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Synthesis, Pharmacology, and Cell Biology of *sn*-2-Aminooxy Analogues of Lysophosphatidic Acid

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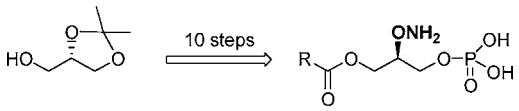
ABSTRACT



An efficient enantioselective synthesis of *sn*-2-aminooxy (AO) analogues of lysophosphatidic acid (LPA) that possess palmitoyl and oleoyl acyl chains is presented. Both *sn*-2-AO LPA analogues are agonists for the LPA_1, LPA_2, and LPA_4 G-protein-coupled receptors, but antagonists for the LPA_3 receptor and inhibitors of autotaxin (ATX). Moreover, both analogues stimulate migration of intestinal epithelial cells in a scratch wound assay.

Lysophosphatidic acid (1- or 2-acyl-*sn*-glycerol 3-phosphate, LPA) is a deceptively simple ligand that elicits a rich palette of biological responses, including platelet aggregation, promotion of cell survival, and cell migration.[1,2] The most important source of LPA is the action of lysophospholipase D (lysoPLD), or autotaxin (ATX), on lysophosphatidyl choline (LPC).[3] ATX is one of the 40 most upregulated genes in invasive cancers[4] and has been implicated in cell motility and tumor invasion, metastasis, and neovascularization.[3] LPA signals through the activation of specific receptors, which in turn leads to distinct cellular events depending on the receptor subtype expressed by the targeted cell. Cell surface LPA receptors belong to the membrane G protein-coupled receptors (GPCRs) protein family, and five mammalian LPA GPCRs have been characterized: LPA_1, LPA_2, LPA_3, LPA_4, and LPA_5.[1,5−8]

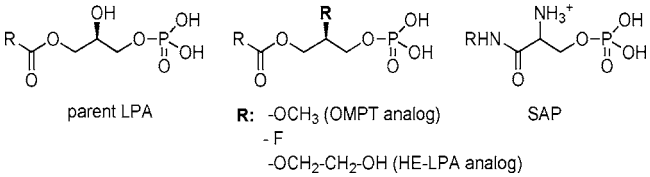


Figure 1. Structures of LPA, OMPT, HE-LPA, and SAP.

Modification of LPA may involve either the phosphate head group, the glycerol backbone, or the acyl groups,

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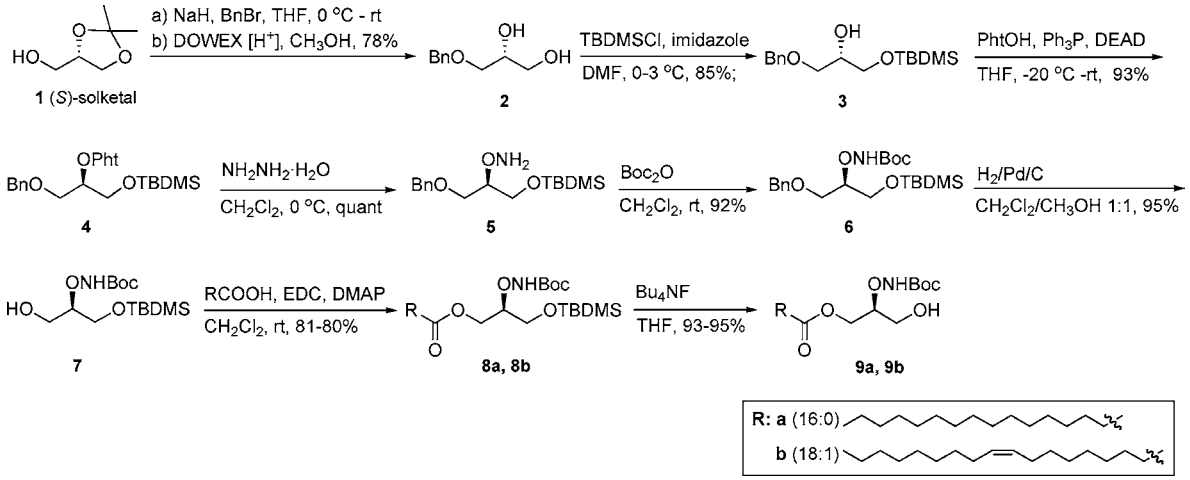
Scheme 1. Synthesis of *sn*-2-AO-LPA Intermediates **9a** and **9b**



leading to many changes in biological activity.[9−12] Only a few analogues of LPA feature modifications only at the *sn*-2 position (Figure 1). For example, OMPT (1-oleoyl-2-*O*-methyl-*rac*-glycerophosphothioate), in either the racemic[13] or optically pure[14] forms, are potent agonists for the LPA_3 receptor. The HE-LPA (hydroxyethoxy-LPA) analogues[15] are modest agonists for LPA_3 with 10-fold lower potency than the parent oleoyl LPA. Finally, the *sn*-2-F LPA analogs[16] show relatively weak agonist activity for all LPA receptor isoforms.

Recently, series of novel cytotoxic phospholipids, LPA-like serine amide phosphates (SAPs) were synthesized and demonstrated to be low micromolar inhibitors of prostate tumor cell proliferation.[17] The key element of this structure was the presence of a primary amine at the *sn*-2 position. Rather than introduce a strongly basic group into the LPA structure, our approach was to introduce the less basic but strongly nucleophilic aminooxy (AO) group at the *sn*-2 position. AO compounds are frequently employed as potent inhibitors of pyridoxal-5-phosphate-dependent enzymes, such as aminotransferases, serine hydroxymethyltransferase, tyrosine decarboxylase, cystationase, and ornithine decarboxylase, whereby the aminooxy moiety forms a stable oxime with the aldehyde group present on the cofactor.[18−22] AO analogues have also been demonstrated to function *in vitro* as potent antimalarial agents.[23]

We used the Mitsunobu reaction to introduce the AO functionality in stereocontrolled manner using straightforward protection and deprotection steps, thus elaborating an efficient synthetic approach to produce enantiomerically pure *sn*-2 AO-LPA analogues. Herein we describe the asymmetric total syntheses of two *sn*-2-AO LPA analogues, as well as evaluation in pharmacological, biochemical, and cell biological assays that reveal unexpected agonist and antagonist activities.

Synthesis. The synthesis of *sn*-2-AO LPA started with benzylation of (*S*)-solketal (**1**) (Scheme 1), followed by removal of the isopropylidene with an acidic ion-exchange resin,[24,25] to give intermediate diol **2**. Next, TBDMS was introduced[26,27] at the primary hydroxyl group at 0−3 °C to produce precursor **3**, which was transformed *via* a Mitsunobu reaction at 0 °C[28,29] into phthalimide derivative **4** with clean

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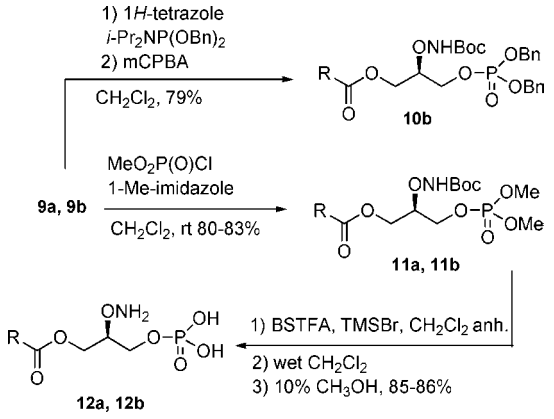
Table 1. Pharmacological Characterization of **12a** and **12b** in LPA GPCR Assays

	LPA_1		LPA_2		LPA_3		LPA_4	
compd	EC_{50} [nM] (E_{max})[a]	IC_{50} [nM] (Inhib. %)[b]	EC_{50} [nM] (E_{max})[a]	IC_{50} [nM] (Inhib. %)	EC_{50} [nM] (E_{max})[a]	IC_{50} [nM] (Inhib. %)	EC_{50} [nM] (E_{max})[a]	IC_{50} [nM] (Inhib. %)
12a	>4630 (57.3)	NE[c]	2450 (68.8)	NE	NE	2670 (63.1)	>10800 (45.8)	NE
12b	>7440 (51.2)	NE	382 (104)	NE	NE	56 (45.9)	1880 (86.8)	NE

[a] E_{max} = maximal efficacy of compound/maximal efficacy of LPA 18:1 × 100. [b] Inhib. % = % maximal inhibition of the response to 200 nM LPA 18:1 [c] NE = no effect was shown at the highest concentration (30 μM) tested.

inversion of configuration at the stereogenic center. Hydrazine monohydrate treatment of compound **4**[29] afforded the aminooxy intermediate **5** in quantitative yield. The enantiomeric purity of the aminooxy compound was verified by conversion to the (*R*)-Mosher amide;[30] ^{19}F NMR showed that intermediate **5** to have >95% enantiomeric purity. Next, the aminooxy group of **5** was reprotected with Boc to produce derivative **6**, and the benzyl ether was hydrogenolyzed to yield alcohol **7** in over 95% yield. Compound **7** was converted to the palmitoyl (**8a**) and oleoyl (**8b**) esters using carbodiimide chemistry, and deprotection of the silyl ether with Bu_4NF[26] in THF smoothly produced intermediates **9a** and **9b** in high yields.

Scheme 2. Conversion of Intermediates to *sn*-2-AO-LPA Analogues **12a** and **12b**



In attempting to remove both methyl and Boc protecting groups, neither an excess of TMSBr nor TMSBr followed by TFA, nor anhydrous HCl gave any desired product. Finally, each ester **11a** and **11b** was treated with BSTFA (*N*,*O*-bis(trimethylsilyl)trifluoroacetamide)[32] and TMSBr for 1 h under strict anhydrous condition in CH_2Cl_2, followed by addition of a wet CH_2Cl_2 (0.5% water) with stirring for 45 min. The crude compounds were then dried *in vacuo*, and 10% aq. methanol was added, which resulted in simultaneous deprotection of Boc group and the phosphate methyl esters. The desired compounds **12a** and **12b** (Scheme 2) were obtained in homogeneous form in good yields after filtration of each methanol solution through a Whatman PTFE Syringe Filter.

Pharmacology of AO-LPA Analogues on LPA Receptors. The pharmacological properties of AO-LPA analogues **12a** and **12b** were evaluated on LPA_{1-3} GPCRs in the McArtl rat hepatoma cell line RH7777, and on LPA_4 in CHO cells. Table 1 summarizes these data, which show that both palmitoyl and oleoyl *sn*-2-AO LPA analogues are agonists for the LPA_1, LPA_2, and LPA_4 G-protein-coupled receptors but are antagonists for the LPA_3 receptor. The oleoyl analogue **12b** is the most potent (382 nM) and also a full agonist for LPA_2. This analogue is also the most potent (56

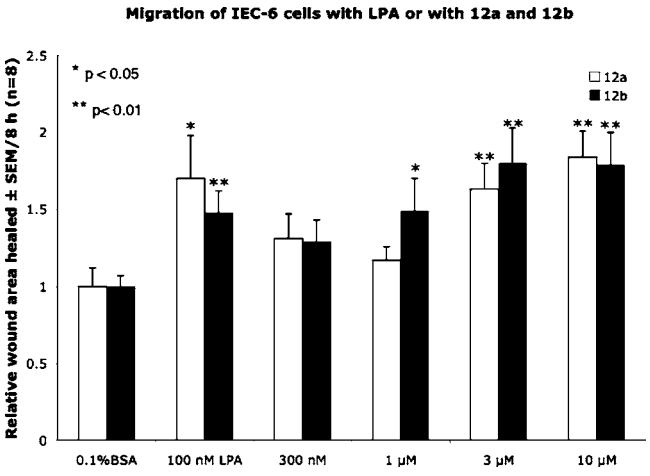


Figure 2. Compounds **12a** and **12b** promote cell migration in a scratch wound assay using IEC-6 cells. The basal rate of cell motility was measured in the presence of 0.1% BSA vehicle for stabilizing the LPA or LPA analogue, and the maximal LPA-induced response was evoked by treatment with 100 nM LPA. The bars represent the mean rate of cell migration in scratch wounds treated with different concentrations of **12a** and **12b**. The results represent the rate of migration over an 8 h period and the mean of 8 experiments.

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nM) partial antagonist for LPA_3. Both **12a** and **12b** inhibit recombinant ATX activity at 10 μM with potencies similar to those of LPA itself (see Supporting information).

Activity of AO-LPA Analogues on Cell Migration. The AO-LPA analogues **12a** and **12b** enhanced rate cell migration in IEC-6 human intestinal endothelial cells in a scratch wound assay (Figure 2). In this assay, the rate of cell migration is measured by the time it takes for the cells to close a gap in the cell monolayer created by "scratching" the monolayer with a pipet tip. This effect is congruent with the agonist activity toward the LPA_2 receptor, which is coupled to the Rho/Rac1 small GTPases and augments cell migration and cell survival.[33]

The replacement of the *sn*-2 hydroxy group of LPA with the more nucleophilic aminooxy function afforded AO-LPA analogues **12a** and **12b**. This small structural change has profound effects on the receptor pharmacology and cell biology of the analogues. Instead of the pan-receptor agonist activity exhibited by LPA, **12a** and **12b** are weak agonists for LPA_1 and LPA_4, strong agonists for LPA_2, and antagonists for the LPA_3. This mixed profile isoform selectivity makes them useful research tools for cell biology. Moreover, their ability to enhance migration of intestinal epithelial cells *in vitro* suggests therapeutic potential for repair of the gastrointestinal epithelium *in vivo*.

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Supporting Information Available: Experimental detailes for the synthesis and characterization of new compounds and protocols for biological assays. This material is available free of charge via the Internet at http://pubs.acs.org

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