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Identification of Darmstoff analogs as selective agonists and antagonists of lysophosphatidic acid receptors

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Abstract—Darmstoff describes a family of gut smooth muscle-stimulating acetal phosphatidic acids initially isolated and characterized from the bath fluid of stimulated gut over 50 years ago. Despite similar structural and biological profiles, Darmstoff analogs have not previously been examined as potential LPA mimetics. Here, we report a facile method for the synthesis of potassium salts of Darmstoff analogs. To understand the effect of stereochemistry on lysophosphatidic acid mimetic activity, synthesis of optically pure stereoisomers of selected Darmstoff analogs was achieved starting with chiral methyl glycerates. Each Darmstoff analog was evaluated for subtype-specific LPA receptor agonist/antagonist activity, PPARγ activation, and autotaxin inhibition. From this study we identified compound **12** as a pan-antagonist and several pan-agonists for the LPA_{1-3} receptors. Introduction of an aromatic ring in the lipid chain such as analog **22** produced a subtype-specific LPA_3 agonist with an EC_{50} of 692 nM. Interestingly, regardless of their $LPA_{1/2/3}$ ligand properties all of the Darmstoff analogs tested activated PPARγ. However, these compounds are weak inhibitors of autotaxin. The results indicate that Darmstoff analogs constitute a novel class of lysophosphatidic acid mimetics.
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Lysophosphatidic acid (LPA) is a growth factor-like lysophospholipid[1] involved in many physiological and pathological processes including neurogenesis,[2] myelination, angiogenesis,[3] wound healing,[4] and cancer progression.[5] LPA is produced by a number of cell types including platelets, adipocytes, fibroblasts, and ovarian cancer cells.[6,7] LPA is a product of lysophospholipase D, also known as autotaxin, an esterase that cleaves lysophosphatidylcholine to LPA and choline.[8,9] LPA acts through three G protein coupled receptors, LPA_1 (EDG-2), LPA_2 (EDG-4), and LPA_3 (EDG-7).[10,11] Recently, two non-EDG LPA receptors, LPA_4/GPR23 and the nuclear transcription factor/receptor PPARγ, have been reported.[12,13] Most cells express a combination of these receptors, making it difficult to dissect the biological effects mediated by an individual receptor subtype. The need to understand the biological function of LPA receptors and the desire to pharmacologically exploit the differences in their ligand recognition require the development of receptor subtype-selective agonists and antagonists.[14,15]

Based on Weiland's observations[16] in 1949, Vogt was first to report the isolation of an acidic phospholipid from the bath fluid of horse intestine that stimulated smooth muscle contraction, which he named Darmstoff.[17] Darmstoff, present not only in the bath but also in the gut extract, was subsequently shown to be a mixture of acetal phosphatidic acids primarily composed of **1a**, **1b**, and **1c** (Fig. 1). Interestingly, all the intestinal smooth muscle contracting activity possessed by Darmstoff resides in the oleyl acetal **1b**.[18,19] These compounds were also shown to display hypotensive and cardiodepressant properties[20] and stimulate platelet aggregation. Vogt was also the first to describe an LPA-induced physiological response. Surprisingly, neither Vogt nor anyone else has published work in which Darmstoff or its analogs were examined as LPA mimics. In our continuing efforts to develop subtype-selective

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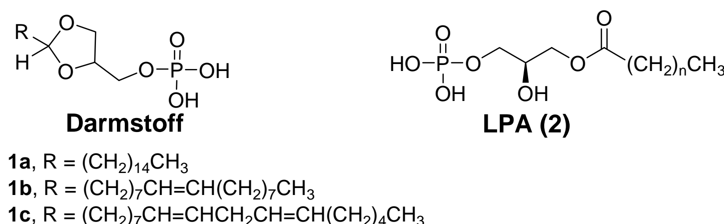


Figure 1. Chemical structures of Darmstoff and LPA.

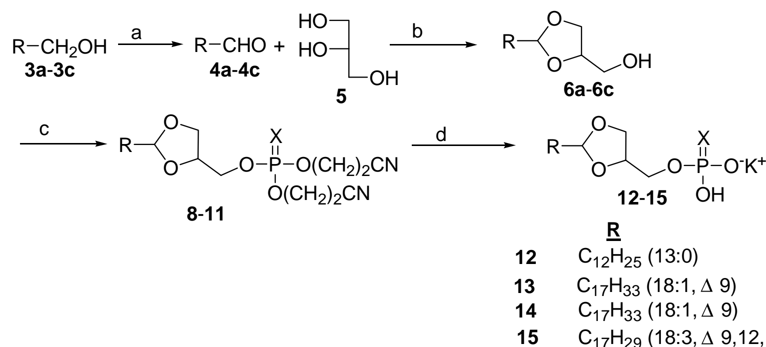
LPA agonists and antagonists, we revisited the possibility that acetal phosphatidates would mimic the effects of LPA and for the first time we show here that Darmstoff analogs constitute a new class of LPA-like ligands that act on multiple LPA targets.[21] The details of synthesis and pharmacological characterization of a series of Darmstoff analogs are discussed herein.

Previously reported methods[19,22] for the synthesis of this compound class have limitations and are particularly unsuitable for the preparation of Darmstoff analogs containing thiophosphate head groups. To facilitate the synthesis of various Darmstoff analogs in an effort to develop a structure–activity relationship, we developed a general method for the preparation of these compounds utilizing phosphoramidite chemistry. Accordingly, PCC mediated oxidation of fatty alcohols produced the corresponding aldehydes, which were condensed with glycerol in the presence of PTSA under reported conditions[23] to give dioxolanes **6a–c** (Scheme 1). Phosphorylation of **6a–c** using bis(cyanoethyl)-*N*,*N*-diisopropylphosphoramidite (**7**) in the presence of 1*H*-tetrazole formed phosphorous acid esters that were converted in situ to phosphate or thiophosphate esters using hydrogen peroxide or sulfur, respectively. Finally, treatment of the phosphate or thiophosphate esters with methanolic KOH at ambient temperature provided potassium salts of Darmstoff analogs.

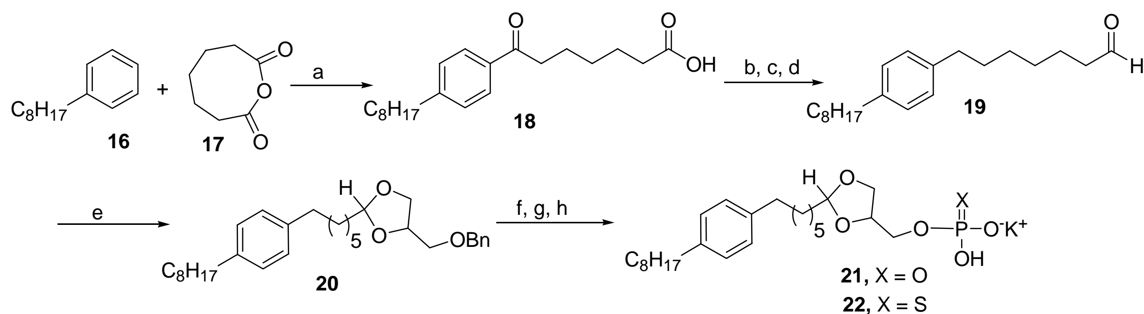
The synthesis of compounds **21** and **22** containing a phenyl ring in the lipid chain is shown in Scheme 2. Friedel–Crafts acylation of *n*-octyl benzene with pimelic anhydride gave arylketo acid **18** that was converted to the required aldehyde **19** in three steps. Condensation of **19** with 3-benzyloxy-propane-1,2-diol under standard conditions formed the dioxolane **20**. Debenzylation of **20** followed by phosphorylation and removal of the protecting groups gave target compounds **21** and **22**.

To investigate the effect of stereochemistry on biological activity, we decided to synthesize all stereoisomers of Darmstoff analogs **13** and **14**. From the chemical structures it is clear that 2,4-disubstituted-1,3-dioxolanes of this type exist as a mixture of four stereoisomers. Previously it was difficult to separate optically pure dioxolanes, although there have been reports that utilize chiral stationary phases[24]or enzymatic methods.[25] Recently, Lin et al. showed the separation of 2,4-disubstituted-1,3-dioxolane stereoisomers utilizing a semi-preparative HPLC method.[26]

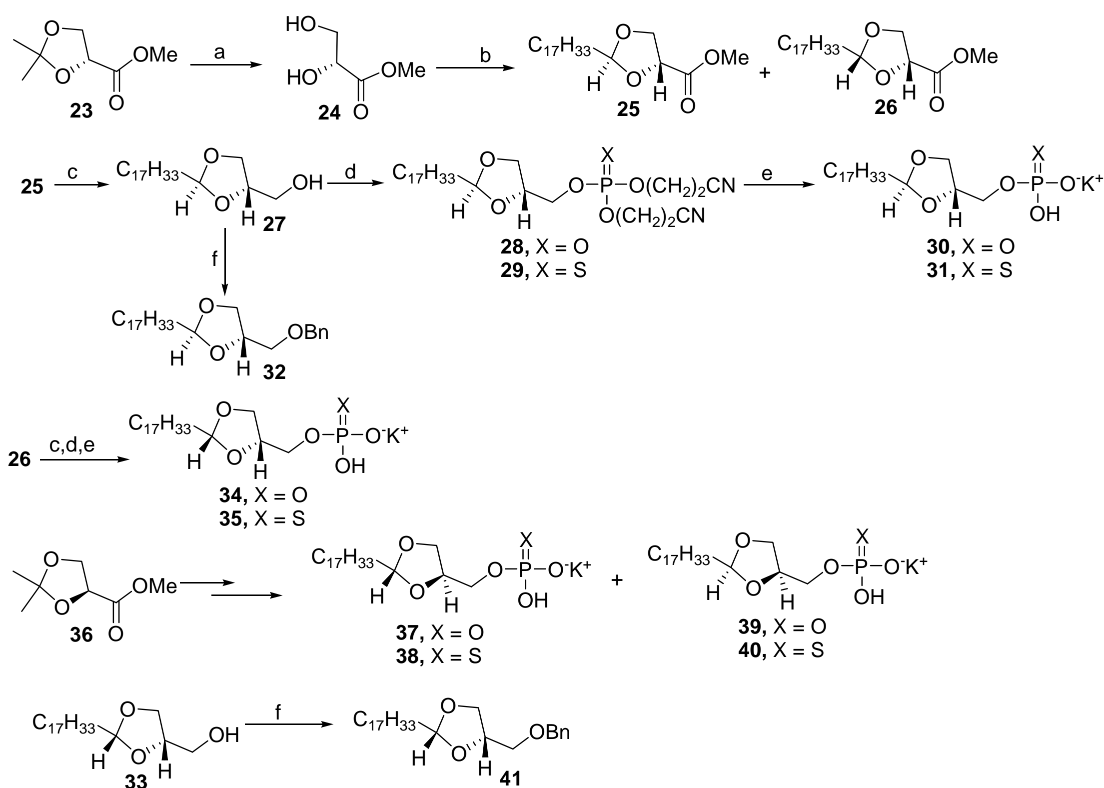
Our synthetic approach for the preparation of the four possible stereoisomers of **13** and **14** is outlined in Scheme 3. Accordingly, acid mediated removal of the isopropylidene group from commercially available **23** (*R*-isomer) gave methyl glycerate (**24**) in a quantitative yield. The acid-catalyzed condensation of *cis*-9-octadecenal with **24** afforded a mixture of dioxolanes **25** and **26**, which were readily separated by column chromatography.[27,28] $LiBH_4$ mediated reduction of the ester functionality of **25** gave alcohol **27** that was phosphorylated using **7** to form phosphate **28** and thiophosphate **29**. Finally, treatment of these esters with methanolic KOH gave the corresponding Darmstoff stereoisomers **30** and **31** as shown in Scheme 3. Similarly, dioxolane intermediate **26** was converted to target compounds **34** and **35** using the same chemistry. Synthesis of the other four Darmstoff stereoisomers (**37–40**) utilized the same procedure but used **36** (*S*-isomer of **23**) as the starting material. To examine the purity of these stereoisomers,



Scheme 1. Reagents and conditions: (a) PCC, CH_2Cl_2, 90–95%; (b) PTSA, C_6H_6, Dean–Stark, reflux, 70–78%; (c) (1) bis(cyanoethyl)-*N*,*N*-diisopropylphosphoramidite (**7**), 1*H*-tetrazole, CH_2Cl_2, (2) 30% H_2O_2, rt or sulfur, reflux, 70–75%; (d) KOH, MeOH, 80–85%.



Scheme 2. Reagents and conditions: (a) $AlCl_3$, CH_2Cl_2, 60%; (b) $N_2H_4{\cdot}H_2O$, KOH, ethylene glycol, MW, 87%; (c) $BH_3{\cdot}THF$, 78%; (d) PCC, CH_2Cl_2, 95%; (e) 3-benzyloxy-propane-1,2-diol, PTSA, C_6H_6, Dean–Stark, reflux, 83%; (f) $H_2/Pd(OH)_2$, EtOAc, 100%; (g) (1) bis(cyanoethyl)-*N*,*N*-diisopropylphosphoramidite, 1*H*-tetrazole, CH_2Cl_2, (2) 30% H_2O_2, rt or sulfur, reflux, 70%; (h) KOH, MeOH, 80%.



Scheme 3. Reagents and conditions: (a) HCl, MeOH, 88%; (b) $C_{17}H_{33}CHO$, PTSA, C_6H_6, Dean–Stark, reflux, 75%; (c) $LiBH_4$, Et_2O, 83%; (d) (1) bis(cyanoethyl)-*N*,*N*-diisopropylphosphoramidite, 1*H*-tetrazole, CH_2Cl_2, (2) 30% H_2O_2, rt or sulfur, reflux, 73%; (e) KOH, MeOH, 81%; (f) BnBr, NaH, THF, reflux, 75%.

HPLC profiles of compounds **32** and **41** were analyzed. Benzyl ethers (**32** and **41**, Scheme 3) were prepared to increase their detection by UV. The HPLC analysis (Chiralpak AS-RH 150×4.6 mm, 1:1 water–acetonitrile) of benzyl ethers confirmed the purity of **32** and **41**. All compounds were fully characterized by spectroscopic methods.[29]

The biological effects of all synthesized compounds were tested using three high-throughput assays. First, intracellular calcium transients in rat hepatoma (RH7777, an LPA receptor null cell line) cell lines individually expressing either LPA_1, LPA_2, and LPA_3 receptors were analyzed to examine agonism and antagonism.[30] Wild type RH7777 cells did not respond to any of the Darmstoff analogs. Second, PPARγ activation was examined in CV1 cells, transfected with an acyl-coenzyme A oxidase-luciferase (PPRE-Acox-Rluc) reporter gene construct as previously reported.[30] Third, inhibition of the lysophospholipase D autotaxin was determined using a previously described procedure.[31]

Compound **12** containing a C13 alkyl chain and no double bond inhibited Ca^{2+} mobilization in cells expressing all three LPA GPCRs there by providing a pan-antagonist of $LPA_{1–3}$ (Table 1). An increase in chain length to C18 and introduction of the $C_9{=}C_{10}$ double bond resulted in analog **13**, which produced $LPA_{1/3}$ antagonist activity. It is interesting to note that oleoyl-LPA is an agonist of $LPA_{1–3}$ while Darmstoff analog **13** containing

Table 1. Ligand properties of Darmstoff analogs at $LPA_{1/2/3}$, PPARγ, and ATX

Compound	LPA_1		LPA_2		LPA_3		PPARγ	ATX IC_{50} (inhibition$_{max}$ %) (nM)
	EC_{50} (E_{max})[a](nM)	IC_{50} (K_i)(nM)	EC_{50} (E_{max}) (nM)	IC_{50} (K_i) (nM)	EC_{50} (E_{max}) (nM)	IC_{50} (K_i) (nM)		
12	NE[b]	1110 (652)	NE	7430 (745)	NE	2870 (681)	Agonist	232 (26)
13	NE	915 (497)	>10,000	NE	NE	527 (548)	Agonist	141 (30)
14	981 (45)	NE	1170 (87)	NE	639 (73)	NE	Agonist	415 (51)
15	3600 (55)	NE	1710 (51)	NE	7590 (29)	NE	Agonist	803 (54)
21	NE	4660 (1930)	NE	NE	NE	504 (171)	Agonist	106 (10)
22	NE	WA[c]	NE	NE	692 (87)	NE	Agonist	449 (55)
30	NE	WA	1170 (39)	NE	NE	WA	Agonist	120 (30)
31	1580 (89)	NE	1300 (77)	NE	127 (127)	NE	Agonist	252 (74)
34	NE	NE	1710 (42)	NE	NE	136 (83)	Agonist	97 (28)
35	1410 (71)	NE	1090 (85)	NE	194 (113)	NE	Agonist	344 (66)
37	>10,000	NE	>10,000	NE	NE	484 (241)	Agonist	238 (46)
38	2260 (68)	NE	1540 (72)	NE	204 (102)	NE	Agonist	363 (64)
39	NE	WA	NE	NE	NE	209 (77)	Agonist	178 (25)
40	1560 (65)	NE	1320 (87)	NE	265 (78)	NE	Agonist	403 (60)

[a] E_{max}, maximal efficacy of drug/maximal efficacy of LPA 18:1, expressed as the percentage.
[b] NE, no effect was shown at the highest concentration (30 μM) tested.
[c] WA, weak antagonist.

an oleoyl chain at C-2 position of the 1,3-dioxolane inhibited LPA_{1-3} receptors indicating the significant role played by the acetal moiety in ligand recognition. The replacement of the phosphate head group with a thiophosphate in our previously reported fatty alcohol phosphate analogs had a significant effect on their ligand properties with regard to the LPA_{1-3} receptors.[30] To examine the effect of this modification with Darmstoff series compound **14** was synthesized. This analog was an agonist at all three LPA receptor subtypes and was most potent at LPA_3 (EC_{50} of 639 nM). The phosphate analog **15** with conjugated double bonds at $C_9{=}C_{10}$, $C_{12}{=}C_{13}$, and $C_{15}{=}C_{16}$ positions was an agonist for all three LPA receptors. Though analog **15** was less potent than **14**, these compounds were identified as two new LPA GPCR pan-agonists. We are currently using these leads to optimize the receptor potency and introduce specificity.

A multitude of aldehydes are produced via oxidative cleavage of unsaturated fatty acids and their phospholipid derivatives.[32] The *cis*-olefinic bond of analogs **13–15** is susceptible to oxidative cleavage. In order to avoid this problem and to examine the effect of structural rigidity on biological activity, we replaced the double bond with an aromatic ring and screened against LPA GPCR, PPARγ, and autotaxin. Incorporation of an aromatic ring in the alkyl chain led to compounds **21** and **22**. Analog **21** was an antagonist of $LPA_{1/3}$ receptors but had no effect against LPA_2. The thiophosphate analog, compound **22**, was a weak LPA_1 antagonist, without any effect on LPA_2 but stimulated LPA_3 with an EC_{50} of 692 nM (E_{max} = 87%).

Next, we examined the importance of stereochemistry on biological activity. Analysis of pure stereoisomers (Table 1) with respect to LPA GPCR activation suggests that regardless of their stereochemistry at C-2 and C-4, Darmstoff analogs (**30**, **34**, **37**, and **39**) with phosphate head groups were LPA_3 antagonists, whereas analogs with thiophosphate groups (**31**, **35**, **38**, and **40**) were pan-agonists. Among the phosphate stereoisomers, analog **34** was identified as the most potent LPA_3 antagonist with an IC_{50} of 136 nM (K_i = 83 nM). Interestingly, compound **30** weakly activated $LPA_{1/3}$ and was a partial LPA_2 agonist with an EC_{50} of 1.17 μM (E_{max} = 39%). It is noteworthy that stereoisomers with a thiophosphate head group were found to be more potent at LPA_3 receptor than parent compound **14**. In this series all other stereoisomers (**31**, **35**, and **38**), with the exception of **40**, were full agonists of LPA_3 receptor, with the most potent being **31** (EC_{50} of 127 nM, E_{max} = 127%) there by indicating the importance of stereochemistry on biological activity of Darmstoff analogs.

PPARγ is a lipid-activated transcription factor that belongs to the nuclear hormone receptor superfamily. Recently, McIntyre et al. showed that LPA is a direct agonist of PPARγ.[13] They demonstrated that LPA competed against the synthetic PPARγ agonist Rosiglitazone and the natural agonist azaleylphosphatidylcholine. LPA binding to PPARγ has a nanomolar Kd and LPA does not bind to PPARα or PPARβ.[13] LPA-induced activation of PPARγ appears to be an important step in the regulation of adipogenesis, macrophage function, neointima formation, and atherogenesis.[33] Zhang et al. showed that unsaturated and alkyl ether analogs of LPA induce neointima formation, an early step that leads to the development of atherogenic plaques, through PPARγ activation.[33] We examined the activity of all synthesized Darmstoff analogs as PPARγ activators in vitro in CV1 cells using a PPRE-Acox-Rluc reporter gene assay. For comparison purposes we also included Rosiglitazone, a known PPARγ agonist, as a positive control in all analyses. The results (Fig. 2) indicate that all tested Darmstoff analogs, regardless of whether they were agonist or antagonist of LPA GPCR, activated the PPARγ reporter construct. These results are consistent with previous

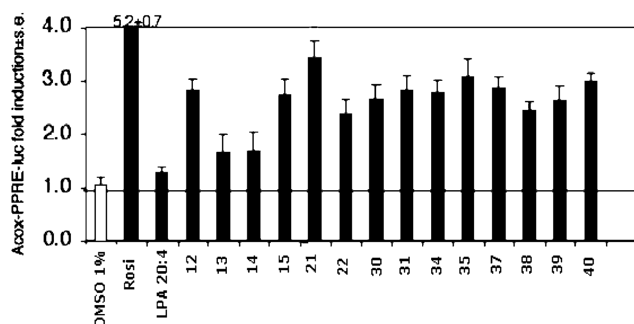


Figure 2. Results of in vitro PPARγ activation by Darmstoff analogs in CV1 cells transfected with PPARγ and PPRE-Acox-Rluc reporter gene. CV1 cells were treated with 1% of DMSO or 10 μM of test compound dissolved in DMSO for 20 h.

reports,[30] establishing that many LPA-like compounds activate PPARγ. These data also emphasize that the SAR of PPARγ activation is distinct from that of GPCR activation/inhibition.

LPA is liberated as the product of lysophosphatidylcholine hydrolysis by the lysophospholipase D autotaxin. Recently, van Meeteren et al. showed that ATX is subjected to mixed type product inhibition.[31] To further characterize the pharmacological behavior of Darmstoff analogs, we screened these compounds for ATX inhibition. The IC_{50} values and percentage of inhibition for Darmstoff analogs are listed in Table 1. These data clearly indicate that all tested analogs are capable of ATX inhibition that is independent of their ligand properties at LPA GPCR and PPARγ. Compound **31**, which is an $LPA_{1–3}$ pan-agonist with preference for LPA_3 emerged as the most effective ATX inhibitor with an IC_{50} of 252 nM.

In conclusion, we have developed a facile method for the preparation of Darmstoff analogs and their stereoisomers. For the first time we showed that Darmstoff represents a novel scaffold for the development of subtype-specific LPA GPCR ligands. The initial SAR study with these analogs led to the discovery of a novel pan-antagonist and several pan-agonists of the $LPA_{1–3}$ receptors. Analysis of the ligand properties of pure stereoisomers indicates that stereochemistry plays an important role in LPA GPCR activity. Further, we found that in addition to their LPA GPCR activity, Darmstoff analogs also activate the nuclear transcription factor PPARγ and inhibit lysophospholipase D/ATX. The LPA receptor subtype-specific agonists and antagonists identified in this study will serve as novel leads for the development of more potent compounds by further synthetic optimization.

Acknowledgments

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References and notes

1. Zhou, D.; Luini, W.; Bernasconi, S.; Diomede, L.; Salmona, M.; Mantovani, A.; Sozzani, S. *J. Biol. Chem.* **1995**, *270*, 25549.
2. Chun, J. *Crit. Rev. Neurobiol.* **1999**, *13*, 151.
3. Sugiura, T.; Nakane, S.; Kishimoto, S.; Waku, K.; Yoshioka, Y.; Tokumura, A. J. *Lipid. Res.* **2002**, *43*, 2049.
4. Sturm, A.; Dignass, A. U. *Biochim. Biophys. Acta* **2002**, *1582*, 282.
5. Mills, G. B.; Moolenaar, W. H. *Nat. Rev. Cancer* **2003**, *3*, 582.
6. Eichholtz, T. K.; Jalink, T.; Fahrenfort, I.; Moolenaar, W. H. *Biochem. J.* **1993**, *291*, 677.
7. Pages, C.; Simon, M.; Valet, P.; Saulnier-Blache, J. S. *Prostaglandins* **2001**, *64*, 1.
8. Umezu-Goto, M.; Kishi, Y.; Taira, A.; Hama, K.; Dohmae, N.; Takio, K.; Yamori, T.; Mills, G. B.; Inoue, K.; Aoki, J.; Arai, H. *J. Cell Biol.* **2002**, *158*, 227.
9. Tokumura, A.; Majima, E.; Kariya, Y.; Tominaga, K.; Kogure, K.; Yasuda, K.; Fukuzawa, K. *J. Biol. Chem.* **2002**, *277*, 39436.
10. Tigyi, G.; Fischer, D. L.; Baker, D.; Wang, D. A.; Yue, J.; Nusser, N.; Virag, T.; Zsiros, Z.; Liliom, K.; Miller, D.; Parrill, A. *Ann. N.Y. Acad. Sci.* **2000**, *905*, 34.
11. Fukushima, N.; Ishii, I.; Contos, J. I. A.; Weiner, J. A.; Chun, J. *Annu. Rev. Pharmacol. Toxicol.* **2001**, *41*, 507.
12. Noguchi, K.; Ishii, S.; Shimizu, T. *J. Biol. Chem.* **2003**, *278*, 25600.
13. McIntyre, T. M.; Pontsler, A. V.; Silva, A. R.; St Hilaire, A.; Xu, Y.; Hinshaw, J. C.; Zimmerman, G. A.; Hama, K.; Aoki, J.; Arai, H.; Prestwich, G. D. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 131.
14. Qian, L.; Xu, Y.; Hasegawa, Y.; Aoki, J.; Mills, G. B.; Prestwich, G. D. *J. Med. Chem.* **2003**, *46*, 5575.
15. Lynch, K. R.; Macdonald, T. L. *Biochim. Biophys. Acta* **2002**, *1582*, 289.
16. Weiland, W. *Pflug. Arch.* **1912**, *147*, 171.
17. Vogt, W. *Arch. Exp. Pathol. Pharmakol.* **1949**, *208*, 221.
18. Vogt, W. *Biochem. Pharmacol.* **1963**, *12*, 415.
19. Wiley, R. A.; Sumner, D. D.; Walaszek, E. J. *Lipids* **1970**, *5*, 803.
20. Milbert, A. N.; Wiley, R. A. *J. Med. Chem.* **1978**, *21*, 245.
21. Gududuru, V.; Walker, M.; Tsukahara, R.; Fujiwara, Y.; Yasuda, S.; Tigyi, G.; Miller, D.D. Abstracts of Papers, 229th National Meeting of the American Chemical Society, San Diego, CA; American Chemical Society: Washington, DC, 2005.
22. Marx, M. H.; Wiley, R. A.; Satchell, D. G.; Maguire, H. M. *J. Med. Chem.* **1989**, *32*, 1319.
23. Piantadosi, C.; Anderson, C. E.; Brecht, E. A.; Yarbro, C. L. *J. Am. Chem. Soc.* **1958**, *80*, 6613.
24. Bernal, J. L.; Toribio, L.; del Nozal, M. J.; Nieto, E. M.; Jiminez, J. J. *J. Chromatogr.* **2000**, *871*, 127.
25. Janes, L. E.; Cimpoia, A.; Kazlauskas, R. J. *J. Org. Chem.* **1999**, *64*, 9019.
26. Lin, S.; Duclos, R. I.; Makriyannis, A. *Chem. Phys. Lipids* **2001**, *113*, 111.
27. Baumann, W. J.; Wedmid, Y. *J. Org. Chem.* **1977**, *42*, 3624.
28. Dioxolanes **25** (49%) and **26** (26%) were separated on a silica gel column (eluted with a mixture of diethyl ether–hexanes, 5:95, v/v). Configurational assignment for the C2 acetal proton of **25** and **26** was based on ^{1}H NMR studies. The C2 acetal proton appeared as a triplet at δ 5.09 ppm for the *trans*-stereoisomer (**25**) and at 4.99 ppm for the *cis*-stereoisomer (**26**). These observations were in complete agreement with the earlier reports[26,27] wherein the C2 proton for the *trans*-stereoisomer of 2,4-disubstituted-1,

3-dioxolanes always appeared in the downfield of ^{1}H NMR spectra.

29. Characteristic data for some compounds: **13**: ^{1}H NMR (300 MHz, CD_3OD) δ 0.86 (m, 3H), 1.27 (m, 24H), 2.0 (m, 4H), 3.77–4.0 (m, 3H), 4.14–4.21 (m, 1H), 4.26–4.35 (m, 1H), 4.89 (t, $J = 4.8$ Hz, 0.5H), 5.0 (t, $J = 4.8$ Hz, 0.5H), 5.35 (m, 2H); ^{13}C NMR (CD_3OD) δ 12.68, 21.68, 22.91, 23.12, 25.99, 26.09, 28.06, 28.25, 28.33, 28.51, 28.60, 28.69, 30.92, 31.43, 32.76, 33.22, 33.72, 63.89, 64.44, 66.17, 69.66, 69.87, 74.54, 74.75, 101.55, 103.80, 104.37, 129.20, 129.25; MS (ESI) *m/z* 419 [M−H]; ^{31}P NMR (CD_3OD) δ 19.68. Compound **14**: ^{1}H NMR (300 MHz, CD_3OD) δ 0.78 (m, 3H), 1.19 (br s, 24H), 1.93 (m, 4H), 3.76–3.89 (m, 3H), 4.05–4.25 (m, 2H), 4.4–4.47 (m, 0.4H), 4.75 (m, 0.75H), 4.97 (m, 0.3H), 5.25 (m, 2H); ^{13}C NMR (CD_3OD) δ 13.36, 22.09, 26.62, 28.81, 29.21, 31.39, 33.09, 64.24, 66.18, 74.86, 74.90, 103.87, 104.50, 129.19, 129.42; MS (ESI) *m/z* 435 [M−H]; ^{31}P NMR (CD_3OD) δ 62.85.
Compound **21**: ^{1}H NMR (300 MHz, CD_3OD) δ 0.90 (t, $J = 6.3$ Hz, 3H), 1.27 (m, 24H), 2.56 (m, 4H), 3.77–3.78 (m, 1H), 3.87–3.95 (m, 3H), 4.22–4.25 (m, 1H), 4.98 (m, 1H), 7.06 (m, 4H); ^{13}C NMR (CD_3OD) δ 12.62, 21.66, 22.86, 23.0, 28.05, 28.10, 28.24, 28.41, 30.54, 30.60, 30.90, 32.78, 34.41, 64.17, 64.69, 64.76, 66.07, 66.18, 74.15, 74.27, 74.44, 103.90, 104.41, 127.44; MS (ESI) *m/z* 455 [M−H]; ^{31}P NMR (CD_3OD) δ 19.26. Compound **31**: ^{1}H NMR (300 MHz, CD_3OD) δ 0.86 (t, $J = 6.3$ Hz, 3H), 1.25 (br s, 24H), 1.98 (m, 4H), 3.64 (m, 1H), 3.89 (t, $J = 6$ Hz, 2H), 4.08–4.13 (m, 1H), 4.30–4.38 (m, 1H), 4.99 (t, $J = 4.8$ Hz, 1H), 5.33 (m, 2H); ^{13}C NMR (CD_3OD) δ 13.04, 21.93, 23.25, 26.44, 28.51, 28.56, 28.78, 28.89, 29.0, 31.20, 31.83, 33.27, 64.24, 66.26, 74.74, 74.85, 103.65, 104.03, 129.05, 129.19; MS (ESI) *m/z* 435 [M−H]; ^{31}P NMR (CD_3OD) δ 63.82.
Compound **34**: ^{1}H NMR (300 MHz, CD_3OD) δ 0.84 (t, $J = 6.3$ Hz, 3H), 1.25 (m, 24H), 1.97 (m, 4H), 3.71–3.80 (m, 2H), 3.85–3.91 (m, 1H), 4.17–4.26 (m, 2H), 4.84 (t, $J = 5.1$ Hz, 1H), 5.32 (m, 2H); ^{13}C NMR (CD_3OD) δ 12.84, 21.86, 23.33, 26.35, 28.45, 28.54, 28.71, 28.81, 28.88, 28.98, 31.16, 31.76, 32.72, 33.16, 38.22, 64.46, 64.52, 66.37, 67.49, 75.04, 75.16, 104.39, 128.97, 129.07; MS (ESI) *m/z* 419 [M−H]; ^{31}P NMR(CD_3OD) δ 20.28.

30. Durgam, G. G.; Virag, T.; Walker, M. D.; Tsukahara, R.; Yasuda, S.; Liliom, K.; van Meeteren, L. A.; Moolenaar, W. H.; Tigyi, G.; Miller, D. D. *J. Med. Chem.* **2005**, *48*, 4919.
31. van Meeteren, L. A.; Ruurs, P.; Christodoulou, E.; Goding, J. W.; Takakusa, H.; Kikuchi, K.; Perrakis, A.; Nagano, T.; Moolenaar, W. H. *J. Biol. Chem.* **2005**, *280*, 21155.
32. Esterbauer, H.; Schaur, R. J.; Zollner, H. *Free Radical Biol. Med.* **1991**, *11*, 81.
33. Zhang, C.; Baker, D. L.; Yasuda, S.; Makarova, N.; Balazs, L.; Johnson, L. R.; Marathe, G. K.; McIntyre, T. M.; Xu, Y.; Prestwich, G. D.; Byun, H. S.; Bittman, R.; Tigyi, G. *J. Exp. Med.* **2004**, *199*, 763.