Synthesis of Symmetric Fluorescently Labeled Poly(ethylene glycols) Using Phosphoramidites of Pyrenebutanol and Their Characterization by MALDI Mass Spectrometry

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ABSTRACT: We report the synthesis and characterization of a series of poly(ethylene oxide) [PEO] polymers in which two pyrene groups are separated by a common EO_{87} chain, and flanked by tails of varying EO_x length. These polymers were prepared by reacting **HO−EO$_{87}$−OH** (M_n = 3841, M_w/M_n = 1.01) with four different *N*,*N*-diisopropylphosphoramidites containing both pyrene (Py) and MeO−EO_x− substituents. In this way, polymers of the form **EO$_x$−Py−EO$_{87}$−Py−EO$_x$** (with MeO end groups) were obtained, with x = 2, 12, 17, and 47. These polymers are particularly useful for studying cyclization kinetics via pyrene excimer formation for two points labeled by pyrenes within the interior of a polymer chain. The polymers were characterized by NMR, size exclusion chromatography, and matrix-assisted laser desorption (MALDI) mass spectroscopy. The MALDI results were particularly informative since each of the oligomers of each of the components could be identified in each mass spectrum.

Introduction

While interest in the cyclization of polymers and linear oligomeric species dates from the early part of this century,[1] it is only recently that appropriate tools have become available to study polymer cyclization with the accuracy and precision needed to test theoretical models. These experiments can now be used to examine issues which are of fundamental interest in polymer science. Effects such as hydrodynamic interactions and excluded volume appear in the dependence of the rate constant for end-to-end cyclization on chain length, type of solvent, and the concentration of the polymer.[2]

One of the most convenient means for studying the rate of end-to-end polymer cyclization is through the kinetics of excimer formation between pyrene groups attached to the ends of the polymer chains.[3] Pyrene (Py), following excitation to form Py*, emits a structured blue fluorescence (at ca. 380 nm) if the pyrene is unassociated. On the other hand, if Py* encounters another pyrene during its relatively long-lived (200 ns) excited state lifetime, the pair have the special property that they form a sandwich-like excimer, which emits a broad green fluorescence (centered at 480 nm). Thus if one has polymers with pyrene groups attached to both ends, the kinetics of intramolecular excimer formation reports on the rate of polymer end-to-end cyclization. The Winnik groups has used this approach to study cyclization kinetics in a broad series of polymers.[3,4]

Here we turn our attention to the question of polymer cyclization between two points in the interior of a polymer chain (cf. Figure 1). The issue we wish to address is whether two points located within the interior of the chain, separated by a common backbone contour length, cyclize at the same rate or a different rate than if those points were located at the chain ends. Theoretical predictions on the dynamics of such systems have appeared recently,[5] and we have been interested in testing these ideas. What we need for this experiment is a series of polymers with two pyrene groups separated by a common polymeric spacer, to which tails of various lengths can be attached. The synthesis of such polymers is a rather daunting task.

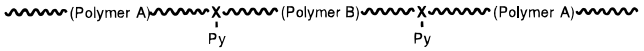

Figure 1. General structure of target polymers labeled with pyrene (Py). X represents the monomer or functional group to which the chromophore is tethered. In a series of samples, the length of polymer B—the central chain—remains fixed while the length of polymer A—the terminal or end chains—varies with each sample. This structure can be considered as an "A−B−A triblock copolymer" labeled at the A−B junctions, where the monomer units of polymer A and polymer B are identical.

Recently, we reported the synthesis of oligomers of oxyethylene glycol labeled with naphthalene probes in the chain interior.[6] Here, in the first part of this paper, we describe the extension of our strategy to the synthesis of polymers which are functionalized at two specific sites, separated by "*y*" monomers, and symmetrically positioned within the chain. An important challenge in the synthesis of these compounds was obtaining convincing characterization data. In the second part of this paper, the analysis of these polymers by matrix-assisted laser desorption/ionization (MALDI) mass spectrometry is described.[7] We have found that this technique provides accurate and reliable mass analysis of the individual chains in our labeled samples. These experiments represent one of the first uses of MALDI mass spectrometry to characterize the polymer products from a multistep synthesis.

Results and Discussion

Synthetic Strategy. The target materials comprise a series of linear homopolymers containing two pyrenes that are separated by a well-defined number of monomer units and that are symmetrically positioned within the chain (Figure 1). These polymers must exhibit a narrow molecular weight distribution and be free from

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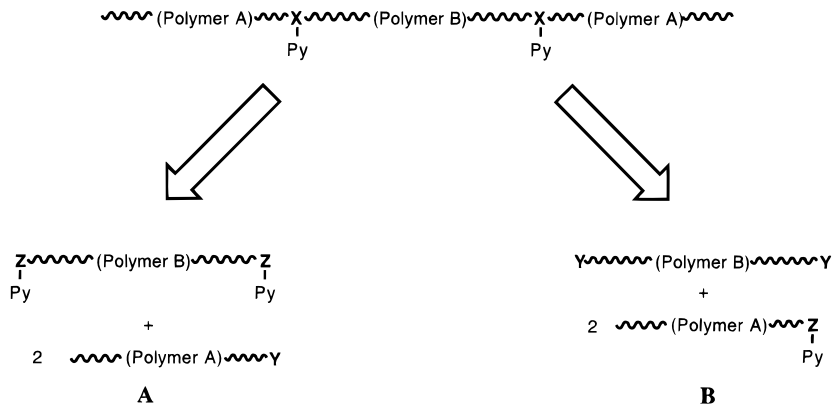
Scheme 1



any adventitious quenchers and fluorescent impurities. Until now, no synthetic strategies have been developed to generate polymers with this specific architecture.

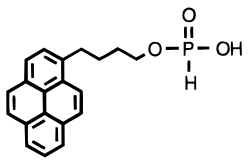
Fluorescent labels are usually incorporated into precise positions of a structurally well-defined polymer by living anionic polymerization[8,9] or by functionalizing the end groups of telechelic monodisperse chains.[3,4,10,11] The former approach has been used to synthesize A−B diblock copolymers labeled at the A−B junction.[9,10] Quirk has demonstrated that A−B−A triblock and A−B diblock copolymers can also be prepared in this manner by using 1-phenyl-1-(1-pyrenyl)ethylene (**1**).[9] However, anionic polymerization has not been successful at incorporating a single label between two "blocks" in homopolymers.

An alternative strategy is required for polymers of the type in Figure 1. The synthons for these target materials are two polymers of different lengths, corresponding to the central and end chains, which are capable of coupling directly with each other. To place the fluorescent label at the junctions between the central and end chains, the functional group(s) Z on the end(s) of one of these polymer chains must incorporate—or be adjacent to—the chromophore. This is shown in Scheme 1. The two proposed strategies are equally effective.[6,12] However, each reaction affords different impurities from hydrolysis (if the chromophore-containing reactant is moisture-sensitive) and incomplete coupling of the starting materials which may adversely affect the subsequent fluorescence experiments if they are not removed completely. The fluorescent impurities from Scheme 1A would include the doubly labeled "polymer B" and "polymer B" labeled with the fluorescent probe at both of its ends, with one end chain attached. Trace amounts of these two impurities would give fluorescene decay measurements which reflect the cyclization rates of up to three different species. From Scheme 1B, the fluorescent impurities would include "polymer A", labeled at one end, and "polymer B" linked to one end chain, with the fluorescent probe situated at their point of attachment. These two singly labeled polymers should exhibit fluorescence that is characteristic of a suitable model compound. The contribution from trace amounts of these impurities to the fluorescence measurements can be extracted,[13] allowing the cyclization rate of the target polymer to be determined. Hence, the synthetic route represented by Scheme 1B is preferred, as it ensures against inadequate product purification.

Our methodology adopts a strategy from polynucleotide chemistry.[14] The well-established synthetic protocols that have been developed for solid-phase DNA and RNA syntheses are based on features which are equally attractive to our work. The key step, involving the reaction of a derivatized phosphoramidite with an appropriate alcohol to form a phosphite bond, proceeds through a rapidly generated and highly reactive tetrazolyl phosphoramidite.[14c,d,15] This is also a particularly efficient reaction, generating internucleotide links in near quantitative yields. The product after oxidative workup is a phosphate triester. Scheme 2 illustrates the use of this reaction to synthesize a linear polymer containing two phosphate groups to which the chromophore is attached.

Poly(ethylene glycol) seemed to be a natural choice for this synthesis. It is a highly flexible polymer that facilitates intramolecular excimer formation readily. It is also stable to the reactive phosphite intermediates involved in phosphoramidite chemistry, and samples of narrow dispersity are available commercially with hydroxyl groups on one or both ends. In a previous work,[6] we have shown that oligoethylene glycols ($x = 1-2$, $y = 3-4$) labeled with naphthalene could be synthesized in this manner and that their fluorescence properties indicated that such compounds were suitable for cyclization studies.

Synthesis of the Phosphoramidites of Poly(ethylene glycol). The phosphoramides **EO$_x$−Py−NR$_2$** were synthesized by the route illustrated in Scheme 3. Reaction of 4-(1-pyrenyl)butanol (**1**) with phosphorus trichloride gave the phosphorodichloridite **2**. This is a moisture-sensitive, white, crystalline solid that melts at 53−54 °C. However, the efficient purification of **2** was problematic. Only one half of the total amount of product was recovered after recrystallization from an anhydrous mixture of ethanol-free chloroform and hexanes. Attempted sublimation of crude **2** at 50 °C under a pressure of 1×10^{-5} mmHg led to considerable polymerization of the product mixture. Consequently, a modification to the usual synthetic procedure[16] was developed. A dilute solution of **1** in tetrahydrofuran (THF) was added slowly to a minimum 6-fold excess of phosphorus trichloride in THF at −78 °C. These conditions reduce the yield of the major impurity **4**, presumably by decreasing the amount of



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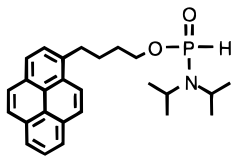

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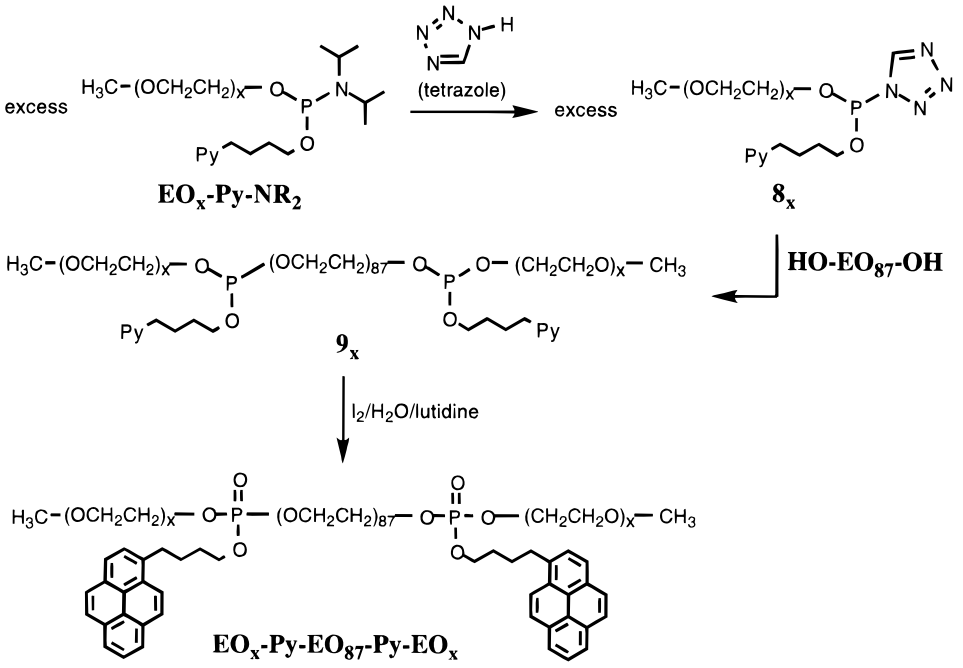
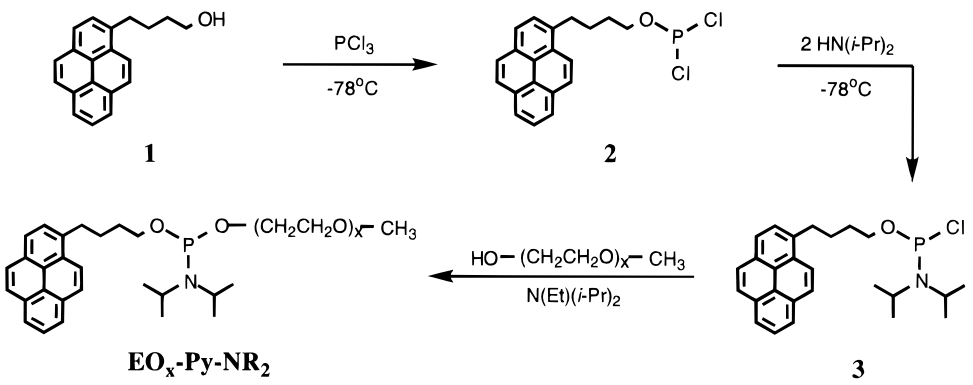
adventitious moisture available for hydrolysis. Crude

Scheme 2



Scheme 3



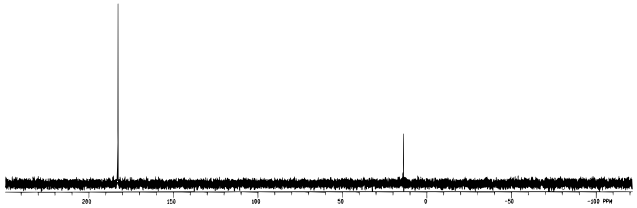


Figure 2. ^{31}P NMR spectrum of crude **3** (182.8 ppm) in C_6D_6. The impurity **5** appears at 13.8 ppm. The ratio of the peak integrals is 8.4:1, respectively.

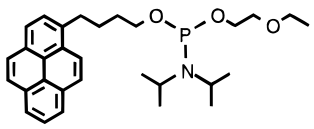
yields of **2** obtained with this procedure were consistently about 95%, as determined by ^{31}P NMR.

The crude phosphorodichloridite **2** was allowed to react with 2 equiv of diisopropylamine to afford the phosphoramidochloridite **3**. The loss of **2** was monitored by ^{31}P NMR to determine the amount of amine required. In all cases, the quantity corresponded to an assumed yield of 100 ± 10% of the phosphorodichloridite **2** from the alcohol **1**. A typical ^{31}P NMR spectrum of the crude product **3** is shown in Figure 2. Only one phosphorus-containing impurity—the phosphoramidous acid[15,17a] **5**—was detected. This was generated from unavoidable traces of water which hydrolyzes **3**, resulting in crude yields based on ^{31}P NMR between 72 and 86%. The phosphoramidochloridite **3** is a viscous oil that is undistillable up to ca. 150 °C at 1×10^{-5} mmHg, where it decomposes.[17b] For the subsequent reaction to phosphitylate the polymers, unpurified **3** could be used, because the phosphoramidous acid **5** is inert to the reactants and is not deleterious to the chemistry.

Shortly after this work was started, we learned from colleagues more experienced with this chemistry that using bis(dialkylamino)phosphines[18] instead of phosphorodichloridites for the synthesis of **EO_x−Py−NR_2** would lead to fewer side products. We feel that this advice is well worth pursuing in any future work.

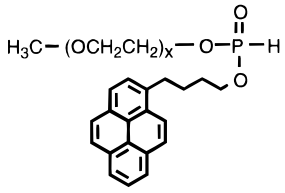
Four samples of poly(ethylene glycol) terminated at one end with a methyl group were purchased for Aldrich Chemical Co. Their approximate molecular weights were listed as 550, 750, 2000, and 5000. The corresponding phosphoramidites, **EO_x−Py−NR_2**, were obtained by adding carefully dried THF solutions of the monomethoxyPEGs in excess to the crude phosphitylating agent **3** in the presence of diisopropylethylamine. The reaction time, monitored by the appearance of the amine salt and by thin layer chromatography, increased with the length of the polymer. With the monomethoxyPEGs 550 and 750, **3** was undetectable after 10 min, while the reactions involving monomethoxyPEGs 2000 and 5000 required over 60 min and 4 h to complete, respectively. These results are consistent with the slower rates of reaction generally observed with longer polymer chains. They may also reflect the greater

segment density of the two longest chains—increasing the steric constraints to reaction—since diisopropylethylamine is a nonsolvent for PEG, causing the polymer coil to collapse. A fifth phosphoramidite, **EO_2−Py−NR_2**, was also synthesized by reacting crude **3** with 2-ethoxyethanol.

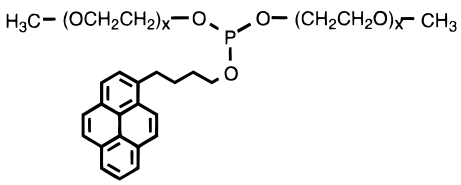


EO_2-Py-NR_2

With the exception of **EO_2−Py−NR_2**, the products **EO_x−Py−NR_2** are surfactants, making aqueous workup impractical. These polymer phosphoramidites were purified by precipitation followed by silica gel chromatography with 10% (v/v) triethylamine in the eluent—which was either 1:1 dichloromethane−ethyl acetate or THF, depending on the molecular weight of the phosphoramidite—to protect against their decomposition by the adsorbent. The weakly polar isopropyl and 4-(1-pyrenyl)butyl groups on these phosphoramidites decreases the interaction between **EO_x−Py−NR_2** and the silica, increasing their rate of migration through the column relative to the other, more polar species such as **6_x** and **7**,which are the major polymeric impurities.



6_x



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It is interesting to note that the extent to which these phosphoramidites are adsorbed depends on their molecular weights: the R_f values decrease with increasing length of the PEG chain. Polymers which can be resolved by TLC due to differences in their molecular weight or end groups are rarely encountered.[19]

The characterization of these phosphoramidites and their precursors, the monomethoxyPEGs, are described in detail below. The results which should be mentioned at this point are that the number average degrees of polymerization, x, for the phosphoramidites of monomethoxyPEGs 550, 750, 2000, and 5000 were determined to be 12, 17, 47, and 128, respectively. Henceforth, these phosphoramidites will be designated as **EO_{12}−Py−NR_2**, **EO_{17}−Py−NR_2**, **EO_{47}−Py−NR_2**, and **EO_{128}−Py−NR_2**.

Coupling the Phosphoramidites to the Central Chain. The poly(ethylene glycol) which constitutes the central chain of the target polymers was a molecular weight standard purchased from Polymer Laboratories Inc. Its number average molecular weight was determined to be 3841 (vide infra), corresponding to an average degree of polymerization of 87. The phosphoramidites **EO_x−Py−NR_2** were coupled to both ends of this polymer, **HO−EO_{87}−OH**, as shown in Scheme 2.

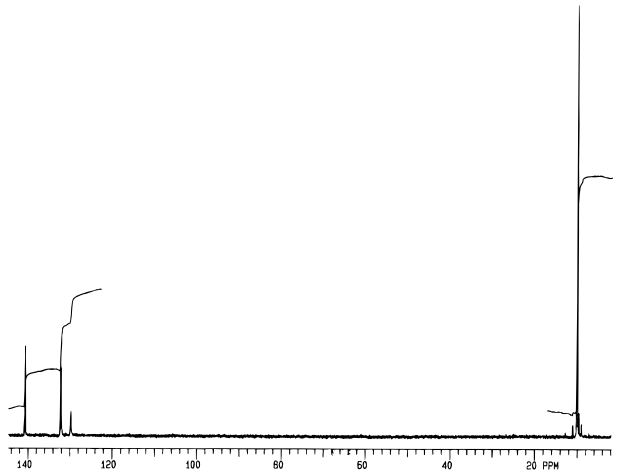
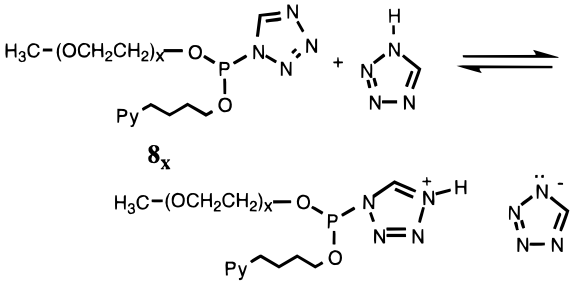


Figure 3. ^{31}P NMR spectrum of the crude product mixture at the completion of the reaction between **EO_{45}−Py−NR_2** and **HO−EO_{87}−OH**.

In a typical reaction, an anhydrous solution of tetrazole in acetonitrile was added to a THF solution of **HO−EO_{87}−OH** and an excess of the phosphoramidite in the presence of some molecular sieves. The mechanism of activation presumably involves protonation of the phosphoramidite nitrogen by tetrazole followed by nucleophilic attack of the tetrazolide anion on the phosphorus.[14c,d,h,15] The resulting intermediate **8_x** is a highly reactive tetrazolylamidite which undergoes nucleophilic displacement in the presence of the α,ω-diol, **HO−EO_{87}−OH**, to form the phosphite **9_x**.

Figure 3 shows a typical ^{31}P NMR spectrum of the crude product mixture at the completion of this reaction. The product phosphite **9_x** and the excess tetrazolide **8_x** give signals at ca. 139 and 128 ppm, respectively. The more intense peak at ca. 9 ppm is due to the phosphonate, **6_x**, that results from the hydrolysis of **8_x**. In a few phosphorylation reactions, an additional species was observed at ca. 131 ppm. This signal disappears upon oxidative workup. One immediate speculation is that it arises from the tetrazolium phosphite **10** (eq 1).



8_x

(1)

10

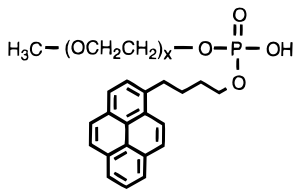
However, the equilibrium between **8_x** and its protonated form should produce some broadening of their ^{31}P resonances which was not observed. Hence, this peak remains unassigned at this time.

Since traces of water were unavoidable under the experimental conditions, an excess of **8_x** ensured that

some depletion of this species through hydrolysis would still leave enough activated phosphoramidite to functionalize both ends of **HO−EO$_{87}$−OH**. The reactants and catalyst were used in typical molar ratios of 140 (tetrazole):22 (phosphoramidite):1 (**HO−EO$_{87}$−OH**). A few reactions performed using less tetrazole indicated that the quantity of this acid could be reduced to a 4-fold excess over the phosphoramidite. On the other hand, when the ratio of **EO$_x$−Py−NR$_2$** to **HO−EO$_{87}$−OH** was reduced to 8:1, the signal at ca. 128 ppm was not observed after the phosphoramidite was activated. With this result, complete phosphorylation could not be assured, even though a peak at ca. 139 ppm provided evidence for the formation of trialkyl phosphite. However, the intensity of the ^{31}P signal due to **8$_x$** relative to that of its hydrolysis product, **6$_x$**, did not change much when the ratio of **EO$_x$−Py−NR$_2$** to **HO−EO$_{87}$−OH** was increased beyond ca. 15:1. Furthermore, the intensity of the **6$_x$** peak, at ca. 9 ppm, from these reactions indicated that more moisture was present than expected. These two results suggest that a considerable amount of water was introduced into the reaction by the polymer **EO$_x$−Py−NR$_2$** itself. It is possible that some water molecules interact with the PEG coils to such an extent that they do not dissociate into the solvent readily, where they could be removed by the molecular sieves.

An important concern during the workup of **9$_x$** to give **EO$_x$−Py−EO$_y$−Py−EO$_x$** was that the electron-rich pyrene groups might also be oxidized. An aqueous solution of iodine in lutidine, which is also used in polynucleotide synthesis, was chosen for the workup. Treatment of pyrenebutanol (**1**) with this reagent (22 °C, <10 min) did not produce any oxidation products that were detectable by mass spectrometry, although traces of a (M + 14) species do appear after ca. 15 min. With two other oxidizing reagents examined—*m*-chloroperoxybenzoic acid (−20 °C, 10 min) and *tert*-butyl hydroperoxide (−20 °C, 10 min)—this same peak was observed at a much greater intensity.

The poly(ethylene glycols) were isolated from the lower molecular weight impurities that were present in the reaction mixture by repeated precipitations. This gave a polymer mixture which was expected to contain chains of two distinct average lengths. The product, **EO$_x$−Py−EO$_{87}$−Py−EO$_x$**, has an average of "2x + 87" oxyethylene units. The derivatives of monomethoxyPEG resulting from the excess phosphoramidite **EO$_x$−Py−NR$_2$**—such as **11$_x$**—have an average of "x" monomer units. This difference in molecular weight allowed the bis(phosphates) **EO$_x$−Py−EO$_{87}$−Py−EO$_x$** to be purified by gel permeation chromatography (GPC).



11$_x$

Figure 4 shows a typical chromatogram of the prepurified product. The highest molecular weight polymer elutes from the columns first. In all cases, the molecular weight corresponding to this peak was determined to be the same as that expected of the product, except for

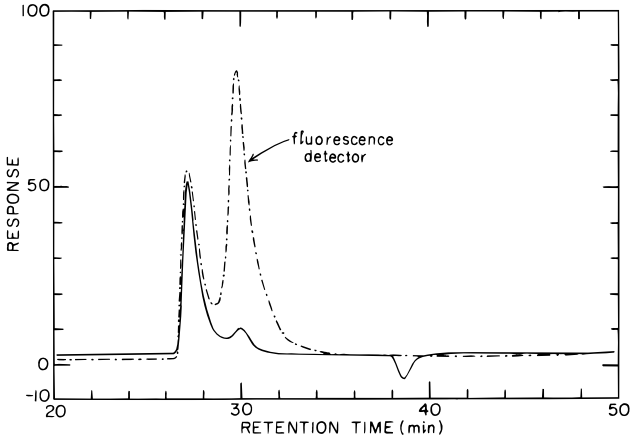
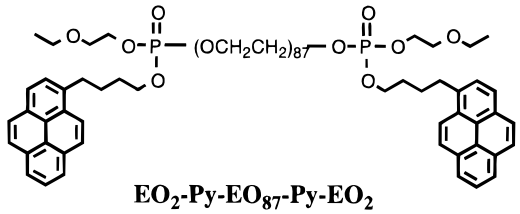


Figure 4. Gel permeation chromatogram of the prepurified **EO$_{15}$−Py−EO$_{87}$−Py−EO$_{15}$**: Waters μStyragel 500 Å, 10^3 Å, and 10^4 Å analytical columns; flow rate, 0.8 mL/min; fluorescence detector, λ_{ex} = 345 nm, λ_{em} = 500 nm (cutoff filter); (−·−) fluorescence detector; (—) refractive index detector.

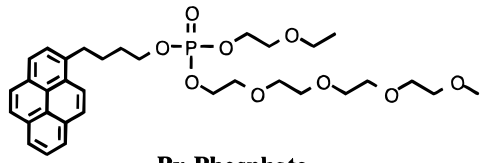
the sample obtained from the synthesis of the longest PEG, **EO$_{128}$−Py−EO$_{87}$−Py−EO$_{128}$** (see next section). The large second peak is due to the PEGs containing "x" monomer units.

The final purification of the product was accomplished by collecting the first fraction that eluted from the chromatograph. This sample produced a single peak when it was reinjected into the chromatograph. Fractionating this peak into several narrower bands was not necessary, because the molecular weights and the fluorescence results obtained from such refractionated samples were found to be identical within experimental error.

The following labeled PEGs were prepared: **EO$_2$−Py−EO$_{87}$−Py−EO$_2$**, **EO$_{12}$−Py−EO$_{87}$−Py−EO$_{12}$**, **EO$_{17}$−Py−EO$_{87}$−Py−EO$_{17}$**, and **EO$_{47}$−Py−EO$_{87}$−Py−EO$_{47}$**. **Py−phosphate** was also synthesized. This was necessary as a model compound for these bis(phosphates) in the fluorescence experiments and for characterization by NMR.



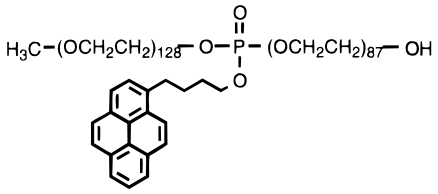
EO$_2$-Py-EO$_{87}$-Py-EO$_2$



Py-Phosphate

Single Functionalization in the Synthesis of EO$_{128}$−Py−EO$_{87}$−Py−EO$_{128}$. The first GPC fraction recovered from the prepurified product of the synthesis of **EO$_{128}$−Py−EO$_{87}$−Py−EO$_{128}$** had a number average molecular weight, M_n, of 9500, corresponding to an average length of ca. "x + 87" monomer units for this product compared to the "2x + 87" oxyethylene segments

found in the other labeled polymers. The expected trialkyl phosphate resonance was observed as the only signal—at −0.08 ppm—in the ^{31}P NMR spectrum of this product. However, the ^{1}H NMR spectrum of a carefully dried sample of this polymer in dimethyl sulfoxide-d_6 showed a small triplet at 4.52 ppm, indicating the presence of a hydroxyl proton.[20] The presence of only one pyrene group per polymer chain was also confirmed with fluorescence measurements.[13] The most likely structure for this product is the singly labeled polymer **EO_{128}−Py−EO_{87}−OH**.



EO_{128}-Py-EO_{87}-OH

Presently, it is not clear why the doubly labeled polymer could not be formed under the reaction conditions used here. It is possible that the interaction between the end groups of **8_{128}** and **EO_{128}−Py−EO_{87}−OH** may be affected by the greater segment density within the coils of these longer chains. It should be noted that **8_x** has a bulky reactive end group which is also larger than the oxyethylene units. Hence, it may be natural to attempt this interpolymeric reaction in a better solvent. As the polymer swells with more solvent, the coil dimensions become less compact, decreasing the barrier against the diffusion of the tetrazolyl end group through the **EO_{128}−Py−EO_{87}−OH** chain, toward the hydroxyl group. However, the excluded volume effect in good solvents also reduces the probability of chain interpenetration—interchain repulsion is favored.[21,22] With these considerations, it is clear that the synthesis of **EO_{128}−Py−EO_{87}−Py−EO_{128}** and longer such bis(phosphates) presents additional challenges yet to be addressed.

MALDI Mass Spectrometry and Characterization of the Products. The number average molecular weights, M_n, of the poly(ethylene glycols) attached to the phosphoramidite moiety of the products **EO_x−Py−NR_2** were determined using ^{1}H NMR. Proton resonances from the oxyethylene segment closest to the phosphorus atom were identified using the lowest molecular weight derivative, **EO_2−Py−NR_2** (c and f in Figure 5); assignments were made with the aid of is ^{1}H−^{1}H COSY and ^{1}H−^{13}C HETCOR spectra (not shown). The PEG chains in the polymer phosphoramidites exhibit a triplet at ca. 3.35 ppm (Figure 6). This signal has been tentatively assigned to the methylene protons adjacent to the methoxy group at the other end of the chain on the basis of the NMR spectra of tetraethylene glycol monomethyl ether and its derivative, **Py−phosphate**. Hence, the characteristic peak of poly(ethylene glycol), observed between ca. 3.41 and 3.50, is due to the intervening methylene protons identified in boldface: $POCH_2CH_2(OC\mathbf{H}_2C\mathbf{H}_2)_{x-2}OC\mathbf{H}_2CH_2OCH_3$. The relative intensity of this peak was established using signals from three separate regions of the molecule; the doublet at ca. 1.17 ppm due to the twelve methyl protons of the isopropyl groups, the two multiplets at ca. 1.71 and 1.93 ppm of the respective C2 and C3 methylene protons from the butyl chain, and the multiplet between ca. 7.70 and 8.23 ppm from the nine pyrenyl protons. This gives three different measures of x which provides an indication of the accuracy of the integration. The lengths of the PEG chains on **EO_x−Py−NR_2** are summarized in Table 1.

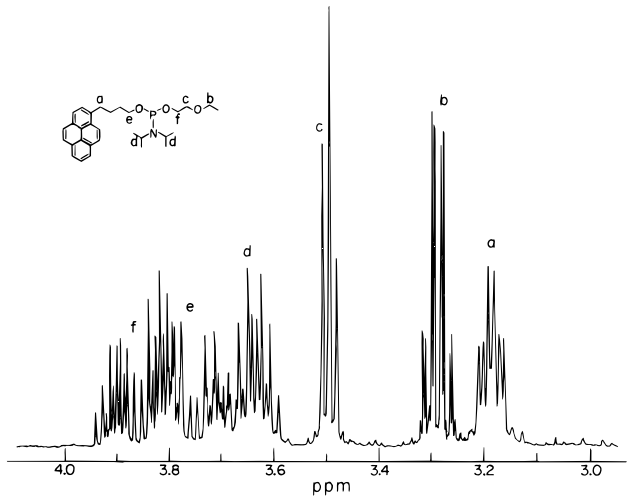


Figure 5. ^{1}H NMR spectrum of **EO_2−Py−NR_2** showing signals from the 3.0−4.1 ppm region.

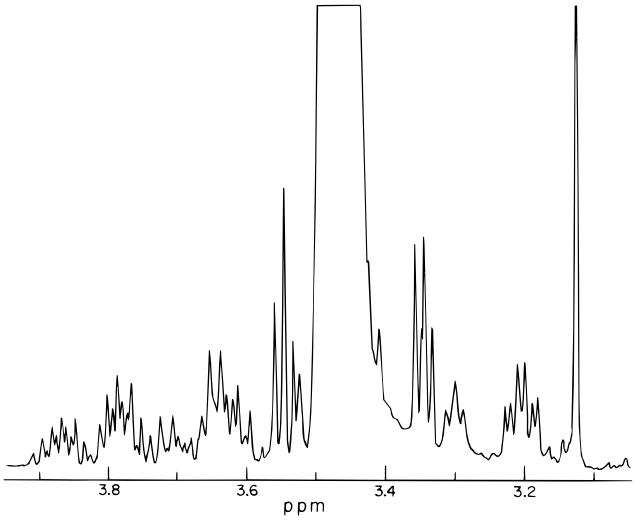


Figure 6. ^{1}H NMR spectrum of the phosphoramidite of monomethoxyPEG 2000 showing signals from the aliphatic protons.

Table 1. Number Average Degree of Polymerization, x, and Molecular Weight, M_n, of the MonomethoxyPEG Chain, $-(OCH_2CH_2)_xOCH_3$, attached to the Phosphoramidite Moiety of EO_x−Py−NR_2 As Determined by Proton NMR

monomethoxyPEG[a]	550	750	2000	5000
x	12.25 ± 1	17.25 ± 3	47.00 ± 4	128.25 ± 4
M_n	571 ± 44	791 ± 110	2102 ± 187	5681 ± 187

[a] Approximate molecular weights of the starting monomethoxyPEGs indicated by the supplier.

The bis(phosphates) synthesized in this work have been examined by using time-lag-focusing MALDI TOF mass spectrometry. The matrix used for the samples whose spectra are shown here was 2-[(4-hydroxyphenyl)-azo]benzoic acid (HABA).[23] All of the observed spectra are the result of the cationization of poly(ethylene glycol) with sodium. The weight and number average molecular weights for the polymers were calculated using eqs 2 and 3, respectively.[24] N_i represents the area under the oligomer peak, and M_i is the observed oligomer

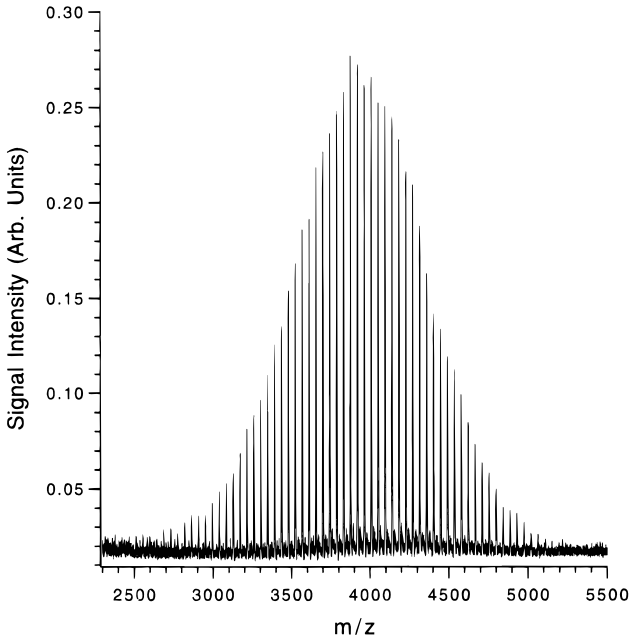


Figure 7. MALDI mass spectrum of the poly(ethylene glycol 3841) molecular weight standard, **HO−EO$_{87}$−OH**, used to construct the central chain.

molecular weight after correcting for the mass of the sodium ion.

$$M_w = \frac{\sum N_i M_i^2}{\sum N_i M_i} \quad (2)$$

$$M_n = \frac{\sum N_i M_i}{\sum N_i} \quad (3)$$

Figure 7 shows the MALDI mass spectrum of the poly(ethylene glycol 3841) molecular weight standard, **HO−EO$_{87}$−OH**, to which the phosphoramidites were coupled. The distribution of chains in the sample is clearly resolved. The peaks are equally spaced at 44 mass units apart, representing the individual polymers which differ by the molecular weight of the repeating oxyethylene unit. A series of smaller peaks are also observed at 16 mass units higher than each of the main peaks. This is due to the corresponding potassiated chains. It is important to note that there is no evidence of fragmentation within this region. This was confirmed by using *trans*-3-indoleacrylic acid and 2,5-dihydroxybenzoic acid as matrices, using potassium and lithium as cationizing agents, and varying the laser power. The relative intensity of these peaks in the spectra was found to be independent of the matrices and laser power used. M_w and M_n were calculated to be 3881 and 3841, respectively. This gives a dispersity, M_w/M_n, of 1.01 and a corresponding number average degree of polymerization of 87.

Table 2 compares the molecular weights determined for this poly(ethylene glycol 3841) with those listed by the supplier. The results agree well with each other. The only interesting observation here is that the dispersity determined by MALDI mass spectrometry is a bit narrower than those which were established using GPC. This is not unreasonable, since the solute band is expected to broaden as it migrates through the GPC columns.

Table 3 summarizes the molecular weights that were determined for the monomethoxypoly(ethylene glycol

Table 2. Molecular Weight Data for the Central Chain Poly(ethylene glycol 3841) Molecular Weight Standard from Polymer Laboratories Inc.

molecular weight[a]	M_w/M_n	technique
$M_v = 4083$		viscometry[b]
$M_n = 3927$		vapor pressure osmometry[b]
$M_w = 3907$	1.03	gel permeation chromatography[b]
$M_n = 3791$		
$M_p = 4084$		
$M_w = 3908$	1.05	gel permeation chromatography[c]
$M_n = 3721$		
$M_p = 4290$		
$M_w = 3881$	1.01_0	MALDI mass spectrometry[c]
$M_n = 3841$		

[a] M_v: viscosity average molecular weight. M_w: weight average molecular weight. M_n: number average molecular weight. M_p: peak average molecular weight (the value corresponding to the peak maximum on the chromatogram). [b] Results were provided by the supplier, Polymer Laboratories Inc. [c] Results were obtained from this work.

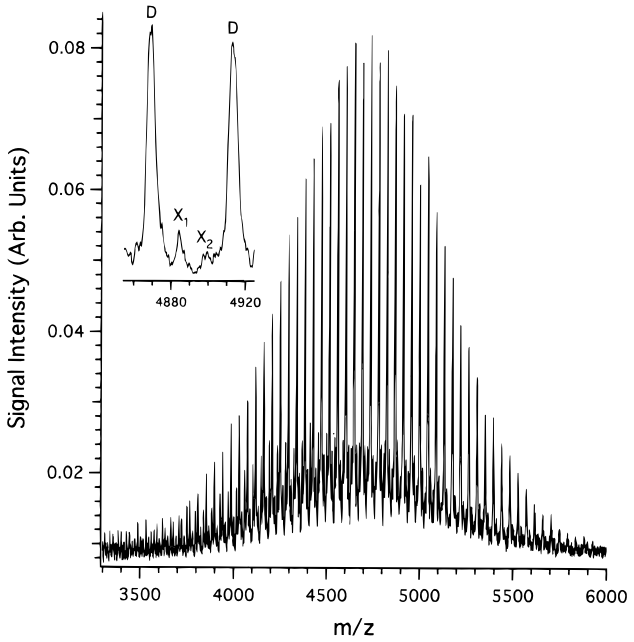


Figure 8. MALDI mass spectrum of **EO$_2$−Py−EO$_{87}$−Py−EO$_2$**. Results of data analysis: $M_w = 4714$, $M_n = 4692$. See text for details.

2108) and its corresponding phosphoramidite. The important result to be noted here is that the functionalization of the PEG chain, through the phosphitylation reaction, does not appear to change its length. The analysis of the other monomethoxyPEGs and their derivatives by MALDI mass spectrometry is currently in progress.

The MALDI mass spectra of the bis(phosphates) are shown in Figures 8−11. Three series of peaks are resolved by the instrument. The peaks in each expanded spectra represented by "D" are from the ions with masses corresponding to the bis(phosphate) (i.e., *M*). Again, no fragmentation of the molecular ions is observed. In all samples, the molecular weight of each peak, after correcting for the mass of the sodium ion, was identical to that of the individual oligomer having the exact structure of the expected bis(phosphate), within experimental error (less than 70 ppm).

The peak labeled "X1" is from the ion with its molecular weight of 14 mass units higher than that of the adjacent peak D (i.e., $M + 14$). The peak "X2" is from the ion with 28 mass units higher than that of peak D (i.e., $M + 28$). A close examination of these series of

Table 3. Molecular Weight Data and Number Average Degree of Polymerization, *x*, for the Starting Monomethoxypoly(ethylene glycol 2108) and Its Derivative, EO_{47}−Py−NR_2

polymer	molecular weight	M_w/M_n	"*x*"	technique
monomethoxyPEG	$M_w = 2138$	1.01_4		MALDI mass spectrometry
	$M_n = 2108$		47.12	
	$M_w = 2357$	1.06		GPC
	$M_u = 2231$		50.2	
	$M_p = 2464$			
EO_{47}−Py−NR_2	$M_n = 2506$[a]		47.0	^{1}H NMR (from Table 1)

[a] This molecular weight includes the mass of the phosphoramidite moiety ($C_{26}H_{31}NOP$), which increases the value of M_n given in Table 1 by 404.

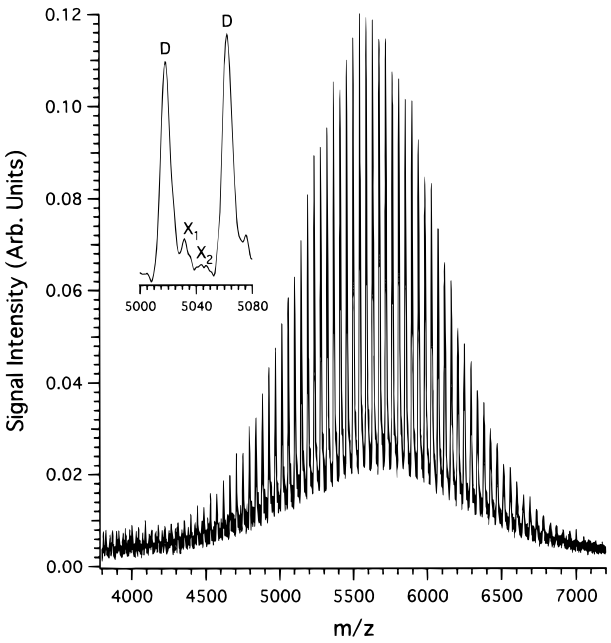


Figure 9. MALDI mass spectrum of **EO_{12}−Py−EO_{87}−EO_{12}**. Results of data analysis: $M_w = 5628$, $M_n = 5607$. See text for details.

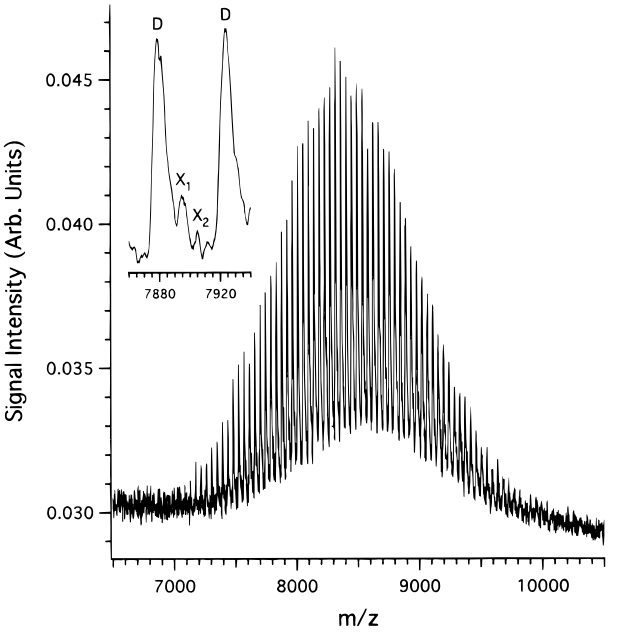


Figure 11. MALDI mass spectrum of **EO_{47}−Py−EO_{87}−Py−EO_{47}**. Results of data analysis: $M_w = 8427$, $M_n = 8409$. See text for details.

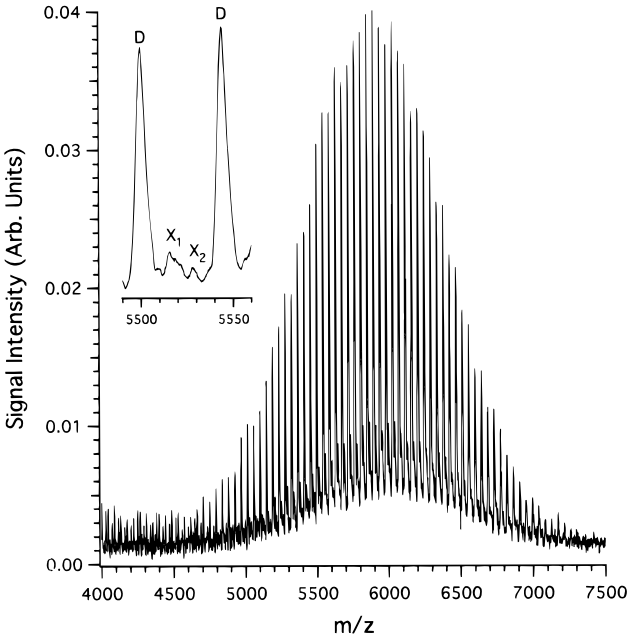


Figure 10. MALDI mass spectrum of **EO_{17}−Py−EO_{87}−Py−EO_{17}**. Results of data analysis: $M_w = 5920$, $M_n = 5897$. See text for details.

peaks reveals that the X1 peaks are actually from partial overlapping of two peaks arising from two different species. For a large number of the peaks in the series X1 we have examined, the major component of this peak is from $M + 14$ and the minor component is from $M + 16$. In some cases, the intensity of the $M + 14$ peak may be smaller than that of the $M + 16$ peak. The $M + 16$ peak is very likely from the potassium-attached adduct ion. Such peaks are often observed at the high sensitivity and resolution of our MALDI system with the sample/matrix preparation protocol used in this work. It should be noted that the X2 series of peaks appear to be mainly from a single component.

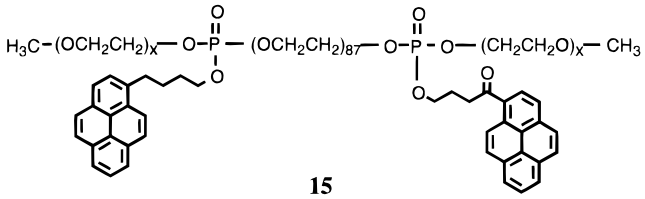
The $M + 14$ and $M + 28$ peaks are most likely to be products with structures that are similar to that formed from the oxidation of the alkyl pyrene moiety during workup, as discussed above. We speculate that these impurities are the bis(phosphates) **15** and **16**. These pyrenyl ketones have very short lifetimes (<1 ns) which renders them undetectable at the time scales used in our fluorescence measurements. At the molecular weights of these polymers, **15** and **16** cannot be separated from their corresponding bis(phosphate) **EO_x−Py−EO_y−Py−EO_x** using any known methods. Hence, the ability of the MALDI technique to detect these two species is significant, as it is informative about the purity of these products and the structure of the impurities.

The M_w and M_n values obtained from the MALDI spectra and from gel permeation chromatography are summarized in Table 4. The number average molecular weights calculated from the ^{1}H NMR spectra of these products are also listed. The M_n values were obtained by comparing the intensity of the peak at ca. 3.39−3.55 ppm—due to the boldfaced protons, $MeOCH_2C\mathbf{H}_2$−

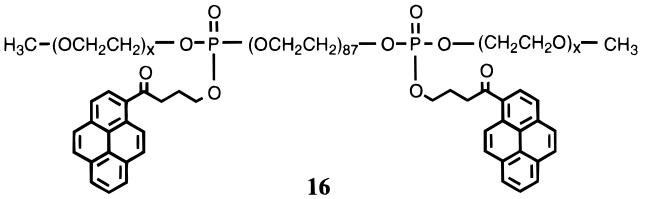
Table 4. Summary of the Molecular Weight Data for the Bis(phosphates), Obtained by MALDI Mass Spectrometry, Gel Permeation Chromatography, and ^{1}H NMR Spectroscopy, and Their Comparison with the Calculated M_n Values

polymer	M_w	M_n	M_w/M_n	technique
EO_2−Py−EO_{87}−Py−EO_2	4714 ± 11	4692 ± 12	1.00_5	MALDI mass spectrometry
	5017	4821	1.04_1	GPC
		4899 ± 452		^{1}H NMR
		4657 ± 12		calculated value[a]
EO_{12}−Py−EO_{87}−Py−EO_{12}	5628 ± 8	5607 ± 8	1.00_4	MALDI mass spectrometry
	5818	5465	1.06_5	GPC
		5818 ± 176		^{1}H NMR
		5621 ± 62		calculated value
EO_{17}−Py−EO_{87}−EO_{17}	5920 ± 6	5897 ± 7	1.00_4	MALDI mass spectrometry
	5156	4983	1.03_5	GPC
		5939 ± 474		^{1}H NMR
		6061 ± 156		calculated value
EO_{47}−Py−EO_{87}−Py−EO_{47}	8427 ± 13	8409 ± 11	1.00_2	MALDI mass spectrometry
	6677	6484	1.03_0	GPC
		8714 ± 705		^{1}H NMR
		8683 ± 264		calculated value

[a] M_n (bis(phosphate)) = $2M_n$(end chain) + M_n(central chain) + 640, where M_n(end chain) was obtained from Table 1 and M_n(central chain) = 3839. The residual mass of 640 corresponds to the two $P(O)O(CH_2)_4C_{16}H_9$ units. The uncertainty in the calculated M_n of the bis(phosphates) represents only those which arise from the end chains.



15

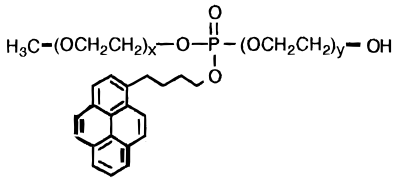


16

$(OCH_2CH_2)_{x-2}OCH_2CH_2OP$ and $POCH_2CH_2(OCH_2CH_2)_{y-2}OCH_2CH_2OP$—with that of the multiplet between 7.69 and 8.20 ppm, representing the 18 pyrenyl protons, for each polymer.

The results in Table 4 compare well with each other and with the expected values calculated from the molecular weights of the monomethoxyPEG end chains and the central chain, **HO−EO_{87}−OH**. Since the GPC data were obtained using poly(ethylene glycol) molecular weight standards, the molecular weights of the PEG derivatives interpolated from the calibration curve might differ somewhat from their true M_w and M_n values. This would occur if the resulting effects of the internal 4-(1-pyrenyl)butyl phosphate and terminal methoxy groups on the hydrodynamic volume of the polymer coils is different from that produced by the oxyethylene segments. Hence, the most useful molecular weight data are those provided by the MALDI mass spectra. A most remarkable result obtained from this technique is that the calculated molecular weight of each peak corresponds exactly to that of the oligo(ethylene glycol) chain containing the expected 4-(1-pyrenyl)butyl and methoxy groups, within experimental error (less than 70 ppm). This provides the most convincing evidence for the structures of the products shown in Schemes 2 and 3.

Fluorescence decay measurements of doubly labeled polymers frequently shows that a small fraction of the chains in the sample contain only one fluorescent group.[4b] A small fraction of chains which do not generate pyrene excimers have also been found in the fluorescence experiments with these bis(phosphates).[13] It would have been reasonable to attribute this observation to those chains where the **HO−EO_{87}−OH** has been phosphorylated with **EO_x−Py−NR_2** at one end only to give **EO_x−Py−EO_y−OH**. However, no molecular ions



EO_x-Py-EO_y-OH

with a mass corresponding to the structure of **EO_x−Py−EO_y−OH** have been observed in the MALDI mass spectra. These chains are more likely to have the structures **15** and **16** (the short-lived fuorescence from traces of 1-ketopyrene derivatives are not detected under the conditions of our measurements). Hence, these characterization results suggest that the coupling reaction was able to functionalize both ends of **HO−EO_{87}−OH** quantitatively with **EO_{12}−Py−NR_2**, **EO_{17}−Py−NR_2**, and **EO_{47}−Py−NR_2**.

Fluorescence measurements on these polymers in solution provide important information about their conformation and dynamic properties. Those results will be presented in a future publication.[13]

Experimental Section

General Procedures. All manipulations with moisture-sensitive compounds were performed using standard Schlenck (with argon) and vacuum line techniques. Operations requiring reduced pressure were performed at 0.01 mmHg or less. Precipitations of the bis(phosphates) were performed in 15 mL Corex No. 8441 centrifuge tubes. The precipitates were isolated by centrifuging at 10 000 rpm for 25 min on a Sorvall RC2 centrifuge at 20 °C.

Materials. Thin layer chromatography was performed on No. 5554 Merck Kieselgel 60 F_{254} analytical silica gel sheets cut into 4 cm × 8 cm strips. Preparative column chromatography was performed on 70−230 mesh silica gel (Aldrich, 60A). THF (Caledon) was refluxed and distilled from sodium benzophenone ketyl. Toluene (Caledon) was refluxed and distilled from sodium. Acetonitrile (Caledon), diisopropylamine (Baker), *N*,*N*-diisopropylethylamine (Aldrich), and 2-ethoxyethanol (Aldrich) were refluxed and distilled from calcium hydride.

Tetraethylene glycol monomethyl ether (Kodak, 98%) was stirred over calcium hydride for 8 h before it was distilled under reduced pressure. Phosphorus trichloride (BDH) and 2,6-lutidine (Aldrich) were freshly distilled before use. Diethyl ether (Caledon), used for precipitating poly(ethylene glycol) from solution, was refluxed and distilled from a 50 wt % sodium dispersion in paraffin. 1*H*-tetrazole (Dalton, 98%) was sublimed under reduced pressure at 60−70 °C. 4-(1-Pyrenyl)-butanol was obtained by lithium aluminum hydride reduction of 4-(1-pyrenyl)butanoic acid (Aldrich). For oxidative workup of the phosphites, a 0.1 M aqueous iodine solution was freshly prepared from iodine (BDH) and 40:20:1 THF−lutidine−water. Solvents for column chromatography were of reagent grade or better and were used as received. PEG 4100 was purchased from Polymer Laboratories. The monomethoxyPEGs were purchased from Aldrich. Before use, all PEG samples were dried by azeotropic distillation with toluene.

Characterization. ^{31}P nuclear magnetic resonance spectra were recorded on a Varian Associates Gemini 300 spectrometer at 121.470 MHz. ^{1}H and ^{13}C NMR were recorded on a Varian Associates VXR-400 spectrometer at 399.952 and 100.577 MHz, respectively. All spectra were obtained at room temperature (21−23 °C), and chemical shifts are quoted in ppm. ^{31}P NMR chemical shifts were referenced to 85% H_3PO_4 using the signal from the deuterated solvent to provide the internal lock. Positive values indicate shifts downfield from the reference. ^{1}H and ^{13}C NMR chemical shifts are quoted downfield from TMS and were referenced to the internal residual proton and d-^{13}C signal of the deuterated solvent, respectively. All deuterated solvents were purchased from Aldrich (except benzene-d_6, from MSD Isotopes) and were used as received. IR spectra were recorded on a Nicolet 5DX FT-IR. KBr pellets were prepared from solid samples, while liquid samples were examined neat. Electron impact and fast atom bombardment (FAB) mass spectra were recorded on a VG 70-250S mass spectrometer. UV−visible absorption spectra were recorded on a Hewlett-Packard 8452A diode array spectrometer.

MALDI Mass Spectra. The MALDI experiment was carried out in a time-lag-focusing linear time-of-flight mass spectrometer developed and constructed at the University of Alberta. The details of the instrument have been described elsewhere.[25] The matrix used for the MALDI mass spectra reported herein was 2-[(4-hydroxyphenyl)azo]benzoic acid (HABA).[23] Poly(ethylene glycol) polymers and modified polymers were dissolved in methanol to a concentration of 1−2 mg/mL. HABA was dissolved in 1,4-dioxane to a concentration of 0.05 M. The polymer solutions were then mixed with the HABA solution to a final concentration of 10^{-4} M. One microliter of 0.02 M sodium chloride or potassium chloride was typically added to 100 μL of the above solution. One microliter of the mixture was placed on the stainless steel sample probe and allowed to dry.

Analytical and Preparative Gel Permeation Chromatography. Samples were run on a Varian Associates 5000 liquid chromatograph pump connected to a Waters R401 differential refractive index detector and a Kratos 970 fluorescence detector. The excitation source was set at 345 nm, and a 370 nm cutoff filter was placed in front of the photodetector. For more concentrated samples, a 500 nm cutoff filter was used. The mobile phase was THF at a flow rate of 0.8 mL/min. For molecular weight determinations, three Waters μStyragel analytical columns (500, 10^3, and 10^4 Å) were employed. Poly(ethylene glycol) standards from Polymer Laboratories were used for the calibration. This system was also used to obtain purified samples of the bis(phosphates). The mobile phase was argon-purged THF, refluxed and distilled from lithium aluminum hydride. The columns used for each bis(phosphate) are listed in the synthesis of the specific polymer. One milligram samples of the polymer in 0.05 mL of THF were injected into the GPC system, and the first fraction from such repeated injections were collected and combined. The THF was removed under an argon flush to leave the purified polymer as a white solid.

4-(1-Pyrenyl)butyl Phosphorodichloridite (2). In a typical synthesis, phosphorus trichloride (9.0 mL, 69 mmol) and THF (100 mL) were added to a 500 mL round bottom flask equipped with a magnetic stir bar, gas inlet adapter, and rubber septum. The solution was cooled to −72 °C (ethanol−dry ice bath), and a solution of 4-(1-pyrenyl)butanol (2.0 g, 7.3 mmol) in THF (90 mL) was added to the flask via a cannula over 2 h. After the addition, the mixture was allowed to warm to room temperature over 5 h with continued stirring. The solvent and excess phosphorus trichloride were removed under reduced pressure to afford an amorphous, pale yellow solid which was used, in the same flask, without further purification. Crude yield: 2.7 g (100%). Mp (uncorrected): 53−54 °C. IR (KBr pellet): 740, 775, 800, 1020, 1390, 1470, 1510, 1600 cm^{-1}. ^{31}P NMR ($CDCl_3$): 178.56 (product), 10.4 (phosphonate) (ratio: >99:1). ^{1}H NMR ($CDCl_3$): 1.95 (m, 4H, $C_{16}H_9CH_2CH_2CH_{2-}$), 3.29 (t, 2H, $^3J_{HH'}$ = 7.41 and 7.69 Hz, $C_{16}H_9CH_{2-}$), 4.27 (hextet, 2H, $^3J_{PH}$ = 8.22 Hz, $^3J_{HH'}$ = 6.15 and 6.26 Hz, $-CH_2OPCl_2$), 7.77−8.25 (9H, m, pyrenyl *H*). ^{13}C NMR ($CDCl_3$): 27.95 ($PyCH_2CH_{2-}$); 29.90 (d, $-CH_2CH_2OPCl_2$, $^3J_{PC}$ = 3.1 Hz); 33.09 ($PyCH_{2-}$); 68.54 (d, $-CH_2OPCl_2$, $^2J_{PC}$ = 9.9 Hz); 123.45, 125.02, 125.07, 125.21, 125.27, 125.37, 126.11, 126.96, 127.45, 127.62, 127.75, 128.87, 130.18, 131.13, 131.69, 136.23 (pyrenyl *C*). Electron impact mass spectrum, *m/e* (relative intensity): 376 (15) $[M + 2]^{\bullet+}$, 374 (21.8) $M^{\bullet+}$, 258 (11), 257 (10), 228 (11), 227 (10), 216 (27), 215 (100).

4-(1-Pyrenyl)butyl *N*,*N*-Diisopropylphosphoramidochloridite (3). THF (90 mL) was added to the previously synthesized crude phosphorodichloridite **2**, and the solution was cooled to −72 °C. Diisopropylamine (2.2 equiv), determined by assuming a quantitative yield from the previous reaction, was added dropwise to this solution via syringe over 5 min with stirring. A white slurry formed immediately. The resulting mixture was allowed to reach room temperature over 5 h with continued stirring. Then, the amine salt was removed by filtration through a medium porosity glass frit. The filtrate was passed into a dry 500 mL two-neck round bottom flask equipped with a gas inlet adapter and a magnetic stir bar. The solvent was removed from the filtrate on a vacuum line to give a thick, orange-red oil. This crude material was left in the flask and used without further purification. Estimated yield (based on ^{31}P NMR): 89%. ^{31}P NMR (benzene-d_6): 182.83 (product), 13.84 (4-(1-pyrenyl)butyl *N*,*N*-diisopropylphosphoramidate) (ratio: 8.4:1). Electron impact mass spectrum, *m/e* (relative intensity): 441 (23) $[M + 2]^{\bullet+}$, 440 (16) $[M + 1]^{\bullet+}$, 439 (46) $M^{\bullet+}$, 421 (17), 360 (32), 304 (23), 258 (32), 257 (75), 256 (43), 229 (32), 228 (77), 227 (28), 216 (29), 215 (100), 86 (47).

4-(1-Pyrenyl)butyl 2-Ethoxyethyl *N*,*N*-Diisopropylphosphoramidite (EO_2−Py−NR_2). THF (90 mL) and diisopropylethylamine (15 mL, 86 mmol, ≈10-fold equiv excess) were added to the previously synthesized crude phosphoramidochloridite **3**. The solution was cooled to 0 °C, and an excess of 2-ethoxyethanol (0.9 mL, 9.3 mmol, 1.3 equiv based on starting pyrenebutanol) was added dropwise to the stirred solution over 1 min with a syringe. The solution was stirred for an additional 0.5 h at room temperature. After this time, the mixture was concentrated to half of its original volume before it was diluted with dichloromethane (50 mL) and washed twice with saturated brine. The organic layer was dried over sodium sulfate, and the solution was concentrated to an orange oil by rotoevaporation. The crude product was flash chromatographed on silica gel using 90:10 pentane−triethylamine eluent to give a clear, colorless oil. This was stored as a frozen 30 wt % solution in benzene at −20 °C. In this form, no appreciable decomposition was detected over 2 years. Just before use, it was thawed at room temperature, and the appropriate quantity, withdrawn from the storage bottle, was concentrated under reduced pressure. Yield (based on pyrenebutanol over three steps): 3.1 g (86%). TLC (silica gel, 90:10 pentane−triethylamine): R_f = 0.62. IR (neat): 845, 975, 1030, 1070, 1125, 1185, 1360, 1400, 1460 cm^{-1}. ^{31}P NMR (benzene-d_6): 147.83 ppm. ^{1}H NMR (benzene-d_6): 1.069 (t, 3H, $^3J_{HH'}$ = 6.958 Hz, CH_2CH_3), 1.156 (d, 6H, $^3J_{HH'}$ = 6.958 Hz, diastereotopic CH_3), 1.164 (d, 6H, $^3J_{HH'}$ = 6.958 Hz, diastereotopic CH_3), 1.687 (m, 2H, $PyCH_2CH_2CH_2$), 1.895 (m, 2H, $PyCH_2CH_2$), 3.128 (t, 1H, $^3J_{HH'}$ = 7.32 and 8.06 Hz, diastereotopic PyCH(*H*)), 3.138 (t, 1H, $^3J_{HH'}$ = 7.69 and 8.06 Hz, diastereotopic PyCH(*H*)), 3.283 (quartet, 1H, $^3J_{HH'}$ = 6.96

Hz, diastereotopic CH(*H*)CH_3), 3.287 (quartet, 1H, $^3J_{HH'} = 6.96$ & 7.32 Hz, diastereotopic CH(*H*)CH_3), 3.490 (t, 2H, $^3J_{HH'} =$ 5.13 and 5.86 Hz, $POCH_2CH_2O$), 3.603 (m, 2H, NC*H*), 3.731 (m, 2H, $Py(CH_2)_3CH_2$), 3.85 (m, 2H, $POCH_2CH_2O$), 7.63−8.15 (m, 9H, pyrenyl *H*). ^{13}C NMR (benzene-d_6): 15.55 (CH_2CH_3), 24.73 (d, $^3J_{PC} = 7.33$ Hz, diastereotopic (CH_3)), 24.84 (d, $^3J_{PC} = 8.05$ Hz, diastereotopic (CH_3)), 28.60 ($PyCH_2CH_2$), 31.72 (d, $^3J_{PC} = 7.32$ Hz, $Py(CH_2)_2CH_2$), 33.41 ($PyCH_2$), 43.25 (d, $^2J_{PC} = 12.45$ Hz, PNCH), 63.15 (d, $^2J_{PC} = 16.85$ Hz, $Py(CH_2)_3CH_2$), 63.53 (d, $^2J_{PC} = 16.11$ Hz, $POCH_2CH_2O$), 66.56 (CH_2CH_3), 71.08 (d, $^3J_{PC} = 7.32$ Hz $POCH_2CH_2O$), 123.83, 124.90, 125.07, 125.12, 125.64, 125.68, 125.93, 126.79, 127.40, 127.42, 127.85, 129.10, 130.24, 131.43, 131.91, 137.12 (pyrenyl *C*). Electron impact mass spectrum, *m/e* (relative intensity): 493 (48) $M^{\bullet+}$, 421 (45), 320 (21), 257 (77), 228 (77), 215 (100). High-resolution mass spectrum: calcd for $C_{30}H_{40}NO_3P$, 493.2746; found, 493.2746. Anal. Calcd for $C_{30}H_{40}NO_3P$: C, 73.00; H, 8.17; N, 2.84; P, 6.27. Found: C, 73.10; H, 8.02; N, 3.01; P, 6.30.

Representative Procedure for the Synthesis of the Phosphoramidites of PEG: 4-(1-Pyrenyl)butyl Monomethoxypoly(ethylene glycol 2000) *N,N*-Diisopropylphosphoramidite (EO_{47}−Py−NR_2). Phosphoramidochloridite **3** was prepared from pyrenebutanol (2.0 g, 7.3 mmol) and isolated in a 500 mL two-neck round bottom flask in the manner described previously. THF (30 mL) and diisopropylethylamine (15 mL, 170 mmol, ≈20-fold equiv excess) were added to the oil by syringe, and the mixture was stirred at room temperature. A solution of poly(ethylene glycol 2000) monomethyl ether (14.6 g) in THF (30 mL) was then added to the flask via a cannula over 4 min. The solution in the flask turned cloudy within 1 h of the addition. After stirring for 2 h, the amine salt was filtered off, and the filtrate was concentrated under reduced pressure. The resulting yellow paste was redissolved in a 50 mL solution of 45:45:10 dichloromethane−ethyl acetate−triethylamine and chromatographed on silica gel, using this solvent mixture as the eluent. Fractions containing the product were combined and concentrated under reduced pressure to a volume of 100 mL. This clear solution was added dropwise to rapidly stirred diethyl ether (750 mL), resulting in the precipitation of a white solid which was filtered out and dried. Stored at −20 °C, the product remains stable for over 1 year. Yield (based on pyrenebutanol over three steps): 13.6 g (77%). TLC (silica gel; 45:45:10 dichloromethane−ethyl acetate−triethylamine): $R_f = 0.42$. ^{31}P NMR (benzene-d_6): 148.18 ppm. ^{1}H NMR (benzene-d_6): 1.173 (d, 12H, $^3J_{HH'} = 6.66$ Hz, $CH(CH_3)_2$), 1.71 (m, 2H, $PyCH_2CH_2CH_2$), 1.94 (m, 2H, $PyCH_2CH_2$), 3.124 (s, 3H, OCH_3), 3.197 (t, 1H, $^3J_{HH'} = 7.32$ and 8.00 Hz, diastereotopic PyCH(*H*)), 3.207 (t, 1H, $^3J_{HH'} = 7.55$ and 7.99 Hz, diastereotopic PyCH(*H*)), 3.299 (t, 2H, $^3J_{HH'} = 5.0$ and 5.6 Hz, CH_2OCH_3), 3.40−3.52 (m, 182 ± 17 H, $POCH_2CH_2(OCH_2CH_2)_{45\pm4}OCH_2CH_2OCH_3$), 3.543 (t, 2H, $^3J_{HH'} = 4.7$ and 5.6 Hz, $POCH_2CH_2O$), 3.64 (m, 2H, NC*H*), 3.78 (m, 2H, $Py(CH_2)_3CH_2$), 3.87 (m, 2H, $POCH_2CH_2O$), 7.71−8.23 (m, 9H, pyrenyl *H*). ^{13}C NMR (benzene-d_6): 24.78 (d, $^3J_{PC} = 7.32$ Hz, diastereotopic CH_3), 24.85 (d, $^3J_{PC} = 6.60$ Hz, diastereotopic CH_3), 28.67 ($PyCH_2CH_2$), 31.72 (d, $^3J_{PC} = 6.59$ Hz, $PyCH_2CH_2CH_2$), 33.45 ($PyCH_2$), 43.28 (d, $^2J_{PC} = 11.72$ Hz, PNCH), 58.69 (CH_3), 63.10 (d, $^2J_{PC} = 16.84$ Hz, $POCH_2CH_2O$), 63.57 (d, $^2J_{PC} = 16.85$ Hz, $Py(CH_2)_3CH_2$), 71.03 ($(CH_2CH_2O)_{45\pm5}CH_2CH_2OCH_3$), 71.76 (d, $^3J_{PC} = 7.32$ Hz, $POCH_2CH_2O$), 72.38 (CH_2OCH_3), 123.93, 124.99, 125.13, 125.20, 125.71, 125.77, 126.02, 126.87, 127.50, 127.56, 128.31, 129.19, 130.32, 131.50, 131.98, 137.24 (pyrenyl *C*).

4-(1-Pyrenyl)butyl Monomethoxypoly(ethylene glycol 750) *N,N*-Diisopropylphosphoramidite (EO_{17}−Py−NR_2). This phosphoramidite was prepared in the same manner as EO_{47}−Py−NR_2 except that the product was obtained by precipitation into diethyl ether at −10 °C. The phosphoramidite is a clear, colorless oil at room temperature. Stored as a glassy polymer at −20 °C, it is stable for over 4 months. Yield (based on pyrenebutanol over three steps): 80%. TLC (silica gel; 45:45:10 dichloromethane−ethyl acetate−triethylamine): $R_f = 0.62$. ^{31}P NMR (benzene-d_6): 147.39 ppm. ^{1}H NMR (benzene-d_6): 1.174 (d, 12H, $^3J_{HH'} = 6.6$ Hz, $CH(CH_3)_2$), 1.71 (m, 2H, $PyCH_2CH_2CH_2$), 1.93 (m, 2H, $PyCH_2CH_2$), 3.121 (s, 3H, OCH_3), 3.194 (t, 1H, $^3J_{HH'} = 7.3$ and 8.1 Hz, diastereotopic PyCH(*H*)), 3.204 (t, 1H, $^3J_{HH'} = 7.7$ and 8.4 Hz, diastereotopic PyCH(*H*)), 3.339 (t, 2H, $^3J_{HH'} = 4.4$ and 5.1 Hz, CH_2OCH_3), 3.42−3.50 (m, 63 ± 10H, $POCH_2CH_2(OCH_2CH_2)_{15\pm3}OCH_2CH_2OCH_3$), 3.544 (t, 2H, $^3J_{HH'} = 5.5$ Hz, $POCH_2CH_2O$), 3.62 (m, 2H, NC*H*), 3.78 (m, 2H, $Py(CH_2)_3CH_2$), 3.87 (m, 2H, $POCH_2CH_2O$), 7.70−8.23 (m, 9H, pyrenyl *H*). ^{13}C NMR (benzene-d_6): 24.80 (d, $^3J_{PC} = 6.6$ Hz, diastereotopic CH_3), 24.87 (d, $^3J_{PC} = 7.3$ Hz, diastereotopic CH_3), 28.68 ($PyCH_2CH_2$), 31.73 (d, $^3J_{PC} = 7.3$ Hz, $PyCH_2CH_2CH_2$), 33.46 ($PyCH_2$), 43.26 (d, $^2J_{PC} = 12.4$ Hz, PNCH), 58.70 (CH_3), 63.09 (d, $^2J_{PC} = 16.8$ Hz, $POCH_2CH_2O$), 63.58 (d, $^2J_{PC} = 16.1$ Hz, $Py(CH_2)_3CH_2$), 71.02 ($POCH_2CH_2O(CH_2CH_2O)_{15\pm3}CH_2CH_2OCH_3$), 71.75 (d, $^3J_{PC} = 7.3$ Hz, $POCH_2CH_2O$), 72.37 (CH_2OCH_3), 123.94, 124.99, 125.13, 125.20, 125.70, 125.75, 126.02, 126.86, 127.50, 127.56, 128.30, 129.18, 130.30, 131.50, 131.97, 137.24 (pyrenyl *C*).

4-(1-Pyrenyl)butyl Monomethoxypoly(ethylene glycol (550) *N,N*-Diisopropylphosphoramidite (EO_{12}−Py−NR_2). This product was purified and stored in the same manner as phosphoramidite EO_{17}−Py−NR_2. Yield (based on pyrenebutanol over three steps): 78%. R_f (silica gel; 45:45:10 dichloromethane−ethyl acetate−triethylamine): 0.64. ^{31}P NMR (benzene-d_6): 148.16 ppm. ^{1}H NMR (benzene-d_6): 1.175 (d, 12H, $^3J_{HH'} = 6.59$ Hz, $CH(CH_3)_2$), 1.71 (m, 2H, $PyCH_2CH_2CH_2$), 1.93 (m, 2H, $PyCH_2CH_2$), 3.119 (s, 3H, OCH_3), 3.194 (t, 1H, $^3J_{HH'} = 7.33$ and 8.05 Hz, diastereotopic PyCH(*H*)), 3.204 (t, 1H, $^3J_{HH'} = 7.32$ and 8.06 Hz, diastereotopic PyCH(*H*)), 3.337 (t, 2H, $^3J_{HH'} = 3.7$ and 5.1 Hz, CH_2OCH_3), 3.41−3.52 (m, 43 ± 4H, $POCH_2CH_2(OCH_2CH_2)_{10\pm1}OCH_2CH_2OCH_3$), 3.547 (t, 2H, $^3J_{HH'} = 5.49$ Hz, $POCH_2CH_2O$), 3.62 (m, 2H, NC*H*), 3.78 (m, 2H, $Py(CH_2)_3CH_2$), 3.87 (m, 2H, $POCH_2CH_2O$), 7.70−8.23 (m, 9H, pyrenyl *H*). ^{13}C NMR (benzene-d_6): 24.79 (d, $^3J_{PC} = 7.32$ Hz, diastereotopic CH_3), 24.85 (d, $^3J_{PC} = 6.60$ Hz, diastereotopic CH_3), 28.67 ($PyCH_2CH_2$), 31.72 (d, $^3J_{PC} = 7.32$ Hz, $PyCH_2CH_2CH_2$), 33.46 ($PyCH_2$), 43.28 (d, $^2J_{PC} = 12.45$ Hz, PNCH), 58.69 (CH_3), 63.10 (d, $^2J_{PC} = 16.84$ Hz, $POCH_2CH_2O$), 63.57 (d, $^2J_{PC} = 16.84$ Hz, $Py(CH_2)_3CH_2$), 71.03 ($POCH_2CH_2O(CH_2CH_2O)_{10\pm1}CH_2CH_2OCH_3$), 71.76 (d, $^3J_{PC} = 7.32$ Hz, $POCH_2CH_2O$), 72.38 (CH_2OCH_3), 123.94, 124.98, 125.13, 125.20, 125.71, 125.77, 126.01, 126.86, 127.50, 127.56, 127.91, 129.19, 130.32, 131.50, 131.98, 137.12 (pyrenyl *C*).

4-(1-Pyrenyl)butyl Monomethoxypoly(ethylene glycol 5000) *N,N*-Diisopropylphosphoramidite (EO_{128}−Py−NR_2). This phosphoramidite was prepared in the same manner as EO_{47}−Py−NR_2 except that the reaction time was increased to 14 h. Since the amine salt could not be removed from the polymer solution efficiently, the entire mixture was concentrated to a yellow solid. This material was chromatographed on silica gel using 45:45:10 THF−ethyl acetate−triethylamine ($R_f = 0.0$−0.2), to remove weakly polar impurities, followed by 90:10 THF−triethylamine to obtain the product. The product fractions were combined and concentrated under reduced pressure to a volume of 250 mL. This solution was added dropwise to rapidly stirred pentane (900 mL), resulting in the precipitation of a white solid which was filtered and dried. Yield (based on recovered polymer): 67%. R_f (silica gel; 90:10 THF−triethylamine): 0.59. ^{31}P NMR (benzene-d_6): 147.37 ppm. ^{1}H NMR (benzene-d_6): 1.161 (d, 12H, $^3J_{HH'} =$ 6.9 Hz, $CH(CH_3)_2$), 1.70 (m, 2H, $PyCH_2CH_2CH_2$), 1.92 (m, 2H, $PyCH_2CH_2$), 3.119 (s, 3H, OCH_3), 3.189 (t, 1H, $^3J_{HH'} = 7.7$ and 7.9 Hz, diastereotopic PyCH(*H*)), 3.194 (t, 1H, $^3J_{HH'} = 7.5$ and 8.0 Hz, diastereotopic PyCH(*H*)), 3.293 (t, 2H, $^3J_{HH'} = 4.4$ and 5.5 Hz, CH_2OCH_3), 3.41−3.49 (m, 507 ± 7 H, $POCH_2CH_2(OCH_2CH_2)_{126\pm2}OCH_2CH_2OCH_3$), 3.518 (t, 2H, $^3J_{HH'} = 5.1$ and 6.2 Hz, $POCH_2CH_2O$), 3.64 (m, 2H, NC*H*), 3.77 (m, 2H, $Py(CH_2)_3CH_2$), 3.85 (m, 2H, $POCH_2CH_2O$), 7.70−8.23 (m, 9H, pyrenyl *H*). ^{13}C NMR (benzene-d_6): 24.72 (d, $^3J_{PC} = 7.3$ Hz, diastereotopic CH_3), 24.79 (d, $^3J_{PC} = 7.3$ Hz, diastereotopic CH_3), 28.62 ($PyCH_2CH_2$), 31.64 (d, $^3J_{PC} = 7.3$ Hz, $PyCH_2CH_2CH_2$), 33.39 ($PyCH_2$), 43.17 (d, $^2J_{PC} = 12.4$ Hz, PNCH), 58.66 (CH_3), 63.03 (d, $^2J_{PC} = 16.8$ Hz, $POCH_2CH_2O$), 63.50 (d, $^2J_{PC} = 16.8$ Hz, $Py(CH_2)_3CH_2$), 70.98 ($(CH_2CH_2O)_{126\pm2}CH_2CH_2OCH_3$), 71.70 (d, $^3J_{PC} = 7.4$ Hz, $POCH_2CH_2O$), 72.33 (CH_2OCH_3), 123.92, 124.99, 125.14, 125.20, 125.71, 125.77, 126.03, 126.86, 127.49, 127.56, 127.91, 129.14, 130.28, 131.47, 131.94, 137.22 (pyrenyl *C*).

Representative Procedure for the Coupling Reaction: 4-(1-Pyrenyl)butyl Methoxytetraethylene Glycol 2-Ethoxyethyl Phosphate (Py−Phosphate). Phosphoramidite **EO_2−Py−NR_2** (0.50 g, 1.0 mmol), tetraethylene glycol monomethyl ether (0.83 g, 4.0 mmol), and THF (10 mL) were placed in a 100 mL two-neck round bottom flask equipped with a magnetic stir bar and gas inlet adapter. Since the phosphoramidite was not dry initially, activated (250 °C under 0.01 mmHg for 8 h) 4 Å molecular sieves (1.5 g) were added to the mixture. After stirring for 30 min, a solution of tetrazole (0.32 g, 4.6 mmol) in THF (30 mL) was added dropwise to the mixture over 5 min with a cannula. A white precipitate formed instantaneously. The suspension was stirred for another 10 min after the addition. Then, a 0.1 M aqueous iodine solution was added dropwise to the mixture, until the red-brown color of the iodine persisted, to oxidize the phosphite. After stirring for 5 min, the excess iodine was reduced by adding 2 drops of triethyl phosphite, returning the suspension to its original faint yellow color. The mixture was filtered, concentrated, and chromatographed on silica gel using diethyl ether as the eluent. This was changed to 50:40:10 dichloromethane−diethyl ether−methanol after the lutidine was isolated. The product fractions were concentrated and rechromatographed on silica gel using 90:10 diethyl ether−methanol eluent to afford a clear, colorless oil. Yield: 0.61 g (98%). TLC: (silica gel, diethyl ether) R_f = 0; (silica gel, 50:40:10 dichloromethane−diethyl ether−methanol) R_f = 0.33; (silica gel, 90:10 diethyl ether−methanol) R_f = 0.26. IR (neat): 780, 800, 1040, 1130, 1275, 1390, 1455, 1510, 1595 cm^{-1}. ^{31}P NMR (benzene-d_6): 0.26 ppm. ^1H NMR ($CDCl_3$): 1.129 (t, 3H, $^3J_{HH'}$ = 6.9 and 7.0 Hz, CH_2CH_3), 1.842 (m, 2H, $PyCH_2CH_2CH_2$), 1.948 (m, 2H, $PyCH_2CH_2$), 3.328 (s, 3H, CH_3), 3.359 (t, 2H, $^3J_{HH'}$ = 7.4 and 8.0 Hz, $PyCH_2$), 3.446 (quartet, 2H, $^3J_{HH'}$ = 6.9 and 7.0 Hz, CH_2CH_3), 3.481 (m, 2H, CH_2OCH_3), 3.558 (m, 12H, $CH_2(OCH_2CH_2)_2OCH_2CH_2OCH_3$), 3.631 (m, 2H, CH_2OEt), 4.12 (m, 6H, $Py(CH_2)_3CH_2$, $POCH_2$-CH_2OEt, and $POCH_2CH_2(OCH_2CH_2)_3OCH_3$), 7.82−8.25 (m, 9H, pyrenyl *H*). ^{13}C NMR (benzene-d_6): 15.11 (CH_2CH_3), 27.65 ($PyCH_2CH_2$), 30.20 (d, $^3J_{PC}$ = 7.3 Hz, $PyCH_2CH_2CH_2$), 32.93 ($PyCH_2$), 58.99 (OCH_3), 66.56 (CH_2CH_3), 66.57 (d, $^2J_{PC}$ = 5.9 Hz, $Py(CH_2)_3CH_2$), 66.71 (d, $^2J_{PC}$ = 5.9 Hz, $POCH_2CH_2OEt$), 67.62 (d, $^2J_{PC}$ = 5.9 Hz, $POCH_2CH_2(OCH_2CH_2)_3OCH_3$), 69.18 (d, $^3J_{PC}$ = 6.6 Hz, $POCH_2CH_2(OCH_2CH_2)_2OCH_3$), 69.97 (d, $^3J_{PC}$ = 7.3 Hz, $POCH_2CH_2OEt$), 70.47 ($CH_2CH_2OCH_3$), 70.54 ($POCH_2CH_2(OCH_2CH_2)_2$), 71.89 (CH_2OCH_3), 123.25, 124.67, 124.75, 124.84, 124.96, 125.04, 125.77, 126.58, 127.21, 127.25, 127.44, 128.56, 129.82, 130.84, 131.37, 136.19 (pyrenyl *C*). FAB mass spectrum (*m*-nitrobenzyl alcohol matrix), *m/e* (relative intensity): 618 (8) $[M + 2]^{\bullet+}$, 617 (25) $[M + 1]^{\bullet+}$, 616 (26) $M^{\bullet+}$, 258 (26), 257 (100), 229 (19), 228 (58), 216 (16), 215 (64). High-resolution mass spectrum: calcd for $C_{33}H_{45}O_9P$ ($M^{\bullet+}$) 616.2801, found 616.2835; calcd for $C_{33}H_{46}O_9P$ ($MH^{\bullet+}$) 617.2879, 617.2914.

α,ω-Poly(ethylene glycol 4100) Bis[4-(1-pyrenyl)butyl 2-ethoxyethyl phosphate] (EO_2−Py−EO_{87}−Py−EO_2). This was prepared by adding a solution of tetrazole (0.64 g, 9.1 mmol) in THF (30 mL) to a mixture of PEG 3841 (0.30 g), phosphoramidite **EO_2−Py−NR_2** (1.0 g), and 4 Å molecular sieves (2 g) in THF (10 mL) over 1 h in the manner described previously. A precipitate formed through the course of the addition. After 8 h of stirring, the mixture was treated with aqueous iodine followed by triethyl phosphite, and then it was filtered and concentrated to approximately 15 mL. Low molecular weight impurities were removed from this mixture by repeated precipitations of the polymer in THF using diethyl ether. Purified samples of the product were obtained by GPC with Waters 500 and 10^3 Å μStyragel analytical columns. TLC: (silica gel, THF) R_f = 0.0−0.09; (silica gel, methanol) R_f = 0.70. ^{31}P NMR (benzene-d_6): 0.24. ^1H NMR (benzene-d_6): 1.019 (t, 6H, $^3J_{HH'}$ = 7.0 and 7.3 Hz, CH_3), 1.64 (m, 4H, $PyCH_2$-CH_2CH_2), 1.81 (m, 4H, $PyCH_2CH_2$), 3.128 (t, 4H, $^3J_{HH'}$ = 7.6 and 7.7 Hz, $PyCH_2$), 3.198 (quartet, 4H, $^3J_{HH'}$ = 6.9 and 7.0 Hz, CH_2CH_3), 3.333 (t, 4H, $^3J_{HH'}$ = 5.1 and 5.5 Hz, CH_2OEt), 3.38−3.54 (m, 365 ± 41 H, $POCH_2CH_2(OCH_2CH_2)_{87\pm10}OCH_2$-$CH_2OP$), 4.12 (m, 12H, $Py(CH_2)_3CH_2$, $POCH_2CH_2OEt$, and $POCH_2CH_2O(CH_2CH_2O)_{87\pm10}CH_2CH_2OP$), 7.67−8.19 (m, 18H, pyrenyl *H*).

α,ω-Poly(ethylene glycol 4100) Bis[methoxy poly(ethylene glycol 2000) 4-(1-pyrenyl)butyl phosphate] (EO_{47}−Py−EO_{87}−Py−EO_{47}). This was synthesized in the manner described for the bis(phosphate) **EO_2−Py−EO_{87}−Py−EO_2** by adding a solution of tetrazole (1.0 g, 14 mmol) in acetonitrile (30 mL) to a mixture of poly(ethylene glycol 3841) (0.31 g), phosphoramidite **EO_{47}−Py−NR_2** (3.8 g), 4 Å molecular sieves (2.5 g), and THF (60 mL). A precipitate started to appear 2 h after the addition of tetrazole was complete. Samples of the purified polymer were obtained by GPC using Waters 500, 500, and 10^3 Å μStyragel analytical columns. ^{31}P NMR (benzene-d_6): 0.23. ^1H NMR (benzene-d_6): 1.67 (m, 4H, $PyCH_2CH_2CH_2$), 1.84 (m, 4H, $PyCH_2CH_2$), 3.134 (s, 6H, CH_3), 3.157 (t, 4H, $^3J_{HH'}$ = 7.3 and 8.1 Hz, $PyCH_2$), 3.31 (t, 2H, $^3J_{HH'}$ = 4.2 and 5.2 Hz, CH_2OCH_3), 3.40−3.56 (m, 714 ± 64 H, $MeOCH_2CH_2(OCH_2CH_2)_{x-2}OCH_2CH_2OP$ and $POCH_2CH_2$-$(OCH_2CH_2)_{y-2}OCH_2CH_2OP$, $2x + y = 176 \pm 16$), 4.13 (m, 12H, $Py(CH_2)_3CH_2$, $POCH_2CH_2O(CH_2CH_2O)_{x-1}Me$, and $POCH_2$-$CH_2O(CH_2CH_2O)_{y-2}CH_2CH_2OP$), 7.70−8.23 (m, 18H, pyrenyl *H*).

α,ω-Poly(ethylene glycol 4100) Bis[methoxy poly(ethylene glycol 740) 4-(1-pyrenyl)butyl phosphate] (EO_{17}−Py−EO_{87}−Py−EO_{17}). This was synthesized in the manner described for the bis(phosphate) **EO_2−Py−EO_{87}−EO_2** by adding a solution of tetrazole (1.0 g, 14 mmol) in acetonitrile (30 mL) to a mixture of poly(ethylene glycol 3841) (0.31 g), phosphoramidite **EO_{17}−Py−NR_2** (2.1 g), 4 Å molecular sieves (2.5 g), and THF (60 mL). A precipitate appeared 1 h after the addition of tetrazole was complete. Samples of the purified polymer were obtained by GPC using Waters 500, 500, and 10^3 Å μStyragel analytical columns. ^{31}P NMR (benzene-d_6): 0.07. ^1H NMR (benzene-d_6): 1.66 (m, 4H, $PyCH_2CH_2CH_2$), 1.83 (m, 4H, $PyCH_2CH_2$), 3.129 (s, 6H, CH_3), 3.155 (t, 4H, $^3J_{HH'}$ = 7.7 and 8.1 Hz, $PyCH_2$), 3.31 (t, 2H, $^3J_{HH'}$ = 4.8 and 5.5 Hz, CH_2OCH_3), 3.40−3.56 (m, 462 ± 43 H, $MeOCH_2CH_2$-$(OCH_2CH_2)_{x-2}OCH_2CH_2OP$ and $POCH_2CH_2(OCH_2CH_2)_{y-2}$-$OCH_2CH_2OP$, $2x + y = 113 \pm 11$), 4.12 (m, 12H, $Py(CH_2)_3CH_2$, $POCH_2CH_2O(CH_2CH_2O)_{x-1}Me$, and $POCH_2CH_2O(CH_2CH_2O)_{y-2}$-$CH_2CH_2OP$), 7.70−8.23 (m, 18H, pyrenyl *H*).

α,ω-Poly(ethylene glycol 4100) Bis[methoxy poly(ethylene glycol 550) 4-(1-pyrenyl)butyl phosphate] (EO_{12}−Py−EO_{87}−Py−EO_{12}). This was synthesized in the manner described for the bis(phosphate) **EO_2−Py−EO_{87}−Py−EO_2** by adding a solution of tetrazole (0.68 g, 9.7 mmol) in THF (25 mL) to a mixture of poly(ethylene glycol 3841) (0.44 g), phosphoramidite **EO_{12}−Py−NR_2** (1.3 g), 4 Å molecular sieves (2.5 g), and THF (50 mL). A precipitate formed through the course of the addition. Samples of the purified polymer were obtained by GPC using Waters 500 and 10^3 Å μStyragel analytical columns. ^{31}P NMR (benzene-d_6): 0.25. ^1H NMR (benzene-d_6): 1.65 (m, 4H, $PyCH_2CH_2CH_2$), 1.83 (m, 4H, $PyCH_2CH_2$), 3.128 (s, 6H, CH_3), 3.150 (t, 4H, $^3J_{HH'}$ = 7.4 and 7.8 Hz, $PyCH_2$), 3.31 (t, 2H, $^3J_{HH'}$ = 4.7 and 5.1 Hz, CH_2OCH_3), 3.38−3.53 (m, 451 ± 16 H, $MeOCH_2CH_2(OCH_2CH_2)_{x-2}$-$OCH_2CH_2OP$ and $POCH_2CH_2(OCH_2CH_2)_{y-2}OCH_2CH_2OP$, $2x + y = 110 \pm 4$), 4.12 (m, 12H, $Py(CH_2)_3CH_2$, $POCH_2CH_2O$-$(CH_2CH_2O)_{x-1}Me$, and $POCH_2CH_2O(CH_2CH_2O)_{y-2}CH_2CH_2OP$), 7.69−8.22 (m, 18H, pyrenyl *H*).

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