Electronic Supplementary Information

Encapsulation of Ionic Liquids inside Cucurbiturils

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1. NMR Experiments:

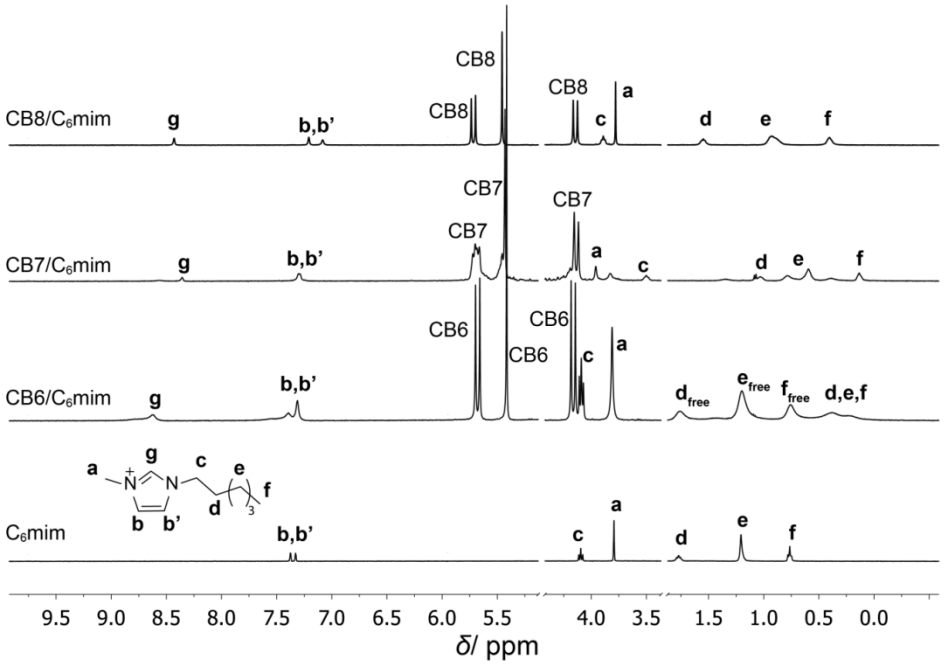

Fig. S1 ^{1}H NMR spectra of C_6mim with CB*n* (1:1 molar ratio), measured in unbuffered D_2O at 25 °C.

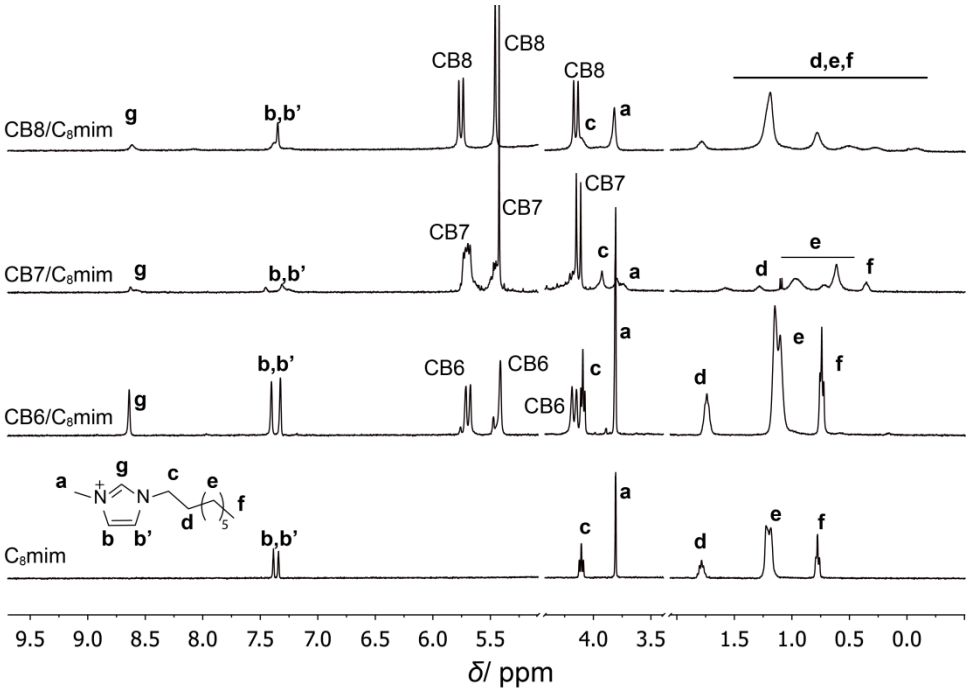

Fig. S2 ^{1}H NMR spectra of C_8mim with CB*n* (1:1 molar ratio), measured in unbuffered D_2O at 25 °C.

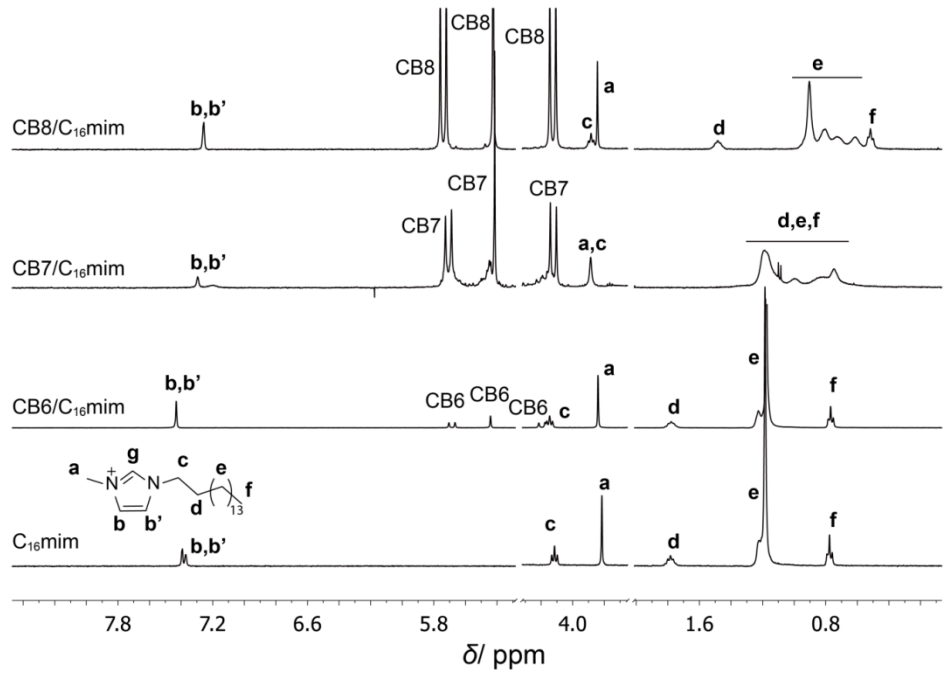

Fig. S3 ^{1}H NMR spectra of C_{16}mim with CB*n* (1:1 molar ratio), measured in unbuffered D_2O at 25 °C.

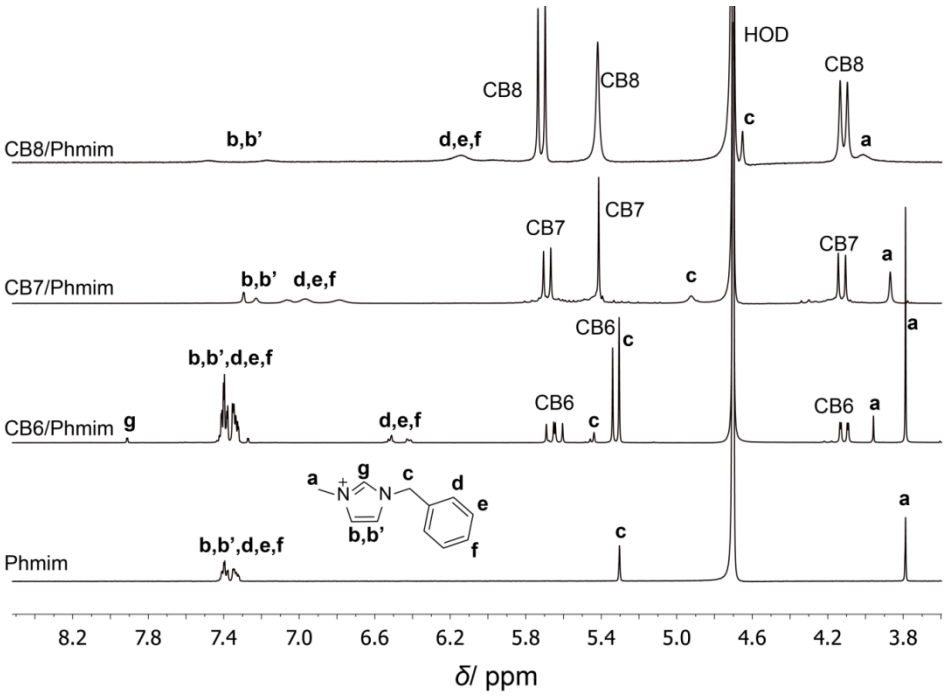

Fig. S4 ^{1}H NMR spectra of Phmim with CB*n* (1:1 molar ratio), measured in unbuffered D_2O at 25 °C.

2. ITC Experiments:

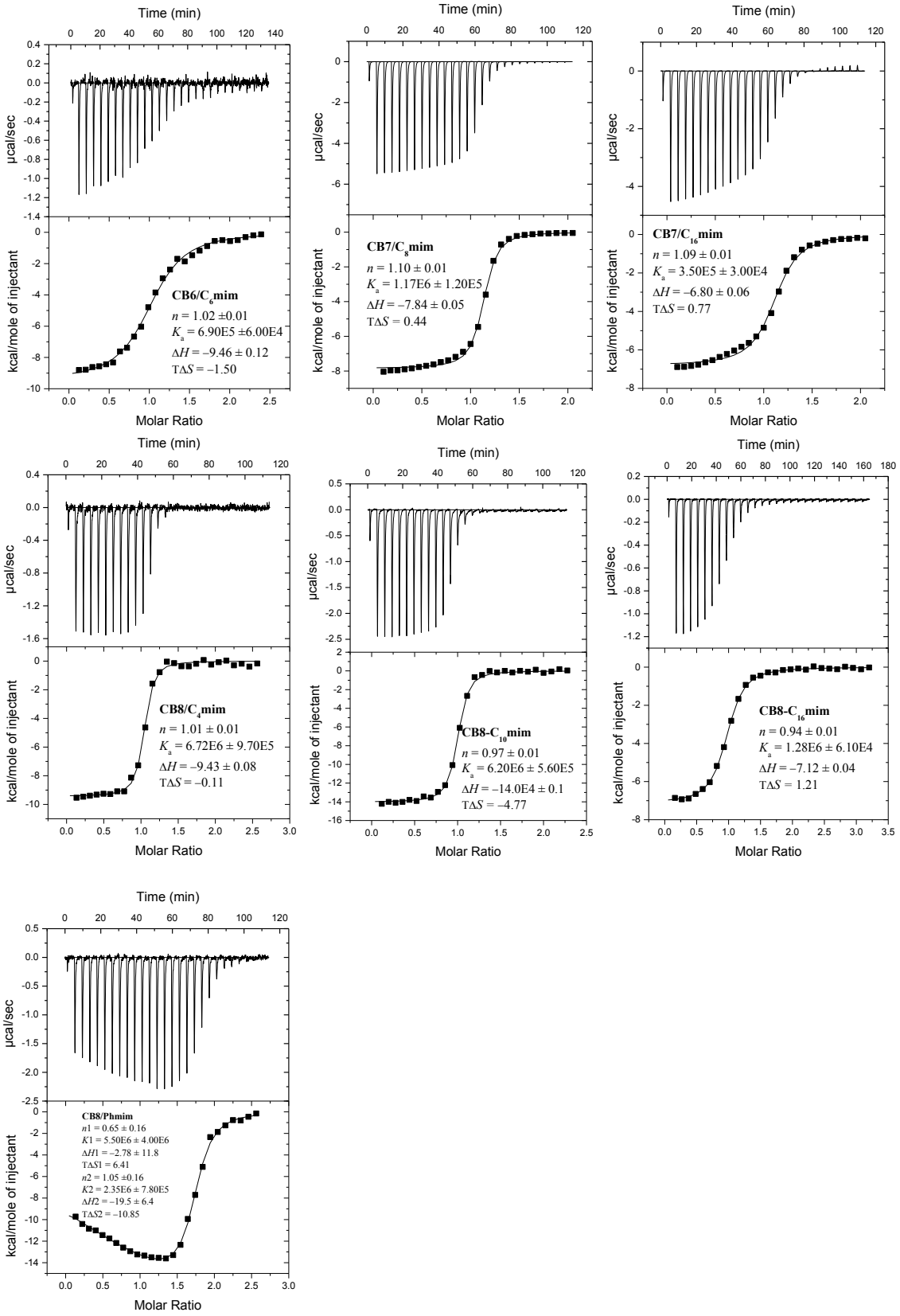

Fig. S5 ITC isotherms for the complexation of selected ILs with CB*n*, as measured in unbuffered water at 25 °C; association constants (K_a) given in M^{-1} and thermodynamic data (ΔG, ΔH, and $T\Delta S$) in kcal mol^{-1}.

3. Dye Displacement Experiments

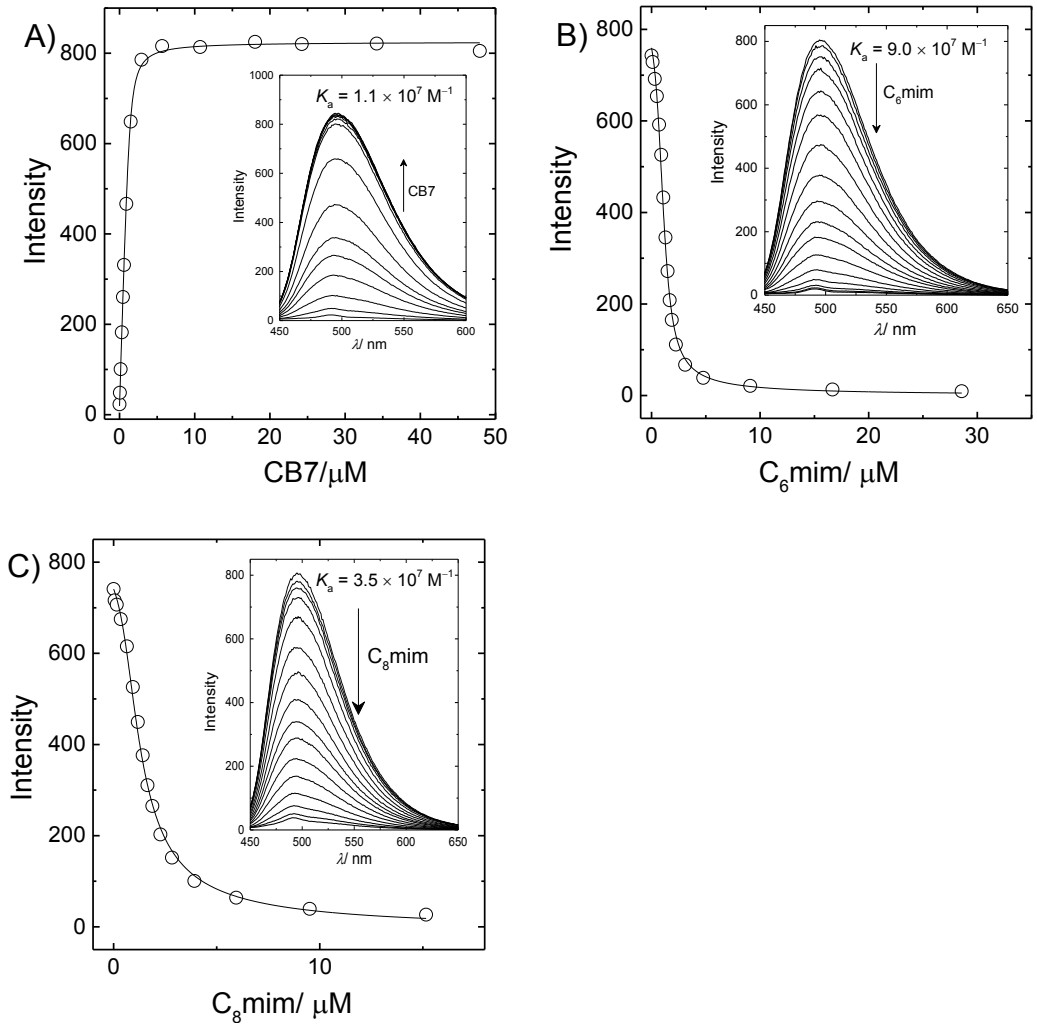

Fig. S6 A) Fluorescence titration of berberine (1 μM) with CB7 in aqueous solution. B and C) Fluorescence displacement titration of CB7• berberine (1.5 μM : 1 μM) with C_6mim and C_8mim. The samples were excited at 345 nm.

Table S1 Association constants (K_a) for the complexation of ILs and CB7, measured by fluorescence displacement titration, see Figure S6.

Host-guest	$K_a/10^6$ M^{-1}
CB7•C_4mim	4.5 [1.3][a]
CB7•C_6mim	90 [2.2][a]
CB7•C_8mim	35 [1.2][a]
CB7•C_{10}mim	7.5 [0.9][a]
CB7•C_{16}mim	1.5 [0.3][a]
CB7•Phmim	420 [2.3][a]

[a] Values in square brackets refer to the ITC values, see Table 2 in the main text.

4. Computational Results

Table S2 Binding Free Energies (kcal/mol) Resulting from MM-PBSA Analysis of the CB6 Complexes.

	C_4mim	C_5mim	C_6mim	C_7mim	C_8mim	C_{10}mim	C_{16}mim	Phmim
BOND	0.24	0.04	0.08	0.16	0.29	0.02	−0.14	0.75
ANGLE	0.06	−0.76	−1.10	−0.76	−0.45	−0.91	−1.41	1.01
DIHED	1.50	1.79	2.21	2.60	2.78	3.21	3.64	4.64
VDWAALS	−19.63	−22.51	−22.39	−23.27	−23.14	−23.74	−25.68	−20.06
EEL	−51.90	−50.99	−46.10	−39.62	−32.53	−36.72	−56.12	−56.62
1−4 VDW	−0.28	−0.22	−0.22	−0.28	−0.25	−0.40	−0.57	−0.39
1−4 EEL	1.78	2.12	2.55	2.93	2.84	3.30	4.24	3.47
EPB	56.20	55.33	50.88	45.77	39.16	43.66	62.53	60.58
ENPOLAR	−2.48	−2.65	−2.64	−2.76	−2.74	−2.83	−3.18	−2.71
ΔG_{gas}	−68.24	−70.53	−64.97	−58.24	−50.46	−55.24	−76.05	−67.20
ΔG_{solv}	53.72	52.68	48.24	43.01	36.42	40.83	59.35	57.87
ΔG	−14.52	−17.86	−16.73	−15.23	−14.04	−14.41	−16.70	−9.33
$\Delta G' = \Delta G - T\Delta S$	−1.77	−3.51	−0.62	−2.25	−0.55	1.88	14.75	1.39
$T\Delta$S	−12.75	−14.35	−16.11	−12.98	−13.49	−16.30	−31.44	−10.72

Table S3 Binding Free Energies (kcal/mol) Resulting from MM-PBSA Analysis of the CB7 Complexes.

	C_4mim	C_5mim	C_6mim	C_7mim	C_8mim	C_{10}mim	C_{16}mim	Phmim
BOND	−0.26	−0.21	−0.17	−0.15	−0.11	−0.02	−0.46	−0.26
ANGLE	0.53	0.74	0.76	0.56	0.30	0.35	−0.76	−0.12
DIHED	0.27	0.71	1.14	1.77	1.93	1.60	1.20	0.16
VDWAALS	−21.86	−23.37	−25.36	−26.89	−27.15	−28.93	−30.01	−23.55
EEL	−53.06	−51.56	−51.75	−48.97	−45.16	−48.03	−49.69	−54.01
1−4 VDW	0.05	0.05	0.01	−0.01	0.01	−0.03	−0.03	0.12
1−4 EEL	0.71	−0.29	0.79	0.88	0.95	1.10	1.02	0.22
EPB	62.02	61.76	61.27	58.47	55.00	61.17	64.07	64.54
ENPOLAR	−2.98	−3.17	−3.34	−3.46	−3.54	−3.79	−3.90	−3.17
ΔG_{gas}	−73.61	−73.92	−74.57	−72.81	−69.22	−73.97	−78.74	−77.44
ΔG_{solv}	59.04	58.59	57.93	55.01	51.47	57.38	60.17	61.37
ΔG	−14.57	−15.33	−16.64	−17.79	−17.75	−16.59	−18.57	−16.07
$\Delta G' = \Delta G - T\Delta S$	−12.39	−14.54	−15.40	−17.53	−17.61	−12.82	−12.58	−10.40
$T\Delta S$	−2.18	−0.79	−1.23	−0.26	−0.14	−3.78	−5.99	−5.67

Table S4 Binding Free Energies (kcal/mol) Resulting from MM-PBSA Analysis of the CB8 Complexes.

	C_4mim	C_5mim	C_6mim	C_7mim	C_8mim	C_{10}mim	C_{16}mim	Phmim
BOND	−0.08	0.01	0.13	0.08	0.38	0.19	0.06	−0.03
ANGLE	0.63	0.97	0.74	1.17	1.26	1.16	0.43	−0.18
DIHED	0.74	0.92	1.00	1.88	2.36	4.20	5.14	0.24
VDWAALS	−26.96	−29.13	−32.30	−35.64	−35.53	−37.68	−39.34	−21.08
EEL	−49.16	−48.86	−50.36	−49.84	−54.26	−52.17	−26.93	−54.58
1−4 VDW	−0.13	−0.05	−0.26	−0.29	−0.43	−0.17	−0.33	0.01
1−4 EEL	0.52	0.07	0.62	0.94	0.59	0.99	1.76	0.23
EPB	58.59	59.43	61.12	60.75	64.01	61.86	36.60	62.12
ENPOLAR	−3.22	−3.47	−3.76	−3.99	−4.23	−4.53	−4.89	−3.10
ΔG_{gas}	−74.45	−76.07	−80.43	−81.70	−85.64	−83.48	−59.21	−75.39
ΔG_{solv}	55.37	55.96	57.36	56.76	59.78	57.34	31.71	59.02
ΔG	−19.08	−20.10	−23.07	−24.94	−25.86	−26.14	−27.50	−16.38
$\Delta G' = \Delta G - T\Delta S$	−19.55	−16.73	−15.52	−23.53	−18.32	−20.21	−27.36	−15.43
$T\Delta$S	0.47	−3.37	−7.55	−1.42	−7.53	−5.93	−0.14	−0.94

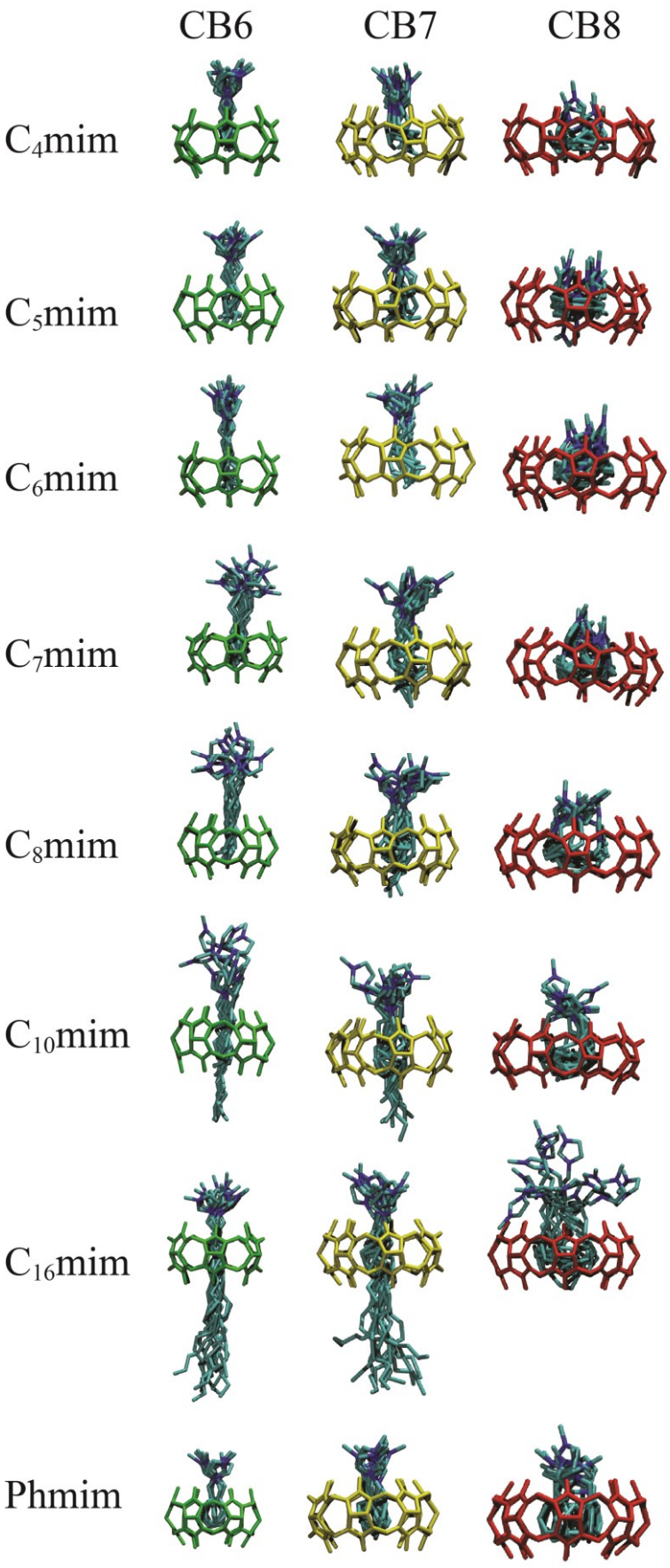

Fig. S7 Dynamics of 1:1 complexes, shown as a clustered molecular display for CB*n*•IL complexes.

5. Conductivity Experiments

In order to investigate the effect of complexation on the conductivity of ILs in aqueous solution, the change in conductivity of an aqueous solution of [C_4mim]Cl and [C_{16}mim]Cl was measured as a function of IL concentration in the absence and presence of CB7 (1.0 mM) (Figure S8). We anticipated that the conductivity of ILs decreased in the presence of CB7 due to the formation of inclusion complexes, which should lower the mobility of the IL. Indeed, CB7 resulted in a reduction of conductivity of the solution, as shown in Figure 8, green data points.

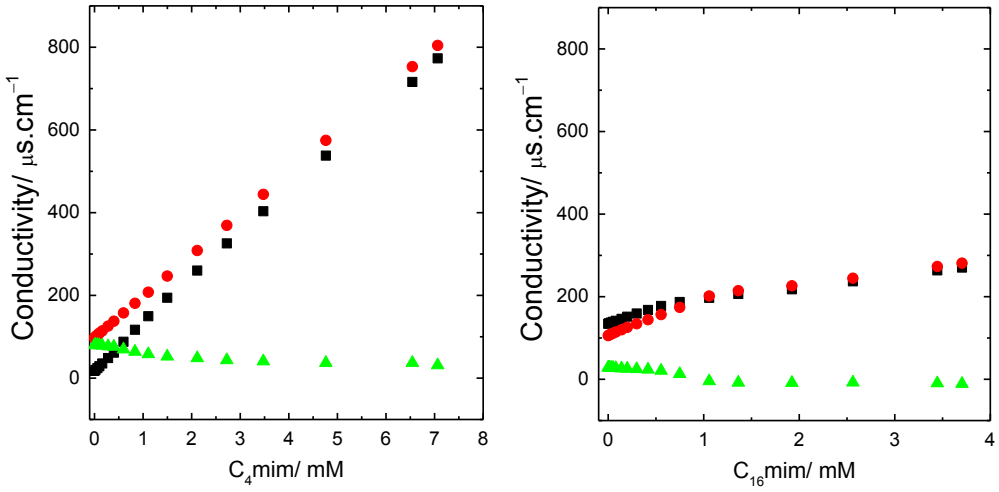

Fig. S8 Change in conductivity of an aqueous solution of [C_4mim]Cl (left) and [C_{16}mim]Cl (right) in the absence (■) and presence (●) of 1.0 mM CB7, at pH 7.0 ± 0.1 and 25.0 ± 0.2 °C. The difference is show as green triangles (▲).

6. Anti-cancer Activity

Three human breast cancer cell lines (MCF-7, T74D, MDA-231) and one human liver cancer cell line (Hep G2) were maintained in DMEM culture medium (Dulbecco's modified essential medium, Gibco), supplemented with 5% (v/v) fetal calf serum (JS Bioscience, Australia), and 1% (v/v) antibiotic (2 mM L-glutamine, 100 U/mL Penicillin and 0.1 mg/mL Streptomycin; Gibco). Cells were cultured at 37ºC in a humidified 5% CO_2 incubator. Enzymatic detachment of the confluent cell layers was carried out using Trypsin/EDTA (Gibco, USA). Trypan blue vital staining (0.4% (w/v); Sigma, USA) was used to assess cell viability with cell number counting done with a regular microscope. All cells were plated at a density of 8 × 10^3 cells per well in 96-well plates and incubated to allow attachment for 24 h. The *In vitro* evaluation of the antiproliferative activities of the examined series was accomplished by using the 3-(4,5-dimethylthiazol-2yl)-2,5-diphenyl tetrazolium bromide (MTT) colorimetric assay, as previously described.[1] In brief, compounds were diluted in culture media to yield the required concentration and applied to test wells for 48 h at 37 °C in a 5 % CO_2 incubator. Three triplicates of each concentration for all tested compounds were evaluated in three independent assays (n = 9). DMEM samples were employed as negative controls, and

Doxorubicin as a positive control. At the end of the exposure period, 20 µL of 0.5 mg/mL of MTT was added to each well and incubated for 4 h. Afterward, its reduction to formazan by metabolically active cells was calculated by measuring the absorbance at 570 nm. Cell viability was calculated based on the measured absorbance relative to the absorbance of cells exposed to the negative control, which represented 100% cell viability.

The anti-cancer activity of the investigated ILs and their corresponding complexes with CB7 were examined against three different human breast cancer cell lines, namely MCF-7, T74D, and MDA-231, as well as one human liver cancer cell line. CB*n* are known to have a sufficiently low toxicity towards normal cells to allow potential pharmaceutical applications.[2-4] In general, the toxicity of $C_n$mim in aqueous solution was found to increase with increasing chain length (Table S5), in accordance with previous reports, which also revealed that the toxicity of ILs depends mainly on their alkyl chain.[5] Interestingly, the activity of ILs against all human cancer cell lines was much higher in the presence of CB7 with small ILs, as revealed by the lower IC_{50} values (Table S5). For example, the anti-cancer activity of C_4mim was found to be enhanced in the presence of CB7 by a factor of eight. However, the activity remained unaffected for the longer ILs ($n > 6$). The complexation of ILs with short alkyl chains ($n < 8$) with CB7 allowed for the full shielding of the hydrophobic part as revealed by the NMR experiments and the simulated structures (Fig. 2 and 3), which is anticipated to improve the IC_{50} values. However, longer alkyl chains can be partially encapsulated inside the cavity of CB7 and, therefore, showed similar IC_{50} values as the free ILs. The complexation of ILs with CB7 is thought to facilitate the uptake in breast cancer cells and, most important, the ILs can be released from the cavity to eventually find their intracellular target. Similar observations were reported by Li *et al.*, namely, the anticancer activity of nitidine chloride was found to be improved upon complexation with CB7, which was attributed to a different cellular uptake behavior.[6]

Table S5. IC_{50} values (in µM) of the examined ILs in the absence and presence of CB7[a] towards human cancer cell lines. Values are expressed as mean; statistical error in data ($n = 9$) is 5%.

	MCF-7	T47D	MDA-231	Hep G2
CB7	>1000	>1000	>1000	>1000
C_4mim	1700	1600	2000	1500
CB7•C_4mim	200	250	370	230
Phmim	1020	950	2900	960
CB7•Phmim	190	160	240	190
C_6mim	780	700	2800	840
CB7•C_6mim	200	280	380	160
C_8mim	120	69	340	190
CB7•C_8mim	130	74	250	150
C_{10}mim	28	29	44	27
CB7•C_{10}mim	28	28	42	27
C_{16}mim	<1	<1	<1	<1
CB7•C_{16}mim	NA[b]	NA[b]	NA[b]	NA[b]

[a] A 1:1 molar ratio was used. [b] Not applicable due to the low IC_{50} of free C_{16}mim.

7. References

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