Electronic Supplementary Information

A Nano-Sized Container for Specific Encapsulation of Isolated Water Molecules

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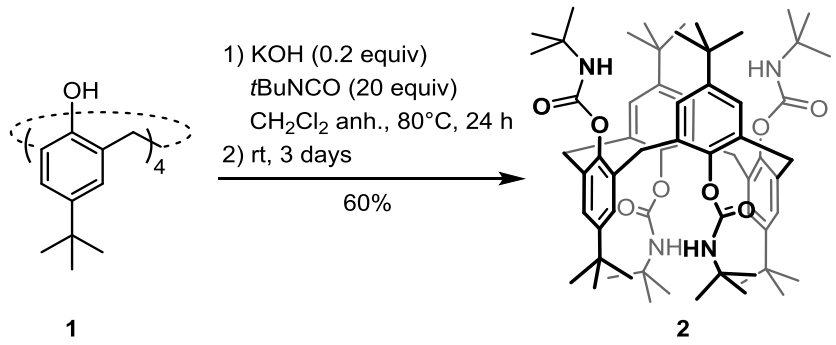
Experimental Section

The reactions were not performed under inert atmosphere unless otherwise stated. Solvents were distilled prior to use. Anhydrous dichloromethane was obtained from distillation over CaH_2. Silica gel (230-400 mesh) was used for flash chromatography. NMR spectra were recorded at either 7.0, 9.4 or 14.1 Tesla. Solvent signals were used for chemical shift referencing: ^1H signal at 7.26 ppm for $CHCl_3$, 3.31 for CHD_2OD and 6.00 ppm for $CDCl_2CHCl_2$; ^{13}C signal at 77.16 ppm for $CDCl_3$ and 73.78 ppm for $(CDCl_2)_2$. Abbreviations: s: singlet, d: doublet, br: broad signal. The high-resolution mass spectra were recorded with Q-TOF ESI+ spectrometer. Melting points (mp) are uncorrected. ATR-FTIR spectra were recorded at room temperature. The starting compounds **1** and **4** were commercial. The abbreviation Bac stands for *tert*-butylaminocarbonyl.

Caution: (i) Reaction described with KOH in CH_2Cl_2 should not be held in any other halogenated solvent which is more acidic than CH_2Cl_2 (*e.g.* $CHCl_3$) due to the possible formation of unstable carbenes. (ii) *t*BuNCO is a toxic reagent and should be trapped and treated appropriately during evaporation processes using a rotary evaporator.

Synthesis of *p*-*t*Bu-calix[4]arene-tetra-Bac **2**



1 **2**

Freshly crushed KOH (11 mg, 0.20 mmol) was added to a suspension of *p*-*t*Bu-calix[4]arene **1** (650 mg, 1.00 mmol) in anhydrous CH_2Cl_2 (10 mL) under inert atmosphere in a closed reactor and the mixture was stirred at room temperature. After 30 min, *t*BuNCO (2.3 mL, 20 mmol) was added and the mixture was stirred at 80°C for 24 h; the pressure in the reactor was then given by the total vapor pressure of the reaction medium. The mixture was cooled down to room temperature, transferred in a 25 mL round-bottomed flask (small amounts of CH_2Cl_2 were used to rinse the reactor) and partially evaporated with a rotary evaporator until ~5 mL were left (approximatively 20 min at 400 mbar in a 24°C bath). The orange mixture was then stirred at room temperature under inert atmosphere. The reaction was monitored by NMR spectroscopy (checking the CH_2 region between 3.0 and 4.5 ppm, see Figure S1). The reaction was stopped after 3 days due to the presence of a major singlet at 3.58 ppm corresponding to the desired compound **2** and quasi-complete disappearance of doublets at 4.05, 3.96, 3.35 and 3.32 ppm corresponding to an intermediate (^1H, 300 MHz, $CDCl_3$, 298 K). The required reaction time at room temperature may vary by few days. The mixture was evaporated under reduced pressure, CH_2Cl_2 was added and evaporated again to remove traces of *t*BuNCO. The crude product was purified by flash chromatography (CH_2Cl_2) affording *p*-*t*Bu-calix[4]arene-tetra-Bac **2** as a white solid (629 mg, 0.602 mmol). Yield: 60%. Further purification can be achieved by crystallization in CH_2Cl_2/CH_3CN (slow evaporation of the CH_2Cl_2).

R_f (CH_2Cl_2) = 0.37. Mp = 284-311°C (sublimation). IR (ATR) ν (cm^{-1}) = 2970, 1744, 1475, 1177, 1115, 1005. ^{1}H NMR (400 MHz, non-anhydrous $CDCl_3$, 298K, bis-aqua complex **2**⊃$2H_2O$) δ (ppm) = 7.05 (s, 8H, Ar*H*), 5.42 (br(s), 4H, N*H*), 3.57 (s, 8H, ArCH_2), 1.43 (s, 36H, N-*t*Bu), 1.33 (s, 36H, Ar-*t*Bu). $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$, 298K) δ (ppm) = 152.5 (*C*=O), 146.3 (*C*-OBac + *C*-*t*Bu), 134.7 (*C*-CH_2), 126.4 (*C*-H), 51.0 (N-*C*-$(CH_3)_3$), 38.2 (CH_2), 34.3 (Ar-*C*-$(CH_3)_3$), 32.1 (Ar-C-$(CH_3)_3$), 29.0 (N-C-$(CH_3)_3$). HRMS (ESI+): calcd for $C_{64}H_{93}N_4O_8$ $[M+H]^+$ 1045.6988, found 1045.6985. Note: Chemical shifts may vary depending on water concentration, especially for the NH signal.

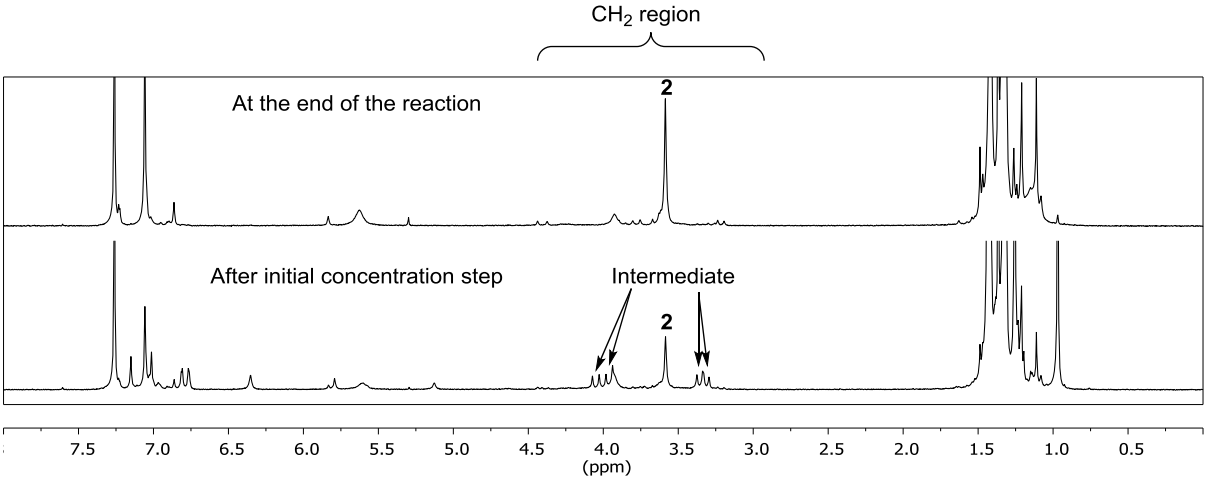
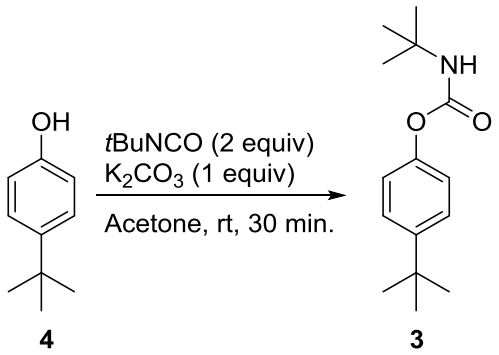


Figure S1. ^{1}H NMR monitoring of the reaction (300 MHz, $CDCl_3$, 298 K). Intense signals are truncated.

Synthesis of *N*-*t*Bu-*O*-(*p*-*t*Bu-phenyl)carbamate **3**



K_2CO_3 (139 mg, 1.01 mmol) and *t*BuNCO (230 µL, 2.01 mmol) were added to a solution of *p*-*t*Bu-phenol **4** (150 mg, 1.00 mmol) in acetone and the mixture was stirred at room temperature for 30 minutes. The mixture was then filtered and concentrated under vacuum to afford *N*-*t*Bu-*O*-(*p*-*t*Bu-phenyl)carbamate **3** (249 mg, 1.00 mmol) as a white solid. Quantitative yield. R_f (CH_2Cl_2) = 0.57. Mp = 67°C. IR (ATR) ν (cm^{-1}) = 3357, 2968, 1715, 1506, 1196, 1020. ^{1}H NMR (400 MHz, $CDCl_3$, 298K) δ (ppm) = 7.36 (d, ^3J = 8.7 Hz, 2H, Ar*H*), 7.03 (d, ^3J = 8.7 Hz, 2H, Ar*H*), 4.96 (br(s), 1H, N*H*), 1.39 (s, 9H, *t*Bu), 1.30 (s, 9H, *t*Bu). $^{13}C\{^1H\}$ NMR (75 MHz, $CDCl_3$, 298K) δ (ppm) = 153.2, 148.7, 148.1, 126.3, 121.3, 51.0, 34.6, 31.6, 29.0. HRMS (ESI+): calcd for $C_{15}H_{24}NO_2$ $[M+H]^+$ 250.1802, found 250.1803.

Optimization regarding the synthesis of compound 2

Conditions screened to optimize the synthesis of compound **2** are given in Table S2. These conditions are not given in chronological order. Some chosen conditions may seem odd at first glance but were based on previous results or held for comparison purpose. Reactions were monitored by TLC, ESI-MS and/or ^{1}H NMR analyses over several days and until no more evolution was observed.

Solvent	Base	Base equiv	Temperature	*t*BuNCO equiv	Major calixarene-based product
Acetone	Cs_2CO_3	5.0	Reflux	20	Starting material **1**
MeCN	Cs_2CO_3	5.0	Reflux	20	Starting material **1**
DMF anh.	KOH	0.1	100°C	20	Starting material **1**
DMF anh.	NaOH	1.5	rt	20	Starting material **1**
DMF anh.	$Ba(OH)_2{\cdot}8H_2O$	2.0	rt	20	Starting material **1**
DMF[a]	NaOH	1.5	rt	20	Starting material **1**
THF anh.	KOH	0.1	Reflux	20	Starting material **1**
Toluene	K_2CO_3	5	80°C	12	Starting material **1**
Toluene	K_2CO_3	5	70°C	72	Mono-carbamated
Toluene	$Ba(OH)_2{\cdot}8H_2O$	2	60°C	40	Starting material **1**
$CHCl_3$	$Ba(OH)_2{\cdot}8H_2O$	2	50°C	40	Starting material **1**
CH_2Cl_2	$Ba(OH)_2{\cdot}8H_2O$	2	rt	10	Starting material **1**
CH_2Cl_2	$Ba(OH)_2{\cdot}8H_2O$	2	rt	20	Starting material **1**
CH_2Cl_2	Na_2CO_3	5.0	rt	40	Starting material **1**
CH_2Cl_2	Cs_2CO_3	5.0	rt	20	*p*-*t*Bu-calix[4]arene-mono-Bac
CH_2Cl_2	Cs_2CO_3	1.0	rt	20	*p*-*t*Bu-calix[4]arene-mono-Bac
CH_2Cl_2[b]	Cs_2CO_3	1.0	rt	20	Starting material **1**
CH_2Cl_2	$NMe_4^+OH^-$	1.3	rt	20	*p*-*t*Bu-calix[4]arene-tri-Bac[c]
CH_2Cl_2	NaOH	1.1	rt	12	*p*-*t*Bu-calix[4]arene-tri-Bac[c]
CH_2Cl_2	NaOH	1.0	rt	20	*p*-*t*Bu-calix[4]arene-tri-Bac[c]
CH_2Cl_2	NaOH	1.1	rt	60	*p*-*t*Bu-calix[4]arene-tri-Bac[c]
CH_2Cl_2	NaOH	1.5	rt	20	*p*-*t*Bu-calix[4]arene-tri-Bac[c]
CH_2Cl_2[b]	NaOH	1.6	rt	20	*p*-*t*Bu-calix[4]arene-tri-Bac[c]
CH_2Cl_2 anh.	NaOH	0.2	80°C	20	Various *p*-*t*Bu-calix[4]arene-tetra-Bac atropisomers
CH_2Cl_2 anh.	KOH	1.5	60°C	20	*p*-*t*Bu-calix[4]arene-tri-Bac[c]
CH_2Cl_2 anh.	KOH	0.2	80°C	20	*p*-*t*Bu-calix[4]arene-tri-Bac[c]
CH_2Cl_2 anh.	KOH	0.2	80°C → rt	20	*p*-*t*Bu-calix[4]arene-tetra-Bac **2**[d]

Table S2. Conditions tested for the synthesis of *p*-*t*Bu-calix[4]arene-tetra-Bac **2**. Bac stands for *tert*-butylaminocarbonyl. Reactions done in anhydrous solvents were held under inert atmosphere. [a] 10 equiv of water were added. [b] Water was added as co-solvent. [c] Cone atropisomer. [d] Compound **2** was accumulated over time at room temperature.

NMR spectra for structural characterization

*p-t*Bu-calix[4]arene-tetra-Bac **2**

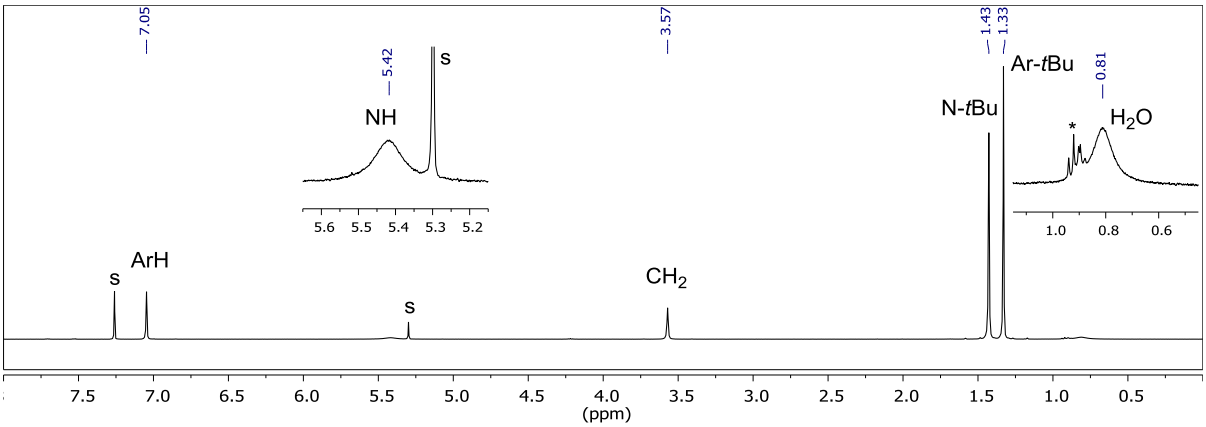


Figure S3. ^{1}H NMR spectrum of compound **2** (400 MHz, $CDCl_3$, 298 K). s: residual solvents. *: minor impurity.

The signal of water is observed at 0.81 ppm. It is significantly upfield shifted ($\Delta\delta$ = -0.75 ppm) with respect to the signal of water in the solvent, which is expected at 1.56 ppm *(see Fulmer et al. Organometallics 2010, 29, 2176–2179)*.

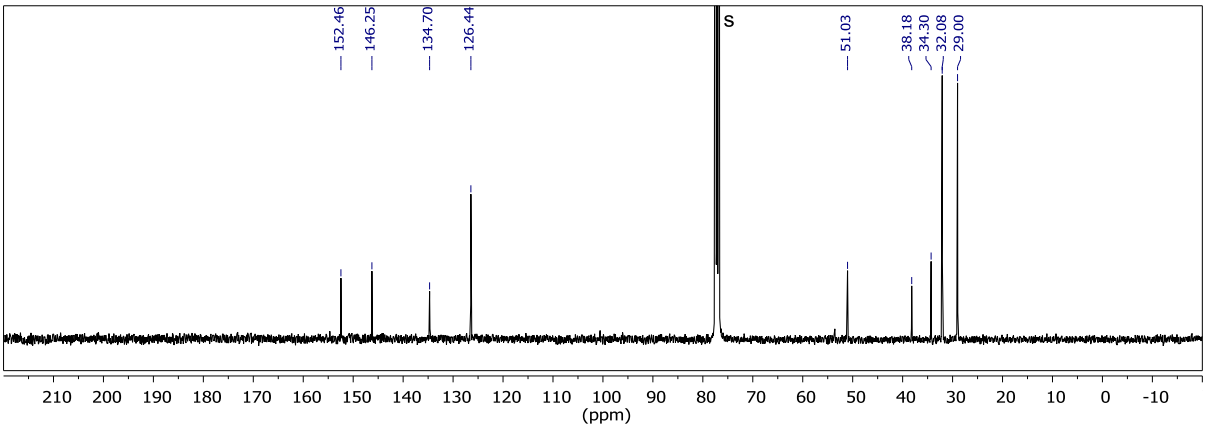


Figure S4. ^{13}C NMR spectrum of compound **2** (100 MHz, $CDCl_3$, 298 K). s: solvent.

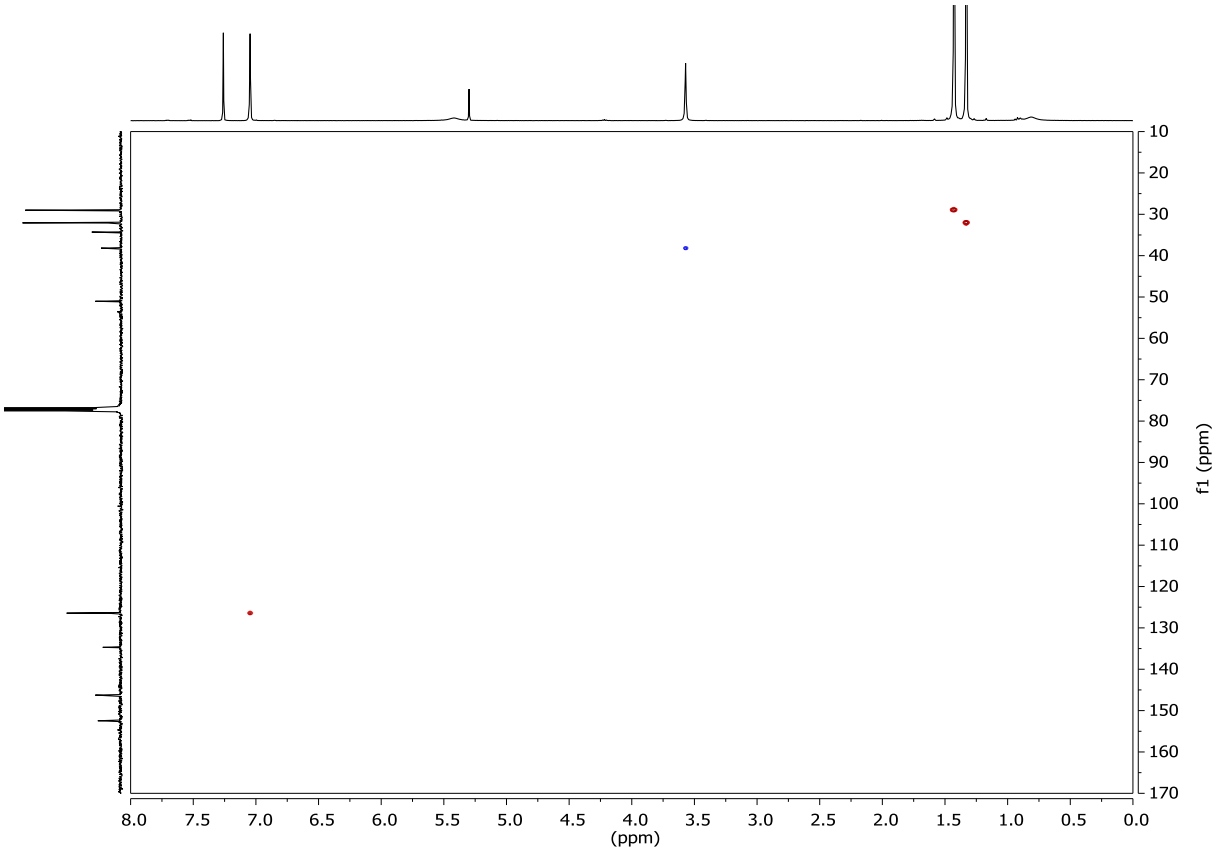


Figure S5. Edited ^{1}H-^{13}C HSQC spectrum of compound **2** (9.4 Tesla, $CDCl_3$, 298 K).

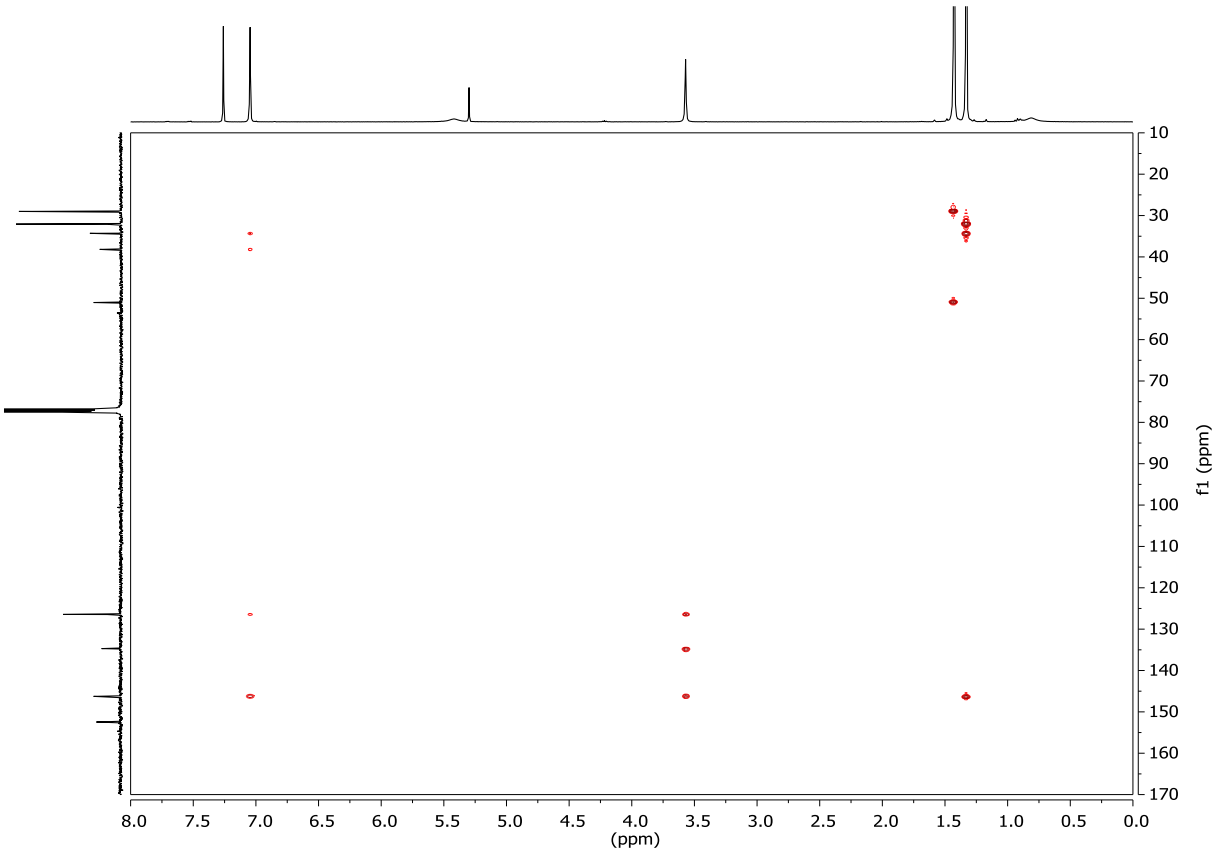


Figure S6: ^{1}H-^{13}C HMBC spectrum of compound **2** (8 Hz, 9.4 Tesla, $CDCl_3$, 298 K).

ROESY analyses of host **2**, **2**⊃H_2O and **2**⊃2H_2O complexes

The free receptor is the 1,3-alternate atropisomer as shown by ^{1}H-^{1}H Overhauser effects (Figure S7a) observed between, on the one hand, the *tert*-butyl groups of the carbamate moieties (N-*tBu*) and, on the other hand, the *tert*-butyl groups and the aromatic rings of the calix[4]arene skeleton (Ar-*tBu* and ArH, respectively).

Because of the in-out exchange process, the ROESY spectrum recorded in the presence of about 1 equiv of water (Figures S7b and c) shows EXSY-type correlations between the signals of free and included water, as well as between the various NH signals. In contrast, no EXSY correlation is detected between the water and NH signals, indicating that proton exchange is not effective.

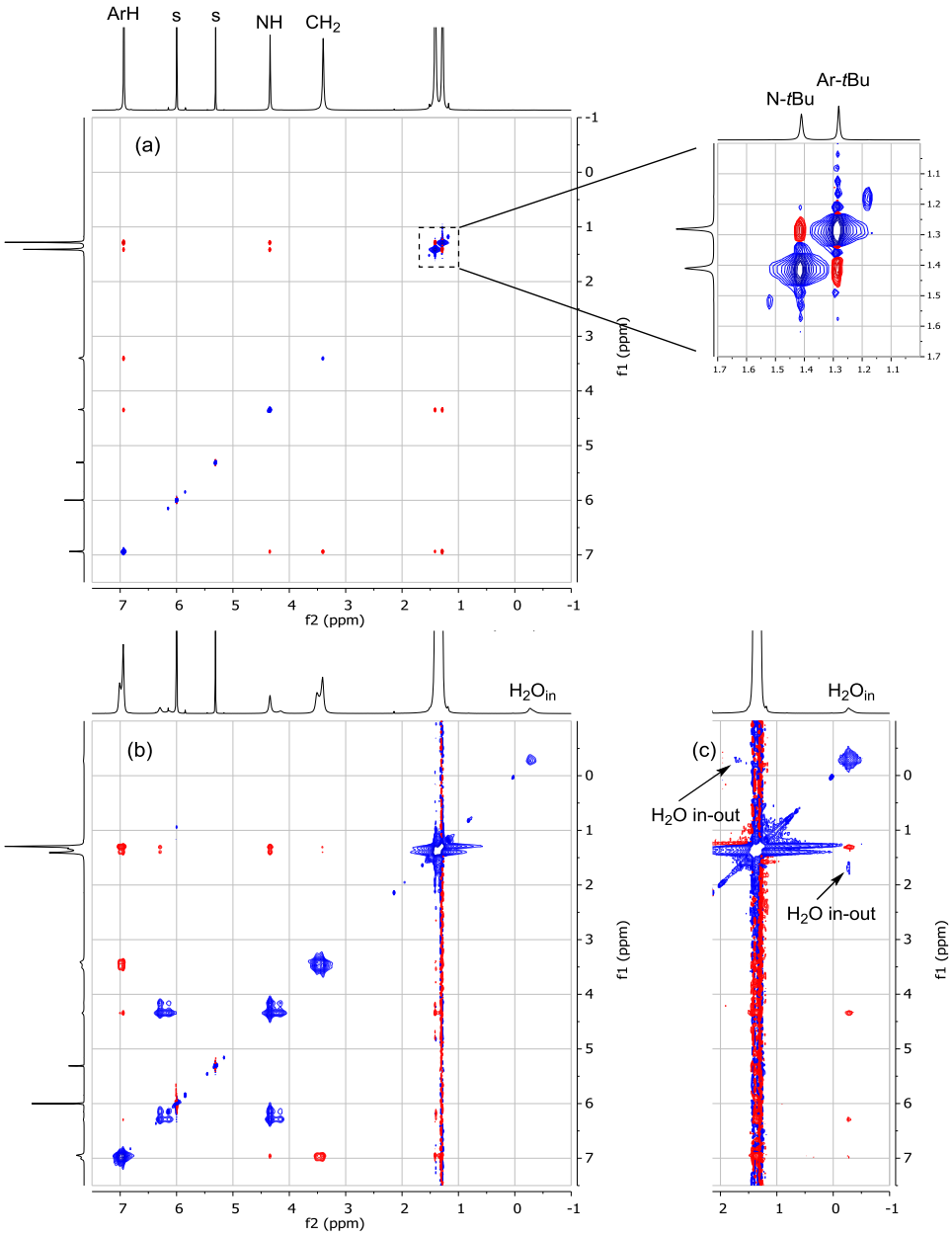


Figure S7. ROESY spectra of compound **2** (600 MHz, $(CDCl_2)_2$, 238 K, [**2**] = 7.0×10^{-4} mol/L) recorded (a) in the absence of water with a mixing time of 250 ms and (b-c) in the presence of about 1 equiv of water with a mixing time of 50 ms. Overhauser effects are shown in red and the correlations due to exchange are in blue. s: residual solvents.

N-*t*Bu-*O*-(*p*-*t*Bu-phenyl)carbamate **3**

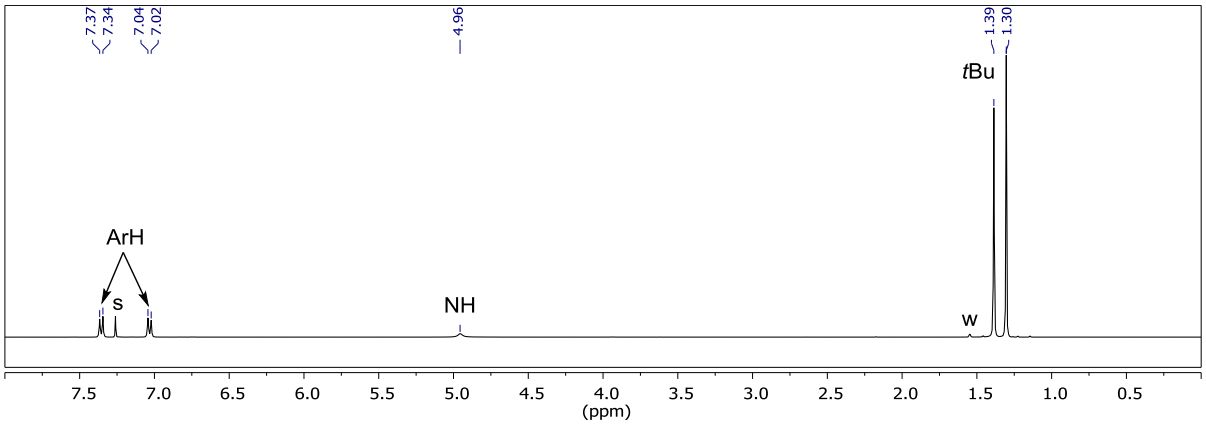


Figure S8. ^{1}H NMR spectrum of compound **3** (400 MHz, $CDCl_3$, 298 K). s: residual solvent. w: water.

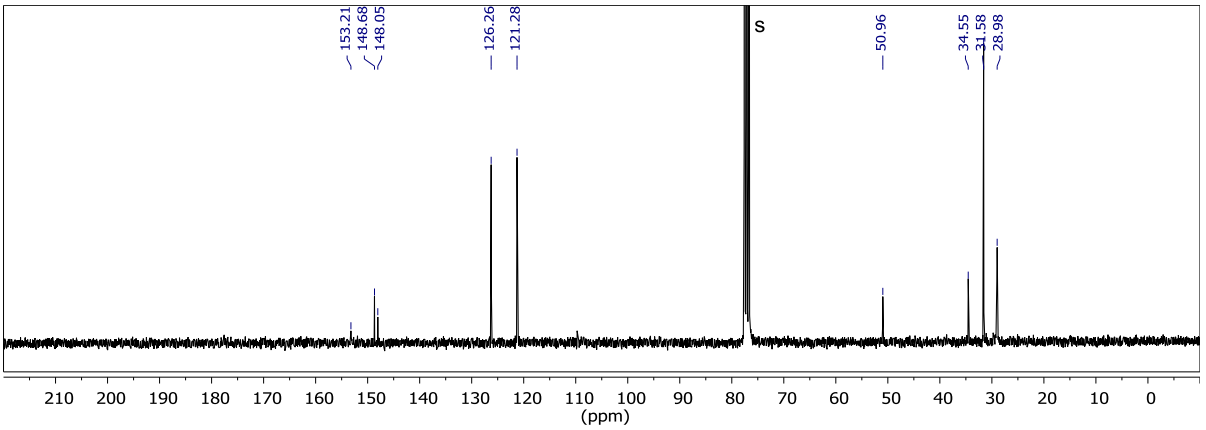


Figure S9. ^{13}C NMR spectrum of compound **3** (75 MHz, $CDCl_3$, 298 K). s: solvent.

Association constants determination

The 1:2 stoichiometry model

The binding of water (guest G) by calixarene **2** (host H) can be described by the following equilibria:

$$H + G \rightleftharpoons HG$$

$$HG + G \rightleftharpoons HG_2$$

The corresponding association constants characterizing the first and second binding processes are :

$$Ka_1 = \frac{[HG]}{[H][G]} \quad \text{, which can be written as} \qquad \kappa_1 = \frac{v}{u\,g}$$

$$Ka_2 = \frac{[HG_2]}{[HG][G]} \text{, which can be written as} \qquad \kappa_2 = \frac{w}{v\,g}$$

where $\kappa_1 = Ka_1 C_H$ and $\kappa_2 = Ka_2 C_H$, with C_H standing for the total molar concentration of host.

u, v and w are, respectively, the mole fraction of free host, the mole fraction of the 1:1 complex HG and the mole fraction of the 1:2 complex HG_2 at equilibrium :

$u=[H]/C_H$, $v=[HG]/C_H$ and $w=[HG_2]/C_H$

with $u + v + w = 1$

g is the number of equivalents of free guest at equilibrium:

$g = [G]/C_H = (R - v - 2w)$

R is the number of equivalents of guest:

$R = C_G/C_H$, with C_G standing for the total molar concentration of guest.

The following equations can then be obtained:

$$\kappa_1\kappa_2\, g^3 + \kappa_1\left(1+\kappa_2(2-R)\right)g^2 + \left(1+\kappa_1(1-R)\right)g - R = 0 \qquad (1)$$

$$u = \frac{1}{1+\kappa_1\, g+\kappa_1\kappa_2\, g^2} \qquad (2)$$

$$v = \frac{\kappa_1\, g}{1+\kappa_1\, g+\kappa_1\kappa_2\, g^2} \qquad (3)$$

$$w = \frac{\kappa_1\kappa_2\, g^2}{1+\kappa_1\, g+\kappa_1\kappa_2\, g^2} \qquad (4)$$

g is thus obtained by solving the cubic polynomial equation (1), considering that $0 \le g < R$ if three real roots are found.

Characterization of the binding of water by calixarene **2** in $(CDCl_2)_2$ at 238 K

Figures S10 and S11 show the ^{1}H NMR spectra used for the determination of Ka_1 and Ka_2. They were recorded at 600 MHz and low temperature (238 K) for a dilute solution of **2** in 1,1,2,2-tetrachloroethane-d_2 (C_2 = 7.0×10^{-4} mol/L) and decreasing amount of water (2.5 ≥ R ≥ 0.2). A 5 mm NMR tube equipped with a J. Young valve was used for this purpose and the concentration of water was decreased by adding freshly dehydrated molecular sieve (3 Å).

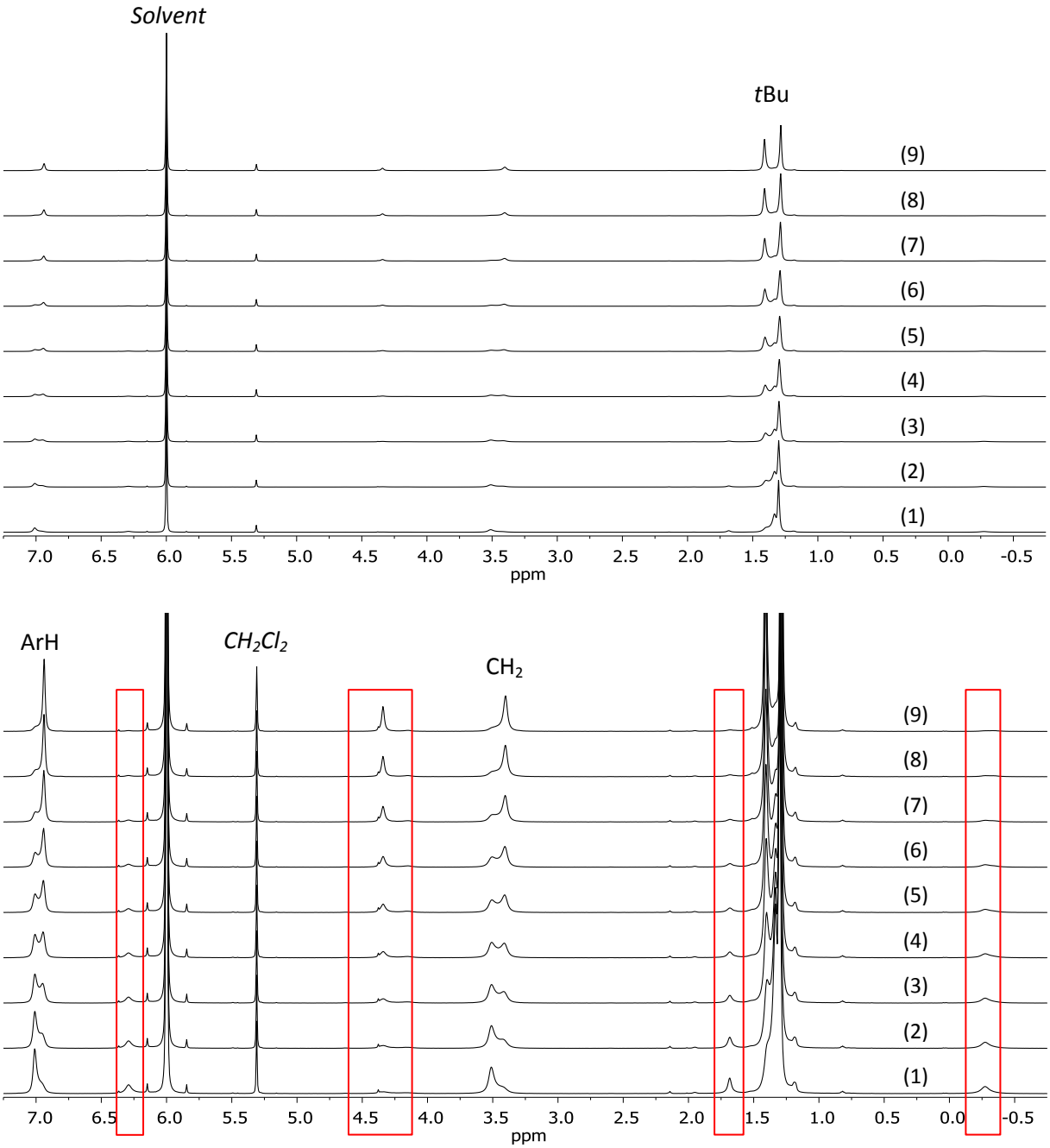


Figure S10. ^{1}H NMR spectra used for the determination of Ka_1 and Ka_2 (600 MHz, $(CDCl_2)_2$, 238 K, C_2 = 7.0×10^{-4} mol/L). R = 2.5, 2.1, 1.8, 1.5, 1.2, 1.0, 0.73, 0.51 and 0.19 for spectra (1) to (9), respectively. The boxed regions are shown in Figure S11.

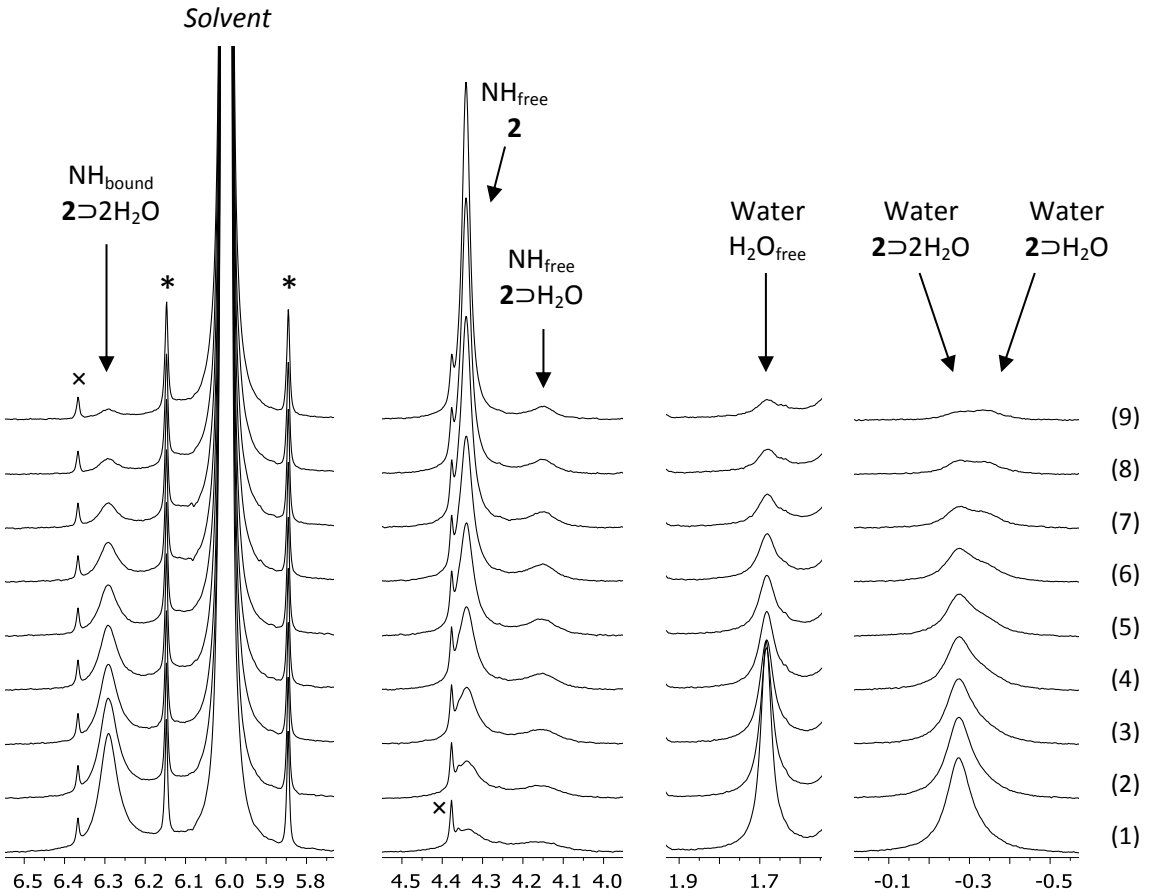


Figure S11. Regions of the ^{1}H NMR spectra used for the determination of Ka_1 and Ka_2 (600 MHz, $(CDCl_2)_2$, 238 K, $C_{\mathbf{2}} = 7.0\times10^{-4}$ mol/L). R = 2.5, 2.1, 1.8, 1.5, 1.2, 1.0, 0.73, 0.51 and 0.19 for spectra (1) to (9), respectively. × = impurity ; * = ^{13}C satellites of the solvent signal.

Both the first and second binding processes are slow on the NMR spectral time scales defined by the the NH and H_2O signals but significant broadening is however observed (Figure S11). For the species **2**⊃H_2O, the broadened signal of the NH groups bound to the water molecule (NH_{bound}) is superimposed to the ^{13}C satellite of the solvent at 6.15 ppm.

The ratio R and the mole fractions u, v and w were obtained from the integrated intensity of the NH and H_2O signals, as determined by deconvolution using lorentzian functions. The integrals of the NH and H_2O signals of the species **2**⊃2H_2O were constrained to be identical (signals at 6.3 and -0.27 ppm, 4 ^{1}H each). Similarly, the integrals of the NH_{free} and H_2O signals of the species **2**⊃H_2O were also constrained to be identical (signals at 4.15 and -0.35 ppm, 2 ^{1}H each). The deconvolution of the spectrum (6), *i.e.* for R = 1.0, is illustrated in Figure S12.

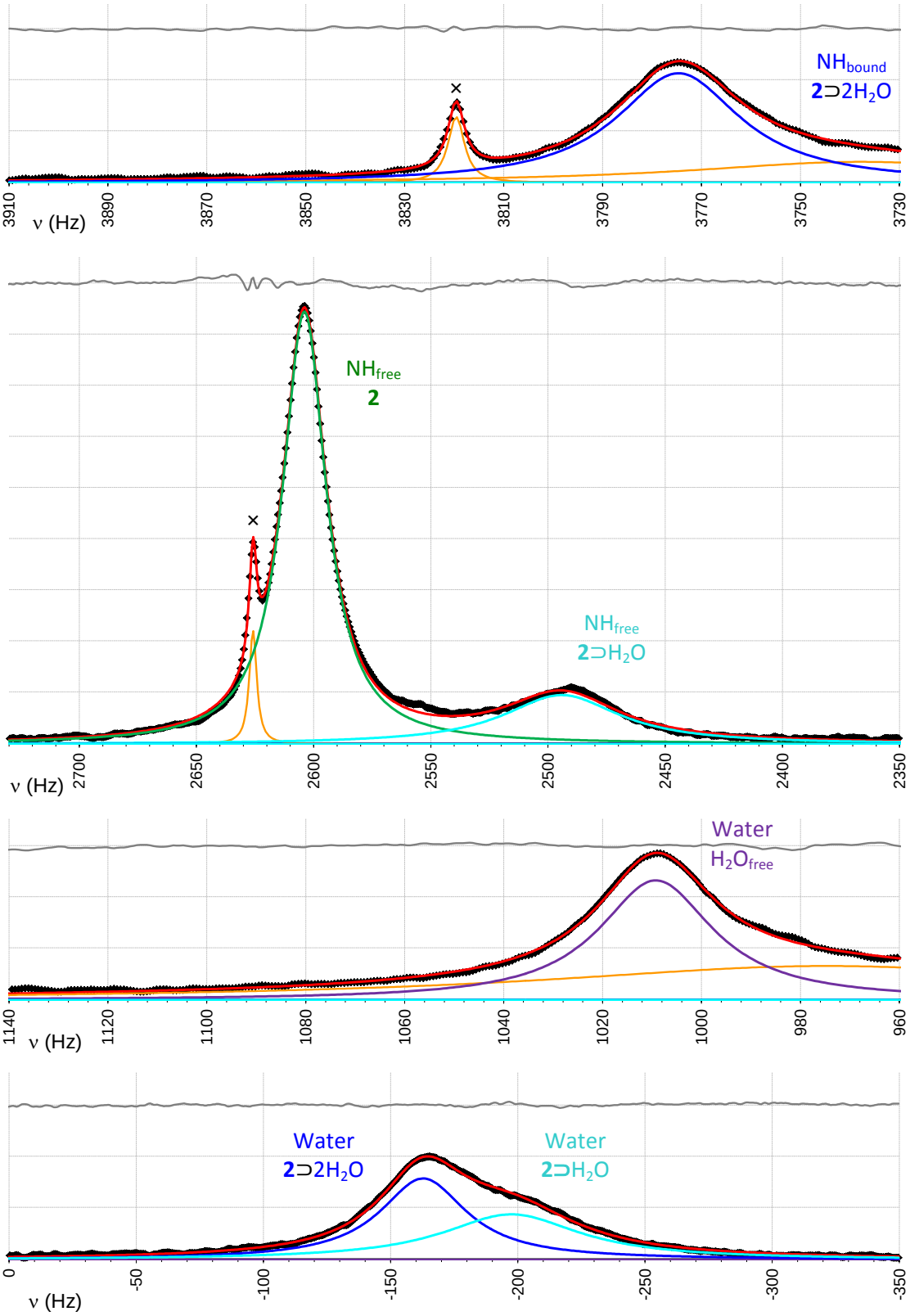


Figure S12. Deconvolution of the spectrum recorded in the presence of 1 equivalent of water (spectrum 6 of Figure S11; 1 ppm ≈ 600 Hz). The experimental data points are shown in dark (♦) and the best-fit is in red. The orange lines are either impurities (×) or local baseline corrections. The gray lines are the residuals.

Additional kinetic considerations

The overall pseudo-first order rate constant characterizing the binding of water depends on the free host and mono-aqua complex concentrations ($k_{in} = k_{in1}[\mathbf{2}] + k_{in2}[\mathbf{2}\supset H_2O]$). Consequently, the linewidth of the signal of free water increases for decreasing water content (Figure S11). In contrast, the linewidth observed for the signal of water pertaining to the bis-aqua complex remains constant, indicating that the escape of water is a dissociative process. The residence time of water in the bis-aqua complex was estimated to be about 10 ms at 238 K in $(CDCl_2)_2$.

Characterization of the binding of water by calixarene **2** in a protic medium at 298 K

^{1}H NMR spectra recorded at 298 K for a 5 mM solution of **2** in $CDCl_3/CD_3OD$ (2:1, v/v) and increasing amounts of D_2O are shown in Figure S13. Fast in-out exchange of water prevails in these conditions. The chemical shift variation observed for the NH signal of **2** is shown in Figures S14 and S15.

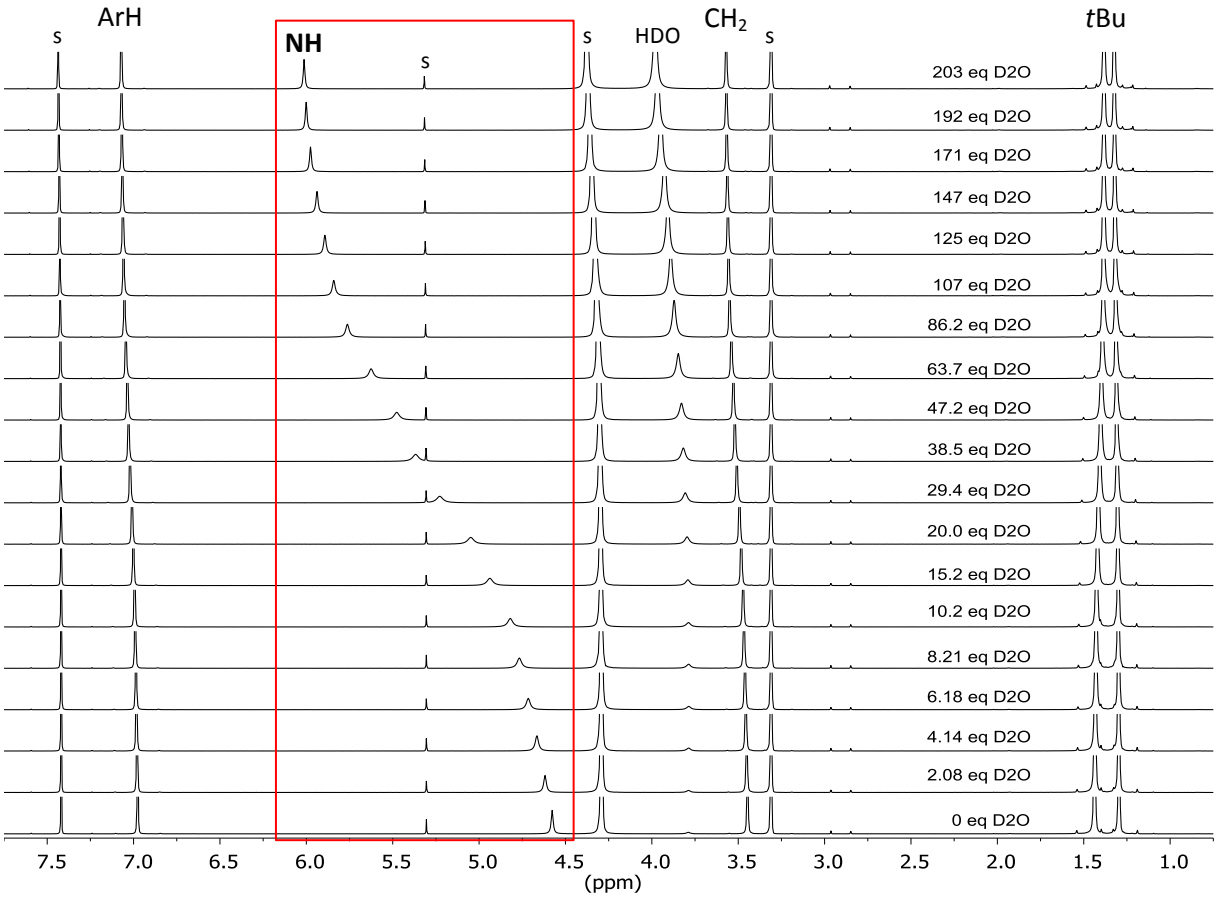


Figure S13. ^{1}H NMR spectra recorded at 298 K for receptor **2** dissolved in $CDCl_3/CD_3OD$ (2:1, v/v) and increasing amounts of D_2O. The initial concentration of the receptor is 5.0 mM. These spectra correspond to the series of measurements displayed with white dots in Figures S14 and S15. Intense signals are truncated. s: residual solvents. These spectra were calibrated on the CHD_2OD signal.

The chemical shift variation was first interpreted considering the two cavities of calixarene **2** as independent receptors. Hence, the 1:1 stoichiometry model was fitted to the experimental data considering that the initial concentration of the receptor is 10 mM (i.e. twice the concentration of **2**)

and, consequently, the numbers of D_2O equivalents was divided by two. Furthermore, the dilution of **2** due to the addition of D_2O was taken into account. The following three parameters were determined by this analysis: an apparent association constant, Ka = 4.8 M^{-1}, an apparent chemical shift for the free NH groups, δ NH_{free} = 4.52 ppm, and an apparent chemical shift for the hydrogen-bonded NH groups, δ NH_{bound} = 6.32 ppm. As can be seen in Figure S14, the 1:1 stoichiometry model globally accounts for the experimental chemical shift variation but systematic deviations are nevertheless observed.

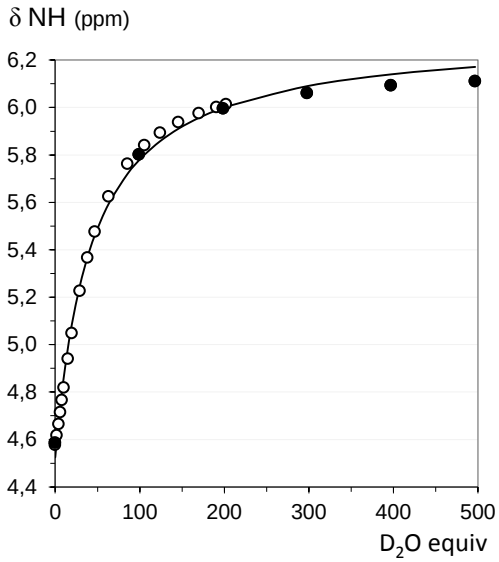


Figure S14. Variation of the chemical shift observed at 298 K for the NH signal of receptor **2** dissolved in $CDCl_3/CD_3OD$ (2:1, v/v) upon addition of D_2O. The initial concentration of the receptor is 5.0 mM. Black and white dots are experimental data from two independent series of measurements. The curve is the fitting of the 1:1 stoichiometry model.

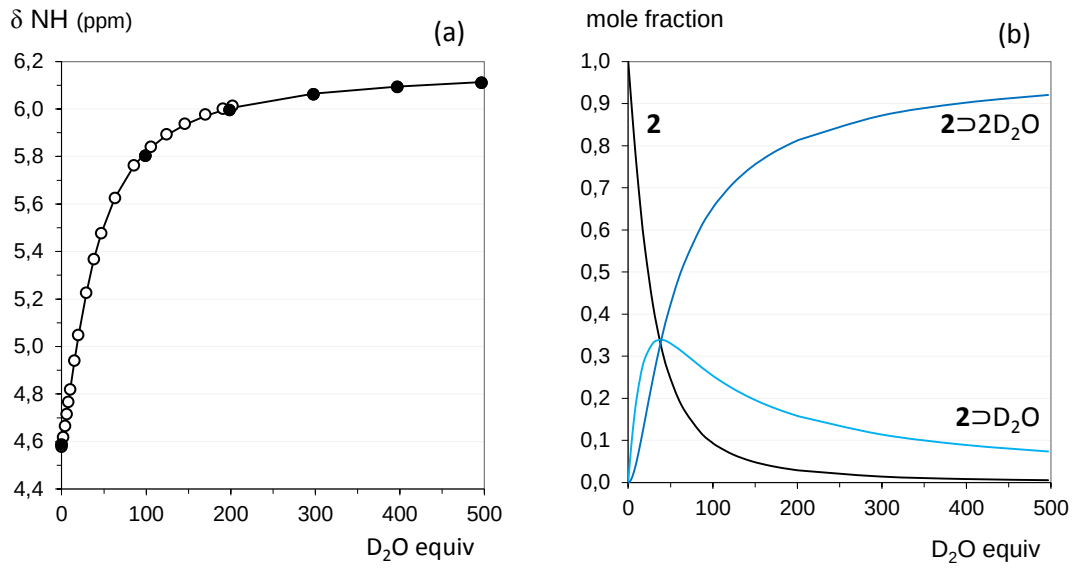


Figure S15. (a) Variation of the chemical shift observed at 298 K for the NH signal of receptor **2** dissolved in $CDCl_3/CD_3OD$ (2:1, v/v) upon addition of D_2O and (b) mole fractions of free host **2**, mono-aqua complex **2**⊃D_2O and bis-aqua complex **2**⊃$2D_2O$, as determined from the best-fit analysis using the 1:2 stoichiometry model. The initial concentration of the receptor is 5.0 mM. Black and white dots are experimental data from two independent series of measurements. In (a), the curve is the fitting of the 1:2 stoichiometry model.

Using the notations introduced above, the chemical shift variation expected in the framework of the 1:2 stoichiometry model can be written as:

$$\delta = \frac{2[H]\delta_{00} + [HG]\delta_{10} + [HG]\delta_{01} + 2[HG_2]\delta_{11}}{2C_H} = u\delta_{00} + v\frac{1}{2}(\delta_{10} + \delta_{01}) + w\delta_{11} \qquad (5)$$

δ_{00} = δ NH_{free} for the free receptor **2**

½(δ_{10} + δ_{01}) is the average value of δ NH_{free} and δ NH_{bound} for the complex **2**⊃D_2O

δ_{11} = δ NH_{bound} for the bis-aqua complex **2**⊃$2D_2O$

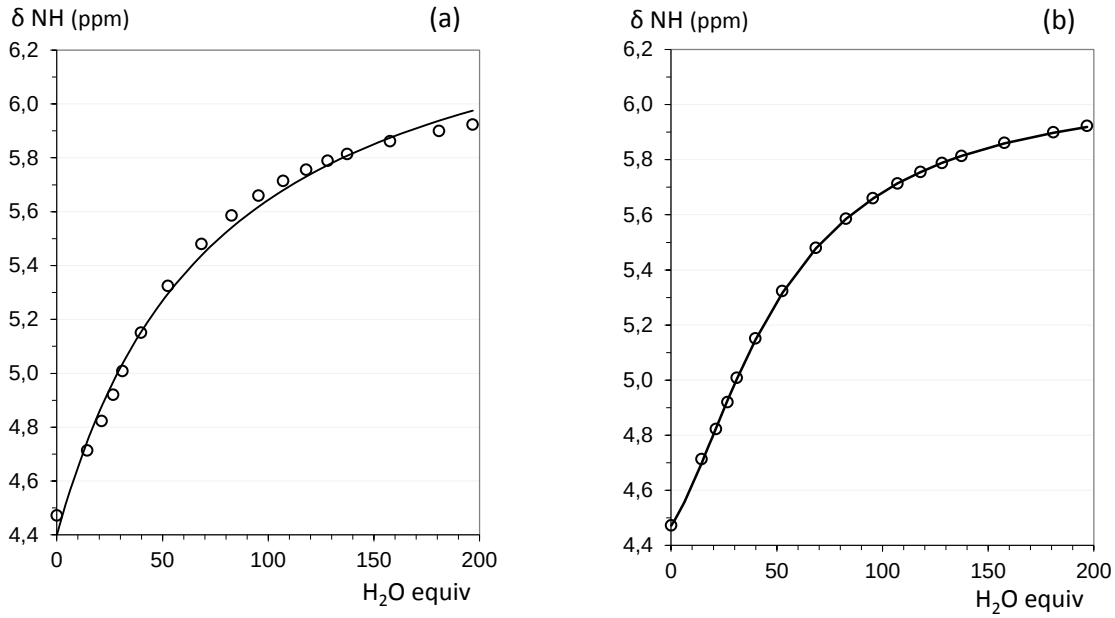
As can be seen in Figure S15, the 1:2 stoichiometry model properly accounts for the observed data. The corresponding parameters are:

	Ka_1	5.5 M^{-1}
	Ka_2	5.2 M^{-1}
2	δ_{00}	4.58 ppm
2⊃D_2O	½ (δ_{10} + δ_{01})	5.34 ppm
2⊃$2D_2O$	δ_{11}	6.18 ppm

Characterization of the binding of water by calixarene **2** in a non-protic polar medium at 298 K

^{1}H NMR spectra were recorded at 298 K for a 1.2 mM solution of **2** in $CDCl_3/CD_3CN$ (2:1, v/v) and increasing amounts of H_2O. Fast in-out exchange of water also prevails in these conditions. The chemical shift variation observed for the NH signal of **2** is shown in Figure S16 with the best-fit of the 1:1 and 1:2 stoichiometry models (see above). The chemical shift of the NH signal measured after extensive drying of the solution with 3 Å molecular sieves is 4.47 ppm. The corresponding value after addition of a large amount of water, leading to saturation, is 6.07 ppm (not included in the best-fit analyses).

As can be seen in Figure S16a, the 1:1 stoichiometry model globally accounts for the experimental chemical shift variation but systematic deviations are observed and the parameter δ NH_{bound} is much larger than the chemical shift measured at saturation. In contrast, the 1:2 stoichiometry model properly accounts for the experimental data, including the chemical shift measured at saturation (Figure S16b).



Ka	12 M^{-1}
δ NH_{free}	4.40 ppm
δ NH_{bound}	6.57 ppm

	Ka_1	11 M^{-1}
	Ka_2	26 M^{-1}
2	δ_{00}	4.47 ppm
2⊃D_2O	½ (δ_{10} + δ_{01})	5.39 ppm
2⊃2D_2O	δ_{11}	6.11 ppm

Figure S16. Variation of the chemical shift observed at 298 K for the NH signal of receptor **2** dissolved in $CDCl_3/CD_3CN$ (2:1, v/v) for increasing amounts of H_2O with (a) the fitting of the 1:1 stoichiometry model and (b) the fitting of the 1:2 stoichiometry model. The concentration of the receptor is constant (about 1.2 mM). The number of H_2O equivalents was determined experimentally based on ^{1}H NMR integral data.

Fluoride binding study with receptor 2

CsF was chosen as a fluoride anions source since it can easily be dried by heating and is soluble enough in a $CDCl_3/CD_3OD$ mixture (i.e. 0.5 M in anhydrous $CDCl_3/CD_3OD$ 9:1, v/v). The titration experiment was meant to be done at constant concentration of receptor **2** (3.6 mM) in anhydrous $CDCl_3/CD_3OD$ (9:1, v/v). Therefore, a stock solution of dehydrated CsF (0.49 M) was prepared by using the solution of receptor **2** as solvent. The titration experiment was then completed by adding small amounts of this stock solution in the NMR tube initially containing the solution of the free receptor (Figure S17). Unexpectedly, decarbamation of **2** occurred in the stock solution overnight and so in the NMR tube but slower. This is attributed to the deprotonation of the NH groups of **2** by bound fluoride anions. Indeed, the downfield shift of the average NH signal at the beginning of the titration strongly suggests the binding of fluoride (or DF) by receptor **2**. The upfield shift observed after the fourth addition is likely to be due to the dilution of the receptor.

Interestingly, no tri-, di- or mono-carbamated intermediate was detected. Only compounds **2** and **1** were observed by NMR spectroscopy, which means that the loss of the first carbamate group is slow and is followed by a very fast degradation of the remaining carbamate groups. The buried NH groups of **2** are thus protected in comparison to the tri-, di- and mono-carbamated intermediates. These observations are consistent with the fact that the NH groups of calixarene **2** do not undergo deuterium exchange in the presence of CD_3OD and D_2O.

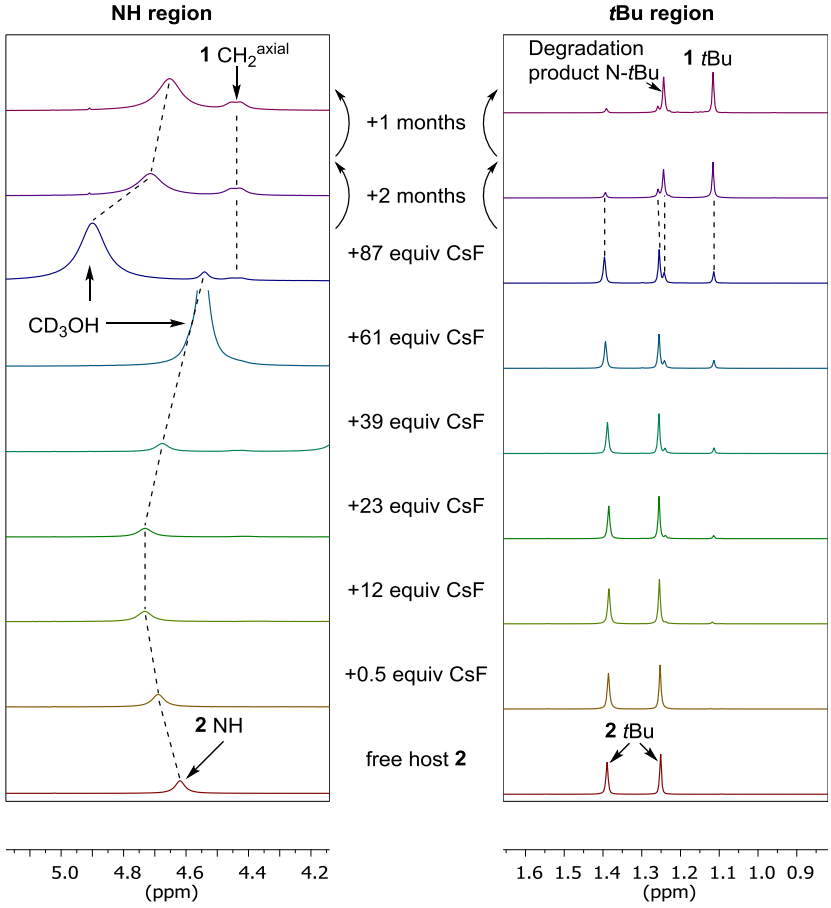


Figure S17. ^1H NMR spectra suggesting weak complexation of F^- by receptor **2** and slow degradation of **2** leading to the formation of *p*-*t*Bu-calix[4]arene **1** (400 MHz, $CDCl_3/CD_3OD$ (9:1, v/v), 298 K). These spectra were calibrated on the $CHCl_3$ signal.

Deuteration of the NH group of compound 3

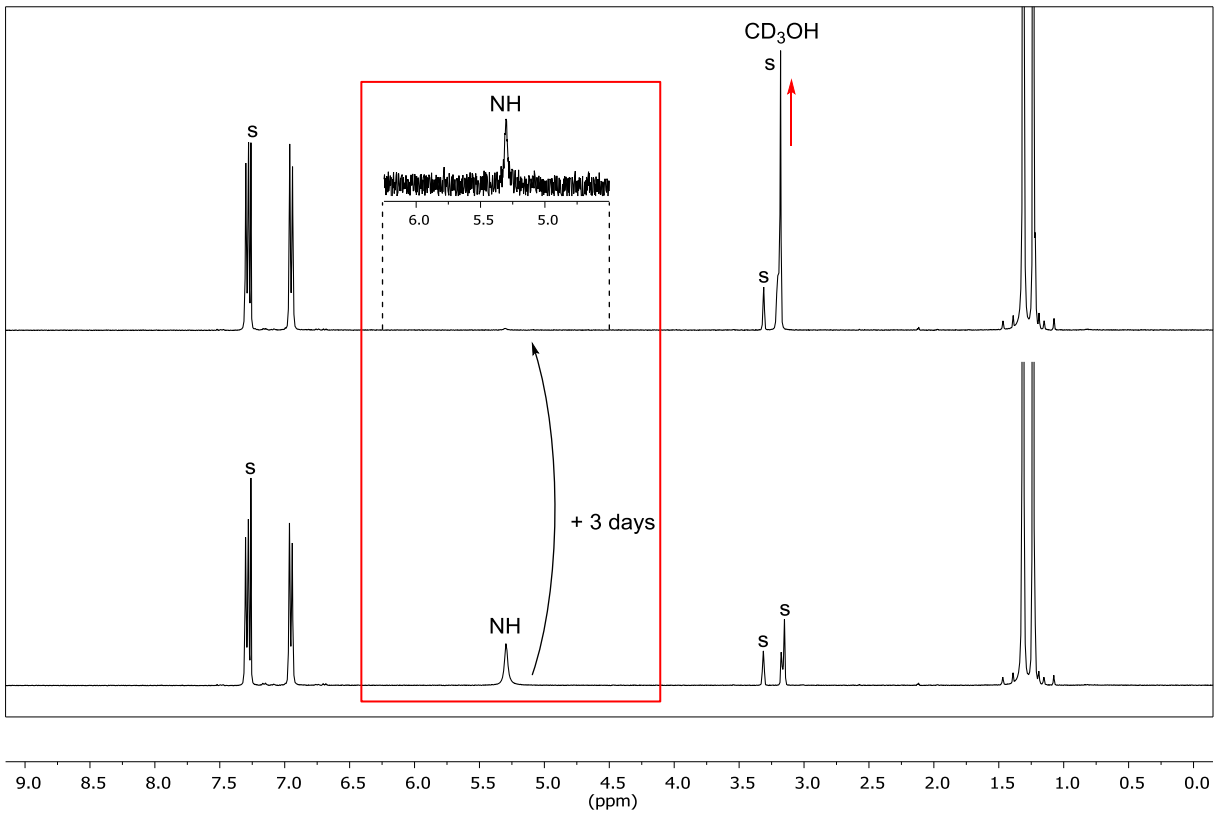


Figure S18. ^{1}H spectra showing the deuteration of the NH group of compound **3** in the presence of CD_3OD (400 MHz, $CDCl_3/CD_3OD$, (9:1, v/v), 298 K). The intense signals of the *t*Bu groups are truncated. s: residual solvents. These spectra were calibrated on the $CHCl_3$ signal.

Crystallographic data

Single crystal diffraction data have been collected at low temperature (145 K) on a Gemini Oxford Ruby diffractometer using Mo Ka radiation. Structure was solved by direct methods and refined using the Shelxl-2014 package.[1] Data have been deposited at CCDC with the following refcode : CCDC 1503962.

Semi-empirical absorption correction has been applied to the data set. A differential Fourier synthesis unambiguously gave the positions of all hydrogen atoms, including on the two encapsulated water molecules. Coordinates and isotropic thermal parameters Uiso were further refined in the riding model for all H atoms, except those of the water molecules. These last H atoms were refined using restraints on O-H covalent bonds (restrained to 0.84 Å).

Main crystallographic data and refinement details are provided in Table S19

Crystal Data			
Formula	C64 H92 N4 O8, 3(C2 H3 N), 2(C H2 Cl2), 2(H2 O)		
Formula Weight			1374.46
Crystal System			Monoclinic
Space group		P21/n	(No. 14)
a, b, c [Angstrom]	14.6718(5)	22.9104(8)	23.5888(9)
alpha, beta, gamma [deg]	90	104.564(4)	90
V [Ang**3]			7674.3(5)
Z			4
D(calc) [g/cm**3]			1.190
Mu(MoKa) [/mm]			0.212
F(000)			2952
Crystal Size [mm]		0.08 x 0.24 x	0.34

Data Collection			
Temperature (K)			145
Radiation [Angstrom]		MoKa	0.71073
Theta Min-Max [Deg]			2.7, 25.0
Dataset		-17: 16 ; -24: 27 ; -27: 28	
Tot., Uniq. Data, R(int)		44255, 13539,	0.037
Observed data [I > 2.0 sigma(I)]			10277

Refinement			
Nref, Npar			13539, 881
R, wR2, S		0.0928, 0.2769,	1.02
w = ^2^(FO^2^)+(0.1503P)^2^+18.9323P] WHERE P=(FO^2^+2FC^2^)/3'			
Max. and Av. Shift/Error			0.00, 0.00
Min. and Max. Resd. Dens. [e/Ang^3]			-0.92, 1.62

Table S19. - Crystal Data and Details of the Structure Determination

[1] Sheldrick, G. M. *Acta Cryst.* **2015**, *C71*, 3–8.

Cavity-volume calculations

Cavity-volume calculations were completed with the PLATON software[2] by using a grid step of 0.1 Å and the following atomic radii: C = 1.70 Å; H = 1.20 Å; N = 1.55 Å; O = 1.52 Å. The smallest probe radius commonly used for such calculations is 1.2 Å but no cavity was found with this value. Since there is no doubt that receptor **2** can accommodate up to two water molecules, the portals were closed by carbon atoms and the calculations were performed for probe radii ranging between 1.1 and 0.5 Å (Figure S21; probes with a radius smaller than 0.8 Å can exit through the portals if these are not closed; the 0.5 Å can exit through small interstices). The results are quoted in Table S20.

Two distinct cavities were found using a probe radius between 1.1 and 0.8 Å. For smaller probes, the hollow space consists in two cavities connected by a short and narrow channel with a total volume of about 65 Å^3. It must be stressed that no guest could pass through this channel. Hence, 65 Å^3 is not the solvent accessible volume; it is an upper bound for the inner volume of calixarene **2**. It is noteworthy that the CPK model of the bis-aqua complex **2**⊃2H_2O shows jammed guest molecules and water included in **2** is maybe an example of a shrinking guest.[3]

probe radius (Å)	Volume (Å^3)
1.2	*no cavity*
1.1	11 + 11
1.0	19 + 15
0.9	22 + 19
0.8	31 + 23
0.7	60
0.6	69
0.5	*probe exits*

Table S20. - Results of the cavity-volume calculations

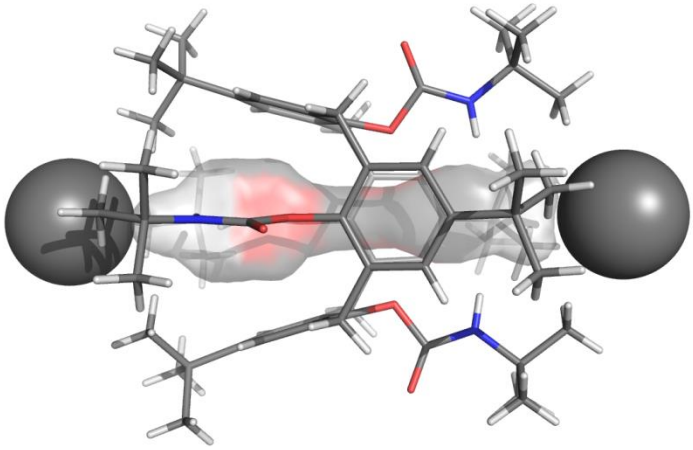


Figure S21. Crystal structure of **2**⊃2H_2O modified for cavity volume calculation. Included water molecules have been removed and carbon atoms have been added to close portals.

[2] Spek, A. L. *Acta Cryst.* **2009**, *D65*, 148–155.
[3] Haberhauer, G.; Woitschetzki, S.; Füten, C. *J. Org. Chem.* **2015**, *80*, 8065–8072.