NEW SYNTHESIS OF 2-THIO-PAF AND RELATED COMPOUNDS AS SUBSTRATES OF PAF ACETYLHYDROLASE AND PHOSPHOLIPASE A₂

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2-Thio-PAF and related compounds (**1a-c**) as substrates of PAF acetylhydrolase and phospholipase A₂ were synthesized stereoselectively from optically active 2-*O*-benzylglycerol monoacetate.

KEYWORDS 2-thio-PAF; PAF acetylhydrolase; phospholipase A₂; arachidonic acid; octanoic acid; stereoselective synthesis

The platelet-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, in addition to 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 A₂ is now thought to be a key enzyme to release arachidonate, which is a precursor of prostanoids, from membrane phospholipids after stimulation in various cells including neutrophils, macrophages and vascular endothelial cells.³⁾

In this study, we developed a new and convenient method to synthesize 1-*O*-hexadecyl-2-thioacetyl-2-deoxy-*sn*-glycero-3-phosphocholine (2-thio-PAF, **1a**), 1-*O*-hexadecyl-2-thiooctanoyl-2-deoxy-*sn*-glycero-3-phosphocholine (**1b**) and 1-*O*-hexadecyl-2-thioarachidonoyl-2-deoxy-*sn*-glycero-3-phosphocholine (**1c**). These were used in simple and reproducible methods for measuring PAF acetylhydrolase activity and phospholipase A₂ activity, respectively.⁴⁾

1a: R = CH₃ (2-thio-PAF), **1b:** R = CH₃(CH₂)₆, **1c:** R = CH₃(CH₂)₄(CH=CHCH₂)₄(CH₂)₂

Stereoselective synthesis of 2-thio-PAF from (*R*)-2,3-isopropylideneglycerol tosylate has been reported by Bhatia and Hajdu.⁵⁾ However, in their synthesis route, racemization at the 2-position and formation of 1-thioacetate were liable 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 esterification, is utilized as a chiral source, and a tetrahydropyranyl group is chosen to protect the primary hydroxyl group to prevent the formation of the epoxide and the S→O acyl migration.

Chart 1 shows the synthesis procedure for 2-thio-PAF and related compounds. Optically pure (*R*)-(-)-3-tosyloxy-2-benzyloxy-1-propanol (**3**) was obtained from a reaction of (*S*)-(+)-2-*O*-benzylglycerol monoacetate⁶⁾ (>90% ee **2**) with *p*-toluenesulfonyl chloride in pyridine, followed by chemoselective deacetylation using NaOH-MeOH and recrystallization of the product from isopropyl ether (90% from **2**). After protection of the hydroxyl group of **3** with dihydropyran and *p*-toluenesulfonic acid, the tosylate (**4**) was treated with hexadecanol in the presence of NaH in DMF to afford an alkylglycerol (**5**) (55% from **3**). Hydrogenation of **6** 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 CHCl₃. The resulting sulfonate (**7**) was treated with potassium thioacetate to give the thioacetate (**8a**). Reduction of **8a** with LiAlH₄ in THF gave the thiol which was then treated with octanoyl chloride or arachidonic acid / diethyl phosphorocyanidate⁷⁾ in the presence of triethylamine to give **8b** or **8c**. Deprotection of the tetrahydropyranyl group of **8a-c** using PPTS in ethanol at 55°C gave the alcohol. The crude alcohol was phosphorylated with 2-bromoethyl phosphorodichloridate in the presence of triethylamine in CHCl₃ then

stirred with H_2O to give the phosphorylate. The crude phosphorylate was heated at 65°C with excess trimethylamine in a sealed tube to afford 2-acylthiophospholipid (**1a-c**).⁸⁾ **1a**: $[\alpha]_{\text{D}} -6.1^\circ$, **1b**: $[\alpha]_{\text{D}} -2.7^\circ$, **1c**: $[\alpha]_{\text{D}} -2.8^\circ$ ($\text{CHCl}_3/\text{MeOH}=4/1$).

Chart 1

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