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Synthesis of Migration-Resistant Hydroxyethoxy Analogues of Lysophosphatidic Acid

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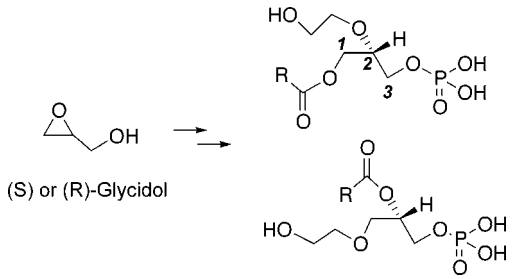
ABSTRACT



The susceptibility of lysophosphatidic acid (LPA) to intramolecular acyl migration impedes the determination of specific receptor activation by the *sn*-1 and *sn*-2 LPA regioisomers. An efficient enantioselective synthesis of hydroxyethoxy (HE)-substituted analogues of *sn*-1-acyl and 2-acyl LPA derivatives that possess palmitoyl and oleoyl chains is described. While the palmitoyl derivatives fail to activate calcium release in cells transfected with LPA_2 or LPA_3 G-protein-coupled receptors, the LPA_3 receptor is activated by both 1-HE and 2-HE oleoyl LPA analogues with a potency 10-fold lower than that of the parent oleoyl LPA.

Lysophosphatidic acid (LPA) is part of a family of bioactive phospholipids with the general form of 1- or 2-*O*-acyl-*sn*-glycero-3-phosphate (*sn*-1 or *sn*-2 LPA) that mediate a variety of biologic effects.[1,2] LPA induces cell proliferation, morphological changes, and has been shown to be involved in many physiological and pathological processes including neurogenesis,[3] myelination, angiogenesis,[4] wound healing,[5] and cancer progression.[6,7]

Normally, LPA is present in serum at low levels and is not detectable in platelet-poor plasma, whole blood, or cerebrospinal fluid. LPA is present at elevated levels, however, in the ascites of ovarian cancer patients, and may thus contribute to the progression of human cancer.[8] While the *sn*-1 LPA acyl regioisomer is preferentially produced in platelets, it appears that the *sn*-2 isomer is also found in

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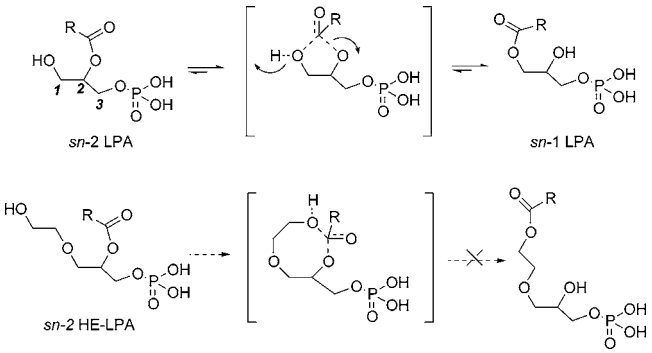
ascites.[9] Therefore, levels of *sn*-2 LPA seem to be associated with the initiation and progression of ovarian cancer.[10] However, *sn*-2 LPA is not stable under physiological conditions; it is readily converted under both acidic and basic conditions to an equilibrium mixture of *sn*-1 and *sn*-2 LPA isomers via intramolecular acyl chain migration (Scheme 1, top).[11] The lability of the acyl group in a 2-acyl-*sn*-glycerol-3-phosphate impedes structure−activity studies of individual LPA species.

Scheme 1. Favorable Intermediate for Acyl Migration for LPA (top) and Unfavorable Intermediate for Migration in HE-LPA Analogues (bottom)



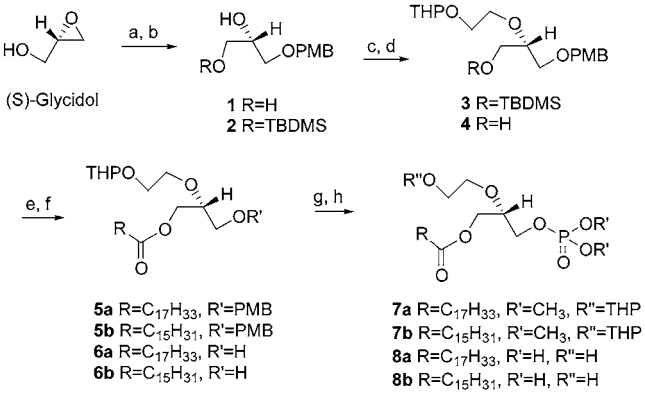
LPA signaling occurs through specific cell surface receptors of the endothelial cell differentiation gene (Edg) family of seven-transmembrane domain G-protein-coupled receptors (GPCR), which includes Edg-2/LPA_1, Edg-4/LPA_2, and Edg-7/LPA_3.[12] Recently, an additional LPA receptor, p2y9/GPR23/LPA_4, only distantly related to the Edg receptors and more closely related to the purinergic receptors, has been identified.[13] However, the function of particular receptors in the mammalian system and the molecular mechanism of LPA actions are still subjects of intensive investigation.[14]

Previously, we described the enantioselective synthesis and biological activities of a variety of fluorinated LPA analogues as metabolically stabilized ligands for LPA receptors.[15−18] Herein, we report an alternative approach to create LPA analogues with improved stability as useful molecular tools. We replaced the hydroxyl group with a hydroxyethoxy (HE) group to render acyl migration unfavorable. The advantage of this design is 2-fold. First, the HE group replacement will maintain a functional "lyso-like" structure, since initial structure−activity relationship (SAR) studies[19] have revealed that a hydroxyl group, an alkyl of more than 12 carbon atoms, and a phosphate monoester are required for the biological activities of LPA. Second, the HE group creates an unfavorable intermediate for the intramolecular acyl[20] migration that compromises the stereochemistry by regioisomerization (Scheme 1, bottom).

Traditionally, GPCRs have shown a preference for the naturally occurring enantiomer of their cognate ligands. However, our preliminary biological results have demonstrated that the unnatural D (2*S*) stereoisomers of some *O*-methylated LPA analogues (OMPT)[21] are more active than naturally occurring L (2*R*) enantiomorphs (Qian, Xu, Mills, Aoki, and Prestwich, submitted for publication). On the basis of these results, we synthesized the (2*S*) enantiomers of *sn*-1 and *sn*-2 acyl HE-LPA as our desired nonmigrating LPA analogues using an efficient and stereoselective chemical synthetic route.

As shown in Scheme 2, regiospecific and stereospecific ring opening of (*S*)-glycidol[22] by 4-methoxybenzyl alcohol (PMB-OH), using diisobutylaluminum hydride (DIBAL), generated the PMB protected glycerol (**1**). After selective

Scheme 2. Synthesis of 1-Acyl, 2-HE LPA Analogues[a]



[a] Reagents and conditions: (a) PMBOH, DIBAL, CH_2Cl_2, 51%; (b) TBDMSCl, DMAP, TEA, CH_2Cl_2, 78%; (c) NaH, TBAI, $BrCH_2CH_2OTHP$, DMF, 56%; (d) TBAF, THF, 95%; (e) Oleic acid (palmitic acid), DCC, DMAP, CH_2Cl_2, 82%; (f) DDQ, CH_2Cl_2, 66%; (g) $(OMe)_2P(O)Cl$, *t*-BuOK, 75%; (h) TMSBr, $MeOH/H_2O$, 95%.

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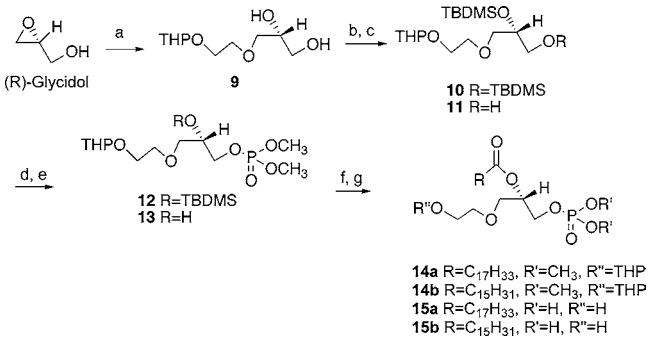
silylation at the primary alcohol by *tert*-butyldimethylsilyl (TBDMS) chloride,[23] the secondary alcohol **2** was alkylated with 2-(2-bromoethoxy)tetrahydro-2*H*-pyran in the presence of NaH and tetrabutylammonium iodide (TBAI) in good yield.[24] The 1-TBDMS ether **3** was then deprotected with tetra(*n*-butyl)ammonium fluoride (TBAF) in THF to give alcohol **4**, which was esterified with oleic acid or palmitic acid with use of DCC and DMAP to produce esters **5a** and **5b**, respectively. Oxidative removal of the PMB group with 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ) produced corresponding alcohols **6a** and **6b**. Phosphorylation with dimethyl chlorophosphate in the presence of *t*-BuOK gave good yields of protected phosphates **7a** and **7b**. The nonreductive deprotection of dimethyl phosphates with bromotrimethylsilane[18] was compatible with the unsaturated acyl chains. The trace amount of acid generated during workup (adding MeOH/H_2O) resulted in elimination of the tetrahydropyranyl group (THP) and generation of the *sn*-1-acyl 2-HE-modified LPA analogues **8a** and **8b**.

The strategy for the synthesis of the nonmigrating *sn*-2 1-HE-LPA analogues (Scheme 3) was similar to that above

Scheme 3. Synthesis of 2-Acyl, 1-HE LPA Analogues[a]



[a] Reagents and conditions: (a) $THPOCH_2CH_2OH$, DIBAL, CH_2Cl_2, 50%; (b) TBDMSCl, imidazole, DMF, 91%; (c) HF-Py/Py, THF, 58%; (d) $(OMe)_2P(O)Cl$, 1-methylimidazole, 87%; (e) TBAF, AcOH, THF, 76%; (f) oleic acid (palmitic acid), DCC, DMAP, CH_2Cl_2, 85%; (g) TMSBr, MeOH/H_2O, 95%.

for *sn*-1 2-HE-LPA. To obtain the (2*S*) enantiomer, (*R*)-glycidol was regioselectively ring-opened and alkylated with 2-(2-bromoethoxy)tetrahydro-2*H*-pyran to give diol **9**, followed by bis-silylation to **10**. Selective deprotection of bis-TBDMS ether **10** with 6.0 equiv of pyridinium hydrofluoride (HF-Py)[25] resulted in 58% yield of alcohol **11** after 18 h at room temperature. The optimal amount of HF-Py was crucial to the reaction, since an excess would cause deprotection of both TBDMS groups. Phosphorylation of **11** with use of dimethyl chlorophosphate in the presence of *N*-methylimidazole gave protected phosphate in 87% yield.[21] Use of *t*-BuOK as base for this reaction gave only 10% yield. The 2-TBDMS ether was further deprotected with TBAF−$3H_2O$ in THF to give alcohol **13**. Neutralization of TBAF with acetic acid allowed the desilylation of the secondary alcohol without migration of the phosphate. After DCC-promoted esterification and deprotection of the phosphate with TMSBr, *sn*-2 LPA analogues **15a** and **15b** were obtained in high yields.

Each of the nonmigrating LPA analogues **8a**, **8b**, **15a**, and **15b** was incubated in Tris-HCl buffer (pH 8.0) at 37 °C for 24 h. No migration or decomposition was observed by ^1H NMR spectroscopy, which demonstrates the good chemical stability of those LPA analogues under biological assay conditions.

The synthetic LPA analogues were evaluated for their ability to activate transmembrane LPA receptors and nuclear PPARγ. First, the LPA analogues were tested in insect Sf9 cells expressing LPA_2 or LPA_3 receptors,[26] and in a cell motility assay with rat hepatoma Rh7777 cells that express LPA_1.[27] None of the analogues tested activated LPA_1 (Figure 1), and only modest calcium release was induced by

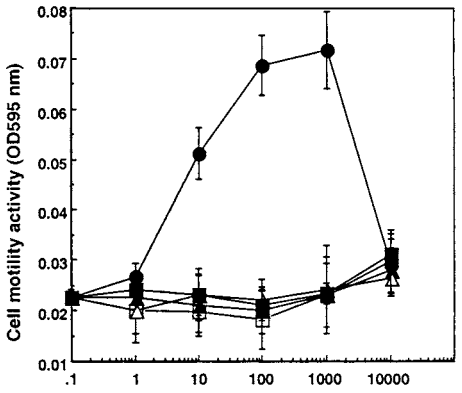


Figure 1. Cell motility assay by activation of LPA_1 in transfected Rh7777 cells: (●) LPA (1-oleoyl); (▲) **8a** (2-HE, 1-oleoyl,); (△) **8b** (2-HE, 1-palmitoyl); (■) **15a** (1-HE, 2-oleoyl); and (□) **15b** (1-HE, 2-palmitoyl).

activation of LPA_2 by any of the compounds (Figure 2). However, the oleoyl HE-LPA analogues **8a** and **15a** activated LPA_3 with potency 30-fold less than that of 1-oleoyl LPA. **8a** and **15a** also showed over 100-fold higher potency than the two palmitoyl HE-LPA analogues **8b** and **15b**, confirming that LPA_3 markedly prefers ligands with an unsaturated fatty-acid ester. Interestingly, little difference was observed between the 1-HE and 2-HE oleoyl regioisomers **8a** and **15a** for LPA_3 activation, which can be attributed to the extended HE group.

In addition, the LPA analogues were evaluated in a nuclear reporter assay in which monocytic cells were transfected with

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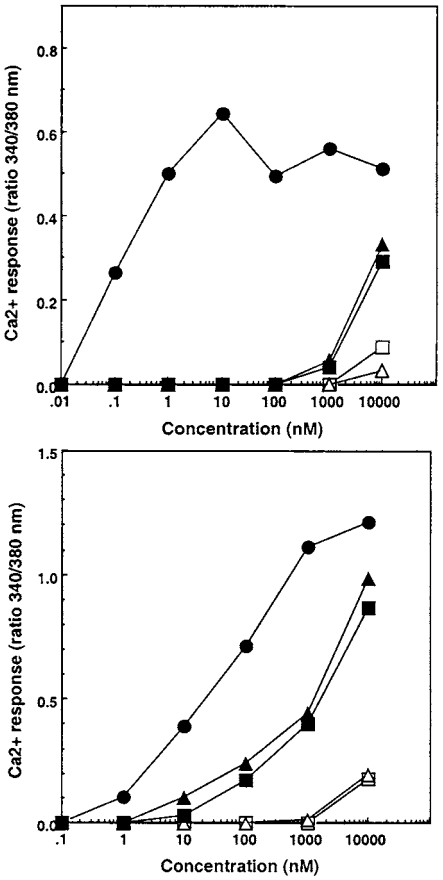

Figure 2. Calcium release by activation of LPA_2 (top) and LPA_3 (bottom) in transfected Sf9 cells: (●) LPA (1-oleoyl); (▲) **8a** (2-HE, 1-oleoyl); (△) **8b** (2-HE, 1-palmitoyl); (■) **15a** (1-HE, 2-oleoyl); and (□) **15b** (1-HE, 2-palmitoyl).

a luciferase construct activated by a PPARγ nuclear receptor response element (Figure 3). In this assay, three of the four analogues, as well as *sn*-1-oleoyl-LPA, were tested. We found that the nuclear transcription factor PPARγ was activated by both the *sn*-1 and *sn*-2 HE-LPA analogues, and did not show a preference for an unsaturated acyl residue.[17,28] PPARγ, therefore, responds to a wide range of LPA

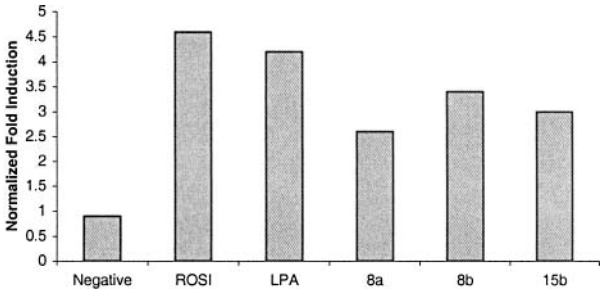

Figure 3. HE-LPA analogues and natural LPA activate the nuclear transcription factor PPARγ: ROSI, rosiglitazone; LPA (1-oleoyl); **8a** (2-HE, 1-oleoyl); **8b** (2-HE, 1-palmitoyl); and **15b** (1-HE, 2-palmitoyl).

homologues consistent with previous results.[28] Moreover, the HE-LPA derivatives are expected to be less susceptible to LPA acyl transferase activity.[29]

In summary, we have established a concise and efficient approach for synthesizing HE-substituted nonmigrating LPA analogues. We have demonstrated that 1-HE and 2-HE oleoyl LPA analogues **8a** and **15a**, at ≤ 1 μM, triggered cellular responses through the LPA_3 but not the LPA_1 or LPA_2 receptors. Neither **8a** nor **15a** were LPA_1 receptor agonists at above 1 μM. Thus, oleoyl HE-LPA analogues are useful probes for the dissection of LPA signaling events in complex mammalian systems. Further application of these LPA analogues is expected to provide additional insight into the complex biology of LPA.

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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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