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Published on Web 11/01/2002

Designing Macromolecules for Therapeutic Applications: Polyester Dendrimer−Poly(ethylene oxide) "Bow-Tie" Hybrids with Tunable Molecular Weight and Architecture

Elizabeth R. Gillies and Jean M. J. Fréchet*

Contribution from the Center for New Directions in Organic Synthesis,† Department of Chemistry, University of California, Berkeley, California 94720-1460

Received August 11, 2002

Abstract: The design and preparation of new polyester dendrimer, poly(ethylene oxide) hybrid systems for drug delivery and related therapeutic applications, are described. These systems consist of two covalently attached polyester dendrons, where one dendron provides multiple functional handles for the attachment of therapeutically active moieties, while the other is used for attachment of solubilizing poly(ethylene oxide) chains. By varying the generation of the dendrons and the mass of the poly(ethylene oxide) chains, the molecular weight, architecture, and drug loading can be readily controlled. The "bow-tie" shaped dendritic scaffold was synthesized using both convergent and divergent methods, with orthogonal protecting groups on the periphery of the two dendrons. Poly(ethylene oxide) was then attached to the periphery of one dendron using an efficient coupling procedure. A small library of eight carriers with molecular weights ranging from about 20 kDa to 160 kDa were prepared and characterized by various techniques, confirming their well-defined structures.

Introduction

In recent decades, there has been increased interest in the preparation of new polymeric materials for drug delivery and other therapeutic or diagnostic applications. This interest is motivated mainly by the problematic properties of some potentially useful low molecular weight (MW) drug candidates. For example, while the beneficial effects of anticancer drugs arise through their interactions with tumor cells, their exposure to other cell types typically leads to undesirable side effects and toxicity.[1] In addition, many low MW drugs suffer from problems such as poor water solubility and bioavailability, as well as rapid elimination.[2]

In natural systems, transport proteins are frequently used to alter the properties of small molecules.[3] The notion of using synthetic, water-soluble polymers to mimic these transport proteins was first introduced by Ringsdorf[4] and Kopeček.[5] Development of this concept has shown that attachment of low MW drugs to a high MW polymeric backbone can lead to several benefits. Polymer attachment can lead to improved solubility as well as increased circulation time of the polymer-drug conjugate in the plasma.[6] This enhancement in circulation time is the direct result of the decreased rate of renal filtration that correlates with molecular size, as molecules with larger hydrodynamic volumes are eliminated more slowly.[7] Moreover, attachment of anticancer drugs to polymers can be an effective means of passively targeting these molecules to solid tumors. Targeting is possible due to the increased permeability of tumor vasculature to macromolecules and limited lymphatic drainage. Combined, these factors lead to the selective accumulation of macromolecules in tumor tissue, a phenomenon known as the enhanced permeation and retention (EPR) effect.[8]

Despite the large number of polymers commercially available, relatively few possess the characteristics that are believed to be important for a drug delivery system. These characteristics include water solubility, lack of both toxicity and immunogenicity, low polydispersity, and the presence of multiple, highly accessible functional handles for drug attachment. One of the systems that has been extensively investigated is based on (2-hydroxypropyl)methacrylamide (HPMA).[6a] This system is currently undergoing clinical trials and has shown promise in terms of its drug delivery capabilities.[9] Some limitations of this system based on a vinyl polymer include its inherent lack of biodegradability and the difficulties encountered in the preparation of low

* To whom correspondence should be addressed. E-mail: frechet@cchem.berkeley.edu.

† The Center for New Directions in Organic Synthesis is supported by Bristol-Myers Squibb as Sponsoring Member.

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polydispersity HPMA.[10] In general, the use of a material with a broad polydispersity index (PDI) is not favored in systemic applications as it may lead to irreproducible or undesired pharmacokinetic behavior because of the presence of species with vastly different MW within a given sample or to variations in molecular weight distribution from preparation to preparation. In addition, the preparation of well-defined drug conjugates of linear polymers is difficult since drug attachment is inevitably statistical in nature, either by incorporation of a comonomer in the polymer preparation or by postsynthetic modification of a homopolymer with a functional handle. Therefore, while HPMA has many attractive features and has shown great promise in early clinical studies, the development of alternative, perhaps more biocompatible polymers, with better-defined molecular weights and architectures enabling enhanced control over drug loading will broaden the scope of applicability of polymers in therapeutic applications.

Dendrimers are very promising candidates as components of drug delivery systems. In contrast to conventional polymers, these molecules with their well-defined architecture possess a very low PDI or even a unique MW, together with a highly regular branching pattern, and a strictly controlled multiplicity of reactive chain ends.[11] Therefore, it is possible to introduce or modify a specific number of functional groups on the periphery of a dendrimer, which can be used to alter its properties, such as its solubility or hydrodynamic volume, or can provide a specified number of readily accessed attachment points for drug loading. Currently, poly(amidoamine) (PAMAM), poly(propyleneimine) (PPI), and low generation polyaryl ether dendrons[11b] are the only dendrimers available commercially. The first two families of dendrimers are relatively toxic in cells and animals because of their polycationic surfaces,[12] while the third is not ideally tuned for water solubility.

We have recently reported the design, synthesis, and biological in vitro and in vivo evaluation of a polyester dendrimer scaffold based on 2,2-bis(hydroxymethyl)propionic acid.[13] This scaffold is a promising candidate for drug delivery applications, as it shows very good biocompatibility and can be easily prepared in large quantities using convergent or divergent strategies.[14] However, both the fourth generation dendrimer with a MW of 3790 Da and an analogous globular macromolecule with MW = 12 183 derived from the same 4th generation dendrimer have a short plasma residence time ($t_{1/2}$ < 10 min) that would limit the scope of their use for some drug delivery applications.[13b] Since prolonged circulation has been observed for higher MW macromolecules,[15] a hybrid structure with MW 22 kDa has also been prepared by functionalizing the periphery of a three-arm poly(ethylene oxide) (PEO) star polymer with polyester dendrons.[13a] Star-shaped PEO was chosen for its water solubility, biocompatibility, and because it is available with low polydispersity (PDI = 1.02), thus providing hybrids of similar narrow polydispersity.[16] After suitable peripheral modification of the dendrons, the anticancer drug doxorubicin was attached to these hybrids via hydrazone formation. Because of their increased MW, these conjugates have an increased plasma residence half-life of 72 min, but an even longer half-life might, in some cases, be beneficial.[13b]

The promising characteristics of these novel polyester-PEO hybrids in initial biological studies both in vitro and in vivo have inspired us to undertake a systematic study of the effect of MW and architecture of these carriers on their pharmacokinetic properties. Therefore, we now report a new "bow-tie" design that allows access to a library of carriers with a range of MWs and architectures. This design consists of two covalently attached and orthogonally protected polyester dendrons. One dendron can be selectively deprotected to allow coupling of solubilizing PEO moieties to one side of the system, while the other dendron can subsequently be deprotected, providing functional handles for further derivatization or drug attachment. A key feature of this system is its multifunctional character, with the ability to control the loading of two different species, one on each side of the "bow-tie". The synthesis of a small library of eight compounds of different molecular weights and architectures is described here.

Results and Discussion

Design. The main advantages of a dendritic scaffold are the well-defined and tunable molecular weight and architecture, as well as the possibility for tailoring the surface functional groups.[11] In the proposed "bow-tie" design, we take advantage of these features to access a range of architectures and MWs efficiently. The target system consists of two covalently linked polyester dendrons where one dendron carries multiple water-soluble PEO chains, while the other dendron has hydroxyl functional handles for later drug or radiolabel attachment. By varying the generation, or the number of branch points on one side of the bow-tie, the number of PEO chains attached can be varied, leading to either a nearly linear polymeric structure at low generations or a highly branched structure at higher generations as illustrated in Figure 1. By varying the mass of the attached PEO as well as the generation of the dendron through the use of dendritic "wedges" or dendrons of different sizes as shown in Figure 1, the MW can be systematically tuned as shown in Table 1. The degree of drug loading can be adjusted by varying the generation number, and hence the number of attachment points, of the second dendron.

A comparison of the previously reported dendrimer and PEO star-dendrimer conjugate with the new bow-tie system is shown

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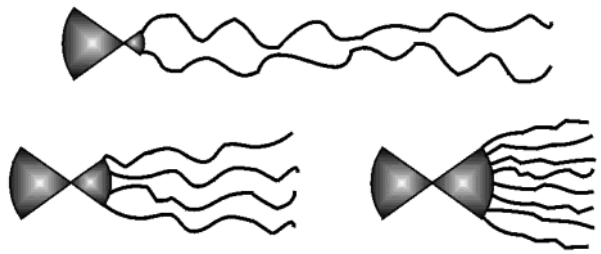

Figure 1. An illustration of different possible architectures obtained by varying the dendrimer generation. The three systems can each have approximately the same total mass but different numbers and masses of PEO chains.

Table 1. Different MW and Architectural Possibilities Available by Varying the Dendrimer Generation and Mass of the PEO Chains

	approximate MW of PEO-dendrimer conjugate (Da)		
MW of PEO chains (Da)	[G-1] (2 arms)	[G-2] (4 arms)	[G-3] (8 arms)
1000	2000	4000	8000
2000	4000	8000	16000
5000	10000	20000	40000
10000	20000	40000	80000
20000	40000	80000	160000

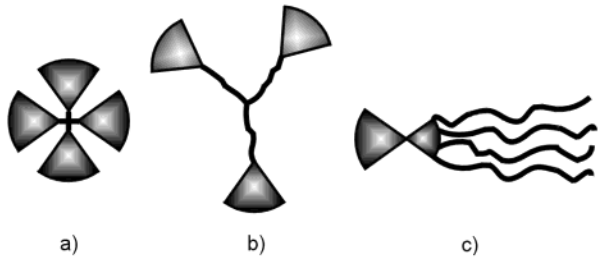

Figure 2. A comparison of the architectures of the previously evaluated carriers (a) a polyester dendrimer and (b) a PEO star-dendrimer conjugate with (c) a dendritic bow-tie PEO conjugate.

in Figure 2. Once again, the bow-tie design is not a true dendrimer as increased residence time would require dendrimer growth to be carried to very high generations thus making the system less synthetically accessible. Key features including the use of biocompatible aliphatic polyester dendrons and PEO chains are preserved but, in contrast to the previously prepared dendrimer-PEO conjugate where the dendrons are on the periphery of the structure,[13] the drug load in this case is near the core of the structure and is therefore expected to be surrounded by the PEO chains once placed in an aqueous environment. This should contribute to the prevention of undesirable interactions of the drug with biological components such as cell surfaces, enzymes, and proteins in the serum.

As in the previously evaluated structures, the dendritic backbone of the "bow-tie" is based on the monomer 2,2-bis-(hydroxymethyl)propionic acid. This is advantageous since polyester bonds are typically characterized by their susceptibility to hydrolysis.[17] Therefore, this provides access to a high MW, at least partly biodegradable PEO based system, which has previously been a challenge to prepare.[18] However, any degradation that might take place is expected to be slow since the sterically hindered ester linkages formed by this monomer make it relatively stable to acid- and base-catalyzed hydrolysis. This feature facilitates synthesis of the functional macromolecules.

The target of this work was the preparation of well-defined carrier macromolecules with a range of MWs and architectures, through variation of the number of branch points on one side of the "bow-tie". Therefore, the dendron carrying PEO will vary from the first to the third generation to provide from two to eight branch points, while the dendron on the other side of the "bow-tie" will be kept at the third generation. Finally, only those carriers with MWs greater than 20 kDa have been considered for this study since one of our objectives was to increase the in vivo circulation time of our previous carriers.[13] Therefore, we will focus here on "bow-tie" structures containing low polydispersity PEO moieties with molecular weights 5 kDa, 10 kDa, and 20 kDa, respectively.

Synthesis. An important consideration in the development of our synthetic strategy toward the "bow-tie" targets was the need to provide orthogonal protecting groups on the periphery of its two constituting dendrons. One option considered was the convergent coupling of two orthogonally protected dendrons. Initial investigation of this strategy showed that the coupling of two relatively large dendrons was difficult to achieve in good yield because of steric factors. Therefore, an alternative strategy was selected involving the preparation of one dendron with a protected periphery, followed by activation of its focal point, and divergent growth of the second dendron from this focal point using a different peripheral protecting group.

Polyester dendrons based on 2,2-bis(hydroxymethyl)propionic acid have been prepared both convergently and divergently.[14] In a typical implementation, the convergent approach has been used to prepare dendrons with isopropylidene acetals protecting the peripheral hydroxyls and a benzyl ester protecting the focal point.[14b] Similarly, the divergent approach makes use of an anhydride monomer with benzylidene acetals as the protecting group for the peripheral hydroxyls.[14d] According to the proposed strategy, it is desirable to have a protected alcohol at the focal point of the first dendron, such that, following deprotection, divergent growth can occur from this point. Hedrick and coworkers have recently reported the use of a multifunctional polyester macroinitiator for the preparation of dendritic-linear miktoarm star polymers by ring opening or atom transfer radical polymeriation.[19] Their approach involves the use of the benzylidene moiety as well as a *tert*-butyldimethylsilyl ether as orthogonal protecting groups. In our approach, an allyl carbonate was used as the protecting group for the focal point hydroxyl group during the convergent synthesis of the first dendron, since it should be selectively removed in the presence of the peripheral isopropylidene acetals. It is then possible to use the benzylidene acetal as an orthogonal protecting group in the divergent growth of the second dendron.

To begin the synthesis, isopropylidene-2,2-bis(oxymethyl)-propionic acid **1** prepared as previously reported[14b] was self-condensed using 1,3-dicyclohexylcarbodiimide (DCC) in CH_2Cl_2 to afford the anhydride monomer **2** as shown in Scheme 1 in 97% yield. This anhydride was then coupled to the hydroxyl

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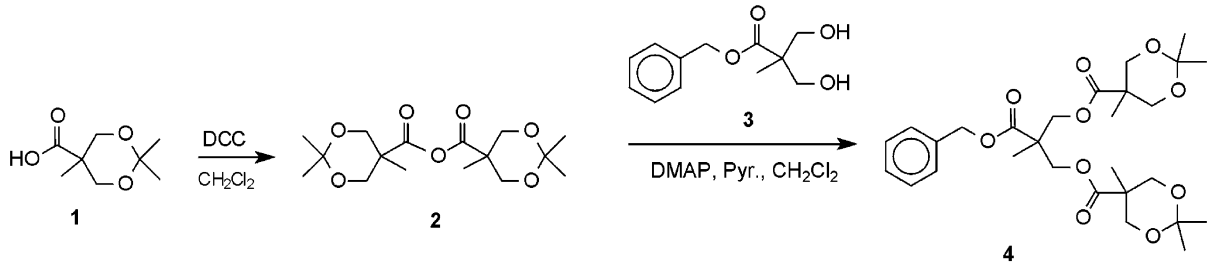
Scheme 1



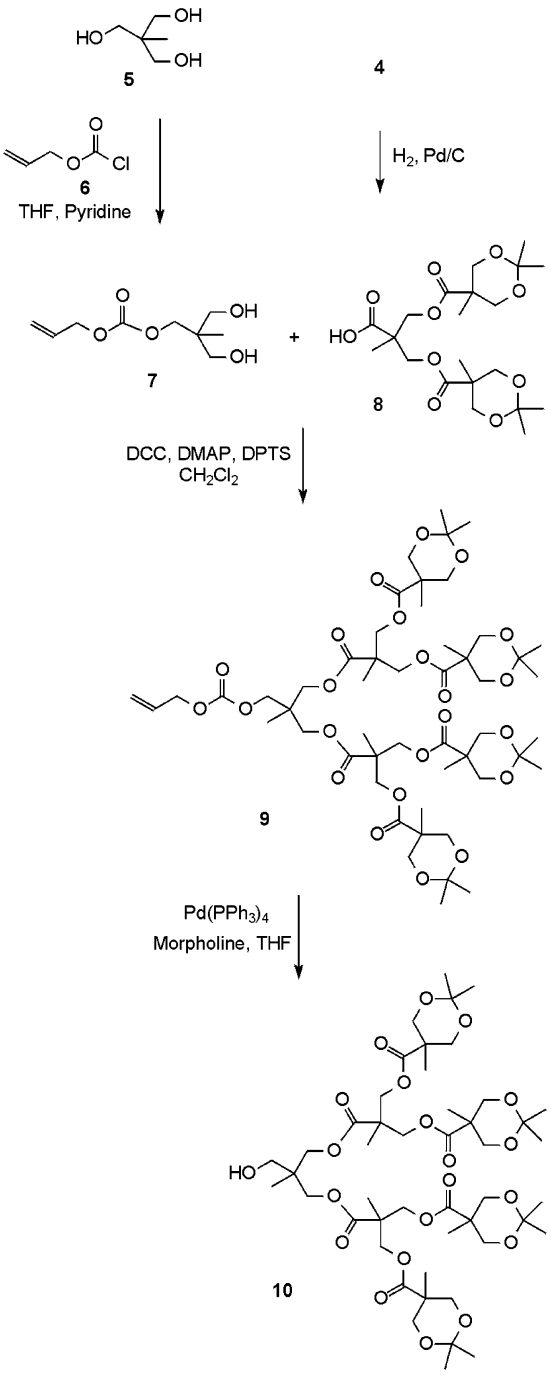
groups of the previously reported benzyl 2,2-bis(hydroxymethyl)propionate **3**,[14b] in the presence of 4-(dimethylamino)pyridine (DMAP) to provide a 95% yield of the isopropylidene-[G-2]-benzyl ester **4**. Although this building block had previously been prepared using a convergent approach,[14b] it was found that it could be conveniently prepared in high yield using this divergent method.

To prepare the protected focal point of the first dendron, excess tris(hydroxymethyl)ethane was reacted with allyl chloroformate to provide the monoprotected triol **7** as shown in Scheme 2 in 64% yield. The isopropylidene-[G-2]-acid **8**, obtained from **4** by the literature procedure,[14b] was then coupled convergently to the remaining alcohol groups of **7** using DCC in the presence of DMAP and 4-(dimethylamino)pyridinium *p*-toluenesulfonate (DPTS) to afford a 95% yield of the isopropylidene-[G-3]-allyl carbonate **9**. The allyl carbonate protecting group in **9** was removed using $Pd(PPh_3)_4$ in the presence of morpholine to provide **10** with an alcohol at the focal point in 91% yield.

The focal point alcohol of the convergent dendron **10** was then reacted with the previously reported benzylidene-2,2-bis-(oxymethyl)propionic anhydride **11**[14d] in the presence of DMAP to afford a 95% yield of the isopropylidene-[G-3]-[G-1]-benzylidene **12**, thus initiating growth of the second part of the "bow-tie" dendrimer as shown in Scheme 3. The benzylidene acetal protecting group was then removed selectively in the presence of the isopropylidene acetals by catalytic hydrogenolysis using Pd/C as catalyst to give **13** quantitatively. It was important to perform the deprotection in an aprotic solvent such as ethyl acetate to avoid undesired removal of the isopropylidene acetals. This coupling and deprotection sequence was then repeated to give the isopropylidene-[G-3]-[G-2]-OH **15** in 90% yield, and once more to give the isopropylidene-[G-3]-[G-3]-OH "bow-tie" dendrimer **17** in 87% yield.

To attach PEO moieties to the dendrons, the free hydroxyl groups of each "bow-tie" dendrimer **13**, **15**, and **17** were activated as *p*-nitrophenyl carbonates by reaction with *p*-nitrophenyl chloroformate **18** in the presence of pyridine as shown in Scheme 4 to provide intermediates **19**, **20**, and **21,** respectively, in good yield. These intermediates were isolated by flash chromatography. The activated compounds were then reacted with 1.2 equiv of amine functionalized poly(ethylene oxide) ($PEO-NH_2$) per carbonate moiety. $PEO-NH_2$ samples of approximate MWs 5 kDa, 10 kDa, and 20 kDa were used to provide a library of eight compounds (**22**−**29**) as shown in Table 2 with a range of MWs as determined by both MALDI-TOF MS and size exclusion chromatography (SEC). This coupling procedure was much more efficient than alternative methods that were also evaluated, such as the DCC coupling of acid functionalized PEO with the peripheral hydroxyls of the "bow-tie" dendrimers. The products could all be precipitated in diethyl ether in high yield.

Scheme 2



Initially, a variety of amine-scavenging resins were explored for the removal of the excess $PEO-NH_2$ used in the coupling

Scheme 3

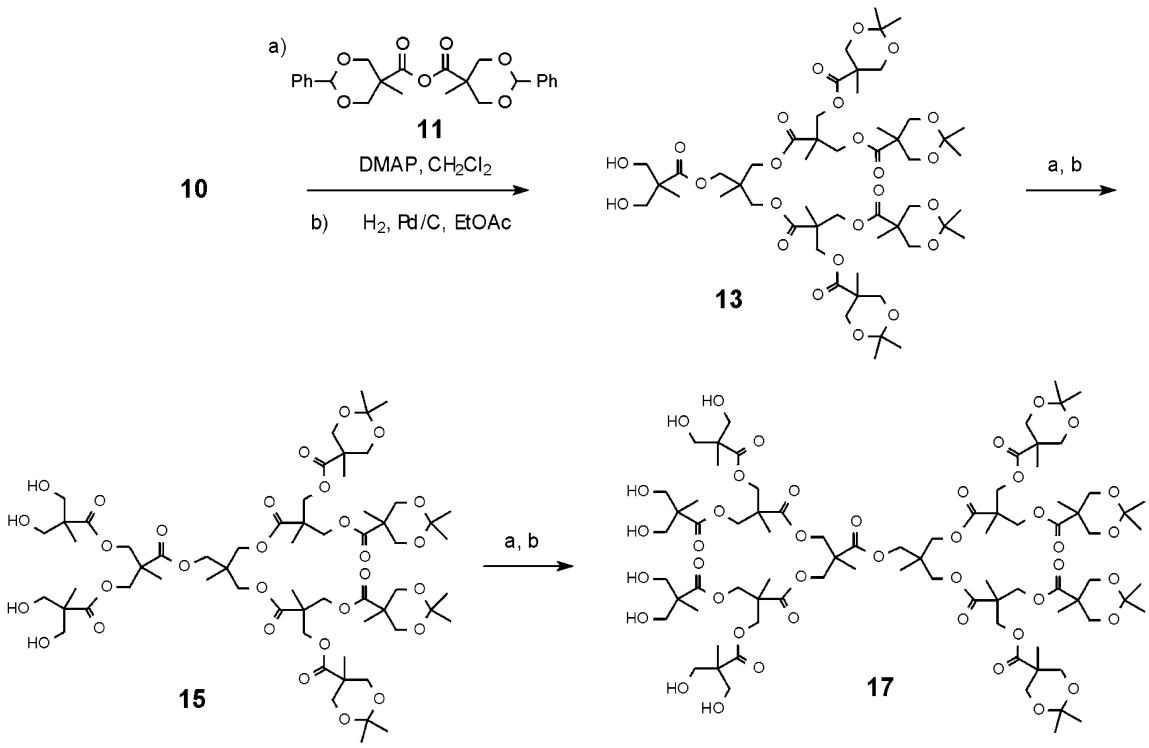


Table 2. Characterization of the Dendrimer-PEO Hybrids

sample	MW (Da) (MALDI-TOF)	M_w (Da) (SEC)	M_n (Da) (SEC)	PDI (Da) (SEC)
22 [G-1]-10 kDa	21800	19900	17900	1.11
23 [G-1]-20 kDa	44200	36000	30900	1.16
24 [G-2]-5 kDa	23000	19600	18400	1.06
25 [G-2]-10 kDa	43000	36900	34900	1.06
26 [G-2]-20 kDa	86800	69700	63700	1.09
27 [G-3]-5 kDa	44700	30500	28300	1.08
28 [G-3]-10 kDa	84900	56600	52100	1.08
29 [G-3]-20 kDa	NA	123000	109000	1.12

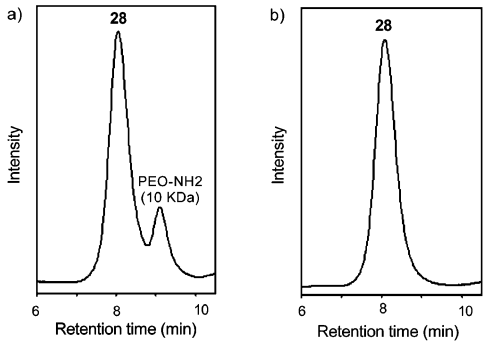


Figure 3. SEC traces of the [G-3]-10 kDa hybrid **28** (a) before purification and (b) after purification by dialysis.

reaction, but as none of these methods proved effective, dialysis or preparative SEC were used to provide complete removal. While SEC is efficient for analytical purposes and for purification of small amounts of material, dialysis was more convenient for larger scale preparations. Using a dialysis membrane with a molecular weight cutoff of 100 kDa, removal of 5 kDa PEO−NH_2 required 18 h of dialysis while removal of PEO−NH_2 of 10 kDa and 20 kDa required 48 and 96 h, respectively. Figure 3 shows SEC traces of the [G-3]-10 kDa hybrid **28** before and after dialysis, illustrating that the free PEO−NH_2 could be completely separated from the desired conjugate by this very simple method.

Given the biocompatibility of PEO, its complete removal may not be necessary for some uses and an alternative method involving capping of the amine groups of the remaining PEO−NH_2 may be used to prevent their coupling with drugs or radiolabels in later experiments.

This was accomplished by reaction of the PEO−NH_2 impurity in the presence of the desired isopropylidene acetal protected polymers **22**−**29** with excess acetic anhydride and pyridine. The amount of free amine both before and after this capping procedure was quantified using the fluorescamine assay.[20] The results, shown in Table 3 indicate that before capping, the amount of amine ranged from 9 to 24% by mass, while following capping, the amount ranged from undetectable to 0.5 %, indicating that the capping procedure was successful. The results of this analysis were also interesting since the amine content before capping provides a measure of the efficiency of coupling of PEO−NH_2 to the dendrimer. Since the expected percentage of amine would be near 17% if the coupling had gone to completion, the results indicate that the coupling efficiency was generally greater than 90%. Samples for which the percentages were lower than the expected 17% may be explained by the presence of PEO chains lacking amine groups in the commercial PEO−NH_2 or by minor variances of the MWs of the PEO−NH_2 from the expected 5 kDa, 10 kDa, and 20 kDa.

The final step in the synthesis was removal of the acetonide protecting groups. As shown in Scheme 5 for the [G-3] hybrid **27**, this was accomplished using a catalytic amount of sulfuric acid in methanol to provide the hydroxyl-terminated dendrimer.

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Scheme 4

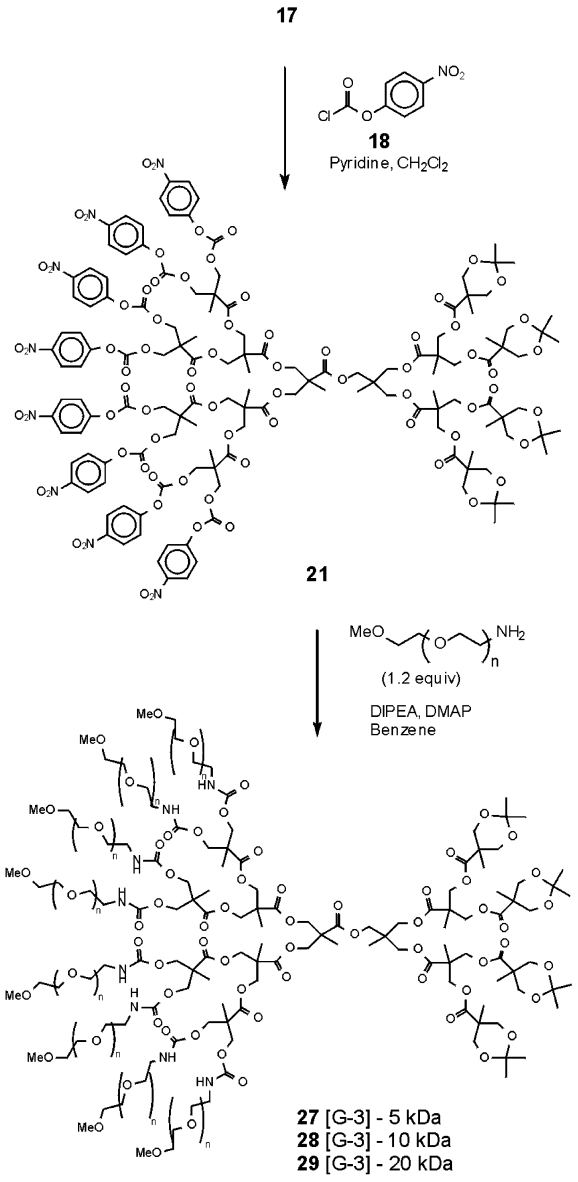


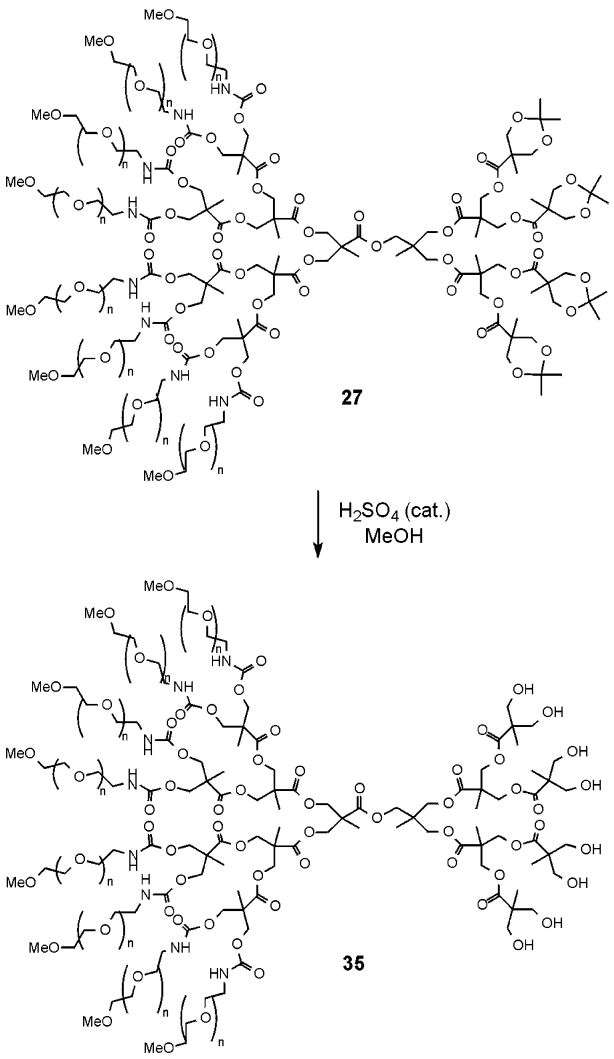
Table 3. Characterization of the Dendrimer-PEO Hybrids Using the Fluorescamine Assay before and after Capping of Excess PEO−NH_2

sample	% amine content prior to capping	% amine content after acylation
22 [G-1]-10 kDa	11	Undetectable
23 [G-1]-20 kDa	15	0.5
24 [G-2]-5 kDa	13	0.1
25 [G-2]-10 kDa	20	Undetectable
26 [G-2]-20 kDa	24	0.4
27 [G-3]-5 kDa	9.1	0.4
28 [G-3]-10 kDa	9.3	0.5
29 [G-3]-20 kDa	19	Undetectable

No degradation of the products was observed in this deprotection step and the expected MWs and PDIs were observed by MALDI-TOF MS and SEC for the deprotected products. The hydroxyl handles of the resulting PEO-modified "bow-tie" dendrimers are now available for the eventual attachment of drugs or radiolabels and the biological evaluation of these carriers.

General Characterization. ^{1}H NMR and MALDI-TOF MS were most useful for monitoring the coupling and deprotection steps during the preparation of dendrimers **9** to **21**, ensuring that the reactions went to completion.

Scheme 5



SEC was also very useful for the characterization of the PEO-dendrimer hybrids **22**−**37**. SEC provides a measure of the hydrodynamic volume of the molecules,[21] a property that is expected to correlate well with the pharmacokinetic properties of the carriers such as tumor uptake and plasma circulation half-life.[22] As polymers are known to assume different conformations in different solvents, the SEC was performed in aqueous conditions to provide a reasonable estimate of the hydrodynamic volumes in vivo. The results of the size exclusion chromatography are summarized in Table 2 and an example is provided in Figure 3. As a result of their more globular structure and lower hydrodynamic volume, the more branched compounds had molecular weights that were underestimated most significantly when compared to linear calibration standards.[23] The low PDIs of the hybrids confirm that the coupling of PEO to the dendrimers proceeded as expected.

MALDI-TOF MS was used to confirm the predicted molecular weights of the conjugates. However, observation of the

(21) Benoit, H.; Grubisic, Z.; Rempp, R. *J. Polym. Sci., Part B: Polym. Phys.* **1967**, *5*, 753.
(22) (a) Tabata, Y.; Murakami, Y.; Ikada, Y. *J. Controlled Release* **1998**, *50*, 123−133. (b) Nakaoka, R.; Tabata, Y.; Yamaoka, T.; Ikada, Y. *J. Controlled Release* **1997**, *46*, 253−261.
(23) Zimm, B. H.; Stockmayer, W. H. *J. Chem. Phys.* **1949**, *17*, 1301.

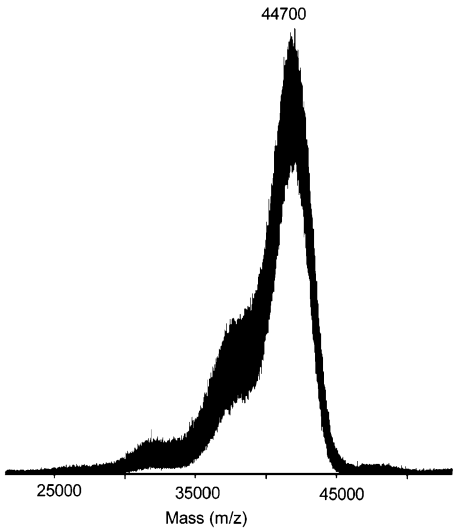


Figure 4. A MALDI-TOF spectrum of the [G-3]-5 kDa conjugate **27** with eight PEO arms. The most intense peak corresponds to the fully coupled product while minor peaks correspond to the products with six and seven arms that may result from incomplete PEO coupling or degradation during MALDI-TOF MS.

molecular ion became increasingly difficult as the molecular weight of the conjugates increased and the degree of branching decreased. This is presumably due to the increased sublimation energies of the high MW polymers, leading to a decrease in their concentrations in the gaseous plume reaching the detector.[24] Particularly in the case of the high MW conjugates, some peaks corresponding to conjugates lacking full coupling were observed. This is likely the result of degradation due to the high laser power necessary to detect these compounds or may be due to a small number of lower MW impurities with relatively increased volatility that are exaggerated by this technique. However, a major peak corresponding to the expected molecular ion was observed in all cases except for **29**, the [G-3]-20 kDa hybrid which has a theoretical MW > 160 kDa and could not be analyzed by MALDI. An example of a MALDI-TOF spectrum is shown in Figure 4 for the [G-3]-5 kDa hybrid **27**, that shows the most intense peak at 44 700, corresponding to the fully coupled product.

Despite the high molecular weight of the PEO-dendrimer conjugates **17**−**32,** ^{1}H NMR (500 MHz instrument) could be used to monitor changes in the dendritic scaffold. In particular, ^{1}H NMR was useful for monitoring the deprotection of the acetonide groups in the final step of the synthesis since the clear signal corresponding to the protons of the isopropylidene acetal groups disappear following the deprotection.

Conclusion

There is little doubt that polymer architecture is an important variable in the design of novel macromolecular therapeutic agents. Given the many desirable structural features of dendrimers as well as the known biocompatibility of poly(ethylene oxide), the small library of polyester dendrimer-PEO hybrids we have designed should prove versatile in exploring application parameters for macromolecular carriers designed for use in targeted drug or gene delivery as well as diagnostics. The macromolecules are designed to enable attachment of the active moieties onto the polymers in a single step to avoid undesirable side-reactions involving the therapeutic or diagnostic agent. The hybrid system selected in this study is attractive as it leads to well-defined and highly functionalized macromolecules with low polydispersity and a range of molecular weights and architectures. The preparation of this dendritic system involving both convergent and divergent methods of synthesis is extremely efficient despite a functionalization approach that involves two different macromolecules. High yields of coupling can be achieved since an excess of PEO−NH_2 can be used and unreacted material later removed by a simple dialysis process. Biological evaluation of the hybrids will follow—first in the context of cancer therapy—and is expected to provide useful information on the effect of MW and architecture on plasma circulation time and tumor uptake.

Experimental Section

General Procedures and Materials. Amine functionalized poly(ethylene oxide) (PEO−NH_2) was purchased from Shearwater. All other chemicals were purchased from Aldrich and used without further purification unless otherwise noted. Solvents (reagent grade) were purchased from Fisher or EM Sciences. Tetrahydrofuran (THF) was distilled under a nitrogen atmosphere from Na/benzophenone immediately prior to use. Dichloromethane and pyridine were distilled from CaH_2 under a nitrogen atmosphere immediately prior to use. ^{1}H NMR spectra were recorded at 500 MHz and ^{13}C NMR spectra were recorded at 125 MHz. NMR chemical shifts are reported in ppm and calibrated against solvent signals. All coupling constants are reported in Hz. FT-IR spectroscopic analyses were performed using a thin film from $CHCl_3$ on a reflective mirror surface. Size-exclusion chromatography (SEC) was performed at 25 °C using a Waters 2690 Separations Module and a Waters 410 differential refractometer with a Suprema 10μ column (10^3 Å) as the stationary phase. Purified water with 0.1 M $NaNO_3$ was used as eluent with a constant flow rate of 1 mL/min, and the instrument was calibrated using PEO standards. High-resolution fast atom bombardment (FABHR MS) and electron impact (EIHR MS) mass spectrometry experiments were performed at the UC Berkeley MS Facilities. MALDI-TOF MS data was collected on a PerSeptive Biosystems Voyager-DE instrument in positive ion mode using a *trans*-indoleacrylic acid matrix and calibration against bovine insulin standards. Elemental analyses were performed by M-H-W Laboratories.

Isopropylidene-2,2-bis(oxymethyl)propionic Anhydride (2). A suspension of isopropylidene-2,2-bis(oxymethyl)propionic acid **1**[14b] (10 g, 57 mmol, 2.0 equiv.) in 30 mL of CH_2Cl_2 was prepared and a solution of 1,3-dicyclohexylcarbodiimide (DCC) (5.9 g, 29 mmol, 1.0 equiv.) in 10 mL of CH_2Cl_2 was added. The reaction mixture was stirred at room temperature for 4 h. The urea DCC byproduct dicyclohexylurea (DCU) was filtered off in a glass filter and washed with a small amount of CH_2Cl_2. The solvent was evaporated and the resulting residue was taken up in EtOAc. Residual DCU was removed by filtering the resulting suspension through a glass filter. The filtrate was evaporated to give 9.2 g (97%) of the anhydride **2** as a viscous oil. IR (cm^{-1}): 1815, 1746. ^{1}H NMR ($CDCl_3$): δ 1.19 (s, 6), 1.35 (s, 6), 1.40 (s, 6), 3.65 (d, 4, J = 12.0), 4.16 (d, 4, J = 12.0). ^{13}C NMR ($CDCl_3$): δ 17.71, 21.59, 25.70, 43.75, 65.75, 98.45, 169.59. Calcd: $[M+H]^+$ ($C_{16}H_{27}O_7$) m/z = 331.1756. Found: FABHR MS: $[M+H]^+$ m/z = 331.1747. Anal. Calcd for $C_{16}H_{26}O_7$: C, 58.2; H, 7.93. Found: C, 58.6; H, 7.76.

Isopropylidene-[G-2]-benzyl Ester (4). Benzyl-2,2-bis(oxymethyl)-propionate **3** (3.0 g, 13 mmol, 1.0 equiv) and 4-(dimethylamino)pyridine DMAP (1.3 g, 11 mmol, 0.80 equiv) were dissolved in 40 mL of CH_2Cl_2 and 6 mL of pyridine was added. Anhydride **2** (13 g, 40 mmol, 3.0 equiv), was added, and the reaction mixture was stirred at room

(24) Ayorinde, F. O.; Elhilo, E. *Rapid Commun. Mass Spectrom.* **1999**, *13*, 2166−2173.

temperature overnight. The excess anhydride was quenched by stirring the reaction mixture with 10 mL of a 1:1 pyridine:water solution overnight. The organic phase was diluted with 100 mL of CH_2Cl_2 and extracted with 1 M $NaHSO_4$ (3 × 60 mL), 10% Na_2CO_3 (3 × 60 mL), and saturated brine (60 mL). The organic phase was dried with $MgSO_4$, filtered, and the filtrate evaporated to give 6.8 g (95%) of **4** as a glassy solid. Spectroscopic data agreed with those reported in the literature.[14b]

(Hydroxy)$_2$-[G-1]-allyl Carbonate (7). 1,1,1-tris(hydroxymethyl)-ethane (8.8 g, 74 mmol, 5.0 equiv) was dissolved in 300 mL of THF. Pyridine (2.5 mL) was added and the solution was cooled to 0 °C under a nitrogen atmosphere. Allyl chloroformate (1.6 mL, 15 mmol, 1.0 equiv) was added dropwise over 0.5 h then the reaction mixture was allowed to come to room temperature and was stirred overnight. The solvent was evaporated and the resulting residue was taken up in ethyl acetate (200 mL). The organic phase was washed with 1 M $NaHSO_4$ (3 × 100 mL) followed by saturated brine (100 mL). The organic phase was dried using $MgSO_4$, filtered, and evaporated to give a colorless oil. The product was purified by flash column chromatography on silica using a solvent gradient from Hex/EtOAc (50:50) to pure EtOAc, yielding 1.9 g (64%) of **7** as a viscous oil. IR (cm^{-1}): 3416, 1746, 1650. ^{1}H NMR ($CDCl_3$): δ 0.86 (s, 3), 2.81 (t, 2, J = 6.0), 3.55−3.61 (m, 4), 4.23 (s, 2), 4.63 (ddd, 2, J = 6.0, 1.5, 1.2), 5.29 (ddt, 1, J = 10.5, 1.2, 1.2), 5.38 (ddt, 1, J = 17.2, 1.5, 1.5), 5.91−5.96 (m, 1). ^{13}C NMR ($CDCl_3$): δ 16.86, 40.97, 67.63, 69.01, 70.32, 119.51, 131.52, 156.01. Calcd: $[M+H]^+$ ($C_9H_{17}O_5$) m/z = 205.10759. Found: EIHR MS: $[M+H]^+$ m/z = 205.10796. Anal. Calcd for $C_9H_{16}O_5$: C, 52.9; H, 7.90. Found: C, 52.9; H, 7.73.

Isopropylidene-[G-2]-COOH (8). This compound was prepared from **4** according to the procedure reported by Ihre et al.[14b] Spectroscopic data agreed with those reported.

Isopropylidene-[G-3]-allyl carbonate (9). The acid **8** (5.0 g, 11 mmol, 3.0 equiv) and 0.76 g (3.7 mmol, 1.0 equiv) of the diol **7** were dissolved in 10 mL of CH_2Cl_2. Following addition of DCC (2.3 g, 11 mmol, 3.0 equiv) and 4-(dimethylamino)pyridinium *p*-toluenesulfonate (DPTS) (0.69 g, 2.2 mmol, 0.60 equiv), the reaction mixture was stirred overnight at room temperature under a nitrogen atmosphere. The reaction mixture was filtered to remove the DCU and the filtrate was concentrated to give a colorless oil. The product was purified by flash column chromatography on silica using a solvent gradient from Hex/EtOAc (80:20) to Hex/EtOAc (50:50) to afford 3.8 g (95%) of **9** as a viscous oil. IR (cm^{-1}): 1738, 1650. ^{1}H NMR ($CDCl_3$): δ 1.03 (s, 3), 1.12 (s, 12), 1.28 (s, 6), 1.33 (s, 12), 1.39 (s, 12), 3.59 (dd, 8, J = 12.2, 1.0), 4.03 (s, 4), 4.05 (s, 2), 4.12 (dd, 8, J = 10.0, 1.9), 4.29−4.34 (m, 8), 4.60 (ddd, 2, J = 6.0, 1.3, 1.3), 5.26 (ddt, 1, J = 10.4, 1.2, 1.2), 5.32 (ddt, 1, J = 17.2, 1.5, 1.5), 5.87−5.94 (m, 1). ^{13}C NMR ($CDCl_3$): δ 17.11, 17.88, 18.70, 22.17, 25.45, 39.18, 42.26, 47.18, 65.19, 66.13, 66.18, 66.33, 68.96, 98.30, 119.44, 131.57, 154.85, 172.25, 173.69. Calcd: $[M+H]^+$ ($C_{51}H_{81}O_{23}$) m/z = 1061.5169. Found: FABHR MS: $[M+H]^+$ m/z = 1061.5192. Anal. Calcd for $C_{51}H_{80}O_{23}$: C, 57.7; H, 7.60. Found: C, 57.8; H, 7.70.

Isopropylidene-[G-3]-OH (10). Isopropylidene-[G-3]-allyl carbonate **9** (1.4 g, 1.3 mmol, 1.0 equiv) was dissolved in THF and $Pd(PPh_3)_4$ (0.074 g, 0.064 mmol, 0.050 equiv) and morpholine (0.23 g, 2.6 mmol, 2.0 equiv) were added. The reaction mixture was stirred in the dark overnight under a nitrogen atmosphere. The THF was evaporated and the resulting residue was purified by flash column chromatography using a solvent gradient from Hex/EtOAc (70:30) to Hex/EtOAc (50:50) to provide 1.2 g (91%) of **10** as a colorless oil. IR (cm^{-1}): 3485, 1738. ^{1}H NMR ($CDCl_3$): δ 0.95 (s, 3), 1.10 (s, 12), 1.29 (s, 6), 1.34 (s, 12), 1.40 (s, 12), 3.12 (t, 1, J = 5.8), 3.40 (d, 2, J = 5.5), 3.61 (d, 8, J = 11.5), 4.02 (d, 2, J = 11.0), 4.06 (d, 2, J = 11.0), 4.10−4.17 (m, 8), 4.30−4.36 (m, 8). ^{13}C NMR ($CDCl_3$): δ 17.00, 17.96, 18.66, 21.70, 25.91, 40.42, 42.33, 47.20, 65.41, 66.18, 66.23, 66.74, 98.40, 172.64, 173.78. Calcd: $[M+H]^+$ ($C_{47}H_{77}O_{21}$) m/z = 977.4957. Found: FABHR MS: $[M+H]^+$ m/z = 977.4962. Anal. Calcd for $C_{47}H_{76}O_{21}$: C, 57.8; H, 7.8. Found: C, 58.0; H, 8.0.

Isopropylidene-[G-3]-[G-1]-Benzylidene (12). The isopropylidene-[G-3]-OH **10** (0.50 g, 0.51 mmol, 1.0 equiv) and DMAP (0.025 g, 0.21 mmol, 0.40 equiv.) were dissolved in 6 mL of CH_2Cl_2 and 2 mL of pyridine was added. Benzylidene-2,2-bis(oxymethyl)propionic anhydride **11** (0.50 g, 0.51 mmol, 2.0 equiv), prepared as previously reported[14d] was added, and the reaction mixture was stirred at room temperature overnight. The excess anhydride was quenched by stirring the reaction mixture with 2 mL of a 1:1 pyridine:water solution overnight. The organic phase was diluted with 60 mL of CH_2Cl_2 and extracted with 1 M $NaHSO_4$ (3 × 30 mL), 10% Na_2CO_3 (3 × 30 mL), and saturated brine (30 mL). The organic phase was dried with $MgSO_4$, filtered, and evaporated to give 0.56 g (99%) of **12** as a glassy solid. IR (cm^{-1}): 3050, 1742. ^{1}H NMR ($CDCl_3$): δ 1.03 (s, 3), 1.04 (s, 3), 1.11 (s, 12), 1.26 (s, 6), 1.34 (s, 12), 1.40 (s, 12), 3.60 (d, 8, J = 12.0), 3.66 (d, 2, J = 11.5), 4.07 (s, 4), 4.13 (d, 8, J = 11.8), 4.17 (s, 2), 4.30−4.37 (m, 8), 4.61 (d, 2, J = 11.5), 5.44 (s, 1), 7.29−7.36 (m, 3), 7.40−7.44 (m, 2). ^{13}C NMR ($CDCl_3$): δ 16.84, 17.58, 17.70, 18.34, 21.76, 25.27, 39.26, 41.91, 42.57, 46.81, 64.76, 65.14, 65.81, 65.88, 73.40, 97.92, 101.56, 126.02, 128.00, 128.75, 137.66, 171.90, 173.25, 173.34. Calcd: $[M+H]^+$ ($C_{59}H_{89}O_{24}$) m/z = 1181.5744. Found: FABHR MS: $[M+H]^+$ m/z = 1181.5710. Anal. Calcd for $C_{59}H_{88}O_{24}$: C, 60.0; H, 7.51. Found: C, 60.2; H, 7.80.

Isopropylidene-[G-3]-[G-1]-OH (13). Isopropylidene-[G-3]-[G-1]-benzylidene **12** (377 mg, 0.341 mmol) was dissolved in EtOAc and 50 mg of 10% Pd/C were added. The apparatus for catalytic hydrogenation was evacuated and filled with H_2 three times. After vigorous stirring overnight and completion of the deprotection according to MALDITOF-MS, the catalyst was filtered off in a glass filter over Celite and was carefully washed with EtOAc. The filtrate was evaporated to give 344 mg (99%) of **13** as a glassy solid. IR (cm^{-1}): 3492, 1738. ^{1}H NMR ($CDCl_3$): δ 1.02 (s, 3), 1.04 (s, 3), 1.08 (s, 12), 1.25 (s, 6), 1.29 (s, 12), 1.35 (s, 12), 3.08 (s, 2), 3.56 (d, 8, J = 12.2), 3.67 (d, 2, J = 11.0), 3.77 (d, 2, J = 11.0), 3.99−4.06 (m, 6), 4.09 (d, 8, J = 11.9), 4.26−4.32 (m, 8). ^{13}C NMR ($CDCl_3$): δ 17.15, 17.27, 17.76, 18.55, 21.90, 25.46, 39.36, 42.14, 47.09, 49.82, 64.78, 65.10, 66.02, 66.09, 67.58, 98.22, 172.25, 173.63, 175.11. Calcd: $[M+Na]^+$ ($C_{52}H_{84}NaO_{24}$) m/z = 1115.59. Found: MALDI-TOF MS: $[M+Na]^+$ m/z = 1115.73. Anal. Calcd for $C_{52}H_{84}O_{24}$: C, 57.1; H, 7.75. Found: C, 56.9; H, 7.58.

Isopropylidene-[G-3]-[G-2]-Benzylidene (14). The isopropylidene-[G-3]-[G-1]-OH **13** (254 mg, 0.250 mmol, 1.0 equiv) and 4-(dimethylamino)pyridine (DMAP) (24 mg, 0.20 mmol, 0.80 equiv.) were dissolved in 6 mL of CH_2Cl_2 and 2 mL of pyridine was added. Anhydride **11** (430 mg, 1.0 mmol, 4.0 equiv) was added, and the reaction mixture was stirred at room temperature overnight. The excess anhydride was quenched by stirring the reaction mixture with 3 mL of a 1:1 pyridine:water solution overnight. The organic phase was diluted with 80 mL of CH_2Cl_2 and extracted with 1 M $NaHSO_4$ (3 × 40 mL), 10% Na_2CO_3 (3 × 40 mL), and saturated brine (40 mL). The organic phase was dried with $MgSO_4$, filtered, and the filtrate evaporated to give 334 mg (94%) of **14** as a glassy solid. IR (cm^{-1}): 3050, 1736. ^{1}H NMR ($CDCl_3$): δ 0.91 (s, 3), 0.97 (s, 6), 1.14 (s, 12), 1.27 (s, 6), 1.28 (s, 3), 1.36 (s, 12), 1.42 (s, 12), 3.61−3.63 (m, 12), 3.91 (s, 2), 3.95 (s, 4), 4.18 (d, 8, J = 11.5), 4.31−4.37 (m, 8), 4.40 (d, 2, J = 11.0), 4.47 (d, 2, J = 11.0), 4.58−4.61 (m, 4), 5.43 (s, 2), 7.30−7.43 (m, 6), 7.88−7.90 (m, 4). ^{13}C NMR ($CDCl_3$): δ 16.83, 17.76, 17.77, 17.79, 18.53, 21.94, 25.42, 39.04, 42.09, 42.65, 46.96, 47.08, 64.90, 65.19, 65.71, 65.79, 65.99, 73.52, 98.13, 101.71, 126.23, 128.16, 128.91, 137.89, 172.00, 172.11, 173.25, 173.53. Calcd: $[M+Na]^+$ ($C_{76}H_{108}NaO_{30}$) m/z = 1523.74. Found: MALDI-TOF MS: $[M+Na]^+$ m/z = 1524.67. Anal. Calcd for $C_{76}H_{108}O_{30}$: C, 60.8; H, 7.25. Found: C, 60.4; H, 6.98.

Isopropylidene-[G-3]-[G-2]-OH (15). Isopropylidene-[G-3]-[G-2]-benzylidene **14** (334 mg, 0.234 mmol) was dissolved in EtOAc and 50 mg of 10% Pd/C was added. The procedure described above was repeated to give 280 mg (96%) of **15** as a white glass. IR (cm^{-1}): 3487, 1740. ^{1}H NMR ($CDCl_3$): δ 1.01 (s, 3), 1.03 (s, 6), 1.08 (s, 12), 1.25 (s, 6), 1.27 (s, 3), 1.29 (s, 12), 1.36 (s, 12), 3.34 (s, 4), 3.57 (d, 8, J =

12.5), 3.64 (dd, 4, $J = 11.2, 4.8$), 3.73 (dd, 4, $J = 11.2, 3.0$), 4.01 (s, 4), 4.02 (s, 2), 4.09 (d, 8, $J = 11.8$), 4.21 (d, 2, $J = 11.1$), 4.26−4.32 (m, 8), 4.36 (d, 2, $J = 11.1$). ^{13}C NMR ($CDCl_3$): δ 16.97, 17.14, 17.72, 18.07, 18.46, 21.70, 25.55, 39.11, 42.10, 46.66, 47.01, 49.89, 64.68, 64.96, 65.94, 65.99, 66.76, 66.83, 98.15, 172.13, 172.44, 173.61, 174.90. Calcd: $[M+Na]^+$ ($C_{62}H_{100}NaO_{30}$) $m/z = 1347.79$. Found: MALDI-TOF MS: $[M+Na]^+$ $m/z = 1350.50$. Anal. Calcd for $C_{62}H_{100}O_{30}$: C, 56.2; H, 7.61. Found: C, 56.0; H, 7.46.

Isopropylidene-[G-3]-[G-3]-Benzylidene (16). Isopropylidene-[G-3]-[G-2]-OH **15** (280 mg, 0.224 mmol, 1.0 equiv) and DMAP (44 mg, 0.36 mmol, 1.6 equiv.) were dissolved in 8 mL of CH_2Cl_2 and 2 mL of pyridine was added. Anhydride **11** (770 mg, 1.8 mmol, 8.0 equiv) was added, and the reaction mixture was stirred at room temperature overnight. The excess anhydride was quenched by stirring the reaction mixture with 5 mL of a 1:1 pyridine:water solution overnight. The organic phase was diluted with 80 mL of CH_2Cl_2 and extracted with 1 M $NaHSO_4$ (3 × 40 mL), 10% Na_2CO_3 (3 × 40 mL), and saturated brine (40 mL). The organic phase was dried with $MgSO_4$, filtered, and the filtrate evaporated to give 430 mg (93%) of **16** as a glassy solid. IR (cm^{-1}): 3045, 1738. ^{1}H NMR ($CDCl_3$): δ 0.92 (s, 12), 0.96 (s, 3), 1.03 (s, 3), 1.12 (s, 12), 1.21 (s, 6), 1.28 (s, 6), 1.34 (s, 12), 1.39 (s, 12), 3.56−3.61 (m, 16), 3.98−4.05 (m, 8), 4.13 (d, 8, $J = 11.5$), 4.30−4.38 (m, 16), 4.55 (d, 8, $J = 9.5$), 5.39 (s, 4), 7.26−7.34 (m, 12), 7.37−7.41 (m, 8). ^{13}C NMR ($CDCl_3$): δ 16.84, 17.18, 17.66, 17.70, 18.47, 21.91, 25.34, 39.05, 42.01, 42.54, 46.58, 46.86, 46.90, 64.82, 65.06, 65.42, 65.76, 65.91, 73.42, 98.05, 101.61, 126.17, 128.09, 128.82, 137.88, 171.55, 171.85, 171.97, 173.16, 173.45. Calcd: $[M+Na]^+$ ($C_{110}H_{148}NaO_{42}$) $m/z = 2164.2$. Found: MALDI-TOF MS: $[M+Na]^+$ $m/z = 2165.4$. Anal. Calcd for $C_{110}H_{148}O_{42}$: C, 61.7; H, 6.96. Found: C, 61.6; H, 6.90.

Isopropylidene-[G-3]-[G-3]-OH (17). Isopropylidene-[G-3]-[G-3]-benzylidene **16** (480 mg, 0.232 mmol) was dissolved in EtOAc and 100 mg of 10% Pd/C was added. The procedure described above was repeated to give 374 mg (94%) of **17** as a white glass. IR (cm^{-1}): 3470, 1740. ^{1}H NMR ($CDCl_3$): δ 1.01 (s, 3), 1.03 (s, 12), 1.06 (s, 15), 1.23 (s, 9), 1.25 (s, 6), 1.28 (s, 12), 1.35 (s, 12), 3.56 (d, 8, $J = 12.0$), 3.62 (d, 8, $J = 11.0$), 3.68 (d, 8, $J = 11.0$), 4.01 (s, 6), 4.08 (d, 8, $J = 12.0$), 4.18−4.32 (m, 20). ^{13}C NMR ($CDCl_3$): δ 17.01, 17.17, 17.59, 17.76, 18.05, 18.50, 21.73, 25.69, 33.88, 39.20, 42.15, 46.61, 47.03, 50.04, 64.78, 64.98, 65.68, 66.01, 66.20, 66.37, 66.47, 98.22, 171.81, 172.21, 172.46, 173.66, 174.99. Calcd: $[M+Na]^+$ ($C_{82}H_{132}NaO_{42}$) $m/z = 1812.0$. Found: MALDI-TOF MS: $[M+Na]^+$ $m/z = 1814.8$. Anal. Calcd for $C_{82}H_{132}O_{42}$: C, 55.0; H, 7.43. Found: C, 55.0; H, 7.58.

Isopropylidene-[G-3]-[G-1]-*p*-nitrophenyl Carbonate (19). Isopropylidene-[G-3]-[G-1]-OH **13** (100 mg, 0.098 mmol, 1.0 equiv) was dissolved in 5 mL of CH_2Cl_2 and 0.15 mL of pyridine was added, followed by *p*-nitrophenyl chloroformate (160 mg, 0.79 mmol, 8.0 equiv). The reaction mixture was stirred overnight under a nitrogen atmosphere and then was diluted with 50 mL of CH_2Cl_2 and washed with 1 M $NaHSO_4$ (2 × 30 mL) and saturated brine (30 mL). The organic phase was dried with $MgSO_4$, filtered, and the filtrate was evaporated. The product was purified by column chromatography using CH_2Cl_2 to elute the excess *p*-nitrophenyl chloroformate followed by a gradient from CH_2Cl_2/EtOAc (80:20) to CH_2Cl_2/EtOAc (50:50) to provide 100 mg (76%) of the product **19** as a colorless glass. IR (cm^{-1}): 3087, 1775, 1738, 1618, 1594, 1529. ^{1}H NMR ($CDCl_3$): δ 1.07 (s, 3), 1.09 (s, 12), 1.27 (s, 6), 1.31 (s, 12), 1.38 (s, 12), 1.39 (s, 3), 3.59 (d, 8, $J = 13.0$), 4.04 (d, 2, $J = 11.0$), 4.08 (d, 2, $J = 11.0$), 4.10−4.13 (m, 10), 4.30 (d, 4, $J = 11.0$), 4.34 (d, 4, $J = 11.0$), 4.47 (d, 2, $J = 11.0$), 4.54 (d, 2, $J = 11.0$), 7.35−7.42 (m, 4), 8.23−8.30 (m, 4). ^{13}C NMR ($CDCl_3$): δ 17.19, 17.86, 17.88, 18.62, 21.80, 25.71, 39.36, 42.24, 46.90, 47.16, 65.02, 65.95, 66.02, 66.12, 69.35, 98.27, 121.98, 125.50, 145.74, 152.31, 155.40, 171.35, 172.22, 173.69. Calcd: $[M+Na]^+$ ($C_{66}H_{90}NaN_2O_{32}$) $m/z = 1445.71$. Found: MALDI-TOF MS: $[M+Na]^+$ $m/z = 1445.44$. Anal. Calcd for $C_{66}H_{90}N_2O_{32}$: C, 55.7; H, 6.37; N, 1.97. Found: C, 55.8; H, 6.50; N, 1.86.

Isopropylidene-[G-3]-[G-2]-*p*-nitrophenyl Carbonate (20). Isopropylidene-[G-3]-[G-2]-OH **15** (110 mg, 0.088 mmol, 1.0 equiv) was dissolved in 5 mL of CH_2Cl_2 and 0.25 mL of pyridine was added, followed by *p*-nitrophenyl chloroformate (280 mg, 1.4 mmol, 16 equiv). The reaction mixture was stirred overnight under a nitrogen atmosphere and then was diluted with 50 mL of CH_2Cl_2 and washed with 1 M $NaHSO_4$ (2 × 30 mL) and saturated brine (30 mL). The organic phase was dried with $MgSO_4$, filtered, and the filtrate was evaporated. The product was purified by column chromatography using CH_2Cl_2 to elute the excess *p*-nitrophenyl chloroformate followed by a gradient from CH_2Cl_2/EtOAc (80:20) to CH_2Cl_2/EtOAc (50:50) to provide 105 mg (63%) of the product **20** as a colorless glass. IR (cm^{-1}): 3090, 1770, 1738, 1616, 1594, 1527. ^{1}H NMR ($CDCl_3$): δ 1.03 (s, 3), 1.09 (s, 12), 1.26 (s, 6), 1.31 (s, 12), 1.34 (s, 3), 1.37 (s, 6), 1.39 (s, 12), 3.60 (d, 8, $J = 12.5$), 4.01−4.07 (m, 8), 4.11 (d, 8, $J = 12.0$), 4.29−4.52 (m, 18), 7.33−7.38 (m, 8), 8.22−8.27 (m, 8). ^{13}C NMR ($CDCl_3$): 14.29, 17.15, 17.85, 18.62, 21.75, 22.82, 25.80, 31.75, 42.26, 46.81, 46.99, 47.14, 64.99, 65.56, 65.92, 66.13, 66.25, 69.31, 98.29, 121.97, 125.52, 145.76, 152.27, 155.38, 171.25, 171.71, 172.27, 173.71. Calcd: $[M+Na]^+$ ($C_{90}H_{112}O_{46}NaN_4$) $m/z = 2007.9$. Found: MALDI-TOF MS: $[M+Na]^+$ $m/z = 2008.0$. Anal. Calcd for $C_{90}H_{112}O_{46}N_4$: C, 54.4; H, 5.69; N, 2.82. Found: C, 54.6; H, 5.77; N, 2.67.

Isopropylidene-[G-3]-[G-3]-*p*-nitrophenyl Carbonate (21). Isopropylidene-[G-3]-[G-3]-OH **17** (150 mg, 0.084 mmol, 1.0 equiv) was dissolved in 5 mL of CH_2Cl_2 and 0.5 mL of pyridine was added, followed by *p*-nitrophenyl chloroformate (541 mg, 2.68 mmol, 32 equiv). The reaction mixture was stirred overnight under a nitrogen atmosphere and then was diluted with 50 mL of CH_2Cl_2 and washed with 1 M $NaHSO_4$ (2 × 30 mL) and saturated brine (30 mL). The organic phase was dried with $MgSO_4$, filtered, and the filtrate was evaporated. The product was purified by column chromatography using CH_2Cl_2 to elute the excess *p*-nitrophenyl chloroformate followed by CH_2Cl_2/EtOAc (85:15) to provide 110 mg (42%) of the product **21** as a colorless glass. IR (cm^{-1}): 3084, 1774, 1738, 1618, 1594, 1528. ^{1}H NMR ($CDCl_3$): δ 1.02 (s, 3), 1.09 (s, 12), 1.24 (s, 6), 1.27 (s, 6), 1.30 (s, 3), 1.31 (s, 12), 1.35 (s, 12), 1.38 (s, 12), 3.60 (d, 8, $J = 12.0$), 4.01−4.04 (m, 6), 4.12 (d, 8, $J = 12.0$), 4.23 (s, 4), 4.29−4.38 (m, 16), 4.42 (d, 8, $J = 11.0$), 4.50 (d, 8, $J = 11.0$), 7.33−7.35 (m, 16), 8.20−8.24 (m, 16). ^{13}C NMR ($CDCl_3$): 14.34, 17.07, 17.56, 17.76, 17.86, 18.59, 21.66, 25.86, 29.83, 39.28, 42.26, 46.79, 46.83, 47.12, 64.95, 65.50, 65.85, 65.92, 66.11, 66.36, 69.26, 98.29, 121.94, 125.48, 145.74, 152.25, 155.35, 171.24, 171.54, 171.65, 172.28, 173.73. Calcd: $[M+H]^+$ ($C_{138}H_{157}N_8O_{74}$) $m/z = 3110.3$. Found: MALDI-TOF MS: $[M+H]^+$ $m/z = 3104.3$. Anal. Calcd for $C_{138}H_{156}N_8O_{74}$: C, 53.3; H, 5.06; N, 3.60. Found: C, 53.5; H, 5.10; N, 3.44.

General Procedure for Attachment of Poly(ethylene oxide) to the *p*-Nitrophenyl Carbonate Activated Dendrimers. A solution of isopropylidene-[G-3]-[G-n]-*p*-nitrophenyl carbonate in benzene was added to PEO−NH_2 (1.2 equiv per *p*-nitrophenyl carbonate) and additional benzene was added for good stirring. A solution in benzene containing DMAP (0.5 equiv per *p*-nitrophenyl carbonate) and diisopropylethylamine (1 equiv per *p*-nitrophenyl carbonate) was added and the reaction mixture was stirred at room temperature overnight. The product was precipitated into diethyl ether, filtered through a glass filter, and washed with ether to afford the product as a white powder.

General Procedure for Purification of Hybrids 22−29 by Dialysis. A Spectra/ Por Cellulose Ester Membrane with a MW cutoff of 100 kDa from Spectrum Laboratories was used for all samples. Polymer solutions (~25 mg/mL) were dialyzed against distilled water, changing the water every 12 h. Dialyses were stopped after 18 h for removal of the 5 kDa PEO−NH_2 and after 48 or 96 h for removal of 10 kDa or 20 kDa PEO−NH_2, respectively. The resulting polymer solutions were lyophilized to provide the pure conjugates.

General Procedure for Acylation of Excess PEO−NH_2. The product from the reaction with PEO−NH_2 was dissolved in a minimum volume of CH_2Cl_2 and pyridine (200 equiv) was added. Acetic

anhydride (100 equiv) was added, followed by DMAP (10 equiv), and the reaction mixture was stirred overnight. The product was precipitated into diethyl ether and filtered through a glass filter two times to provide the product as a white powder.

Isopropylidene-[G-3]-[G-1]-PEO 10 kDa (22). IR (cm^{-1}): 3352 (w), 2885 (s), 1738, 1727. ^1H NMR ($CDCl_3$): δ 1.01 (s, 3), 1.09 (s, 12), 1.18 (s, 3), 1.26 (s, 6), 1.31 (s, 12), 1.37 (s, 12), 1.94 (s, ~1), 3.34 (s, ~7), 3.46−3.79 (m, ~2200), 3.96−4.03 (m, 6), 4.09−4.19 (m, 12), 4.28−4.33 (m, 8), 5.42 (s, ~2). Anal. Found: C, 54.5; H, 9.30; N, 0.19.

Isopropylidene-[G-3]-[G-1]-PEO 20 kDa (23). IR (cm^{-1}): 3468, 2888, 1736, 1730. ^1H NMR ($CDCl_3$): δ 1.02 (s, 3), 1.10 (s, 12), 1.19 (s, 3), 1.27 (s, 6), 1.32 (s, 12), 1.38 (s, 12), 1.94 (s, ~1), 3.35 (s, ~7), 3.46−3.76 (m, ~5000), 4.00−4.29 (m, 6), 4.10−4.20 (m, 12), 4.29−4.34 (m, 8), 5.45 (s, ~2). Anal. Found: C, 54.7; H, 9.37; N, 0.17.

Isopropylidene-[G-3]-[G-2]-PEO 5 kDa (24). IR (cm^{-1}): 3375, 2882, 1741, 1727. ^1H NMR ($CDCl_3$): δ 1.00 (s, 3), 1.07 (s, 12), 1.13 (s, 6), 1.20 (s, 3), 1.24 (s, 6), 1.28 (s, 12), 1.35 (s, 12), 1.92 (s, ~3), 3.31 (s, ~12), 3.43−3.77 (m, ~2100), 3.97−4.01 (m, 6), 4.07−4.18 (m, 18), 4.25−4.31 (m, 10), 5.47 (s, ~4). Anal. Found: C, 54.8; H, 9.33; N, 0.26.

Isopropylidene-[G-3]-[G-2]-PEO 10 kDa (25). IR (cm^{-1}): 3350, 2884, 1738, 1732. ^1H NMR ($CDCl_3$): δ 1.00 (s, 3), 1.07 (s, 12), 1.12 (s, 6), 1.20 (s, 3), 1.24 (s, 6), 1.28 (s, 12), 1.34 (s, 12), 1.91 (s, ~3), 3.31 (s, ~12), 3.43−3.72 (m, ~4200), 3.98−4.02 (m, 6), 4.06−4.18 (m, 18), 4.24−4.31 (m, 10), 5.45 (s, ~4). Anal. Found: C, 54.6; H, 9.22; N, 0.15.

Isopropylidene-[G-3]-[G-2]-PEO 20 kDa (26). IR (cm^{-1}): 2884, 1738, 1727. ^1H NMR ($CDCl_3$): δ 1.01 (s, 3), 1.08 (s, 12), 1.14 (s, 6), 1.21 (s, 3), 1.25 (s, 6), 1.29 (s, 12), 1.36 (s, 12), 1.91 (s, ~ 3), 3.33 (s, ~12), 3.45−3.77 (m, ~9000), 3.99−4.03 (m, 6), 4.07−4.19 (m, 18), 4.23−4.32 (m, 10), 5.47 (s, ~4). Anal. Found: C, 54.7; H, 9.36; N, 0.20.

Isopropylidene-[G-3]-[G-3]-PEO 5 kDa (27). IR (cm^{-1}): 3344, 2889, 1736, 1730. ^1H NMR ($CDCl_3$): δ 1.02 (s, 3), 1.09 (s, 12), 1.13 (s, 12), 1.20 (s, 6), 1.24 (s, 3), 1.26 (s, 6), 1.30 (s, 12), 1.36 (s, 12), 1.94 (s, ~3), 3.34 (s, ~30), 3.47−3.78 (m, ~4800), 4.01−4.34 (m, 50), 5.52 (s, ~8). Anal. Found: C, 54.6; H, 9.28; N, 0.28.

Isopropylidene-[G-3]-[G-3]-PEO 10 kDa (28). IR (cm^{-1}): 3345, 2885, 1737, 1730. ^1H NMR ($CDCl_3$): δ 1.02 (s, 3), 1.09 (s, 12), 1.13 (s, 12), 1.20 (s, 6), 1.24 (s, 3), 1.26 (s, 6), 1.30 (s, 12), 1.36 (s, 12), 1.94 (s, ~3), 3.33 (s, ~30), 3.45−3.75 (m, ~8600), 4.01−4.26 (m, 50), 5.52 (s, ~8). Anal. Found: C, 54.66; H, 9.03; N, 0.19.

Isopropylidene-[G-3]-[G-3]-PEO 20 kDa (29). IR (cm^{-1}): 2885, 1736, 1730. ^1H NMR ($CDCl_3$): δ 1.02 (s, 3), 1.08 (s, 12), 1.13 (s, 12), 1.20 (s, 6), 1.24 (s, 3), 1.26 (s, 6), 1.29 (s, 12), 1.36 (s, 12), 1.94 (s, ~4), 3.33 (s, ~33), 3.45−3.78 (m, ~18 000), 4.01−4.26 (m, 50), 5.53 (s, ~8). Anal. Found: C, 55.1; H, 9.15; N, 0.40.

General Procedure for Acetonide Deprotection. The above PEO-modified product was dissolved in methanol (~30 mg/mL) and concentrated sulfuric acid was added to a concentration of 2% v/v. The reaction mixture was stirred at room temperature for 3 h. The sulfuric acid was neutralized with ammonia in methanol, resulting in ammonium sulfate as a white precipitate. The precipitate was removed by filtration over glass wool and the filtrate evaporated to provide the product as a white solid. Residual salt was removed by dialysis using GibcoBRL tubing with a MW cutoff of 12−14 kDa from Life Technologies against distilled water. The resulting polymer solution was lyophilized to give the product as a white powder.

HO-[G-3]-[G-1]-PEO 10 kDa (30). IR (cm^{-1}): 3440, 2887, 1734, 1726. ^1H NMR ($CDCl_3$): δ 1.04 (s, 3), 1.06 (s, 12), 1.21 (s, 3), 1.30 (s, 6), 1.96 (s, ~1), 3.36 (s, ~7), 3.47−3.80 (m, ~2100), 4.04 (s, 6), 4.09−4.20 (m, 4), 4.28−4.35 (m, 8), 5.74 (m, ~2). Anal. Found: C, 54.4; H, 9.3; N, 0.21.

HO-[G-3]-[G-1]-PEO 20 kDa (31). IR (cm^{-1}): 3460, 2884, 1737, 1732. ^1H NMR ($CDCl_3$): δ 1.04 (s, 3), 1.06 (s, 12), 1.21 (s, 3), 1.29 (s, 6), 1.93 (s, ~1), 3.32 (s, ~7), 3.46−3.76 (m, ~4600), 4.04 (s, 6), 4.10−4.19 (m, 4), 4.28−4.35 (m, 8), 5.75 (m, ~2). Anal. Found: C, 54.4; H, 8.82; N, 0.11.

HO-[G-3]-[G-2]-PEO 5 kDa (32). IR (cm^{-1}): 3450, 3340, 2884, 1737, 1727. ^1H NMR ($CDCl_3$): δ 1.06 (s, 3), 1.07 (s, 12), 1.19 (s, 6), 1.25 (s, 3), 1.30 (s, 6), 1.97 (s, ~2), 3.36 (s, ~12), 3.47−3.77 (m, ~2200), 4.06−4.15 (m, 12), 4.24−4.36 (m, 10), 5.66 (s, ~4). Anal. Found: C, 54.4; H, 9.25; N, 0.26.

HO-[G-3]-[G-2]-PEO 10 kDa (33). IR (cm^{-1}): 3441, 3353, 2885, 1738, 1732. ^1H NMR ($CDCl_3$): δ 1.06 (s, 3), 1.07 (s, 12), 1.18 (s, 6), 1.25 (s, 3), 1.30 (s, 6), 1.96 (s, ~3), 3.36 (s, ~14), 3.47−3.81 (m, ~4400), 4.06−4.15 (m, 12), 4.24−4.35 (m, 10), 5.68 (s, ~4). Anal. Found: C, 54.7; H, 9.30; N, 0.18.

HO-[G-3]-[G-2]-PEO 20 kDa (34). IR (cm^{-1}): 3202, 2884, 1738, 1730. ^1H NMR ($CDCl_3$): δ 1.04 (s, 3), 1.05 (s, 12), 1.16 (s, 6), 1.23 (s, 3), 1.28 (s, 6), 1.94 (s, ~3), 3.33 (s, ~16), 3.45−3.75 (m, ~8800), 4.06−4.15 (m, 12), 4.24−4.35 (m, 10), 5.68 (s, ~4). Anal. Found: C, 54.2; H, 9.20; N, 0.19.

HO-[G-3]-[G-3]-PEO 5 kDa (35). IR (cm^{-1}): 3453, 3348, 2884, 1738, 1728. ^1H NMR ($CDCl_3$): δ 1.06 (s, 15), 1.15 (s, 12), 1.22 (s, 6), 1.26 (s, 3), 1.28 (s, 6), 1.95 (s, ~4), 3.34 (s, ~25), 3.47−3.78 (m, ~4200), 4.02−4.36 (m, 36) 5.64 (s, ~8). Anal. Found: C, 54.4; H, 9.27; N, 0.26.

HO-[G-3]-[G-3]-PEO 10 kDa (36). IR (cm^{-1}): 3440, 3360, 2887, 1730, 1737. ^1H NMR ($CDCl_3$): δ 1.08 (s, 15), 1.17 (s, 12), 1.23 (s, 6), 1.28 (s, 3), 1.30 (s, 6), 1.94 (s, ~4), 3.36 (s, ~24), 3.45−3.80 (m, ~8000), 4.01−4.38 (m, 36), 5.66 (s, ~8). Anal. Found: C, 54.7; H, 9.32; N, 0.15.

HO-[G-3]-[G-3]-PEO 20 kDa (37). IR (cm^{-1}): 2886, 1741, 1726. ^1H NMR ($CDCl_3$): δ 1.07 (s, 15), 1.16 (s, 12), 1.22 (s, 6), 1.27 (s, 3), 1.29 (s, 6), 1.96 (s, ~5), 3.33 (s, ~26), 3.46−3.76 (m, ~16 000), 4.02−4.37 (m, 36), 5.65 (s, ~8). Anal. Found: C, 56.0; H, 9.51; N, 0.10.

Determination of Amine Content Using the Fluorescamine Assay. A calibration curve was prepared using commercial PEO−NH_2 as a standard at concentrations ranging from 0 to 180 μM. The MW corresponded to that used for preparation of the hybrid system being analyzed (e.g., 5 kDa, 10 kDa, or 20 kDa) and the mass of polymer used was calculated on the basis of the MW determined by MALDI-TOF MS. The polymer standard or sample was dissolved in 3.45 mL of pH 9 buffer (1 M K_2CO_3/$KHCO_3$), and 150 μL of a 3.0 mg /mL solution of fluroescamine in acetone was added. After mixing, the solutions of standards and samples were allowed to stand at room temperature for 10 min before taking the fluorescence reading.

Acknowledgment. We thank the National Institute of Health (GM 65361) for support of this research. Thanks are also due to Professors Matthew Francis (UCB) and Frank Szoka, Jr. (UCSF) for useful discussions on the design of this family of macromolecules.

JA028100N