ACS OMEGA

Open Access

http://pubs.acs.org/journal/acsodf

Article

Synthesis and Biological Evaluation of Lysophosphatidic Acid Analogues Using Conformational Restriction and Bioisosteric Replacement Strategies

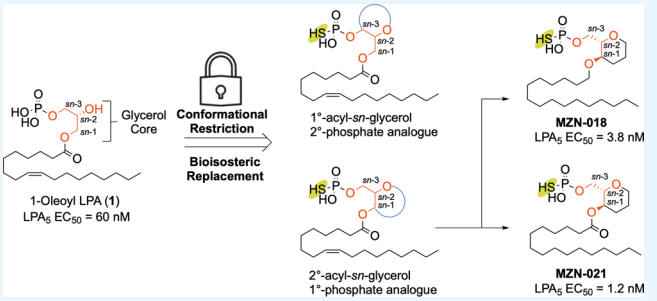
Mazin A. S. Abdelwahid, Kosuke Ohsawa, Akiharu Uwamizu, Kuniyuki Kano, Junken Aoki,* and Takayuki Doi*

Cite This: *ACS Omega* 2023, 8, 49278−49288

Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information

ABSTRACT: Lysophosphatidic acid (LPA) is a key player in many physiological and pathophysiological processes. The biological activities of LPA are mediated through interactions with—at least—six subtypes of G-protein-coupled receptors (GPCRs) named LPA_{1-6}. Developing a pharmacological tool molecule that activates LPA subtype receptors selectively will allow a better understanding of their specific physiological roles. Here, we designed and synthesized conformationally restricted 25 1-oleoyl LPA analogues **MZN-001** to **MZN-025** by incorporating its glycerol linker into dihydropyran, tetrahydropyran, and pyrrolidine rings and variating the lipophilic chain. The agonistic activities of these compounds were evaluated using the TGFα shedding assay. Overall, the synthesized analogues exhibited significantly reduced agonistic activities toward LPA_1, LPA_2, and LPA_6, while demonstrating potent activities toward LPA_3, LPA_4, and LPA_5 compared to the parent LPA. Specifically, **MZN-010** showed more than 10 times greater potency (EC_{50} = 4.9 nM) than the standard 1-oleoyl LPA (EC_{50} = 78 nM) toward LPA_5 while exhibiting significantly lower activity on LPA_1, LPA_2, and LPA_6 and comparable potency toward LPA_3 and LPA_4. Based on the **MZN-010** scaffold, we synthesized additional analogues with improved selectivity and potency toward LPA_5. Compound **MZN-021**, which contains a saturated lipophilic chain, exhibited 50 times more potent activity (EC_{50} = 1.2 nM) than the natural LPA against LPA_5 with over a 45-fold higher selectivity when compared to those of other LPA receptors. Thus, **MZN-021** was found to be a potent and selective LPA_5 agonist. The findings of this study could contribute to broadening the current knowledge about the stereochemical and three-dimensional arrangement of LPA pharmacophore components inside LPA receptors and paving the way toward synthesizing other subtype-selective pharmacological probes.

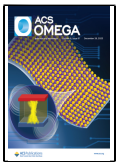


INTRODUCTION

Lysophosphatidic acid (LPA) is a kind of bioactive lipid that acts as an extracellular signaling molecule, eliciting wide arrays of physiological and pathophysiological functions. Most of the LPA effects are mediated via at least six G-protein-coupled receptors (GPCRs),[1] named LPA_{1-6}. The first subtype of this family (LPA_1) was discovered in the mid-1990s from studies using neural cells for its ability to induce cell rounding.[2] Following this, five LPA receptors were identified in the late 1990s and early 2000s. LPA_{1-3} are classified as part of the endothelial differentiation gene (Edg) subfamily of GPCRs, whereas LPA_{4-6} are categorized as non-Edg family members of LPA receptors and belong to the purinergic GPCR cluster.[3] LPA is involved in many biological events including cellular proliferation, cancer cell invasion, calcium mobilization, platelet activation, brain development, and hair follicle formation, among others.[4−10] Hence, developing potent LPA receptor modulators that can selectively activate or block specific receptors would enhance our understanding of the distinct functions of each receptor and uncover its potential benefits for therapeutic applications.

1-Oleoyl LPA (**1**) (Figure 1) is the most widely and extensively used LPA molecular species reported in research with potent biological effects[1,3] and agonism toward all six LPA receptors. Structurally, it has a glycerol backbone with a phosphate group at the *sn*-3 position and an oleoyl fatty chain at the *sn*-1 position.[11] Generally, LPA exists as two isomers, *sn*-1 and *sn*-2 acyl LPA, depending on the relative position of the acyl chain. Several studies have been conducted by different groups in an attempt to establish a structure−activity relationship (SAR) of LPA toward LPA receptors and to

Received: October 3, 2023
Revised: November 8, 2023
Accepted: November 13, 2023
Published: December 11, 2023



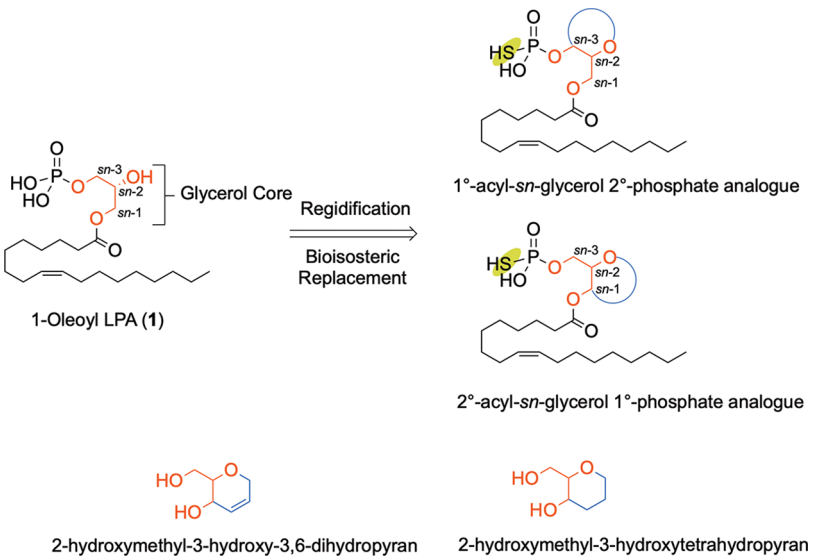


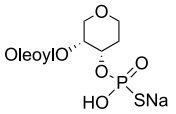
Figure 1. Molecular design of the LPA analogs.

obtain a better understanding of its pharmacophore. These studies have concluded that the phosphate group or phosphate isosteres that have the capability of retaining the negative charge under physiological pH are essential for agonistic activity.[12] Only two classes of phosphate isosteres proved to be active in this context, namely, phosphonate derivatives especially those containing α-hydroxy, keto, and fluorophosphonate[13,14] and phosphorothioate analogues,[12,15,16] indicating its direct involvement in receptor activation, which has been proven by X-ray crystallography and single-particle cryo-EM analysis of LPA_6 and LPA_1, respectively.[17−19] Recently, a nonlipid LPA_2-selective agonist was developed. The sulfamoyl benzoic acid (SBA) group in this agonist was predicted to occupy the phosphate group binding site, based on the model used in the study.[20−22] However, no report on the use of this SBA head in glycerol-based LPA analogues has been published.

Regarding the lipophilic chain, the change in this part appeared to be more tolerated.[23] Naturally occurring LPA species consist of LPA having varied acyl chains of saturated and unsaturated types with lengths ranging from 14 to 22 carbons. These LPA species have differential biological activities, implying that different degrees of selectivity toward LPA receptors can be achieved by changing the acyl chain moiety.[24,25] Furthermore, the more stable LPA analogues based on amide and ether isosteres proved to be active and potent LPA receptor agonists.[13,23]

The *sn*-2 hydroxy group does not seem to be essential for the activation of, at least, some LPA receptors. Analogues having no hydroxy group at this position or masked OH group in form of methyl ether, short-chain ester, or cyclic phosphatidic acid have shown potent and sometimes selective activity.[17,26−31] The glycerol backbone itself as a linker between the lipophilic tail and the phosphate head is not required for optimal agonistic activity since it was replaced with many scaffolds without much loss of ability to activate receptors. For example, oleyl thiophosphate, a fatty alcohol thiophosphate, has been reported to have partial agonistic activity toward $LPA_{1,3}$ and full agonistic activity toward LPA_2, indicating the importance but not essentiality of the glycerol linker.[27] Replacing the glycerol core with other linkers, such as unsubstituted and small-sized substituted ethanolamines, has been reported as a successful strategy for developing LPA receptors agonists.[13,32,33]

Being a highly flexible molecule, LPA has several conformations to adopt, which explains the ability of LPA to activate many receptors. Strategies to restrict this flexibility by modifying the glycerol core into more rigid substructures have been reported in the field of lipid mediators. For example, LPA analogues containing conformationally restricted aryl substructures such as resorcinol and aminophenols have been synthesized and evaluated for their agonistic activity. However, none of these compounds showed equipotent activity to LPA.[34] In another study, a linker that secures a nonflattened three-dimensional arrangement of the LPA pharmacophore compared to the flattened aromatic systems previously examined was used by employing a tetrahydrofuran scaffold as a linker to synthesize a set of 2-oleoyl LPA analogues. Among this series, compound **T13** showed a very potent and selective LPA_3 agonistic activity (Figure 2).[35] A recent



T13

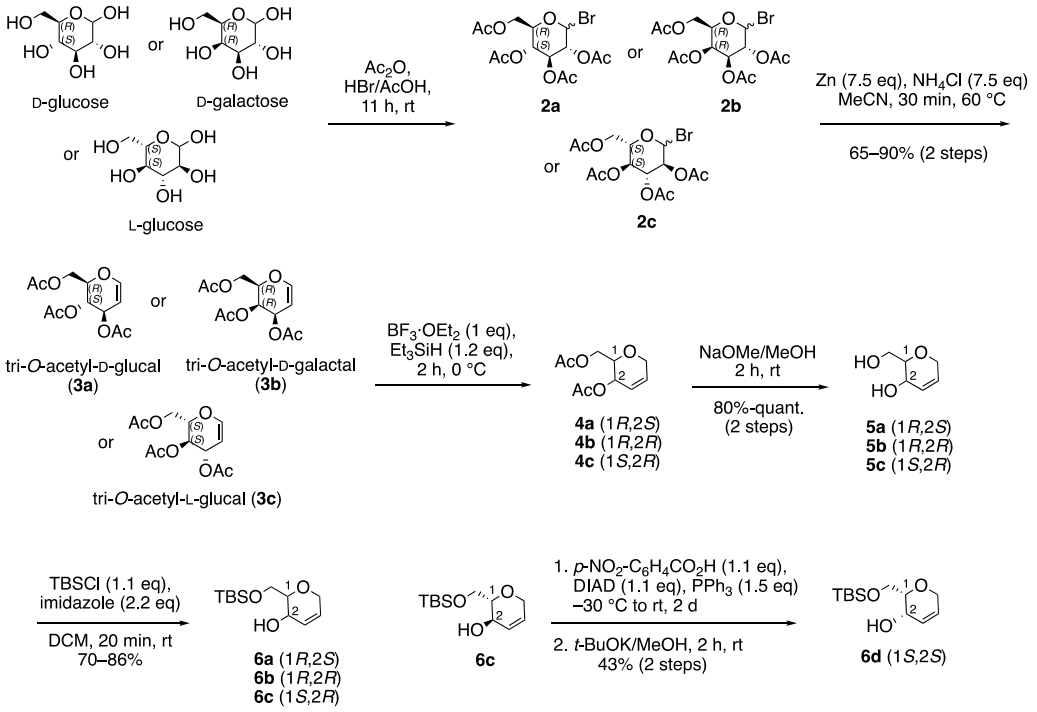
Figure 2. Structure of **T13**.

evaluation of **T13** activity against LPA_{1-6} using a more robust assay, TGFα shedding assay, showed that although **T13** has a subnanomolar activity on LPA_3; it also has more potent activity than 1-oleoyl LPA on LPA_5.[36] Aside from cyclic phosphatidic acids that contain a cyclic phosphate group,[31] no previous report has been published using this strategy to design 1-oleoyl LPA (rather than 2-oleoyl LPA) analogues. Besides, many ligands have been designed and synthesized for LPA_{1-3}, but ligands for LPA_{4-6} are still limited.

Seeking to enhance our understanding of the three-dimensional arrangement of the LPA pharmacophore components and its relation to the LPA agonistic activity and trying to develop a selective and potent LPA_5 pharmacological probe, here, we describe the design and synthesis of a number of conformationally restricted 1-oleoyl

https://doi.org/10.1021/acsomega.3c07668
ACS Omega 2023, 8, 49278−49288

Scheme 1. Synthesis of the Common Intermediates 6a−d



LPA analogues by employing 2-hydroxymethyl-3-hydroxytetrahydropyran and 2-hydroxymethyl-3-hydroxy-3,6-dihydropyran as glycerol substitute linkers (Figure 1). These skeletons have two chiral centers and two regioisomers arising from the functionalization of two different hydroxy groups. A total of 16 compounds of all possible stereoisomers and regioisomers were initially synthesized to evaluate their selectivity, and then more analogues have been synthesized based on the molecular structure of the most potent one toward LPA_5.

RESULTS AND DISCUSSION

Molecular Design. As shown in Figure 1, 1-oleoyl LPA (**1**) is composed of two substructures, a polar phosphate moiety and a lipophilic chain, connected via a glycerol linker through ester bonds. Both substructures proved to be essential for LPA activity. Owing to its numerous rotatable bonds and, therefore, conformational flexibility, LPA can adopt many active conformations, which may explain its pan-agonistic effect.[35] We hypothesize that restricting this flexibility and removing the H-bond donating potential at the *sn*-2 hydroxy group by incorporating its glycerol moiety into more rigid skeletons than glycerol (namely, 2-hydroxymethyl-3-hydroxytetrahydropyran and 2-hydroxymethyl-3-hydroxy-3,6-dihydropyran) could generate molecules with selective agonistic activity. In addition, these conformational constraints could reduce the entropic cost arising from freezing the ligand inside the receptor into a specific conformation during the binding event, increasing its potency.[37]

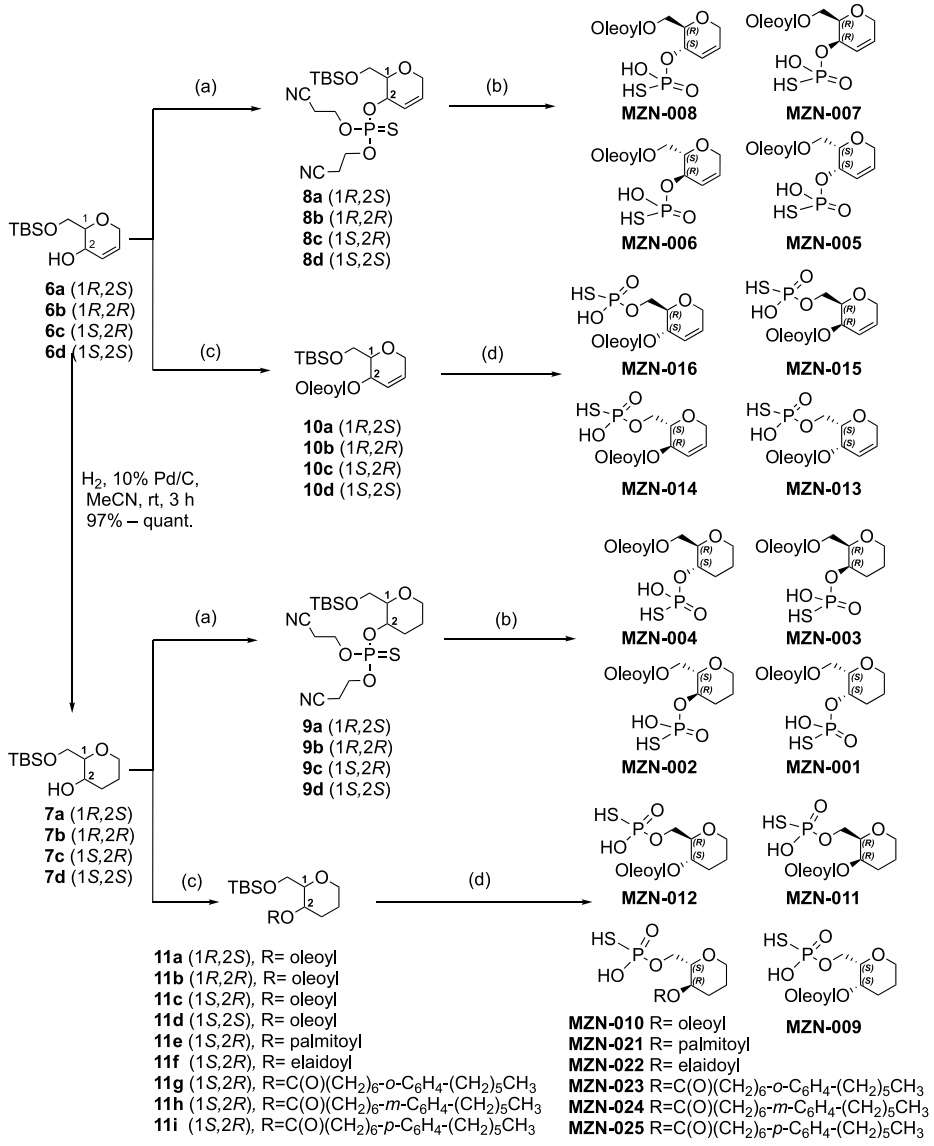
Incorporating glycerol into these two scaffolds could be achieved in two ways, generating two types of analogues, as depicted in Figure 1. Connecting carbon 3 of the glycerol core to the *sn*-2 hydroxy group produces 1°-acyl-*sn*-glycerol 2°-phosphate analogues while linking the hydroxy group with carbon 1 generates 2-acyl-*sn*-glycerol-1-phosphate analogues.[38] In addition, cyclization produces another stereogenic center in either case. We considered synthesizing all possible stereoisomers of both types of each scaffold (16 compounds) and evaluating their LPA_{1-6} agonistic activity. Bioisosteric replacement of phosphate into phosphorothioate has been previously reported to generate less readily hydrolyzable molecules.[16] Using this strategy is expected to bring more metabolic stability to the designed molecules. Further analogues were synthesized based on the most potent analogues toward LPA_5 by varying the lipophilic chain or/and the linker.

Chemistry. *Synthesis of 2-Hydroxymethyl-3-hydroxytetrahydropyran and 2-Hydroxymethyl-3-hydroxy-3,6-dihydropyran-Based LPA Analogues.* We started the synthesis by employing enantiomerically pure monosaccharides, namely, D-glucose, D-galactose, and L-glucose, to synthesize the corresponding glycals **3a**, **3b**, and **3c** (Scheme 1). Treating these monosaccharides separately with acetic anhydride followed by the addition of HBr/AcOH solution generated bromides **2a**, **2b**, and **2c**, which in turn reduced to the corresponding tri-*O*-acetylated glycals using the Zn/NH_4Cl mixture.[39,40]

The glycals **3a**, **3b**, and **3c** were subjected to Ferrier rearrangement employing $BF_3{\cdot}OEt_2/Et_3SiH$, followed by alcoholysis using NaOMe/MeOH to produce diols **5a**, **5b**, and **5c**.[38] Selective protection of the primary alcohol of **5a**, **5b**, and **5c** using a TBS-protecting group generated **6a**, **6b**, and **6c**. To invert the stereochemistry of the carbon bearing secondary alcohol in **6c** to produce its epimer **6d**, Mitsunobu reaction was employed for this step, followed by base-promoted hydrolysis of ester product.[41] The corresponding saturated analogues of **7a−7d** were obtained by catalytic hydrogenation of **6a−6d** over Pd/C (Scheme 2), respectively. The formation of thiophosphate diesters **8** and **9** was achieved by treating compounds **6** and **7**, respectively, with phosphorodiamidite $(CNCH_2CH_2OP[N(iPr)_2]_2)$/1*H*-tetrazole, followed by oxidation using elemental sulfur. Acid-catalyzed deprotection of silyl ethers **8** and **9** was carried out producing alcohols, which, in turn, were esterified by oleoyl chloride/DMAP, followed by *β*-

https://doi.org/10.1021/acsomega.3c07668
ACS Omega 2023, 8, 49278−49288

Scheme 2. Synthesis of MZN-001−MZN-016 and MZN-021−MZN-025[a]



[a]Reaction conditions: (a) $CNCH_2CH_2OP[N(iPr)_2]_2$ (2 equiv), ethylene cyanohydrin (1.6 equiv), 1*H*-tetrazole (2.5 equiv), then S (2 equiv), DCM, RT, 4 h, 66-88%. (b) 1. Amberlyst 15 (H^+), MeOH, 14 h, RT; 2. Oleoyl chloride (1.5 equiv) (or carboxylic acid, DIC), DMAP (1.5 equiv), DCM, RT; 3. DBU, MSTFA, Pyridine, RT, 1 h, 21−58% (three steps). (c) Oleoyl chloride (1.5 equiv), DMAP (1.5 equiv), DCM, RT, or RCOOH, DIC, DMAP, DCM, RT, 55−97%. (d) 1. HF/Py, THF, RT, 16 h; 2. $CNCH_2CH_2OP[N(iPr)_2]_2$ (2 equiv), ethylene cyanohydrin (1.6 equiv), 1*H*-tetrazole (2.5 equiv), and then S (2 equiv), DCM, RT, 4 h; 3. DBU, MSTFA, pyridine, RT, 1 h, 34−81% (three steps).

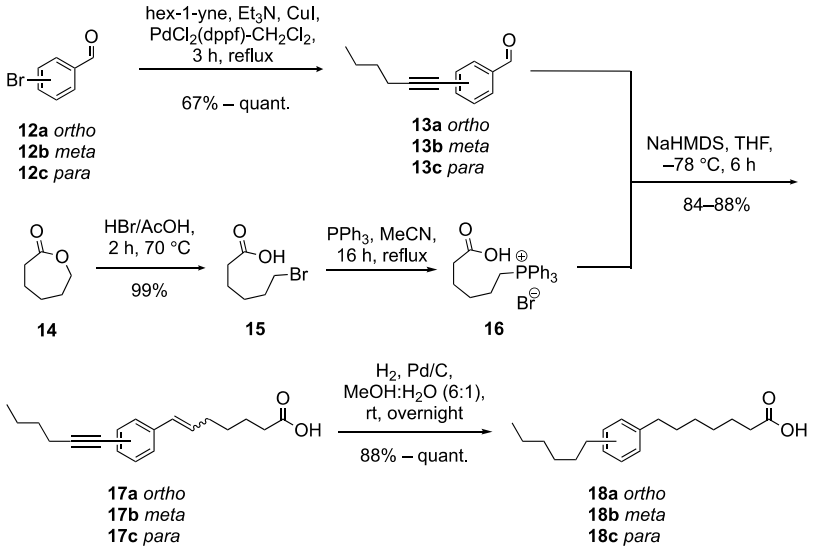
elimination of the cyanoethyl group to provide the target compounds 1°-acyl-*sn*-glycerol-2°-phosphate analogues **MZN-001** to **MZN-008**. To synthesize the 2°-acyl-*sn*-glycerol-1°-phosphate analogues (Scheme 2), we changed the reaction sequence, starting with the esterification of compounds **6** and **7** by the appropriate carboxylic acid or acid chloride followed by deprotection of the TBS group using HF/Py, and then thiophosphorylation of free alcohol and *β*-elimination of the cyanoethyl group, generating compounds **MZN-009** to **MZN-016, MZN-021**, and **MZN-022**.

Synthesis of Unnatural Fatty Acids. The synthesis of benzene-containing fatty acids **18a**, **18b**, and **18c** is summarized in Scheme 3.[18] Aldehydes **13a**, **13b**, and **13c** were constructed by utilizing Sonogashira coupling of the corresponding aldehydes **12a−12c** and 1-hexyne. Stirring lactone **14** with 30% HBr/AcOH at room temperature for 1 h produced compound **15**, which was treated with PPh_3 in refluxing MeCN to obtain triphenylphosphonium bromide **16** as the second component for the following Wittig reaction. A dropwise addition of **16** to a THF solution of NaHMDS at −78 °C, followed by the addition of aldehydes **13**, furnished the corresponding alkenes **17a**, **17b**, and **17c**. Catalytic hydrogenation of these enynes over Pd/C afforded the target acids **18a**, **18b**, and **18c**. These acids were utilized to synthesize **MZN-023** to **MZN-025** in a similar way that **MZN-009** to **MZN-016** were synthesized. However, rather than using the acid chloride for the esterification step, acids **18a−c** were directly esterified by stirring them with alcohol **7c** and DIC in DCM (Scheme 2).

Synthesis of Pyrrolidine-Based LPA Analogues. Because of the nucleophilicity difference between amine and alcohol functionalities, the synthesis of pyrrolidine-based LPA was

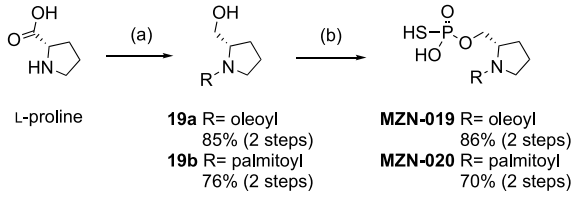
https://doi.org/10.1021/acsomega.3c07668
ACS Omega 2023, 8, 49278−49288

Scheme 3. Synthesis of Benzene-Containing Fatty Acids 18



much simpler, with no need for protecting groups. The synthesis commenced by reducing the amino acid L-proline using $LiAlH_4$ to generate L-prolinol, which was then directly coupled with oleoyl chloride and palmitoyl chloride to obtain compounds **19a** and **19b**, respectively (Scheme 4). Thiophosphorylation using the phosphorodiamidite/sulfur oxidation method, followed by the removal of the cyanoethyl group, yielded **MZN-019** and **MZN-020**.

Scheme 4. Synthesis of MZN-019 and MZN-020[a]

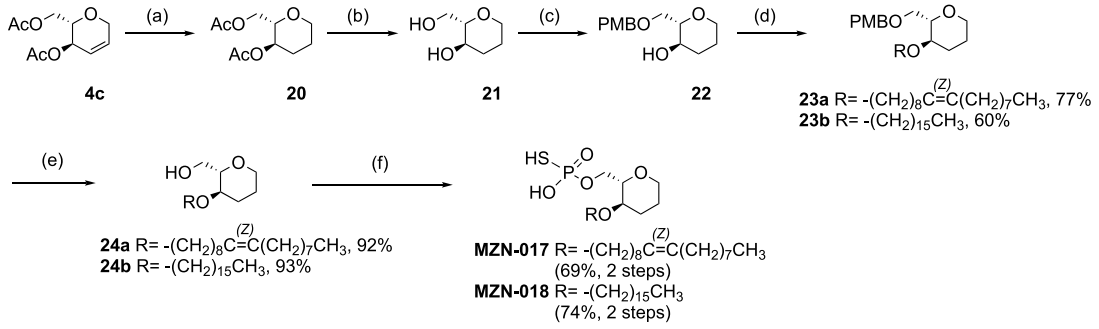


[a]Reactions conditions: (a) 1. $LiAlH_4$, THF, reflux, 2 h; 2. Acid chloride, Et_3N (3 equiv), 0 °C to RT, 6 h. (b) 1. $CNCH_2CH_2OP[N(iPr)_2]_2$ (2 equiv), ethylene cyanohydrin (1.6 equiv), 1*H*-tetrazole (2.5 equiv), then S (2 equiv), DCM, RT, 4 h; 2. DBU, MSTFA, pyridine, RT, 30 min.

*Synthesis of **MZN-010** Ether Analogues.* To synthesize **MZN-010** ether analogues, selective protection of the primary alcohol of diol **21**, readily prepared from **4c** in two steps, was required. We chose to use the PMB group for this step, which can withstand harsh basic conditions without risk of transfer in the etherification step and can be easily removed under acidic conditions. Treating diol **21** with *n*-Bu_2SnO, followed by *p*-methoxybenzyl chloride, provided the benzyl ether **22** (Scheme 5). *O*-Alkylation of the protected diol **22** with oleyl bromide and cetyl bromide, separately, followed by acid-catalyzed deprotection of the PMB group afforded alcohols **24a** and **24b**.[42,43] The two alcohols were then converted to **MZN-017** and **MZN-018**, respectively, using the phosphorodiamidite method mentioned above, followed by removal of the cyanoethyl groups.

Biological Evaluation. The TGFα shedding assay results of the agonistic activities of the synthesized LPA analogues (**MZN-001** – **MZN-016**) toward LPA_{1-6} are summarized in Table 1. Aside from **MZN-016**, all other analogues showed a significant drop of activity toward $LPA_{1,2,6}$. This drop could be attributed to the deviation of these compounds from the active conformation adopted by the natural ligand that is required to activate these receptors, as well as to the loss of the H-bond

Scheme 5. Synthesis of MZN-017 and MZN-018[a]



[a]Reactions conditions: (a) H_2, 10% Pd/C, MeOH, 99%. (b) NaOMe, MeOH, RT, 97%. (c) *n*-Bu_2SnO (1.3 equiv), MS3A, toluene, reflux, overnight, and then PMBCl (1.1 equiv), TBAI (1.3 equiv), reflux, 5 h, 73%. (d) KOH (4 equiv), RBr (2 equiv), toluene, reflux, overnight. (e) TfOH (0.5 equiv), resorcinol dimethyl ether (3 equiv), DCM, RT, 20 min. (f) 1. $CNCH_2CH_2OP[N(iPr)_2]_2$ (2 equiv), ethylene cyanohydrin (1.6 equiv), 1*H*-tetrazole (2.5 equiv), then S (2 equiv), DCM, RT, 4 h; 2. DBU, MSTFA, pyridine, RT, 30 min.

https://doi.org/10.1021/acsomega.3c07668
ACS Omega 2023, 8, 49278−49288

Table 1. Biological Evaluation of MZN-001−MZN-016 on LPA_{1-6} Using the TGFα Shedding Assay

	LPA_1	LPA_2	LPA_3	LPA_4	LPA_5	LPA_6
	EC_{50} (pEC_{50}) [Emax] <RIA>	EC_{50} (pEC_{50}) [Emax] <RIA>	EC_{50} (pEC_{50}) [Emax] <RIA>	EC_{50} (pEC_{50}) [Emax] <RIA>	EC_{50} (pEC_{50}) [Emax] <RIA>	EC_{50} (pEC_{50}) [Emax] <RIA>
18:1-LPA (1)	7.6 nM (−8.12) [30%] <1>	13 nM (−7.90) [37%] <1>	73 nM (−7.14) [50%] <1>	21 nM (−7.70) [20%] <1>	78 nM (−7.11) [43%] <1>	35 nM (−7.45) [28%] <1>
MZN-001	1500 nM (−5.83) [27%] <0.0045>	1700 nM (−5.77) [29%] <0.0056>	41 nM (−7.38) [42%] <1.5>	170 nM (−6.76) [11%] <0.067>	390 nM (−6.41) [46%] <0.21>	NA (NA) [NA] <NA>
MZN-002	NA (NA) [NA] <NA>	2300 nM (−5.63) [11%] <0.0016>	13 nM (−7.89) [48%] <5.4>	160 nM (−6.80) [17%] <0.11>	110 nM (−6.96) [50%] <0.84>	NA (NA) [NA] <NA>
MZN-003	1600 nM (−5.79) [9.5%] <0.0015>	>10 μM (>−4) [ND] <ND>	57 nM (−7.25) [30%] <0.77>	180 nM (−6.74) [17%] <0.095>	230 nM (−6.64) [46%] <0.37>	NA (NA) [NA] <NA>
MZN-004	530 nM (−6.27) [22%] <0.01>	1600 nM (−5.81) [16%] <0.0036>	37 nM (−7.43) [42%] <1.7>	130 nM (−6.89) [22%] <0.17>	73 nM (−7.14) [30%] <0.75>	980 nM (−6.01) [10%] <0.013>
MZN-005	250 nM (−6.60) [32%] <0.032>	740 nM (−6.13) [30%] <0.014>	11 nM (−7.97) [46%] <6.3>	85 nM (−7.07) [15%] <0.18>	64 nM (−7.19) [53%] <1.5>	490 nM (−6.31) [13%] <0.034>
MZN-006	440 nM (−6.36) [25%] <0.014>	1900 nM (−5.73) [28%] <0.0051>	4.7 nM (−8.33) [54%] <17>	48 nM (−7.32) [17%] <0.37>	36 nM (−7.45) [51%] <2.6>	590 nM (−6.23) [4.1%] <0.0087>
MZN-007	1300 nM (−5.9) [18%] <0.0035>	>10 μM (>−4) [ND] <ND>	11 nM (−7.94) [53%] <6.8>	300 nM (−6.53) [19%] <0.064>	160 nM (−6.79) [54%] <0.61>	1500 nM (−5.81) [26%] <0.021>
MZN-008	71 nM (−7.15) [27%] <0.095>	500 nM (−6.30) [35%] <0.024>	2.6 nM (−8.59) [54%] <30>	26 nM (−7.59) [17%] <0.69>	59 nM (−7.23) [37%] <1.1>	680 nM (−6.17) [28%] <0.051>
MZN-009	470 nM (−6.33) [25%] <0.013>	8200 nM (−5.09) [74%] <0.0031>	73 nM (−7.13) [45%] <0.89>	300 nM (−6.52) [4.6%] <0.016>	34 nM (−7.46) [54%] <2.9>	NA (NA) [NA] <NA>
MZN-010	590 nM (−6.23) [21%] <0.009>	>10 μM (>−4) [ND] <ND>	51 nM (−7.29) [42%] <1.2>	24 nM (−7.62) [18%] <0.74>	4.9 nM (−8.31) [52%] <19>	1100 nM (−5.96) [35%] <0.041>
MZN-011	210 nM (−6.68) [7.2%] <0.0086>	>10 μM (>−4) [ND] <ND>	50 nM (−7.30) [37%] <1.1>	13 nM (−7.89) [19%] <1.5>	34 nM (−7.47) [53%] <2.9>	110 nM (−6.98) [29%] <0.35>
MZN-012	310 nM (−6.51) [31%] <0.025>	1400 nM (−5.85) [33%] <0.0079>	12 nM (−7.91) [48%] <5.7>	36 nM (−7.44) [18%] <0.5>	23 nM (−7.65) [51%] <4.1>	190 nM (−6.71) [25%] <0.16>
MZN-013	710 nM (−6.15) [33%] <0.012>	2000 nM (−5.70) [26%] <0.0045>	43 nM (−7.37) [51%] <1.8>	390 nM (−6.41) [15%] <0.04>	26 nM (−7.58) [55%] <3.8>	700 nM (−6.15) [6.9%] <0.012>
MZN-014	290 nM	1300 nM	18 nM	2.3 nM	9.1 nM	370 nM

https://doi.org/10.1021/acsomega.3c07668
ACS Omega 2023, 8, 49278−49288

Table 1. continued

	LPA_1	LPA_2	LPA_3	LPA_4	LPA_5	LPA_6
	(−6.54)	(−5.88)	(−7.75)	(−8.64)	(−8.04)	(−6.44)
	[26%]	[41%]	[46%]	[17%]	[50%]	[13%]
	<0.023>	<0.011>	<3.8>	<7.6>	<9.9>	<0.045>
MZN-015	230 nM	860 nM	10 nM	8.1 nM	27 nM	100 nM
	(−6.63)	(−6.06)	(−7.98)	(−8.09)	(−7.57)	(−7.00)
	[27%]	[39%]	[49%]	[22%]	[53%]	[32%]
	<0.029>	<0.015>	<6.9>	<2.8>	<3.6>	<0.39>
MZN-016	61 nM	270 nM	2.5 nM	24 nM	9.5 nM	49 nM
	(−7.22)	(−6.57)	(−8.60)	(−7.62)	(−8.02)	(−7.31)
	[32%]	[39%]	[55%]	[22%]	[53%]	[32%]
	<0.13>	<0.049>	<32>	<0.93>	<10>	<0.82>

donating potential of *sn*-2-OH of these compounds. In contrast, all these compounds exhibited overall potent agonistic activities toward $LPA_{3,4,5}$ with those having *trans* configuration (even numbers) exhibiting more potent activity compared to the corresponding *cis* isomers (odd numbers). It is also noted that dihydropyran-containing compounds generally showed more potent agonistic activities toward all LPA receptors than the tetrahydropyran-containing analogues (**MZN-001−004** vs **MZN-005−008**; **MZN-009−012** vs **MZN-013−016**).

1°-Acyl-*sn*-glycerol-2°-phosphate analogues **MZN-001−008** were more potent toward LPA_3 and less potent or equipotent toward other receptors than **1**. Specifically, **MZN-007** had an EC_{50} of 11 nM toward LPA_3 compared to that of 73 nM for **1**. It was significantly less potent toward $LPA_{1,2,4,6}$ and twofold less potent toward LPA_5 than **1**. **MZN-002** demonstrated a close activation potency toward the six LPA receptors to **MZN-007** while **MZN-008** showed very strong activity at LPA_3 (EC_{50}= 2.6 nM) with comparable activity to parent LPA at $LPA_{4,5}$. Again, those analogues of this type with *trans* configuration were more potent toward LPA_3. On the other hand, 2°-acyl-*sn*-glycerol-1°-phosphate analogues showed more potent activities toward $LPA_{3,4,5}$ and less potent activities toward $LPA_{1,2,6}$ than 1-oleoyl LPA. One such analogue is **MZN-014**, which exhibited a strong potency on $LPA_{3,4,5}$, approximately 4- to 10-fold more potent than parent LPA. This difference in selectivity between the two types could be attributed to the free movement of the phosphate head in 2°-acyl-*sn*-glycerol-1°-phosphate analogues compared to 1°-acyl-*sn*-glycerol-2°-phosphate analogues, which could adjust itself precisely inside the binding pocket, activating potently more than one receptor. Among all these analogues, **MZN-010** was the most potent LPA_5 agonist with EC_{50} = 4.9 nM, approximately 16 times more potent than the standard **1** with comparable potency toward $LPA_{3,4}$.

Interestingly, **MZN-005** and its enantiomer **MZN-007** were equally effective at activating LPA_3, and similarly, **MZN-014** and its enantiomer **MZN-016** were equally effective against LPA_5. Other enantiomer pairs showed very close values (**MZN-006** and **MZN-008** on LPA_3, and **MZN-013** and **MZN-015** on LPA_3), which may indicate a lack of stereospecific recognition of this set of compounds by the mentioned receptors. A similar pattern of lacking stereospecific recognition has been noticed for the natural LPA where the natural one and its unnatural enantiomer demonstrated almost equal activity in some assays.[44] On the contrary, nonglycerol-based LPA analogues tend to exhibit a stereoselective preference for LPA receptors.[12]

Motivated by the fact that there are no selective pharmacological tools for LPA_5, we chose the skeleton of **MZN-010**, being the most potent at LPA_5, to be a starting point to synthesize more analogues in an attempt to enhance the potency and selectivity at LPA_5. We hypothesized that the dihedral angle between the phosphate group and the lipophilic chain might be an important determinant for selectivity. Therefore, the synthesis of other analogues bearing a more rigid structure with a relatively close dihedral angle to that shown by **MZN-010** should enhance both selectivity and potency. To examine this hypothesis, we synthesized **MZN-019** and **MZN-020** utilizing a pyrrolidine ring, which could achieve a close dihedral angle, as depicted in Figure 3, and

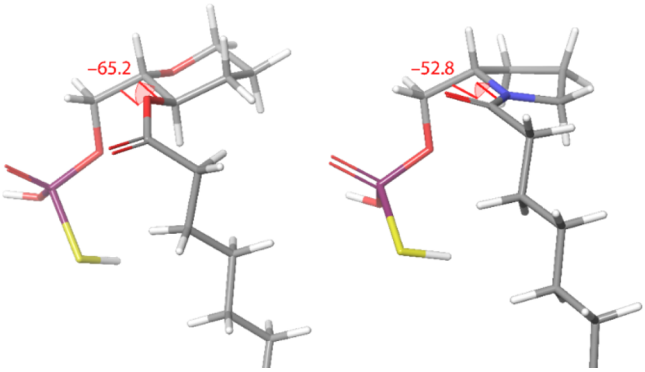


Figure 3. Dihedral angles of compounds **MZN-010** (left) and **MZN-020** (right) (measured after being energy-minimized using Maestro).

examined their potency against LPA_{1-6}. As expected, both ligands showed very potent activity toward LPA_5 compared to **1** and **MZN-010** with a slight preference for the ligand bearing the saturated fatty acid chain (**MZN-020** EC_{50} at LPA_5 = 1.1 nM) over the unsaturated one **MZN-019** (see Tables 2 and 3). However, the enhancement of activity at LPA_5 was associated with simultaneous enhancement at $LPA_{3,4}$. A previous report demonstrated that LPA_5 had a preference for alkyl LPA over acyl LPA;[45] hence, we speculated that the high electron density of the amide and ester functionalities could contribute to the activation of $LPA_{3,4}$ but not LPA_5, which may explain the enhancement shown by **MZN-019** and **MZN-020** at $LPA_{3,4}$. Accordingly, we considered the synthesis of both saturated (16:0) and unsaturated (18:1) chain ether analogues based on the **MZN-010** structure. We also considered changing the lipophilic chain of the ester analogues by changing the alkene configuration in the oleic acid, examining another unsaturated system, namely, a benzene ring, which could interact with the

https://doi.org/10.1021/acsomega.3c07668
ACS Omega 2023, 8, 49278−49288

Table 2. Biological Evaluation of the First Set of MZN-010 Analogues on LPA_{1-6} Using the TGFα Shedding Assay

	LPA_1	LPA_2	LPA_3	LPA_4	LPA_5	LPA_6
	EC_{50} (pEC_{50}) [Emax] <RIA>	EC_{50} (pEC_{50}) [Emax] <RIA>	EC_{50} (pEC_{50}) [Emax] <RIA>	EC_{50} (pEC_{50}) [Emax] <RIA>	EC_{50} (pEC_{50}) [Emax] <RIA>	EC_{50} (pEC_{50}) [Emax] <RIA>
18:1-LPA (1)	28 nM (−7.55) [30%] <1>	32 nM (−7.50) [38%] <1>	220 nM (−6.67) [45%] <1>	18 nM (−7.76) [15%] <1>	160 nM (−6.80) [40%] <1>	42 nM (−7.38) [25%] <1>
MZN-017	940 nM (−6.03) [5.4%] <0.0054>	>10 μM (>−4) [ND] <ND>	85 nM (−7.07) [34%] <1.9>	94 nM (−7.03) [13%] <0.16>	13 nM (−7.89) [44%] <13>	260 nM (−6.58) [24%] <0.16>
MZN-018	NA (NA) [NA] <NA>	1000 nM (−5.98) [16%] <0.012>	170 nM (−6.77) [23%] <0.65>	39 nM (−7.4) [6.4%] <0.19>	3.8 nM (−8.42) [43%] <44>	330 nM (−6.48) [12%] <0.06>
MZN-019	270 nM (−6.58) [26%] <0.09>	1600 nM (−5.80) [42%] <0.022>	26 nM (−7.58) [52%] <9.4>	8.2 nM (−8.08) [16%] <2.3>	3 nM (−8.52) [43%] <55>	340 nM (−6.47) [34%] <0.17>

Table 3. Biological Evaluation of the Second Set of MZN-010 Analogues on LPA_{1-6} Using the TGFα Shedding Assay

	LPA_1	LPA_2	LPA_3	LPA_4	LPA_5	LPA_6
	EC_{50} (pEC_{50}) [Emax] <RIA>	EC_{50} (pEC_{50}) [Emax] <RIA>	EC_{50} (pEC_{50}) [Emax] <RIA>	EC_{50} (pEC_{50}) [Emax] <RIA>	EC_{50} (pEC_{50}) [Emax] <RIA>	EC_{50} (pEC_{50}) [Emax] <RIA>
18:1-LPA (1)	9.5 nM (−8.02) [28%] <1>	17 nM (−7.78) [48%] <1>	70 nM (−7.15) [57%] <1>	15 nM (−7.83) [21%] <1>	60 nM (−7.23) [44%] <1>	13 nM (−7.88) [31%] <1>
MZN-020	450 nM (−6.35) [22%] <0.017>	630 nM (−6.20) [44%] <0.024>	30 nM (−7.53) [56%] <2.4>	20 nM (−7.70) [19%] <0.67>	1.1 nM (−8.96) [52%] <64>	290 nM (−6.54) [24%] <0.036>
MZN-021	320 nM (−6.49) [7.5%] <0.008>	>10 μM (>−4) [ND] <ND>	140 nM (−6.84) [31%] <0.27>	55 nM (−7.26) [21%] <0.27>	1.2 nM (−8.91) [51%] <55>	380 nM (−6.42) [19%] <0.022>
MZN-022	480 nM (−6.32) [16%] <0.011>	>10 μM (>−4) [ND] <ND>	78 nM (−7.11) [48%] <0.77>	34 nM (−7.47) [23%] <0.49>	6.3 nM (−8.20) [51%] <11>	450 nM (−6.35) [11%] <0.011>
MZN-023	1100 nM (−5.97) [15%] <0.0049>	>10 μM (>−4) [ND] <ND>	310 nM (−6.50) [27%] <0.11>	380 nM (−6.42) [18%] <0.034>	44 nM (−7.35) [55%] <1.7>	>10 μM (>−4) [ND] <ND>
MZN-024	630 nM (−6.20) [18%] <0.0097>	>10 μM (>−4) [ND] <ND>	240 nM (−6.63) [40%] <0.21>	58 nM (−7.24) [22%] <0.28>	8.1 nM (−8.09) [53%] <9>	>10 μM (>−4) [ND] <ND>
MZN-025	370 nM (−6.43) [13%] <0.012>	>10 μM (>−4) [ND] <ND>	260 nM (−6.58) [10%] <0.05>	49 nM (−7.31) [23%] <0.34>	11 nM (−7.95) [51%] <6.1>	>10 μM (>−4) [ND] <ND>

target to form π-stacking or CH−π interactions, as well as testing saturated analogues.

The results of the new set of compounds are summarized in Table 3. The elaidoyl derivative **MZN-022** showed almost equipotent activity to **MZN-10** at LPA_{1-6}, indicating that the configuration of the double bond in the lipophilic chain had no significant effect on the activity. However, replacing this unsaturated system with a benzene moiety results in different

https://doi.org/10.1021/acsomega.3c07668
ACS Omega 2023, 8, 49278−49288

patterns of activity and potency. All benzene derivatives **MZN-023−025** lost their activities against $LPA_{2,6}$. Furthermore, the *ortho* analogue **MZN-023** exhibited much weaker activity at $LPA_{1,3,4}$ and slightly stronger activity at LPA_5 with an EC_{50} value of 44 nM for LPA_5 compared to 1-oleoyl LPA's EC_{50} value of 60 nM. On the other hand, *meta* derivative **MZN-024** and *para* derivative **MZN-025** showed much stronger activity at $LPA_{4,5}$ than **MZN-023**. Replacing the unsaturated chain with the saturated one had a positive effect on LPA_5 activation in both ether and ester analogues with approximately three- to fourfold increases in potency compared to the unsaturated ones. For example, the palmityl derivative **MZN-018** showed a potent LPA_5 agonistic activity (EC_{50} = 3.8 nM compared to 13 nM for oleyl ether **MZN-017**) with a partial agonistic effect on both $LPA_{3,4}$. Its ester analogue **MZN-021** was the most potent analogue at LPA_5 with an EC_{50} value of 1.2 nM, 50 times more potent than **1**. In addition, it was approximately 2 and 3.5 times less potent at $LPA_{3,4}$ than **1** with a partial agonistic effect at LPA_3 and no activity at LPA_2. The results demonstrate that **MZN-021** displayed robust and selective agonistic activity targeting LPA_5, exhibiting over a 45-fold higher selectivity when compared with other LPA receptors. Ether derivatives **MZN-017** and **MZN-018** exhibited lower maximum effects (Emax) on LPA_4 than the corresponding ester analogues **MZN-10** and **MZN-021**, suggesting that groups with high electron densities, such as esters and amides, could contribute to a higher Emax by increasing the stability of the ligand/LPA_4 complex by acting as hydrogen bond acceptors.

CONCLUSIONS

In this study, a group of LPA analogues containing dihydropyran and tetrahydropyran skeletons were designed, synthesized, and biologically evaluated for their agonistic effect using the TGFα shedding assay. By employing conformational restriction, bioisosteric replacement, and lipophilic chain variation, we succeeded in developing compounds **MZN-018** and **MZN-021** with improved potency and selectivity toward LPA_5 compared to 1-oleoyl LPA (**1**) (EC_{50} = 3.8, 1.2, and 60 nM, respectively). The results of this study support the idea that the high flexibility of LPA is responsible for its pan-agonistic activity. Restricting this flexibility by using ring systems as a core linker between the phosphate and the acyl or alkyl lipophilic chain alters the agonistic activity toward LPA_{1-6}. The findings also clearly indicate that different lipophilic chains have different levels of agonistic activity. This could potentially have important implications for further study and understanding of compounds' selectivity toward LPA receptors. Research is ongoing in our laboratory to enhance the potency further by varying the lipophilic chains.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.3c07668.

Experimental procedures, dose−response curves, and copies of ^1H and ^{13}C NMR spectra (PDF)

AUTHOR INFORMATION

Corresponding Authors

Junken Aoki − *Graduate School of Pharmaceutical Sciences, The University of Tokyo, Tokyo 113-0033, Japan*; Email: jaoki@mol.f.u-tokyo.ac.jp

Takayuki Doi − *Graduate School of Pharmaceutical Sciences, Tohoku University, Sendai 980-8578, Japan*; orcid.org/0000-0002-8306-6819; Email: doi_taka@mail.pharm.tohoku.ac.jp

Authors

Mazin A. S. Abdelwahid − *Graduate School of Pharmaceutical Sciences, Tohoku University, Sendai 980-8578, Japan*

Kosuke Ohsawa − *Graduate School of Pharmaceutical Sciences, Tohoku University, Sendai 980-8578, Japan*

Akiharu Uwamizu − *Graduate School of Pharmaceutical Sciences, The University of Tokyo, Tokyo 113-0033, Japan*

Kuniyuki Kano − *Graduate School of Pharmaceutical Sciences, The University of Tokyo, Tokyo 113-0033, Japan*; orcid.org/0000-0002-4539-5750

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.3c07668

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This work was supported by JST [Moonshot R&D Program] Grant Numbers JPMJMS2023-11 and JPMJMS2023-15 for J.A. and T.D. and JSPS KAKENHI Grant Number22H00438 for J.A. The first author (M.A.S.A) thanks the Ministry of Education, Culture, Sports, Science and Technology − Japan (MEXT) for financial support.

REFERENCES

(1) Kihara, Y.; Maceyka, M.; Spiegel, S.; Chun, J. Lysophospholipid receptor nomenclature review: IUPHAR Review 8. *Br. J. Pharmacol.* **2014**, *171*, 3575−3594.

(2) Im, D. S. Pharmacological tools for lysophospholipid GPCRs: development of agonists and antagonists for LPA and S1P receptors. *Acta Pharmacol. Sin.* **2010**, *31*, 1213−1222.

(3) Geraldo, L. H. M.; Spohr, T. C. L. S.; Amaral, R. F. D.; Fonseca, A. C. C. D.; Garcia, C.; Mendes, F. A.; Freitas, C.; dosSantos, M. F.; Lima, F. R. S. Role of lysophosphatidic acid and its receptors in health and disease: novel therapeutic strategies. *Signal Transduction Targeted Ther.* **2021**, *6* (1), 45.

(4) Willier, S.; Butt, E.; Grunewald, T. G. Lysophosphatidic acid (LPA) signalling in cell migration and cancer invasion: a focussed review and analysis of LPA receptor gene expression on the basis of more than 1700 cancer microarrays. *Biol. Cell.* **2013**, *105*, 317−333.

(5) Goldsmith, Z. G.; Ha, J. H.; Jayaraman, M.; Dhanasekaran, D. N. Lysophosphatidic Acid Stimulates the Proliferation of Ovarian Cancer Cells via the gep Proto-Oncogene Gα12. *Genes Cancer.* **2011**, *2*, 563−575.

(6) Xu, Y. J.; Saini, H. K.; Cheema, S. K.; Dhalla, N. S. Mechanisms of lysophosphatidic acid-induced increase in intracellular calcium in vascular smooth muscle cells. *Cell Calcium* **2005**, *38*, 569−579.

(7) Siess, W.; Zangl, K. J.; Essler, M.; Bauer, M.; Brandl, R.; Corrinth, C.; Bittman, R.; Tigyi, G.; Aepfelbacher, M. Lysophosphatidic acid mediates the rapid activation of platelets and endothelial cells by mildly oxidized low density lipoprotein and accumulates in human atherosclerotic lesions. *Proc. Natl. Acad. Sci. U. S. A.* **1999**, *96*, 6931−6936.

(8) Geach, T. J.; Faas, L.; Devader, C.; Gonzalez-Cordero, A.; Tabler, J. M.; Brunsdon, H.; Isaacs, H. V.; Dale, L. An essential role for LPA signalling in telencephalon development. *Development.* **2014**, *141*, 940−949.

(9) Takahashi, T.; Kamimura, A.; Hamazono-Matsuoka, T.; Honda, S. Phosphatidic Acid Has a Potential to Promote Hair Growth In Vitro and In Vivo, and Activates Mitogen-Activated Protein Kinase/

Extracellular Signal-Regulated Kinase Kinase in Hair Epithelial Cells. *J. Invest. Dermatol.* **2003**, *121*, 448−456.
(10) Choi, J. W.; Herr, D. R.; Noguchi, K.; Yung, Y. C.; Lee, C. W.; Mutoh, T.; Lin, M. E.; Teo, S. T.; Park, K. E.; Mosley, A. N.; Chun, J. LPA Receptors: Subtypes and Biological Actions. *Annu. Rev. Pharmacol. Toxicol.* **2010**, *50*, 157−186.
(11) Yung, Y. C.; Stoddard, N. C.; Chun, J. LPA receptor signaling: pharmacology, physiology, and pathophysiology. *J. Lipid Res.* **2014**, *55*, 1192−1214.
(12) Qian, L.; Xu, Y.; Simper, T.; Jiang, G.; Aoki, J.; Umezu-Goto, M.; Arai, H.; Yu, S.; Mills, G. B.; Tsukahara, R.; Makarova, N.; Fujiwara, Y.; Tigyi, G.; Prestwich, G. D. Phosphorothioate analogs of alkyl lysophosphatidic acid as LPA_3 receptor-selective agonists. *ChemMedChem.* **2006**, *1*, 376−383.
(13) Hooks, S. B.; Santos, W. L.; Im, D. S.; Heise, C. E.; Macdonald, T. L.; Lynch, K. R. Lysophosphatidic Acid-induced Mitogenesis is Regulated by Lipid Phosphate Phosphatases and is Edg-receptor Independent. *J. Biol. Chem.* **2001**, *276*, 4611−4621.
(14) Xu, Y.; Aoki, J.; Shimizu, K.; Umezu-Goto, M.; Hama, K.; Takanezawa, Y.; Yu, S.; Mills, G. B.; Arai, H.; Qian, L.; Prestwich, G. D. Structure-activity relationships of fluorinated lysophosphatidic acid analogs. *J. Med. Chem.* **2005**, *48*, 3319−3327.
(15) Jiang, G.; Inoue, A.; Aoki, J.; Prestwich, G. D. Phosphorothioate analogs of *sn*-2 radyl lysophosphatidic acid (LPA): Metabolically stabilized LPA receptor agonists. *Bioorg. Med. Chem. Lett.* **2013**, *23*, 1865−1869.
(16) Hasegawa, Y.; Erickson, J. R.; Goddard, G. J.; Yu, S.; Liu, S.; Cheng, K. W.; Eder, A.; Bandoh, K.; Aoki, J.; Jarosz, R.; Schrier, A. D.; Lynch, K. R.; Mills, G. B.; Fang, X. Identification of a phosphothionate analogue of lysophosphatidic acid (LPA) as a selective agonist of the LPA_3 receptor. *J. Biol. Chem.* **2003**, *278*, 11962−11969.
(17) Taniguchi, R.; Inoue, A.; Sayama, M.; Uwamizu, A.; Yamashita, K.; Hirata, K.; Yoshida, M.; Tanaka, Y.; Kato, H. E.; Nakada-Nakura, Y.; Otani, Y.; Nishizawa, T.; Doi, T.; Ohwada, T.; Ishitani, R.; Aoki, J.; Nureki, O. Structural insights into ligand recognition by the lysophosphatidic acid receptor LPA_6. *Nature.* **2017**, *548*, 356−360.
(18) Akasaka, H.; Tanaka, T.; Sano, F. K.; Matsuzaki, Y.; Shihoya, W.; Nureki, O. Structure of the active G_i-coupled human lysophosphatidic acid receptor 1 complexed with a potent agonist. *Nat. Commun.* **2022**, *13*, 5417.
(19) Liu, S.; Paknejad, N.; Zhu, L.; Kihara, Y.; Ray, M.; Chun, J.; Liu, W.; Hite, R. K.; Huang, X.-Y. Differential activation mechanisms of lipid GPCRs by lysophosphatidic acid and sphingosine 1-phosphate. *Nat. Commun.* **2022**, *13*, 731.
(20) Kiss, G. N.; Fells, J. I.; Gupte, R.; Lee, S.-C.; Liu, J.; Nusser, N.; Lim, K. G.; Ray, R. M.; Lin, F.-T.; Parrill, A. L.; Sümegi, B.; Miller, D. D.; Tigyi, G. Virtual Screening for LPA_2-Specific Agonists Identifies a nonlipid Compound with Antiapoptotic Actions. *Mol. Pharmacol.* **2012**, *82*, 1162.
(21) Patil, R.; Fells, J. I.; Szabó, E.; Lim, K. G.; Norman, D. D.; Balogh, A.; Patil, S.; Strobos, J.; Miller, D. D.; Tigyi, G. J. Design and synthesis of sulfamoyl benzoic acid analogues with subnanomolar agonist activity specific to the LPA_2 receptor. *J. Med. Chem.* **2014**, *57*, 7136−7140.
(22) Patil, R.; Szabó, E.; Fells, J. I.; Balogh, A.; Lim, K. G.; Fujiwara, Y.; Norman, D. D.; Lee, S. C.; Balazs, L.; Thomas, F.; Patil, S.; Emmons-Thompson, K.; Boler, A.; Strobos, J.; McCool, S. W.; Yates, C. R.; Stabenow, J.; Byrne, G. I.; Miller, D. D.; Tigyi, G. J. Combined Mitigation of the Gastrointestinal and Hematopoietic Acute Radiation Syndromes by a Novel LPA_2 Receptor-specific Non-lipid Agonist. *Chem. Biol.* **2015**, *22*, 206−216.
(23) Xu, Y.; Tanaka, M.; Arai, H.; Aoki, J.; Prestwich, G. D. Alkyl lysophosphatidic acid and fluoromethylene phosphonate analogs as metabolically-stabilized agonists for LPA receptors. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 5323−5328.
(24) Aikawa, S.; Hashimoto, T.; Kano, K.; Aoki, J. Lysophosphatidic acid as a lipid mediator with multiple biological actions. *J. Biochem.* **2015**, *157*, 81−89.
(25) Bandoh, K.; Aoki, J.; Taira, A.; Tsujimoto, M.; Arai, H.; Inoue, K. Lysophosphatidic acid (LPA) receptors of the EDG family are differentially activated by LPA species. Structure-activity relationship of cloned LPA receptors. *FEBS Lett.* **2000**, *478*, 159−165.
(26) Lynch, K. R.; Hopper, D. W.; Carlisle, S. J.; Catalano, J. G.; Zhang, M.; Macdonald, T. L. Structure/Activity Relationships in Lysophosphatidic Acid: The 2-Hydroxyl Moiety. *Mol. Pharmacol.* **1997**, *52*, 75−81.
(27) Durgam, G. G.; Virag, T.; Walker, M. D.; Tsukahara, R.; Yasuda, S.; Liliom, K.; van Meeteren, L. A.; Moolenaar, W. H.; Wilke, N.; Siess, W.; Tigyi, G.; Miller, D. D. Synthesis, Structure-Activity Relationships, and Biological Evaluation of Fatty Alcohol Phosphates as Lysophosphatidic Acid Receptor Ligands, Activators of PPARγ, and Inhibitors of Autotaxin. *J. Med. Chem.* **2005**, *48*, 4919−4930.
(28) Tokumura, A.; Kume, T.; Fukuzawa, K.; Tsukatani, H. Cardiovascular Effects of Lysophosphatidic Acid and Its Structural Analogs in Rats. *J. Pharmacol. Exp. Ther.* **1981**, *219*, 219−224.
(29) Jalink, K.; Hengeveld, T.; Mulder, S.; Postma, F. R.; Simon, M. F.; Chap, H.; van der Marel, G. A.; van Boom, J. H.; van Blitterswijk, W. J.; Moolenaar, W. H. Lysophosphatidic acid-induced Ca2+ mobilization in human A431 cells: structure-activity analysis. *Biochem. J.* **1995**, *307*, 609−616.
(30) Bandoh, L.; Aoki, J.; Hosono, H.; Kobayashii, S.; Kobayashi, T.; Murakami-Murofushi, K.; Tsujimoto, M.; Arai, H.; Inoue, K. Molecular cloning and characterization of a novel human G-protein-coupled receptor, EDG7, for lysophosphatidic acid. *J. Biol. Chem.* **1999**, *274*, 27776−27785.
(31) Fukasawa, K.; Gotoh, M.; Uwamizu, A.; Hirokawa, T.; Ishikawa, M.; Shimizu, Y.; Yamamoto, S.; Iwasa, K.; Yoshikawa, K.; Aoki, J.; Murakami-Murofushi, K. 2-Carba-lysophosphatidic acid is a novel β-lysophosphatidic acid analogue with high potential for lysophosphatidic acid receptor activation and autotaxin inhibition. *Sci. Reports.* **2021**, *11*, 17360.
(32) Sugiura, T.; Tokumura, A.; Gregory, L.; Nouchi, T.; Weintraub, S. T.; Hanahan, D. J. Biochemical Characterization of the Interaction of Lipid Phosphoric Acids with Human Platelets: Comparison with Platelet Activating Factor. *Arch. Biochem. Biophys.* **1994**, *311*, 358−368.
(33) Heise, C. E.; Santos, W. L.; Schreihofer, A. M.; Heasley, B. H.; Mukhin, Y. V.; Macdonald, T. L.; Lynch, K. R. Activity of 2-Substituted Lysophosphatidic Acid (LPA) Analogs at LPA Receptors: Discovery of a LPA_1/LPA_3 Receptor Antagonist. *Mol. Pharmacol.* **2001**, *60*, 1173−1180.
(34) Hopper, D. W.; Ragan, S. P.; Hooks, S. B.; Lynch, K. R.; Macdonald, T. L. Structure activity relationships of lysophosphatidic acid: Conformationally restricted backbone mimetics. *J. Med. Chem.* **1999**, *42*, 963−970.
(35) Tamaruya, Y.; Suzuki, M.; Kamura, G.; Kanai, M.; Hama, K.; Shimizu, K.; Aoki, J.; Arai, H.; Shibasaki, M. Identifying specific conformations by using a carbohydrate scaffold: Discovery of subtype-selective LPA-receptor agonists and an antagonist. *Angew. Chem., Int. Ed.* **2004**, *43*, 2834−2837.
(36) Aikawa, S.; Kano, K.; Inoue, A.; Wang, J.; Saigusa, D.; Nagamatsu, T.; Hirota, Y.; Fujii, T.; Tsuchiya, S.; Taketomi, Y.; Sugimoto, Y.; Murakami, M.; Arita, M.; Kurano, M.; Ikeda, H.; Yatomi, Y.; Chun, J.; Aoki, J. Autotaxin−lysophosphatidic acid− LPA_3 signaling at the embryo-epithelial boundary controls decidualization pathways. *EMBO J.* **2017**, *36*, 2146−2160.
(37) Fang, Z.; Song, Y.; Zhan, P.; Zhang, Q.; Liu, X. Conformational restriction: an effective tactic in 'follow-on'-based drug discovery. *Future Med. Chem.* **2014**, *6*, 885−901.
(38) Jung, S.; Inoue, A.; Nakamura, S.; Kishi, T.; Uwamizu, A.; Sayama, M.; Ikubo, M.; Otani, Y.; Kano, K.; Makide, K.; Aoki, J.; Ohwada, T. Conformational Constraint of the Glycerol Moiety of Lysophosphatidylserine Affords Compounds with Receptor Subtype Selectivity. *J. Med. Chem.* **2016**, *59*, 3750−3776.
(39) Mangunuru, H. P. R.; Yerabolu, J. R.; Liu, D.; Wang, G. Synthesis of a series of glucosyl triazole derivatives and their self-assembling properties. *Tetrahedron Lett.* **2015**, *56*, 82−85.

https://doi.org/10.1021/acsomega.3c07668
ACS Omega 2023, 8, 49278−49288

(40) Chen, H.; Xian, T.; Zhang, W.; Si, W.; Luo, X.; Zhang, B.; Zhang, M.; Wang, Z.; Zhang, J. An efficient method for the synthesis of pyranoid glycals. *Carbohydr. Res.* **2016**, *431*, 42−46.
(41) De Oliveira, R. N.; Cottier, L.; Sinou, D.; Srivastava, R. M. Stereocontrolled palladium(0)-catalyzed preparation of unsaturated azidosugars: An easy access to 2- and 4-aminoglycosides. *Tetrahedron.* **2005**, *61*, 8271−8281.
(42) Miyazaki, H.; Ohkawa, N.; Nakamura, N.; Itou, T.; Sada, T.; Oshima, T.; Koike, H. Lactone and Cyclic Ether Analogs of Platelet-Activating Factor: Synthesis and Biological Activities. *Chem. Pharm. Bull.* **1989**, *37*, 2379−2390.
(43) Jung, M. E.; Koch, P. Mild, selective deprotection of PMB ethers with triflic acid/1,3-dimethoxybenzene. *Tetrahedron Lett.* **2011**, *52*, 6051−6054.
(44) Yokoyama, K.; Baker, D. L.; Virag, T.; Liliom, K.; Byun, H. S.; Tigyi, G.; Bittman, R. Stereochemical properties of lysophosphatidic acid receptor activation and metabolism. *Biochim. Biophys. Acta - Mol. Cell Biol. Lipids.* **2002**, *1582*, 295−308.
(45) Williams, J. R.; Khandoga, A. L.; Goyal, P.; Fells, J. I.; Perygin, D. H.; Siess, W.; Parrill, A. L.; Tigyi, G.; Fujiwara, Y. Unique Ligand Selectivity of the GPR92/LPA_5 Lysophosphatidate Receptor Indicates Role in Human Platelet Activation. *J. Biol. Chem.* **2009**, *284*, 17304−17319.

https://doi.org/10.1021/acsomega.3c07668
ACS Omega 2023, 8, 49278−49288