Electronic Supplementary Material (ESI) for New Journal of Chemistry.

Supplementary Material

Understanding the behaviour of carnosine in aqueous solution: an experimental and quantum-based computational investigation on acid-base properties and complexation mechanisms with Ca^{2+} and Mg^{2+}

Chiara Abate,[a] Giuseppe Cassone,[b,*] Massimiliano Cordaro,[a] Ottavia Giuffrè,[a] Viviana Mollica-Nardo,[b] Rosa Celeste Ponterio,[b] Franz Saija,[b] Jiri Sponer,[c] Sebastiano Trusso[b] and Claudia Foti[a,*]

[a]*Dipartimento di Scienze Chimiche, Biologiche, Farmaceutiche e Ambientali, Università di Messina, Viale F. Stagno d'Alcontres 31, 98166 Messina, Italy*

[b]*Institute for Chemical-Physical Processes, National Research Council of Italy (IPCF-CNR), Viale F. Stagno d'Alcontres 37, 98158 Messina, Italy*

[c]*Institute of Biophysics of the Czech Academy of Sciences (IBP-CAS), Kràlovopolskà 135, 61265 Brno, Czechia*

Table S1. Chemical shifts for each nucleus

δ	L	LH	LH_2	LH_3
CH2	7.64(1)	7.70(1)	8.58(1)	8.61(1)
CH4	6.91(38)	6.93(48)	7.26(38)	7.32(38)
CH6a	3.12(9)	3.11(9)	3.25(9)	3.34(9)
CH6b	2.93(4)	2.95(4)	3.10(4)	3.22(4)
CH10	2.46(34)	2.66(34)	2.68(34)	2.72(33)
CH11	2.92(8)	3.24(8)	3.23(8)	3.25(8)

Table S2. Literature protonation constant values of carnosine

Ionic	T /K (°C)	I /mol L^{-1}	$\log\beta_1$	$\log\beta_2$	$\log\beta_3$	Ref
$NaClO_4$	298	0.1	9.397	16.20	17.493	a
	298	0.1	9.35	16.09	18.72	b
	298	0.1	9.32	15.89	-	c
KCl	298	0.1	9.372	16.146	18.740	d
	298	0.2	9.30	16.14	18.67	e
	298	0.2	9.39	16.23	18.76	f
KNO3	298	0.1	9.372	16.15	18.74	g
	298	0.1	9.466	16.30	18.90	h
	298	0.1	9.36	16.12	-	i
	310	0.15	9.04	15.83	18.37	J
	310	0.15	9.04	15.62	18.26	k

a. P Surdy,P Rubini,T Gajda; Inorg.Chem.,38,346 (1999)
b. T Gajda,B Henry,J-J Delpuech; J.Chem.Soc.,Perkin Trans.II,157 (1994)
c. T Kaden,A Zuberbuhler; Helv.Chim.Acta,49,2189 (1966)
d. P Daniele,E Prenesti,G Ostacoli; J.Chem.Soc.,Dalton Trans.,3269 (1996)
e. I Sovago,E Farkas,A Gergely; J.Chem.Soc.,Dalton Trans.,2159 (1982)
f. E Farkas,I Sovago,A Gergely; J.Chem.Soc.,Dalton Trans.,1545 (1983)
g. P Daniele,E Prenesti,V Zelano,G Ostacoli; Spectrochim.Acta,49A,1299 (1993)
h. G Brookes,L Pettit; J.Chem.Soc.,Dalton Trans.2112 (1975)
i. Lenz,A Martell; Biochemistry,3,745;750 (1964)
j. P Daniele,P Amico,G Ostacoli; Inorg.Chim.Acta,66,65 (1982)
k. R Agarwal,D Perrin; J.Chem.Soc.,Dalton Trans.268 (1975)

Table S3. Calculated values of protonation and Ca^{2+} and Mg^{2+} complex formation at different temperatures and ionic strengths

Reaction	**logβ**				
	T = 298K				
	***I* = 0.15**	***I* = 0.25**	***I* = 0.5**	***I* = 0.75**	***I* = 1**
$H^+ + L^- = HL^0$	9.38±0.03[a]	9.34±0.03[a]	9.28±0.03[a]	9.25±0.05[a]	9.23±0.07[a]
$2H^+ + L^- = H_2L^+$	16.17±0.03	16.14±0.03	16.10±0.04	16.09±0.06	16.08±0.08
$3H^+ + L^- = H_3L^{2+}$	18.86±0.04	18.84±0.04	18.80±0.04	18.76±0.06	18.72±0.08
$Ca^{2+} + L^- = CaL^+$	2.99±0.04	3.02±0.04	3.20±0.04	3.43±0.07	3.68±0.10
$Ca^{2+} + L^- + H^+ = CaLH^{2+}$	12.07±0.03	12.13±0.03	12.31±0.02	12.51±0.03	12.73±0.05
$Ca^{2+} + L^- + 2H^+ = CaLH_2^{3+}$	18.74±0.04	18.83±0.04	19.00±0.04	19.15±0.07	19.30±0.09
$Mg^{2+} + L^- = MgL^+$	2.70±0.02				
$Mg^{2+} + L^- + H^+ = MgLH^{2+}$	11.75±0.08	11.74±0.07	11.75±0.04	11.79±0.04	11.85±0.06
$Mg^{2+} + L^- + 2H^+ = MgLH_2^{3+}$	18.32±0.08	18.40±0.08	18.56±0.05	18.67±0.04	18.82±0.06
	***I* = 0.15**				
	278 K	**288 K**	**308 K**	**310 K**	**318 K**
$H^+ + L^- = HL^0$	9.9±0.1	9.65±0.08	9.12±0.07	9.07±0.08	8.9±0.1
$2H^+ + L^- = H_2L^+$	17.0±0.1	16.58±0.08	15.80±0.08	15.73±0.08	15.4±0.1
$3H^+ + L^- = H_3L^{2+}$	19.3±0.2	19.08±0.10	18.66±0.09	18.62±0.09	18.5±0.2
$Ca^{2+} + L^- = CaL^+$	2.77±0.10	2.88±0.07	3.08±0.06	3.10±0.07	3.17±0.10
$Ca^{2+} + L^- + H^+ = CaLH^{2+}$	11.86±0.08	11.97±0.05	12.17±0.04	12.19±0.05	12.26±0.07
$Ca^{2+} + L^- + 2H^+ = CaLH_2^{3+}$	18.40±0.12	18.58±0.07	18.89±0.07	18.92±0.07	19.04±0.11
$Mg^{2+} + L^- = MgL^+$	3.06±0.06	2.88±0.03	2.53±0.03	2.50±0.04	2.38±0.05
$Mg^{2+} + L^- + H^+ = MgLH^{2+}$	12.45±0.06	12.07±0.04	11.38±0.04	11.32±0.04	11.07±0.06
$Mg^{2+} + L^- + 2H^+ = MgLH_2^{3+}$	19.51±0.05	18.92±0.03	17.85±0.03	17.75±0.03	17.36±0.04

[a] ± 3 std. dev.

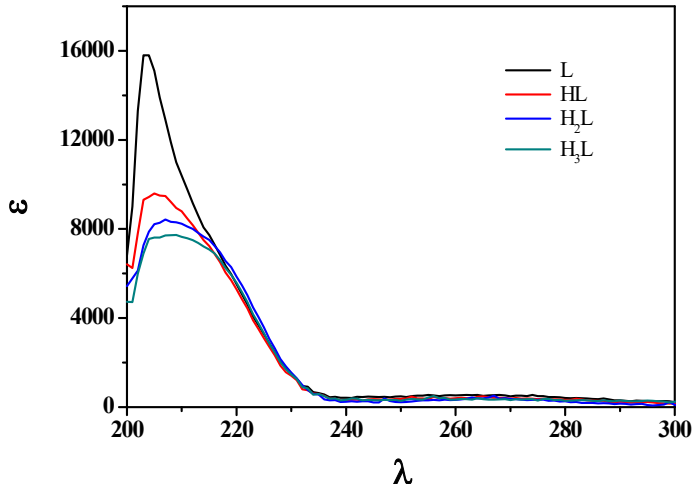

Fig. S1. Molar absorption coefficients of the H^+-Carnosine (L) species as a function of the wavelength.

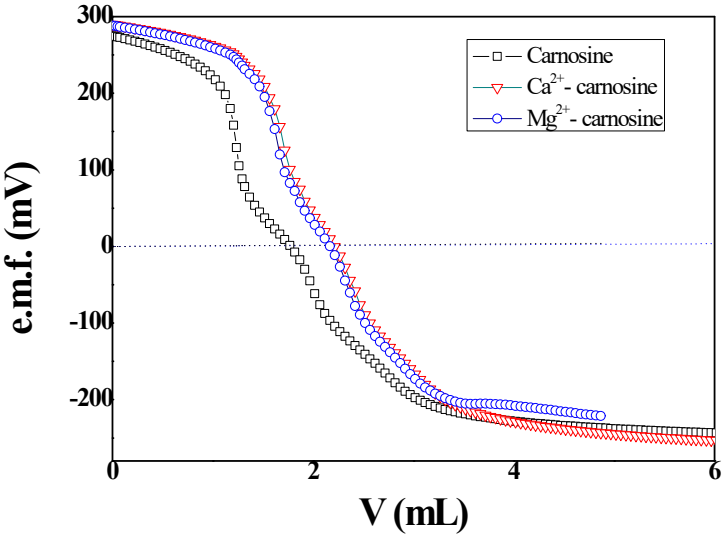

Fig. S2. Potentiometric titrations of the free ligand and Ca^{2+} and Mg^{2+}- carnosine mixtures in NaCl at *I* = 0.15 mol L^{-1} and T = 310 K (C_M = 1 mmol L^{-1}, C_L = 2 mmol L^{-1}).

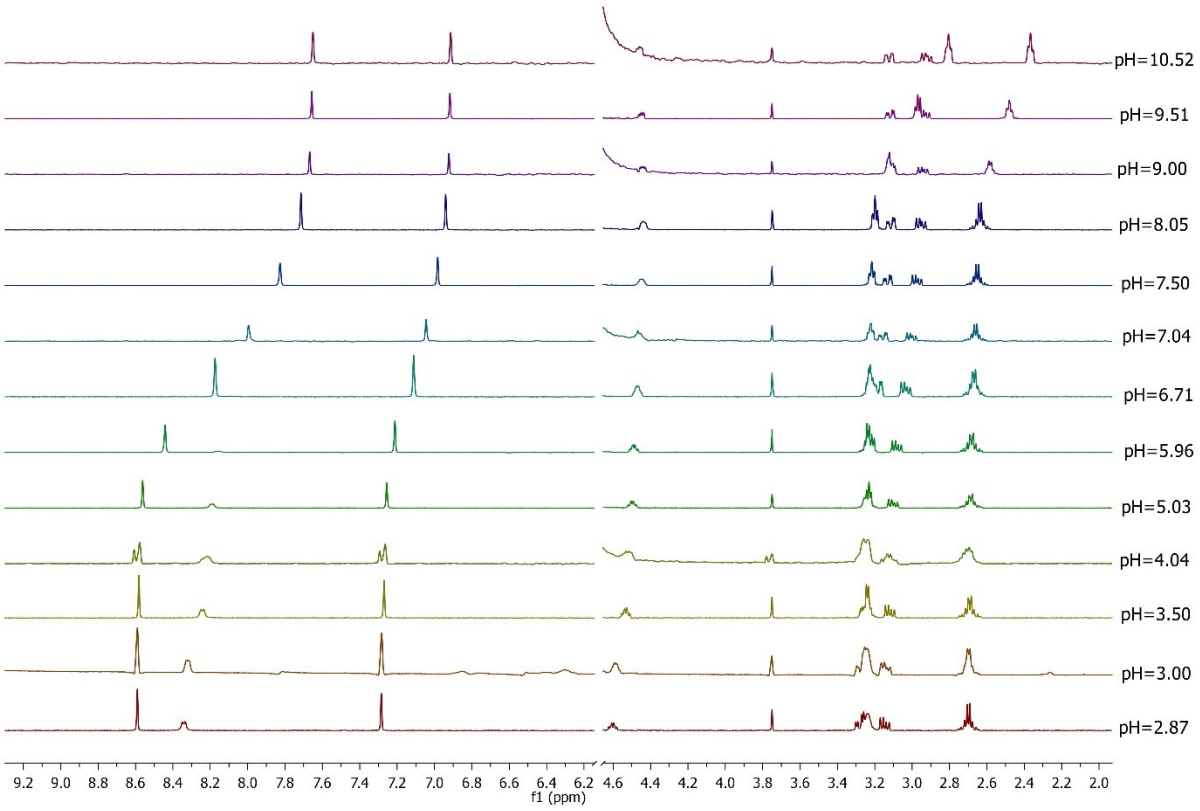

Fig. S3. ^{1}H NMR titration of Ca^{2+}-carnosine mixture in water, from pH 2.5 to pH 10.

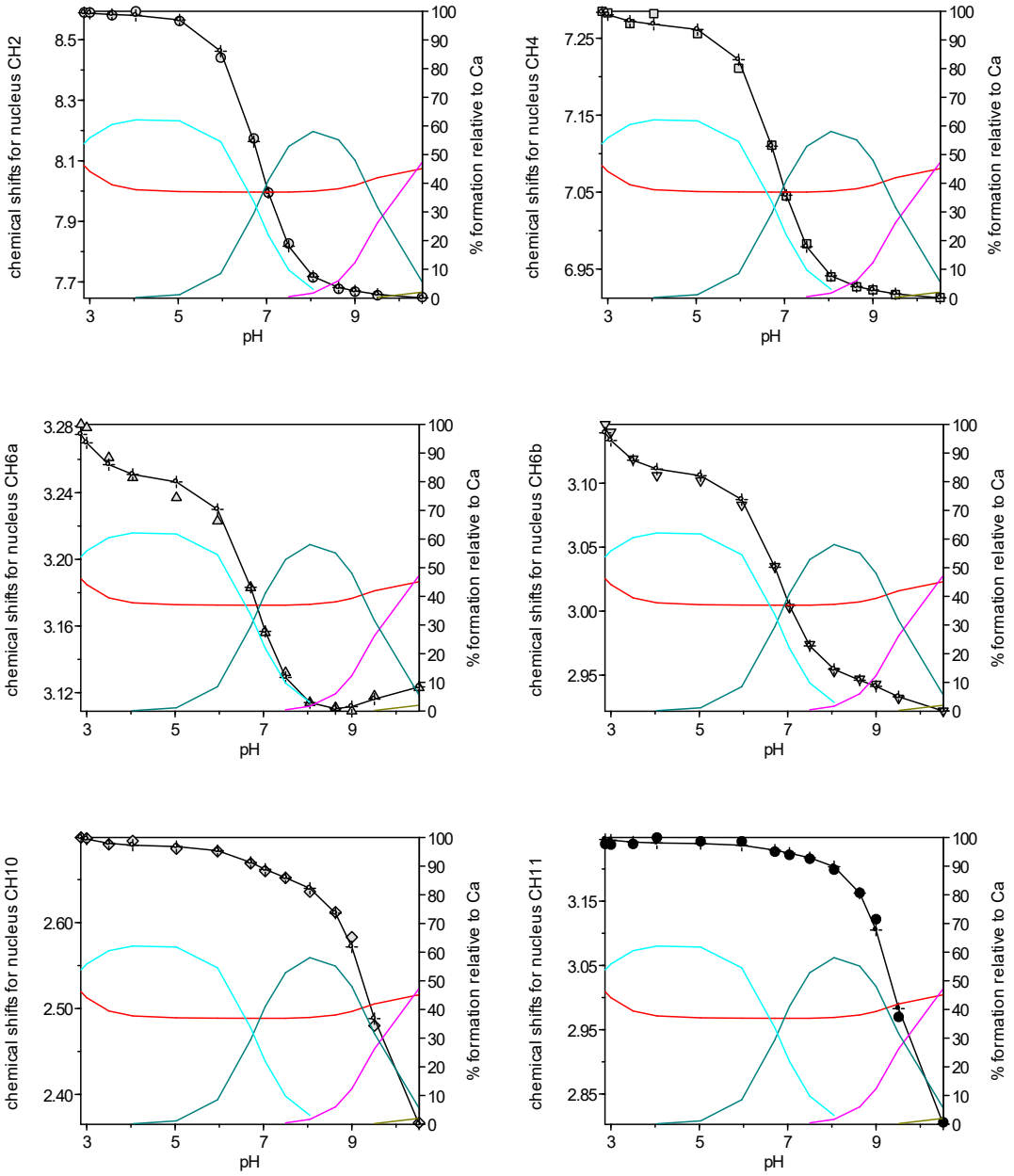

Fig. S4. Experimental and calculated chemical shift *vs.* pH of Ca^{2+}-carnosine system together with formation percentages of species: $CaLH_2$ (cyan), CaLH (green), CaL (magenta), free Ca^{2+} (red).

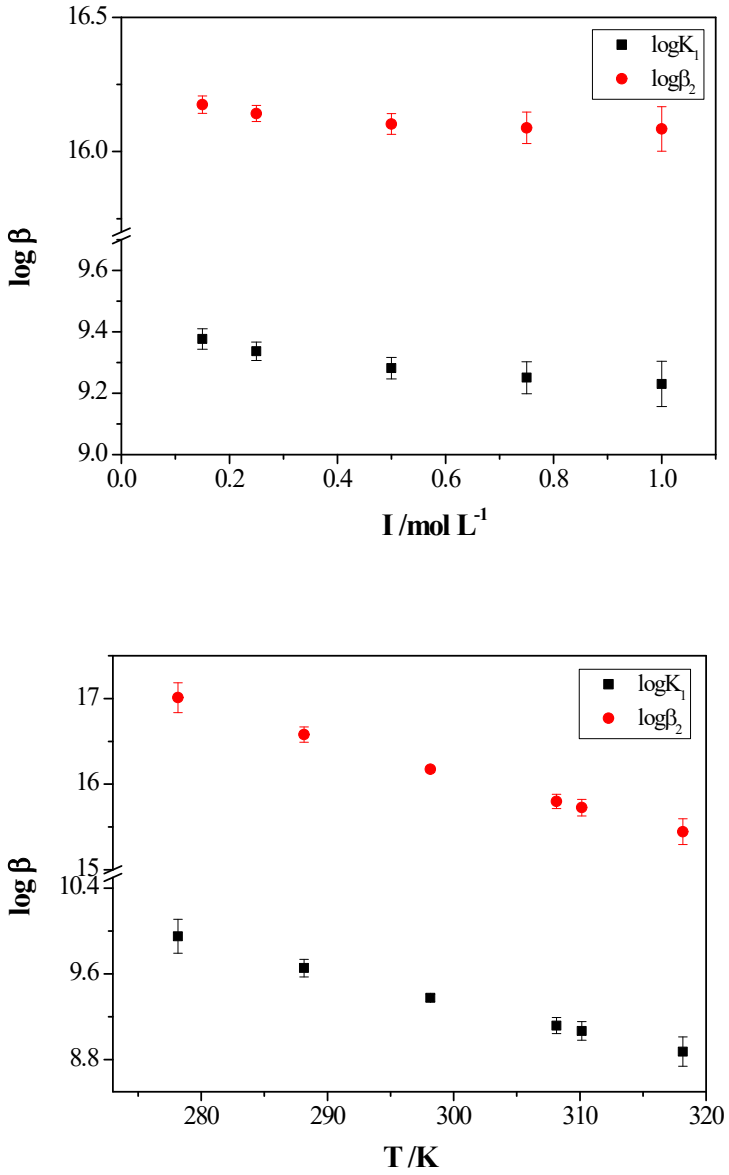

Fig. S5. Calculated protonation constant values of carnosine vs. ionic strength and temperature.

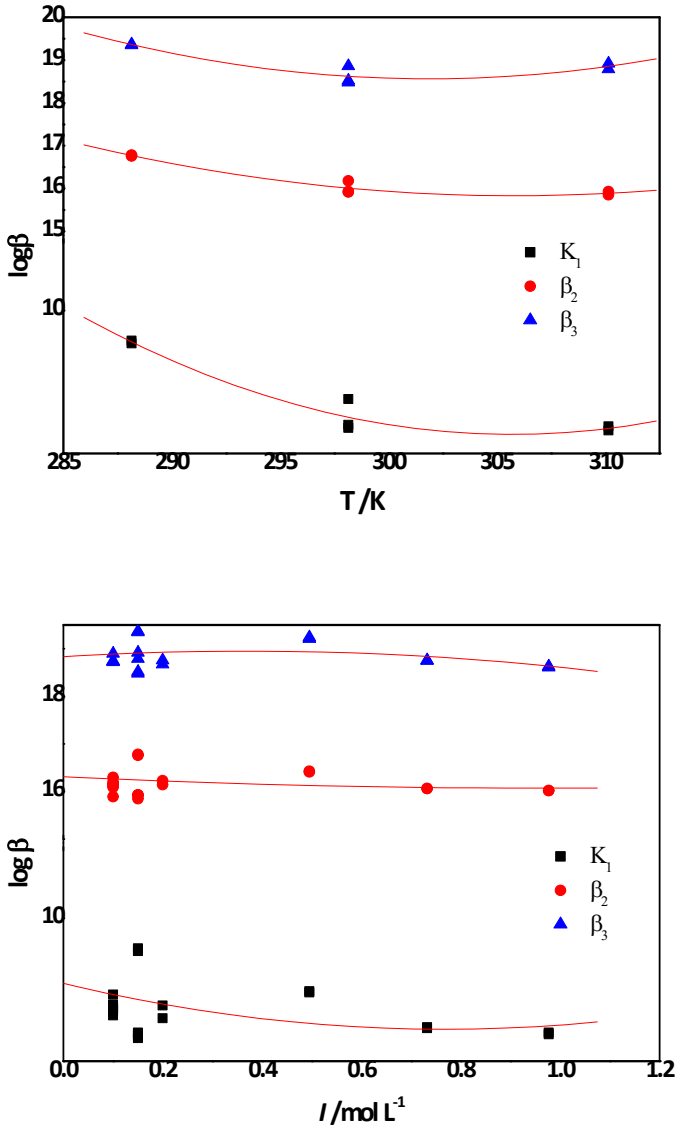

Fig. S6. Fitting curves obtained in calculating parameters of Tables 3 and 4 of the main text for the protonation constants of carnosine.