Synthesis of Didocosaheaxenoylphosphatidylserine

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1,2-Di-O-isopropylidendeglycerophosphorochloridate prepared from isopropyldiene glycerol and phosphorus oxychloride, was allowed to react with Z-l-serine-N-phthalimidomethyl ester to obtain a derivative of phosphatidylserine. Then, after the isopropyldiene group was removed by Amberlite IR-120 (H+), and the phosphate group was also blocked as a Ba-salt, this derivative was coupled with docosahexaenoic acid, applying the method of activated ester. Removal of both protective groups of serine was finally done by dry hydrogen chloride in chloroform.

Key words: docosahexaenoic acid; phosphatidylserine; didocosaheaxenoylphosphatidylserine

A number of phosphatidylserines with saturated acyl moieties and a few of those with unsaturated acyl moieties have been synthesized by a variety of methods.1–6 The anti-coagulation activity of these compounds on coagulating system of blood have been investigated. These researches suggested that more unsaturated compounds may be more active. Thus, obtaining more highly unsaturated derivatives is desired.4,7,11 Recently, phosphatidylserine has become an object of attention in regard to effects on brain function, such as promoting learning in rats and preventing brain deterioration.8–13

A further complicated derivative, didocosaheaxenoylphosphatidylserine exists in brains of animals13 and in the phospholipids of the retina.14

Although these substances are isolated from bovine brain, isolation from natural sources, and purification are considerably difficult.15,16

On the other hand, synthesis of phosphatidylserine having an arachidonic acid (5,8,11,14-eicosatetraenoic acid) moiety have been attempted by Speer and Ridgway.17 In the synthesis of diarachidonylphosphatidylserine, to protect the double bonds of arachidonic acid, it was first brominated. In the last step of the synthesis the bromo atoms were eliminated with zinc in acetic acid. This elimination reaction, however, gave rise to the formation of considerable amounts of trans, besides the proper cis double bonds.

The synthesis of naturally occurring phosphatidylserine containing (SZ9Z)-5,9-hexacosadienoic acid was reported by Mena and Djerassi.18 The final product was prepared from the corresponding phosphatidylcholine and serine by enzymatic transphosphatidylcholylase catalyzed by phospholipase D. Although that method seems to be an attractive biochemical synthesis, the yields is small and the resultant phosphatidylserine is contaminated by large amounts of phosphatidic acid,19 because the transphosphatidylcholylase takes place in competition with hydrolysis. Furthermore, the reaction is done under quite drastic conditions, so the applicability to fragile compounds containing highly unsaturated fatty acids may be uncertain.

Therefore, to obtain satisfactorily and conveniently much highly unsaturated phosphatidylserine, this work was undertaken.

The adopted method for the synthesis was as follows: 1,2-di-O-isopropylidendeglycerophosphorochloridate was allowed to react with serine, which was protected at the amino group with benzoxycarbonyl, and at the carboxyl group with N-phthalimidoethyl. After removing the isopropyldiene group, docosahexaenoic acid was coupled with it at 0°C by the activated ester method. Removal of both protective groups of serine was done last.

Results and Discussion

Acylation of 3-O-tetrahydropyran-glycerol by acyl imidazolides20 is not suitable for use for highly unsaturated fatty acids, because of the alkaline conditions and high reaction temperature. Therefore, 1,2-isopropyldiene glycerol21 was treated with phosphorus oxychloride in the presence of quinoline to obtain phosphorochloridate (4).

Then, according to the method of Turner et al.,4,7 this chloridrate was allowed to react with serine, which was protected at the amino and the carboxyl groups with a benzoxycarbonyl and with a phthalimidoethyl group, respectively.

The reaction product (5), a derivative of phosphatidylserine, was treated with Amberlite IR-120 (H+) to remove the isopropyldiene group.4,7 Subsequently, the free OH group of phosphate was blocked as a Ba-salt.22

The resulting derivative (6), was coupled with docosahexaenoic acid (C22:6 (n = 3)) by the method of activated ester with 1-hydroxybenzotriazole (HOBT)23,24 to get 1,2-didocosaheaxenoylglycerol-3-phospho-N-benzoxycarbonyl-l-serine phthalimidoethyl ester (8). The yields in each step were 52%, 43%, and 77%, respectively.

One of the problems encountered in devising a procedure for the chemical synthesis of highly unsaturated phosphatidylserine is the reaction condition in removal of the protective groups for serine. In general, blocking groups are removed by hydrazine, then hydrogen chloride.35 However, the two successive reactions result in lower yields of the final product.

Combinations of amino and carboxyl protective groups

Abbreviations: Z, benzoxycarbonyl; HOBT, 1-hydroxybenzotriazole; Boc, r-butoxycarbonyl; TMS, tetramethylsilane; DMF, dimethylformamide; DCC, N,N'-dicyclohexylcarbodiimide.
that can simultaneously be split off in the presence of unsaturated acyl moieties have been described by Haas et al., but the coupling of these groups to serine is difficult.25

The simultaneous elimination of the benzyl group protecting carboxyl and N-benzoxoacylcarbonyl groups of protected phosphatidylycerine by treatment with triethylsilane in the presence of palladium chloride and triethyl amine was reported by Stepanov and Shvets,26 and Vasilenko et al.27

Hermetter et al. also reported that the protective groups of N-t-butoxycarbonylphosphatidylycerine benzyl ester were simultaneously removed by treatment with hydrogen chloride in chloroform at 0°C.29 Woolley and Eibl treated the appropriate N-Boc-serine-t-buty! ester in methylene chloride with trifluoroacetic acid and 70% perchloric acid at a temperature below 10°C to simultaneously remove both protective groups.28 The compounds treated, however, were only saturated derivatives except oleic acid.

We attempted to remove the protective groups of compound 8 by the method of Hermetter et al.,29 but the yield of 9 was very low.

Since it was thought that the low yield may be due to incomplete removal and formation of by-products during the reaction, we scrutinized adequate conditions for removing them, pursuing reaction products by a gas chromatography. As the result, the procedure described in the experimental part was appropriate, so that the yield of 9 was much increased.

Thus, it seems that this paper might contribute to the routine synthesis of phosphatidylserines having moieties of highly unsaturated fatty acids.

We are now planning to examine the effects on the coagulating system of blood by the synthesized compound, along with other similar compounds having less unsaturated fatty acids.

Experimental

Materials and methods. Chemicals were mostly purchased from the Wako Pure Chemicals Co., Ltd. Docosahexaenoic acid was obtained from Nippon Suisan Kaisha, Ltd.

Solvents were distilled in the presence of metallic sodium, after it had been dried with anhydrous calcium chloride. 1H-NMR spectra were measured on a Hitachi model R-24 B spectrometer at 60 MHz, adopting TMS in chloroform-d as an internal standard. IR spectra were obtained on a model FT/IR-500 instrument of Japan Spectroscopic Co., Ltd. Analysis of fluorescent X-rays was done by a SEA-2001 model of Seiko Instruments Inc. Differential scanning calorimetry was done by DSC 220 model of Seiko Instruments Inc. Thermogravimetry/differential thermal analysis (TG/DTA) was done by TG/DTA 320 model of Seiko Instruments Inc. Preparative liquid chromatography was practiced by using LC-908 of Japan Analytical Industry Co., Ltd.

1) Synthesis of N-benzoxoacylserine phthalimidomethyl ester (2). DMF solution (150 ml) containing diethylamine (9.1 g, 50 mmol) was added to DMF solution (300 ml) containing Z-serine (12 g, 50 mmol) and N-(chloromethyl)phthalimide (9.8 g, 50 mmol) under stirring slowly for 60 min at 37-40°C. The reaction mixture was further stirred for 3 d under an atmosphere of previously dried nitrogen at 37-40°C. After we filtered off precipitates, the filtrate was evaporated in vacuo at 28°C, 66 Pa. The residue was poured into ethanol to obtain a crude product. After this was left overnight at 0°C, the crude product was washed with cold ethanol, then dried in a silica gel desiccator for 3 d in vacuo. Finally 13.8 g of 2 was obtained as a colorless fine powder. Yield: 69%. mp: 136°C (DCS).

2) Synthesis of 1,2-di-O-isopropyldenglycerolglycerophosphochloridate (4). Chloroform solution (30 ml) containing quinoline (3.3 g, 55 mmol) was added under stirring for 60 min of elapsed time to cooled (at -12°C to -15°C) chloroform solution (30 ml) containing isopropyldiene glycerol (2.8 g, 21 mmol) and freshly distilled phosphorus oxychloride (3.3 g, 21 mmol). Then the mixture was stirred for 60 min more at 25°C, finally for 30 min at 35°C (the above procedures were done under nitrogen atmosphere within a glove box).

2b) Synthesis of 1,2-di-O-isopropyldenglycerol-3-phospho-N-benzoxoacylserine phthalimidomethyl ester (5). To a chloroform solution of 4, were added a chloroform solution (150 ml) of 2 (8.5 g, 21 mmol) and pyridine (6.7 g, 85 mmol) under stirring at 10°C during 60 min. The reaction mixture was further stirred at room temperature for 18 h. Then it was stirred further for 1 h, after water was added (0.48 ml, 27 mmol). The solvent was removed in vacuo at 40°C. The residue was extracted 3 times with pet. ether, once with ethyl ether, finally 6 times with benzene. The benzene extracts were concentrated in vacuo to obtain 5. Yield was 6.3 g (52%).

Scheme. Synthetic Route for Docosahexaenoylphosphatidylserine.
C-CH-CH-OH, 3.55-4.70 (7H, m, C-CH-OH, C-CH=O serine), 5.00 (2H, s, CH2-Ph), 5.70 (2H, s, CH2-N), 6.10 (1H, d, J = 8 Hz, =NH), 7.19 (5H, t, J = 2 Hz, C6H5), 7.65-7.85 (4H, m, C6H4H) IR \nu_{max} (KBr disk) cm⁻¹: 2980 (C-H), 1730 (ester C=O), 1605 (P=O), 1050 (P-O-C) 

3) Synthesis of barium salt of glycerol-3-phospho-N-benzoylcarboxyl-L-serine phthalimidomethyl ester (6). Eighty % methanolic solution (100 ml) containing 1,2-di-O-isopropylideneglycerol-3-phospho-N-benzoylcarboxyl-L-serine phthalimidomethyl ester (5) (3.5 g, 5.9 mmol) was stirred with 50 g of Amberlite IR-120 (H⁺) for 3 h. After the resin was removed by filtration, the filtrate was evaporated in vacuo. The evaporated residue was mixed with 200 ml of water and warmed to 40°C under vigorous stirring. After it was left standing at room temperature for 1 h, the solution was mixed with a fine powder of BaCO₃ (10 g) and stirred for 1 h. The excess BaCO₃ was removed by filtration, and the filtrate was concentrated in vacuo at 35-40°C. The evaporated residue was dissolved in methanol. After further removal of insoluble substances by filtration, the filtrate was evaporated in vacuo, then dried in a P₂O₅ desiccator for 3 d in vacuo. Finally 1.0 g of 6 was obtained as a colorless amorphous solid. Yield 43 %, mp: 178°C (decomp.) (TG-DTA). IR \nu_{max} (KBr disk) cm⁻¹: 3400 (OH), 2940 (CH₃), 1730 (ester C=O), 1220 (P=O), 1050 (P-O-C). Ratio of P:Ba by fluorescent X-ray, 2:1 (calcled., 1:9.1) (found). 

4) Synthesis of 1,2-didocosahexaenoylglycero-3-phospho-N-benzoylcarboxyl-L-serine phthalimidomethyl ester (8). Five ml of DMF solution containing HOBT (0.37 g, 2.7 mmol) and DCC (0.56 g, 2.7 mmol) was added slowly for 30 min of elapsed time (to 5 ml of DMF solution containing didocosahexaenoic acid (7) (0.9 g, 2.7 mmol) at 0°C. The reaction mixture was stirred further for 3 h at 37°C to obtain the activated ester of 7. To this reaction mixture, 10 ml of DMF solution containing 6 (0.92 g, 1.4 mmol) was added dropwise at 0°C for 30 min of elapsed time. Thereafter, the final solution was continuously stirred for 2 h at 0°C, then was filtered at 0°C. The filtrate was carefully, under stirring, poured into 800 ml of ice-cooled diluted sulfuric acid (0.02 mol/liter), then was added 80 g of NaCl to the solution and extracted with 200 ml ethyl ether. The ether layer was washed twice with 400 ml of NaCl solution (10%), dried with anhydrous Na₂SO₄, then evaporated in vacuo. The residue was dried over P₂O₅ in a desiccator overnight in vacuo. Finally, 8 was obtained as a pale yellow transparent liquid. Yield 1.2 g (77%). \(^{1}H-NMR \delta \text{ (CDCl}_3): \text{0.96 (6H, t, J = 8 Hz, CH}_3\text{), 1.90-2.55 (12H, m, C-CH}_2\text{-O), 2.56-3.05 (20H, m, =C-CH}_2\text{-C, 3.06-4.10 (7H, m, C-CH}_2\text{-O serine, C-CH}_2\text{-O glycerol), 5.33 (24H, t, J = 5 Hz, cis HC=CH), 7.00-7.50 (5H, m, C6H_4H), 7.51-8.50 (4H, m, C6H_4H). IR \nu_{max} (NaCl disk) cm^{-1}: 3070 (C=C-H), 2960 (C-H), 1730 (ester C=O), 1495 (C=O), 1180 (P=O-C), 720 (cis HC=CH) 

5) Synthesis of 1,2-didocosahexaenoylglycero-3-phospho-L-serine. Twenty ml of chloroform solution containing 8 (400 mg, 0.34 mmol) was added to 50 ml of cooled (0°C) chloroform solution saturated with dried hydrogen chloride for 10 min of elapsed time. The mixed solution was stirred for 10 h at 0°C, then further 2 h at 10°C. The solvent was then taken off in vacuo, after hydrogen chloride was removed by adding Amberlite IRA-410 (2 g). Finally the product was purified through a preparative gel permeation chromatography (column: JAIHEL 1H+2H, eluent: chloroform, detector: RI and UV) to obtain 9 as pale yellow transparent liquid. Yield 125 mg (41%). \(^{1}H-NMR \delta \text{ (CDCl}_3): \text{0.98 (6H, t, J = 7 Hz, CH}_3\text{), 1.80-2.55 (12H, m, C-CH}_2\text{-C, 2.56-3.10 (20H, m, =C-CH}_2\text{-C), 3.11-4.30 (7H, m, C-CH}_2\text{-O serine, C-CH}_2\text{-O glycerol, 5.33 (24H, t, J = 4 Hz, cis HC=CH), IR \nu_{max} (NaCl disk) cm^{-1}: 3015 (C=C-H, C=H), 1740 (ester C=O), 1260 (P=O), 1160, 1060 (P-O-C), 715 (cis HC=CH) 

IR spectroscopic datum of synthesized didocosahexaenoylglycero-3-phospho-L-serine (Sigma Chemical Co.) and glycero-3-phosphono lipid, except that of peaks attributable to the unsaturated fatty acid moiety. MS m/z: 28 (C₂H₄⁺, 71%), 29 (C₂H₅⁺, 21%), 44 (CH₃CH⁺=NH₂⁺, 21%), 83 (C₅H₁₁⁺, 7%). Anal. Found: C, 67.85; H, 8.75; N, 1.36; P: 3.34. Calcd. for C₃₆H₅₃NO₅P: C, 68.26; H, 8.42; N, 1.58; P: 3.53.

References