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Synthesis and biological evaluation of phosphonic and thiophosphoric acid derivatives of lysophosphatidic acid

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Abstract—Using an *N*-oleoyl ethanolamide scaffold, a series of phosphate polar head group analogues of LPA comprised of various α-substituted phosphonates and thiophosphates was prepared. In a broken cell GTP[γ^{35}S] binding assay, agonist activity was evaluated at the three LPA receptors of the endothelial differentiation gene (Edg) family. This study has resulted in the discovery of a nonhydrolyzable LPA_1-selective agonist (**11**). Additionally, thiophosphate **19** bears an isosteric phosphate mimetic that confers agonism at the LPA_1 receptor but not LPA_2.

Lysophosphatidic acid (LPA, 1- or 2-*O*-acyl-*sn*-glycero-3-phosphate) is an intercellular signaling phospholipid that induces an array of receptor-mediated biological effects.[1] However, lipid phosphate phosphatase (LPP)-catalyzed hydrolysis of the phosphomonoester polar head group of LPA results in functional inactivation at LPA receptors.[2] In recent years, progress has been made toward the development of phosphatase-resistant pharmacologic agents that modulate LPA signaling. The metabolically stabilized LPA analogue, 1-oleoyl-2*S*-*O*-methyl-glycerophosphothioate (2*S*-OMPT) is a potent and selective agonist for the LPA_3 receptor.[3] Further development of nonhydrolyzable molecular entities such as this may provide the necessary tools to define the receptor specific pathophysiological responses to LPA stimulation in intact animals (Fig. 1).

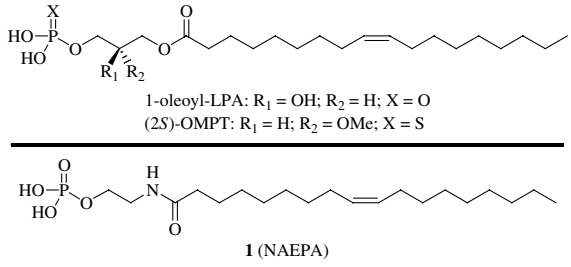

Figure 1. Structures of LPA receptor agonists.

Replacement of the bridging oxygen of the phosphate group with carbon renders phosphonates hydrolytically stable. Not only does a phosphonate possess the same spatial distribution of functionality as a phosphate but it also allows similar conformations to be accessed. However, the strong electronegative property of the oxygen clearly affects the electronic attributes of a phosphate group. As a result, the second phosphate pK_a value is 1-log order more acidic than the corresponding α-methylene phosphonate (~6.4 compared to ~7.6).[4] This discrepancy may strongly affect receptor–ligand binding interactions wherein charge density on the polar head group is favorable. Electronegative substituents on the α-carbon will inductively influence charge density thereby increasing acidity. Indeed, the introduction of α,α-difluoro decreases the second phosphonate pK_a by as much as 2-log orders.[4] Alternatively, the substitution of sulfur for one of the nonbridging oxygen atoms of a phosphate yields the metabolically stable[5]

Keywords: Lysophosphatidic acid; Phosphonic acid; Thiophosphoric acid; Phosphatase.
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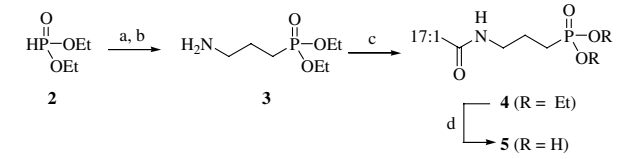
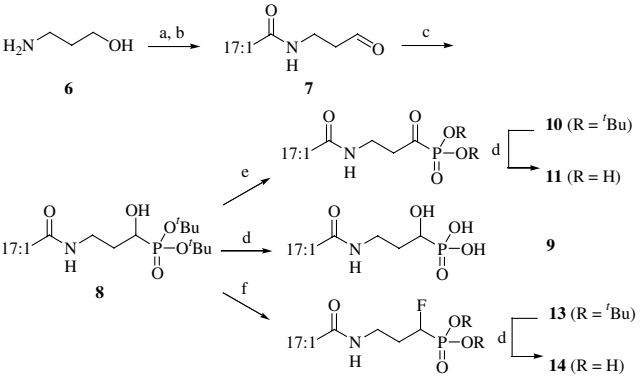
phosphothioate, which exhibits different steric and geometric properties as well as charge distribution in the respective dianions.[6] Thiol phosphoric acids, in which the bridging oxygen of the phosphate is replaced by sulfur, have been explored to a lesser extent as biologically active thio-analogues. This isostere is similarly charged and has the same hydrogen-bonding capabilities as the phosphate ester, except for the bridging atom.

To explore the potential of phosphonates and thiophosphates as potential nonhydrolyzable analogues of LPA, we now report the synthesis and biological evaluation of a series of phosphate-mimetic derivatives of the known LPA receptor agonist *N*-acyl ethanolamide phosphoric acid (NAEPA, **1**).[7]

Syntheses of all compounds listed in Table 1 except **20**[8] are described in Schemes 1–3. As outlined in Scheme 1, phosphite anion condensed with acrylonitrile in Micheal fashion to provide the resulting nitrile in good yield. Cobalt(II) chloride-catalyzed sodium borohydride reduction afforded the amine **3**, which underwent routine acylation followed by transesterification of the phosphonodiester using bromotrimethylsilane (TMSBr) and subsequent desilylation with aqueous methanol to give α-methylene phosphonate **5**.



Scheme 1. Synthesis of phosphonate **5**. Reagents and conditions: (a) Na, EtOH, acrylonitrile, 70%; (b) $NaBH_4$, $CoCl_2$, −30 to 25 °C, 53%; (c) oleoyl chloride, DIEA, 80%; (d) (i) TMSBr, CH_3CN, 4 h; (ii) 95% MeOH, 1 h, 95%.

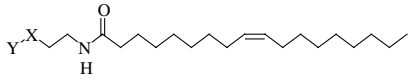


Scheme 2. Synthesis of α-substituted phosphonates **9**, **11**, **14**. Reagents and conditions: (a) oleoyl chloride, DIEA, 81%; (b) PCC, 47%; (c) di-*tert*-butyl phosphite, NaH, 51%; (d) TFA–CH_2Cl_2 (1:1), 100%; (e) PCC, 28%; (f) DAST, 0 °C, 15%.

As outlined in Scheme 2, condensation of propanolamine **6** with oleoyl chloride followed by pyridinium chlorochromate (PCC) oxidation generated the aldehyde **7**. Phosphite addition to **7** afforded the racemic-hydroxy phophonodiester **8**, which is converted to the phosphonic acid by treatment with trifluoroacetic acid (TFA). Additionally, **8** is a common intermediate used to generate the α-keto and α-fluoro derivatives, **11** and **14**, respectively. PCC oxidation of **8** followed by TFA deprotection afforded α-keto phosphonate **11**. For the latter, reaction of **8** with (diethylamino)sulfur trifluoride (DAST) at ice-bath temperature provided the racemic α-fluorophosphonodiester in modest yield. Subsequent TFA deprotection gave **14**.

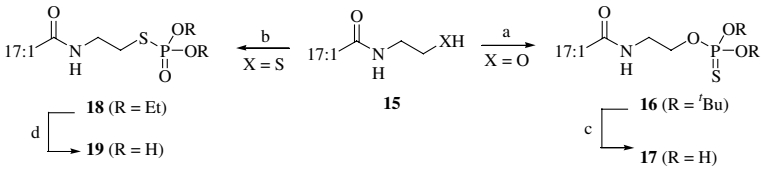
As outlined in Scheme 3, two thio-analogues were also prepared. Either ethanolamine or 2-aminoethane thiol condensed selectively with oleoyl chloride to generate **15**. Phosphitylation of the alcohol **15** followed by in situ sulfurization with elemental sulfur provided the phosphothioate triester **16** in good yield. Notably, TFA deprotection in this case required the addition of at least 20 equiv of the scavenger reagent triethylsilane so as to prevent intra- or intermolecular migration of the intermediate *tert*-butyl cation onto sulfur. An analogous alkyl migration has been noted elsewhere.[9] Removal of volatiles under reduced pressure afforded the pure phosphothioate **17**. The thiol phosphoric triester **18** was prepared by an Arbuzov-type redox reaction of trimethyl phosphite with the thiol **15** in the presence of

Table 1. Agonist activities of LPA analogues at individual LPA receptors using GTP[γ^{35}S] binding assay



Compound	X	Y	LPA_1		LPA_2		LPA_3	
			EC_{50} (nM)	E_{max}	EC_{50} (nM)	E_{max}	EC_{50} (nM)	E_{max}
LPA	NA	NA	17	100	6.8	100	263	100
1	–O–	$–PO_3H_2$	197	100	30	110	>5000	NA
5	$–CH_2–$	$–PO_3H_2$	1950	50	>5000	NA	>5000	NA
9	–CH(OH)–	$–PO_3H_2$	1225	80	2066	90	>5000	NA
11	–C(O)–	$–PO_3H_2$	221	80	1089	90	>5000	NA
14	–CH(F)–	$–PO_3H_2$	>5000	NA	1960	100	>5000	NA
20	–C(OH)–	$–(PO_3H_2)_2$	>5000	NA	>5000	NA	>5000	NA
17	–O–	$–P(S)O_2H_2$	40	80	108	46	>5000	NA
19	–S–	$–PO_3H_2$	318	50	>5000	NA	>5000	NA

E_{max} = maximal efficacy of drug/maximal efficacy of 1-oleoyl-LPA; expressed as the percentage. NA, not applicable.



Scheme 3. Synthesis of thiophosphoric acids **17**, **19**. Reagents and conditions: (a) (i) di-*tert*-butyl diisopropyl phosphoramidite, tetrazole; (ii) sulfur, reflux, 86%; (b) trimethyl phospite, $TeCl_4$, 2,6-lutidine, 67%; (c) TFA–CH_2Cl_2 (1:2), triethylsilane (20 equiv), 95%; (d) (i) TMSBr, CH_2Cl_2, 4 h; (ii) MeOH, 1 h, 100%.

$TeCl_4$ and 2,6-lutidine according to the method of Watanbe et al.[10] TMSBr deprotection afforded the thiol phosphoric acid **19**.

All compounds were characterized by ^1H and ^{13}C NMR and mass spectroscopy.[11] A membrane-based GTP[$\gamma^{35}S$] binding assay was adapted to assess in vitro activity at the LPA_1, LPA_2, and LPA_3 receptors.[12] It has been demonstrated that NAEPA (**1**) is an excellent LPA mimetic, indistinguishable from LPA in aggregating human platelets and mobilizing calcium in mammalian cells.[13,14] However, this activity is primarily elicited by activation of the LPA_1 and LPA_2 receptors, but not LPA_3, where potency is markedly diminished.[7] We conserved the ethanolamide scaffold of **1** and replaced the phosphate moiety with a series of phosphate isosteres. The resulting GTP[$\gamma^{35}S$] binding data for all compounds are presented in Table 1.

Compared to NAEPA (**1**), α-methylene phosphonate **5** is less active by 1-log order at the LPA_1 receptor and inactive at LPA_2 and LPA_3. To modulate the second p*K*a value and thus more effectively mimic the electronics of the phosphate, electron-withdrawing heteroatom substituents were introduced on the α-carbon of phosphono-NAEPA derivatives **9**, **11**, **14**, and **20**. The phosphonate rank order potency at the LPA_1 receptor is α-keto > α-hydroxy > α-methylene > α-fluoro. Given the improved agonism of compounds **9** and **11**, electron density and pK_a of the polar head group does seem to impact activity at LPA receptors. However, the relative inactivity of α-fluoro phosphonate **14** is not consistent with this hypothesis; this moiety has been reported to most closely approximate the electronics, acidity, and conformation of phosphate,[4] but results in a dramatic loss of LPA receptor activation. This unexpected result remains unresolved and will require additional studies. Notably, **14** is fully efficacious at the LPA_2 receptor albeit at a relatively high concentration. α-Hydroxy bisphosphonate **20** is inactive at all three receptors, perhaps due to steric and/or electrostatic factors. Thus, α-keto phosphonate **11** displays potency and efficacy at the LPA_1 receptor comparable to that of lead compound NAEPA. However, **11** is greater than 1-log order less potent than NAEPA at LPA_2 and therefore represents a nonhydrolyzable LPA_1-selective agonist.

Two thiophosphate analogues were analyzed for agonist activity at LPA receptors. Phosphothioate **17** displayed agonist potency comparable to that of lead compound NAEPA. Maximal efficacy of this derivative, however, is diminished by twofold at the LPA_2 receptor, compared

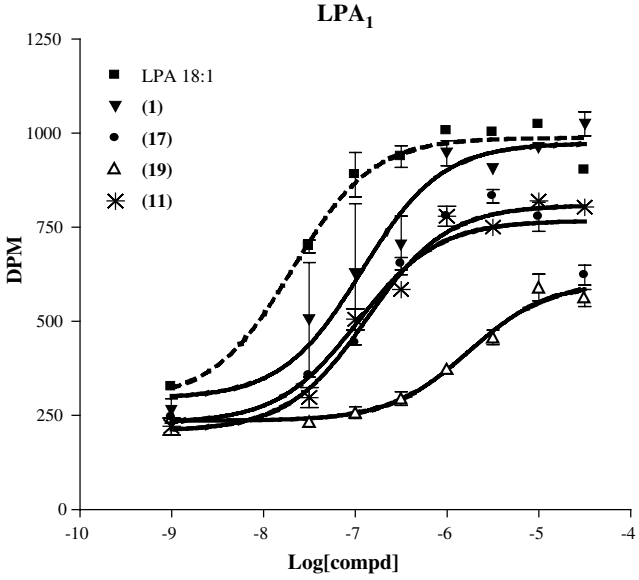


Figure 2. Agonist-induced GTP[$\gamma^{35}S$] binding[11] at the LPA_1 receptor. Points are in triplicate and are representative of at least two experiments.

to LPA or NAEPA. Hence **17** is a phosphatase-resistant[5] dual LPA agonist, displaying EC_{50}s at LPA_1, and LPA_2 of 40 and 108 nM, respectively. Additionally, thiol phosphoric acid **19**, unlike dual-agonist NAEPA, is an LPA_1-selective agonist of relatively good potency and efficacy. This selectivity is presumably due to a lack of compatibility between this phosphate isostere, perhaps due to sterics or geometry, and the LPA_2 receptor binding pocket. At least one group has reported on the chemical properties and enzymatic transformations of the phosphorylated thiol (Fig. 2).[15]

In summary, a series of polar head group analogues of LPA have been prepared and evaluated at three individual LPA receptors. This study has resulted in the discovery of a nonhydrolyzable LPA_1-selective agonist bearing an α-keto phosphonate head group as the phosphate mimic. The resistance of this new pharmacologic agent to phosphatase-catalyzed degradation renders **11** a useful tool to elucidate the functions of the LPA_1 receptor in vivo models. Additionally, thiol phosphoric acid **19** is a relatively potent and efficacious agonist at LPA_1 whose head group is not tolerated at the LPA_2 receptor. Finally, a phosphothioate analogue (**17**) of NAEPA is highly potent at the LPA_1 and LPA_2 receptors. The lack of activity at LPA_3 of the head group isosteres presented here is not surprising given the

inactivity of lead compound NAEPA itself at this receptor. Studies to determine binding affinity at additional LPA receptors are ongoing and will be published in due course.

Acknowledgements

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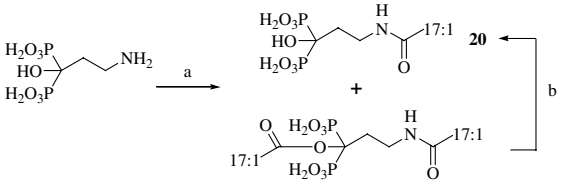
8. In brief, Pamidronate® (phosphoric acid (3-amino-1-hydroxypropylidene)bisdisodium salt) was condensed with oleoyl chloride in 2 M aqueous K_2CO_3. This afforded a mixture of mono- and di-acylated bisphosphonates, which were exposed to a solution of 1:1 2 M LiOH–THF. Upon addition of methanol to the reaction mixture, a precipitate formed which was isolated by filtration and washed with excess methanol. The white solid **20** was judged as pure by ^1H and ^{13}C NMR and mass spectroscopy analysis.



Synthesis of α-OH bisphosphonate **20**. (a) oleoyl chloride, 2M K_2CO_3 (aq); (b) 2M LiOH (aq)/THF, 80% over two steps.

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