Electronic Supplementary Information

Selective Monoalkylation of Primary Amines on Solid Phase: A New Route to Structurally Diverse Peptoid Tertiary Amides

Kevin Pels and Thomas Kodadek

Departments of Chemistry and Cancer Biology

The Scripps Research Institute

130 Scripps Way, Jupiter, FL 33458

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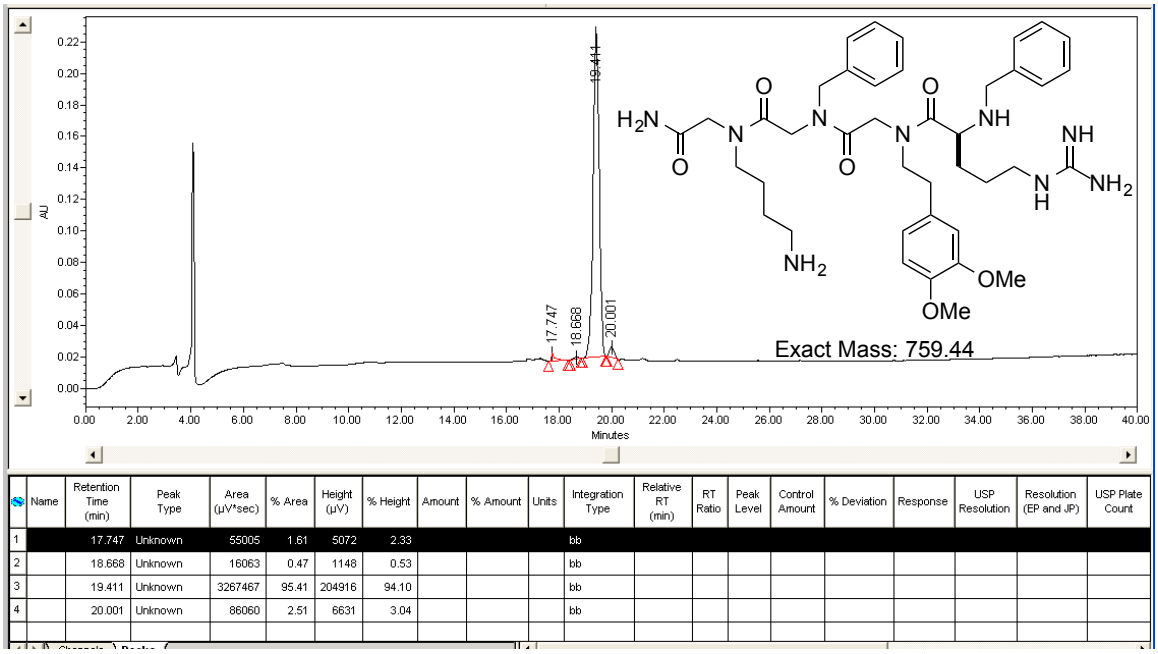
HPLC chromatograms for compounds **1a-l**

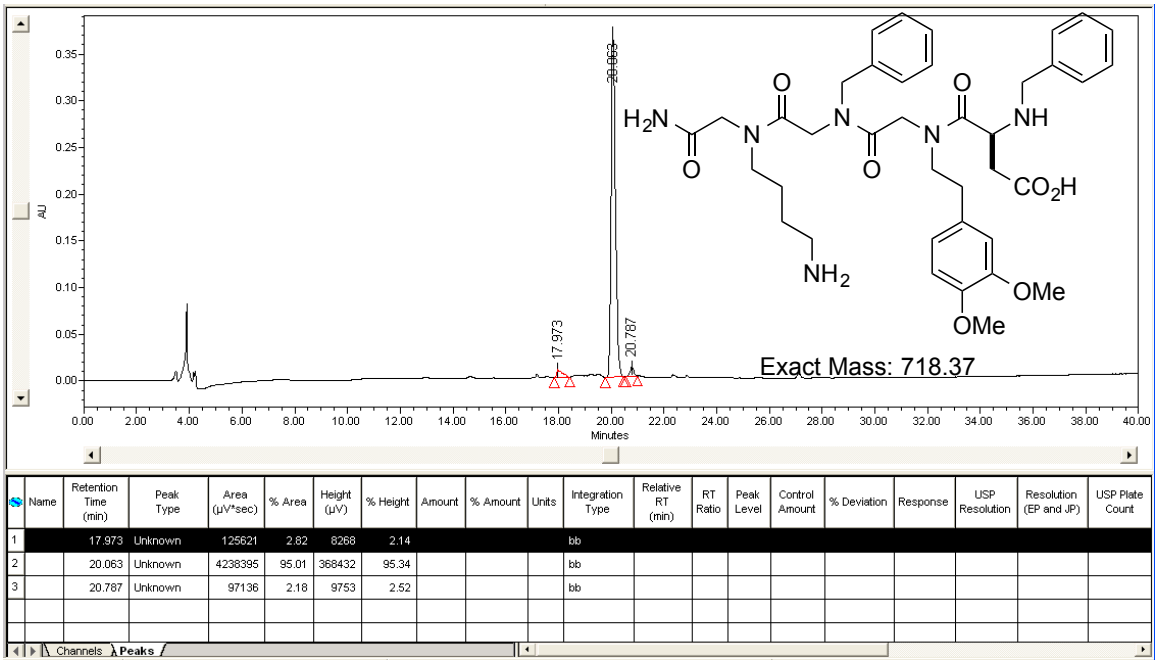
HPLC chromatograms are shown. In each case the major peak is the compound indicated, as confirmed by MALDI-TOF.

1a



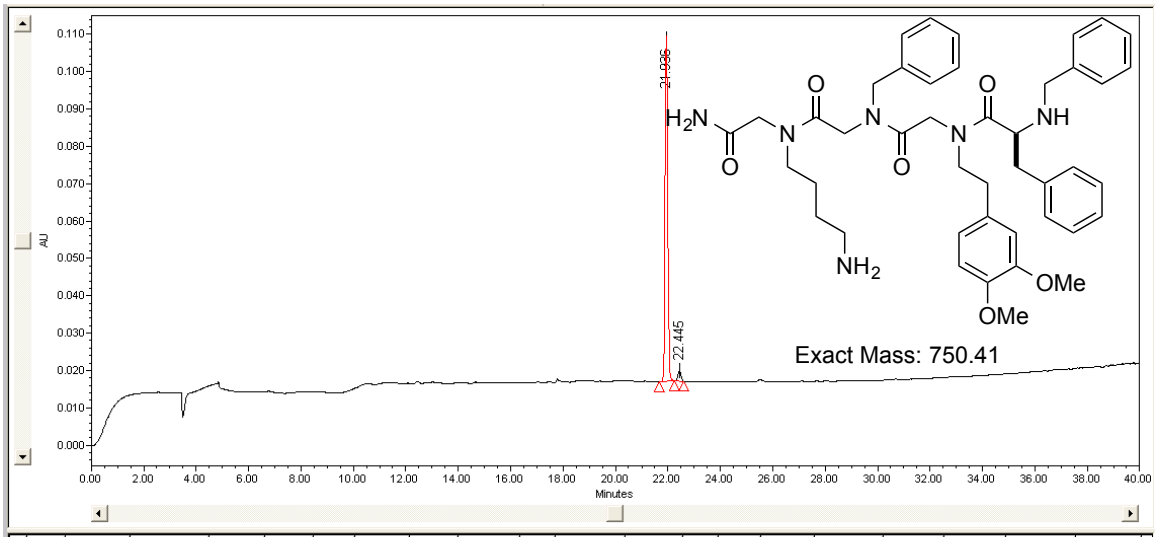
	Name	Retention Time (min)	Peak Type	Area (µV*sec)	% Area	Height (µV)	% Height	Amount	% Amount	Units	Integration Type	Relative RT (min)	RT Ratio	Peak Level	Control Amount	% Deviation	Response	USP Resolution	Resolution (EP and JP)	USP Plate Count
1		17.747	Unknown	55005	1.61	5072	2.33				bb									
2		18.668	Unknown	16063	0.47	1148	0.53				bb									
3		19.411	Unknown	3267467	95.41	204916	94.10				bb									
4		20.001	Unknown	86060	2.51	6631	3.04				bb									

1b



	Name	Retention Time (min)	Peak Type	Area (µV*sec)	% Area	Height (µV)	% Height	Amount	% Amount	Units	Integration Type	Relative RT (min)	RT Ratio	Peak Level	Control Amount	% Deviation	Response	USP Resolution	Resolution (EP and JP)	USP Plate Count
1		17.973	Unknown	125621	2.82	8268	2.14				bb									
2		20.063	Unknown	4238395	95.01	368432	95.34				bb									
3		20.787	Unknown	97136	2.18	9753	2.52				bb									

1c



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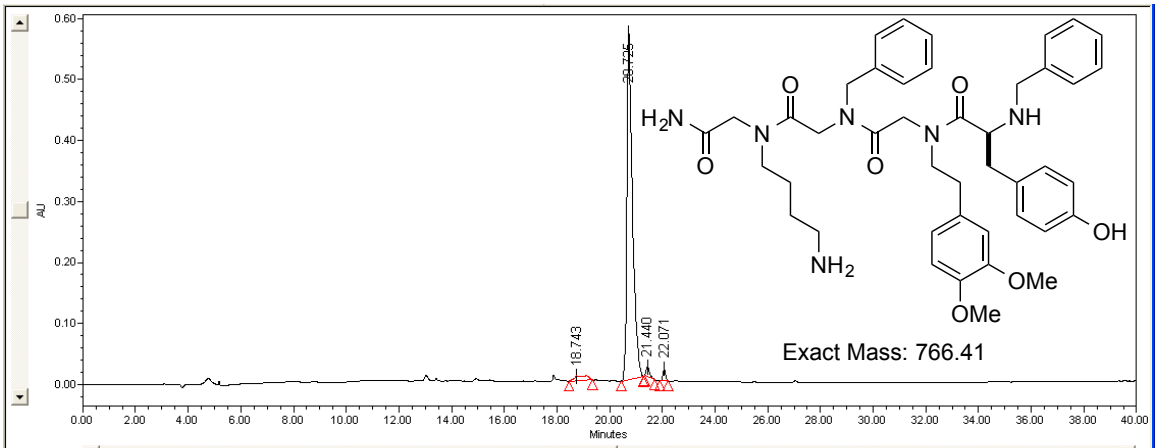
1		21.936	Unknown	663936	96.55	92284	96.83				bb									
2		22.445	Unknown	23726	3.45	3023	3.17				bb									

Channels / Peaks

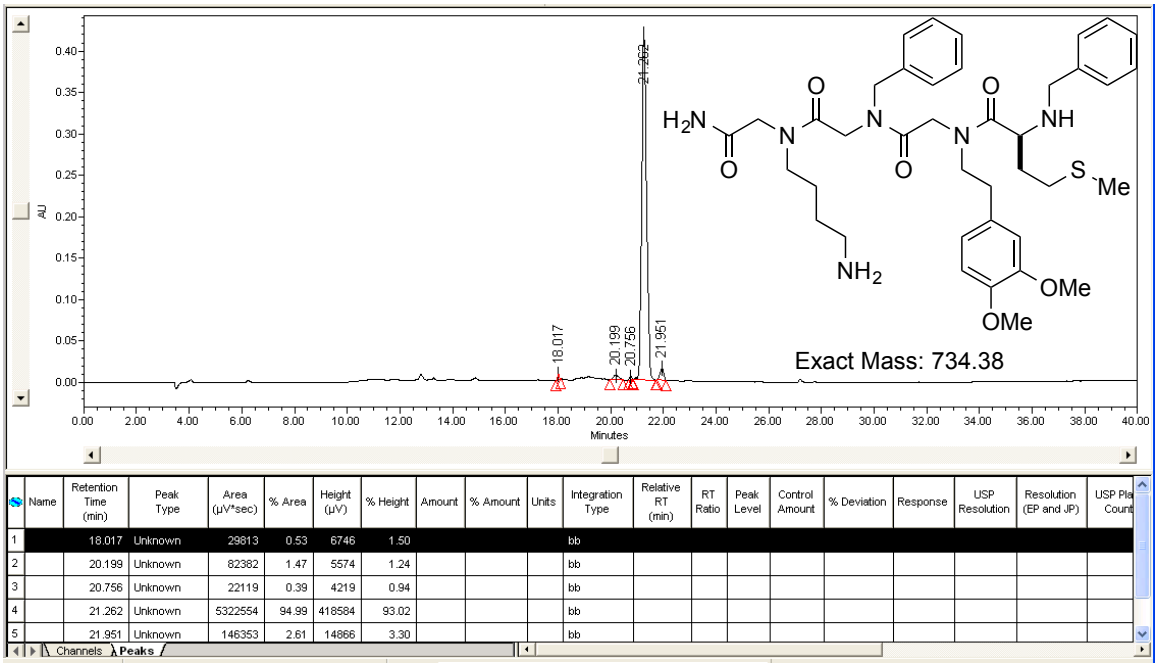
1d



	Name	Retention Time (min)	Peak Type	Area (µV*sec)	% Area	Height (µV)	% Height	Amount	% Amount	Units	Integration Type	Relative RT (min)	RT Ratio	Peak Level	Control Amount	% Deviation	Response	USP Resolution	Resolution (EP and JP)	USP Plate Count
1		18.743	Unknown	257982	2.93	8130	1.31				bb									
2		20.725	Unknown	8257611	93.82	573348	92.52				bb									
3		21.440	Unknown	148976	1.69	17041	2.75				bb									
4		22.071	Unknown	137254	1.56	21179	3.42				bb									

Channels / Peaks

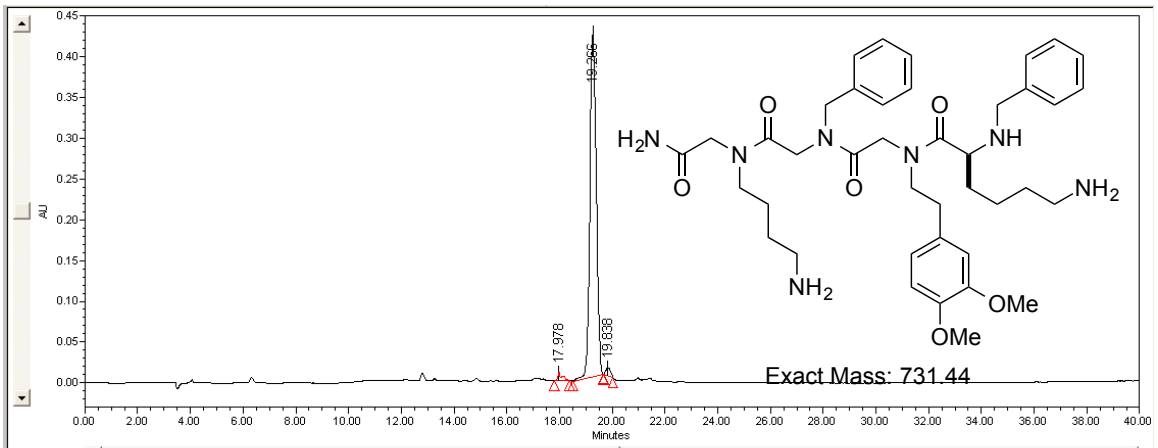
1e



	Name	Retention Time (min)	Peak Type	Area (µV*sec)	% Area	Height (µV)	% Height	Amount	% Amount	Units	Integration Type	Relative RT (min)	RT Ratio	Peak Level	Control Amount	% Deviation	Response	USP Resolution	Resolution (EP and JP)	USP Pla Count
1		18.017	Unknown	29813	0.53	6746	1.50				bb									
2		20.199	Unknown	82382	1.47	5574	1.24				bb									
3		20.756	Unknown	22119	0.39	4219	0.94				bb									
4		21.262	Unknown	5322554	94.99	418584	93.02				bb									
5		21.951	Unknown	146353	2.61	14866	3.30				bb									

Channels | Peaks

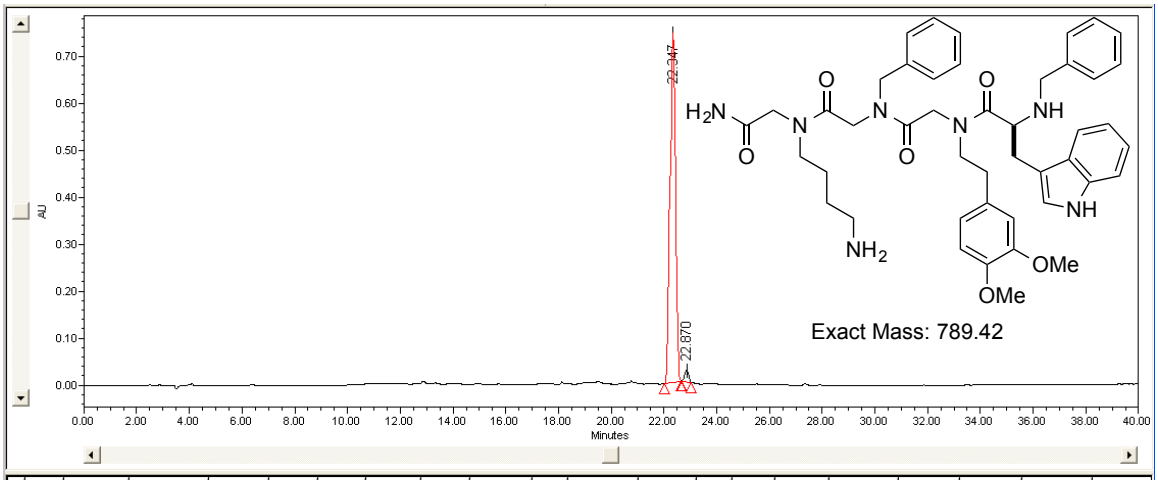
1f



	Name	Retention Time (min)	Peak Type	Area (µV*sec)	% Area	Height (µV)	% Height	Amount	% Amount	Units	Integration Type	Relative RT (min)	RT Ratio	Peak Level	Control Amount	% Deviation	Response	USP Resolution	Resolution (EP and JP)	USP Plate Count
1		17.978	Unknown	109305	1.51	9511	2.16				bb									
2		19.266	Unknown	7025366	96.85	420518	95.64				bb									
3		19.838	Unknown	118841	1.64	9642	2.19				bb									

Channels | Peaks

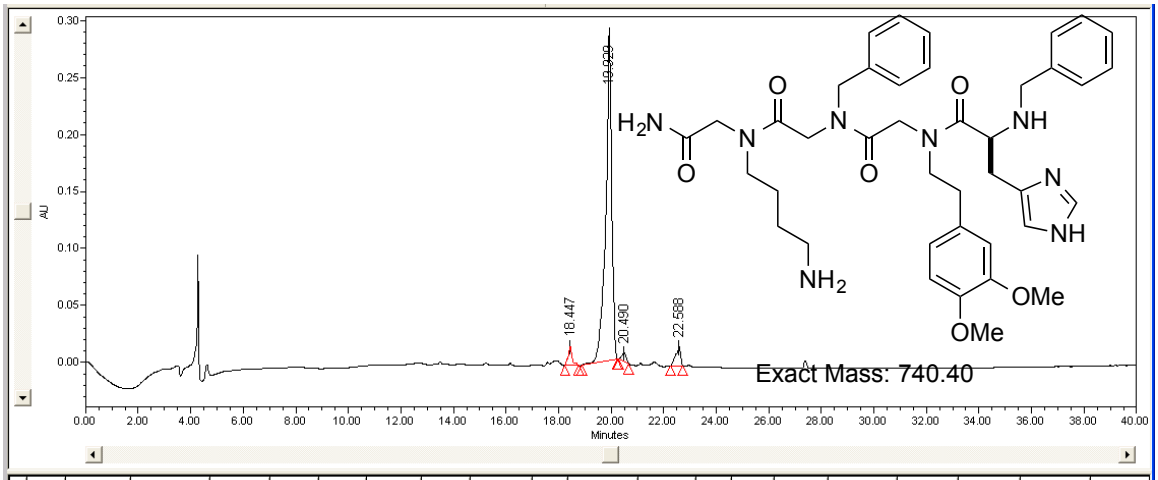
1g



	Name	Retention Time (min)	Peak Type	Area (µV*sec)	% Area	Height (µV)	% Height	Amount	% Amount	Units	Integration Type	Relative RT (min)	RT Ratio	Peak Level	Control Amount	% Deviation	Response	USP Resolution	Resolution (EP and JP)	USP Plate Count
1		22.347	Unknown	10645000	97.62	742373	96.80				bb									
2		22.870	Unknown	259491	2.38	24575	3.20				bb									

Channels | Peaks

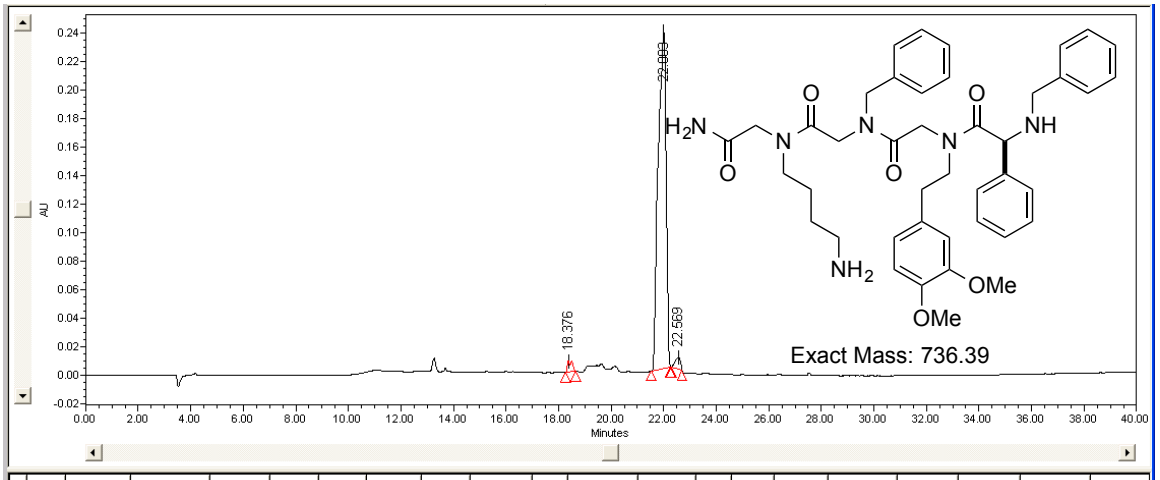
1h



	Name	Retention Time (min)	Peak Type	Area (µV*sec)	% Area	Height (µV)	% Height	Amount	% Amount	Units	Integration Type	Relative RT (min)	RT Ratio	Peak Level	Control Amount	% Deviation	Response	USP Resolution	Resolution (EP and JP)	USP Plate Count
1		18.447	Unknown	168586	3.37	16410	5.02				bb									
2		19.929	Unknown	4521224	90.50	286186	87.59				bb									
3		20.490	Unknown	91138	1.82	7640	2.34				bb									
4		22.588	Unknown	215000	4.30	16506	5.05				bb									

Channels | Peaks

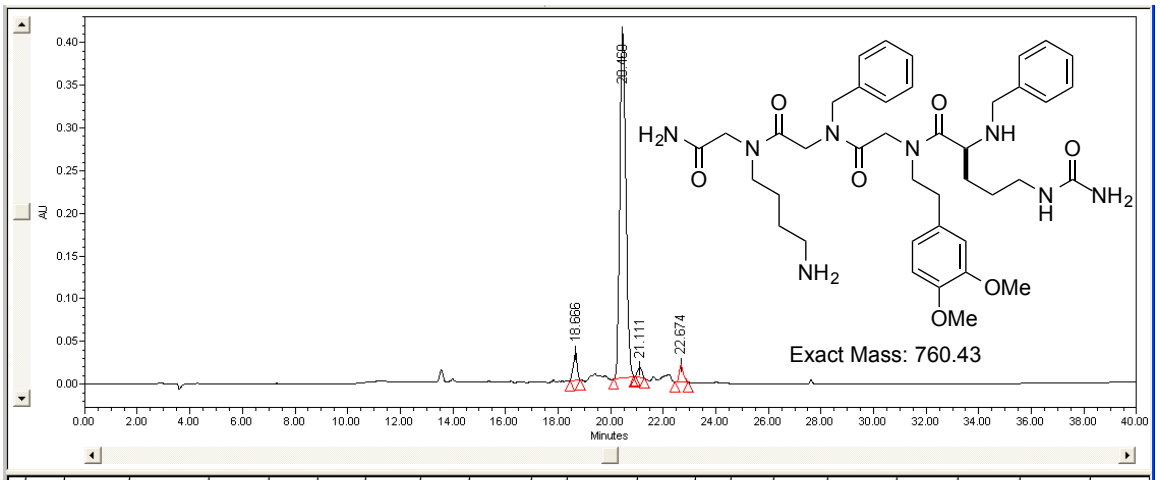
1i



	Name	Retention Time (min)	Peak Type	Area (µV*sec)	% Area	Height (µV)	% Height	Amount	% Amount	Units	Integration Type	Relative RT (min)	RT Ratio	Peak Level	Control Amount	% Deviation	Response	USP Resolution	Resolution (EP and JP)	USP Plate Count
1		18.376	Unknown	94302	1.83	7947	3.15				bb									
2		22.003	Unknown	4958355	95.96	235703	93.47				bb									
3		22.569	Unknown	114343	2.21	8509	3.37				bb									

Channels | Peaks

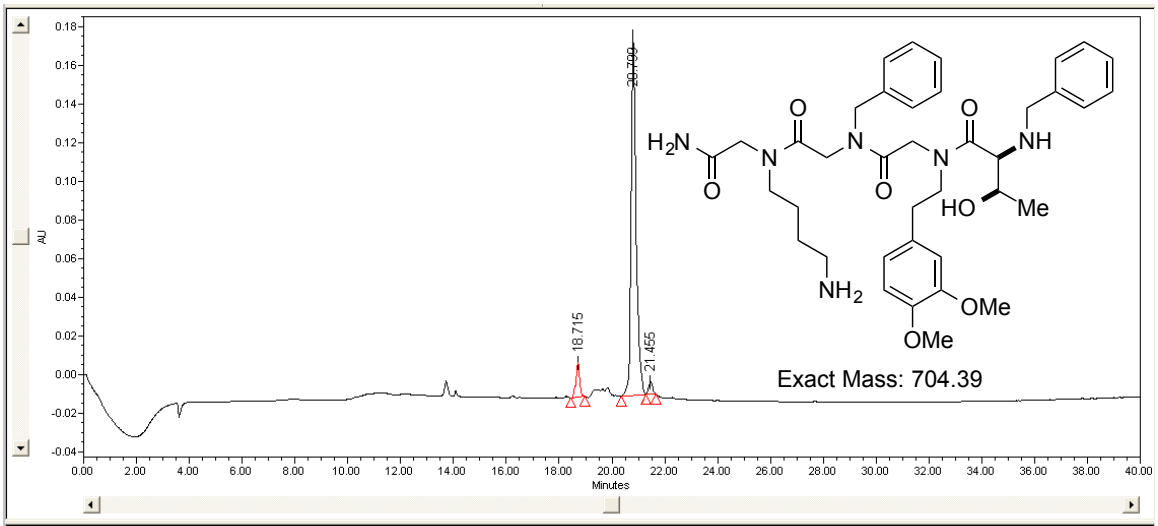
1j



	Name	Retention Time (min)	Peak Type	Area (µV*sec)	% Area	Height (µV)	% Height	Amount	% Amount	Units	Integration Type	Relative RT (min)	RT Ratio	Peak Level	Control Amount	% Deviation	Response	USP Resolution	Resolution (EP and JP)	USP Plate Count
1		18.666	Unknown	327690	4.62	32740	7.00				bb									
2		20.460	Unknown	6412701	90.38	403297	86.26				bb									
3		21.111	Unknown	128913	1.82	11000	2.35				bb									
4		22.674	Unknown	226184	3.19	20485	4.38				bb									

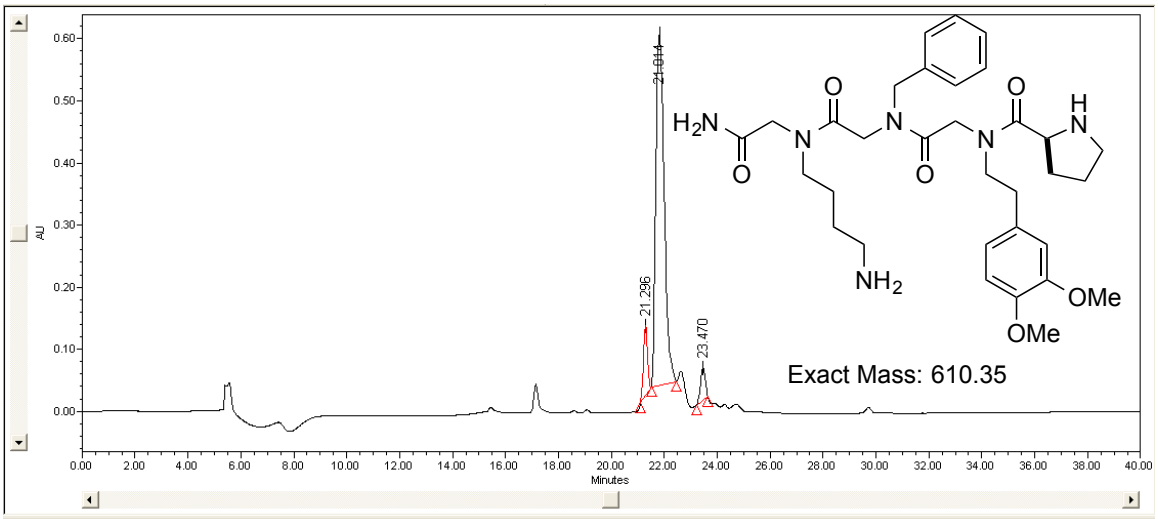
Channels | Peaks

1k



	Name	Retention Time (min)	Peak Type	Area (µV*sec)	% Area	Height (µV)	% Height	Amount	% Amount	Units	Integration Type	Relative RT (min)	RT Ratio	Peak Level	Control Amount	% Deviation	Response	USP Resolution	Resolution (EP and JP)	USP Plate Count
1		18.715	Unknown	173857	6.38	17982	8.55				bb									
2		20.799	Unknown	2483595	91.11	185531	88.25				bb									
3		21.455	Unknown	68399	2.51	6711	3.19				bb									

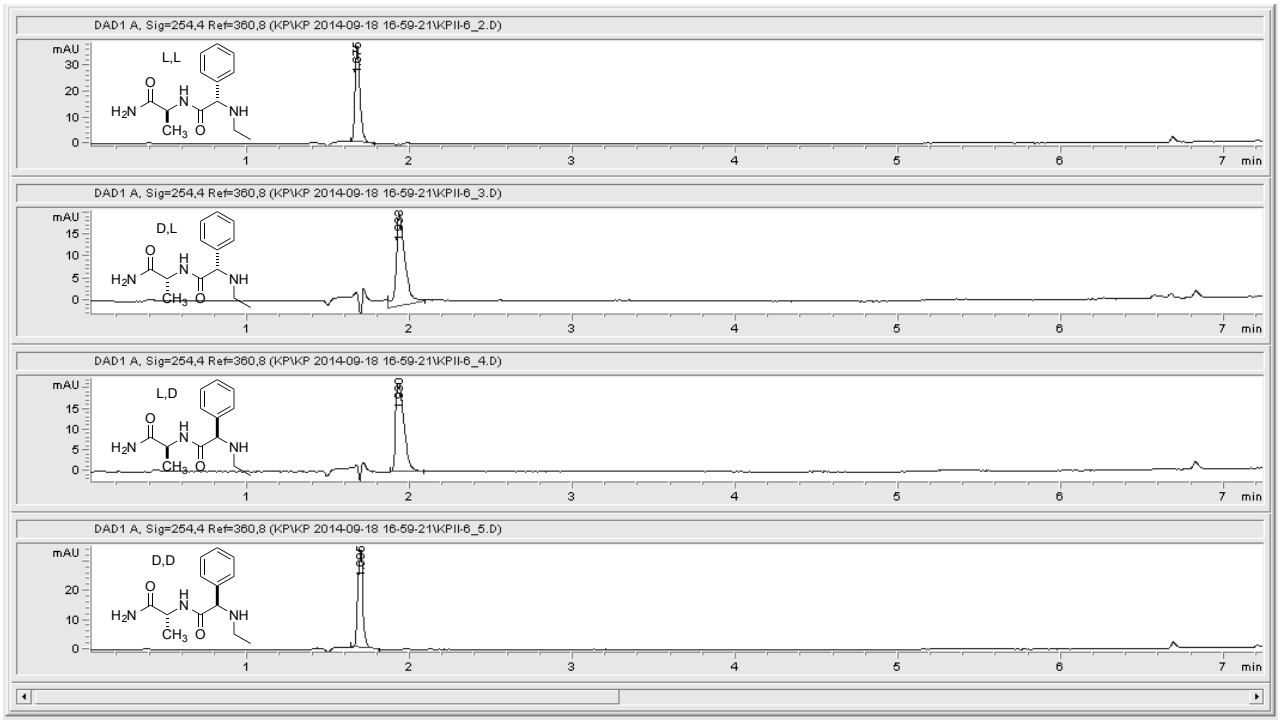
1l



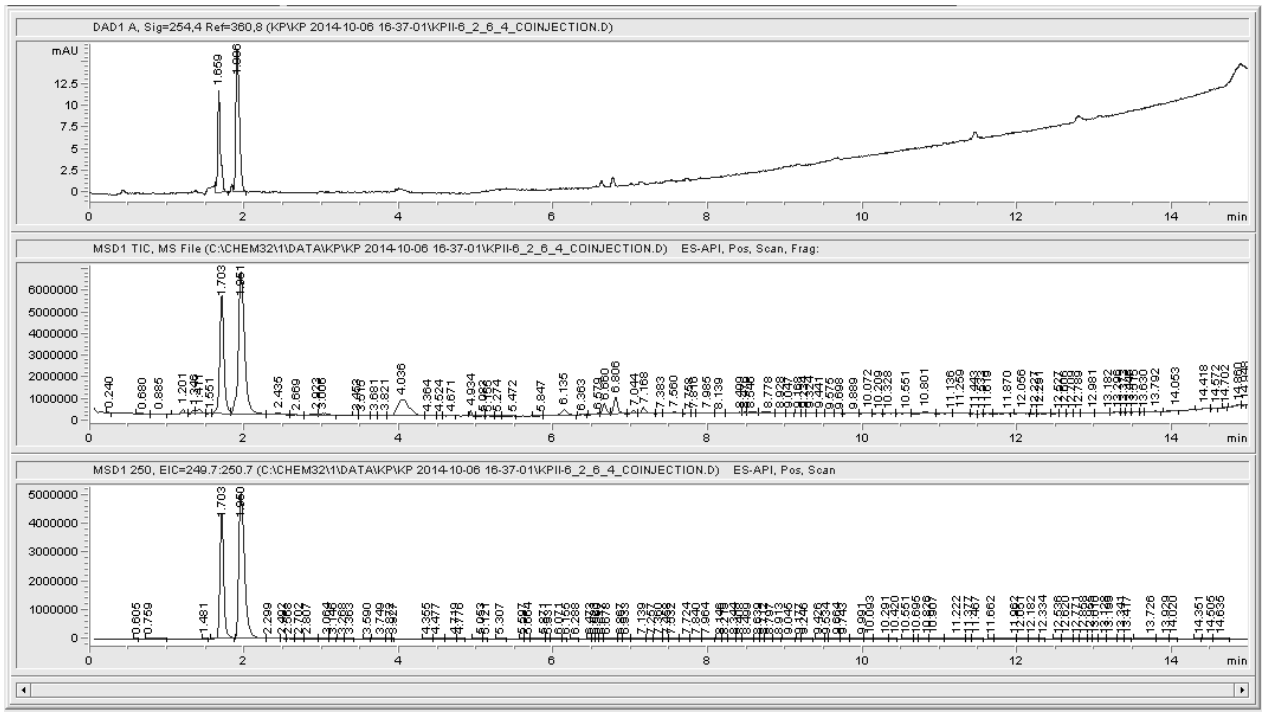
	Name	Retention Time (min)	Peak Type	Area (µV*sec)	% Area	Height (µV)	% Height	Amount	% Amount	Units	Integration Type	Relative RT (min)	RT Ratio	Peak Level	Control Amount	% Deviation	Response	USP Resolution	Resolution (EP and JP)	USP Plate Count
1		21.296	Unknown	1184065	8.23	112307	15.41				bb									
2		21.814	Unknown	12552968	87.28	564630	77.47				bb									
3		23.470	Unknown	645936	4.49	51886	7.12				bb									

*no alkylated product observed with conditions indicated in Fig. 1

LC-MS data for Et-Phg-Ala-NH_2 diastereomers



Coinjection of L,L and L,D diastereomers, with EIC of Et-Phg-Ala-NH_2 M+H (MW = 249, M+H = 250)

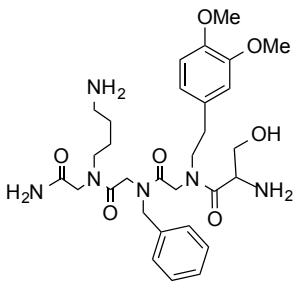


LCMS data for compounds **2a-t**

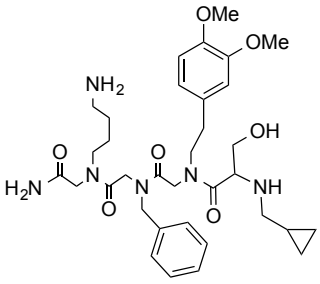
Full structures of each compound are provided, along with 4 chromatograms: A^{280}; TIC; EIC (product); EIC (starting material)

In many chromatograms, a peak appears after the product peak that has a mass of Pdt + 56. This is an artifact from incomplete scavenging of the isobutylene cation from Ser(tBu) deprotection, and because it is Pdt+56, this peak is included in the integration of product for the calculation of purity. Its presence is annotated on the A^{280} chromatogram when present.

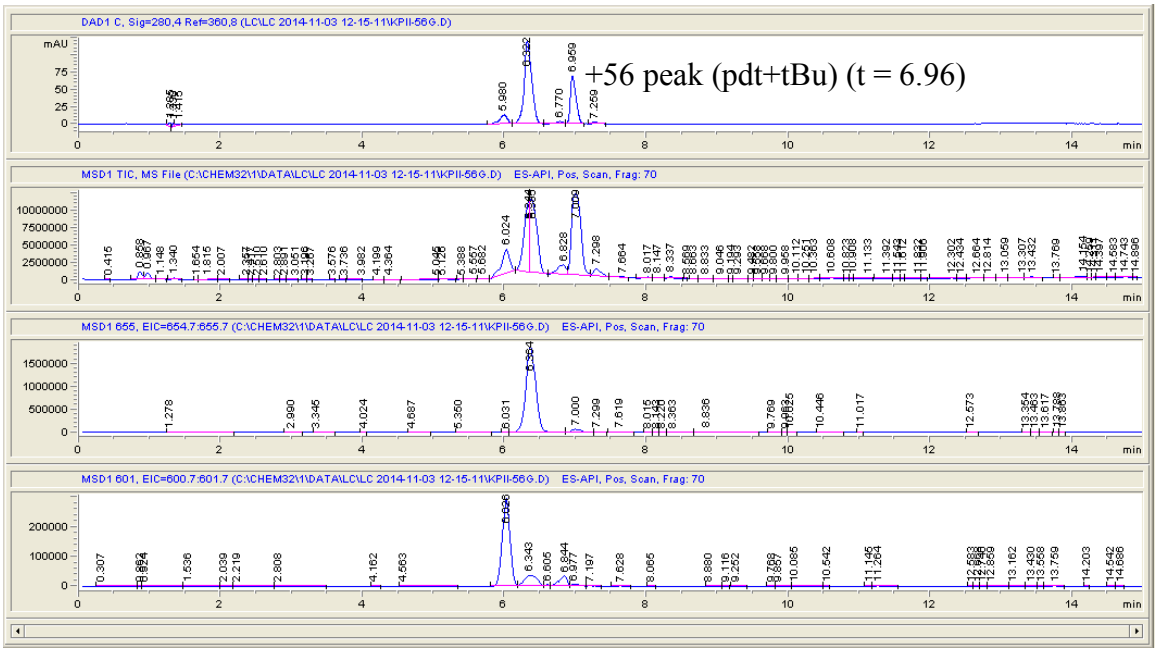
2a



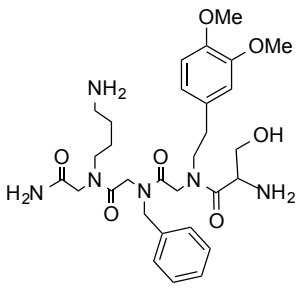
Exact Mass: 600.33



Exact Mass: 654.37



2b



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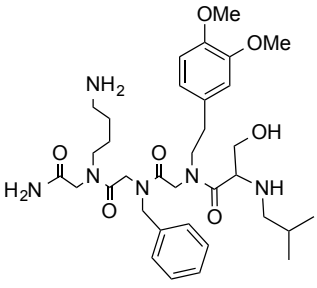

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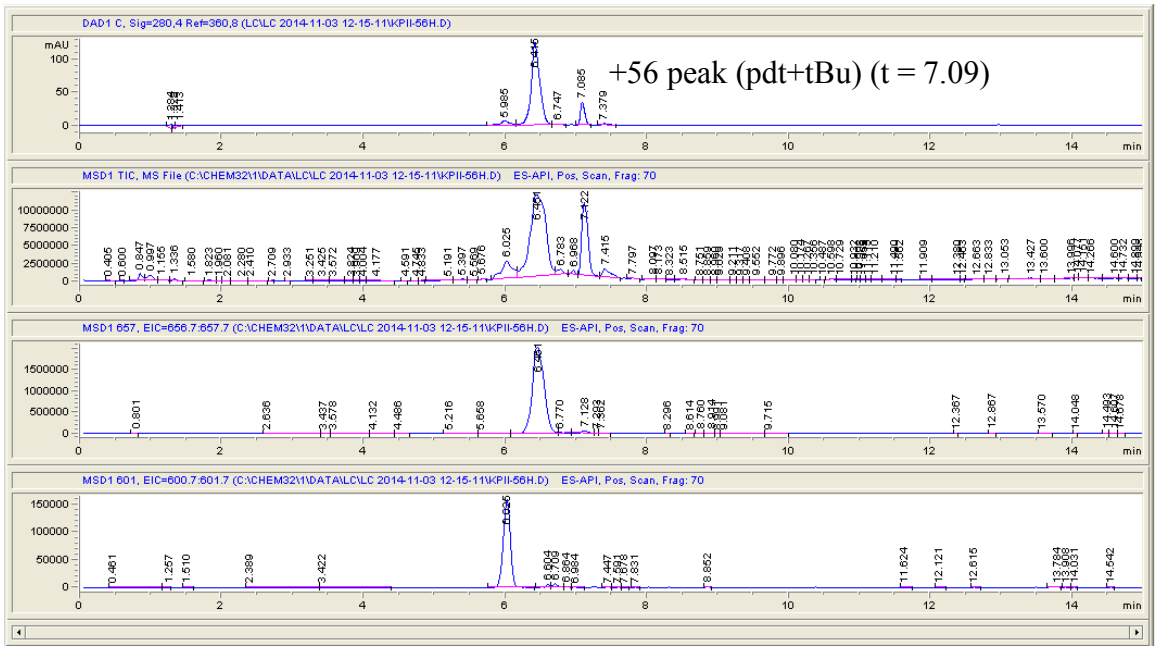

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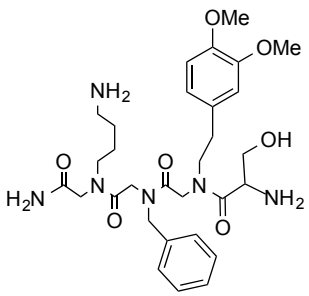

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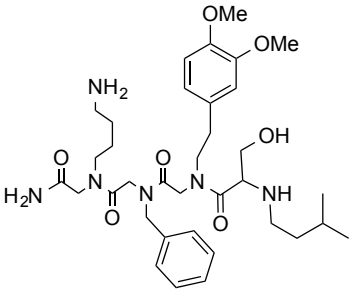

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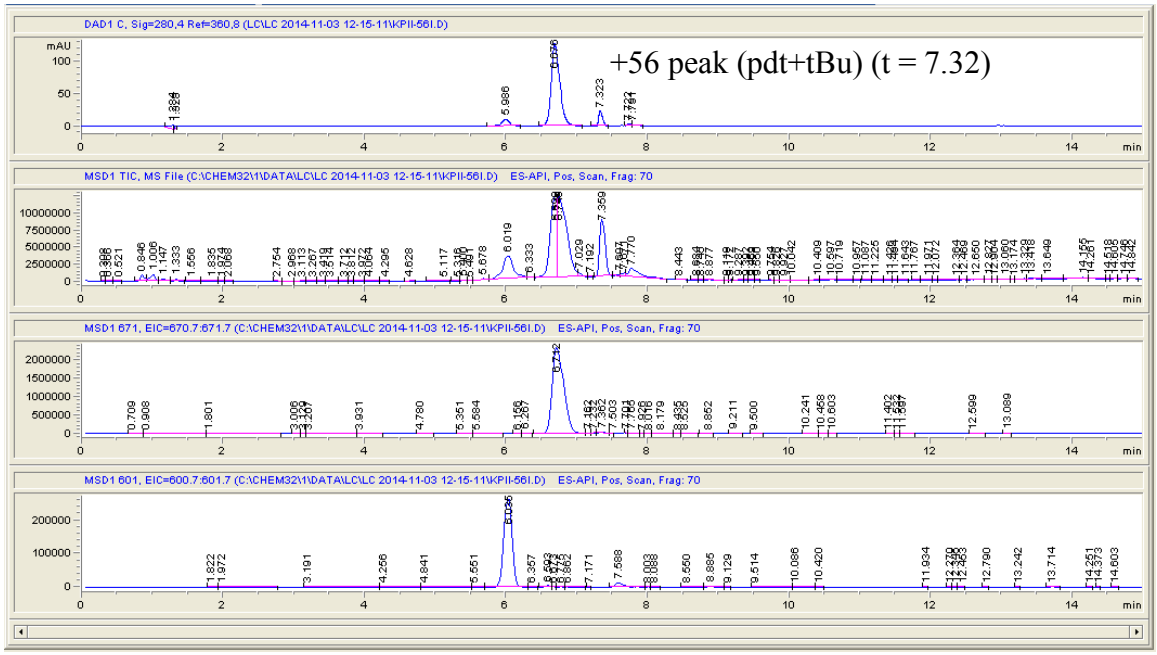

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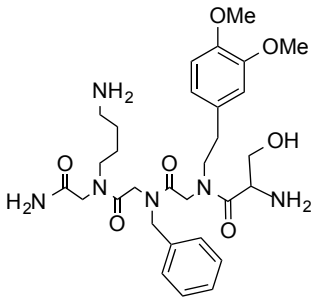

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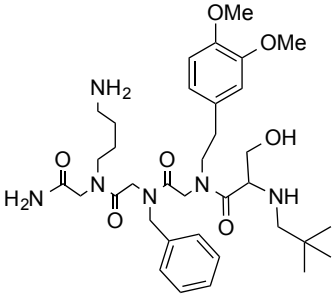

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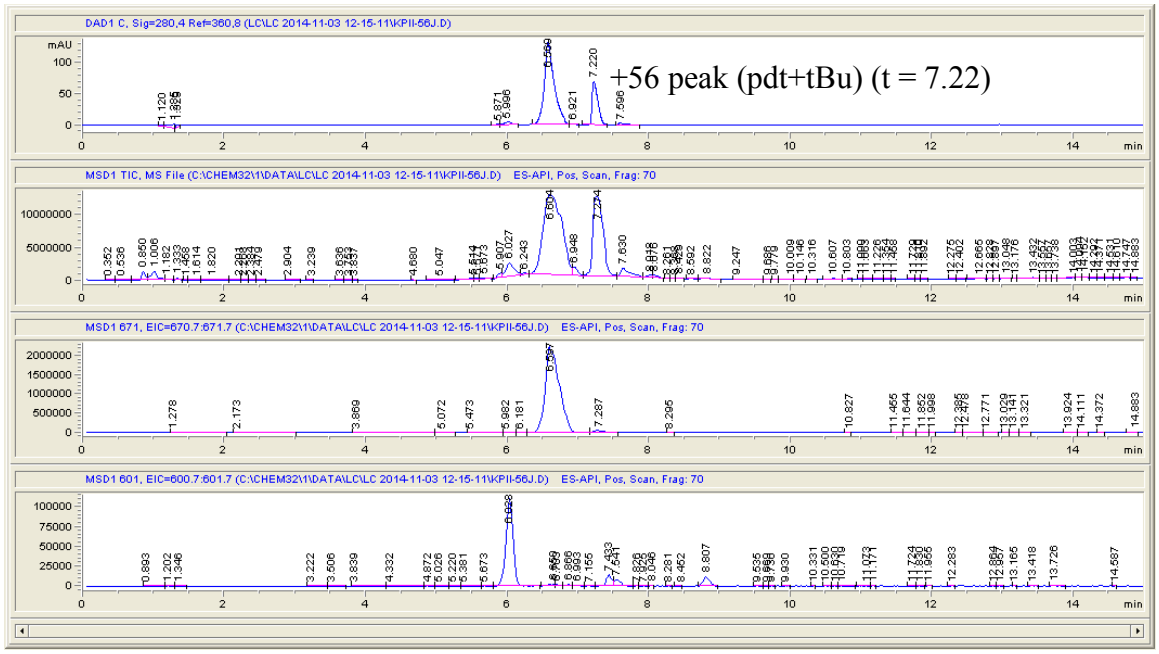

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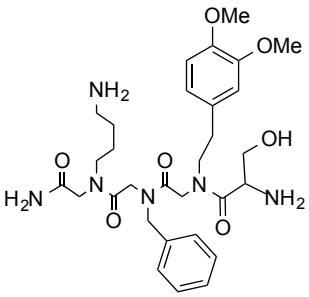

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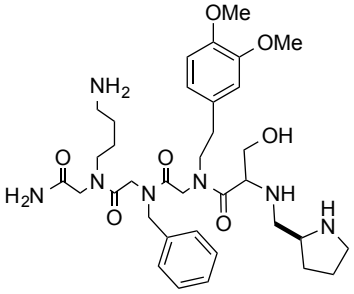
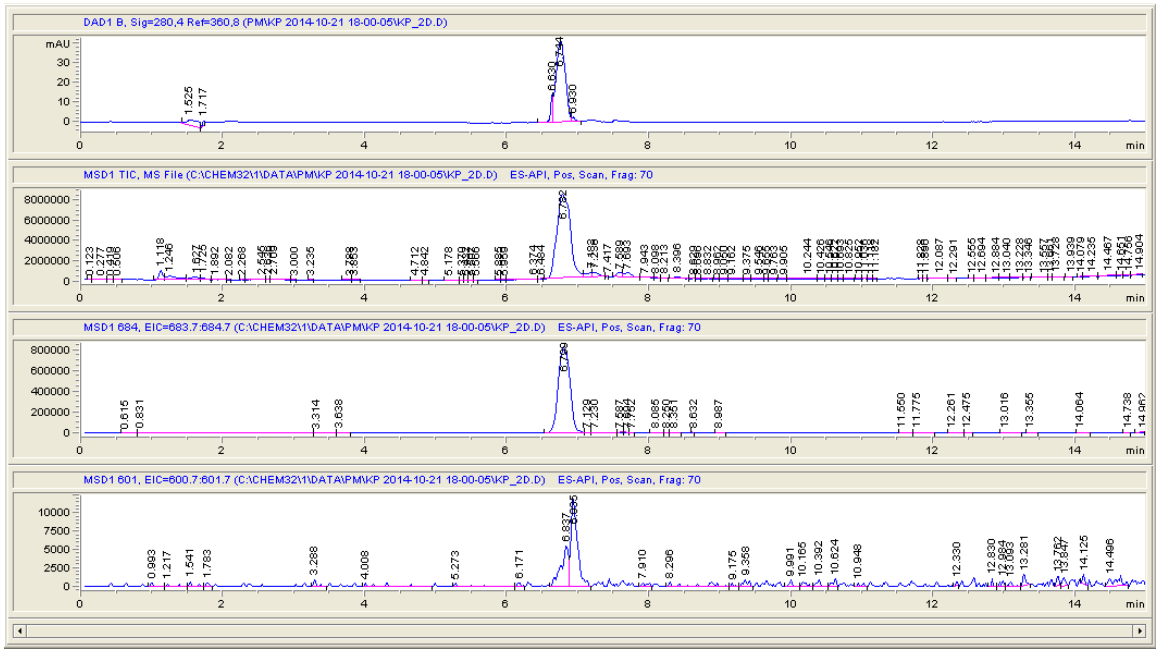


Exact Mass: 683.40



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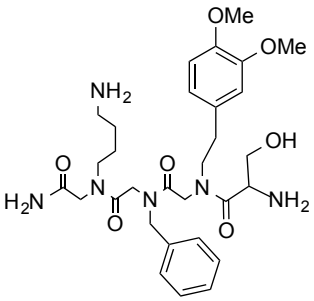
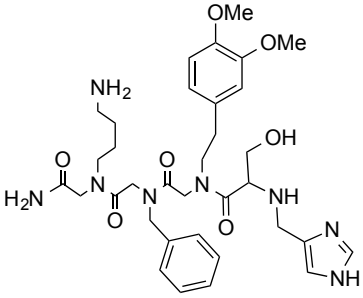


Exact Mass: 600.33



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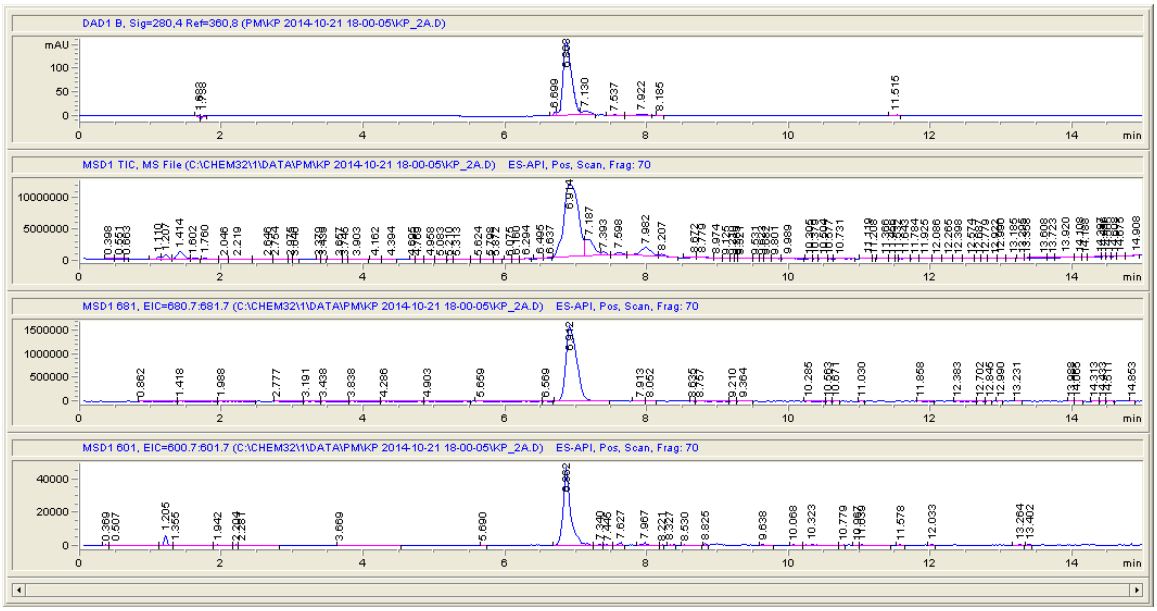
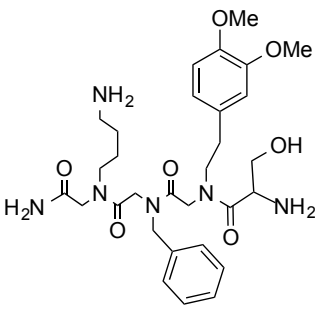
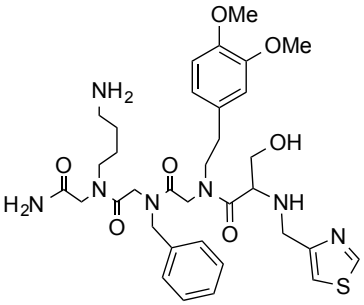


2g



Exact Mass: 600.33



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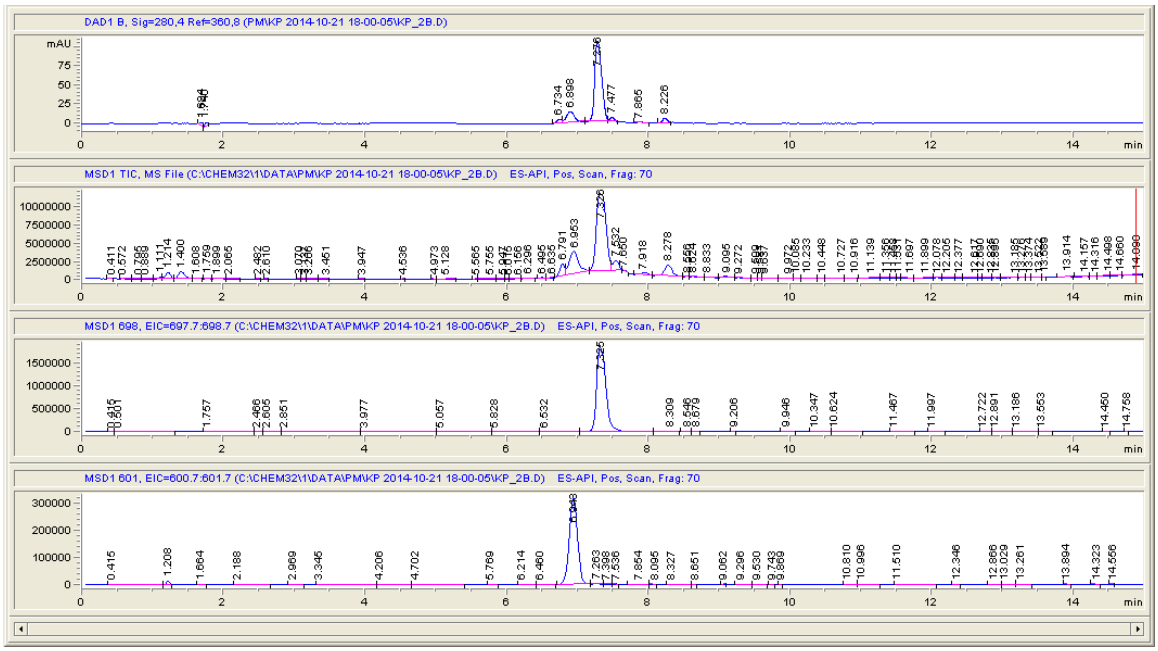
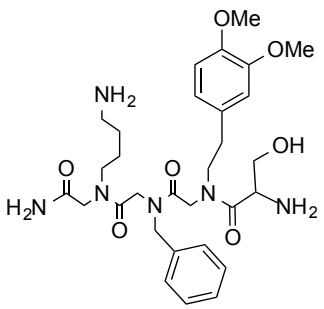
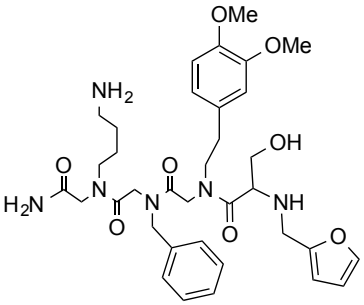


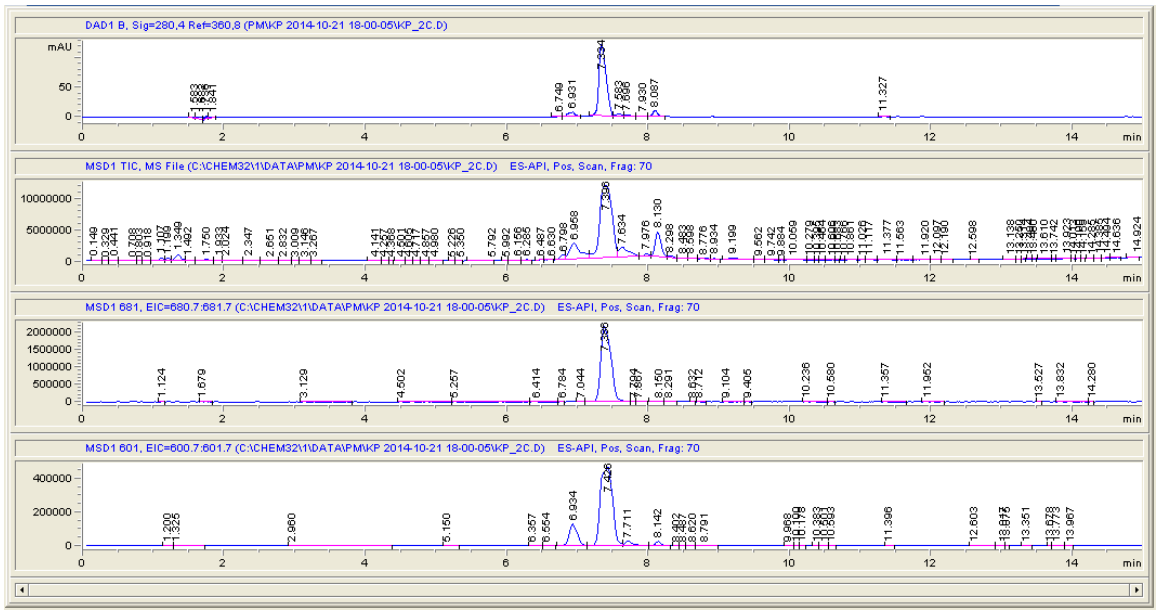
2h



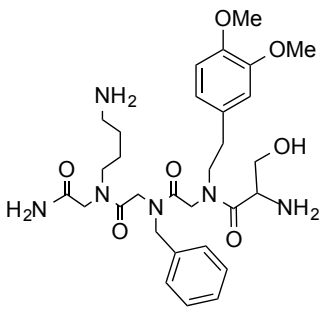
Exact Mass: 600.33



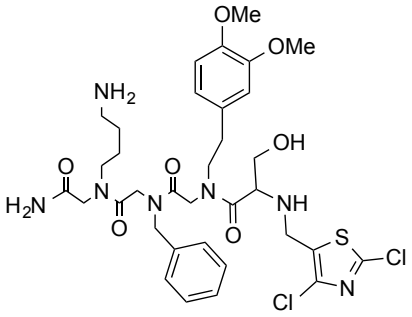
Exact Mass: 680.35



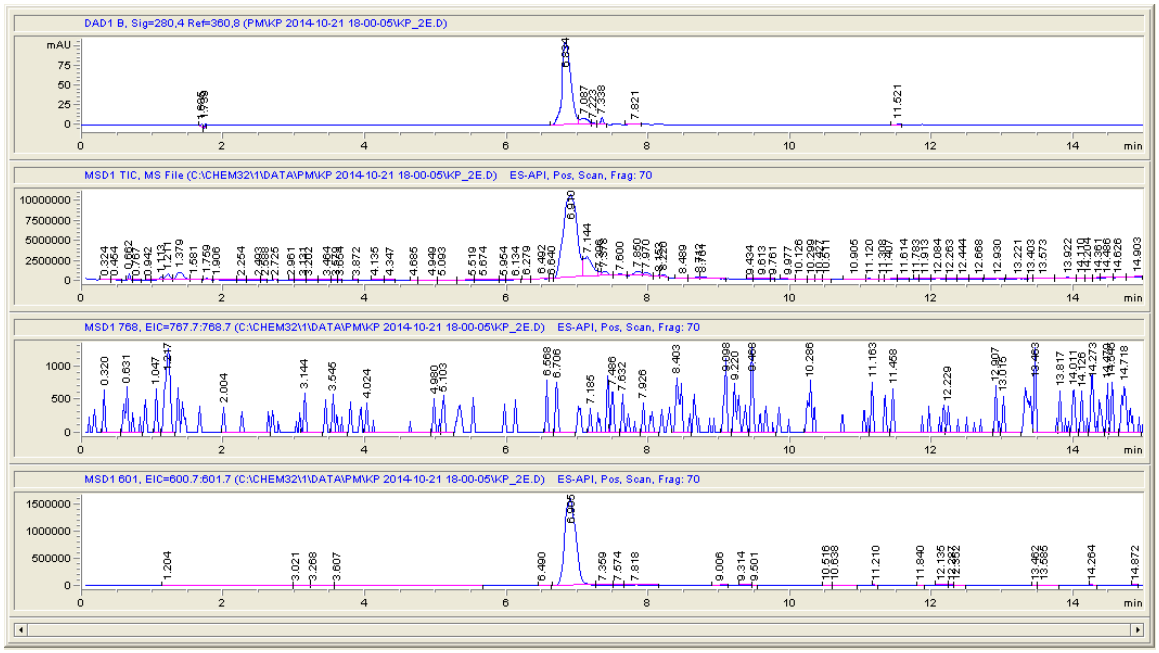
2i



Exact Mass: 600.33



Exact Mass: 765.25



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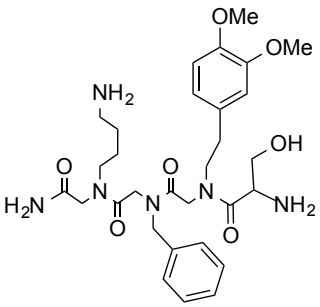

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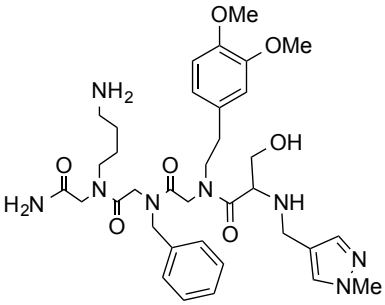

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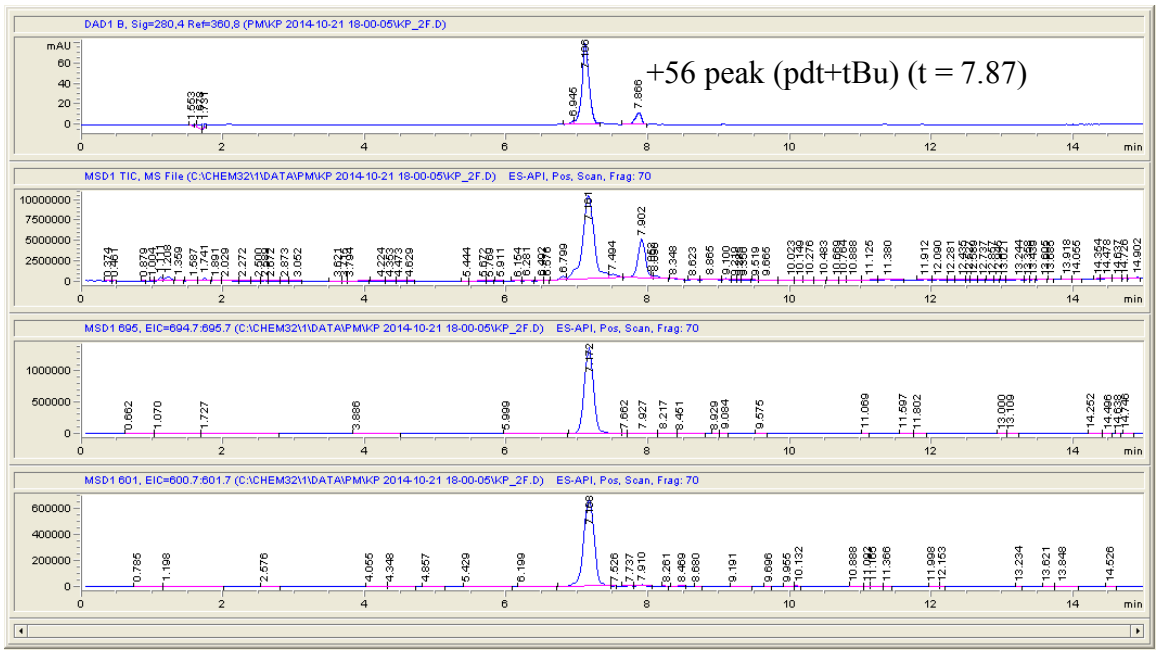

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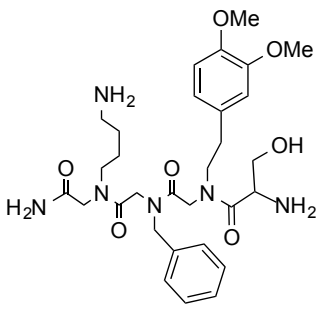

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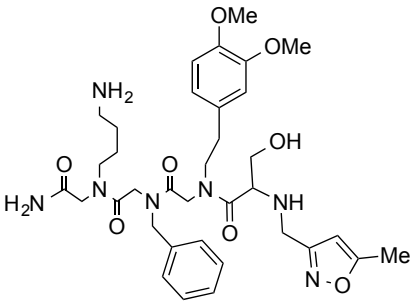

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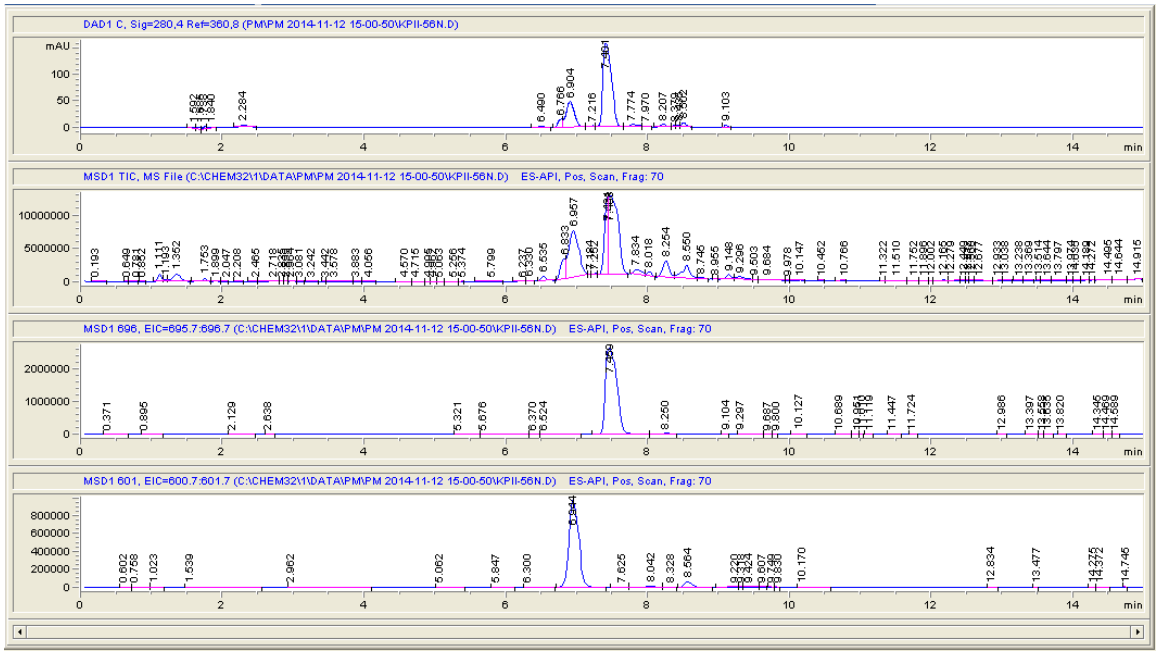

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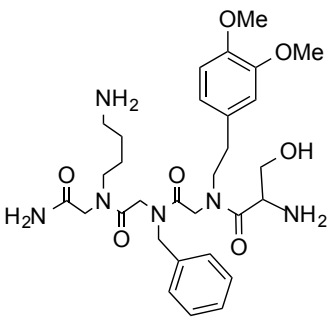

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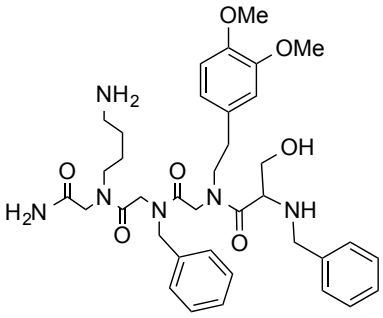

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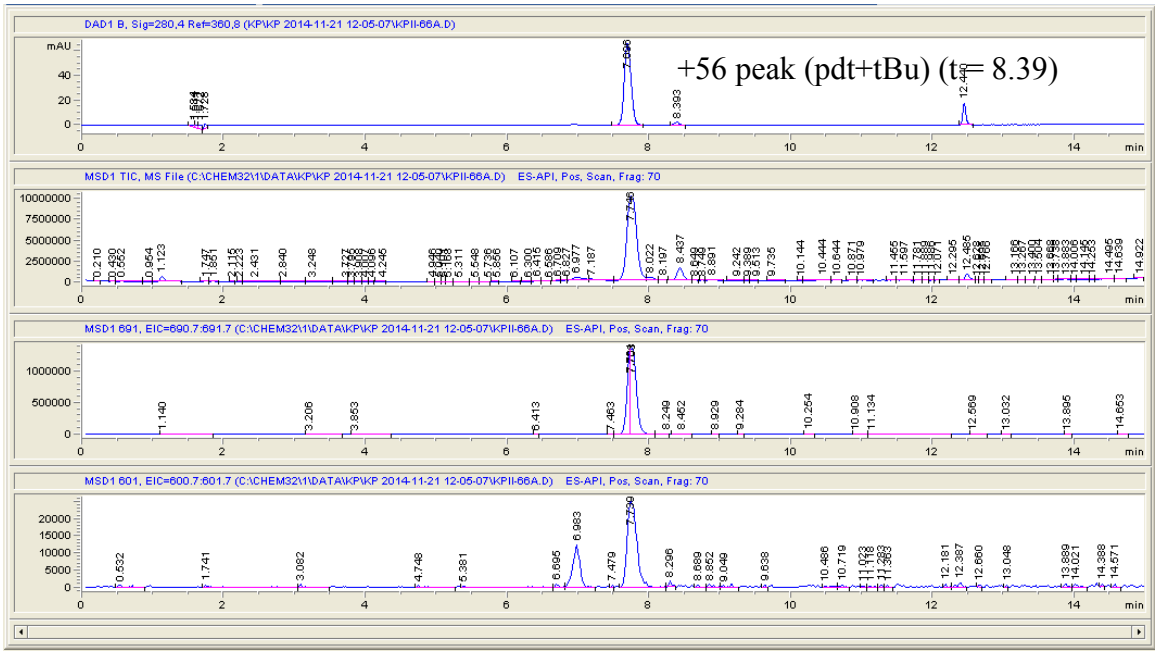

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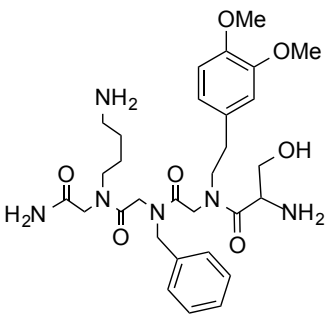

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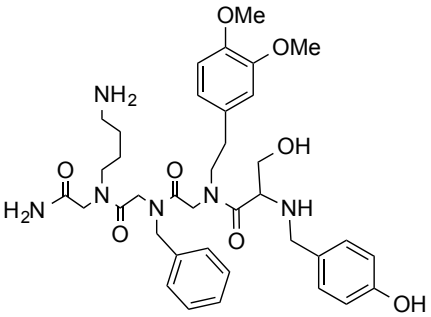

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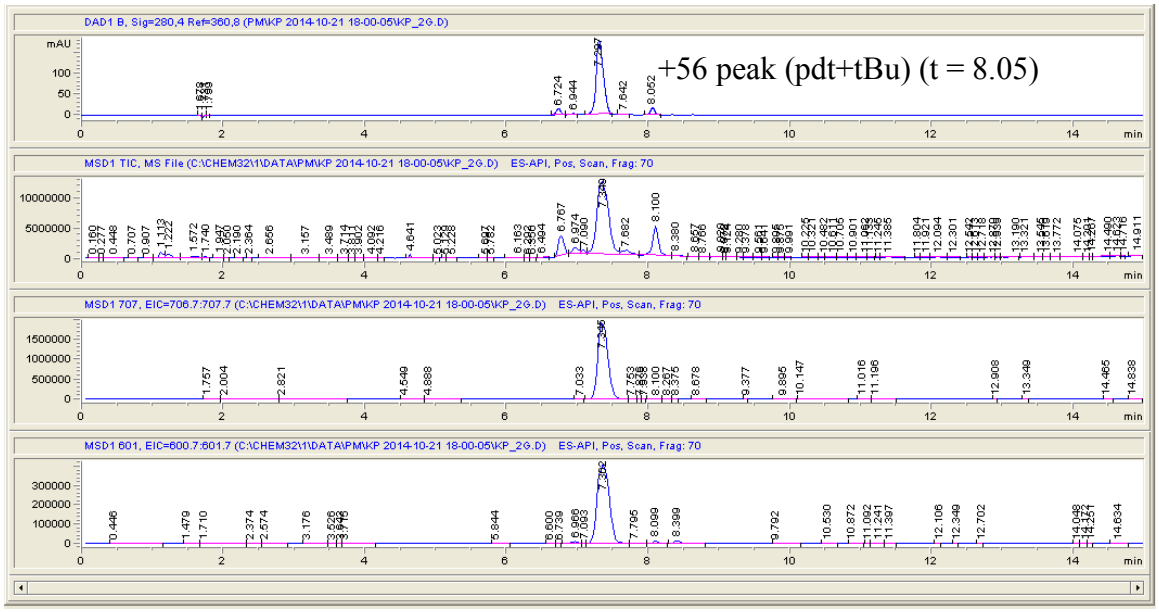

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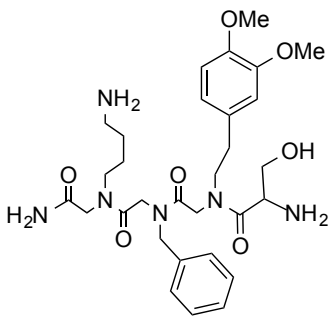

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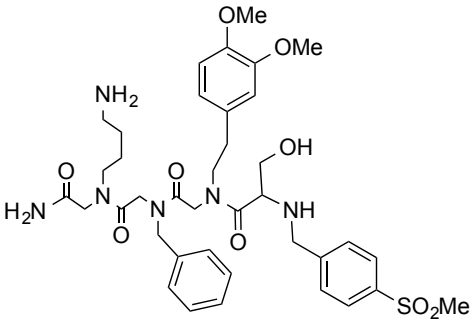

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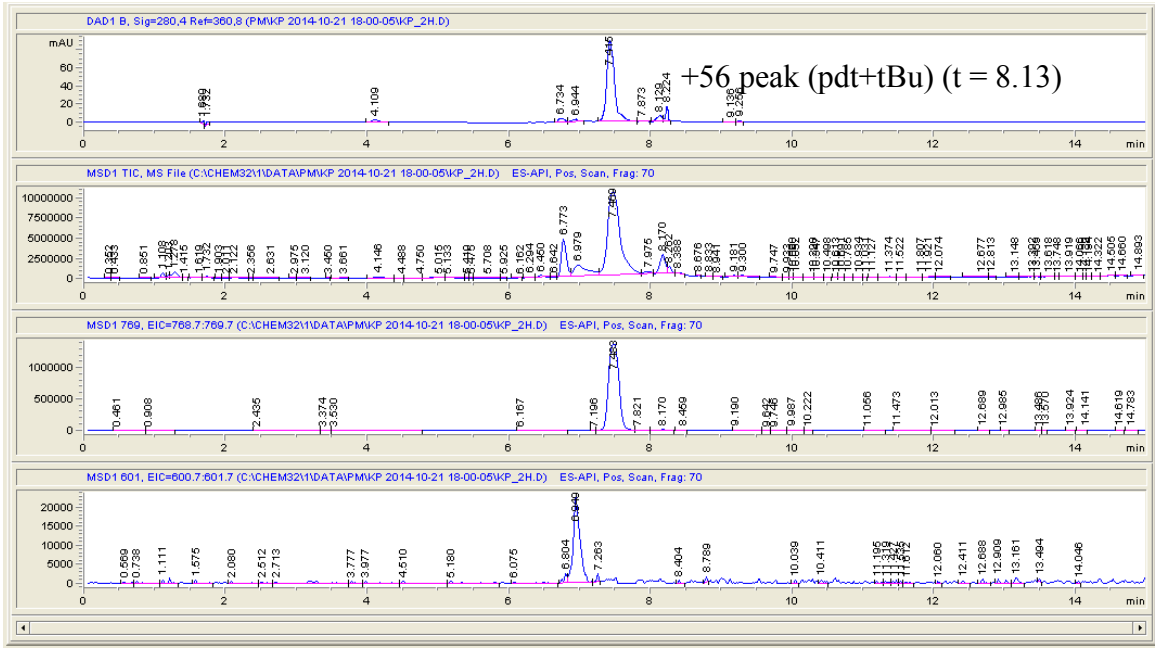

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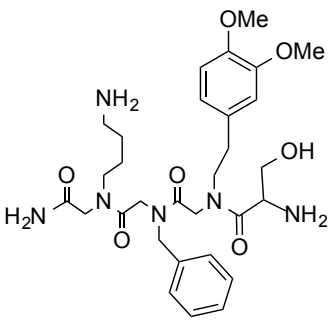

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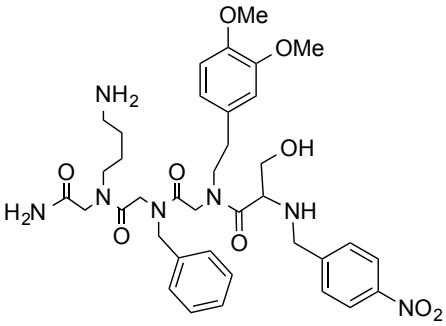

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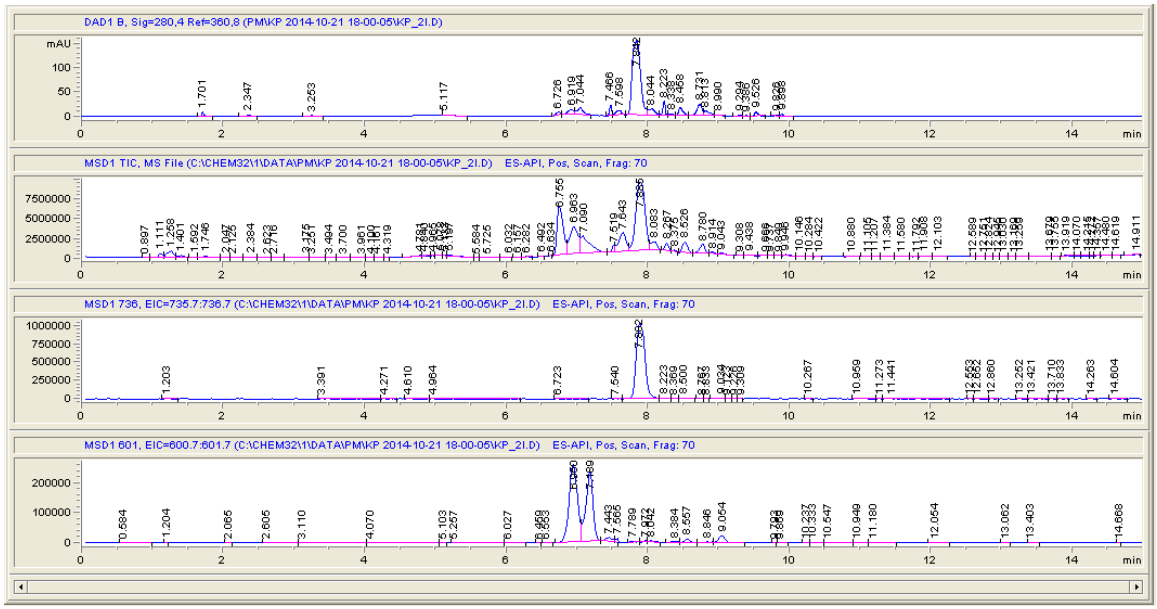

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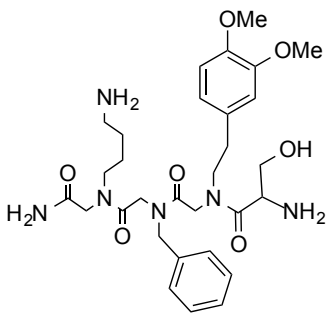

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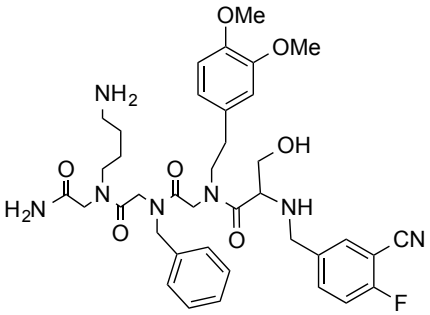

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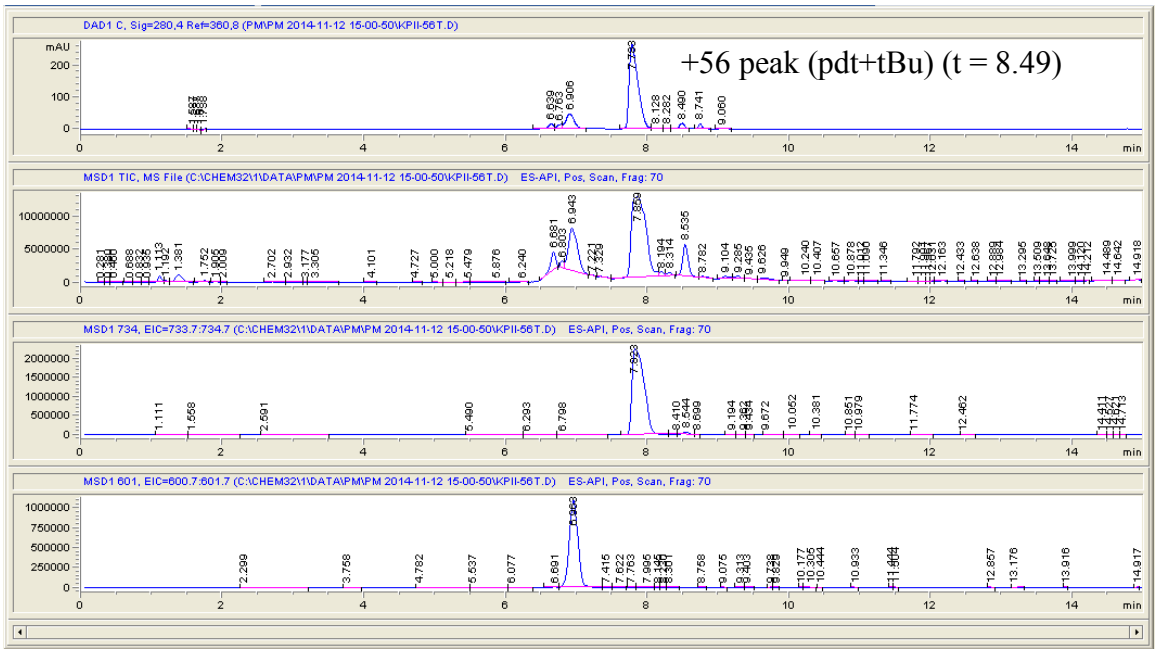

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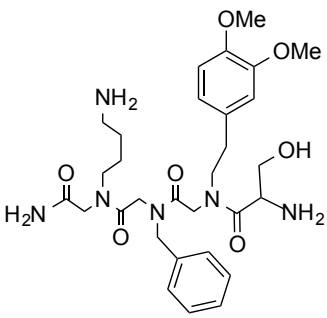

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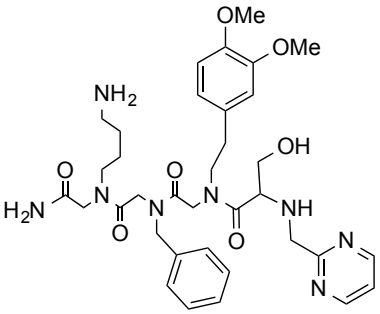

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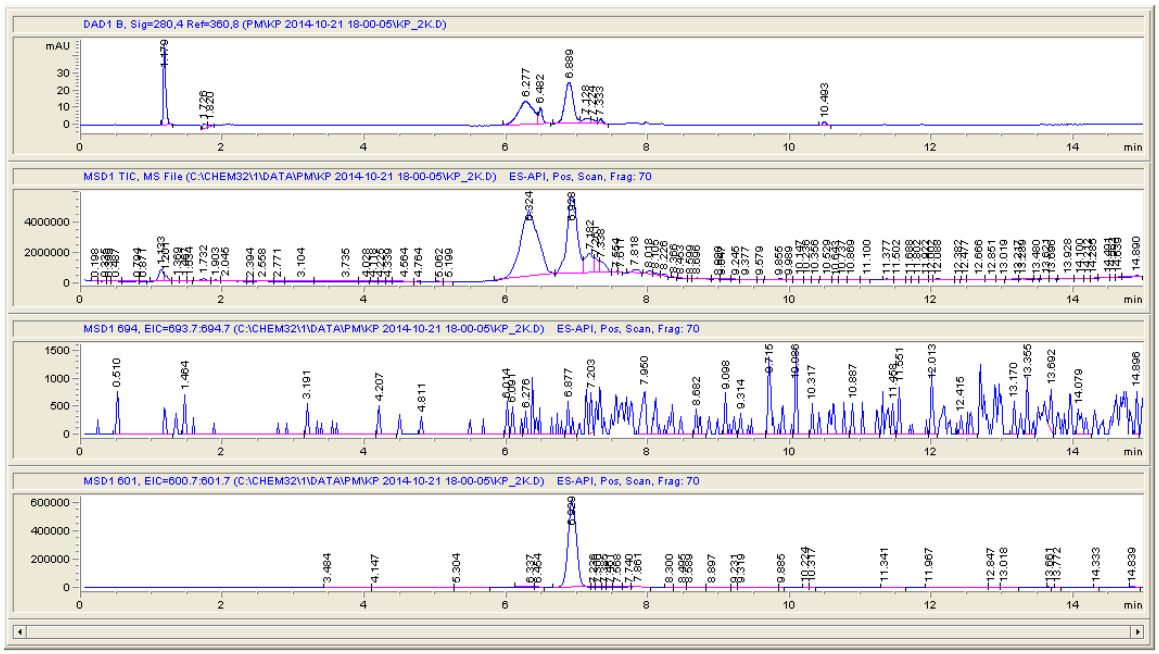

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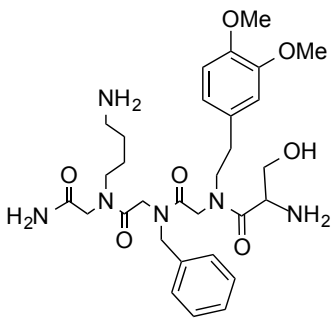

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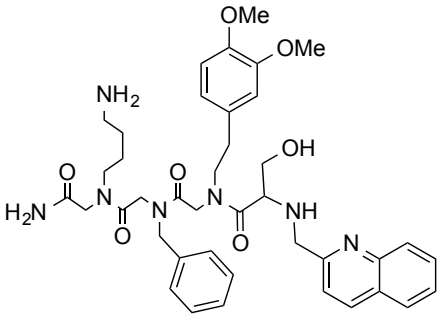

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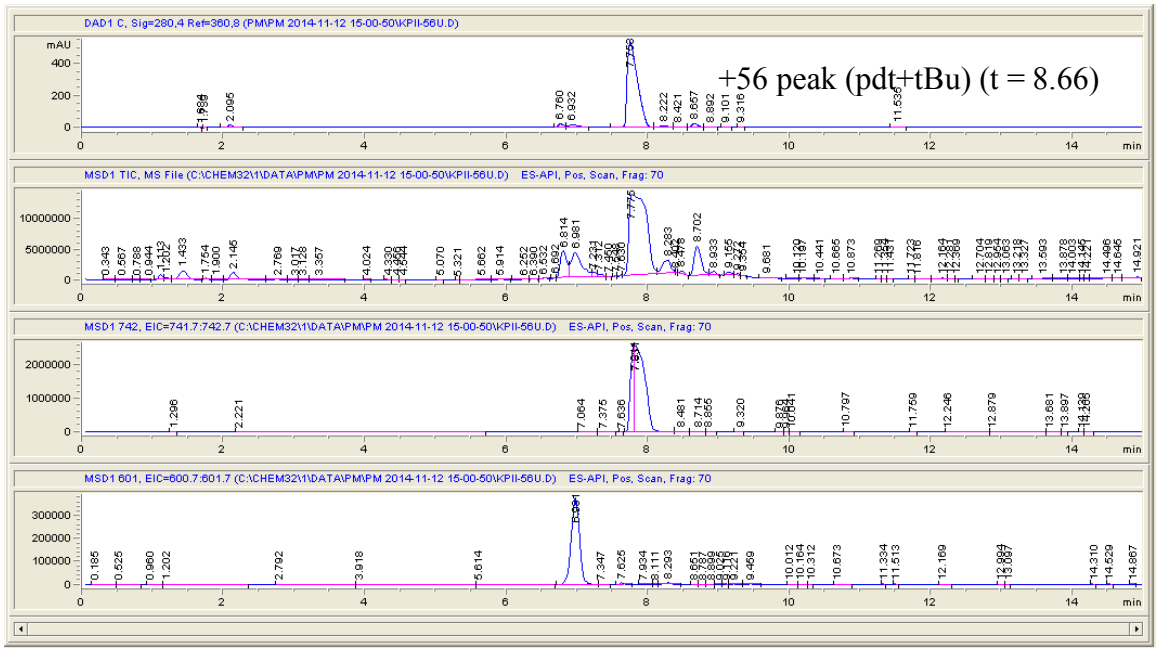

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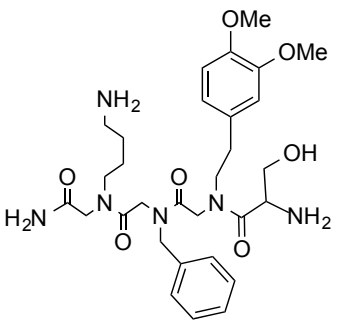

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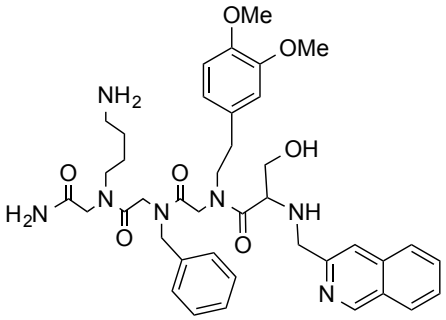

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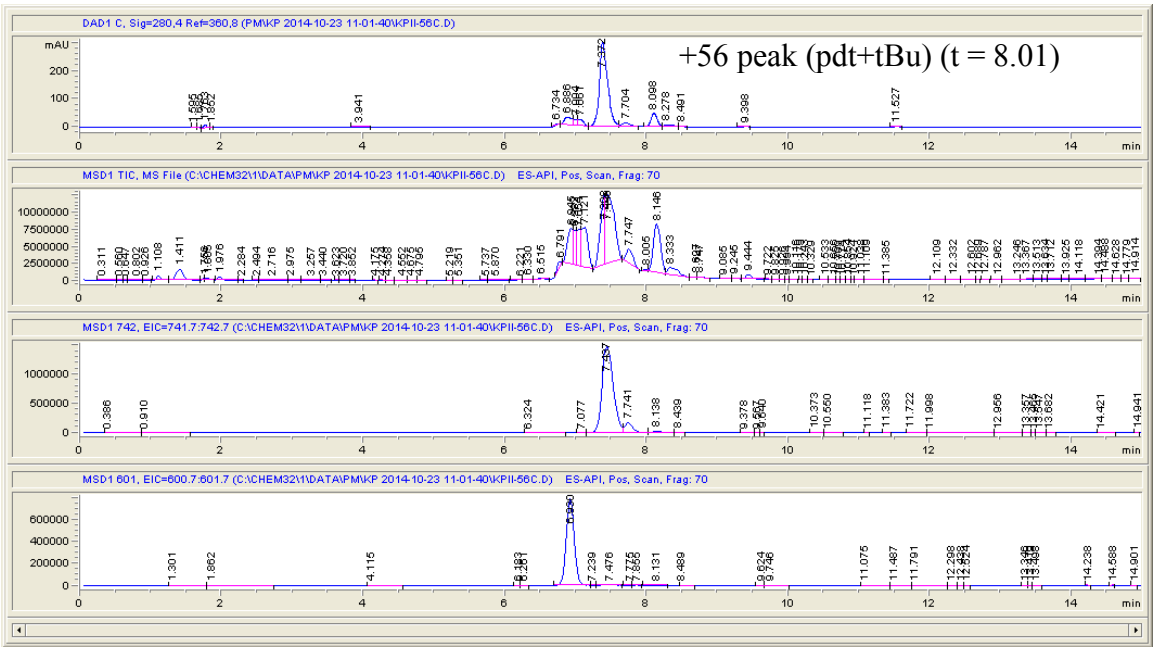

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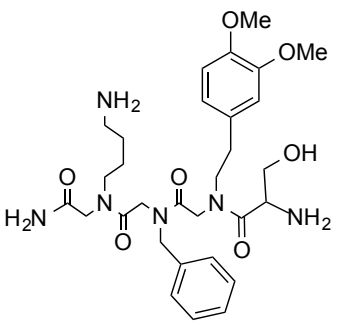

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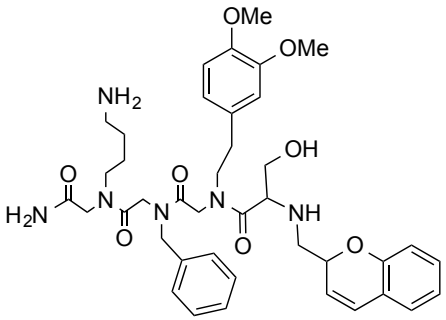
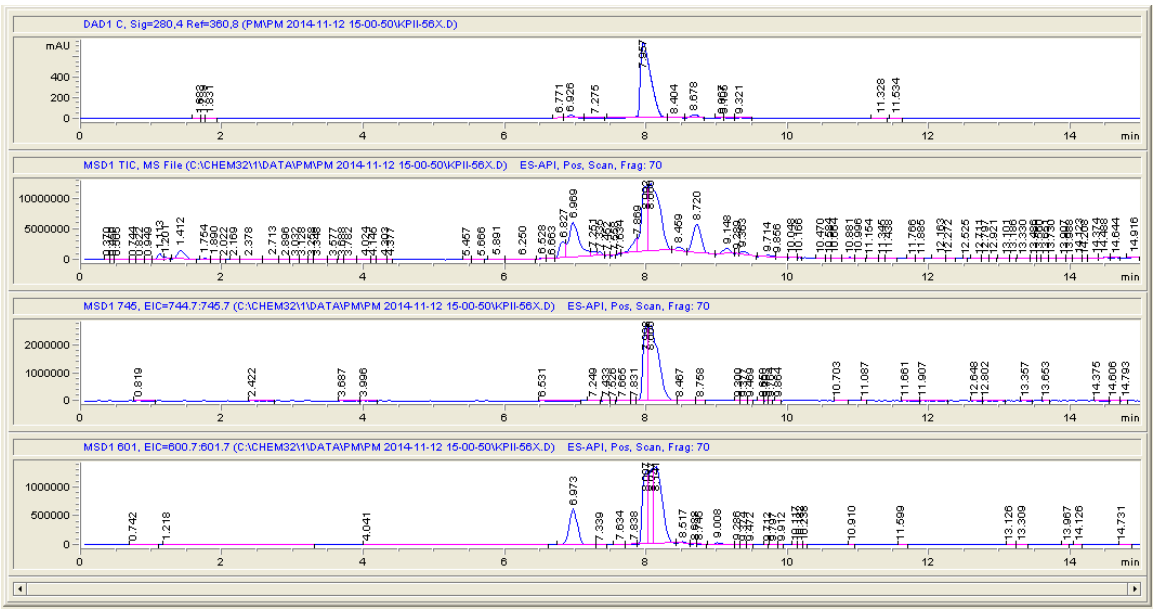


Exact Mass: 744.38

+56 peak (pdt+tBu) (t = 8.68)



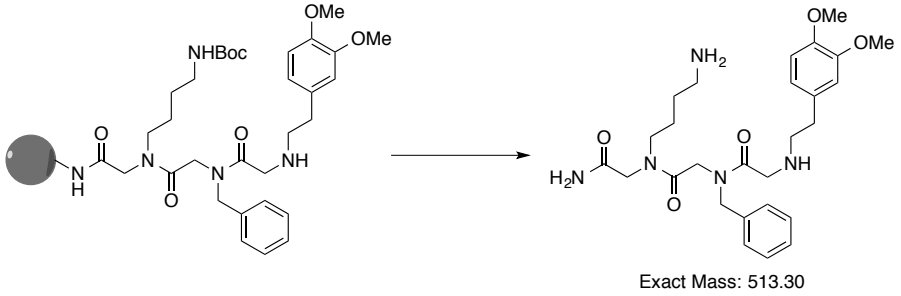
Materials and Methods

All the chemical reagents and solvents from commercial sources were used without further purification unless otherwise noted. Fmoc-Tyr(tBu)-H was synthesized in two steps from commercial Fmoc-Tyr(tBu)-OH according to the literature procedure[13]. Rink amide MBHA resin (100-200 mesh, 0.69 mmol/g) resin was purchased from EMD Millipore. TentaGel R NH_2 resin (90 μm, 0.29 mmol/g) resin was purchased from Rapp Polymere Gmbh. Disposable polypropylene reaction columns (2 mL and 5 mL, Torviq, Niles, MI, www.torviq.com) were used as reaction vessels for solid phase synthesis. Wash volumes equate to 1 mL of solvent per 100 mg of resin. Analytical HPLC was carried out on Waters 1525 binary HPLC pumps equipped with Waters FlexInject (20 μL analytical loop), Grace Apollo 250 mm x 4.6 mm C18 column, and a Waters 2487 Dual λ Absorbance detector. The mobile phase comprised of buffer A (H_2O containing 0.1% TFA) and buffer B (CH_3CN containing 0.1% TFA). LCMS was carried out on an Agilent 1100 system equipped with LC/MSD SL. The mobile phase comprised of buffer A (95/5 H_2O/CH_3CN containing 0.1% formic acid) and buffer B (95/5 CH_3CN/H_2O containing 0.1% formic acid) 1H NMR spectra were recorded at 25 °C on Bruker 400 instrument operating at 400 MHz. 1H chemical shifts were referenced to residual solvent signal δH 2.50 for DMSO-d6. MS and MS/MS (MALDI-TOF) were performed on a 4800 Proteomics Analyzer (Applied Biosystems) with α-cyano-4-hydroxycinnamic acid (CHCA) as a matrix.

Assembly of common linker for test compounds

The tripeptide linker common to each of the test compounds was assembled using standard thermal peptoid synthesis conditions. Knorr amide MBHA resin (1g, 0.69 mmol) was swelled for 5 minutes in 1:1 DCM/DMF and then Fmoc was deprotected by 2x incubations in

20% piperidine in DMF. After washing 5x DMF, 3 mL each of 2 M bromoacetic acid in anhydrous DMF and 2 M N,N'-diisopropylcarbodiimide (DIC) in anhydrous DMF were added to the reaction vessel, which was capped and incubated at 37 C for 5 minutes. The resin was washed 5x DMF and 5 mL of a 1 M solution of 1-Boc-1,4-diaminobutane (Boc-Nlys) was added to the reaction vessel, which was capped and incubated at 37 C for 1 h. This cycle of acylation and nucleophilic displacement was repeated twice more with benzylamine and 3,4-dimethoxyphenethylamine to provide the common linker, which can be cleaved from resin to provide a peptoid trimer (MW = 513, M+H = 514).



General procedure for the monoalkylation of resin-bound primary amines

Resin displaying $-NH_2$ ligands was washed 5x with DMF, then incubated for 1 h with 1 M (10-15 eq.) of an aldehyde solution in anhydrous DMF. Aldehyde solution was filtered off and the resin was rapidly washed 2x DMF and 2x DCM. A freshly prepared 75/25 DCM/MeOH solution was added to the resin, and 1-2 large pellets (~8 mg each on average) of $NaBH_4$ were added to the open reaction vessel, and the reduction was carried out open to the atmosphere. Effervescence of the reaction, which agitates the resin via solvolysis of the reducing agent, was usually observed a few minutes after adding the $NaBH_4$ pellet(s). After 30 m, the resin was purged of solution and washed with MeOH until bubbling ceased, indicating that the reducing agent had been quenched. The resin was further washed 5x DCM and 5x DMF. A few beads are

picked for chloranil test (positive) and trinitrobenzotoluylsulfonic acid (TNBS) test (negative) to confirm the complete transformation of starting material before continuing the synthesis or cleavage from resin.

General procedure for the alkylation of resin-bound secondary amines

Resin displaying –NHR ligands was washed 5x with DMF, then incubated for 10 m with 1 M (10-15 eq.) of an aldehyde solution in anhydrous DMF with 1% AcOH as an additive. The reaction vessel was opened and a freshly prepared 1 M solution of $NaCNBH_3$ in 75/25 DCM/MeOH with 1% AcOH as an additive was added to the resin, to give a final concentration of 0.5 M with respect to both aldehyde and reducing agent. Reaction was again capped and allowed to shake for 1 h, at which point the resin was purged of solution and washed with 5x MeOH, 5x DCM and 5x DMF. A few beads are picked for chloranil test (negative) to confirm the complete transformation of starting material before continuing the synthesis or cleavage from resin. If the reaction was incomplete (positive chloranil), the procedure was repeated once more.

Peptoid chain extension from sterically hindered PTA secondary amine

Resin displaying N-terminal PTA (α-branched secondary amine) was washed 5x with DCM, then incubated for 10 m with 6 eq. of 2,4,6-trimethylpyridine (TMP) in minimal DCM. To a solution of 4 eq. of chloroacetyl chloride in DCM (0.2 M) cooled to -20 C was added 6 eq. of TMP and the cloudy mixture was applied directly to the beads in the reaction vessel. The resin was shaken at 4 C for 1 h, then washed with DCM and chloranil checked for completion of coupling. Often this acylation had to be repeated 1-2 more times at room temperature to see completion of reaction on chloranil, at which point the resin was washed 5x DCM and a 1 M

amine solution in anhydrous N-methylpyrollidine (NMP) was added, and the vessel incubated at 37 C overnight.

Note: For very hindered PTAs the reaction yield never became quantitative, leveling out around 85-95% products even with repeated acylations.

Et-Phg-Ala-NH_2

Knorr amide MBHA resin (150 mg, 0.69 mmol) was swelled for 5 minutes in 1:1 DCM/DMF and then Fmoc was deprotected by 2x incubations in 20% piperidine in DMF. After washing 5x DMF, 1.5 mL of a preactivated (5 m) solution of Fmoc-Ala-OH (0.2 M), ethyl (hydroxyimino)cyanoacetate (Oxyma, 0.2 M), and DIC (0.2 M) in DMF was applied to the resin and shaken for 40 m at rt. The resin was washed 5x with DMF and Fmoc was deprotected. Coupling and Fmoc deprotection was repeated for Fmoc-Phg-OH. The general reductive amination procedure for primary amines was used with acetaldehyde in excess, and the product was cleaved in 95/2.5/2.5 TFA/H_2O/TIPS for 2.5 h. The crude TFA salt which was not quantified was used without purification.

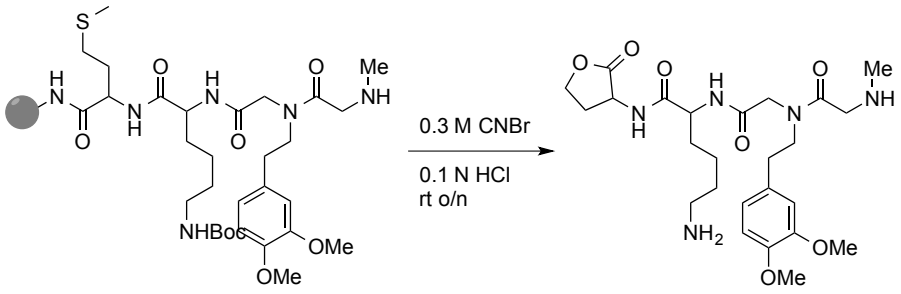
(L,D) Et-Phg-Ala-NH_2 NMR spectral characterization

^{1}H NMR (400 MHz, DMSO-d_6): δ 9.45 (s, 2H, amide-N**H₂**), 8.91 (d, 1H, amide-N**H**), 7.58-7.54 (m, 2H, Ar-**H**), 7.52 (s, 1H, amine-N**H**$_2$+), 7.48-7.42 (m, 3H, Ar-**H**), 7.13 (s, 1H, amine-N**H**$_2$+), 5.04 (br s, 1H, Phg-C**H**), 4.24-4.17 (quint, 1H, Ala-C**H**), 2.90-2.79 (br m, 2H, Et-C**H₂**), 1.21-1.18 (t, 3H, Et-C**H₃**), 1.11-1.09 (d, 3H, Ala-C**H₃**)

Library synthesis

The library was synthesized on 100 mg TentaGel R resin (90 μm, 0.29 mmol/g). The resin

was swelled for 5 minutes in 1:1 DCM/DMF and then Fmoc was deprotected by 2x incubations in 20% piperidine in DMF. After washing 5x DMF, 1 mL of a preactivated (5 m) solution of Fmoc-Met-OH (0.2 M), Oxyma (0.2 M), and DIC (0.2 M) in DMF was applied to the resin and shaken for 40 m at rt. The resin was washed 5x with DMF and Fmoc was deprotected. Coupling and Fmoc deprotection was repeated for Fmoc-Lys(Boc)-OH. After washing 5x DMF, 0.5 mL each of 2 M bromoacetic acid in anhydrous DMF and 2 M DIC in anhydrous DMF were added to the reaction vessel, which was capped and incubated at 37 C for 5 minutes. The resin was washed 5x DMF and 5 mL of a 1 M solution of 1-Boc-1,4-diaminobutane (Boc-Nlys) was added to the reaction vessel, which was capped and incubated at 37 C for 1 h. Finally, Fmoc-Sar-OH was coupled with Oxyma/DIC and deprotected as previously described. This represents the common linker for the library, which has a mass of 521 upon cyanogen bromide (CNBr) cleavage.



Exact Mass: 521.28

At the first diversity position R_1, half the residues come from amine displacement and half from reductive amination. The resin was split into two vessels. The first was bromoacetylated as previously described, then split into 5 vessels (10 mg each) for amine displacement with 5 different primary amines. The other half of the resin was coupled with Fmoc-Gly-OH, Fmoc deprotected, split into 5 vessels and monoalkylated via the general procedure with 5 aldehydes. All 10 vessels were then recombined and washed 5 times with DMF. The beads were split again

into 4 vessels and coupled to Fmoc-Gly-OH, Fmoc-Ala-OH, Fmoc-His(Boc)-OH, or Fmoc-Lys(Boc)-OH (R_2 diversity). After coupling the beads were recombined, Fmoc deprotected, and split into 5 vessels and monoalkylated via the general procedure with the 5 aldehydes used in this library (R_3 diversity). The resulting PTA position, being substituted at the α-carbon as well as the amine, is highly hindered and difficult to acylate, so the pooled resin was washed 5 times with DCM and incubated with 8 eq. of TMP for a brief period before a freshly prepared, cooled solution of 4 eq. chloroacetyl chloride and 8 eq. TMP in DCM was added to the basified bead slurry. The acylating solution was allowed to mix for 1 h at rt, and after this step the acylation was repeated until beads tested negative with chloranil (2x). The resin was split into 10 vessels and each was incubated with a 1 M primary amine solution in NMP overnight (16 h) at 37 C (R_4 diversity). The next day the resin was pooled, washed, and submitted to the general reductive amination procedure for secondary amines with the 5 aldehydes to provide the tertiary amine terminus of the library (R_5 diversity). The completed library beads were pooled and washed thoroughly with 5 x DMF (5 x 10 mL), 5 x DCM, and then incubated in DCM for 30 minutes. Most of the library was dried under vacuum and stored for future use at 4 C. For quality control, a small aliquot of beads was subjected to TFA cleavage (95/2.5/2.5 TFA/H_2O/TIPS rt 2.5 h), and after washing with DCM, the beads were suspended in EtOH for individual selection with a micropipetter. After picking 60 beads under a light microscope and depositing into individual wells of a 96-well plate, the beads were dried of EtOH and to each well was added 20 μL of a 0.3 M CNBr solution in 0.1 N HCl. The plate was covered and placed on a shaker at rt overnight (16 h). The next morning the solvent was removed via speedvac, and the crude residue redissolved in 10 μL of a 1:1 H_2O:acetonitrile solution, and these solutions in the plate were used for MALDI-TOF analysis.