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Synthesis and self-assembly properties of a novel [poly(ethylene glycol)]–fluorocarbon–phospholipid triblock copolymer

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Abstract—The synthesis of a novel poly(ethylene glycol)–fluorocarbon–phospholipid conjugate that self-assembles into hyper-stable micelles characterized by an internal fluorous phase is described. Physical characterization of the micelles formed by this polymer in aqueous solution is included. Dynamic light scattering (DLS) measurements indicate a mean diameter of 15 nm (±3 nm), while pyrene fluorescence studies show a critical micelle concentration (CMC) of only 0.65 μM.

Amphiphilic block copolymers that assemble into micelles in aqueous solutions have found several applications in drug delivery. Biocompatible micelles can be used to solubilize, stabilize, and deliver pharmaceutical agents.[1,2] The use of micelles is advantageous due to their unique hydrophobic inner core that can sequester sparingly soluble hydrophobic molecules. These nanoparticles can find use in drug delivery for highly hydrophobic drugs, for drugs that are not very stable under physiological conditions, and as a tool to direct delivery to specific tissues or organs. Polymeric micelles with phospholipid or poly-amino acid core blocks have been shown to encapsulate pharmaceuticals such as doxorubicin, amphotericin-B, and paclitaxel.[3–5] Also, they have been shown to preferentially accumulate in the leaky vasculature of cancerous tumors due to their relatively small size (under 100 nm).[5] Polymeric PEG-based micelles exhibit potential as delivery vessels for pharmaceuticals by likely overcoming obstacles such as poor water solubility of drug candidates, drug bioavailability, and other harmful side-effects via encapsulation of hydrophobic compounds in the micelle inner core hydrophobic environment.

Although micelles have the potential for sustained release formulations, the issue of micelle stability in vivo has yet to be fully resolved so that they may see broad applicability in the area. In order for a micelle to realize potential as a delivery vessel, it must be stable in the presence of blood proteins, such as albumin. Unfortunately, many micelles do not satisfy this condition. Also, the ability of a micelle to maintain its integrity when diluted in vivo is an important factor. Micelles formed from PEG–lipid conjugates are characterized by CMCs on the order of 10^{-5} M and half-lives in the blood stream between 1.2 and 2.0 h, depending on the size of the PEG incorporated into the polymer.[5] We have addressed the problem of the in vivo stability of phospholipid conjugates by designing a novel triblock copolymer that adds a perfluorinated group between the water-solubilizing PEG and the phospholipid (Fig. 1).

A perfluorinated group has peculiar physical and chemical properties. It is both hydrophobic and lipophobic at the same time. As a matter of fact, perfluorocarbons prefer to form a separated fluorous phase rather than mix with either hydrophilic or hydrophobic molecules. This phenomenon, known as the fluorophobic effect, allows the self-assembly of highly fluorinated molecules in strict analogy to the hydrophobic effect.[6,7] In the case of polymer **1**, the resulting micelles become stabilized by two different effects. The internal phospholipid forms a classical hydrophobic inner core while the intermediate fluorocarbon assembles with the corresponding perfluorocarbon chains from vicinal polymer molecules to generate an intermediate fluorous shell that

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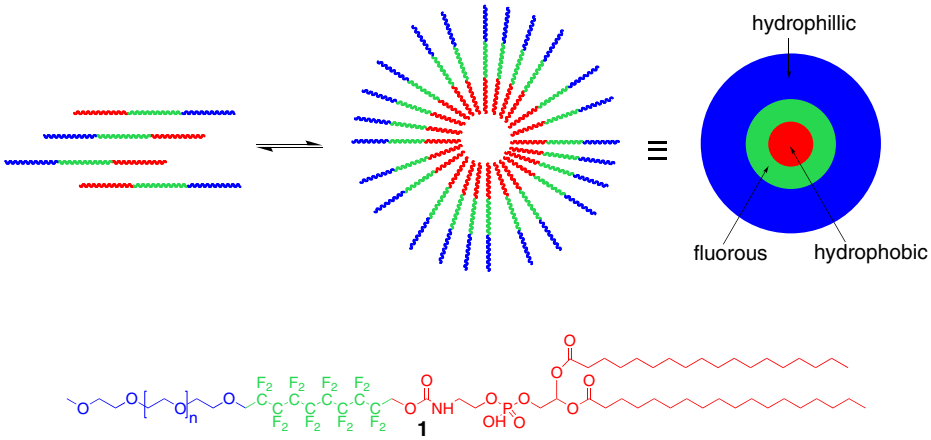


Figure 1. Poly(ethylene glycol) monomethyl ether–fluorocarbon–DSPE conjugate **1** and the corresponding micelles characterized by an intermediate fluorous shell and a hydrophobic inner core.

contributes to the stability of the micelle and protects a drug encapsulated in the inner core. The advantage of using semi-fluorinated polymers in drug delivery is multifold: (i) It allows the formation of hyper-stable micelles due to a combination of hydrophobic and fluorophobic effects. (ii) It allows more direct control of the rate of delivery through the size of the fluorocarbon. (iii) It allows better protection of the encapsulated pharmaceutical. (iv) It confers exceptional resistance to micellar dissociation induced by binding to blood proteins. Therefore, this new approach combines the usefulness of micellar delivery with the flexibility of rational design that can control both the rate of release and the stability of the micelles.

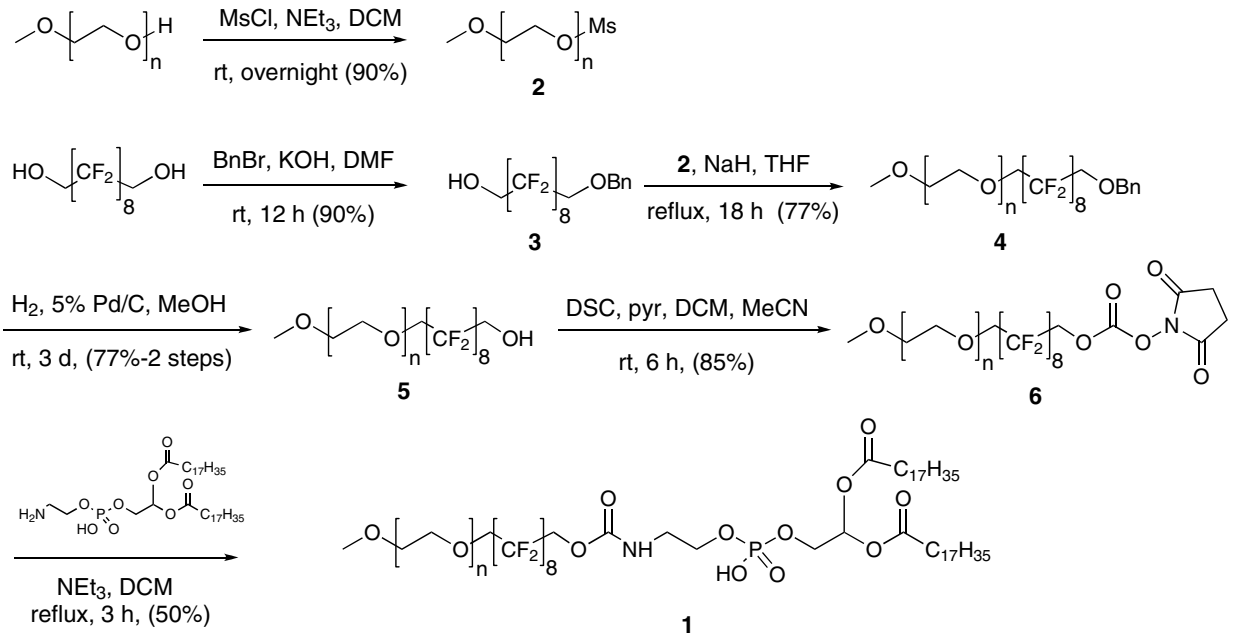
The use of fluorinated surfactants for biomedical purposes has been explored extensively for liposomes and vesicles.[8–10] Recently, micelles formed by a semi-fluorinated diblock copolymer have been shown to encapsulate highly fluorinated molecules such as volatile anesthetics.[11] In the design of molecule **1**, we are taking advantage of the ability of perfluorocarbons to self-assemble in aqueous solutions to generate a PEG–fluorocarbon–phospholipid conjugate that can form hyper-stable micelles with the potential of encapsulating classical hydrophobic drugs.

Polymer **1**, composed of a monomethylated [poly(ethylene glycol)] characterized by an average molecular weight of 5000 g/mol, a perfluorooctyl group, and the phospholipid 1,2-distearoyl-*S*,*N*-glycero-3-phosphoethanolamine (DSPE) has been synthesized through a five-step synthesis. Preliminary characterization of micelles formed from the tri-block conjugate shows a mean diameter of 15 nm (±3 nm) and a critical micelle concentration (CMC) of 0.65 mM. This is a considerably lower CMC than that of the analogous PEG–DSPE conjugate (6.2 mM).[5]

Design of the mPEG–fluorocarbon–lipid conjugate **1** was modeled on earlier PEG–lipid conjugates. Polyethylene glycol (PEG) is by far the most common choice for the hydrophilic component of micelles and liposomes. Its behavior is well studied both in vitro and in vivo, and it is one of the few polymers approved by the FDA for use in cosmetics and pharmaceuticals. PEG is known to lack immunogenicity, and it has been shown that pegylation of liposomes increases the half-life, decreases drug leaking, and decreases uptake by the reticuloendothelial system.[12]

The phospholipid DSPE was chosen because it is known to assist in encapsulation of compounds such as the anti-cancer drugs doxorubicin and paclitaxel as well as the toxic fungicide amphotericin-B.[5,13,14] Its use in the synthesis of PEG–lipid conjugates for the assembly into micelles is well established.[15–17]

Synthesis of the mPEG–fluorocarbon–DSPE conjugate **1** is described in Scheme 1.[18] Initially, the hydroxyl functionality of mPEG (MW = 5000 g/mol) was activated by reaction with methanesulfonyl chloride in dichloromethane at room temperature to give compound **2** (90%). Subsequently, 2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9-hexadecafluorodecane-1,10-diol was mono-protected with benzyl bromide using crushed KOH in *N*,*N*-dimethylformamide at room temperature to give benzyl ether **3** (90%). To ensure that mono- versus di-protection was favored, the perfluorinated diol was used in a 3:1 excess during the reaction. A trace amount of di-protected material was detected by thin-layer chromatography, but this by-product was easily separated from the desired mono-protected diol via flash column chromatography. The mono-protected alcohol **3** was coupled to the activated mPEG **2** using an excess of sodium hydride in refluxing tetrahydrofuran to give the benzyl-protected mPEG–hexadecafluorodecanol **4**. After quenching with water, the reaction mixture was filtered to remove salts. Isolation of PEG derivatives is often accomplished by precipitation of the hydrophilic polymer from a mixture of solvents. In this specific case, a solution of tetrahydrofuran and cold diethyl ether was used to isolate intermediate conjugate **4**, which was carried on to the next step without further purification. Hydrogenolysis of **4** removed the benzyl group and yielded the corresponding de-protected alcohol **5**. The



Scheme 1. Synthesis of poly(ethylene glycol) monomethyl ether–fluorocarbon–DSPE conjugate (**1**).

alcohol was activated with an excess of *N*,*N*′-disuccinimidyl carbonate (DSC) to give the desired succinimidyl derivative **6**.[19] ^{1}H NMR showed a signal for un-reacted DSC at 2.65 ppm (<10%). In order to remove the excess DSC, the mixture was dissolved in acetone and the product precipitated by adding diethyl ether. A slight excess of 1,2-distearoyl-*sn*-glycero-3-phospho-ethanolamine (DSPE) and triethylamine was used in an attempt to drive the coupling of **5** to the lipid. The excess amine was removed by quenching with acetic acid, followed by removal of dichloromethane in vacuo. Any excess lipid was removed by taking up the remaining residue in water and filtering out the resulting solids. The filtrate was lyophilized to give a white fluffy solid. HPLC analysis of the solid indicated that a minor amount (<15%) of PEG–perfluoroalkyl conjugate **4** was still present. The mixture was purified by preparative HPLC using a Jordi-Gel reverse phase divinylbenzene column (500 Å 22 × 100 mm).

The mean diameter of the micelles formed by the polymer in aqueous solutions was measured using dynamic light scattering (DLS).[20] The volume-weighted distribution obtained by NICOMP analysis indicated a micellar mean diameter of 15 ± 3 nm.

Pyrene is often used to determine the onset of aggregation in micelles.[21] According to the py scale, the ratio of the intensities (I_1/I_3) of the first to the third peaks in the vibronic fluorescence spectrum of pyrene depends on the polarity of the environment.[22] In water, this ratio is approximately 1.87 and in hexanes it is approximately 0.58. Figure 2 illustrates the change in I_1/I_3 intensity with changing of the polymer concentration.[23] From this plot of I_1/I_3 versus co-polymer concentration of the CMC was determined to be 0.65 mM by taking the first point where the curve becomes non-linear. In

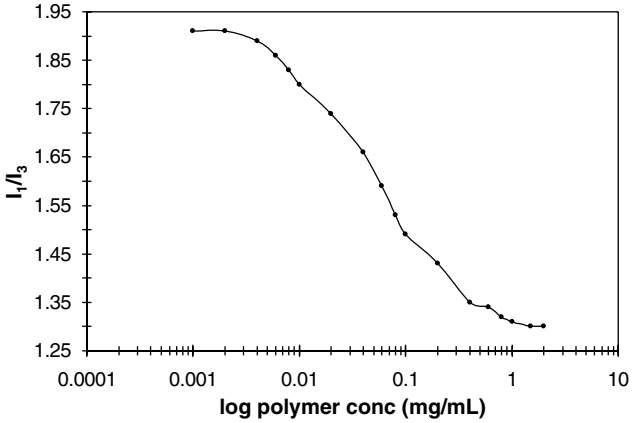


Figure 2. Change in ratio of the intensities (I_1/I_3) of the first to the third peaks in the vibronic fluorescence spectrum of pyrene at different polymer concentrations. From this plot, the CMC was determined to be 0.65 mM by taking the first point where the curve becomes non-linear.

summary, we have successfully synthesized a novel poly(ethylene glycol)–fluorocarbon–lipid conjugate **1** capable of self-assembling into micelles with a lower CMC than analogous PEG–lipid conjugates. This preliminary physical characterization points toward increased stability of the micelles, which can eventually lead to the use of these micelles as delivery vessels for sparingly soluble pharmaceuticals. Further stability, encapsulation, and release studies are currently underway in our laboratory.

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Supplementary data

Synthetic procedures and HPLC trace showing the purity of polymer **1**. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2007.03.148.

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