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ENANTIOSELECTIVE SYNTHESIS OF INOSITOLS AS INTERMEDIATES FOR THE PREPARATION OF DEOXY-INOSITOL PHOSPHATES FROM D-GALACTOSE

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Abstract:

Optically pure 6-deoxy-inososes, 6-deoxy-inositols and 6-deoxy-inositol-1,4,5-trisphosphates were synthesized from D-galactose by the carbohydrate-inosose Ferrier rearrangement. 6-Deoxy-inositolphosphates exhibit a tight binding to the Ins-P_3-receptor making such compounds an interesting tool for studying the intracellular signalling.

It is now well established that receptor stimulated hydrolysis of inositol phospholipids is a common mechanism for transmembrane signalling when cells respond to external stimuli such as hormones, neurotransmitters, antigens, light, growth factors and insulin[1]. It was also shown that phosphatidylinositol-4,5-bisphosphate [(Ptd)Ins(4,5)P_2] is a major inositol lipid hydrolysed by activated phospholipase C, resulting into the simultaneous generation of two "second messengers", D-*myo*-inositol-1,4,5-trisphosphate [Ins(1,4,5)P_3] and diacylglycerol (DG).[2] Ins(1,4,5)P_3 triggers the mobilization of Ca^{++} from intracellular stores and DG stimulates protein phosphorylation *via* the activation of protein kinase C.[3,4] In addition (Ptd)Ins(4,5)P_2 contains a high percentage of arachidonic acid in the sn-2 position, which is released for lipoxygenase and cyclooxygenase pathways. These "second messengers" and their metabolites control and modulate vital physiological processes by their independent, additive and synergestic effects.[5]

Therefore, it is conceivable that inhibitors of the key enzymes of the phosphoinositide cascade could be of medicinal interest and could be also useful tools to elucidate the individual role of the inositol metabolites in regulation of cell functions.[6,7]

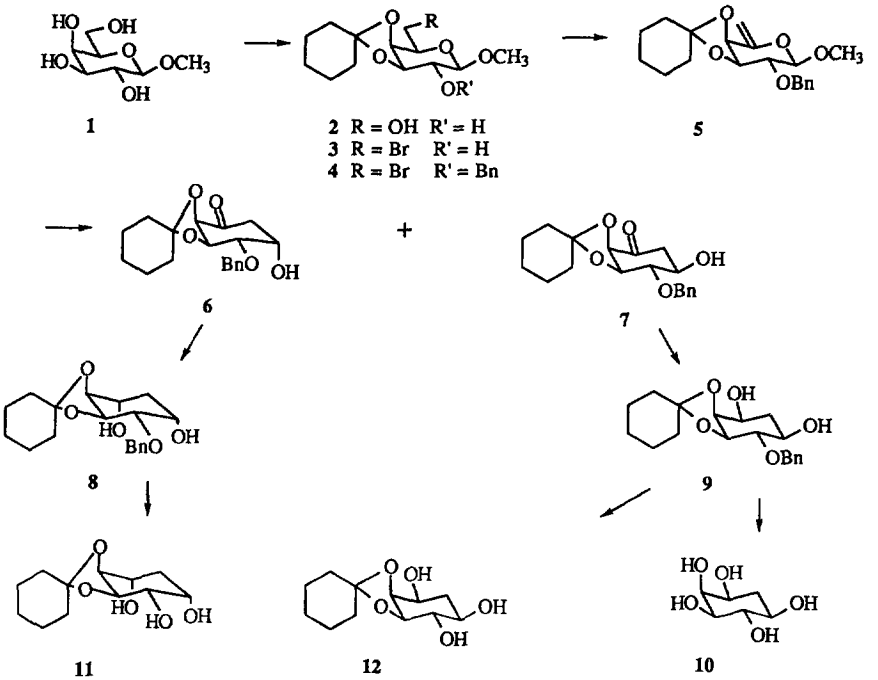
In view of difficulties in the isolation of inositol phosphate metabolites from natural sources and the need for structural analogues, several synthetic studies have been reported[8]. However, these have employed mostly the optically inactive *myo* inositol as a logical and cheap starting material. The crucial role of the phosphate esters at positions 1, 3, 4 and 5 of the *myo* inositol nucleus in the "second messengers" Ins(1,4,5)P_3 and Ins(1,3,4,5)P_4 is well established. For analogue synthesis, modifications of the centers C-1, C-3, C-4 and C-5 or alteration of the hydroxyl functions at neighboring carbons (C-2 or C-6), not involved at first glance in cellular processes, seemed justified.

With these considerations in mind we have initiated a synthetic program aimed to provide access to the hitherto unknown partially protected 6-deoxy-cyclitols **8** and **9**, appropriate precursors of a variety of chiral deoxy-inositol phosphates[9]. Our approach to the deoxy-inositols **8** and **9** has been envisaged from the chiral deoxy-inososes **6** and **7** which could be obtained by mercury(II) mediated carbohydrate-inosose Ferrier rearrangement[10] from hex-5-*ene*-pyranoside **5**.

Olefin **5** was readily prepared in a four steps sequence from methyl-β-D-galactopyranoside **1** in 60% overall yield.[11] Treatment of **1** with 1,1-dimethoxycyclohexane in DMF in presence of sulfuric acid afforded acetal **2** in 90% yield. The latter was selectively brominated with triphenylphosphine-carbontetrabromide, leading to **3** (m.p. 122-123°C). Benzylation of **3** by a phase transfer process (powdered KOH, benzyltriethylammonium chloride, benzyl bromide in CH_2Cl_2) furnished benzyl ether **4** (m.p. 94-95°C, $[\alpha]_D^{20}$ + 46). Access to olefin **5** (m.p. 61-62°C, $[\alpha]_D^{20}$ - 55) was achieved by two methods. Initially the bromo compound **4** was dehydrobrominated with sodium hydride in DMF (3h, 100°C) giving **5** in 90% yield[12]. An alternative route for the

dehydrohalogenation of **4** in the same yield was the use of phase transfer catalysis[13] (DMF, cesium fluoride and benzyltriethylammonium chloride; 4h, 120°C). Under well established Ferrier conditions, olefin **5** did not undergo the expected rearrangement into 6-deoxy-inososes **6** and **7**. However, in the presence of $HgCl_2$ (1.5 eq) in acetone-water (2:1) addition to the crude reaction mixture of an excess of thiourea (4 eq.) assured the cyclisation of the acyclic mercury-complex intermediate to give **6** and **7** (75% in 2:1 ratio). Similarly, **5** in presence of $Hg(OAc)_2$ or $Hg(NO_2)_2$ (1.7 eq.) and subsequent mercury decomplexation with thiourea afforded a 2:1 ratio of **6** and **7** in 75% yield.

Cerium chloride ($CeCl_3$, $7H_2O$) mediated sodium borohydride reduction[14] of **6** in methanol resulted into the formation of 1-*O*-benzyl 5,6-*O*-cyclohexylidene-3-deoxy-L-*chiro*-inositol **8** (m.p. 35-36°C, $[\alpha]_D^{20}$ + 48 (C = 0,95, $CHCl_3$)) in 65% yield. Whereas 6-deoxy-*myo*-inosose **7** was stereoselectively reduced to 4-*O*-benzyl-2,3-*O*-cyclohexylidene-6-deoxy-D-*myo*-inositol **9** (m.p. 124-125°C, $[\alpha]_D^{20}$ - 50 (C = 0,8, $CHCl_3$)) with lithium borohydride in THF in 85% yield.



Hydrogenolysis of compound **8** using 10% Pd/C in 95% ethanol (3.5 bars) furnished the crystalline triol **11** (m.p. 136-137°C, $[\alpha]_D^{20}$ - 38 (C = 0,9, CH_3OH)). Surprisingly in the same conditions, diol **9** gave 6-deoxy-D-*myo*-inositol **10** in 90% yield. However, treatment of **9** with 10% $Pd(OH)_2/C$ (Pearlman's catalyst) (3.5 bars) yielded the crystalline triol **12** (m.p. 134°C, $[\alpha]_D^{20}$ + 42 (C = 0,85, CH_3OH)) in 95% yield.

The structures of the cyclohexanepentols **9** and **11** were rigorously established by X-ray diffraction[15].

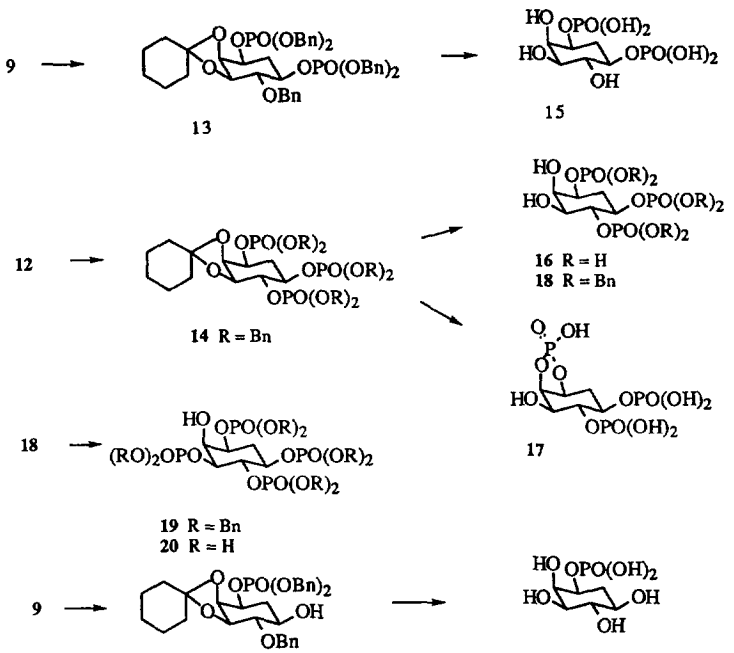
These 6-deoxy-D-inositols **9**, **11**, **12** were appropriate intermediates for access to a series of poly-phosphate analogues, metabolites of the phosphoinositide cascade.

Phosphorylation of diol **9** and triol **12** using dibenzyl-N,N-diisopropylaminophosphoramidite in presence of 1-H-tetrazole in acetonitrile[16], followed by oxydation of P(III) to P(V) with t-butylhydroperoxide in CH_2Cl_2 was achieved to produce respectively the protected 1,5-bisphosphate **13** (70% yield) and crystalline 1,4,5-trisphosphate **14** (m.p. 76°C, 70% yield). Hydrogenolysis of **13** and **14** using 10% Pd/C in 95% ethanol and

evaporation in presence of tris-(hydroxymethyl)-aminomethan gave directly the 6-deoxy-D-*myo*-inositol-1,5-bisphosphate **15** and 6-deoxy-D-*myo*-inositol-1,4,5-trisphosphate **16** isolated respectively as tetra-TRIS and hexa-TRIS salts[17]. However, hydrogenolysis of **14** followed evaporation - in the absence of tris-(hydroxymethyl)-aminomethane - provided 6-deoxy-D-*myo*-inositol-1,2-cyclic-4,5-trisphosphate **17** in quantitative yield.

In order to understand the role of D-*myo*-inositol-1,3,4,5-tetrabisphosphate in the phosphoinositide pathway[18], we have elaborated a synthesis of 6-deoxy-D-*myo*-inositol-1,3,4,5- tetrabisphosphate **20** from **14**. Acid hydrolysis of the cyclohexylidene acetal of **14** followed by selective phosphorylation of the equatorial hydroxyl of diol **18** with tetrabenzylpyrophosphate (1.1 eq) and butyllithium (2 eq) in THF furnished the protected 1,3,4,5-tetrabisphosphate **19** (70%). Hydrogenolysis of **19** in presence of 10% Pd/C produced the tetrabisphosphate **20**, isolated as an octa-TRIS salt.

The 6-deoxy-D-*myo*-inositol-1-phosphate **22** was an interesting target as a potential inhibitor for *myo*-inositol-monophosphatase[19,20]. Recently the 3,5,6-trideoxy derivative of *myo*-inositol-1-monophosphate was identified as a potent inhibitor of inositol-monophosphatase[19]. The monophosphate **22** was obtained in two steps from the diol **9**. Selective phosphorylation of the 1-hydroxyl of **9** by tetrabenzylpyro-phosphate (1.1 eq), in presence of butyllithium (2 eq) in THF, (- 40°C, 1h) furnished **21**. The usual hydrogenolysis of **21** (10% Pd/C, 3.5 bars in ethanol) afforded **22**[20] isolated as a bis-TRIS salt.



$InsP_3$ receptor binding: The displacement of specifically bound $InsP_3$ from purified porcine cerebellum membranes was measured according to Willcocks et al.[24]. Compound **16** exhibited a tight binding to the receptor and displaced $Ins(1,4,5)P_3$ with an IC_{50} of 1.5×10^{-6} mol/l indicating that the hydroxy group in position 6 is not strictly necessary for receptor recognition. [$Ins(1,4,5)P_3$] is displaced in the same assay with an IC_{50} of 2×10^{-8} mol/l. None of the other compounds showed activity in this assay.

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