Supporting Information

Antimicrobial peptides with potential for biofilm eradication: Synthesis and structure activity relationship studies of Battacin peptides.

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Figure S22: 500 MHz ^{1}H NMR spectrum of **30** in H_2O:D_2O (90:10) at 278K

Table S1: Physical Characteristics of peptides

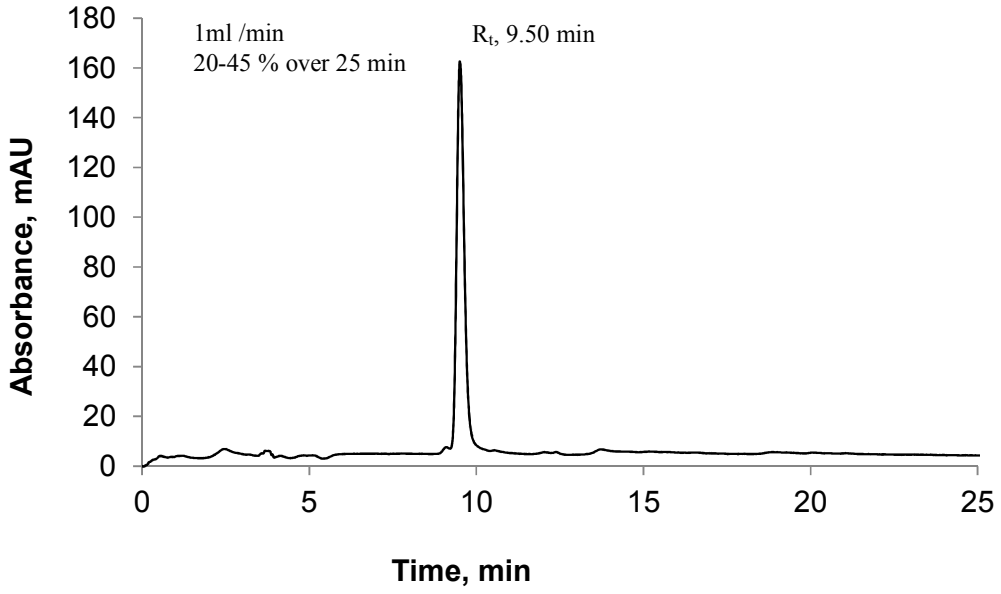
Peptide		HPLC gradient (% solvent B; Time)	HPLC t_R(min) and % Purity	Molecular Formula	Calculated MW (Da) for $[M+H]^+$	Observed m/z
Cyclic	**10**	20-45%; 25mins	9.50; 96.87 %	$C_{50}H_{88}N_{13}O_{10}$	1030.6772	$[M+H]^+$1030.6743
	12	10-60 %; 30mins	18.09; 96.92 %	$C_{56}H_{82}N_{13}O_{10}$	1096.6302	$[M+H]^+$ 1096.6268
	13	10-60 %; 30 mins	15.68; 98.50 %	$C_{48}H_{84}N_{13}O_9$	986.6509	$[M+H]^+$986.6452
	14	10-60 %; 30 mins	16.81; 96.69%	$C_{51}H_{86}N_{13}O_9$	1024.6666	$[M+H]^+$1024.6647
	15	10-80 %; 30 mins	19.59; 95.98%	$C_{55}H_{98}N_{13}O_9$	1084.7605	$[M+H]^+$ 1084.7586
	16	10-80 %; 30 mins	18.69; 97.45%	$C_{65}H_{118}N_{13}O_9$	1224.9170	$[M+H]^+$1224.9117
Linear	**17**	10-55 %; 30 mins	20.93; 99.50%	$C_{48}H_{87}N_{14}O_9$	1003.6775	$[M+H]^+$ 1003.6786
	18	10-60 %; 30 mins	14.00; 98.19%	$C_{51}H_{89}N_{14}O_9$	1041.6931	$[M+H]^+$ 1041.6917
	19	10-60 %; 30 mins	22.86; 98.07 %	$C_{55}H_{101}N_{14}O_9$	1101.7870	$[M+H]^+$1101.7798
	20	10-60 %; 30mins	22.12; 98.12 %	$C_{65}H_{121}N_{14}O_9$	1241.9435	$[M+H]^+$ 1241.9326
	29	10-60 %; 30 mins	11.63; 99.17 %	$C_{41}H_{75}N_{14}O_8$	891.5887	$[M+H]^+$ 891.5919
	30	10-60 %; 30mins	17.30; 97.44 %	$C_{34}H_{60}N_9O_6$	690.4661	$[M+H]^+$ 690.6484

Table S2: ^{1}HNMR chemical shifts along with $^{3}J_{NHC^{\alpha}H}$ and temperature coefficient values (dδ/dT) of 1**7** in H_2O (70%) + TFE (30%) recorded on 800 MHz spectrometer.

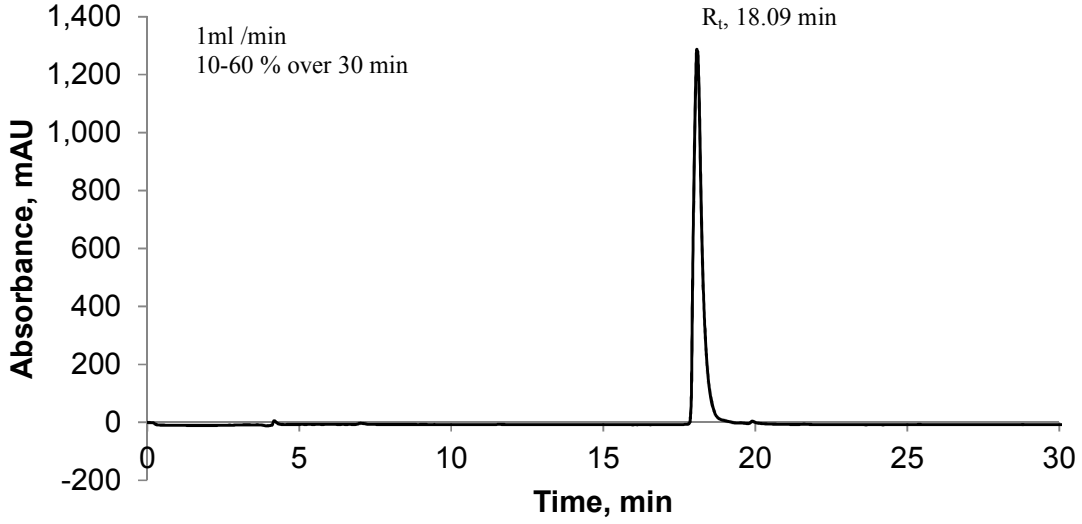
Residue	Chemical Shift (ppm)						$^{3}J_{NH-C^{\alpha}H}$ (Hz)	dδ/dT (ppb)
	NH	$C^{\alpha}H$	$C^{\beta}H$	$C^{\gamma}H$	$C^{\delta}H$	Others		
Fatty acid		2.32	1.61	$C^{\gamma}H$:1.17	1.33/1.40 $C^{\delta}H_3$: 0.87	$C^{\varepsilon}H_3$: 0.85		
Dab 1 (X1)	8.46	4.38	2.20/2.10	3.13 /3.08		7.83	6.6	-4.8
Dab 2 (X2)	8.76	4.45	2.13/2.25	3.09		7.83	7.0	-6.1
Dab 3 (X3)	8.51	4.40	2.22/2.13	3.12		7.83	7.6	-8.3
Leu 4 (L4)	8.04	4.26	1.39	1.33	0.84/0.80		7.0	-7.0
Phe 5 (F5)	8.37	4.65	3.19/3.03			7.30, 7.40, 7.35	7.0	-9.0
Dab 6 (X6)	8.53	4.46	2.77/2.61	2.16/1.93		7.74	7.6	-4.1
Dab 7 (X7)	8.54	4.39	3.12	2.23		7.83	7.3	-6.7
Leu 8 (L8)	8.39	4.33	1.71, 1.67	1.60	0.95/0.90		7.2	-6.9
C-ter. NH_2						7.67/7.05		

Figure S1: Analytical RP-HPLC traces (absorbance at 214 nm) and ESI-MS spectra of all peptides

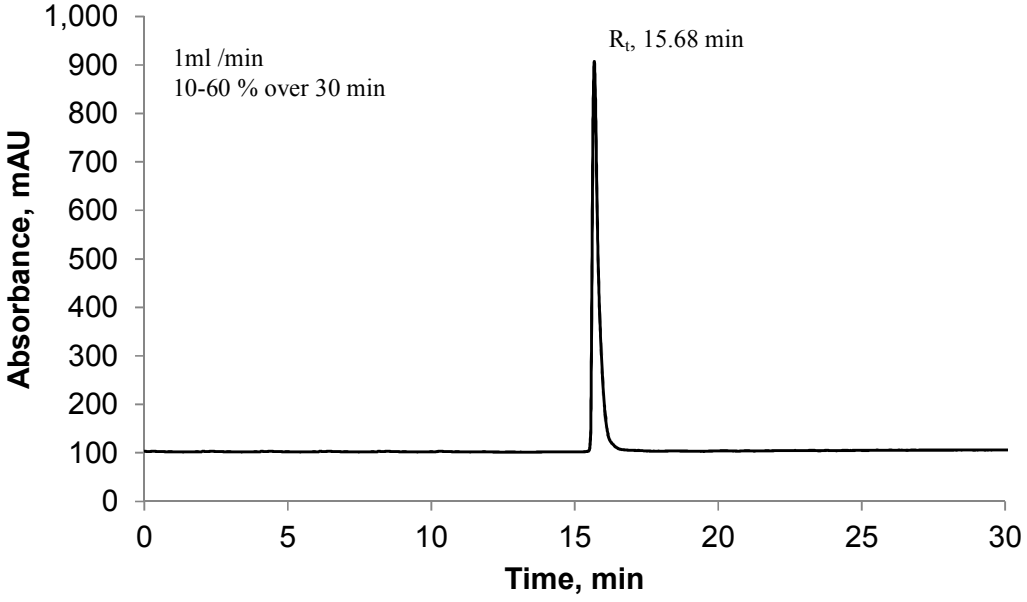
Battacin (**10**)



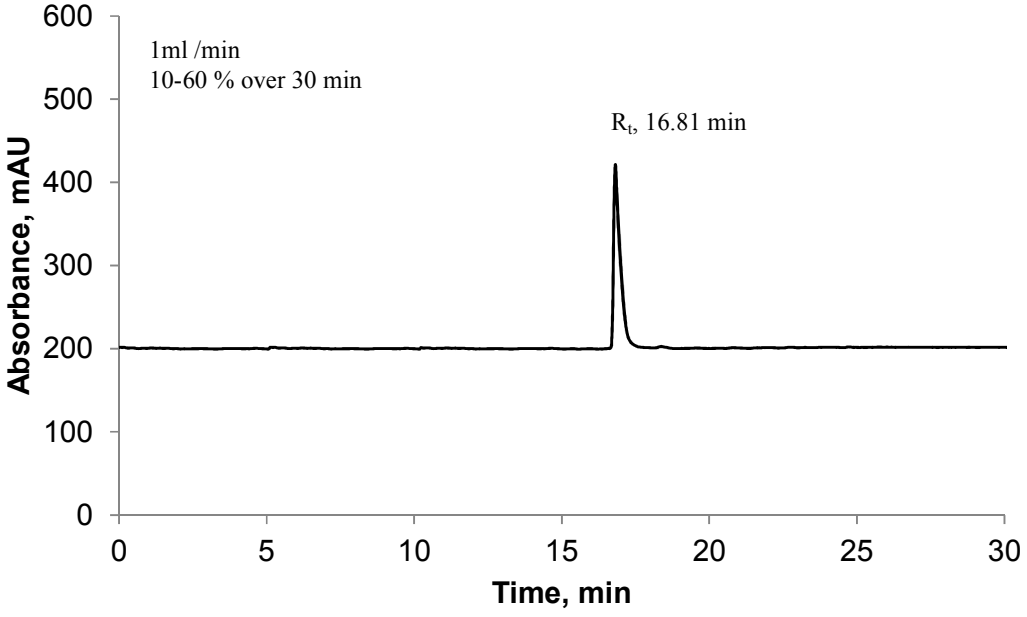
Fmoc-D-Dab-*cyclo*[Dab-Dab-Leu-D-Phe-Dab-Dab-Leu] (**12**)



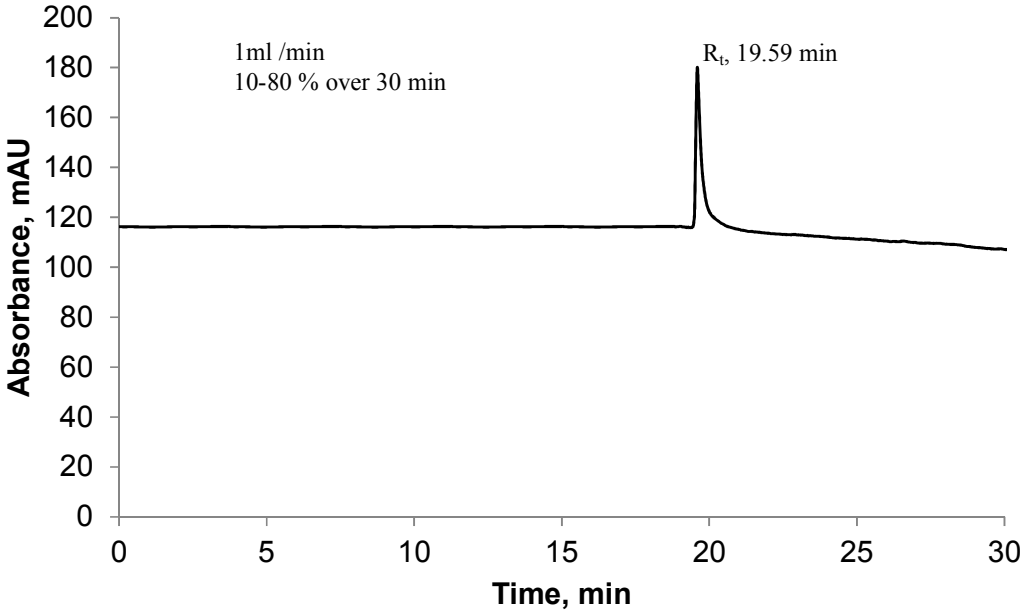
R^1-D-Dab-*cyclo*[Dab-Dab-Leu-D-Phe-Dab-Dab-Leu] (**13**)



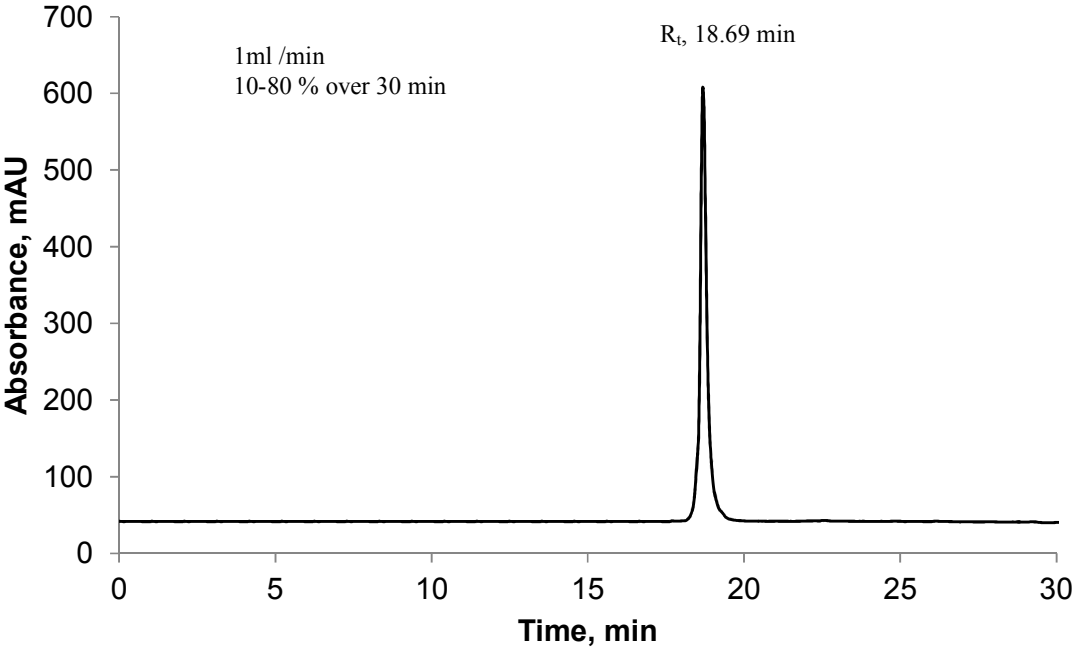
R^2-D-Dab-*cyclo*[Dab-Dab-Leu-D-Phe-Dab-Dab-Leu] (**14**)



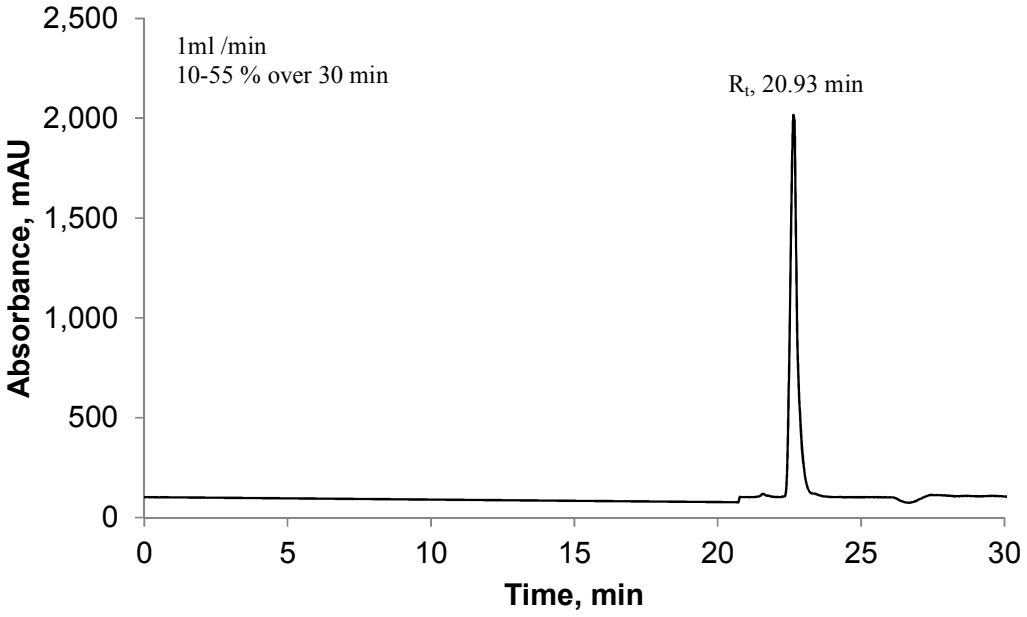
R^3-D-Dab-*cyclo*[Dab-Dab-Leu-D-Phe-Dab-Dab-Leu] (**15**)



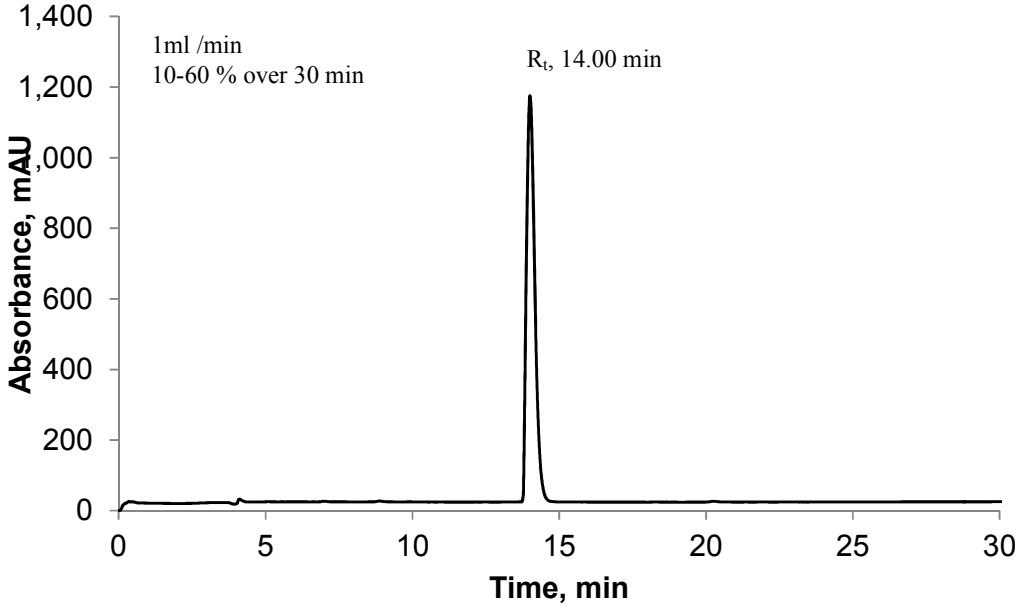
R^3-D-Lys-*cyclo*[Lys-Lys-Leu-D-Phe-Lys-Lys-Leu] (**16**)



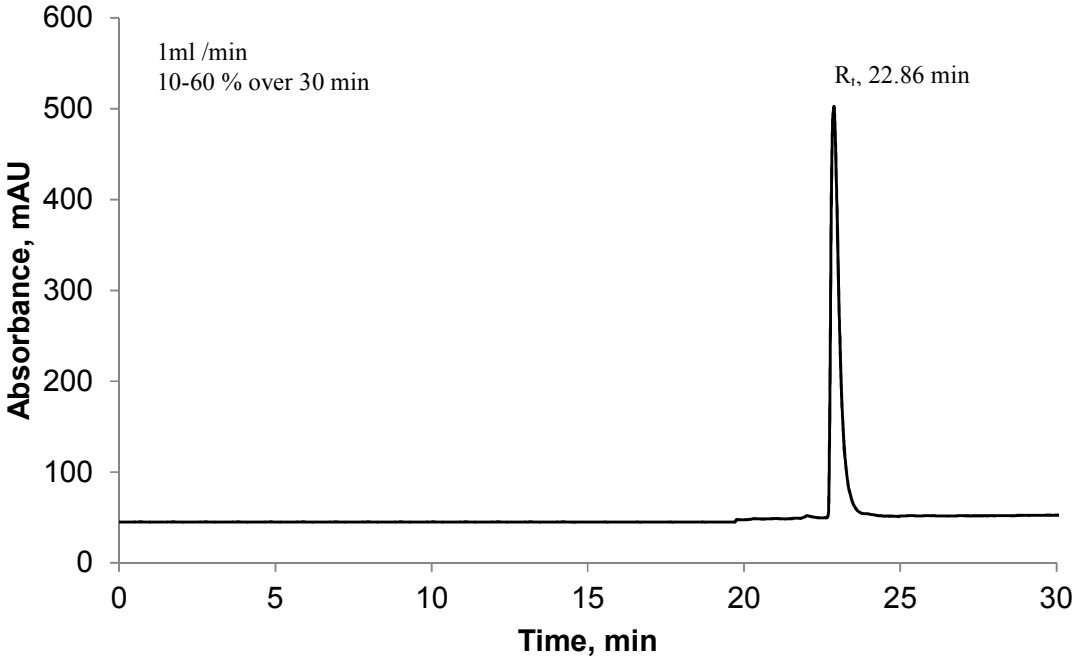
R^1-D-Dab-Dab-Dab-Leu-D-Phe-Dab-Dab-Leu-NH_2 (**17**)



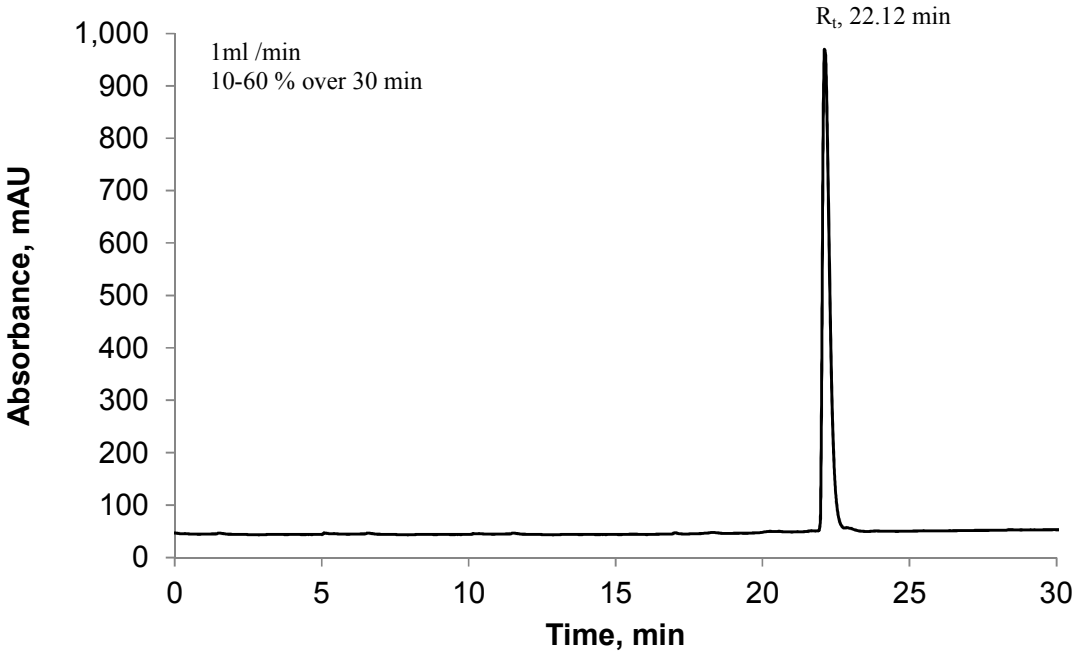
R^2-D-Dab-Dab-Dab-Leu-D-Phe-Dab-Dab-Leu-NH_2 (**18**)



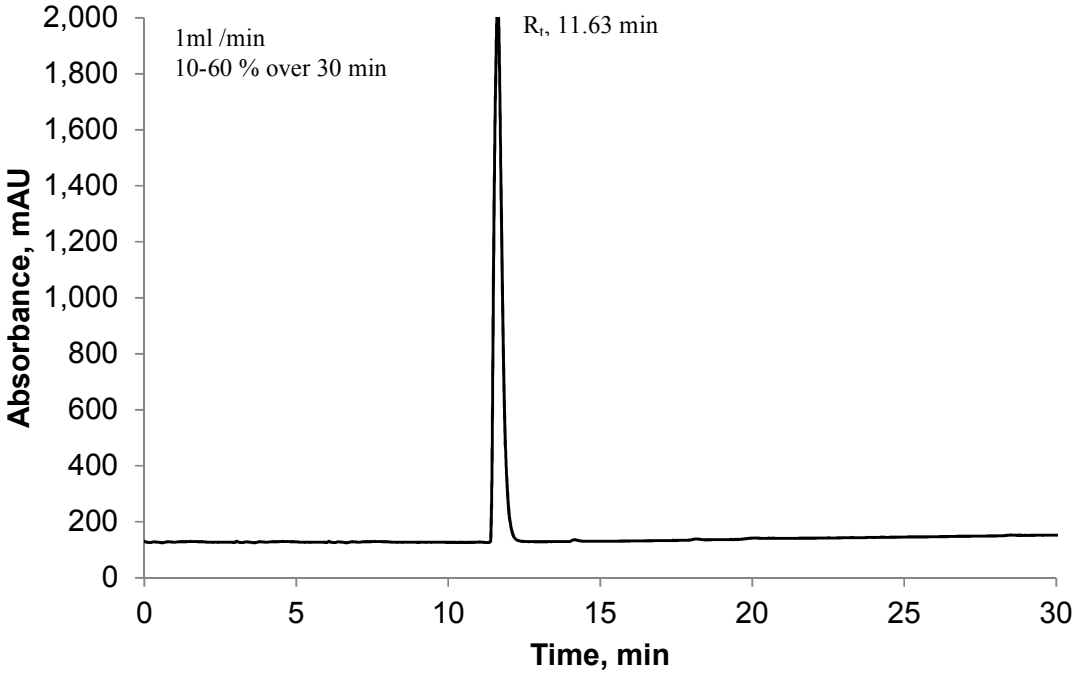
R^3-D-Dab-Dab-Dab-Leu-D-Phe-Dab-Dab-Leu-NH_2 (**19**)



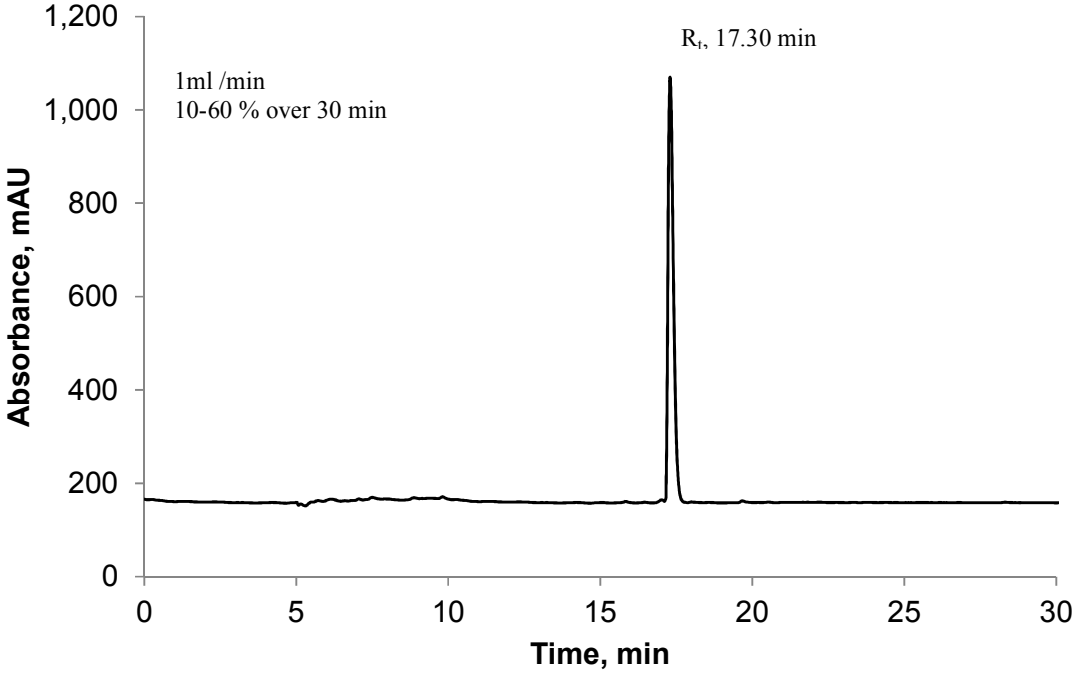
R^3-D-Lys-Lys-Lys-Leu-D-Phe-Lys-Lys-Leu-NH_2 (**20**)



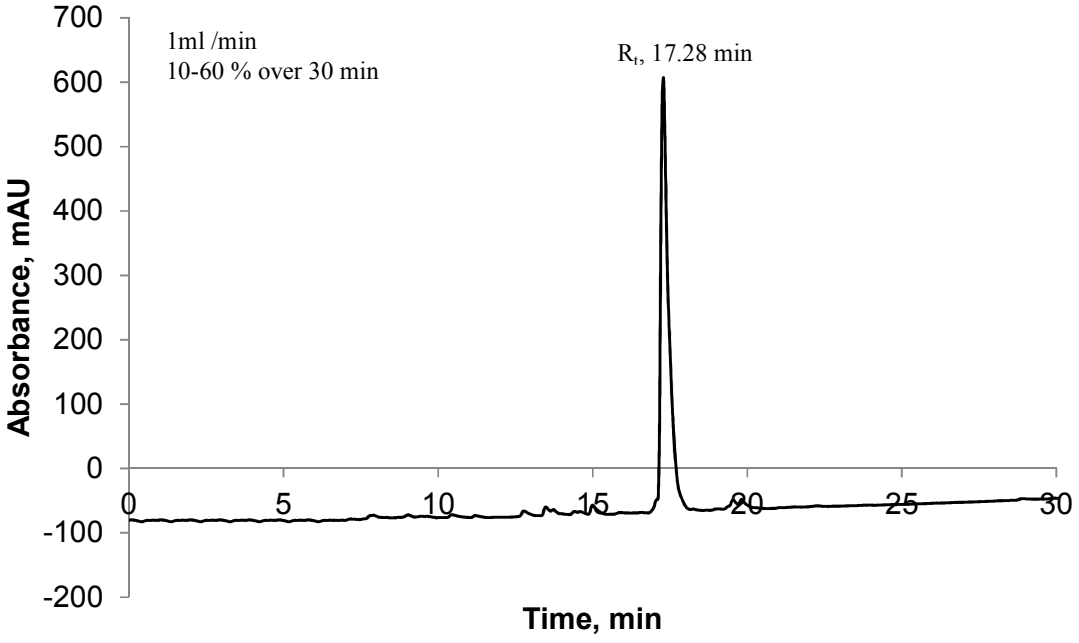
NH_2-D-Dab-Dab-Dab-Leu-D-Phe-Dab-Dab-Leu-NH_2 (**29**)



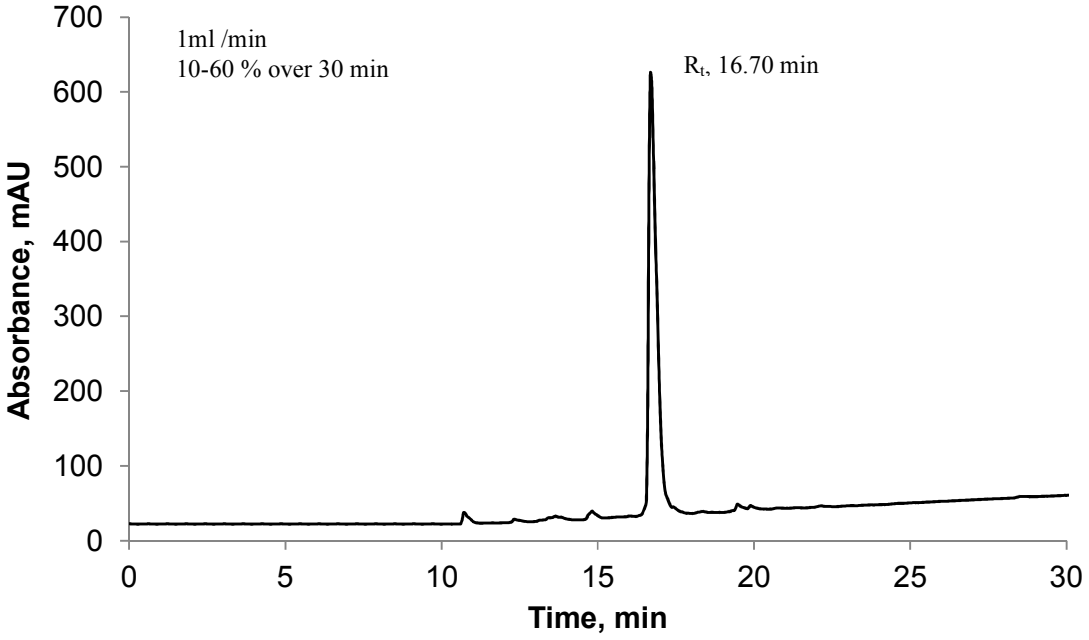
R^1-D-Dab-Dab-Leu-D-Phe-Dab-NH_2 (**30**)



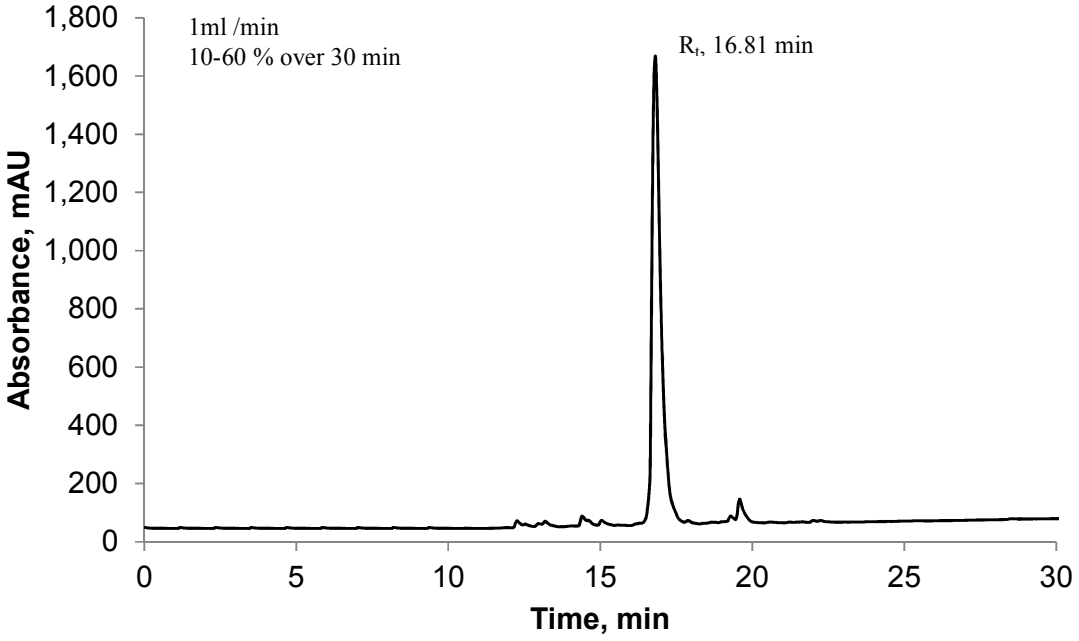
R^1-Ala-Dab-Dab-Leu-D-Phe-Dab-Dab-Leu-OH (**28**)



R^1-D-Dab-Ala-Dab-Leu-D-Phe-Dab-Dab-Leu-OH (**27**)



R^1-D-Dab-Dab-Ala-Leu-D-Phe-Dab-Dab-Leu-OH (**26**)



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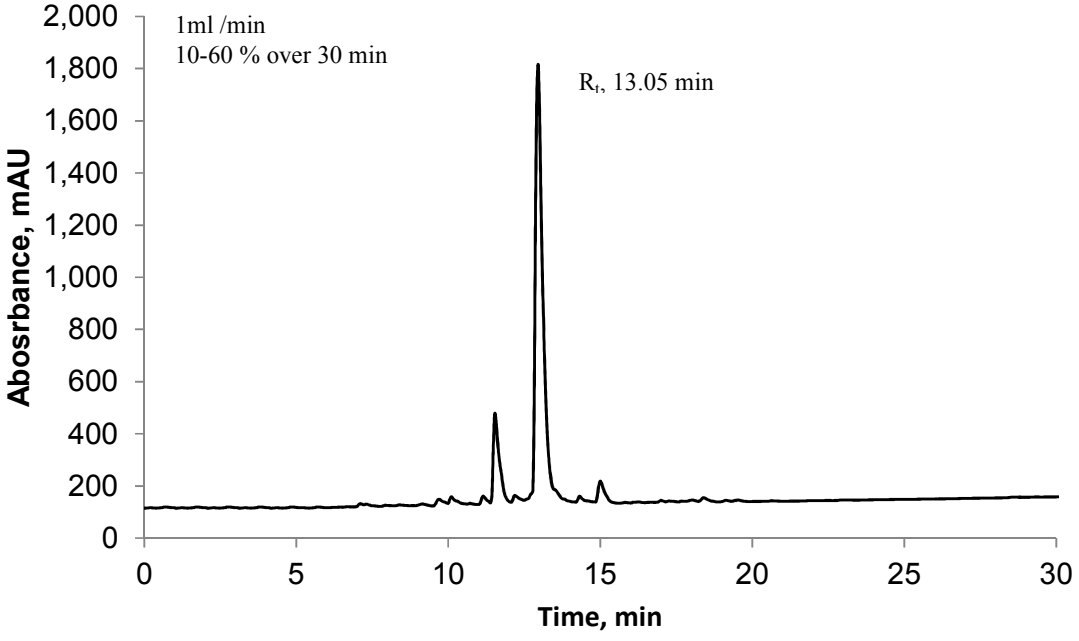
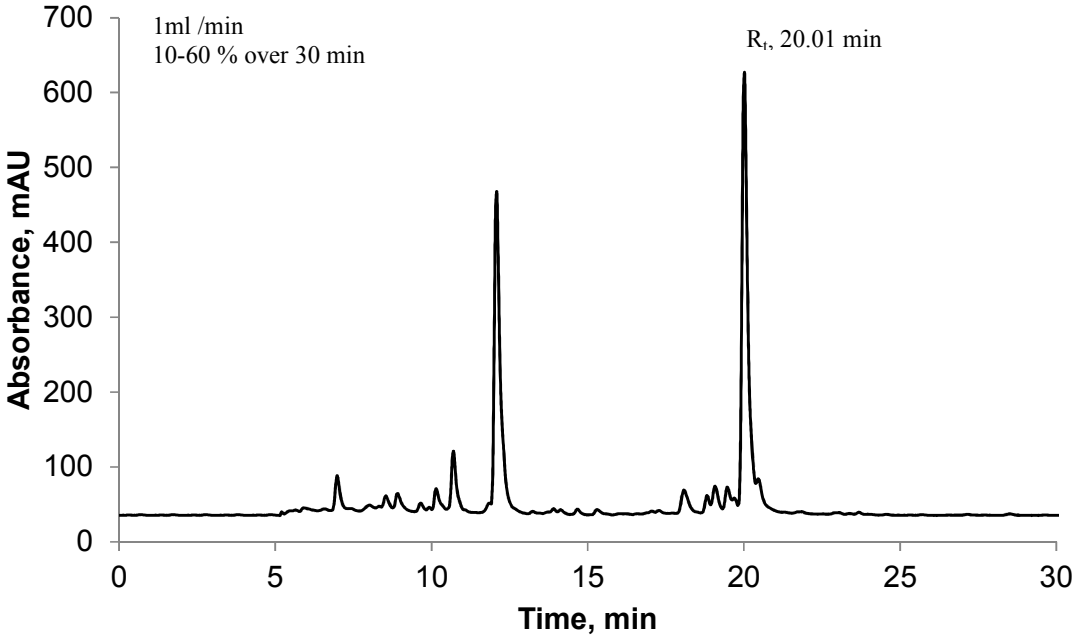


R^1-D-Dab-Dab-Dab-Leu-Ala-Dab-Dab-Leu-OH (**24**)



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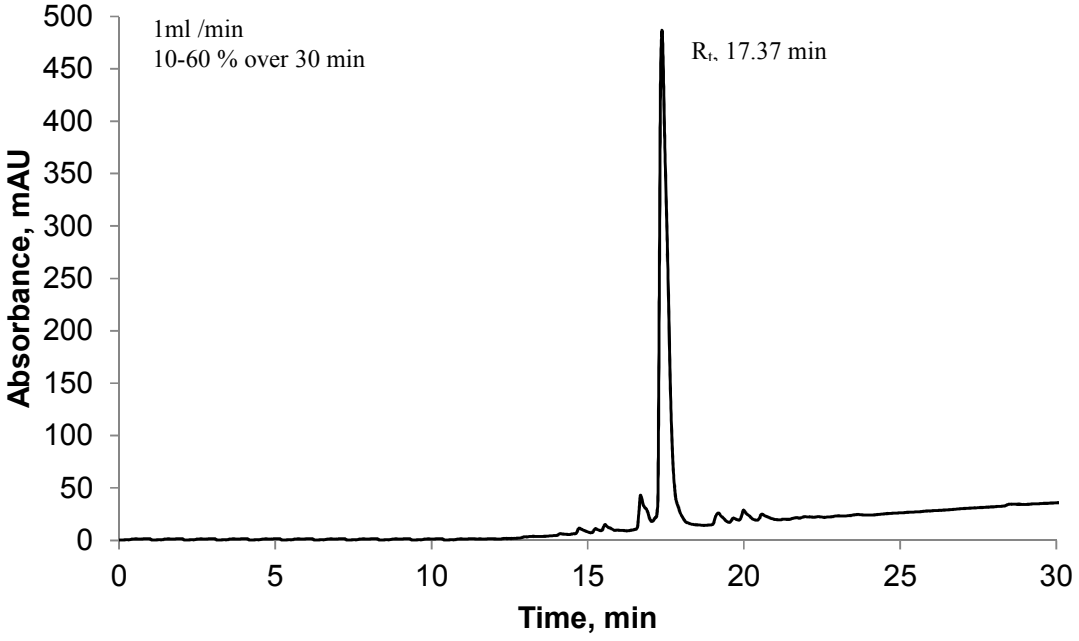

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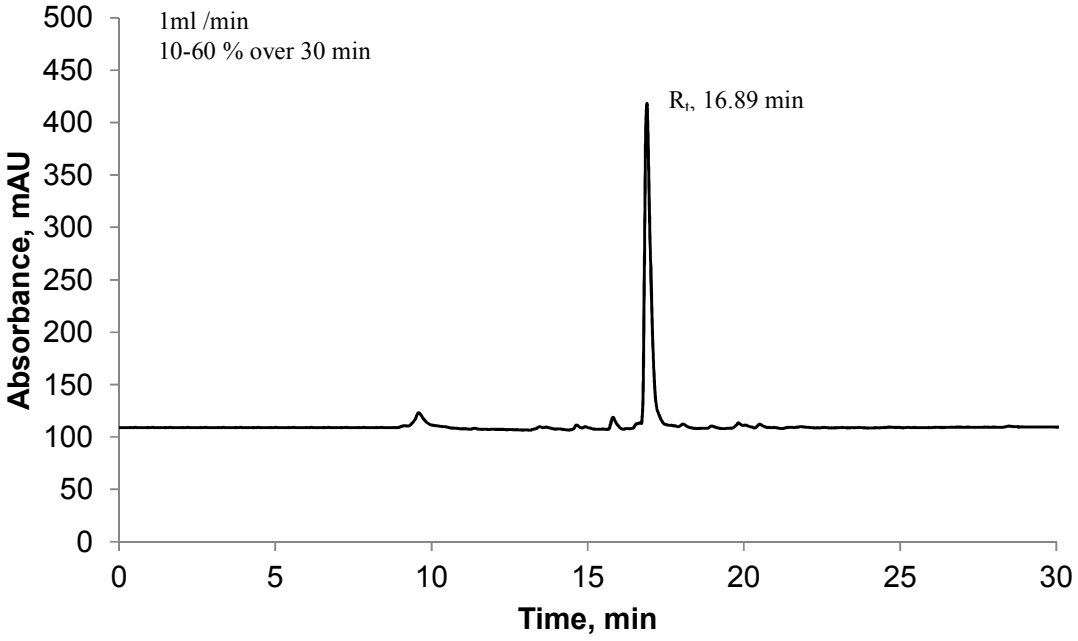

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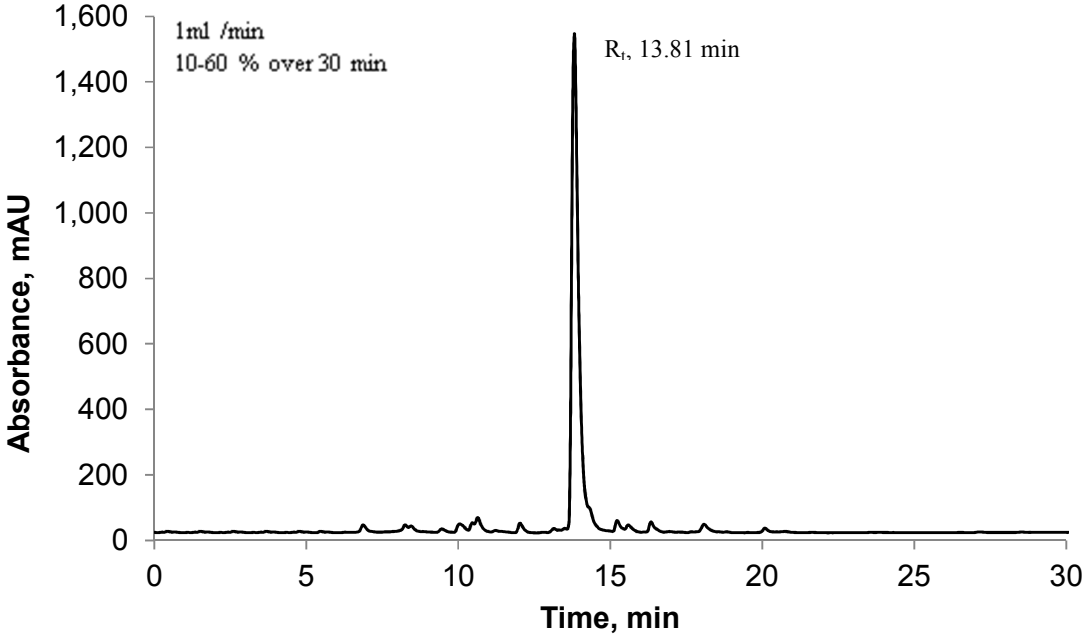
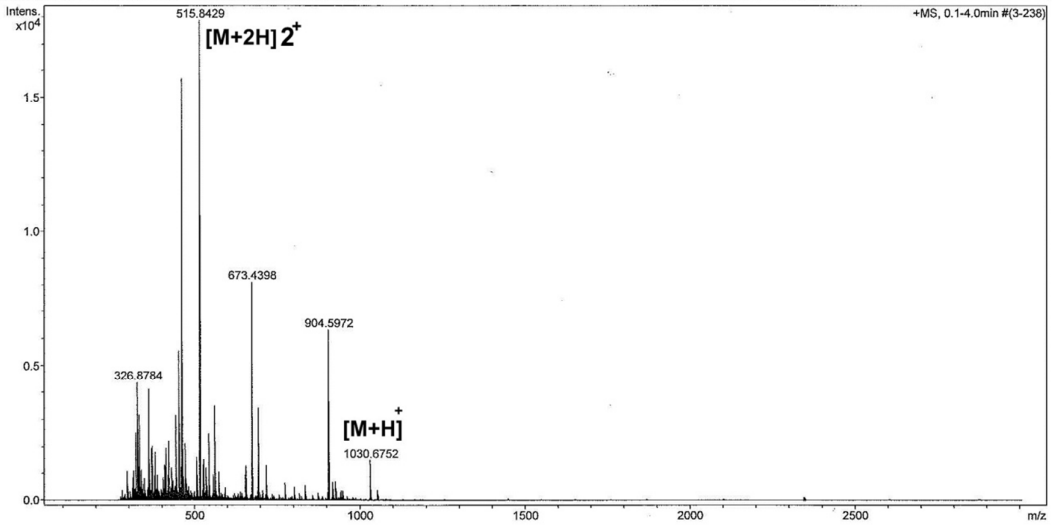


ESI-MS Spectra

Battacin (**10**) ESI-MS (Exact mass = 1029.6699)



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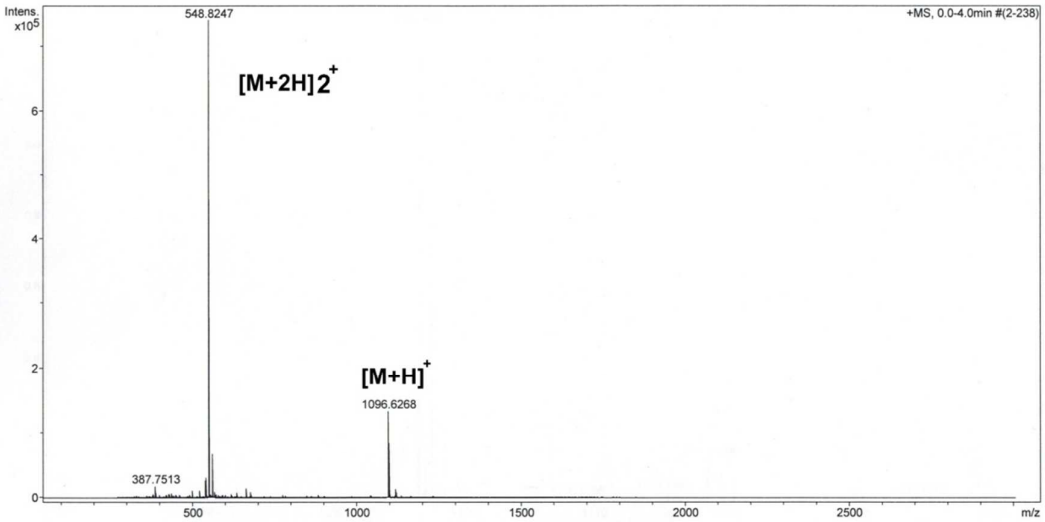

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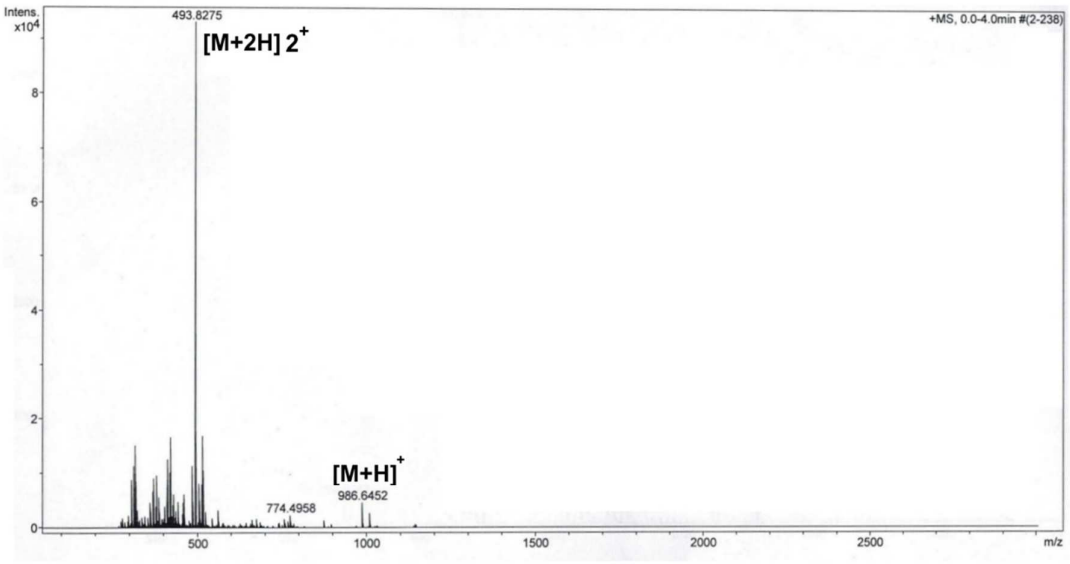

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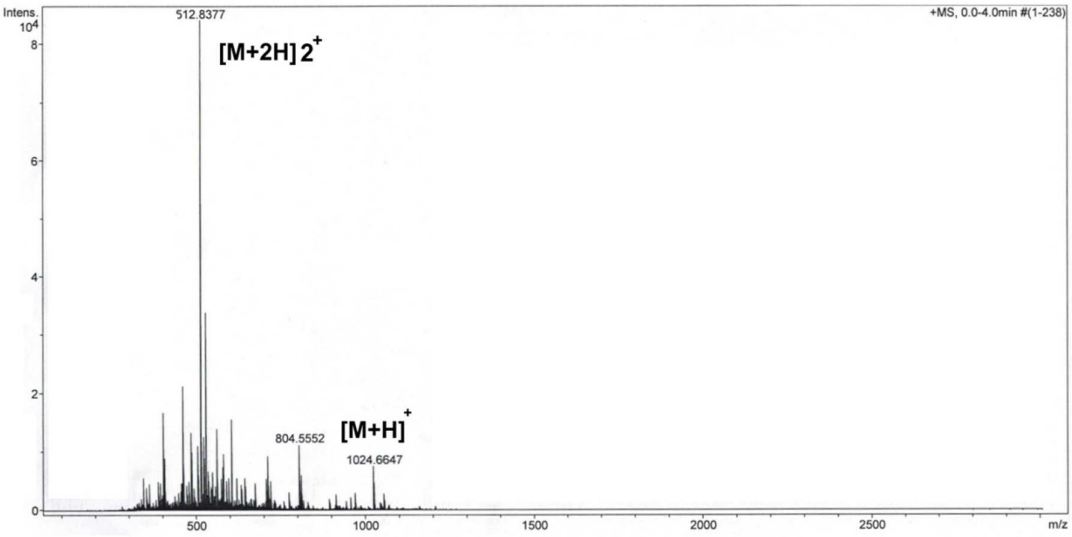

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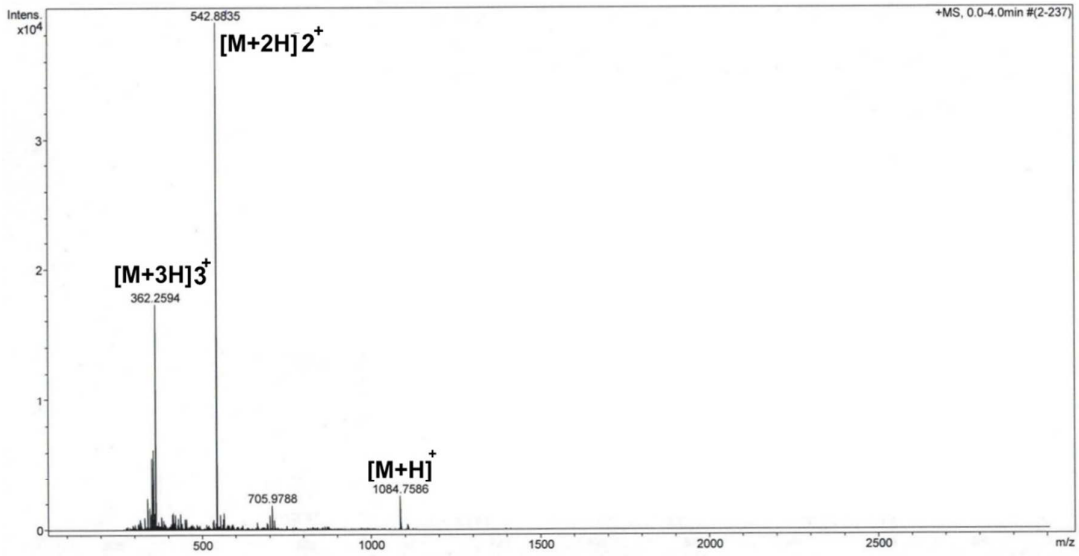

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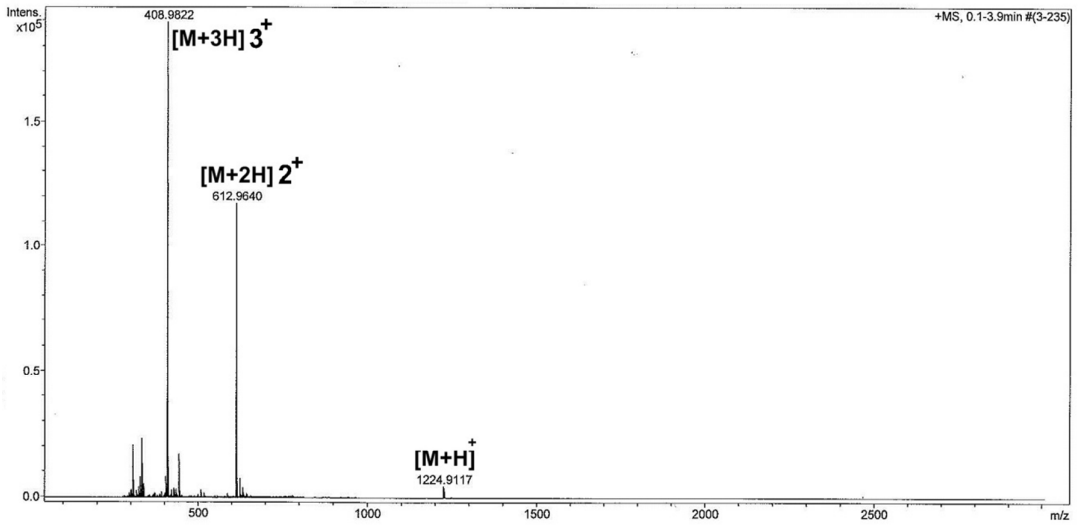

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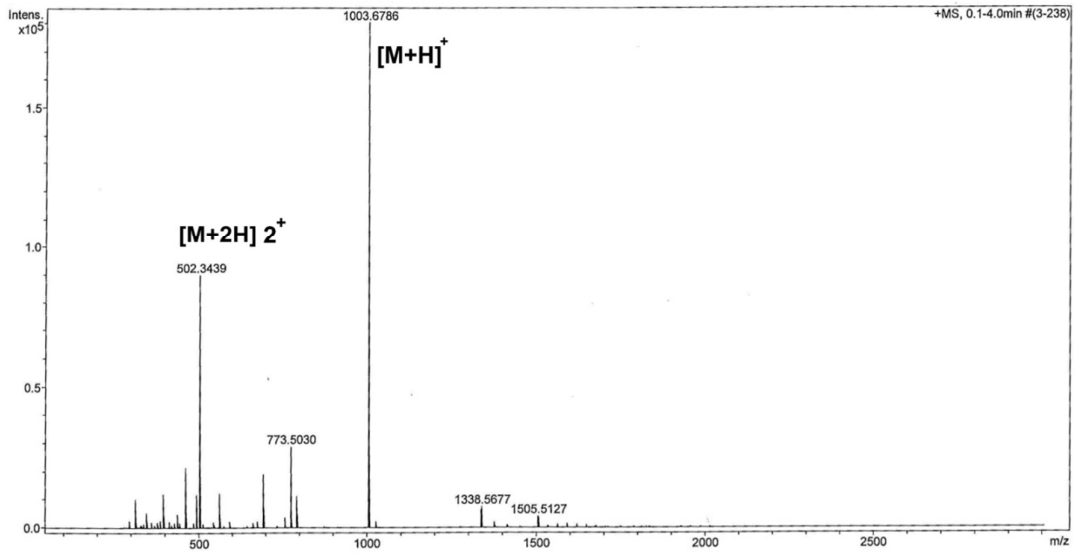

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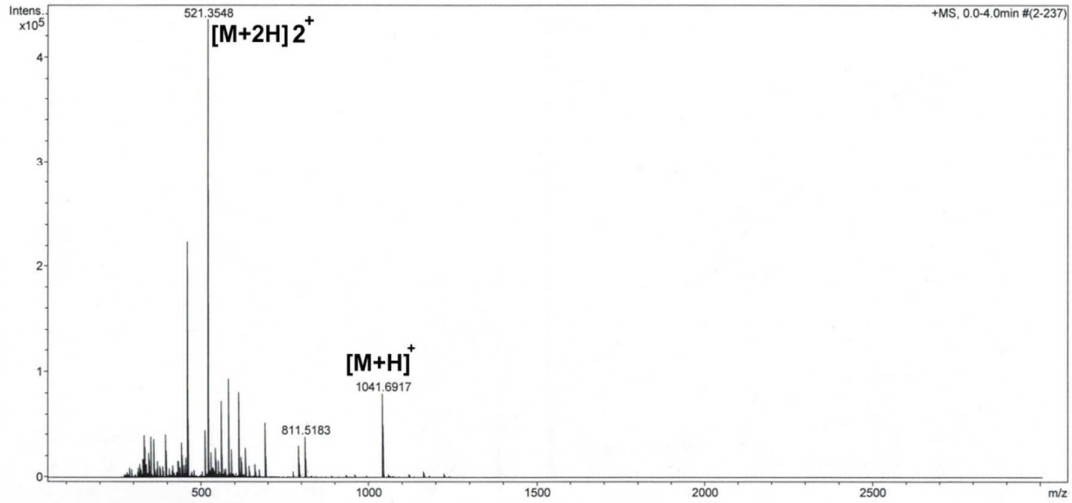

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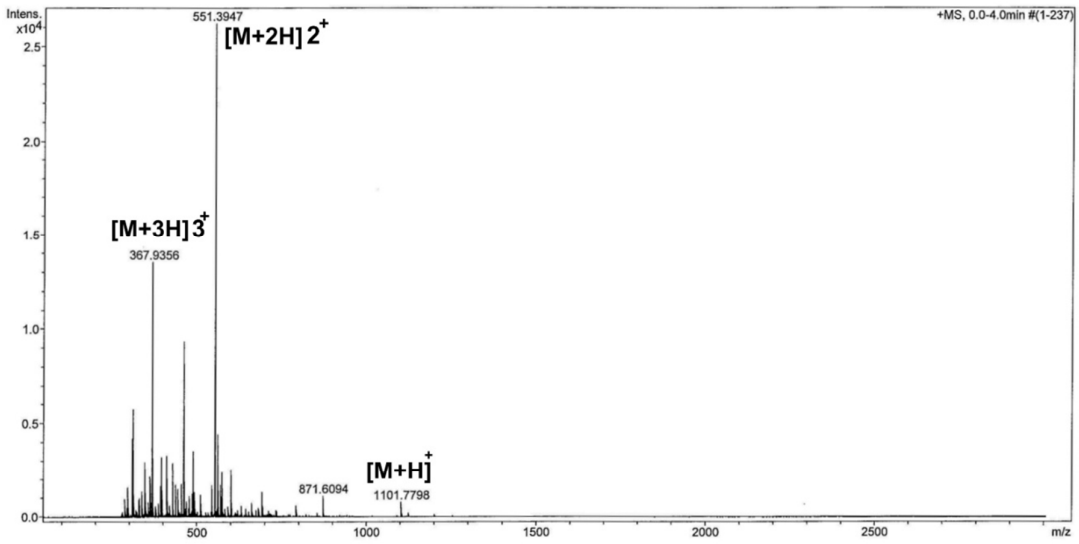

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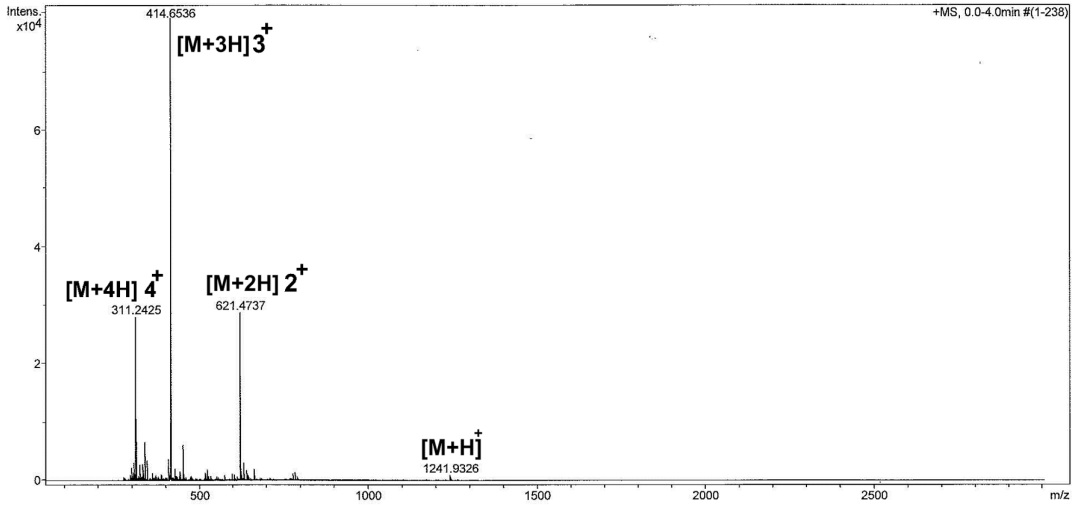

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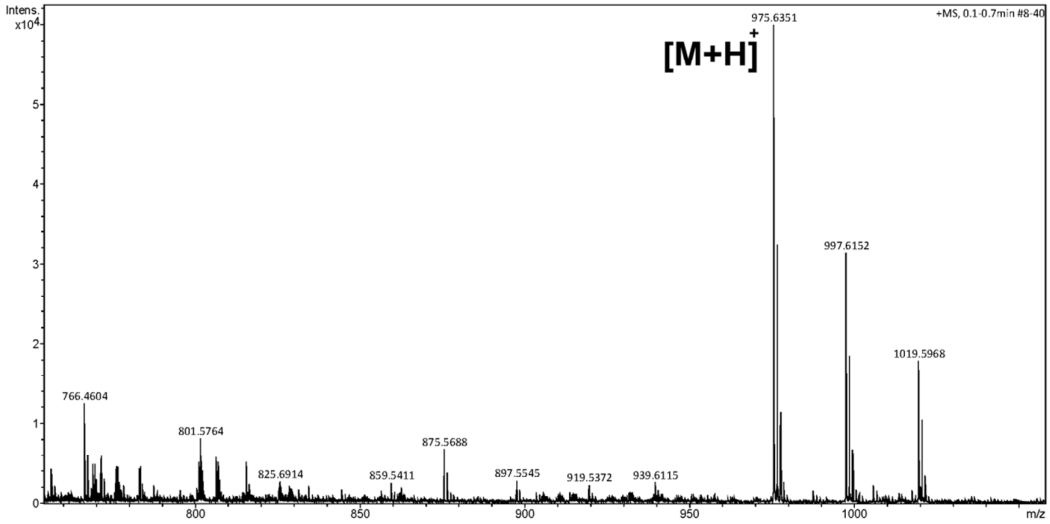

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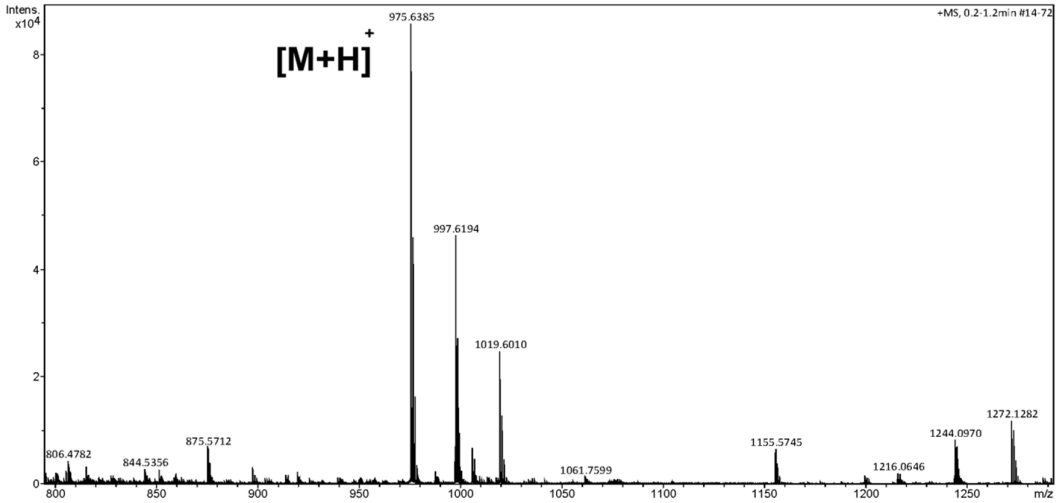
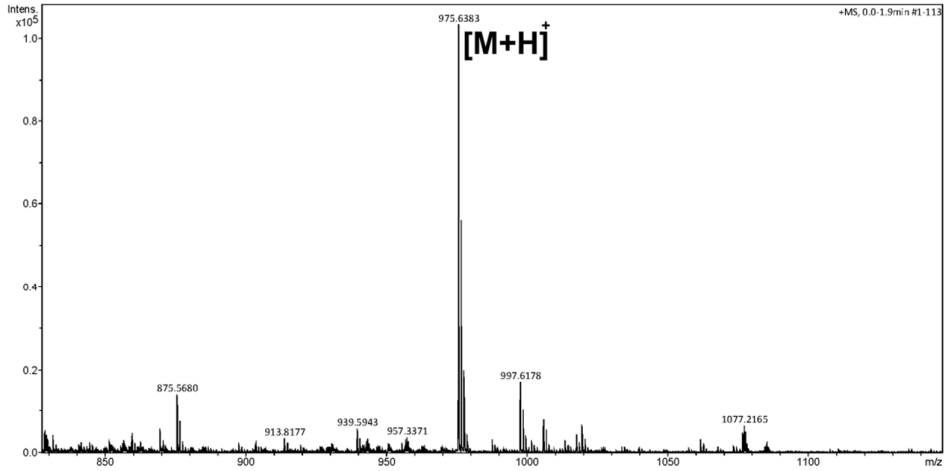
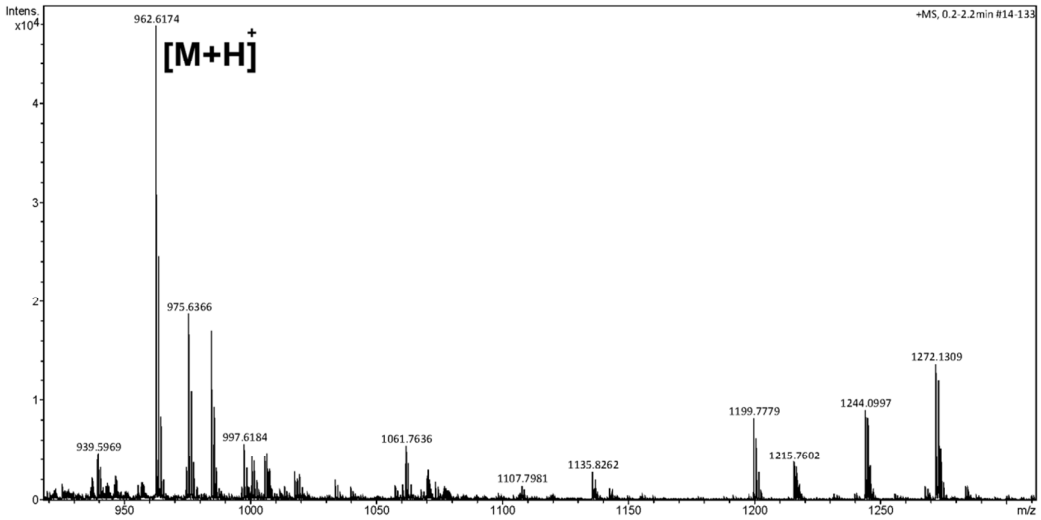


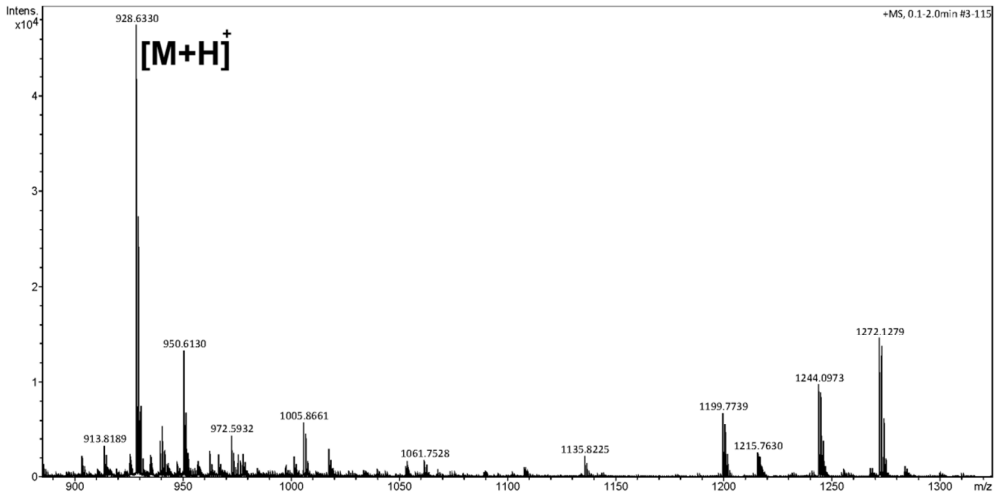
26 ESI-MS $[M+H]^+$ = 975.6350



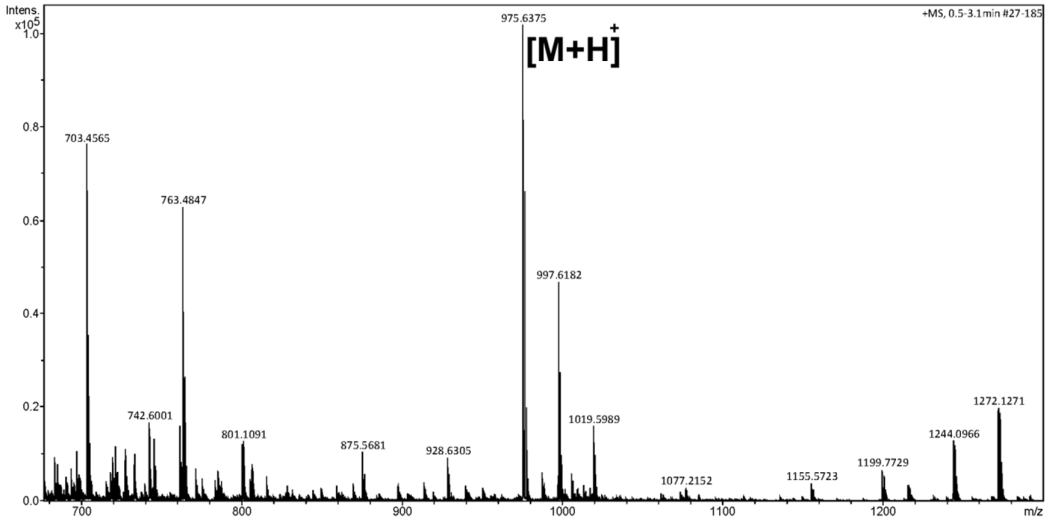
25 ESI-MS $[M+H]^+$ = 962.6146



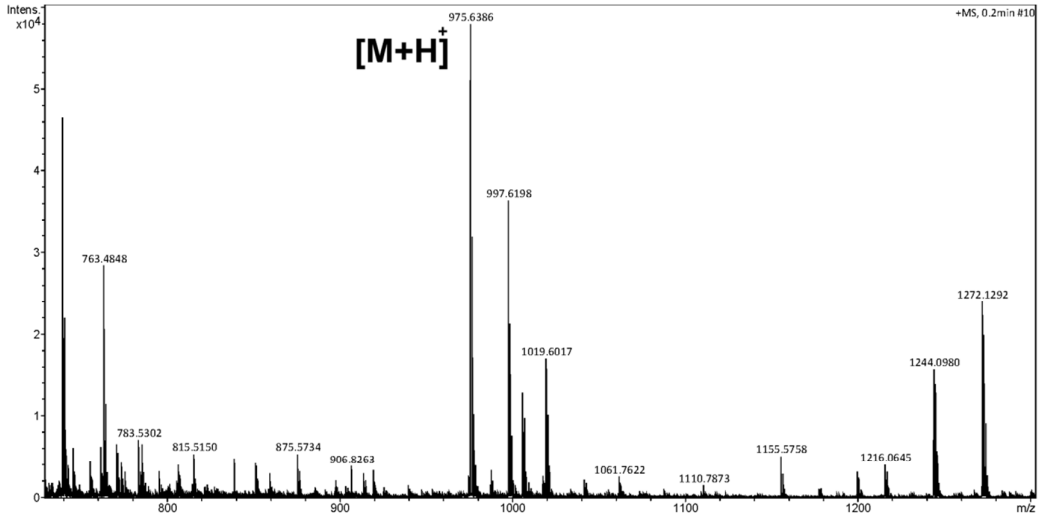
24 ESI-MS $[M+H]^+$ = 928.6302



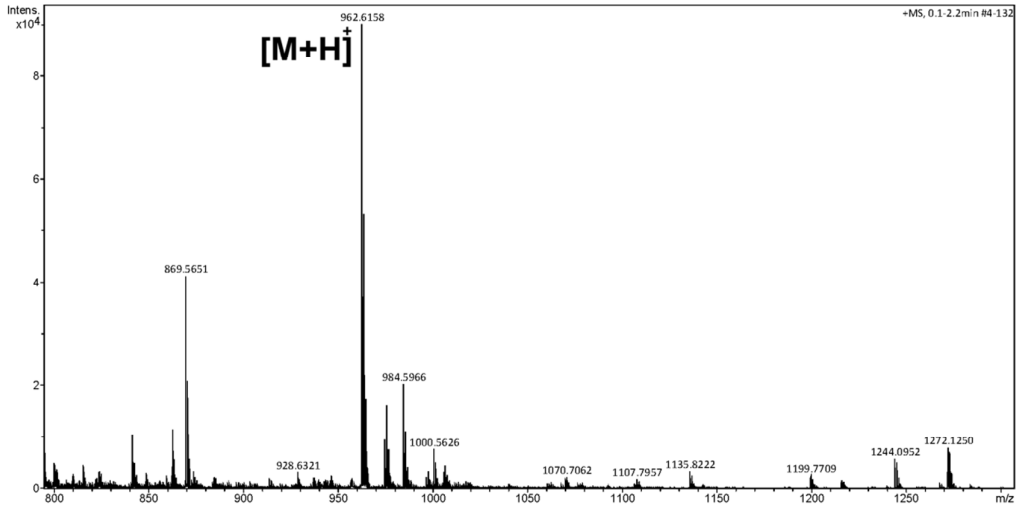
23 ESI-MS $[M+H]^+$ = 975.6350



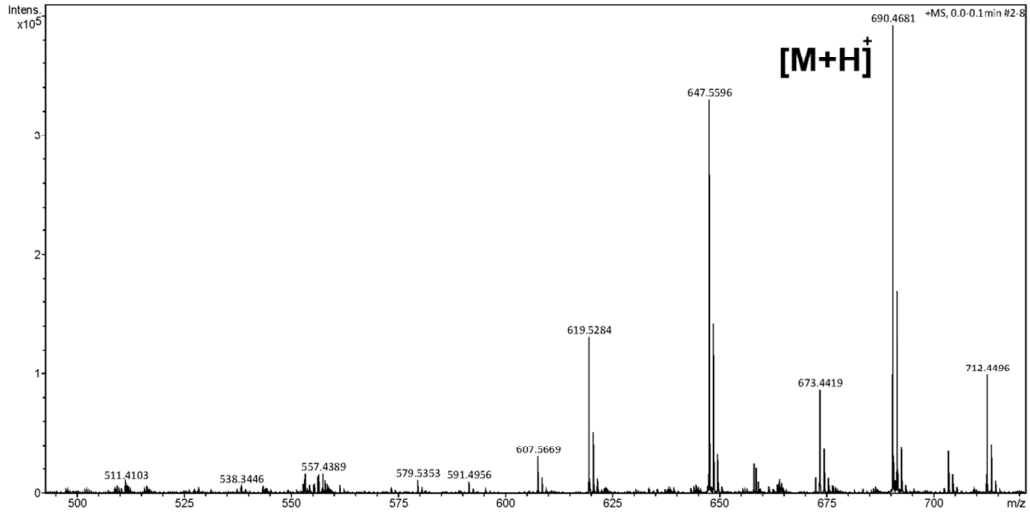
22 ESI-MS $[M+H]^+$ = 975.6350



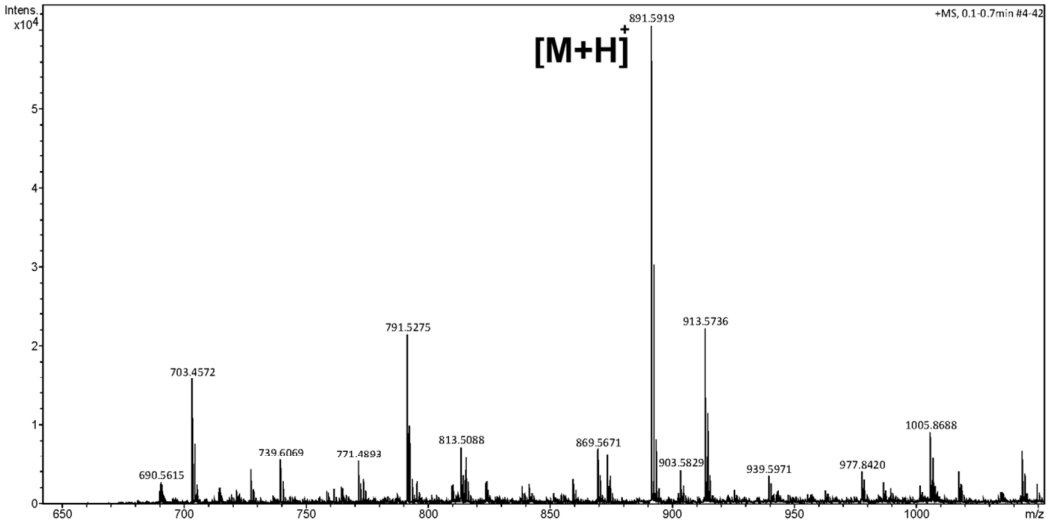
21 ESI-MS $[M+H]^+$ = 962.6146



30 ESI-MS $[M+H]^+$ = 690.4661



29 ESI-MS $[M+H]^+$ = 891.5887



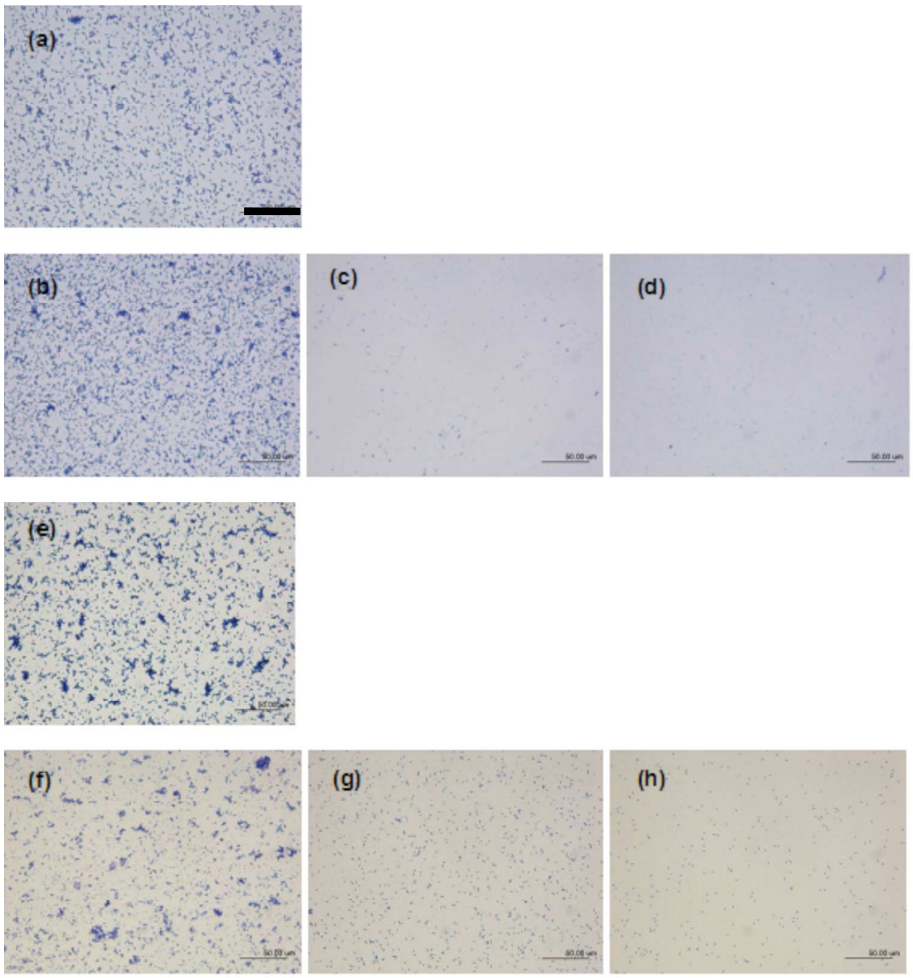


Figure S2: Representative crystal violet stained images of Psa 16207 biofilms at 48 hours in the absence (a) and presence (b-d) of the lipopeptide **17** at (b) 1 μM, (c) 5 μM and (d) 50 μM respectively. The effect of the lipopeptide at 1, 5 and 50 μM on preformed (for 48 hours) Psa16027 biofilms is shown in images (f) to (h) respectively. (e) Untreated preformed biofilms of Psa16027. Bar = 50 μm.

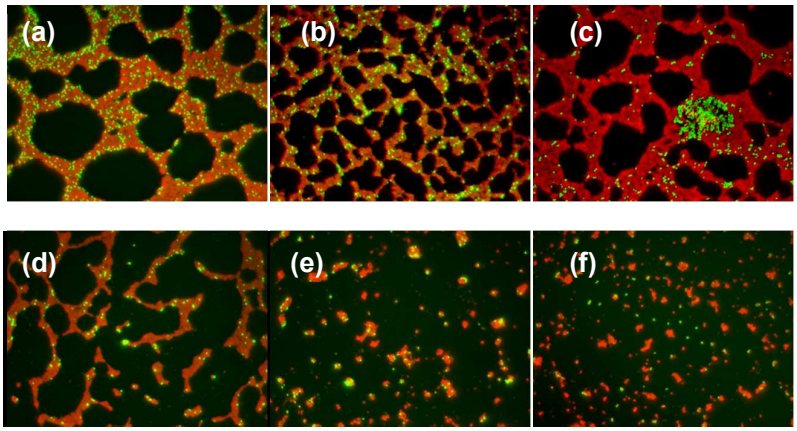


Figure S3: Effect of treating *S. aureus* biofilms with **17** at various concentrations. (a) Control biofilm with no peptide; (b) 5 μM; (c) 10 μM; ; (d) 25 μM; (e) 50 μM; ; (f) 100 μM. Biofilms were allowed to form for 48 hours in the presence of the various peptide concentrations.

R^1-D-Dab-Dab-Dab-Leu-D-Phe-Dab-Dab-Leu-NH_2 (**17**)

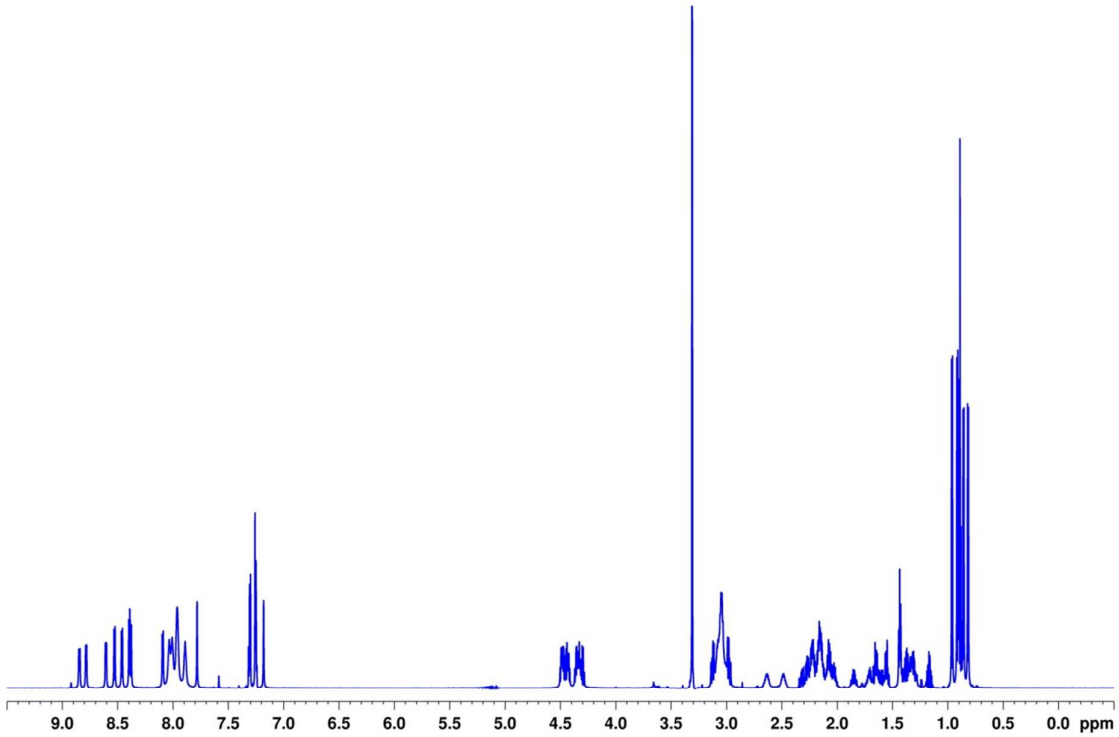


Figure S4: ^{1}H NMR spectrum of **17** in CD_3OH at 278K

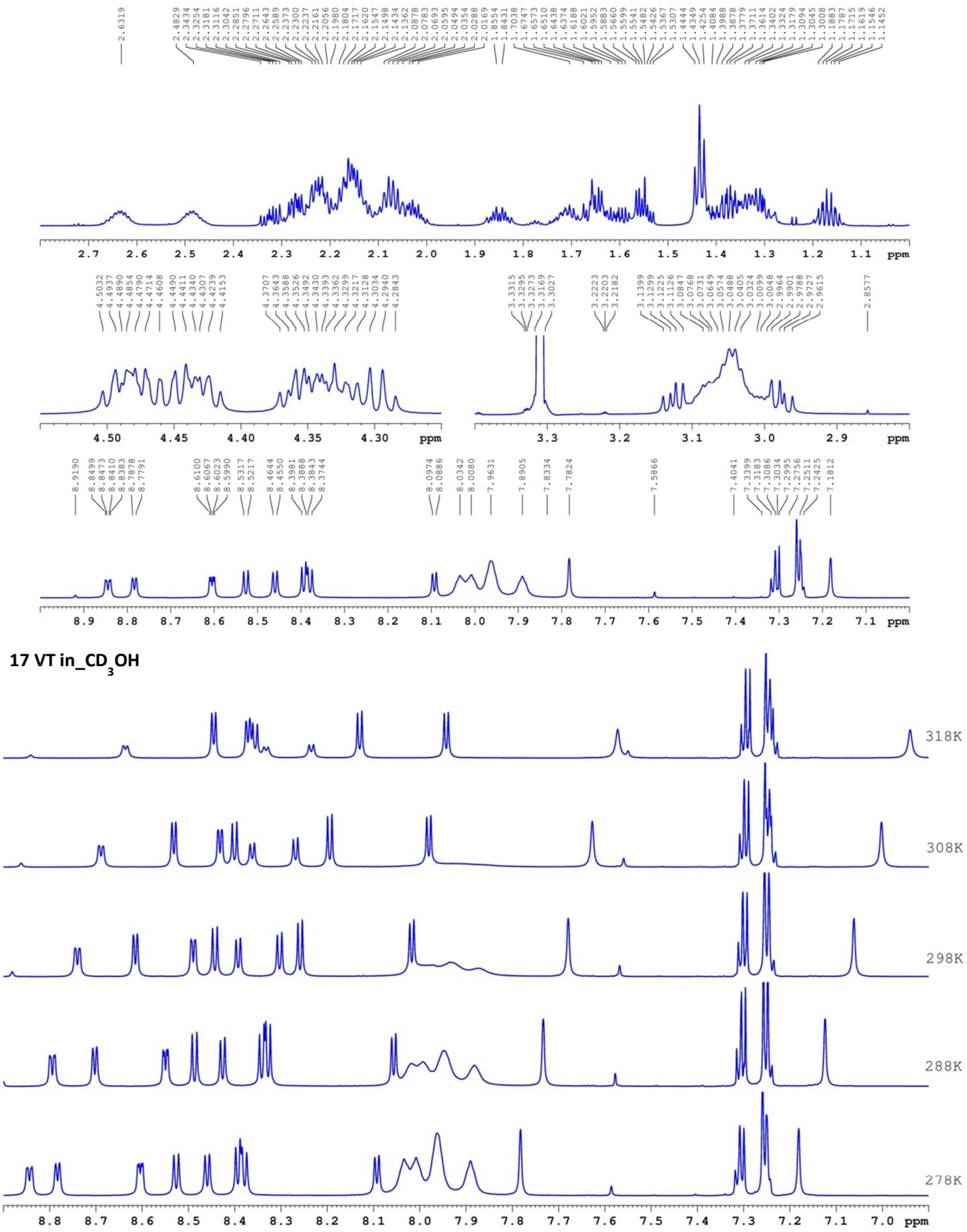


Figure S5: (top) Expansion of the various regions of the ^{1}H NMR spectrum of **17** in CD_3OH at 278K. (Bottom) Variation of the amide proton chemical shifts with temperature

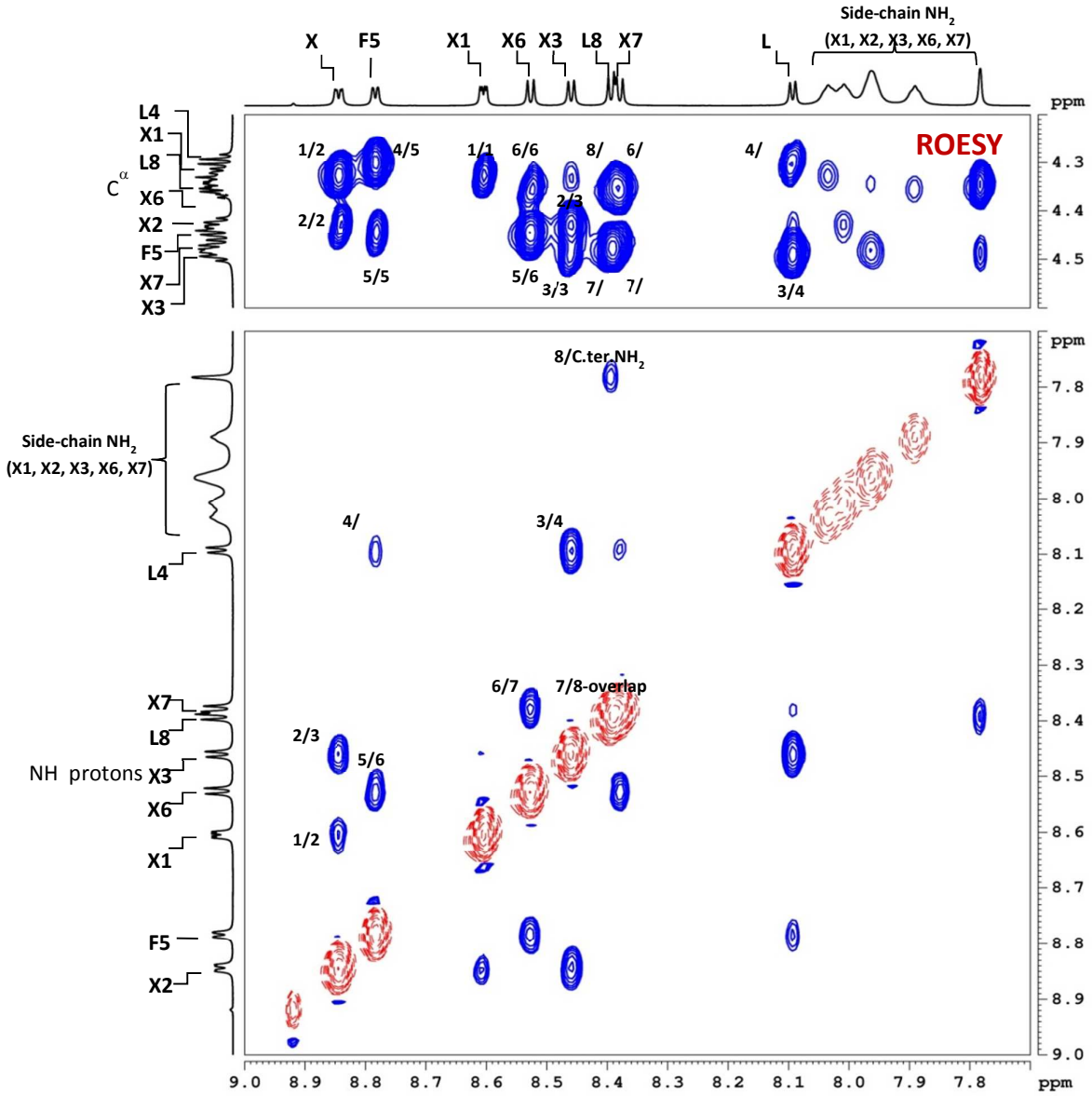


Figure S6: Partial ROESY spectrum of **17** in CD_3OHshowing $C^{\alpha}H$-NH and NH-NH NOEs

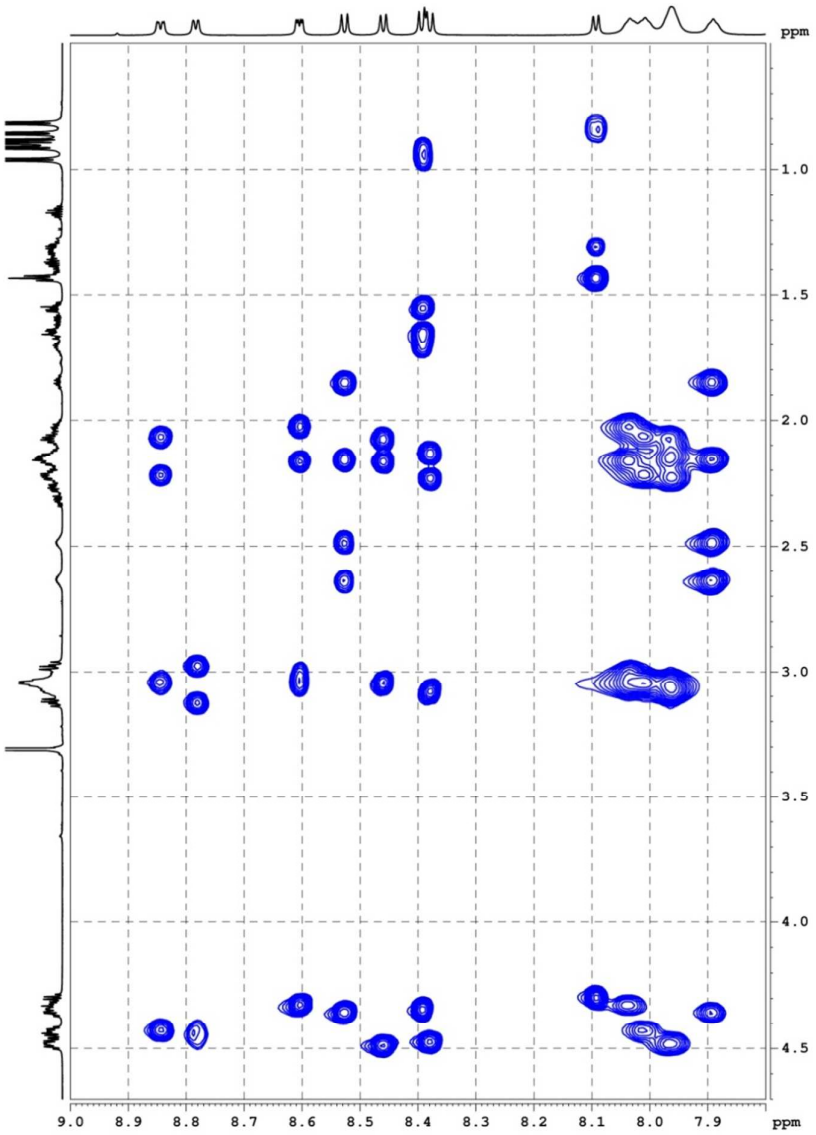


Figure S7: Partial 800 MHz TOCSY spectrum of **17** in CD_3OH at 278K

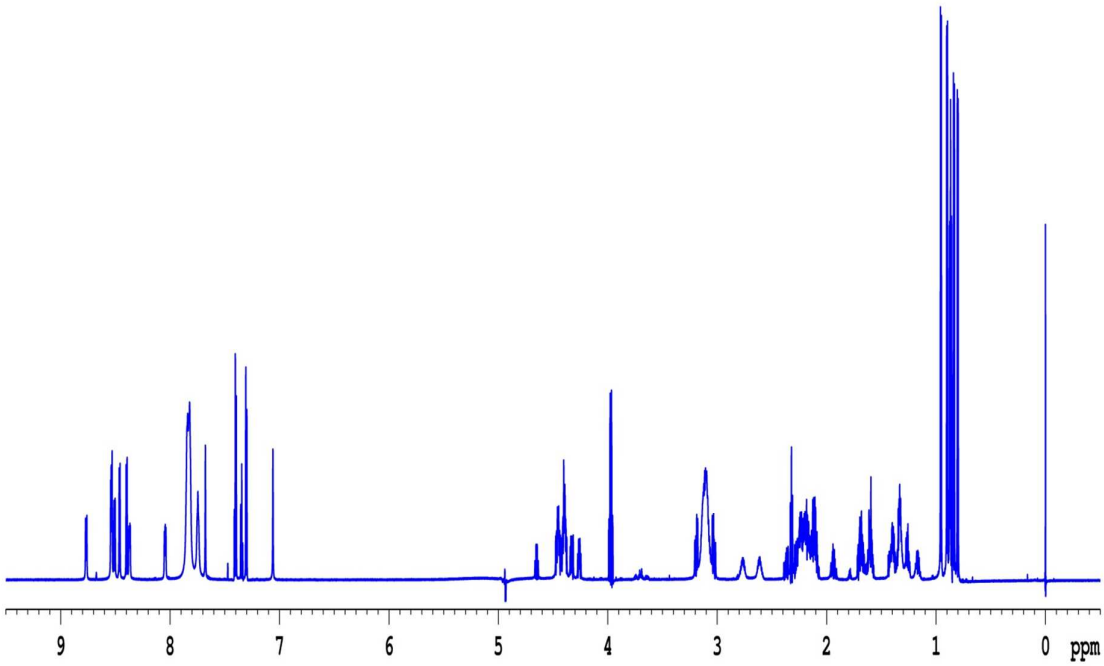


Figure S8: ^{1}H NMR spectrum of **17** in water (70%) and TFE (30%) at 278K

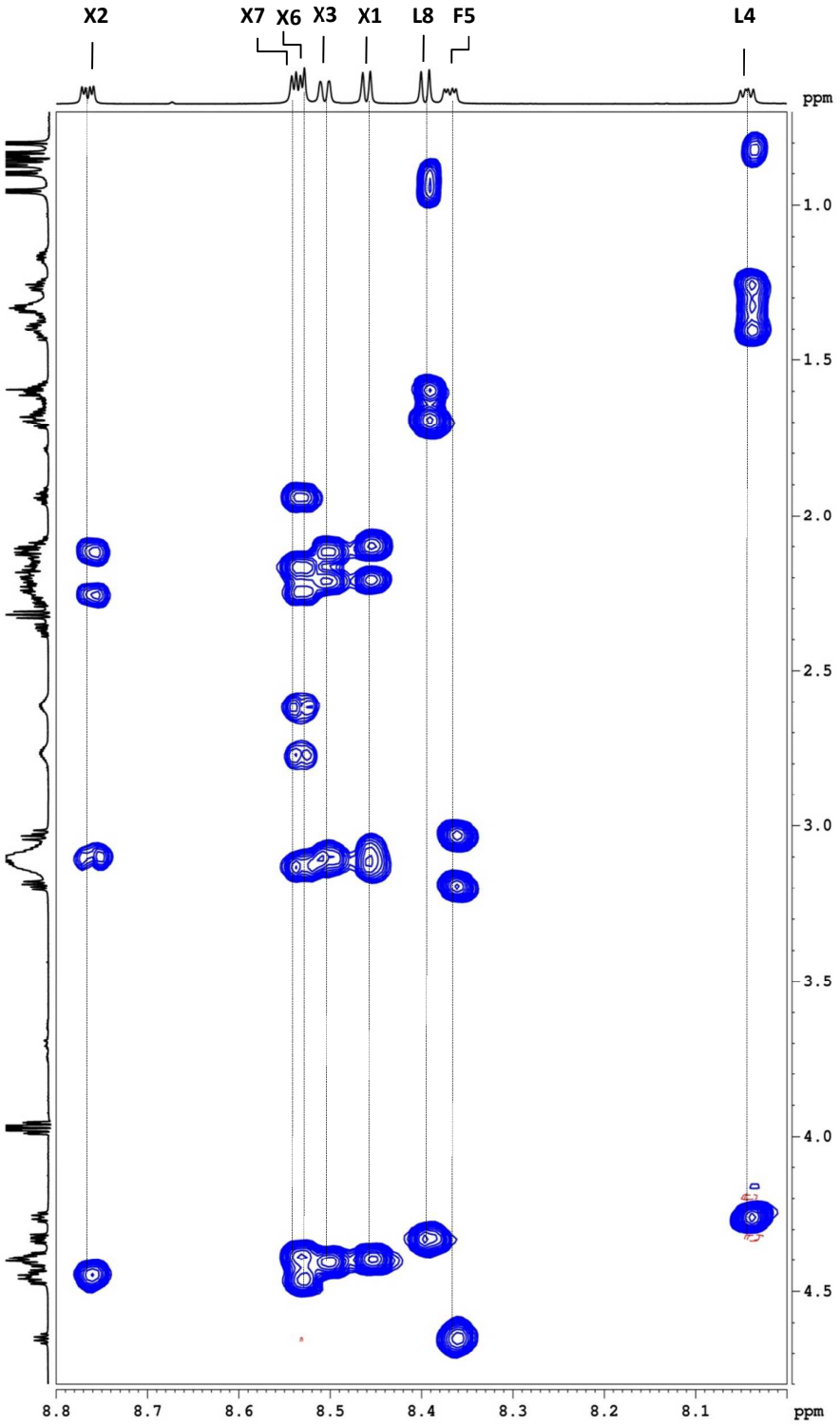


Figure S9: Partial 800 MHz TOCSY spectrum of **17** in H_2O (70%) and TFE (30%) showing residue specific assignments.

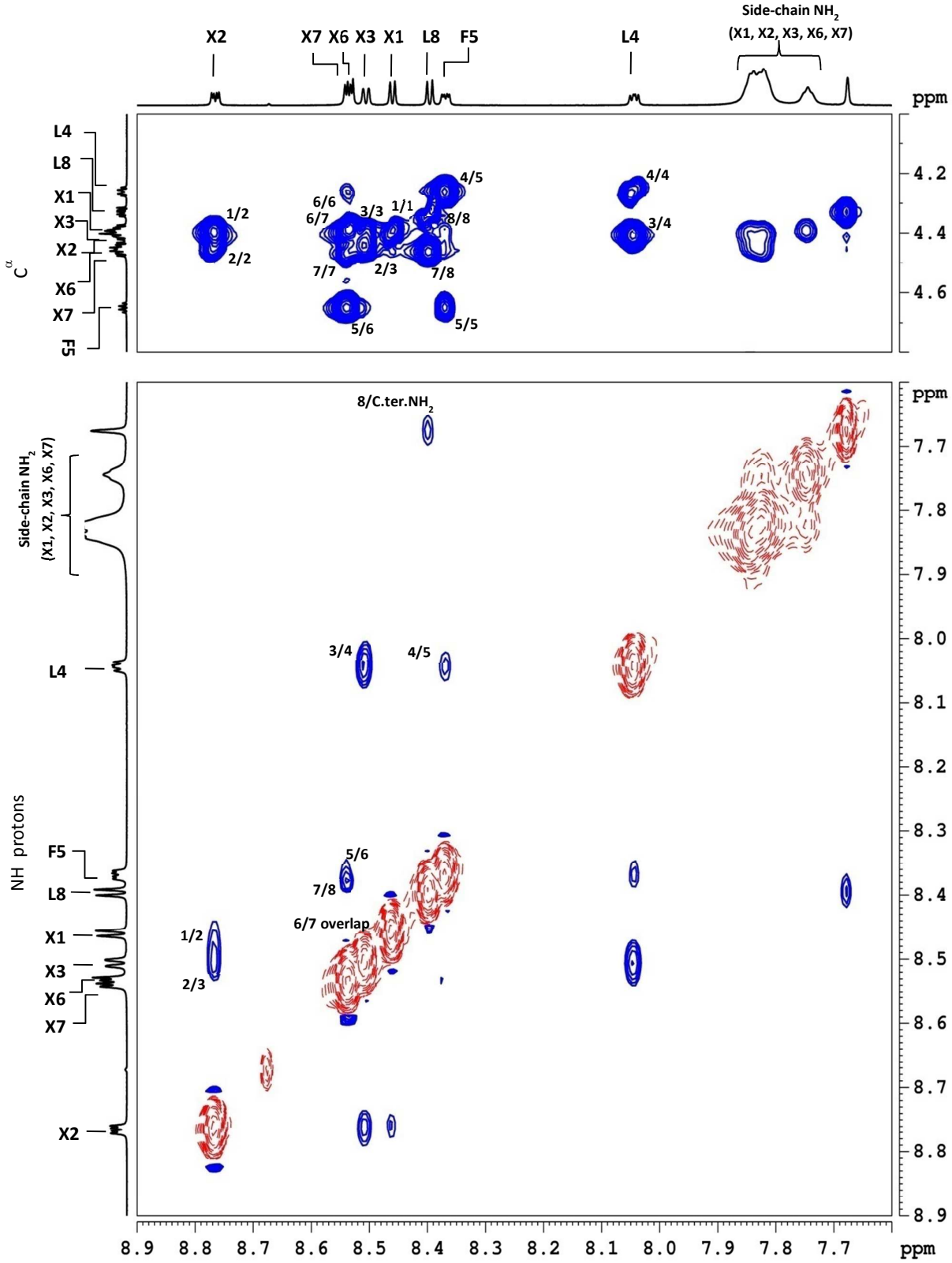


Figure S10: Partial 800 MHz ROESY spectrum of **17** in H_2O (70%) and TFE (30%). (Bottom) Weak d_{NN} and (Top) strong $d_{\alpha N}$ NOEs

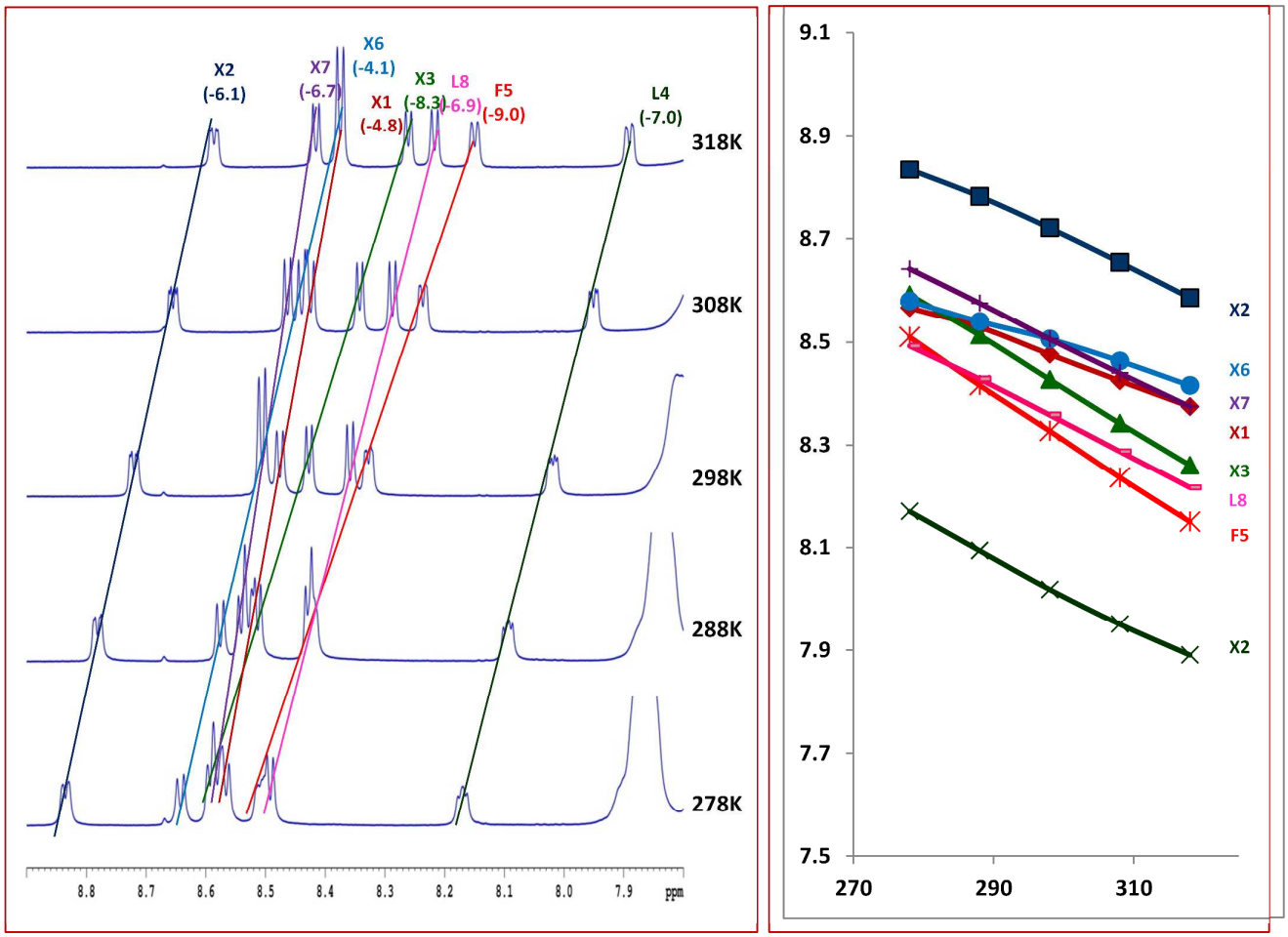


Figure S11: Amide proton shifts of **17** with respect to temperature. Temperature coefficients (dδ/dT) of amide protons are mentioned on top of each trace. Linear plots of amide chemical shifts vs temperature shown on the right is indicative of no structural variations throughout the temperature range. Experiment carried out at 700 MHz spectrometer.

Battacin (**10**)

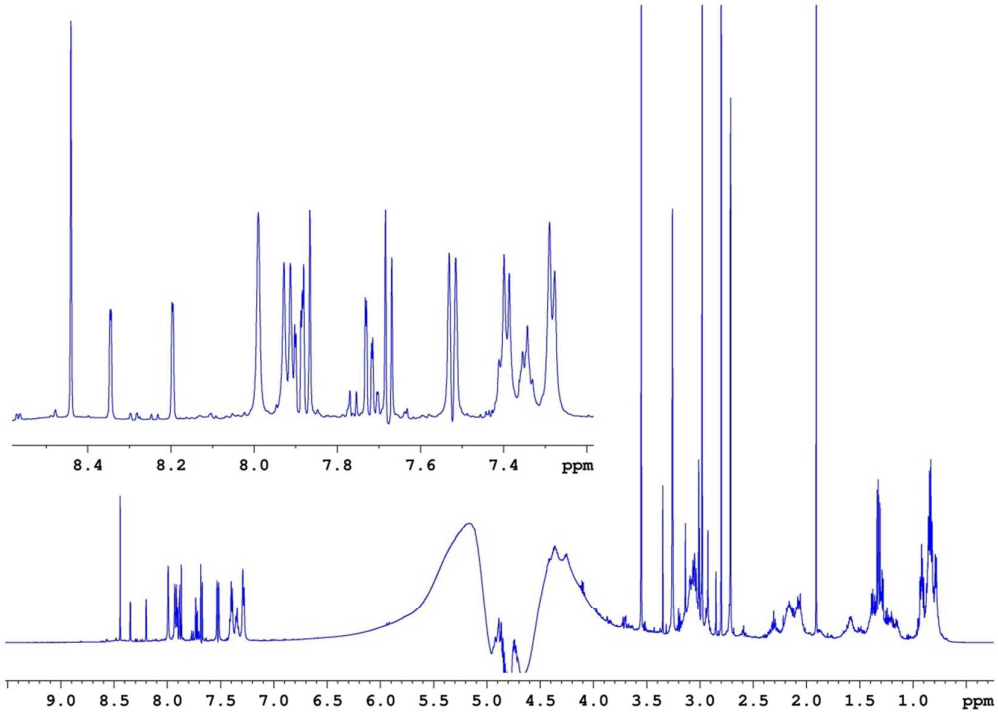


Figure S12: 600 MHz ^{1}H NMR spectrum of **10** in H_2O:D_2O (90:10) at 300K

Fmoc-D-Dab-*cyclo*[Dab-Dab-Leu-D-Phe-Dab-Dab-Leu] (**12**)

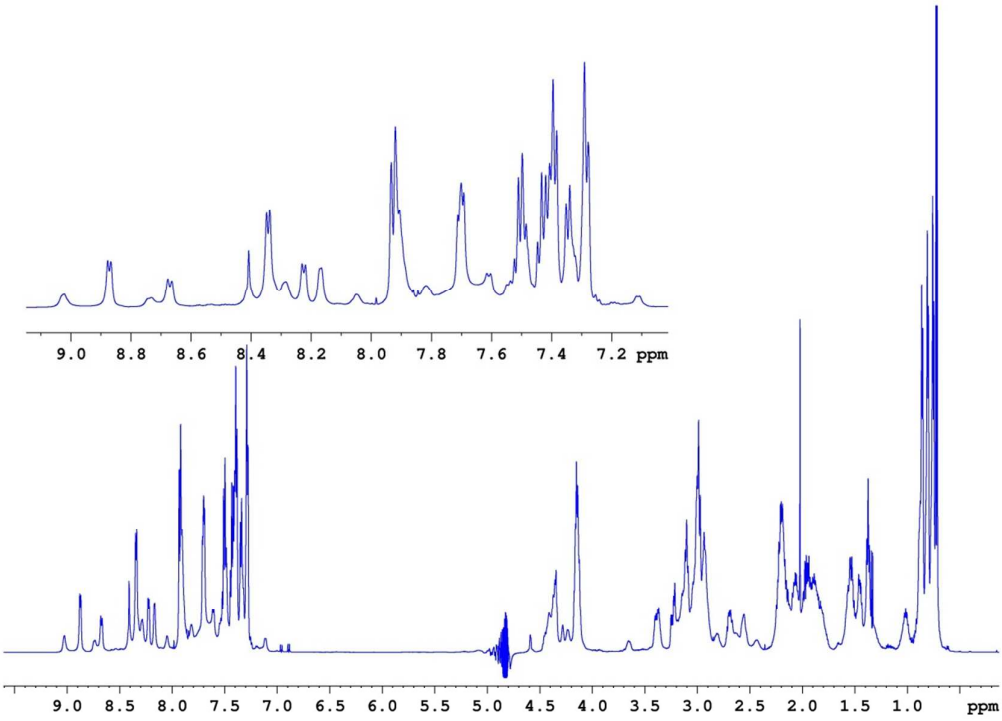


Figure S13: 600 MHZ ^{1}H NMR spectrum of **12** in H_2O:D_2O (90:10) at 300K

R^1-D-Dab-*cyclo*[Dab-Dab-Leu-D-Phe-Dab-Dab-Leu] (**13**)

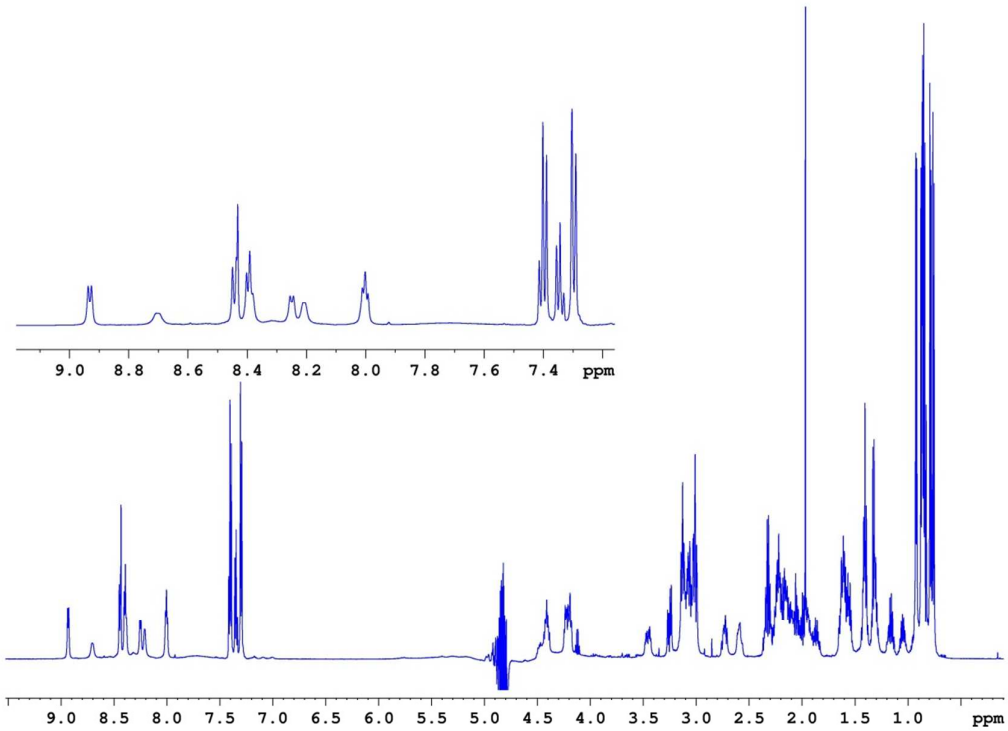


Figure S14: 600 MHz ^{1}H NMR spectrum of **13** in H_2O:D_2O (90:10) at 300K

R^2-D-Dab-*cyclo*[Dab-Dab-Leu-D-Phe-Dab-Dab-Leu] (**14**)

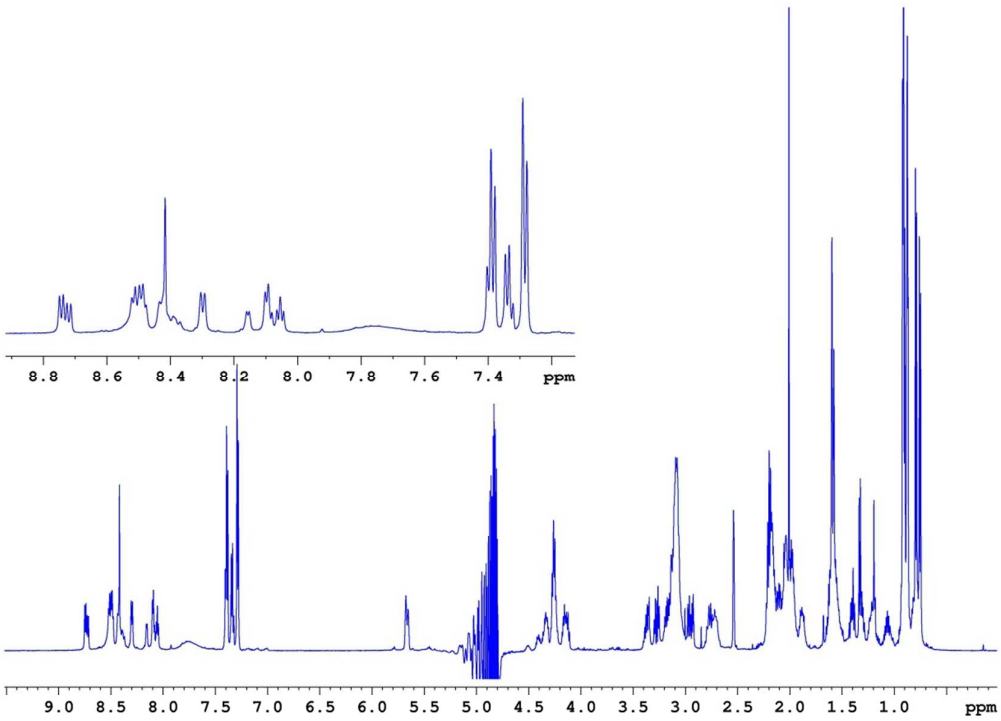


Figure S15: 600 MHz ^{1}H NMR spectrum of **14** in H_2O:D_2O (90:10) at 300K

R^3-D-Dab-*cyclo*[Dab-Dab-Leu-D-Phe-Dab-Dab-Leu] (**15**)

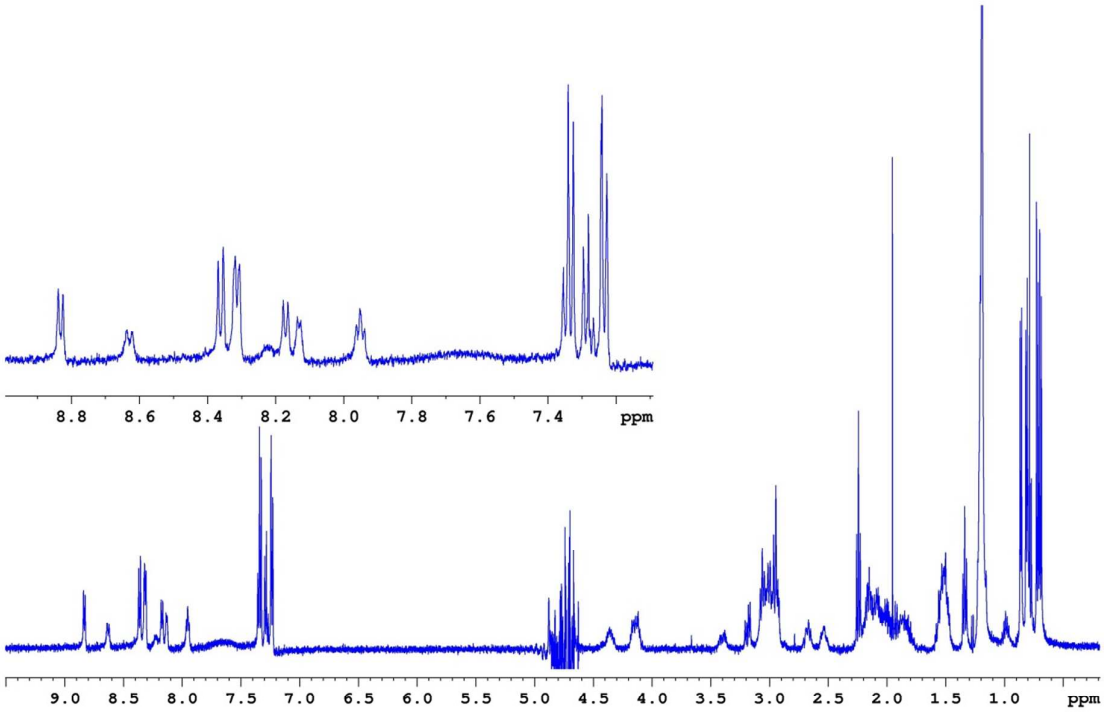


Figure S16: 500 MHZ ^{1}H NMR spectrum of **15** in H_2O:D_2O (90:10) at 278K

R^3-D-Lys-*cyclo*[Lys-Lys-Leu-D-Phe-Lys-Lys-Leu] (**16**)

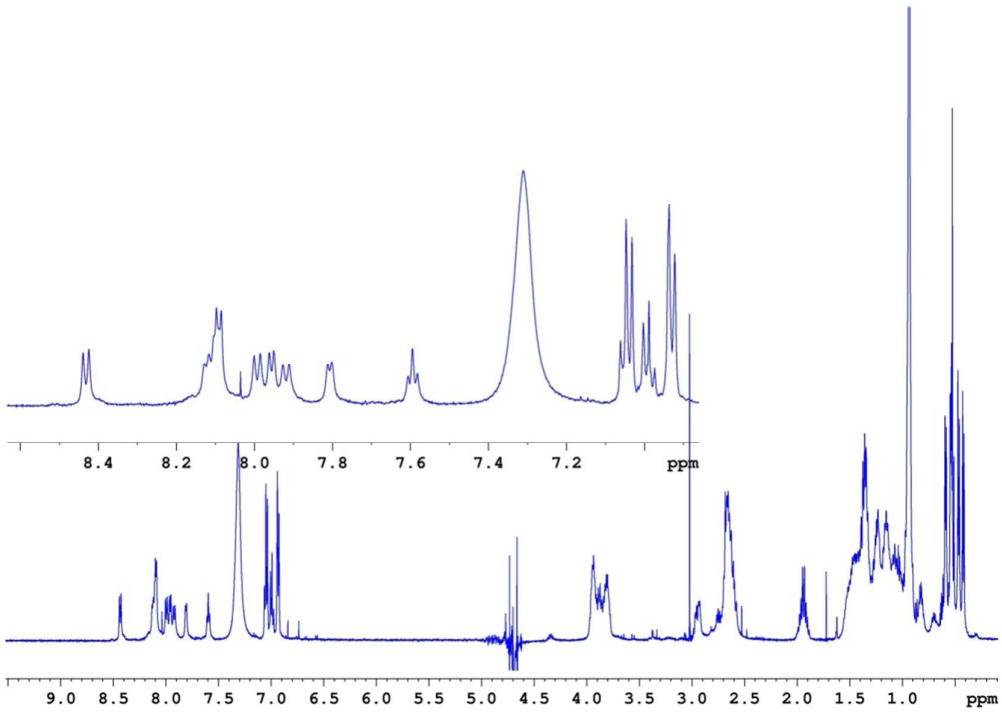


Figure S17: 500 MHz ^{1}H NMR spectrum of **16**) in H_2O:D_2O (90:10) at 278K

R^2-D-Dab-Dab-Dab-Leu-D-Phe-Dab-Dab-Leu-NH_2 (**18**)

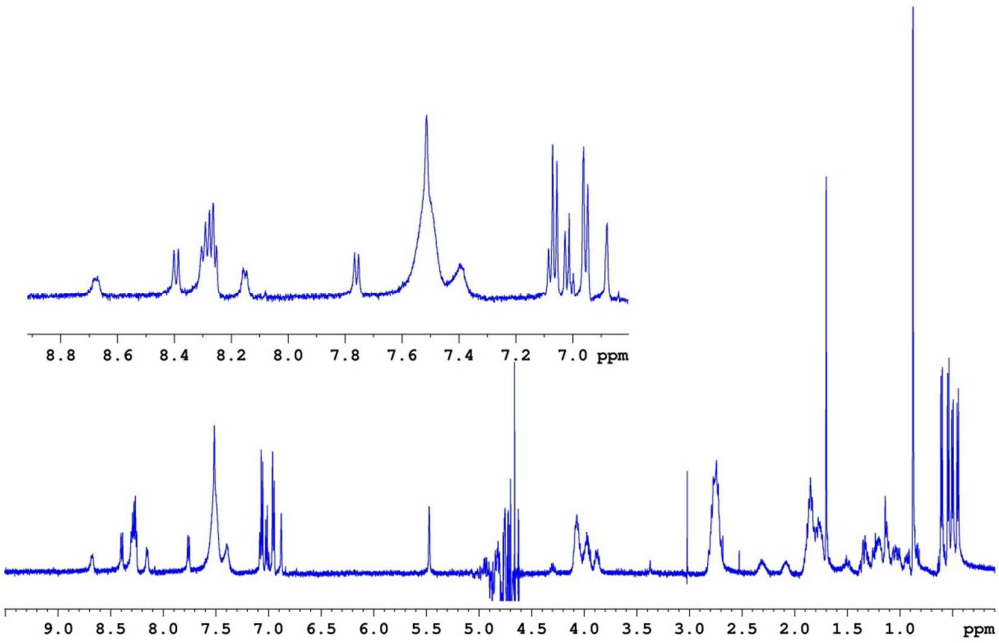


Figure S18: 500 MHz ^{1}H NMR spectrum of **18** in H_2O:D_2O (90:10) at 278K

R^3-D-Dab-Dab-Dab-Leu-D-Phe-Dab-Dab-Leu-NH_2 (**19**)

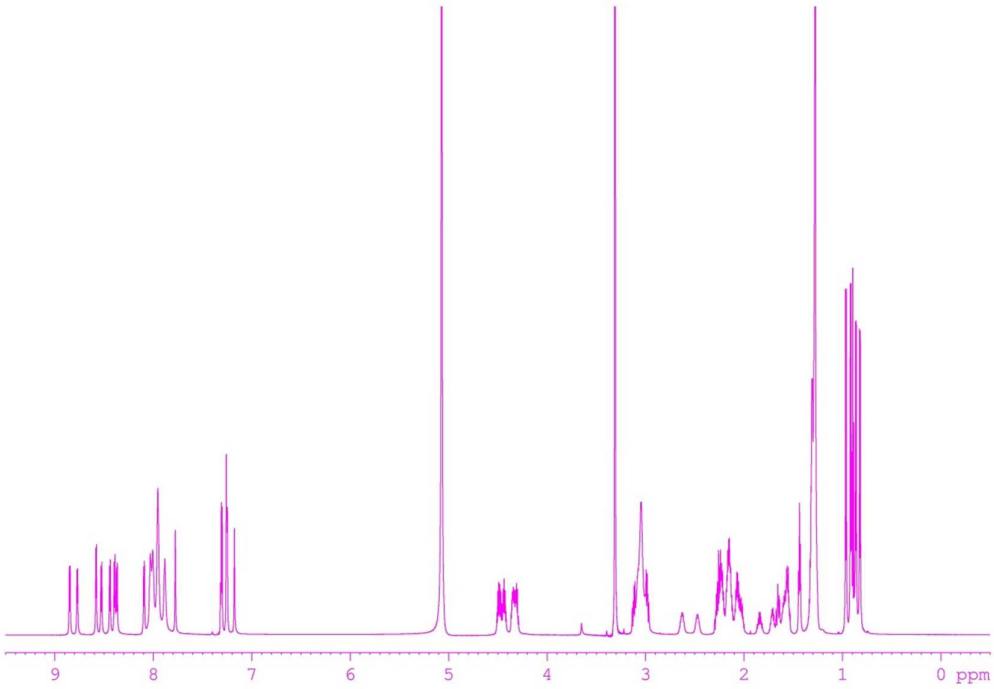


Figure S19: 800 MHz ^{1}H NMR spectrum of **19** in CD_3OH at 278K

R^3-D-Lys-Lys-Lys-Leu-D-Phe-Lys-Lys-Leu-NH_2 (**20**)

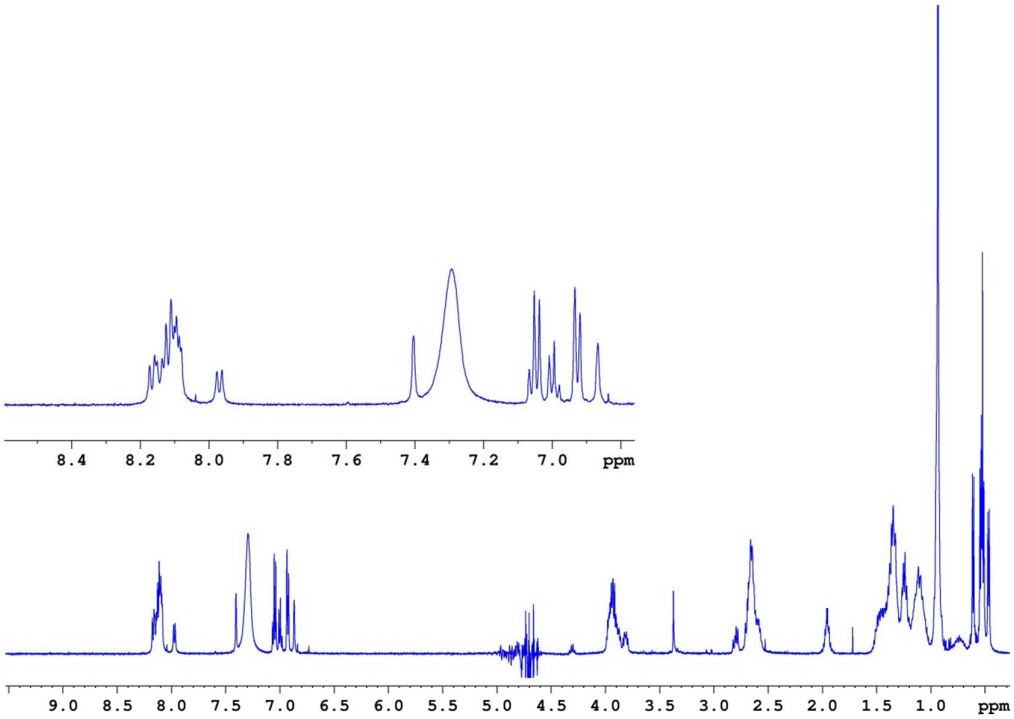


Figure S20: 500 MHz ^{1}H NMR spectrum of GZ3.37 (**20**) in H_2O:D_2O (90:10) at 278K

NH_2-D-Dab-Dab-Dab-Leu-D-Phe-Dab-Dab-Leu-NH_2 (**29**)

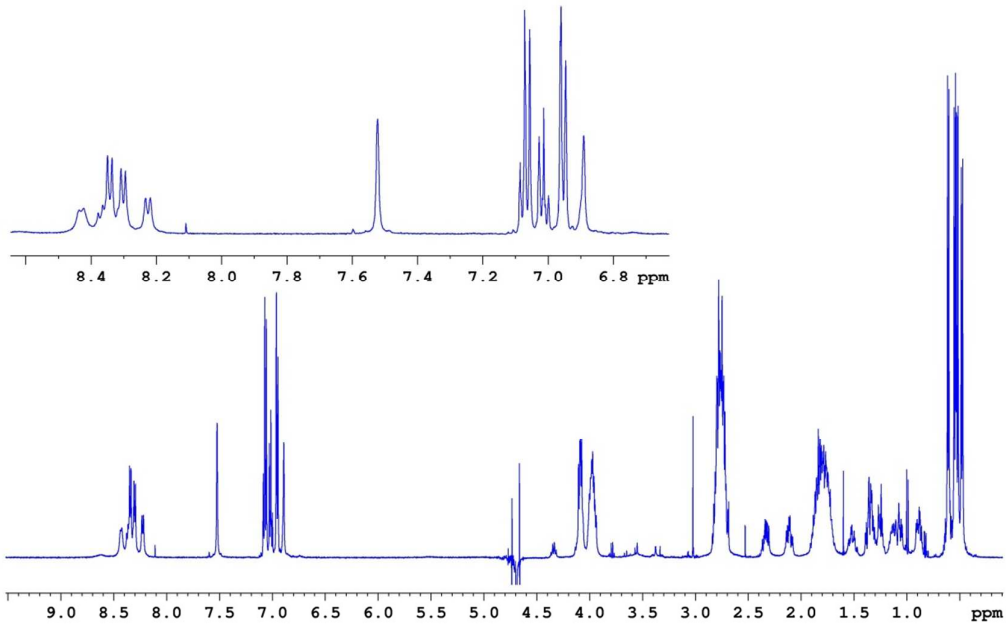


Figure S21: 500 MHz ^{1}H NMR spectrum of **29** in H_2O:D_2O (90:10) at 278K

R^1-D-Dab-Dab-Leu-D-Phe-Dab-NH_2 (**30**)

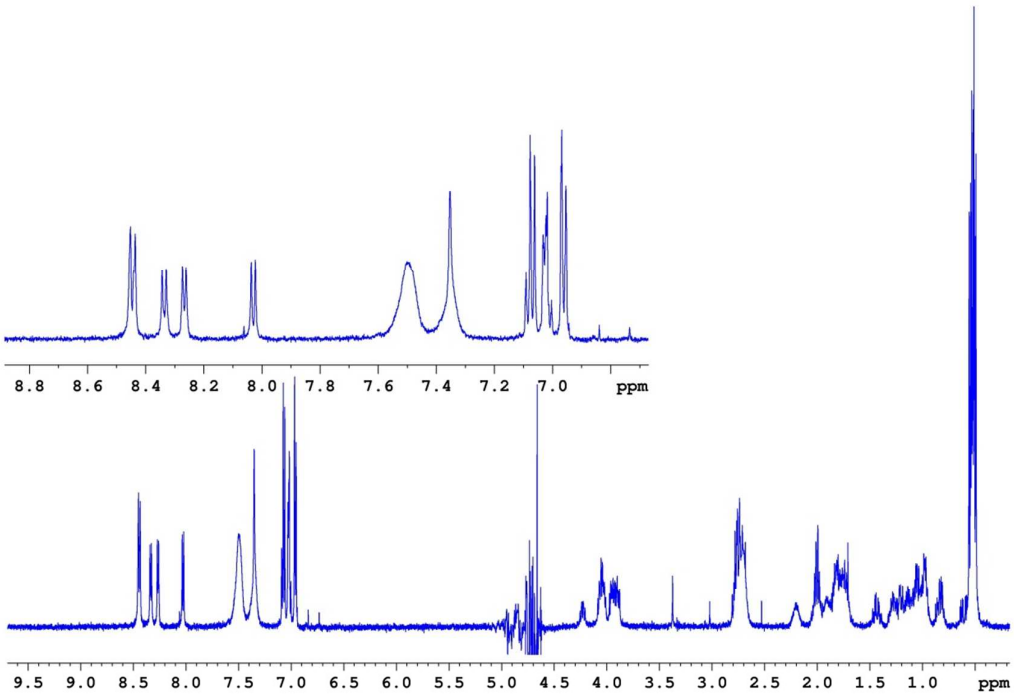


Figure S22: 500 MHz ^{1}H NMR spectrum of **30** in H_2O:D_2O (90:10) at 278K