SUPPORTING INFORMATION

Aqueous self-assembly of short hydrophobic peptides containing norbornene amino acid into supramolecular structure of spherical shape

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General Experimental Methods

Chemicals were obtained from commercial sources and used without further purification. Preparative RP-HPLC analyses were performed using a DENALI C-18 column (10 mm, 250_22 mm). Two mobile phases were used: A=94.9% water, 5% MeCN, 0.1% TFA; B=95% MeCN, 4.9% water, 0.1% TFA. ESI mass spectra were recorded on an LCQ Advantage spectrometer. NMR spectroscopic analysis: NMR spectroscopic experiments were carried out on either 200 MHz spectrometer (200 and 50 MHz for ^{1}H and ^{13}C, respectively), 500 MHz spectrometer (500 and 125 MHz for ^{1}H and ^{13}C, respectively) or 300 MHz spectrometer (300 and 75 MHz for ^{1}H and ^{13}C, respectively) To take advantage of the magnetic field value, measurements that required temperatures higher than room temperature for observing coalescence were performed in an apparatus with a ^{1}H resonance frequency of 300 MHz spectrometer. 2D-NOESY experiments on peptides **2a** and **2b** were performed at different mixing times (300, 500 ms). Chemical shifts are given in ppm relative to $CDCl_3$ or CD_3CN, CD_3OD as internal standards, and coupling constants J are reported in hertz (Hz). The MW mediated reaction were performed using MW reactor with IR temperature detector. The Dynamic Light Scattering (DLS) measurements were performed in low volume disposable cuvettes using a Malvern Zetasizer Nano ZS90 instrument, equipped with a light source wavelength at 633 nm and a fixed scattering angle of 90°. For Transmission Electron Miscroscopy (TEM) analysis, measurements were run with a FEI Tecnai G2 (FEI, Eindhoven, NL) instrument with an accelerating voltage of 200kV.

NOESY and ROESY experiments in CD_3OH and in H_2O/D_2O

NOESY and ROESY experiments in CD_3OH show a strong spatial proximity between NH,NH(i+1) for peptide **1** except for Aib4 and Ala5 NHs that are overlapped (Figure SI1a).
The analysis of $C^{\alpha}H$-NH region for peptide **1** allowed to identify strong $C^{\alpha}H_i$-NH_{i+1} (Ala1-NRBNH; Ala3-AibNH; Ala5- COH_2) and $C^{\alpha}H_i$-NH_{i+3} diagnostic signals (Ala1-Aib4 and Ala3-$CONH_2$) characterizing the helical structure. $C^{\alpha}H_i$-NH_{i+2} signals between Ala1-Ala3 and Ala3-Ala5[1] (Figure SI2a) confirmed the formation of a 3_{10}-helix.
Unfortunately NRB2, Aib4 and Ala3 NHs signals are overlapped in peptide **2**. NOEs are detected both at N-terminus (NH-Ala1/NH-NBR2, s) and at C-terminus (NH-Ala5/$CONH_2$, s) (Figure SI1b) as well as $C^{\alpha}H_i$-NH_{i+3} and $C^{\alpha}H_i$-NH_{i+2} proximity between Ala3-$CONH_2$ and Ala3-Ala5, respectively (SI2b). Taking together these data we hypothesize the formation of a 3_{10}-helix structure also for the peptide **2**

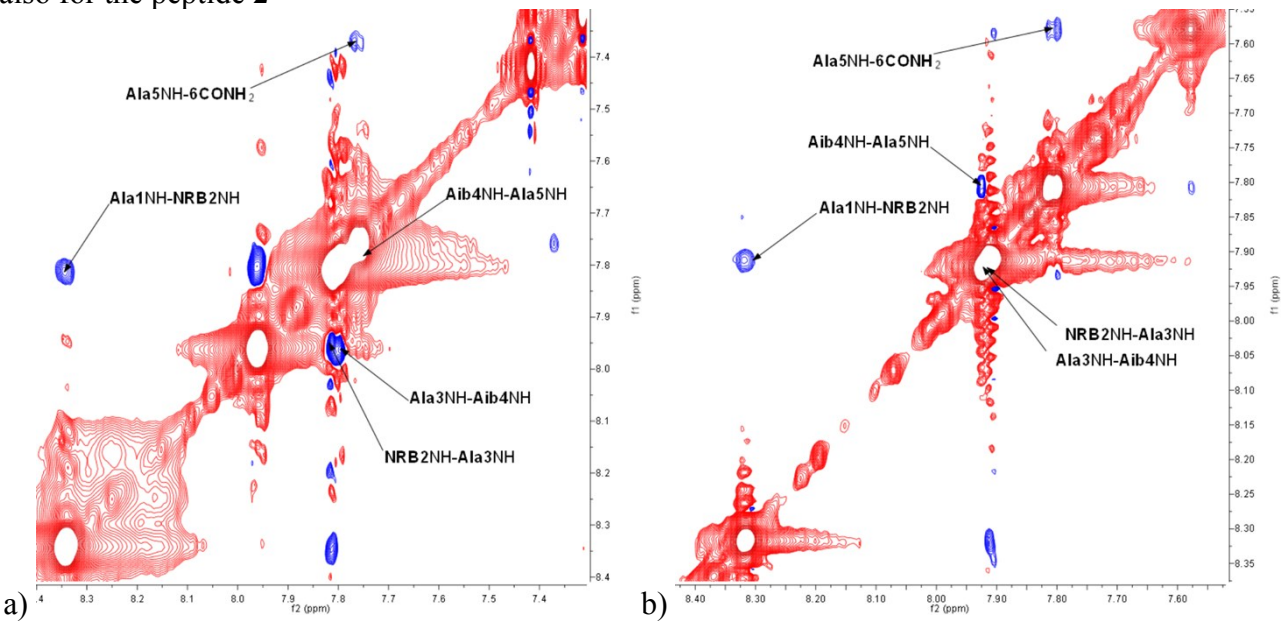


Figure SI1 ROESY experiment analysis of N_i,N_{i+1} region in CD_3OH for a) peptide **1** (16 mg/mL); b) peptide **2** (18 mg/mL)

[1] K. Wutricht, NMR of Protein and Nucleic Acids, Wiley:New York, 1986;

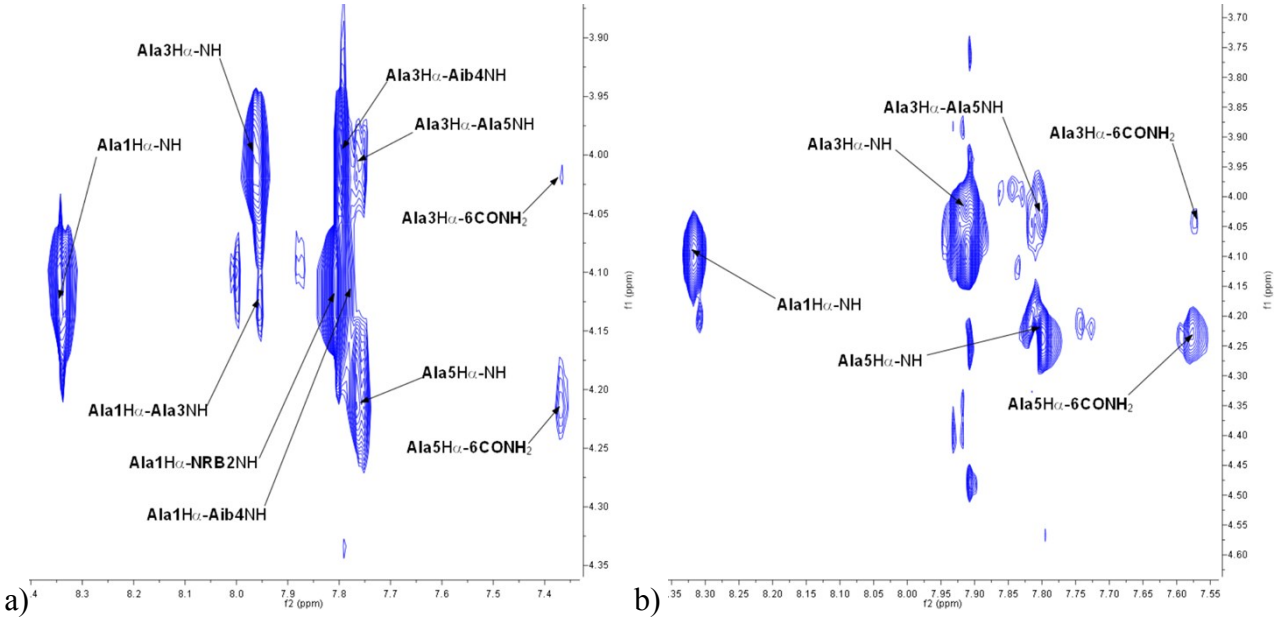


Figure SI2. NOESY experiment analysis of $C^{\alpha}H$-NH region for peptide **1** (16 mg/mL)and **2** in CD_3OH (18 mg/mL)a) peptide **1** ; b) peptide **2**

The experiment in H_2O/D_2O for peptide **1** showed the NH-NH proximity between Aib4–Ala5 and Ala5–$CONH_2$ and the lack of cross peak between Ala1-NRB2 (Figure SI3a). As a confirmation of a helical conformation only at C terminus, $C^{\alpha}H_i$-NH_{i+3} and $C^{\alpha}H_i$-NH_{i+2} spatial proxymities (Ala3-$CONH_2$ and Ala3-Ala5, respectively) are detected (Figure SI4a).

Regarding peptide **2**, all the NH-NH cross peaks are visible (Figure SI3b). $C^{\alpha}H_i$-NH_{i+3} cross peak is detected only for Ala3-$CONH_2$ (signals of Ala1H^{α} and Ala3H^{α} are overlapped, Figure SI4b). These data confirm the formation of a helical structure but the less intensity of the signals in H_2O/D_2O compared to CD_3OH indicates a less stable helix secondary structure.

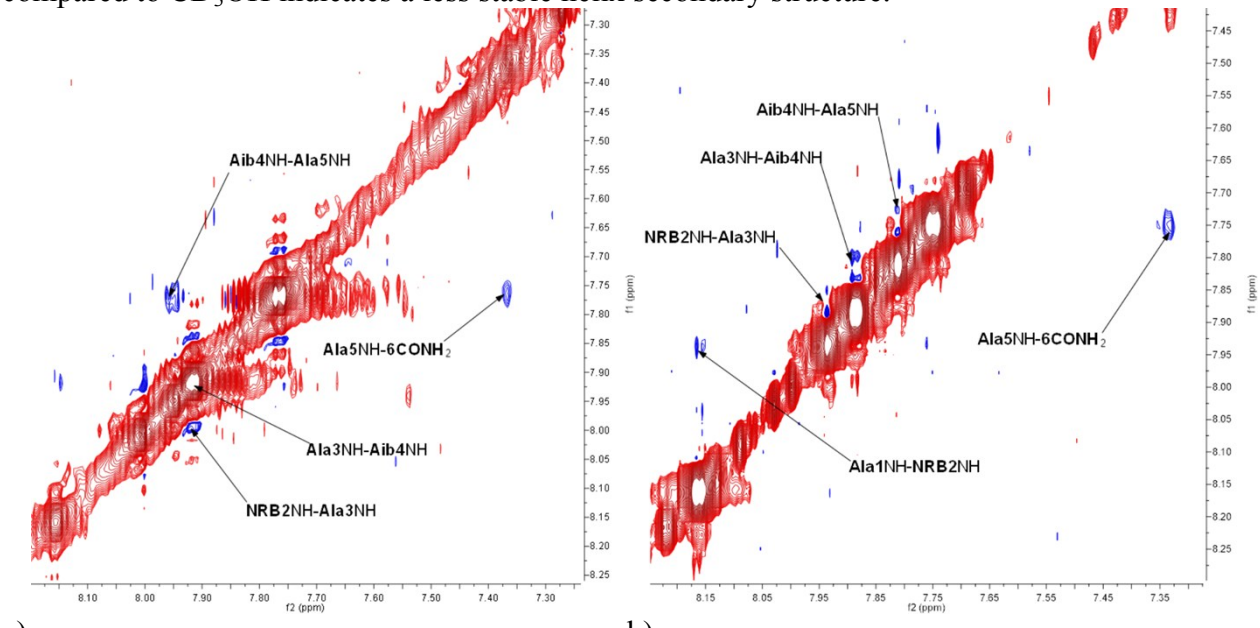


Figure SI3. NOESY experiment analysis of N,N i,i+1 for peptide **1** in D_2O/H_2O; (16 mg/mL) a) peptide **1**; ROESY experiment analysis of N_i,N_{i+1} region in for peptide **2** in D_2O/H_2O; (16 mg/mL) b) peptide **2**

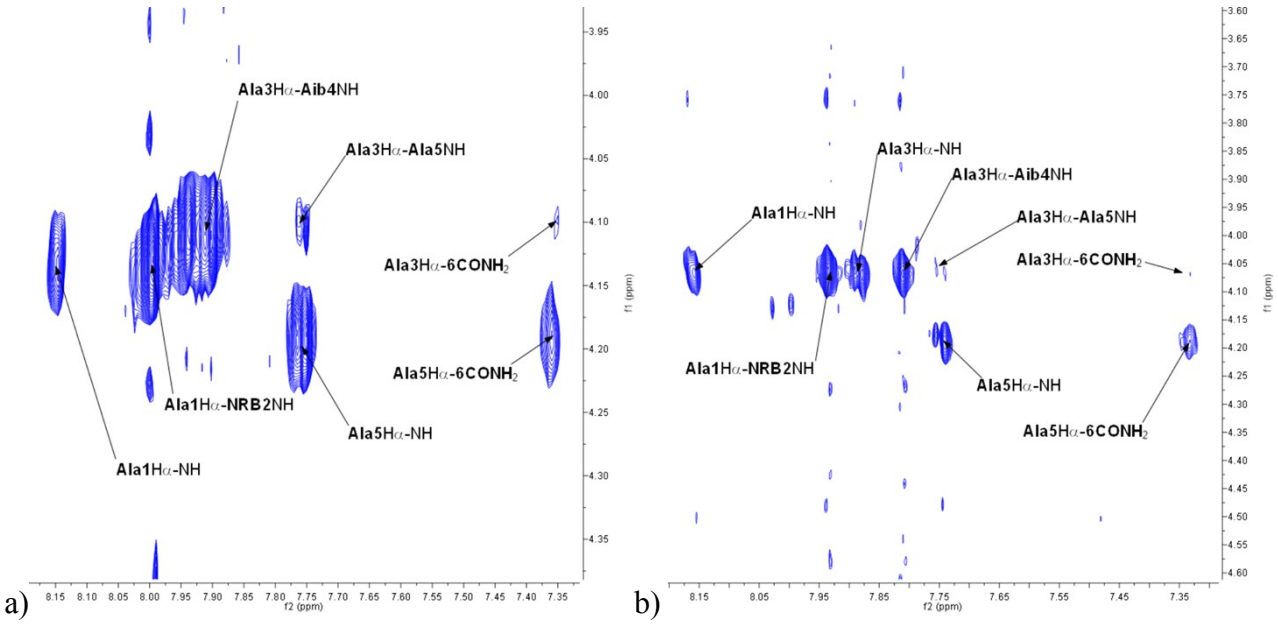


Figure SI4: NOESY experiment analysis of $C^{\alpha}H$-NH region for peptide **1** and **2** in D_2O/H_2O., (16 mg/mL) a) peptide **1**; b) peptide **2**

Further evidences of the helical conformation in CD_3OH are given by $C^{\alpha}H_i$-C^{β}_{i+3} cross peaks between Ala1-Aib4 (Figure SI5a and b), respectively for **1** and **2**. Only peptide **2** presents such signals in H_2O/D_2O (Figure SI5c)

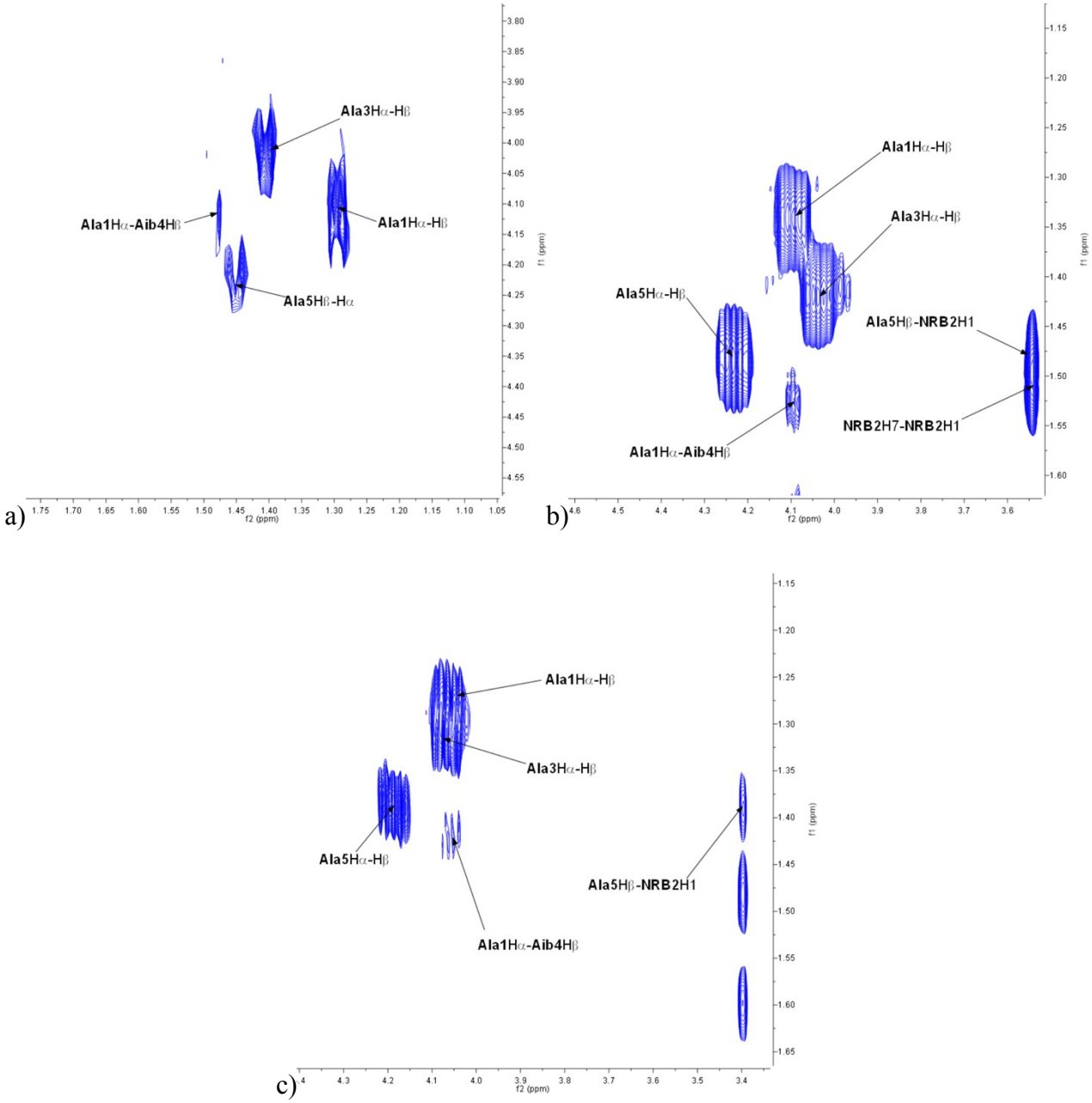


Figure SI5. NOESY experiment analysis of $C^{\alpha}H_i$- $C^{\beta}H_{i+3}$ cross peaks a) peptide **1** in CD_3OH, (16 mg/mL); b) peptide **2** in CD_3OH, (18 mg/mL) c) peptide **2** in D_2O/H_2O (16 mg/mL).

Assignment of the stereochemistry of the norbornene scaffold

The NOE analysis (CD_3OH and D_2O/H_2O, Figure SI6) of norbornene core allowed to tentatively assign the absolute stereochemistry of this scaffold in peptide **1** and **2.** Peptide **1** shows a spatial proximity between Ala5NH-NRB2H3 but no proximity between Ala5NH-NRB2H1 that, as expected, is detected in compound **2**. This is in agreement with the crystal structure of similar peptides already published by us RSC Advances (2015), 5(41), 32643-32656 and the computational model of the two peptides **1** and **2**.[2] In fact, in compound **1**, the distances between Ala5NH-NRB2H3 is 3.4 Å while for Ala5NH-NRB2H1 ≈ 5.7 Å. On the other hand, the distances between Ala5NH-NRB2H3 and Ala5NH-NRB2H1 in compound **2** are 5.4 Å and ≈ 3.7 Å, respectively.

[2] I. Maffucci, S. Pellegrino, J. Clayden, A. Contini J. Phys. Chem. B, 2015, 119,1350–1361

As a further confirmation, peptide **1** shows a very intense NOE between Ala3NH-NRB2H1 (computed distance: 2.9 Å) and signals of very low intensity between Ala3NH-NRB2H3 and Ala3NH-NRB2H7 (computed distance: 4.4 Å). Peptide **2** does not present any Ala3NH-NRB2H7 cross peak (computed distance: 5.0 Å) but NOEs, comparable in intensity, between Ala3NH-NRB2H1 and Ala3NH-NRB2H3 (computed distance: 3.9 Å and 3.1 Å, respectively). Finally, the NOE between Ala5H$^{\beta}$-NRB2H1 is suitable only for **2** (computed distance: 3.8 Å compared to 5.9 Å for **1**)

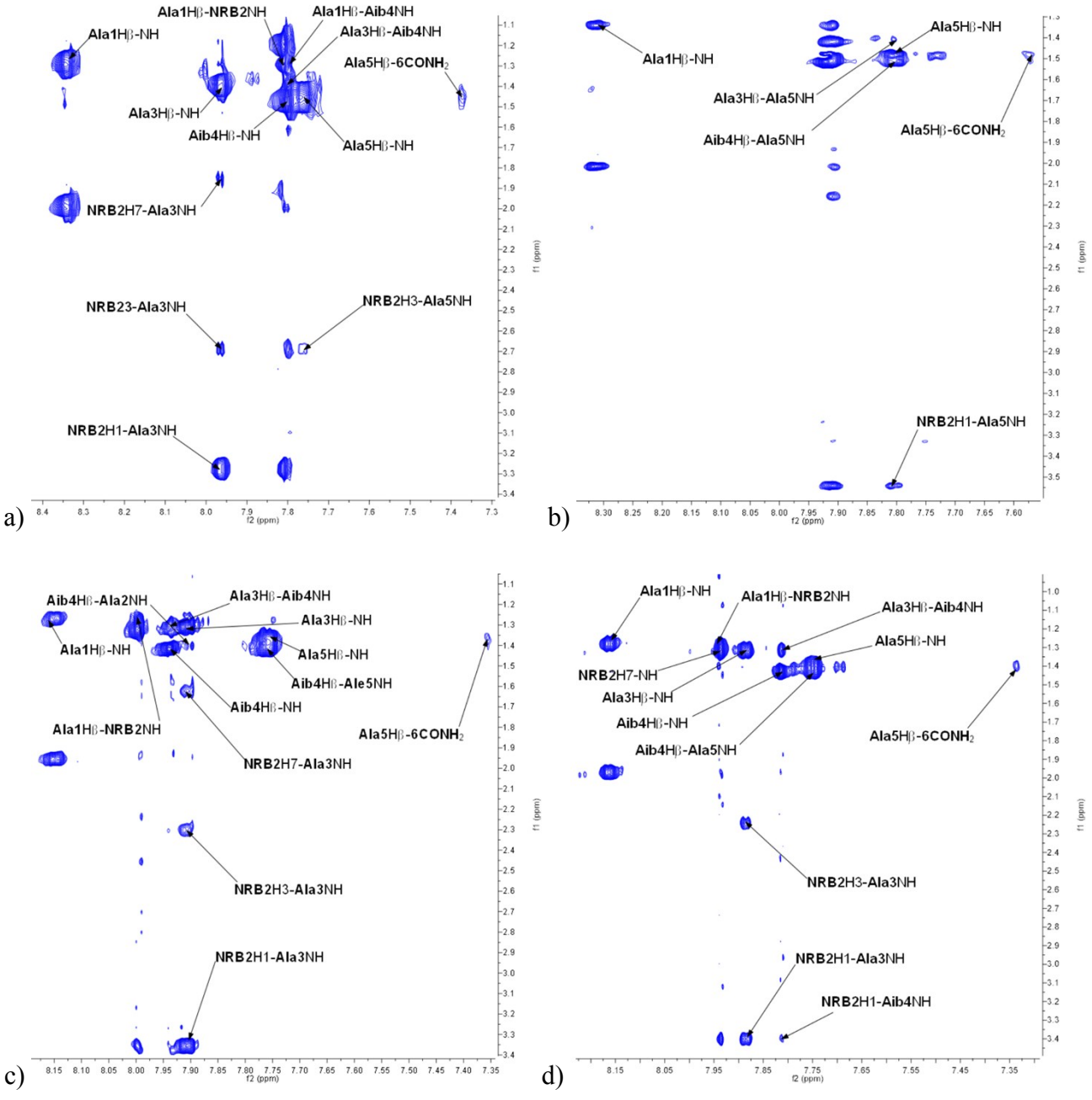


Figure SI6 NOESY experiment analysis a) peptide **1** in CD_3OH; (16 mg/mL) b) ROESY experiment analysis peptide **2** in CD_3OH, (16 mg/mL) c) NOESY experiment analysis of peptide **1** in D_2O/H_2O; (16 mg/mL) d) NOESY experiment analysis peptide **2** in D_2O/H_2O (16 mg/mL)

Evaluation of $^3J_{NH\text{-}H\alpha}$ at 300 K° in CD_3OH and H_2O/D_2O for peptides 1 and 2

Table TS1. $^3J_{NH\text{-}H\alpha}$ values (Hz) for Ala1, Ala3, Ala5 of peptides **1** and **2** in CD_3OH and H_2O/D_2O

solvent	$^3J_{NH\text{-}H\alpha}$ values (Hz)		
	Ala1	Ala3	Ala5
1 CD_3OH	5.00	5.10	7.31
1 H_2O/D_2O	5.68	5.58	6.48
2 CD_3OH	4.21	-[a]	6.87
2 H_2O/D_2O	5.10	5.16	6.51

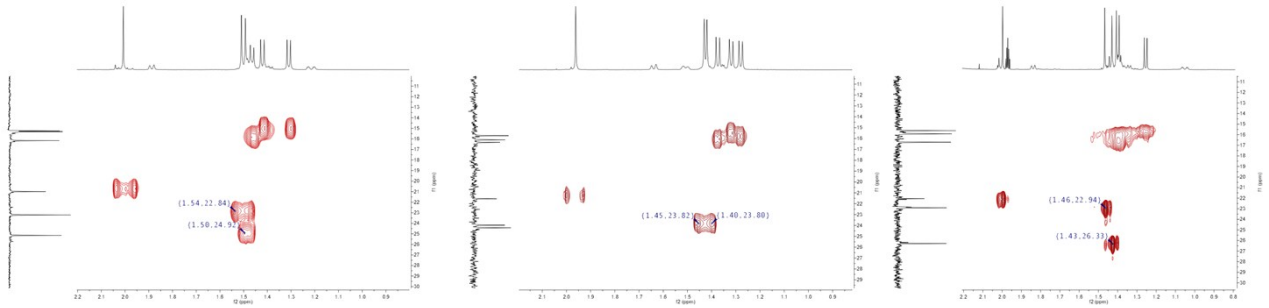
[a]: overlapped

Magnetic nonequivalence

The evaluation of the ^{13}C-magnetic nonequivalence (MNE) of the signals related to the diastereotopic methyl groups of Aib4 was performed by HSQC experiments both in CD_3CN, CD_3OD and D_2O at critical aggregate concentration (13 mg/ mL) as shown in Table TS2. The values reported largely demonstrate how in CD_3CN and CD_3OD both peptides present a stable asymmetric secondary structure that fits perfectly with the hypothesized helix. The same analysis in D_2O gave very low $\Delta\delta$ values. According to literature data,[3] taking in account that chiral residues of Ala3 and Ala5 could induce an MNE in Aib4 not higher than 0.5 ppm in D_2O, these results confirm the absence of a stable helix structure for **1** and a transition helix/random coil structure for **2**.

Table TS2. ^{13}C magnetic nonequivalence (MNE) of Aib4

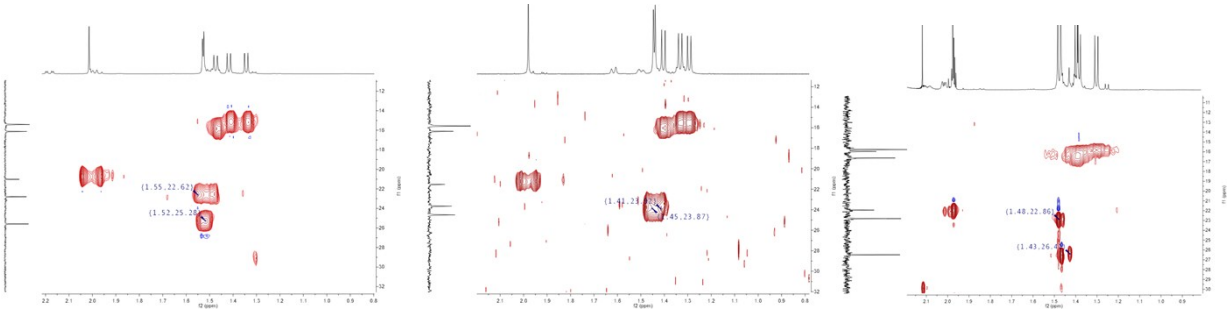
solvent	Peptide **1**		Peptide **2**	
	Aib4 δ	MNE δ	Aib4 δ	MNE δ
CD_3CN	26.22, 22.92	3.38	26.50, 22.81	3.69
CD_3OD	25.15, 23.21	1.94	25.59, 22.80	2.79
D_2O	24.22, 23.97	0.25	24.50, 23.65	0.85



a) b) c)

Figure SI7 HSQC experiment analysis of peptide **1** in a) CD_3OD, b) D_2O, c) CD_3CN; (16 mg/mL)

[3] G. Jung, H. Bruckner, R. Bosch, V. Winter, H. Schaal,J. Strahle, Liebigs Ann. Chem., 1983, 7, 1096–1106.



a) b) c)

Figure SI8 HSQC experiment analysis of peptide **2** in a) CD_3OD, b) D_2O, c) CD_3CN, (16 mg/mL)

Temperature dependence of amide chemical shift (*Δδ/ΔT*)

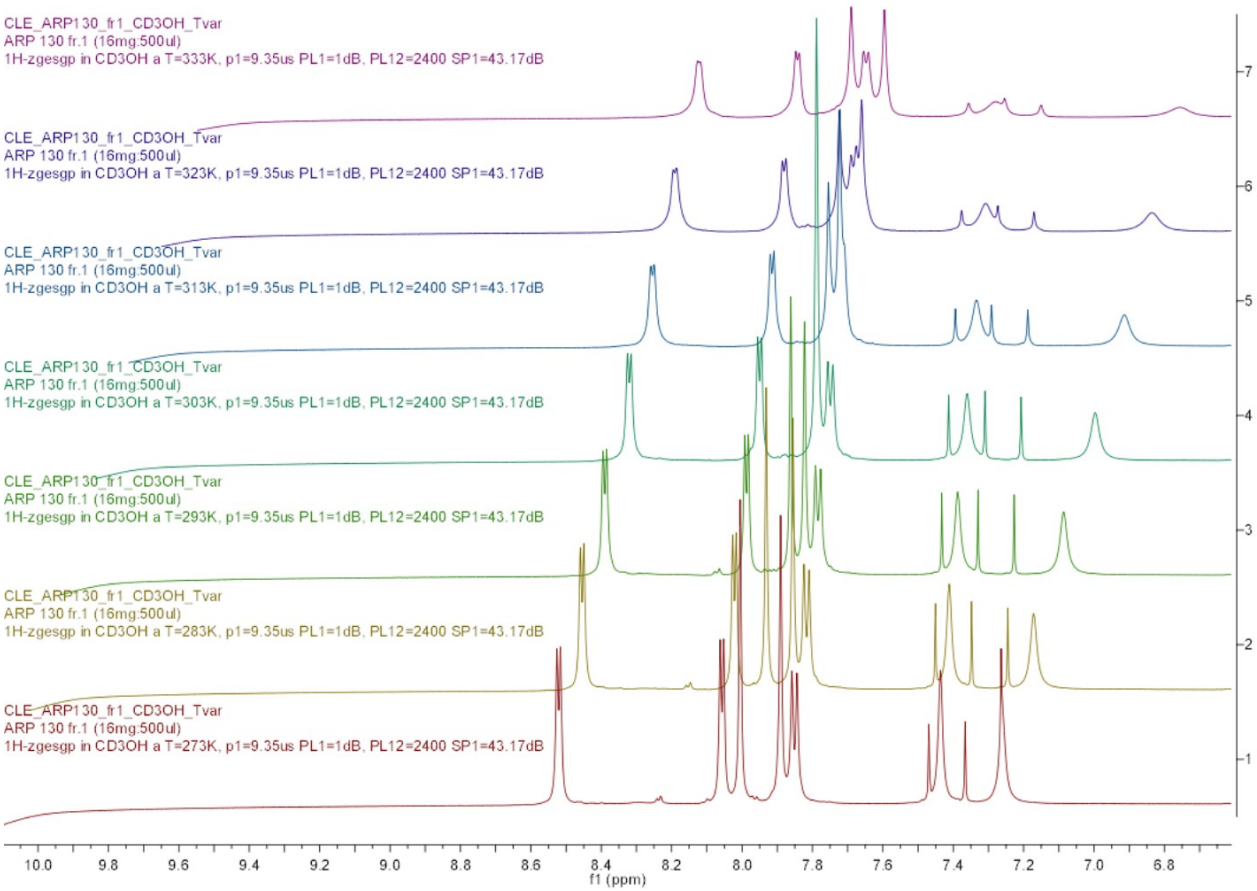


Figure SI9 ^{1}H NMR of peptide **1** in CD_3OH *Δ*T 273 °K- 333 °K , (32 mg/mL)

Table TS3. *Δδ/ΔT* of peptide **1** in CD_3OH

°K	Ala-1	NBR F1	Ala-3	Aib-4	Ala-5	CONH2	CONH2
273	8,52	8,01	8,06	7,89	7,85	7,44	7,26
283	8,45	7,93	8,02	7,86	7,82	7,41	7,17
293	8,39	7,86	7,99	7,82	7,78	7,39	7,09
303	8,32	7,79	7,95	7,79	7,75	7,36	7
313	8,25	7,72	7,91	7,75	7,72	7,33	6,91
323	8,19	7,66	7,88	7,72	7,68	7,31	6,84
333	8,12	7,6	7,84	7,69	7,65	7,28	6,76
Δδ/ΔT	0,006666667	0,006833333	0,003666667	0,003333333	0,003333333	0,002666667	0,008333333
	7	7	4	3	3	3	8

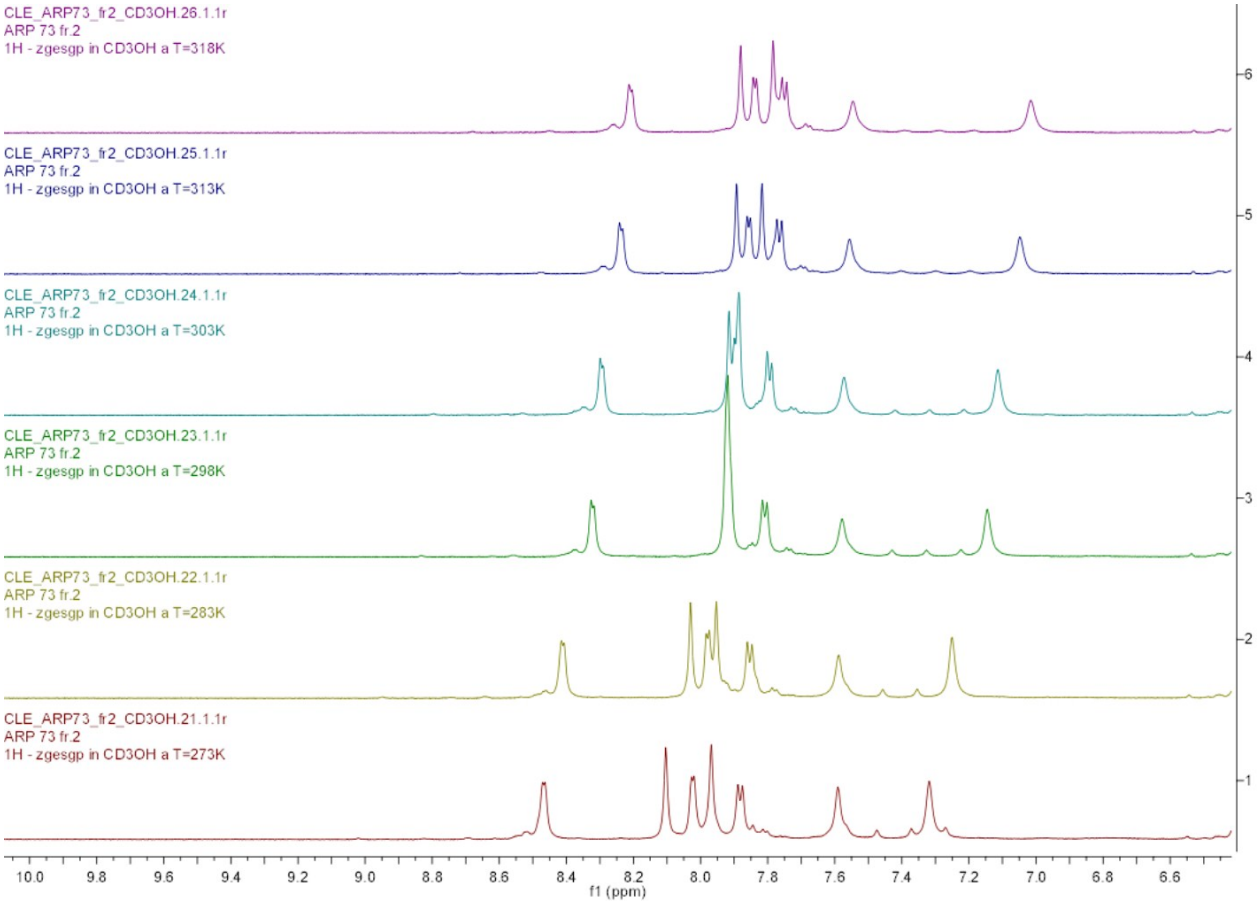


Figure SI10 ^{1}H NMR of peptide **2** in CD_3OH ΔT 273 °K- 318 °K , (26 mg/mL)

Table TS4. $\Delta\delta/\Delta T$ of peptide **2** in CD_3OH

°K	Ala-1	NBR F2	Ala-3	Aib-4	Ala-5	CONH2	CONH2
273	8,47	8,1	8,02	7,97	7,88	7,59	7,32
283	8,41	8,03	7,98	7,95	7,85	7,59	7,25
298	8,32	7,92	7,92	7,91	7,81	7,58	7,15
303	8,29	7,88	7,89	7,92	7,79	7,57	7,11
313	8,24	7,82	7,86	7,89	7,77	7,56	7,05
318	8,21	7,78	7,84	7,88	7,75	7,55	7,02
$\Delta\delta/\Delta T$	0,005777778	0,007111111	0,004	0,002	0,002888889	0,000888889	0,006666667
	6	7	4	2	3	1	7

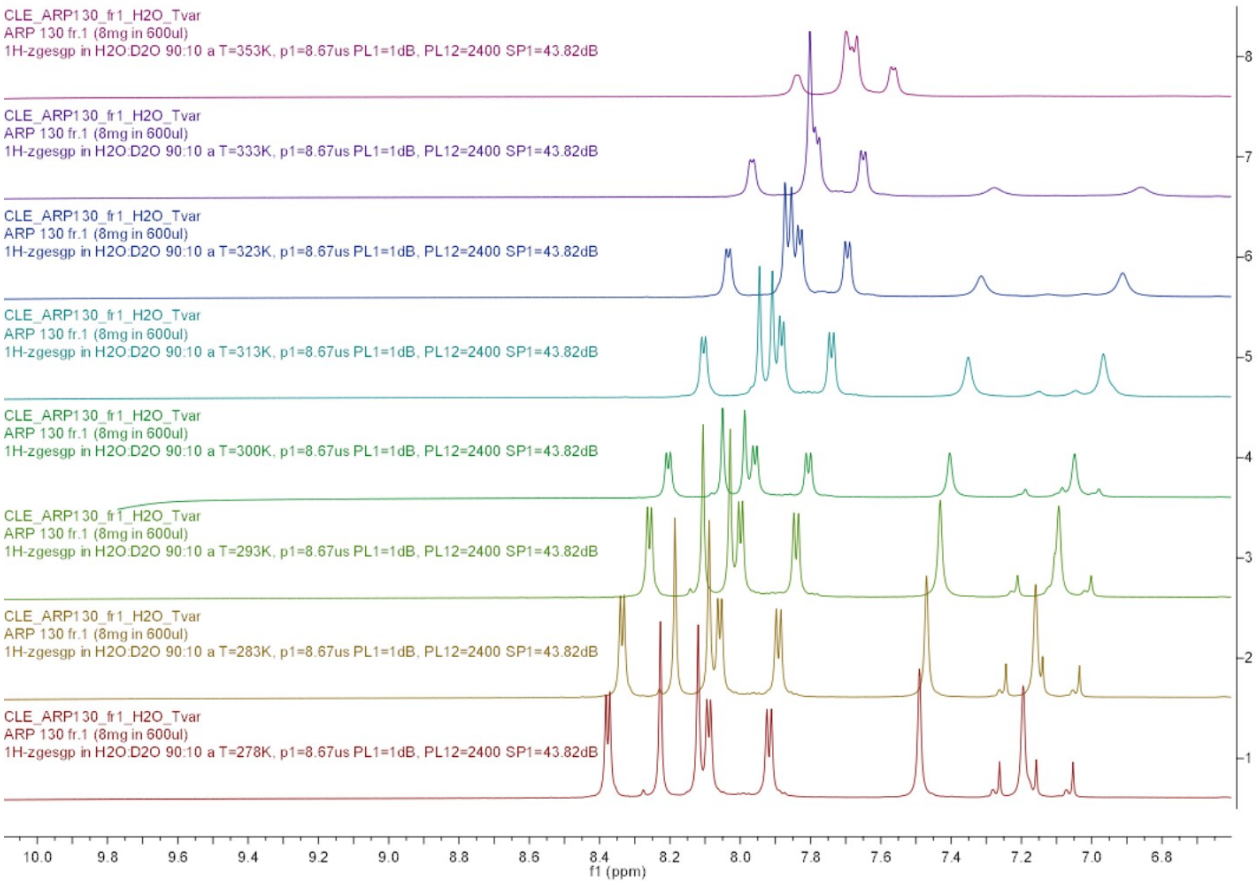


Figure SI11 ^{1}H NMR of peptide **1** in H_2O/ D_2O ΔT 278 °K- 353 °K , (13 mg/mL)

Table TS5. $\Delta\delta/\Delta T$ of peptide **1** in H_2O/ D_2O

°K	Ala-1	NBR F1	Ala-3	Aib-4	Ala-5	CONH2	CONH2
278	8,38	8,23	8,09	8,12	7,92	7,49	7,19
283	8,34	8,19	8,06	8,09	7,89	7,47	7,16
293	8,26	8,11	8	8,03	7,84	7,43	7,09
300	8,2	8,05	7,96	7,99	7,81	7,4	7,05
313	8,1	7,95	7,88	7,91	7,74	7,35	6,97
323	8,03	7,87	7,83	7,85	7,69	7,31	6,91
333	7,97	7,8	7,78	7,8	7,65	7,27	6,86
353	7,84	7,67	7,68	7,7	7,56		
$\Delta\delta/\Delta T$	0,0072	0,007466667	0,005466667	0,0056	0,0048	0,004	0,006
	7	7	5	6	5	4	6

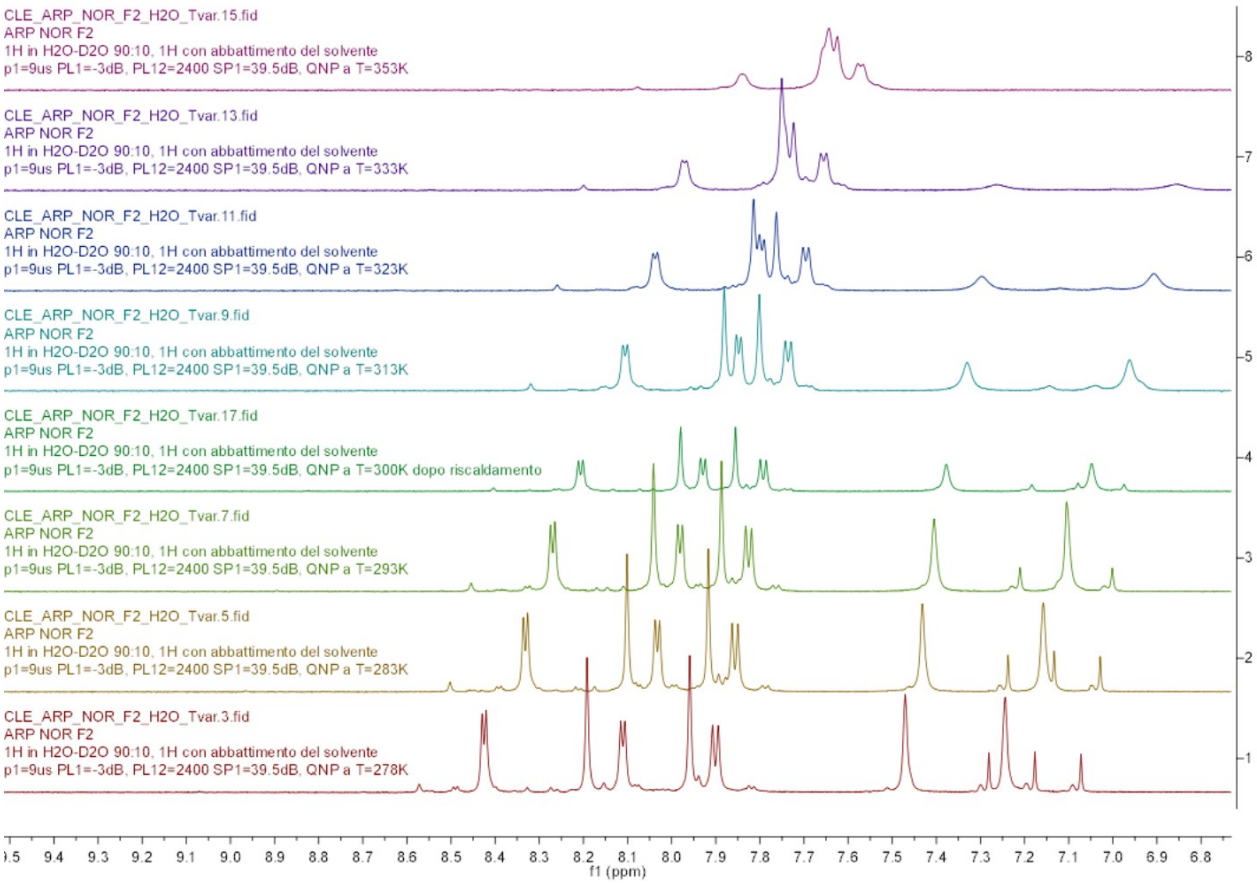


Figure SI12 ^{1}H NMR of peptide **2** in H_2O/ D_2O ΔT 278 °K- 353 °K , (16 mg/mL)

Table TS6. $\Delta\delta/\Delta T$ of peptide **2** in H_2O/ D_2O

	Ala-1	NBR F2	Ala-3	Aib-4	Ala-5	CONH2	CONH2
278	8,42	8,19	8,11	7,96	7,9	7,47	7,24
283	8,33	8,1	8,03	7,92	7,86	7,43	7,16
293	8,27	8,04	7,98	7,89	7,83	7,41	7,1
300	8,21	7,98	7,93	7,86	7,78	7,38	7,05
313	8,11	7,88	7,85	7,8	7,74	7,33	6,96
323	8,04	7,81	7,8	7,76	7,7	7,3	6,91
333	7,97	7,75	7,74	7,72	7,66	7,27	6,85
353	7,84	7,63	7,65	7,63	7,57		
75	0,007733333	0,007466667	0,006133333	0,0044	0,0044	0,003636364	0,007090909
	8	7	6	4	4	4	7

Preparation and characterization of peptide assemblies

The oligopeptides **1** and **2** were previously frozen by dipping in liquid nitrogen and then freeze-dried (Telstar Cryodos 50). After the addition of 1.5 mg of either **1** or **2** or their mixture (1:1) in 300 μL of bidistilled water (MilliQ, Millipore), a clear solution appeared, indicating the high solubility of the tested oligopeptides. The mixture was stirred for 1 minute at room temperature (RT), then the product was filtered (pore size 1.2 μm, Sartorius filters) in order to remove any large impurities that could interfere with further analyses. Finally the sample was allowed to stabilize for few seconds.
The particle size measurements were repeated for three runs at RT and the data reported as the average hydrodynamic diameter. The stability of self-assembled supramolecular structures in foetal bovine serum (FBS) was tested by DLS. To this aim, the mixture of **1** and **2** was added with pure FBS. As a control, FBS was analyzed (orange line). For Transmission Electron Miscroscopy (TEM) analysis, the mixture of **1** and **2** was further diluted 1:100 in water and deposited on a formvar-coated copper grid, then negative staining was performed using saturated uranyl acetate in 20% ethanol.

Determination of critical aggregation concentrations (CACs) of 1 and 2.

A series of solutions were prepared varying the concentration of either **1** or **2** and then analyzed by DLS. The mean size and the count rate of all the tested solutions of **1** and **2** are reported in Tables TS7 and TS8, respectively.

Table TS7.

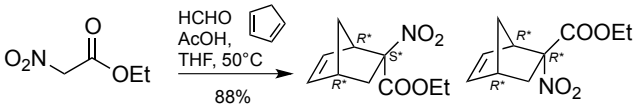
concentration (mg/mL)	mean size (nm)	count rate (kcps)
26.6	478.1	10.8
13.3	155.9	140.2
5.0	357.0	405.2
2.5	167.9	71.5
1.25	207.4	37.5
0.5	354.3	20.0

Table TS8.

concentration (mg/mL)	mean size (nm)	count rate (kcps)
42.5	1004	702.7
26.0	330.5	1096.3
13.0	536.5	496.5
5.0	322.9	145.8
2.0	420.9	145.3
0.5	181.5	28.4

Synthetic procedure

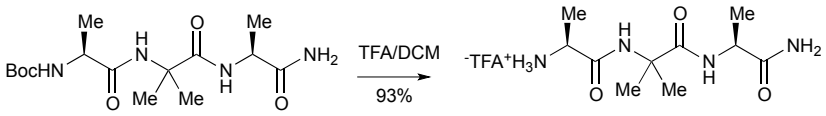
(*1R*,2R*,4R)-ethyl 2-nitrobicyclo[2.2.1]hept-5-ene-2-carboxylate (4); (*1R*,2S*,4R**)-ethyl 2-nitrobicyclo[2.2.1]hept-5-ene-2-carboxylate (5)**



4 5

To a solution of ethyl 2-nitroacetate in THF (0.1 M), freshly distilled cyclopentadiene (5 eq.), formaldehyde (5 eq.) and acetic acid (5 eq.) were added The reaction was brought to 50 ° C and allowed to react until completion (12h; TLC: AcOEt: n-hexane, 1: 4, detected by *Pancaldi* reagent: $(NH_4)_6MoO_4$, $Ce(SO_4)_2$, H_2SO_4, H_2O). The solvent was evaporated and the crude was taken up with H_2O (saturated NaCl solution). The aqueous phase was extracted 3 times with ethyl acetate. The combined organic phases were dried over Na_2SO_4, filtered and the solvent was evaporated under reduce pressure. The crude was purified by flash column chromatography (*n*-hexane 100% to AcOEt / *n*-hexane 1:10) affording compounds **4** and **5** in mixture (ratio: 85:15; 83-88% yield) as yellow oil on a scale of 2,75 g.[4]

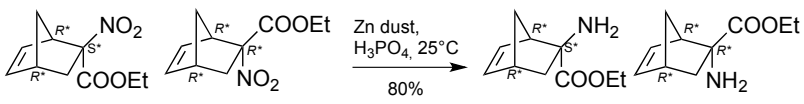
2,2,2-trifluoroacetate(*S*)-1-((1-(((*S*)-1-amino-1-oxopropan-2-yl)amino)-2-methyl-1-oxopropan-2-yl)amino)-1-oxopropan-2-aminium (9)



9

A modification of the procedure previously reported by our group [5] was required for the obtainment of **9**. Trifluroacetic acid 99% (TFA, 30 eq.) was added dropwise under stirring to a solution of tripeptide BocNHAla-Aib-AlaNH$_2$ (1 eq.) in dry DCM (0.1 M) at 0°C. The solution was warmed up at room temperature and let to react for 4 hours. The reaction was monitored by Tlc (MeOH : DCM, 1 : 10; detected by ninhydrin). Upon consumption of the starting material the solvent was evaporated at reduced pressure and wash several time with *n*-hexane / DCM / Et_2O to afford the desired compound **9** as white solid (93% yield) on a scale of 1g. Characterization already reported[6]

(*1R*,2S*,4R)-ethyl 2-aminobicyclo[2.2.1]hept-5-ene-2-carboxylate (6); (*1R*,2R*,4R**)-ethyl 2-aminobicyclo[2.2.1]hept-5-ene-2-carboxylate (7)**



4 5 6 7

Compounds **4** and **5** were dissolved in THF (0.1M) and zinc in powder (20 eq.) was added under vigorous stirring. The reaction was brought to 0 ° C and H_3PO_4 (1M, 20 eq.) was added dropwise. Then the reaction was warmed to room temperature and left to react until disappearance of the starting material (12 hours; TLC: AcOEt / n-hexane 1: 1, detected by ninhydrin). The resulting

[4] Wade, P. A.; Murray, Jr., K. J.; Shah-Patela, S.; Carroll, P. J. *Tetrahedron Letters*. **2002**, 43, 2585–2588

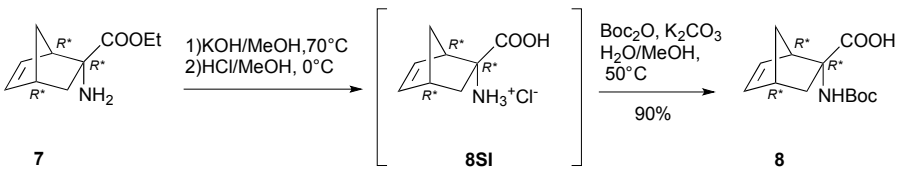
[5] Ruffoni A.; Contini A.; Soave R.; Lo Presti L.; Esposto I.; Maffucci I.; Nava D.; Pellegrino S.; Gelmi M. L. and Clerici F. *RSC Adv*., **2015**, 5, 32643–32656

[6] Pellegrino S.; Contini A.; Clerici F.; Gori A.; Nava D. and Gelmi M. L. *Chem.–Eur. J*., **2012**, 18, 8705;

suspension was filtered under vacuum and the solvent was evaporated under reduced pressure. The remaining aqueous phase was basified to pH 8 with solid $NaHCO_3$ and then extracted three times with AcOEt. The combined organic phases were dried over Na_2SO_4, filtered and the solvent evaporated under reduced pressure. The crude was purified by flash column chromatography (EtOAc : *n*-hexane, 1 : 6 + 0,8% TEA) affording two separated fraction **6** (15%) and **7** (85%) as yellow oils with a yield of 70-80% on a scale of 2.00 g.

Compound **6:** δ_H (200 MHz, $CDCl_3$) 6.22 (1 H, dd, J 5.7, 3.0), 5.93 (1 H, dd, J 5.7, 2.9), 4.12 (2 H, dq, J 0.9, 7.1), 2.87 (1 H, s), 2.79 (1 H, s), 2.30 (2 H, s), 2.05 (1 H, dd, J 2.8, 12.6), 1.94 (1 H, d, J 8.6), 1.60–1.46 (2 H, m), 1.25 (3 H, t, J 7.1); δ_C (50 MHz, $CDCl_3$) 175.50, 139.81, 133.46, 65.28, 60.94, 53.10, 47.64, 42.45, 39.12, 14.42. IR(NaCl): v = 2971, 2243, 1732, 1473, 1383 $cm^{-1;}$ (+)ESI-MS (m/z) : $[M+H]^+$ 182.0. Anal.Calcd for $C_{10}H_{15}NO_2$ (181.1): C, 66.27; H, 8.34; N, 7.73; O, 17.66; found C, 66.32; H, 8.39; N, 7.78.
Compound **7**: δ_H (200 MHz, $CDCl_3$) 6.42 (1 H, dd, J 5.7, 3.0), 6.18 (1 H, dd, J 5.7, 3.1), 4.20 (2 H, q, J 7.1), 2.97 (1 H, s), 2.88 (1 H, s), 2.56 (1 H, dd, J 12.3, 3.8), 1.83 (2 H, s), 1.68 (1 H, d, J 8.9), 1.49 (1 H, ddt, J 1.7, 3.3, 8.8), 1.29 (3 H, t, J 7.1), 0.93 (1 H, dd, J 12.3, 3.2); δ_C (50 MHz, $CDCl_3$) 176.41, 140.54, 133.40, 65.20, 61.31, 52.69, 49.14, 43.22, 41.05, 14.37. IR(NaCl): v = 2975, 2237, 1734, 1476, 1393 cm^{-1} ; (+)ESI-MS (m/z) : $[M+H]^+$ 182.2; Anal.Calcd for $C_{10}H_{15}NO_2$ (181.1): C, 66.27; H, 8.34; N, 7.73; O, 17.66; found C, 66.25; H, 8.31; N, 7.77.

(*1S*,2S*,4S)-2-((*tert*-butoxycarbonyl)amino)bicyclo[2.2.1]hept-5-en-2-carboxylic acid (8)**



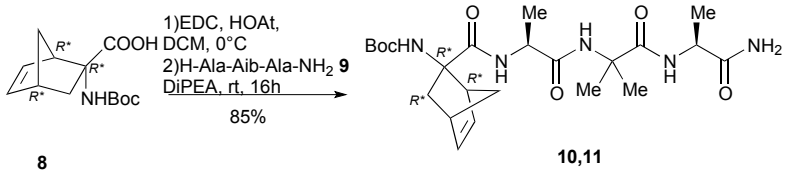
7 **8SI** **8**

Compound **7** (0.1M) was dissolved in a KOH saturated MeOH solution. The temperature was brought to 70 °C and the mixture was left to react under stirring until disappearance of the starting material (4 h; TLC MeOH : DCM, 1:5 ; detected by ninhydrin). The solvent was evaporated and wash a couple of time with a mixture of EtOAc : *n*-hexane 1 : 1, than the dry solid was treated with HCl (saturated methanol solution) at 0°C until pH 2. The precipitate (KCl) was filtered under vacuum and the solvent was removed under reduced pressure at room temperature. The crude so obtained (compound **8SI**) was dissolved in a mixture of MeOH: H_2O = 1 : 1 (0.1M) and Boc_2O (1.2 eq.) and K_2CO_3 (2.5 eq.) were added. The reaction was warmed up to 50°C and monitored by TLC (MeOH : DCM, 1 : 6). Two additions of the same equivalents of Boc_2O and K_2CO_3 were made every 6 hours. Upon the consumption of starting material the organic solvent was evaporated at reduced pressure and the crude was dissolved at 0°C in HCl 1M until pH = 2. The precipitate was filtered under vacuum providing compound **8** in a pure as white solid on a scale of 1.72 g (Yield: 90% over two steps). (In alternative if is not possible precipitate the compound the crude was dissolved in HCl 1M and extracted 3 times with EtOAc. The crude was purified by flash column cromatography (MeOH : DCM , 1 : 50 to 1 : 30), providing compound **8** as a pure white solid(Yield: 82% over two steps)).

Compound **8SI, (*1R*,2R*,4R**)-2-carboxybicyclo[2.2.1]hept-5-en-2-aminium chloride**. white solid (m.p. 199-197 °C), δ_H (200 MHz, D_2O) 6.41 (1 H, dd, *J* 5.6, 3.1), 6.07 (1 H, dd, J 5.6, 2.9), 2.95 (1 H, s), 2.90 (1 H, s), 2.25 (1 H, dd, J 13.0, 3.5), 1.90 (1 H, d, J 9.0), 1.41 (1 H, dd, J 9.0, 1.4), 1.12 (1 H, dd, J 12.9, 3.1). δ_C (50 MHz, D_2O) 177.80, 142.81, 132.58, 66.45, 50.71, 49.20, 42.71, 37.19. (+)ESI-MS (m/z) : $[M+H]^+$ 189.8 Anal.Calcd for $C_8H_{12}ClNO_2$ (189.6): C, 50.67; H, 7.56; N, 7.39; O, 16.87; found C, 50.55; H, 7.63; N, 7.31.

Compound **8,** white solid (m.p. 178-179 °C), δ_H (200 MHz, CD_3OD) 6.33 (1 H, dd, J 5.4, 3.0), 6.07 (1 H, s), 3.30 (1 H, s), 2.85 (1 H, s), 2.44 (1 H, dd, J 12.5, 3.7), 1.80 (1 H, d, J 8.8), 1.47 – 1.41 (10 H, m), 1.26 (1 H, dd, J 12.5, 3.2). δ_C (50 MHz, CD_3OD) 177.45, 156.50, 139.45, 133.53, 79.05, 64.90, 50.29, 47.51, 42.19, 39.63, 27.54. (+)ESI-MS (m/z) : $[M+Na]^+$ 276.1. Anal.Calcd for $C_{13}H_{19}NO_4$ (253.1): C, 61.64; H, 7.56; N, 5.53; O, 25.27; found C, 61.69; H, 7.61; N, 5.58. IR(KBr): v = 3308, 2992, 2975, 2957, 1699, 1619 cm^{-1}

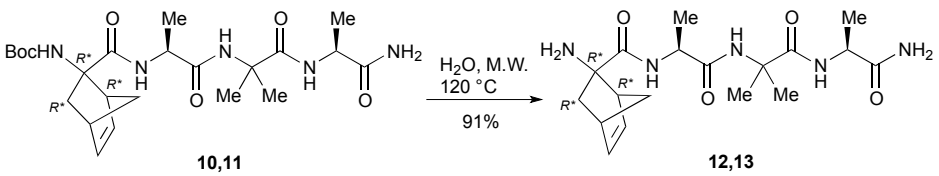
Tert-butyl ((1S*,2S*,4S*)-2-(((R)-1-((1-(((R)-1-amino-1-oxopropan-2-yl)amino)-2-methyl-1-oxopropan-2-yl)amino)-1-oxopropan-2-yl)carbamoyl)bicyclo[2.2.1]hept-5-en-2-yl)carbamate (10 and 11)



To a solution of compound **8** in dry DCM (0,1 M), HOAt (1.15 eq.) and EDC (1.15eq.) were added under nitrogen at 0°C and let react under stirring at 0°C for 1h 30 min. Afterwards the TFA salt of tripeptide **9** (1.15 eq.) and DIPEA (2.3 eq.) were added. Additional DIPEA was used, if needed, to reach pH = 8 and then the reaction mixture was let react at room temperature for 16 h (TLC: MeOH : DCM, 1 : 8, detected by ninhydrin). Upon the consumption of starting material **8** the reaction mixture was washed with saturated NH_4Cl, saturated $NaHCO_3$ and brine. The NH_4Cl, saturated aqueous phase was extracted 3 time with DCM and the combined organic phases dried over Na_2SO_4 and the solvent evaporated at reduced pressure. The crude was recrystallized with DCM/ *n*-hexane affording a mixture of the two diastereoisomers **10** and **11** as white solid (85-90% yield) on a scale of 0.5 g. (in alternative was purified by flash chromatography (DCM : MeOH, 30 : 1 to 10 : 1).

Compounds **10** and **11** δ_H (200 MHz, $CDCl_3$) 7.72 (2 H, s, **10** + **11**), 7.57 (1 H, d, J 7.9, **11**), 7.52 (1 H, d, J 7.3, **10**), 7.33 (2 H, bs, **10**+**11**), 6.99 (2 H, bs, **10**+**11**), 6.59 (1 H, dd, J 5.0, 2.3, **10**), 6.40 (1 H, dd, J 5.7, 2.9, **11**), 6.19 (1 H, dd, J 5.3, 2.5, **10**), 6.03 (1 H, dd, J 5.5, 3.9, **11**), 5.37 (2 H, bs, **10**+**11**), 5.20 (1 H, s, **11**), 5.10 (1 H, s, **10**), 4.37 (2 H, dq, J 7.4, 7.4 , **10**+**11**), 4.08 – 3.93 (2 H, m, **10**+**11**), 3.48 (1 H, bs, **10**), 2.98 (1 H, bs, **11**), 2.92-2.91 (2 H, m, **10**+**11**), 2.17-2.06 (4 H, m, **10**+**11**), 1.89 (2 H, d, J 8.8, **10**+**11**), 1.57 – 1.42 (44 H, m, **10**+**11**). δ_C (50 MHz, $CDCl_3$) 176.66 (**10**), 176.51 (**11**), 175.69 (**10**+**11**), 174.83 (**10**+**11**), 174.08 (**11**), 173.93 (**10**), 156.81 (**10**), 156.70 (**11**), 144.58 (**10**), 140.28 (**11**), 134.92 (**11**), 131.12 (**10**), 81.64 (**11**), 81.52 (**10**), 67.05 (**10**), 64.65 (**11**), 57.43 (**10**+**11**), 52.73 (**10**+**11**), 52.15 (**10**+**11**), 49.93 (**11**), 49.73 (**10**), 47.76 (**10**+**11**), 43.08 (**10**), 42.45 (**11**), 42.13 (**10**), 39.79 (**10**), 28.45 (**10**+**11**), 27.85, 23.78 (**10**), 27.76, 23.73 (**11**), 17.59 (**10**), 17.50 (**11**), 17.34 (**10**), 17.18 (**11**). (+)ESI-MS (m/z) : $[M+Na]^+$ 502.3. Anal.Calcd for $C_{23}H_{37}N_5O_6$ (479.2): C, 57.60; H, 7.78; N, 14.60; O, 20.02; found C, 57.65; H, 7.79; N, 14.65. IR(KBr): v = 3426, 3313, 2981, 2941, 1660, 1531 cm^{-1}

(1S,2S,4S)-2-amino-N-((R)-1-((1-(((R)-1-amino-1-oxopropan-2-yl)amino)-2-methyl-1-oxopropan-2-yl)amino)-1-oxopropan-2-yl)bicyclo[2.2.1]hept-5-ene-2-carboxamide (12 and 13)

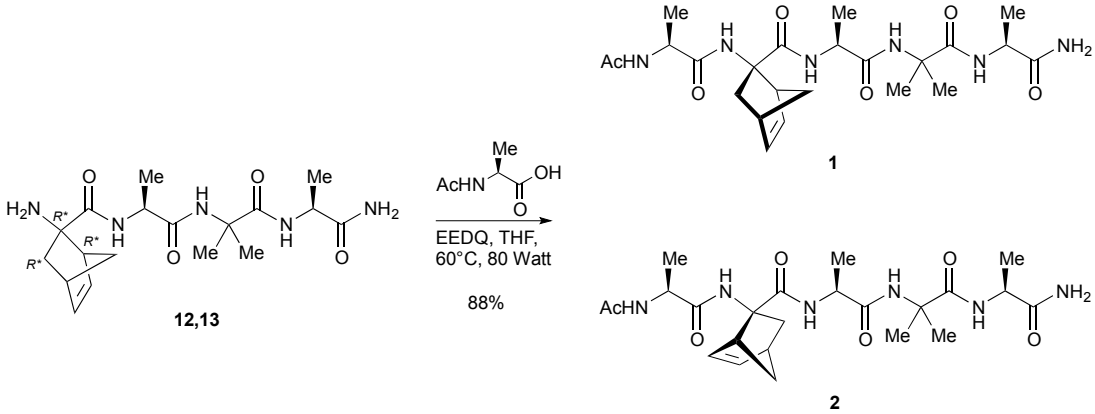


Compounds **10** and **111** were suspended in H_2O (0.1 M from the tap) in a sealed tube for micro waves reactor. The sample was irradiated under magnetic stirring by microwaves for 20 minutes at 120°C. The reaction was monitored by TLC (MeOH : DCM, 1 : 8, detected by ninhydrin). If not

finished, another round of 20 minutes was performed. Upon consumption of the starting material, the solvent was evaporated at reduced pressure. The oily residue was treated with cold Et_2O affording a mixture of compounds **12** and **13** as white solid (91%) on a scale of 0.25 g.

Compounds **12** and **13** δ_H (200 MHz, CD_3OD) 6.45 (2 H, dd, *J* 5.6, 3.0, **12**+**13**), 6.21 (2 H, dd, *J* 4.0, 1.6, **12**+**13**), 4.22 (2 H, dd, *J* 14.8, 7.4, **12**+**13**), 4.13 (2 H, dd, *J* 14.5, 7.2, **12**+**13**), 2.94 (1 H, s, **12**), 2.87 (3H, s, **12**+**13**+**13**), 2.59 – 2.42 (2 H, m, **12**+**13**), 1.93 (2 H, d, *J* 8.4, **12**+**13**), 1.52 – 1.34 (26 H, m, **12**+**13**), 0.93 (2 H, dd, *J* 12.1, 2.6, **12**+**13**). δ_C (50 MHz, CD_3OD) 178.63 (**12**), 178.61 (**13**), 176.93 (**12**), 176.92 (**13**), 175.47 (**12**), 175.39 (**13**), 174.46 (**12**), 174.40 (**13**), 140.78 (**12**), 140.78 (**13**), 133.46(**12**), 133.38 (**13**), 64.30 (**12**), 64.27 (**13**), 56.66 (**12**), 56.61 (**13**), 52.85 (**12**), 52.62 (**13**), 51.07 (**12**), 50.91 (**13**), 49.67 (**12**+**13**), 48.92 (**12**), 48.74 (**13**), 43.06 (**12**), 43.03 (**13**), 41.39 (**12**), 41.31 (**13**), 24.81 (**12**), 24.75 (**13**), 23.83(**12**+**13**), 16.50 (**12**+**13**), 16.07 (**12**), 15.95 (**13**). (+)ESI-MS (m/z) : $[M+Na]^+$ 402.4. Anal.Calcd for $C_{18}H_{29}N_5O_4$ (379.2): C, 56.97; H, 7.70; N, 18.46; O, 16.87; found C, 56.99; H, 7.75; N, 18.51. IR(KBr): v = 3312, 3060, 2980, 2939, 1657, 1529 cm^{-1}

(1S*,2S*,4S*)-2-((S)-2-acetamidopropanamido)-N-((R)-1-((1-(((R)-1-amino-1-oxopropan-2-yl)amino)-2-methyl-1-oxopropan-2-yl)amino)-1-oxopropan-2-yl)bicyclo[2.2.1]hept-5-ene-2-carboxamide (1, 2)

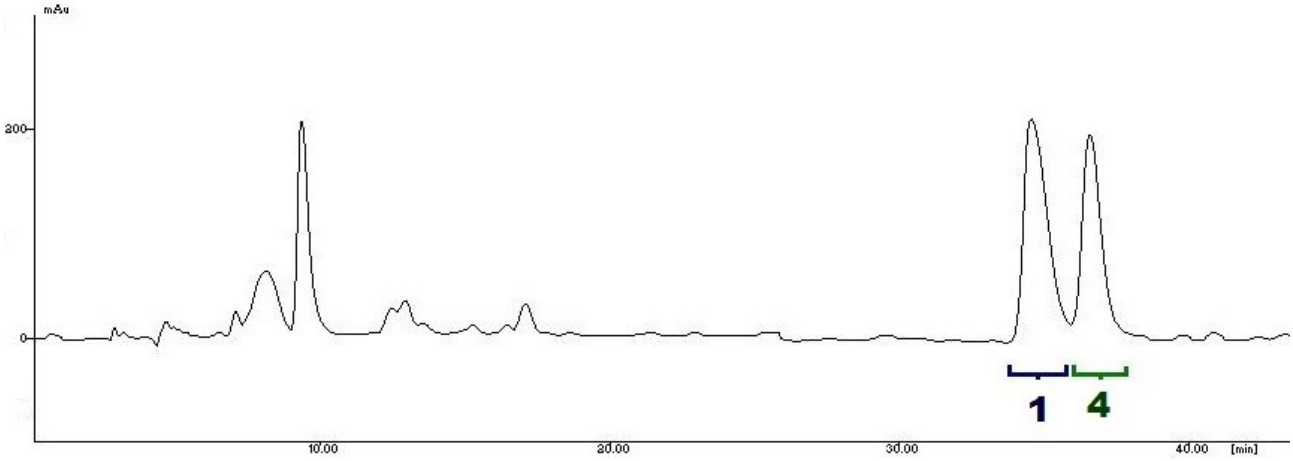


The inseparable mixture of compounds **12** and **13** was dissolved in THF (0.1 M) in a sealed tube for microwaves reactor and EEDQ (1.1 eq.) and (L)-NHAc-Ala-OH (1. eq.) were added. The sample was irradiated under magnetic stirring by microwaves for 30 minutes at 60°C (80 Watt) using the air compressing cooling system to keep down the temperature. The reaction was monitored by TLC (MeOH : DCM, 1 :8, detected by ninhydrin). If not finished another round of 30 minutes was performed. Upon consumption of the starting material, cold Et_2O was added to precipitate the mixture of peptide and was decanted affording compounds **1** and **2** in mixture as a white solid (88%). The two diastereoisomers were separated by HPLC (inverse phase, gradient from 95% H_2O, 5% CH_3CN +0,1 % of TFA (5min) to 80% H_2O, 20% CH_3CN +0,1 % of TFA (40min.) affording two fractions: **1** (retention time 35') and **2** (retention time 37') The reaction was performed with comparable yield from 50mg to 100mg.

1(first fraction) α_D^{MeOH} = -8.3. δ_H (500 MHz, H_2O+D_2O) 8.14 (1 H, d, *J* 5.6), 7.97 (1 H, s), 7.90 (2 H, s,), 7.74 (1 H, d, *J* 6.4), 7.34 (1 H, s), 6.98 (1 H, s), 6.40 (1 H, dd, *J* 5.7, 3.1), 6.02 (1 H, dd, *J* 5.8, 3.2), 4.23 – 4.02 (3 H, m), 3.33 (1 H, d, *J* 1.0), 2.87 (1 H, s), 2.29 (1 H, dd, *J* 13.2, 3.7), 1.93 (3 H, s), 1.60 (1 H, d, *J* 9.1), 1.47 (1 H, dd, *J* 9.2, 1.5), 1.39 (6 H, d, *J* 5.8), 1.34 (3 H, d, *J* 7.4), 1.31 (1 H, d, *J* 3.2), 1.29 (3 H, d, *J* 7.3), 1.25 (3 H, d, *J* 7.3). δ_C (126 MHz, H_2O+D_2O) 178.27, 177.11, 176.95, 175.49, 175.21, 174.03, 141.04, 133.04, 65.38, 56.70, 50.91, 50.08, 49.83, 49.04, 48.16, 41.81, 40.42, 24.35, 24.00, 21.67, 16.47, 16.21, 15.82. (+)ESI-MS (m/z) : $[M+Na]^+$ 515.4. Anal.Calcd for $C_{23}H_{36}N_6O_6$ (492.3): C, 56.08; H, 7.37; N, 17.06; O, 19.49; found C, 56.01; H, 7.32; N, 17.10.IR(KBr) v = 3429, 3066, 2981, 2945, 1658, 1533, 1198 cm^{-1}

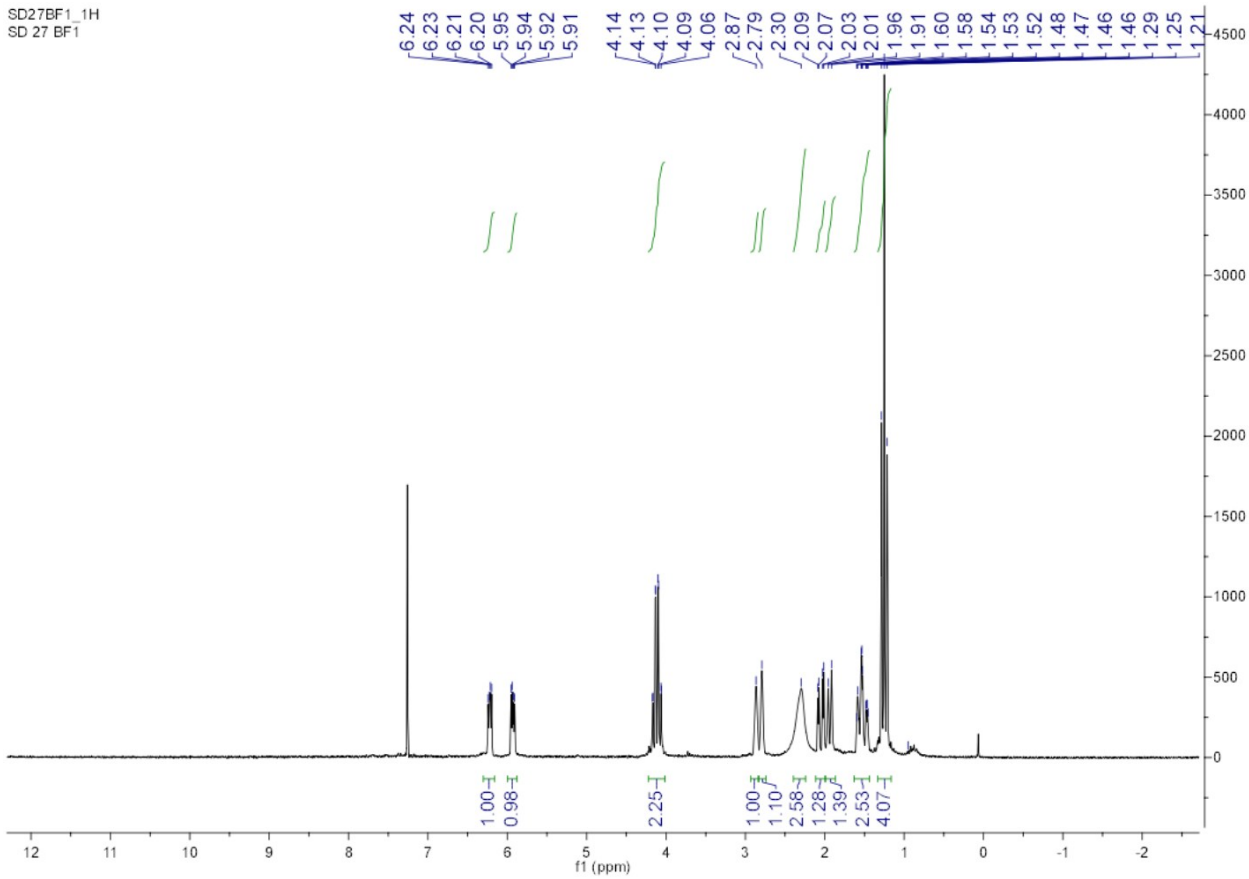
2 (second fraction) α_D^{MeOH} = +2. δ_H (500 MHz, H_2O+D_2O) 8.16 (1 H, d, *J* 5.1), 7.93 (1 H, s), 7.88 (1 H, d, *J* 5.2), 7.81 (1 H, s), 7.75 (1 H, d, *J* 6.5), 7.33 (1 H, s), 7.00 (1 H, s), 6.41 (1 H, dd, *J* 5.8, 3.1), 6.04 (1 H, dd, *J* 5.8, 3.1), 4.22 – 4.15 (1 H, m), 4.10 – 4.04 (2 H, m,), 3.40 (1 H, s), 2.89 (1 H, s), 2.24 (1 H, dd, *J* 13.4, 3.7), 1.97 (3 H, s), 1.60 (1 H, d, *J* 9.2), 1.48 (1 H, d, *J* 9.1), 1.43 (6 H, d, *J* 3.8), 1.41-1.40 (1 H, m), 1.39 (3 H, d, *J* 7.4), 1.32 (3 H, d, *J* 7.4), 1.28 (3 H, d, *J* 7.4). δ_C (126 MHz, D_2O) 178.25, 177.22, 177.13, 175.90, 175.44, 174.21, 140.75, 133.19, 65.05, 56.63, 51.16, 50.38, 49.96, 49.03, 48.09, 41.73, 40.21, 24.50, 23.65, 21.53, 16.35, 15.83, 15.70. (+)ESI-MS (m/z) : $[M+Na]^+$ 515.4. Anal.Calcd for $C_{23}H_{36}N_6O_6$ (492.3): C, 56.08; H, 7.37; N, 17.06; O, 19.49; found C, 56.12; H, 7.35; N, 16.98.IR(KBr) v = 3430, 3066, 2981, 2943, 1656, 1534, cm^{-1}

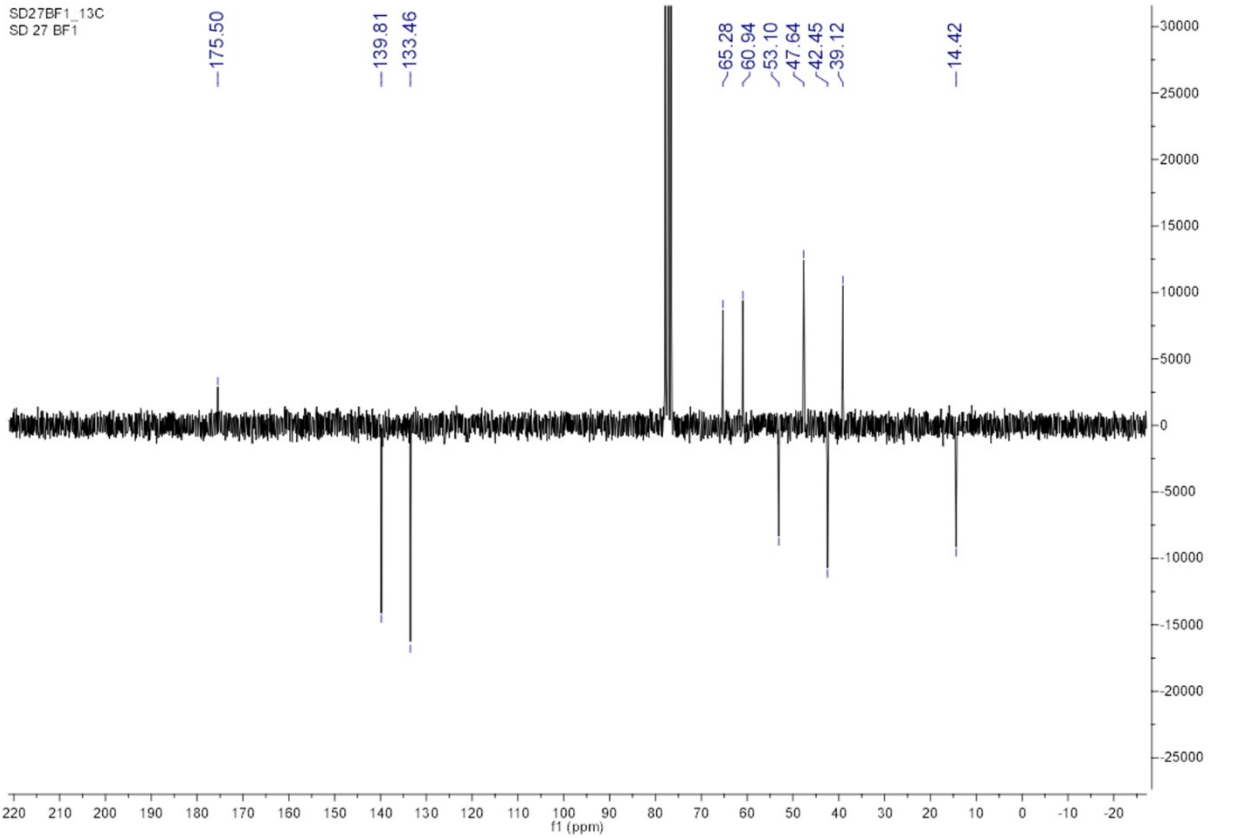
RF-HPLC 1-2



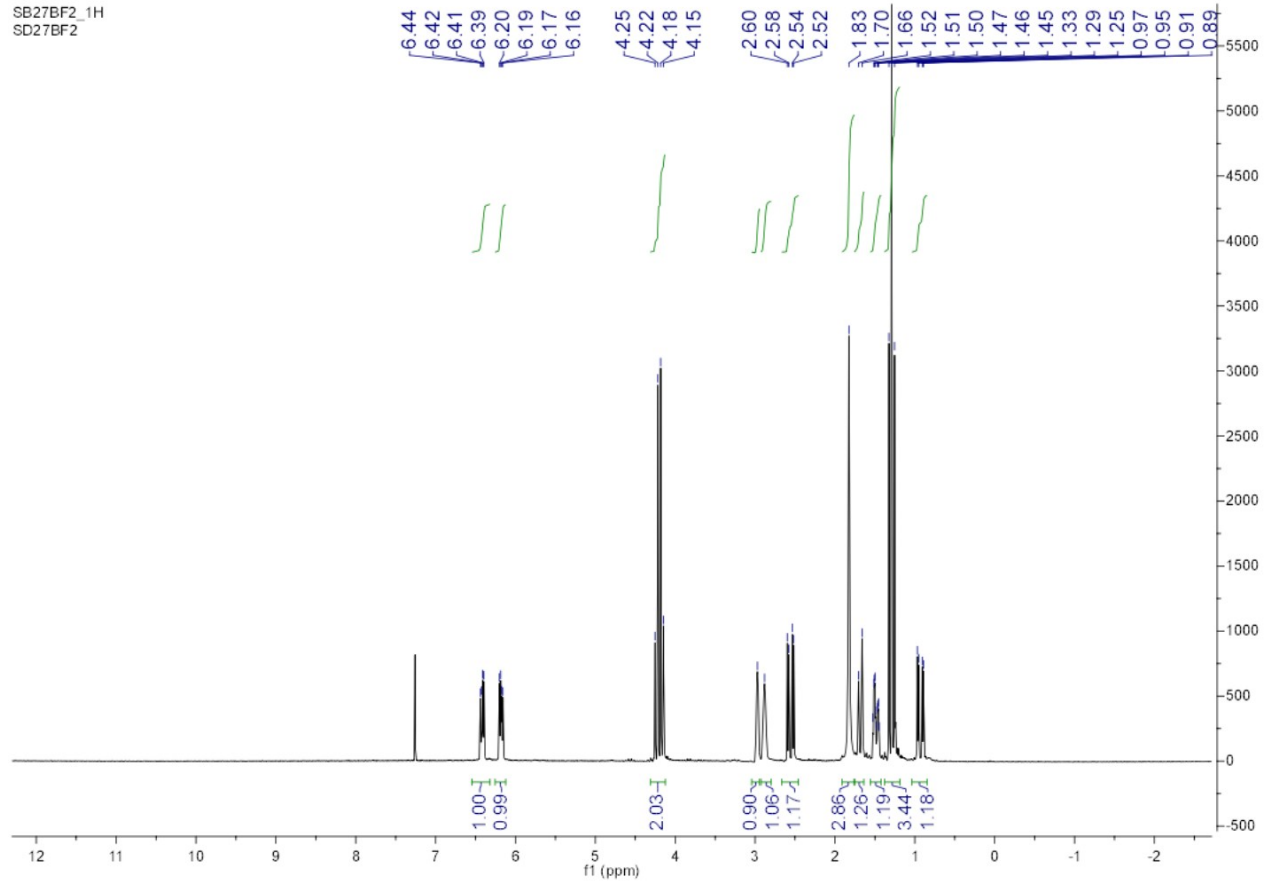
Fid 1H-NMR, 13C-NMR

Compound 6





Compound 7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1.46
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1.33
1.29
1.25
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0.95
0.91
0.89
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1.18
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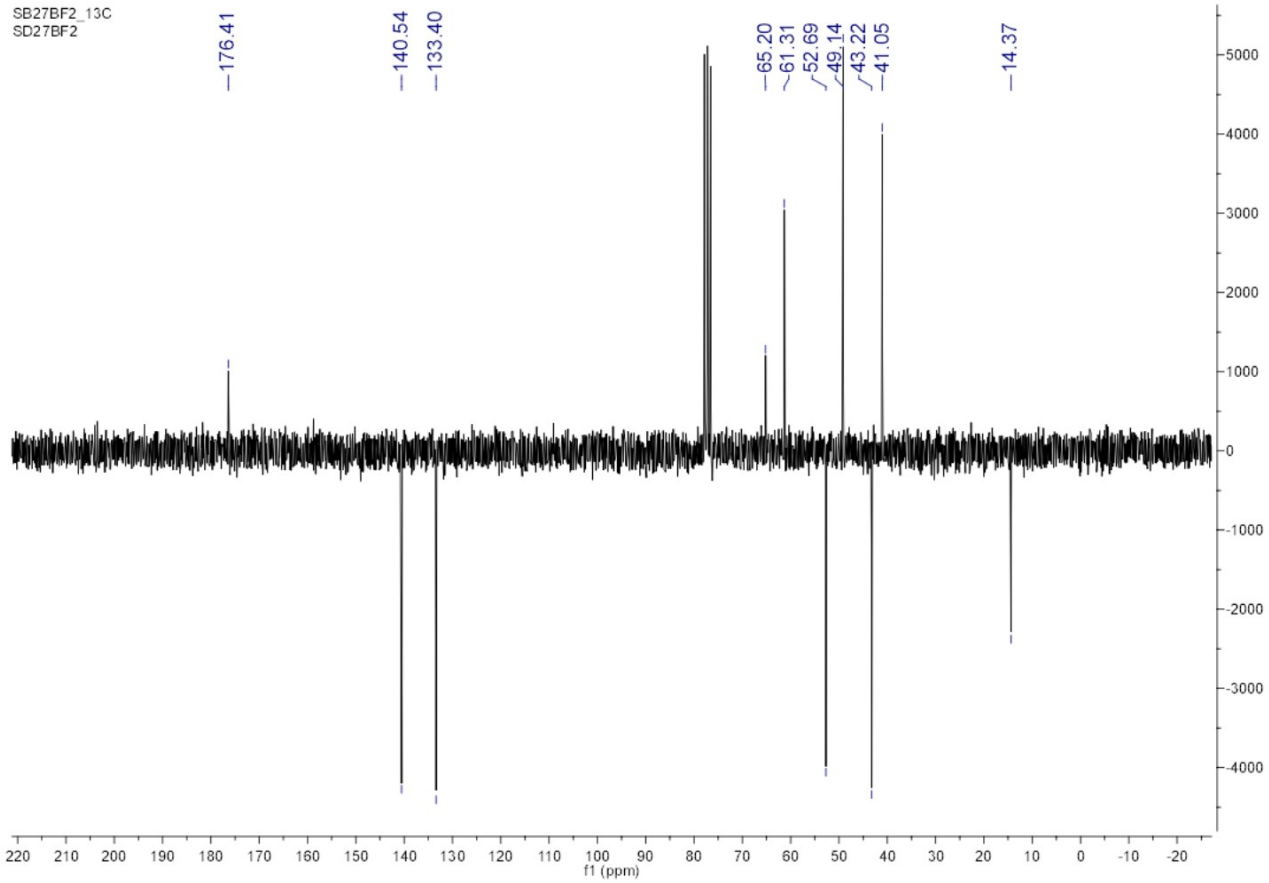
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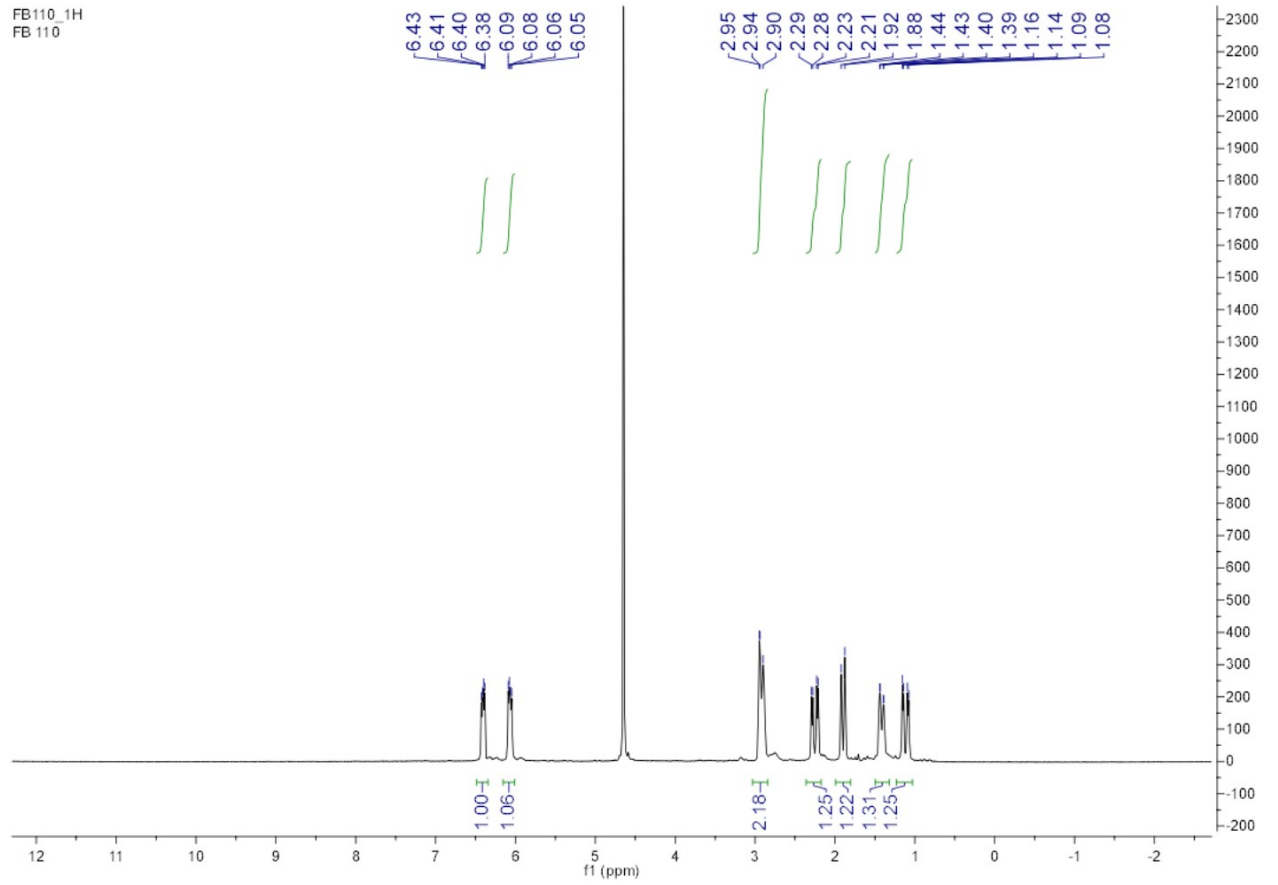
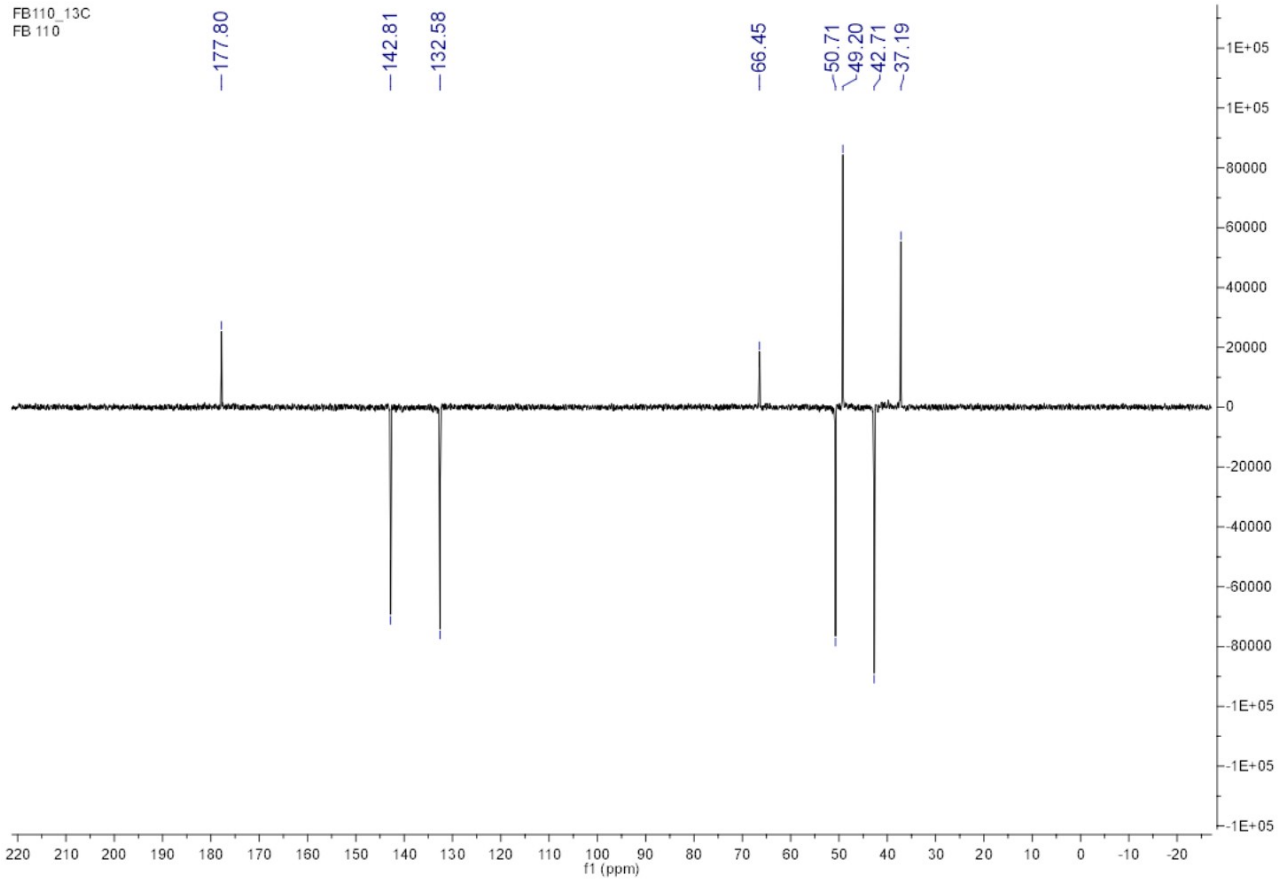
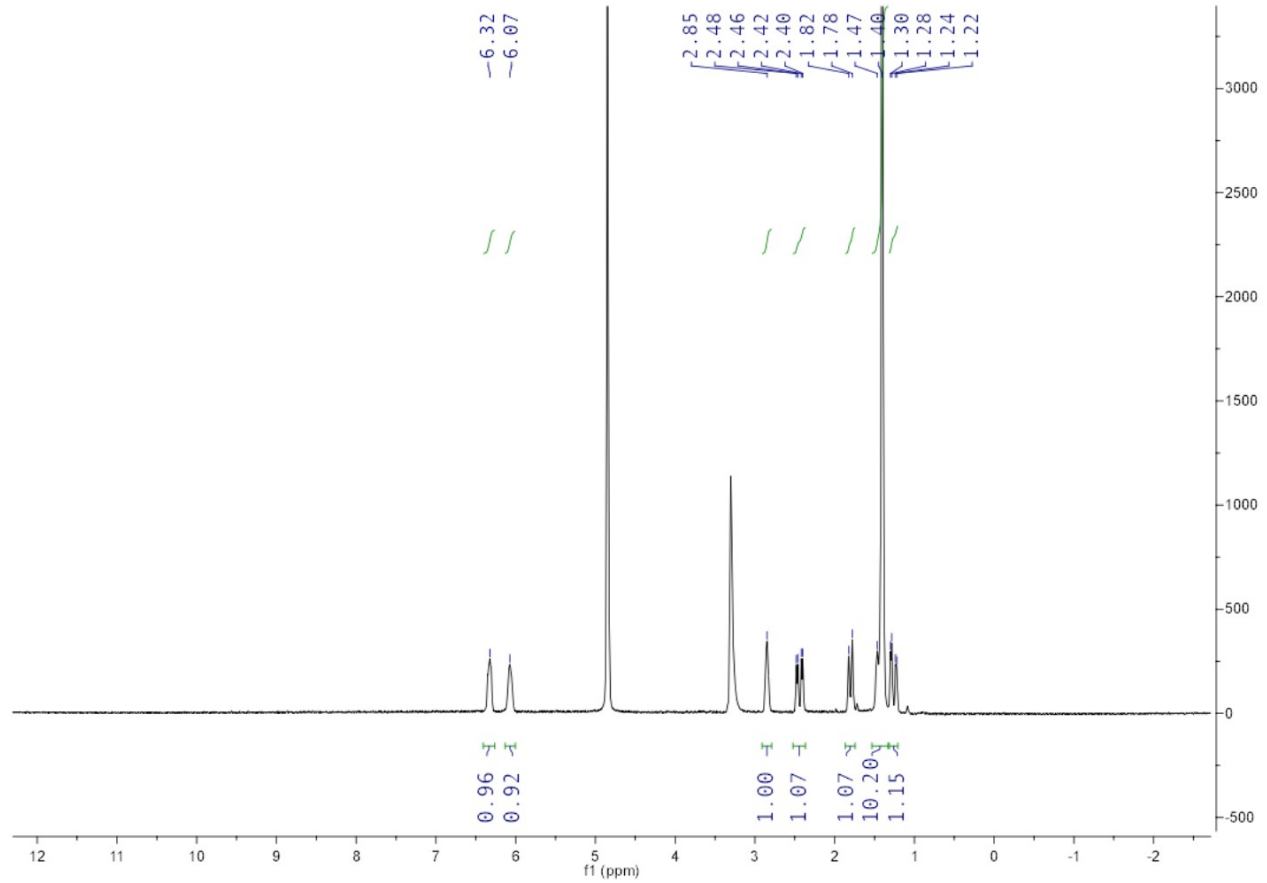
Compound 8SI





Compound 8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1.47
1.40
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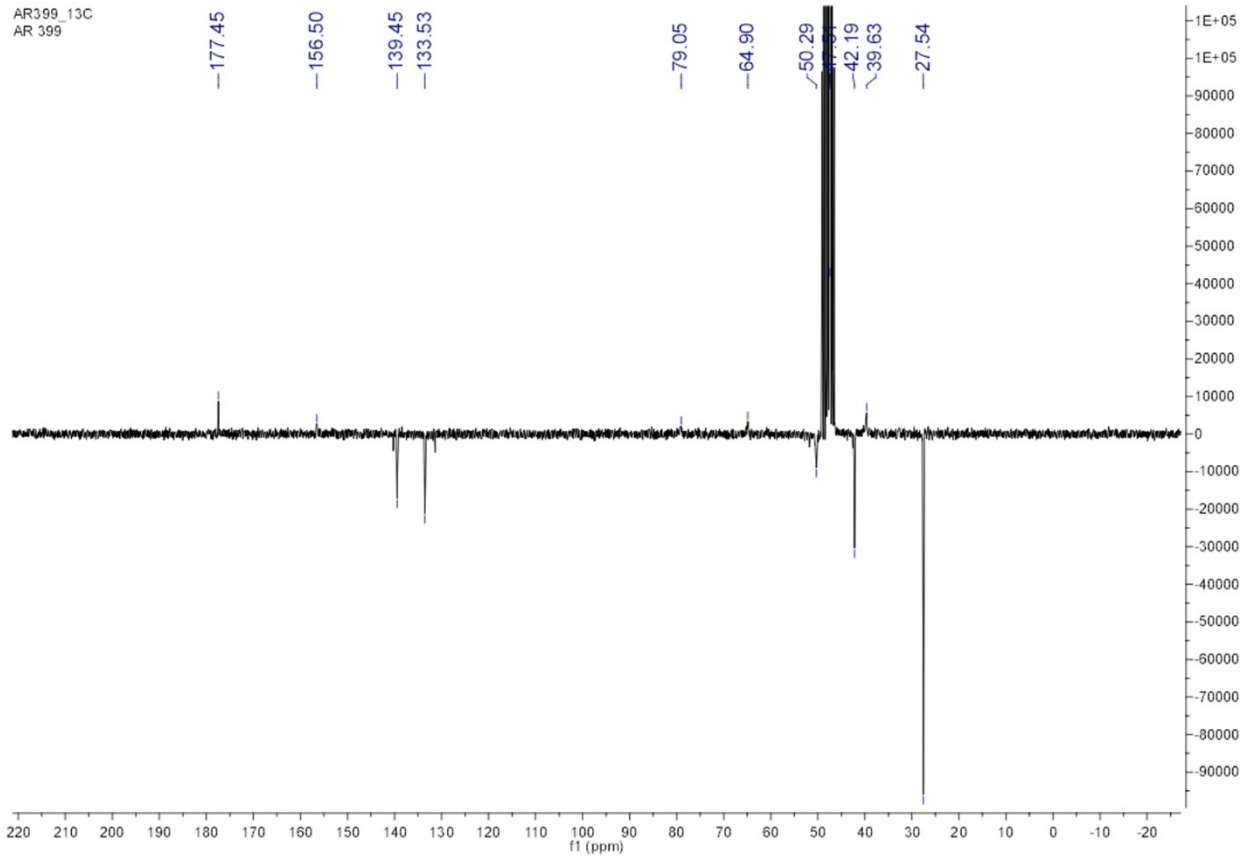
1.15
f1 (ppm)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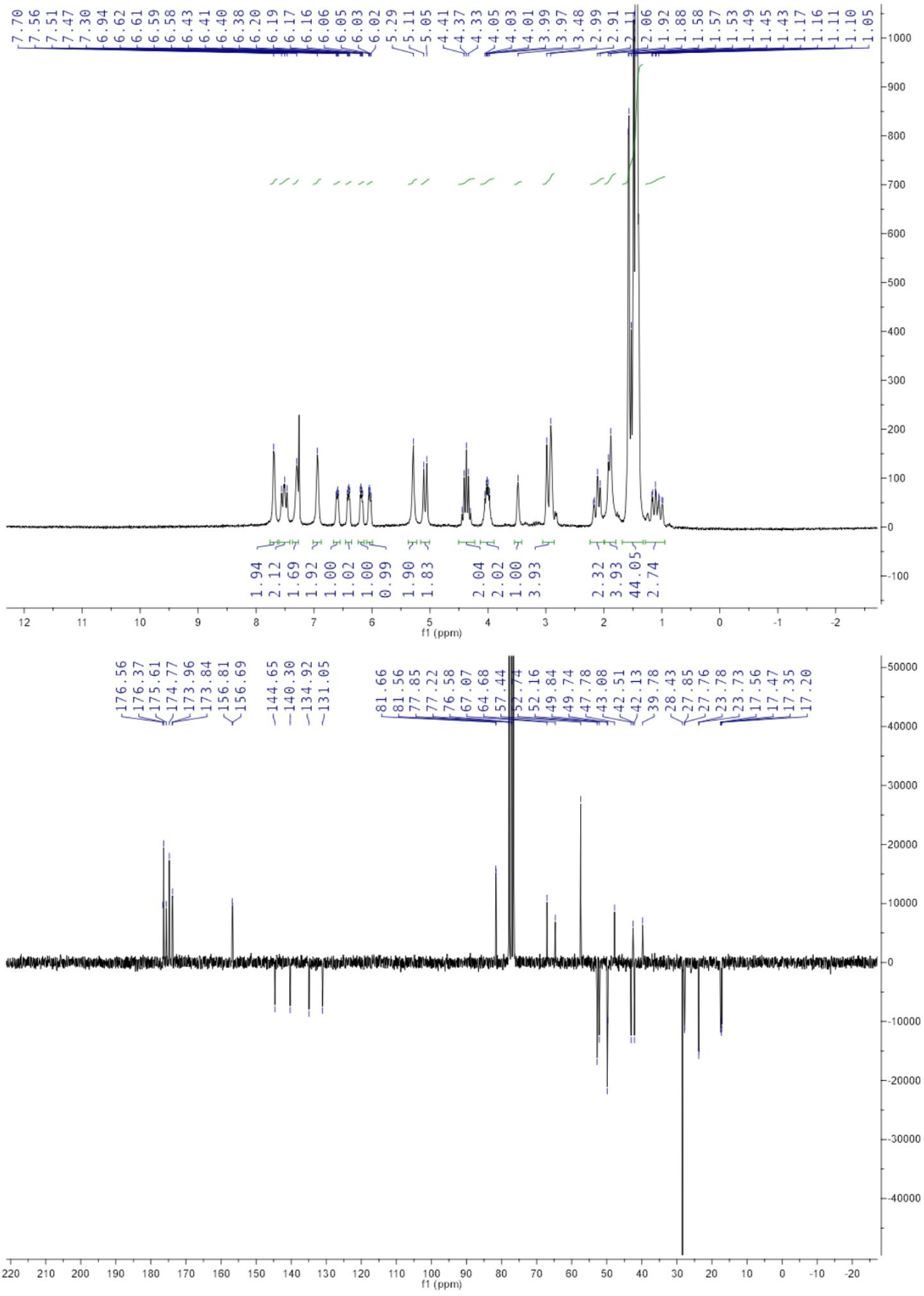
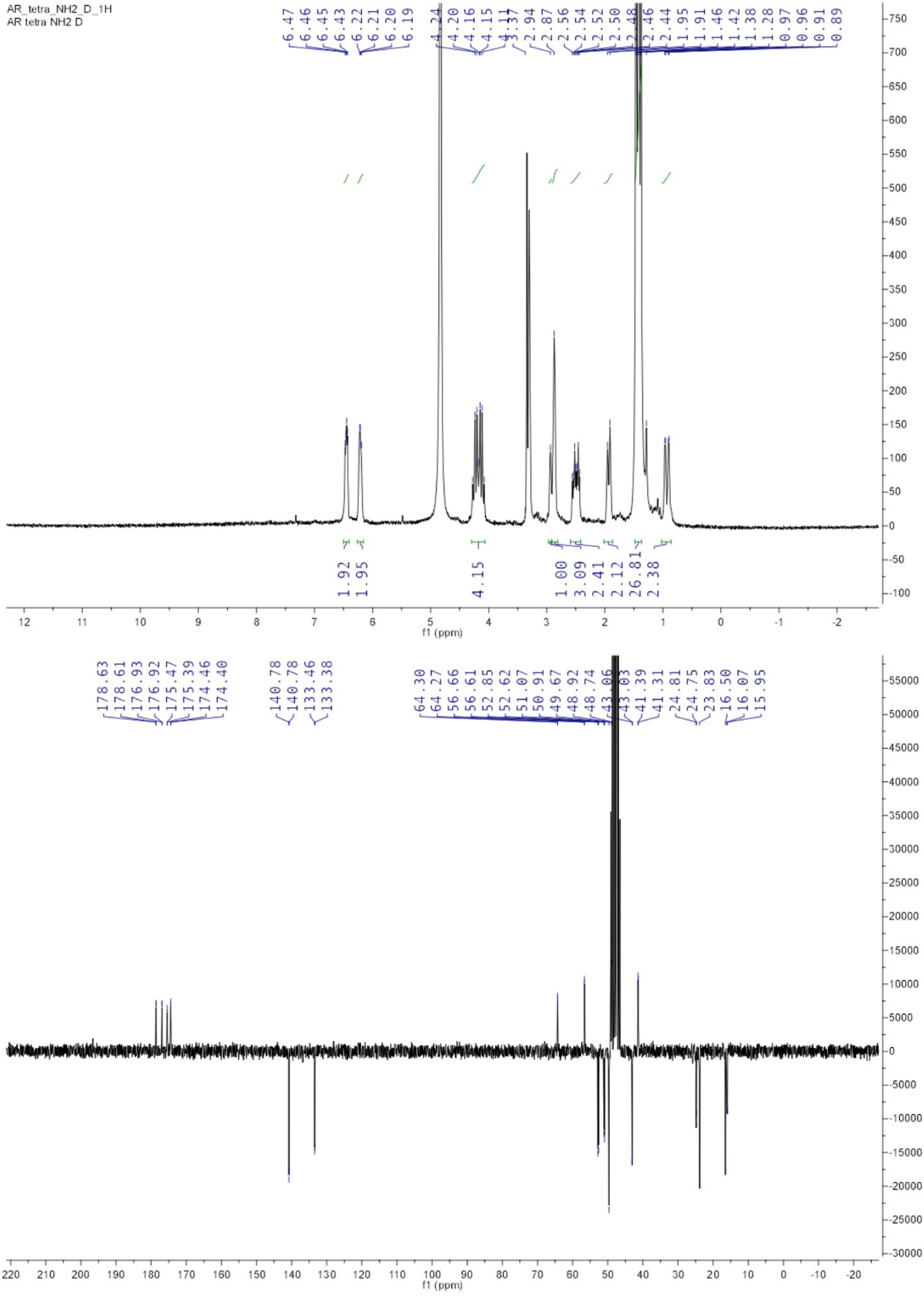
f1 (ppm)

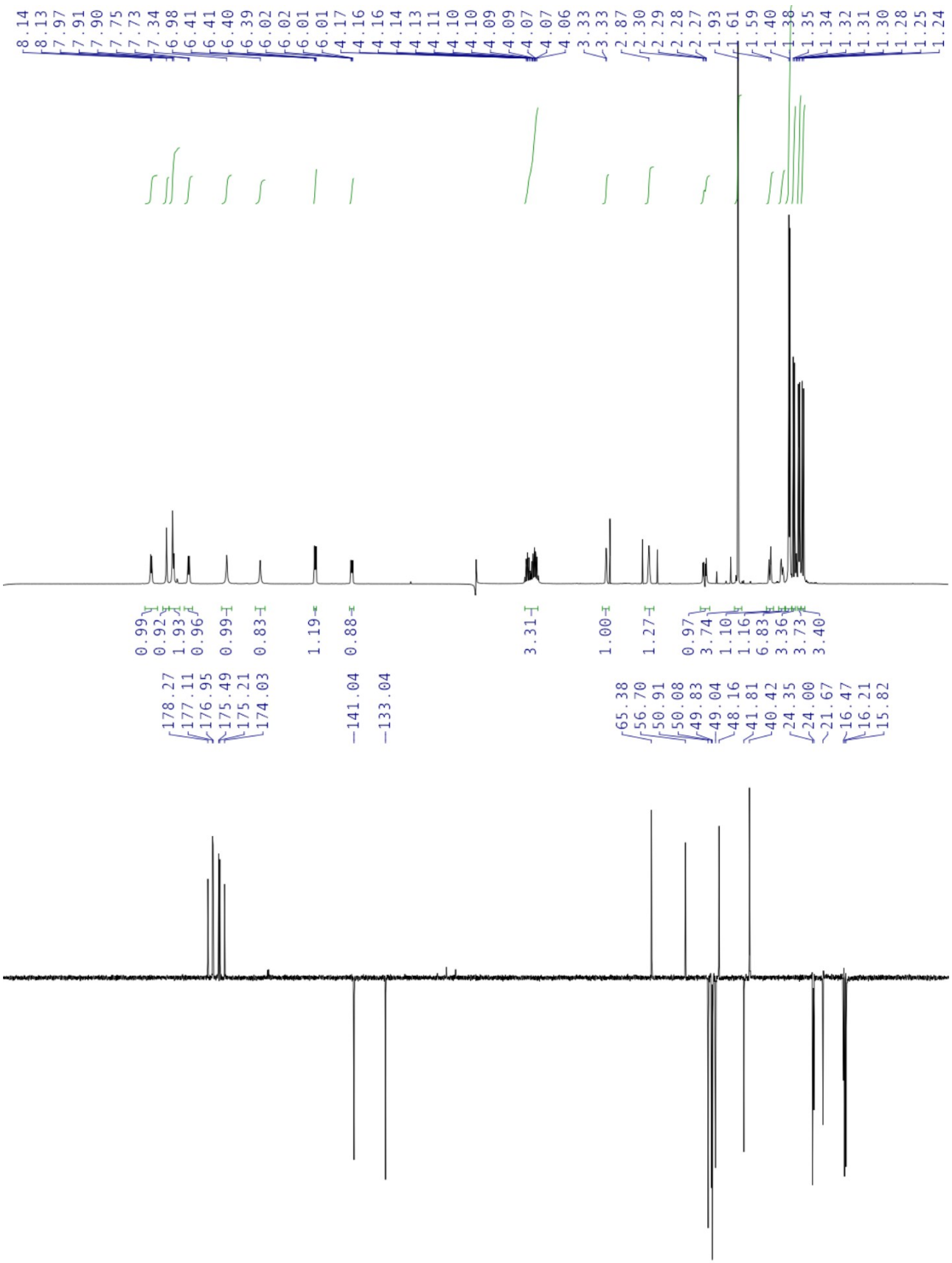
Compound 10-11



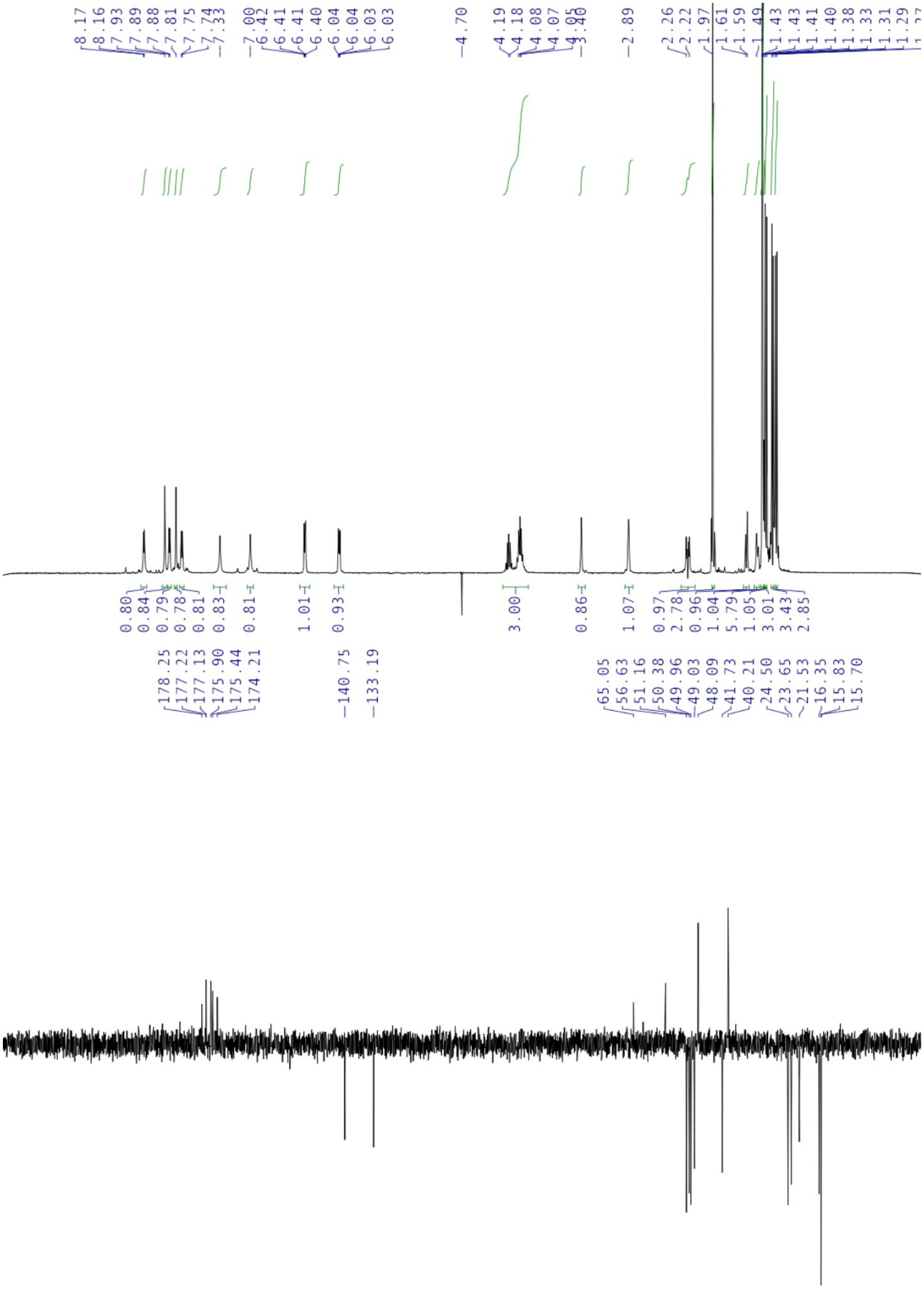
Compound 12-13



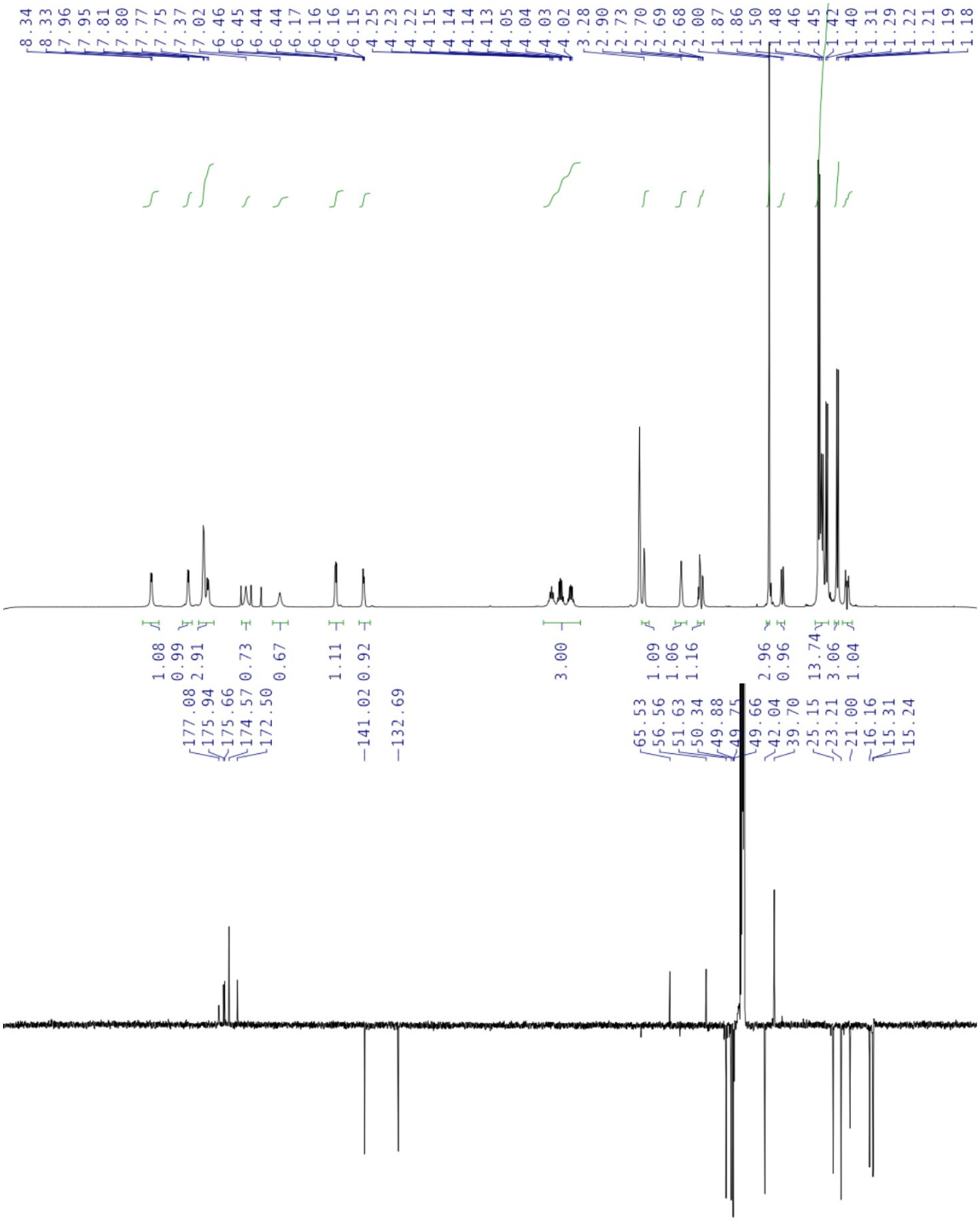
Compound 1 D_2O/H_2O



Compound 2 D_2O/H_2O



Compound 1 CD_3OH



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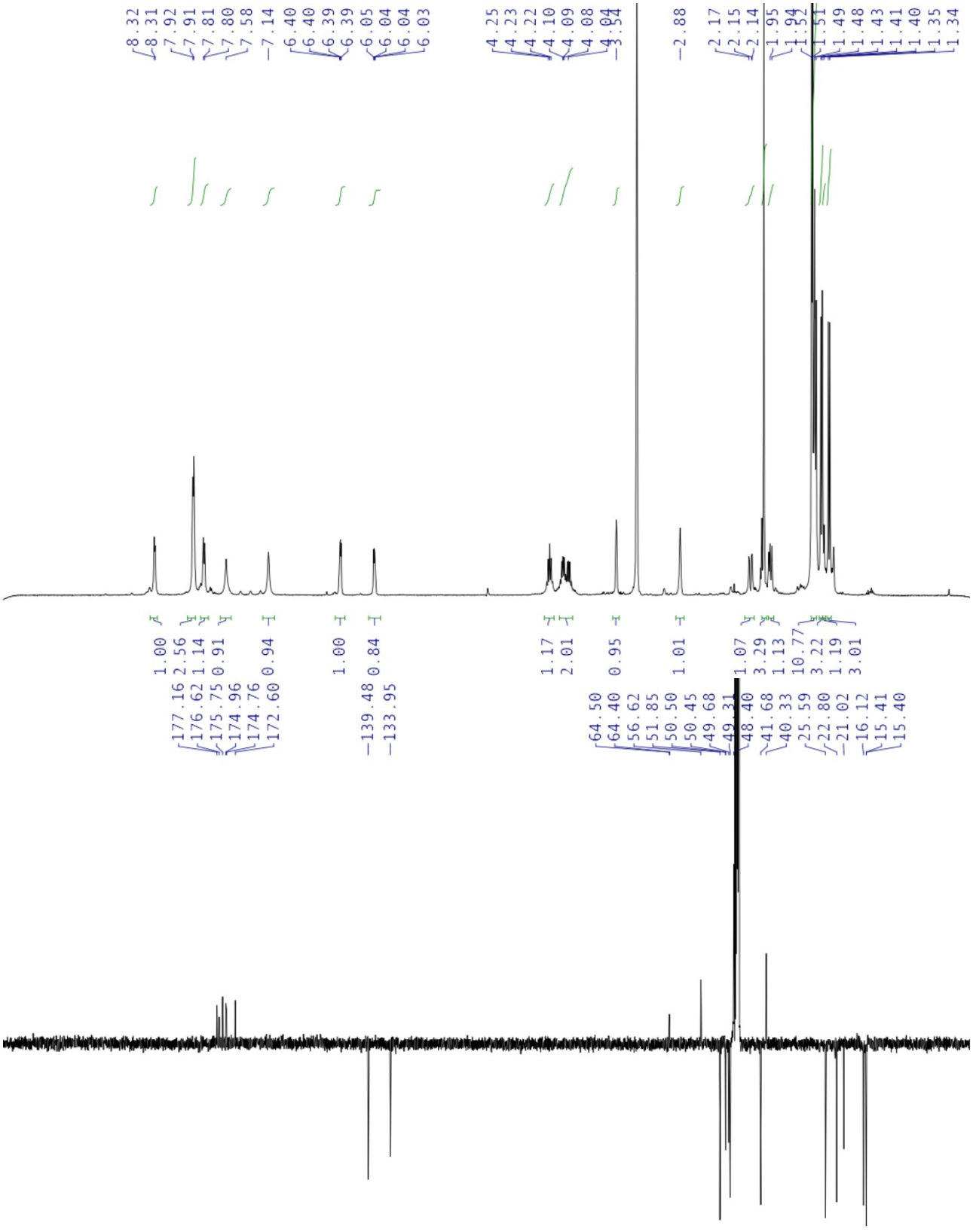
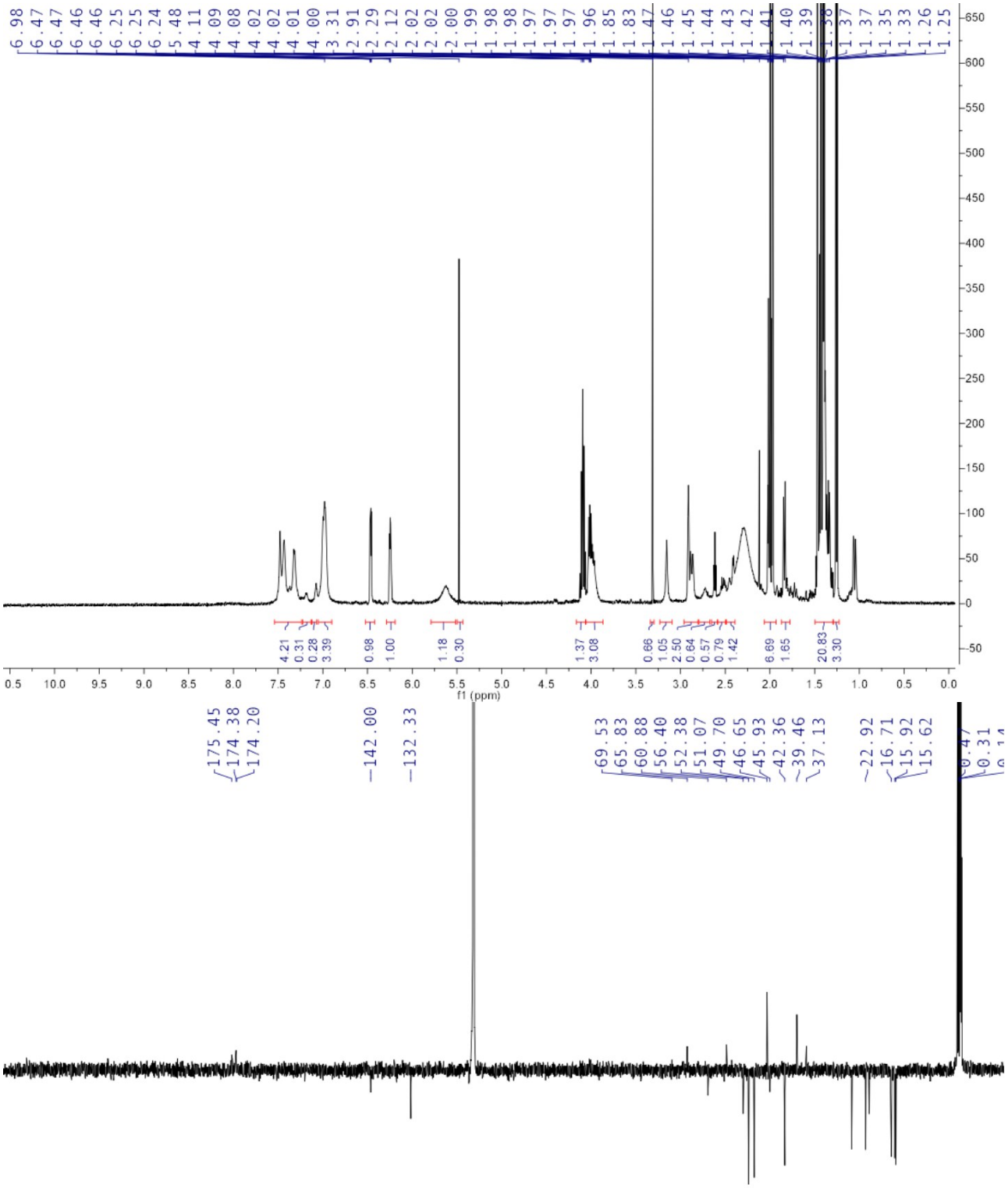


Compound 1 CD_3CN



Compound 2 CD_3CN