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Polyamidoamines as Drug Carriers: Synthesis of Polymers Featuring Extrachain-type Primary Amino Groups as Drug-anchoring Sites

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ABSTRACT

The versatile polymerization of bisacrylamides with mono- and difunctional amines, first investigated and greatly expanded in Ferruti's laboratory,[1–3] is utilized in the present project for the synthesis of macromolecular drug carriers. Specifically, we report on the preparation of linear polyamidoamines possessing primary amino groups as terminals of short side chains, designed to function as drug attachment sites. In the first reaction step, performed in aqueous medium, methylenebisacrylamide (MBA) is copolymerized with two types of comonomer: (1) primary amines bearing solubilizing functionality, such as *tert*-amine or hydroxyl groups, and (2) a variety of mono-N-Boc-protected primary diamines (Boc = *tert*-butoxycarbonyl). In other reactions, MBA is allowed to react with a mono-N-protected diamine to give a macromonomer, which is polymerized with an oligo- or poly(ethylene oxide) terminated at both ends by a primary amino group. The intermediary polymers so obtained, as yet featuring N-protected amino side groups, are treated with trifluoroacetic acid for deprotection. Further work-up by aqueous dialysis (25 000 mwco tubing) and freeze-drying affords the target polymers as water- and methanol-soluble solids in ultimate yields of 10–25%. ^{1}H NMR spectroscopy serves to confirm the structural assignments 1–12**. In order to demonstrate the drug-carrying potential of these polymers, an exemplifying polyamidoamine (**11**) is allowed to react with an active ester of 4-ferrocenylbutanoic acid in methanolic solution. A water-soluble conjugate (**11-Fc**) is thus obtained, in which 93% of available primary amine side-chain terminals are acylated by the ferrocenylation agent.**

KEYWORDS

Polyamidoamines., methylenebisacrylamide, macromolecular drug carriers, primary amine side functionality, 4-ferrocenylbutanoic acid.

1. Introduction

For a forthcoming polymer-drug conjugation project we were in need of carrier polymers that would provide primary amino groups as side-chain terminals for drug attachment, while possessing complete solubility not only in aqueous media but also, quite critically for certain drug coupling reactions, in methanol. The synthetic versatility inherent in polyamidoamines of the structural type pioneered by Ferruti[1–3] suggested that polymer type to serve our purpose. This prompted a study aiming at the synthesis of bisacrylamide-derived polymers containing various solubilizing groups as side-chain or main-chain components in addition to short side chains terminated with a primary amine functionality as the drug conjugation site. These synthetic efforts are reported in the present communication. While this work was in progress, a recent paper from Ferruti's laboratory describing similar synthetic approaches came to our attention[4]

2. Experimental

2.1. General Procedures

Aqueous polymer product solutions were dialysed in Spectra/Por 4 cellulose membrane tubing (12 000–14 000 molecular mass cut-off) and Spectra/Por 6 wet tubing (25 000 molecular mass cut-off) against stirred and repeatedly exchanged batches of H_2O. Solutions were freeze-dried in a Virtis Bench Top 3 freeze-drier operating at –30°C, 10–15 Pa. Freeze-dried material was routinely post-dried in a Sartorius Thermo Control Infrared Drying System programmed for 2 × 2 min at 65°C. Analytical samples were additionally dried in an Abderhalden tube at 65°C under reduced pressure. ^{1}H NMR spectra (400 MHz) were taken on D_2O solutions adjusted to pH 10 (NaOH) in order to eliminate protonation effects; they were referenced against sodium 3-(trimethylsilyl)-2,2,3,3-d_4-propionate (integration error limits ±12%). In the spectra of polymeric compounds, the brackets contain the expected proton counts. Viscometric measurements were conducted in Cannon-Fenske viscometers at 30.0 ± 0.1°C (c = 0.2 g/100 mL); inherent viscosities, η_{inh}, are given in mL/g.

2.2. Solvents, Reagents and Reactants

Deionized H_2O was used for all preparative and work-up operations. N,N-Dimethylformamide (DMF) was distilled under reduced pressure in a faint stream of N_2; a forerun (5%) was discarded. Methylenebisacrylamide (MBA), puriss., was used as received, and so were di-*tert*-butyl dicarbonate and the amine monomers, ethylenediamine, 1,3-diaminopropane, diethylenetriamine, 1,2-bis(2-aminoethoxy)ethane, 4,7,10-trioxa-1,13-tridecanediamine, and O,O′-bis(3-aminopropyl) poly(ethylene glycol) 1500 (Fluka Chemie AG, Aldrich Chemie GmbH).

For the preparation of N-(*tert*-butoxycarbonyl)-1,2-diaminoethane, **13** (Scheme 1), the solution of di-*tert*-butyl dicarbonate,

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Table 1 Molar feed ratios and compositions of polyamidoamines **1**–**12**.

Reactants (equivalents)					Polymers					
MBA	R_1 [a]		R_2 [b]		Designation	x/y	*n*	Yield/%	Composition	M [c]
(2)	$-(CH_2)_3NMe_2$	(1)	$-(CH_2)_2-$	(1)	**1**	1		18.1	$C_{21}H_{42}N_8O_4$	(470.6)
(2)	$-(CH_2)_3NMe_2$	(1)	$-(CH_2)_3-$	(1)	**2**	1		10.3	$C_{22}H_{44}N_8O_4$	(484.6)
(5)	$-(CH_2)_3NMe_2$	(4)	$-(CH_2)_3-$	(1)	**3**	4		17.0	$C_{58}H_{116}N_{20}O_{10}$	(1253.7)
(2)	$-(CH_2)_3NMe_2$	(1)	$-(CH_2)_2O(CH_2)_2O(CH_2)_2-$	(1)	**4**	1		18.3	$C_{25}H_{50}N_8O_6$	(558.7)
(5)	$-(CH_2)_3NMe_2$	(4)	$-(CH_2)_2O(CH_2)_2O(CH_2)_2-$	(1)	**5**	4		18.6	$C_{61}H_{122}N_{20}O_{12}$	(1327.7)
(5)	$-(CH_2)_3NMe_2$	(4)	$-(CH_2)_2NH(CH_2)_2-$	(1)	**6**	4		17.5	$C_{59}H_{119}N_{21}O_{10}$	(1282.7)
(5)	$-(CH_2)_2NMe_2$	(4)	$-(CH_2)_3-$	(1)	**7**	4		22.2	$C_{54}H_{108}N_{20}O_{10}$	(1197.5)
(5)	$-(CH_2)_2NMe_2$	(4)	$-(CH_2)_2O(CH_2)_2O(CH_2)_2-$	(1)	**8**	4		15.6	$C_{57}H_{114}N_{20}O_{12}$	(1271.6)
(2)	$-(CH_2)_2O(CH_2)_2OH$	(1)	$-(CH_2)_3-$	(1)	**9**	1		11.0	$C_{21}H_{41}N_7O_6$	(487.6)
(2)	$-(CH_2)_2O(CH_2)_2OH$	(1)	$-(CH_2)_2O(CH_2)_2O(CH_2)_2-$	(1)	**10**	1		10.8	$C_{24}H_{47}N_7O_8$	(561.7)
(2)	$-(CH_2)_3-$	(1)			**11**		3	10.7	$C_{27}H_{54}N_8O_7$	(602.8)
(2)	$-(CH_2)_3-$	(1)			**12**		32	25.2	$C_{85}H_{170}N_8O_{36}$	(1880.3)

[a] R_1 in Scheme 2 for **1** to **10**; in Scheme 3 for **11** and **12.**
[b] R_2 in Scheme 2 for **1** to **10.**
[c] Base molecular mass.

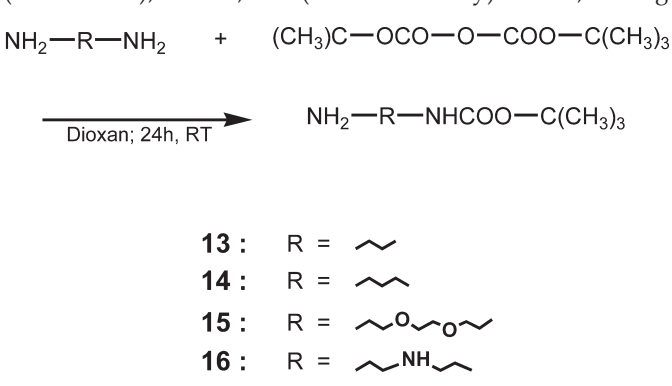
10.9 g (50 mmol) in 70 mL of dioxan, was added dropwise to 1,2-diaminoethane, 21 g (350 mmol), predissolved in 60 mL of the same solvent. The mixture was stirred for 1 d at ambient temperature, and the solvent, together with excess 1,2-diaminoethane, was distilled off by rotary evaporation at 50°C bath temperature. Upon the addition of 50 mL of H_2O, a small portion of insoluble N,N′-bisprotected amine was removed by filtration, and the filtrate was extracted with several 40-mL portions of methylene chloride, leaving any residual unreacted diaminoethane as the most hydrophilic constituent in the aqueous phase. From the combined extracts, dried over anhydrous $MgSO_4$, crude mono-Boc-protected amine **13** was obtained after solvent removal as a yellow, oily liquid in a yield of 6.72 g (84%). The compound, just like the subsequently described Boc derivatives **14**–**16**, gave a very clean ^{1}H NMR spectrum and was used without further purification.

^{1}H NMR, *δ*/ppm: 3.1 t, 2H (CONHC*H*$_2$); 2.7 t, 2H (C*H*$_2$NH$_2$); 1.4 s, 9H (CH_3).

In an analogous fashion, N-(*tert*-butoxycarbonyl)-1,3-diaminopropane, **14** (Scheme 1), was prepared from di-*tert*-butyl dicarbonate, 10 g (45.8 mmol), and 1,3-diaminopropane, 30.4 g (410 mmol), in a total of 130 mL of dioxan. Work-up as in the preceding experiment afforded crude mono-Boc-protected diamine **14** as an oily liquid in a yield of 7.55 g (95%).

^{1}H NMR, *δ*/ppm: 3.1 t, 2H (CONHC*H*$_2$); 2.65 t, 2H (C*H*$_2$NH$_2$); 1.6 m, 2H ($CH_2CH_2CH_2$); 1.45 s, 9H (C*H*$_3$).

The preparation of N-(*tert*-butoxycarbonyl)-4,7-dioxa-1,10-diaza-decane **15** (Scheme 1) from di-*tert*-butyl dicarbonate, 15.0 g (68.7 mmol), and 1,2-bis(2-aminoethoxy)ethane, 69.3 g (468 mmol), in 260 mL of dioxan followed the same basic procedure. Again, the unreacted, very hydrophilic bis(aminoethoxy)ethane present in excess partitioned entirely into the aqueous phase and escaped co-extraction with the chlorocarbon solvent. There was obtained 15.6 g (91%) of crude mono-protected compound as an oily liquid.



Scheme 1

^{1}H NMR, *δ*/ppm: 3.7 s, 4H (O-CH_2CH_2O); 3.6 m, 4H (N-CH_2C*H*$_2$O); 3.2 t, 2H (CONHC*H*$_2$); 2.75 t, 2H (C*H*$_2$NH$_2$); 1.45 s 9H (CH_3).

Di-*tert*-butyl dicarbonate, 10 g (45.8 mmol), and diethylenetriamine, 32.0 g (310 mmol), in 130 mL of dioxan, treated as before, gave 8.8 g (94.5%) of crude N-(*tert*-butoxycarbonyl)-1,4,7-triaza-heptane, **16** (Scheme 4), as an oily liquid.

^{1}H NMR, *δ*/ppm: 3.2 t, 2H (CONHC*H*$_2$); 2.6 m, 6H (remaining CH_2); 1.45 s, 9H (CH_3).

The ferrocenylation agent, 4-ferrocenylbutanoic acid N-succinimidyl ester, was synthesized as previously described[12–14].

2.3. Polyamidoamines 1–10

Amounts of polymeric educts and products are given as base moles and thus refer to the simplest recurring units, defined by structures **1**–**10** normalized to y = 1.

Polymer 1. The procedure given in the following for the synthesis of 1 is representative of the experiments providing the first ten target polymers of this study.

A solution was prepared from MBA, 2.47 g (16 mmol), in 15 mL of hot H_2O. Upon the addition of the mono-Boc derivative **13**, 1.28 g (8 mmol), the N_2-saturated solution was stirred for 1 d at room temperature and for another 1 d at 60°C. 3-(Dimethylamino)propylamine, 817 mg (8 mmol), was added. Stirring of the solution, resaturated with N_2, was continued for 3 d at 60°C and, upon the addition of ethanolamine, 49 mg (0.8 mmol), for another 2 h at that temperature. The last step served to eliminate terminal vinyl groups as potential causes of delayed cross-linking. The volatiles were now removed by rotating evaporation (60°C bath temperature), and the residual intermediary polymer was treated with 5 mL of trifluoroacetic acid (1 h, room temperature). Removal of the acid under reduced pressure at 30°C bath temperature was followed by precipitation of the product polymer with Et_2O-EtOH-hexane (2:1:2), thorough washing with hot toluene, and redissolution in 20 mL of H_2O. The pH was adjusted to 7, and the solution was dialysed for 2 d in Spectra/Por 4 tubing and for another 2 d in Spectra/Por 6

tubing. For the last 4 h of this operation, the pH of the tube contents was raised to 8.5–9 (NH_4OH) to eliminate protonation effects. The retentate was freeze-dried and post-dried, to give 0.68 g (18.1%) of solid, water- and methanol-soluble **1**; η_{inh}, 16.0 mL g^{-1}.

^{1}H NMR, δ/ppm: 4.5, 4H (4H; NHCH_2NH); 2.8–2.25, 25H (24H; NHCOCH_2, $CH_2N(CH_2)(CH_2)$, CH_2NH$_2$, CH_2N$(CH_3)_2$); 2.2, 5.5H (6H; CH_3); 1.6, 1.8H (2H; $CH_2CH_2CH_2$).

In a separate experiment performed as above yet only to the point of ethanolamine treatment, the product remaining after solvent removal was washed with hot toluene, then redissolved in 5 mL of H_2O and passed through a 25 × 2.5 cm Sephadex G10 column. Freeze-drying of the eluate afforded 2.95 g of solid, water-soluble intermediary polymer still featuring the Boc-protected primary amine side-chain terminals.

^{1}H NMR, δ/ppm: 4.5, 4H (4H; NHCH_2NH); 3.1, 1.8H (2H; CONHCH_2CH_2); 2.8–2.2, 32H (28H; NHCOCH_2, $CH_2N(CH_2)(CH_2)$, $CH_2N(CH_3)_2$); 1.6, 2H (2H; $CH_2CH_2CH_2$); 1.4, 8H (9H; $(CH_3)_3C$).

Polymer 2. By the basic procedure described for the synthesis of **1**, polyamidoamine **2** was prepared from MBA, 2.47 g (16 mmol), mono-Boc derivative **14**, 1.39 g (8 mmol), and 3-(dimethylamino)propylamine, 817 mg (8 mmol). The target polymer **2** was collected as a water- and methanol-soluble solid in a yield of 0.4 g (10.3%); η_{inh}, 26.0 mL g^{-1}.

^{1}H NMR, δ/ppm: 4.5, 4H (4H; NHCH_2NH); 2.75, 8.5H (8H; NHCOCH_2); 2.6–2.2, 17H (16H; $CH_2N(CH_2)(CH_2)$, CH_2NH$_2$, CH_2N$(CH_3)_2$); 2.18, 6.1H (6H; CH_3); 1.55, 3.9H (4H; $CH_2CH_2CH_2$).

Polymer 3.This polyamidoamine, a variant of **2**, was prepared as described in the preceding experiment except that the molar feed ratios were changed. Thus, MBA, 2.47 g (16 mmol), was allowed to react with **14**, 558 mg (3.2 mmol), and 3-(dimethylamino)propylamine, 1.31 g (12.8 mmol). This gave 0.68 g (17.0%) of water-soluble, solid **3**.

^{1}H NMR, δ/ppm: 4.55, 10H (10H; NHCH_2NH); 2.8, 21H (20H; NHCOCH_2); 2.7–2.25, 43H (40H; $CH_2N(CH_2)(CH_2)$, CH_2NH$_2$, CH_2N$(CH_3)_2$); 2.2, 24.3H (24H; CH_3); 1.6, 10.3H (10H; $CH_2CH_2CH_2$).

Polymer 4. The reaction of MBA, 2.47 g (16 mmol), N-Boc derivative **15**, 1.99 g (8 mmol), and 3-(dimethylamino)propylamine, 817 mg (8 mmol), by the standard procedure gave 820 mg (18.3%) of solid, water- and methanol-soluble **4**.

^{1}H NMR, δ/ppm: 4.5, 4H (4H; NHCH_2NH); 3.6–3.5, 8H (8H; CH_2OCH_2); 2.8–2.2, 24.5H (24H; NHCOCH_2, $CH_2N(CH_2)(CH_2)$, CH_2NH$_2$, CH_2N$(CH_3)_2$); 2.15, 6.5H (6H; CH_3); 1.55, 2H (2H; $CH_2CH_2CH_2$).

Polymer 5. This polyamidoamine, a variant of **4**, was synthesized by the general procedure from MBA, 2.47 g (16 mmol), N-Boc derivative **15**, 795 mg (3.2 mmol), and 3-(dimethylamino) propylamine, 1.31 g (12.8 mmol). The polymer was isolated as a water- and methanol-soluble solid in a yield of 790 mg (18.6%).

^{1}H NMR, δ/ppm: 4.52, 10H (10H; NHCH_2NH); 3.65-35., 8.8H (8H; CH_2OCH_2); 2.8–2.25, 62H (60H; NHCOCH_2, $CH_2N(CH_2)(CH_2)$, CH_2NH$_2$, CH_2N$(CH_3)_2$); 2.2, 24.4H (24H; CH_3); 1.6, 8.1H (8H; $CH_2CH_2CH_2$).

Polymer 6.The standard synthesis method was used for the preparation of **6**, except that isopropanol-water (4:1), 25 mL, was employed as the solvent, and the heating period of the second step was reduced to 36 h. The following amounts of reactants were used: MBA, 2.47 g (16 mmol), N-Boc derivative **16**, 651 mg (3.2 mmol), and 3-(dimethylamino)propylamine, 1.31 g (12.8 mmol). The water- and methanol-soluble, solid **6** was collected in a yield of 0.72 g (17.5%); η_{inh}, 16.8 mL g^{-1}.

^{1}H NMR, δ/ppm: 4.55, 10H (10H; NHCH_2NH); 2.8, 21H (20H; NHCOCH_2); 2.75–2.25, 49H (44H; $CH_2N(CH_2)(CH_2)$, CH_2NHCH_2CH_2NH$_2$, CH_2N$(CH_3)_2$); 2.2, 21.5H (24H; CH_3); 1.6, 7H (8H; $CH_2CH_2CH_2$).

Polymer 7. In the two polyamidoamines **7** and **8**, 2-(dimethylamino)ethylamine was employed in lieu of the propylamine derivative serving as the solubilizing factor in **1**–**6**. Polymer **7** was thus prepared as described for **3**, except that 3-(dimethylamino)propylamine was replaced by 2-(dimethylamino) ethylamine, 1.13 g (12.8 mmol). Conventional work-up gave 850 mg (22.2%) of water- and methanol-soluble, solid **7**; η_{inh}, 24.3 mL g^{-1}.

^{1}H NMR, δ/ppm: 4.55, 10H (10H; NHCH_2NH); 2.8, 21H (20H; NHCOCH_2); 2.7–2.3, 41.7H 40H; $CH_2N(CH_2)(CH_2)$, CH_2NH$_2$, CH_2N$(CH_3)_2$); 2.2, 23H (24H; CH_3); 1.6, 2.3H (2H; $CH_2CH_2CH_2$).

Polymer 8.This polymer was synthesized as described for **4**, except that 3-(dimethylamino)-propylamide was replaced by 2-(dimethylamino)ethylamine, 1.13 g (12.8 mmol). The target polymer was isolated as a water- and methanol-soluble solid in a yield of 636 mg (15.6%); η_{inh}, 21.0 mL g^{-1}.

^{1}H NMR, δ/ppm: 4.55, 10H (10H; NHCH_2NH); 3.7–3.5, 8.9H (8H, CH_2OCH_2); 2.8, 23H (20H; NHCOCH_2); 2.75–2.3, 39.7H (40H; $CH_2N(CH_2)(CH_2)$, CH_2NH$_2$, CH_2N$(CH_3)_2$); 2.2, 24.4H (24H; CH_3).

Polymer 9. Polyamidoamines **9** and **10** are counterparts to **2** and **4**, with the 3-(dimethylamino)-propylamine solubilizing group replaced by 2-(2-hydroxyethoxy)ethylamine. Thus, 9 was prepared from MBA, 2.47 g (16 mmol), Boc-derivative **14**, 1.39 g (8 mmol), and the hydroxyethoxyethylamine, 841 mg (8 mmol). The water- and methanol-soluble solid **9** was obtained in a yield of 430 mg (11.0%); η_{inh}, 16.3 mL g^{-1}.

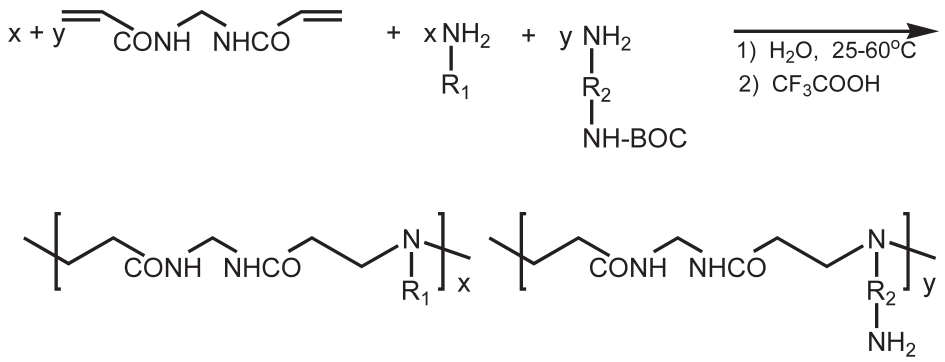
^{1}H NMR, δ/ppm: 4.5, 4H (4H; NHCH_2NH); 3.7–3.5, 6.3H (6H; CH_2OCH_2CH_2OH); 2.8–2.2, 23H (22H; NHCOCH_2, CH_2NH$_2$, $CH_2N(CH_2)(CH_2)$); 1.6, 1.55H (2H; $CH_2CH_2CH_2$).

Polymer 10.The reaction of MBA, 2.47 g (16 mmol), Boc-derivative **15**, 1.99 g (8 mmol), and 2-(2-hydroxyethoxy)ethylamine, 841 mg (8 mmol), gave **10** as a water- and methanol-soluble solid in a yield of 475 mg (10.8%).

^{1}H NMR, δ/ppm: 4.5, 4H (4H; NHCH_2NH); 3.7–3.5, 13.3H (14H; CH_2OCH_2CH_2OH); 2.6–2.2, 22.4H (22H; NHCOCH_2, CH_2NH$_2$, $CH_2N(CH_2)(CH_2)$).

Polymer 11.MBA, 2.47 g (16 mmol), was dissolved in 25 mL of hot isopropanol-H_2O (4:1). The mono-N-Boc derivative **14**, 1.39 g (8 mmol), dissolved in 5 mL of isopropanol, was added. Upon saturation with N_2, the resulting solution was stirred for 3 d at ambient temperature. Following solvent removal by rotatory evaporation, the residual macromonomer was redissolved in 80 mL of the same solvent blend, thus providing the high dilution ([MBA] = 0.2 M) required for the second reaction step. After cooling in an ice bath, 4,7,10-trioxa-1.13-tridecanediamine, 1.76 g (8 mmol), was added, followed by NEt_3, 810 mg (8 mmol), and stirring of the solution, resaturated with N_2, was continued for 1 d at room temperature and another 2 d at 60°C. The solvent was removed again under reduced pressure, 10 mL of trifluoroacetic acid was added, and stirring was continued for 1 h at ambient temperature. The acid was removed under reduced pressure, and the residual material was washed with hot toluene to remove traces of unreacted oligo(ethylene oxide). Polymer precipitation and further work-up was as described for polymer **1**. There was obtained 516 mg (10.7%) of water- and methanol-soluble solid **11**; η_{inh}, 19.8 mL g^{-1}.

^{1}H NMR, δ/ppm: 4.55 , 4H (4H; NHCH_2NH); 3.7–3.6, 12.4H (12H; CH_2OCH_2); 3.1, 7.5H (8H; NHCOCH_2); 2.7–2.4, 14.8H



Polymer	R_1	R_2	x/y
1	NMe_2		1
2	NMe_2		1
3	NMe_2		4
4	NMe_2	O O	1
5	NMe_2	O O	4
6	NMe_2	NH	4
7	NMe_2		4
8	NMe_2	O O	4
9	O OH		1
10	O OH	O O	1

Scheme 2

(16H; $CH_2N(CH_2)(CH_2)$, CH_2NH_2, CH_2NHCH_2); 1.7, 6.2H (6H; $CH_2CH_2CH_2$).

Polymer 12. For the preparation of **12** the same procedure was used as in the preceding experiment, except that the trioxatridecanediamine was replaced by O,O′-bis(3-aminopropyl)poly(ethylene glycol) 1500, 12 g (8 mmol), and the solvent volume in the second reaction step was increased to 100 mL. The solid, water- and methanol-soluble **12** was collected in a yield of 3.79 g (25.2%).

^{1}H NMR, δ/ppm: 4.55 , 4H (4H; $NHCH_2NH$); 3.8–3.5, 138H (128H; CH_2OCH_2); 2.8, 8.4H (8H; $NHCOCH_2$); 2.6–2.3, 14.5H (16H; $CH_2N(CH_2)(CH_2)$, CH_2NH_2, CH_2NHCH_2); 1.8–1.5, 5.2H (6H; $CH_2CH_2CH_2$).

2.4. Conjugate 11-Fc

A solution was prepared from **11**, 184 mg (0.305 mmol), in 5 mL of MeOH. N-Succinimidyl 4-ferrocenylbutanoate[12–14], 146 mg (0.396 mmol), was added and dissolved. The solution, flushed with N_2 and protected from light, was stirred for 3 d at ambient temperature. After solvent removal by rotatory evaporation, the conjugate was precipitated in a resinous state by treatment with Et_2O-Me_2CO (2:1), washed with precipitant, and redissolved in 5 mL of H_2O. Upon the addition of L-ascorbic acid (10 mg) for reduction of any ferricenium species present, the pH was adjusted to 10, and the solution was passed through a column (2 × 30 cm) charged with Sephadex G25. The eluate, after pH readjustment to 8, was dialysed for 2 d in Spectra/Por 6 tubing, and the retentate was freeze-dried to give 102 mg (39%) of orange-brown, water-soluble conjugate **11-Fc**.

^{1}H NMR, δ/ppm: 4.55, 4H (4H; $NHCH_2NH$); 4.2, 8.4H (9H; CH, cyclopentadienyl); 3.7–3.5, 13.5H (12H; CH_2OCH_2).

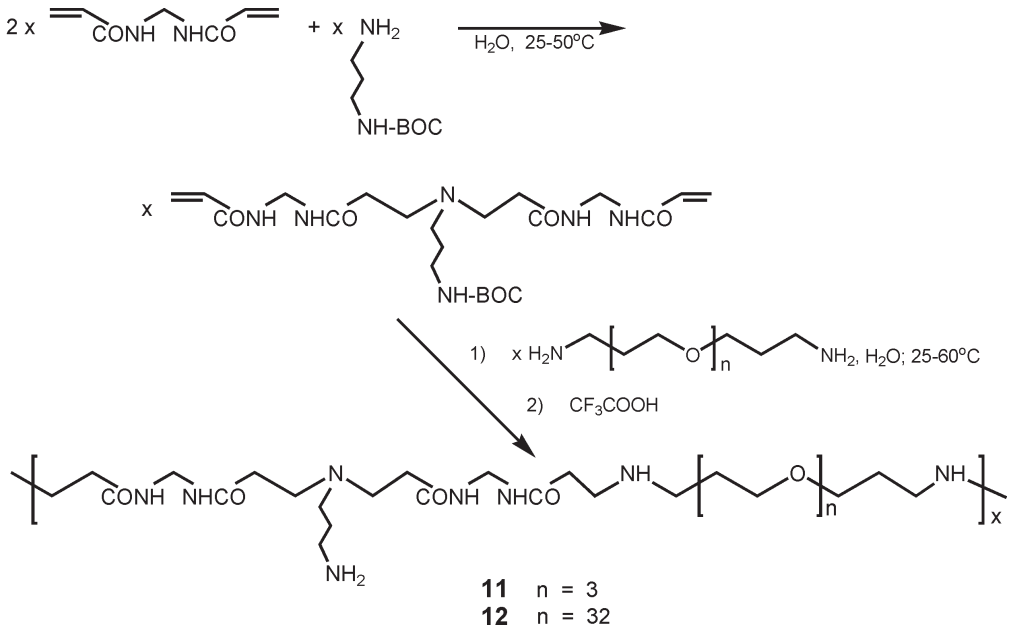
3. Results and Discussion

Methylenebisacrylamide (MBA), used in some of our earlier[5,6] and more recent[7] investigations, was chosen as the bifunctional acrylic acid derivative, to be copolymerized in various feed ratios with functionalized monoamines and diamines mono-N-protected by the *tert*-butoxycarbonyl substituent. Deprotection of the primary amino groups in the intermediary structures so obtained would then give the target polymers **1**–**10** (Scheme 2).

The substituent R_1 in these constructs is typically represented, and exemplified by polymers **1**–**8**, by a dimethylaminoalkyl residue, imparting cationic behaviour to the molecule under physiological pH conditions. This feature entails potential pharmacokinetic benefits, allowing for facilitated pinocytotic cell entry of the macromolecule in biomedical applications[8,9]. Introduced into related polyamide-based drug conjugates the dimethylaminoalkyl functionality has been found to provide generally superior cytotoxic activity in cell culture tests against human cancer lines[10,11].

For comparison a hydroxyl-terminated side chain incapable of adding to the polymers' cationic behaviour has been introduced in **9** and **10**. Various short-chain aliphatic spacers designed to provide spacing between main chain and drug represent the segment R_1 as depicted in Scheme 1.

The polymerizations, conducted in aqueous medium, were performed in two steps. The first step involved reaction of MBA with the protected amine, NH_2-R_2-NH-Boc, for 2 d at 25–50°C. Upon the addition of the second amine comonomer, NH_2-R_1, the experiments were then continued for 2–3 d at 50–60°C. A brief treatment with ethanolamine to eliminate any terminal unsaturation was followed by solvent removal, treatment with trifluoroacetic acid for N-deprotection, and acid removal under



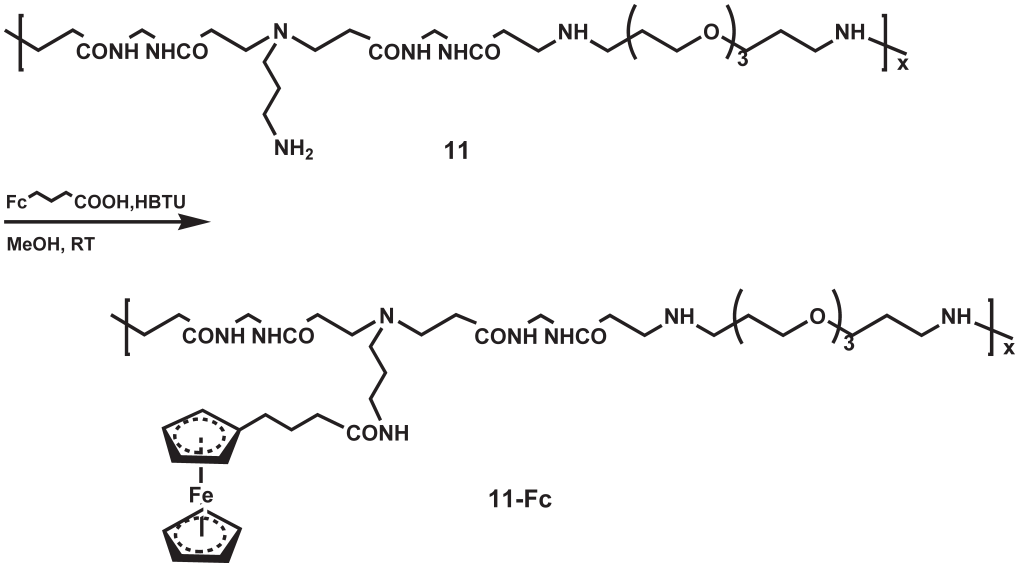
Scheme 3

reduced pressure. The crude target polymers were worked up by aqueous dialysis in tubing with 12 000–14 000 and 25 000 molecular mass cut-off and were isolated in the solid state upon freeze-drying. The products possessed complete solubility in water and, as demanded, in methanol. They were structurally characterized by comparison of the NH-CH_2-NH methylene proton resonance near 4.5 ppm with other prominent bands in the ^{1}H NMR spectra.

Contrasting with polymers **1**–**10**, in which side groups served as the solubilizing entities, polymeric structures comprising the solubilizing units as main chain segments are exemplified by **11** and **12** (Scheme 3). The synthesis again involved a two-step process. In the first step, MBA was treated with 0.5 equivalents of a mono-N-protected diamine. The bis(acrylamido)-terminated macromonomer so generated was allowed in the second step to copolymerize with primary diamines of the type NH_2-CH_2 $(CH_2CH_2O)_n(CH_2)_3NH_2$, where n = 3 and 32, thus giving polymers **11** and **12**, respectively. Polymerization and work-up conditions were similar to those leading to **1**–**10**, and the ultimate polymer products retained water and methanol solubility.

The ultimate yields in these polymerization experiments were quite low, ranging from 10 to 25%. It was emphasized previously[1,5–7] that the polymerization process of bisacrylamides with amines by a Michael addition mechanism is inherently inefficient. Aqueous or partly aqueous media are necessary for an efficacious addition step, and in this solvent hydrolytic chain fission involving the rather labile amide links invariably militates against the propagation sequence. As a result the growing chain will become increasingly vulnerable to fragmentation, and the ensuing molecular mass distribution of the polymeric product will be unduly wide. The rigorous fractionation step by dialysis in 25 000 tubing will therefore remove the lower-molecular material as the predominant product portion, leaving a very minor fraction in the retentate. As in previous work, we accept the resultant low yields as a price to be paid for having at hand a collection of polymer samples with molecular masses in the desired range of 20 000–40 000, sufficiently high to retard renal excretion, yet low enough to avoid toxicological problems.

In order to demonstrate the proneness of these NH_2-substi-



Scheme 4

tuted polyamidoamines to conjugation with bioactive agents in methanolic solution, polymer **11** was chosen to serve as a drug carrier. The compound, dissolved in methanol, was treated (72 h, room temperature) with 1.2 equivalents of N-succinimidyl 4-ferrocenylbutanoate[12–14] (Scheme 4). Ferrocene, di-η^5-cyclopentadienyl-iron(II), is an experimental drug, which in polymer-bound form has shown highly promising antiproliferative properties,[15] and the active ferrocenylbutanoate ester has been the ferrocenylation agent of choice in this laboratory. Following solvent removal, an aqueous work-up similar to that employed for the isolation of **1** to **12** afforded the ferrocene conjugate **11-Fc** as a water-soluble polymer in 72% yield. A ferrocene content corresponding to 93% acylation of primary amino groups was determined from ^{1}H NMR data.

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Supplementary Material

The proton NMR spectra of the different polymers are available as supplementary material.

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