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Initial structure–activity relationships of lysophosphatidic acid receptor antagonists: discovery of a high-affinity LPA_1/LPA_3 receptor antagonist

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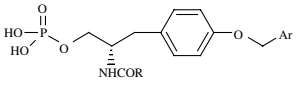
Abstract—A recently reported dual LPA_1/LPA_3 receptor antagonist (VPC12249, **1**) has been modified herein so as to optimize potency and selectivity at LPA receptors. Compounds containing variation in the acyl lipid chain and linker region have been synthesized and screened for activity at individual LPA receptors. LPA_1-selective (**14b**) and LPA_3-selective (**10g**,**m**) compounds of modest potency have been discovered. Additionally, 2-pyridyl derivative **10t** exhibits a K_i value of 18 nM at the LPA_1 receptor and is significantly more potent than **1** at the LPA_3 receptor. This paper describes the synthetic methods, biological evaluation, and structure–activity relationships (SARs) of LPA receptor antagonists.

Lysophosphatidic acid (LPA, 1- or 2-*O*-acyl-*sn*-glycero-3-phosphate) is an endogenous glycerophospholipid that can be generated by many cell types to elicit varied biological responses including cellular proliferation, platelet aggregation, smooth muscle contraction, and changes in cell morphology and mitogenesis.[1–4] G-protein coupled receptors of the endothelial differentiation gene (Edg) family transduce many of the biological effects of LPA.[5] This family consists of eight members, which are further divided into two subfamilies based upon their specificity for LPA or a related lysophospholipid sphingosine-1-phosphate (S1P). Three of the eight Edg receptors, LPA_1, LPA_2, and LPA_3 (formerly Edg2, Edg4, and Edg7, respectively), are high affinity LPA receptors. Recent evidence has emerged suggesting at least two non-Edg receptors also bind LPA with high affinity.[6,7]

Lysophosphatidic acid (LPA) antagonists have therapeutic potential as inhibitors of inflammation, neoplasms, and ischemic reperfusion injury. LPA can be detected in various biological fluids such as serum, plasma, tears, malignant ascites, and saliva, and its levels are elevated in various physiological and pathological conditions such as pregnancy and ovarian cancer. LPA, a potent fibroblast growth factor, is now known to be an 'ovarian cancer activating factor' in ascitic fluid from ovarian cancer patients.[8] Elevated levels of LPA are present both at early and late stages in ovarian cancer and may play a role in tumor cell proliferation and invasion. LPA has also been identified as pathogenic in the development and progression of atherosclerosis.[9] Finally, it was demonstrated recently that LPA is protective at low doses against renal ischemia-reperfusion (IR) injury.[10] Furthermore, a dual LPA_1/LPA_3 antagonist (VPC12249, **1**) produced a profound decrease in IR injury, a protective effect that was reversed by a selective LPA_3 agonist.

Despite potential applications of LPA receptor antagonists as therapeutic agents, the detailed physiological implications of blockade of individual LPA receptors are largely unknown because subtype-selective antagonists are unavailable currently. This paper describes the synthetic methods, biological evaluation, and initial structure–activity relationships (SARs) of LPA receptor antagonists.



1 : R = 17:1 (oleoyl); Ar = Ph (K_i137 nM LPA_1, 428 nM LPA_3)

Figure 1. Structure of dual LPA_1/LPA_3 antagonist, VPC12249 (**1**).

Keywords: Lysophosphatidic acid; Antagonist.
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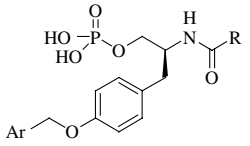
Recently, a series of 2-substituted *N*-acyl ethanolamine phosphoric acid (NAEPA) derivatives were synthesized and evaluated at LPA receptors.[11] From this series of NAEPA derivatives emerged a dual LPA_1/LPA_3 antagonist containing a bulky benzyl-4-oxybenzyl substitution at the 2-position in the linker region (VPC12249, **1**, Fig. 1). To more thoroughly elucidate the structural parameters that contribute to potency and selectivity in LPA antagonism, a series of VPC12249 (**1**) analogues were prepared and analyzed.

Syntheses of all the compounds listed in Tables 1–3 are described in Schemes 1–3. As outlined in Scheme 1, *N*-acyl tyrosine methyl esters **3a**–**c**,**e** were prepared by esterification and subsequent *N*-acylation of protected tyrosine **2**. Methyl ester reduction and phosphitylation of the resulting alcohol followed by in situ oxidation and subsequent trifluoroacetic acid (TFA) deprotection afforded phosphates **4a**–**c**,**e**. In the case of **4d**, catalytic hydrogenolysis of **3c** afforded the phenol, which was *O*-alkylated to the 3-methoxybenzyl aryl ether using Mitsunobu conditions.

As outlined in Scheme 2, the amide **7** was obtained by selective acylation of tyrosine methyl ester followed by protection of the phenol as the silyl ether. Methyl ester reduction and phosphorylation followed by desilylation with tetrabutylammonium fluoride (TBAF) afforded the phosphotriester **9**. Compound **9** was a common intermediate used to generate a series of ethers/esters by the Mitsunobu reaction or standard PyBOP® chemistry, respectively. TFA deprotection afforded phosphates **10a**–**u**. In the case of glucuronide **10u**, catalytic hydrogenolysis using Pearlmann's catalyst deprotected the tetra-*O*-benzyl-D-glucopyranose moiety prior to TFA deprotection of the phosphate ester. Compound **10u** is therefore the only compound in this series, which contains a saturated acyl chain.

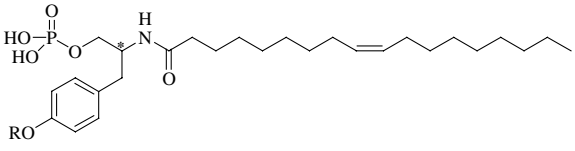
Table 1. Optimization of the *N*-acyl moiety as compared with lead compound **1**: biological evaluation of derivatives **4a**–**e**



Compounds	R	Ar	LPA_1		LPA_3 IC_{50} (nM)
			IC_{50} (nM)	K_i (nM)	
4a	n-C_7H_{15}	Phenyl	7970	N/D	>10,000
4b	n-$C_{13}H_{27}$	Phenyl	9030	N/D	>10,000
4c	n-$C_{15}H_{31}$	Phenyl	>10,000	N/D	6840
4d	n-$C_{15}H_{31}$	3-OMe-phenyl	>10,000	N/D	7220
1[11]	Oleoyl, 17:1[a]	Phenyl	5210	137	6450
4e	Linoleoyl, 17:2[b]	Phenyl	4250	N/D	>10,000

[a] 18 carbon chain; *cis* double bond located between C-9 and C-10 from the carbonyl.
[b] 18 carbon chain; two *cis* double bonds located between C-9/C-10 and C-12/C-13 from the carbonyl.

Table 2. Biological evaluation of ether/ester derivatives **10a**–**u**



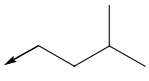
Compounds	R	*S*/*R*	LPA_1		LPA_3 IC_{50} (nM)
			IC_{50} (nM)	K_i (nM)	
10a	$\leftarrow CH_3$	*R*	>10,000	N/D	>10,000
10b	$\leftarrow CH_2CH_3$	*S*	>10,000	N/D	6930
10c	$\leftarrow CH_2CH_3$	*R*	>10,000	N/D	6410
10d	$\leftarrow CH_2CH=CH_2$	*S*	>10,000	N/D	>10,000
10e		*S*	>10,000	N/D	>10,000
10f		*S*	>10,000	N/D	5920

Table 2 (*continued*)

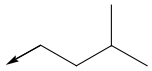
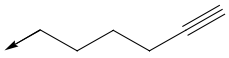
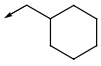
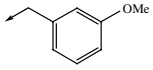
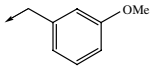
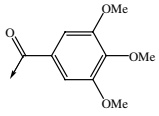
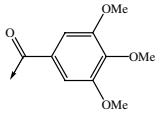
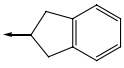
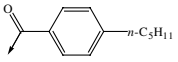
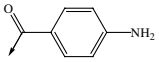
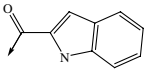
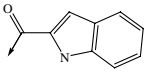
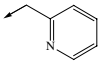
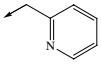
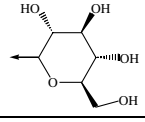
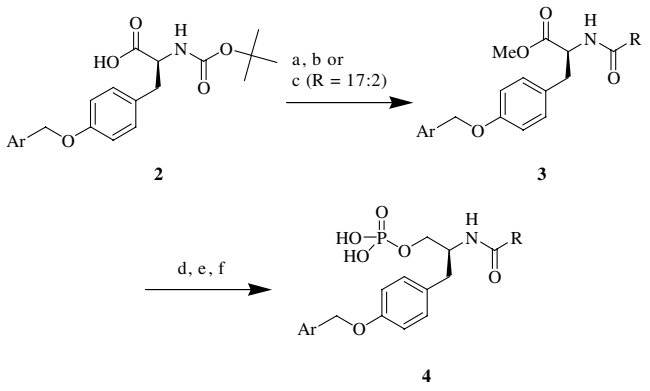
Compounds	R	*S*/*R*	LPA_1		LPA_3 IC_{50} (nM)
			IC_{50} (nM)	K_i (nM)	
10g		*R*	>10,000	N/D	351
10h		*R*	>10,000	N/D	3280
10i		*R*	>10,000	N/D	4030
10j		*S*	5660	358	642
10k		*R*	1070	34	35.6
10l		*S*	N/D	N/D	N/D
10m		*R*	>10,000	N/D	427
10n		*R*	2930	134	2310
10o		*S*	>10,000	N/D	>10,000
10p		*S*	>10,000	N/D	>10,000
10q		*S*	>10,000	N/D	9260
10r		*R*	7000	73	2630
10s		*S*	604	156	940
10t		*R*	109	18	175
10u[17]		*R*	>10,000	N/D	>10,000

Table 3. Comparison of ketone derivatives **14a**–**b** with tyrosine-based lead compound **1**

Compounds	*S*/*R*	LPA_1		LPA_3 IC_{50} (nM)
		IC_{50} (nM)	K_i (nM)	
1[11]	*S*	5210	137	6450
14a	*S*	>10,000	N/D	>10,000
14b	*R*	2490	N/D	>10,000

The synthesis of ketone derivatives **14a**–**b** is outlined in Scheme 3. Serine was converted in three steps into Weinreb's hydroxamate **11**,[12] whose condensation with the Grignard reagent 4-benzyloxyphenyl magnesium iodide proceeded to give the expected ketone **12** in modest yield. TFA deprotection afforded the ammonium salt **13**, which underwent selective acylation followed by phosphorylation. TFA deprotection provided ketones **14a**–**b**.

All compounds were characterized by ^1H and ^{13}C NMR, mass spectroscopy and, in certain cases, elemental analysis.[13]



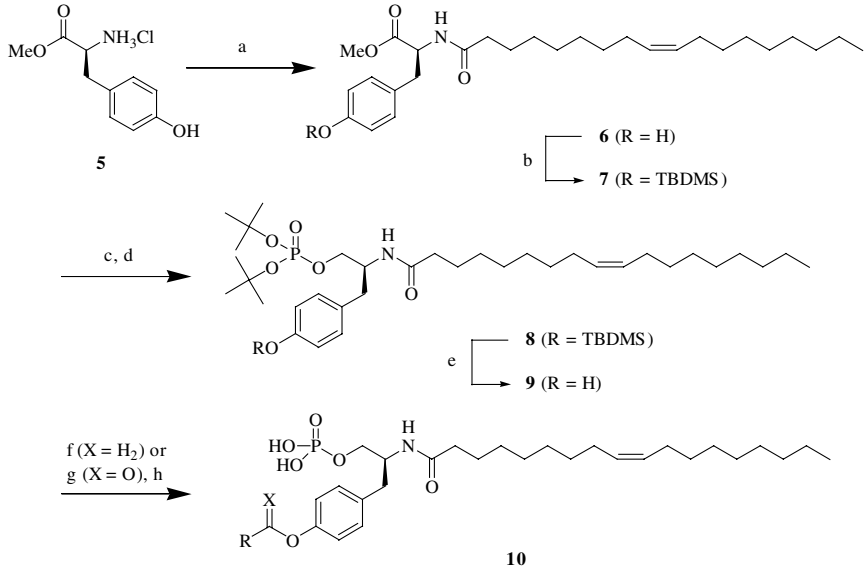
Scheme 1. Synthesis of compounds **4a**–**e**. Reagents and conditions: (a) $SOCl_2$, MeOH, 18 h, 100%; (b) acyl halide, DIEA, CH_2Cl_2, 4 h, 74–85%; (c) linoleic acid, PyBOP, DIEA, 18 h, 70%; (d) $NaBH_4$, $CaCl_2$, EtOH–THF (2:1), 18 h, 95%; (e) di-*tert*-butyldiisopropyl phosphoramidite, tetrazole, 4 h; then 30% H_2O_2, 4 h, 75–80%; (f) TFA–CH_2Cl_2 (1:2), 1 h, 95–100%.

A GTP[γ^{35}S] binding assay was adapted to assess in vitro activity.[14] The compounds presented in Tables 1–3 were assayed for their ability to antagonize LPA-evoked GTP[γ^{35}S] binding. However, the differential affinity of LPA_1 versus LPA_3 for 1-oleoyl-LPA prohibited a uniform analysis at each receptor. Specifically, the relatively low affinity of the LPA_3 receptor for 1-oleoyl-LPA impeded the determination of accurate K_i values for each antagonist. The detergent-like properties of the ligands prohibit the use of concentrations greater than 30 μM, which are required to achieve a statistically significant rightward shift in the sigmoidal curve requisite to the K_i determination. Consequently, the relative potencies, in the form of IC_{50} values,[15] are shown for the LPA_3 receptor in Tables 1–3. The K_i values as determined by Schild regression[11] are shown for the LPA_1 receptor. That is, compounds that produced rightward, parallel shifts in the concentration–response curves as a function of antagonist concentration were considered surmountable antagonists for which a reliable K_i value could be calculated for the LPA_1 receptor. LPA_1 IC_{50} values are also listed for comparison with LPA_3 data. The compounds presented here were devoid of any significant activity at the LPA_2 receptor.

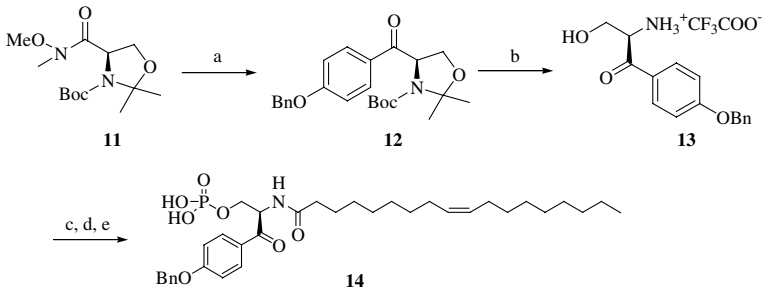
Optimization of the *N*-acyl moiety (Table 1), the outermost benzyl substituent and stereochemistry of **1** (Table 2) led to the development of an SAR as described below. In addition, two ketone derivatives of **1** (Table 3) were evaluated.

Phospholipid chain length plays a critical role in LPA receptor antagonist binding (Table 1). *N*-Oleoyl lead compound **1** displays potent LPA_1/LPA_3 dual antagonism.[11] At LPA_1, active compounds generally contained relatively long chain length and unsaturation (**1** and **4e**) in the *N*- acyl region. Palmitoyl derivatives **4c**–**d** showed no activity at LPA_1 while the unsaturated derivative **4e** displayed potency comparable to lead compound **1**. At LPA_3, palmitoyl derivatives **4c**–**d** were comparable in activity to **1** while shorter chain lengths and increased unsaturation led to a marked reduction in inhibitory activity. Thus compound **4e**, which contains a linoleoyl-amide shows selectivity for LPA_1 while palmitoyl derivatives **4c**–**d** were active only at LPA_3.

As described in Table 2, the outermost benzyl substituent of LPA antagonists can be modified with various ether/ester linkages to improve the potency and selectivity of lead compound **1**. LPA receptors also clearly exhibit stereochemical recognition as has been documented elsewhere.[11,16] The *N*-oleoyl lipid chain is held constant among derivatives **10a**–**u** as this feature has been shown



Scheme 2. Synthesis of compounds **10a**–**u**. Reagents and conditions: (a) oleoyl chloride, DIEA, CH_2Cl_2, 4 h, 85%; (b) TBDMSCI, DIEA, DMF, 4 h, 96%; (c) $NaBH_4$, $CaCl_2$, EtOH–THF (2:1), 18 h, 95%; (d) di-*tert*-butyldiisopropyl phosphoramidite, tetrazole, 4 h; then 30% H_2O_2, 4 h, 83%; (e) TBAF·$3H_2O$, THF, 1 h, 95%; (f) ROH, PPh_3, DIAD, CH_2Cl_2, 18 h, 73–88%; (g) RCOOH, DIEA, PyBOP, 18 h, 50–70%; (h) TFA–CH_2Cl_2 (1:2), 1 h, 95–100%.



Scheme 3. Synthesis of compounds **14a**–**b**. Reagents and conditions: (a) 4-benzyloxyphenyl iodide, Mg^0, Et_2O, 25 °C; (b) TFA–CH_2Cl_2 (1:2), 1 h, then Et_2O precipitation, 26% over two steps; (c) oleoyl chloride, DIEA, CH_2Cl_2, 4 h, 77%; (d) di-*tert*-butyldiisopropyl phosphoramidite, tetrazole, 4 h; then 30% H_2O_2, 4 h, 75%; (e) TFA–CH_2Cl_2 (1:2), 1 h, 100%.

to afford dual LPA_1/LPA_3 antagonism and good potency (Table 1). When the benzyl moiety of **1** is replaced with small alkyl substituents as in compounds **10a**–**e**, inhibitory activity is generally diminished. Relatively bulkier alkyl ethers **10f**–**i**, display varying degrees of antagonism at LPA_3 but are inactive at LPA_1. Electron-rich aromatic systems in place of the outermost benzyl moiety confer activity to LPA antagonists. This is exemplified by derivatives **10j**–**m**, which show good to excellent potency, particularly at LPA_3. Sterically demanding electron-rich aromatic substituents, in general, retain the potency of **1** at LPA_3 but exhibit diminished activity at LPA_1. This trend is particularly evident in compounds **10m** and **10r**. However, 4-*n*pentylbenzoyl derivative **10o** was inactive at both receptors, a result which implies some limitation to the steric tolerance of the LPA_3 antagonist binding pocket. Polar groups such as 4-aminobenzoyl (**10p**) and glucuronide **10u**[17] were also devoid of activity. To our delight, 2-pyridyl derivatives **10s**–**t** were significantly more potent than lead compound **1** at LPA_1 and LPA_3 receptors. *R* Enantiomer **10t** improves on the antagonist activity of **1** by approximately one log order at each receptor (Figs. 2 and 3). Indeed, all of the *R* derivatives in Table 2 are more potent than their *S* counterparts at LPA receptors.

Thus, in comparison to **1**, electron-rich aromatic systems in place of the outermost benzyl moiety improve antagonist activity at both receptors (**10k**). LPA_3 is more tolerant of sterically bulky substituents, which provides a strategy to realize LPA_3-selective antagonists with modest potency such as **10g** and **10m**. Finally, *N*-heterocycle derivative **10t** is a high affinity dual antagonist, with a K_i value of 18 nM at the LPA_1 receptor and IC_{50} of 175 nM at LPA_3.

Two ketone derivatives of **1**, oxidized at the innermost benzylic carbon atom, were evaluated at LPA receptors (Table 3). In this series (**14a**–**b**), a stereochemical preference for the *R* enantiomer is apparent at the LPA_1 receptor. Installation of the carbonyl in derivatives **14a**–**b** completely diminishes activity at the LPA_3 receptor while **14b** retains potency comparable to **1** at LPA_1. Thus, **14b** represents an LPA_1-selective blocker of modest potency.

In summary, an initial SAR of benzyl-4-oxybenzyl-substituted NAEPA analogues is described with regard

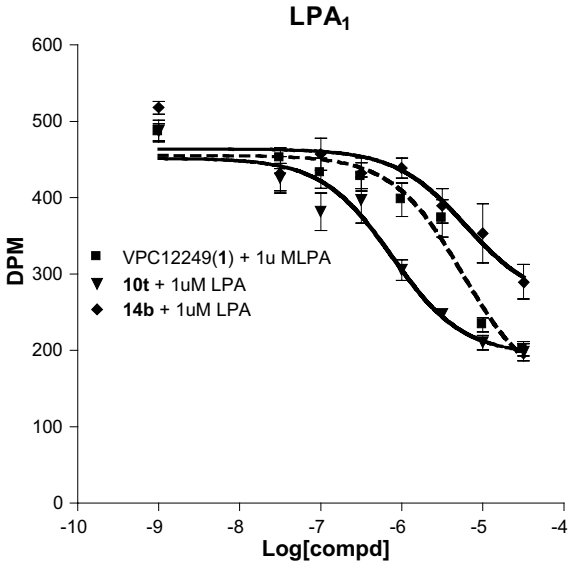


Figure 2. Effect of LPA antagonists on LPA_1 receptor GTP[γ^{35}S] binding.[15]

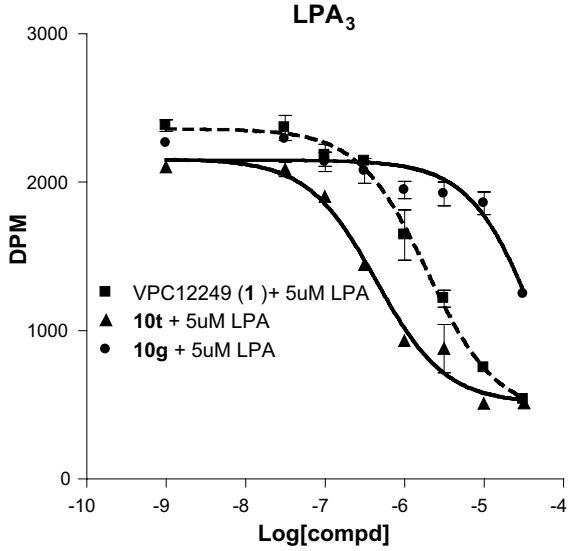


Figure 3. Effect of LPA antagonists on LPA_3 receptor GTP[γ^{35}S] binding.[15]

to LPA receptor antagonism. This study has resulted in the discovery of a high-affinity LPA_1/LPA_3 receptor antagonist (**10t**), which exhibits a K_i value of 18 nM at LPA_1 in our GTP[γ^{35}S] in vitro binding assay. The findings presented herein will be a useful platform for further optimization of lead derivative **10t**. Additionally, it will be critical to improve on the metabolic stability of the hydrolytically-labile phosphate head group so that in vivo studies might be employed. These efforts will be published in due course.

Acknowledgements

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References and notes

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13. (*S*)-*Phosphoric acid mono-{2-octadec-9-enoylamino-3-[4-(pyridin-2-ylmethoxy)-phenyl]-propyl} ester* (**10s**): ^{1}H NMR (300 MHz, $CD_3OD/CDCl_3$) δ 0.62 (t, 3H, $J = 7.2$ Hz), 0.95–1.10 (m, 11H), 1.22–1.32 (m, 2H), 1.70–1.79 (m, 4H), 1.89 (t, 2H, $J = 7.9$ Hz), 2.51 (dd, 1H, $J = 7.7$, 13.6 Hz), 2.61 (dd, 1H, $J = 7.7$, 13.6 Hz), 3.68–3.73 (m, 2H), 3.92–4.03 (m, 1H), 4.91 (s, 2H), 5.07 (t, 2H, $J = 5.5$ Hz), 6.66 (d, 2H, $J = 7.9$ Hz), 6.91 (d, 2H, $J = 7.9$ Hz), 7.09 (t, 1H, $J = 6.4$ Hz), 7.84 (d, 1H, $J = 8.1$ Hz), 7.69 (t, 1H, $J = 8.6$ Hz), 8.29 (d, 1H, $J = 5.0$ Hz); ^{13}C NMR (300 MHz, $CD_3OD/CDCl_3$) δ 13.19, 22.01, 25.21, 26.52, 28.52, 28.68, 28.87, 29.11, 31.27, 35.15, 35.68, 50.16, 50.26, 66.21, 69.15, 114.15, 121.58, 122.75, 129.11, 129.27, 129.80, 130.10, 137.68, 147.62, 148.31, 156.19, 156.43; MS (ESI) m/z 601.74 [M−H$^+$, 100%]; anal. calcd for $C_{33}H_{51}N_2O_6P$: C, 65.76; H, 8.53; N, 4.65; found: C, 65.06; H, 8.57; N, 4.75.
(*R*)-*Phosphoric acid mono-{2-octadec-9-enoylamino-3-[4-(pyridin-2-ylmethoxy)-phenyl]-propyl} ester* (**10t**): This product had spectroscopic properties identical to its enantiomer.
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15. DNAs encoding LPA_1, LPA_2, or LPA_3 receptors were transfected into HEK293T cells along with DNAs encoding $G\alpha_{i2}$, $G\beta$, and $G\gamma_2$ proteins. After 48 h, microsomes were prepared and used in a GTP[γ^{35}S] binding assay, as described.[14] For determination of IC_{50} values, a concentration of 1-oleoyl-LPA equal to the EC_{80} value (LPA_1: 1 μM; LPA_3: 10 μM) was collided with antagonists at concentrations ranging from 0.1 to 30,000 nM. Points are in triplicate and are representative of at least two experiments.
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17. For reasons mentioned in the text above, **10u** is the only compound in Table 2 which contains a saturated 18 carbon length acyl chain.