms





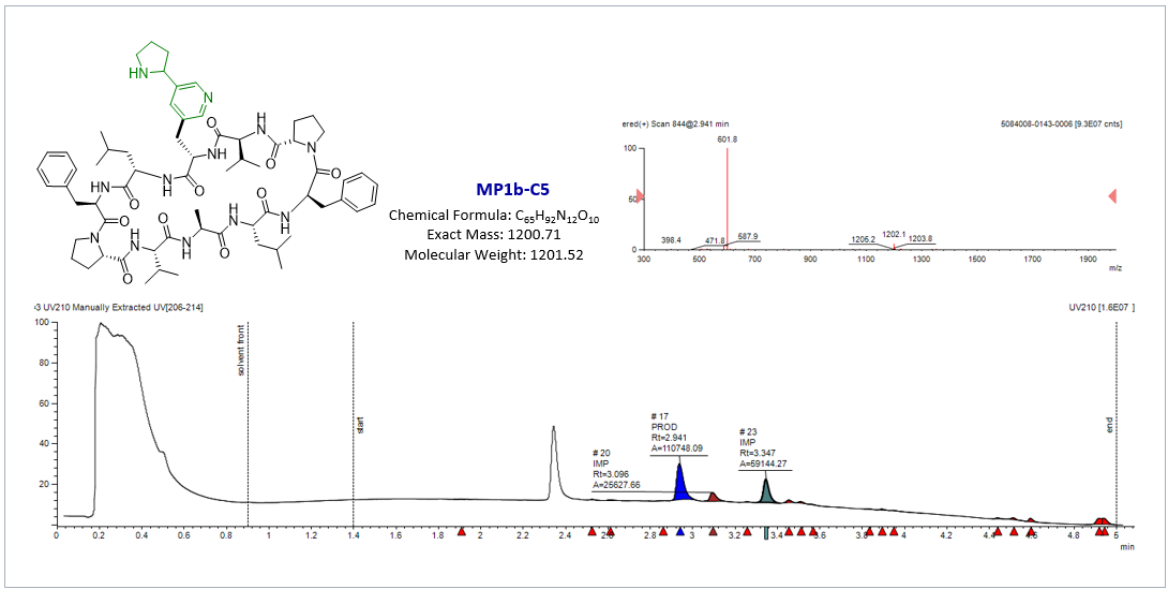
MP1b-B6

Chemical Formula: $C_{66}H_{88}N_{10}O_{14}$
Exact Mass: 1244.65
Molecular Weight: 1245.49



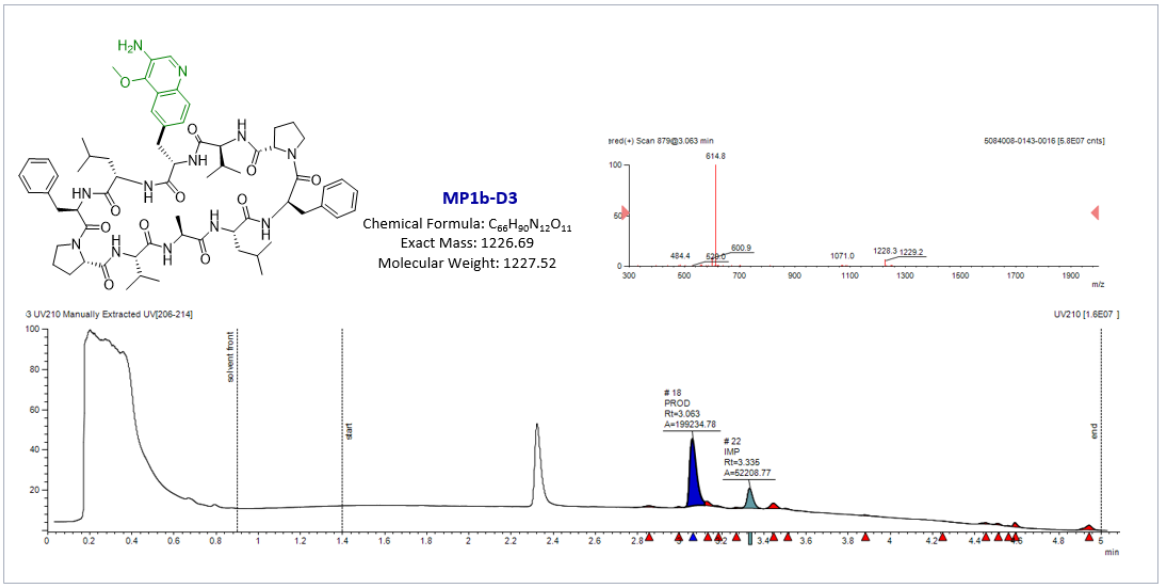
MP1b-B11

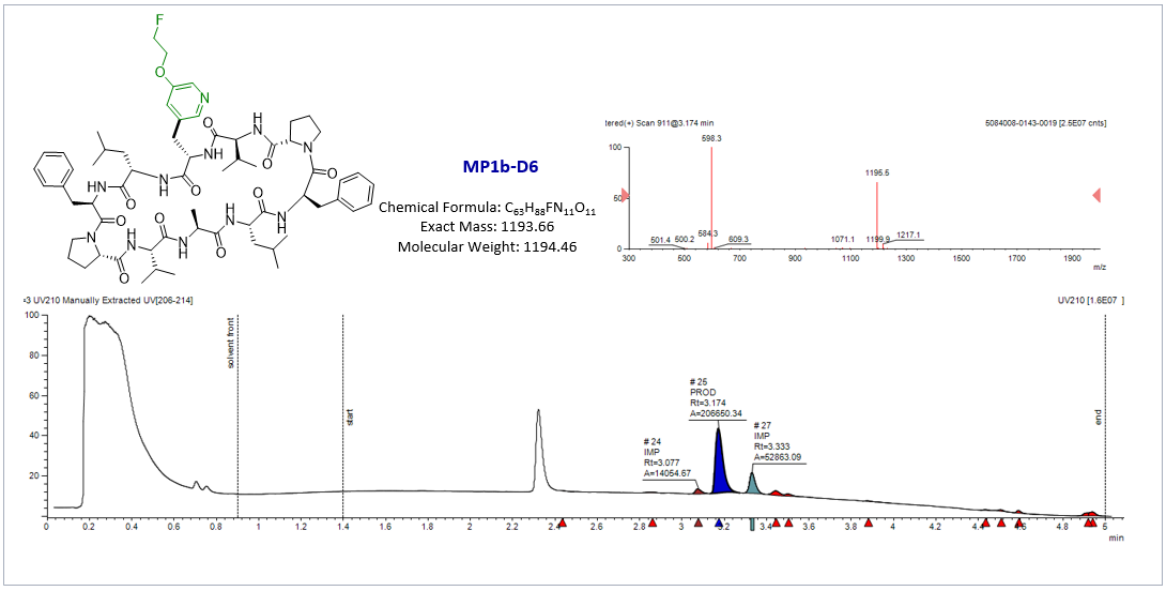
Chemical Formula: $C_{65}H_{87}FN_{12}O_{11}$
Exact Mass: 1230.66
Molecular Weight: 1231.48

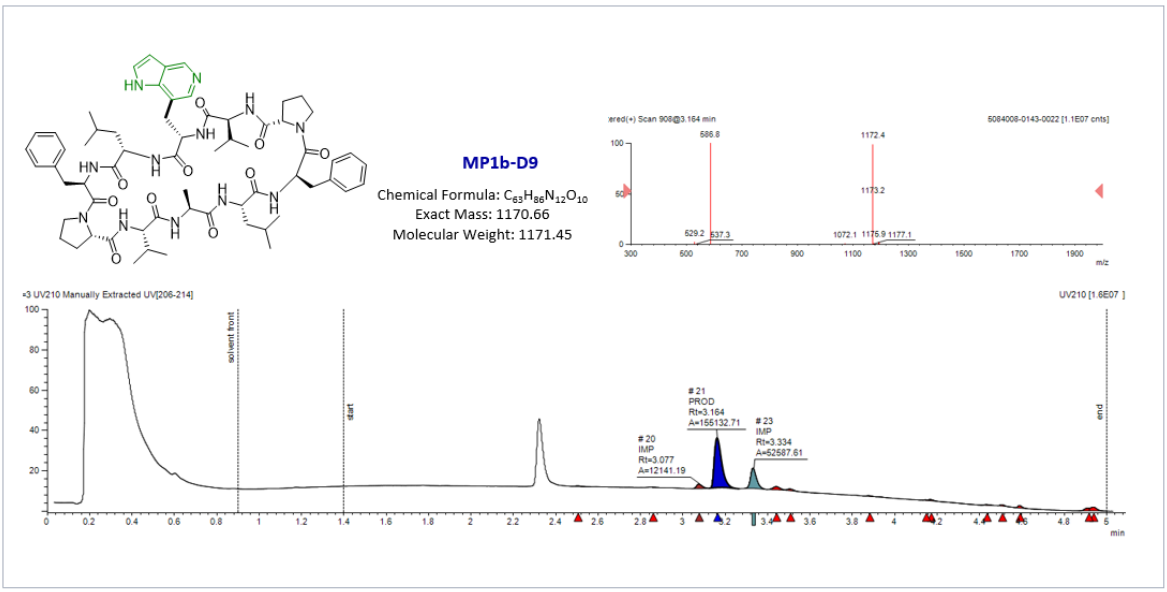


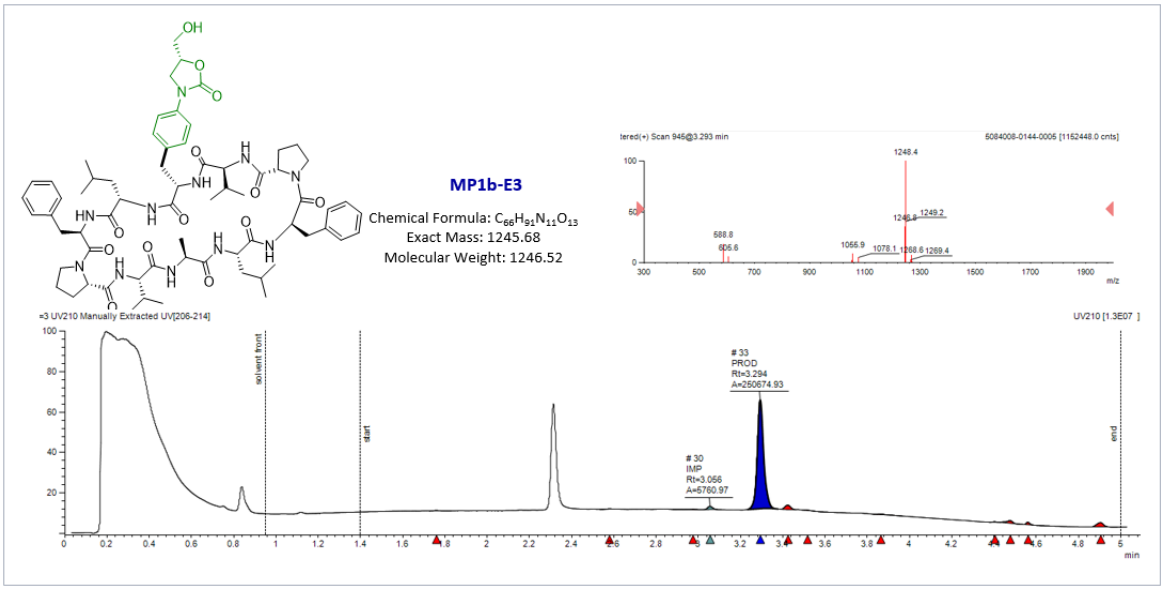
MP1b-C5

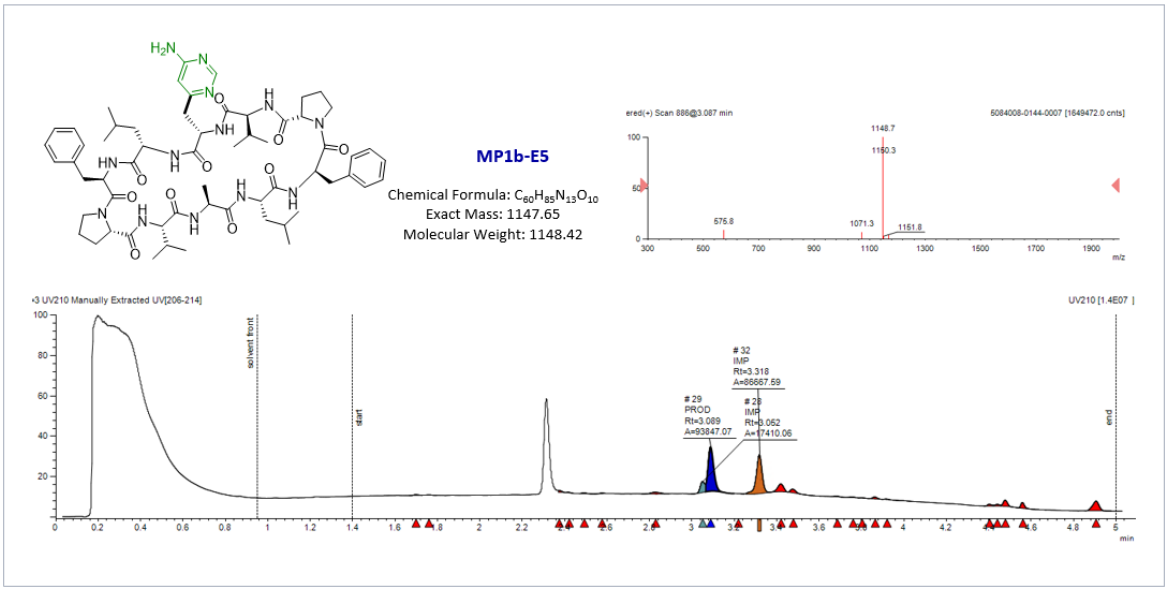
Chemical Formula: $C_{65}H_{92}N_{12}O_{10}$
Exact Mass: 1200.71
Molecular Weight: 1201.52

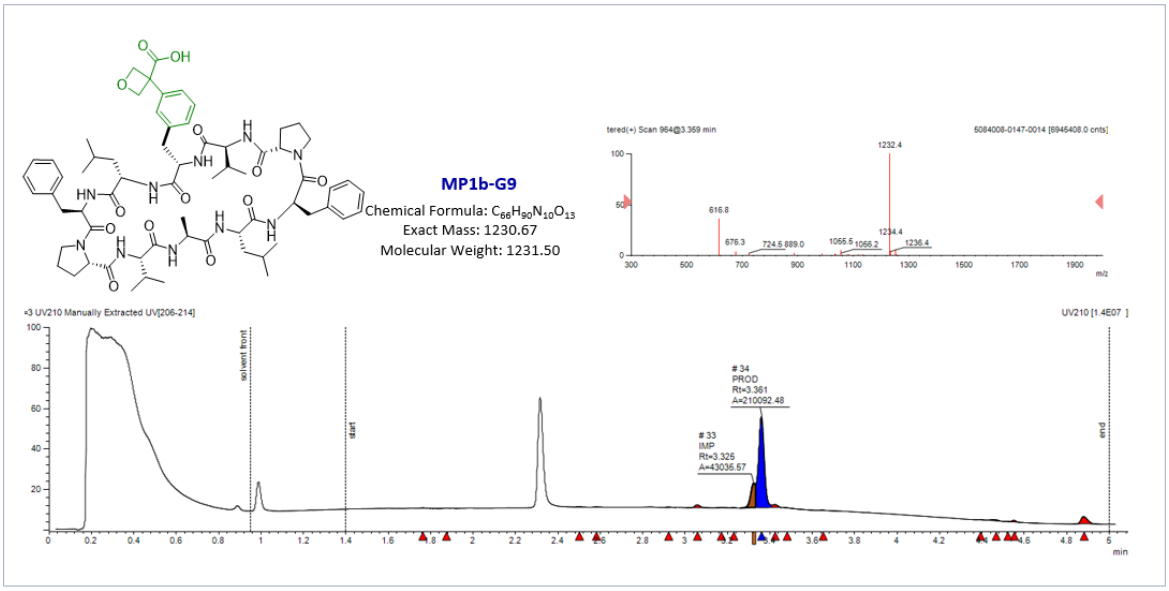

MP1b-D3
Chemical Formula: $C_{66}H_{90}N_{12}O_{11}$
Exact Mass: 1226.69
Molecular Weight: 1227.52
18
PROD
Rt=3.063
A=199234.78
# 22
IMP
Rt=3.335
A=52208.77
solvent front
start
end
min

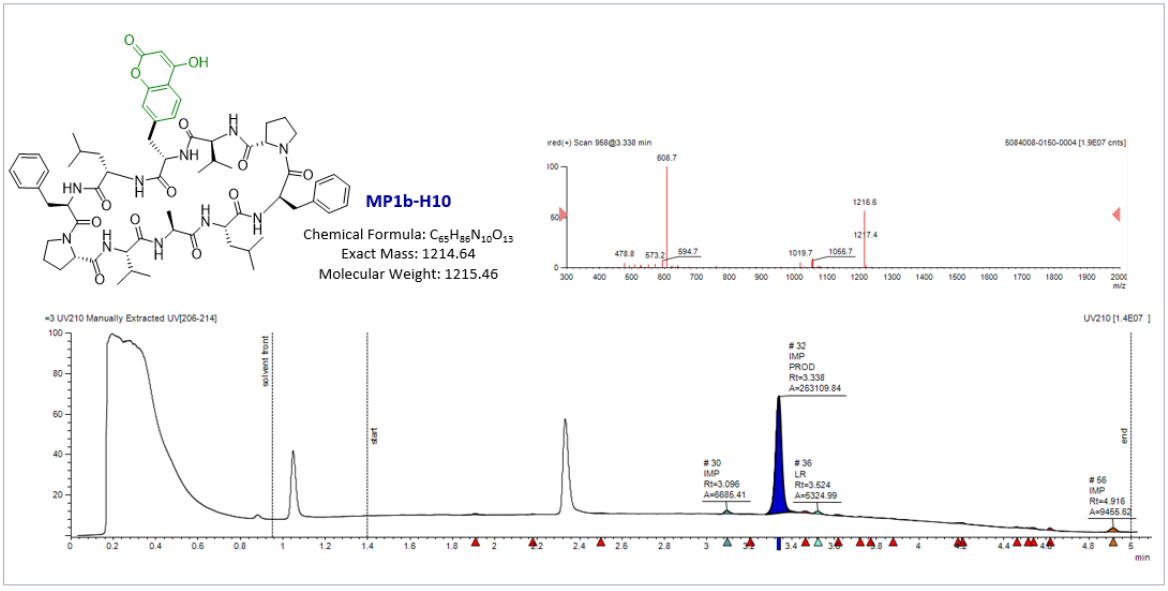

MP1b-D6
Chemical Formula: $C_{63}H_{88}FN_{11}O_{11}$
Exact Mass: 1193.66
Molecular Weight: 1194.46
25
PROD
Rt=3.174
A=206650.34
# 24
IMP
Rt=3.077
A=14054.67
# 27
IMP
Rt=3.333
A=52863.09
solvent front
start
end
min

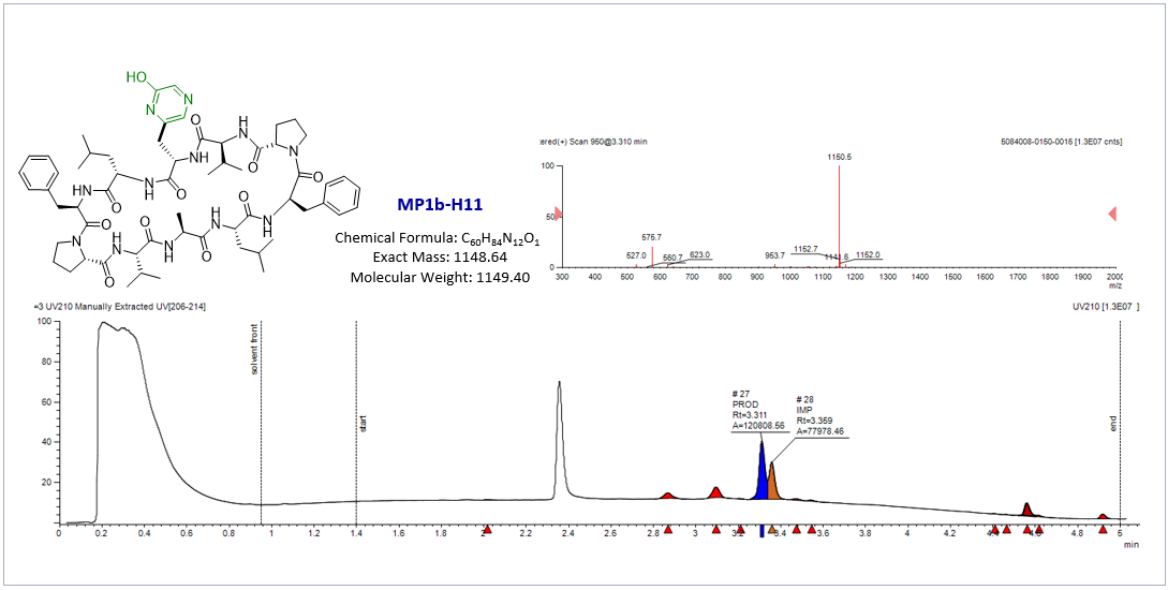

MP1b-D9
Chemical Formula: $C_{63}H_{86}N_{12}O_{10}$
Exact Mass: 1170.66
Molecular Weight: 1171.45
21
PROD
Rt=3.164
A=155132.71
# 20
IMP
Rt=3.077
A=12141.19
# 23
IMP
Rt=3.334
A=52587.61
solvent front
start
end
min


MP1b-E3
Chemical Formula: C66H91N11O13
Exact Mass: 1245.68
Molecular Weight: 1246.52
33
PROD
Rt=3.294
A=250674.93
# 30
IMP
Rt=3.056
A=6760.97
solvent front
start
end
min

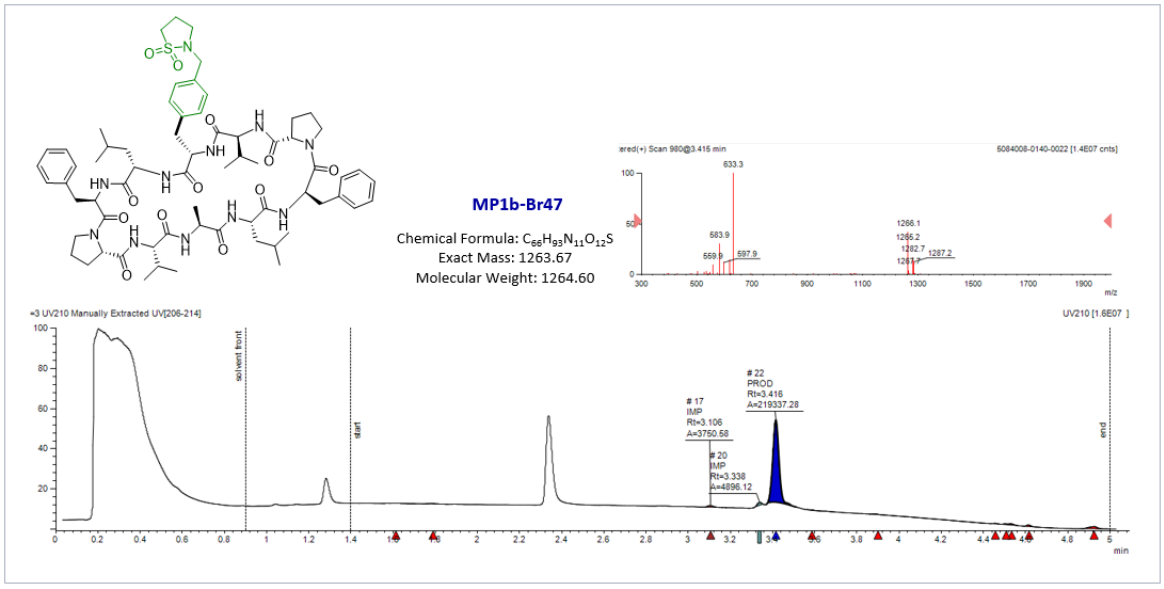

MP1b-E5
Chemical Formula: C60H85N13O10
Exact Mass: 1147.65
Molecular Weight: 1148.42
32
IMP
Rt=3.318
A=86667.59
# 29
PROD
Rt=3.089
A=93847.07
# 28
IMP
Rt=3.062
A=17410.06
solvent front
start
end
min

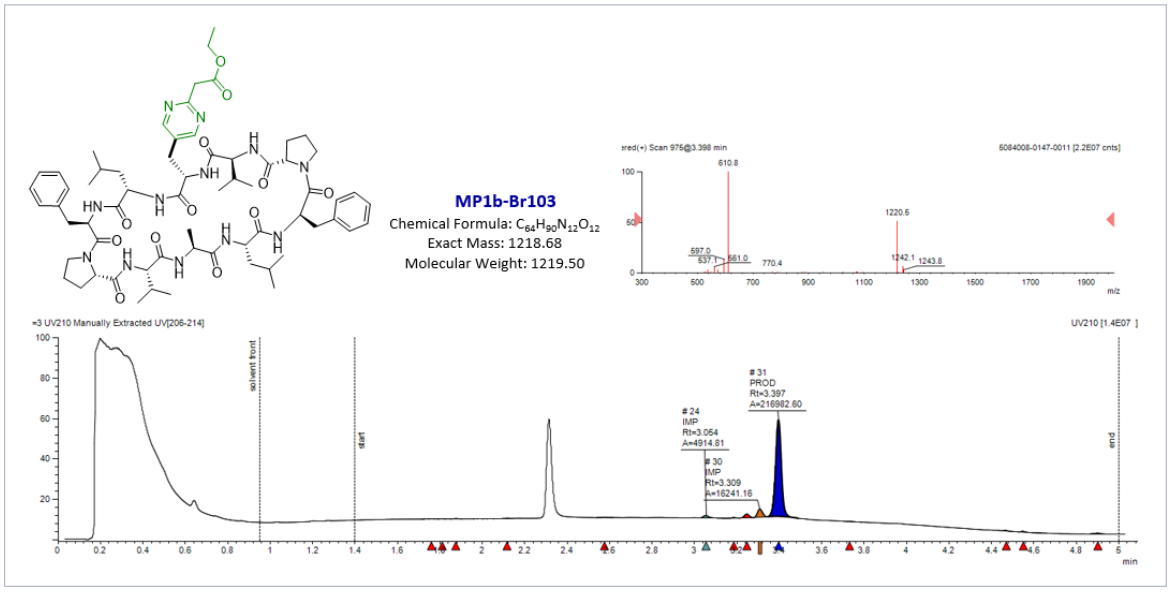

MP1b-G9
Chemical Formula: C66H90N10O13
Exact Mass: 1230.67
Molecular Weight: 1231.50
34
PROD
Rt=3.361
A=210092.48
# 33
IMP
Rt=3.325
A=43036.57
solvent front
start
end
min





Manuscript Figure 2 representative chromatograms





MP1b-Br103
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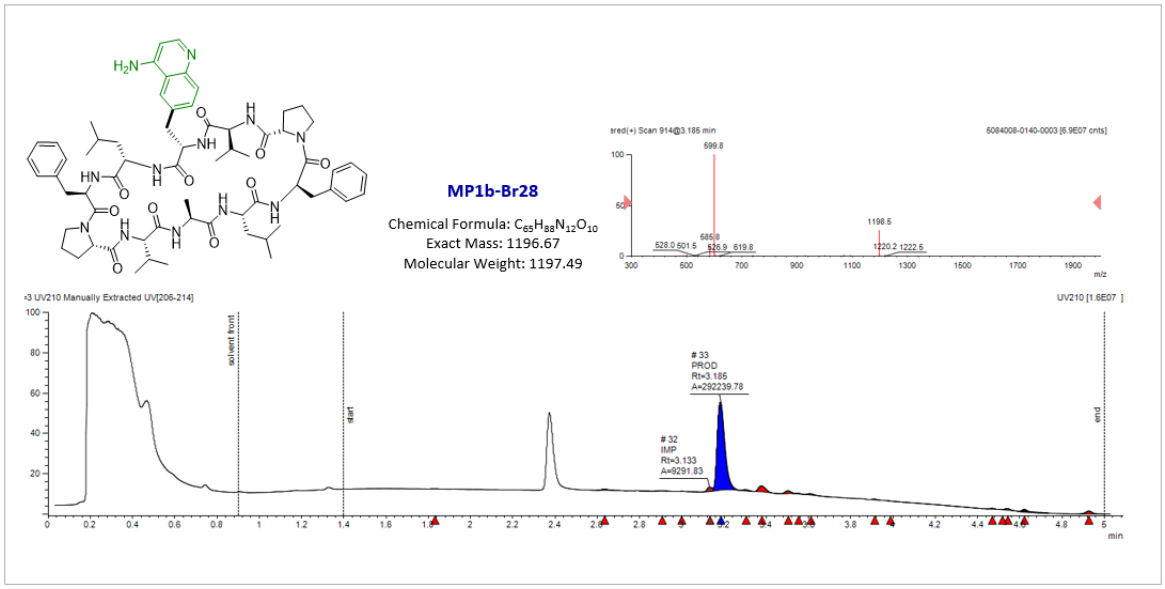
Exact Mass: 1218.68
Molecular Weight: 1219.50



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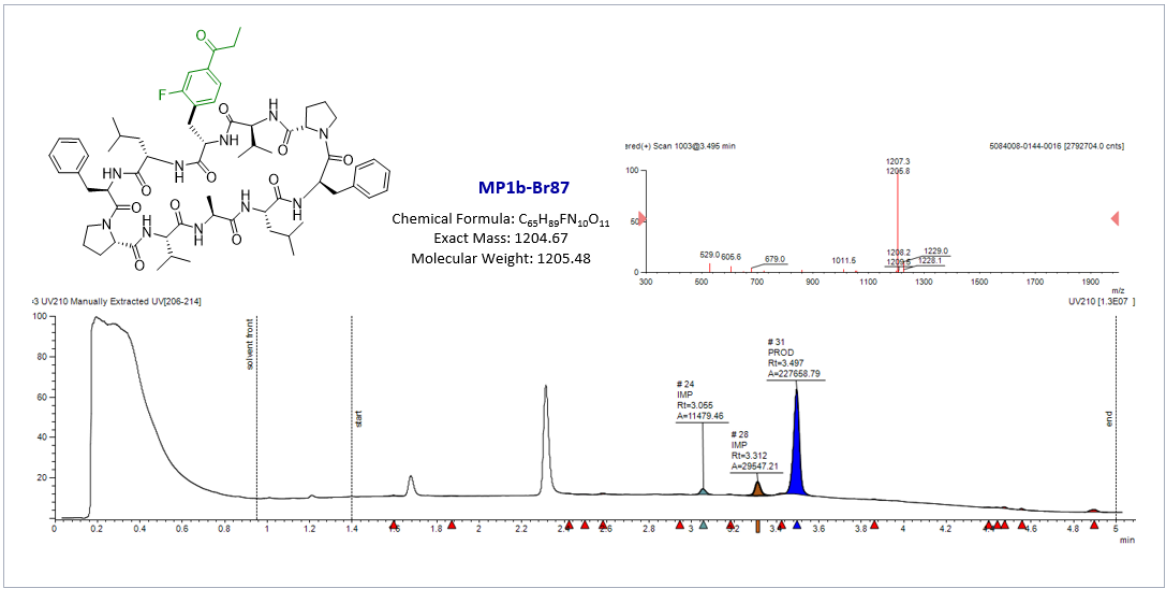
Molecular Weight: 1197.49



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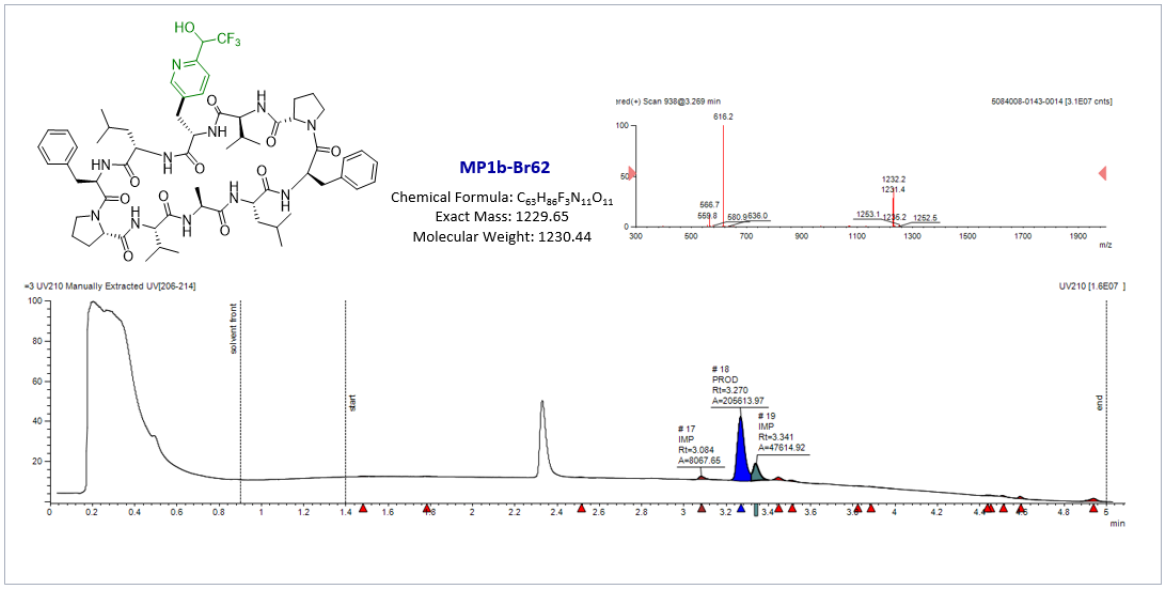
Molecular Weight: 1205.48


MP1b-Br62
Chemical Formula: $C_{63}H_{86}F_3N_{11}O_{11}$
Exact Mass: 1229.65
Molecular Weight: 1230.44
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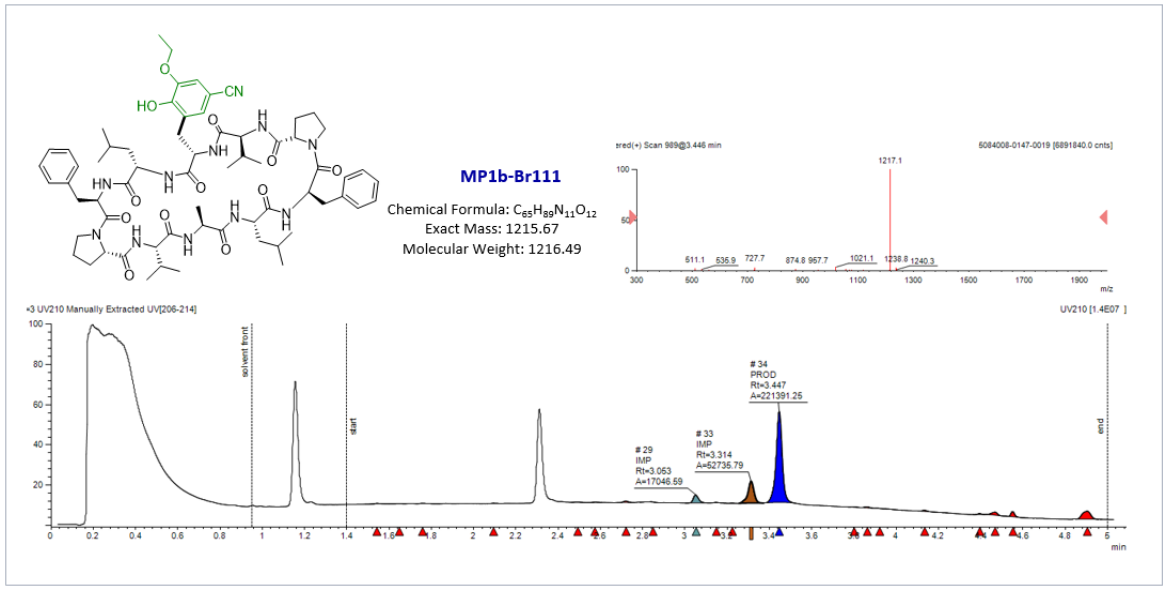
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Chemical Formula: $C_{65}H_{89}N_{11}O_{12}$
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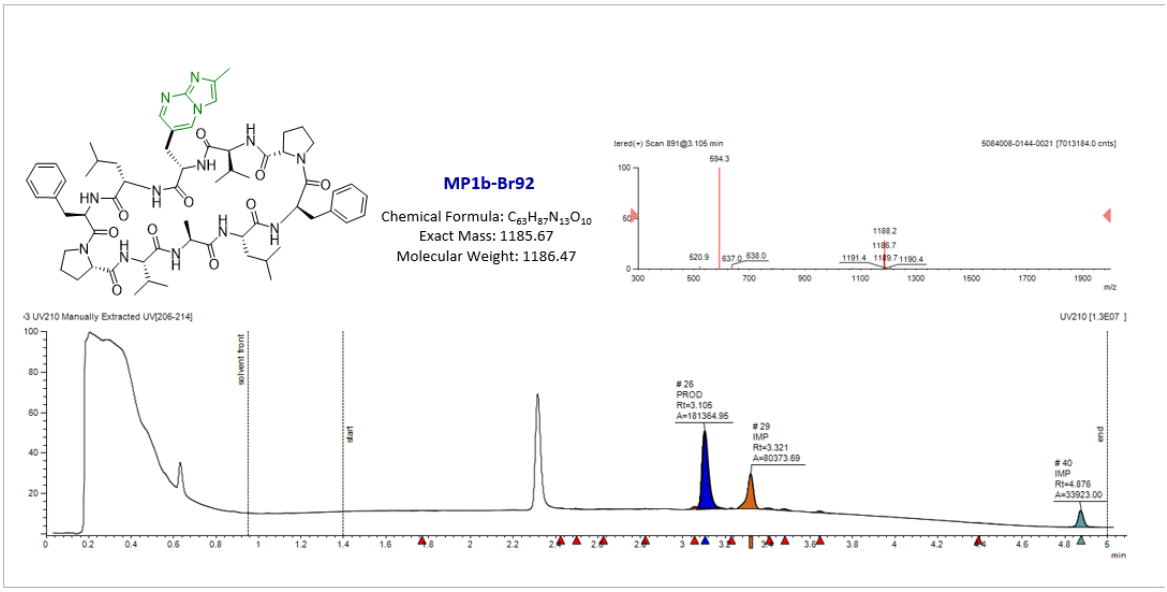
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Molecular Weight: 1186.47
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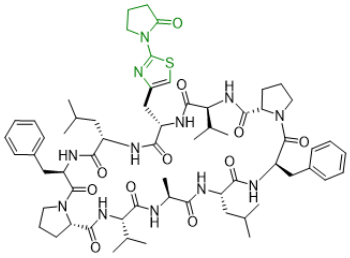

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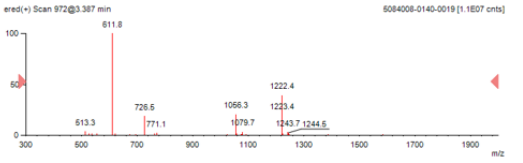
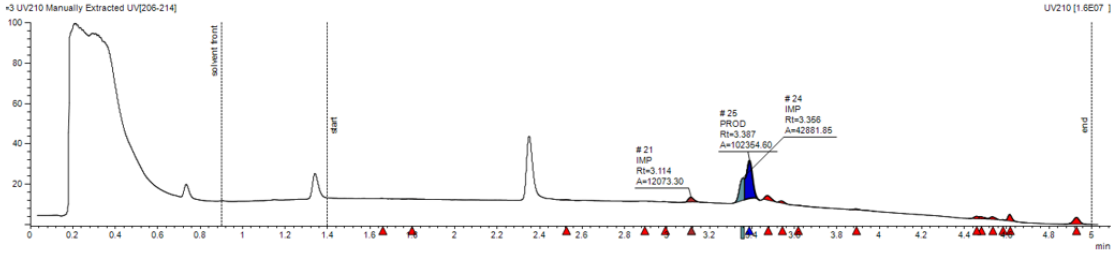
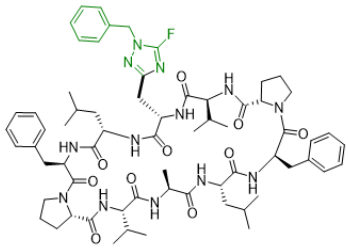
Molecular Weight: 1221.53

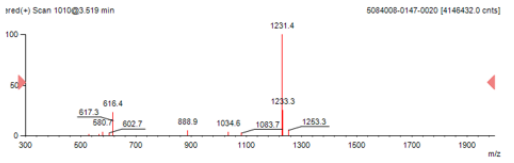


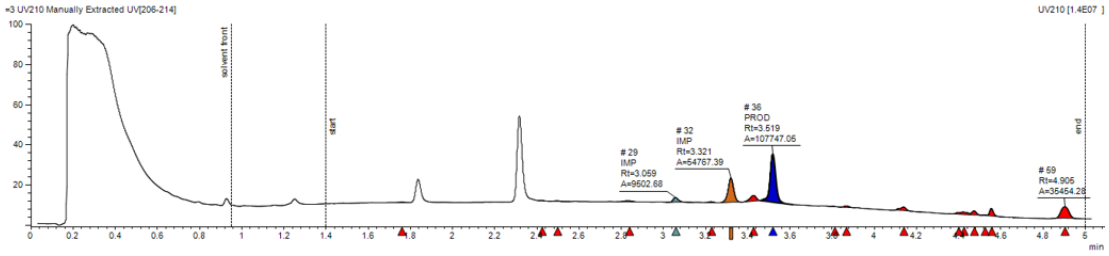


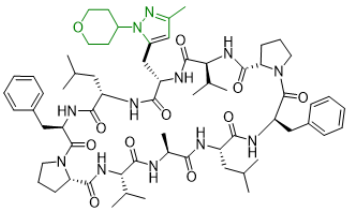


MP1b-Br112

Chemical Formula: $C_{65}H_{88}FN_{13}O_{10}$
Exact Mass: 1229.68
Molecular Weight: 1230.50

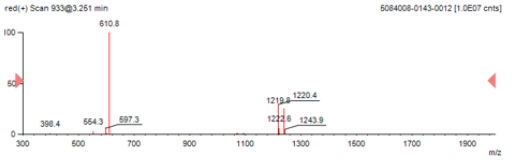


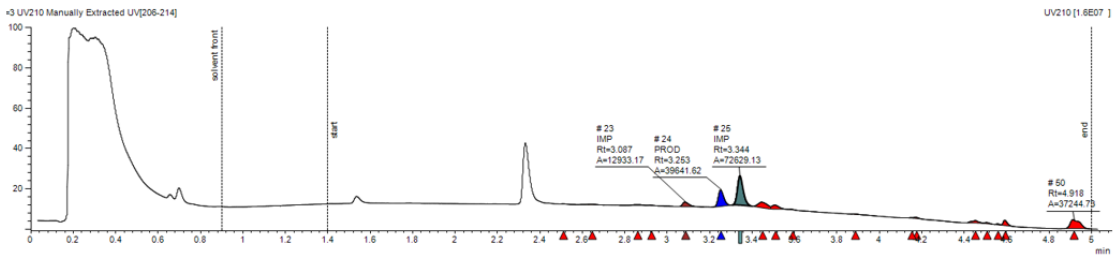


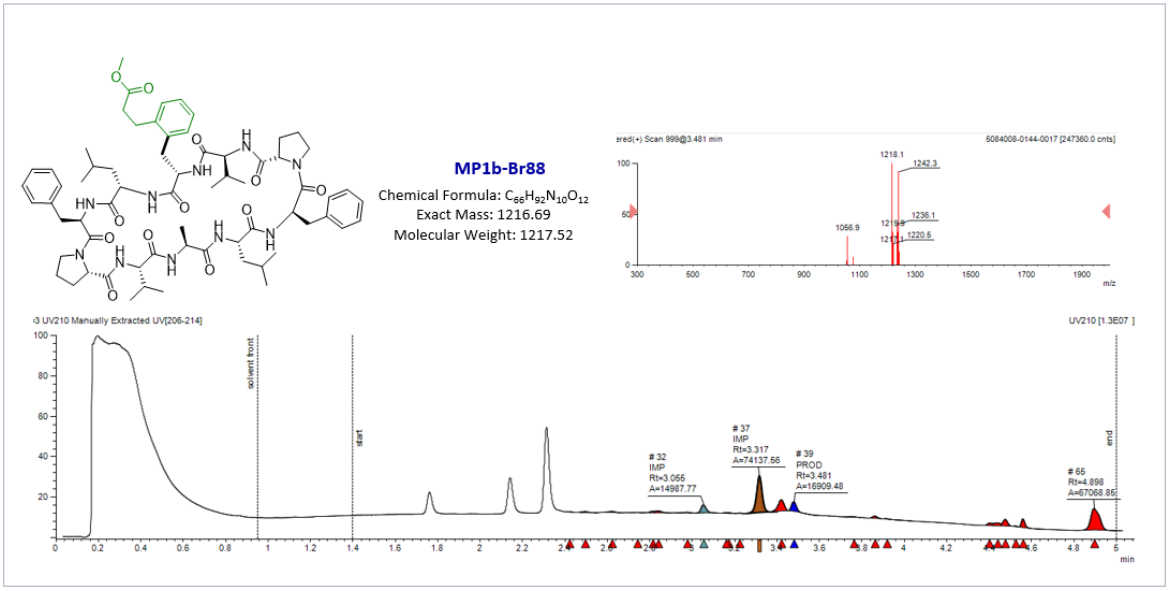


MP1b-Br60

Chemical Formula: $C_{65}H_{94}N_{12}O_{11}$
Exact Mass: 1218.72
Molecular Weight: 1219.54

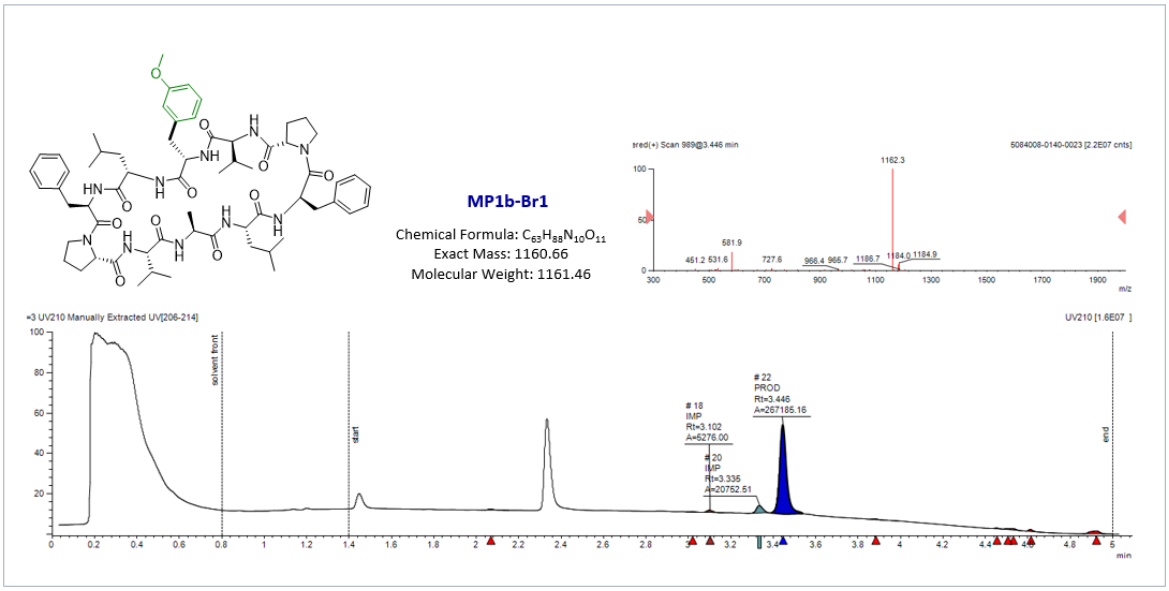






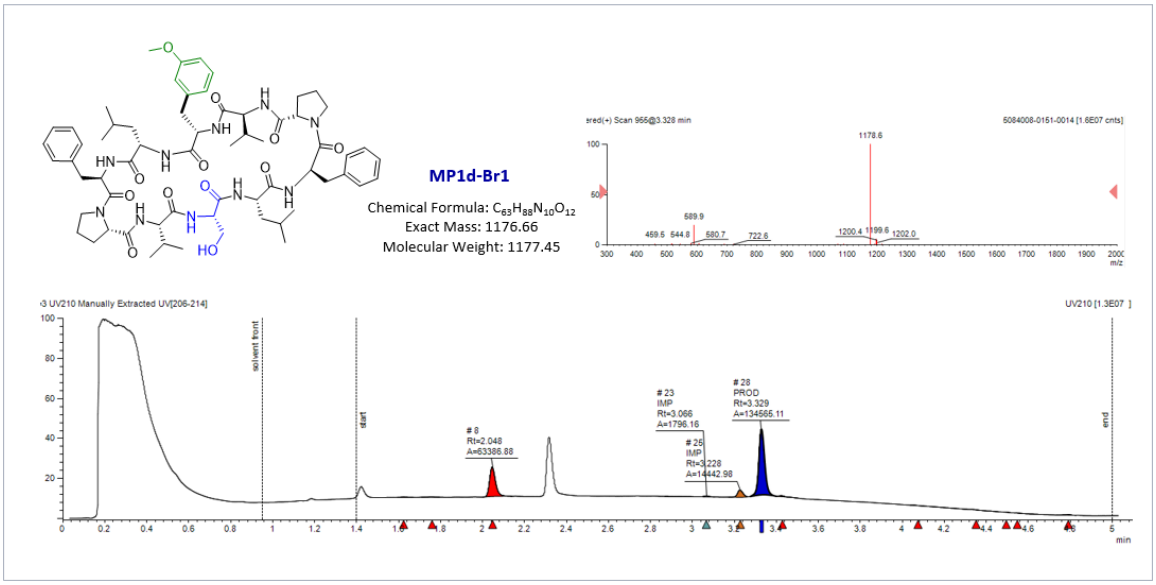
MP1b-Br88

Chemical Formula: $C_{66}H_{92}N_{10}O_{12}$
Exact Mass: 1216.69
Molecular Weight: 1217.52



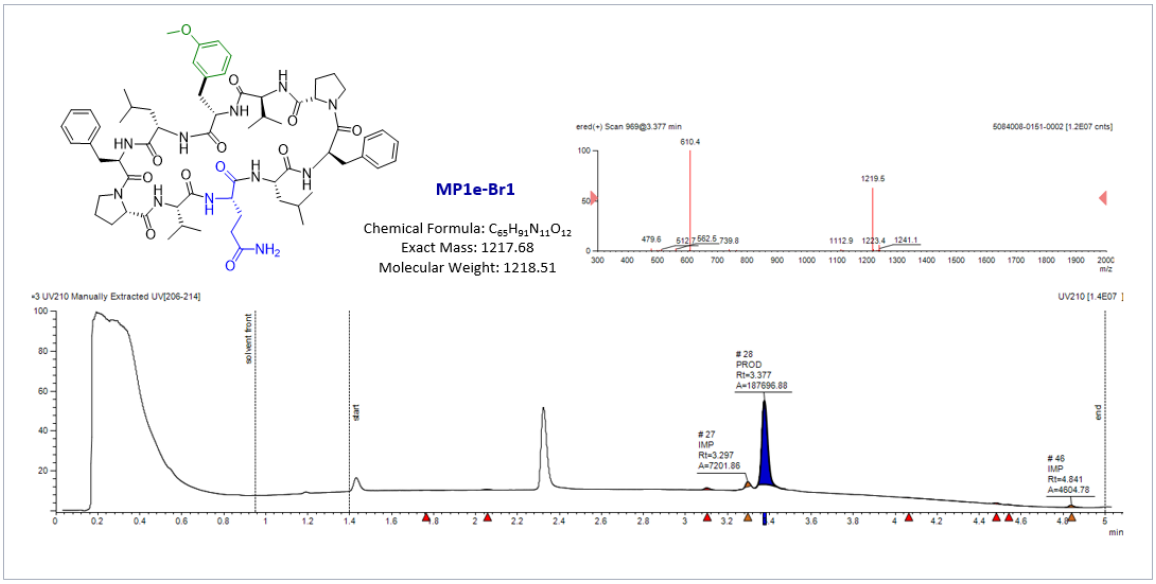
MP1b-Br1

Chemical Formula: $C_{63}H_{88}N_{10}O_{11}$
Exact Mass: 1160.66
Molecular Weight: 1161.46



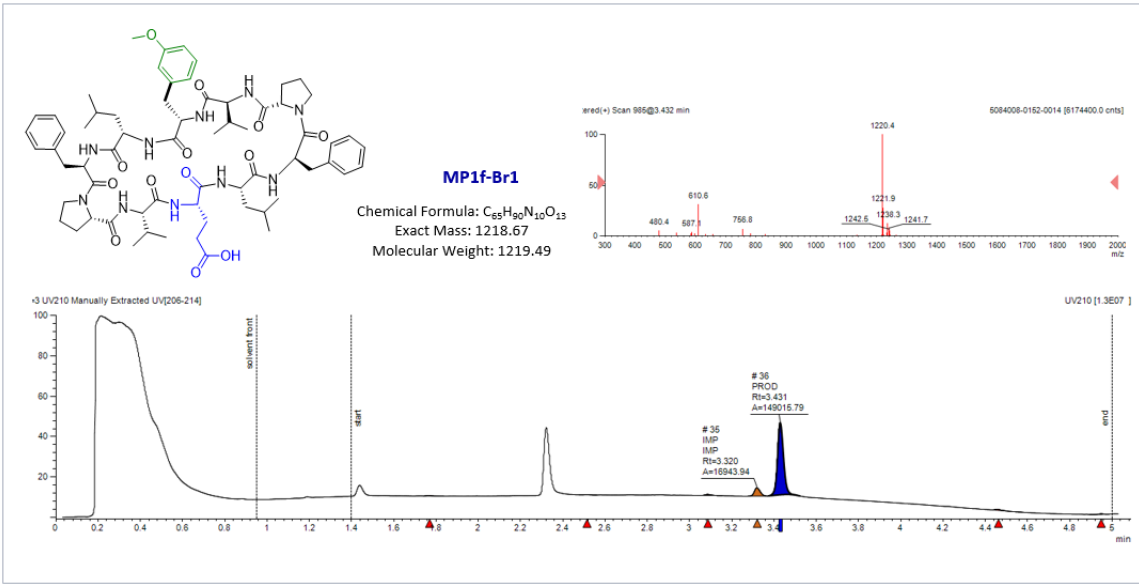
MP1d-Br1

Chemical Formula: $C_{63}H_{88}N_{10}O_{12}$
Exact Mass: 1176.66
Molecular Weight: 1177.45



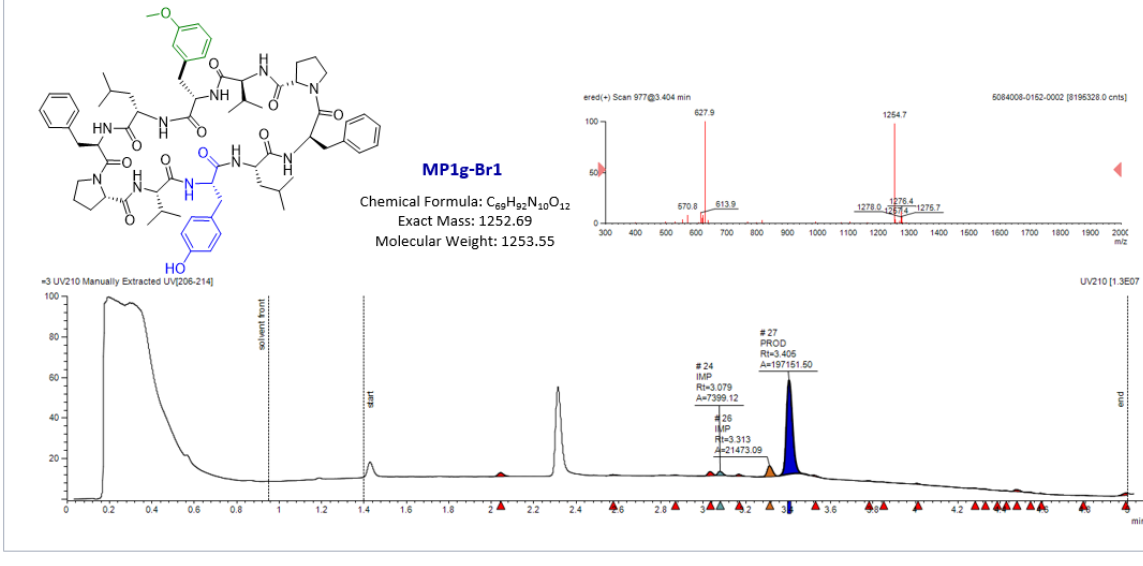
MP1e-Br1

Chemical Formula: $C_{65}H_{91}N_{11}O_{12}$
Exact Mass: 1217.68
Molecular Weight: 1218.51



MP1f-Br1

Chemical Formula: $C_{65}H_{90}N_{10}O_{13}$
Exact Mass: 1218.67
Molecular Weight: 1219.49



MP1g-Br1

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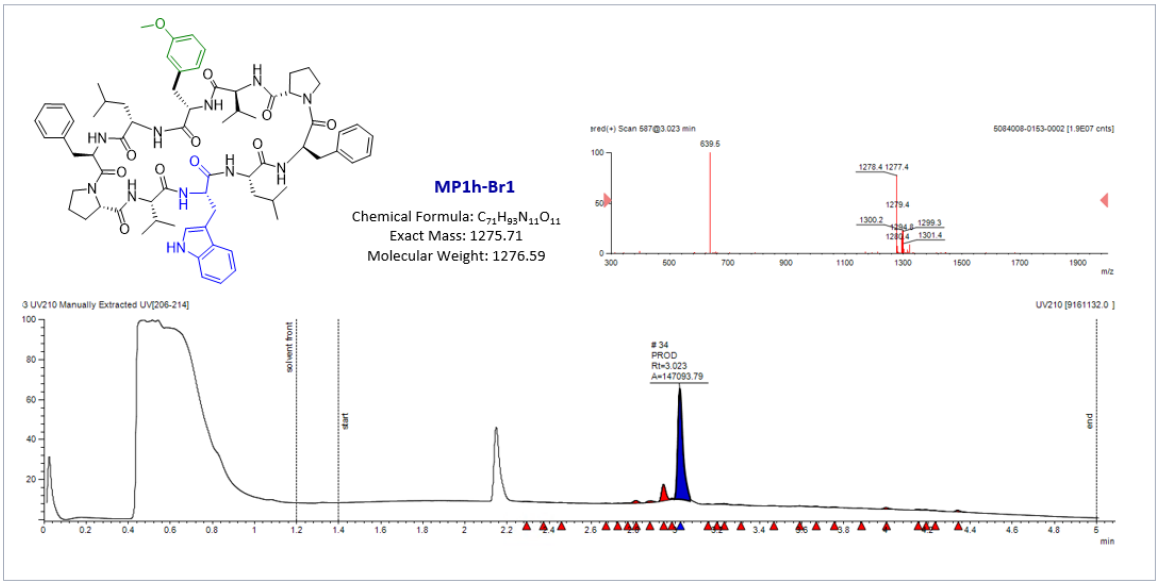
Exact Mass: 1252.69
Molecular Weight: 1253.55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Chemical Formula: $C_{71}H_{93}N_{11}O_{11}$
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Molecular Weight: 1276.59
639.5
1278.4 1277.4
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1301.4
34
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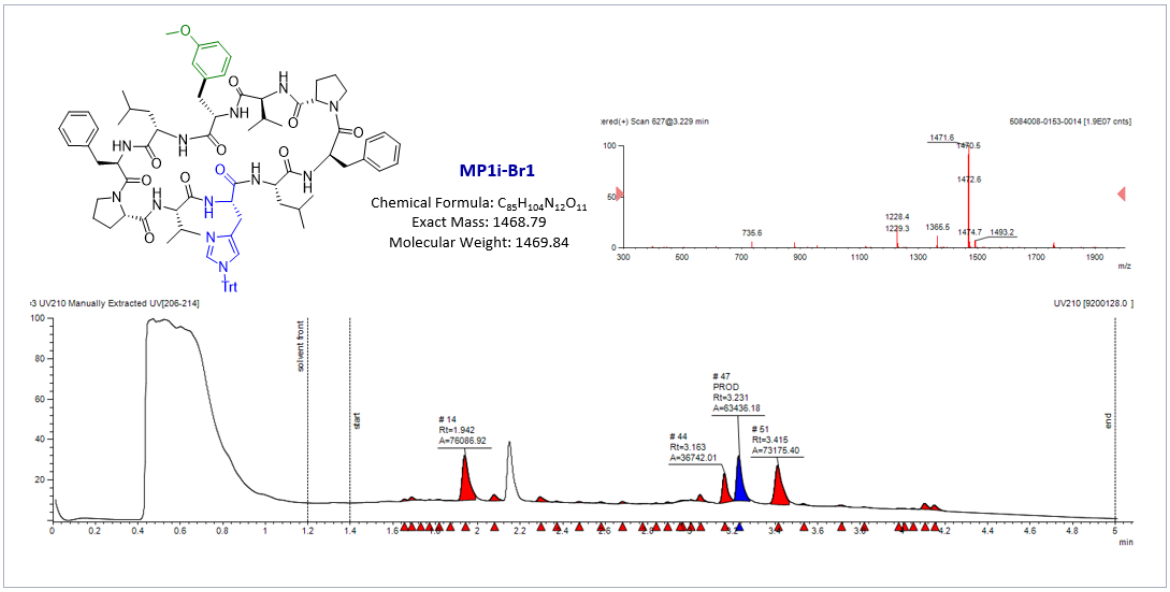
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Exact Mass: 1468.79
Molecular Weight: 1469.84
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1472.6
736.6
1228.4
1229.3
1365.5
1474.7
1493.2
# 14
Rt=1.942
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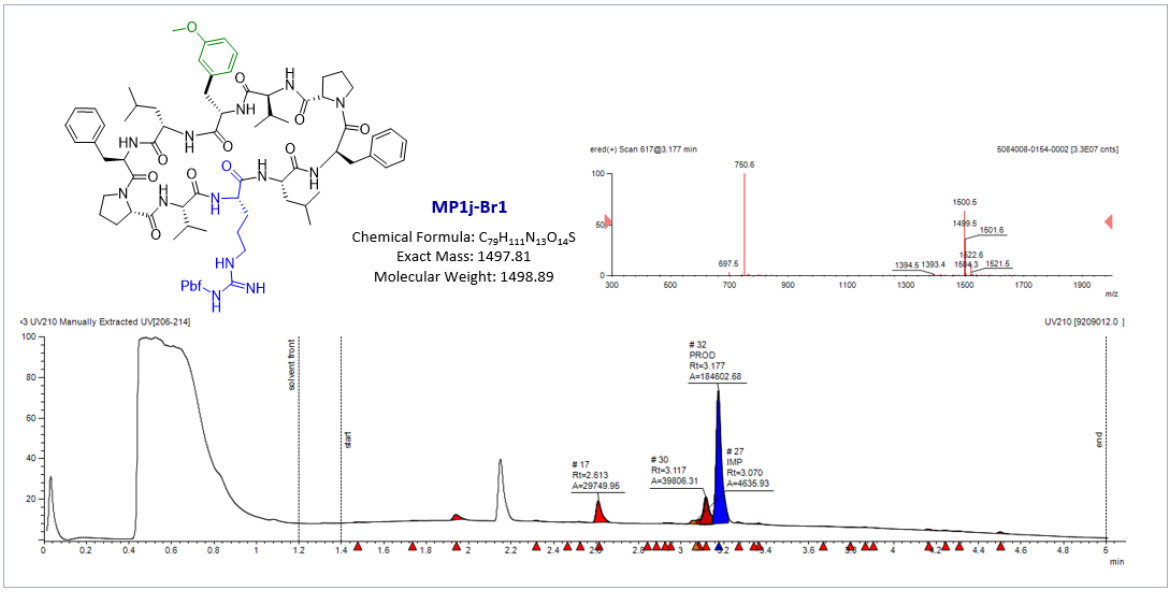
MP1j-Br1
Chemical Formula: $C_{79}H_{111}N_{13}O_{14}S$
Exact Mass: 1497.81
Molecular Weight: 1498.89
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697.5
1500.5
1499.5
1501.6
1522.6
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Rt=2.613
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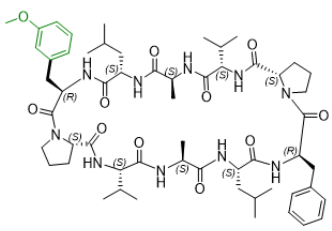
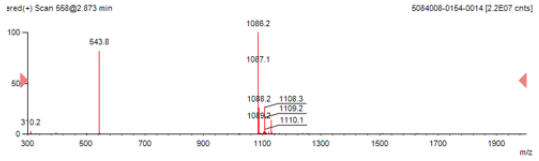
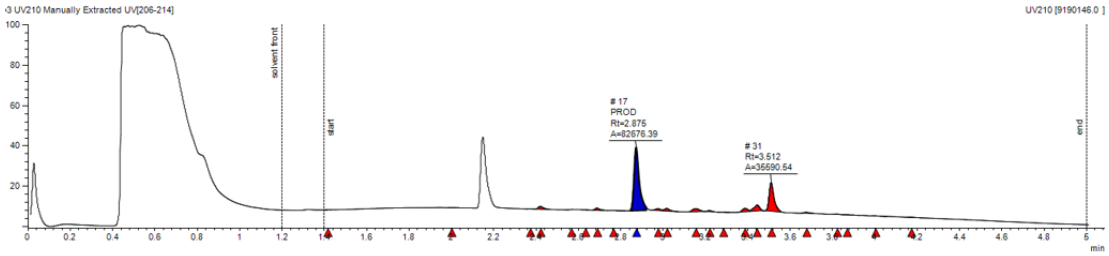
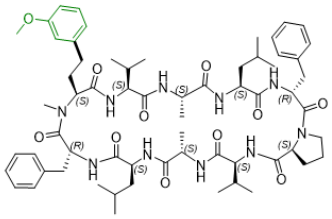


MP1k-Br1

Chemical Formula: $C_{57}H_{84}N_{10}O_{11}$
Exact Mass: 1084.63
Molecular Weight: 1085.36

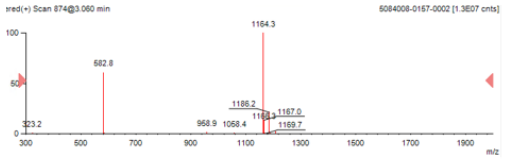


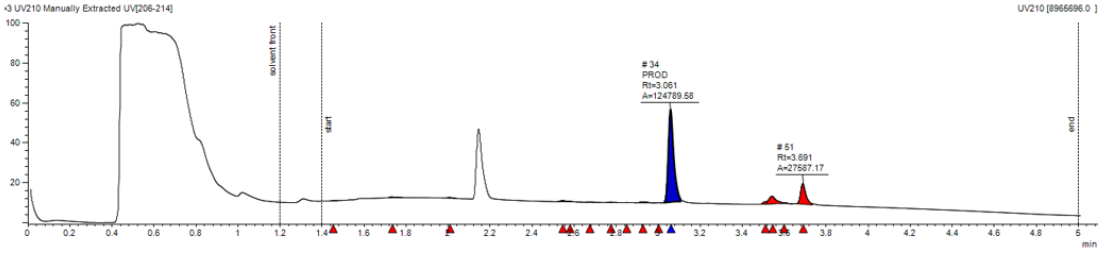


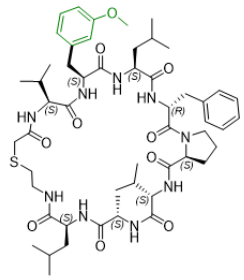


MP1l-Br1

Chemical Formula: $C_{63}H_{90}N_{10}O_{11}$
Exact Mass: 1162.68
Molecular Weight: 1163.47

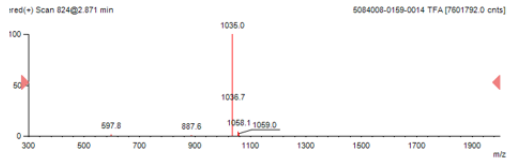


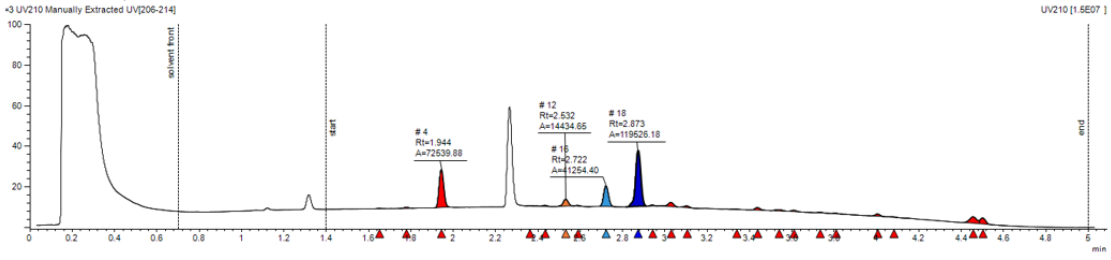


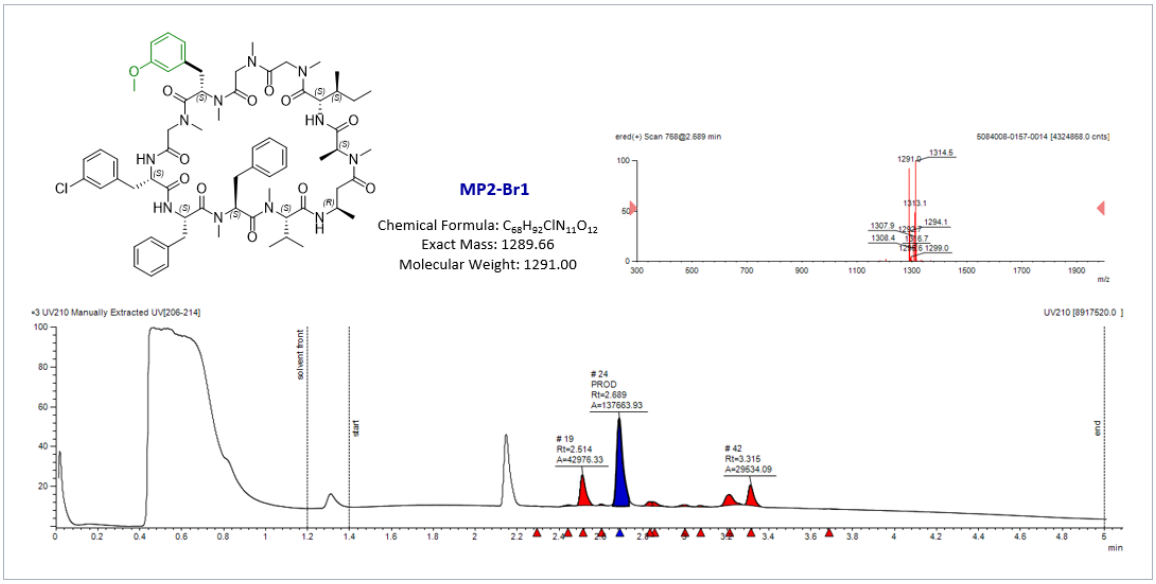


MP1m-Br1

Chemical Formula: $C_{53}H_{79}N_9O_{10}S$
Exact Mass: 1033.57
Molecular Weight: 1034.33







4 Cross-coupling of MP-pyridiniums (25-50 µmol scale) procedures.

General Procedure A:

In a nitrogen-filled glovebox, to 4-mL vial containing stir bar, and MP-pyridinium, (28-44 µmol, 1 eq), was added 3-bromoanisole (0.12 M solution in DMA, 42-66 µmol, 1.5 eq), pre-complexed $NiBr_2$.glyme/2-pyridylcarboxamidine ligand mixture* (0.1 M mixture in DMA, 5.6-8.8 µmol, 0.2 eq.) and Zn or Mn (0.6 M slurry in DMA, 112-176 µmol, 4eq.) sequentially. The reaction concentration is 0.05 M. The vial was sealed, placed on a tumble stirrer preheated to 60°C for 90 minutes. The reaction crude was cooled to room temperature, taken out of the glovebox, diluted with DMSO (750 µL), and filtered through Nalgene PTFE 0.48 µm filter. The filter was rinsed with DMSO (500 µL). The crude mixture was then directly purified on reverse phase HPLC.

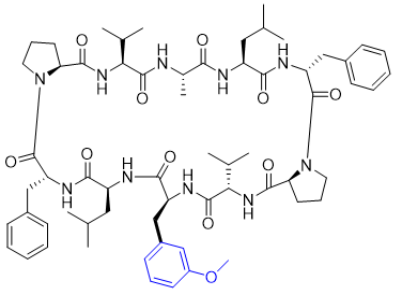
*Preparation of 0.1M stock solution of $NiBr_2$.glyme/2-pyridylcarboxamidine ligand mixture: In a nitrogen-filled glovebox, a 4-mL vial (equipped with stir bar) was charged with $NiBr_2$.glyme (0.049 mmol) and pyridine-2,6-bis(carboximidamide)dihydrochloride (0.058 mmol) in 472.2 µL of DMA. The mixture was stirred for ~30 minutes (resulting in a green or blue slurry) before being dosed to the reaction plate. Catalyst, Zn and Mn stock slurries were dosed while stirring at 800 rpm.

General Procedure B:

In a nitrogen-filled glovebox, to a 4-mL vial was added pre-complexed $NiBr_2$.glyme/2-pyridylcarboxamidine ligand mixture* (59µL, 0.1M mixture in DMA, 5.9 µmol, 0.2 eq.) and Zn (197 µL, 0.6 M slurry in DMA, 0.118 mmol, 4 eq.) The resulting mixture was heated to 60°C. To a separate 4 mL vial was added MP-Pyridinium (0.03 mmol, 1 eq), 3-bromoanisole (0.044 mmol, 1.5 eq), and DMA (285 µL). The MP-pyridinium, 3-bromoanisole mixture was then added portion wise (57 µL x 5 x every 20 minutes) to the 4 mL vial containing activated catalyst and Zn mixture. The resulting mixture was stirred at 60° C. The reaction crude was cooled to room temperature, taken out of the glovebox, diluted with DMSO (750µL) and filtered through Nalgene PTFE 0.48 µm filter. The filter was rinsed with DMSO (500 µL). The crude mixture was then directly purified on reverse phase HPLC.

*Preparation of 0.1 M stock solution of $NiBr_2$.glyme/2-pyridylcarboxamidine ligand mixture: In a nitrogen-filled glovebox, a 4-mL vial (equipped with stir bar) was charged with $NiBr_2$.glyme (0.049 mmol) and pyridine-2,6-

bis(carboximidamide)dihydrochloride (0.058mmol) in 472.2 µL of DMA. The mixture was stirred for ~30 minutes (resulting in a green or blue slurry) before being dosed to the reaction plate. Catalyst, Zn and Mn stock slurries were dosed while stirring at 800 rpm.

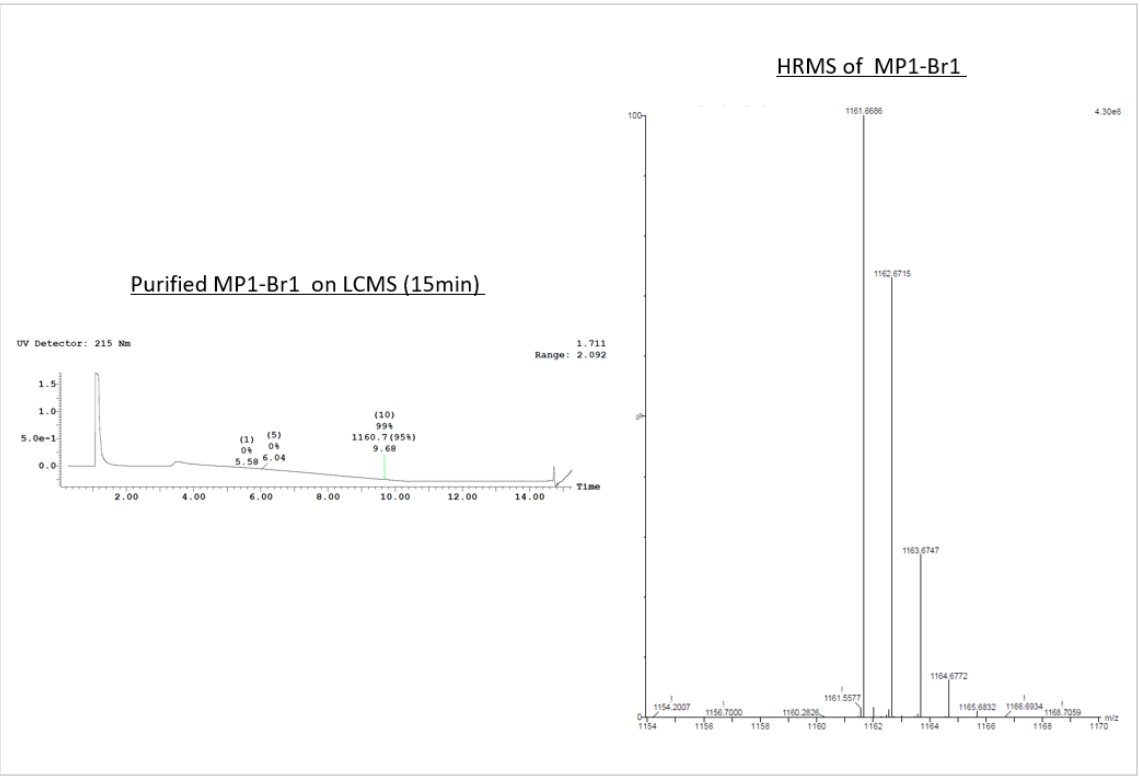


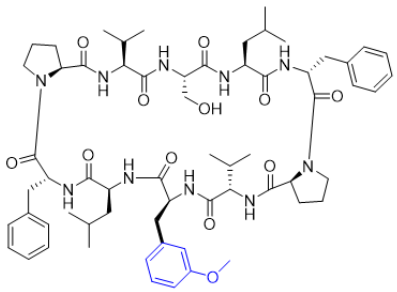
MP1-Br-1: Prepared via General Procedure A in section 4 using **MP-Pyridinium-1b** (40 µmol). The crude mixture was purified by preparative HPLC Method-A (40-100 B in A) to give **MP1-Br-1** (27.1 mg, 58%) as a white solid: ^{1}H NMR (500 MHz, DMSO-D6) δ 8.72 (d, *J* = 4.6 Hz, 1H), 8.66 (d, *J* = 5.1 Hz, 1H), 8.57 (d, *J* = 8.6 Hz, 2H), 8.49 (d, *J* = 8.9 Hz, 1H), 8.16 (d, *J* = 8.7 Hz, 1H), 7.30 – 7.18 (m, 10H), 7.08 (t, *J* = 7.8 Hz, 1H), 7.08 (t, *J* = 7.8 Hz, 1H), 6.80 – 6.74 (m, 2H), 6.70 – 6.66 (m, 1H), 6.53 (br s, 1H), 4.95 (td, *J* = 9.7, 3.0 Hz, 1H), 4.58 – 4.46 (m, 3H), 4.44 – 4.34 (m, 4H), 4.32 (d, *J* = 7.0 Hz, 1H), 4.27 (d, *J* = 6.6 Hz, 1H), 3.65 (s, 3H), 3.60 – 3.50 (m, 2H), 3.10 (d, *J* = 11.4 Hz, 1H), 2.96 – 2.81 (m, 4H), 2.76 (dd, *J* = 13.9, 10.5 Hz, 1H), 2.70 – 2.62 (m, 1H), 2.60 – 2.53 (m, 1H), 2.14 – 1.89 (m, 4H), 1.59 – 1.18 (m, 12H), 0.96 (d, *J* = 6.9 Hz, 3H), 0.85 – 0.73 (m, 24H).

^{13}C NMR (126 MHz, DMSO-D6) δ 171.82, 171.60, 171.53, 170.94, 170.73, 170.48, 170.40, 170.19, 170.15, 158.99, 140.00, 136.72, 136.60, 129.31, 129.27, 128.90, 128.21, 128.17, 126.74, 126.68, 121.48, 113.83, 112.48, 59.75, 59.71, 56.61, 55.91, 54.87, 53.74, 53.59, 53.34, 50.10, 50.04, 47.68, 45.78, 40.98, 40.64, 38.00, 35.92, 35.79, 31.87, 31.24, 29.08, 24.26, 24.16, 23.25, 22.67, 22.58, 22.56, 22.54, 19.38, 19.11, 18.15, 17.96, 17.02.

Note: Less than 63 peaks seen in the C13 NMR because some carbon resonances are coincidentally overlapping.

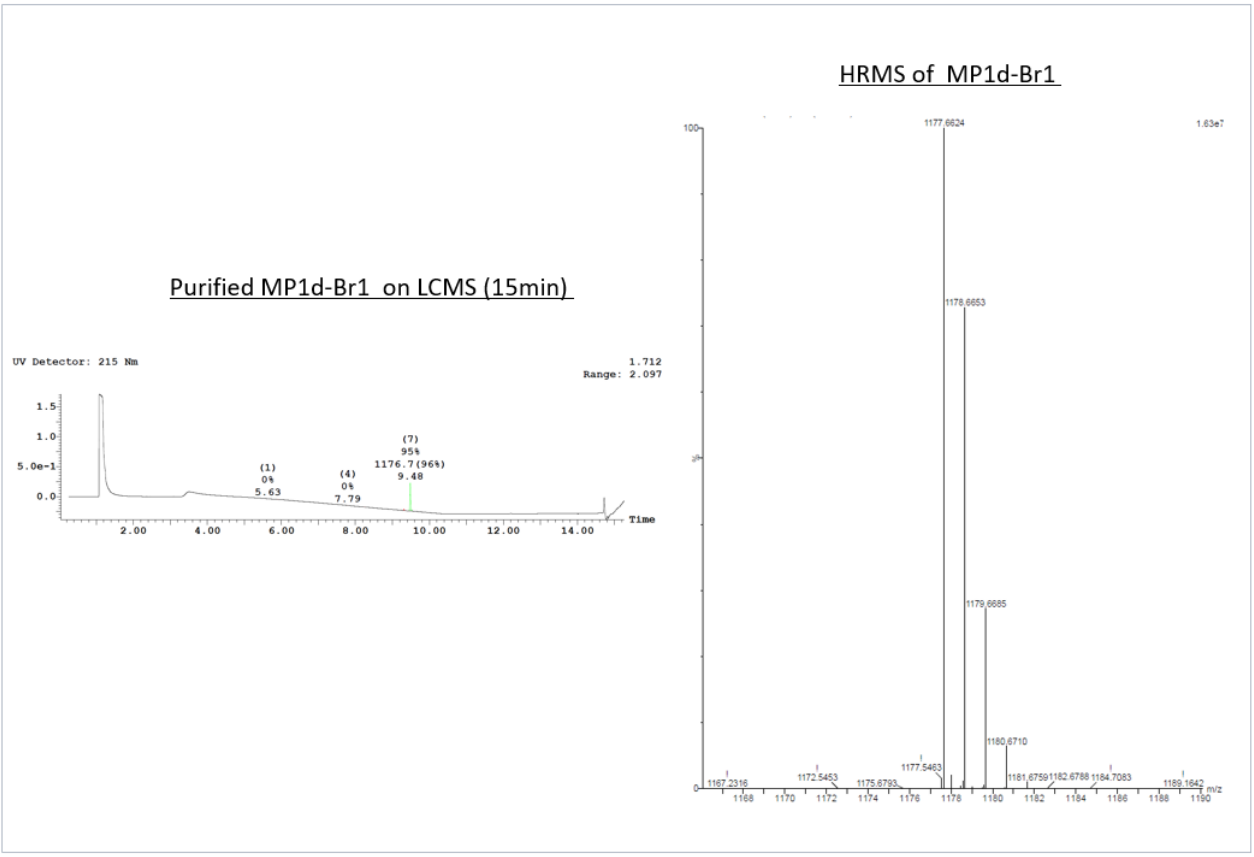
HRMS: calculated for $C_{63}H_{88}N_{10}O_{11}$: 1161.6707, observed 1161.6686.

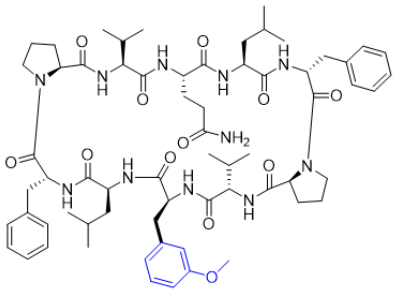




MP1d-Br-1: Prepared via General Procedure A in section 4 using **MP-Pyridinium-1d** (41 μmol). The crude mixture was purified by preparative HPLC Method-A (40-100 B in A) to give **MP1d-Br-1** (24.2 mg, 50%) as a white solid: ^{1}H NMR (500 MHz, DMSO) δ 8.99 (d, *J* = 3.6 Hz, 1H), 8.69 (dd, *J* = 16.3, 8.9 Hz, 2H), 8.49 (d, *J* = 8.7 Hz, 1H), 8.39 (d, *J* = 9.2 Hz, 1H), 8.13 (d, *J* = 8.8 Hz, 1H), 7.36 (d, *J* = 9.6 Hz, 1H), 7.30 – 7.09 (m, 11H), 6.83 – 6.78 (m, 2H), 6.74 (d, *J* = 8.7 Hz, 1H), 6.50 (d, *J* = 8.6 Hz, 1H), 5.33 (br, s, 1H), 5.02 (t, *J* = 8.3 Hz, 1H), 4.75 (q, *J* = 8.5 Hz, 1H), 4.56 – 4.47 (m, 2H), 4.43 – 4.24 (m, 6H), 3.68 (s, 3H), 3.61 (q, *J* = 8.4 Hz, 1H), 3.56 – 3.39 (m, 1H), 3.35 – 3.27 (m, 1H), 3.18 (d, *J* = 11.6 Hz, 1H), 2.99 – 2.71 (m, 5H), 2.48 – 2.42 (m, 1H), 2.12 (dp, *J* = 20.4, 6.8 Hz, 2H), 1.99 – 1.80 (m, 4H), 1.74 – 1.63 (m, 1H), 1.59 – 1.14 (m, 9H), 0.89 – 0.64 (m, 24H)

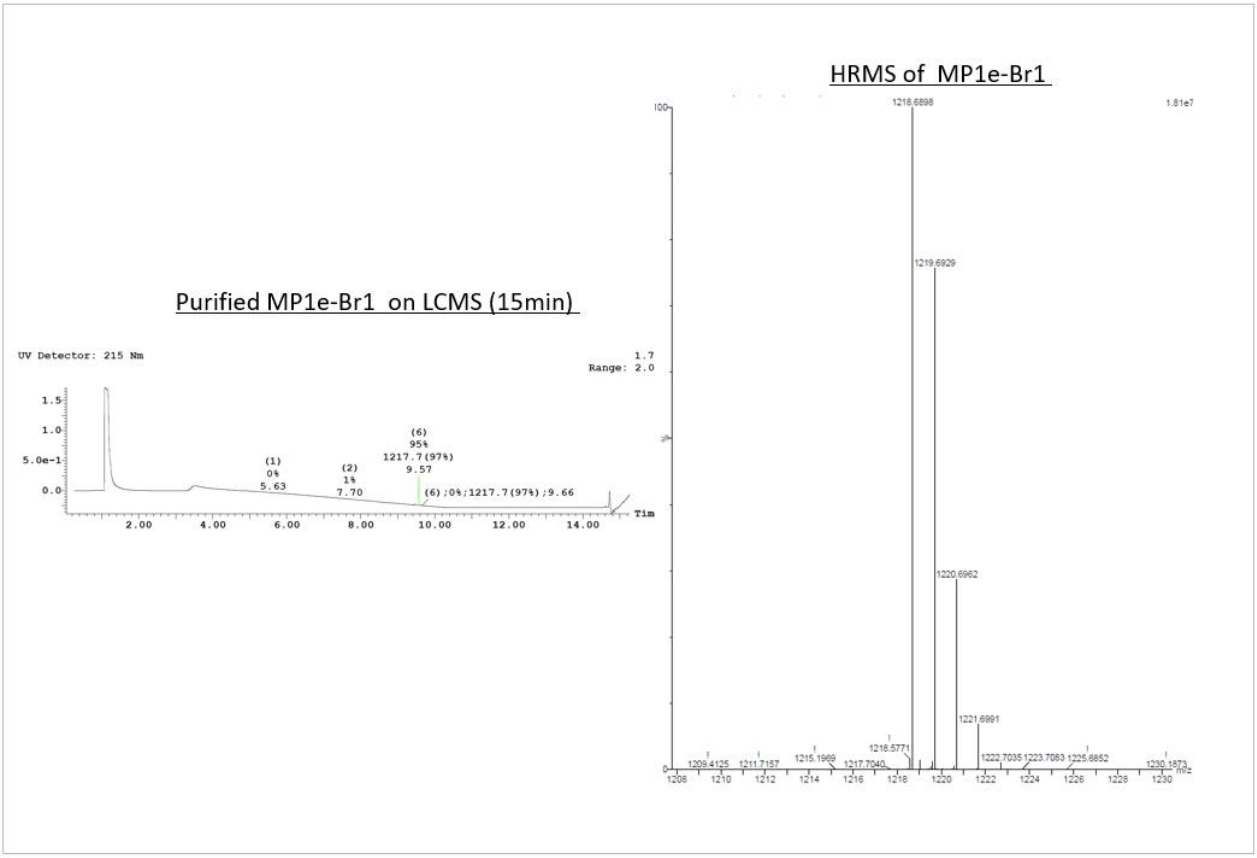
HRMS: calculated for $C_{63}H_{88}N_{10}O_{12}$: 1177.6656, observed 1177.6624.

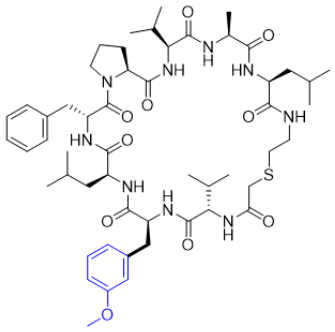




MP1e-Br-1: Prepared via General Procedure A in section 4 using **MP-Pyridinium-1e** (37 μmol). The crude mixture was purified by preparative HPLC Method-A (40-100 B in A) to give **MP1e-Br-1** (22.4 mg, 49%) as a white solid: ^{1}H NMR (500 MHz, DMSO) δ 8.86 (d, *J* = 3.8 Hz, 1H), 8.80 (d, *J* = 4.3 Hz, 1H), 8.63 (d, *J* = 8.6 Hz, 1H), 8.50 (t, *J* = 10.4 Hz, 2H), 8.36 (d, *J* = 9.0 Hz, 1H), 7.30 – 7.18 (m, 12H), 7.10 – 7.01 (m, 2H), 6.72 (s, 1H), 6.68 (d, *J* = 7.6 Hz, 1H), 6.62 (dd, *J* = 8.2, 2.0 Hz, 2H), 5.05 (t, *J* = 11.2 Hz, 1H), 4.57 (dd, *J* = 9.6, 4.9 Hz, 1H), 4.51 (p, *J* = 7.5 Hz, 2H), 4.41 – 4.32 (m, 4H), 4.27 – 4.17 (m, 2H), 3.65 (s, 3H), 3.64 – 3.58 (m, 2H), 3.05 (d, *J* = 11.7 Hz, 1H), 2.97 – 2.77 (m, 5H), 2.73 (dd, *J* = 13.8, 10.5 Hz, 1H), 2.56 (dd, *J* = 18.0, 8.9 Hz, 1H), 2.14 – 1.90 (m, 5H), 1.72 – 1.62 (m, 2H), 1.59 – 1.18 (m, 13H), 0.84 – 0.74 (m, 24H).

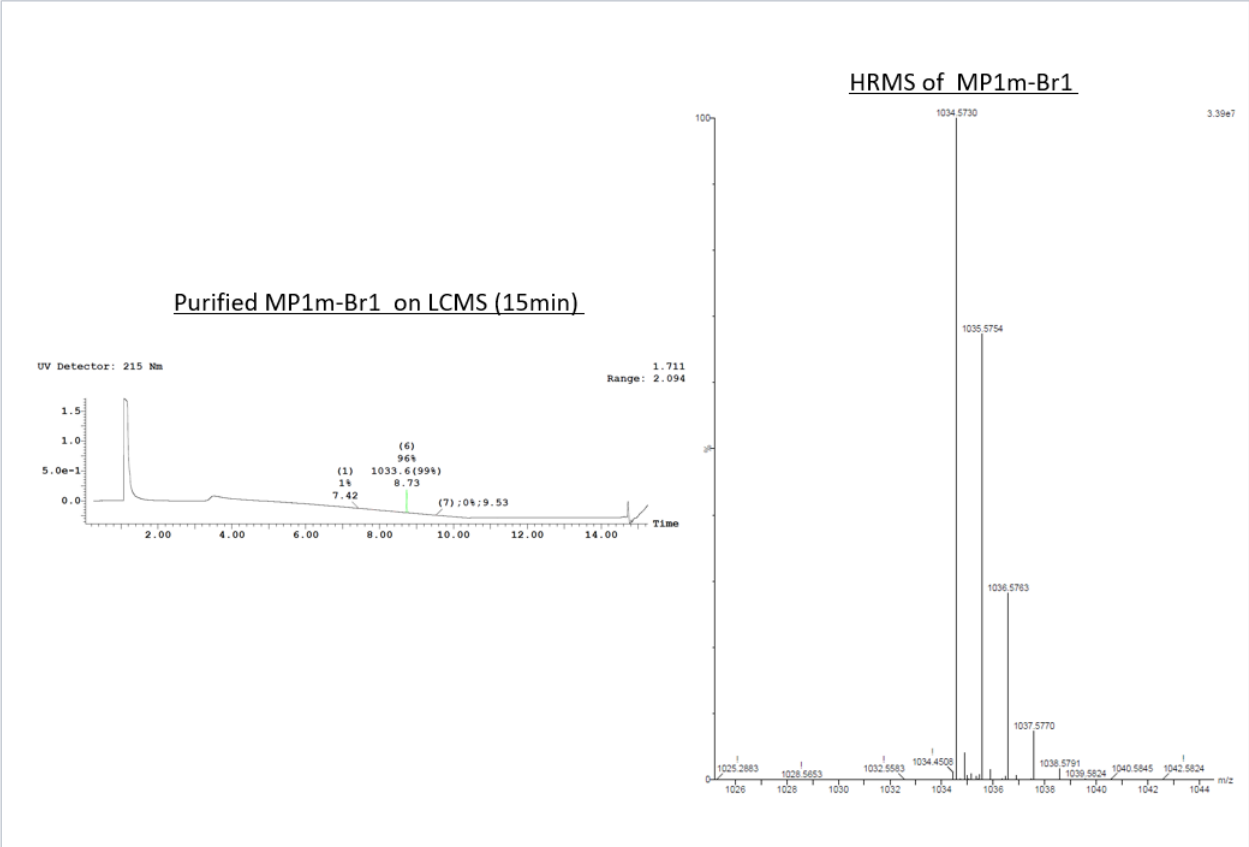
HRMS: calculated for $C_{65}H_{91}N_{11}O_{12}$: 1218.6922, observed 1218.6898.

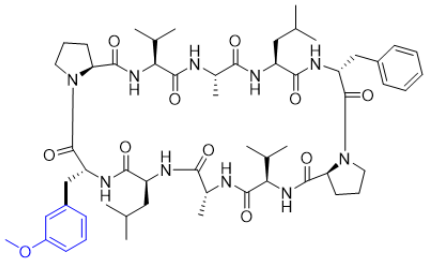




MP1m-Br-1: Prepared via General Procedure A in section 4 using **MP-Pyridinium-1m** (28 μmol). The crude mixture was purified by preparative HPLC Method-A (40-100 B in A) to give **MP1m-Br-1** (11.7 mg, 40%) as a white solid: ^{1}H NMR (500 MHz, DMSO) δ 8.74 (br s, 1H), 8.62 (d, *J* = 7.9 Hz, 1H), 8.52 (d, *J* = 7.7 Hz, 1H), 8.33 (d, *J* = 8.2 Hz, 1H), 8.06 (d, *J* = 8.4 Hz, 1H), 7.99 (br s, 1H), 7.63 (d, *J* = 8.3 Hz, 1H), 7.35 (d, *J* = 8.4 Hz, 1H), 7.30 – 7.19 (m, 4H), 7.09 (t, *J* = 7.5 Hz, 1H), 6.83 – 6.76 (m, 2H), 6.70 (br s, 1H), 6.53 (s, 1H), 6.53 (br s, 1H), 4.56 – 4.20 (m, 7H), 3.64 (s, 3H), 3.62-3.40 (m, 4H), 3.20-3.05 (m, 3H), 2.95-2.85 (m, 2H), 2.84 – 2.74 (m, 1H), 2.70-2.58 (m, 2H), 2.12-1.90 (m, 3H), 1.65-1.05 (m, 9H), 0.94 (d, *J* = 6.1 Hz, 3H), 0.86 – 0.73 (m, 24H).

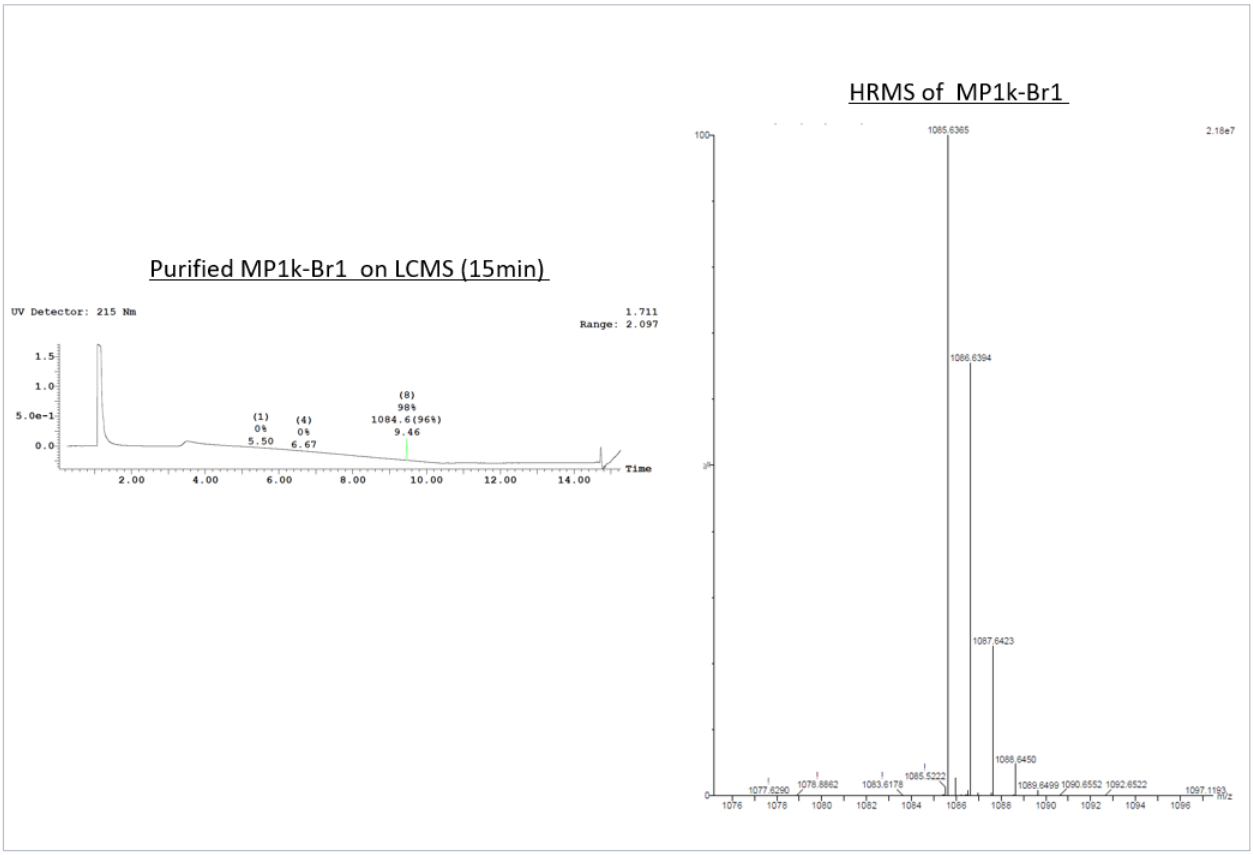
HRMS: calculated for $C_{53}H_{79}N_9O_{10}S$: 1034.5744, observed 1034.5730.

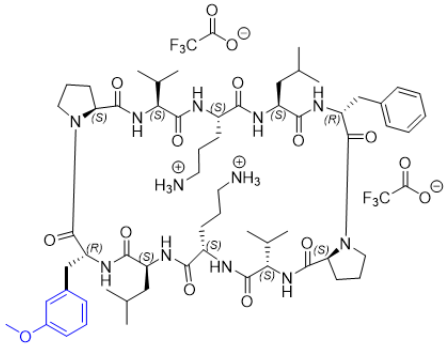




MP1k-Br-1: Prepared via General Procedure B in section 4 using **MP-Pyridinium-1k** (30μmol). The crude mixture was purified by preparative HPLC Method-A (30-100 B in A) to give **MP1k-Br-1** (16.1 mg, 50%) as a white solid: ^{1}H NMR (500 MHz, DMSO) δ 8.79 (dd, *J* = 9.8, 5.2 Hz, 2H), 8.55 (dd, *J* = 8.6, 4.1 Hz, 2H), 8.18 (dd, *J* = 8.3, 3.7 Hz, 2H), 7.30 – 7.07 (m, 8H), 6.83 – 6.77 (m, 3H), 4.76 (dq, *J* = 14.4, 6.8 Hz, 2H), 4.49 – 4.36 (m, 6H), 4.33-4.28 (m, 2H), 3.72 (s, 3H), 3.63 – 3.55 (m, 2H), 2.88 (dd, *J* = 12.4, 7.8 Hz, 4H), 2.79 – 2.69 (m, 2H), 2.05-1.91 (m, 4H), 1.67-1.53 (m, 6H), 1.45-1.31 (m, 6H), 1.23 (dd, *J* = 6.7, 3.4 Hz, 6H), 0.83 – 0.73 (m, 24H).

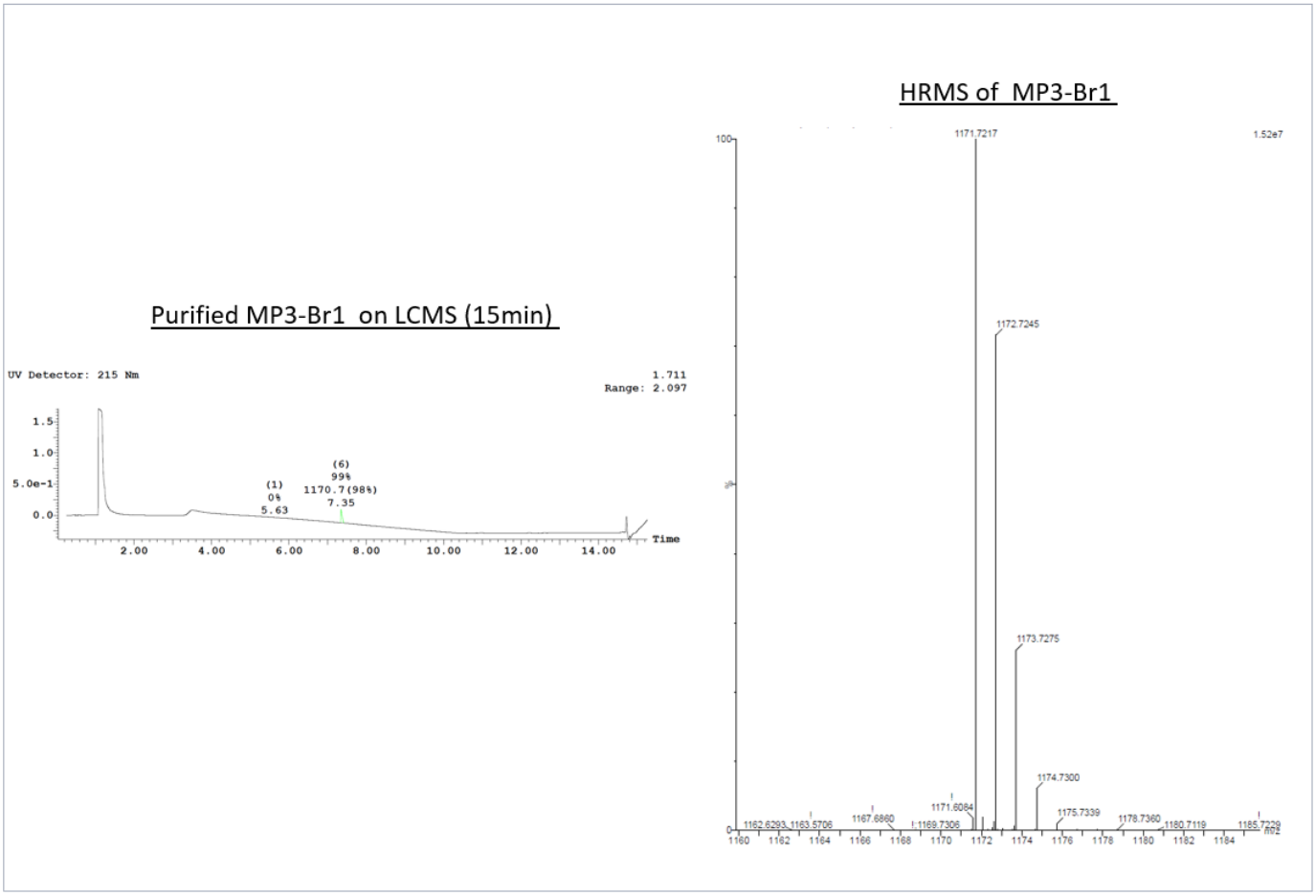
HRMS: calculated for $C_{57}H_{84}N_{10}O_{11}$: 1085.6394, observed 1085.6365.

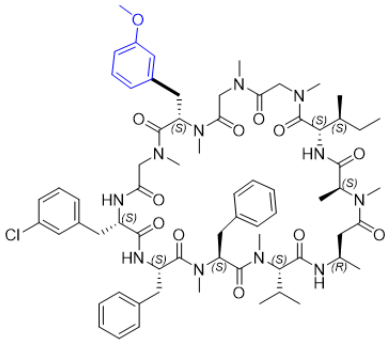




MP3-Br-1: Prepared via General Procedure B in section 4 using **MP-Pyridinium-1n** (31 μmol). The crude mixture was purified by preparative HPLC Method-B (46-61 B in A) to give **MP1n-Br-1** (9 mg, 21%) as a white solid: ^{1}H NMR (500 MHz, DMSO) δ 9.09 (s, 2H), 8.74 (d, *J* = 9.1 Hz, 2H), 8.35 (d, *J* = 7.4 Hz, 2H), 7.69 (s, 6H), 7.33 – 7.19 (m, 6H), 6.86 – 6.78 (m, 3H), 6.54 (s, 2H), 4.82 – 4.72 (m, 2H), 4.59 (q, *J* = 7.8 Hz, 2H), 4.44 – 4.34 (m, 4H), 4.33-4.29 (m, 2H), 3.73 (s, 3H), 3.65-3.57 (m, 2H), 2.97 (ddd, *J* = 17.5, 12.8, 5.2 Hz, 2H), 2.90 – 2.69 (m, 6H), 2.55-2.41 (m, 2H), 2.09 (dq, *J* = 13.5, 6.7 Hz, 2H), 2.02-1.92 (m, 2H), 1.82-1.72 (m, 2H), 1.67 – 1.26 (m, 18H), 0.85 – 0.75 (m, 24H).

HRMS: calculated for $C_{61}H_{94}N_{12}O_{11}$: 1171.7238, observed 1171.7217.

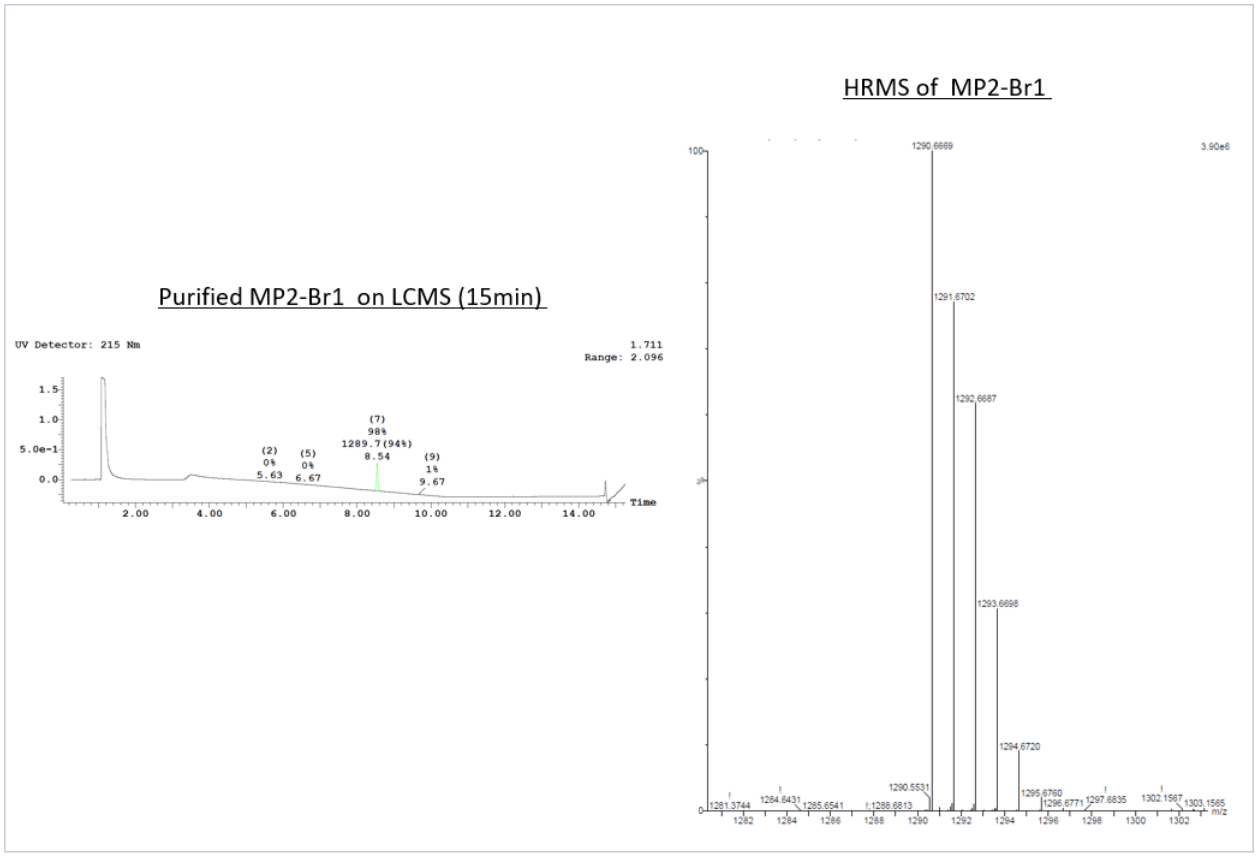




MP2-Br-1: Prepared via General Procedure A in section 4 using **MP-Pyridinium-2** (44 µmol). The crude mixture was purified by preparative HPLC Method-A (30-100 B in A) to give **MP2-Br-1** (22.2 mg, 39%) as a white solid:

Note : ^{1}H NMR spectrum is included without integration because presence of multiple rotamers making integration un-interpretable.

HRMS: calculated for $C_{68}H_{92}ClN_{11}O_{12}$: 1290.6688, observed 1290.6669.



5 References

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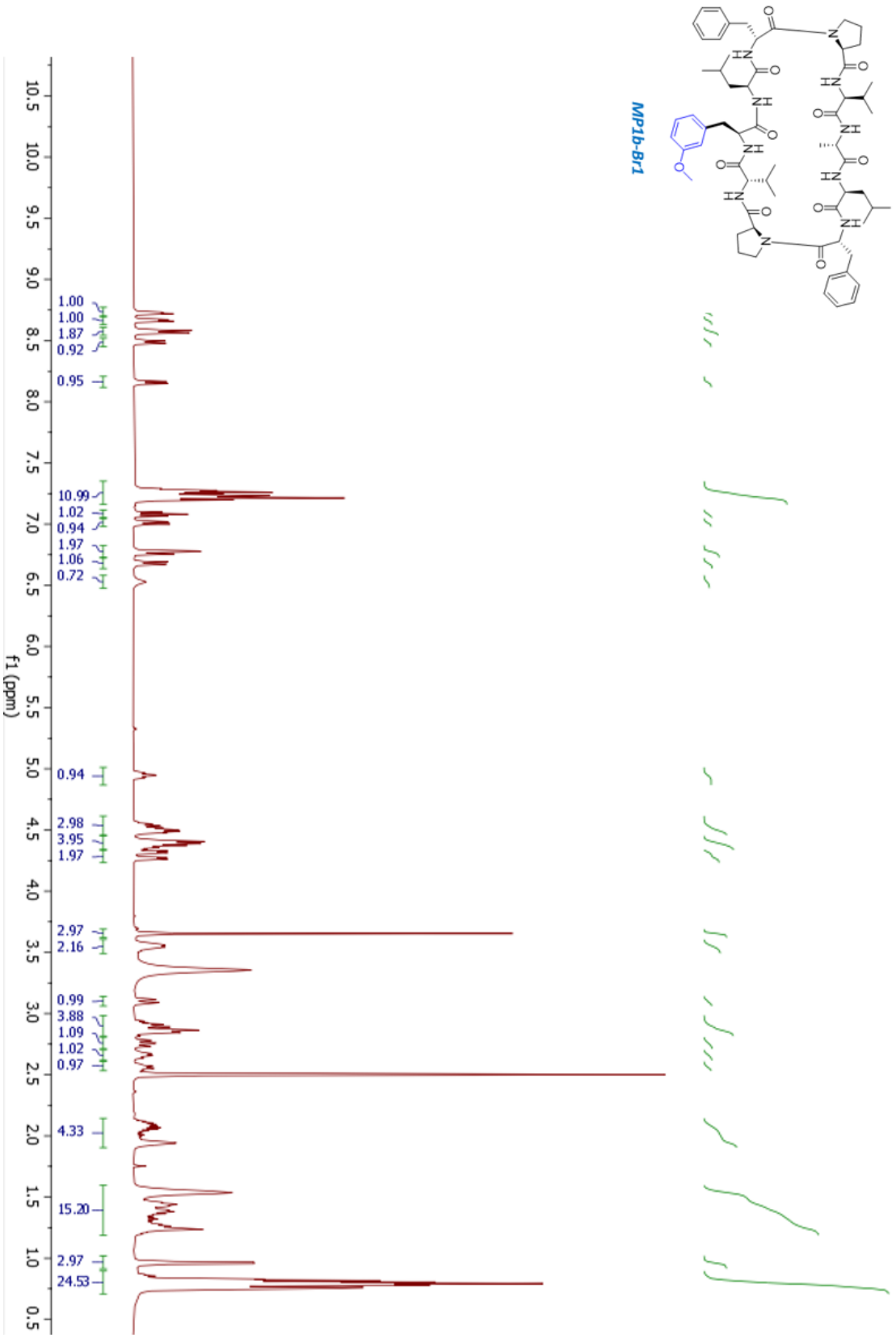
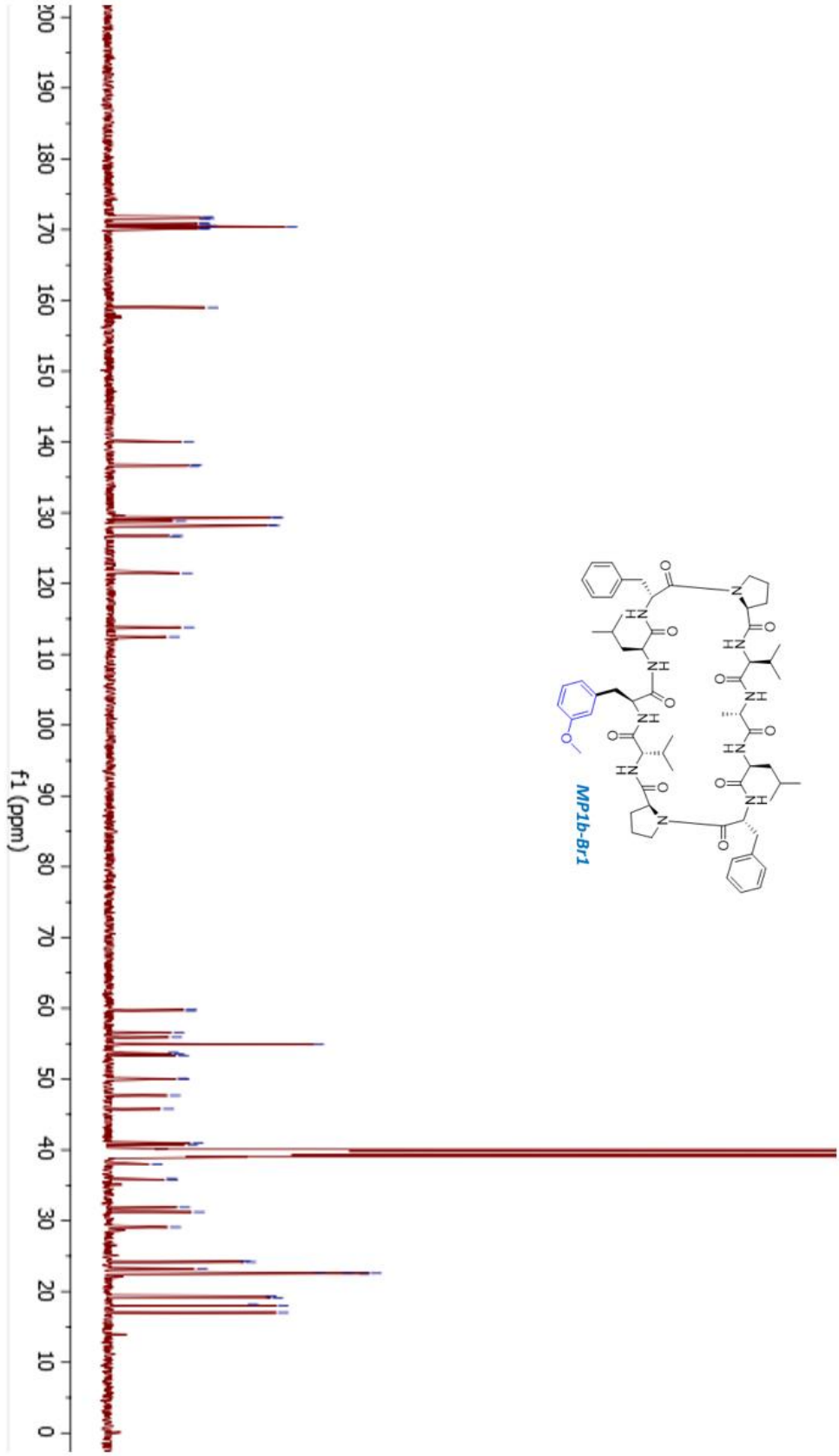
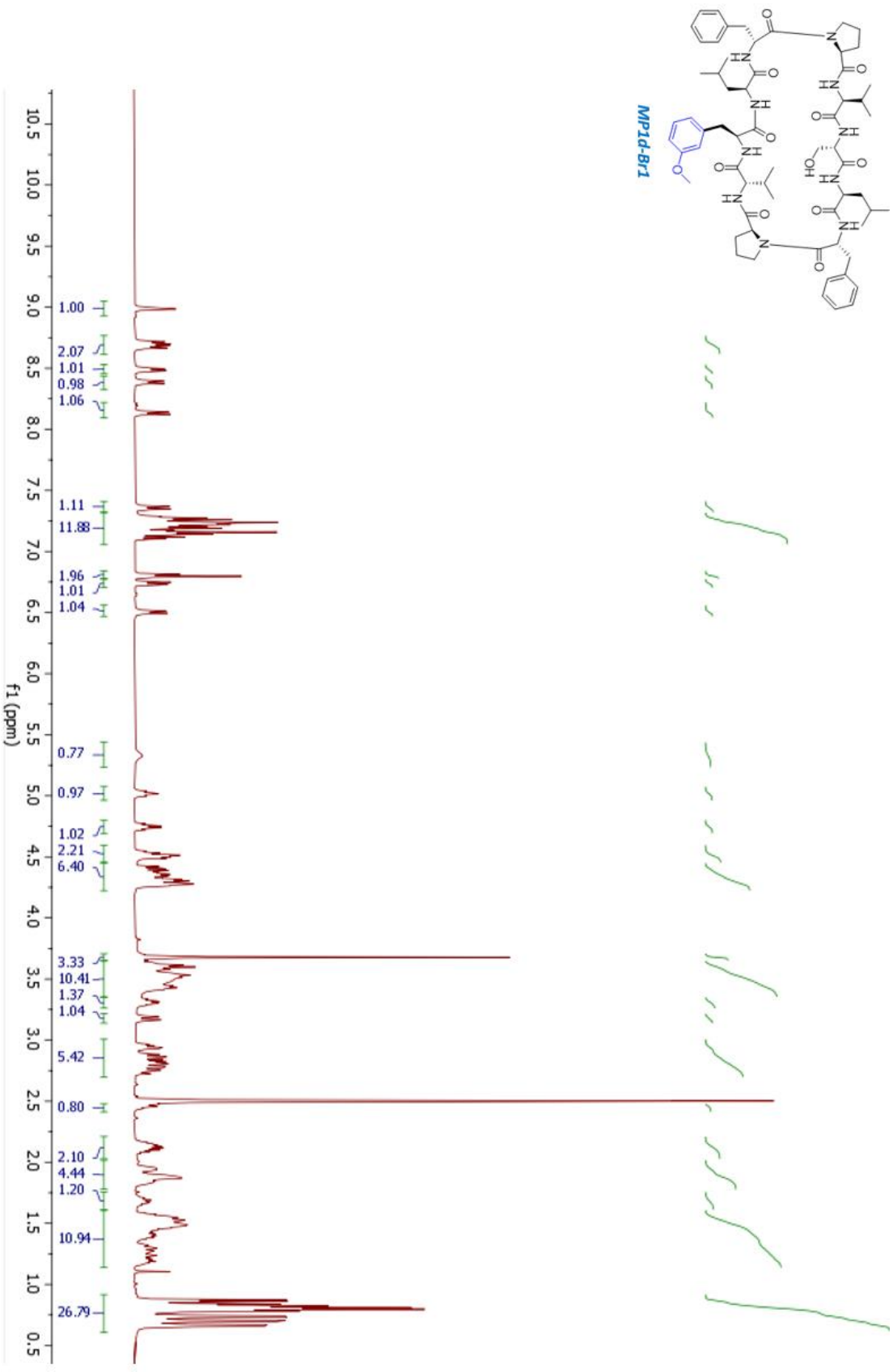
2- Jayanta Chatterjee, Burkhardt Laufer, Horst Kessler. Synthesis of N-Methylated cyclic peptides. Nature protocols, 2012, Vol.7, No.3, 432-444.

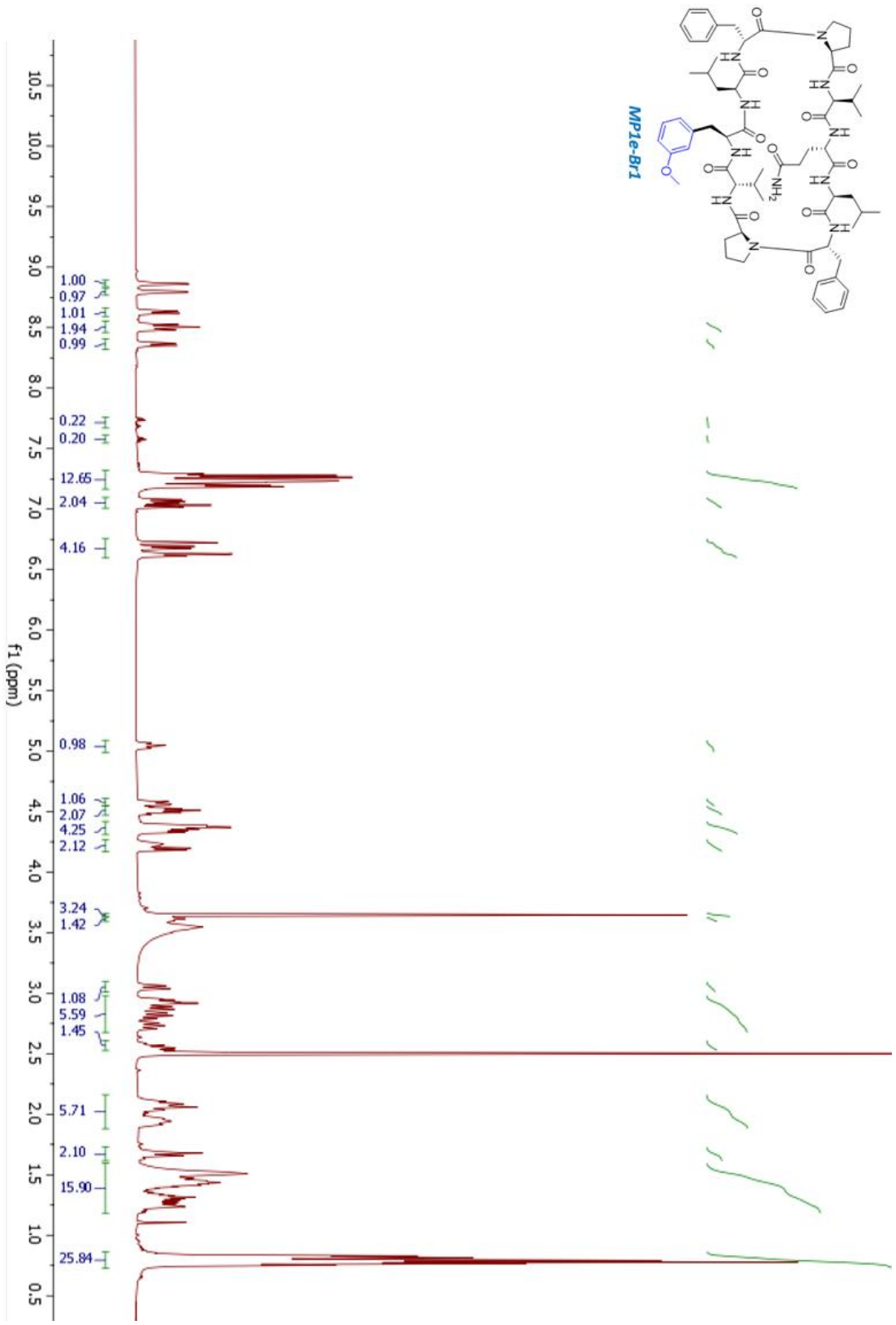
3- Scikit-learn: Machine Learning in Python, Pedregosa et al., JMLR 12, pp. 2825-2830, 2011.

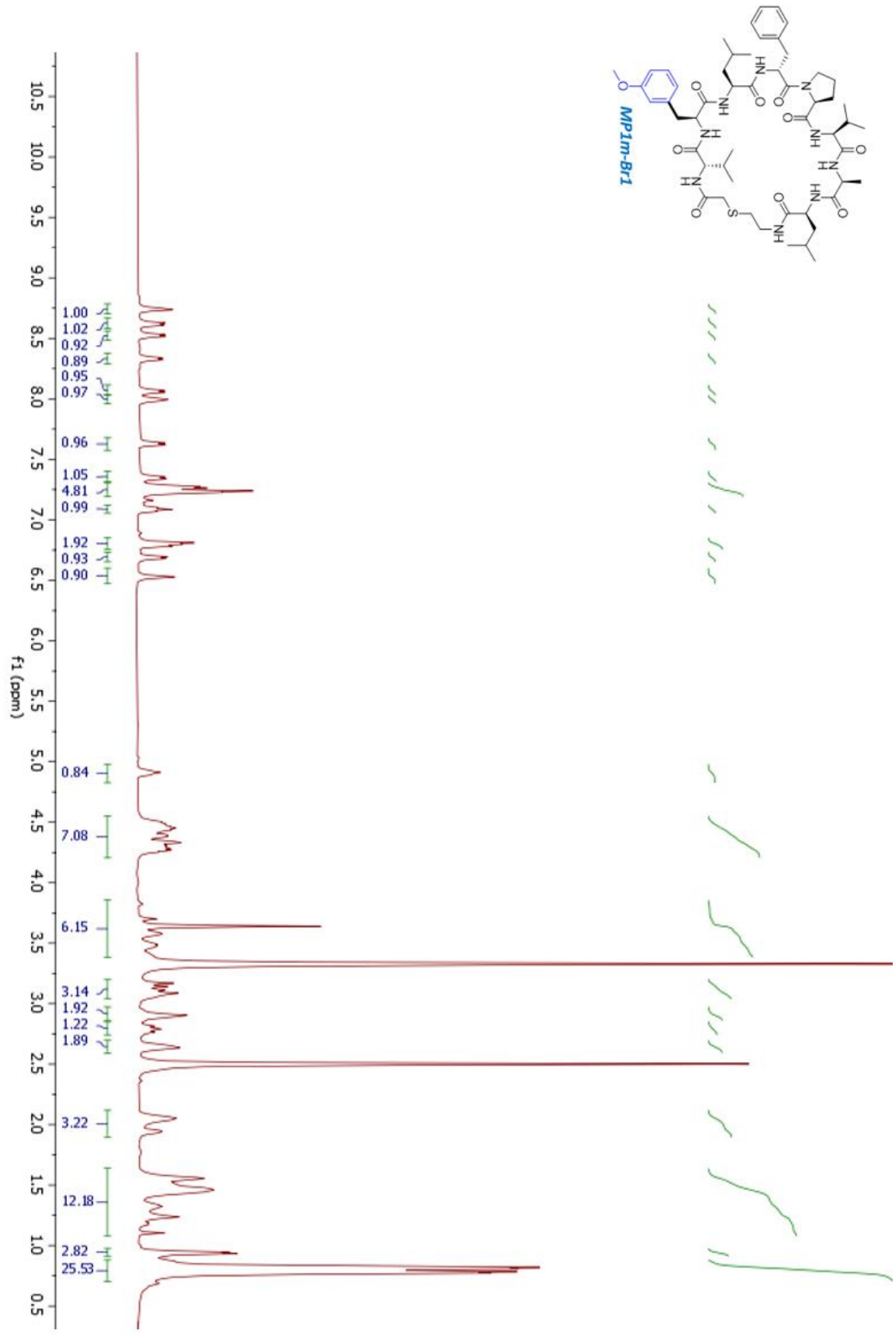
4- RDKit: Open-source cheminformatics. https://www.rdkit.org.

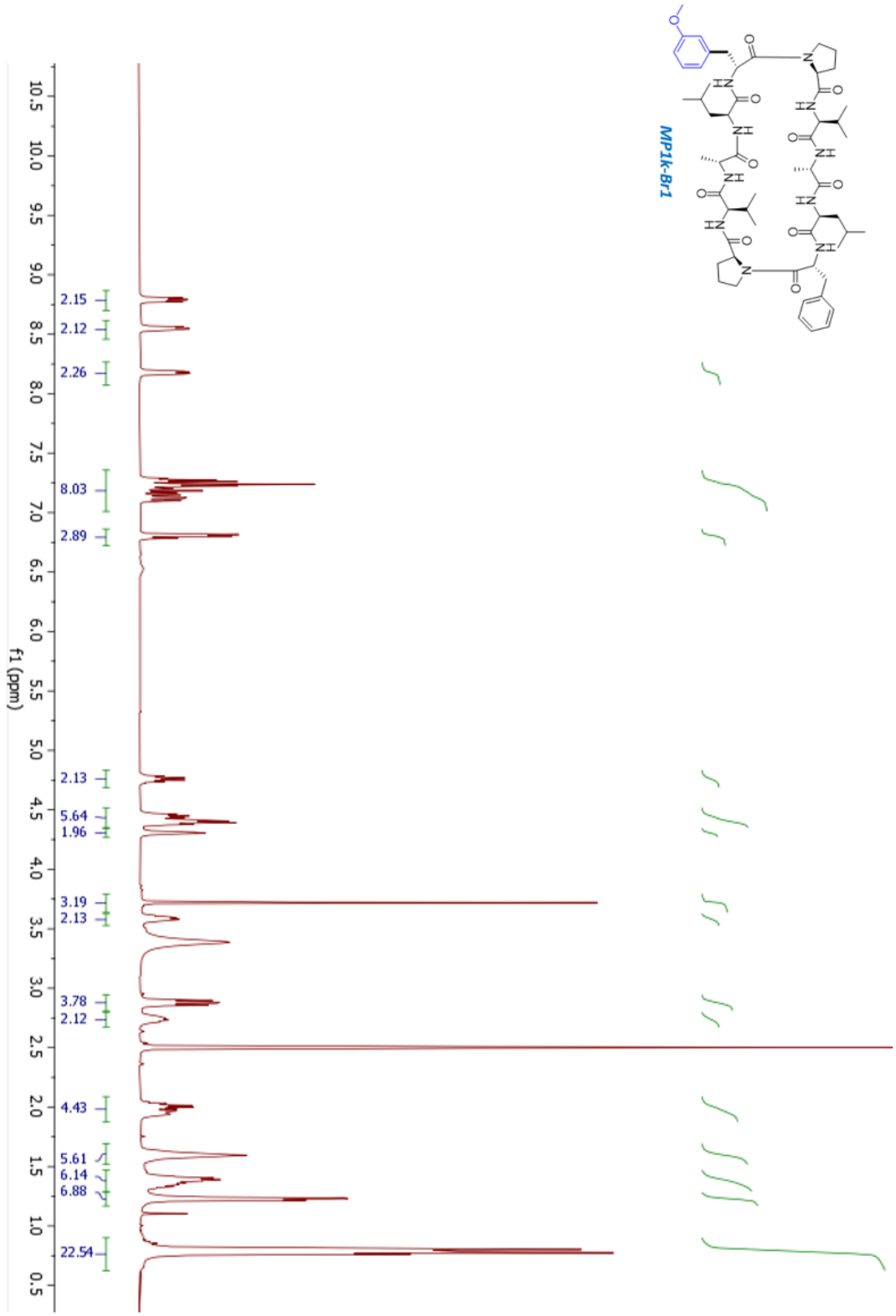

MP1b-Br1
f1 (ppm)
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
1.00
1.00
1.87
0.92
0.95
10.99
1.02
0.94
1.97
1.06
0.72
0.94
2.98
3.95
1.97
2.97
2.16
0.99
3.88
1.09
1.02
0.97
4.33
15.20
2.97
24.53


MP1b-Br1
f1 (ppm)
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0


MP1d-Br1
f1 (ppm)
10.5
10.0
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
1.00
2.07
1.01
0.98
1.06
1.11
11.88
1.96
1.01
1.04
0.77
0.97
1.02
2.21
6.40
3.33
10.41
1.37
1.04
5.42
0.80
2.10
4.44
1.20
10.94
26.79


MP1e-Br1
f1 (ppm)
10.5
10.0
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
1.00
0.97
1.01
1.94
0.99
0.22
0.20
12.65
2.04
4.16
0.98
1.06
2.07
4.25
2.12
3.24
1.42
1.08
5.59
1.45
5.71
2.10
15.90
25.84


MP1m-Br1
f1 (ppm)
10.5
10.0
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
1.00
1.02
0.92
0.89
0.95
0.97
0.96
1.05
4.81
0.99
1.92
0.93
0.90
0.84
7.08
6.15
3.14
1.92
1.22
1.89
3.22
12.18
2.82
25.53


MP1k-Br1
f1 (ppm)
2.15
2.12
2.26
8.03
2.89
2.13
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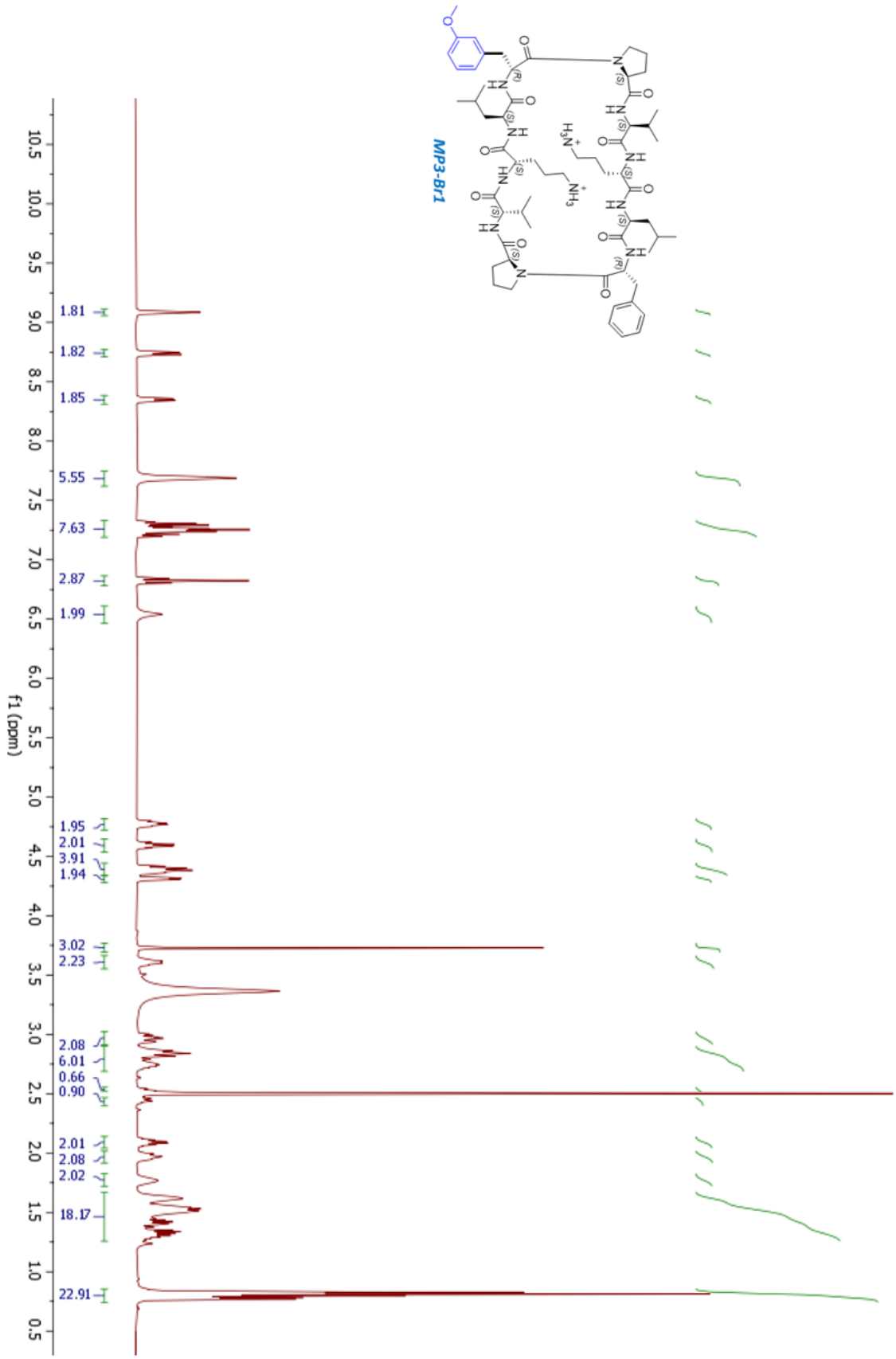
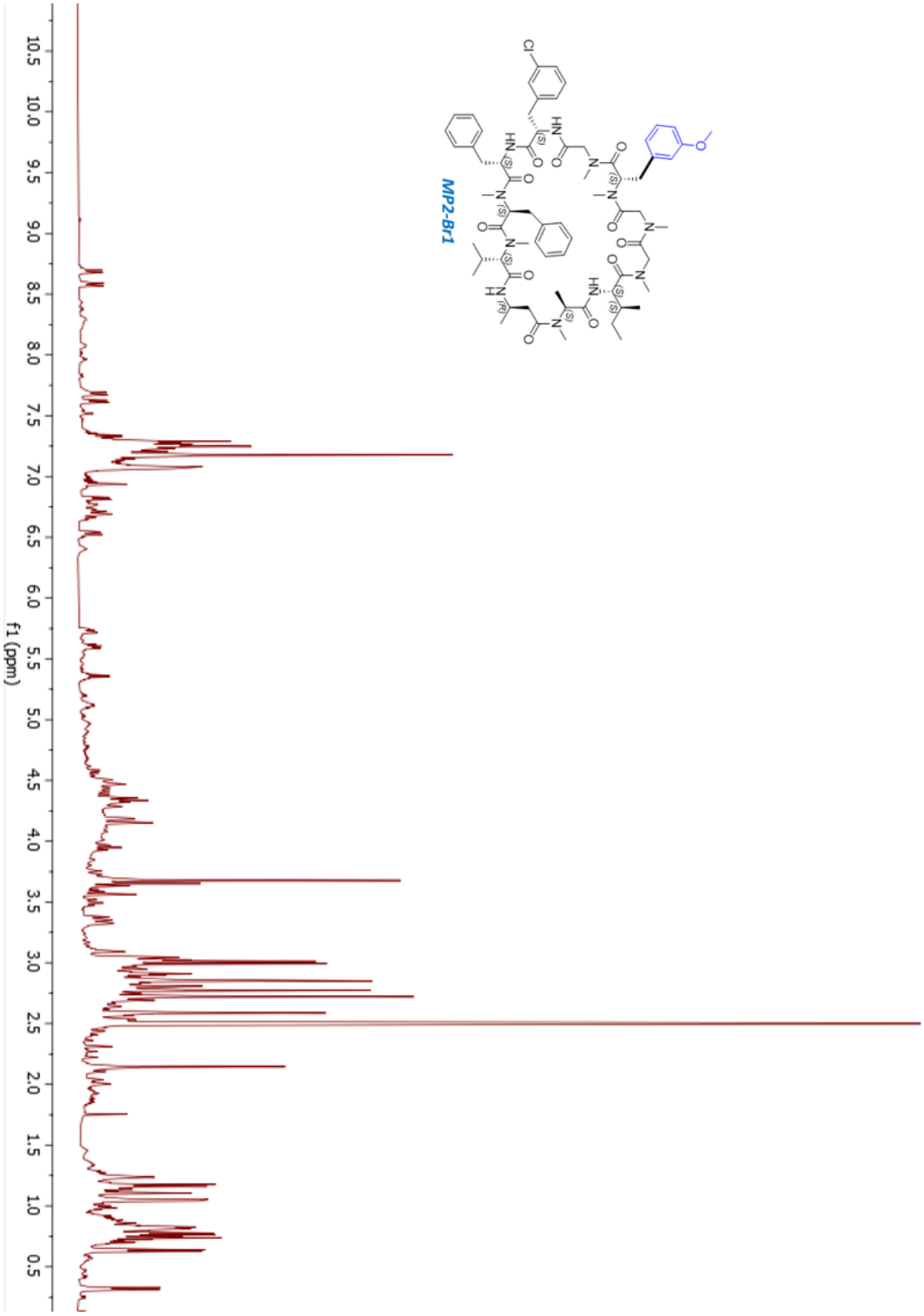
1.96
3.19
2.13
3.78
2.12
4.43
5.61
6.14
6.88
22.54


MP3-Br1
f1 (ppm)
1.81
1.82
1.85
5.55
7.63
2.87
1.99
1.95
2.01
3.91
1.94
3.02
2.23
2.08
6.01
0.66
0.90
2.01
2.08
2.02
18.17
22.91


MP2-Br1
f1 (ppm)
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10.0
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