Synthesis, Characterization, and Properties of Phosphoryl Choline Functionalized Poly ϵ-caprolactone and Charged Phospholipid Analogues

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ABSTRACT: Here, we introduce the synthesis, characterization, and surface orientation properties of a new generation of biodegradable and biomimetic polymers. These phospholipid-mimetic, biodegradable polymers are synthesized by combining poly ϵ-caprolactone (PCL) with various functional polar end-groups, including zwitterionic phosphoryl choline (PC), anionic succinic acid, and cationic quaternary ammonium. The polyester backbone provides mechanical stability and biodegradability, whereas the various headgroups provide a variety of functions. The careful evaluation of the synthesis has allowed reaction conditions to be optimized, leading to complete conversion at each step and, subsequently, high yields of PCL−PC. Analysis of the ability for trimethylamine to cause any adverse transesterification reactions has shown that no molecular scrambling occurs either with two low-molecular-weight esters or with PCL. Therefore, the PCL initially present is retained throughout the synthesis. In parallel to the polymer synthesis, a model synthesis was conducted using benzyl 6-hydroxy hexanoate as a starting point, to aid the conversion studies and spectroscopic characterization. We ascertained from this feature that such synthesis allows the formation of any molecular architecture with targeted functionalities. The film properties of the PCL−PC amphiphile product revealed surface enrichment behavior, because the polar PC headgroup is oriented toward the surface. This followed the treatment of a cast film in a hydrophilic environment, as observed by electron spectroscopy for chemical analysis.

1. Introduction

The need to develop new materials to provide solutions to biomedical problems is currently one of the most challenging areas within polymer research. The research involved in developing, optimizing, and, therefore, fulfilling this need must be given much attention. Biodegradable and biocompatible polymers are frequently used to provide solutions to such biomedical problems. Indeed, it is because of their remarkable properties that they are, for instance, used in sutures and bioactive membranes.[1] Promising future applications include their use as scaffolds for tissue regeneration,[2] coatings for increased hemeocompatability,[3] replacement materials for eye lenses,[4] and various cosmetic solutions.[5] Other applications involve the requirement for new sophisticated and "smart" materials for active drug delivery.[6] In the United States, sales in this area alone amounted to 14 billion dollars by 1997.[7] Nano vectors for drug delivery could, for instance, have a pronounced effect by increasing the efficiency of drug release in cancer therapy. By releasing drugs specifically to the target tumor site, the amount of drugs and the risk of adverse side reactions could be decreased.

Using the cell membrane as a source of inspiration, we propose a new concept in the development of tailor-made biomaterials to meet some of these new needs. Just as phospholipids in the cell membrane enable dialogue with the cell's surroundings, our biodegradable polymeric amphiphile is equipped with biomimetic functional groups that may induce changes in local organization and influence biological functions. In so doing, we propose the introduction of a new type of biodegradable material. Surface activity and the ability to self-assemble/self-organize are properties that make such materials candidates for the formation of biomimetic membranes or particles with surfaces that are chemically identical to those of vesicles, which are the organelles that facilitate cellular communication in vivo. We believe that by designing our material from a cellular perspective, using biomimetic functional groups, the resulting material may interact favorably with biological systems. For example, the fate of blood-contacting implants is largely determined by their interaction with proteins, which mediate cellular interactions with synthetic substrates. Therefore, one way to improve the performance of polymers for medical applications is to control protein adsorption. This may be achieved by reducing the amount of protein adsorbed onto a surface.[3] A reduction in protein adsorption, in turn, reduces the activation of the immune system and platelet deposition. As a result, the initial coating of the foreign material with components that would lead to phagocytosis is prevented or at least delayed. Clotting of blood vessels and opsonization of drug-delivering particles in the blood stream could thereby be prevented.

Strategies for such improvements include heparin immobilization,[8] adsorption or grafting of hydrophilic polymers such as poly(ethylene glycol)[9] and poly(vinylpyrrolidone).[10] Another approach has been to introduce biomimetic groups in combination with a polymer. One of the most notable has been the use of phosphoryl choline (PC), which is a hydrophilic moiety in naturally occurring phospholipids present in the cell membrane. Chapman introduced a diacytelenic PC-containing monomer that was polymerized, which resulted in a polyconjugated polymer with enhanced blood compatability.[11] In parallel to this development, Kadoma[12] and later Umeda and Nakaya[13] introduced a methacrylic PC-

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containing monomer, 2-methacryloyloxyethyl phosphoryl choline (MPC), which was further used and exploited by Nakabayashi and co-workers.[14] A range of MPC-based copolymers has been developed with significantly improved blood compatibility that is due to reduced plasma protein adsorption.[15] In the quest for more-complicated architectures and compositions, Lobb and Ma have recently published work on enhancing the control of polymerization, using newly developed atom transfer radical polymerization (ATRP) techniques on the same monomer.[16] Furthermore, Ishihara and co-workers have, in recent publications, introduced biodegradability into PC-containing polymers.[17]

Ishihara and co-workers have given a plausible reason for reduced protein adsorption using these biomimetic polymers, and two different explanations have been given for this enhanced hemeocompatability. In both cases, the critical factor is the minimal interaction of plasma proteins with the polymer surface, such that activation of the blood cascade system is suppressed. It has been shown that the MPC moiety, in combination with polymers, such as methacrylates, has an ability to interact with phospholipids. This interaction has a pronounced stabilizing effect on the resulting biomembrane, thereby minimizing the protein−polymer interaction.[18] However, in a more recent publication, it is believed that the PC moiety has a strong hydrating property, also known as “free water”, which causes the hydrated PC group to act as a buffer to prevent protein adsorption.[19] In both cases, the ability of the plasma proteins to interact with the surface is limited, which results in a nonthrombogenic material with enhanced hemeocompatability.

Even if the immune response, using these techniques, can be suppressed for some time, most permanent implants are eventually recognized as foreign bodies. Therefore, it would be advantageous to develop materials with biomimetic and biodegradable surfaces, which, by the gradual detachment of attached proteins, during the degradation of the polymer, would be capable of withstanding any protein attack. Importantly, the surface must regenerate to maintain a low blood contact activation throughout its degradation, i.e., the biomimetic group should minimize its free energy by constantly emerging toward the surface. Therefore, the introduction of nonthrombogenic properties into biodegradable polymers is highly interesting and allows the development of materials with self-regenerating, antifouling surfaces with drug-releasing capabilities. Moreover, the possibility of drugs from loaded particles of such molecules reaching the target cell should increase, thereby facilitating a more precise and controlled transport and drug release. This should reduce unwanted side and release effects. In addition, the degradation leads to nontoxic products, which are readily metabolized by the human body.

In this study, we report the novel synthesis of a “new generation” of phospholipid analogues. Their synthesis combines the use of biodegradable polyesters, such as poly ϵ-caprolactone (PCL), with phosphoryl choline (PC), which is the hydrophilic moiety in the cell membrane. This report will focus on the synthetic aspects of this novel approach. Initially, we present the general synthetic scheme with additional studies on the possibility of base-catalyzed transesterfication from the nucleophilic trimethylamine (Me_3N) ring-opening agent used in the synthesis. The polymer synthesis and end-group functionalization is then presented with the supportive model synthesis, with regard to conversion studies and spectroscopic analysis.

Furthermore, we introduce phospholipid analogous with different charged ionic groups, such that one should be able to combine specific interactions provided by the charged “phospholipid-like” polymer with the phosphoryl choline-terminated poly ϵ-caprolactone (PCL−PC) that prevents nonspecific interaction. Various combinations of these should promote the binding of charged hydrophilic compounds, in addition to the incorporation of hydrophobic water-insoluble compounds into the PCL.[20] This also bears a similarity to the biological environment, where, for instance, cell-membrane-bound phosphatidyl serine has a negative charge.

The combination of hydrophilic PC and hydrophobic polymer segments makes the material amphiphilic. The surface activity of PC-terminated polycaprolactone, as supported from ESCA analysis, is presented, demonstrating the possibility that these functionalities are spontaneously enriched at the surface of films and that this is dependent on the polarity of the surrounding media.

If one, in a latter situation, would optimize these biomimetic and biodegradable molecules for a drug-releasing event, it is the author’s ambition to tailor the architecture of the drug-releasing particle or membrane as precisely as the drug that it should carry.

2. Experimental Section

2.1. Materials. Prior to use, ϵ-caprolactone, acetonitrile, pyridine, and triethylamine (all from Aldrich), and benzyl alcohol (Lancaster) were refluxed over calcium hydride (CaH_2), distilled, and stored under argon. Chloroform and dichloromethane (VWR) were washed over a basic aluminum oxide (Al_2O_3) column and distilled over CaH_2. Succinic anhydride (Aldrich) was recrystallized from dry chloroform and stored in a glovebox prior to use. Trimethylamine$_{(g)}$ and 4-chlorobutyryl chloride (Aldrich) were used as received. $Sn(OTf)_2$ (Aldrich) was azeotropically dried from toluene and stored in a glovebox. $Sn(Oct)_2$ (Aldrich) was distilled at reduced pressure and also stored in a glovebox. 2-Chloro-1,3,2-dioxaphospholane-2-oxide (ethylene chloro phosphate) (Lancaster) was distilled at reduced pressure, and the second fraction was collected at a pressure of 0.011 mm Hg and a temperature of 48 °C and then stored in a freezer under argon. Butyl propionate and hexyl acetate (Lancaster) were used as received.

2.2. Instrumentation. ^1H NMR and ^{31}P NMR spectra were recorded using a JEOL model ECP 400 MHz spectrometer, with the solvent proton signal as an internal standard.

Size exclusion chromatography (SEC) measurements were performed (Waters Alliance GPCV 2000 with three Styragel HR columns, 7.8 mm × 300 mm, HR1/4/5) to determine the molecular weights and molecular-weight distributions. Tetrahydrofuran (THF) was used as an eluent (40 °C, 1.0 mL/min), and a universal calibration with polystyrene standards (Shodex, Showa Denko) was performed. Millennium 32 was used to process the data from both the refractive index detector and the viscometer.

Electron spectroscopy for chemical analysis, coupled with X-ray photoelectron spectroscopy (ESCA/XPS), was performed on a Phi Electronics model Quantum 2000 instrument, using a monochromatic Al X-ray source (with an energy of $h\nu = 1486.86$ eV). An initial survey was conducted (0−1400 eV), and scans on C, O, N, and P followed.

Gas chromatography (GC) was performed on a Perkin−Elmer Instruments Auto System XL gas chromatograph, using a capillary column (5% diphenyl/95% dimethyl polysiloxane, length of 25 m, inner diameter of 0.32 mm, 0.52 μm df). The injector and detector temperature was set at 250 °C. Samples were heated from 40 °C to 190 °C, at a heating rate of 10 °C/min.

2.3. Synthesis. *2.3.1. General Procedure for Bulk Polymerization of Polycaprolactone (PCL) (**1**).* A stir bar was added to a 50-mL two-necked Schlenk flask, and the flask was sealed with a septum. The flask was carefully flame-dried under vacuum and purged with nitrogen. For polymerization, 10.0 g (87.6 mmol) of ϵ-caprolactone and 0.063 g (0.11 mmol) of $Sn(OTf)_2$ catalyst, with 5 mol % of initiator, were combined in a glovebox and sealed with a rubber septum. Outside the glovebox, 0.22 mL (2.2 mmol) of the initiator (benzyl alcohol) for a degree of polymerization (DP) of 40 was introduced with a syringe into the flask under the protecting gas. The reaction mixture was stirred at 35 °C using an oil bath. Following completion of the reaction ($t = 60$ min), the polycaprolactone (PCL) was dissolved in THF and precipitated in 500 mL of cold methanol. The precipitate was filtered and washed repeatedly with methanol and then dried under vacuum at 40 °C until it reached a constant weight. Yield: 95%. ^{1}H NMR ($CDCl_3$) $\delta = 1.35$ (m, 2H, $-CH_2-$, poly), 1.65 (m, 2H, $-CH_2-$, poly), 1.65 (m, 2H, $-CH_2-$, poly), 2.30 (t, 2H, $-O(O)CCH_2-$, poly), 3.63 (q, 2H, $-CH_2OH$, ω-end), 4.04 (t, 2H, $-CH_2OC(O)-$), poly), 5.10 (s, 2H, $PhCH_2$-, α-end), 7.34 (m, 5H, ArH-, α-end).

*2.3.2. General Procedure for Ethylene Phosphate-Terminated Polycaprolactone (**2**).* For phosphorylation, 4.0 g (0.86 mmol) of **1** was weighed in a predried Schlenk flask and dissolved in 20 mL of dry dichloromethane (CH_2Cl_2). Thereafter, 1.5 equiv of dry pyridine (0.11 mL, 1.29 mmol) were added under nitrogen. The flask was attached to a predried dropping funnel and attached to a nitrogen inlet and cooled to −5 °C. Five milliliters of dry CH_2Cl_2 and 2 equiv of ethylene chloro phosphate (0.90 μL, 1.028 mmol) were added to the dropping funnel. The solution was slowly added dropwise, stirred for ∼2 h, and gradually allowed to reach ambient temperature, after which point it was stirred for another 4 h. When the reaction was complete, the solution was diluted with an additional 50 mL of CH_2Cl_2 and then extracted twice with distilled water (50 mL) and twice with a 1 M $NaHCO_{3(aq)}$ (50 mL) solution, to remove the pyridinium salt that was formed during the reaction and excess ethylene chloro phosphate reagent. The organic phase was then separated and dried by stirring for 30 min with sodium sulfate and filtered. Toluene (50 mL) was added and the organic phase and trace amounts of pyridine removed by rotational evaporation at ambient temperature. Yield: 90%. ^{1}H NMR ($CDCl_3$) $\delta = 1.35$ (m, 2H, $-CH_2-$, poly), 1.63 (m, 2H, $-CH_2-$, poly), 1.63 (m, 2H, $-CH_2-$, poly), 2.30 (t, 2H, $-O(O)CCH_2-$, poly), 4.04 (t, 2H, $-CH_2OC(O)-$, poly), 4.32−4.48 (m, 4H, $-CH_2-CH_2-$, ω-end), 5.10 (s, 2H, $PhCH_2-$, α-end), 7.34 (m, 5H, ArH−, α-end). ^{31}P NMR ($CDCl_3$) $\delta = 18.3$ (s).

*2.3.3. General Procedure for Phosphoryl Choline-Terminated Polycaprolactone (PCL−PC, **3**).* For the formation of PC-terminated PCL, 1.0 g (0.21 mmol) of **2** was weighed and placed into a 50-mL predried round-bottom flask and dissolved in 10 mL of dry acetonitrile. The solution was transferred to a pressure tube with two stopcocks, purged with nitrogen, sealed, and cooled to −10 °C. Approximately 2 equiv (or 39 μL, 0.42 mmol) of trimethylamine$_{(g)}$ to PCL polymer was carefully condensed into the pressure tube and slowly heated to 60 °C. (Caution: Reaction under pressure!) The solution was stirred for 45 h and left to cool to ambient temperature. The reaction product was precipitated in cold methanol, and the precipitate was collected and dried until constant weight was reached. Alternatively, the formation of PC-terminated polycaprolactone (PCL−PC) could be performed in situ in a one-step ("one pot") synthesis. This can be achieved by direct ring opening of the intermediate phosphate group without any isolation and purification of the intermediate by altering the solvent from dichloromethane to acetonitrile and transferring the intermediate solution into a pressure tube. The trimethylamine ring-opening agent can then be added and the same reaction conditions and purification procedure used as outlined previously. Yield: 30%−40% (isolated intermediate), 90% ("in situ/one pot"). ^{1}H NMR ($CDCl_3$) $\delta = 1.35$ (m, 2H, $-CH_2-$, poly), 1.63 (m, 2H, $-CH_2-$, poly), 1.63 (m, 2H, $-CH_2-$, poly), 2.30 (t, 2H, $-O(O)CCH_2-$, poly), 3.40 (s, 9H, $-NCH_3$, ω-end), 3.90 (m, 2H, $-CH_2-$, ω-end), 3.90 (m, 2H, $-CH_2-$, ω-end), 4.05 (t, 2H, $-CH_2OC(O)-$, poly), 4.40 (m, 2H, $-CH_2-$, ω-end), 5.10 (s, 2H, $PhCH_2-$, α-end), 7.35 (m, 5H, ArH−, α-end). ^{31}P NMR ($CDCl_3$) $\delta = -1.0$ (s).

*2.3.4. Synthesis of Benzyl 6-Hydroxy Hexanoate (**4**).* A 100-mL predried nitrogen flask that was equipped with a stir bar and sealed with a septum was charged with 5.0 g (44 mmol) of dry ϵ-caprolactone, 22.7 mL (0.44mol) of dry benzyl alcohol, and 8.9 g (0.022 mol) of dry $Sn(Oct)_2$. The solution was stirred at 110 °C for 1 h and allowed to cool. Monoester product was purified and separated from the excess alcohol by fractionated distillation at reduced pressure. The fraction at a pressure of $p = 1.1 \times 10^{-2}$ Torr and a temperature of $T = 108-114$ °C was collected. Yield: 50%. ^{1}H NMR ($CDCl_3$) $\delta = 1.37$ (m, 2H, $-CH_2-$), 1.55 (m, 2H, $-CH_2-$), 1.66 (m, 2h, $-CH_2-$), 2.36 (t, 2H, $-O(O)CCH_2-$), 3.60 (t, 2H, $-CH_2OH$), 5.10 (s, 2H, $PhCH_2-$), 7.33 (m, 5H, ArH−).

*2.3.5. Synthesis of Benzyl 6-[(2-Oxido-1,3,2-dioxaphospholan-2-yl)oxy]hexanoate (Benzyl 6-Ethylene Phosphate Hexanoate, **5**).* For phosphorylation, 0.2 g (0.9 mmol) of **4** and 0.11 mL (1.35 mmol) of dry pyridine were dissolved in 5 mL of dry dichloromethane in a predried 50-mL Schlenk flask. The flask was attached to a predried dropping funnel, attached to a nitrogen inlet, and cooled to −5 °C. Three milliliters of dry dichloromethane and 17 μL (0.18 mmol) of ethylene chloro phosphate was added to the dropping funnel. The solution was slowly added dropwise, stirred for ∼2 h, and slowly allowed to reach ambient temperature, followed by more stirring for an additional 4 h. The reaction was monitored by thin-layer chromatography (TLC), eluting with a 1:1 mixture of ethyl acetate and hexane. When the reaction was complete the solution was charged with an additional 25 mL of CH_2Cl_2 and extracted twice with distilled water (25 mL) and twice with a 1 M $NaHCO_{3(aq)}$ (25 mL) solution, to remove the pyridinium salt reagent that was formed during the reaction and excess ethylene chloro phosphate. The organic phase was then separated and dried by stirring for 30 min with sodium sulfate, after which point it was filtered. Toluene (50 mL) was then added and the organic phases with trace amounts of pyridine were removed azeotropically by rotational evaporation at ambient temperature. Yield: 90%. ^{1}H NMR (CDCl$_3$) δ = 1.39 (m, 2H, $-CH_2-$), 1.66 (m, 2H, $-CH_2-$), 1.66 (m, 2H, $-CH_2-$), 2.33 (t, 2H, $-O(O)CCH_2-$), 4.09 (q, 2H, $-CH_2OC(O)-$), 4.22−4.41 (m, 4H, $-CH_2-CH_2-$), 5.08 (s, 2H, $PhCH_2-$), 7.31 (m, 5H, $ArH-$). ^{31}P NMR (CDCl$_3$) δ = 18.1 (s).

2.3.6. Synthesis of ([6-Benzyloxy-6-oxohexyl]oxy) Phosphatidyl Choline (Benzyl 6-Phosphatidyl Choline Hexanoate, ***6****).* For the synthesis of **6**, 0.2 g (0.61 mmol) of **5** was dissolved in 5 mL of dry acetonitrile and transferred into a 50-mL pressure tube that was equipped with a stir bar and cooled to −20 °C. Approximately 113 μL (1.22 mmol) of trimethylamine$_{(g)}$ (Me$_3$N) was carefully condensed into the pressure tube and properly sealed. (Caution: Reaction under pressure!) The solution was heated and maintained at 60 °C for 45 h to allow the reaction to complete. Following completion, the product was filtered over a silica plug, eluting with a 70/30 mixture of water and ethanol, according to a published procedure.[21] Yield: 80%. ^{1}H NMR (CDCl$_3$) δ = 1.35 (m, 2H, $-CH_2-$), 1.59 (m, 4H, $-CH_2-$), 1.59 (m, 4H, $-CH_2-$), 2.32 (t, 2H, $-O(O)CCH_2-$), 3.33 (s, 9H, $-NCH_3$), 3.76 (m, 2H, $-CH_2-$), 3.78 (m, 2H, $-CH_2-$), 4.22 (m, 2H, $-CH_2-$), 5.08 (s, 2H, $PhCH_2-$), 7.31 (m, 5H, $ArH-$). ^{31}P NMR (CDCl$_3$) δ = −0.9 (s).

*2.3.7. Synthesis of α-Benzyl-ω-4-oxobutanoic Acid−Polycaprolactone (****7****).* For the synthesis of **7**, 2.0 g (0.44 mmol) of **1** and 88 mg (0.88 mmol) of succinic anhydride was added to a 50-mL predried two-necked round-bottom flask that was equipped with a stir bar and purged with nitrogen. The compounds were dissolved in 15 mL of dry chloroform, a dropping funnel was attached, and the solution cooled to 0 °C. The funnel was charged with 89 μL (0.88 mmol) of triethylamine to which was added 5 mL of dry chloroform. This was slowly added dropwise to the cooled solution over a 30-min period. The solution was slowly allowed to reach ambient temperature and stirred for an additional 3 h. When conversion was complete, the polymer was precipitated in cold methanol, filtered, and dried until it reached a constant weight. Yield: 97%. ^{1}H NMR (CDCl$_3$) δ = 1.35 (m, 2H, $-CH_2-$, poly), 1.65 (m, 2H, $-CH_2-$, poly), 1.65 (m, 2H, $-CH_2-$, poly), 2.30 (t, 2H, $-O(O)CCH_2-$, poly), 2.62 (t, 2H, $-CH_2-$, ω-end), 2.62 (t, 2H, $-CH_2-$, ω-end), 4.04 (t, 2H, $-CH_2OC(O)-$, poly), 5.10 (s, 2H, $PhCH_2-$, α-end), 7.34 (m, 5H, $ArH-$, α-end).

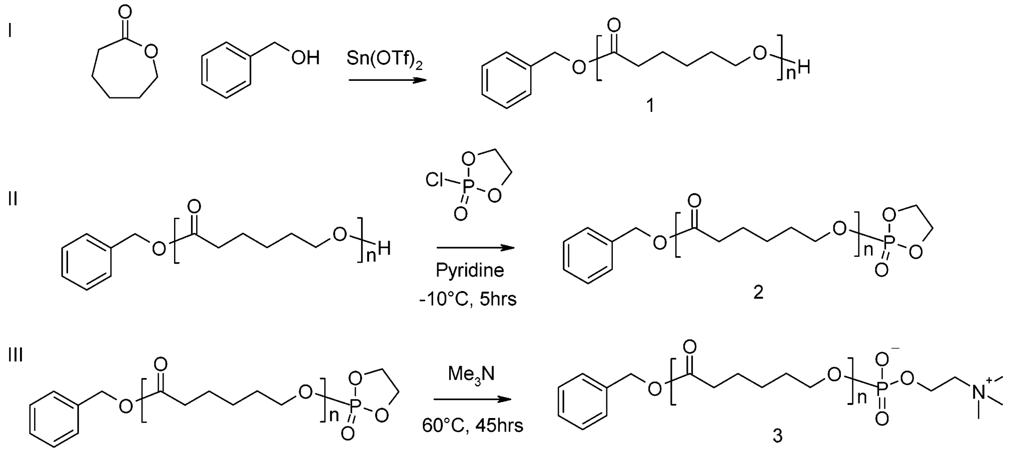
*2.3.8. Synthesis of 4-([6-(Benzyloxy)-6-oxohexyl]oxy)−4-Oxobutanoic Acid (****8****).* For the synthesis of **8**, 0.2 g (0.9 mmol) of **4** and 0.135 g (1.35 mmol) of succinic anhydride were added to a 50-mL predried two-necked round-bottom flask that was equipped with a stir bar and purged with nitrogen. The compounds were dissolved in 5 mL of dry chloroform, with a dropping funnel attached, and the solution cooled to 0 °C. The funnel was charged with 0.17 mL (1.8 mmol) of triethylamine to which was added 3 mL of dry chloroform. This mixture was slowly added dropwise to the cooled solution over a 30-min period. The solution was slowly allowed to reach ambient temperature, after which it was stirred for an additional 3 h. The conversion was monitored by TLC and eluted with a 1:1 mixture of hexane/ethyl acetate. Yield (by NMR): 95%. ^{1}H NMR (CDCl$_3$) δ = 1.25 (m, 2H, $-CH_2-$), 1.65 (m, 2H, $-CH_2-$), 1.65 (m, 2H, $-CH_2-$), 2.32 (t, 2H, $-O(O)CCH_2-$), 2.53 (t, 2H, $-CH_2-$), 2.56 (t, 2H, $-CH_2C(O)OH$), 4.00 (t, 2H, $-CH_2OC(O)-$), 5.08 (s, 2H, $PhCH_2-$), 7.34 (m, 5H, $ArH-$, α-end).

*2.3.9. Synthesis of α-Benzyl-ω-4-chloro-butanoyl−Polycaprolactone (****9****).* For the synthesis of **9**, 2.0 g (0.44 mmol) of **1** and 87 μL (1.10 mmol) of pyridine were added to a 50-mL predried two-necked round-bottom flask that was equipped with a stir bar and purged with nitrogen. The compounds were dissolved in 15 mL of dry chloroform and a dropping funnel was attached and the solution cooled to −10 °C. A funnel was charged with 92 μL (1.10 mmol) of 4-chlorobutyryl chloride to which was added 5 mL of dry chloroform. This was slowly added dropwise to the cooled solution over a 30-min period. The solution was slowly allowed to reach ambient temperature and stirred for an additional 3 h. When conversion was complete, the polymer was precipitated in cold methanol, filtered, and dried until a constant weight was reached. Yield: 91%. ^{1}H NMR (CDCl$_3$) δ = 1.35 (m, 2H, $-CH_2-$, poly), 1.65 (m, 2H, $-CH_2-$, poly), 1.65 (m, 2H, $-CH_2-$, poly), 2.05 (m, 2H, $-CH_2-$, ω-end), 2.30 (t, 2H, $-O(O)CCH_2-$, poly), 2.47 (t, 2H, $-CH_2-$, ω-end), 3.58 (t, 2H, $-CH_2-$, ω-end), 4.04 (t, 2H, $-CH_2$-OC(O)$-$, poly), 5.10 (s, 2H, $PhCH_2-$, α-end), 7.34 (m, 5H, $ArH-$, α-end).

*2.3.10. Synthesis of α-Benzyl-ω-N,N,N-trimethyl-4-oxobutan-1-aminium−Polycaprolactone (****10****).* For the synthesis of **10**, 1.0 g (0.21 mmol) of **9** was weighed in a 50-mL predried round-bottom flask and dissolved in 10 mL of dry acetonitrile. The solution was transferred to a pressure tube with two stopcocks, purged with nitrogen, sealed, and cooled to −10 °C. Approximately 2 equiv (39 μL, 0.42 mmol) of trimethylamine$_{(g)}$ to PCL polymer were carefully condensed into the pressure tube and slowly heated to 60 °C. (Caution: Reaction under pressure!) The solution was stirred for 45 h and allowed to cool to ambient temperature. The compound formed was precipitated in cold methanol, and the precipitate was collected and dried until it reached a constant weight. Yield: 80%. ^{1}H NMR (CDCl$_3$) δ = 1.35 (m, 2H, $-CH_2-$, poly), 1.65 (m, 2H, $-CH_2-$, poly), 1.65 (m, 2H, $-CH_2-$, poly), 2.10 (m, 2H, $-CH_2-$, ω-end), 2.30 (t, 2H, $-O(O)CCH_2-$, poly), 2.50 (t, 2H, $-CH_2-$, ω-end), 3.43 (s, 9H, $-NCH_3$, ω-end), 3.72 (t, 2H, $-CH_2-$, ω-end), 4.04 (t, 2H, $-CH_2OC(O)-$, poly), 5.10 (s, 2H, $PhCH_2-$, α-end), 7.34 (m, 5H, $ArH-$, α-end).

*2.3.11. Synthesis of Benzyl 6-[(4-chlorobutanoyl) oxy] Hexanoate (****11****).* For the synthesis of **11**, 0.2 g (0.9 mmol) of **4** and 0.14 g (1.80 mmol) of pyridine were added to a 50-mL predried two-necked round-bottom flask that was equipped with a stir bar and purged with nitrogen. The compounds were dissolved in 5 mL of dry chloroform, a dropping funnel was attached, and the solution was cooled to −10 °C. A funnel was charged with 0.11 mL (1.35 mmol) of 4-chlorobutyryl chloride, to which was added 5 mL of dry chloroform. This was slowly added dropwise to the cooled solution over a 30-min period. The solution was allowed to reach ambient temperature and stirred for an additional 5 h. TLC using a 1:1 mixture of hexane and ethyl acetate monitored the conversion. The product was purified by flash chromatography and eluted with the same system. Yield: 70%. ^{1}H NMR (CDCl$_3$) δ = 1.38 (m, 2H, $-CH_2-$), 1.65 (m, 2H, $-CH_2-$), 1.65 (m, 2H, $-CH_2-$), 2.05 (m, 2H, $-CH_2-$), 2.35 (t, 2H, $-O(O)CCH_2-$), 2.47 (t, 2H, $-CH_2-$), 3.58 (t, 2H, $-CH_2-$), 4.07 (t, 2H, $-CH_2OC(O)-$), 5.10 (s, 2H, $PhCH_2-$), 7.34 (m, 5H, $ArH-$).

Scheme 1: Synthesis of Phosphatidyl Choline-Terminated Polycaprolactone (PCL−PC)



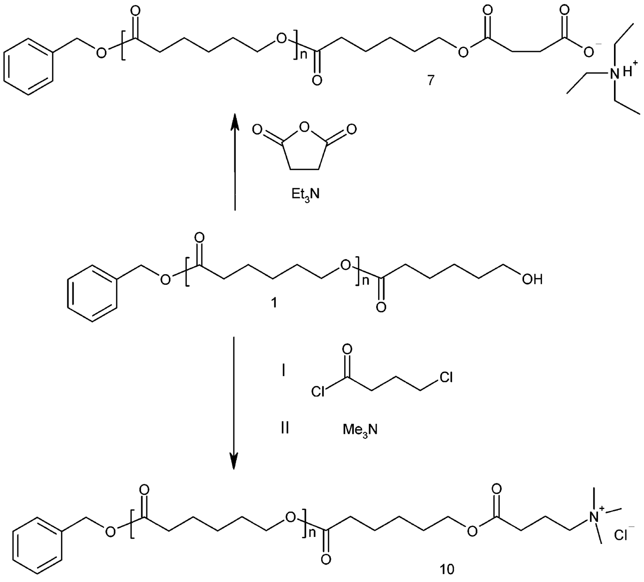
*2.3.12. Synthesis of 4-[{6-(Benzyloxy)-6-oxohexyl} oxy]−N,N,N-Trimethyl-4-oxobutan-1-aminium (**12**).* For the synthesis of **12**, 0.1 g (0.34 mmol) of **11** was weighed in a 50-mL predried round-bottom flask and dissolved in 5 mL of dry acetonitrile. The solution was transferred to a pressure tube with two stopcocks, purged with nitrogen, sealed, and cooled to −10 °C. Approximately 2 equiv (59 μL, 0.68 mmol) of trimethylamine$_{(g)}$ to **11** were carefully condensed into the pressure tube and slowly heated to 60 °C. (Caution: Reaction under pressure!) The pressure tube was stirred for 45 h and then allowed to cool to ambient temperature. Yield: 95%. ^{1}H NMR (CDCl$_3$) $\delta = 1.38$ (m, 2H, $-CH_2-$), 1.65 (m, 2H, $-CH_2-$), 1.65 (m, 2H, $-CH_2-$), 2.08 (m, 2H, $-CH_2-$), 2.38 (t, 2H, $-O(O)CCH_2-$), 2.49 (t, 2H, $-CH_2-$), 3.42 (s, 9H, $-NCH_3$), 3.70 (t, 2H, $-CH_2-$), 4.08 (t, 2H, $-CH_2-$), 5.10 (s, 2H, $PhCH_2-$), 7.34 (m, 5H, ArH−).

2.3.13. Transesterification Studies. For this analysis, either of the two esters (butyl propionate and hexyl acetate) or PCL were dissolved in acetonitrile and transferred to a pressure tube with two stopcocks, purged with nitrogen, sealed, and cooled to −10 °C. Two equivalents of trimethylamine$_{(g)}$ to either the total amount of esters or the PCL were carefully condensed in the pressure tube and thereafter slowly heated to 60 °C. (Caution: Reaction under pressure!) The solution was stirred for the duration of the study, cooled, and then analyzed using GC and SEC, respectively.

3. Results and Discussion

3.1. General Synthetic Approach and Analysis of Base-Catalyzed Transesterification. Phospholipid-mimetic, biodegradable polymers were synthesized by combining the use of poly ϵ-caprolactone (PCL) with various functional polar end-groups, including zwitterionic phosphoryl choline (PC), anionic succinate, and cationic quaternary ammonium salt. The polyester backbone provides mechanical stability and biodegradability, whereas the various headgroups add various functions. For example, PC provides biomimetic and hemeocompatible properties such that protein adsorption is suppressed.[3] To increase the versatility of the product, ionic polymers could be used, either separately or in combination with PCL−PC, to modify the electrostatic interactions. Scheme 1 (PC end) and Scheme 2 (cationic and anionic end) show the general synthetic routes used to prepare the different polymers.

Scheme 2: Synthesis of Anionic-Terminated PCL (7) and Cationic-Terminated PCL (10)



Regardless of the target functionality, the starting material is a biodegradable polymer, which is prepared in advance. One or two additional steps are then needed to modify the ω-hydroxyl end of the polymer to the required zwitterion, anion, or cation. To provide the terminal PC, the ω-hydroxyl group of a PCL is first phosphorylated using ethylene chloro phosphate and, in a final step, the intermediate ethylene phosphate-terminated PCL ring opened, using the nucleophilic base trimethylamine (Me$_3$N) as the ring-opening agent. To form a terminal anion, the ω-hydroxyl group of the polymer is reacted with succinic anhydride in the presence of triethylamine, to produce the wanted succinic acid with the triethylammonium as a gegenion. Finally, the reaction of the ω-hydroxyl with a ω-halo-α-acid halide, after which point the intermediate is treated with Me$_3$N to yield the wanted quaternary ammonium with the Cl$^-$ ion as a gegen ion, forms the terminal cation. The treatment of the intermediates with the nucleophilic base Me$_3$N is the key step, as shown in Schemes 1 and 2, and provides the zwitterionic PC and the cationic ammonium salt.

It is widely known that both acids and bases catalyze ester formation and transesterification reactions.[22] de Groot et al. have shown the sensitivity of lactides toward aminolysis during attempts to prepare polyurethanes.[23] Furthermore, recent studies have demonstrated the

Table 1. Conditions during the Transesterification Study on PCL

solvent	time (h)	base used, 2 equiv to PCL	temperature (°C)	polydispersion index, PDI
acetonitrile	6	Me_3N	60	1.15[a]
acetonitrile	24	Me_3N	60	1.16[a]
acetonitrile	72	Me_3N	60	1.14[a]
acetonitrile	45	pyridine	60	1.20[b]

[a] Initial PCL had a PDI of 1.16, obtained from SEC measurements. [b] Initial PCL had a PDI of 1.19, obtained from SEC measurements.

feasibility of using nucleophilic organic catalysts, such as (dimethylamino)pyridine (DMAP) and *N*-heterocyclic carbenes, in the ring-opening polymerization of cyclic esters.[24] Such nucleophiles promote the polymerization of lactides and lactones with precise molecular weight and narrow distributions. In addition, controlled chain scission provides an alternative route to controlled molecular weight, functionality, and architecture, using DMAP to mediate organocatalytic transesterification.[25] This prompted us to (i) investigate the stability and, therefore, the integrity of the polymers that were to be functionalized at elevated temperature and pressure and (ii) use prolonged reaction times in the presence of the base trimethylamine (Me_3N).

For this investigation, two types of systems were used. The first consisted of two low-molecular-weight esters and the second was a polymer system. In the first system, the two esters (butyl propionate and hexyl acetate) were used and GC analysis was performed. The investigation focused on the treatment of esters with the addition of Me_3N at different temperatures and times. Any adverse transesterification reactions would appear as additional peaks in the GC spectra both at lower and higher temperatures, because both butyl acetate and ethyl heptanoate would be present as a result of scrambling between the two starting esters. In each investigation, the same molar equivalent of esters was used, with 2 equiv of Me_3N to the total amount of esters. Three different reaction times were used: 6, 24, and 72 h. To mimic the reaction conditions used for the functionalization step, a temperature of 60 °C and the solvent acetonitrile were selected. Of the combinations that were investigated, no evidence of transesterification or any other side reaction was noticed; i.e., only the two GC peaks from the starting butyl propionate and hexyl acetate were observed. This indicated that the ring-opening agent was not prone to any ester activation that would lead to a loss of molecular-weight control. This finding encouraged us to continue investigating a polymer system. For this system, the method of analysis was altered from GC to SEC. Any adverse side or transesterification reaction would appear as a broadening of the molecular-weight distribution. A premade PCL with a ω-hydroxyl end group was used for this investigation. When compared to the synthesis of zwitterionic- or cationic-terminated PCLs, this polymer is even more nucleophilic than the intermediate ethylene phosphate-terminated PCL; thus, it would be more prone for alcoholysis with an activated ester than the intermediate during the final functionalization step. The reaction conditions used on this polymer were the same as those used for the previous esters. The polymer used was a PCL with a DP value of 30 and a polydispersity index (PDI) of 1.16. For all analyzed samples, no change was observed from the SEC measurements, as can be seen for the observed PDI values in Table 1.

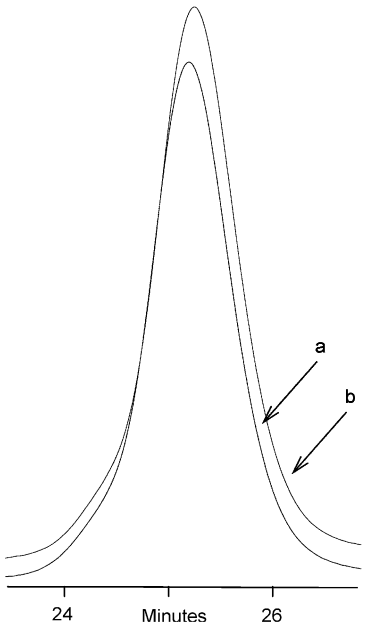


Figure 1. Size exclusion chromatography (SEC) traces of PCL ((a) reference and (b) treated with Me_3N); PDI = 1.16 for both PCL traces.

On the contrary, all the measured PDI values were in the vicinity of the PCL reference and no broadening occurred. A typical SEC example of a base-treated PCL is shown in Figure 1.

Moreover, no signs of shoulders or additional peaks were observed for any of the measurements that were performed. Through the use of the measurements and the results from the low-molecular-weight esters and the PCL, it was ascertained that no adverse side reaction, originating from the heat treatment with Me_3N, was competing with the desired transformations.

3.2. Polymer Synthesis and Formation of PCL−PC. The synthesis process for the preparation of PCL−PC requires three separate steps. The first step constitutes the preparation of the polymer and the last two steps equip the end-group with the desired PC. Because we are attempting to synthesize, characterize, and modify the properties of amphiphilic polymers with one, two, or more functionalities, it is important to develop reaction conditions that lead to high conversions rates at each step. The following section will focus on various aspects of the synthesis to ascertain the optimal synthetic conditions.

Ring-opening polymerization) techniques, using recently developed tin catalysts that are initiated from primary alcohols, afforded polymers with precise molecular weight and low polydispersity,[25] thus formulating the platform for the following synthesis. The polymerization conditions and functionalization results are summarized in Table 2.

The ω-hydroxyl end-group of these polymers was converted by phosphorylation to ethylene phosphate. To optimize the reaction conditions, the solvent conditions were altered and additional proton scavengers were used. The use of halogenated solvents (i.e., dichloromethane), in combination with pyridine, provided the conditions under which high conversion rates within a low reaction time occurred. ^1H NMR analysis could be

Table 2: Results of the Formation of PCL−PC

Data from the Ring-Opening Polymerization Step (DP^a = 30)

initiator	catalyst	temperature (°C)	time (h)	conversion (%)^b	DP
benzyl-OH	$Sn(OTf)_2$	35	4	6	3
benzyl-OH	$Sn(OTf)_2$	35	15	40	14
benzyl-OH	$Sn(OTf)_2$	35	20	48	16
benzyl-OH	$Sn(OTf)_2$	35	40	98	30, PDI ≈ 1.15^c

Data from the Phosphorylation Step

solvent	proton scavenger	time (h)	conversion (%)^d	comment
THF	triethylamine	24	26	modest
dichloromethane	triethylamine	14	98	modest
dichloromethane	pyridine	2	48	best
dichloromethane	pyridine	5	96	best

Data from the Ring-Opening Step

solvent	Me_3N (equiv to polymer)	temperature (°C)	time (h)	conversion (%)^e	yield (%)^f
acetonitrile, ***“one-pot”***	2	60	45	100	90
acetonitrile, **“isolated”**	2	60	45	100	35
THF	2	60	45		

[a] Degree of polymerization. [b] Conversion of the monomer, as judged by ^1H NMR. [c] From SEC. [d] Conversion of the OH group as judged by ^1H NMR. [e] Conversion of the ethylene phosphate as judged by ^1H NMR. [f] Of PCL−PC product.

used to monitor the transformation of hydroxyl to ethylene phosphate as the proton group adjacent the hydroxyl group at 3.62 ppm diminished while, at the same time, an increase in the resonances from the ethylene protons in the phosphate was observed at 4.32 ppm. However, the repeat units of the polymer probably obscured the shifted proton group, which was now adjacent to the ethylene phosphate group at 4.10 ppm. ^{31}P NMR analysis provided a second spectroscopic analysis with which the formation of the ethylene phosphate-terminated PCL could be tracked as the ^{31}P NMR signal of the starting material shifted from 23.1 ppm to 18.0 ppm in the case of ethylene phosphate. Finally, after altering the solvent from dichloromethane to acetonitrile, trimethylamine$_{(g)}$ (Me_3N) was at −5 °C carefully condensed in the reaction vessel and slowly heated to 60 °C, allowing ring opening of the ethylene phosphate group and resulting in the targeted PC ω-end. This step was also optimized using various reaction conditions, such as altering the reaction time, solvent, and temperature. A 100% excess of Me_3N to polymer was used to ensure reactivity of the intermediate throughout the reaction. Using a reaction temperature of <60 °C only gave partial conversion, even if prolonged reaction times were used, and higher temperatures resulted in a pressure buildup that was difficult to control with the equipment used. Acetonitrile, which was also used by Ishihara et al.,[3] was a good solvent to maintain the product in solution throughout the reaction. Other solvents, such as THF, caused precipitation at low conversions, which resulted in a heterogeneous system with only modest yields (see Table 2); therefore, such solvents were excluded. ^1H NMR and ^{31}P NMR analysis was also used to monitor the formation of the targeted PC. ^1H NMR analysis showed that the ethylene signals at 4.32 ppm of the phosphate group were diminished and the most-obvious new signal was the distinct singlet from the choline methyl groups at 3.40 ppm. Resonances from the two ethylene protons in the phosphoryl group and the ethylene group now adjacent the PC end were probably observed within the range of 3.7−4.30 ppm. These signals were difficult to observe, partly because of aggregation of the zwitterions in the nonpolar deuterated chloroform ($CDCl_3$), resulting in dipolar line broadening, and partly due to the low intensity of the end-group, as seen in Figure 2.

Increasing the polarity, by adding a drop of deuterated methanol (CD_3OD), resulted not only in more easily detected resonances but also in a slight shift of the signals. For example, the choline group at 3.40 ppm was, in this case, shifted upfield to 3.20 ppm. This is indicative of a structural rearrangement of the polar PC segments due to the change in polarity, as observed by Ruiz et al.[15] The higher polarity of the $CDCl_3/CD_3OD$ mixture solvates the polar PC end and thereby the molecular mobility increases. In the case of ^{31}P NMR analysis, the investigation was more straightforward and the phosphorus signal from the PC group was easily detected at −1.1 ppm, compared to the intermediate phosphorus signal at 18.0 ppm.

Initially, the yield of the PCL−PC product was disappointingly low—typically ~30%−40%—although the conversion of the intermediate ethylene phosphate group was complete in the ring-opening step. This assumed that the choline singlet at 3.40 ppm, used to determine the conversion, was a discrete peak. The remaining 60%−70% was shown to consist of the starting polymer, as indicated by the distinct ethylene signal adjacent to the hydroxyl group, which reappeared at 3.62 ppm. We asked ourselves how this could happen and investigations were made in regard to why this event occurred.

The reappearance of the shift indicated that hydrolysis of the intermediate had occurred, thus explaining the poor yield. An explanation for this may lie in the intermediate ethylene phosphate-terminated PCL isolation process. Two different extraction series with water were made to remove residual reactants from the reaction-formed salts. The reason is probably the instability of the intermediate phosphotriester, which, upon heating, is susceptible to hydrolysis by adsorbed water from the workup, as shown in Table 2. However, one could increase the yield of the targeted PC to ~50% if the ring-opening step was conducted immediately after the purification. Even at room temperature, stor-

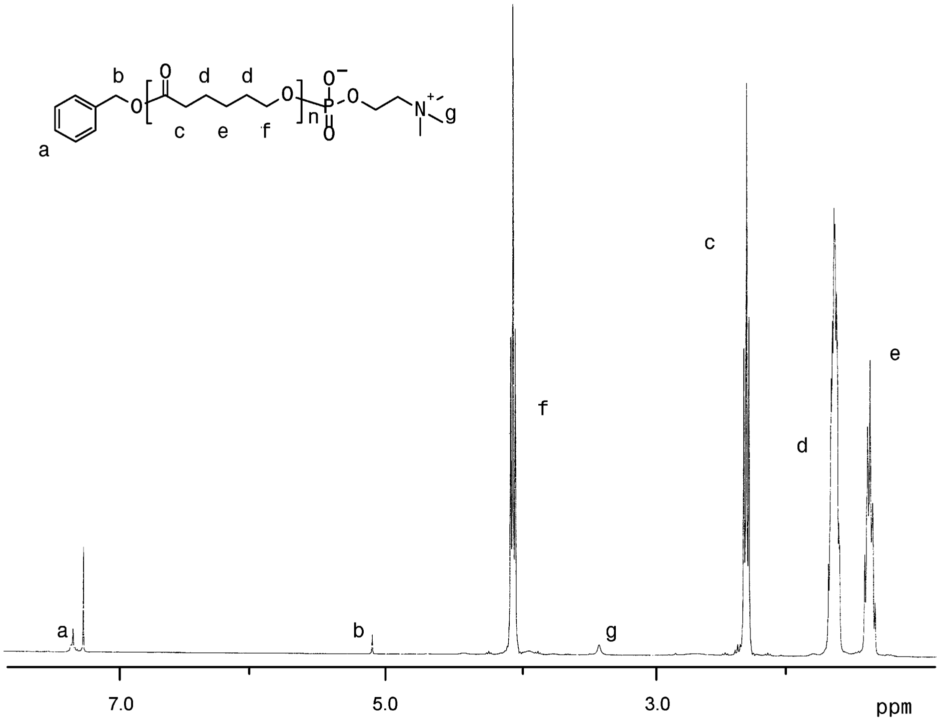


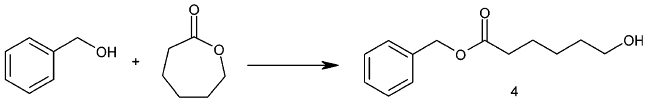
Figure 2. ^{1}H NMR ($CDCl_3$) of PCL−PC (**3**). Inset shows the respective chemical structure.

age of the intermediate for a week or longer resulted in complete hydrolysis, which clearly demonstrates its instability. Although the washed product was thoroughly dried using sodium sulfate, water was carried over to subsequent steps, resulting in hydrolysis. To overcome this problem, a one-pot procedure was developed where the intermediate purification step was avoided. The ring-opening step was, instead, performed without any isolation or purification of the intermediate. The only modification was a solvent alteration from dichloromethane to acetonitrile. This resulted in complete conversion, as judged by NMR, of the hydroxyl to PC. Therefore, the product could be worked up close to quantitative yields. Importantly, there was no adverse effect on the product from the excess reagents, i.e., salts or excess pyridine, as seen in Table 1.

Even though reaction conditions had been developed to give high conversions and high yields, a complete conversion could not be assured, because of the low intensity of NMR peaks and, hence, difficulties in integration. In addition, the spectroscopic analysis was inadequate, in regard to the complete assignment of protons that constitute the zwitterion. Therefore, a synthetic model series was performed to aid the synthesis of PCL−PC.

3.3. PCL−PC Model Series as a Support to Observed Spectroscopy Data and Conversion to PC. The work conducted so far has involved polymers (sometimes with DP ≥ 100), in combination with a three-step synthetic end-group procedure that converted hydroxyl to PC; therefore, it was difficult to clearly locate all the structural changes using NMR spectroscopy and to ascertain whether complete conversion to PC had occurred. There are three reasons for this observation: (i) the intensity of the end-groups was low, compared to the polymer; (ii) the repeat unit of the polymer backbone obscured the resonance of the modified end-groups; and finally (iii) spectroscopic assignment was difficult, because of line broadening of the polar ends. In addition, to ascertain the high yields in the synthesis, the spectroscopic analysis could be made more qualitatively, thus making the synthesis and structural analysis complete.

Scheme 3: Synthesis of Benzyl 6-Hydroxy Hexanoate (4).



To do this, a synthetic model series that mimicked the actual polymer system was performed, to elucidate all the chemical shifts during the transition from hydroxyl to PC thoroughly. The model was chosen to have the same functionalities as the polymer, such that it would not alter its chemical appearance but, instead, lead to a more easily interpreted spectroscopic analysis. The linear monoester from one ring-opened ϵ-CL monomer, benzyl 6-hydroxy hexanoate, was prepared as a starting substance, as seen in Scheme 3.

The use of a 10-fold excess of the initiator (benzyl alcohol), relative to the monomer, and a higher catalyst concentration resulted in mono opening of the cyclic ϵ-CL monomer. The rest of the synthesis followed the procedure developed for the polymer. For the phosphorylation step, pyridine, in combination with dichloromethane, gave complete conversion at short reaction times. Also, in this case, the conversion of the reaction was monitored using ^{1}H NMR ($CDCl_3$), where the sensitive proton group closest to the hydroxyl group shifted from 3.62 ppm in the alcohol to 4.10 ppm in the ethylene phosphate, as seen in Figure 3. This information could not be obtained during the polymer synthesis, because of the overlapping polymer repeat unit at 4.08 ppm. As mentioned previously, one could also track the formation of resonance from the ethylene protons in the phosphate group at 4.32 ppm. Following the formation of PC with the ring-opening step, the monoester proton group closest to the phosphate group shifted to 3.75

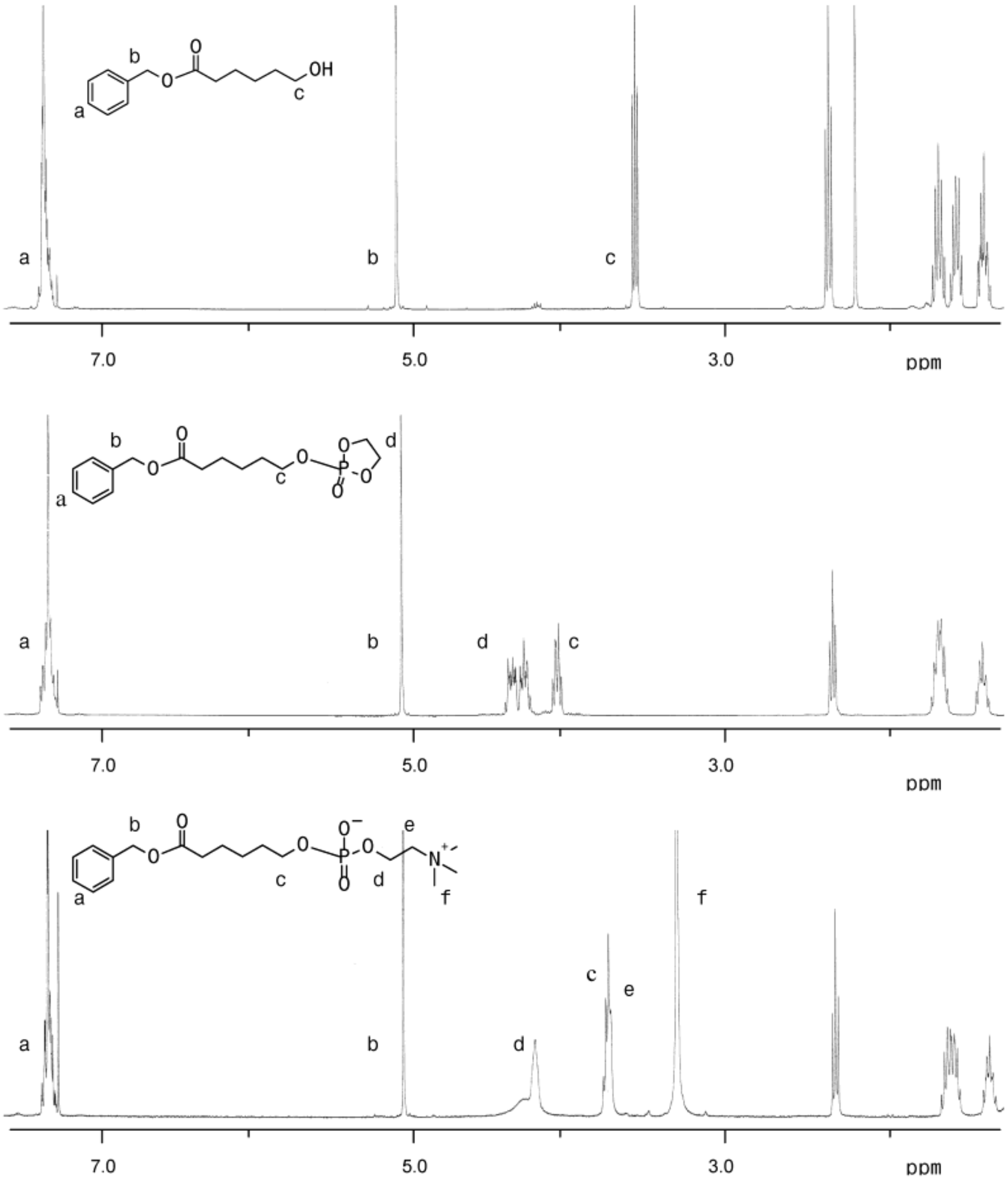


Figure 3. ^{1}H NMR ($CDCl_3$) of model synthesis for **1** (top), **2** (middle), and **3** (bottom). Insets show respective chemical structures.

ppm. The multiple from the ethylene resonances in the phosphate group were separated, giving resonances at 3.75 and 4.20 ppm. The distinct singlet from the choline group was observed at 3.32 ppm, as observed in Figure 3.

Homonuclear correlation spectroscopy (COSY) was used to assign the overlapping signals at 3.75 ppm. From the signal at 4.20 ppm to the signal at 3.75 ppm, one crosspeak was observed. Subsequently, one crosspeak was observed from the signal at 3.75 to the one at 4.20 ppm; in addition, one crosspeak was observed in regard to the aliphatic downfield region at 1.60 ppm, as seen in Figure 4.

The conclusion was drawn that the overlapping signal results from the ethylene proton group adjacent the PC end and from one of the ethylene proton groups in the phosphoryl, with the other ethylene group appearing at 4.20 ppm. To assign the separated ethylene protons in the phosphoryl group, a long-range ($^1H-^{31}P$) heteronuclear correlation spectroscopy (COLOC) experiment was performed. From this experiment, one crosspeak was observed between the phosphorus signal and the proton group at 4.20 ppm, which meant that the proton group at 4.20 ppm was the one adjacent to the phosphodiester, whereas the proton group at 3.75 ppm was adjacent the choline group. ^{31}P NMR analysis facilitated further characterization as the phosphorus signal at 18.3 ppm in the phosphorylated ethylene phosphate intermediate was shifted to −0.9 ppm in the PC.[21] The results can be summarized as follows. The complete structural assignment and the complete conversion, including the synthesis of PCL−PC and benzyl 6-phosphatidyl choline hexanoate, from hydroxyl to PC was obtained from ^{1}H NMR and ^{31}P NMR spectroscopy data. On the basis of the appearance of the PC ethylene protons from the model synthesis, it can now be stated that the reaction conditions that were used lead to the complete conversion of PCL−PC.

3.4. Synthetic Versatility and the Synthesis of Charged Phospholipid-Like Analogous. Having established a route for the synthesis of a "phospholipid-like" PCL−PC polymer, the scope of the synthesis was broadened to include charged "phospholipid-like" polymers with a net anionic or cationic charge, as shown in

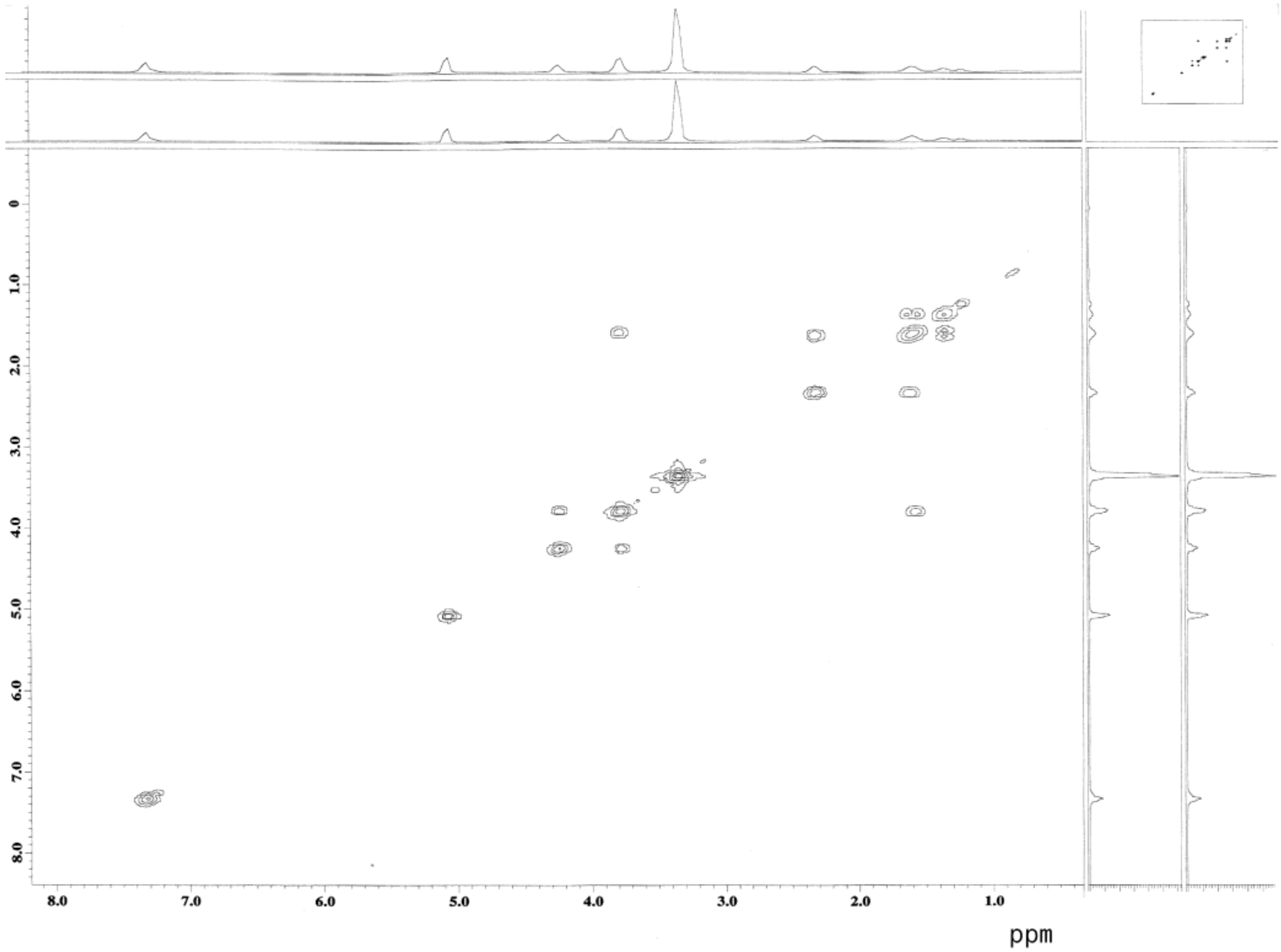


Figure 4. COSY spectra of the model product.

Scheme 2. For example, the use of a charged phospholipid-like polymer in combination with PCL−PC would make it possible to modify the electrostatic interactions of a particle or membrane, whereas the biomimetic PCL−PC would prevent nonspecific interactions. Phospholipid-like polymers with a net charge would attract oppositely charged bioactive substances.[20] Such combinations could be made to mimic the surface of cells that contain negatively charged phosphatidyl serine.

To provide a phospholipid analogue with a net anionic charge, a PCL with a terminal hydroxyl group was reacted with succinic anhydride in the presence of triethylamine, resulting in the wanted terminal succinic acid monoester. ^{1}H NMR analysis was used to monitor this conversion and the buildup of the succinate protons; i.e., two triplets were formed at 2.65 ppm, whereas the ethylene protons adjacent to the hydroxyl were shifted to 4.12 ppm. This synthetic step was also supported by the model series; 4-([6-(benzyloxy)-6-oxohexyl]oxy)-4-oxobutanoic acid had the same spectroscopic correlation, according to ^{1}H NMR analysis.

For the formation of a cationic phospholipid analogue, the synthesis was somewhat more complex and consisted of two separate steps. In the first step, 4-chlorobutyryl chloride was reacted with the terminal hydroxyl group of the polymer. Following purification, the intermediate was redissolved in acetonitrile and reacted with Me_3N at 60 °C to substitute the alkyl chloride for a quaternary ammonium salt with the Cl^- ion as a gegenion. ^{1}H NMR was used to characterize the product that was obtained, and the methyl resonance of the quaternary ammonium salt was observed at 3.43 ppm. The proton group adjacent to the quaternary ammonium was observed at 3.72 ppm. The ethylene group adjacent to the butyryl monoester on the polymer side was obscured by the polymer repeat units at 4.08 ppm, which is supported by the same model synthesis conducted on the zwitterionic and anionic PCL.

3.5. Film Properties. The mechanism that explains the low protein adsorption and cell attachment for nondegradable PC functional polymers involves both surface enrichment of the PC unit[15] and the attraction of phospholipids to this surface to form a biomembrane-like structure.[18] Therefore, it is conceivable that the biodegradable amphiphile PCL−PC functions in the same way, in addition to being biodegradable.

Films of the PCL−PC (DP = 45) oligomers discussed previously were not stable in water. PCL (molecular weight of ∼80 000 g/mol) blended with PCL−PC were good film formers and could be cast into homogeneous films. The surface composition of cast films of PCL/PCL−PC (90/10 wt %) were quite similar to pure PCL, as shown by XPS, with a C/O ratio of 73/27 and no signs of phosphorus or nitrogen. The contact angle of cast PCL/PC films was 65°, which is only slightly less than the 69° angle that has been measured for pure PCL.[26] This is not surprising, considering the low content of PC end groups and the hydrophobic nature of PCL. As

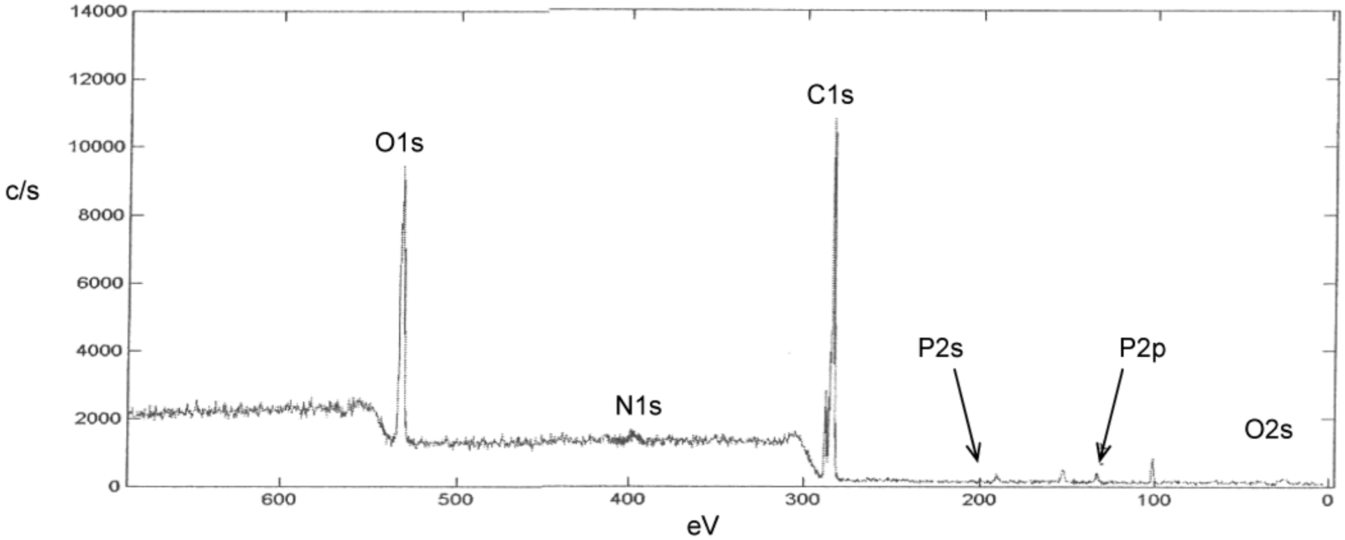


Figure 5. ESCA spectrum of a blend of PCL and PCL−PC after surface rearrangement in heated water. Nonlabeled peaks originate from silicon impurities.

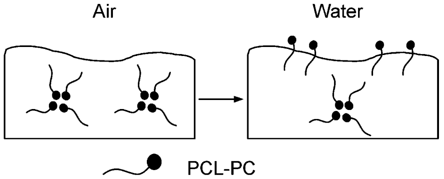


Figure 6. Schematic model of the possible molecular arrangement in a PCL−PC blend as cast films (left) and after heat treatment in water (right).

the system strives to minimize its interfacial energy, the PC chain ends will be buried in the bulk, exposing pure PCL to the polymer/air interface.

In a hydrophilic environment, however, the interfacial free energy is minimized when the hydrophilic PC is enriched at the polymer/water interface. Therefore, the PCL/PCL−PC blend film was immersed into heated water at 90 °C (which is above the melting temperature for PCL crystallites), to provide molecular mobility for migration. The film first became transparent because of melting of the crystalline PCL. Prior to cooling, the film again became opaque, because of water uptake by micellar domains of PC in the bulk at 90 °C. During cooling, further opaqueness appeared as the polymer recrystallized.

Migration of PCL−PC oligomers to the surface was indeed confirmed by contact angle measurements. The contact angle (advancing) was decreased to 40°, which is indicative of an enrichment of polar groups at the surface toward water.[27] Importantly, the contact angle of the reference PCL remained at 69° after the same treatment. The ESCA spectrum shown in Figure 5 reveals the appearance of both nitrogen (N 1s, 2.4%) and phosphorus (P 2p, 1.5%), arising from the polar, surface-oriented PC. The theoretical concentration in pure PCL−PC (DP = 45) is only 0.3%.

It is likely that some surface rearrangement still occurs in the amorphous top layer, when the samples are dried prior to ESCA and contact angle measurements. Surface dynamics should therefore be investigated further, using the dynamic contact angle. The overall mechanism is summarized in Figure 6, where PCL is oriented toward the air surface with PC forming micellar domains in the bulk of cast films. Upon heating in water, however, the surface rearranges to drive PC toward the polymer/water interface.[28]

Future studies will focus on the bulk properties of PC in an amorphous polymer, including surface enrichment and hemeocompatability. A completely amorphous polymer would enhance the rearrangement kinetics, compared to PCL−PC, allowing spontaneous surface enrichment at ambient temperatures.

Summary

Here, the synthesis, characterization, and surface orientation properties of a new generation of biodegradable and biomimetic polymers are introduced. These phospholipid-mimetic, biodegradable polymers are synthesized by combining the use of poly ϵ-caprolactone (PCL) with various functional polar end-groups, including zwitterionic phosphoryl choline (PC), anionic succinic acid, and cationic quaternary ammonium. The polyester backbone provides mechanical stability and biodegradability, whereas the various headgroups add a variety of functions. Careful evaluation of their synthesis has allowed reaction conditions that lead to complete conversion in each step and high yields of the PCL−PC product. Analysis of the ability of trimethylamine to cause any adverse transesterification reactions has shown, on two low-molecular-weight esters and a polymer, that no molecular scrambling occurs. Moreover, benzyl 6-hydroxy hexanoate was synthesized as a model for the polymer synthesis, which leads to a more easily interpreted ^{1}H NMR spectrum with additional characterization. Film properties of the PCL−PC amphiphile that was obtained revealed surface enrichment behavior; the polar PC headgroup is oriented toward the surface after a cast film was treated in water, as observed by electron spectroscopy for chemical analysis.

The properties of the obtained polymers that were synthesized suggest that these molecules may be used in biological environments as new candidates for drug delivery or as temporary coatings for blood-contacting biomaterials.

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