Supporting Information

Solution Structure of Polytheonamide B, a Highly Cytotoxic Non-Ribosomal Polypeptide from Marine Sponge

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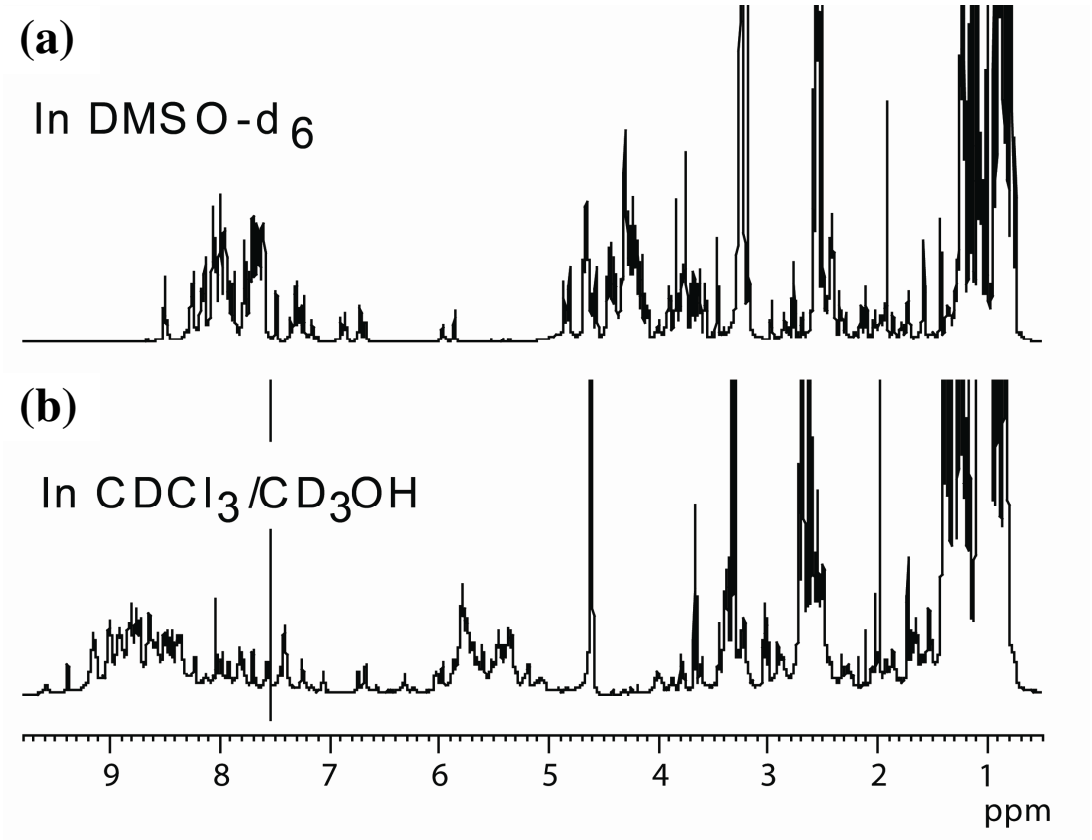
The file contains Supporting Table T1 and Supporting Figures and Legends S1-S8. The Supporting Table T1 provides the ^{1}H NMR chemical shift assignments of pTB in $CDCl_3/CD_3OH$ (1:1, v/v). The Supporting Figures show ^{1}H-NMR spectra in $CDCl_3/CD_3OH$ (1:1, v/v) and DMSO-d_6 (S1), DQF-COSY (S2), TOCSY (S3), and NOESY spectra (S4) together with secondary structure information of pTB (S5), representations of the NMR structures of pTB (S6 and S7) and a structural comparison of pTB with a gA channel (S8).

Table T1. ^{1}H NMR chemical shifts of pTB in $CDCl_3/CD_3OH$ (1:1, v/v)

residue	NH	$C_\alpha H$	$C_\beta H$	others
Gly-1	9.38	4.03,4.22		
Mil-2	7.70	4.84		$C_\gamma H_3$0.90; $C_\gamma H_3$ 0.92; $C_\gamma H_2$ 1.33; $C_\delta H_3$ 0.82
Gly-3	8.57	3.42, 4.53		
Tle-4	7.41	5.43		$C_\gamma H_3$ 0.98
Tle -5	8.02	4.50		$C_\gamma H_3$ 1.08
Tle -6	7.58	5.34		$C_\gamma H_3$ 0.99
Ala-7	8.62	5.07	1.38	
Tle -8	7.82	5.37		$C_\gamma H_3$ 1.06
Tle -9	8.49	4.65		$C_\gamma H_3$ 1.07
Ala-10	8.65	5.60	1.28	
Gly-11	8.23	3.25, 5.36		
Ala-12	8.52	5.80	1.40	
Tle -13	8.40	5.20		$C_\gamma H_3$ 1.03
Ala-14	8.81	5.68	1.31	
Asm-15	8.45	5.68	2.53, 3.25	NH 7.92; $C_\delta H_3$ 2.62
Mth-16	8.90	5.79		$C_\gamma H_3$ 1.34
Gly-17	8.76	3.37, 5.21		
Ala-18	8.66	5.53	1.37	
Gly-19	8.77	3.40, 5.48		
Tle -20	9.01	5.11		$C_\gamma H_3$ 0.96
Asm-21	8.82	5.73	2.58, 3.21	NH 8.36; $C_\delta H_3$ 2.69
Mgn-22	9.58	5.12	2.49	$C_\gamma H_3$ 1.07; $C_\gamma H_2$ 1.59, 2.62; NH_2 5.97, 7.25
Mth-23	9.02	5.46		$C_\gamma H_3$ 1.40; $C_\gamma H_3$ 1.40
Ala-24	8.74	5.43	1.25	
Gly-25	8.83	3.40, 5.46		
Gly-26	8.81	3.42, 5.24		
Asm-27	9.03	5.75	2.50, 3.27	NH 8.46; $C_\delta H_3$ 2.70
Ile-28	9.12	5.38	1.52	$C_\gamma H_3$ 0.96; $C_\gamma H_2$ 1.06, 1,65; $C_\delta H_3$ 0.90
Ham-29	8.98	5.79	4.43	NH 8.12; $C_\delta H_3$ 2.68
Tle -30	8.51	5.59		$C_\gamma H_3$ 1.03
Mth-31	9.17	5.34		$C_\gamma H_3$ 1.22; $C_\gamma H_3$ 1.22
Gly-32	8.47	3.41, 5.42		
Asm-33	9.00	5.87	2.51, 3.25	NH 8.38; $C_\delta H_3$ 2.72
Ile-34	9.16	5.08	1.54	$C_\gamma H_3$ 0.84; $C_\gamma H_2$ 1.68; $C_\delta H_3$ 0.74
Asm-35	8.90	5.76	2.53, 2.67	NH 7.78; $C_\delta H_3$ 2.59
Val-36	8.77	5.09	1.99	$C_\gamma H_3$ 0.83; $C_\gamma H_3$ 1.01
Ham-37	8.93	5.76	4.02	NH 7.97; $C_\delta H_3$ 2.67
Ala-38	8.57	5.37	1.19	
Asm-39	8.62	5.79	2.43, 2.90	NH 7.82; $C_\delta H_3$ 2.62
Val-40	9.14	4.90	1.86	$C_\gamma H_3$ 0.80; $C_\gamma H_3$ 0.83
Ser-41	8.73	5.70	3.60, 3.85	
Val-42	8.92	4.48	1.92	$C_\gamma H_3$ 0.88; $C_\gamma H_3$ 0.88
Asn-43	7.97	6.03	2.78, 2.59	NH_2 6.65, 7.42
Mms-44	8.82	4.73		$C_\gamma H_3$ 1.40; $C_\gamma H_3$ 1.17; $C_\gamma H_2$ 2.58, 2.89; $C_\varepsilon H_3$ 2.52
Asn-45	8.33	5.79	2.59, 2.88	NH_2 6.71, 7.46
Gln-46	9.13	4.68	1.78	$C_\gamma H_2$ 2.05, 2.23; NH_2 6.29, 7.06
Ath -47	8.35	5.29	3.98	$C_\gamma H_3$ 1.23
Thr-48	8.72	4.71	4.30	$C_\gamma H_3$ 1.26

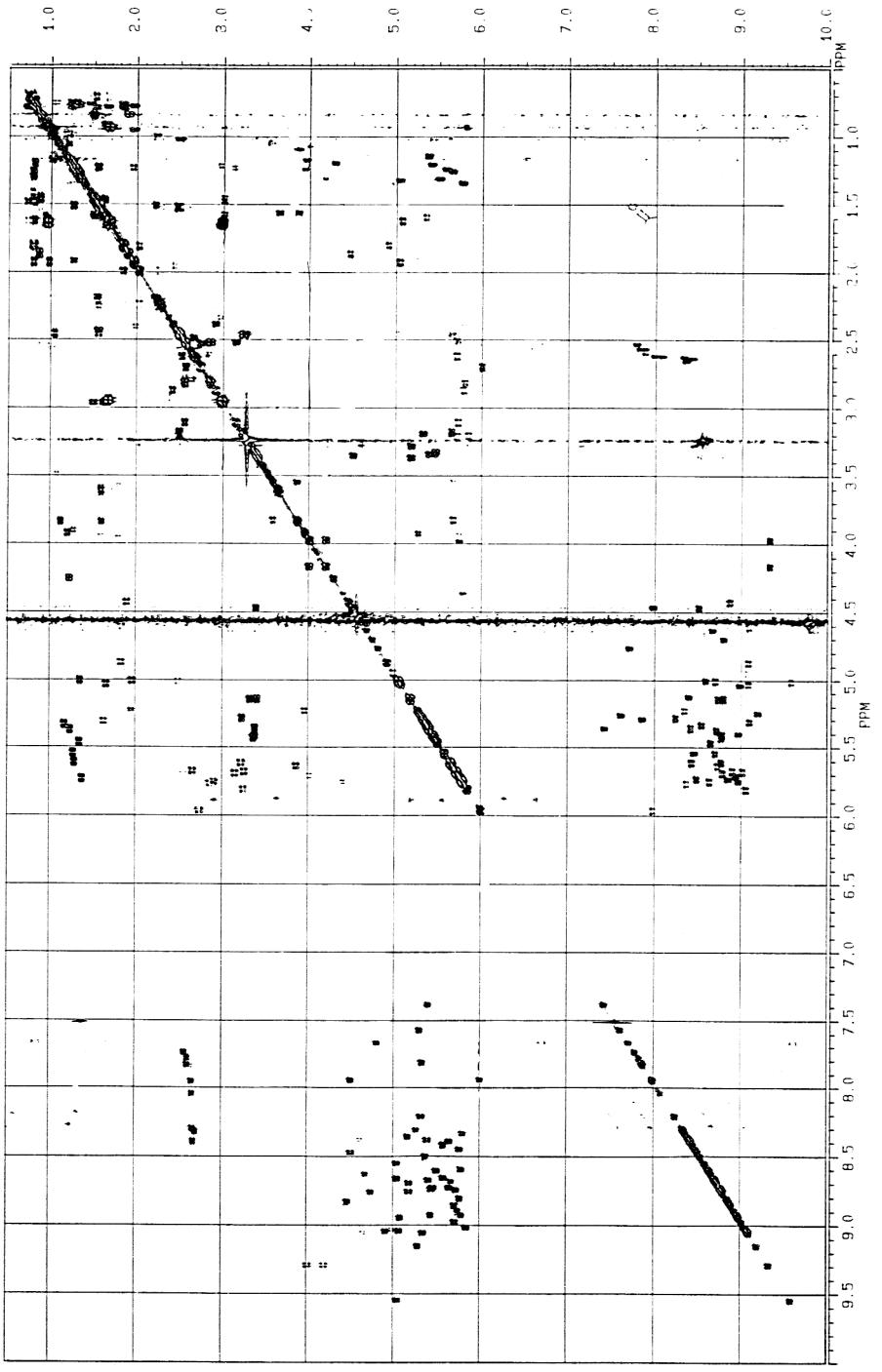
Figure S1



^{1}H NMR spectra of pTB in DMSO-d_6 (a) and in $CDCl_3$/CD_3OH (1:1, v/v) (b).

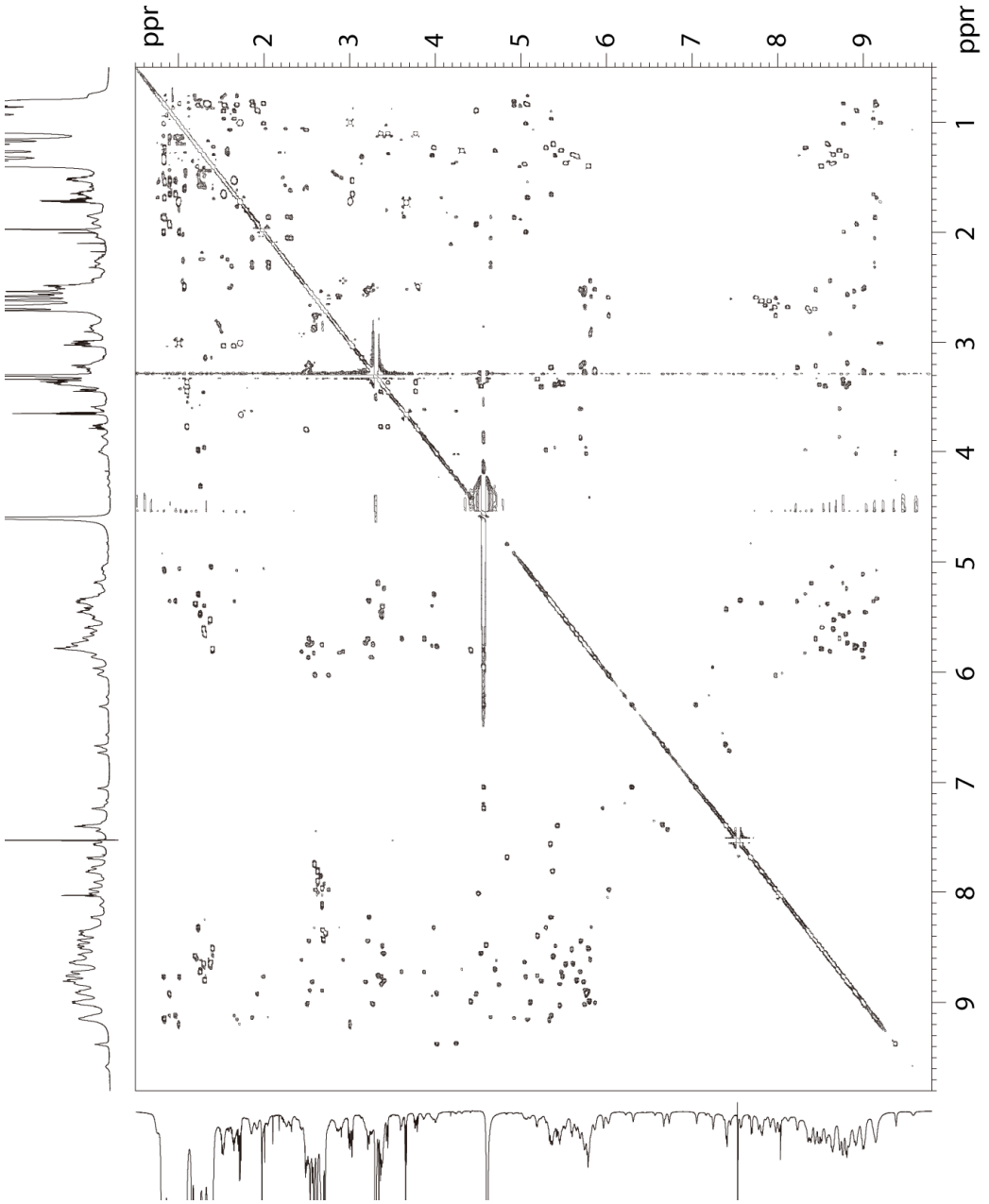
The larger chemical shift dispersion in the ^{1}H-NMR spectrum of pTB in $CDCl_3$/CD_3OH (1:1, v/v) than in DMSO-d_6 indicates a well established structure of the protein in methanol/chloroform mixture.

Figure S2



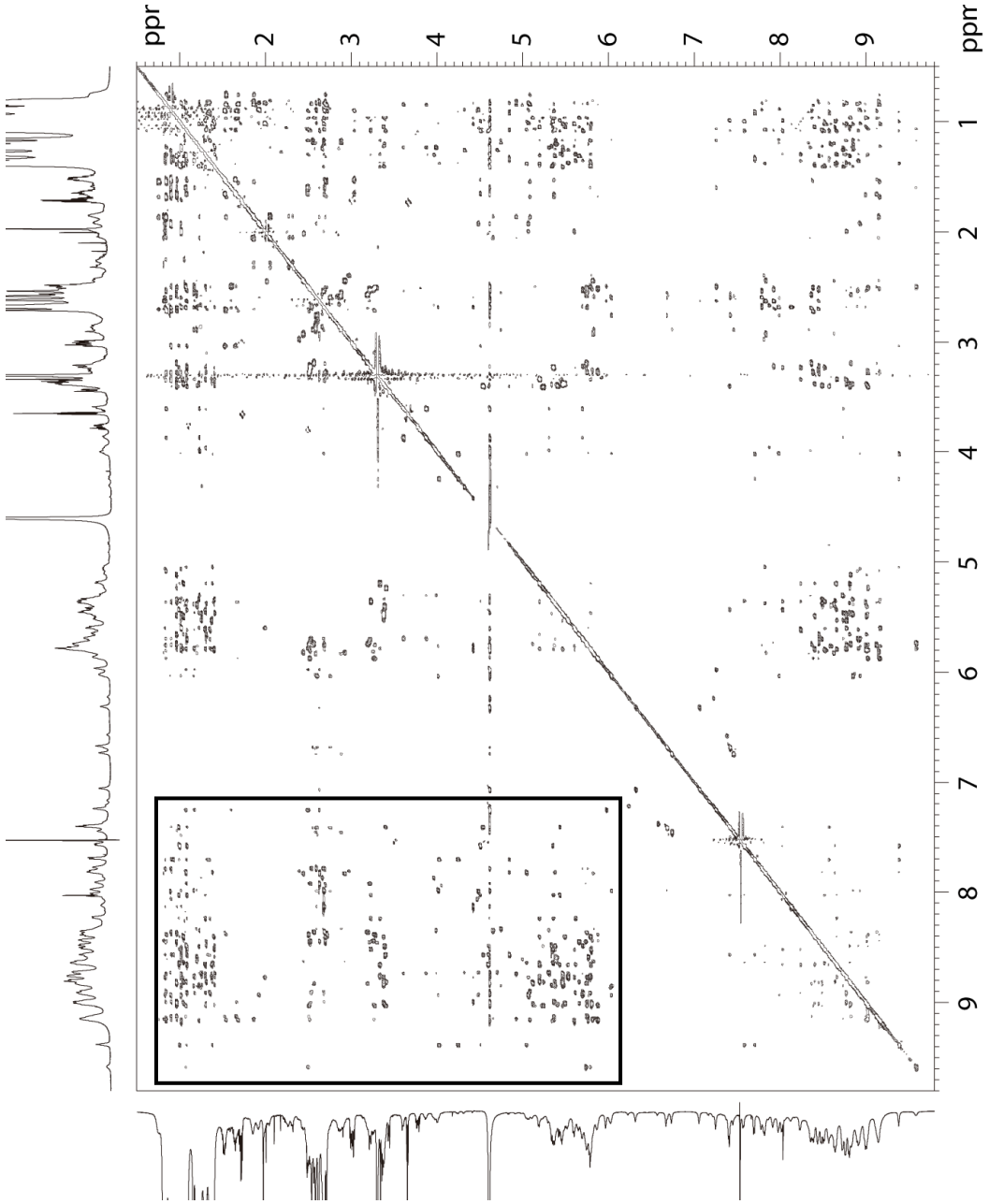
DQF-COSY spectrum of pTB in $CDCl_3/CD_3OH$ (1:1, v/v).

Figure S3



TOCSY spectrum of pTB in $CDCl_3/CD_3OH$ (1:1, v/v) with a spin-lock time of 100 ms.

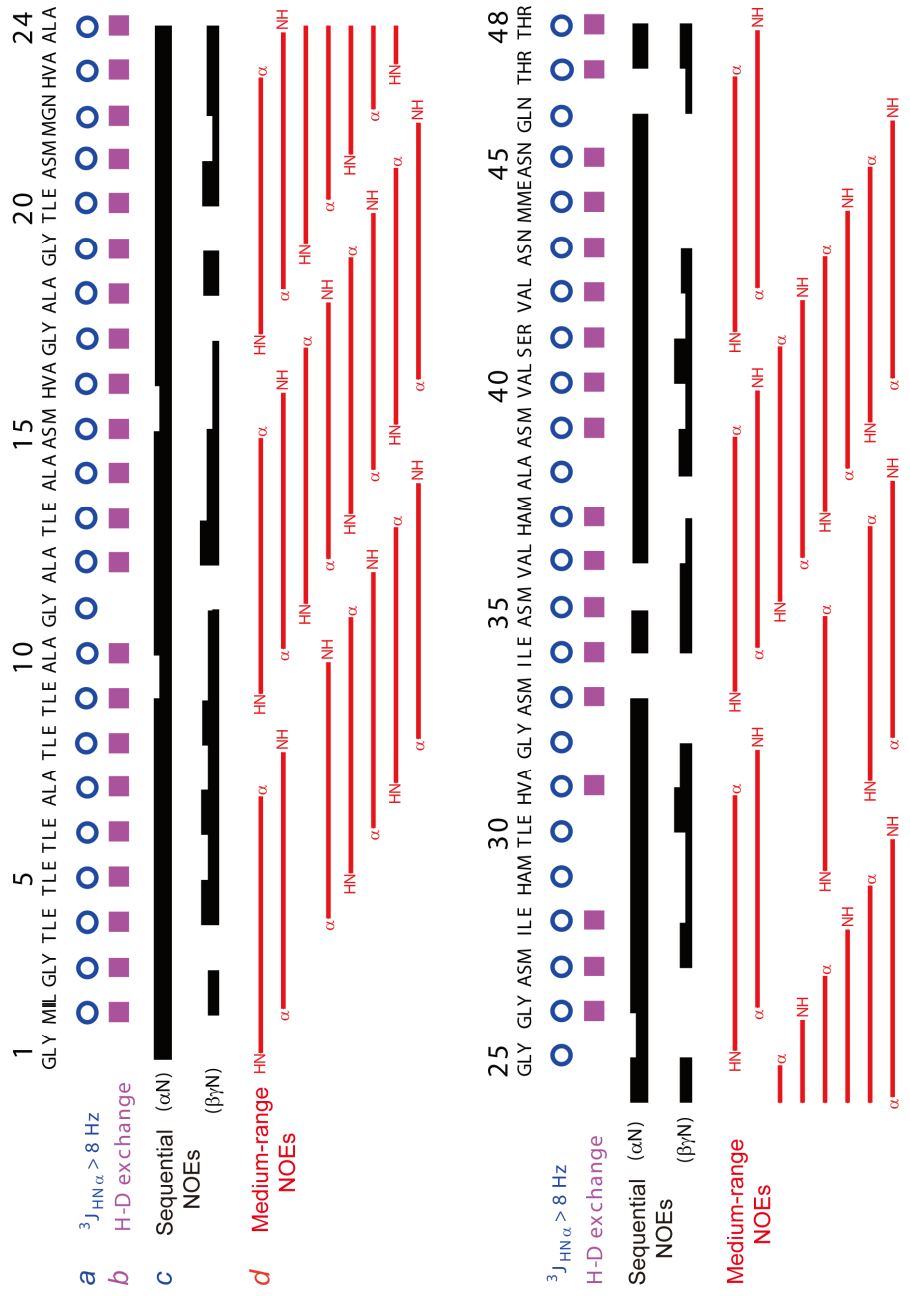
Figure S4



NOESY spectrum of pTB in $CDCl_3/CD_3OH$ (1:1, v/v)

The mixing time in the experiment was 200 ms. The boxed region of the NOESY spectrum is shown in Fig. 2 of this article.

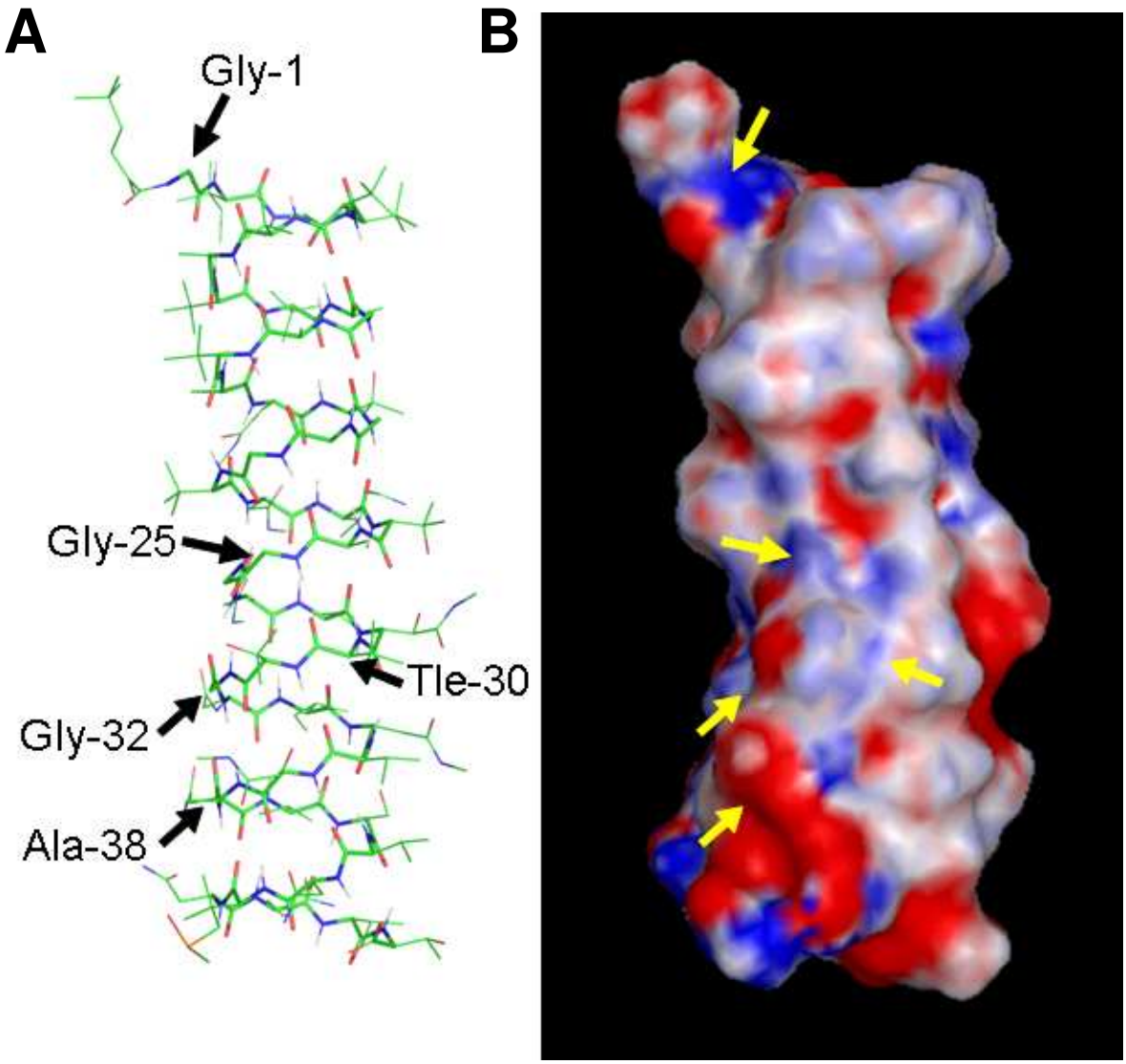
Figure S5



Summary of secondary structure information.

(a) $^3J_{HN\alpha}$ scalar coupling constant values between the backbone amide proton and the α proton of each amino acid residue. Circles indicate coupling constants larger than 8 Hz. (b) Summary of the deuterium exchange experiment. Filled squares indicate slowly exchanging backbone amide protons in pTB whose TOCSY signals were still detectable 9.5 hours after the solubilization of pTB in $CDCl_3/CD_3OD$ (1:1, v/v). (c) Sequential NOE connectivities. Thin, medium, and thick lines indicate weak, medium, and strong NOE peak intensities, respectively. (d) Medium-range NOE connectivities between backbone amide protons (HN) and α protons (α).

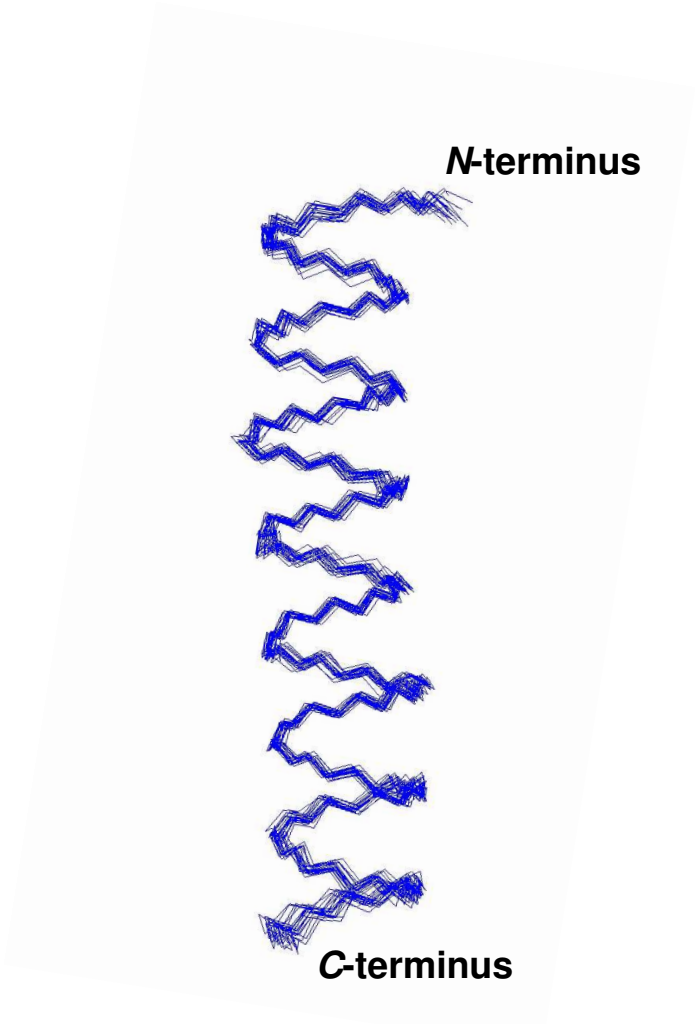
Figure S6



A. Representation of the solution structure of pTB. Five residues with fast backbone amide proton exchange are labeled.

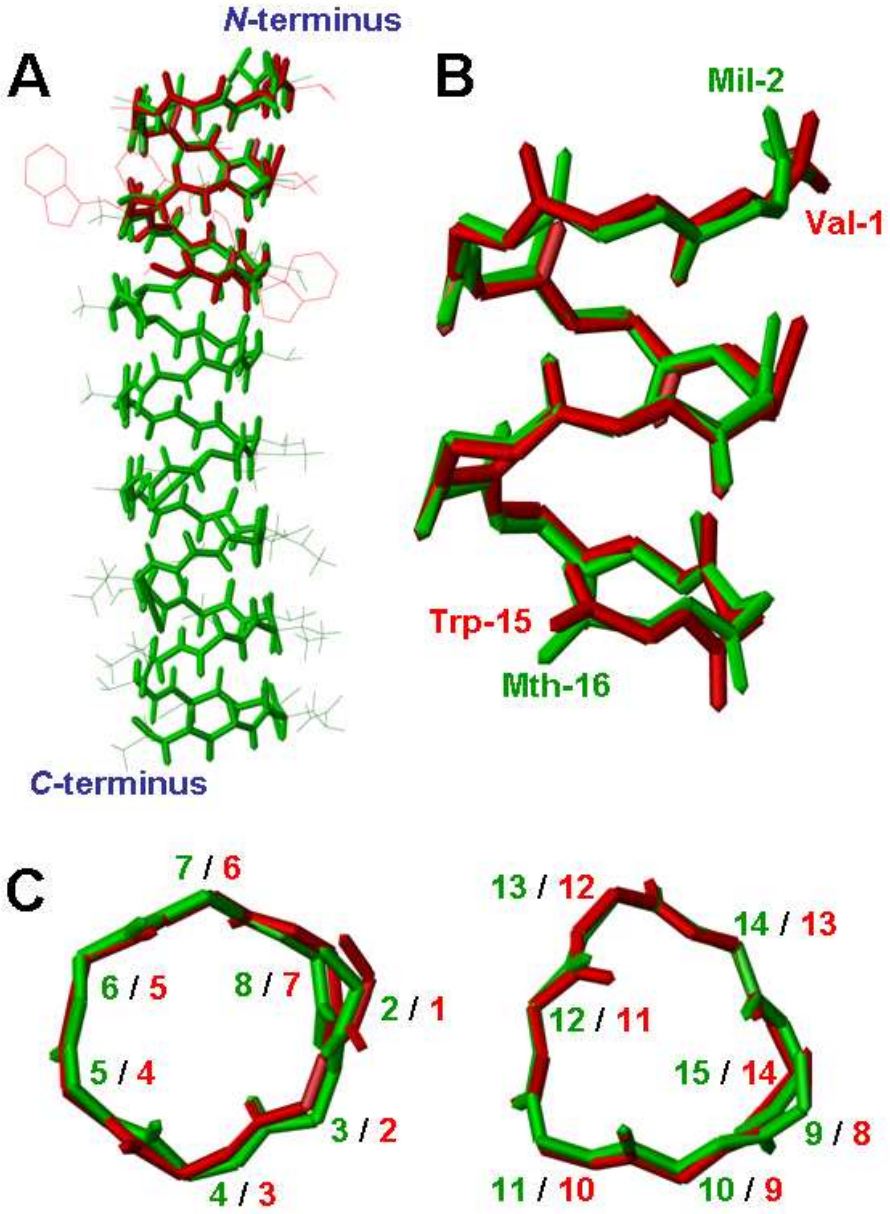
B. Electrostatic surface potential of pTB depicted in the same orientation as in A. The positions of the five residues in A indicated by arrows.

Figure S7



Representation of the final 20 conformers of pTB after optimal superposition of the backbone heavy atoms of residues 3 to 44.

Figure S8



The backbone heavy atom superposition of residues 3 to 15 in pTB (green) with residues 2 to 14 in gA (red; PDB accession code 1JNO).[12b] The RMSD value between the mean coordinates is 0.30 Å for the backbone atoms in the above-mentioned region. **A.** Side view showing the complete structures of pTB and gA. **B.** Side view of the superimposed region in **A**. **C.** Top views of the superimposed region in **A**.