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Short communication

Synthesis of *rac*-1-deoxy-1-thio-dihydroceramide-1-phosphate

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Abstract

The synthesis of racemic 1-deoxy-1-thio-dihydroceramide-1-phosphate **6** from *rac*-3-benzoyl-dihydroceramide **1** as a substrate analogue for ceramide 1-phosphate phosphatase, was developed using an Arbuzov-type reaction to form the S–P bond.

Keywords: Ceramide phosphate; Synthesis; Thiophosphate

1. Introduction

Phosphosphingolipids are not limited to sphingomyelin, sphingoethanolamine, and their phosphonate analogues. In sphingolipid-containing fractions from microorganisms, other sphingolipids containing glycerol and glycerol phosphate [1,2], or 1,2-dihydroxy-3-aminopropane [3] have been identified. Ceramide-1-phosphates may be intermediates in sphingolipid biosynthesis in microorganisms [4]. Ceramide-1-phosphates were proposed as precursors of cytidinediphosphoceramides, which in turn are precursors in the biosynthesis of ceramide phosphoglycerols, ceramide phosphoserines, and possibly other complex sphingophospholipids [4,5]. In 1990, Dressler and Kolesnick showed that ceramide phosphates exist in leukemia cells (HL-60) and discussed their metabolic pathways [6]. Ceramide 1-phosphate phosphatase was described by Shinghal et al. [7] and Boudker and Futerman [8]. The chemical synthesis of ceramide phosphates and their analogues will further aid the study of these compounds and the above-mentioned enzymes.

Earlier, one of the authors reported the synthesis of optically-active and racemic ceramide-1-

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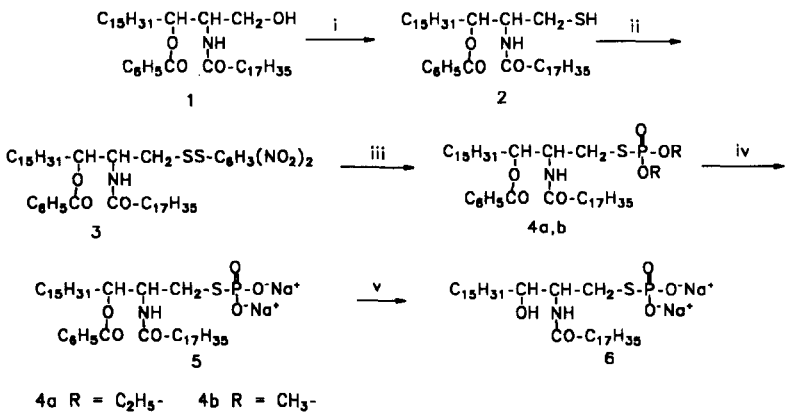

Scheme 1. Synthesis of ceramide thiophosphate. Reagents and conditions: (i) P_2S_5/xylene, reflux, 2 h [10]; AcOH/H_2O (1:50), reflux, 4 h; (ii) 2,4$(NO_2)_2C_6H_3SCl$/Et_3N/toluene, rt, 2 h; (iii) $(EtO)_3P$ or $(MeO)_3P$/toluene, rt, 72–80 h; (iv) Me_3SiI/CH_2Cl_2, rt, 2 h; (v) MeONa/MeOH-$CHCl_3$, rt, 4–6 h.

phosphates [9]. Here we report the synthesis of a racemic thio analogue, 1-deoxy-1-thio-dihydroceramide-1-phosphate (**6**, Scheme 1), with the sulfur atom between the ceramide and phosphoric acid residues. This thiophosphate analogue may be useful in a spectrophotometric assay of ceramide-1-phosphatase, similar to that used for phosphatidylinositol-specific phospholipase C [25].

2. Experimental

2.1. Materials and analytical procedures

3-Benzoyl-2-stearoyl-1-deoxy-1-*rac*-sphinganin-1-thiol **2** was synthesized according to the method of Karpyshev et al. [10]. Alufolien Kieselgel 60 F_{254} (Merck) was used for TLC-analysis. Compounds were detected by spraying with chromic acid in 55% H_2SO_4 or (for phosphorus-containing compounds) with ammonium molybdate in 10% aqueous sulfuric acid 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 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 as a film with mineral oil on a sodium chloride disc on a MIDAC FT-IR spectrometer; only the structurally important peaks are listed. The positive ion FAB mass spectrum of disulfide **3** and negative ion FAB mass spectra of thiophosphates **5** and **6** were determined using a JEOL HX-110 double-focusing mass spectrometer (Jeol Ltd., Tokyo).

2.2. 3-Benzoyl-2-stearoyl-1-deoxy-rac-sphinganin-1-(2,4-dinitrophenyl)disulfide 3

A solution of racemic 3-benzoylthioceramide **2** (200 mg, 0.30 mmol) in THF (8 ml) was added to a solution of 2,4-dinitrophenylsulfenyl chloride (280 mg, 1.20 mmol) and triethylamine (0.160 ml, 116 mg, 1.15 mmol) in THF (16 ml) at 0°C for 15–20 min. The reaction mixture was then stirred at room temperature for 2 h, the precipitate filtered and washed with THF, the filtrate concentrated under vacuum, and the residue chromatographed on a silica gel column with $CHCl_3$. Yield 240 mg, 92%; mp 112–113°C; R_f 0.40 ($CHCl_3$), and 0.75 (hexane-acetone, 6:4). IR 3294 (NH), 2350 (SS), 1714 (ester C=O), 1646 (amide I), 1538 (amide II), 1593, 1524 and 1337 (2,4-dinitrophenyl), 1272 (benzoate C–O) cm^{-1}. ^{1}H-NMR ($CDCl_3$): δ 9.04 (1H, s), 8.40 (2H, dd), 8.00 (2H, m), 7.60 (1H, m), 7.45 (2H, m), 6.08 (1H, m),

5.10 (1H, m), 4.50 (1H, m), 3.08 (2H, m), 2.24 (2H, m), 1.6–1.7 (br., 4H, m), 1.24 (54H, m), 0.87 (6H, m). Mass spectrum, *m/z* 886 (MH^+) ($C_{49}H_{80}N_3O_7S_2$ requires 886), 764 ($MH^+ - PhCO_2H$), 686 ($MH^+ - (NO_2)_2C_6H_3SH$), 654 ($MH^+ - (NO_2)_2C_6H_3S_2H$).

2.3. 3-Benzoyl-2-stearoyl-1-deoxy-rac-sphinganin-1-thiophosphoric acid, diethyl ester **4a**

A solution of disulfide **3** (182 mg, 0.205 mmol) and triethylphosphite (1.4 ml, 8.2 mmol) in toluene (5 ml) was stirred at room temperature for 72–80 h. The reaction mixture was concentrated under vacuum and the residue chromatographed on a silica gel column with $CHCl_3$. Yield 58%. R_f 0.62 (hexane-acetone, 6:4). IR 3305 (NH), 1716 (benzoate C=O), 1646 (amide I), 1538 (amide II), 1268 (benzoate C–O and P=O), 1020 and 972 (P–O–C) cm^{-1}. ^{1}H-NMR ($CDCl_3$): δ 8.03 (2H, dd), 7.56 (1H, t), 7.44 (2H, m), 6.63 (1H, d), 5.22 (1H, dd), 4.43 (1H, m), 4.12 (4H, m), 3.10 (2H, br.m), 2.20 (2H, m), 1.70 (2H, m), 1.61 (2H, m), 1.21–1.38 (60H, m), 0.87 (6H, m). ^{31}P-NMR ($CDCl_3$): δ 27.29.

2.4. 3-Benzoyl-2-stearoyl-1-deoxy-rac-sphinganin-1-thiophosphoric acid, dimethyl ester **4b**

This was prepared similarly to **4a** with trimethylphosphite in a yield of 73%. R_f 0.59 (hexane-acetone, 6:4). IR 3353 (NH), 1714 (benzoate C=O), 1652 (amide I), 1531 (amide II), 1280–1252 (benzoate C–O and P=O), 1070 and 1042 (P–O–C) cm^{-1}. ^{1}H-NMR ($CDCl_3$): δ 8.02 (2H, dd), 7.57 (1H, t), 7.46 (2H, m), 6.60 (1H, d), 5.22 (1H, dd), 4.46 (1H, m), 3.77 (6H, m), 3.10 (2H, br.m), 2.22 (2H, m), 1.70 (2H, m), 1.64 (2H, m), 1.21–1.37 (54H, m), 0.88 (6H, m). ^{31}P-NMR ($CDCl_3$): δ 30.60.

2.5. 3-Benzoyl-2-stearoyl-1-deoxy-rac-sphinganin-1-thiophosphoric acid, disodium salt **5**

A solution of the dimethyl ester **4b** (220 mg, 0.28 mmol) and Me_3SiI (200 μl, 1.40 mmol) in CH_2Cl_2 (5 ml) was stirred at room temperature for 2 h. Two ml of methanol were added; after 2 h the reaction mixture was neutralized with saturated sodium bicarbonate solution and concentrated under vacuum. The residue was suspended in acetone/water 1:1, the precipitate filtered on a sintered filter and washed with acetone. Yield 175 mg, 78%. R_f 0.53 ($CHCl_3$-MeOH-AcOH-H_2O, 32:6:2:0.1). IR 3290 (br., NH), 1715 (benzoate C=O), 1647 (amide I), 1541 (amide II), 1274 (benzoate C–O), 1111 and 969 (PO_2^-) cm^{-1}. ^{1}H-NMR ($CDCl_3$-CD_3OD-D_2O, 2:1:0.1): δ 8.02 (2H, dd), 7.55 (1H, t), 7.44 (2H, m), 5.19 (1H, m), 2.9 (2H, br.m), 2.21 (2H, m), 1.71 (2H, m), 1.65 (2H, m), 1.20–1.36 (54H, m), 0.88 (6H, m). ^{31}P-NMR ($CDCl_3$-CD_3OD-D_2O, 2:1:0.1): δ 18.86. Mass spectrum, *m/z* 766 ($M-H^+$) ($C_{43}H_{77}NO_6PS$ requires 766).

2.6. 2-Stearoyl-1-deoxy-rac-sphinganin-1-thiophosphoric acid, disodium salt **6**

A solution of disodium salt **5** (40 mg, 0.05 mmol) in $CHCl_3$-MeOH-2 M MeONa/MeOH 5:5:1 (1.6 ml) was stirred at room temperature for 4–6 h. The reaction mixture was diluted with acetone/water (1:1) (2 ml), the precipitate filtered on a sintered filter and washed with acetone. Yield 26 mg, 74%. R_f 0.25 ($CHCl_3$-MeOH-AcOH-H_2O, 32:6:2:0.1). IR 3280 (br., NH and OH), 1648 (amide I), 1548 (amide II), 1098 and 974 (PO_2^-) cm^{-1}. ^{31}P-NMR ($CDCl_3$-CD_3OD-D_2O, 1:1:0.1): δ 19.59. Mass spectrum, *m/z* 662 ($M-H^+$) ($C_{36}H_{73}NO_5PS$ requires 662), 644 ($M-H^+-H_2O$).

3. Results and discussion

The main problem in the synthesis of **6** (Scheme 1) was formation of the S–P bond. Several methods have been reported to form this bond: phosphorylation of thiols [11–17] or lithium derivatives of thiols [18,19] with chlorophosphates, phosphatidylation of thiols [20,21], and use of an Arbuzov reaction of activated thiols

with trialkyl phosphites [22,23] or diaryl alkyl phosphites [24]. The Arbuzov reaction was used by Hendrickson et al. [25,26] and Alisi et al. [19] to synthesize alkyl analogues of thiophosphatidylinositol according to the method of Mûller and Roth [27].

For the synthesis of thioceramide-1-phosphate **6**, we chose a scheme involving the intermediate preparation of dialkyl esters **4a,b** followed by a two-step removal of protecting groups. We began with racemic 1-deoxy-1-thio-3-benzoylceramide **2**, synthesized earlier by Karpyshev et al. in two steps from *rac*-3-benzoylceramide **1** [10]. To activate compound **2**, the 2,4-dinitrophenylsulfenate derivative **3** was made by reaction with 2,4-dinitrophenylsulfenyl chloride [28,29] in the presence of triethylamine. The Arbuzov reaction of **3** with triethyl or trimethylphosphite gave triesters **4a,b**. To remove the alkyl protecting groups, triester **4b** was reacted with trimethylsilyliodide in CH_2Cl_2 at room temperature. This method of deprotection was used earlier by one of the authors in the synthesis of phosphonic analogs of sphingomyelin [30], and the possibility of using this method to deprotect thiophosphate derivatives without destroying the S–P bond was reported by Mlotkowska and Markowska [22], and Alisi et al. [18,19]. 3-Benzoyl-1-deoxy-1-thioceramide-1-phosphoric acid **5**, owing to its instability, was prepared as a disodium salt and transformed to the final product by reaction with sodium methylate in MeOH-$CHCl_3$.

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