Synthesis of D-*myo*-Inositol 3,4,5,6- and 1,4,5,6-Tetrakisphosphate Analogues and their Membrane-permeant Derivatives

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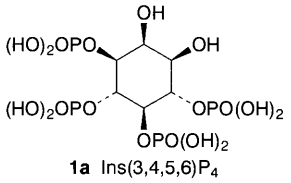
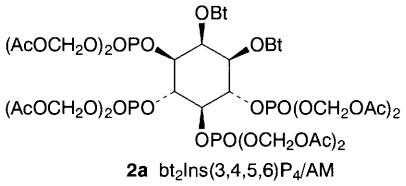
A set of D-*myo*-inositol 3,4,5,6- and 1,4,5,6-tetrakisphosphates [D-Ins(3,4,5,6)P_4 and D-Ins(1,4,5,6)P_4, respectively] analogues with modifications of the hydroxy groups is synthesized and subsequently converted to the corresponding uncharged, bioactivatable acetoxymethyl esters.

It has become firmly established that the agonist-stimulated hydrolysis of phosphatidylinositols plays an important role in intracellular signal transduction processes.[1] Apart from the well-documented formation of *myo*-inositol 1,4,5-trisphosphate [Ins(1,4,5)P_3] from phosphatidylinositol 4,5-bisphosphate, there is evidence that *myo*-inositol 1,3,4,5,6-pentakisphosphate is hydrolysed, by a thus far unknown mechanism, to *myo*-inositol 3,4,5,6-tetrakisphosphate [Ins(3,4,5,6)P_4, **1a**] upon receptor activation.[2,3] While the turnover of Ins(1,4,5)P_3 and its effect on Ca^{2+} mobilization from internal stores is known to be very rapid, the intracellular levels of Ins(3,4,5,6)P_4 appear to be elevated for longer periods of time. It was recently suggested that Ins(3,4,5,6)P_4 might be involved in the uncoupling of chloride secretion from the Ca^{2+} signal in T_{84} cells, a human epithelial cell line.[4,5] Strong support for this hypothesis was provided by the use of membrane-permeant derivatives of Ins(3,4,5,6)P_4, namely DL-1,2-di-*O*-butyryl *myo*-inositol 3,4,5,6-tetrakisphosphate octakis(acetoxymethyl)ester [bt_2Ins(3,4,5,6)P_4/AM, **2a**]. Extracellularly-applied doses of racemic **2a** were shown to have a potent inhibitory effect on thapsigargin-induced chloride secretion without altering intracellular Ca^{2+} levels themselves. This effect was highly specific, because the enantiomerically pure membrane-permeant derivative of D-*myo*-inositol 1,4,5,6-tetrakisphosphate [Ins(1,4,5,6)P_4] was inactive.[5]



1a Ins(3,4,5,6)P_4



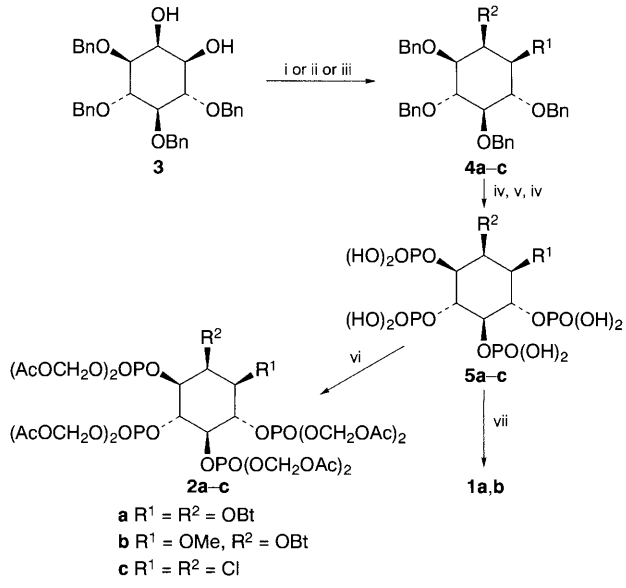
2a bt_2Ins(3,4,5,6)P_4/AM

Here we report the synthesis of enantiomerically pure bt_2Ins(3,4,5,6)P_4/AM **2a**. Furthermore, we describe the synthesis of the first set of analogues of **1a** and its enantiomer Ins(1,4,5,6)P_4 carrying modifications of their hydroxy groups. The analogues were subsequently converted to their potentially membrane-permeant acetoxymethyl esters (Scheme 1). Acetoxymethyl esters have previously been shown to greatly enhance the permeability of other biologically interesting phosphates.[6,7]

The common precursor DL-1,4,5,6-tetra-*O*-benzyl *myo*-inositol was regioselectively esterified to its 3(1)-camphanate and the resulting diastereoisomers were separated by crystallization, as described previously.[5] Hydrolysis of the esters by treatment with MeOH–KOH at room temperature yielded gram quantities of D-1,4,5,6-tetra-*O*-benzyl *myo*-inositol and D-3,4,5,6-tetra-*O*-benzyl *myo*-inositol **3**. This method avoided the use of dibutyl tin oxide for the regioselective introduction of the esters as was described by Anejo and Parra.[8]

Both enantiomers of **3** were either esterified to give the dibutyrate **4a**, or initially converted regioselectively to the 1-*O*-methyl ether and subsequently butyrylated to yield **4b**. In a third approach, both hydroxy groups of racemic **3** were replaced by the method of Appel[9] to afford the 1,2-dichloro derivative **4c**. Catalytic hydrogenolysis of **4a–c** gave 3,4,5,6-tetrahydroxy derivatives, which were phosphitylated conveniently with *N,N*-diethyl dibenzylphosphoramidite,[10] followed by oxidation with peracetic acid. The resulting, fully-protected inositol tetrakisphosphates were purified by preparative HPLC (50 × 250 mm, LiChrospher 100, RP-18, 10 μm) and subsequently deprotected by hydrogenolysis to yield the 3,4,5,6-tetrakisphosphates **5a–c**† as the free acids. Treatment of the tetrakisphosphates with acetoxymethyl bromide[11] and diisopropylethylamine in dry acetonitrile gave the corresponding octakis(acetoxymethyl)-esters **2a–c**‡ in good yields (70–90%). Alternatively, **5a** and **5b** were hydrolysed in aqueous KOH to yield Ins(3,4,5,6)P_4 **1a**§ and 1-*O*-methyl Ins(3,4,5,6)P_4 **1b**,¶ respectively as well as the enantiomeric 1,4,5,6-tetrakisphosphates.

The Ins(3,4,5,6)P_4 analogues and the corresponding acetoxymethyl esters described above may become important tools for future studies concerning the role of Ins(3,4,5,6)P_4 as well as its putative binding proteins. Furthermore, **5b** should be resistant to phosphorylation at the 1-position and, if active, might exhibit prolonged biological activity. Investigations of the effect of the acetoxymethyl esters on the Cl^- secretion of



a $R^1 = R^2 =$ OBt
b R^1 = OMe, R^2 = OBt
c $R^1 = R^2 =$ Cl

Scheme 1 *Reagents and conditions*: i, Bt_2O, pyridine, 50 °C, 1 d, 95% yield; ii, (*a*) Bu_2SnO, MeOH, Soxhlet/molecular sieves 3 Å, reflux, 20 h, (*b*) MeI, DMF, 50 °C, 2 d, 72%, (*c*) Bt_2O, pyridine, 50 °C, 1 d, 95%; iii, dry CCl_4, Ph_3P, reflux, 12 h, 65%; iv, H_2, Pd/C (10%), AcOH, 4 h, 99%; v, (*a*) $(BnO)_2PNEt_2$, 1*H*-tetrazole, MeCN, room temp., 18 h, (*b*) MeCOOOH, MeCN, −40 °C, 1 h, 50–70%; vi, $MeCO_2CH_2Br$, diisopropylethylamine, MeCN, 2 d, 70–90%; vii, (*a*) 0.1 mol dm^{-3} KOH, pH 12.8, 18 h, (*b*) Dowex 50W-X8, 95%

T_{84} cells are in progress. The results will be published elsewhere.

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Footnotes

† *Selected data:* **5c** ^{1}H NMR (D_2O, 360 MHz, free acid) δ 4.81 (dd, *J* 3.0 Hz, 1H, H-2), 4.60 (2 ddd, *J* 9.5 Hz, 2H, H-4/6), 4.46 (dd, *J* 9.5, 3.0 Hz, 1H, H-1), 4.44 (ddd, *J* 9.5, 3.0 Hz, 1H, H-3), 4.31 (dd, *J* 9.5 Hz, 1H, H-5); ^{31}P NMR (D_2O, 145.8 MHz, ^{1}H decoupled) δ 1.15 (s, 1P), 0.47 (s, 1P), −0.27 (s, 1P), −0.46 (s, 1P); FAB–MS (neg. mode) *m/z* 535 [M − H^+]$^-$.

‡ *Selected data*: **2a** $[\alpha]_D^{20}$ +1.9 (*c*. 1.9, toluene); ^{1}H NMR ([$^2H_8$]toluene, 360 MHz) δ 6.01 (dd, *J* 2.8 Hz, 1H, H-2), 5.59–5.60 (m, 16H, 8 CH_2), 5.75 (dd, *J* 9.5, 2.8 Hz, 1H, H-1), 5.07 (ddd, *J* 9.5 Hz, 1H, H-4), 4.97 (ddd, *J* 9.5 Hz, 1H, H-6), 4.81 (ddd, *J* 9.5, 2.8 Hz, 1H, H-3), 4.79 (ddd, *J* 9.5 Hz, 1H, H-5), 2.44 (m, 2H, α-CH_2), 2.17 (m, 2H, α-CH_2), 1.90–1.76 (8s, 24 H, 8 COMe), 1.70 (m, 2H, β-CH_2), 1.60 (m, 2H, β-CH_2), 0.95 (t, 3H, γ-Me), 0.88 (t, 3H, γ-Me); ^{31}P NMR ([$^2H_8$]toluene, 145.8 MHz, ^{1}H decoupled) δ −3.56 (s, 1P), −3.82 (s, 1P), −4.40 (s, 1P), −4.72 (s, 1P); FAB–MS (neg. mode) *m/z* 1143 [M − CH_2OAc^+]$^-$. **2b** $[\alpha]_D^{20}$ −3.9 (*c* 0.9, toluene); ^{1}H NMR ([$^2H_8$]toluene, 360 MHz) δ 6.05 (dd, *J* 3.0 Hz, 1H, H-2), 5.97–5.68 (m, 16H, 8 CH_2), 5.06 (ddd, *J* 9.5 Hz, 1H, H-4), 4.88–4.27 (m, 3H, H-3/5/6), 3.38 (s, 3H, OMe), 3.21 (m, 1H, H-1), 2.09 (m, 2H, α-CH_2), 1.86–1.75 (8s, 24H, 8 COMe), 1.55 (m, 2H, β-CH_2), 0.84 (t, *J* 7.5 Hz, 3H, γ-Me); ^{31}P NMR ([$^2H_8$]toluene, 145.8 MHz, ^{1}H decoupled) δ −3.66 (s, 1P), −3.72 (s, 1P), −4.07 (s, 1P), −4.80 (s, 1P); FAB–MS (neg. mode) *m/z* 1087 [M − CH_2OAc^+]$^-$. All corresponding L-enantiomers gave similar NMR and FAB–MS data, and $[\alpha]_D$ values were consistent. **2c** ^{1}H NMR ([$^2H_8$]toluene, 360 MHz) δ 5.96–5.61 (m, 16H, 8 CH_2), 5.14 (ddd, *J* 9.0, 3.0 Hz, 1H, H-3), 5.05 (2 ddd, *J* 9.0 Hz, 2H, H-4/6), 4.98 (dd, *J* 3.0 Hz, 1H, H-2), 4.88 (ddd, *J* 9.0 Hz, 1H, H-5), 4.43 (dd, *J* 9.0, 3.0 Hz, 1H, H-1), 1.85–1.73 (m, 24H, 8 COMe); ^{31}P NMR ([$^2H_8$]toluene, 145.8 MHz, ^{1}H decoupled) δ −3.67 (s, 1P), −3.78 (s, 1P), −4.08 (s, 1P), −5.16 (s, 1P); FAB–MS (neg. mode) *m/z* 1039 [M − CH_2OAc^+]$^-$.

§ *Selected data*: **1a** $[\alpha]_D^{20}$ −3.0 (*c* 1.0, H_2O, free acid); ^{1}H NMR (D_2O, 360 MHz, free acid, data consistant with lit.[12]) δ 4.48 (ddd, 1H, *J* 9.7 Hz, H-4), 4.36 (ddd, 1H, *J* 9.5 Hz, H-6), 4.2 (m, 3H, H-5/3/2), 3.72 (dd, 1H, *J* 9.7, 2.7 Hz, H-1); ^{31}P NMR (D_2O, 145.8 MHz, free acid, ^{1}H decoupled) δ 0.82 (s, 1P), 0.55 (br s, 2P), −0.11 (s, 1P); FAB–MS (neg. mode) *m/z* 499 [M − H^+]$^-$.

¶ *Selected data*: **1b** $[\alpha]_D^{20}$ +2.7 (*c* 0.5, H_2O free acid); ^{1}H NMR (D_2O, 360 MHz, free acid) δ 4.51 (ddd, *J* 9.5 Hz, 1H, H-4), 4.45 (dd, *J* 2.8 Hz, 1H, H-2), 4.42 (ddd, *J* 9.5 Hz, 1H, H-6), 4.27 (ddd, *J* 9.5 Hz, 1H, H-5), 4.21 (ddd, *J* 9.5, 2.8 Hz, 1H, H-3), 3.43 (dd, *J* 9.5, 2.8 Hz, 1H, H-1), 3.42 (s, 3H, OMe); ^{31}P NMR (D_2O, 145.8 MHz, free acid, ^{1}H decoupled) δ 1.3 (s, 1P), 0.9 (s, 1P), 0.4 (s, 2P); FAB–MS (neg. mode) *m/z* 513 [M − H^+]$^-$.

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