Synthesis of 2-Thio-platlet Activating Factor and Related Compounds

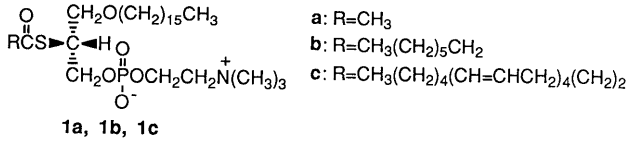
Masakazu MURATA, Hiroshi UCHIDA and Kazuo ACHIWA*

School of Pharmaceutical Sciences, University of Shizuoka, 52–1 Yada, Shizuoka 422, Japan. *Received March 16, 1992*

2-Thio-platlet-activating factor and related compounds as substrates of platlet activating factor acetylhydrolase and phospholipase A_2 were synthesized from optically active 2-*O*-benzylglycerol monoacetate.

Keywords 2-thio-platlet activating factor; acetylhydrolase; arachidonic acid; octanoic acid; stereoselective synthesis

Platlet-activating factor (PAF) is a potent lipid mediator with a wide spectrum of biological activities. The amount of PAF in tissues and body fluids is considered to be regulated by PAF acetylhydrolase, as well as by PAF-synthesize enzyme.[1] It has been reported that the level of PAF acetylhydrolase activity in blood/serum is correlated with respiratory symptoms in asthmatic children.[2] On the other hand, phospholipase A_2 is now thought to be a key enzyme in the release of arachidonate, a precursor to prostanoids, from membrane phospholipids after stimulation in various cells including neutrophils, macrophages and vascular endothelial cells.[3] PAF acetylhydrolase and phospholipase A_2 activity has been measured using labeled glycerolipid as substrates. From the standpoint of clinical analysis, the development of nonlabeled substrates is needed for a simple and reproducible method of measuring these enzyme activities. We desired 2-thio-PAF and related compounds (**1a**—**c**) as substrates of these enzymes. The measurement of activity using 2-thio-PAF and related compounds is easy because of the formation of thiol, which is readily detected, catalyzed by enzyme-hydrolysis. We describe here details of the preparation of **1a**—**c**.



1a, 1b, 1c

Chart 1

Stereoselective synthesis of 2-thio-PAF from (*R*)-2,3-isopropylideneglycerol tosylate has been reported by Bhatia and Hajdu.[4] However, in their synthesis route, racemization at the 2-position and formation of 1-thioacetate were likely to occur *via* the formation of an epoxide during the introduction of the thioacetyl group. We developed a new synthesis route to prevent these side reactions. Our approach is as follows: (*S*)-1-*O*-acetyl-2-*O*-benzylglycerol, which is easily obtained by lipase-catalyzed transesterification of 2-*O*-benzylglycerol, is utilized as a chiral source, and a tetrahydropyranyl group is chosen to protect the primary hydroxy group in order to prevent the formation of the epoxide and the $S \rightarrow O$ acyl migration.

Chart 2 shows the synthesis procedure for 2-thio-PAF and related compounds. Optically pure (*R*)-(−)-1-*O*-tosyl-2-*O*-benzylglycerol (**3**) was obtained from a reaction of (*S*)-(+)-2-*O*-benzylglycerol monoacetate[5] (**2**) with *p*-toluenesulfonyl chloride in pyridine, followed by deacetylation with NaOH–MeOH and recrystallization of the product from diisopropyl ether. After protection of the hydroxy group of **3** with dihydropyran, the tosylate (**4**) was heated

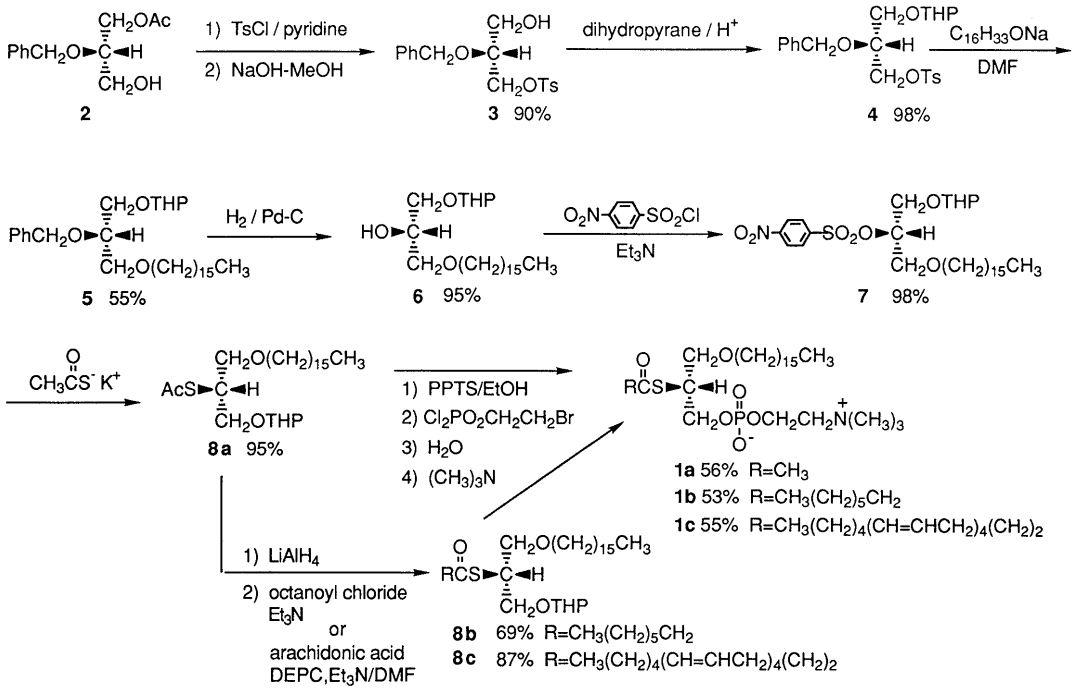


Chart 2

with sodium cetylate in DMF at 60 °C to give an alkylglycerol (**5**). Hydrogenation of **5** over 5% Pd–C in ethanol gave the alcohol (**6**) in a quantitative yield which was then treated with *p*-nitrobenzenesulfonyl chloride in the presence of triethylamine in $CHCl_3$. The resulting sulfonate (**7**) was heated with potassium thioacetate to give the thioacetate (**8a**). Reduction of **8a** with $LiAlH_4$ in tetrahydrofuran (THF) gave the thiol, which was then treated with octanoyl chloride or arachidonic acid/diethyl phosphorocyanidate[6] in the presence of triethylamine to give **8b** and **8c**. Deprotection of the tetrahydropyranyl group of **8a**—**c** with pyridinium *p*-toluenesulfonate in ethanol gave the corresponding alcohol. The crude alcohol was phosphorylated with 2-bromoethyl phosphorodichloridate in the presence of triethylamine in $CHCl_3$, and then stirred with H_2O to give the phosphorylate. The crude phosphorylate was heated with excess triethylamine in a sealed tube at 65 °C to afford 2-acylthiophospholipids (**1a**—**c**), respectively.

Thus, a new convenient method for the preparation of optically pure 2-thio-PAF (**1a**) and related compounds (**1b** and **1c**) were clarified.[7,8]

Experimental

(*R*)-2-*O*-Benzyl-1-*O*-tosylglycerol (3) This compound was prepared from (*S*)-(+)-1-*O*-acetyl-2-*O*-benzylglycerol as described.[9]

(*R*)-2-*O*-Benzyl-3-*O*-tetrahydropyranyl-1-*O*-tosylglycerol (4) A solution of **3** (18.43 g, 54.8 mmol), dihydropyran (6.92 g, 82.3 mmol) and *p*-toluenesulfonic acid (100 mg) in dichloromethane (100 ml) was stirred at 0 °C for 2 h. The reaction mixture was washed with sat. $NaHCO_3$ solution, washed with brine, dried, and concentrated *in vacuo*. The residue was chromatographed on a short silica gel column with AcOEt–hexane (1 : 3) to give **4** (22 g, 98%).

4: Colorless oil, $[\alpha]_D^{22}$ +2.44° (c=1.12, $CHCl_3$). IR (neat) cm^{-1}: 1360, 1175 (SO_2). ^{1}H-NMR ($CDCl_3$) δ: 1.40—1.75 (6H, m, $CH_2CH_2CH_2$), 2.43 (3H, s, Ar-CH_3), 3.40—3.50 (2H, m, CH_2O), 3.73—3.81 (3H, m, CH_2O, CH–O), 4.06—4.25 (2H, m, CH_2O), 4.52—4.58 (1H, m, O–CH–O), 4.58 (2H, s, CH_2Ph), 7.24—7.33 (5H, m, Ar-H), 7.31 (2H, d, J=8.1 Hz, Ar-H), 7.78 (2H, d, J=8.1 Hz, Ar-H).

(*S*)-2-*O*-Benzyl-3-*O*-hexadecyl-1-*O*-tetrahydropyranylglycerol (5) To a stirred suspension of oil-free sodium hydride (1.55 g, 64.5 mmol) in dry *N*,*N*-dimethylformamide (DMF) (100 ml) was added hexadecanol (15.6 g, 64.5 mmol). The reaction mixture was heated at 60 °C for 1 h, and then a solution of **4** (22 g, 53.8 mmol) in dry DMF (20 ml) was added. After stirring at 60 °C for 3 h, the reaction mixture was cooled and poured into ice-water, and extracted with ether three times. The combined extracts were washed with brine, dried, and concentrated. The residual oil was chromatographed on a silica gel column with AcOEt–hexane (1 : 8) to give **5** (13.2 g, 50%).

5: Colorless oil, $[\alpha]_D^{22}$ +0.80° (c=1.03, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ: 1.15 (28H, m, CH_2 × 14), 1.40—1.80 (6H, m, $CH_2CH_2CH_2$), 3.30—4.20 (9H, m, CH_2O × 4, CH–O), 4.6 (1H, m, O–CH–O), 4.68 (2H, s, CH_2Ph), 7.3 (5H, s, Ar-H).

(*S*)-3-*O*-hexadecyl-1-*O*-tetrahydropyranylglycerol (6) **5** (13.2 g, 26.9 mmol) was hydrogenated over 5% Pd–C (1 g) in ethanol at room temperature. The catalyst was filtered off and the filtrate was evaporated *in vacuo* to give **6** (10.54 g, 98%).

6: Colorless oil, $[\alpha]_D^{22}$ +2.2° (c=4.0, MeOH). IR (neat) cm^{-1}: 3400 (OH). ^{1}H-NMR: 0.88 (3H, br t, CH_3), 1.15—1.35 (28H, m, CH_2 × 14), 1.40—1.80 (6H, m, $CH_2CH_2CH_2$), 3.40—4.14 (10H, m, CH_2O × 4, CH–O, OH), 4.58—4.59 (1H, m).

(*S*)-3-*O*-Hexadecyl-2-*O*-(4-nitrobenzenesulfonyl)-1-*O*-tetrahydropyranylglycerol (7) To an ice-cooled and stirred solutio of **6** (10.54 g, 26.4 mmol) and 4-dimethylaminopyridine (30 mg) in dry pyridine (50 ml) was added 4-nitrobenzenesulfonyl chloride (7 g, 31.6 mmol). The mixture was stirred for 12 h. After removal of pyridine, the reaction mixture was dissolved in dichloromethane and washed with water, washed with brine, dried, and concentrated *in vacuo*. The residual oil was chromatographed on a silica gel column with AcOEt–hexane (6 : 1) to give **7** (12.4 g, 80%).

7: Colorless oil, $[\alpha]_D^{22}$ +0.90° (c=1.03, $CHCl_3$). IR (neat) cm^{-1}: 1350, 1190 (SO_2). ^{1}H-NMR: 0.87 (3H, br t, CH_3), 1.15—1.35 (28H, m, CH_2 × 14), 1.40—1.80 (6H, m, $CH_2CH_2CH_2$), 3.20—4.00 (9H, m, CH_2O × 4, CH–O), 4.70 (1H, m, O–CH–O), 8.00 (2H, d, J=9.0 Hz, Ar-H), 8.26 (2H, d, J=9.0 Hz, Ar-H). *Anal.* Calcd for $C_{30}H_{51}NO_8S$: C, 61.51; H, 8.78; N, 2.39. Found: C, 61.32; H, 8.84; N, 2.42.

(*R*)-1-*O*-Hexadecyl-3-*O*-tetrahydropyranyl-2-thioacetyl-2-deoxyglycerol (8a) A mixture of **7** (12.4 g, 21.2 mmol), potassium thioacetate (3.63 g, 31.8 mmol) and acetonitrile (50 ml) was refluxed for 3 h. After removal of acetonitrile, the reaction mixture was partitioned between dichloromethane and water. The organic layer was washed with brine and dried. After removal of the solvent, the residue was chromatographed on a short silica gel column with AcOEt–hexane (10 : 1) to give **8a** (9.2 g, 95%).

8a: Oil, $[\alpha]_D^{22}$ −2.26° (c=1.00, $CHCl_3$). IR (neat) cm^{-1}: 1690 (thioester). ^{1}H-NMR: 0.88 (3H, br t, CH_3), 1.15—1.35 (28H, m, CH_2 × 14), 1.40—1.80 (6H, m, $CH_2CH_2CH_2$), 2.31 (3H, s, $SCCH_3$), 3.27—4.30 (9H, m, CH_2O × 4, CH–O), 4.61 (1H, m, O–CH–O). FAB-MS m/z: $(M+H)^+$ 459.

(*R*)-1-*O*-Hexadecyl-3-*O*-tetrahydropyranyl-2-thiooctanoyl-2-deoxyglycerol (8b) To an ice-cooled and stirred suspension of lithium aluminum hydride (228 mg, 6 mmol) in dry THF (30 ml) was added a solution of **8a** (916 mg, 2 mmol) in THF (20 ml). The mixture was stirred at room temperature for 2 h. The mixture was cooled with an ice-bath and decomposed by a 10% sodium hydroxide solution, and filtered. The resulting cake was washed with THF. The combined filtrate was concentrated under reduced pressure to give crude thiol. To a solution of this thiol and triethylamine (202 mg, 2 mmol) in dry dichloromethane (20 ml) was added a solution of octanoyl chloride (325 mg, 2 mmol) in dichloromethane (5 ml) at 0 °C. The mixture was stirred at room temperature for 2 h. The reaction mixture was washed with water and washed with brine, and dried. After removal of the solvent, the residue was chromatographed on a short silica gel column with AcOEt–hexane (1 : 10) to give **8b** (748 mg, 69%) as an oil. IR (neat) cm^{-1}: 1690 (thioester).

(*R*)-1-*O*-Hexadecyl-3-*O*-tetrahydropyranyl-2-thioarachidonyl-2-deoxyglycerol (8c) To an ice-cooled and stirred suspension of lithium aluminum hydride (228 mg, 6 mmol) in dry THF (30 ml) was added a solution of **8a** (916 mg 2 mmol) in THF (20 ml). The mixture was stirred at room temperature for 2 h. The mixture was cooled with an ice-bath and decomposed by a 10% sodium hydroxide solution, and filtered. The resulting cake was washed with THF. The combined filtrate was concentrated under reduced pressure to give crude thiol. To a solution of the thiol and arachidonic acid (609 mg, 2 mmol) in dry DMF (20 ml) was added diethyl phosphorocyanidate (652 mg, 4 mmol) at 0 °C, followed by triethylamine (404 mg, 4 mmol). The mixture was stirred at room temperature for 2 h. The reaction mixture was poured into water (100 ml) and extracted with diethyl ether (20 ml) three times. The combined extracts were washed with brine and dried. After removal of the solvent, the residue was chromatographed on a short silica gel column with AcOEt–hexane (1 : 10) to give **8c** (1.22 g, 87%) as an oil. IR (neat) cm^{-1}: 1690 (thioester).

1-*O*-Hexadecyl-2-thioacyl-2-deoxy-*sn*-glycerol-3-phosphocoline (1a—c) A mixture of **8a**—**c** (1 mmol) and pyridinium *p*-toluenesulfonate (25 mg, 0.1 mmol) in ethanol (50 ml) was heated at 55 °C for 12 h. After removal of ethanol, the residue was dissolved in ether. The solution (50 ml) was washed with water and brine, dried, and concentrated. A solution of the resulting crude alcohol in dichloromethane (5 ml) was added to a solution of 2-bromoethyl phosphorodichloridate (390 mg, 1.64 mmol) and triethylamine (370 mg, 3.67 mmol) in dichloromethane (30 ml) at 0 °C. The mixture was stirred at room temperature for 12 h. The reaction mixture was washed with water, washed with brine, dried, and concentrated. The resulting phosphorylate was added to a solution of trimethylamine in chloroform which was transferred into a pressure-bottle. The bottle was sealed and heated at 65 °C for 12 h. The mixture was cooled to room temperature and methanol was added to dissolve any precipitate. After removal of the solvent, the residue was chromatographed on a silica gel column with $CHCl_3$–MeOH–H_2O (65 : 25 : 4) to give **1a**—**c**.

1a: Amorphous powder, yield 56% (302 mg), $[\alpha]_D^{22}$ −6.1° (c=0.91, $CHCl_3$: MeOH = 4 : 1). IR (neat) cm^{-1}: 1690 (thioester), 1080 (P=O). ^{1}H-NMR ($CDCl_3$) δ: 0.88 (3H, br t, CH_3), 1.26 (28H, s, CH_2 × 14), 2.34 (3H, s, $SCCH_3$), 3.32 (9H, s, $N(CH_3)_3$), 3.45—4.30 (11H, m, CH_2O × 3, CH–O, $POCH_2CH_2N$). FAB-MS m/z: $(M+H)^+$ 540.

1b: Amorphous powder, yield 53% (330 mg), $[\alpha]_D^{22}$ −2.7° (c=1.74, $CHCl_3$: MeOH = 4. : 1). IR (neat) cm^{-1}: 1690 (thioester), 1080 (P=O). ^{1}H-NMR ($CDCl_3$) δ: 0.88 (6H, br t, CH_3 × 2), 1.26 (38H, s, CH_2 × 19), 2.52—2.57 (2H, m, CH_2), 3.43 (9H, s, $N(CH_3)_3$), 3.59—4.46 (11H, m, CH_2O × 3, CH–O, $POCH_2CH_2N$). FAB-MS m/z: $(M+H)^+$ 624. *Anal.*

Calcd for $C_{32}H_{66}NO_6PS \cdot 2H_2O$: C, 58.24; H, 10.69; N, 2.12. Found: C, 58.16; H, 10.88; N, 1.98.

1c: Amorphous powder, yield 55% (431 mg), $[\alpha]_D^{22}$ $-2.8°$ ($c=2.45$, $CHCl_3:MeOH=4:1$). IR (neat) cm^{-1}: 1690 (thioester), 1090 (P=O). ^1H-NMR ($CDCl_3$) δ: 0.85—0.91 (6H, m, $CH_3 \times 2$), 1.26 (36H, m, $CH_2 \times 18$), 2.01—2.13 (4H, m, $CH_2 \times 2$), 2.53—2.58 (2H, m, CH_2), 2.78—2.86 (6H, m, $CH_2 \times 3$), 3.42 (9H, s, $N(CH_3)_3$), 3.35—4.36 (11H, m, $CH_2O \times 3$, CH–O, $POCH_2CH_2N$), 5.26—5.45 (8H, m, $CH=CH \times 4$). FAB-MS m/z: $(M+H)^+$ 785.

References and Notes

1) *a*) R. N. Pinckard, R. S. Farr and D. J. Hanahan, *J. Immunol.*, **123**, 1847 (1979); *b*) M. L. Wardlow, C. P. Cox, K. E. Meng, D. E. Greene and R. S. Farr, *ibid.*, **136**, 3441 (1986).
2) M. Miwa, T. Miyake, T. Yamanaka, J. Sugatani, Y. Suzuki, S. Sakata, Y. Araki and M. Matsumoto, *J. Clin. Invest.*, **82**, 1983 (1988).
3) J. Y. Channon and C. C. Leslie, *J. Biol. Chem.*, **265**, 5409 (1990).
4) S. K. Bhatia and J. Hajdu, *Tetrahedon Lett.*, **28**, 1729 (1987).
5) M. Murata, Y. Terao, K. Achiwa, T. Nishio and K. Seto, *Chem. Pharm. Bull.*, **37**, 2670 (1989).
6) Y. Yokoyama, T. Shioiri and S. Yamada, *Chem. Pharm. Bull.*, **25**, 2423 (1977).
7) The optical purity of **1a**—**c** was confirmed by comparison of the optical rotation of **1a** ($[\alpha]_D^{22}$ $-6.1°$) with that of the optically pure sample ($[\alpha]_D^{23}$ $-6.07°$).[8)]
8) S. K. Bhatia and J. Hajdu, *Synthesis*, **1989**, 16.
9) M. Kurimura, M. Takemoto and K. Achiwa, *Chem. Pharm. Bull.*, **39**, 2590 (1991).