Four Generations of Water Soluble Dendrimers with 9 to 243 Benzoate Tethers: Synthesis and Dendritic Effects on Their Ion Pairing with Acetylcholine, Benzyltriethylammonium and Dopamine in Water

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General data.
All reactions were carried out using Schlenk techniques or in a nitrogen-filled Vacuum Atmosphere drylab. $^{1}$H NMR spectra were recorded at 25°C with a Brucker AC 300 (300 MHz) spectrometer. $^{13}$C NMR spectra were obtained in the pulsed FT mode at 75.0 MHz with a Brucker AC 300 spectrometer and $^{29}$Si NMR spectra were obtained in at 59.6 MHz with a Brucker AC 300 spectrometer. All chemical shifts are reported in parts per million (d, ppm) with reference to Me$_4$Si (TMS). The MALDI TOF mass spectra were recorded with a PerSeptive Biosystems Voyager Elite (Framingham, MA) time-of-flight mass spectrometer. Elemental analyses were performed by the Center of Microanalyses of the CNRS at Lyon Villeurbanne, France. Diffusion measurements were performed at different AC concentrations using a $^{1}$H NMR pulsed-gradient experiment: the simulated spin-echo sequence which leads to the measurement of the diffusion coefficient $D$, where $D$ is the slope of the straight line obtained when ln($I$) is displayed against the gradient-pulse power’s square according to the following equation: ln($I$) = - $?^2G^2d^2/3$, where $I$ is the relative intensity of a chosen resonance, $?^2$ is the proton gyromagnetic ratio, $?^2$ is the intergradient delay (150 ms), $d$ is the gradient pulse duration (5 ms), and $G$ is the gradient intensity. The diffusion constant of water ($2.3 \times 10^{-9}$ m$^2$/s) was used to calibrate the instrument.
Synthesis of dendri-9-benzoate (4):

The dendrimer nonaiodide 3 (1.1 g, 0.482 mmol), the methyl 4-hydroxybenzoate (1.32 g, 8.68 mmol), K$_2$CO$_3$ (6.10 g, 43.4 mmol) and dry DMF (30 mL) were introduced in a Schlenk flask. The reaction mixture was stirred at 80°C for 48 h. The DMF was removed, the crude product was washed in 30 mL of dichloromethane and washed with water in order to remove the K$_2$CO$_3$. The organic layer was dried with Na$_2$SO$_4$, filtered and the solvent was removed in vacuo. The product was washed with methanol and precipitated twice in CH$_2$Cl$_2$/methanol in order to remove the excess of methyl 4-hydroxybenzoate. The dendrimer-nona-benzoate de methyl was obtained as a colourless waxy product (1.089 g, 90% yield).

$^1$H NMR (CDCl$_3$, 250MHz): 7.93 and 6.88 (d, 18H, arom), 7.01 (s, 3H, arom. core), 3.86 (s, 27H, COOCH$_3$), 3.50 (s, 18H, SiCH$_2$O), 1.65 (s, 18H, CH$_2$CH$_2$CH$_2$Si), 1.13 (s, 18H, CH$_2$CH$_2$CH$_2$Si), 0.57 (s, 18H, CH$_2$CH$_2$CH$_2$Si), 0.040 (s, 54H, Si(CH$_3$)$_2$). $^{13}$C NMR (CDCl$_3$, 62 MHz): 165.8 (COOCH$_3$), 164.2 (arom. CqO), 144.8 (CH, arom. core), 130.4 and 112.7 (CH, arom), 121.2 (arom. CqCOOCH$_3$), 59.7 (SiCH$_2$O), 50.7 (COOCH$_3$), 42.9 (CH$_2$CH$_2$CH$_2$Si), 41.0 (CqCH$_2$), 16.8 (CH$_2$CH$_2$CH$_2$), 13.6 (CH$_2$CH$_2$CH$_2$Si), -5.6 (SiMe$_2$). $^{29}$Si NMR (CDCl$_3$, 59.62 MHz): 0.55 (SiCH$_2$O). MS (MALDI-TOF; m/z) Calcd. for C$_{135}$H$_{192}$O$_{27}$Si$_9$Na : 2 522.71 ; found: 2 520.21 (MNa$^+$). Anal. Calc. for C$_{135}$H$_{192}$O$_{27}$Si$_9$: C 64.86, H 7.74; found: C 64.37, H 7.56. Infrared C=O: 1 719 cm$^{-1}$.
Synthesis of dendri-27-benzoate (7):

Dendri-27-iodide (0.1 g, 0.016 mmol), methyl 4-hydroxybenzoate (0.134 g, 0.882 mmol), K$_2$CO$_3$ (0.609 g, 4.40 mmol) and dry DMF (20 mL) were introduced in a Schlenk flask. The reaction mixture was stirred at 80°C for 48 h. DMF was removed, the crude product was solved in 30 mL of dichloromethane and washed with water in order to remove the K$_2$CO$_3$. The organic layer was dried with Na$_2$SO$_4$, filtered, and the solvent was removed in vacuo. The product was washed with methanol and precipitated twice in CH$_2$Cl$_2$/methanol in order to remove the excess of methyl 4-hydroxybenzoate. The dendri-81-benzoate was obtained as a colourless waxy product (0.140 g, 92% yield).

$^1$H NMR (CDCl$_3$, 250MHz): 7.94 and 6.88 (d, 54H, outer arom), 7.10 and 6.80 (d, 18H, inner arom), 3.84 (s, 81H, COOCH$_3$), 3.51 (s, 72H, SiCH$_2$O), 1.60 (s, 72H, CH$_2$CH$_2$CH$_2$Si), 1.11 (s, 72H, CH$_2$CH$_2$CH$_2$Si), 0.55 (s, 72H, CH$_2$CH$_2$CH$_2$Si), 0.025 (s, 216H, Si(CH$_3$)$_2$). $^{13}$C NMR (CDCl$_3$, 62 MHz): 165.8 (COOCH$_3$), 164.2 (outer arom. CqO), 158.1 (inner arom. CqO), 130.4 and 112.8 (CH, arom.), 121.1 (arom. CqCOOCH$_3$), 59.7 (SiCH$_2$O), 50.7 (COOCH$_3$), 41.9 (CH$_2$CH$_2$CH$_2$Si), 41.9 (CqCH$_2$), 16.6 (CH$_2$CH$_2$CH$_2$), 13.5 (CH$_2$CH$_2$CH$_2$Si), -5.5 (SiMe$_2$). $^{29}$Si NMR (CDCl$_3$, 59.62 MHz) $\delta$ ppm: 0.53 (SiCH$_2$O). MS (MALDI-TOF; m/z) Calcd. For C$_{504}$H$_{732}$O$_{90}$Si$_{36}$: 9264.9816; found: 9265.4374. Infrared $\nu$C=O: 1719 cm$^{-1}$. Mass spectrum of the dendri-27-ester.
Synthesis of dendri-81-benzoate (9):

Dendri-81-iodide 6 (0.30 g, 0.011 mmol), methyl 4-hydroxybenzoate (0.27 g, 1.78 mmol), K$_2$CO$_3$ (1.25 g, 8.91 mmol) and dry DMF (30 mL) were introduced in a Schlenk flask. The reaction mixture was stirred at 80°C for 48 h. DMF was removed, the crude product was solved in 30 mL of dichloromethane and washed with water in order to remove the K$_2$CO$_3$. The organic layer was dried with Na$_2$SO$_4$, filtered, and the solvent was removed in vacuo. The product was washed with methanol and precipitated twice in CH$_2$Cl$_2$/methanol in order to remove the excess of methyl 4-hydroxybenzoate. The dendri-81-benzoate was obtained as a colourless waxy product (0.289 g, 89% yield).

$^1$H NMR (CDCl$_3$, 250MHz): 7.94 and 6.88 (d, 162H, outer arom), 7.10 and 6.80 (d, 72H, inner arom), 3.84 (s, 243H, COOCH$_3$), 3.51 (s, 234H, SiCH$_2$O), 1.60 (s, 234H, CH$_2$CH$_2$CH$_2$Si), 1.11 (s, 234H, CH$_2$CH$_2$CH$_2$Si), 0.55 (s, 234H, CH$_2$CH$_2$CH$_2$Si), 0.034 (s, 702H, Si(CH$_3$)$_2$). $^{13}$C NMR (CDCl$_3$, 62 MHz): 167.2 (C$_{OOCH_3}$), 164.2 (outer arom. CqO), 159.4 (inner arom. CqO), 131.8 and 114.2 (CH, arom.), 122.5 (arom. CqCOOCH$_3$), 61.1 (SiCH$_2$O), 52.2 (COOCH$_3$), 43.4 (CH$_2$CH$_2$CH$_2$Si), 42.3 (CqCH$_2$), 18.0 (CH$_2$CH$_2$CH$_2$), 14.9 (CH$_2$CH$_2$CH$_2$Si), -4.3 (SiMe$_2$). $^{29}$Si NMR (CDCl$_3$, 59.62 MHz) $\delta$ ppm: 0.53 (SiCH$_2$O). MS (MALDI-TOF; m/z) Calcd. For C$_{1611}$H$_{2352}$O$_{279}$Si$_{117}$: 29 469.75; found: 29 471.00. Anal. Calc. for C$_{1611}$H$_{2352}$O$_{279}$Si$_{117}$: C 65.63, H 7.99; found: C 65.58, H 8.04. Infrared ?C=O: 1 719 cm$^{-1}$. 

Mass spectrum of the dendri-81-ester.
Synthesis of dendri-243-benzoate (11):

Dendri-243-iodide 6 (0.10 g, 0.001 mmol), methyl 4-hydroxybenzoate (0.088 g, 0.576 mmol), K$_2$CO$_3$ (0.398 g, 2.88 mmol) and dry DMF (20 mL) were introduced in a Schlenk flask. The reaction mixture was stirred at 80°C for 48 h. DMF was removed, the crude product was solved in 30 mL of dichloromethane and washed with water in order to remove the K$_2$CO$_3$. The organic layer was dried with Na$_2$SO$_4$, filtered, and the solvent was removed in vacuo. The product was washed with methanol and precipitated twice in CH$_2$Cl$_2$/methanol in order to remove the excess of methyl 4-hydroxybenzoate. The dendri-81-benzoate was obtained as a colourless waxy product (0.289 g, 89% yield).

$^1$H NMR (CDCl$_3$, 250MHz): 7.94 and 6.88 (d, 486H, outer arom), 7.10 and 6.80 (d, 234H, inner arom), 3.79 (s, 729H, COOC$_3$H$_7$), 3.47 (s, 720H, SiCH$_2$O), 1.59 (s, 720H, CH$_2$CH$_2$CH$_2$Si), 1.01(s, 720H, CH$_2$CH$_2$CH$_2$Si), 0.53 (s, 720H, CH$_2$CH$_2$CH$_2$Si), -0.012 (s, 2160H, Si(CH$_3$)$_2$). $^{13}$C NMR (CDCl$_3$, 62 MHz): 165.7 (COOCH$_3$), 164.2 (outer arom. CqO), 159.4 (inner arom. CqO), 130.4 and 112.8 (CH, arom.), 121.1 (arom. CqCOOCH$_3$), 59.6 (SiCH$_2$O), 50.7 (COOCH$_3$), 42.0 (CH$_2$CH$_2$CH$_2$Si), 40.9 (CqCH$_2$), 16.6 (CH$_2$CH$_2$CH$_2$), 13.5 (CH$_2$CH$_2$CH$_2$Si), -5.7 (SiMe$_2$). $^{29}$Si NMR (CDCl$_3$, 59.62 MHz) δ ppm: 0.47 (SiCH$_2$O). Infrared ?$_{c=O}$: 1 719 cm$^{-1}$. 
Synthesis of dendri-9-benzoic (5):
The dendri-9-benzoate \(6\) (0.50 g, 0.20 mmol), was dissolved in dioxane (40 mL) and 10 mL of an aqueous solution of NaOH (18 mmol, 10 equiv. per branch) was added. The reaction mixture was stired at 60°C for 48 h. Dioxane was removed under vacuum and the aqueous solution was acidified with HCl. The dendri-9-acid precipitated as a white powder. The solution was filtrated and the powder was washed twice with ether. The product was recovered from filter by dissolving it with methanol. The methanol was removed \textit{in vacuo} and the product was obtained as a white powder with 72% yield. \(^1H\) NMR (MeOD, 250MHz): 7.93 and 6.91 (d, 18H, arom), 7.06 (s, 3H, arom-core), 3.52 (s, 18H, Si\(CH_2\)O), 1.64 (s, 18H, \(CH_2CH_2CH_2Si\)), 1.16 (s, 18H, \(CH_2CH_2CH_2Si\)), 0.54 (s, 18H, \(CH_2CH_2CH_2Si\)), -0.003 (s, 54H, Si(CH_3)_2). \(^1^3\)C NMR (MeOD, 62 MHz): 169.8 (COOH), 166.7 (arom. CqO), 147.2 (CH, arom. core), 132.9 and 115.0 (CH, arom), 123.0 (arom. CqCOOCH_3), 61.9 (SiCH_2O), 45.2 (CH_2CH_2CH_2Si), 43.3 (CqCH_2), 19.3 (CH_2CH_2CH_2), 15.9 (CH_2CH_2CH_2Si), -4.1 (SiMe_2). \(^2^9\)Si NMR (MeOD, 59.62 MHz) \(\delta\) ppm: 0.36 (SiCH_2O). MS (MALDI-TOF; m/z) Calcd. for C_{126}H_{174}O_{27}Si_9Na: 2396.47; found: 2394.44 (MNa\(^+\)). Anal. Calc. for C_{126}H_{174}O_{27}Si_9: C 63.76, H 7.39; found: C 62.71, H 7.22. Infrared \(\nu_{C=O}\): 1686 cm\(^{-1}\).

\(^1H\) NMR of 7 (\(D_2O + NaOH, 250\) MHz): 7.82 and 6.79 (d, 18H, arom), 7.08 (s, 3H, arom-core), 3.41 (s, 18H, Si\(CH_2\)O), 1.68 (s, 18H, \(CH_2CH_2CH_2Si\)), 1.16 (s, 18H, \(CH_2CH_2CH_2Si\)), 0.52 (s, 18H, \(CH_2CH_2CH_2Si\)), -0.045 (s, 54H, Si(CH_3)_2).
Synthesis of dendri-27-benzoic (8):

Dendri-27-benzoate 7 (0.070 g, 0.007 mmol), was dissolved in dioxane (45 mL), and 5 mL of an aqueous solution of NaOH (2.5 mmol, 12 equiv. per branch) was added. The reaction mixture was stirred at 60°C for 48 h. Dioxane was removed under vacuum, and the aqueous solution was acidified with HCl. Dendri-81-acid precipitated as a white powder. The solution was filtrated, and the powder was washed twice with ether. The product was recovered from filter by dissolving in methanol. The methanol was removed in vacuo, and the product was obtained as a white powder in 67 % yield.

$^1$H NMR (MeOD, 250MHz): 7.91 and 6.86 (d, 54H, outer arom), 7.10 and 6.79 (d, 18H, inner arom), 3.48 (s, 81H, SiC$_2$H$_2$O), 1.60 (s, 72H, CH$_2$CH$_2$CH$_2$Si), 1.15 (s, 72H, CH$_2$CH$_2$CH$_2$Si), 0.53 (s, 72H, CH$_2$CH$_2$CH$_2$Si), -0.056 (s, 216H, Si(CH$_3$)$_2$). $^{13}$C NMR (MeOD, 62 MHz): 168.5 (COOH), 165.4 (outer arom. CqO), 159.1 (inner arom. CqO), 131.5 and 113.6 (CH, arom.), 122.3 (arom. CqCOOCH$_3$), 60.4 (SiCH$_2$O), 42.9 (CH$_2$CH$_2$CH$_2$Si), 41.8 (CqCH$_2$), 17.6 (CH$_2$CH$_2$CH$_2$), 14.4 (CH$_2$CH$_2$CH$_2$Si), -4.9 (SiMe$_2$). $^{29}$Si NMR (MeOD, 59.62 MHz) δ ppm: 1.57 (SiCH$_2$O). MS (MALDI-TOF; m/z) Calcd. For C$_{477}$H$_{678}$O$_{90}$Si$_{36}$: 8886.3; found: 8886.4. Infrared ?C=O: 1 686 cm$^{-1}$.
Synthesis of dendri-81-benzoic (10):

Dendri-81-benzoate 9 (0.20 g, 0.0068 mmol), was dissolved in dioxane (50 mL), and 5 mL of an aqueous solution of NaOH (5.51 mmol, 10 equiv. per branch) was added. The reaction mixture was stired at 60°C for 48 h. Dioxane was removed under vacuum, and the aqueous solution was acidified with HCl. Dendri-81-acid precipitated as a white powder. The solution was filtrated, and the powder was washed twice with ether. The product was recovered from filter by dissolving in methanol. The methanol was removed in vacuo, and the product was obtained as a white powder in 67% yield.

$^1$H NMR (MeOD, 250MHz): 7.90 and 6.83 (d, 162H, outer arom), 7.08 and 6.75 (d, 72H, inner arom), 3.43 (s, 234H, SiCH$_2$O), 1.58 (s, 234H, CH$_2$CH$_2$CH$_2$Si), 1.10 (s, 234H, CH$_2$CH$_2$CH$_2$Si), 0.49 (s, 234H, CH$_2$CH$_2$CH$_2$Si), -0.056 (s, 702H, Si(CH$_3$)$_2$). $^{13}$C NMR (MeOD, 62 MHz): 169.9 (COOH), 166.8 (outer arom. CqO), 160.5 (inner arom. CqO), 133.0 and 115.1 (CH, arom.), 123.8 (arom. CqCOOCH$_3$), 61.8 (SiCH$_2$O), 44.3 (CH$_2$CH$_2$CH$_2$Si), 43.4 (CqCH$_2$), 19.0 (CH$_2$CH$_2$CH$_2$), 15.8 (CH$_2$CH$_2$CH$_2$Si), -4.0 (SiMe$_2$). $^{29}$Si NMR (MeOD, 59.62 MHz) $\delta$ ppm: 0.26 (SiCH$_2$O). Anal. Calc. for C$_{1530}$H$_{2190}$O$_{279}$Si$_{117}$: C 64.86, H 7.79; found: C 64.25, H 7.68. Infrared $\nu$C=O: 1686 cm$^{-1}$. 
Synthesis of dendri-243-benzoic (12):

Dendri-243-benzoate 7 (0.06 g, 0.0007 mmol), was dissolved in dioxane (45 mL), and 5 mL of an aqueous solution of NaOH (2.5 mmol, 15 equiv. per branch) was added. The reaction mixture was stirred at 60°C for 48 h. Dioxane was removed under vacuum, and the aqueous solution was acidified with HCl. Dendri-81-acid precipitated as a white powder. The solution was filtrated, and the powder was washed twice with ether. The product was recovered from filter by dissolving in methanol. The methanol was removed in vacuo, and the product was obtained as a white powder in 67 % yield.

$^1$H NMR (MeOD, 250MHz): 7.94 and 6.84 (d, 486H, outer arom), 7.08 and 6.75 (d, 234H, inner arom), 3.36 (s, 720H, SiCH$_2$O), 1.62 (s, 720H, CH$_2$CH$_2$CH$_2$Si), 1.10 (s, 720H, CH$_2$CH$_2$CH$_2$Si), 0.54 (s, 720H, CH$_2$CH$_2$CH$_2$Si), -0.020 (s, 2160H, Si(CH$_3$)$_2$). $^{13}$C NMR (MeOD, 62 MHz): 168.6 (COOH), 165.3 (outer arom. CqO), 160.5 (inner arom. CqO), 131.6 and 113.6 (CH, arom.), 122.3 (arom. CqCOOCH$_3$), 60.4 (SiCH$_2$O), 44.3 (CH$_2$CH$_2$CH$_2$Si), 43.4 (CqCH$_2$), 19.0 (CH$_2$CH$_2$CH$_2$), 14.3 (CH$_2$CH$_2$CH$_2$Si), -5.3 (SiMe$_2$). Infrared $\nu$$_{C=O}$: 1 686 cm$^{-1}$. 

Chemical Shift Variations of the AC proton signals with the dendri-9-carboxylate (5).

NMR titrations have been performed by titrating dendrimer solution with cation solutions.

The number $n$ of AC molecules bound to the dendrimer is a function of the variation $\Delta \delta$ of chemicals shift (equation 1):

$$\Delta \delta = \frac{1}{2} \Delta \delta_{\text{max}} \left[ \left( 1 + \frac{K_d}{n[D_0]} + \frac{[AC]}{n[D_0]} \right) - \left( \frac{1 + \frac{K_d}{n[D_0]} + \frac{[AC]}{n[D_0]}}{\left( 1 + \frac{K_d}{n[D_0]} + \frac{[AC]}{n[D_0]} \right)^2 - 4 \frac{[AC]}{n[D_0]} \right)^{1/2} \right]$$

$n$: number of AC molecules bound to the dendri-9-carboxylate; $[D_0]$: total concentration of the dendrimer; $[AC]$: concentration of AC; $K_d$: dissociation constant; $\Delta \delta_{\text{max}}$: the highest chemical shift variation.

1H NMR titration of dendri-9-carboxylate solution with AC solution: a) dendri-9-carboxylate as its Na+ salt; b) dendri-9-carboxylate + 1 eq. of AC; c) dendri-9-carboxylate + 2 eq. of AC; d) dendri-9-carboxylate + 3 eq. of AC; e) dendri-9-carboxylate + 4 eq. of AC; f) dendri-9-carboxylate + 5 eq. of AC; g) dendri-9-carboxylate + 6 eq. of AC; h) dendri-9-carboxylate + 7 eq. of AC; i) dendri-9-carboxylate + 8 eq. of AC; j) dendri-9-carboxylate + 9 eq. of AC; k) dendri-9-carboxylate + 10 eq. of AC; l) dendri-9-carboxylate + 12 eq. of AC; m) dendri-9-carboxylate + 14 eq. of AC; n) dendri-9-carboxylate + 16 eq. of AC; o) dendri-9-carboxylate + 20 eq. of AC; p) AC alone.

$\Delta \delta_{\text{max}} = 0.32$

$q_2 = 0.06$

For the first 9 molecules of AC bound to dendri-9-carboxylate: $K_{d1} = 20 (\pm 2) \times 10^{-3}$ M

For the other 9 molecules of AC bound to dendri-9-carboxylate: $K_{d2} = 1 (\pm 0.1)$ M
Chemical Shift Variations of the AC proton signals with the dendri-27-carboxylate (8).

NMR titrations have been performed by titrating dendrimer solution with cation solutions.

$$\Delta \delta_{\text{max}} = 0.35$$

$$\sigma_2 = 0.02$$

For the first 27 molecules of AC bound to dendri-27-carboxylate: $K_d_1 = 18 \pm 0.15 \times 10^{-3}$ M

For the other 27 molecules of AC bound to dendri-27-carboxylate: $K_d_2 = 8 \pm 0.8 \times 10^{-1}$ M
Chemical Shift Variations of the AC proton signals with the dendri-81-carboxylate (10).

NMR titrations have been performed by titrating dendrimer solution with cation solutions.

\[ \Delta \delta_{\text{max}} = 0.6 \]

\[ \varphi_2 = 0.04 \]

For the first 81 molecules of AC bound to dendri-81-carboxylate: \[ K_d_1 = 17 \pm 2 \times 10^{-3} \text{ M} \]

For the other 81 molecules of AC bound to dendri-81-carboxylate: \[ K_d_2 = 230 \pm 20 \times 10^{-3} \text{ M} \]
Chemical Shift Variations of the AC proton signals with the dendri-243-carboxylate (12).

NMR titrations have been performed by titrating dendrimer solution with cation solutions.

Δδ_{max} = 0.46

?_2 = 0.08

For the first 243 molecules of AC bound to dendri-243-carboxylate: Kd₁ = 13 (±1.3) x 10^{-3} M

For the other 243 molecules of AC bound to dendri-243-carboxylate: Kd₂ = 8 (0.8) x 10^{-2} M
Chemical Shift Variations of the BTEA proton signals with the dendri-9-carboxylate (5).

NMR titrations have been performed by titrating dendrimer solution with cation solutions.

\[ \Delta \delta_{\text{max}} = 0.9 \]
\[ K_d^1 = 8 \pm 0.8 \times 10^{-3} \text{ M} \]
\[ K_d^2 = 3 \pm 0.3 \times 10^{-1} \text{ M} \]

\( 1^H \) NMR titration of dendri-9-carboxylate solution with BTEA solution: a) dendri-9-carboxylate as its Na+ salt; b) dendri-9-carboxylate + 2 eq. of BTEA; c) dendri-9-carboxylate + 4 eq. of BTEA; d) dendri-9-carboxylate + 6 eq. of BTEA; e) dendri-9-carboxylate + 8 eq. of BTEA; f) dendri-9-carboxylate + 9 eq. of BTEA; g) dendri-9-carboxylate + 10 eq. of BTEA; h) dendri-9-carboxylate + 12 eq. of BTEA; i) dendri-9-carboxylate + 14 eq. of BTEA; j) dendri-9-carboxylate + 15 eq. of BTEA; k) dendri-9-carboxylate + 18 eq. of BTEA; l) BTEA alone.
Chemical Shift Variations of the BTEA proton signals with the dendri-27-carboxylate (8).

NMR titrations have been performed by titrating dendrimer solution with cation solutions.

\[ \Delta \delta_{\text{max}} = 1.1 \]
\[ \gamma_2 = 0.02 \]

For the first 27 molecules of BTEA bound to dendri-27-carboxylate: \( K_d_1 = 7 (\pm 0.7) \times 10^{-3} \text{ M} \)

For the other 27 molecules of BTEA bound to dendri-27-carboxylate: \( K_d_2 = 3 (\pm 0.3) \times 10^{-1} \text{ M} \)
Chemical Shift Variations of the BTEA proton signals with the dendri-81-carboxylate (10).

NMR titrations have been performed by titrating dendrimer solution with cation solutions.

\[ \Delta \delta_{\text{max}} = 1.16 \]

\[ \beta_2 = 0.03 \]

For the first 81 molecules of BTEA bound to dendri-81-carboxylate: \( K_d = 6 (\pm 0.6) \times 10^{-3} \) M

For the other 81 molecules of BTEA bound to dendri-81-carboxylate: \( K_d = 3 (\pm 0.3) \times 10^{-1} \) M
Chemical Shift Variations of the BTEA proton signals with the dendri-243-carboxylate (12).

NMR titrations have been performed by titrating dendrimer solution with cation solutions.

\[ \Delta \delta_{\text{max}} = 0.6 \]
\[ ?_2 = 0.08 \]

For the first 243 molecules of BTEA bound to dendri-243-carboxylate: \( K_d_1 = 5 (\pm 0.5) \times 10^{-3} \text{ M} \)

For the other 243 molecules of BTEA bound to dendri-243-carboxylate: \( K_d_2 = 3 (\pm 0.3) \times 10^{-1} \text{ M} \)
Chemical Shift Variations of the Dopamine proton signals with the dendri-9-carboxylate (5).

NMR titrations have been performed by titrating dendrimer solution with cation solutions.

\[ n = 9 \]
\[ \Delta \delta_{\text{max}} = 0.35 \]
\[ \beta_2 = 0.009 \]

For the 9 molecules of Dopamine bound to dendri-9-carboxylate: \( K_d = 5 (\pm 0.5) \times 10^{-4} \text{ M} \)
Chemical Shift Variations of the Dopamine proton signals with the dendri-27-carboxylate (8).

NMR titrations have been performed by titrating dendrimer solution with cation solutions.

\[ n = 27 \]
\[ \Delta \delta_{\text{max}} = 0.37 \]
\[ ?^2 = 0.007 \]

For the 27 molecules of Dopamine bound to dendri-27-carboxylate: \[ K_d = 4 (\pm 0.4) \times 10^{-4} \text{ M} \]
Chemical Shift Variations of the Dopamine proton signals with the dendri-81-carboxylate (10).  

NMR titrations have been performed by titrating dendrimer solution with cation solutions.

\[ \Delta \delta_{\text{max}} = 0.54 \]
\[ \sigma^2 = 0.009 \]

For the 81 molecules of Dopamine bound to dendri-81-carboxylate: \( K_d = 3 (\pm 0.3) \times 10^{-4} \text{ M} \)
Chemical Shift Variations of the Dopamine proton signals with the dendri-243-carboxylate (12).

NMR titrations have been performed by titrating dendrimer solution with cation solutions.

\[ \Delta \delta_{\text{max}} = 0.41 \]

\[ K_d = 2.0 \times 10^{-4} \text{ M} \]

For the 243 molecules of Dopamine bound to dendri-243-carboxylate: Kd = 2 (±0.2) x 10^{-4} M
Table of the Kd values: \( \Delta \delta_{\text{max}} \) is the maximum of the observed chemical displacement; \( \delta_2 \) translated the difference between the experimental points and the numerical values extracted from a theoretical curve; \( n_1 \) is the number of cationic molecules fixed with the first association constant; \( Kd_1 \) is the first dissociation constant; \( Ka_1 \) is the first association constant; \( n_2 \) is the number of cationic molecules fixed with the second association constant; \( Kd_2 \) is the second dissociation constant and \( Ka_2 \) is the second association constant. The error of all the values of this table is 10%.

<table>
<thead>
<tr>
<th>Supramolecular assembly</th>
<th>( \Delta \delta_{\text{max}}^a ) (ppm)</th>
<th>( \delta_2^b )</th>
<th>( n_1^c )</th>
<th>( Kd_1^d ) (M)</th>
<th>( Ka_1^e ) (M(^{-1}))</th>
<th>( n_2^f )</th>
<th>( Kd_2^g ) (M)</th>
<th>( Ka_2^h ) (M(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dendri-9-carboxylate + AC</td>
<td>0.32</td>
<td>0.06</td>
<td>9</td>
<td>20 x 10(^{-3})</td>
<td>50</td>
<td>9</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Dendri-27-carboxylate + AC</td>
<td>0.35</td>
<td>0.02</td>
<td>27</td>
<td>18 x 10(^{-3})</td>
<td>56</td>
<td>27</td>
<td>8 x 10(^{-1})</td>
<td>1</td>
</tr>
<tr>
<td>Dendri-81-carboxylate + AC</td>
<td>0.6</td>
<td>0.04</td>
<td>81</td>
<td>17 x 10(^{-3})</td>
<td>59</td>
<td>81</td>
<td>23 x 10(^{-2})</td>
<td>4</td>
</tr>
<tr>
<td>Dendri-243-carboxylate + AC</td>
<td>0.46</td>
<td>0.08</td>
<td>243</td>
<td>13 x 10(^{-3})</td>
<td>77</td>
<td>243</td>
<td>8 x 10(^{-2})</td>
<td>12</td>
</tr>
<tr>
<td>Dendri-9-carboxylate + BTEA</td>
<td>0.9</td>
<td>0.007</td>
<td>9</td>
<td>8 x 10(^{-3})</td>
<td>125</td>
<td>9</td>
<td>3 x 10(^{-1})</td>
<td>3</td>
</tr>
<tr>
<td>Dendri-27-carboxylate + BTEA</td>
<td>1.1</td>
<td>0.02</td>
<td>27</td>
<td>7 x 10(^{-3})</td>
<td>143</td>
<td>27</td>
<td>3 x 10(^{-1})</td>
<td>3</td>
</tr>
<tr>
<td>Dendri-81-carboxylate + BTEA</td>
<td>1.16</td>
<td>0.03</td>
<td>81</td>
<td>6 x 10(^{-3})</td>
<td>167</td>
<td>81</td>
<td>3 x 10(^{-1})</td>
<td>3</td>
</tr>
<tr>
<td>Dendri-243-carboxylate + BTEA</td>
<td>0.6</td>
<td>0.08</td>
<td>243</td>
<td>5 x 10(^{-3})</td>
<td>200</td>
<td>243</td>
<td>3 x 10(^{-1})</td>
<td>3</td>
</tr>
<tr>
<td>Dendri-9-carboxylate + Dopamine</td>
<td>0.35</td>
<td>0.009</td>
<td>9</td>
<td>5 x 10(^{-4})</td>
<td>2000</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Dendri-27-carboxylate + Dopamine</td>
<td>0.37</td>
<td>0.007</td>
<td>27</td>
<td>4 x 10(^{-4})</td>
<td>2500</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Dendri-81-carboxylate + Dopamine</td>
<td>0.54</td>
<td>0.009</td>
<td>81</td>
<td>3 x 10(^{-4})</td>
<td>3333</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Dendri-243-carboxylate + Dopamine</td>
<td>0.41</td>
<td>0.002</td>
<td>243</td>
<td>2 x 10(^{-4})</td>
<td>5000</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
Measurements of diffusion coefficient by $^1$H NMR upon titration of dendrimer-81-carboxylate (10) with cations (AC, BTEA and dopamine).

The goal of this series of experiments is to measure the diffusion coefficient (noted D) by $^1$H NMR. The studied molecules are the dendrimer-81-carboxylate and the three cations (AC, BTEA and dopamine). The sample are those used during the titration by $^1$H NMR.

First, the measurement of D allows to calculate the hydrodynamic diameter of a molecule. Then the $^1$H NMR experiment focuses on the diffusion that is mathematically treated according to a process DOSY (Diffusion Ordered SpectroscopY) in order to obtain the equivalent of a spectral chromatography. The objective is thus double: measure the size of the two free and bound molecules in solution by $^1$H NMR, and obtain a DOSY spectrum that will account for the purity of the product.

The dendri-81-carboxylate is considered as a spherical molecular object, and characterized by an apparent diffusion coefficient. The application of the Stokes-Einstein law gives an estimate of the diameter of the molecule.

Stokes-Einstein law:

$$D = \frac{K_B T}{6\pi \eta r_H}$$

D: diffusion constant; $K_B$: Boltzman’s constant; T: temperature (K); $\eta$: solvent viscosity; $r_H$: hydrodynamic radius of the species.
DOSY spectrum of dendrimer-81-carboxylate (10) in D$_2$O

The four signals on the line (top) represent the log(D) of the dendri-81-carboxylate, and the last signal below the line represents the log(D) of water.

$D_d = 4.441 \pm 0.1 \times 10^{-11}$ m$^2$/s

$R_{Hd} = 5.517$ nm

$D_d$: diffusion coefficient of the dendri-81-carboxylate; $R_{Hd}$: hydrodynamic radius of the dendri-18-carboxylate.
The four signals on the line (top) represent the log(D) of one molecule of acetylcholine (AC), and the last signal below the line represents the log(D) of water.

\[ D_{AC} = 5.948 \pm 0.1 \times 10^{-10} \text{ m}^2/\text{s} \]

\[ R_{H, AC} = 0.594 \text{ nm} \]

\( D_{AC} \): diffusion coefficient of AC; \( R_{H, AC} \): hydrodynamic radius of AC.
Superposition of the three DOSY spectra: free dendrimer (10), free acetylcholine (AC), and assembly dendrimer +AC in D₂O

The four black signals on the line (top) represent the log(D) of the free dendrimer; the four red signals on the line (top) represent the log(D) of the bound to AC dendrimer; the four black signals on the medium line represent the log(D) of a molecule of AC bound to the dendrimer; the four blue signals (medium) represent the log(D) of a free molecule of AC. The last multicolor signal on the line below represents the log(D) of water.

\[ D_\text{d} = 4.998 \pm 0.1 \times 10^{-11} \text{ m}^2/\text{s} \]

\[ R_{H,d} = 4.902 \text{ nm} \]

\( D_\text{d} \): diffusion coefficient of the dendrimer; \( R_{H, d} \): hydrodynamic radius of the dendrimer.
Evolution of diffusion coefficients of AC and dendrimer (10) as a function of the concentration of AC in water.
DOSY spectrum of assembly dendrimer (10) + BTEA in D$_2$O

The two signals on the line (top) represent the log(D) of the bound to BTEA dendrimer; the four black signals on the medium line represent the log(D) of a molecule of BTEA bound to the dendrimer; the last signal on the line below represents the log(D) of water.

\[ D_d = 5.4 \times 10^{-11} \text{ m}^2/\text{s} \]

\[ R_{H_d} = 4.537 \text{ nm} \]

\( D_d \): diffusion coefficient of the dendrimer; \( R_{H_d} \): hydrodynamic radius of the dendrimer.
DOSY spectrum of assembly dendrimer (10) + dopamine in D$_2$O

The two signals on the line (top) represent the log(D) of the bound to dopamine dendrimer; the four black signals on the medium line represent the log(D) of a molecule of dopamine bound to the dendrimer; the last signal on the line below represents the log(D) of water.

\[ D_d = 7 \, (\pm 1) \times 10^{-11} \, \text{m}^2/\text{s} \]

\[ R_{Hd} = 3.5 \, \text{nm} \]

\( D_d \): diffusion coefficient of the dendrimer; \( R_{Hd} \): hydrodynamic radius of the dendrimer.
The ROESY spectrum shows that there is no dipolar interaction between the molecules of acetylcholine (AC) and the cavity of the dendrimer; this confirms that the molecules of AC are bound to the dendrimer at its periphery.
Chemical shift variation of the AC proton signals with the dendri-81-carboxylate (10) by $^{13}$C NMR

NMR titrations have been performed by titrating dendrimer solution with cation solutions.

$^{13}$C NMR titration of dendri-81-carboxylate solution with AC solution: a) dendri-81-carboxylate as its Na$^+$ salt; b) dendri-81-carboxylate + 81 eq. of AC; c) dendri-81-carboxylate + 162 eq. of AC; d) AC alone.