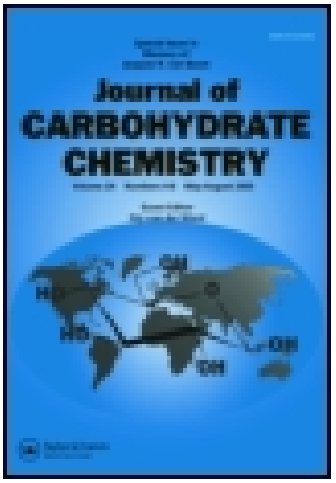

Journal of Carbohydrate Chemistry
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/lcar20

Syntheses of D-Myo-Inositol-1,2,6-Trisphosphate and -2,6-bisphosphate

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Published online: 15 Aug 2006.

To cite this article: Sung-Kee Chung , Seok-Ho Yu & Young-Tae Chang (1998) Syntheses of D-Myo-Inositol-1,2,6-Trisphosphate and -2,6-bisphosphate, Journal of Carbohydrate Chemistry, 17:3, 385-390, DOI: 10.1080/07328309808002899

To link to this article: http://dx.doi.org/10.1080/07328309808002899

J. CARBOHYDRATE CHEMISTRY, 17(3), 385-390 (1998)

SYNTHESES OF D-*MYO*-INOSITOL-1,2,6-TRISPHOSPHATE AND -2,6-BISPHOSPHATE

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Received August 24, 1997 - Final Form December 24, 1997

ABSTRACT

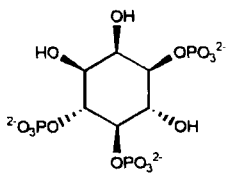
A D-*myo*-inositol derivative (**3**), obtained from methyl α-D-glucopyranoside by Ferrier rearrangement, was efficiently transformed to D-*myo*-inositol 1,2,6-trisphosphate (**1**, α-trinositol) and D-*myo*-inositol 2,6-bisphosphate (**2**).

INTRODUCTION

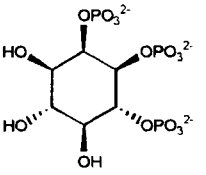
D-*myo*-Inositol 1,4,5-trisphosphate [D-I(1,4,5)P_3], released into the cytosol of cells by the phospholipase C (PLC)-catalyzed cleavage of phosphatidylinositol 4,5-bisphosphate [PI(4,5)P_2], is known to mobilize intracellular calcium.[1] A number of other *myo*-inositol phosphates have also been implicated as either second messengers or key metabolic intermediates in the intracellular signal transduction pathways. D-*myo*-Inositol 1,2,6-trisphosphate (**1**, α-trinositol; PP56), obtained from $InsP_6$ (phytic acid) by enzymatic hydrolysis with yeast phytase, was shown to possess no appreciable agonist activity in biological systems, but to have an inhibitory effect on neuropeptide Y-evoked vasoconstriction in many vascular assays, and was also shown to be a potent

antiinflammatory agent.[2] More recently it has been suggested that α-trinositol has a specific and possibly unique binding site in membranes from several rat tissues although it also shares common binding characteristics with Ins(1,3,4,5)P_4 and, to a lesser degree, also with $InsP_6$ and Ins(1,4,5)P_3.[3] A couple of chemical[4] and enzyme assisted[5] syntheses of α-trinositol and its analogues[6] were reported.

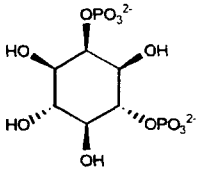
As a part of our efforts to understand the molecular recognition aspect of inositol phosphate-dependent signal transduction including phospholipase C, inositol 1,4,5-trisphosphate receptor, and the metabolic enzymes, we have been studying syntheses and biological activities of various natural and unnatural inositols and their phosphate analogues.[7] Synthesis of modified *myo*-inositol derivatives might be carried out with *myo*-inositol as the starting material, but this approach suffers from the inherent disadvantage of having to resolve the racemates at a suitable stage of the synthesis, if an optically active compound is desired. Use of chiral natural products as the starting material has some advantage in this respect. Ferrier rearrangement[8] has been sporadically employed for the conversion of a sugar derivatives into chiral inositol derivatives.[9] We report here the application of the Ferrier methodology to the synthesis of D-*myo*-inositol 1,2,6-trisphosphate (**1**) and its 1-dephosphorylated analogue, D-*myo*-inositol 2,6-bisphosphate (**2**).



I(1,4,5)P_3



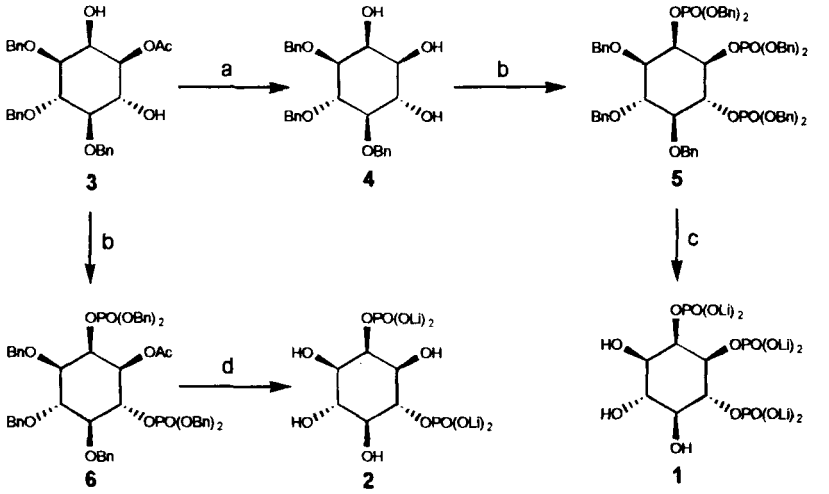
I(1,2,6)P_3 (**1**)



I(2,6)P_2 (**2**)

RESULTS AND DISCUSSION

1-*O*-Acetyl-3,4,5-tri-*O*-benzyl-D-*myo*-inositol (**3**) was prepared by the Ferrier rearrangement from D-glucose essentially according to the reported procedure.[9a] Base catalyzed solvolysis of **3** using MeONa/MeOH gave D-*myo*-I(3,4,5)Bn_3 (**4**) in 95% yield. Compound **4** was phosphorylated by successive treatments with *N,N*-diisopropyldibenzylphosphoramidite and 1H-tetrazole, and then H_2O_2 to give protected



Scheme. a. NaOMe, MeOH. b. (i) *N,N*-diisopropyldibenzylphosphoramidite, 1H-tetrazole, DMF, (*ii*) 30% H_2O_2. c. (*i*) H_2 (1 atm), $Pd(OH)_2$, MeOH. (*ii*) pH 10 (LiOH). d. H_2, $Pd(OH)_2$. (ii) 1N LiOH, (iii) H^+ ion exchange, (iv) pH 10 (LiOH).

I(1,2,6)P_3, **5**. Hydrogenolysis of **5** in the presence of $Pd(OH)_2$ catalyst was followed by addition of LiOH to pH 10 to give the lithim salt of α-trinositol, **1**.

Direct phosphorylations of **3** with the same procedure as described for **6** gave protected D-I(2,6)P_2. Hydrogenolysis and the base catalyzed hydrolysis of **6** followed by ion-exchange chromatography and pH adjustment to 10 gave the lithium salt of D-I(2,6)P_2.

Thus, we accomplished the chemical synthesis of biologically interesting natural product, α-trinositol and the first synthesis of D-*myo*-inositol 2,6-bisphosphate (**2**) which might be useful in evaluating the importance of the 2-phosphate group in the action of α-trinositol.

EXPERIMENTAL

3,4,5-Tri-*O*-benzyl-D-*myo*-inositol (4). A solution of **3** (80.8 mg, 0.16 mmol) and sodium methoxide (20.3 mg, 0.36 mmol) in anhydrous methanol (2 mL) was stirred at room temperature for 30 min. The mixture was poured into a saturated aq $NaHCO_3$

solution and extracted with CH_2Cl_2. The combined organic layer was dried over anhydrous Na_2SO_4 and concentrated to give a white solid **4** (70.2 mg, 95%). ^1H NMR ($CDCl_3$): δ 3.30 (t, J = 9.4, 1H, H-5), 3.38 (dd, J = 9.4, 3.1 Hz, 1H, H-1), 3.49 (dd, J = 9.4, 3.1 Hz, 1H, H-3), 3.87 (t, J = 9.4 Hz, 1H, H-6), 3.93 (t, J = 9.4 Hz, 1H, H-4), 4.18 (t, J = 3.1 Hz, 1H, H-2), 4.70-4.95 (m, 6H, 3PhC**H**$_2$), 7.29-7.32(m, 15H, Ph); ^{13}C NMR ($CDCl_3$): δ 69.21, 71.83, 72.78, 72.84, 75.36, 75.76 (inositol ring carbons), 80.19, 81.22, 82.58 (3PhC**H**$_2$), 127.04-135.57 (Ph); Mass Spectrum (FAB), *m/z* 473 (M^++ 23); $[\alpha]_D^{32}$ -2.7 (*c* 1.79, $CHCl_3$).

3,4,5-Tri-*O*-benzyl-D-*myo*-inositol 1,2,6-tris(dibenzyl phosphate) (5). To a solution of **4** (46 mg, 0.102mmol) and 1H-tetrazol (28 mg, 0.40 mmol) in dimethylformamide (2 mL), was added *N,N*-diisopropyldibenzylphosphoramidite (352.6 mg, 1.02 mmol), and the mixture was stirred for 5 h at room temperature. Sodium phosphate buffer (1N, pH 7, 2 ml) and 30% aq H_2O_2 (3 mL) were added and the solution was stirred overnight. Ethyl acetate was added to the mixture, and the organic layer was separated and washed with 1% aq $KHSO_4$, 1% aq $NaHCO_3$, and then saturated aq NaCl. The organic layer was dried over anhydrous $MgSO_4$, filtered and the filtrate was concentrated under reduced pressure. The residue was separated by silica gel chromatography to give **5** as an oil (76.8 mg, 65%). ^1H NMR ($CDCl_3$): δ 3.47 (dd, J = 9.3, 3.1 Hz, 1H, H-3), 3.49 (app.t, J = 9.3 Hz, 1H, H-5), 3.88 (app.t, J = 9.3 Hz, 1H, H-4), 4.34 (app.t, J = 8.7 Hz, 1H, H-1), 4.43-4.85 (m, 6H, 3PhC**H**$_2$), 4.88-5.19 (m, 13H, 6PhC**H**$_2$ & H-6), 5.48(br d, J = 8.7 Hz, 1H, H-2), 7.01-7.42 (m, 45H, Ph); ^{13}C NMR ($CDCl_3$) δ 69.09-69.90 [3P(O)(OC**H**$_2$Ph)$_2$], 72.71, 74.62, 74.69, 75.29, 75.81, 77.40, 78.24, 80.39, 80.67 (inositol ring carbons & 3PhC**H**$_2$), 138.8-127.96 (Ph); ^{31}P NMR ($CDCl_3$) : δ -1.42, -0.46, -0.06; $[\alpha]_D^{29}$ = -2.1 (*c* 2.5, $CHCl_3$). Crude **5** was used in the next step without further purification.

Lithium salt of α-trinositol (1). A mixture of **5** (63.7 mg, 55 μmol) and $Pd(OH)_2$ (100 mg) in methanol (5 mL) was stirred under H_2 gas (1 atm) at room temperature. After 3 days the mixture was filtered through celite and washed with water. The filtrate was concentrated under reduced pressure, and the residual aqueous solution was adjusted to pH 10 with 1N aqueous LiOH, and then lyopilized to give **1** as a white powder. ^1H NMR (D_2O, pH 10) δ 3.42 (dd, J = 10.0, 1.9 Hz, 1H, H-3), 3.48(app. t, J = 9.4 Hz, 1H,

H-5), 3.81 (app. t, J = 10.0 Hz, 1H, H-4), 3.92 (m, 1H, H-1), 4.23 (app. q, J = 8.7 Hz, 1H, H-6), 4.68 (br d, J = 6.8 Hz, 1H, H-2); ^{13}C NMR (D_2O, pH 10): δ 74.64, 75.15, 75.96, 77.75, 78.12 (2C); ^{31}P NMR (D_2O, pH 10) δ 5.08, 5.44, 5.86; $[\alpha]_D^{29}$ = -16.9 (c 0.56, H_2O, pH 10), lit.[5b] $[\alpha]_D^{25}$ -19.5 (*c* 1.1, H_2O).

1-*O*-Acetyl-3,4,5-tri-*O*-benzyl-D-*myo*-inositol 2,6-bis(dibenzyl phosphate) (6) To a solution of **3**[9a] (90 mg, 0.18 mmol) and 1H-tetrazol (50 mg) in CH_2Cl_2 (10 mL), was added *N,N*-diisopropyldibenzylphosphoramidite (330 mg, 0.95 mmol), and the mixture was stirred for 3 h at room temperature. Sodium phosphate buffer (1N, pH 7, 3 mL) and H_2O_2 (30%, 3 ml) were added and the solution was stirred overnight. Ethyl acetate was added to the mixture, and the organic layer was separated and washed with 1% aq $KHSO_4$, 1% aq $NaHCO_3$, and then saturated aq NaCl. The organic layer was dried over anhydrous $MgSO_4$, filtered and the filtrate was concentrated under reduced pressure. The product mixture was separated by silica gel chromatography to give **6** as an oil (87 mg, 48 %). ^{1}H NMR ($CDCl_3$) δ 1.78 (s, 3H, CH_3CO), 3.56-3.64 (m, 2H, H-3 & H-5), 3.92 (dd, J = 9.4, 9.3 Hz, 1H, H-4), 4.55-5.26 (m, 17H, 7PhCH_2, H-1, H-2 & H-6), 7.06-7.44 (m, 35H, Ph); ^{13}C NMR ($CDCl_3$) δ 20.52 (CH_3CO), 69.14–69.44 [2P(O)(OCH_2Ph)$_2$], 70.23, 74.18, 77.59, 78.34, 80.49, 81.12 (inositol ring carbons), 72.58, 75.41, 75.89 (3PhCH_2, assigned by DEPT-135), 170.26 (CH_3CO); ^{31}P NMR ($CDCl_3$) δ -1.09, -0.61; $[\alpha]_D^{25}$ = + 0.33 (*c* 1.64, $CHCl_3$). Crude **6** was used in the next step without further purification.

Lithium salt of D-*myo*-inositol 2,6-bisphosphate (2). A mixture of **6** (32.7 mg, 32.3 μmol) and $Pd(OH)_2$ (68 mg) in methanol (3 mL) was stirred under H_2 gas (1 atm) at room temperature. After 3 days the mixture was filtered through celite, and washed with water. 1N LiOH (3 mL) was added to the filtrate and the mixture was stirred at 80 °C. After 3 h the mixture was cooled to room temperature and then passed through the ion exchange resin (Dowex 50W-hydrogen, strongly acidic). The aqueous layer was adjusted to pH 10 with 1N aqueous LiOH and lyophilized to give compound **2**. ^{1}H NMR (D_2O, pH 10) δ 3.45 (app t, J = 9.3 Hz, 1H, H-5), 3.49 (dd, J = 10.0, 2.5 Hz, 1H, H-3), 3.63 (br d, J = 9.3 Hz, 1H, H-1), 3.82 (app t, J = 10.0 Hz, 1H, H-4), 4.20 (app. q, J = 9.3 Hz, 1H, H-6), 4.52 (br d, J = 9.3 Hz, 1H, H-2); ^{31}P NMR (D_2O, pH 10) δ 5.55, 5.61; $[\alpha]_D^{22}$ = -4.3 (*c* 0.7, H_2O, pH 10). The spectral data of **2** were satisfactorily compared with those of DL-*myo*-inositol 2,6-bisphosphate which was prepared by a different synthetic route.[10]

ACKNOWLEDGEMENT

This work was supported by research grants from the Korea Science and Engineering Foundation/Center for Biofunctional Molecules, and the Ministry of Education/Basic Science Research Institute Fund (3437-96).

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