Supporting information

Influence of the Amino Acid Side Chain on Peptide Bond Hydrolysis Catalyzed by a Dimeric Zr(IV)-Substituted Keggin Type Polyoxometalate

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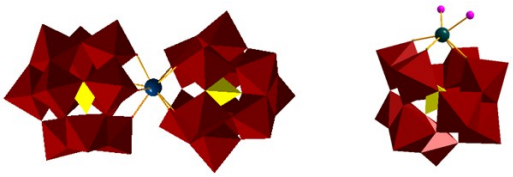
Synthesis of $(Et_2NH_2)_8[\{\alpha\text{-}PW_{11}O_{39}Zr\text{-}(\mu\text{-}OH)(H_2O)\}_2]\cdot 7H_2O$ (1):

The synthesis of **1** was accomplished by a 1:1 molar ratio reaction of the *in situ*-generated $[Zr(\alpha\text{-}PW_{11}O_{39})_2]^{10-}$ with $ZrOCl_2$ in an aqueous HCl solution, followed by addition of an excess amount of solid Et_2NH_2Cl. $H_3PW_{12}O_{40}\cdot 23H_2O$ (4.94 g) was dissolved in 25 mL of H_2O, followed by addition of 1.0 M $NaHCO_3$ until a pH value of 5.25 was obtained. The volume was adjusted to 50 mL by adding H_2O. $ZrOCl_2$ (0.256 g) was added and the solution was stirred for 5 minutes at room temperature. HCl (1 mL of 1.0 M) was added dropwise under vigorous stirring. The solution was stirred for an additional 30 minutes at room temperature. To this solution $ZrOCl_2$ (0.256 g dissolved in 4 mL of 1 M HCl) was added dropwise. The solution was stirred for 30 minutes and filtered with a folded filter paper. The colourless filtrate was evaporated at 40 °C to 20 mL. After stirring for 1 minute at 95 °C, 2.5 g of Et_2NH_2Cl was added and the mixture was stirred for 5 minutes. The white suspension was cooled to room temperature and stirred for 1 hour. White powder was collected and washed with EtOH (30 mL) and Et_2O (3 × 50 mL), and dried *in vacuo* for 2 hours. A water-soluble white powder (4.18 g - 88 %) was obtained.

The ^{31}P NMR spectrum of 2.0 mM **1** in D_2O at pD 7.4 shows one signal at -13.49 ppm.

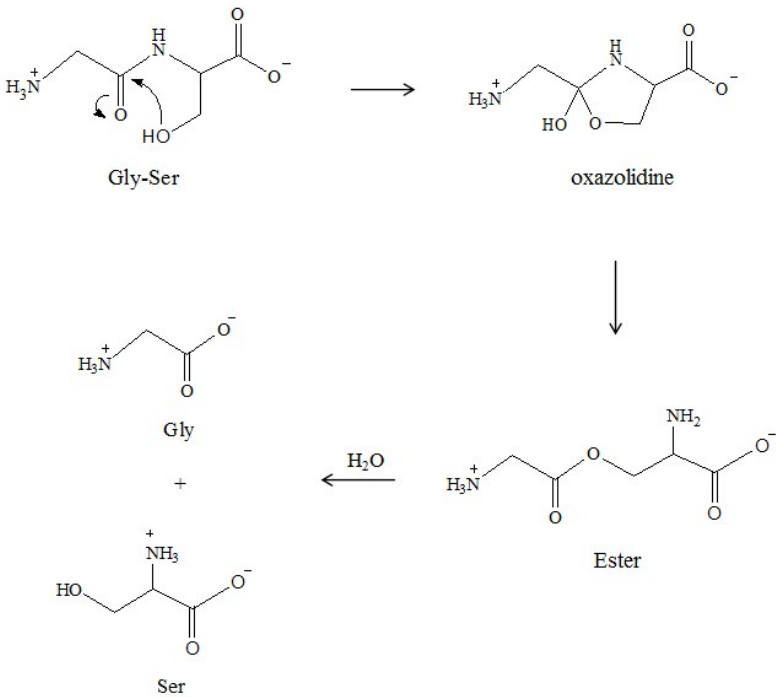


(a) (b)

Fig. S1 Schematical representation of (a) the 1:2 Zr(IV) - Keggin complex and (b) the 1:1 Zr(IV) - Keggin species.

Table S1. Grouping of dipeptides based on the nature of their side chains.

Dipeptides with hydroxyl group	Dipeptides with carbonyl group	Dipeptides with positively charged residue	Dipeptids containing a sulfur atom
Gly-Ser	Gly-Asp	Gly-His	Cys-Gly
Leu-Ser	Asp-Gly	His-Gly	Gly-Met
Ile-Ser	Gly-Glu	Gly-Lys	
Tyr-Ser	Gly-Asn	Gly-Arg	
Ser-Gly	Gly-Gln		
Gly-Thr			
Gly-Tyr			



Scheme S1. Formation of an ester intermediate via intramolecular attack of the Ser OH group.

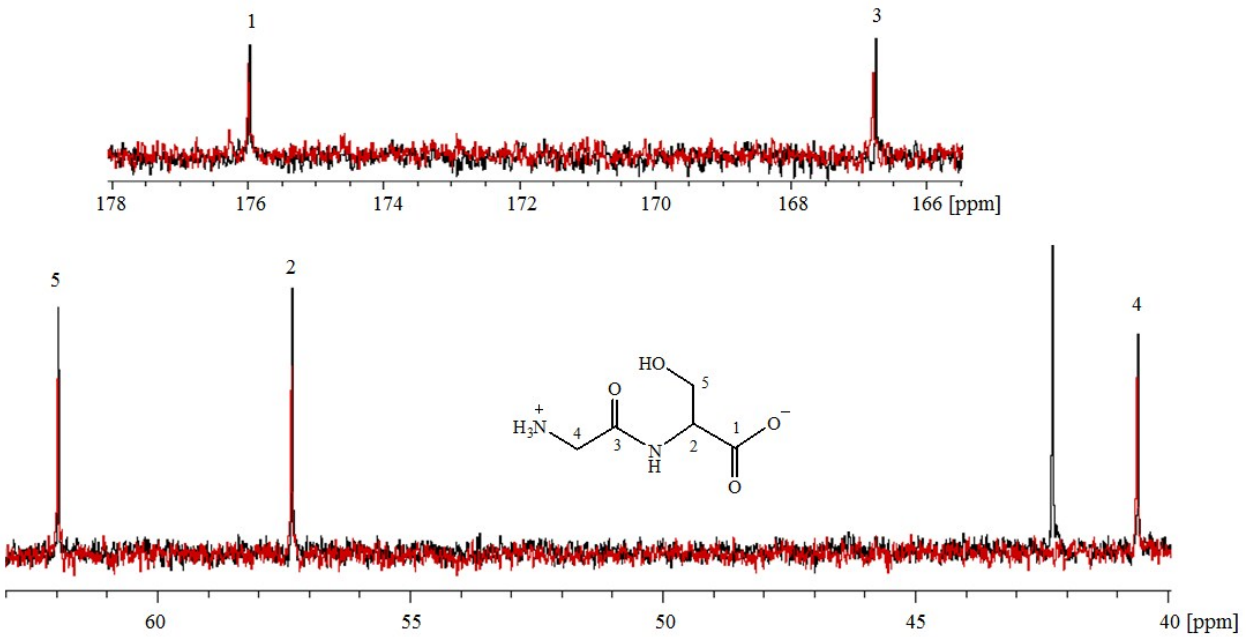


Fig. S2 ^{13}C NMR spectra of 20.0 mM Gly-Ser in the absence (red) and in the presence of 32.0 mM Et_2NH_2Cl (black) at pD 6.0. (The signal at 42.25 ppm belongs to the CH_2 carbon of the ethyl group)

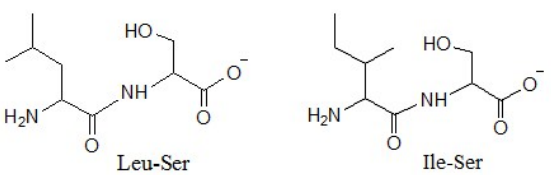


Fig. S3 Structures of Leu-Ser and Ile-Ser.

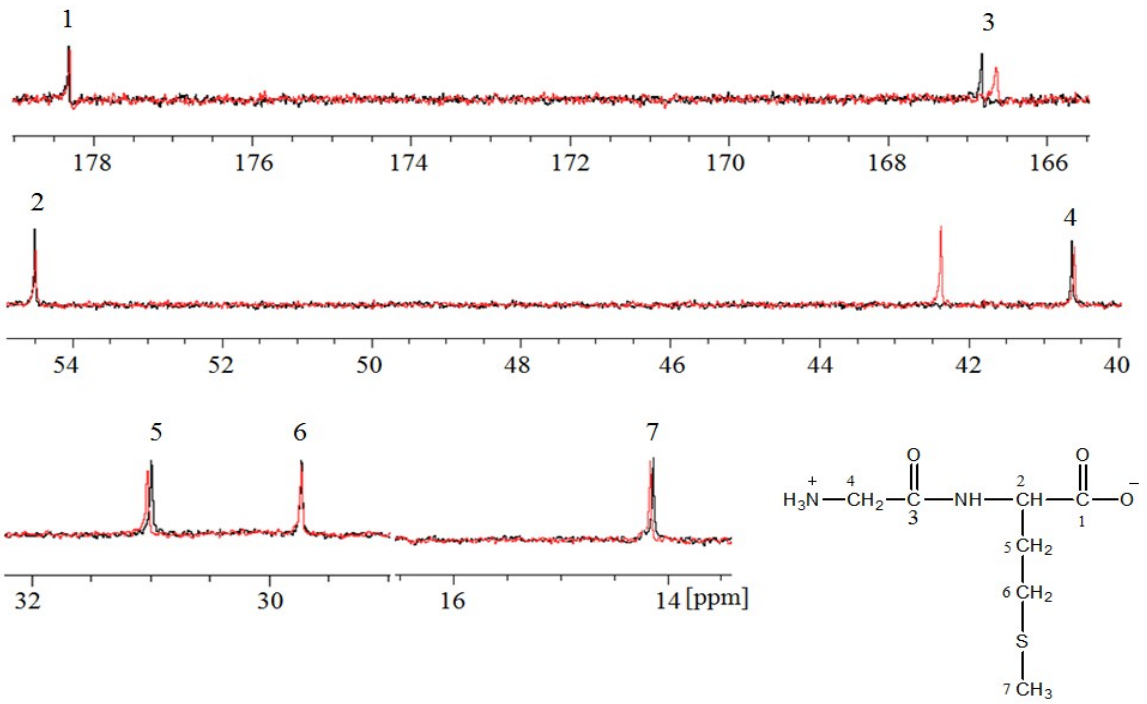


Fig. S4 ^{13}C NMR spectra of Gly-Met in the presence (red) and absence (black) of **1** at pD 7.0 (the signal at 42.4 ppm belongs to the CH_2 carbon of the ethyl group in the counter ion).

Table S2. ^{13}C NMR chemical shift values (ppm) of Gly-Met in the presence and in the absence of **1** at pD 7.0

^{13}C NMR	15.0 mM Gly-Met	15.0 mM Gly-Met + 2.0 mM **1**	Δδ (ppm)
δ_1	178.29	178.28	0.01
δ_2	54.50	54.49	0.01
δ_3	166.80	166.61	0.19
δ_4	40.62	40.54	0.08
δ_5	30.98	31.00	0.02
δ_6	29.72	29.72	0.00
δ_7	14.13	14.15	0.02

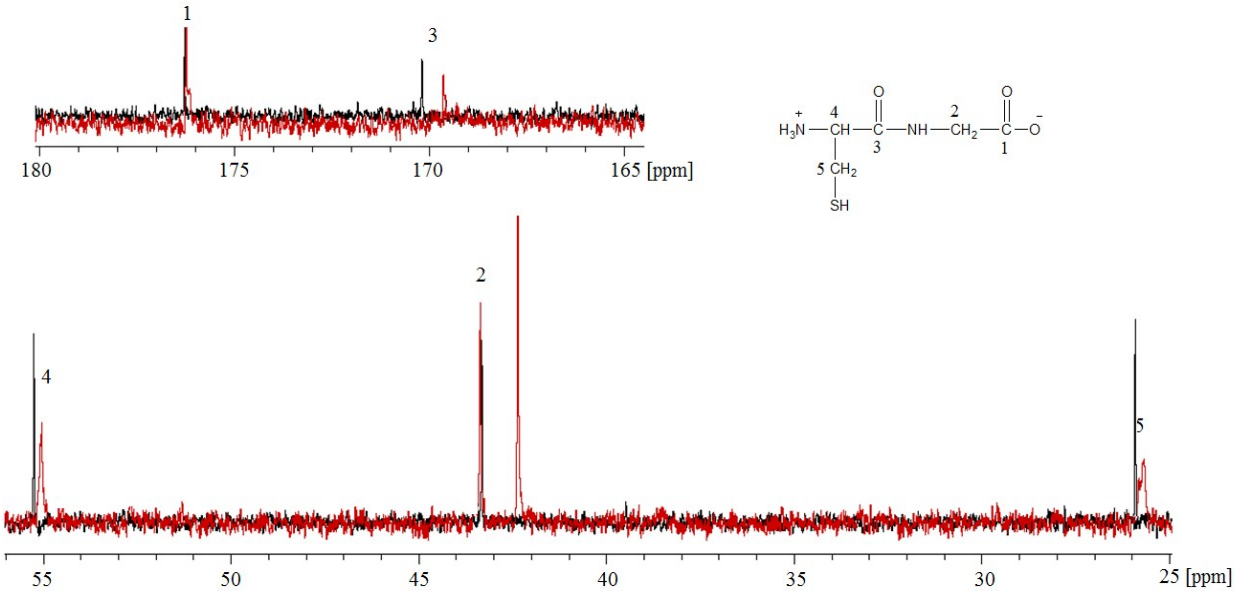


Fig. S5 ^{13}C NMR spectra of Cys-Gly in the absence (black) and in the presence (red) of 1 at pD 7.4 (the signal at 42.36 ppm belongs to the CH_2 carbon of the ethyl group in the counter ion).

Table S3. ^{13}C NMR chemical shift values (ppm) of 20.0 mM Cys-Gly in the absence and in the presence of 2.0 mM **1** at pD 7.4

^{13}C NMR	20.0 mM Cys-Gly	20.0 mM Cys-Gly + 2.0 mM **1**	Δδ (ppm)
δ_1	176.25	176.22	0.03
δ_2	43.31	43.35	0.04
δ_3	170.18	169.67	0.51
δ_4	55.24	55.03	0.21
δ_5	26.00	25.67	0.33

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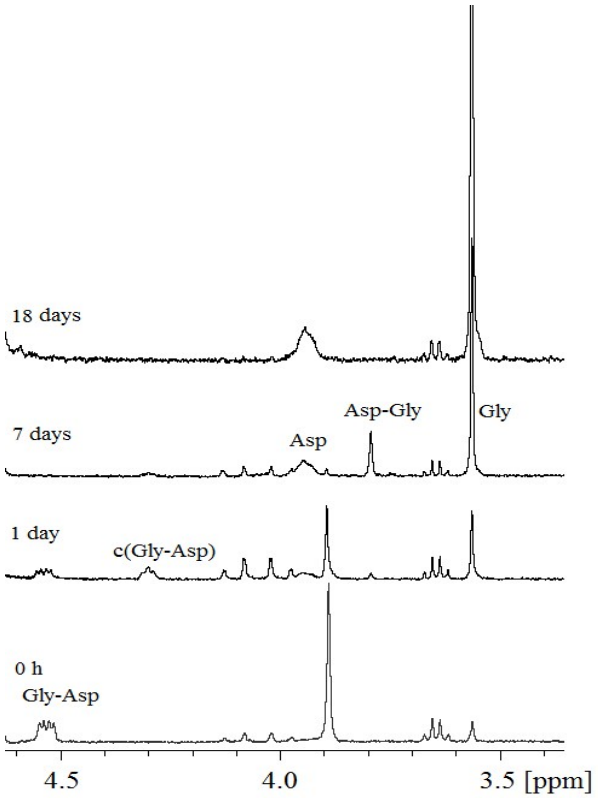


Fig. S6 ^{1}H NMR spectra of Gly-Asp recorded at different time increments for the reaction between 2.0 mM Gly-Asp and 2.0 mM **1** at pD 5.4 and 80 °C.

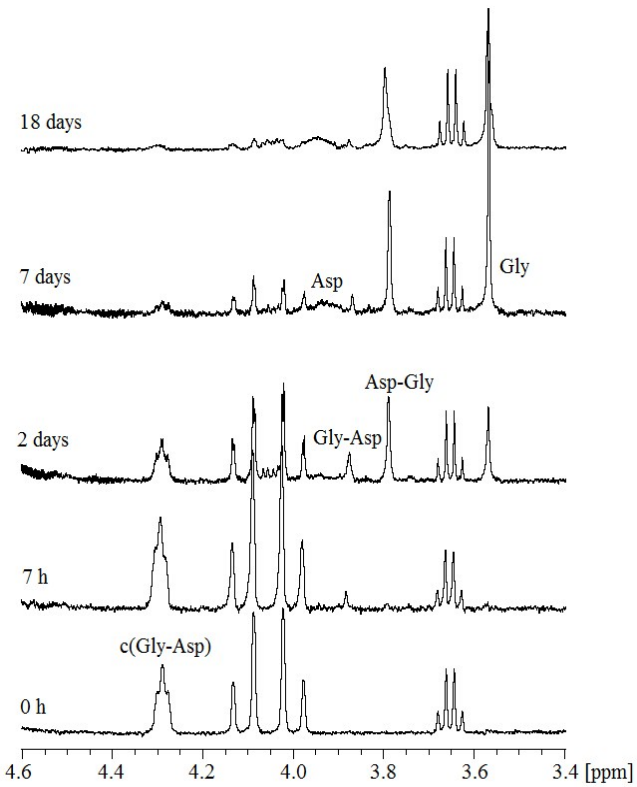


Fig. S7 ^{1}H NMR spectra of c(Gly-Asp) recorded at different time increments for the reaction between 2.0 mM c(Gly-Asp) and 2.0 mM **1** at pD 5.4 and 80 °C.

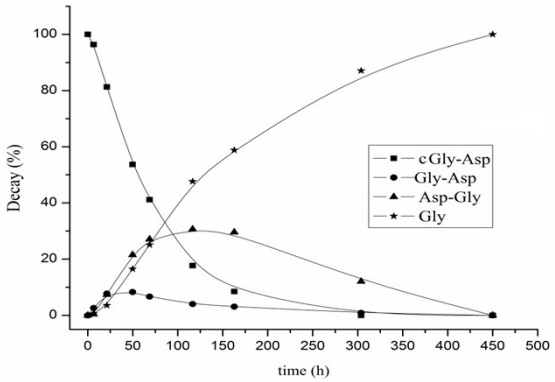
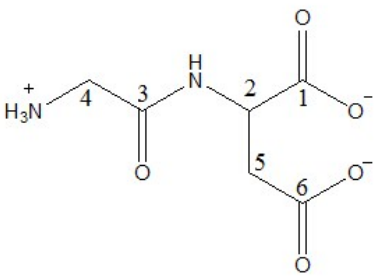


Fig. S8 Fraction of cGly-Asp, Gly-Asp, Asp-Gly, and Gly as a function of time in the reaction of 2.0 mM cGly-Asp and 2.0 mM **1** at pD 5.4 and 80 °C.

Table S4. ^{13}C NMR chemical shift values (ppm) of 20.0 mM Gly-Asp in the absence and in the presence of 2.0 mM **1** at different pD values.

pD	^{13}C NMR	20.0 mM Gly-Asp	20.0 mM Gly-Asp + 2.0 mM **1**	Δδ (ppm)
5.4	δ_1	177.91	177.86	0.05
	δ_2	52.95	52.80	0.15
	δ_3	166.37	166.38	0.01
	δ_4	40.53	40.54	0.01
	δ_5	39.04	38.94	0.10
	δ_6	178.12	178.50	0.38
6.9	δ_1	178.31	178.28	0.03
	δ_2	53.41	53.38	0.03
	δ_3	166.46	166.42	0.04
	δ_4	40.60	40.59	0.01
	δ_5	39.62	39.59	0.03
	δ_6	178.82	178.81	0.01
7.4	δ_1	178.31	178.27	0.04
	δ_2	53.41	53.38	0.03
	δ_3	166.57	166.42	0.15
	δ_4	40.64	40.59	0.05
	δ_5	39.63	39.59	0.04
	δ_6	178.83	178.81	0.02
8.1	δ_1	178.36	178.33	0.03
	δ_2	53.37	53.39	0.02
	δ_3	167.17	166.57	0.6
	δ_4	40.86	40.63	0.23
	δ_5	39.62	39.61	0.01
	δ_6	178.86	178.84	0.02



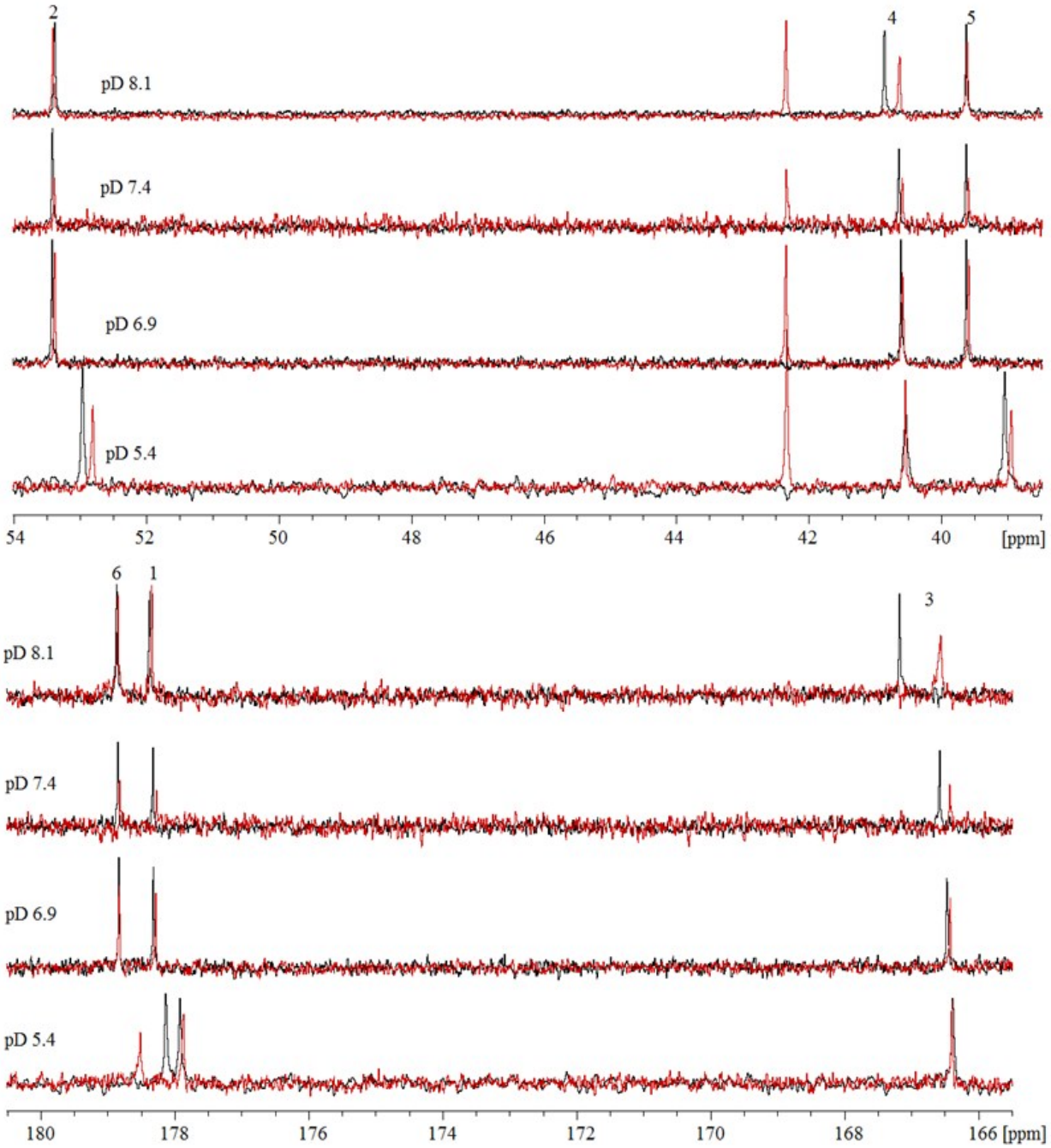


Fig. S9 ^{13}C NMR spectra of 20.0 mM Gly-Asp in the absence (black) and in the presence (red) of 2.0 mM **1** at different pD values (the signal at 42.4 ppm belongs to the CH_2 carbon of the ethyl group in the counter ion).

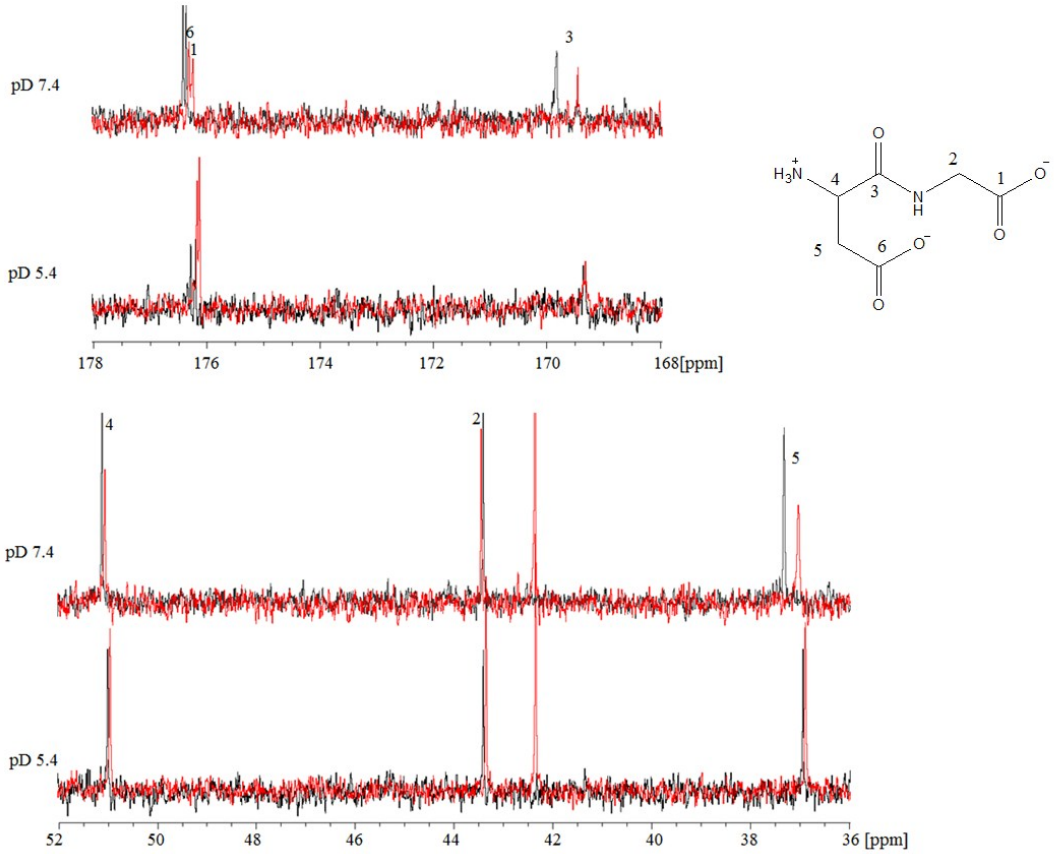


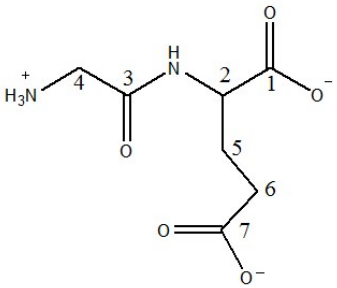
Fig. S10 ^{13}C NMR spectra of Asp-Gly in the absence (red) and presence (black) of 1 at different pD values (the signal at 42.35 ppm belongs to the CH_2 carbon of the ethyl group in the counter ion).

Table S5. ^{13}C NMR chemical shift values (ppm) of 20.0 mM Asp-Gly in the absence and presence of 2.0 mM **1** at different pD values

pD	^{13}C NMR	20.0 mM Asp-Gly	20.0 mM Asp-Gly + 2.0 mM **1**	Δδ (ppm)
5.4	δ_1	176.17	176.11	0.06
	δ_2	43.39	43.34	0.05
	δ_3	169.30	169.32	0.02
	δ_4	51.00	50.95	0.05
	δ_5	36.91	36.87	0.04
	δ_6	176.27	176.15	0.12
7.4	δ_1	176.34	176.23	0.11
	δ_2	43.40	43.43	0.03
	δ_3	169.82	169.43	0.39
	δ_4	51.12	51.06	0.06
	δ_5	37.31	37.01	0.30
	δ_6	176.40	176.29	0.11

Table S6. ^{13}C NMR chemical shift values (ppm) of 20.0 mM Gly-Glu in the absence and in the presence of 2.0 mM **1** at pD 5.4 and 7.8

pD	^{13}C NMR	20.0 mM Gly-Glu	20.0 mM Gly-Glu + 2.0 mM **1**	Δδ (ppm)
5.4	δ_1	178.38	178.35	0.03
	δ_2	55.25	55.20	0.05
	δ_3	166.46	166.48	0.02
	δ_4	40.50	40.52	0.02
	δ_5	28.02	27.93	0.09
	δ_6	33.45	33.32	0.13
	δ_7	181.29	181.37	0.08
7.8	δ_1	178.56	178.53	0.03
	δ_2	55.40	55.41	0.01
	δ_3	167.09	166.59	0.50
	δ_4	40.76	40.58	0.18
	δ_5	28.32	28.30	0.02
	δ_6	34.10	34.08	0.02
	δ_7	182.13	182.10	0.03



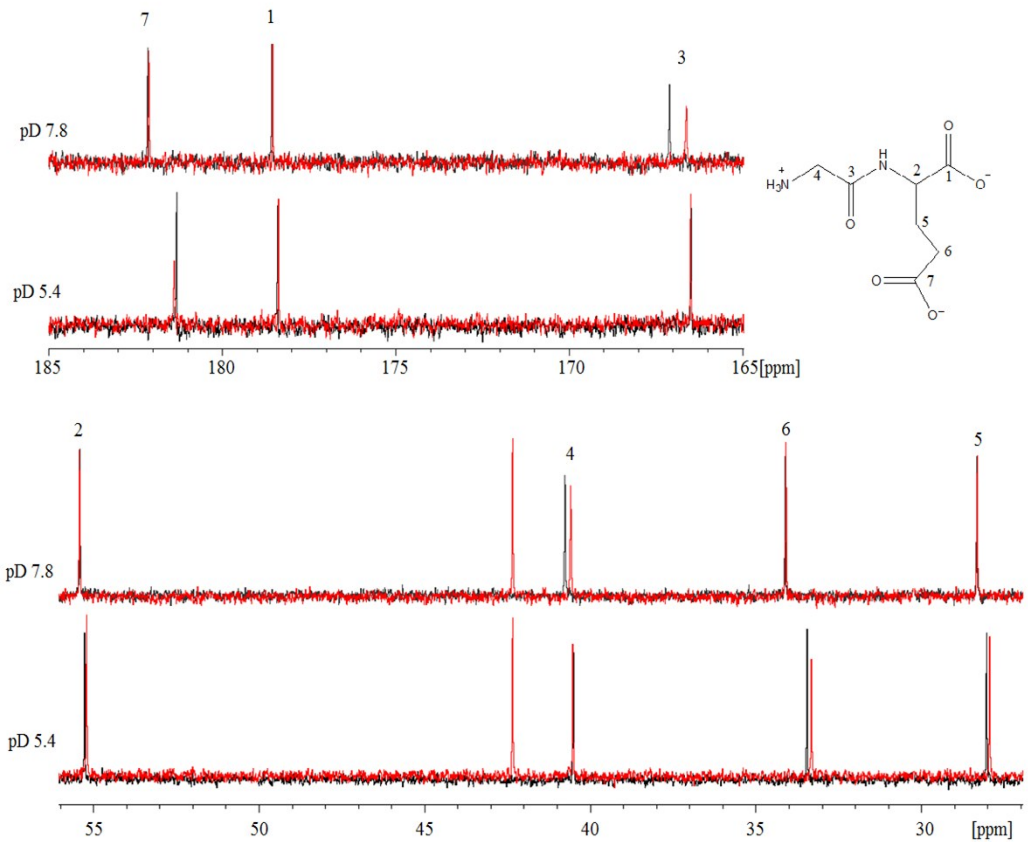


Fig. S11 ^{13}C NMR spectra of 20.0 mM Gly-Glu in the absence (black) and in the presence (red) of 2.0 mM **1** at pD 5.4 and 7.8 (the signal at 42.33 ppm belongs to the CH_2 carbon of the ethyl group in the counter ion).

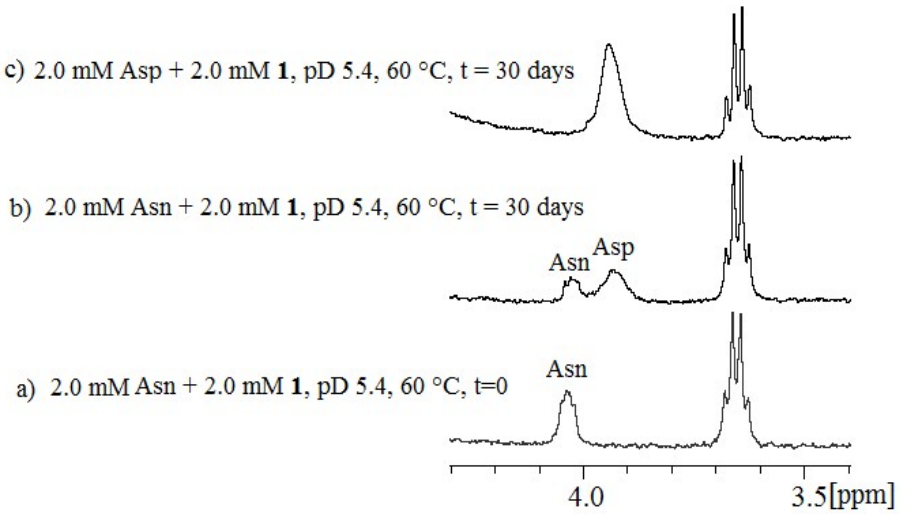
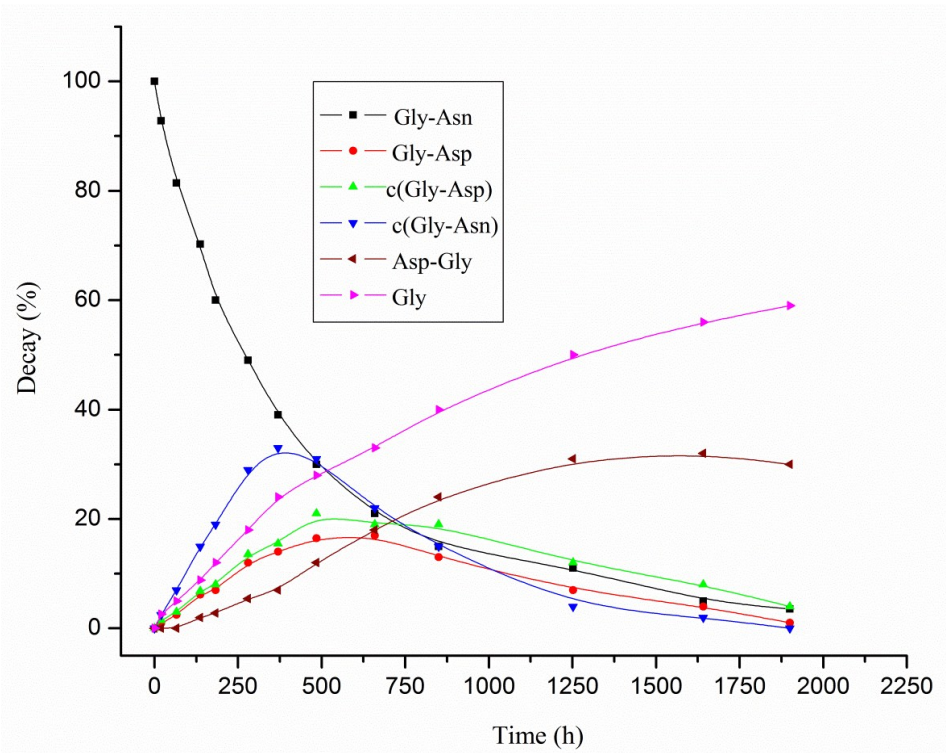


Fig. S12 ^{1}H NMR spectra of 2.0 mM Asn (a, b) and Asp (c) in the presence of 2.0 mM **1** at pD 5.4 and 60 °C.



5 **Fig. S13** Fraction of Gly-Asn, Gly, c(Gly-Asn), c(Gly-Asp), Gly-Asp, and Asp-Gly as a function of time in the reaction between 2.0 mM Gly-Asn and 2.0 mM **1** at pD 5.4 and 60 °C.

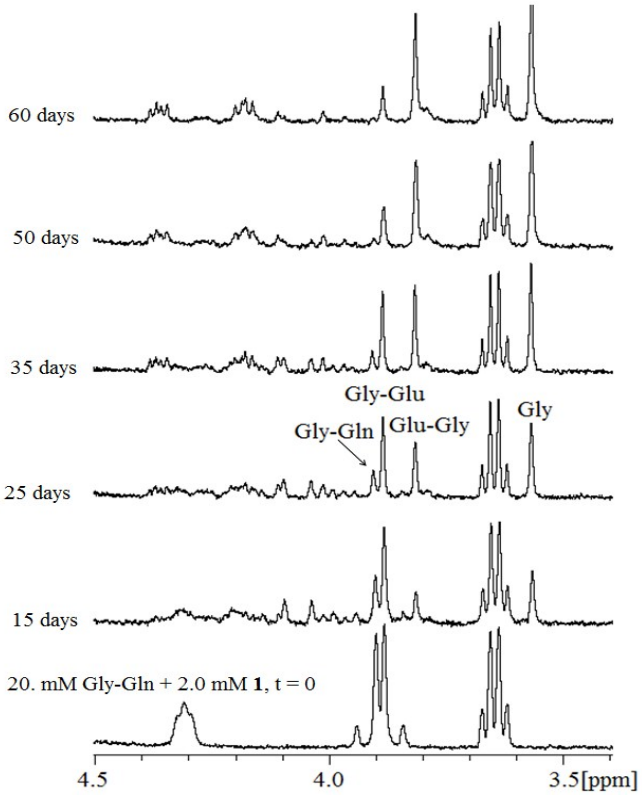


Fig. S14 ^{1}H NMR spectra of Gly-Gln recorded at different time increments for the reaction between 2.0 mM Gly-Gln and 2.0 mM **1** at pD 5.4 and 60 °C.

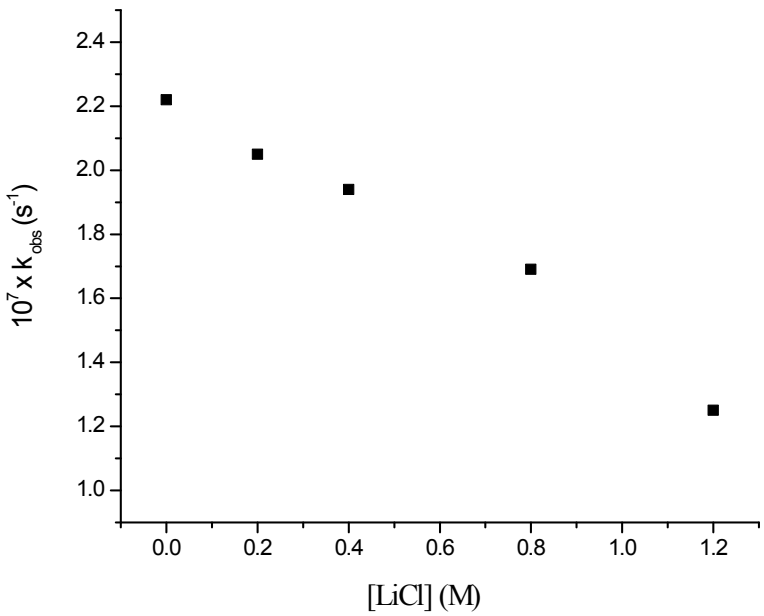


Fig. S15 Influence of the concentration of LiCl on the rate constant for the reaction between 2.0 mM Gly-Lys and 2.0 mM 1 at pD 5.4 and 60 °C.

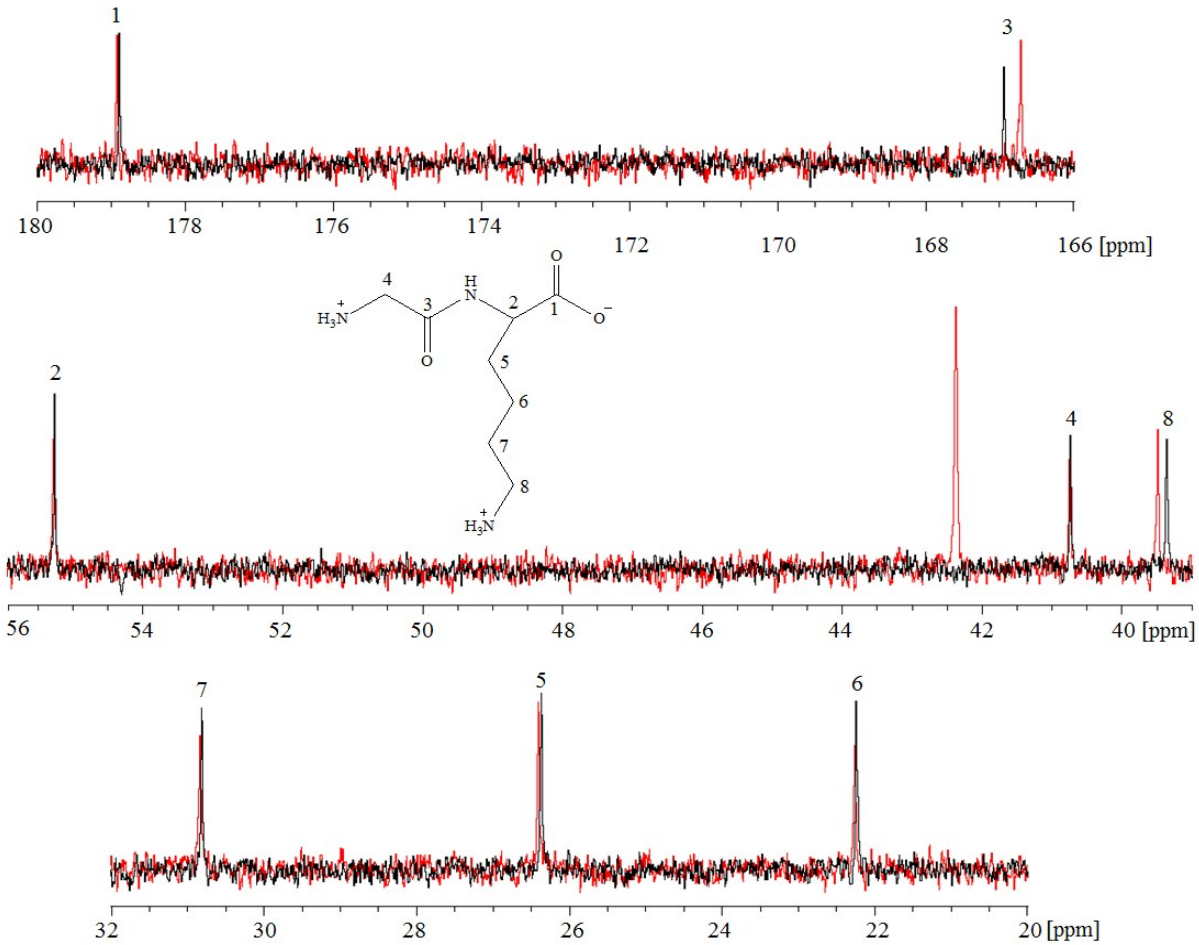


Fig. S16 ^{13}C NMR spectra of Gly-Lys in 1.2 M LiCl and in the absence (black) and presence (red) of 1 at pD 7.4 (the signal at 42.4 ppm belongs to the CH_2 carbon of the ethyl group in the counter ion).

Table S7. ^{13}C NMR chemical shift values (ppm) of 15.0 mM Gly-Lys in 1.2 M LiCl and in the absence and presence of 2.0 mM **1** at pD 7.4

^{13}C NMR	15.0 mM Gly-Lys	15.0 mM Gly-Lys + 2.0 mM **1**	Δδ (ppm)
δ_1	178.88	178.91	0.03
δ_2	55.25	55.27	0.02
δ_3	166.93	166.70	0.23
δ_4	40.72	40.73	0.01
δ_5	26.36	26.40	0.04
δ_6	22.23	22.25	0.02
δ_7	30.81	30.83	0.02
δ_8	39.35	39.48	0.13

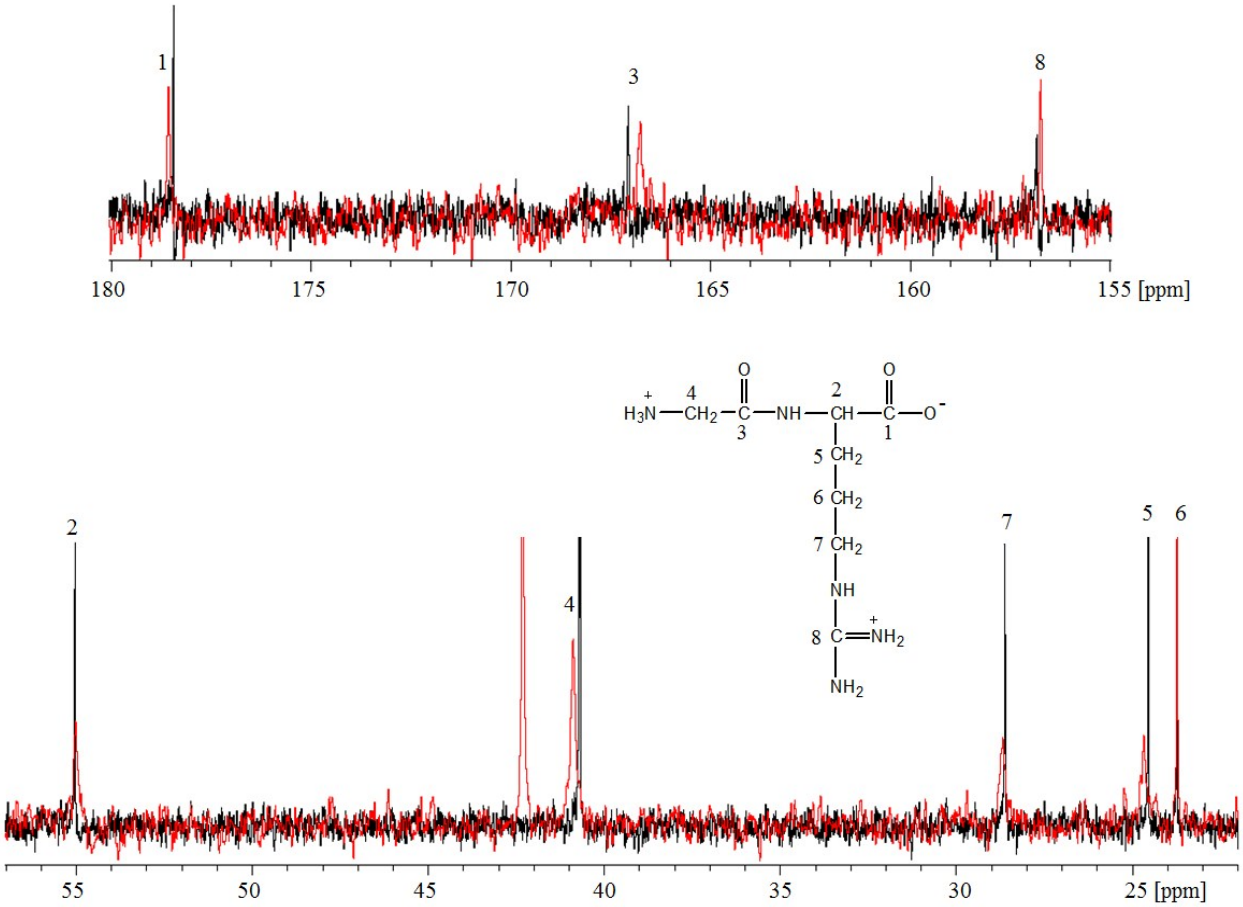


Fig. S17 ^{13}C NMR spectra of Gly-Arg in the absence (red) and in the presence (black) of 1 at pD 7.4 (the signal at 42.4 ppm belongs to the CH_2 carbon of the ethyl group in the counter ion).

Table S8. ^{13}C NMR chemical shift values (ppm) of 20.0 mM Gly-Arg in the absence and in the presence of 2.0 mM **1** at pD 7.4

^{13}C NMR	20.0 mM Gly-Arg	20.0 mM Gly-Arg + 2.0 mM **1**	Δδ (ppm)
δ_1	178.42	178.51	0.09
δ_2	55.02	55.02	0.00
δ_3	167.03	166.85	0.23
δ_4	40.69	40.90	0.21
δ_5	24.54	24.66	0.12
δ_6	23.74	23.74	0.00
δ_7	28.61	28.72	0.11
δ_8	156.81	156.75	0.06

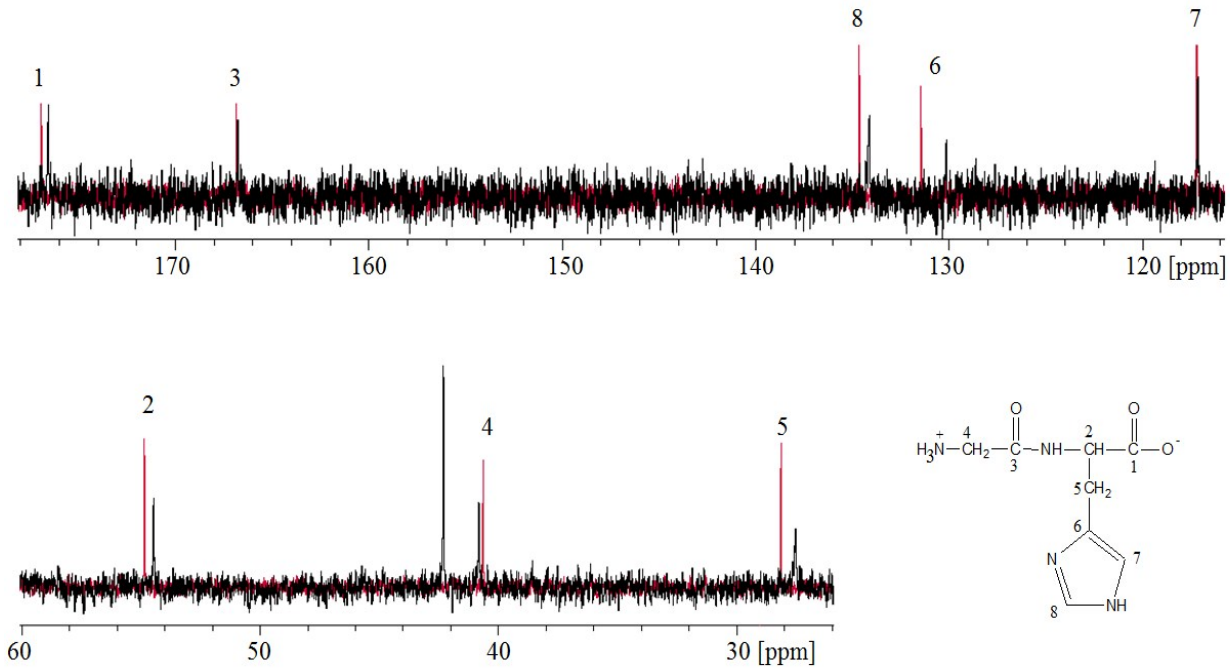


Fig. S18 ^{13}C NMR spectra of Gly-His in the absence (red) and in the presence (black) of **1** at pD 7.4 (the signal at 42.4 ppm belongs to the CH_2 carbon of the ethyl group in the counter ion).

Table S9. ^{13}C NMR chemical shift values (ppm) of 20.0 mM Gly-His in the absence and in the presence of 2.0 mM **1** at pD 7.4

^{13}C NMR	20.0 mM Gly-His	20.0 mM Gly-His + 2.0 mM **1**	Δδ (ppm)
δ_1	176.88	176.50	0.38
δ_2	54.84	54.45	0.39
δ_3	166.79	166.71	0.08
δ_4	40.63	40.81	0.18
δ_5	28.13	27.52	0.58
δ_6	131.41	130.10	1.31
δ_7	117.16	117.09	0.07
δ_8	134.60	134.07	0.53

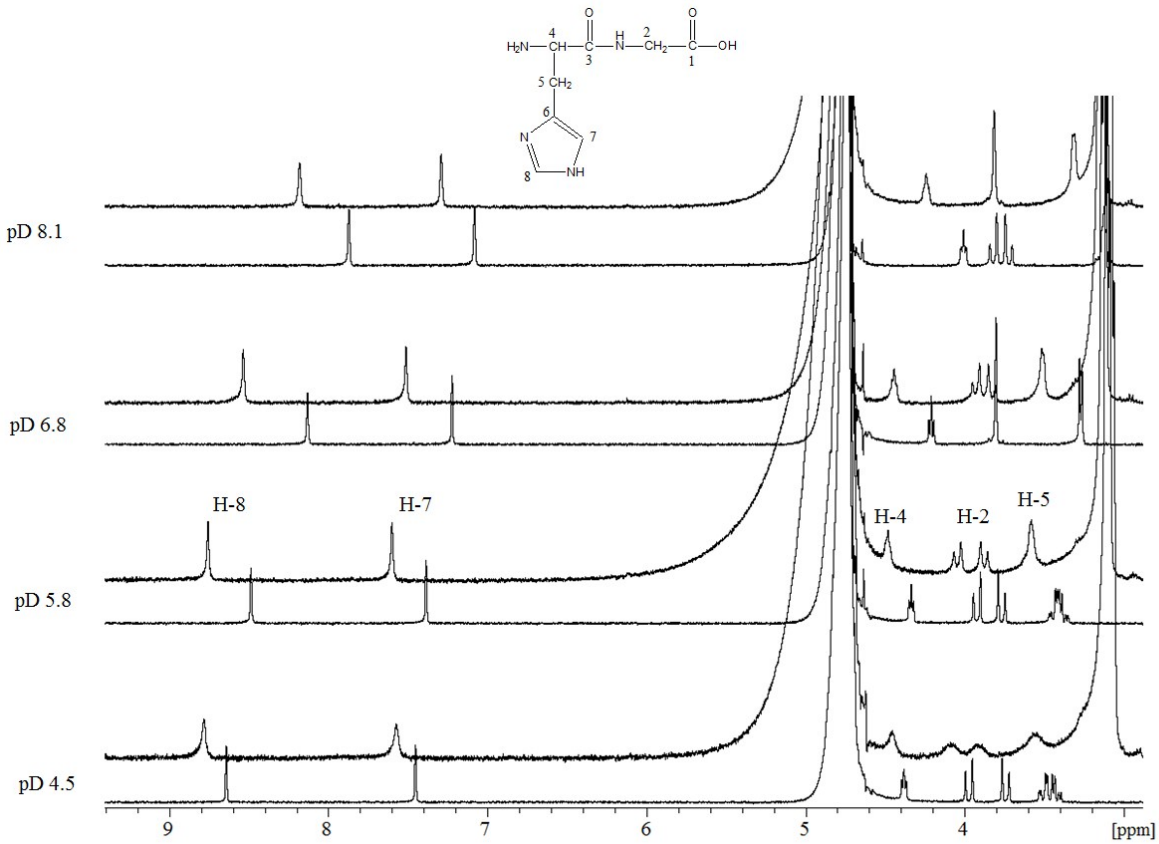


Fig. S19 [1]H NMR spectra of 2.0 mM His-Gly in the absence (lower) and in the presence (upper) of 2.0 mM **1** at different pD values.

Table S10. [1]H NMR chemical shift differences (ppm) of 2.0 mM His-Gly in the absence and in the presence of 2.0 mM **1** at different pD values

[1]H NMR	Δδ (ppm)			
	pD 4.5	pD 5.8	pD 6.8	pD 8.1
δ_2	0.14	0.12	0.08	0.05
δ_4	0.08	0.15	0.23	0.24
δ_5	0.10	0.17	0.24	0.29
δ_7	0.12	0.21	0.29	0.21
δ_8	0.14	0.27	0.40	0.31

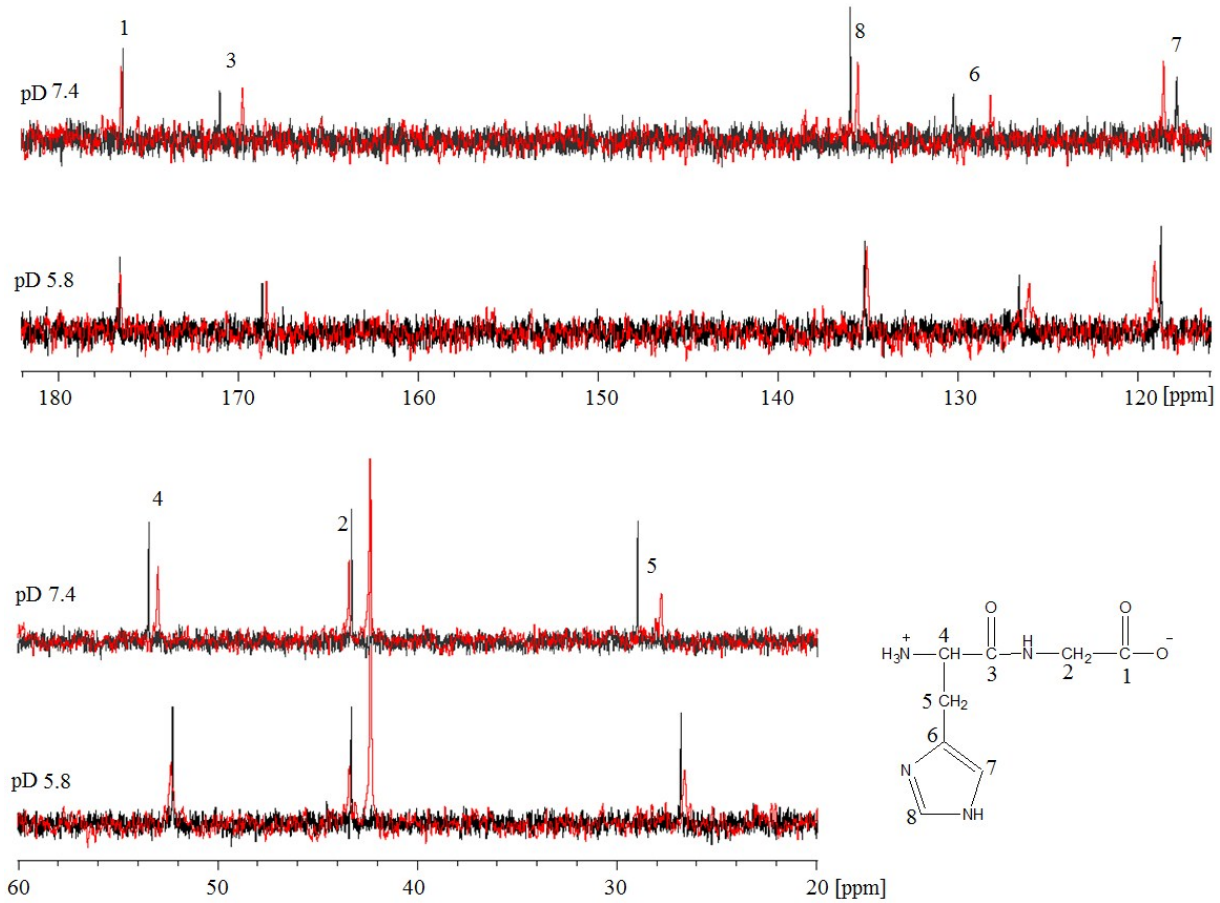


Fig. S20 ^{13}C NMR spectra of His-Gly in the absence (black) and in the presence (red) of **1** at pD 5.8 and 7.4 (the signal at 42.34 ppm belongs to the CH_2 carbon of the ethyl group in the counter ion).

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Table S11. ^{13}C NMR chemical shift values (ppm) of 15.0 mM His-Gly in the absence and in the presence of 2.0 mM **1** at pD 5.8 and pD 7.4

pD	^{13}C NMR	15.0 mM His-Gly	15.0 mM His-Gly + 2.0 mM **1**	Δδ (ppm)
5.8	δ_1	176.55	176.53	0.02
	δ_2	43.29	43.35	0.06
	δ_3	168.63	168.40	0.23
	δ_4	52.25	52.34	0.09
	δ_5	26.76	26.56	0.20
	δ_6	126.51	125.97	0.54
	δ_7	118.63	118.98	0.35
	δ_8	135.10	135.00	0.10
7.4	δ_1	176.39	176.47	0.08
	δ_2	43.27	43.39	0.12
	δ_3	171.00	169.72	1.32
	δ_4	53.44	52.97	0.47
	δ_5	28.93	27.73	1.20
	δ_6	128.02	130.20	2.18
	δ_7	117.75	118.48	0.73
	δ_8	135.92	135.49	0.43

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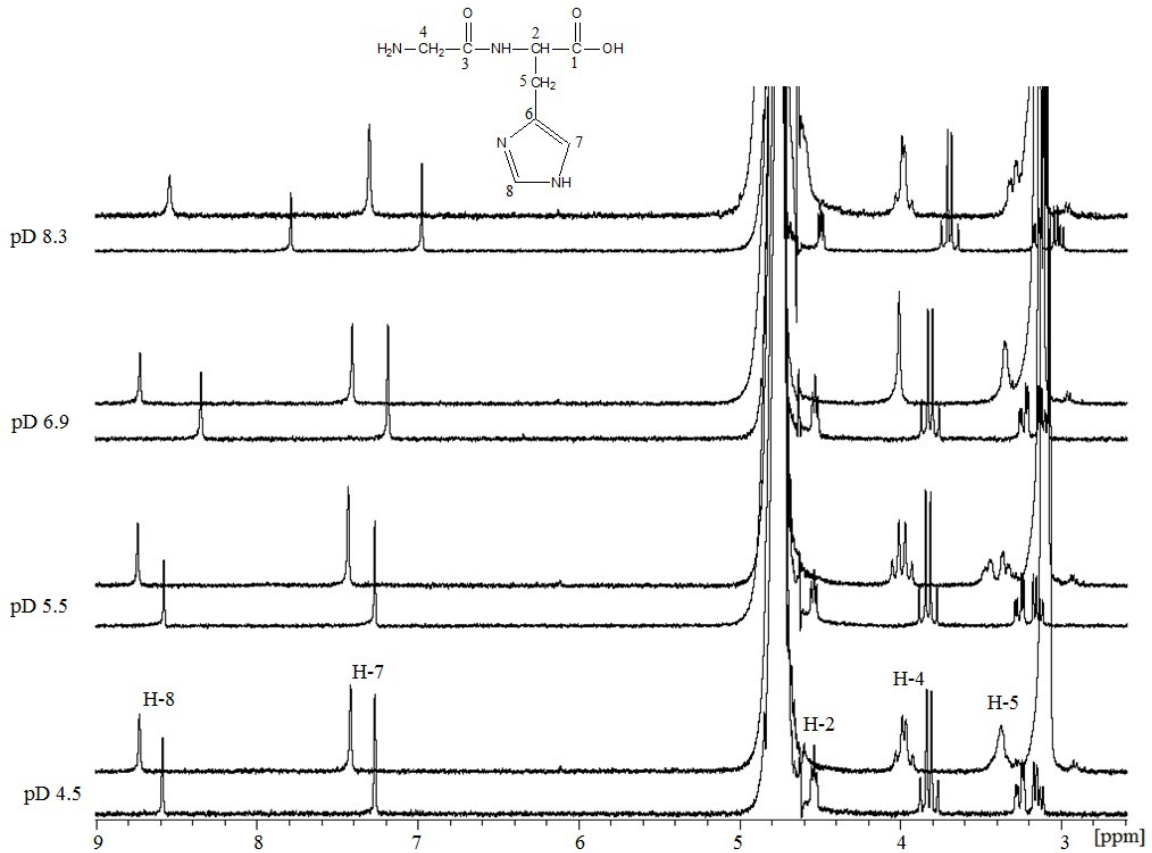

Fig. S21 ^{1}H NMR spectra of 2.0 mM Gly-His in the absence (lower) and in the presence (upper) of 2.0 mM **1** at different pD values.

Table S12. ^{1}H NMR chemical shift differences (ppm) of 2.0 mM Gly-His in the absence and in the presence of 2.0 mM **1** at different pD values

^{1}H NMR	Δδ (ppm)			
	pD 4.5	pD 5.5	pD 6.9	pD 8.3
δ_2	nd	nd	nd	nd
δ_4	0.15	0.17	0.20	0.28
δ_5	0.14	0.19	0.14	0.15
δ_7	0.15	0.17	0.22	0.33
δ_8	0.14	0.16	0.38	0.75