Electronic Supplementary Information

Application of (*4R*)-aminoproline in peptide engineering: conformational bias and pH-responsiveness revisited

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Supplementary figure

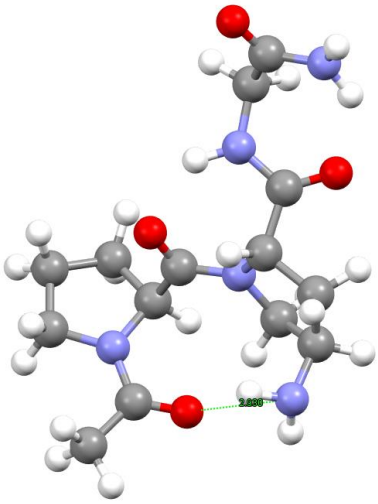


Fig. S1 Molecular modelling of peptide Ac-Pro-Amp-Gly-NH_2 (**3**). The distance between the acetyl oxygen and side-chain nitrogen in Amp is 2.9 Å as highlighted in green.

The modeling was performed using Scigress Modelling Suite (Fujitsu, Poland) using the PM6 algorithm provided in the MOPAC package.

Description of experiments

General experimental information

pH of aqueous solutions was read-out by pre-calibrated standard glass electrodes at 295-297 K. NMR spectra were recorded on a spectrometer machine operating at 500, 471 and 126 MHz frequencies for ^{1}H, ^{19}F and ^{13}C nuclei, respectively. The temperature was set as either 298 or 310 K according to a methanol calibration sample. The spectra assignment was performed using standard 2D experiments: $^{1}H\{^{13}C\}$ HSQC, $^{1}H^{13}C$ HMBC, ^{1}H NOESY (used as EXSY), and/or ^{1}H HOHAHA (60 ms of dipsi2 sequence).

Peptide synthesis

The peptides were prepared using conventional manual Fmoc-based peptide synthesis. Fmoc-Pro-OH, Fmoc-Gly-OH, and Fmoc-(Boc)Amp-OH were collected from commercial sources. For C-terminally free peptides, an amino acid was pre-loaded onto clorotrityl-polystyrene resin to the loading of about 0.45 mmol g^{-1}, terminally amidated peptides were prepared on Rink amide-polystyrene resin with 0.5 mmol g^{-1} loading. *N*-Fmoc amino acids were activated by mixing with 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-b]pyridinium 3-oxide hexafluorophosphate (HATU) and *N,N*-diisopropylethylamine in *N,N*-dimethylformamide (DMF). The coupling was performed in DMF for 1-2 hours. Fioc was removed by treatment with 20 w% piperidine in DMF. N-terminal acetylation was performed by treatment with excess acetic anhydride and *N,N*-diisopropylethylamine in DMF. Peptides were cleaved off by treatment with 30 vol% hexafluoro-2-propanol in dichloromethane for chlorotrityl resin and 95 vol% aqueous trifluoroacetic acid for Rink amide. In the former case, the Boc-group was removed by treatment with 4 M hydrogen chloride in dioxane. All peptides except **10** (see below) were freeze-dried from acetonitrile-water and used for measurements without further purification. The identity and purity of the peptides was attested by ^{1}H NMR analysis. Peptide **10** with the sequence Ac-(Pro-Amp-Gly)$_6$-OH was prepared by standard manual Fmoc(Boc) peptide synthesis scheme. Side chain Boc-group was removed by treatment with trifluoroacetic acid. Crude peptide **10** was purified by a standard semi-preparative reverse phase high performance liquid chromatography using a C18 column, water-acetonitrile gradient elution with 0.1 vol% trifluoroacetic acid as an ion paring agent. Resulting peptide was a trifluoroacetate salt. The purity was examined by analytical HPLC, ^{1}H NMR, and diffusion ordered spectra (see below). Electrospray – time-of-flight spectra: Ac-Pro-Amp-Gly-NH_2 (peptide **3**) $[M+H]^+$ 327, Ac-(Pro-Amp-Gly)$_6$-OH (peptide 10) $[M+2H]^{2+}$ 830, Ac-(Pro-Hyp-Gly)$_6$-OH $[M+H]^+$ 1664, $[M+2H]^{2+}$ 832.

Acidity measurements

A peptide (20 mg) and potassium monohydrogen phosphate (10-15 mg) were dissolved in deionized water (15 ml). The solution was titrated by a concentrated potassium hydroxide solution to 10-15 distinct pH values, while 500 µl aliquots were taken to NMR tubes containing 50 µl deuterium oxide each. ^{1}H NMR spectra were measured using W5 water suppression scheme at 700 or 500 MHz frequencies and 298 K. Additional ^{1}H{^{13}C} HSQC spectra were measured to clarify the resonance assignments. Resonances undergoing transitions were analysed according to Henderson–Hasselbalch equation. The values originating from analysis of distinct resonances were averaged to deliver the final pK_a value and standard deviation. See copies of the spectra in a section below.

Table S1 Acid-base transitions in peptides.

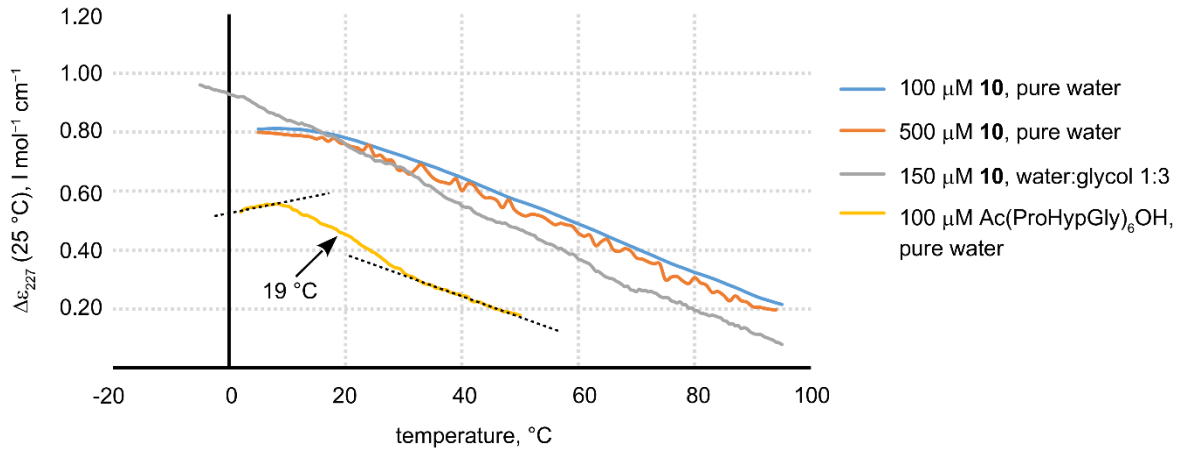
Structure	pK_a1	pK_a2	Intercharge distance, Å
Ac-Amp-Pro-Pro-Pro-Pro-Pro-OH	3.66±0.04	7.74±0.06	19.4
Ac-Pro-Amp-Pro-Pro-Pro-Pro-OH	3.57±0.08	7.80±0.04	15.9
Ac-Pro-Pro-Amp-Pro-Pro-Pro-OH	3.60±0.03	7.79±0.06	12.9
Ac-Pro-Pro-Pro-Amp-Pro-Pro-OH	3.49±0.04	7.84±0.05	10.3
Ac-Pro-Pro-Pro-Pro-Amp-Pro-OH	3.40±0.03	7.93±0.04	7.4
Ac-Pro-Pro-Pro-Pro-Pro-Amp-OH	2.91±0.04	8.11±0.03	4.7
Ac-Amp-Pro-Pro-Pro-Pro-Amp-NH_2 (**1**)	-	7.68±0.06	16.5
Ac-Pro-Pro-Amp-Amp-Pro-Pro-NH_2 (**2**)	-	7.49±0.07	7.5
Ac-Pro-Amp-Gly-NH_2 (**3**)	-	7.52±0.10	-

Circular dichroism for peptides 10 and 3

Circular dichroism measurements were performed on a standard laboratory spectropolarimeter operating with a xenon lamp under a nitrogen gas flow. The spectra were acquired between 280-180 nm using the following parameters: band width 1 nm, response 2 s, data pitch 0.2 nm, scan speed 20 nm min^{-1}, 2 accumulations. The samples were thermostated using a Peltier type temperature controller. Melting curves were measured at 227 nm in a 5-95 °C temperature scan with 1 °C min^{-1} speed for peptide **10** and 1 °C min^{-1} for **3**. Additional CD spectra were recorded at 5 (before scan) and 95 °C (after the scan) temperatures.

The peptide concentration was 100 µM for **10** and 600 µM for **3** (1.8 mM amide), the spectra were measured in 1 nm path length quartz cells. The buffers were 50 mM with the following compositions: glycine-HCl (pH 3), MES-HCl (pH 6), potassium phosphate (pH 7), Tris-HCl (pH 8), potassium borate (pH 9) and potassium phosphate (pH 11). Buffers were prepared at 150 mM, and added during preparation of the sample along with the peptide from a stock solution. The spectra were converted to circular dichroism units as mean residue values, thus using the amide bond concentration rather than peptide.

Control measurements were performed with 100 µM **10** in pure water and compared with: 1) 500 µM **10** in pure water to exclude the influence of concentration; 2) 150 µM **10** in water:ethylene glycol 1:3 to promote formation of the triple helix under hydrogen bond inducing conditions. Results showed consistently the absence of a notable melting transition. Melting curves were also recorded for 100 µM of peptide Ac-(Pro-Hyp-Gly)-OH in water. The curve shows a weak melting transition with a midpoint about 19 °C.



Dipeptide models

Methyl esters of *N*-acetylamino acids and difluoroethyl esters of *N*-Fmoc amino acids were prepared as previously described.[S1] Lipophilicity values were previously reported.[S2] Distribution coefficient for Amp derivatives were measured in octan-1-ol partitioning against 150 mM buffers at pH 6 (2-(*N*-morpholino)ethanesulfonic acid buffer, MES-HCl), 7 (potassium phosphate buffer), 8 (tris(hydroxymethyl)aminomethane buffer, Tris-HCl). Solutions of model compounds **4**-**8** were prepared by dissolving 5 mg of the substance in 550 µl deuterium oxide. ^{1}H NMR spectra were measured at 298 and 310 K for reading out the multiplicity of the α-CH resonances and equilibrium *trans/cis* amide ratios. Rotation velocities were measured in two-dimensional ^{1}H EXSY spectra at 310 K using mixing times 2 and 3 s. The rotation rate values were calculated as described.[S2]

Table S2 Experimental (log*D*) and extrapolated (log*D**) lipophilicity for Amp and lysine derivatives.

Xaa		logD_{pH} for Fmoc-Xaa-OCH_2CHF_2	logD_{pH}* for Ac-Xaa-OCH_3
Amp	pH 6	+1.35±0.09	[−3.46±0.27]
	pH 7	+2.16±0.06	[−2.65±0.24]
	pH 8	+2.57±0.10	[−2.24±0.28]
Lys	pH 6	+1.00±0.08	[−3.81±0.26]
	pH 7	+1.31±0.04	[−3.50±0.22]
	pH 8	+1.60±0.03	[−3.21±0.21]

Table S3 Summarized experimental properties of model compounds **4**-**9**.

compound	logP/D_{pH}	$K_{trans/cis}$ at 298 K	$k_{trans\text{-to-}cis}$ at 310 K, s^{-1}	$k_{cis\text{-to-}trans}$ at 310 K, s^{-1}
4	−0.66±0.03	7.03±0.20	0.013±0.001	0.090±0.008
5	+0.95±0.03	6.61±0.10	0.009±0.001	0.050±0.008
6	−1.27±0.03	6.43±0.18	0.006±0.001	0.038±0.003
7	+0.66±0.04	5.51±0.19	0.010±0.001	0.053±0.005
8	−1.23±0.08	5.54±0.10	0.009±0.001	0.051±0.009
9, pH 6	[−3.46±0.27]	3.56±0.10	0.026±0.002	0.092±0.014
9, pH 7	[−2.65±0.24]	3.82±0.15	0.021±0.002	0.079±0.008
9, pH 8	[−2.24±0.28]	4.98±0.31	0.013±0.003	0.056±0.013
9, pH 9	-	5.51±0.19	0.008±0.001	0.045±0.012

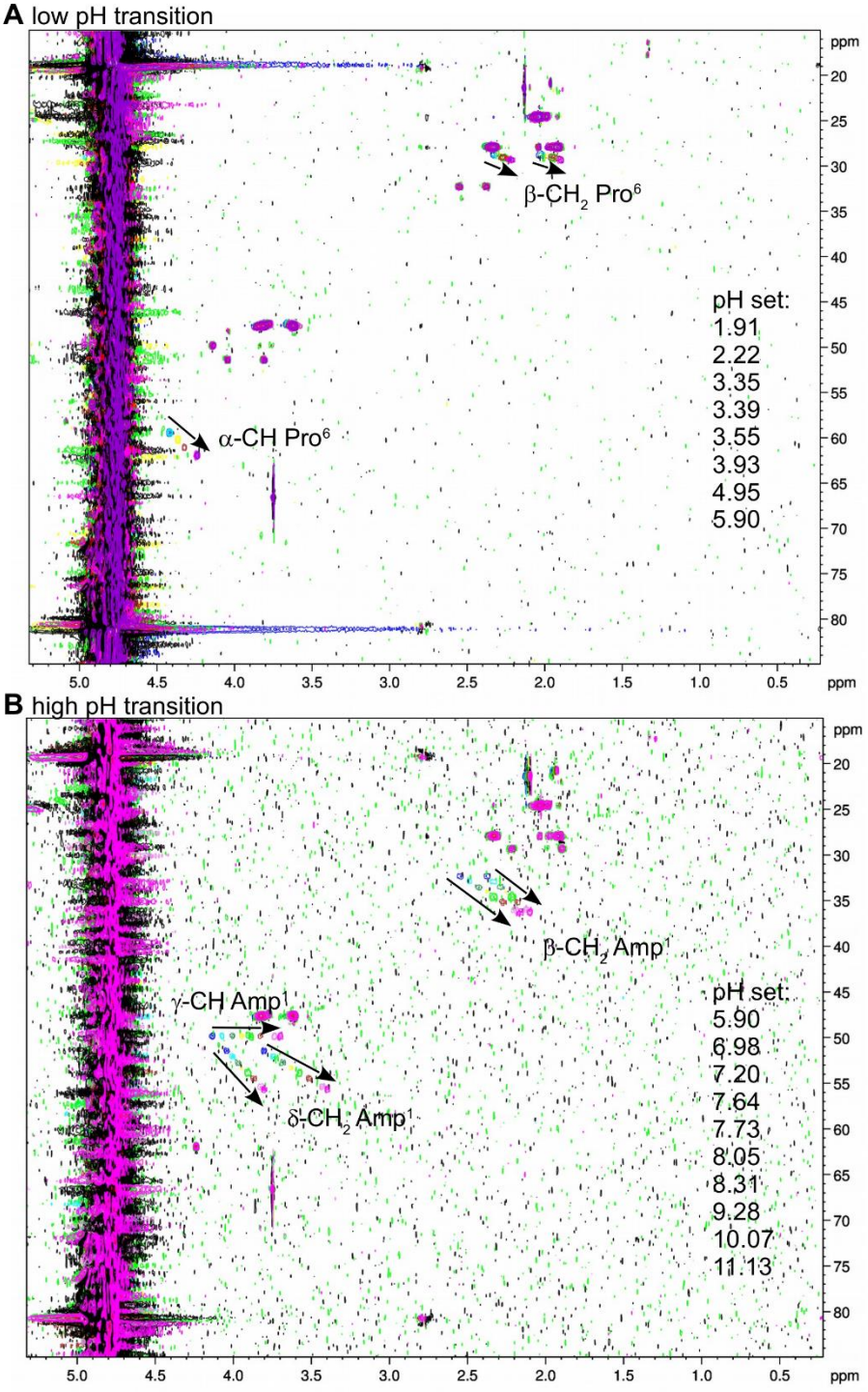
Table S4 Comparison of the *trans/cis* equilibrium ratios experimentally determined here (by ^{1}H NMR at 298 K), and previously reported by another group.[S3]

compound	in deuterium oxide		in deuterochloroform	
	this work	from reference[S3]	this work	from reference[S3]
4	7.03±0.20	-	4.23±0.08	-
5	6.61±0.10	-	4.22±0.05	-
6	6.43±0.18	6.1	4.10±0.08	4.2
7	5.51±0.19	5.2	3.98±0.13	3.5
8	5.54±0.10	5.8	4.13±0.20	5.2

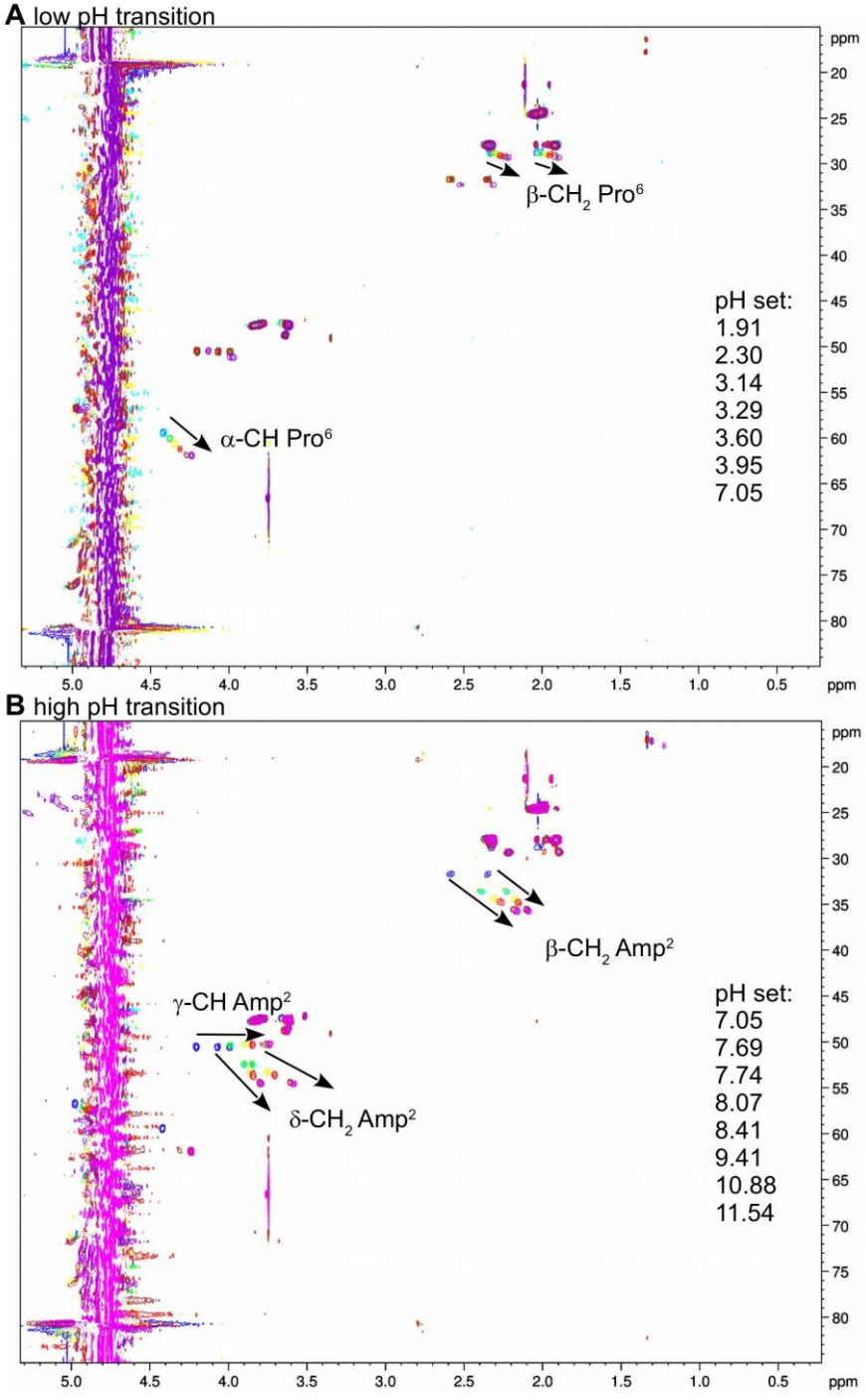
Spectral information

Ammonium transition

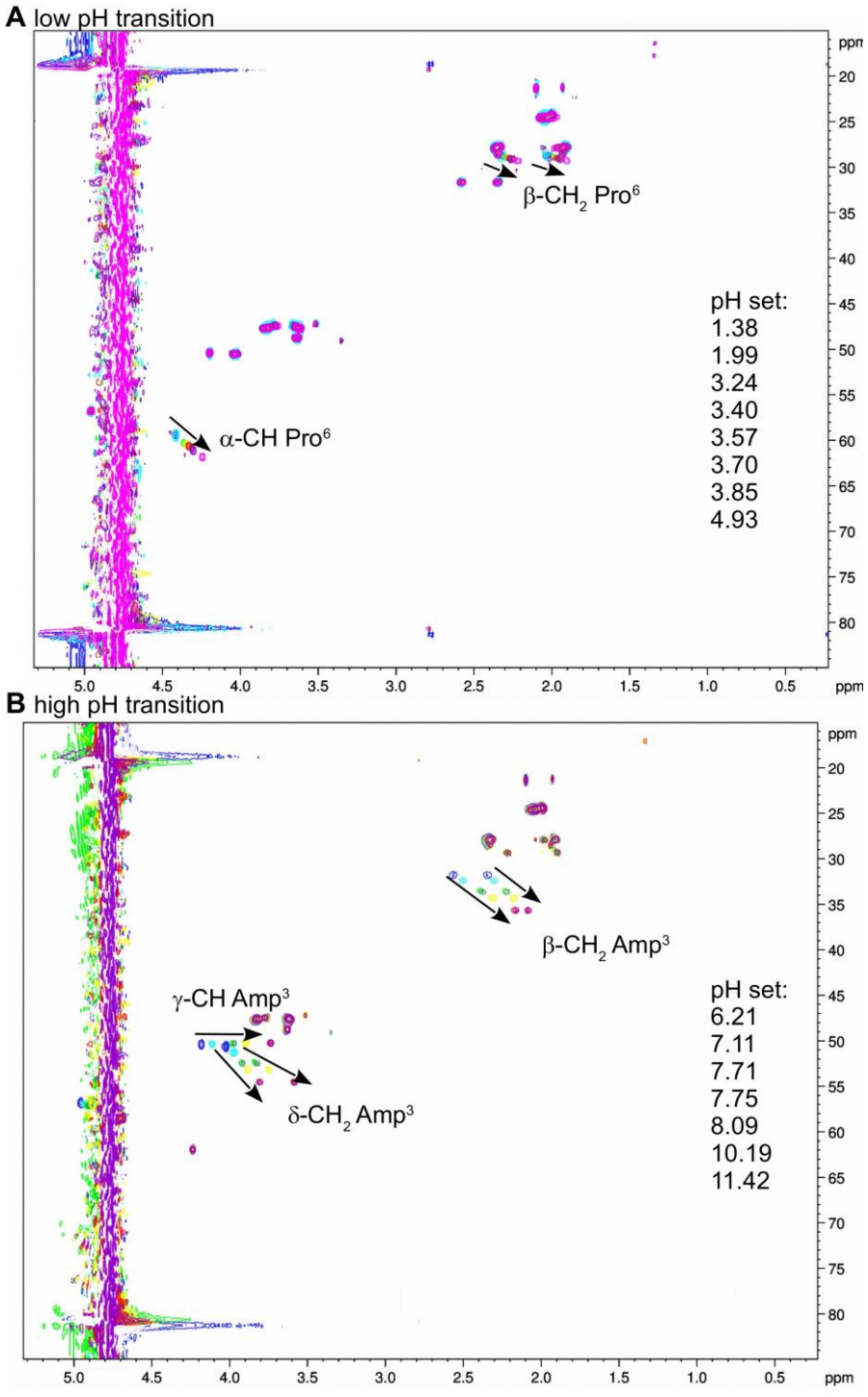
Ac-Amp[1]-Pro-Pro-Pro-Pro-Pro-OH transitions in $^{1}H\{^{13}C\}$ HSQC spectra:



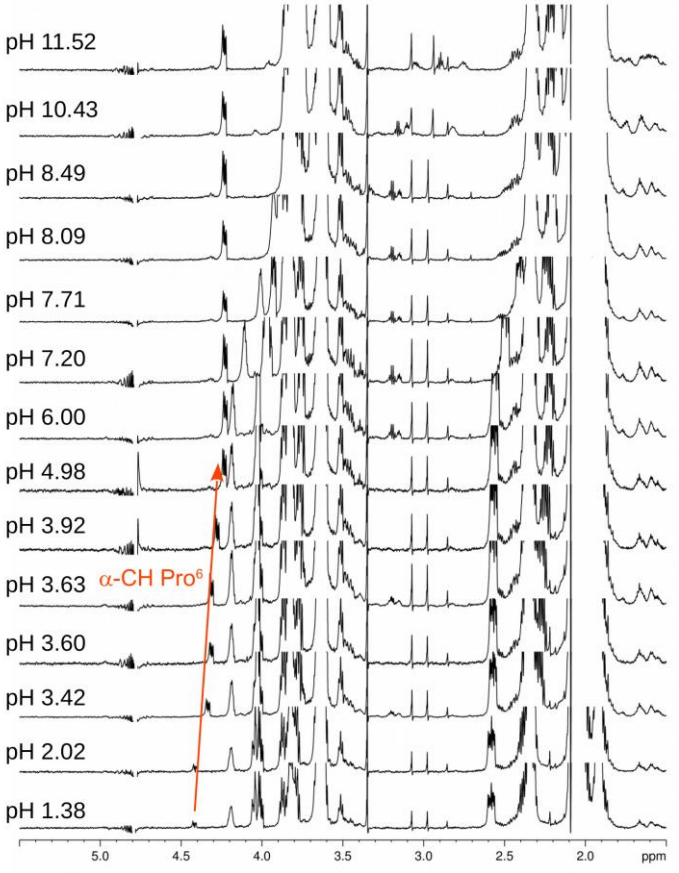
Ac-Pro-Amp^{2}-Pro-Pro-Pro-Pro-OH transitions in $^{1}H\{^{13}C\}$ HSQC spectra:



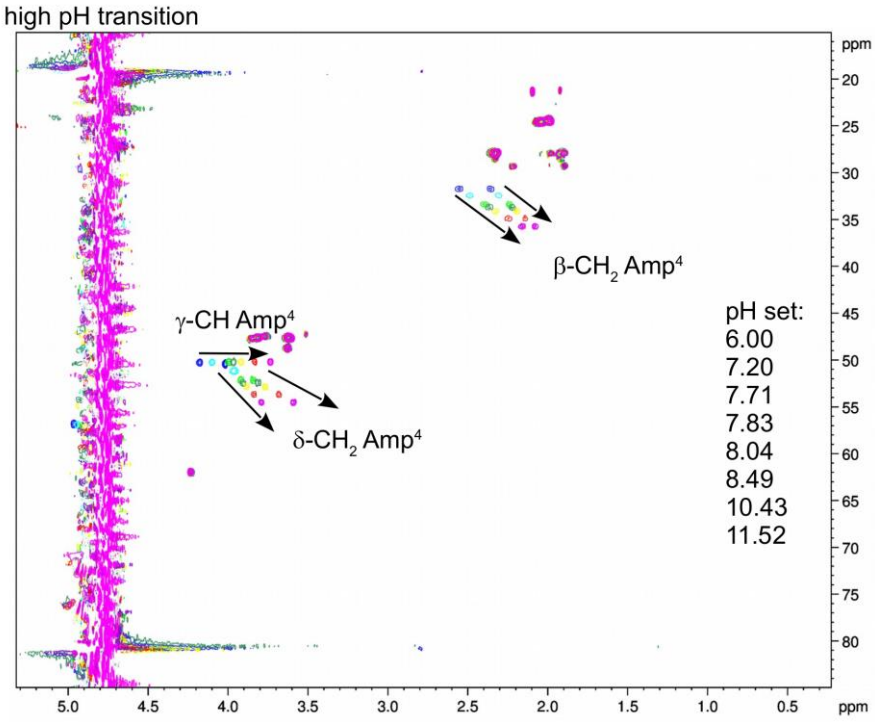
Ac-Pro-Pro-Amp^{3}-Pro-Pro-Pro-OH transitions in $^{1}H\{^{13}C\}$ HSQC spectra:



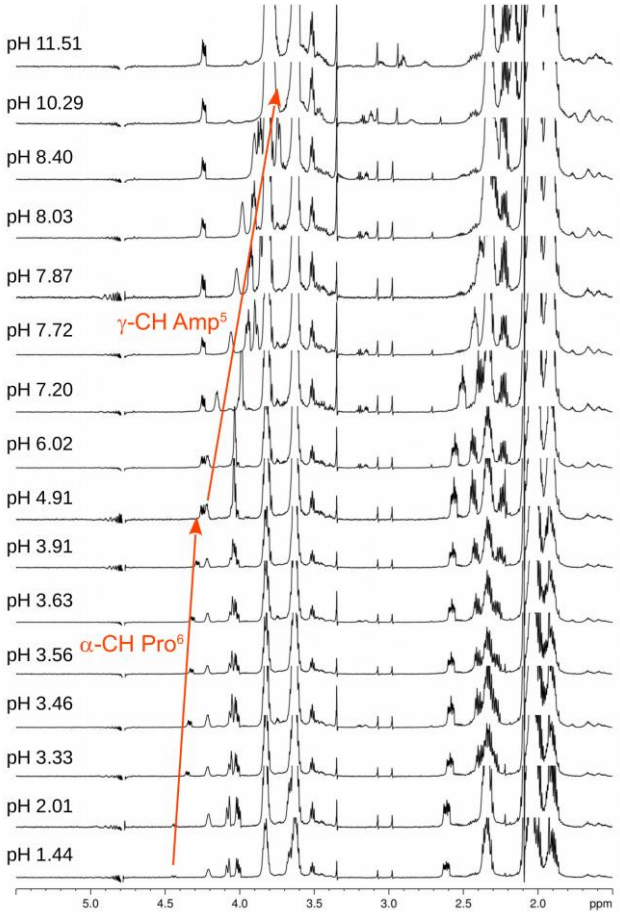
Ac-Pro-Pro-Pro-Amp^4-Pro-Pro-OH transitions in ^{1}H W5 spectra:



in ^{1}H{^{13}C} HSQC spectra:

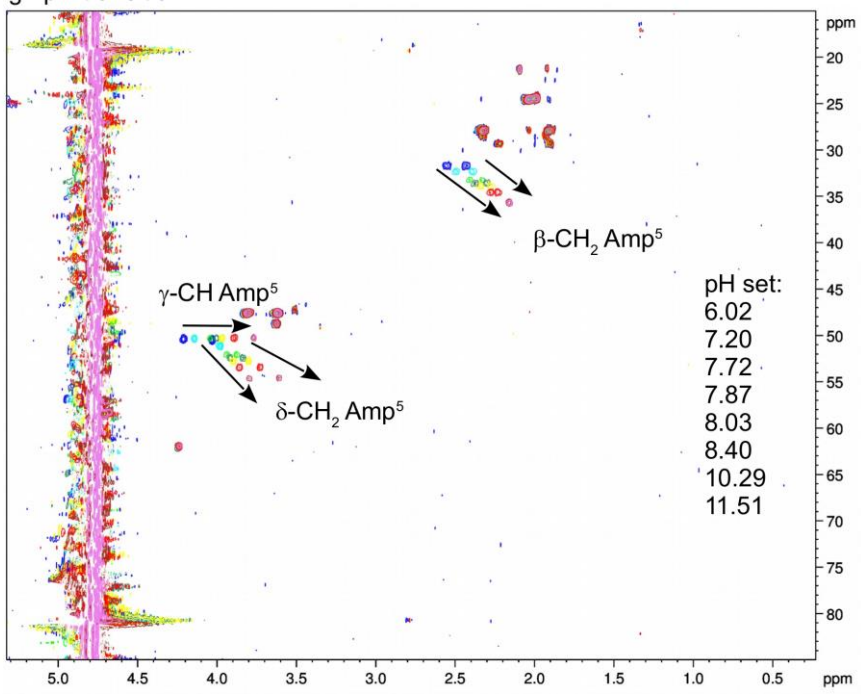


Ac-Pro-Pro-Pro-Pro-Amp^{5}-Pro-OH transitions in ^{1}H W5 spectra:

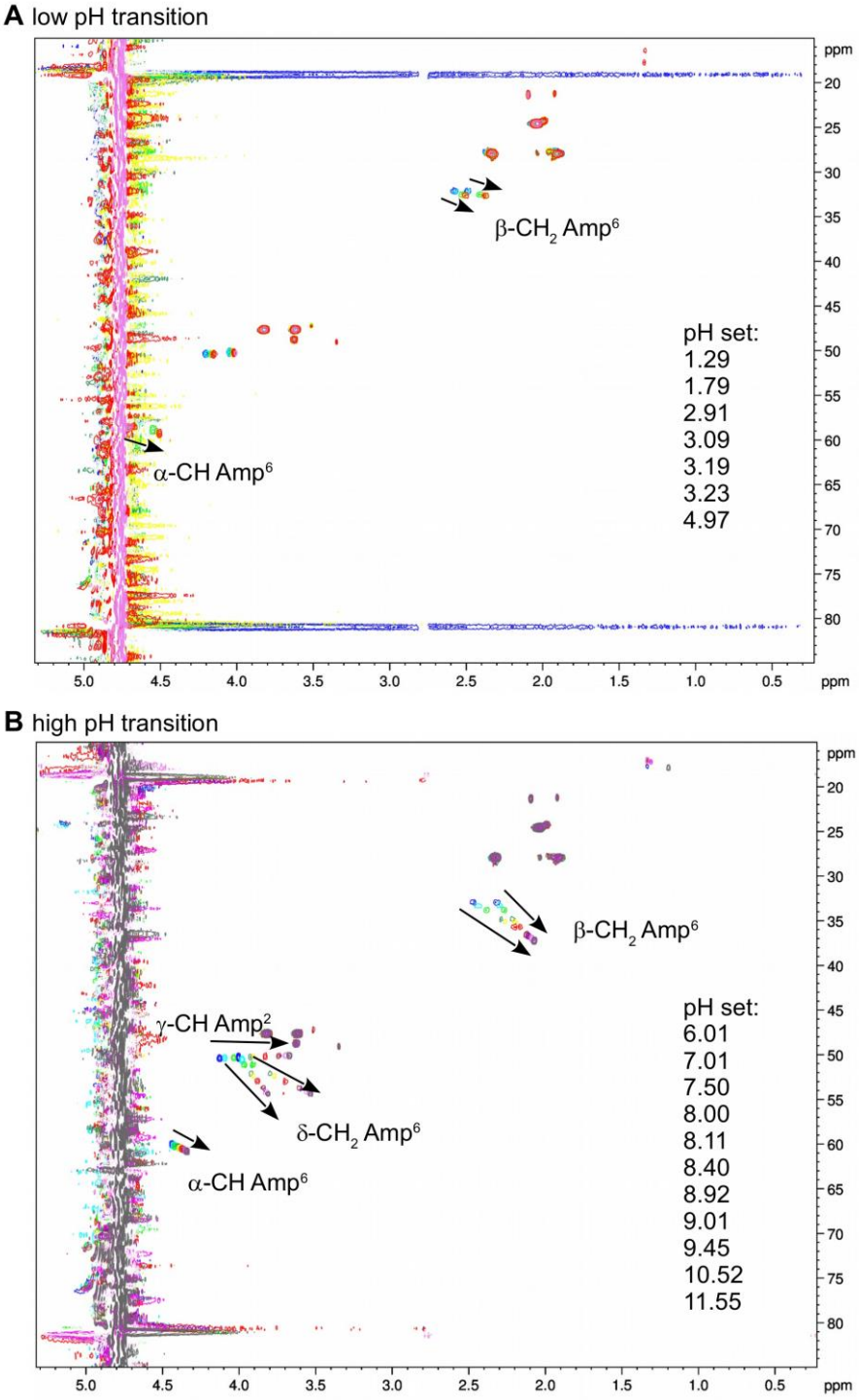


in ^{1}H{^{13}C} HSQC spectra:

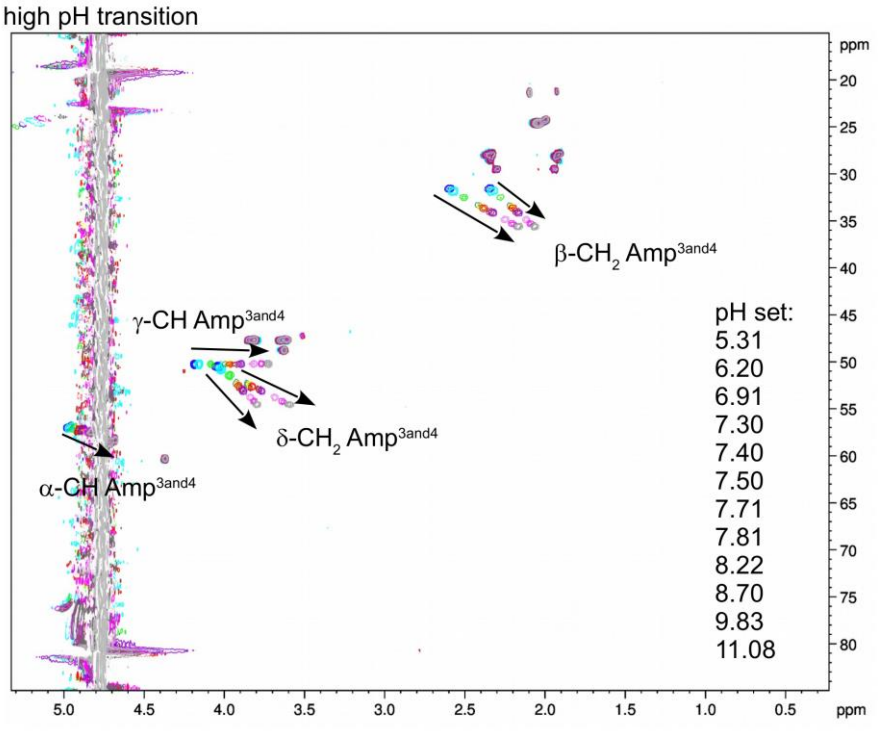
high pH transition



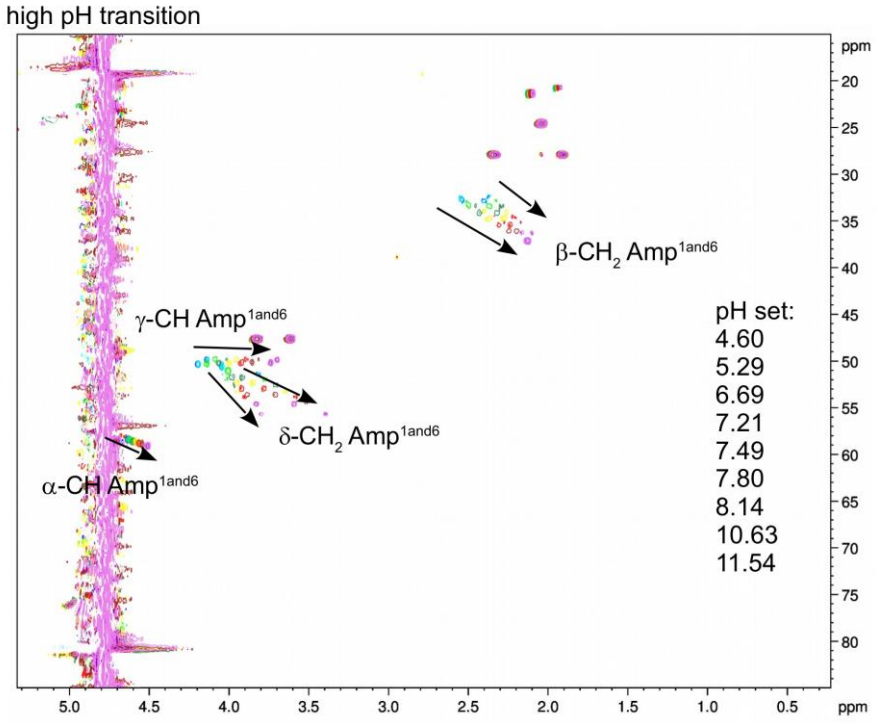
Ac-Pro-Pro-Pro-Pro-Pro-Amp^{6}-OH transitions in $^{1}H\{^{13}C\}$ HSQC spectra:



Ac-Pro-Pro-Amp^{3}-Amp^{4}-Pro-Pro-NH$_2$ (**1**) transitions in ^{1}H{^{13}C} HSQC spectra:

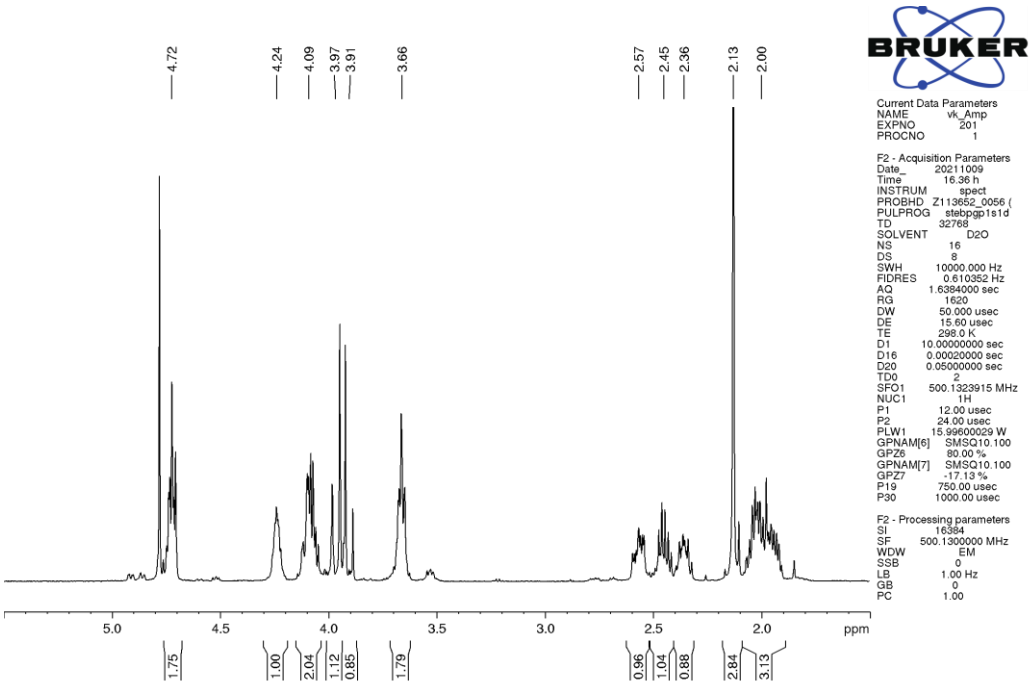


Ac-Amp^{1}-Pro-Pro-Pro-Pro-Amp^{6}-NH$_2$ (**2**) transitions in ^{1}H{^{13}C} HSQC spectra:

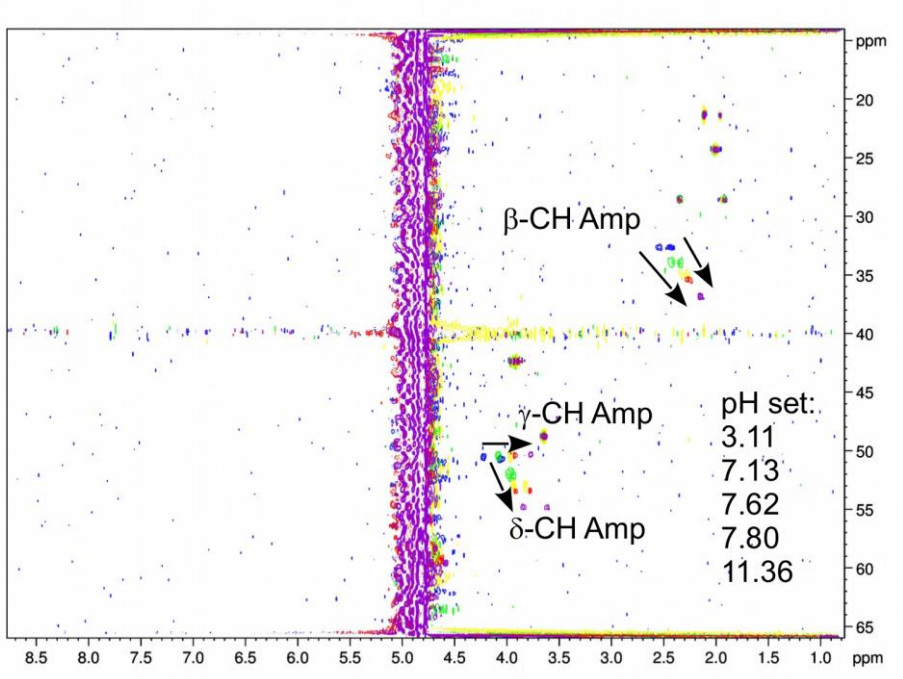


Ac-Pro-Amp-Gly-OH × CF_3CO_2H (**3**):

^{1}H NMR (500 MHz, D_2O), main rotamer only: 4.72 (m, 2H, Amp α-CH and Pro α-CH), 4.25 (m, 1H, Amp γ-CH), 4.09 (m, 2H, Amp δ-CH_2), 3.96 (d, *J* = 17.4 Hz, Gly), 3.90 (d, *J* = 17.2 Hz, Gly), 3.67 (m, 2H, Pro δ-CH_2), 2.57 (m, 1H, Amp β-CH), 2.46 (m, 1H, Amp β-CH), 2.36 (m, 1H, Pro β-CH), 2.13 (s, 3H, CH_3CO), 2.07-1.90 (m, 3H, Pro β-CH and Pro γ-CH_2).



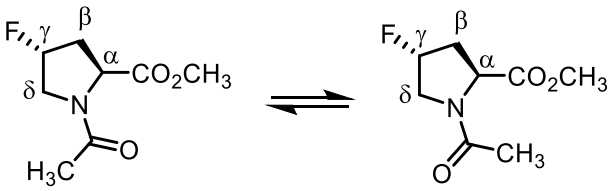
The ammonium transition was followed by a series of ^{1}H spectra with W5 water suppression scheme and ^{1}H{^{13}C} HSQC at certain pH points as shown below:



NMR data for compounds 4-9

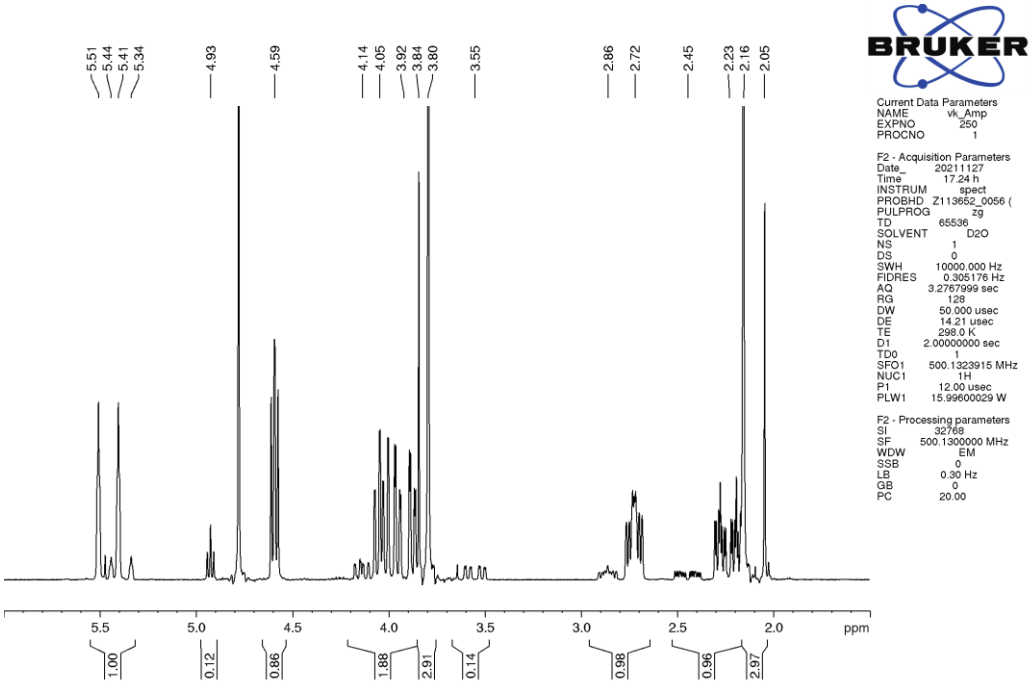
AcFlpOCH$_3$, **4**:

4

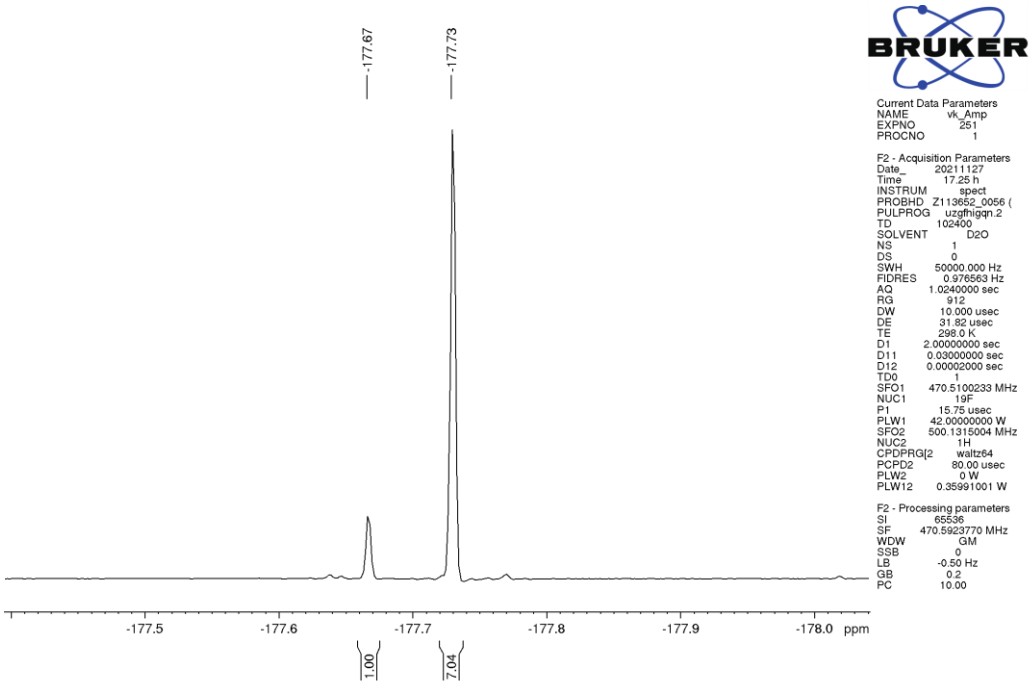


trans-amide *cis*-amide

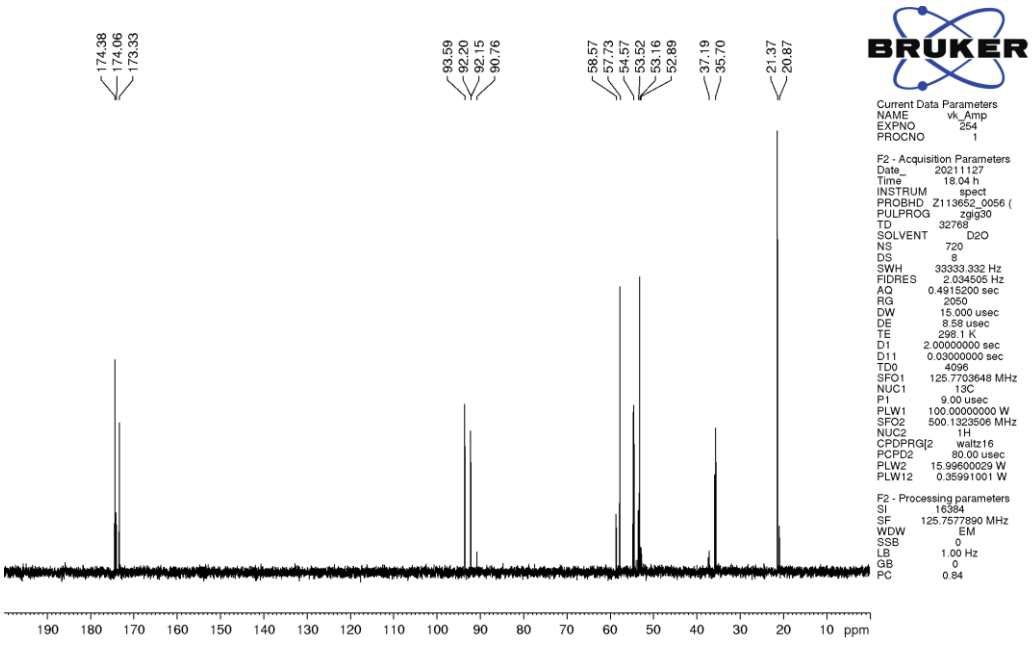
^{1}H NMR (500 MHz, D_2O): *trans*-amide: 5.46 (dt, *J* = 51.7, 2.9 Hz, 1H, γ-CH), 4.59 (dd, *J* = 9.4, 8.4 Hz, 1H, α-CH), 4.04 (ddd, *J* = 21.8, 12.9, 1.7 Hz, 1H, δ-CH), 3.91 (ddd, *J* = 38.1, 12.8, 2.8 Hz, 1H, δ-CH), 3.79 (s, 3H, CH_3O), 2.72 (dddt, *J* = 18.2, 15.0, 7.8, 1.0, 1H, β-CH), 2.23 (dddd, *J* = 42.0, 14.9, 10.2, 3.9 Hz, 1H, β-CH), 2.16 (s, 3H, CH_3); *cis*-amide: 5.39 (dt, *J* = 52.3, 3.1 Hz, 1H, γ-CH), 4.93 (t, *J* = 8.5 Hz, 1H, α-CH), 4.14, (ddd, *J* = 21.0, 14.1, 2.3 Hz, 1H, δ-CH), 3.84 (s, 3H, CH_3O), 3.55 (ddd, *J* = 37.3, 14.0, 3.0 Hz, 1H, δ-CH), 2.86 (dddt, *J* = 20.9, 15.4, 8.4, 1.2 Hz, 1H, β-CH), 2.45 (dddd, *J* = 39.1, 15.0, 8.2, 4.4 Hz, 1H, β-CH), 2.05 (s, CH_3).



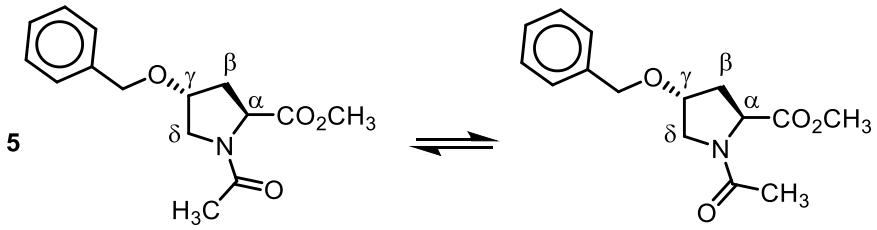
$^{19}F\{^{1}H\}$ NMR (471 MHz, D_2O): *trans*-amide: −177.73 (s); *cis*-amide: −177.67 (s).



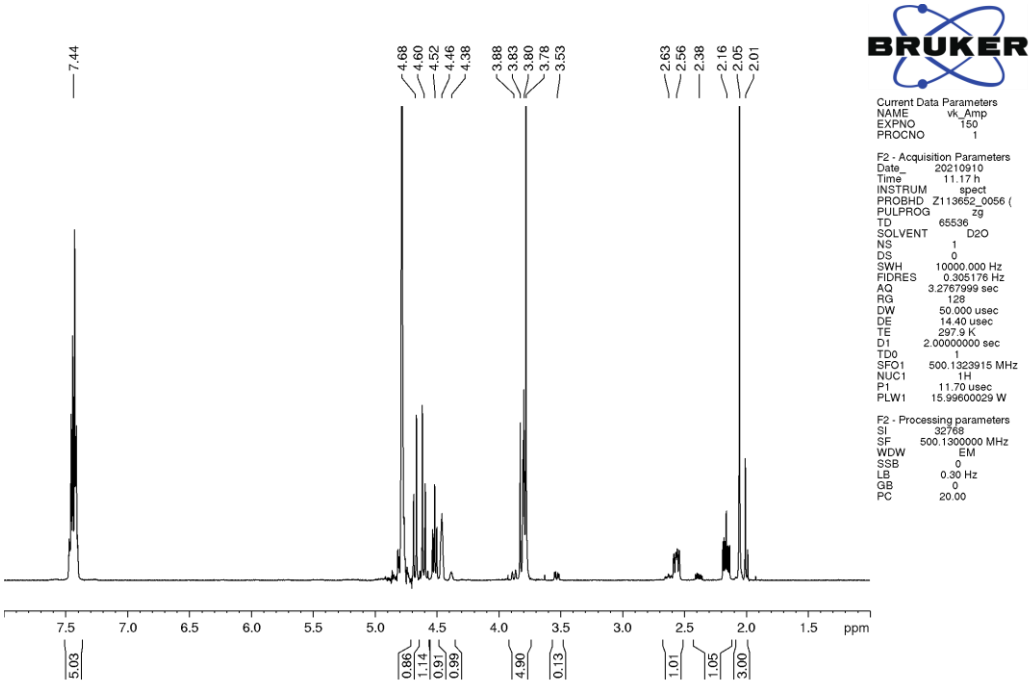
$^{13}C\{^{1}H\}$ NMR (126 MHz, D_2O): *trans*-amide: 174.4 (s, CO_2Me), 173.3 (s, CNO), 92.9 (d, J = 175 Hz, γ-CH), 57.7 (s, α-CH), 54.6 (d, J = 22 Hz, δ-CH_2), 53.2 (s, CH_3O), 35.7 (d, J = 22 Hz, β-CH_2), 21.4 (s, CH_3); *cis*-amide: 174.1 (two s, CO_2Me and CON), 91.5 (d, J = 175 Hz, γ-CH), 58.6 (s, α-CH), 53.5 (s, CH_3O), 52.9 (d, J = 22 Hz, δ-CH_2), 37.2 (d, J = 23 Hz, β-CH_2), 20.9 (s, CH_3).



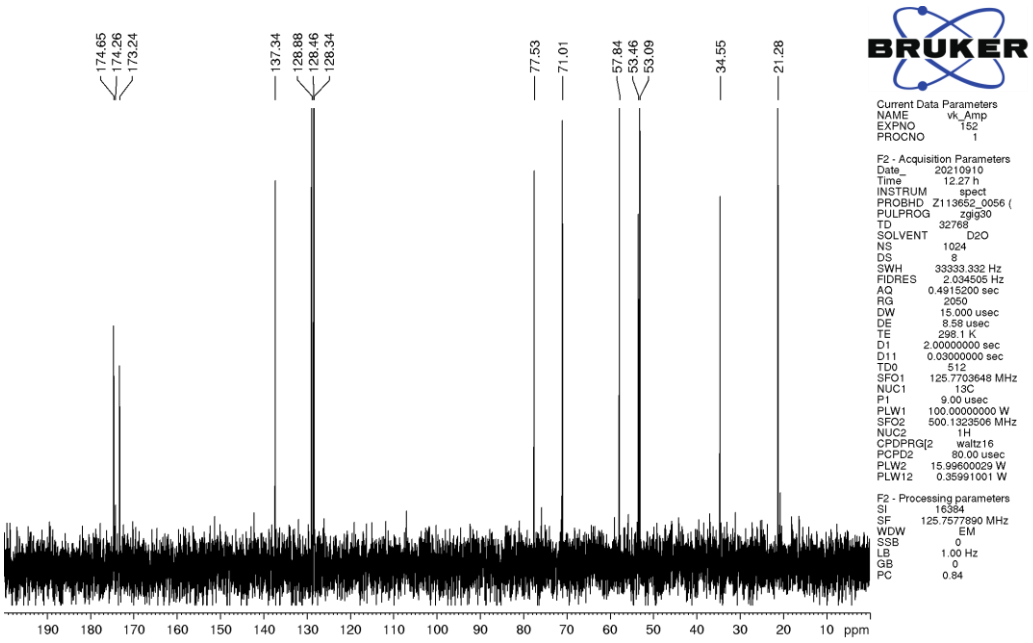
Ac(Bn)HypOCH$_3$, **5**:



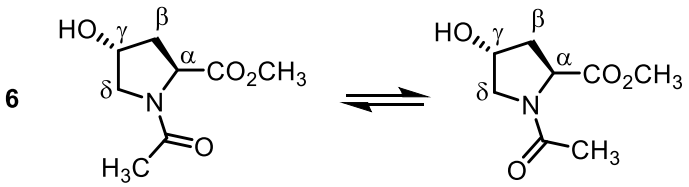
^{1}H NMR (500 MHz, D_2O): *trans*-amide: 7.47-7.40 (m, 5H, Ph), 4.68 (d, *J* = 11.7 Hz, 1H, CHPh), 4.60 (d, *J* = 11.6 Hz, 1H, CHPh), 4.52 (t, *J* = 8.4 Hz, 1H, α-CH), 4.46 (m, 1H, γ-CH), 3.80 (m, 2H, δ-CH_2), 3.78 (s, 3H, CH_3O), 2.56 (dddd, *J* = 13.8, 8.0, 2.2, 1.3 Hz, 1H, β-CH), 2.16 (ddd, *J* = 13.8, 8.8, 4.6 Hz, 1H, β-CH), 2.06 (s, 3H, CH_3); *cis*-amide: 7.47-7.40 (m, 5H, Ph), 4.81 (m, 1H, α-CH), 4.64-4.57 (m, 2H, CH_2Ph), 4.38 (m, 1H, γ-CH), 3.88 (dt, *J* = 12.8, 2.0 Hz, 1H, δ-CH), 3.83 (s, 3H, CH_3O), 3.53 (dd, *J* = 12.7, 4.6 Hz, 1H, δ-CH), 2.63 (dddd, *J* = 13.7, 8.7, 3.6, 1.7 Hz, β-CH), 2.38 (ddd, *J* = 13.8, 6.3, 5.2 Hz, 1H, β-CH), 2.01 (s, 3H, CH_3).



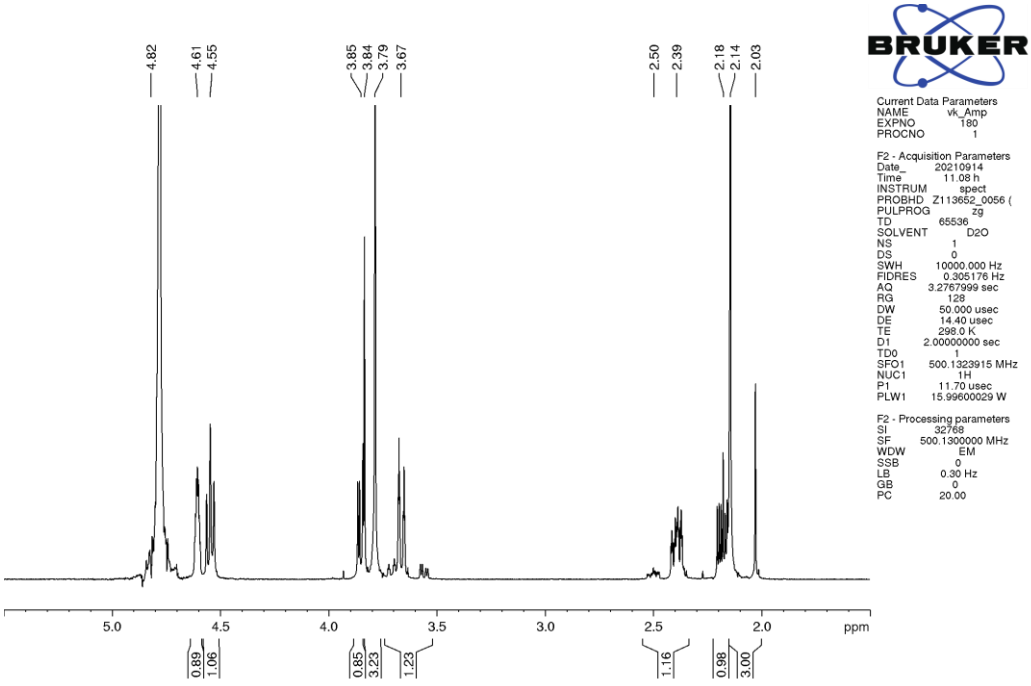
$^{13}C\{^{1}H\}$ NMR (126 MHz, D_2O): *trans*-amide: 174.6 (CO_2Me), 173.2 (CON), 137.3 (C, Ph), 128.9 (CH, Ph), 128.5 (CH, Ph), 128.3 (CH, Ph), 77.5 (γ-CH), 71.0 (CH_2Ph), 57.8 (α-CH), 53.5 (δ-CH_2), 53.2 (CH_3O), 34.6 (β-CH_2), 21.3 (CH_3); *cis*-amide (only resolved signals): 174.2 (CO_2Me), 173.8 (CON), 76.0 (γ-CH), 59.0 (α-CH), 53.5 (CH_3O), 51.3 (δ-CH_2), 36.2 (β-CH_2), 20.7 (CH_3).



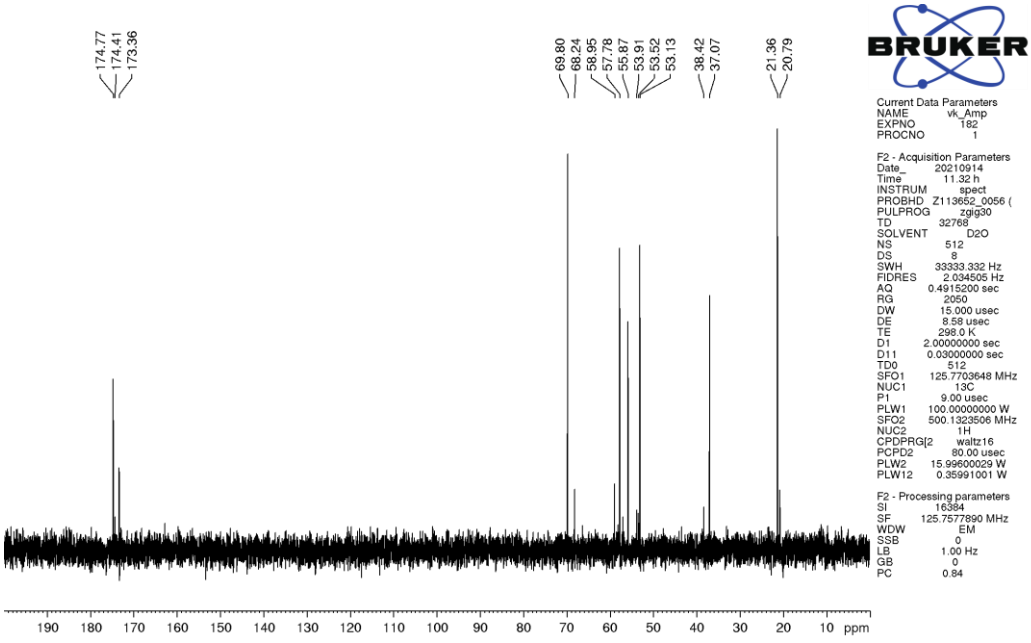
AcHypOCH$_3$, **6**:



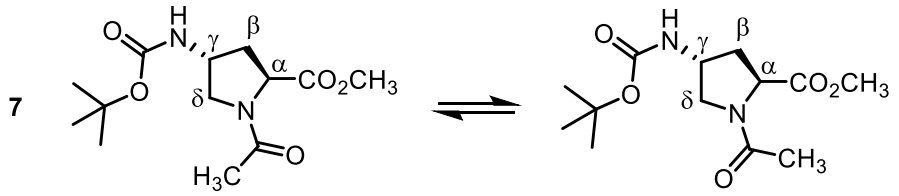
^{1}H NMR (500 MHz, D_2O): *trans*-amide: 4.61 (m, 1H, γ-CH), 4.55 (t, *J* = 8.5 Hz, 1H, α-CH), 3.85 (dd, *J* = 11.7, 4.1 Hz, 1H, δ-CH), 3.79 (s, 3H, CH_3O), 3.67 (dt, *J* = 11.8, 1.7 Hz, 1H, δ-CH), 2.39 (ddt, *J* = 13.9, 7.9, 2.2 Hz, 1H, β-CH), 2.18 (ddd, *J* = 13.7, 8.9, 4.5 Hz, 1H, β-CH), 2.15 (s, 3H, CH_3); *cis*-amide: 4.83 (m, 1H, α-CH), 4.54 (m, 1H, γ-CH),3.83 (s, 3H, CH_3O), 3.71 (dt, *J* = 12.6, 2.2 Hz, 1H, δ-CH), 3.56 (dd, *J* = 12.6, 4.6 Hz, 1H, δ-CH), 2.50 (dddd, *J* = 13.9, 8.6, 3.6, 1.9 Hz, 1H, β-CH), 2.37 (m, 1H, β-CH), 2.03 (s, 3H, CH_3).



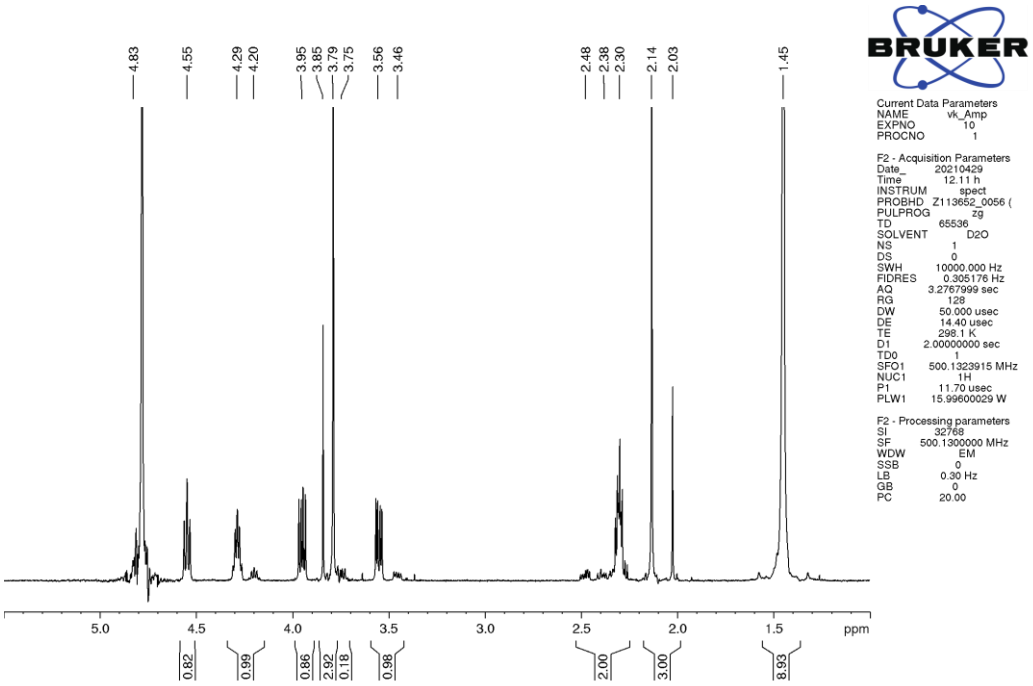
$^{13}C\{^{1}H\}$ NMR (126 MHz, D_2O): *trans*-amide: 174.8 (CO_2Me), 173.4 (CON), 69.8 (γ-CH), 57.8 (α-CH), 55.9 (δ-CH_2), 53.1 (CH_3O), 37.1 (β-CH2), 21.4 (CH_3); *cis*-amide: 174.4 (CO_2Me), 174.1 (CON), 68.2 (γ-CH), 58.9 (α-CH), 53.9 (δ-CH_2), 53.5 (CH_3O), 38.4 (β-CH_2), 20.8 (CH_3).



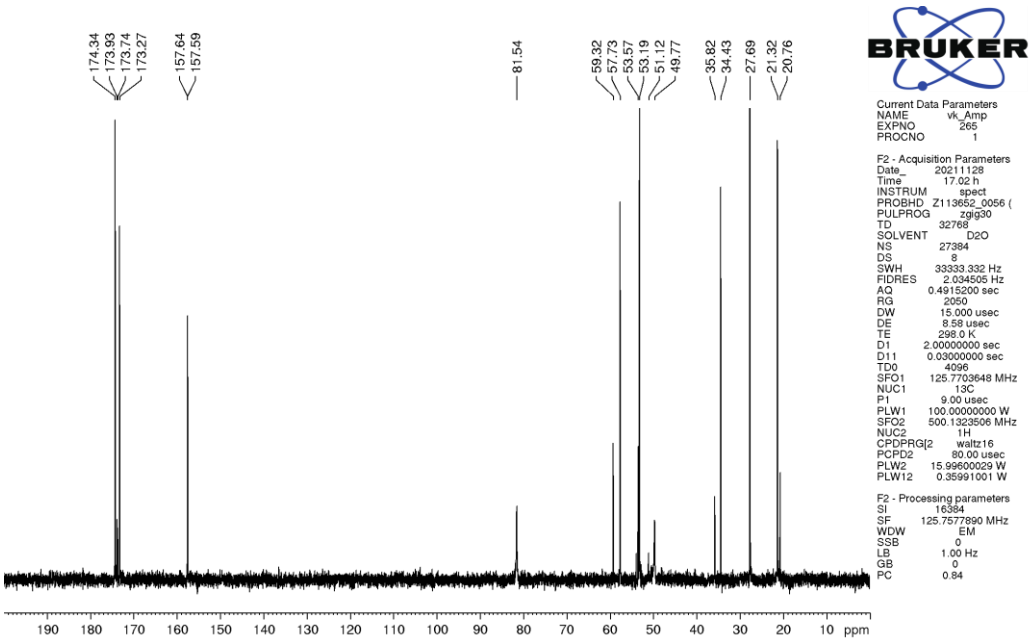
Ac(Boc)AmpOCH$_3$, **7**:



^{1}H NMR (500 MHz, D_2O): *trans*-amide: 4.55 (t, *J* = 7.4 Hz, 1H, α-CH), 4.29 (m, 1H, γ-CH), 3.95 (dd, *J* = 11.0, 6.1 Hz, 1H, δ-CH), 3.79 (s, 3H, CH_3O), 3.55 (dd, *J* = 11.0, 4.7 Hz, 1H, δ-CH), 2.30 (m, 2H, β-CH_2), 2.14 (s, 3H, CH_3CO), 1.45 (s, 9H, Boc); *cis*-amide: 4.82 (m, 1H, α-CH), 4.20 (m, 1H, γ-CH), 3.84 (s, 3H, CH_3O), 3.75 (dd, *J* = 11.7, 6.9 Hz, 1H, δ-CH), 3.46 (dd, *J* = 11.7, 6.0 Hz, 1H, δ-CH), 2.48 (m, 1H, β-CH), 2.38 (m, 1H, β-CH), 2.03 (s, CH_3CO), 1.45 (s, 9H, Boc).

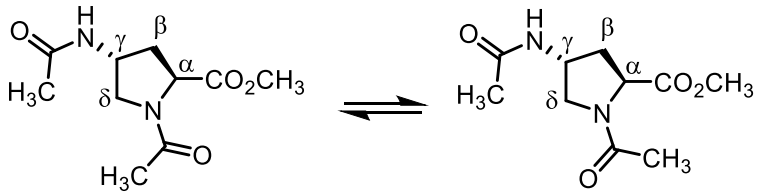


$^{13}C\{^{1}H\}$ NMR (126 MHz, D_2O): *trans*-amide: 174.3 (ester CO), 173.3 (amide CO), 157.59 (carbamate CO), 81.5 (CMe_3), 57.7 (α-CH), 53.2 (CH_3O and δ-CH_2), 49.8 (γ-CH), 34.4 (β-CH_2), 27.7 (3×CH_3), 21.3 (acetyl CH_3); *cis*-amide: 173.3 (ester CO), 173.7 (amide CO), 157.64 (carbamate CO), 81.5 (CMe_3), 59.3 (α-CH), 53.6 (CH_3O), 51.1 (δ-CH_2), 48.0 (γ-CH), 35.8 (β-CH_2), 27.7 (3×CH_3), 20.8 (acetyl CH_3).



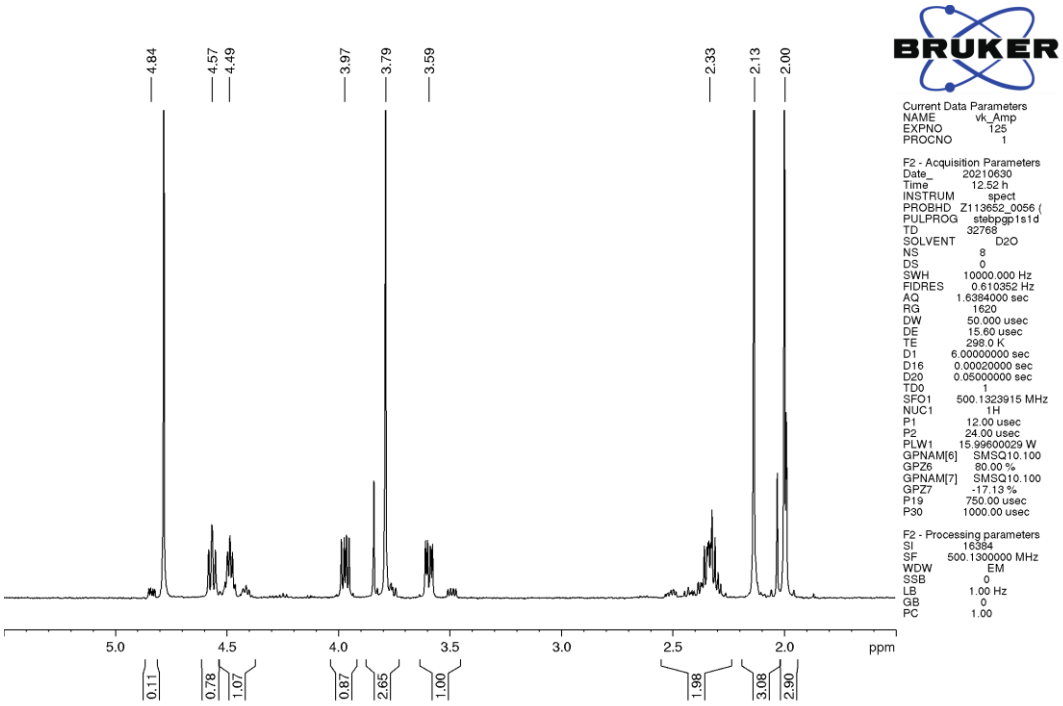
Ac(Ac)AmpOCH$_3$, **8**:

8

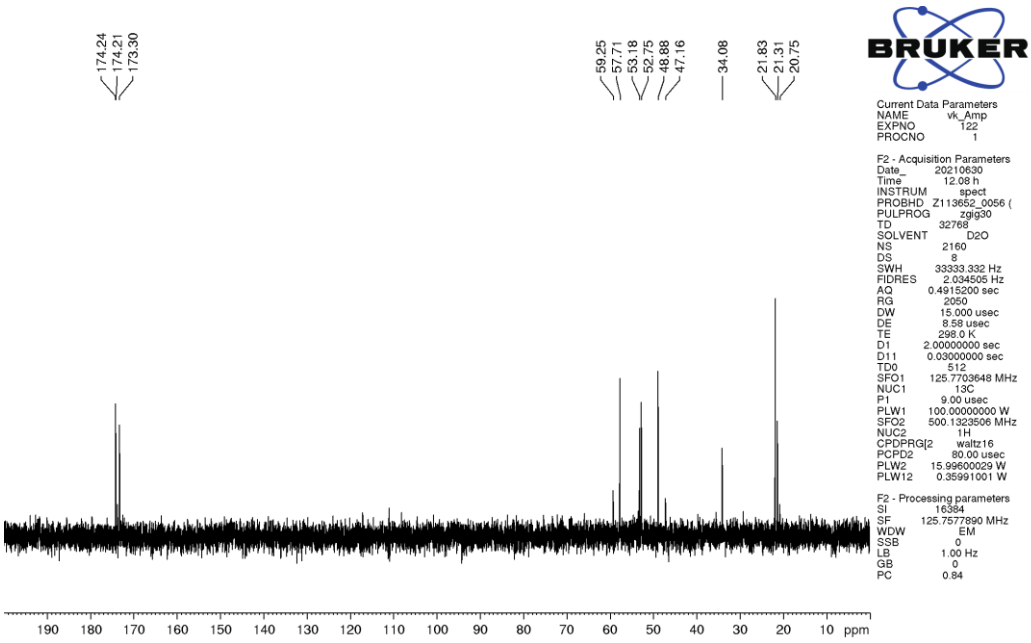


trans-amide *cis*-amide

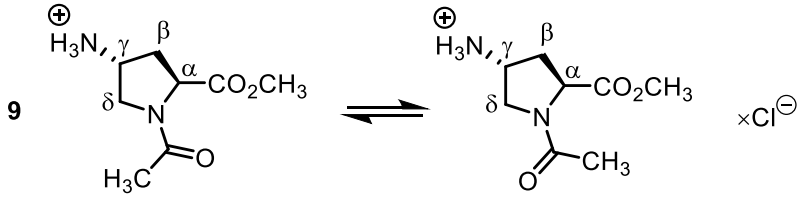
^{1}H NMR (500 MHz, D_2O): *trans*-amide: 4.57 (dd, *J* = 8.0, 7.2 Hz, 1H, α-CH), 4.49 (m, 1H, γ-CH), 3.97 (dd, *J* = 11.3, 6.1 Hz, δ-CH), 3.79 (s, 3H, CH_3O), 3.59 (dd, *J* = 11.3, 4.4 Hz, 1H, δ-CH), 2.33 (m, 2H, β-CH_2), 2.13 (s, 3H, Ac), 2.00 (s, 3H, γ-Ac); *cis*-amide: 4.84 (dd, *J* = 8.9, 4.3 Hz, 1H, α-CH), 4.42 (m, 1H, γ-CH), 3.90 (m, 1H, δ-CH), 3.84 (s, 3H, CH_3O), 3.49 (dd, *J* = 12.3, 6.2 Hz, δ-CH), 2.50 (m, 1H, β-CH), 2.42 (m, 1H, β-CH), 2.03 (s, 3H, Ac), 1.99 (s, 3H, γ-Ac).



$^{13}C\{^{1}H\}$ NMR (126 MHz, D_2O): *trans*-amide: 174.24 (CO_2Me), 174.21 (γ-CON), 173.3 (CON), 57.7(α-CH), 53.2 (CH_3O), 52.7 (δ-CH_2), 48.9 (γ-CH), 34.1 (β-CH_2), 21.8 (γ-CH_3), 21.3 (CH_3); *cis*-amide: 174.17 (γ-CON), 173.79 (CON), 173.74 (CO_2Me), 59.3 (α-CH), 53.6 (CH_3O), 50.7 (δ-CH_2), 47.3 (γ-CH), 35.5 (β-CH_2), 21.8 (γ-CH_3), 20.8 (CH_3).

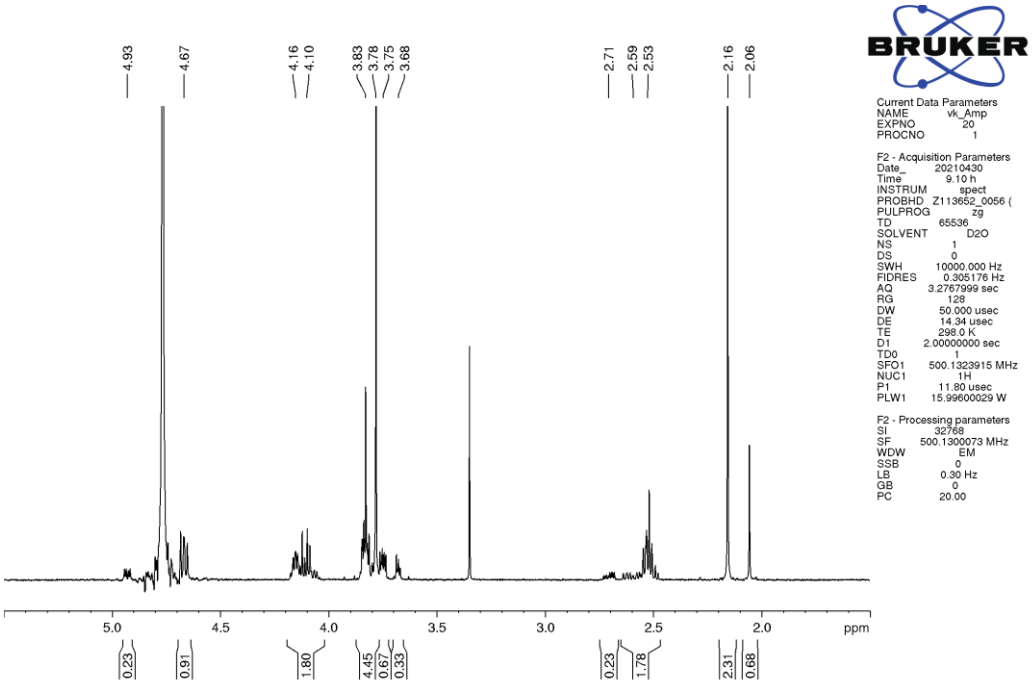


AcAmpOCH$_3$×HCl, **9**:

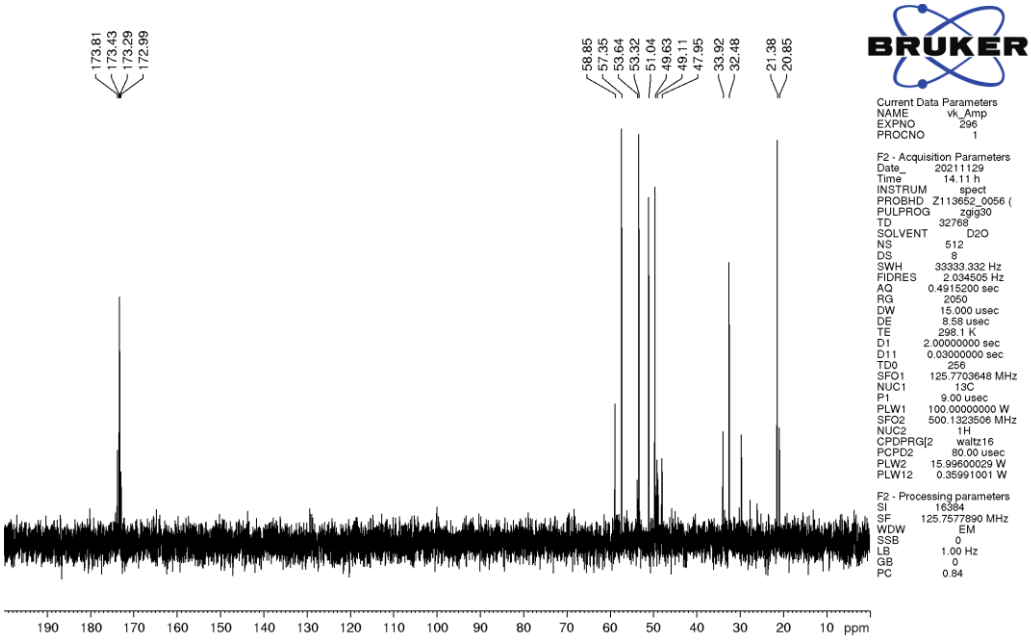


trans-amide *cis*-amide

^{1}H NMR (500 MHz, D_2O): *trans*-amide: 4.69 (dd, *J* = 8.4, 7.0 Hz, 1H, α-CH), 4.17 (m, 1H, γ-CH), 4.12 (dd, *J* = 11.7, 6.1 Hz, 1H, δ-CH), 3.84 (dd, *J* = 11.8, 4.0 Hz, 1H, δ-CH), 3.80 (s, 3H, CH3O), 2.54 (m, 2H, β-CH_2), 2.17 (s, 3H, CH_3CO); *cis*-amide: 4.95 (dd, *J* = 9.0, 4.2 Hz, 1H, α-CH), 4.09 (m, 1H, γ-CH), 3.77 (m, 1H, δ-CH), 3.69 (m, 1H, δ-CH), 2.72 (m, 1H, β-CH), 2.62 (m, 1H, β-CH), 2.07 (s, 3H, CH_3CO).

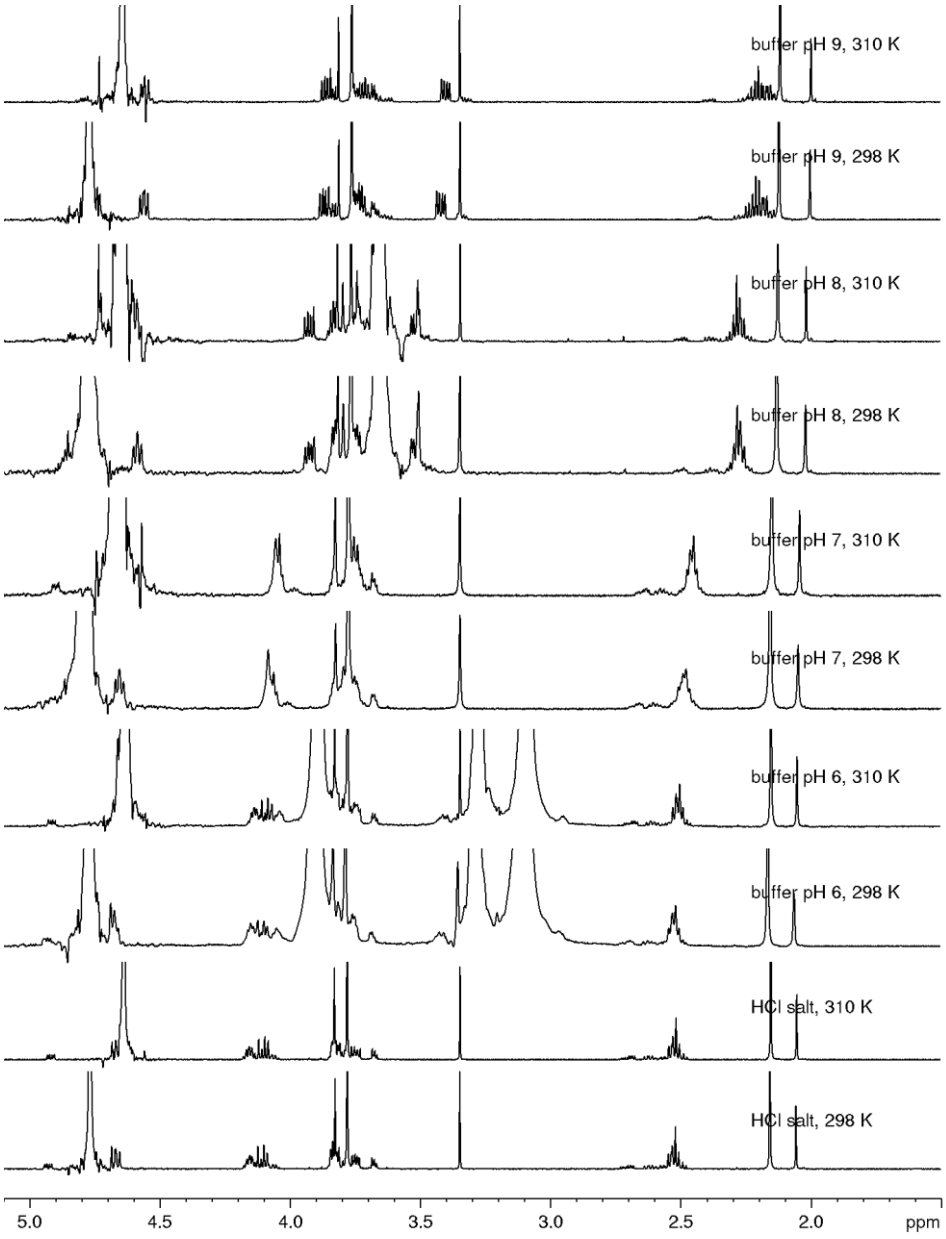


$^{13}C\{^{1}H\}$ NMR (126 MHz, D_2O): *trans*-amide: 173.4 (CO2Me), 173.3 (CON), 57.3 (α-CH), 53.3 (CH_3O), 51.0 (δ-CH_2), 49.6 (γ-CH), 32.5 (β-CH_2), 21.4 (CH_3); *cis*-amide: 173.8 (CON), 173.0 (CO_2Me), 58.9 (α-CH), 53.6 (CH_3O), 49.1 (δ-CH_2), 48.0 (γ-CH), 33.9 (β-CH_2), 20.9 (CH_3).

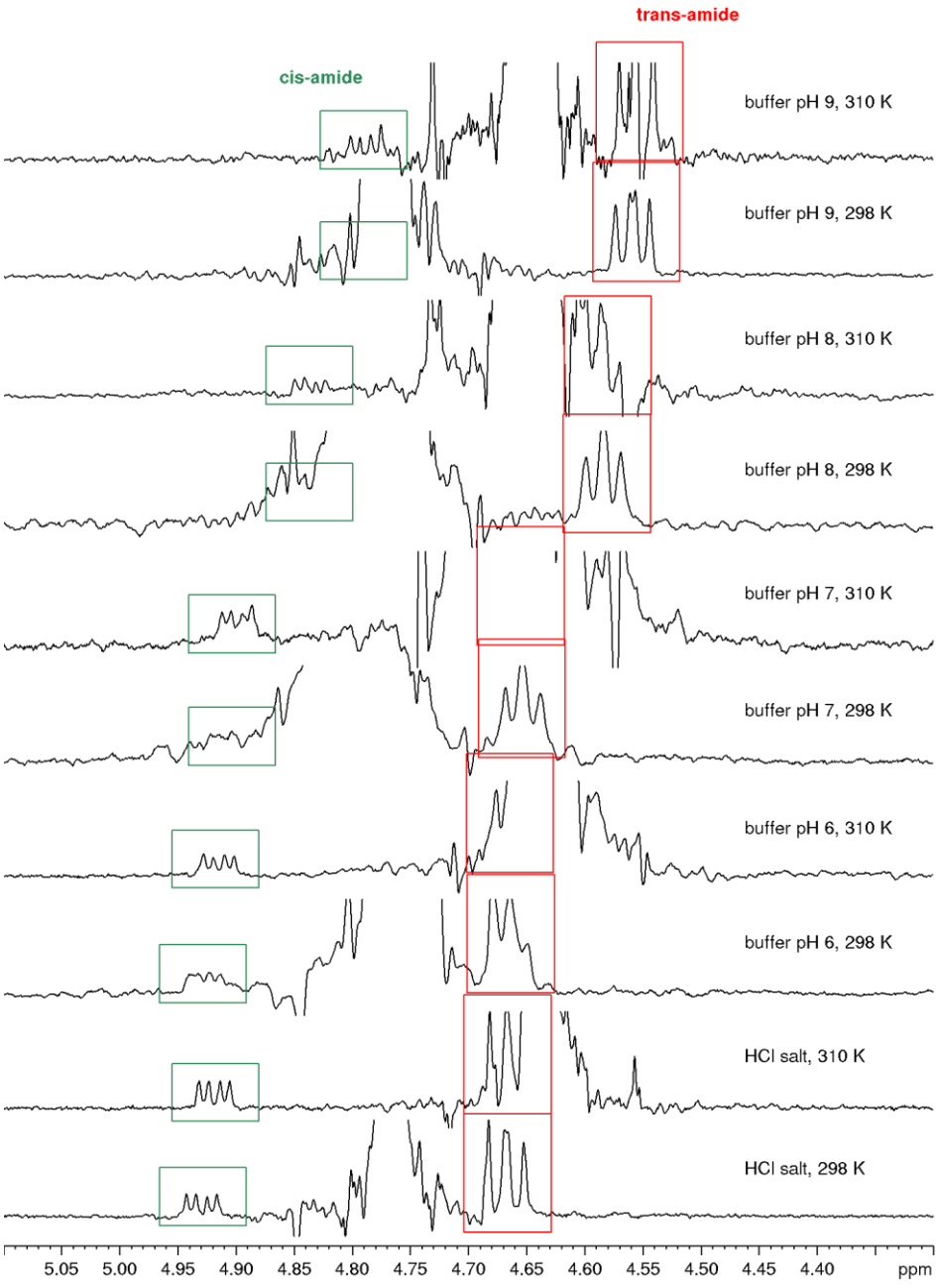


NMR spectra of compound 9 at different pH

Aliquotes of 550 µl of 150 mM buffers: MES-HCl (pH 6), KH_2PO_4-KOH (pH 7), Tris-HCl (pH 8), and H_3BO_3-KOH (pH 9) – were taken to glass vials and dried over weekend followed by dissolving in 450 µl deuterium oxide each. Compound **9** was freshly prepared from compound **7** (25 mg) by 1-hour treatment with acidic methanol. After removing the volatiles, it was dissolved in 500 µl of deuterium oxide; 100 µl of resulting solution was added to the buffers, and these samples were used for measurements (final sample volume 550 µl). NMR spectra were recorded using fresh samples, between the measurements the samples were stored at 277 K fridge. Full ^{1}H NMR spectra are shown below (residual methanol resonance was used for calibration):



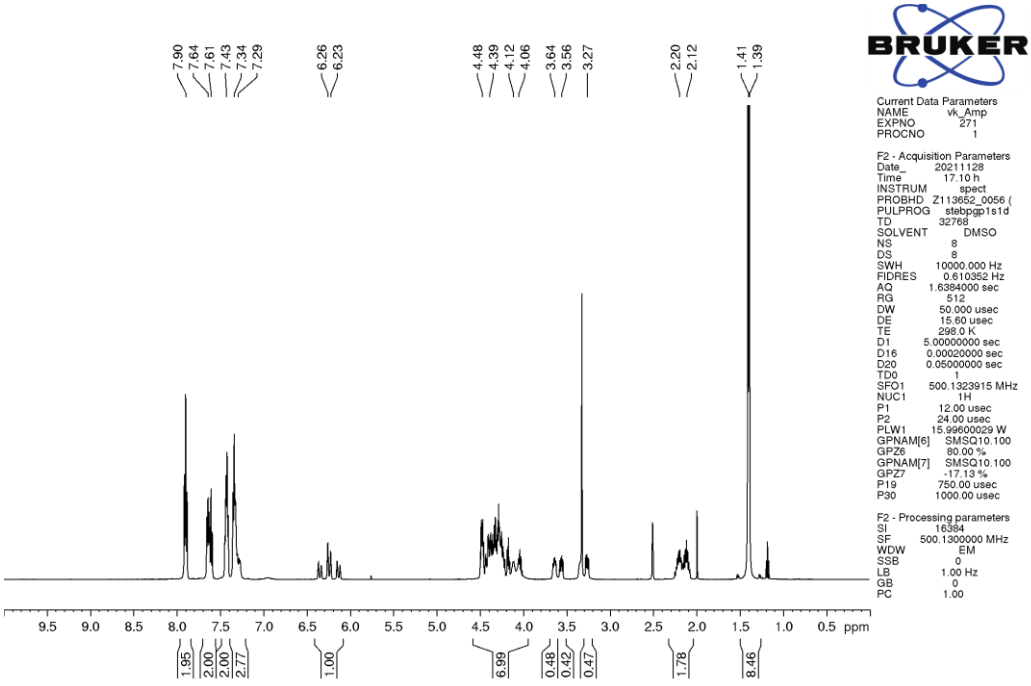
Fragments of ^{1}H NMR spectra containing α-CH resonances are shown below:



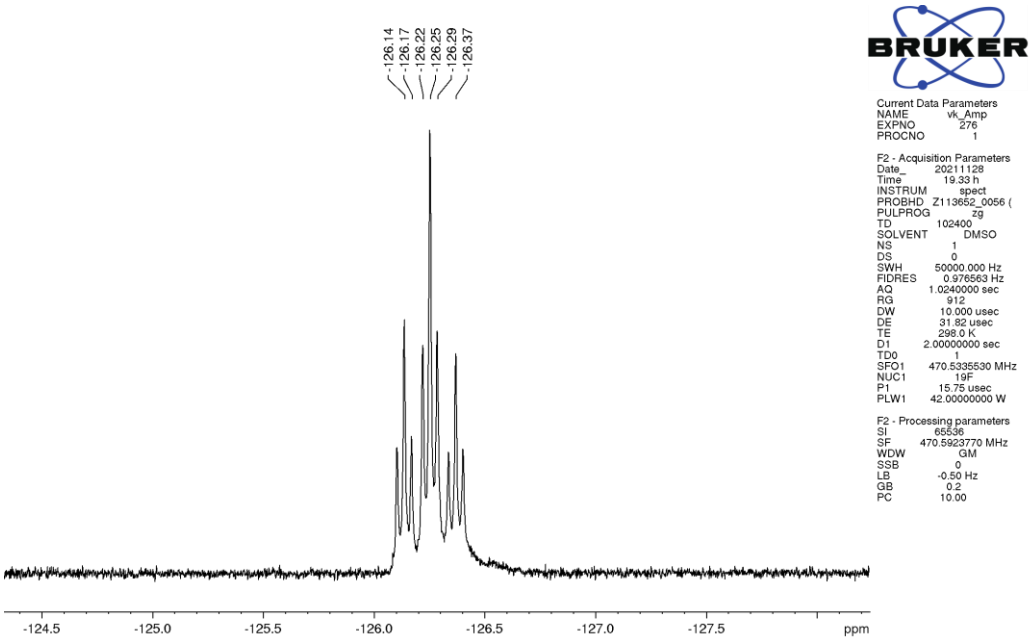
NMR spectra of Fmoc-derivatives

Fmoc-(Boc)Amp-OCH_2CHF_2:

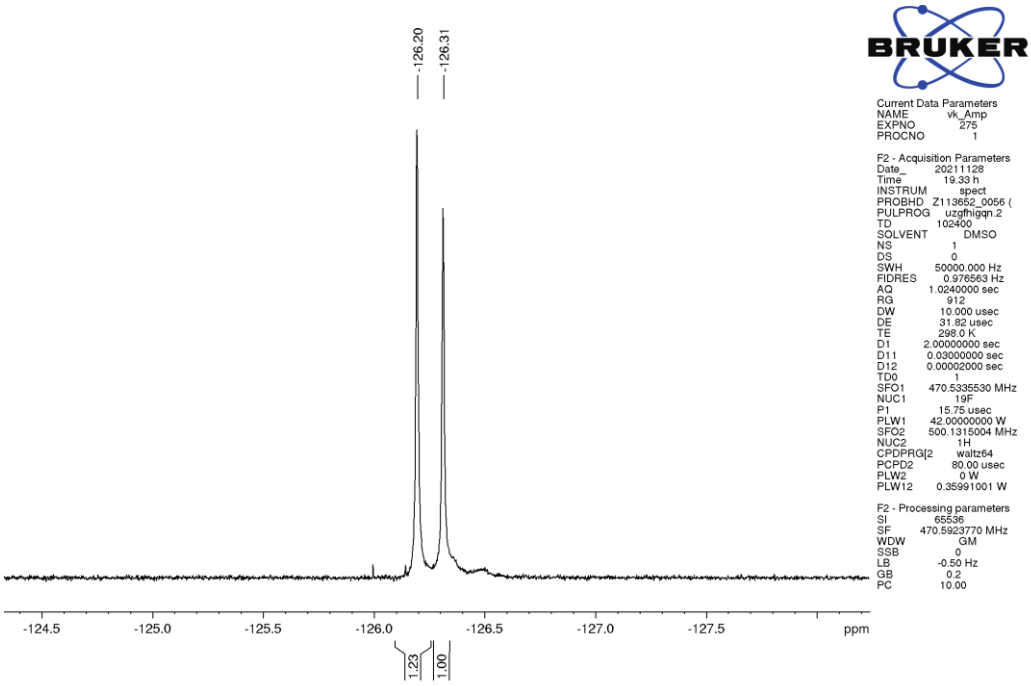
^{1}H NMR (500 MHz, DMSO-d_6), two rotamers 1.2:1 ratio: 7.90 (t, *J* = 7.5 Hz, 2H, Fmoc), 7.64 and 7.61 (two m, 2H, Fmoc), 7.43 (m, 2H, Fmoc), 7.34 (m, 2H, Fmoc), 7.31 and 7.28 (two d, *J* = 6.6 Hz, 1H, NHBoc), 6.26 (major, tt, *J* = 54.0, 3.0 Hz) and 6.23 (minor, tt, *J* = 54.2, 3.1 Hz, 1H in total, CHF_2), 4.48 (m, 1H, α-CH), 4.45-4.23 (m, 1H, OCH_2), 4.35-4.20 (m, 2H, CH_2O in Fmoc), 4.29 and 4.17 (two t, *J* = 6.5 Hz, CH in Fmoc), 4.11 and 4.05 (two m, 1H, γ-CH), 3.64 and 3.56 (two dd, *J* = 10.7, 6.4 Hz, 1H, δ-CH), 3.34 and 3.26 (two dd, *J* = 10.7, 5.3 Hz, 1H, δ-CH), 2.20 and 2.11 (two m, 2H, β-CH_2), 1.41 and 1.39 (two s, 9H, 3×CH_3 in Boc).



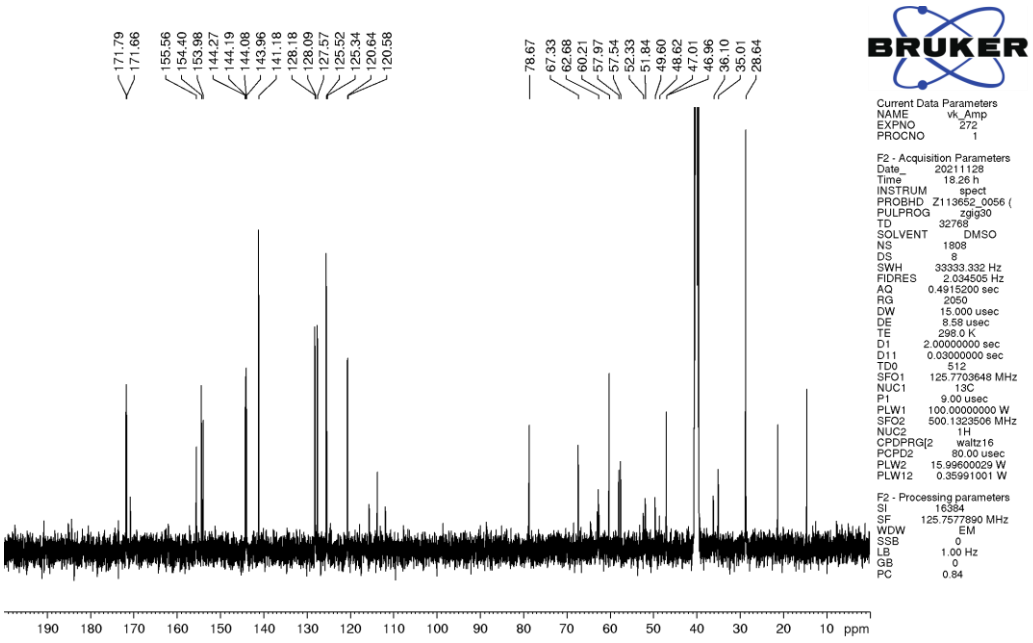
^{19}F NMR (471 MHz, DMSO-d_6), two rotamers: −126.2 (dt, *J* = 54, 16 Hz) and −126.3 (dt, *J* = 54, 16 Hz).



^{19}F NMR (471 MHz, DMSO-d_6), two rotamers 1.2:1 ratio: −126.20 (s, major) and −126.31 (s, minor).

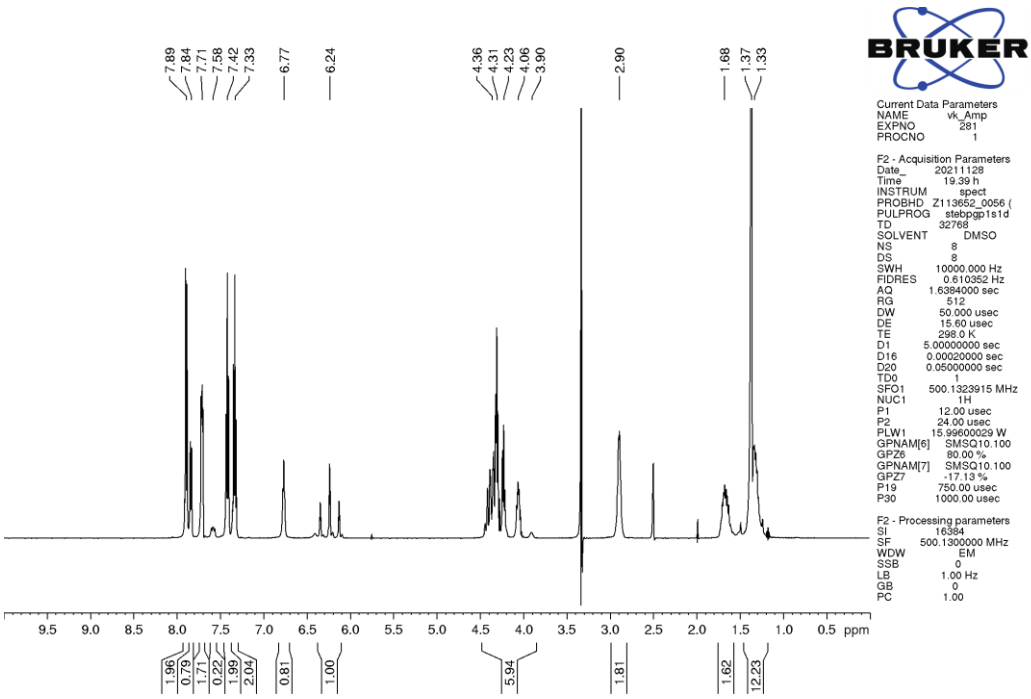


$^{13}C\{^{1}H\}$ NMR (126 MHz, DMSO-d_6), only major rotamer: 171.8 and 171.7 (two s, ester CO), 155.6 (broad s, CO in Boc), 154.4 and 154.0 (two s, CO in Fmoc), 144.3, 144.2, 144.1 and 144.0 (four s, two C from Fmoc), 141.2 (broad s, C in Fmoc), 128.2 and 128.1 (two s, CH in Fmoc), 127.6 (broad s, CH in Fmoc), 125.5 and 125.3 (two s, CH in Fmoc), 120.64 and 120.58 (two s, CH in Fmoc), 113.8 and 113.7 (two t, J = 239 Hz, CHF_2), 78.7 (s, CMe_3), 67.3 (s, CH_2O in Fmoc), 62.74 and 62.68 (two t, J = 27 Hz, OCH_2), 58.0 and 57.5 (two s, α-CH), 52.3 and 51.8 (two broad s, δ-CH_2), 49.6 and 48.6 (two broad s, γ-CH), 47.01 and 46.95 (two s, CH in Fmoc), 36.1 and 35.0 (two s, β-CH_2), 28.6 (s, 3×CH_3 in Boc).

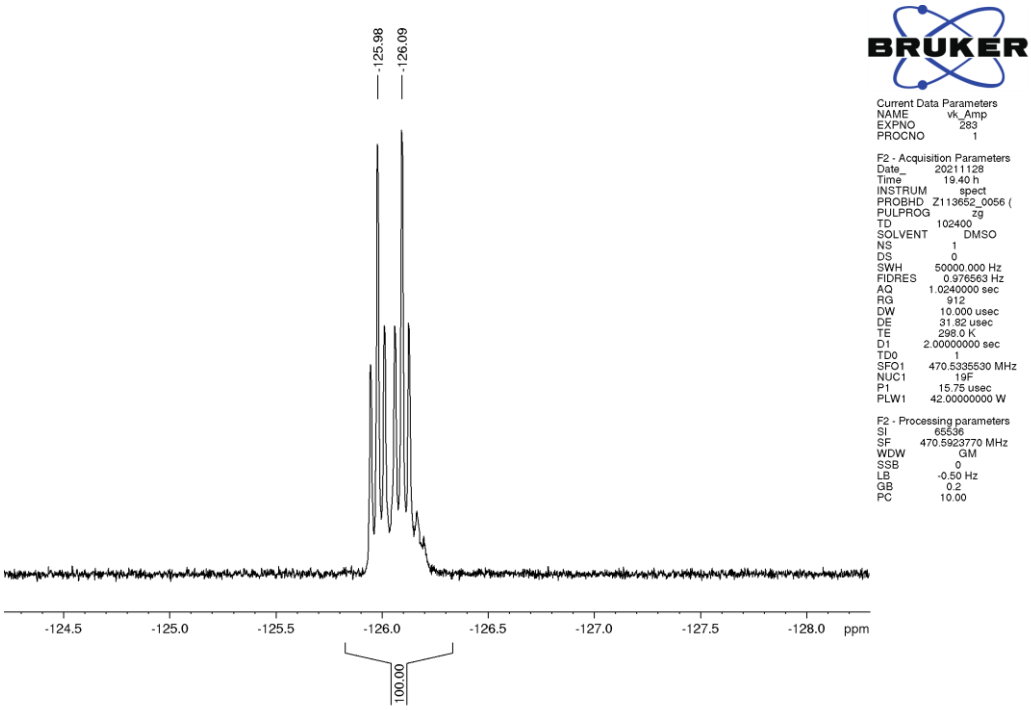


Fmoc-(Boc)Lys-OCH_2CHF_2:

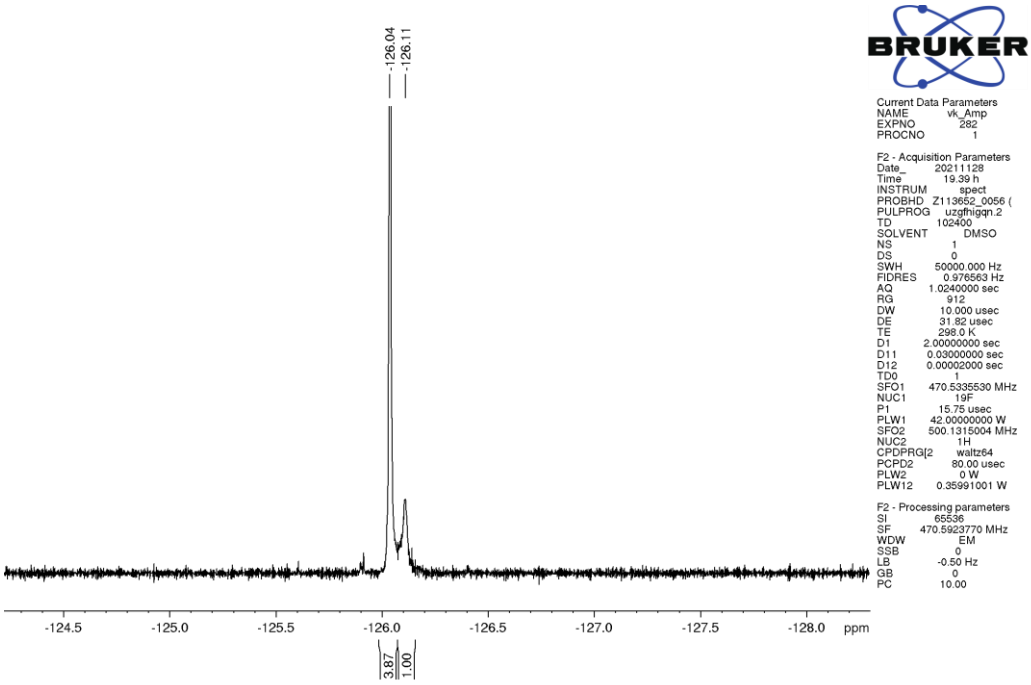
^{1}H NMR (500 MHz, DMSO-d_6), only major rotamer: 7.90 (d, *J* = 7.8 Hz, 2H, Fmoc), 7.85 (d, *J* = 7.5, 1H, NHFmoc), 7,72 (dd, *J* = 7.3, 3.8 Hz, 2H, Fmoc), 7.43 (t, *J* = 7.5 Hz, 2H, Fmoc), 7.34 (t, *J* = 7.5 Hz, 2H, Fmoc), 6.77 (t, *J* = 5.5 Hz, 1H, NHBoc), 6.24 (tt, *J* = 54.2, 3.1 Hz, 1H, CHF_2), 4.44-4.29 (m, 2H, OCH_2), 4.31 (m, 2H, CH_2O in Fmoc), 4.24 (t, *J* = 6.8 Hz, 1H, CH in Fmoc), 4.06 (m, 1H, α-CH), 2.90 (m, 2H, CH_2N), 1.68 (CH_2), 1.37 (s, 9H, 3×CH_3), 1.33 (m, 4H, 2×CH_2).



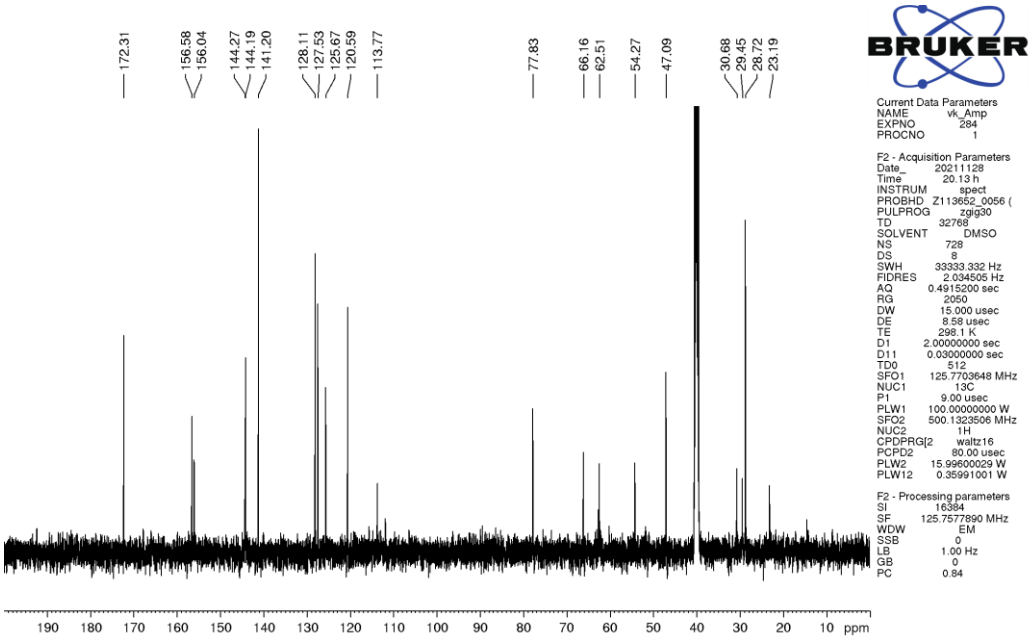
^{19}F NMR (471 MHz, DMSO-d_6), only major rotamer: −126.0 (dt, J = 54, 16 Hz).



^{19}F{^{1}H} NMR (471 MHz, DMSO-d_6), two rotamers 4:1 ratio: −126.04 (s, major), −126.11 (broad s, minor).

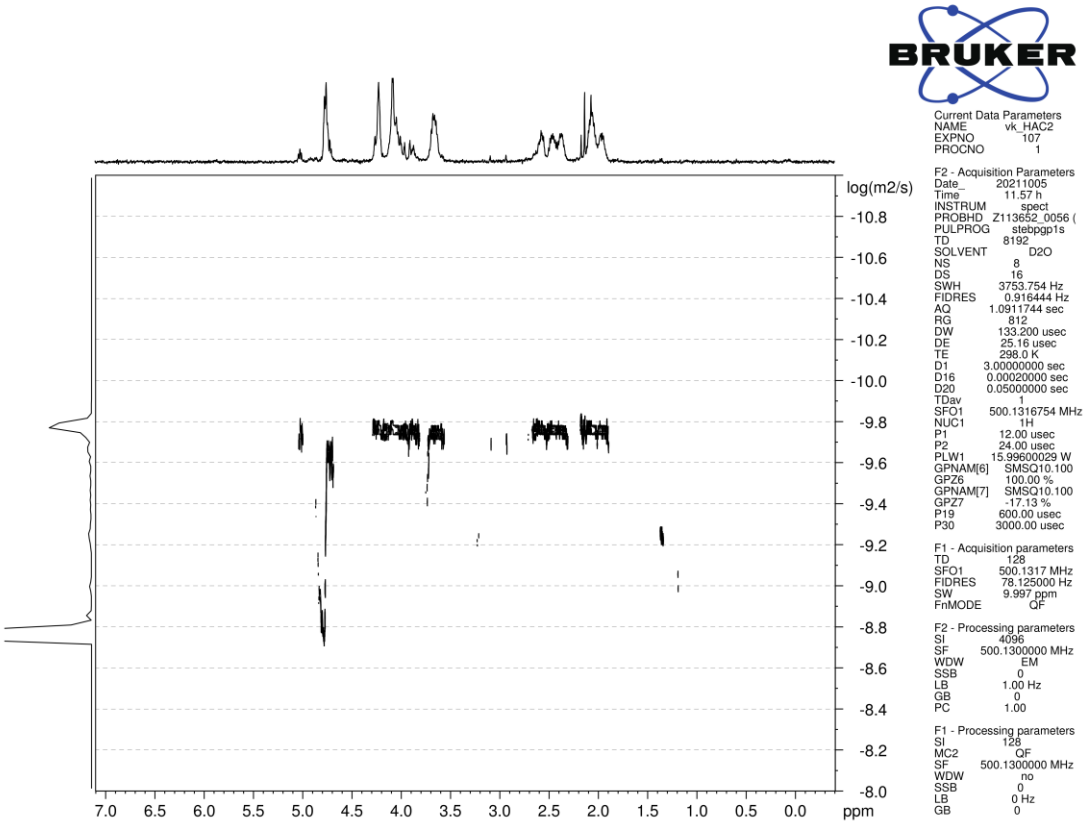


$^{13}C\{^{1}H\}$ NMR (126 MHz, DMSO-d_6), only major rotamer: 172.3 (s, ester CO), 156.6 (s, carbamate CO), 156.0 (s, carbamate CO), 144.3 (s, Fmoc C), 144.2 (s, Fmoc C), 141.2 (s, Fmoc C), 128.1 (s, Fmoc CH), 127.5 (s, Fmoc CH), 125.7 (s, Fmoc CH), 120.6 (s, Fmoc CH), 113.8 (t, J = 238 Hz, CHF_2), 77.8 (s, Boc C), 66.2 (s, CH_2O in Fmoc), 62.5 (t, J = 26 Hz, OCH_2), 54.3 (s, α-CH), 47.1 (s, CH in Fmoc), 40.3 (s, NCH_2), 30.7 (s, CH_2), 29.5 (s, CH_2), 28.7 (s, CH_3 in Boc), 23.2 (s, CH_2).



Diffusion spectrum for peptide 10

Diffusion properties of peptide **10** were examined in ^{1}H DOSY experiment recorded in deuterium oxide solution at 298 K. The experiment was conducted using diffusion time 50 ms and gradient pulse 3 ms. The diffusion ordering demonstrates presence of homogenous species with diffusion coefficient log$D$ at −9.76 log m^2 s^{-1}.

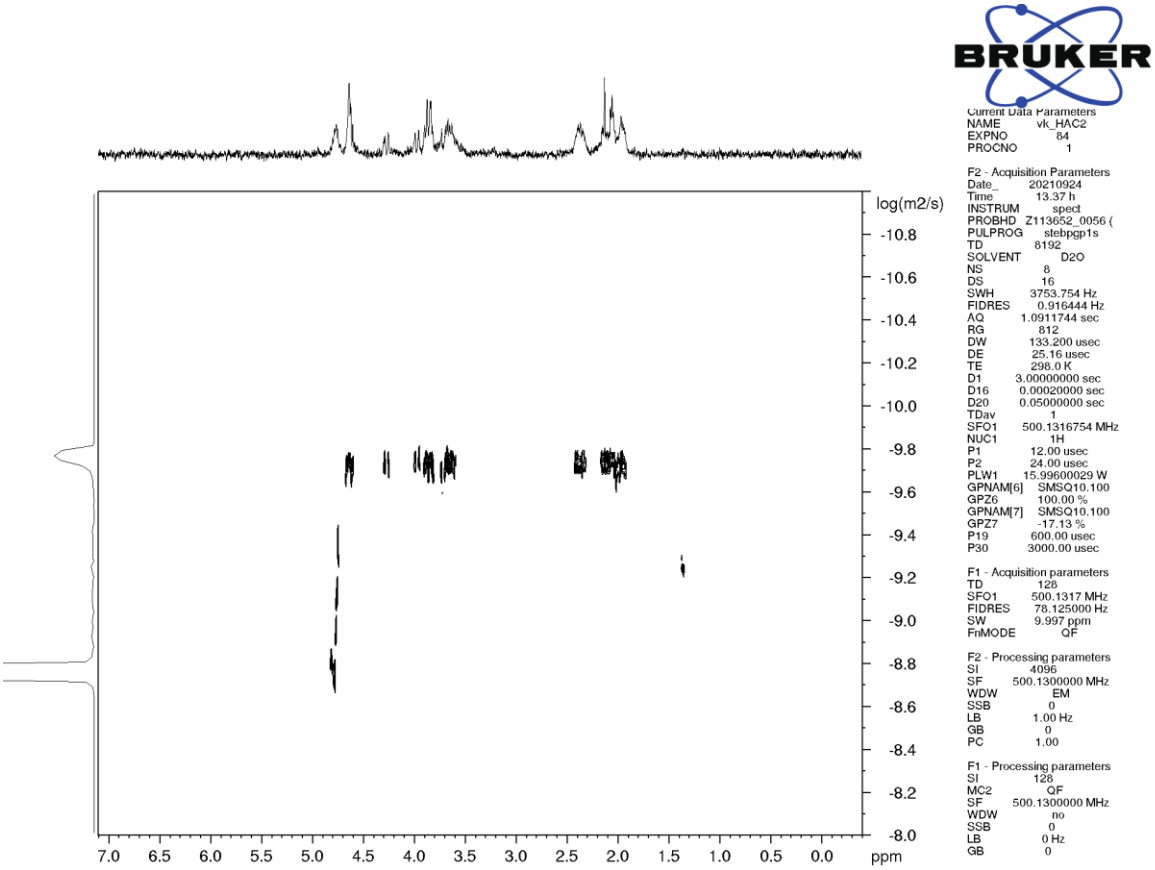


Theoretical value for the peptide was calculated from MW = 1664 (excluding trifluoroacetate counterions) using eq. S1[S4] as −9.60 log m^2 s^{-1}. Higher experimental value indicates deviation from spherical shape, which is common for peptides that lack a persistent structure.

$$logD = -8.524 - \frac{1}{3} logMW \quad \text{(eq. S1)}$$

For reference, the diffusion of residual deuterium oxide was expected at −8.73 and found at −8.75 log m^2 s^{-1}.

Diffusion experiment with peptide Ac-(Pro-Hyp-Gly)$_6$-OH was conducted with the same experimental parameters delivering the diffusion values −9.76 log m^2 s^{-1} for the peptide resonances and −8.75 log m^2 s^{-1} for residual water:



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[S3] M. Kuemin, Y. A. Nagel, S. Schweizer, F. W. Monnard, C. Ochsenfeld and H. Wennemers, *Angew. Chem. Int. Ed.* **2010**, 49, 6324-6327. Doi: 10.1002/anie.201001851

[S4] V. Kubyshkin and N. Budisa, *Beilstein J. Org. Chem.* **2017**, 13, 2442-2457. Doi: 10.3762/bjoc.13.241