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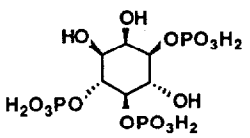
AN EFFICIENT SYNTHESIS OF OPTICALLY ACTIVE D-MYO-INOSITOL 1,4,5-TRIPHOSPHATE

Yeuk-Chuen Liu and Ching-Shih Chen*

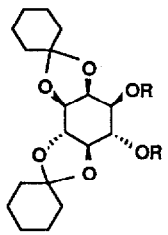
College of Pharmacy, University of Rhode Island
Kingston, RI 02881, USA

Summary: An effective synthesis of D-myo-inositol 1,4,5-triphosphate was developed using a chiral inositide precursor which can be prepared via a facile enzymatic or chemical method.

Recently, much attention has been focused on the metabolic cascade of membrane phosphoinositides, which regulates intracellular Ca^{+2} mobilization in response to a variety of extracellular signals[1]. This complex information-transducing process is initiated by a receptor-mediated breakdown of phosphatidyl inositol 4,5-bisphosphate with the formation of D-myo-inositol 1,4,5-triphosphate [Ins(1,4,5)P_3] (1) and 1,2-diacylglycerol, both of which function as second messengers eliciting specific intracellular responses. It has also become clear that Ins(1,4,5)P_3 is implicated in the generation of two other biologically active inositol polyphosphates[1], Ins(1,3,4)P_3 and Ins(1,3,4,5)P_4, whose in vivo functions still remain unclear. As part of our interest in exploring this intriguing signal-transducing mechanism, we have developed an effective synthetic procedure which allowed the preparation of optically pure 1 in multi-mmol quantities.

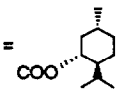


1



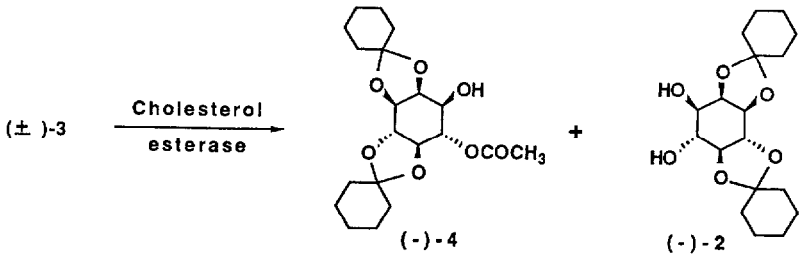
2 R = H

3 R = $COCH_3$

5 R = 

In the past[2], one general problem that has impeded the preparation of enantiomeric 1 in quantities was the optical resolution of inositide intermediates. Cumbersome procedures entailing chiral resolving agents[2] or a chiral HPLC column[3] had to be adopted in the synthesis to separate individual enantiomers in small quantities. Herein, we report a facile synthetic route to optically active 1 using a chiral precursor, (+)-1,2:5,6-di-O-cyclohexylidene-myo-inositol ((+)-2) which can be easily prepared via a facile enzymatic or chemical method.

The diacetate of (±)-2[4] ((±)-3) was readily digested by a number of hydrolytic enzymes. Of these, cholesterol esterase (Sigma, from bovine pancreas) exhibited high regio- and enantio-preference in removing the acetate functions. In a typical experiment, 1 g of (±)-3, dissolved in 2

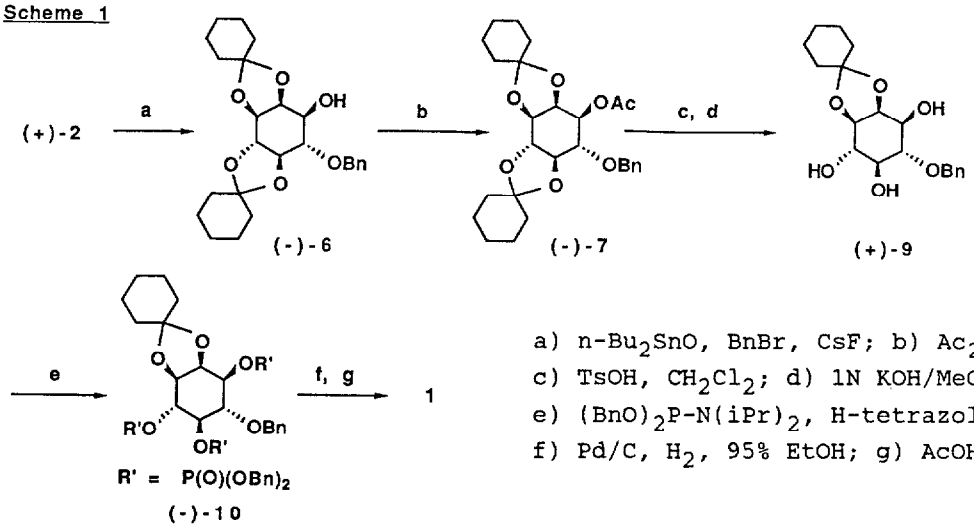


ml of DMF, was finely suspended in 80 ml of phosphate buffer (250 mM, pH 7.5) containing 0.5% Tween 80 and 120 units of cholesterol esterase. The suspension was incubated at 28°C on a rotary shaker (250 rpm) for 168 hr, which yielded a mixture of (-)-2 (412 mg; 85% ee[5]; $[\alpha]_D^{20}$ = -15.3, c = 1.5, $CHCl_3$). (-)-4 (345 mg; 86% ee[5]; $[\alpha]_D^{20}$ = -8.4, c = 1.2, $CHCl_3$), and a small amount of 3-acetyl-1,2:5,6-di-O-cyclohexylidene myo-inositol (30 mg, optical purity not determined). Alkaline hydrolysis of the acetate of (-)-4 (10 equiv. of 1N KOH/MeOH, 25°C, 1h) afforded (+)-2 which was subsequently recrystallized from ether-hexane (1:3) to give the diol with optical purity greater than 98% ($[\alpha]_D^{20}$ = +18.4, c = 1.0, $CHCl_3$; recovery: 280 mg; combined yield: 70%).

Alternatively, the racemic mixture, (±)-2, could also be resolved chemically after being converted to the corresponding di-l-menthyl carbonates[6] (5a and 5b)[7]. The two diastereomers exhibited quite different chromatographic mobility (silica gel TLC (0.25 mm); ether/hexane, 1:3; R_f: 5a, 0.87; 5b, 0.74), and thus were readily separated by SiO_2 chromatography (ether/hexane, 1:20 to 1:15). Alkaline hydrolysis of the carbonates of 5a and 5b gave optically active diols (-)-2 (>98% ee; $[\alpha]_D^{20}$ = -18°, c = 1.0, $CHCl_3$), and (+)-2 (>98% ee; $[\alpha]_D^{20}$ = +18.4°, c = 1.0, $CHCl_3$), respectively, in nearly quantitative yields. Retrospectively, optically pure 2 may serve as a versatile precursor to various inositol phosphates, which can be illustrated by the efficient synthesis of Ins(1,4,5)P_3 (Scheme 1).

(+)-2 (2.54 mmol) underwent selective partial benzylation by reacting with di-n-butyltin oxide (2.8 mmol), followed by benzyl bromide (3.8 mmol) in the presence of cesium fluoride[8] (6.4 mmol) to afford (-)-6[9] ($[\alpha]_D^{20}$ = -4.2°,c = 1.0, $CHCl_3$) in 91% yield. To prevent the migration of the

Scheme 1



a) n-Bu_2SnO, BnBr, CsF; b) Ac_2O, DMAP;
c) TsOH, CH_2Cl_2; d) 1N KOH/MeOH;
e) $(BnO)_2P$-N(iPr)$_2$, H-tetrazole, MCPBA
f) Pd/C, H_2, 95% EtOH; g) AcOH

cis-ketal from C-2,3 to C-1,2, which accompanied the direct selective hydrolysis of the trans-cyclohexylidene of **(-)-6**, the compound was first converted to **(-)-7**[10] ($[\alpha]_D^{20}$ = -14°, c = 1, $CHCl_3$) in quantitative yield. The fully protected inositol (2 mmol) was treated with a catalytic amount of p-toluenesulfonic acid (0.2 mmol) in acetone (40 ml) under reflux for 18 min. The resulting compound, without further purification, was subjected to alkaline hydrolysis to give the key intermediate **(+)-9**[11] ($[\alpha]_D^{20}$ = +20.8°, c = 1.0, $CHCl_3$; lit.[2c] $[\alpha]_D^{20}$ = +21°, c = 0.1, $CHCl_3$) in 60% total yield (based on **6**). Phosphorylation[12] of triol **(+)-9** (1.1 mmol) was achieved by the use of N,N-diisopropyl dibenzyl phosphoramidite (6.6 mmol), 1-H-tetrazole (6.6 mmol) and MCPBA (6.6 mmol) in CH_2Cl_2 to afford **(-)-10**[13] ($[\alpha]_D^{20}$= -4.3°, c = 2.42, $CHCl_3$; lit.[2c] $[\alpha]_D^{20}$= -4.2°, c = 0.1, $CHCl_3$) in 91% yield. Hydrogenolysis of the benzyl groups of **(-)-10** (0.9 mmol) (H_2, 50 psig, 10% Pd/C, 95% EtOH, 5 hr) and the subsequent acid hydrolysis of the cyclohexylidene ketal resulted in D-myo-inositol 1,4,5-triphosphate **1** (hexasodium salt; $[\alpha]_D^{20}$= -30°, c = 0.5, H_2O (pH 9.5); lit.[2c] $[\alpha]_D^{20}$= -30°, c = 0.16, H_2O (pH 9.5)) in 90% yield. Thus, enantiomeric **1** was prepared from **(+)-2** in 45% overall yield. The ^1H and ^{31}P NMR spectra of the synthetic compound **1** were identical to those recently reported[14].

It should be noted that, in addition to Ins(1,4,5)P_3, enantiomerically pure **2** also provides a facile entry into a variety of optically active phosphoinositides. Currently, syntheses of enantiomeric Ins(1,3,4)P_3 and Ins(1,3,4,5)P_4 using a similar approach are undergoing in this laboratory.

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5. The optical purity of individual inositides was determined by HPLC analysis of the corresponding diastereomeric (+)-MTPA esters using a Whatman Partisil (10μm) column (4.6 mm i.d. x 25 cm). The column was eluted with a solvent mixture of hexane-ether (3:1) at a flow rate of 1 ml/min. Retention times: **(-)-2 di-MTPA ester:** 5 min 50 sec; **(+)-2 di-MTPA ester:** 7 min 25 sec; **(+)-4 MTPA ester:** 8 min 20 sec; **(-)4 MTPA ester:** 8 min 50 sec.
6. The enantiomeric mixture can also be chromatographically separated via the corresponding diastereomeric di-L-menthoxyacetic ester, or the di-S-camphanic ester.
7. Optical rotation: **5a**: $[\alpha]_D^{20}$ = -31.8°, c = 1.0, $CHCl_3$; **5b**: $[\alpha]_D^{20}$ =-61.1°, c = 1.02, $CHCl_3$.
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9. For **(-)6**, δ_H (300 MHz; $CDCl_3$) 1.41-1.74 (2H, m), 2.6 (1H, s), 3.48-3.60 (1H, m), 3.90 (1H, t, J = 6.6 Hz), 4.04 (1H, d, J = 2.5 Hz), 4.18 (1H, dd, J = 5.4, 7.3Hz), 4.35 (1H, t, J = 7.3 Hz), 4.44 (1H, dd, J = 3.6 Hz), 4.73 (2H, ABq, J = 11.7, 34.7 Hz), 7.27-4.41 (5H, m).
10. For **(-)7**, δ_H (300 MHz, $CDCl_3$) 1.39-1.65 (2H, m), 2.09 (3H, s), 3.59 (1H, dd, J = 6.8, 9.7 Hz), 3.78 (1H, dd, J = 2.0, 5.8 Hz), 4.01 (1H, dd, J = 7.4 Hz), 4.37 (1H, pseudo t, J = 7.4 Hz), 4.51 (1H, dd, J = 3.9, 5.8 Hz), 4.76 (2H, s), 5.27 (1H, dd, J = 2.5, 5.3 Hz), 7.26-7.36 (5H, m).
11. For **(+)-9**, δ_H (300 MHz, DMSO-d_6) 1.38-1.66 (10H, m), 3.2-3.23 (1H, m), 3.3 (1H, m), 3.46-3.54 (2H, m), 3.75 (1H, m), 3.9 (1H, dd, J = 5.7, 7.4 Hz), 4.25 (1H, dd, J = 3.9, 5.3 Hz), 4.68 (1H, d, J = 4.5 Hz), 4.79 (2H, ABq, J = 10.8, 16.8 Hz), 4.85 (1H, dd, J = 4.6, 7.4 Hz), 7.23 - 7.43 (5H, m).
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13. For **(-)-10**, δ_H (300 MHz, $CDCl_3$) 1.2-1.8 (10H, m), 4.07 (1H, pseudo t, J = 8.2 Hz), 4.2 (1H, pseudo t, J = 8.2 Hz), 4.5-4.60 (3H, m), 4.65 - 5.03 (15H, m), 7.0-7.3 (35H, m).
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