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Synthesis of Enantiomerically Pure D-*myo*-Inositol 1,5,6-Trisphosphate

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Abstract: The synthesis of enantiomerically pure D-*myo*-inositol 1,5,6-trisphosphate from *myo*-inositol involving two sequential regioselective protections of hydroxyl groups in the intermediate selfresolving *myo*-inositol camphor monoacetal has been accomplished

Recent investigations of the phosphoinositide signal transduction systems have revealed that enormous number of inositol phosphates and related phospholipids are present in eukaryotic cells.[1] The chemical synthesis of the naturally occurring inositol phosphates and more recently of analogs designed as tools to study the signaling cascade is one of the major themes in organic chemistry today.[2,3] In this communication, we wish to report the first synthetic route to enantiomerically pure D-*myo*-inositol 1,5,6-trisphosphate [Ins(1,5,6)P_3]. This trisphosphate [or/and Ins(3,4,5)P_3] has recently been found in avian erythrocytes[4] and in stimulated rat mammary cells.[5] Its occurrence in plants is also probable.[6]

The developed synthetic route to Ins(1,5,6)P_3 is presented in Scheme 1 which commences with D-2,3-*O*-(D-1,7,7-trimethyl[2.2.1]bicyclohept-2-ylidene)-*myo*-inositol (*O3 endo*) (1) available promptly from the parent cyclitol and D-camphor dimethyl acetal in one step in 65-70% yield as described previously.[7-9]

Scheme 1
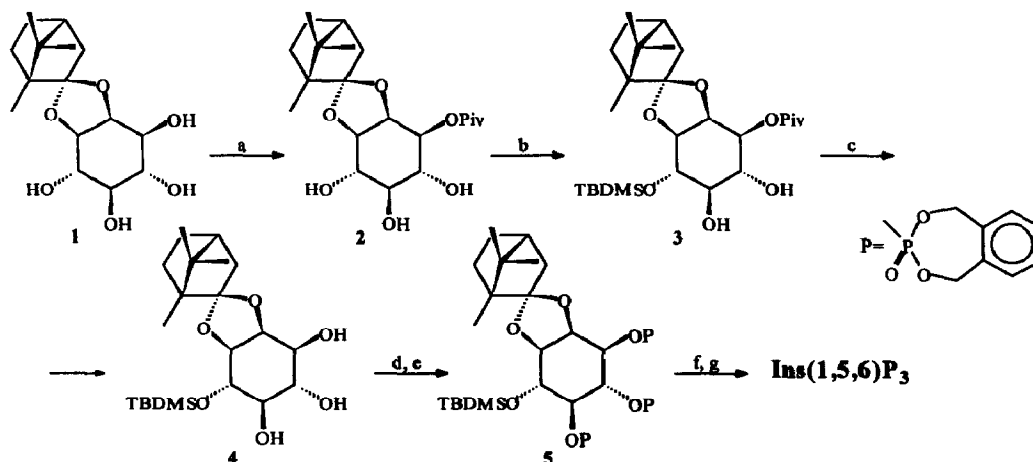
Reagents: a: $(CH_3)_3CC(O)Cl$, pyridine; b: Bu^tMe_2SiCl, DMF, Et_3N, DMAP; c: DIBAL-H, THF, d: 2-dimethylamino-5,6-benzo-1,3,2-dioxaphosphepane, tetrazole, CH_2Cl_2; e: MCPBA, CH_2Cl_2; f: H_2, Pd/C, methanol; g: H_2O

Following published procedure with a slight modification 1 was reacted with pivaloyl chloride (1.2 equiv., pyridine, 0°C, 4h) to give the expected C1-O-monoester 2 in 65% isolated yields.[7c,8] Subsequent reaction of 2 with *tert*-butylchlorodimethylsilane (1.2 equiv., DMF, -20°C, 24h) in the presence of DMAP[10] resulted in the selective protection of C4-OH group and yielded diol 3 in 60% yield.[11] Cleavage of pivaloyl ester in 3 without migration of silyl group was achieved by means of DIBAL-H (5 equiv., tetrahydrofuran, rt, 1h) which provided triol 4 [mp 150-2°C, $[\alpha]_D$ =+15.3° (c 2.2, CHCl₃)] in 89% isolated yields. Phosphorylation of 4 by sequential treatment with 2-dimethylamino-5,6-benzo-1,3,2-dioxaphosphepane[12] [4.5 equiv., tetrazole (9 equiv.), methylene chloride, rt, 1h] and MCPBA (5.1 equiv., -60°C, 10 min; rt, 10 min) gave trisphosphate 5, as a colorless glass, $\delta^{31}P(C_6D_6)$ -3.80, -2.54, -1.72 ppm, (85%). Finally, hydrogenolysis of the *o*-xylil phosphate groups over 10% Pd/C (methanol, 1h), followed by cleavage of camphor acetal and silyl ether (H₂O, 10 min.) afforded Ins(1,5,6)P₃ which was isolated as its hexasodium salt in 97% yield.[13]

In summary, the synthesis of enantiomerically pure Ins(1,5,6)P₃ from *myo*-inositol in six steps and in 15% overall yield has been accomplished. This synthesis further illustrates the versatility that is generated by the selective functionalization of *myo*-inositol initiated by its transformation into D- or L-camphor monoacetal. Use of the latter in the above synthetic sequence secures equally straightforward access to Ins(3,4,5)P₃.[14]

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11. Diol 3 was freed from minor contaminants (probably regioisomers, not identified) by chromatography on silica gel using CCl₄-Et₂O 7:2 as eluent, R_F=0.18. 3: mp 155-7°C; $[\alpha]_D$ = -39° (c 2.1, CHCl₃); MS(CI) m/z: 512.3161, calc. 512.3169; NMR; δ_H (CDCl₃) 0.13(s, 3H), 0.17(s, 3H), 0.84(s, 6H), 0.91(s, 9H), 0.93(s, 3H), 1.22(s, 9H), 1.41-2.00(m, 7H), 2.41(brs, OH), 2.49(d, J = 3.0 Hz, OH), 3.36(dt, J = 3.0, 9.0 Hz, 1H), 3.58(dd, J = 6.1, 9.0 Hz, 1H), 3.87(t, J = 6.1 Hz, 1H), 3.92(brt, J ~ 9.5 Hz, 1H), 4.42(dd, J = 4.7, 6.1 Hz, 1H), 4.93(dd, J = 4.7, 9.9 Hz, 1H) ppm; δ_C (C₆D₆) -3.43, -3.28, 10.9, 19.2, 21.2(2C), 26.9(3C), 28.0(3C), 30.4, 39.7, 46.1(2C), 48.8, 52.4, 71.6, 72.6, 74.8, 75.6, 78.1, 79.2, 118.5, 178.8 ppm.
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13. **Ins(1,5,6)P₃**: $[\alpha]_D$ = -2.8° (c 2.3 H₂O); NMR(D₂O) δ_P 4.11, 4.83, 5.94 ppm; δ_C 73.7, 74.6, 76.2, 77.6(d, $^2J_{PC}$ = 4.4 Hz), 78.5(d, $^2J_{PC}$ = 5.6 Hz), 80.3(d, $^2J_{PC}$ ~ 2.5 Hz) ppm; δ_H 3.63(dt, J ~ 2.4, 8.8 Hz, 1H), 3.85-3.94(m, 2H), 3.88(dt, J ~ 2.7, 7.5 Hz, 1H), 4.34(q, J ~ 8.8 Hz, 1H), 4.42(t, J= 2.5 Hz, 1H) ppm.
14. For recent synthesis of Ins(3,4,5)P₃, see: Falck, J.R.; Abdali, A. *Bioorg.Med.Chem.Lett.* **1993**, *3*, 717.