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SYNTHESIS OF 1,2-DIPALMITOYL-sn-GLYCER-3-YL-D-myo-INOSITOL 1-PHOSPHATE.

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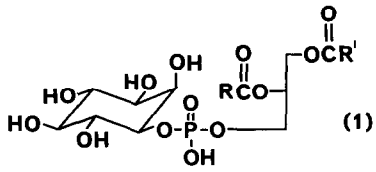
Summary: A convenient route for the synthesis of pure 1,2-dipalmitoyl-sn-glycer-3-yl-D-myo-inositol 1-phosphate is presented.

Phosphatidylinositol (1a), which is one of a number of phospholipids found in the membranes of animal cells, is a key intermediate for the production of the intracellular second messengers, inositol 1,4,5-trisphosphate and diacylglycerol.[1]



(1)

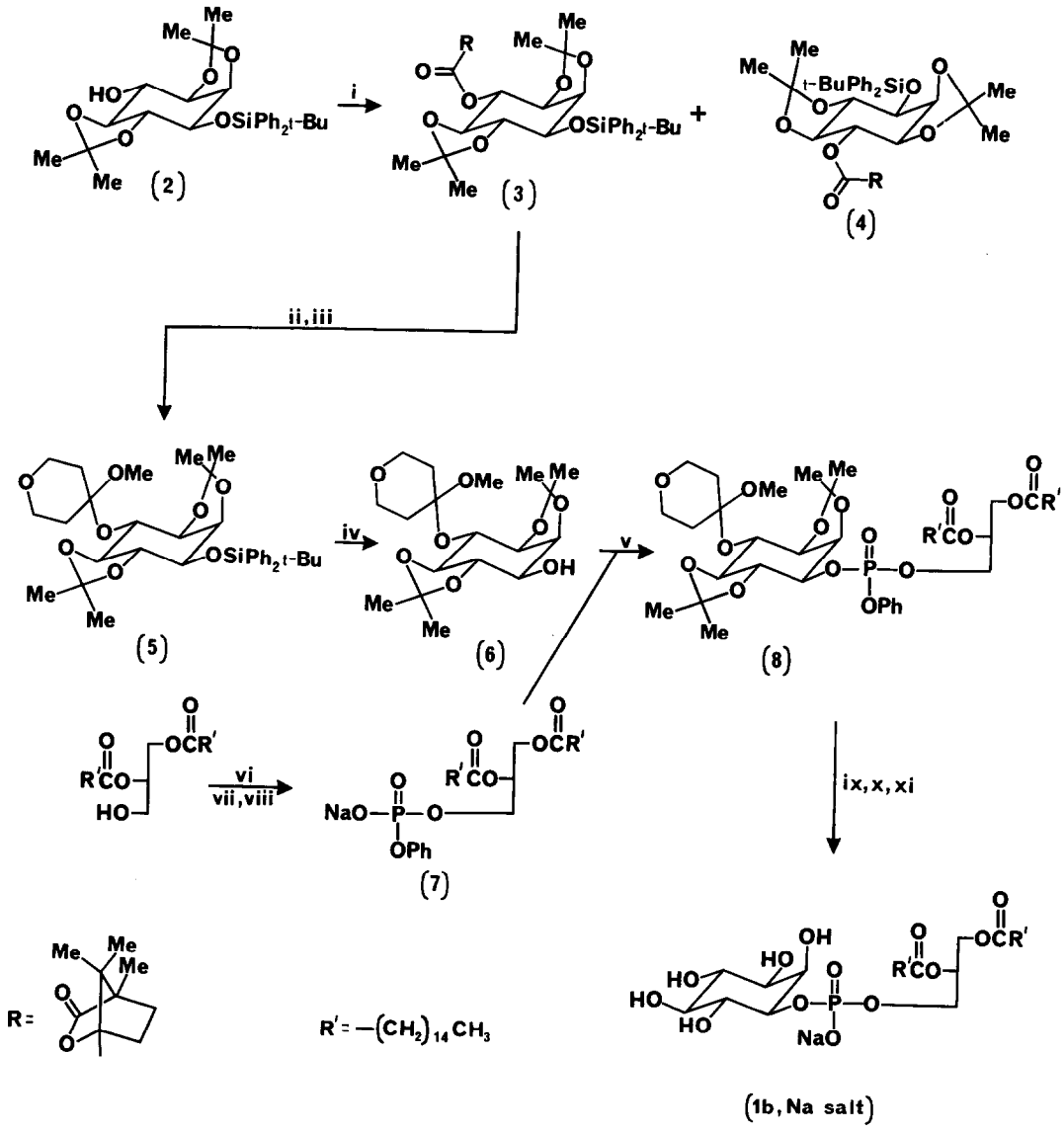
a) R= $-(CH_2)_2(CH_2CH=CH)_4(CH_2)_4CH_3$
R'= $-(CH_2)_{16}CH_3$

b) R=R'= $-(CH_2)_{14}CH_3$

Sequential phosphorylation of (1a) at the inositol 4- and 5- positions by specific kinases generates phosphatidylinositol 4,5-bisphosphate, which is cleaved by a receptor-coupled phospholipase C on stimulation by numerous neurotransmitters, hormones and growth factors.[2] In addition, (1a) is a substrate for other enzymes, including phospholipases A and C, and a recently discovered 3-kinase.[3] The relative importance of these different enzymes in controlling the production of second messengers is, thus far, unclear, and more detailed studies utilizing chemically well-defined phospholipids are needed.

Various routes for the synthesis of phosphatidylinositol analogues have appeared over the last twenty years, and these were reviewed in 1980.[4] Although synthetic, optically active analogues have been reported, many of the stages in their synthesis have been hampered by low yields and lack of reproducibility. Moreover, rigorous high-field NMR analysis which is essential for establishing their identity and purity is lacking.

Here we report a convenient and reproducible method for the preparation of 1,2-dipalmitoyl-sn-glycer-3-yl-D-myo-inositol 1-phosphate (1b) as the sodium salt. Our synthetic strategy required a suitably protected and resolved inositol building block and as starting material we chose the racemic 1(3)-silyl inositol diacetonide (±)-(2).[5] The optical resolution of this racemate was effected by esterification with (-)- camphanic acid chloride,[6] to give a 97 % yield of a mixture of diastereoisomers (3) and (4) which were separated by HPLC on silica gel (15-25 μm); 7 % THF in hexane.



Reagents: i, (-)-camphanic acid choride, pyridine, dioxan; ii, KOH, EtOH; iii, 5,6-dihydro-4-methoxy-2H-pyran, pyridinium p-toluenesulphonate, CH_2Cl_2; iv, TBAF, THF; v, MSNT, pyridine; vi, $PhOP(O)Cl_2$, pyridine; vii, H_2O; viii, amberlite IRC-50 (Na form), EtOH aq.; ix, H_2, PtO_2, EtOH; x, H_2O, EtOH; xi, amberlite IRC-50 (Na form), EtOH aq.

The absolute stereochemistry of the less polar isomer (3) was determined by single crystal X-ray analysis and is as shown in the scheme.[7] Basic hydrolysis of (3) in alcoholic potassium hydroxide gave the optically pure alcohol (-)-(2) in 94 % yield.[8] The 4-OH group was then protected[9] as its 4-methoxytetrahydropyran-4-yl derivative (5) in 87 % yield.[10] Subsequent desilylation by treatment with tetrabutylammonium fluoride in THF gave the building block (6) in 85 % yield.

Condensation of the alcohol (6) with 1,2-dipalmitoyl-sn-glycer-3-yl phenylphosphate sodium salt[12] (7) in the presence of 1-(mesitylene-2-sulphonyl)-3- nitro-1,2,4-triazole (MSNT)[13] gave (8) in 80 % yield.[14] Sequential deprotection of (8) firstly by hydrogenolysis of the phenyl group, and then hydrolysis in aqueous ethanol of the acid labile protecting groups[15] gave the phosphatidylinositol (1b), which was obtained as a white solid by concentration and centrifugation. The sodium salt was obtained in 48 % overall yield by treatment of a solution of (1b) in aqueous ethanol with Amberlite IRC-50 (Na form). Again, concentration and centrifugation gave the product as a white solid.[16]

References and Notes.

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2. Downes, C.P. and Michell, R.H., 1985, in Molecular Mechanisms of Transmembrane Signalling, Cohen, P. and Houslay, M. eds. p 3-56, Elsevier, North Holland.
3. Whitman, M., Downes, C.P., Keeler, M., Keller, T. and Cautley, L., Nature, 1988, 332, 644-646.
4. Gigg, R., Chem. Phys. Lipids, 1980, 26, 287-404.
5. The silyl inositol (±)-2: m.p. 142-5° C, was obtained in 60 % yield by selective silylation of 2,3(1): 5,6(4) di-O-isopropylidene-myo-inositol.[17] We thank Dr Martyn Voyle and Ms Lucy Hyatt, SK&F Welwyn, for supplying a sample of this compound. Its preparation will be described by these authors elsewhere.
6. Billington, D.C., Baker, R., Kulagowski, J.J. and Mawer, I.M., J. Chem. Soc. Chem. Commun. 1987, 314.
7. The following data were obtained for compound (3): m.p. 179-80 °C, $[\alpha]_D^{20}$ = +4.4° (c 1.54, in CH_3CN), found: C, 67.11; H, 7.46. $C_{38}H_{50}O_9Si$ requires: C, 67.23; H, 7.42 %, δ_H (250 MHz; $CDCl_3$) 0.93 (3H, s), 1.01 (3H, s), 1.08 (9H, s), 1.09 (3H, s), 1.27 (3H, s), 1.34 (3H, s), 1.46 (3H, s), 1.60 (3H, s), 1.65 (1H, m), 1.89 (1H, m), 2.03 (1H, m), 2.43 (1H, m), 3.24 (1H, dd, J = 9.2, 11.1 Hz), 3.87 - 4.02 (3H, m), 4.15 (1H, dd, J = 9.5, 9.5 Hz), 5.33 (1H, dd, J = 6.4, 11.1 Hz), 7.34 - 7.47 (6H, m), 7.78 - 7.84 (4H, m).
8. The following data were obtained for the compound (-) - (2): m.p. 127-8 °C, $[\alpha]_D^{20}$ = -2.5 ° (c 2.02, in CH_3CN); found: C, 67.24; H, 7.66. $C_{28}H_{38}O_6Si$ requires: C, 67.44; H, 7.68 %, δ_H (250 MHz, $CDCl_3$) 1.07 (9H,

s), 1.27 (3H, s), 1.42 (3H, s), 1.49 (3H, s), 1.55 (3H, s), 2.35 (1H, d, J=2.9 Hz), 3.13 (1H, m), 3.72 (1H, dd, J=4.8, 6.6 Hz), 3.82-3.94 (2H, m), 3.98-4.10 (2H, m), 7.33-7.47 (6H, m), 7.78-7.86 (4H, m).

9. Reese, C.B., Saffhill, R. and Sulston, J.E., Tetrahedron, 1970, 26, 1023.
10. For (5): δ_H (200 MHz, $CDCl_3$) 1.07 (9H, s), 1.26 (3H, s), 1.36 (3H, s), 1.42 (3H, s), 1.58 (3H, s), 1.65-2.00 (4H, m), 3.11 (1H, dd, J=9.2, 10.5 Hz), 3.27 (3H, s), 3.55-3.85 (5H, m), 3.85-4.10 (4H, m), 7.30-7.50 (6H, m), 7.75-7.90 (4H, m).
11. The following data were obtained for compound (6): m.p. 122-22.5 °C, $[\alpha]_D^{20}$ = +34.9 ° (c 0.53 in CH_3CN); δ_H (250 MHz, $CDCl_3$) 1.35 (3H, s), 1.41 (3H, s), 1.43 (3H, s), 1.53 (3H, s), 1.70-2.00 (4H, m), 2.41 (1H, d, J=8.7 Hz), 3.30 (3H, s), 3.33 (1H, dd, J=9.5, 10.4 Hz) 3.59-3.80 (3H, s), 3.33 (1H, dd, J=9.5, 9.5 Hz) 3.93-4.05 (2H, m), 4.13 (1H, dd, J=5.6, 5.6 Hz), 4.46 (1H, dd, J=4.9, 4.9 Hz). FAB-MS: m/z = 375 $(M+H)^+$.
12. Prepared by phosphorylation of 1,2-dipalmitoyl-sn-glycerol with phenyl phosphorodichloridate followed by aqueous hydrolysis and treatment with Amberlite IRC-50 (Na form). The following data were obtained for (7): m.p. begins to soften at 65 °C, liquifies over a large range, $[\alpha]_D^{20}$ = +4.6° (c 2.0, $CHCl_3$); found: C, 65.64, H, 9.61. $C_{41}H_{72}NaPO_8$ requires: C, 65.92; H, 9.72%, δ_H (250 MHz; DMSO-d_6/CD_2Cl_2; 50:50) 0.87 (6H, t, J=6.3 Hz), 1.25 (48H, br.s), 1.51 (4H, m), 2.21 (4H, m), 3.88 (2H, m), 4.07 (1H, dd, J = 6.8, 11.9 Hz), 4.27 (1H, dd, J = 3.2, 11.9 Hz), 5.09 (1H, m), 6.89-6.96 (1H, m), 7.13 - 7.23 (4H, m).
13. Jones, S.S., Rayner, B., Reese, C.B., Ubasawa, A. and Ubasawa, M., Tetrahedron, 1980, 36, 3075.
14. For (8), a mixture of two diastereoisomers by virtue of the chiral phosphorus atom; δ_H (250 MHz, CD_2Cl_2) inter alia 0.88 (6H, t, J = 6.3 Hz), 1.68-1.83 (4H, m), 2.28 (4H, m), 3.25 and 3.26 (3H, 2xs), 3.36 (1H, dd, J = 10.2, 10.2 Hz), 3.48-3.63 (2H, m), 3.64-3.75 (2H, m), 3.98-4.20 (4H, m), 4.26-4.35 (3H, m), 4.43 and 4.58 (1H, 2xdd, in both cases J=4.5, 4.5 Hz), 4.67-4.82 (1H, m), 5.22 (1H, m), 7.18-7.38 (5H, m).
15. The inherent acidity of the intermediate phosphodiester was sufficient to effect this deprotection.
16. The following data were obtained for the sodium salt of (1b): m.p. 214-7 °C dec. (begins to soften at ~125 °C), δ_H (360 MHz; 72 °C; DMSO-d_6) inter alia (some signals obliterated by the water peak) 0.85 (6H, t, J=6.6 Hz), 1.24, (48H, br. s), 1.52 (4H, br. m), 2.26 (4H, m), 2.93 (1H, dd, J=8.8, 8.8 Hz), 3.39 (1H, dd, J=9.1, 9.1 Hz), 3.56 (1H, m), 3.62 (1H, dd, J=9.2, 9.2 Hz), 3.75-3.90 (3H, m), 3.95 (1H, br. s), 4.10 (1H, dd, J=7.1 and 12 Hz) 4.17 (2H, br. s); 4.30 (1H, dd, J=3.1 and 12 Hz) 4.94 (1H, br.s), 5.08 (1H, m). δ_{31P} (360 MHz, DMSO-d_6) 2.30.
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