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Synthesis of Novel Polyethyleneimine Derivatives by Stepwise, Reversible Blocking of Primary and Secondary Amines

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In the design of polyethyleneimine derivatives for use as catalysts and binding media, the placement of reactive and hydrophobic groups previously has been limited by the specificity of the addition reaction. In this paper is described a protocol to limit the sites of addition of nucleophiles and long-chain alkanes to tertiary amines and the less reactive of the secondary amines. Three blocking groups for the primary and secondary amines were tested, but only trifluoroacteylating reagents left the polymer reactive to substitution on the tertiary amines with halogenated alkanes. The secondary amines that resisted trifluoroacetylation were blocked with either *tert*-butyloxy carbonate or trimethylsilyl carbonate. The tertiary amines were quaternized with iodododecane or dodecyl benzyl chloride. After removal of the trifluoroacetyl groups, the polymer amines were inactivated by methylation, which proceeded to 93% completion. The 7% of the amines that were not quaternized were largely tertiary, since propylene sulfide, which reacts only with secondary and primary amines, was substituted onto the polymer only to the extent of 0.2% total amine, as quantified by the indirect method of P. H. Butterworth, F. Baum, and J. W. Porter (1967, *Arch. Biochem. Biophys.* **118,** 716–723). The sulfhydryl group did not oxidize over at least 14 days. This is the first stable sulfhydryl-containing synthetic polymer that has been reported. © 1990 Academic Press, Inc.

INTRODUCTION

There are two general approaches in the design of new polymers. The first is to vary the monomeric subunits prior to polymerization such that they contain the chemical group of interest or a reversibly protected form of it. The second is to introduce the group subsequent to polymerization. In the latter approach, placement is usually dictated by the selectivity of the reaction. Indeed, while an impressive array of polymers have been synthesized, little has been reported on multistep modifications subsequent to polymerization (*1*). Here we describe the site-directed substitution of a branched polymer, polyethyleneimine, with alkyl and acyl groups. This has been achieved by blocking most of the amines with a protecting group. A second or differential blocking group allows temporary protection of sites that later serve as loci for chemically susceptible groups.

Polyethyleneimine (PEI) is a highly branched, water-soluble polyamine having

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primary, secondary, and tertiary amines in a relative ratio of 1 : 2 : 1 (*2*). PEI has the greatest potential of all synthetic polymers thus far studied to serve as a matrix for catalytic groups (*3*). Also, it has an impressive binding affinity and capacity for various small neutral and anionic aromatic compounds (*3*), including nucleoside phosphates (*4*). Previous modifications onto PEI have been of surface (primary) amines or have involved extensive modification of virtually all the amines. This is because the basicity of PEI's amines increase from tertiary to secondary to primary, which is opposite that which is observed with small molecule aliphatic amines (*5*).

In an effort to depart from surface modification on PEI and obtain a more compact core we have redirected the site of hydrophobe substitution to tertiary amines. The primary amines and most of the secondary amines have been blocked reversibly by trifluoroacetylation. A subclass of the secondary amines, those resisting trifluoroacetylation, have been reversibly blocked with a differential blocking group. These secondary amines later serve as sites for chemically reactive groups, with which they are substituted at the end of the reaction scheme. This makes it possible to place even chemically susceptible nucleophiles onto the polymer after all other modifications have been performed.

This reaction scheme demonstrates that multistep, site-directed modifications are achievable on synthetic branched polymers. Such modifications may facilitate generation of microenvironments in synthetic polymers that contain both binding and nucleophilic groups.

MATERIALS

Polyethyleneimine 600, of average molecular weight 60,000, was provided by Dow Chemicals (Midland, MN). Trifluoroacetic anhydride and methyl trifluoroacetate were obtained from Alpha Chemicals (Danover, MA). Di-*tert.*-buytloxy dicarbonate (di-t-doc) and 2-(trimethyl silyl) ethyl carbonate, *para*-nitrophenyl ester (TEOC-*O*-Np) were obtained from Fluka, A.G. (Buchs SG, Switzerland). Methyl and dodecyl iodides were purchased from Eastman (Westport, CT). Dodecyl benzyl chloride was obtained from Stauffer Chemicals (Shelton, CT). Propylene sulfide, diisopropylethylamine (DIEA), *tert.*-butylammonium fluoride, and deuterated solvents were purchased from Aldrich (Milwaukee, WI). *N*-Succinimidyl *para*-nitrophenyl acetate (SNPA) and *n*-methyl bis(trifluoroacetate) (MB-TFA) were from Pierce (Rockford, IL). Piperidine, 5,5′ dithiobis(2-nitrobenzoic acid) (DTNB), sodium arsenate, Dowex-1 X8-200, and Sephadex LH-20 were from Sigma Chemical Company (St. Louis, MO). Organic solvents, of HPLC grade, were purchased from Aldrich (Milwaukee, WI) and were dried over 3-, 5-, and 15-Å molecular sieves from Davison Chemicals (Baltimore, MD). Fluorescent-treated thin-layer plates were obtained from Whatman, Ltd. (Clifton, NY) or Analtech (Newark, DL). Spectra/por dialysis tubing (6000–8000 molecular weight cutoff) was purchased from Spectrum Medical Industries (Los Angeles, CA).

METHODS

Ethanolic stock solutions of PEI (1 monomolar) were prepared as described previously (*6*).

Preparation of Trifluoroacetyl PEI

For synthesis of 100 mmol of trifluoroacetyl PEI (TFA PEI),[2] 100 ml of 1 monomolar ethanolic stock of Dow PEI 600 was vacuum dried 1.5 h at 45° C, redissolved in chloroform, and twice dried to remove small amounts of water. The polymer was then redissolved in chloroform to a final volume of 100 ml. Seventy-five millimoles of methyl trifluoroacetate was added. The solution was shaken at 23°C for 24 h. (Flocculency of the solution is due to insolubility of the derivatized polymer in chloroform.) The solution was then vacuum dried and sufficient acetonitrile was added to bring the final volume to 100 ml. An additional 75 mmol of methyl trifluoroacetate was then added and the solution was shaken at 23°C for 24 h.

The solution was brought to −20°C and 10 mmol of diisopropyl ethylamine (DIEA) was added, followed by 15 mmol of trifluoroacetic anhydride or methyl bis(trifluoroacetate). The solution was kept at −20°C for 24 h. It was dried by vacuum concentration at 45°C and stored at −20°C in a desiccated container. Correction of the molecular weight for the presence of the base was made in subsequent modifications.

Preparation of TEOC PEI

For 10 mmol of TEOC PEI, 10 ml of 1 M stock ethanolic PEI was vacuum dried and redissolved in 10 ml of chloroform. The solution was brought to 4°C. Ten millimoles of molecular sieve-dried DIEA was added, and 7.0 mmol of TEOC-*O*-Np was then added with continuous stirring. The solution was allowed to react at 4°C for 1 h, followed by 24 h at 23°C. An additional 7.0 mmol of TEOC-*O*-Np was then added and the solution was stirred continuously at 23°C for 24 h. Unreacted TEOC-*O*-Np was detected by thin-layer chromatography against hexane:ethyl acetate, 2 : 1 (R_f = 0.95).

Preparation of t-boc PEI

For 10 mmol of t-boc PEI, 10 ml of 1 M stock ethanolic PEI was vacuum dried and dissolved in 10 ml of chloroform. To this was added 7.5 mmol of di-t-boc slowly and dropwise with continuous stirring. The solution was stirred for 24 h at 23°C with a small aperature to the atmosphere to permit the release of carbon dioxide. An additional 2.5 mmol of di-t-boc was then added. After an additional 24 h of continuous stirring, the polymer was vacuum dried and stored at −20° in desiccant.

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[2] For simplicity, amounts of PEI are given as molar equivalents of monomer, *i.e.*, amine, be it primary, secondary, or tertiary.

Reaction of TFA PEI with Di-tert.-butyloxy Dicarbonate, Trimethylsilyl Ethoxy Carbonate Nitrophenyl Ester, or N-Succinimidyl para-Nitrophenylacetate

TFA PEI was dissolved to a 1 M concentration in acetonitrile for modification with di-t-doc or TEOC-*O*-Np, or in tetrahydrofuran for modification with *n*-succinimidyl *para*-nitrophenyl acetate (SNPA). Molecular sieve-dried DIEA (0.2 mole equivalents) was then added. TEOC-*O*-Np, di-*tert.*-butyloxy dicarbonate, or SNPA was then added at a twofold excess of the estimated number of available reactive amines (i.e., primary or secondary). The TEOC and t-boc modifications were allowed to proceed for 20 h at 23°C. Modification by SNPA was carried out at 40°C for 40 min (*7*).

Reaction of TFA PEI with Epoxides

PEI that had been reacted with the stronger trifluoroacetylating reagents, MB-TFA and trifluoroacetic anhydride, as well those reacted only with methyl trifluoroacetate, could be substituted on their remaining secondary amines with propylene oxide, styrene oxide, decyl oxide, and, presumably other epoxides. For these substitutions a one monomer molar solution of TFA PEI was made in THF (nitromethane was also employed successfully). Twenty-five percent total nitrogen of DIEA was added, followed by 25% total amine of the epoxide. Reaction with long-chain epoxides required continuous stirring and heating at 55°C for 24 h. Short-chain epoxides were reacted at ambient temperature for 24 h.

Purification of TFA PEI Derivatives.

TFA PEI. TFA PEI (10 mmol) was vacuum dried and dissolved in absolute ethanol (2 ml). It was then passed through an ethanol-equilibrated Dowex-1 X8-200 column (1 × 10 cm) having chloride as the counterion. The polymer was then concentrated by vacuum evaporation to a final volume of 2 ml and passed through a Sephadex LH-20/ethanol column (25 × 2 cm) to remove neutral DIEA. Ethanol was removed by vacuum evaporation followed by 20-h drying in a drying piston with refluxing.

TFA–TEOC PEI. The *para*-nitrophenolate ion was removed from 10 ml of 1 M TFA–TEOC PEI by ion-exchange chromatography in ethanol on a 2 × 10-cm Dowex-1 X8-200 column. The eluted polymer was concentrated under vacuum and was applied to a 25 × 2-cm column of Sephadex LH-20 in absolute ethanol. The void volume was free of DIEA as well as *para*-nitrophenol.

TFA–t-boc PEI and TFA–epoxide PEI The polymer was dissolved in ethanol to a concentration of *ca.* 1 M amine and run on a Sephadex LH-20 column. A 25 × 2-cm column was used for polymer samples of 10 mmol or less. Removal of ethanol was achieved by the method described for TFA PEI.

TFA–SNPA PEI The reaction mixture was vacuum dried and redissolved in ethanol to about 0.5 M amine. The reagent was poorly soluble in this solvent and was partially removed by centrifugation or filatration through glass wool. The polymer was then passed through Dowex-1 X-8 200/chloride column and LH-20 columns as described for TFA PEI.

Tertiary Amine Quaternization of TFA PEI with Iodododecane and Dodecyl Benzyl Chloride

The TFA PEI derivatives were dissolved to a 0.75 to 1.0 M concentration in acetonitrile. DIEA and alkyl halide were added at stoichiometries of 0.2 and 0.3 total amine, respectively. The solution was reacted at 55–60°C for 24 h with continuous stirring to maintain a homogeneous solution. The solution was vacuum dried. Unreacted alkyl halides were removed by LH-20 ethanol chromatography as described in the purification of TFA PEI. The purified polymer eluted in the void volume. Uneacted alkyl halides were detected by thin-layer chromatography with hexane as the mobile phase ($R_f = 0.9$).

If the desired degree of alkylation was not achieved, unreacted alkylating reagents and side products were removed by LH-20 chromatography in ethanol (as described for TFA PEI purification). Ethanol was removed under reduced pressure and the polymer was redissolved to 1 M in nitromethane, in which it was reacted again with the alkyl halide as described for the reaction in acetonitrile.

Hydrolysis of TFA PEI Derivatives

The trifluoroacetamide bond is labile to strong acid and to mildly basic solutions (*8*), but the t-boc (*9*) and TEOC (*10*) derivatives are only acid labile. Therefore, base permits differential deblockage of TFA groups.

Base-catalyzed deblockage. TFA PEI was dissolved in 95% ethanol to a monomer concentration of 0.33 M. To this was added 100% total amine each of piperidine and deionized water. After 24 h, an additional 100% total amine of water was added and this is repeated daily until the polymer is found to be freely soluble in deionized water (5 to 6 days). Continuous shaking was necessary for polymers with substantial hydrophobe substitution. The polymer was then diluted approximately tenfold and dialyzed against 0.1 M NaCl, followed by deionized water. The polymer was vacuum concentrated at 50°C, lyophilized, and stored desiccated at −20°C. The hydrophobe-containing polymers were particularly hygroscopic.

Acid-catalyzed deblockage. The weighed polymer was dissolved in 95% ethanol to a concentration of 0.5 M. To this was added 37% HCl with a molar content of acid sufficient to protonate all primary, secondary, and tertiary amines. After 24 h, the ethanol was removed by vacuum drying and the polymer was dissolved in glacial acetic acid to a final amine concentration of 0.5 M. The solution was shaken at 23°C for 7 days, or until a drop of the solution was found to give a clear solution in deionized water. The polymer was then carefully brought to pH 7 with 0.5 M NaOH and is dialyzed against first 0.1 M NaCl, then deionized water, changing each solution several times. This removed trifluoroacetate, diisopropyl ethylammonium salts, and acetate. The product was then lyophilized and stored at −20°C.

Quaternization of PEI Derivatives with Methyl Iodide

The lyophilized polymer was dissolved to 0.1 M in a two-to-one anhydrous solution of *n*-methyl pyrrolidinone, methanol, or anhydrous methanol and the solution was brought to −20°C. To this was added molecular sieve-dried DIEA of a volume whose molar content of base was equal to the proton content on all amines, assuming complete protonation. This was followed by the same molar amount of methyl iodide. After 8 h of reaction at −20°C, an additional 1.0 nitrogen equivalent of methyl iodide was added and the reaction mixture was shaken at 23°C for an additional 24 h. The quaternized polymer was sparingly soluble in both of these solvent systems. Both DIEA and unreacted methyl iodode were removed by dialysis, first against 0.10 M NaCl, then against deionized water.

Removal of TEOC from Secondary Amines of Quaternized PEI Derivatives

The lyophilized polymer was dissolved in HMPA:DMF (10 : 1) to a final monomer concentration of 0.5–0.75 M. The solution was brought to −4°C. Meanwhile, tetramethylammonium fluoride (0.3 mmol/mmol polymer nitrogen) was diluted in 0.5 ml of absolute methanol (*10*). This solution was also cooled to −4°C. Aliquots of the fluoride solution were added slowly to the polymer over a 1- to 2-h period. After 5 h the solution was brought to 23°C and, for 24 h, maintained under atmospheric pressure to permit carbon dioxide release.

Removal of t-boc Groups from Secondary Amines of Quaternized PEI Derivatives

The lyophilized, quaternized polymer was dissolved in a 2 : 1 solution of *n*-methylpyrrolidinone:methanol to a final concentration of 0.1 M. To this was added 5 vol % of deionized water, followed by a 3 : 1 excess of trifluoroacetic acid over t-boc amines. The solution was stirred for 24 h under atmospheric pressure, allowing for carbon dioxide release. The solution was dialyzed first against 0.1 M NaCl, then deionized water, changing each solution two to three times over a 48-h period.

Reaction of Residual Secondary Amines with Propylene Sulfide

An aqueous solution of the permethylated polymer was carefully neutralized with 0.5 M NaOH to pH 7.2. It was then lyophilized and dissolved in absolute methanol, after which was added 0.05 equivalent in total amine of propylene sulfide. This was stirred at 23°C for 3 to 4 h under a slight positive pressure of argon. The polymer was purified by LH-20/ethanol chromatography as described for the purification of TFA PEI.

Assay of Mercaptan Content on the Polymer

The indirect dithionitrobenzene (DTNB) method of Butterworth *et al.* (*11*) was modified slightly for use on the polymer. Fifty to one hundred microliters of a 0.6 to 0.8 M PEI solution was diluted with 2.80 ml of 0.02 M barbital buffer, pH 8.7, and 100 μl of stock DTNB in water (40 mg DTNB/10 ml water) was added. The reaction was allowed to proceed for 15 min. Unreacted DTNB, buffer, and released *para*-nitrothiophenolate were removed by dialysis against 0.10 M sodium chloride, and then against deionized water. The polymer was lyophilized in a preweighed vial. It was then weighed and brought up in deionized water to a final volume of 3.0 ml. The pH was checked and adjusted to 8.0 with 0.1 M NaOH. Its background absorption at 412 nm was read. The pH of the solution was then brought to 11.0 with approximately 50 μl of 1 M NaOH. The solution's absorbance at 412 nm was read and the background absorbance measured at pH 8.0 was subtracted. The molar concentation of sulfhydryl was determined based on an ε_{412} of *para*-nitrothiophenolate of 13,000 OD ml/mg-cm (*12*) since exposure to base released the *para*-nitrothiophenolate previously bound to the polymer's thiol groups through a disulfide bond to the polymer thiols.

RESULTS

Of the three blocking groups tested, trifluoroacetic, trimethylsilylethoxy carbonate, and *tert.*-butyloxy carbonate, only the TFA derivatized polymer was capable of being alkylated on tertiary amines. None of the three trifluoroacetylating reagents used reacted completely with the secondary amines, whereas secondary amines could not be detected on t-boc and TEOC PEI (Table 1). The trifluoroacetylation of PEI was also unique in that it was sufficiently exothermic that the reaction had to be performed at −20°C to prevent discoloration, presumably due to amine oxidation (*13*). Reaction of PEI with methyl trifluoroacetate left as many as 25% of the total amines unprotected. MB-TFA or trifluoroacetic anhydride treatment reduced this to 5–8%, all being of the secondary type (Table 1). These remaining secondary amines were reacted with an epoxide or, for subsequent introduction of a chemically susceptible nucleophile, with TEOC-*O*-Np or

TABLE 1

Reversible Blockage of PEI Amines

	Percentage of each amine type modified by the Various Blocking Reagents		
	Primary	Secondary	Tertiary
Methyl TFA[a]	95	60	—
TFA anhydride[a]	N.T.[b]	84–90	—
TEOC-*O*-Np[c]	98+	98+	—
Di-t-boc[c]	98+	98+	—

[a] As determined by modification of unblocked amines with TEOC-*O*-Np, di-t-boc, and SNPA. The extent of reaction with these acylating reagents was measured using proton NMR (60 mHz Varian).

[b] Not tested due to amine oxidation by this reagent.

[c] Quantitated by proton NMR integration of the polymer's methylene carbon signal and the trimethylsilyl signal of the TEOC or the *tert.*-butyl signal of the t-boc group.

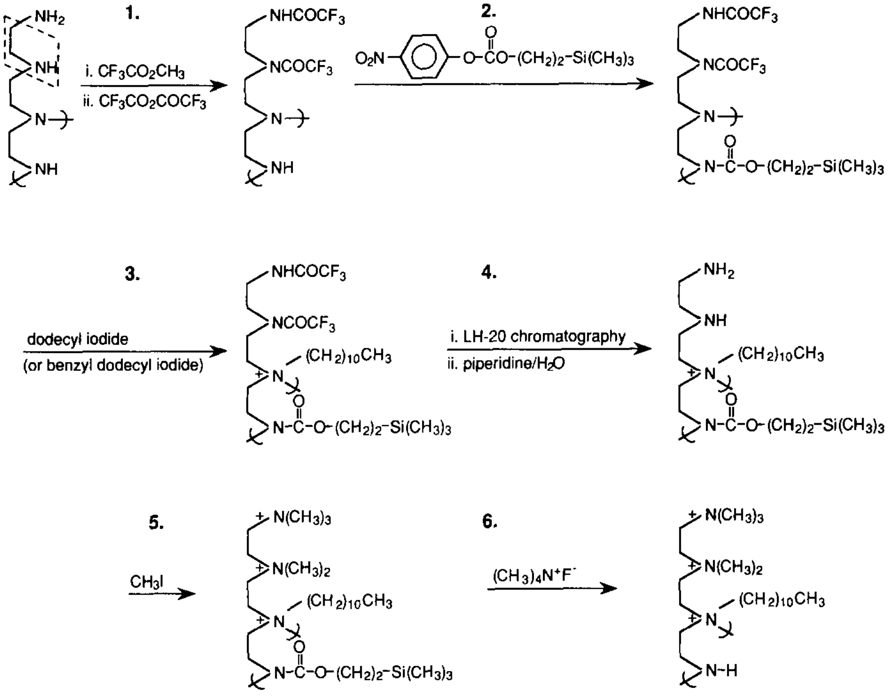

FIG. 1.

di-t-boc, as outlined in Fig. 1. Reaction of TFA PEI with TEOC-*O*-Np rendered it inert to substitution with di-t-boc; similarly, di-t-boc-modified TFA PEI was not seen to react with TEOC-*O*-Np as assessed by proton and carbon-13 NMR (results not shown).

That these differentially blocked amines are secondary was demonstrated by comparing the carbon-13 NMR spectrum of TFA-t-boc polymer (Fig. 2) to that of 75% t-boc PEI (Fig. 3). The positions of the carbonyl carbon and the central carbon of the *tert.*-butyl function in the TFA-containing polymer are consistent with derivatization by di-*tert.*-butyloxy dicarbonate of a secondary amine. Each of these signals is further downfield than are those of t-boc groups on primary amines (Fig. 3).

The tertiary amines were then substituted with either dodecyl benzyl chloride or dodecyl iodide (Fig. 1). The extent of reaction varied depending on the molar excess of the alkylating reagent. Substitution was never quantitative, unlike substitution onto the primary amines of the parent polymer (*14*). Removal of unreacted reagent and repeated substitution with excess reagent in the presence of base permitted as much as 25% alkylation, the theoretical maximum for quaternization of the tertiary amines (*2*).

An ethanolic solution of piperidine removes the TFA groups without affecting TEOC, t-boc, dodecyl, or hydroxyl-containing substituents, as determined by

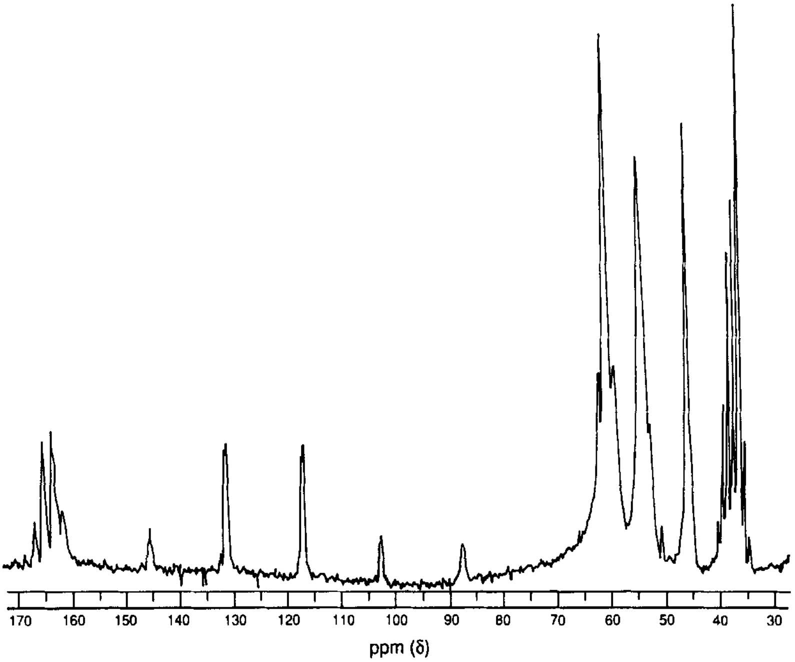

FIG. 2. Carbon-13 NMR spectrum of TFA PEI that has been reacted with di-*tert.*-butyl dicarbonate. Spectrum taken in deuteroacetone. The polymer backbone gives three asymmetric signals between 45 and 65 ppm. The methyl carbons of the solvent are upfield of the polymer. The carbon of the trifluoromethyl group is split by the three fluorines into signals at 146, 132, 117, and 103 ppm. Those on primary versus secondary amines of the polymer overlap extensively. The carbonyl functions of the trifluoroacetyl groups are split into two sets of quartets as well, and are located between 161 and 169 ppm. Note the single t-boc methyl signal at 88 ppm, consistent with its being on a secondary amine of the polymer. Also, a single signal from the carbonyl function of the t-boc group overlaps with the carbonyl quartet of the trifluoroacetyl group at 164 ppm, which is also consistent with its being on a secondary amine of the polymer. The spectra were performed on a multinuclear 24K Varian FT-80 NMR spectrometer operating with a 10-mm broadband probe at 20.0 MHz and with proton decoupling.

proton NMR (see Table 2). Both acid- and base-catalyzed hydrolyses of the TFA groups were complete as demonstrated by carbon-13 NMR (results not shown). TFA hydrolysis was particularly slow when hydrophobe substitution exceeded 15% total amine.

The quaternization of the TFA-deblocked amines with methyl iodide typically proceeds to 93% completion as assessed by the increase in the polymer proton content relative to the trimethylsilyl or *tert.*-butyl peaks, located upfield from the polymer backbone. Quaternization renders the polymer inert to further TEOC or t-boc substitution, as assessed by proton NMR. However, propylene sulfide reacted to the extent of 0.2% total amine. Since epoxides and their sulfur analogs react with primary and secondary amines but not tertiary amines, quaternization

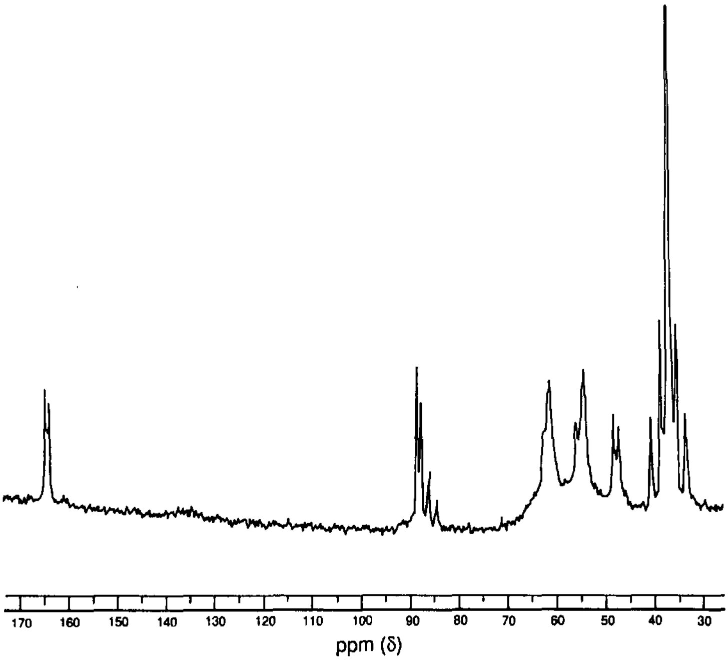

FIG. 3. Carbon-13 NMR spectrum of 75% t-boc PEI in 99% d_6-DMSO. The polymer's methylene carbons are located between 45 and 65 ppm. d_6-DMSO is upfield of the polymer. At 84–86 ppm is a doublet corresponding to the methyl group of t-boc on primary amines, and at 88–89 ppm is a doublet of a t-boc on secondary amines. At 163 ppm is the signal from the carbonyl carbon of a t-boc function on a primary amine, and at 164 ppm is the carbonyl signal of a t-boc group on a secondary amine of the polymer.

TABLE 2

Degree of Polymer Substitution of Several PEI Derivatives before and after Piperidine-Catalyzed Trifluoroacetyl Removal as Quantitated by Proton NMR[a]

With trimethylsilylethoxy carbonate (using TEOC-*O*-Np)	
On TFA tertiary *n*-dodecyl PEI	12.7 ± 0.7
On piperidine-deblocked TFA-dodecyl PEI	11.4 ± 0.7
With *tert.*-butyloxy carbonate (using di-t-boc)	
On TFA PEI	8.3 ± 0.4
On piperidine-deblocked methylated PEI	8.8 ± 0.6
With *n*-dodecane (using *N*-dodecyl iodide)	
On TFA, 8% (1-OH-2-phenyl) ethyl PEI	17.9 ± 0.9
On piperidine-deblocked methylated, 8% (1-OH-2-phenyl) ethyl PEI	17.5 ± 1.3
With (1-OH-2-phenyl)ethane (using styrene oxide)	
On TFA PEI	8.8 ± 0.4
On piperidine-deblocked TFA PEI	8.8 ± 0.4

[a] Expressed as percentage total polymer amine

of these PEI derivatives with methyl iodide eliminates greater than 99% of the primary and secondary amines. This is important since it ensures that the secondary amines subsequently regenerated by removal of differential blocking groups are the predominant sites for the final placement onto the polymer of chemically susceptible nucleophiles, such as sulfhydryls and peroxides, when these are introduced as acid chlorides, epoxides, or other functional groups that react with primary and secondary but not tertiary amines.

The sulfhydryl content of a polymer preparation having 0.2% monomolar sulfhydryl was assayed by the indirect method over more than 2 weeks and was found to be constant when stored at ambient temperature as a 0.6–0.8 M solution in deionized water (pH 8). This indicates that the mercaptans were not oxidizing to form disulfides. The indirect method of assay ensures no possible catalytic breakdown of Ellman's reagent by the polymer itself, either through sequestering of hydroxyl ions or direct attack by the amines to form a sulfenamide (*15*). If sulfenamides are formed with our highly quaternized polymer, they must be turned over since polymers not containing a mercaptan gave no increase in absorbance at 412 nm upon being subjected to base.

DISCUSSION

To further the use of this polymer as a chromatographic matrix or synthetic catalyst, the efficacy of stepwise and site-directed chemical modifications had to be established. This work clearly illustrates that multistep site-directed modification of PEI, a polyelectrolyte of molecular weight 40,000–60,000 (*2*), may be achieved readily. Exotic catalysts, unusually reactive alkylating reagents, and harsh conditions were not required. The modifications were monitored and quantitated by rather simple spectroscopic and analytical techniques. The reactions were also readily reproducible. The unreacted reagents and side products were removed easily from the polymer using a combination of ion exchange, gel permeation chromatography, and dialysis. The prevailing view regarding polyelectrolyte modification is that the degree of substitution is rather limited and that the reactions are extremely difficult to follow (*1*). This work demonstrates that such an assumption is incorrect, at least for PEI. Each modified polymer has been effectively purified, so that the vast recent advances in physical techniques such as NMR spectroscopy and dynamic light scattering as well as in chemical modification can now be applied to monitor and analyze various polymer derivatives.

Reactions on PEI are slow, particularly the base-catalyzed removal of TFA. This may be due to slow polymer disaggregation as solvents are changed during the modification scheme. The light scattering properties of a lyophilized quaternized PEI derivative have been shown not to be stable until some 96 h after dissolution into an aqueous buffer (unpublished results). Prior to that time, there is a slow but significant increase in the fractional content of monodispered polymer. In this reaction sequence, the polymer is dried numerous times from several solvents both by vacuum concentration and by lyophilization.

Despite their importance as biological catalysts and covalent linkers, sulfhydryls on synthetic macromolecules remain little studied due to their tendency to oxidize. Polyvinyl mercaptan (*16*), for example, and primary-substituted PEI containing dodecyl and episulfide moieties (*17*), both undergo oxidation and crosslinking, often resulting in precipitation. However, in this polymer, only 0.2% of the monomers have sulfhydryl groups; this corresponds to about 2.6 sulfhydryls per polymer molecule (60,000 molecular weight, 43 g/mol weight per subunit). In this work, we have strived to synthesize a polymer in which the sulfhydryl groups would be isolated from one another and hopefully from the water interface, to decrease the likelihood of intermolecular as well as intramolecular disulfide bond formation. The fact that the amount of assayable sulfhydryl does not change with time on the polymer described indicates that a stable sulfhydryl-containing polymer is now available. The extraordinary degree of acylation, alkylation, and deacylation achieved on PEI indicates that this highly branched polymer is quite reactive despite possible steric constraints and a high charge density.

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