Supporting Information

Quantitative Determination of Ala-Ala Conformer Ratios in Solution by Decomposition of Raman Optical Activity Spectra

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Contents

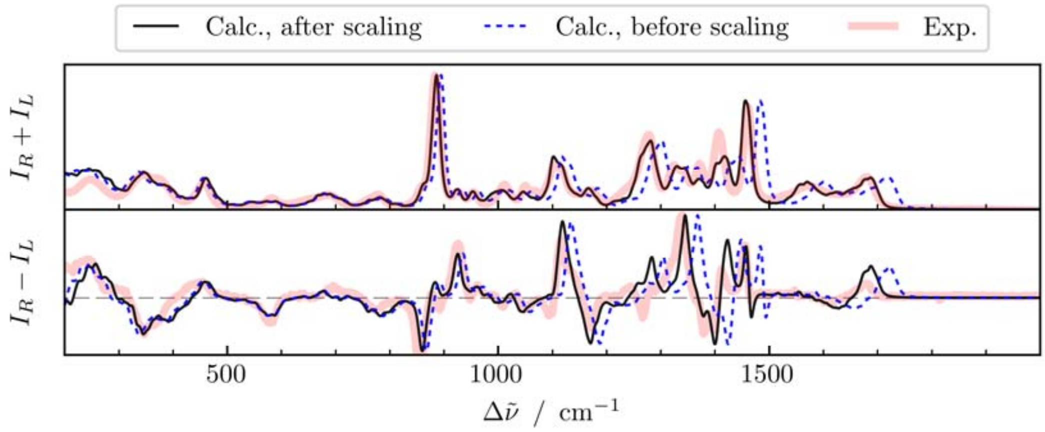
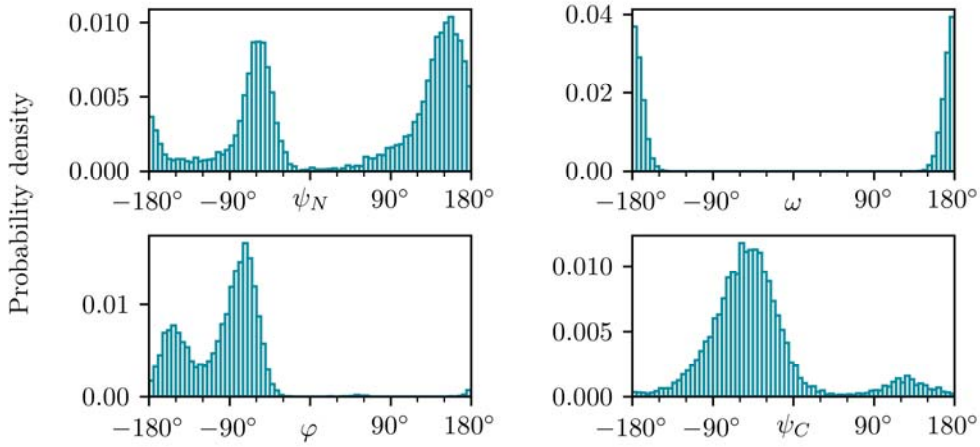
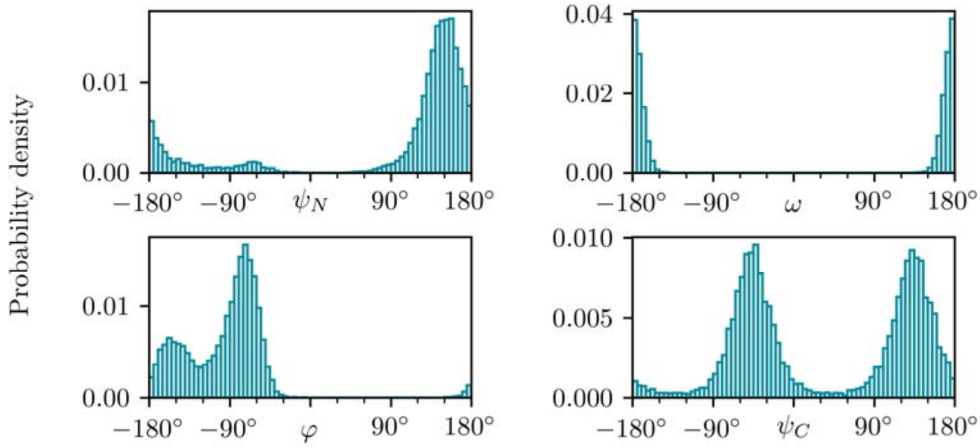


Figure S1. Effect of the wavenumber scaling for Raman and ROA intensities for zwitterionic Ala-Ala.

Protonated Ala-Ala:



Zwitterionic Ala-Ala :



Deprotonated Ala-Ala:

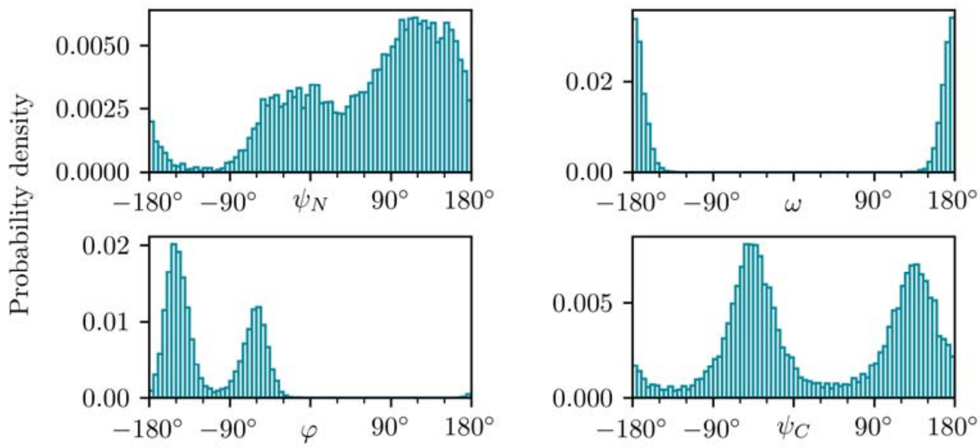


Figure S2. Histograms of the principal Ala-Ala torsion angles as obtained from unconstrained MD.

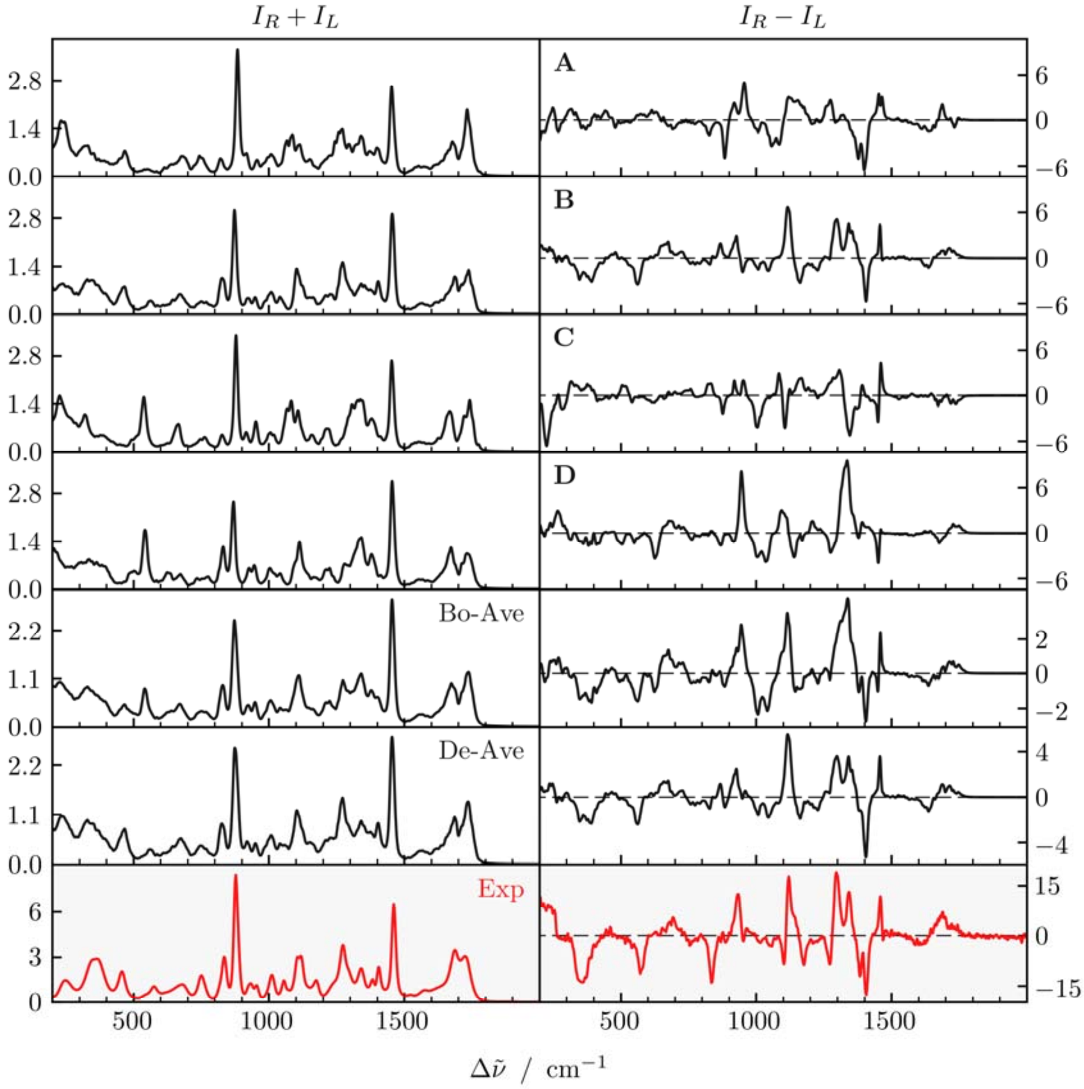


Figure S3a. Protonated Ala-Ala, calculated Raman (left) and ROA (right) spectra of individual conformers, Boltzmann average (integrated), decomposition fit, and the experiment. The experimental intensities are multiplied by a factor of 10^{-10} (Raman) and 10^{-6} (ROA) to approximately match the calculated (arbitrary) scale and avoid large numbers.

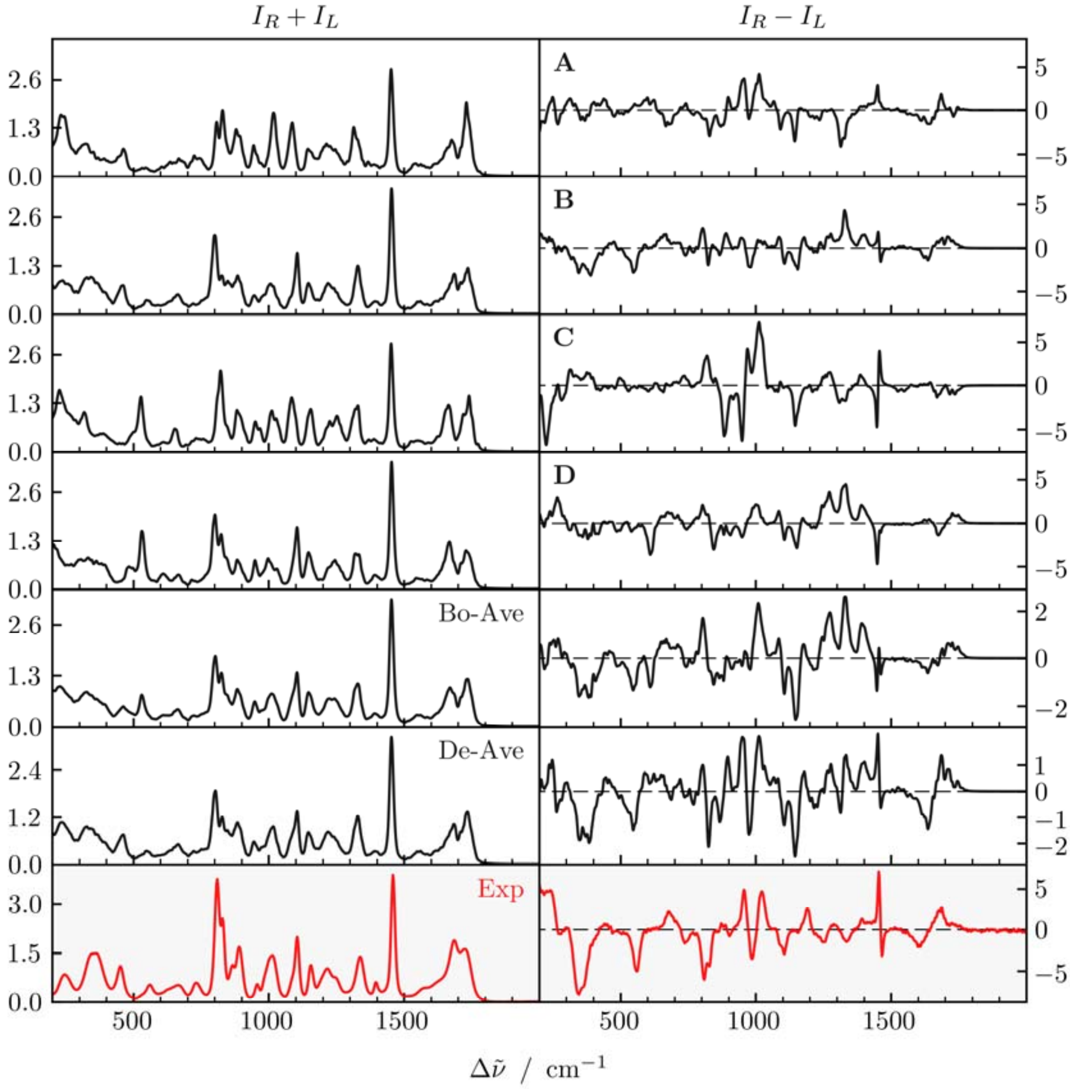


Figure S3b. Raman (left) and ROA (right) spectra of protonated Ala-d_1-Ala-d_1, cf. **Figure S3a** for the scale and spectra layout.

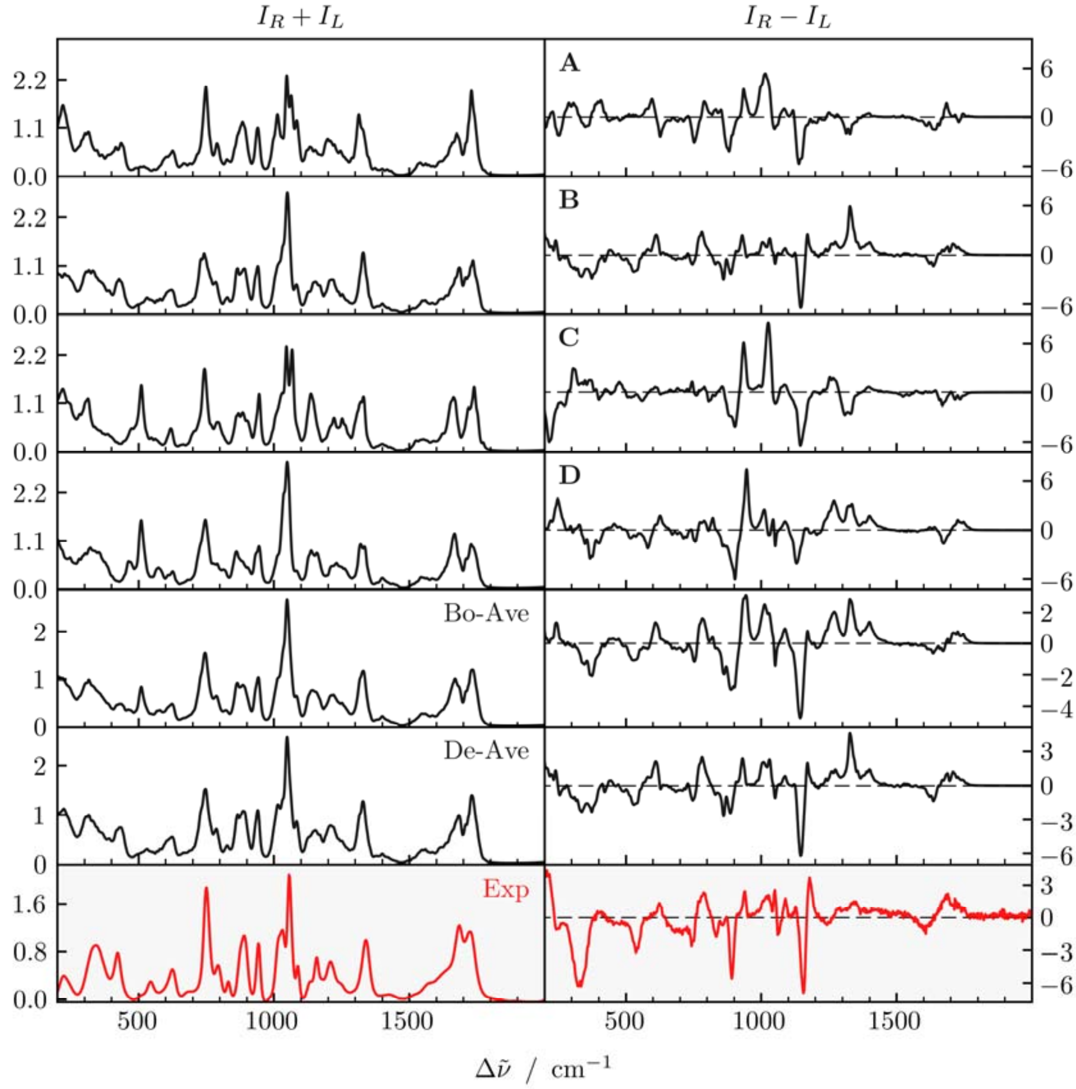


Figure S3c. Raman and ROA spectra of protonated Ala-d_4-Ala-d_4, cf. **Figure S3a** for the scale and spectra layout.

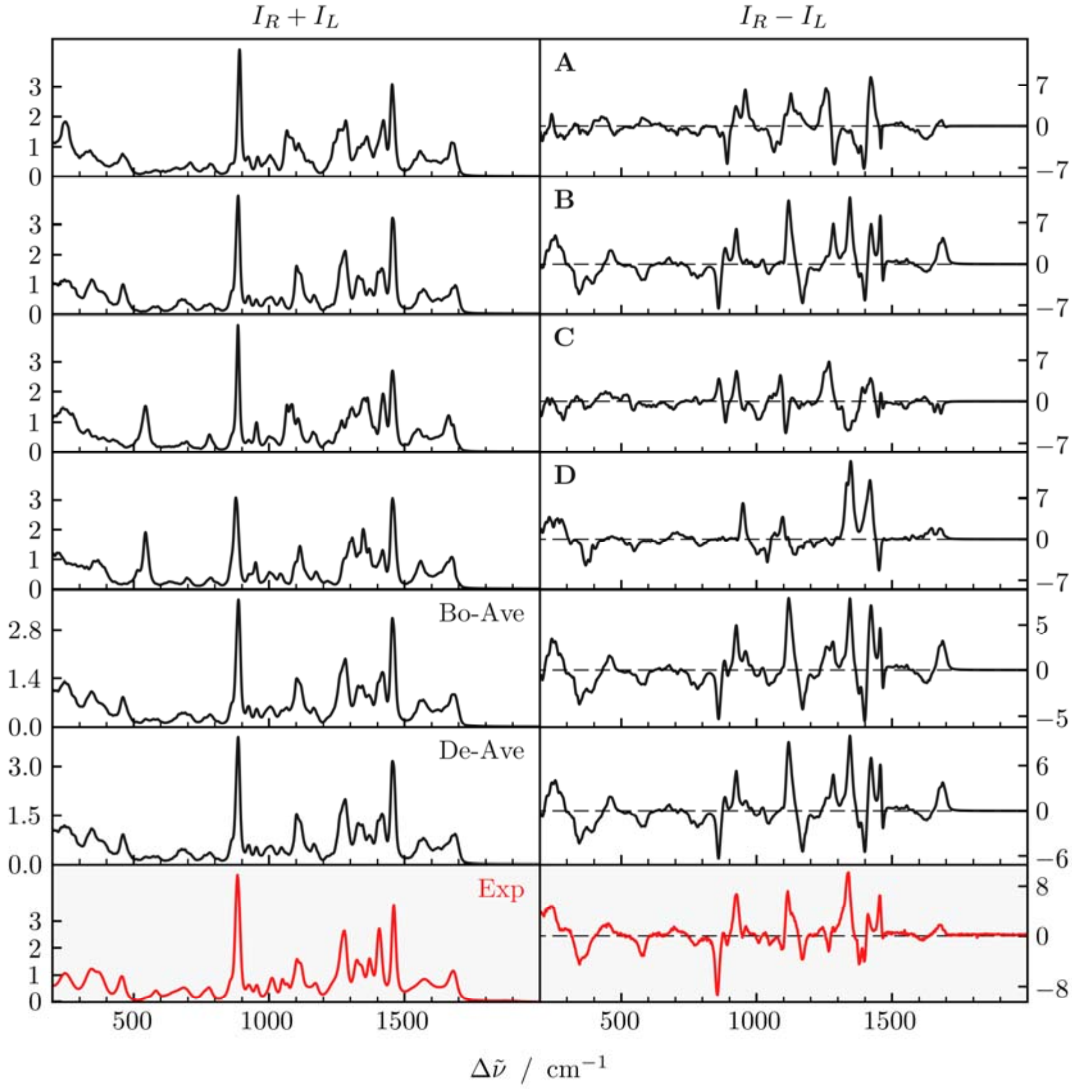


Figure S3d. Raman and ROA spectra of zwitterionic Ala-Ala, cf. **Figure S3a** for the scale and spectra layout.

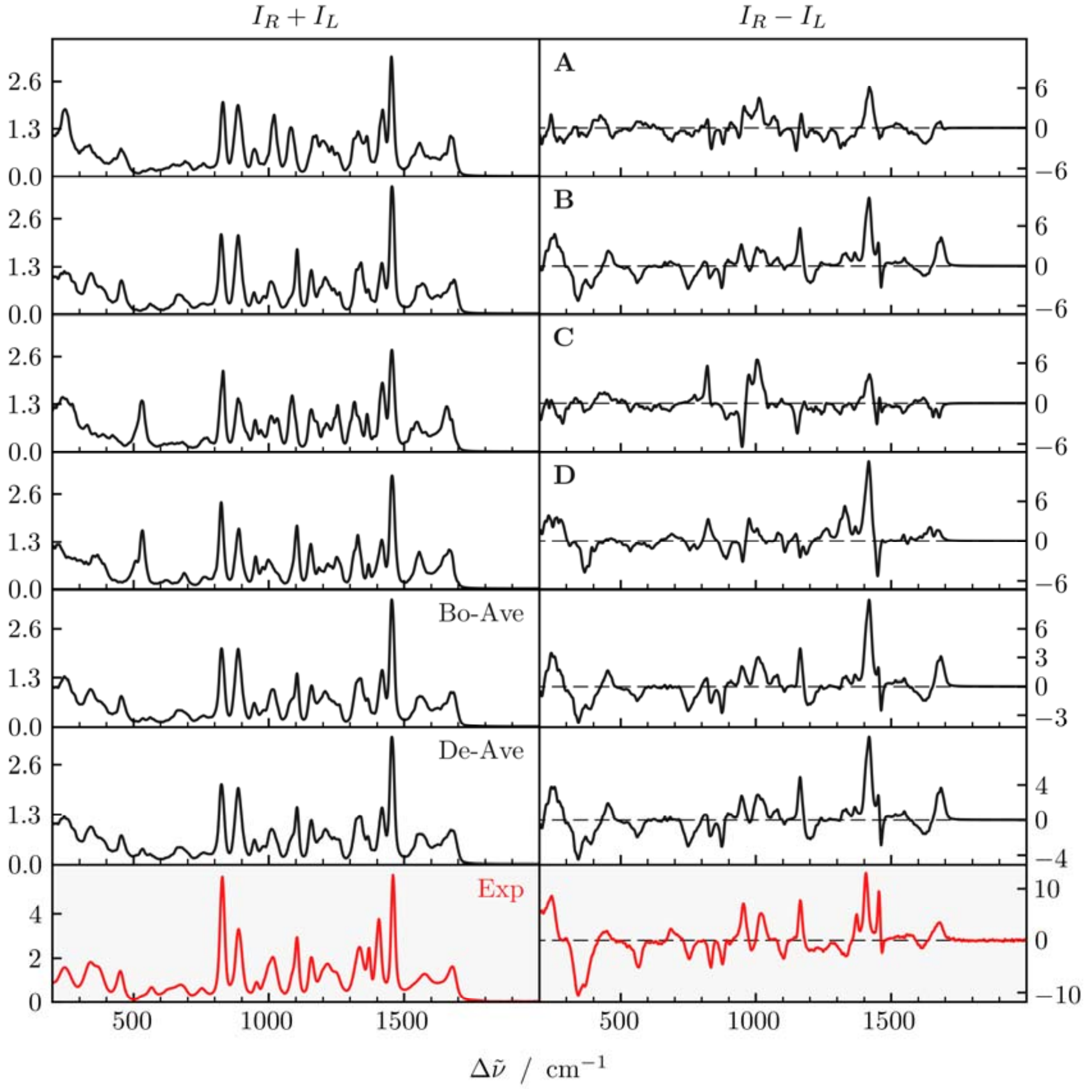


Figure S3e. Raman and ROA spectra of zwitterionic Ala-d_1-Ala-d_1, cf. **Figure S3a** for the scale and spectra layout.

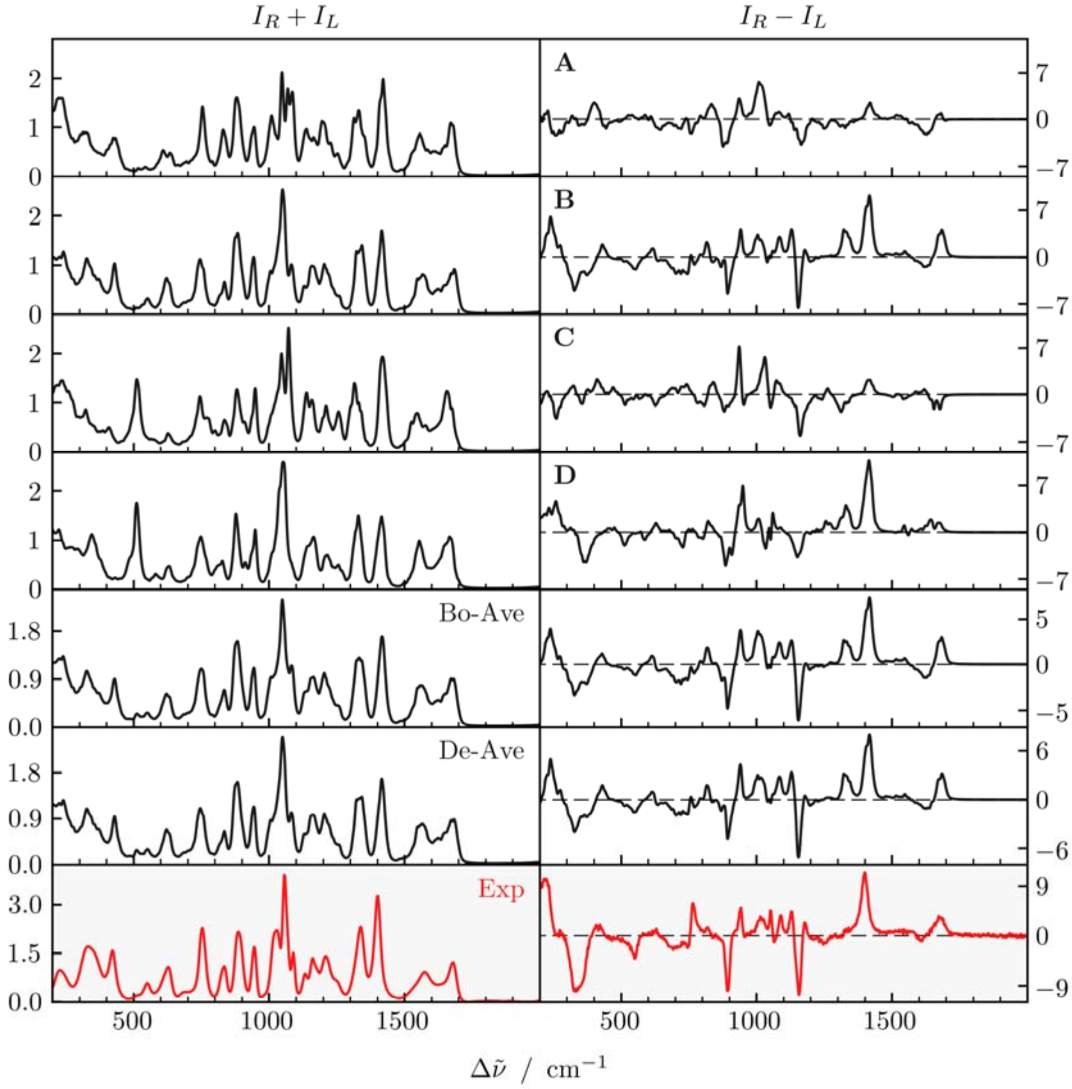


Figure S3f. Raman and ROA spectra of zwitterionic Ala-d_4-Ala-d_4, cf. **Figure S3a** for the scale and spectra layout.

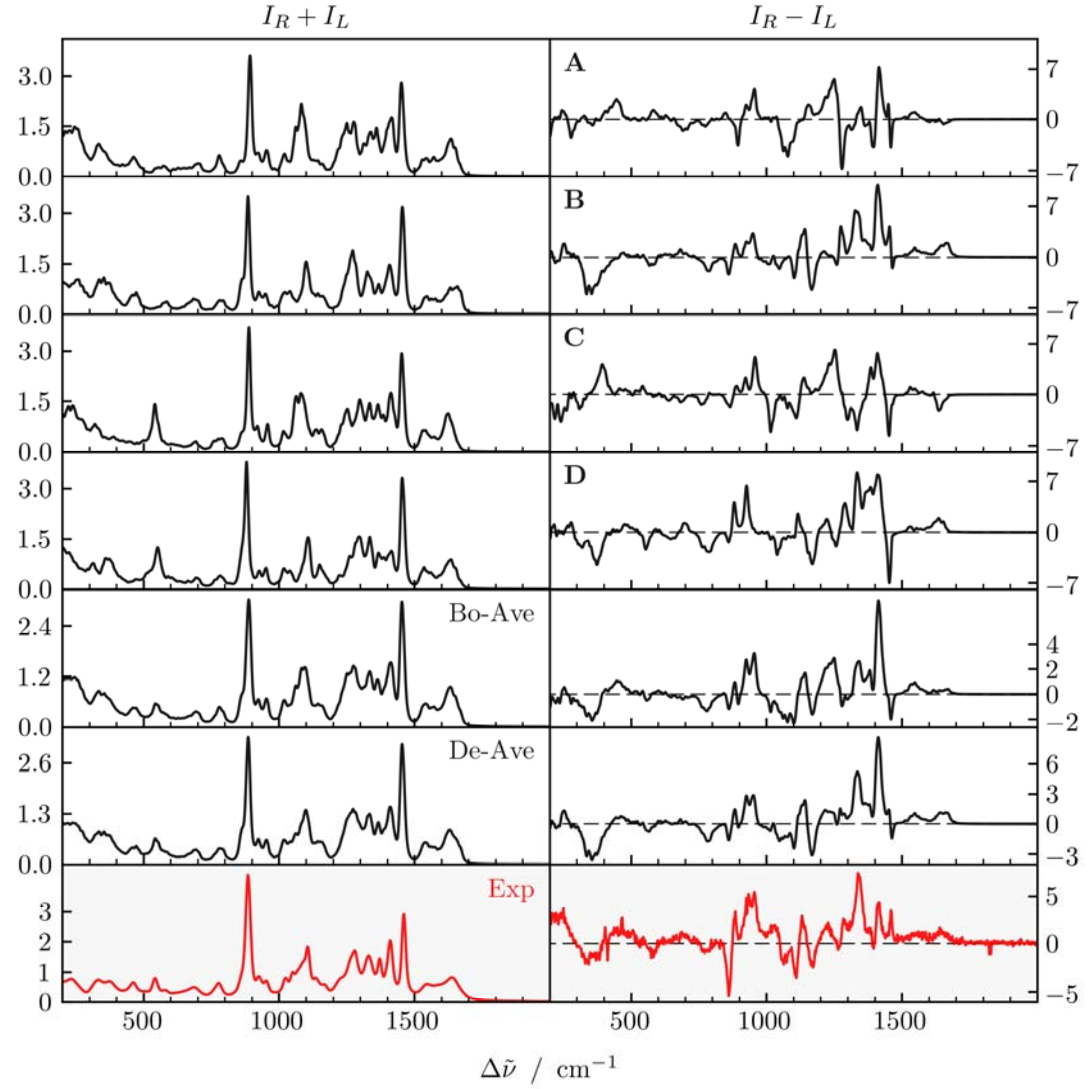


Figure S3g. Raman and ROA spectra of deprotonated Ala-Ala, cf. **Figure S3a** for the scale and spectra layout.

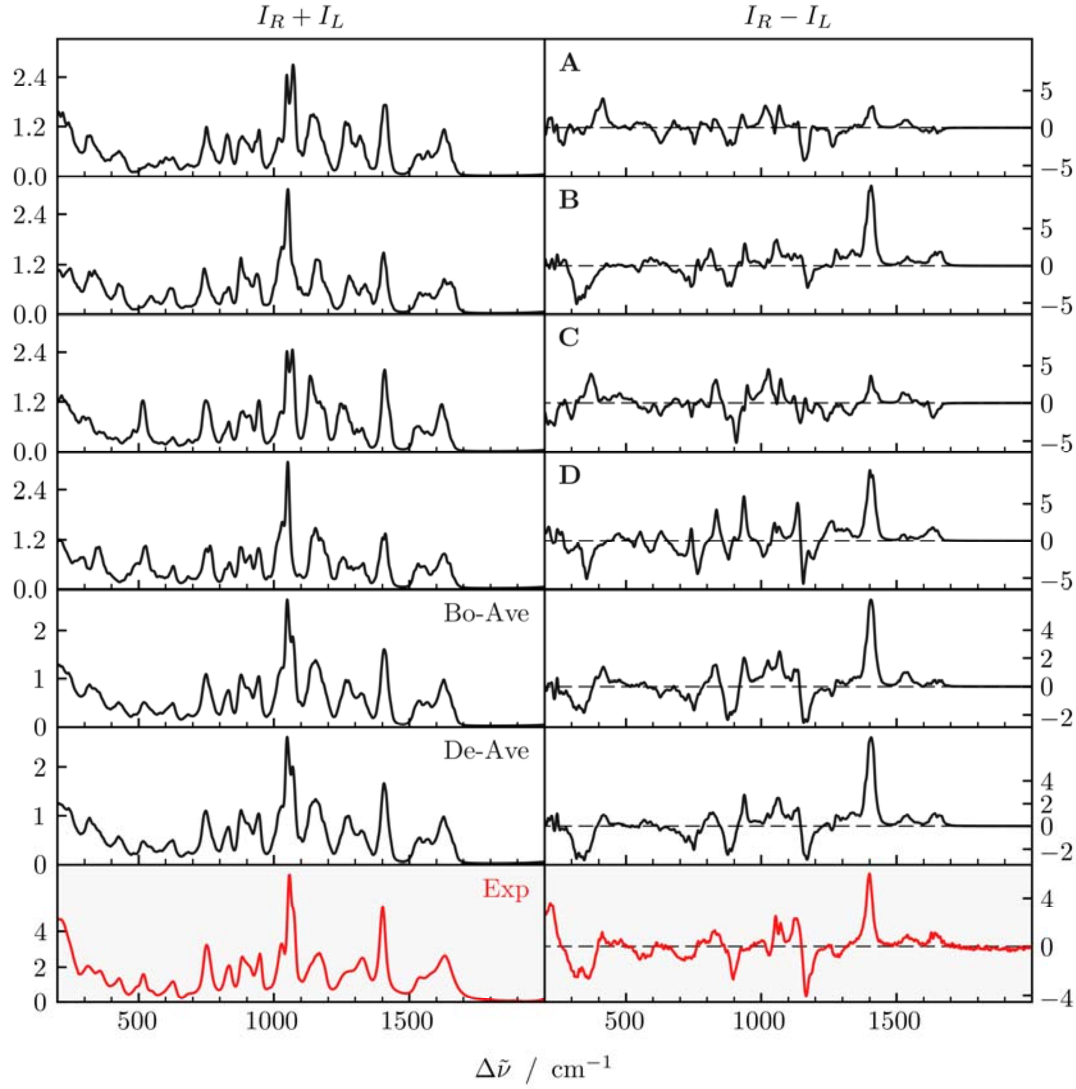


Figure S3h. Raman and ROA spectra of deprotonated Ala-d_1-Ala-d_1, cf. **Figure S3a** for the scale and spectra layout.

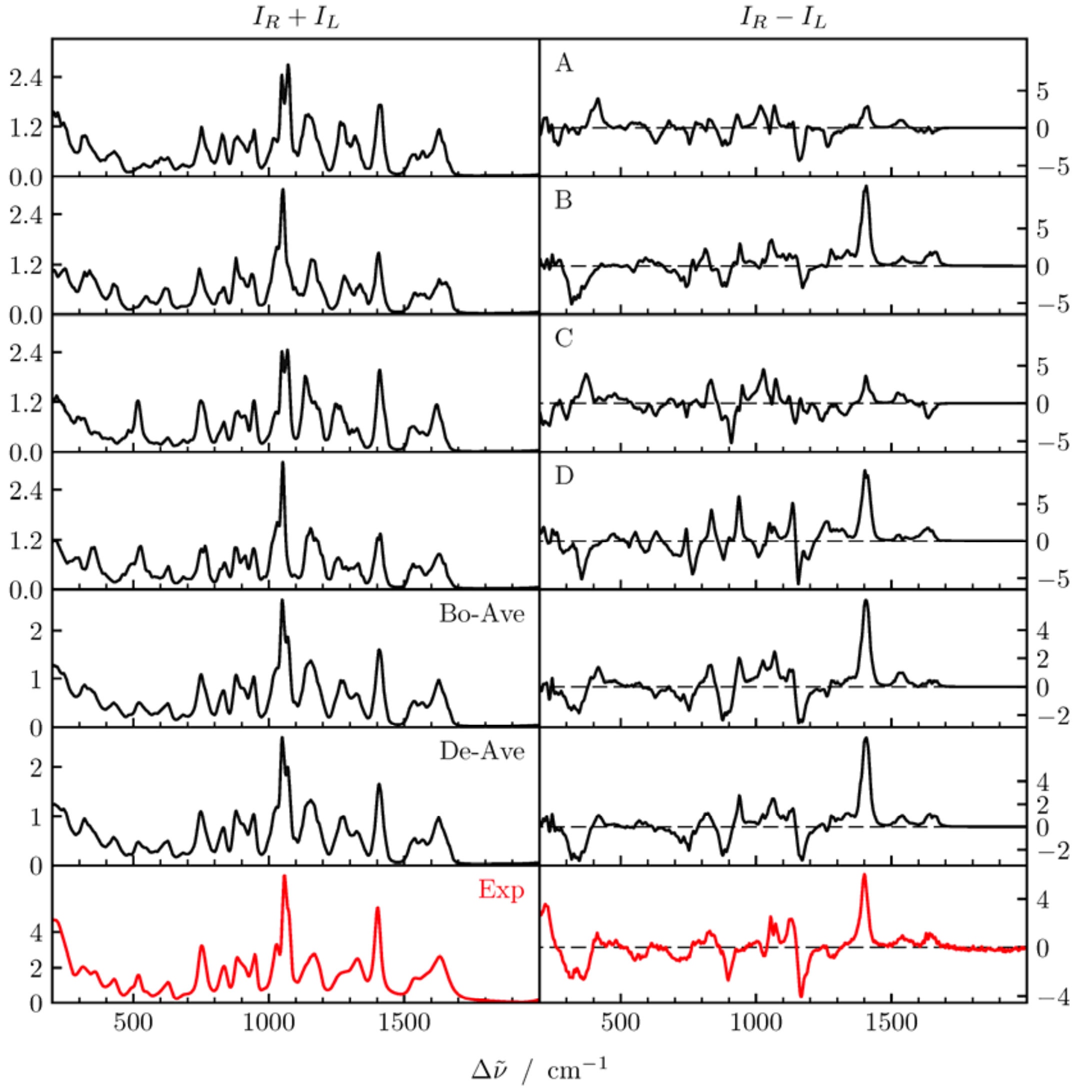


Figure S3i. Raman and ROA spectra of deprotonated Ala-d_4-Ala-d_4, cf. **Figure S3a** for the scale and spectra layout.

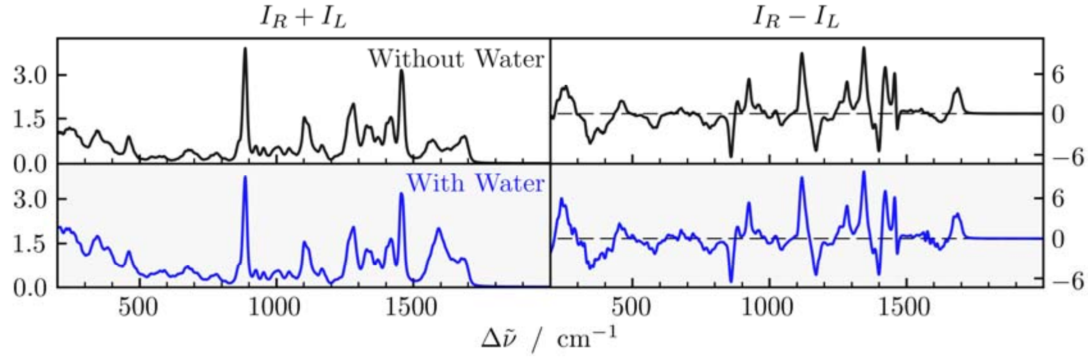


Figure S4. Raman and ROA zwitterionic Ala-Ala spectra simulated from the clusters with and without contribution of the water atoms to spectral intensities.

Table S1. Torsion angles and relative energies of the main Ala-Ala zwitterion's conformers as suggested by different works.

		φ / deg.	ψ_N / deg.	*E*/ kcal/mol	*Remark*
This work:					
	A	-155	152	0.61	WHAM-MD
	B	-73	152	0.00	
	C	-157	-74	2.12	
	D	-69	-64	1.71	
	E	62	148	2.75	
	F	61	-75	2.54	
Mohammady, M. K.; Jalkanen, K. J.; Nardi, F.; Wade, R.C.; Suhai, S. *Chem. Phys.* **1999**, *240*, 63-77:					
	I,II	-75	15/-17	0	B3LYP/6-31G*/Onsager solvent model
	III	-158	16	1.48	
	IV	67	16	1.63	
	V	-157	-18	1.66	
		66	-16	1.84	
	A	-157	-158		
	B	-159	160		
Jalkanen, K. J.; Nieminen, R. M.; Mohammady, M. K; Suhai, S. *Int. J. Quantum Chem.* **2003**, *92*, 239-259:					
		-64.77	-163.57	0	
Sychrovský, V.; Buděšínský, M.; Benda, L.; Špirko, V.; Vokáčová, Z.; Šebestík, J.; Bouř, P. *J. Phys. Chem. B* **2008**, *112*, 1796-1805:					NMR study, BPW91/PCM/6-311++G**
	A	-153	147	0	different naming of conformers than this work
	B	66	150	3.6	
	C	-150	-51	5.1	
	D	64	-48	9.2	
Šebek, J.; Kapitán, J.; Šebestík, J.; Baumruk, V.; Bouř, P. *J. Phys. Chem. A* **2009**, *113*, 7760–7768:					
		-155	148	0	
Fletterick, R. J.; Tsai, C.; Hughes, R. E. *J. Phys. Chem.* **1971**, *75*, 918-922:					x-ray (solid state)
		67.1	-14.6	0	

Table S2. Torsion angles (φ and ψ_N, in degrees) and relative conformer populations of three Ala-Ala forms varying in the protonation as obtained by WHAM.

Protonation, conformer	φ	ψ_N	c_{int} [a]	c [a]
Protonated				
A	-154	153	0.13	0.11
B	-71	157	0.40	0.31
C	-155	-57	0.12	0.14
D	-73	-58	0.35	0.43
E	59	158	0.00	0.00
F	57	-62	0.00	0.01
Zwitterionic				
A	-155	152	0.25	0.25
B	-73	152	0.67	0.69
C	-157	-74	0.02	0.02
D	-69	-64	0.06	0.04
E	62	148	0.00	0.00
F	61	-75	0.00	0.01
Deprotonated				
A	-150	113	0.36	0.32
B	-60	94	0.29	0.27
C	-151	-5	0.19	0.22
D	-59	-1	0.16	0.18
E	46	107	0.00	0.01
F	45	2	0.00	0.00

[a] c_{int} were calculated by dividing the (φ, ψ_N) conformation space into 6 parts and integrating the probabilities, whereas c are Boltzmann weights obtained from energy minima.

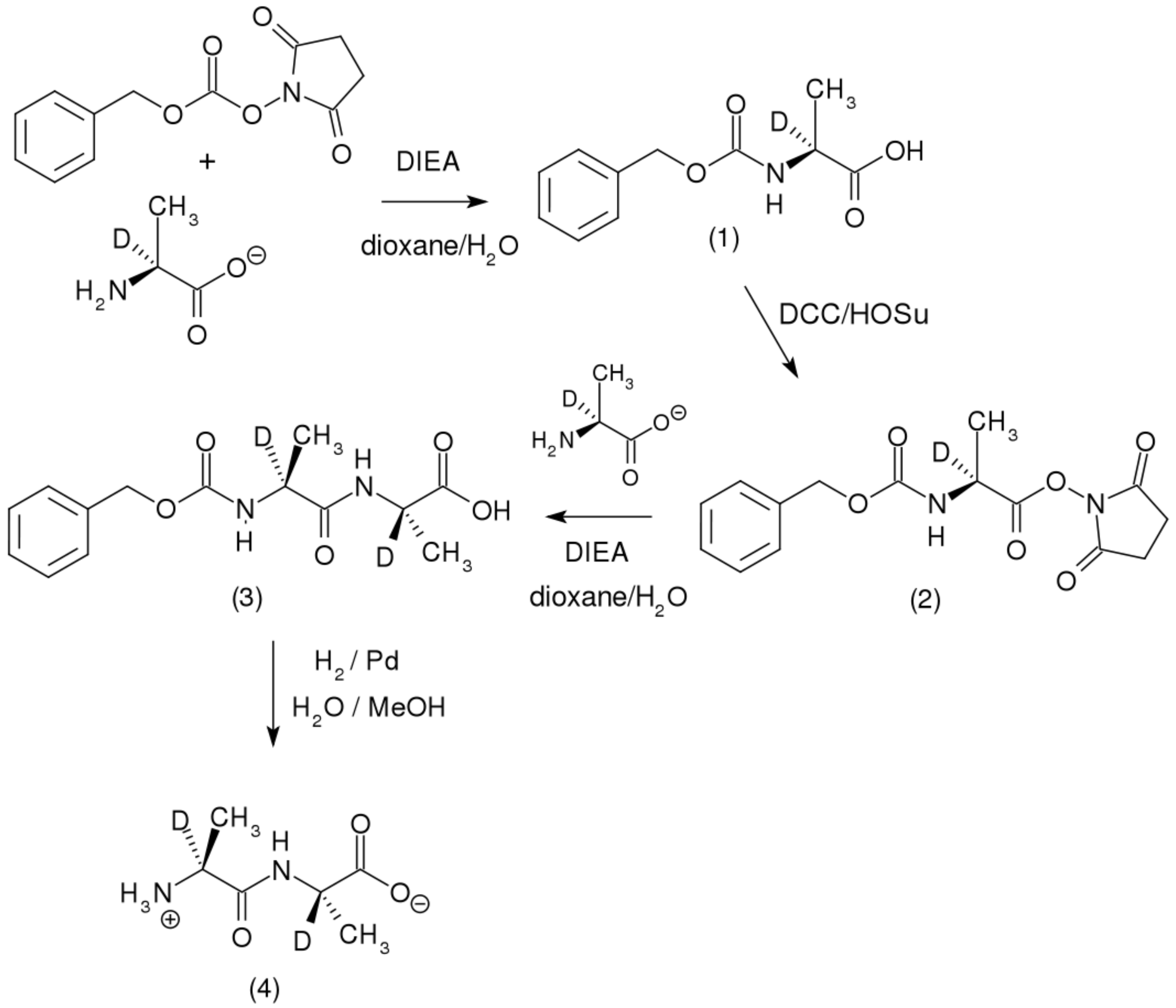
Table S3. Unscaled calculated and experimental positions (cm^{-1}) of most intense Ala-Ala Raman bands and their assignment.

Protonated		Zwitterion		Deprotonated		Major vibrations[a]
Calc.	Exp.	Calc.	Exp.	Calc.	Exp.	
359	365	337	346	327	333	water, deloc
				366	373	
462	457	456	457	469	462	water, CH_3 rot, NH_3 / NH_2 rot
560	576	588	583	541	543	water, deloc
676	680	680	685	695	687	deloc
753	751	787	778	787	778	CO oop (amide)
833	836					deloc, at C-end
882	878	895	884	895	886	deloc, ν(CC), ν(CN)
961	955	964	955	964	953	CH_3 wag, NH_3 / NH_2 wag
1021	1011	1018	1012	1031	1025	deloc
1119	1107	1117	1104	1114	1106	ν(CC), ν(CN), deloc
	1118		1115			
1167	1175	1183	1172			ν(N^{α}C), CH_3 wag, NH_3 wag, δ(C^{α}H)
1293	1274	1302	1278	1294	1279	CH_3 wag, NH_3/ NH_2 wag
1364	1341	1352	1325	1357	1331	δ($^{\alpha}$CH)
1398	1383	1395	1372	1391	1372	δ($^{\alpha}$CH)
1429	1406	1443	1407	1436	1411	δ(CH_3) "umbrella", δ($^{\alpha}$CH)
1482	1462	1483	1461	1481	1461	δ(CH_3), "scissoring"
1582	1577	1600	1573	1568	1547	ν(NC_O) + δ(NH) (= "amide II"), δ(NH_3)
1719	1688	1721	1680	1661	1639	ν(CO), "amide I", δ(NH_3)
1756	1728					ν(CO), COOH

[a] deloc – delocalized mode, oop – out of plane deformation, rot – rotation, wag – wagging motion, ν - stretching, δ - bending

Organic Synthesis and Compound Characterization

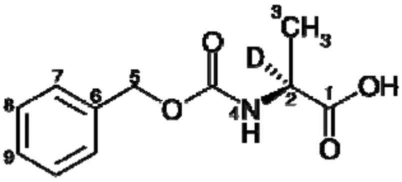
The deuterated peptides were prepared according to procedure developed earlier (announced, but not completely specified in Bouř, P.; Buděšínský, M.; Špirko, V.; Kapitán, J.; Šebestík, J.; Sychrovský, V. *J. Am. Chem. Soc.* **2005**, *127*, 17079-17089), according to Scheme 1:



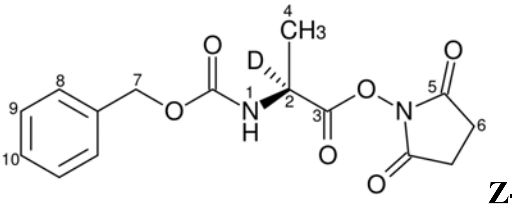
Scheme 1. Synthesis of *d2*-deuterated dipeptide. The *d8*-compound was synthesized accordingly.

Commercially available deuterated amino acids (Sigma–Aldrich) were used without further purification. Thin layer chromatography on silica gel-coated aluminium plates was used to visualize by-products by 254 nm UV light. Alternatively, they were stained by Cl_2/N,N,N',N'-tetramethyl-4,4'-diaminodiphenylmethane. Products were dried in a vacuum drying box at room temperature for 16 h. Molecular weights were determined using the electrospray ionization mass spectroscopy, ^{1}H-NMR and ^{13}C-NMR, spectra were measured on a Bruker Avance II™ 400 MHz spectrometer, analytical HPLC was carried out on the Poroshell 120 SB-C18 2.7 mm, 3.0 x 50 mm column using flow rate 1 mL/min and (method A) gradient 10–10–100% of ACN in 0.05% aqueous TFA within 0-1-10 min or (method B) gradient 1–1–100% of ACN in 0.05% aqueous TFA within 0-5-10 min. Protected amino acids and protected peptides were purified by flash column chromatography on silica gel 60 (Merck) using $CHCl_3$–MeOH–AcOH (9:1:0.1) mobile phase.

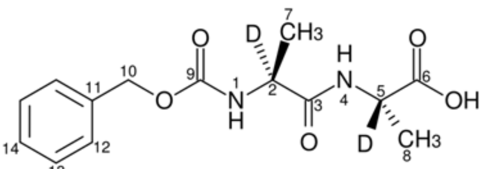
Synthesized Compounds



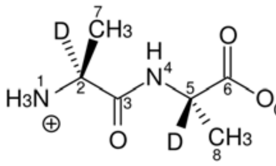
Z-Ala-d_1-OH (**1**) ^{1}H-NMR (400 MHz, DMSO-*d6*) δ 12.52 (s, 1H, COOH), 7.60 (s, 1H, H4), 7.35 (s, 5H, H7-H9), 5.02 (s, 2H, H5), 1.25 (s, 3H, H3). ^{13}C-NMR (100 MHz, DMSO-*d6*) δ 174.43 (C1), 155.85 (O**C**ONH), 137.04 (C6), 128.36 (C_{Ar}), 127.82 (C_{Ar}), 127.77 (C_{Ar}), 65.36 (C5), 48.94 (t, *J* = 21.4 Hz, C2), 16.99 (C3). **ESI HRMS** (m/z): for $[M+Na]^+$ $C_{11}H_{11}D_2O_4N^{23}Na$ calc. 248.08680; found 248.09264 (0.11656 ppm). **TLC Rf** ($CHCl_3$–MeOH–AcOH, 9:1:0.1) 0.46. **HPLC Rt** (method A, 220 nm, 99% purity) 4.1 min.



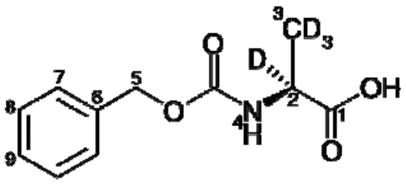
Z-Ala-d_1-OSu (**2**) The NMR spectrum did not contain only one compound but mixture of compound **2** and various impurities. ^{1}H-NMR (400 MHz, DMSO-*d6*) δ 8.09 (s, 1H, H1), 7.47 – 7.26 (m, 5H, H8-H10), 5.06 (d, J = 1.5 Hz, 2H, H7), 2.81 (s, 4H, H6), 1.44 (s, 3H, H4). **ESI HRMS** (m/z): for $[M+Na]^+$ $C_{15}H_{15}DO_6N_2{}^{23}Na$ calc. 344.09633; found 344.09637 (0.11656 ppm). **TLC Rf** ($CHCl_3$–MeOH–AcOH, 9:1:0.1) 0.76. **HPLC Rt** (method A, 220 nm, 89.7% purity) 4.8 min.



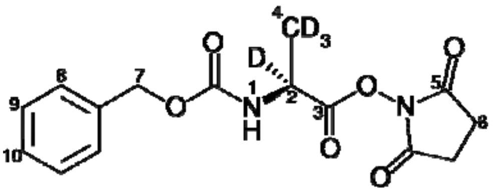
Z-Ala-d_1-Ala-d_1-OH (**3**) ^{1}H-NMR (400 MHz, DMSO-*d6*) δ 8.11 (s, 1H, H4), 7.40 (s, 1H, H1), 7.38 – 7.28 (m, 5H, H12-H13), 5.01 (d, *J* = 2.3 Hz, 2H, H10), 1.26 (s, 3H, H8), 1.19 (s, 3H, H7). ^{13}C-NMR (100 MHz, DMSO-*d6*) δ 174.07 (C6), 172.30 (C3), 155.61 (C9), 137.06 (C11), 128.35 (C_{Ar}), 127.79 (C14), 127.73 (C_{Ar}), 65.33 (C10), 49.40 (C2), 47.14 (C5), 18.07 (C7), 17.09 (C8). **ESI HRMS** (m/z): for $[M+H]^+$ $C_{14}H_{17}D_2O_5N_2$ calc. 297.14140; found 297.14152 (0.39843 ppm). **TLC Rf** ($CHCl_3$–MeOH–AcOH, 9:1:0.1) 0.32. **HPLC Rt** (method A, 220 nm, 88.6% purity) 3.2 min.



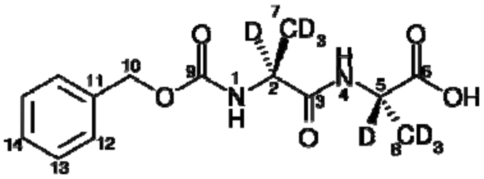
H-Ala-*d*$_1$-Ala-*d*$_1$-OH (**4**) ^{1}H-NMR (400 MHz, D_2O) δ 1.54 (s, 3H, H7), 1.34 (s, 3H, H8). ^{13}C-NMR (100 MHz, D_2O) δ 182.80 (C6), 172.83 (C3), 54.00 (t, *J* = 21.4 Hz, C5), 51.77 (t, *J* = 22.2 Hz, C2), 19.96 (C8), 19.29 (C7). **ESI HRMS** (m/z): for $[M+H]^+$ $C_6H_{11}D_2O_3N_2$ calc. 163.10462; found 163.10464 (0.10496 ppm). **TLC Rf** (CH□CN–25% NH_3 /H_2O, 7:2) 0.27. **HPLC Rt** (method B, 220 nm, 98.4% purity) 0.25 min.



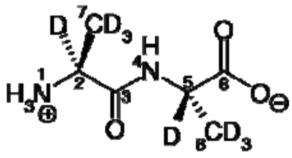
Z-Ala-*d*$_4$-OH (5) ^{1}H-NMR (400 MHz, DMSO-*d6*) δ 12.51 (s, 1H, COOH), 7.59 (s, 1H, H4), 7.35 (m, 5H, H7-H9), 5.02 (s, 2H, H5). ^{13}C-NMR (100 MHz, DMSO-*d6*) δ 174.43 (C1), 155.85 (O**C**NH), 137.03 (C6), 128.35 (C_{Ar}), 127.81 (C_{Ar}), 127.76 (C_{Ar}), 65.35 (C5). **ESI HRMS** (m/z): for $[M+Na]^+$ C11H9D4O4N^{23}Na calc 250.09879; found 250.09880 (0.03965 ppm). **HPLC Rt** (method A, 220 nm, 98.1% purity) 4.1 min.



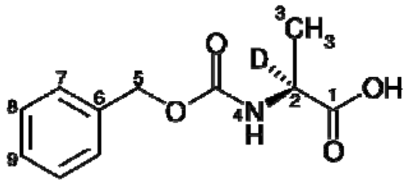
Z-Ala-*d*$_4$-OSu (6) The NMR spectrum did not contain only one compound but mixture of compound **6** and various impurities. ^{1}H-NMR (400 MHz, DMSO-*d6*) δ 8.10 (s, 1H, H1), 7.40 – 7.31 (m, 5H, H8-H10), 5.06 (s, 2H, H7), 2.81 (s, 4H, H6). ^{13}C-NMR (100 MHz, DMSO-*d6*) δ 169.97 (C5), 169.36 (C3), 155.70 (NH**C**O), 136.70 (C_{Ar}), 128.40 (CH_{Ar}), 127.94 (CH_{Ar}), 127.86 (CH_{Ar}), 65.77 (C7). **ESI HRMS** (m/z): for $[M+Na]^+$ C15H12D4O6N2^{23}Na calc 347.11516; found 347.11522 (0.169326 ppm). **HPLC Rt** (method A, 220 nm, 75.2% purity) 5.2 min.



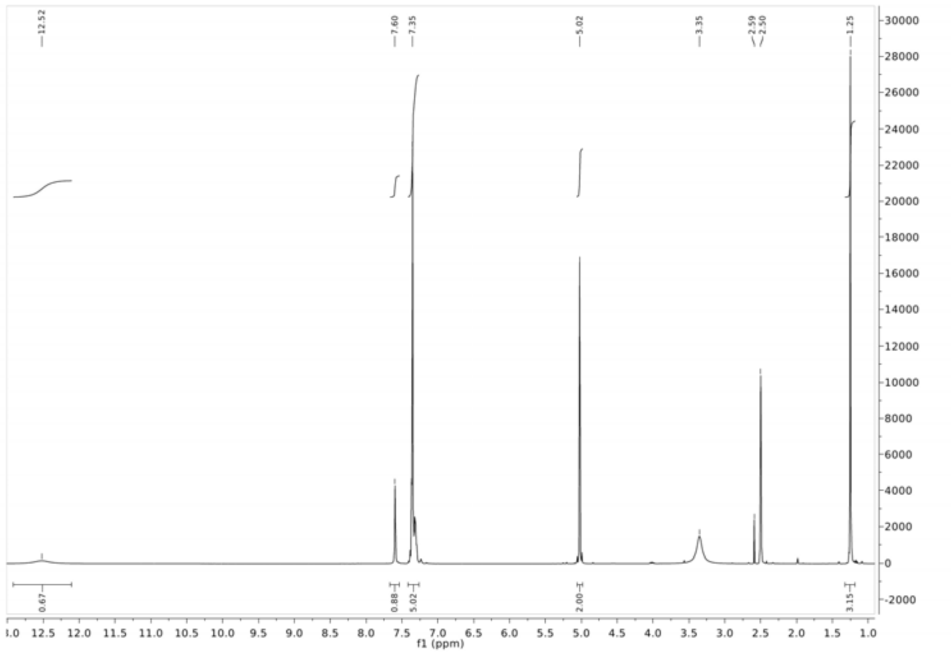
Z-Ala-*d*$_4$-Ala-*d*$_4$-OH (7) ^{1}H-NMR (400 MHz, DMSO-*d6*) δ 8.10 (s, 1H, H1), 7.40 – 7.30 (m, 6H, H12-H13, H4), 5.01 (s, 2H, H10). ^{13}C-NMR (100 MHz, DMSO-*d6*) δ 174.11 (C6), 172.34 (C3), 155.63 (C9), 137.07 (C11), 128.35 (C_{Ar}), 127.79 (C14), 127.73 (C_{Ar}), 65.33 (C10). **ESI HRMS** (m/z): for $[M+H]^+$ C14H10D8O5N2^{23}Na calc 325.16101; found 325.16104 (0.111057 ppm). **HPLC Rt** (method A, 220 nm, 85.8% purity) 3.9 min.

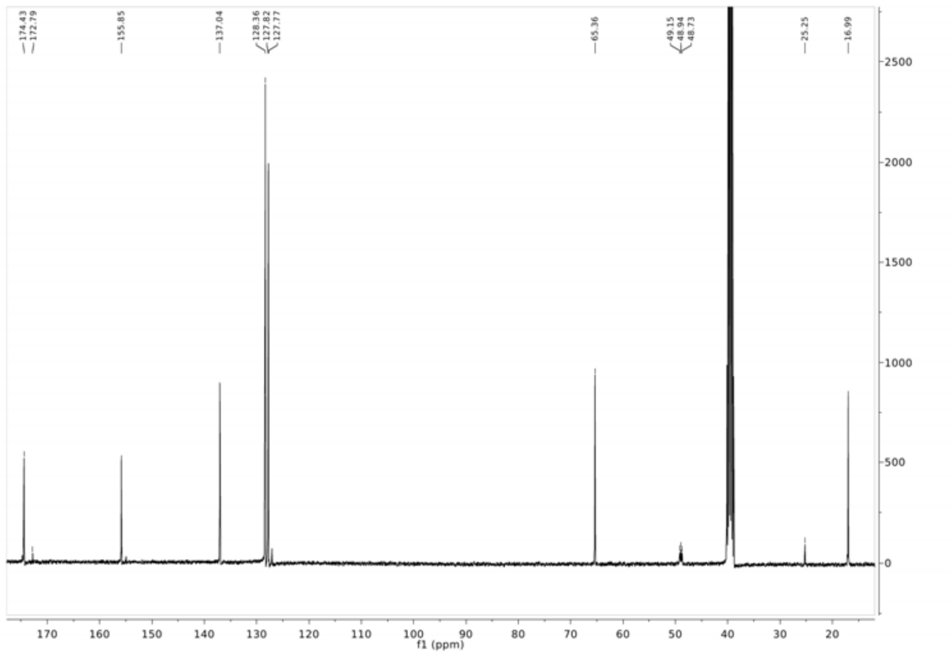
 **H-Ala-d_4-Ala-d_4-OH (8)** ^{1}H-NMR (400 MHz, D_2O) very weak signals. ^{13}C-NMR (100 MHz, D_2O) δ 182.72 (C6), 172.80 (C3), 53.79 (m, C5), 51.46 (m, C2), 18.69 (m, C7, C8). **ESI HRMS** (m/z): for $[M+H]^+$ C6H4D8O3N2^{23}Na calc 191.12423; found 191.12425 (0.12259 ppm); C6H5D8O3N2 calc 169.14228; found 169.14233 (0.26566 ppm). **HPLC Rt** (method B, 220 nm, 97.6% purity) 0.28 min.

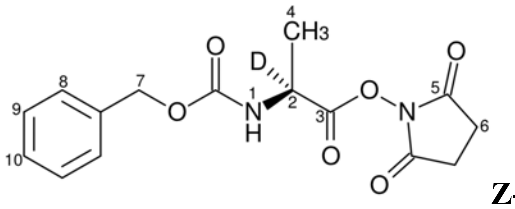
NMR Spectra



Z-Ala-d_1-OH (1)







CH3
D
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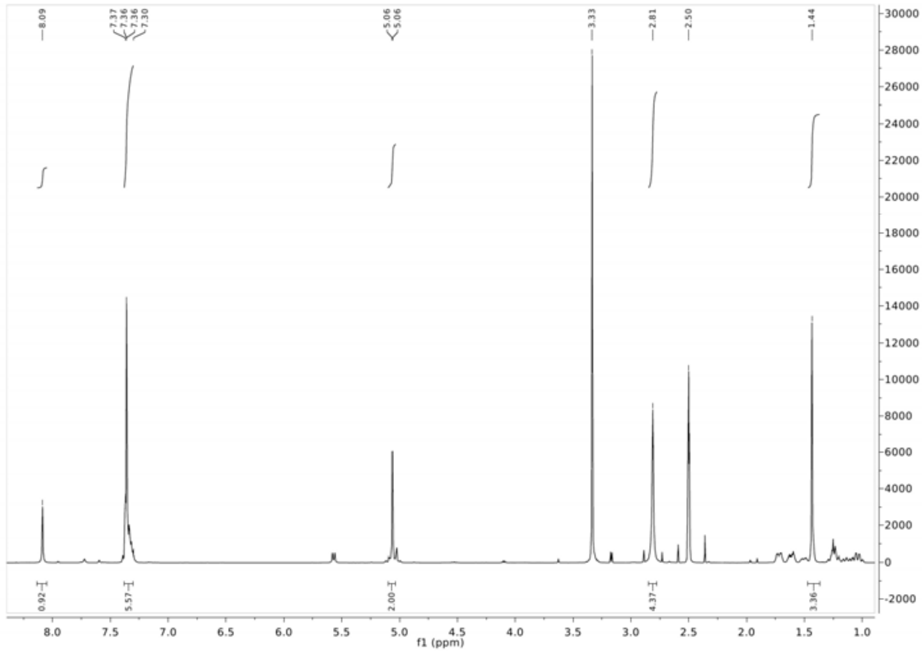
Z-Ala-d_1-OSu (2)



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7.36
7.36
7.30
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5.06
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2.50
1.44
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5.57
2.00
4.37
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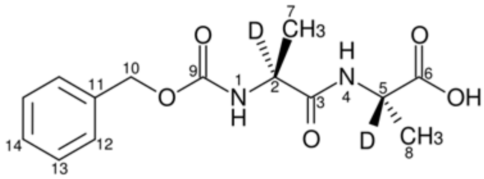
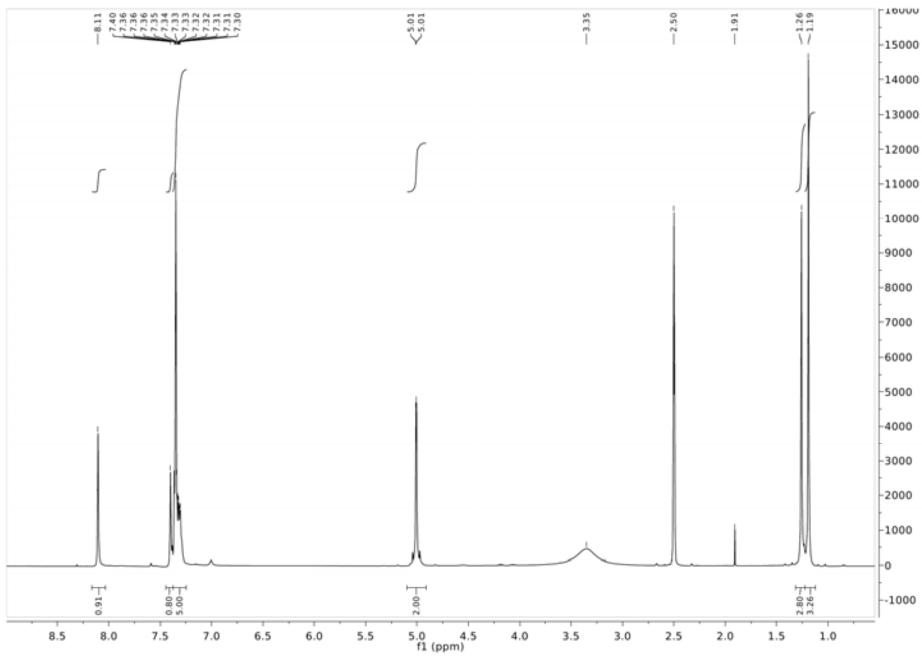
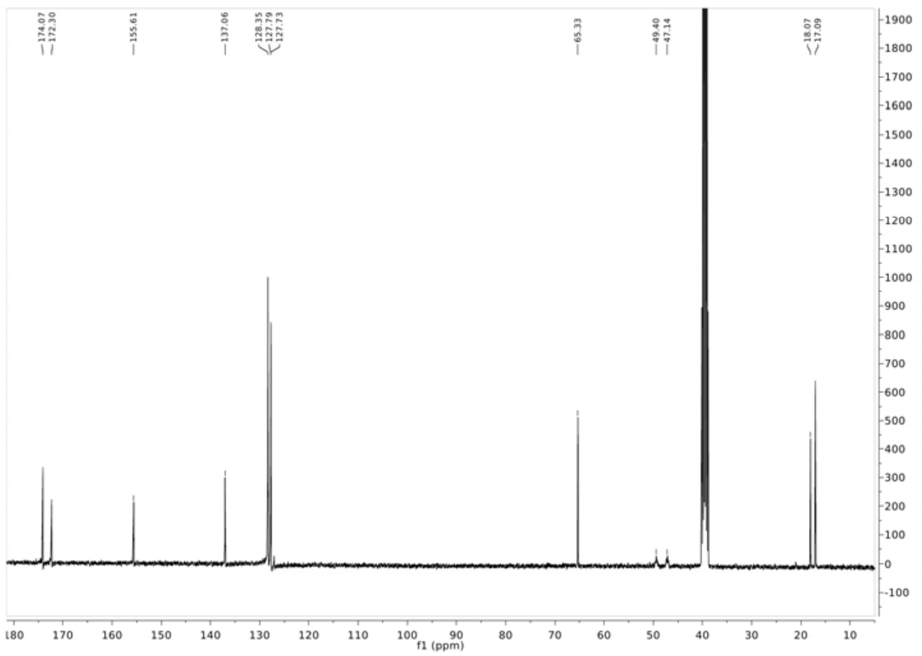
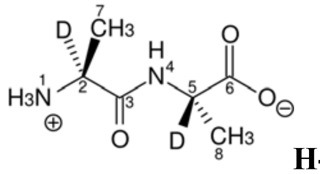
f1 (ppm)



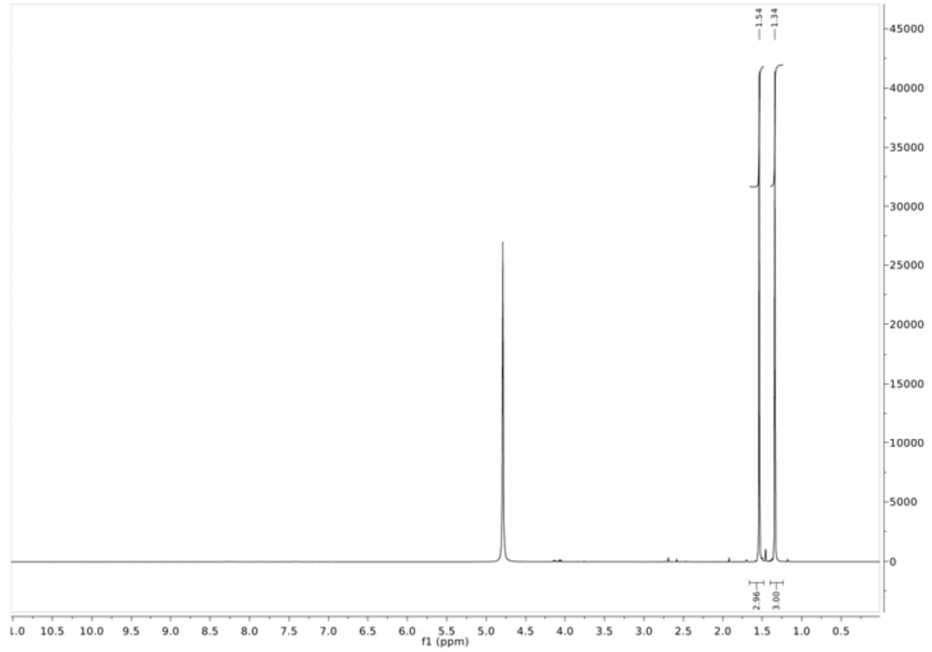
Z-Ala-d_1-Ala-d_1-OH (3)

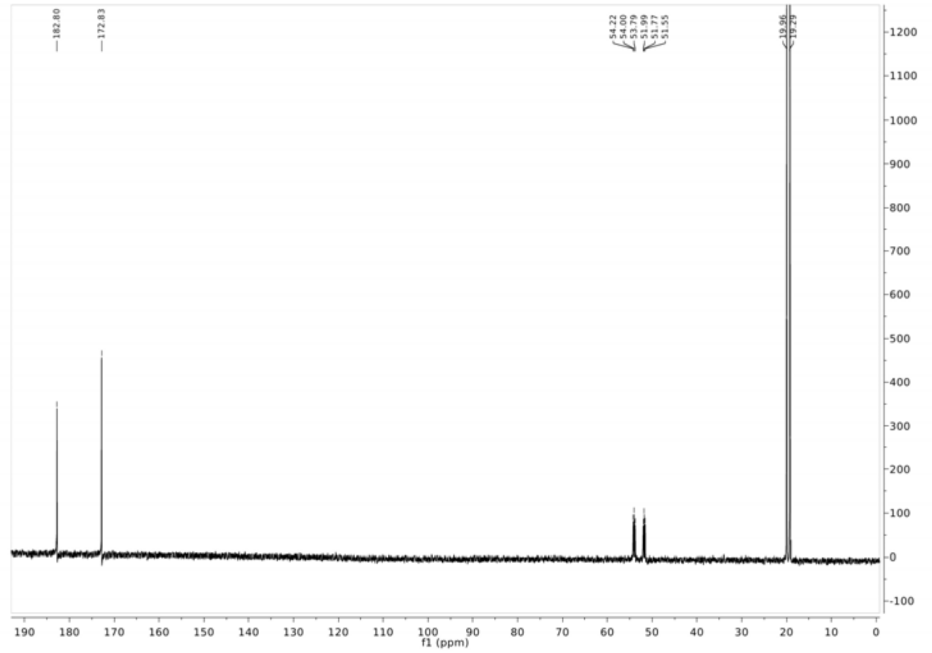


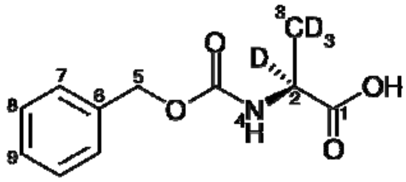




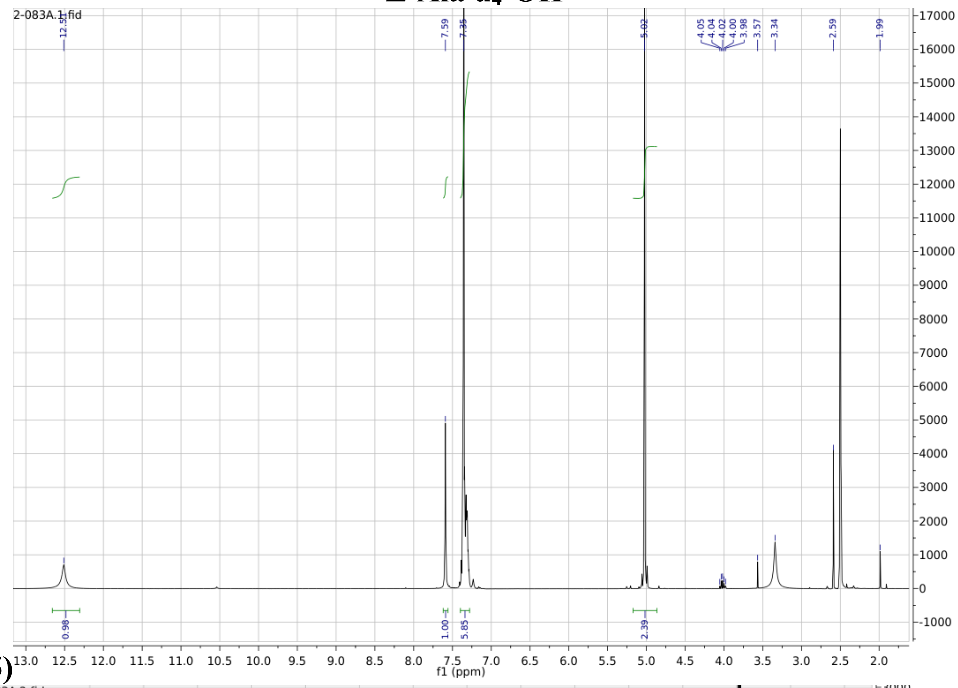
H-Ala-d_1-Ala-d_1-OH (4)



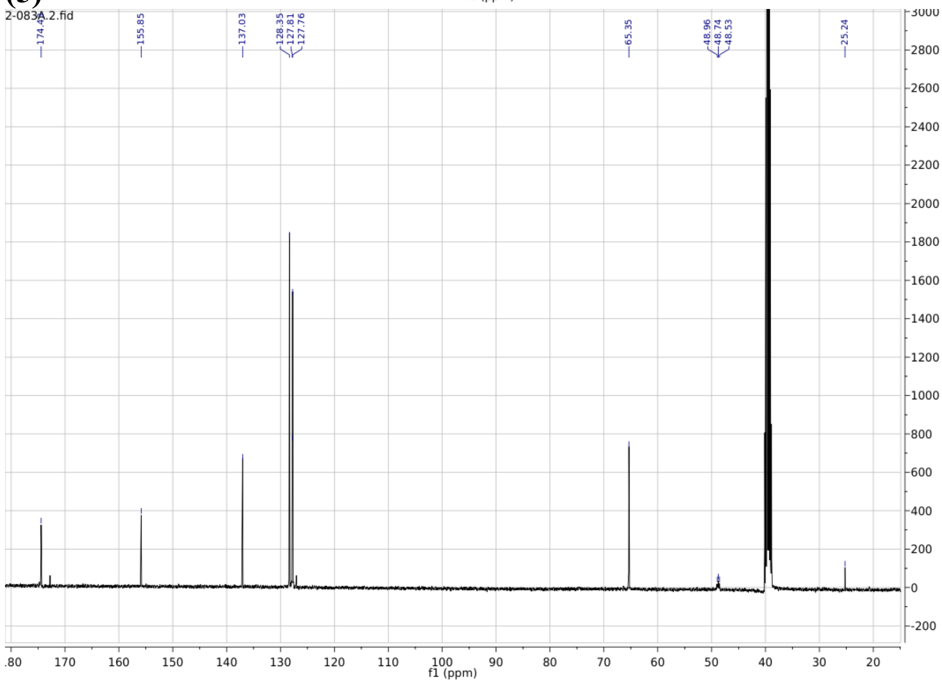


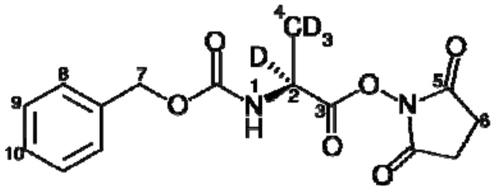

D
D
D
OH
O
O
N
H
D
1
2
3
4
5
6
7
8
9

Z-Ala-d_4-OH

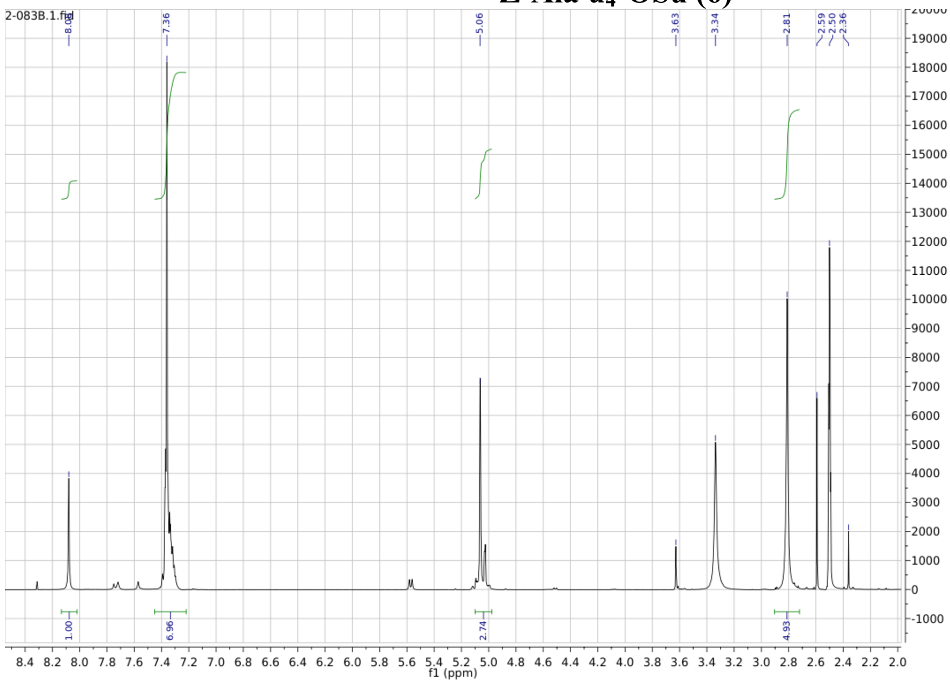

2-083A.1.fid
12.55
7.59
7.35
5.02
4.05
4.04
4.02
4.00
3.98
3.57
3.34
2.59
1.99
0.98
1.00
5.85
2.39
f1 (ppm)

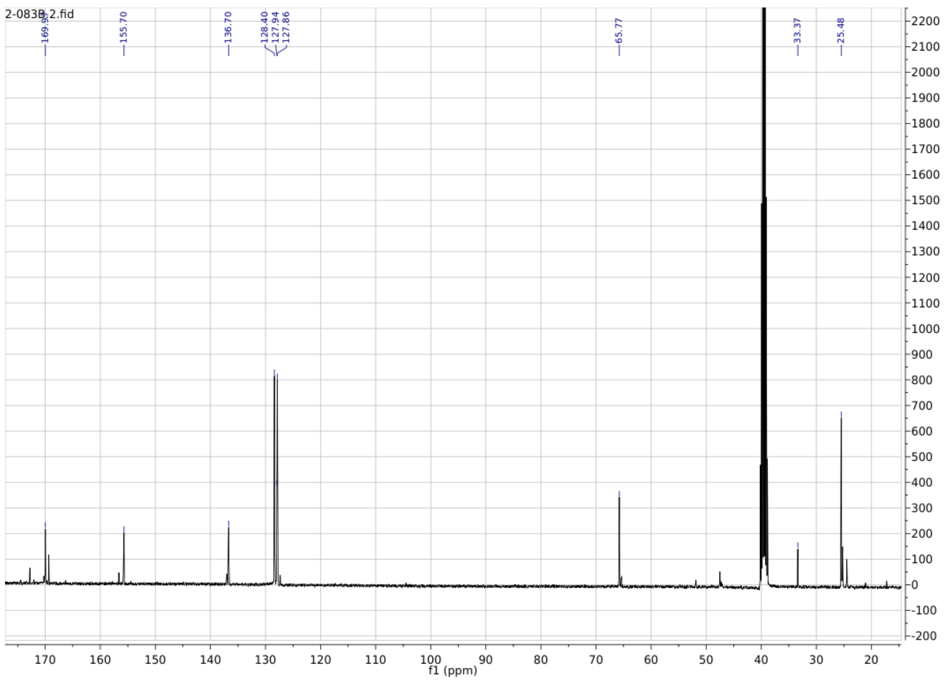
(5)

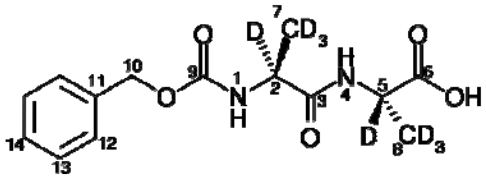

2-083A.2.fid
174.40
155.85
137.03
128.35
127.81
127.76
65.35
48.96
48.74
48.53
25.24
f1 (ppm)



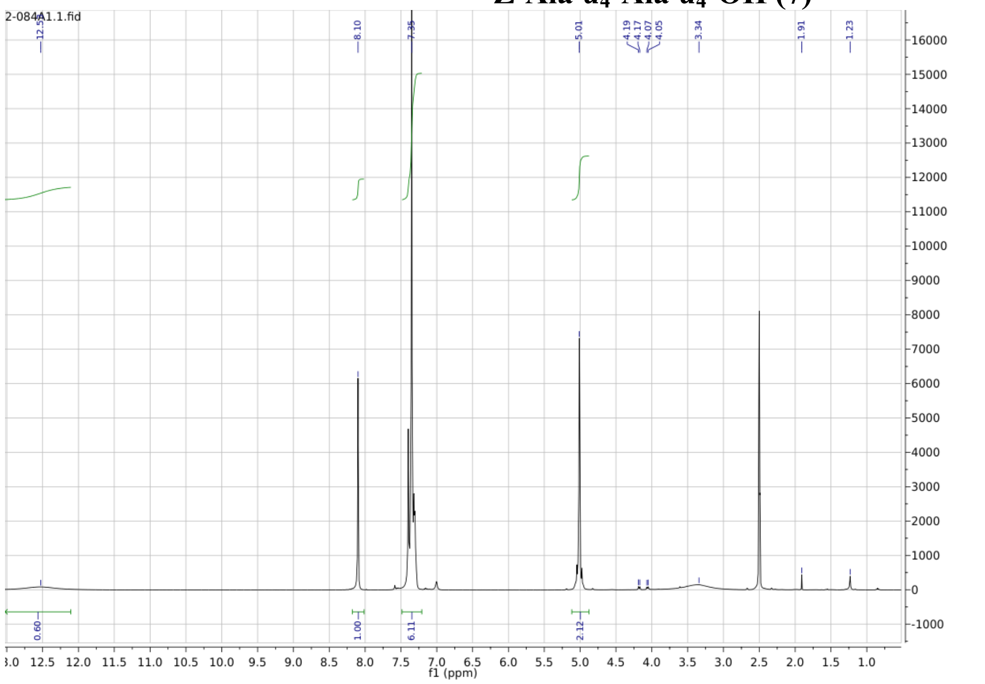
Z-Ala-d_4-OSu (6)

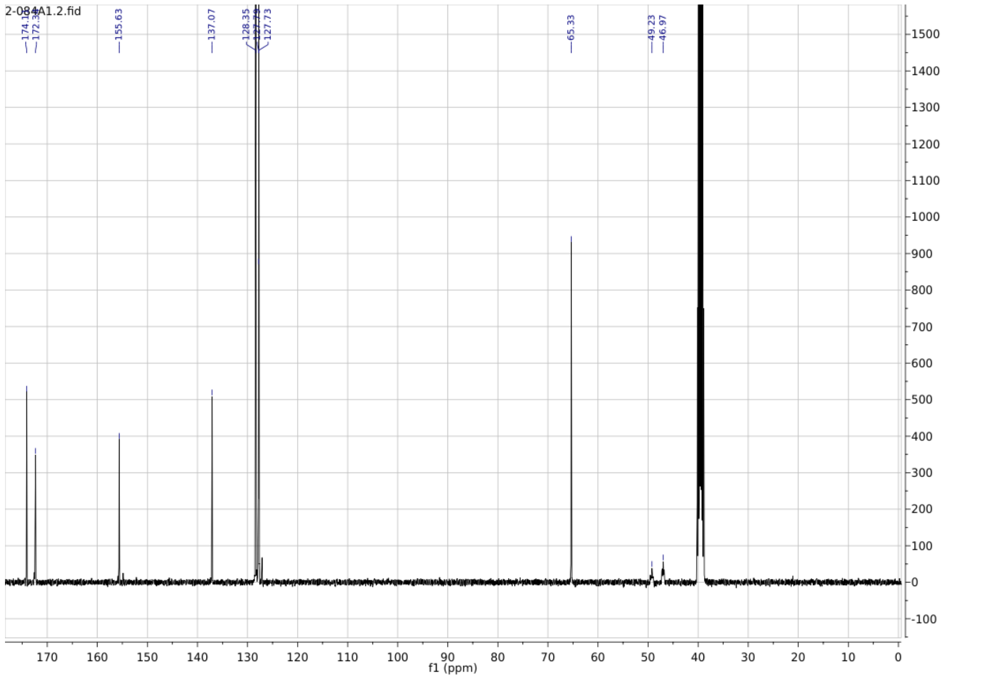


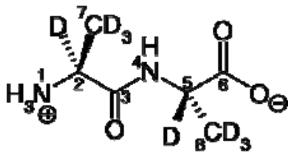




Z-Ala-d_4-Ala-d_4-OH (7)







H-Ala-d_4-Ala-d_4-OH (8)