Molecular Recognition

Identifying Specific Conformations by Using a Carbohydrate Scaffold: Discovery of Subtype-Selective LPA-Receptor Agonists and an Antagonist**

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Lysophosphatidic acids (LPA) are a group of important extracellular signaling molecules that elicit a wide variety of fundamental biological responses, such as cell-growth stim-

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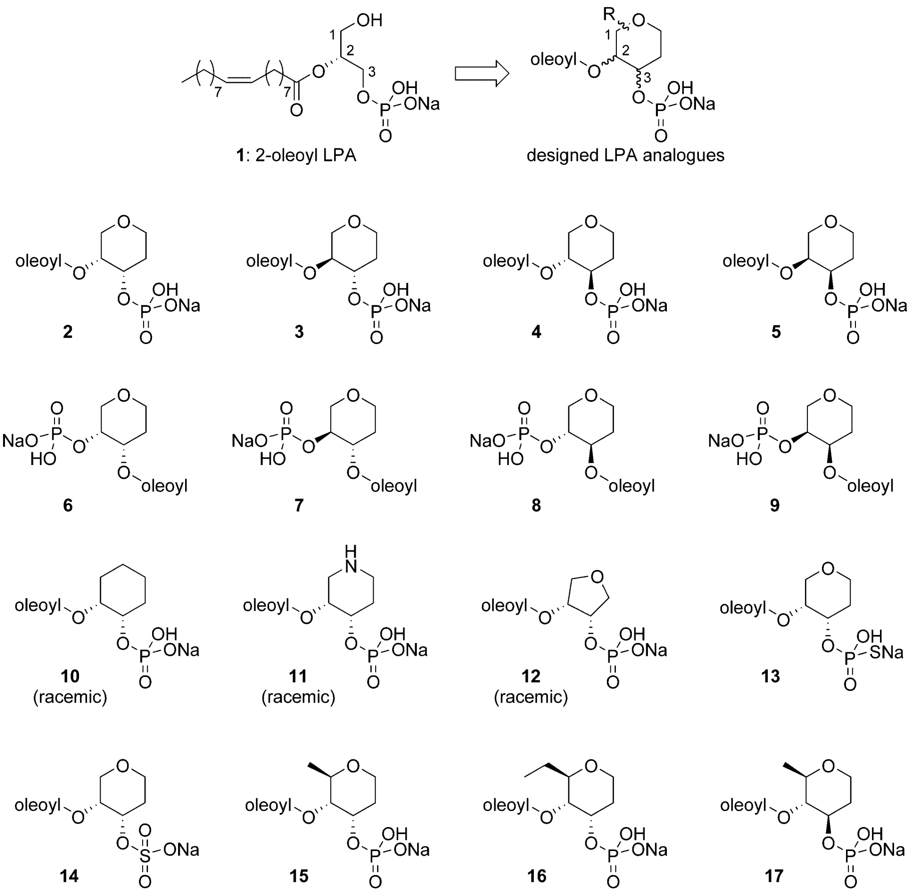
ulation, calcium mobilization, escape from apoptosis, tumor-cell invasion, and smooth-muscle contraction.[1] The diverse properties are mediated through interactions of LPA with G-protein-coupled receptors named LPA_1, LPA_2, and LPA_3. Studies to explore the specific role of each subtype are in progress, and are essential for further advances, for example, for designing drugs to modulate a specific LPA-mediated signal-transduction pathway. Studies with subtype-selective agonists and antagonists are very powerful for this purpose. Herein, we describe a new strategy for the discovery of subtype-selective ligands for LPA receptors, based on the identification of specific active conformations of LPA by using carbohydrates as scaffolds.

2-Oleoyl LPA (**1**) is the most potent physiological molecule in the LPA family. Studies on this LPA-receptor ligand have focused mainly on the generation of stable analogues by preventing acyl migration between the 1- and 2-hydroxy groups.[2–4] These studies led to the proposed LPA pharmacophore,[5] as well as to several subtype-selective agonists and antagonists.[6] The activity and selectivity of these analogues, however, are not yet satisfactory for their use as biological tools. This might be partly because of the conformational flexibility of these analogues. The population and/or lifetime of analogues in the active three-dimensional conformation might not be sufficient for selective receptor activation as a result of a thermal disturbance. Therefore, we planned to restrict the conformational flexibility by introducing a ring structure.[7]



Scheme 1. Design of LPA analogues and selected synthesized compounds.

Our molecular design for LPA analogues is summarized in Scheme 1. Based on the hypothesis that different LPA-receptor subtypes distinguish different three-dimensional arrangements of a negative charge (phosphate anion), a long hydrophobic tail (oleoyl group), and a hydrogen-bond acceptor (oxygen atom at the 1-position),[8] we synthesized an array of molecules that display these recognition motifs at various relative positions defined by core carbohydrate scaffolds.[9] By employing a variety of readily available enantiomerically pure carbohydrate configurational isomers, the arrangement can be finely tuned. The diversity of the arrangements can be further increased by introducing substituents on the ring to modulate the ring conformation. For each arrangement, a particular recognition motif can be modified independently to examine the effect of the motif. Furthermore, problematic acyl migration is prevented in these analogues, because the oxygen atom corresponding to the 1-hydroxy group of 2-oleoyl LPA is incorporated into the pyran ring. Based on this idea, we synthesized approximately 40 molecules; selected compounds are shown in Scheme 1.[10]

First, the agonist activity of **2**–**9** was examined. Upon stimulation with the LPA analogues, the increase in Ca^{2+} concentration in insect Sf9 cells that express LPA_3 receptors was assessed (Figure 1a).[11] Compounds **2**–**9** include all possible stereo- and regioisomers derived from the carbohydrate template. The isomer **2** had 5- to 10-fold higher agonist activity ($EC_{50} \approx 10$ nM) relative to the commonly used agonist 1-oleoyl LPA ($EC_{50} \approx 50$–100 nM). The other isomers **3**–**9** were 10- to 500-fold less potent than **2**. Specifically, the natural (2*R*)-oleoyl LPA analogue **2** was 50-fold more active than the non-natural 2*S* analogue **3** ($EC_{50} > 500$ nM).[12]

Next, we investigated the effect of individual recognition motifs on LPA_3 activation (compounds **10**–**14**) by fixing the stereochemistry to match that of the superior agonist **2**. Analogues **10**, **11**, and **12** did not show any agonist activity, which indicated the essential role of the oxygen atom in the six-membered ring as a hydrogen-bond acceptor.[10] On the other hand, the activity of the thiophosphate analogue **13** was approximately 100- to 500-fold higher ($EC_{50} \approx 0.5$ nM) than 1-oleoyl LPA. Thus, **13** is one of the most potent LPA_3 agonists reported to date.[13] Moreover, **13** had only very weak agonist activity for LPA_1 (500- to 1000-fold weaker than 1-oleoyl LPA, Figure 1b), and no agonist activity for LPA_2.[10,14] Thus, **13** is a highly potent LPA_3-selective agonist that can be used as a biological tool.[15]

We next targeted the discovery of LPA_1-selective agonists. An LPA_1-selective agonist would be highly desirable, because of the importance of this receptor. No agonist has been reported that can selectively activate the LPA_1 receptor in the

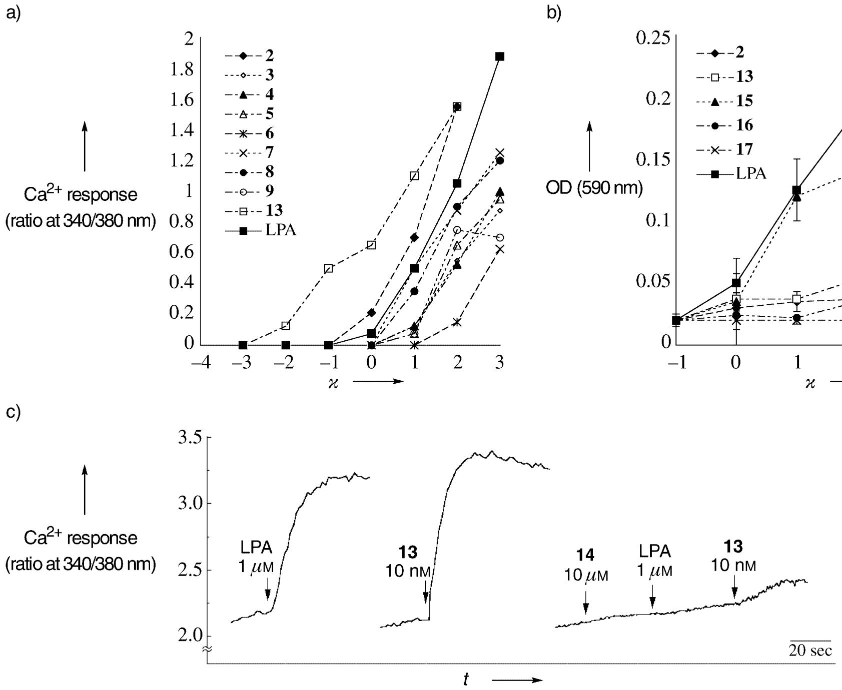


Figure 1. a) LPA_3-agonist activities. Assays were performed at least three times; representative data are shown. The change in the intracellular concentration of Ca^{2+} ions (Ca^{2+} response; *y* axis) upon activation by the agonists was determined by the emission ratio of Fura-2 AM fluorescence at an excitation wavelength of 340 nm (Ca^{2+}-bound form) and 380 nm (Ca^{2+}-free form); x = dose (10^x nM). b) LPA_1-agonist activities. The numbers of migrated cells upon activation by the agonists was determined by measuring the optical density (OD) at 590 nm (*y* axis). c) LPA_3-antagonist activity of **14**.

presence of the LPA_2 receptor. As compounds **2**–**14** had no significant agonist activity for LPA_1, we attempted to introduce a substituent on the six-membered ring to adjust the relative position of the recognition motifs for LPA_1 through fine-tuning of the ring conformation. Thus, we synthesized **15**–**17** and found that **15** had equivalent or stronger potency as an agonist for LPA_1 relative to 1-oleoyl LPA (Figure 1b).[14,16] As **15** did not activate LPA_2,[10] it is the first compound that can be used to distinguish between LPA_1- and LPA_2-agonist activity.[15,17]

The subtype-selective agonist activity of **2**, **13** (LPA_3-selective), and **15** (LPA_1-selective) might be partly rationalized based on the hypothesis that a specific LPA receptor distinguishes a specific three-dimensional arrangement of the recognition motifs. To test this hypothesis, we determined the ring conformation in a solution state by using NMR techniques. All NOE data and coupling constant values suggested that **2** exists in a skewed-boat conformation with both the phosphate and oleoyl groups in pseudoequatorial positions (Figure 2a). On the other hand, **15** exists in a chair conformation with the methyl and oleoyl groups in equatorial positions and the phosphate group in an axial position (Figure 2b).[18] The observed recognition-motif arrangements in **2** and **15** might correspond to the active binding structures of flexible 2-oleoyl LPA to LPA_1 and LPA_3, respectively, and the arrangements might be recognized selectively by each receptor.

Finally, we found that the analogue **14**, which contains a sulfate instead of a phosphate group, inhibited LPA_3 (Figure 1c).[10,19] No response was observed upon activation of

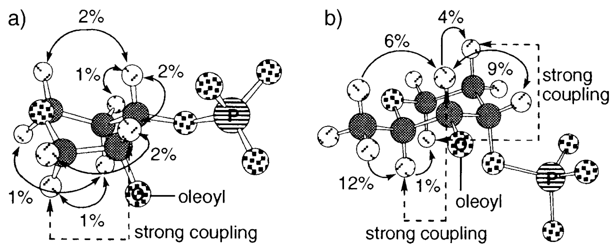


Figure 2. Observed conformations of **2** (a) and **15** (b) in solution in CD_3OD.

LPA_3 with 1-oleoyl LPA after pretreatment with **14** (10 μM), and only weak activation was observed even with the highly potent agonist **13**. Moreover, **14** did not antagonize the 1-oleoyl LPA induced migration of LPA_1-expressing cells (MDA, PC3, and 203g), nor the 1-oleoyl LPA-induced mobilization of Ca^{2+} ions in LPA_2-expressing cells (HT29). Thus, the antagonist activity of **14** is LPA_3-selective. Although the inhibitory activity was not very strong, **14** can be used as a lead LPA_3-selective antagonist for further structural optimization.

In conclusion, potent and subtype-selective agonists (**2**, **13**, and **15**) for LPA_1 and LPA_3 were developed by using carbohydrates as a core structure. The basic concept for ligand discovery was the selective extraction of active three-dimensional recognition-motif arrangements from conformationally flexible 2-oleoyl LPA. The concept allowed the discovery of a lead compound **14** for subtype-selective antagonists. To our knowledge, this is the first example of receptor subtype-selective recognition by an array of small molecules by changing the relative three-dimensional arrangement of pharmacophores attached to a carbohydrate core. These compounds can be synthesized on a gram scale, and are stable for at least several months at −20 °C. Physiological studies on these subtype-selective agonists, as well as studies toward the development of potent and selective LPA antagonists are in progress.

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[13] The analogue of Prestwich and co-workers is the only example with this level of activity (see reference [4c]). Compound **13** also had high potency (100- to 500-fold relative to 1-oleoyl LPA) in Ca^{2+}-mobilization assays with several cells that express LPA_3.

[14] The LPA_1-agonist activity was assessed by determining MDA-cell migratory activity: M. Umezu-Goto, Y. Kishi, A. Taira, K. Hama, N. Dohmae, K. Takio, T. Yamori, G. B. Mills, K. Inoue, J. Aoki, H. Arai, *J. Cell Biol.* **2002**, *158*, 227. The LPA_2-agonist activity was assessed based on Ca^{2+}-releasing activity from Sf9 cells transfected with LPA_2 (reference [11]).

[15] Physiological studies with these analogues are ongoing.

[16] A cell-migration assay with PC3 cells indicated that **15** is 10 times more potent than 1-oleoyl LPA as an LPA_1 agonist; see Supporting Information.

[17] Compound **15** activates the LPA_3 receptor with comparable efficacy to 1-oleoyl LPA; see Supporting Information.

[18] The ring in compound **16** should occupy the same conformation as that in **15**; however, **16** might not fit into the binding pocket of LPA_1 as a result of the bulky substituent.

[19] Only **14** showed inhibitory activity among compounds **2–17**.

