R_f 0.30; MS, z/e 301 $(M + 1)^+$; UV λ_{max} pH 1 263 (15.2), pH 7 265 (15.4), pH 13 264 (15.7); ^1H NMR (Me_2SO-d_6, 300 MHz) δ 1.53 (br s, 2, 2'-NH_2), 3.52 (apparent t, 1, H-2'), 3.63 (dd, 1, J = 4 Hz, J = 12.5 Hz, H-5'), 3.73 (m, 2, $J_{3',4'}$ = 6 Hz, H-4', H-5'), 4.03 (br dd, 1, H-3'), 5.10 (br, 1, 5'-OH), 5.44 (d, 1, J = 5 Hz, 3'-OH), 6.11 (d, 1, $J_{1',2'}$ = 6.5 Hz, H-1'), 7.75 (br s, 2, 6-NH_2), 8.30 (s, 1, H-8); ^{13}C NMR (Me_2SO-d_6, 300 MHz) δ 60.20 (C-2',5'), 74.65 (C-3'), 84.10, 84.49 (C-1',4'), 117.44 (C-5), 140.43 (C-8), 150.27 (C-4), 152.61 (C-2), 156.51 (C-6). Anal. ($C_{10}H_{13}ClN_6O_3$) C, H, N.

2-Fluoro-9-(2-azido-2-deoxy-β-D-arabinofuranosyl)purin-6-amine (4l). A solution of **4e** (220 mg, 0.398 mmol) in 2 mL of dry THF (Aldrich, Sure/Seal) was treated at room temperature with 1 M tetra-*n*-butylammonium fluoride in THF (796 μL, 0.796 mmol). A 0.5-h TLC aliquot showed complete reaction. The solution was concentrated and applied to one silica gel thick plate (Analtech, GF, 2000 μm) that was developed with 4:1 $CHCl_3$–MeOH. The product was extracted with warm MeOH and crystallized from boiling water (20 mL) to give pure **4l**, which was dried in vacuo at 56 °C for 22 h: yield 106 mg (86%); mp 212 °C; TLC 4:1 $CHCl_3$–MeOH, R_f 0.55; HPLC 99%, 90:10 H_2O–MeCN; MS, z/e 311 $(M + 1)^+$; IR 2120 cm^{-1} (N_3); UV λ_{max} pH 1 261.5 (14.5), 270 (sh), pH 7 262 (15.2), 270 (sh), pH 13, 262 (15.5), 270 (sh); ^1H NMR (Me_2SO-d_6, 300 MHz) δ 3.62–3.85 (m, 3, H-4', 2 H-5'), 4.37 (m, 1, H-3'), 4.61 (dd, 1, $J_{1',2'}$ = 7 Hz, $J_{2',3'}$ = 8 Hz, H-2'), 5.16 (t, 1, 5'-OH), 6.01 (d, 1, J = 6 Hz, 3'-OH), 6.29 (d, 1, H-1'), 7.93 (br s, 2, NH_2), 8.32 (s, 1, H-8); ^{13}C NMR (Me_2SO-d_6, 300 MHz) δ 59.47 (C-5'), 67.20 (C-2'), 71.26 (C-3'), 81.54, 83.08 (C-1',4'), 116.78 (d, C-5, $J_{5,F}$ = 3.8 Hz), 139.37 (d, C-8, $J_{8,F}$ = 2.6 Hz), 150.39 (d, C-4, $J_{4,F}$ = 20.3 Hz), 157.45 (d, C-6, $J_{6,F}$ = 22.3 Hz), 158.51 (d, C-2, $J_{2,F}$ = 204.0 Hz). Anal. ($C_{10}H_{11}FN_8O_3$) C, H, N.

2-Fluoro-9-(2-chloro-2-deoxy-β-D-arabinofuranosyl)-purin-6-amine (4m). A solution of **4f** (126 mg, 0.23 mmol) in 1 mL of dry THF (Aldrich, Sure/Seal) was treated at room temperature with 2 equiv of 1 M tetra-*n*-butylammonium fluoride in THF (0.46 mL). A 3-h TLC aliquot showed complete reaction. The solution was evaporated, and the residue was crystallized from MeOH to give crude **4m**, 70 mg. This solid was recrystallized from hot MeOH (35 mL) to give pure material that was dried in vacuo at 56 °C for 16 h: yield 63 mg (89%); mp 205–207 °C; TLC 5:1 $CHCl_3$–MeOH, R_f 0.45; HPLC 98%, 90:10 H_2O–MeCN; MS, z/e 304 $(M + 1)^+$; UV λ_{max} pH 1 262 (14.8), 270 (sh), pH 7 261 (15.6), 270 (sh), pH 13 262 (16.1), 270 (sh); ^1H NMR (Me_2SO-d_6, 300 MHz) δ 3.66–3.86 (m, 3, H-4', 2 H-5'), 4.44 (m, 1, H-3'), 4.77 (dd, 1, $J_{1',2'}$ = 7 Hz, $J_{2',3'}$ = 8 Hz, H-2'), 5.20 (t, 1, 5'-OH), 6.07 (d, 1, J = 5.5 Hz, 3'-OH), 6.37 (d, 1, H-1'), 7.88 (br s, 2, NH_2), 8.36 (s, 1, H-8); ^{13}C NMR (Me_2SO-d_6, 300 MHz) δ 59.69 (C-5'), 63.48 (C-2'), 73.72 (C-3'), 82.39, 83.40 (C-1',4'), 116.67 (d, C-5, $J_{5,F}$ = 4.1 Hz), 139.31 (d, C-8, $J_{8,F}$ = 2.3 Hz), 150.39 (d, C-4, $J_{4,F}$ = 20.3 Hz), 157.46 (d, C-6, $J_{6,F}$ = 21.4 Hz), 158.46 (d, C-2, $J_{2,F}$ = 204.3 Hz). Anal. ($C_{10}H_{11}ClFN_5O_3$·0.8CH_3OH) C, H, N.

2-Fluoro-9-(2-amino-2-deoxy-β-D-arabinofuranosyl)-purin-6-amine (4n). To a solution of **4l** (60 mg, 0.193 mmol) in 15 mL of EtOH was added 19 mg of 5% platinum-on-carbon powder (Engelhard), and the mixture was stirred under hydrogen at atmospheric pressure and room temperature. A 16-h TLC aliquot showed essentially complete reaction. The catalyst was filtered off and washed with EtOH, and the filtrate was evaporated to dryness. This residue in hot MeOH was applied to two Analtech, GF, 1000 μm layer plates with Celite zones. After one development in 4:1 $CHCl_3$–MeOH + 5% NH_4OH, the product band was eluted with 1:1 $CHCl_3$–MeOH + 10% NH_4OH. The plate extract was evaporated to dryness, and the residue was crystallized from 20 mL of hot 99:1 EtOH–H_2O. The solid was collected after chilling, washed with cold EtOH, and dried in vacuo at 56 °C for 16 h to give pure **4n**: yield 25 mg (45%); mp 210 °C dec; TLC 3:1 $CHCl_3$–MeOH + 5% NH_4OH, R_f 0.30; HPLC 98%, pH 3.6, 90:10 0.1 M NaOAc–MeCN; MS, z/e 285 $(M + 1)^+$; UV λ_{max} pH 1 261 (14.9), 268 (sh), pH 7 262 (15.4), 270 (sh), pH 13 262 (15.4), 270 (sh); ^1H NMR (Me_2SO-d_6, 300 MHz) δ 3.32 (br s, 2, 2'-NH_2), 3.62–3.84 (m, 4, H-2', H-4', 2 H-5'), 4.29 (m, 1, H-3'), 5.56 (v br s, 1, 5'-OH), 5.77 (br s, 1, 3'-OH), 6.19 (d, 1, $J_{1',2'}$ = 6 Hz, H-1'), 7.82 (br s, 2, 6-NH_2), 8.29 (s, 1, H-8); ^{13}C NMR (Me_2SO-d_6, 300 MHz) δ 58.80 (C-2'), 59.82 (C-5'), 72.62 (C-3'), 82.80, 83.96 (C-1',4'), 117.04 (d, C-5, $J_{5,F}$ = 4.0 Hz), 139.94 (C-8), 150.43 (d, C-4, $J_{4,F}$ = 21.3 Hz), 157.38 (d, C-6, $J_{6,F}$ = 20.2 Hz), 158.37 (d, C-2, $J_{2,F}$ = 203.8 Hz). Anal. ($C_{10}H_{13}FN_6O_3$·0.7H_2O) C, H, N.

Acknowledgment. This investigation was supported by the National Cancer Institute, National Institutes of Health, DHHS, Grant P01 CA34200. We thank Dr. L. L. Bennett, Jr., B. J. Bowdon, and D. A. Adamson for the cytotoxicity data in normal and mutant cell lines, Dr. W. M. Shannon and G. Arnett for the antiviral data, S. Campbell for HPLC analysis, and members of the Molecular Spectroscopy Section of the Southern Research Institute for spectral data.

Registry No. **3a**, 146-77-0; **3b**, 111556-90-2; **3c**, 111581-33-0; **3d**, 146-78-1; **3e**, 111556-91-3; **3f**, 111556-92-4; **4a**, 111556-93-5; **4b**, 111556-94-6; **4c**, 111556-95-7; **4d**, 111556-96-8; **4e**, 111556-97-9; **4f**, 111556-98-0; **4g**, 111556-99-1; **4h**, 111615-20-4; **4i**, 111557-00-7; **4j**, 111557-01-8; **4k**, 111557-02-9; **4l**, 111557-03-0; **4m**, 111557-04-1; **4n**, 111557-05-2.

Structure-Activity Relationship in PAF-acether. 4.[1] Synthesis and Biological Activities of Carboxylate Isosteres

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The synthesis and biological characterization of some 3-carboxylate isosteres of PAF-acether structurally modified in positions 1 (ether, carbamate), 2 (acetoyl, ethoxy), and 3 (chain length and polar head group) are reported. All derivatives present antagonist activities against PAF-acether-induced effects in vitro (platelet aggregation) and in vivo (bronchoconstriction and thrombocytopenia in guinea pig and, to a lesser extent, hypotension in rat). The functional modifications presented here do not modify dramatically the potency of antagonist activities, and there is no enantioselectivity. All of the isosteres are specific PAF-acether antagonists, except the 1-carbamoyl analogue, which is also potent against acetylcholine-induced hypotension and bronchoconstriction.

Platelet activating factor (PAF-acether) is a phospholipid mediator[2-4] of anaphylaxis that is released by a number of stimulated cells including platelets, macrophages, basophils, neutrophils, endothelium cells, and isolated tissue preparation (for reviews, see ref 5–8). It

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(1) For part 3 and references of previous papers, see ref 21.
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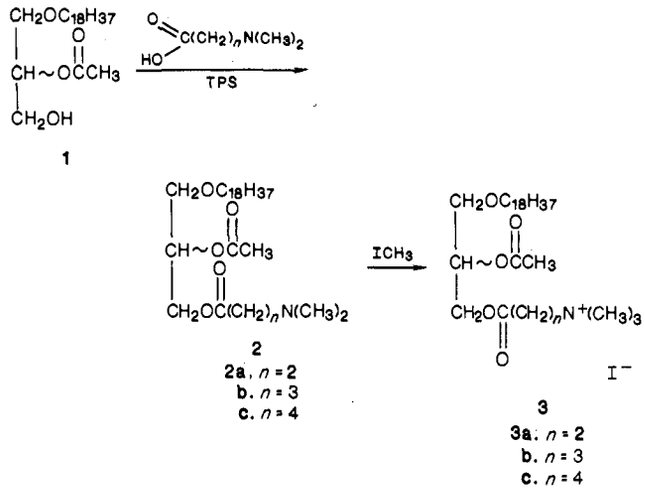
0022-2623/88/1831-0410$01.50/0

Scheme I

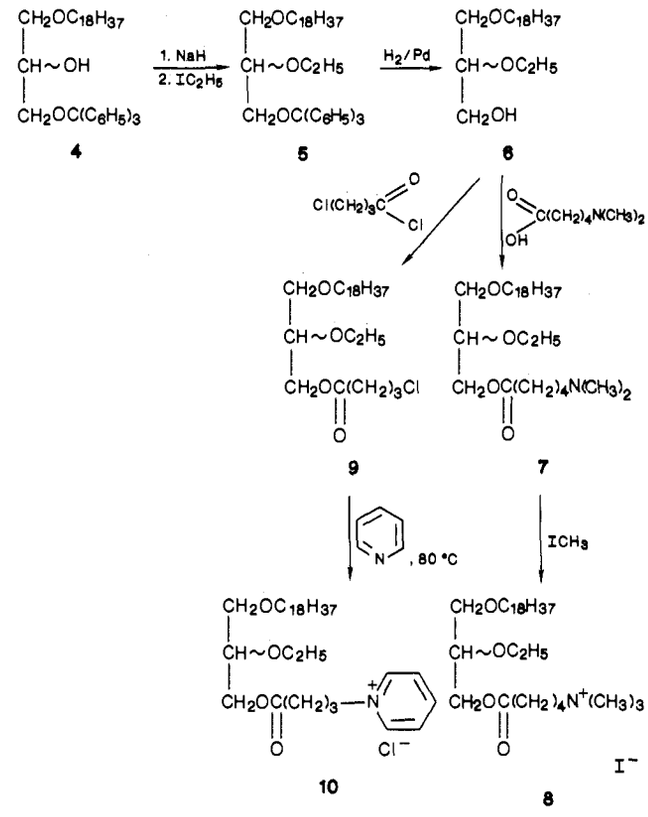


also induces chemotaxis,[3] vasopermeability,[10] and hypotension.[4] It may be involved in various inflammatory, respiratory, and cardiovascular disorders.[11-15]

Investigations of PAF-acether analogues have clarified some structural features necessary for platelet and polymorphonuclear leukocyte stimulation and other agonist activities (for reviews, see ref 7, 8, 16, and 17). It now seems clear that the interaction of PAF-acether with its specific site is a key step in its biological functions.[16-19] Specific antagonists of PAF-acether capable of inhibiting this first step would further clarify the physiopathological role of this mediator. The first molecule described as a PAF antagonist was CV 3988 (Takeda), 3-(4-oxy-7-methoxy-10-oxo-3,5,9-trioxa-11-aza-4-phosphanonacos-1-yl)-thiazolium *p*-oxide, which was first described in the investigation of an ether phospholipid series for cytotoxic and antifungal agents.[20]. Replacement of phosphate by another function yielded a new group of antagonists synthesized by Sandoz, Hoffmann-La Roche, and Ono researchers. These results were presented at various international symposiums (for review, see ref 17 and 19).

We report here the synthesis and biological activities of carboxylate isosteres in position 3 that also contain several structural modifications in positions 1, 2, and 3.

Scheme II



Chemistry

1-*O*-Octadecyl-2-*O*-acetyl-3-*O*-[ω-(*N*,*N*,*N*-trimethylammonio)acyl]glycerol iodides **3** were synthesized according to Scheme I.

1-*O*-Octadecyl-2-*O*-acetylglycerol (1) prepared as described previously[21] was esterified according to the procedure of Aneja[22] with the corresponding ω-*N*,*N*-dimethylamino acid, with triisopropylbenzenesulfonyl chloride (TPS) as a condensing agent. The resulting 1-*O*-octadecyl-2-*O*-acetyl-3-*O*-[ω-(*N*,*N*-dimethylamino)-acyl]glycerol **2** was converted into its quaternary ammonium salt **3** by classical treatment of **2** with methyl iodide. The two enantiomers, *R* and *S*, were prepared by using the same procedure.

Scheme II describes the synthesis of 1-*O*-octadecyl-2-*O*-ethyl-3-*O*-[5-(*N*,*N*,*N*-trimethylammonio)pentanoyl]-glycerol iodide (**8**) and of 1-*O*-octadecyl-2-*O*-ethyl-3-*O*-(4-*N*-pyridiniobutanoyl)glycerol chloride (**10**). Treatment of 1-*O*-octadecyl-3-*O*-tritylglycerol (**4**) with sodium hydride followed with ethyl iodide in dimethylformamide (DMF) gave the triether **5**. The trityl group was removed by hydrogenolysis, and esterification of the resulting diether

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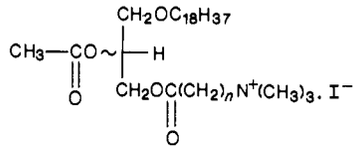
Table I. In Vitro Aggregant Activity and Antagonist Effect against PAF-acether-Induced Platelet Aggregation: Influence of the Chain Length between the Carboxylate Function and the Ammonium Group



no.	n	platelet aggregation:[a,b] EC_{50}, μM	antiaggregation after 2 min:[a,c] EC_{50}, μM
CV 3988[d]		>100	30.5 (18–57)
3a	2	>100	24 (15–28)
3b	3	>100	12 (9–14)
3c	4	>100	18 (14–24)

[a] See Experimental Section for details. [b] All these compounds are also inactive against all in vivo agonist activities at doses close to 1 μM/kg, i.e., bronchoconstriction, hypotension, hypotension, and thrombocytopenia. [c] Values in parentheses are 95% confidence intervals. [d] 3-(4-Oxy-7-methoxy-10-oxo-3,5,9-trioxa-11-aza-4-phosphanonacos-1-yl)thiazolium *P*-oxide as reference compound.[20]

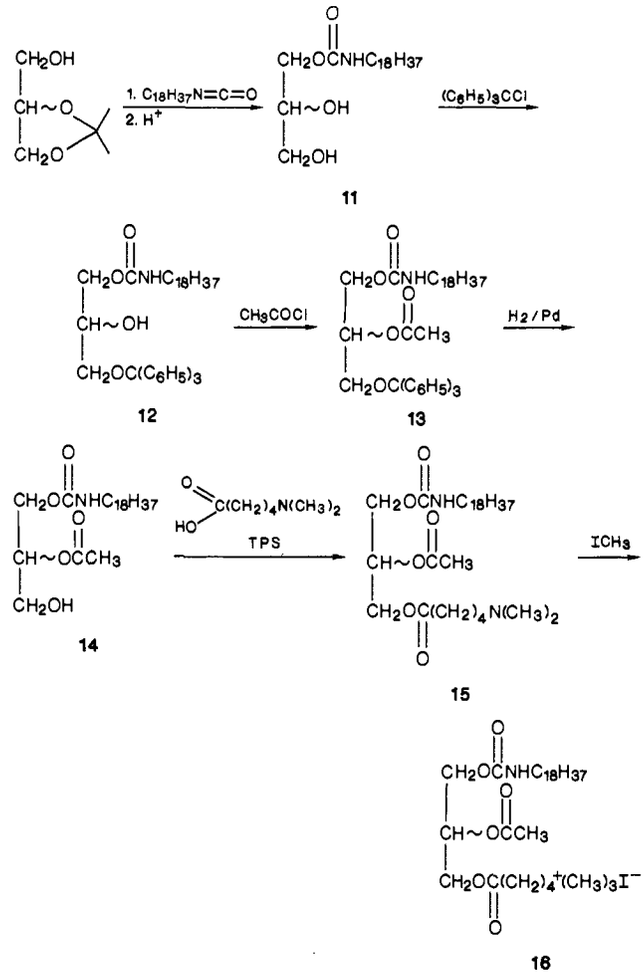
alcohol **6** by 5-(*N*,*N*-dimethylamino)valeric acid yielded **7**. Reaction of methyl iodide with **7** as above gave **8**.

Esterification of **6** by 4-chlorobutyryl chloride yielded 1-*O*-octadecyl-2-*O*-ethyl-3-*O*-(4-chlorobutanoyl)glycerol (**9**), which was converted to **10** by treatment with pyridine (80 °C).

Compound **14** was obtained in three steps (Scheme III, tritylation, acetylation, detritylation) from 1-*O*-(octadecylcarbamoyl)glycerol (**11**) prepared by condensing 1,2-*O*-isopropylideneglycerol with *N*-octadecyl isocyanate followed by acid hydrolysis of the acetal.

1-*O*-(Octadecylcarbamoyl)-2-*O*-acetyl-3-*O*-[5-(*N*,*N*,*N*-trimethylammonio)pentanoyl]glycerol iodide (**16**) was synthesized from 1-*O*-(octadecylcarbamoyl)-2-*O*-acetylglycerol (**14**) according to the same procedure as for preparation of **3**.

Scheme III

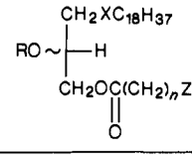


Biological Results and Structure–Activity Relationships

None of the synthesized compounds **3**, **8**, **10**, and **16** present agonist properties in vitro at 10^{-4} M and in vivo at around 1 μM/kg (see Tables I and II). In contrast, all have an antagonist activity on the effects induced by PAF-acether.

(a) Platelet Aggregation in Vitro. The antagonist activity of the analogues against PAF-acether occurs between 1.3×10^{-5} and 1.5×10^{-6} M. Several points can be made with regard to inhibition of PAF-acether by these compounds. (i) It is to be noted that the effect of the chain length in position 3 (Table I) is not very pronounced for methylenes between 2 and 4, compounds **3a–3c**. (ii) In terms of stereospecificity, there is no significant difference between the two enantiomers **3c**(*R*) and **3c**(*S*) (Table II). (iii) Similarly, the functional modification in position 1, i.e., change from an ether function, compound **3c**, to a carbamate function, compound **16**, does not lead to significant variations in antagonist activity (Tables I and II). (iv) Neither is there a great difference in activity between

Table II. In Vivo Aggregant Effect and Antagonist Activities of Selected Carboxylate Isosteres of PAF-acether against Inducers of Aggregation Such as PAF, Collagen, and Arachidonic Acid



no.	X	R	n	Z	platelet aggregation:[a,b] ED_{50}, μM	antagonism:[a,b] EC_{50}, μM PAF, 5×10^{-8} M	collagen, 40 μg/mL	AA, 2×10^{-4} M
CV 3988[c]					>100	30.5 (18–57)	89 (56–220)	–
3c(*R*)	O	$COCH_3$	4	$N^+(CH_3)_3$, I^-	>100	20 (13–28)	≃90	>100
3c(*S*)	O	$COCH_3$	4	$N^+(CH_3)_3$, I^-	>100	11 (9–13)	84 (58–150)	>100
8[d]	O	Et	4	$N^+(CH_3)_3$, I^-	>100	8 (7–9)	60 (26–750)	>100
10	O	Et	3	$C_5H_5N^+$, Cl^-	>100	1.5 (1–4)	≃100	>100
16	OCONH	$COCH_3$	4	$N^+(CH_3)_3$, Cl^-	>100	13 (11–16)	70 (33–480)	>100

[a] See Experimental Section for details. [b] All the compounds are also inactive against all in vivo agonist activities, i.e., bronchoconstriction, hypotension, and thrombocytopenia, at doses close to 1 μM/kg. Values in parentheses are 95% confidence intervals. [c] See Table I. [d] RU-45703.

Table III. In Vivo Antagonist Activities of Selected Carboxylate Isosteres of PAF-acether against Some Inducers of Hypotension, Bronchoconstriction, and Thrombocytopenia

no.	dose, mg/kg iv, 5 min	bronchoconstriction, % protection			thrombocytopenia % protection: PAF iv	hypotension, % protection	
		PAF iv	Ach[f] iv	Hist[g] iv		PAF iv	Ach iv
CV 3988[h]	0.5	53	–	–	32	–	–
	1	72	–	–	62	45	0
	2	85	–	–	69	–	–
3c(*R*)	0.5	79	–	–	–	–	–
	1	90	21	35	38	18	18
	2	88	0	20	57	32	5
3c(*S*)	0.5	61	–	–	–	–	–
	1	91	0	0	63	12	16
	2	93	0	0	49	34	20
8[i]	0.5	69	–	–	–	–	–
	1	77	0	0	24	–	–
	2	73	9	0	55	20	17
10	0.5	60	–	–	–	–	–
	1	100	34	16	52	51	20
	2	–	16	8	60	53	22
16	1	88	–	–	21	–	–
	2	90	79	17	38	46	63
	5	–	80	–	–	58	74

[a] See Table II for formulas. [b] See Experimental Section for details; n = 5–8 animals. [c] On guinea pig. [d] On rat. [e] PAF-acether at 5 mg/kg all compounds present agonist effects, i.e., bronchoconstriction, except compound **16**. [f] Acetylcholine. [g] Histamine. [h] See Table I. [i] RU-45703.

an acetate function, compound **3c**, and an ethoxy function, compound **8**, in position 2 (Table I and II). (v) The introduction of a charge transfer group, i.e., pyridinium group, compound **10**, modifies the activity by a factor close to 6 with respect to the trimethylammonium analogue, compound **8**, and by 10 with respect to compound **3c** (Table I). (vi) The inhibition of platelet aggregation induced by PAF-acether is the most pronounced for compound **10**. The other isosteres selected (**3c**(*R*), **3c**(*S*), **8**, and **16**) are more active on the aggregation induced by the PAF-acether pathway than on the aggregation induced by collagen. All are ineffective on aggregation induced by arachidonic acid.

(b) Antagonist Effects in Vivo. A very significant antagonism of PAF-acether activity is noted on the bronchoconstriction induced in the guinea pig at 1 and 2 mg/kg iv for all isosteres selected, **3**, **8**, **10**, and **16** (Table III), i.e., between 77% and 100% protection. The compounds are still significantly active at 0.5 mg/kg iv, i.e., between 60% and 79% protection; however, it is to be noted that enantioselectivity is not apparent. The antagonist activity is less clear on the hypotension induced in the anesthetized rat. It is highest for compound **10**, 51% protection at 1 mg/kg (Table III). Concerning the thrombocytopenia induced by PAF-acether, the protection is close to 50% at 1 or 2 mg/kg iv for the isosteres **3c**(*R*), **3c**(*S*), **8**, and **10** (Table III), the 1-carbamoyl analogue **16** being the least active. In contrast, this isostere (**16**) appears to be the least specific since it presents a significant antagonist activity at 2 mg/kg iv on bronchoconstriction and hypotension induced by acetylcholine (i.e., 79% and 63% protection respectively). None of the compounds display any significant antagonist effect on the bronchoconstriction induced by histamine (Table III).

Conclusion

3-Carboxylate isosteres of PAF-acether are antagonists against PAF-induced effects in vitro (platelet aggregation) and in vivo (bronchoconstriction and thrombocytopenia and, to a lesser extent, hypotension). The structural variations studied here in positions 1, 2, and 3 do not lead to great differences in the antagonist activities against PAF-acether, in vivo and in vitro, except for compound **10**, which possesses a "charge-transfer" pyridinium group. Furthermore, there is no stereospecificity displayed by these compounds in their antagonist activity, a fact already observed by several authors (for review, see ref 17 and 19). It appears that the antagonism against PAF-acether expressed by structural analogues of this mediator may require interaction at two levels on the hypothetical receptor site: in the hydrophobic pocket believed to be situated in the membrane and in an interaction point situated outside the membrane in the polar heads, with the target protein involved, possibly by a charge-transfer process.

Experimental Section. Materials and Methods

A. Chemistry. Solvents were all reagent grade. The purity of each product was checked by TLC on silica gel plates (Kieselgel 60 F_{254}, thickness 0.2 mm). Column chromatography was performed on silica gel (Merck, particle size 0.063–0.200 mm for normal chromatography, middle particle size 15 μm for flash chromatography), without any special treatment. Melting points were determined on a hot-stage microscope (Reichert Thermovar). The structures of all compounds were consistent with their IR spectra (Pye-Unicam SP3-200) and ^{1}H NMR (Varian EM 360 or Brucker 250 MHz) spectra in $CDCl_3$ with TMS as an internal standard.[23] Chemical ionization mass spectra were obtained at 220 °C on a modified AEI spectrometer.[24] Elemental analyses were within ±0.4% of calculated values (C, H, N).

1-*O*-Octadecyl-2-*O*-acetyl-3-*O*-[5-(*N*,*N*-dimethylamino)pentanoyl]glycerol (2c). A solution of 1-*O*-octadecyl-2-*O*-acetylglycerol (1) (7.2 mmol), 5-(*N*,*N*-dimethylamino)valeric acid (10.8 mmol),[25] and TPS (14.4 mmol) in anhydrous ethanol-free $CHCl_3$ (20 mL) and dry pyridine (10 mL) was stirred for a night at room temperature.

The reaction was quenched by the addition of water and extracted several times with $CHCl_3$. The organic layer was washed with H_2O until pH 7, dried (Na_2SO_4), and evaporated in vacuum. The residue was chromatographed on silica gel (flash chromatography) by using, successively, $CHCl_3$ and then 1–2% MeOH in $CHCl_3$ to give **2c**, as a viscous oil (yield 42%): R_f 0.47 ($CHCl_3$/MeOH, 80:20 v/v); IR (film) 2950–2860 (CH), 2810, 2770 ($N(CH_3)_2$), 1745 (C=O), 1240 (C—O—C ether), 1130 (C—O—C ester) cm^{-1}; ^{1}H NMR ($CDCl_3$, 80 MHz) δ 0.9 (t, 3 H, CH_3), 1.24 (large s, 32 H, $(CH_2)_{16}$), 1.6 (m, 4 H, $COCH_2CH_2CH_2CH_2N$), 2.04 (s, 3 H, $COCH_3$), 2.34 (large s, 6 H, $N(CH_3)_2$), 2.50 (t, 4 H, $COCH_2$ and CH_2N), 3.45 (m, 4 H, CH_2OCH_2), 4.23 (m, 2 H, CH_2OCO),

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(25) Olomucki, M. *Bull. Soc. Chim. Fr.* **1963**, 2067.

5.15 (quintet, 1 H, HCOCO); mass spectrum, *m/z* 514 (MH^+), 454 ($MH^+ - CH_3CO_2H$), 146 ($HOOC(CH_2)_4N(CH_3)_2$). Anal. ($C_{30}H_{59}NO_5$) C, H, N. By the same procedure, **2a** and **2b** were obtained. **2a**: R_f 0.35 ($CHCl_3$/MeOH, 90:10 v/v). Anal. ($C_{28}H_{55}NO_5$) C, H, N. **2b**: R_f 0.47 ($CHCl_3$/MeOH, 80:20 v/v). Anal. ($C_{29}H_{57}NO_5$) C, H, N.

1-*O*-Octadecyl-2-*O*-acetyl-3-*O*-[5-(*N*,*N*,*N*-trimethylammonio)pentanoyl]glycerol Iodide (3c). A mixture of **2c** (0.4 mmol), ICH_3 (4 mmol), and Na_2CO_3 (0.4 g) in 20 mL of dry acetone was stirred overnight at room temperature. After removal of Na_2CO_3 by filtration, solvent and the excess of ICH_3 were evaporated. The residue was purified on a silica gel column (flash chromatography) by using $CHCl_3$/MeOH (97:3 v/v) as eluent to yield **3c** (45%) as a yellow powder: mp 87–88 °C; IR (Nujol) 1745 (C=O), 1240 (C—O—C ester), 1130 (C—O—C ether) cm^{-1}; ^1H NMR ($CDCl_3$, 250 MHz) δ 0.90 (t, 3 H, CH_3), 1.30 (large s, 30 H, $(CH_2)_{15}$), 1.55 (m, 2 H, OCH_2CH_2), 1.68 (m, 2 H, $COCH_2CH_2$), 1.85 (m, 2 H, $CH_2CH_2N^+$), 2.05 (s, 3 H, $COCH_3$), 2.49 (t, 2 H, $COCH_2$), 3.17 (s, 9 H, $N^+(CH_3)_3$), 3.45 (m, 4 H, OCH_2 and CH_2N^+), 3.57 (d, 2 H, CH_2O), 4.3 (m, 2 H, CH_2OCO), 5.20 (m, 1 H, HCOCO); mass spectrum, *m/z* 528 (MH^+ - I), 468 ((MH^+ - I) - CH_3CO_2H), 160 ($HOOC(CH_2)_4N^+(CH_3)_3$). By the same procedure, **3a** and **3b** were obtained. **3a**: R_f 0.25 ($CHCl_3$/MeOH, 80:20 v/v); dec. **3b**: R_f 0.26 ($CHCl_3$/MeOH, 80:20 v/v); mp 76–77 °C; mass spectrum, *m/z* 514 (MH^+ - I), 454 ((MH^+ - I) - CH_3CO_2H). Compounds **3c**(*R*) and **3c**(*S*) were obtained from (*R*)- and (*S*)-1-*O*-octadecyl-2-*O*-acetylglycerol, by the same procedure as that for **3c**.

1-*O*-Octadecyl-2-*O*-ethyl-3-*O*-tritylglycerol (5). NaH (20 mmol, dispersion 50% paraffin oil) was added to 1-*O*-octadecyl-3-*O*-tritylglycerol (**4**) (10 mmol) in 100 mL of dry DMF. The mixture was stirred for 30 min at room temperature under anhydrous conditions, after which ethyl iodide (1.1 mmol) was added in a single dose. The mixture was stirred at 50 °C for 5 h, then diluted with 200 mL of H_2O, and extracted with $CHCl_3$. The organic phase was washed once with H_2O, dried ($MgSO_4$), and evaporated. The remaining oil was chromatographed on a silica gel column, with petroleum ether/ether (95:5 v/v) as eluent to yield **5** (75%): R_f 0.44 (petroleum ether/ether, 85:15 v/v); IR (film) 1600 (C_6H_5), 1080 (C-O-C) cm^{-1}.

1-*O*-Octadecyl-2-*O*-ethylglycerol (6). Catalytic hydrogenolysis of the trityl group of **5** was performed in $CHCl_3$ at 40 °C for 18 h (20 psi) by using Pd/C (10%). After filtraton and evaporation, **6** was purified on a silica gel column, with petroleum ether/ether (85:15 v/v) as eluent (yield 80%): R_f 0.17 (petroleum ether/ether, 70:30 v/v); IR (film) 3450 (OH), 1090 (C-O-C) cm^{-1}.

1-*O*-Octadecyl-2-*O*-ethyl-3-*O*-[5-(*N*,*N*-dimethylamino)pentanoyl]glycerol (7). Esterification of **6** was performed by using the same procedure as that for **2c** and led to the title compound **7**: R_f 0.33 ($CHCl_3$/MeOH, 90:10 v/v); viscous oil; IR (film) 2810–2730 ($N(CH_3)_2$), 1735 (C=O), 1240 (C—O—C ester), 1130 (C—O—C ether) cm^{-1}; ^1H NMR ($CDCl_3$, 80 MHz) δ 0.9 (t, 3 H, CH_3), 1.2 (large s, 35 H, $(CH_2)_{16}$ and OCH_2CH_3), 1.6 (m, 4 H, $COCH_2CH_2CH_2CH_2N$), 2.34 (large s, 6 H, $N(CH_3)_2$), 2.50 (t, 4 H, $COCH_2$ and CH_2N), 3.45 (m, 4 H, CH_2OCH_2), 3.7 (quadruplet, 2 H, OCH_2CH_3), 3.5–3.7 (m, 1 H, HCO), 4.23 (m, 2 H, CH_2OCO). Anal. ($C_{30}H_{61}NO_4$) C, H, N.

1-*O*-Octadecyl-2-*O*-ethyl-3-*O*-[5-(*N*,*N*,*N*-trimethylammonio)pentanoyl]glycerol Iodide (8). Quaternization of **7** was perfomed by using the same procedure as that for **3c** and led to the title compound **8**: R_f 0.24 ($CHCl_3$/MeOH, 80:20 v/v); mp 68–69 °C; IR (Nujol) 1735 (C=O), 1240 (C—O—C ester), 1140 (C—O—C ether) cm^{-1}; ^1H NMR ($CDCl_3$, 250 MHz) (like **3c** except for $HCOCOCH_3$ changed into $HCOCH_2CH_3$) δ 1.18 (t, 3 H, CH_3), 3.7 (quadruplet, 2 H, OCH_2), and 3.6 (m, 1 H, HCO); mass spectrum, *m/z* 514 (MH^+ - I), 160 ($HOOC(CH_2)_4N^+(CH_3)_3$).

1-*O*-Octadecyl-2-*O*-ethyl-3-*O*-(4-chlorobutanoyl)glycerol (9). 1-*O*-Octadecyl-2-*O*-ethylglycerol (**6**) (4 mmol) and Et_3N (6 mmol) in 15 mL of $CHCl_3$ were added dropwise to a solution of 4-chlorobutyryl chloride (6 mmol) in 6 mL of $CHCl_3$, cooled to 0 °C. The mixture was stirred at room temperature for 12 h. Then, 15 mL of $CHCl_3$ was added, the organic phase was washed twice with 15 mL of NaOH and then with water and dried (Na_2SO_4), and solvent was eliminated in vacuum. The crude product was chromatographed on silica gel with petroleum ether/ether (80:20, then 70:30 v/v) as eluent and gave **9** (yield 84%): R_f 0.58 (petroleum ether/ether, 70:30 v/v); IR (film) 1735 (C=O), 1210 (C—O—C ester), 1120 (C—O—C ether) cm^{-1}; ^1H NMR ($CDCl_3$, 80 MHz) δ 0.9 (t, 3 H, CH_3), 1.20 (large s, 35 H, $(CH_2)_{16}$ and OCH_2CH_3), 2.05 (m, 2 H, $COCH_2CH_2CH_2Cl$), 2.25 (t, 2 H, $COCH_2$), 3.45 (m, 8 H, 3 CH_2O and CH_2Cl), 3.7 (m, 1 H, CHO), 4.1 (m, 2 H, CH_2OCO).

1-*O*-Octadecyl-2-*O*-ethyl-3-*O*-(4-*N*-pyridiniobutanoyl)glycerol Chloride (10). A mixture of **9** (2 mmol) in 10 mL of pyridine was heated (80 °C) for 24 h and then evaporated in vacuum with use of toluene to eliminate traces of pyridine. The residue was purified by column chromatography with $CHCl_3$ and then $CHCl_3$/MeOH (90:10, then 80:20, v/v) as eluent to yield **10** (77%) as a viscous product: R_f 0.41 ($CHCl_3$/MeOH, 70:30 v/v); IR (film) 1735 (C=O), 1630 ($C_5H_5N^+$), 1230 (C—O—C ester), 1210 (C—O—C ether) cm^{-1}; ^1H NMR ($CDCl_3$, 80 MHz) δ 0.9 (t, 3 H, CH_3), 1.20 (large s, 35 H, $(CH_2)_{16}$ and OCH_2CH_3), 2.30 (m, 4 H, $COCH_2CH_2CH_2N^+$), 3.25 (m, 6 H, CH_2O), 3.7 (m, 1 H, CHO), 4.0 (m, 2 H, CH_2OCO), 4.9 (t, 2 H, CH_2N^+), 7.8 (t, 2 H, $CH{=}CHN^+$), 8.25 (t, 1 H, $CHCH{=}CHN^+$), 9.2 (d, 2 H, ${=}CHN^+$); mass spectrum, *m/z* 521 (MH^+ - Cl). Anal. ($C_{32}H_{58}NO_4Cl$) C, H, N, Cl.

1-*O*-(Octadecylcarbamoyl)glycerol (11). A mixture of isopropylideneglycerol (0.1 mol), octadecyl isocyanate (0.1 mol), and pyridine (6 mL) in 15 mL of $CHCl_3$ was heated with stirring, for 3 days at 50–60 °C. After cooling, the reaction was quenched by the addition of water and extracted with $CHCl_3$. The organic phase was washed with diluted HCl and then with water, dried (Na_2SO_4), and evaporated in vacuum. The remaining solid was recrystallized from hexane: mp 65 °C, yield 86%. Opening of the acetal ring was performed by refluxing the product in a mixture of 100 mL of MeOH and 10 mL of concentrated HCl for 2 h. After cooling, **11** was filtered and recrystallized from MeOH (yield 89%): mp 96 °C; IR (Nujol) 3500–3330 (OH, NH), 1700 (CONH) cm^{-1}.

1-*O*-(Octadecylcarbamoyl)-3-*O*-tritylglycerol (12). Tritylation of **11** (50 mmol) was performed with $(C_6H_5)_3CCl$ (60 mmol) and Et_3N (0.1 mol) in boiling toluene (150 mL) for 3 h. After the usual treatment, the residue was purified by flash chromatography with $CHCl_3$ as eluent and yielded pure **12** (75%): mp 84 °C; R_f 0.28 ($CHCl_3$/MeOH, 99:1 v/v); IR (Nujol) 3500–3320 (OH, NH), 1700 (CONH), 1600 (C_6H_5) cm^{-1}.

1-*O*-(Octadecylcarbamoyl)-2-*O*-acetyl-3-*O*-tritylglycerol (13). Acetylation of **12** was performed with CH_3COCl and pyridine. Compound **13** was flash chromatographed with $CHCl_3$ as eluent (yield 70%): mp 69–70 °C; R_f 0.29 ($CHCl_3$); IR (Nujol) 3340 (NH), 1740 (C=O ester), 1700 (NHCO), 1600 (C_6H_5), 1240 (C—O—O ester) cm^{-1}.

1-*O*-(Octadecylcarbamoyl)-2-*O*-acetylglycerol (14). A mixture of **13** (30 mmol), $CaCO_3$ (2 g), and Pd/C (10%) in 100 mL of $CHCl_3$ was hydrogenated with stirring at 40 °C for 18 h (20 psi). After filtration and evaporation, the residue (including **14** and $(C_6H_5)_3CH$) was used without purification in the next step to avoid acetyl migration. **14**: R_f 0.12 (petroleum ether/ether, 70:30 v/v).

1-*O*-(Octadecylcarbamoyl)-2-*O*-acetyl-3-*O*-[5-(*N*,*N*-dimethylamino)pentanoyl]glycerol (15). Esterification of 14 was carried out by using the same procedure as that described for compound **2c** and resulted in **15**: R_f 0.33 ($CHCl_3$/MeOH, 90:10 v/v); viscous oil; IR (Nujol) 3340 (NH), 1740 (large C=O ester), 1700 (NHCo), 1240 (C—O—C ester) cm^{-1}; ^1H NMR ($CDCl_3$, 80 MHz) (same spectrum as **3c** except for CH_2OR in 1-position, $CH_2OCONHCH_2(CH_2)_{16}CH_3$) δ 3.15 (m, 2 H, $NHCH_2$), 4.10 (m, 2 H, CH_2OCONH). Anal. ($C_{31}H_{60}N_2O_6$) C, H, N.

1-*O*-(Octadecylcarbamoyl)-2-*O*-acetyl-3-*O*-[5-(*N*,*N*,*N*-trimethylammonio)pentanoyl]glycerol Iodide (16). Quaternization of **15** was carried out by using the same procedure as that described for compound **3c** and resulted in **16**: R_f 0.26 ($CHCl_3$/MeOH, 80:20 v/v); mp 84–85 °C; IR (Nujol) 3340 (NH), 1740 (C=O ester), 1700 (C=O carbamate), 1550 (CONH), 1240 (C—O—C ester) cm^{-1}; ^1H NMR ($CDCl_3$, 250 MHz) δ 0.90 (t, 3 H, CH_3), 1.30 (large s, 30 H, $(CH_2)_{15}$), 1.5 (m, 2 H, $CONHNCH_2CH_2$), 2.05 (s, 3 H, $COCH_3$), 3.14 (m, 2 H, $NHCH_2$), 3.20 (s, 9 H, $N^+(CH_3)_3$), 4.11 (d, 2 H, CH_2OCONH), 4.3 (m, 2 H, CH_2OCO), 5.20 (m, 1 H, CHOCO); mass spectrum, *m/z* 571 (MH^+ - I), 511 ((MH^+ - I) - CH_3CO_2H).

B. Biology. For all experiments, PAF-acether and other compounds were solubilized in ethanol at 10 mg/mL and diluted

in saline. Activities (EC_{50}) were calculated by a polynomial adjustment method followed by a linear least squares regression analysis (GLM procedure from SAS (Statistical Analysis System)).

(a) Platelet Aggregation. (i) Agonist effect. Platelet-aggregation studies were performed by the method of Born.[26] Blood was collected into 3.2% sodium citrate (1 mL for 9 mL of blood) by cardiac puncture from conscious male New Zealand rabbits (≃3 kg). Platelet-rich plasma (PRP) was obtained by centrifugation of blood at 300*g* for 10 min at room temperature. The platelet concentration was adjusted to 300 000 μL^{-1} with platelet-poor plasma. Platelet aggregation was monitored by continuous recording of light transmission in a dual-channel aggregometer (Chronolog) coupled with a two-channel recorder. Various concentrations of PAF-acether or analogues (40 μL) were added to 0.4 mL of incubated and stirred PRP. Aggregation induced by analogues was compared to that obtained with PAF-acether which produced 100% aggregation at 5–8 × 10^{-8} M. The concentrations of drugs required to produce 50% aggregation (EC_{50}) were calculated.

(ii) Antagonist effect on aggregation. Various concentrations of antagonists were added to incubated and stirred PRP 2 min before PAF-acether or other aggregating agents were added. Inhibition of aggregation was measured and compared to a control aggregation induced by PAF (5 × 10^{-8} M), collagen (40 μg/mL), or arachidonic acid (AA) (2 × 10^{-4} M). The concentrations of drugs producing 50% inhibition (IC_{50}) of aggregation were calculated from dose–response curves.

(b) Rat Hypotension. (i) Agonist effect. Male albino rats (Sprague–Dawley) weighing 280–300 g were anesthetized with sodium pentobarbital (50 mg/kg) by intraperitoneal injection. PAF-acether and analogues were injected via a cannulated jugular vein, and arterial pressure was registered from a cannulated carotid artery by means of a Gould Statham P 23 ID pressure transducer. Drugs were injected at three successive doses at 20-min intervals, to obtain a lowering of arterial diastolic blood pressure (BP) to 40% of the initial value. Hypotensive activity was maximal 1 min after administration of compounds, and the doses required to lower BP by 30% (ED_{30}) were calculated.

(ii) Antagonist effect. Using the same rat preparation as employed above, we investigated the effect of PAF-acether isosteres against PAF-acether (0.3 μg/kg iv) induced hypotension. After PAF-acether injection, the hypotensive response was measured, the control response was noted, and 15 min later, PAF-acether antagonist was injected iv. After a further 5 min, PAF-acether (0.3 μg/kg) was injected again and the hypotensive response again noted. In order to ascertain the specificity of the response, we also investigated the effect of PAF-acether isosteres on acetylcholine (Ach) induced hypotension. The protocol employed was identical with that used for PAF-acether, the dose of Ach administered being 1 μg/kg. Only one dose of antagonist was investigated per rat. Percent inhibition was calculated for each dose.

(c) Bronchoconstriction and Thrombocytopenia in the Guinea Pig. (i) Agonist effect. Male Hartley guinea pigs (450–500 g) were anesthetized with ethyl carbamate (1.25 g/kg ip). The jugular vein was cannulated for administration of agonists, the carotid artery for blood sampling, and the trachea for artificial ventilation. Respiration was arrested with pancuronium bromide (2 mg/kg), and the animal was respirated by means of a Harvard pump (50 strokes/min, 10 mL/kg). Resistance to lung inflation was measured by a modification of the Konzett–Rossler overflow technique.[27] The resistance to lung inflation (in cm H_2O) was measured 1 min after drug administration. The dose that induced a bronchoconstriction of 3 cm H_2O was calculated (five animal/dose). This effect was obtained with 0.18 nM PAF-acether. Each animal received only one dose of test compound since guinea pigs show tachyphylaxis to PAF-acether. One minute before and after injection of PAF-acether, a blood sample was taken for platelet counting. The doses inducing 50% thrombocytopenia were calculated (ED_{50}).

(ii) Antagonist effect. Using the same guinea pig preparation as employed above, we investigated the effect of PAF-acether antagonists on PAF-acether (100 ng/kg iv) induced bronchoconstriction. PAF-acether antagonist was injected 5 min before PAF-acether. Bronchoconstriction response was compared to that obtained with a control group. Percent inhibition was calculated for each dose. One minute before and after PAF-acether injection, a blood sample was taken for platelet counting. The inhibiting effect on thrombocytopenia was calculated for each dose with respect to reverse control group. In order to ascertain the specificity of the response, we also investigated the effect of PAF-acether antagonists on acetylcholine- and histamine-induced bronchoconstriction. The protocol employed was identical with that used for PAF-acether, the doses administered being 20–30 μg/kg for acetylcholine and 2–5 μg/kg for histamine.

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Registry No. **1**, 89496-75-3; **2a**, 111325-02-1; **2b**, 111325-03-2; **2c**, 111325-01-0; **3a**, 111325-05-4; **3b**, 111325-06-5; **3c**, 111325-04-3; (*R*)-**3c**, 111408-47-0; (*S*)-**3c**, 111408-48-1; **4**, 86334-56-7; **5**, 111325-07-6; **6**, 111325-08-7; **7**, 111325-09-8; **8**, 111325-10-1; **9**, 111325-11-2; **10**, 111325-12-3; **11**, 111325-13-4; **12**, 111325-14-5; **13**, 111325-15-6; **14**, 111325-16-7; **15**, 111325-17-8; **16**, 111325-18-9; $HOOC(CH_2)_4NMe_2$, 89855-60-7; $HOOC(CH_2)_2NMe_2$, 6300-04-5; $HOOC(CH_2)_3NMe_2$, 693-11-8; $Cl(CH_2)_3COCl$, 4635-59-0; $C_{18}H_{37}NCO$, 112-96-9; isopropylideneglycerol, 22323-83-7.

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