Cascade Polymers.[1] pH Dependence of Hydrodynamic Radii of Acid Terminated Dendrimers

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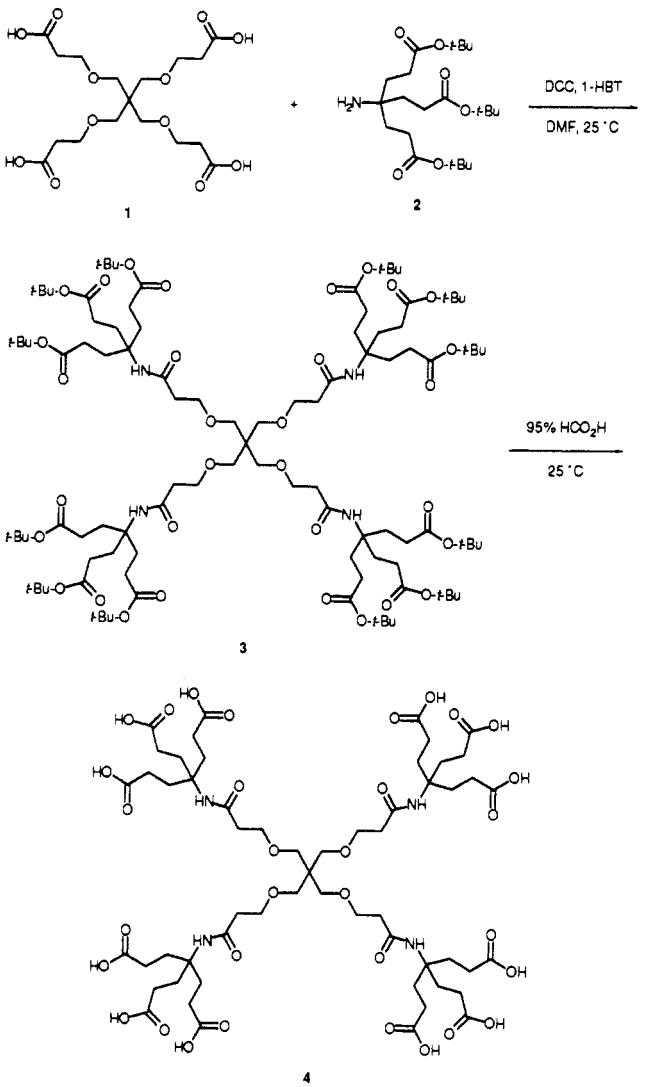
The unique physical properties inherent to cascade (dendritic) molecules and polymers have spawned a burst of interest in this relatively new field. Pertinent aspects of cascade syntheses,[2] as well as the variety of novel branched building blocks (monomers) and initiator cores, prepared in our laboratories,[3] have been reviewed. We herein report the preliminary physical studies of a cascade series, *Z*-Cascade:methane[4]:(3-oxo-6-oxa-2-aza-heptylidyne):(3-oxo-2-azapentylidyne)$^{G-1}$:propanoic acids,[4] which was readily prepared from the tetraacid core **1**[5] and branched amine **2** (Scheme I).[6]

The iterative, stepwise synthesis was comprised of amide formation followed by surface polarity reversal via facile hydrolysis to yield the desired polyacid at each generation. Thus, coupling[7] of tetraacid **1** with amine **2** via treatment with 1-hydroxybenzotriazole and dicyclohexylcarbodiimide in *N*,*N*-dimethylformamide at 25 °C gave (70%) dodecaester **3**,[8] which was hydrolyzed using 95% formic acid at 25 °C to give (72%) the dodecaacid **4**.[9] Five generations were prepared by repetition of this two-step sequence; after purification, the yields ranged from 30 to 70% for the esters and from 65 to 85% for the acids.[10] Table I shows the expected nominal formula weights for the acid-terminated series. After the third generation, all the cascade polyacids showed nearly identical, yet assignable, NMR patterns; only the relative peak intensities varied from generation to generation. With the third generation and larger acids, the peak for the central quaternary carbon (^{13}C NMR: δ 45.0) of the initiator core becomes unobservable; however in the ^{1}H NMR spectra, the OCH_2 moieties of the initiator core (δ 3.2 and 3.5) were observable through the fourth generation and gave proper relative integration.

The hydrodynamic properties of the third generation 108-acid were initially examined via size-exclusion chromatography (SEC). These experiments indicated large size changes with pH variance. Figure 1A shows the elution profile of a partially purified sample of this 108-acid at pH 6.8, where the first eluted peak was determined (NMR) to be the desired acid; the later eluting peaks (ca. position 28 and position 46) were synthetic impurities that were readily eliminated by dialysis and HPLC (see the Supplementary Material). These impurities have no effect on the elution position of the 108-acid, which elutes at position 20 at pH 6.8, corresponding to the elution position of a globular protein of approximately 29.6 kDa. The peak fractions containing the 108-acid were combined, and the *pH was reduced to 2.0*; reapplication of this sample onto the SEC column resulted in a significant shift in the elution position (Figure 1B), with the majority of the material eluting at position 24, corresponding to the elution of a

Scheme I



globular protein of approximately 17.3 kDa. This apparent molecular contraction for this typical member of the cascade acid series is also operative in the reverse direction, as shown in Figure 1 (C and D).[11]

The molecular size distribution for this polymer acid series was also examined by means of diffusion ordered 2D-NMR spectroscopy (DOSY) incorporating the inversion program SPLMOD.[12-14] The 250-MHz ^{1}H DOSY display for the 108-acid at neutral pH is shown in Figure 2. Only the polymer and HOD peaks are present, and each shows a single component, indicating that the sample is pure and monodisperse within the limits of detection. Similar results were obtained in the DOSY analyses of the other generations. Diffusion coefficients were conveniently obtained with pulsed field gradient NMR for each polymer generation at ca. 1 mM concentration by using only the integral A of the major polymer peaks. The integral was fit by nonlinear least-squares regression to the equation[15]

$$A = A_0 \exp[-K^2(\Delta - \delta/3)D] \qquad (1)$$

with A_0 and the tracer diffusion coefficient D as the free parameters. In eq 1, $K = \gamma g \delta$, where γ is the magnetogyric ratio, g and δ are the amplitude and duration of the gradient pulse, respectively, and Δ is the diffusion time (i.e., the time between the leading edges of the gradient pulses).

Effective hydrodynamic radii were calculated from measured D values with the Stokes–Einstein equation,

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Table I
Observed Diffusion Coefficients and Calculated Hydrodynamic Radii for the Cascade Polymers

generation (G)	no. of terminal acids (Z)	formula wt	[Cascade] (mM)	D ($cm^2\ s^{-1}$) [hydrodynamic radius (Å)] acidic pH[a]	neutral pH[a]	basic pH[a]
1	12	1 341	1.00	2.41×10^{-6} [8.24]	1.62×10^{-6} [12.3]	1.68×10^{-6} [11.8]
2	36	4 092	1.00	1.74×10^{-6} [11.4]	1.15×10^{-6} [17.3]	1.26×10^{-6} [15.8]
3	108	12 345	1.00	1.15×10^{-6} [17.3]	8.32×10^{-7} [23.9]	9.09×10^{-7} [21.9]
4	324	37 102	0.966	8.79×10^{-7} [22.6]	6.01×10^{-7} [33.1]	6.87×10^{-7} [28.9]
5	972	111 373	0.339	7.83×10^{-7} [25.4]	5.35×10^{-7} [37.1]	6.17×10^{-7} [32.3]

[a] Solution pH ranges 3.16–4.02, 6.95–7.30, and 13.24–13.50 for the acidic, neutral, and basic solutions, respectively.

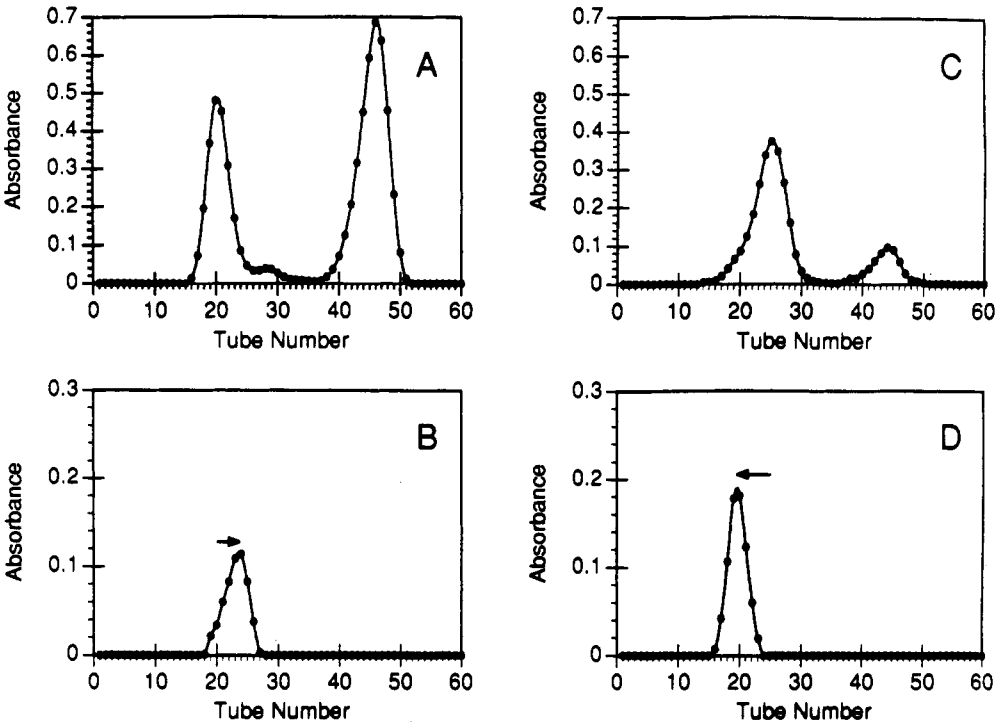

Figure 1. Size-exclusion profiles of 108-acid chromatographed under high and low initial pH conditions: (A) crude 108-acid (900 μg) dissolved in 25 mM sodium phosphate buffer (900 μL; adjusted to pH = 6.8) and chromatographed at pH 6.8 in the same buffer; (B) material from the first peak in chromatogram A adjusted to pH 2.0 with HCl, allowed to stand for at least 30 min, and then chromatographed as in A; (C) crude 108-acid (900 μg) dissolved in H_2O (900 μL), adjusted to pH 2.0 with HCl, and then chromatographed as in A; (D) material from the first peak in chromatogram C adjusted to pH 6.8 with NaOH, allowed to stand for at least 30 min, and then chromatographed as in A. The arrows in B and D indicate the shift in elution position for the 108-acid.

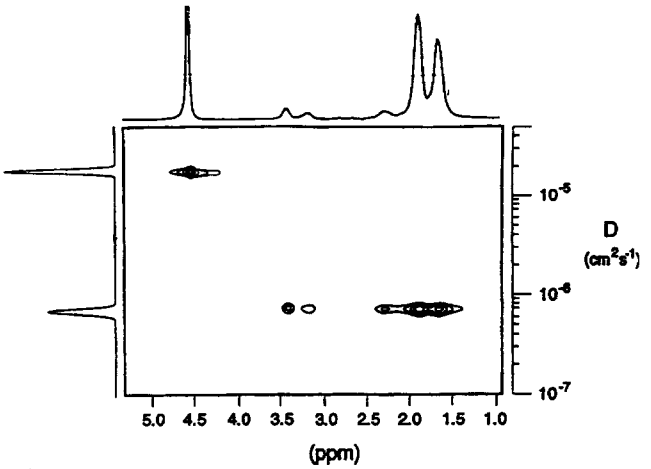

Figure 2. 250-MHz ^1H DOSY display for a 5.00 mM solution of the 108-acid in D_2O at pH 7.12 (28 spectra collected at 25 °C with an LED pulse sequence with K values ranging from 83.5 to $1.34 \times 10^3\ cm^{-1}$). The diffusion coefficients are 1.79×10^{-5} and $7.24 \times 10^{-7}\ cm^2\ s^{-1}$ for HOD and the polymer, respectively.

$R_H = k_BT/(D6\pi\eta)$, where k_B is the Boltzmann constant, T is the absolute temperature, and $\eta = 1.098$ cP is the viscosity of D_2O at 298 K.[16] The measured diffusion coefficients and hydrodynamic radii are listed in Table I. For each cascade generation, the hydrodynamic radius was largest at neutral pH and smallest at acidic pH.

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Supplementary Material Available: Experimental details of the size-exclusion chromatography, chromatograms of calibration standards, and chromatograms of crude and purified 108-acid as well as the details of the DOSY NMR studies (3 pages). Ordering information is given on any current masthead page.

References and Notes

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(4) Z represents the number of terminal moieties and G refers to the number of layers of repeated units (i.e., the number of generations). For details, see: Newkome, G. R.; Baker, G. R.;

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(8) The esters were purified via column chromatography (SiO_2). The ^{13}C NMR spectrum exhibited the expected 11 resonances.
(9) The acids were purified via dialysis [Spectra Por 6 dialysis membrane (MWCO 1000)] and then reverse-phase HPLC [ODS: H_2O/CH_3CN (60:40)]. Transformation was evidenced by the loss of (^{13}C NMR) resonances at δ 28.1 and 80.4 corresponding to the *tert*-butyl group.
(10) ^1H and ^{13}C NMR, IR, MP, and combustion data were obtained for all compounds. Combustion data (C, H, N) were within $\pm 0.2\%$ of the theoretical values.
(11) There is a slight discrepancy in the elution position of the 108-cascade evident in the chromatograms of parts B and C of Figure 1, which is likely due to a modest difference in ionic strength. Preliminary studies varying the ionic strength at constant pH show that the smallest apparent size (radius) occurs at low ionic strength; however, all observed size variations due to ionic strength changes are small relative to variations due to pH changes.
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