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Synthesis of Chiral (α,α-Difluoroalkyl)phosphonate Analogues of (Lyso)phosphatidic Acid via Hydrolytic Kinetic Resolution

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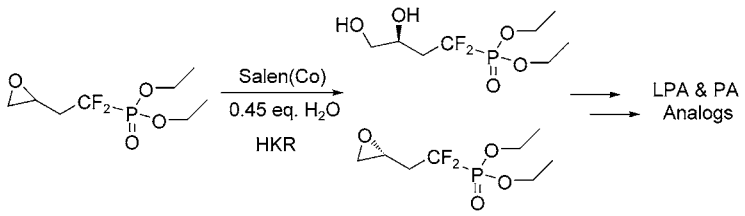
ABSTRACT



The hydrolytic kinetic resolution of 1,1-difluoro-3,4-epoxy-butylphosphonate using a chiral salen–Co complex was employed as a key step to obtain enantiomeric diols in 99% ee as key intermediates. The enantiomerically homogeneous (α,α-difluoroalkyl)phosphonates were obtained after selective esterification and deprotection of the corresponding phosphonates. These compounds are novel phosphatase-resistant analogues of lysophosphatidic acid and phosphatidic acid.

Current interest in (α,α-difluoroalkyl)phosphonates as hydrolytically stable bioisosteres[1] of phosphate esters includes incorporation of this functionality into phospholipids, nucleosides, isoprenoids, phosphotyrosine, and phosphoserine analogues.[2−6] The isoelectronic and isosteric replacement of oxygen by difluoromethylene in phosphate analogues confers metabolic stability and imparts important features for receptor binding. Many difluorinated organophosphate derivatives have been studied as potential enzyme inhibitors and as probes for the elucidation of biochemical processes.[7−10] Indeed, the difluoromethylene analogue of glycerol 3-phosphate is a substrate for glycerol 3-phosphate dehydrogenase,[11] and 9-(5′,5′-difluoro-5′-phosphonopentyl)guanine is a potent bisubstrate analogue inhibitor for purine nucleoside phosphorylase.[12] Earlier, work from our laboratories also demonstrated the preparation of a nonhydrolyzable phosphonate analogues of phosphatidylinositol 4,5-bisphosphate.[13]

Lysophosphatidic acid (LPA, 1- or 2-acyl-*sn*-glycerol-3-phosphate) is an important lysophospholipid mediator produced by activated platelets.[14] LPA elicits a variety of

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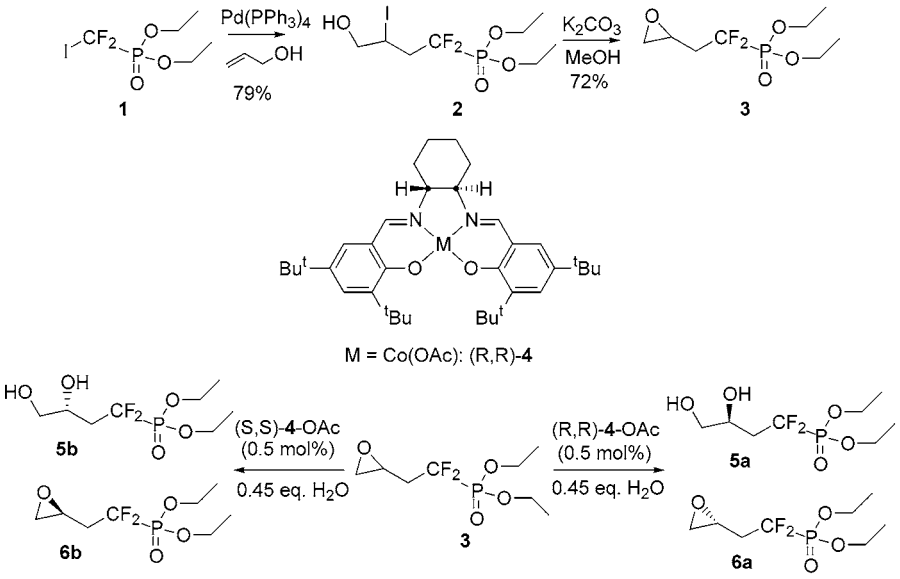
Scheme 1



biological effects, which include platelet aggregation, smooth muscle contraction, changes in cell morphology, and stimulation of cell growth and proliferation.[15−18] Moreover, the observation that LPA is the key cell proliferation factor overproduced in ascites of human ovarian cancer patients[19] has led to the validation of the G-protein-coupled seven-transmembrane domain LPA receptors[20] as targets for cancer therapy.[21−23] In addition, phosphatidic acid (PA), the product of the action of phospholipase D on phosphatidylcholine and other phospholipids, is well-established as an important intermediate in the biosynthesis of phosphoglycerides. PA also regulates phosphoinositide metabolism and plays key roles in cell growth and protein trafficking.[24] PA is thus an important lipid mediator of cellular physiology, and its metabolism[25] and interactions with PA-binding proteins deserve continued attention.[26] Since the phosphate moiety is readily hydrolyzed by the action of lipid phosphate phosphatases,[27−29] the strategic substitution of oxygen by difluoromethylene PA and LPA would be expected to prolong biological activity by altering pharmacokinetics, metabolism, and ligand binding. The resulting CF_2 analogues may prove to be long-lasting agonists, antagonists, or enzyme inhibitors.

Specifically, we describe herein a new synthetic approach to the preparation of difluoromethylene analogues of 1-acyl-*sn*-glycerol-phosphate and the 1,2-diacyl derivative that has two key features. First, the construction of PCF_2−C bond was carried out by a palladium-catalyzed addition of diethyl difluoroiodomethylphosphonate to an allylic alcohol, with subsequent base-catalyzed ring closure of the iodohydrin to an epoxide. Second, highly enantiomerically enriched diols (up to 99% ee) were obtained by hydrolytic kinetic resolution (HKR) of the epoxide catalyzed by a chiral salen−Co complex.[30−32]

Current approaches to difluoromethylene phosphonates include electrophilic fluorination (Selectorfluor, FN−$(SO_2$-Ph$)_2$),[33,34] nucleophilic fluorination (DAST),[35] Arbuzov reactions with fluorohalomethanes,[36] alkylation of (diethoxy-

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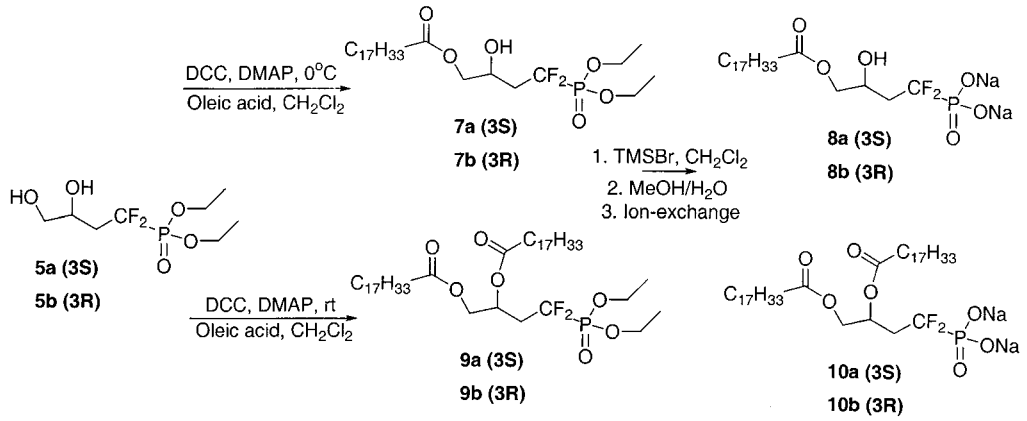
Scheme 2



phosphoryl)difluoromethyllithium,[37−39] addition of difluoro(trimethylsilyl)methylphosphonate to carbonyl compounds under fluoride catalysis,[40,41] and transition metal-catalyzed addition of diethyl difluoroiodomethylphosphonate to alkene.[42,43] In the preparation of difluoromethylene phosphonate, the key step is the formation of a carbon−carbon bond between the PCF_2-containing synthon and the remainder of the target molecule. Many examples employing dialkyl lithiodifluoromethylenephosphonates have been reported for the construction of a PCF_2−C bond.[37−39] However, these organolithium reagents are unstable and difficult to handle. Yang and Burton reported a facile and practical method for the construction of a PCF_2−C bond by the addition of iododifluoromethylenephosphonate to an alkene.[42] This led us to synthesize the 1,1-difluoro-3,4-epoxy-butylphosphonate by addition of iododifluoromethylenephosphonate to allyl alcohol and subsequent base-induced cyclization of the iodohydrin to an epoxide. We then planned to employ the recently described HKR reaction of the terminal epoxide to obtain access to enantiomerically enriched diols that could be mono- or bisacylated to afford LPA and PA analogues, respectively.

Thus, the addition reaction of diethyl iododifluoromethylenephosphonate **1** to allyl alcohol catalyzed by tetrakis(triphenylphosphine)palladium in hexane gave the corresponding iodohydrin **2** in 79% yield (Scheme 1). Initial attempts to prepare the epoxide from iodohydrin **2** with powered potassium hydroxide in diethyl ether failed.[44] However, treatment of the iodohydrin **2** with diluted K_2CO_3/MeOH solution for 5 min at room temperature provided the desired epoxide **3** in good yield (72%).[45] Next, terminal epoxide **3** was employed for the HKR reaction, constituting the first application of HKR in a substrate containing both fluorine and phosphonate functionalities. Few examples of HKR with fluorine-containing epoxides were found,[32] and no HKR substrates have been reported for phosphonate or phosphate-containing epoxides. The reaction of racemic epoxide with 0.45 equiv of H_2O in a minimum volume of THF in the presence of 1.0 mol % (*R*,*R*)-**4**-OAc gave diol **5a** in 99% ee and 69% isolated yield (Scheme 1). Similarly, catalyst (*S*,*S*)-**4**-OAc provided the opposite configuration of diol **5b** in 99% ee and 70% yield. The epoxide and diol were readily separated by flash chromatography, providing an excellent example of the scope and utility of the HKR process.

Regioselective acylation at the primary hydroxyl of the 1,2-diol was readily accomplished, as shown in Scheme 2.[46,47] Thus, treatment of **5a** with 0.95 equiv of oleic acid and 1.2 equiv of DCC and DMAP in CH_2Cl_2 at 0 °C gave **7a** in 42% yield after chromatography, accompanied by a small amount of diester. When the reaction was performed at room temperature, the ratio of primary ester to diester decreased. Diesters bearing identical acyl chains, e.g., **9a** and **9b**, could be obtained in 73% yield, with 2.4 equiv of oleic acid in the presence of excess DCC and DMAP in CH_2Cl_2. Dealkylation of phosphonic acid diethyl esters was achieved by treatment with excess bromotrimethylsilane (10.0 equiv) for 8 h at room temperature; interestingly, use of only 3.0 equiv of TMSBr did not result in complete dealklylation.[48,49] After hydrolysis by aqueous methanol (95%) followed by ion exchange chromatography, the sodium salts of LPA ana-

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logues **8** and PA analogues **10** were obtained in essentially quantitative yield (Scheme 2).

The enantiomeric purity of diols **5a** and **5b** was determined by Mosher's ester method,[50,51] and optical purities were measured by integration of the ^{1}H NMR. The double doublet at δ 4.35 ppm in **11a** was shifted to δ 4.44 ppm in **11b**. There was no detectable signal at δ 4.44 ppm in **11a** or at δ 4.35 ppm in **11b**, indicating that each diol had been obtained in >99% ee.

In summary, we have described a concise and straightforward method for the synthesis of CF_2-substituted enantiopure LPA and PA from a racemic epoxide. The present strategy capitalizes on the palladium-catalyzed PCF_2-C bond formation and the high enantioselectivity of the HKR reaction. Moreover, this is the first report using a substrate containing a phosphonate or difluoroiodomethylene phosphonate for the HKR process. This is the second type of difluorinated analogue of LPA prepared thus far, the first being the migration-resistant 1,1-difluoro *sn*-2-acyl analogues of LPA, which exhibited selective activation of intracellular with little activity toward extracellular receptors.[52] In preliminary experiments, compounds **10a** and **10b** were 90 and 70% as potent, respectively, as 1-palmitoyl-2-oleoyl *sn*-PA in activation of mTOR signaling in quiescent HEK 293 cells (J. Fang and J. Chen, unpublished results). Full descriptions of the utility of analogues **8** and **10** as hydrolytically stable LPA and PA mimics will be reported in due course.

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Supporting Information Available: Experimental procedures and characterization for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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