unlabeled ligands. Incubation was carried out for 20 min (at which time, binding was at equilibrium) at 30 °C and was terminated by centrifugation as previously described.[8] Assays were carried out in quadruplicate, and the nonspecific binding was defined as the radioactivity bound or entrapped in the pellet when the incubation medium contained 10^{-6} M 3-quinuclidinyl benzilate.

Partition Coefficients. The partition coefficients were determined between 1-octanol and an aqueous buffer solution at pH 7.4 (20 °C). The determination of concentrations in each phase was performed spectrophotometrically.

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Registry No. **1**, 108295-86-9; **3**, 80880-93-9; **4**, 21098-65-7; **5**, 885-70-1; **6**, 96437-72-8; **6a**, 28797-48-0; **7**, 108295-85-8; **8**, 113600-38-7; **8**·HCl, 69548-70-5; **8a**, 69541-21-5; **9**, 113600-39-8; **9a**, 69541-24-8; **10**, 113600-48-9; **11**, 113600-49-0; **12**, 113600-50-3; **13**, 87571-94-6; **14**, 87571-89-9; **15**, 87571-92-4; **16**, 113600-53-6; **17**, 69541-27-1; **17a**, 31265-81-3; **18**, 113600-51-4; **19**, 113600-52-5; **21** (p = 1, q = 0), 113600-40-1; **21** (p = 1, q = 0, acid chloride), 113600-44-5; **21** (p = 2, q = 0), 87571-48-0; **21** (p = 2, q = 0, acid chloride), 87571-47-9; **21** (p = 2, q = 1), 113600-41-2; **21** (p = 2, q = 1, acid chloride), 113600-45-6; **21** (p = 1, q = 1), 113600-42-3; **21** (p = 1, q = 1, acid chloride), 113600-46-7; **21** (p = 0, q = 2), 113600-43-4; **21** (p = 0, q = 2, acid chloride), 113600-47-8; **22**, 87571-90-2; *N*-methylpiperazine, 109-01-3; *N*-methylpiperidin-4-ol, 106-52-5; 4-amino-*N*-methylpiperidine, 41838-46-4; 1-amino-4-methylpiperazine, 6928-85-4.

Derivatives of 2-Methylenepropane-1,3-diol as New Antagonists of Platelet Activating Factor

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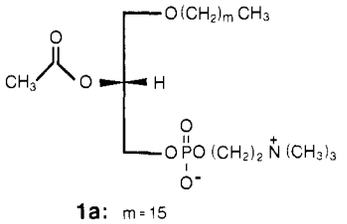
Two new achiral platelet activating factor (PAF) antagonists, *N*-[5-[[2-methylene-3-[[(octadecylamino)carbonyl]-oxy]propoxy]carbonyl]pentyl]pyridinium bromide (**9**) and 3-[6-[[2-methylene-3-[[(octadecylamino)carbonyl]oxy]-propoxy]carbonyl]hexyl]thiazolium bromide (**10**) were synthesized from 2-methylenepropane-1,3-diol (**5**). Platelet aggregation in platelet-rich plasma from rabbits, induced by racemic C_{16}-PAF, was competitively antagonized by **9** or **10**. At concentrations $\leq 10^{-4}$ M, neither compound **9** nor compound **10** caused platelet aggregation, nor did they inhibit platelet aggregation induced by collagen or adenosine diphosphate. Bronchoconstriction in the guinea pig and hypotension in the rat, induced by racemic C_{16}-PAF, were also effectively antagonized by **9** and **10**. Both appear to be more potent as PAF antagonists than Takeda's CV-3988.

Platelet activating factor (PAF) (**1**), an endogenous ether phospholipid identified as 1-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine (alkyl = hexadecyl, octadecyl),[1] exerts via receptor binding[2] a wide range of biological actions.[3] It induces platelet aggregation and smooth-muscle contraction, and in vivo experiments have demonstrated PAF's role in pathological processes,[3] such as asthma,[4] anaphylactic shock,[5] gastric ulceration,[6] and transplant rejection.[7]



1a: m = 15
1b: m = 17

A strong interest in the mode of action of PAF has led to the discovery of several structurally different PAF antagonists.[8] Most of the specific PAF receptor antagonists with PAF-like structures have retained a chiral carbon atom in the moiety that mimics the glycerol backbone of PAF. Such antagonists can be evaluated either as a racemate or as separate enantiomers. Generally, racemates are more readily synthesized than its separate enantiomeric components, but the pharmacological evaluation of a racemate may be a complicated and delicate matter.[9] In contrast to PAF and its unnatural enantiomer, which exhibit very different biological activities,[10] some chiral specific PAF antagonists with PAF-like structures demonstrate almost equal antagonist activity in either enantiomeric form.[11] Thus the chirality at the carbon atom

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(1) Demopoulos, C. A.; Pinckard, R. N.; Hanahan, D. J. *J. Biol. Chem.* **1979**, *254*, 9355–9358. Benveniste, J.; Tencé, M.; Varenne, P.; Bidault, J.; Boullet, C.; Polonsky, J. *C. R. Seances Acad. Sci., Ser. D* **1979**, *289*, 1037–1040.
(2) Godfroid, J. J.; Braquet, P. *Trends Pharmacol. Sci.* **1986**, *7*, 368–373.
(3) *New Horizons in Platelet Activating Factor Research*; Winslow, C. M., Lee, M. L., Eds., Wiley: New York, 1987. Braquet, P.; Touqui, L.; Shen, T. Y.; Vargaftig, B. B. *Pharmacol. Rev.* **1987**, *39*, 97–145. Chustecka, Z. *An Overview of R and D Involving Platelet Activating Factor (PAF)*; PJB Publications: Richmond, Surrey, England, 1986. Hanahan, D. J. *Ann. Rev. Biochem.* **1986**, *55*, 483–509. Snyder, F. *Med. Res. Rev.* **1985**, *5*, 107–140. Venuti, M. C. *Annu. Rep. Med. Chem.* **1985**, *20*, 193–202.
(4) Page, C. P. *Developments in Asthma. A View of Current Research*; PJB Publications: Richmond, Surrey, England, 1987. Mencia-Huerta, J. M.; Benhamou, M. In *Asthma. Clinical Pharmacology and Therapeutic Progress*; Kay, A. B., Ed.; Blackwell Scientific: Oxford, 1986; pp 237–250.
(5) Feuerstein, G.; Hallenbeck, J. M. *Annu. Rev. Pharmacol. Toxicol.* **1987**, *27*, 301–313.
(6) Rosam, A.-C.; Wallace, J. L.; Whittle, B. J. R. *Nature (London)* **1986**, *319*, 54–56.
(7) Foegh, M. L.; Khirabadi, B. S.; Rowles, J. R.; Braquet, P.; Ramwell, P. W. *Transplantation* **1986**, *42*, 86–88.
(8) Saunders, R. N.; Handley, D. A. *Annu. Rev. Pharmacol. Toxicol.* **1987**, *27*, 237–255. Braquet, P.; Godfroid, J. J. *Trends Pharmacol. Sci.* **1986**, *7*, 397–403.
(9) Williams, K.; Lee, E. *Drugs* **1985**, *30*, 333–354.
(10) Wykle, R. L.; Craig, H. M.; Lewis, J. C.; Schmitt, J. D.; Smith, J. A.; Jefferson, J. R.; Piantadosi, C.; O'Flaherty, J. T. *Biochem. Biophys. Res. Commun.* **1981**, *100*, 1651–1658.

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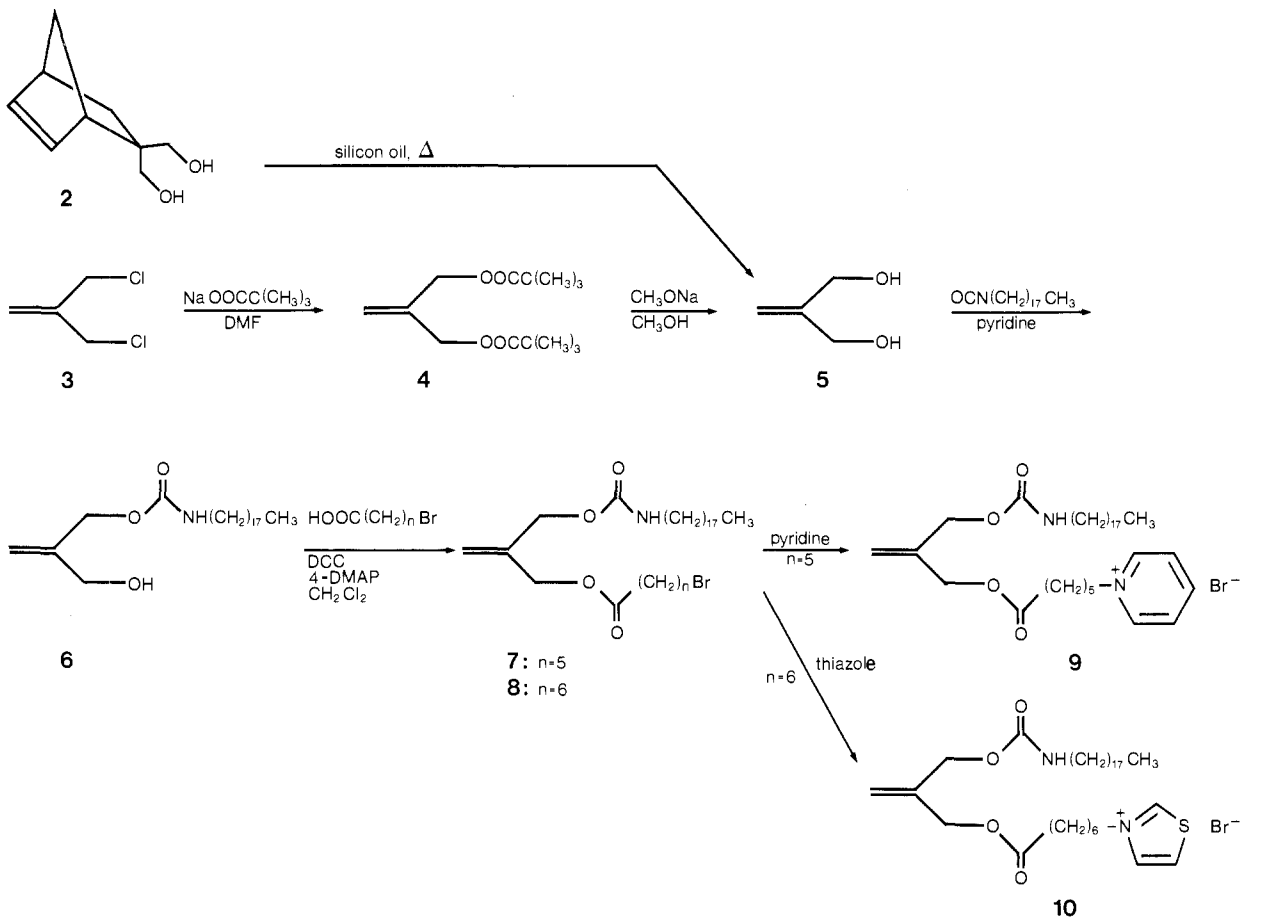
Scheme I



which mimics C-2 of glycerol is, at least in some cases, not important to PAF antagonist activity. This would speak in favor of testing achiral PAF analogues as antagonists. Such achiral analogues could profit by their synthetic simplicity and would not suffer from the disadvantage of being a racemate, i.e., compound mixture. We report some of our chemical and biological findings regarding two representatives, **9** and **10**, of a number of tested achiral PAF analogues, and, for comparison, we have included Takeda's racemic PAF antagonist, CV-3988,[12] in our studies.

Chemistry

PAF analogues **9** (GS-1065-180) and **10** (GS-1160-180) were prepared (Scheme I) from the diol **5**.[13-15] In an attempt to develop a convenient method for a multigram preparation of **5** we have modified existing literature procedures. Two methods have emerged. The first method (A), a retro-Diels–Alder reaction[13,14] of 5-norbornen-2-ylidenedimethanol[13,16] (**2**), was carried out in simple glass equipment by heating in silicone oil, maintaining strict temperature control (235–237 °C). Although the yield (54%) of isolated **5** with this method was not higher than in the previous methods, it required no special thermolysis equipment, which seriously limits the convenience of producing multigram quantities of **5**.

The second method (B) utilized 2-methylene-1,3-dichloropropane (**3**) (cf. ref 15) in a nucleophilic substitution with pivalate ion to give 2-methylene-1,3-propylene dipivalate (**4**), followed by base-catalyzed methanolysis to produce the diol **5** in 76% overall yield. Methyl pivalate, formed by the methanolysis, had to be removed from the equilibrium mixture (by evaporation) in order to convert 4/3-hydroxy-2-methylenepropyl pivalate to the diol **5**. Any attempt to convert **3** directly into **5** in satisfactory yield with hydroxide ion was unsuccessful, due to competing intramolecular displacement reaction of 2-methylene-3-chloro-1-propanolate ion to give 3-methyleneoxetane (cf. ref 17).

The diol **5** was reacted with 1 equiv of octadecyl isocyanate in pyridine to give the monocarbamate **6** together with minor amounts of the less soluble 2-methylene-1,3-propylene *N,N'*-dioctadecyldicarbamate and unreacted **5**. Purification of **6** was achieved by chromatography and the final yield was 42%. With use of dicyclohexylcarbodiimide

(11) Barner, R.; Hadvary, P.; Burri, K.; Hirth, G.; Cassal, J.-M.; Müller, K. Eur. Patent 147 768, 1984; *Chem. Abstr.* **1986**, *104*, 88 939. Hadvary, P.; Baumgartner, H. R.; Barner, R.; Burri, K.; Cassal, J.-M.; Hirth, G.; Müller, K.; Weller, T. *Abstracts of Lecture Presentations*; Second International Conference on Platelet-Activating Factor and Structurally Related Ether Lipids; Gatlinburg, TN, 1986, p 28. Wichrowski, B.; Jonqney, S.; Heymans, F.; Broquet, C.; Godfroid, J. J.; Fichelle, J.; Worcel, M. *Abstracts of Poster Communications*; Second International Conference on Platelet-Activating Factor and Structurally Related Ether Lipids; Gatlinburg, TN, 1986; No. 29, p 117.

(12) Terashita, Z.; Tsushima, S.; Yoshioka, Y.; Nomura, H.; Inada, Y.; Nishikawa, K. *Life Sci.* **1983**, *32*, 1975–1982.

(13) Weiss, F.; Bensa, R. Fr. Patent 1 350 723, 1962; *Chem. Abstr.* **1964**, *60*, 13 206. Weiss, F.; Isard, A.; Bensa, R. *Bull. Soc. Chim. Fr.* **1965**, 1355–1358.

(14) Corey, E. J.; Suggs, J. W. *Tetrahedron Lett.* **1975**, 3775–3778.

(15) Ducharme, Y.; Latour, S.; Wuest, J. D. *Organometallics* **1984**, *3*, 208–211.

(16) Cambell, T. W.; Foldi, V. S. *J. Org. Chem.* **1961**, *26*, 4654–4658.

(17) Searles, S. In *Comprehensive Heterocyclic Chemistry*; Lwowski, W.; Katritzky, A. R., Rees, C. W., Eds.; Pergamon: Oxford, 1984; Vol. 7, Chapter 5.13.

Table I. PAF Antagonist Activities

compd	platelet aggregation:[a] EC_{50},[b] μM	bronchoconstriction:[c] ED_{50},[d] mg/kg iv	blood pressure:[e] ED_{50},[f] mg/kg iv
9	0.5	0.2	0.5
10	0.25	0.1	0.5
CV-3988	20	3.5	1.0

[a] Antagonists were added to platelet-rich plasma from rabbits 3 min before PAF (0.06 μM). [b] Concentration required to reduce to 50% of maximum aggregation. [c] Antagonists were dosed to anesthetized guinea pigs 3 min before PAF (30 ng/kg). [d] Dose required to reduce the bronchoconstriction caused by PAF by 50%. [e] Antagonists were dosed to anaesthetized rats 10 min before PAF (25 ng/kg). [f] Dose required to reduce the lowering of the arterial blood pressure caused by PAF by 50%.

as a dehydrating agent and 4-(dimethylamino)pyridine as a catalyst, the alcohol **6** was esterified with 6-bromohexanoic acid to give **7** (84% yield) or with 7-bromoheptanoic acid to give **8** (79% yield).

The bromo compound **7** was reacted with pyridine to give the final pyridinium compound **9** in 84% yield. Similarly, **8** was reacted with thiazole to give the final thiazolium compound **10** in 90% yield.

HPLC analysis of **9** and **10** was achieved by ion-pair chromatography. The retention time was rather sensitive to small changes in eluent composition ($MeOH/H_2O$ ratio). A tailing peak was avoided by a sufficient concentration of ion-pairing agent (tetramethylammonium perchlorate).

Biological Results

We report the results of the biological testing of **9** and **10** as PAF antagonists in a comparative study with CV-3988 (Table I). The compounds were tested (i) in vitro for their ability to inhibit platelet aggregation caused by PAF, collagen, or adenosine diphosphate in platelet-rich plasma (PRP) from rabbits and (ii) in vivo for their ability to inhibit PAF-induced bronchoconstriction in guinea pigs and PAF-induced hypotension in rats.

The in vitro platelet aggregation experiments with PRP from rabbits revealed **10** as the most effective PAF antagonist (EC_{50} = 0.25 μM), twice as active as **9** and 80 times more active than CV-3988, which recently[18] has been characterized as a specific and competitive antagonist to PAF.

Neither compound **9** nor **10** induced platelet aggregation at concentrations $\leq 10^{-4}$ M, and at these concentrations neither **9** nor **10** exhibited any inhibitory effect on platelet aggregation induced by collagen or adenosine diphosphate (ADP). To investigate whether **9** and **10** are competitive antagonists to PAF, various concentrations of PAF were added to PRP treated with fixed concentrations of **9** (10^{-7} M), **10** (10^{-7} M), or CV-3988 (10^{-6} M) 3 min before PAF. The dose–response curves are shown in Figure 1.

In vivo experiments with PAF-induced bronchoconstriction in guinea pigs demonstrated that **10** (ED_{50} = 0.1 mg/kg iv) was twice as active as **9** and 35 times more active than CV-3988 as PAF antagonist.

The difference in activity was less marked from the experiments with PAF-induced hypotension in rats. Compounds **9** and **10** were equipotent (ED_{50} = 0.5 mg/kg iv) but only twice as active as CV-3988.

Discussion

Our aim was to produce compounds that are nonracemic, easy to synthesize, and effective as specific PAF antagonists. PAF is a chiral compound, but we thought that the conservation of this chirality in PAF analogues was not a prerequisite for good PAF antagonist activity. Indeed, several chiral PAF analogues are equally active as antagonists in either enantiomeric form.[11] The inherent chirality of PAF analogues that are derivatives of glycerol may cause some difficulties as far as the synthesis (of optically pure compounds) or the biological evaluation (of racemates) is concerned. By choosing an achiral substitute for glycerol, both synthetic simplicity and straightforward biological evaluation can be achieved. We decided to synthesize readily obtainable achiral PAF analogues on the basis of 2-methylene-1,3-propanediol (**5**) as a "glycerol substitute".

A great number of PAF analogues have been prepared and tested for PAF-related activity and considerable knowledge about structure/activity relationships has been gained.[2,3,8] The structural analysis of a good first generation PAF antagonist of the PAF analogue type predicts the following general features. The compound should possess (i) a quaternary (preferentially aromatic) ammonium (e.g., pyridinium or thiazolium) ion, (ii) a polar group (e.g., phosphate, ether, ester) at position *sn*-C-3 of glycerol or similar fragment, (iii) a chain of five to eight atoms connecting position *sn*-C-3 of glycerol or similar fragment with the ammonium ion, (iv) a glycerol moiety or similar fragment, with a variety of substituents at nonterminal position(s) of this fragment, (v) a polar group (e.g., carbamate) at position *sn*-C-1 of glycerol or similar fragment, and (vi) attached to this group a nonpolar chain (saturated or unsaturated) similar to that of PAF (**1**). Structural combinations fulfilling any of these requirements will not always give compounds with PAF antagonist activity. This investigation demonstrates, however, that some of the most readily obtainable achiral compounds of the PAF analogue type, **9** and **10**, meeting the above criteria are powerful PAF antagonists.

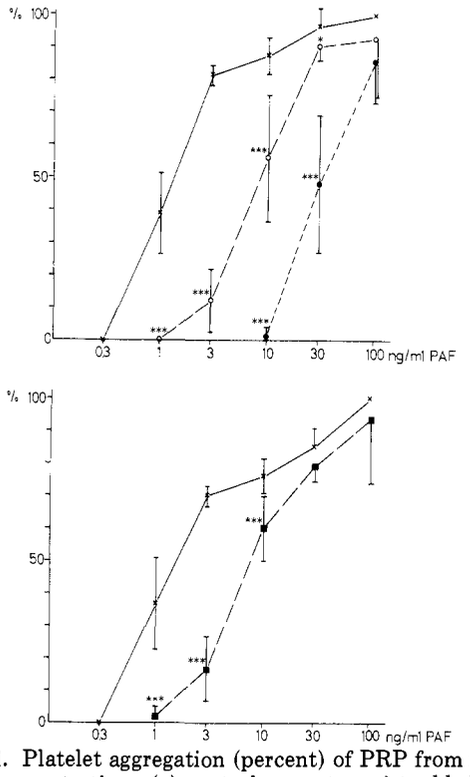


Figure 1. Platelet aggregation (percent) of PRP from rabbits vs PAF concentration: (x) control, no antagonist added, (○) **9** (10^{-7} M) added 3 min before PAF, (●) **10** (10^{-7} M) added 3 min before PAF, (■) CV-3988 (10^{-6} M) added 3 min before PAF. Values are means ± SD, n = 6. Student's t test: (***) $p < 0.01$, (*) $p < 0.05$.

(18) Hayashi, M.; Kimura, J.; Oh-Ishi, S.; Tsushima, S.; Nomura, H. *Jpn. J. Pharmacol.* **1987**, *44*, 127–134.

Platelet aggregation studies have demonstrated that **9** and **10** are competitive antagonists of PAF (Figure 1), being 40 and 80 times, respectively, more active than CV-3988 (Table I). Compounds **9** and **10** showed no sign of platelet aggregating (i.e., agonist) activity at $\leq 10^{-4}$ M. At these concentrations no inhibition of platelet aggregation was observed when collagen or ADP was added 3 min later. Thus, compounds **9** and **10** are specific and competitive antagonists of PAF.

PAF-induced bronchoconstriction in the guinea pig and hypotension in the rat were effectively inhibited by—in decreasing order of potency—**10**, **9**, and CV-3988. In the guinea pig the activity of **9** or **10** is more than 1 order of magnitude greater than the activity of CV-3988, whereas the difference in the activities between all three compounds is much smaller in the rat. Whether this change in relative potency of **9**, **10**, and CV-3988 reflects a difference between the receptor affinities in the two animal species or is caused by other factors, such as differences in transport and metabolism, is not known.

Further investigations of PAF analogues will result in a greater understanding of the structure/activity relationship and eventually lead to powerful PAF antagonists, surpassing those of today. Such investigations are in progress.

Experimental Section

A. Chemistry. Melting points are uncorrected. IR spectra were recorded on a Perkin-Elmer 457/783 spectrometer. NMR spectra were recorded at 100 MHz on a JEOL FX 100 instrument with Me_4Si as internal reference. TLC was performed on silica gel 60 F_{254} plates (Merck), and the spots were visualized by spraying with 2 M H_2SO_4 and 10% (w/v) phosphomolybdic acid in EtOH, ca. 150 °C. Column chromatography was performed on a Waters PrepLC/system 500A by using a PrepPAK-500/silica cartridge with a flow rate at 250 mL/min. To prolong the lifetime of the prepacked Waters columns, all samples were washed through silica gel 60 (in amounts adequate to remove TLC base line material) with the eluent to be used on the Waters column. Elemental analyses were performed by the Microanalytical Laboratory, Leo Pharmaceutical Products.

2-Methylene-1,3-propanediol (5). Method A. A stirred mixture of 5-norbornen-2-ylidenedimethanol[13,16] (**2**) (40 g, 0.26 mol) and silicone oil (Aldrich, *d* 1.05, 60 mL) in a distillation flask was heated to 235–237 °C[19] under a gentle stream of nitrogen. After ca. 4 h, when product collection had ceased, the temperature was gradually raised during 1 h to 300 °C. Pentane (20 mL) was added to the distillate, and the mixture was extracted with water (2 × 20 mL). The water was removed in vacuo, and the residue (16.7 g) was distilled at 125–128 °C (20 mm) (lit.[14] bp 127–129 °C (32 mm)) to give 12 g (0.14 mol, 54%) of **5** as a colorless oil: NMR ($CDCl_3$) δ 2.15 (s, 2 H, 2 OH), 4.22 (t, 4 H, J = 1 Hz, 2 CH_2O), 5.13 (p, 2 H, J = 1 Hz, H_2C=C).

Method B. A mixture of 1,3-dichloro-2-methylenepropane (**3**) (135 g, 1.08 mol) and sodium pivalate (335 g, 2.7 mol) was stirred in DMF (1.5 L) at 80 °C for 7 h. After cooling, water (1.5 L) was added, and the mixture was extracted with Et_2O (3 × 1.5 L). The combined extracts were washed with water (1 L), dried ($MgSO_4$), and evaporated to dryness in vacuo. The crude 2-methylene-1,3-propylene dipivalate (**4**) (270 g) was used in the following reaction without further purification. An analytical sample was obtained by distillation at 90–91 °C (0.5 mm): mp 29–30 °C; bp 257–259 °C (760 mm); NMR ($CDCl_3$) δ 1.22 (s, 18 H, 6 CH_3), 4.60 (t, 4 H, J = 1 Hz, 2 CH_2O), 5.24 (p, 2 H, J = 1 Hz, H_2C=C); IR ($CHCl_3$) 2975, 1725, 1480, 1285, 1150 cm^{-1}. Anal. ($C_{14}H_{24}O_4$) C, H. Crude **4** (from above) (270 g) was stirred with CH_3ONa (54 g, 1.0 mol) in CH_3OH (1.5 L) at room temperature for 20 h. The reaction mixture was evaporated at 40 °C (15 mm) to remove CH_3OH and methyl pivalate. The residue was redissolved in CH_3OH (1.5 L) and stirred at room temperature for 4 h. After neutralization with 4 M HCl to pH ca. 7, the reaction mixture was evaporated at 40 °C (15 mm). The residue was extracted with $CHCl_3$ (3 × 250 mL), and the extracts were evaporated in vacuo. The diol **5** was obtained by distillation (see method A), overall yield 72.2 g (0.82 mol, 76%).

3-Hydroxy-2-methylenepropyl *N*-Octadecylcarbamate (6). Octadecyl isocyanate (59.1 g, 200 mmol) and 2-methylenepropane-1,3-diol (**5**) (17.7 g, 200 mmol) were stirred in pyridine (100 mL) at 22 °C for 24 h. Water (200 mL) was added, and the mixture was extracted with $CHCl_3$ (3 × 200 mL). The $CHCl_3$ extracts were washed with water (200 mL), dried ($MgSO_4$), and evaporated to dryness in vacuo. The residue was extracted with 100-mL portions of $CHCl_3/Et_2O$, 1:1, v/v, until all of the desired product had been extracted as checked by TLC ($CHCl_3/Et_2O$, 1:1, v/v), R_f 0.5. The combined extracts were chromatographed on a preparative Waters chromatograph with $CHCl_3/Et_2O$/pentane, 1:1:3, v/v/v, as eluent. The product was recrystallized from acetone: yield 32.0 g (83 mmol, 42%); mp 71–73 °C; NMR ($CDCl_3$) δ 0.9 (t, 3 H, J = 6 Hz, CH_3), 1.1–1.7 (m, 32 H, CCH_2C), 2.4 (t, 1 H, J = 6 Hz, OH), 3.17 (m, 2 H, CH_2N), 4.12 (d, 2 H, J = 6 Hz, CH_2OH), 4.64 (s, 2 H, CH_2OC), 4.75 (m, 1 H, NH), 5.17 (m, 2 H, H_2C=C); IR ($CHCl_3$) 3450, 2925, 1710, 1518 cm^{-1}. Anal. ($C_{23}H_{45}NO_3$) C, H, N.

2-Methylene-3-[[(octadecylamino)carbonyl]oxy]propyl 6-Bromohexanoate (7). 3-Hydroxy-2-methylenepropyl *N*-octadecylcarbamate (**6**) (1.62 g, 4.2 mmol), *N*,*N*′-dicyclohexylcarbodiimide (1.08 g, 5.2 mmol), 4-(dimethylamino)pyridine (52 mg, 0.43 mmol), and 6-bromohexanoic acid (0.91 g, 4.7 mmol) were stirred in CH_2Cl_2 (50 mL) at 22 °C for 20 h. Ether (50 mL) was added, and the mixture was filtered. The filtrate was chromatographed on a preparative Waters chromatograph with $CHCl_3/Et_2O$/pentane, 1:1:3, v/v/v, as eluent. TLC ($CHCl_3/Et_2O$/pentane, 1:1:1, v/v/v) R_f 0.75. Recrystallization from MeOH gave the desired product: 1.97 g (3.5 mmol, 84%); mp 50–51 °C; NMR ($CDCl_3$) δ 0.9 (t, 3 H, J = 6 Hz, CH_3), 1.2–2.0 (m, 38 H, CCH_2C), 2.37 (t, 2 H, J = 7 Hz, CH_2C=O), 3.17 (m, 2 H, CH_2N), 3.40 (t, 2 H, J = 7 Hz, CH_2Br), 4.60 (s, 4 H, 2 CH_2O), 4.7 (m, 1 H, NH), 5.24 (br s, 2 H, H_2C=C). Anal. ($C_{29}H_{54}BrNO_4$) C, H, Br, N.

2-Methylene-3-[[(octadecylamino)carbonyl]oxy]propyl 7-Bromoheptanoate (8). Via the above procedure for the synthesis of **7**, with 6-bromohexanoic acid replaced with 7-bromoheptanoic acid, the desired product was obtained in 79% yield: TLC ($CHCl_3/Et_2O$/pentane, 1:1:1, v/v/v) R_f 0.75; mp 49 °C (methanol); NMR ($CDCl_3$) δ 0.87 (t, 3 H, J = 6 Hz, CH_3), 1.1–2.0 (m, 40 H, CCH_2C), 2.35 (t, 2 H, J = 7 Hz, CH_2C=O), 3.14 (q, 2 H, J = 6 Hz, CH_2N), 3.40 (t, 2 H, J = 7 Hz, CH_2Br), 4.60 (s, 4 H, 2 CH_2O), 4.65 (m, 1 H, NH), 5.24 (m, 2 H, H_2C=C). Anal. ($C_{30}H_{56}BrNO_4$) C, H, N.

***N*-[5-[[2-Methylene-3-[[(octadecylamino)carbonyl]oxy]propoxy]carbonyl]pentyl]pyridinium Bromide (9).** 2-Methylene-3-[[(octadecylamino)carbonyl]oxy]propyl 6-bromohexanoate (**7**) (0.84 g, 1.50 mmol) was dissolved in dry pyridine (20 mL) and kept at 100 °C for 7 h. After the mixture was cooled, excess pyridine was removed in vacuo. The residue was recrystallized from acetone/Et_2O, yield 0.82 g (1.27 mmol, 84%). The purity was checked by analytical HPLC on a Merck LiChrosorb RP-18 column eluting with 5 mM tetramethylammonium perchlorate in MeOH/H_2O, 9:1, v/v, with detection at 260 nm: mp 57–58 °C; hygroscopic; NMR ($CDCl_3$) δ 0.9 (t, 3 H, J = 6 Hz, CH_3), 1.1–1.9 (m, 36 H, CCH_2C), 1.9–2.25 (m, 2 H, CH_2CN^+), 2.36 (t, 2 H, J = 7 Hz, CH_2C=O), 3.16 (m, 2 H, CH_2NH), 4.57 (s, 4 H, 2 CH_2O), 5.03 (t, 3 H, J = 6 Hz, CH_2N, NH), 5.23 (m, 2 H, H_2C=C), 8.17 (t, 2 H, J = 7 Hz, H-3, H-5 of pyridine), 8.57 (t, 1 H, J = 7 Hz, H-4 of pyridine), 9.61 (d, 2 H, J = 7 Hz, H-2, H-6 of pyridine). Anal. ($C_{34}H_{59}BrN_2O_4 \cdot 0.5H_2O$) C, H, Br, N, H_2O.

3-[6-[[2-Methylene-3-[[(octadecylamino)carbonyl]oxy]propoxy]carbonyl]hexyl]thiazolium Bromide (10). 2-Methylene-3-[[(octadecylamino)carbonyl]oxy]propyl 7-bromoheptanoate (**8**) (0.57 g, 1.00 mmol) was dissolved in thiazole (2.0 mL) and kept at 100 °C for 7 h. After cooling, excess thiazole was removed in vacuo. The residue was recrystallized from acetone/Et_2O or from MeOH, yield 0.61 g (0.90 mmol, 90%). The purity was checked by analytical HPLC as described for **2** with detection at 238 nm: mp 55–56 °C; hygroscopic; NMR ($CDCl_3$) δ 0.88 (t, 3 H, J = 6 Hz, CH_3), 1.0–2.0 (m, 38 H, CCH_2C), 2.1 (m,

(19) This is the boiling point of **5** at 760 mm; at higher temperatures unreacted starting compound (**2**) distilled.

2 H, CH_2CN^+), 2.35 (t, 2 H, J = 7 Hz, $CH_2C{=}O$), 3.14 (q, 2 H, J = 6 Hz, CH_2NH), 4.58 (s, 4 H, 2 CH_2O), 4.88 (t, 2 H, J = 6 Hz, CH_2N^+), 5.02 (m, 1 H, NH), 5.24 (s, 2 H, $H_2C{=}C$), 8.43 (m, 1 H, H-5 of thiazole), 8.69 (d, 1 H, J = 3 Hz, H-6 of thiazole), 11.15 (s, 1 H, H-2 of thiazole). Anal. ($C_{33}H_{59}BrN_2O_4S{\cdot}H_2O$) C, H, Br, N, S, H_2O.

B. Biological Methods. Platelet Aggregation in Platelet-Rich Plasma (PRP). Platelet aggregation studies were performed by the method of Born and Cross.[20] Blood was collected in 3.8% sodium citrate (1 mL for 9 mL of blood) by cardiac puncture from conscious Chinchilla rabbits (≃3 kg). Platelet-rich plasma was obtained by centrifugation of blood at 290g for 10 min at room temperature. Platelet aggregation was monitored by continuous recording of light transmission in a dual-channel Payton aggregometer. Various concentrations of PAF (racemic C_{16}-PAF, Sigma), collagen, or adenosine diphosphate (ADP) (in 30 μL of H_2O) were added to 0.3 mL of incubated and stirred PRP. The dose producing 100% aggregation (6×10^{-8} M (30 ng/mL) PAF (see Figure 1), 60 μg/mL collagen, or 6×10^{-6} M ADP) was used in the testing of antagonists. The compounds to be tested were added in 30 μL of H_2O in various concentrations 3 min before PAF, collagen, or ADP. The concentrations (EC_{50}) causing 50% inhibition of PAF-induced aggregation were determined (Table I) by linear regression from a log concentration vs inhibitory effect plot generated by three concentrations (each repeated four times). The correlation coefficient for all regression lines was >0.93. Neither compound **9** nor compound **10** caused any platelet aggregation at concentrations ≤ 10^{-4} M nor did they antagonize aggregation caused by collagen or ADP at these concentrations. Various concentrations of PAF were added to PRP treated with **9** (10^{-7} M), **10** (10^{-7} M), or CV-3988 (10^{-6} M) 3 min before PAF. The dose–response curves are shown in Figure 1.

Bronchoconstriction in the Guinea Pig. Female Dunkin–Hartley guinea pigs (400–500 g) were anesthetized with ethyl carbamate (1.3 g/kg ip). The jugular vein was cannulated for administration of drugs and the trachea for artificial ventilation with a rodent ventilator (Ugo Basile, Comerio-Varese, Italy) (60 strokes/min, 10 mL/kg). Resistance to lung inflation was measured by a modification of the Konzett Rössler overflow technique[21] by a bronchial transducer (Ugo Basile) (8 cm of H_2O). The animals were given the β-adrenergic blocking agent propranolol (0.1 mg/kg) 10 min before PAF and the muscle depolarizing agent suxamethonium chloride (1.2 mg/kg) 5 min before PAF. The dose of PAF (30 ng/kg in 0.5 mL of H_2O/kg) giving 80% of maximal obtainable bronchoconstriction was used in the testing of antagonists. Each animal received only one dose of test compound since guinea pigs show tachyphylaxis to PAF. The antagonist compounds were dosed (in 0.5 mL of H_2O/kg) 3 min before PAF, and the doses (ED_{50}) inducing 50% inhibition of the PAF bronchoconstriction were determined (Table I) by linear regression from a log concentration vs percent inhibitory effect plot generated by three concentrations (five animals/dose). The correlation coefficient for all regression lines was >0.93.

Rat Hypotension. Female Sprague–Dawley albino rats weighing about 200 g were anesthetized with sodium pentobarbital (40 mg/kg) by intraperitoneal injection. PAF and the antagonists were injected via a cannulated femural vein, and arterial pressure was registered from a cannulated artery by means of a Statham P 23 IB pressure transducer. PAF (25 ng/kg) was injected (in 1.0 mL H_2O/kg) twice at 20-min intervals to obtain about 50-mmHg lowering of arterial mean blood pressure. The antagonists were dosed (in 1.0 mL of H_2O/kg) 10 min before the second PAF dose. The doses (ED_{50}) required to reduce the PAF hypotension by 50% were determined (Table I) by linear regression from a log concentration vs percent reduction of hypotension plot generated by three concentrations (six animals/dose). The correlation coefficient for all regression lines was >0.91.

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(20) Born, G. V. R.; Cross, M. J. *J. Physiol. (London)* **1963**, *168*, 178–195.

(21) Konzett, H.; Rössler, R. *Naunyn-Schmiedeberg's Arch. Exp. Pathol. Pharmakol.* **1940**, *195*, 71–74.