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Note

Synthesis of the 3-deoxy-3-*C*-(phosphonomethyl) analogue of 1D-*myo*-inositol 3-(dihydrogenphosphate) *

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Interest in the role of phosphatidylinositols (PtdIns) in the cell-signaling process has exploded in the last few years with the discovery of the phosphatidylinositol 3-kinase [PtdIns 3-kinase] that is responsible for the production of 3-phosphorylated phosphatidylinositols, namely PtdIns(3)*P*, PtdIns(3,4)P_2, and PtdIns(3,4,5)P_3. Such lipids have been found in many cells[1], especially in transformed fibroblasts[2,3] where they have been associated with stimulation of cell proliferation and the onset of oncogenesis.

Phosphonates have found considerable use as long-lived agonists or antagonists of natural phosphates in that they possess unique stabilities toward the actions of hydrolase enzymes such as phosphatases. The biochemical stability of phosphonates has been exploited in the study of a number of enzymes including dinucleoside triphosphate hydrolase[4] and phosphatidyltransferases[5]. Many nonhydrolyzable analogues of inositol phosphates have recently been used to probe the process of Ca^{2+} mobilization[6,7]. Recently, Kulagowski[8] has reported the synthesis of a phosphonate analogue of (±)-*myo*-inositol 1-(dihydrogenphosphate) as a biochemical probe to aid in the study of inositol monophosphatase, the enzyme responsible for the hydrolysis of (±)-*myo*-inositol 1-(dihydrogenphosphate) to *myo*-inositol.

It has recently been demonstrated[9–11] that certain 3-modified analogues of 1D-*myo*-inositol enter the PtdIns cycle[12] and serve as substrates for PtdIns synthase, the first enzyme of that pathway. As part of our ongoing program[9,10] to develop 3-modified 1D-*myo*-inositol analogues as fraudulent substrates for PtdIns synthase, as well as probes for other enzymes of the PtdIns cycle, we have

* Nomenclature used herein conforms to the "Nomenclature of Cyclitols" (1973 Recommendations), *Pure Appl. Chem.*, 37 (1974) 285–297.

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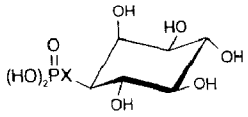
synthesized 1D-3-deoxy-3-*C*-(phosphonomethyl)-*myo*-inositol * (**2**), which is a close analogue of the 3-(dihydrogenphosphate) **1**



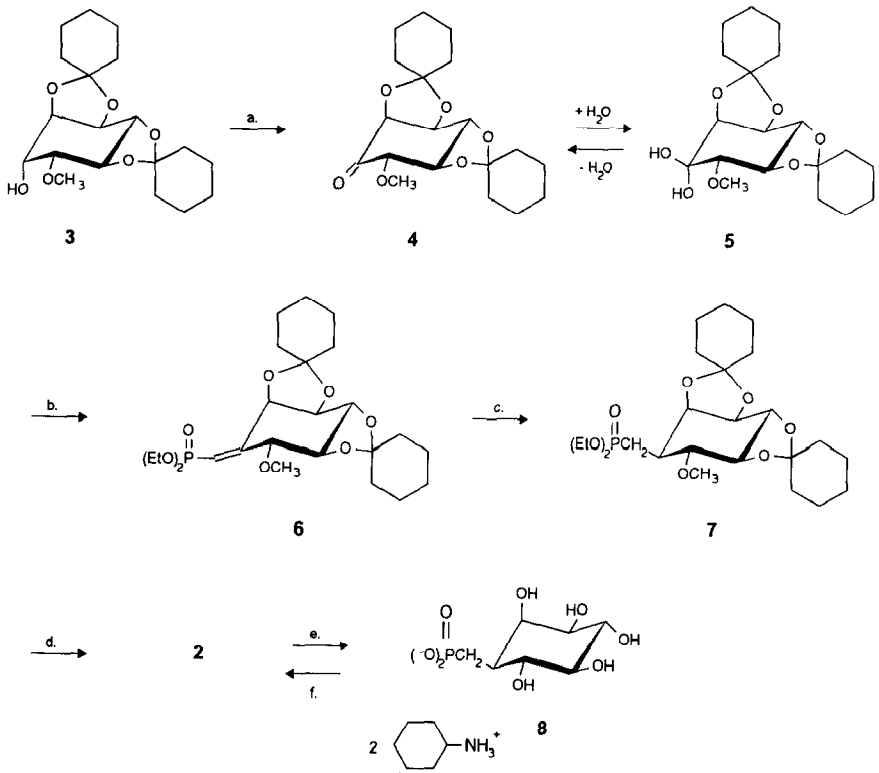
1 X = O

2 X = CH_2

and is the optically active 1D form of the racemic compound synthesized by Kulagowski[8]. (Note that since submission of this paper, Kozikowski, Powis, and co-workers have reported a synthesis and evaluation of the 1D-3-deoxy-3-*C*-(phosphonomethyl) analogue **2** as a cell-growth inhibitor[13]. The synthetic route, also from L-quebrachitol, is considerably more lengthy than that presented herein.) This analogue, which substitutes a P–C–C linkage for the normal P–O–C linkage of the phosphate derivative, might provide insight into the physiological effects of 3-phosphorylated 1D-*myo*-inositol phospholipids.

In order to prepare the optically active phosphonate analogue, naturally occurring L-quebrachitol was utilized as a starting material. Protection of L-quebrachitol was accomplished as its bis(cyclohexylidene)acetal, 1L-1,2:3,4-di-*O*-cyclohexylidene-5-*O*-methyl-*chiro*-inositol (**3**) (ref 14). Alcohol **3** was converted to the ketone, 2L-2,3:4,5-di-*O*-cyclohexylidene-6-*O*-methyl-2,3,5/4,6-pentahydroxycyclohexanone (**4**), via RuO_4 oxidation in the presence of K_2CO_3 (Scheme 1). Other methods of oxidation were found to give incomplete yields and resulted in hydrolysis of the cyclohexylidene acetals. Hydration of **4** to the *gem*-diol **5** (as exemplified by ^1H NMR spectroscopy and loss of the carbonyl stretching frequency in the IR spectrum) occurred readily on standing. Regeneration of **4** via dehydration of **5** was accomplished in refluxing toluene with azeotropic removal of water. The phosphinylmethylene moiety was introduced utilizing Horner–Emmons methodology by addition of the sodium salt of tetraethyl methylenediphosphonate to the ketone (**4**). Although Kulagowski[8] has reported a similar coupling of a racemic *myo*-inosose with the lithium salt of tetramethyl methylenediphosphonate in tetrahydrofuran, we obtained superior results in our example by employing an analogous sodium salt in toluene. Only one isomer, 1L-1,2:3,4-di-*O*-cyclohexylidene-6-[(diethoxyphosphinyl)methylene]-5-*O*-methyl-1,2,4/3,5-cyclohexanepentol (**6**, stereochemistry unspecified for the exocyclic double bond), was evident by TLC and NMR spectroscopy. Hydrogenation of the resulting vinyl phosphonate **6** over platinum resulted in addition of hydrogen from the α face at C-6 of the molecule to yield exclusively the C-3 (1D-*myo* numbering) phosphonate **7** having the desired

* Compound **2** may also be considered as 1L-1-deoxy-1-*C*-(phosphonomethyl)-*myo*-inositol.



Scheme 1. Reagents: (a) RuO_2, $NaIO_4$, K_2CO_3, H_2O, CH_2Cl_2; (b) NaH, tetraethyl methylenephosphonate, toluene; (c) H_2, PtO_2, EtOAc; (d) BBr_3, CH_2Cl_2, MeOH; (e) cyclohexylamine, H_2O; (f) DEAE Sephadex A-25, TEAB buffer.

myo-inositol stereochemistry. The three-step transformation (**3** → **7**) occurs with net inversion of stereochemistry at C-6 (C-3, 1D-*myo* numbering) as evidenced by a single downfield resonance (a "pseudo" triplet) for an equatorial proton at C-2 having $J_{1,2} = J_{2,3} = 4.6$ Hz. (The other resonances for H-3 and H-4 were overlapping and complex.) Subsequent deprotection with boron tribromide in dichloromethane, followed by workup in methanol, resulted in demethylation, phosphonate deprotection, and concomitant acetal hydrolysis to yield **2**, which was purified as the bis(cyclohexyl)ammonium salt **8**. The structure for **2** is supported by ^1H and ^{31}P NMR data, together with high-resolution mass spectrometry. The ^1H NMR spectrum was found to be dependent on the pH of the solution, with variations in chemical shift being noted for changes in pH (actually, pD in D_2O as estimated with pHydrion paper). Reports on the biological evaluations of this analogue will be forthcoming.

EXPERIMENTAL

General methods.—^1H NMR spectra were recorded at 250 MHz as solutions that were typically 1–2% (w/v) using a Bruker AC 250 spectrometer. ^1H Chemical shifts are reported in δ-units downfield from an internal tetramethylsilane (Me_4Si)

standard in chloroform-*d*, while those in deuterium oxide are reported downfield from an external 4,4-dimethyl-4-silapentane-1-sulfonate (DSS) standard. Multiplicities are first-order values in Hz and are indicated as: b, broad; s, singlet; d, doublet; dd, doublet of doublets; m, multiplet; p, pentet; q, quartet; t, triplet; ψt, "pseudo" triplet (i.e., a doublet of doublets having nearly equal *J* values). ^{1}H-Decoupled ^{13}C NMR spectra were recorded at 62.5 MHz. Chemical shifts (reported in δ units) for solutions in chloroform-*d* were measured using the solvent peaks as the standard, while those in deuterium oxide were measured against external DSS. For compounds **6** and **7** the data are reported as observed resonance lines with no attempt being made to discern ^{13}C–^{31}P spin–spin splitting. ^{1}H-Decoupled ^{31}P NMR spectra were recorded at 36.19 MHz using a Jeol FX90Q spectrometer. ^{31}P Chemical shifts are reported in δ-units with respect to an external 85% H_3PO_4 standard. Infrared spectroscopy was conducted using a Perkin–Elmer model 710B spectrophotometer. Electron-impact (EI^+) and negative-ion fast-atom bombardment (FAB) mass spectrometry were carried out on a VG ZAB-EQ (VG Analytical, Manchester, UK) instrument. Optical rotations were measured in the indicated solvent and concentration at the sodium D-line in a 1-dm cell at ambient temperatures (20–27°C), on a Perkin–Elmer model 241 spectropolarimeter. Adsorption chromatography was carried out using E. Merck Silica Gel-60 products: (*a*) TLC on 0.2-mm aluminum backed plates; (*b*) open column chromatography using 38–63 μm silica gel. TLC visualizations were carried out using an anisaldehyde–H_2SO_4 dip with heating[15]. All solvents and reagents were reagent grade and were used directly unless noted otherwise. Tetrahydrofuran (THF) was distilled from potassium–benzophenone ketyl at atmospheric pressure. Solvents, unless otherwise stated, were evaporated at ca. 40°C/30 torr on a Büchi Rotovapor. Elemental analyses were carried out by Atlantic Microlab, Inc., Norcross, GA.

2L-2,3:4,5-Di-O-cyclohexylidene-6-O-methyl-2,3,5/4,6-pentahydroxycyclohexanone (**4**).—To a vigorously stirred suspension of RuO_2 (37 mg) in an aq solution of $NaIO_4$ (2.2 g, 45 mL) was added K_2CO_3 (5 mL, 0.4 M) at ambient temperature. When the resulting yellow color persisted and a solution had formed (RuO_4), a solution of **3** (ref 14) (1.31 g, 3.70 mmol) in CH_2Cl_2 (45 mL) was added dropwise over 25 min. The resulting two-phase suspension was allowed to stir for 24 h, maintaining the pH at ca. pH 9.0 by periodic addition of aq K_2CO_3. The reaction was quenched by the addition of 2-propanol (15 mL) and was allowed to stir for an additional 30 min. The mixture was then filtered to give a two-phase suspension. The organic phase was dried ($MgSO_4$) and evaporated to give a colorless glass. Column chromatography, eluting with CH_2Cl_2, separated the desired ketone that was isolated as a colorless glass (1.06 g, 82%): R_f 0.39 (1:4 EtOAc–hexanes); $[\alpha]_D^{21}$ −15.5° (*c* 1.24, $CDCl_3$); IR: 1740 cm^{-1} (C=O); ^{1}H NMR data ($CDCl_3$, 250 MHz): δ 1.61–1.87 (m, 20 H, CH_2, cyclohexyl), 3.53 (s, 3 H, OCH_3), 3.57–3.69 (m, 2 H, H-4, H-6), 3.98 (dd, 1 H, $J_{5,6}$ 8.2, $J_{4,5}$ 11.1 Hz, H-5), 4.62–4.73 (m, 2 H, H-2, H-3). Anal. Calcd for $C_{19}H_{28}O_6 \cdot 0.2H_2O$: C, 64.09; H, 8.04. Found: C, 63.82; H, 7.87.

1L-1,2:3,4-Di-O-cyclohexylidene-6-[(diethoxyphosphinyl)methylene]-5-O-methyl-1,2,4/3,5-cyclohexanepentol (**6**).—A solution of **4** (1.20 g, 3.40 mmol) in toluene (40 mL) was heated under reflux for 12 h with a Dean–Stark apparatus to collect the azeotrope. When the generation of water was complete, the solution was cooled to ambient temperature under anhydrous conditions. A separate solution of NaH (120 mg, 80% suspension in mineral oil, washed with hexanes) in toluene (5 mL) was prepared and treated with tetraethyl methylenediphosphonate dropwise, and the mixture was allowed to stand for 30 min under N_2. The solution containing the phosphonate salt was added to the solution containing the ketone that was maintained in an ice-bath, and the mixture was allowed to stir for 20 min at 0°C, followed by 20 h at ambient temperature. The mixture was concentrated, and the resulting syrup was partitioned between CH_2Cl_2 (20 mL) and H_2O (20 mL). The organic phase was dried ($MgSO_4$), concentrated, and percolated through silica gel, eluting with 1:4 EtOAc–hexanes to separate the ketone starting-material from **6**. The latter was isolated as a colorless glass (1.13 g, 68%): R_f 0.21 (1:3 EtOAc–hexanes); $[\alpha]_D^{20}$ +14.6° (*c* 2.2, CH_2Cl_2); ^{1}H NMR data ($CDCl_3$, 250 MHz): δ 1.25–1.40 (m, 6 H, CH_3CH_2O), 1.49–1.74 (m, 20 H, CH_2, cyclohexyl), 3.45 (dd, 1 H, $J_{4,5}$ 11.0, $J_{3,4}$ 7.4 Hz, H-4), 3.53 (s, 3 H, OCH_3), 3.80 (dd, 1 H, $J_{5,6}$ 6.8 Hz, H-5), 4.03–4.14 (m, 4 H, CH_3CH_2O), 4.45 (dψt, 1 H, $J_{2,3} = J_{3,4} = 7.5$ Hz, H-3), 4.82–4.85 (m, 1 H, H-6), 5.03 (d, 1 H, H-2), 6.13 (dd, 1 H, *J* 15.6, *J* 2.07 Hz, CH=C); ^{13}C NMR data ($CDCl_3$, 62.5 MHz): δ 15.2, 15.3, 15.4, 22.5, 22.5, 22.7, 22.9, 23.9, 24.0, 33.5, 35.5, 35.9, 56.5, 60.4, 60.5, 60.7, 60.8, 73.2, 73.5, 74.5, 74.6, 75.6, 76.6, 111.5, 113.2, 114.6, 117.6, 155.2, 155.3; HRMS: Calcd for $C_{24}H_{39}O_8P$: 486.2380; found: 486.2375.

1D-1,2:5,6-Di-O-cyclohexylidene-3-deoxy-3-C-[(diethoxyphosphinyl)methyl]-4-O-methyl-myo-*inositol* (**7**).—To a solution of **6** (1.00 g, 2.06 mmol) in EtOAc (15 mL) was added a catalytic amount of PtO_2. This mixture was stirred under 50 psi H_2 for 20 h. Successive filtration, concentration, and drying of the residue in vacuo for 14 h at ambient temperature gave **7** (939 mg, 93%) as a colorless syrup; R_f 0.45 (EtOAc); ^{1}H NMR data (D_2O, 250 MHz): δ 1.33 (t, 6 H, OCH_2CH_3), 1.54–1.68 (m, 20 H, CH_2, cyclohexyl), 2.21–2.33 (m, 2 H, CH_2), 3.23–3.28 (m, 2 H), 3.50–3.55 (m, 2 H), 3.56 (s, 3 H, OCH_3), 4.05–4.17 (m, 5 H, OCH_2CH_3, CH), 4.55 (ψt, 1 H, $J_{2,3} = J_{1,2} = 4.64$ Hz, H-2); ^{13}C NMR data (D_2O, 62.5 MHz): δ 15.2, 20.7, 22.5, 22.8, 23.8, 33.7, 35.2, 35.3, 37.0, 37.1, 57.4, 60.3, 74.1, 75.4, 78.8, 79.6, 79.7, 108.5, 110.7; HRMS: Calcd for $C_{24}H_{41}O_8P$: 488.2537; found: 488.2534.

1D-3-Deoxy-3-C-(phosphonomethyl)-myo-*inositol* (**2**).—To a solution of **7** (925 mg, 1.89 mmol) in CH_2Cl_2 (10 mL) was added dropwise a solution of BBr_3 (9.45 mL of a M solution in CH_2Cl_2; 9.45 mmol). The resulting solution was stirred at 0°C under N_2 for 50 min, followed by stirring at ambient temperature for 16 h, at the end of which time the mixture was concentrated to give a brown residue. The residue was successively dissolved in dry MeOH (3 × 15 mL), and the solvent was evaporated to eliminate boron-containing byproducts. The residual gum was partitioned between H_2O (20 mL) and CH_2Cl_2 (20 mL), and the aq phase was

separated, filtered through Celite, concentrated to 10 mL volume, and treated dropwise with cyclohexylamine until pH 9.0 was attained. The deep-brown mixture was kept for 30 min at ambient temperature under N_2, at the end of which time the mixture was extracted with EtOAc (15 mL), and the aq phase was separated, decolorized with carbon, filtered through Celite, and lyophilized to yield 589 mg (68%) of the bis(cyclohexyl)ammonium salt **8**. Column chromatography on DEAE Sephadex A-25 using aq triethylammonium hydrogencarbonate (TEAB) as eluent, followed by decationization with Dowex-50 × 2 (H^+), gave **2** (309 mg, 63% from **7**) after lyophilization.

Physicochemical data for **2**: $[\alpha]_D^{22}$ +1.8° (*c* 0.67, H_2O); ^1H NMR data (D_2O, pD ~ 6, 250 MHz): δ 1.73–2.04 (m, 3 H, H-3, CH_2), 3.13–3.29 (m, 2 H, H-4, H-5), 3.30–3.54 (m, 2 H, H-1, H-6), 4.01 (bs, 1 H, H-2); ^{31}P NMR data (D_2O, pD ~ 3, 36.19 MHz): δ 27.259; HRMS: Calcd for $C_7H_{15}O_8P$: 257.0426; found: 257.0437.

Physicochemical data for **8**: ^1H NMR data (D_2O, 250 MHz): δ 1.66–1.96 (m, 20 H, CH_2, cyclohexyl), 3.02–3.21 (m, 3 H), 3.25–3.38 (m, 2 H), 3.52–3.59 (2 H, AB of an ABX, CH_2P), 3.91 (p, 2 H, CH, cyclohexyl), 4.18 (bs, 1 H, H-2).

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REFERENCES

1 P.W. Majerus, T.S. Ross, T.W. Cunningham, K.K. Caldwell, A.B. Jefferson, and V.S. Bansal, *Cell*, 63 (1990) 459–465.
2 M. Whitman, C.P. Downes, M. Keeler, T. Keller, and L. Cantley, *Nature*, 332 (1988) 644–646.
3 D.L. Lips, P.W. Majerus, F.R. Gorga, A.T. Young, and T.L. Benjamin, *J. Biol. Chem.*, 264 (1989) 8759–8763.
4 G.M. Blackburn, G.R. Thatcher, G.E. Taylor, M. Prescott, and A.G. McLennan, *Nucleic Acids Res.*, 15 (1987) 6991–7004.
5 L.A. Vargas, L.X. Miao, and A.F. Rosenthal, *Biochim. Biophys. Acta*, 796 (1984) 123–128.
6 C.E. Dreef, W. Schiebler, G.A. van der Marel, and J.H. van Boom, *Tetrahedron Lett.*, 32 (1991) 6021–6024.
7 J.P. Lyssikatos and M.D. Bednarski, *Bioorg. Med. Chem. Lett.*, 3 (1993) 685–688.
8 J.J. Kulagowski, *Tetrahedron Lett.*, 30 (1989) 3869–3872.
9 T.-L. Shih, S.C. Johnson, D.J.A. Schedler, D.C. Baker, J. Dahl, K. Dower, T.L. Benjamin, and L. Anderson, *Abstr. Papers 201st ACS Natl. Meeting*, Atlanta, GA, American Chemical Society, Washington, DC, 1991, CARB 38.
10 S.C. Johnson, J. Dahl, T.-L. Shih, D.J.A. Schedler, L. Anderson, T.L. Benjamin, and D.C. Baker, *J. Med. Chem.*, 36 (1993) in press.
11 (a) A.P. Kozikowski, A.H. Fauq, G. Powis, and D.C. Melder, *J. Am. Chem. Soc.*, 112 (1990) 4528–4531; (b) G. Powis, I.A. Aksoy, D.C. Melder, S. Aksoy, H. Eichinger, A.H. Fauq, and A.P. Kozikowski, *Cancer. Chemother. Pharmacol.*, 29 (1991) 95–104; (c) A.P. Kozikowski, A.H. Fauq, G. Powis, and D.C. Melder, *Med. Chem. Res.*, 1 (1991) 277–282.
12 D.E. Vance, in G. Zubay (Ed.), *Biochemistry*, 2nd ed., Macmillan, New York, 1988, pp 698–701.

13 A.P. Kozikowski, G. Powis, A. Gallegos, and W. Tückmantel, *Bioorg. Med. Chem. Lett.*, 3 (1993) 1323–1326.
14 (a) S.D. Gero, *Tetrahedron Lett.*, (1966) 591–595; (b) S.J. Angyal, G.C. Irving, D. Rutherford, and M.E. Tate, *J. Chem. Soc.*, (1965) 6662–6664.
15 J.P. Schaumberg, G.C. Hokanson, J.C. French, E. Smal, and D.C. Baker, *J. Org. Chem.*, 50 (1985) 1651–1656.