Electronic Supplementary Information

Effect of Na^+ and K^+ on the cucurbituril-mediated hydrolysis of a phenyl acetate

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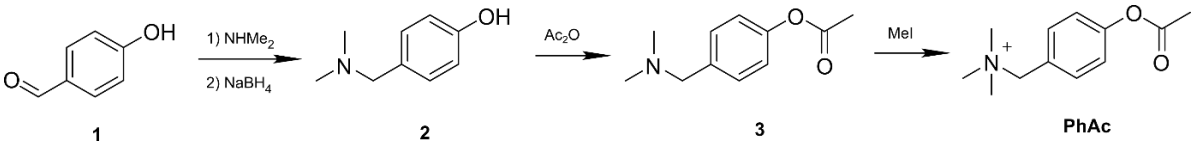
1. General information

All chemicals were purchased as reagent grade from commercial suppliers (Sigma-Aldrich, AlfaAesar) and used without further purification. The solvents used (Merck, ChemPur, PoCh) were of analytical grade quality. Deuterated solvents were purchased from Eurisotop. Experiments were performed at room temperature unless otherwise noted. The progress of organic reactions was monitored by thin layer chromatography (TLC) using Merck silica gel 60 F254 (0.2 mm) on alumina plates. The products were purified by column chromatography (CC) using Merck silica gel 60 (230-400 mesh ASTM). NMR spectra were recorded on Varian 400 MHz instruments. The chemical shifts (δ) are given in ppm relative to TMS, coupling constants are (J) in Hz. High-resolution ESI mass spectra were recorded on a SYNAPT spectrometer. The pH was measured using HI 3220 pH Meter equipped with InLab® Micro glass electrode (Mettler Toledo).

2. Substrate synthesis



Scheme 1. Substrate synthesis

*Synthesis of **2***. Compound **2** was synthesised following the procedure described previously[1]. To a solution of 1 (2.44 g, 20 mmol) in 10 ml of methanol, 33% ethanol solution of NMe_2 (7 ml, 40 mmol) was added. The mixture was stirred 10 min at rt, then solid $NaBH_4$ was added portionwise to the orange solution for 1 h. The stirring was continued for 1h at rt. About 10 ml of 2 M HCl solution was added to adjust pH to 2. Methanol was evaporated and 30 ml of 2 M HCl was added. The obtained solution was washed two times with Et_2O (2x20ml). The conc. NH_3 aq. solution was added to achieve pH of about 10. The product was collected by extraction with EtOAc (3x30ml). The organic phase was washed with brine (2x20ml) and dried over Na_2SO_4. The solvent was evaporated and the resultant white amorphous solid was dried under reduced pressure to give 2.3 mg of product. The product was utilised in the next step without further purification. Yield 95%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.24 (s, 1H), 7.04 (d, J = 8.4 Hz, 2H), 6.69 (d, J = 8.4 Hz, 2H), 3.23 (s, 2H), 2.08 (s, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 156.68, 130.33, 129.44, 128.47, 115.26, 115.22, 63.43, 45.24.

*Synthesis of **3***. Compound **2** (755 mg, 5 mmol) was dissolved in 16 ml of DCM and trimethylamine (3 ml, 22 mmol) was added. Acetic anhydride (1 ml, 11 mmol) was added to the mixture under stirring. The reaction mixture was stirred for 20 h at rt. The solution was washed with saturated $NaHCO_3$ solution (30 ml) and water (30 ml). After drying under Na_2SO_4, the solvent was evaporated to give a yellow liquid. The crude product was utilised in the next step.

*Synthesis of **PhAc***. Compound **3** was dissolved in 5 ml of dry ACN. The solution was cooled by ice to 5 ºC, and MeI (400 µL, 6,5 mmol) was added dropwise. The reaction mixture was stirred for 30 min at rt. The addition of a few drops of acetone caused the precipitation of the product. The residue was filtrated and washed with Et_2O (2x10 ml) to give 1.36 g of a white powder (yield 82%). ^{1}H NMR (400 MHz, DMSO-d6) δ 7.60 (d, J = 8.4 Hz, 2H), 7.29 (d, J = 8.4 Hz, 2H), 4.57 (s, 2H), 3.04 (s, 9H), 2.29 (s, 3H). ^{13}C NMR (101 MHz, dmso) δ 169.40, 152.35, 134.56, 126.28, 122.87, 67.38, 52.20, 21.37. HRMS (ESI) m/z: 208.1338 calc for: $C_{12}H_{18}NO_2$ $[M]^+$; found: 208.1342.

3. Complexation of PhAc with CB7

The complexation of **PhAc** with **CB7** was studied by NMR titration of **PhAc** with **PhAc@CB7** (4:1). The **PhAc** substrate (4.53 mg) was dissolved in D_2O (1.69 ml). A portion of this solution (787 µL) was used to dissolve 2.36 mg of **CB7**. Then 500 µL of a **PhAc@CB7** mixture was transferred into a NMR tube and titrated with 8 mM solution of the substrate. For NMR spectra, see Fig. 1. The titration data points were fitted using BindFit software (http://supramolecular.org). to give the binding constant $K = (1.5 \pm 0.42) \cdot 10^5$ (Figure 2).

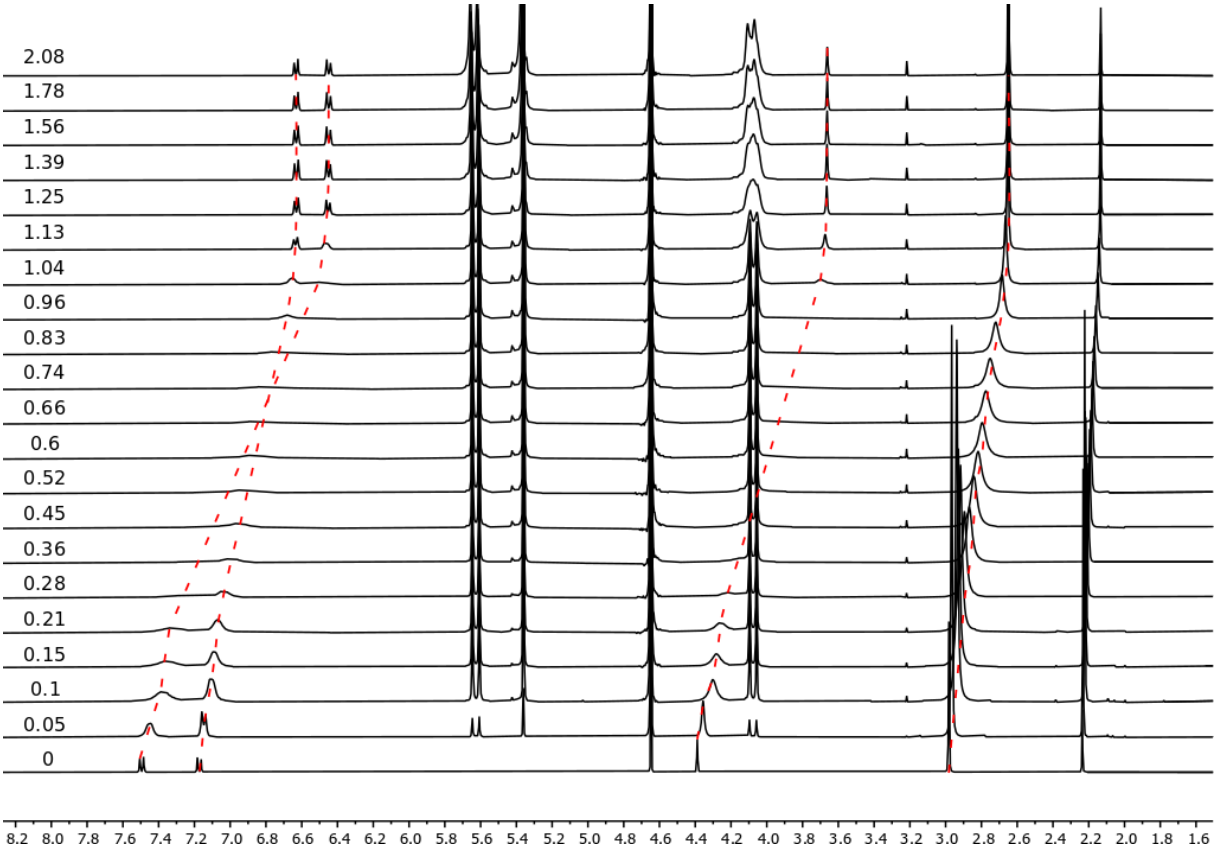


Figure S1. ^{1}H NMR spectra of **PhAc** with increasing amounts of **CB7** (D_2O, 298 K).

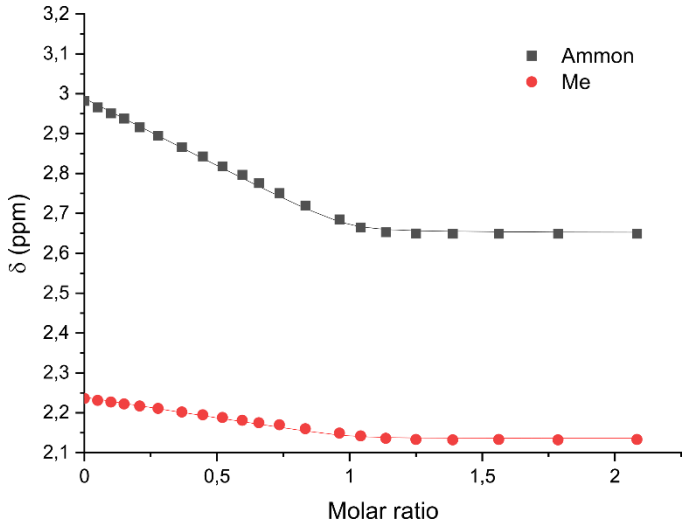


Figure S2. Non-linear fits of the data obtained from the titration of **PhAc** with **CB7**. The shifts of ammonium (Ammon) and acetyl (Me) proton signals were used for fitting.

Protons were assigned by analysis of NOESY, ROESY and HMBC spectra (Fig S3-S5).

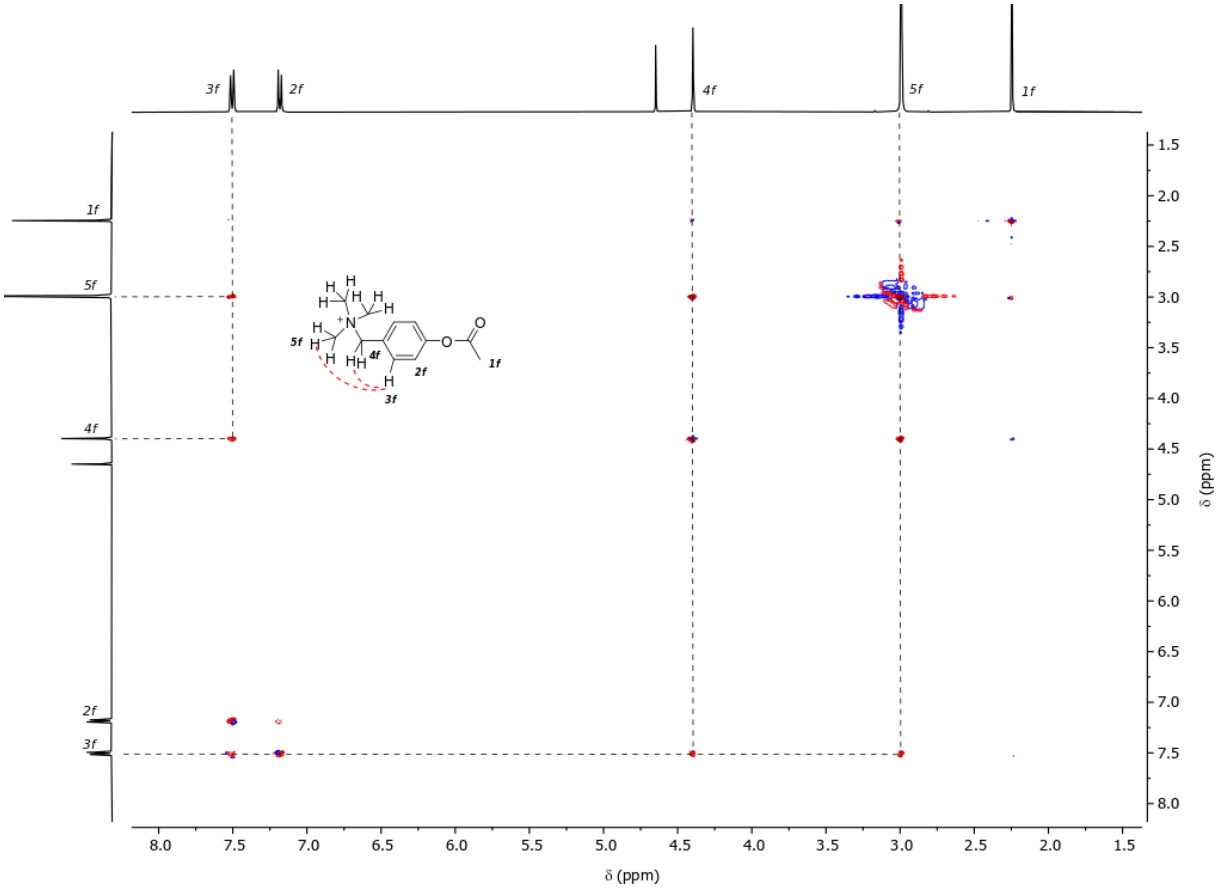


Figure S3. NOESY spectrum of **PhAc** in D_2O (298 K).

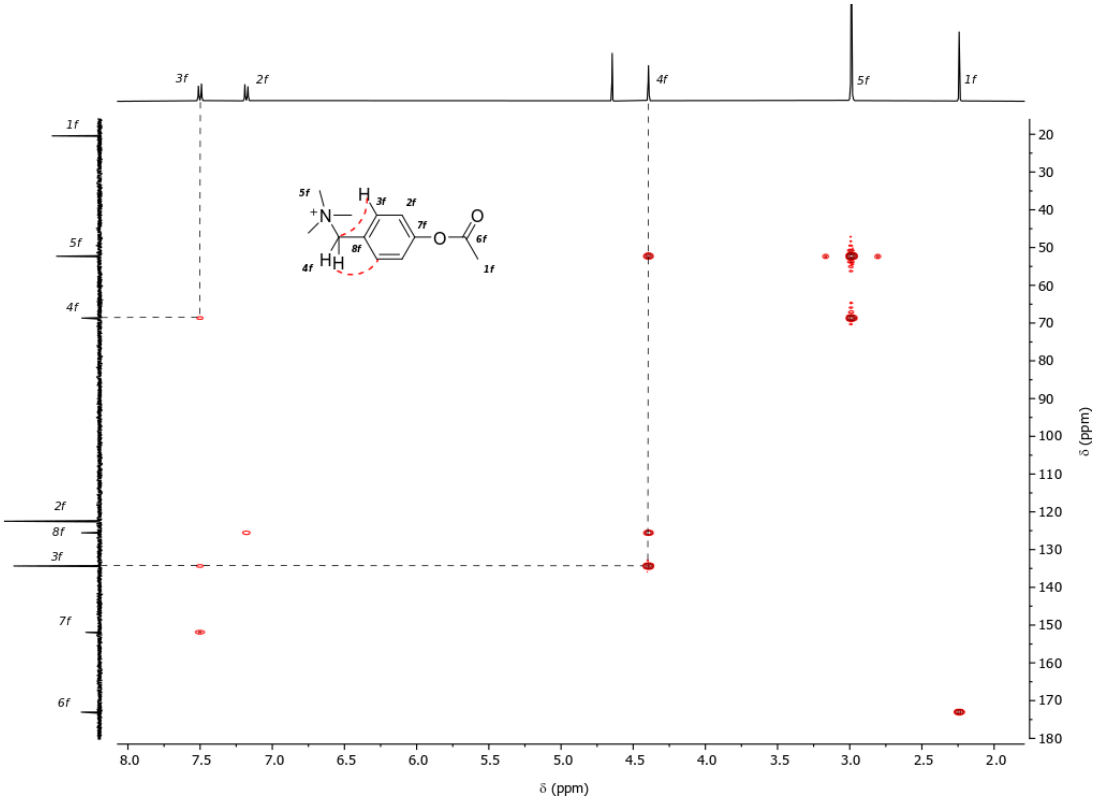


Figure S4. HMBC spectrum of **PhAc** in D_2O (298 K).

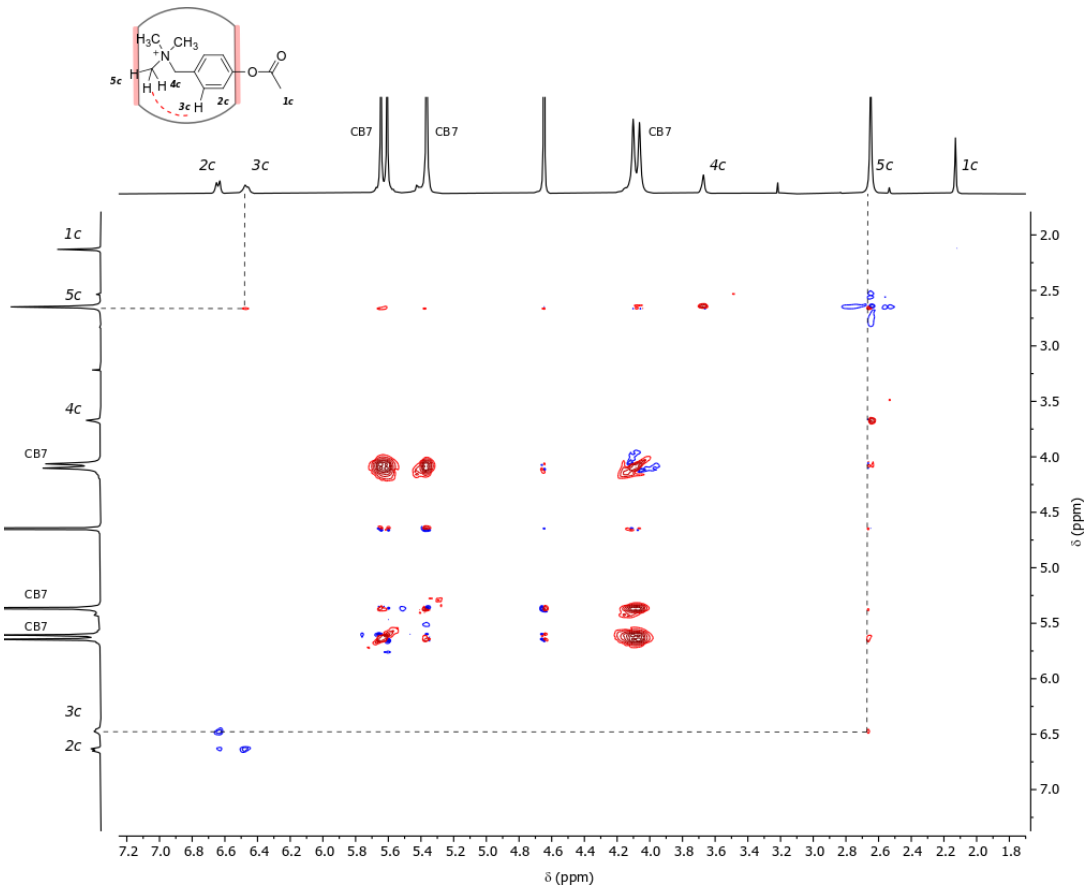


Figure S5. ROESY spectrum of **PhAc@CB7** complex in D_2O (298 K). Diagonal peaks were suppressed.

4. Salt-free condition

The hydrolysis of **PhAc** was observed using NMR spectroscopy in solutions with pD from 0.75 to 2.5. The DCl solution was prepared by dilution of 0.2 M DCl with D_2O. To 300 µL of the acid solution was added 200 µL of D_2O or 4.95 mM solution of **CB7** (19.86 mg of **CB7** dissolved in 2.675 ml of D_2O). The reaction was started by addition 100 µL of 9 mM solution of **PhAc** (6.74 mg of **PhAc** was dissolved in 2.236 mL of D_2O).

In all cases, the hydrolysis rate constants were determined from the slopes of $\ln\frac{1}{1-Y}$ against *t*. The conversions *Y* at time *t* were calculated using integrals of ammonium group signals in the ^{1}H NMR spectra.

We found that rate constant linearly depends on the concentration of deuterium ions and ionic strength of the solution following the equation 1:

$$k_f = k_f^0 \cdot [D^+] \cdot 10^{-2\cdot\left(-\frac{0.509\cdot\sqrt{I}}{1+\sqrt{I}}+0.14\cdot I\right)} \quad (1)$$

where the coefficient *c=0.14* (responsible for non-electrostatic ion-ion and ion-solvent interactions) was found numerically by minimisation of the deviation ($\overline{x}$) of each rate constant (k_{fi}^0) from the average independent rate constant $\bar{k}_f^0$ following the equations 2-4.

$$I_i = \frac{1}{2} \cdot ([D^+]_i + [Cl^-]_i + [AcAm^+] + [I^+]) \quad (2)$$

$$\gamma_i^{-2} = 10^{-2 \cdot \left(-\frac{0.509 \cdot \sqrt{I_i}}{1+\sqrt{I_i}} + c \cdot I_i \right)} \quad (3)$$

$$k_{fi}^0 = \frac{k_{fi}}{[D^+]_i \cdot \gamma_i^{-2}} \quad (4)$$

The relative average deviation from the mean of the data set $\overline{x}$ is equal to 1.14 %.

The rate constants of the hydrolysis of the substrate by **CB7** depend on the acid concentration. However, these constants are insensitive to the ionic strength of the solution. The **CB7**-promoted hydrolysis could be satisfactorily described by equation 7. The deviation from the mean $\overline{x}$ is equal to 4.7 %:

$$k_b = k_b^0 \cdot [D^+] \quad (5)$$

The acceleration factor was calculated as rate constants of the encapsulated and free substrate (equation 8). The average ion-strength-independent accelerator factor α_{CB7}^0 of **CB7** is equal to 263±12.

$$\alpha_{CB7} = \frac{k_b}{k_f} \quad (6)$$

Table S1. Hydrolysis rate constants for free and bound **PhAc**.

pD	$k_f \cdot 10^6$, $M^{-1} \cdot c^{-1}$	$k_{f/\gamma} \cdot 10^6$, $M^{-1} \cdot c^{-1}$	$k_b \cdot 10^6$, $M^{-1} \cdot c^{-1}$	α_{CB7}	α_{CB7}^0
0.75	10.458	5.821	1490.00	142	256
1.00	5.556	3.359	927.67	167	276
1.25	2.781	1.831	514.82	185	281
1.5	1.474	1.049	264.52	179	252
1.75	0.748	0.569	138.52	185	244
2.5	0.404	0.324	86.89	215	268
2.25	0.221	0.185	46.52	210	251
2.5	0.119	0.103	28.19	237	274

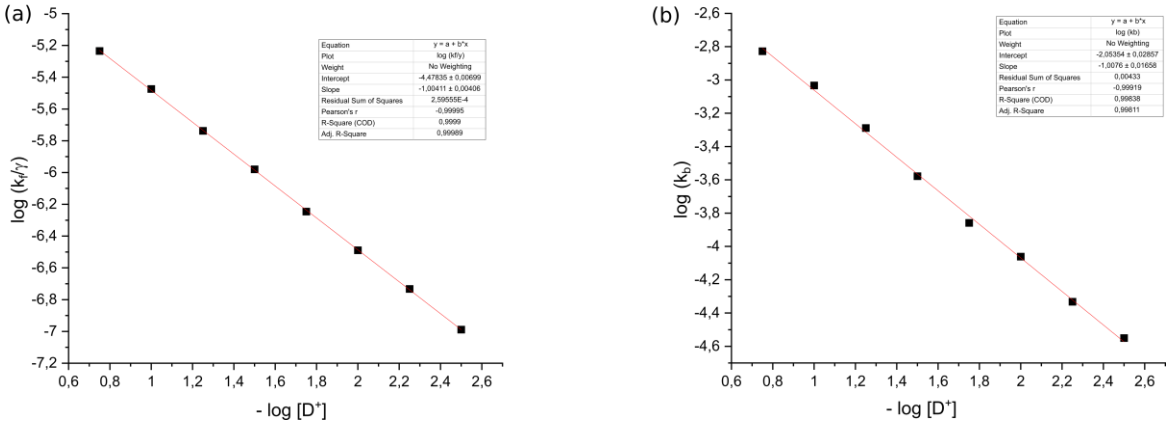


Figure S6. pD-profile of **PhAc** (a) and **PhAc@CB7** (b) hydrolysis in the pD range of 0.75 to 2.5.

5. The variation of salts

The rate constants of the hydrolysis of **PhAc** and **PhAc@CB7** complex were determined at pD=1.55. The acidic buffer was prepared by mixing 889 μL of 0.2M DCl and 4.111 μL of D_2O.

Hydrolysis in the presence of different salts

The NMR tube was filled with 150 μL of buffer, 150 μL of the appropriate salt solution (C=40mM) and 200 μL of D_2O or 4.95 mM **CB7** solution. The reaction was started by the addition of 100 μL of 9 mM solutions of substrate **PhAc**.

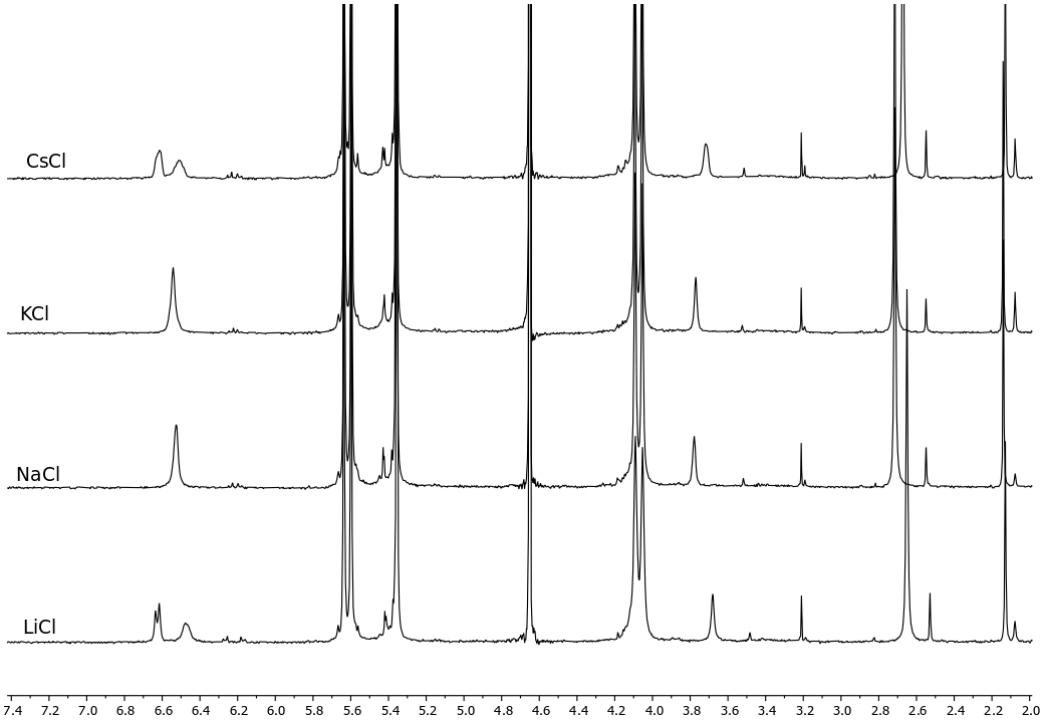


Figure S7. ^{1}H NMR spectra of **PhAc@CB7** in the presence of different chloride salts.

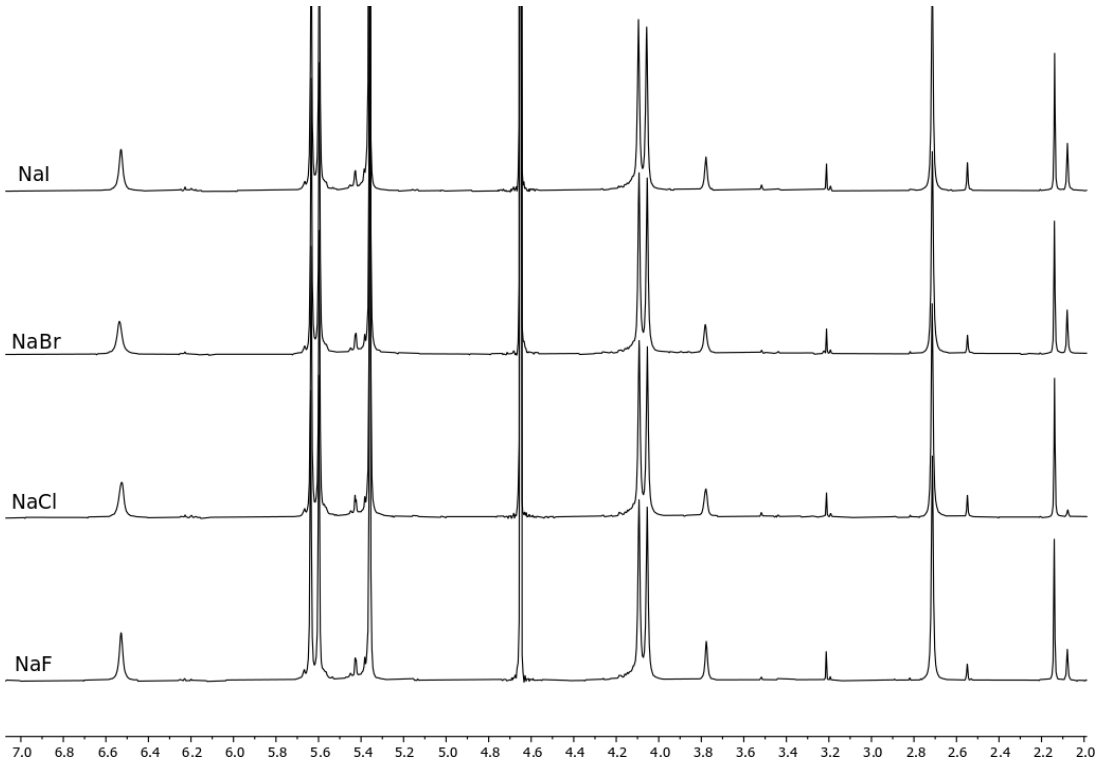


Figure S8. ^{1}H NMR spectra of **PhAc@CB7** in the presence of different sodium salts.

6. Sodium ion effect

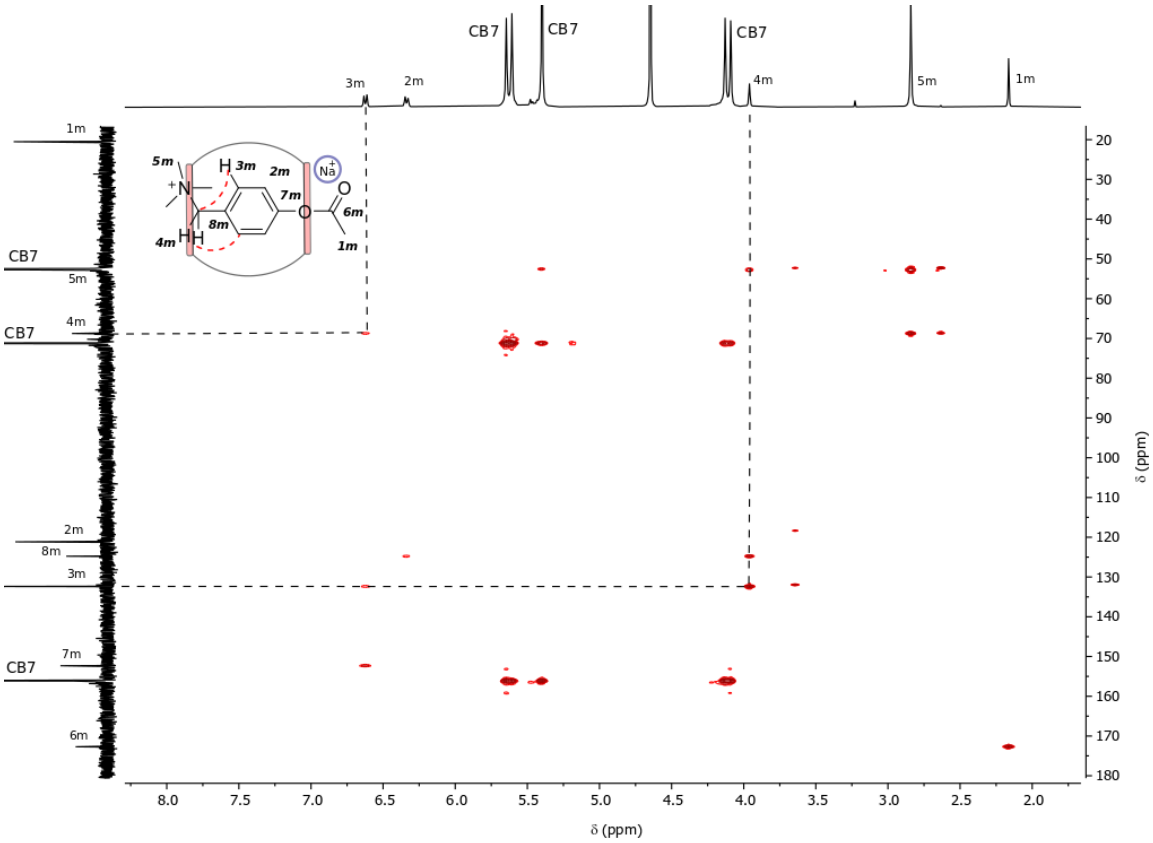


Figure S9. HMBC spectrum of **PhAc@CB7** complex with NaCl in D_2O (298 K). The spectrum shows the correlation between 1H-signal of *3m* and ^{13}C-signal of *4m*. ^{1}H-signals at *2.6* and *3.6* ppm correspond to the product of hydrolysis (**PhOH@CB7**) formed during the measurement.

*Hydrolysis of **PhAc@CB7***

The NMR tube was filled with 150 µL of buffer, 150 µL of NaCl solution and 200 µL of D_2O or 4.95 mM **CB7** solution. The reaction was started by the addition of 100 µL of 9 mM solutions of substrate **PhAc**.

The hydrolysis rate constants of the free substrate were determined in the same way as in salt-free conditions (Table S2). The rate equation (10) was determined by the minimisation of relative average deviation of rate constant from the mean $\overline{x}$ following to the equations 9 and 3-6.

$$I_i = \frac{1}{2} \cdot \left([Na^+]_i + [Cl^-]_i + [D^+] + [AcAm^+] + [I^+] \right) \quad (7)$$

$$k_{f,Na} = k_f^0 \cdot [D^+] \cdot 10^{-2 \cdot \left(-\frac{0.509 \cdot \sqrt{I}}{1+\sqrt{I}} + 0.19 \cdot I \right)} \quad (8)$$

Table S2. Hydrolysis rate constants of **PhAc** and **PhAc@CB7** at different concentration of NaCl.

C(NaCl), mM	$k_f \cdot 10^6$, $M^{-1} \cdot c^{-1}$	$k_{f/\gamma} \cdot 10^6$, $M^{-1} \cdot c^{-1}$	$k_{b,Na} \cdot 10^6$, $M^{-1} \cdot c^{-1}$	$\alpha_{CB7,Na}$
0	1.30	0.97	269.4	207
5	1.32	0.96	204.7	156
10	1.35	0.97	176.4	131
20	1.39	0.97	136.3	98
50	1.46	0.96	75.3	54
100	1.59	0.97	45.4	29
150	1.62	0.95	32.8	21

*Model of competitive inhibition of **CB7***

We assumed that sodium competes with the substrate for the cucurbit[7]uril portal. In this model, the product could be formed in two ways: from the free substrate (**S**) and the encapsulated substrate (**S@CB7**). However, the attachment of sodium cation to **CB7** portal prevents the substrate complexation. As a result, the addition of sodium ions increases the fraction of free substrate.

$$S \xrightarrow{k_f} P$$

$$S + CB7 = S@CB7 \xrightarrow{k_{b,Na}} P$$

$$Na^+ + CB7 = CB7 \cdot Na^+$$

where **S** = **PhAc**.

The binding constant of sodium (K = 130±10 M^{-1}) was determined previously by C.Bohne[2].

$$K_S = \frac{[S@CB7]}{[CB7] \cdot [S]} = 1.5 \cdot 10^5 M^{-1} \quad (9)$$

$$K_{Na} = \frac{[CB7 \cdot Na]}{[CB7] \cdot [Na]} = 130\ M^{-1} \quad (10)$$

$$C_S = [S] + [S@CB7] \quad (11)$$

$$C_{Na} = [Na] + [CB7 \cdot Na] \quad (12)$$

$$[S@CB7] = \frac{K_s \cdot [CB7] \cdot C_S}{1 - K_S \cdot [CB7]} \quad (13)$$

$$[S \cdot Na] = \frac{K_{Na} \cdot [CB7] \cdot C_{Na}}{1 - K_{Na} \cdot [CB7]} \quad (14)$$

$$C_{CB7} = [CB7] + [S@CB7] + [CB7 \cdot Na] \quad (15)$$

$$C_{CB7} = [CB7] + \frac{K_s \cdot [CB7] \cdot C_S}{1 - K_S \cdot [CB7]} + \frac{K_{Na} \cdot [CB7] \cdot C_{Na}}{1 - K_{Na} \cdot [CB7]} \quad (16)$$

$$K_S \cdot K_{Na} \cdot [CB7]^3 + \quad (17)$$

$$(K_S + K_{Na} + K_S \cdot K_{Na} \cdot C_S + K_S \cdot K_{Na} \cdot C_{Na} - K_S \cdot K_{Na} \cdot C_{CB7}) \cdot [CB7]^2 + (K_S \cdot C_S - K_S \cdot C_{CB7} + K_{Na} \cdot C_{Na} - K_{Na} \cdot C_{CB7}) \cdot [CB7] - C_{CB7} = 0$$

The solution of the cubic equation 17 gives the concentration of free macrocycle [CB7]. The equilibrium concentration of encapsulated substrate [S] was found from eq. 13. The rate constant at different concentrations of sodium chloride was calculated from eq. 18.

$$k_{Na}^{calc} = \frac{[S]}{C_S} \cdot k_f + \frac{[S@CB7]}{C_S} \cdot k_{b,Na} \quad (18)$$

where k_f and $k_{b,Na}$ are hydrolysis rates of the free and bound substrate in the absence of sodium ions. The plot of k_{Na}^{calc} against C_{Na} is given in the main text.

Model of uncompetitive inhibition of ***CB7***

In the model of uncompetitive inhibition, the sodium ion attaches to the free **CB7** portal, on the opposite side from the ammonium group of the substrate. The localisation of the sodium ion near the reaction centre inhibits the reaction.

$$B^+ \xrightarrow{k_b^0} P$$

$$B^+ + Na^+ = B^+ \cdot Na^+ \xrightarrow{k_{b,Na}^0} no\ reaction$$

where $B^+ = \boldsymbol{AcPh@CB7}$

From the kinetic data, the dissociation constant of the ternary complex K_{Na}^0 could be found by numerical minimisation of the relative average deviation of calculated rate constant from experiment following the equations 19-25.

$$K_{i,Na} = \frac{a_i(B^+) \cdot a_i(Na^+)}{a_i(BNa^{2+})} = \frac{C(B^+) \cdot C_i(Na^+)}{C_i(BNa^{2+})} \cdot \frac{\gamma_{i,B} \cdot \gamma_{i,Na}}{\gamma_{i,BNa}} = K_{Na}^0 \frac{\gamma_{i,B} \cdot \gamma_{i,Na}}{\gamma_{i,BNa}} \quad (19)$$

$$\gamma_{i,B} = \gamma_{i,Na} = 10^{\frac{-0.509 \cdot \sqrt{I_i}}{1+\sqrt{I_i}} + 0.19 \cdot I_i} \quad (20)$$

$$\gamma_{i,BNa} = 10^{\frac{-0.509 \cdot 4 \cdot \sqrt{I_i}}{1+\sqrt{I_i}} + 0.19 \cdot I_i} \quad (21)$$

$$C_i(BNa^{2+}) = \frac{\left(K_{i,Na} + C(B^+) + C_i(Na^+)\right) - \sqrt{(K_{i,Na} + C(B^+) + C_i(Na^+))^2 - 4 \cdot C(B^+) \cdot C_i(Na^+)}}{2} \quad (22)$$

$$X_i(BNa^{2+}) = \frac{C_i(BNa^+)}{C(B^+) + C_i(BNa^{2+})} \quad (23)$$

$$X_i(B^+) = 1 - X_i(BNa^{2+}) \quad (24)$$

$$k_{bi,Na}^{calc} = X_i(B^+) \cdot k_b^0 + X_i(BNa^{2+}) \cdot k_{b,Na}^0 = X_i(B^+) \cdot k_b^0 \quad (25)$$

where $C(B^+) = 0{,}0015 \cdot 0{,}96 = 0{,}00144\ M$ is constant for all experiments.

We did not find a reliable dissociation constant when ionic strength was involved in the calculation. However, the assumption that the effect of the ionic strength on the dissociation constant is negligible ($I_i = 0$) gives the binding constant $K^0_{b,Na} = {}^1/_{K^0_{Na}} = 45.5\ M^{-1}$ with a relative average deviation $\bar{x} = 2.0$ % (Table 1). The plot of $k^{calc}_{b,Na}$ against C_{Na} is given in the main text (figure 3a).

*NMR spectra of sodium binding to **PhAc@CB7***

The binding constant of sodium to **PhAc@CB7** complex was also found from the ^{1}H NMR spectra. The chemical shifts were taken from the ^{1}H NMR spectra at the start of the reaction (Fig. S10). The data were fitted using the BindFit program.

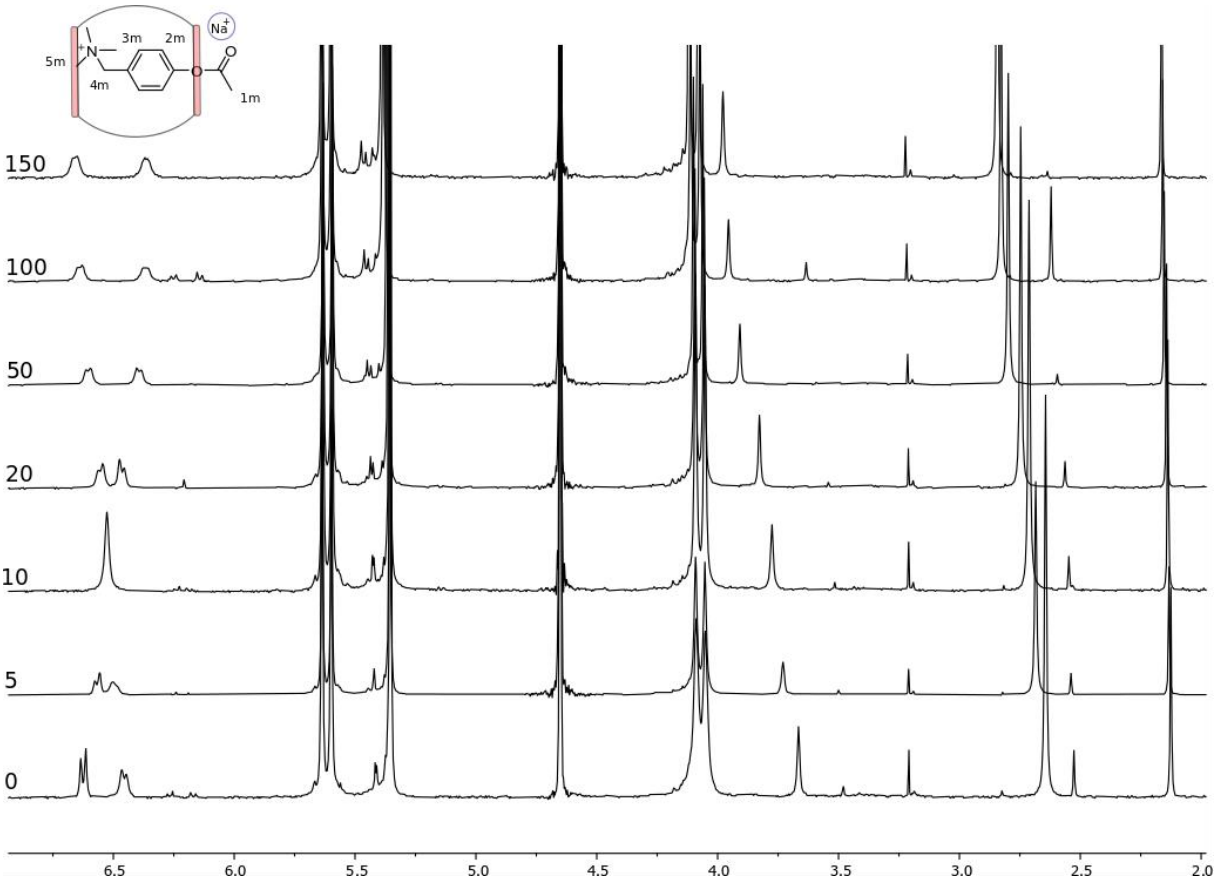


Figure S10. ^{1}H NMR spectra of **PhAc@CB7** complex with increasing amounts of NaCl (D_2O, 298 K).

The application of activities of **PhAc@CB7** and sodium ion, calculated from eq. 26-27, gives binding constant $K^0_{b,Na} = 54.2$ with deviation 4.7%. However, in the ionic-strength-independent model $K^0_{b,Na} = 46.1$ with deviation 4.8%.

$$a(B^+) = C(B^+) \cdot \gamma_B \quad (26)$$

$$a(Na^+) = C(Na^+) \cdot \gamma_{Na} \quad (27)$$

where γ_B and γ_{Na} were found from eq (22).

*Sodium complex with hydrolysis product (**PhOH@CB7**)*

The binding constant of the product complex **PhOH@CB7** with sodium ion was found from ^{1}H NMR spectra when the hydrolysis was completed (Fig. S11). The data were fitted using the BindFit program. The ionic-strength-independent binding constant of sodium to the product complex **PhOH@CB7** is $K^0_{p,Na} = 19.4$ (error is 3.6%)

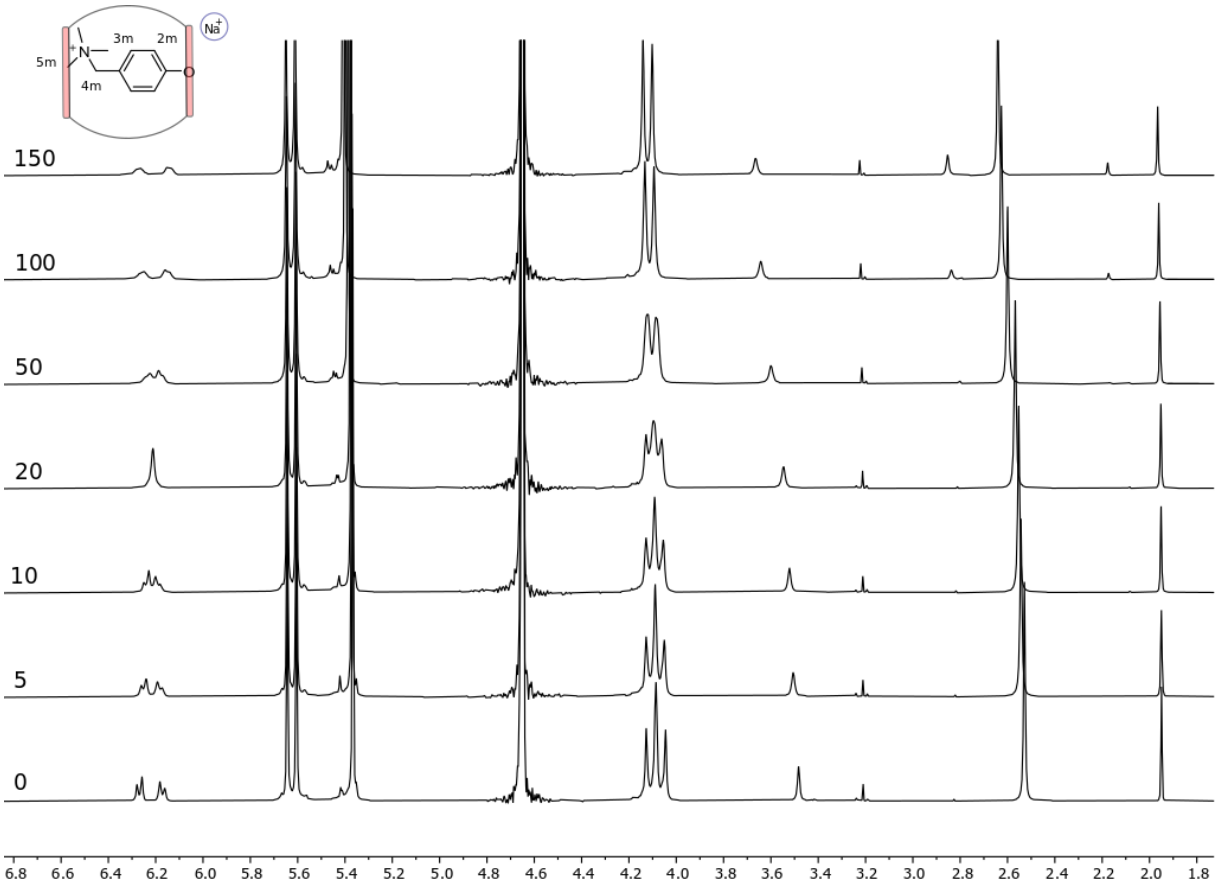


Figure S11. ^{1}H NMR spectra of **PhOH@CB7** complex with increasing amounts of NaCl (D_2O, 298 K).

7. Effects of other alkali metal cations

*Hydrolysis of **PhAc@CB7***

The NMR tube was filled with 150 μL of buffer, 150 μL of salts solution at different concentration and 200 μL 4.95 mM **CB7** solution. The reaction was started by the addition of 100 μL of 9 mM solutions of substrate **PhAc**.

The rate constants of the hydrolysis of the free substrate (k_f) at different pD were found from the slopes of $\ln\frac{1}{1-Y}$ against *t*. The conversions *Y* at time *t* were calculated using the integrals of the ammonium group signals in the ^{1}H NMR spectra.

Table S3. The hydrolysis rate constants of **PhAc@CB7** with different metal ions.

C(MCl), mM	$k_{b,Li}\cdot10^6$, $M^{-1}\cdot c^{-1}$	$k_{b,K}\cdot10^6$, $M^{-1}\cdot c^{-1}$	$k_{b,Cs}\cdot10^6$, $M^{-1}\cdot c^{-1}$
0	248.8	234.2	236.4
5	-	193.4	-
10	-	166.4	-
20	225.0	135.3	156.4
50	204.6	79.3	108.5
100	191.5	42.9	84.8
150	235.1	32.1	205.1

Based on the kinetics data (Table S3), we found the binding constant of different metal ions to the **PhAc@CB7** complex using numerical minimisation of deviation from eq 21-28. In all cases, the concentrations of species were used. The use of the activities instead of concentrations led to significant deviations.

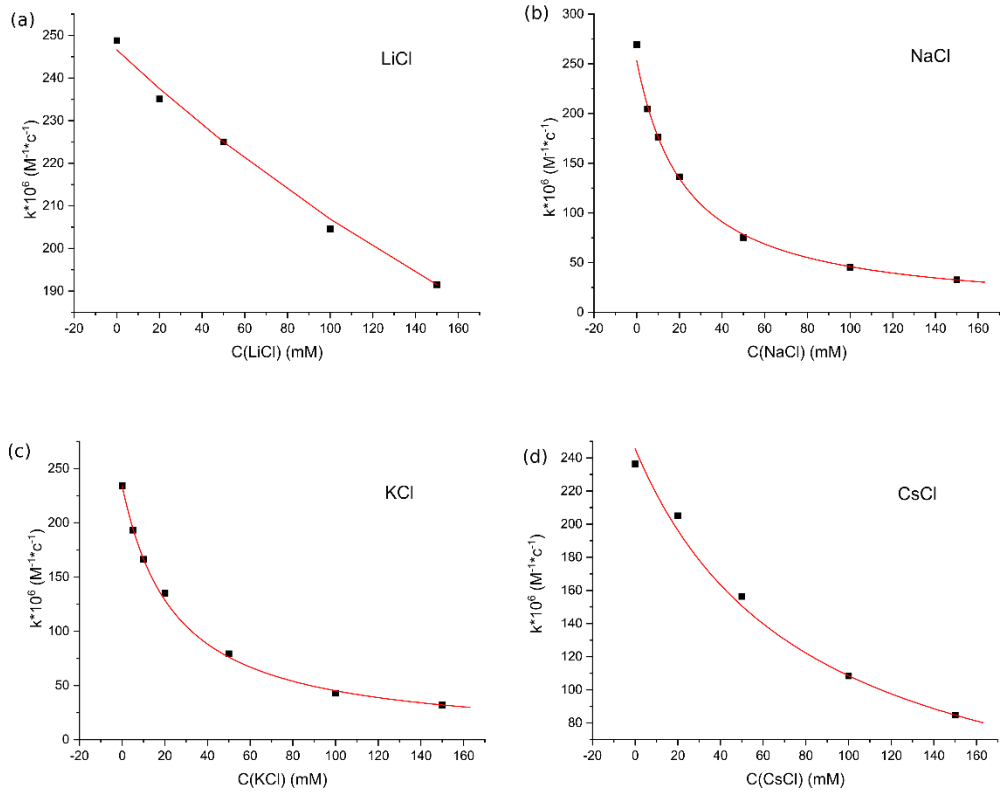


Figure S12. The dependence of the hydrolysis rate constants on salt concentrations. The black dots denote the experimental data. The red line is a fit to the uncompetitive inhibition model.

*Binding constants of ions with **PhAc@CB7** and **PhOH@CB7** from NMR spectra*

We found the binding constants of metal ions with **PhAc@CB7** from the ^{1}H NMR spectra at the start of the reactions (Fig. S13, S15 and S17). The data points were fitted using BindFit software. The chemical shifts at the end of the reactions (Fig. S14, S16 and S18) were used to find the binding constants of metal ions with the product complex **PhOH@CB7**. All binding constants are summarised in table 1.

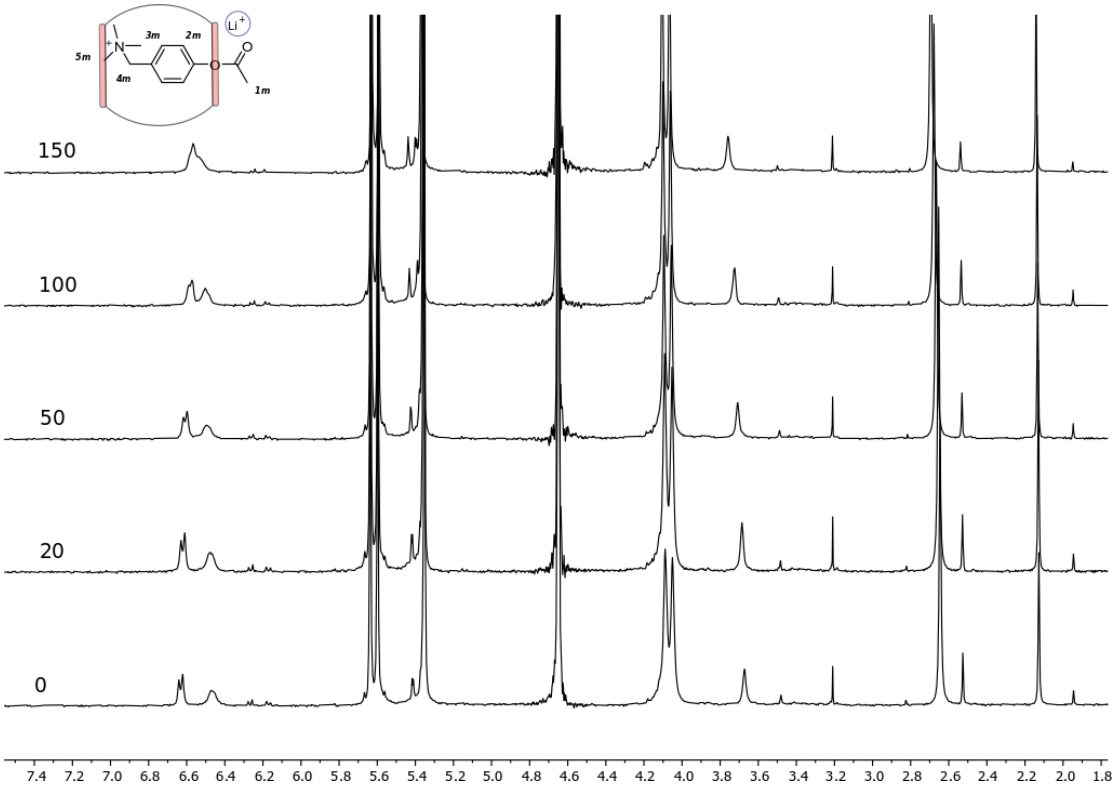


Figure S13. ^{1}H NMR spectra of **PhAc@CB7** complex with increasing amounts of LiCl (D_2O, 298 K).

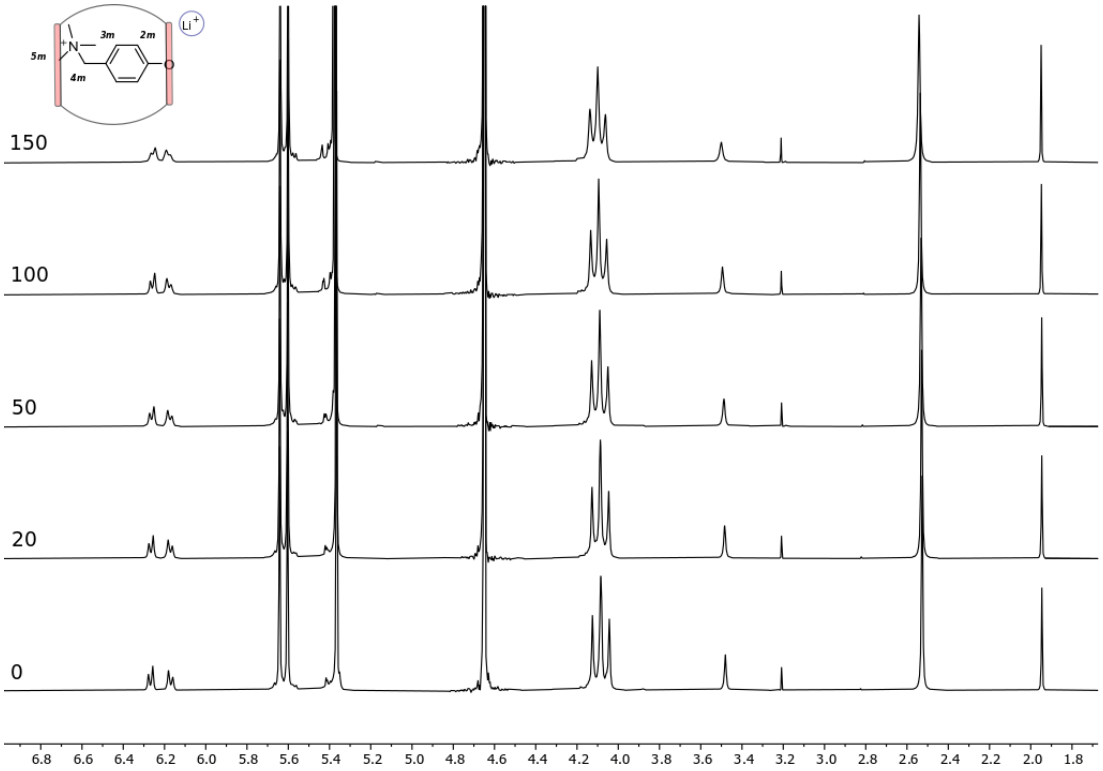


Figure S14. ^{1}H NMR spectra of **PhOH@CB7** complex with increasing amounts of LiCl (D_2O, 298 K).

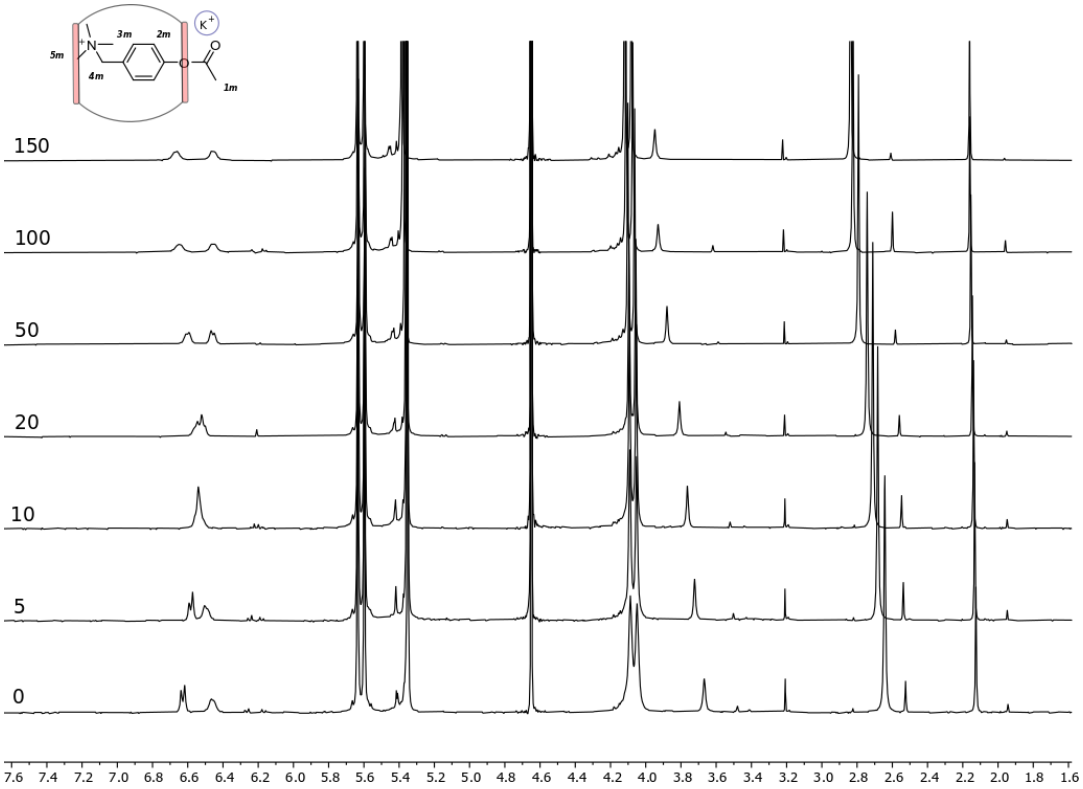


Figure S15. ^{1}H NMR spectra of **PhAc@CB7** complex with increasing amounts of KCl (D_2O, 298 K).

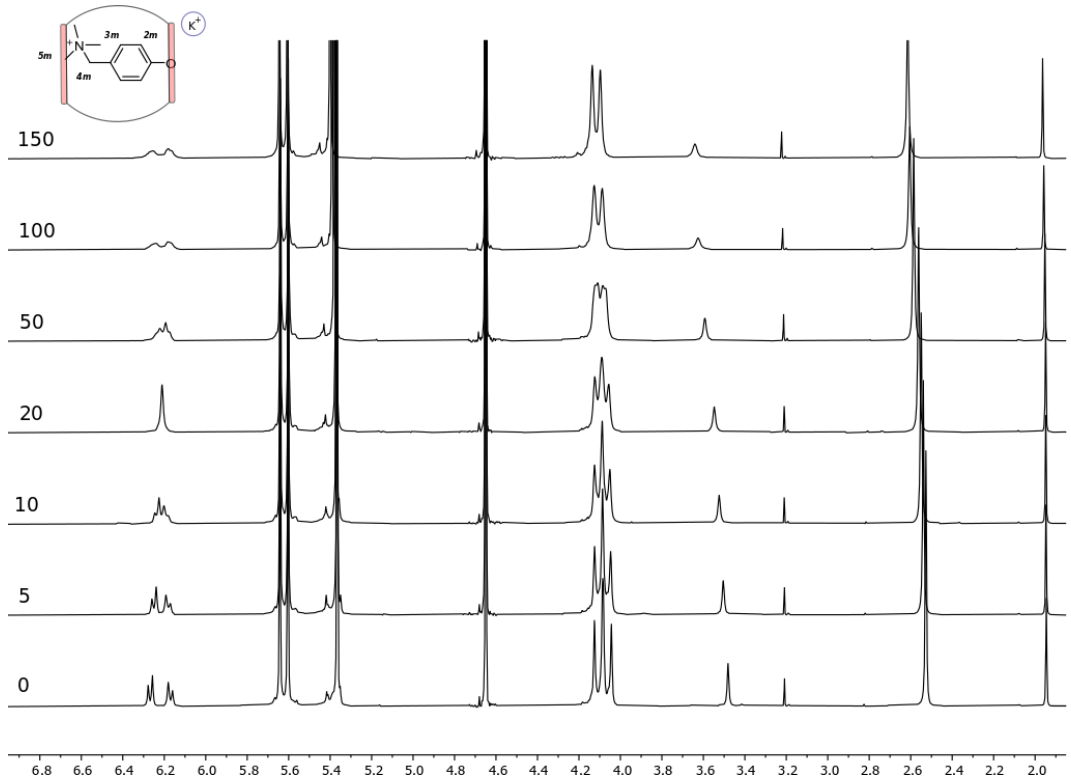


Figure S16. ^{1}H NMR spectra of **PhOH@CB7** complex with increasing amounts of KCl (D_2O, 298 K).

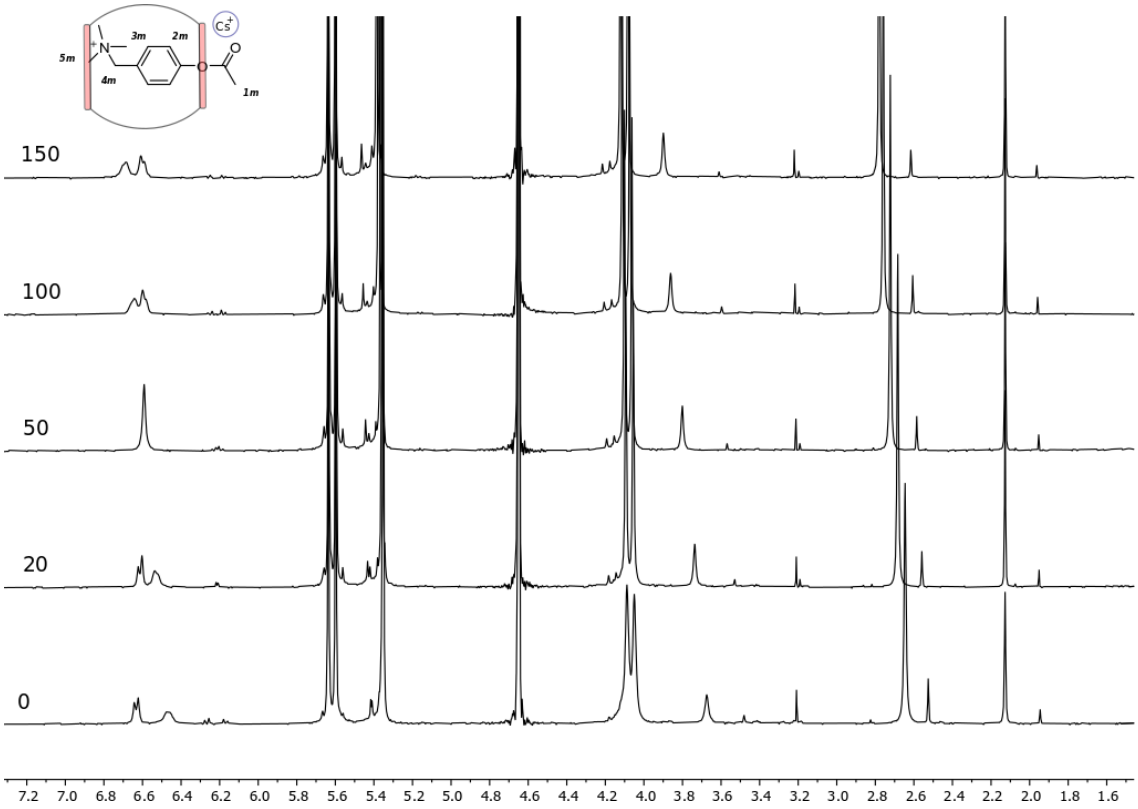


Figure S17. ^{1}H NMR spectra of **PhAc@CB7** complex with increasing amounts of CsCl (D_2O, 298 K).

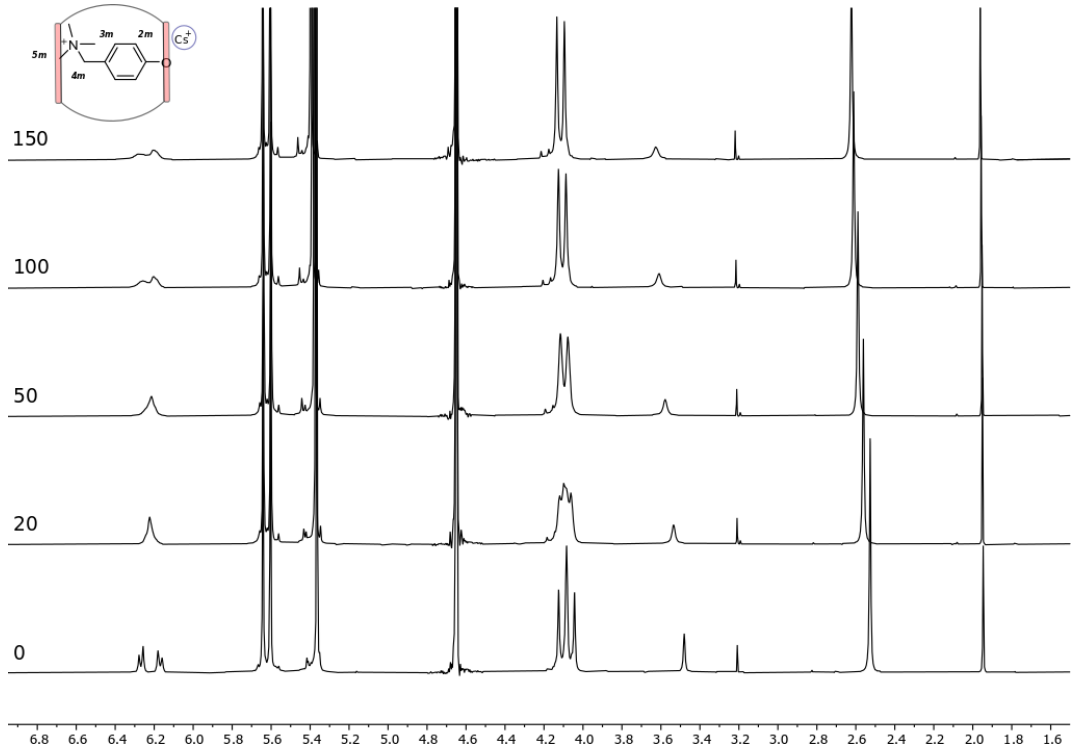


Figure S18. ^{1}H NMR spectra of **PhOH@CB7** complex with increasing amounts of CsCl (D_2O, 298 K).

8. Additional spectra

HR ESI spectra

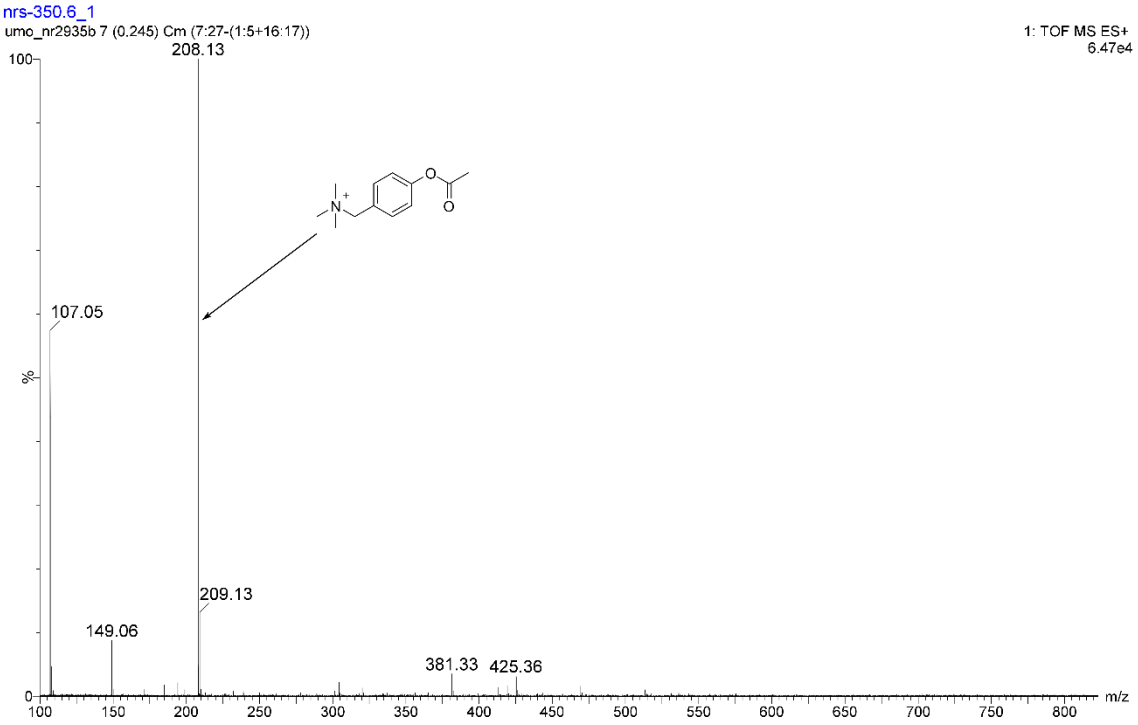


Figure S19. HR ESI spectra of the substrate **PhAc**.

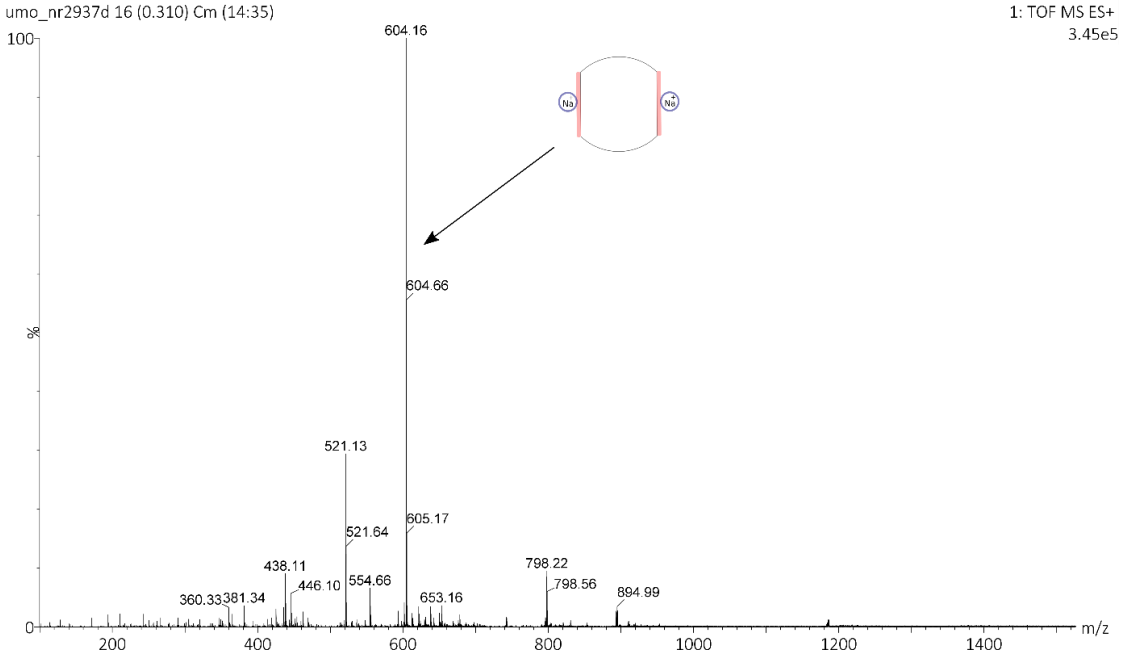


Figure S20. HR ESI spectra of the $\boldsymbol{CB7 \cdot 2Na^{+}}$complex.

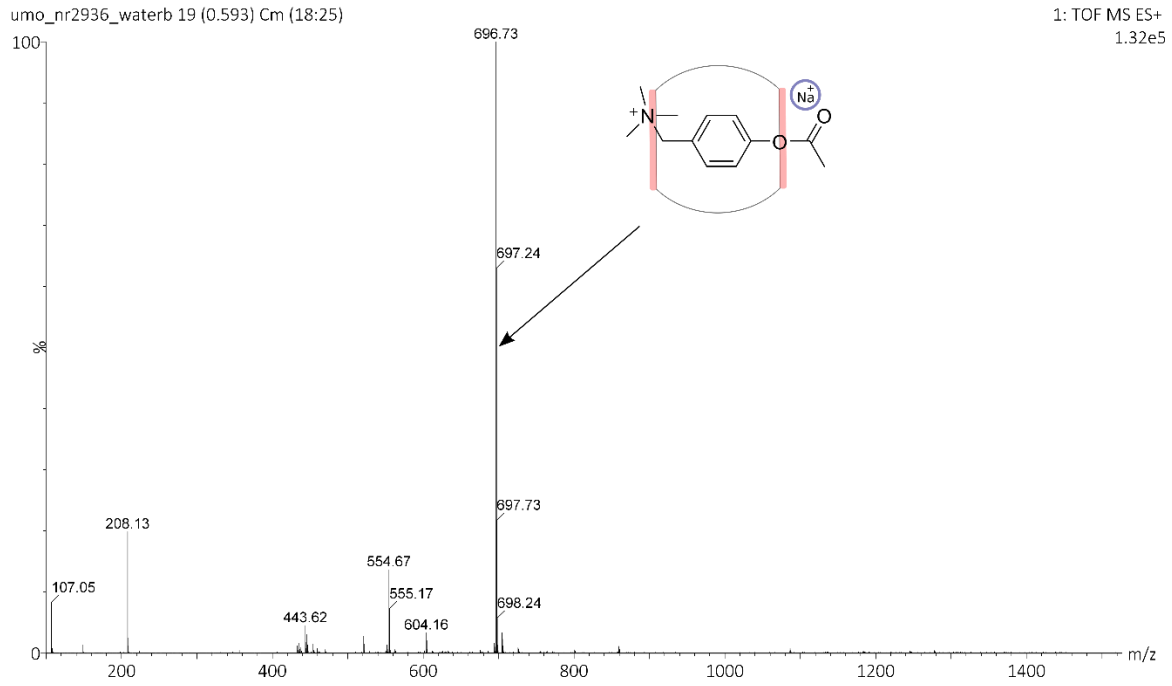


Figure S21. HR ESI spectra of the $\boldsymbol{PhAc@CB7 \cdot Na^{+}}$complex.

NMR spectra

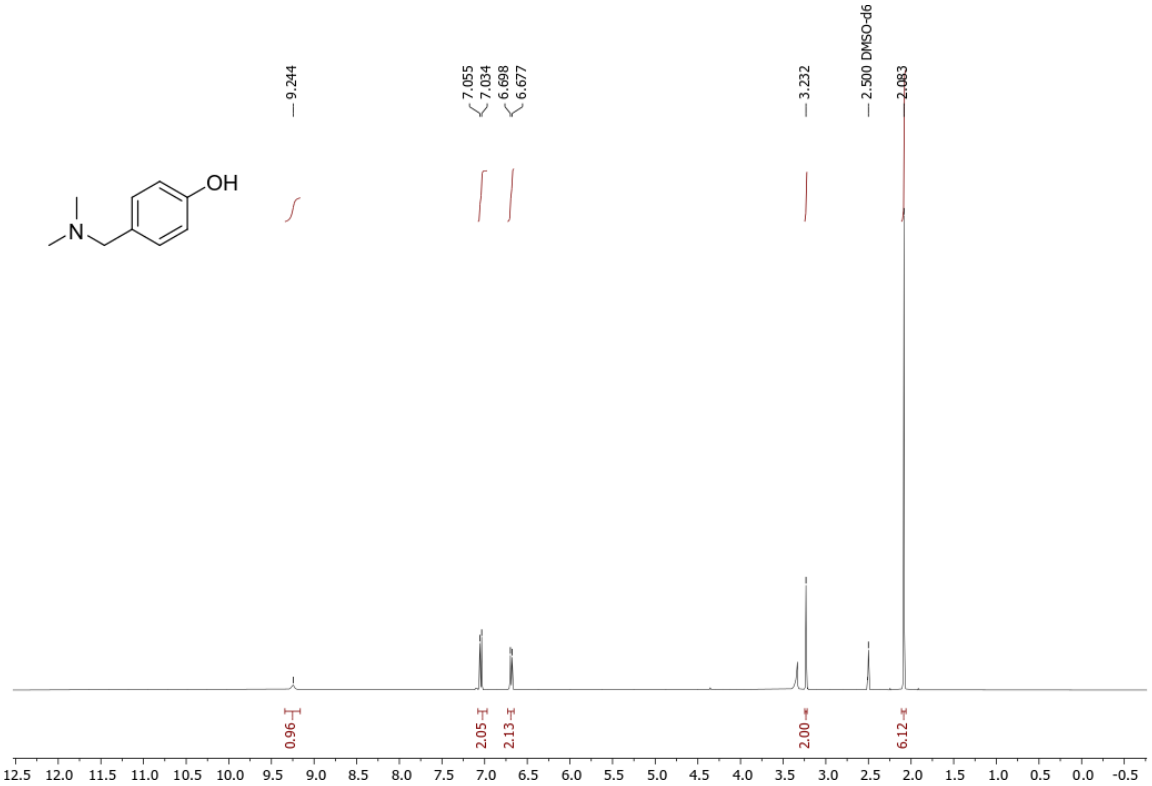


Figure S22. ^{1}H NMR spectrum of **2** in DMSO-*d6*(298 K).

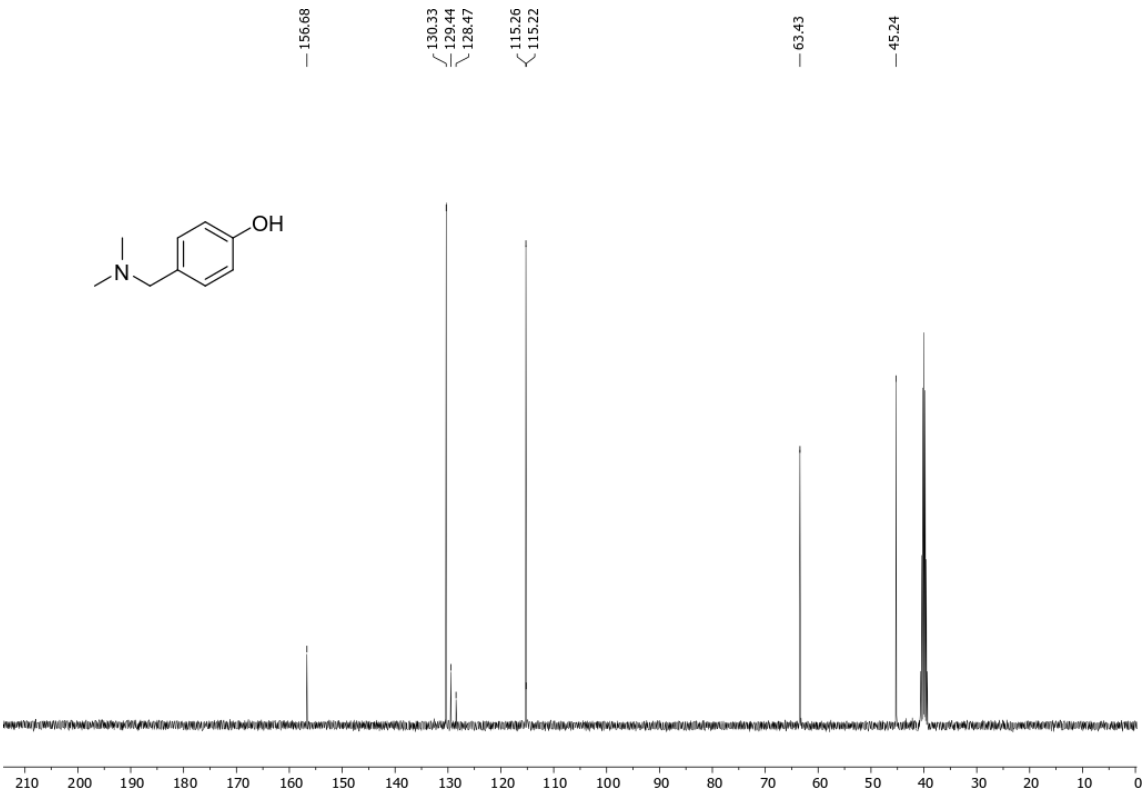


Figure S23. ^{13}C NMR spectrum of **2** in DMSO-*d*6(298 K).

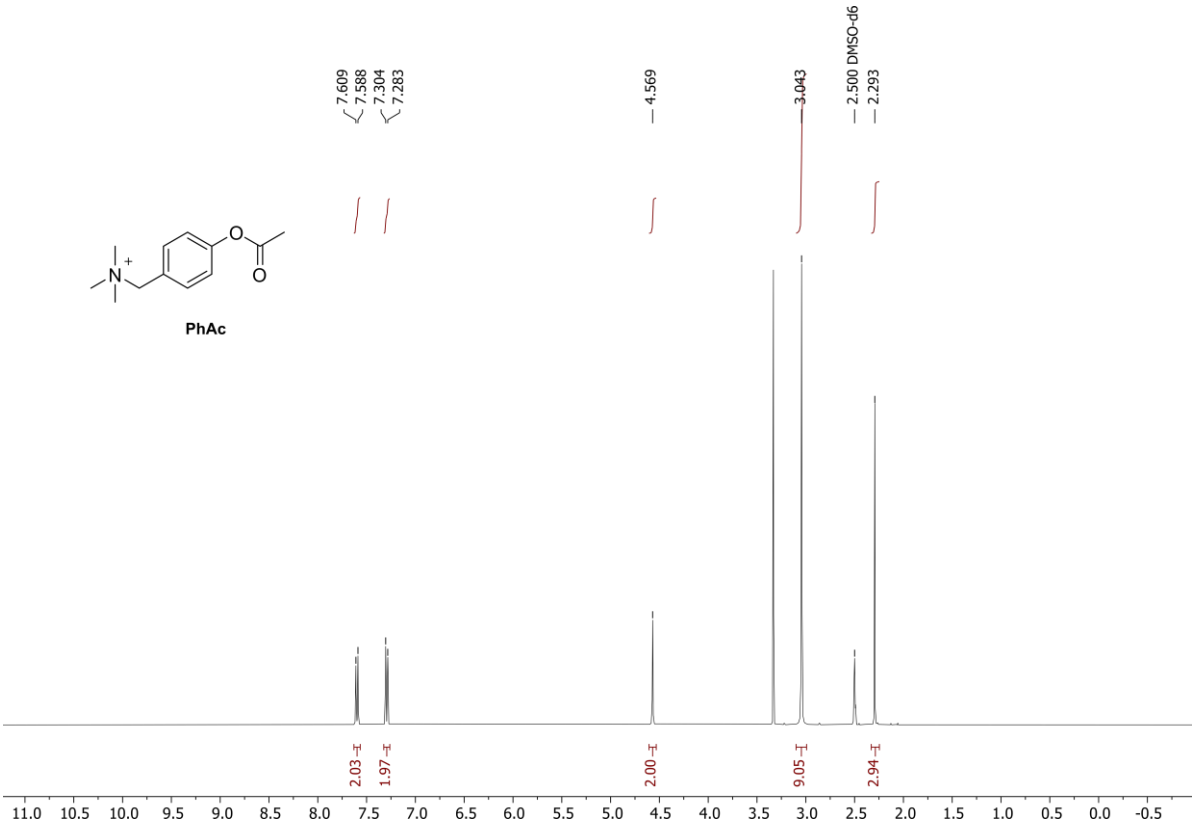


Figure S24. ^{1}H NMR spectrum of **PhAc** in DMSO-*d*6 (298 K).

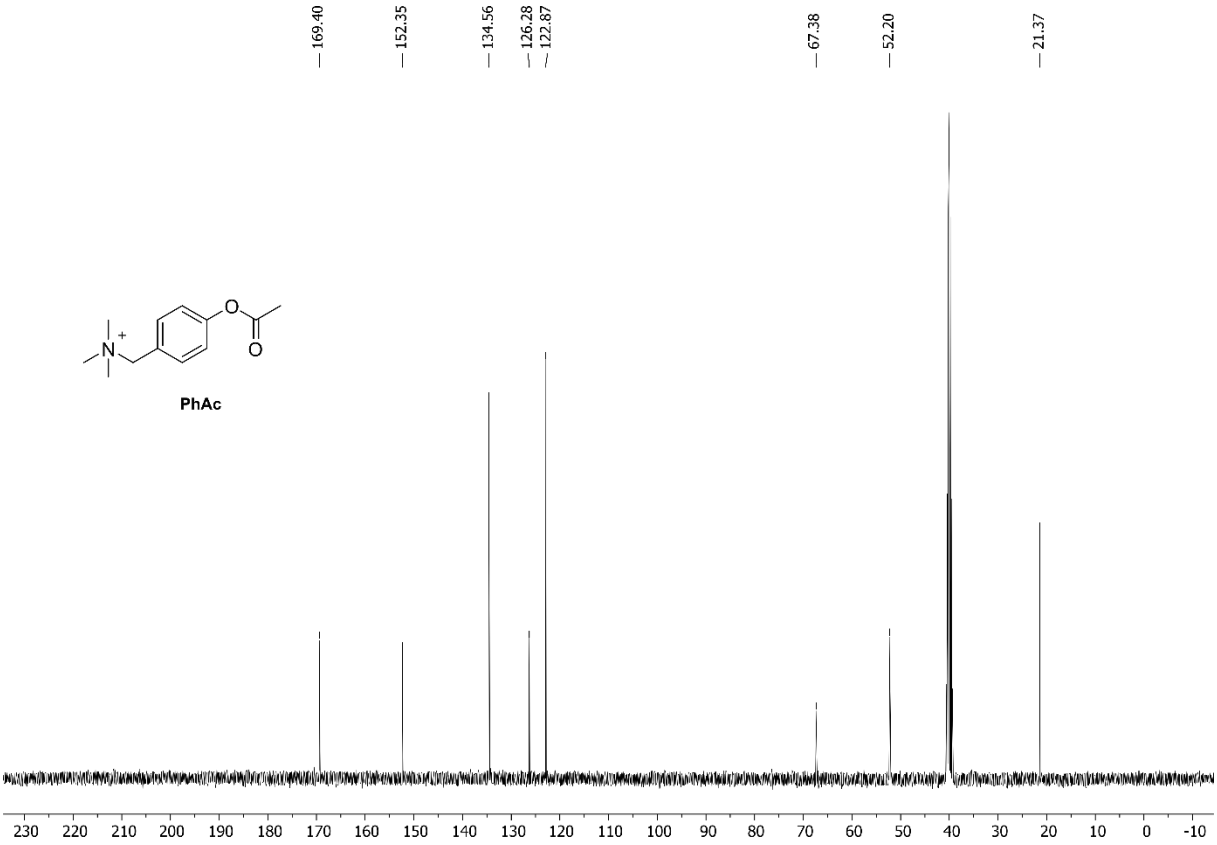


Figure S25. ^{13}C NMR spectrum of **PhAc** in DMSO-*d*6 (298 K).

References:

1. P. Jia, R. Sheng, J. Zhang, L. Fang, Q. J. He, B. Yang and Y. Z. Hu, *Eur J Med Chem*, 2009, **44**, 772-784.
2. H. Tang, D. Fuentealba, Y. H. Ko, N. Selvapalam, K. Kim and C. Bohne, *J Am Chem Soc*, 2011, **133**, 20623-20633.