Expedient Synthetic Routes to [^{3}H]-D-3-Azido-3-deoxy-*myo*-inositol and D-3-Azido-3-deoxy-*myo*-inositol 2,4,5-Trisphosphate

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Expedient routes to a tritiated derivative of the v-*sis* oncogene-transformed cell specific growth inhibitor 3-azido-3-deoxy-*myo*-inositol and the unlabelled 2,4,5-trisphosphate derivative are described together with the effects of the latter compound in binding to the IP_3 receptor.

The phosphatidylinositol (PI) signalling pathway plays a key role in the control of cellular growth through the mediation of the action of growth factors and oncogenes. In an effort to develop novel, non-DNA targeted agents for the treatment of cancer, we have focused our attention on the design of agents capable of disrupting steps in the PI intracellular signalling pathway.[1]

We have recently shown, for example, that the 3-azido-3-deoxy isostere **1** of *myo*-inositol is a selective inhibitor of the growth of v-*sis* transformed NIH 3T3 cells exhibiting a selectivity of over 1200-fold for the transformed cells compared to the wild type NIH 3T3 cells.[2] This azidoinositol displays an IC_{50} of 40 μmol dm^{-3} in experiments carried out in a *myo*-inositol free medium. In order to better understand the

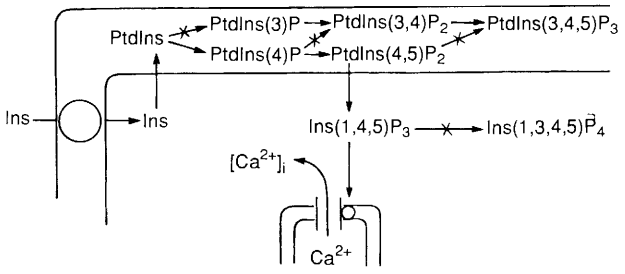
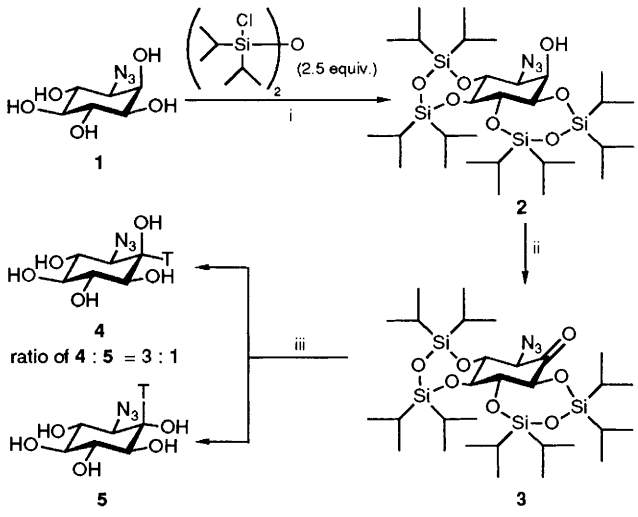


Fig. 1 Possible pathways for D-3-substituted *myo*-inositol analogues in the cell. Ins is the analogue which is taken up by the *myo*-inositol transport process. It is then incorporated into inositol phospholipids and may give rise to D-3-substituted inositol phosphates or D-3'-substituted phosphatidylinositols. The formation of some of the phosphatidylinositol phosphates and inositol 1,3,4,5-tetrakisphosphate is blocked (as indicated by the X) because of the substitution at the D-3-position.

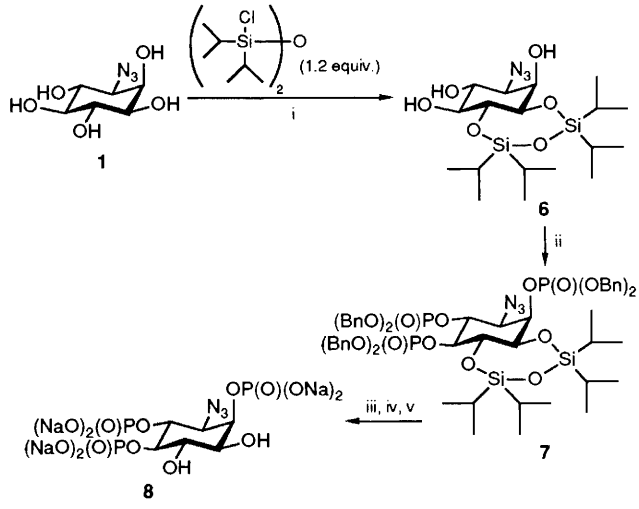
mechanism of action of these and related inositol isosteres, we required an efficient method to achieve the labelling of this compound with high specific activity. In efforts to achieve this end, we also discovered an expedient route to the 3-azido-3-deoxy analogue of *myo*-inositol 2,4,5-trisphosphate [Ins(2,4,5)P_3]. Since Ins(2,4,5)P_3 has recently been shown to serve as an important probe in elucidating further details of calcium signalling associated with the phosphatidylinositol pathway,[3] we believe the present results to be of considerable general interest.

In order to achieve the introduction of a radiolabel into the azido compound **1** so as to obtain a compound of high specific activity (>5 Ci mmol^{-1}), we decided to oxidize the C-2 hydroxy group to ketone, and then to carry out a [^{3}H]$NaBH_4$ reduction. This process required that we protect selectively four of the hydroxy groups of **1**. Quite remarkably, we found that the reaction of **1** with 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane (2.5 equiv.) in pyridine at 73 °C gave exclusively inositol derivative **2**.[4] The structure of this compound was confirmed by an NMR analysis of the derived acetate in which the methine proton on the acetate-bearing carbon appeared as a doublet of doublets with two small couplings (δ 5.48, $J_{1,2}$ = $J_{2,3}$ 2.9 Hz). This observation rigorously defines the structure of **2**, since any other acetate derivative would have exhibited large–large, or large–small couplings. Next, the alcohol was oxidized to ketone **3** by the action of acetic anhydride–Me_2SO, and the ketone was reduced with $NaBT_4$ (New England Nuclear, specific activity 54 Ci mmol^{-1}) in a 3 : 1 MeOH–THF (tetrahydrofuran) mixture at −78 °C to 0 °C to give a 3 : 1 mixture of the *myo*-inositol analogue with an axial C-2 hydroxy group and its stereoisomer. Desilyation of the crude mixture was carried out with aqueous HF in acetonitrile to provide the radiolabelled derivatives **4** and **5**, which were separated by HPLC using a RP μ Bondapak C18 column with water as the eluent. Azide **4** was found to have a specific activity of >6 Ci mmol^{-1}.

When the inositol **1** was treated with 1.2 equiv. of 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane in pyridine at room temperature for 10 h, the 1,6-protected inositol derivative **6** was obtained in 95% yield (Scheme 1). The structure of this compound was confirmed by converting **6** to its triacetate derivative, and examining the coupling constants of the protons attached to the acetate-bearing carbons. The presence of two protons with two large couplings (H-4 at δ 5.37, $J_{3,4}$ = $J_{4,5}$ 10.2 Hz; H-5 at δ 5.07, $J_{5,6}$ 9.6, $J_{4,5}$ 10.2 Hz) and one proton with two small couplings (H-2 at δ 5.61, $J_{1,2}$ = $J_{2,3}$ 2.9 Hz) confirmed the location of the newly introduced silyl protecting group. Next, intermediate **6** was phosphorylated using sodium hydride and tetrabenzylpyrophosphate in dimethylformamide (DMF) to provide compound **7** (Scheme 2). Deprotection of **7** was achieved by treatment with TMSBr,



Scheme 1 Synthesis of the tritiated form of 3-azido-3-deoxy-*myo*-inositol. *Reagents and conditions*: i, pyridine, 73 °C, 40 h, 80% yield; ii, Ac_2O, Me_2SO, room temp., overnight, 95% yield; iii, $NaBT_4$, MeOH–THF (3 : 1), −78 °C to room temp., 90% overall yield calculated for the cold products.



Scheme 2 Synthesis of 3-azido-3-deoxy-*myo*-inositol-2,4,5-trisphosphate. *Reagents and conditions*: i, pyridine, room temp., 10 h, 95% yield; ii, NaH, tetrabenzylpyrophosphate, DMF, 0 °C, 8 h, 61% yield; iii, TMSBr, $CHCl_3$, room temp.; iv, H_2O; v, aq. NaOH, 68% overall yield.

followed by aqueous workup and conversion of the phosphorylated derivative to its hexasodium salt **8** by the action of NaOH. To our knowledge compound **8** represents the first synthesis of an bioisosterically substituted analogue of Ins(2,4,5)P_3.[5]

The tritiated azide **4** is currently being studied in cell culture systems. As regards the biological activity of the trisphosphate **8**, we note that in calcium release studies,[5] it was found to be considerably less potent than Ins(1,4,5)P_3. In preliminary experiments carried out in saponin permeabilized human colon carcinoma HT29 cells loaded with $^{45}Ca^{2+}$, analogue **8** released 21% of the Ca^{2+} after two minutes (addition at six minutes) at a concentration of 100 μmol dm^{-3}, whereas natural Ins(1,4,5)P_3 caused 53% release of Ca^{2+} at a concentration of 10 μmol dm^{-3}.[6] In competition binding experiments using bovine adrenal gland P_2 membrane the azide exhibited a K_i of 18.9 μmol dm^{-3} in comparison with a K_i of 10 nmol dm^{-3} for Ins(1,4,5)P_3.

In summation, highly efficient routes for the synthesis of [^{3}H]3-azido-3-deoxy-*myo*-inositol and 3-azido-3-deoxy-*myo*-

inositol 2,4,5-trisphosphate have been developed. The former compound is of key importance to acquiring an understanding of the cell selectivity exhibited by this analogue, and further biological studies will be reported elsewhere.[7,†]

† *Selected spectral data* for compounds **2, 4, 6** and **8**: Compound **2**: $[\alpha]_D^{23}$ + 0.11 (c = 27 mg ml^{-1}, $CHCl_3$); IR (thin film) ν/cm^{-1} 2108, 1060 and 989; ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.13 (t, 1H, J 9.7 Hz), 4.03 (t, 1H, J 2.81 Hz), 3.91 (t, 1H, J 8.6 Hz), 3.64 (dd, 1H, J 8.6, 2.8 Hz), 3.59 (t, 1H, J 8.5 Hz), 3.37 (dd, 1H, J 9.9, 2.8 Hz), 2.53 (1H, OH), 1.2–0.8 (m, 56 H).

Compound **4**: $[\alpha]_D^{23}$ −5.0 (c = 8 mg ml^{-1}, H_2O); IR (KBr disc) ν/cm^{-1} 3400, 2100, 1558 and 1419; ^{1}H NMR (300 MHz, D_2O) δ 4.14 (t, 1H, J 2.5 Hz), 3.73 (dd, 1H, J 10.4, 9.4 Hz), 3.59 (t, 1H, J 9.9 Hz), 3.51 (dd, 1H, J 9.9, 2.5 Hz), 3.41 (dd, 1H, J 10.4, 2.5 Hz), 3.25 (t, 1H, J 9.4 Hz).

Compound **6**: $[\alpha]_D^{23}$ + 7.0 (c = 16 mg ml^{-1}, $CHCl_3$); IR (thin film) ν/cm^{-1} 3481, 2108, 1041 and 987; ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.12 (t, 1H, J 3.0 Hz), 4.08 (t, 1H, J 9.8 Hz), 3.88 (t, 1H, J 8.9 Hz) 3.65 (dd, 1H, J 8.9, 3 Hz), 3.38 (t, 1H, J 9.8 Hz), 3.26 (dd, 1H, J 9.8, 3 Hz), 1.2–1.0 (m, 28 H).

Compound **8**: $[\alpha]_D^{23}$ + 7.8 (c = 1.15 mg ml^{-1}, $CHCl_3$); IR (KBr disc) ν/cm^{-1} 3450, 2125, 1662 and 1114; ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.58 (dt, 1H, J 7.9, 2.5 Hz), 4.31 (q, 1H, J 9.2 Hz), 3.9–3.8 (m, 2H), 3.48 (dd, 1H, J 9.4, 2.5 Hz), 3.35 (dt, 1H J 7.8, 2.5 Hz); ^{31}P NMR ($CDCl_3$, 85% H_3PO_4 external standard) δ 2.60 (d, J 8.9 Hz), 2.50 (d, J 9.1 Hz), 1.99 (d, J 7.8 Hz).

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