Phosphonolipids. 3. Phosphonic acid analogues of phosphatidylinositol and related materials[1,2]

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A convergent synthesis of an isosteric phosphonic acid analogue of phosphatidylinositol has been accomplished in which a non-hydrolyzable P—C—C linkage is present in place of the normal P—O—C esteric linkage joining the phosphate and diacylglycerol portions of the molecule. The synthetic route used provides the configuration at each stereogenic center to correspond to that present in the biologically generated phospholipid. In addition, the approach provides asymmetric introduction of acyl functions, placing saturated and unsaturated acyl groups in the terminal and internal positions respectively of the backbone portion of the analogue, corresponding to that present in the biologically generated phospholipid.

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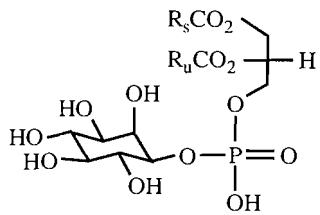
On a réalisé une synthèse convergente d'un acide phosphonique isostère analogue du phosphatidylinositol dans lequel on rencontre une liaison P—C—C à la place de la liaison normale P—O—C de l'ester qui relie les portions phosphate et diacylglycérol de la molécule. La voie de synthèse utilisée permet d'établir que la configuration de chacun des centres stéréogènes correspond à celle qui est présente dans la phospholipide généré d'une façon biologique. De plus, cette approche permet d'introduire des fonctions acyles d'une façon asymétrique, plaçant des groupes saturés et insaturés respectivement dans les positions terminales et internes du squelette de l'analogue, comme on les retrouve dans le phospholipide généré d'une façon biologique.

[Traduit par la rédaction]

Introduction

Phosphoinositides have been the subject of intensive investigation in recent years owing to the recognition of their role for intracellular signalling in response to extracellular stimuli (3). Following the recognition of this biological role for phosphoinositides have come efforts toward the laboratory synthesis of not only phosphatidylinositol (a phosphate diester) (4), but of a variety of analogues designed to probe details of the mechanism of signalling and the potential for regulation of the biochemical processes involved (1, 5).

In the continuing effort of our laboratory concerning the synthesis and investigation of isosteric phosphonic acid analogues of biological phosphates (2, 6), the preparation of an analogue of phosphatidylinositol (**1**) that incorporates all but one of the functional and stereochemical characteristics of the biological material has been undertaken. The structural variation of this analogue (**2**), which is of particular interest and described herein, involves the introduction of a P—C—C linkage in place of the normal P—O—C linkage connecting the phosphate with the diacylglycerol backbone of phosphatidylinositol. In **2** the entire remaining structural elements have been maintained in correspondence with the biological material. Specifically, the configuration at each stereogenic site in **2**, both in the inositol and modified diacylglycerol portions, matches that of the corresponding site of the biological material. Further, the analogue **2** is generated with regiospecific disposition of the acyl groups, saturated (R_s) at the terminal position and unsaturated (R_u) at the internal position. This disposition is in accord with phosphatidylinositol exhibiting preferential interaction with phosphatidylinositol-specific phospholipase C (7).



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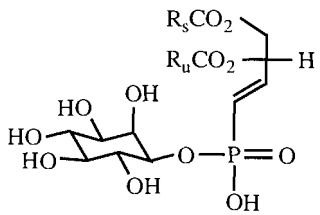

2

The route used for the preparation of **2** is a convergent one, separate syntheses of the protected inositol and lipid portions being performed, followed by a coupling and final deprotection sequence. Two approaches are described for the synthesis of the lipid portion, itself an analogue of phosphatidic acid, these being a classical protection/deprotection route and a regioselective epoxide-opening procedure. The latter general approach has also been used for the preparation of a phosphonic acid analogue of PAF.[4]

The analogue **2**, bearing a non-hydrolyzable ester linkage in the phospholipid portion of the molecule, is anticipated to be of use in the investigation of phospholipase C mediated processes of phosphatidylinositol.

Results and discussion

The synthesis of the lipid component of **2**, an isosteric phosphonic acid analogue of phosphatidic acid, has been

[1]For preliminary reports of this work see ref. 1.

[2]Paper 2 in this series is ref. 2.

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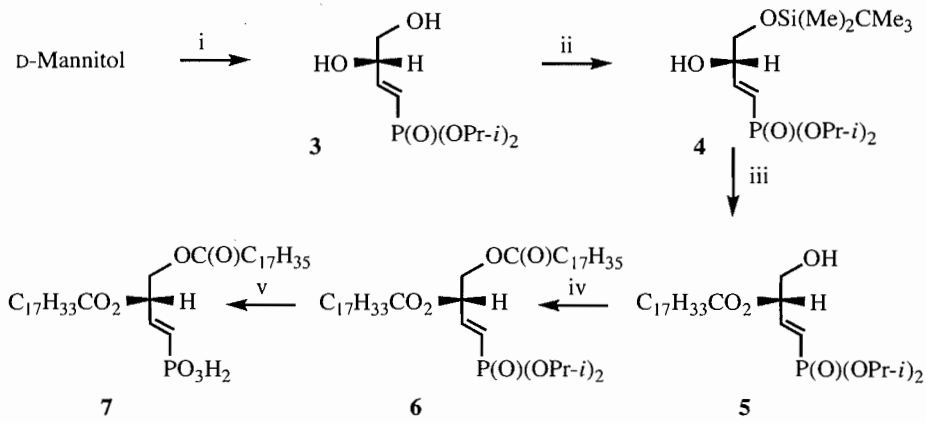
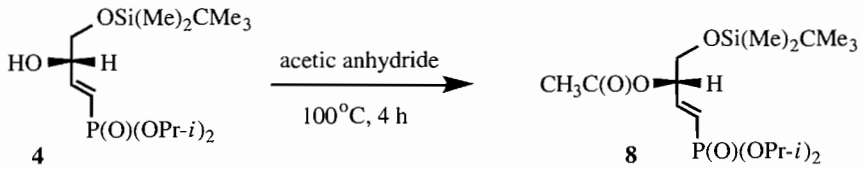
[4]Y.-j. Liu, B. E. Tropp, and R. Engel, unpublished results of this laboratory.



i. acetone, $ZnCl_2$; $Pb(OAc)_4$; tetraisopropyl methylenebisphosphonate/BuLi; aq. acid: ii. *tert*-butyldimethylsilyl chloride, imidazole, DMF (66% yield): iii. oleoyl chloride, pyridine, DMAP, 0°C; Bu_4NF, THF (36% yield): iv. stearic anhydride, pyridine, DMAP, 0°C–r.t. (68% yield): v. TMSBr, ether; water (75% yield)

SCHEME 1



98.5% yield

SCHEME 2

accomplished by two routes. The first of these, involving a classical approach of protection/deprotection, is illustrated in Scheme 1.

Standard procedures were used for the conversion of D-mannitol to **3**, including the initial protection and cleavage to D-glyceraldehyde acetonide (8), the preparation of the tetraisopropyl methylenebisphosphonate (9), and its use for the synthesis of the diisopropyl (*R*)-(*E*)-3,4-dihydroxybut-1-enyl-1-phosphonate (10).

Selective protection of the terminal hydroxyl group of **3** was accomplished using a 3% excess of *tert*-butyldimethylsilyl chloride with imidazole. Small amounts of the internally silylated and disilylated product were removed easily using flash chromatography.

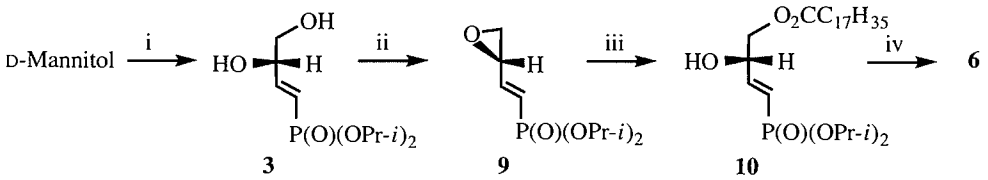
The verification of the position of silylation in **4** was accomplished by the preparation of a derivative and examination of its ^{1}H NMR spectrum. In the 200 MHz ^{1}H NMR spectrum of **3** the signals for the hydrogens attached to C-3 and C-4 were virtually indistinguishable, appearing as a multiplet at δ 3.7 and integrating for three hydrogens. A sample of the material **4** was acetylated using acetic anhydride to give **8** in nearly quantitative yield (Scheme 2).

The acetate **8** exhibited a downfield shift (to δ 5.3) for a one-hydrogen multiplet corresponding to the C-3 hydrogen while a two-hydrogen multiplet remained at δ 3.7. Differentiation of sites of acylation on a phosphonate lipid backbone have been noted previously by similar shifts for related systems, the differentiation having been confirmed by specific enzymatic action (11).

The material **4** was acylated using oleoyl chloride in pyridine with 4-(dimethylamino)pyridine (DMAP) at 0°C, conditions slightly modified from those known not to permit significant acyl migration (12). Deprotection of the terminal hydroxyl group was performed to generate **5** prior to complete structural and purity analysis. The ^{1}H NMR spectrum of **5** verified that no acyl migration had occurred during the work-up, exhibiting an upfield multiplet for the pair of hydrogens at C-4 and a downfield multiplet for the single hydrogen at C-3. Further acylation placing a saturated fatty-acyl function at the terminal hydroxyl position was accomplished, again using conditions known not to permit significant acyl migration, and cleavage of the phosphonate esters was accomplished using a standard procedure (13).

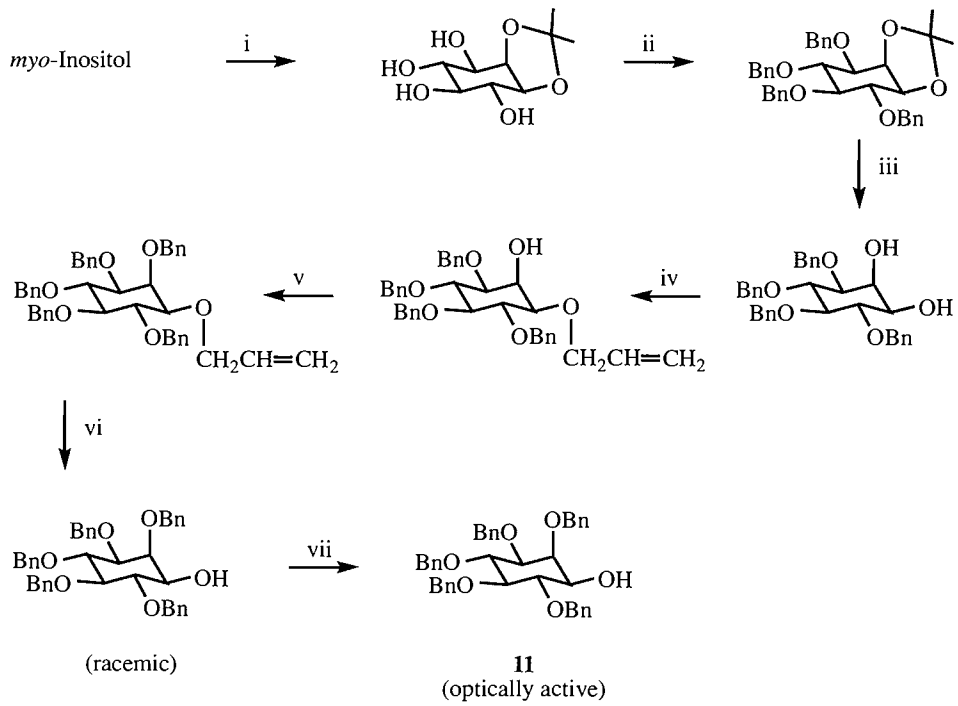
In light of the success demonstrated for the preparation of symmetric-chain glycerophospholipids starting with optically active glycidyl tosylate (14), an alternative synthesis of **6**, and subsequently **7**, was developed as illustrated in Scheme 3.

The generation of the epoxide **9** from the diol **3** was accomplished in a two-step procedure, the tosylate being formed first, followed by intramolecular displacement to effect ring closure. Although IR and NMR spectra could be obtained which were in accord with the proposed structures of the tosylate and epoxide **9** species, these compounds proved too reactive for storage or satisfactory elemental analysis and were used immediately upon isolation in continuing reactions. Regiospecific ring opening was accomplished using a modification of the previously reported technique (14) facilitated by boron trifluoride etherate. The ^{1}H NMR spectrum of **10** verified the introduction of the acyl function at the terminal position of the chain. (The regiospecificity of this type of reaction has been in-



i. acetone, $ZnCl_2$; $Pb(OAc)_4$; tetraisopropyl methylenebisphosphonate/BuLi; aq. acid: ii. *p*-toluenesulfonyl chloride, pyridine; K_2CO_3, methanol (76% yield): iii. BF_3 etherate, stearic acid (70% yield): iv. oleic anhydride, DMF, pyridine, DMAP, 0°C–r.t. (84% yield)

SCHEME 3



i. DMSO, 2,2-dimethoxypropane, *p*-TsOH (50% yield): ii. DMF, 97% NaH, benzyl bromide (70% yield): iii. 0.9 M HCl in methanol (75% yield): iv. benzene, NaOH, allyl bromide (58% yield): v. benzene, 97% NaH, benzyl bromide (90% yield): vi. aqueous methanol, *p*-TsOH, 10% Pd/C (38% yield): vii. methylene chloride, triethylamine, (*S*)-(−)-camphanic acid chloride (95% yield); HPLC separation of diastereoisomers, Lichrosorb Si-60, 1% diethyl ether in methylene chloride eluent; 0.1 M KOH in ethanol (98% yield).

SCHEME 4

vestigated and is to be published separately.[5]) Finally, **10** was acylated at the internal position using a procedure known not to involve acyl migration in structurally related systems (12). The materials **6** and **7** from the two approaches were identical.

Once prepared, the analogue **7** of phosphatidic acid was ready for coupling with a suitably protected, optically active derivative **11** of inositol for the generation of the target material **2**. The overall route for the preparation of the protected inositol **11** is one that involves modifications of inositol-related and allyl ether cleavage processes previously reported (15–17) and is outlined in Scheme 4.

Resolution of the pentabenzyl-*myo*-inositol is accomplished by the generation of a diastereoisomeric ester mixture using (*S*)-camphanic acid chloride followed by separation of the diastereoisomers using HPLC. The synthesis of **2** is completed by the coupling of **11** with the independently prepared **7** and deprotection as shown in Scheme 5.

[5]Y.-j. Liu and R. Engel, unpublished results of this laboratory.

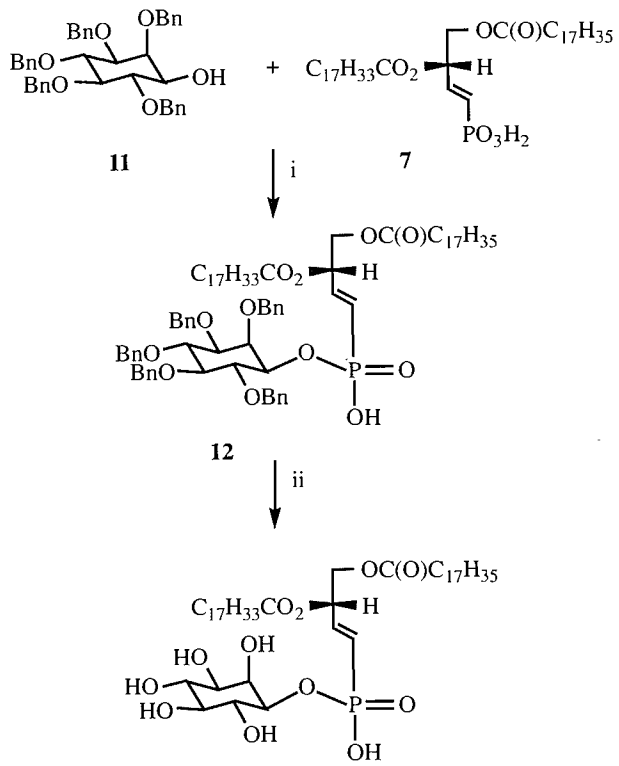
The use of (*S*)-camphanic acid chloride for the resolution of 2,3,4,5,6-penta-*O*-benzyl-*myo*-inositol has been discussed recently (18). The coupling using trichloroacetonitrile is an adaptation of a procedure used previously in this laboratory and elsewhere (19). The deprotection using iodotrimethylsilane (20) rapidly cleaves benzyl ether linkages selectively compared to other ether linkages and ester linkages, liberating the target material without reduction of the olefinic linkages.

This procedure represents the first preparation of a nominally isosteric phosphonic acid analogue of phosphatidyl inositol in which the esteric oxygen of the normal diacylglycerol backbone has been replaced by a carbon–phosphorus linkage.

Experimental

General

All chemicals were of reagent quality and used without further purification with the following exceptions: chloroform was distilled over phosphorus pentoxide; pyridine was dried over calcium hydride and distilled; dimethylformamide (DMF) was dried over



i. pyridine, trichloroacetonitrile, 50°C, 72 h (20% yield): ii. chloroform, iodotrimethylsilane, 20 min (85% yield).

SCHEME 5

molecular sieves 4A prior to use; oleoyl chloride (commercial source) was kept in the sealed ampoule until immediately prior to use; methanol for chromatographic purposes was distilled prior to use.

(*S*)-(*E*)-3,4-Dihydroxybut-1-enylphosphonic acid diisopropyl ester (**3**) and its precursors were prepared as previously described (10). Racemic 1-*O*-allyl-2,3,4,5,6-tetra-*O*-benzyl-*myo*-inositol and its precursors were prepared as previously described (15). Thin-layer chromatography (TLC) was performed using Polygram Sil N-HR sheets (Brinkmann)(PS) or Kodak Chromagram sheets (Eastman) (KC). Silica gel for flash chromatography was from EM Science (230–300 mesh). HPLC was performed using a Waters 6000A instrument with either a Lichrosorb Si60 (10 μm) column or a Bakerbond Chiral DNBPG (Coval) (5 μm) column. Infrared spectra were measured using a Perkin–Elmer 1600 FTIR instrument, and ^{1}H NMR spectra were measured using an IBM–Bruker WP200SY instrument. Optical rotations were measured using a JASCO DIP-140 digital polarimeter. Elemental analyses were performed by Desert Analytics of Tucson, Arizona, and by Schwarzkopf Microanalytical Laboratories of Woodside, New York.

Preparation of (S)-(E)-*4*-tert-*butyldimethylsiloxy-3-hydroxybut-1-enylphosphonic acid diisopropyl ester (4)*

The (*S*)-(*E*)-3,4-dihydroxybut-1-enylphosphonic acid diisopropyl ester (**3**)(16.14 g, 64 mmol) was dissolved in dry DMF (200 mL) and there was added to it with stirring at room temperature *tert*-butyldimethylsilyl chloride (9.96 g, 66 mmol) and imidazole (10.90 g, 160 mmol). The reaction mixture was stirred at room temperature for 72 h after which time the volatile materials were evaporated under reduced pressure. Water (150 mL) was added to the residue and the aqueous solution was extracted with ethyl acetate (3 × 200 mL). The extracts were combined, dried over calcium chloride, filtered, and volatile materials were evaporated under reduced pressure. The residue was subjected to flash chromatography on a silica gel column (120 g, 5 cm diameter) packed with methylene chloride. The elution of the column was performed with the following order of solvents: 525 mL methylene chloride; 375 mL 1:1 methylene chloride:chloroform; 75 mL chloroform; 150 mL chloroform:ethyl acetate; 775 mL ethyl acetate. Fractions from the ethyl acetate portion of the elution exhibiting a single spot of R_f = 0.67 on TLC (KC, ethyl acetate) as visualized with phosphomolybdate spray reagent were combined to give the pure desired material (15.48 g, 0.042 mol, 66%), which exhibited IR and NMR spectra in accord with the proposed structure (**4**). Specific rotation: $[\alpha]_D$ −34.0 (0.16 M, ethanol). The ^{1}H NMR ($CDCl_3$) exhibited the following signals: δ 0.1, singlet, 6H; δ 0.9, singlet, 9H; δ 1.4, doublet of doublets, 12H; δ 3.5–3.9, multiplet, 3H; δ 4.3, broad, 1H; δ 4.7, multiplet, 2H; δ 5.8–7.5, multiplet, 2H. Anal. calcd. for $C_{16}H_{35}O_5PSi$: C 52.43, H 9.62%; found: C 52.46, H 9.69%. A small amount of material eluted immediately prior to the desired material and exhibited ^{1}H NMR and IR spectra indicating it to be the result of silylation at both hydroxyl groups of the starting diol.

Preparation of (S)-(E)-*4*-tert-*butyldimethylsiloxy-3-acetoxybut-1-enylphosphonic acid diisopropyl ester (8)*

A sample of **4** (100 mg, 0.27 mmol) was dissolved in acetic anhydride (10 mL) and heated at 100°C for 4 h. After this time all volatile materials were removed under reduced pressure. The solid residue was found to exhibit a single spot of R_f = 0.15 on TLC (KC, chloroform) visualized with phosphomolybdate spray reagent. This material exhibited IR and ^{1}H NMR spectra in accord with the proposed structure (**8**) (112 mg, 98.5%). Specific rotation: $[\alpha]_D$ −1.1 (0.054 M, ethanol). The ^{1}H NMR ($CDCl_3$) spectrum exhibited the following signals: δ 0.1, singlet, 6H; δ 0.9, singlet, 9H; δ 1.4, doublet of doublets, 12H; δ 2.2, singlet, 3H; δ 3.7, multiplet, 2H; δ 4.4, multiplet, 2H; δ 4.7, multiplet, 1H; δ 5.8–7.5, multiplet, 2H. Anal. calcd. for $C_{18}H_{37}O_6PSi$: C 52.92, H 9.13%; found: C 53.22, H 9.19%.

Preparation of (S)-(E)-*4-hydroxy-3-oleoyloxybut-1-enylphosphonic acid diisopropyl ester (5)*

The material (**4**) (2.16 g, 5.89 mmol) was dissolved in chloroform (40 mL) and cooled to 0°C in an ice bath. There was then added to it pyridine (0.9 g, 11.38 mmol) and a catalytic amount of 4-dimethylaminopyridine (0.050 g) followed by a solution of oleoyl chloride (2.95 g, 9.8 mmol) in chloroform (5 mL). The reaction mixture was allowed to come to room temperature and was stirred for 4 days. The volatile materials were then removed under reduced pressure, the residue was dissolved in tetrahydrofuran (10 mL), and there was added tetrabutylammonium fluoride (0.7 g, 3.23 mmol) and the reaction mixture was stirred at room temperature for 12 h. Chloroform (100 mL) and water (50 mL) were then added and the mixture was stirred vigorously. The layers were separated and the aqueous layer was washed with chloroform (50 mL). The organic solutions were combined, dried over sodium sulfate, filtered, and volatile materials were removed under reduced pressure. The residue exhibited a single spot of R_f = 0.70 on TLC (KC, ethyl acetate) and IR and ^{1}H NMR spectra in accord with the proposed structure (**5**) (1.09 g, 36%). Specific rotation: $[\alpha]_D$ −1.6 (0.05 M, ethanol). The ^{1}H NMR ($CDCl_3$) spectrum exhibited the following signals: δ 0.9, broad triplet, 3H; δ 1.2–1.7, broad, 34H; δ 2.0, multiplet, 5H (including OH); δ 2.2, multiplet, 2H; δ 3.9, multiplet, 2H; δ 4.7, multiplet, 2H; δ 5.1, multiplet, 2H; δ 5.3, triplet, 1H; δ 5.8–7.5, multiplet, 2H. Anal. calcd. for $C_{28}H_{53}O_6P$: C 65.09, H 10.34%; found: C 64.89, H 10.51%.

Preparation of (S)-(E)-*3,4-epoxybut-1-enylphosphonic acid diisopropyl ester (9)*

The material (**3**) (2.0 g, 7.93 mmol) was dissolved in chloroform (7.8 mL) and cooled to 0°C with an ice bath. Pyridine (1.26 mL) was then added followed by *p*-toluenesulfonyl chloride

(2.22 g, 11.6 mmol) and the reaction mixture was stirred for 2 h. After this time ether (25 mL) and water (6 mL) were added to the reaction mixture. The organic layer was separated and washed with water (2 × 16 mL), dried over calcium chloride, filtered, and the volatile materials were removed under reduced pressure. The ^{1}H NMR ($CDCl_3$) of the crude material (2.2 g) indicated the desired material to be present, exhibiting the following signals: δ 1.4, doublet of doublets, 12H; δ 2.2, broad, 1H; δ 2.5, singlet, 3H; δ 3.5–3.9, multiplet, 3H; δ 4.8, multiplet, 2H; δ 5.8–7.5, multiplet, 2H; δ 7.8 AA′BB′, 4H. The material exhibited one major spot of R_f = 0.25 on TLC (KC, 1:15 ethyl acetate:hexane) as visualized with phosphomolybdate spray reagent and was used without further purification. The isolated tosylate (2.2 g) was dissolved in methanol (24 mL) under nitrogen and potassium carbonate (0.98 g, 7.08 mmol) was added. The reaction mixture was stirred for 2 h at −10°C. After this time ether (24 mL) was added and the mixture was filtered twice through a pad of silica gel. The crude material (1.41 g, 76%) exhibited one major spot of R_f = 0.59 on TLC (KC, ethyl acetate) visualized with phosphomolybdate spray reagent and IR and ^{1}H NMR spectra in accord with the proposed structure. The ^{1}H NMR ($CDCl_3$) spectrum exhibited the following signals: δ 1.4, doublet of doublets, 12H; δ 2.7, multiplet, 1H; δ 3.1, multiplet, 1H; δ 3.4, multiplet, 1H; δ 4.7, multiplet 2H; δ 6.0–7.8, multiplet, 2H. The material proved to be too reactive for storage or elemental analysis and was used immediately upon isolation.

Preparation of (S)-(E)-*3-hydroxy-4 stearoyloxybut-1-enylphosphonic acid diisopropyl ester (**10**)*

Stearic acid (0.34 g, 1.20 mmol), (*S*)-(*E*)-3,4-epoxybut-1-enylphosphonic acid diisopropyl ester (**9**) (0.28 g, 1.20 mmol), and boron trifluoride etherate (0.082 g, 1.20 mmol) in ether (total volume 11 mL) were stirred overnight at room temperature. Volatile materials were removed under reduced pressure and the residue was subjected to flash chromatography (20 g, 2 cm diameter) packed and eluted with chloroform. Fractions exhibiting a single spot of R_f = 0.18 on TLC (KC, chloroform) as visualized with phosphomolybdate spray reagent were combined to give the pure desired material (0.436 g, 70%) which exhibited IR and ^{1}H NMR spectra in accord with the proposed structure (**10**). The ^{1}H NMR ($CDCl_3$) exhibited the following signals: δ 0.9, triplet, 3H; δ 1.2, broad, 42H; δ 2.3, triplet, 2H; δ 3.3, broad, 1H; δ 3.7, multiplet, 1H; δ 4.1–4.9, multiplet, 4H; δ 5.8–7.6, multiplet, 2H. Analysis, $C_{28}C_{55}O_6P$ requires C 64.83%, H 10.69%; found: C 64.71%, H 10.82%. Specific rotation: $[\alpha]_D$ = 8.9 (0.176 M, chloroform).

Preparation of (S)-(E)-*3-oleoyloxy-4-stearoyloxybut-1-enylphosphonic acid diisopropyl ester (**6**)*

*Method A: from **5***

To (*S*)-(*E*)-4-hydroxy-3-oleoyloxybut-1-enylphosphonic acid diisopropyl ester (**5**)(1.66 g, 3.21 mmol) dissolved in chloroform (15 mL) cooled to 0°C with an ice bath was added pyridine (0.9 g, 11.38 mmol) with a catalytic amount of 4-dimethylaminopyridine (0.050 g). There was then added stearic anhydride (1.57 g, 5.52 mmol) under a nitrogen atmosphere and the reaction mixture was stirred for 3 days, being allowed to come to room temperature. The volatile materials were removed under reduced pressure and the residue was subjected to flash chromatography (20 g, 2 cm diameter) packed with chloroform and eluted with ethyl acetate. Fractions exhibiting a single spot of R_f = 0.33 on TLC (KC, ethyl acetate) as visualized with phosphomolybdate spray reagent were combined to give the pure desired material (1.71 g, 68%), which exhibited IR and NMR spectra in accord with the proposed structure (**5**). Specific rotation $[\alpha]_D$ 0.18 (0.2 M, ethyl acetate). The ^{1}H NMR ($CDCl_3$) exhibited the following signals: δ 0.9, broad, 6H; δ 1.3, broad, 64H; δ 1.8–2.5, multiplet, 8H; δ 4.1–5.1, multiplet, 5H; δ 5.3, triplet, 2H; δ 5.8–7.5 multiplet, 2H. Anal. calcd. for $C_{46}H_{87}O_7P$: C 70.55, H 11.20%; found: C 70.41, H 11.24%.

*Method B: from **10***

To (*S*)-(*E*)-3-hydroxy-4-stearoyloxybut-1-enylphosphonic acid diisopropyl ester (**10**) (160 mg, 0.305 mmol) in dry dimethyl formamide (2.5 mL) with pyridine (0.25 mL) and 4-dimethylaminopyridine (0.05 g) at 0°C was added oleic anhydride (0.2 g, 0.366 mmol). The reaction mixture was stirred and allowed to come to room temperature overnight. Volatile materials were removed under reduced pressure and the residue was subjected to flash chromatography (20 g, 2 cm diameter) packed with chloroform and eluted with ethyl acetate:chloroform 3:1. Fractions exhibiting a single spot of R_f = 0.33 on TLC (KC, ethyl acetate) as visualized with phosphomolybdate spray reagent were combined to give the pure desired material (201 mg, 84%), which exhibited IR and ^{1}H NMR spectra and optical rotations identical to those noted for **6** prepared in Method A as noted previously.

Preparation of (S)-(E)-*oleoyloxy-4-stearoyloxybut-1-enlyphosphonic acid (**7**)*

Under a nitrogen atmosphere (*S*)-(*E*)-3-oleoyloxy-4-stearoyloxybut-1-enylphosphonic acid diisopropyl ester (**6**) (0.70 g, 0.89 mmol) was added to diethyl ether (15 mL). To the solution was added dropwise bromotrimethylsilane (0.46 g, 3.0 mmol) and the resultant mixture was stirred at room temperature overnight. The reactant mixture was then washed with water (3 × 35 mL) and the organic layer was separated, dried over anhydrous sodium sulfate, filtered, and evaporated under reduced pressure. The resulting solid was recrystallized from a minimum volume of methanol and the precipitate was subjected to flash chromatography (20 g, 5 cm diameter) packed with chloroform and eluted with chloroform (200 mL) followed by ethyl acetate (4 × 200 mL). Fractions of the ethyl acetate eluent exhibiting a single spot of R_f = 0.14 on TLC (KC, chloroform) as visualized with phosphomolybdate spray reagent were combined to give the pure desired material (466 mg, 75%), which exhibited IR and ^{1}H NMR spectra in accord with the proposed structure (**7**). Specific rotation: $[\alpha]_D$ 1.42 (0.13 M, chloroform). The ^{1}H NMR ($CDCl_3$) exhibited the following signals: δ 0.9, broad triplet, 6H; δ 1.2, broad, 52H; δ 1.8–2.5, multiplet, 8H; 4.3–4.9, multiplet, 3H; δ 5.3, triplet, 2H; δ 5.8–7.5, multiplet, 2H; δ 11.5, singlet, 2H. Anal. calcd. for $C_{40}H_{75}O_7P$: C 68.73, H 10.82%; found: C 68.73, H 11.01%.

Preparation of (±)-*2,3,4,5,6-penta-*O-*benzyl-*myo-*inositol* (±)-(**11**)*

The racemic 1-*O*-allyl-2,3,4,5,6-penta-*O*-benzyl-*myo*-inositol (4.38 g, 6.72 mmol) was dissolved in methanol (10 mL) to which water (10 mL) was added. There was then added 10% Pd/C (0.44 g) along with *p*-toluenesulfonic acid monohydrate (0.44 g) and the resulting mixture was heated at reflux for 24 h. Solid materials were removed by filtration and the volatile materials of the filtrate were removed under reduced pressure to leave an oily residue. This residue was partitioned between a water/ether mixture (80 mL of each) and the ether layer was collected, dried over anhydrous magnesium sulfate, filtered, and evaporated under reduced pressure. The oily residue was subjected to flash chromatography (40 g, 2 cm diameter) packed and eluted with chloroform:ether 50:1. Fractions exhibiting a single spot of R_f = 0.62 on TLC (PS, chloroform:ether 50:1) as visualized with iodine were combined to give the pure desired material (1.55 g, 38%) as a clear oil, which crystallized on standing to give white crystals that exhibited IR and ^{1}H NMR spectra in accord with the proposed structure and a melting point in agreement with that previously reported (15). The ^{1}H NMR ($CDCl_3$) exhibited the following signals: δ 2.05–2.46, broad singlet, 1H; δ 3.33–3.62, multiplet, 3H; δ 3.74–3.94, multiplet, 1H; δ 3.96–4.20 multiplet, 2H; δ 4.56–5.13, multiplet, 10H; δ 7.10–7.62, multiplet, 25H.

Resolution of (±)-*2,3,4,5,6-penta-*O-*benzyl-*myo-*inositol* (±)-(**11**)*

The (±)-2,3,4,5,6-penta-*O*-benzyl-*myo*-inositol (1.55 g, 2.46 mmol) was dissolved in methylene chloride (37 mL) and to it was added DMAP (0.033 g, 0.27 mmol), triethylamine (0.75 g, 7.43 mmol), and (−)-camphanic acid chloride (0.64 g, 2.95 mmol). The reaction mixture was stirred at room temperature for 24 h, after which time it was washed with water (2 × 30 mL) and saturated

aqueous sodium chloride solution (20 mL). It was then dried over anhydrous magnesium sulfate, filtered, and the volatile materials were evaporated under reduced pressure. The residual solid was subjected to flash chromatography (40 g, 2 cm diameter) packed and eluted using methylene chloride:ether 99:1. Material exhibiting R_f between 0.5 and 0.75 on TLC (PS, methylene chloride:ether 99:1) as visualized using iodine was separated into two components using HPLC (Lichrosorb Si-60 10 μm) eluting with methylene chloride:ether 99:1. In this way two components (less polar ester, R_f = 0.73, 1.03 g, and more polar ester, R_f = 0.60, 0.99 g, using the above noted TLC conditions) were isolated as pure materials, which exhibited IR and ^{1}H NMR spectra in accord with their proposed structures and which were separately subjected to hydrolysis. The ^{1}H NMR ($CDCl_3$) exhibited the following signals: (less polar ester) δ 0.83–0.96, singlet, 3H; δ 0.96–1.05, singlet, 3H; δ 1.05–1.19, singlet, 3H; δ 1.48–2.02, multiplet, 3H; δ 2.15–2.43, multiplet, 1H; δ 3.50–3.73, multiplet, 2H; δ 4.01–4.32, multiplet, 3H; δ 4.50–5.17, multiplet, 11H; δ 6.90–7.54, multiplet, 25H; (more polar ester) δ 0.74–0.90, singlet, 3H; δ 0.90–1.03, singlet, 3H; δ 1.03–1.22, singlet, 3H; δ 1.43–2.10, multiplet; 3H; δ 2.15–2.46, multiplet, 1H; δ 3.43–3.80, multiplet, 2H; δ 3.90–4.40, multiplet, 3H; δ 4.44–5.70, multiplet, 11H; δ 6.93–7.68, multiplet, 25H.

The more polar and less polar esters were each separately hydrolyzed using the procedure as described here for the more polar ester. The more polar ester (0.99 g, 1.22 mmol) was added to a 0.1 M solution of potassium by hydroxide in ethanol (100 mL) and the mixture was stirred at room temperature for 24 h. After this time the volatile materials were evaporated under reduced pressure and the residue was partitioned between ether:water 10:7 (170 mL). The layers were separated and the aqueous layer was extracted with ether (35 mL). The organic layers were combined, dried over magnesium sulfate, filtered, and volatile materials were evaporated under reduced pressure. The oily residue was subjected to flash chromatography (20 g, 2 cm diameter) packed and eluted with hexane:ether 1:3. The fractions exhibiting a single spot of R_f = 0.81 (PS, hexane:ether 1:3) were combined to give the pure desired material (0.725 g, 94.2%), which exhibited IR and ^{1}H NMR spectra in accord with the proposed structure (−)-(**11**). Specific rotation: $[\alpha]_D$ −9.0 (0.11 M, ethanol). The ^{1}H NMR ($CDCl_3$) exhibited the following signals: δ 2.02–2.26, broad doublet, 1H; δ 3.25–3.60, multiplet, 3H; δ 3.60–3.87, multiplet, 1H; δ 3.87–4.14, multiplet, 2H; δ 4.46–5.08, multiplet, 10H; δ 6.94–7.55, multiplet, 25H.

The equivalent procedure was performed using the less polar ester (1.03 g, 1.27 mmol) leading to the isolation of (+)-(**11**) (0.726 g, 90.9%), which exhibited IR, TLC, and NMR data in accord with the proposed structure. Specific rotation: $[\alpha]_D$ +8.8 (0.11 M, ethanol). The ^{1}H NMR ($CDCl_3$) exhibited the following signals: δ 2.08–2.25, broad doublet, 1H; δ 3.22–3.53, multiplet, 3H; δ 3.60–3.84, multiplet, 1H; δ 3.86–4.08, multiplet, 2H: δ 4.40–5.04, multiplet, 10H; δ 6.88–7.47, multiplet, 25H.

The observed specific rotations were in accord with those previously reported (16).

Preparation of (S)-(E)*-3-oleoyloxy-4-stearoyloxybut-1-enyl-phosphonic acid 2,3,4,5,6-penta-*O*-benzyl-*myo*-inosit-1-yl ester (12)*

To (*S*)-(*E*)-3-oleoyloxy-4-stearoyloxybut-1-enylphosphonic acid (**7**) (70 mg, 0.10 mmol) dissolved in freshly distilled pyridine (0.5 mL) was added (−)-2,3,4,5,6-penta-*O*-benzyl-*myo*-inositol (−)-(**11**) (50 mg, 0.08 mmol), followed by an excess of trichloroacetonitrile (0.45 g). The reaction flask was stoppered under a nitrogen atmosphere and the reaction mixture was stirred for 3 days using a controlled temperature water bath at 51°C. After this time volatile materials were evaporated under reduced pressure and the residue was purified by flash chromatography (10 g, 2 cm diameter) packed and eluted using methylene chloride:ether 50:1. The fractions exhibiting R_f = 0.85 on TLC (PS, methylene chloride:ether 50:1) as visualized with phosphomolybdate spray reagent were combined to give the pure desired material (21 mg, 20%), which exhibited IR and ^{1}H NMR spectra in accord with the proposed structure (**12**). Specific rotation: $[\alpha]_D$ −2.47 (0.0014 M, CCl_4). The ^{1}H NMR ($CDCl_3$) exhibited the following signals: δ 0.9, broad multiplet, 6H; δ 1.1–1.4, broad multiplet, 52H; δ 1.9–2.5, multiplet, 8H; δ 3.3–3.6, multiplet, 3H; δ 3.8–4.0, multiplet, 2H; δ 4.0–5.0, multiplet, 13H; δ 5.2, multiplet, 1H; δ 5.4, triplet, 2H; δ 5.6–7.8, multiplet, 2H; δ 7.0–7.4, multiplet, 25H; δ 12.1, singlet, 1H. Anal. calcd. for $C_{81}H_{115}O_{12}P$: C 74.17, H 8.84%; found: C 73.91, H 9.14%.

Preparation of (S)-(E)*-3-oleoyloxy-4-stearoyloxybut-1-enylphosphonic acid* myo*-inosit-1-yl ester (2)*

The material (*S*)-(*E*)-3-oleoyloxy-4-stearoyloxybut-1-enylphosphonic acid 2,3,4,5,6-penta-*O*-benzyl-*myo*-inosit-1-yl ester (**12**) (7.3 mg, 0.0056 mmol) was dissolved in chloroform (0.5 mL), an excess of iodotrimethylsilane (0.25 mL) was added, and the reaction mixture was stirred at room temperature for 15 min. The reaction was quenched by the addition of methanol (0.75 mL). Volatile materials were removed under reduced pressure and diethyl ether (1 mL) was added to the residue. Saturated aqueous sodium bisulfite solution (2 drops) was added until the material became clear. Volatile materials were removed again under reduced pressure and the residue was subjected to flash chromatography (20 g, 2 cm diameter) packed and eluted with chloroform. Those fractions exhibiting a single spot of R_f = 0.31 (PS, chloroform) were combined to give the pure desired material (3.7 mg, 76.7%), which exhibited IR and ^{1}H NMR spectra in accord with the proposed structure (**2**). Specific rotation: $[\alpha]_D$ −6.38 (0.0021 M, $CHCl_3$). The ^{1}H NMR exhibited the following signals: δ 0.9, broad multiplet, 6H; δ 1.0–1.6, broad multiplet, 52H; δ 1.6–2.5, broad, 8H; δ 3.6, 6H, singlet; δ 3.7–4.5, broad multiplet, 9H; δ 5.4,, triplet, 2H; δ 5.7–7.8, multiplet, 2H. Anal. calcd. for $C_{46}H_{85}O_{12}P$: C 64.16, H 9.95%; found: C 63.90, H 10.10%.

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