Supplementary Material (ESI) for Chemical Communications

New Water-soluble Polyanionic Dendrimers and Transport of Acetylcholin in Water By Means of Supramolecular Interactions

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Supplementary Information

Experimental section.

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 (δ, ppm) with reference to Me_4Si (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: $\ln(I) = -\gamma^2G^2D\delta^2(\Delta-\delta/3)$, where I is the relative intensity of a chosen resonance, γ is the proton gyromagnetic ratio, Δ is the intergradient delay (150 ms), δ is the gradient pulse duration (5 ms), and G is the gradient intensity. The diffusion constant of water (2.3×10^{-9} m²/s) was used to calibrate the instrument.

Synthesis of dendri-81-benzoate, 7:

Dendri-81-iodide **6** (0.30 g, 0.011 mmol), methyl 4-hydroxybenzoate (0.27 g, 1.78 mmol), K_2CO_3 (1.25 g, 8.91 mmol) and dry DMF (30 mL) were indroduced 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_2CO_3. The organic layer was dried with Na_2SO_4, filtered, and the solvent was removed *in vacuo.* The product was washed with methanol and precipitated twice in CH_2Cl_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, $SiCH_2O$), 1.60 (s, 234H, $CH_2CH_2CH_2Si$), 1.11 (s, 234H, $CH_2CH_2CH_2Si$), 0.55 (s, 234H, $CH_2CH_2CH_2Si$), 0.034 (s, 702H, $Si(CH_3)_2$). ^{13}C NMR ($CDCl_3$, 62 MHz): 167.2 ($COOCH_3$), 164.2 (outer arom. CqO), 159.4 (*inner* arom. CqO), 131.8 and 114.2 (CH, arom.), 122.5 (arom. $CqCOOCH_3$), 61.1 ($SiCH_2O$), 52.2 ($COOCH_3$), 43.4 ($CH_2CH_2CH_2Si$), 42.3 ($CqCH_2$), 18.0 ($CH_2CH_2CH_2$), 14.9 ($CH_2CH_2CH_2Si$), -4.3 ($SiMe_2$). ^{29}Si NMR ($CDCl_3$, 59.62 MHz) δ ppm: 0.53 ($SiCH_2O$). 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 $\upsilon_{C=O}$: 1 719 cm^{-1}.

Synthesis of dendri-81-acid, 8:

Dendri-81-benzoate **7** (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.

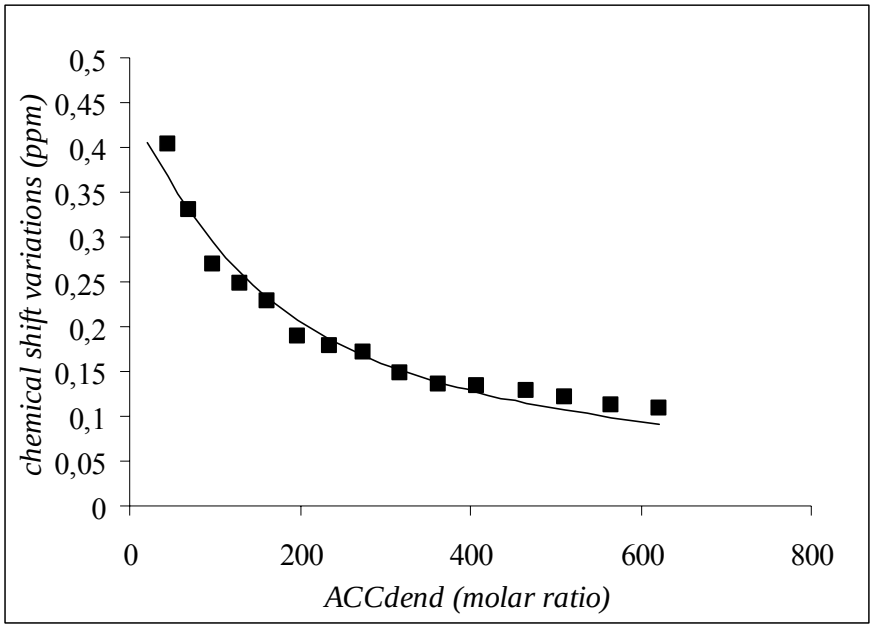
^{1}H NMR (MeOD, 250MHz): 7.90 and 6.83 (d, 162H, *outer arom*), 7.08 and 6.75 (d, 72H, *inner arom*), 3.43 (s, 234H, SiCH_2O), 1.58 (s, 234H, $CH_2CH_2CH_2$Si), 1.10 (s, 234H, $CH_2CH_2CH_2$Si), 0.49 (s, 234H, $CH_2CH_2CH_2$Si), -0.056 (s, 702H, Si(CH_3)$_2$). ^{13}C NMR (MeOD, 62 MHz): 169.9 (*C*OOH), 166.8 (*outer* arom. *C*qO), 160.5 (*inner* arom. *C*qO), 133.0 and 115.1 (*C*H, arom.), 123.8 (arom. *C*q$COOCH_3$), 61.8 (SiCH_2O), 44.3 ($CH_2CH_2CH_2$Si), 43.4 (*C*qCH_2), 19.0 ($CH_2CH_2CH_2$), 15.8 ($CH_2CH_2CH_2$Si), -4.0 ($SiMe_2$). ^{29}Si NMR (MeOD, 59.62 MHz) δ ppm: 0.26 (*Si*CH_2O). 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 $\upsilon_{C=O}$: 1 686 cm^{-1}.

Chemical Shift Variations of the AC proton signals.

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

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

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



$\Delta\delta_{max} = 0{,}6$

$\chi_2 = 0{,}04$

For the first 81 molecules of AC bound to **9:** $Kd_1 = 17\ (\pm 2) \times 10^{-3}$ M

For the other 81 molecules of AC bound to **9:** $Kd_2 = 230\ (\pm 20) \times 10^{-3}$ M

Measurements of diffusion coefficient by ^{1}H NMR upon titration of acetylcholine (AC) with the dendrimer-81-benzoate 9.

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-benzoate **9** and the acetylcholine (AC).

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.

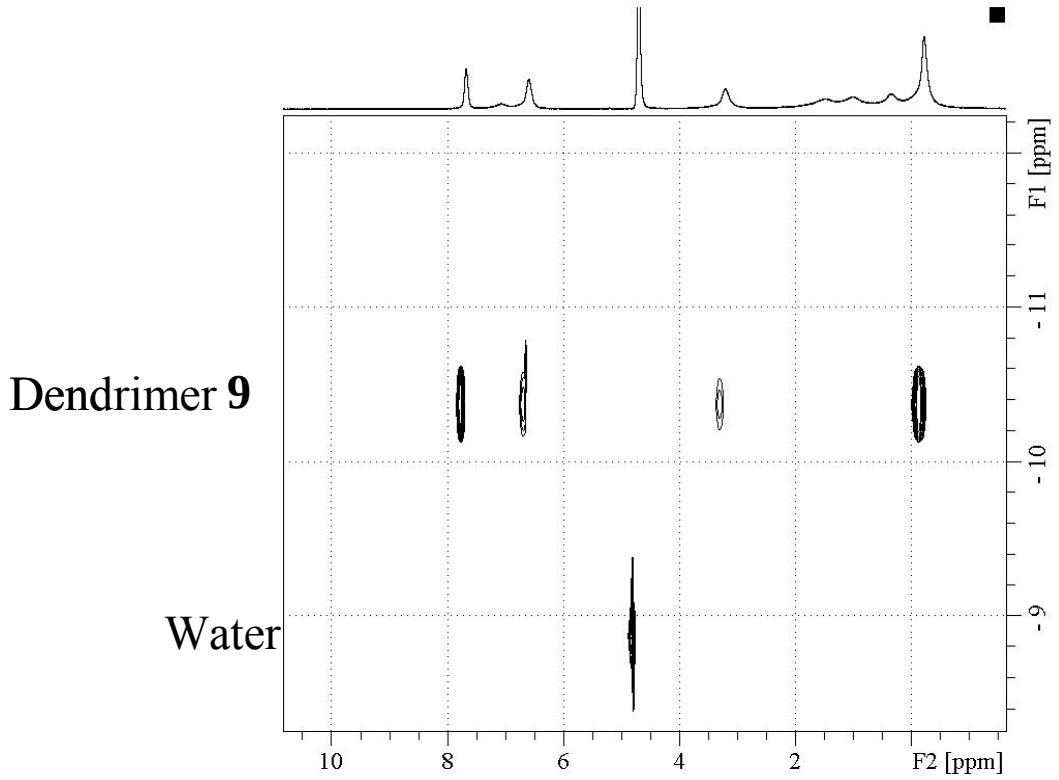
The dendrimer **9** is considered as a spherical molecular object, and characterized by an apparent diffusion coefficient. The application of the Stokes-Einstein law gives an estimate of the diameter of the molecule.

Stokes-Einstein law:

$D = K_B T / 6\pi\eta r_H$

D: diffusion constant; K_B: Boltzman's constant; T: temperature (K); η: solvent viscosity; r_H: hydrodynamic radius of the species.

DOSY spectrum of the dendrimer-81- benzoate 9 in D_2O



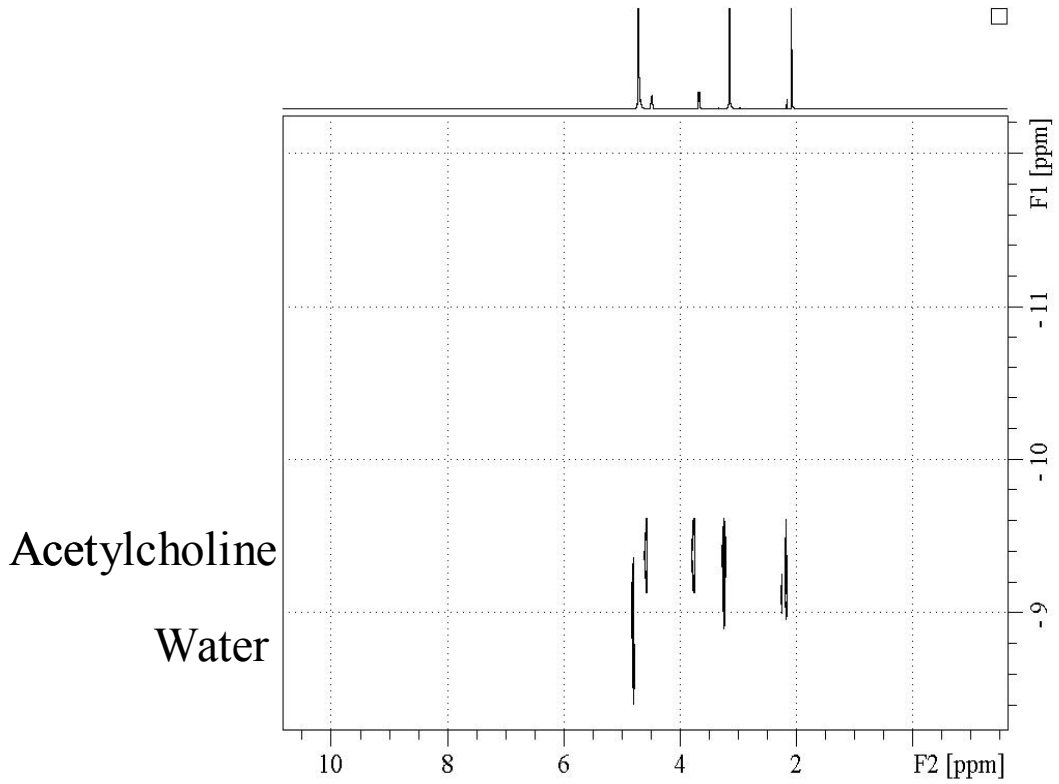
The four signals on the line (top) represent the log(D) of **9,** and the last signal below the line represents the log(D) of water.

$D_{\mathbf{9}} = 4.441 \times 10^{-11}$ m²/s (SD = 8.819×10^{-5})

$R_{H\,\mathbf{9}} = 5.517$ nm

$D_{\mathbf{9}}$: diffusion coefficient of the dendrimer **9**; $R_{H\,\mathbf{9}}$: hydrodynamic radius of the dendrimer **9**; SD: standard deviation.

DOSY spectrum of acetylcholine (AC) in D_2O



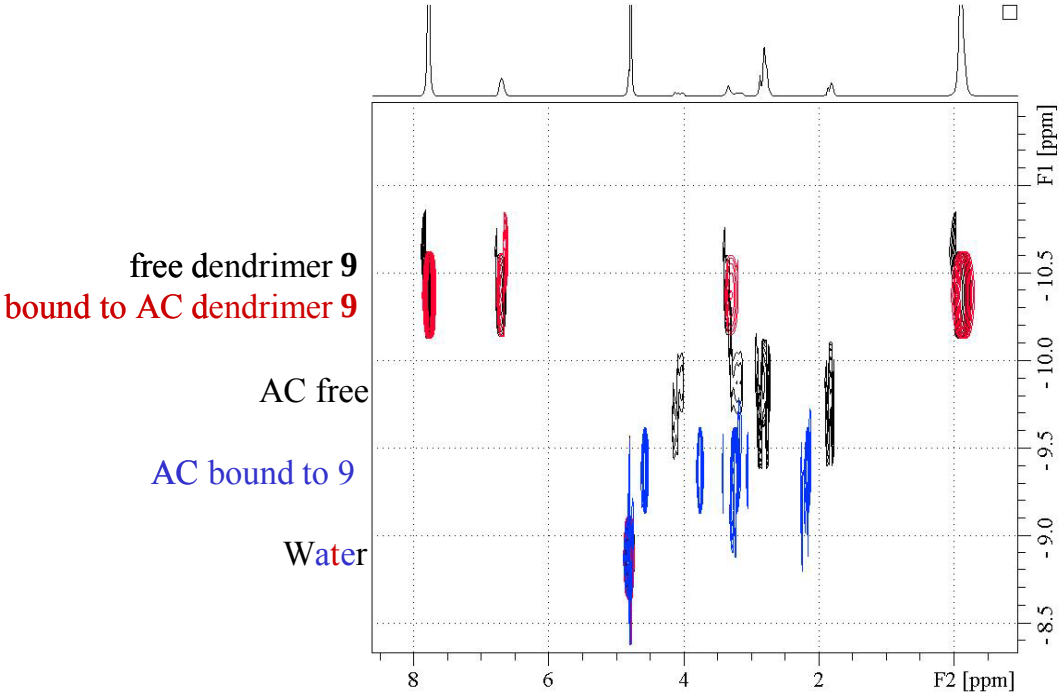
The four signals on the line (top) represent the log(D) of one molecule of acetylcholine (AC)**,** and the last signal below the line represents the log(D) of water.

$D_{\mathbf{AC}} = 5.948 \times 10^{-10}$ m²/s (SD = 9.308×10^{-4})

$R_{H\,\mathbf{AC}} = 0.594$ nm

$D_{\mathbf{AC}}$: diffusion coefficient of AC; $R_{H\,\mathbf{AC}}$: hydrodynamic radius of AC; SD: standard deviation.

Superposition of the three DOSY spectra: free dendrimer 9, free acetylcholine (AC), and assembly 9 +AC in D_2O:



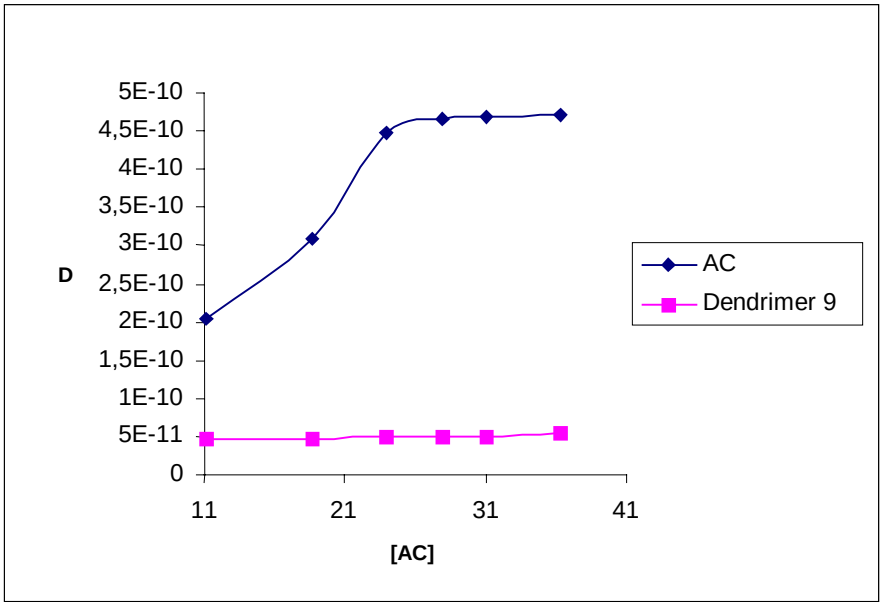
The four black signals on the line (top) represent the log(D) of the free dendrimer **9**; the four red signals on the line (top) represent the log(D) of the bound to AC dendrimer **9**; the four black signals on the medium line represent the log(D) of a free molecule of AC; the four blue signals (medium) represent the log(D) of a molecule of AC bound to the dendrimer. The last multicolor signal on the line below represents the log(D) of water.

$D_{\mathbf{9}} = 4.441 \times 10^{-11}$ m²/s (SD = 8.819×10^{-5})

$R_{H\,\mathbf{9}} = 5.517$ nm

$D_{\mathbf{9}}$: diffusion coefficient of the dendrimer **9**; $R_{H\,\mathbf{9}}$: hydrodynamic radius of the dendrimer **9**; SD: standard deviation.

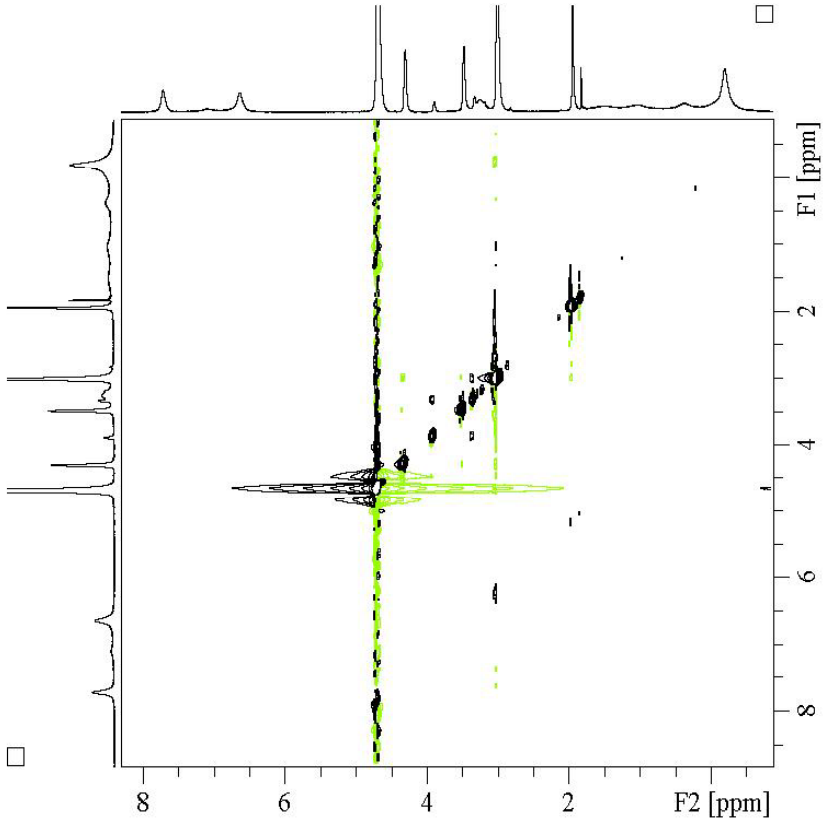
Evolution of the diffusion coefficient of AC as a function of its concentration in water



The diffusion coefficient of the dendrimer **9** does not vary the titration; this information means that the dendrimer has approximately the same size alone and in the complex.

The diffusion coefficient of AC very clearly increases until a concentration of 27.9 mM, that corresponds to 162 AC per dendrimer, before stagnating at higher concentrations. This means that there is a real interaction between the AC molecules and the dendrimer until a molar ratio of 162 AC per dendrimer. After this stage, the diffusion coefficient observed is an average between the free AC and the AC bound to the dendrimer, and its value slowly approaches that of free AC.

ROESY spectrum of acetylcholine (AC) in D_2O



The ROESY spectrum shows that there is no dipolar interaction between the molecules of acetylcholine (AC) and the cavity of the dendrimer **9**; this confirms that the molecules of AC are bound to the dendrimer **9** at its periphery.