

Structure and activity of dicarba-ImI, an α -conotoxin containing a non-reducible cystine analogue

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Table S1. Assigned chemical shifts for isomer I of dicarba-ImI at pH 5.9, 15°C

Residue	Chemical Shift (ppm)			
	NH (N)	H ^α (C ^α)	H ^β (C ^β)	Others
Gly 1				
Δ4Das 2		4.69 (56.2)	2.72, 2.39 (31.9)	γ: 5.54 (75.9)
Cys 3	8.08 (114.0)	4.26 (56.8)	3.36, 2.90 (40.4)	
Ser 4	8.50 (114.2)	4.35 (58.9)	3.95 (63.0)	
Asp 5	8.05	5.07 (50.9)	3.09, 2.65 (43.1)	
Pro 6	--	4.35 (64.9)	2.41, 1.97 (32.5)	γ: 2.06 (27.5), δ: 3.94 (51.4)
Arg 7	8.51 (116.1)	4.24 (57.3)	1.81, 1.90 (27.3)	γ: 1.69 (30.3), δ: 3.25, 3.18 (43.3), ε: 7.57 (84.4)
Δ4Das 8	7.89 (119.7)	4.19 (55.3)	2.49, 2.54 (32.7)	γ: 5.50 (73.6)
Ala 9	8.05	4.08 (54.5)	1.32 (18.6)	
Trp 10	7.72 (118.2)	4.58 (56.5)	3.33, 3.28 (28.7)	δ1: 7.32 (127.1), ε3: 7.56 (120.9), ε1: 10.12 (129.6), ζ3: 7.17 (122.2), ζ2: 7.49 (114.6), η2: 7.24 (124.8)
Arg 11	7.99 (120.0)	4.03 (56.4)	1.51 (29.8)	γ: 0.97 (26.8), δ: 2.92 (43.3), ε: 7.08 (84.7)
Cys 12	8.19 (119.7)	4.58 (57.2)	3.21, 3.18 (28.7)	
	6.90, 6.60			

Table S2. Amide temperature coefficients for isomer I of dicarba-ImI at pH 5.9

Residue	Temperature coefficient (ppb/K)
Cys 3	-6.7
Ser 4	-9.8
Asp 5	-5.6
Pro 6	
Arg 7	-2.0
Δ 4Das 8	-2.8
Ala 9	-5
Trp 10	-6.6
Arg 11	-9.3
Cys 12	-7.3

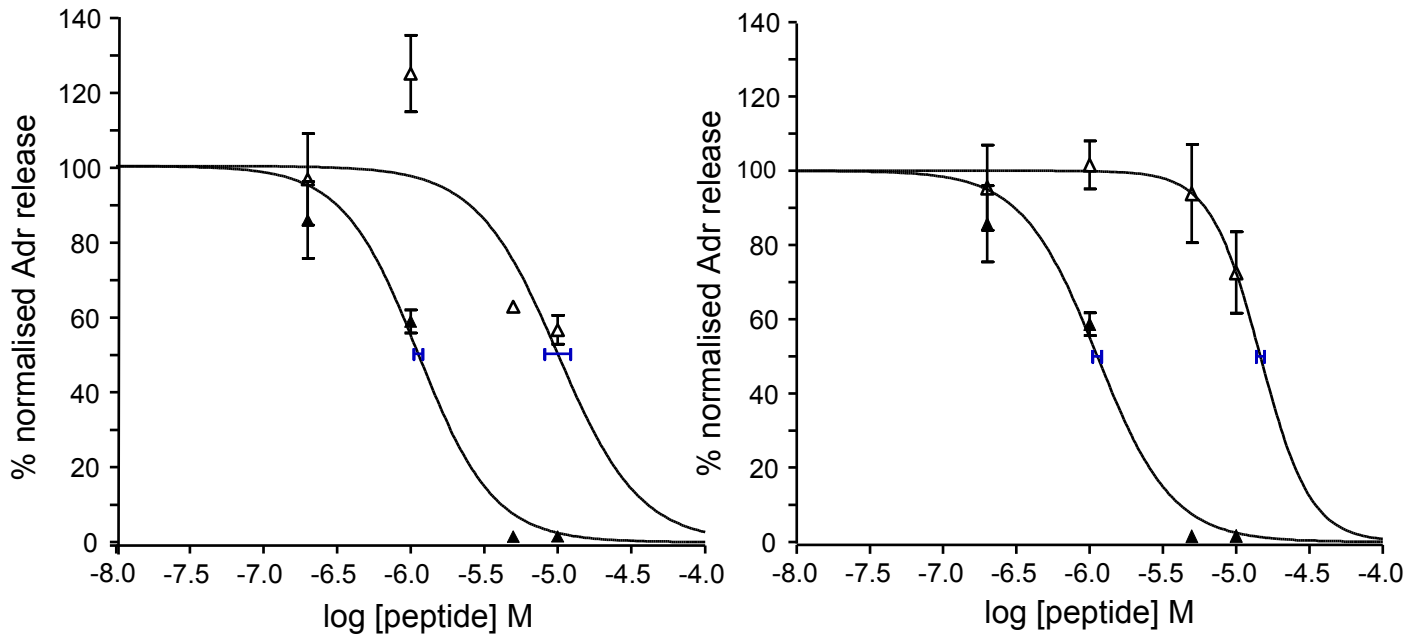


Figure S1. Normalised adrenaline release from bovine adrenal chromaffin cells on stimulation with 4.0 μ M nicotine in the presence of α -ImI (closed symbols) dicarba α -ImI isomer I (A, open symbols) or dicarba α -ImI isomer II (B, open symbols).

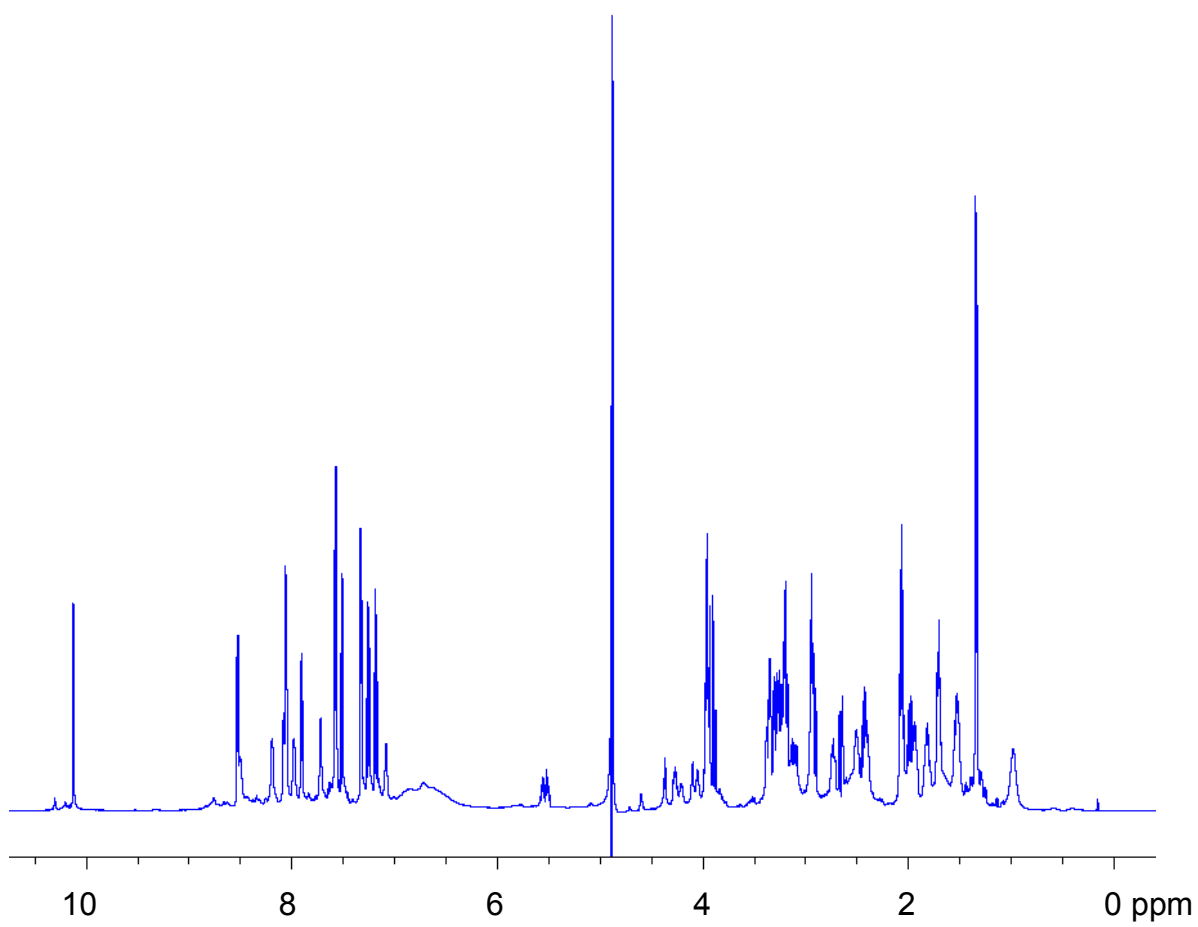


Figure S2. One-dimensional ^1H NMR spectrum of dicarba α -ImI isomer I at pH 5.9 and 288 K

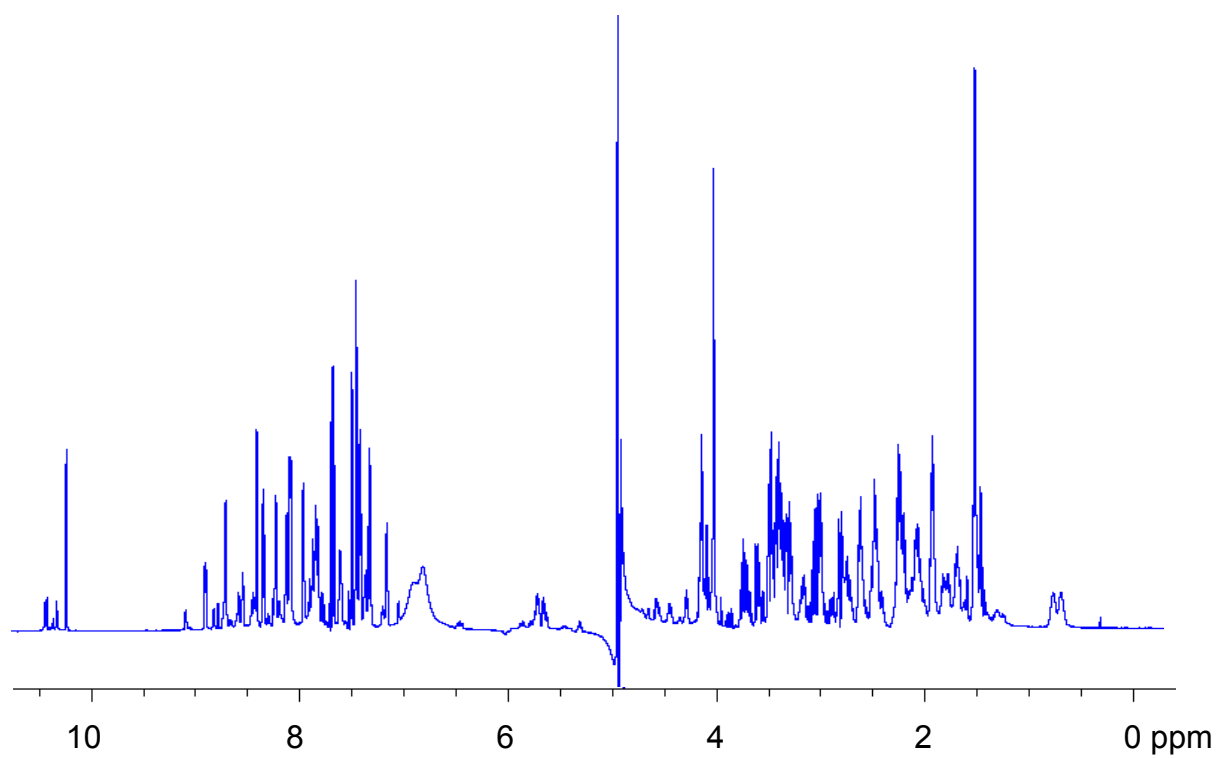


Figure S3. One-dimensional ^1H NMR spectrum of dicarba α -ImI isomer II at pH 3.5 and 298 K.

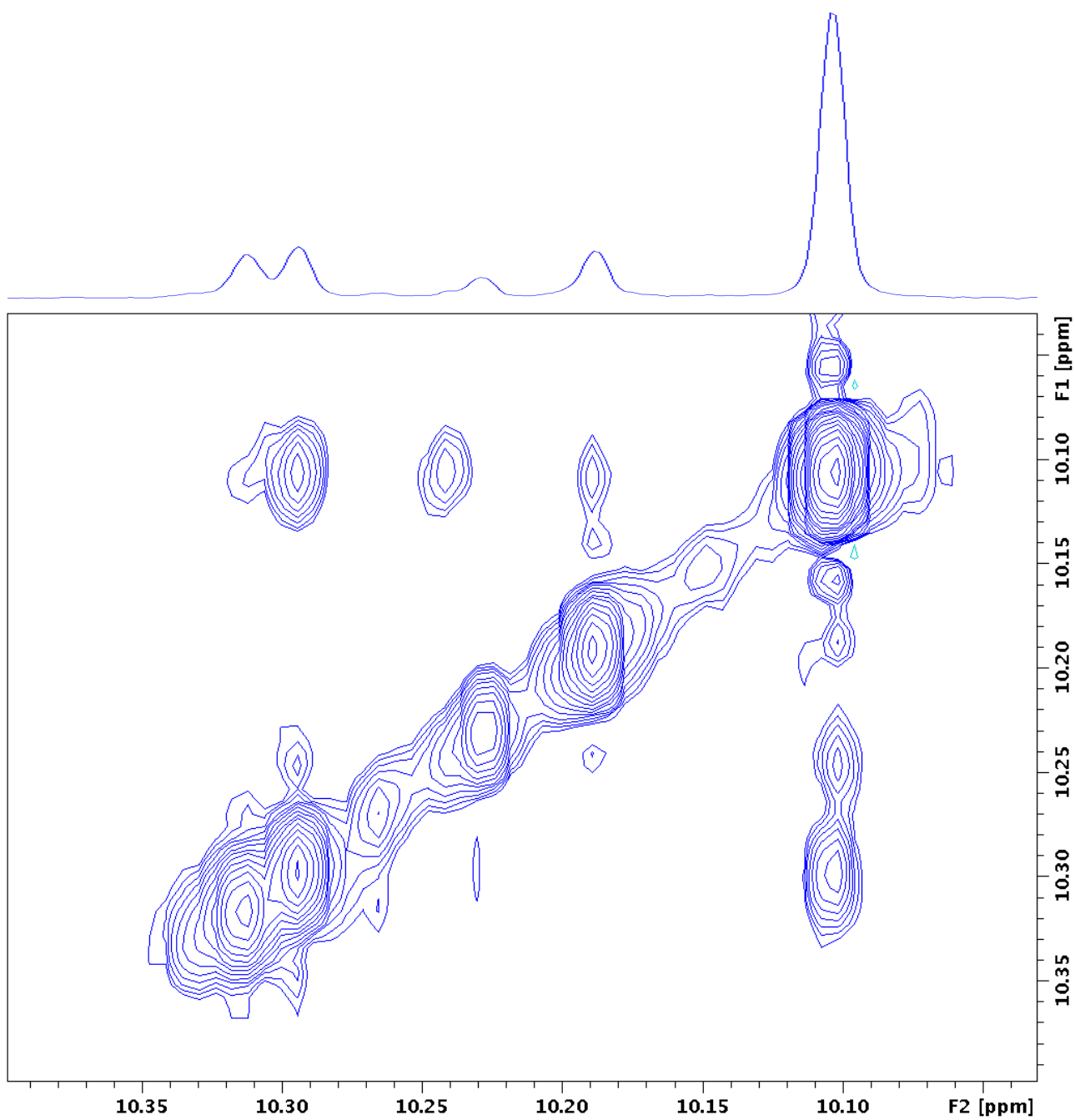


Figure S4. NOESY (400 ms mixing time) and 1D ¹H NMR spectrum of Trp indole NH resonances of dicarba α -ImI isomer II at pH 3.5 and 298 K, showing exchange-derived cross peaks in the NOESY spectrum.

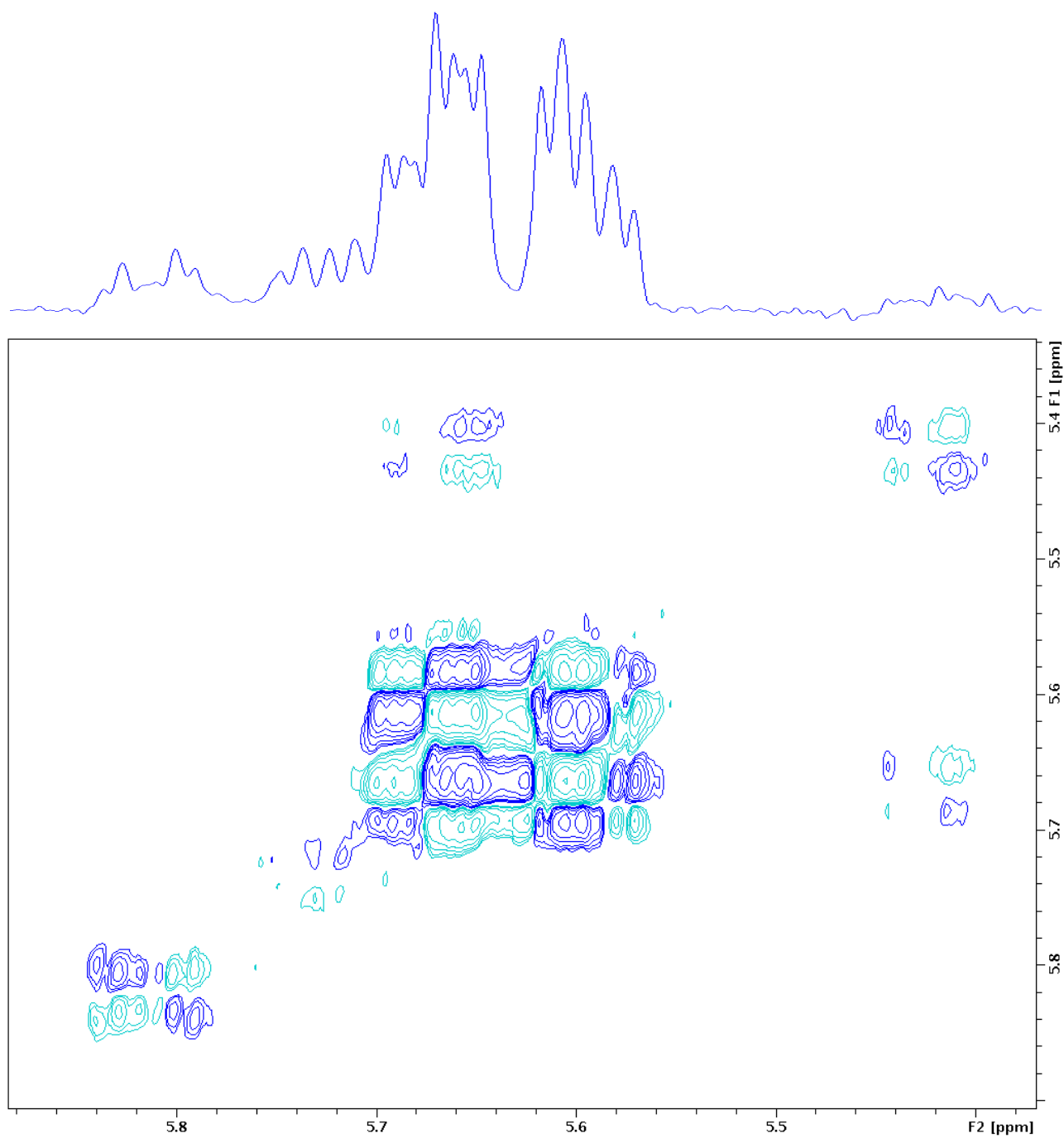


Figure S5. DQF-COSY and 1D ¹H NMR spectrum of dicarba α -ImI isomer II in H₂O at 298 K, showing the strong (>15 Hz) 3-bond coupling across the dicarba double bond.

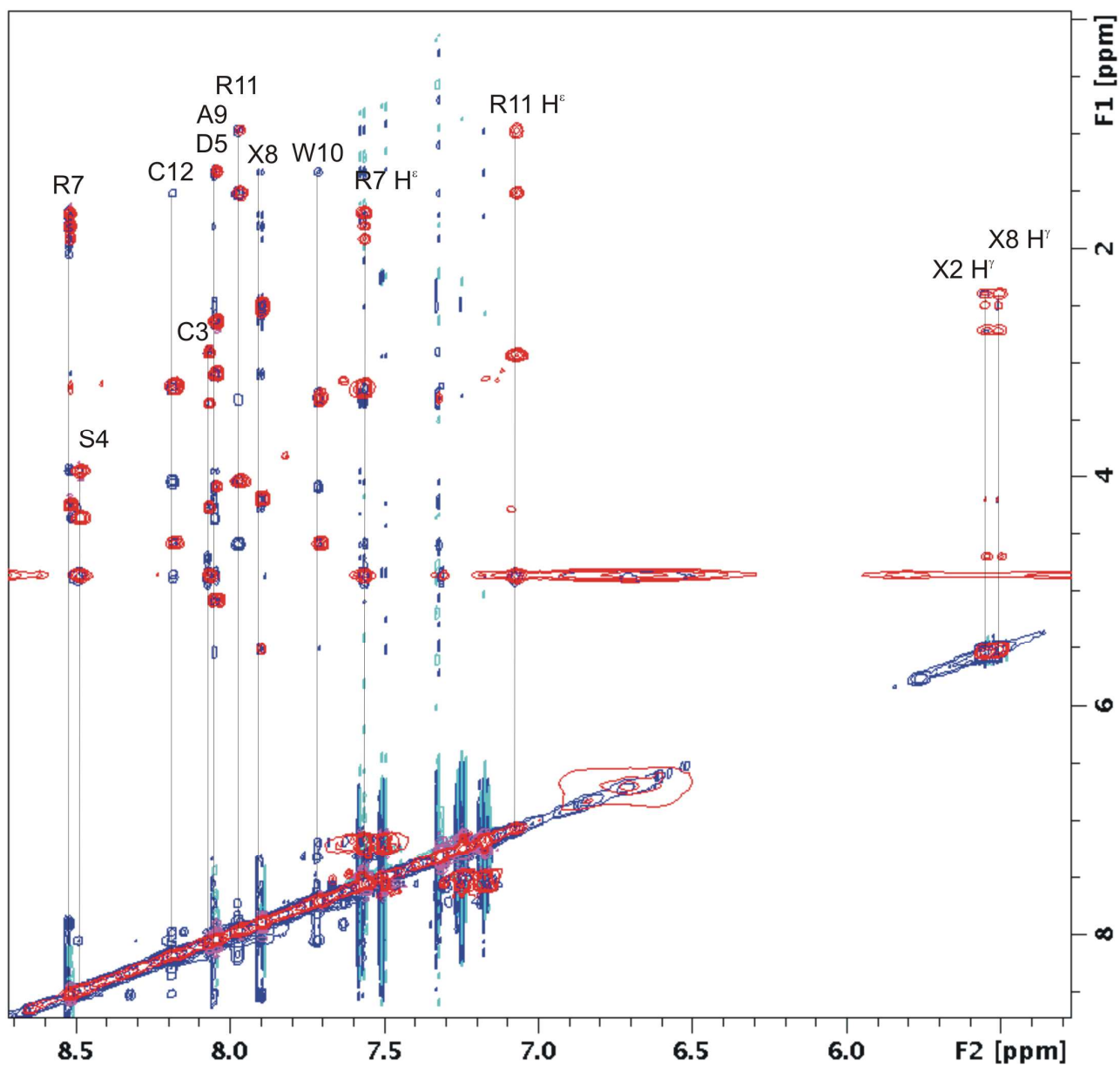


Figure S6. Representative NOESY (blue) and TOCSY (red) spectra of dicarba ImI isomer I in H₂O at pH 5.9, 288 K. Assignments for backbone NH, arginine side-chain H^e and olefinic dicarba H^γ protons in the F2 dimension are labelled.

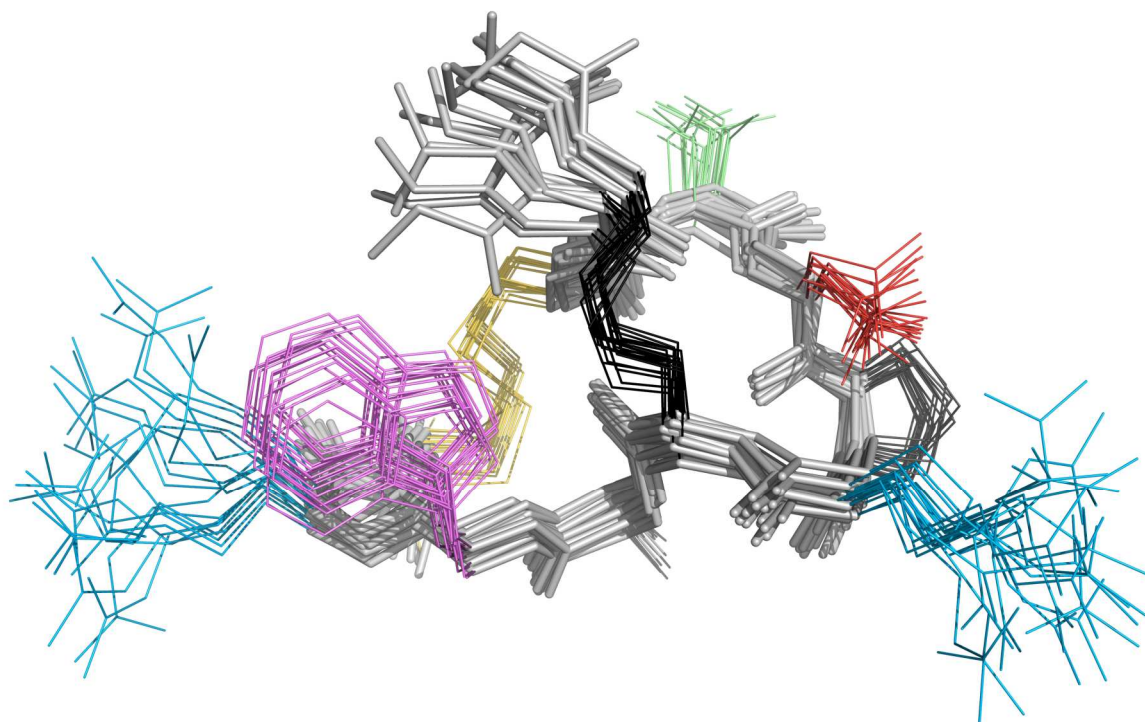


Figure S7. The ensemble of 20 structures determined for dicarba α -ImI.