Water Soluble Polyamides as Potential Drug Carriers. IX. Polyaspartamides Grafted with Amine-Terminated Poly(ethylene oxide) Chains

GREGG CALDWELL, EBERHARD W. NEUSE, AXEL G. PERLWITZ

Department of Chemistry, University of the Witwatersrand, WITS 2050, Republic of South Africa

Received 17 May 1996; accepted 1 February 1997

ABSTRACT: The synthesis of side-chain-functionalized polyaspartamides as potential carrier polymers for medicinal agents is described. The nucleophilic ring opening in poly-D,L-succinimide, mediated by *O*,*O*′-*bis*(2-aminopropyl)poly(ethylene oxide) (nominal molecular mass is 600) and ethanolamine under carefully controlled experimental conditions leads to the formation of aspartamide polymers bearing hydrosolubilizing hydroxyethyl side groups in addition to variable proportions of poly(ethylene oxide) (PEO) side chains terminated with primary amino groups. The side chain terminals represent functionalities for drug binding, whereas the PEO constituents contribute to overall hydrophilicity and biocompatibility of the carriers and to enhance their biomedical performance by imparting resistance to protein binding and increasing central circulation lifetime. The water-soluble polymeric products are isolated by dialysis (molecular mass cutoff: 25,000) and freeze-drying in typical yields of 40–60%, with inherent viscosities in the range of 10–18 mL g^{-1}. Polymer compositions are determined by ^{1}H NMR spectroscopy and microanalysis. A selected carrier is modified by *N*-acylation with 4-ferrocenylbutanoic acid as a model drug, giving a ferrocene-containing, water-soluble conjugate, thus demonstrating the accessibility of the terminal amino groups on the PEO side chains to acylating agents and other potential reactants.
 J Appl Polym Sci **66:** 911–919, 1997

Key words: polymeric drug carriers; polyaspartamide; poly(ethylene oxide) grafts

INTRODUCTION

In a previous article from this laboratory[1] we reported on the grafting of poly(ethylene oxide) (PEO) chains onto drug carrier-type polyaspartamides. It was the purpose of such grafting to increase the carriers' hydrophilicity, reduce immunogenicity, and enhance resistance to both protein binding and capture by the reticuloendothelial system with concomitant prolongation of residence time in serum circulation. Such features can be of outstanding biomedical benefit for drug carrier molecules.[2–4] In addition to the PEO grafts, the polymers contained amine-functionalized side groups for drug conjugation. With these amine functions linked to the polymer backbone by very short (less than 10 atomic constituents) spacers, any attached drug species would thus reside in close proximity to the main chain and remain embedded in the protective layer of PEO "tentacles" surrounding the backbone. While this spatial arrangement offers certain pharmacokinetic advantages, it also creates difficulties associated with poor accessibility of the biofissionable polymer-drug tether to proteolytic enzymes instrumental in the intracellular (lysosomal) release of the drug from the carrier. The present

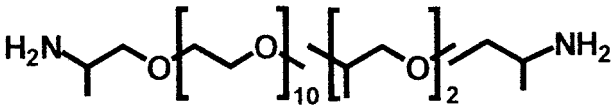
For part VIII of this series, see Neuse et al.[1]
Correspondence to: E. W. Neuse.

Journal of Applied Polymer Science, Vol. 66, 911–919 (1997)
 CCC 0021-8995/97/050911-09

investigation is concerned with drug carriers modified with PEO side chains, which, in turn, are functionalized with terminal amino groups. Drug "anchoring" to carriers of this type will take place at the graft terminals, rendering the biofissionable amide linking group in the spacer more readily susceptible to enzymatic attack.

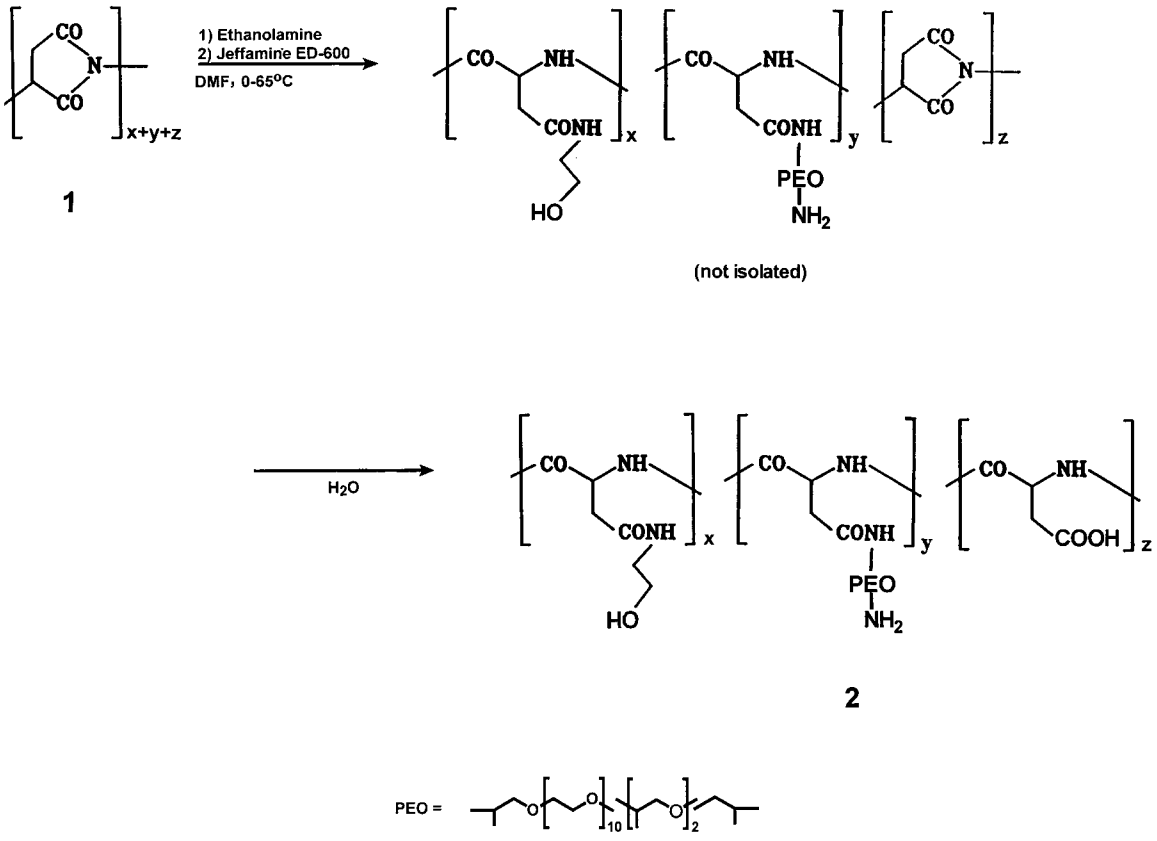
RESULTS AND DISCUSSION

The "workhorse" polymer, as in previous investigations,[1,5] was poly-D,L-succinimide (**1**), obtained by high-temperature solution polymerization of D,L-aspartic acid.[6] Stepwise aminolytic ring-opening in **1,** leading to the polyaspartamide targets, was routinely brought about in DMF solution. Ethanolamine as the majority reactant was employed for the introduction of the hydrosolubilizing 2-hydroxyethyl side group. The minority reactant, an *O*,*O′-bis*(2-aminopropyl)poly(ethylene oxide) commercially available as Jeffamine ED-600, served as the source of the ω-amino-PEO grafts; for this Jeffamine type, spectroscopic (^{1}H NMR) and microanalytical results supported the following structure:



Two different experimental approaches were explored in this work. In the first approach (method A), the substrate polyimide **1** was treated under strictly anhydrous conditions with the highly reactive aminoalcohol in the feed ratios precisely conforming to the desired product compositions. This resulted in partial ring opening with *N*-(2-hydroxyethyl)amidation. The remaining imide rings were then, in the second reaction step, attacked by the Jeffamine employed in appropriate excess and, ultimately, at elevated temperature (65°C). Even under these forcing conditions, however, the reactivity of the Jeffamine α-terminals proved to be insufficient to achieve complete substitution of the relatively small proportion of remaining imide units. As a result, the product polymers, after the usual aqueous work up, were found to contain aspartic acid subunits in addition to the expected *N*-substituted aspartamide units, thus conforming to the polyampholyte structure **2** (Scheme 1; random sequence of repeat units, α-peptide forms[7] shown only). Typically, in copolymers with x representing a 90 mol % content of hydroxyethyl-modified units, nuclear magnetic resonance (NMR) data (*vide infra*) indicated the remaining 10 mol % to be made up of y = 2.5 mol % NH_2-PEO-substituted units and z = 7.5 mol % aspartic acid units. Showing ampholytic behavior as a consequence of the introduced carboxyl functions, the product polymers prepared by method A featured substantially higher inherent viscosities (35–45 mL g^{-1}) than shown by the products of method B (10–18 mL g^{-1}), to be discussed further below.

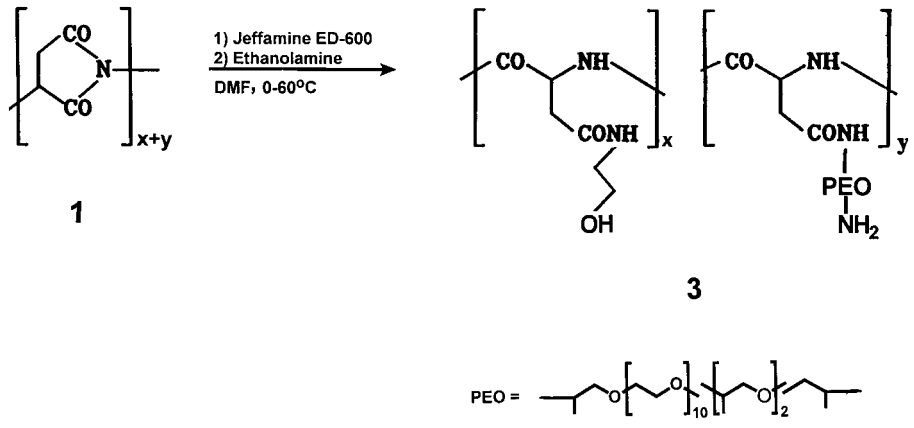
The ampholytic nature of the polyaspartamides **2** entailed the risk of crosslinking side reactions in subsequent drug binding work. This prompted us to abandon the approach of method A and, instead, explore the counterpart approach, method B, in which the sequence of amine addition was reversed. In the first step, **1** was treated with given quantities of the Jeffamine, resulting in partial incorporation of this reactant; and the ring-opening process was now completed in the second step by treatment of the intermediary polymer with ethanolamine (Scheme 2). Temperatures were initially kept low (0–25°C), and so was the substrate concentration ([polysuccinimide] $\approx$ 0.2 mol L^{-1}), in order to restrict Jeffamine reactivity to the monofunctional mode and thus preclude crosslinking. In addition, in view of the required low temperatures and the low reactivity of the Jeffamine terminals, it proved necessary to use the diamine in a large excess over the desired stoichiometry, with a molar feed-to-incorporation ratio of typically 4 : 5. (It will be seen further below that this requirement led to unavoidable run-to-run variability in the percentage of Jeffamine incorporated, necessitating accurate determination of Jeffamine contents in every polyaspartamide synthesized.) Rigorously anhydrous conditions, as before, were maintained in both reaction steps so as to prevent spurious concurrent hydrolysis of unreacted imide rings with resultant formation of aspartic acid units. Purification and crude fractionation by precipitation and aqueous dialysis, followed by freeze-drying, provided the target polymers **3** as water-soluble solids in typical yields of 40–60%. Inherent viscosities were in the 10–18 mL g^{-1} range. In view of the biomedical requirement that polymer–drug conjugates should possess molecular masses high enough to avoid rapid renal clearance, the dialysis operation as part of this work-up procedure was



Scheme 1

performed in two steps, the ultimate one utilizing membrane tubing with a molecular mass cut-off of 25,000.

Tables I and II record the experimental variables and results for a series of polyaspartamides **3** prepared in various feed ratios. The parenthetic ratio ($x : y$) in the target polymer designation used in the tables reflects the contents, in mol %, of the randomly placed hydroxyethyl- and NH_2-PEO-modified repeat units. Polymer compositions were ascertained by microanalysis and from relative band intensities of the ^1H NMR spectra (*vide infra*). The found carbon and nitrogen percentages in Table II invariably show combined deficiencies by some 2%, whereas the hydrogen contents are well within expected ranges. The product polymers **3,** only mildly



Scheme 2

Table I Synthesis of Polyaspartamides 3[a]

	Amine Reactants in Feed (mol %)[b]		Polyaspartamides **3**		
Exp. No.	ED-600	Ethanolamine	Designation[c]	Ultimate Yield/%[d]	η_{inh}[e]/mL g^{-1}
1	20	140	**3**(95 : 5)	50.8 (57.1)	17
2	30	135	**3**(92 : 8)	47.5 (56.9)	12
3	35	130	**3**(91 : 9)	55.8 (69.8)	12
4	35	130	**3**(90 : 10)	57.7 (64.1)	10
5	40	130	**3**(90 : 10)	50.5 (56.1)	13
6	40	130	**3**(91 : 9)	50.3 (57.2)	18
7	45	130	**3**(87 : 13)	48.6 (58.6)	13
8	45	130	**3**(89 : 11)	54.3 (61.0)	14
9	50	128	**3**(88 : 12)	54.1 (58.8)	12
10	50	128	**3**(88 : 12)	58.7 (64.5)	10
11	55	125	**3**(85 : 15)	42.8 (55.6)	14
12	65	120	**3**(85 : 15)	52.1 (57.2)	12
13	75	120	**3**(83 : 17)	38.0 (43.8)	9
14	90	115	**3**(80 : 20)	44.3 (49.2)	11
15	95	110	**3**(76 : 24)	57.0 (64.0)	12
16	120	105	**3**(71 : 29)	47.2 (47.2)	10

[a] Prepared by method B. Step 1: 24 h, 0°C. Step 2: 4 h, 0°C; 18 h, RT; 2 h, 60°C.
[b] Moles of amine reactant per 100 base moles of 1. ED-600 = Jeffamine ED-600.
[c] Parenthetic ratio indicates contents, in mol %, of ethanolamine and ED-600, in that order.
[d] After ultimate (25,000 molecular mass cutoff) dialysis; in parentheses, the yield after the first (12,000–14,000 cutoff) dialysis step. In addition, 20–25% of lower molecular polyaspartamide **3** collected by redialysis of outer phase in 6000 molecular mass cutoff tubing (not tabulated).
[e] At 30,000 ± 0.05°C, in H_2O; c = 0.2 g/100 mL.

Table II Analytical Data for Polyaspartamides 3

			Base Molecular Mass[b]	Anal Found[c] (Calcd)		
Exp. No.	Polymer Designation[a]	Molecular Formula		C	H	N
1	**3**(95 : 5)	$(C_{150}H_{261}N_{41}O_{72})_n$	3791	46.22 (47.52)	7.20 (6.94)	14.33 (15.15)
2	**3**(92 : 8)	$(C_{105}H_{186}N_{26}O_{49.5})_n$	2605	47.03 (48.41)	7.42 (7.20)	13.65 (13.98)
3	**3**(91 : 9)	$(C_{96.7}H_{172.1}N_{23.2}O_{45.3})_n$	2385	47.34 (48.70)	7.35 (7.27)	13.40 (13.63)
4	**3**(90 : 10)	$(C_{90}H_{161}N_{21}O_{42})_n$	2209	47.64 (48.92)	7.58 (7.35)	13.07 (13.31)
5	**3**(90 : 10)	$(C_{90}H_{161}N_{21}O_{42})_n$	2209	47.44 (48.92)	7.45 (7.35)	13.15 (13.31)
6	**3**(91 : 9)	$(C_{96.7}H_{172.1}N_{23.2}O_{45.3})_n$	2385	46.98 (48.70)	7.43 (7.27)	13.11 (13.63)
7	**3**(87 : 13)	$(C_{76.2}H_{137.9}N_{16.4}O_{35.1})_n$	1845	48.08 (49.59)	7.69 (7.53)	12.17 (12.45)
8	**3**(89 : 11)	$(C_{84.5}H_{151.9}N_{19.2}O_{39.3})_n$	2066	47.96 (49.13)	7.61 (7.41)	12.88 (13.02)
9	**3**(88 : 12)	$(C_{80}H_{144.3}N_{17.7}O_{37})_n$	1946	47.25 (49.37)	7.69 (7.47)	12.57 (12.74)
10	**3**(88 : 12)	$(C_{80}H_{144.3}N_{17.7}O_{37})_n$	1946	47.56 (49.37)	7.59 (7.47)	12.21 (12.74)
11	**3**(85 : 15)	$(C_{70}H_{127.7}N_{14.3}O_{32})_n$	1682	48.75 (49.99)	7.67 (7.65)	11.51 (11.91)
12	**3**(85 : 15)	$(C_{70}H_{127.7}N_{14.3}O_{32})_n$	1682	48.11 (49.99)	7.78 (7.65)	11.35 (11.91)
13	**3**(83 : 17)	$(C_{65.3}H_{119.8}N_{12.8}O_{29.6})_n$	1558	48.72 (50.34)	7.79 (7.75)	11.34 (11.51)
14	**3**(80 : 20)	$(C_{60}H_{111}N_{11}O_{27})_n$	1419	49.06 (49.37)	7.81 (7.47)	11.69 (12.74)
15	**3**(76 : 24)	$(C_{55}H_{102.7}N_{9.3}O_{24.5})_n$	1286	48.87 (51.35)	8.12 (8.05)	10.10 (10.13)
16	**3**(71 : 29)	$(C_{50.7}H_{95.5}N_{7.9}O_{22.3})_n$	1173	50.54 (51.93)	8.39 (8.21)	9.51 (9.44)

[a] See footnote c in Table I.
[b] Molecular mass of repeat unit (**3**, normalized to y = 1).
[c] Average of duplicate determinations.

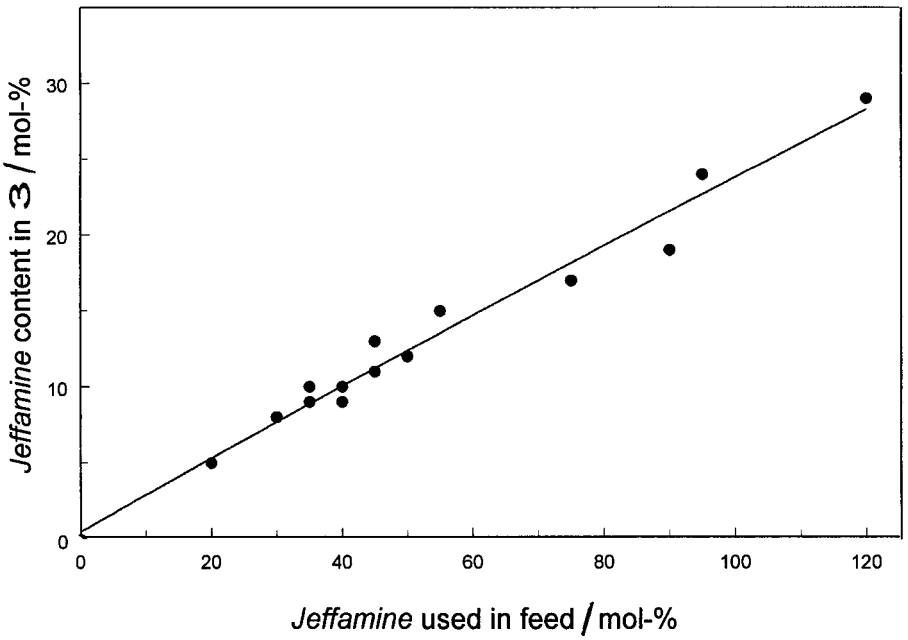


Figure 1 Fraction of aspartamide submits in **3** modified with Jeffamine ED-600, in mol %, versus moles of Jeffamine ED-600 per 100 base moles of **1** in the feed, in mol %.

dried in order to avoid degradative changes, are strongly hygroscopic and tenaciously retain a high percentage of water, and this moisture content is reflected in the proportionately reduced *C* and *N* values found. Similar residual moisture contents had earlier been reported for hygroscopic polyaspartamides and other water-soluble polymers prepared in our laboratory.

The relationship between the extent of Jeffamine incorporation into the polymer (expressed as mol % of aspartamide subunits modified with Jeffamine ED-600) and Jeffamine in the feed (expressed as moles of Jeffamine per 100 base moles of **1**) is graphically represented in Figure 1. The curve confirms the trend, observed previously,[1] of growing feed-to-incorporation ratio with increasing content of the bulky, space-filling Jeffamine substituents in the polymer. The data points also illustrate the aforementioned run-to-run variability in the extent of Jeffamine incorporation and underline the need for accurate compositional characterization of every single polymer synthesized.

In a number of subsequent experiments (again by method B) conducted for comparison, the first step involving Jeffamine incorporation was modified by using higher reactant concentrations (educt **1** : 0.6 mol L^{-1}) and/or adding a heating period (4 h, 65°C) in the first ring-opening step to increase the reactivity of the Jeffamine terminals. Both experimental variations, not unexpectedly, led to significantly larger degrees of Jeffamine incorporation. For example, 35 mol % (based on substrate **1**) of Jeffamine in the feed, which under conventional conditions gave a polymer with 10 mol % of Jeffamine-containing subunits (Table I), entailed a 12.5 mol % incorporation of the Jeffamine when the 65°C treatment was included in the ring-opening step; and 15 mol % Jeffamine incorporation was achieved with a combination of elevated temperature and triple reactant concentrations. In a similar experiment, with 90 mol % (based on **1**) of Jeffamine in the feed and a 4 h heating period of 65°C added in the first ring-opening step, the resultant **3** had a Jeffamine content of 25 mol %, as against 19 mol % achieved under the conventional conditions. The product polymers isolated in these modified experiments were still completely soluble in water and possessed viscosities not substantially different from those of the tabulated conventional products. Despite the obvious improvement of economy, these experimental versions were not considered further for routine carrier preparation in view of the potentially greater risk of hidden crosslink formation not immediately evident from the solubility and viscosity behavior.

The solid-state infrared spectra of **3** are dominated by the strong amide I and amide II bands at 1650 and 1530 cm^{-1} and the equally intense

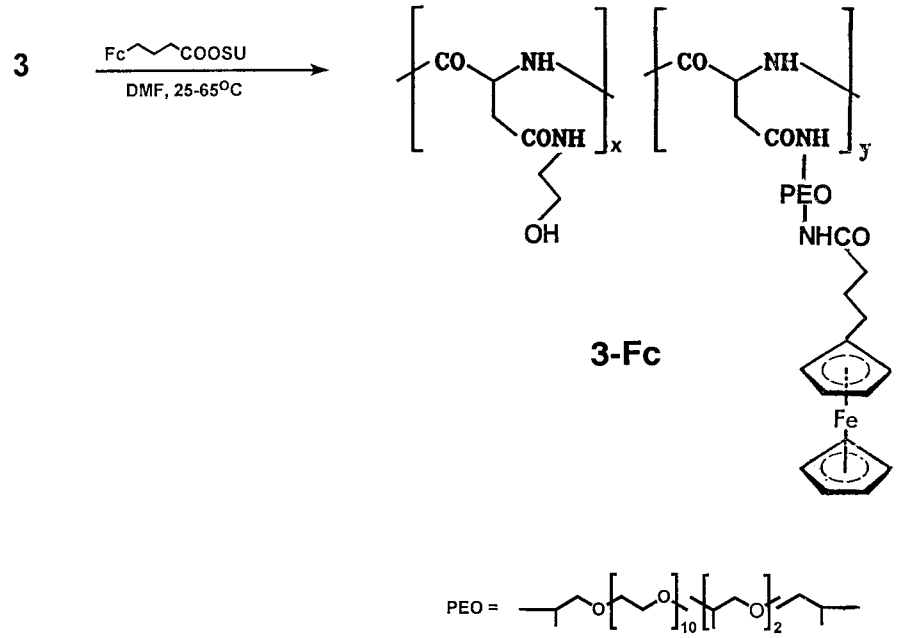
Table III ^{1}H NMR Data for Polyaspartamides 3[a]

Exp. No.	Polymer Designation[b]	Number of Protons Counted[c] (exptd.[d])					
		δ 4.7–4.5[e]	δ 4.1–3.4	δ 3.3	δ 3.1	δ 2.9–2.6	δ 1.2–1.0
1	**3**(95 : 5)	20 (20)	87 (87)	40.1 (40)	—	38.9 (40)	10.3 (12)
2	**3**(92 : 8)	11.7 (12.5)	72 (72)	23.6 (25)	—	23.7 (25)	9.9 (12)
3	**3**(91 : 9)	9.9 (11.1)	69.2 (69.2)	23.5 (22.2)	1 (1.0)	22.1 (22.2)	10.0 (12)
4	**3**(90 : 10)	9.5 (10)	67 (67)	20.4 (20)	—	18.0 (20)	11.7 (12)
5	**3**(90 : 10)	9.9 (10)	67 (67)	20.1 (20)	(0.9)	20.1 (20)	9.8 (12)
6	**3**(91 : 9)	11.0 (11.1)	69.2 (69.2)	22.4 (22.2)	—	22.0 (22.2)	9.2 (12)
7	**3**(87 : 13)	7.2 (7.7)	62.4 (62.4)	16.1 (15.4)	—	14.5 (15.4)	9.0 (12)
8	**3**(89 : 11)	7.9 (9.1)	65.2 (65.2)	17.8 (18.2)	—	12.1 (18.2)	8.9 (12)
9	**3**(88 : 12)	9.2 (8.4)	63.7 (63.7)	15.9 (16.7)	1 (1.2)	15.9 (16.7)	9.9 (12)
10	**3**(88 : 12)	8.9 (8.4)	63.7 (63.7)	16.9 (16.7)	1 (1.3)	15.8 (16.7)	10.5 (12)
11	**3**(85 : 15)	7.1 (6.7)	60.3 (60.3)	13.7 (13.3)	—	13.4 (13.3)	9.5 (12)
12	**3**(85 : 15)	6.2 (6.7)	60.3 (60.3)	12.8 (13.3)	1 (0.9)	12.8 (13.3)	10.3 (12)
13	**3**(83 : 17)	5.4 (5.9)	58.8 (58.8)	12.1 (11.8)	1 (1.2)	10.8 (11.8)	9.9 (12)
14	**3**(80 : 20)	4.2 (5)	57 (57)	9.7 (10)	1 (1.1)	10.3 (10)	10.3 (12)
15	**3**(76 : 24)	4.3 (4.2)	55.3 (55.3)	8.6 (8.3)	1 (0.7)	7.9 (8.3)	8.7 (12)
16	**3**(71 : 29)	3.1 (3.5)	53.9 (53.9)	71 (6.9)	1 (1.0)	6.4 (6.9)	8.5 (12)

[a] In D_2O, pD is 10–11. Chemical shifts, δ/ppm, referenced against internal sodium 3-trimethylsilyl-2,2,3,3-d_4-propionate.
[b] See footnote[c] in Table I.
[c] Integration error limits ± 15%.
[d] Expected for composition in accordance with designation **3**, normalized to $y = 1$.
[e] Proton assignments. 4.7–4.5 ppm: methine (α, β aspartyl; variable because of the partial overlap with the HOD band). 4.1–3.4 ppm: methylene and CONH—C*H* methine (Jeffamine), C*H*$_2$—OH methylene. 3.3 ppm: CH_3—C*H*—O methine (Jeffamine), C*H*$_2$—C*H*$_2$—OH methylene. 3.1 ppm: H_2N—C*H* methine (Jeffamine). 2.9–2.6 ppm: methylene (α, β aspartyl). 1.2–1.0 ppm: C*H*$_3$—CH—O and C*H*$_3$—CH—N methyl (Jeffamine).

absorption near 1100 cm^{-1} due to the PEO ether system. In the spectra of the carboxyl-containing polymers **2,** an additional strong shoulder emerges at 1720 cm^{-1} in the carboxyl region. The ^{1}H NMR spectra of both types **2** and **3** (D_2O; *pD* adjusted to 10–11 in order to eliminate spurious *N* protonation) display the two methine proton signals (α and β peptide forms[7]) of the aspartyl system at 4.7 and 4.5 ppm, and the main feature, a cluster of resonances in the range of 4.1 to 3.4 ppm, represents the methylene and CONH—CH methine protons of the Jeffamine substituent, as well as the hydroxyl-substituted methylene protons of the *N*-(2-hydroxyethyl) group. The second methylene group of the last-named substituent gives rise to an apparent singlet at 3.3 ppm, which also includes the methine proton resonance of the propylene oxide units in the Jeffamine constituent. The Jeffamine NH_2—C*H* methine resonance appears at 3.1 ppm (difficult to assess quantitatively because of the low proton count and considerable line broadening), and the aspartyl methylene protons resonate at 2.9–2.6 ppm. Lastly, the Jeffamine methyl protons (C*H*$_3$—CH—O and C*H*$_3$—CH—N) give the characteristic doublet signals at 1.2 and 1.0 ppm. The NMR data for **3** are summarized in Table III. Most of the signals tabulated lend themselves to an acceptable fit of proton counts. It is only in the region of 1.2–1.0 ppm where, rather consistently, a reduction in signal intensity relative to the expected proton count is noticed, indicative of a slight loss of methyl groups. The implications of this observation with respect to the structural integrity of the Jeffamine side chains (and attached amine terminals) need further study.

The polyaspartamides **3,** all completely water-soluble upon isolation in the solid state, have retained this feature over an observation period of more than a year now. Even repeated cycles of redissolution, reprecipitation, and sharp drying have not caused any settlement of insolubles. The polymers should therefore lend themselves appropriately for conversion to water-soluble drug conjugates, and several drug binding approaches are currently being investigated in this laboratory. In a typical experiment, demonstrating the susceptibility of the amine terminals to drug conjugation, the carrier **3**(87 : 13) was *N*-acylated with 4-ferrocenylbutanoic acid via the active *N*-hydroxysuc-



Scheme 3

cinimide ester of that acid (Scheme 3). The water-soluble conjugate **3**(87 : 13)-**Fc** obtained in 56% yield was found (^{1}H NMR) to be completely *N*-acylated under the chosen experimental conditions. The ferrocene [di-η^5-cyclopentadienyl-iron(II)] complex and its one-electron oxidation product, the ferricenium cation, are both of interest in the biomedical field, notably as antineoplastic agents,[8,9] and the conjugation of this organometallic complex system to water-soluble carrier polymers lends itself as a potential means of enhancing the system's therapeutic effectiveness.

EXPERIMENTAL

General Procedure

Solid-state infrared (IR) spectra ((KBr pellets) were recorded over the region of 4000–200 cm^{-1}. ^{1}H NMR spectra (200 MHz) were taken on D_2O solutions, and chemical shifts, δ, were referenced against sodium 3-(trimethylsilyl)-2,2,3,3-d_4-propionate (integration limits ± 15%). In order to eliminate protonation effects, spectral solutions were adjusted to *pD* 10–11. Inherent viscosities, η_{inh}, were determined at 30.00 ± 0.05°C in Cannon–Fenske tubes, with deionized water used as the solvent (c = 0.2 g/100 mL), and results, averaged from two runs, are given in units of mL g^{-1}. Cellulose tubing, types Spectra/Por 6 wet tubing and Spectra/Por 4 dry tubing (Spectrum Industries, Los Angeles, CA) with (mass-average) molecular-mass cut-off limits of 25,000 and 12,000–14,000, respectively, were employed for dialysis; operations were performed against several batches of stationary, deionized water. Aqueous polymer solutions were freeze-dried with the aid of a Virtis Bench Top 3 freeze-drier operating at −30°C, at 10–15 Pa. Freeze-dried material was routinely post-dried in a Sartorius Thermo Control IR drying system with a heating program of 10 min at 65°C. Analytical samples were additionally dried for 2 d at 75°C in an Abderhalden tube (P_4O_{10} drying agent). This drying treatment generally left 1–3% of moisture in the samples; however, more rigorous drying conditions were avoided in order to preclude structural changes. Microanalyses were performed by Robertson Microlit Laboratories, Inc., Madison, NJ. Determinations were made in duplicate, and the results averaged.

Solvents and Reagents

N,N-Dimethylformamide (DMF) was predried over Molecular Sieves 4A and redistilled under reduced pressure in a faint stream of N_2. Deionized water was used for dialysis and viscometric work. Ethanolamine, reagent grade, (Fluka Chemie), was used as received. The commercial

amine-terminated poly(ethylene oxide), Jeffamine ED-600 (Fluka Chemie), a technical-grade *O,O′-bis*(2-aminopropyl)poly(ethylene, propylene glycol), nominal molecular mass 600, was found (^{1}H NMR) to comprise two propylene oxide units in addition to 10 ethylene oxide units.

ANAL. Found: C, 54.02; H, 9.84; N, 4.36. Calcd for H_2N - $CH(CH_3)CH_2O$ - $(CH_2CH_2O)_{10}(CH(CH_3)$ - $CH_2O)_2$-$CH_2CH(CH_3)NH_2$ ($C_{32}H_{68}N_2O_{13}$) (688.9): C, 55.79; H, 9.95; N, 4.07. ^{1}H NMR, δ/ppm (D_2O; expected proton count in parentheses): 3.7, 44 H (43 H); 3.5, 4 H (6.5 H); 3.3, 2.1 H (2 H); 3.1, 2.1 H (2 H); 1.2–1.0, 12 H (12 H).

Poly-D,L-succinimide (**1**) was prepared by the method of Neri and Antoni[6]; products of approximately equal viscosity were pooled from several preparations and thoroughly mixed to give a master batch with η_{inh} (DMF) of 39 mL g^{-1}, corresponding[10] to a mass-average molecular mass of approximately 36,800. In this communication, overall nominal amounts of polymeric compounds refer to the repeat unit and thus are given as base moles. For the target polymers, the base mole accordingly corresponds to structures **2** and **3,** each normalized to $y = 1$.

Copolymers 2

The experiment described below for the preparation of **2** with $x/(y + z) = 9$ is representative of the general procedure for method A.

Ethanolamine [550 mg (9 mmol)], dissolved in DMF (5 mL), was added in one dash to the stirred and N_2-saturated solution of polyimide **1** (970 mg [10 mmol]) in the same solvent (40 mL). In the stoppered flask, the solution was stirred for 24 h at ambient temperature, then cooled in an ice bath and resaturated with N_2, before Jeffamine ED-600 [2.067 g (3 mmol)], dissolved in DMF (10 mL), was added rapidly; and stirring was continued for 12 h in an ice bath, 10 h at room temperature, and another 2 h at 65°C. Following removal of most of the solvent at 60°C under reduced pressure (residual volume was approximately 15 mL), the polymeric product was precipitated with EtOH–Et_2O (1 : 1; 40 mL), washed with 3 × 20 mL portions of boiling Me_2CO for extraction of unreacted Jeffamine and oligomers, and dissolved in H_2O (50 mL). The clear solution was dialyzed for two days in Spectra/Por 4 dry tubing against H_2O, and the retentate solution was freeze-dried and post-dried to give 1.18 g (69%) of **2**(90 : 2.5 : 7.5) as a water-soluble solid (η_{inh}, 44 mL g^{-1}).

IR/cm^{-1}: 1720 (s sh), 1650 (vs), 1537 (s), 1075 (s), 1102 (s sh). ^{1}H NMR, δ/ppm (expected proton count in parentheses): 4.7–4.5, 34 H (40 H); 3.9–3.4, 121 H (121 H); 3.3, 74 H (74 H); 3.1, intensity variable (1 H); 2.9–2.6, 76 H (80 H); 1.2–1.0, 11 H (12 H).

In a similar fashion, the copolymers **2**(85 : 3.5 : 11.5) and **2**(95 : 2 : 3) were prepared using, 8.5 and 9.5 mmol, respectively, of ethanolamine. Yields were 60–65% (η_{inh}, 35–45 mL g^{-1}).

Polyaspartamides 3

Illustrating the general procedure for method B, the experiment below describes the preparation of **3** with $x/y = 88 : 12$.

Polysuccinimide **1**, 970 mg (10 mmol), was dissolved in DMF (40 mL). To the stirred solution, cooled in an ice bath with introduction of a fast stream of dry N_2, Jeffamine ED-600, (3.44 g [5 mmol]), dissolved in the same solvent (10 mL), was added. Stirring in the stoppered flask was continued for 24 h at 0°C and another 3 d at ambient temperature. The solution was then recooled in an ice bath, and ethanolamine, (782 mg [12.8 mmol]), was added with stirring, while the solution was resaturated with N_2. Continued stirring for 4 h at 0°C, 18 h at room temperature, and 2 h at 60°C was followed by volume reduction to approximately 15 mL under reduced pressure. The polyaspartamide was precipitated with EtOH-Et_2O and washed with boiling Me_2CO as before; it was then dissolved in H_2O (50 mL) and dialyzed for 50 h in Spectra/Por 4 tubing. The retentate solution was freeze-dried and post-dried. Of the crude solid product so obtained, 1.385 g (59%), a 1 g portion was washed with several portions of boiling Me_2CO and redialyzed for 45 h in Spectra/Por 6 wet tubing. Freeze-drying and post-drying gave 916 mg (54%, based on total crude product) of ultimate product polymer **3** (88 : 12) as a cream-colored, water-soluble solid; η_{inh}, 12 mL g^{-1}, IR/cm^{-1}, 1655 (vs), 1540 (s), 1080 (s).

The same procedure, with appropriately varied feed ratios, was used for the preparation of other members of the series **3**, where $x/y \geq 85 : 15$. Polymers **3**, where $x/y < 85 : 15$, were washed with a boiling Me_2CO-Et_2O mixture (1 : 1) instead of neat Me_2CO because of increasing solubility in the last-named solvent. The experimental variables and results, including analytical data, are listed in Tables

I and II, and the summarized nuclear magnetic resonance (NMR) data are in Table III.

Ferrocene Conjugate 3(87 : 13)-Fc

To the solution of carrier **3**(87 : 13), (369 mg [0.2 mmol]), in DMF (4 mL), was added triethylamine (61 mg [0.6 mmol]), and *N*-succinimidyl 4-ferrocenylbutanoate, (148 mg [0.6 mmol]). After saturation with N_2, the solution was stirred for 24 h at room temperature and another 8 h at 65°C. Precipitation of the polymeric product with Et_2O–hexane (2 : 1) was followed by dialysis in Spectra/Por 4 tubing for 2 h against H_2O at pH 8 (Na_2CO_3) and another 30 h against plain H_2O. Freeze-drying of the retentate left 240 mg (57%) of a light tan, fluffy solid completely soluble in H_2O.

^{1}H NMR, δ/ppm: 4.25–4.05, 8.9 H (9 H; ferrocenyl); 3.9–3.4, 63.4 H (63.4 H, CH_2—O—CH_2, CONH—C*H*, CH_2OH). The active *N*-succinimidyl ester used in this ferrocenylation reaction was prepared from 4-ferrocenylbutanoic acid, *N*-hydroxysuccinimide, and *N*,*N*′-dicyclohexylcarbodimide, 1 : 1.2 : 1.1 equivalents, in ethyl acetate [4 h at 0°C, 10 h at 20–25°C; mp 90°C (from isopropanol)].

The authors gratefully acknowledge the generous support provided by the Cancer Association of South Africa, by Western Platinum Refinery Ltd., and by the Council of this University. A.G.P. and G.C. express their gratitude to the Medical Research Council and the Council for Scientific and Industrial Research for doctoral bursary grants. Mrs. Anna Barbosa is thanked for her dedicated assistance with some of the experimental work.

REFERENCES

1. E. W. Neuse, A. G. Perlwitz, and A. P. Barbosa, *J. Appl. Polym. Sci.,* **54,** 57 (1994).
2. *Poly(Ethylene Glycol) Chemistry: Biotechnical and Biomedical Applications,* J. M. Harris, Ed., Plenum, New York, 1992.
3. S. Herman, G. Hooftman, and E. Schacht, *J. Bioact. Compat. Polym.,* **10,** 145 (1995).
4. Y. Ohya, H. Kuroda, K. Hirai, and T. Ouchi, *J. Bioact. Compat. Polym.,* **10,** 51 (1995).
5. M. de L. Machado, E. W. Neuse, and A. G. Perlwitz, *Angew. Makromol. Chem.,* **195,** 35 (1992) (and preceding articles in this series).
6. P. Neri and G. Antoni, *Macromol. Synt.,* **8,** 25 (1982).
7. H. Pivcová, V. Saudek, and H. Drobník, *Polymer,* **23,** 1237 (1982), and refs. cited.
8. E. W. Neuse, *Macromol. Symp.,* **80,** 111 (1994).
9. V. N. Babin, P. M. Raevskii, K. G. Shchitkov, L. V. Snegur, and Yu. S. Nekrasov, *Mendeleev Chem. J.,* **39,** 17 (1995).
10. J. Flasák, F. Rypáček, J. Drobník, and V. Saudek, *J. Polym. Sci., Polym. Symp.,* **66,** 59 (1979).