Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Supplementary Information

Cyclopeptides containing the DEKS motif as conformationally restricted collagen telopeptide analogues: synthesis and conformational analysis

Robert Wodtke,[a,d] Gloria Ruiz-Gómez,[b] Manuela Kuchar,[a,d] M. Teresa Pisabarro,[b] Pavlina Novotná,[c] Marie Urbanová,[c] Jörg Steinbach,[a,d] Jens Pietzsch[a,d] and Reik Löser[a,d]

[a]Institute of Radiopharmaceutical Cancer Research, Helmholtz-Zentrum Dresden-Rossendorf, Bautzner Landstraße 400, 01328 Dresden, Germany

[b]Structural Bioinformatics, BIOTEC, Technische Universität Dresden, Tatzberg 47/49, 01307 Dresden, Germany

[c]Department of Physics and Measurements, Institute of Chemical Technology, 166 28 Prague, Czech Republic

[d]Department of Chemistry and Food Chemistry, Technische Universität Dresden, Bergstraße 66, 01069 Dresden, Germany

Table of Contents

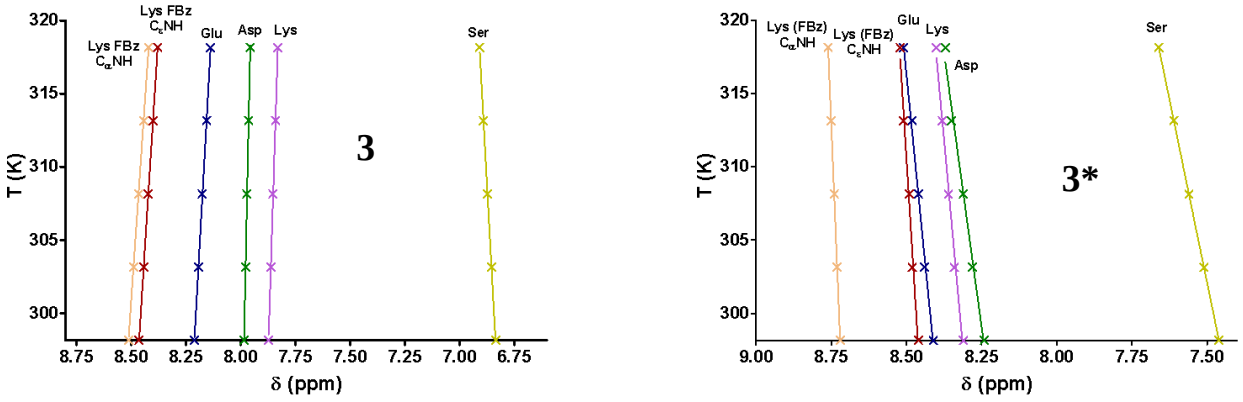
Figure S1.

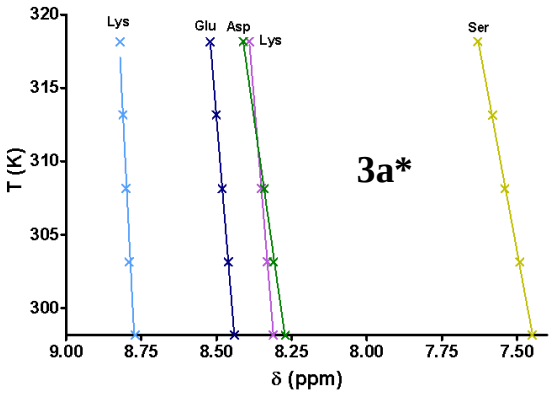
A. δ_{NH}- and $\Delta\delta$/T-values and $^3J_{NH,C\alpha H}$-coupling constants for the amide protons of the cyclohexapeptides 3, 3a, 8, 9 and the linear hexapeptide 10 in DMSO or water (marked with *).

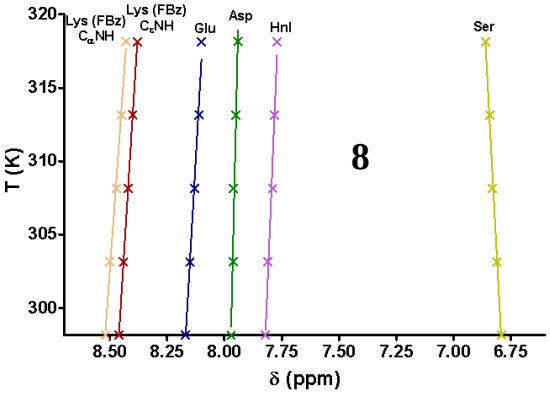
compound	δ_{NH} (ppm)[a] / $\Delta\delta$/T (ppb/K) / $^3J_{NH,C\alpha H}$ (Hz)[a]						
	Lys (FBz) C_αNH	Lys (FBz) C_εNH	Asp	Glu	Xaa	Ser	Lys
3 (Xaa=Lys)	8.51/-4.6/8.4	8.46/-4.2/5.6	7.98/-1.4/9.3	8.21/-3.7/3.0	7.87/-2.2/8.6	6.83/3.6/6.0	-
3* (Xaa=Lys)	8.72/2.0/7.4	8.46/3.0/5.8	8.24/6.6/9.1	8.41/4.8/4.0	8.31/4.4/7.5	7.46/10.0/6.5	-
3a* (Xaa=Lys)	-	-	8.27/6.9/9.1	8.44/4.0/4.0	8.31/4.0/7.4	7.45/9.0/6.7	8.77/2.4/7.5
8 (Xaa=Hnl)	8.52/-4.6/8.2	8.46/-4.0/5.4	7.97/-1.4/9.4	8.17/-3.6/2.6	7.82/-2.6/8.4	6.79/3.4/6.1	-
9 (Xaa=Aly)	8.51/-5.0/8.2	8.46/-5.0/5.4	7.97/-1.6/9.3	8.19/-4.0/2.5	7.86/-2.6/8.8	6.83/4.0/6.2	-
10 (Xaa=Lys)	8.09/-4.3/6.9	8.46/-4.7/5.5	8.29/-5.2/7.7	7.65/-2.0/8.1	7.85/-4.7/7.8	8.20/-6.0/6.8	-

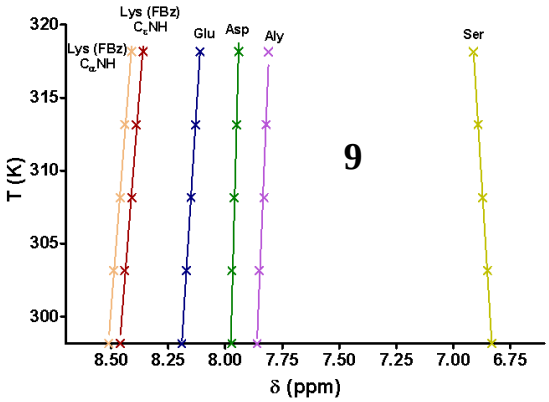
[a] **at 298 K.**

B. Temperature dependence of chemical shifts for the amide protons of 3, 3a, 8, 9 and 10 in DMSO or water (marked with *).




Lys
Glu
Asp
Lys
Ser
3a*
T (K)
δ (ppm)


Lys (FBz) Cα NH
Lys (FBz) Cε NH
Glu
Asp
Hnl
Ser
8
T (K)
δ (ppm)


Lys (FBz) Cε NH
Lys (FBz) Cα NH
Glu
Asp
Aly
Ser
9
T (K)
δ (ppm)

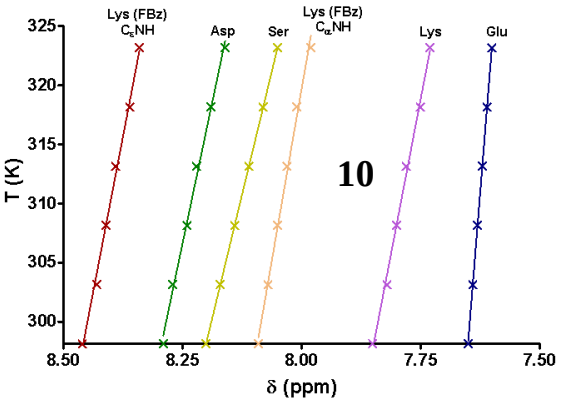
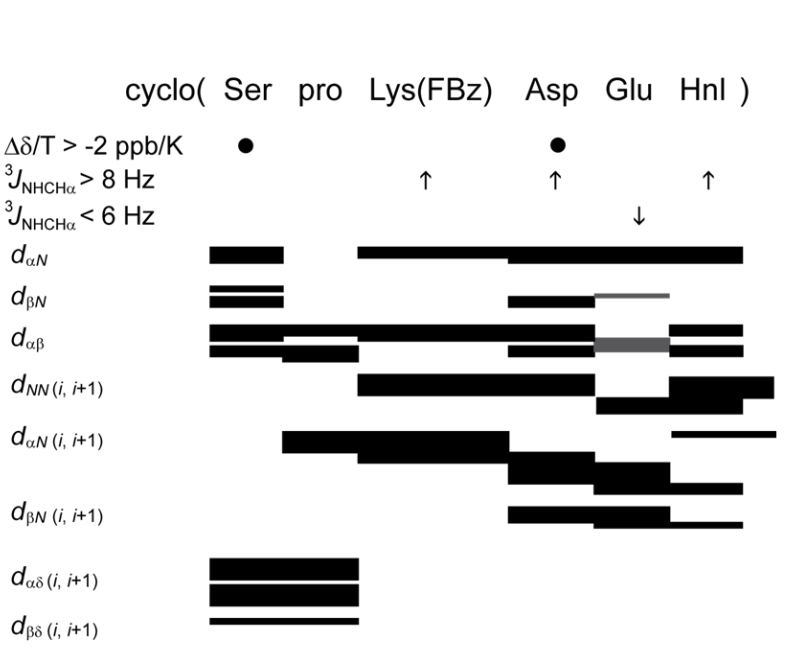
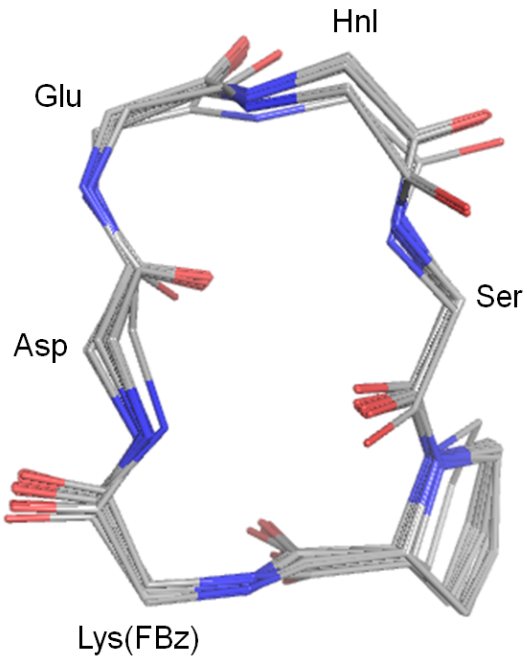
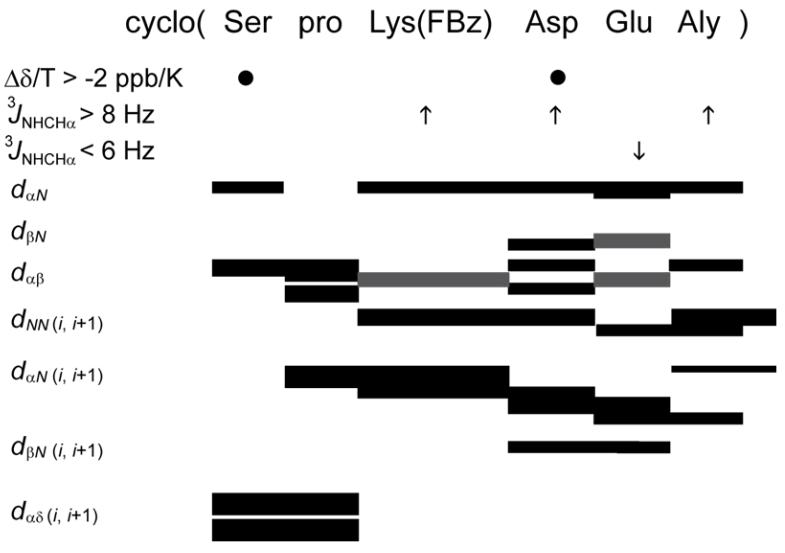

Lys (FBz) Cε NH
Asp
Ser
Lys (FBz) Cα NH
Lys
Glu
10
T (K)
δ (ppm)

Figure S2.

Left: summary of Δδ/T amide NH temperature coefficients (> -2 ppb/K shown by ●), $^{3}J_{NHCH\alpha}$ coupling constants (> 8 Hz represented by ↑ and < 6 Hz shown by ↓) and ROEs correlations summary in DMSO at 298 K; right: 20-lowest energy structures of 8 (top) and 9 (bottom). Grey bars indicate protons not stereospecifically assigned. For clarity only the side chain of pro is shown.







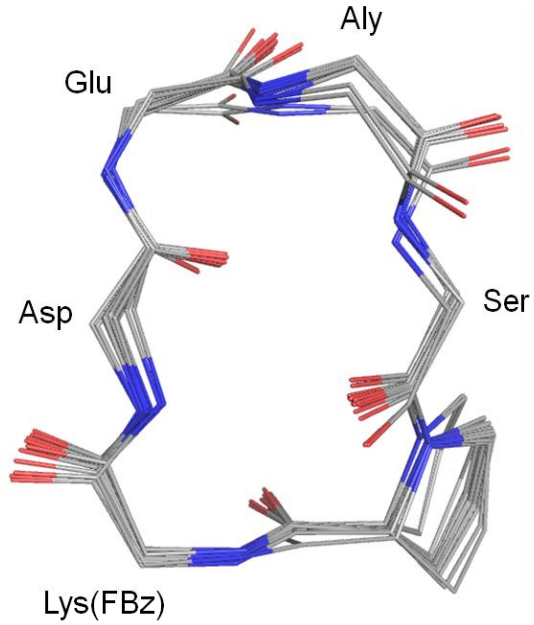


Table S1.

Distance restraints used in the structure calculation of compound 3

A. ROE derived distance restraints used for calculating the solution structure of compound **3** cyclo(Ser-D-Pro-Lys(FBz)-Asp-Glu-Lys) in DMSO-d_6 at 298 K.

Atom A	Atom B	Upper distance restraint (Å) and comment
Ser1 Hα	Ser1 HN	≤ 5.0 Å, weak
Ser1 Hα	Ser1 Hβ1	≤ 3.5 Å, medium
Ser1 Hα	Ser1 Hβ2	≤ 5.0 Å, weak
Ser1 Hβ1	Ser1 HN	≤ 6.0 Å, very weak
Ser1 Hβ2	Ser1 HN	≤ 5.0 Å, weak
Ser1 Hα	D-Pro2 HD1	≤ 2.7 Å, strong
Ser1 Hα	D-Pro2 HD2	≤ 3.5 Å, medium
Ser1 Hβ1	D-Pro2 HD1	≤ 6.0 Å, very weak
Ser1 HN	Asp4 HN	≤ 6.0 Å, very weak
D-Pro2 Hα	Lys(FBz)3 HN	≤ 3.5 Å, medium
D-Pro2 Hα	Lys(FBz)3 H^{ortho}*	≤ 6.0 Å, very weak (+ 0.5 Å correction)
Lys(FBz)3 Hα	Lys(FBz)3 HN	≤ 5.0 Å, weak
Lys(FBz)3 HE*	Lys(FBz)3 HZ	≤ 3.5 Å, medium (+ 1 Å correction)
Lys(FBz)3 HZ	Lys(FBz)3 H^{ortho}*	≤ 2.7 Å, strong (+ 1 Å correction)
Lys(FBz)3 Hα	Asp4 HN	≤ 5.0 Å, weak
Lys(FBz)3 HN	Asp4 HN	≤ 3.5 Å, medium
Asp4 Hα	Asp4 HN	≤ 5.0 Å, weak
Asp4 Hβ2	Asp4 HN	≤ 5.0 Å, weak
Asp4 Hα	Asp4 Hβ1	≤ 5.0 Å, weak
Asp4 Hα	Asp4 Hβ2	≤ 5.0 Å, weak
Asp4 Hα	Glu5 HN	≤ 3.5 Å, medium
Asp4 Hβ1	Glu5 HN	≤ 3.5 Å, medium
Glu5 Hα	Glu5 HN	≤ 3.5 Å, medium
Glu5 Hα	Glu5 Hβ1	≤ 3.5 Å, medium
Glu5 Hα	Glu5 Hβ2	≤ 3.5 Å, medium
Glu5 Hα	Glu5 HG*	≤ 3.5 Å, medium (+ 1 Å correction)

Glu5 Hβ1	Glu5 HN	≤ 5.0 Å, weak
Glu5 Hβ2	Glu5 HN	≤ 6.0 Å, very weak
Glu5 HG*	Glu5 HN	≤ 5.0 Å, weak (+ 1 Å correction)
Glu5 Hα	Lys6 HN	≤ 5.0 Å, weak
Glu5 HN	Lys6 HN	≤ 3.5 Å, medium
Glu5 Hβ1	Lys6 HN	≤ 6.0 Å, very weak
Lys6 Hα	Lys6 HN	≤ 5.0 Å, weak
Lys6 Hβ1	Lys6 HN	≤ 6.0 Å, very weak
Lys6 Hβ2	Lys6 HN	≤ 6.0 Å, very weak
Lys6 Hα	Ser1 HN	≤ 6.0 Å, very weak
Lys6 HN	Ser1 HN	≤ 2.7 Å, strong

*Represents protons not stereospecifically assigned. Their distance restraints have been adjusted with standard pseudoatom corrections. (K. Wüthrich, M. Billeter and W. Braun, *J. Mol. Biol.*, 1983, **169**, 949-961.)

B. ϕ angle restrains (Å) used for calculating the solution structure of compound **3** cyclo(Ser-D-Pro-Lys(FBz)-Asp-Glu-Lys) in DMSO-d_6 at 298 K.

Residue	$^3J_{NHCH\alpha}$ **(Hz)**	**ϕ restraints**
Ser	6.0	
D-Pro	-	
Lys(FBz)	8.4	-120˚ ± 30
Asp	9.3	-120˚ ± 30
Glu	3.0	-65˚ ± 30
Lys	8.6	-120˚ ± 30

C. H-bonds distance restrains (Å) used for calculating the solution structure of compound **3** cyclo(Ser-D-Pro-Lys(FBz)-Asp-Glu-Lys) in DMSO-d_6 at 298 K.

Carbonyl group	**Amide NH group**	**Distance**	**Lower bound**	**Upper bound**
Ser1-CO	Asp4-NH	1.88	0.30	0.32
Ser1-CO	Asp4-N	2.88	0.30	0.32
Asp4-CO	Ser1-NH	1.88	0.30	0.32
Asp4-CO	Ser1-N	2.88	0.30	0.32

Table S2.

Distance restraints used in the structure calculation of compound 8

A. ROE derived distance restraints used for calculating the solution structure of compound **8** in cyclo(Ser-D-Pro-Lys(FBz)-Asp-Glu-Hnl) in DMSO-d_6 at 298 K.

Atom A	Atom B	Upper distance restraint (Å) and comment
Ser1 Hα	Ser1 HN	≤ 3.5 Å, medium
Ser1 Hα	Ser1 Hβ1	≤ 3.5 Å, medium
Ser1 Hα	Ser1 Hβ2	≤ 5.0 Å, weak
Ser1 Hβ1	Ser1 HN	≤ 6.0 Å, very weak
Ser1 Hβ2	Ser1 HN	≤ 5.0 Å, weak
Ser1 Hα	D-Pro2 HD1	≤ 2.7 Å, strong
Ser1 Hα	D-Pro2 HD2	≤ 2.7 Å, strong
D-Pro2 Hα	D-Pro2 Hβ1	≤ 5.0 Å, weak
D-Pro2 Hα	D-Pro2 Hβ2	≤ 3.5 Å, medium
D-Pro2 Hα	Lys(FBz)3 HN	≤ 3.5 Å, medium
Lys(FBz)3 Hα	Lys(FBz)3 HN	≤ 5.0 Å, weak
Lys(FBz)3 Hα	Lys(FBz)3 Hβ1	≤ 3.5 Å, medium
Lys(FBz)3 HE*	Lys(FBz)3 HZ	≤ 3.5 Å, medium (+ 1 Å correction)
Lys(FBz)3 HZ	Lys(FBz)3 H^{ortho}*	≤ 2.7 Å, strong (+ 1 Å correction)
Lys(FBz)3 Hα	Asp4 HN	≤ 5.0 Å, weak
Lys(FBz)3 HN	Asp4 HN	≤ 2.7 Å, strong
Asp4 Hα	Asp4 HN	≤ 3.5 Å, medium
Asp4 Hβ2	Asp4 HN	≤ 5.0 Å, weak
Asp4 Hα	Asp4 Hβ1	≤ 3.5 Å, medium
Asp4 Hα	Asp4 Hβ2	≤ 5.0 Å, weak
Asp4 Hα	Glu5 HN	≤ 2.7 Å, strong
Asp4 Hβ1	Glu5 HN	≤ 3.5 Å, medium
Glu5 Hα	Glu5 HN	≤ 3.5 Å, medium
Glu5 Hα	Glu5 Hβ*	≤ 3.5 Å, medium (+ 1 Å correction)
Glu5 Hα	Glu5 HG*	≤ 3.5 Å, medium (+ 1 Å correction)
Glu5 Hβ*	Glu5 HN	≤ 6.0 Å, very weak (no correction)

Glu5 HG*	Glu5 HN	≤ 5.0 Å, weak (+ 1 Å correction)
Glu5 Hα	Hnl6 HN	≤ 5.0 Å, weak
Glu5 HN	Hnl6 HN	≤ 3.5 Å, medium
Hnl6 Hα	Hnl6 HN	≤ 3.5 Å, medium
Hnl6 Hα	Hnl6 Hβ1	≤ 5.0 Å, weak
Hnl6 Hα	Hnl6 Hβ2	≤ 5.0 Å, weak
Hnl6 Hα	Ser1 HN	≤ 6.0 Å, very weak
Hnl6 HN	Ser1 HN	≤ 3.5 Å, medium

*Represents protons not stereospecifically assigned. Their distance restraints have been adjusted with standard pseudoatom corrections. (K. Wüthrich, M. Billeter and W. Braun, *J. Mol. Biol.*, 1983, **169**, 949-961.)

B. ϕ angle restrains (Å) used for calculating the solution structure of compound **8** in cyclo(Ser- D-Pro-Lys(FBz)-Asp-Glu-Hnl) in DMSO-d_6 at 298 K.

Residue	$^3J_{NHCH\alpha}$ (Hz)	ϕ restraints
Ser	6.1	
D-Pro	-	
Lys(FBz)	8.2	-120˚ ± 30
Asp	9.4	-120˚ ± 30
Glu	2.6	-65˚ ± 30
Hnl	8.4	-120˚ ± 30

C. H-bonds distance restrains (Å) used for calculating the solution structure of compound **8** in cyclo(Ser- D-Pro-Lys(FBz)-Asp-Glu-Hnl) in DMSO-d_6 at 298 K.

Carbonyl group	Amide NH group	Distance	Lower bound	Upper bound
Ser1-CO	Asp4-NH	1.88	0.30	0.32
Ser1-CO	Asp4-N	2.88	0.30	0.32
Asp4-CO	Ser1-NH	1.88	0.30	0.60
Asp4-CO	Ser1-N	2.88	0.30	0.48

Table S3.

Distance restraints used in the structure calculation of compound 9

A. ROE derived distance restraints used for calculating the solution structure of compound **9** in cyclo(Ser-D-Pro-Lys(FBz)-Asp-Glu-Aly) in DMSO-d_6 at 298 K.

Atom A	Atom B	Upper distance restraint (Å) and comment
Ser1 Hα	Ser1 HN	≤ 5.0 Å, weak
Ser1 Hα	Ser1 Hβ1	≤ 3.5 Å, medium
Ser1 Hα	D-Pro2 HD1	≤ 2.7 Å, strong
Ser1 Hα	D-Pro2 HD2	≤ 2.7 Å, strong
D-Pro2 Hα	D-Pro2 Hβ1	≤ 2.7 Å, strong
D-Pro2 Hα	D-Pro2 Hβ2	≤ 3.5 Å, medium
D-Pro2 Hα	Lys(FBz)3 HN	≤ 2.7 Å, strong
Lys(FBz)3 Hα	Lys(FBz)3 HN	≤ 5.0 Å, weak
Lys(FBz)3 Hα	Lys(FBz)3 Hβ*	≤ 3.5 Å, medium (+ 1 Å correction)
Lys(FBz)3 HE*	Lys(FBz)3 HZ	≤ 3.5 Å, medium (+ 1 Å correction)
Lys(FBz)3 HZ	Lys(FBz)3 H^{ortho}*	≤ 2.7 Å, strong (+ 1 Å correction)
Lys(FBz)3 Hα	Asp4 HN	≤ 5.0 Å, weak
Lys(FBz)3 HN	Asp4 HN	≤ 3.5 Å, medium
Asp4 Hα	Asp4 HN	≤ 5.0 Å, weak
Asp4 Hβ2	Asp4 HN	≤ 5.0 Å, weak
Asp4 Hα	Asp4 Hβ1	≤ 5.0 Å, weak
Asp4 Hα	Asp4 Hβ2	≤ 5.0 Å, weak
Asp4 Hα	Glu5 HN	≤ 3.5 Å, medium
Asp4 Hβ1	Glu5 HN	≤ 5.0 Å, weak
Glu5 Hα	Glu5 HN	≤ 3.5 Å, medium
Glu5 Hα	Glu5 Hβ*	≤ 3.5 Å, medium (+ 1 Å correction)
Glu5 Hβ*	Glu5 HN	≤ 3.5 Å, medium (+ 1 Å correction)
Glu5 HG*	Glu5 HN	≤ 5.0 Å, weak (+ 1 Å correction)
Glu5 Hα	Aly6 HN	≤ 5.0 Å, weak
Glu5 HN	Aly6 HN	≤ 5.0 Å, weak
Aly6 Hα	Aly6 HN	≤ 5.0 Å, weak

Aly6 Hα	Aly6 Hβ1	≤ 5.0 Å, weak
Aly6 Hα	Ser1 HN	≤ 6.0 Å, very weak
Aly6 HN	Ser1 HN	≤ 3.5 Å, medium

*Represents protons not stereospecifically assigned. Their distance restraints have been adjusted with standard pseudoatom corrections. (K. Wüthrich, M. Billeter and W. Braun, *J. Mol. Biol.*, 1983, **169**, 949-961.)

B. ϕ angle restrains (Å) used for calculating the solution structure compound **9** in cyclo(Ser- D-Pro-Lys(FBz)-Asp-Glu-Aly) in DMSO-d_6 at 298 K.

Residue	$^3J_{NHCH\alpha}$ **(Hz)**	**ϕ restraints**
Ser	6.2	
D-Pro	-	
Lys(FBz)	8.2	-120˚ ± 30
Asp	9.3	-120˚ ± 30
Glu	2.5	-65˚ ± 30
Aly	8.8	-120˚ ± 30

C. H-bonds distance restrains (Å) used for calculating the solution structure of compound **9** in cyclo(Ser- D-Pro-Lys(FBz)-Asp-Glu-Aly) in DMSO-d_6 at 298 K.

Carbonyl group	**Amine group**	**Distance**	**Lower bound**	**Upper bound**
Ser1-CO	Asp4-NH	1.88	0.30	0.32
Ser1-CO	Asp4-N	2.88	0.30	0.32
Asp4-CO	Ser1-NH	1.88	0.30	0.32
Asp4-CO	Ser1-N	2.88	0.30	0.32

Figure S3.

2D ROESY spectrum for the amide region of cyclohexapeptide 3 in water. The highlighted cross peaks indicate the observed $d_{NN}(i,i+1)$ correlations.

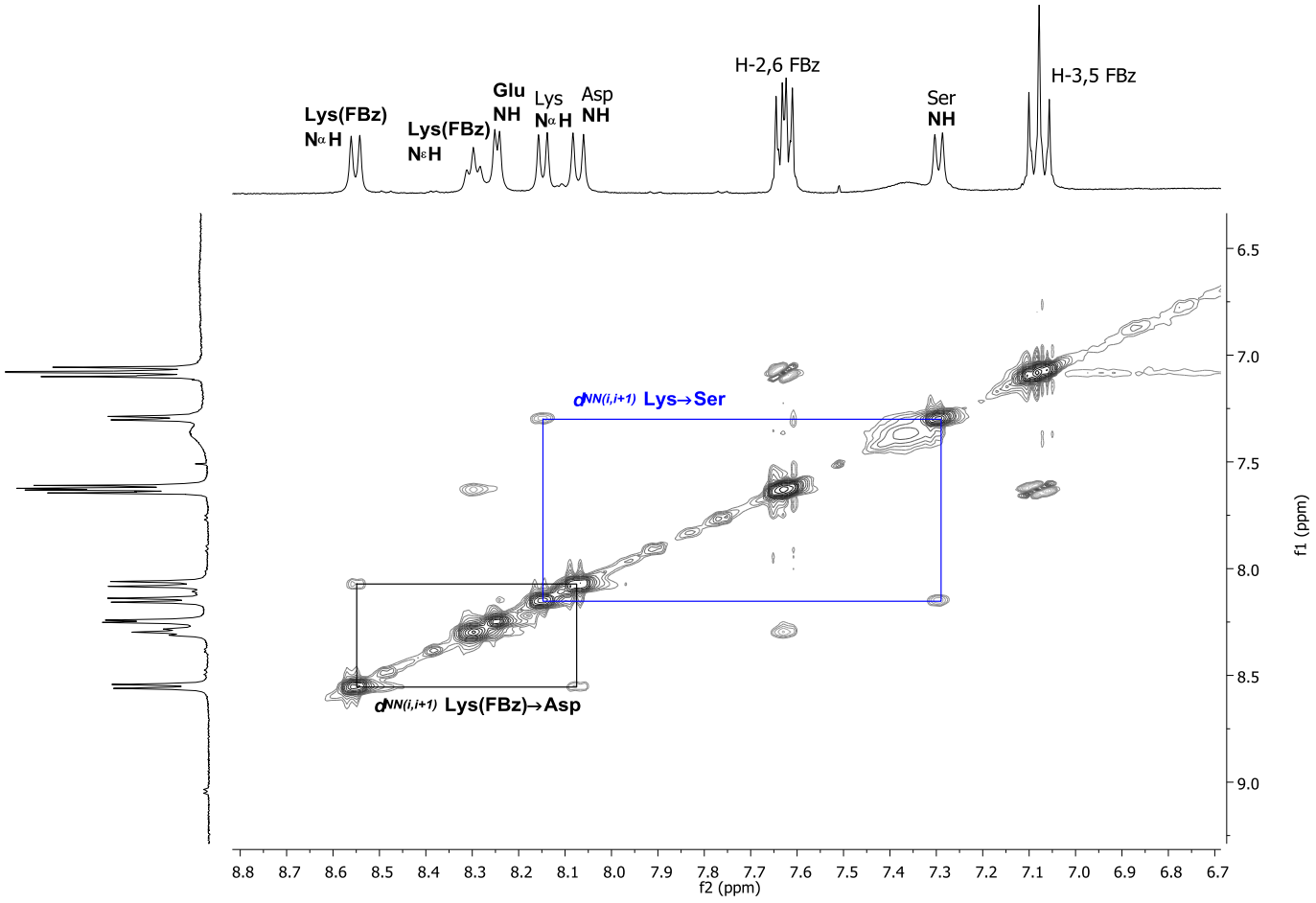


Figure S4.

Comparison of ECD spectra of 3, 8 and 10 at varying concentrations. Similar shapes and molar ellipticities at different concentrations indicate no aggregation in solution. Compound **9** was only measured at a concentration of 0.125 mg/mL in order to reduce substance consumption.

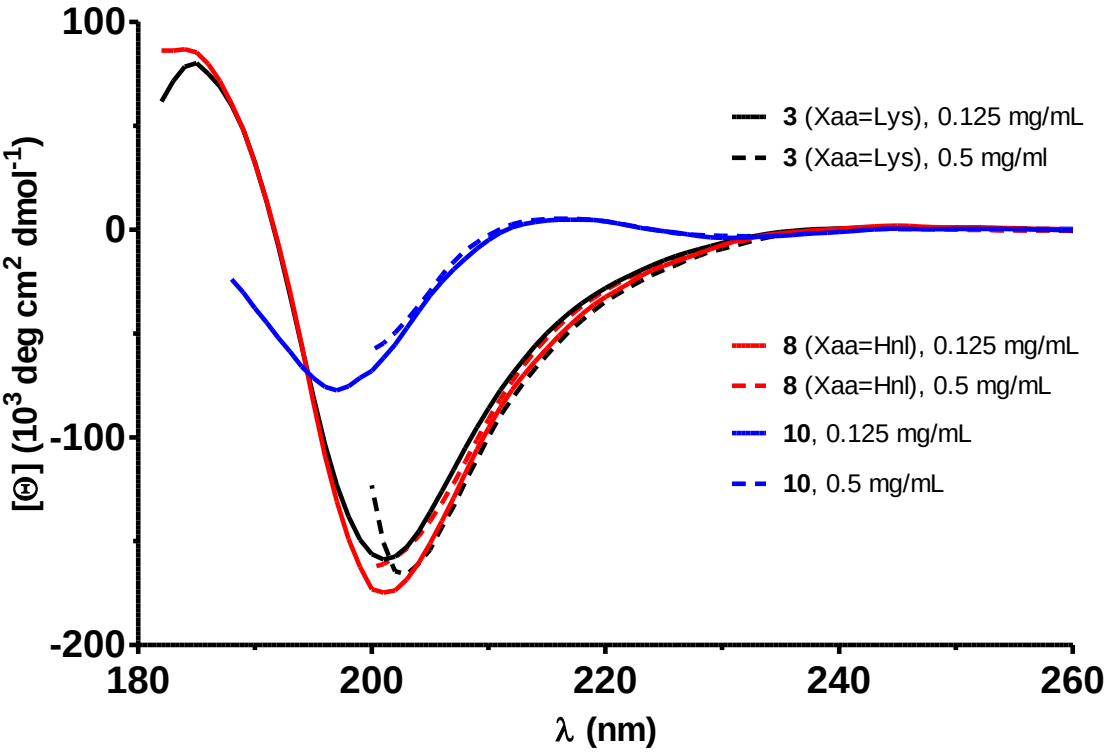
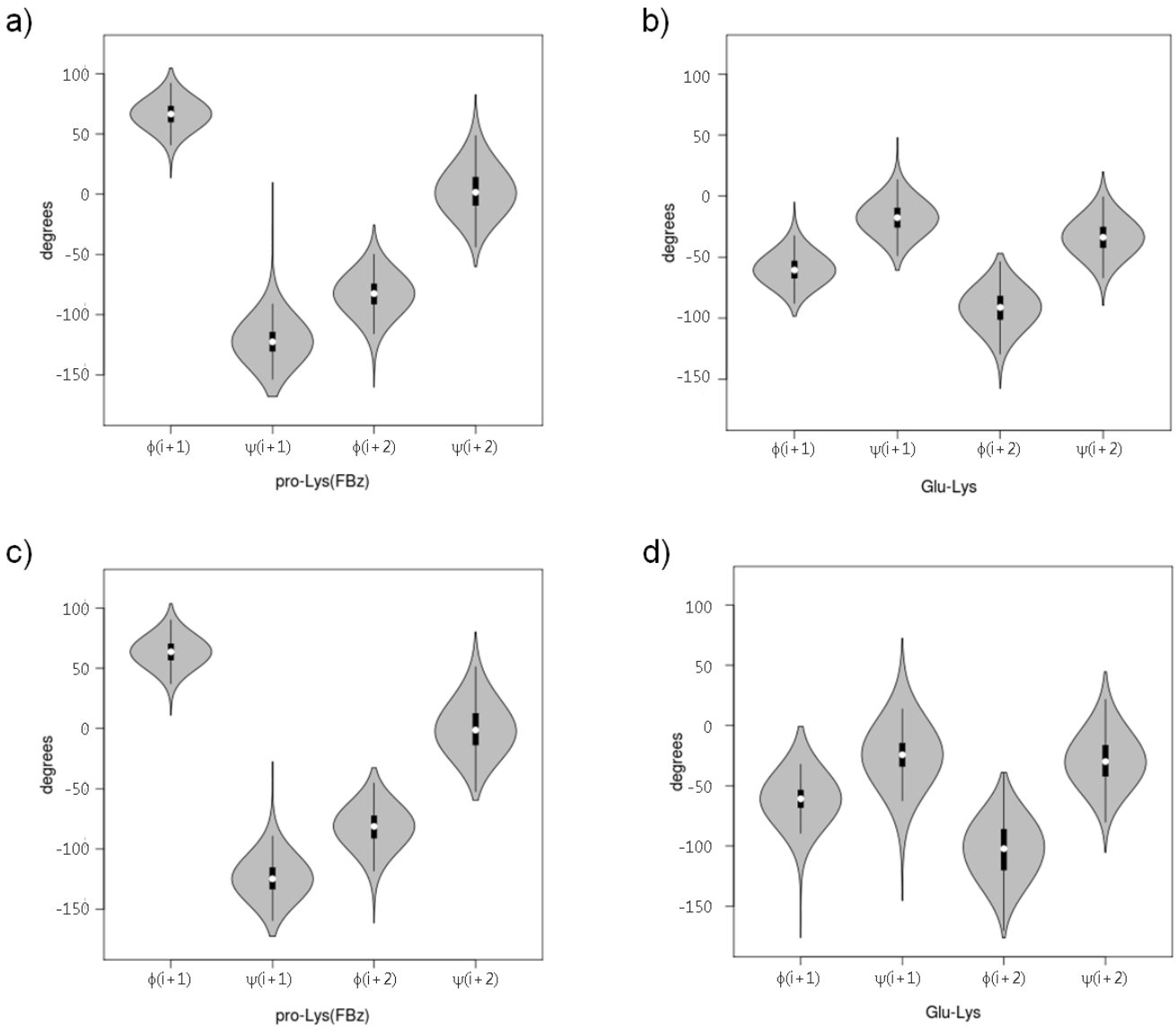
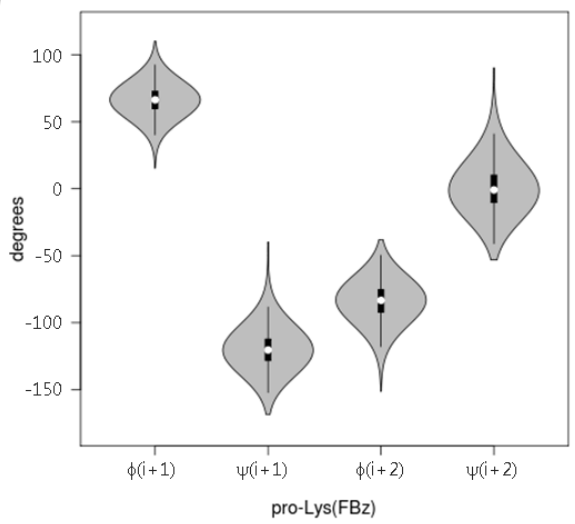


Figure S5.

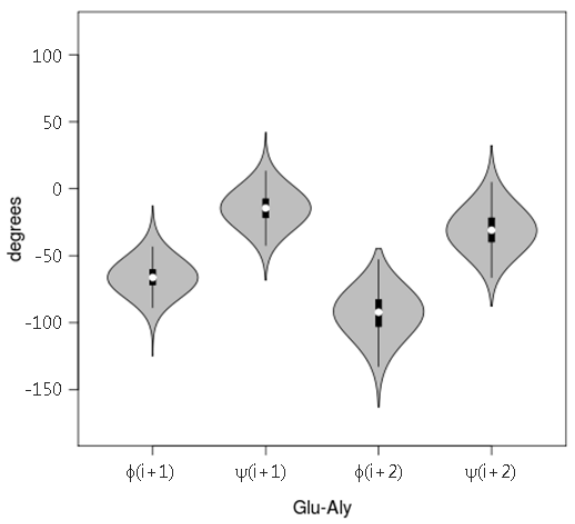
Violin plot representation of Phi (ϕ) and Psi (ψ) dihedral angles of cyclo(Ser-pro-Lys(FBz)-Asp-Glu-Xaa) involved in β-turns around pro (D-Pro) and Lys(FBz), and Glu and Xaa, extracted from four 100 ns MD simulations. Xaa = Lys (3): a) and b) in DMSO, c) and d) in water; Xaa = Aly (9): e) and f) in DMSO, g) and h) in water.



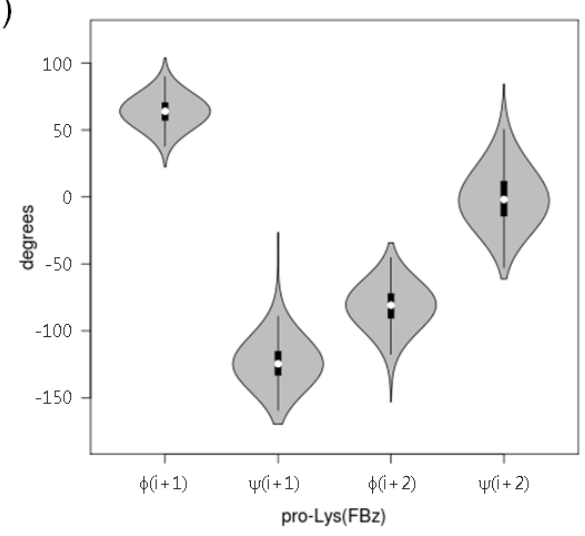
e)


100
50
0
-50
-100
-150
degrees
ϕ(i+1)
ψ(i+1)
ϕ(i+2)
ψ(i+2)
pro-Lys(FBz)

f)


100
50
0
-50
-100
-150
degrees
ϕ(i+1)
ψ(i+1)
ϕ(i+2)
ψ(i+2)
Glu-Aly

g)


100
50
0
-50
-100
-150
degrees
ϕ(i+1)
ψ(i+1)
ϕ(i+2)
ψ(i+2)
pro-Lys(FBz)

h)

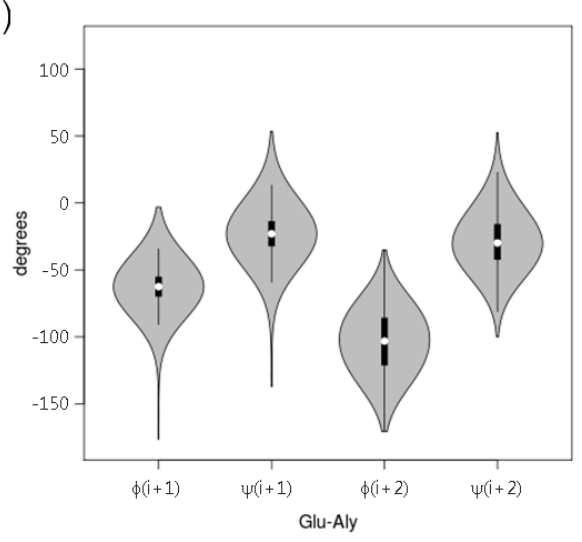
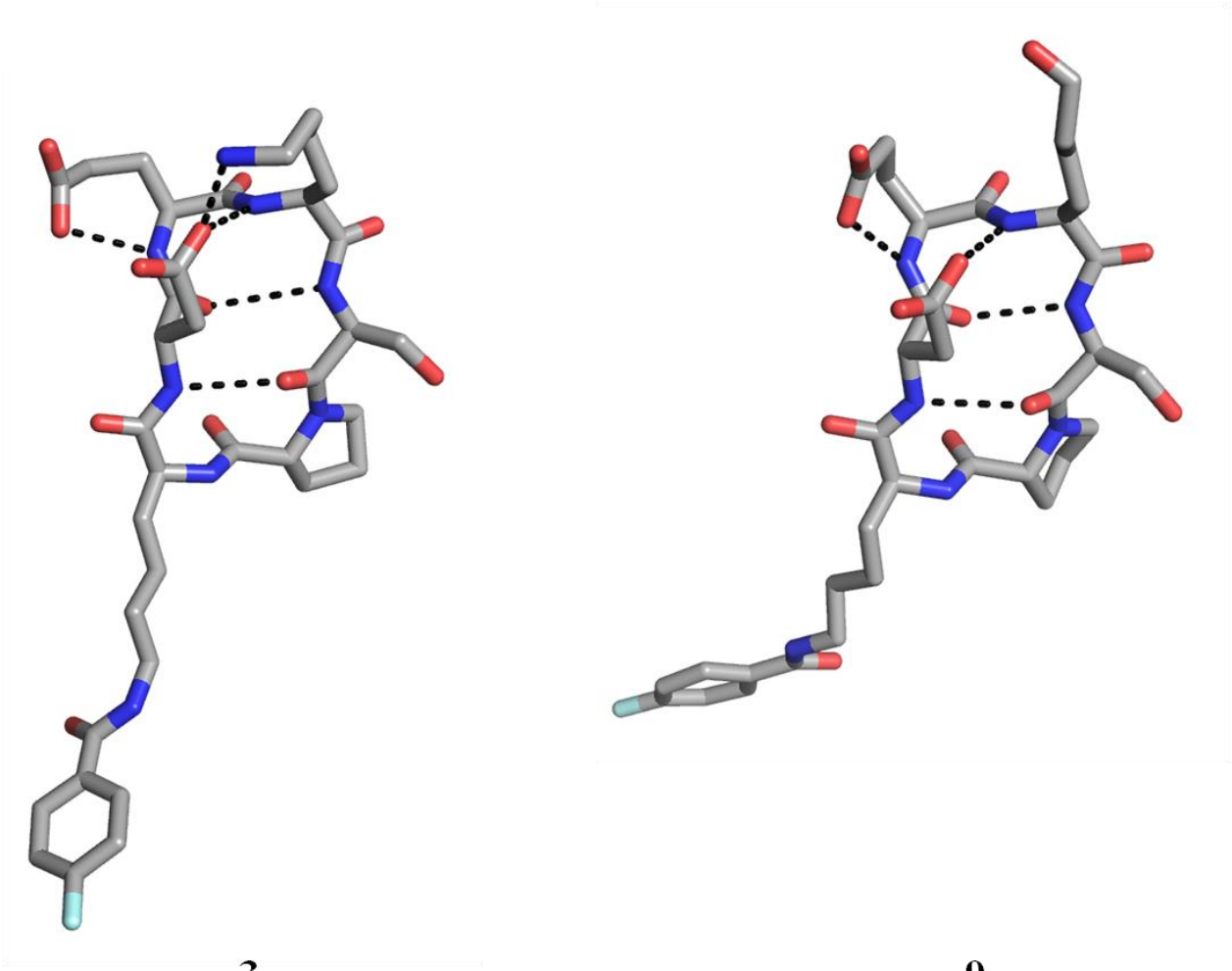

100
50
0
-50
-100
-150
degrees
ϕ(i+1)
ψ(i+1)
ϕ(i+2)
ψ(i+2)
Glu-Aly

Figure S6.

Representative structures of the cyclohexapeptides 3 and 9 from the clusters obtained using the means algorithm* along 100 ns MD simulations in DMSO. Dashed black lines stand for hydrogen bond contacts.



3 **9**

*J. Shao, S. W. Tanner, N. Thompson and T. E. Cheatham, III. *J. Chem. Theory Comput.* 2007, **3**, 2312-2334.

Figure S7.

B-factors representation derived from 100 ns trajectory of cyclo(Ser-pro-Lys(FBz)-Asp-Glu-Lys) (3) and cyclo(Ser-pro-Lys(FBz)-Asp-Glu-Aly) (9) in DMSO and water. Low B-factors represent high structural order.

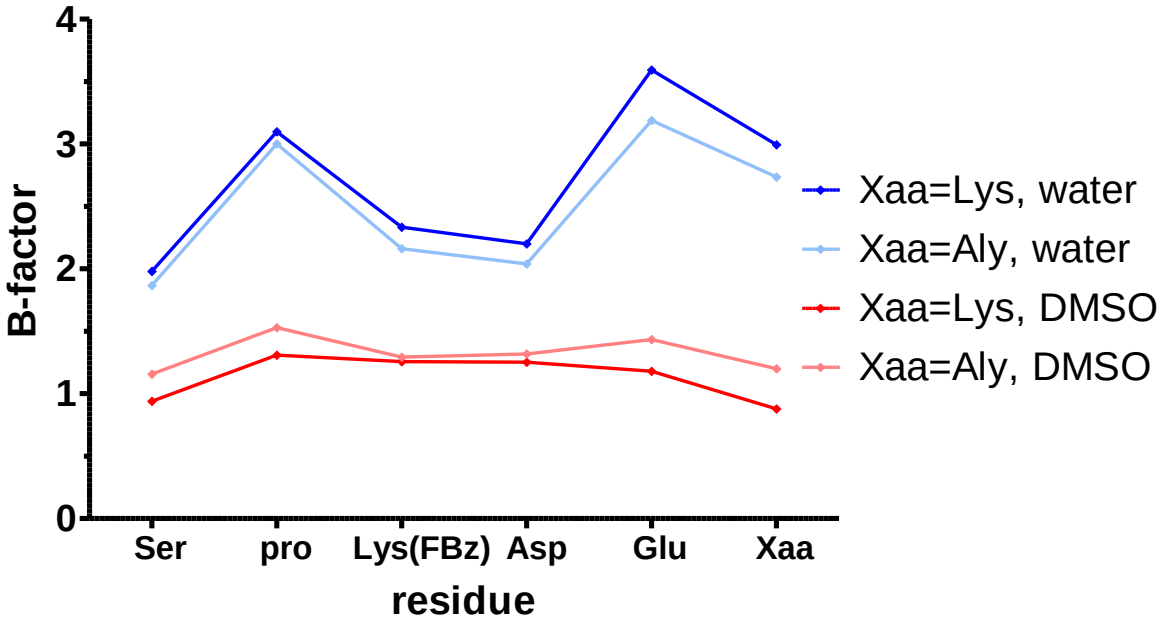
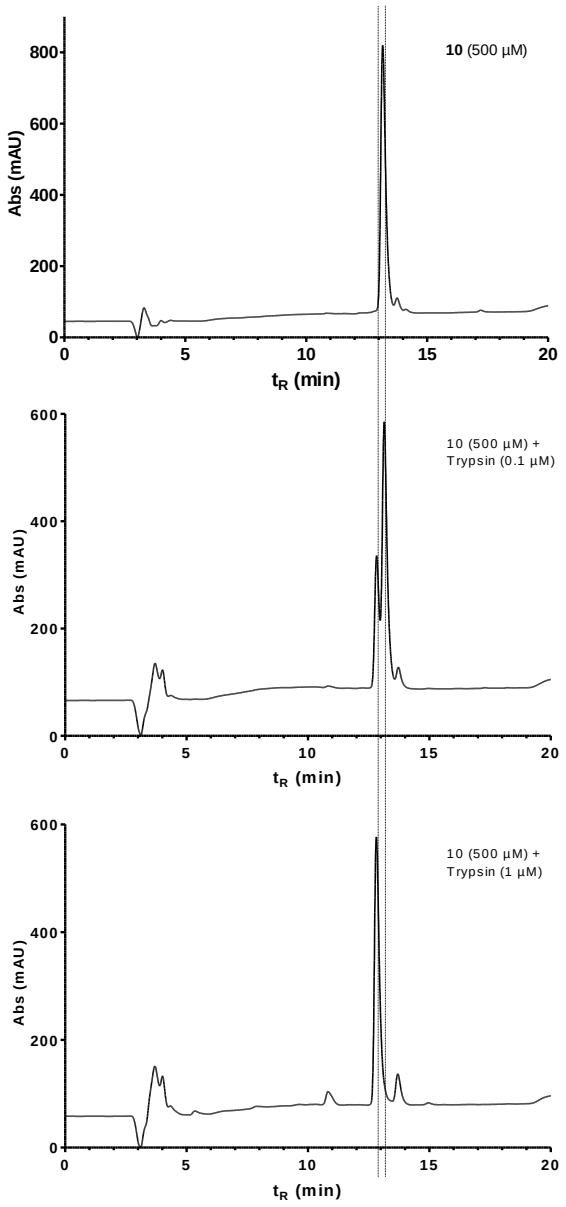
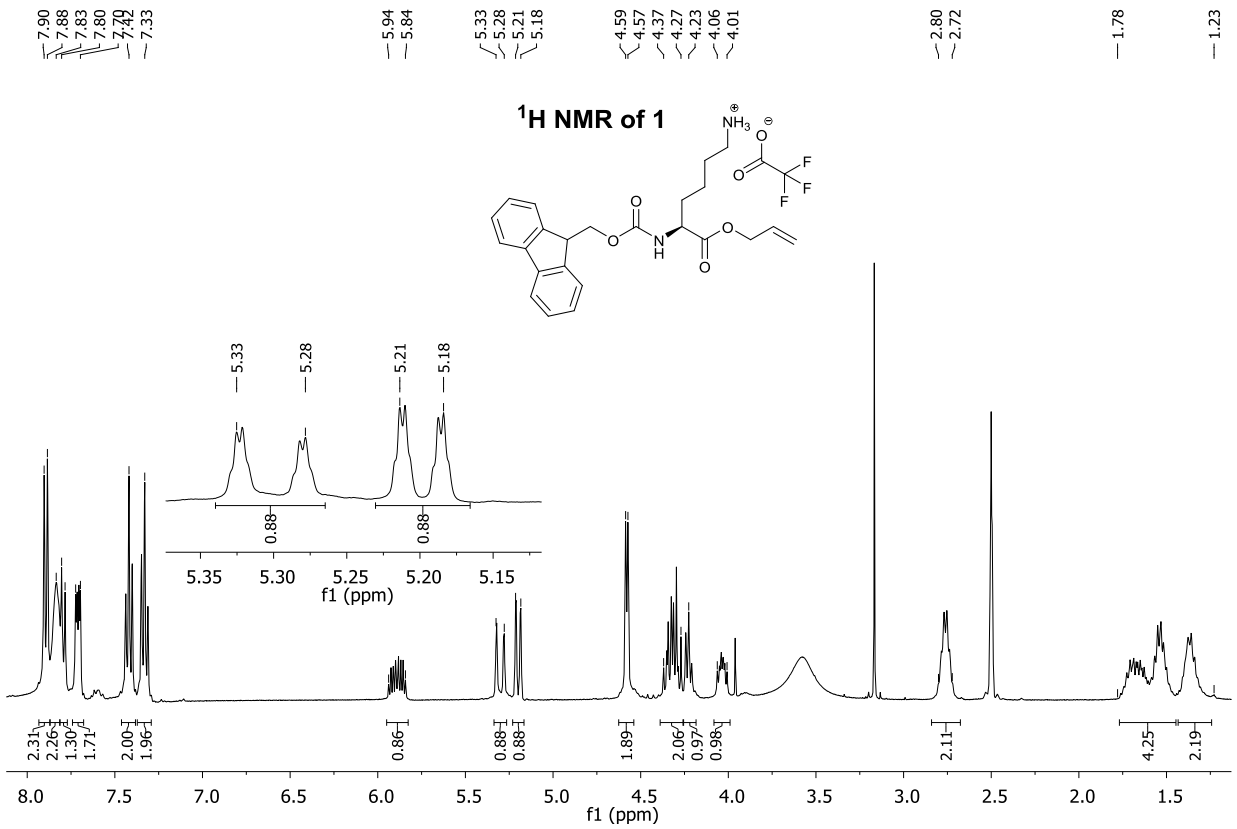


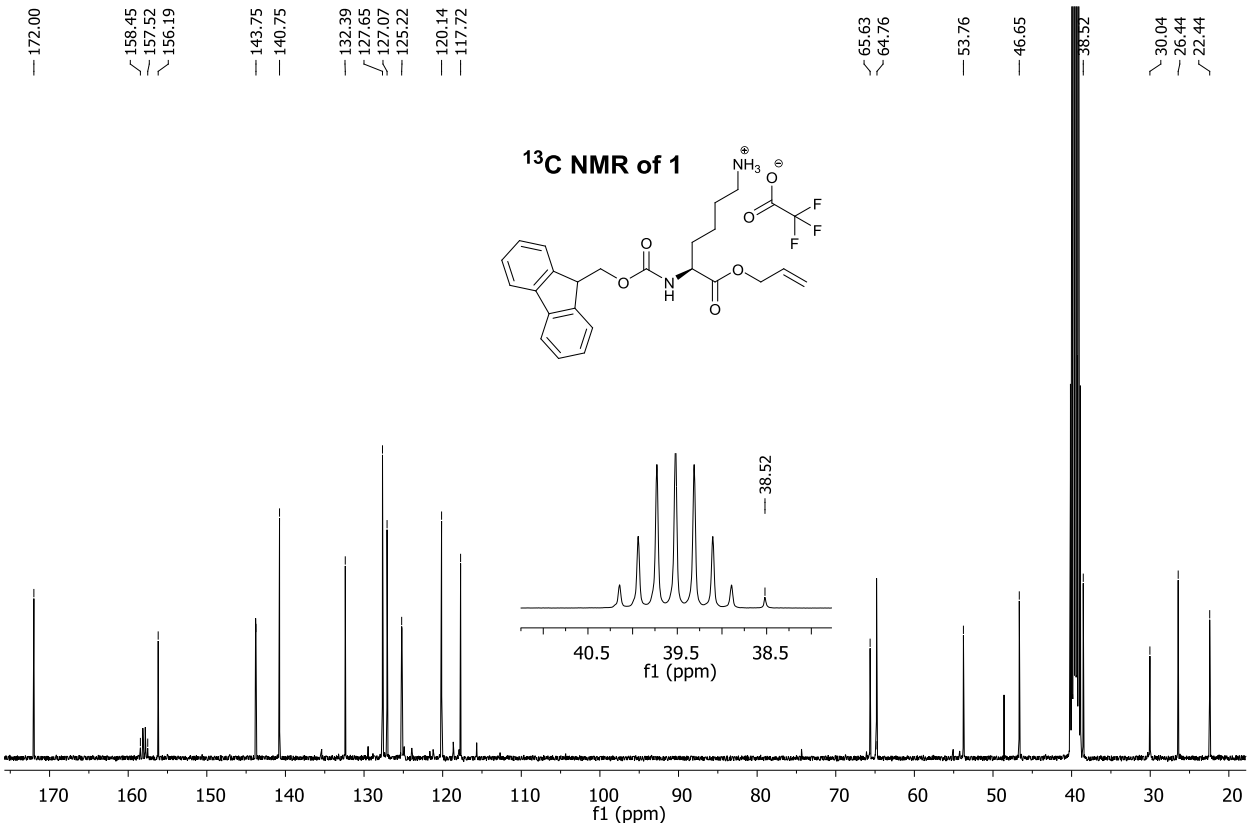
Figure S8.

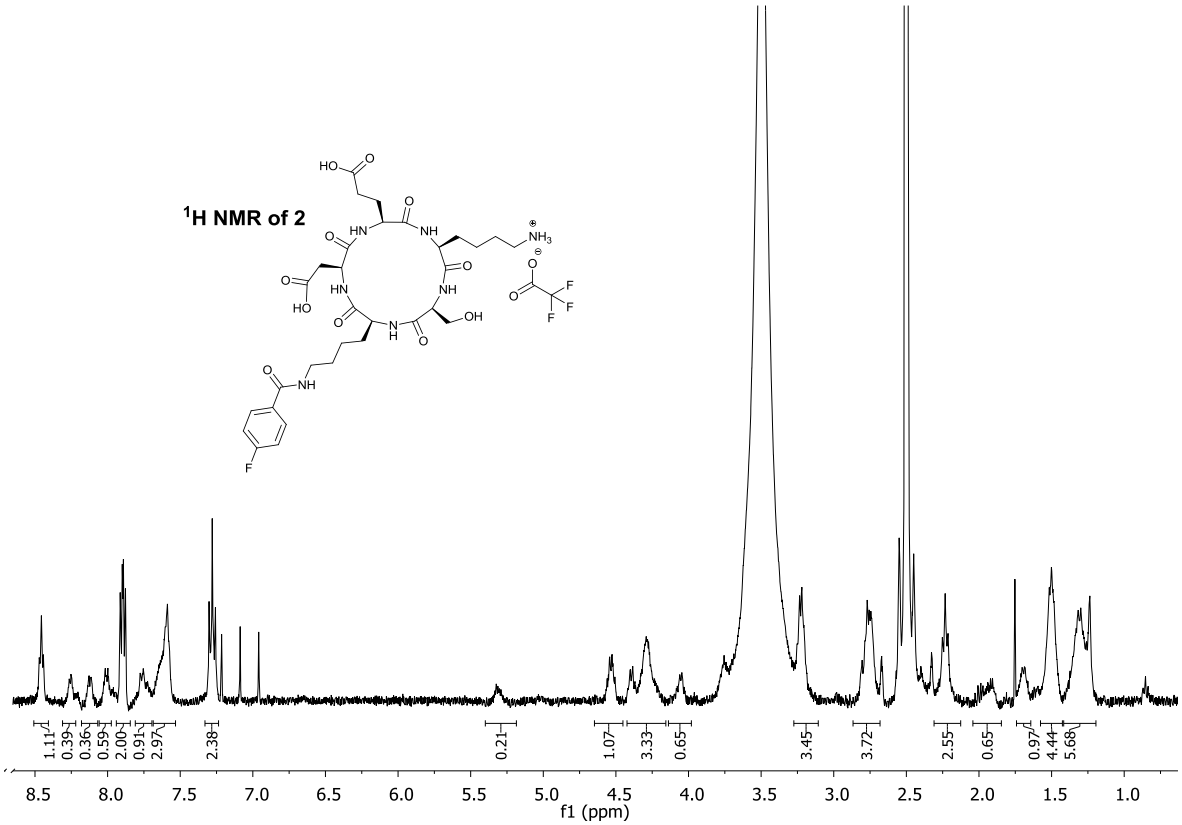
Stability of linear hexapeptide 10 against cleavage by trypsin as determined by RP-HPLC. Compound 10 was incubated with bovine trypsin at varying concentrations over a time of 30 min.

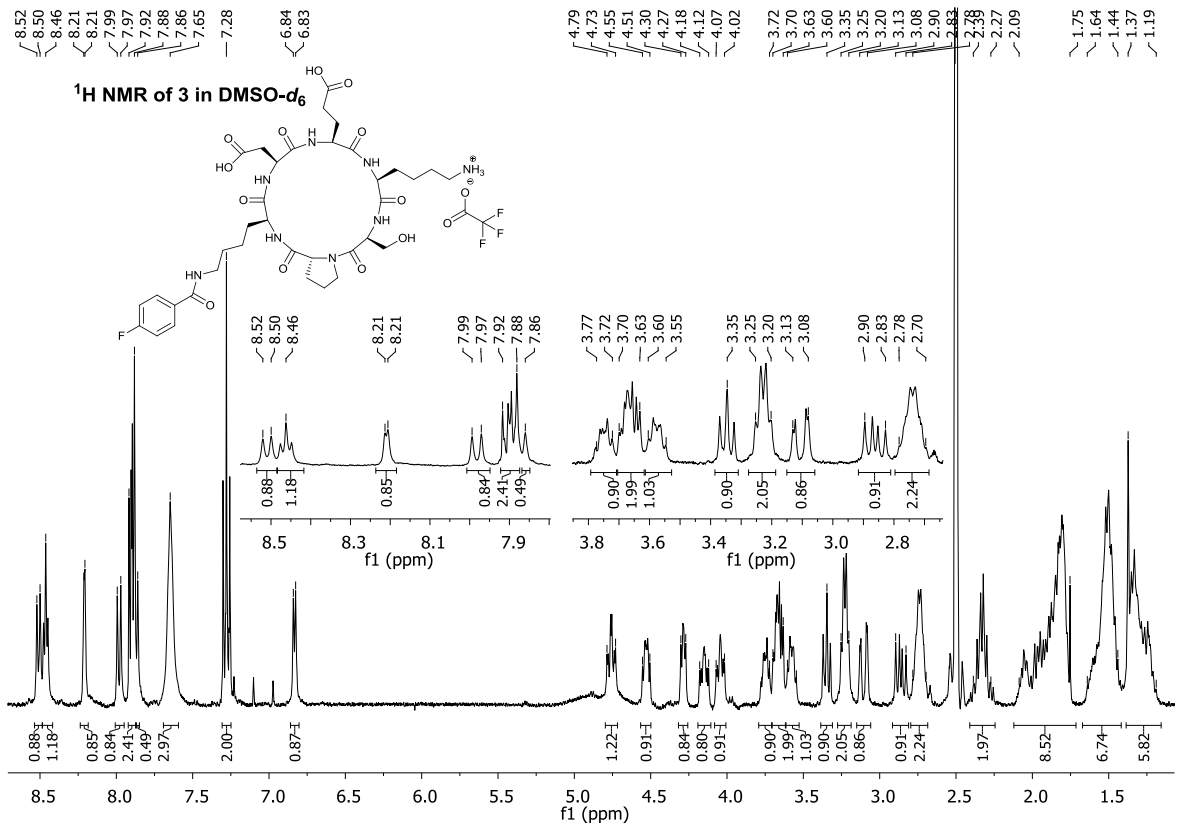


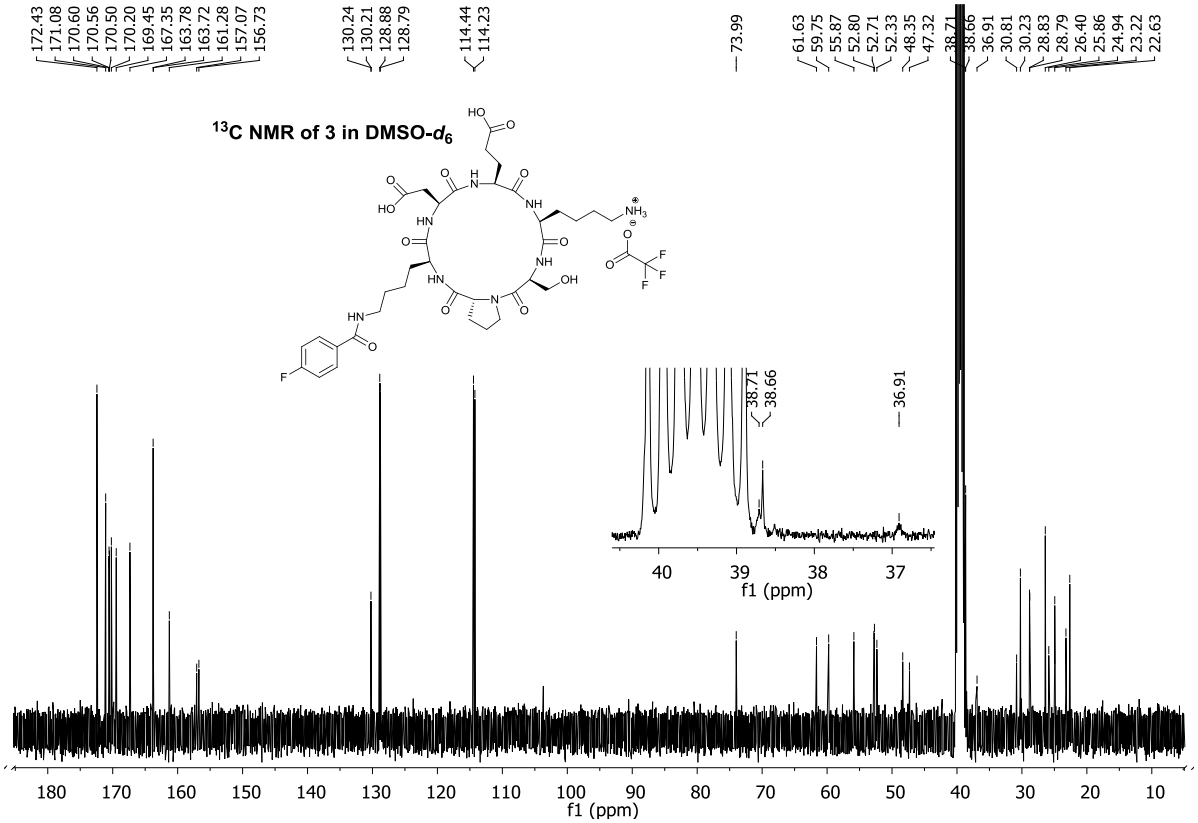
NMR Spectra of Products

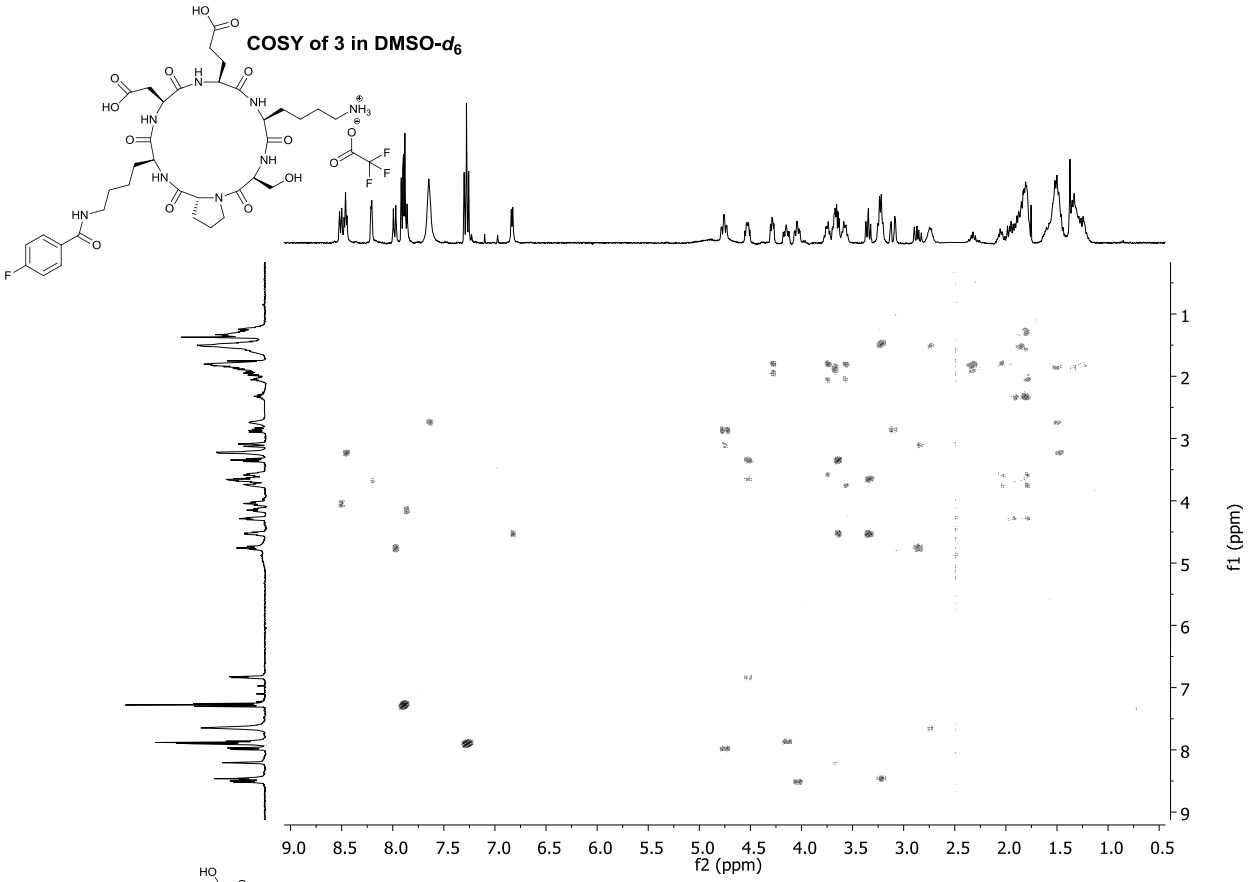


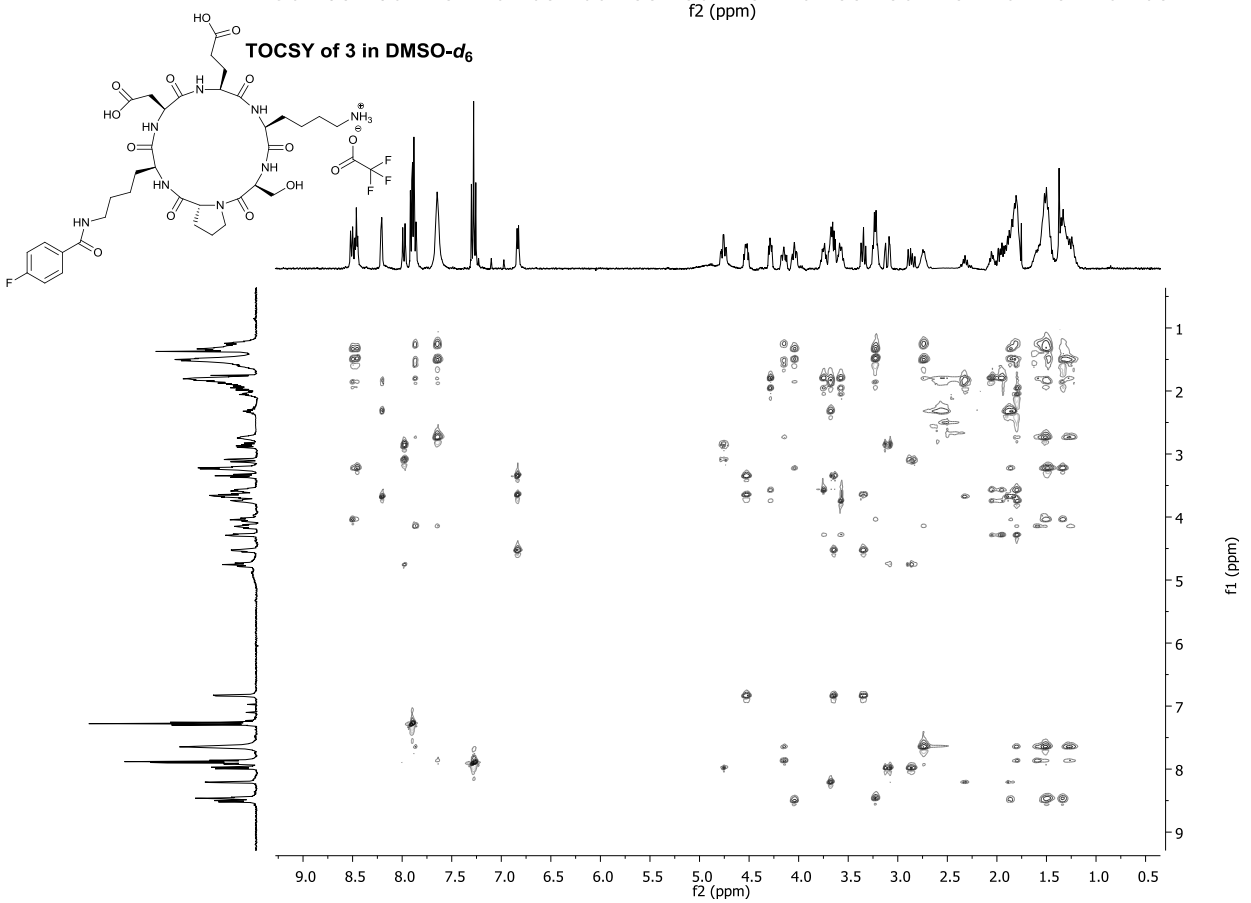


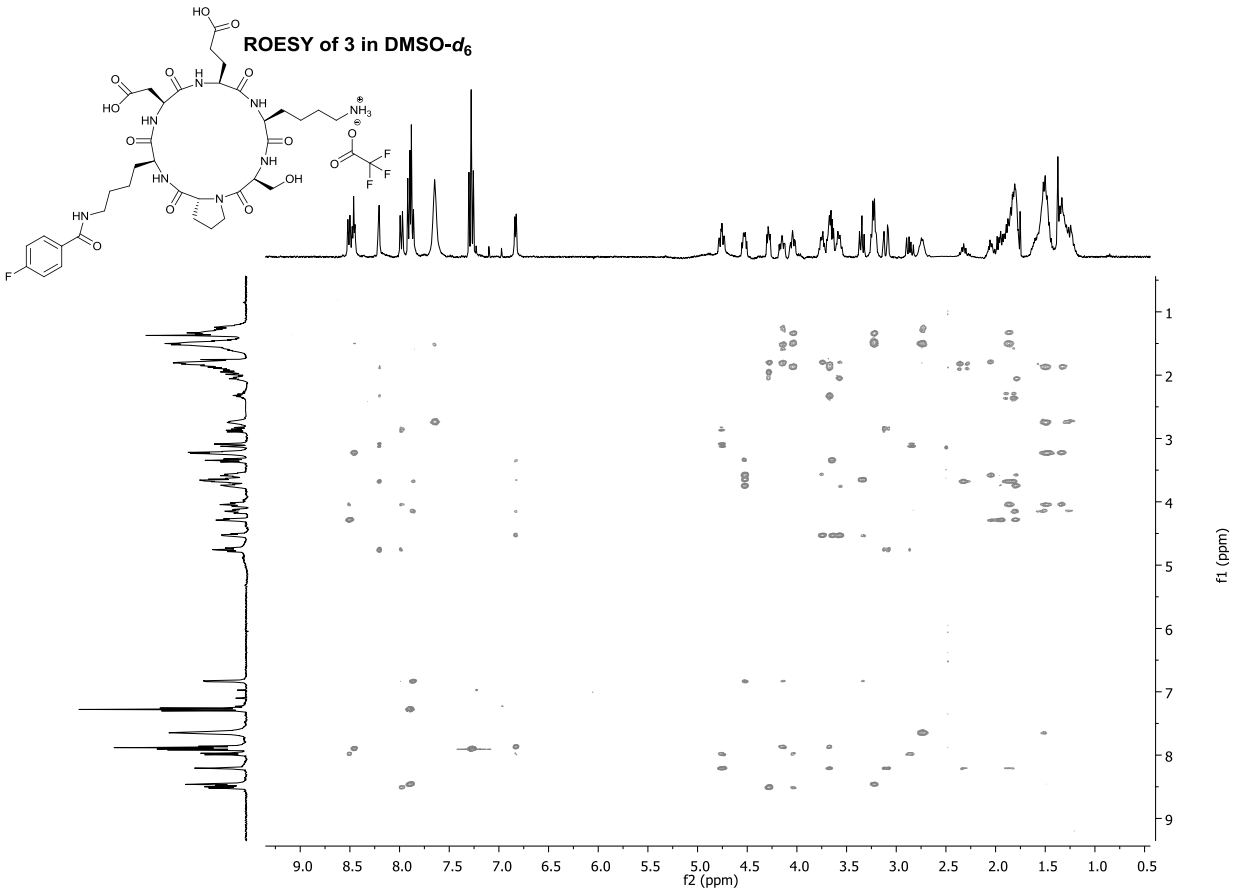

1H NMR of 2
1.11
0.39
0.36
0.59
2.00
0.91
2.97
2.38
0.21
1.07
3.33
0.65
3.45
3.72
2.55
0.65
0.97
4.44
5.68
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
f1 (ppm)

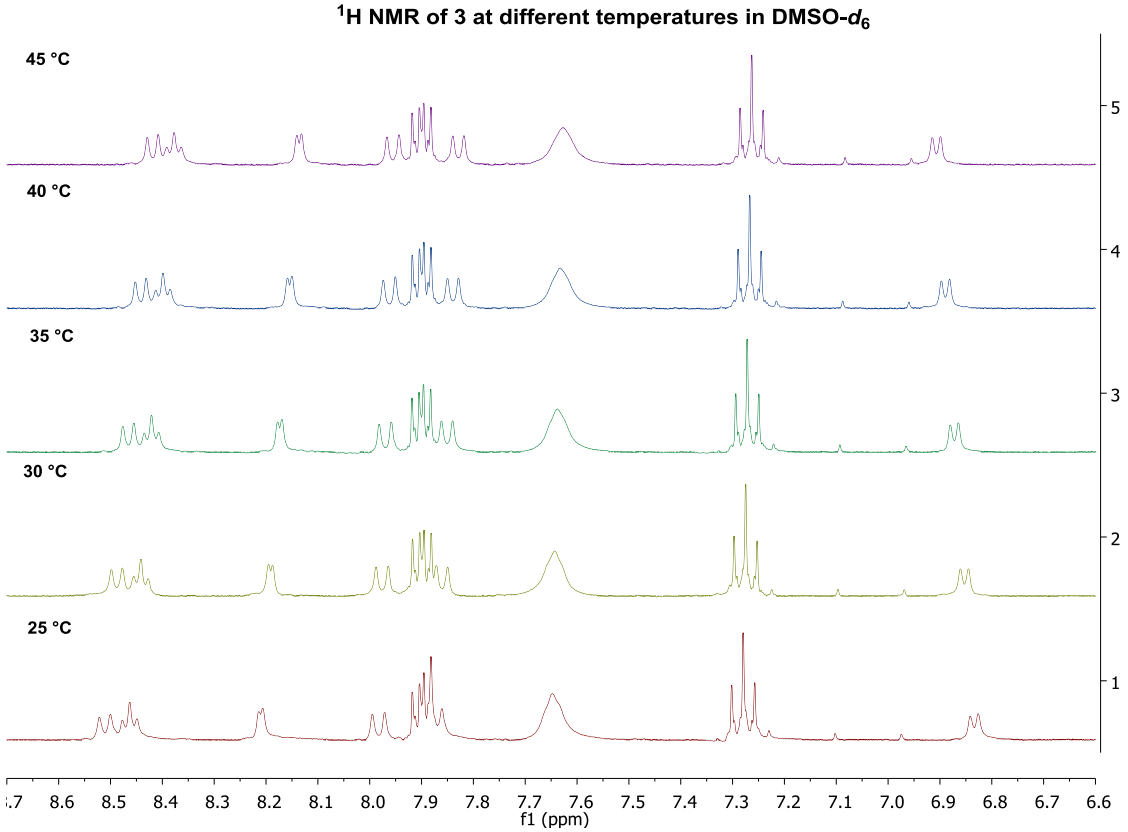

^{1}H NMR of 3 in DMSO-d_6
f1 (ppm)

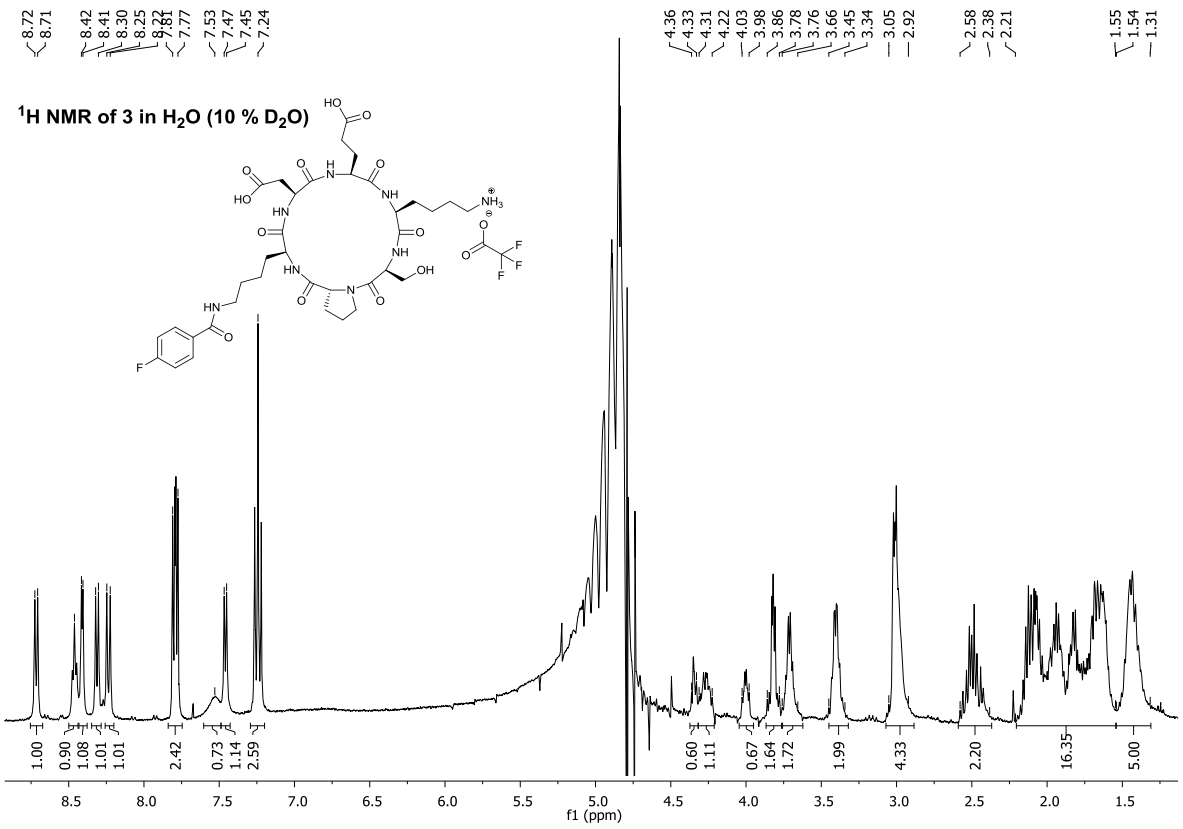

^{13}C NMR of 3 in DMSO-d_6
f1 (ppm)

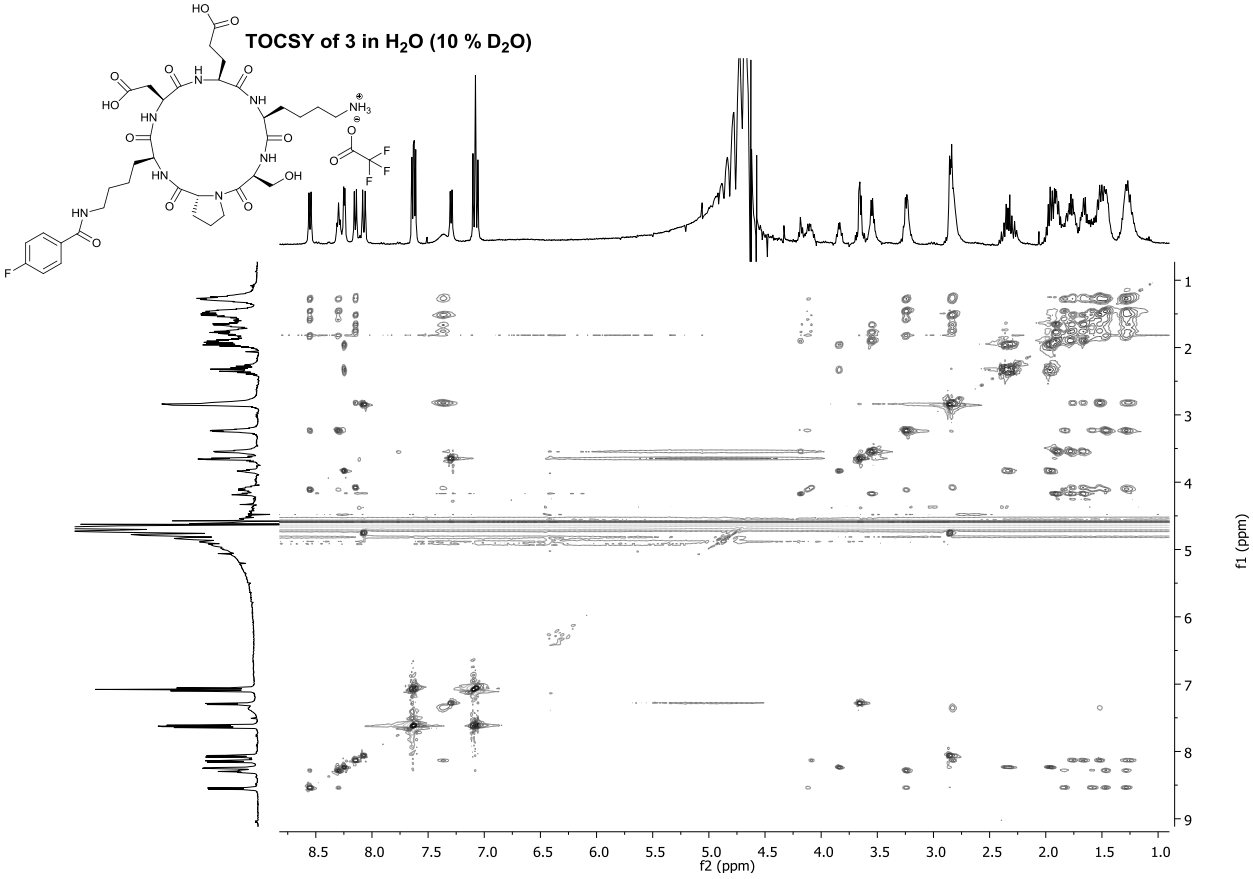

COSY of 3 in DMSO-d_6
f2 (ppm)
f1 (ppm)

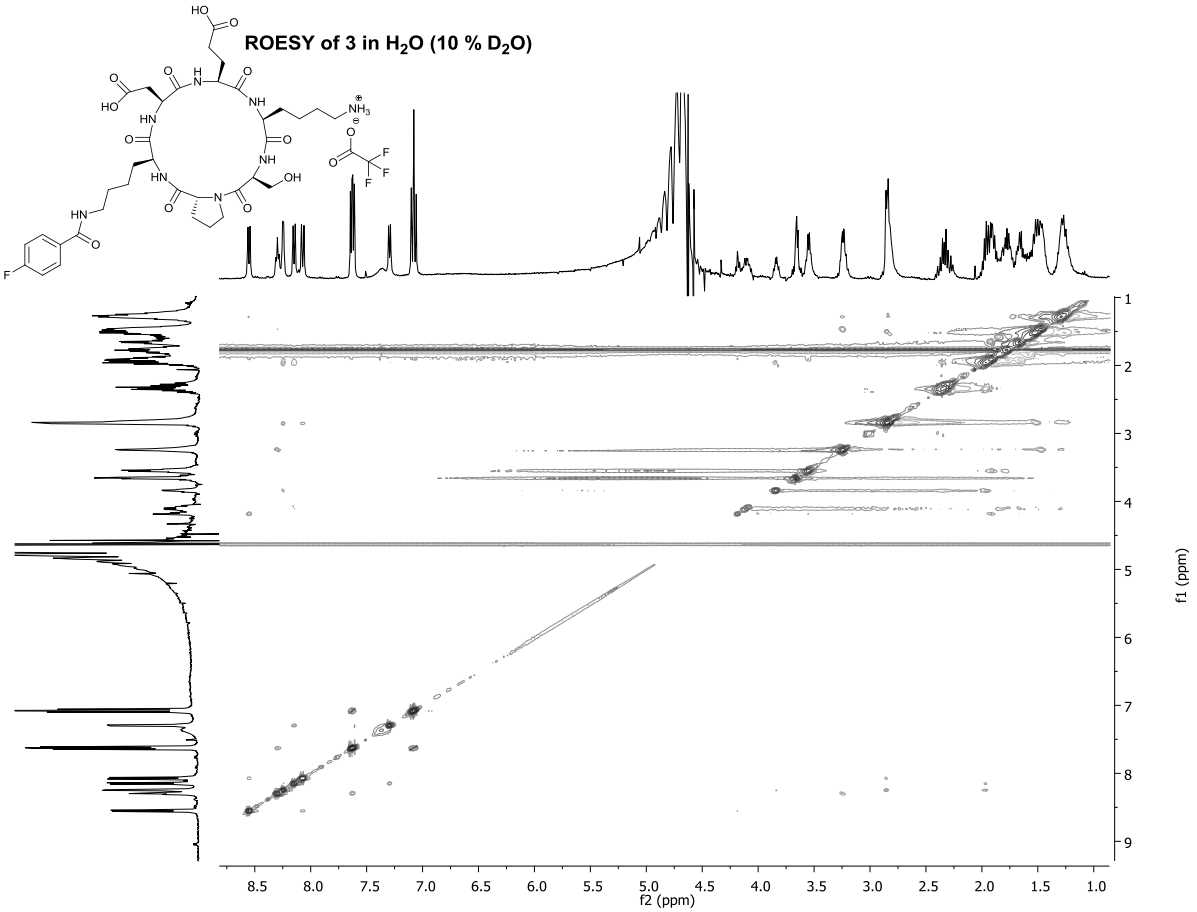

TOCSY of 3 in DMSO-d_6
f2 (ppm)
f1 (ppm)

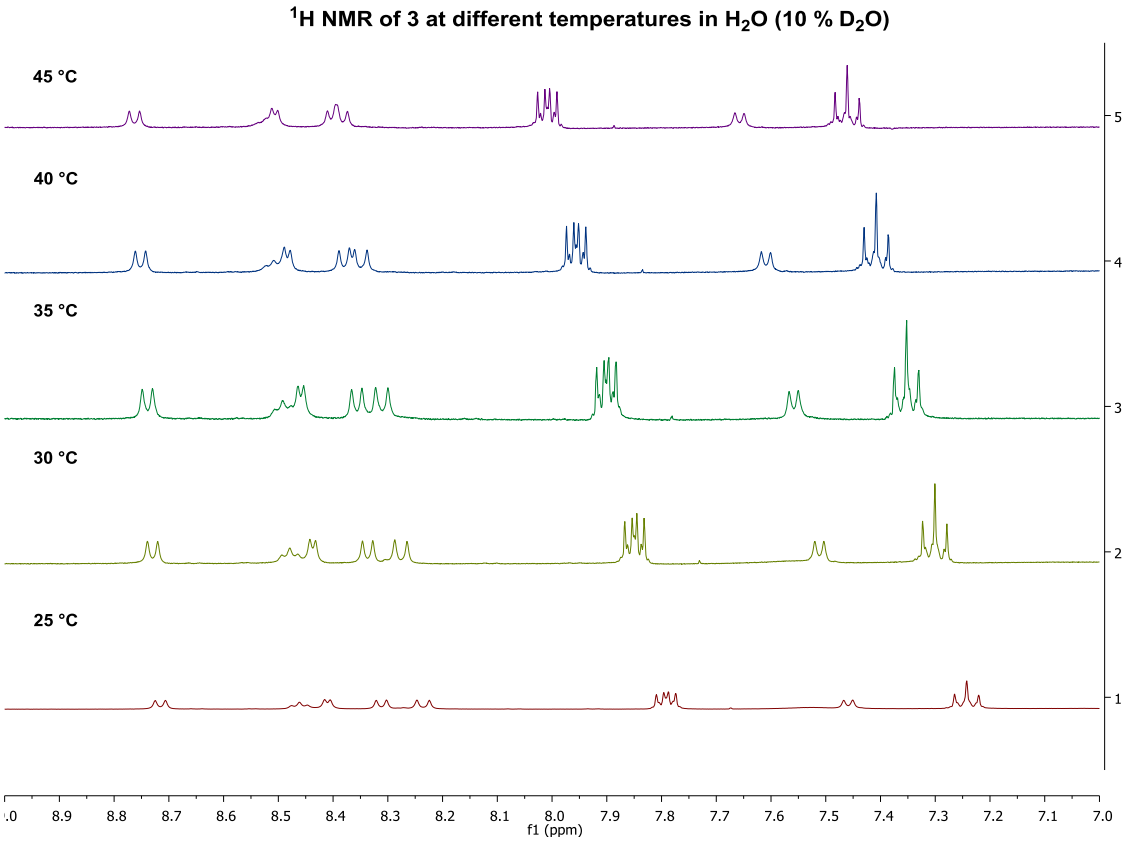

ROESY of 3 in DMSO-d_6
f2 (ppm)
f1 (ppm)

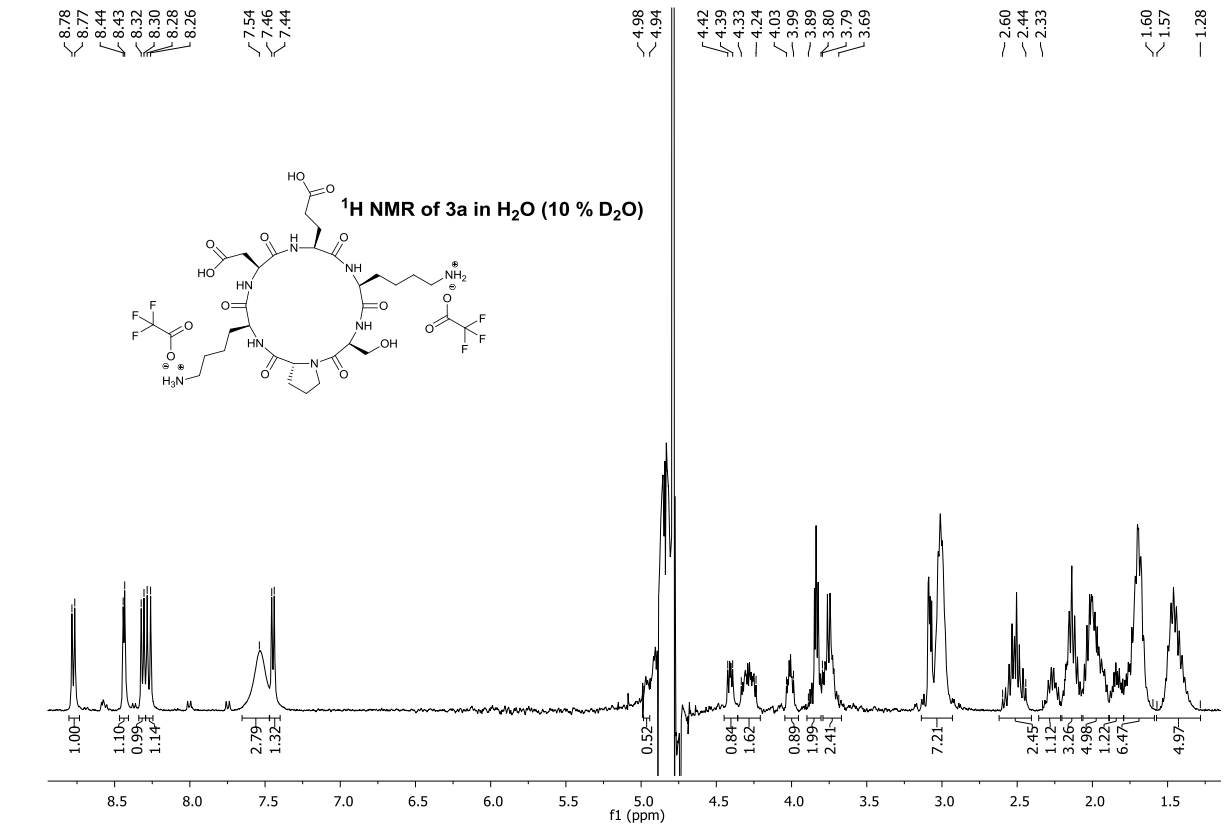

^{1}H NMR of 3 at different temperatures in DMSO-d_6
45 °C
40 °C
35 °C
30 °C
25 °C
f1 (ppm)

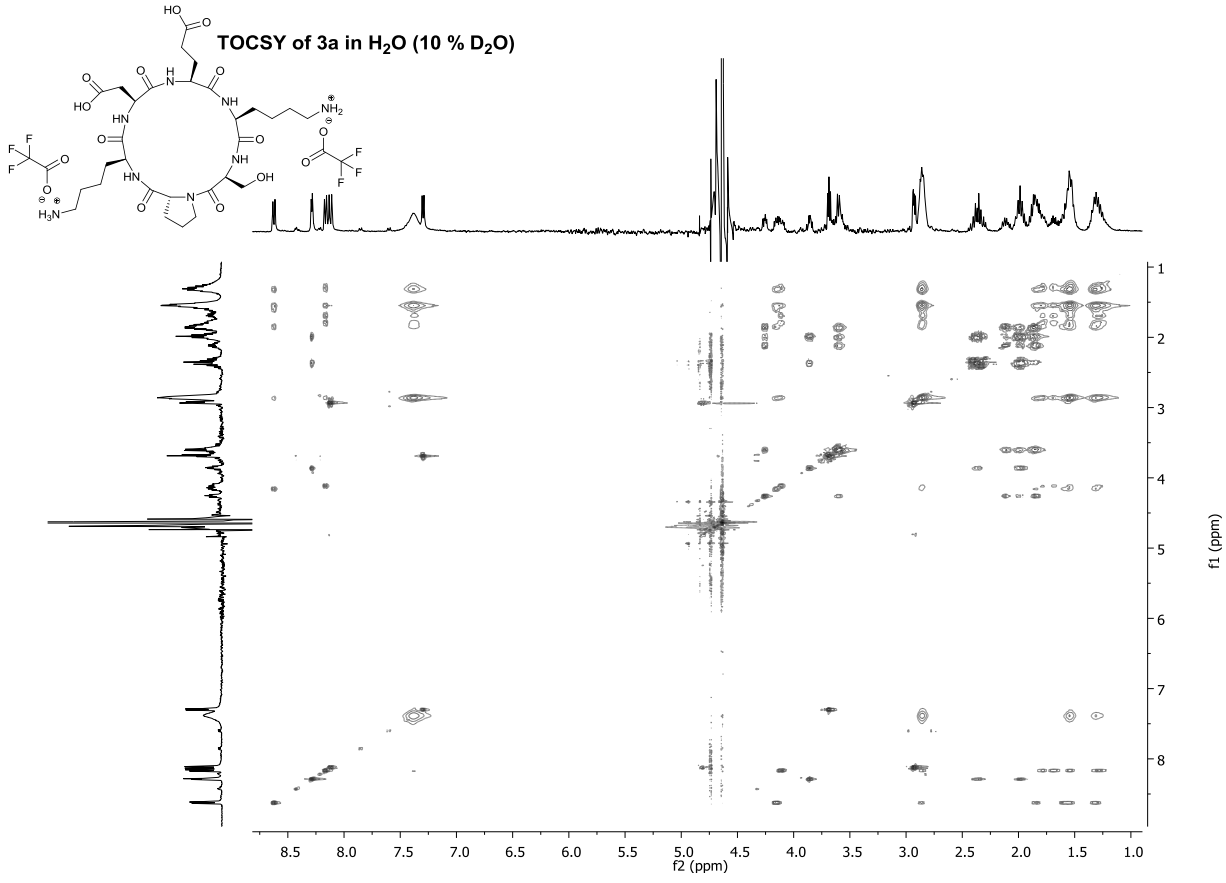

^{1}H NMR of 3 in H_2O (10 % D_2O)
f1 (ppm)

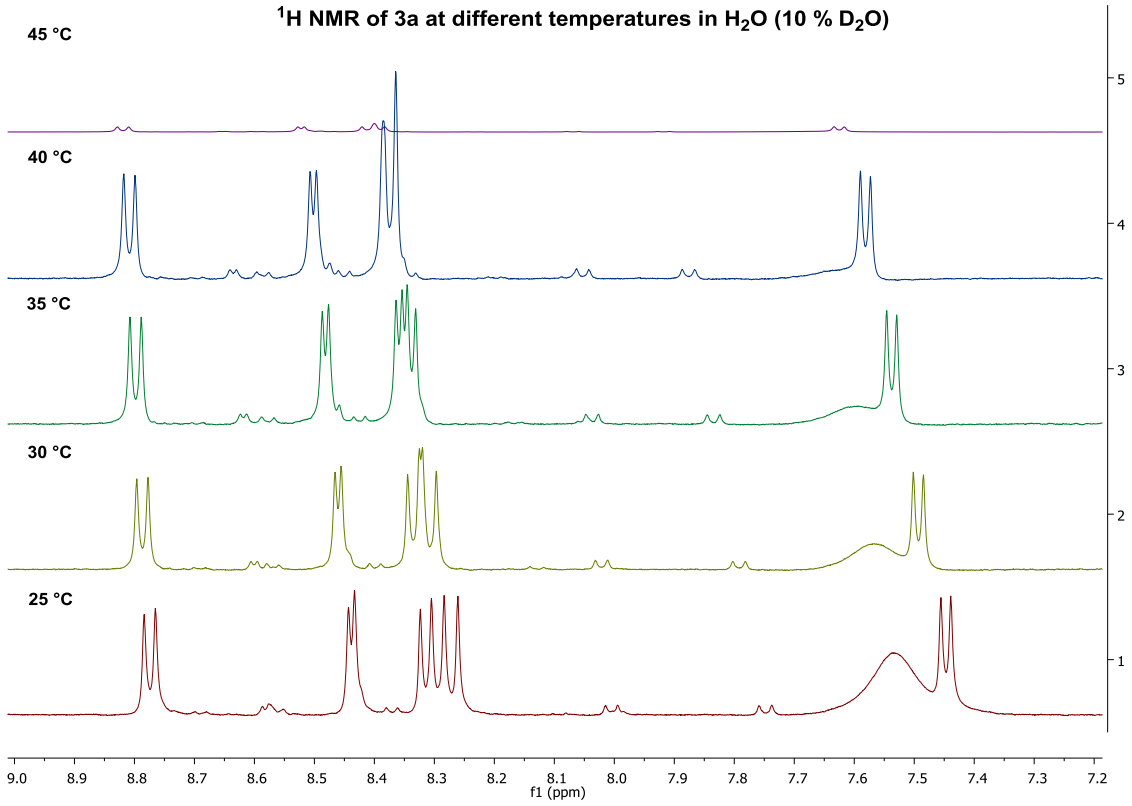

TOCSY of 3 in H_2O (10 % D_2O)
f2 (ppm)
f1 (ppm)

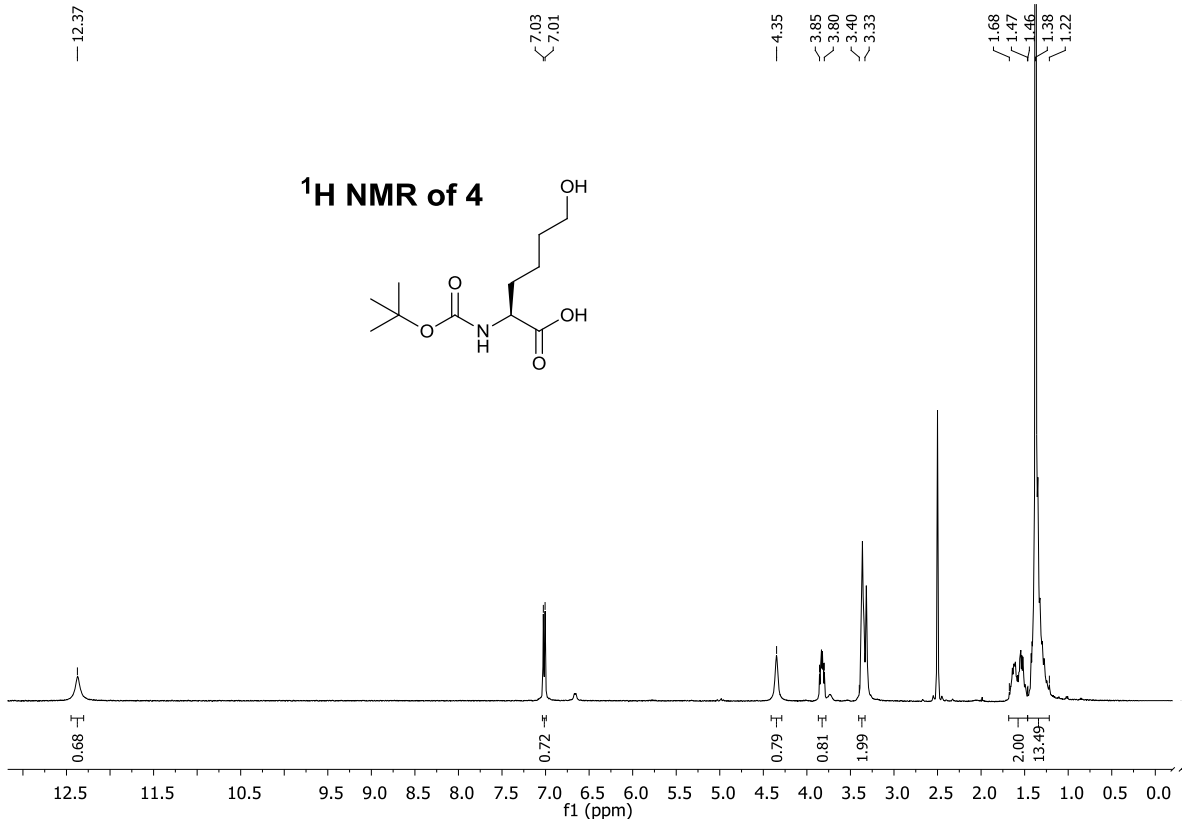

ROESY of 3 in H_2O (10 % D_2O)
f2 (ppm)
f1 (ppm)


^{1}H NMR of 3 at different temperatures in H_2O (10 % D_2O)
45 °C
40 °C
35 °C
30 °C
25 °C
f1 (ppm)

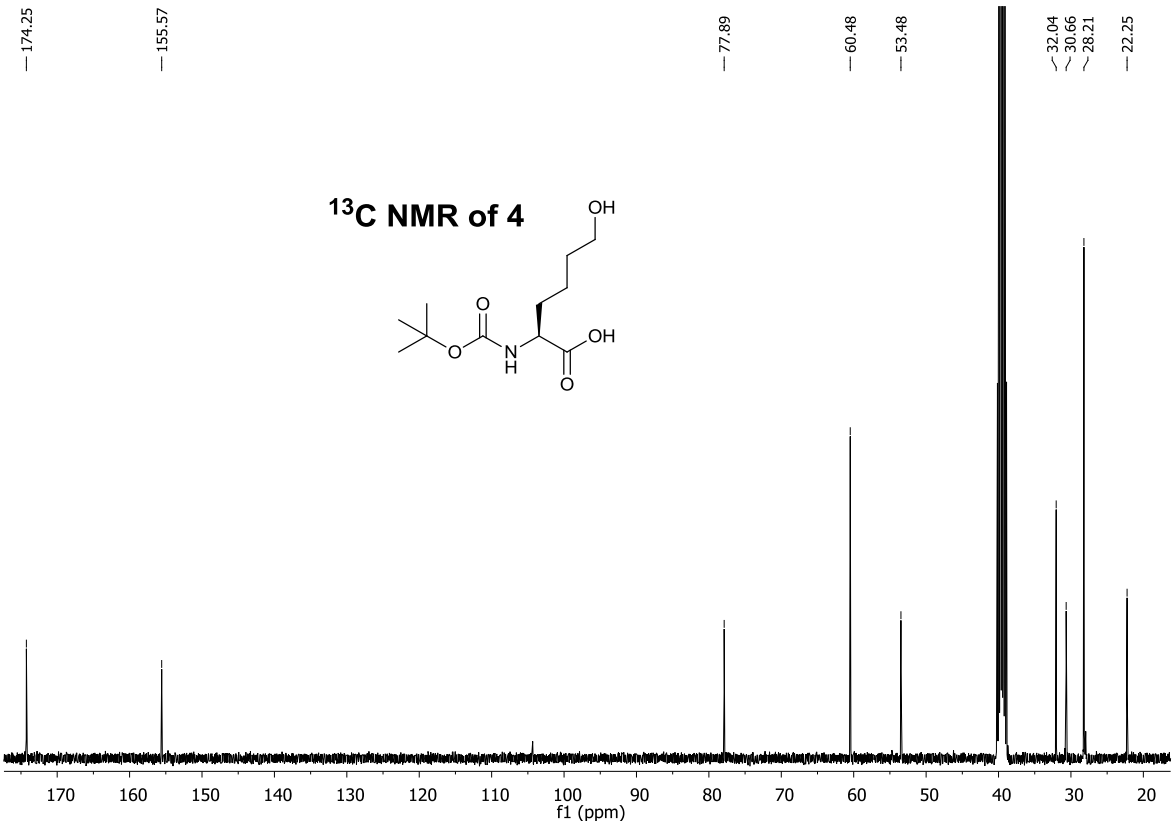

8.78
8.77
8.44
8.43
8.32
8.30
8.28
8.26
7.54
7.46
7.44
4.98
4.94
4.42
4.39
4.33
4.24
4.03
3.99
3.89
3.80
3.79
3.69
2.60
2.44
2.33
1.60
1.57
1.28
1H NMR of 3a in H2O (10 % D2O)
1.00
1.10
0.99
1.14
2.79
1.32
0.52
0.84
1.62
0.89
1.99
2.41
7.21
2.45
1.12
3.26
4.98
1.22
6.47
4.97
f1 (ppm)


TOCSY of 3a in H2O (10 % D2O)
f2 (ppm)
f1 (ppm)

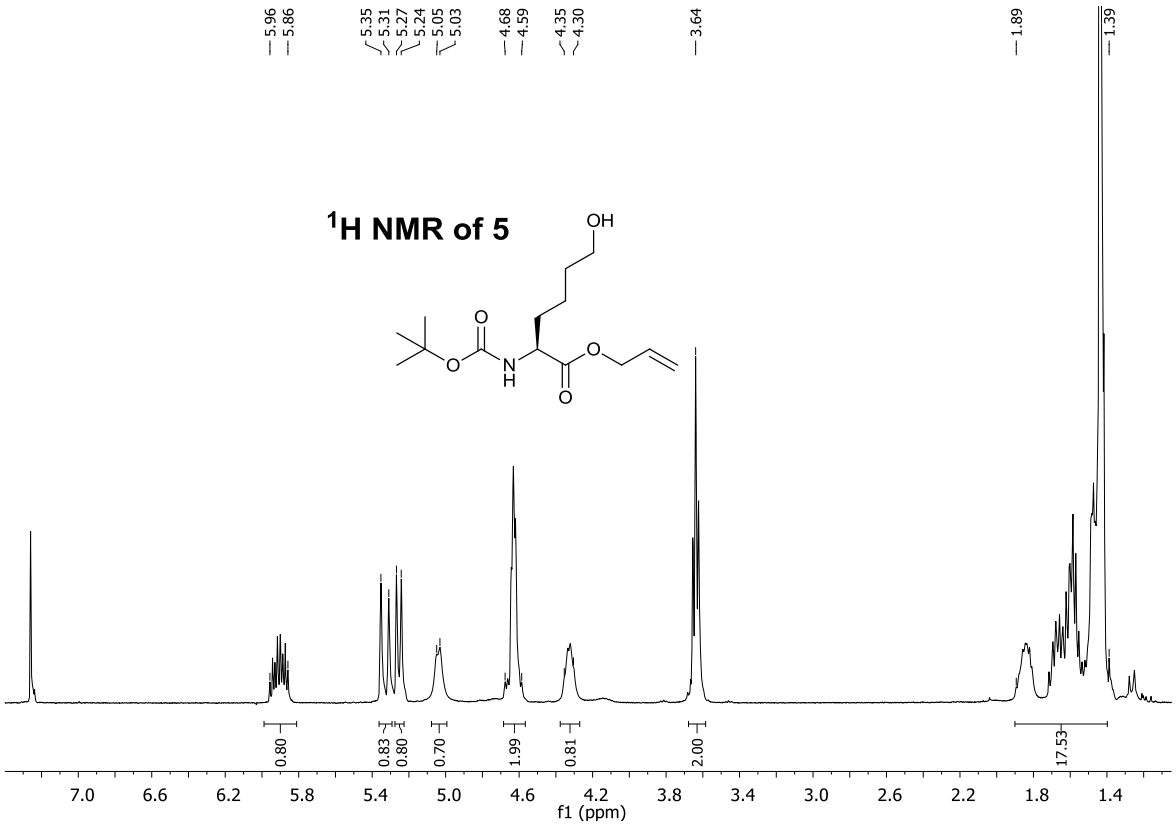

^{1}H NMR of 3a at different temperatures in H_2O (10 % D_2O)
45 °C
40 °C
35 °C
30 °C
25 °C
5
4
3
2
1
9.0
8.9
8.8
8.7
8.6
8.5
8.4
8.3
8.2
8.1
8.0
7.9
7.8
7.7
7.6
7.5
7.4
7.3
7.2
f1 (ppm)

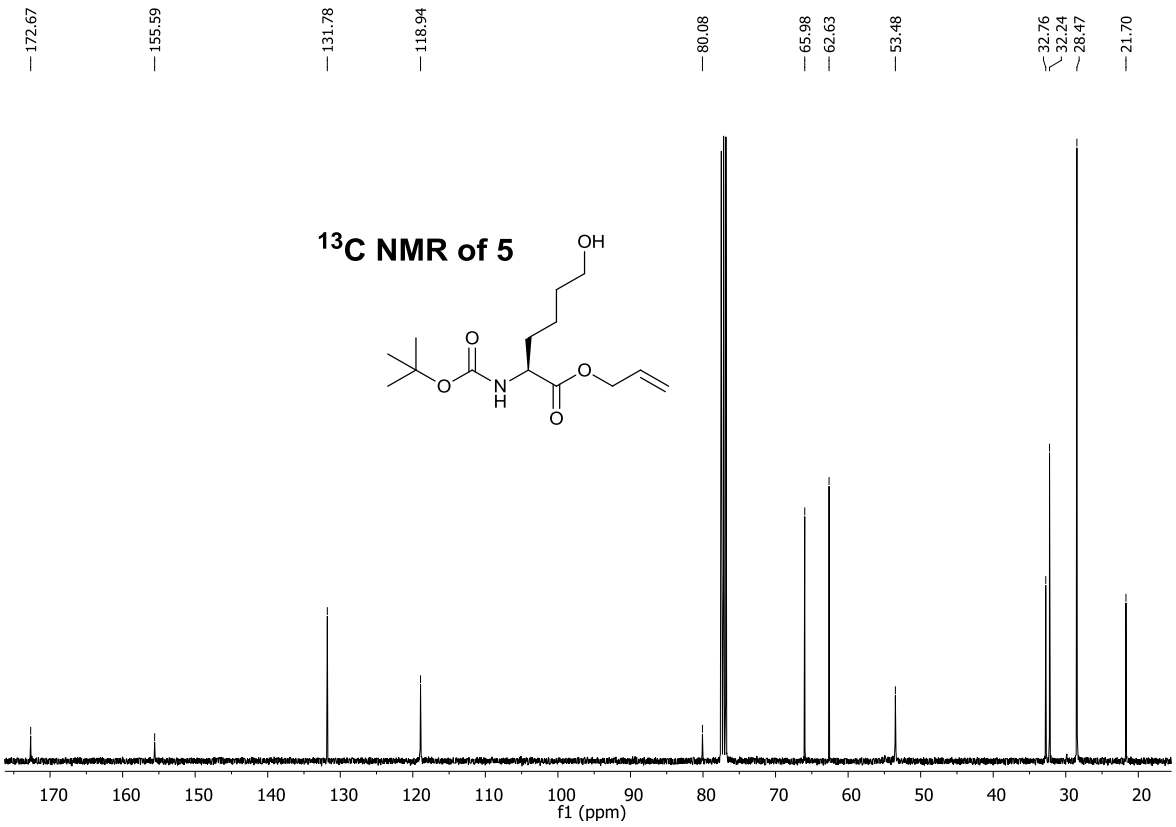


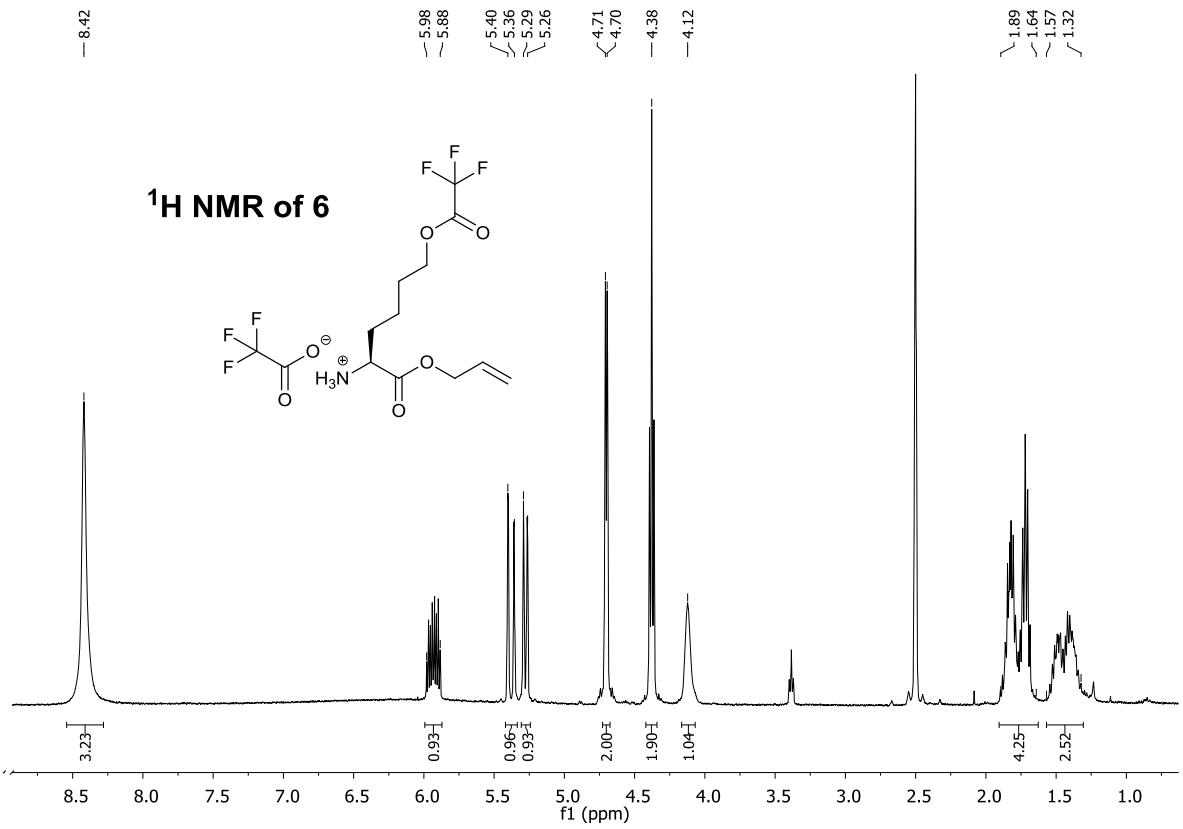
^{1}H NMR of 4

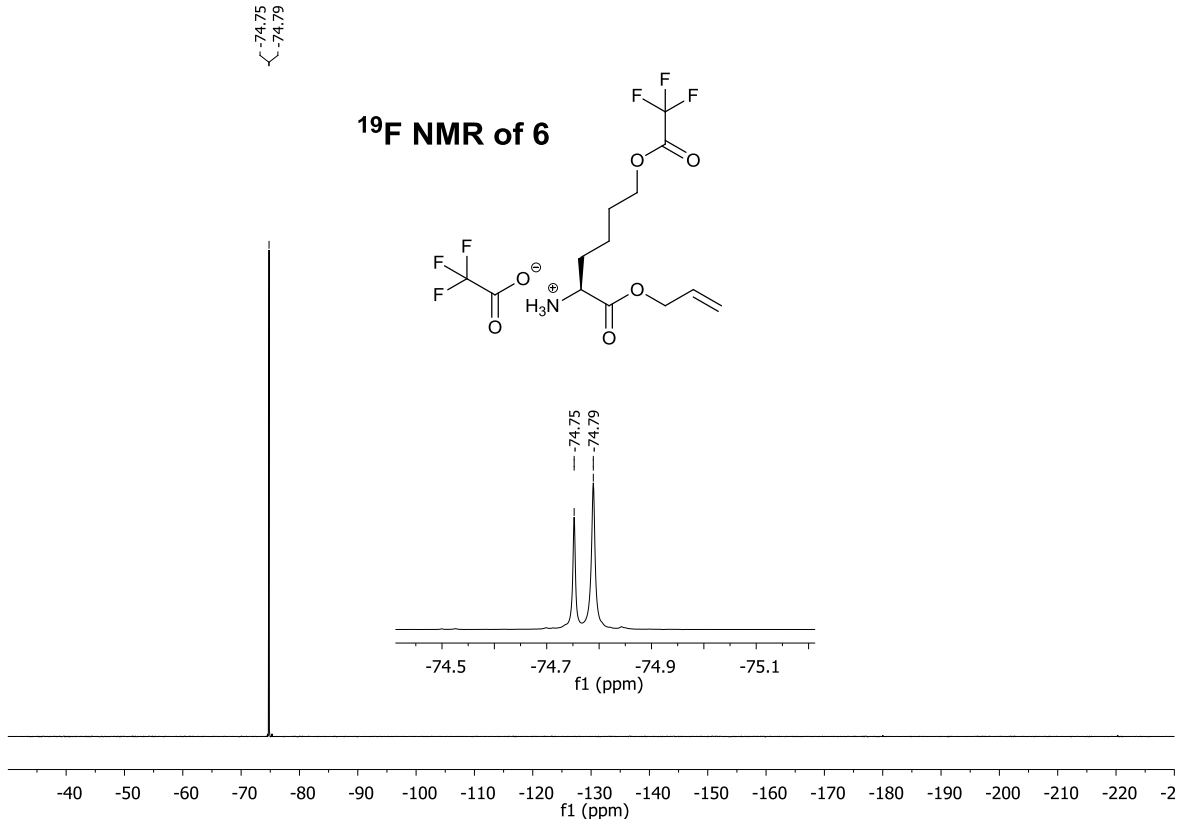


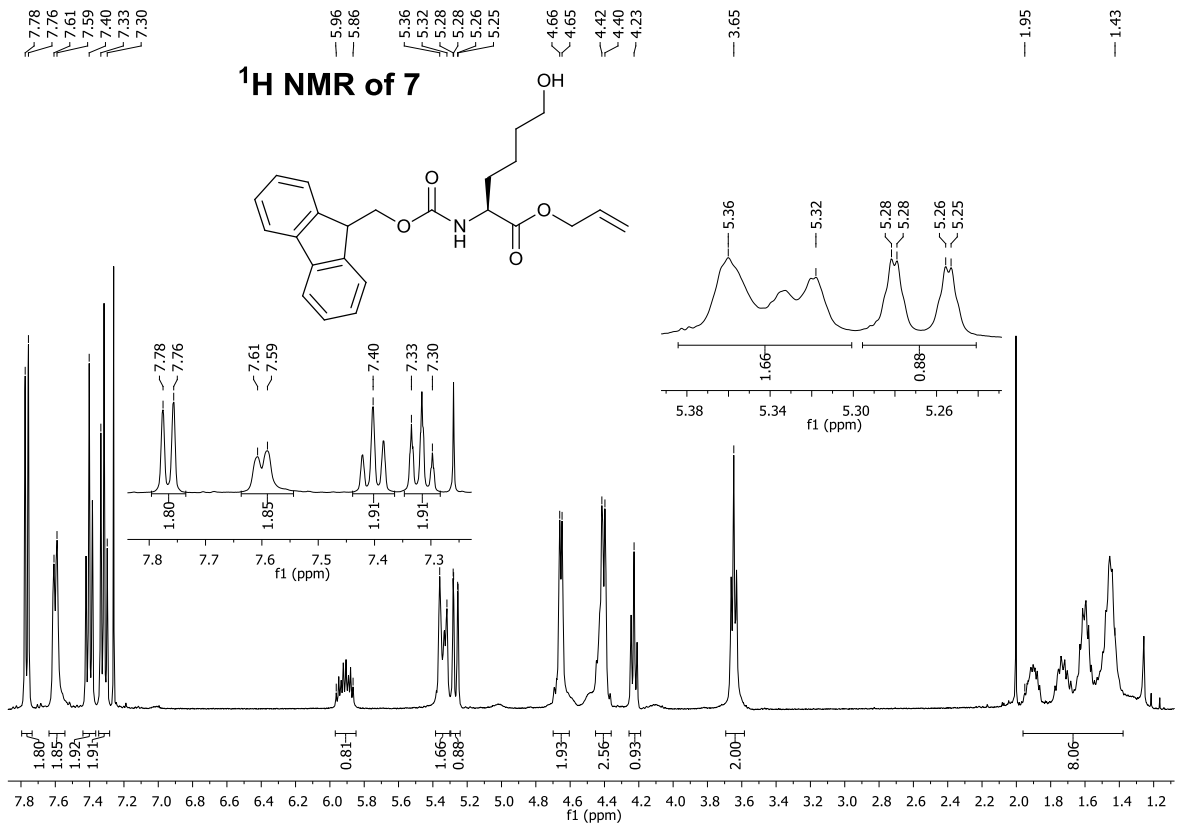
^{13}C NMR of 4

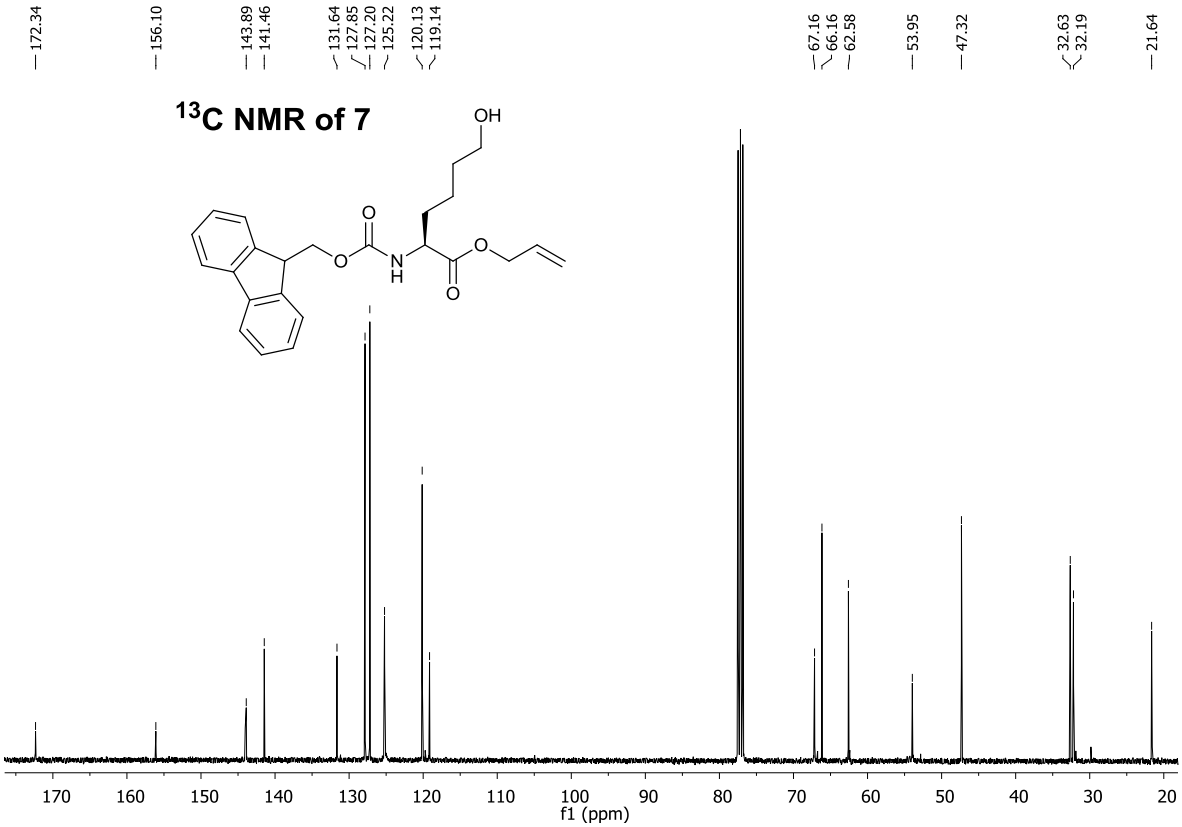

5.96
5.86
5.35
5.31
5.27
5.24
5.05
5.03
4.68
4.59
4.35
4.30
3.64
1.89
1.39
^{1}H NMR of 5
OH
O
O
N
H
O
O
O
0.80
0.83
0.80
0.70
1.99
0.81
2.00
17.53
7.0
6.6
6.2
5.8
5.4
5.0
4.6
4.2
3.8
3.4
3.0
2.6
2.2
1.8
1.4
f1 (ppm)

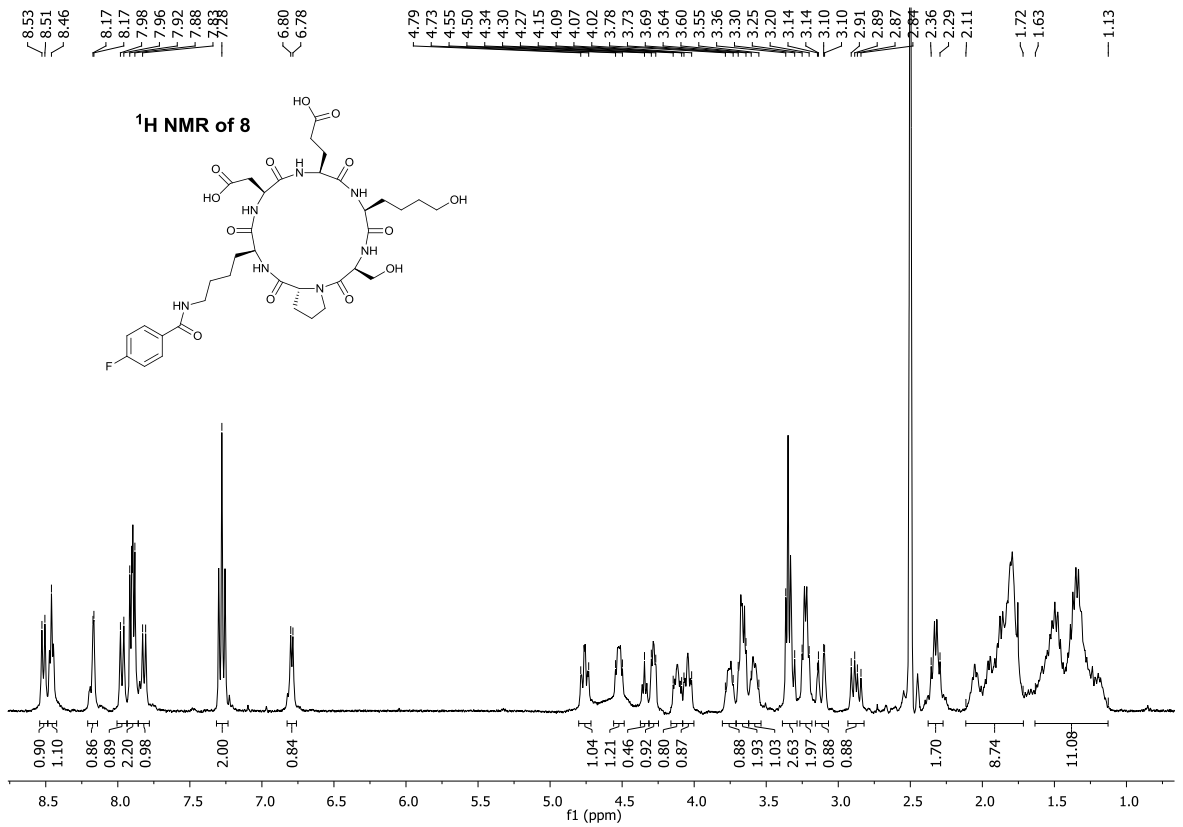

172.67
155.59
131.78
118.94
80.08
65.98
62.63
53.48
32.76
32.24
28.47
21.70
^{13}C NMR of 5
OH
O
O
N
H
O
O
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
f1 (ppm)

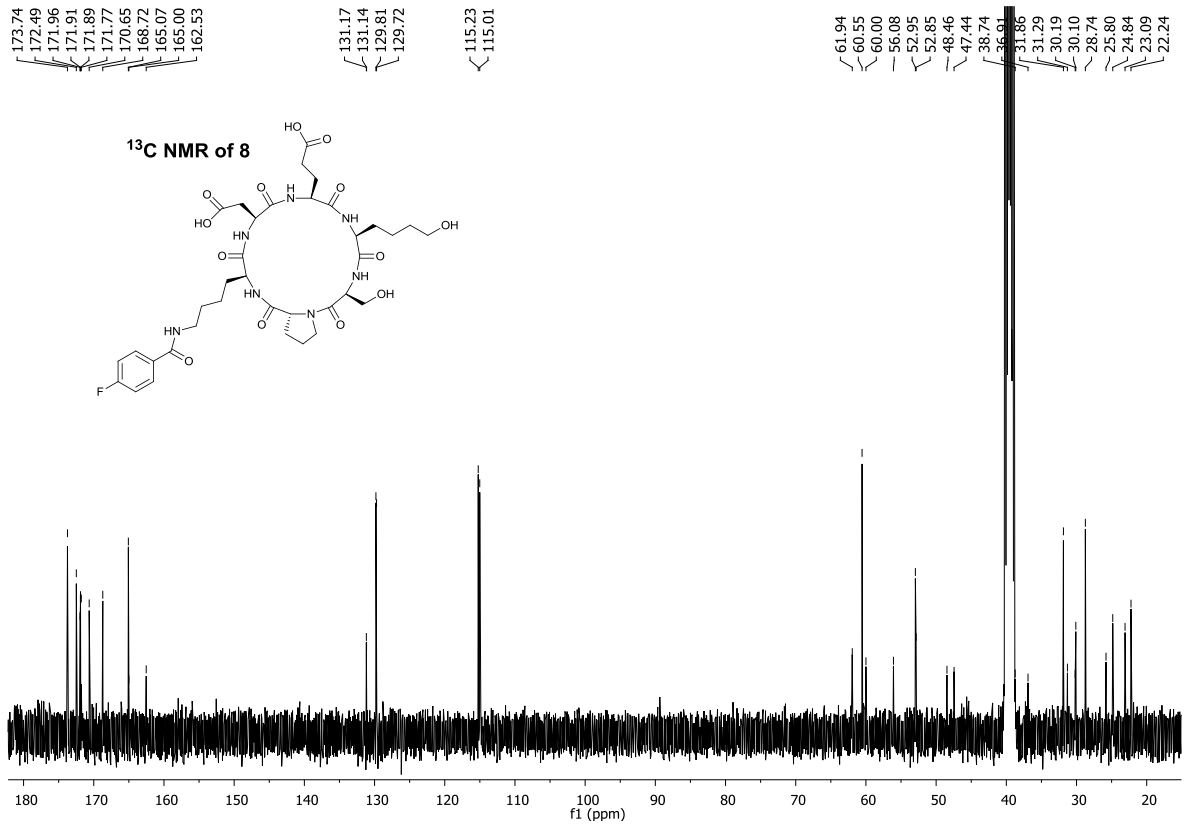

8.42
5.98
5.88
5.40
5.36
5.29
5.26
4.71
4.70
4.38
4.12
1.89
1.64
1.57
1.32
1H NMR of 6
3.23
0.93
0.96
0.93
2.00
1.90
1.04
4.25
2.52
8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0
f1 (ppm)

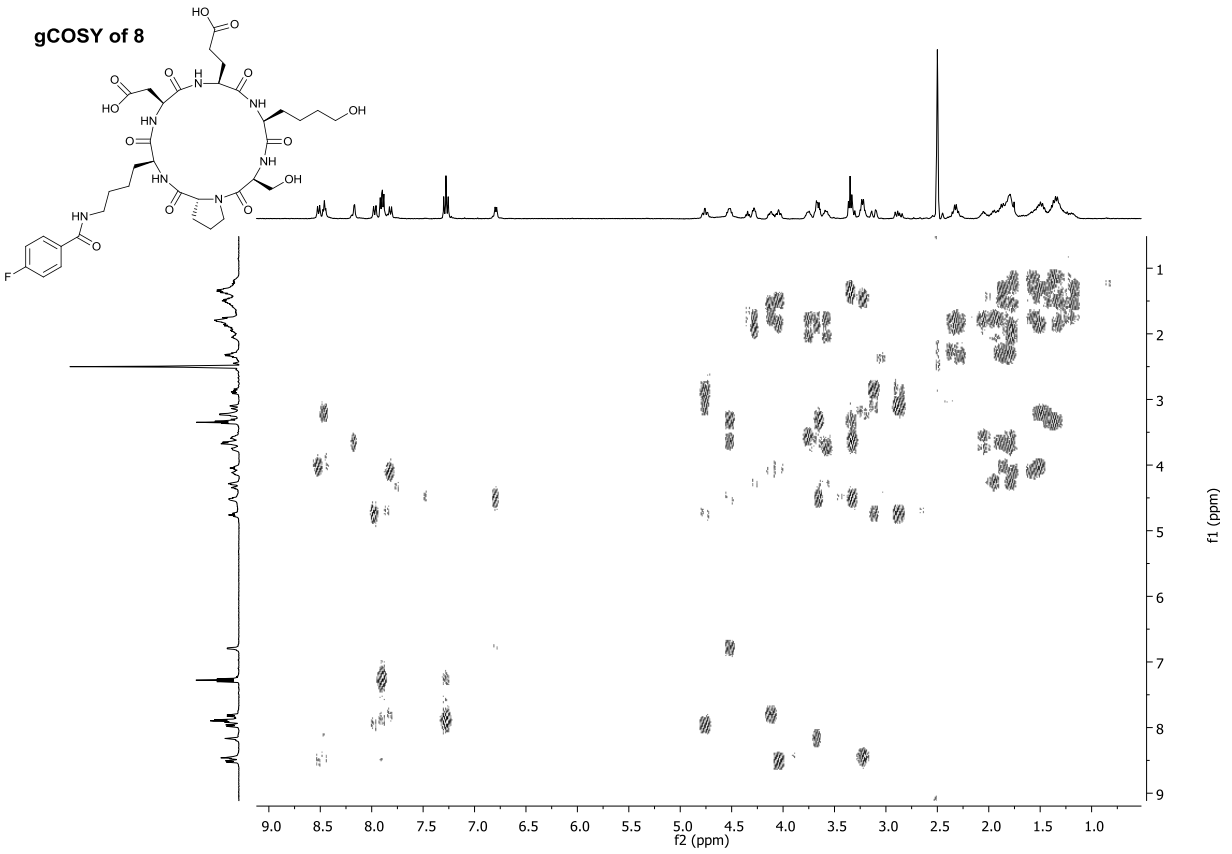

-74.75
-74.79
19F NMR of 6
-74.5 -74.7 -74.9 -75.1
f1 (ppm)
-40 -50 -60 -70 -80 -90 -100 -110 -120 -130 -140 -150 -160 -170 -180 -190 -200 -210 -220
f1 (ppm)

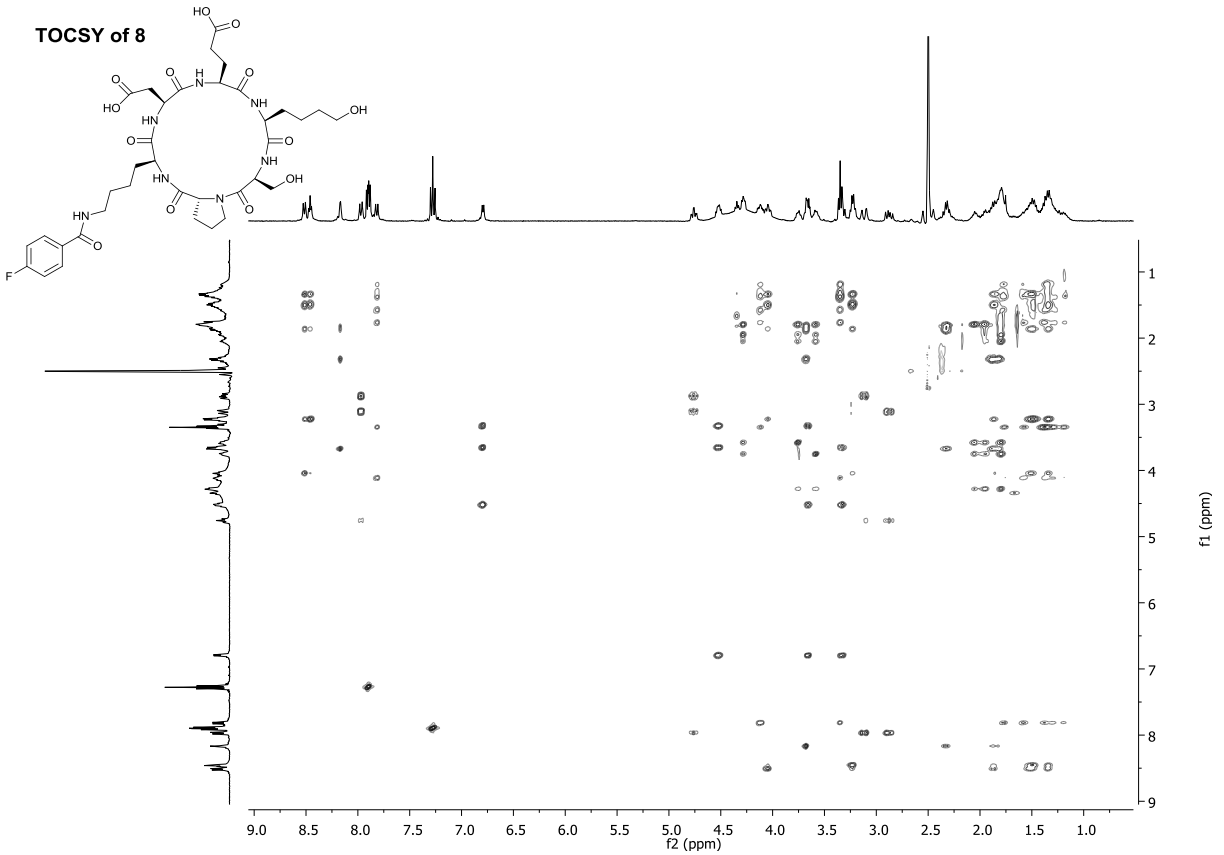

^{1}H NMR of 7
OH
O
N
H
O
O
O
7.78
7.76
7.61
7.59
7.40
7.33
7.30
5.96
5.86
5.36
5.32
5.28
5.28
5.26
5.25
4.66
4.65
4.42
4.40
4.23
3.65
1.95
1.43
1.80
1.85
1.92
1.91
0.81
1.66
0.88
1.93
2.56
0.93
2.00
8.06
f1 (ppm)

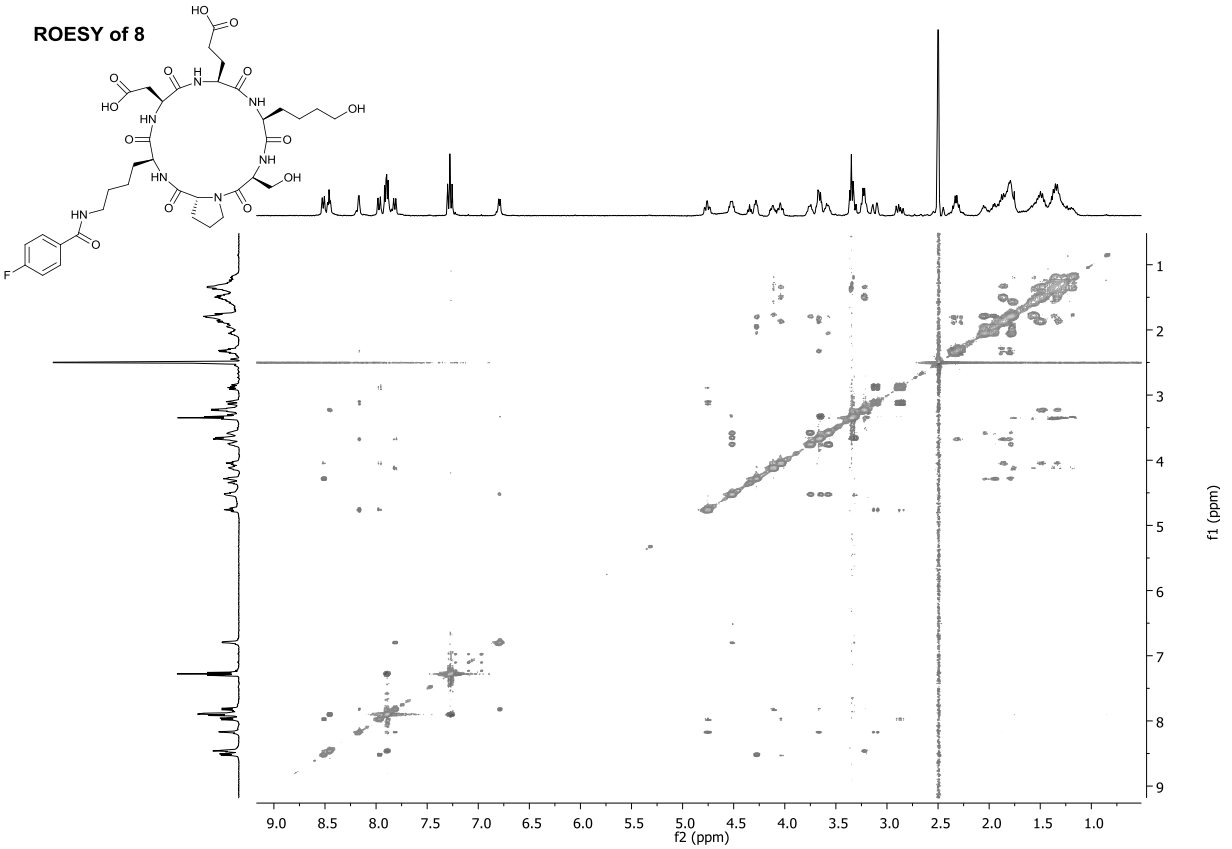

^{13}C NMR of 7
OH
O
N
H
O
O
O
172.34
156.10
143.89
141.46
131.64
127.85
127.20
125.22
120.13
119.14
67.16
66.16
62.58
53.95
47.32
32.63
32.19
21.64
f1 (ppm)

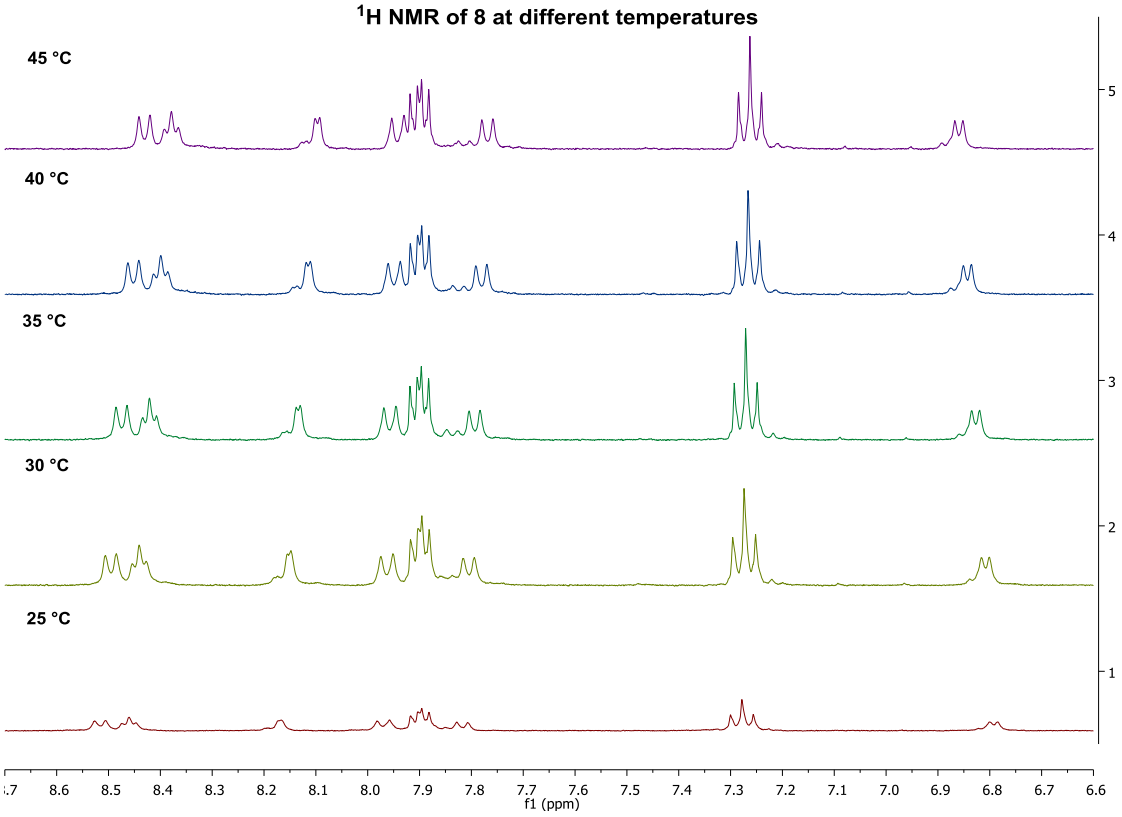

^{1}H NMR of 8
f1 (ppm)

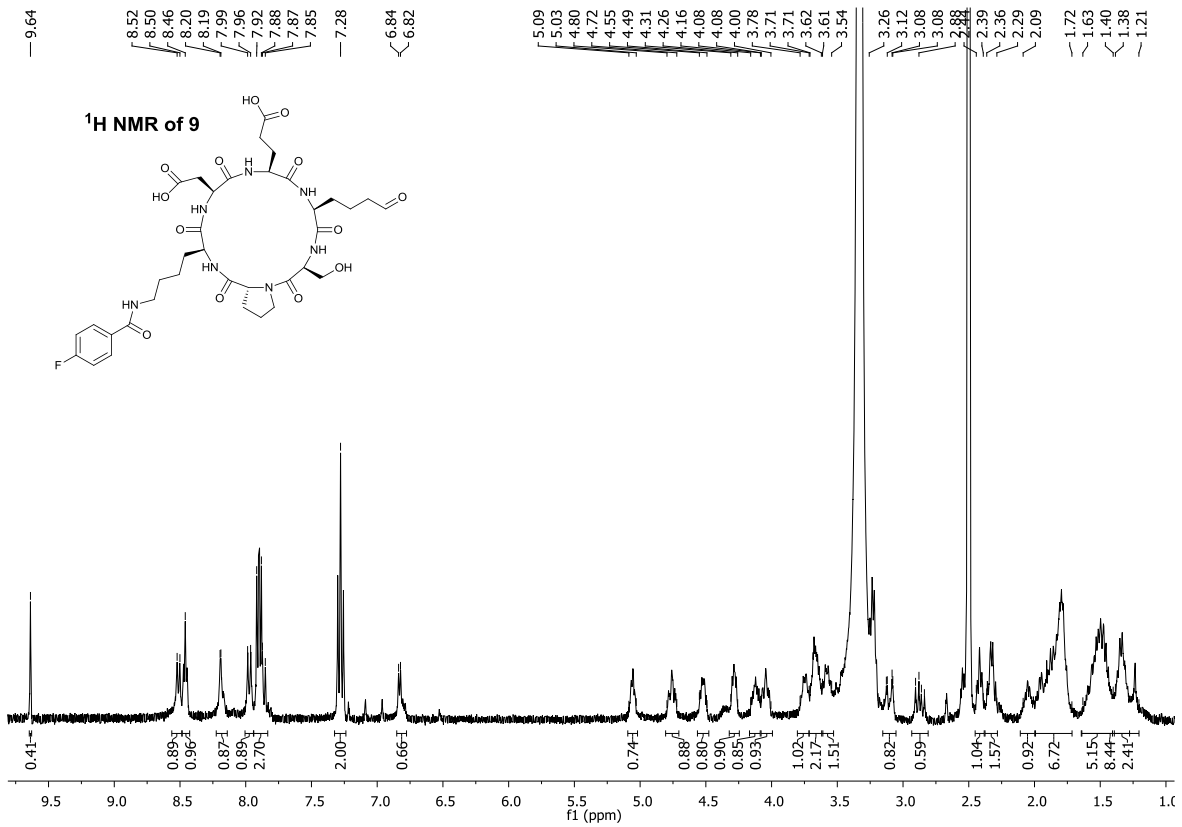

^{13}C NMR of 8
f1 (ppm)

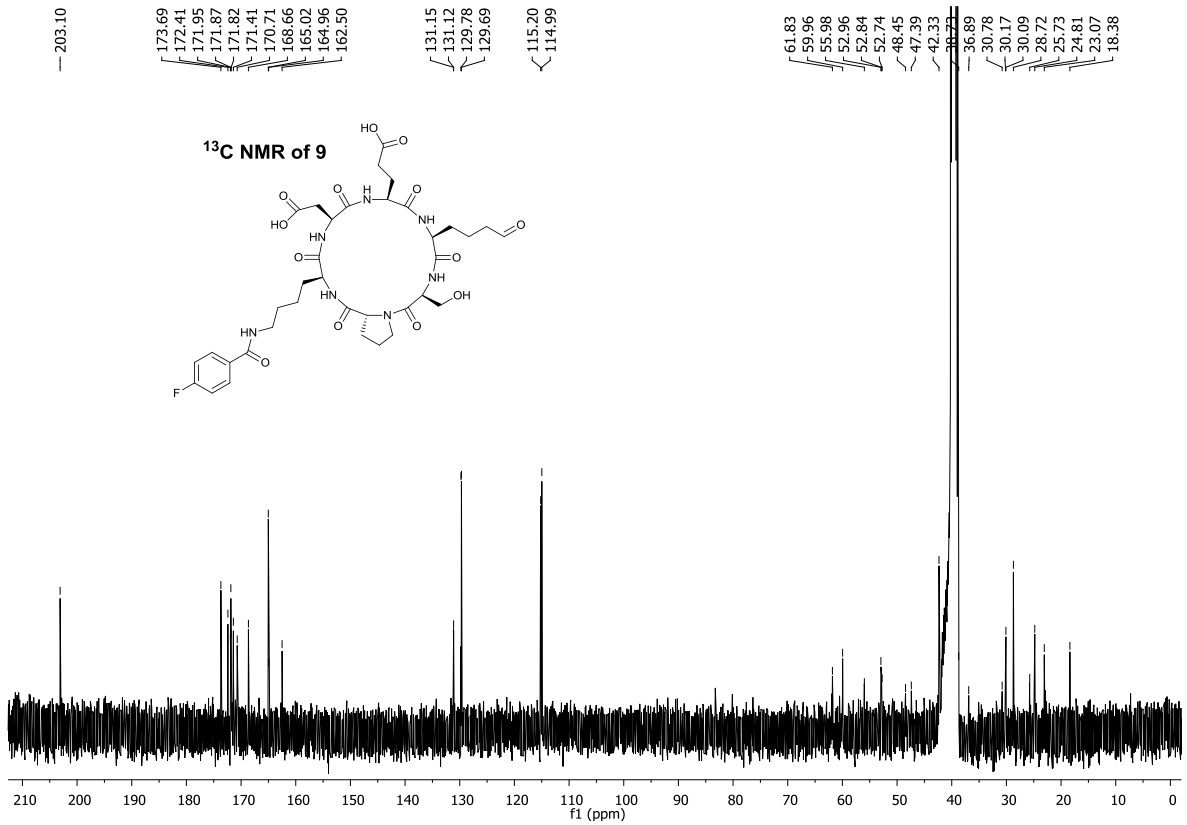

gCOSY of 8
f2 (ppm)
f1 (ppm)


TOCSY of 8
f2 (ppm)
f1 (ppm)

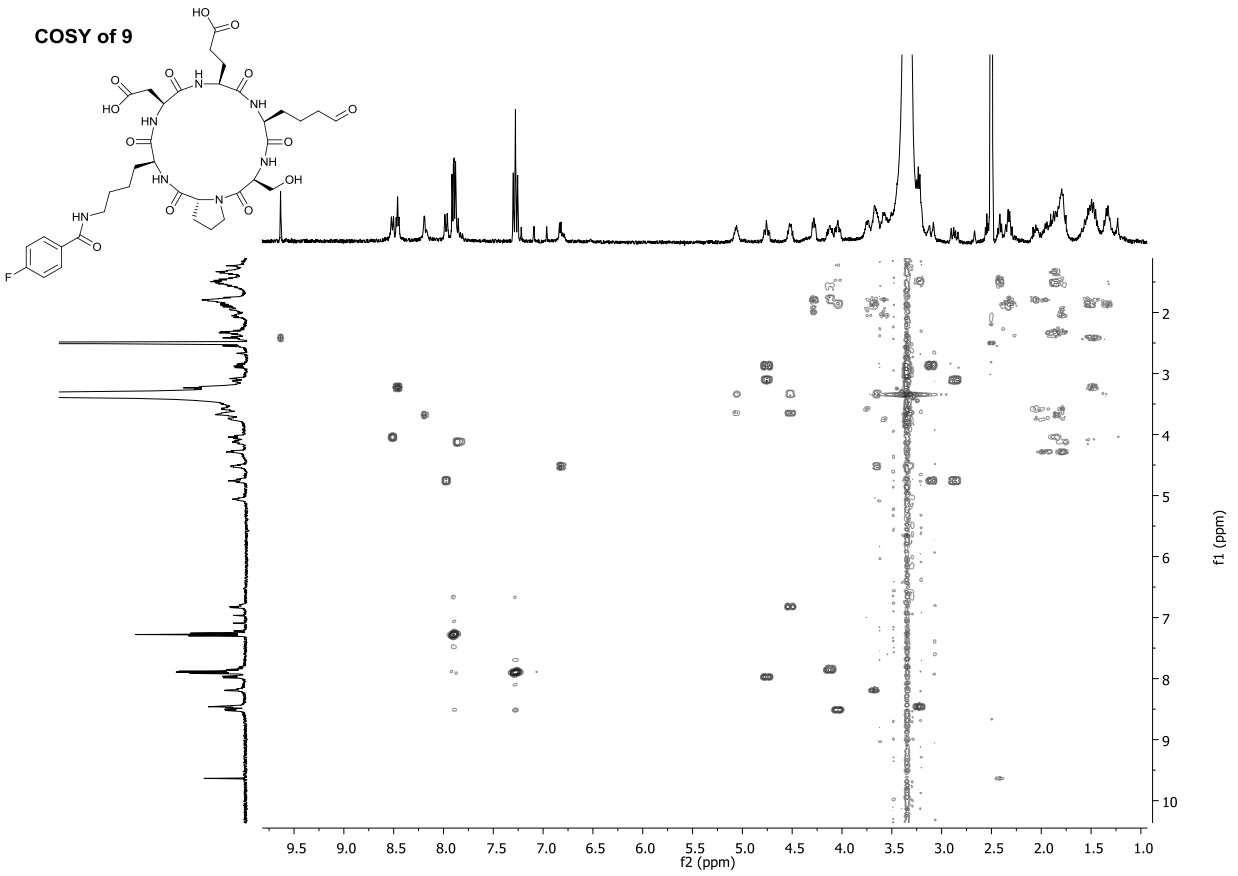

ROESY of 8
f2 (ppm)
f1 (ppm)


^{1}H NMR of 8 at different temperatures
45 °C
40 °C
35 °C
30 °C
25 °C
f1 (ppm)

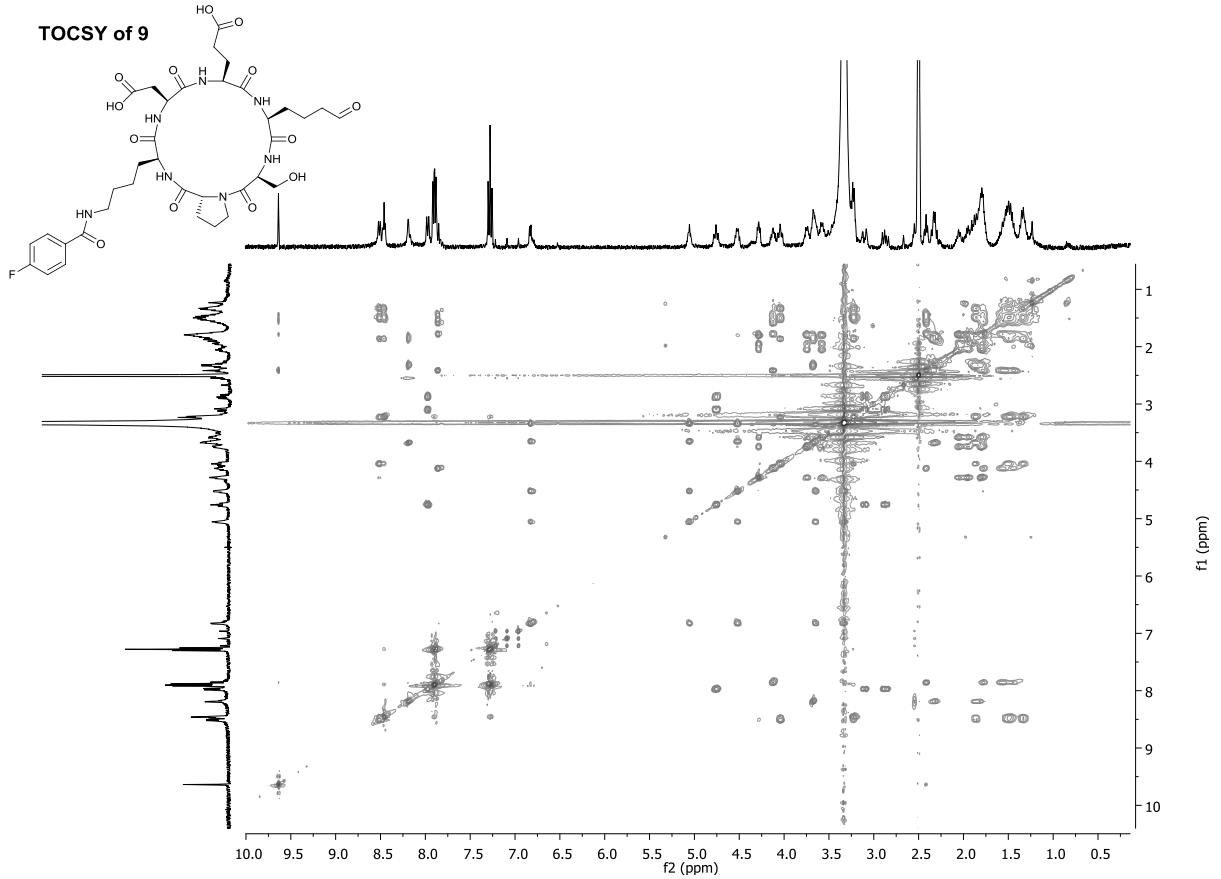

9.64
8.52
8.50
8.46
8.20
8.19
7.99
7.96
7.92
7.88
7.87
7.85
7.28
6.84
6.82
5.09
5.03
4.80
4.72
4.55
4.49
4.31
4.26
4.16
4.08
4.08
4.00
3.78
3.71
3.71
3.62
3.61
3.54
3.26
3.12
3.08
3.08
2.88
2.39
2.36
2.29
2.09
1.72
1.63
1.40
1.38
1.21
0.41
0.89
0.96
0.87
0.89
2.70
2.00
0.66
0.74
0.88
0.80
0.90
0.85
0.93
1.02
2.17
1.51
0.82
0.59
1.04
1.57
0.92
6.72
5.15
8.44
2.41
f1 (ppm)


203.10
173.69
172.41
171.95
171.87
171.82
171.41
170.71
168.66
165.02
164.96
162.50
131.15
131.12
129.78
129.69
115.20
114.99
61.83
59.96
55.98
52.96
52.84
52.74
48.45
47.39
42.33
36.89
30.78
30.17
30.09
28.72
25.73
24.81
23.07
18.38
f1 (ppm)

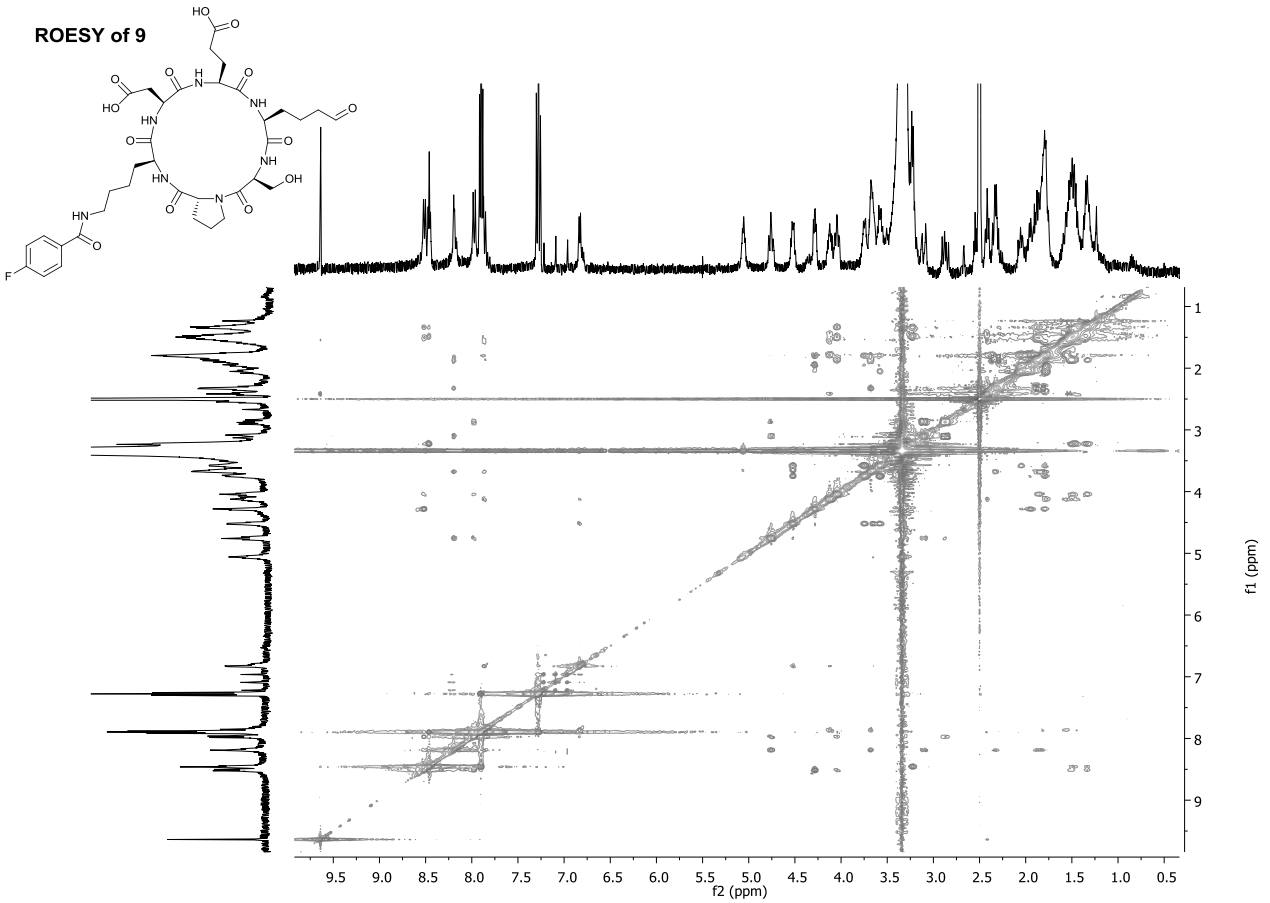
COSY of 9



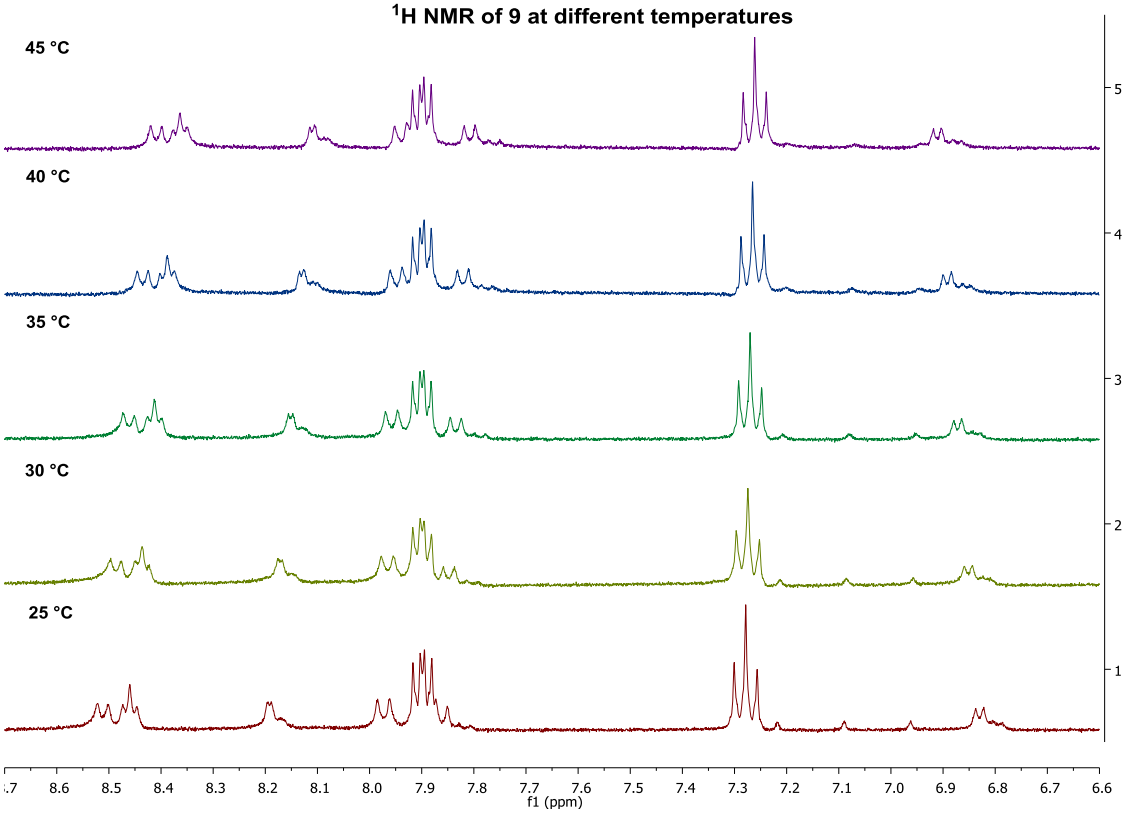
TOCSY of 9

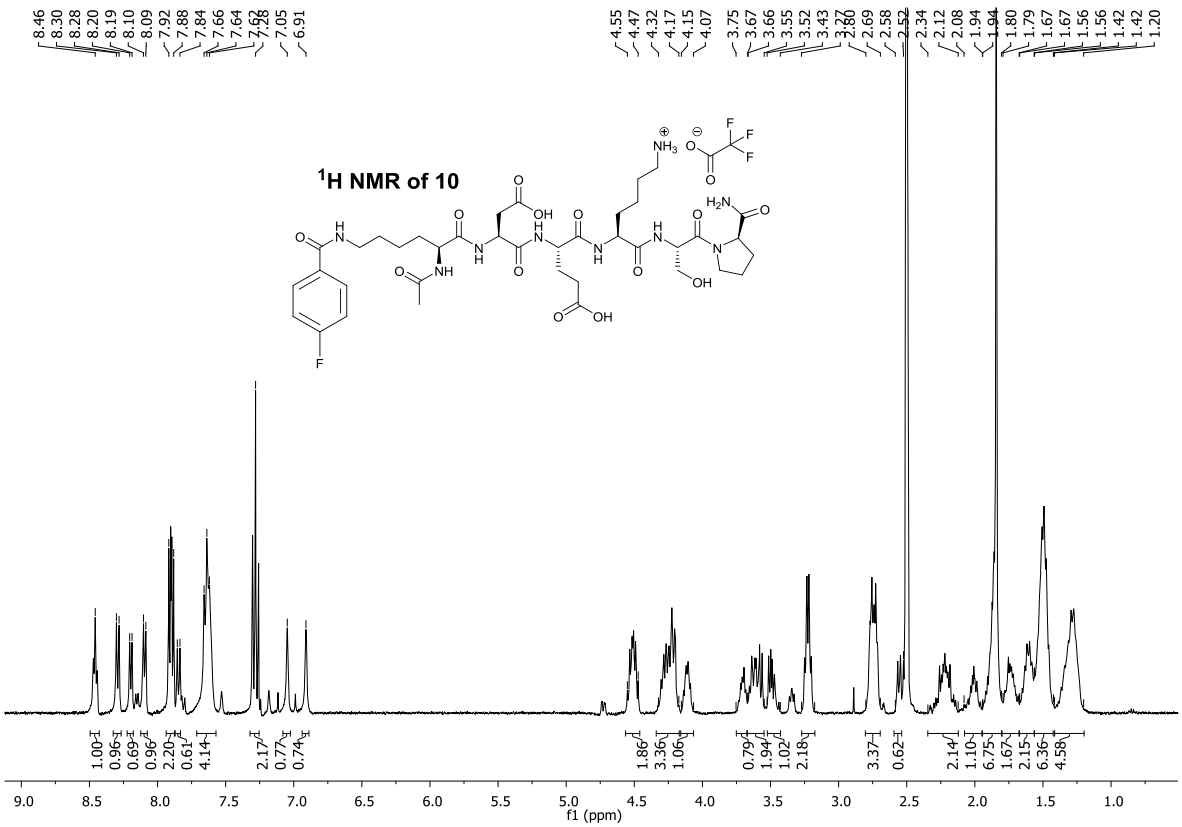


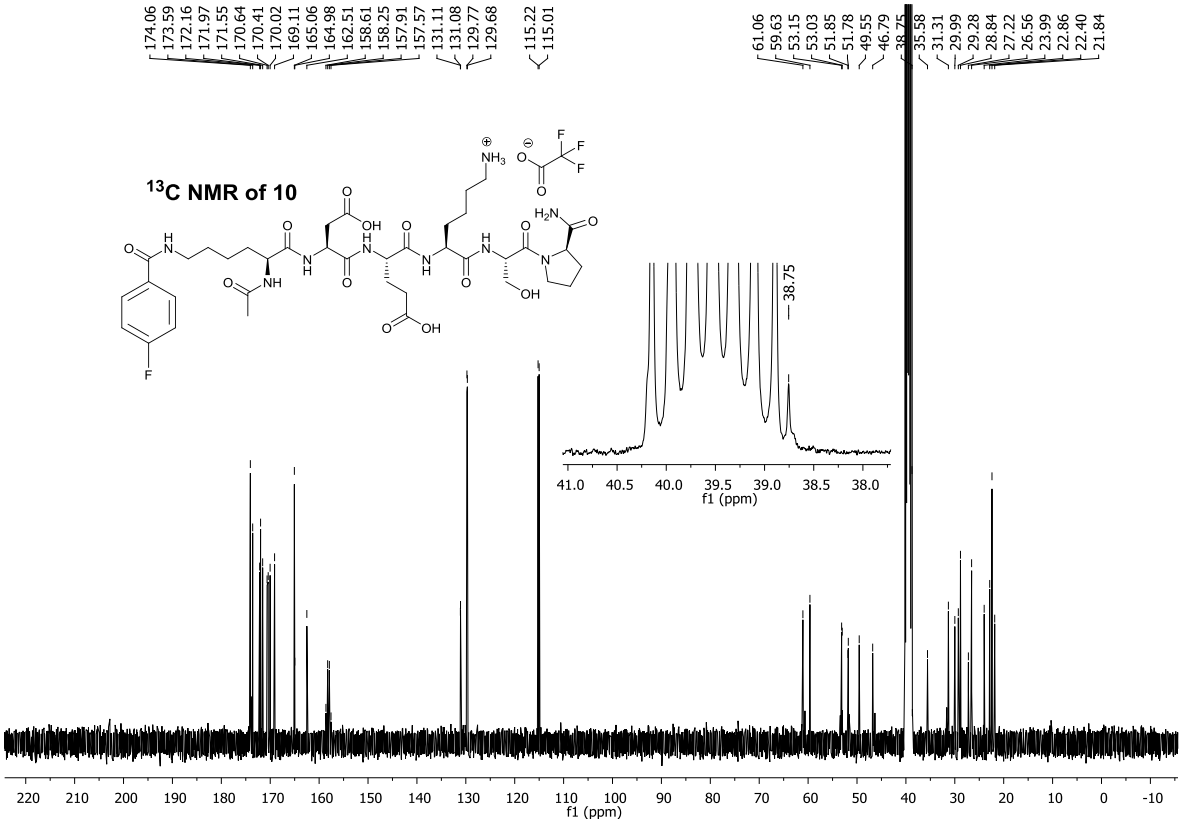
ROESY of 9



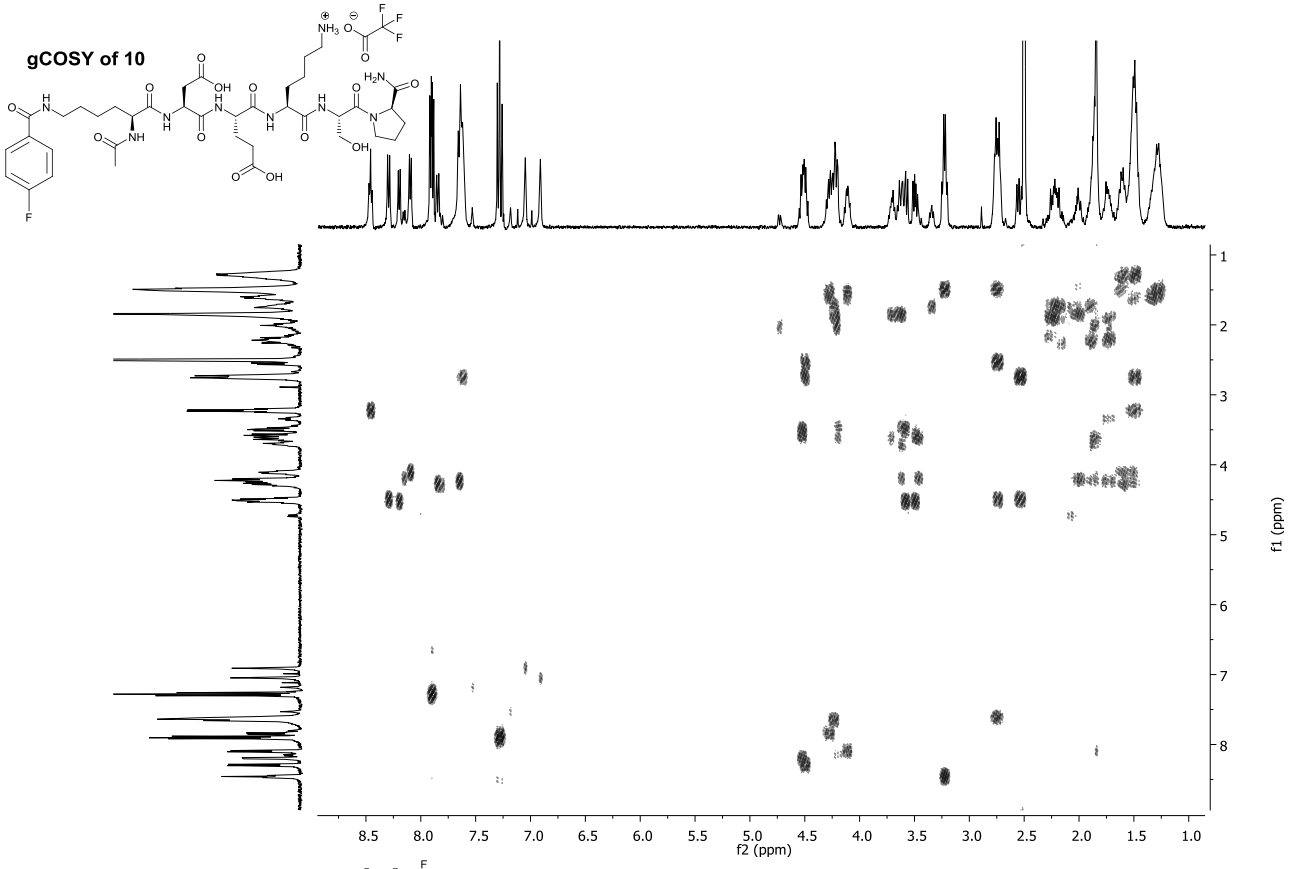
^{1}H NMR of 9 at different temperatures



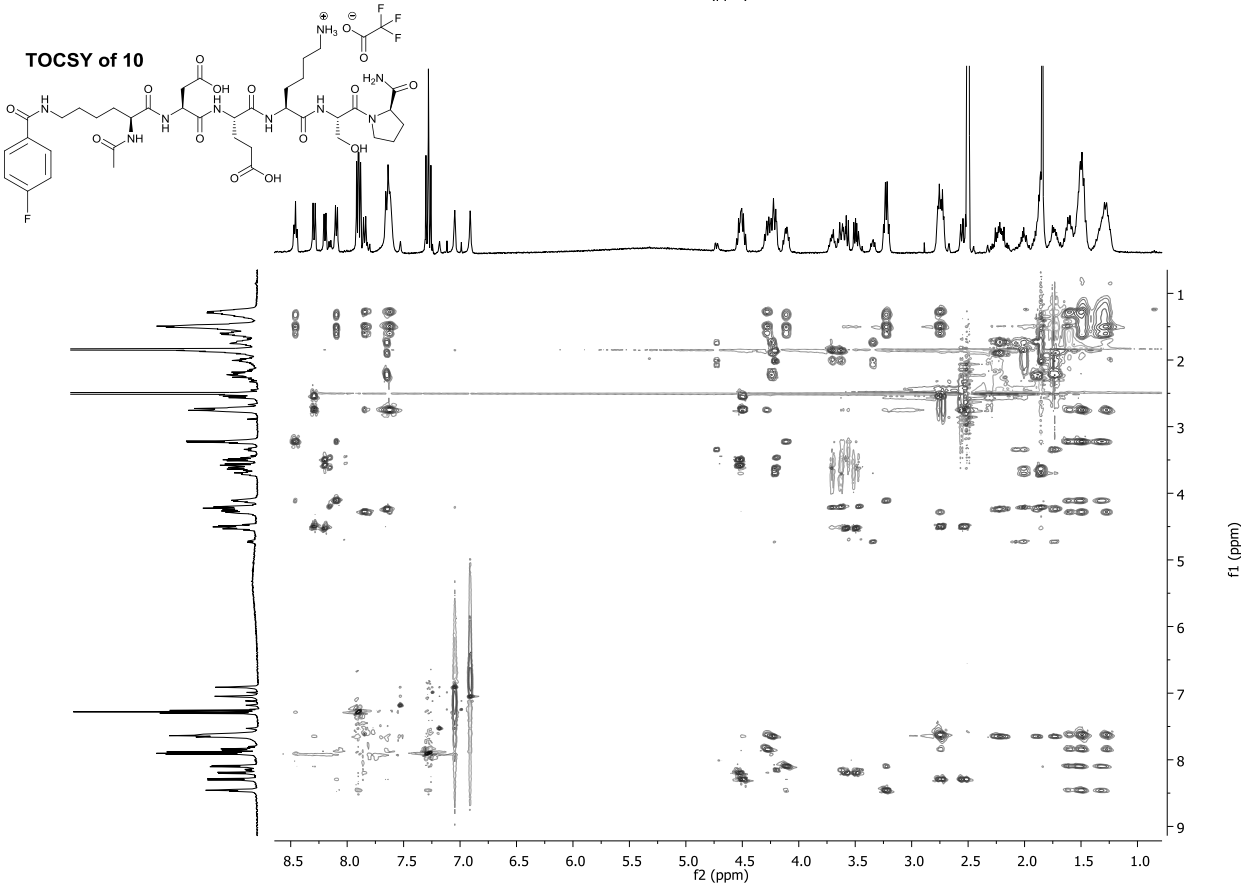

8.46
8.30
8.28
8.20
8.19
8.10
8.09
7.92
7.88
7.84
7.66
7.64
7.62
7.28
7.05
6.91
4.55
4.47
4.32
4.17
4.15
4.07
3.75
3.67
3.66
3.55
3.52
3.43
3.27
2.80
2.69
2.58
2.52
2.34
2.12
2.08
1.94
1.94
1.80
1.79
1.67
1.67
1.56
1.56
1.42
1.42
1.20
1H NMR of 10
1.00
0.96
0.69
0.96
2.20
0.61
4.14
2.17
0.77
0.74
1.86
3.36
1.06
0.79
1.94
1.02
2.18
3.37
0.62
2.14
1.10
6.75
1.67
2.15
6.36
4.58
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
f1 (ppm)

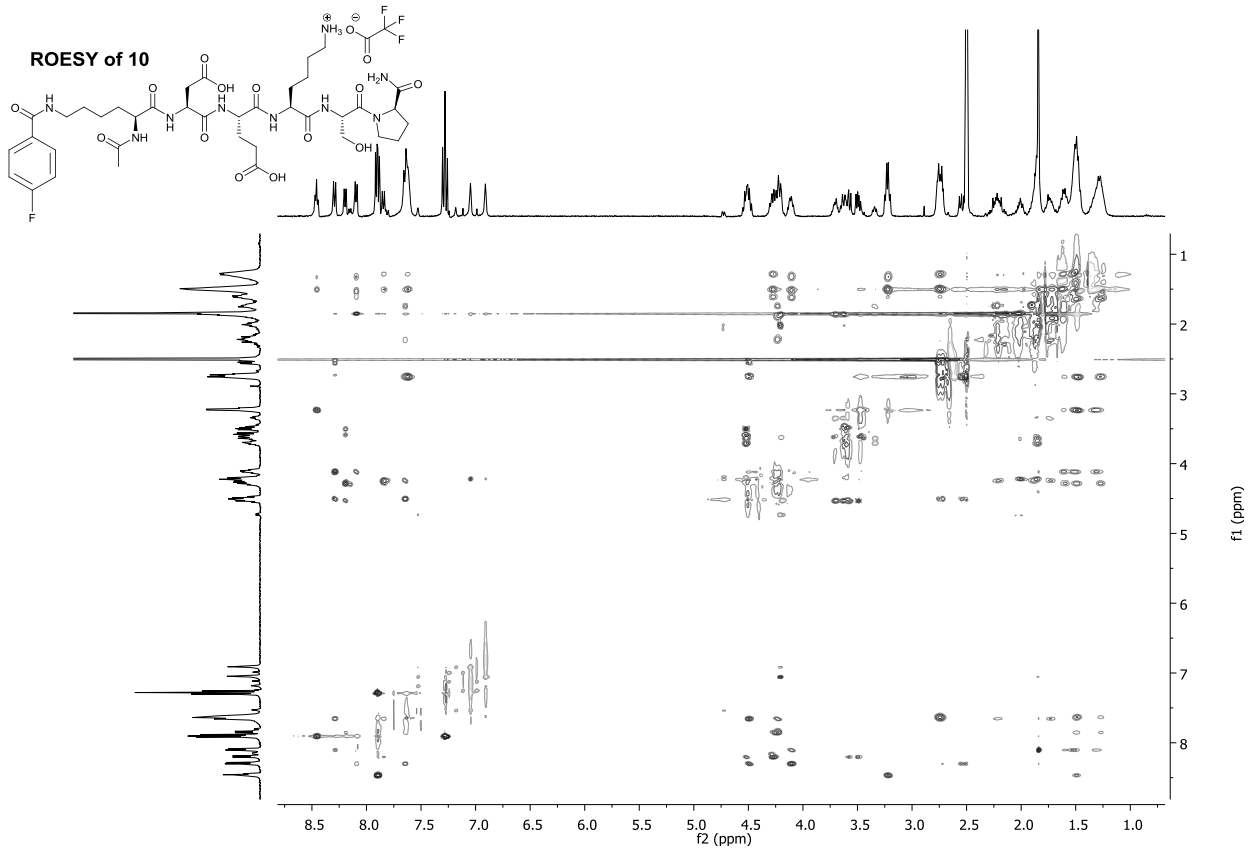

174.06
173.59
172.16
171.97
171.55
170.64
170.41
170.02
169.11
165.06
164.98
162.51
158.61
158.25
157.91
157.57
131.11
131.08
129.77
129.68
115.22
115.01
61.06
59.63
53.15
53.03
51.85
51.78
49.55
46.79
38.75
35.58
31.31
29.99
29.28
28.84
27.22
26.56
23.99
22.86
22.40
21.84
13C NMR of 10
38.75
41.0
40.5
40.0
39.5
39.0
38.5
38.0
f1 (ppm)
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)

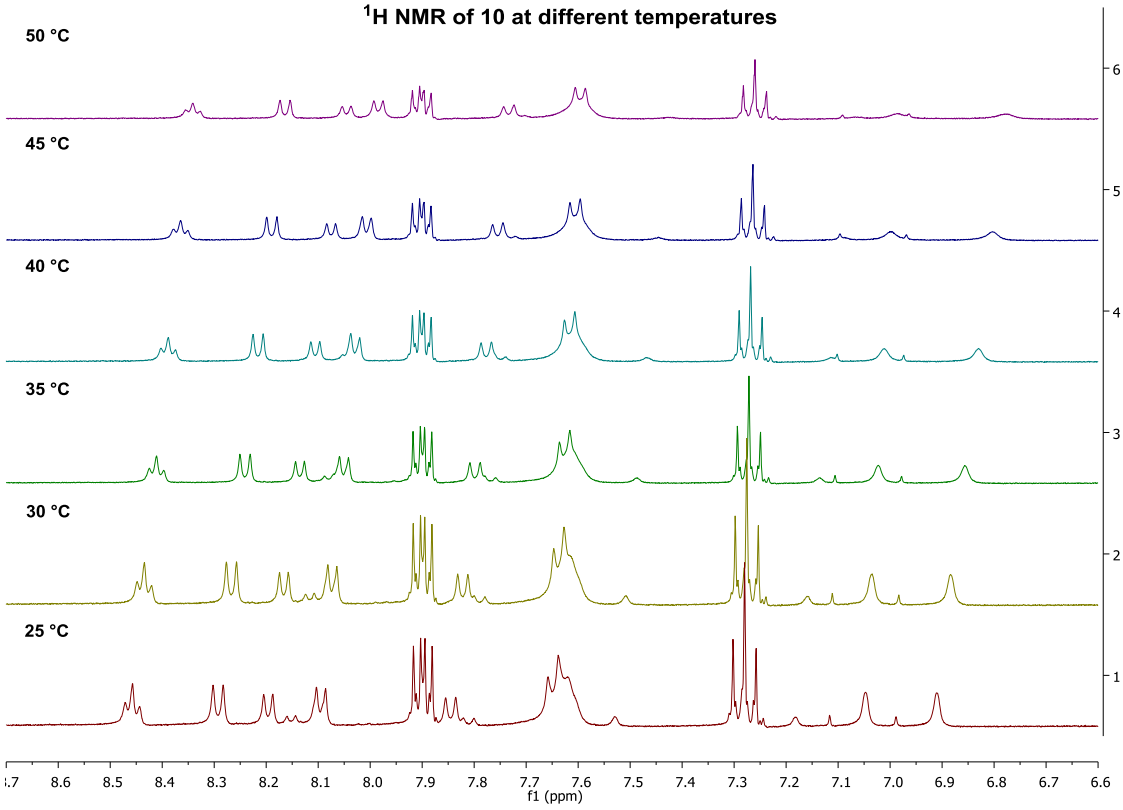
gCOSY of 10



TOCSY of 10




ROESY of 10
f2 (ppm)
f1 (ppm)


^{1}H NMR of 10 at different temperatures
50 °C
45 °C
40 °C
35 °C
30 °C
25 °C
f1 (ppm)