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GENERAL SYNTHESIS OF PHOSPHATIDYLINOSITOL 3-PHOSPHATES

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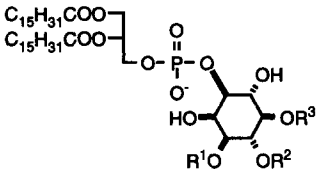
Abstract: Uniform synthetic approach to phosphatidylinositol 3-phosphate, 3,4-bisphosphate and 3,4,5-trisphosphate has been elaborated starting from 1D-1-*O*-tert-butyldiphenylsilyl- and 1,6-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-*myo*-inositols using regioselective benzoylation at the 3-, 3,4- and 3,4,5-positions of inositol.

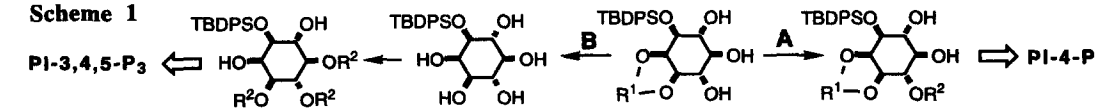
Phosphatidylinositol 4-phosphate (PI-4-P) and 4,5-bisphosphate (PI-4,5-P_2) play a key role in signal transduction involving phosphatidylinositol-specific phospholipase C (PI-PLC).[1] In contrast, the 3-phosphorylated PI are refractory to PI-PLC,[2] and their cellular function involves signal transduction of growth factors.[3] The cellular content of these phospholipids is low,[2b] therefore their isolation from biological sources is impractical. To date only a few reports on the synthesis of phosphatidylinositol phosphates have been published.[4,5,6]



1, $R^1 = PO_3^{2-}$, $R^2 = R^3 = OH$
2, $R^1 = R^2 = PO_3^{2-}$, $R^3 = OH$
3, $R^1 = R^2 = R^3 = PO_3^{2-}$

This paper describes a uniform route to 3-phosphorylated inositol phospholipids: phosphatidylinositol 3-phosphate (**1**, PI-3-P), 3,4-bisphosphate (**2**, PI-3,4-P_2) and 3,4,5-trisphosphate (**3**, PI-3,4,5-P_3) starting from the readily available inositol intermediates, 1D-1-*O*-tert-butyldiphenylsilyl-*myo*-inositol (TBDPS-inositol, **4**) and 1D-1,6-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-*myo*-inositol (TIPDS-inositol, **5**).[7,8] As we have shown earlier[7,9,10] inositol 2,3-acetals can be regioselectively protected at the 1-position. The resulting 1-acyl or 1-silyl derivatives can be further protected at the 4- and 4,5-positions (**Scheme 1**, path A).[7,9] The use of different protective groups for these reactions (i.e. $R^2 \neq$TBDPS) is the basis for differentiation of hydroxyl groups at the 1- and 4-positions, leading to a precursor of PI-4-P.[11] On the other hand, if the 2,3-acetal group is cleaved off first (path B), the subsequent triple protection takes place exclusively at the 3,4,5-positions to generate the precursor of PI-3,4,5-P_3.[7]

Scheme 1



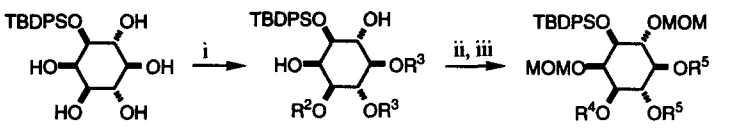
R^1 = bornane-2,2-diyl, R^2 = benzoyl

The synthetic strategy devised here provides a set of precursors of phospholipids **1-3** differing only by virtue of the protection of the hydroxyl groups to be further converted into phosphate monoesters (**Schemes 2-4**).

Synthesis of Precursors of PI-3-P, PI-3,4-P_2 and PI-3,4,5-P_3.

TBDPS-inositol **4** was treated with benzoyl chloride in pyridine at -40°C (**Scheme 2**). The reaction of the pentol **4** with 1 equiv. of BzCl gave mainly 3-benzoate **6** (73%) and smaller amounts of 4-benzoate (7%), 3,4-bisbenzoate (12%) and 3,5-bisbenzoate (8%). The analogous reaction with 3 equiv. of BzCl provided exclusively 3,4,5-trisbenzoate **7**, without any detectable amounts of 2- or 6-benzoylated products.[7] The reaction of the pentol **4** with 2 equiv. of BzCl afforded the mixture of the above products in which 3,4-bisbenzoate was a main product, but constituted only 39% of the product mixture. Methoxymethylation of **6** and **7** and further debenzoylation produced the 3-alcohol **8** and the 3,4,5-triol **9**, further used in synthesis of PI-3-P and PI-3,4,5-P_3, respectively.

Scheme 2



4

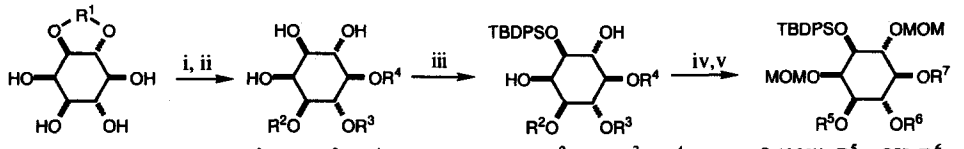
6, R^2 = Bz, R^3 = OH
7, $R^2 = R^3$ = Bz

8, R^4 = OH, R^5 = MOM
9, $R^4 = R^5$ = OH

i: BzCl/Py, -40°C; ii: MOM-Cl/EtiPr$_2$N, DMF; iii: NaOMe/MeOH

Benzoylation of the tetrol **5** (**Scheme 3**) under the above conditions produced slightly different results. Benzoylation of the tetrol **5** with 1 equiv. BzCl followed by desilylation with 10% HF in MeCN gave mainly inositol 3-benzoate (**10**, 81%), with 4-benzoate (6%) and 3,4-bisbenzoate (**11**, 13%) as by-products. Bisbenzoylation (2 equiv. BzCl) gave mainly 3,4-bisbenzoate **11** (88%) with small amounts of 3-benzoate **10** (6%) and the 3,4,5-trisbenzoate **12** (6%). Trisbenzoylation (3 equiv. BzCl) afforded the desirable 3,4,5-trisbenzoate **12** (60%) as a main product along with 3,4-bisbenzoate (12%), 2,3,4-trisbenzoate (6%) and 2,3,4,5-tetrabenzoate (15%). The obtained mixtures were readily separated into individual compounds by chromatography. Benzoates **10**, **11** and **12** were resilylated with TBDPS-Cl/imidazole in DMF at 0°C to yield the corresponding 1-silyl ethers **6**, **13** and **7**, and further converted into the 3-alcohol **8**, the 3,4-diol **14** and the 3,4,5-triol **9**, respectively, as shown above.

Scheme 3



5

10 (81%), R^2 = Bz, $R^3 = R^4$ = OH
11 (88%), $R^2 = R^3$ = Bz, R^4 = OH
12 (60%), $R^2 = R^3 = R^4$ = Bz

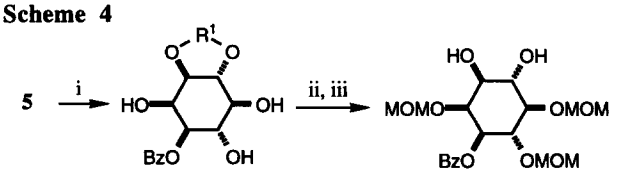
6 (90%), R^2 = Bz, $R^3 = R^4$ = OH
13 (60%), $R^2 = R^3$ = Bz, R^4 = OH
7 (90%), $R^2 = R^3 = R^4$ = Bz

8 (66%), R^5 = OH, $R^6 = R^7$ = MOM
14 (72%), $R^5 = R^6$ = OH, R^7 = MOM
9 (87%), $R^5 = R^6 = R^7$ = OH

R^1=-iPr$_2$SiOSiiPr$_2$-; i: BzCl/Py; ii: HF/MeCN; iii: TBDPS-Cl/ imidazole, DMF;
iv: MOM-Cl/EtiPr$_2$N, DMF; v: NaOMe/MeOH

Monobenzoylation of the tetrol **5** followed by methoxymethylation of the 3-benzoate **15** and final desilylation afforded the diol **16**, the functional equivalent of the enantiomer of the diol **14** (Scheme 4).

Scheme 4



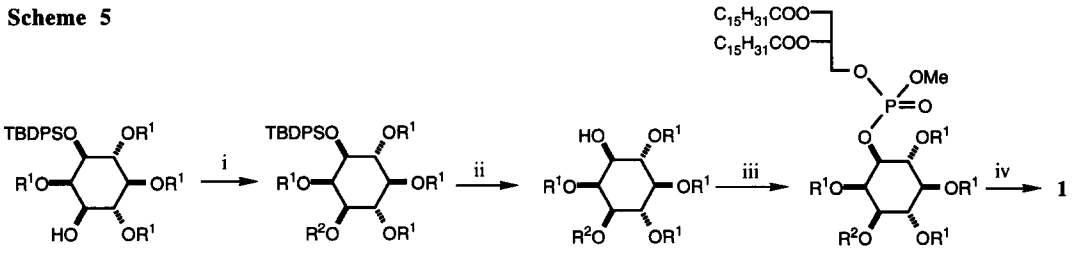
15 16

R^1: -iPr_2Si-O-$SiiPr_2$-; i: BzCl/Py, -40°C; ii: MOM-Cl/$EtiPr_2N$, DMF; iii: Bu_4N^+,F^-

Synthesis of Phosphatidylinositol Phosphates.

The above compounds **8**, **14** and **9** served as substrates for syntheses of PI phosphates **1**, **2** and **3**, respectively. The procedure is illustrated in **Scheme 5** using synthesis of PI-3-P as an example. The assembly of the phosphomonoester groups was achieved by phosphorylation of the 3-hydroxyl group with *O,O*-bisbenzyl-*N,N*-diisopropylphosphoramidite in the presence of tetrazole followed by MCPBA oxidation.[12] The initially formed phosphate **17** was desilylated with tetra-*n*-butylammonium fluoride and the resulting alcohol **18** was subsequently reacted with (1) *N,N*-diisopropyl-*O*-methylphosphonamidic chloride[13] in the presence of ethyldiisopropylamine, (2) dipalmitoylglycerol in the presence of tetrazole, and finally with (3) MCPBA to form the phosphate triester **19**. At this point the product was chromatographically purified and further subjected to a sequence of (1) phosphate demethylation in neat trimethylamine at 45°C (24 h), (2) removal of benzyl ester group by hydrogenolysis over 10% Pd/C under atmospheric pressure (12 h), and (3) cleavage of MOM groups with ethanethiol and boron trifluoride etherate as a catalyst at 23°C (8 h) to give the final PI-3-P (**1**).[14] Synthesis of PI-3,4-P_2 (**2**)[15] and PI-3,4,5-P_3 (**3**)[16] was accomplished analogously starting from the diol **14** and the triol **9**, respectively. The final products were purified as ammonium salts by acetone precipitation from methanol-chloroform solutions. Alternatively, synthesis of PI-3,4-P_2 (**2**) can be also achieved staring from the enantiomer of the diol **16**. This precursor could be obtained analogously as shown in Scheme 4 starting from the readily available enantiomer of the tetrol **5**.

Scheme 5



8 17 18 19

R^1 = MOM, R^2 = -P(O)$(OBn)_2$; i: (a) $(BnO)_2$P-$NiPr_2$/tetrazole, (b) MCPBA; ii: $Bu_4N^+F^-$; iii: (a) Cl-P(OMe)$NiPr_2$/$EtiPr_2N$, (b) 1,2-dipalmitoylglycerol/tetrazole, (c) MCPBA; iv: (a) Me_3N, (b) H_2/Pd, (c) EtSH/BF_3-Et_2O

The synthetic approach described here takes advantage of different nucleophilic reactivity of inositol hydroxyl groups. The origin of these differences in unprotected inositol is unclear,[17] however, the enhancement of regioselectivity observed in benzoylation reactions of **4** and **5** could be explained by a steric hindrance imposed on the hydroxyl groups at the 2- and 6-positions in the pentol **4**, and at the 2- and 5-positions in the tetrol **5** by the bulky protective groups. Thus, with limiting amount of benzoyl chloride, none or very little benzoylation was observed at positions adjacent to these protective groups in **4** and **5**. The approach to 3-phosphorylated inositol phospholipids described here offers several advantages: (i) it starts with substrates readily available from inositol in

only 3 steps;[7] (ii) syntheses of **1-3** are accomplished using analogous pathways and the same reagents; (iii) all 3-phosphorylated phosphatidylinositols are synthesized in the enantiomerically pure form.

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14. PI-3-P (ammonium salt): ^{1}H NMR (500 MHz, CD_3OD-$CDCl_3$, 2:1) δ 5.27 (m, 1H), 4.46 (dd, J 3.0, 11.9 Hz, 1H), 4.36 (tr, J 2.7 Hz, 1H), 4.20 (dd, J 6.9, 12.2 Hz, 1H), 4.07 (m, 2H), 3.95 (ddd, J 2.5, 8.0, 10.0 Hz, 1H), 3.91 (ddd, J 2.5, 7.5, 10.0 Hz, 1H), 3.84 (tr, J 9.4 Hz, 1H), 3.82 (tr, J 9.4 Hz, 1H), 3.30 (tr, J 9.4 Hz, 1H), 2.34 (tr, J 7.4 Hz, 2H), 2.31 (tr, J 7.1 Hz, 1H), 1.60 (m, 4H), 1.28 (m, 48H), 0.89 (tr, 6H). ^{31}P NMR ($CDCl_3$-CH_3OH-H_2O, 1:1:0.3) δ 0.3, -1.47 ppm. LC ESMS (negative ions): m/z 889 (M-H).
15. PI-3,4-P_2 (ammonium salt): ^{1}H NMR (CD_3OD-$CDCl_3$-D_2O, 1:1:0.3) δ 5.29 (m, 1H), 4.45 (dd, J 2.5, 12.3 Hz, 1H), 4.38 (tr, J 2.5 Hz, 1H), 4.26 (brq, J 9.5 Hz, 1H), 4.22 (dd, J 7.6, 12.0 Hz, 1H), 4.06 (m, 3H), 3.97 (m, 1H), 3.82 (tr, J 9.5 Hz, 1H), 3.49 (tr, J 9.5 Hz, 1H), 2.36 (tr, J 7.3 Hz, 2H), 2.32 (m, 2H), 1.61 (m, 4H), 1.28 (m, 48 H), 0.90 (tr, 6H). ^{31}P NMR ($CDCl_3$-CH_3OH-H_2O, 1:1:0.3) δ 2.8, 1.7, -0.1 ppm. LC ESMS: m/z 969 (M-H).
16. PI-3,4,5-P_3 (ammonium salt): ^{1}H NMR (CD_3OD-$CDCl_3$-D_2O, 3:2:1) δ 5.30 (m, 1H), 4.46 (dd, J 2.6, 11.9 Hz, 1H), 4.43 (tr, J 9.5 Hz, 1H), 4.39 (tr, J 2.7 Hz, 1H), 4.22 (dd, J 7.9, 12.3 Hz, 1H), 4.13 (dtr, J 2.6, 9.8 Hz, 1H), 4.10-4.02 (m, 4H), 3.93 (tr, J 9.5 Hz, 1H), 2.37 (tr, J 7.4 Hz, 2H), 2.33 (brtr, J 7.9 Hz, 2H), 1.61 (m, 4H), 1.29 (m, 48H), 0.89 (tr, J 6.8 Hz, 1H). ^{31}P NMR ($CDCl_3$-CH_3OH-H_2O, 1:1:0.3) δ 0.55, 0.38, -0.3, -1.67 ppm. LC ESMS m/z 1049 (M-H).
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