SYNTHESIS OF ALKYL GLYCEROPHOSPHOLIPIDS THROUGH 1-O-BENZYL-2-O-METHYL-rac-GLYCEROL

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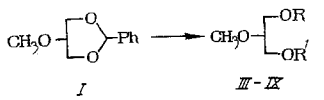
1-O-Alkyl-2-O-methylglycero-3-phosphocholines are the synthetic analogs of natural alkyllysolecithins. They display high biological activity [13] and, especially importantly, selective cytotoxicity relative to tumor cells of various etiologies. This property has been repeatedly confirmed both in experiments with cell cultures and in experimental animals [3, 4, 6, 11, 15]. One of the most active analogs (according to literature data), 1-O-octadecyl-2-O-methyl-rac-glycero-3-phosphocholine (ET-18-OCH_3) is undergoing clinical testing at the present time [5, 6].

Known methods for synthesis of 1-O-alkyl-2-O-methyl-rac-glycero-3-phosphocholines, including ET-18-OCH_3, are based on monoalkylation of higher alkyl halides of 2-O-methylglycerol, which is obtained by hydrogenolysis of 2-O-methyl-1, 3-O-O-benzylideneglycerol [16, 18]. In this case, the yield of the target monoalkylation product is not more than 40-45% due to the low regioselectivity of the process. In this paper, we suggest a different synthetic approach based on selective protection of one of the primary hydroxyl groups of the glycerol residue. It involves using the method of reductive cleavage of acetals [8] for directed transformation of 2-O-methyl-1, 3-O-O-benzylideneglycerol (II) to 1-O-benzyl-2-O-methyl-rac-glycerol (III).



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This statement is demonstrated in this paper for the example of synthesis of ET-18-OCH_3 and its C_{16} homolog. The starting 2-O-methyl-1,3-O-O-benzylideneglycerol (II), obtained in quantitative yield upon methylation of 1,3-O-O-benzylideneglycerol (I) by methyl iodide according to the method in [14], was reduced using a BH_3 solution in THF and 1-O-benzyl-2-O-methyl-rac-glycerol (III) was isolated in 96% yield; the structure of the latter was confirmed by spectral methods. We should note that the latter compound can be then used as the starting material for synthesis of different types of racemic lipids containing a methyl group or another alkyl substituent in the 2 position of the glycerol residue. The corresponding 1-O-benzyl-2-O-methyl-3-O-alkyl-rac-glycerols (IV) and (V) were obtained in 95% yield by alkylation of III by octadecyl- and hexadecyl bromides in DMF. In this stage, we used an excess of alkyl bromide due to the simultaneous occurrence of the process of elimination of hydrogen bromide [9]. Further transformation of the protected 2-O-methyl-3-O-alkyl-rac-glycerols (IV) and (V) was done using methods successfully certified previously in lipid chemistry. Thus catalytic debenzylation of IV and V led to 1-O-alkyl-2-O-methyl-rac-glycerols (VI) and (VII), which then were transformed to the corresponding phosphocholine derivatives (VIII) and (IX) by successive action of 2-chloro-2-oxo-1,3,2-dioxaphospholan and a solution of trimethylamine in acetonitrile [2, 7].

Thus the method we have proposed allows us to synthesize 1-O-alkyl-2-O-methyl-rac-glycero-3-phosphocholines in amounts sufficient for clinical testing.

EXPERIMENTAL

1,3-O-O-Benzylideneglycerol was obtained according to the method in [17]. A solution of solution of borane in tetrahydrofuran (1 M) was obtained according to the method in [1], passing a stream of BH_3 into abs. THF cooled down to 0°C. The PMR spectra were obtained

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on the Bruker W-250 instrument (West Germany) with operating frequency 250 MHz in deuterochloroform; the IR spectra were obtained on the Shimadzu IR-435 instrument (Japan) in carbon tetrachloride. We used Kieselgel 60 (40-63 μm) (Merck, West Germany) for the column chromatography.

2-O-Methyl-1,3-O-O-benzylideneglycerol (I). To an 80% suspension of pentane-washed sodium hydride (0.33 g, 11 millimoles) in 7 ml abs. THF, we added freshly distilled methyl iodide (2 ml, 32 millimoles). We heated the mixture with stirring up to 55°C and added dropwise a solution of 1,3-O-O-benylideneglycerol (II) (1 g, 5.5 millimoles) in 10 ml THF. We stirred the reaction mixture for 4 h at 55°C, cooled it down to 0°C, carefully added water (1 ml), and evaporated. To the residue, we added water (50 ml) and extracted the material with ether (3 × 80 ml), washed the combined extract with water to pH 7, dried with Na_2SO_4, evaporated, and chromatographed the residue on a column (8 × 3.5 cm) with Kieselgel, which was washed successively with hexane and a 1:1 hexane–ether mixture. After evaporation of the eluate, we obtained 1.05 g (97%) of the methyl ether II, m.p. 49.5-50.5°C (from benzene–hexane, uncorr.) (lit. [10] 52°C). PMR (δ, ppm): 3.2 (CH–OMe, 1H; m); 3 (CH_3O, 3H, s); 4.05 (ax CH_2, 2H dm, J13 Hz); 4.4 (eq- CH_2, 2H, dm J13 Hz); 5.55 (PhCH, 1H, s); 7.35 (arom., meta- and para-h, 3H, m); 7.5 (arom. ortho-H, 2H, m).

1-O-Benzyl-2-O-methyl-rac-glycerol (III). To compound I (2.4 g, 2.4 millimoles) we added 18 ml (18 millimoles) of a 1 M complex of borane-THF at 0°C. We held the solution at room temperature for 12 h, then at 40-45°C until the starting compound disappeared according to TLC (2 days). We then cooled it down to 0°C and slowly added water (3 ml) to decompose the excess borane, then evaporated the solvent. To the residue we added water (50 ml) and extracted the material with ether (3 × 100 ml), washed the combined extract with water, dried with (Na_2SO_4). After evaporation, we obtained 2.32 g (96%) of compound II as a colorless oil. The material was used in the next stage without additional purification. The analytical sample was chromatographed on Kieselgel 60 in chloroform. n_D^{21} 1.5104. PMR (δ, ppm): 2.1 (OH, 1H. dd, J_1 8 Hz), J_2 6 Hz); 3.4-3.8 (CH_2CHCH_2, 5H, m); 3.45 (OCH_3, 3H, s); 4.55 ($PhCH_2$, 2H, s); 7.3 C_6H_5, 5H, m). IR (ν, cm^{-1}): 3604 (m), (OH); 3032 (w), 1496 (w) (arom); 2932 (s), 2868 (s), 1456 (s) (C-H and CH_3); 1096 (s) (C-O-C); 1046 (s) (C-OH). Calculated (%): 67.32, H 8.22. Found: C 66.55, H 8.00.

1-O-Benzyl-2-O-methyl-3-O-hexadecyl-rac-glycerol (IV). To an 80% suspension of pentane-washed sodium hydride (1.8 g, 60 millimoles) we added dimethylformamide (30 ml); and cooling it with ice, we added in small portions a solution of compound III (3.25 g, 16.6 millimoles) in 15 ml THF. Then at +10°C we added dropwise 1-bromo-hexadecane (11 ml, 36 millimoles); 30 min after we finished adding the reagent, we heated the reaction mixture up to 20°C and held it for 24 h. At 0°C we decomposed the mixture by addition of 100 ml water and extracted the material with petroleum ether. After chromatography (25 × 3.5 cm) in the system petroleum ether-ether (95:5), we obtained 6.61 g (95%) of the triether IV as a colorless oil. IR (ν cm^{-1}): 2922 (s), 2854 (s), 1467 (s) (C-H and CH_3); 3032 (w), 1500 (w) (arom.); 1365 (w) (CH_3), 1119 (s), (C–O–C). Ref. [12].

1-O-Benzyl-2-O-methyl-3-O-octadecyl-rac-glycerol (V) was obtained analogously to IV with only the difference that we added 1-bromooctadecane as a 50% solution in THF.

1-O-Hexadecyl-2-O-methyl-rac-glycerol (VI) and 1-O-octadecyl-2-O-methyl-rac-glycerol (VII) were obtained in quantitative yield by hydrogenolysis of respectively compound IV and compound V in ethanol at 45°C in the presence of 10% Pd/C. IR (ν, cm^{-1}): 3602 (m) (O-H); 2928 (s), 2854 (s), 1467 (m) (C-H); 1378 (w) (CH_3-); 1118 (s) (C-O-C); 1046 (m) (C-OH). Ref. [12].

1-O-Octadecyl-2-O-methyl-rac-glycero-3-phosphocholine (IX). To a solution of compound (VII) (2.85 g, 7.95 millimoles) in 15 ml dry benzene, we added at 0°C with stirring 2-chloro-2-oxo-1,3,2-dioxaphospholan (Fluka) (0.73 ml, 7.95 millimoles) and freshly distilled triethylamine (1.22 ml, 8.7 millimoles). After 30 min, we heated the reaction mixture to 20°C and held it for 24 h. We filtered off the triethylammonium hydrochloride residue, evaporated the solvent, dried it for 1 h at 20°C and 1 torr. To the residue we added with stirring 8 ml of a solution of trimethylamine in acetonitrile (14 g/100 ml acetonitrile) and held it for 48 h at 65°C. We added 50 ml acetone and held it for 1 h at –20°C. We filtered off the

residue and chromatographed it on a column (25 × 3.5 cm) in the system 65:25:4 chloroform–methanol–water. We obtained 2.56 g (60%) of the crystalline compound IX. PMR (δ, ppm.): 0.85 (ω-CH_3, 3H, br.t); 1.25 ($(CH_2)_{15}$, 3OH, br.s); 1.5 (β-CH_2, 2H, m); 3.35 ($N(CH_3)_3$, 9H, s); 3.45 (CH_3O, 3H, s); 3.3-3.55 (CH_2CHCH_2, OCH_2 $(CH_2)_{16}$, 5H, m), 3.8-3.95 (CH_2N, $CHCH_2OP$, 4H, m), (4.3 $POCH_2$, 2H, br.t). Ref. [4, 6].

1-O-Hexadecyl-2-O-methyl-rac-glycero-3-phosphocholine (VIII). We obtained it from VI in 63% yield under the synthesis conditions for IX.

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