Synthesis of Water-Soluble Polyamidoamines for Biomedical Applications. II. Polymers Possessing Intrachain-Type Secondary Amino Groups Suitable for Side-Chain Attachment

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SYNOPSIS

As part of a program to synthesize water-soluble polymeric carriers suitable for drug binding, the polyaddition reaction of methylenebisacrylamide with comonomers containing two primary amino groups is investigated. The copolymerization of the bisacrylamide with equimolar quantities of primary diamines under properly controlled experimental conditions is found to proceed in a linear propagation, giving rise to the formation of polyamides comprising two or more secondary amino groups in the recurring unit. Selected diamine monomers include ethylenediamine, diethylenetriamine, triethylenetetramine, 1,2-bis(3-aminopropylamino)ethane, and three 0,0'-bis-(2-aminopropyl) derivatives of poly(ethylene glycol) of different chain length, the last three monomers being chosen because of their outstanding hydrosolubilizing properties. Use of two different diamines in the proper stoichiometry leads to corresponding copolymers. The reactions are conducted in aqueous phase over periods of 1–3 days at 65°C, and the polymeric products, possessing the linear polyamidoamine structures 1 and 2, are fractionated by dialysis in membrane tubing with 12000–14000 molecular-mass cutoff and are isolated by freeze-drying as solid or resinous materials possessing complete solubility in water. Inherent viscosities are in the range of 8–40 mL g^{-1}. Microanalytical and spectroscopic data confirm the proposed structures. The suitability of the intrachain secondary amine functions for side chain attachment and drug coupling is demonstrated in model reactions involving N-substitution. © 1993 John Wiley & Sons, Inc.

INTRODUCTION

As part of a major synthetic program in the area of water-soluble polymeric drug carriers we have exploited the addition polymerization process involving nucleophilic addition of the amino group across the activated double bond (Michael addition), pioneered by Ferruti.[1] These workers demonstrated the practicability of polymerizing equimolar quantities of primary monoamines or secondary diamines with bisacrylamides, such as ethylenebisacrylamide and bisacryloylpiperazine, preferably in aqueous so-

lution, thereby obtaining linear and water-soluble polyamidoamines with secondary amide and tertiary amine functions in the main chain. Several representatives of this polymer class were found to be of interest in biomedical applications. The topic has been reviewed.[2–4] In the preceding paper of this series[5] we described a synthetic project in which the polymerization method developed by Ferruti and his collaborators was utilized for the preparation of water-soluble, carboxyl-functionalized polyamidoamines possessing drug-coupling capabilities. The structural prerequisites for the biological functioning of these drug carriers were briefly discussed in that communication. In subsequent work we modified the bisacrylamide–amine polyaddition reaction to permit the synthesis of polyamidoamines comprising

intrachain-type secondary amino groups amenable to substitution and introduction of side-chain functionalization for the purpose of drug binding. A portion of this work is described in the present communication.
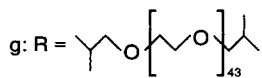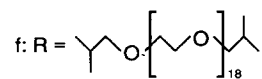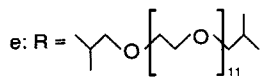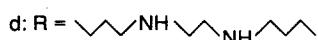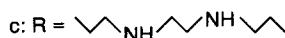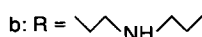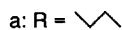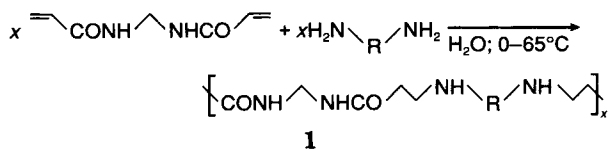
RESULTS AND DISCUSSION

The synthetic strategy chosen for the preparation of the target polymers **1** involved the step-growth addition copolymerization of methylenebisacrylamide (MBA) with primary diamines (Scheme 1; end groups neglected).

The diamine comonomers were ethylenediamine; diethylenetriamine; triethylenetetramine; 1,2-bis-(3-aminopropylamino)ethane; 0,0'-bis(2-aminopropyl)poly(ethylene glycol) 500 (Jeffamine ED 600), 0,0'-bis(2-aminopropyl)poly(ethylene glycol) 800 (Jeffamine ED 900), and 0,0'-bis(2-aminopropyl)poly(ethylene glycol) 1900 (Jeffamine ED 2001). The last three amine-terminated poly-(oxyethylene) derivatives were included in this project as a means of introducing hydrosolubilizing blocks of various lengths into the backbone. The excellent solubility of oxyethylene chains in aqueous media has been utilized in numerous laboratories.[6-8]

Under nonselective conditions, a monomer bearing two primary amino groups would be expected to react tetrafunctionally in these polyaddition reac-

tions because the secondary amino groups generated in the first addition step (Scheme 1), although inherently less reactive, are prone to further addition. As a consequence, the ultimate products should come down as gels generated by three-dimensional cross-linking. Such cross-link formation was indeed observed in Ferruti's laboratory whenever primary diamines served as comonomers.[9,10] We confirmed these findings in exploratory experiments performed under comparable conditions in aqueous solution (bisacrylamide concentration typically 1 mol L^{-1}) for the monomer pair methylenebisacrylamide–ethylenediamine, where gelling set in after less than 6 h at ambient temperature. However, under the more selective conditions employed in the presently described work, linear propagation was achieved in conformance with Scheme 1. These conditions included low reactant concentrations and low initial temperatures. Reactions were typically performed by allowing bisacrylamide and diamine, 0.2–0.4 mol L^{-1} each, to copolymerize in aqueous solution for 20 h at ice bath temperature, 4 h at ambient temperature, and 2 days at 65°C. In a terminal step, a 5 mol % excess of ethylenediamine was added to react with, and thus inactivate, acryloyl end groups, which had been implicated previously in a number of experiments to cause solid-state cross-linking upon extended sample storage. The polymeric products were fractionated and purified by stepwise dialysis in membrane tubing possessing molecular mass cutoff limits of 3500 and 12000–14000 and were collected in yields of 25–50% as solids or highly viscous resins possessing excellent solubility in water. Inherent viscosities typically ranged from 8 to 40 mL g^{-1}. From the external dialysis phase additional polymeric material of smaller molecular size was obtained in yields of 35–50%. Not unexpectedly in light of earlier observations with related polyamidoamines,[5] the second fractions had viscosities only insignificantly lower than determined for the main fractions, indicating the effect of molecular size on viscosity to be quite small as a result of efficacious solvation in the aqueous medium.

Polymerization experiments performed as before, yet at lower temperatures or over considerably shorter periods of heating time, generally afforded lower yields of dialyzed product polymer. On the other hand, attempts to increase the overall yields of (dialyzed) polymer by extending the heating period proved unsuccessful. Yields remained substantially in the same range after 3–4 days, and actually decreased after 5–7 days at that temperature. As in the preceding investigation[5] and elsewhere,[1] we conclude that side reactions involving main chain

$$x \; \overset{=}{\diagup}\text{CONH}\diagup\text{NHCO}\diagup^{=} \;+\; x\text{H}_2\text{N}\diagdown_{\text{R}}\diagup\text{NH}_2 \; \xrightarrow{\text{H}_2\text{O; 0–65°C}}$$

$$\left\{ \text{CONH}\diagup\text{NHCO}\diagup\diagdown^{\text{NH}}\diagdown_{\text{R}}\diagup^{\text{NH}}\diagdown\diagup \right\}_x$$

1

a: R = /\/\

b: R = /\/\NH/\/

c: R = /\/\NH/\/NH/\/

d: R = /\/\/NH/\/\NH/\/\

e: R = (structure with O and O repeat unit subscript 11)

f: R = (structure with O and O repeat unit subscript 18)

g: R = (structure with O and O repeat unit subscript 43)

Scheme 1

fission, notably by hydrolysis, effectively counteract propagation at the elevated temperature level of our experiments. The chosen experimental standard conditions for polymerization, 2 days at 65°C, represent a reasonable compromise between the two opposing types of reaction.

Several polymerization experiments were conducted in nonaqueous solvents under otherwise unchanged conditions. Here again, as in the preceding study,[5] considerably lower yields resulted from use of ethanol or N,N-dimethylformamide as the reaction media, and these did not improve on further extended (8–10 days) heating. Ferruti's group had earlier reported unsatisfactory results with non-aqueous solvents.[1] The experimental results discussed in the foregoing are summarized in Table I. The data represent averages derived from three identically performed runs for each set of variables.

The product polymers corresponded in elemental composition (C/N atomic ratio[5]) to the structures **1**. Further corroboration was obtained spectroscopically. The IR spectra displayed the powerful carbonyl absorptions at 1650 (amide I) and 1535 cm^{-1} (amide II). The Jeffamine containing polymers, in accordance with their prevalent oxyethylene contents, gave spectra in which the aliphatic CH stretching ($\sim$ 2950 cm^{-1}) and ether (1100 cm^{-1})

bands dominated the absorption pattern. The ^{1}H NMR spectra of **1a–d,** within experimental integration error ($\pm 15\%$), showed the proton signals of the MBA methylene bridge (4.6–4.5 ppm) and of the remaining methylene groups (3.1–1.5 ppm) in the expected area ratios. The Jeffamine monomers, being technical-grade products, as such give rather erratic NMR spectra; in particular the ethylene/methyl intensity ratios in the three types, inconsistent with the manufacturer's structural descriptions, indicate the presence (variable from batch to batch) of 1–2 additional methyl groups in the molecule, probably in the form of propyleneoxy segments. Although not considered in the structural representations of the schemes, these inconsistencies in the monomer compositions are reflected in the proton counts extracted from the spectra of the derived polymers **1(e–g)**. Pertinent microanalytical and spectroscopic data are collected in Table II.

In order to demonstrate the feasibility of incorporating two different diamine monomers in the polymeric backbone, several experiments were carried out in which MBA was copolymerized with:

1. ethylenediamine and Jeffamine ED-600, giving the copolyamidoamine **2a;**

Table I Polyamidoamindes 1 and Copolyamidoamines 2

Row Designation	Polymer Designation	Monomer Feed (mmol)[a]								H$_2$O (mL)	Yield (%)	η_{inh} (mL g^{-1})
		MBA	EDA	DET	TET	BAPA	ED-600	ED-900	ED-2001			
1	**1a**	15	15							60^c	28	8^d
2	**1b**	15		15						60	33	8
3	**1c**	10			10					40	31	12^d
4	**1d**	10				10				40	44	10
5	**1e**	5					5			20	34	19
6	**1f**	5						5		20	39	31^d
7	**1g**	5							5	20	50	41
8	**2a**	10	5				5			35	31	7^d
9	**2b**	10	5					5		35	46	11
10	**2c**	10	5						5	40	55	13^d
11	**2d**	10		5				5		30	33	12
12	**2e**	10			5			5		40	26	12

Experiments performed in triplicate over the following reaction periods: 20 h at 0–5°C; 4 h at room temp.; 2 days at 65 $\pm$ 2°C; addition of 0.5 mol % (based on MBA) EDA, followed by another 6 h at 65 $\pm$ 2°C.

^{a} See Experimental for abbreviations.

^{b} Ultimate yield and viscosity data (averaged from three identically performed runs for each set of variables) after dialysis in 12000–14000 cutoff tubing. Data on polymeric material collected from the outer dialysis phase (25–50%) not tabulated.

^{c} Same experiment conducted in EtOH (5 days at reflux temp.): yield, 23%; η_{inh}, 12 mL g^{-1}; in DMF (6 days at 65 $\pm$ 2°C): yield, 20%; η_{inh}, 8 mL g^{-1}.

^{d} Similar yield and viscosity data in experiments conduced for 3 days at 65°C under otherwise identical conditions.

Table II   Analytical and NMR Data for Polyamidoamines 1 and 2

Polymer Designation	C/N[a]		Shift, δ[b] (ppm)	^{1}H NMR Number of Protons		Assignment
	Found	Calcd.		Found[c]	Expected	
1a	2.3	2.2$_5$	4.57	2	2	N—CH$_2$—N
			3.1–2.3	12	12	Remaining CH$_2$
1b	2.2	2.2	4.55	2	2	N—CH$_2$—N
			3.1–2.3	15	16	Remaining CH$_2$
1c	2.3	2.2	4.57	2	2	N—CH$_2$—N
			3.1–2.3	19	20	Remaining CH$_2$
1d	2.5	2.5	4.55	2	2	N—CH$_2$—N
			3.1$_5$–1.5	21	24	Remaining CH$_2$
1e	9.0	8.75	4.57	2	2	N—CH$_2$—N
			3.8–2.4	55	58	Remaining CH, CH$_2$
			1.2–1.0	10	6	CH$_3$
1f	12.3	12.2$_5$	4.56	2	2	N—CH$_2$—N
			3.8–2.3$_5$	92	86	Remaining CH, CH$_2$
			1.2–1.0	10	6	CH$_3$
1g	27.8	24.7$_5$	4.56	2	2	N—CH$_2$—N
			3.8–2.4	190	186	Remaining CH, CH$_2$
			1.2–1.0	11	6	CH$_3$
2a	5.7	5.5	4.55	4	4	N—CH$_2$—N
			3.7–2.4	87	70	Remaining CH, CH$_2$
			1.2–1.0	11	6	CH$_3$
2b	7.3	7.2$_5$	4.56	4	4	N—CH$_2$—N
			3.8–2.3$_5$	85	98	Remaining CH, CH$_2$
			1.2–1.0	10	8	CH$_3$
2c	14.9	13.5	4.56	4	4	N—CH$_2$—N
			3.8–2.3$_5$	202	198	Remaining CH, CH$_2$
			1.2–1.0	10	6	CH$_3$
2d	7.0	6.7	4.56	4	4	N—CH$_2$—N
			3.8–2.3$_5$	122	102	Remaining CH, CH$_2$
			1.2–1.0	12	6	CH$_3$
2e	6.0	6.2	4.5	4	4	N—CH$_2$—N
			3.8–2.3	93	106	Remaining CH, CH$_2$
			1.2–1.0	8	6	CH$_3$

[a] Carbon/nitrogen atomic ratio.

[b] In D$_2$O, with sodium 3-(trimethylsilyl)-2,2,3,3-d_4-propionate as internal standard; listed shifts (centroids or ranges) and intensities averaged from two or more individual samples of each kind.
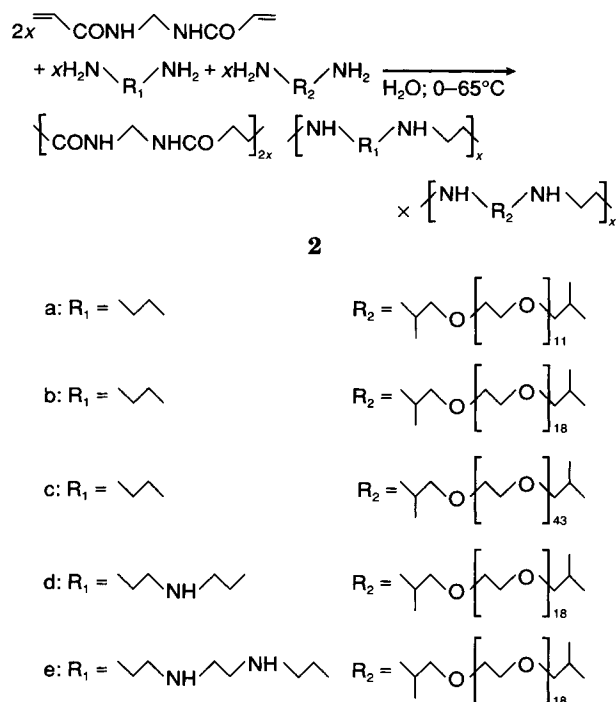
[c] Rounded off to full integer; integration error limits ± 15%.

2. ethylenediamine and Jeffamine ED-900, to afford the copolymer **2b**;

3. ethylenediamine and Jeffamine ED-2001, to provide the copolymer **2c**;

4. diethylenetriamine and Jeffamine ED-900, to give the copolymer **2d**; and

5. triethylenetetramine and Jeffamine ED-900, giving rise to the formation of the copolymer **2e** (Scheme 2; random placement of diamine units; end groups neglected).

Experimental conditions were similar to those used in the preceding experiments (Table I). The

copolymers **2** were obtained in yields of typically 25–55% as completely water-soluble, viscous resins. Microanalytical and spectroscopic results were in reasonable agreement with the presented structures (Table II).

The secondary amine segment groups in the chain of the target polymers are susceptible to substitution with side groups that may comprise drug molecules proper or may represent spacers equipped for drug attachment in subsequent reaction steps. This was demonstrated by treatment of polyamidoamine **1a** with acryloyl chloride in a mixed water–chloroform solvent system at pH $\sim$ 7, thereby introducing the

2x $\equiv$ CONH NHCO $\equiv$

+ xH_2N R_1 NH_2 + xH_2N R_2 NH_2 $\xrightarrow{H_2O;\ 0-65°C}$

$\{CONH\ NHCO\}_{2x}$ $\{NH\ R_1\ NH\}_x$

$\times$ $\{NH\ R_2\ NH\}_x$

2

a: R_1 = R_2 = ... $O[...O]_{11}$

b: R_1 = R_2 = ... $O[...O]_{18}$

c: R_1 = R_2 = ... $O[...O]_{43}$

d: R_1 = ...NH... R_2 = ... $O[...O]_{18}$

e: R_1 = ...NH...NH... R_2 = ... $O[...O]_{18}$

Scheme 2

functional acryloyl group, which in turn may be allowed to react further by nucleophilic addition of amine-functionalized spacers or prodrugs. The water-soluble acylation products **3** were obtained in yields of 35–55% (Scheme 3; choice of substituent position arbitrary). With 3 equivalents of acylating agent per repeat unit of the substrate polymer a product of the average composition **3** ($y = 0.7x$) was obtained, and four equivalents were required in order to attain a degree of substitution of 50%, in accordance with an average composition of **3** ($y = x$). The extent of acryloylation in the product polymers **3** was determined by comparison of the intensities of the vinyl proton signals at 6.3–5.7 ppm and the singulet at 4.5 ppm associated with the $N-CH_2-N$ methylene protons.

In another exemplifying approach toward substitution of intrachain $-NH-$ groups, polyamidoamine **1a** was treated in ethanolic solution with
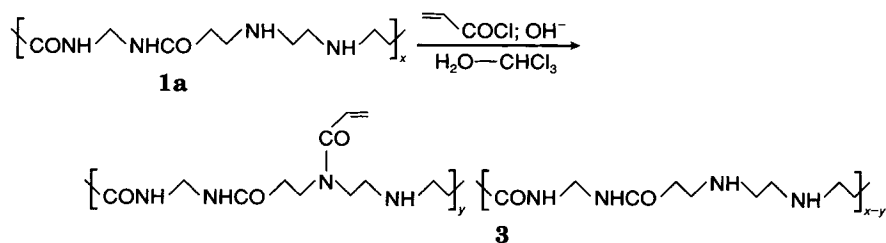
the active N-hydroxysuccinimide ester of phenylacetic acid used here as a representative drug model. Equimolar quantities of polymer and active ester, allowed to interact for 20 h at room temperature, gave rise to the formation of coupling product **4a** ($y = 0.5x$), isolated after dialysis and freeze-drying in 60% yield (Scheme 4). The analogous reaction with **1e** as the substrate gave **4b** ($y = 0.6x$) in 38% yield. With 1.5 equivalents of active ester in the last reaction, the degree of acylation increased to 35%; accordingly, the resulting substitution product possessed the average composition **4b** ($y = 0.7x$). The degree of substitution in these phenacetylated polymers was assessed from the relative intensities of the aromatic proton peak at 7.3 ppm and the $N-CH_2-N$ proton signal near 4.6 ppm in the ^1H NMR spectra. The conjugates **4** dissolved readily in water; inherent viscosities ranged from 7 to 12 mL g^{-1}.

It is apparent from the exemplifying acylation reactions of Schemes 3 and 4 that the intrachain secondary amino groups of the amidoamine-type carrier polymers synthesized in this study are sufficiently accessible to permit a major extent of substitution. Carriers of this type thus are promising candidate substrates for drug anchoring.
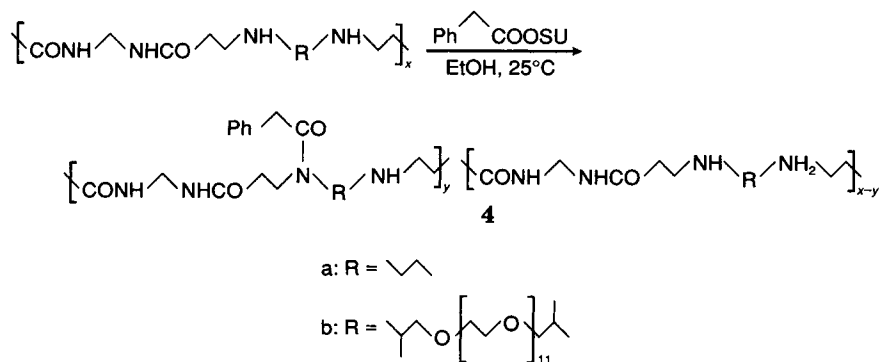
EXPERIMENTAL

General

$\{CONH\ NHCO\ NH\ NH\}_x$ $\xrightarrow[H_2O-CHCl_3]{\equiv COCl;\ OH^-}$

1a

$\overset{\underset{\displaystyle CO}{|}}{}$

$\{CONH\ NHCO\ N\ NH\}_y$ $\{CONH\ NHCO\ NH\ NH\}_{x-y}$

3

Scheme 3

$\{CONH \frown NHCO \diagdown\diagup NH \diagdown_R \diagup NH \diagdown\diagup\}_x$ $Ph \frown COOSU$
$\xrightarrow{EtOH, 25°C}$

$Ph \frown CO$
$|$
$\{CONH \frown NHCO \diagdown\diagup N \diagdown_R \diagup NH \diagdown\diagup\}_y$ $\{CONH \frown NHCO \diagdown\diagup NH \diagdown_R \diagup NH_2 \diagdown\diagup\}_{x-y}$

4

a: R = $\diagdown\diagup\diagdown$

b: R = $\diagup\diagdown\diagup O \left[\diagdown\diagup O\right]_{11} \diagup\diagdown$

Scheme 4

geles, CA) with 3500 and 12000–14000 (mass-average) molecular-mass cutoff limits against several batches of stationary, deionized, and N_2-saturated H_2O. Freeze-drying of aqueous polymer solutions was achieved with the aid of a VIRTIS Bench Top 3 freeze-drier operating at −40°C, 10–15 Pa. Freeze-dried polymer material was routinely postdried for further dehydration in a SARTORIUS Thermo Control infrared drying assembly programmed for 2 × 2 min at 85°C. Analytical samples were additionally dried for 2 days at 75°C in an Abderhalden tube (P_4O_{10} drying agent; pressure reduced to 200–250 Pa). Although this treatment still left up to 4% moisture in the samples, more rigorous drying was avoided to preclude structural changes and degradation. Microanalyses were performed by Robertson Laboratory, Inc., Madison, NJ. Determinations were made in duplicate; the results were averaged and are tabulated as C/N atomic ratios so as to avoid variations due to moisture contents.

Solvents, Monomers, and Reagents

N,N-Dimethylformamide (DMF) was freshly distilled under reduced pressure in a faint stream of N_2 (forerun discarded). Deionized H_2O was used in polymerization and dialysis work. Methylenebisacrylamide, puriss, (MBA), N,N'-dicyclohexylcarbodiimide (DCC), ethylenediamine (EDA), diethylenetriamine (DET), triethylenetetramine (TET, supplied as the disulfate dihydrate, $M = 378.4$), 1,2-bis(3-aminopropylamino)ethane (BAPA), 0,0'-bis(2-aminopropyl)poly(ethylene glycol) 500 (Jeffamine ED-600), 0,0'-bis(2-aminopropyl)-poly(ethylene glycol) 800 (Jeffamine ED-900), and 0,0'-bis(2-aminopropyl)poly(ethylene glycol) 1900 (Jeffamine ED-2001) were all used as received (Fluka AG, Aldrich Chemie G.m.b.H.). The last three compounds were technical grade, and the molecular masses stated by the supplier (∼ 600, 900,

and 2000, respectively) were used for the stoichiometric calculations.

Phenylacetic Acid N-Hydroxysuccinimide Ester

The active HSU ester of phenylacetic acid was prepared by the addition of DCC (52.5 mmol) in THF (30 mL) to the solution of phenylacetic acid (50 mmol) in the same solvent (10 mL) cooled in an ice bath. After 30 min of stirring, HSU (52.5 mmol) suspended in THF (20 mL) was added, and stirring at ice bath temperature was continued for 4 h, whereupon the mixture was stored for 24 h at 5°C and stirred for another 4 h at ambient temperature. Glacial acetic acid (0.5 mL) was added for decomposition of excess coupling agent, and the precipitated urea derivative was removed by filtration and thoroughly washed with warm THF. Stepwise volume reduction and cooling of the combined mother liquor and organic washings afforded several fractions of the active ester as colorless crystals, mp 115–120°C, in 65% total yield. Recrystallization from isopropanol raised the mp to 122–123°C. IR/cm^{-1}: 1800, 1780, 1720 vs. (ν_{CO}). Anal. found: C, 61.29; H, 5.14; N, 6.06. Calcd. for $C_{12}H_{11}NO_4$ (233.2): C, 61.80; H, 4.75; N, 6.01.

Polymerizations

The polymerization runs of this study were performed in triplicate for each set of variables, and the tabulated data represent average results. All product fractions were hygroscopic and dissolved readily in H_2O. The experiments described in the following are representative.

Polyamidoamines 1

The procedure described below for the polymerization of MBA and EDA, to afford 1a, illustrates the preparation of polyamidoamines 1. The solution of MBA, 2.313 g (15 mmol), in H_2O (60 mL) was

cooled in an ice bath while a faint stream of N_2 was introduced into the liquid phase. A portion of the bisacrylamide crystallized out at this temperature. EDA, 902 mg (15 mmol), was added with continued introduction of N_2, and the mixture was stirred in the stoppered flask for 4 h at ice bath temperature, followed by 20 h of storage at 0–3°C in a refrigerator. This gave a clear solution, which was now stirred for 4 h at ambient temperature and for 2 days at 65 ± 2°C in an air-circulating incubator. After the addition, in a flush of N_2, of another 45 mg (0.75 mmol) of EDA to the solution for inactivation of terminal vinyl groups, heating at 65°C was continued for a further 8 h. Concentration of the solution to 2–3 mL under reduced pressure was followed by product precipitation with Me_2CO — Et_2O (3:1, 50 mL). The precipitated polymer was washed with Me_2CO and redissolved in H_2O (30 mL). The solution was dialyzed for 24 h in spectra/Por 3 tubing (outer phase discarded) and for another 30 h in Spectra/Por 4 tubing (combined outer-phase portions collected). The tube contents were freeze-dried and postdried, to yield 953 mg (29.7%) of solid; η_{inh}, 10 mL g^{-1}. Anal. found: C, 50.98; H, 8.56; N, 22.55; C/N, 2.64. Calcd. for $(C_9H_{18}N_4O_2)_n$ (**1a**): C, 50.45; H, 8.47; N, 26.15; C/N, 2.25.

From the combined outer-phase portions collected from 12000–14000 cutoff dialysis, 1.55 g (48.2%) of lower-molecular polymer was isolated after freeze-drying and postdrying; η_{inh}, 7 mL g^{-1}. The results, averaged with data from two repeat runs conducted in an identical manner, are in Table I (row 1).

In analogous polymerization experiments, summarized in Table I (row 2), use of DET, 1.548 g (15 mmol), in place of EDA gave polyamidoamine **1b** in a yield of 33%; η_{inh}, 8 mL g^{-1} (data averaged). Typical anal. found: C, 49.01; H, 8.92; N, 24.44; C/N, 2.34. Calcd. for $(C_{11}H_{23}N_5O_2)_n$ (**1b**): C, 51.34; H, 9.01; N, 27.22; C/N, 2.20. The combined outer-phase portions afforded lower-molecular **1b** (38%); η_{inh}, 6 mL g^{-1}.

In this, as well as in the following five sets of polymerization experiments, EDA (5 mol %, based on MBA), was the reactant added after the 2-day heating period for inactivation of terminal vinyl groups.

A mixture of MBA, 1.542 g (10 mmol), TET disulfate dihydrate, 3.784 g (10 mmol), and Na_2CO_3, 2.12 g (20 mmol), in H_2O (40 mL), saturated with N_2, was prepared at ice-bath temperature. The thick suspension was stirred for 1 h at 0°C and stored for 20 h at 0–3°C. It was then stirred for 4 h at ambient temperature, causing the majority of suspended material to dissolve. Stirring was continued for 2 days

at 65°C. To the resulting solution a 0.5 mmol portion (30 mg) of EDA was added, and all subsequent operations were performed as described for **1a**. This gave 995 mg (33.1%) of solid polyamidoamine **1c**; η_{inh}, 11 mL g^{-1}. Anal. found: C, 50.43; N, 9.22; N, 26.11; C/N, 2.27. Calcd. for $(C_{13}H_{28}N_6O_2)_n$ (**1c**): C, 51.97; H, 9.40; N, 27.98; C/N, 2.17. From the combined outer-phase solutions a second fraction of lower-molecular **1c** was collected in 46% yield; η_{inh}, 8 mL g^{-1}. Averaged data are in Table I (row 3).

Reaction of MBA, 1.542 g (10 mmol), with BAPA 1.743 g (10 mmol), in H_2O (40 mL) in the same manner as described for **1a** gave amidoamine **1d**. In a typical experiment, the yield was 1.53 g (46.6%); η_{inh}, 9 mL g^{-1}. Anal. found: C, 51.95; H, 9.71; N, 22.48; C/N, 2.69. Calcd. for $(C_{15}H_{32}N_6O_2)_n$ (**1d**): C, 54.85; H, 9.82; N, 25.59; C/N, 2.50. Lower-molecular **1d** was collected from the combined outer-phase portions in 33% yield; η_{inh}, 6 mL g^{-1}. Averaged results are in Table I, row 4.

The polymerization of MBA, 771 mg (5 mmol), and Jeffamine ED-600, 3.0 g (5 mmol), in H_2O (20 mL) in the same fashion afforded polyamidoamine **1e** as a highly viscous resin. A representative run gave a yield of 1.26 g (32.7%); η_{inh}, 17 mL g^{-1}. Anal. found: C, 53.84; H, 9.48; N, 6.97; C/N, 9.01. Calcd. for $(C_{35}H_{70}N_4O_{14})_n$ (**1e**): C, 54.52; H, 9.15; N, 7.27; C/N, 8.75. In this and the following two reaction types involving Jeffamine monomers, lack of efficacious precipitability of the product polymers dictated a modification of the work-up procedure omitting the precipitation step. The reaction mixtures were directly dialyzed in Spectra/Por 3 and Spectra/Por 4 tubing, the dialysis periods being extended to 26 and 38 h, respectively, to ensure complete removal of any unreacted Jeffamines.

Analogous reactions of MBA (5 mmol) with Jeffamine Ed-900, 4.5 g (5 mmol), and of MBA (5 mmol) with Jeffamine ED-2001, 10.0 g (5 mmol), each in H_2O (20 mL), gave polyamidoamines **1 f** (viscous resin) and **1g** (solid) in averaged yields of, respectively, 39 and 50%; η_{inh}, 30–40 mL g^{-1}. Typical anal. found for **1f**: C, 53.15; H, 9.22; N, 4.99; C/N, 12.42; for **1g**: C, 54.48; H, 9.13; N, 2.82; C/N, 22.52. Calcd. for $(C_{49}H_{98}N_4O_{21})_n$ (**1 f**): C, 54.53; H, 9.15; N, 5.19; C/N, 12.25; for $(C_{103}H_{206}N_4O_{48})_n$ (**1g**): C, 54.53; H, 9.15; N, 2.47; C/N, 25.75. From the outer-phase solutions in these three experiments, lower-molecular fractions of **1e–g** were isolated in yields of 30–40%. Averaged data for the main fractions are in Table I (rows 5–7).

Copolyamidoamines 2

The method described in the following for the preparation of **2a** is representative of the copolymeriza-

tion reactions of MBA with two different diamine comonomers.

To the stirred suspension of MBA, 1.542 g (10 mmol), in H_2O (35 mL), cooled in an ice bath and saturated with N_2, was added Jeffamine ED-600, 3.0 g (5 mmol), and EDA, 300 mg (5 mmol), in that sequence. The N_2 flow was continued for 10 min, and the mixture was stirred in the stoppered flask for 2 h at ice-bath temperature and subsequently stored for 20 h at 3–5°C. The clear solution obtained at this point was stirred for 4 h at ambient temperature and for 2 days at 65°C. After the addition of EDA, 30 mg (0.5 mmol), stirring at 65°C was continued for another 8 h. The solution, diluted by addition of another 50 mL of H_2O and filtered, was dialyzed and the solute collected as described for **1a**, to give 1.43 g (29.0%) of highly viscous resin; η_{inh}, 8 mL g^{-1}. Anal. found: C, 53.37; H, 8.81; N, 10.84; C/N, 5.74. Calcd. for $(C_{44}H_{88}N_8O_{16})_n$ (**2a**): C, 53.64; H, 9.00; N, 11.37; C/N, 5.50.

The same type of reaction, yet with Jeffamine ED-600 replaced by Jeffamine ED-900, 4.5 g (5 mmol), gave resinous copolymer **2b** in a yield of 2.89 g (44.7%); η_{inh}, 10 mL g^{-1}. Anal. found: C, 50.26; H, 8.89; N, 7.96; C/N, 7.36. Calcd. for $(C_{58}H_{116}N_8O_{23})_n$ (**2b**): C, 53.85; H, 9.04; N, 8.66; C/N, 7.25.

In experiments carried out as before, but with Jeffamine ED-2001, 10.0 g (5 mmol), used in place of the lower Jeffamine members and the volume of solvent increased to 40 mL, solid copolymer **2c** was obtained in a yield of 6.47 g (53.3%); η_{inh}, 12 mL g^{-1}. Anal. found: C, 52.30; H, 9.19; N, 3.98; C/N, 15.33. Calcd. for $(C_{108}H_{216}N_8O_{50})_n$ (**2c**): C, 53.45; H, 8.97; N, 4.62; C/N, 13.50.

By an analogous procedure, the copolymerization of MBA (10 mmol), DET, 516 mg (5 mmol), and Jeffamine ED-900 (5 mmol) in H_2O (30 mL) gave **2d**, 2.33 g (34.9%), as a resinous material; η_{inh}, 10 mL g^{-1}. Anal. found: C, 51.77; H, 9.01; N, 9.03; C/N, 6.69. Calcd. for $(C_{60}H_{121}N_9O_{23})_n$ (**2d**): C, 53.91; H, 9.13; N, 9.43; C/N, 6.67.

With DET replaced by TET disulfate dihydrate, 1.892 g (5 mmol), and Na_2CO_3, 1.06 g (10 mmol), added for neutralization of the sulfuric acid component, all other conditions being unchanged, there was obtained 1.89 g (27.4%) of **2e** as a highly viscous resin; η_{inh}, 11 mL g^{-1}. Anal. found: C, 51.07; H, 8.97; N, 9.11; C/N, 6.54. Calcd. for $(C_{62}H_{126}N_{10}O_{23})_n$ (**2e**): C, 53.97; H, 9.21; N, 10.15; C/N, 6.20. Averaged data for the last five copolymers are in Table I (rows 8–12). From the outer-phase solutions in these polymerizations, lower-molecular polymer fractions of **2a–e** were obtained in yields of 25–40%; η_{inh}, 7–12 mL g^{-1}.

N-Acryloylation of Polyamidoamine 1a

The acryloylation experiments described in the following illustrate the substitution behavior of polymers **1** in acid chloride-mediated acylation reactions.

Polyamidoamine **1a**, 215 mg (1 mmol), was dissolved in H_2O (2 mL). To the rapidly stirred solution, cooled in an ice-NaCl bath ($-5°C$), was dropwise and simultaneously added a 1.6 M solution of acryloyl chloride in $CHCl_3$, 1.88 mL (3 mmol), and a solution of NaOH, 120 mg (3 mmol), in H_2O (3 mL), the alkali addition being such as to maintain a pH of 6–7 in the aqueous phase. After completed addition, the pH was adjusted to 7–8 (NaOH), and the mixture was stirred for 2 h at ice-bath temperature and another 1 h at 20–25°C. Separation of the phases was followed by washing of the aqueous layer with several portions of $CHCl_3$ (organic layer and washings discarded). Upon the addition of t-butyl-catechol inhibitor (10 mg), the aqueous phase was dialyzed for 48 h in Spectra/Por 4 tubing. Another portion (5 mg) of inhibitor was added and the solution freeze-dried, to give 133 mg (52.8%) of water-soluble, solid **3** ($y = 0.7x$); η_{inh}, 11 mL g^{-1}. ^{1}H NMR, δ: 6.3–5.7 ppm, 2.1H (vinyl); 4.54 ppm, 2H ($N-CH_2-N$).

Use of 4 mmol of acylating agent and alkali under otherwise identical conditions gave water-soluble **3** ($y = x$) in a yield of 127 mg (47.4%); η_{inh}, 9 mL g^{-1}. ^{1}H NMR, δ: 6.3–5.7 ppm, 3H (vinyl); 4.54 ppm, 2H ($N-CH_2-N$).

N-Phenylacetylation of Polyamidoamines 1a and 1e

Acid–amine coupling reactions leading to N-substitution in polyamidoamines are exemplified by the phenylacetylation of **1a** and **1e** via the active N-hydroxysuccinimide ester of phenylacetic acid.

To the solution of polyamidoamine **1a**, 215 mg (1 mmol) in EtOH (96%; 2 mL) was added phenylacetic acid hydroxysuccinimide ester, 257 mg (1 mmol), suspended and partly dissolved in EtOH (2 mL). The mixture was stirred for 20 h at room temperature, whereupon most of the suspended material had dissolved. After solvent removal under reduced pressure the residual solid was dissolved in H_2O (20 mL), and the filtered solution was dialyzed for 40 h in Spectra/Por 4 tubing and freeze-dried. This gave 150 mg (45.1%) of coupling product **4a** ($y = 0.5x$) as a water-soluble solid; η_{inh}, 5 mL g^{-1}. ^{1}H NMR, δ: 7.3 ppm, 2.5H (phenyl); 4.56 ppm, 2H ($N-CH_2-N$).

In a similar fashion, the reaction of **1e**, 771 mg (1 mmol), dissolved in EtOH (96%; 2 mL), with

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the active ester, 257 mg (1 mmol), in the same solvent (2 mL), afforded coupling product **4b** (y = 0.6x) as a water-soluble, resinous solid, 321 mg (38.1%); η_{inh}, 12 mL g^{-1}. ^{1}H NMR, δ: 7.3 ppm, 3H (phenyl); 4.56 ppm, 2H (N—CH$_2$—N). Use of 1.5 mmol of active ester under otherwise unchanged conditions afforded water-soluble **4b** (y = 0.7x), 273 mg (32.0%); η_{inh}, 8 mL g^{-1}. ^{1}H NMR, δ: 7.3 ppm, 3.5H (phenyl); 4.56 ppm, 2H (N—CH$_2$—N).

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