Chiral syntheses of D- and L-*myo*-inositol 1,4,5-trisphosphate

(*chiro*-inositol/selective benzylation/trifluoromethane sulfonate displacement/phosphite triester oxidation)

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ABSTRACT Chiral inositols (D-*chiro*-inositol from D-pinitol and L-*chiro*-inositol from L-quebrachitol) were converted to the 3,4-di-*O*-benzyl ethers, which were selectively benzyolated to yield the 1,2,5-tri-*O*-benzoyl-3,4-di-*O*-benzyl-*chiro*-inositols. The free hydroxyl group in each derivative was inverted by way of the trifluoromethane sulfonate ester to provide D- and L-1,2,4-tri-*O*-benzoyl-5,6-di-*O*-benzyl-*myo*-inositol. Hydrogenolysis to remove the benzyl ether groups gave the enantiomeric 1,2,4-tri-*O*-benzoyl-*myo*-inositols, which were phosphorylated by a dibenzylphosphite triester method. After hydrogenolysis and saponification of the derivatives, the D- and L-*myo*-inositol 1,4,5-trisphosphates were isolated as the crystalline cyclohexylammonium salts in gram quantity.

myo-Inositol-containing phospholipids were isolated from bovine brain by Folch in 1942 (1) and from soybeans by Woolley in 1943 (2), and a more highly phosphorylated form was obtained from brain tissue in 1949 (3). Folch called the latter preparation "diphosphoinositide" because it yielded an "inositol metadiphosphate" following strong acid hydrolysis, but the known susceptibility of inositol phosphates to acid-catalyzed migration suggests that the product must have been a mixture. The general nature of the inositol phosphates from brain lipids was reported by Grado and Ballou (4) and Dittmer and Dawson (5) in 1960, who concluded that it was composed of *myo*-inositol mono-, di-, and triphosphates. Characterization of the *myo*-inositol monophosphate from soybean inositol phospholipid (6), synthesis of its enantiomer (7), and characterization of the *myo*-inositol polyphosphates from brain inositol phospholipid (8, 9) followed, and the exact nature of the phospholipid complex was reported by Brocknerhoff and Ballou in 1961 (10). These results were confirmed by Brown and Stewart (11).

Although the brain inositol phospholipid complex was shown to have an active metabolism in tissue slices with respect to the turnover of the monoesterified phosphate groups (12–15), studies reported by Hokin and Hokin (16) failed to establish a specific physiological role for the inositides. Regardless, these workers are recognized for promoting the hypothesis that the inositol phospholipids were involved in specific cellular processes. Developments of the last decade that have elucidated the role of *myo*-inositol polyphosphates as second messengers have given new focus to this field of lipid metabolism (17), and the renewed interest has stimulated the development of synthetic procedures for the preparation of such derivatives. In studies published to date (18–23), the syntheses have started with *myo*-inositol, a *meso* compound, and have yielded racemic products that must be resolved to provide the natural isomer. In this report, we describe chiral syntheses of *myo*-inositol 1,4,5-trisphosphate that yield the optical antipodes directly and that follow a synthetic strategy that should facilitate the preparation of other useful derivatives.

MATERIALS AND METHODS

Chemicals. D-Pinitol was isolated from sugar pine (*Pinus lambertiana* Dougl) heartwood (24), while L-quebrachitol came from an exudate of the rubber tree (*Hevea brasiliensis*) (25). Both compounds were demethylated with 50% HI, and the free inositols were crystallized after dilution of the reaction mixture with ethanol. 1,2:5,6-Di-*O*-isopropylidene-D-*chiro*-inositol [mp, 148–150°C, $[\alpha]_D +4.5^\circ$ (ethanol); reported: mp, 149–151°C, $[\alpha]_D +4.8^\circ$] was from previous studies in this laboratory (26). 1,2:5,6-Di-*O*-cyclohexylidene-L-*chiro*-inositol [mp, 204–206°C, $[\alpha]_D -16^\circ$ (chloroform) (27)] was prepared by the procedure of Jiang and Baker (28). Cyclohexanone dimethylketal (1,1-dimethoxycyclohexane) was from CTC Organics (Atlanta) and *m*-chloroperoxybenzoic acid was from Sigma. Dichloro(diisopropylamino) phosphine, for preparation of di-*O*-benzyl-di-*N*-isopropylphosphoramidite, and most other organic reagents were from Aldrich. Pyridine, dimethylformamide, and dimethyl sulfoxide were distilled from CaH₂, while tetrahydrofuran and diethyl ether were distilled from sodium wire. All were stored over molecular sieve 4A. Benzyl alcohol was dried over K₂CO₃, distilled, and stored over molecular sieve 3A.

Analytical and Preparative Methods. Analytical high-performance liquid chromatography (HPLC) was done on a Supelco LC18 (5 μ m) stainless steel column (0.4 $\times$ 25 cm) by elution with methanol/water at a flow rate of 1.5 ml/min. An LKB 2138 Uvicord S, equipped with a microflow cell and a 254-nm filter, was used to monitor the effluent. For preparative HPLC, a stainless steel column (5.1 $\times$ 50 cm) (HT Chemicals, Saint Louis) was packed manually with Merck LiChroprep RP-18 (25–40 μ m) (EM Scientific, Cherry Hill, NJ) and eluted with methanol/water at a flow rate of 20 or 25 ml/min. The UV detector was equipped with a preparative flow cell, and solvents were mixed and delivered by a Waters Prep LC3000 system fitted with a 15-ml injection loop.

Melting points were determined in capillaries on a Thomas-Hoover apparatus and are uncorrected, whereas optical activity was measured on a Bendix 1100 Automatic Polarimeter with a Hg lamp (546 nm). The reported rotations are converted to approximate values for the sodium D line by multiplying them by the fraction 0.85, derived from the rotations of sucrose at 589 and 546 nm. ³¹P NMR spectra, referenced to external 85% phosphoric acid, were determined on a Bruker 400 MHz spectrometer and ¹H NMR spectra were determined on a Bruker 500 MHz spectrometer in the Chemistry Department on this campus, while combustion analyses were performed in the Chemistry Department Microchemical Analysis laboratory. Total phosphate was determined according to Ames (29).

For hydrogenations, 5% PdCl₂ on charcoal (30) was reduced just before use by shaking it with 1 atmosphere of H₂ in ethanol in a boat mounted on an Eberbach reciprocal shaker until uptake ceased, at which time the catalyst was collected by centrifugation and washed twice with ethanol to

remove HCl. The washed catalyst was shaken with H₂ for 10 min before adding the compound to be reduced.

RESULTS

Benzylation of 1,2:5,6-Di-*O*-cyclohexylidene-*L*-chiro-inositol and 1,2:5,6-Di-*O*-isopropylidene-*D*-chiro-inositol. A mixture of dicyclohexylidene- or diisopropylidene-*chiro*-inositol (50 mmol), benzyl chloride (200 ml), KOH pellets (55 g), and 18-Crown-6 phase transfer catalyst (250 mg) was stirred at 100°C for 6 hr. The reaction mixture was cooled, diluted with toluene, and washed with water in a separatory funnel until the extract was neutral, after which the organic layer was dried (anhydrous Na₂SO₄) and concentrated to remove toluene and excess benzyl chloride. The 3,4-di-*O*-benzyl-1,2:5,6-di-*O*-cyclohexylidene-*L*-chiro-inositol crystallized from ethanol (76% yield) and had a mp of 182–184°C (reported mp, 191–192°C) (31).

The crude 3,4-di-*O*-benzyl-1,2:5,6-di-*O*-isopropylidene-*D*-chiro-inositol was purified by preparative HPLC by elution at 20 ml/min with a methanol/water step-gradient starting at 82%. The product, eluted at 240 min after reaching 88% methanol, gave a syrup (84% yield) after evaporation of the solvents that solidified on standing. It melted at 50–53°C and had $[\alpha]_{546} +22.8^\circ$ (ethanol). Analysis calculated for C₂₆H₃₂O₆ (440): C, 70.61; H, 7.27. Found: C, 70.55; H, 7.19.

¹H NMR (C²HCl₃) δ 7.37–7.40 (10 H, m, aromatic), 4.79–4.88 (4H, m, —CH₂—), 4.28–4.33 (2H, m, inositol ring H), 4.23–4.26 (2H, m, inositol ring H), 3.56–3.61 (2H, m, inositol ring H), 1.46–1.50 (6H, s, —CH₃), 1.32–1.37 (6H, s, —CH₃).

Isolation of 3,4-Di-*O*-benzyl Ethers of *D*- and *L*-chiro-Inositol. The cyclohexylidene and isopropylidene derivatives of the dibenzyl ethers were heated in 80% acetic acid at 100°C for 2 hr to hydrolyze the acetal or ketal bonds, the reaction mixtures were concentrated to dryness under vacuum, and the product was crystallized from benzene. The *D*-isomer had a mp of 133–134°C, $[\alpha]_{546} -9.8^\circ$ (dimethylformamide), while the *L*-isomer had a mp of 134–135°C and $[\alpha]_{546} +10.6^\circ$ (dimethylformamide). Analysis calculated for C₂₀H₂₄O₆ (360.4): C, 66.67; H, 6.67. Found: C, 66.06; H, 6.46.

1,2,5-Tri-*O*-benzoyl-3,4-di-*O*-benzyl-*chiro*-inositols. 3,4-Di-*O*-benzyl-*D*-chiro-inositol (1) (20.6 g, 57 mmol) in dry pyridine (200 ml) was cooled below 0°C, and benzoyl chloride (20 ml, 171 mmol, 3 M equivalents) was added to the stirred solution. The mixture was stirred for 2 days at 5°C, for 1 day at 23°C, and for 18 hr at 80°C. The cooled solution was filtered to remove pyridine hydrochloride, evaporated at reduced pressure to remove pyridine, and a toluene solution of the residue was washed with dilute HCl. The organic layer was dried with MgSO₄, filtered, and concentrated at reduced pressure to a syrup. The product was dissolved in 75 ml of acetonitrile, and 15-ml portions were fractionated on the preparative HPLC column by elution at 20 ml/min with a step-gradient of methanol/water. The dibenzoyl derivative was eluted at 70 min with 83% methanol, the tribenzoyl derivative was eluted at 170 min after shifting to 87% methanol, and the tetrabenzoyl derivative was eluted at 220 min after shifting to 100% methanol. Each fraction was collected and evaporated to dryness under vacuum. The 1,2,5-tri-*O*-benzoyl-3,4-di-*O*-benzyl-*D*-chiro-inositol (2) (15.5 g) was recrystallized from 95% ethanol and had a mp of 146–149°C and $[\alpha]_{546} +99^\circ$ (chloroform). Analysis calculated for C₄₁H₃₆O₉ (672.7): C, 73.19; H, 5.40. Found: C, 73.09; H, 5.32. The *L*-chiro-derivative, made in a similar manner, had a mp of 145–148°C and $[\alpha]_{546} -96.1^\circ$ (chloroform). Found: C, 73.08; H, 5.39.

¹H NMR (C²HCl₃) δ 7.14–8.08 (25H, m, aromatic), 5.850–5.858 (1H, dd, $J_{1,2} = 3.4$ Hz, $J_{2,3} = 8.9$ Hz, H-2), 5.825–5.850 (1H, dd, $J_{1,2} = 3.6$ Hz, $J_{1,6} = 4.6$ Hz, H-1), 5.64–5.68 (1H, dd, $J_{5,6} = 3.2$ Hz, $J_{4,5} = 9.2$ Hz, H-5), 4.73–4.87 (4H, 2AB,

—CH₂—), 4.48–4.515 (1H, dd, $J_{5,6} = 3.2$ Hz, $J_{1,6} = 4.8$ Hz, H-6), 4.295–4.35 (1H, dd, $J_{3,4} = J_{4,5} = 8.8$ Hz, H-4), 4.20–4.25 (1H, dd, $J_{2,3} = J_{3,4} = 8.8$ Hz, H-3). Decoupling of H-6 converted H-5 to a doublet ($J_{4,5} = 9.2$ Hz) and H-1 to a doublet ($J_{1,2} = 3.25$ Hz), while decoupling of H-4 converted H-3 to a doublet ($J_{2,3} = 8.5$ Hz) and H-5 to a broad singlet.

The 2,5-di-*O*-benzoyl-3,4-di-*O*-benzyl-*D*-chiro-inositol was crystallized from diethylether-hexane and had a mp of 166–168°C and $[\alpha]_{546} +120.1^\circ$ (dioxane). Analysis calculated for C₃₄H₃₂O₈ (568.7): C, 71.81; H, 5.68. Found: C, 71.65; H, 5.64. The corresponding *L*-chiro derivative had a mp of 168–169°C and $[\alpha]_{546} -138.2^\circ$ (dioxane). Found: C, 71.70; H, 5.72.

¹H NMR (C²HCl₃) δ 7.10–8.07 (20H, m, aromatic), 5.64–5.69 (2H, m, H-2 and H-5), 4.71–4.80 (4H, m, —CH₂—), 4.37–4.39 (2H, d, H-1 and H-6), 4.19–4.24 (2H, m, H-3 and H-4), 2.50–2.60 (2H, broad s, —OH).

A computer simulation (PANIC program) of the second-order system of the inositol ring protons gave the same multiplicity observed. Decoupling of the H-2 and H-5 signals changed those for all remaining ring protons, whereas decoupling of the other two ring proton signal groups had an effect only on the signals for H-2 and H-5, which shows that the benzoyl groups are located on the ring between the hydroxyl and the benzyl groups.

1,2,5,6-Tetra-*O*-benzoyl-3,4-di-*O*-benzyl-*D*-chiro-inositol was crystallized from methanol. It had a mp of 112–114°C and $[\alpha]_{546} +31.4^\circ$ (dioxane). Analysis calculated for C₄₈H₄₀O₁₀ (776.9): C, 74.21; H, 5.20. Found: C, 74.44; H, 5.13. The corresponding *L*-chiro derivative had a mp of 112–114°C and $[\alpha]_{546} -36.5^\circ$ (dioxane). Found: C, 74.16; H, 5.20.

¹H NMR (C²HCl₃) δ 7.16–8.07 (30H, m, aromatic), 5.95–5.60 (2H, s, H-1 and H-6 or H-2 and H-5), 5.86–5.92 (2H, d, H-2 and H-5 or H-1 and H-6), 4.77–4.87 (4H, m, —CH₂—), 4.28–4.33 (2H, m, H-3 and H-4).

1,2,4/1,3,4-Tri-*O*-benzoyl-5,6-di-*O*-benzyl-*myo*-inositols. 1,2,5-Tribenzoyl-3,4-dibenzyl-*D*-chiro-inositol (2) (15.3 g, 23 mmol) was dissolved in dry pyridine (250 ml) and the solution was evaporated to dryness under vacuum (oil pump) to remove traces of water. The residue was redissolved in dry pyridine (250 ml), the solution was cooled to 0°C, and trifluoromethane sulfonic acid anhydride (20 ml, 120 mmol) was added through a septum from a syringe. During the addition, the temperature was maintained at 5–15°C, and the mixture was then warmed to 30–35°C. After 30 min, HPLC analysis showed that the sulfonylation reaction was complete, so most of the pyridine was evaporated at 25°C and reduced pressure (oil pump) until a solid mass remained, and a solution of the product (3) in 1500 ml of 95% acetic acid containing 3.7 g of sodium acetate was stirred for 48 hr at 40°C to effect displacement of the sulfonate group (32). The reaction was concentrated to dryness under vacuum, and a solution of the product in toluene was washed several times with water. The dried organic layer (anhydrous Na₂SO₄) was evaporated to dryness under vacuum and the residue, dissolved in acetonitrile, was fractionated by preparative HPLC by stepwise elution with methanol/water, starting at 84% methanol. The two isomeric tribenzoyl-dibenzyl-*L*-*myo*-inositols were eluted together and after evaporation of the solvents, the product was obtained as a syrup, which crystallized on standing. Subsequent analysis revealed it to be a 3:1 mixture of the 1,2,4- (4a) and 1,3,4-isomers (4b) (see below). Inversion of the 1,2,5-tribenzoyl-3,4-dibenzyl-*L*-chiro-inositol was carried out in a similar fashion to give the enantiomeric derivatives in 70% yield. [Note that because the direction for numbering the ring and assignment of configuration change with structure (33), 1,2,5-tribenzoyl-*D*-chiro-inositol yields 1,2,4-tribenzoyl-*L*-*myo*-inositol that leads to *D*-*myo*-inositol 1,4,5-trisphosphate.]

For preparative purposes, the mixed isomers were not separated before reductive removal of the benzylether groups

(see below), but some HPLC fractions contained relatively pure isomers that crystallized from ethanol. The 1,2,4-*tri-O*-benzoyl-5,6-di-*O*-benzyl-D-*myo*-inositol had a mp of 140–142°C and $[\alpha]_{546} -81.2^\circ$ (dioxane). Analysis calculated for $C_{41}H_{36}O_9$ (672.7): C, 73.19; H, 5.40. Found: C, 73.04; H, 5.38.

1H NMR (C^2HCl_3) δ 7.10–8.10 (25H, m, aromatic), 5.96–5.98 (1H, dd, $J_{1,2} = J_{2,3} = 2.9$ Hz, H-2), 5.73–5.78 (1H, dd, $J_{3,4} = J_{4,5} = 10.0$ Hz, H-4), 5.41–5.45 (1H, dd, $J_{1,2} = 3.1$ Hz, $J_{1,6} = 10.2$ Hz, H-1), 4.72–4.88 (4H, m, $-CH_2-$), 4.25–4.30 (1H, dd, $J_1 = J_2 = 9.8$ Hz, H-5 or H-6), 4.11–4.16 (1H, br d, $J = 9.8$ Hz, H-3), 3.89–3.94 (1H, dd, $J_1 = J_2 = 9.5$ Hz, H-6 or H-5), 2.50–2.58 (1H, br s, $-OH$).

1,3,4-*Tri-O*-benzoyl-5,6-di-*O*-benzyl-D-*myo*-inositol had a mp of 196°C and $[\alpha]_{546} -30.9^\circ$ (dioxane). Analysis calculated for $C_{41}H_{36}O_9$ (672.7): C, 73.19; H, 5.40. Found: C, 73.22; H, 5.31.

1H NMR (C^2HCl_3) δ 7.08–8.09 (25H, m, aromatic), 6.10–6.15 (1H, dd, $J_{3,4} = J_{4,5} = 9.96$ Hz, H-4), 5.36–5.40 (1H, dd, $J_{ax,eq} = 2.58$ Hz, $J_{ax,ax} = 10.1$ Hz, H-1 or H-3), 5.35–5.38 (1H, dd, $J_{ax,eq} = 2.58$ Hz, $J_{ax,ax} = 10.5$ Hz, H-3 or H-1), 4.66–4.70 (4H, 2 s, $-CH_2-$), 4.59–4.61 (1H, d, $J = 2.46$ Hz, H-2), 4.39–4.44 (1H, dd, $J = 9.72$ Hz, H-5 or H-6), 3.86–3.91 (1H, dd, $J = 9.35$ Hz, H-6 or H-5), 2.30–2.35 (1H, d, $-OH$).

1,2,4-*Tri-O*-benzoyl-*myo*-inositol and 1,3,4-*Tri-O*-benzoyl-*myo*-inositol. The L-enantiomers of these compounds were prepared by hydrogenolysis of the mixed L-isomers of 1,2,4-/1,3,4-tribenzoyl-5,6-dibenzyl-*myo*-inositol (15.3 g, 23 mmol) in ethanol (500 ml) with 5% $PdCl_2$ on charcoal (5 g) that had been reduced and washed free of HCl as described in *Materials and Methods*. After shaking the mixture with hydrogen gas for 3 days, the starting material was converted to more polar products revealed by analytical HPLC. The catalyst was removed by filtration and the solvent was removed by rotary evaporation; the product was dissolved in acetonitrile and fractionated in portions by preparative HPLC by elution at 20 ml/min with 60% methanol/water in which the water was first adjusted to pH 3 with HCl to avoid acyl migration. The 1,2,4-tribenzoyl-L-*myo*-inositol (**5a**) was eluted at 110 min and the 1,3,4-tribenzoyl-L-*myo*-inositol (**5b**) was eluted at 230 min. The fractions of the 1,2,4-isomer were evaporated to yield 6.85 g of a crystalline residue that was recrystallized from 95% ethanol. It had a mp of 188–191°C and $[\alpha]_{546} +5.4^\circ$ (tetrahydrofuran). Analysis calculated for $C_{27}H_{24}O_9$ (492.5): C, 65.85; H, 4.92. Found: C, 65.42; H, 4.84. The 1,2,4-tribenzoyl-D-*myo*-inositol had a mp of 186–190°C and $[\alpha]_{546} -6.5^\circ$ (tetrahydrofuran). Found: C, 65.92; H, 4.92.

1H NMR (C^2HCl_3) δ 7.33–8.18 (15H, m, aromatic), 5.21–5.24 (1H, dd, $J_{1,2} = J_{2,3} = 2.5$ Hz, H-2), 4.95–5.00 (1H, dd, $J_{3,4} = J_{4,5} = 8.5$ Hz, H-4), 4.60–4.64 (1H, dd, $J_{1,2} = 2.7$ Hz, $J_{1,6} = 8.7$ Hz, H-1), 4.45–4.47 (1H, d, $J = 5.3$ Hz, $-OH$), 4.23–4.25 (1H, d, $J = 4.1$ Hz, $-OH$), 4.20–4.21 (1H, d, $J = 3.7$ Hz, $-OH$), 3.72–3.78 (1H, ddd, $J_1 = J_2 = 8.6$ Hz, $J_{OH} = 4.1$ Hz, H-5 or H-6), 3.64–3.69 (1H, ddd, $J_{2,3} = 2.7$ Hz, $J_{3,4} = 8.5$ Hz, $J_{OH} = 5.5$ Hz, H-3), 3.35–3.40 (1H, ddd, $J_1 = J_2 = 8.1$ Hz, $J_{OH} = 4.0$ Hz, H-5 or H-6).

Since the 1,2,4-isomer was preponderant in the mixture, the inversion reaction occurs mainly without migration of a benzoyl group. After saponification of the recovered 1,2,4-tribenzoyl-*myo*-inositol, paper chromatography revealed that it contained exclusively *myo*-inositol.

The 1,3,4-tribenzoyl-L-*myo*-inositol (2.1 g) was crystallized from methanol. It had a broad melting point of 184–194°C, although it appeared to be pure by HPLC and NMR. $[\alpha]_{546} -38.3^\circ$ (dioxane). Analysis calculated for $C_{27}H_{24}O_9$ (492.5): C, 65.85; H, 4.92. Found: C, 65.80; H, 4.90.

1H NMR (C^2HCl_3) δ 7.30–8.17 (15H, m, aromatic), 5.87–6.00 (1H, dd, $J_{3,4} = J_{4,5} = 10.5$ Hz, H-4), 5.44–5.49 (1H, dd, $J_{ax,eq} = 2.2$ Hz, $J_{ax,ax} = 10.6$ Hz, H-1 or H-3), 5.24–5.29 (1H, dd, $J_{ax,eq} = 2.2$ Hz, $J_{ax,ax} = 10.6$ Hz, H-1 or H-3), 4.63–4.68

(1H, s, H-2), 4.41–4.48 (1H, dd, $J_1 = J_2 = 10.3$ Hz, H-5 or H-6), 3.89–3.94 (1H, dd, $J_1 = J_2 = 10.0$ Hz, H-5 or H-6), 3.26–3.31 (1H, br s, $-OH$), 3.10–3.18 (1H, br s, $-OH$); 2.80–2.87 (1H, br s, $-OH$).

Phosphorylation of 1,2,4-*Tri-O*-benzoyl-*myo*-inositol. To a stirred suspension of the L-isomer (**5a**) (1 g, 2.03 mmol) in dry dichloromethane (100 ml), cooled in ice water, was added tetrazole (2.14 g, 30.5 mmol) and di-*O*-benzyl-di-*N*-isopropyl phosphoramidite (4.2 ml, 12.1 mmol) (34, 35). The reaction mixture was stirred at room temperature for 30 min, at which time the suspension had turned clear and HPLC analysis revealed that the starting material was converted completely to a single less polar product. The reaction was cooled to $-78^\circ C$ (acetone/dry ice), *m*-chloroperoxybenzoic acid (6.7 g, 38.8 mmol) was added, and the mixture was stirred and allowed to warm up to room temperature overnight. The solvent was evaporated, dimethylformamide was added, the mixture was filtered to remove salts, and the product was isolated by preparative HPLC using a step-gradient starting at 80% methanol/water with elution at 25 ml/min. The 1,4,5-tris-*O*-diphenylphosphoryl-2,3,6-tri-*O*-benzoyl-D-*myo*-inositol was eluted at 400 min with 90% methanol/water to give 2.43 g (94%) of a syrup with $[\alpha]_{546} -11.3^\circ$ (ethanol). Analysis calculated for $C_{69}H_{63}O_{18}P_3$ (1273.1): C, 65.10; H, 4.99; P, 7.31. Found: C, 64.95; H, 5.10; P, 7.32. The L-isomer showed $[\alpha]_{546} +7.60^\circ$ (ethanol). Found: C, 65.27; H, 5.00; P, 7.47.

1H NMR (C^2HCl_3) δ 6.78–8.15 (45H, aromatic), 6.19–6.22 (1H, dd, $J_{1,2} = J_{2,3} = 3.03$ Hz, H-2), 6.12–6.18 (1H, dd, $J_{1,6} = J_{5,6} = 10.10$ Hz, H-6), 5.50–5.55 (1H, dd, $J_{2,3} = 3.20$ Hz, $J_{3,4} = 10.27$ Hz, H-3), 5.30–5.37 (1H, ddd, $J_1 = J_2 = 9.5$ Hz, $J_{H-P} = 9.25$ Hz, H-4 or H-5), 4.36–5.05 (14H, m, $-CH_2-$, H-1 and H-4 or H-5).

^{31}P NMR (C^2HCl_3 , H decoupled): three signals at δ -0.95 , -1.12 , and -1.42 ppm.

D-*myo*-Inositol 1,4,5-Trisphosphate. The 1,4,5-tris-diphenylphosphoryl-2,3,6-tribenzoyl D-*myo*-inositol (**6**) (2.1 g) was hydrogenated in ethanol (250 ml) with 5% $PdCl_2$ on charcoal (250 mg) that had been reduced and washed free of HCl. The calculated hydrogen uptake (6 M equivalents) was complete in 2 hr, when the catalyst was removed by filtration, 40 ml of 0.5 M NaOH was added, the ethanol was evaporated at reduced pressure, and the aqueous solution was left overnight to saponify the benzoyl ester groups. The cations were removed by treatment with Dowex 50 (H^+), the acidic solution was extracted with chloroform to remove benzoic acid, and cyclohexylamine was added to bring the pH to 11. The solution was concentrated to dryness, the solid was dissolved in 5 ml of water, and isopropanol was added to cause crystallization of the D-*myo*-inositol 1,4,5-trisphosphate hexacyclohexylamine salt dihydrate (**7**) after a day at $5^\circ C$ (1.5 g, 87%). The salt showed $[\alpha]_{546} -35.1^\circ$ (calculated for the free acid).

Analysis calculated for $C_6H_{15}O_{15}P_3(C_6H_{11}NH_2)_6 \cdot 2H_2O$ (1051): C, 47.99; H, 9.30; N, 7.99; P, 8.85. Found: C, 47.97; H, 9.58; N, 7.96; P, 9.43.

1H NMR (2H_2O , cyclohexylammonium salt, pH 8.5) δ 4.32–4.34 (1H, d, H-2); 4.11–4.18 (1H, ddd, H-4); 3.84–3.93 (3H, m, H-1, H-5, and H-6); 3.68–3.72 (1H, dd, H-3); 3.07–3.18 (6H, m, cyclohexylamine H-1); 1.12–2.04 (60H, m, cyclohexylamine).

^{31}P NMR (2H_2O , cyclohexylammonium salt, pH 8.5, H decoupled) δ 3.61 (2P, s); 4.91 (1P, s); pH 12, δ 5.08 (1P, s, P-1 or P-5); 5.95 (1P, s, P-4); 6.17 (1P, s, P-1 or P-5); pH 12, not decoupled, δ 5.00–5.04 (1P, d, $J = 7.3$ Hz); 5.84–5.89 (1P, d, $J = 7.2$ Hz); 6.07–6.12 (1P, d, $J = 7.6$ Hz) (36).

The L-*myo*-inositol 1,4,5-trisphosphate was made in the same manner in 88% yield from the tribenzoyl-D-*myo*-inositol, and a FAB mass spectrum (negative mode) of the free acid gave a pseudomolecular ion at m/z 419 $[M - H^+]^-$ and

at m/z 839 ($2M - H^+$)⁻. The free acid gave $[\alpha]_{546} +39.0^\circ$ (pH 9.5). A paper chromatogram of the *myo*-inositol 1,4,5-trisphosphate preparations showed single phosphate-containing spots with the R_f reported for the inositol phospholipid-derived material (8).

In a separate experiment, the intermediate 2,3,6-tribenzoyl-*myo*-inositol 1,4,5-trisphosphate formed by reduction of 6 was recovered as the sodium salt after adjusting the ethanolic solution to pH 8. The 1H and ^{31}P NMR spectra were consistent with the assigned structure and confirmed that the product was a single isomer. Such derivatives, with different acyl groups, have potential interest as lipophilic analogs of *myo*-inositol 1,4,5-trisphosphate.

1H NMR (2H_2O , pH 8.3) δ 7.44–8.27 (15H, m, aromatic), 6.20–6.25 (1H, s, H-2), 5.85–5.92 (1H, dd, $J_{5,6} = J_{1,6} = 10.0$ Hz, H-6), 5.46–5.52 (1H, dd, $J_{2,3} = 3.0$ Hz, $J_{3,4} = 10.0$ Hz, H-3), 4.97–5.05 (1H, ddd, $J_1 = J_2 = 10.0$ Hz, $J_{H-P} = 9.75$ Hz, H-4 or H-5), 4.74–4.81 (1H, ddd, $J_{eq,ax} = 2.95$ Hz, $J_2 = J_{H-P} = 10.25$ Hz, H-1), 4.64–4.71 (1H, ddd, $J_1 = J_2 = 9.75$ Hz, $J_{H-P} = 9.85$ Hz, H-4 or H-5).

^{31}P NMR (2H_2O , pH 8.3, H decoupled) δ 1.48 (2P, br s); 1.09 (1P, s), pH 0.3, δ -0.59 (1P, s), -0.68 (1P, s), -1.24 (1P, s), pH 0.3, not decoupled, δ -0.58 to -0.64 (1P, d, $J = 9.5$ Hz); -0.67 to -0.73 (1P, s, $J = 9.7$ Hz); -1.22 to -1.28 (1P, s, $J = 9.5$ Hz).

DISCUSSION

The chiral synthesis of D-*myo*-inositol 1,4,5-trisphosphate is summarized in Fig. 1. Two of the steps are notable: namely, the selective benzylation of 1 to 2, which is accomplished in excellent yield after separating the products by preparative HPLC and recycling the di- and tetrabenzoyl derivatives; and the conversion of 3 to 4, which occurs predominantly without migration of the neighboring axial benzoyl ester during displacement of the trifluoromethane sulfonate group (37) to give the desired isomer 4a. It has been shown for other inositol derivatives that equatorial hydroxyl groups are benzyloylated faster than axial positions (31) and, by conducting the reaction at low temperature, the crude tribenzoyl derivative we obtain is >90% the desired isomer. The preferential opening of benzoyloxonium ions on fused ring systems to yield the axial benzoyl ester has also been reported (38). The phosphorylation of neighboring hydroxyl groups in a cyclitol with formation of a cyclic phosphate (39) also posed a problem prior to the development of the phosphite triester method (40). In our hands, the reagent di-*O*-benzyl-di-*N*-isopropyl phosphoramidite (34) reacted smoothly and gave an excellent yield of the phosphate esters after oxidation.

The molecular rotation, $[M] = M_r[\alpha]/100$, of the synthetic L-*myo*-inositol 1,4,5-trisphosphate measured at 546 nm was $+164^\circ$ (NaOH, pH 9.5). When multiplied by the factor 0.85 (see *Materials and Methods*), a $[M]_{589} +140^\circ$ is obtained. The cyclohexylamine salt of the D-isomer (pH 8.5) gave $[M]_{589} -125^\circ$. Literature values cited for the natural isomer isolated from the inositol phospholipid are $[M]_{589} -115^\circ$ (8) and $[M]_{596} -134^\circ$ (11), both at pH 9.5. Of the reported syntheses of enantiomeric *myo*-inositol trisphosphates, only one records optical rotations. Vacca *et al.* (20) give values of $[M]_{589} -118^\circ$ and $+147^\circ$ for the D- and L-isomers, respectively, determined at pH 9.5 and pH 10.

The synthetic strategy we have developed has several advantages. Firstly, because it starts with a chiral inositol, the tedious and unpredictable resolution of racemic mixtures is avoided. Although pinitol is not available commercially, it can be isolated on a large scale with relative ease (24). Secondly, the methodology allows for selective modification of the substituent at position 1 while still permitting normal phosphorylation at the two biologically critical ring positions

FIG. 1. Synthesis of D-*myo*-inositol 1,4,5-trisphosphate. A, benzoyl chloride (three equivalents) in dry pyridine; B, trifluoromethane sulfonic acid anhydride in dry pyridine, 30°C; C, 95% acetic acid/sodium acetate, 40°C; D, H₂-Pd in ethanol; E, di-*O*-benzyl-di-*N*-isopropyl phosphoramidite in tetrahydrofuran, 23°C, followed by *m*-chloroperoxybenzoic acid; F, H₂-Pd in ethanol, followed by NaOH. Bn, benzyl; Bz, benzoyl; Tf, trifluoromethane sulfonyl (triflate).

4 and 5 (41). Thirdly, feasible schemes exist for synthesis of other trisphosphates and some bis- and tetrakisphosphates of *myo*-inositol by appropriate use of the intermediates. Finally, since it is reasonable to expect that the selective acylation and inversion can be carried out with different acyl groups, the synthetic pathway allows the formation of *myo*-inositol polyphosphates with various ester groups on the ring. Such substituents should enhance the lipophilicity and membrane permeability of the molecules, which, once inside the cell, would be subject to deacylation by cellular esterases.

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