DOI: 10.1002/chem.200701607

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

Elodie Boisselier,[a] Cátia Ornelas,[a] Isabelle Pianet,[b] Jaime Ruiz Aranzaes,[a] and Didier Astruc*[a]

Abstract: Water-soluble benzoate-terminated dendrimers of four generations (from G_0 with 9 branches to G_3 with 243 branches) were synthesized and fully characterized. They form water-soluble assemblies by ion-pairing interactions with three cations of medicinal interest (acetylcoline, benzyltriethylammonium, and dopamine), which were characterized and investigated by ^{1}H NMR spectroscopy, whereas such interactions do not provoke any significant shift of ^{1}H NMR signals with the monomeric benzoate anion. The calculated association constants confirm that the dendritic carboxylate termini reversibly form ion pairs and aggregates. Diffusion coefficients and hydrodynamic diameters of the dendrimers, as well as changes thereof on interaction with the cations, were evaluated by DOSY experiments. The lack of increase of dendrimer size on addition of the cations and the upfield shifts of the ^{1}H NMR signals of the cation indicate encapsulation within the hydrophobic dendrimer interiors together with probable backfolding of the benzoate termini.

Keywords: aggregation · cations · dendrimers · ion pairs · supramolecular chemistry

Introduction

An attractive property of dendrimers is their supramolecular facet,[1] and they have indeed been used as unimolecular micelles,[2] molecular boxes,[3] exoreceptors,[4] and sensors.[5] Applications of water-soluble dendrimers as drug vectors are most promising.[6,7] Polycationic dendrimers are considered to be toxic, but polyanionic dendrimers usually exhibit acceptable biocompatibility.[8]

We have reported, in preliminary form, the ionic interaction of a single benzoate-terminated dendrimer (81 branches) with acetylcholine.[9] We have now extended this study to four generations of benzoate-terminated dendrimers (from G_0 with 9 branches to G_3 with 243 branches) and their supramolecular interactions with three cations of medicinal interest: acetylcholine (AC), benzyltriethylammonium (BTEA), and dopamine.

Acetylcholine 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 on oral ingestion because of hydrolysis in the digestive tract. Therefore, transport by dendrimers may be useful. Acetylcholine chloride has various pharmacological properties: it can be used as a parasympathomimetic,[10a] a peripheral vasodilator, an antihypertensive, a myotic, or a coronarodilator.[10b] Its muscarinic parasympathomimetic action consists of contracting the smooth fiber in the digestive tract,[10c] the eye, and the bronchi.[10d] It is used in a drug marketed as a parasympathomimetic preparation for an intraocular use, although aqueous solutions are unstable and must thus be prepared just before use.[11]

As a quaternary ammonium compound, benzyltriethylammonium chloride shows a variety of physical, chemical, and biological properties. It can disrupt the cell processes of microorganisms, and it is used as a phase-transfer catalyst, antimicrobial agent, emulsifying agent, and pigment disperser.

[a] E. Boisselier, C. Ornelas, Dr. J. R. Aranzaes, Prof. D. Astruc
Nanosciences and Catalysis Group
Institut des Sciences Moléculaires, UMR CNRS N° 5255
Université Bordeaux 1
351, Cours de la Libération, 33405 Talence Cedex (France)
Fax: (+33) 5-4000-6994
E-mail: d.astruc@ism.u-bordeaux1.fr

[b] I. Pianet
CESAMO
Université Bordeaux 1
351, Cours de la Libération, 33405 Talence Cedex (France)

Supporting information for this article is available on the WWW under http://www.chemistry.org or from the author.

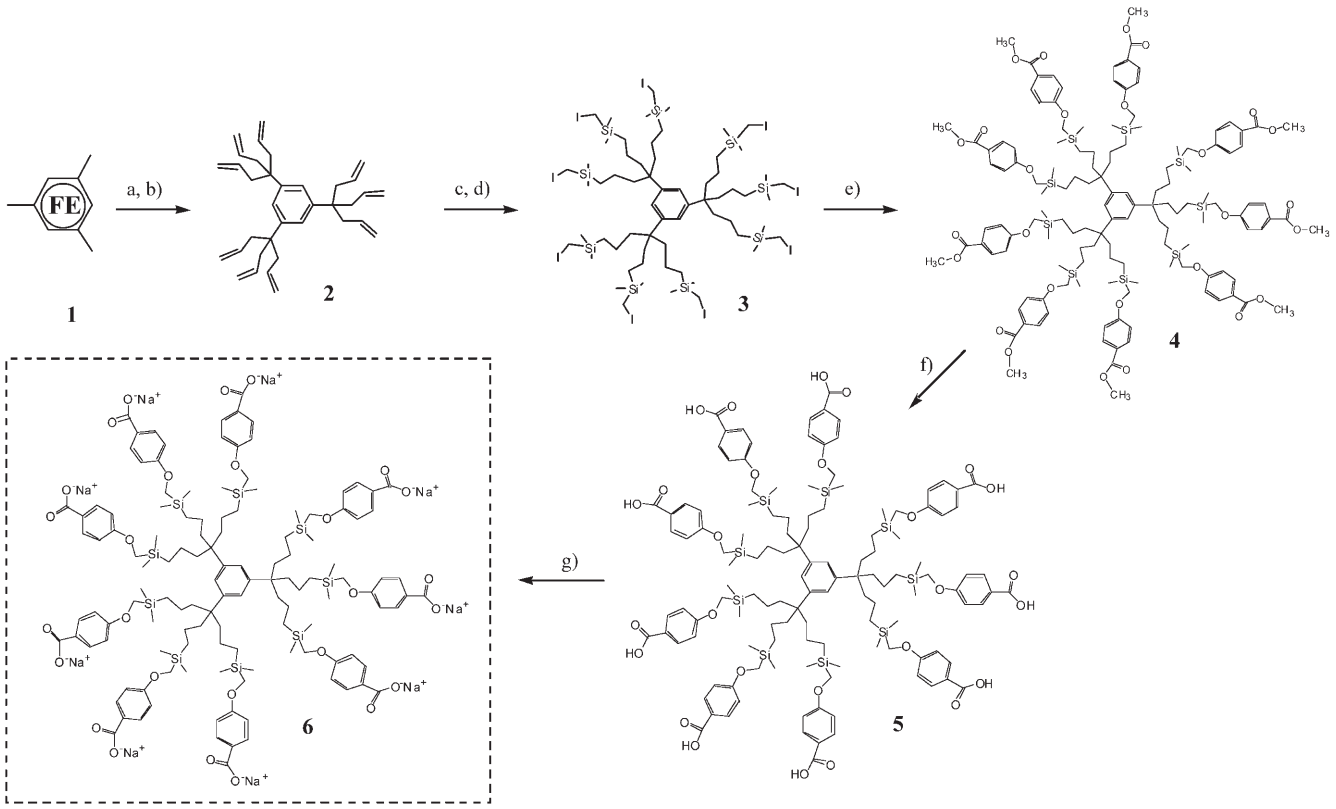
Its interaction with dendrimers may allow it to be stabilized in various formulations.[12,13] Moreover, together with alkyldimethylbenzylammonium chloride, it belongs to a large category of chemical compounds whose surface-active and biocidal properties are widely used in the pharmaceutical industry.

Dopamine is a natural catecholamine formed by decarboxylation of 3,4-dihydroxyphenylalanine (DOPA). It is a precursor to norepinephrine in noradrenergic nerves and is also a neurotransmitter in certain areas of the central nervous system.[14] Dopamine produces positive chronotropic and inotropic effects on the myocardium, which result in increased heart rate and cardiac contractility.[15,16] Dopamine hydrochloride is indicated for the correction of hemodynamic imbalances present in the Shock syndrome.[17] The half-life of dopamine in plasma is about two minutes when it is intravenously administrated. Use of a dendrimer/dopamine assembly might lead to a longer half-life that would allow dopamine to better penetrate the target area. Supramolecular interactions of the new benzoate dendrimers with AC, BTEA, and dopamine in water were investigated by ^{1}H NMR spectroscopy, and the calculated association constants and dendritic effects, that is, comparison of the influence of benzoate monomer and dendrimers and that of the dendritic generation, are discussed.

Results and Discussion

Water-soluble polyanionic dendrimers with 3^{n+2} benzoate termini (generation number $n=0$–3) were synthesized as described below, and their assembly with AC, BTEA, and dopamine was investigated by ^{1}H NMR spectroscopy, as were their solubility properties and ion-pair behavior in water. Titration of the three compounds with each generation of dendrimers (i.e., twelve complexes is described and analyzed. Diffusion ordered spectroscopy (DOSY), dipolar correlation ROESY experiments, and a titration by ^{13}C NMR are provided for the interaction between 81-benzoate G_2 and AC.

Synthesis of benzoate-terminated dendrimers: For dendrimer construction, we used the 1→3 C connectivity pioneered by Newkome et al.[18] (Schemes 1 and 2). It starts with the known nonaallylation of [FeCp(η^6-mesitylene)][PF_6] (**1**), which quantitatively yields nonaallyl dendritic core 1,3,5-[$C(CH_2CH{=}CH_2)_3]_3C_6H_3$ (**2**) on a large scale after to visible-light photolysis to removes the metal moiety.[19] Hydrosilylation of terminal olefinic bonds, pioneered in dendrimer synthesis by van Leeuwen et al.,[20] was carried out on **2** by using chloromethyldimethylsilane and Karstedt catalyst to regioselectively give a nonakis(chloromethyldimethylsilyl) intermediate that reacts with NaI to form nonaiodide **3**.



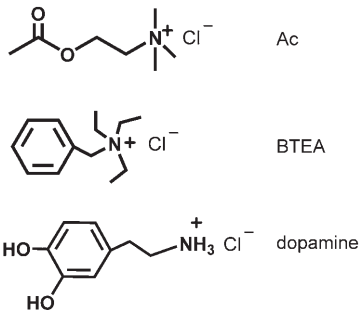
Scheme 1. Synthesis of water-soluble nonabenzoate G_0 dendrimer **6**. FE = [η^5-CpFe]$^+$[PF_6]$^-$. a) $CH_2{=}CHCH_2Br$, KOH, THF, RT, 3 days; b) $h\nu_{vis}$, MeCN; c) $HSiMe_2CH_2Cl$, Karsted cat., RT, 1 day; d) NaI, butanone, 80 °C, 16 h; e) $HOC_6H_4COOCH_3$, K_2CO_3, DMF, 80 °C, 2 days; f) NaOH, dioxane/water (9:1), 60 °C, 16 h, HCl; g) H_2O, NaOH.

We functionalized nine-branch G_0 dendrimer **3** with carboxylate termini to solubilize it in water. This was achieved in two steps: 1) Williamson reaction of dendri-9-iodide (dendrimer with 9 iodide termini) **3** with methyl 4-hydroxybenzoate yielded dendri-9-benzoate **4**, which was characterized by its molecular peak at 2520.21 $[M+Na]^+$ in the MALDI-TOF mass spectrum (calcd for $C_{135}H_{192}O_{27}Si_9Na$: 2522.71); 2) basic hydrolysis of dendri-9-benzoate **4** to form dendri-9-acid **5**, which was characterized by its molecular peak at 2394.44 $[M+Na]^+$ in the MALDI-TOF mass spectrum (calcd for $C_{126}H_{174}O_{27}Si_9Na$: 2396.47). Dendrimer **5** was easily solubilized in water as its sodium carboxylate form **6** in the presence of a stoichiometric amount of NaOH (Scheme 1).

Dendritic progression was achieved by using the known "phenoltriallyl" dendronic brick p-$HOC_4H_4C(CH_2CH{=}CH_2)_3$, obtained by one-pot reaction of $[FeCp(\eta^6\text{-}p\text{-chlorotoluene})]PF_6$ with allyl bromide and tBuOK (Scheme 2).[19]

The same synthetic strategy was then applied to G_1 with 27 branches, G_2 with 81 branches, and G_3 with 243 branches. All new dendrimers were fully characterized by ^1H, ^{13}C, and ^{29}Si NMR spectroscopy, mass spectrometry (except G_3), and elemental analysis. The MALDI-TOF mass spectra show molecular peaks of the products (see Figure 1 and Supporting Information): dendri-27-$COOCH_3$ **7** (found: 9265.44 $[M]^+$; calcd for $C_{504}H_{732}O_{90}Si_{36}$: 9264.98), dendri-27-COOH **8** (found: 8886.4 $[M]^+$; calcd for $C_{477}H_{678}O_{90}Si_{36}$: 8886.3), and dendri-81-$COOCH_3$ **9** (found: 29 471 $[M]^+$; calcd for $C_{1611}H_{2352}O_{279}Si_{117}$: 29469.75). The ^1H NMR spectra of the benzoate dendrimers in MeOD show all the expected signals and thus confirm their structure. However, the ^1H NMR spectra of the higher generations in D_2O only show the peripheral proton signals. The synthesis of water-soluble 81-benzoate G_2 dendrimer **10** is shown in Scheme 2.

^1H NMR experiments and determination of association constants: Intermolecular interactions in solution play a key role in molecular recognition. NMR spectroscopy is a very useful technique to analyze them, because it allows the estimation of the association constants. It also gives information on the formation of aggregates, ion pairing, encapsulation, and size variations. This technique was used here to analyze three compounds that all contain ammonium moieties: AC, BTEA, and dopamine.



We investigated the supramolecular interactions of the three cations with the four generations of benzoate dendrimers to evaluate the role of dendrimer size and number of carboxylate groups at the periphery. The major advantage of these dendrimers is that they all become water-soluble on addition of a stoichiometric amount of NaOH relative to the number of acid groups. Thus, the supramolecular interactions can be tested at a pH close to neutrality, a condition for applications in biological systems (here pH 7.6 at $c = 10^{-3}$ M for all sodium carboxylate dendrimers).

The dendrimer with nine sodium carboxylate groups reversibly reacts with AC chloride to form water-soluble supramolecular assemblies whose structure can be examined by ^1H NMR spectroscopy.[21] The interaction between dendri-9-carboxylate and AC is characterized in the ^1H NMR spectrum by a large upfield shift of the four AC signals. The dendrimer signals also move, but to a lesser extent; the average shift is $\Delta\delta = 0.06$ ppm in water for peripheral protons H^5–H^8 (see Figure 2 for numbering scheme). Indeed, the protons at the periphery of the dendrimers that are detectable by ^1H NMR spectroscopy are too far away from the carboxylate groups, and this small shift is not representative of the interaction. The titration of AC was performed to quantify the number of AC molecules that can possibly be transported by dendri-9-carboxylate.

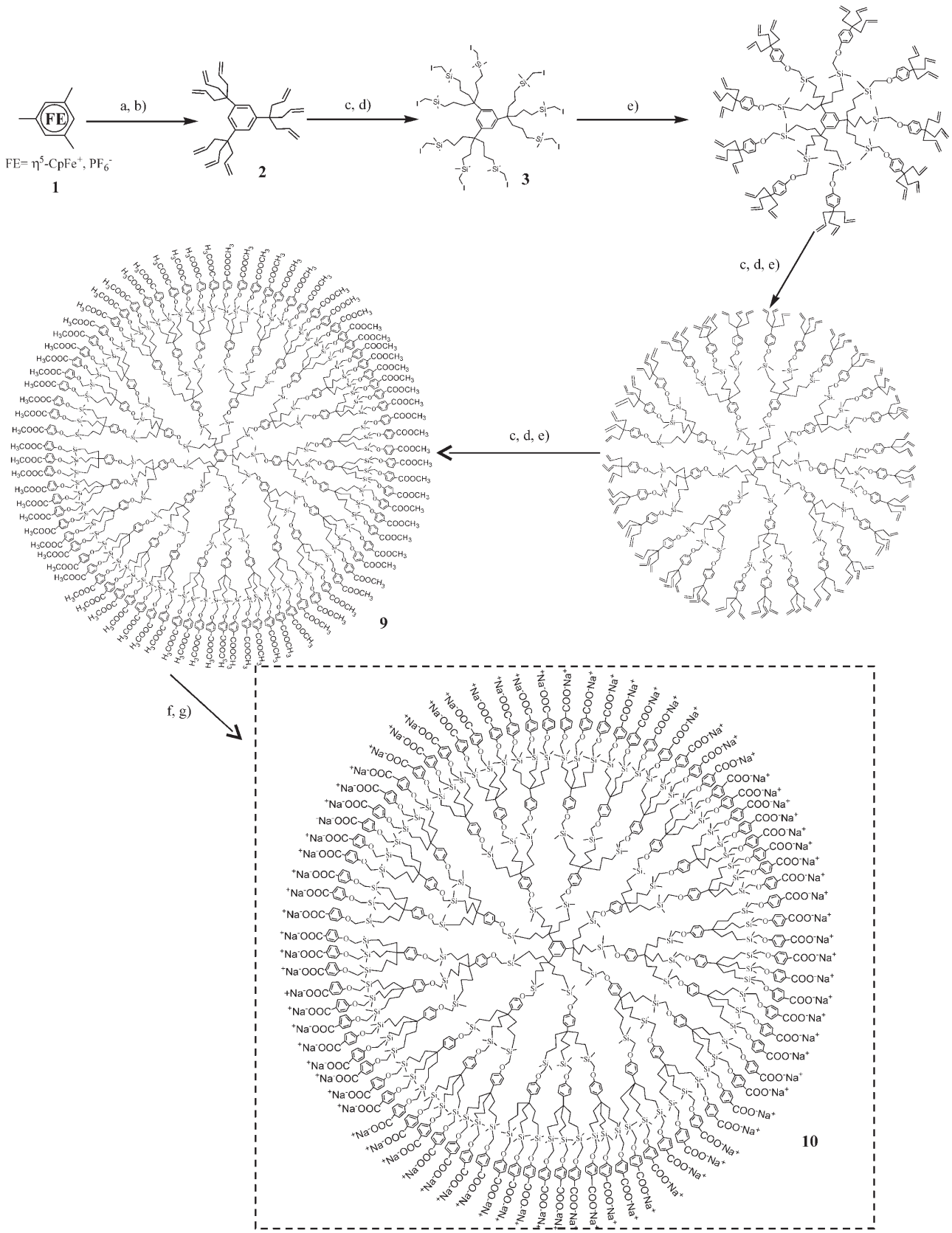
When the first equivalent of AC is added, the AC signals shift from $\delta = 4.56$ to 4.33 ppm for CH_2CH_2N proton H^3, from $\delta = 3.75$ to 3.30 ppm for CH_2N protons H^2, from $\delta = 3.23$ to 3.03 ppm for the CH_3N protons H^1, and from $\delta = 2.16$ to 2.00 ppm for the CH_3COO protons. These results correspond to an average displacement of $\Delta\delta = 0.26$ ppm. The four signals of AC shift during this titration because of interactions of the whole molecule with dendri-9-carboxylate. When AC interacts with this dendrimer, the ammonium AC group should be located at the dendrimer periphery, where it reversibly forms a contact ion pair and aggregates with the carboxylate ion (see Figure 2).

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 Equation (1)[21]

$$\Delta\delta = {}^1/_2\Delta\delta_{max}\,[(1 + K_d/n[D_0] + [AC]/n[D_0]) - \{(1 + K_d/n[D_0] + [AC]/n[D_0])^2 - 4\,[AC]/n[D_0]\}^{1/2}] \quad (1)$$

where $[D_0]$ is the total concentration of dendrimer, [AC] the total concentration of acetylcholine, and K_d the dissociation constant.

The data fit best with dendri-9-carboxylate interacting on average (equilibrium) with 18 ± 2 molecules of AC. The first nine molecules interact electrostatically at the dendrimer periphery. The first dissociation constant K_{d1} of $(20 \pm 2) \times 10^{-3}$ M involves an equilibrium with nine AC molecules. A second weaker interaction in equilibrium with nine other AC molecule is obtained from the best fit, with a second dissociation constant K_{d2} of 1 ± 0.1 M [Eq. (2); R = dendrimer, R′ = Ac].



Scheme 2. Synthesis of the water-soluble G_2 dendri-81-benzoate **10**. FE = $[\eta^5\text{-CpFe}]^+[PF_6]^-$. a) $CH_2{=}CHCH_2Br$, KOH, THF, RT, 3 days; b) $h\nu_{vis}$, MeCN; c) $HSiMe_2CH_2Cl$, Karsted cat., RT, 1 day; d) NaI, butanone, 80 °C, 16 h; e) $HOC_6H_4C(CH_2CH{=}CH_2)_3$, K_2CO_3, DMF, 80 °C, 2 days; f) NaOH, dioxane/water (9:1), 60 °C, 16 h, HCl; g) H_2O, NaOH.

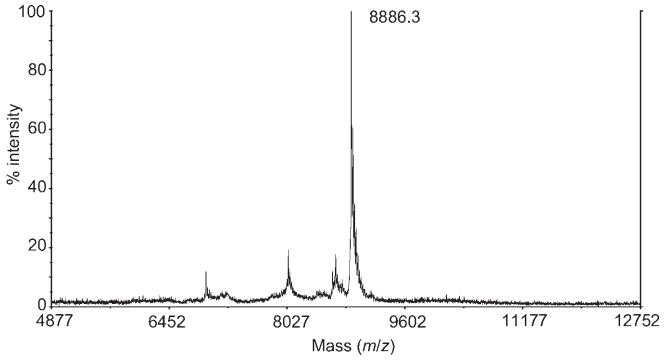


Figure 1. MALDI-TOF mass spectrum of G_1 dendri-27-benzoic acid **8**.

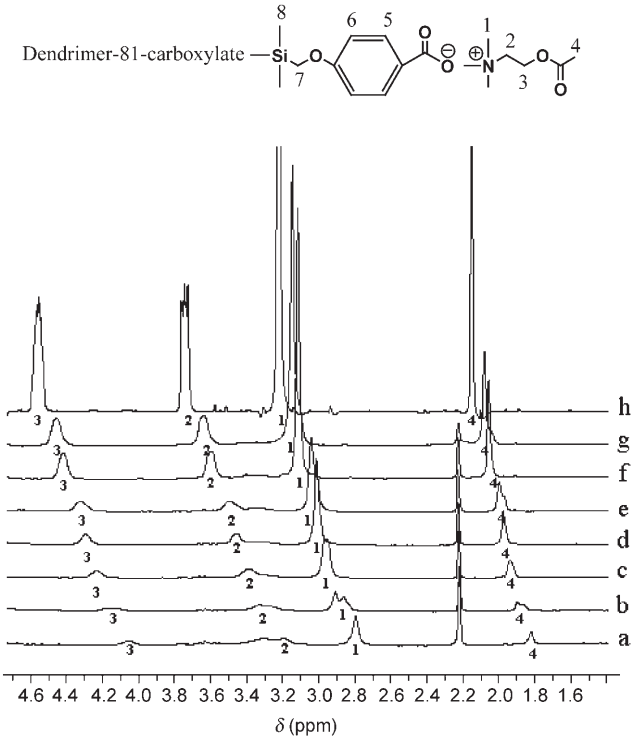


Figure 2. ^{1}H NMR titration of dendri-81-carboxylate with AC. AC proton signals: a) dendri-81-carboxylate + 20 equivalents of AC; b) dendri-81-carboxylate + 40 equivalents of AC; c) dendri-81-carboxylate + 60 equivalents of AC; d) dendri-81-carboxylate + 80 equivalents of AC; e) dendri-81-carboxylate + 100 equivalents of AC; f) dendri-81-carboxylate + 200 equivalents of AC; g) dendri-81-carboxylate + 300 equivalents of AC; h) AC alone.

$$[RCO_2^-Na^+] + [R'N^+Me_3Cl^-] \overset{K_{d1}}{\rightleftharpoons} [RCO_2^-R'N^+Me_3] + [Na(H_2O)_x^+Cl(H_2O)_y^-] \overset{K_{d2}}{\underset{H_2O}{\rightleftharpoons}} [RCO_2^-(R'N^+Me_3)_2Cl^-] + [Na(H_2O)_x^+ Cl(H_2O)_y^-] \quad (2)$$

As AC is added to a D_2O solution of dendri-9-carboxylate, the ^{1}H NMR signals of the dendrimer benzoate termini progressively broaden, in confirmation of a reversible exchange interaction, and the signals of AC follow the same trend when the dendrimer is progressively added to a solution of AC (Figure 2).

The interactions of AC with dendrimers of higher generations are similar and were also analyzed, as were the interactions of all dendrimers with BTEA and dopamine (Table 1).

For the three cations, $\Delta\delta_{max}$ varies with dendrimer generation and has the largest value for dendri-81-carboxylate. The $\Delta\delta_{max}$ value is 0.6 for AC, 1.16 for BTEA, and 0.54 ppm for dopamine. These values show the large change in environment of each cation when it is in the presence of a dendrimer terminated by benzoate groups.

All AC proton signals are shielded upfield as the dendrimer concentration increases. This is best taken into account by incoming electron density near these AC protons due to the negatively charged carboxylate groups and by penetration of AC into the hydrophobic interior of the dendrimer, that is, AC is encapsulated in the dendrimer near its periphery.[22] A similar situation is found for the ammonium protons of the two other cations.

We can distinguish two different interactions, one for AC and BTEA (Figure 3a), and another for dopamine (Figure 3b). The evolution curves of the ^{1}H NMR signals have different shapes and thus reflect different interactions. In the cases of AC and BTEA, the cation interacts with the dendrimer in two steps, with a first association constant K_{a1} for a first number of molecules n_1 and a second association constant a_1 for a second number of molecules n_2 (Table 1).

The observed behavior of the dendrimer/cation assembly is best taken into account by reversible formation of ionic bonds between the dendrimers and the three cations located near the dendrimer periphery but inside the dendrimer.[23] The second stage most probably involves agglomeration of additional charges of chloride salt to reversibly form an aggregate at each tether terminus that is backfolded into the dendrimer interior.

This should be due to the dual location of the anionic charge delocalized on both carboxylate oxygen atoms of the carboxylate group, which can form a five-component aggregate (one chloride anion in addition to the two oxygen atoms and the two cations, see Figures 4 and 5).

This reasoning is also valid for AC and BTEA. The first association constant increases with increasing dendrimer generation, from 50 to 77 M^{-1} for AC, and from 125 to 200 M^{-1} for BTEA. The association constants are slightly smaller for AC than for BTEA, that is, the dendrimer/BTEA assembly is more strongly bound than the dendrimer/AC assembly. There is no significant difference between K_{a2} values of the two cations, which are both between 1 and 12 M^{-1}.

Dopamine has only one association constant that describes the interaction between a molecule of dopamine and a carboxylate group (Figure 6). Each dendrimer interacts with a number of dopamine molecules equal to the number of dendrimer carboxylate groups, and the association constant also increases from 2000 M^{-1} to 5000 M^{-1} with increasing dendrimer generation. This corresponds to a positive dendritic effect, which is observed in both cases.

The $\Delta\delta_{max}$ value observed in each case confirms the existence of the interaction, and the evolution of $\Delta\delta$ leads to an

Table 1. Results obtained from ^{1}H NMR titration of the three cations with the benzoate-terminated dendrimers.

Supramolecular assembly	$\Delta\delta_{max}$ [a] [ppm]	χ_2 [b]	n_1 [c]	K_{d1} [d] [M]	K_{a1} [e] [M^{-1}]	n_2 [f]	K_{d2} [g] [M]	K_{a2} [h] [M^{-1}]
Dendri-9-carboxylate + AC	0.32	0.06	9	20×10^{-3}	50	9	10×10^{-1}	1
Dendri-27-carboxylate + AC	0.35	0.02	27	18×10^{-3}	56	27	8	1
Dendri-81-carboxylate + AC	0.60	0.03	81	17×10^{-3}	59	81	23×10^{-2}	4
Dendri-243-carboxylate + AC	0.46	0.08	243	13×10^{-3}	77	243	8×10^{-2}	12
Dendri-9-carboxylate + BTEA	0.9	0.007	9	8×10^{-3}	125	9	3×10^{-1}	3
Dendri-27-carboxylate + BTEA	1.1	0.02	27	7×10^{-3}	143	27	3×10^{-1}	3
Dendri-81-carboxylate + BTEA	1.16	0.03	81	6×10^{-3}	167	81	3×10^{-1}	3
Dendri-243-carboxylate + BTEA	0.6	0.08	243	5×10^{-3}	200	243	3×10^{-1}	3
Dendri-9-carboxylate + dopamine	0.35	0.009	9	5×10^{-4}	2000	–	–	–
Dendri-27-carboxylate + dopamine	0.37	0.007	27	4×10^{-4}	2500	–	–	–
Dendri-81-carboxylate + dopamine	0.54	0.009	81	3×10^{-4}	3333	–	–	–
Dendri-243-carboxylate + dopamine	0.41	0.002	243	2×10^{-4}	5000	–	–	–

[a] $\Delta\delta_{max}$ is the maximum observed chemical shift variation. [b] χ_2 is the difference between the experimental points and the numerical values extracted from a theoretical curve. [c] n_1 is the number of cationic molecules revealed by the first association constant. [d] K_{d1} is the first dissociation constant. [e] K_{a1} is the first association constant. [f] n_2 is the number of cationic molecules involved with the second association constant. [g] K_{d2} is the second dissociation constant. [h] K_{a2} is the second association constant. The attenuated maximum error for all the values of this table is $\pm10\%$.

estimation of the association constants. By comparison, monomeric sodium benzoate hardly shows any interaction with the three cations ($\Delta\delta<0.1$ ppm), which demonstrates a positive dendritic effect (i.e., comparison of the dendrimers with the monomer). Moreover, a study on a carboxylate dendrimer and the neutral amine benzylamine did not reveal any interaction that could be characterized by ^{1}H NMR spectroscopy.

In conclusion, in the studies on association constants, the data fits clearly show a plateau for dopamine, that is, the benzoate terminus interacts with a single dopamine molecule. Such a plateau is not found in titrations with AC and BTEA cations, for which the best fit is a sinusoidal curve involving two distinct association constants, the second of which very weak and thus only significant in its order of magnitude. These association constants are small, because water molecules also strongly interact with these ions. It is their order of magnitude that is informative, while their exact values have little meaning given that they are small. It is remarkable that dopamine behaves so differently from the two other cations given the similar structures of BTEA and dopamine. The association constant of the dendrimers with dopamine are indeed about 20 times larger that those with BTEA and 50 times larger than those with AC. In addition, the data only fit single 1:1 association between the benzoate

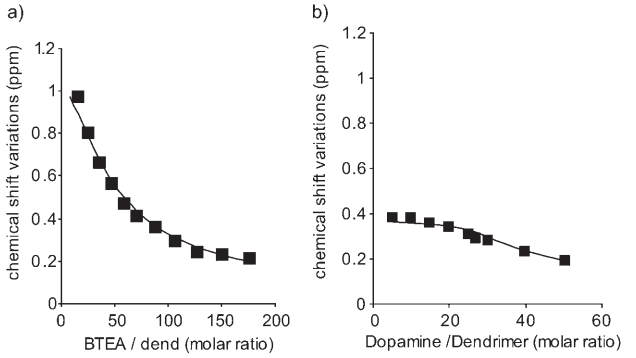


Figure 3. a) Titration of dendri-27-carboxylate with BTEA. b) Titration of dendri-27-carboxylate with dopamine.

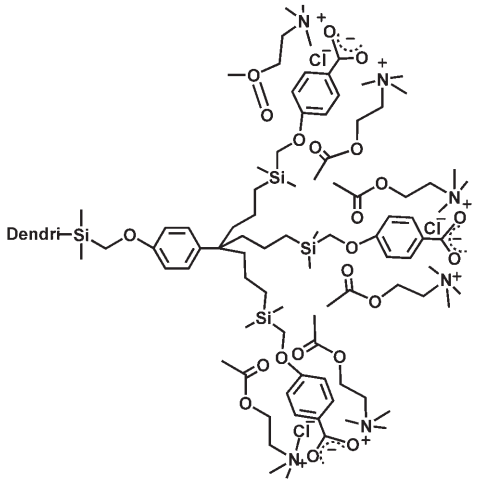


Figure 4. Representation of the ionic aggregates of carboxylate dendrimers with two AC molecules (close contacts between the whole AC groups and benzoate are omitted for clarity).

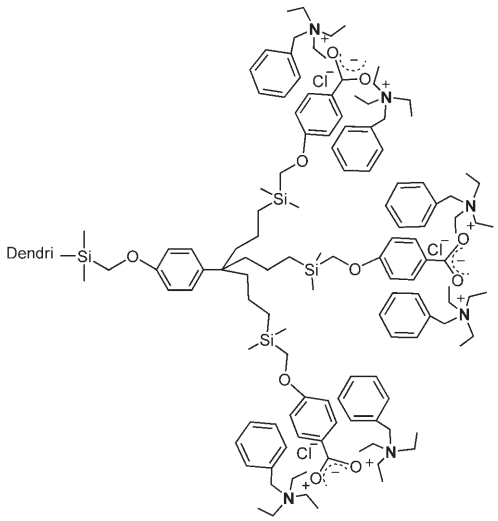


Figure 5. Representation of the ionic aggregates of carboxylate dendrimers with two BTEA molecules.

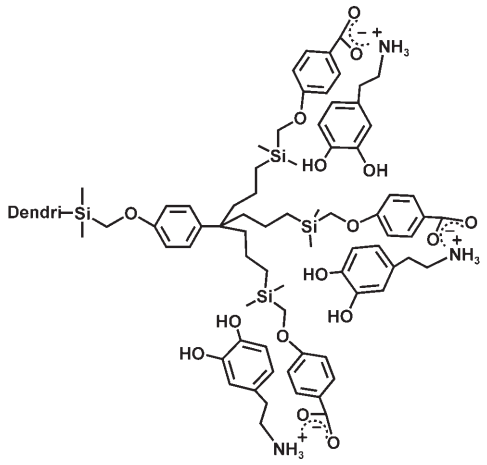


Figure 6. Representation of the ion pair between dopamine and carboxylate-terminated dendrimers.

termini and dopamine. Clearly, this completely specific behavior of dopamine is due to the fact that it is a primary ammonium compound, whereas the two other cations are quaternary ammonium compounds. Thus, dopamine has acidic hydrogen atoms on the ammonium center that can form hydrogen bonds with benzoate groups in synergy with the electrostatic attraction between the two oppositely charged ions. We know from recognition studies with ATP that synergistic interactions between two oppositely charged ions is considerably strengthened by such favorable hydrogen bonding.[4] Although the hydroxyl protons of the catechol moiety of dopamine are less acidic than the ammonium protons, they may also be involved in hydrogen bonding with one or two oxygen atoms of the bidentate carboxylate group. However, distinction between these two possible modes of hydrogen bonding is not feasible at this stage.

DOSY experiments to determine diffusion coefficients and ^{13}C NMR experiments: DOSY experiments were carried out for the dendrimer/cation assemblies involving dendri-81-carboxylate and the three cations in order to follow the evolution of the diffusion coefficient of the free dendrimer on addition of each cation. The main goal of these experiments was to measure the diffusion coefficient D by ^{1}H NMR spectroscopy. The D value allows the hydrodynamic diameter of a molecule to be calculated. In the ^{1}H NMR experiment diffusion is mathematically treated as a DOSY (diffusion-ordered spectroscopy) process in order to obtain the equivalent of "spectral" chromatography. The objective is thus double: measuring the size of the free and bound molecules in solution by ^{1}H NMR, and obtaining a DOSY spectrum that reflects the purity of the assembly.

Dendri-81-carboxylate is regarded as a spherical molecular object and characterized by an apparent diffusion coefficient. The Stokes–Einstein law [Eq. (3)] gives an estimate for the diameter of the molecule

$$D = k_B T/6\pi\eta r_H \quad (3)$$

where D is the diffusion coeficient, k_B the Boltzman constant, T the absolute temperature, η the solvent viscosity, and r_H the hydrodynamic radius of the species.

Figures 7–9 show the DOSY spectra of free dendri-81-carboxylate, free AC, and dendri-81-carboxylate/AC. Free dendri-81-carboxylate in water has a diffusion coefficient of $(4.4\pm0.2)\times10^{-11}\,m^2s^{-1}$ and a hydrodynamic diameter of 11 ± 1 nm. The AC, BTEA, and dopamine molecules have diffusion coefficients of $(5.9\pm0.03)\times10^{-10}$, $(5.0\pm0.04)\times10^{-10}$, and $(5.0\pm0.03)\times10^{-10}\,m^2s^{-1}$, and hydrodynamic diameters of 1.2 ± 0.1, 1.0 ± 0.1, and 1.0 ± 0.1 nm, respectively. When dendri-81-carboxylate is bound to 162 AC, 162 BTEA, or 81 dopamine molecules, it has diffusion coefficients of $(5.0\pm0.1)\times10^{-11}$, $(5.4\pm0.1)\times10^{-11}$, and $(7.0\pm0.1)\times10^{-11}\,m^2s^{-1}$, and its hydrodynamic diameter is to (9.8 ± 0.1), (9.0 ± 0.1), and (7.0 ± 0.1) nm, respectively. The diffusion co-

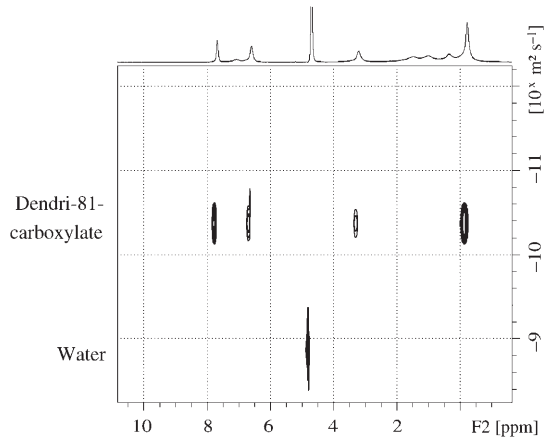


Figure 7. The four signals on the top line represent lg$D$ for the dendri-81-carboxylate, and the last signal below the line lg$D$ for water. $D_d=(4.4\pm0.1)\times10^{-11}\,m^2s^{-1}$ and $R_{H,d}=5.517$ nm, where D_d is the diffusion coefficient of the dendri-81-carboxylate, and $R_{H,d}$ its hydrodynamic radius.

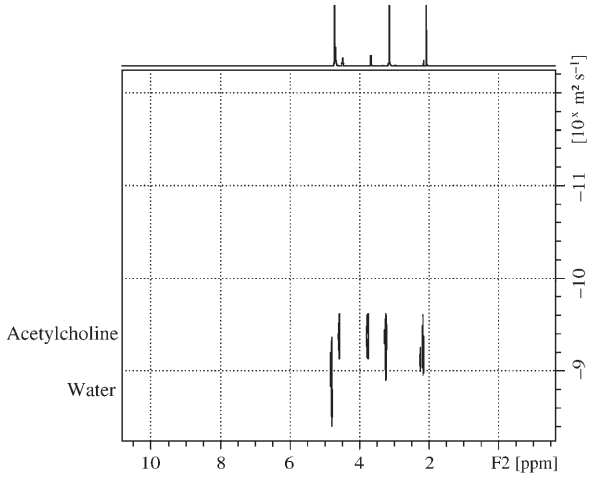


Figure 8. The four signals on the line (top) represent lg$D$ of one molecule of acetylcholine (AC), and the last signal below the line represents lg$D$ for water. $D_{AC}=(5.9\pm0.1)\times10^{-10}\,m^2s^{-1}$, $R_{H,AC}=0.59$ nm.

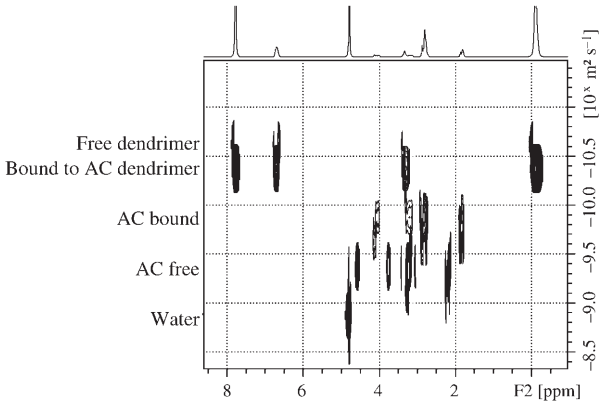


Figure 9. Four lines are identifiable from to bottom: the four signals on the first line (top) represent $\lg D$ for the free dendri-81-carboxylate; the four other signals on the same line (top) represent $\lg D$ for dendri-81-carboxylate bound to AC; the four signals on the second line represent $\lg D$ for an AC molecule bound to the dendrimer; the four signals on the third line represent $\lg D$ for a free molecule of AC. The last signal on the line below represents $\lg D$ for water. $D_d = (4.998 \pm 0.1) \times 10^{-11}\ m^2 s^{-1}$, $R_{H,d} = 4.902$ nm.

efficient of dendri-81-carboxylate increases on titration, that is, dendrimer size decreases in the dendrimer/guest assemblies. This is in accord with our above interpretation of $\Delta\delta$ in terms of encapsulation. For instance, although the diffusion coefficient of AC significantly increases on titration up to a concentration of 27.9 mM, which corresponds to 162 AC molecules per dendrimer, it stagnates at higher concentrations (plateau in Figure 10). This confirms interaction between the AC molecules and the dendrimer up to a molar ratio of 162 AC molecules per dendrimer. After this stage, the diffusion coefficient observed is an average between free AC and AC bound to the dendrimer, and its value slowly approaches that of free AC when the proportion of AC increases.

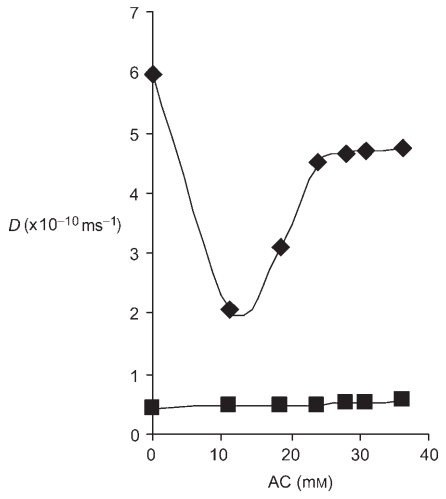


Figure 10. Evolution of the diffusion coefficients of AC (◆) and dendri-81-carboxylate **10** (■) as a function of their concentration in water.

The DOSY experiments show the evolution of the diffusion coefficients of each molecule and of the assembly. The dendrimer and the AC cation have their own diffusion coefficients in the free state that reflect their molecular weight. When a complex is formed with fast exchange on the NMR timescale, however, the observed diffusion coefficient is a weighted average of the diffusion coefficients for the free and bound species.

The diffusion coefficient of AC decreases at a a low concentration of AC, and then increases up to a concentration corresponding to 162 ± 5 AC molecules per dendrimer (i.e., [AC] = 28 mM), which shows the interaction between the two species, before stagnating at higher AC concentrations. In the beginning of AC addition to the dendrimer, the diffusion coefficient of AC becomes close to that of the dendrimer. Then, it becomes close to its initial value in the free state, as the amount of excess AC increases. The two other cations (BTEA and dopamine) follow approximately the same trend.

An increase in diffusion coefficient on titration is found for all three cations, but it is more pronounced for dopamine, which interacts more strongly with the dendrimers than the other two cations, as indicated by association constants that are one order of magnitude larger than those of AC and BTEA. These observations are in accord with the proposed encapsulation of the guest cations deduced from the shielding of their protons on interaction with the dendrimers. This encapsulation on ionic bonding forces the benzoate branch termini to be more strongly backfolded as the strength of this interaction increases. The free dendrimer is surrounded by a layer of hydrogen-bonded water molecules, and it appears that this layer is significant given the rather large hydrodynamic diameter of 11 nm. The decreased diameter of the dendrimers in the presence of cationic guests may not only signify backfolding of the dendrimer branch termini, but possibly also significant decrease of the water layer around the dendrimer, which is involved in the hydrodynamic diameter. This smaller surrounding water layer would itself be also due to the bakfolding of the ionic benzoate groups. Altogether, backfolding of the benzoate termini that is enforced by encapsulation of the ammonium guest most probably makes the dendrimers more compact.

Supramolecular interactions were investigated by ^{13}C NMR only for the single case of dendri-81-carboxylate and AC, because the ^{13}C NMR technique requires a considerably longer recording time than ^{1}H NMR and is thus impractical. In ^{1}H NMR spectra, the shifts of the dendrimer protons describing the effect of the dendrimer interaction are rather small ($\delta = 0.06$ ppm), because the closest protons are four bonds away from the carboxylate group that interacts with the AC molecules.

In the ^{13}C NMR experiment, when 81 equivalents of AC are added to dendri-81-carboxylate, the signal of the carbon atom of the carboxylate group shifts from $\delta = 174.4$ to 173.3 ppm, whereas the other carbon signals of the dendrimer do not move. Under the same conditions, the AC signals shift from $\delta = 60.9$ to 57.9 (CH_2CH_2N), from 67.2 to

64.2 (CH_2N), from 56.5 to 53.5 (CH_3N), from 23 to 20 (CH_3COO), and from 175.7 to 172.4 ppm (COO). These results correspond to an average shift of $\Delta\delta = 3$ ppm. The five signals of AC shift during titration because of interactions of the whole molecule with dendri-81-carboxylate, consistent with encapsulation of AC in the dendrimer.

When 81 additional equivalents are added, the AC signals always show an average shift of $\Delta\delta = 3$ ppm, and the signal of the carboxylate group of the dendri-81-carboxylate shifts from $\delta = 174.4$ to 172.0 ppm. These results confirm those obtained in the ^{1}H NMR experiments and the fact that the ammonium group of AC should be located at the dendrimer periphery. It forms an ion pair and aggregates with the carboxylate ion, which backfolds into the dendrimer interior so that the guest be encapsulated.

Conclusions

New water-soluble dendrimers were synthesized, characterized, and used as sensors of three cations of biological interest. The supramolecular interactions between the two entities were investigated by ^{1}H NMR spectroscopy.

The protons of dendritic benzoate tethers are deshielded (downfield shifts) on addition of any ammonium cation due to decreased electron density in ion-pairing interactions. Those of the ammonium cations are all shielded (upfield shift) due to encapsulation within the hydrophobic dendrimer interior.

The $\Delta\delta_{max}$ value observed in each case confirms the existence of the reversible interaction, and the evolution of $\Delta\delta$ led to an estimate of the association constants. By comparison, the monomeric sodium benzoate hardly shows any interaction with the three cations ($\Delta\delta < 0.1$ ppm), and this demonstrates a positive dendritic effect.

Two distinct cases were found: AC and BTEA on the one hand, and dopamine on the other. In the former case, the dendrimer acts in two steps: it first equilibrates with a stoichiometric number of cations by ionic association, and then form aggregates with the same number of cations with much weaker association. Dopamine has only one association constant characterizing the interaction between a dopamine molecule and a carboxylate group (i.e., 9 molecules of dopamine for the dendri-9-carboxylate, 27 for the dendri-27-carboxylate, 81 for the dendri-81-carboxylate, and 243 for the dendri-243-carboxylate). This interaction between dopamine and the dendrimers is much stronger than those between the dendrimers and AC and BTEA, as shown by the relative values of the association constants, because for dopamine hydrogen-bonding between the primary ammonium protons and the carboxylate termini acts in synergy with ion paring, which is not the case with the two other ammonium cations, which are quaternary.

For dendrimers of different generations, $\Delta\delta_{max}$ increases in all the cases with increasing generation number up to the dendri-81-carboxylate, then slightly decreases from the dendri-81-carboxylate to the dendri-243-carboxylate. For the association constants, the same trend is observed. Thus, the dendritic effect for the three ammonium cations is positive, that is, the $\Delta\delta_{max}$ and association constant increase as the dendrimer generation increases up to the dendri-81-carboxylate.

Experimental Section

General: All reactions were carried out by Schlenk techniques or in a nitrogen-filled Vacuum Atmosphere drylab. ^{1}H NMR spectra were recorded at 25 °C with a Brucker AC 250 (250 MHz) spectrometer. ^{13}C NMR spectra were obtained in pulsed FT mode at 62.0 MHz with a Brucker AC 250 spectrometer, and ^{29}Si NMR spectra were obtained at 59.6 MHz with a Brucker AC 300 spectrometer. All chemical shifts δ are reported in parts per million (ppm) relative 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. Syntheses of the precursor iodomethyltrimethylsilyl dendrimers were described previously.[19]

Synthesis of dendri-9-benzoate 4: Nonaiodide dendrimer **3** (1.1 g, 0.482 mmol), methyl 4-hydroxybenzoate (1.32 g, 8.68 mmol), K_2CO_3 (6.10 g, 43.4 mmol), and dry DMF (30 mL) were introduced into a Schlenk flask. The reaction mixture was stirred at 80 °C for 48 h. DMF was removed, and the crude product was dissolved in dichloromethane (30 mL) and washed with water to remove 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 to remove excess methyl 4-hydroxybenzoate. Nonabenzoate dendrimer **4** was obtained as a colorless waxy material in 90% yield (1.089 g). ^{1}H NMR ($CDCl_3$, 250 MHz): $\delta = 7.93$ and 6.88 (d, 18H, arom.), 7.01 (s, 3H, arom. core), 3.86 (s, 27H, $COOCH_3$), 3.50 (s, 18H, $SiCH_2O$), 1.65 (s, 18H, $CH_2CH_2CH_2Si$), 1.13 (s, 18H, $CH_2CH_2CH_2Si$), 0.57 (s, 18H, $CH_2CH_2CH_2Si$), 0.040 ppm (s, 54H, $Si(CH_3)_2$); ^{13}C NMR ($CDCl_3$, 62 MHz): 165.8 ($COOCH_3$), 164.2 (arom. C_qO), 144.8 (CH, arom. core), 130.4 and 112.7 (CH, arom.), 121.2 (C_qCOOCH_3, arom.), 59.7 ($SiCH_2O$), 50.7 ($COOCH_3$), 42.9 ($CH_2CH_2CH_2Si$), 41.0 (C_qCH_2), 16.8 ($CH_2CH_2CH_2$), 13.6 ($CH_2CH_2CH_2Si$), −5.6 ppm ($Si(CH_3)_2$); ^{29}Si NMR ($CDCl_3$, 59.62 MHz): $\delta = 0.55$ ppm ($SiCH_2O$); MS (MALDI-TOF): m/z calcd for $C_{135}H_{192}O_{27}Si_9Na$: 2522.71; found: 2520.21 $[M+Na]^+$; elemental analysis (%) calcd for $C_{135}H_{192}O_{27}Si_9$: C 64.86, H 7.74; found: C 64.37, H 7.56; IR: $\tilde{\nu} = 1719$ ($\nu_{C=O}$) cm^{-1}.

Synthesis of dendri-9-benzoic acid 5: Dendri-9-benzoate **4** (0.50 g, 0.20 mmol) was dissolved in dioxane (40 mL), and an aqueous solution of NaOH (10 mL, 18 mmol, 10 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. The dendri-9-acid precipitated as a white powder. The solution was filtered, and the powder was washed twice with diethyl ether. The product was recovered from the filter by dissolving it with methanol. Methanol was removed in vacuo, and the product was obtained as a white powder in 72% yield. ^{1}H NMR (MeOD, 250 MHz): $\delta = 7.93$ and 6.91 (d, 18H, arom.), 7.06 (s, 3H, arom. core), 3.52 (s, 18H, $SiCH_2O$), 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 ppm (s, 54H, $Si(CH_3)_2$); ^{13}C NMR (MeOD, 62 MHz): $\delta = 169.8$ ($COOH$), 166.7 (arom. C_qO), 147.2 (CH, arom. core), 132.9 and 115.0 (CH, arom.), 123.0 (arom. C_qCOOCH_3), 61.9 ($SiCH_2O$), 45.2 ($CH_2CH_2CH_2Si$), 43.3 (C_qCH_2), 19.3 ($CH_2CH_2CH_2$), 15.9 ($CH_2CH_2CH_2Si$), −4.1 ppm ($Si(CH_3)_2$); ^{29}Si NMR (MeOD, 59.62 MHz) $\delta = 0.36$ ppm ($SiCH_2O$); MS (MALDI-TOF): m/z calcd for $C_{126}H_{174}O_{27}Si_9Na$: 2 396.47; found: 2 394.44 $[M+Na]^+$; elemental analysis (%) calcd for $C_{126}H_{174}O_{27}Si_9$: C 63.76, H 7.39; found: C 62.71, H 7.22; IR: $\tilde{\nu} = 1686$ ($\nu_{C=O}$) cm^{-1}; ^{1}H NMR of **6** (D_2O + NaOH, 250 MHz): $\delta = 7.82$ and 6.79 (d, 18H, arom.), 7.08 (s, 3H, arom. core), 3.41 (s, 18H, $SiCH_2O$), 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 ppm (s, 54H, $Si(CH_3)_2$).

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_2CO_3 (0.609 g, 4.40 mmol), and dry DMF (20 mL) were introduced into a Schlenk flask. The reaction mixture was stirred at 80 °C for 48 h, then DMF was removed, the crude product was dissolved in 30 mL of dichloromethane and washed with water to remove K_2CO_3. The organic layer was dried with Na_2SO_4, filtered, and the solvent removed in vacuo. The product was washed with methanol and precipitated twice in CH_2Cl_2/methanol to remove excess methyl 4-hydroxybenzoate. The dendri-27-benzoate was obtained as a colorless waxy material (0.140 g, 92% yield). ^{1}H NMR ($CDCl_3$, 250 MHz): $\delta = 7.94$ and 6.88 (d, 54 H, outer arom.), 7.10 and 6.80 (d, 18 H, inner arom.), 3.84 (s, 81 H, $COOCH_3$), 3.51 (s, 72 H, $SiCH_2O$), 1.60 (s, 72 H, $CH_2CH_2CH_2Si$), 1.11 (s, 72 H, $CH_2CH_2CH_2Si$), 0.55 (s, 72 H, $CH_2CH_2CH_2Si$), 0.025 (s, 216 H, $Si(CH_3)_2$). ^{13}C NMR ($CDCl_3$, 62 MHz): 165.8 ($COOCH_3$), 164.2 (outer arom. C_qO), 158.1 (inner arom. C_qO), 130.4 and 112.8 (CH, arom.), 121.1 (arom. C_qCOOCH_3), 59.7 ($SiCH_2O$), 50.7 ($COOCH_3$), 41.9 ($CH_2CH_2CH_2Si$), 41.9 (C_qCH_2), 16.6 ($CH_2CH_2CH_2$), 13.5 ($CH_2CH_2CH_2Si$), −5.5 ppm ($Si(CH_3)_2$); ^{29}Si NMR ($CDCl_3$, 59.62 MHz): $\delta = 0.53$ ppm ($SiCH_2O$); MS (MALDI-TOF): m/z calcd for $C_{504}H_{732}O_{90}Si_{36}$: 9264.9816; found: 9265.4374; IR: $\tilde{\nu} = 1719$ ($\nu_{C=O}$) cm^{-1}.

Synthesis of dendri-27-benzoic acid 8: Dendri-27-benzoate **7** (0.070 g, 0.007 mmol) was dissolved in dioxane (45 mL), and an aqueous solution of NaOH (5 mL, 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 filtered, and the powder was washed twice with diethyl ether. The product was recovered from the 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, 250 MHz): $\delta = 7.91$ and 6.86 (d, 54 H, outer arom.), 7.10 and 6.79 (d, 18 H, inner arom.), 3.48 (s, 81 H, $SiCH_2O$), 1.60 (s, 72 H, $CH_2CH_2CH_2Si$), 1.15 (s, 72 H, $CH_2CH_2CH_2Si$), 0.53 (s, 72 H, $CH_2CH_2CH_2Si$), −0.056 ppm (s, 216 H, $Si(CH_3)_2$); ^{13}C NMR (MeOD, 62 MHz): $\delta = 168.5$ (COOH), 165.4 (outer arom. C_qO), 159.1 (inner arom. C_qO), 131.5 and 113.6 (CH, arom.), 122.3 (arom. C_qCOOCH_3), 60.4 ($SiCH_2O$), 42.9 ($CH_2CH_2CH_2Si$), 41.8 (C_qCH_2), 17.6 ($CH_2CH_2CH_2$), 14.4 ($CH_2CH_2CH_2Si$), −4.9 ppm ($Si(CH_3)_2$); ^{29}Si NMR (MeOD, 59.62 MHz) $\delta = 1.57$ ppm ($SiCH_2O$); MS (MALDI-TOF): m/z calcd for $C_{477}H_{678}O_{90}Si_{36}$: 8886.3; found: 8886.4; IR: $\tilde{\nu} = 1686$ ($\nu_{C=O}$) cm^{-1}.

Synthesis of dendri-81-benzoate 9: Dendri-81-benzoate **9** was synthesized from dendri-81-iodide (0.30 g, 0.011 mmol) following the same procedure as for the synthesis of **4**, in 89% yield. ^{1}H NMR ($CDCl_3$, 250 MHz): $\delta =$ 7.94 and 6.88 (d, 162 H, exterior arom.), 7.10 and 6.80 (d, 72 H, interior arom.), 3.84 (s, 243 H, $COOCH_3$), 3.51 (s, 234 H, $SiCH_2O$), 1.60 (s, 234 H, $CH_2CH_2CH_2Si$), 1.11 (s, 234 H, $CH_2CH_2CH_2Si$), 0.55 (s, 234 H, $CH_2CH_2CH_2Si$), 0.034 ppm (s, 702 H, $Si(CH_3)_2$); ^{13}C NMR ($CDCl_3$, 62 MHz): $\delta = 167.2$ ($COOCH_3$), 164.2 (exterior arom. C_qO), 159.4 (interior arom. C_qO), 131.8 and 114.2 (CH, arom.), 122.5 (arom. C_qCOOCH_3), 61.1 ($SiCH_2O$), 52.2 ($COOCH_3$), 43.4 ($CH_2CH_2CH_2Si$), 42.3 (C_qCH_2), 18.0 ($CH_2CH_2CH_2$), 14.9 ($CH_2CH_2CH_2Si$), −4.3 ppm ($Si(CH_3)_2$); ^{29}Si NMR ($CDCl_3$, 59.62 MHz): $\delta = 0.53$ ppm ($SiCH_2O$); MS (MALDI-TOF): m/z calcd for $C_{1611}H_{2352}O_{279}Si_{117}$: 29 469.75; found: 29 471.00; elemental analysis (%) calcd for $C_{1611}H_{2352}O_{279}Si_{117}$: C 65.63, H 7.99; found: C 65.58, H 8.04; IR: $\tilde{\nu}_{C=O} = 1719$ cm^{-1}.

Synthesis of dendri-81-benzoate 10: Dendri-81-acid **10** was synthesized from dendri-81-benzoate **9** (0.20 g, 0.0068 mmol) by the same procedure as for the synthesis of **5**, in 67% yield. ^{1}H NMR (MeOD, 250 MHz): $\delta =$ 7.90 and 6.83 (d, 162 H, exterior arom.), 7.08 and 6.75 (d, 72 H, interior arom.), 3.43 (s, 234 H, $SiCH_2O$), 1.58 (s, 234 H, $CH_2CH_2CH_2Si$), 1.10 (s, 234 H, $CH_2CH_2CH_2Si$), 0.49 (s, 234 H, $CH_2CH_2CH_2Si$), −0.056 (s, 702 H, $Si(CH_3)_2$). ^{13}C NMR (MeOD, 62 MHz): 169.9 (COOH), 166.8 (exterior arom. C_qO), 160.5 (interior arom. C_qO), 133.0 and 115.1 (CH, arom.), 123.8 (arom. C_qCOOCH_3), 61.8 ($SiCH_2O$), 44.3 ($CH_2CH_2CH_2Si$), 43.4 (C_qCH_2), 19.0 ($CH_2CH_2CH_2$), 15.8 ($CH_2CH_2CH_2Si$), −4.0 ppm ($Si(CH_3)_2$); ^{29}Si NMR (MeOD, 59.62 MHz): $\delta = 0.26$ ppm ($SiCH_2O$); elemental analysis (%) calcd for $C_{1530}H_{2190}O_{279}Si_{117}$: C 64.86, H 7.79; found: C 64.25, H 7.68; IR: $\tilde{\nu} = 1686$ ($\nu_{C=O}$) cm^{-1}.

Synthesis of dendri-243-benzoate 11: Dendri-243-iodide (0.10 g, 0.001 mmol), methyl 4-hydroxybenzoate (0.088 g, 0.576 mmol), K_2CO_3 (0.398 g, 2.88 mmol), and dry DMF (20 mL) were introduced into a Schlenk flask. The reaction mixture was stirred at 80 °C for 48 h. DMF was removed, and the crude product dissolved in dichloromethane (30 mL) and washed with water to remove 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 to remove excess methyl 4-hydroxybenzoate. Dendri-81-benzoate was obtained as a colorless waxy material (0.289 g, 89% yield). ^{1}H NMR ($CDCl_3$, 250 MHz): $\delta = 7.94$ and 6.88 (d, 486 H, outer arom.), 7.10 and 6.80 (d, 234 H, inner arom.), 3.79 (s, 729 H, $COOCH_3$), 3.47 (s, 720 H, $SiCH_2O$), 1.59 (s, 720 H, $CH_2CH_2CH_2Si$), 1.10(s, 720 H, $CH_2CH_2CH_2Si$), 0.53 (s, 720 H, $CH_2CH_2CH_2Si$), −0.012 ppm (s, 2160 H, $Si(CH_3)_2$); ^{13}C NMR ($CDCl_3$, 62 MHz): $\delta = 165.7$ ($COOCH_3$), 164.2 (outer arom. C_qO), 159.4 (inner arom. C_qO), 130.4 and 112.8 (CH, arom.), 121.1 (arom. C_qCOOCH_3), 59.6 ($SiCH_2O$), 50.7 ($COOCH_3$), 42.0 ($CH_2CH_2CH_2Si$), 40.9 (C_qCH_2), 16.6 ($CH_2CH_2CH_2$), 13.5 ($CH_2CH_2CH_2Si$), −5.7 ppm ($Si(CH_3)_2$); ^{29}Si NMR ($CDCl_3$, 59.62 MHz): $\delta = 0.47$ ppm ($SiCH_2O$); IR: $\tilde{\nu} = 1719$ ($\nu_{C=O}$) cm^{-1}.

Synthesis of dendri-243-benzoic acid 12: Dendri-243-benzoate (0.06 g, 0.0007 mmol) was dissolved in dioxane (45 mL), and an aqueous solution of NaOH (5 mL, 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 filtered, and the powder was washed twice with diethyl ether. The product was recovered from the 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, 250 MHz): $\delta = 7.94$ and 6.84 (d, 486 H, outer arom.), 7.08 and 6.75 (d, 234 H, inner arom.), 3.36 (s, 720 H, $SiCH_2O$), 1.62 (s, 720 H, $CH_2CH_2CH_2Si$), 1.10 (s, 720 H, $CH_2CH_2CH_2Si$), 0.54 (s, 720 H, $CH_2CH_2CH_2Si$), −0.020 ppm (s, 2160 H, $Si(CH_3)_2$); ^{13}C NMR (MeOD, 62 MHz): $\delta = 168.6$ (COOH), 165.3 (outer arom. C_qO), 160.5 (inner arom. C_qO), 131.6 and 113.6 (CH, arom.), 122.3 (arom. C_qCOOCH_3), 60.4 ($SiCH_2O$), 44.3 ($CH_2CH_2CH_2Si$), 43.4 (C_qCH_2), 19.0 ($CH_2CH_2CH_2$), 14.3 ($CH_2CH_2CH_2Si$), −5.3 ppm ($Si(CH_3)_2$); IR: $\tilde{\nu} = 1686$ ($\nu_{C=O}$) cm^{-1}.

General procedure for titration: In an NMR tube, carboxylate dendrimer (3 mg) was introduced into D_2O (0.4 mL), then one of the cations was progressively added. Titrations spanned from 0 to the $2x$ equivalents of the cation per dendrimer, where x is the number of carboxylate groups in each dendrimer.

Synthesis of all dendrimers and detailed ^{1}H NMR, ^{13}C NMR, DOESY, and ROESY spectra and data are available as Supporting Information.

Acknowledgements

We are grateful to the referees for helpful comments, to the Fundação para a Ciência e a Tecnologia (FCT), Portugal (Ph.D. grant to C.O.), the Institut Universitaire de France (IUF, DA), the CNRS, and the Université Bordeaux 1 for financial support.

[1] a) V. Percec, G. Johansson, G. Ungar, J. P. Zhou, *J. Am. Chem. Soc.* **1996**, *118*, 9855; F. Zeng, S. C. Zimmermann, *Chem. Rev.* **1997**, *97*, 1681; b) V. Balzani, S. Campana, G. Denti, A. Juris, S. Serroni, M. Venturi, *Acc. Chem. Res.* **1998**, *31*, 26; c) O. A. Mattews, A. N. Shipway, F. Stoddart, *Prog. Polym. Sci.* **1998**, 23; d) G. R. Newkome, C. N. Moorefield, *Chem. Rev.* **1999**, *99*, 1689; e) G. R. Newkome, C. N. Moorefield, F. Vögtle, *Dendritic Molecules*, Wiley-VCH, Weinheim, **2001**.

[2] a) G. R. Newkome, Z. Yao, G. R. Baker, V. K. Gupta, *J. Org. Chem.* **1985**, *50*, 2003; b) G. R. Newkome, *C. R. Chim.* **2003**, *6*, 715 (review).

[3] a) J. F. G. A. Jansen, E. M. M. de Brabander-van den Berg, E. W. Meijer, *Science* **1994**, *266*, 1226; b) A. W. Bosman, E. W. Jensen, E. W. Meijer, *Chem. Rev.* **1999**, *99*, 1665.
[4] a) C. Valério, J.-L. Fillaut, J. Ruiz, J. Guittard, J.-C. Blais, D. Astruc, *J. Am. Chem. Soc.* **1997**, *119*, 2588; b) S. Nlate, J. Ruiz, J.-C. Blais, D. Astruc, *Chem. Commun.* **2000**, 417; c) D. Astruc, M.-C. Daniel, J. Ruiz, *Chem. Commun.* **2004**, 2637; d) M-C. Daniel, D. Astruc, *Chem. Rev.* **2004**, *104*, 293.
[5] a) M. Albrecht, N. J. Hovestad, J. Boersma, G. van Koten, *Chem. Eur. J.* **2001**, *7*, 1289; b) A. W. Kleij, A. Ford, J. T. B. H. Jastrzebski, G. van Koten in *Dendrimers and Other Dendritic Polymers* (Eds.: J. M. J. Fréchet, D. A. Tomalia), Wiley, New York, **2002**, 185.
[6] a) D. Astruc, *C. R. Acad. Sci. Ser. IIb* **1996**, *322*, 757 (review); b) C. Kojima, K. Kono, K. Maruyama, T. Takagishi, *Bioconjugate Chem.* **2000**, *11*, 910; c) K. Sadler, J. P. Tam, *J. Biotechnol.* **2002**, *90*, 195; d) M. J. Cloninger, *Curr. Opin. Chem. Biol.* **2002**, *6*, 742; e) S. E. Stiriba, H. Frey, R. Haag, *Angew. Chem.* **2002**, *114*, 1385; *Angew. Chem. Int. Ed.* **2002**, *41*, 1329; f) M. W. Grinstaff, *Chem. Eur. J.* **2002**, *8*, 2838; g) U. Boas, P. M. H. Heegarard, *Chem. Soc. Rev.* **2004**, *33*, 43; h) C. C. Lee, J. A. Mac Kay, J. M. J. Fréchet, F. Szoka, *Nat. Biotechnol.* **2005**, *23*, 1517.
[7] For the first water-soluble dendrimers, see ref. [2a] and: a) D. A. Tomalia, H. Baker, J. Dewald, M. Hall, G. Kallos, S. Martin, J. Roeck, P. Smith, *Macromolecules* **1986**, *19*, 2466; b) D. A. Tomalia, A. N. Naylor, N. A. Goddard III, *Angew. Chem.* **1990**, *102*, 119; *Angew. Chem. Int. Ed. Engl.* **1990**, *29*, 138.
[8] a) N. Malik, R. Wiwattanapatapee, R. Klopsch, K. Lorenz, H. Frey J. W. Weener, *J. Controlled Release* **2000**, *65*, 133; b) R. Jevprasesphant, J. Penny, R. Jalal, D. Attwood, N. B. McKown, A. D'Emmanuele, *Int. J. Pharm.* **2003**, *252*, 263.
[9] C. Ornelas, E. Boisselier, V. Martinez, I. Pianet, J. Ruiz Aranzaes, D. Astruc, *Chem. Commun.* **2007**, 5093.
[10] a) O. Voss, *Archiv Experiment. Pathol. Pharmakol.* **1926**, *116*, 367–382; b) E. A. Carmichael, F. R. Fraser, *Heart* **1933**, *16*, 263; c) M. Beauvallet, *C. R. Seances Soc. Biol. Ses Fil.* **1938**, *127*, 213; d) B. A. Houssay, O. Orias, *C. R. Seances Soc. Biol. Ses Fil.* **1934**, *117*, 61.
[11] D. M. Sletten, K. K. Nickander, P. A. Low, *J. Neurol. Sci.* **2005**, *234*, 1.
[12] Y. Jia, H. Zhou, M. Wang, X. Huang, T. Dong, *Fudan Xuebao Ziran Kexueban* **1986**, *25*, 57.
[13] H. Koma, Y. Igarashi, H. Nobeshima, *Jpn. Kokai Tokkyo Koho* **2005**, 16.
[14] A. Bjoerklund, S. B. Dunnett, *Neuroscience* **2007**, *30*, 185.
[15] A. Dahan, D. S. Ward, *Adv. Exp. Med. Biol.* **1998**, *450*, 173.
[16] D. Horwitz, S. M. FoxIII, L. I. Goldberg, *Circ. Res.* **1962**, *10*, 237.
[17] A. Aronski, A. Kubler, M Sliwinski, A. Paszkowska, *Anaesthesist* **1978**, *27*, 183.
[18] G. R. Newkome, Z. Yao, G. R. Baker, V. K. Gupta, *J. Org. Chem.* **1985**, *50*, 2003.
[19] a) V. Sartor, L. Djakovitch, J.-L. Fillaut, F. Moulines, F. Neveu, V. Marvaud, J. Guittard, J.-C. Blais, D. Astruc, *J. Am. Chem. Soc.* **1999**, *121*, 2929; b) J. Ruiz, G. Lafuente, S. Marcen, C. Ornelas, S. Lazare, J.-C. Blais, E. Cloutet, D. Astruc, *J. Am. Chem. Soc.* **2003**, *125*, 7250; c) C. Ornelas, D. Méry, J. Ruiz, J.-C. Blais, E. Cloutet, D. Astruc, *Angew. Chem.* **2005**, *117*, 7565; *Angew. Chem. Int. Ed.* **2005**, *44*, 7399; C. Ornelas, L. Salmon, J. Ruiz Aranzaes, D. Astruc, *Chem. Eur. J.* **2007**, DOI: 10.1002/chem.200701410.
[20] A. W. van der Made, P. W. N. M. van Leeuwen, J. C. de Wilde, R. A. C. Brandes, *Adv. Mater.* **1993**, *5*, 466.
[21] a) A. J. Charlton, N. J. Baxter, M. L. Khan, A. J. G. Moir, E. Haslam, A. P. Davies, M. P. Williamson, *J. Agric. Food Chem.* **2002**, *50*, 1593; b) C. Simon, K. Barathieu, M. Laguerre, J. M. Schmitter, E. Fouquet, I. Pianet, E. J. Dufourc, *Biochemistry* **2003**, *42*, 10385.
[22] For seminal exemples of intradendritic encapsulation of guests, see refs. [3,7a] and G. R. Newkome, *Pure Appl. Chem.* **1998**, *70*, 2337.
[23] a) J. Smid in *Ions and Ion Pairs in Organic Reactions, Vol. 1* (Ed.: M. Schwarc), Wiley, New York, **1972**, Chap. 3; b) *The Organic Chemistry of Electrolyte Solutions* (Ed.: J. E. Gordon), Wiley, New York, **1975**; c) J. D. Simon, K. S. Peters, *Acc. Chem. Res.* **1984**, *17*, 277; d) A. Loupy, B. Tchoubar, D. Astruc, *Chem. Rev.* **1992**, *92*, 1141.

Received: October 11, 2007
Revised: January 31, 2008
Published online: April 2, 2008

