

**A SIMPLE SYNTHESIS OF *D-MYO*-INOSITOL 4-PHOSPHATE  
AND *D-MYO*-INOSITOL 6-PHOSPHATE**

Sarah Iacobucci and Marc d'Alarcao\*

*Michael Chemistry Laboratory, Department of Chemistry  
Tufts University, Medford, MA 02155*

**ABSTRACT**

A simple synthesis of the title compounds from *myo*-inositol is reported. The procedure involves the regioselective dibutyltin oxide-promoted acylation of racemic 1,2:5,6-dicyclohexylidene-*myo*-inositol (**2**) with 1-(*S*)-(-)-camphanil chloride followed by chromatographic separation of the resulting diastereomers.

The discovery that inositol phosphates are intimately involved in various biological signal transduction processes<sup>1,2</sup> has rekindled considerable interest in inositol chemistry in recent years. Of particular interest has been the preparation of optically pure *myo*-inositols in which the six hydroxyl groups are differentially protected.<sup>3</sup> As part of a program aimed at the synthesis of inositol glycans implicated in insulin signal transduction,<sup>2,4</sup> we needed access to large amounts of an optically pure *D-my*o-inositol derivative maintaining a free 6-hydroxyl group and protected elsewhere by a scheme that would allow selective deprotection at the 1-hydroxyl group. In this communication we describe a simple synthesis of inositols **3a** and **3b** which meet these requirements, and their respective elaboration to *D-my*o-inositol 4-phosphate (**1**) or its enantiomer<sup>5</sup> *D-my*o-inositol 6-phosphate,<sup>6</sup>

---

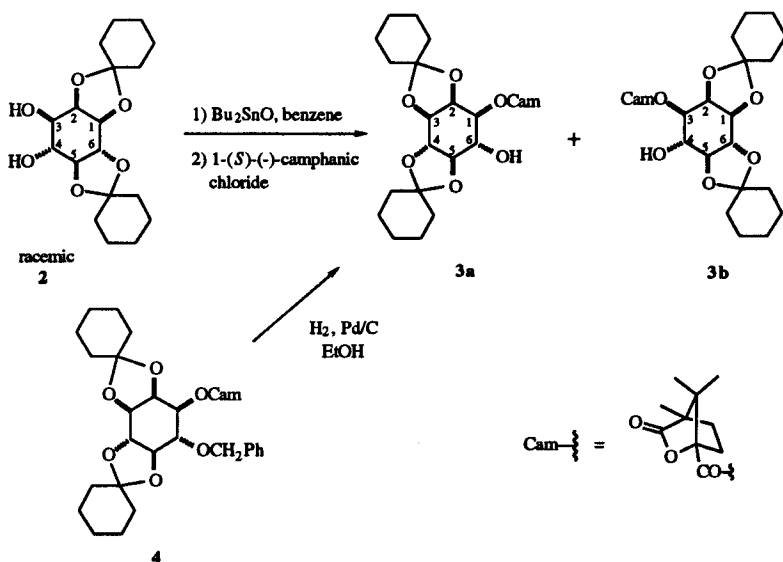
\* To whom correspondence may be addressed

an intermediate in the biological dephosphorylation cascade of 1,4,5-inositol triphosphate.<sup>1</sup>

### Results and Discussion

Racemic 1,2:5,6-dicyclohexylidene-*myo*-inositol (**2**, FIG 1) was prepared from *myo*-inositol by a variation of the known procedure.<sup>7</sup> Treatment of diol **2** with  $\text{Bu}_2\text{SnO}$  in benzene to produce the dibutylstannylene derivative<sup>8</sup> followed by addition of 1-(*S*)-(-)-camphanic chloride resulted in selective acylation at the C-3(1) hydroxyl to produce alcohols **3** in quantitative yield.

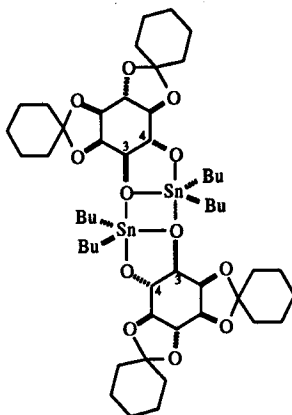
FIG 1



It is interesting to note that benzylation of the stannylene derivative of **2** has also been reported<sup>8</sup> to be regioselective, but with the *opposite* regiochemistry; i.e. benzylation occurs predominantly at the C-4(6)-hydroxyl. These data may be reconciled, as suggested by David and Hanessian<sup>9</sup> for other carbohydrate stannylenes, if the reactive stannylene species in solution is a dimer such as **5**

(FIG 2).<sup>10</sup> Faster reaction of electrophiles at the oxygen atoms which occupy only the apical positions of the trigonal bipyramids would be expected to produce the 4-acylated or alkylated inositol as the kinetic product. Subsequent vicinal acyl (but not alkyl) migration would account for the ultimate formation of the observed 3-acyl product.

FIG 2



5

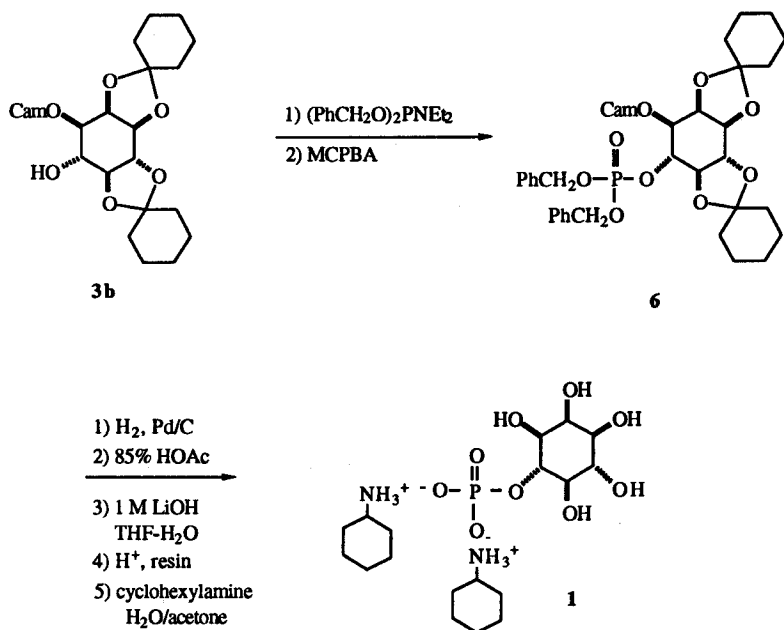
It has been suggested<sup>9</sup> that in stannylene complexes of diols the more electronegative oxygen atom occupies the apical position. If we assume that the oxygen at C-4 of **2** is more electronegative than that at C-3, then the proposed stannylene structure **5** and the apparent thermodynamic preference for 3-acylation follow consistently. However, we do not know why the C-4 oxygen of **2** may be more electronegative.

Separation of the camphanyl diastereomers **3** was accomplished by silica gel chromatography. The identity of the 1-camphanyl-*D*-myo-inositol-containing diastereomer **3a** was established by its preparation from known inositol **4** whose structure has been determined unequivocally<sup>11</sup> (FIG 1).

The synthesis of *D*-myo-inositol-4-phosphate (**1**) was completed as shown in FIG 3. Phosphitylation and oxidation by the Fraser-Reid procedure<sup>8</sup> afforded fully

protected phosphoinositol **6** in quantitative yield. Deprotection was accomplished by catalytic hydrogenolysis (Pd/C), acidic deketalization (85% HOAc), and LiOH deacylation to afford **1** in overall 41% of the theoretical yield (from **2**). Similar treatment of the 1-camphanyl-*D*-myo-inositol diastereomer **3a** produced *D*-myo-inositol 6-phosphate in 41% yield from **2**.

FIG 3



### Experimental Section

All moisture sensitive reactions were carried out under an argon or nitrogen atmosphere. Organic extracts were dried with anhydrous magnesium sulfate. Solvents were removed *in vacuo* on a Büchi rotary evaporator. Solvents and reagents obtained from commercial sources were used without further purification with the following exception: benzene was distilled from sodium-benzophenone.

Reactions were routinely monitored by TLC on Baker glass backed silica gel plates (0.25  $\mu\text{m}$  thickness) with a 254 nm fluorescent indicator. The chromatograms were visualized by dipping in an ethanolic solution of 2.5% *p*-anisaldehyde, 3.5% sulfuric acid and 1% acetic acid followed by heating. Preparative separations were performed either by flash chromatography on Baker silica gel (40  $\mu\text{m}$ ), or by gravity chromatography on Baker silica gel (60-200  $\mu\text{m}$ ). Melting points are uncorrected. Phosphate assays were performed colorimetrically by the phosphomolybdate method.<sup>12</sup> NMR data were obtained on a Bruker AM-300FT NMR spectrometer. TMS (0.03%) was used as an internal standard for <sup>1</sup>H NMR and H<sub>3</sub>PO<sub>4</sub>-d<sub>3</sub> (85% in D<sub>2</sub>O) was used as an external standard for <sup>31</sup>P NMR. High resolution mass spectrometry data were obtained on a JEOL AX-505 or JEOL SX-102 mass spectrometer using FAB as the ionization method.

**1-(1-(*S*)-Camphanyl)-2,3:4,5-dicyclohexylidene-*D*-myo-inositol (3a) and 3-(1-(*S*)-camphanyl)-1,2:5,6-dicyclohexylidene-*D*-myo-inositol (3b).** A suspension of 1.42 g (4.18 mmol) racemic 1,2:5,6-dicyclohexylidene-*myo*-inositol (2)<sup>7</sup> and 1.07 g (4.29 mmol) dibutyltin (IV) oxide in 40 mL of benzene was fitted with a distillation head and placed in an oil bath at 110 °C until most of the benzene had distilled. An additional portion of benzene (40 mL) was added to the residue. To this was added 1.13 g (5.21 mmol) of (*1S*)-(-)-camphanic chloride and the reaction was stirred at 20 °C for 1 h. The mixture was poured into 100 mL of 1M aqueous NaHCO<sub>3</sub> and extracted with CHCl<sub>3</sub> (3 X 100 mL). The extracts were dried, evaporated, and carefully chromatographed on a 500 g silica gravity column (compound was loaded by dry packing) eluting with ether:hexane (7:3). This provided pure **3a** and **3b**, combined yield: 2.2 g (100 %). Alternatively, the two diastereomers may be separated by preparative HPLC: 1" diameter silica column, elution with 1:9 *i*-PrOH : hexane, flow rate 25 mL/min. Under these conditions **3b** eluted at 6.08 min and **3a** eluted at 6.95 min.

For **3a**:  $R_f = 0.37$  (70% ether-hexane)  $^1\text{H NMR}$  ( $\text{CDCl}_3$ )  $\delta$  1.00 (s, 3H,  $\text{CH}_3$ ), 1.13 (s, 3H,  $\text{CH}_3$ ), 1.26 (s, 3H,  $\text{CH}_3$ ), 1.40, 1.50 - 1.76 (2m, 21H), 1.96 (m, 1H), 2.09 (m, 1H), 2.47 (m, 1H), 2.67 (br s, 1H, OH), 3.40 (wt, 1H, H5), 3.74 (dd, 1H,  $J = 8.6, 10.1$  Hz, H4), 4.17 (dd, 1H,  $J = 7.2, 9.6$  Hz, H6), 4.32 (dd, 1H,  $J = 5.4, 8.6$  Hz, H3), 4.66 (wt, 1H, H2), 5.04 (dd, 1H,  $J = 5.0, 7.2$ , H1). MS (high resolution) found 521.2764 (calcd for  $\text{C}_{28}\text{H}_{41}\text{O}_9$   $[\text{M}+\text{H}]^+$  521.2750).

For **3b**:  $R_f = 0.45$  (70% ether-hexane)  $^1\text{H NMR}$  ( $\text{CDCl}_3$ )  $\delta$  1.04 (s, 3H,  $\text{CH}_3$ ), 1.07 (s, 3H,  $\text{CH}_3$ ), 1.13 (s, 3H,  $\text{CH}_3$ ), 1.40, 1.54 - 1.74 (2m, 21H), 1.95 (m, 1H), 2.07 (m, 1H), 2.50 (m, 1H), 2.81 (d, 1H,  $J = 3.4$  Hz OH), 3.40 (wt, 1H, H5), 3.73 (dd, 1H,  $J = 8.6, 10.2$  Hz, H6), 4.20 (ddd, 1H,  $J = 3.4, 7.2, 9.7$  Hz, H4), 4.32 (dd, 1H,  $J = 5.4, 8.6$  Hz, H1), 4.67 (wt, 1H, H2), 5.00 (dd, 1H,  $J = 5.0, 7.2$ , H3). MS (high resolution) found 521.2768 (calcd for  $\text{C}_{28}\text{H}_{41}\text{O}_9$   $[\text{M}+\text{H}]^+$  521.2750).

**3 - (1-(S)-Camphanyl)-1,2:5,6-dicyclohexylidene-4-(dibenzylphosphoryl)-D-myo-inositol (6)**. Alcohol **3b** (20 mg, 0.038 mmol) and 1*H*-tetrazole (32 mg, 0.46 mmol) were combined and dried by coevaporation with toluene (2 X 5 mL), then dissolved in  $\text{CH}_2\text{Cl}_2$  (1.5 mL). After adding the dibenzyl *N,N*-diethylphosphoramidite (36  $\mu\text{L}$ , 0.1152 mmol) the mixture was stirred at 20  $^\circ\text{C}$  and the progress of the reaction was monitored by TLC (85% ether-hexane). When the starting alcohol was consumed ( $\sim 1$  h) the reaction was cooled to -40  $^\circ\text{C}$  and a solution of *m*-chloroperbenzoic acid (66 mg, 0.31 mmol) in  $\text{CH}_2\text{Cl}_2$  (0.7 mL) was added. The reaction was warmed to 0  $^\circ\text{C}$ , allowed to stir for 30 min, then allowed to warm to 20  $^\circ\text{C}$ .  $\text{CH}_2\text{Cl}_2$  (5.5 mL) was added and the organic phase was washed with 10% aqueous  $\text{Na}_2\text{SO}_3$  (2 X 3 mL), 10% aqueous  $\text{NaHCO}_3$  (2 X 2 mL),  $\text{H}_2\text{O}$  (2 mL), and brine (2 mL), then dried ( $\text{MgSO}_4$ ) and chromatographed (flash, elution with 85% ether-hexane) to produce **6** (31 mg, crude product): This material may be purified to homogeneity by further chromatography (100% yield) or used crude in the next step.

R<sub>f</sub> = 0.67 (85% ether-hexane); <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 0.93 (s, 3H, CH<sub>3</sub>), 1.04 (s, 3H, CH<sub>3</sub>), 1.08 (s, 3H, CH<sub>3</sub>), 1.36, 1.56-1.72 (2m, 21H), 1.87 (m, 2H), 2.41(m1H), 3.57 (vt, 1H, J = 10 Hz), 3.83 (dd, 1H, J = 9, 10 Hz), 4.32 (dd, 1H, J = 6, 9 Hz), 4.61 (vt, 1H, J = 5 Hz), 4.92 (m, 1H), 5.02 (d, 2H, J = 8 Hz), 5.08 (dd, 1H, J = 7, 2 Hz), 5.16 (d, 1H, J = 8 Hz), 5.30 (dd, 1H, J = 5, 6 Hz), 7.32-7.38 (m, 10 H), characteristic impurities 7.45 (t), 7.65 (dd), 7.95 (dd), 8.15 (s). MS (high resolution) found 781.3361(calcd for C<sub>42</sub>H<sub>54</sub>O<sub>12</sub>P [M+H]<sup>+</sup> 781.3353).

**D-myo-Inositol 4-phosphate (1).** To a solution of the crude fully protected phosphate **6** (31 mg) in ethanol : CH<sub>2</sub>Cl<sub>2</sub> (1 : 0.4 mL) containing a few drops of acetic acid was added 10% Pd on C (20 mg) and the mixture was shaken on a Parr apparatus under an atmosphere of 50 PSIG H<sub>2</sub> for 24 h at 20 °C. The catalyst was removed by centrifugation and the solvent was removed *in vacuo*. To the residue was added 1.0 mL of 85% HOAc and the reaction was stirred for 24 h at 20 °C. The solvent was removed by repeated coevaporation with heptane. TLC of the residue indicated a single material (R<sub>f</sub> = 0.43, 1-butanol : EtOH : H<sub>2</sub>O, 5:4:1). This residue was dissolved in THF (1 mL) and 1M LiOH (1 mL) and stirred overnight at 20 °C. The solution was then acidified using 1M HCl and extracted with CH<sub>2</sub>Cl<sub>2</sub> (7 X 2 mL). To make the cyclohexylammonium salt BIORAD AG 50W-X4, H<sup>+</sup>, 200-400 mesh resin was added to the sample and stirred for 10 min and then filtered. Cyclohexylamine (100 μL) was added to the solution and stirred for 10 min and then concentrated. The residue was then dissolved in a minimum amount of H<sub>2</sub>O and acetone was added until the precipitation was complete. The <sup>1</sup>H NMR spectrum was identical to that previously reported.<sup>6</sup> MS (high resolution) found 259.0234 (calcd for C<sub>6</sub>H<sub>12</sub>O<sub>9</sub>P<sup>-</sup> [M-H]<sup>-</sup> 259.0219).

***D*-myo-Inositol 6-phosphate.** Treatment of alcohol **3a** (20 mg, 0.038 mmol) exactly as described above provided *D*-myo-inositol 6-phosphate (0.016mmol, 41% yield from **3a** by phosphate assay).

#### Acknowledgement

We are grateful to the Warner-Lambert Company and to the National Institute of Health (grant # DK44589-02) for financial support of this work. Mass spectra were obtained at the Harvard University Chemistry Department Mass Spectrometry Facility.

#### References

- (1) Berridge, M. J.; Irvine, R. F. *Nature* **1989**, *341*, 197.
- (2) Saltiel, A. R.; Cuatrecasas, P. *Am. J. Physiol.* **1988**, *255*, C1.
- (3) Billington, D. C. *Chem. Soc. Rev.* **1989**, *18*, 83; Bruzik, K. S.; Myers, J.; Tsai, M.-D. *Tetrahedron Lett.* **1992**, *33*, 1009; Aguiló, A.; Martín-Lomas, M.; Penadés, S. *Tetrahedron Lett.* **1992**, *33*, 401.
- (4) Plourde, R.; d'Alarcao, M.; Saltiel, A. R. *J. Org. Chem.* **1992**, *57*, 2606.
- (5) In this communication we are using the stereospecific numbering system for *myo*-inositol described by the International Union of Biochemistry: *Eur. J. Biochem.* **1989**, *180*, 485. By this system all *myo*-inositol derivatives are named and numbered as *D*-*myo*-inositols regardless of whether the substituents occupy the lowest numbered positions in the resulting name. For example, the enantiomer of *D*-*myo*-inositol 1-phosphate is *D*-*myo*-inositol 3-phosphate (not *L*-*myo*-inositol 1-phosphate).
- (6) Compound **1** and its enantiomer have been prepared enantioselectively previously: Vacca, J. P.; deSolms, S. J.; Huff, J.R.; Billington, D. C.; Baker, R.; Kulagowski, J. J.; Mawer, I. M. *Tetrahedron* **1989**, *45*, 5679, corrected optical rotation measurements were reported later *Tetrahedron* **1991**, *47*, 907.
- (7) Garegg, P. J.; Iversen, T.; Johansson, R.; Lindberg, B. *Carbohydr Res.* **1984**, *130*, 322. We substituted commercially available (American Tokyo

- Kasei, Inc., Portland OR.) 1,1-dimethoxycyclohexane for 1-ethoxycyclohexene in the synthesis of **2**. See also: Jiang, C.; Baker, D. C. *J. Carbohydr Chem.* **1986**, *5*, 615.
- (8) Yu, K. -L.; Fraser-Reid, B. *Tetrahedron Lett.* **1988**, *29*, 979.
- (9) David, S.; Hanessian, S. *Tetrahedron* **1985**, *41*, 643.
- (10) The dimer shown, **5**, incorporates two molecules of inositol diol **2** with the same absolute configuration. It is also possible that the dimer formed in the the presence of the racemic **2** incorporates two diols of opposite absolute configuration. This possibility does not affect the rationalization suggested to account for the observed regiochemistry of acylation and alkylation.
- (11) Vacca, J. P.; deSolms, S. J.; Huff, J. R. *J. Am. Chem. Soc.* **1987**, *109*, 3478.
- (12) Clark, T. M.; Switzer, R. L. *Experimental Biochemistry*, 2ed.; W. H. Freeman: San Francisco, 1977; pp 161-2.

(Received in the USA 13 September 1993)