Supporting Information

Selective recognition of quaternary ammonium ions and zwitterions by a biomimetic bis-calix[6]arene-based receptor

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SI1. Synthesis of G6

![Chemical Reaction](image)

Ethyl isocyanate (226 µL, 2.86 mmol) was added to a solution of (2-aminoethyl)trimethylammonium chloride hydrochloride (50 mg, 0.286 mmol) and triethylamine (80 µL, 0.574 mmol) in acetonitrile (3 mL). The reaction mixture was stirred for 16 h at room temperature and then concentrated under reduced pressure. The solid was dissolved in methanol (3 mL). Then, dichloromethane (5 mL) was added to the solution and the mixture was sonicated and filtered to afford the desired ammonium G6 as a white powder (56.1 mg, 94%).

$^1$H NMR (D$_2$O, 298 K, 400 MHz): $\delta$ (ppm) = 1.09 (t, $^3$J = 7.2 Hz, 3H, NCH$_2$CH$_3$), 3.14 (q, $^3$J = 7.2 Hz, 2H, NCH$_2$CH$_3$), 3.20 (s, 9H, N'(CH$_3$)$_3$), 3.46 (t, $^3$J = 6.8 Hz, 2H, N'CH$_2$CH$_2$), 3.64 (t, $^3$J = 6.8 Hz, 2H, N'CH$_2$CH$_2$). 13C NMR (D$_2$O, 298 K, 100 MHz, in presence of 5 µL of CH$_3$OH as internal reference): $\delta$ (ppm) = 15.04, 34.79, 35.54, 54.02, 65.80, 166.28. FTIR: $\nu$ (cm$^{-1}$): 2920, 1657, 1630, 1567, 1435, 1379. m.p.: > 260 °C.

$^1$H NMR (298 K, 400 MHz) spectrum of G6 in D$_2$O; W: Water.

$^{13}$C NMR (298 K, 100 MHz) spectrum of G6 in D$_2$O; r: reference (CH$_3$OH).
Dodecyl isocyanate (400 µL, 1.66 mmol) was added to a solution of choline chloride (232 mg, 1.66 mmol) in anhydrous acetonitrile (25 mL). The reaction mixture was stirred for 24 h at 85°C. After cooling to room temperature, the precipitate was isolated by suction filtration and was washed with diethyl ether (15 mL) to afford N-dodecylcarbamylcholine G7 as a white powder (582 mg, quant).

$^1$H NMR (CD$_3$OD, 298 K, 400 MHz): $\delta$ (ppm) = 0.90 (t, $^3J = 6.8$ Hz, 3H, CH$_3$), 1.29 (s, 18 H, CH$_3$(CH$_2$)$_9$), 1.50 (s, 2H, NHCH$_2$CH$_2$), 3.11 (t, $^3J = 7.0$ Hz, 2H, NHCH$_2$), 3.22 (s, 9H, N$^+$CH$_3$), 3.68 (s, 2H, N$^+$CH$_2$), 4.50 (s, 2H, OCH$_2$). $^{13}$C NMR (CD$_3$OD, 298 K, 100 MHz): $\delta$ (ppm) = 14.43, 23.72, 27.86, 30.45, 30.73 (m), 30.83, 33.06, 41.95, 54.50, 59.09, 66.59, 157.41. FTIR: $\nu$ (cm$^{-1}$): 2921, 2851, 1713, 1613, 1572, 1539, 1468, 1253, 1146. m.p.: 112 °C (dec).

$^1$H NMR (298 K, 400 MHz) spectrum of G7 in CD$_3$OD; S: Solvent; W: Water.

$^{13}$C NMR (298 K, 100 MHz) spectrum of G7 in CD$_3$OD; S: Solvent.
SI3. $^1$H NMR, COSY NMR, HMBC NMR and ROESY NMR spectra of 1 with acetylcholine G1 in CDCl$_3$.

$^1$H NMR (298K, 600MHz) spectrum of 1$\supset$G1 in CDCl$_3$; S: Solvent; W: Water; *: residual grease.

COSY NMR (298K, 300MHz) spectrum of 1$\supset$G1 in CDCl$_3$. 
HMBC NMR (298K, 600MHz) spectrum of 15G1 in CDCl₃.
ROESY NMR (273K, 600MHz) spectra of \(\text{15G1}\) in CDCl\(_3\).
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$^1$H NMR (298K, 300MHz) spectrum of 1⊃G2 in CDCl$_3$; S: Solvent; W: Water.

COSY NMR (298K, 300MHz) spectrum of 1⊃G2 in CDCl$_3$. 
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$^1$H NMR (263K, 600MHz) spectrum of 1 + 10 equiv. of choline G2 in CD$_3$OD/CDCl$_3$ (1:50); S: Solvent; W: Water.

HSQC NMR (263K, 600MHz) spectrum of 1·G2 in CD$_3$OD/CDCl$_3$ (1:50).
SI7. $^1$H NMR, COSY NMR and HSQC NMR spectra of 1 with carbamylcholine G3 in CD$_3$OD/CDCl$_3$ (1:50)

$^1$H NMR (298K, 600MHz) spectrum of 1$\supset$G3 in CD$_3$OD/CDCl$_3$ (1:50); S: Solvent; W: Water; $\ast$: residual grease.

COSY NMR (298K, 600MHz) spectrum of 1$\supset$G3 in CD$_3$OD/CDCl$_3$ (1:50).
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$^1$H NMR (298K, 600MHz) spectrum of 1⊂G4 in CD$_3$OD/CDCl$_3$ (1:50); S: Solvent; W: Water; *: residual grease.

COSY NMR (298K, 600MHz) spectrum of 1⊂G4 in CD$_3$OD/CDCl$_3$ (1:50).
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$^1$H NMR (298K, 600MHz) spectrum of 1⊃G5 in CD$_3$OD/CDCl$_3$ (1:50); S: Solvent; W: Water.

COSY NMR (298K, 600MHz) spectrum of 1⊃G5 in CD$_3$OD/CDCl$_3$ (1:50).
HSQC NMR (298K, 600MHz) spectrum of 1G5 in CD₃OD/CDCl₃ (1:50).
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$^1$H NMR (298K, 600MHz) spectrum of 1⊃G6 in CD$_3$OD/CDCl$_3$ (1:50); S: Solvent; W: Water; *: residual grease.

COSY NMR (298K, 600MHz) spectrum of 1⊃G6 in CD$_3$OD/CDCl$_3$ (1:50).
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$^1$H NMR (258K, 600MHz) spectrum of 1⊃G7 in CDCl$_3$; S: Solvent; W: Water.

COSY NMR (258K, 600MHz) spectrum of 1⊃G7 in CDCl$_3$. 
HSQC NMR (298K, 600MHz) spectrum of 1\(\text{G7} \) in CDCl\(_3\).

ROESY NMR (258K, 600MHz) spectrum of 1\(\text{G7} \) in CDCl\(_3\).
SI12. $^1$H NMR, COSY NMR and HSQC NMR spectra of 1 with trimethylglycine G11 in CDCl$_3$

$^1$H NMR (298K, 600MHz) spectrum of 1 $\supset$ G11 in CDCl$_3$; S: Solvent; W: Water; *: residual grease.

COSY NMR (298K, 600MHz) spectrum of 1 $\supset$ G11 in CDCl$_3$. 
HSQC NMR (298K, 600MHz) spectrum of 15G11 in CDCl₃.
SI13. $^1$H NMR spectrum of 1 with trimethylglycine G11 in CD$_3$OD/CDCl$_3$ (1:50)

$^1$H NMR (298K, 600MHz) spectrum of 1{$>$}G11 in CD$_3$OD/CDCl$_3$ (1:50); S: Solvent; W: Water; *: residual grease.
SI14. $^1$H NMR, COSY NMR and HSQC NMR spectra of 1 with $\beta$-alanine betaine G12 in CD$_3$OD/CDCl$_3$ (1:50)

$^1$H NMR (298K, 600MHz) spectrum of 1⊂G12 in CD$_3$OD/CDCl$_3$ (1:50); S: Solvent; W: Water; *: residual grease.

COSY NMR (298K, 600MHz) spectrum of 1⊂G12 in CD$_3$OD/CDCl$_3$ (1:50).
HSQC NMR (298K, 600MHz) spectrum of 12G12 in CD$_3$OD/CDCl$_3$ (1:50).
SI15. $^1$H NMR, COSY NMR and HSQC NMR spectra of 1 with deoxycarnitine G13 in CD$_3$OD/CDCl$_3$ (1:50)

$^1$H NMR (298K, 600MHz) spectrum of 1⊃G13 in CD$_3$OD/CDCl$_3$ (1:50); S: Solvent; W: Water; *: residual grease.

COSY NMR (298K, 600MHz) spectrum of 1⊃G13 in CD$_3$OD/CDCl$_3$ (1:50).
HSQC NMR (298K, 600MHz) spectrum of 1\textsuperscript{G}13 in CD\textsubscript{3}OD/CDCl\textsubscript{3} (1:50).
SI16. ¹H NMR, COSY NMR and HSQC NMR spectra of 1 with sulfobetaine-18 G14 in CDCl₃

¹H NMR (298K, 600MHz) spectrum of 1 + 1.9 equiv. of G14 in CDCl₃; S: Solvent; W: Water.

COSY NMR (298K, 600MHz) spectrum of 1+G14 in CDCl₃.
HSQC NMR (298K, 600MHz) spectrum of 1G14 in CDCl₃.
SI17. $^1$H NMR spectra of 1 with acetylcholine$^+$BARF$^-$ in CDCl$_3$

$^1$H NMR (CDCl$_3$, 258K, 300MHz) spectra of: a) 1, b) 1 + 0.8 equiv. of acetylcholine$^+$BARF$^-$; S: Solvent; W: Water; *: residual grease.
SI18. $^1$H NMR (298K, 600MHz) spectrum of 1 with carbamylcholine G3 in CDCl$_3$/D$_2$O (5:1).

$^1$H NMR (298K, 600MHz) spectrum of 1 with G3 in CDCl$_3$/D$_2$O (5/1) obtained after 24h of stirring; ○: Signals corresponding to free G3 which is solubilized in the water phase; S: Solvent; W: Water; *: residual grease.
SI19. Theoretic model for the determination of the kinetic association constant $k_{on}$

Complexation of sulfobetaines (G) by bis-calix[6]arene 1 proceeds with a 1 + 1 stoichiometry. This is an equilibrium process. Kinetic association and dissociation constants can be described respectively as $k_{on}$ and $k_{out}$. The kinetics of complex 1$\rightarrow$G formation depends on those two concurrent processes. It can be described as:

$$\frac{d[1\rightarrow G]}{dt} = k_{on}[1][G] - k_{out}[1\rightarrow G] \quad (1)$$

The dissociation kinetic constant can be defined as:

$$k_{out} = \frac{k_{on}}{K_a} \quad (2)$$

The studies were conducted with a large excess of the sulfobetaines G so their concentration remained almost unchanged, while the calix[6]arene 1 is partially consumed. Therefore, the concentration of active species can be expressed as:

$$[1] = [1]_0 - [1\rightarrow G]; \quad [G] \approx \text{const} = [G]_0 \quad (3)$$

Using the equations (1), (2) and (3), the kinetics of complex 1$\rightarrow$G formation can be written as:

$$\frac{d[1\rightarrow G]}{dt} = k_{on}([1]_0 - [1\rightarrow G])[G]_0 - \frac{k_{on}}{K_a}[1\rightarrow G]$$

$$= -k_{on}\left([G]_0 + \frac{1}{K_a}\right)[1\rightarrow G] + k_{on}[1]_0[G]_0 \quad (4)$$

Given that at $\tau = 0$ the complex is absent, i.e. $[1\rightarrow G]_0 = 0$, the solution of this differential equation can be written as:

$$[1\rightarrow G] = \frac{[1]_0[G]_0}{([G]_0 + \frac{1}{K_a})([G]_0 + \frac{1}{K_a})} - \frac{[1]_0[G]_0}{([G]_0 + \frac{1}{K_a})([G]_0 + \frac{1}{K_a})} e^{-k_{on}([G]_0 + \frac{1}{K_a})\tau}$$

$$\frac{[1\rightarrow G]}{[1]_0} = \frac{1}{1 + \frac{1}{K_a[G]_0}} - \frac{1}{1 + \frac{1}{K_a[G]_0}} e^{-k_{on}([G]_0 + \frac{1}{K_a})\tau} \quad (5)$$

Thus, the kinetic constant $k_{on}$ can be determined from an exponential fit of the 1$\rightarrow$G concentration over the reaction time.
SI20. Selected $^1$H NMR spectra of 1 with sulfobetaine-8 G14 in CDCl$_3$ and determination of $k_{on}$

Selected $^1$H NMR (333K, 600MHz) spectra of 1 with G14 in CDCl$_3$ at different times. Only aromatic, axial ArC$_2$H$_2$Ar and high field signals are shown for clarity. Starting concentrations of bis-calix[6]arene 1 and sulfobetaine-8 G14 are 1 mM and 9.4 mM respectively.

Singlet at 0.34 ppm corresponds to $\alpha$-CH$_3$ protons of the guest included into the calixarene cavity. It was used to estimate the concentration of the complex $1\supset G14$. Peaks in the 4.3-4.8 ppm region correspond to signals of axial ArCH$_2$Ar protons of free host 1 ($d$ at 4.51 ppm) and of the complex $1\supset G14$ ($2d$ at 4.68 ppm and 4.70 ppm). Based on integration of those signals, the $1\supset G14$ concentration – time dependence was estimated as shown on figure below.
Relative $\frac{[\text{G14}]}{[1]_0}$ concentration over time and fitted exponential growth.

Using founded fitting parameters, the kinetic constant $k_{\text{con}}$ was calculated accordingly to equation (5), giving a value of $1.86 \text{ M}^{-1} \cdot \text{min}^{-1} = 3.1 \cdot 10^{-2} \text{ M}^{-1} \cdot \text{s}^{-1}$. 
SI21. Selected $^1$H NMR spectra of 1 with sulfobetaine-10 G15 in CDCl$_3$ and determination of $k_{on}$

Selected $^1$H NMR (333K, 600MHz) spectra of 1 with G15 in CDCl$_3$ at different times. Only aromatic, axial ArC$_2$H$_2$Ar and high field signals are shown for clarity. Starting concentrations of bis-calix[6]arene 1 and sulfobetaine-10 G15 are 1 mM and 9.9 mM respectively.

Similarly to the case of sulfobetaine-8 G14 (vide supra), based on NMR peaks integration (regions 0.30-0.38 ppm and 4.30-4.80 ppm), the 1$\supset$G15 concentration – time dependence was estimated as shown on figure below.
Relative $\text{1G15}$ concentration over time and fitted exponential growth.

Using founded fitting parameters, the kinetic constant $k_{on}$ was calculated accordingly to equation (5) giving a value of $0.0609 \text{ M}^{-1} \cdot \text{min}^{-1} = 1.02 \cdot 10^{-3} \text{ M}^{-1} \cdot \text{s}^{-1}$. 


SI22. Selected $^1$H NMR spectra of 1 with sulfobetaine-18 G16 in CDCl$_3$ and determination of $k_{on}$

Selected $^1$H NMR (333K, 600MHz) spectra of 1 with G16 in CDCl$_3$ at different times. Only aromatic, axial ArCH$_2$Ar and high field signals are shown for clarity. Starting concentrations of bis-calix[6]arene 1 and sulfobetaine-18 G16 are 1 mM and 10.9 mM respectively.

Similarly to the case of sulfobetaine G14 (vide supra), based on NMR peaks integration (regions 0.30-0.38 ppm and 4.30-4.80 ppm), the 1$\supset$G16 concentration – time dependence was estimated as shown on figure below.
Relative $1\rightarrow G16$ concentration over time and fitted exponential growth.

Using founded fitting parameters, the kinetic constant $k_{on}$ was calculated accordingly to equation (5) giving a value of $0.000795 \text{ M}^{-1}\cdot\text{min}^{-1} = 1.33 \cdot 10^{-5} \text{ M}^{-1}\cdot\text{s}^{-1}$.
SI23. $^1$H NMR spectra of the complex $\text{1} \rightarrow \text{G15}$ in presence of a competing guest $\text{G3}$ and in a polar environment

$^1$H NMR (298 K, 600MHz) spectra of: (a) 1 with ca. 9 equiv. of G15 in CDCl$_3$; (b) 16h after addition of ca. 9 equiv. of G3 (in 10 μL of CD$_3$OD); (c) after evaporation and dissolution in CDCl$_3$/CD$_3$OD (2:3); (d) after 2 weeks at room temperature; ●: signals corresponding to complex $\text{1} \rightarrow \text{G15}$; ○: signals corresponding to complex $\text{1} \rightarrow \text{G3}$; □: signals corresponding to free receptor 1. Only selected regions are shown for clarity. The intensity of the high field signals as well as those of the ArCH$_2$Ar protons are adjusted as indicated at the right side of spectra.