Analogues of Platelet Activating Factor. 6. Mono- and Bis-Aryl Phosphate Antagonists of Platelet Activating Factor

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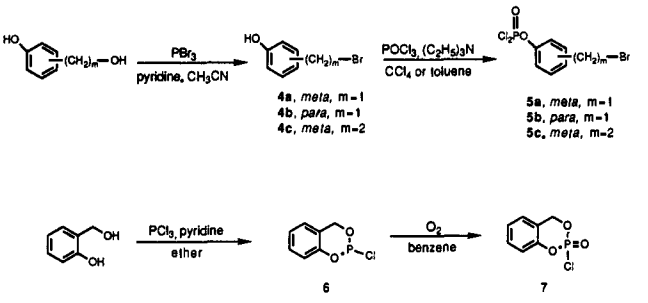
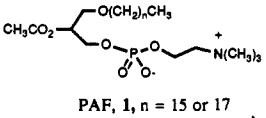
A series of aryl phosphoglyceride (3, 19–61) and bis-aryl phosphate (67–135) antagonists of platelet activating factor (PAF) were prepared. A group of four bifunctional phosphorus reagents (5a–c and 7) were developed that allowed the preparation of these aryl phosphates in which the position of aromatic substitution can be varied. These compounds were examined for their ability to inhibit PAF-induced platelet aggregation of rabbit platelets. Selected compounds were also evaluated for their ability to displace [^{3}H]PAF from its receptor on rabbit platelets. These in vitro data were compared to similar data obtained for a number of known PAF antagonists. The compounds were evaluated in vivo, in both the mouse and rabbit, for their ability to prevent death induced by a lethal challenge of PAF. The relationships between the biological activity and the nature, lipophilicity, and position of substituents of the aromatic rings were studied. Compound 105 (CL 184005) has been selected to undergo further development as a potential therapeutic agent for the treatment of septic shock in man.

Platelet activating factor (PAF, 1) is a naturally occurring alkyl ether phospholipid that was first described by Benveniste in 1972.[1] The structure determination of this substance followed seven years later.[2] Over the ensuing years, a vast amount of information has become available concerning the role of PAF in a number of pathological conditions. Evidence has accumulated that implicates PAF as a mediator in asthma, septic (endotoxic) shock, and gastric ulceration.[3] It is therefore possible that an antagonist to this substance may prove useful in the treatment of these and other inflammatory diseases. Because of this, a number of groups have been engaged in the design and preparation of PAF antagonists with the result that quite a few antagonists of varying structural type have been described.[4]

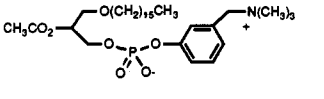
In an earlier investigation,[5] we studied the effects of structural modifications of the phosphocholine portion of PAF on the ability of the resulting molecules to aggregate platelets and to lower blood pressure. In the course of this study, we made the interesting observation that racemic 2 is essentially equivalent to racemic C16-PAF (1, n = 15) with respect to its agonist properties. At the time, we were aware of the structure of Takeda's PAF antagonist CV-3988, the first specific antagonist to appear in the literature.[6] It occurred to us that it would be of interest to combine the novel feature of 2, namely the meta-substituted aromatic moiety, with some of the structural features of the Takeda antagonist, in particular those that we felt were important for antagonist activity. Our expectation was that the resulting compound would be at least as potent as CV-3988 in PAF antagonist assays. The first compound of this type, 3, combined our meta-substituted aromatic spacer with the thiazolium and methoxy groups of CV-3988, and at the same time, retained the lipophilic alkoxy chain as in PAF. We were pleased to find that 3 is actually about 20 times more potent than CV-3988 in inhibiting PAF induced platelet aggregation. We therefore decided to further explore this series of monoaryl phosphoglycerides; this has resulted in the preparation of the compounds listed in Tables II–IV.

Scheme I

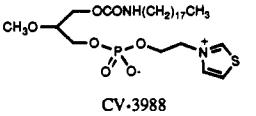




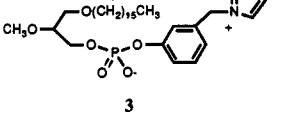
PAF, 1, n = 15 or 17



2



CV-3988



3

(1) Benveniste, J.; Henson, P. M.; Cochrane, C. G. Leukocyte-dependent histamine release from rabbit platelets. The role of IgE, basophils, and a platelet-activating factor. *J. Exp. Med.* **1972**, *136*, 1356–1377.

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(3) For some recent reviews of the biological properties of PAF and its involvement in human disease see: (a) *Platelet Activating Factor and Human Disease*; Barnes, P. J., Page, C. P., Henson, P. M., Eds.; Blackwell Scientific: Oxford, 1989. (b) Page, C. P. *Developments in Asthma. A View of Current Research*; PJB Publications: Richmond, 1987. (c) Feuerstein, G.; Siren, A.-L. Platelet-activating factor and shock. *Prog. Biochem. Pharmacol.* **1988**, *22*, 181–190. (d) Braquet, P.; Touqui, L.; Shen, T. Y.; Vargaftig, B. B. Perspectives in platelet-activating factor research. *Pharmacol. Rev.* **1987**, *39*, 97–145.

(4) For reviews see: (a) Handley, D. A. Development and therapeutic indications for PAF receptor antagonists. *Drugs Future* **1988**, *13*, 137–152. (b) Chang, M. N. PAF and PAF antagonists. *Ibid.* **1986**, *11*, 869–875. (c) Also see: Miyazaki, H.; Nakamura, N.; Ito, T.; Sada, T.; Oshima, T.; Koike, H. Synthesis and antagonistic activities of enantiomers of cyclic platelet-activating factor analogs. *Chem. Pharm. Bull.* **1989**, *37*, 2391–2397 and references therein. (d) *Handbook of PAF and PAF Antagonists*, Braquet, P., Ed.; CRC Press: Boca Raton, 1991.

(5) Wissner, A.; Schaub, R. E.; Sum, P.-E.; Kohler, C. A.; Goldstein, B. M. Analogs of platelet activating factor. 4. Some modifications of the phosphocholine moiety. *J. Med. Chem.* **1986**, *29*, 328–333.

(6) Terashita, Z.; Tsushima, S.; Yoshioka, Y.; Nomura, H.; Inada, Y.; Nishikawa, N. CV-3988 – a specific antagonist of platelet activating factor (PAF). *Life Sci.* **1983**, *32*, 1975–1982.

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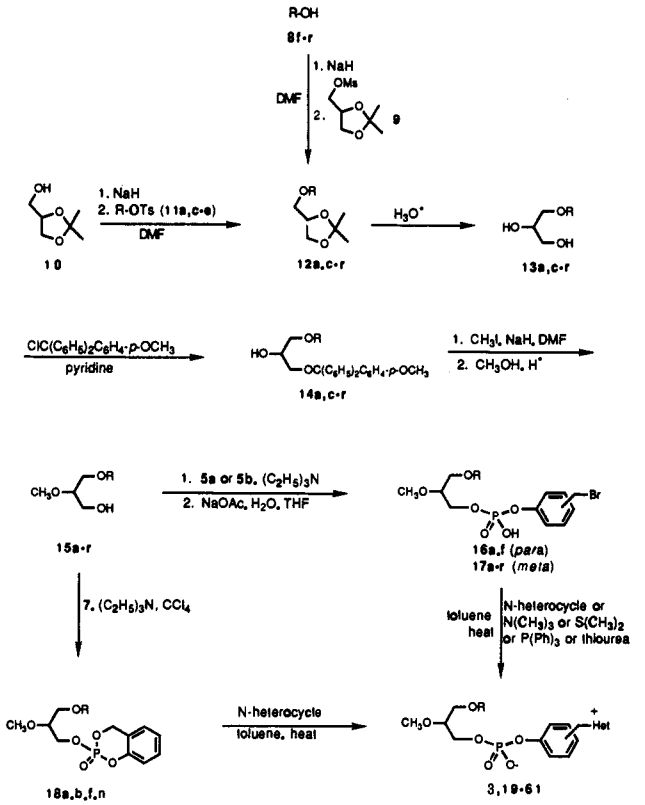
It is well known that the biological properties of PAF are highly stereospecific;[7] only the *R* enantiomer shows significant biological activity. In contrast to this, there has appeared a number of reports that describe glycerol-based types, as well as other structural types, of PAF antagonists in which the configuration of the compound has little influence on the antagonist properties.[8] In addition, a compound has been described in which the stereocenter has been completely removed by replacing it with a methylene unit; nevertheless, this compound still retains significant antagonist activity.[9] These literature observations suggested to us that since an asymmetric center with a defined configuration is not a requirement for antagonist activity, that perhaps we could dispense with the glycerol backbone all together. We therefore decided to replace the glycerol backbone of our aryl phosphoglyceride antagonists such as **3** with a simple aromatic backbone. This has resulted in the series of bis-aryl phosphates listed in Tables V and VI that also display excellent PAF antagonist activity.

In this report we would like to describe the relationships between structure and PAF antagonist activity that have been uncovered for these mono- and bis-aryl phosphate derivatives. Furthermore, we would like to point out that on the basis of the studies reported herein and on the basis of additional studies involving models of septic (endotoxic) shock that will be reported at a future date, one of the compounds described in this discourse, namely compound **105** (CL 184005), has been chosen to undergo further development as a potential therapeutic agent for the treatment of septic shock in man.

Chemistry

Three bifunctional phosphorus reagents **5a,b** and **7** (Scheme I) were developed which allow the preparation of antagonists in which the position of the aromatic substitution can be varied from meta, to para, to ortho, respectively. In addition, **5c** (a homologue of **5a**) allows the preparation of a compound in which the distance of the charged heterocycle from the aromatic ring is increased.

Scheme II



	R
a	$C_{16}H_{33}$
b	$CONHC_{18}H_{37}$
c	$(CH_2)_2O(CH_2)_2O(CH_2)_9CH_3$
d	$(CH_2)_8CH{=}CHCH_2CH{=}CH(CH_2)_4CH_3$
e	C_5H_{11}

R =

	2	3	4
f	H	$C_{14}H_{29}$	H
g	H	H	$C_{14}H_{29}$
h	H	$OC_{10}H_{21}$	H
i	H	$OC_{12}H_{25}$	H
j	H	$OC_{14}H_{29}$	H
k	H	$OC_{16}H_{33}$	H
l	$C_{16}H_{33}$	H	H
m	H	H	$OC_{16}H_{33}$
n	CH_3	$OC_{12}H_{25}$	H
o	CH_3	$OC_{16}H_{33}$	H
p	H	H	$OCH_2C_6H_5$
q	H	$OCH_2C_6H_5$	H
r			$OC_{12}H_{25}$

The synthesis of **5a** has been described.[5] The reagent **5b** needed to synthesize the para-substituted compounds was prepared in a similar manner from the bromophenol **4b** by the reaction of **4b** with phosphorus oxychloride in toluene. In contrast to **4a**, precautions had to be taken in handling **4b** because of its greater instability. The instability of **4b** presumably is the result of a facile elimination of HBr and the formation of *p*-quinone methide; **4b** is best stored in solution in the cold.

The cyclic chlorophosphate **7** needed to synthesize the ortho-substituted antagonists was prepared by the reaction of 2-hydroxybenzyl alcohol with phosphorus trichloride and pyridine in ether to give **6** followed by oxidation with molecular oxygen in benzene.

The antagonists that have a glycerol backbone (Tables II–IV) are prepared as outlined in Scheme II. The syn-

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(8) (a) Nishikawa, N.; Terashita, Z.; Yoshioka, Y.; Tsushima, S. Discovery of a specific PAF antagonist CV-3988, and an analysis of the pathophysiological roles of PAF. *J. Takeda Res. Lab.* **1987**, *46*, 1–19. (b) Terashita, Z.; Imura, Y.; Takatani, M.; Tsushima, S.; Nishikawa, N. CV-6209, a highly potent antagonist of platelet activating factor in vitro and in vivo. *J. Pharmacol. Exp. Ther.* **1987**, *242*, 263–268. (c) Miyamoto, T.; Ohno, H.; Yano, H.; Okada, T.; Hamanaka, N.; Kawasaki, A. ONO-620: A new potent antagonist of platelet-activating factor. *Adv. Prostaglandin Thromboxane Leukotriene Res.* **1985**, *15*, 719–720. (d) Takatani, M.; Yoshioka, Y.; Tasaka, A.; Terashita, Z.; Imura, Y.; Nishikawa, K.; Tsushima, S. Platelet activating factor (PAF) antagonists: synthesis and structure-activity studies of novel PAF analogs modified in the phosphorylcholine moiety. *J. Med. Chem.* **1989**, *32*, 56–64. (e) Corey, E. J.; Chen, Chung Pin; Parry, M. J. Dual binding modes to the receptor for platelet activating factor (PAF) of anti-PAF trans-2,5-diarylfurans. *Tetrahedron Lett.* **1988**, *29*, 2899–2902. (f) Handley, D. A.; Anderson, R. C.; Saunders, R. N. Inhibition by SRI 63-072 and SRI 63-119 of PAF-acether and immune complex effects in the guinea pig. *Eur. J. Pharmacol.* **1987**, *141*, 409–416.

(9) Grue-Soerensen, G.; Nielsen, I.; Nielsen, C. Derivatives of 2-methylenepropane-1,3-diol as new antagonists of platelet activating factor. *J. Med. Chem.* **1988**, *31*, 1174–1178.

thesis of the intermediates **12a,c–r** can be approached in one of two ways. For those compounds that contain a phenoxy group attached to the 1-position of the glycerine moiety, alkylation of the mesylate of solketal **9** with the appropriate phenol **8f–r** using sodium hydride in dimethylformamide results in **12f–r**. Alternatively, for the non-phenoxy-containing compounds, alkylation of the tosylates **11a,c–e** with **10** under similar conditions results in the ketals **12a,c–e**, respectively. Acid-catalyzed hydrolysis of **12a,c–r** then furnishes the diols **13a,c–r**. The preparation of diols **13a**,[10] **13c**,[11] and **13f,g**[12] have already been described as has the preparation of **15b**.[8c]

The primary hydroxyl group of diols **13a,c–r** were selectively protected as the *p*-methoxytrityl derivatives using *p*-methoxytrityl chloride in pyridine. The protected compounds **14a,c–r** were then alkylated with methyl iodide (or in one case with ethyl iodide) to give, after solvolytic removal of the protecting group in methanol, the intermediates **15a,c–r**. The aromatic phosphate groups were introduced by reacting the alcohols **15a–r** with reagents **5a** or **5b** and triethylamine in carbon tetrachloride followed by hydrolysis to give **17a–r** in the meta series and **16a,f** in the para series, respectively. Introduction of the positively charged group was accomplished by alkylating **17a–r** or **16a,f**, with a nitrogen-containing heterocycle, and in the case of **17a**, with trimethylamine, dimethyl sulfide, triphenylphosphine, or various thioureas in toluene at 65–70 °C for about 18 h to give the antagonists of the meta series **3, 21, 23, 24, 26, 29,** and **31–61** and the antagonists of para series **20, 25,** and **27**, respectively.

The aryl phosphoglyceride antagonists in the ortho series were prepared by the reaction of **15a,b,f,n** with reagent **7** and triethylamine in carbon tetrachloride to give the cyclic phosphates **18a,b,f,n**. The reaction of these cyclic phosphates with thiazole is somewhat more sluggish compared to the reaction of the benzyl bromides and requires heating in toluene at 80–90 °C for 3 days to furnish to ortho series antagonists **19, 22, 28,** and **30**.

Table I. Inhibition of PAF-Induced Platelet Aggregation: Literature Standards

compound	inhibition of platelet agg: IC_{50} (μM)[a]	ref
CV-3988	25.9 (n = 4)	6
CV-6209	0.02 (n = 3)	8b
triazolam	11.1 (n = 3)	13
alprazolam	41.2 (n = 2)	13
WEB-2086	0.34 (n = 6)	14
kadsurenone	3.3 (n = 2)	15
L-652731	1.6 (n = 3)	16
SRI-63072	16.7 (n = 1)	17
SRI-63441	2.1 (n = 1)	18

[a] Concentration needed to inhibit PAF-induced platelet aggregation in rabbit PRP by 50%; the PAF challenge concentration was 5.0E^{-8} M; the n value in parentheses is the number of determinations.

Although all the antagonists described herein which contain a glycerol backbone have been prepared in racemic form, the syntheses described above can (in principle) be applied to the preparation of compounds with either the *R* or *S* configuration by starting the synthesis with optically active **10**, both enantiomers of which are readily available commercial products.

The bis-aryl phosphate antagonists (Tables V and VI) are prepared as shown in Scheme III. The synthesis starts with the preparation of an appropriately substituted phenol. The phenols that contain a lipophilic alkoxy chain are prepared from the corresponding dihydroxybenzenes by alkylation of the diol with 1 equiv of an alkyl bromide in dimethylformamide using sodium hydride and a catalytic amount of sodium iodide. As might be expected, the usual outcome of this reaction is a near-statistical mixture composed of the desired phenol **63**, the dialkylated product **64**, and recovered starting phenol **62**. Fortunately, there is a wide variation in the physical properties of the components of these mixtures, and therefore the desired monoalkylated products **63** can usually be separated on a large scale by utilizing a combination of Kugelrohr distillation under reduced pressure and recrystallization or chromatography. By this method, the phenols **63a–e,g–j,l–i′,o′,r′,s′** were prepared. The preparation of **63f** has been described.[12] The preparations of **63k,j′** are described in the Experimental Section. The remaining phenols are commercially available.

The reaction of phenols **63a–s′** with phosphorus reagents **5a** and **5c** utilizing triethylamine in carbon tetrachloride followed by hydrolysis gives the intermediates **64a–s′** and **65o** in the meta series. Introduction of the positively charged group into the molecule to give the desired antagonists **67, 69–85, 87, 89–96,** and **98–135** was accomplished by heating these intermediates with the desired heterocycle (or in one case with a triethylamine) in toluene at 70–80 °C overnight. As might be expected, the reaction of **65o** is much more sluggish compared to the benzyl halides **64a–s′** and requires heating for 6 days to give **86**, a compound in which the distance between the charged heterocycle and the aromatic ring is extended by one methylene unit.

The antagonists that contain the ortho substitution pattern in the right hand aromatic ring (**68, 88,** and **97**) were prepared by the reaction of phenols **63a,o,v** with reagent **7** in the presence of triethylamine. The resulting cyclic phosphate intermediates **66a,o,v** were alkylated with the desired heterocycle by heating in acetonitrile con-

(10) Stoffel, W.; Le Kim, D. Biosynthesis of plasmalogens. Precursors in the biosynthesis of plasmalogens. Stereospecificity of the biochemical dehydrogenation of the 1-0-alkylglyceryl to the 1-0-alk-1′-enylglyceryl ether bond. *Hoppe-Seyler's Z. Physiol. Chem.* 1971, *352*, 501–511.
(11) Wissner, A.; Kohler, C. A.; Goldstein, B. M. Analogs of platelet activating factor. 5. Multiple oxygen substitution of the alkoxy chain. *J. Med. Chem.* 1986, *29*, 1315–1319.
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(13) Kornecki, E.; Ehrlich, Y. H.; Lenox, R. H. Platelet-activating factor-induced aggregation of human platelets specifically inhibited by triazolobenzodiazepines. *Science* 1984, *226*, 1454–1456.
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(16) Biftu, T.; Gamble, N. F.; Doebber, T. W.; Hwang, S.-B.; Shen, T.-Y.; Snyder, J. Conformation and activity of tetrahydrofuran lignans and analogs as specific platelet-activating-factor antagonists. *J. Med. Chem.* 1987, *29*, 1917–1921.
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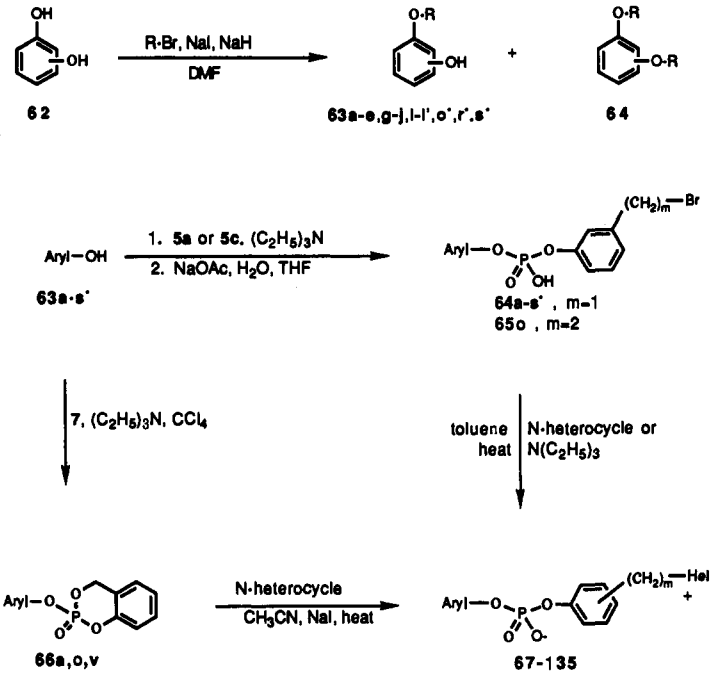
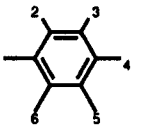
(18) Handley, D. A.; Tomesch, J. C.; Saunders, R. N. Inhibition of PAF-induced systemic responses in the rat, guinea pig, dog, and primate by the receptor antagonist SRI 63-441. *Thromb. Haemostasis* 1986, *56*, 40–44.

Scheme III



Aryl =



	2	3	4	5	6		2	3	4	5	6
a	H	$OC_{16}H_{33}$	H	H	H	a'	Cl	H	$OC_{14}H_{29}$	Cl	H
b	H	$OC_{14}H_{29}$	H	H	H	b'	H	$OC_{14}H_{29}$	Cl	H	Cl
c	H	$OC_{12}H_{25}$	H	H	H	c'	CO_2CH_3	$OC_{14}H_{29}$	H	H	H
d	H	H	$OC_{16}H_{33}$	H	H	d'	$COCH_3$	$OC_{14}H_{29}$	H	H	H
e	$OC_{14}H_{29}$	H	H	H	H	e'	$COCH_3$	H	H	$OC_{14}H_{29}$	H
f	H	$C_{14}H_{29}$	H	H	H	f'	$COCH_3$	H	$OC_{14}H_{29}$	H	H
g	CH_3	$OC_{16}H_{33}$	H	H	H	g'	H	$OC_{14}H_{29}$	H	$COCH_3$	H
h	CH_3	CH_3	$OC_{14}H_{29}$	H	CH_3	h'	COC_6H_5	H	H	$OC_{14}H_{29}$	H
l	H	CH_3	$OC_{14}H_{29}$	CH_3	CH_3	l'	$CO(CH_2)_4CH_3$	H	$OC_{12}H_{25}$	H	H
j	C_2H_5	H	$OC_{14}H_{29}$	H	H	j'	$COCH_3$	H	$C_{14}H_{29}$	H	H
k	C_2H_5	H	$C_{14}H_{29}$	H	H	k'	H	H	Cl	H	H
l	C_6H_5	H	$OC_{14}H_{29}$	H	H	l'	Cl	H	H	H	H
s	H	C_6H_5	$OC_{14}H_{29}$	H	H	s'	H	Cl	H	H	H
n	OCH_3	$OC_{12}H_{25}$	H	H	H	n'	H	C_6H_5	H	H	H
o	OCH_3	$OC_{14}H_{29}$	H	H	H	o'	H	H	$OCH_2C_6H_5$	H	H
p	OCH_3	$OC_{14}H_{29}$	H	Br	H	p'	H	$C(CH_3)_3$	H	$C(CH_3)_3$	H
q	OCH_3	H	$OC_{14}H_{29}$	H	H	q'	H	H	$C(CH_3)_3$	H	H
r	H	OCH_3	$OC_{14}H_{29}$	H	H	r'	H	$C(CH_3)_3$	$OC_{12}H_{25}$	H	H
s	OCH_3	R'[a]	H	H	H	s'	H	$OC_{14}H_{29}$	H	OCH_3	H
t	OCH_3	R''[b]	H	H	H						
u	H	$OC_{12}H_{25}$	$C(CH_3)_3$	H	$C(CH_3)_3$						
v	$C(CH_3)_3$	H	$OC_{12}H_{25}$	$C(CH_3)_3$	H						
w	$C(CH_3)_3$	H	$OC_{12}H_{25}$	H	H						
x	Cl	H	$OC_{14}H_{29}$	H	H						
y	H	Cl	$OC_{14}H_{29}$	H	H						
z	Cl	Cl	$OC_{14}H_{29}$	Cl	Cl						

[a] R' = $O(CH_2)_2O(CH_2)_2O(CH_2)_7CH_3$ [b] R'' = $O(CH_2)_6O(CH_2)_2O(CH_2)_2OCH_3$

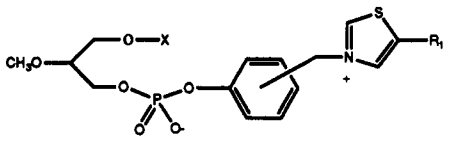
taining a catalytic amount of sodium iodide at 85 °C for 19 h.

Biology

The test compounds were evaluated for their PAF antagonist properties both in vitro and in vivo. In one assay, we examined their ability to inhibit PAF-induced platelet aggregation in rabbit platelet rich plasma (PRP). The data are expressed as a molar IC_{50}, the concentration of antagonist needed to inhibit platelet aggregation induced by a standard challenge concentration (usually 5.0×10^{-8} M) of PAF by 50%. Multiple determinations of the IC_{50} values were averaged to give the values shown in Tables I–VI.

In order to facilitate the comparison of the new compounds presented herein with other antagonists in the

Table II. Inhibition of PAF-Induced Platelet Aggregation: Aryl Phosphoglycerides. Influence of the Lipophilicity of the Alkyl Chain and Variation of the Position of Aromatic Substitution



compd	X	aromatic substn	R_1	inhibn of platelet agg: IC_{50} (μM)[a]	formula[c]	anal.[d]
3	$C_{16}H_{33}$	meta	H	1.21 (n = 4)	$C_{30}H_{50}NO_6PS(1.5H_2O)$	C,H,N,P,S
19	$C_{16}H_{33}$	ortho	H	5.90 (n = 1)	$C_{30}H_{50}NO_6PS$	N,P,S;C[g],H[h]
20	$C_{16}H_{33}$	para	H	3.91 (n = 2)	$C_{30}H_{50}NO_6PS(1.25H_2O)$	C,H,N,P,S
21	$CONHC_{16}H_{37}$	meta	H	0.92 (n = 4)	$C_{33}H_{55}N_2O_7PS(0.5H_2O)$	C,H,N,P,S
22	$CONHC_{18}H_{37}$	ortho	H	0.61 (n = 7)	$C_{33}H_{55}N_2O_7PS$	C,N,P;H[i],S[j]
23	$CONHC_{18}H_{37}$	meta	CH3	0.17 (n = 4)	$C_{34}H_{57}N_2O_7PS(1.0H_2O)$	C,H,N,P,S
24	$C_{16}H_{33}$	meta	CH3	0.68 (n = 5)	$C_{31}H_{52}NO_6PS(0.75H_2O)$	C,H,N,P,S
25	$C_{16}H_{33}$	para	CH3	68.0 (n = 1)[b]	$C_{31}H_{52}NO_6PS(1.5H_2O)$	C,N,P,S;H[k]
26	C_6H_4-m-$C_{14}H_{29}$	meta	H	0.65 (n = 5)	$C_{34}H_{50}NO_6PS(0.5H_2O)$	C,H,N,P,S
27	C_6H_4-m-$C_{14}H_{29}$	para	H	91.2 (n = 1)[b]	$C_{34}H_{50}NO_6PS(1.25H_2O)$	C,H,N,P,S
28	C_6H_4-m-$C_{14}H_{29}$	ortho	H	5.73 (n = 1)[b]	$C_{34}H_{50}NO_6PS(1.0H_2O)$	C,H,N,P,S
29	C_6H_3-o-CH_3-m-$OC_{12}H_{25}$	meta	H	2.89 (n = 7)	$C_{33}H_{48}NO_7PS(1.0H_2O)$	C,H,N,P,S
30	C_6H_3-o-CH_3-m-$OC_{12}H_{25}$	ortho	H	3.30 (n = 1)	$C_{33}H_{48}NO_7PS$	H,N,S;C[l],P[m]
31	C_5H_{11}	meta	H	NA (n = 1)[f]	$C_{19}H_{28}NO_6PS(0.75H_2O)$	C,H,N,P,S
32	$((CH_2)_2O)_2(CH_2)_9CH_3$	meta	H	6.97 (n = 1)	$C_{28}H_{48}NO_6PS(0.5H_2O)$	C,H,N,P,S
33	$((CH_2)_2O)_2(CH_2)_9CH_3$	meta	CH3	4.05 (n = 2)	$C_{28}H_{48}NO_6PS(1.0H_2O)$	C,H,N,P,S
34	R'[e]	meta	H	6.40 (n = 1)	$C_{32}H_{50}NO_6PS(4.0H_2O)$	C,N,P,S;H[n]

[a] Concentration needed to inhibit PAF-induced platelet aggregation in rabbit PRP by 50%; unless indicated, the PAF challenge concentration was 5.0E^{-8} M; the n value in parentheses is the number of determinations. [b] PAF challenge concentration was 1.0E^{-7} M. [c] Empirical formula with amount of water of hydration. All compounds showed the expected M + H ion in the FAB mass spectrum. [d] Analytical results for the indicated elements are within ±0.4% of the calculated values, unless indicated otherwise. [e] R' = E,E-$(CH_2)_8CH{=}CHCH_2CH{=}CH(CH_2)_4CH_3$. [f] No inhibition observed at a compound concentration up to 10 μM. [g] C: calcd, 61.73; found, 58.33. [h] H: calcd, 8.63; found, 8.22. [i] H: calcd, 8.57; found, 7.90. [j] S: calcd, 4.69; found, 5.25. [k] H: calcd, 8.87; found, 8.38. [l] C: calcd, 62.54; found, 57.76. [m] P: calcd, 4.89; found, 5.74. [n] H: calcd, 8.59; found, 7.71.

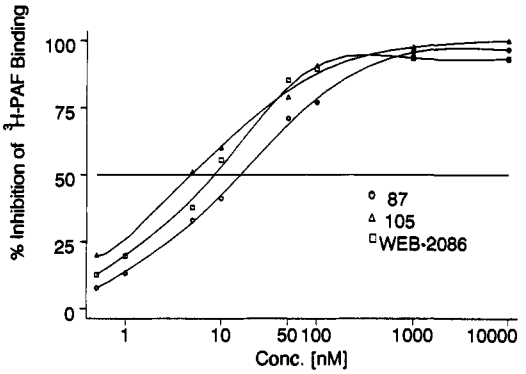


Figure 1. Inhibition of [^{3}H]PAF binding to rabbit platelet receptor by compounds **87** (CL 181919), **105** (CL 184005), and WEB-2086. Specific binding (SB) was calculated as described in the Experimental Section. [^{3}H]PAF concentration was 2.5 nM, and specific binding was >10 000 cpm in the absence of displacer, and ranged from <1000 to 10 000 cpm in the presence of various concentrations of test compounds. Percent inhibition values were calculated with the equation %ihb = 100 × ($SB_{control}$ − SB_{cmpd})/$SB_{control}$. Calculated IC_{50} values are 19.4 nM for **87**, 9.1 nM for WEB-2086, and 5.6 nM for **105**.

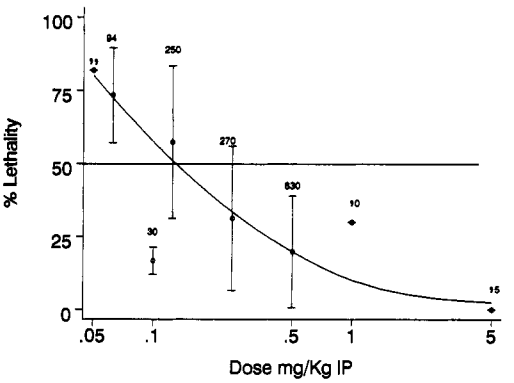


Figure 2. Dose–response relationship for the protective ability of **105** (CL 184005) against PAF-induced lethality in mice. Using the same protocol for mouse PAF-induced lethality as described in the Experimental Section, compound **105** was given at dose range 0.05–5.0 mg/kg. The values shown are the mean lethality ±SD for each dose. Under these conditions **105** shows an ED_{50} of 0.13 mg/kg. The data shown in Table VII is a subset of this data.

literature, we have included similar data generated in our laboratory for a number of these antagonists (see Table I). For comparisons of our IC_{50} values with those in the literature to be meaningful, it is important to note the PAF challenge concentrations used and whether washed platelets or, as is the case in this study, PRP was used. We generally find that the IC_{50} values are about 10-fold lower when washed platelets are used.

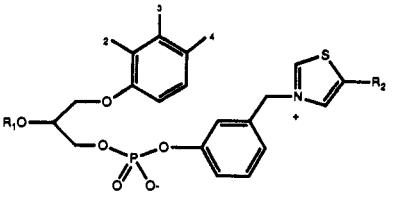
To further examine PAF antagonism at the receptor site, a few selected compounds (**87**, **105**, and WEB-2086) were evaluated for their ability to inhibit binding of [^{3}H]PAF to its receptor located on rabbit platelets. The competitive displacement curves and the calculated IC_{50} values are shown in Figure 1.

For selected compounds, we also evaluated their potential for PAF antagonism in vivo. We evaluated the compounds for their ability to prevent death resulting from a lethal challenge of PAF in both the mouse and rabbit. These results are presented in Table VII. A full dose–response curve of the mouse data for one of the compounds, **105** (CL 184005), is shown in Figure 2.

Results and Discussion

In Table II, which encompasses the monoaryl phosphate antagonists having a glycerine backbone, one of the questions we address concerns the lipophilicity of the group in the 1-position of the glycerine. It is quite clear that a highly lipophilic substituent in the 1-position is essential for antagonist activity in this series of compounds since shortening the chain length from C_{16} to C_5 (**3** and **31**, re-

Table III. Inhibition of PAF-Induced Platelet Aggregation: Aryl Phosphoglycerides Having an Additional Aromatic Substituent in the 1-Position



compd	aromatic substituents 2	3	4	R_1	R_2	inhibn of platelet agg: IC_{50} (μM)[a]	formula[c]	anal.[d]
35	H	$OC_{12}H_{25}$	H	CH_3	H	1.62 (n = 5)	$C_{32}H_{46}NO_7PS(1.3H_2O)$	C,H,N,P,S
36	H	$OC_{14}H_{29}$	H	CH_3	H	2.60 (n = 1)	$C_{34}H_{50}NO_7PS(1.0H_2O)$	C,H,N,P,S
37	H	$OC_{16}H_{33}$	H	CH_3	H	9.08 (n = 2)	$C_{36}H_{54}NO_7PS(0.5H_2O)$	C,H,N,P,S
38	$C_{16}H_{33}$	H	H	CH_3	H	21.5 (n = 1)[b]	$C_{36}H_{54}NO_6PS(2.1H_2O)$	C,N,P,S;H[f]
39	H	H	$OC_{16}H_{33}$	CH_3	H	5.20 (n = 1)	$C_{36}H_{54}NO_7PS(1.0H_2O)$	C,H,N,P,S
26	H	$C_{14}H_{29}$	H	CH_3	H	0.65 (n = 5)	$C_{34}H_{50}NO_6PS(0.5H_2O)$	C,H,N,P,S
40	H	H	$C_{14}H_{29}$	CH_3	H	2.85 (n = 1)	$C_{34}H_{50}NO_6PS(1.0H_2O)$	C,H,N,P,S
29	CH_3	$OC_{12}H_{25}$	H	CH_3	H	2.67 (n = 7)	$C_{33}H_{46}NO_7PS(1.0H_2O)$	C,H,N,P,S
41	CH_3	$OC_{12}H_{25}$	H	CH_3	CH_3	4.70 (n = 2)[b]	$C_{34}H_{50}NO_7PS(1.0H_2O)$	C,H,N,P,S
42	CH_3	$OC_{16}H_{33}$	H	CH_3	H	1.79 (n = 2)	$C_{37}H_{56}NO_7PS(0.75H_2O)$	C,H,N,P,S
43	CH_3	$OC_{12}H_{25}$	H	C_2H_5	CH_3	0.23 (n = 1)	$C_{35}H_{52}NO_7PS(0.5H_2O)$	C,H,N,P,S
44	H	$OC_{12}H_{25}$	H	CH_3	CH_3	1.57 (n = 2)	$C_{33}H_{48}NO_7PS(1.0H_2O)$	C,H,N,P,S
45	H	$OC_{14}H_{29}$	H	CH_3	CH_3	4.08 (n = 2)[b]	$C_{35}H_{52}NO_7PS(0.75H_2O)$	C,H,N,P;S[g]
46	H	$OCH_2C_6H_5$	H	CH_3	H	NA (n = 1)[e]	$C_{27}H_{28}NO_7PS(1.3H_2O)$	C,H,N,P,S
47	H	H	$OCH_2C_6H_5$	CH_3	H	14.9 (n = 2)	$C_{27}H_{28}NO_7PS(1.0H_2O)$	C,H,N,P,S
48			$OC_{12}H_{25}$	CH_3	H	3.80 (n = 1)	$C_{36}H_{48}NO_7PS(0.75H_2O)$	C,H,N,P,S

[a] Concentration needed to inhibit PAF-induced platelet aggregation in rabbit PRP by 50%; unless indicated, the PAF challenge concentration was $5.0E^{-8}$ M; the n value in parentheses is the number of determinations. [b] PAF challenge concentration was $1.0E^{-7}$ M. [c] Empirical formula with amount of water of hydration. All compounds showed the expected M + H ion in the FAB mass spectrum. [d] Analytical results for the indicated elements are within ±0.4% of the calculated values, unless indicated otherwise. [e] No inhibition observed at a compound concentration up to 10 μM. [f] H: calcd, 8.41; found, 7.95. [g] S: calcd, 4.75; found, 5.35.

spectively), completely eliminated the antagonist activity. In a related observation, decreasing the lipophilicity of the 1-substituent by incorporating additional oxygen atoms within the chain, yet keeping the chain length constant, also leads to a decrease in potency (compared **3** with **32** and **24** with **33**). A similar reduction in potency resulting from oxygen incorporation into the lipophilic chain was reported earlier by us for a series of PAF agonists.[11] Additionally, the unsaturated compound **34** s about 5 times less active then the fully saturated C_{16} compound **3**. It is also clear that compounds that contain a C_{18} carbamate group in the 1-position show better activity than the corresponding compounds with a simple alkyl chain (compare **3** with **21**, **19** with **22**, and **23** with **24**).

In Table II, we also address the question concerning the position of aromatic substitution in the aryl phosphate ring. There does not appear to be any overruling preference for a particular substitution pattern. For the compounds containing a simple alkyl group in the 1-position (**3**, **19**, and **20**), there seems to be a 5-fold preference for meta substitution compared to ortho substitution with para substitution resulting in intermediate potency. In contrast to this, the meta and ortho carbamates **21** and **22**, respectively, have nearly identical potency as do the meta and ortho compounds **29** and **30**. For the series isomeric compounds **26–28**, a full comparison is not possible since the compounds were tested using different PAF challenge concentrations.

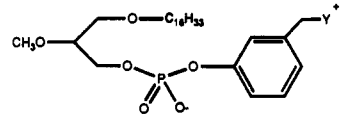
Table III contains the inhibition of PAF-induced platelet aggregation data for a series of monoaryl phosphoglycerides that also have an additional aromatic ring located as a substituent at the 1-position of the glycerine. For best antagonist activity, this additional aromatic ring must have a substituent with sufficient lipophilicity; the compounds with the smaller benzyloxy groups **46** and **47** show either no activity or, at best, low potency compared to the compounds that have more lipophilic alkoxy chains **35–37**. Within the homologous series **35–37**, it appears that the best activity is observed for the C_{12} homologue which is about 5 times more potent then the C_{16} homologue. Removal of the oxygen atom, resulting in an alkyl substituent, yields an improvement in the antagonist activity (compare **26** with **36**). With respect to the position of aromatic substitution on the ring located at the 1-position, there does not appear to be clear preference for a particular substitution pattern; for example, in comparing **37** and **39**, we find about a 2-fold difference in activity in favor of the para isomer **39** and conversely, comparing **26** and **40**, there is about a 4-fold preference for the meta isomer **26**.

In Table IV, the nature of the charged moiety attached to the benzylic position and its effect on antagonist activity is shown. It is clear that it is important for the benzylic group to have an explicit positive charge for maximal activity; the neutral imidazole compound **57** is clearly less potent then its methylated counterpart **58**. Interestingly, compound **49** which has a trimethylammonium group, as do PAF and **2**, shows very poor antagonist activity. The sulfonium salt **50**, the phosphonium salt **51**, and the thiouronium salts **59–61** also show reduced potency compared to some of the heterocyclic-substituted compounds. With respect to the heterocyclic-substituted compounds, the thiazoles are usually consistent with the best activity; there appears to be a slight bias in favor of a 5-methylthiazolium over a thiazolium substituent (compare **3** with **24**, **21** with **23**, **32** with **33**, and **35** with **44**).

Comparing the activities of CV-3988 (Table I) and **21** clearly illustrates the importance of the aromatic ring that separates the phosphate from the charged group; removing this ring and replacing it with a two-carbon methylene bridge (as is present in PAF as well as CV-3988) decreases the activity almost 30-fold.

Of the aryl phosphoglycerides exemplified in Tables II–IV, the most potent antagonists are **22**, **23**, **24**, **26**, and **43** all of which have IC_{50} values under 1 μM. Thus, these

Table IV. Inhibition of PAF-Induced Platelet Aggregation: Aryl Phosphoglycerides. Variation of the Heteroadduct



compd	heteroadduct (Y)	inhibn of platelet agg: IC_{50} (μM)[a]	formula[b]	anal.[c]
49	$N(CH_3)_3$	351 (n = 1)	$C_{30}H_{56}NO_6P(1.0H_2O)$	C,H,N,P
50	$S(CH_3)_2$	12.9 (n = 1)	$C_{28}H_{53}O_6PS(0.75H_2O)$	C,H,P,S
51	$P(C_6H_5)_3$	13.6 (n = 1)	$C_{45}H_{63}O_6P_2Br(1.0H_2O)$[e]	C,H,P,Br
3	thiazole	1.21 (n = 4)	$C_{30}H_{50}NO_6PS(1.5H_2O)$	C,H,N,P,S
24	5-methylthiazole	0.68 (n = 5)	$C_{31}H_{52}NO_6PS(0.75H_2O)$	C,H,N,P,S
52	4-methylthiazole	6.67 (n = 1)	$C_{31}H_{52}NO_6PS(0.75H_2O)$	C,H,N,P,S
53	4,5-dimethylthiazole	1.31 (n = 1)	$C_{32}H_{54}NO_6PS(0.5H_2O)$	C,H,N,P,S
54	2-isobutylthiazole	5.30 (n = 1)	$C_{34}H_{58}NO_6PS$	C,H,N,P,S
55	pyridine	3.99 (n = 3)	$C_{32}H_{52}NO_6P(0.25H_2O)$	C,H,N,P
56	isoquinoline	23.1 (n = 1)	$C_{36}H_{54}NO_6P(3.75H_2O)$	C,N,P;H[f]
57	imidazole	157 (n = 1)	$C_{30}H_{51}N_2O_6P(1.0H_2O)$	P;C[g],H[h],N[i]
58	N-methylimidazole	9.32 (n = 1)	$C_{31}H_{58}N_2O_6P(1.8H_2O)$	C,H,N,P
59	1-methyl-2-thiourea	3.89 (n = 1)	$C_{29}H_{54}N_2O_6PSBr(0.5H_2O)$[e]	C,H,N,P,S,Br
60	1,3-dimethylthiourea	15.7 (n = 1)	$C_{30}H_{56}N_2O_6PSBr(1.0H_2O)$[e]	C,H,N,P,S,Br
61	1-phenyl-2-thiourea	d (n = 1)	$C_{34}H_{56}N_2O_6PSBr(0.5H_2O)$[e]	C,H,N,P,S,Br

[a] Concentration needed to inhibit PAF-induced platelet aggregation in rabbit PRP by 50%; the PAF challenge concentration was $5.0E^{-8}$ M; the n value in parentheses is the number of determinations. [b] Empirical formula with amount of water of hydration. All compounds showed the expected M + H ion in the FAB mass spectrum. [c] Analytical results for the indicated elements are within ±0.4% of the calculated values, unless indicated otherwise. [d] Only 22% inhibition at a dose of 10 μM. [e] Isolated as the HBr salt. [f] H: calcd, 8.91; found, 7.90. [g] C: calcd, 61.52; found, 60.99. [h] H: calcd, 9.29; found, 8.57. [i] N: calcd, 4.78; found, 5.27.

compounds compare quite favorably with the literature standards listed in Table I.

Tables V and VI list the data for inhibition of PAF-induced platelet aggregation for the series of bis-aryl phosphate antagonists. Table V addresses questions concerning the effects on activity of substituents and their relative positions on the aromatic rings. For the most part, we have added substituents at various positions only to the left hand aromatic ring. With respect to the right hand ring, we have varied the position of aromatic substitution of the benzylic group that attaches the charged heterocycle.

The importance of the lipophilicity of the chain on the left hand ring can be addressed by noting the homologous series consisting of compounds **67**, **69**, and **71**, for this series, we find a steady improvement in activity with increasing chain length with the extremes of the series differing in activity by a factor of 28. Also, for compounds that have an aromatic ring which does not have a long lipophilic chain, we find either low activity (see **117** and **119–121**) or none at all (see **115**, **116**, and **118**). Attaching the long lipophilic chain to the aromatic ring via an oxygen atom gives a more active compound than when the chain is attached directly via carbon (compared **69** and **75**). Inserting additional oxygen atoms into the chain, and thereby decreasing the lipophilicity of the chain, without altering the chain length, results in deceased activity (compare **92** and **93** with **87**), and in addition, this observed decrease in activity is greatest when the oxygen atoms are inserted near the end of the chain. We previously observed a similar dependence of activity on the degree and position of oxygen insertion in the lipophilic chain in a series of PAF agonists.[11]

Clearly, within the bis-aryl phosphate series, the position of aromatic substitution of the lipophilic chain is an important factor. While there does not appear to be any significant difference in activity between the 3- and 4-substituted isomers **67** and **72**, respectively, there is a prominent difference between the 2- and 3-substituted isomers **74** and **69**, respectively. Presumably, the large lipophilic chain ortho to the phosphate moiety precludes good binding to the receptor. With respect to the position of aromatic substitution on the right hand ring, the answers are less clear. While there is no significant difference between the meta compound **87** and its ortho counterpart **88**, there is an important difference (about 72-fold) between the isomeric pair **96** and **97** in favor of the meta substitution pattern. Unfortunately, an accurate comparison of the other isomeric pair **67** and **68** is not possible since these compounds were tested at different PAF challenge concentrations.

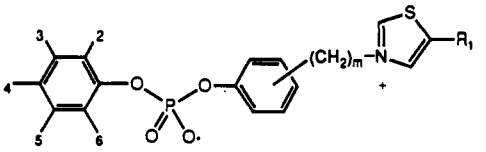
The number of methylene groups interposed between the charged heterocycle and the right hand aromatic ring is also a critical factor; increasing the number of methylene groups from one to two completely abolishes the antagonist activity (compare **86** with **87**).

We have prepared compounds that contain, in addition to a lipophilic chain, a variety of different substituents on the left hand aromatic ring. These substituents included both electron-releasing as well as electron-withdrawing functionalities. If compounds are examined in which the size and orientation of the lipophilic chain is kept constant, there is no obvious important difference between compounds that contain either electron-releasing or electron-withdrawing groups.

Compounds that contain multiple *tert*-butyl groups comprise the most active compounds of this series (see **95** and **96**); however, this activity depends strongly on the relative position of the substituents on the aromatic ring. This is illustrated dramatically in comparing **94**, a compound which is nearly inactive, with its positional isomer **95**, one of the most active compounds that we have made. We see a similar, but not quite as dramatic, dependence on the relative position of the substituents in comparing the dichloro compounds **102** and **103**. While the *tert*-butyl-substituted compounds **95** and **96** are the most active compounds that we have found so far in this series, their physical properties, most notably poor solubility in an aqueous environment, have precluded further development of these compounds as potential drug candidates.

Additionally, we examined the effects of modifying the terminal charged group on the activity exhibited by these bis-aryl phosphate antagonists. These results are shown in Table VI. As was evident in the monoaryl series discussed above, an explicit positive charge is needed for

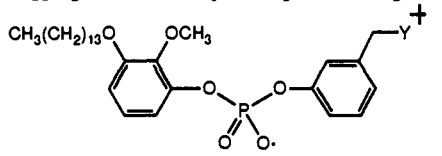
Table V. Inhibition of PAF-Induced Platelet Aggregation: Bis-Aryl Phosphate Antagonists. Variation of the Aromatic Substituents



compd	aromatic substituents 2	3	4	5	6	substn[a]	R_1	inhibn of platelet agg: IC_{50} (μM)[b]	formula[c]	anal.[d]
67	H	$OC_{16}H_{33}$	H	H	H	meta	H	1.81 (n = 3)	$C_{32}H_{46}NO_5PS(1.5H_2O)$	C,H,N,P,S
68	H	$OC_{16}H_{33}$	H	H	H	ortho	H	6.36 (n = 2)[e]	$C_{32}H_{48}NO_5PS$	C,H,N,S;P[k]
69	H	$OC_{14}H_{29}$	H	H	H	meta	H	6.15 (n = 2)	$C_{30}H_{42}NO_5PS(0.75H_2O)$	C,H,N,P,S
70	H	$OC_{14}H_{29}$	H	H	H	meta	CH_3	9.92 (n = 1)	$C_{31}H_{44}NO_5PS(1.25H_2O)$	C,H,N,P,S
71	H	$OC_{12}H_{25}$	H	H	H	meta	H	51.9 (n = 1)	$C_{28}H_{38}NO_5PS(0.5H_2O)$	C,H,N,P,S
72	H	H	$OC_{16}H_{33}$	H	H	meta	H	1.61 (n = 1)	$C_{32}H_{46}NO_5PS(1.0H_2O)$	C,H,N,S;P[l]
73	H	H	$OC_{16}H_{33}$	H	H	meta	CH_3	9.68 (n = 1)	$C_{33}H_{48}NO_5PS$	C,H,N,P,S
74	$OC_{14}H_{29}$	H	H	H	H	meta	H	NA (n = 1)[f]	$C_{30}H_{42}NO_5PS(1.0H_2O)$	C,H,N,P,S
75	H	$C_{14}H_{29}$	H	H	H	meta	H	16.5 (n = 1)	$C_{30}H_{42}NO_4PS(0.7H_2O)$	C,H,N,S;P[m]
76	CH_3	$OC_{16}H_{33}$	H	H	H	meta	H	1.79 (n = 2)	$C_{33}H_{48}NO_5PS(2.0H_2O)$	C,N,P;H[n],S[o]
77	CH_3	CH_3	$OC_{14}H_{29}$	H	CH_3	meta	CH_3	0.21 (n = 1)	$C_{34}H_{50}NO_5PS(1.25H_2O)$	C,H,N,P,S
78	H	CH_3	$OC_{14}H_{29}$	CH_3	CH_3	meta	CH_3	1.94 (n = 1)	$C_{34}H_{50}NO_5PS(1.0H_2O)$	C,H,N,P,S
79	C_2H_5	H	$OC_{14}H_{29}$	H	H	meta	CH_3	0.81 (n = 2)	$C_{33}H_{48}NO_5PS(0.25H_2O)$	C,H,N,P,S
80	C_2H_5	H	$C_{14}H_{29}$	H	H	meta	CH_3	1.21 (n = 2)	$C_{33}H_{48}NO_4PS(1.5H_2O)$	C,H,N,P,S
81	C_6H_5	H	$OC_{14}H_{29}$	H	H	meta	CH_3	0.20 (n = 2)	$C_{37}H_{48}NO_5PS(0.5H_2O)$	C,H,N,P,S
82	H	C_6H_5	$OC_{14}H_{29}$	H	H	meta	CH_3	2.16 (n = 1)	$C_{37}H_{48}NO_5PS(1.5H_2O)$	C,H,N,P,S
83	OCH_3	$OC_{12}H_{25}$	H	H	H	meta	H	17.2 (n = 1)[e]	$C_{29}H_{40}NO_6PS(1.0H_2O)$	C,H,N,P,S
84	OCH_3	$OC_{12}H_{25}$	H	H	H	meta	CH_3	10.5 (n = 1)[e]	$C_{30}H_{42}NO_6PS(0.25H_2O)$	C,H,N,P,S
85	OCH_3	$OC_{14}H_{29}$	H	H	H	meta	H	4.31 (n = 2)	$C_{31}H_{44}NO_6PS(1.8H_2O)$	C,H,N,S;P[p]
86	OCH_3	$OC_{14}H_{29}$	H	H	H	meta[j]	CH_3	NA (n = 2)[f]	$C_{33}H_{48}NO_6PS(1.0H_2O)$	C,H,N,P,S
87	OCH_3	$OC_{14}H_{29}$	H	H	H	meta	CH_3	1.14 (n = 9)	$C_{32}H_{46}NO_6PS(0.5H_2O)$	C,H,N,P,S
88	OCH_3	$OC_{14}H_{29}$	H	H	H	ortho	CH_3	0.90 (n = 2)	$C_{32}H_{46}NO_6PS(1.1H_2O)$	C,H,N,P,S
89	OCH_3	$OC_{14}H_{29}$	H	Br	H	meta	CH_3	0.57 (n = 2)	$C_{32}H_{45}NBrO_6PS(1.0H_2O)$	C,H,N,Br,P,S
90	OCH_3	H	$OC_{14}H_{29}$	H	H	meta	CH_3	0.64 (n = 2)	$C_{32}H_{46}NO_6PS(1.25H_2O)$	C,H,N,P,S
91	H	OCH_3	$OC_{14}H_{29}$	H	H	meta	CH_3	1.84 (n = 2)	$C_{32}H_{46}NO_6PS(1.75H_2O)$	C,N,P,S;H[q]
92	OCH_3	R'[h]	H	H	H	meta	CH_3	7.90 (n = 1)	$C_{30}H_{42}NO_6PS(1.25H_2O)$	C,H,N,P,S
93	OCH_3	R''[i]	H	H	H	meta	CH_3	NA (n = 2)[f]	$C_{29}H_{40}NO_6PS(0.75H_2O)$	C,H,N,P,S
94	H	$OC_{12}H_{25}$	$C(CH_3)_3$	H	$C(CH_3)_3$	meta	H	g (n = 1)	$C_{36}H_{54}NO_5PS$	C,H,N,P,S
95	$C(CH_3)_3$	H	$OC_{12}H_{35}$	$C(CH_3)_3$	H	meta	H	0.13 (n = 4)	$C_{38}H_{54}NO_5PS(0.5H_2O)$	C,H,N,P,S
96	$C(CH_3)_3$	H	$OC_{12}H_{35}$	$C(CH_3)_3$	H	meta	CH_3	0.09 (n = 5)	$C_{37}H_{56}NO_5PS(0.5H_2O)$	C,H,N,P,S
97	$C(CH_3)_3$	H	$OC_{12}H_{35}$	$C(CH_3)_3$	H	ortho	CH_3	6.52 (n = 1)	$C_{37}H_{56}NO_5PS(1.5H_2O)$	C,H,N,P,S
98	$C(CH_3)_3$	H	$OC_{12}H_{25}$	H	H	meta	CH_3	0.46 (n = 2)	$C_{33}H_{48}NO_5PS(1.25H_2O)$	C,H,N,P,S
99	Cl	H	$OC_{14}H_{29}$	H	H	meta	CH_3	2.00 (n = 1)	$C_{31}H_{43}NClO_5PS(1.25H_2O)$	C,H,N,Cl,P,S
100	H	Cl	$OC_{14}H_{29}$	H	H	meta	CH_3	2.93 (n = 1)	$C_{31}H_{43}NClO_5PS(0.5H_2O)$	C,H,N,Cl,P,S
101	Cl	Cl	$OC_{14}H_{29}$	Cl	Cl	meta	CH_3	11.8 (n = 1)	$C_{31}H_{40}NCl_4O_5PS(1.6H_2O)$	C,N,P,S;H[r],Cl[s]
102	Cl	H	$OC_{14}H_{29}$	Cl	H	meta	CH_3	0.56 (n = 3)	$C_{31}H_{42}NCl_2O_5PS(1.0H_2O)$	C,H,N,Cl,P,S
103	H	$OC_{14}H_{29}$	Cl	H	Cl	meta	CH_3	9.90 (n = 1)	$C_{31}H_{42}NCl_2O_5PS(0.25H_2O)$	C,H,N,Cl,P,S
104	CO_2CH_3	$OC_{14}H_{29}$	H	H	H	meta	H	0.67 (n = 2)	$C_{32}H_{44}NO_7PS(0.5H_2O)$	C,H,N,P,S
105	CO_2CH_3	$OC_{14}H_{29}$	H	H	H	meta	CH_3	0.54 (n = 12)	$C_{33}H_{46}NO_7PS(0.25H_2O)$	C,H,N,P,S
106	$COCH_3$	$OC_{14}H_{29}$	H	H	H	meta	H	1.33 (n = 1)	$C_{32}H_{44}NO_6PS(1.0H_2O)$	C,H,N,P,S
107	$COCH_3$	$OC_{14}H_{29}$	H	H	H	meta	CH_3	0.88 (n = 3)	$C_{33}H_{46}NO_6PS(2.0H_2O)$	C,H,N,P;S[t]
108	$COCH_3$	H	H	$OC_{14}H_{29}$	H	meta	CH_3	1.52 (n = 1)	$C_{33}H_{46}NO_6PS(1.25H_2O)$	C,H,N,P,S
109	$COCH_3$	H	$OC_{14}H_{29}$	H	H	meta	CH_3	0.30 (n = 1)	$C_{33}H_{46}NO_6PS(4.0H_2O)$	N,P,S;C[u],H[v]
110	H	$OC_{14}H_{29}$	H	$COCH_3$	H	meta	CH_3	2.62 (n = 2)	$C_{33}H_{46}NO_6PS(0.25H_2O)$	C,H,N,P,S
112	COC_6H_5	H	H	$OC_{14}H_{29}$	H	meta	CH_3	2.09 (n = 1)	$C_{38}H_{48}NO_6PS(0.5H_2O)$	C,H,N,P,S
113	COC_5H_{11}	H	$OC_{12}H_{25}$	H	H	meta	CH_3	0.61 (n = 1)	$C_{35}H_{50}NO_5PS(0.25H_2O)$	C,H,N,P,S
114	$COCH_3$	H	$C_{14}H_{29}$	H	H	meta	CH_3	0.66 (n = 2)	$C_{33}H_{46}NO_5PS(2.0H_2O)$	C,N,P,S;H[w]
115	H	H	Cl	H	H	meta	H	NA (n = 2)[f]	$C_{16}H_{13}NClO_4PS(0.5H_2O)$	C,H,N,Cl,P,S
116	Cl	H	H	H	H	meta	H	NA (n = 2)[f]	$C_{16}H_{13}NClO_4PS(0.5H_2O)$	C,H,N,Cl,P,S
117	H	Cl	H	H	H	meta	H	22.0 (n = 2)	$C_{16}H_{13}NClO_4PS(1.5H_2O)$	C,H,N,P,S
118	H	C_6H_5	H	H	H	meta	H	NA (n = 2)[f]	$C_{22}H_{18}NO_4PS(1.2H_2O)$	C H N;P[x],S[y]
119	H	H	$OCH_2C_6H_5$	H	H	meta	CH_3	54.0 (n = 1)	$C_{24}H_{22}NO_5PS(1.5H_2O)$	C,H,N,P,S
120	H	$C(CH_3)_3$	H	$C(CH_3)_3$	H	meta	H	41.0 (n = 1)[e]	$C_{24}H_{30}NO_4PS(1.5H_2O)$	C,H,N,P,S
121	H	H	$C(CH_3)_3$	H	H	meta	H	27.0 (n = 1)	$C_{20}H_{22}NO_4PS(1.0H_2O)$	C,H,N,P;S[z]
122	H	$C(CH_3)_3$	$OC_{12}H_{25}$	H	H	meta	CH_3	2.11 (n = 2)	$C_{33}H_{48}NO_5PS(1.0H_2O)$	C,H,N,P,S
123	H	$OC_{14}H_{29}$	H	OCH_3	H	meta	H	1.21 (n = 1)	$C_{31}H_{44}NO_6PS(2.0H_2O)$	C,H,N,P,S

[a] Refers to the position of substitution in the right hand aromatic ring; unless indicated, the value of m is 1. [b] Concentration needed to inhibit PAF-induced platelet aggregation in rabbit PRP by 50%; unless indicated, the PAF challenge concentration was $5.0E^{-8}$ M; the n value in parentheses is the number of determinations. [c] Empirical formula with amount of water of hydration. All compounds showed the expected M + H ion in the FAB mass spectrum. [d] Analytical results for the indicated elements are within ±0.4% of the calculated values, unless indicated otherwise. [e] PAF challenge concentration was $1.0E^{-7}$ M. [f] No inhibition observed at a compound concentration up to 10 μM. [g] Only 15% inhibition at a dose of 50 μM. [h] R' = $O(CH_2)_2O(CH_2)_2O(CH_2)_7CH_3$. [i] R'' = $O(CH_2)_6O(CH_2)_2O(CH_2)_2OCH_3$. [j] The value of m is 2. [k] P: calcd, 5.27; found, 5.80. [l] P: calcd, 5.11; found, 4.55. [m] P: calcd, 5.57; found, 4.64. [n] H: calcd, 8.22; found, 7.59. [o] S: calcd, 5.03; found, 5.58. [p] P: calcd, 4.98; found, 4.46. [q] H: calcd, 7.84; found, 7.24. [r] H: calcd, 5.88; found, 5.10. [s] Cl: calcd, 19.16; found, 19.66. [t] S: calcd, 4.92; found, 5.40. [u] C: calcd, 57.62; found, 56.35. [v] H: calcd, 7.91; found, 6.53. [w] H: calcd, 7.92; found, 7.39. [x] P: calcd, 6.96; found, 6.44. [y] S: calcd, 7.20; found, 6.50. [z] S: calcd, 7.61; found, 8.13.

Table VI. Inhibition of PAF-Induced Platelet Aggregation: Bis-Aryl Phosphate Antagonists. Variation of the Heterocycle

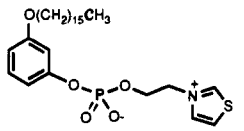


compd	heteroadduct (Y)	inhibn of platelet agg: IC_{50} (μM)[a]	formula[c]	anal.[d]
85	thiazole	4.30 (n = 2)	$C_{31}H_{44}NO_6PS(1.8H_2O)$	C,H,N,S;P[f]
87	5-methylthiazole	1.15 (n = 9)	$C_{32}H_{46}NO_6PS(0.5H_2O)$	C,H,N,P,S
111	5-ethylthiazole	5.10 (n = 1)	$C_{33}H_{48}NO_6PS(1.5H_2O)$	C,H,N,P,S
124	4-methylthiazole	3.39 (n = 1)	$C_{32}H_{46}NO_6PS$	H,N,P,S;C[g]
125	2,4-dimethylthiazole	19.5 (n = 2)	$C_{33}H_{48}NO_5PS(0.5H_2O)$	C,H,N,P,S
126	4,5-dimethylthiazole	9.50 (n = 3)	$C_{33}H_{48}NO_6PS(0.5H_2O)$	C,H,N,P,S
127	2,4,5-trimethylthiazole	1.40 (n = 2)	$C_{34}H_{50}NO_6PS(1.0H_2O)$	C,H,N,P,S
128	2-isobutylthiazole	3.00 (n = 1)	$C_{35}H_{52}NO_6PS$	C,H,N,P,S
129	pyridine	9.09 (n = 1)	$C_{33}H_{46}NO_6P(4.0H_2O)$	C,N,P,S;H[h]
130	quinoline	1.99 (n = 1)	$C_{37}H_{48}NO_6P(2.0H_2O)$	C,N,P;H[i]
131	isoquinoline	18.3 (n = 1)	$C_{37}H_{48}NO_6P(2.0H_2O)$	C,H,N,P
132	imidazo[1,2-a]pyridine	1.49 (n = 1)	$C_{35}H_{47}N_2O_6P(0.3H_2O)$	C,H,N,P
133	N-methylimidazole	14.4 (n = 1)[b]	$C_{32}H_{47}N_2O_6P(0.5H_2O)$	C,H,N,P
134	imidazole	e (n = 1)	$C_{31}H_{45}N_2O_6P(1.25H_2O)$	C,H,N,P
135	triethylamine	35.0 (n = 1)	$C_{34}H_{56}NO_6P(1.25H_2O)$	C,H,N,P

[a] Concentration needed to inhibit PAF-induced platelet aggregation in rabbit PRP by 50%; the PAF challenge concentration was $5.0E^{-8}$ M; the n value in parentheses is the number of determinations. [b] The PAF challenge concentration was $1.0E^{-7}$ M. [c] Empirical formula with amount of water of hydration. All compounds showed the expected M + H ion in the FAB mass spectrum. [d] Analytical results for the indicated elements are within ±0.4% of the calculated values, unless indicated otherwise. [e] Only 12% inhibition at a dose of 50 μM. [f] See Table V. [g] C: calcd, 63.65; found, 64.20. [h] H: calcd, 8.30; found, 7.23. [i] H: calcd, 7.83; found, 7.16.

maximal activity (compare neutral **134** with its methylated derivative **133**). The compound with a triethylammonium group, **135**, showed significantly less activity than any of the charged heterocyclic-containing compounds. Of the thiazolium-substituted compounds shown in Table VI, the best activity was observed for the 5-methylthiazolium-containing compound **87**; however, in general, there is no clear preference for the 5-methylthiazolium group over the simple thiazolium group (see Table V). Some of the compounds that contain a charged bicyclic heterocycle show reasonable activity (see **130** and **132**).

Finally, in order to evaluate the importance of the aromatic ring that separates the phosphate from the charged group, we prepared **136**, a compound in which the aromatic ring has been replaced with a simple two-carbon methylene bridge (as is present in PAF); we have determined that **136** has an IC_{50} = 560 μM, and it is therefore about 300 times less potent then its aromatic-containing counterpart **67**.



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In order to examine whether the inhibition of platelet aggregation that we have observed for these compounds is specific to PAF, three compounds (**87**, **105**, and **43**) were evaluated for their ability to inhibit platelet aggregation induced by the agonists adenosine diphosphate, collagen, and arachidonate. No inhibition was observed at a compound concentration of 1×10^{-5} M to 5×10^{-5} M which is about 2 orders of magnitude greater than the IC_{50} values for inhibiting PAF-induced aggregation. Thus, the inhibition exhibited by these compounds appears to be specific for PAF.

In studies not shown, we found that the IC_{50} values for **87**, **105**, and **43** shifted to higher values as the PAF challenge concentration increased. Additional studies showed that with increasing concentrations of these three antagonists, the aggregation dose–response curve for PAF is shifted to the right. These results suggest that the antagonists are competing with PAF at the receptor site.

More definitive data for competition at the receptor site were obtained using a [^{3}H]PAF binding assay. As shown in Figure 1, compounds **87** and **105** displace [^{3}H]PAF from its binding site on the rabbit platelet receptor with IC_{50} values of 19.4 nM and 5.6 nM, respectively. For comparison, WEB-2086, had an IC_{50} value of 9.1 nM.

We assessed selected antagonists for their ability to prevent death resulting from a lethal challenge of PAF in both the mouse and rabbit. These results are presented in Table VII. In vivo PAF has a multitude of inflammatory effects including platelet aggregation, activation of other inflammatory cells and release of mediators, disruption of vascular permeability, hypotension, and death. In the rabbit, intravenous PAF administration results in death within 2–5 min, presumably due to a sequence of platelet aggregation, production of emboli in the lungs, and cardiovascular collapse. In vitro activity was predictive of activity in the rabbit; inactivity in vitro (e.g. compound **94**) was also correlated with inactivity in vivo. A dose–response pattern was also evident for compounds **87**, **105**, and **43** in the rabbit. In the rabbit, the ED_{50} value for compound **105** was about 1 mg/kg; for compound **87** it was between 1 and 5 mg/kg.

Although, the pattern of PAF-induced death in the mouse is more delayed than in the rabbit and may be related to vascular leakage rather than platelet aggregation as a proximal cause of hypotension and shock, compounds active in vitro were also active in the mouse lethality assay. Compound **105** was examined in a dose–response manner in this species, and an ED_{50} value of 0.13 mg/kg was calculated from the dose–response curve shown in Figure 2. This represents approximately a 1:1 agonist–antagonist ratio. The greater in vivo potency of compound **105** in the mouse compared with the rabbit may reflect the differences in the PAF receptor and the mechanism of death in the two species.

In conclusion, a series of aryl phosphoglyceride (**3**, **19–61**) and bis-aryl phosphate (**67–135**) antagonists of PAF were prepared; many members of both series of compounds show excellent PAF antagonist activity both in vitro as well as in vivo compared to other antagonists already described

Table VII. Protection of PAF-Induced Lethality in the Mouse and Rabbit

compd	dose (mg/kg)	mouse[a] (dead/total)	rabbit[b] (dead/total)
control		2345/2599	30/30
3	1.25	2/10	
	2.5		1/2
19	2.5		1/2
22	2.5	0/10	
24	1.25	4/10	
	2.5		1/2
26	0.5	0/10	
	2.5		1/2
29	0.5	1/11	
43	0.5	23/49	
	1.0		1/2
	2.5	0/16	1/2
	10.0	6/12	0/2
44	2.5		1/2
85	0.5	4/9	
87	0.5	80/220	
	1.0	24/88	2/3
	5.0		1/3
	10.0	40/74	0/5
90	0.5	16/41	
94	2.5		2/2
95	0.5	2/10	
	2.5		0/2
96	2.5		0/2
104	0.5	11/11	
	1.0	5/10	
105	0.5	165/830	
	1.0	3/10	6/11
	2.5		0/4
	5.0		0/5
107	0.5	18/38	
109	0.5	6/23	
112	1.0	3/10	
	10.0	0/10	
127	1.0	9/12	
	10.0	6/13	
CV-3988	0.5	11/12	
CV-6209	0.5	4/22	
	1.0	1/10	0/2

[a] Compound was given ip in saline at the indicated dose 0.5 h before the lethal iv PAF challenge (75–150 μg/kg). Survival was assessed 90 min after challenge and did not change thereafter. The control is an accumulated group of saline-treated mice that showed 90% lethality from PAF. [b] Compound was given iv at the indicated doses 15 min before the lethal iv PAF challenge (150 μg/kg). Survival was assessed 60 min after challenge and did not change thereafter. The control is an accumulated group of saline-treated rabbits that showed 100% lethality from PAF.

in the literature. In addition to the PAF antagonist data that has been presented herein, we have evaluated most of the active antagonists in models of septic (endotoxic) shock in various species (rats, mice, and monkeys); these data will be reported at a future date. On the basis of these combined studies, one of the compounds described in this discourse, namely compound **105** (CL 184005), has been chosen to undergo further development as a potential therapeutic agent for the treatment of septic shock in man. Additional factors that have entered in the decision to pursue development of this particular compound included the results of an extensive toxicological study and a consideration of the physical properties of the compound that allowed the design of an appropriate intravenous formulation.

Experimental Section

Inhibition of PAF-Induced Platelet Aggregation Assay. About 60–100 mL of blood was collected by cardiac puncture or ear bleeding from unanesthetized male New Zealand rabbits with the use of sodium citrate anticoagulant (1 part of 3.2% citrate for 10 parts of blood). All syringes and pipets were plastic. Platelet-rich plasma (PRP) was recovered from 20 mL blood centrifuged at 800 rpm × 15 min at room temperature. Dilutions (1:3000) of PRP in Isoton diluent were made and the platelet count was determined on a Coulter Thrombocounter. Platelet counts ranged from 350 000 to 550 000 per μL.

The L-isomer of C16-PAF (1) was obtained from Boehringer-Mannheim, and diluted in CH_3OH to give a 10 mg/mL solution. Aliquots were used to make working serial dilutions in saline. The test compounds were diluted in CH_3OH to give 1 mg/mL stock solutions and then serially diluted in saline. All working solutions were made fresh in plastic tubes and kept on ice.

Incubation mixtures consisted of 400 μL of PRP, 50 μL of saline or test compound, and 50 μL of L-PAF agonist. Briefly, 400 μL of PRP was stabilized in a cuvette for 1–2 min at 37 °C in a Chronolog aggregometer to achieve a stable baseline. Fifty microliters of saline or test compound (final concentration ranging from 10^{-8} to 10^{-5} M) was added and incubated for 5 min. Controls and test samples were run in parallel. At 5 min, the challenge concentration of PAF was added (5×10^{-8} M or 1×10^{-7} M) as determined from a PAF dose–response. The dose–response for each test compound was determined, and the IC_{50} value (the dose required for 50% inhibition of the magnitude of aggregation) was determined. The aggregation response was recorded and analyzed as previously described.[12]

Receptor Binding Assay: Displacement of Radiolabeled PAF from Its Receptor. Freshly washed rabbit platelets were used for the radiolabeled ligand binding study. PRP was centrifuged at 2800 rpm for 10 min to obtain a platelet pellet and platelet-poor plasma (PPP). The pellet was gently suspended and washed in calcium-free, albumin-free Tyrode's buffer at pH 6.3. The suspension was recentrifuged, and the washed pellet was resuspended in normal Tyrode's with 0.25% BSA at pH 7.4. Approximately 10^7 to 10^8 cells were used per assay tube. The L-isomer of C16-PAF (1) was from Boehringer-Mannheim and labeled [^{3}H]PAF was from New England Nuclear (specific activity of 56 Ci/mmol). [^{3}H]PAF binding was measured by a radioisotopic method where free and platelet-bound ligand are separated by vacuum filtration on a Brandel cell harvester. Platelets were incubated for 30 min at room temperature in Tyrode's-BSA buffer pH 7.4 with 1–2.5 nM [^{3}H]PAF and various concentrations (1–1000 nM) of test compound. Parallel tubes were made up with and without excess unlabeled PAF (10–100 μM) for the determination of nonspecific and total binding, respectively. Specific binding was calculated as the difference between total and nonspecific binding. Displacement curves were plotted and IC_{50} values (dose required to inhibit binding by 50%) were calculated from the dose–response curve.

Prevention of PAF-Induced Lethality in Mice. A stock solution containing 7.5–15 μg/mL of racemic C16-PAF in saline was prepared. Female Swiss-Webster mice weighing 20–25 g (Charles River Labs Inc.) were dosed ip with test compound in saline at 10 mL/kg. Control animals were dosed only with saline. Thirty minutes later the mice were injected with PAF stock solution as an intravenous bolus in a tail vein at 10 mL/kg (resulting in a dose of 75–150 μg/kg of PAF). Survival was monitored at 1.5 h and did not change thereafter. There was a day to day variability in the sensitivity of mice to the PAF challenge; several small test groups were done to select the appropriate dose for the experiment. The racemic PAF was indistinguishable from the stereochemically pure compound when the overall concentration was adjusted for the inactive isomer.

Prevention of PAF-Induced Lethality in Rabbits. Male New Zealand white rabbits (Hare Marland or Hazelton) were used. Intravenous PAF at 150 μg/kg induces lethality in a rabbit within 3–5 min, due to profound platelet aggregation which produces respiratory distress and cardiovascular collapse. A 10 mg/mL stock solution of L-C16-PAF in methanol was diluted in saline to administer a lethal dose of 150 μg/kg. The test compounds were given intravenously at various doses (1.0–10.0 mg/kg) 15 min before an intravenous PAF challenge. Survival was monitored at 1 h and did not change thereafter.

Chemistry. Melting points were determined on a Mel-Temp apparatus and are uncorrected. The melting points of the internal salts generally were not determined since these compounds are usually composed of amorphous powders with no well-defined melting range. Fast atom bombardment (FAB) mass spectra were determined on a VG-ZAB SE mass spectrometer. Electron impact (EI) and chemical ionization (CI) mass spectra were determined

on a Finnigan MAT-90 mass spectrometer. IR spectra were recorded on a Nicolet 20SXB FT-IR spectrometer. ^{1}H NMR spectra were determined at 300 MHz using a Nicolet QE-300 WB spectrometer; chemical shifts (δ) are in parts per million relative to tetramethylsilane. NMR spectra of all internal salts were determined in DMSO-d_6; it was usually found that adding several drops of CF_3CO_2D to the sample improved the resolution of the resulting spectrum. Where analyses are indicated only by symbols of the elements, analytical results obtained for these elements were within ±0.4% of the theoretical value. Unless otherwise noted all reagents and solvents obtained from commercial suppliers were used without further purification.

4-(Bromomethyl)phenyl Phosphorodichloridate (5b). To a solution of 9.1 g (33.6 mmol) of PBr_3 in 10 mL of dry THF at −5 °C was added a solution of 1.32 mL (16.4 mmol) of pyridine in 2.5 mL THF. A solution of 12.4 g (100 mmol) of 4-hydroxybenzyl alcohol in 150 mL of THF was added dropwise at −5 °C with stirring. The mixture was then allowed to stand at room temperature for 18 h. The mixture was diluted with THF and filtered through Celite. Solvent was removed, and the residue was redissolved in 100 mL of toluene. The solution was maintained at −20 °C for 2 h and then filtered through Celite to give a solution of **4b** in toluene. To this solution was added 10.1 mL of $POCl_3$. The mixture was stirred in an ice bath and a solution of 15.3 mL of triethylamine in 15 mL of toluene was added dropwise during 1.5 h. After standing overnight, the mixture was filtered through Celite. Solvent was removed, and the residue was dried under vacuum giving 10.0 g of **5b** as a yellow liquid was used without additional purification: ^{1}H NMR ($CDCl_3$) δ 7.35 (m, 4 H, aromatic), 4.48 (s, 2 H, CH_2).

3-(2-Bromoethyl)phenyl Phosphorodichloridate (5c). To a solution of 18.4 g (91.5 mmol) of 3-(2-bromoethyl)phenol, **4c**[19] in 100 mL CCl_4 at 0 °C was added 11.8 mL (128 mmol) of $POCl_3$. The solution was stirred in an ice bath, and a solution of 13.6 mL (128 mmol) of triethylamine in 25 mL of CCl_4 was added dropwise during 1.5 h. The mixture was stirred an additional 2 h and then stored at −10 °C for 2 days. The mixture was filtered, the solvent was removed, and the residue was dried under vacuum giving 22.1 g (76%) of **5c** as a orange oil: ^{1}H NMR ($CDCl_3$) δ 7.18 (m, 4 H, aromatic), 3.58 (m, 2 H, CH_2), 3.20 (m, 2 H, CH_2).

2-Chloro-4*H*-1,3,2-benzodioxaphosphorin 2-Oxide (7). To a solution of 100 g (0.81 mol) of 2-hydroxybenzyl alcohol and 121.7 g (0.87 mol, 77.3 mL) of phosphorus trichloride in 1.0 L of ether at −10 °C was added dropwise with stirring a solution of 133.8 g (1.69 mol, 136.8 mL) of pyridine in 200 mL of ether over 1.5 h. The mixture was then stirred at room temperature for another 1.5 h and then stored at 10 °C overnight. The mixture was filtered, and the solvent was removed. Hexane was added, and the mixture was filtered. The solvent was removed. The residue was distilled in a Kugelrohr apparatus (1 mm, 80–90 °C) giving 81g of **6** as a colorless liquid. A solution of this material in 200 mL of benzene was stirred as dry oxygen was bubbled slowly in over a period of 20 h. The benzene was removed giving 87.5 (53%) of **7** as a pale yellow liquid: ^{1}H NMR ($CDCl_3$) δ 7.5–7.0 (m, 4 H, aromatic), 5.5 (m, 2 H, CH_2).

3-(Hexadecyloxy)-2-methylphenol (8o). To a suspension of 25.0 g (625 mmol) of 60% NaH (hexane washed) in 215 mL of DMF was added dropwise over 1.5 h under argon, with stirring, a solution of 60.0 g (483 mmol) of 2-methylresorcinol in a mixture of 145 mL of DMF and 72 mL of THF. The mixture was cooled in an ice-bath and 7.3 g (48.3 mmol) of NaI was added followed by the dropwise addition of 147.5 g (483 mmol) of 1-bromohexadecane over 1 h. The mixture was then stirred at room temperature overnight. The mixture was poured into dilute hydrochloric acid, and ether was added. Insoluble dialkylated product (93 g) was removed by filtration. The organic layer was washed with saturated NaCl solution and dried ($MgSO_4$), and the solvent was removed. The residue was then purified by silica gel column chromatography to give 54.5 g (33%) of **8o** as a white solid: mp 53–54 °C; IR (KBr) 3320 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 6.99 (t, 1 H, CH), 6.44 (m, 2 H, CH), 4.68 (bs, 1 H, OH), 3.94 (t, 3 H, OCH_2), 2.12 (s, 3 H, CH_3), 1.80 (m, 2, CH_2CH_2O), 1.55–1.18 (m's, 26 H, CH_2's), 0.88 (t, 3 H, terminal CH_3); mass spectrum (CI) m/e 349 (M + H). Anal. ($C_{23}H_{40}O_2$) C, H.

3-[3-(Hexadecyloxy)-2-methylphenoxy]-1,2-propanediol (13o). A sample of 4.3 g (71.7 mmol) of 60% sodium hydride was washed with hexanes and then suspended in 120 mL of DMF. To this suspension was added dropwise under argon a solution of **8o** in 85 mL of THF over 1 h. NaI (0.86 g, 5.7 mmol) was added followed by the dropwise addition of a solution of 13.3 g (57.4 mmol) of **9**[12] in 15 mL of THF. The mixture was then stirred at 80 °C for 18 h. The solution was cooled, poured into ice–water, and extracted with ether. The ether solution was washed with brine and dried ($MgSO_4$). The solvent was removed giving **12o** as a crystalline solid. This material was stirred at reflux in a mixture of 250 mL of methanol, 40 mL of H_2O, and 3 mL of H_2SO_4 for 1.5 h. The reaction mixture was then cooled in an ice bath, and product was collected by filtration. After recrystallization from methanol, 19.3 g (82%) of **13o** was obtained as a colorless solid: mp 80–82 °C; ^{1}H NMR ($CDCl_3$) δ 7.08 (t, 1 H, CH), 6.52 (d's, 2 H, CH), 3.38 (m, 2 H, CH_2O), 3.94 (t, 2 H, OCH_2), 4.05 (m, 2 H, CH_2O), 4.12 (m, 1 H, CHO), 2.14 (bs, 2 H, OH), 2.13 (s, 3 H, CH3), 1.79 (m, 2, CH_2CH_2O), 1.53–1.18 (m's, 26 H, CH_2's), 0.88 (t, 3 H, terminal CH_3); mass spectrum (CI) m/e 423 (M + H). Anal. ($C_{26}H_{46}O_4$) C, H.

3-[3-(Hexadecyloxy)-2-methylphenoxy]-2-methoxy-1-propanol (15o). To a solution of 16.7 g (54 mmol) of *p*-anisylchlorodiphenylmethane in 120 mL of THF was added 19 g (45 mmol) of **13o** and 50 mL of pyridine. After 18 h, the solvent was removed, 300 mL of ice–H_2O was added, and the mixture was extracted with ether. The extract was dried (Na_2SO_4), and the solvent was removed giving **14o** which was used without additional purification.

To a suspension of 2.7 g (67.5 mmol) of hexane-washed 60% NaH dispersion in 125 mL of DMF containing 8.4 mL (134.9 mmol) of CH_3I was added a solution of the **14o** prepared above in 65 mL of THF over a 1-h period. After stirring for 18 h, the mixture was diluted with 500 mL of ice–H_2O and extracted with ether. The extracts were washed with brine and dried ($MgSO_4$). Solvent was removed. The residue was heated to boiling in a mixture of 200 mL of methanol, 100 mL of $CHCl_3$, and 20 g of Amberlyst-15 strongly acidic resin and then stirred at room temperature for 1.5 h. The mixture was filtered, and the solvent was removed immediately before the chromatography commenced. The residue was purified by silica gel chromatography, eluting with hexane–ethyl acetate 4:1 to give 1.8 g of **15o** as a colorless solid: mp 46–47 °C; IR (KBr) 3405 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.08 (t, 1 H, CH), 6.51 (d's, 2 H, CH), 4.10–3.68 (m's, 7 H, CH_2O's, CHO), 3.56 (s, 3 H, OCH_3), 2.10 (s, 3 H, CH3), 2.04 (bt, 1 H, OH), 1.78 (m, 2, CH_2CH_2O), 1.53–1.18 (m's, 26 H, CH_2's), 0.88 (t, 3 H, terminal CH_3); mass spectrum (CI) m/e 437 (M + H). Anal. ($C_{27}H_{48}O_4$) C, H.

3-(Bromomethyl)phenyl 3-[3-(Hexadecyloxy)-2-methylphenoxy]-2-methoxy-1-propyl Phosphoric Acid Diester (17o). To a solution of 1.8 g (4.12 mmol) of **15o** in 30 mL of CCl_4 was added 1.5 g (4.95 mmol) of phosphorus reagent **5a**[5] in 5 mL of CCl_4 followed by 0.5 g (4.95 mmol) of triethylamine in 5 mL of CCl_4. The mixture was stirred at room temperature for 17 h. Toluene (30 mL) was added, and the mixture was filtered. The solvent was removed from the filtrate and the resulting oil was stirred in a mixture of 65 mL of THF and 65 mL of 0.5 M sodium acetate for 2 h. The THF was removed at reduced pressure, and the aqueous mixture was cooled and acidified with concentrated HCl. The mixture was extracted with ether. The solution was dried ($MgSO_4$). The solvent was removed, and the residue was chromtographed on Florisil, eluting first with $CHCl_3$ to remove the less polar impurities and then with $CHCl_3$–CH_3OH (9:1) to elute the product **17o**, obtained as a thick oil (1.9 g, 67%): ^{1}H NMR ($CDCl_3$) δ 7.30–6.40 (m's, 7 H, aromatic), 4.30–3.40 (m's, 9 H, CH_2O's, CH_2Br, CHO), 3.27 (s, 3 H, OCH_3), 1.94 (s, 3 H, CH_3), 1.74 (m, 2, CH_2CH_2O), 1.53–1.18 (m's, 26 H, CH_2's), 0.88 (t, 3 H, terminal CH_3). Anal. ($C_{34}H_{54}O_6PBr$) C, H, P, Br.

3-[[3-[[[3-[3-(Hexadecyloxy)-2-methylphenoxy]-2-methoxypropoxy]hydroxyphosphinyl]oxy]phenyl]methyl]thiazolium Hydroxide, Inner Salt (42). A stirred solution of 1.8 g (2.69 mmol) of **17o** and 1.83 g (21.5 mmol) of thiazole in 15 mL of toluene was maintained at 65 °C under argon for 24 h. The solvent was removed, and the residue was stirred with 1.8 g of Amberlite-IR 4B ion-exchange resin (weakly basic) in 40 mL of

(19) Weibel, P. A., Hesse, M. Mass spectrometric behavior of nitrogen-containing compounds. 19. Substituent effect on mass spectrometric fragmentation. *N*-methyl-β,β'-diphenyldiethylamines. *Helv. Chim. Acta* **1973**, *56*, 2460–2479.

methanol for 2 h. The solution was filtered and solvent was removed. The product was purified by chromatography on silica gel (125 mL dry volume), eluting first with $CHCl_3$–CH_3OH (2:1) to remove less polar impurities and then with $CHCl_3$–CH_3OH–H_2O (14:6:1) to elute product. Product fractions were combined, and solvent was removed. The residue was mixed with ether, and the solid product was collected via filtration and dried in vacuum to give 1.28 g (69%) of **42** as a white hygroscopic solid: ^1H NMR (DMSO-d_6 + TFA) δ 10.41 (m, 1 H, NCHS), 8.60, 8.36 (m's, 2 H, NCHCHS), 7.48–6.53 (m's, 7 H, aromatic), 5.81 (s, 3 H, CH_2N), 4.33–3.73 (m's, 7 H, CH_2O's, CHO), 3.41 (s, 3 H, OCH_3), 2.01 (s, 3 H, CH_3), 1.73 (m, 2, CH_2CH_2O), 1.50–1.15 (m's, 26 H, CH_2's), 0.88 (t, 3 H, terminal CH_3); mass spectrum (FAB) m/e 690 (M + H). Anal. ($C_{37}H_{56}NO_7SP\cdot0.75H_2O$) C, H, N, P, S.

4-Tetradecylphenol Acetate. A mixture of 0.29 g of 4-tetradecylphenol,[12] 2 mL of acetic anhydride, and 5 mL of pyridine was stirred a room temperature for 24 h. The mixture was poured into dilute HCl and extracted with ether. The ether solution was washed with saturated $NaHCO_3$ solution and then dried ($MgSO_4$). The solvent was removed giving 0.26 g of the desired acetate: mp 32–34 °C; mass spectrum (EI) m/e 332 (M^+).

1-(2-Hydroxy-5-tetradecylphenyl)ethanone (63j'). A 0.9 g (2.71 mmol)-portion of 4-tetradecylphenol acetate was maintained at 70 °C under argon as 0.72 g (5.41 mmol) of $AlCl_3$ was added portionwise over 2 h. The mixture was then heated to 120 °C for 45 min. The mixture was cooled and diluted with $CHCl_3$. The resulting solution was washed first with 50% HCl and then with H_2O. The solution was dried ($MgSO_4$), solvent was removed, and the residue was recrystallized from ether–CH_3OH giving 0.85 g (94%) of **63j'**: mp 39–40 °C; IR (KBr) 1645 cm^{-1}; ^1H NMR ($CDCl_3$) δ 12.1 (s, 1 H, OH), 7.60–6.90 (m, 3 H, aromatic), 2.62 (s, 3 H, $COCH_3$), 2.57 (m, 2 H, benzylic CH_2), 1.25 (m, 24 H, CH_2's), 0.95 (m, 3 H, terminal CH_3).

2-Ethyl-4-tetradecylphenol (63k). A mixture of 23 g (69.2 mmol) of **63j'**, 5 g of 5% palladium on carbon catalyst, 10 mL of concentrated HCl, 100 mL of H_2O, and 100 mL of acetic acid was warmed to 50 °C and then hydrogenated in a Parr shaker for 24 h. The reaction mixture was filtered and the solvent was removed. The residue was taken up in ether, and the resulting solution was washed with H_2O followed by dilute $NaHCO_3$. The solution was dried ($MgSO_4$), and the solvent was removed. The residue was purified by silica gel chromatography giving 16.1 g (73%) of **63k**: mp 46–47 °C; ^1H NMR ($CDCl_3$) δ 7.30–6.65 (m, 3 H, aromatic), 4.45 (s, 1 H, OH), 2.54 (m, 4 H, benzylic CH_2's), 1.25 (m, 27 H, CH_2's, CH_3), 0.95 (m, 3 H, terminal CH_3); mass spectrum (EI) m/e 318 (M^+). Anal. ($C_{22}H_{38}O$) C, H.

2,6-Dihydroxybenzoic Acid Methyl Ester. To a stirred solution of 300 g (1.95 mol) of 2,6-dihydroxybenzoic acid in 2 L of methanol was added cautiously 87 mL of H_2SO_4. The resulting solution was stirred under argon at reflux for 7 days, and the solvent was removed. The residue was dissolved in 2 L of ethyl acetate, and the solution was washed with saturated NaCl solution and saturated $NaHCO_3$ solution and dried ($MgSO_4$), and taken to dryness. The residue was chromatographed on 1500 g of silica gel, eluting with 5% CH_3OH in $CHCl_3$. Product fractions were combined, and the solvent was removed. The residue was mixed with hexanes and collected to give 214.5 g (67%) of product as white crystals: mp 69–70 °C; IR (KBr) 3400, 1680 cm^{-1}; ^1H NMR ($CDCl_3$) δ 9.65 (s, 2 H, OH), 7.40–7.25 (m, 1 H, aromatic), 6.50 (d, 2 H, aromatic), 4.10 (s, 3 H, CO_2CH_3); mass spectrum (EI) m/e 168 (M^+). Anal. ($C_8H_8O_4$) C, H.

2-(Methoxycarbonyl)-3-(tetradecyloxy)phenol (63c'). To a suspension of 66.3 g (1.7 mol) of 60% NaH (hexane washed) in 500 mL of DMF was added under argon at 0 °C, with stirring, a solution of 214.5 g (1.3 mol) of 2.6-dihydroxybenzoic acid methyl ester in 900 mL of DMF dropwise over 1 h. To the mixture was added 19 g (0.17 mol) of NaI. A solution of 361 g (2.3 mol) of 1-bromotetradecane in 240 mL of DMF and 100 mL of THF was added at 0 °C over 30 min. The mixture was then stirred at room temperature overnight. The mixture was poured into dilute hydrochloric acid, and ethyl acetate was added. Insoluble dialkylated product (48 g) was remove by filtration. The organic layer was washed with saturated NaCl solution and saturated $NaHCO_3$ solution and dried ($MgSO_4$), and the solvent was removed. The residue was then distilled in a Kugelrohr apparatus. The fraction 80–120 °C (0.1 mm) was collected from which 84 g (39%) of unreacted 2,6-dihydroxybenzoic acid methyl ester was recovered by trituration with methanol. The pot residue was dissolved in 600 mL of hexanes and placed in the freezer for 24 h. Filtration furnished an additional 48 g of dialkylated product. Solvent was removed, and the residue was distilled in a Kugelrohr apparatus. The fraction 130–200 °C (0.5 mm) was collected. Recrystallization from 500 mL of methanol gave 78.8 g (28% based on recovered starting material) of product as a white solid. A sample was purified by dry column chromatography to give white needles: mp 43–44 °C; IR (KBr) 3329, 1668 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.30–6.39 (m's, 3 H, aromatic), 3.97 (t, 2 H, CH_2O), 3.93 (s, 3 H, CO_2CH_3), 1.80 (m, 2, CH_2CH_2O), 1.55–1.26 (m's, 22 H, CH_2's), 0.87 (t, 3 H, terminal CH_3); mass spectrum (EI) m/e 364 (M^+). Anal. ($C_{22}H_{36}O_4$) C, H.

3-(Bromomethyl)phenyl 2-(Methoxycarbonyl)-3-(tetradecyloxy)phenyl Phosphoric Acid Diester (64c'). To a solution of 33.3 g (91.35 mmol) of phenol **63c'** and 35.9 (118.1 mmol) of phosphorus reagent **5a**[5] in 190 mL of CCl_4 was added with stirring at 0 °C under argon a solution of 20.6 mL (118.2 mmol) of *N,N*-diisopropylethylamine in 20 mL of CCl_4. The mixture was then stirred at room temperature for 4 h. The mixture was filtered through Celite, and the solvent was removed. The residue was stirred in a mixture of 450 mL of 0.5 M sodium acetate and 450 mL of THF for 1.5 h. Most of the THF was removed, and the resulting aqueous mixture was chilled in an ice bath and acidified with concentrated HCl. The mixture was extracted three times with ether. The ether solution was dried ($MgSO_4$), and the solvent was removed. The residue was purified by chromatography on 530 g of Florisil, eluting first with $CHCl_3$ to remove less polar impurities and then with $CHCl_3$–methanol (8:2) to elute the product. Solvent was removed from the product fractions to give 38.2 g (70%) of **64c'** as a thick oil: ^1H NMR ($CDCl_3$) δ 7.27–6.92 (m's, 7 H, aromatic), 4.29 (s, 2 H, CH_2Br), 3.80 (t, 2 H, CH_2O), 3.73 (s, 3 H, CO_2CH_3), 1.65–1.25 (m, 24 H, CH_2's), 0.88 (t, 3 H, terminal CH_3). Anal. ($C_{29}H_{42}BrOP$) H, Br, P; C: calcd, 56.77; found, 56.07.

3-[[3-[[Hydroxy[2-(methoxycarbonyl)-3-(tetradecyloxy)phenoxy]phosphinyl]oxy]phenyl]methyl]-5-methylthiazolium Hydroxide, Inner Salt (105). A stirred solution of 48.8 g (79.5 mmol) of **64c'** and 40 g (403 mmol) of 5-methylthiazole in 400 mL of toluene was maintained at 70 °C under argon for 24 h. The solvent was removed, and the residue was stirred with 50 g of Amberlyst A-21 ion-exchange resin (weakly basic) in 400 mL of CH_3OH for 1.5 h. The solution was filtered and solvent was removed. The product was purified by HPLC on silica gel eluting with CH_3OH–CH_2Cl_2 (1:1). Product fractions were combined, and solvent was removed. The residue was mixed with ether, and the solid product was collected via filtration and dried in vacuum to give 43.5 g (86%) of **105** as an off-white hygroscopic solid: ^1H NMR (DMSO-d_6 + TFA) δ 10.23 (s, 1 H, thiazole), 8.30 (s, 1 H, thiazole), 7.45–6.87 (m's, 7 H, aromatic), 5.75 (s, 2 H, CH_2N), 4.0 (t, 2 H, CH_2O), 3.70 (s, 3 H, CO_2CH_3), 2.51 (s, 3 H, CH_3), 1.65 (m, 2 H, CH_2CH_2O), 1.5–1.15 (m's, 22 H, CH_2's), 0.86 (t, 3 H, terminal CH_3); mass spectrum (FAB) m/e 632 (M + H). Anal. ($C_{33}H_{46}NO_7SP\cdot0.25H_2O$) C, H, N, P, S.

3-[[2-[[Hydroxy[2-methoxy-3-(3-tetradecylphenoxy)propoxy]phosphinyl]oxy]phenyl]methyl]thiazolium Hydroxide, Inner Salt (28). A solution of 1.8 g (4.75 mmmol) of **15f**, 1.07 g (5.23 mmol) of reagent **7**, and 0.53 g (5.23 mmol) of triethylamine was stirred in 25 mL of CCl_4 for 18 h. The mixture was diluted with ether and filtered. The solvent was removed, and the residue **18f** was heated with 4.05 g (47.5 mmol) of thiazole in 30 mL of toluene at 85 °C for 3 days under argon. The mixture was cooled, and the solvent was removed. The residue was purified by silica gel chromatography, eluting first with $CHCl_3$–CH_3OH (8:2) to remove less polar impurities and then with $CHCl_3$–CH_3OH–H_2O (14:6:1) to elute the product. The product fractions were combined, and the solvent was removed giving 0.5 g (17%) of **28** as a hygroscopic solid: ^1H NMR (DMSO-d_6 + TFA) δ 10.21 (m, 1 H, NCHS), 8.47, 8.33 (m, 2 H, NCHCHS), 7.50–6.70 (m's, 8 H, aromatic), 5.82 (s, 2 H, CH_2N), 4.30–3.70 (m's, 5 H, CH_2O, CHO), 3.37 (s, 3 H, OCH_3), 2.49 (m, 2 H, benzylic CH_2), 1.60–1.15 (m's, 24 H, CH_2's), 0.88 (t, 3 H, terminal CH_3); mass spectrum (FAB) m/e 632 (M + H). Anal. ($C_{34}H_{50}NO_6PS\cdot1.0H_2O$) C, H, N, P, S.

2-Methoxy-3-(tetradecyloxy)phenol (63o). To a suspension of 3.8 g (94.9 mmol) of 60% NaH dispersion in 25 mL of DMF was added dropwise under argon at 0 °C, with stirring, a solution of 10 g (71.4 mmol) of 2-methoxyresorcinol in 50 mL of DMF over

20 min. To the mixture was added 1.07 g (7.1 mmol) of NaI followed by the dropwise addition of a solution of 19.8 g (71.4 mmol) of 1-bromotetradecane in 25 mL of DMF over 20 min. The mixture was stirred a room temperature 18 h. The mixture was poured into dilute HCl and extracted with ether. The ether solution was dried ($MgSO_4$), and the solvent was removed. The residue was then distilled in a Kugelrohr apparatus at 0.1 mm. The fraction binding at 80–120 °C consisted mostly of unreacted 2-methoxyresorcinol; the product fraction 130–180 °C was collected. The pot residue consisted largely of dialkylated product. The product fraction was further purified by HPLC on silica gel eluting with hexane–ether (9:1) to give 8.7 g (36%) of **63o** as a thick oil: IR (neat) 3330, 1600 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.89 (t, 1 H, CH), 6.57 (d, 1 H, CH), 6.45 (d, 1 H, CH), 5.80 (s, 1 H, OH), 3.99 (t, 2 H, CH_2O), 3.92 (s, 3 H, OCH_3), 1.83 (m, 2 H, CH_2CH_2O), 1.5–1.2 (m, 22 H, CH_2's), 0.88 (t, 3 H, terminal CH_3); mass spectrum (CI) m/e 337 (M + H). Anal. ($C_{21}H_{36}O_3$) C, H.

3-[2-[3-[[Hydroxy[2-methoxy-3-(tetradecyloxy)phenoxy]phosphinyl]oxy]phenyl]ethyl]-5-methylthiazolium Hydroxide, Inner Salt (86). To a solution of 3.0 g (8.9 mmol) of phenol **63o** and 3.4 g (10.7 mmol) of phosphorus reagent **5c** in 40 mL of CCl_4 was added with stirring at 0 °C under argon a solution of 1.5 mL (10.8 mmol) of triethylamine in 10 mL of CCl_4. The mixture was then stirred at room temperature for 17 h. The mixture was filtered through Celite, and the solvent was removed. The residue was stirred in a mixture of 95 mL of 0.5 M sodium acetate and 95 mL of THF for 2 h. Most of the THF was removed, and the resulting aqueous mixture was chilled in an ice bath and acidified with concentrated HCl. The mixture was extracted three times with ether. The ether solution was washed with brine and dried ($MgSO_4$), and the solvent was removed. The residue was purified by chromatography an 100 g of Florisil, eluting first with $CHCl_3$ to remove less polar impurities and then with $CHCl_3$–CH_3OH (8:2) to elute the product. Solvent was removed from the product fractions to give 3.6 g (67%) of **65o** as a glass: mass spectrum (FAB) m/e 597 (M – H), 519 (M – Br).

A stirred solution of **65o** and 3 g (29.3 mmol) of 5-methylthiazole in 30 mL of toluene was maintained at 80 °C under argon for 6 days. The solvent was removed, and the residue was stirred with 4 g of Amberlyst A-21 ion-exchange resin (weakly basic) in 60 mL of CH_3OH for 1.5 h. The solution was filtered, and solvent was removed. The product was purified by chromatography on silica gel (180 mL dry volume), eluting with CH_3OH–$CHCl_3$ (1:2) to remove less polar impurities and then with CH_3OH–$CHCl_3$–H_2O (65:35:6) to elute product. Product fractions were combined, and solvent was removed. The residue was mixed with ether, and the solid product was collected via filtration and dried in vacuum to give 0.37 g of **86** as a white hygrosopic solid: ^1H NMR (DMSO-d_6 + TFA) δ 9.90 (s, 1 H, thiazole), 8.37 (s, 1 H, thiazole), 7.50–6.85 (m's, 7 H, aromatic), 4.77 (t, 2 H, NCH_2), 4.0 (t, 2 H, CH_2O), 3.72 (s, 3 H, OCH_3), 3.26 (t, 2 H, NCH_2CH_2), 2.54 (s, 3 H, CH_3), 1.75 (m, 2 H, CH_2CH_2O), 1.5–1.10 (m's, 22 H, CH_2's), 0.87 (t, 3 H, terminal CH_3); mass spectrum (FAB) m/e 618 (M + H). Anal. ($C_{33}H_{48}NO_6PS \cdot 1.0H_2O$) C, H, N, P, S.

3-[[2-[[Hydroxy[2-methoxy-3-(tetradecyloxy)phenoxy]phosphinyl]oxy]phenyl]methyl]-5-methylthiazolium Hydroxide, Inner Salt (88). A solution of 2.5 g (7.4 mmol) of **63o**, 1.67 g (8.2 mmol) of reagent **7**, and 0.9 g (8.9 mmol) of triethylamine was stirred in 30 mL of CCl_4 for 18 h. The mixture was diluted with ether and filtered. The solvent was removed to give **66o** which was used in the next step without additional purification.

A solution of **66o**, 3.68 g (37.1 mmol) of 5-methylthiazole, and 0.11 g (0.74 mmol) of NaI in 40 mL of CH_3CN was maintained in a pressure vessel under argon at 85 °C for 20 h. The mixture was cooled, and the solvent was removed. The residue was stirred in 100 mL of CH_3OH containing 10 g of Amberlite IR-45 (weakly basic) resin for 10 min. The mixture was filtered, and the solvent was removed. The residue was purified by silica gel chromatography, eluting first with $CHCl_3$–CH_3OH (8:2) to remove less polar impurities and then with $CHCl_3$–CH_3OH–H_2O (14:6:1) to elute the product. The product fractions were combined, and the solvent was removed. The residue was mixed with ether, and 2.6 g (58%) of **88** was collected as a hygroscopic white powder: ^1H NMR (DMSO-d_6 + TFA) δ 10.06 (s, 1 H, thiazole), 8.20 (s, 1 H, thiazole), 7.60–6.80 (m's, 7 H, aromatic), 5.77 (s, 2 H, CH_2N), 3.99 (t, 2 H, CH_2O), 3.67 (s, 3 H, OCH_3), 2.50 (s, 3 H, CH_3), 1.75 (m, 2 H, CH_2CH_2O), 1.5–1.15 (m's, 22 H, CH_2's), 0.86 (t, 3 H, terminal CH_3); mass spectrum (FAB) m/e 604 (M + H). Anal. ($C_{32}H_{46}NO_6PS \cdot 1.1H_2O$) C, H, N, P, S.

3-[2-[[[3-(Hexadecyloxy)phenoxy]hydroxyphosphinyl]oxy]ethyl]thiazolium Hydroxide, Inner Salt (136). To a solution of 3.0 g (8.96 mmol) of phenol **63a** and 2.82 g (11.7 mmol) of 2-bromoethyl phosphorodichloridate[20] in 40 mL of CCl_4 was added with stirring under argon a solution of 1.63 mL (11.7 mmol) of triethylamine in 5 mL of CCl_4. The mixture was then stirred at room temperature for 3 h and then stored at 5 °C overnight. The mixture was filtered through Celite, and the solvent was removed. The residue was stirred in a mixture of 75 mL of 0.5 M sodium acetate and 75 mL of THF for 1.5 h. Most of the THF was removed, and the resulting aqueous mixture was chilled in an ice bath and acidified with concentrated HCl. The mixture was extracted three times with ether. The ether solution was washed with brine and dried ($MgSO_4$), and the solvent was removed. The residue was purified by chromatography on 100 g of Florisil, eluting first with $CHCl_3$ to remove less polar impurities and then with $CHCl_3$–methanol (9:1) to elute the product. Solvent was removed from the product fractions to give 3.7 g of a glassy residue. This material was dissolved in 20 mL of toluene containing 4.7 g (55.2 mmol) of thiazole. The mixture was maintained at 65–85 °C under argon for 8 days. The solvent was removed, and the residue was stirred with 4 g of Amberlite IR-4B ion-exchange resin (weakly basic) in 75 mL of CH_3OH for 2 h. The solution was filtered, and solvent was removed. The product was purified by chromatography on silica gel (180 mL dry volume), eluting with CH_3OH–$CHCl_3$ (1:2) to remove less polar impurities and then with CH_3OH–$CHCl_3$–H_2O (65:35:6) to elute product. Product fractions were combined, and solvent was removed to give 1.6 g (34%) of **136** as a white hygroscopic solid: ^1H NMR (DMSO-d_6 + TFA) δ 10.2 (m, 1 H, NCHS), 8.49, 8.37 (m's, 2 H, NCHCS), 7.13 (m, 1 H, aromatic), 6.58 (m, 2 H, aromatic), 4.81 (m, 2 H, NCH_2), 4.42 (m, 2 H, CH_2O), 3.86 (m, 2 H, OCH_2), 2.54 (s, 3 H, CH_3), 1.75 (m, 2 H, CH_2CH_2O), 1.5–1.10 (m's, 26 H, CH_2's), 0.88 (t, 3 H, terminal CH_3); mass spectrum (FAB) m/e 526 (M + H). Anal. ($C_{27}H_{44}NO_5PS \cdot 0.5H_2O$) C, H, N, P, S.

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