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MICROBIAL OXIDATION IN SYNTHESIS: PREPARATION FROM BENZENE OF THE CELLULAR SECONDARY MESSENGER *MYO*-INOSITOL-1,4,5-TRISPHOSPHATE (IP_3) AND RELATED DERIVATIVES

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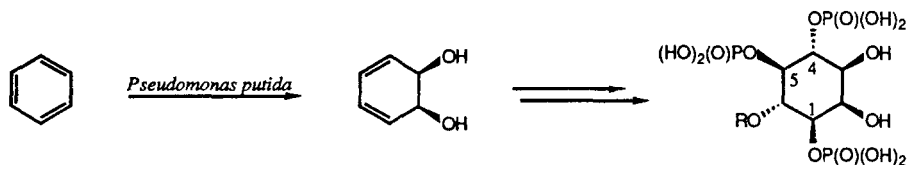
Summary: *Following microbial oxidation of benzene with Pseudomonas putida the resulting cis-1,2-dihydroxycyclohexa-3,5-diene may be converted to the cellular secondary messenger myo-inositol -1,4,5-trisphosphate (IP_3) and its 6-methyl derivative.*

The cellular secondary messenger inositol-1,4,5-trisphosphate IP_3 (1) has stimulated intense interest owing to its involvement in many cellular processes.[1] In view of its low bioavailability and the urgent need for structural analogues several synthetic studies have been reported.[2-4] However, to date these syntheses have employed the logical application of *myo*-inositol as the starting material.

We report here a conceptually different approach starting from benzene and making use of a microbial oxidation using *Pseudomonas putida*. We previously demonstrated the strategic importance of this microbial oxidation which affords *cis*-1,2-dihydroxycyclohexa-3,5-diene (2)[5] as a key intermediate during the efficient synthesis of the natural product pinitol.[6] Here we show its pivotal role in the preparation of IP_3 (1) and the 6-methyl derivative (3) (Scheme 1).



Scheme 1

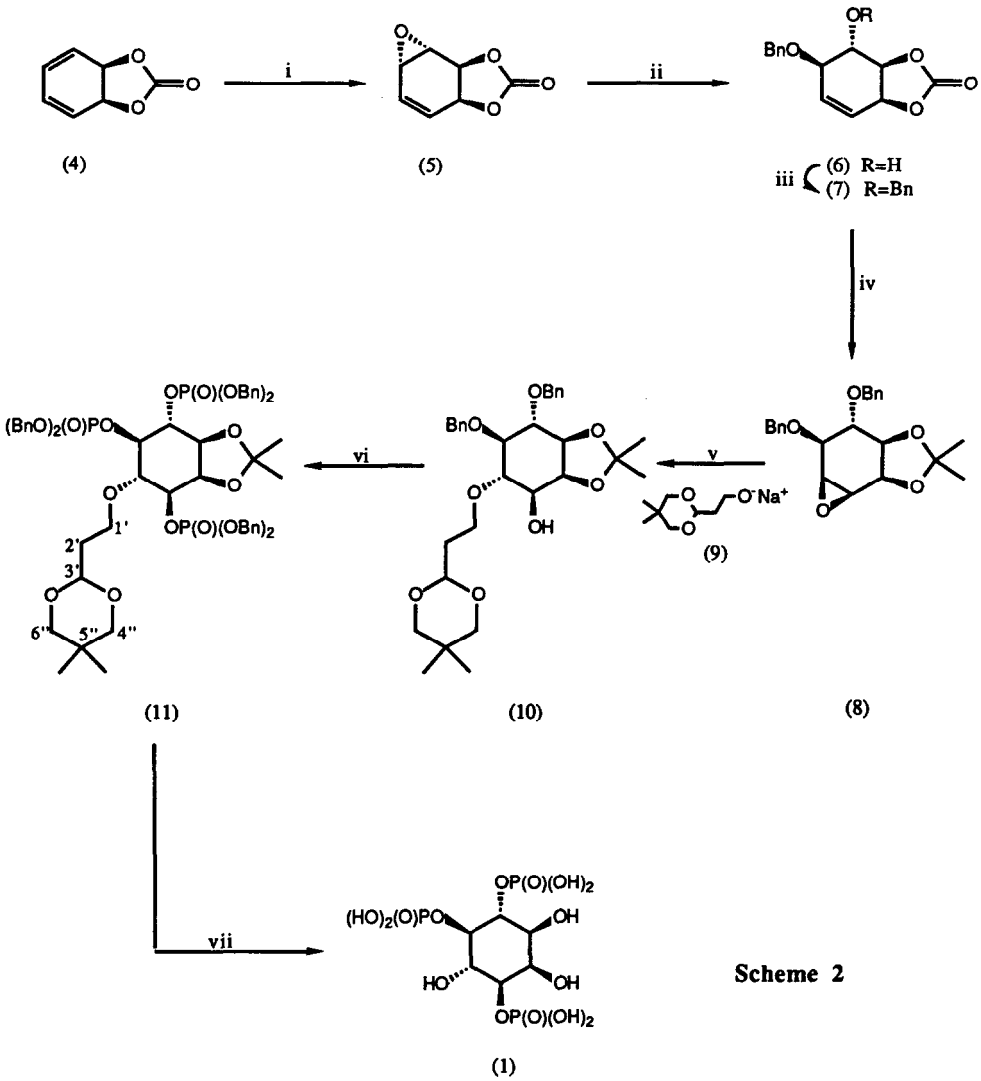
(2)

(1) R=H
(3) R=Me

The cyclic carbonate (4) formed from (2) in 65% yield by treatment with phosgene and triethylamine in dichloromethane/toluene at low temperature (-30° to RT)[7] was epoxidised with m-chloroperbenzoic acid to give the α-epoxide (5) as the major product together with the β-epoxide in a ratio of 4.6:1 in 95% combined yield. Regioselective ring opening of (5) with benzyl alcohol catalysed by camphorsulphonic acid gave (6) which upon benzylation produced the protected cyclohexene derivative (7) in excellent overall yield. In this way four of the required chiral centres were established.

In order to set up the remaining hydroxyl substituents it was necessary to effect a hydroxyl group directed epoxidation of the cyclohexene bond. Removal of the carbonate group in (7) by hydrolysis with aqueous methanol/triethylamine followed by directed epoxidation and subsequent reprotection of the *cis*-glycol functionality as the acetonide produced (8) in 72% overall yield. A small amount (8%) of the undesired α-epoxy acetonide was also produced during this sequence of reactions but could be readily removed by silica-gel chromatography.

Epoxide opening of (8) with oxygen nucleophiles in which the oxygen protection was compatible with later synthetic steps proved to be much more difficult than anticipated. Consequently we have developed the use of sodium-2β-propoxy-5,5-dimethyl-1,3-dioxane (9) as a new hydroxide equivalent.[8] This stereochemically unencumbered alkoxide was designed such that at a later stage in the synthesis acid hydrolysis would release a β-alkoxy aldehyde which would spontaneously undergo β-elimination under the acidic conditions to afford the free hydroxy group.[9] Thus opening of (8) in HMPA/THF solution at 95°C proceeded regioselectively to afford (10) as the only adduct in acceptable yield (Scheme 2). This product constitutes a key intermediate since the hydroxyl groups are now suitably differentiated to facilitate the final stages of the sythesis.

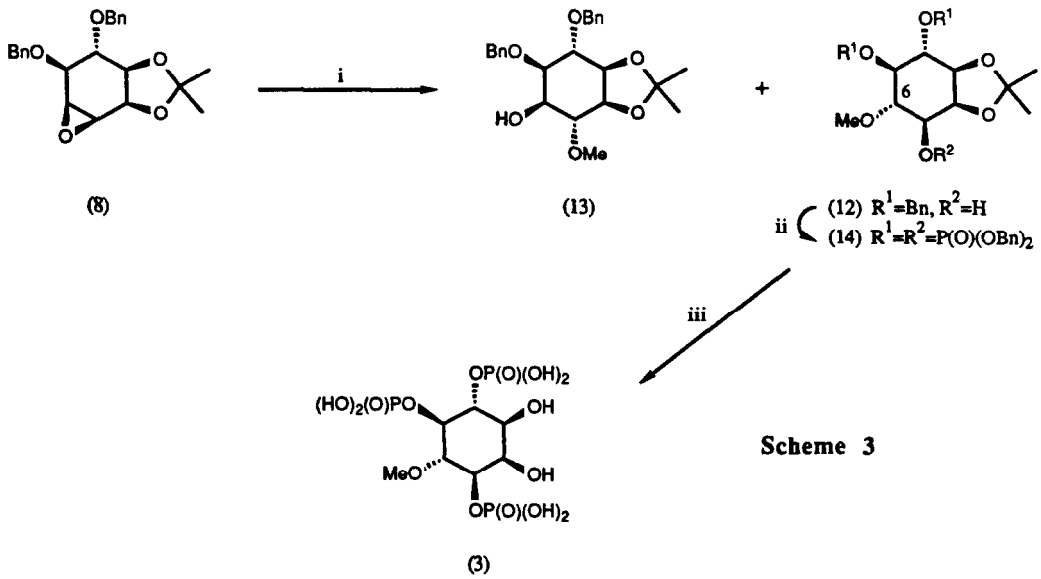


Scheme 2

i) mCPBA,DCM, 0°-rt, 95%; ii) BnOH, CSA, DCM, 85%; iii)BnBr, Ag_2O, DMF, 81%; iv)a. $Et_3N/MeOH/H_2O$; b. mCPBA, pH8, DCM; c. 2,2-dimethoxypropane, CSA, DCM, 72% overall; v) (9), HMPA/THF, 95°C, 43%; vi)a. H_2, Pd-C, EtOH; b. n-BuLi, DIPA, tetrabenzylpyrophosphate, THF, 62%overall; vii) H_2, Pd-C, EtOH then TFA-H_2O, 74%.

Debenzylation of (10) by standard methods and subsequent phosphorylation with tetrabenzylpyrophosphate[10] provided the 1,4,5-trisphosphate (11) in good overall yield.[11,12] Removal of the protecting groups in (11) was readily achieved by hydrogenolysis and treatment with moist trifluoroacetic acid to afford inositol-1,4,5-trisphosphate (1), identical with an authentic sample[13] (Scheme 2). Purification of (1) was accomplished by HPLC (Spherisorb S5SAX column, 0.2M ammonium formate buffer at pH4).

The need to prepare novel analogues of IP_3 for biological evaluation led us to exploit further the epoxy acetonide (8). Thus nucleophilic ring opening using sodium methoxide gave alcohol (12) with the required *myo*-inositol stereochemistry in 75% yield but was also accompanied by formation of some of the alternative ring opened product (13) (21%). In a similar fashion to that used for preparation of (1) compound (12) was hydrogenolysed and phosphorylated to afford (14)[14] which was then deprotected to produce the novel 6-methyl derivative of IP_3 (3).[15] (Scheme 3).



Scheme 3

i) MeO^-Na^+, MeOH, 65°C, combined yield 95%; ii)a. H_2, Pd-C, EtOH; b. n-BuLi, DIPA, tetrabenzylpyrophosphate, THF, 62% overall; iii)H_2, Pd-C, EtOH then TFA-H_2O, 45%.

We believe that the routes to inositol phosphates described above further exemplify the usefulness of the microbial arene oxidation process. Additionally we have developed a potentially very versatile sequence towards a range of novel IP_3 analogue compounds not readily available by the previously reported approaches from *myo*-inositol.

Acknowledgements

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3. For the synthesis of the only 1,4,5-IP_3 analogue reported to date see: A.M. Cooke, R. Gigg and B.V.L. Potter, *J. Chem. Soc., Chem. Commun.*, 1987, 1525.
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6. S.V. Ley, F. Sternfeld and S. Taylor, *Tetrahedron Lett.*, 1987, **28**, 225.
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8. Prepared in 76% yield from acrolein by treatment with 2,2-dimethylpropane-1,3-diol (1.5 eq), benzyl alcohol (3.3 eq) and tosic acid (1.6 mol%)[16] and subsequent catalytic hydrogenolysis.
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10. H.G. Khorana and A.R. Todd, *J. Chem. Soc.*, 1953, 2257; Y. Watanabe, H. Nakahira, M. Bunya and S. Ozaki, *Tetrahedron Lett.*, 1987, **28**, 4179.
11. All compounds reported here are racemic; our approaches to optically pure materials will be presented in a later publication.
12. ^1H NMR: δ (500 MHz, $CDCl_3$): 7.45-7.15 (30H, m, Ph), 5.11-4.96 (12H, m, PhC$\underline{H_2}$O-), 4.89 (1H, q, J = 7.9 Hz, H_4), 4.72 (1H, td, J = 8.1 and 3.2 Hz, H_1), 4.56-4.50 (2H, m, H_2 and H_5), 4.41 (1H, t, J = 5.2 Hz, $H_{3'}$), 4.24 (1H, t, J = 6.8 Hz, H_3), 3.98 (1H, dd, J = 7.2 and 4.9 Hz, H_6), 3.77 (1H, dt, J= 9.2 and 6.6 Hz, $H_{1'}$), 3.68 (1H, dt, J = 9.2 and 6.9 Hz, $H_{1'}$) 3.46 (2H, d, J = 11.2 Hz, $H_{4''}$ and $H_{6''}$), 3.30 (2H, d, J = 10.9 Hz, $H_{4''}$ and $H_{6''}$), 1.83 (2H, m, $H_{2'}$), 1.47 (3H, s, $\underline{Me}_2$CO-), 1.24 (3H, s, $\underline{Me}_2$CO-), 1.10 (3H, s, $\underline{Me}_2$C-C), 0.63 (3H, s, $\underline{Me}_2$C-C).
13. We thank Professor Raymond Baker and Dr. David Billington (Merck, Sharp and Dohme) for an authentic sample of IP_3 and valuable advice concerning HPLC conditions for its purification.
14. ^1H NMR: δ (500 MHz, $CDCl_3$): 7.45-7.15 (30H, m, Ph). 5.11-4.98 (12H, m, PhC$\underline{H_2}$O), 4.80 (1H, td, J =8.8 and 6.8 Hz, H_5 or H_4), 4.67 (1H, td, J=8.2 and 3.8Hz, H_1), 4.52 (1H, dd, J=6.2 and 3.8 Hz, H_2), 4.47 (1H, td, J = 8.8 and 6.6 Hz, H_4 or H_5), 4.20 (1H, t, J = 6.4 Hz, H_3), 3.81 (1H, dd, J = 8.2 and 6.8 Hz, H_6) 3.38 (3H, s, $\underline{Me}$O-), 1.51 (3H, s, -C$\underline{Me}_2$), 1.24 (3H, s, -C$\underline{Me}_2$).
15. ^1H NMR: δ (500 MHz, D_2O, pH 9.1): 4.16 (1H, br s, H_2), 4.05 (1H, q, J = 8.5 Hz, H_4 or H_5), 3.88 (1H, q, J = 8.5 Hz, H_5 or H_4), 3.80 (1H, t, J = 8.2 Hz, H_6), 3.49 (1H, br d, J = 8.5 Hz, H_3), 3.45-3.35 (4H, m including s at δ 3.42, H_1 and $\underline{Me}$O). Resonances quoted relative to $\underline{H}$OD = 4.63.
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