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TOTAL SYNTHESES OF CHIRAL *sn-myo*-INOSITOL-1,4,5-TRISPHOSPHATE[1] AND ITS ENANTIOMER.

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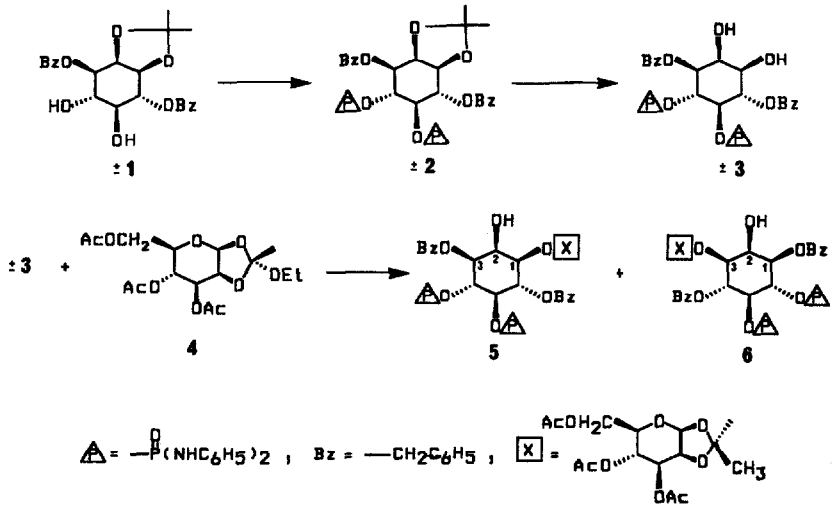
sn-myo-Inositol-1,4,5-trisphophate (Ins(1,4,5)P_3) and its enantiomers are prepared by synthesis of suitably protected *myo*-inositols, separation of enantiomers via the formation of D-mannose diastereomeric derivatives and selective phosphorylations.

sn-myo-Inositol-1,4,5-trisphosphate Ins(1,4,5)P_3 (-)-8 is a well known second messenger in living cells.[2] To date, only chemical approaches could provide preparative quantities of inositol-phosphates for physico-chemical and biological investigations. Biological probes, labelled and modified inositol-phosphates can also be obtained only by synthetic means. During the last three years, several syntheses of chiral Ins(1,4,5)P_3 have been reported. The separation of the enantiomers was achieved by the formation of diastereomeric esters using chiral acids such as l-menthoxyacetic acid[3] or camphanic acid[4] or by the use of glycosyl derivatives.[5,6] Some separations of enantiomers were also performed by chiral HPLC methods.[7] More recently a method using an enzymatic reaction has been proposed.[8] The aim of our program is to develop convenient methods for the total synthesis of Ins(1,4,5)P_3 and derivatives

which would subsequently be used for biological studies or to study their complexation properties towards cations of biological interest. We want to report here a new synthesis of Ins(1,4,5)P_3 enantiomers.

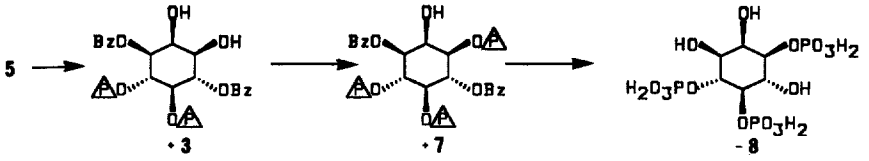
For our synthesis, we used 1(3),4(6)-di-O-benzyl-2,3(1)-isopropyliden- *sn-myo* -inositol **(±)-1)** as starting material. The synthesis of this compound has been reported by Gigg.[9] The free hydroxyl groups of diol **(±)-1)** were then phosphorylated using dianilidophosphoryl chloride.[10,11] (8 equ, pyridine, -10°C, then 48 h RT, yield 96 % after chromatography, recrystallization from ethanol m.p. 110-111°C). This method gave an excellent yield in the phosphorylation of the vicinal 4,5-diol **(±)-2**. The next step of the synthesis involved the selective removal of the ketal group[9], leading to the 1(3),2-dihydroxy derivative **(±)-3**. Optical resolution of **(±)-3** was achieved by means of the formation of diastereomers using a conveniently protected D-mannose. Thus the reaction of 3,4,6-tri-O-acetyl-1,2,0-ethylorthoacetyl -β-D-mannopyranose[12] (D-mannose ethylorthoacetate) **4** with compound **(±)-3** under standard conditions[13] resulted in esterification the enantiotopic positions 1 and 3 of the diol **(±)-3** and yielded a mixture of the diastereomers **5** and **6** (Scheme 1).



Scheme 1

These isomers were separated by preparative HPLC to give individual diastereomers[14] (Lichrospher 10 µM 150x15, acetonitrile/water/triethylamine : 1500/500/1, 9.9 ml. min^{-1}).

Chiral Ins(1,4,5)P_3 **(-)-8** was obtained from compound **5** using the following steps (Scheme 2). The D-mannose moiety was removed from diastereomer **5** under mild acidic conditions[13] to yield 4,5-bisdianilidophosphate-3,6-di-O-benzyl *sn-myo*-inositol **(+)-3** (82 % recrystallization from ethanol m.p. 181°C, $[\alpha]_D$ = +34 ±1 c=2.3 in chloroform).



+3 **+7** **-8**

Scheme 2

Phosphorylation of **(+)-3** again using dianilidophosphoryl chloride leads to the selective phosphorylation of the equatorial hydroxyl group and gave the protected Ins(1,4,5)P_3 **(+)-7** which was purified by column chromatography (silica gel, chloro form/acetone : 50/1, yield 44.5 %, recristallization from ethanol m.p. 230 -231°C, $[\alpha]_D$= +16 ±1 c=3.5 in chloroform). The complete deprotection was achieved in two steps but in a one pot reaction. First, the anilino groups of the phosphates were removed by reaction with isoamyl nitrite in pyridine, acetic acid and acetic anhydride[13,15] (1/1/1). Then the reaction mixture was evaporated to dryness and the residue was dissolved in a mixture of ethanol and water (6/1). This solution was hydrogenolysed in the presence of palladium. The crude product was purified by HPLC (Versapack C_{18} 250x4.6, gradient 0-60% methanol in 0.1 M ammonium formate, pH 5.7, 1 ml. min^{-1}). The elution of Ins(1,4,5)P_3 **(-)-8**, as its ammonium salt, was monitored by the use of (^{3}H)-Ins(1,4,5)P_3 (Amersham)(12.1 %, $[\alpha]_D$= -28 ±3 c=0.37 in water pH 10, lit. $[\alpha]_D$= -27.4 in water pH 9.5[16], ^{31}P-NMR : 3.37 (d), 5.09(d, 5.18(d)). Our sample was compared with an authentic sample of natural Ins(1,4,5)P_3 isolated from beef

heart muscle (paper chromatography, HPLC, ion exchange chromatography) and showed the same chromatographic properties.

The enantiomer of compound **(-)-8** Ins(3,5,6)P_3 was obtained using the same sequence of reactions as for Ins(1,4,5)P_3 but starting from diastereomer **6** (9 %, $[\alpha]_D$ =+30 ±3 c=0.29 in water pH 10).[17] The use of D-mannose diastereomeric derivatives could be a general method of preparation of chiral inositol-phosphates.

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