Supplementary Materials: Effects of Halide Ions on the Carbamidocyclophane Biosynthesis in *Nostoc* sp. CAVN2

Michael Preisitsch, Stefan E. Heiden, Monika Beerbaum, Timo H. J. Niedermeyer, Marie Schneefeld, Jennifer Herrmann, Jana Kumpfmüller, Andrea Thürmer, Inga Neidhardt, Christoph Wiesner, Rolf Daniel, Rolf Müller, Franz-Christoph Bange, Peter Schmieder, Thomas Schweder and Sabine Mundt

Table S1. Composition of Z½ medium used in this study.

Ingredient	mg/L H_2O
Macronutrients [a]	
$NaNO_3$	233.5
$Ca(NO_3)_2 \cdot 4H_2O$	29.5
K_2HPO_4	15.5
$MgSO4 \cdot 7H_2O$	12.5
Na_2CO_3	10.6
Micronutrients [b]	
H_3BO_3	0.24800
$MnSO_4 \cdot 4H_2O$	0.17840
KBr	0.00952
KI	0.00664
$ZnSO_4 \cdot 7H_2O$	0.02296
$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$	0.00704
$Na_2WO_4 \cdot 2H_2O$	0.00264
$KCr(SO_4)_2 \cdot 12H_2O$	0.00400
$AlK(SO_4)_2 \cdot 12H_2O$	0.07584
$Cd(NO_3)_2 \cdot 4H_2O$	0.01232
$Co(NO_3)_2 \cdot 6H_2O$	0.01168
$CuSO_4$	0.00640
$NiSO_4 \cdot 7H_2O$	0.01128
NH_4VO_3	0.00184
Other Components [c]	
$FeSO_4 \cdot 7H_2O$	5.0
Na_2EDTA	20.0

[a] Half quantity of macronutrients found in Zehnder-Medium according to Falch *et al.* [1]; [b] Micronutrient according to Gaffron's solution reported by Hughes *et al.* [2] in modified form; [c] Fe-EDTA-supplement according to Meffert *et al.* [3] in modified form.

Table S2. Biomass dry weights of *Nostoc* sp. CAVN2 cultures grown in the presence of different halogen salts at varying concentrations for 20–30 days. Values are presented as mean ± standard error of the mean (SEM), $n = 2$.

CAVN2 Culture	Biomass Dry Weight (g/L)		
	20 Days	25 Days	30 Days
Control	0.39 ± 0.03	0.40 ± 0.05	0.47 ± 0.00
+0.001% KF	0.40 ± 0.02	0.46 ± 0.02	0.50 ± 0.02
+0.01% KF	0.42 ± 0.01	0.42 ± 0.04	0.50 ± 0.04
+0.1% KF	0.28 ± 0.03	0.33 ± 0.03	0.39 ± 0.03
+1.0% KF	0.17 ± 0.01	0.20 ± 0.02	0.23 ± 0.02
+0.001% KI	0.36 ± 0.00	0.39 ± 0.01	0.44 ± 0.05
+0.01% KI	0.36 ± 0.03	0.43 ± 0.04	0.43 ± 0.03
+0.1% KI	0.20 ± 0.00	0.25 ± 0.02	0.38 ± 0.11
+1.0% KI	0.17 ± 0.03	0.15 ± 0.05	0.19 ± 0.05
+0.001% KBr	0.38 ± 0.04	0.51 ± 0.04	0.45 ± 0.05
+0.01% KBr	0.36 ± 0.02	0.46 ± 0.05	0.51 ± 0.01
+0.1% KBr	0.32 ± 0.04	0.43 ± 0.00	0.40 ± 0.02
+1.0% KBr	0.44 ± 0.03	0.47 ± 0.02	0.48 ± 0.07
+0.001% KCl	0.45 ± 0.08	0.65 ± 0.06	0.52 ± 0.00
+0.01% KCl	0.41 ± 0.04	0.49 ± 0.11	0.54 ± 0.06
+0.1% KCl	0.38 ± 0.05	0.42 ± 0.04	0.52 ± 0.05
+1.0% KCl	0.50 ± 0.05	0.54 ± 0.01	0.63 ± 0.00

Table S3. ^{1}H (600 MHz,) and ^{13}C (150 MHz) NMR spectroscopic data for compounds **1–5** in MeOH-d_4 [a].

No. [b]	1		2		3		4		5	
	δ_C, Type	δ_H (*J* in Hz)	δ_C, Type	δ_H (*J* in Hz)	δ_C, Type	δ_H (*J* in Hz)	δ_C, Type	δ_H (*J* in Hz)	δ_C, Type	δ_H (*J* in Hz)
1	84.9, CH	4.93, *d* (10.1)	84.9, CH	4.90, *d* (10.4)	84.9, CH	4.93, *d* (10.1)	84.6, CH	4.90, *d* (10.1)	84.8, CH	4.90, *d* (10.2)
2	41.7, CH	1.81, *m*	41.7, CH	1.81, *m*	41.8, CH	1.84, *m*	41.8, CH	1.81, *m*	41.7, CH	1.81, *m*
3	35.9, CH_2	0.87, 0.79, *m*	36.0, CH_2	0.87, 0.79, *m*	35.9, CH_2	0.91, 0.82, *m*	35.8, CH_2	0.87, 0.80, *m*	35.8, CH_2	0.87, 0.80, *m*
4	31.0, CH_2	1.52, n.o., *m*	31.0, CH_2	1.52, n.o., *m*	31.0, CH_2	1.56, 0.92, *m*	30.9, CH_2	1.53, 0.92, *m*	30.9, CH_2	1.53, 0.92, *m*
5	31.7, CH_2	1.03, 0.81, *m*	31.8, CH_2	1.04, 0.81, *m*	31.8, CH_2	1.07, 0.84, *m*	31.8, CH_2	1.05, 0.81, *m*	31.8, CH_2	1.05, 0.81, *m*
6	36.6, CH_2	2.12, 1.41, *m*	36.6, CH_2	2.13, 1.41, *m*	36.6, CH_2	2.15, 1.44, *m*	36.6, CH_2	2.13, 1.42, *m*	36.6, CH_2	2.13, 1.42, *m*
7	37.9, CH	3.27, *m*	37.9	3.27, *m*	37.7, CH	3.31, *m*	37.7, CH	3.28, *m*	37.7, CH	3.28, *m*
8	119.1, C		119.1, C		119.7, C		119.9, C		118.7, C	
9	158.4, C		160.2, C		160.2, C		161.2, C		160.2, C	
10	106.6, CH	6.28, *s*	106.6, CH	6.28, *s*	106.6, CH	6.24, *s*	106.8, CH	6.22, *s*	106.6, CH	6.22, *s*
11	141.2, C		141.3, C		141.4, C		141.8, C		141.5, C	
12	110.7, CH	6.21, *s*	110.7, CH	6.21, *s*	110.8, CH	6.31, *s*	110.8, CH	6.28, *s*	110.8, CH	6.28, *s*
13	158.4, C		158.5, C		158.4, C		158.3, C		158.4, C	
14	84.9, CH	4.93, *d* (10.1)	84.9, CH	4.90, *d* (10.4)	84.9, CH	4.93, *d* (10.1)	84.6, CH	4.90, *d* (10.1)	84.8, CH	4.90, *d* (10.2)
15	41.7, CH	1.81, *m*	41.7, CH	1.81, *m*	41.8, CH	1.84, *m*	41.8, CH	1.81, *m*	41.7, CH	1.81, *m*
16	35.9, CH_2	0.87, 0.79, *m*	36.0, CH_2	0.87, 0.79, *m*	35.9, CH_2	0.91, 0.82, *m*	35.8, CH_2	0.87, 0.80, *m*	35.8, CH_2	0.87, 0.80, *m*
17	31.0, CH_2	1.52, n.o., *m*	31.0, CH_2	1.52, n.o., *m*	31.0, CH_2	1.56, 0.92, *m*	30.9, CH_2	1.53, 0.92, *m*	30.9, CH_2	1.53, 0.92, *m*
18	31.7, CH_2	1.03, 0.81, *m*	31.8, CH_2	1.04, 0.81, *m*	31.8, CH_2	1.07, 0.84, *m*	31.8, CH_2	1.05, 0.81, *m*	31.8, CH_2	1.05, 0.81, *m*
19	36.6, CH_2	2.12, 1.41, *m*	36.6, CH_2	2.13, 1.41, *m*	36.6, CH_2	2.15, 1.44, *m*	36.6, CH_2	2.13, 1.42, *m*	36.6, CH_2	2.13, 1.42, *m*
20	38.1, CH	3.24, *m*	37.9	3.27, *m*	38.2, CH	3.27, *m*	37.7, CH	3.28, *m*	37.7, CH	3.28, *m*
21	119.7, C		119.1, C		118.7, C		119.9, C		118.7, C	
22	158.4, C		160.2, C		160.2, C		161.2, C		160.2, C	
23	106.6, CH	6.28, *s*	106.6, CH	6.28, *s*	106.6, CH	6.24, *s*	106.8, CH	6.22, *s*	106.6, CH	6.22, *s*
24	141.2, C		141.3, C		141.4, C		141.8, C		141.5, C	
25	110.7, CH	6.21, *s*	110.7, CH	6.21, *s*	110.8, CH	6.31, *s*	110.8, CH	6.28, *s*	110.8, CH	6.28, *s*
26	158.4, C		158.5, C		158.4, C		158.3, C		158.4, C	
27	35.4, CH_2	2.06, 1.60, *m*		2.06, 1.59, *m*	34.7, CH_2	2.14, 1.64, *m*	34.7, CH_2	2.11, 1.61, *m*	34.7, CH_2	2.11, 1.61, *m*
28	29.3, CH_2	1.39, 1.32, *m*	29.3, CH_2	1.39, 1.32, *m*	29.2, CH_2	1.64, 1.49, *m*	29.2, CH_2	1.61, 1.46, *m*	29.2, CH_2	1.61, 1.46, *m*
29	35.8, CH_2	1.90, 1.84, *m*	35.8, CH_2	1.91, 1.83, *m*	48.3, CH_2	2.50, 2.38, *m*	48.3, CH_2	2.47, 2.35, *m*	48.3, CH_2	2.47, 2.35, *m*
30	35.8, CH_2	3.42, *td* (7.0, 0.9)	35.8, CH_2	3.42, *td* (7.1, 0.8)	49.3, CH	5.91, *t* (6.3)	49.3, CH	5.88, *t* (6.4)	49.3, CH	5.88, *t* (6.3)

Table S3. *Cont.*

31	36.2, CH_2	2.00, 1.58, *m*	35.4, CH_2	2.06, 1.59, *m*	36.2, CH_2	2.03, 1.61, *m*	35.4, CH_2	2.06, 1.60, *m*	34.7, CH_2	2.11, 1.61, *m*
32	33.0, CH_2	1.29, 1.19, *m*	29.3, CH_2	1.39, 1.32, *m*	33.0, CH_2	1.29, 1.19, *m*	29.1, CH_2	1.46, 1.33, *m*	29.2, CH_2	1.61, 1.46, *m*
33	25.3, CH_2	1.33, n.o., *m*	35.8, CH_2	1.91, 1.83, *m*	25.3, CH_2	1.40, 1.34, *m*	35.7, CH_2	1.91, 1.84, *m*	48.3, CH_2	2.47, 2.35, *m*
34	15.8, CH_3	0.90, *t* (7.2)	35.8, CH_2	3.42, *td* (7.1, 0.8)	15.9, CH_3	0.93, *t* (7.2)	35.8, CH_2	3.42, *td* (7.0, 1.2)	49.3, CH	5.88, *t* (6.3)
35	17.9, CH_3	1.09, *d* (6.4)	17.9, CH_3	1.09, *d* (6.4)	17.9, CH_3	1.12, *d* (6.4)	17.9, CH_3	1.09, *d* (6.4)	17.9, CH_3	1.09, *d* (6.4)
36	17.9, CH_3	1.09, *d* (6.4)	17.9, CH_3	1.09, *d* (6.4)	17.9, CH_3	1.12, *d* (6.4)	17.9, CH_3	1.09, *d* (6.4)	17.9, CH_3	1.09, *d* (6.4)
37	161.3, C		161.2, C		161.3, C		162.3, C		161.2, C	
38	161.3, C		161.2, C		161.3, C		162.3, C		161.2, C	

[a] ^{13}C chemical shifts obtained from HMQC and HMBC spectra; Abbreviation: n.o. = not observed; [b] Numbering of the carbamidocyclophane framework, see Figure 4A.

Table S4. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR spectroscopic data for compounds **6–9** in MeOH-d_4. [a]

No.[b]	6		7		8		9	
	δ_C, Type	δ_H (*J* in Hz)	δ_C, Type	δ_H (*J* in Hz)	δ_C, Type	δ_H (*J* in Hz)	δ_C, Type	δ_H (*J* in Hz)
1	84.9, CH	4.93, *d* (10.4)	84.9, CH	4.90, *d* (10.3)	84.9, CH	4.90, *d* (10.6)	85.0, CH	4.89, *d* (10.4)
2	41.8, CH	1.85, *m*	41.8, CH	1.82, *m*	41.8, CH	1.82, *m*	41.7, CH	1.82, *m*
3	35.9, CH_2	0.89, 0.79, *m*	35.9, CH_2	0.88, 0.79, *m*	35.9, CH_2	0.88, 0.80, *m*	35.9, CH_2	0.88, 0.79, *m*
4	31.2, CH_2	1.54, n.o., *m*	31.2, CH_2	1.51, 0.91, *m*	31.1, CH_2	1.51, 0.90, *m*	31.0, CH_2	1.53, 0.90, *m*
5	31.9, CH_2	1.03, 0.80, *m*	31.9, CH_2	1.04, 0.80, *m*	31.8, CH_2	1.03, 0.81, *m*	31.8, CH_2	1.03, 0.81, *m*
6	36.8, CH_2	2.15, 1.43, *m*	36.7, CH_2	2.13, 1.41, *m*	36.7, CH_2	2.13, 1.41, *m*	36.7, CH_2	2.14, 1.41, *m*
7	37.9, CH	3.30, *m*	37.7, CH	3.28, *m*	37.8, CH	3.28, *m*	37.7, CH	3.28, *m*
8	119.2, 119.7, C		119.2, 119.7, C		119.2, 119.7, C		119.9, C	
9	160.3, C		160.3, C		160.2, C		160.3, C	
10	106.6, CH	6.32, *s*	106.7, CH	6.31, *s*	106.6, CH	6.30, *s*	106.9, CH	6.29, *s*
11	141.2, C		141.3, C		141.4, C		141.8, C	
12	110.8, CH	6.20, *s*	110.9, CH	6.24, *s*	110.8, CH	6.22, *s*	111.0, CH	6.22, *s*
13	158.4, C		158.4, C		158.4, C		n.o.	
14	83.1, CH	3.86, *d* (9.8)	83.1, CH	3.84, *d* (9.6)	83.1 CH	3.84, *d* (9.5)	83.2, CH	3.83, *d* (9.5)
15	43.4, CH	1.66, *m*	43.4, CH	1.63, *m*	43.4, CH	1.63, *m*	43.4, CH	1.63, *m*
16	36.6, CH_2	0.85, 0.74, *m*	36.6, CH_2	0.83, 0.72, *m*	36.5, CH_2	0.82, 0.71, *m*	36.5, CH_2	0.83, 0.73, *m*

Table S4. *Cont.*

17	31.2, CH_2	1.54, n.o., *m*	31.2, CH_2	1.51, 0.91, *m*	31.1, CH_2	1.51, 0.91, *m*	31.1, CH_2	1.51, n.o., *m*
18	31.9, CH_2	1.03, 0.80, *m*	31.9, CH_2	1.04, 0.80, *m*	31.8, CH_2	1.03, 0.81, *m*	31.8, CH_2	1.03, 0.81, *m*
19	36.8, CH_2	2.15, 1.43, *m*	36.7, CH_2	2.13, 1.41, *m*	36.7, CH_2	2.13, 1.41, *m*	36.7, CH_2	2.14, 1.41, *m*
20	38.2, CH	3.27, *m*	38.2, CH	3.24, *m*	37.8, CH	3.28, *m*	37.7, CH	3.28, *m*
21	118.5, 119.0, C		118.5, 119.0, C		118.5, 119.0, C		118.3, C	
22	160.2, C		160.4, C		160.3, C		160.3, C	
23	106.4, CH	6.18, *s*	106.5, CH	6.36, *s*	106.4, CH	6.34, *s*	106.6, CH	6.33, *s*
24	145.4, C		145.5, C		145.6, C		146.0, C	
25	110.2, CH	6.35, *s*	110.3, CH	6.19, *s*	110.2, CH	6.16, *s*	110.4, CH	6.16, *s*
26	158.4, C		158.5, C		158.4, C		n.o.	
27	35.4, CH_2	2.11, 1.61, *m*	35.4, CH_2	2.06, 1.59, *m*	34.8, CH_2	2.13, 1.60, *m*	34.7, CH_2	2.12, 1.60, *m*
28	29.2, CH_2	1.38, 1.33, *m*	29.2, CH_2	1.60, 1.45, *m*	29.2, CH_2	1.60, 1.45, *m*	29.1, CH_2	1.60, 1.45, *m*
29	35.8, CH_2	1.93, n.o., *m*	48.3, CH_2	2.49, 2.35, *m*	48.2, CH_2	2.48, 2.34, *m*	48.3, CH_2	2.48, 2.34, *m*
30	35.9, CH_2	3.43, *m*	49.4, CH	5.89, *t* (6.3)	49.4, CH	5.89, *td* (6.3, 1.4)	49.3, CH	5.88, *td* (6.3, 1.4)
31	36.2, CH_2	2.03, 1.59, *m*	36.2, CH_2	2.01, 1.57, *m*	35.3, CH_2	2.08, 1.59, *m*	34.7, CH_2	2.12, 1.60, *m*
32	33.1, CH_2	1.28,1.17, *m*	33.2, CH_2	1.26, 1.19, *m*	29.2, CH_2	1.42, 1.35, *m*	29.1, CH_2	1.60, 1.45, *m*
33	25.3, CH_2	1.40, 1.32, *m*	25.3, CH_2	1.37, 1.28, *m*	35.5, CH_2	1.91, 1.84, *m*	48.3, CH_2	2.48, 2.34, *m*
34	15.9, CH_3	0.92, *m*	15.9, CH_3	0.90, *m*	35.9, CH_2	3.41, *m*	49.3, CH	5.88, *td* (6.3, 1.4)
35	18.0, CH_3	1.12, *d* (6.1)	18.0, CH_3	1.09, *d* (6.3)	17.9, CH_3	1.09, *d* (6.4)	17.9, CH_3	1.09, *d* (6.4)
36	18.3, CH_3	1.17, *d* (6.1)	18.3, CH_3	1.15, *d* (6.3)	18.3, CH_3	1.14, *d* (6.4)	18.3, CH_3	1.15, *d* (6.4)
37	161.3, C		161.3, C		161.2, C		161.5, C	

[a] ^{13}C chemical shifts obtained from HMQC and HMBC spectra; Abbreviation: n.o. = not observed; [b] Numbering of the carbamidocyclophane framework, see Figure 4A.

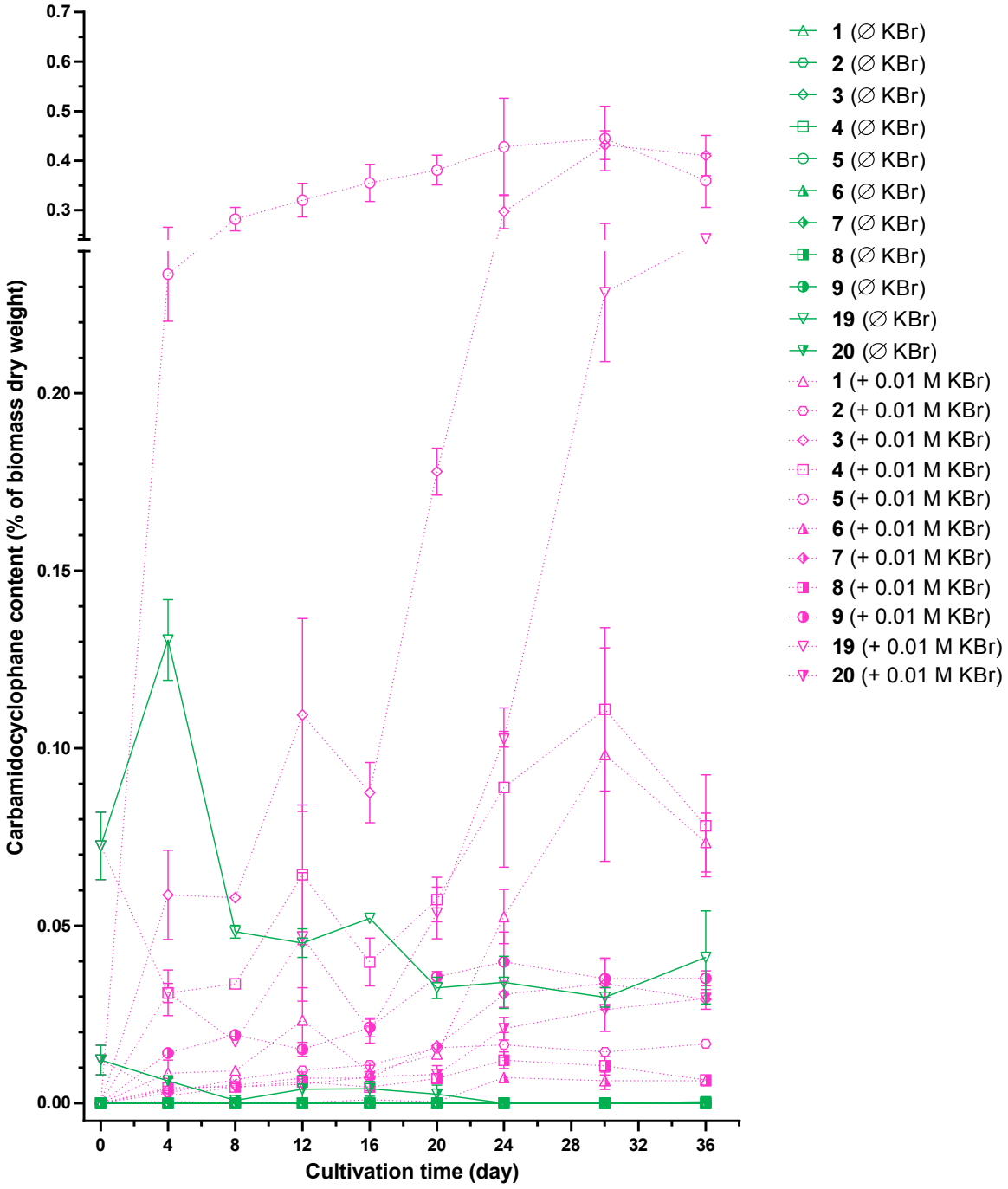

Figure S1. Intracellular contents of brominated (**1**–**9**) and non-halogenated (**19** and **20**) carbamidocyclophanes of *Nostoc* sp. CAVN2 cultivated in Z½ medium as control containing <0.1 μM halide ions (Ø KBr) or in bromide-enriched (+0.01 M KBr) Z½ medium. Values shown are expressed as the mean ± SEM, $n = 3$.

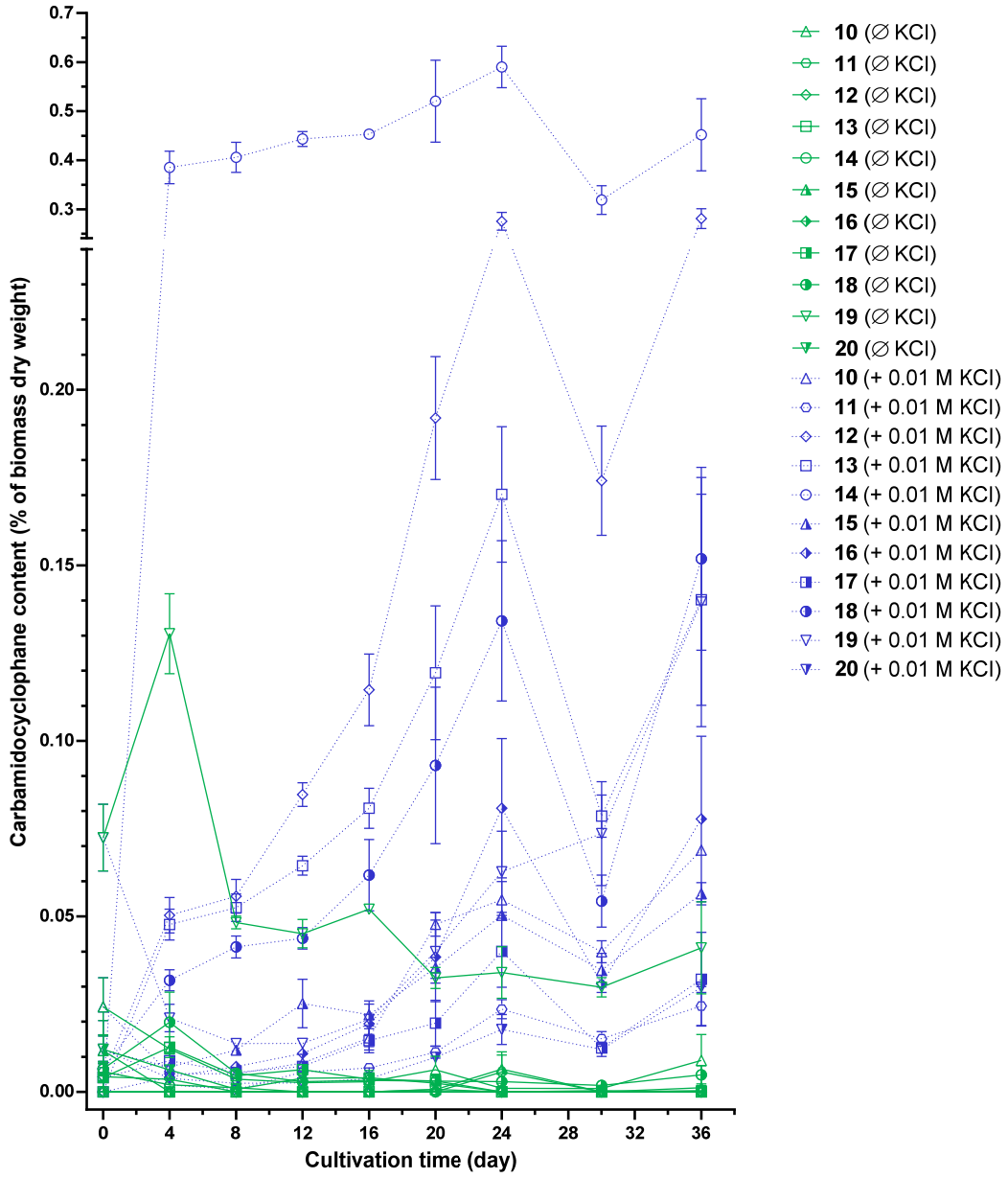

Figure S2. Intracellular contents of chlorinated (**10**–**18**) and non-halogenated (**19** and **20**) carbamidocyclophanes of *Nostoc* sp. CAVN2 cultivated in Z½ medium as control containing <0.1 µM halide ions (Ø KCl) or in chloride-enriched (+ 0.01 M KCl) Z½ medium. Values shown are expressed as the mean ± SEM, $n = 3$.

Table S5. Biomass dry weights of *Nostoc* sp. CAVN2 cultures grown in the presence of KCl and KBr at different mixing ratios for 20 days. Values are presented as mean ± SEM, $n = 3$.

CAVN2 Culture	Biomass Dry Weight (g/L)	Statistical Significance *versus* the Control [a]
Control	0.35 ± 0.01	
+0.01 M KCl and 0.01 M KBr	0.77 ± 0.03	**
+0.01 M KCl and 0.001 M KBr	0.68 ± 0.02	No
+0.001 M KCl and 0.01 M KBr	0.64 ± 0.03	No

[a] Two asterisks (**) indicate a significant difference of $p < 0.01$ versus the control; Data were tested for Gaussian distribution using the Kolmogorov-Smirnov test; Statistical analysis was performed using the Kruskal-Wallis test followed by Dunn's multiple comparisons test.

Table S6. List of predicted open reading frames (ORF) of the *cab* gene cluster. [a]

ORF	Annotation	Protein	Amino Acids	Top BlastP Result Protein [Organism] (Acc. No.)	Ident/Sim/Qcov (%), E-Value	InterPro Results
1	Caspase-like domain-containing protein		220	Hypothetical protein [*Scytonema hofmanni* UTEX B 1581] (WP_051502726)	56/68/87.27, 8.16e-57	Caspase-like domain(IPR029030)
2	Long-chain-fatty-acid—CoA ligase	CabA	604	AMP-dependent synthetase/ligase [*Cylindrospermum licheniforme* UTEX B 2014] (AFV96135)	88/94/99.83, 0	AMP-dependent synthetase/ligase (IPR000873)
3	Acyl carrier protein	CabB	103	Acyl carrier protein [*C. licheniforme* UTEX B 2014] (AFV96136)	87/90/100, 1.61e-57	Acyl carrier protein-like (IPR009081)
4	Hypothetical protein	CabC	471	Hypothetical protein [*C. licheniforme* UTEX B 2014] (AFV96137)	96/97/100, 0	None
5	Polyketide synthase	CabD	1,385	Polyketide synthase [*C. licheniforme* UTEX B 2014] (AFV96138)	88/94/77.11, 0	Polyketide synthase, beta-ketoacyl synthase domain (IPR020841), acyl transferase domain (IPR020801), 3× phosphopantetheine-binding domain (IPR020806)
6	Beta-ketoacyl synthase	CabE	413	Beta-ketoacyl synthase [*C. licheniforme* UTEX B 2014] (AFV96139)	92/96/100, 0	Polyketide synthase, beta-ketoacyl synthase domain (IPR020841)
7	Hydroxymethylglutaryl-CoA synthase	CabF	419	3-Hydroxy-3-methylglutaryl CoA synthase [*Cylindrospermum stagnale*] (WP_015207399)	94/98/100, 0	Hydroxymethylglutaryl-coenzyme A synthase, N-terminal (IPR013528), C-terminal domain (IPR013746)
8	Enoyl-CoA hydratase	CabG	258	Enoyl-CoA hydratase/isomerase [*C. licheniforme* UTEX B 2014] (AFV96141)	90/95/98.45, 1.41e-162	Crotonase superfamily (IPR001753)

Table S6. *Cont.*

9	Polyketide synthase	CabH	2077	Polyketide synthase family protein [*C. stagnale*] (WP_015207397)	84/91/68.85, 0	Crotonase superfamily (IPR001753); Polyketide synthase, enoylreductase domain (IPR020843), beta-ketoacyl synthase domain (IPR020841), acyl transferase domain (IPR020801), phosphopantetheine-binding domain (IPR020806); Thioesterase (IPR001031)
10	Type III polyketide synthase	CabI	373	Naringenin-chalcone synthase [*C. stagnale*] (WP_015207396)	92/97/100, 0	FAE1/Type III polyketide synthase-like protein (IPR013601); Chalcone/stilbene synthase, C-terminal (IPR012328)
11	Isoprenylcysteine carboxyl methyltransferase	CabJ	199	Phospholipid methyltransferase [*C. licheniforme* UTEX B 2014] (AFV96144)	84/94/100, 3.66e-122	Isoprenylcysteine carboxyl methyltransferase (IPR007269)
12	Beta-propeller repeat-containing protein	CabK	687	Hemolysin-type calcium-binding region [*C. licheniforme* UTEX B 2014] (AFV96145)	79/90/92.87, 0	Quinonprotein alcohol dehydrogenase-like superfamily (IPR011047); Beta-propeller repeat (IPR010620)
13	Carbamoyltransferase	CabL	581	Hypothetical protein [Methylococcaceae bacterium Sn10-6] (WP_052700247)	52/68/71.08, 6.74e-150	Carbamoyltransferase (IPR003696)
14	Rieske [2Fe-2S] iron-sulphur domain-containing protein	CabM	502	Ring-hydroxylating dioxygenase [*C. stagnale*] (WP_015207391)	89/95/100, 0	Rieske [2Fe-2S] iron-sulphur domain (IPR017941); Pheophorbide a oxygenase (IPR013626)

[a] Abbreviations: Acc. No. = accession number; Ident = identity, Sim = similarity, Qcov = query coverage, E-value = expectation value.

Table S7. Results of the MegaBLAST search of the *C. licheniforme* UTEX 'B 2014' *cyl* gene cluster (query) against the *Nostoc* sp. CAVN2 *cab* gene cluster (subject).

Identity (%)	Alignment Length	Mismatches	Gap Opens	Query		Subject		E-Value	Bit Score
				Start	End	Start	End		
88.78	1880	206	4	1005	2879	1337	3216	0	2298.0
84.60	448	51	10	3345	3788	3275	3708	4e-121	429.0
91.08	4954	423	9	3924	8865	3833	8779	0	6682.0
85.01	467	68	2	8478	8943	8791	9256	2e-134	473.0
89.56	5767	573	19	8886	14646	9276	15019	0	7287.0
83.51	97	16	0	14691	14787	14923	15019	2e-19	91.6
76.68	223	36	7	14832	15054	14923	15129	5e-25	110.0
87.72	6148	687	40	15058	21189	15044	21139	0	7108.0
85.66	1980	273	10	21414	23388	21468	23441	0	2073.0

Table S8. Results of the MegaBLAST search of the *Nostoc* sp. CAVN2 *cab* gene cluster (query) against the *C. stagnale* PCC 7417 gene cluster (subject).

Identity (%)	Alignment Length	Mismatches	Gap Opens	Query		Subject		E-Value	Bit Score
				Start	End	Start	End		
88.46	1881	207	6	1337	3216	797	2668	0	2263.0
89.75	5979	565	25	3275	9223	2853	8813	0	7601.0
86.36	396	54	0	8384	8779	8372	8767	3e-122	433.0
85.27	387	57	0	8791	9177	7981	8367	3e-122	399.0
89.64	5744	565	20	9300	15019	8799	14536	0	7284.0
82.47	97	17	0	14923	15019	14581	14677	1e-17	86.1
83.51	97	16	0	14923	15019	14722	14818	2e-19	91.6
87.07	6282	726	43	14923	21139	14863	21123	0	7023.0
86.34	1999	264	8	21468	23461	21349	23343	0	2170.0
91.51	106	8	1	23775	23880	23650	23754	2e-35	145.0
95.31	128	6	0	23871	23998	24026	24153	3e-53	204.0
87.25	659	55	13	25853	26482	24185	24843	0	725.0
90.80	1630	135	4	26727	28341	25970	27599	0	2165.0

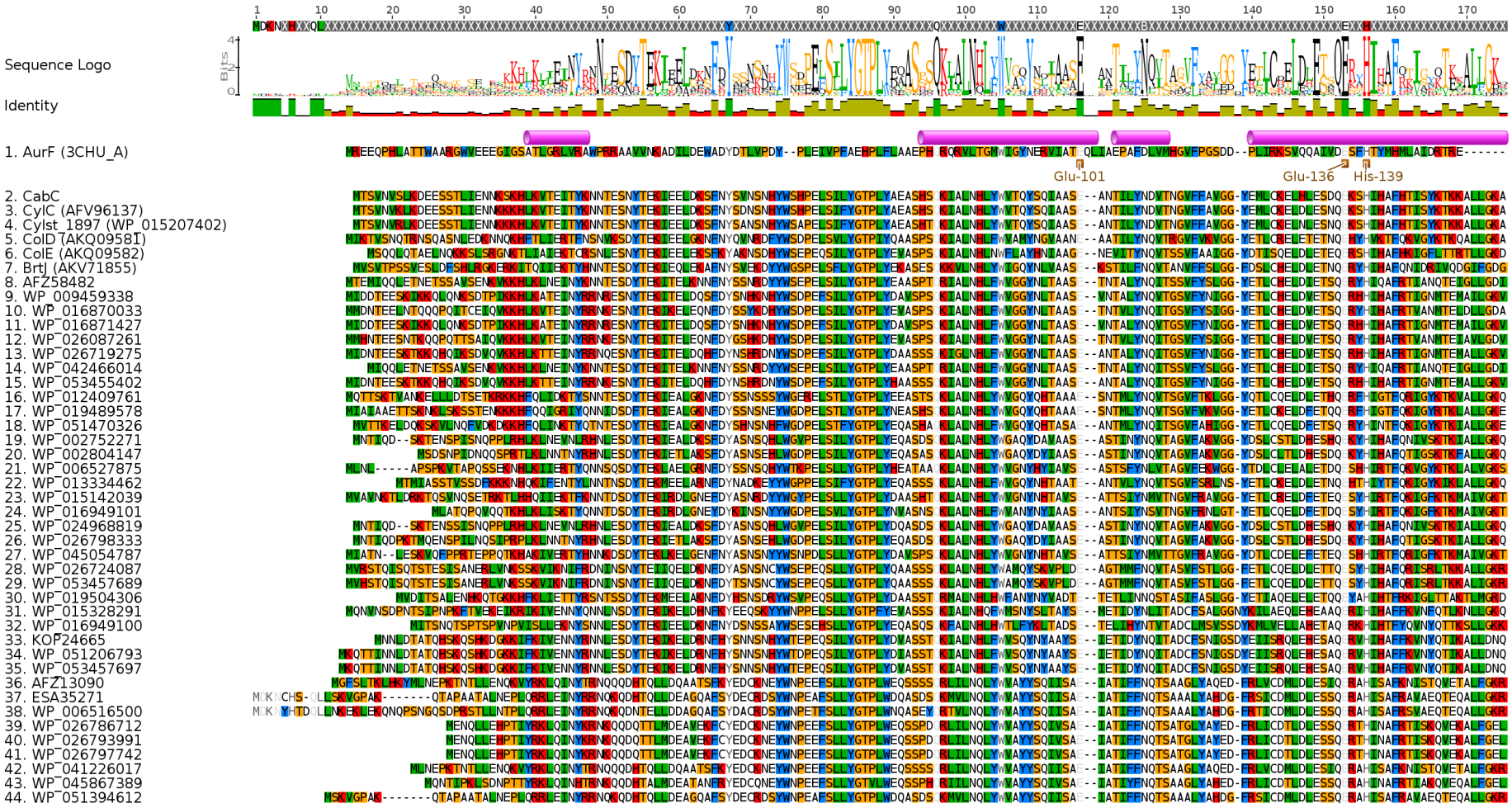

Figure S3. *Cont.*

Figure S3. *Cont.*

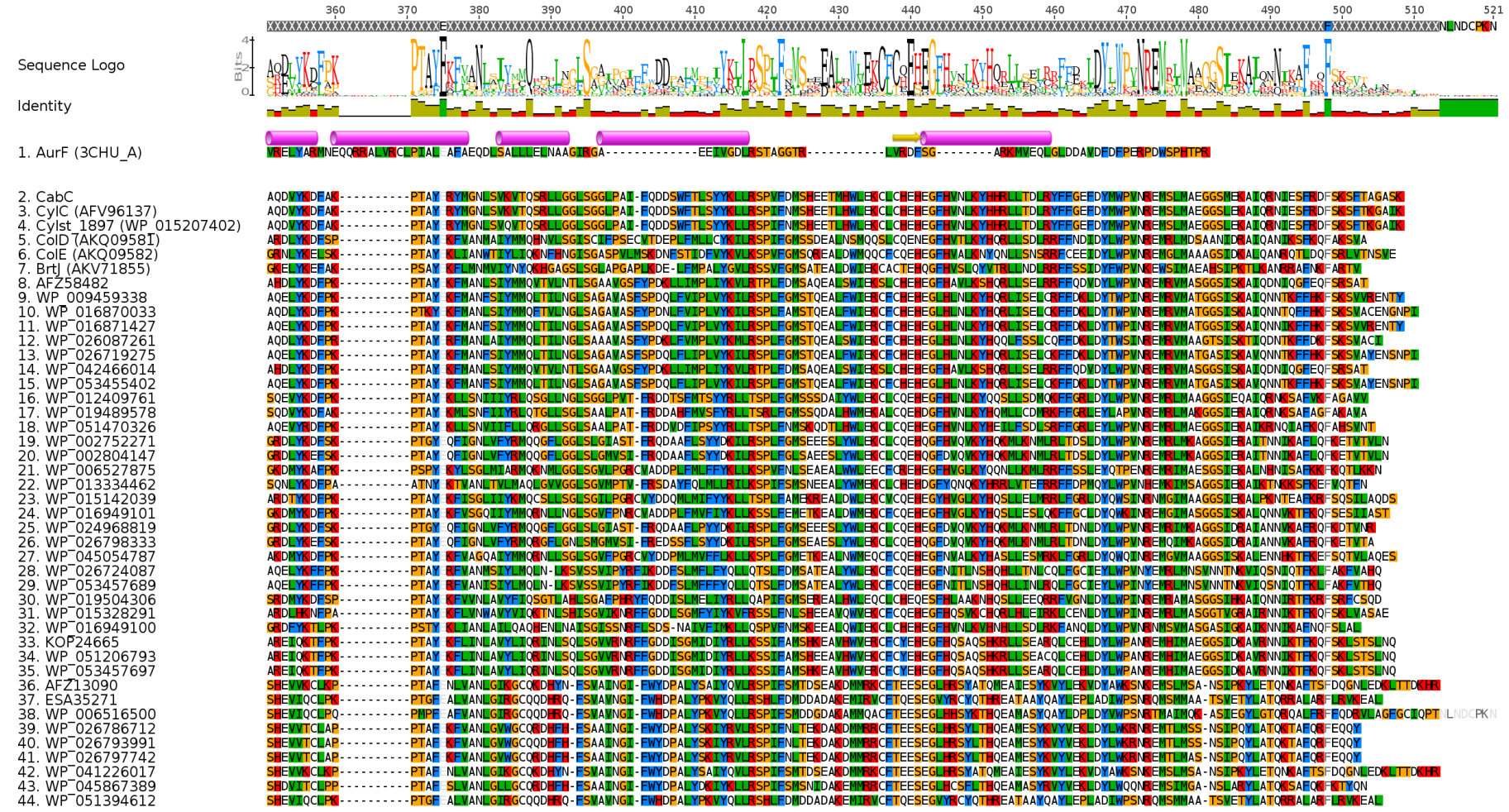

Figure S3. Protein sequence alignment of CabC and homologues. The consensus sequence based on a 100% conservation rule along with a sequence logo and an identity graph is depicted at the top. The first sequence shows the *p*-aminobenzoate *N*-oxygenase (AurF) Chain A of *Streptomyces thioluteus*. Conserved glutamic acid and histidine residues responsible for di-iron coordination are depicted below this sequence.

Table S9. Oligonucleotide primers used for gap-closure and frameshift refutation.

Name	Sequence 5′–3′	Usage [a]	Comment
MP01_129-for	GGAAATTTGAACCACCGAC	A	Gap-closure between contig00129 and contig00638
MP02_129-for_seq	CCTTGAACTTTACTACTAG	S	
MP03_638-rev_seq	AGGTATTGGATTACGAATC	S	
MP04_638-rev	AGTAGCAATAGCCGCCATC	A	
MP05_638-for	ATCTGCTTATGATGTAGCG	A	Gap-closure between contig00638 and contig00697
MP06_638-for_seq	AGCATAGTGAACTGCGACC	S	
MP07_697-rev_seq	TCCCTTTGTCTAGCAGGAC	S	
MP08_697-rev	GCTAAACCAACAGCATATG	A	
MP09_129-for_seq2	GAGATTCAGCAGGTTCGG	S	Gap-closure between contig00129 and contig00638
SH1_CAVN2-F	CACCCCCTTTAGAAGACCTGG	A	Refutation of two putative frameshifts in 454-based assembly
SH2_CAVN2-R	GTGCATCTTCCCAAGCCTCT	A	
SH3_CAVN2-F	GTTACAAGCCCCTAAGTTCG	S	
SH4_CAVN2-F	CGTCTAGCTGGTGTTAGTGC	S	
SH5_CAVN2-F	TCCCAAGAGAATGCAGCCAG	A, S	
SH6_CAVN2-R	GCCATCAAGCTCATTTCCCG	A, S	
SH7_CAVN2-F	GTAAGGCGCTGCAAGAACTG	A, S	
SH8_CAVN2-R	ATCTGGTTTGATGCCCCAGG	A, S	

[a] Abbreviations: A = amplification, S = sequencing.

Table S10. Basic data of biological activity for compounds **1–30** against Gram-positive as well as Gram-negative bacteria and HaCaT cells.[a]

#	MIC (µg/mL)														IC$_{50}$ (µg/mL)
	E. faecium DSM-20477	*E. faecium* DSM-17050 (VREF)	*M. tuberculosis* ATCC 25618 (H37Rv)	*S. aureus* Newman (MSSA)	*S. aureus* N315 (MRSA)	*S. aureus* 1 (MRSA)[b]	*S. aureus* Mu50 (MRSA/VISA)	*S. pneumoniae* 7 (ATCC 49619)[b]	*S. pneumoniae* DSM-20566	*S. pneumoniae* DSM-11865 (PRSP)	*E. coli* 13[b]	*E. coli* TolC-deficient	*E. coli* TolC-deficient + PMBN	*K. pneumoniae* 18 (KRKP)[b]	*P. aeruginosa* 22 (MDR)[b]	HaCaT[b]
1	8	2–8	8–12	1	0.25–0.5	0.08	0.5	0.2	1	2	>50	>64	2	>50	>50	2.9
2	8	4–8	2–5	0.25	0.25–0.5	n.t.	0.25–0.5	n.t.	1	0.25–0.5	n.t.	>64	1–2	n.t.	n.t.	n.t.
3	8	8	1–1.5	0.125–0.25	0.125	0.63	0.125–0.25	0.2	0.5	0.25–0.5	>50	>64	2	>50	>50	2.1
4	8	4–8	0.5–1.5	0.5	0.25–0.5	0.08	0.25	0.2	1	0.5	>50	>64	1	>50	>50	3.5
5	32	16–32	2–4	0.25	0.25	0.16	0.125	0.2	1	0.5–2	>50	>64	8–16	>50	>50	7.4
6	n.t.	8–16	4–8	n.t.	0.5	n.t.	n.t.	n.t.	n.t.	1	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.
7	2	2	0.5–1.5	0.125	0.06–0.125	0.04	0.125	0.2	0.25	0.25	>50	>64	1	>50	>50	2.4
8	4	4	2–5	0.25	0.125–0.25	n.t.	0.25	n.t.	0.5–1	0.5	n.t.	>64	1	n.t.	n.t.	n.t.
9	8	4	0.5–2	0.125–0.25	0.125	0.16	0.5	0.2	0.25–0.5	0.25	>50	>64	1	>50	>50	7.5
10	4	4	2–3	0.125–0.25	0.125	0.08	0.125–0.25	0.2	0.5–1	0.25–0.5	>50	>64	1–2	>50	>50	4.0
11	n.t.	8	2–4	n.t.	0.25	0.08	n.t.	0.2	n.t.	0.25	>50	n.t.	n.t.	>50	>50	2.2
12	4	2–4	2–4	0.125–0.25	0.125	0.08	0.125	0.2	0.25–0.5	0.25	>50	>64	1	>50	>50	2.1
13	4	4	1–1.5	0.125–0.25	0.125	0.08	0.125	0.2	0.25–0.5	0.125–0.25	>50	>64	1	>50	>50	3.4
14	4	4	32–64	0.25	0.125	0.08	0.125–0.5	0.2	0.25–0.5	0.25–0.5	>50	>64	1–2	>50	>50	3.9
15	n.t.	4	2–3	n.t.	0.25	0.08	n.t.	0.2	n.t.	0.5	>50	n.t.	n.t.	>50	>50	3.8
16	2	2	1.5–2	0.125–0.25	0.06–0.125	0.08	0.125	0.2	0.25	0.5	>50	>64	0.5–1	>50	>50	2.7
17	2	2	2–5	0.125–0.25	0.06–0.125	0.08	0.125	0.2	0.25	0.25	>50	>64	0.5	>50	>50	3.4
18	2–4	2	1–1.5	0.125	0.06	0.08	0.125	0.2	0.25	0.25	>50	>64	0.5–1	>50	>50	3.6
19	4–8	8	2–3	0.125	0.125	0.08	0.125	0.2	0.5	0.25	>50	>64	1–4	>50	>50	2.5
20	n.t.	2	1.5–2	n.t.	0.125–0.25	0.08	n.t.	0.2	n.t.	0.25	>50	n.t.	n.t.	>50	>50	4.8
21	n.t.	8	>8	1–2	0.5	0.63	8	1.3	n.t.	2	>50	>64	4–8	>50	>50	7.0
22	n.t.	n.t.	>8	n.t.	n.t.	0.63	n.t.	1.3	n.t.	n.t.	>50	n.t.	n.t.	>50	>50	7.5
23	n.t.	4–8	2–4	0.5	0.5	0.31	1	0.63	n.t.	n.t.	>50	>64	2	>50	>50	5.9
24	n.t.	n.t.	>8	n.t.	n.t.	0.31	n.t.	0.63	n.t.	n.t.	>50	n.t.	n.t.	>50	>50	6.7
25	4	2	2–4	0.125	0.125	0.26	0.125	0.57	0.25	0.25	>50	>64	1–2	>50	>50	2.9
26	4	2	0.5–1	0.125	0.125	0.08	0.125	0.2	0.5	0.125	>50	>64	2–4	>50	>50	1.8
27	8	2	1–2	1–4	0.5–2	0.63	2–8	1.6	2–8	4–8	>50	>64	64	>50	>50	7.3
28	16	16	>16	4	4–8	3.2	4–8	6.23	4	4	>50	>64	4	>50	>50	37.3
29	64	64	>16	2–4	4	>50	4–8	>50	2–8	2	>50	>64	8–32	>50	>50	16.5
30	>64	>64	>16	4–8	>64	>50	>64	>50	4	2–4	>50	>64	>64	>50	>50	17.5
POS	2.0[c]	>64[c]	0.024–0.036[d] 0.125–0.25[e] 0.02–0.08[f] 0.2–0.8[g]	0.5[c]	1.0[c]	2.0[c,h]	16[c]	2.0[c,h]	<0.03[i]	>64[i]	0.0062[j,k,l] 62.5[c]	0.003[j]	0.003[j]	1.25[j] 0.62[k]	0.025[l] 250[c]	3.9[m]

[a] Abbreviations: VREF = vancomycin-resistant *E. faecium*, MSSA = methicillin-sensitive *S. aureus*, MRSA = methicillin-resistant *S. aureus*, VISA = vancomycin-intermediate *S. aureus*, PRSP = penicillin-resistant *S. pneumoniae*, PMBN = polymyxin B nonapeptide, KRKP = kanamycin-resistant *K. pneumoniae*, MDR = multi-drug resistant (for detailed resistance profile, see Pretsch *et al*. [4]), n.t. = not tested, POS = positive control; [b] Equivalent data for **10–30** have previously been reported; For further details, see Preisitsch *et al*. [5] (**10–25**) and Preisitsch *et al*. [6] (**26–30**); [c] vancomycin; [d] delamanid; [e] pretomanid (formerly known as PA-824); [f] isoniazid; [g] rifampicin; [h] fusidic acid; [i] ampicillin; [j] ciprofloxacin; [k] moxifloxacin; [l] levofloxacin; [m] mitoxantrone.

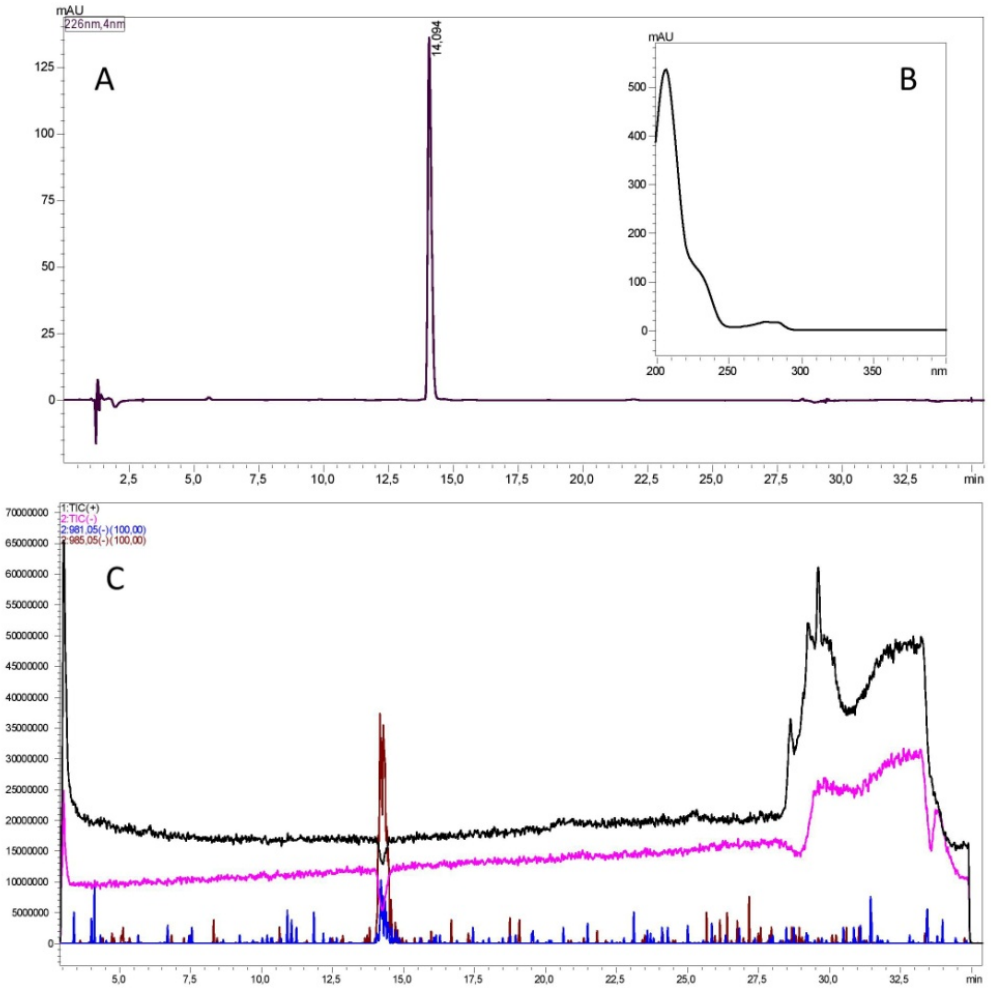

Figure S4. *Cont.*

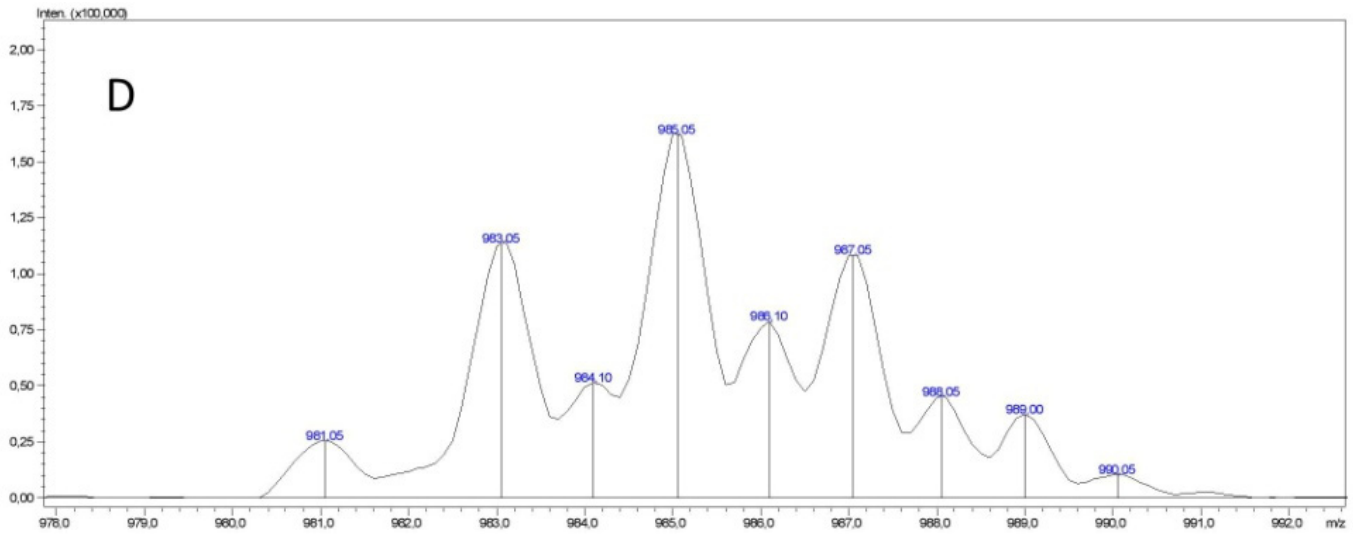

Figure S4. Identity and stability control of carbamidocyclophane Q (**5**) after the antimycobacterial bioactivity assay. HPLC-UV-MS analysis was performed on a Shimadzu LC-20A Prominence liquid chromatography system with a SPD-M20A diode array detector (DAD) coupled to a Shimadzu LCMS-8030 triple quadrupole (QqQ) mass spectrometer using a Phenomenex Kinetex PFP column (100 × 4.6 mm, 2.6 µm, 100 Å) and a binary gradient of MeOH in deionized H_2O with a flow rate of 0.8 mL/min from 60% to 80.3% MeOH in 26 min at 40 °C. Sample: 0.1 mg of **5** in 1.0 mL MeOH, 10 µL injection, solvent flow split of 10:1 after the DAD analysis and prior to QqQ measurement. (**A**) HPLC chromatogram of **5** detected at wavelength λ = 226 nm; (**B**) Online UV spectrum (λ from 200 to 400 nm) of **5** at retention time 14.1 min; (**C**) Total ion chromatograms (TICs) of **5** (positive mode in black and negative mode in magenta) and its extracted ion chromatograms (EICs) for *m*/*z* 981.05 and 985.05 (both negative mode), consistent with the monoisotopic mass peak $[M - H]^-$ and the most abundant isotopic mass peak $[M + 4 - H]^-$, respectively; (**D**) Measured isotopic distribution pattern of **5** referring to the $[M - H]^-$ ion.

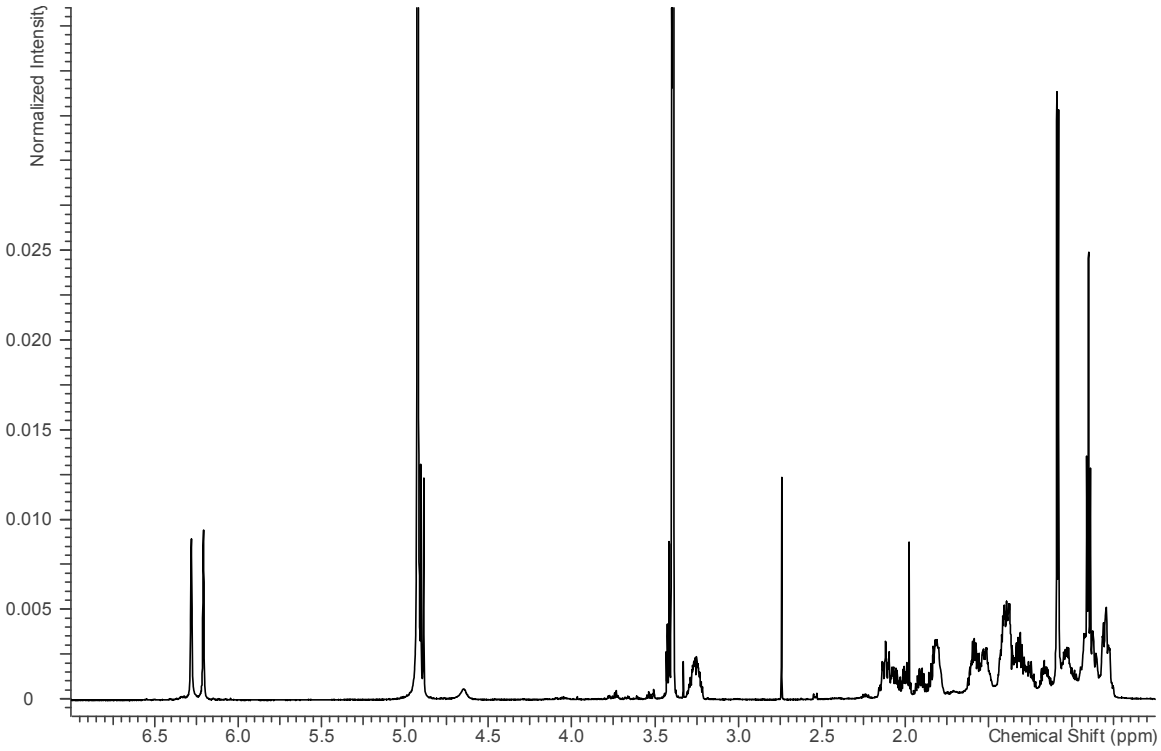

Figure S5. ^{1}H NMR spectrum (600 MHz, MeOH-d_4) of carbamidocyclophane M (**1**).

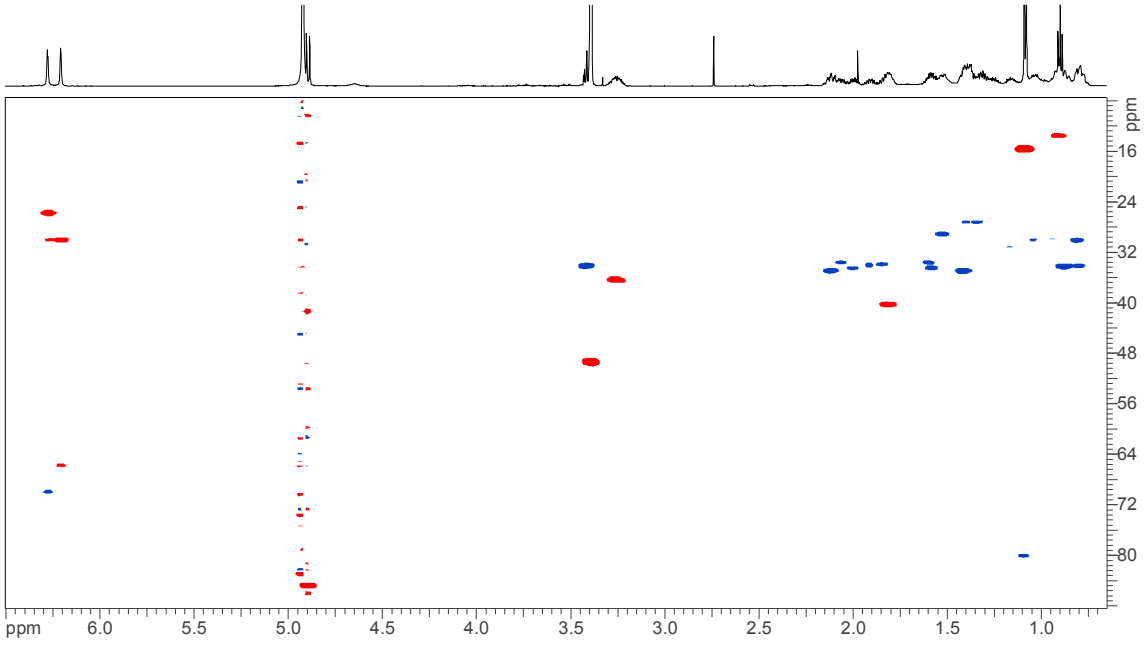

Figure S6. HMQC-DEPT spectrum (600 MHz, MeOH-d_4) of carbamidocyclophane M (**1**). Red signals are attributed to CH or CH_3 groups (positively phased) and blue signals to CH_2 groups (negatively phased).

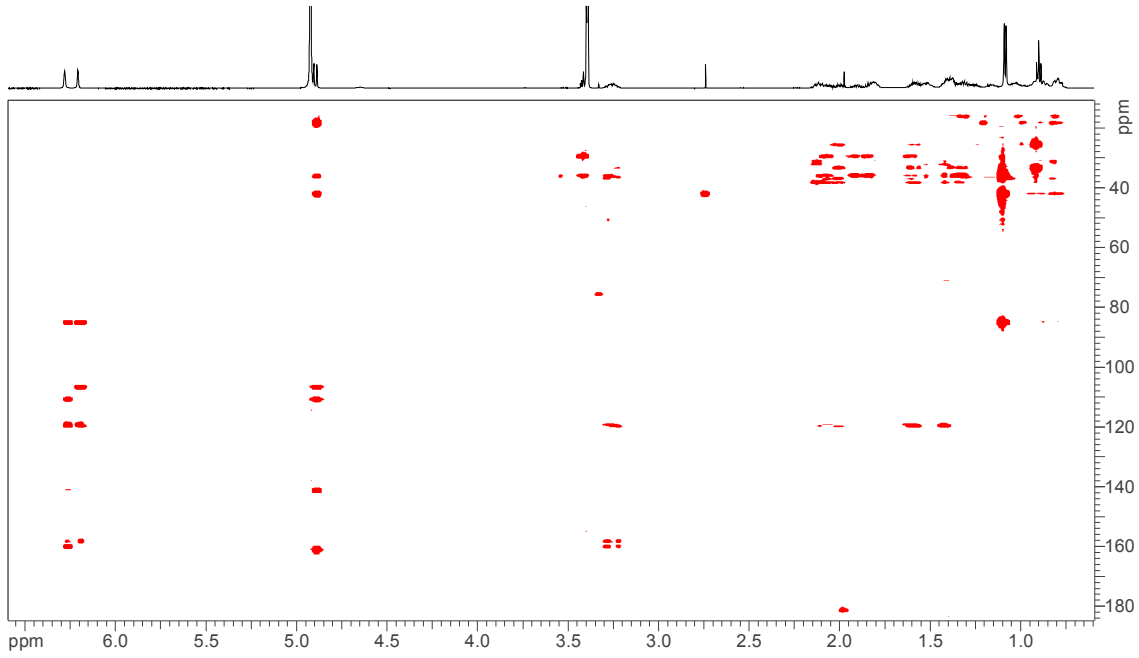

Figure S7. HMBC spectrum (600 MHz, MeOH-d_4) of carbamidocyclophane M (**1**).

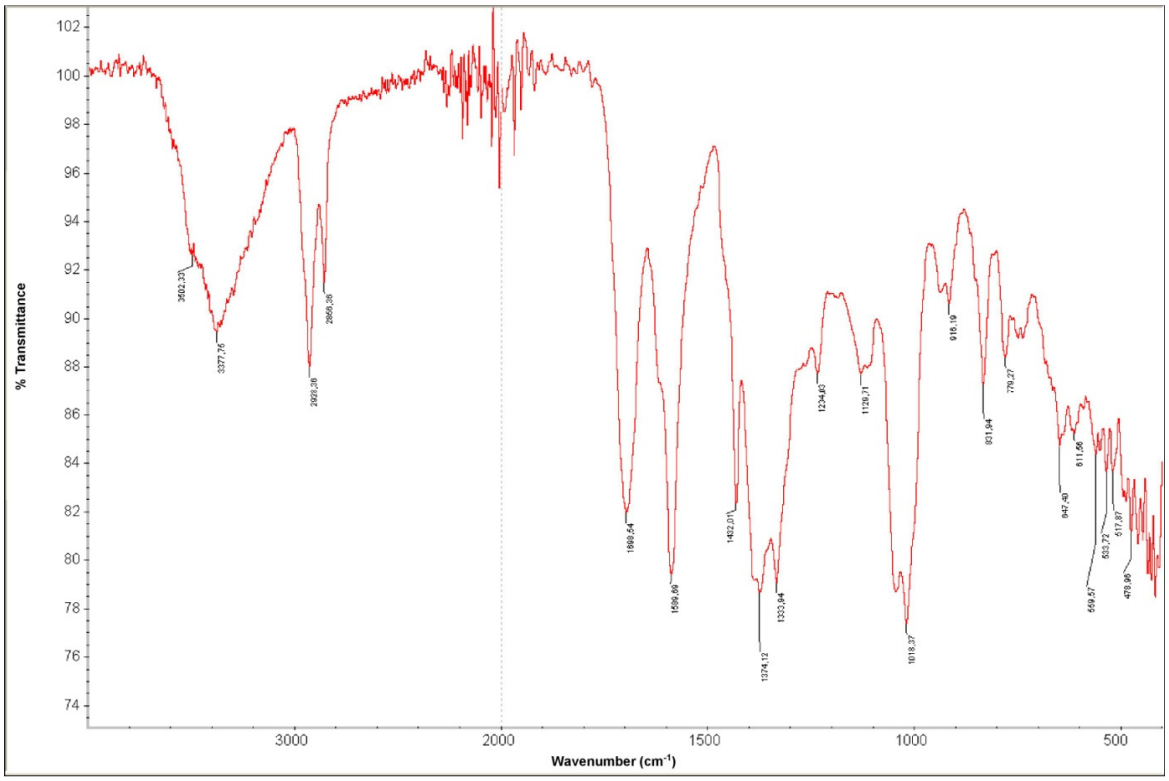

Figure S8. ATR-IR (film) spectrum of carbamidocyclophane M (**1**).

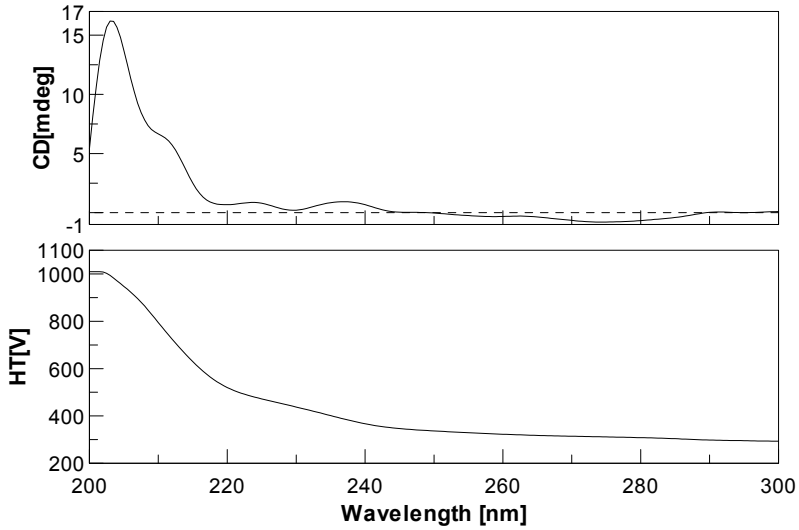

Figure S9. ECD spectrum of carbamidocyclophane M (**1**).

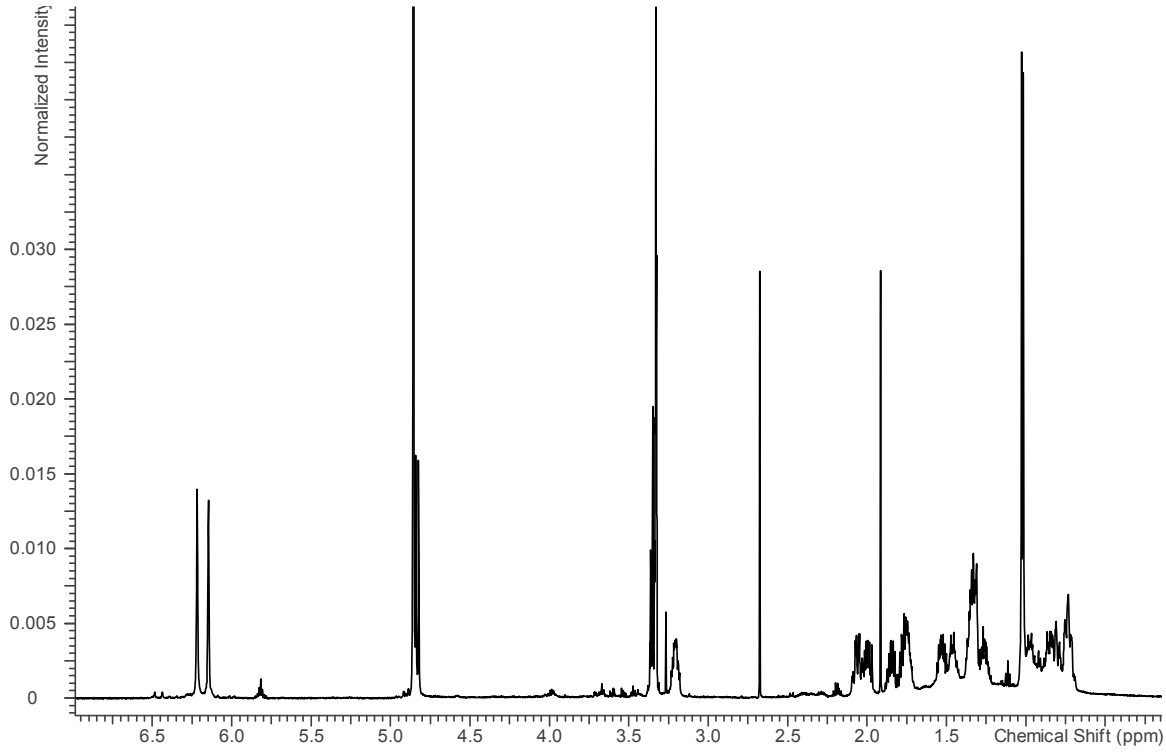

Figure S10. ^{1}H NMR spectrum (600 MHz, MeOH-d_4) of carbamidocyclophane N (**2**).

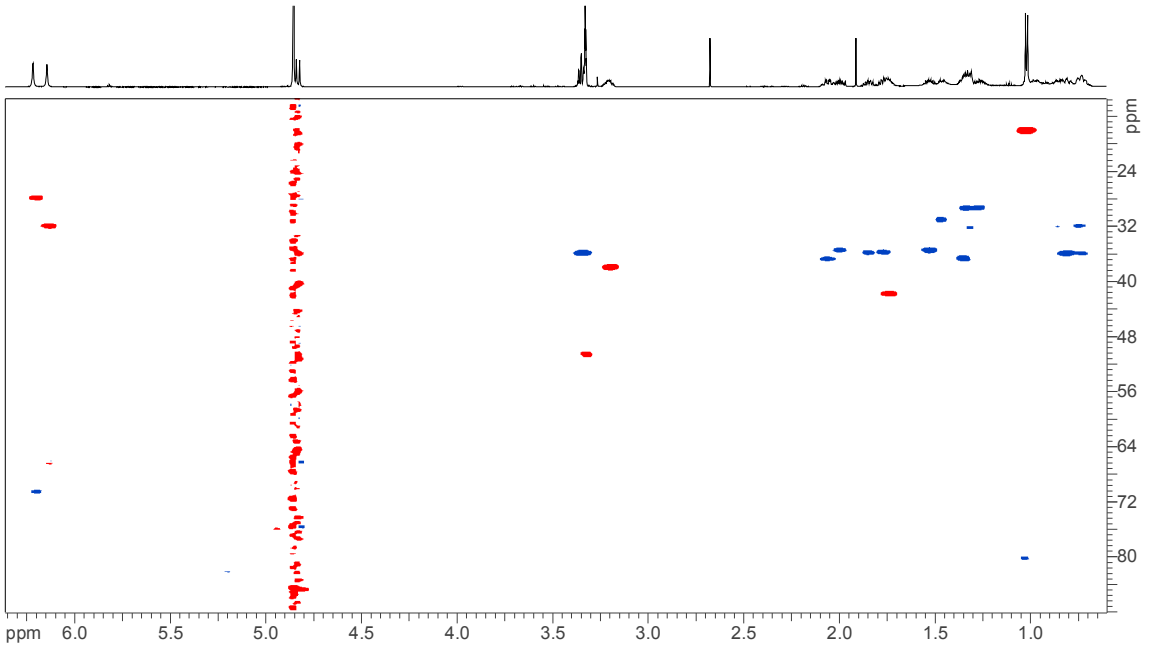

Figure S11. HMQC-DEPT spectrum (600 MHz, MeOH-d_4) of carbamidocyclophane N (**2**). Red signals are attributed to CH or CH_3 groups (positively phased) and blue signals to CH_2 groups (negatively phased).

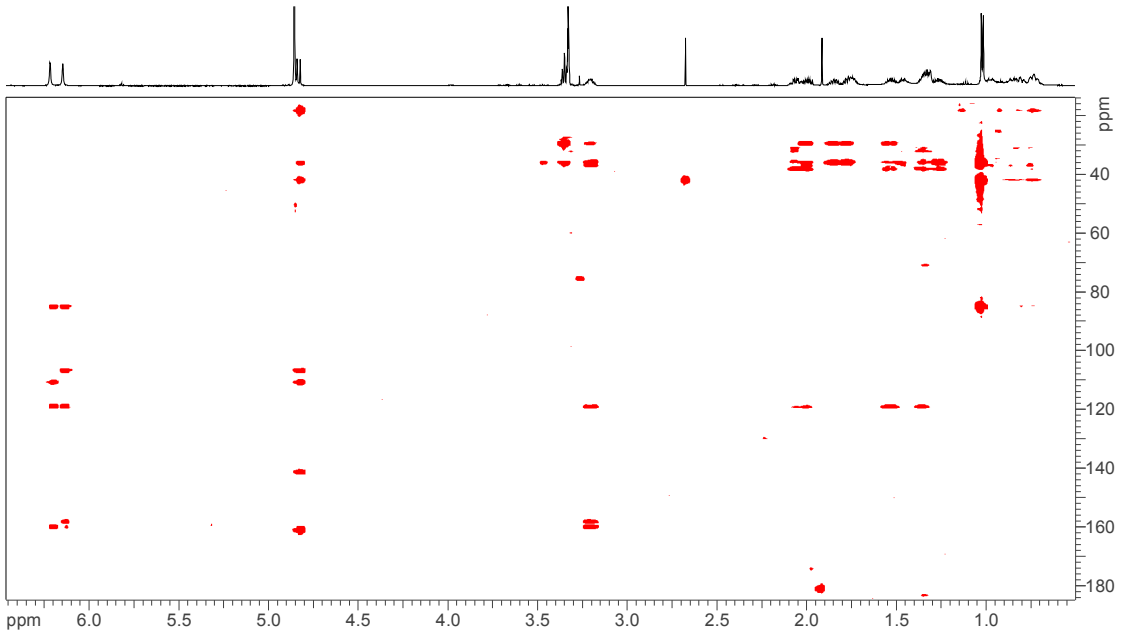

Figure S12. HMBC spectrum (600 MHz, MeOH-d_4) of carbamidocyclophane N (**2**).

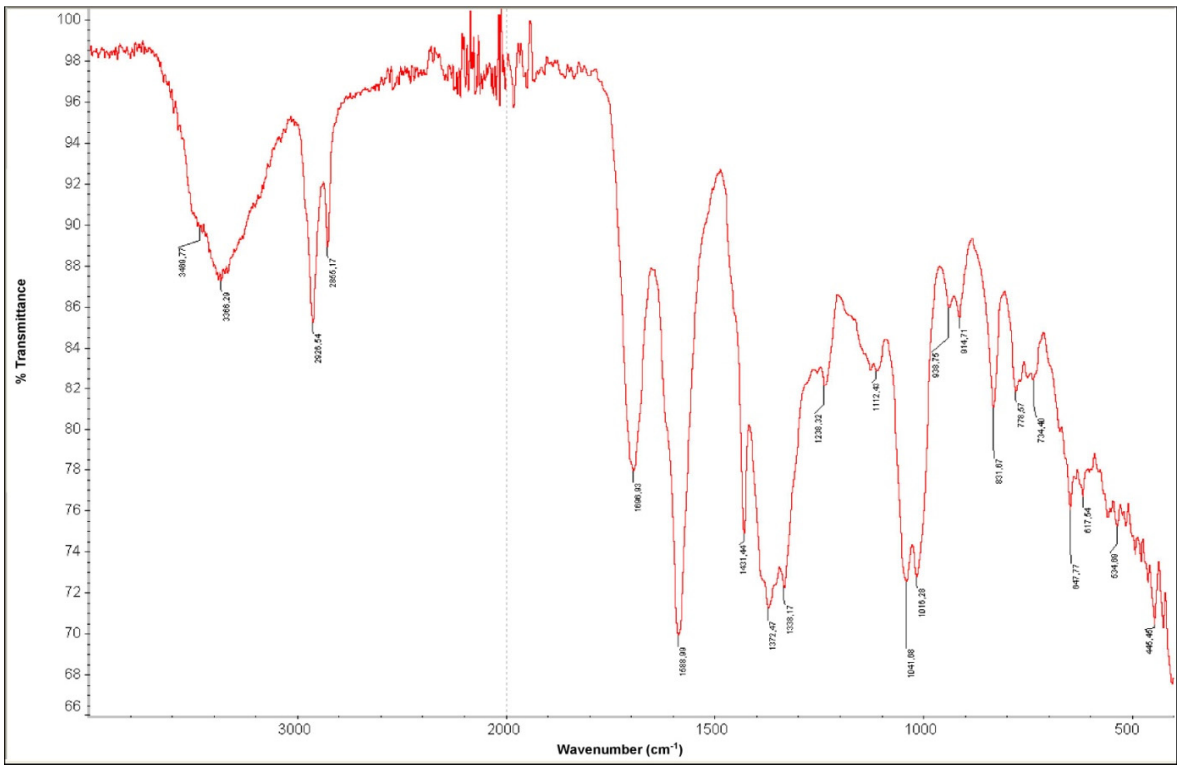

Figure S13. ATR-IR (film) spectrum of carbamidocyclophane N (**2**).

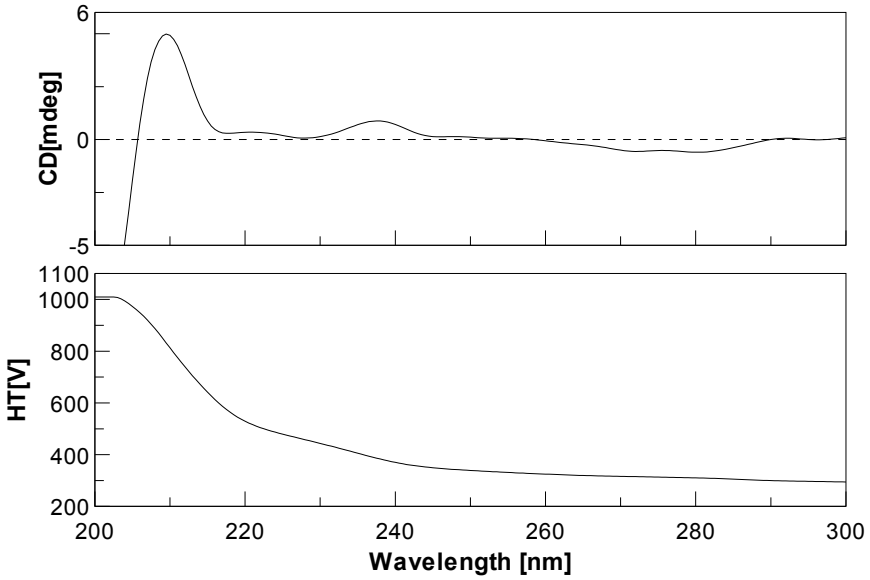

Figure S14. ECD spectrum of carbamidocyclophane N (**2**).

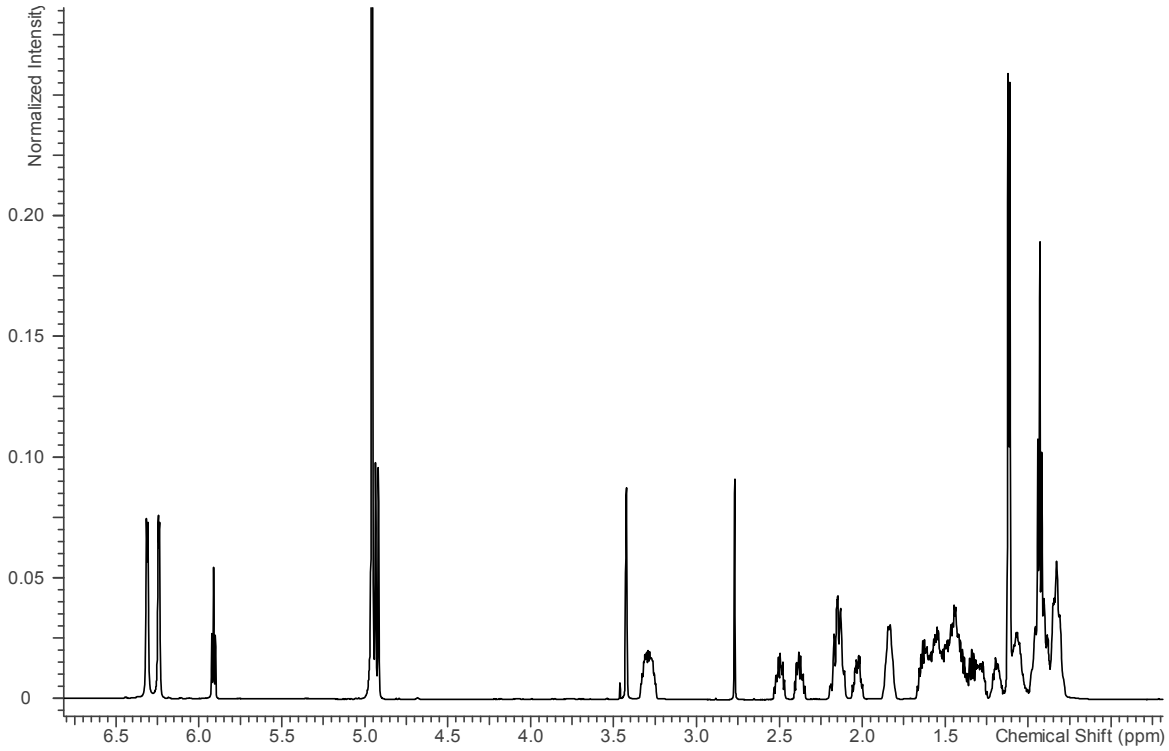

Figure S15. ^{1}H NMR spectrum (600 MHz, MeOH-d_4) of carbamidocyclophane O (**3**).

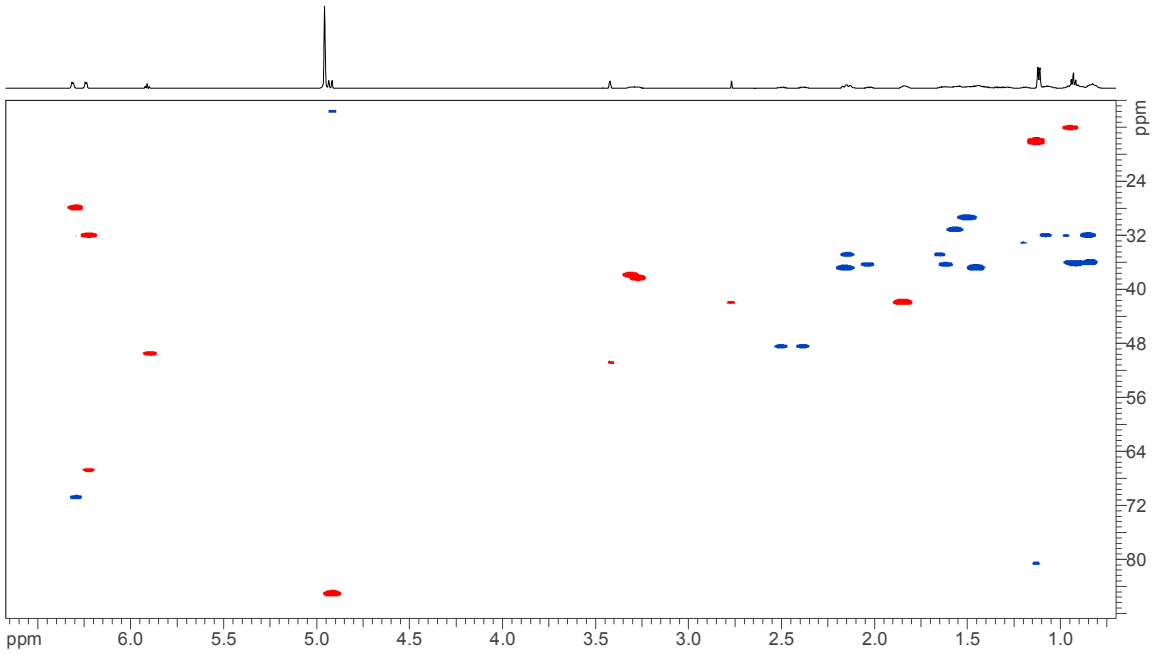

Figure S16. HMQC-DEPT spectrum (600 MHz, MeOH-d_4) of carbamidocyclophane O (**3**). Red signals are attributed to CH or CH_3 groups (positively phased) and blue signals to CH_2 groups (negatively phased).

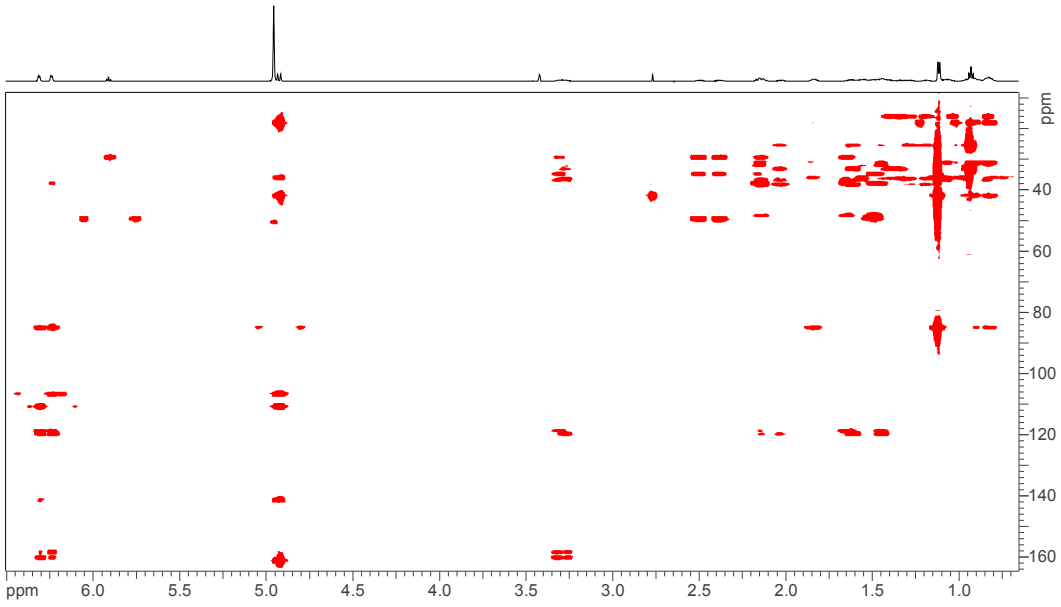

Figure S17. HMBC spectrum (600 MHz, MeOH-d_4) of carbamidocyclophane O (**3**).

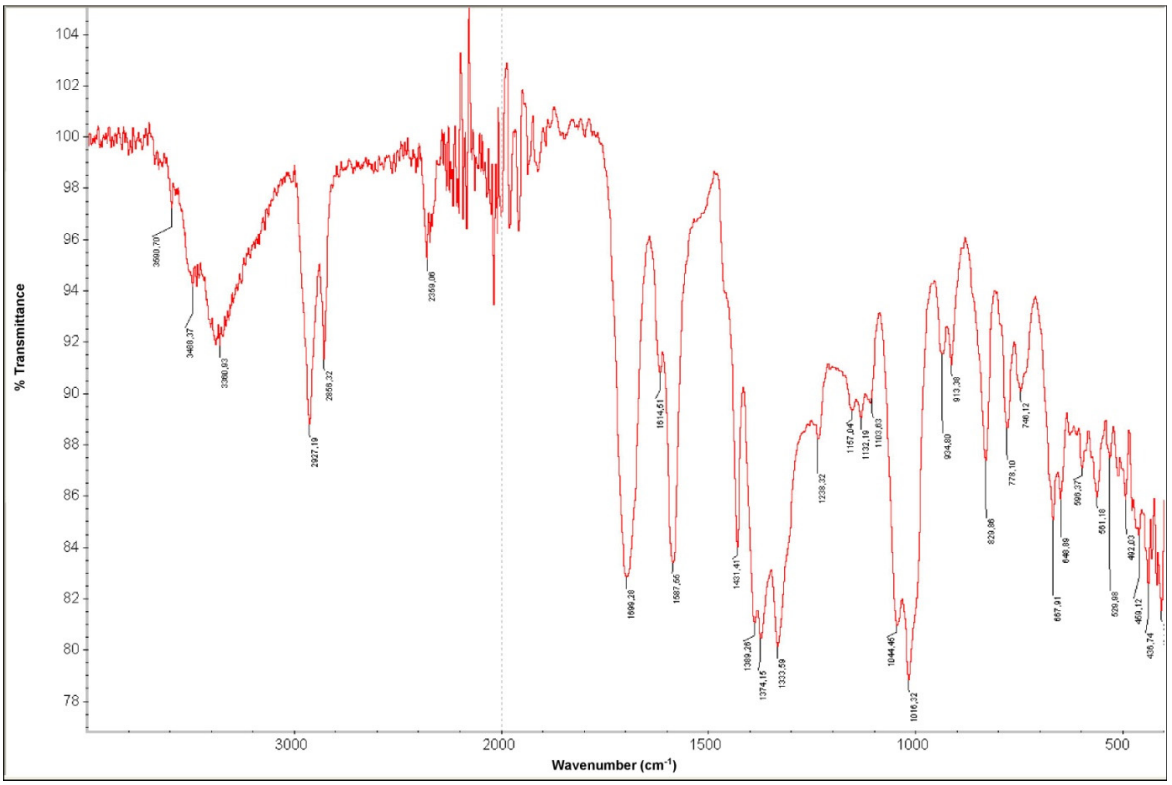

Figure S18. ATR-IR (film) spectrum of carbamidocyclophane O (**3**).

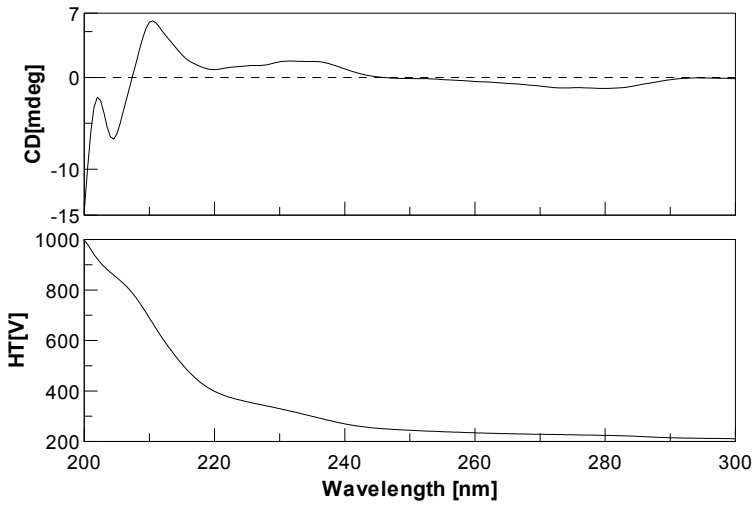

Figure S19. ECD spectrum of carbamidocyclophane O (**3**).

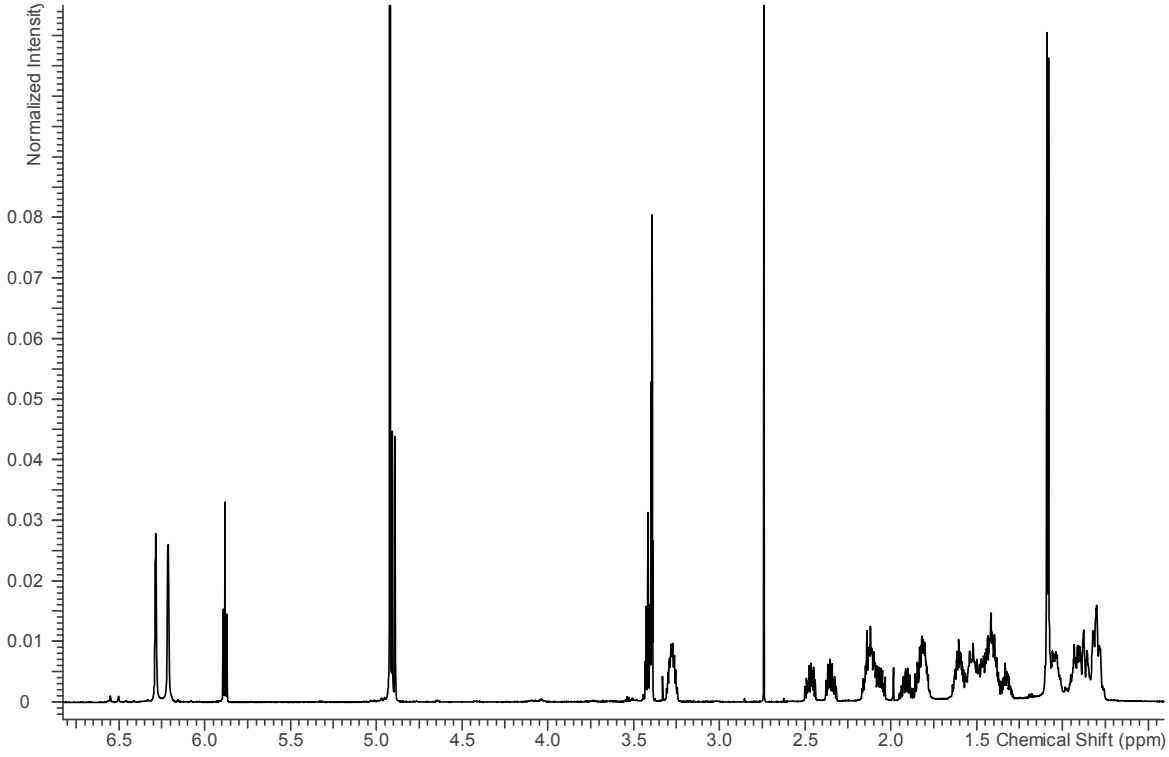

Figure S20. ^{1}H NMR spectrum (600 MHz, MeOH-d_4) of carbamidocyclophane P (**4**).

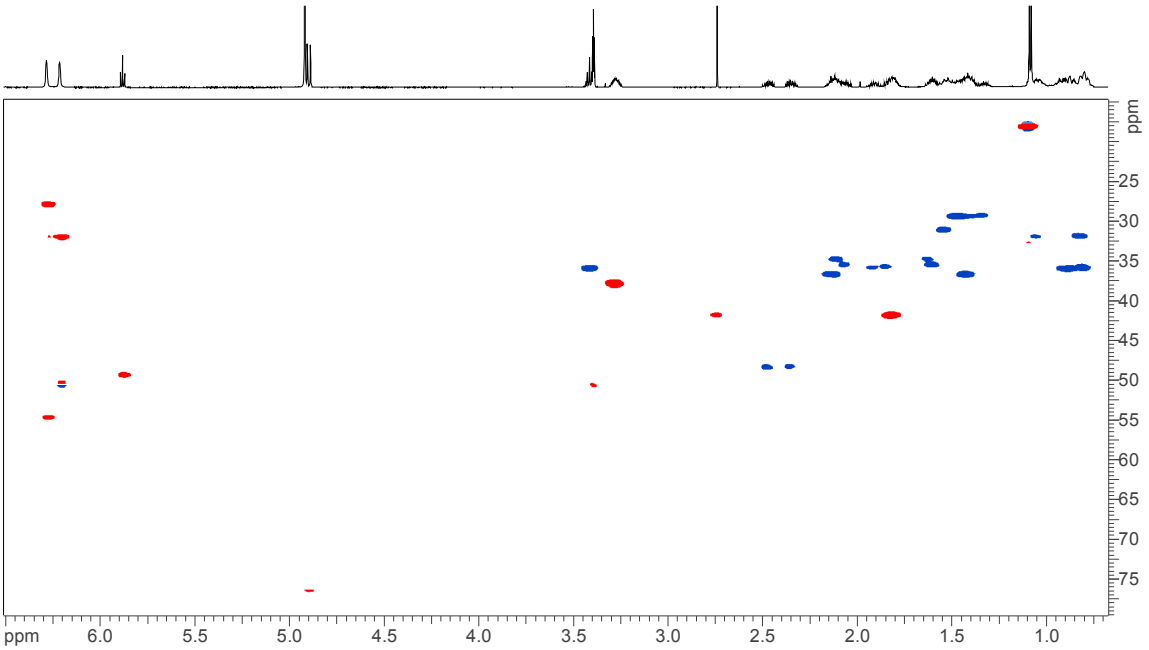

Figure S21. HMQC-DEPT spectrum (600 MHz, MeOH-d_4) of carbamidocyclophane P (**4**). Red signals are attributed to CH or CH_3 groups (positively phased) and blue signals to CH_2 groups (negatively phased).

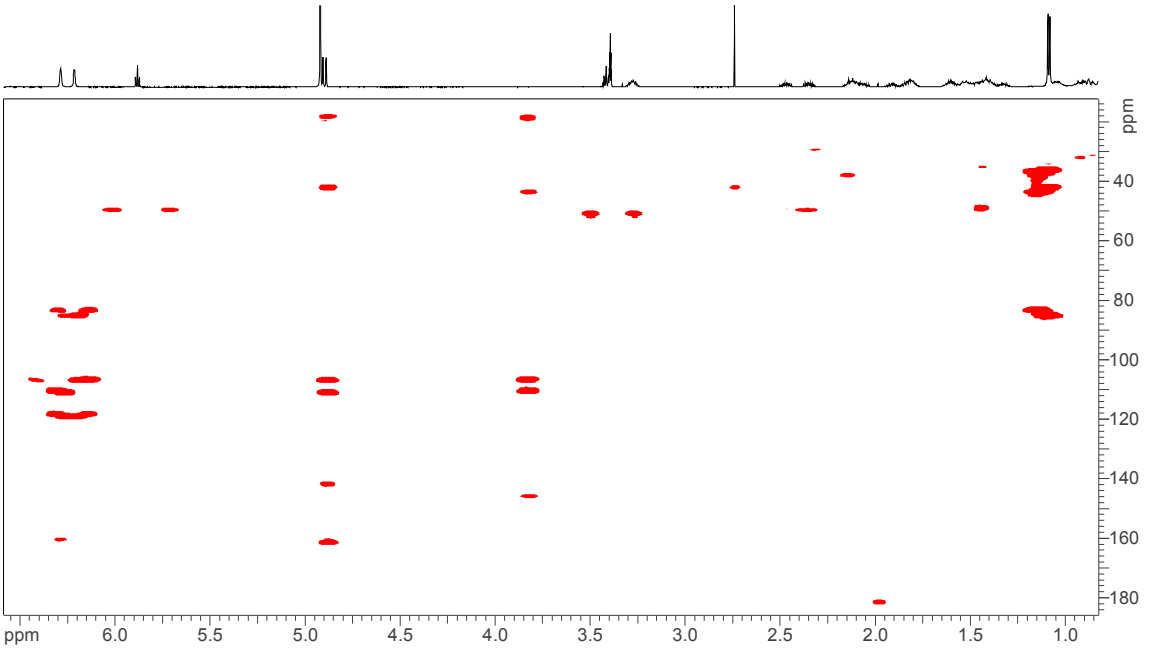

Figure S22. HMBC spectrum (600 MHz, MeOH-d_4) of carbamidocyclophane P (**4**).

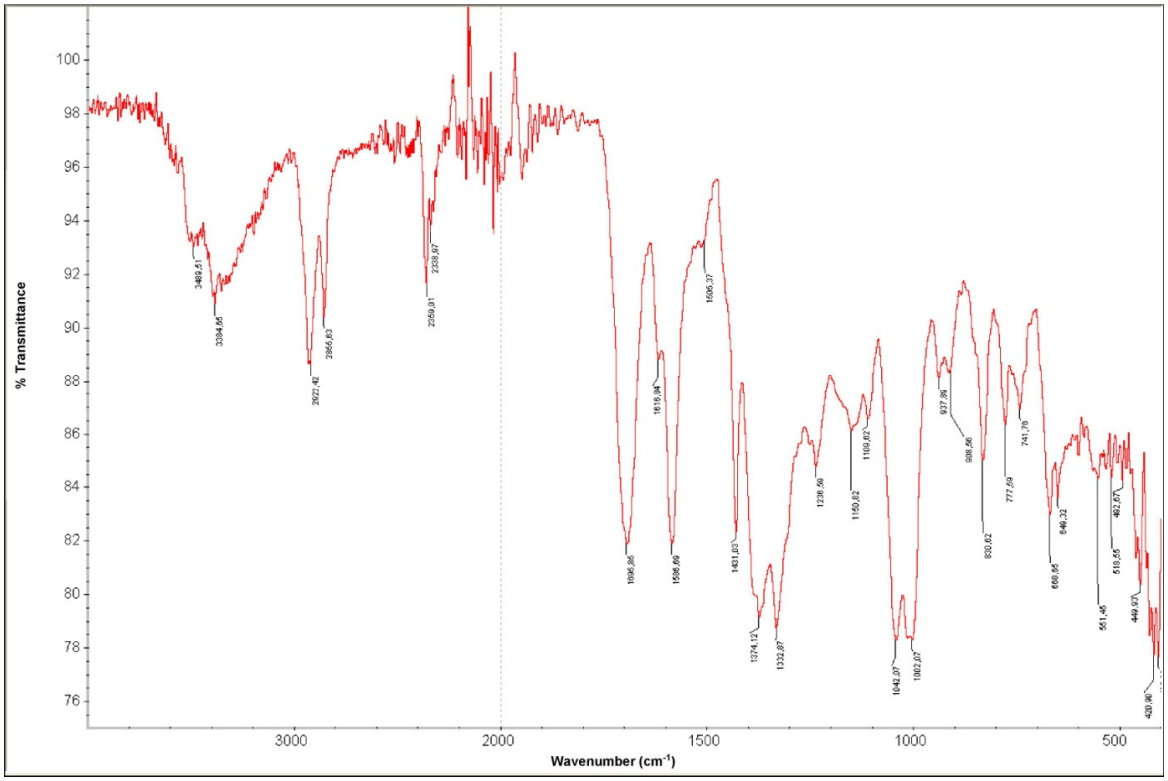

Figure S23. ATR-IR (film) spectrum of carbamidocyclophane P (**4**).

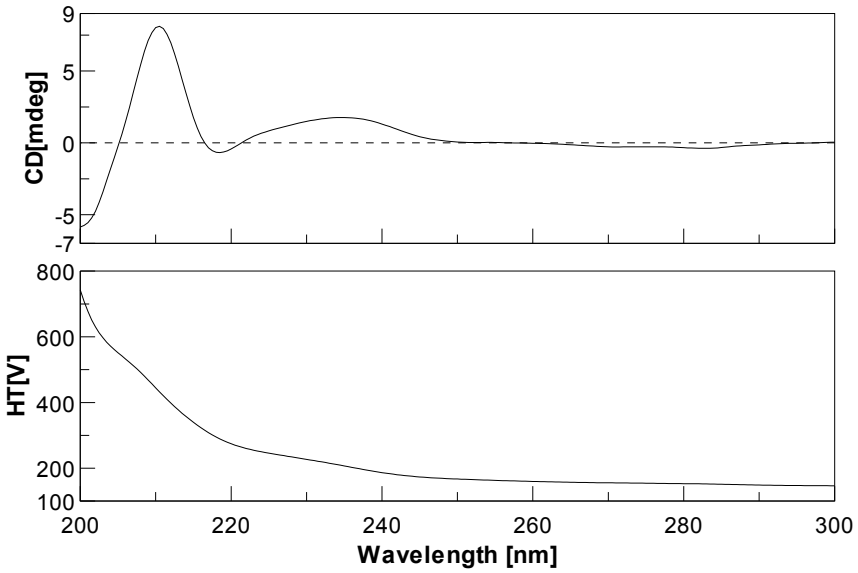

Figure S24. ECD spectrum of carbamidocyclophane P (**4**).

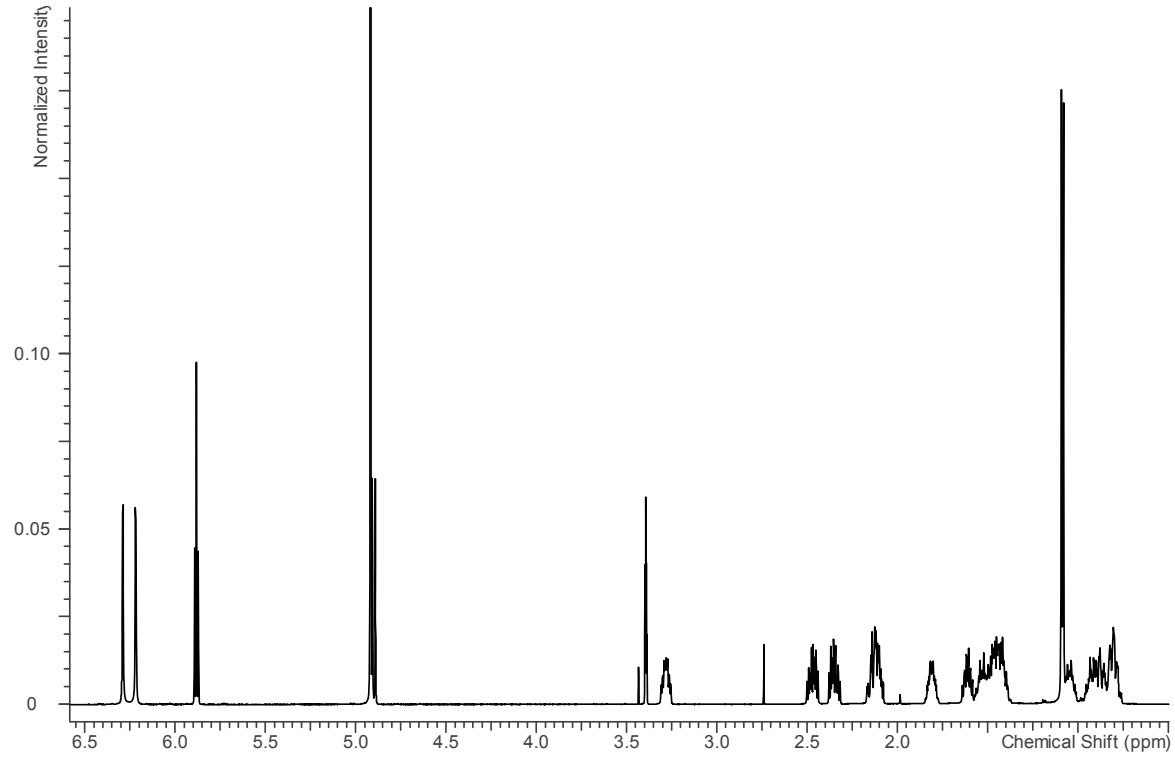

Figure S25. ^{1}H NMR spectrum (600 MHz, MeOH-d_4) of carbamidocyclophane Q (**5**).

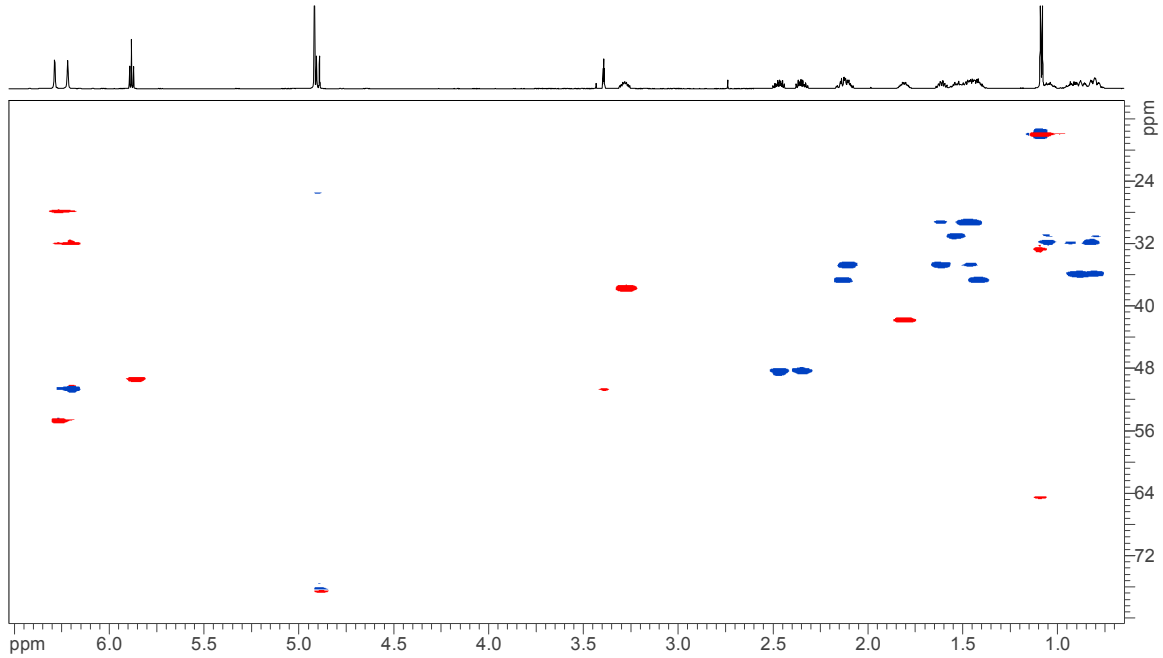

Figure S26. HMQC-DEPT spectrum (600 MHz, MeOH-d_4) of carbamidocyclophane Q (**5**). Red signals are attributed to CH or CH_3 groups (positively phased) and blue signals to CH_2 groups (negatively phased).

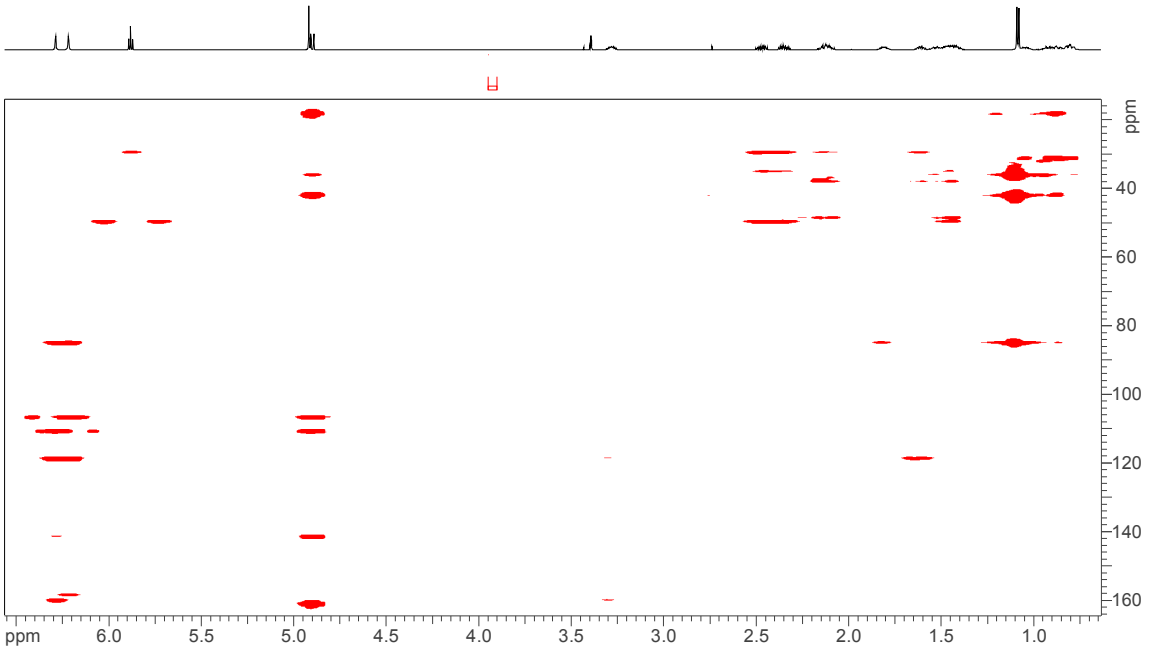

Figure S27. HMBC spectrum (600 MHz, MeOH-d_4) of carbamidocyclophane Q (**5**).

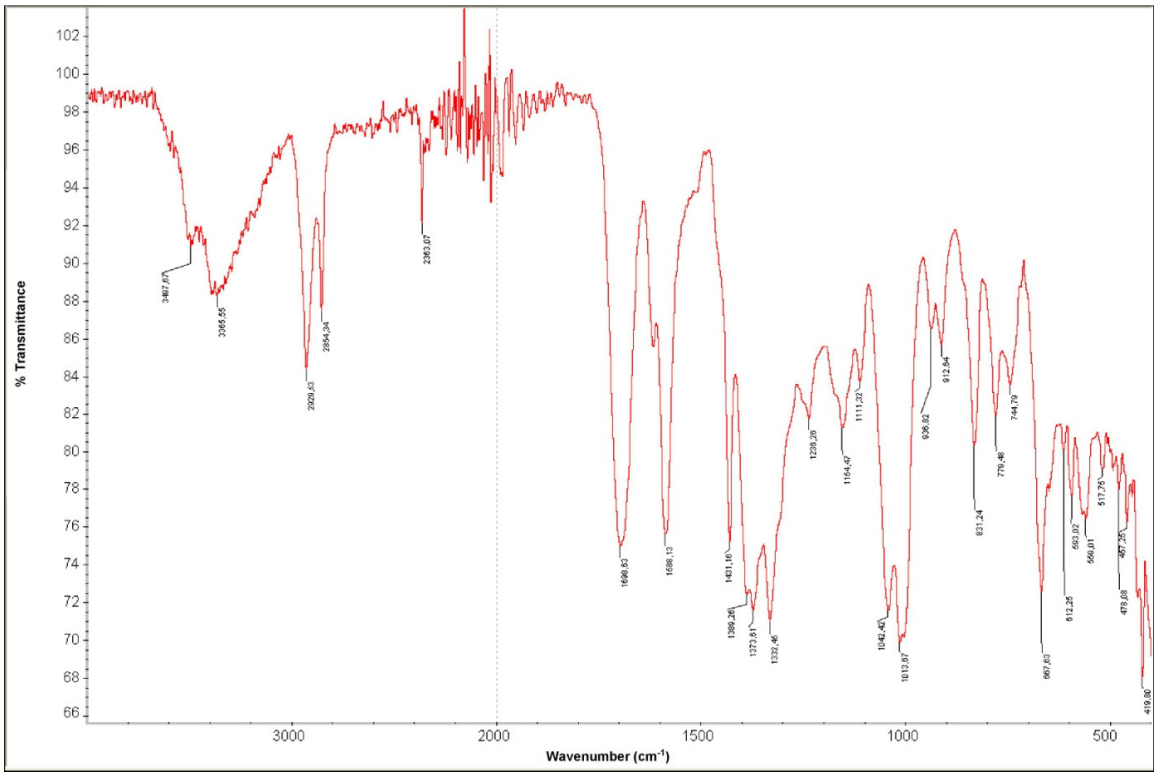

Figure S28. ATR-IR (film) spectrum of carbamidocyclophane Q (**5**).

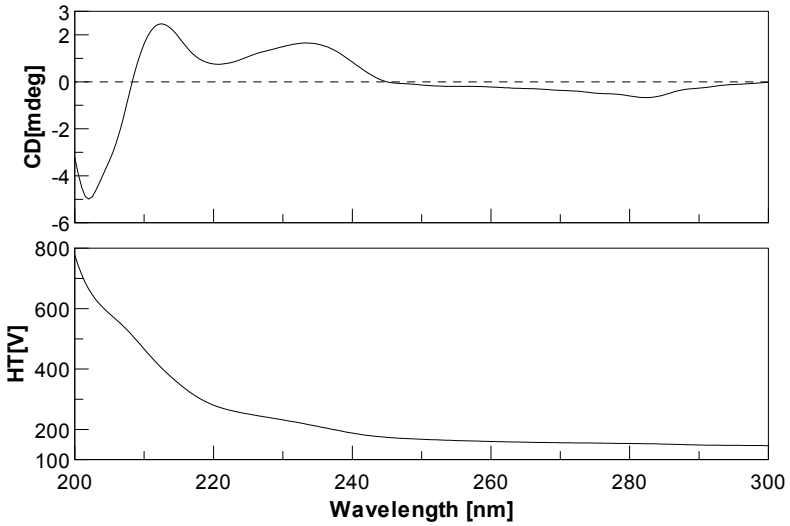

Figure S29. ECD spectrum of carbamidocyclophane Q (**5**).

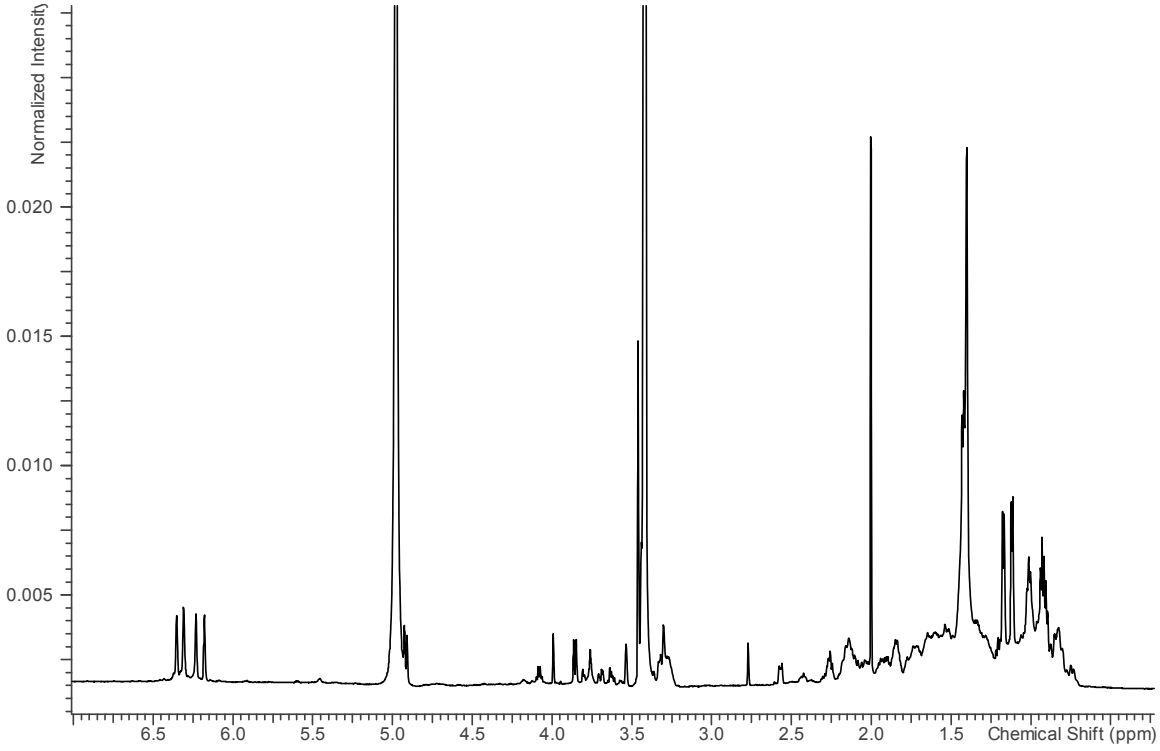

Figure S30. ^{1}H NMR spectrum (600 MHz, MeOH-d_4) of carbamidocyclophane R (**6**).

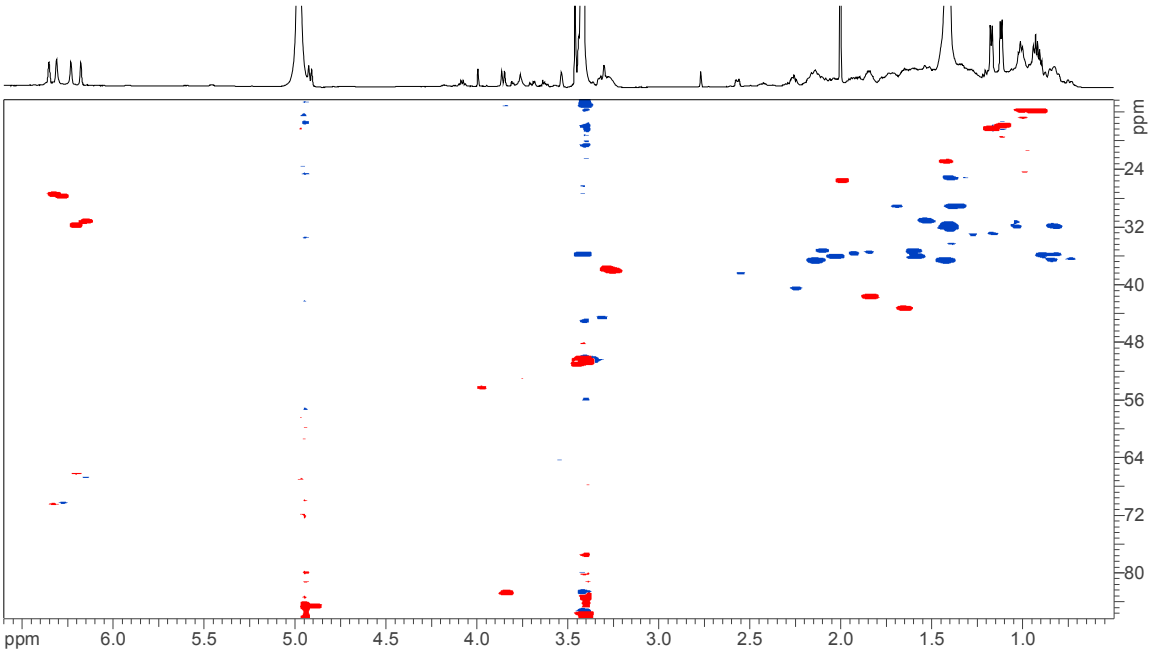

Figure S31. HMQC-DEPT spectrum (600 MHz, MeOH-d_4) of carbamidocyclophane R (**6**). Red signals are attributed to CH or CH_3 groups (positively phased) and blue signals to CH_2 groups (negatively phased).

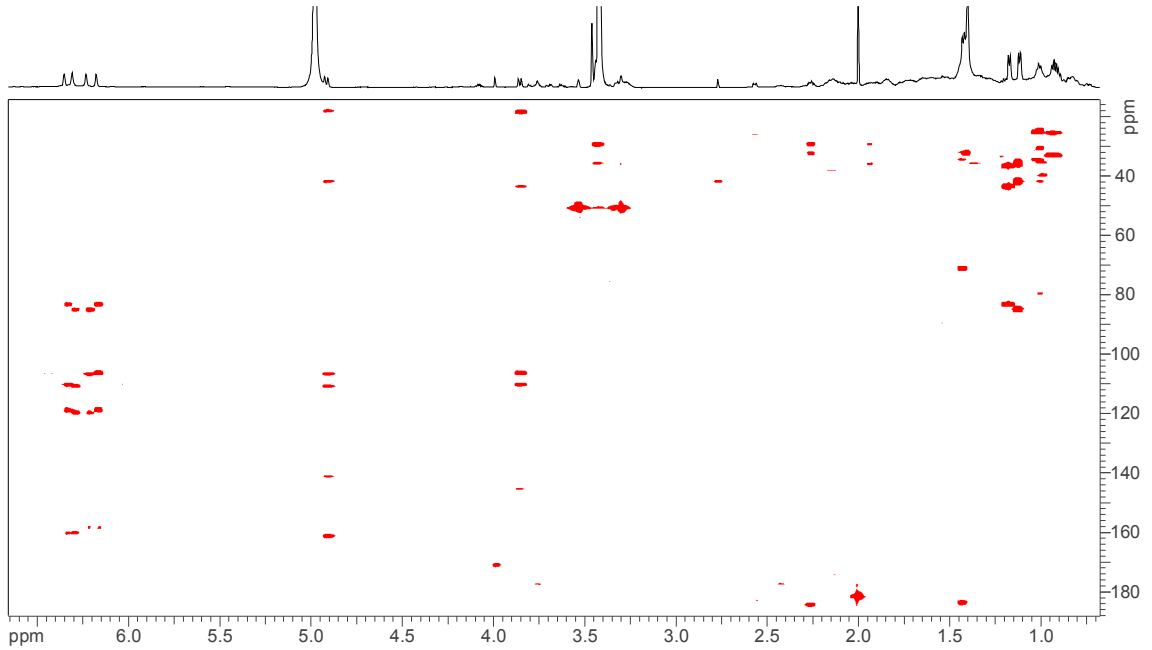

Figure S32. HMBC spectrum (600 MHz, MeOH-d_4) of carbamidocyclophane R (**6**).

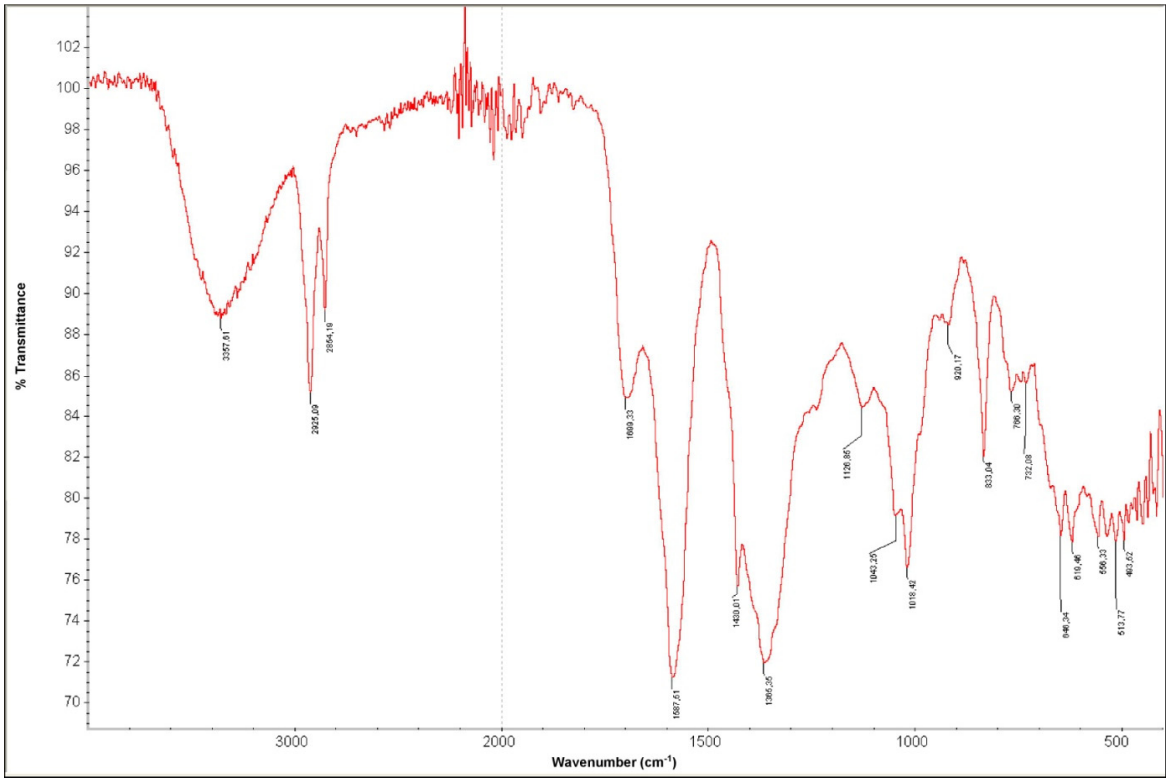

Figure S33. ATR-IR (film) spectrum of carbamidocyclophane R (**6**).

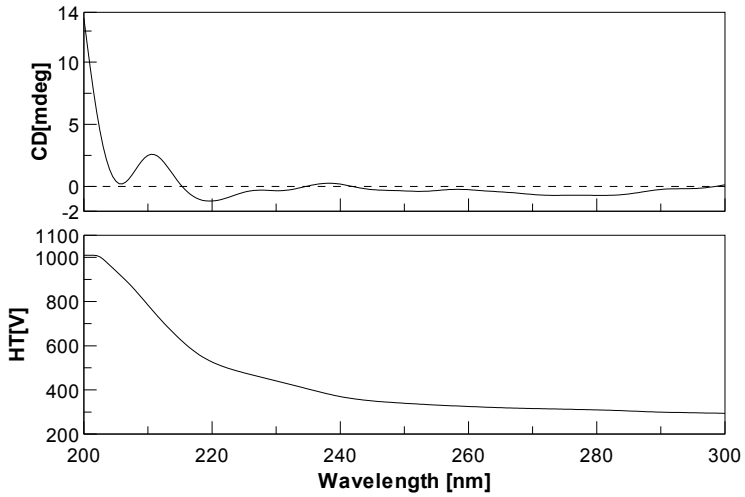

Figure S34. ECD spectrum of carbamidocyclophane R (**6**).

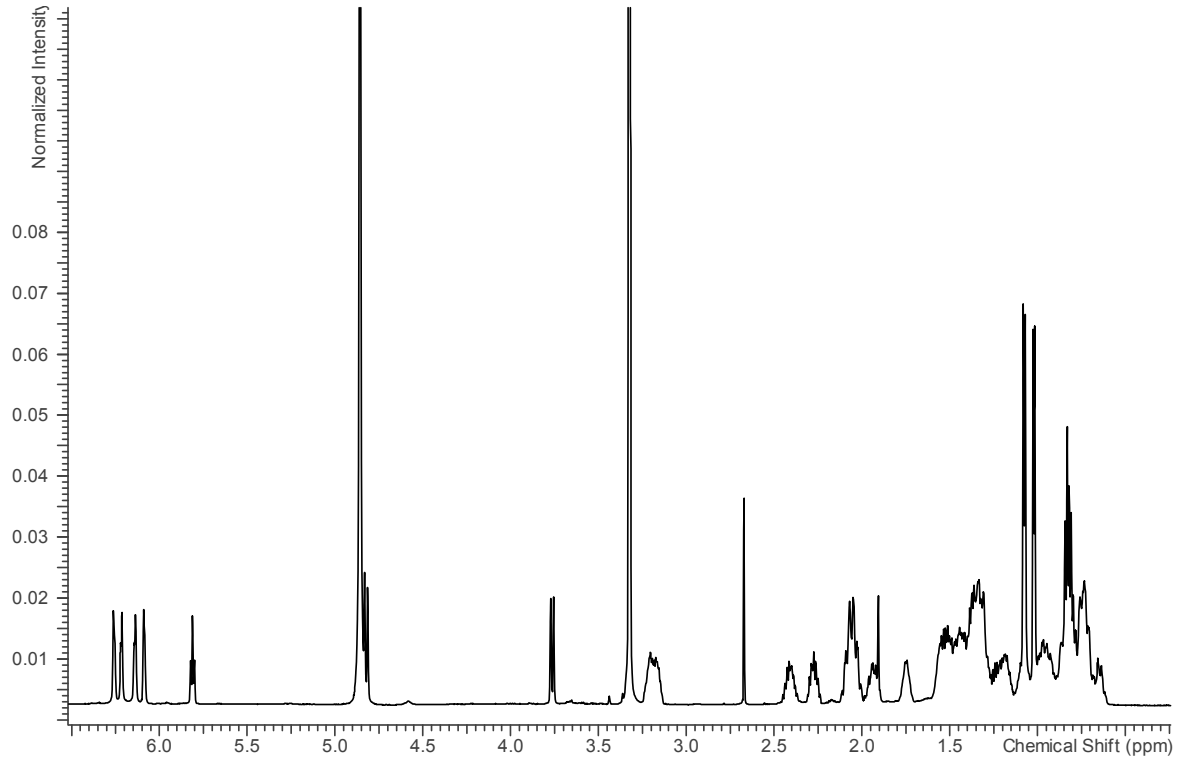

Figure S35. ^{1}H NMR spectrum (600 MHz, MeOH-d_4) of carbamidocyclophane S (**7**).

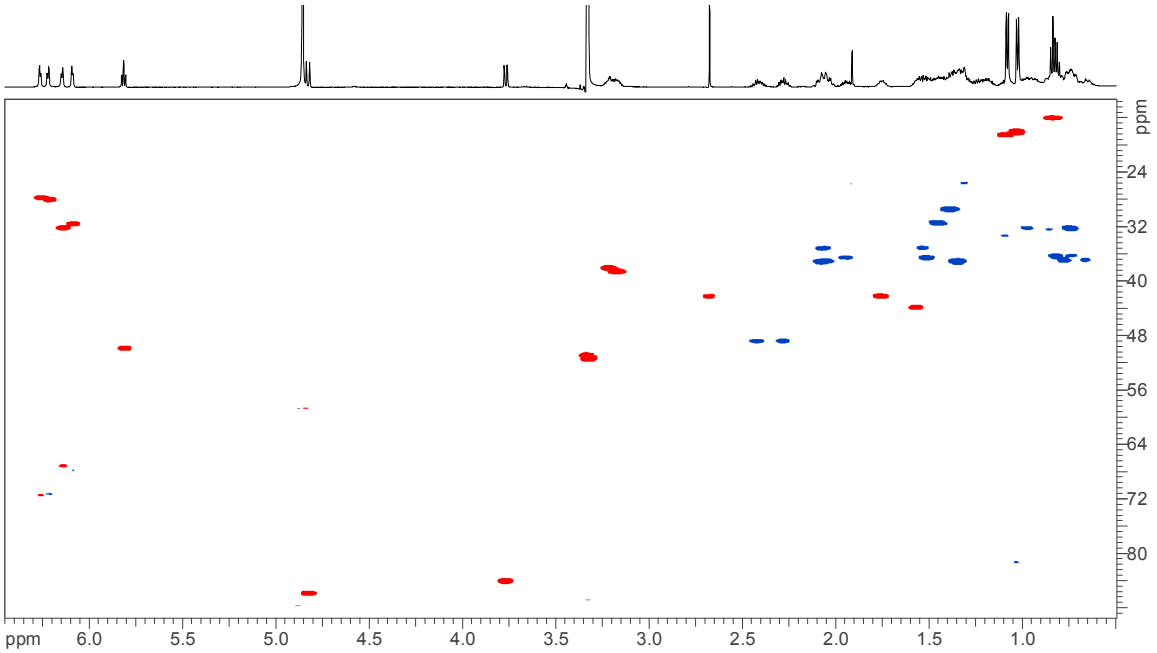

Figure S36. HMQC-DEPT spectrum (600 MHz, MeOH-d_4) of carbamidocyclophane S (**7**). Red signals are attributed to CH or CH_3 groups (positively phased) and blue signals to CH_2 groups (negatively phased).

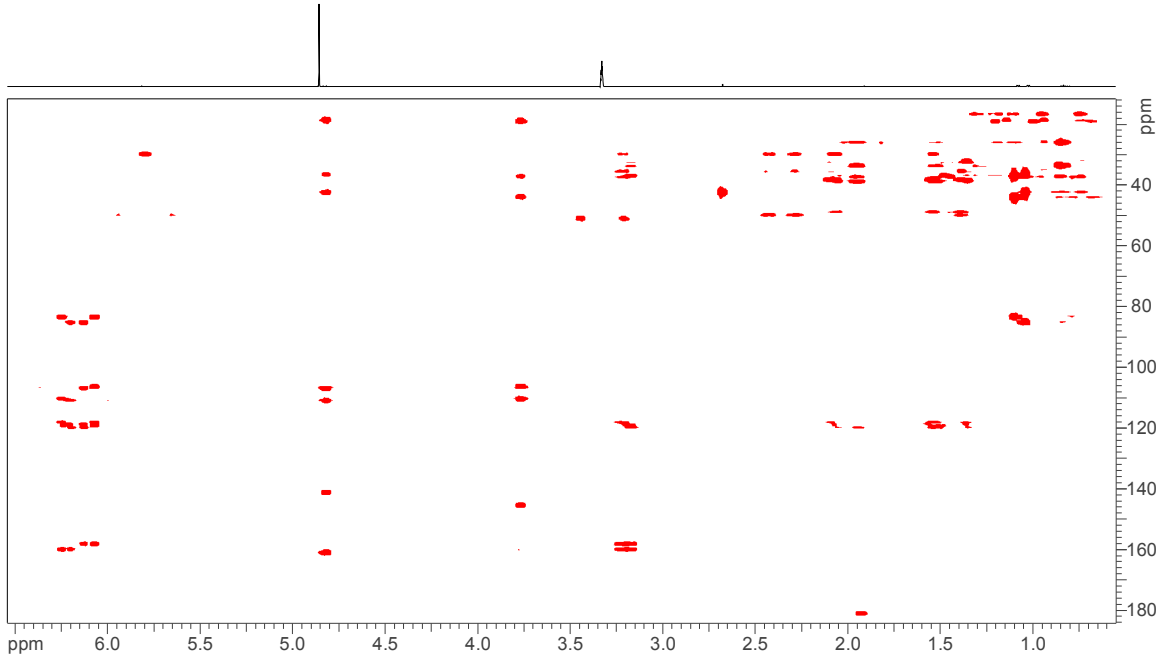

Figure S37. HMBC spectrum (600 MHz, MeOH-d_4) of carbamidocyclophane S (**7**).

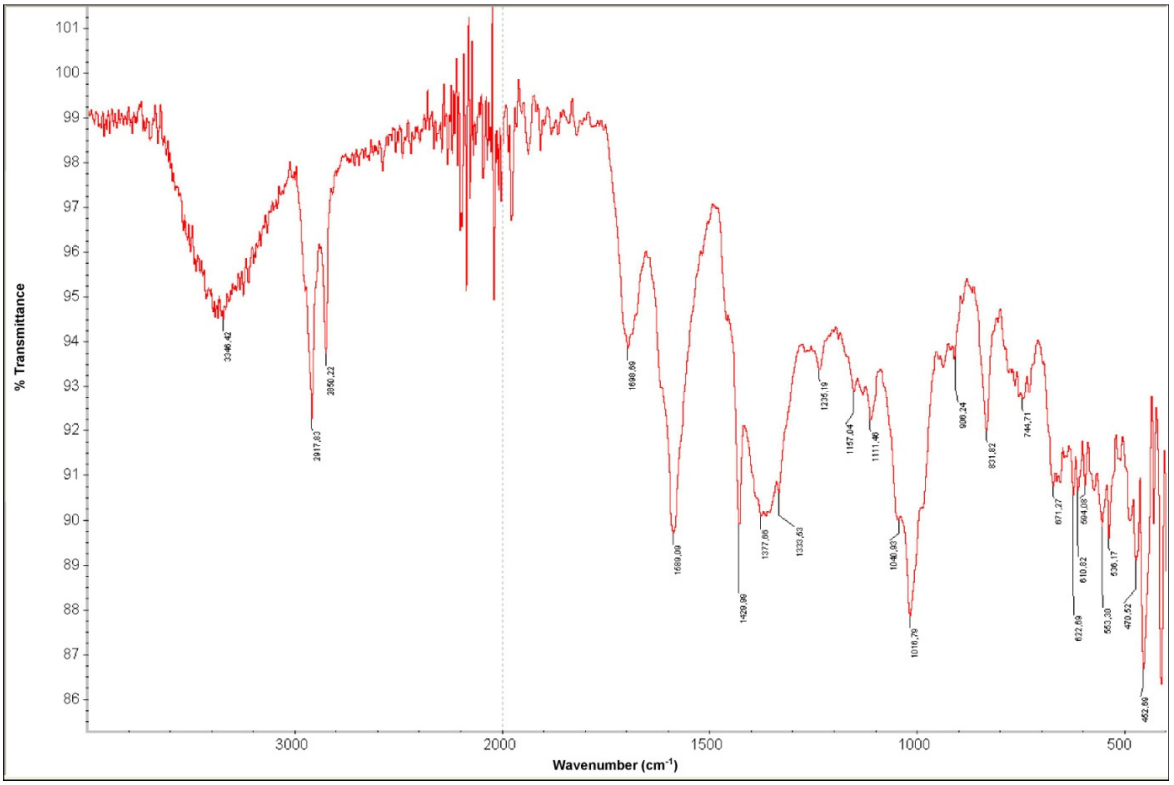

Figure S38. ATR-IR (film) spectrum of carbamidocyclophane S (**7**).

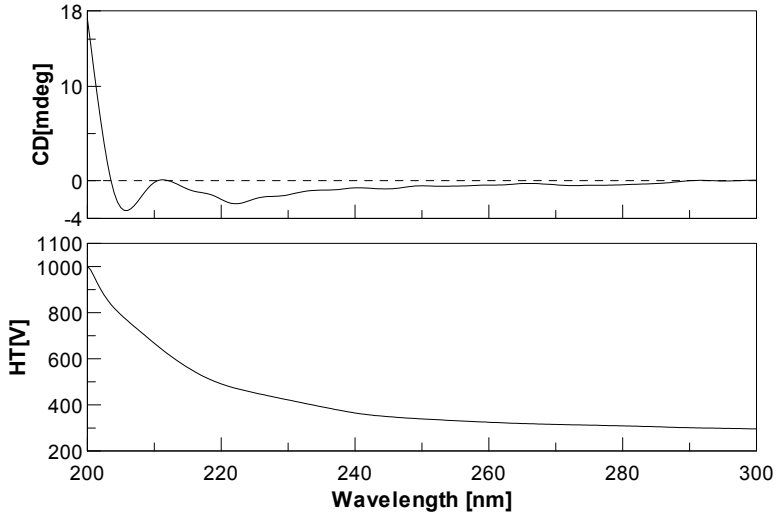

Figure S39. ECD spectrum of carbamidocyclophane S (**7**).

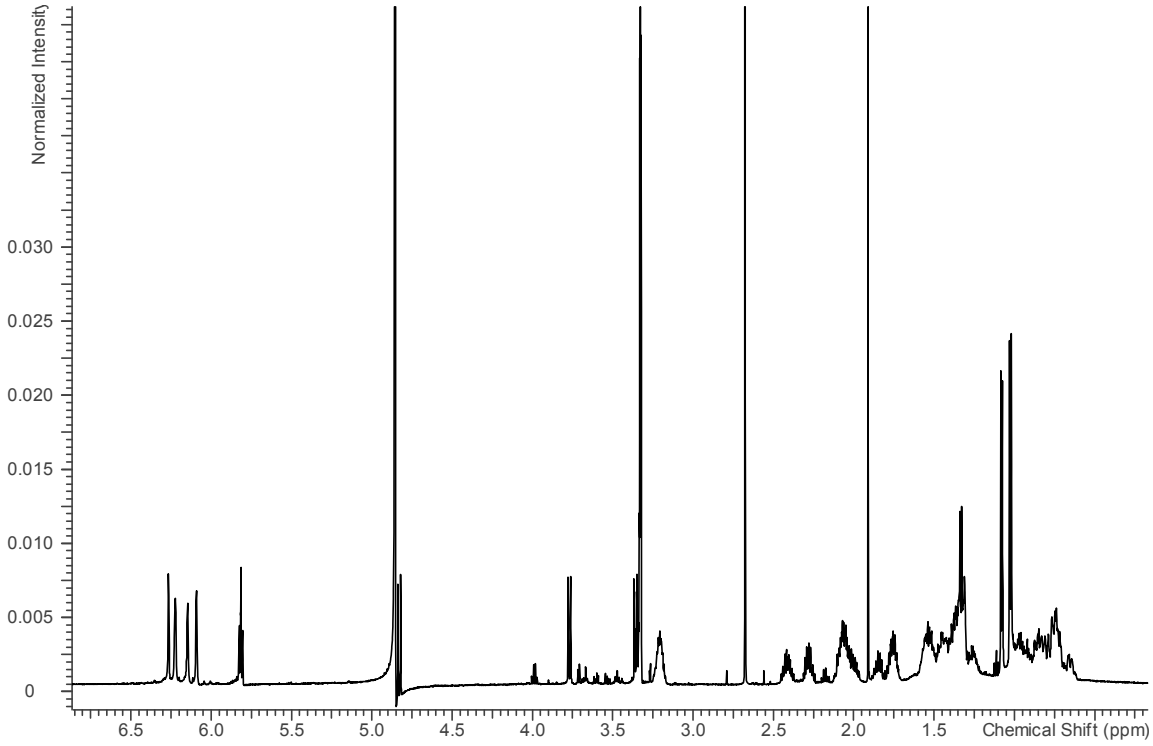

Figure S40. ^{1}H NMR spectrum (600 MHz, MeOH-d_4) of carbamidocyclophane T (**8**).

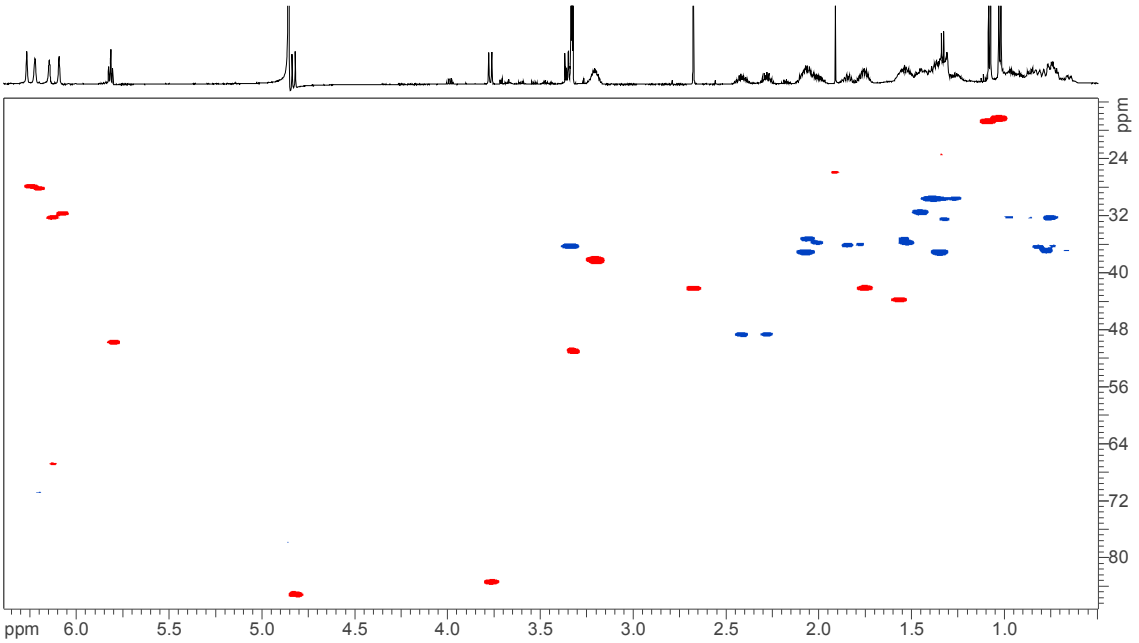

Figure S41. HMQC-DEPT spectrum (600 MHz, MeOH-d_4) of carbamidocyclophane T (**8**). Red signals are attributed to CH or CH_3 groups (positively phased) and blue signals to CH_2 groups (negatively phased).

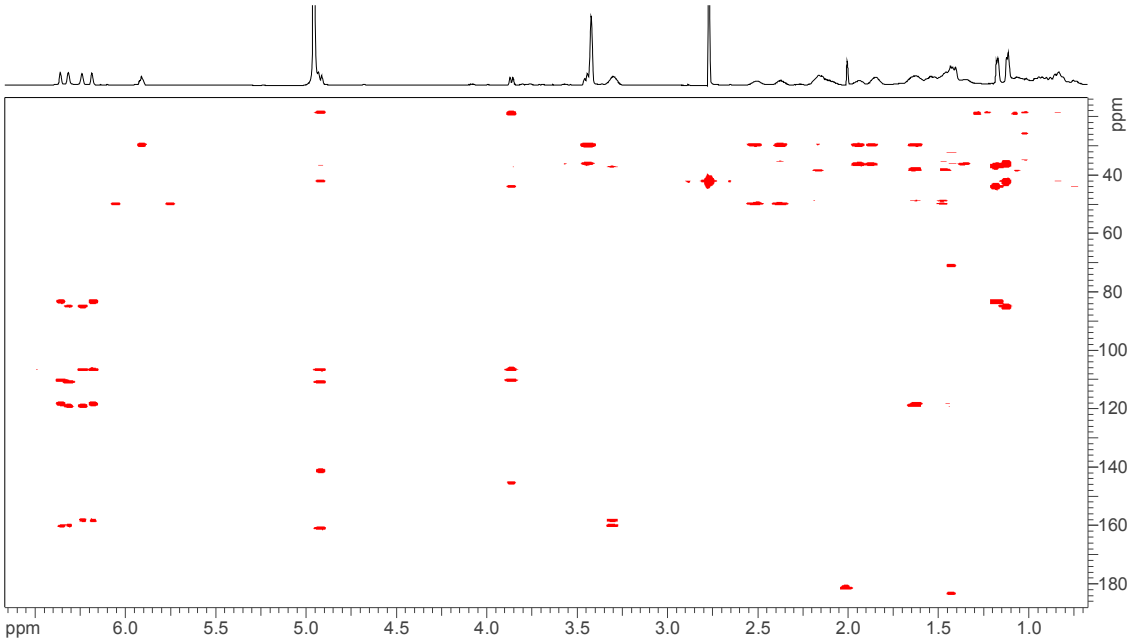

Figure S42. HMBC spectrum (600 MHz, MeOH-d_4) of carbamidocyclophane T (**8**).

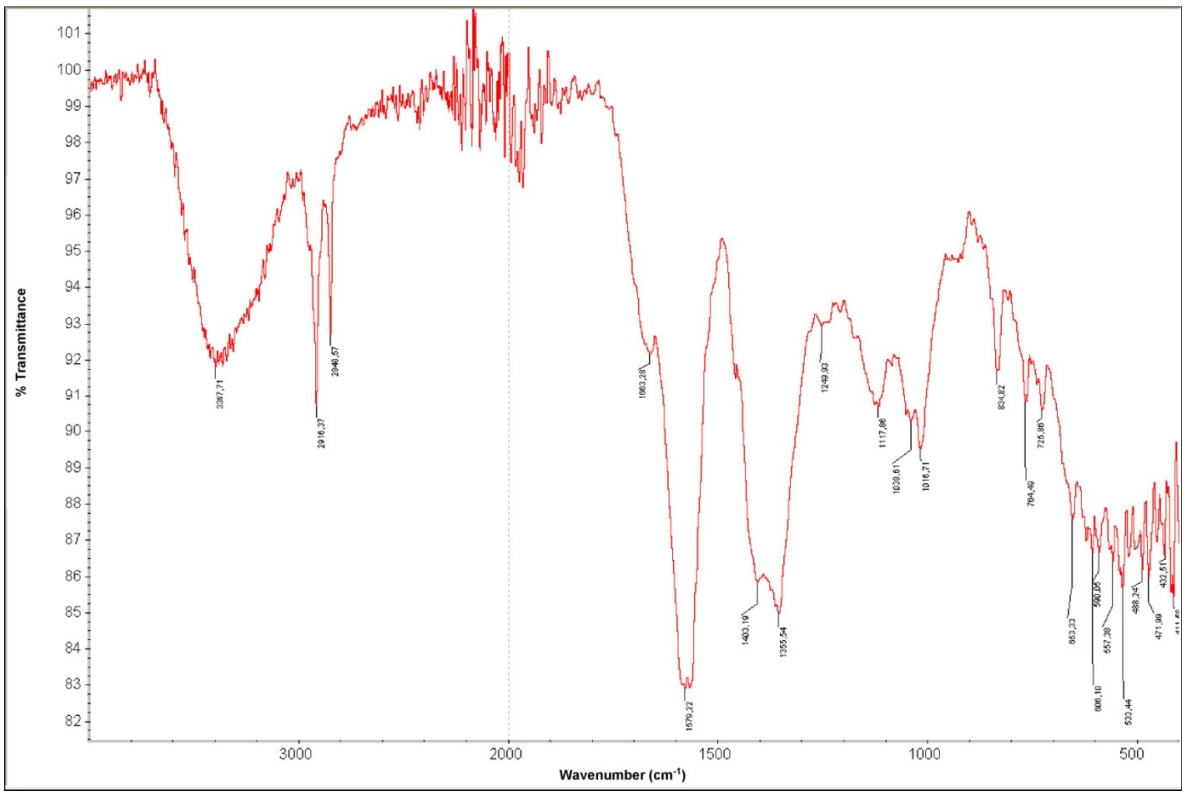

Figure S43. ATR-IR (film) spectrum of carbamidocyclophane T (**8**).

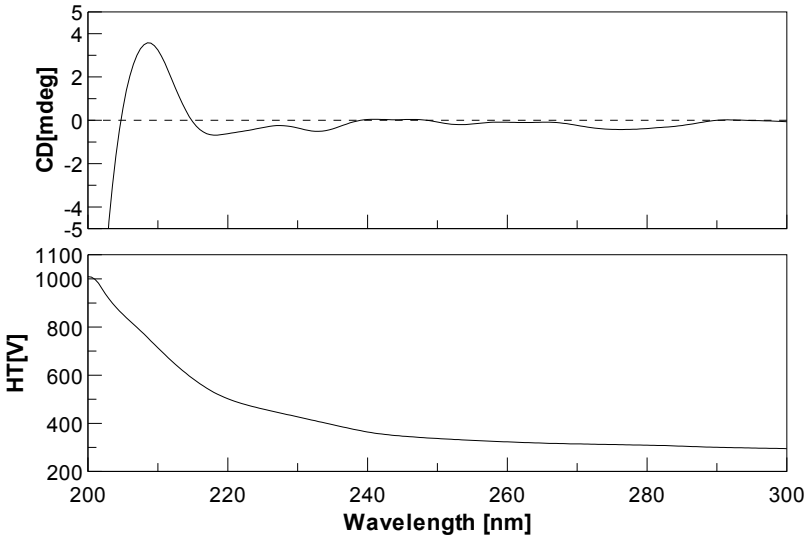

Figure S44. ECD spectrum of carbamidocyclophane T (**8**).

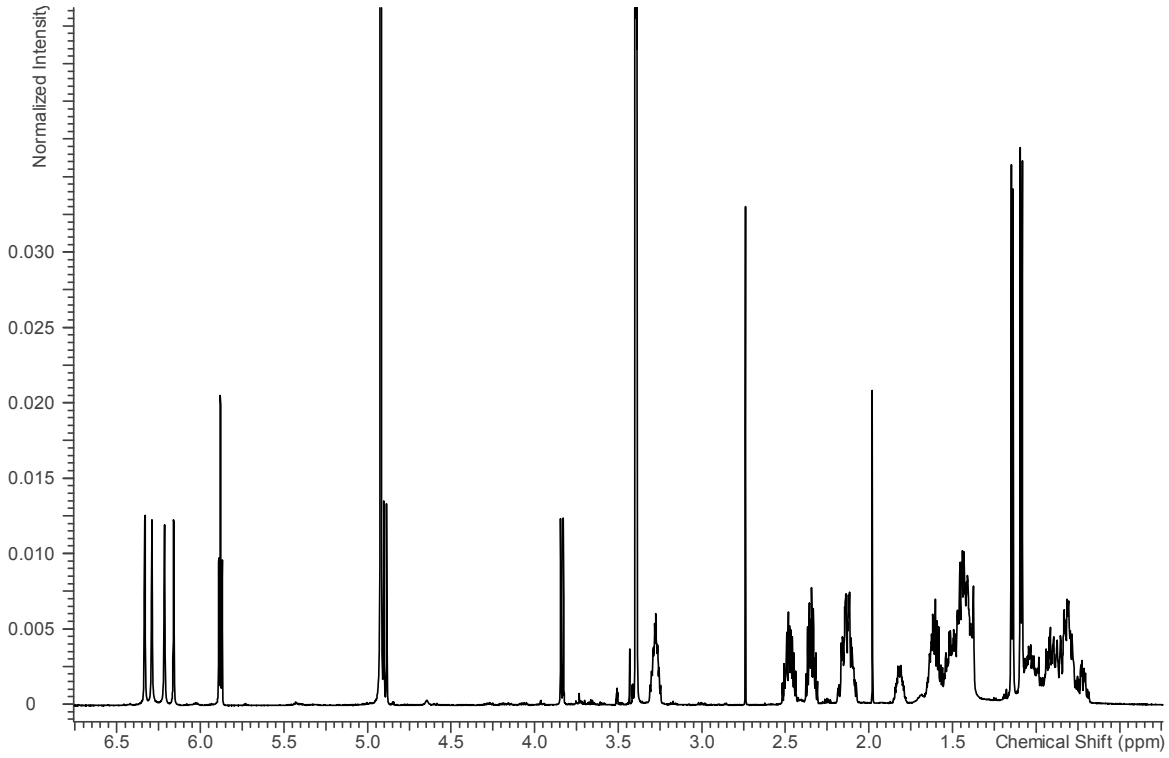

Figure S45. ^{1}H NMR spectrum (600 MHz, MeOH-d_4) of carbamidocyclophane U (**9**).

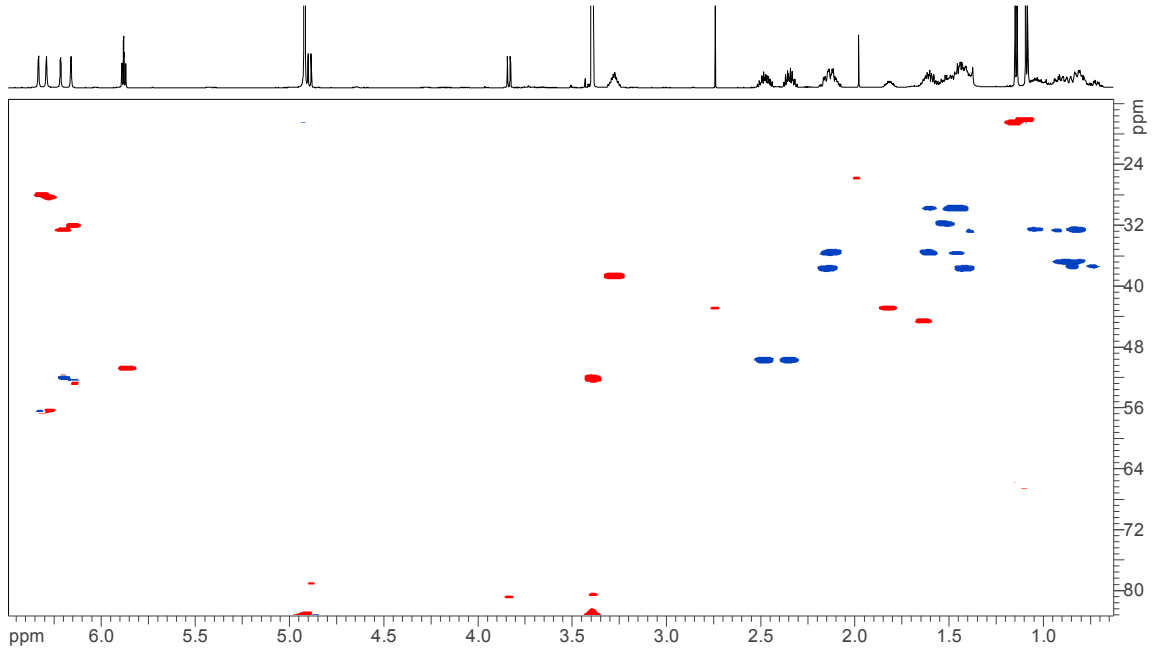

Figure S46. HMQC-DEPT spectrum (600 MHz, MeOH-d_4) of carbamidocyclophane U (**9**). Red signals are attributed to CH or CH_3 groups (positively phased) and blue signals to CH_2 groups (negatively phased).

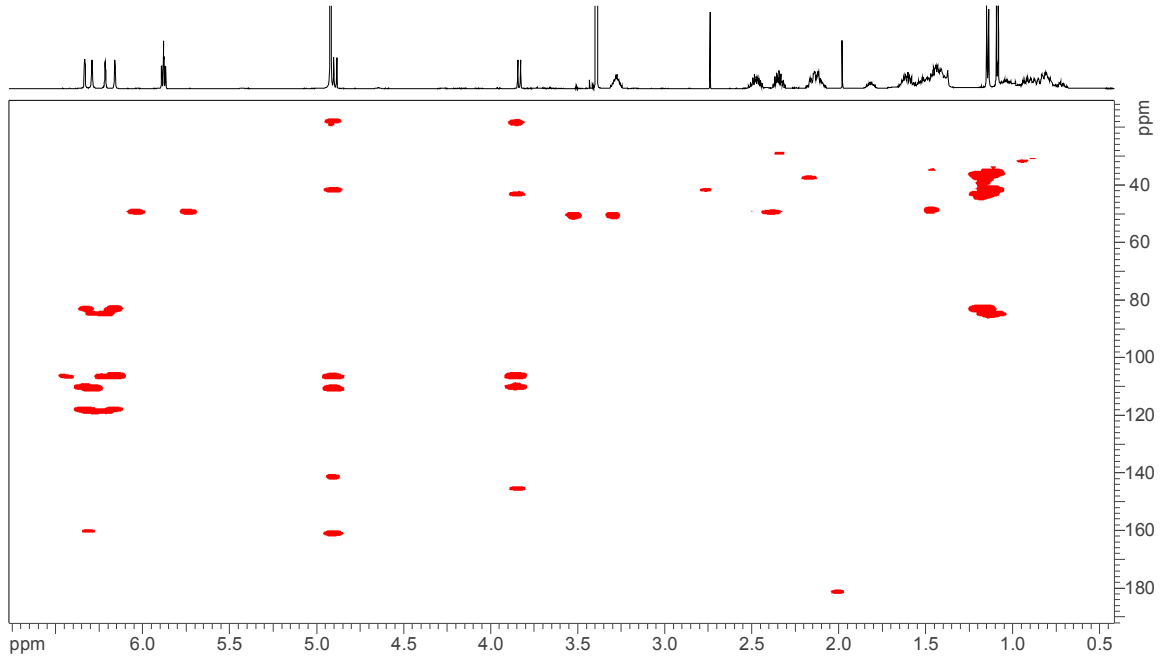

Figure S47. HMBC spectrum (600 MHz, MeOH-d_4) of carbamidocyclophane U (**9**).

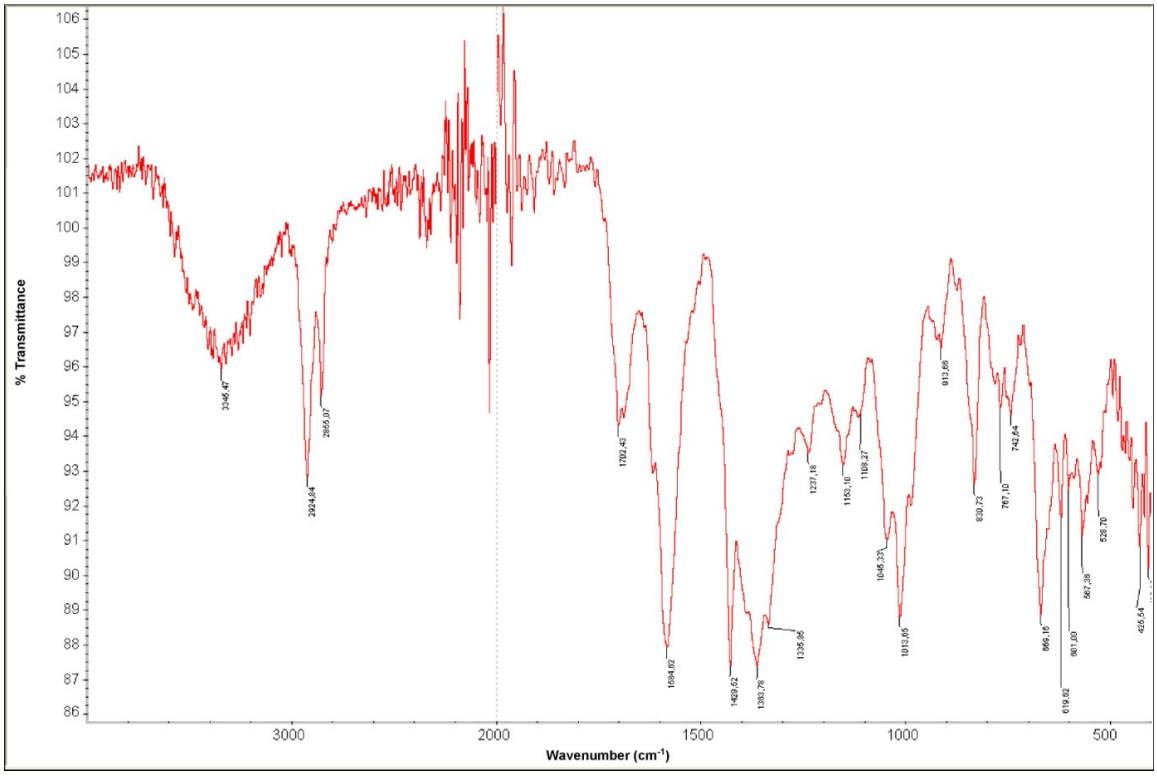

Figure S48. ATR-IR (film) spectrum of carbamidocyclophane U (**9**).

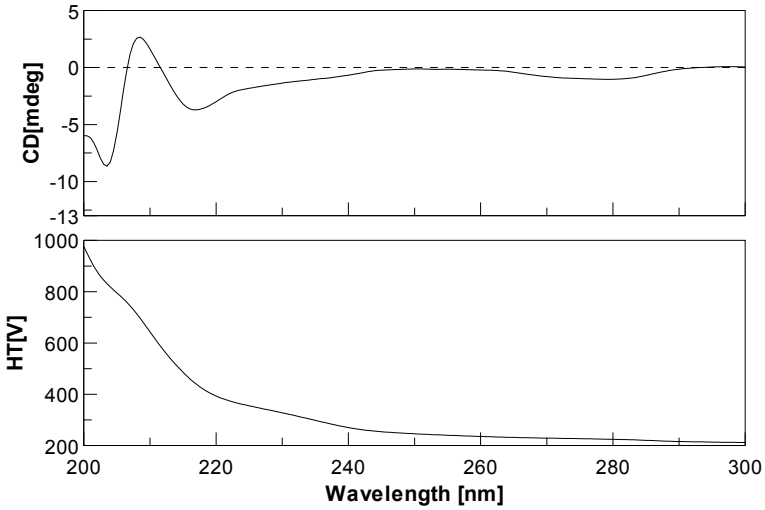

Figure S49. ECD spectrum of carbamidocyclophane U (**9**).

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