Pergamon

Bioorganic & Medicinal Chemsitry, Vol. 2, No. 3, pp. 147-151, 1994

0968-0896/94 $6.00 + .00

0968-0896(94)E0004-L

Synthesis of Optically-Active Hexadecyl Thiophosphoryl-1-D-*myo*-inositol: A Thiophosphate Analog of Phosphatidylinositol

Anatoliy S. Bushnev,[a,b] Elizabeth K. Hendrickson,[a] Vitaliy I. Shvets,[b] and H. Stewart Hendrickson[a,*]

[a]*Department of Chemistry, St Olaf College, Northfield, MN, U.S.A.*

[b]*The Lomonosov Institute of Fine Chemical Technology, Moscow, Russia*

Abstract—The synthesis of optically-active hexadecyl thiophosphoryl-1-D-*myo*-inositol **11** was accomplished from *2,3-O-(D-1',7',7'-trimethyl[2.2.1]bicyclohept-2'-ylidene)-4,5,6-O-tris(methoxymethyl)-D-myo-inositol* **6** *or 2,3,4,5,6-O-pentakis(methoxymethyl)-D-myo-inositol* **14**, using the Arbusov reaction of their dimethyl phosphite derivatives **7** and **15** with *N*-hexadecyl thiophthalimide **8**. This product was a substrate for phosphatidylinositol-specific phospholipase C from *Bacillus cereus*.

Introduction

There are many reasons for interest in thio analogs of natural phospholipids. One of them is the spectrophotometric determination of released thiols to measure enzyme activity. This method, as applied to phospholipase C (from *Clostridium perfringens*), was first reported by Cox *et al.*[1] Snyder[2,3] used a racemic thiophosphate analog of dioctanoylphosphatidylcholine for the assay of phospholipase C. Later this method was used by Nyquist[4] for the spectrophotometric analysis of phosphomonoesterase with a thiophosphate analog of phosphatidic acid. Young *et al.*[5,6] studied the inhibition of phospholipase C from *Clostridium perfringens* using 1-*S*-phosphocholine-2-*O*-hexadecanoyl-1-mercapto-2-ethanol. Hendrickson *et al.*[7,8] used racemic alkyl thiophosphate analogs of phosphatidyl inositol to study the kinetics of phosphatidylinositol-specific phospholipase C from *Bacillus cereus* (PI-PLC; EC 3.1.4.10).

To continue our studies on PI-PLC from *Bacillus cereus*[7,8] we were interested in obtaining an optically-active thiophosphate substrate analog. Lewis *et al.*[9] showed that phosphatidylinositol containing the L-isomer of *myo*-inositol was neither a substrate nor inhibitor of this enzyme. Here we report the synthesis of optically-active hexadecyl thiophosphoryl-1-D-*myo*-inositol **11** by two methods (Schemes I and II).

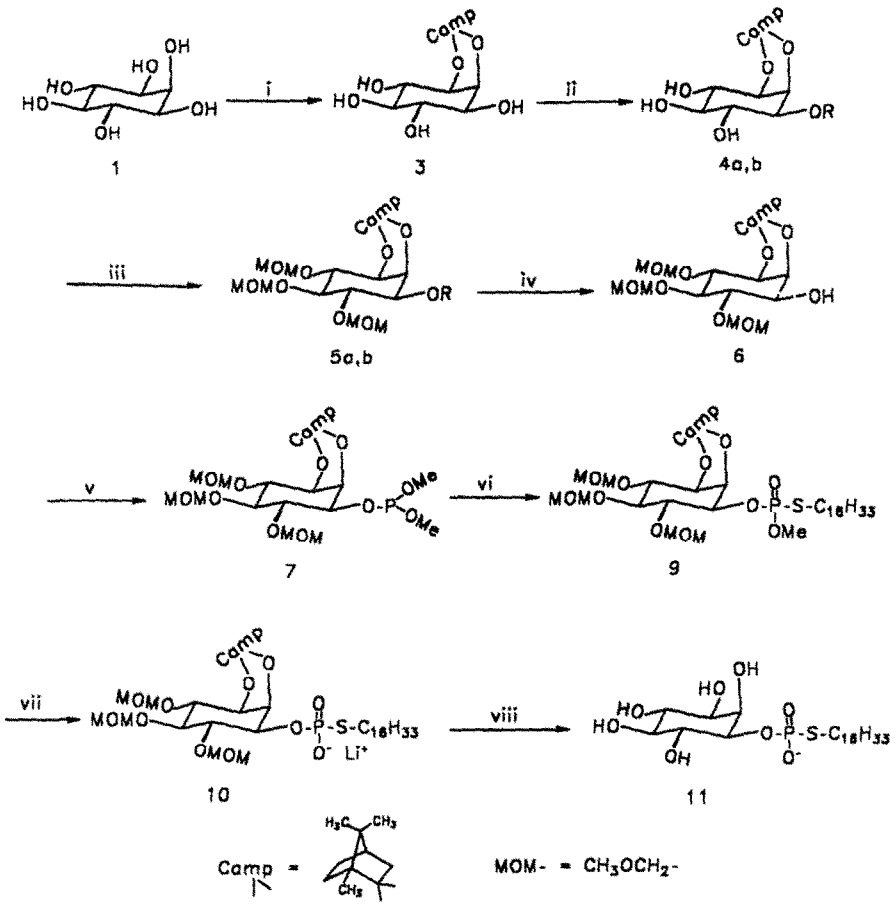
Results and Discussion

There were two major problems to overcome in these syntheses: the method of preparation of an optically-active *myo*-inositol derivative, and the method of introducing the phosphate group into the compound. To solve the first problem we chose a kinetically-controlled preparation of optically-active *myo*-inositol derivatives using chiral camphor **2** as the chiral auxiliary-mediating agent (Scheme I). This method was first proposed by Bruzik and Salamonczyk[10] and was used to prepare a variety of substituted *myo*-inositol phosphates[11–14] and to synthesize phosphatidylinositols.[15]

There are two slightly different methods for the preparation of the camphor ketal **3**.[13,14] Pietrusiewicz *et al.*[13] used sulfuric acid as the catalyst in DMSO at 50–55 °C, and *p*-toluene sulfonic acid to isomerize the diastereomeric products to one diastereomer in chloroform–methanol–water 50:5:1 with 65% yield. Bruzik and Tsai[14] carried out the ketal formation at 90 °C for 3 h with trimethylsilyl-triflate, followed by the addition of ethylene glycol in chloroform to partially cleave the bis addition products, and then *p*-toluene sulfonic acid at room temperature for 12 h to give a 25–31% yield of **3**. We used the first method to prepare **3**. Purification of products **4a,b**, **5a,b** and **6** by flash chromatography facilitated the removal of other isomers present with **3**.

Compound **3** was transformed to **4a** and **4b** following the method of Bruzik and Tsai.[13,14] In the next transformations we used both **4a** and **4b** for the preparation of **6**. The preparation of **6** from the tetrabutyldiphenylsilyl (TBDPS) derivative **5b** was described by Bruzik and Tsai,[14] but preparation of **6** from the pivaloyl derivative **5a** is first reported here. Compound **4a** was reacted with a five-fold excess of methoxymethyl chloride in the presence of diisopropylethylamine. After flash chromatography, the hexaprotected optically-active *myo*-inositol derivative **5a** was isolated with a yield of over 80%. To transform **5a** to **6**, the pivaloyl group was removed by KOH hydrolysis in methanol. The diastereomeric purity and structure of products were confirmed by ^{1}H-NMR spectroscopy. The presence of a pivaloyl group in **5a** and its absence in **6** were confirmed by IR spectra.

*Author to whom correspondance should be addressed.



Scheme I. a) **R = Piv**, b) **R = TBDPS**. Reagents and conditions: i, (+)-(1*R*)-dimethylcamphoketal 2/DMSO/H_2SO_4(cat), 50–55 °C, 2 h; ii, *t*-BuCOCl/Pyridine, 0 °C, 20–30 min; iii, $MeOCH_2Cl$/*i*-Pr_2NEt, THF, 40–50 °C, 72 h; iv, KOH/MeOH, 20–25 °C, 36 h; v, $(MeO)_2PCl/Et_3N/CH_2Cl_2$, -15 °C, 15 min, then 20–25 °C, 1 h; vi, $C_{16}H_{33}SNPhthalimide$ **8**/toluene, 20–25 °C, 1 h; vii, LiBr/acetone, reflux 4 h; viii, $HOCH_2CH_2SH/BF_3$ Et_2O/CH_2Cl_2, 20–25 °C, 3 h.

The next step in the synthesis of compound **11** involved the formation of the C–S–P bond. We chose the method of Muller and Roth[16] used by Hendrickson *et al.*[7] for the synthesis of alkyl thiophosphate analogs of phosphatidyl inositol—an Arbusov-type reaction of *N*-phthalimide thiol derivatives with a pentaprotected inositol dimethyl phosphite **7**. This compound was prepared *in situ* from dimethyl chlorophosphite and the pentaprotected inositol **6**; the reaction was followed by TLC and ^{31}P-NMR spectroscopy (δP 139.33 ppm from H_3PO_4). The Arbusov reaction between **7** and *N*-hexadecyl thiophthalimide **8** resulted in **9**; the structure was confirmed by ^{31}P-NMR spectroscopy—there were two signals, δP 29.31 and 28.90 of the same intensity, which corresponded to the two diastereoisomers at the phosphate center. TLC confirmed the presence of two diastereometric compounds with R_f 0.50 and 0.53 in hexane–acetone 7:3. The methyl group was easily removed by refluxing with LiBr in acetone. The product **10** gave only one spot on TLC and showed only one signal in ^{31}P-NMR, δP 18.47.

The method used to remove the protecting groups of the diester **10** was critical due to the instability of the S–P bond under acidic conditions. Methoxymethyl (MOM) groups are usually removed by weak acids such as 80% acetic acid[15] or 6N HCl in THF,[17] sometimes warming the mixture to 95 °C.[7,18] Bruzik *et al.*[19] reported the removal of MOM groups spontaneously at room temperature after deblocking three phosphate residues on a protected tris(dibenzylphosphate)inositol. Under the same conditions cyclohexylidene groups can be removed, but stronger conditions are required to remove the camphor ketal, which is otherwise similar to the cyclohexylidene group—warming in trifluoroacetic acid–methanol 1:1 at 70 °C[20] or treatment with 10–20% trifluoroacetic acid in chloroform at 25 °C.[14] Application of these methods in this work did not lead to complete removal of the camphor ketal group and was often accompanied by destruction of the product. Perhaps steric hindrance made removal of this group more difficult. Another method of removing the camphor ketal is by treatment with BF_3-etherate in the presence of thiophenol[20] or β-mercaptoethanol.[14] This last method gave good results. The chiral product **11** prepared according to Scheme I was similar to the racemic compound[7] by TLC and ^{1}H-NMR spectra. ^{31}P-NMR spectra confirmed the presence of a thiophosphate diester structure in **11** (δP 24.51).

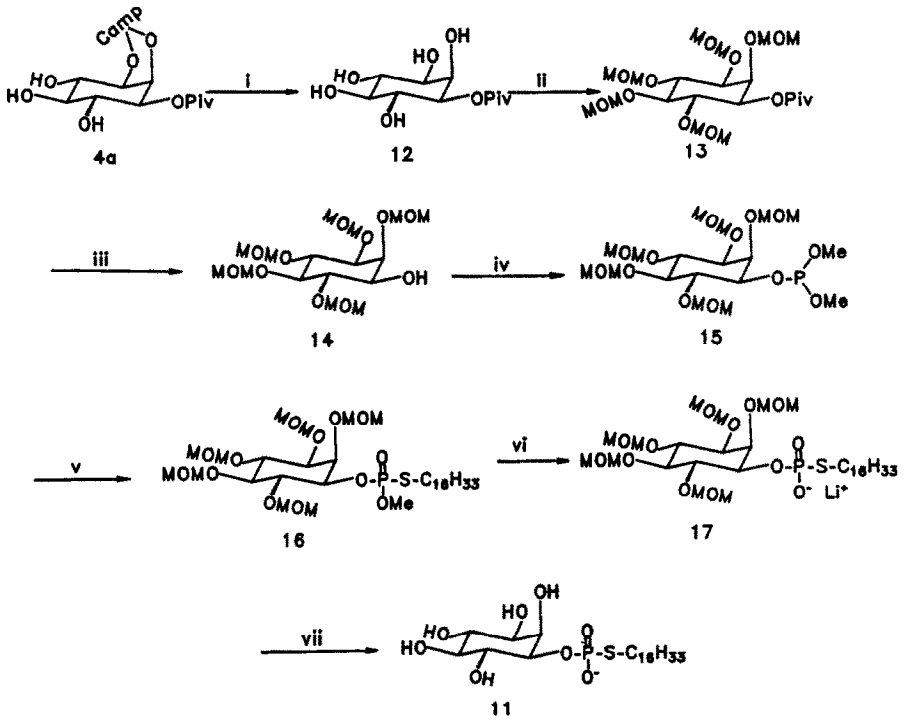
To avoid using the camphor ketal blocking group in the last step of the synthesis, we developed another method to

prepare product **11** (Scheme II) using a kinetically-controlled preparation of a chiral inositol derivative and 2,3,4,5,6-*O*-penta-MOM-D-*myo*-inositol **14** as a key intermediate. According to this scheme, after selective blocking of the 1-position of campho-*myo*-inositol **3** with the pivaloyl group and removal of the camphor group with BF_3–etherate and β-mercaptoethanol, complete methoxymethylation of 1-*O*-pivaloyl-*myo*-inositol **12** led to the completely-protected inositol **13**. Treatment of **13** with sodium methoxide in methanol gave 2,3,4,5,6-*O*-penta-MOM-D-*myo*-inositol **14**. The structures of these products were confirmed by IR and ^{1}H-NMR spectroscopy and the diastereometric purity by TLC and ^{1}H-NMR spectroscopy. The reactions **4a** →**12**→**13**→**14** each gave yields of 90–95%. It is possible to use this method for the preparation of known optically-active 2,3,4,5,6-*O*-pentabenzyl-D-*myo*-inositol from **4a** and 2,3,4,5,6-*O*-pentaacetyl-D-*myo*-inositol from **4b**.

Both products **11** showed identical kinetics with PI-PLC. The concentration giving half-maximal activity (K_M') with *B. cereus* PI-PLC was one-half that with the racemic hexadecyl thiophosphoryl-1-*myo*-inositol .[8]

Experimental

2,3-O-(D-1',7',7'-Trimethyl[2.2.1]bicyclohept-2'-ylidene)-D-myo-inositol **3** *and 1-O-(trimethylacetyl)-2,3-O-(D-1',7',7'trimethyl[2.2.1]bicyclohept-2'-ylidene)-D-myo-inositol* ***4a*** were synthesized according to the method of Pietrusiewicz *et al.*,[13] 1-*O*-(*tert*-butyldiphenylsilyl)-2,3-*O*-(D-1',7',7'-trimethyl [2.2.1] bicyclohept-2'-ylidene)-D-*myo*-inositol **4b** and *1*-O-(tert-*butyldiphenylsilyl)-2,3*-O-*(D-1',7',7'-trimethyl[2.2.1]bicyclohept-2'-ylidene)-4,5,6-O-tris-(methoxymethyl)-D-*myo-*inositol* ***5b*** by that according to Bruzik and Tsai.[14] Alufolien Kieselgel 60 F_{254} (Merck)



Scheme II. Reagents and conditions: i, $HOCH_2CH_2SH/BF_3Et_2O/CHCl_3$-MeOH (10:1), 20–25 °C, 7 h; ii, $MeOCH_2Cl$/i-Pr_2NEt, CH_2Cl_2, 20–25 °C, 12 h, then 50 °C, 24 h; iii, MeONa/MeOH, 20–25 °C, 4–6 h; iv, $(MeO)_2PCl/Et_3N/CH_2Cl_2$, -15 °C, 15 min, then 20–25 °C, 1.5 h; v, **8**/toluene, 20–25 °C, 1 h; vi, LiBr/acetone, reflux 2 h; vii, AcOH-H_2O, 4:1, 95 °, 40 min.

Phosphitidylation of **14** with dimethyl chlorophosphite and subsequent Arbusov reaction of **15** (δP 139.41) with *N*-hexadecyl thiophthalimide **8** gave triester **16** (δP 29.58 and 29.28) with a 65% yield. However, sometimes this reaction leads to the formation of other phosphorus-containing compounds (δP 9.36, 8.37 and 7.84, 6.76) with one or more deprotected hydroxy groups (IR 3450 cm^{-1}), possibly due to instability of the MOM group. Removal of the methyl group from the phosphate **16** with LiBr, and MOM groups from the diester **17** with acetic acid gave the final product **11** which was identical with the final product from Scheme I as determined by TLC, ^{1}H- and ^{31}P-NMR spectra.

was used for TLC analysis. Compounds were detected by spraying with a solution of ammonium molybdate in 10% aqueous sulfuric acid or by spraying with a solution of chromic acid in 55% H_2SO_4 followed by charring at 150–200 °C. Column chromatography was performed on Silica Gel 60 (75–150 μm, Analtech). ^{1}H- and ^{31}P-NMR spectra were recorded on a Varian UNITY-300 spectrometer. ^{1}H-chemical shifts are given in ppm (δ), relative to tetramethylsilane (TMS) as the internal standard. ^{31}P-NMR spectra were proton-decoupled, chemical shifts are given in ppm (δ) relative to 85% H_3PO_4. IR spectra were recorded neat (for **5a**, **6**, **13** and **14**) or as film with mineral oil (for **12**) on a sodium chloride disc on a MIDAC FT-IR

spectrometer and only the structurally important peaks are listed. Optical rotation of **14** was measured on an Autopol III automatic polarimeter. Kinetics of **11** with PI-PLC were obtained according to Hendrickson *et al.*[8]

*1-O-(Trimethylacetyl)-2,3-O-(D-1',7',7'-trimethyl[2.2.1]-bicyclohept-2'-ylidene)-4,5,6-O-tris(methoxymethyl)-D-*myo*-inositol* **5a**.

Camphor-pivaloyl-*myo*-inositol **4a** (0.9 g, 2.3 mmol), MOM-Cl (1.6 g, 20.0 mmol), and diisopropylethylamine (3.5 mL, 20.0 mmol) in CH_2Cl_2 (40 mL) were stirred at 40 °C for 72 h. Reaction mixture was concentrated and the residue was chromatographed on silica gel with hexane–ether 5:1. Yield 1.0 g, 83.3%. Oil, R_f 0.63 (hexane–acetone, 7:3). IR 1730 (C=O) cm^{-1}. ^{1}H-NMR ($CDCl_3$): δ 0.85 (s, 3H), 0.90 (s, 3H), 0.95 (s, 3H), 1.18–1.23 (m, 10H), 1.40–1.44 (m, 2H), 1.71 (m, 2H), 1.83–1.93 (m, 2H), 3.43 (m, 9H), 3.60 (tr, 1H), 3.88 (tr, 1H), 3.99 (m, 2H), 4.21 (dd, 1H), 4.74–4.83 (m, 6H), 5.04 (dd, 1H).

*2,3-O-(D-1'7'7'-Trimethyl[2.2.1]bicyclohept-2'-ylidene)-4,5,6-O-tris(methoxymethyl)-D-*myo*-inositol* **6**

From **5a**: A solution of **5a** (1.0 g, 1.9 mmol) and KOH (0.7 g) in MeOH (13 mL) was stirred at room temperature for 36 h. The reaction mixture was diluted with CH_2Cl_2 (80 mL) and water (30 mL), the organic layer was separated and washed with water and dried over Na_2SO_4, and concentrated. The residue was chromatographed on silica gel with hexane–acetone from 0 to 20% acetone. Yield 0.7 g, 79.5%.

From **5b**: Prepared according to Bruzik and Tsai[14] with yield 90.0%. Oil, R_f 0.31 (hexane–acetone, 7:3). IR 3460 (O–H) cm^{-1}. ^{1}H-NMR ($CDCl_3$): δ 0.86 (s, 3H), 0.89 (s, 3H), 1.01 (s, 3H), 1.22 (m, 1H), 1.40–1.48 (m, 2H), 1.70–1.76 (m, 2H), 1.90–2.05 (m, 2H), 3.43 (m, 9H), 3.68 (tr, 1H), 3.86 (tr, 1H), 3.97 (dd, 1H), 4.12 (tr, 1H), 4.22 (tr, 1H), 4.34 (tr, 1H), 4.74–4.86 (m, 6H).

*Methyl 2,3-O-(D-1',7',7'-trimethyl[2.2.1]bicyclohept-2'-ylidene)-4,5,6-O-tris(methoxymethylene)D-*myo*-inositol 1-(hexadecylthio)phosphate* **9**

A solution of dimethyl chlorophosphite (290 mg, 2.26 mmol) in CH_2Cl_2 (1.35 mL) was added to a solution of camphor-MOM-D-*myo*-inositol **6** (690 mg, 1.48 mmol) and triethylamine (300 mg, 3.0 mmol) in CH_2Cl_2 (5.4 mL) at -15 °C. The reaction mixture was stirred at the same temperature for 15 min, then at room temperature for 1 h. After dilution with CH_2Cl_2 (100 mL) the reaction mixture was washed with 5% $NaHCO_3$, and then water, dried over Na_2SO_4 and concentrated *in vacuo*. The crude product phosphite **7** [δP 139.33, R_f 0.38 (hexane–acetone, 7:3)] in toluene (2.5 mL) was stirred with a solution of *N*-hexadecylthiophthalimide **8** (510 mg, 1.26 mmol) in toluene (2 mL) at room temperature for 1 h. The reaction mixture was concentrated *in vacuo* and the residue was chromatographed on silica gel with hexane–acetone 7:3. Yield 475 mg, 48.3%. Oil, R_f 0.41 and 0.45 (hexane–acetone, 7:3). ^{31}P-NMR ($CDCl_3$): δ 29.31 and 28.90.

*D-*myo*-Inositol 1-(hexadecylthio)phosphate* **11**

From **9**: A solution of triester **9** (475 mg, 0.61 mmol) and LiBr (80 mg, 0.90 mmol) in acetone (10 mL) was refluxed for 4 h. A precipitate, which formed after the solution was cooled to 0 °C, was separated by filtration and washed with acetone. The crude product, *lithium 2,3-O-(D-1',7',7'-trimethyl[2.2.1]bicyclohept-2'-ylidene)-4,5,6-O-tris(methoxymethyl)-D-*myo*-inositol 1-(hexadecylthio)phosphate* **10** [δP 18.47, R_f 0.53 ($CHCl_3$–MeOH–H_2O, 96:33:4), m.p. 209–210 °C] in CH_2Cl_2 (9 mL), was stirred with β-mercaptoethanol (1.8 mL) and BF_3–etherate (0.215 mL) at 20–25 °C for 3 h, the reaction mixture was concentrated, the residue was suspended in acetone, filtered, and washed with acetone. Yield 216 mg, 70.1 % (Li-salt).

From **16**: A solution of triester **16** (90 mg, 0.126 mmol) and LiBr (40 mg, 0.45 mmol) in acetone (3 mL) was refluxed for 2 h. The crude product, *lithium 2,3,4,5,6-O-pentakis(methoxymethylene)-D-*myo*-inositol 1-(hexadecylthio)phosphate* **17** [δP 18.51, R_f 0.47 ($CHCl_3$–MeOH–25%NH_4OH, 30:6:1)] without purification, was heated in AcOH–H_2O, 4:1 (3 mL) at 95 °C for 40 min. The reaction mixture was concentrated, and the residue was purified on a silica gel column with $CHCl_3$–MeOH, 2:1. Yield 36 mg, 58.3 %. R_f 0.23 ($CHCl_3$–MeOH–H_2O, 65:35:3). ^{31}P-NMR ($CDCl_3$-CD_3OD, 1:1): δ 24.51.

*1-O-(Trimethylacetyl)-D-*myo*-inositol* **12**

One mL of β-mercaptoethanol and 0.2 mL of BF_3–etherate were added to camphor–pivaloyl–inositol **4a** (0.42 g, 1.05 mmol) in $CHCl_3$–MeOH 10:1 (5 mL). The reaction mixture was stirred at room temperature for 7 h and rotovaped. The residue was suspended in $CHCl_3$ and washed with $CHCl_3$ in a sintered glass filter. Yield 0.271 g, 100 %. m.p. 202–204 °C, R_f 0.36 ($CHCl_3$–MeOH, 3:1). IR 3415 (O–H), 1715 (C=O) cm^{-1}. ^{1}H-NMR (CD_3OD): δ 1.24 (s, 9H), 3.21 (tr, 1H), 3.40 (dd, 1H), 3.63 (tr, 1H), 3.83 (tr, 1H), 4.01 (tr, 1H), 4.59 (dd, 1H).

*2,3,4,5,6-O-Pentakis(methoxymethyl)-1-O-(trimethylacetyl)-D-*myo*-inositol* **13**

Diisopropylethylamine (2.0 g, 2.7 mL, 15.0 mmol) and MOM–Cl (1.2 g, 1.15 mL, 15.0 mmol) were added to a solution of pivaloyl–inositol **12** (0.39 g, 1.5 mmol) in CH_2Cl_2 (15 mL). The reaction was stirred at room temperature for 12 h and then at reflux for 24 h. The reaction mixture was concentrated *in vacuo*, taken up in $CHCl_3$ (250 mL), washed with water, and rotovaped. The residue was chromatographed on silica gel with hexane–ether 1:1. Yield 0.63 g, 88.7%. Oil, R_f 0.60 (hexane–acetone, 7:3). IR 1730 (C=O) cm^{-1}. ^{1}H-NMR ($CDCl_3$): δ 1.23 (s, 9H), 3.39–3.41 (m, 9H), 3.45–3.50 (m, 7H), 3.55–3.58 (dd, 1H), 3.91–4.02 (m, 2H), 4.06 (tr, 1H), 4.67–4.89 (m, 12H).

*2,3,4,5,6-O-Pentakis(methoxymethyl)-D-*myo*-inositol* **14**

A solution of *1-O-pivaloyl-2,3,4,5,6-O-penta-MOM-D-*myo*-inositol* **13** (0.75 g, 15.5 mmol) in 1 N $NaOCH_3$ in

methanol (15 mL) was stirred at room temperature for 4–6 h. After neutralization with 25% acetic acid–H_2O the reaction mixture was diluted with chloroform (150 mL), washed with water, and concentrated under vacuum. The residue was chromatographed on silica gel with hexane–ether (from 50 to 100% ether). Yield 0.60 g, 96.8%. Oil, R_f 0.30 (hexane–acetone, 7:3). IR 3430 (O–H) cm^{-1}. ^1H-NMR ($CDCl_3$): δ 3.40–3.53 (m, 17H), 3.67 (tr, 1H), 3.90–4.09 (m, 3H), 4.74–4.89 (m, 10H). $[\alpha]^{25}_D$ -37.31° (c = 2.0, $CHCl_3$).

*Methyl 2,3,4,5,6-*O*-pentakis(methoxymethylene)-*D*-*myo*-inositol 1-(hexadecylthio)phosphate* ***16***

A solution of dimethyl chlorophosphite (54 mg, 0.42 mmol) in 1 mL of CH_2Cl_2 was added to a solution of **14** (110 mg, 0.27 mmol) and triethylamine (55 mg, 0.54 mmol) in CH_2Cl_2 (2 mL) at -15 °C. The reaction was stirred at -15 °C for 10–15 min then at room temperature for 1.5 h. Methylene dichloride (75 mL) was added and the reaction mixture was washed with 5% $NaHCO_3$ (3 x 25 mL), water (2 x 30 mL), concentrated *in vacuo* and evaporated twice with toluene. *N*-Hexadecyl thiophthalimide **8** (95 mg, 0.24 mmol) in 2.5 mL of toluene was added to the above residue [*1*-O-*(dimethylphosphino)-2,3,4,5,6-*O*-pentakis(methoxymethylene)-*D*-myo-inositol 15*, δP 139.00, R_f 0.75 (hexane–acetone 7:3)] and the reaction mixture was stirred at room temperature for one hour. Toluene was evaporated and the residue was chromatographed on silica gel with hexane–acetone 7:3. Yield 111 mg, 65.7 %. R_f 0.40 (hexane–acetone 7:3). ^{31}P-NMR ($CDCl_3$): δ 29.58 and 29.28.

Acknowledgements

This work was supported by a grant (to H. S. H.) from the National Science Foundation (DMB-9103973). Anatoliy S. Bushnev was a Visiting Scholar at St Olaf College, 1992–1994.

References

1. Cox, J. W.; Snyder, W. R.; Horrocks, L. A. *Chem. Phys. Lipids* **1979**, *25*, 369–380.

2. Snyder, W. R. *J. Lipid Res.* **1987**, *28*, 949–954.

3. Snyder, W. R. *Anal. Biochem.* **1987**, *164*, 199–206.

4. Nyquist, D. A. *Lipids* **1988**, *23*, 989–992.

5. Young, P. R.; Snyder, W. R.; McMahon, R. F. *Lipids* **1991**, *26*, 957–959.

6. Young, P. R.; Snyder, W. R.; McMahon, R. F. *Biochim. Biophys. Acta* **1992**, *1121*, 297–300.

7. Hendrickson, E. K.; Johnson, J. L.; Hendrickson, H. S. *BioMed. Chem. Lett.* **1991**, *1*, 615–618.

8. Hendrickson, H. S.; Hendrickson, E. K.; Johnson, J. L.; Khan, T. H.; Chial, H. J. *Biochemistry* **1992**, *31*, 12169–12172.

9. Lewis, K. A.; Garigapati, V. R.; Zhou, C.; Roberts, M. F. *Biochemistry* **1993**, *32*, 8836–8841.

10. Bruzik, K. S.; Salamonczyk, G. M. *Carbohydr. Res.* **1989**, *195*, 67–73.

11. Salamonczyk, G. M.; Pietrusiewicz, K. M. *Tetrahedron Lett.* **1991**, *32*, 4031–4032.

12. Salamonczyk, G. M.; Pietrusiewicz, K. M. *Tetrahedron Lett.* **1991**, *32*, 6167–6170.

13. Pietrusiewicz, K. M.; Salamonczyk, G. M.; Bruzik, K. S.; Wieczorek, W. *Tetrahedron* **1992**, *48*, 5523–5542.

14. Bruzik, K. S.; Tsai, M.-D. *J. Am. Chem. Soc.* **1992**, *114*, 6361–6374.

15. Bruzik, K. S.; Lin, G.; Tsai, M.-D. In *Inositol Phosphates and Derivatives. Synthesis, Biochemistry, and Therapeutic Potential*, pp. 172–185, Reitz, A. B. Ed.; 200th National Meeting of the American Chemical Society; Washington, DC, August 26–31, 1991.

16. Muller, C. E.; Roth, H. J. *Tetrahedron Lett.* **1990**, *31*, 501–502.

17. Lin, G.; Bennett, C. F.; Tsai, M.-D. *Biochemistry* **1990**, *29*, 2747–2757.

18. Hendrickson, E. K.; Johnson, J. L.; Hendrickson, H. S. *BioMed. Chem. Lett.* **1991**, *1*, 619–622.

19. Bruzik, K. S.; Myers, J.; Tsai, M.-D. *Tetrahedron Lett.* **1992**, *33*, 1009–1012.

20. Bruzik, K. S.; Morocho, A. M.; Jhon, D.-Y.; Rhee, S. G.; Tsai, M.-D. *Biochemistry* **1992**, *31*, 5183–5193.

(*Received* 17 *December* 1993; *accepted* 11 *January* 1994)