JOURNAL OF PHYSICAL ORGANIC CHEMISTRY
J. Phys. Org. Chem. 2001; **14:** 392–399
DOI:10.1002/poc.368

Do dendritic amphiphiles self-assemble in water? A Fourier transform pulse-gradient spin-echo NMR study†

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Received 21 October 2000; revised 10 December 2000; accepted 2 January 2001

ABSTRACT: Amphiphilic poly(amidoamine) (PAMAM) dendrimers were synthesized having an average of 7–46 hydrophobic chains of varying lengths (C_{10}–C_{14}) attached to the periphery of 64 amine groups. The synthesis was performed in three steps: (1) protection of a desired number of amines with BOC groups; (2) reaction of the remaining amine groups with long-chain acid chlorides; and (3) deprotection from the BOC groups to produce the amphiphilic dendrimers as HCl salts. The behavior of the dendrimers in aqueous media was examined by pulse-gradient spin-echo (FT-PGSE) NMR and dynamic light scattering. Self-diffusion data on dendrimers with 22 or fewer chains, along with dynamic light scattering on concentrations of $\leq$16 wt%, gave no indication of dendrimer–dendrimer self-assembly via hydrocarbon chains exposed on the dendrimer surface. It is concluded that dendrimers with 7–22 chains, each having 10–14 carbons, behave as unimolecular entities with chains coiled largely within the dendrimer periphery. Only when the number of chains becomes very large (ca $\geq$34) are chains forced externally where they can promote hydrophobically induced self-assembly. Monomeric dendrimers possessing the full range of functional polarity have great potential in enzyme modeling. Copyright © 2001 John Wiley & Sons, Ltd.

KEYWORDS: dendrimers; amphiphilic dendrimers; self-assembly; Fourier transform pulse-gradient spin-echo NMR

INTRODUCTION

Following the initial synthesis of molecules with dendritic architectures in the late 1970s,[1] many different structural classes of dendrimers have been constructed, including poly(amidoamine) (PAMAM) dendrimers,[2,3] poly(propyleneimine) dendrimers,[4] polyether dendrimers[5] and various metallodendrimers.[6,7] Supramolecular compounds with dendritic formats are also known and include iptycenes[8] and hydra-amphiphiles.[9] Poly(amidoamine) and poly(propyleneimine) dendrimers were among the first to be synthesized in large quantities and become available commercially.

Tomalia and co-workers, the developers of PAMAM dendrimers, reported a synthesis of a hydrophobically modified PAMAM dendrimers and their properties at the air–water interface.[10] Each amine group of the PAMAM dendrimers had been derivatized with a long-chain epoxide, thus converting the hydrophilic PAMAM dendrimers into hydrophobic substances. These compounds were capable of transporting copper(II) sulfate from an aqueous solution into an organic phase. The authors proposed two models for the behavior of amphiphilic PAMAM dendrimers at the air–water interface. One model considers the dendrimers to be flexible molecules capable of placing their hydrophilic interior in contact with aqueous subphase and extending their chains into the air above the air–water interface. The second model views the dendrimers as 'hydrophobic spheroids' floating on the water surface. The authors concluded that 'it is difficult to tell which of these models is the most probable. Perhaps there is a transition from the first model which may be operable for the lower generations to the floating hydrophobic spheroid model for the more congested higher generations.'

Another group headed by Meijer investigated the self-assembly of the hydrophobically modified poly(propyleneimine) dendrimers in which all the amine groups were modified with palmitoyl chains.[11] They observed the formation of small spherical aggregates (20 –200 nm in diameter) which, based on x-ray diffraction data and osmotic experiments, were thought to be vesicular in nature. Once again the results reflect a significant flexibility of the dendrimers which allows the hydrophobic moieties to form a region resembling a parallel-packed bilayer, while the dendritic interior is redirected externally toward the water.

Research into amphiphilic dendrimers has been progressing at a rapid rate. Important contributions to the area include the synthesis and investigation of

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†Dedicated to Professor Hans-Jörg Schneider in recognition of his 65th birthday.
Contract/grant sponsor: National Institutes of Health.

unimolecular dendritic micelles based on a neopentyl core,[12] glucose-persubstituted (PAMAM) dendrimers,[13] carbosilane dendrimers,[14] poly(propyleneimine) dendrimers with both octyl and triethylenoxy methyl ether chain termini[15] and polyether amphiphilic dendrimers,[16] among others.[17–22]

This paper describes (a) the synthesis of amphiphilic PAMAM dendrimers with controlled degrees of surface hydrophobicity and (b) the behavior of such dendrimers in aqueous solutions as studied by means of pulse-gradient spin-echo (FT-PGSE) NMR and dynamic light scattering (DLS). A schematic diagram of the target amphiphilic dendrimers is shown.

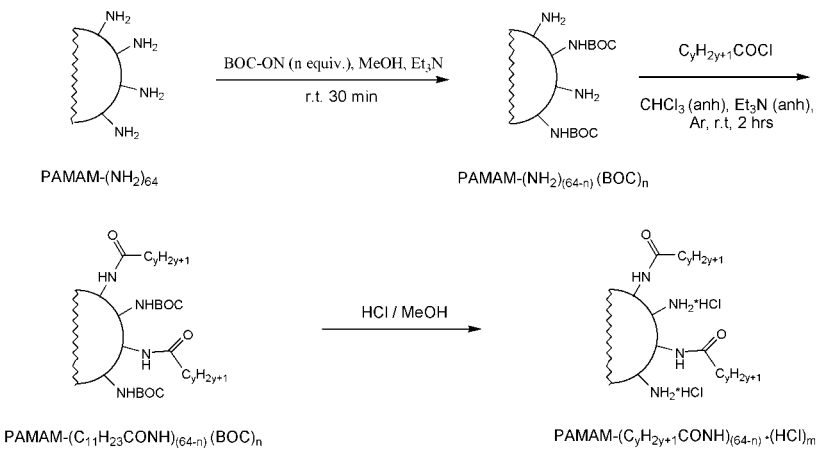
It should be noted that the drawing represents an 'idealized' picture of the amphiphilic dendrimer because, as we shall see, the true state of the hydrophobic chains in water is far different from the one portrayed.

RESULTS AND DISCUSSION

Despite considerable research on hydrophobically modified dendrimers, there are few reports, to the best of our knowledge, about the effect of partial modification of the terminal functionalities with long hydrophobic chains on the behavior in solution.[23–27] Meijer's group attempted to modify only a finite number of terminal amine groups of poly(propyleneimine) with long hydrophobic chains, but this led to two products only: fully reacted dendrimer and totally unmodified dendrimer.[28] (similar 'all or nothing' substitution was also noticed with calixarenes).[29] On the other hand, Tomalia and co-workers alluded to unpublished results[10] where PAMAM dendrimers modified with epoxyalkanes do not reveal an 'all or nothing' behavior (L. T. Piehler and D. A. Tomalia, unpublished results).

We chose commercially available (Aldrich) poly(amidoamine) (PAMAM) dendrimers of the fourth generation with 64 terminal primary amine groups (MW≈14 215). Attempts to synthesize the amphiphilic PAMAM dendrimers with various degrees of terminal substitution in one step using long-chain acid chlorides proved fruitless. Similar to the results of Meijer's group, we isolated only a mixture of the unsubstituted product and the fully substituted product. In order to circumvent these difficulties, we developed a three-stage procedure as shown in Scheme 1.

The first step allowed us to obtain dendrimers with controlled amounts of BOC protecting groups on the surface. Total substitution, in this case, did not take place. We note in this regard that when Meijer's group derivatized dendrimers with pivaloyl chloride instead of palmitoyl chloride, the 'all or nothing' effect did not apply, and partially acylated material was formed.[28] We assume that the BOC groups distributed themselves randomly among the excess dendrimer amine groups. The resulting BOC-protected dendrimers, in contrast to unmodified dendrimers, were soluble in organic solvents such as chloroform and dichloromethane. The combined second and third steps involve the reaction of the BOC-protected dendrimers with a slight excess of long-chain acid chlorides followed by deprotection with HCl–MeOH to produce the desired dendrimers in moderate to excellent (37–100%) isolated yields as HCl salts.



Scheme 1. Synthesis of amphiphilic PAMAM dendrimers

Based on the above procedure, we were able to prepare modified dendrimers with various degrees of substitution. Nine amphiphilic dendrimers were synthesized which, for brevity, are symbolized as P-0, P-C_{10}(9), P-C_{10}(23), P-C_{12}(7), P-C_{12}(16), P-C_{12}(34), P-C_{12}(46), P-C_{14}(8) and P-C_{14}(22), where P-0 stands for the HCl salt of unmodified PAMAM dendrimer with zero chains and, e.g., P-C_{10}(9) has nine decanoic (C_{10}) residues on the dendrimer surface. The average number of chains, which agreed well with the reaction stoichiometries, was determined by integrating the NMR proton signals from the dendrimer nucleus and the alkyl chains. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometric (MALDI-TOFMS) data, also used to obtain the number of chains per dendrimer molecule, agreed fairly well with the NMR-based values. It should be emphasized that the cited 'number of chains' represents in fact a distribution. Although broadened MALDI peaks made it impossible to define this distribution, dendrimers with, for example, 10 chains greater or fewer than an average of 22 are no doubt present at low levels.

Our dendrimers are colorless hygroscopic powders that are soluble in water and methanol [except for P-C_{12}(46), which is insoluble in water but soluble in chloroform, producing a gel-like solution]. Most dendrimers gave clear aqueous solutions with stable foams upon shaking, indicating the surface-active nature of the compounds. P-C_{12}(34) solutions are slightly turbid owing to the presence of larger aggregates, as confirmed by DLS (see discussion below).

The principal question that engaged our curiosity concerned the effect of the multiple aliphatic chains on properties in the aqueous media. Would the chains face outward forcing a hydrophobically based self-assembly, or would the chains hide themselves inside the dendrimer interior? The answer to this question should, of course, depend on the degree of substitution. Various techniques including NMR (self-diffusion PGSE) and DLS were applied to the problem.

PGSE NMR is a powerful technique which allowed us to monitor the translational diffusion of molecules in solution. Reviews have been written on the subject (for reviews on FT-PGSE, see Ref. 30); suffice it to say here that with sufficient care one can extract reliable diffusion coefficients and that these parameters reflect the molecular aggregation state. Data on the self-diffusion of amphiphilic dendrimers as a function of their chain content and concentration are provided in Fig. 1.

The graphs reveal that the diffusion coefficients of the dendrimers depend upon the number of hydrophobic chains. For example, the three compounds P-C_{12}(7), P-C_{12}(16), and P-C_{12}(34) with 7, 16 and 34 C_{12} chains, respectively, have diffusion coefficients at 2 wt% dendrimer of 4.8×10^{-11}, 4.1×10^{-11} and 2.8×10^{-11} $m^2\ s^{-1}$, respectively. A similar sequence was found for the C_{10} and C_{14} derivatives. However, as seen, the

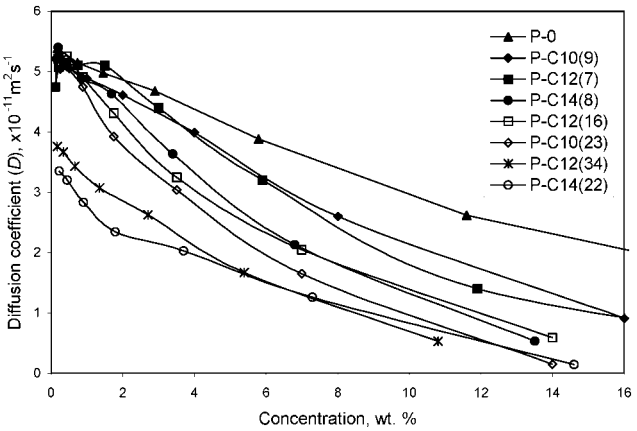


Figure 1. Self-diffusion measurements of amphiphilic dendrimers by FT-PGSE NMR

changes in diffusion with substitution are modest, and none of the values for the dendrimer derivatives differ by more than a factor of two from that of the parent dendrimer (4.8×10^{-11} $m^2\ s^{-1}$). If the chains were in fact inducing self-assembly, much larger differences in diffusion would have been expected as the number of chains was varied from 7 to 34. A lack of self-assembly is also evident from a comparison of two dendrimers with the same number of chains but with differing lengths such as P-C_{10}(23) and P-C_{14}(22). Their diffusion coefficients differ by less than a factor of two despite the fact that the latter possesses 78 additional carbons. Thus, the picture painted by Fig. 1 is one of a monomeric species whose diffusion at the relatively high concentrations slowly decreases owing to particle–particle interactions.

Self-diffusion PGSE NMR experiments allowed us to calculate the diameters of the aggregates from the diffusion coefficients using the Stokes–Einstein equation, $d = kT/3\pi\eta D$, where d = diameter of a spherical particle (cm), k = Boltzmann's constant (1.38×10^{-16} erg K^{-1}), T = temperature (K), η = diluent viscosity (P) and D = diffusion coefficient ($cm^2\ s^{-1}$). Since the diameters were calculated at low concentrations (0.2 wt%), the viscosity was assumed to be that of pure water. The calculations showed that the diameters of all dendrimers, except that of P-C_{14}(22) and P-C_{12}(34), are 8 ± 2 nm. This value also applies to the parent unsubstituted dendrimer, indicating that substitution has little effect on the dendrimer size. Only P-C_{14}(22) and P-C_{12}(34) show an increase in diameter (12 ± 2 nm). This could result from a minor expansion of the dendrimer or, alternatively, from a small fraction of aggregated dendrimer contributing to the diffusion properties.

The theoretical maximum diameter of unmodified dendrimer is ca 6 nm.[2,3] We presume that the larger value obtained from the diffusion data relates in part to the cationic nitrogens which electrostatically repel each other and swell the polymer. Moreover, the periphery of the dendrimers is replete with counterions and water of

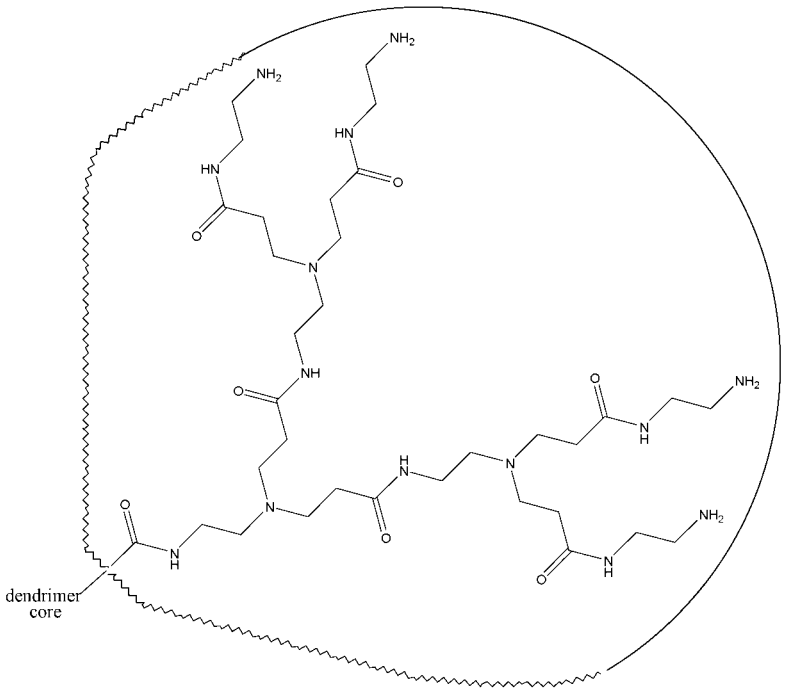


Figure 2. The structure of one of 16 dendrons present in PAMAM dendrimer

hydration, all of which contribute to the dendrimer size (and to the diffusion properties) over and above that calculated from simple geometric considerations alone.

DLS was also used to obtain information on dendrimer aggregation. By this means it could be shown that at ca 4–8 wt% all water-soluble dendrimers, except the most highly substituted one, P-C_{12}(34), have hydrodynamic diameters less than 10 nm (the limit of our instrument). Thus, the FT-PGSE and DLS results agree: no dendrimer aggregation occurs even when there are as many as 22 chains of 14 carbons each speckling the dendrimer surface. Consistent with this notion is the fact that aqueous solutions of P-C_{14}(22) remain fluid at concentrations up to 16 wt%. Moreover, significant broadening of alkyl chain protons in the NMR spectra, indicative of aggregation, was not observed in the 0–16 wt% concentration range.

How can we explain the reluctance of highly hydrophobized dendrimers to self-assemble via chain–chain contact or, possibly, via contact between external hydrophobic patches? We seek refuge, as have others in the past, in dendrimer flexibility.[10,11] The situation may be distantly reminiscent of the polyether copolymers of Fréchet and co-workers, which change their shape significantly depending upon the solvent.[17,20] In our case, conformational changes are induced by hydrocarbon substitution rather than solvation effects. Apparently, up to 22 C_{14} chains can be housed by a dendrimer without the chains being flagrantly exposed to the surface where they can interact hydrophobically with chains of another dendrimer. This hydrocarbon 'absorption' process can only occur if the hydrophilic dendrimer groups readjust themselves to accommodate hydrocarbon chains which, conceivably, assist the process by 'curling up' as they are likely to do in aqueous media. In order to judge whether this is a reasonable model, we performed the simple arithmetic calculations below.

The parent PAMAM dendrimer used in our work has (a) 64 terminal primary ammonium groups, (b) 62 tertiary ammonium groups and (c) 124 amide groups. When 22 C_{14} groups are placed on the dendrimer via amide groups, the compound now has (a) 42 terminal primary ammonium groups, (b) 62 tertiary ammonium groups, (c) 146 amide groups and (d) 286 methyl + methylene carbons from the chains. This means, therefore, that in P-C_{14}(22) there are only about three carbons for each protonated ammonium group, and there are only two carbons for each amide group. In this light it is hardly surprising that P-C_{14}(22) is water soluble. It is also not surprising that internal space is available to accommodate the chains. Figure 2, showing one of the 16 dendrons of the parent PAMAM dendrimer, helps clarify the point. Each dendron must encompass, and protect from the external bulk solvent, an average of 1.4 chains. Surrounding this number of carbons would not seem to be an insurmountable task.

Of course, we do not have a precise molecular picture of our dendrimer in water. We do not know, for example, if chain–chain contact within the dendrimer is a possible route to reducing unfavorable chain–water interactions. We also do not know if in fact hydrocarbon is, to some extent, exposed to the bulk water, and only the

electrostatic repulsion among the ammonium groups prevents aggregation. We do know, however, that our dendrimers remain monomeric, similar to many proteins which also are water soluble and monomeric despite large regions of hydrophobicity. Here again hydrophilic functionalities manage to encase the hydrophobic regions and impart solubility.

Despite the reasonableness of the above model, we were, admittedly, surprised and pleased that a single molecule can solubilize hundreds of methylenes in water. One would expect these carbons to change dramatically the properties of the dendrimer, e.g. the binding ability toward hydrophobic guests. Indeed, our results suggest the possibility of designing non-colloidal enzyme-like models that combine high-capacity hydrophobic binding with catalytic functionalities, and therein may lie the most useful ramifications of this research.

Ultimately, of course, one can overtax the hydrophilicity of the dendrimer. When the number of chains becomes large ($\geq$34 chains), self-assembly does indeed occur. At still larger numbers of chains (46), the dendrimer fails to dissolve in water. It is interesting in this regard that P-C_{12}(46) can be sonicated in water to produce large aggregates 50–700 nm in diameter according to DLS. When such a suspension was passed through a 100 nm filter, the 50–700 nm size distribution persisted. Obviously, the dendrimer aggregates were able to disassemble, pass through the filter and then reassemble. The nature of the aggregates was not explored in detail because, as mentioned, the dendrimers with 22 or fewer chains incorporate a full range of functional polarity and yet remain water soluble and monomeric; such molecules are more attractive from the point of view of enzyme modeling, a continuing interest of ours.[31,32]

EXPERIMENTAL

General. ^{1}H NMR and ^{13}C NMR spectra were acquired on a Varian Inova 400 (400 MHz ^{1}H, 100 MHz ^{13}C) spectrometer in D_2O (4.80 ppm, ^{1}H) as an internal reference and with CH_3OH in D_2O (49.15 ppm, ^{13}C) as an external reference. Data are reported in the following order: chemical shifts are given (δ); multiplicities are indicated by br (broadened) and m (multiplet); integration is provided. MALDI-TOFMS was performed by the Microchemical Facility of Emory University School of Medicine. Analytical thin-layer chromatography (TLC) was performed on Merck silica gel plates with an F-254 indicator; visualization was accomplished with iodine and UV irradiation. Solvents used as reaction media were dried under 4 Å molecular sieves and titrated for water content prior to use with a Fisher Coulomatic K-F titrator.

Fourier transform pulsed-gradient spin-echo (FT-PGSE) measurements of dendrimer self-diffusion. Experiments for measuring self-diffusion were performed on a Varian Inova 600 spectrometer equipped with a *Z*-gradient probe and a PFG amplifier. Self-diffusion coefficients were determined based on a procedure which was first developed by Stejskal and Tanner[33] and later summarized by various authors.[30] The basic spin-echo sequence 90°–τ–180° originally developed by Hahn[34] consists of two r.f. pulses (a 90° pulse followed by a 180° pulse) and two gradient pulses (one before and one after the 180° degree pulse). Owing to molecule self-diffusion during the gradient pulses, the spins were not able to refocus perfectly, leading to reduced echo attenuation which can be expressed as follows:

$$\ln(A/A_0) = -\gamma^2 D\delta^2 G^2(\Delta - \delta/3)$$

where G = pulsed field gradient strength (G cm^{-1}), δ = length of the field gradient pulse (ms), D = diffusion coefficient, Δ = time between the start of the first gradient pulse and the second gradient pulse, A_0 = echo attenuation without field gradient and A = echo attenuation with field gradient. The self-diffusion coefficients were determined from the slope of ln (A/A_0) vs G^2 while keeping δ (60 ms) constant with Δ = 62 ms. Signals from dendrimer protons were generally used for determining diffusion coefficients, although alkyl chain protons gave identical results. Absolute values of D were determined on a reference sample (10 mol% H_2O–D_2O) for which the diffusion coefficient is known.[35] Values of self-diffusion coefficients obtained for water and cyclooctane were found to be 2.3×10^{-9} and 0.53×10^{-9} m^2 s^{-1}, respectively, at 25 °C. In the case of slow diffusion ($\leq 10^{-10}$ m^2 s^{-1}), longer gradients were required (δ = 40–60 ms) and a secondary calibration standard was developed (10 mol% ethylene glycol–ethylene glycol-d_4) for which the self-diffusion coefficient (D) was determined as 9.2×10^{-11} m^2 s^{-1} at 25 °C. All standards were stored in NMR tubes sealed with Parafilm, to avoid any water contamination. Shigemi NMR tubes supplied by Shigemi were used for all diffusion measurements, allowing excellent reproducibility ($\leq$5%). Samples were equilibrated for 40 min prior to measurements to allow for temperature equilibration. The volume of the samples in D_2O was ca 150 µl. Internal deuterium lock was used for field/frequency stabilization.

Dynamic light scattering (DLS). DLS measurements were performed on an N4 PLUS Coulter particle sizer. Measurements were taken at a 90° angle and were repeated 3–4 times (30 min each run).

Starting materials. Starburst (PAMAM) dendrimer (Generation 4, 10 wt% solution in CH_3OH, contains 64 surface primary amino groups, FW = 14 215), BOC-ON [2-(*tert*-butoxycarbonyloxyimino)-2-phenylacetonitrile], myristoyl chloride, decanoyl chloride and lauroyl chloride were purchased from Aldrich, triethylamine

and methanol from Fisher and Sephadex G-10 and lypophilic Sephadex LH-20 from Sigma. All reagents were used without further purification.

General procedure for the synthesis of amphiphilic dendrimers. *Step I. Synthesis of BOC-protected dendrimers* [$P-(BOC)_n$] *with n-number of amines protected with BOC groups*. A solution of Starburst (PAMAM) dendrimer (Generation 4, 10 wt% solution in CH_3OH, 10 g, 0.07 mmol, 1.0 equiv.) and triethylamine ($1.5n \times 0.07$ mmol, $1.5n$ equiv.) was stirred at room temperature under an open atmosphere. A solution of BOC-ON ($n \times 0.07$ mmol, n equiv.) in methanol (ca 30 ml) was added dropwise over 30 min at room temperature. The reaction was complete within 5–10 min after the addition, as monitored by the disappearance of the BOC-ON TLC spot [UV visualization, hexane–ethyl acetate (2:1) as eluent] with $R_f = 0.7$ and the appearance of a new spot with $R_f = 0.5$; the reaction was stirred for an additional 2–3 h at room temperature. The reaction mixture was concentrated by rotary evaporation and the resulting green oil was subjected to size exclusion chromatography using Sephadex LH-20 as a stationary phase (50 g of Sephadex were used for column preparation) and methanol as eluent. Fractions 1–7 were collected (60 ml total volume, dendrimer eluting first), methanol was removed with the rotary evaporator and the residue was dried under vacuum to give compounds of the general formula P-$(BOC)_n$ in 80–90% overall yield as colorless, hygroscopic powders. The purity of BOC-substituted dendrimers was evaluated by TLC (as well as NMR): (a) by the absence of spots with $R_f = 0.5$ and 0.7 [UV, hexane–ethyl acetate (2:1)] and (b) by the absence of the Et_3N spot with $R_f = 0.3$ [I_2, methanol–acetic acid (20:1)]. Dendrimers have $R_f = 0$. The resulting dendrimers are soluble in chloroform, in contrast to unsubstituted dendrimers, which are insoluble. The number of BOC groups (n) can be determined by ^{1}H NMR ($CDCl_3$) assuming that the dendrimer protons appear as a multiplet from 2.2 to 3.4 ppm [number of protons = $996 + 2(64 - n)$] and the signal from the BOC group appears at 1.4 ppm (number of protons = $9n$). Knowing n, it is possible to calculate the molecular weight of the partially protected dendrimers.

Steps II and III. Reaction of BOC-protected dendrimers [$P-(BOC)_n$] *with acid chlorides followed by the cleavage of BOC groups*. P-$(BOC)_n$ obtained in the first step was dissolved in 30 ml of dry $CHCl_3$ and the solution was stirred under argon at room temperature. Dry triethylamine [$1.3 \times (64 - n)$ equiv.] was added and the reaction mixture was cooled to 0 °C. A solution of an appropriate long-chain acid chloride [$1.1 \times (64 - n)$ equiv.] in 10 ml of dry $CHCl_3$ was added dropwise over 20–30 min. After the addition was complete, the reaction mixture was stirred for 4 h at room temperature under argon. Methanol (5 ml) was added and the reaction mixture was stirred for an additional 1 h, after which the solvents were evaporated with the rotary evaporator followed by drying *in vacuo*. The resulting solid was washed with Et_2O (3×100 ml) and filtered, then dissolved in 10 ml of methanol followed by a quick (2 min) addition of 30 ml of approximately 1 M HCl–CH_3OH solution (prepared by bubbling HCl gas in methanolic solution) under vigorous stirring at room temperature. After stirring for 30 min, the solution was evaporated, washed with Et_2O (3×100 mL), then with a large amount of $CHCl_3$ (5×300 ml) [washing with chloroform was not performed in the case of P-C_{12}(46) because of its solubility in $CHCl_3$], followed by filtration and drying under vacuum. The resulting solid was further purified by size exclusion chromatography using 50 g of Sephadex LH-20 as a stationary phase and methanol as eluent [in the case of P-C_{12}(46), chloroform was used as eluent, because the compound was only partially soluble in methanol]. Fractions 1–7 were collected (60 ml total volume, dendrimer eluting first) and methanol was removed with the rotary evaporator. The residue was dried under vacuum to give compounds of the general formula P-C_N(M) as HCl salts in 37–100% overall yields as colorless, very hygroscopic powders that are soluble in water and methanol [with the exception of P-C_{12}(46), which is insoluble in H_2O but is soluble in $CHCl_3$]. The purity of the modified dendrimers can also be checked by TLC (as well as NMR) by the absence of an Et_3N–HCl (Et_3N) spot with $R_f = 0.3$ [I_2, methanol–acetic acid (20:1)]. Amphiphilic dendrimers have $R_f = 0$.

The number of chains can be determined by ^{1}H NMR assuming that the dendrimer protons appear as a multiplet from ca 2.0 to 3.7 ppm (number of protons = $996 + 2(64 - M)$, where M is the number of chains) and that the long-chain protons appear as a set of signals from ca 0.5 to 2.2 ppm. Knowing M, it is then possible to calculate the molecular weight of the obtained amphiphilic dendrimers (without HCl). The final number of chains was estimated using MALDI-TOFMS and NMR data.

Dendrimers. *P-0 (HCl salt of unmodified dendrimer prepared with excess HCl in methanol).* Yield, 100%; ^{1}H NMR (400 MHz, D_2O, 25 °C), $\delta = 2.80$–3.75 (br m); ^{13}C NMR (100 MHz, D_2O, 25 °C), $\delta = 173.2$, 172.8, 172.5, 172.4, 172.3, 52.6, 52.4, 52.3, 50.3, 50.0, 49.8, 49.5, 39.8, 39.7, 39.5, 37.4, 34.9, 34.7, 29.6, 29.5, 29.2; MALDI-TOFMS, MW = 14 300 (literature MW = 14 215).

P-C_{12}(7). Yield (overall), 62%; ^{1}H NMR (400 MHz, D_2O, 25 °C), $\delta = 2.75$–3.75 (br m, 1110H), 2.20–2.28 (br m, 14H), 1.50–1.62 (br m, 14H), 1.20–1.32 (br m, 112H), 0.82–0.90 (br m, 21H); number of chains determined by NMR = 7 [calculated MW = 15 490 (without HCl)]; ^{13}C NMR (100 MHz, D_2O, 25 °C), $\delta = 173.1$, 172.7, 172.5, 172.3, 52.4, 52.3, 50.2, 50.0, 49.9, 47.2, 39.6, 39.3, 39.1,

38.9, 37.3, 36.5, 34.8, 34.7, 32.3, 30.1, 29.5, 29.2, 26.2, 23.1, 14.4; MALDI-TOFMS, MW = 15 360 (calculated number of chains = 6).

P-C$_{12}$(16). Yield (overall), 55%; ^{1}H NMR (400 MHz, D_2O, 25 °C), δ = 2.50–3.65 (br m, 1092 H), 1.98–2.10 (br m, 32H), 1.30–1.45 (br m, 2H), 0.95–1.25 (br m, 256H), 0.60–0.71 (br m, 48H); number of chains determined by NMR = 16 [calculated MW = 17 130 (without HCl)]; ^{13}C NMR (100 MHz, D_2O, 25 °C), δ = 176.3, 173.2, 172.9, 172.7, 172.4, 52.4, 50.1, 49.8, 39.7, 39.6, 39.2, 37.3, 36.5, 34.9, 32.4, 30.2, 29.9, 29.3, 26.2, 23.1, 14.4; MALDI-TOFMS, MW = 16 850 (calculated number of chains = 15).

P-C$_{12}$(34). Yield (overall), 66%; ^{1}H NMR (400 MHz, D_2O, 25 °C), δ = 2.50–3.75 (br m, 1048H), 2.15–2.30 (br m, 76H), 1.30–1.50 (br m, 76H), 1.10–1.40 (br m, 608H), 0.75–0.90 (br m, 114H); number of chains determined by NMR = 38 [calculated MW = 21 100 (without HCl)]; ^{13}C NMR (100 MHz, D_2O, 25 °C), δ = 176.2, 173.2, 172.9, 172.7, 172.4, 52.3, 50.1, 49.3, 39.5, 39.2, 37.3, 36.6, 34.8, 32.5, 30.2, 30.1, 29.3, 23.1, 14.4; MALDI-TOFMS, MW = 19 700 (calculated number of chains = 30).

P-C$_{12}$(46). Yield (overall), 37% [the yield was diminished because of poor separation on the Sephadex column (LH-20, $CHCl_3$ eluent), possibly on account of unsatisfactory packing of the gel due to the high density of chloroform]; ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C), δ = 2.60–4.40 (br m, 1026H), 2.10–2.15 (br m, 98H), 1.55–1.65 (br m, 98H), 1.05–1.20 (br m, 784H), 0.85–0.90 (br m, 147H); number of chains determined by NMR = 49 [calculated MW = 23 100 (without HCl)]; MALDI-TOFMS, MW = 21 600 (calculated number of chains = 41).

P-C$_{10}$(9). Yield (overall) 65%; ^{1}H NMR (400 MHz, D_2O, 25 °C), δ = 2.75–3.80 (br m, 1106H), 2.15–2.30 (br m, 18H), 1.50–1.63 (br m, 18H), 1.20–1.37 (br m, 108H), 0.80–0.91 (br m, 27H); number of chains determined by NMR = 9 [calculated MW = 15 600 (without HCl)]; ^{13}C NMR (100 MHz, D_2O, 25 °C), δ = 173.2, 173.1, 172.8, 172.5, 172.4, 52.4, 52.3, 50.2, 49.9, 39.6, 39.3, 39.1, 38.8, 37.3, 36.4, 34.8, 32.3, 31.7, 29.6, 29.2, 28.7, 25.9, 23.1, 22.6, 14.4, 14.1; MALDI-TOFMS, MW = 15 800 (calculated number of chains = 10).

P-C$_{10}$(23). Yield 46%; ^{1}H NMR (400 MHz, D_2O, 25 °C), δ = 2.50–3.80 (br m, 1076H), 2.15–2.30 (br m, 48H), 1.50–1.63 (br m, 48H), 1.20–1.37 (br m, 288H), 0.80–0.91 (br m, 72H); number of chains determined by NMR = 24 [calculated MW = 17 900 (without HCl)]; ^{13}C NMR (100 MHz, D_2O, 25 °C), δ = 176.4, 173.1, 173.0, 172.7, 172.2, 52.4, 50.1, 49.8, 39.7, 39.5, 39.3, 39.1, 37.3, 36.5, 34.8, 32.4, 29.6, 29.2, 26.2, 23.1, 14.3; MALDI-TOFMS, MW = 17 500 (calculated number of chains = 21).

P-C$_{14}$(8). Yield 52%; ^{1}H NMR (400 MHz, D_2O, 25 °C), δ = 2.75–3.70 (br m, 1106H), 2.18–2.28 (br m, 18H), 1.50–1.60 (br m, 18H), 1.19–1.31 (br m, 180H), 0.80–0.90 (br m, 27H); number of chains determined by NMR = 9 [calculated MW = 16 100 (without HCl)]; ^{13}C NMR (100 MHz, D_2O, 25 °C), δ = 173.2, 172.9, 172.6, 172.1, 52.4, 52.2, 50.1, 49.8, 39.7, 39.6, 39.3, 39.1, 38.9, 37.3, 36.5, 34.8, 32.4, 30.2, 29.7, 29.3, 26.2, 23.1, 14.4; MALDI-TOFMS, MW = 15 430 (calculated number of chains = 6).

P-C$_{14}$(22). Yield, 51%; ^{1}H NMR (400 MHz, D_2O, 25 °C), δ = 2.75–3.75 (br m, 1080H), 2.18–2.28 (br m, 48H), 1.50–1.65 (br m, 48H), 1.20–1.35 (br m, 480H), 0.78–0.90 (br m, 72H); number of chains determined by NMR = 24 [calculated MW = 19 250 (without HCl)]; ^{13}C NMR (100 MHz, D_2O, 25 °C), δ = 176.2, 173.1, 172.9, 172.7, 172.0, 52.4, 50.2, 49.4, 39.7, 39.6, 39.4, 38.9, 37.3, 36.6, 34.8, 32.5, 30.5, 30.1, 29.6, 29.2, 26.3, 23.2, 14.4; MALDI-TOFMS, MW = 18 400 (calculated number of chains = 20).

Acknowledgement

This work was supported by the National Institutes of Health.

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