Synthesis of Analogues of Platelet Activating Factor: 1(3)-*O*-[2′-(*R*,*S*)-Methoxyhexadecyl]-2-*O*-acetyl-*sn*-glycero-3(1)-phosphocholines

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Analogues of platelet activating factor, 1-*O*-[2′-(*R*,*S*)-methoxyhexadecyl]-2-*O*-acetyl-*sn*-glycero-3-phosphocholine and 3-*O*-[2′-(*R*,*S*)-methoxyhexadecyl]-2-*O*-acetyl-*sn*-glycero-1-phosphocholine, have been synthesized.

1-*O*-Alkyl-2-*O*-acetyl-*sn*-glycero-3-phosphocholine, platelet activating factor (PAF), is known to be a potent wide range bioregulator. PAF has been found to be a mediator of a variety of biological processes stimulating platelet aggregation and degranulation, increasing vascular permeability and modulating intracellular signalling. It is also thought to play an important role in some physiological and pathological reactions, namely inflammation, allergy, anaphylaxis, hypotension, *etc*.[1]

By now a large number of structural PAF analogues have been obtained. PAF agonists as well as effective PAF antagonists have been synthesized. Modifications of the structure of the PAF molecule have allowed the separation of different biological effects. For example, compounds of higher hypotensive effect but of much lower aggregatory activity compared to PAF have been prepared.[2]

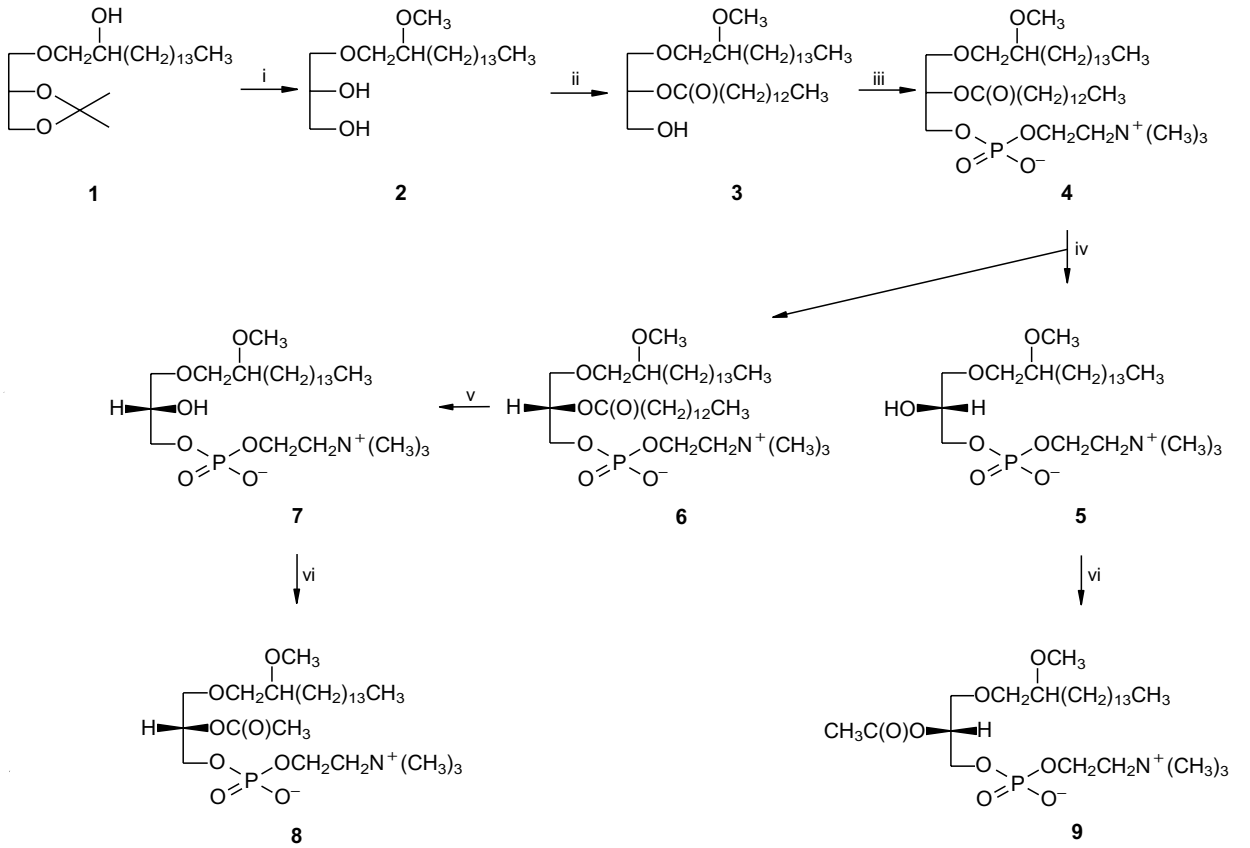
To contribute to these studies we have synthesized two novel PAF analogues: 3-*O*-[2′-(*R*, *S*)-ethoxyhexadecyl]-2-*O*-acetyl-*sn*-glycero-1-phosphocholine **8** and 1-*O*-[2′-(*R*, *S*)-ethoxyhexadecyl]-2-*O*-acetyl-*sn*-glycero-3-phosphocholine **9**.

Initial 1-*O*-[2′-(*R*, *S*)-hydroxyhexadecyl]-2,3-di-*O*-isopropylidene-*rac*-glycerol **1**[3] was converted to its potassium salt by refluxing with potassium hydroxide in benzene and then alkylated with methyl iodide (Scheme 1). The isopropylidene protective group of the derivative obtained was removed by methanolysis in the presence of a catalytic amount of toluene-4-sulfonic acid to give **2**.

1-*O*-[2′-(*R*, *S*)-Methoxyhexadecyl]-*rac*-glycerol **2** was treated with diphenylmethylchlorosilane in the presence of pyridine in anhydrous toluene to protect the primary hydroxyl function and the silyl derivative obtained *in situ* was acylated with myristoyl chloride. The silyl ether was then hydrolysed by refluxing in the presence of $NH_4F{\cdot}HF$ in a mixture of acetone, pyridine and water, 100:3:4 (vol.), to give 1-*O*-[2′-(*R*, *S*)-ethoxyhexadecyl]-2-*O*-myristoyl-*rac*-glycerol **3**.[4]

Diglyceride **3** was converted to the corresponding phosphocholine **4** by subsequent treatment with phosphorus oxychloride in the presence of triethylamine and with choline toluene-4-sulfonate in the presence of pyridine following the procedure of Brokerhoff *et al*.[5]

1-*O*-[2′-(*R*, *S*)-Methoxyhexadecyl]-2-*O*-myristoyl-*rac*-glycero-3-phosphocholine **4** was treated with phospholipase A_2 from *Echis multusqu amatus venom*,[4] which cleaved the enantiomer of natural configuration at the glycerol chiral centre to give 1-*O*-[2′-(*R*, *S*)-methoxyhexadecyl]-*sn*-glycero-3-phosphocholine **5** and did not affect 3-*O*-[2′-(*R*, *S*)-methoxy-



Scheme 1 *Reagents and conditions:* i, MeI, KOH, benzene, 80 °C; toluene-4-sulfonic acid, methanol; yield of **2**, 91%; ii, $SiPh_2MeCl$, pyridine, toluene; $C_{13}H_{27}COCl$; $NH_4F{\cdot}HF$, acetone–pyridine–water, 100:3:4; yield of **3**, 50%; iii, $POCl_3$, Et_3N, chloroform; choline toluene-4-sulfonate, pyridine; H_2O; yield of **4**, 65%; iv, *Echis multusqu amatus venom*, buffer solution (100 mM Tris-OH, 50 mM $CaCl_2$, pH 8.00), chloroform; yield of **5**, 80%; yield of **6**, 98%; v, NaOMe, chloroform–methanol–water, 10:10:3; yield of **7**, 91%; vi, Ac_2O, $HClO_4$, chloroform; yield of **8**, 63%; yield of **9**, 56%.

hexadecyl]-2-*O*-myristoyl-*sn*-glycero-1-phosphocholine **6** of unnatural configuration at the glycerol chiral centre. Compound **6** was then hydrolysed with sodium methylate in a mixture of chloroform, methanol and water, 10:10:3 (vol.) to yield 3-*O*-[2′-(*R*, *S*)-methoxyhexadecyl]-*sn*-glycero-1-phosphocholine **7**.

Lysophospholipids **5** and **7** were acetylated with acetic anhydride in the presence of perchloric acid[6] to give 1-*O*-[2′-(*R*, *S*)-methoxyhexadecyl]-2-*O*-acetyl-*sn*-glycero-3-phosphocholine **9** and 3-*O*-[2′-(*R*, *S*)-methoxyhexadecyl]-2-*O*-acetyl-*sn*-glycero-1-phosphocholine **8**. Using an acid catalysis approach allowed us to avoid the acyl migration that occurred in the presence of organic bases (*e.g.* triethylamine or *N,N*-dimethylaminopyridine) and led to a structural isomer with the phosphocholine group at the C_2-position of the glycerol moiety.[4]

The identity and structure of the compounds obtained were confirmed by TLC, IR, ^{1}H and ^{31}P NMR spectroscopy and mass spectrometry data.†

† **5**: *m*/*z* (plasma desorption mass spectrometry) Calc. for $[M+H]^+$ 512.69, observed 512.5. ^{1}H NMR (Bruker MSL-200, 200.13 MHz, $CDCl_3$–CD_3OD–D_2O, 1:1:0.15, internal standard $SiMe_4$) δ 0.86 [t, *J* 7 Hz, 3H, $(CH_2)_{12}CH_3$], 1.25 [br.s, 24H, $(CH_2)_{12}$], 1.45 [m, 2H, CH(OH)CH_2], 3.21 [s, 9H, $N^+(CH_3)_3$], 3.35–3.75 (m, 7H, CH_2N, CH_2OCH_2, C*H*–OCH_3), 3.39 (s, 3H, OCH_3), 3.78–4.0 (m, 3H, C*H*–OH, CH_2–OP), 4.27 (m, 2H, PO–CH_2); ^{31}P NMR (Bruker MSL-200, 81 MHz, broad-band proton decoupling, $CDCl_3$–CD_3OD–D_2O, 1:1:0.15, external standard H_3PO_4) δ 2.12; $[\alpha]_D^{25}$ (Perkin-Elmer 241 MC) −3.87 (*c* 1.5, $CHCl_3$).

7: *m*/*z* Calc. for $[M]^+$ 511.68, observed 511.2. ^{1}H NMR δ 0.86 [t, *J* 7 Hz, 3H, $(CH_2)_{12}CH_3$], 1.25 [br.s, 24H, $(CH_2)_{12}$], 1.45 [m, 2H, CH(OH)CH_2], 3.21 [s, 9H, $N^+(CH_3)_3$], 3.35–3.75 (m, 7H, CH_2N, CH_2OCH_2, C*H*–OCH_3), 3.39 (s, 3H, OCH_3), 3.78–4.0 (m, 3H, C*H*–OH, CH_2–OP), 4.27 (m, 2H, PO–CH_2); ^{31}P NMR δ 1.52; $[\alpha]_D^{25}$ +3.73 (*c* 1.5, $CHCl_3$).

8: *m*/*z* Calc. for $[M+H]^+$ 554.73, observed 554.2. ^{1}H NMR δ 0.86 [t, *J* 7 Hz, 3H, $(CH_2)_{12}CH_3$], 1.24 [br.s, 24H, $(CH_2)_{12}$], 1.43 [m, 2H, CH(OH)CH_2], 2.07 [s, 3H, OC(O)CH_3], 3.20 [s, 9H, $N^+(CH_3)_3$], 3.35–3.75 (m, 7H, CH_2N, CH_2OCH_2, C*H*–OCH_3), 3.37 (s, 3H, OCH_3), 3.98 (m, 2H, CH_2–OP), 4.24 (m, 2H, PO–CH_2), 5.13 (m, 1H, C*H*–OAc); ^{31}P NMR δ 0.73; $[\alpha]_D^{25}$ +0.85 (*c* 0.71, $CHCl_3$).

9: *m*/*z* Calc. for $[M+H]^+$ 554.73, observed 554.4. ^{1}H NMR δ 0.86 [t, *J* 7 Hz, 3H, $(CH_2)_{12}CH_3$], 1.25 [br.s, 24H, $(CH_2)_{12}$], 1.43 [m, 2H, CH(OH)CH_2], 2.07 [s, 3H, OC(O)CH_3], 3.18 [s, 9H, $N^+(CH_3)_3$], 3.35–3.75 (m, 7H, CH_2N, CH_2OCH_2, C*H*–OCH_3), 3.38 (s, 3H, OCH_3), 3.97 (m, 2H, CH_2–OP), 4.24 (m, 2H, PO–CH_2), 5.13 (m, 1H, C*H*–OAc); ^{31}P NMR δ 0.96; $[\alpha]_D^{25}$ −0.75 (*c* 1.2, $CHCl_3$).

Compounds **5**, **7**, **8** and **9** were obtained in amounts close to 100 mg.

Detailed synthesis and studies on biological activity of the compounds obtained will be published elsewhere.

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References

1 K. Ju. Gordeev, G. A. Serebrennikova and R. P. Evstigneeva, *Bioorg. Khim.*, 1984, **10**, 1559 (in Russian).
2 M. A. Sablina, I. P. Ushakova and G. A. Serebrennikova, *Khim. Farm. Zh.*, 1994, **28**, 9 (in Russian).
3 E. A. Parfenov, G. A. Serebrennikova and N. A. Preobrazhenskii, *Zh. Org. Khim.*, 1966, **2**, 633 [*J. Org. Chem. USSR (Engl. Transl.)*, 1966, **2**, 633].
4 V. V. Chupin, O. V. Octapenko, V. N. Klykov, M. V. Anikin and G. A. Serebrennikova, *Bioorg. Khim.*, 1993, **19**, 1111 (*Russ. J. Bioorg. Chem.*, 1993, **19**, 667).
5 H. Brokerhoff and N. K. N. Ayengar, *Lipids*, 1979, **14**, 88.
6 R. Kumar, S. T. Weintraub, L. M. McManus, R. N. Pinckard and R. N. Hanahan, *J. Lipid Res.*, 1984, **25**, 198.

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