New water-soluble polyanionic dendrimers and binding to acetylcholine in water by means of contact ion-pairing interactions†

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A new water-soluble polyanionic dendrimer containing 81 benzoate termini (diameter: 11 ± 1 nm from DOSY NMR spectroscopy) has been synthesized; it interacts with acetylcholine cations in water-soluble assemblies in which each carboxylate terminus reversibly forms contact ion pairs and aggregates at the tether termini, as shown by ^{1}H NMR spectroscopy.

The supramolecular facets of dendrimers[1] have been largely considered for uses as molecular boxes,[2] exoreceptors,[3] and sensors.[4] Indeed, applications of water-soluble dendrimers as drug vectors are most promising.[5,6] A possible drawback of polycationic dendrimers, however, is their toxicity. On the other hand, it has been recognized that polyanionic dendrimers usually display acceptable biocompatibility.[7,8] Therefore, we have investigated the route to polybenzoate-terminated dendrimers synthesized from polyiodomethylsilyl precursors, and their ability to transport cations of biomedical interest such as acetylcholine.

Acetylcholine (AC) chloride is produced naturally by the nervous system. It is also used as an active ingredient in some drugs, but it is not very active upon oral ingestion because of hydrolysis in the digestive tube. AC chloride has various pharmacological properties: it can be used as a parasympathomimetic,[9a] a peripheral vasodilator, an antihypertensive, a miotic or a coronarodilator.[9b] Its muscarinic parasympathomimetic action consists in contracting the smooth fibre in the digestive tube,[10b] the eye and the bronchi.[10a] It is used in a drug marketed as a parasympathomimetic preparation for intraocular use, although aqueous solutions are unstable and must thus be prepared just before use.[11]

We now report the assembly of polyanionic dendrimers and dendrimer–acetylcholine molecular architectures, including their water-solubility properties and ion-pair behavior in water.

For the dendritic construction, we used the 1 → 3 C connectivity, pioneered by Newkome *et al.*,[12] as shown in Scheme 1. It starts with the known nona-allylation of [FeCp(η^6-mesitylene][PF_6], **1**, that quantitatively yields the nona-allyl dendritic core 1,3,5-[$C(CH_2CH{=}CH_2)_3$]C_6H_3, **2**, on a large scale subsequent to visible-light photolysis that removes the metal moiety.[13] Hydrosilylation of the terminal olefinic bonds, a reaction pioneered in dendrimer synthesis by van Leeuwen *et al.*,[14] is carried out on **2**, using chloromethyldimethylsilane and Karsted catalyst, regioselectively giving the nona-chloromethyldimethylsilyl intermediate that, upon reaction with NaI, provides the nona-iodide **3**. The dendritic progression was achieved using the known phenoltriallyl dendron *p*-(HO)$C_4H_4C(CH_2CH{=}CH_2)_3$, obtained by one-pot reaction of [FeCp(η^6-*p*-chlorotoluene][PF_6] with allyl bromide and *t*-BuOK (Scheme 1).[13]

Williamson's reaction of the dendri-81-iodide, **6**, with methyl 4-hydroxybenzoate yielded the dendri-81-benzoate, **7**, that was characterized by its molecular peak at 29 471 (M^+) in the MALDI TOF mass spectrum (calcd for $C_{1611}H_{2352}O_{279}Si_{117}$: 29 469.75). The ^{1}H NMR spectrum of the dendrimer-81-acid, **8**, in MeOD shows all the signals of the molecule, including all the protons of the dendritic core, confirming the expected structure. When this dendrimer is solubilized in water upon addition of a stoichiometric amount of NaOH yielding **9**, the ^{1}H NMR spectrum in D_2O shows the proton signals of the periphery only.

The dendrimer **9**, containing 81 sodium carboxylate groups, reversibly reacts with AC chloride to form water-soluble assemblies whose structure can be examined by ^{1}H NMR spectroscopy.[15] The interaction between the dendrimer **9** and AC is characterized in the ^{1}H NMR spectrum by the large upfield shift of the four AC signals upon interaction with **9**. The dendrimer signals also move, but to a lesser extent, the average shift being 0.06 ppm in water for the peripheral protons numbered from 5 to 8. The titration of AC was achieved in order to tentatively quantify the number of AC that can be transported by **9**.‡

When the first 20 equivalents of AC are added, the AC signals are shifted from 4.56 ppm to 4.04 ppm for the C**H$_2$**–CH_2–N protons numbered 3, from 3.75 ppm to 3.18 ppm for the C**H$_2$**–N protons numbered 2, from 3.23 ppm to 2.78 ppm for the C**H$_3$**–N numbered 1, and from 2.16 ppm to 1.81 ppm for the C**H$_3$**–COO protons (see Fig. 1). These results correspond to an average displacement of 0.5 ppm. The four signals of AC are shifted during this titration because of the interactions of the whole molecule with **9**. Indeed, when AC interacts with **9**, the ammonium AC group should be located at the dendrimer periphery, reversibly forming contact ion pairs and aggregates with the carboxylate ion.

The number *n* of AC molecules bound to the dendrimer is determined as a function of the variation $\Delta\delta$ of chemical shifts according to eqn (1):[15]

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

n: number of AC molecules bound to **9**; $[D_0]$: total concentration of **9**; [AC]: total concentration of AC; K_d: dissociation constant.

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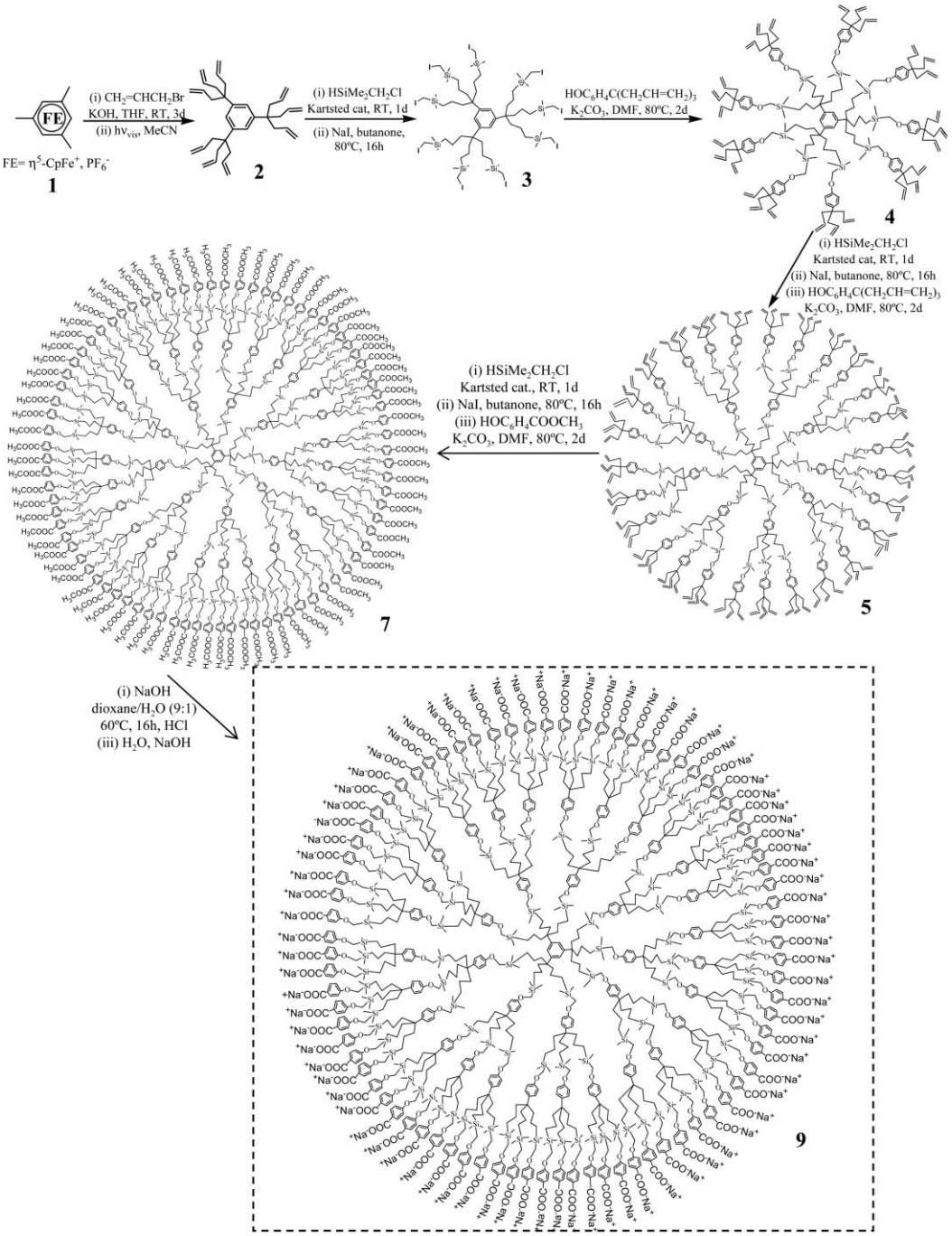
† Electronic supplementary information (ESI) available: Synthesis and detailed ^{1}H NMR data including DOSY and ROESY experiments. See DOI: 10.1039/b710391c



Scheme 1 Synthesis of the water soluble dendrimer-81-carboxylate G_2 (**9**).

The best fit is that for which dendrimer **9** interacts with 2 ($\pm$0.1) molecules of AC per dendritic branch. Indeed, the first AC molecule (per branch) interacts at the dendrimer periphery by electrostatic interaction with a dissociation constant K_{d1} of 17 ($\pm$2) $\times$ 10^{-3} M. Then, the other AC molecule (per branch) also interacts, with seemingly weaker interactions, with a dissociation constant K_{d2} of 230 ($\pm$20) $\times$ 10^{-3} M (eqn (2), R = dendrimer, R′ = AC).

$$\mathrm{RCO_2^-Na^+ + R'N^+Me_3Cl^- \overset{K_{d1}}{\rightleftharpoons} RCO_2^-R'N^+Me_3 \overset{K_{d2}}{\rightleftharpoons} RCO_2^-\left(R'N^+Me_3\right)_2} \quad (2)$$

$[Na(H_2O)_x]^+[Cl(H_2O)_y]^-$ is also formed in eqn (2).

DOSY experiments were carried out in order to follow the evolution of the diffusion coefficient of the dendrimer without AC and with an increasing concentration of AC. The dendrimer without AC in water has a diffusion coefficient of 4.4 $\times$ 10^{-11} m^2 s^{-1}, and a hydrodynamic diameter of 11 $\pm$ 1 nm; a molecule of AC has a diffusion coefficient of 5.9 $\times$ 10^{-10} m^2 s^{-1}, and a hydrodynamic diameter of 1.2 $\pm$ 0.1 nm (see the ESI†). The diffusion coefficient of **9** does not vary during the titration, meaning that the dendrimer has approximately the same size whether it is free or bound. This lack of dendrimer size increase upon AC binding, together with the upfield shift of the AC NMR

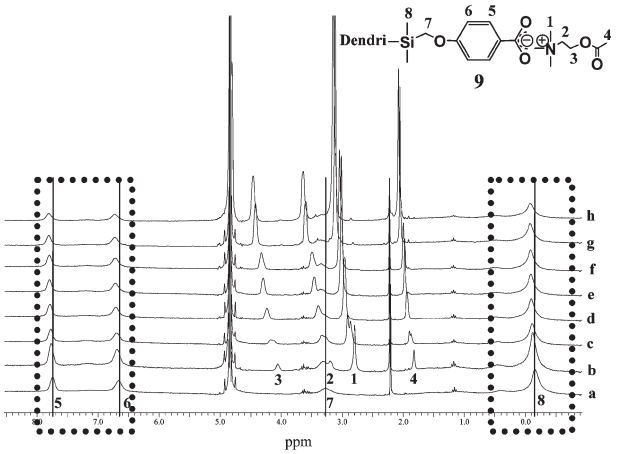


Fig. 1 ^{1}H NMR titration of AC with **9**: (a) **9** as its Na^+ salt; (b) **9** + 20 eq. of AC; (c) **9** + 40 eq. of AC; (d) **9** + 60 eq. of AC; (e) **9** + 80 eq. of AC; (f) **9** + 100 eq. of AC; (g) **9** + 200 eq. of AC; (h) **9** + 300 eq. of AC (similar to AC).

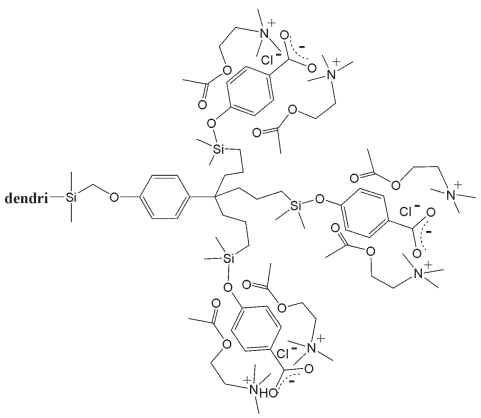


Fig. 2 Arbitrary representation of the ionic aggregates with 2 AC at the termini of **9**, taking into account the mutual influence of the AC and benzoate groups on the ^{1}H NMR shifts and lack of dendrimer size increase with AC (the AC molecules, whose ammonium parts form an aggregate with the carboxylate and chloride anions, are also folding back towards the hydrophobic dendritic interior).

signals upon dendrimer binding, suggest encapsulation of AC in the hydrophobic dendrimer interior (near the periphery, back folding of the dendrimer tethers being not excluded).

The diffusion coefficient of AC increases until a concentration matching approximately 162 ($\pm$5) AC per dendrimer, confirming the interaction of each carboxylate termini with two AC molecules, before stagnating at higher AC concentrations.

The observed behavior of the assembly **9** + AC is best taken into account by the reversible formation of ionic bonds between the dendrimer-81-carboxylate **9** and the AC cations as contact ion pairs.[16] The second stage most probably also involves agglomeration of additional charges of AC chloride to reversibly form an aggregate at each tether terminus. This should be due to the dual location of the anionic charge, delocalized onto both carboxylate oxygen atoms of the carboxylate group, that can form a five-component aggregate (one chloride anion in addition to the two oxygen atoms and the two AC cations, see Fig. 2). By comparison, $PhCO_2^-Na^+$ hardly shows any interaction with AC ($\Delta\delta < 0.1$ ppm), which demonstrates the positive dendritic effect.

Notes and references

‡ In an NMR tube, 5 mg of **9** were introduced in 0.5 mL D_2O, then AC was progressively added. The titration spans from 0 to 320 equivalents of AC *per* dendrimer **9** (Fig. 1, the shifts of the peripheral protons of **9** are framed). The shifts of the four signals of AC are represented and numbered from 1 to 4.

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