and to the Deutsche Forschungsgemeinschaft, who supported this work by grants (SFB 234-85). The technical assistance of Maria Beer and Gabi Seidl is gratefully acknowledged. We thank Dr. A. E. Bogden, EG & G Bogden Laboratories, Worcester, MA, for providing us with the MXT tumor and for helpful discussions in setting up the MXT-tumor model.

Registry No. 2, 28714-19-4; 2a, 28714-18-3; 2b, 711-38-6; 3, 74944-95-9; 4, 99749-15-2; 4a, 99749-11-8; 4b, 30724-22-2; 5, 2962-14-3; 6, 99749-16-3; 6a, 99749-13-0; 6b, 28714-28-5; 7, 78682-42-5; 8, 99749-17-4; 8a, 99749-14-1; 8b, 99749-12-9; F_3CCO_2H, 76-05-1; 4-$MeOC_6H_4MgBr$, 13139-86-1; 3-$MeOC_6H_4MgBr$, 36282-40-3.

Supplementary Material Available: ^1H NMR data (Table VII) of compounds 2, 2a, 4, 4a, 6, 6a, 8, and 8a (2 pages). Ordering information is given on any current masthead page.

Analogues of Platelet Activating Factor. 4.[1] Some Modifications of the Phosphocholine Moiety

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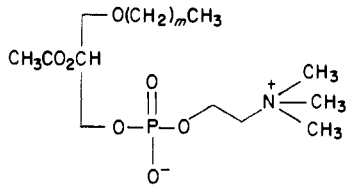
Racemic analogues of platelet activating factor (PAF) in which the methylene bridge separating the phosphate and trimethylammonium moieties is altered in length (7a–f) have been prepared. Increasing the length of this bridge results in a progressive decrease in the hypotensive and platelet aggregation responses. Analogues in which the phosphocholine group is substituted with a methyl group (7h and 7i) or a phenyl group (5j) or in which the methylene bridge is replaced with a meta-substituted benzyl group (5k) have been prepared. With respect to both the blood pressure and platelet aggregation responses, 7i and 5k showed little if any changes in potency compared to racemic C_{16}-PAF (1a). While 7h is more potent than 1a with respect to both the hypotensive and platelet aggregation properties, 5j is less potent. Replacement of the phosphate moiety of C_{18}-PAF (1b) with a phosphonate group (7g) leads to decreased activity in both assays. Analogue 11, in which the phosphocholine group has been replaced with a 4-(trimethylammonio)butoxy group, exhibited no detectable hypotensive or platelet aggregating activity. None of the analogues exhibited a separation of the blood pressure and platelet aggregation activities.

Platelet activating factor (PAF), a mixture of homologous alkyl ether phospholipids of structure 1, possesses a

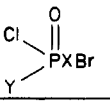


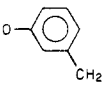
1a, m = 15
1b, m = 17

variety of biological activities. It is one of the important mediators of anaphylaxis and inflammation and has the ability to activate various inflammatory cells such as basophils, neutrophils, and platelets. PAF is also very potent in its ability to lower blood pressure.[2]

Over the last few years we have been involved in structure–activity studies of this important phospholipid molecule. Previously, we have reported compounds containing modifications of the alkyl ether chain[1a] and glycerine backbone.[1b] We[1c] and others[3] have reported compounds in which the phosphocholine portion of the PAF molecule has been altered. In this report we describe the preparation and structure–activity relationships of

Table I. Phosphorus Reagents Used



no.	X	Y	method	reactn time, h	ref
3a	$O(CH_2)_3$	Cl	A	18	5
3b	$O(CH_2)_4$	Cl	A	18	5
3c	$O(CH_2)_6$	Cl	A	18	5
3d	$O(CH_2)_8$	Cl	A	18	5
3e	$O(CH_2)_{10}$	Cl	A	18	5
3f	$(CH_2)_2$	OH	*a*	18	6
3g	$OCH_2CH(CH_3)$	Cl	A	18	*b*
3h	$OCH(CH_3)CH_2$	Cl	A	18	*b*
3i	$OCH_2CH(C_6H_5)$	Cl	A	76	*b*
3j		Cl	B	20	*b*

[a] See ref 6. [b] This work.

(1) For previous publications in this series see: (a) Wissner, A.; Sum, P.-E.; Schaub, R. E.; Kohler, C. A.; Goldstein, B. M. *J. Med. Chem. 1984, 27,* 1174. (b) Wissner, A.; Sum, P.-E.; Schaub, R. E.; Kohler, C. A.; Goldstein, B. M. *Ibid.* **1985**, *28,* 1181. (c) Wissner, A.; Kohler, C. A.; Goldstein, B. M. *Ibid.* **1985**, *28,* 1365.

(2) For recent reviews of the biological properties of PAF see: (a) Pinckard, R. N.; McManus, L. M.; Hanahan, D. J. *Adv. Inflammation Res.* **1982**, *4,* 147. (b) Snyder, F. *Ann. Rep. Med. Chem.* **1982**, *17,* 243. (c) Vargaftig, B. B.; Chegnord, M.; Benveniste, J.; Lefort, J.; Wal, F. *Ann. N.Y. Acad. Sci.* **1981**, *370,* 119. (d) O'Flaherty, J. T.; Wykle, R. L. *Clin. Rev. Allergy,* **1983**, *1,* 353. (e) Vargaftig, B. B.; Benveniste, J. *Trends Pharmacol. Sci.* **1983**, *4,* 341.

(3) (a) Hanahan, D. J.; Munder, P. G.; Satouchi, K.; McManus, L.; Pinckard, R. N. *Biochem. Biophys. Res. Commun.* **1981**, *99,* 183. (b) Tence, M.; Coeffier, E.; Heymans, F.; Polonsky, J.; Godfroid, J.; Benveniste, J. *Biochim.* **1981**, *63,* 723. (c) Satouchi, K.; Pinckard, R. N.; McManus, L. M.; Hanahan, J. T. *J. Biol. Chem.* **1981**, *256,* 4425. (d) Moschidis, M. C.; Demopoulos, C. A.; Kritikou, L. G. *Chem. Phys. Lipids* **1983**, *33,* 87. (e) Nakomura, N.; Miyazaki, H.; Ohkawa, N.; Koike, H.; Sada, T.; Asai, F.; Kobayoshi, S. *Chem. Pharm. Bull.* **1984**, *32,* 2452. (f) Kasuya, Y.; Masuda, Y.; Shigenobu, K. *Can. J. Physiol. Pharmacol.* **1983**, *62,* 457.

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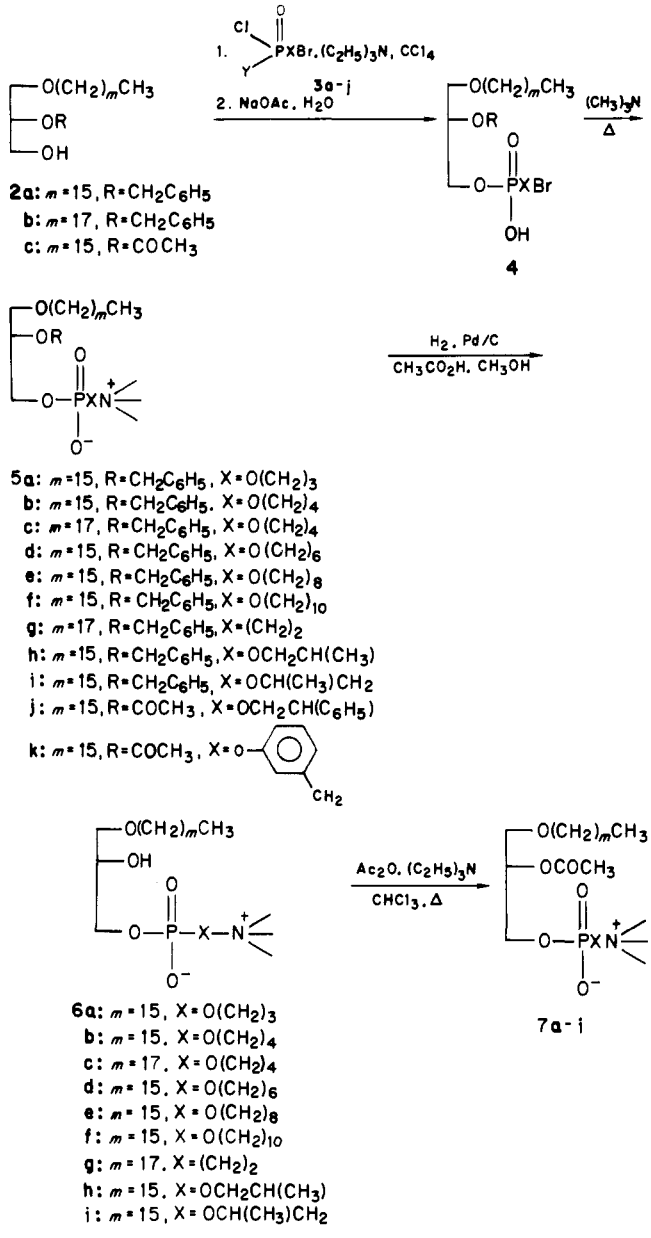
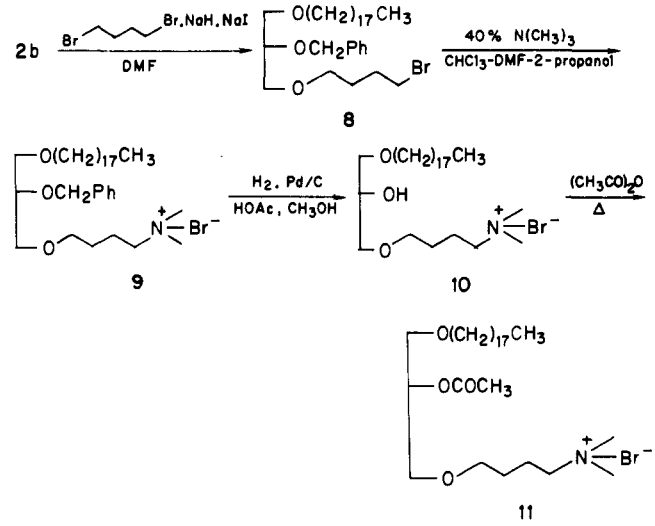
Scheme I



some PAF analogues containing additional modifications of the phosphocholine moiety. In particular, we now describe some racemic PAF analogues in which the methylene bridge separating the phosphate and trimethylammonium moieties is altered in length, is substituted with methyl or phenyl groups, or is replaced with an aromatic ring. In addition, compounds in which the phosphate group has been replaced by a phosphonate moiety or has been totally removed will be described.

Chemistry. The preparation of the various PAF analogues (Scheme I) involves the condensation of an appropriately protected glycerol alkyl ether **2a–c**[4] with the phosphorus reagents **3a–j** (Table I). The preparations of the homologous series of ω-bromoalkylphosphoric acid dichlorides **3a–e**[5] and the (2-bromoethyl)phosphonic acid monochloride **3f**[6] have been described previously. The

Scheme II



remaining reagents **3g–j** have been prepared for this study by the reaction of an appropriate bromo alcohol or phenol with an excess of phosphorus oxychloride in carbon tetrachloride in the absence (method A) or the presence (method B) of 1 equiv of triethylamine.

The reaction of alcohols **2a–c** with an excess of phosphorus reagents **3a–j** and triethylamine at room temperature gives, after hydrolysis in a sodium acetate buffer, a series of bromoalkyl phosphorus compounds **4** that can be used without additional purification. The trimethylammonium groups are introduced by the overnight reaction of **4** with a large excess of anhydrous trimethylamine in a mixture of chloroform and acetonitrile at reflux (**5a–g,i**) or, for more sterically hindered compounds (**5h,j**), at a higher reaction temperature (70–100 °C) in a glass-lined pressure reactor. Milder conditions (DMF–acetonitrile, 45 °C, 20 min) were used to prepare the benzyl compound **5k**.

Use of the benzyl-protected intermediates **2a,b** or the acetate **2c** depends on the nature of the phosphorus group introduced. Usually we found the use of **2a,b** to be more convenient than **2c**. Despite the fact that the use of **2c** results in a shorter synthesis, giving the PAF analogues directly, we found that the lengthy silicic–boric acid chromatography needed to prepare **2c**[4b,c] and its instability on storage with respect to acyl migration make it a less useful intermediate than **2a,b**. However, for those cases in which the quaternary ammonium group would be a benzylic position, the PAF analogues (**5j,k**) were prepared directly from **2c** since it was anticipated that cleavage of the benzylic ammonium group would be concurrent with hydrogenolysis of the benzyl ether protecting group.

The benzyl ether groups were removed from **5a–i** by hydrogenolysis using a palladium catalyst in a mixture of acetic acid and methanol. Finally, the resulting lyso compounds (**6a–i**) were acetylated with an excess of acetic anhydride and triethylamine in refluxing chloroform to give the PAF analogues **7a–i** listed in Table II.

In order to investigate the contribution of the phosphate moiety to the biological activity of PAF, we prepared the analogue **11** (Scheme II) in which the phosphate group has been removed and the methylene bridge has been extended to maintain, approximately, the spatial relationship of the quaternary ammonium group with the other functionality. Alkylation of **2b** with an excess of 1,4-dibromobutane using sodium hydride and a catalytic amount of sodium iodide in DMF gives the bromide **8**. The trimethylammonium group is introduced by heating **8** with a large excess of 40%

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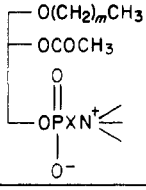
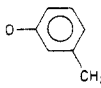
Table II. Platelet Activating Factor Analogues[a]



compd	m	X	MABP[b]	platelet[c] EC_{50}	max[d] aggregn resp	ratio[e]	formula[f]	anal.[g]
1a	15	$O(CH_2)_2$	1.25 (0.30, 4.98)	2.65×10^{-8} (n = 9)	71 (6.8×10^{-6})	0.47		
1b	17	$O(CH_2)_2$	3.17 (1.03, 10.22)	2.36×10^{-8} (n = 27)	75 (1.48×10^{-5})	1.34		
7a	15	$O(CH_2)_3$	1.43 (0.10, 12.50)	2.5×10^{-8} (n = 1)	65 (1×10^{-5})	0.57	$C_{27}H_{56}NO_7P$ (0.65 H_2O)	C, N, P; H[h]
7b	15	$O(CH_2)_4$	4.84 (1.59, 12.14)	7.5×10^{-8} (n = 1)	68 (1.8×10^{-4})	0.65	$C_{28}H_{58}NO_7P$ (1.25 H_2O)	C, H, N, P
7c	17	$O(CH_2)_4$	15.85 (3.72, 55.12)	4.4×10^{-7} (n = 1)	71 (1×10^{-5})	0.36	$C_{30}H_{62}NO_7P$ (1 H_2O)	C, H, N, P
7d	15	$O(CH_2)_6$	164.90 (36.7, 753.0)	2.7×10^{-5} (n = 1)	35 (1.7×10^{-4})	0.06	$C_{30}H_{62}NO_7P$ (1 H_2O)	C, H, N, P
7e	15	$O(CH_2)_8$	>4000	3.7×10^{-5} (n = 1)	41 (1.7×10^{-4})		$C_{32}H_{66}NO_7P$ (0.5 H_2O)	C, H, N, P
7f	15	$O(CH_2)_{10}$	>4000	5.9×10^{-5} (n = 1)	18 (1.6×10^{-4})		$C_{34}H_{70}NO_7P$ (0.5 H_2O)	C, H, N, P
7g	17	$(CH_2)_2$	76.54 (13.3, 380.5)	2.0×10^{-7} (n = 1)	82 (1×10^{-5})	3.83	$C_{28}H_{58}NO_6P$ (0.75 H_2O)	C, H; N, P[i]
7h	15	$OCH_2CH(CH_3)$	0.15 (0.02, 1.03)	3.2×10^{-9} (n = 1)	66 (1×10^{-6})	0.47	$C_{27}H_{56}NO_7P$ (1.5 H_2O)	C, H, N, P
7i	15	$OCH(CH_3)CH_2$	1.16 (0.20, 6.13)	4.2×10^{-8} (n = 1)	74 (1×10^{-5})	0.28	$C_{27}H_{56}NO_7P$ (1 H_2O)	C, H, N, P
5j	15	$OCH_2CH(C_6H_5)$	3.93 (1.68, 9.10)	7.0×10^{-7} (n = 1)	69 (5×10^{-5})	0.06	$C_{32}H_{58}NO_7P$ (1 H_2O)	C, H, N, P
5k	15		1.36 (0.17, 10.58)	5.8×10^{-8} (n = 1)	64 (5×10^{-5})	0.23	$C_{31}H_{56}NO_7P$ (1.25 H_2O)	C, H, N, P

[a] All compounds exhibited IR and ^1H NMR spectra consistent with the assigned structures; the mass spectrum (FAB) of each compound showed the expected M + H peak. [b] Dose (μg/kg, iv) required to decrease mean arterial blood pressure (MABP) 50 mmHg. Values in parentheses are 95% confidence limits. [c] Molar concentration required to produce 50% of maximum aggregation. The n values are the number of experiments in which a dose–response curve was determined using three to four replicates per dose level and six to eight doses tested over the range 10^{-9}–10^{-4} M. [d] The maximum aggregation response observed at the specified minimum molar concentration. [e] (MABP/platelet EC_{50}) × 10^{-8}. [f] Parentheses contain the moles of water of hydration. [g] Analytical results are within ±0.4% of theoretical values unless indicated. [h] H: calcd, 10.51; found, 10.04. [i] N: calcd, 2.55; found, 2.09. P: calcd, 5.65; found, 4.81.

trimethylamine in a mixture of chloroform, 2-propanol, and DMF. The resulting salt **9** is deprotected by hydrogenolysis using a palladium catalyst in a mixture of acetic acid and methanol. The lyso compound **10** is then acetylated with acetic anhydride, giving the desired analogue **11** lacking a phosphate moiety.

Biology. Over the last few years we have explored the possibility of preparing a PAF analogue that would retain the ability to lower blood pressure but would have minimal effects on inflammatory cell activation and release. Since the various biological properties of PAF need not be mediated by a common receptor and since the receptor responsible for the hypotensive activity and those responsible for cell activation may differ in their structural requirements, a PAF analogue with the appropriate structural modifications may exhibit the desired separation of activities. Evidence that at least some of the biological effects of PAF are the result of a receptor interaction include the stereospecificity of action of PAF,[7] the discovery of specific antagonists,[8] and the demonstration of specific PAF binding sites.[9]

To examine the effect of PAF analogues on mediator cell activation, we have chosen to study platelet aggregation in rabbit platelet rich plasma. The platelets of ths species are very sensitive to PAF.[2c,10,11] The data (Table II) are presented as the molar concentration of the analogue required to obtain 50% of its maximum aggregation response (EC_{50}, a measure of potency) and as the maximum aggregation response of the analogue obtained at the indicated minimum concentration (a measure of efficacy); this latter value is of use in distinguishing partial from full agonists.

For blood pressure studies we used spontaneously hypertensive rats since it has been determined that rat platelets do not respond well to PAF and that the hypotensive effect of PAF is not mediated by platelets in this species.[2c,11] The hypotensive data are expressed as the intravenous dose of the analogue needed to reduce the mean arterial blood pressure (MABP) 50 mm Hg as determined from the dose–response curve.

As a measure of the degree of separation of the two biological activities for a particular compound, we determine the ratio of the blood pressure and the platelet aggregation values. A value of this ratio that is significantly smaller than that obtained for the standard compounds **1a,b** is an indication of selectivity in favor of the hypotensive effect while a ratio larger than that of the standards would suggest selectivity in favor of platelet aggregation.

Results and Discussion

The hypotensive and platelet aggregation data obtained for racemic C_{16} and C_{18} PAF (**1a**, **1b**) and the racemic PAF analogues are presented in Table II. It is evident that increasing the length of the methylene bridge separating the phosphate and quaternary ammonium moieties from two to 10 carbon atoms results in a progressively decreasing

(7) (a) Wykle, R. L.; Miller, C. H.; Lewis, J. C.; Schmitt, J. D.; Smith, J. A.; Surles, J. R.; Piantadosi, C.; O'Flaherty, J. T. *Biochem. Biophys. Res. Commun.* **1981**, *100*, 1651. (b) Tence, M.; Coeffies, E.; Polonsky, J.; Benveniste, J. *Biochem. Biophys. Acta* **1983**, *735*, 526.

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(11) Cargell, D. I.; Cohen, D. S.; Von Valen, R. G.; Klimek, J. J.; Leven, R. P. *Thromb. Haemostasis* **1983**, *49*, 204.

response with respect to both blood pressure and platelet aggregation. However, it is also apparent that this decline in biological activities is gradual; compared to **1a** increasing the methylene bridge by one carbon atom (**7a**) leads to essentially no change in biological activity while increasing the bridge length by two methylene units (**7b**) results in approximately a fourfold decrease in the blood pressure response and only about a threefold decrease in the platelet aggregation response. Larger increases in the length of the methylene bridge (**7d–f**) result in even greater decreases of the hypotensive and platelet aggregation responses; for platelet aggregation this is evident both in the EC_{50} as well as the maximum response values. With the possible exception of **7d**, the ratios of the blood pressure and platelet aggregation values obtained for the members of this homologous series (**1a**, **7a,b,d–f**) are comparable to each other, suggesting that variation of the length of the methylene bridge separating the phosphate and trimethylammonium groups results in little change in selectivity of the biological response. Previous structure–activity studies of PAF have established that maximal biological responses are obtained with increasing methyl substitution of the amine base of the polar head group.[3c] Therefore, in the present series of homologues, it appears that while a quaternary ammonium group plays an important role in the interaction of PAF with its receptor, the lack of sensitivity of the response to small changes in the length of the methylene bridge separating the phosphate and quaternary groups suggests that the interaction of the ammonium group need not be with a specific group of atoms but is a nonspecific hydrophilic interaction either with an aqueous environment or with a number of different hydrophilic sites on the receptor.

While methyl substitution of the phosphocholine group adjacent to the phosphate moiety (**7i**) results in little change in the biological responses, methyl substitution adjacent to the quaternary ammonium group (**7h**) results in an eightfold increased response with respect to both blood pressure lowering and platelet aggregation as compared to **1a**. Phenyl substitution adjacent to the trimethylammonium group (**5j**), however, leads to a decreased response in both assays. Replacement of the two-carbon bridge of the phosphocholine group with a meta-substituted benzyl moiety (**5k**) results in only a slight if any decrease in the two biological activities. The ratios of the hypotensive dose and platelet aggregation EC_{50} values obtained for these substituted analogues (**7h,i**, **5j,k**) are not dramatically different from that observed for **1a**, indicating that these particular alterations of the phosphocholine group have resulted in little gain in selectivity.

The phosphonate analogue **7g** is considerably less potent than **1b** in both assays. A similarly decreased platelet response was also observed for the C_{16} homologue of **7g**.[3d]

The analogue **11**, in which the phosphocholine group is replaced with a 4-(trimethylammonio)butoxy group, showed no detectable platelet aggregation activity at concentrations as high as 1×10^{-4} M and no hypotensive activity at a dose as high as 3 mg/kg (iv). Since the spatial relationship of the quaternary ammonium group of **11** with the other functionality is approximately the same as in **1a**, the total lack of biological activity of **11** is likely due to the absence of the phosphate group. This observation is consistent with our previous supposition that a negatively charged species such as a phosphate group is necessary for a good biological response.[1c]

Experimental Section

General Methods. Unless otherwise stated, the following are implied. Melting points were determined on a Mel-Temp capillary melting point apparatus and are uncorrected. The nuclear magnetic resonance (NMR) spectra were recorded on either a Varian EM-390, a varian FT-80, or a Nicolet-300 spectrometer, and chemical shifts in parts per million (ppm) are reported with tetramethylsilane (Me_4Si) or chloroform as internal references. Infrared spectra (IR) were recorded on a Nicolet FT-7000 spectrophotometer. Electron impact (EI) mass spectra were determined on a Finnegan MAT Model CH-7 mass spectrometer. The field desorption (FD) and fast atom bombardment (FAB) mass spectra were obtained on a Kratos MS-50 mass spectrometer. Where analyses are indicated only by symbols of the elements, analytical results obtained for thee elements were within ±0.4% of the theoretical value.

Biological Assays. The methods used in the platelet aggregation and blood pressure assays have been described in detail perviously.[1a,12]

2-Bromopropyl Phosphodichlorodate (3g). Method A. To a solution of $POCl_3$ (42 g, 0.27 mol) in 110 mL of CCl_4 was added dropwise with stirring a solution of 2-bromopropanol (25 g, 0.179 mol) in 25 mL of CCl_4 at a rate that did not let the reaction temperature exceed 30 °C. The solution was stirred for 18 h. Solvent was removed, and the residue was distilled in a Kugelrohr apparatus (75 °C (0.05 mm)), giving **3g** as a colorless liquid: 26.1 g (57%); NMR ($CDCl_3$) δ 4.66–4.00 (m, 3 H, CHBr, CH_2O), 1.75 (d, 3 H, J = 6.0 Hz, CH_3).

In a similar manner **3h** and **3i** were prepared with the exception that **3i** was not distilled but used directly as obtained.

3-(Bromomethyl)phenyl Phosphodichlorodate (3j). Method B. To a solution of 3-(bromomethyl)phenol (17 g, 91 mmol) in 150 mL of CCl_4 was added a solution of $POCl_3$ (25.5 g, 166 mmol) in 25 mL of CCl_4. The solution was placed in a water bath, and triethylamine (9.2 g, 91 mmol) in 35 mL of CCl_4 was added over 40 min. The solution was stirred at 25 °C for 20 h. The mixture was filtered, and solvent was removed, giving **3j** as a light yellow liquid (23.3 g, 84%) that was not purified further: NMR ($CDCl_3$) δ 7.35 (m, 4 H, C_6H_4), 4.47 (s, 2 H, CH_2).

6-Hydroxy-*N*,*N*,*N*-trimethyl-9-(phenylmethoxy)-5,7,11-trioxa-6-phosphaheptacosan-1-aminium 6-Oxide, Hydroxide, Inner Salt (5b). A solution of **3b** (5.3 g, 19.5 mmol) and triethylamine (10.6 mL, 79.4 mmol) in 140 mL of CCl_4 was stirred at 0 °C under argon as a solution of **2a** (5.0 g, 12.3 mmol) in 15 mL of CCl_4 was added dropwise. The mixture was then stirred at 25 °C for 2 h and then diluted with 100 mL of toluene. The mixture was filtered, and the solvent was removed. The residue was stirred in a mixture of 110 mL of THF and 110 mL of 0.5 M sodium acetate for 2.5 h. Most of the THF was removed. The residue was acidified with dilute HCl, and the mixture was extracted several times with ether. The combined extracts were washed with brine and dried ($MgSO_4$). The solvent was removed, and the residue was chromatographed on 100 g of Florisil, eluting first with $CHCl_3$ to remove the less polar impurities and then with $CHCl_3$–CH_3OH (9:1) to elute product, giving 5.4 g (71%) of (4-bromobutyl)-3-(hexadecyloxy)-2-(phenylmethoxy)propyl phosphoric acid ester: Anal. ($C_{30}H_{54}BrO_6P$) H, Br, P; C: Calcd, 57.96; found, 57.13.

This compound was stirred at reflux in a mixture of 90 mL of $CHCl_3$, 100 mL of CH_3CN, and 50 mL of anhydrous trimethylamine for 17 h. The solvents were removed, and the residue was stirred in 50 mL of CH_3OH containing 1.2 g of Ag_2CO_3 (to remove the trimethylamine hydrobromide). After 3 h, the mixture was filtered and solvent was removed. The residue was mixed with ether and allowed to stand at 0 °C overnight. Solid was collected and washed with cold ether, giving **5b** (3.6 g, 49%) as a white powder with no well-defined melting point: NMR ($CDCl_3$–CD_3OD) δ 7.31 (m, 5 H, C_6H_5), 4.70 (s, 2 H, $CH_2C_6H_5$), 4.15–3.00 (m, 11 H, CH_2OCH_2, CHO, CH_2N, CH_2OPOCH_2), 3.05 (s, 9 H, $N(CH_3)_3$), 2.10–1.10 (m, 32 H, $(CH_2)_{14}$, CH_2CH_2), 0.85 (m, 3 H, terminal CH_3). Anal. ($C_{33}H_{62}NO_6P \cdot 0.75H_2O$) C, H, N, P.

In a similar manner **5a,c–f,i** were prepared.

6,9-Dihydroxy-*N*,*N*,*N*-trimethyl-5,7,11-trioxa-6-phosphaheptacosan-1-aminium 6-Oxide, Hydroxide, Inner Salt (6b). A solution of **5b** (3.5 g, 5.8 mmol) in a mixture of 35 mL of CH_3CO_2H and 35 mL of CH_3OH containing 0.6 g of 5% Pd/C

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was shaken in a Parr apparatus at an initial pressure of 25 psi of hydrogen for 20 h. The mixture was filtered, and solvents were removed at reduced pressure. The residue was stirred with ether and cooled. Solid was collected and washed with ether, giving **6b** (2.6 g, 87%) as a white powder with no well-defined melting point: NMR ($CDCl_3$ + CD_3OD) δ 3.94 (m, 4 H, CH_2OPOCH_2), 3.60–3.36 (m, 8 H, OH, CH_2OCH_2, CHO, CH_2N), 3.11 (s, 9 H, $N(CH_3)_3$), 1.95, 1.72 (m, 4 H, $CH_2CH_2CH_2N$), 1.54 (m, 2 H, OCH_2CH_2), 1.23 (m, 26 H, $(CH_2)_{13}$), 0.88 (m, 3 H, terminal CH_3); IR (KBr) 3400 cm^{-1}. Anal. ($C_{26}H_{56}NO_6P\cdot1.5H_2O$) C, H, N, P.

In a similar manner **6a,c–i** were prepared.

9-(Acetyloxy)-6-hydroxy-*N,N,N*-trimethyl-5,7,11-trioxa-6-phosphaheptacosan-1-aminium 6-Oxide, Hydroxide, Inner Salt (7b). A solution of **6b** (2.0 g, 3.9 mmol), acetic anhydride (10 g, 97.9 mmol), and triethylamine (3.97 g, 39.2 mmol) in 100 mL of $CHCl_3$ was stirred at reflux for 3 h. Solvents and excess reagents were removed at reduced pressure. The residue was chromatographed on silica gel (250 mL dry volume), eluting first with $CHCl_3$ and then with $CHCl_3$–CH_3OH (7:3) to remove less polar impurities followed by elution with $CHCl_3$–CH_3OH–water (65:35:6) to elute product. Solvent was removed from product fractions. The residue was triturated with cold ether, giving 1.07 g (50%) of **7b** as a white powder with no well-defined melting point: NMR ($CDCl_3$ + CD_3OD) δ 5.13 (m, 1 H, CHOAc), 3.90 (m, 4 H, CH_2OPOCH_2), 3.55, 3.43 (m, 6 H, CH_2OCH_2, CH_2N), 3.26 (s, 9 H, $N(CH_3)_3$), 2.06 (s, 3 H, $COCH_3$), 1.95, 1.72 (m, 4 H, $CH_2CH_2CH_2N$), 1.52 (m, 2 H, OCH_2CH_2), 1.25 (m, 26 H, $(CH_2)_{13}$), 0.88 (m, 3 H, terminal CH_3); IR (KBr) 1730 cm^{-1}; MS (FAB) m/z 552 (M + H). Anal. ($C_{28}H_{58}NO_7P\cdot1.25H_2O$) C, H, N, P.

In a similar manner **7a,c–i** were prepared.

3-[[[2-(Acetyloxy)-3-(hexadecyloxy)propoxy]hydroxyphosphinyl]oxy]-*N,N,N*-trimethylbenzenemethanaminium, Hydroxide, Inner Salt (5k). A solution of **2c** (2.86 g, 7.98 mmol and **3j** (2.43 g, 8.0 mmol) in 30 mL of CCl_4 was stirred under argon at 0 °C as a solution of triethylamine (0.81 g, 8.0 mmol) in 5 mL of CCl_4 was added dropwise. The mixture was then stirred at 25 °C for 2 h. Toluene was added, and the mixture was filtered. Solvent was removed, and the residue was stirred in a mixture of 40 mL of THF and 40 mL of 0.5 M sodium acetate solution for 24 h. Most of the THF was removed. The aqueous mixture was acidified with 0.5 N HCl and extracted several times with ether. The combined ether extracts were dried ($MgSO_4$) and solvent was removed. The residue was chromatographed on Florisil, eluting first with $CHCl_3$ to remove the less polar impurities and then with $CHCl_3$–CH_3OH (9:1) to elute 2.2 g of 2-(acetyloxy)-3-(hexadecyloxy)propyl 3-(bromomethyl)phenyl phosphoric acid ester. Anal. ($C_{28}H_{48}BrO_7P$) C, H, Br, P.

This compound was dissolved in 4 mL of DMF, and 25 mL of a 33% solution of trimethylamine in CH_3CN was added. After the mixture was stirred at 25 °C for 20 min and 45 °C for another 20 min, the solvents were removed at reduced pressure. The residue was chromatographed on silica gel, eluting with $CHCl_3$–CH_3OH–H_2O (65:35:3). Product was isolated and triturated with ether to give 1.2 g (26%) of **5k** as a white powder with no well-defined melting point: NMR ($CDCl_3$ + CD_3OD) δ 7.29 (m, 4 H, aromatic), 5.16 (m, 1 H, CHO), 4.34 (s, 2 H, CH_2N), 4.12 (m, 2 H, CH_2OP), 3.61, 3.41 (m, 4 H, CH_2OCH_2), 3.14 (s, 9 H, $N(CH_3)_3$), 2.03 (s, 3 H, $COCH_3$), 1.52 (m, 2 H, OCH_2CH_2), 1.25 (m, 26 H, $(CH_2)_{13}$), 0.89 (m, 3 H, terminal CH_3); IR (KBr) 1730 cm^{-1}; MS (FAB) m/z 586 (M + H). Anal. ($C_{31}H_{56}NO_7P\cdot1.25H_2O$) C, H, N, P.

2-[Hydroxy[3-(octadecyloxy)-2-(phenylmethoxy)propoxy]phosphinyl]-*N,N,N*-trimethylethanaminium Hydroxide, Inner Salt (5g). To a solution of **3f**[6] (4.2 g, 20 mmol) in 50 mL of $CHCl_3$ was added at 0 °C a solution of **2b** (4.35 g, 10 mmol) and triethylamine (3.0 mL, 22 mmol) in 50 mL of $CHCl_3$ over 1 h. After the mixture was stirred at 25 °C for 48 h, the solvent was removed. The residue was dissolved in ether. The ether solution was washed with brine and dried ($MgSO_4$). Solvent was removed, giving 5.54 g of an oil.

A 3.0-g portion of this material was placed in a glass-lined pressure reactor containing 50 mL of 33% trimethylamine in CH_3CN. Another 50 mL of CH_3CN was added, and the mixture was heated to 65 °C for 48 h. The solvent and excess trimethylamine were removed. The residue was dissolved in a mixture of 25 mL of $CHCl_3$, 25 mL of CH_3OH, and 2.5 mL of H_2O and stirred 2 h with 1.0 g of Ag_2CO_3. The mixture was filtered, and solvent was removed, giving 1.42 g of **5g** as a white powder after trituration with cold ether: NMR ($CDCl_3$–CD_3OD) δ 7.30 (m, 5 H, C_6H_5), 4.62 (s, 2 H, $CH_2C_6H_5$), 4.15–2.90 (m, 1 H, CH_2O, CHO, CH_2CH_2N), 2.90 (s, 9 H, $N(CH_3)_3$), 1.20 (m, 32 H, $(CH_2)_{16}$), 0.90 (m, 3 H, terminal CH_3); MS (FAB) m/z 584 (M + H). Anal. ($C_{33}H_{62}NO_5P\cdot2H_2O$) C, H, N, P.

5-Hydroxy-*N,N,N*-trimethyl-8-(phenylmethoxy)-4,6,10-trioxa-5-phosphahexacosan-2-aminium 5-Oxide, Hydroxide, Inner Salt (5h). To a solution of **3g** (4.4 g, 17.2 mmol) and triethylamine (9.8 mL, 70.1 mmol) in 140 mL of CCl_4 was added under argon with stirring at 0 °C a solution of **2a** (5.0 g, 12.3 mmol) in 20 mL of CCl_4. After the mixture was stirred for 18 h, 100 mL of toluene was added and the mixture was filtered. Solvents were removed. The residue was stirred for 2 h in a mixture of 110 mL of 0.5 M sodium acetate solution and 110 mL of THF. Most of the THF was removed, and the aqueous mixture was acidified with 1 N HCl. The mixture was extracted with ether several times. The combined extracts were washed with brine and dried ($MgSO_4$). Solvent was removed, and the residue was chromatographed on 100 g of Florisil, eluting first with $CHCl_3$ to remove the less polar impurities and then with $CHCl_3$–CH_3OH 9:1 to elute 6.5 g of 2-(bromopropyl)-3-(hexadecyloxy)-2-(phenylmethoxy)propyl phosphoric acid ester. Anal. ($C_{29}H_{52}O_6BrP\cdot H_2O$) C, H; Br: calcd, 12.77; found, 13.41; P: calcd, 4.95; found, 5.40.

This material was mixed with 50 mL of DMF, 25 mL of CH_3CN, and 50 mL of precooled anhydrous trimethylamine in a glass-lined steel pressure reactor. The mixture was heated to 65 °C for 18 h. Solvents were removed, and the residue was dissolved in a mixture of 25 mL of $CHCl_3$, 25 mL of CH_3OH, and 2.5 mL of H_2O. The solution was stirred with 1.5 g of Ag_2CO_3 (to remove the trimethylamine hydrobromide) for 2.5 h and then filtered through Celite. The solvent was removed. The residue was chromatographed on silica gel (300-mL dry volume), eluting first with $CHCl_3$–CH_3OH (7:3) to remove less polar impurities and then with $CHCl_3$–CH_3OH–H_2O (65:35:6) to elute product that was triturated with cold ether to give 0.84 g of **5h** as a white powder with no well-defined melting point: NMR ($CDCl_3$, CD_3OD) δ 7.30 (m, 5 H, C_6H_5), 4.68 (s, 2 H, $CH_2C_6H_5$), 4.35–2.95 (m, 10 H, CH_2OCH_2, CH_2OPOCH_2, CHN, CHO), 3.05 (s, 9 H, $N(CH_3)_3$), 1.80–1.05 (m, 31 H, $(CH_2)_{14}$, CH_3), 0.90 (m, 3 H, terminal CH_3); MS (FAB) m/z 586 (M + H). Anal. ($C_{32}H_{60}NO_6P\cdot1.25H_2O$) C, H, N, P.

In a similar manner **5j** was prepared, starting with **2c**.

[[1-[(4-Bromobutoxy)methyl]-2-(octadecyloxy)ethoxy]methyl]benzene (8). To a suspension of prewashed (hexanes) NaH 50% mineral oil dispersion (3.2 g, 66.7 mmol) in 25 mL of DMF were added 1,4-dibromobutane (28.8 g, 133.4 mmol) and NaI (0.5 g, 3.3 mmol). The mixture was stirred under argon as a solution of **2b** (14.5 g, 33.4 mmol) in 25 mL of DMF was added dropwise. After the mixture was stirred for 17 h, an aliquot indicated incomplete reaction. Additional NaH dispersion (3.2 g, 66.7 mmol) and 1,4-dibromobutane (28.8 g, 133.4 mmol) were added, and stirring was continued for another 24 h. Water was added slowly, and the mixture was extracted with ether. The ether solution was dried ($MgSO_4$), and solvent was removed. Unreacted dibromobutane was removed at reduced pressure (70 °C (0.3 mm)). The residue was chromatographed on a dry column of silica gel (700 g), eluting with $CHCl_3$ to give 11.09 g (58%) of **8** as a pale yellow oil: NMR ($CDCl_3$) δ 7.40 (m, 5 H, C_6H_5), 4.78 (s, 2 H, $CH_2C_6H_5$), 3.75–3.40 (m, 11 H, CH_2OCH_2, CHO, CH_2Br), 2.0–1.23 (m, 36 H, $(CH_2)_{16}$, $(CH_2)_2$), 0.93 (m, 3 H, terminal CH_3). Anal. ($C_{32}H_{57}BrO_3$) C, H, Br.

4-[3-(Octadecyloxy)-2-(phenylmethoxy)propoxy]-*N,N,N*-trimethyl-1-butanaminium Bromide (9). A solution of **8** (6.3 g, 11.9 mmol) in 170 mL of $CHCl_3$–2-propanol–DMF (3:5:5) containing 105 mL of 40% trimethylamine was stirred at 55 °C for 6 h and at 25 °C for 16 h. The solvents were removed at reduced pressure. The residue was mixed with ether, and 6.49 g (87%) of **9** was collected as a white powder: NMR ($CDCl_3$) δ 7.35 (m, 5 H, C_6H_5), 4.70 (m, 2 H, $CH_2C_6H_5$), 3.80–3.40 (m, 11 H, CHO, CH_2OCH_2, CH_2N), 3.30 (s, 9 H, $N(CH_3)_3$), 2.05–1.20 (m, 36 H, $(CH_2)_{16}$, $(CH_2)_2$), 0.90 (m, 3 H, terminal CH_3). Anal. ($C_{35}H_{66}BrNO_3\cdot H_2O$) C, H, N; Br: calcd, 12.35; found, 12.95.

4-[2-Hydroxy-3-(octadecyloxy)propoxy]-*N,N,N*-trimethyl-1-butanaminium Bromide (10). A solution of **9** (6.4 g, 10.2 mmol) in a mixture of 50 mL of CH_3OH and 50 mL of

CH_3CO_2H containing 2 g of 5% Pd/C was hydrogenated in a Parr apparatus for 48 h. The mixture was filtered, and solvent was removed at reduced pressure. The residue was triturated with ether, giving 4.81 g (88%) of **10** as a white powder: NMR ($CDCl_3$) δ 3.90–3.30 (m, 11 H, CH_2OCH_2, CH_2N, CHO), 3.20 (s, 9 H, $N(CH_3)_3$), 2.85 (s, 1 H, OH) 2.20–1.10 (m, 36 H, $(CH_2)_{16}$ $(CH_2)_2$), 0.90 (m, 3 H, terminal CH_3); IR (KBr) 3300 cm^{-1}; MS (FD) *m/z* 458 (M–Br). Anal. ($C_{28}H_{60}O_3NBr \cdot H_2O$) C, N, Br; H: calcd, 11.22; found, 10.70.

4-[2-(Acetyloxy)-3-(octadecyloxy)propoxy]-*N*,*N*,*N*-trimethyl-1-butanaminium Bromide (11). A mixture of acetic anhydride (5 mL) and **10** (0.5 g, 0.93 mmol) was refluxed under argon with stirring for 15 min. Excess acetic anhydride was removed at reduced pressure. Toluene was added and removed several times. Ether was added, and the mixture was cooled to 0 °C, giving 0.5 g (93%) of **11** as a white powder with no well-defined melting point: NMR ($CDCl_3$–CD_3OD) δ 5.10 (m, 1 H, C*H*OAc), 3.80–3.10 (m, 10 H, CH_2OCH_2, CH_2N), 3.15 (s, 9 H, $N(CH_3)_3$), 2.01 (s, 3 H, $COCH_3$), 1.85–1.08 (m, 36 H, $(CH_2)_{16}$, $(CH_2)_2$), 0.90 (m, 3 H, terminal CH_3); IR (KBr) 1730 cm^{-1}; MS (FD) *m/z* 500 (M – Br). Anal. ($C_{30}H_{62}BrNO_4 \cdot 1.25H_2O$) C, H, N, Br.

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Synthesis and Hypolipidemic Activities of 5-Thienyl-4-oxazoleacetic Acid Derivatives[1]

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A series of 2,5-disubstituted 4-oxazoleacetic acid derivatives was synthesized and evaluated for hypolipidemic activity. Among them, those with a thienyl group at C-5 of the oxazole ring exerted highly potent hypolipidemic effects in rats. 2-(4-Fluorophenyl)-5-(3-thienyl)-4-oxazoleacetic acid (**88**) was the most potent derivative: it was about 2 times as active in normal SD male rats and about 4 times as active in hereditary hyperlipidemic rats (THLR/1) as clofibrate with an improved antiarterioschlerosis index (HDL-Cho/Total-Cho). In addition, it showed inhibition of platelet aggregation ex vivo.

In recent years, as the recognition of the role of hyperlipidemia as a risk factor for coronary heart diseases gained more momentum,[2] much attention has been paid to developing more satisfactory hypolipidemic agents such as an agent effective for Type IIa hyperlipidemia.

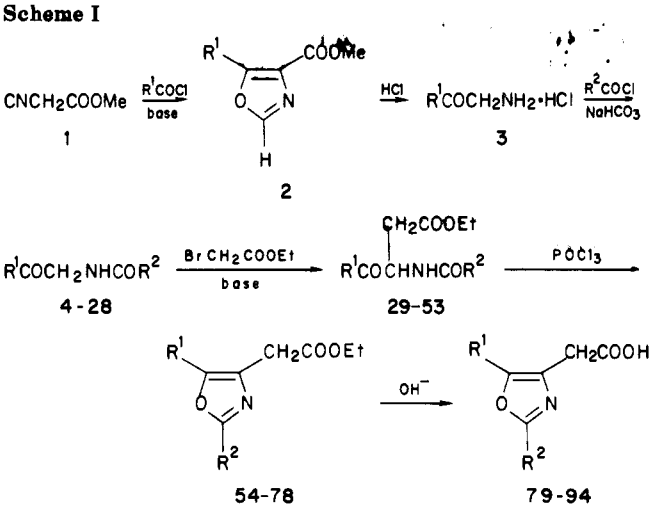
On the other hand, syntheses of biologically active compounds from amino acids continued to be of interest in our laboratory. Using methyl α-isocyanoacetate, which is a key reactive species of glycine, we have synthesized a number of heterocycles and amino acid derivatives.[3] In particular, we have systematically synthesized 5-aryl-4-oxazolecarboxylic acid derivatives having inhibitory activities on platelet aggregation,[4] and we found that some of them often showed hypolipidemic activities and did not exhibit marked toxicities.

In the present study, we synthesized more of the oxazole analogues and evaluated their hypolipidemic activities. From these compounds was found a series of 5-thienyl-4-oxazoleacetic acid derivatives that possess more potent hypocholesterolemic and hypotriglyceridemic activities than clofibrate [ethyl 2-(*p*-chlorophenoxy)isobutyrate].

Chemistry

A general synthetic method of 2,5-disubstituted 4-oxazoleacetic acid derivatives (**54–94**) is shown in Scheme I. The initial conversion of methyl α-isocyanoacetate (**1**) to α-amino ketones (**3**) was carried out as described in the previous reports:[5,6] the reaction of **1** with acyl halides under basic conditions followed by treatment with hydrochloric acid of the resulting oxazolecarboxylates (**2**)[7,8] afforded the α-amino ketone hydrochlorides (**3**) in good yields. After N-acylation of the α-amino ketone with a second acyl halide by the Schotten–Baumann reaction, an acetic acid

Scheme I



moiety was selectively introduced to the active methylene group of the α-(*N*-acylamino) ketones (**4–28**) by base-as-

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