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IMPROVED SYNTHESES OF INOSITOL PHOSPHOLIPID ANALOGUES

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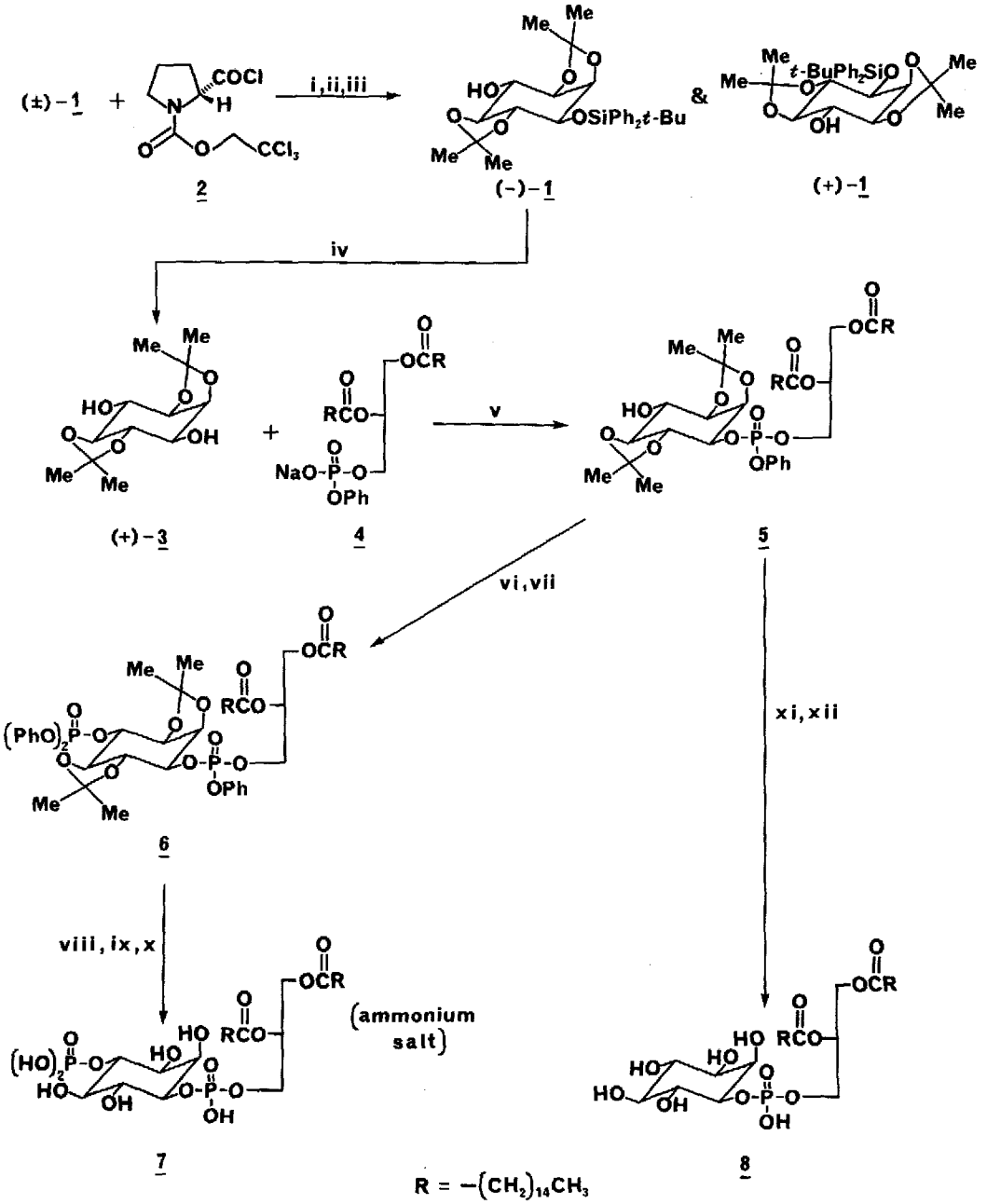
Summary: Short and convenient syntheses of PtdIns 4P and PtdIns analogues based on selective phosphorylation of (+)-2,3:5,6-di-O-isopropylidene-myo-inositol are presented.

Continued interest in the metabolism of myo-inositol phospholipids has led to numerous investigations concerning the synthesis of phospholipids and polyphosphates derived from myo-inositol.[1] For our work in this field we required chemically and optically pure well characterised samples of the phosphatidylinositol (PtdIns) analogue 8 and the analogue of phosphatidylinositol 4-phosphate (PtdIns 4P) 7.

The observation that the racemic diacetonide (±)-3 may be selectively silylated[2] at the 1(3)-OH group led us to conjecture that a similar selective phosphorylation of this racemate or, by implication, of either of the pure enantiomers would be possible. Syntheses of the optically pure PtdIns 4P analogue 7 and the PtdIns analogue 8 will be reported, in which such a selective phosphorylation is a key step.

As in all syntheses of optically pure inositol phosphate derivatives, it was first necessary to resolve a suitable inositol precursor. In our previous synthesis[1b] of the PtdIns analogue 8 (Na salt) we described the resolution of the silyl ether (±)-1 by esterification with (-)-camphanic acid chloride, followed by chromatographic separation of the diastereoisomeric esters so produced. This separation was however difficult and tedious. Subsequently we have found that the corresponding diastereoisomers formed by reaction with the (R)-proline derivative 2[3] may be separated by silica gel chromatography with much greater facility.[4] The less polar of these diastereoisomers was saponified with alcoholic potassium hydroxide and the absolute stereochemistry of the

Scheme



Reagents: i, pyridine; ii, chromatographic separation[4]; iii, KOH, EtOH; iv, TBAF, THF; v, MSNT, pyridine; vi, $(PhO)_2PNEt_2$ tetrazole, CH_2Cl_2; vii, Et_3N, ^tBuOOH; viii, H_2, PtO_2, EtOH-EtOAC (83:17); ix, EtOH, EtOAc, H_2O (70:15:15) 25°C→40°C; x, 25% NH_3 aq.; xi, H_2, PtO_2, EtOH; xii, EtOH, H_2O (70:30).

product demonstrated to correspond to the structure (-)-1, as shown in the scheme, by correlation of its optical rotation with that of a sample of (-)-1 prepared by alkaline cleavage of its optically pure camphanate ester,[5] the structure of which had been determined by single crystal X-ray analysis.[6] Treatment of (-)-1 with tetrabutylammonium fluoride (TBAF) in THF afforded the enantiomerically pure diacetonide (+)-3.[7]

This compound was selectively phosphorylated at the 1-OH group by treatment with the sodium salt of 1,2-di-O-palmitoyl-sn-glycer-3-yl phenylphosphate (4)[1b] (1.0 equiv.) and 1-(mesitylene-2-sulphonyl)-3-nitro-1,2,4-triazole (MSNT; 3.0 equiv.)[8] in dry pyridine. After work-up and flash chromatography on silica gel to remove higher R_f impurities a 60% yield of the phosphotriester 5[9] was obtained. Three inositol containing components were detected in the higher R_f fractions. On the basis of its nmr spectrum the major component was shown to be 2,3:5,6-di-O-isopropylidene-D-myo-inositol-1-O-mesitylenesulphonate (ca 20% yield). The minor components were tentatively identified, again on the basis of nmr, as the 4-phosphorylated counterpart of 5 (ca 5% yield), and the 1,4-bis phosphorylated compound (<1% yield). Phosphitylation of the free 4-OH group was accomplished by reaction with diphenyl N,N-diethylphosphoramidite[10] (ca. 5.8 equiv.) in CH_2Cl_2 in the presence of tetrazole (3.9 equiv.) and the resulting phosphite oxidized in situ with excess t-butyl hydroperoxide, triethylamine having been added to buffer the reaction mixture. Standard extractive work-up (CH_2Cl_2/H_2O) followed by flash chromatography on silica gel gave compound 6[11] in 73% yield.

Deprotection was effected by platinum oxide catalysed hydrogenolysis of the phenyl groups, the acetonides being then hydrolysed by allowing the acidic compound thus produced to stand in solution in ethanol-ethyl acetate-water (70:15:15) overnight at 25°C and then at 40°C for 3.5 hours. Treatment of the solution with 25% aqueous ammonia and subsequent freeze drying gave a 47% yield of the PtdIns 4P analogue 7 as a white solid.[12]

Platinum oxide catalysed hydrogenolysis of the phosphotriester 5 in ethanol, followed by hydrolysis in ethanol-water (70:30) for 3 days at 25°C and then at 40°C for 10 hours gave, after concentration, a 40% yield of the PtdIns analogue 8[13] as a white solid which was collected by centrifugation. This shortened route to compound 8 obviates the need for an additional acid-labile protecting group at the 4-OH group.[1b]

Notes and References

1. a, D.C. Billington, Chem. Soc. Rev., 1989, 18, 83; b, J.G. Ward and R.C. Young, Tetrahedron Lett., 1988, 29, 6013; c, C.E. Dreef, C.J.J. Elie, P. Hoogerhout, G.A. van der Marel and J.H. van Boom, Tetrahedron Lett., 1988, 29, 6513.

2. This selective silylation was first observed in the course of work carried out by Dr. Martyn Voyle and Ms. Lucy Hyatt, SK&F Welwyn. Experimental details will be described by these authors elsewhere.

3. Our attention was drawn to this resolving agent by Dr. W. Bondinell (SK&F, King of Prussia, Philadelphia) who had used the corresponding (S)-enantiomer[14] to resolve some unrelated racemic alcohols.

4. The separation was carried out on preparative scale using a Jobin-Yvon Autoprep-100 preparative liquid chromatography system having a silica (Merck Kieselgel 60; 15-40μm) column (80 x 1000mm) with stepwise gradient elution from 5% to 9% isopropyl acetate in cyclohexane over ca. 18hr. and a uniform flow rate of 60 ml min^{-1}.

5. A sample of (-)-1 prepared from its N-(2,2,2-trichloroethoxycarbonyl)-(R)-prolyl ester had $[\alpha]_D^{20}$ = -2.5° (C 2.02, in CH_3CN). A sample prepared from the corresponding (-)-camphanate ester[6] had $[\alpha]_D^{20}$ = -2.5° (C 2.01, in CH_3CN).

6. The single crystal X-ray structure of this camphanate ester was determined by Dr. D. Eggleston (SK&F, King of Prussia, Philadelphia) and will be published with a full description of our earlier work: J. Med. Chem. in press.

7. The diacetonide (+)-3 had: m.p. 159-61°C, $[\alpha]_D^{20}$ = +22.0° (C 1.08, in CH_3CN).

8. S.S. Jones, B. Rayner, C.B. Reese, A. Ubasawa and M. Ubasawa, Tetrahedron, 1980, 36, 3075.

9. For compound 5, a mixture of two diasteroisomers by virtue of the chiral phosphorus atom: δ_H (360 MHz; DMSO-d_6) 0.85 (6H, t), 1.17-1.44 (60H, m), 1.51 (4H, m), 2.25 (4H, m), 3.43 (1H, m) 3.63 (1H, m), 3.85 (1H, m), 3.99 (1H, m), 4.14 (1H, m), 4.28 (3H, m), 4.39 and 4.47 (1H, 2xdd, in both cases J≈4.7, 4.7 Hz), 4.90 (1H, m), 5.20 (1H, m), 5.43 and 5.44 (1H, 2xd, in both cases J≈5.2 Hz, OH), 7.19-7.25 (3H, m), 7.35-7.40 (2H, m). δ_{31P} (145.8 MHz; DMSO-d_6) -6.21, -6.65.

10. This reagent was prepared by treating N,N-diethylphosphoramidous dichloride with 2 equivalents of phenol and 2.1 equivalents of triethylamine in dry ether at 0°C. After filtration to remove the solid $Et_3NH^+Cl^-$ the solution was evaporated to a pale yellow oil which was used for phosphitylation without further purification.

11. For compound 6, a mixture of two diasteroisomers by virtue of the chiral phosphorus atom: δ_H (250 MHz; DMSO-d_6) 0.85 (6H, t), 1.23-1.47 (60H, m), 1.50 (4H, m), 2.24 (4H, m), 3.84 (1H, m), 4.02 (1H, m), 4.14 (1H, m), 4.25-4.39 (4H, m), 4.46 and 4.55 (1H, 2xdd, in both cases J≈4.6, 4.6 Hz), 4.69 (1H, m), 5.00 (1H, m), 5.20 (1H, m), 7.19-7.25 (3H, m), 7.35-7.43 (2H, m). δ_{31P} (145.8 MHz; DMSO-d_6) -6.45, -6.85, -11.86.

12. For compound 7: δ_H (360 MHz; DMSO-d_6; recorded using inversion recovery to suppress the H_2O peak) 0.85 (6H, t), 1.24 (48H, m), 1.51 (4H, m), 2.26 (4H, m), 3.26 (1H, dd, J≈3.4, 9.5 Hz), 3.58 (1H, m), 3.64 (1H, dd (pseudo triplet), J≈9.0, 9.0 Hz), 3.70-3.87 (3H, m), 3.99 (1H, m), 4.10 (1H, dd, J≈7.1, 12.1 Hz), 4.30 (1H, dd, J≈3.1, 12.0 Hz), 5.06 (1H, m), one of the inositol ring proton signals is obscured by the residual H_2O peak which could not be fully suppressed. δ_{31P} (145.8 MHz; DMSO-d_6) 2.40, 3.33.

13. For compound 8: δ_H (360MHz; DMSO-d_6; recorded using inversion recovery to suppress the H_2O peak) 0.86 (6H, t) 1.25 (48H, m), 1.51 (4H, m), 2.27 (4H, m), 2.97 (1H, dd, (pseudo triplet), J≈8.9, 8.9 Hz) 3.15 (1H, dd, J≈2.5, 9.5 Hz), 3.40 (1H, dd, (pseudo triplet) J≈9.3, 9.3 Hz), 3.60 (1H, dd (pseudo triplet), J≈9.4, 9.4 Hz), 3.75 (1H, m), 3.97 (3H, m), 4.11 (1H, dd, J≈6.9, 12.0 Hz), 4.29 (1H, dd, J≈3.2, 12.0 Hz), 5.11 (1H, m). δ_{31P} (145.8 MHz; DMSO-d_6) 18.69.

14. S.A. Boyd and W.J. Thompson, J. Org. Chem., 1987, 52, 1790.

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