

# Design and Modeling of New Platelet-Activating Factor Antagonists. 1. Synthesis and Biological Activity of 1,4-Bis(3',4',5'-trimethoxybenzoyl)-2-[[substituted carbonyl and carbamoyl]oxy]methyl]piperazines

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To further investigate our hypothesis on the structure of the platelet-activating factor (PAF) receptor, 35 compounds derived from 1,4-bis(3',4',5'-trimethoxybenzoyl)piperazine were synthesized and their in vitro antagonistic effect was measured. Substitution of the compounds in position 2, by ester or carbamate groups, giving increased steric hindrance and hydrophobicity, increased the platelet aggregation inhibitory activity from 2  $\mu$ M (without substitution, compound 2) to 0.07  $\mu$ M (compound 1h) and gave a maximum displacement of [<sup>3</sup>H]PAF from platelet membrane of 0.05  $\mu$ M (compound 1k). It appears that the PAF antagonistic effect is only weakly enantiospecific, as observed in many cases including antagonists structurally related or not to PAF. 3D electrostatic potential maps (calculated at -10 kcal/mol) of such compounds revealed a double "Cache-oreilles" (ear-muffs) system. One of these systems has been previously described (distance between atoms generating negative wells, 11-14 Å). The second shorter "Cache-oreilles" (6-7 Å) system appears to be required for increased PAF antagonistic activity. This short distance between groups generating the negative wells is present in the ginkgolides, a series of naturally occurring PAF antagonists. The present study indicates that the structure of the PAF receptor may be more complicated than our initial hypothesis and may be a tetrapolarized structure, with alternants of electropositive and hydrophobic areas. This modified hypothesis is in agreement with recent publications concerning PAF antagonists bearing a cationic moiety.

After characterization<sup>1-3</sup> of platelet-activating factor (PAF), elucidation of its structure by three independent research groups<sup>4-6</sup> and its stereospecific synthesis,<sup>7-11</sup> sufficient material became available to allow evaluation of the pharmacology and pathophysiology of this agent. PAF is now recognized as a powerful autacoid mediator of inflammation and allergy and may play a physiopathological role in a variety of clinical conditions such as asthma and pulmonary dysfunction, acute inflammation, cardiac anaphylaxis, thrombosis, gastrointestinal ulceration, endotoxin shock, allergic skin diseases, transplanted organ rejection, ovariectomy in pregnancy, and retinal and corneal diseases (reviewed in refs 12-20).

Consequently, the search for molecules able to antagonize the effects of PAF has been focused on the synthesis of analogues structurally related to PAF, screening of natural products, and biological evaluation of a large variety of synthetic compounds, some of them having other well-known pharmacological activities<sup>13</sup> (reviewed in refs 21-30). The unusual structural differences of PAF antagonists and the existence of specific binding sites of high affinity for the mediator renders necessary a comparison between the common geometric and electronic features generated by these derivatives. After 3D electrostatic map calculations of seven non-PAF-like molecules (including terpenoids such as ginkgolides and triazolothienobenzodiazepines), the authors<sup>31</sup> observed a major electronic effect named "Cache-oreilles" (ear-muffs), i.e. two negatives wells at -10 kcal/mol located at 180° from each other (reviewed in ref 32). The first step toward constructing a mirror

image of the PAF receptor is to take into account these results. We have proposed a simple model for the PAF receptor comprising a bipolarized cylinder 10-12 Å in diameter, containing a hydrophobic subsite. The distance between the atoms generating the negative wells was calculated at 12-13 Å after evaluation of antagonistic activities of flexible simple molecules bearing two trimethoxyphenyl groups generating the "Cache-oreilles" system.<sup>33</sup>

Starting from this simple hypothesis we have chosen as a semirigid system the piperazine ring substituted as shown in the general formula in Table I, the distance between the *p*-methoxy groups being in the range of that determined by preliminary molecular modeling studies.<sup>31-33</sup>

In the present study, we examine that influence of variations in the Z group on platelet aggregation and consider the necessity of this hydrophobic anchorage through an ester function (compound 1, Table I) with regard to the molecule not substituted on the ring, i.e. the 1,4-bis(3',4',5'-trimethoxybenzoyl)piperazine (2) (Table I). In order to analyze the importance of the carbonyl functions of the trimethoxybenzoyl substituents carried by the nitrogen atoms, the 1,4-bis(3',4',5'-trimethoxyphenyl)piperazine (3) (Table I) was resynthesized in which the carbonyls are absent. To keep the same range of distance in one of the terms of the more active compounds substituted on the ring, the same carbonyl groups were replaced by methylenes: 1,4-bis(3',4',5'-trimethoxybenzyl)-2-[[2'-ethylbutanoyl]oxy]methyl]piperazine (4) (Figure 2).

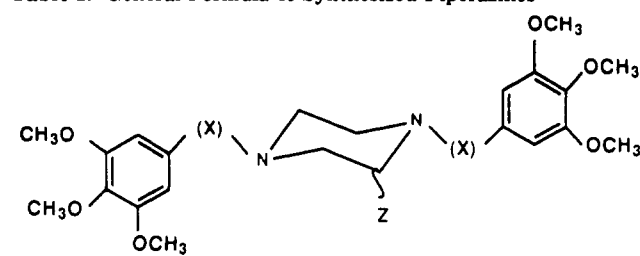
In order to study the enantiospecificity in this species, three of the more potent compounds, 1g, 1h, and 1k (Table

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Table I. General Formula of Synthesized Piperazines



no.	(X)	Z
1	>C=O	CH <sub>2</sub> OCOR
2	>C=O	H
3 <sup>a</sup>	deleted	H
4	CH <sub>2</sub>	CH <sub>2</sub> OCOCHEt <sub>2</sub>
5	>C=O	CH <sub>2</sub> OCONHR
6	>C=O	CH <sub>2</sub> OCONR <sub>2</sub>

<sup>a</sup> 1,4-Bis(3',4',5'-trimethoxyphenyl)piperazine.

II), were selected, and each of their enantiomers was synthesized and tested.

The more active compounds in series 1 were modified by replacing the ester by a carbamoyl function while maintaining the same level of lipophilicity. With this aim a series of mono- or disubstituted carbamates of general formulas 5 and 6 (Table I) were prepared.

Studies on these compounds have allowed us to reconsider our simplified model of the PAF binding site and indicate that the structure may be a multipolarized cylinder.

## Chemistry

All compounds of formula 1 were synthesized according to Scheme I, starting from 1,4-dibenzyl-2-(hydroxymethyl)piperazine (7) prepared according to Jucker et al.<sup>34</sup> Esterification of 7 with acyl chloride gave the corresponding 1,4-dibenzyl-2-[(acyloxy)methyl]piperazine (8) which was debenzylated using catalytic hydrogenolysis into the 2-[(acyloxy)methyl]piperazine (9). Fixation of the "Cache-oreilles" groups was obtained by reacting 3,4,5-trimethoxybenzoyl chloride on the 2-substituted piperazine 9, leading to the final 1,4-bis(3',4',5'-trimethoxybenzoyl)-2-[(acyloxy)methyl]piperazine (1) (Table II). Enantiomeric resolution was carried out on the racemic alcohol (±)-7 by a modified method of Jaeger et al.<sup>35</sup> by means of fractional crystallization with the chiral (+)-mandelic acid. The enantiomeric purity was determined on the alcohols (+)-7 and (-)-7 thus obtained by means of a new 400-MHz <sup>1</sup>H NMR method developed by Dr. G. Cahiez not yet patented and published (laboratoire de Chimie des Organoéléments, University of Paris 6). These alcohols were then treated separately as described above for (±)-7; these methods do not induce epimerizations. The final enantiomeric purity cannot be determined by high-field <sup>1</sup>H NMR method using chiral europium complexes because of difficulties of resolution of the piperazinic protons (and <sup>13</sup>C): their signals were near coalescence at room temperature (cf. Discussion). The simple 1,4-bis(3',4',5'-trimethoxybenzoyl)piperazine (2)<sup>36,37</sup> was prepared by treating commercial piperazine with 3,4,5-trimethoxybenzoyl chloride while the 1,4-bis(3',4',5'-trimethoxyphenyl)piperazine (3) was synthesized from 3,4,5-trimethoxyaniline and 1-bromo-2-chloroethane according to the method of Benington et al.<sup>38</sup> Compound 4 was prepared starting from the piperazinic intermediate 9h (R = CHEt<sub>2</sub>) by reacting with 2 equiv of 3,4,5-trimethoxybenzyl chloride.

Substituted carbamates 5 and 6 were synthesized via the respective 1,4-dibenzyl-2-[(N-substituted carbamoyl)oxy]methyl]piperazines 11 and 12 prepared following Scheme II, starting from the same alcohol 7, by different pathways depending of the availability of reagents. (i) The reaction of 7 with an isocyanate led to the mono-substituted carbamate 11. Isocyanates could be prepared "in situ" following the Ninomiya et al.<sup>39</sup> process (ii) using a carboxylic acid and diphenyl phosphorazidate (DPPA) or (iii) by Curtius rearrangement of an acyl azide.<sup>40</sup> Production of the intermediate 1,4-dibenzyl-2-[(phenoxycarbonyl)oxy]methyl]piperazine (13) according to the method of Takatani et al.<sup>41</sup> allowed preparation of either the N-monosubstituted carbamate 11 by reacting the primary amine (iv) or the N,N-disubstituted carbamate 12 with a secondary amine (v). Debenzylation and fixation of the trimethoxybenzoyl groups led to the final 1,4-bis(3',4',5'-trimethoxybenzoyl)-2-[(N-alkylcarbamoyl)oxy]methyl]piperazine (5) (Table III) and 1,4-bis(3',4',5'-trimethoxybenzoyl)-2-[(N,N-dialkylcarbamoyl)oxy]methyl]piperazine (6) (Table IV).

## Biological Results and Discussion

PAF-induced rabbit platelet aggregation was used to evaluate the target compounds as potential PAF antagonists. The structure-activity strategy was based only on these data. Indeed, it has been demonstrated<sup>42</sup> that this primary screening is closely correlated with in vivo activities such as PAF-induced bronchoconstriction and hypotension in rat. The ability to displace [<sup>3</sup>H]PAF from the high affinity binding sites on the platelets was used to classify some selected compounds as specific PAF receptor antagonists.

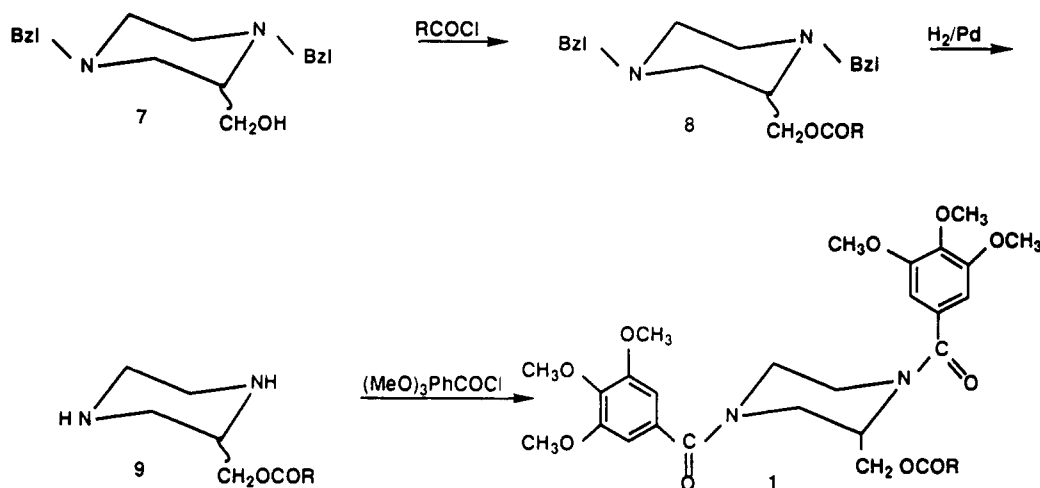
### Initial Hypotheses Based on Molecular Modeling.

As stated in the introduction, the piperazine ring was chosen as the semirigid system bearing the "Cache-oreilles" generated by the trimethoxyphenyl groups. Direct branching of both trimethoxyphenyl groups in positions 1,4 gives a distance of 14 Å between the oxygens of *p*-methoxy substituents in an expected extended conformation optimized by MM2 (cf. Experimental Section) (Figure 1). Our previous studies<sup>31-33</sup> demonstrated that the distance is not favourable and the corresponding compound 3 is a weak antagonist (IC<sub>50</sub> = 1.25 × 10<sup>-5</sup> M, see Table V). Placement of trimethoxybenzoyl groups in the same position (compound 2) enhances the antagonistic activity (IC<sub>50</sub> = 1.78 × 10<sup>-6</sup> M). It is well-known that such piperazines 1,4-(or *N,N'*)-disubstituted by strong electronegative groups are found in the most extended geometry: a chair form and a transoid conformation<sup>43,44</sup> as verified by conformational optimization by AM1 (cf. Experimental Section) (Figure 2). Although the difference of energy between both rotamers is small in this case: Δ*E* (AM1) = 0.1 kcal/mol). Consequently, another "Cache-oreilles" system appears at the carbonyl groups (Figure 2). The distance between the oxygens of the *p*-methoxy substituents remains at an unfavorable distance of 14.9 Å (transoid conformation) and the higher antagonistic potency can be only explained by the new "Cache-oreilles", giving a distance between the oxygen of amide groups of 6.8 Å. In an attempt to verify this assumption we have synthesized the analogue with methylene groups instead of carbonyl groups, compound 4, of one of the more active antagonists 1h. Comparison of compound 1h (IC<sub>50</sub> = 7 × 10<sup>-8</sup> M) and compound 4 (IC<sub>50</sub> = 2.7 × 10<sup>-6</sup> M) reveals the importance of the new "Cache-oreilles" system. An

**Table II.** Physicochemical Data of 1,4-Bis(3',4',5'-trimethoxybenzoyl)-2-[(acyloxy)methyl]piperazines (1) (See Table I)

no.	R	mp, °C	% yield	$R_f^a$	$^1\text{H NMR (CDCl}_3\text{)} \delta \text{ ppm}^b$	formula <sup>c</sup>
1a	CH <sub>3</sub>	59 <sup>d</sup>	70	0.12	1.92 (s, 3 H, CH <sub>3</sub> )	C <sub>27</sub> H <sub>34</sub> N <sub>2</sub> O <sub>10</sub>
1b	(CH <sub>2</sub> ) <sub>2</sub> CH <sub>3</sub>	82.4 <sup>d</sup>	72	0.40	2.3 (t, 2 H, CH <sub>2</sub> CO <sub>2</sub> ), 1.77–1.35 (m, 2 H, CH <sub>2</sub> ), 0.87 (t, 3 H, CH <sub>3</sub> )	C <sub>29</sub> H <sub>38</sub> N <sub>2</sub> O <sub>10</sub>
1c	(CH <sub>2</sub> ) <sub>4</sub> CH <sub>3</sub>	56 <sup>d</sup>	75	0.45	2.27 (t, 2 H, CH <sub>2</sub> CO <sub>2</sub> ), 1.82–0.95 (m, 6 H, (CH <sub>2</sub> ) <sub>3</sub> ), 0.8 (t, 3 H, CH <sub>3</sub> )	C <sub>31</sub> H <sub>42</sub> N <sub>2</sub> O <sub>10</sub>
1d	(CH <sub>2</sub> ) <sub>6</sub> CH <sub>3</sub>	resin	78	0.44	2.2 (t, 2 H, CH <sub>2</sub> CO <sub>2</sub> ), 1.52 (m, 2 H, CH <sub>2</sub> CCO <sub>2</sub> ), 1.33 (large s, 8 H, (CH <sub>2</sub> ) <sub>4</sub> ), 0.82 (t, 3 H, CH <sub>3</sub> )	C <sub>33</sub> H <sub>46</sub> N <sub>2</sub> O <sub>10</sub>
1e	(CH <sub>2</sub> ) <sub>8</sub> CH <sub>3</sub>	resin	75	0.48	2.12 (t, 2 H, CH <sub>2</sub> CO <sub>2</sub> ), 1.42 (m, 2 H, CH <sub>2</sub> CCO <sub>2</sub> ), 1.27 (m, 12 H, (CH <sub>2</sub> ) <sub>6</sub> ), 0.75 (t, 3 H, CH <sub>3</sub> )	C <sub>35</sub> H <sub>50</sub> N <sub>2</sub> O <sub>10</sub>
1f	(CH <sub>2</sub> ) <sub>16</sub> CH <sub>3</sub>	resin	77	0.54	2.2 (t, 2 H, CH <sub>2</sub> CO <sub>2</sub> ), 1.47 (m, 2 H, CH <sub>2</sub> CCO <sub>2</sub> ), 1.21 (large s, 28 H, (CH <sub>2</sub> ) <sub>14</sub> ), 0.77 (t, 3 H, CH <sub>3</sub> )	C <sub>43</sub> H <sub>66</sub> N <sub>2</sub> O <sub>10</sub> <sup>e</sup>
1g	C(CH <sub>3</sub> ) <sub>3</sub>	164.3 <sup>d</sup>	71	0.43	1.06 (s, 9 H, CH <sub>3</sub> )	C <sub>30</sub> H <sub>40</sub> N <sub>2</sub> O <sub>10</sub>
1h	CH(CH <sub>2</sub> CH <sub>3</sub> ) <sub>2</sub>	170.2 <sup>d</sup>	72	0.43	2.12 (quintet, 1 H, CH <sub>2</sub> CO <sub>2</sub> ), 1.7–1.12 (m, 4 H, CH <sub>2</sub> ), 0.78 (t, 6 H, CH <sub>3</sub> )	C <sub>31</sub> H <sub>42</sub> N <sub>2</sub> O <sub>10</sub>
1i	cyclohexyl	resin	74	0.57	2.6–2.23 (m, 1 H, CHCO <sub>2</sub> ), 1.93–0.9 (m, 10 H, cyclohexyl CH <sub>2</sub> )	C <sub>32</sub> H <sub>42</sub> N <sub>2</sub> O <sub>10</sub>
1j	CH <sub>2</sub> C(CH <sub>3</sub> ) <sub>3</sub>	86.6 <sup>d</sup>	69	0.44	2.05 (s, 2 H, CH <sub>2</sub> CO <sub>2</sub> ), 0.92 (s, 9 H, CH <sub>3</sub> )	C <sub>31</sub> H <sub>42</sub> N <sub>2</sub> O <sub>10</sub>
1k	<i>o</i> -Cl-C <sub>6</sub> H <sub>4</sub>	81 <sup>d</sup>	72	0.40	7.63–7.13 (m, 4 H, C <sub>6</sub> H <sub>4</sub> )	C <sub>32</sub> H <sub>35</sub> ClN <sub>2</sub> O <sub>10</sub>
1l	<i>p</i> -F-C <sub>6</sub> H <sub>4</sub> OCH <sub>2</sub>	96.5 <sup>d</sup>	71	0.42	4.6–5.32 (m, 2 H, OCH <sub>2</sub> CO <sub>2</sub> ), 6.37–7.30 (m, 8 H, C <sub>6</sub> H <sub>4</sub> and C <sub>6</sub> H <sub>2</sub> )	C <sub>33</sub> H <sub>37</sub> FN <sub>2</sub> O <sub>11</sub>
1m	<i>p</i> -Cl-C <sub>6</sub> H <sub>4</sub> OCH <sub>2</sub>	172 <sup>d</sup>	69	0.42	4.6–5.32 (m, 2 H, OCH <sub>2</sub> CO <sub>2</sub> ), 6.37–7.30 (m, 8 H, C <sub>6</sub> H <sub>4</sub> and C <sub>6</sub> H <sub>2</sub> )	C <sub>33</sub> H <sub>37</sub> ClN <sub>2</sub> O <sub>11</sub>

<sup>a</sup> MeOH/CHCl<sub>3</sub>, 3:97, v/v. <sup>b</sup> Specific signals other than common ones described in Experimental Section. <sup>c</sup> All compounds were analyzed for C, H, N; analytical results were within  $\pm 0.4\%$  theoretical values unless specified. <sup>d</sup> Crystallization in Et<sub>2</sub>O. <sup>e</sup> N: calcd, 3.63; found, 3.21.

**Scheme I<sup>a</sup>**

<sup>a</sup> Bzl = benzyl; (MeO)<sub>3</sub>PhCO = 3,4,5-trimethoxybenzoyl.

alternative explanation for the decreased antagonistic activity of this compound could be a possible protonation of piperazine moiety hindering the interaction with the receptor.

**Influence of R Substitution.** Our first simple receptor model implies a hydrophobic pocket as a third point of interaction in the binding site (reviewed in ref 32). Thus, it was logical to branch hydrophobic chains on the piperazine ring. In this paper these appendices are derived from 1,4-bis(3',4',5'-trimethoxybenzoyl)-2-(hydroxymethyl)piperazine. Structural variations are introduced via ester or carbamate groups including a steric effect and a hydrophobic effect which is quantified via hydrophobic fragmental constant system calculation ( $f$ ) after Rekker and De Koort.<sup>45</sup> The first notable feature is that inhibition of PAF-induced aggregation is closely related to [<sup>3</sup>H]PAF displacement from the high affinity binding sites with the more active antagonists in the ester and in the carbamate series (Table V).

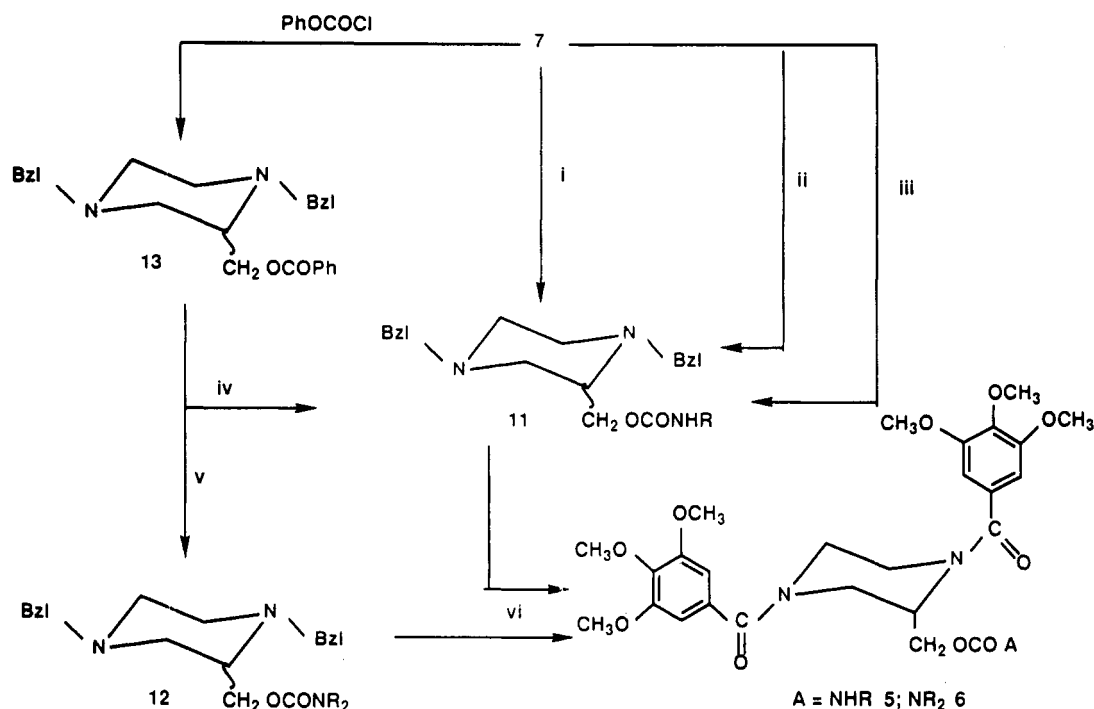
In ester series ( $Z = \text{CH}_2\text{OCOR}$ ), Table V shows that bulky groups such as  $R = \text{tBu}$ ,  $\text{CH}(\text{Et})_2$ ,  $\text{tBuCH}_2$ , or *o*-ClPh, compounds 1g, 1h, 1j, or 1k, respectively, induce the highest antagonistic activity at a lower or equal hydrophobicity than that of their linear equivalent in number of carbons. Thus, at isolipophilicity the bulky substituent produces a higher antagonistic activity. For example, in compounds 1h and 1j bulky appendices which present the

same hydrophobicity ( $f(R) = 2.78$ ) as the *n*-pentyl substituent included in compound 1c enhance antagonistic activity from 2 to 4 times. In strictly linear substitution, antiaggregant activity is maximum with *n*-pentyl or *n*-heptyl chain (compounds 1c and 1d),  $\text{IC}_{50} = 2.9$  and  $3.1 \times 10^{-7}$  M. A shorter (compound 1b) and a longer chain equivalent to that of PAF (compound 1f) gives lower activities (Table V).

Similar features are observed in the *N,N*-disubstituted carbamate series ( $Z = \text{CH}_2\text{OCONR}_2$ ): the maximum antagonistic activity is observed at a lipophilicity of  $f(R)_2 = 3.48$ , compound 6b,  $\text{IC}_{50} = 3.8 \times 10^{-7}$  M, compared with compound 1d from the ester series,  $f(R) = 3.81$ ,  $\text{IC}_{50} = 3.1 \times 10^{-7}$  M.

These latter two results are characteristic of an interaction with a hydrophobic area in the receptor and are observed in other PAF antagonist series. Heuer and Birke<sup>46</sup> found the same characteristics in tetrazepine series: WEB 2347 14a, which is a very potent PAF antagonist ( $\text{IC}_{50} = 8 \times 10^{-8}$  M) presents a hydrophobic *N,N*-dialkyl subsite ( $f(n\text{-propyl})_2 = 3.48$ ) equivalent to that in our carbamate series.

In addition, we have shown<sup>47</sup> that in a cationic PAF antagonist series structurally related to PAF (2-oxo-5-substituted-tetrahydrofuran 15a, 15b) such a hydrophobic limit occurs in the same range corresponding to a C<sub>6</sub>–C<sub>7</sub> linear chain ( $f(R) = 3.1$ – $3.6$ ), compound 15a. In this case,

Scheme II<sup>a</sup>

<sup>a</sup> (i) RN=C=O; (ii) RCO<sub>2</sub>H, diphenyl phosphorazidate (DPPA); (iii) RCOCl, NaN<sub>3</sub>, Δ; (iv) RNH<sub>2</sub>; (v) R<sub>2</sub>NH; (vi) (1) H<sub>2</sub>/Pd, (2) (MeO)<sub>3</sub>PhCOCl.

**Table III.** Physicochemical Data of 1,4-Bis(3',4',5'-trimethoxybenzoyl)-2-[[N-monoalk(aryl)carbamoyl]oxy]methylpiperazines (5) (See Table I)

no.	R	route <sup>a</sup>	mp, °C	% yield	R <sub>f</sub> <sup>b</sup>	<sup>1</sup> H NMR (CDCl <sub>3</sub> ) δ ppm <sup>c</sup>	formula <sup>d</sup>
5a	(CH <sub>2</sub> ) <sub>3</sub> CH <sub>3</sub>	(i)	87.6	71	0.30	3.42–2.7 <sup>e</sup> (m, 2 H, CH <sub>2</sub> N), 1.3 (m, 4 H, CH <sub>2</sub> ), 0.8 (t, 3 H, CH <sub>3</sub> )	C <sub>30</sub> H <sub>41</sub> N <sub>3</sub> O <sub>10</sub>
5b	C(CH <sub>3</sub> ) <sub>3</sub>	(iv)	resin	75	0.33	1.22 (s, 9 H, CH <sub>3</sub> )	C <sub>30</sub> H <sub>41</sub> N <sub>3</sub> O <sub>10</sub>
5c	CH <sub>2</sub> C(CH <sub>3</sub> ) <sub>3</sub>	(iv)	118.8	72	0.32	2.9 (d, 2 H, CH <sub>2</sub> ), 0.75 (s, 9 H, CH <sub>3</sub> )	C <sub>31</sub> H <sub>43</sub> N <sub>3</sub> O <sub>10</sub>
5d <sup>f</sup>	CHMe(CH <sub>2</sub> ) <sub>2</sub> CH <sub>3</sub>	(iv)	resin	78	0.29	3.67–2.90 <sup>e</sup> (m, 1 H, CH), 1.27 (m, 4 H, CH <sub>2</sub> ), 1.02 (dd, 3 H, Me), 0.85 (t, 3 H, CH <sub>3</sub> )	C <sub>31</sub> H <sub>43</sub> N <sub>3</sub> O <sub>10</sub> <sup>g</sup>
5e <sup>f</sup>	CHMeC(CH <sub>3</sub> ) <sub>3</sub>	(iv)	resin	80	0.30	3.65–2.9 <sup>e</sup> (m, 1 H, CH), 0.97 (m, 3 H, Me), 0.81 (s, 9 H, CH <sub>3</sub> )	C <sub>32</sub> H <sub>45</sub> N <sub>3</sub> O <sub>10</sub>
5f <sup>f</sup>	CHMeCH <sub>2</sub> CH(CH <sub>3</sub> ) <sub>2</sub>	(iv)	resin	78	0.35	3.42–2.7 <sup>e</sup> (m, 1 H, CHN), 2.13 (m, 1 H, CH), 1.18 (m, 2 H, CH <sub>2</sub> ), 0.98 (d, 3 H, Me), 0.78 (d, 6 H, CH <sub>3</sub> )	C <sub>32</sub> H <sub>45</sub> N <sub>3</sub> O <sub>10</sub>
5g	C(Me) <sub>2</sub> CH <sub>2</sub> CH <sub>3</sub>	(iv)	resin	69	0.34	1.2 (m, 8 H, CH <sub>2</sub> and Me), 0.8 (t, 3 H, CH <sub>3</sub> )	C <sub>31</sub> H <sub>43</sub> N <sub>3</sub> O <sub>10</sub>
5h <sup>f</sup>	CHMeCH(CH <sub>3</sub> ) <sub>2</sub>	(iv)	resin	77	0.32	3.65–2.90 <sup>e</sup> (m, 1 H, CHN), 1.55 (m, 1 H, CH), 0.97 (dd, 3 H, Me), 0.78 (d, 6 H, CH <sub>3</sub> )	C <sub>31</sub> H <sub>43</sub> N <sub>3</sub> O <sub>10</sub>
5i	(CH <sub>2</sub> ) <sub>2</sub> CH(CH <sub>3</sub> ) <sub>2</sub>	(iv)	resin	66	0.33	3.35–2.8 <sup>e</sup> (m, 2 H, CH <sub>2</sub> N), 1.58 (m, 1 H, CH), 1.27 (m, 2 H, CH <sub>2</sub> ), 0.80 (d, 6 H, CH <sub>3</sub> )	C <sub>31</sub> H <sub>43</sub> N <sub>3</sub> O <sub>10</sub>
5j	CH <sub>2</sub> CHEt(CH <sub>2</sub> ) <sub>3</sub> CH <sub>3</sub>	(iv)	resin	65	0.35	3.5–2.8 <sup>e</sup> (m, 2 H, CH <sub>2</sub> N), 1.78 (m, 1 H, CH), 1.2 (large s, 8 H, CH <sub>2</sub> ), 0.8 (t, 6 H, CH <sub>3</sub> )	C <sub>34</sub> H <sub>49</sub> N <sub>3</sub> O <sub>10</sub>
5k	(CH <sub>2</sub> ) <sub>7</sub> CH <sub>3</sub>	(iv)	resin	82	0.32	3.4–2.75 <sup>e</sup> (m, 2 H, CH <sub>2</sub> N), 1.22 (large s, 12 H, CH <sub>2</sub> ), 0.85 (t, 3 H, CH <sub>3</sub> )	C <sub>34</sub> H <sub>49</sub> N <sub>3</sub> O <sub>10</sub>
5l <sup>f</sup>	CH <sub>2</sub> CH(CH <sub>3</sub> )CH <sub>2</sub> CH <sub>3</sub>	(iv)	resin	72	0.31	3.45–2.75 <sup>e</sup> (m, 2 H, CH <sub>2</sub> N), 1.7 (m, 1 H, CH), 1.22 (m, 2 H, CH <sub>2</sub> ), 0.85 (m, 6 H, CH <sub>3</sub> )	C <sub>31</sub> H <sub>43</sub> N <sub>3</sub> O <sub>10</sub>
5m	CH(CH <sub>2</sub> CH <sub>3</sub> ) <sub>2</sub>	(ii)	90.2	70	0.30	3.55–2.92 <sup>e</sup> (m, 1 H, CH), 1.37 (m, 4 H, CH <sub>2</sub> ), 0.82 (t, 6 H, CH <sub>3</sub> )	C <sub>31</sub> H <sub>43</sub> N <sub>3</sub> O <sub>10</sub>
5n	3,4,5-(MeO) <sub>3</sub> C <sub>6</sub> H <sub>2</sub>	(iii)	resin	58	0.37	6.7–6.55 <sup>e</sup> (m, 2 H, ArH), 3.95–3.6 <sup>e</sup> (large s, 9 H, CH <sub>3</sub> O)	C <sub>35</sub> H <sub>43</sub> N <sub>3</sub> O <sub>13</sub> <sup>h</sup>

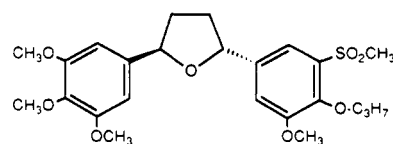
<sup>a</sup> Process used for preparation of the intermediate 11. <sup>b</sup> MeOH/CHCl<sub>3</sub>, 3:97, v/v. <sup>c</sup> Specific signals other than common ones described in Experimental Section. <sup>d</sup> All compounds were analyzed for C, H, N; analytical results were within ±0.4% of theoretical values unless specified.

<sup>e</sup> Part of this signal with piperazine CH<sub>2</sub>N. <sup>f</sup> Diastereoisomeric mixture, only one spot is observed in TLC. <sup>g</sup> N: calcd 6.80; found, 7.24. <sup>h</sup> N: calcd, 5.89; found, 5.48.

increasing the number of carbons does not modify the antiaggregant activity. When a trimethoxyphenyl group, compound 15b, replaces the hydrophobic subsite in position 5, the same activity is observed in spite of lower hydrophobicity.

These features indicate that this hydrophobic pocket in the receptor is located near a positive counterpart area of the binding site.<sup>47</sup> Indeed, some potent PAF antagonists bearing functions generating a "Cache-oreilles" system also present a weak hydrophobic appendice in one electronegative well (*n*-propyl), for instance L-659,989, 16,<sup>48</sup> inhibits

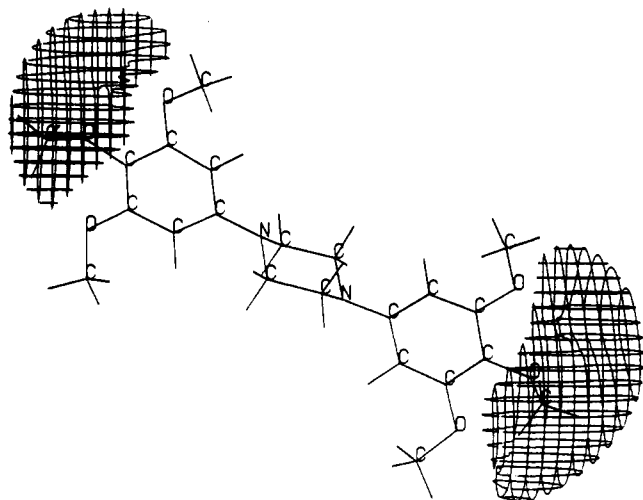
[<sup>3</sup>H]PAF binding to rabbit platelet membranes with an IC<sub>50</sub> = 3 × 10<sup>-9</sup> M (for other examples also see reviews in refs. 29, 30).



**Table IV.** Physicochemical Data of 1,4-Bis(3',4',5'-trimethoxybenzoyl)-2-[(*N,N*-dialkylcarbamoyl)oxy]methyl]piperazines (**6**) (See Table I)

no.	R	mp, °C	% yield	$R_f^a$	$^1\text{H NMR}$ ( $\text{CDCl}_3$ ) $\delta$ ppm <sup>b</sup>	formula <sup>c</sup>
<b>6a</b>	$\text{CH}_2\text{CH}_3$	128.6	82	0.35	3.15 (q, 4 H, $\text{CH}_2$ ), 1.0 (t, 6 H, $\text{CH}_3$ )	$\text{C}_{30}\text{H}_{41}\text{N}_3\text{O}_{10}$
<b>6b</b>	$(\text{CH}_2)_2\text{CH}_3$	109.3	80	0.36	3.4–2.8 <sup>d</sup> (m, 4 H, $\text{CH}_2\text{N}$ ), 1.3 (m, 4 H, $\text{CH}_2$ ), 0.8 (t, 6 H, $\text{CH}_3$ )	$\text{C}_{32}\text{H}_{45}\text{N}_3\text{O}_{10}$
<b>6c</b>	$(\text{CH}_2)_3\text{CH}_3$	130.2	80	0.38	3.4–2.75 <sup>d</sup> (m, 4 H, $\text{CH}_2\text{N}$ ), 1.25 (m, 8 H, $\text{CH}_2$ ), 0.82 (t, 6 H, $\text{CH}_3$ )	$\text{C}_{34}\text{H}_{49}\text{N}_3\text{O}_{10}$
<b>6d</b>	$(\text{CH}_2)_4\text{CH}_3$	131.8	68	0.39	3.4–2.7 <sup>d</sup> (m, 4 H, $\text{CH}_2\text{N}$ ), 1.2 (m, 12 H, $\text{CH}_2$ ), 0.8 (t, 6 H, $\text{CH}_3$ )	$\text{C}_{36}\text{H}_{53}\text{N}_3\text{O}_{10}$

<sup>a</sup> MeOH/ $\text{CHCl}_3$ , 3:97, v/v. <sup>b</sup> Specific signals other than common ones described in Experimental Section. <sup>c</sup> All compounds were analyzed for C, H, N; analytical results were within  $\pm 0.4\%$  of theoretical values. <sup>d</sup> Part of this signal with piperazine  $\text{CH}_2\text{N}$ .

**Figure 1.** 3D electrostatic potential map (isocountours drawn at  $-10$  kcal/mol) calculated for 1,4-bis(3',4',5'-trimethoxyphenyl)-piperazine (**3**).

In the monosubstituted carbamate series ( $Z = \text{CH}_2\text{-OCONHR}$ ), we have explored the substitution effect (steric effect) along a linear chain of 4–5 carbons. Despite the presence of diastereoisomeric mixtures the results are similar to those obtained in the preceding series, from  $4.7 \times 10^{-7}$  M (compound **5i**) to  $8 \times 10^{-8}$  M (compound **5h**). The same bulky substituents *t*Bu and  $\text{CH}(\text{Et})_2$  induce a similar activity in the carbamate series as in the ester series (compounds **5b** and **5m**). When the lipophilicity of the R moiety is excessive (compound **5j**,  $f(\text{R}) > 4.0$ ) the antiaggregant activity decreases, as observed in other series.

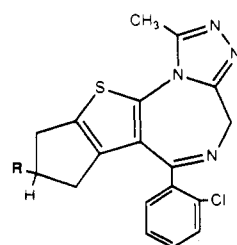
An aromatic moiety rich in electrons without steric effects does not give potent antagonistic activity, compounds **1l**, **1m**, and **5n** ( $\text{IC}_{50} \approx (5\text{--}14) \times 10^{-6}$  M).

**Consequences of the Monosubstitution.** (i) **Conformation.** N-containing six-membered rings have been known to form the chair conformation<sup>43,44,49</sup> except for molecules possessing internal hydrogen bonds which stabilize a twisted ring (reviewed in ref 49) and for a few constrained compounds such as *cis*-2,5-disubstituted-1,4-dibenzoylpiperazines which present a twist-boat conformation as demonstrated in the literature by both NMR and X-ray studies.<sup>50–52</sup> Introduction of monosubstitution at the piperazine ring implies the theoretical existence of four rotamers, *cisoid* 1 and 2 and *transoid* 3 and 4 (Figure 3). The conformational stability depends obviously on a balance between steric interactions of phenyl groups and the ring (with vicinal equatorial hydrogens) and also between the ring and the R substituent. The optimum position for Z is axial as described in the literature for 1,4-dibenzoylpiperazines.<sup>50–52</sup> We examined the geometry of the series presented in this paper and selected one of the more representative compounds, **1g**, which possesses a bulky substituent, i.e.,  $\text{R} = \text{tBu}$ , representative of the sterically hindered compounds such as **1h**, **1i**, **1k**, **5e**, **5f**,

**Table V.** Biological Data and Hydrophobic Fragmental Constant of R Group in  $Z = \text{CH}_2\text{OCOR}$ ,  $\text{CH}_2\text{OCONHR}$ , and  $\text{CH}_2\text{OCONR}_2$  (See Table I for General Formulas)

no. <sup>a</sup>	$\text{IC}_{50}$ ( $\mu\text{M}$ ) <sup>b</sup>	$\text{EC}_{50}$ ( $\mu\text{M}$ ) <sup>c</sup>	$f(\text{R})$ or $f(\text{R}_2)$ <sup>d</sup>
<b>17</b> <sup>e</sup>	0.74	0.18	
<b>1a</b>	0.43	0.40	0.70
<b>1b</b>	0.42		1.74
<b>1c</b>	0.29		2.78
<b>1d</b>	0.31		3.81
<b>1e</b>	2.82		4.85
<b>1f</b>	45		9.00
<b>1g</b> <sup>f</sup>	0.10	0.30	2.26
	(+) 0.12 <sup>g</sup>	(+) 0.42	
	(-) 0.10	(-) 0.30	
<b>1h</b>	0.07	0.25	2.78
	(+) 0.06 <sup>g</sup>	(+) 0.22	
	(-) 0.04	(-) 0.09	
<b>1i</b>	0.29		2.93
<b>1j</b>	0.12		2.78
<b>1k</b>	0.17	0.45	2.58
	(+) 0.88 <sup>g</sup>	(+) 0.61	
	(-) 0.08	(-) 0.05	
<b>1l</b>	10.3		2.99
<b>1m</b>	5.0		3.53
<b>2</b>	1.78		
<b>3</b>	12.5		
<b>4</b>	2.7		
<b>5a</b>	0.24	0.70	2.26
<b>5b</b>	0.13		2.26
<b>5c</b>	0.10		2.78
<b>5d</b>	0.10		2.78
<b>5e</b>	0.17		3.29
<b>5f</b>	0.28	0.18	3.29
<b>5g</b>	0.32		2.78
<b>5h</b>	0.08	0.07	2.78
<b>5i</b>	0.14		2.78
<b>5j</b>	3.9		4.33
<b>5k</b>	0.37		4.33
<b>5l</b>	0.47		2.78
<b>5m</b>	0.13		2.78
<b>5n</b>	13.8		2.08
<b>6a</b>	25.8		2.44
<b>6b</b>	0.38	0.68	3.48
<b>6c</b>	1.25		4.52
<b>6d</b>	11.4		5.55

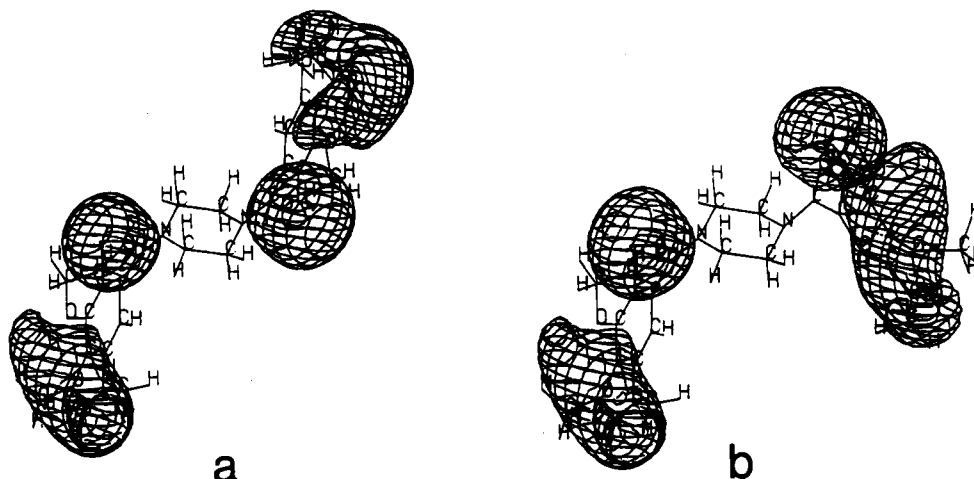
<sup>a</sup> Biological data for racemic or diastereoisomeric mixtures, except for compounds **1g**, **1h**, and **1k**. <sup>b</sup> Inhibition of PAF-induced platelet aggregation. <sup>c</sup> [ $^3\text{H}$ ]PAF displacement on platelet membranes (cf. Experimental Section). <sup>d</sup> Hydrophobic fragmental constant of R group(s). <sup>e</sup> BN 52021, **14**, used as reference. <sup>f</sup> PMS 536-BN 54062. <sup>g</sup> Biological data of (+) and (–) enantiomer.



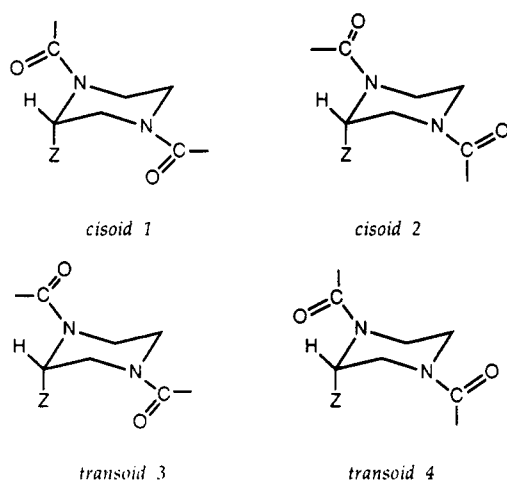
$\text{R} = \text{---C---N}(\text{C}_3\text{H}_7)_2$  WEB 2347 **14 a**

$\text{R} = \text{---C---N---O}$  WEB 2170 **14 b**

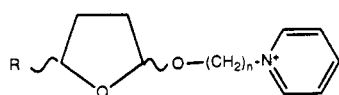
**5g**, **5h**, and **5m**, for further investigation. X-ray studies revealed that the ring is chair, that the Z substituent ( $\text{CH}_2\text{-OCOtBu}$ ) is axial, and that only the *cisoid* 1 rotamer is present in the crystal.<sup>53</sup> These features are in agreement



**Figure 2.** 3D electrostatic potential maps of 1,4-bis(3',4',5'-trimethoxybenzoyl)piperazine (**2**) (a) in cisoid conformation and (b) in transoid conformation (isocontours drawn at  $-10$  kcal/mol).



**Figure 3.** Four possible conformations of 1,4-bis-benzoyl 2-substituted piperazine.



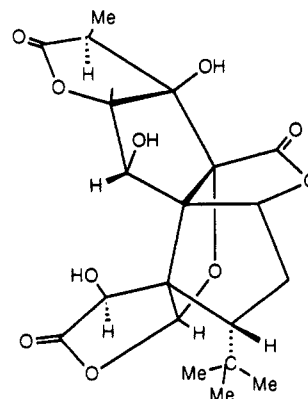
R =  $n$  C<sub>6</sub>H<sub>1,3</sub> **15 a**  
R = 3,4,5-trimethoxyphenyl **15 b**

with previous results.<sup>49–52</sup> Considering the relatively high level of rotation energy around the amide N–C bond (reviewed in ref 49), only ring reversal can be taken into account in solution in these series. These features were verified by 400-MHz <sup>13</sup>C NMR studies which demonstrate that the <sup>13</sup>C signals of the ring are large, close to coalescence at room temperature and that the <sup>13</sup>C signals of the amide carbonyls are weak and well separated (unpublished results<sup>54</sup>). Conformational optimization by AM1 (or MM2) of the four expected conformers demonstrate that the cisoid 1 and transoid 4 are more stable.<sup>54</sup> As observed by X-ray studies,<sup>53</sup> the optimized cisoid 1 conformer presents the *tert*-butyl chain folded under one of the trimethoxybenzoyl groups which explains the chemical nonequivalence of methoxy groups in 400-MHz <sup>1</sup>H and particularly <sup>13</sup>C NMR.<sup>54</sup>

Consequently, the studied compound **1g** presents a 3D electrostatic potential map (isocontoured at  $-10$  kcal/mol) with two major “Cache-oreilles” systems, generated by both methoxy groups at a distance of  $11$  Å (*p*-methoxy) and carbonyl groups of amides (C=O, O=C) at a distance of

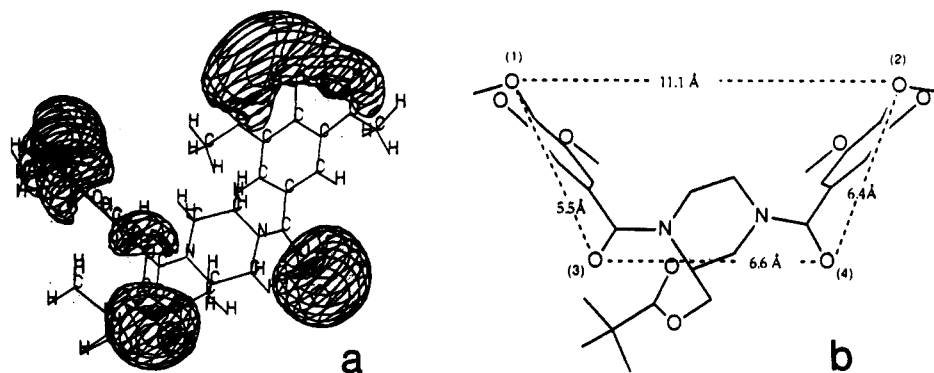
$6.6$  Å. A secondary well appears at the carbonyl of the ester group, opposite to one of the neighboring amide groups (dipole–dipole interaction) and a small capsule is present, which is almost fused with the well generated by the amide group.

It seems that the bulky groups induce an uniquely favorable conformation (cisoid 1), giving a potent antagonist activity. The role of both “Cache-oreilles” systems is determinant and leads us to believe that at least a tetrapolarized interaction with the receptor takes place. The presence of a “Cache-oreilles” system at a short distance generated by the C=O of amide groups of  $6.6$  Å (Figure 4) reveals that this distance ( $6.5$ – $7.2$  Å) found between atoms generating the “Cache-oreilles” system in the ginkgolides<sup>31</sup> is not exceptional. Moreover, such distances are found between the extremities of each “Cache-oreilles” system in the ginkgolide, BN 52 021 17.



BN 52021 17

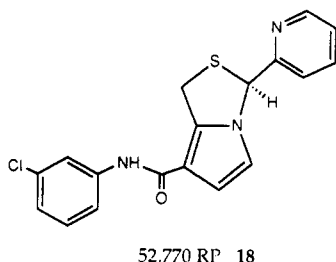
(ii) **Enantiomery.** It appears that in these series enantiospecificity is not present. No significant difference of inhibitory activity is displayed by (+) and (–) enantiomers of compounds **1g** and **1h**. Nevertheless, the (–) isomer of **1k** is 10 times more active than the (+) isomer in both biological responses (Table V), i.e., inhibition of PAF-induced platelet aggregation and in displacing [<sup>3</sup>H]-PAF from platelet membrane. Generally no significant enantiospecificity is observed for antagonistic activities either for PAF-like antagonists<sup>55</sup> or for compounds not structurally related to PAF<sup>56,57</sup> (see also reviews in refs 13, 24, 29, 30 and included references in ref 55). The results



**Figure 4.** (a) 3D electrostatic potential map (isocontours drawn at  $-10$  kcal/mol) of 1,4-bis(3',4',5'-trimethoxybenzoyl)-2-[(*tert*-pentanoyloxy)methyl]piperazine (**3g**) and (b) characteristic distances between atoms generating the negative wells.

in 2,5-diaryltetrahydrofurans are also ambiguous: the enantiospecificity can be weak<sup>56,57</sup> or relatively high (reviewed in ref 58). Indeed, Guthrie et al.<sup>59</sup> obtained the same results as ours in a series of pentadienyl carboxamide derivatives: the difference of inhibitory activity is included between 0 and 10 times.

When one of the negative wells of the unique "Cache-oreilles" is associated with chiral carbon, the enantiospecificity seems to be more significant. For example, displacement of [<sup>3</sup>H]-52,770 RP, a pyrrolo[1,2-*c*]thiazole derivative, by the (+) and (−) enantiomers of **18** shows a difference of 300 between the enantiomers<sup>60</sup> (reviewed in ref 30).



This is also the case for the hetrazepine series (reviewed in ref 61), where the presence of a chiral carbon bearing one of the two negative wells induces an enantiospecificity, the *S*-(−) isomer of compound **14b** (WEB 2170) being 50 times more active than *R*-(+) isomer.

However, this is still low and not essential with regard to the difference in agonistic enantiospecificity: (*R*)-PAF, natural form, is  $10^4$  more active than (*S*)-PAF<sup>9,62</sup> (reviewed in ref 63).

## Conclusion

In this paper we have presented our reasoning which allowed us to obtain high potency PAF antagonists. The presence of a second system of "Cache-oreilles" in the piperazine analogues synthesized in the present study indicates that this feature is required for high potency PAF antagonism. 3D potential electrostatic map calculations of **1g** reveals the importance of the shorter "Cache-oreilles" system which can be compared to the system present in the ginkgolides.<sup>31</sup> Consequently, it appears that the PAF receptor may be tetrapolarized as we proposed in a recent short communication.<sup>64</sup> The hydrophobic appendix is important to enhance the antagonistic activity: such a feature may be provided by a bulky group or a C<sub>6</sub>–C<sub>7</sub> linear alkyl substituent or its hydrophobic equivalent (thioether, aryl, etc). These facts are confirmed

qualitatively in aryl carboxamide series<sup>58,65</sup> and with ketopiperazine analogues.<sup>66</sup> This hydrophobic limitation is revealed in very different series of PAF antagonists, including molecules which present a cationic moiety.<sup>47</sup> Hydrophobic pockets may occur near an electropositive part of the receptor, and at least two areas can be proposed for such pockets, their being opposite each other<sup>64</sup> explaining the moderate enantiospecificity of the receptor versus antagonists where a hydrophobic appendice is linked to a chiral carbon. Conversely, electronegative areas may alternate with hydrophobic and (or) electropositive zones. Conformational and electronic properties of PAF agonists and antagonists structurally related to PAF have been recently studied<sup>67</sup> and are compatible with our proposed model. Further studies will be performed on other PAF antagonists presenting one or several negative wells, enabling us to present a more complete hypothesis. Indeed, our model corresponds to rabbit platelet high affinity binding sites, and it is currently thought that this G-protein coupled receptor (cloned from guinea pig lung<sup>68</sup>) is not homogeneous<sup>69,70</sup> and differs according to cell origin (platelet versus polymorphonuclear leukocytes<sup>71</sup> and platelets versus macrophages<sup>72</sup>). These features are not in contradiction with our model: the polarization can vary according to membrane state and to its composition. However, this does not preclude the possibility that an antagonist presenting several adjusted negative wells may present the same antagonistic activities in several cells. Indeed, in a preliminary study, compound **1g** which is a potent PAF antagonist in platelets ( $IC_{50} = 1.0 \times 10^{-7}$  M) was also found to be a potent inhibitor of PAF-induced neutrophil aggregation ( $IC_{50} = 3 \times 10^{-8}$  M). Further studies are required on compound **1g** (PMS 536-BN 54062) in this context and also to further investigate its dual activity.

A recent paper<sup>73</sup> examining heterocyclic nitrogen PAF antagonists proposes a partial pharmacophore for the receptor. Contrary to the latter author's assertions, our hypothesis of a multipolarized system fits not only with "weakly" active natural products such as ginkgolides and kadsurenone but also with high affinity synthetic PAF receptor antagonists such as hetrazepine WEB 2086 as well as neolignans derived from THF series. Moreover, the pharmacophore map presented by Hodgkin et al.<sup>73</sup> shows a "Cache-oreilles" system between N (heterocyclic) and C=O at the same distance (calculated by the latter authors) as found in our model, i.e.  $\approx 12$  Å. Indeed this is not surprising as one molecule (WEB 2086) is common in both studies.



## Experimental Section

**Chemistry. General Methods.** The purity of each compound was checked by thin-layer chromatography on TLC plastic sheets (silica gel 60F<sub>254</sub>, layer thickness 0.2 mm) from Merck. Column chromatography purification was carried out on silica gel 60 (particle size 0.063–0.200 mm) from Merck, without any special treatment. All melting points were determined in a digital melting point apparatus (Electrothermal) and are uncorrected. The structures of all compounds were confirmed by IR and <sup>1</sup>H NMR spectra. IR spectra were obtained with a PYE-UNICAM SP3-200 infrared spectrometer, and <sup>1</sup>H NMR spectra were recorded in CDCl<sub>3</sub> on a BRUKER WP 80 spectrometer using hexamethyldisiloxane (HMDS) as an internal standard. Specific rotations, [ $\alpha$ ]<sub>D</sub>, were recorded on a Perkin-Elmer 141 polarimeter at 20 °C temperature and a concentration of 25 mg/mL in CHCl<sub>3</sub>. All elemental analyses were within  $\pm 0.4\%$  of theoretical values.

**1,4-Dibenzyl-2-[(acyloxy)methyl]piperazine (8). General Procedure.** A solution of 2 g (6.8 mmol) of 7 prepared according to a published procedure<sup>34</sup> in 30 mL of dry benzene and 1 mL of Et<sub>3</sub>N was added dropwise with 6.8 mmol of the desired acyl chloride in 10 mL of dry benzene. After the mixture was stirred overnight at room temperature, the solvent and excess Et<sub>3</sub>N were eliminated under vacuum, and the crude residue, taken up in CHCl<sub>3</sub>, was washed with H<sub>2</sub>O, 5% dilute NaHCO<sub>3</sub>, and H<sub>2</sub>O. The organic layer was then dried (MgSO<sub>4</sub>), evaporated, and chromatographed on a silica gel column using Et<sub>2</sub>O/petroleum ether (10:90 v/v) as eluent, giving about a 70% yield of the title compound 8 as an oil. IR (film): all derivatives 8a–m displayed common bands at 3090, 3070, 3030 ( $\nu$  aromatic CH), 2940–2810 ( $\nu$  CH), 1735 ( $\nu$  C=O), and 1600 ( $\nu$  C=C) cm<sup>-1</sup>. <sup>1</sup>H NMR: all derivatives 8a–m displayed common signals at average  $\delta$  ppm 7.27 (large s, 10 H, aromatic H), 4.27 (m, 2 H, CH<sub>2</sub>OCO), 3.95–3.41 (2 d,  $J$  = 14 Hz, 2 H, CH<sub>2</sub>Ph), 3.42 (s, 2 H, CH<sub>2</sub>Ph), 2.95–2.02 (m, 7 H, piperazine).

**2-[(Acyloxy)methyl]piperazine (9). General Procedure.** A solution of 4 mmol of 8 and 50 mg Pd (10%)/charcoal in 50 mL of absolute EtOH and 0.3 mL of concentrated HCl was treated with H<sub>2</sub> under pressure (40 psi) with shaking at 40 °C overnight. After filtration, the ethanol was evaporated to dryness. The crude residue was taken up in the minimum volume of MeOH to dissolve. The addition of Et<sub>2</sub>O allowed precipitation of 9-HCl which was used just as it is in the next step.

**1,4-Bis(3',4',5'-trimethoxybenzoyl)-2-[(acyloxy)methyl]piperazine (1). General Procedure.** A solution of 2.2 mmol of 9-HCl in 30 mL of dry benzene and 1.5 mL of Et<sub>3</sub>N was added dropwise with 1 g (4.6 mmol) of 3,4,5-trimethoxybenzoyl chloride in 10 mL of dry benzene. The mixture was kept overnight with stirring at room temperature. After evaporation of the solvents in vacuum, the residue was taken up in CHCl<sub>3</sub> and washed with H<sub>2</sub>O, 5% dilute NaHCO<sub>3</sub>, and H<sub>2</sub>O until pH 7. The organic layer was dried (MgSO<sub>4</sub>) and concentrated, and the crude product was purified on a silica gel column using first CHCl<sub>3</sub> and then MeOH/CHCl<sub>3</sub> (0.5:99.5, v/v) as eluents, yielding about 70% of 1 with aspect depending on R (see Table II). IR (in paraffin oil): all compounds 1a–m displayed common bands at 3050–3000 ( $\nu$  aromatic CH), 2940–2860 ( $\nu$  CH), 1730 ( $\nu$  C=O ester), 1640 ( $\nu$  C=O amide), 1585 ( $\nu$  C=C) cm<sup>-1</sup>. <sup>1</sup>H NMR: all final products 1a–m displayed common signals at average  $\delta$  ppm 7.15–6.57 (one or two s, depending on R, 4 H, aromatic H), 5.07–4.30 (m, 2 H, CH<sub>2</sub>OCO), 3.86–3.77 (large s, 18 H, CH<sub>3</sub>O), 4.45–3.97, 3.5–2.66 (2 m, 7 H, piperazine CH<sub>2</sub>N and CHN). Specific signals, mp, and morphologies are reported in Table II.

**1,4-Bis(3',4',5'-trimethoxybenzoyl)piperazine (2).** This compound was prepared as 1 starting from piperazine (Aldrich Chemie). Mp: 224.6 °C (lit. mp: 220–222 °C,<sup>36</sup> 218–220 °C<sup>37</sup>). IR (paraffin oil): 1630 ( $\nu$  C=O), 1590 ( $\nu$  C=C) cm<sup>-1</sup>. <sup>1</sup>H NMR:  $\delta$  ppm 6.62 (s, 4 H, aromatic H), 3.87 (s, 18 H, CH<sub>3</sub>O), 3.62 (s, 8 H, CH<sub>2</sub>). Anal. (C<sub>24</sub>H<sub>30</sub>N<sub>2</sub>O<sub>8</sub>) C, H, N.

**1,4-Bis(3',4',5'-trimethoxyphenyl)piperazine (3).** This compound prepared according to published procedure<sup>38</sup> was purified on a silica gel column using 20% ether in petroleum ether as eluent. Mp: 225 °C (lit. mp: 201–202 °C). IR (paraffin oil): 1590 ( $\nu$  C=C) cm<sup>-1</sup>. <sup>1</sup>H NMR:  $\delta$  ppm 5.95 (s, 4 H, aromatic H), 3.87 (s, 18 H, CH<sub>3</sub>O), 3.67 (s, 8 H, CH<sub>2</sub>). Anal. (C<sub>22</sub>H<sub>30</sub>N<sub>2</sub>O<sub>6</sub>) C, H, N.

**1,4-Bis(3',4',5'-trimethoxybenzyl)-2-[(2'-ethylbutanoyl)-oxy]methylpiperazine (4).** 9h-HCl (2.5 g, 8.7 mmoles), prepared via the general procedure for 9, was neutralized with stirring in a mixture of dry C<sub>6</sub>H<sub>6</sub> (50 mL) and Et<sub>3</sub>N (4 mL) at room temperature for 1 h. After filtration of Et<sub>3</sub>N, HCl, C<sub>6</sub>H<sub>6</sub>, and the excess of Et<sub>3</sub>N were evaporated to leave 1.5 g (80%) of crude diamine 9h as a wax. IR (film): 3340 ( $\nu$  NH), 2960–2860 ( $\nu$  CH), and 1730 ( $\nu$  C=O) cm<sup>-1</sup>. Diamine 9h (1.25 g, 5.8 mmol) and 2.52 g (11.6 mmol) of 3,4,5-trimethoxybenzyl chloride were refluxed with stirring in 60 mL of dry xylene for 4 h. After evaporation of the solvent, the residue was taken up with a saturated solution of NaHCO<sub>3</sub> and then extracted with CHCl<sub>3</sub>, and the organic layer was washed with H<sub>2</sub>O until pH 7. Drying (MgSO<sub>4</sub>), filtration, and evaporation of CHCl<sub>3</sub> led to a mixture which was chromatographed on a silica gel column using CHCl<sub>3</sub> as eluent. This purification yielded 2 g (60%) of the title compound as a yellow oil. IR (film): 3000 ( $\nu$  aromatic CH), 2960–2820 ( $\nu$  CH), 1730 ( $\nu$  C=O ester), 1590 ( $\nu$  C=C) cm<sup>-1</sup>. <sup>1</sup>H NMR:  $\delta$  ppm 6.56 (s, 4 H, aromatic H), 4.72–3.97 (m, 4 H, CH<sub>2</sub>OCO and (MeO)<sub>3</sub>PhCH<sub>2</sub>), 3.80 (large s, 18 H, CH<sub>3</sub>O), 3.40 (s, 2 H, (MeO)<sub>3</sub>-PhCH<sub>2</sub>), 3.0–2.0 (m, 8 H, piperazine H and CHCO<sub>2</sub>), 1.47 (m, 4 H, CH<sub>2</sub>), 0.77 (t, 6 H, CH<sub>3</sub>). Anal. (C<sub>31</sub>H<sub>46</sub>N<sub>2</sub>O<sub>8</sub>) C, H, N.

**1,4-Dibenzyl-2-(hydroxymethyl)piperazines (+)-7 and (-)-7.** A warmed (60 °C) solution of 100 g (33.8 mmol) of ( $\pm$ )-7 (mp 81.3 °C, lit. bp 174–175 °C/0.02 Torr<sup>34</sup>) in 100 mL of AcOEt was added with a warmed (60 °C) solution of 10.6 g (67.6 mmol) of (+)-mandelic acid in 100 mL of AcOEt. The mixture was kept until cooled at room temperature without stirring and then put in the refrigerator for 2 h, during which the diastereoisomeric salt (+)-10 crystallized. It was recovered by filtration and recrystallized in AcOEt until the melting point and [ $\alpha$ ]<sub>D</sub> remained constant, mp 123–124 °C, [ $\alpha$ ]<sub>D</sub> = +85.2°. The collected filtrates were evaporated to dryness to give a viscous product containing (-)-10 and small amounts of (+)-10 which were eliminated by successive crystallizations in AcOEt and filtrations. The two diastereoisomeric salts solid (+)-10 and viscous (-)-10 were taken up separately in CHCl<sub>3</sub> and washed with a saturated NaHCO<sub>3</sub> solution then with H<sub>2</sub>O until pH 7 to give respectively the alcohols (+)-7 (mp 81.3 °C (hexane), [ $\alpha$ ]<sub>D</sub> = +28.1°) and (-)-7 (mp 81.2 °C (hexane), [ $\alpha$ ]<sub>D</sub> = -27.6°). The enantiomeric purity was respectively 96% (+) and 92% (-) (cf. Chemistry).

**1,4-Bis(3',4',5'-trimethoxybenzoyl)-2-[(tert-pentanoyloxy)-methyl]piperazines [(+)- and (-)-1g].** Alcohols (+)-7 and (-)-7 were treated as ( $\pm$ )-7 following general procedures described above, i.e. esterification with tertipentanoyl chloride, debenzoylation, and amidification with 3,4,5-trimethoxybenzoyl chloride led to the title compounds (+)-1g (mp 156 °C, [ $\alpha$ ]<sub>D</sub> = +18.5°) and (-)-1g (mp 155 °C, [ $\alpha$ ]<sub>D</sub> = -17.95°).

**1,4-Bis(3',4',5'-trimethoxybenzoyl)-2-[(2'-ethylbutanoyloxy)methyl]piperazines [(+)- and (-)-1h].** According to the general procedures described above, (+)-7 and (-)-7 after esterification with 2-ethylbutanoyl chloride, debenzoylation, and amidification gave the enantiomers (+)-1h (mp 159.7 °C, [ $\alpha$ ]<sub>D</sub> = +18.1°) and (-)-1h (mp 158.2 °C, [ $\alpha$ ]<sub>D</sub> = -17.5°).

**1,4-Bis(3',4',5'-trimethoxybenzoyl)-2-[(o-chlorobenzoyloxy)methyl]piperazines [(+)- and (-)-1k].** According to the general procedures described above, (+)-7 and (-)-7 after esterification with o-chlorobenzoyl chloride, debenzoylation, and amidification gave the enantiomers (+)-1k (mp 80 °C, [ $\alpha$ ]<sub>D</sub> = +19.7°) and (-)-1k (mp 80.8 °C, [ $\alpha$ ]<sub>D</sub> = -19.1°).

**1,4-Dibenzyl-2-[(N-n-butylcarbamoyl)oxy]methylpiperazine (11a) (route i).** A mixture of 1 g (3.4 mmol) of 7 and 0.4 g (4.1 mmol) of butyl isocyanate in 30 mL of dry C<sub>6</sub>H<sub>6</sub> was refluxed with stirring for 48 h. After evaporation of solvent and excess isocyanate, the residue was chromatographed on a silica gel column using Et<sub>2</sub>O/petroleum ether (30:70, v/v) as eluent, leading to 11a (0.67 g, 50%) as a yellow viscous oil. *R*<sub>f</sub>: 0.25 (Et<sub>2</sub>O/petroleum ether, 50:50, v/v). IR (film): 3350 ( $\nu$  NH), 3080, 3060, 3020 ( $\nu$  aromatic CH), 2980–2820 ( $\nu$  CH), 1720 ( $\nu$  C=O). <sup>1</sup>H NMR:  $\delta$  ppm 7.25 (s, 10 H, aromatic H), 4.62 (m, 1 H, NH), 4.25 (m, 2 H, CH<sub>2</sub>OCO), 3.98 and 3.35 (2 d,  $J$  = 14 Hz, 2 H, CH<sub>2</sub>Ph), 3.46 (s, 2 H, CH<sub>2</sub>Ph), 3.11 (m, 2 H, CH<sub>2</sub>NCO), 2.90–2.10 (m, 7 H, piperazine H), 1.27 (m, 4 H, CH<sub>2</sub>), 0.81 (t, 3 H, CH<sub>3</sub>).

**1,4-Dibenzyl-2-[(N-(diethylmethyl)carbamoyl)oxy]methylpiperazine (11m) (route ii).** A mixture of 1 g (3.4 mmol) of 7, 0.5 g (5 mmol) of Et<sub>3</sub>N, 0.92 g (3.4 mmol) of DPPA, and 0.5



g (4.3 mmol) of 2-ethylbutanoic acid in 40 mL of 1,4-dioxane was refluxed with stirring for 14 h. Evaporation of solvent left a residue which was taken up in hexane. This organic layer was washed with a concentrated  $\text{NaHCO}_3$  solution, with  $\text{H}_2\text{O}$ , and then with a saturated  $\text{NaCl}$  solution. Drying ( $\text{MgSO}_4$ ), filtration, and evaporation gave a crude product which was purified by chromatography on a silica gel column using  $\text{Et}_2\text{O}$ /petroleum ether (30:70, v/v) as eluent to yield 0.9 g (64%) of 11m as a yellow viscous oil.  $R_f$ : 0.20 ( $\text{Et}_2\text{O}$ /petroleum ether, 50:50, v/v). IR (film): the same as for 11a.  $^1\text{H}$  NMR:  $\delta$  ppm 7.30 (s, 10 H, aromatic H), 4.51 (m, 1 H, NH), 4.32 (m, 2 H,  $\text{CH}_2\text{OCO}$ ), 4.05 and 3.4 (2 d,  $J = 14$  Hz, 2 H,  $\text{CH}_2\text{Ph}$ ), 3.48 (m, 3 H,  $\text{CH}_2\text{Ph}$  and  $\text{CHNCO}$ ), 2.93–2.16 (m, 7 H, piperazine H), 1.41 (m, 4 H,  $\text{CH}_2$ ), 0.85 (t, 6 H,  $\text{CH}_3$ ).

**1,4-Dibenzyl-2-[[[N-(3',4',5'-trimethoxyphenyl)carbamoyl]oxy]methyl]piperazine (11n)** (route iii). To a stirred solution of 7.8 g (0.12 mol) of  $\text{NaN}_3$  in 50 mL of  $\text{H}_2\text{O}$  was added dropwise a solution of 23 g (0.1 mol) of 3,4,5-trimethoxybenzoyl chloride in 30 mL of  $(\text{CH}_3)_2\text{CO}$ . After 0.5 h of stirring at 25 °C, the precipitate of 3,4,5-trimethoxybenzoyl azide was filtered, dried under vacuum, and obtained in a 80% yield as white crystals (mp 87.5 °C). An aliquot (3.2 g, 17 mmol) of this azide and 5 g (17 mmol) of 7 in 100 mL of dry benzene were refluxed for 2.5 h. After evaporation, the residue was chromatographed on a silica gel column using  $\text{Et}_2\text{O}$ /petroleum ether (30:70, v/v) as eluent to leave 4.4 g (55%) of yellow viscous 11n.  $R_f$ : 0.20 ( $\text{Et}_2\text{O}$ /petroleum ether, 50:50, v/v). IR (film): 3320 ( $\nu$  NH), 3080, 3060, 3020 ( $\nu$  aromatic CH), 2950–2800 ( $\nu$  CH), 1725 ( $\nu$  C=O)  $\text{cm}^{-1}$ .  $^1\text{H}$  NMR:  $\delta$  ppm 7.20 (s, 10 H, aromatic H), 6.55 (s, 2 H, trimethoxyphenyl aromatic H), 4.26 (d,  $J = 5$  Hz, 2 H,  $\text{CH}_2\text{OCO}$ ), 3.92 and 3.35 (2 d,  $J = 14$  Hz, 2 H,  $\text{CH}_2\text{Ph}$ ), 3.75 (s, 9 H,  $\text{CH}_3\text{O}$ ), 3.38 (s, 2 H,  $\text{CH}_2\text{Ph}$ ), 2.92–2.05 (m, 7 H, piperazine H).

**1,4-Dibenzyl-2-[[[N-(phenoxycarbonyl)oxy]methyl]piperazine (13)**. To an ice bath cooled solution of 10 g (33.7 mmol) of 7 in 200 mL of  $\text{CH}_2\text{Cl}_2$  was added dropwise a solution of 6.4 g (42 mmol) of phenoxycarbonyl chloride in 50 mL of  $\text{CH}_2\text{Cl}_2$ , and the mixture was stirred for 1 h. After 3 h more of stirring at room temperature, the reaction was stopped by addition of a saturated  $\text{NaHCO}_3$  solution and the organic layer was separated, washed with water until pH 7, and then dried ( $\text{MgSO}_4$ ). After filtration and evaporation, the residue was chromatographed on a silica gel column using  $\text{Et}_2\text{O}$ /petroleum ether as eluent to give 8 g (57%) of 13 as a syrup.  $R_f$ : 0.32 ( $\text{Et}_2\text{O}$ /petroleum ether, 50:50, v/v). IR (film): 3060–3040 ( $\nu$  aromatic CH), 2960–2820 ( $\nu$  CH), 1760 ( $\nu$  C=O), 1600 ( $\nu$  C=C)  $\text{cm}^{-1}$ .

**1,4-Dibenzyl-2-[[[N-(monoalkylcarbamoyl)oxy]methyl]piperazine (11)** (route iv). **General Procedure.** 13 (1 g, 2.4 mmol) in 10 mL of primary amine was refluxed using an oil bath for 48 h. After cooling, the excess of amine and  $\text{PhOH}$  were eliminated under vacuum, and the residue taken up in  $\text{CHCl}_3$  was washed with  $\text{H}_2\text{O}$  until pH 7. After drying ( $\text{MgSO}_4$ ), filtration, and evaporation, purification on a silica gel column using  $\text{Et}_2\text{O}$ /petroleum ether (30:70, v/v) as eluent led to the carbamate 11 in a 50% mean yield. IR (film): all derivatives 11b–l displayed common bands same as 11a described above.  $^1\text{H}$  NMR: all derivatives 11b–l displayed common signals at average  $\delta$  ppm 7.3 (s, 10 H, aromatic H), 4.6 (m, 1 H, NH), 4.2 (m, 2 H,  $\text{CH}_2\text{OCO}$ ), 4 and 3.4 (2 d,  $J = 14$  Hz, 2 H,  $\text{CH}_2\text{Ph}$ ), 3.46 (s, 2 H,  $\text{CH}_2\text{Ph}$ ), 2.90–2.10 (m, 7 H, piperazine H).

**1,4-Dibenzyl-2-[[[N,N-dialkylcarbamoyl]oxy]methyl]piperazine (12)** (route v). **General Procedure.** The process was the same as for 11 (route iv) described above but using a secondary amine and led to the carbamate 12 in a 40% mean yield. IR (film): compounds 12a–d displayed common bands at 3090, 3070, 3040 ( $\nu$  aromatic CH), 2970–2820 ( $\nu$  CH), 1710 ( $\nu$  C=O), 1600 ( $\nu$  C=C)  $\text{cm}^{-1}$ .  $^1\text{H}$  NMR: compounds 12a–d displayed common signals at average  $\delta$  ppm 7.27 (s, 10 H, aromatic H), 4.30 (m, 2 H,  $\text{CH}_2\text{OCO}$ ), 4.07 and 3.35 (2 d,  $J = 14$  Hz, 2 H,  $\text{CH}_2\text{Ph}$ ), 3.45 (s, 2 H,  $\text{CH}_2\text{Ph}$ ), 3.15 (m, 4 H,  $\text{CH}_2\text{NCO}$ ), 2.91–2.07 (m, 7 H, piperazine H).

**1,4-Bis(3',4',5'-trimethoxybenzoyl)-2-[[[N-monosubstituted carbamoyl]oxy]methyl]piperazine (5).** **General Procedure.** Compounds 11a–n were debenzylated using the same procedure as for obtention of the esters 9 from 8. The corresponding piperazine derivatives were then reacted with 3,4,5-trimethoxybenzoyl chloride following the same process as for 1

and purified in the same manner to lead to 5 with aspect depending of R (see Table III). IR (in paraffin oil): all compounds 5a–n displayed common bands at 3340 ( $\nu$  NH), 3080–3060 ( $\nu$  aromatic CH), 2960–2860 ( $\nu$  CH), 1720 ( $\nu$  carbamic C=O), 1640 ( $\nu$  amidic C=O), 1590 ( $\nu$  C=C)  $\text{cm}^{-1}$ .  $^1\text{H}$  NMR: all compounds 5a–n displayed common signals at average  $\delta$  ppm 6.62 (s, 4 H, aromatic H), 4.72 (m, 2 H,  $\text{CH}_2\text{OCO}$ ), 4.65–3.92 (m, 3 H, piperazine  $\text{CH}_2$  and CH), 3.87 (large s, 18 H,  $\text{CH}_3\text{O}$ ), 3.42–2.70 (m, 4 H, piperazine  $\text{CH}_2\text{N}$ ), 1.85 (s, 1 H, NH). Specific signals, mp, and morphologies are reported in Table III.

**1,4-Bis(3',4',5'-trimethoxybenzoyl)-2-[[[N,N-dialkylcarbamoyl]oxy]methyl]piperazine (6).** **General Procedure.** The same procedures were used for 12 as for 11 in debenzylation and amidification processes to lead disubstituted 6 with aspects depending on R (see Table IV). IR (in paraffin oil): all compounds 6a–d displayed common bands at 3060 ( $\nu$  aromatic CH), 2960–2860 ( $\nu$  CH), 1700 ( $\nu$  carbamic C=O), 1640 ( $\nu$  amidic C=O), 1590 ( $\nu$  C=C)  $\text{cm}^{-1}$ .  $^1\text{H}$  NMR: all compounds 6a–d displayed common signals at average  $\delta$  ppm 6.62 (s, 4 H, aromatic H), 5–4.07 (m, 5 H,  $\text{CH}_2\text{OCO}$  and piperazine  $\text{CH}_2$  and CH), 3.8 (large s, 18 H,  $\text{CH}_3\text{O}$ ), 3.62–2.92 (m, 8 H,  $\text{CH}_2\text{N}$ ). Specific signals, mp, and morphologies are reported in Table IV.

**Biological Studies. Platelet Aggregation.** The inhibition of platelet aggregation was determined using platelet-rich plasma (PRP) of New Zealand rabbits by the method of Cazenave et al.<sup>74</sup> Blood samples were collected from the auricular artery into a citrate buffer (3.8%, pH 7.4), and PRP was obtained by centrifugation for 15 min at 1200 rpm. The antagonists were solubilized in DMSO at concentrations from  $10^{-2}$  to  $10^{-7}$  M and added to the incubate and stirred PRP for 1 min before PAF (2.5 nM) challenge. Platelet aggregation induced by PAF in the presence of the antagonist was monitored by continuous recording of light transmission in a dual-channel recorder (Cronolog Coultronics Apparatus) and was compared to a control aggregation induced by PAF alone. The drug concentration required to produce 50% inhibition ( $\text{IC}_{50}$ ) was calculated from dose-response curves (number of determinations: 5–6).

**Inhibition of PAF Binding.** The inhibition of the binding of [ $^3\text{H}$ ]PAF to platelet membranes by piperazine antagonist was measured according to the method of Domingo et al.<sup>75</sup> adapted to our use. Platelet membrane homogenate was prepared as described and used as follow for the binding assays. Aliquots of 60–100  $\mu\text{g}$  of membrane proteins were added to a final volume of 1 mL in plastic tubes containing 1 mM [ $^3\text{H}$ ]PAF (specific activity of 59.5 ci/mmol) in Tris-HCl 10 mM,  $\text{MgCl}_2$  5 mM, pH 7 buffer containing 0.025% bovine serum albumine. The samples were incubated 1.5 h at 0 °C with or without unlabeled PAF (in ethanol solution) or PAF antagonist (antagonists were dissolved in DMSO and diluted with buffer to obtain a final DMSO concentration lower than 1/5000). Immediately at the end of incubation time, the bound [ $^3\text{H}$ ]PAF was separated from free [ $^3\text{H}$ ]PAF by filtration through Whatman GF/C glass fiber filters under vacuum (Brandel System). The filters were washed three times with 5 mL of ice-cold buffer and then put into polyethylene vials filled up with 10 mL of liquid scintillation fluid (Instagel, Packard). The radioactivity was measured by an LKB  $\beta$  Countner with 45% efficiency.

The nonspecific binding was determined in the presence of  $10^{-6}$  M unlabeled PAF. The specific binding was calculated by subtracting nonspecific binding from the total binding. The inhibition by piperazine antagonists on the specific [ $^3\text{H}$ ]PAF binding was normalized as the percent inhibition by the equation

$$\% \text{ inhibition} = \frac{([\text{^3H}]\text{PAF total bound}) - ([\text{^3H}]\text{PAF bound in presence of antagonist})}{[\text{^3H}]\text{PAF specifically bound}} \times 100$$

The dose-response curves were performed by mean of 6–8 determinations.  $\text{EC}_{50}$  is the effective antagonist concentration required to produce 50% of inhibition of the [ $^3\text{H}$ ]PAF binding.

**Molecular Modeling.** Molecular geometries were obtained by the energy minimization MM2 (molecular mechanics force field)<sup>76</sup> (compound 3) and AM1 (Austin Model 1)<sup>77</sup> for compound 2. The crystallographic data<sup>53</sup> of compound 1g were used for a MNDO<sup>77</sup> (mean neglect of differential overlap) calculation in order to obtain a more relaxed geometry.

**Electrostatic Potential.** The calculations were performed within a complete CNDO (complete neglect of differential orbitals)<sup>78</sup> scheme (i) by neglecting the off-diagonal matrix elements and the nuclear attraction approximated by subtracting the repulsion integrals between s orbitals (approximation  $I_{\gamma\alpha H}$ ) (for compound 3) or (ii) by evaluation of the electrostatic potential map (approximation IV)<sup>79</sup> without any simplification into the calculation of the mono-electronic integrals<sup>80</sup> (compounds 2 and 1g). For details see refs 31, 33, and 67.

A regular 3D grid was calculated around each molecule. The dimension of the box were defined as the dimensions of the molecule increased by 5 Å in each direction. From this data set a contouring algorithm (PSI 77)<sup>81</sup> was applied joining points of the same energy.

**Computational Methods.** The MM2 minimizations were conducted on a Gould NP1 at the Paris 6 computer center (ccrp6) and on a Apple Macintosh using the Chem-3D-Plus<sup>82</sup> program. The other calculations (AM1, CNDO, MNDO electrostatic potential programs) were performed at the "Centre d'Ingénierie des Protéines" (University of Liège) on an attached processor FPS 164 linked to a VAX 11/780. The program PSI 77 was interfaced with a DI 3000 Library implemented on a Data General MV 7800.

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