Electronic Supplementary Information for:

Cucurbit[7]uril encapsulation completely protects reactive imine in weak acid

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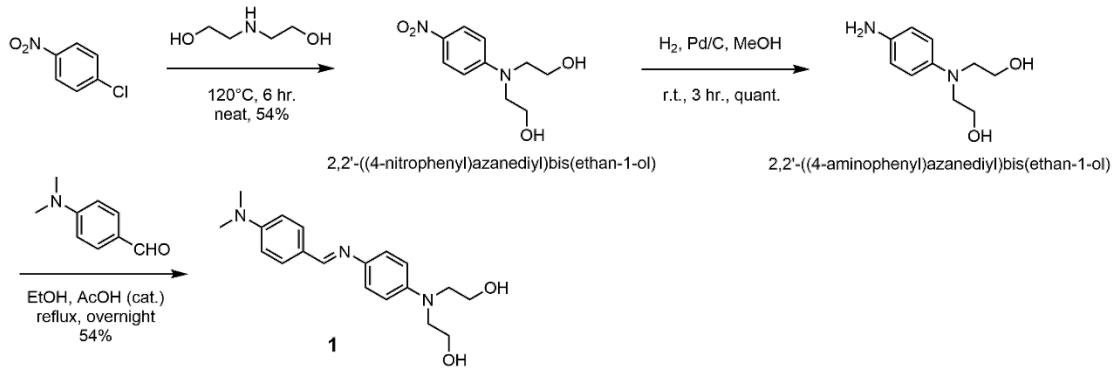
1. Materials and general methods

All commercially available compounds were purchased from Sigma-Aldrich, Oakwood Chemicals, and AK Scientific unless noted otherwise. **CB7** was purchased from BOC Sciences. All the compounds have a purity of at least 97% and were used without any purification. All absorption spectra were collected using quartz cuvettes (1 mL, 1 cm path length). Flash column chromatography was performed using Biotage SNAP Ultra columns. ^{1}H NMR spectra were recorded on a Bruker 400 or Bruker 500 NMR spectrometer. The NMR spectra were processed using MestreNova software v9.0. Chemical shifts are presented in ppm and referenced by residual solvent peak.

2. Compound synthesis and characterization

Synthesis of imine 1

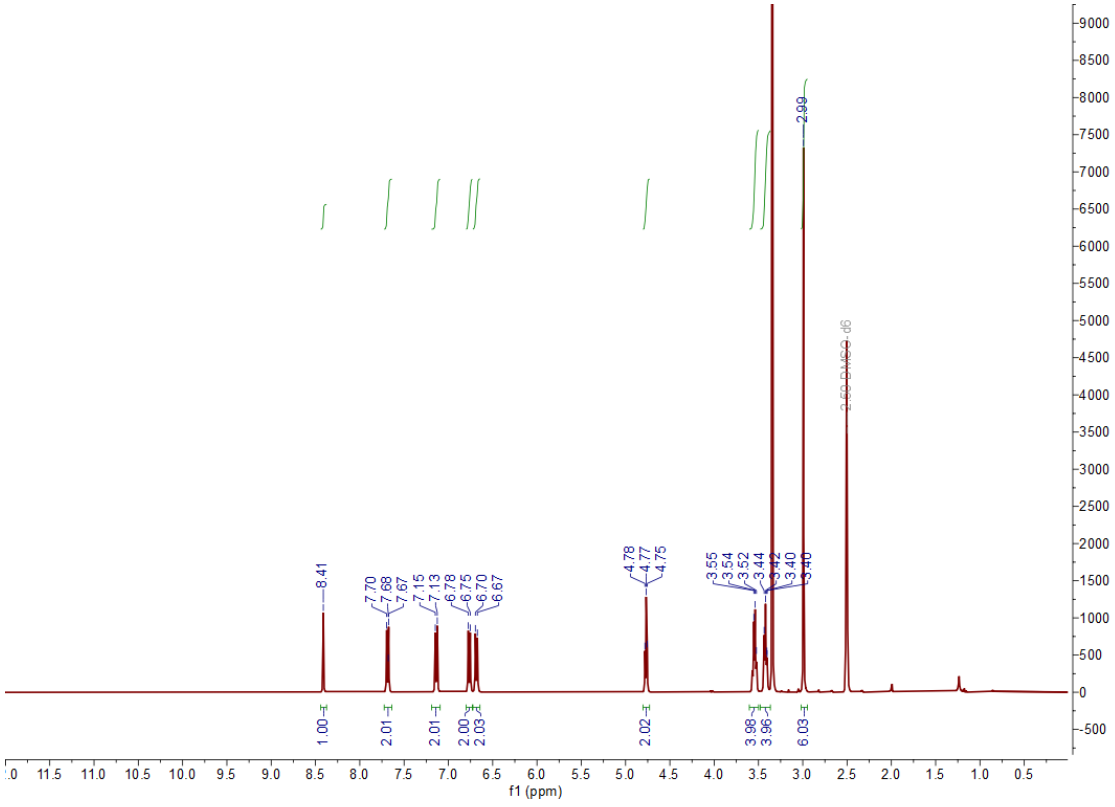


A mixture of 4-chloronitrobenzene 1.57 g (10 mmol) and diethanolamine (2.10 g, 20 mmol) was heated with stirring at 120 °C for 6 hours. The mixture was poured into water and extracted multiple times with EtOAc, and the combined organic layers were washed with brine, dried over anhydrous Na_2SO_4. The crude product was further purified with column chromatography on silica using EtOAc/Hexanes as eluent to give 2,2'-((4-nitrophenyl)azanediyl)bis(ethan-1-ol) as orange solid (1.22 g, 54%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.10 (d, *J* = 9.5 Hz, 2H), 6.66 (d, *J* = 9.4 Hz, 2H), 3.95 (t, *J* = 5.0 Hz, 4H), 3.73 (t, *J* = 4.9 Hz, 4H), 2.89 (s, 2H).

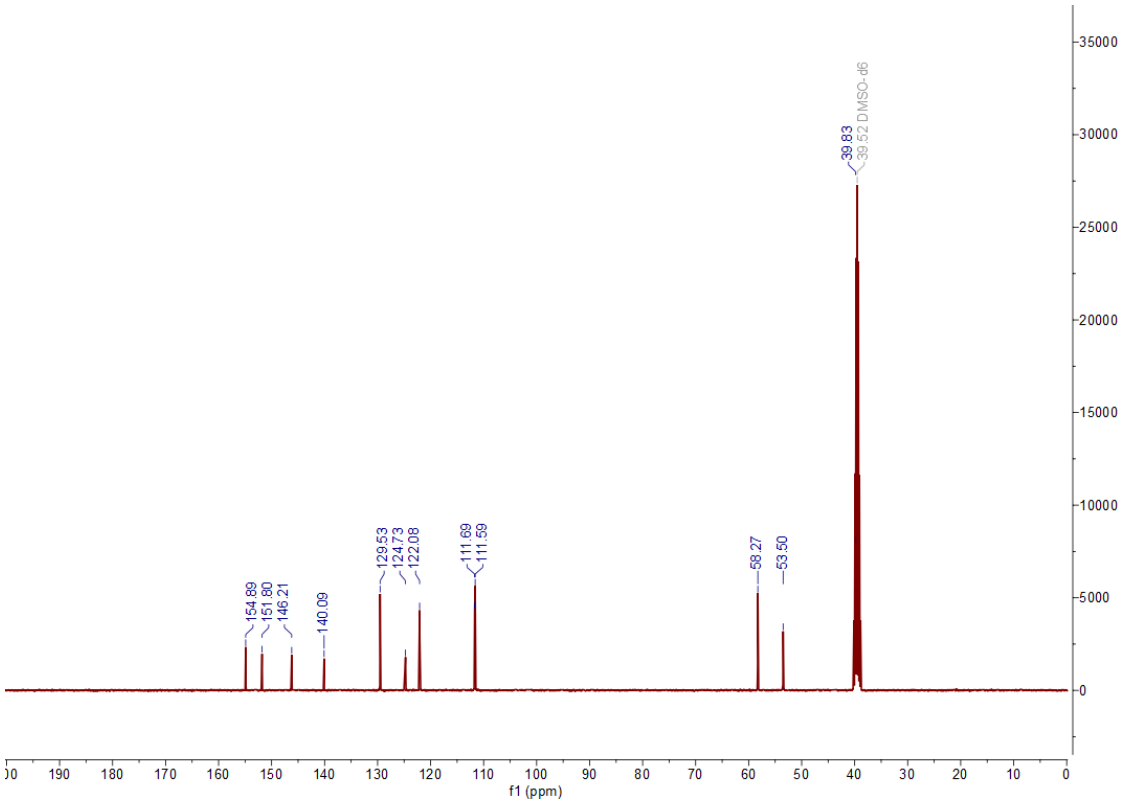
To a solution of 2,2'-((4-nitrophenyl)azanediyl)bis(ethan-1-ol) (226 mg, 1 mmol) in methanol (20 mL), 10% Pd/C (200 mg) was added. A balloon filled with H_2 gas was attached to the flask of reaction mixture. After stirring overnight, the reaction mixture was filtered over celite. The filtrate was then dried *in vacuo*, giving 2,2'-((4-aminophenyl)azanediyl)bis(ethan-1-ol) as product (196 mg, quant.). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 6.71 (d, *J* = 8.9 Hz, 2H), 6.68 – 6.62 (m, 2H), 3.74 (t, *J* = 5.1 Hz, 4H), 3.45 – 3.34 (m, 6H), 2.80 (d, *J* = 4.5 Hz, 2H).

One drop of acetic acid was added as catalyst to a solution of 2,2'-((4-aminophenyl)azanediyl)bis(ethan-1-ol) (98 mg, 0.5 mmol) and *p*-dimethylamino-

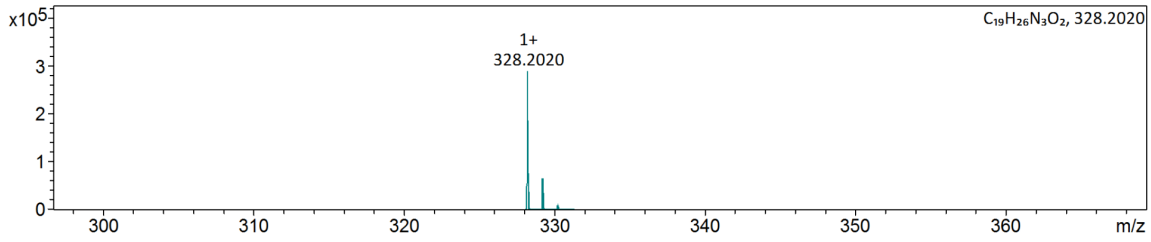
benzaldehyde (75 mg, 0.5 mmol) in anhydrous ethanol (20 mL) and the resulting solution was refluxed overnight. After cooling to room temperature, the solvent was removed *in vacuo*. The crude product was further purified with column chromatography on silica using EtOAc/Hexanes as eluent to give imine **1** as orange solid (88 mg, 54%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.41 (s, 1H), 7.69 (d, J = 8.9 Hz, 2H), 7.14 (d, J = 9.0 Hz, 2H), 6.76 (d, J = 9.0 Hz, 2H), 6.68 (d, J = 9.1 Hz, 2H), 4.77 (t, J = 5.4 Hz, 2H), 3.54 (t, J = 5.9 Hz, 4H), 3.42 (t, J = 6.2 Hz, 4H), 2.99 (s, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 154.89, 151.80, 146.21, 140.09, 129.53, 124.73, 122.08, 111.69, 111.59, 58.27, 53.50, 39.83. HRMS (ESI-TOF) m/z: $[M + H]^+$ calculated for $C_{19}H_{26}N_3O_2^+$ 328.201954, found 328.2020.



^{1}H NMR spectrum of **1** in DMSO-d_6



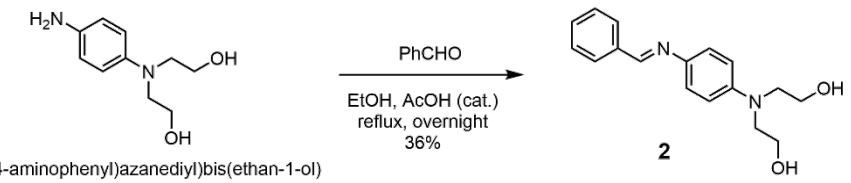
^{13}C NMR spectrum of **1** in DMSO-d_6



Meas. m/z	#	Ion Formula	m/z	err [ppm]	Mean err [ppm]	rdb	N-Rule	e¯ Conf
328.202282	1	C19H26N3O2	328.201954	-1.0	-1.0	8.5	ok	even

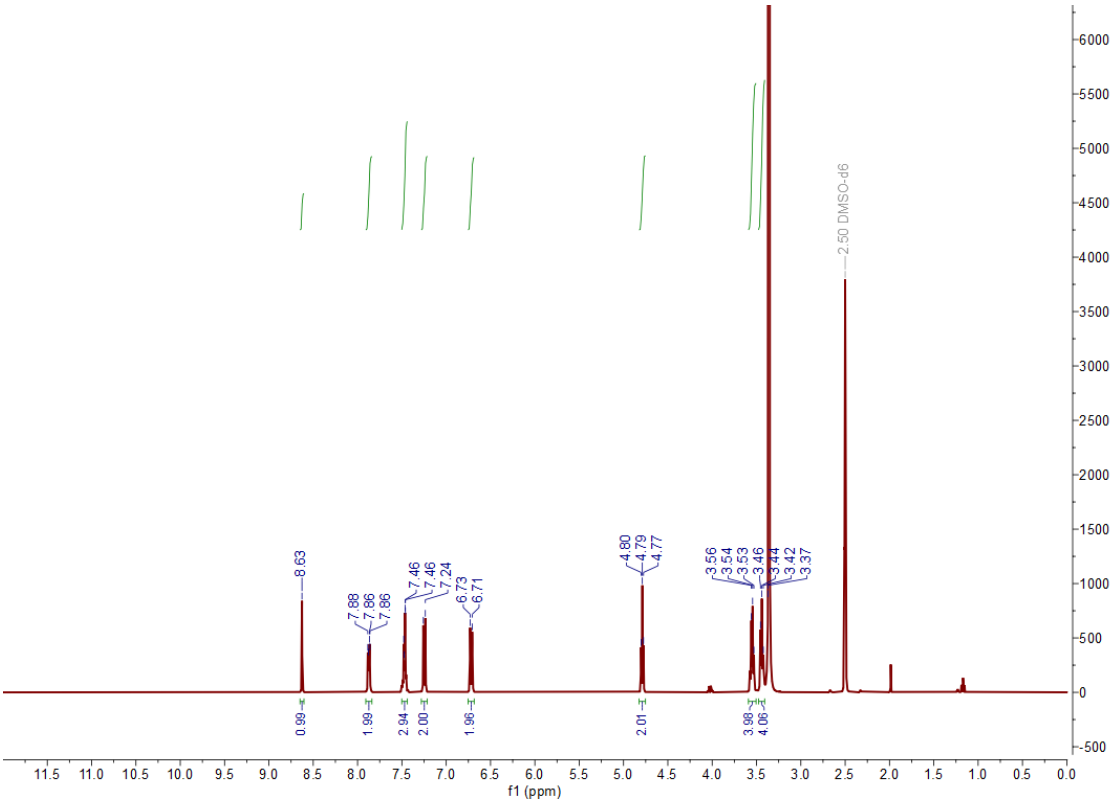
Mass spectrum of **1**

Synthesis of imine 2

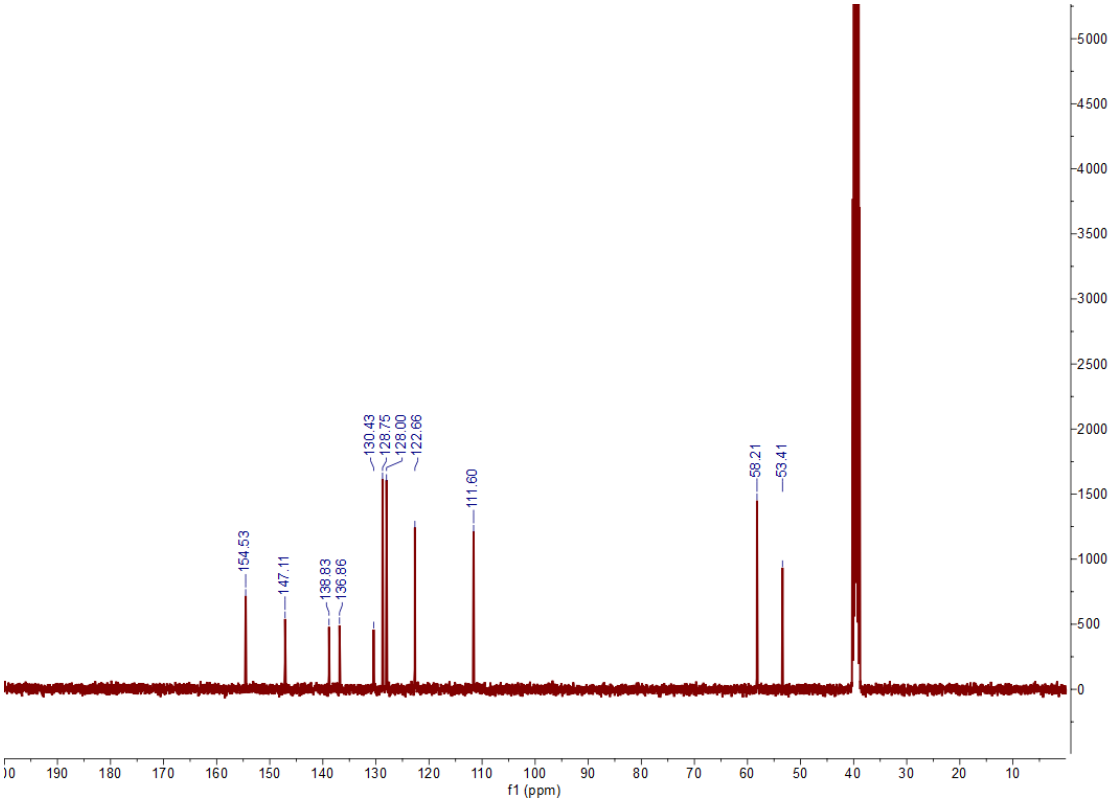


2,2'-((4-aminophenyl)azanediyl)bis(ethan-1-ol)

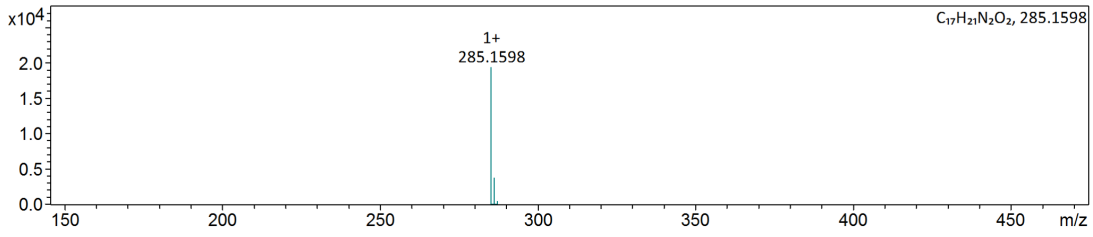
One drop of acetic acid was added as catalyst to a solution of 2,2'-((4-aminophenyl) azanediyl)bis(ethan-1-ol) (196 mg, 1 mmol) and benzaldehyde (212 mg, 2 mmol) in anhydrous ethanol (40 mL) and the resulting solution was refluxed overnight. After cooling to room temperature, the solvent was removed in vacuo. The crude product was further purified with column chromatography on silica using EtOAc/Hexanes as eluent to give **2** as yellow solid (103 mg, 36%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.63 (s, 1H), 7.87 (dd, J = 7.5, 2.2 Hz, 2H), 7.47 (dd, J = 6.0, 1.6 Hz, 3H), 7.25 (d, J = 9.0 Hz, 2H), 6.72 (d, J = 9.0 Hz, 2H), 4.79 (t, J = 5.4 Hz, 2H), 3.54 (t, J = 5.9 Hz, 4H), 3.44 (t, J = 6.3 Hz, 4H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 154.53, 147.11, 138.83, 136.86, 130.43, 128.75, 128.00, 122.66, 111.60, 58.21, 53.41. HRMS (ESI-TOF) m/z: $[M + H]^+$ calculated for $C_{17}H_{21}N_2O_2^+$ 285.159754, found 285.1598.



^{1}H NMR spectrum of **2** in DMSO-d_6



^{13}C NMR spectrum of **2** in DMSO-d_6



Meas. m/z	#	Ion Formula	m/z	err [ppm]	Mean err [ppm]	rdb	N-Rule	e¯ Conf
285.159952	1	C17H21N2O2	285.159754	-0.7	-2.5	8.5	ok	even

Mass spectrum of **2**

3. Characterization of 1·H⁺@CB7 complex

The spectra in **Fig. S1** and **S2** were obtained by preparing separate NMR samples. Each solution was generated by adding an aliquot of **1** in DMSO-d_6 to an appropriate solution of **CB7** in D_2O containing acetic acid.

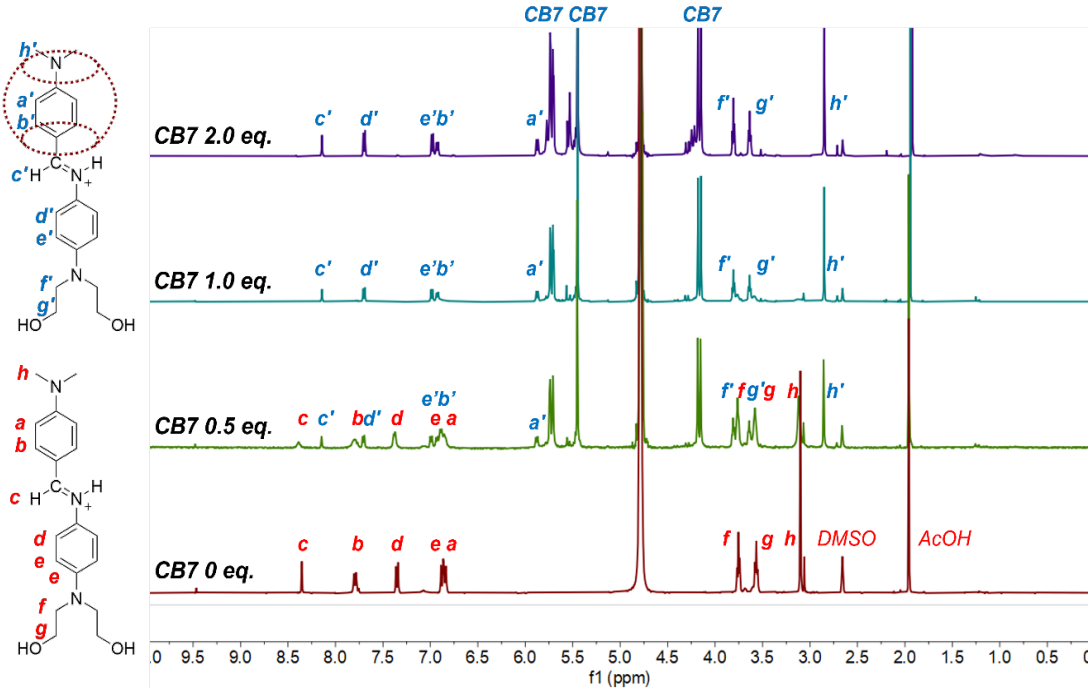


Fig. S1 Full scale ^{1}H NMR spectra (400 MHz) and atom assignments for solutions of imine **1** (1.0 mM) mixed with 0 – 2.0 molar equivalents of **CB7** in D_2O containing acetic acid and 2% DMSO-d_6 (pD = 4.70) at room temperature. Note, the very minor peaks at 9.5 ppm and the region 8.5 to 6.5 ppm reflects the minute amounts of imine hydrolysis that occurs in the short time between sample preparation and spectral acquisition.

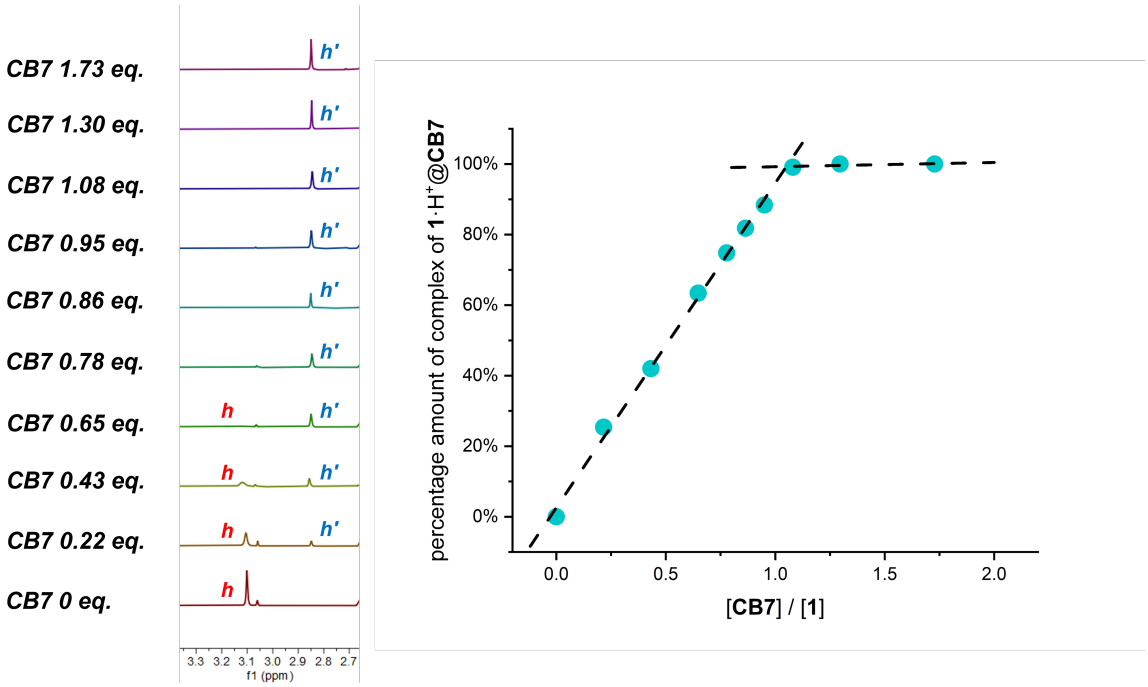


Fig. S2 (*left*) Partial ^{1}H NMR spectra, for ten separate samples, showing dimethylamino peaks *h* and *h'* for **1** and **1**·H^{+}@CB7 complex, respectively. Peak integration gives the percentage amount of **1**·H^{+}@CB7 complex. (*right*) Plot identifies 1:1 stoichiometry for **1**·H^{+}@CB7 complex.

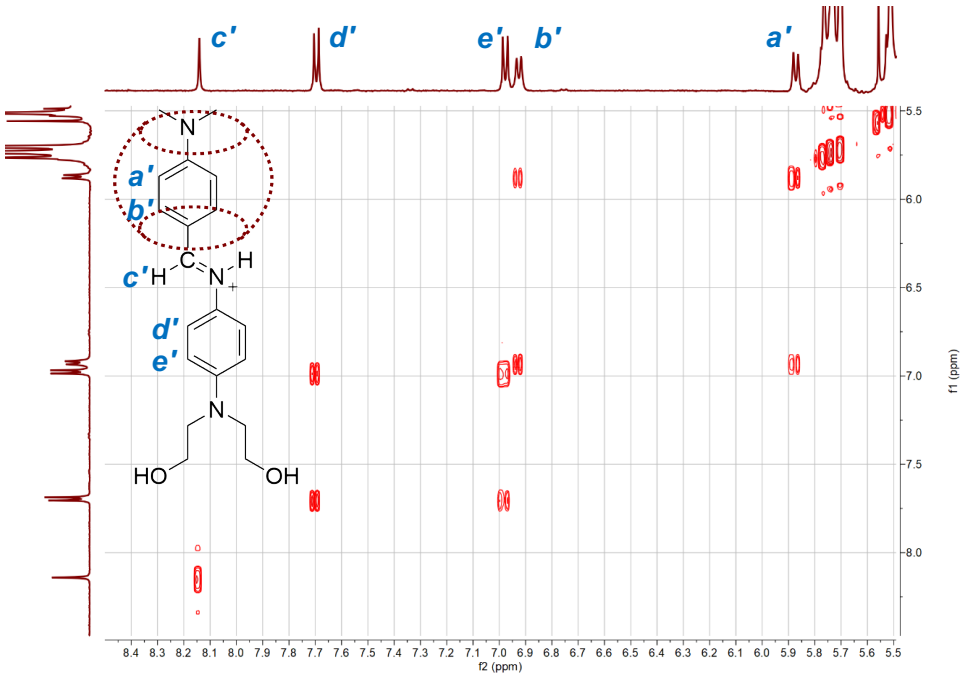


Fig. S3 Partial COSY spectra of **1**·H^+@CB7 complex in D_2O with acetic acid (2.0 mM) and 2% DMSO-d_6 (pD = 4.70) at room temperature.

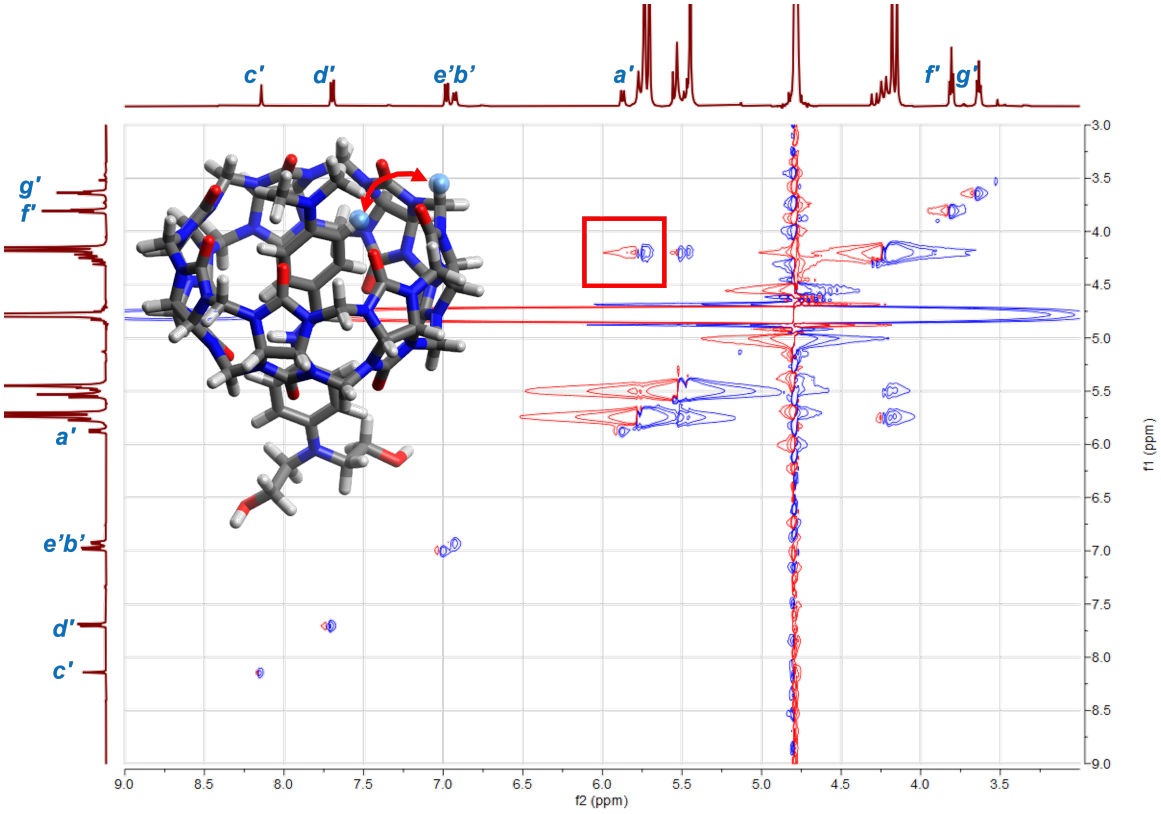


Fig. S4 Partial NOESY spectrum of **1**·H^+@**CB7** complex in D_2O with acetic acid (2.0 mM) and 2% DMSO-d_6 (pD = 4.70) at room temperature reveals a single intermolecular cross-peak (highlighted in the red box) reflecting cross-relaxation of imine proton $H_{a'}$ and an inward-pointing hydrogen of the surrounding **CB7** (see double headed arrow drawn on the molecular model). The sparsity of intermolecular cross-peaks is a common feature of NOESY spectra of **CB7** complexes due in part to the outwardly directed **CB7** hydrogens.

4. Determination of pKa and association constant for formation of 1·H⁺@CB7 Complex

Determination of pKa: A series of phosphate buffer solutions was made with a pH range of 5.0 to 12.0. Aliquots of **1** in DMSO were added to each solution and a spectrum quickly acquired. An analogous set of absorption experiments were conducted in phosphate buffer solutions containing **CB7** (500 μM). The pKa of **1**·H⁺ and **1**·H⁺@**CB7** were obtained by plotting the absorbance at 468 nm (**Fig. S5**).

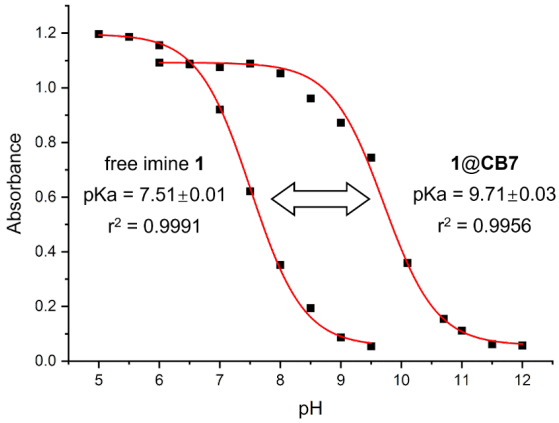


Fig. S5 Titration experiment measured absorbance at 468 nm for **1**·H⁺ (25 μM) or absorption at 465 nm for **1**·H⁺ (25 μM) and **CB7** (500 μM), in phosphate buffer with 0.5% DMSO.

Determination of Association Constant: A series of solutions composed of 10 mM $NaHCO_3$ – Na_2CO_3 buffer (pH 8.80) containing 0 – 250 μM **CB7** were made. Aliquots of **1** in DMSO were added to each solution and a spectrum quickly acquired. The absorbance values at 383 nm and 468 nm were plotted and the following logic was used to calculate the association constant for formation of **1**·H⁺@**CB7** complex.

Two major chemical processes exist in the solution:

$$[\mathrm{G}]_0 = [\mathrm{G_{np}}] + [\mathrm{G_p}] + [\mathrm{HG_p}]$$

$$[\mathrm{H}]_0 = [\mathrm{H}] + [\mathrm{HG_p}]$$

where the total concentration of guest molecules $[\mathrm{G}]_0$ equals the sum of free non-protonated guest $[\mathrm{G_{np}}]$, free protonated guest $[\mathrm{G_p}]$, and encapsulated protonated guest $[\mathrm{HG_p}]$. The total concentration of host molecules $[\mathrm{H}]_0$ equals the sum of free host $[\mathrm{H}]$ and complex $[\mathrm{HG_p}]$. Whereas two sets of equilibria exist in the system:

$$K_a = \frac{[\mathrm{HG_p}]}{[\mathrm{H}][\mathrm{G_p}]}$$

$$K_{iminium} = \frac{[\mathrm{G_{np}}]}{[\mathrm{G_p}][\mathrm{H^+}]}$$

K_a, the binding constant of $\mathrm{G_p}$ and host. $K_{iminium}$, acid dissociation constant of the iminium. Therefore, when measure in buffer where pH = 8.8, then $K_{iminium}$ = $10^{-7.5}$, and $[\mathrm{G_{np}}]/[\mathrm{G_p}]$ = 20. The following equations can be obtained:

$$[\mathrm{HG_p}]^2 - \left([\mathrm{H}]_0 + [\mathrm{G}]_0 + \frac{21}{K_a}\right)[\mathrm{HG_p}] + [\mathrm{H}]_0[\mathrm{G}]_0 = 0$$

$$[\mathrm{HG_p}] = \frac{1}{2}\left([\mathrm{H}]_0 + [\mathrm{G}]_0 + \frac{21}{K_a}\right) - \sqrt{\left([\mathrm{H}]_0 + [\mathrm{G}]_0 + \frac{21}{K_a}\right)^2 - 4[\mathrm{H}]_0[\mathrm{G}]_0}$$

The absorbance data then can be fitted with the following equation:

$$\Delta \mathrm{A_{obs}} = \varepsilon[\mathrm{HG_p}]$$

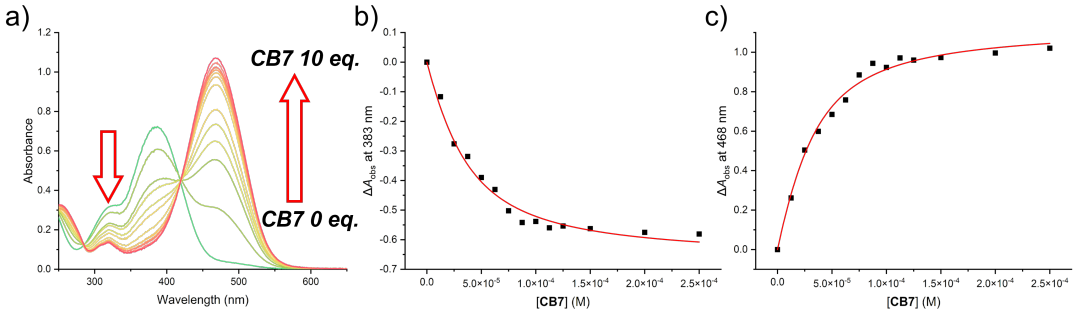


Fig. S6 a) UV-Vis spectra of imine **1** (25 μM) upon **CB7** addition (0 – 10 eq.) in a 10 mM $NaHCO_3$ – Na_2CO_3 buffer (pH 8.80). b) Association constant fitted with ΔA_{obs} at 383 nm vs. [**CB7**], obtained K_a = (0.92 ± 0.14) × 10^6 M^{-1}, r^2 = 0.983. b) Association constant fitted with ΔA_{obs} at 468 nm vs. [**CB7**], obtained K_a = (1.09 ± 0.14) × 10^6 M^{-1}, r^2 = 0.989. The average of the two association constants is 1.0 × 10^6 M^{-1}.

5. Hydrolysis of imines monitored using NMR spectroscopy

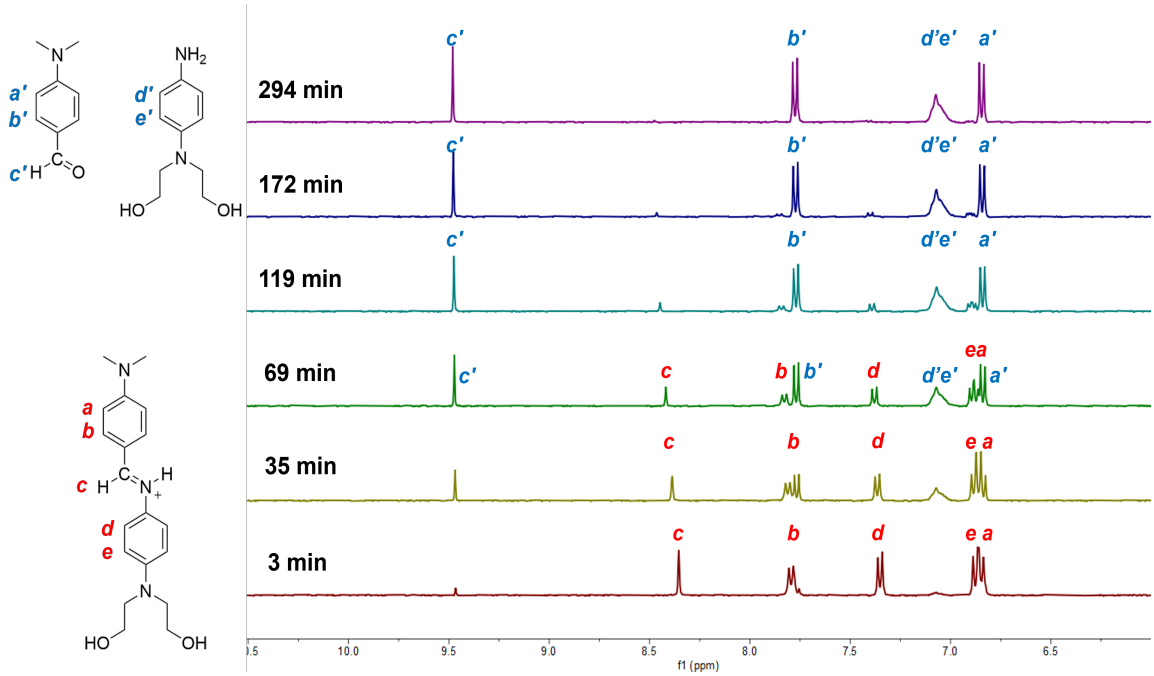


Fig. S7 ^{1}H NMR spectra of **1**·H$^+$ (1.0 mM) in D_2O with acetic acid (2.0 mM) and 2% DMSO-d_6 (pD = 4.70) over time at room temperature.

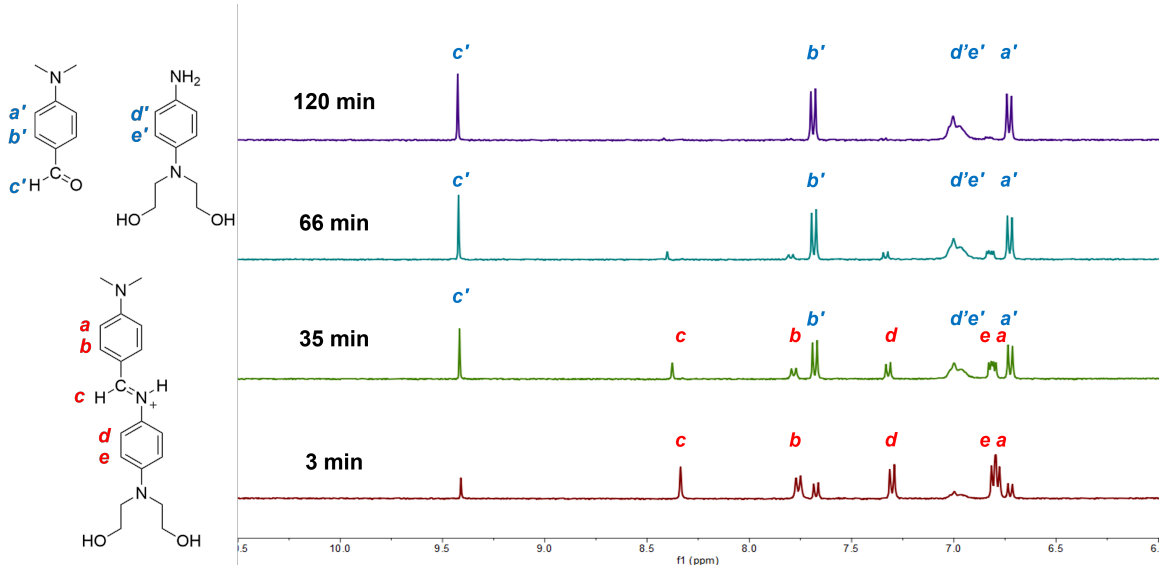


Fig. S8 ^{1}H NMR spectra of **1**·H$^+$ (1.0 mM) and **β-CD** (2.0 mM) in D_2O with acetic acid (2.0 mM) and 2% DMSO-d_6 (pD = 4.70) over time at room temperature.

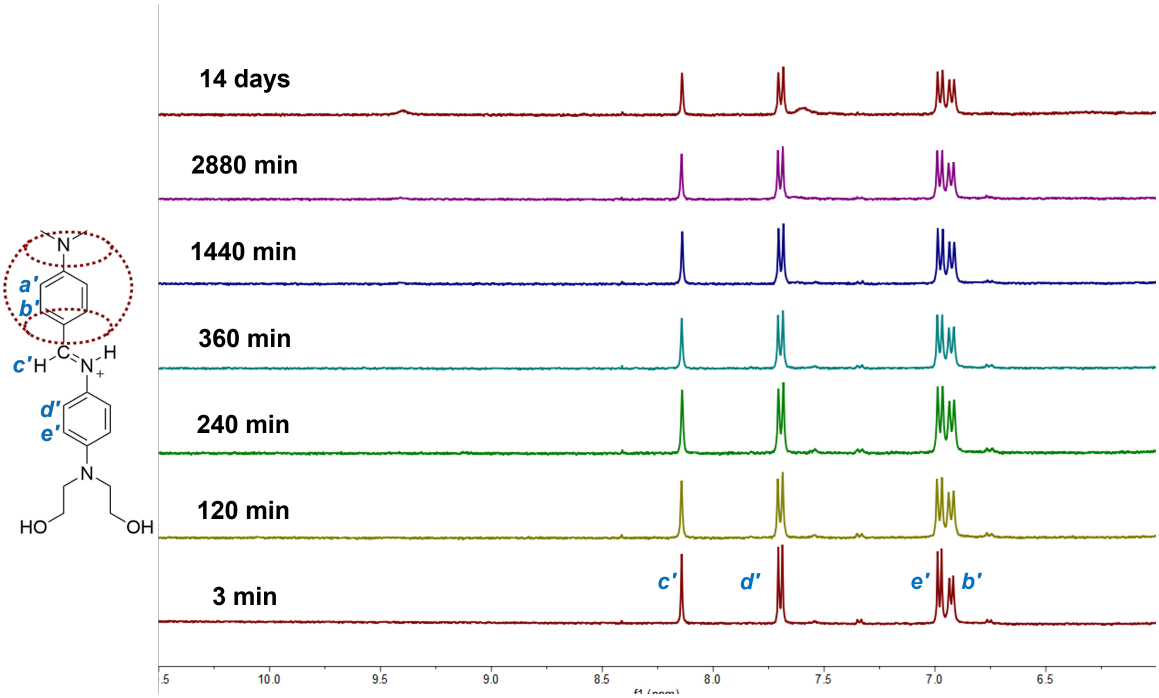


Fig. S9 ^{1}H NMR spectra of **1**·H^{+} (1.0 mM) and **CB7** (2.0 mM) in D_2O with acetic acid (2.0 mM) and 2% DMSO-d_6 (pD = 4.70) over time at room temperature.

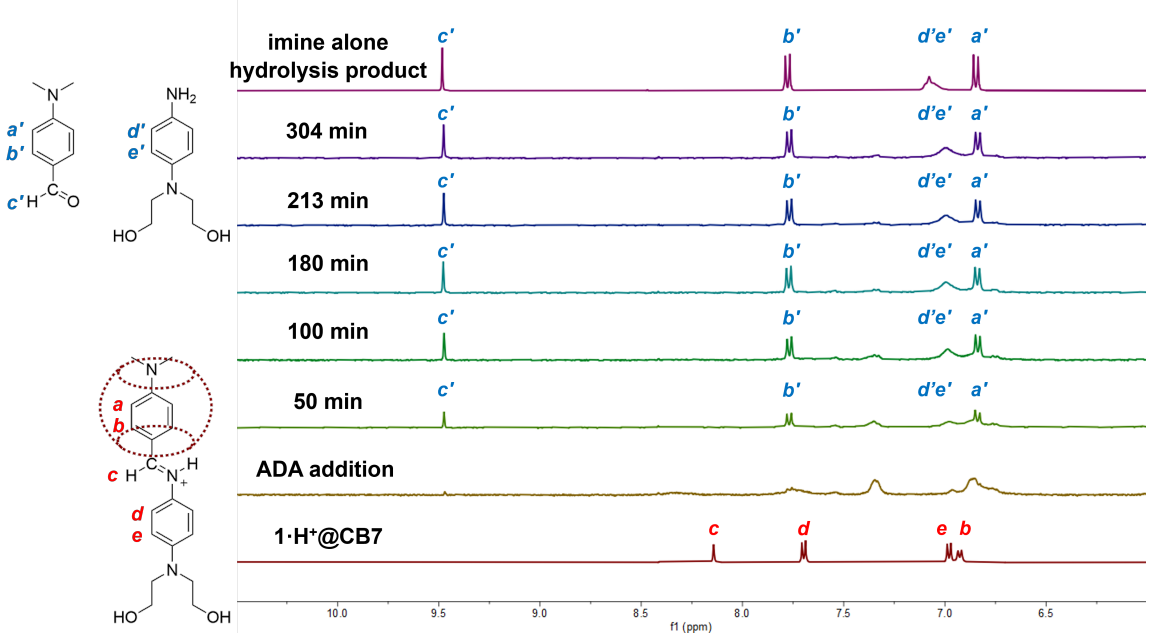


Fig. S10 ^{1}H NMR spectra for **ADA** displacement experiment over time. An aliquot of **ADA** acetate (2.0 mM) in D_2O with acetic acid (2.0 mM) was added to a solution of **1** (1.0 mM) and **CB7** (2.0 mM) in D_2O with acetic acid (2.0 mM) and 2% DMSO-d_6 (pD = 4.70). The time points listed denote time after addition of the **ADA**. ^{1}H NMR spectra acquired at room temperature.

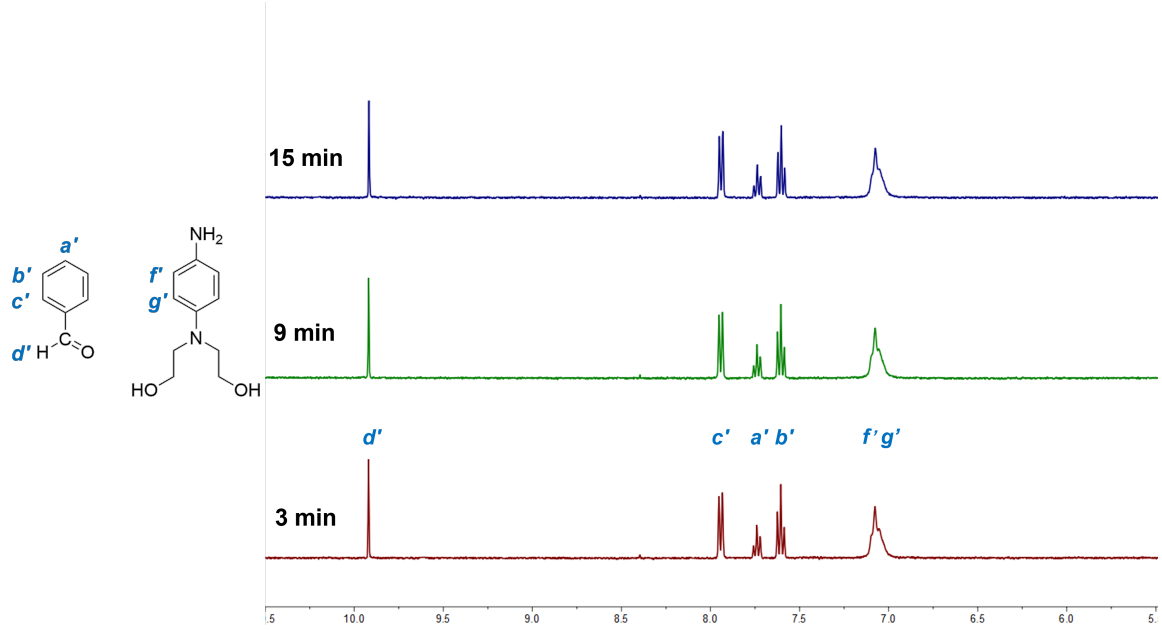


Fig. S11 ^{1}H NMR spectra showing that imine **2**·H$^+$ (1.0 mM) is instantly hydrolyzed in D_2O with acetic acid (2.0 mM) and 2% DMSO-d_6 (pD = 4.70) at room temperature.

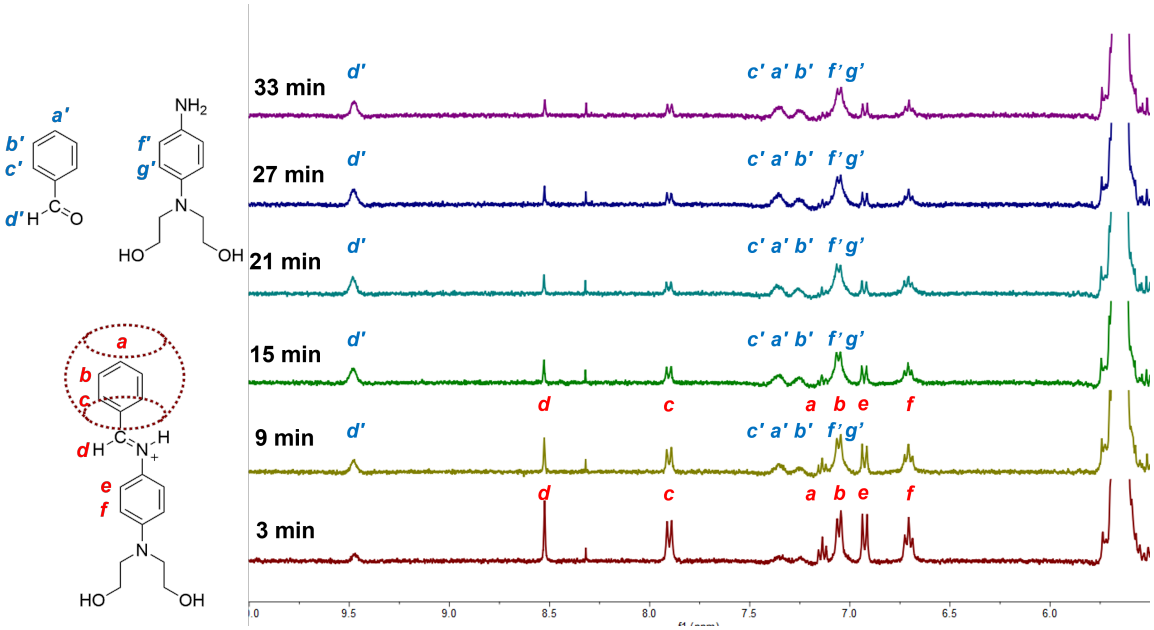


Fig. S12 ^{1}H NMR spectra of **2**·H$^+$ (1.0 mM) and **CB7** (2.0 mM) in D_2O with acetic acid (2.0 mM) and 2% DMSO-d_6 (pD = 4.70) at room temperature shows hydrolysis of imine **2** with half-life of ~ 3 mins.

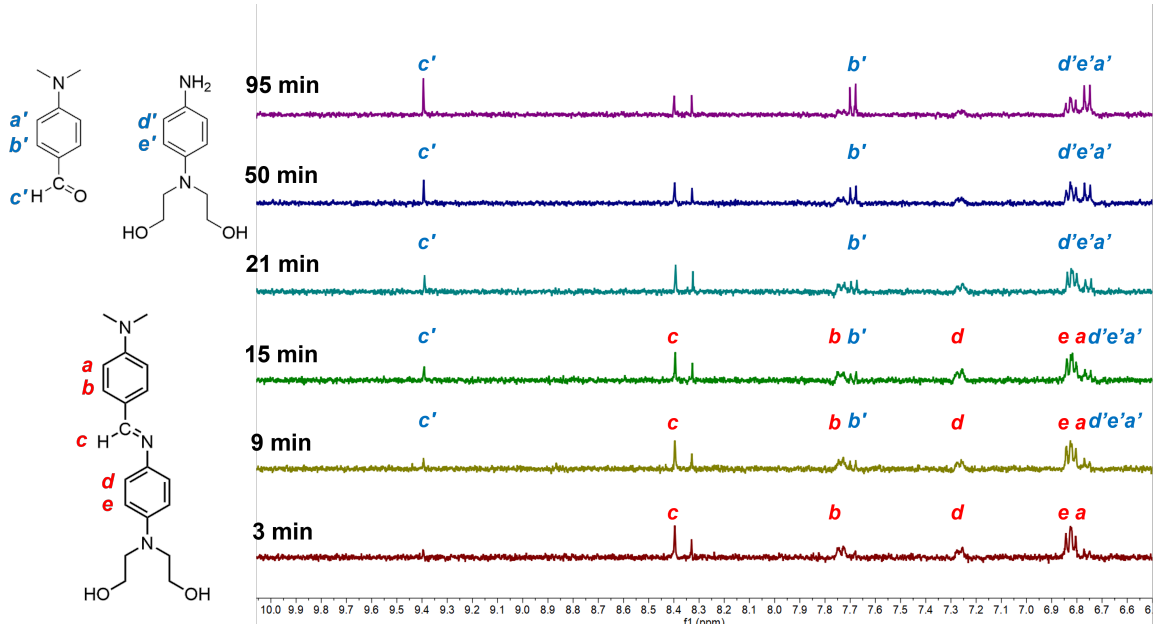


Fig. S13 ^{1}H NMR spectra of **1** (1.0 mM) in neutral D_2O with 2% DMSO-d_6 at room temperature shows hydrolysis of imine **1** with half-life of ~ 50 mins.

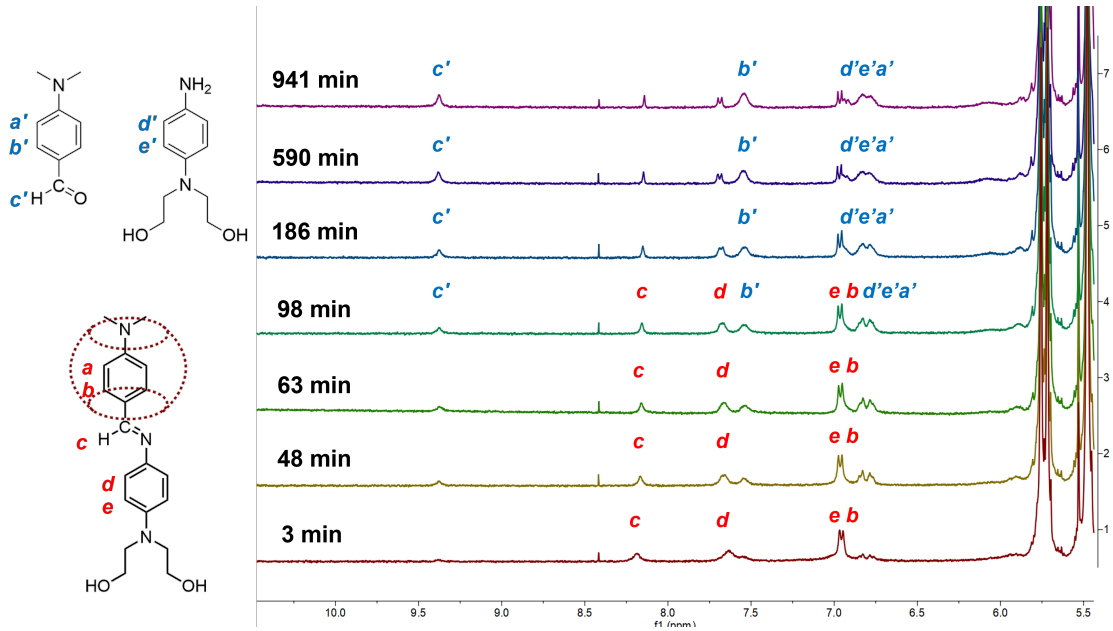


Fig. S14 ^{1}H NMR spectra of **1** (1.0 mM) and **CB7** (2.0 mM) in neutral D_2O with 2% DMSO-d_6 at room temperature shows hydrolysis of imine **1** with half-life of ~ 120 mins.

6. DFT calculated molecular models of 1•H$^+$@CB7

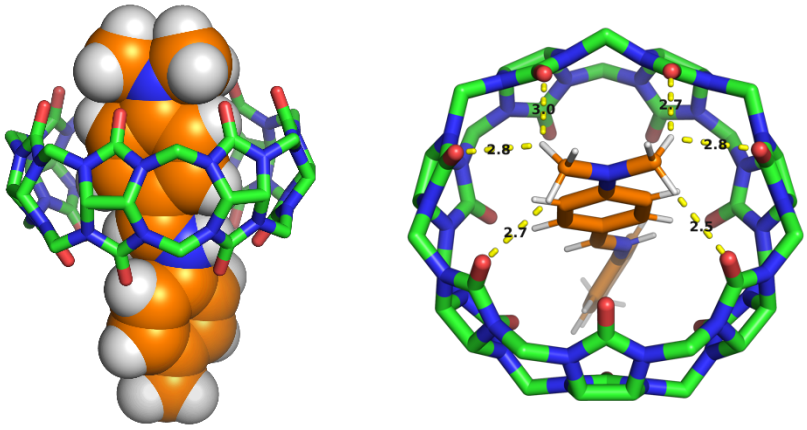


Figure S15. Models of the DFT-calculated, energy-minimized **1**·H$^+$@**CB7** complex in water at room temperature (B3LYP/6-31G**). Stabilizing CH···O interactions between the six *N*,*N*-dimethylamino hydrogens of **1**·H$^+$and the oxygens within the top portal of **CB7** are highlighted. **CB7** hydrogens have been omitted for clarity.

Calculation Method = B3LYP, Basis Set = 6-31G(d, p), Charge = 1

Solvation = scrf = (cpcm, solvent = water), Electronic Energy (Hartree) = − 4914.03

Dipole Moment (Debye) = 3.014 (No imaginary frequency)

No.	Atom	X	Y	Z
1	C	-0.901	-5.699	-0.502
2	C	0.644	-5.732	-0.453
3	N	0.946	-5.166	0.837
4	C	-0.176	-4.908	1.568
5	O	-0.204	-4.539	2.713
6	N	-1.262	-5.179	0.789
7	N	1.032	-4.932	-1.585
8	C	-0.044	-4.447	-2.272
9	O	0	-3.816	-3.295
10	N	-1.181	-4.835	-1.621
11	H	-1.349	-6.673	-0.656
12	H	1.059	-6.728	-0.536
13	C	3.948	-4.276	-0.244
14	C	4.948	-3.104	-0.12

15	N	4.459	-2.38	1.028
16	C	3.455	-3.047	1.669
17	O	2.95	-2.722	2.712
18	N	3.148	-4.158	0.943
19	N	4.825	-2.424	-1.38
20	C	3.862	-2.973	-2.174
21	O	3.602	-2.638	-3.301
22	N	3.266	-3.991	-1.486
23	H	4.422	-5.249	-0.286
24	H	5.971	-3.423	0.039
25	C	-5.079	-2.861	-0.3
26	C	-4.142	-4.083	-0.42
27	N	-3.42	-4.072	0.821
28	C	-3.698	-2.973	1.576
29	O	-3.249	-2.73	2.666
30	N	-4.607	-2.211	0.898
31	N	-3.364	-3.778	-1.598
32	C	-3.868	-2.707	-2.279
33	O	-3.525	-2.344	-3.374
34	N	-4.853	-2.141	-1.523
35	H	-6.125	-3.123	-0.203
36	H	-4.667	-5.022	-0.545
37	C	-5.419	2.193	-0.18
38	C	-5.812	0.695	-0.171
39	N	-5.062	0.165	0.94
40	C	-4.328	1.12	1.569
41	O	-3.616	0.936	2.531
42	N	-4.538	2.311	0.954
43	N	-5.391	0.232	-1.467
44	C	-4.838	1.226	-2.22
45	O	-4.48	1.125	-3.365
46	N	-4.789	2.361	-1.465
47	H	-6.261	2.865	-0.075
48	H	-6.871	0.524	-0.029
49	C	-1.639	5.56	-0.386
50	C	-3.047	4.933	-0.293
51	N	-3.154	4.161	-1.499
52	C	-1.994	4.165	-2.219
53	O	-1.825	3.642	-3.289
54	N	-1.065	4.898	-1.533
55	N	-2.98	4.167	0.928

56	C	-1.841	4.425	1.636
57	O	-1.597	4.035	2.748
58	N	-1.046	5.232	0.881
59	H	-1.65	6.633	-0.532
60	H	-3.844	5.665	-0.239
61	C	5.868	0.405	-0.048
62	C	5.571	1.921	-0.116
63	N	4.754	2.153	1.044
64	C	4.468	1.005	1.718
65	O	3.821	0.923	2.731
66	N	5.062	-0.036	1.065
67	N	4.902	2.066	-1.387
68	C	4.925	0.913	-2.116
69	O	4.565	0.793	-3.258
70	N	5.454	-0.075	-1.339
71	H	6.911	0.174	0.125
72	H	6.46	2.539	-0.083
73	C	3.357	4.794	-0.303
74	C	1.975	5.478	-0.389
75	N	1.38	5.187	0.886
76	C	2.169	4.391	1.662
77	O	1.93	4.044	2.789
78	N	3.295	4.087	0.952
79	N	1.359	4.835	-1.525
80	C	2.23	4.017	-2.187
81	O	2.018	3.463	-3.234
82	N	3.395	3.966	-1.478
83	H	4.186	5.491	-0.305
84	H	2.03	6.548	-0.545
85	C	-5.697	-1.073	-2.001
86	H	-6.723	-1.316	-1.755
87	H	-5.591	-1.019	-3.07
88	C	-5.308	-1.127	1.531
89	H	-6.374	-1.319	1.49
90	H	-4.994	-1.09	2.559
91	C	-2.485	-4.731	-2.229
92	H	-2.351	-4.426	-3.252
93	H	-2.953	-5.71	-2.208
94	C	-2.608	-5.162	1.299
95	H	-3.094	-6.091	1.027
96	H	-2.552	-5.09	2.37

97	C	2.367	-4.919	-2.128
98	H	2.303	-4.643	-3.165
99	H	2.777	-5.919	-2.051
100	C	2.26	-5.185	1.424
101	H	2.72	-6.147	1.229
102	H	2.145	-5.056	2.486
103	C	-4.397	3.637	-2.012
104	H	-4.293	3.515	-3.075
105	H	-5.178	4.36	-1.809
106	C	-4.135	3.566	1.54
107	H	-4.973	4.251	1.472
108	H	-3.904	3.394	2.576
109	C	0.161	5.335	-2.154
110	H	0.191	6.419	-2.143
111	H	0.155	4.992	-3.173
112	C	0.178	5.808	1.376
113	H	0.196	6.857	1.104
114	H	0.174	5.718	2.448
115	C	4.592	3.342	-1.983
116	H	4.467	3.186	-3.039
117	H	5.422	4.019	-1.817
118	C	4.438	3.459	1.563
119	H	4.233	3.36	2.614
120	H	5.305	4.096	1.428
121	C	5.731	-1.4	-1.839
122	H	6.736	-1.675	-1.544
123	H	5.671	-1.364	-2.912
124	C	5.206	-1.333	1.677
125	H	4.857	-1.26	2.692
126	H	6.257	-1.602	1.68
127	C	-1.246	0.175	-4.545
128	N	0.018	0.037	-3.828
129	C	1.247	-0.101	-4.6
130	C	0.046	-0.024	-2.492
131	C	1.267	-0.186	-1.786
132	C	1.279	-0.234	-0.423
133	C	-1.153	0.062	-1.72
134	C	-1.115	0.02	-0.361
135	C	0.102	-0.119	0.344
136	C	0.225	-0.144	1.751
137	N	-0.709	0.048	2.632

138	C	-0.504	-0.04	4.043
139	C	0.188	-1.102	4.593
140	C	0.377	-1.15	5.968
141	C	-0.133	-0.165	6.801
142	C	-0.842	0.886	6.22
143	C	-1.028	0.958	4.854
144	H	-1.036	0.256	-5.599
145	H	-1.773	1.067	-4.235
146	H	-1.885	-0.684	-4.384
147	H	1.008	-0.027	-5.648
148	H	1.717	-1.061	-4.422
149	H	1.953	0.682	-4.354
150	H	-2.097	0.15	-2.215
151	H	-2.045	0.073	0.171
152	H	-1.64	0.298	2.35
153	H	0.564	-1.892	3.97
154	H	0.919	-1.978	6.388
155	C	0.065	-0.219	8.297
156	H	-1.248	1.663	6.845
157	H	-1.563	1.779	4.414
158	H	0.61	-1.109	8.589
159	H	-0.889	-0.223	8.815
160	H	0.621	0.646	8.646
161	H	2.189	-0.275	-2.32
162	H	2.222	-0.362	0.076
163	H	1.204	-0.33	2.156