Supplemental informations

Experimental Section

The samples of mr3c, *cis*-mr3c and *cis*-conomarphin were chemically synthesized by SBS Co. Ltd.(Beijing, China) and were purified and identified by HPLC and MS with more than 95% certainty. Samples for NMR studies were prepared in either 9:1(v/v) H_2O/D_2O or 99.99% D_2O(CIL, Germany) with 0.01% Trifluoroacetic acid(TFA, St. Louis, USA) at pH 3 with a final concentration of 4 mM approximately.

NMR spectra were recorded on Bruker-DRX 600 MHz spectrometer at 283K. 2D NOESY, DQF-COSY and TOCSY spectra were acquired with the transmitter set at 4.7ppm and a spectrum width of 7200Hz. Data were processed with Topspin 2.1 and analyzed by SPARKY. The distance restraints were generated and used as input for CYANA(V. 2.1). Phi dihedral angles were derived from DQF-COSY spectrum. The hydrogen bond acceptors for the slowly exchanged amide protons were identified by H-D exchange experiments and analysis of the preliminary calculated structures.

The 20 lowest energy conformers from 100 calculated structures were submitted to a molecular dynamics refinement procedure using the Sander module of Amber 9 program. The three-dimensional structures of conopeptides were generated by MOLMOL software and assessed by Procheck-NMR. The data including constraints and chemical shifts have been submitted to the BMRB database with access code 16874 for mr3c, 16875 for cis-mr3c and 16876 for cis-conomarphin.

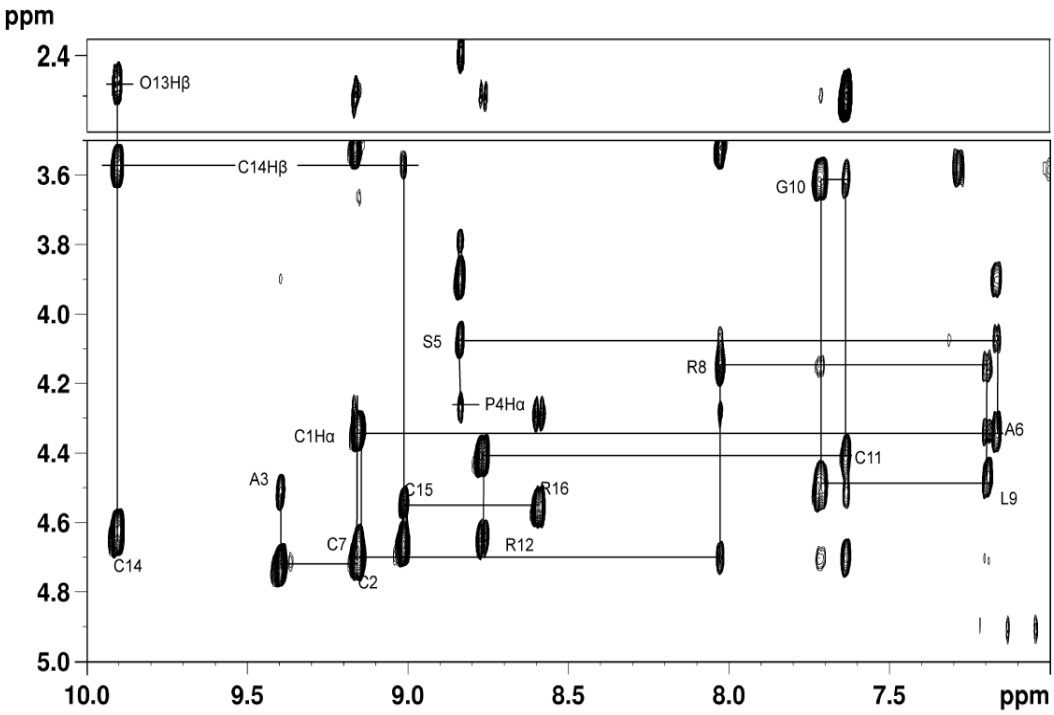

Figure S1 Sequential connectivities of mr3c at 283K, pH 3.0.

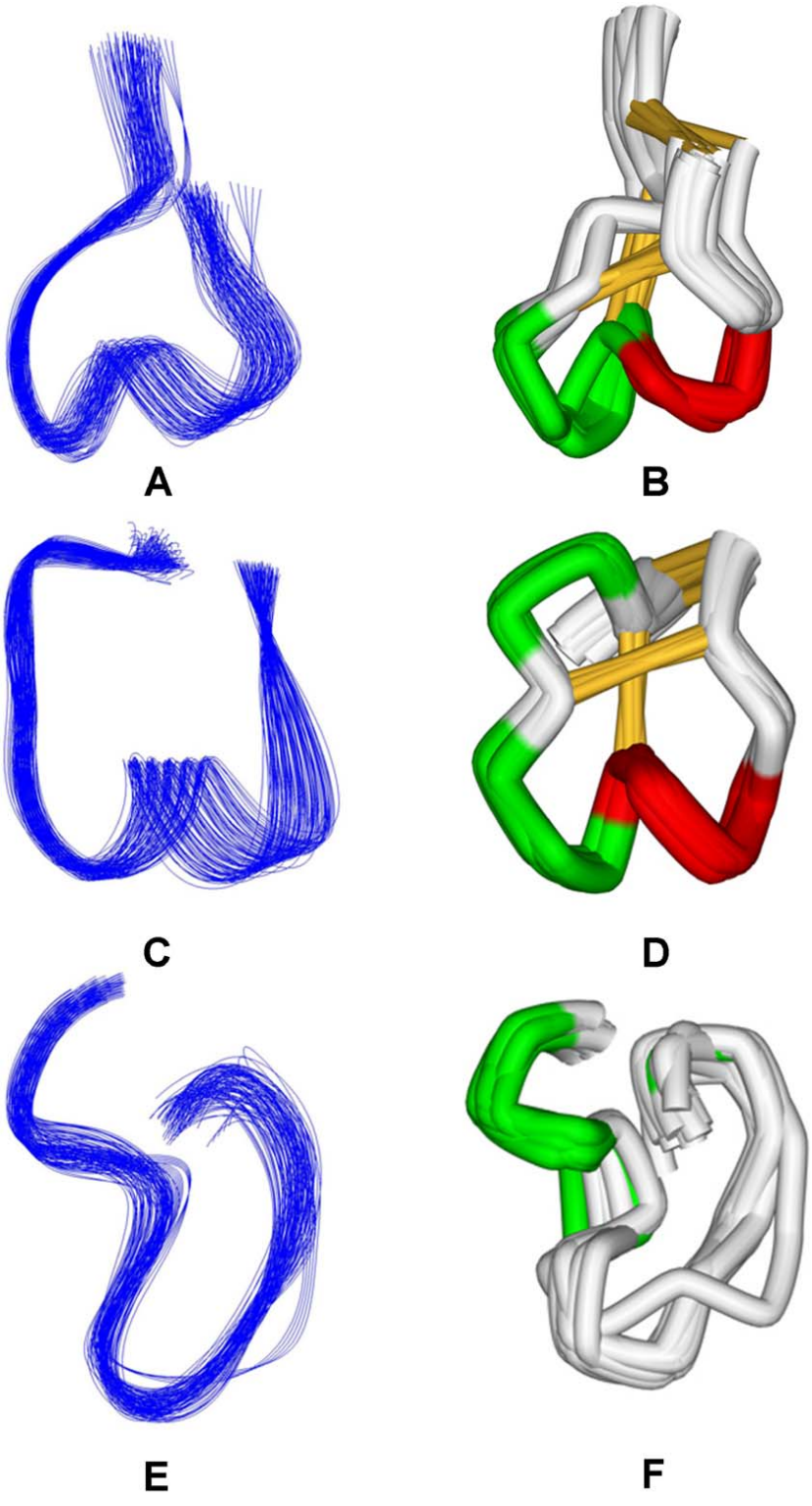

Figure S2. Overlays of backbone atoms for the 20 converged structures of mr3c (A,B), cis-mr3c (C,D) and cis-conomarphin (E,F) in line ribbon and tube style respectively.

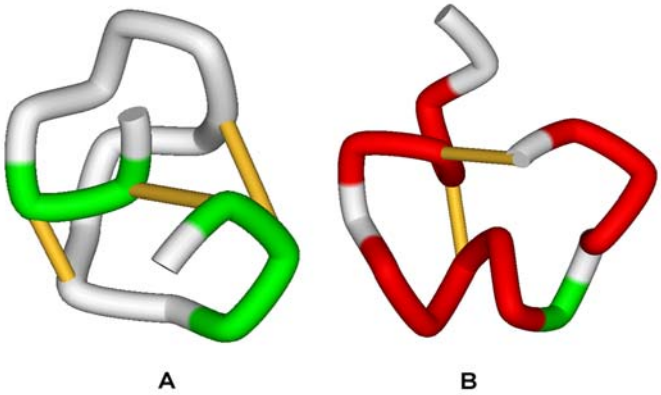

Figure S3 The backbone structures of M-2 conotoxin mr3a (A, ref [17]) and α-conotoxin EI (B, PDB code: 1k64). The conformation of mr3c (Fig.2A) shows difference from mr3a and similarity with α-conotoxin EI.

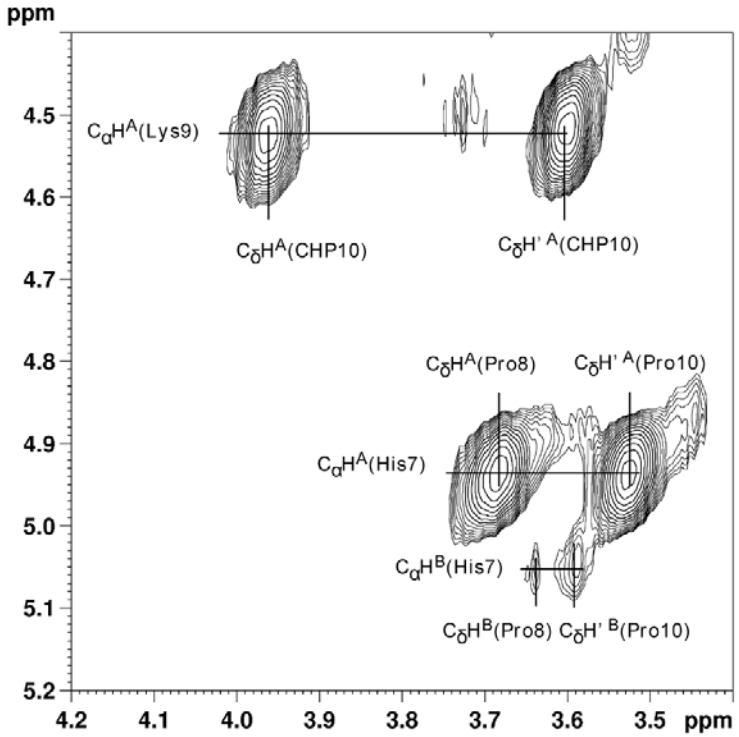

Figure S4 Portion of 2D NOESY spectrum in *cis*-conomarphin to show the NOE contacts between His7 and Pro8(Isomer A and B), and NOE contacts between Lys9 and CHP10(Isomer A only). No NOE contact was found between Lys9 and CHP10 for isomer B.

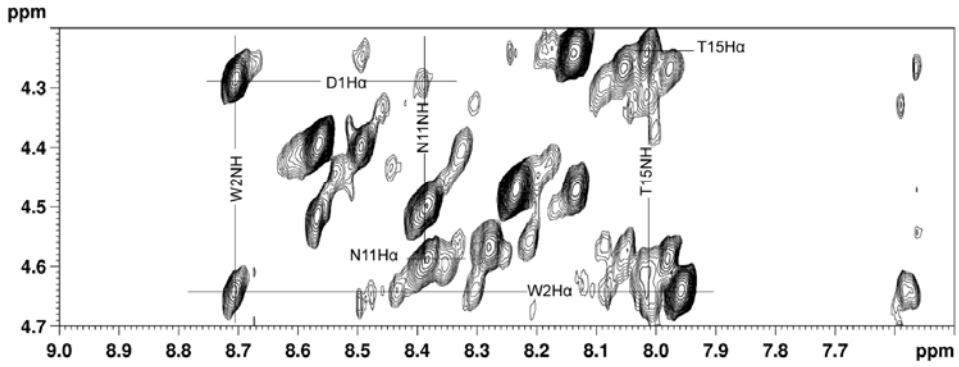

Figure S5 Some crucial and long range NOE contacts observed in *cis*-conomarphin.

Table S1 Proton chemical shifts for mr3c and *cis*-mr3c

Residue	Proton	mr3c	cis-mr3c
Cys1	CαH	4.34	4,37
	CβH	3.24, 2.79	3,06, 2.90
Cys2	NH	9.16	9.46
	CαH	4.72	4.76
	CβH	3.30, 2.79	3.33, 2.79
Ala3	NH	9.40	9.41
	CαH	4.51	4.51
	CβH	1.57	1.57
Pro4	CαH	4.28	4.26
	CβH	2.40, 2.01	2.40, 2.00
	CγH	2.23, 2.04	
	CδH	3.90, 3.81	3.89, 3.80
Ser5	NH	8.84	8.84
	CαH	4.08	4.08
	CβH	3.90	3.90
Ala6	NH	7.17	7.18
	CαH	4.34	4.35
	CβH	1.55	1.55
Cys7	NH	9.16	9.14
	CαH	4.71	4.74
	CβH	3.52, 3.01	3.55, 2.96
Arg8	NH	8.03	8.06
	CαH	4.15	4.16
	CβH	1.95, 1.77	1.96, 1.76
	CγH	1.67	1.67
	CδH	3.25	3.72, 3.22
	NεH	7.31	6.98, 6.69
Leu9	NH	7.20	7.25
	CαH	4.47	4.46
	CβH	1.88, 1.82	1.86
	CγH	1.80	1.77
	CδH	0.93, 0.88	0.93, 0.88
Gly10	NH	7.72	7.75
	CαH	3.61	4.50, 3.36
Cys11	NH	7.64	7.65
	CαH	4.41	4.42
	CβH	2.92, 2.50	2.89, 2.57
Arg12	NH	8.77	8.79
	CαH	4.65	4.62
	CβH	1.75	

	CγH	1.68	1.69
	CδH	3.59, 3.22	3.57, 3.17
	NεH	7.28, 6.96, 6.68	6.97, 6.68
Hyp/CHP13	CαH	4.52	4.54
	CβH	2.41	2.70
	CγH	2.16	2.08
	CδH	3.67	3.88, 3.50
Cys14	NH	9.91	7.65
	CαH	4.63	4.42
	CβH	3.58, 3.24	2.89, 2.57
Cys15	NH	9.01	9.02
	CαH	4.63	4.55
	CβH	3.58, 3.24	3.37, 2.63
Arg16	NH	8.59	8.60
	CαH	4.29	4.26
	CβH	1.90, 1.75	1.90, 1.74
	CγH	1.63	1.64
	CδH	3.24	
	NεH	7.23	

Table S2 Crucial and long-range NOE constraints observed in mr3c and *cis*-mr3c

	native mr3c		*cis*-mr3c
C2HB3-A6QB	m*	C1HB3-C15HN	s
C2HN-O13QD	w	C2HB2-C14HA	s
P4HD2-O13HA	w	C2HB2-C7HN	w
P4HD3-O13HA	w	C2HB3-C7HN	w
A6HA-O13QD	w	A3HN-C11HB3	w
P4HA-C7HN	s	P4HA-C7HB3	m
P4HA-C7HB3	w	S5QB-L9QD2	w
C7HN-C11HB3	w	C7HN-C11HB3	s
C11HB3-C2HB3	s	C11HB3-C2HB2	s
C7HA-C11HB3	m	C7HA-C11HB3	s
O13HB2-C14HN	m	O13HB2-C14HN	w

*NOE constraints, s, strong; m, medium; and w, weak.

Table S3 Proton chemical shifts for *cis*-conomarphin.

Residue	Proton	cis-conomarphin (major)	cis-conomarphin (minor)
Asp1	CαH	4.30	4.40
	CβH	2.92	2.84
Trp2	NH	8.71	8.70
	CαH	4.64	4.61
	CβH	3.23	3.19
	CδH	7.58	
	NεH	7.21	7.16
	NηH	10.11	
Glu3	NH	7.96	8.01
	CαH	4.11	4.10
	CβH	1.77, 1.66	1.76, 1.65
	CγH	2.08	2.07
Tyr4	NH	8.02	8.18
	CαH	4.25	4.51
	CβH	2.88, 2.80	3.09, 2.95
	CδH	7.04	7.14
	CεH	6.78	6.73
His5	NH	8.14	8.17
	CαH	4.47	4.51
	CβH	3.09, 2.95	3.15, 2.98
	CδH	7.14	
	CεH	8.51	8.50
Ala6	NH	8.24	8.17
	CαH	4.12	4.14
	CβH	1.31	1.20
His7	NH	8.54	8.50
	CαH	4.93	5.08
	CβH	3.20, 3.10	3.17, 3.05
	CδH	7.30	7.25
	CεH	8.58	8.56
Pro8	CαH	4.40	4.40
	CβH	2.22	2.23
	CγH	1.95, 1.86	1.96, 1.87
	CδH	3.69, 3.54	3.65, 3.59
Lys9	NH	8.56	8.49
	CαH	4.52	4.25
	CβH	1.84, 1.73	1.76, 1.69
	CδH	1.48	1.39
CHP10	CαH	4.57	4.57
	CβH	2.50	2.52

	CγH	2.04	2.06
	CδH	3.97, 3.60	3.45
Asn11	NH	8.39	8.44
	CαH	4.60	4.64
	CβH	2.77, 2.73	2.71, 2.64
	NδH	7.58	7.57
Ser12	NH	8.30	
	CαH	4.27	
	CβH	3.68	
Phe13	NH	8.05	8.09
	CαH	4.57	4.57
	CβH	2.88, 2.77	2.89, 2.81
	CδH	6.77	7.86
	CεH	7.02	
	CζH	7.23	
Trp14	NH	8.28	8.35
	CαH	4.75	4.76
	CβH	3.13, 3.29	3.29, 3.10
	NεH	7.60	7.61
Thr15	NH	8.01	8.11
	CαH	4.24	4.24
	CβH	4.11	4.17
	CγH	1.14	0.91

Table S4 Statistics s for the family of 20 structures of mr3c, *cis*-mr3c and *cis*-conomarphin

	mr3c	cis-mr3c	cis-conomarphin
Experimental constraints		Number	
Intraresidual	113	106	101
Sequential (|*i*-*j*|=1)	45	63	47
Medium range	31	35	8
Long range	10	15	3
Dihedral angle	5	5	5
Hydrogen bond	4	4	2
AMBER energeies, kcal·mol^{-1}			
Total	-626.54±2.95	-636.59±2.86	-390.41±4.62
Bond	11.85±0.30	9.77±0.41	8.56±0.41
Angle	34.26±1.16	27.54±0.97	69.95±1.47
Dihedral	137.02±1.58	139.18±1.40	127.13±2.74
VDW	-77.55±1.64	-85.75±2.38	-100.94±3.22
EEL	-735.31±22.32	-698.44±30.81	-1056.26±47.72
EGB	-379.81±21.68	-366.51±30.10	-417.96±46.17
Constraints	2.48±0.93	2.80±0.58	4.79±0.95
Rmsd to mean coordinates Å			
Backbone atoms	0.26±0.11	0.15±0.08	0.88±0.3
Nonhydrogen heavy atoms	1.41±0.47	1.03±0.32	1.73±0.45
Rachandran statistics from PROCHECK-NMR			
Most favored regions, %	80.7	88.9	32.2
Additional allowed regions, %	19.3	11.1	53.3
Generously allowed regions, %	0	0	14.4
Disallowed regions, %	0	0	0