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An Efficient and Scalable Synthesis of Perfluorinated Phosphatidylcholines

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Abstract:

Perfluorinated glycerophosphocholine (1), which is useful as an emulsifier in fluorocarbon suspensions, was prepared in multikilogram scale in 38% overall yield. The desired phosphocholine was prepared from diacylglycerol (6) by sequential phosporylation by $POCl_3$, reaction with choline *p*-toluenesulfonate, and aqueous hydrolysis. Purification was accomplished by treatment of crude 1 with ion-exchange resin. Diacylglycerol (6) was prepared by esterification of *rac*-1-benzyloxy-2,3-propanediol with perfluorinated acid followed by hydrogenolysis of the protective group.

Introduction

Fluorocarbon-based oxygen delivery systems are evolving as promising substitutes for whole blood. Such a delivery system should have, in addition to oxygen carrying capability, controllable formulation and biocompatibility.[1] Formulation of the fluorocarbons is complicated by the fact that they are immiscible with aqueous media and thus require emulsifiers which impart desirable characteristics, such as uniform particle size and stability. Recently, several classes of surfactants were described which were effective emulsifiers of fluorocarbon suspensions.[2] One novel class, the perfluorinated diacylglycerophosphocholine surfactants, not only provides for the preparation of stable fluorocarbon emulsions but also exhibits promising biocompatibility (LD50 i.v. in mice >2750 mg/kg).

To further investigate the surfactant and biological properties of this class of molecules, we required a synthetic process which would produce large (>1 kg) amounts of the desired phospholipid **1**.

Several synthetic approaches were considered.[3] Diacylation of glycerophosphocholine has been utilized; however, the products require purification by column chromatography, conditions under which the perfluorinated diacylglycerophosphocholines are not stable.[4] Phosphorylation of 1,2-diacylglycerols can be accomplished using various acyclic or cyclic prebuilt phosphorylating agents. Most of these methods have, however, some drawbacks which preclude their use for large scale preparations.

Considering further the low stability of the desired 1,2-diacylglycerols (2,3-acyl migration), it was decided to proceed through esterification of $POCl_3$ and then condensation with choline tolsylate. This approach was reported to afford dialkylglycerophosphocholines in good yields from the corresponding dialkylglycerols.[5] The diacylglycerols would be derived from the perfluorinated acid **4c**.

Results and Discussion

A radical atom-transfer reaction followed by reduction was used to couple the perfluorohexyl iodide **2** to the olefinic ester **3**.[6] In model systems, the radical addition was found to be efficient on small (<100 g) scale when initiated by $Na_2S_2O_4$ and sonication.[7] When the reaction was conducted on larger scale, the rate of reaction slowed considerably, and low yields were obtained even with increased sonication power. Alternatively, initiation of the radical reaction of ester **3** and perfluoroalkyliodide **2** with Et_3B[8] in hexanes gave

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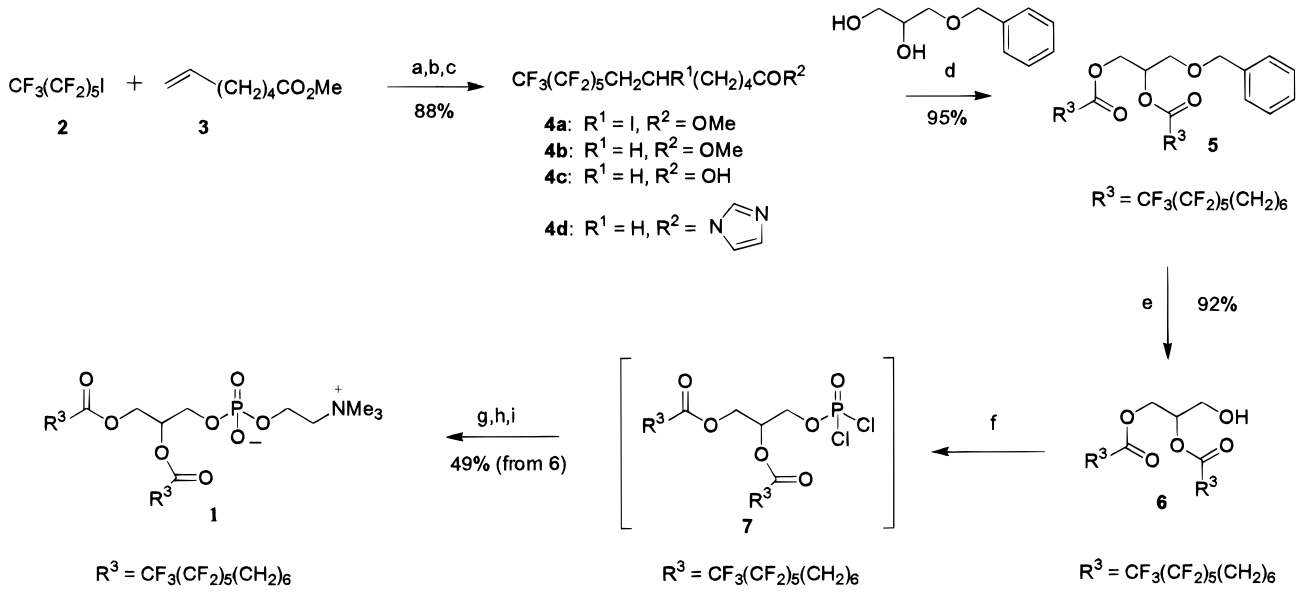
Scheme 1[a]



[a] Conditions: (a) Et_3B, hexane, 5 °C; (b) H_3PO_2, Et_3N, AIBN, dioxane, reflux; (c) LiOH, dioxane, H_2O, 80 °C, then HCl; (d) Im_2CO, THF, 45−50 °C, then DBU, 45−50 °C; (e) 10% Pd/C, H_2, 1:1 THF:H_2O; (f) $POCl_3$, Et_3N, isopropyl ether −20 °C-rt; (g) choline tosylate, pyridine, $CHCl_3$, 0−25 °C, (h) H_2O, 25 °C; (i) TMD-8 ion exchange resin, EtOH.

reproducibly high yields of the desired iodide **4a** (Scheme 1). On laboratory scale, sufficient oxygen was present in the solvent to consistently initiate the reaction; however, upon scale-up to 100-gal pilot plant equipment, two charges of 1 M Et_3B were necessary for completion of the reaction. This is presumably due to the efficiency with which oxygen and moisture can be excluded from fixed equipment, thereby interfering with reaction initiation. A small amount of air was added to the large scale reactions to initiate the radical process.[9]

The iodide **4a** was directly reduced via Barton's procedure,[10] which employed phosphinic acid as the reducing reagent, thus allowing the reaction to be carried out on large (>35 mol) scale. Hydrolysis with LiOH afforded perfluorinated acid **4c** in 88% overall yield from the starting olefin.

Numerous methods potentially could be utilized for synthesis of benzyldiacylglycerol **5** from acid **4c**. The reaction conditions chosen for larger scale reactions, however, must result in complete conversion of the *rac*-benzyloxypropanediol to the corresponding diester with few impurities as the products are nondistillable oils, and on large scale, chromatography was impractical. For initial small scale experiments, carbodiimide methodology gave yields in the 60−90% range after column chromatography. The acyl chloride derived from acid **4c** also underwent esterification, but small quantities (5−10%) of unremovable monoacylated material remained even after extended (>24 h) reaction times. Eventually, reaction of *rac*-1-benzyloxy-2,3-propanediol with 2.5 equiv of the preformed acylimidazolide **4d** afforded excellent yields of the protected diacylglycerol.[11] The product was obtained relatively free (usually <2%) of monoacylated material and was taken directly on to the next step.

The hydrogenolysis of benzylated diacylglycerol **5** proceeded slowly with use of 10% Pd/C purchased from Aldrich, and often a catalyst recharge was necessary after 24 h. This reaction was complicated by the propensity of the product diacylglycerol **6** to undergo 1−2 acyl migration even at ambient temperature. This problem was entirely avoided by the use of Degussa type 10% Pd/C, which catalyzed the hydrogenolysis within 1 h at 5−10 °C, thus avoiding the lengthy reaction times and associated acyl migration.

The conditions under which the final steps of the synthesis were carried out were to be crucial, as the emulsification properties of the desired phospholipid **1** made any extractive workup unfeasible, as was column chromatography. In the event, a solution of the diacylglycerol **6** in isopropyl ether was added to a cold (−15 °C) solution of $POCl_3/Et_3N$ in isopropyl ether. The phosphomonoester **7** was obtained in high purity, provided the usual precautions were taken to avoid moisture. Solvent choice for this reaction was critical, as most solvents, with the exception of ethyl ether and isopropyl ether, gave phosphomonoester **7** of low quality. The process was monitored by ^{31}P NMR, which showed the phosphomonoester as a triplet at about 8 ppm (Ph_3P standard = 0 ppm). Under these reaction conditions, we obtained little if any diesterification of the $POCl_3$. The labile phosphomonoester was taken directly on to the product **1** by sequential reaction with choline tosylate in chloroform and water. Dichloromethane could be used as solvent in place of chloroform with an accompanying 10−15% decrease in yield. Use of choline chloride in place of the tosylate led to significant 1-chloro-2,3-diacylglycerol formation via chloride

(9) Although the reaction initiation was initially carried out by briefly (1−2 s) opening a reactor valve to the atmosphere, we do not recommend nor plan to utilize this method in the future. For any future runs, the amount of O_2 needed for reaction initiation should be chosen on the basis of small scale laboratory runs. The safest method for O_2 charging on scale should be carefully considered prior to running on pilot scale.

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reaction with phosphomonoester **7**. Purification of the products was accomplished by stirring with TMD-8 mixed-bed ion-exchange resin to remove any acidic or basic impurities, followed by precipitation from toluene. The yield for the final step was 49% on a 5-kg scale, with product purity above 98%, as determined by reversed-phase HPLC analysis.

Conclusion

An efficient large scale preparation of perfluoroalkylated phosphatidylcholines has been described which should provide the basis for commercial manufacture of a variety of synthetic highly fluorinated phospholipids. The yield for the six-step procedure was 38% based on methyl-6-hepteneoate.

Experimental Section

NMR spectra were measured on a Bruker AMX 300 or AMX 400 spectrometer. Chemical shifts are reported in parts per million (δ). Multiplicities are given as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), and b (broad). Infrared spectra were obtained on a Nicolet 20 DBX FTIR spectrometer. Elemental combustion analysis was performed by Oneida Research Services, One Halsey Rd., Whitesboro, NY 13492, or by Atlantic Microlab Inc., P.O. Box 2288, Norcross, GA 30091. Unless otherwise noted, materials and solvents were obtained from commercial suppliers and used without further purification.

8,8,9,9,10,10,11,11,12,12,13,13,13-Tridecafluorotridecanoic acid (4c). To a 100-gal glass-lined reaction vessel under a stream of N_2 were added 5.0 kg (35.2 mol) of methyl 6-heptenoate (**3**), 18.9 kg (42.4 mol) of perfluorohexyl iodide (**2**), and 45.0 kg of hexane. The contents of the vessel were cooled to 0−5 °C, and then a solution of 474.4 g (702.8 mL, 0.7082 mol) of 1 M Et_3B in hexanes diluted with 1.0 kg of hexane[12] was added over 10 min (note: it was necessary to introduce a small amount of air into the reaction vessel to initiate the reaction). The contents of the vessel were stirred for 30 min at 20−25 °C, and ^{1}H NMR of the reaction mixture indicated that the reaction was not complete. The contents were cooled to 0−5 °C, and an additional 474.4 g charge of 1 M Et_3B in hexanes was made. After warming the vessel contents to 20−25 °C, ^{1}H NMR of the reaction mixture indicated completion of the reaction. The reaction was quenched by the addition of 1 N HCl (29.5 kg), and then the layers were separated. The organic layer containing **4a** was evaporated to a minimum under reduced pressure at 20−25 °C.

Dioxane[13] (57 kg) was added, and the solvent volume was reduced to approximately 50 L. Subsequently, a premixed solution of 16.0 kg (158.1 mol) of triethylamine and 16.0 kg (121.2 mol) of 50% aqueous hypophosphorous acid in 32.6 kg of dioxane was added, followed by 35.9 g (0.218 mol) of AIBN. The reaction mixture was heated to reflux for 2 h and then cooled to ambient temperature before separation of the layers. The aqueous phase was discarded, and the upper organic phase containing **4b** was carried directly into the next reaction.

To the mixure was added 28.5 kg (52.3 mol) of 2 N LiOH (aqueous). The reaction mixture was refluxed for 2 h and then cooled to 35 °C. The mixture was brought to pH 1 by the addition of 3 N HCl (aquous) and then transferred to a 200-gal glass-lined reactor vessel containing 800 kg of water at 5 °C. The mixture was stirred at 5 °C for 30 min, and then the product was isolated by filtration. Drying at 35 °C under reduced pressure afforded 13.9 kg (88.0%) of perfluorinated acid **4c**: mp 61−63 °C; IR (KBr) 3100−2800 (br), 1710, 1430, 1250, 1190, 1120, 600 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.50 (t, 2 H, J = 7 Hz), 2.28−2.05 (m, 2 H), 1.88−1.66 (m, 4 H), 1.63−1.41 (m, 4 H); ^{19}F NMR (376 MHz, $CFCl_3$ as reference, $CDCl_3$) δ −0.50 (s, 3 F), −34.13 (s, 2 F), −41.6 (s, 2 F), −42.43 (s, 2 F), −43.20 (s, 2 F), −45.77 (s, 2 F). Anal. Calcd for $C_{13}H_{13}F_{13}O_2$: C, 34.84; H, 2.92. Found: C, 34.84; H, 2.87.

***rac*-3,5,9-Trioxa-4-phosphadocosan-1-aminium-17,17,18,18,19,19,20,20,21,21,22,22,22-tridecafluoro-4-hydroxy-*N*,*N*,*N*-trimethyl-10-oxo-7-[(8,8,9,9,10,10,11,11,12,12,13,13,13-tridecafluorooxotridecyl)oxy]-, Inner Salt, 4-Oxide (1).** A suspension of 13.90 kg (31.0 mol) of **4c** and 5.03 kg (31.0 mol) of 1,1-carbonyldiimidazole in 80.5 kg of THF was heated to 48 °C for 1 h, and then a solution of 2.51 kg (13.8 mol) of *rac*-3-benzyloxy-1,2-propanediol and 4.72 kg (31.0 mol) of DBU in 5 kg of THF was added. The reaction mixture was stirred for 2 h at 50 °C and then cooled to ambient temperature. The mixture was partitioned between 102 kg of 1 M HCl and 70.6 kg of MTBE, and the layers were separated. The organic phase was washed with water (100.5 kg), and then saturated aqueous $NaHCO_3$ (136.3 kg) was added. The organics were distilled out of the mixture and replaced with 75 kg of MTBE. The layers were separated, and the organic phase was washed with 50 kg of water and 50 kg of brine. The solvent was evaporated and replaced with 80 kg of THF. The solution of **5** was carried directly into the next step without further purification (the yield of the reaction was 92% based on a small representative sample which was evaporated to dryness).

To a suspension of 4.6 kg of 50% wet 10% Pd/C (Degussa type) in 10.5 kg of THF was added 30.7 kg (11.0 mol) of a 37.5% solution of benzyl ether **5** in THF. The reaction mixture was stirred under an atmosphere of H_2 until the uptake of hydrogen had ceased. The reaction mixture was filtered through a bed of Celite, and the filter cake was washed with 13.0 kg of isopropyl ether.[14] The solution was dried with sodium sulfate (12.0 kg), and the solvent was evaporated and replaced with isopropyl ether to afford a 49.3% (w/w) solution of diacylglycerol **6** in isopropyl ether, which was carried on to the next step without further

(12) The 1 M Et_3B in hexanes were charged to the reactor by the use of a Pope pressure vessel. The Pope vessel was charged with the 1 M Et_3B in a laboratory hood, and the vessel was swept with N_2 and then pressurized with N_2. The contents of the Pope vessel were then charged to the reactor.

(13) Charging of dioxane to the reactor, as well as filtration of solutions containing dioxane, was performed using appropriate PPE, including a powered air-purifying respirator.

(14) Isopropyl ether can form peroxides over time when exposed to air. The solvent was handled safely on plant scale and periodically monitored for peroxide formation by testing with E. Merck peroxide test paper.

purification (the yield of the reaction was 92% based on a small representative sample which was evaporated to dryness).

To a −20 °C solution of 1.64 kg (10.7 mol) of $POCl_3$ and 1.30 kg (12.8 mol) of NEt_3 in 8.0 kg of anhydrous isopropyl ether was added a solution of 9.68 kg (10.2 mol) of diacylglycerol **6** in 40 kg of isopropyl ether so that the reaction temperature remained below −15 °C. After completion of the addition, the reaction mixture was warmed to ambient temperature to afford phosphomonoester **7**. A sample removed from the reaction vessel indicated clean monoesterification by ^{31}P NMR analysis. The salts were filtered, and the solvent was evaporated and replaced with 60 kg of $CHCl_3$.[15] The reaction mixture was cooled to 0 °C, and then 3.09 kg (11.2 mol) of choline tosylate was added, followed by a solution of 4.02 kg of pyridine in 15 kg of $CHCl_3$. The suspension was warmed to ambient temperature and stirred for 12 h before the addition of 0.96 kg (53.3 mol) of water. The reaction was stirred for 5 h, at which time ^{31}P NMR indicated conversion to phosphate diester. The reaction vessel was charged with 137.5 kg of TMD-8 ion-exchange resin (which had previously been washed thrice with 82.8 kg of ethanol) and 73.2 kg of ethanol. The mixture was stirred for 2 h at ambient temperature, and then the resin was removed by filtration. The resin bed was washed with ethanol (3 × 64.8 kg), and then the solvent was evaporated and replaced with 140.4 kg of toluene. The solvent was evaporated to a total volume of about 80 L, during which time the product precipitated out of solution, and then an additional 140.4 kg of toluene was added. The solvent was evaporated to a volume of about 80 L in order to further remove water from the product. The solids were isolated on a single plate filter and dried under vacuum with a nitrogen bleed to afford 5.60 kg (49.0%) of the fluorinated phosphatidylcholine **1** as a hygroscopic white solid: mp 224−224 °C dec; IR (KBr) 3700−3100 (br, absorbed H_2O), 2940, 2850, 1730, 1230, 1200, 1140 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3/CD_3OD$) δ 5.17−5.08 (m, 1 H), 4.31 (dd, 1 H, J = 3.4 Hz, 13 Hz), 4.19−4.09 (br s, 2 H), 4.09−3.97 (m, 1 H, obscured by solvent), 3.88 (t, 2 H, J = 5.3 Hz), 3.55−3.47 (m, 2 H), 3.12 (s, 9 H), 2.22 (q, 4 H, J = 14.5 Hz), 2.03−1.86 (m, 4 H), 1.52 (m, 8 H), 1.29 (m, 8 H); ^{31}P NMR (162 MHz, $CDCl_3/CD_3OD$) δ −0.10 (s, H_3PO_4 reference). Anal. Calcd for $C_{34}H_{42}F_{26}NO_8P$ (corrected for 1.8 mol water/mole of product): C, 35.51; H, 4.00; N, 1.22. Found: C, 35.73; H, 4.05; N, 1.22.

(15) Chloroform was charged to the reactor with the aid of local air extraction, and no exposure of workers to the solvent was detected, as measured by Glaxo Wellcome Environmental Safety Department.

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