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Protection of Phosphate with the 9-Fluorenylmethyl Group. Synthesis of Unsaturated-acyl Phosphatidylinositol 4,5-Bisphosphate

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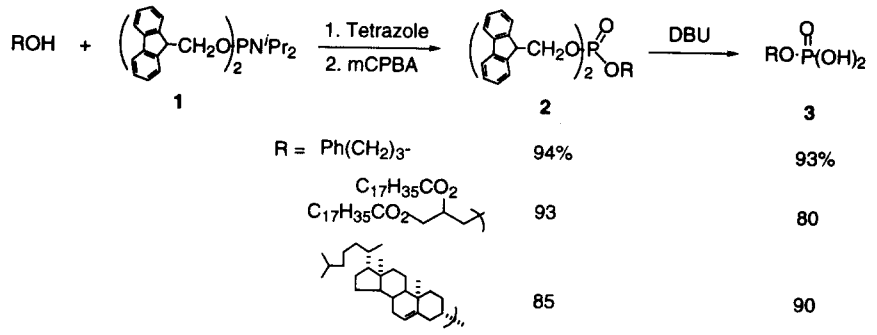
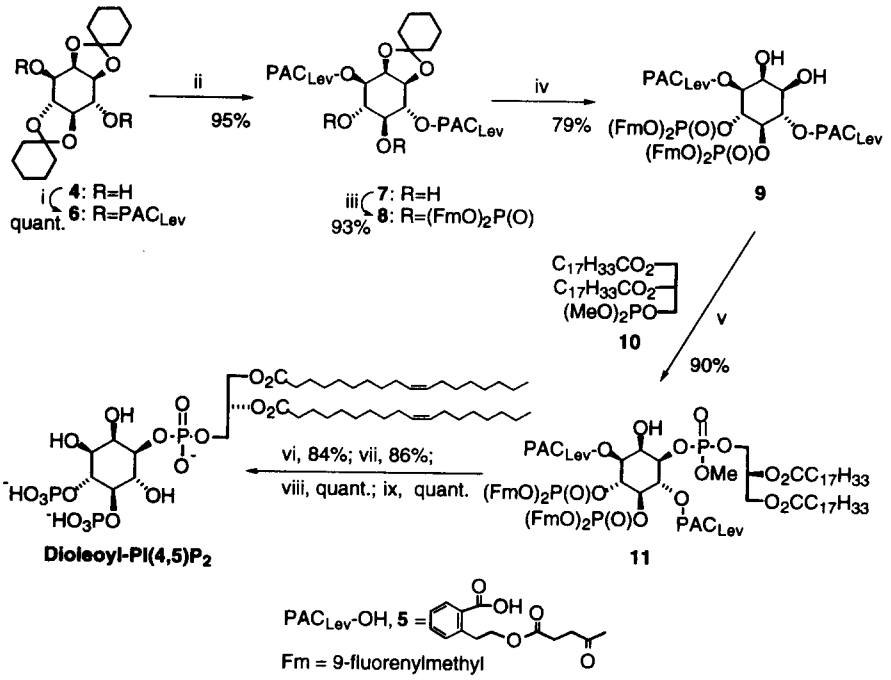
Abstract: Alkyl di-(9-fluorenylmethyl) phosphates obtained via phosphitylation were found to be suitable for protection of phosphoric monoesters, which were generated by treatment of the triesters with DBU or triethylamine. This strategy and a hydroxyl protecting group, 2-[2-(levulinoyloxy)ethyl]benzoyl were applied to synthesis of a dioleoyl analog of PI(4,5)P_2. © 1997 Elsevier Science Ltd.

Natural phosphatidylinositol 4,5-bisphosphate [PI(4,5)P_2], which has been recognized as not only a parent molecule producing two second messengers, inositol 1,4,5-trisphosphate and 1,2-diacylglycerol, but also a modulator by binding to cytoskeletal and signaling molecules,[1] has stearoyl and arachidonoyl groups at the *sn*-1 and 2 positions in the glyceryl moiety. Although chemical syntheses of PI(4,5)P_2 analogs bearing saturated fatty acid chains have been reported,[2] neither the natural product nor any unsaturated-type analog has been so far synthesized. To prepare this type of lipids, benzyl and related groups which have been used for the protection of phosphate functions in the synthesis of saturated phosphoinositides, can not be removed generally by the hydrogenolysis procedure, since it reduces also the olefinic function.[3] To solve this problem, we have searched for protecting groups for phosphates which may be removed by a β-elimination mode. Eventually, the 9-fluorenylmethyl (Fm) group was found to be promising for our purposes. This group was used for converting nucleoside H-phosphonodithioates to the phosphorodithioates via the transient mono-*O*-fluorenylmethyl esters, which were removed by the reaction with concentrated ammonia.[4] We describe here that alkyl difluorenylmethyl phosphates can be efficiently utilized as protected derivatives of phosphoric monoesters. Synthesis of dioleoyl PI(4,5)P_2 using this protecting group is also reported.

Difluorenyl phosphoramidite[5] **1** was prepared according to a conventional procedure via phosphoramidous dichloride.[6] The reaction of alcohols with **1** in the presence of 1*H*-tetrazole (r.t., 3.5 h) followed by oxidation of the resultant phosphites with mCPBA (r.t., 1 h after addition of the oxidant at -78 °C) proceeded smoothly to give phosphoric triesters **2** in high yields (Scheme 1). Complete deprotection of **2** was



Scheme 1



i) PAC_{Lev}-OH, DCC, DMAP; ii) TFA, MeOH, CH_2Cl_2; iii) $(FmO)_2PN^iPr_2$, 1*H*-tetrazole then mCPBA; iv) 80% aq. AcOH, refl; v) $PyHBr_3$, 2,6-Lutidine; vi) NaI, acetone, refl.; vii) Et_3N, CH_3CN/CH_3CH_2CN (3:1), r.t. (overnight) to refl. (7 h); viii) NH_2NH_2, pyridine, AcOH; ix) *t*-BuOH

Scheme 2

accomplished by using 3 molar equivalents of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU, r.t., 3 to 5 h) or large excess of triethylamine (r.t., 1 day) while, in both cases, the first fluorenyl group was removed smoothly as expected.

The preliminary results thus obtained prompted us to apply the protecting strategy to synthesis of unsaturated-acyl phosphatidylinositol phosphates. As shown in Scheme 2, synthesis of a DL-1,2-di-*O*-oleoyl-*sn*-glyceryl phosphate analog of PI(4,5)P_2 has now been accomplished where each step proceeded in excellent yield. This route involved protection of the 3,6-dihydroxyl groups with a 2-[2-(levulinoyloxy)ethyl]benzoyl group (PAC_{Lev})[7] which could be chemoselectively deprotected and is also expected to function as an auxiliary for kinetic resolution of inositol derivatives using an acylating agent, tartaric monoester.[8] In fact, the 4,5-bis(dibenzyl phosphoryl) inositol derivative analogous to **9** was obtained in 98% ee,[9] though optical resolution of **9** has not been done yet. Phosphorylation of 1,2-diol **9** via the phosphite method[10] using **10** and pyridinium tribromide proceeded smoothly to afford the completely regioselective 1-phosphate **11**. The final deprotection stage commenced at the 1-phosphate group and then the fluorenylmethyl phosphates at the 4- and 5-positions were deblocked as follows. The first Fm group in each phosphate was removed at room temperature by the reaction with triethylamine in acetonitrile and propionitrile (3:1) to avoid any unexpected side reaction and then the second under refluxing conditions to give the product with deprotected phosphate groups in high yield. The levulinoyl groups in the PAC groups were removed by the reaction with hydrazine in pyridine and acetic acid, and the resulting hydroxyethylbenzoates were treated with *t*-BuOK in dichloromethane after strict removal of a trace of water with pyridine to afford the final product.[11]

In conclusion, the 9-fluorenylmethyl group is promising for protection of phosphate functions in the synthesis of unsaturated phosphatidylinositol phosphates.

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11. R_f 0.2 (silica gel, $CHCl_3$/Acetone/MeOH/AcOH/H_2O, 40:15:13:12:8); ^{1}H-NMR [400 MHz, Et_3N salt, 22.2 mg in $CDCl_3$ (0.5 ml), CD_3OD (0.05 ml), and D_2O (1 drop), pH ca. 7] 0.88 (6H, t, *J*=7.7 Hz, CH_3), 1.98 (8H, br , allylic H), 2.15 (4H, br, oleoyl α-H), 3.0-4.39 (9H, Ins-Hs, glyceryl α- and γ-H), 5.36 (5H, br, glyceryl β-H and olefinic H); ^{13}C-NMR (100 MHz, same as in ^{1}H-NMR) 27.11 and 27.71 (2C, allylic), 33.97 (2C, oleoyl α-C), 64.80 (2C, br, glyceryl α- and γ-C), 68.50 (2C, br, Ins-$C_{2,6}$), 70.00-71.50 (2C, br, Ins-C_3 and glyceryl β-C), 127.20, 127.60, 129.90 and 130.35 (4C, olefinic C), 174.00 (2C, CO); ^{31}P-NMR [109 MHz, Et_3N salt, 53.5 mg in $CDCl_3$ (2.5 ml), CD_3OD (0.3 ml), and D_2O (1 drop), pH ca. 7] 2.00, 2.08, 4.10, 4.14, 5.27, 5.64; FAB-MS (negative, diethanolamine) *m/z* 1021 [M-1].

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