Supporting Information

NMR Studies of Protonation and Hydrogen Bond States of Internal Aldimines of Pyridoxal 5'-Phosphate Acid-Base in Alanine Racemase, Aspartate Aminotransferase and Poly-L-Lysine

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Preparation and characterization of ^{15}N-PLL$_l$

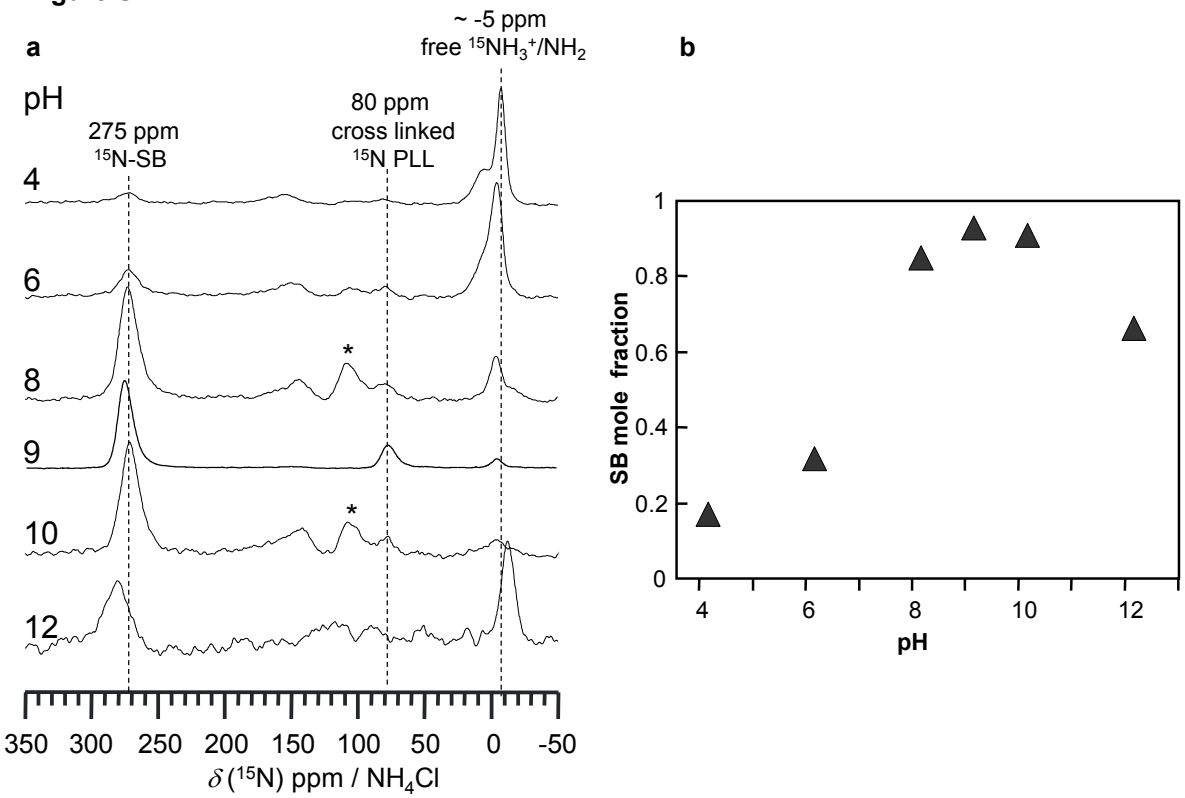
A thoroughly dried flask, which was purged with argon, was charged with a suspension of **N^{ε},N^{ε}-Di-(*tert*-butoxycarbonyl)-L-lysine** (2.9 g, 8 mmol, 3 eq) in EtAc (100 mL). Then, triphosgene (766 mg, 2.7 mmol, 1 eq) was added, while the suspension was vigorously stirred at room temperature. After 10 min, freshly distilled triethylamine (0.38 mL, 2.8 mmol, 1.05 eq) was added. Upon addition of triethylamine, precipitation of triethylamine hydrochloride was observed. After stirring for 5 h, the reaction mixture was cooled down to – 18 °C to allow complete precipitation of triethylamine hydrochloride. The precipitate was removed by filtration, and the cold organic phase was washed with ice-cold water and 0.5% $NaHCO_3$ (aqueous). The organic phase was separated, dried over $MgSO_4$, filtered and concentrated to approximately 1/3 of the initial volume. The addition of hexane resulted in the precipitation of the desired product, which was filtered, dried, and recrystallized at least one more time from EtAc/hexane before polymerization. The reaction yielded 1.4 g (68 %) of ^{15}N-NCA as a white crystal. **^{13}C {^{1}H} -NMR** (125 MHz, DMSO-d6): δ = 171.63 (s, C-4), 155.56 (s, C-11), 151.95 (s, C-2), 77.36 (s, C-12), 57.00 (d, C-5), 30.62 (t, C-6), 28.84 (t, C-7 or C-8), 28.23 (q, C-13), 21.57 (t, C-7 or C-8).**^{1}H-NMR** (500 MHz, DMSO-d6): δ = 7.5 (s, 1H, H-6), 5.9 (s, 1H, H-4'), 4.7 (s, 2H, H-3'), 1.9 (s, 3H, H-2'). **MS** (ESI-FT ICR MS): m/z (%) = 312.0958 ($[M + K]^+$, M_{calc} = 312.09741), 296.1228 ($[M + Na]^+$, M_{calc} = 296.12348), 291.1682 ($[M + NH_4]^+$, M_{calc} = 291.16808), 274.1407 ([M + H]+, M_{calc} = 274.14153), 218.0785 ($[M - {}^{t}Bu]^+$), 174.0888 ($[M - Boc]^+$). Polymerization to ^{15}N-Boc-poly-L-lysine was undertake in a dried flask and under Argon atmosphere, NCA (600 mg, 2.2 mmol) was dissolved in dry 1,4-dioxane (20 mL). Freshly distilled triethylamine (2.2 mg, 3 µL, A/I=100 = 22 10^{-6} mol) was added to the mixture. After 7 days stirring with closed flask, the reaction mixture was poured inside water (200-400 mL) which resulted in a white precipitate. After filtration on a Büchner, the solid was lyophilzed for 2 days.

Table S1. Acquisition parameters of the NMR spectra shown in the main text.

Figure	molecule	experiment	90 ° pulse ^{1}H in µs	contact time CP in ms	90 ° pulse of ^{15}N or ^{13}C in µs	pulse delay in second	MAS in kHz	magnetic field	T in K	rotor diameter in mm
1a	2	$^{15}N\{^{1}H\}$			11			12	130	
1b	3	$^{15}N\{^{1}H\}$			11			12	300	
1c		^{15}N CPMAS	6.5	2		3 s	8	14	108	4
1d		^{15}N CPMAS	2.79	2		4 s	8	14	108	4
2a	2	$^{15}N\{^{1}H\}$			11			12	130	
2b		^{15}N CPMAS	4			3 s	7	14	225	4
2c		$^{15}N\{^{1}H\}$			11			14	282	
3a	1s"	^{13}C CPMAS	3.5	2		4 s	6	14	298	4
3a	1s"	^{15}N CPMAS	3.8	2		3 s	7	14	298	4
3b	1s"	^{13}C CPMAS	3.5	2		3 s	6	14	298	4
3b	1s"	^{15}N CPMAS	3.8	2		3 s	7	14	298	4
3c	1s'	^{15}N CPMAS	3.8	2		3 s	7	14	298	4
3d	1s*	^{13}C CPMAS	3.8	2		3 s	7	14	298	4
3d	1s*	^{15}N CPMAS	3.8	2		3 s	7	14	298	4
4a	1s"	^{13}C CPMAS	3.8	2		3 s	7	14	298	4
4a	1s"	^{15}N CPMAS	3.8	2		3 s	7	14	298	4
4b	1s"	^{13}C CPMAS	5.5	0.7		4 s	6	7	298	6
4b	1s"	^{15}N CPMAS	3.5	0.7		4 s	7	7	298	6
4c	1s"	^{13}C CPMAS	5.5	0.7		4 s	6	7	298	6
4c	1s"	^{15}N CPMAS	3.5	0.7		4 s	7	7	298	6
4d	1s"	^{13}C CPMAS	3.5	2		4 s	6	7	298	6
4d	1s"	^{15}N CPMAS	3.5	2		4 s	6	7	298	6
4e	1s*	^{15}N CPMAS	3.8	2		3 s	7	14	298	4
4f	1s"	$^{13}C\{^{1}H\}$			3.5	4 s	6	7	298	6
4f	1s"	$^{15}N\{^{1}H\}$			4.5	2 s	5	7	298	6
5	1s'-e/k	^{13}C CPMAS	2	0.5		3 s	12	14	298	4
5	1s'-e/k	^{15}N CPMAS	2	2		3 s	12	14	298	4

Figure S1. ^{15}N CP-MAS 7 kHz 14 T, spectra of a 1:1 mixture of ^{15}N-PLL_h with PLP lyophilized from aqueous solutions (6 mM) at different pH values recorded at room temperature. Figure 1b. Plot of the Schiff base mole fraction depending on the pH value of the aqueous solution before lyophilisation.

Figure S1


a
~ -5 ppm
free $^{15}NH_3^+/NH_2$
pH
275 ppm
^{15}N-SB
80 ppm
cross linked
^{15}N PLL
4
6
8
9
10
12
*
*
350 300 250 200 150 100 50 0 -50
δ (^{15}N) ppm / NH_4Cl
b
SB mole fraction
0
0.2
0.4
0.6
0.8
1
4
6
8
10
12
pH