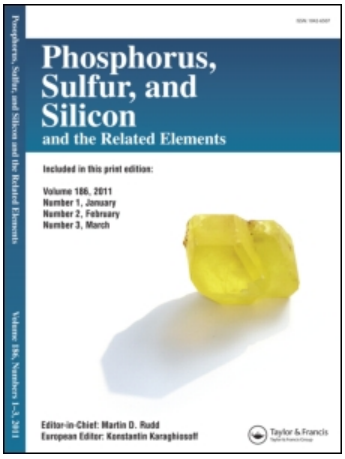

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SYNTHESIS OF PHOSPHOLIPID CONJUGATES OF N¹-(2-FURANIDYL)-N³-(2-HYDROXYETHYL)-5-FLUOROURACIL

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SYNTHESIS OF PHOSPHOLIPID CONJUGATES OF N^1-(2-FURANIDYL)-N^3-(2-HYDROXYETHYL)-5-FLUOROURACIL

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The synthesis of phospholipid conjugates of N^1-(2-furanidyl)-N^3-(2-hydroxyethyl)-5-fluorouracil is reported. The strategy for the synthesis is using hexaethylphosphorous triamide, activated by a catalytic amount of iodine, as the phosphorylating reagent in a one-pot reaction resulting in a number of new types of phospholipid-drug conjugates.

Keywords: Phospholipids; glyceroamidothiophosphates; conjugates; 5-fluorouracil derivatives; hexaethylphosphorous triamide

INTRODUCTION

Phospholipid-nucleoside conjugates have demonstrated potent antitumor activity *in vitro* and *in vivo*[1,2,3]. The conjugates are not only prodrugs or molecular depots of nucleoside antitumor agents but also may generate two cytotoxic groups with different target sites, membrance and synthesis of nucleic acid, inside a neoplastic cell[4]. These interesting biological activities lead us to synthesize new types of phospholipid conjugates of 5-fluorouracil derivatives. Various types of 5-fluorouracil derivatives have been widely used as effective chemotherapeutics against neoplastic cells. For example, 1,3-bis(hydroxyalkyl)-5-fluorouracil have been described as antitumor compounds[5], and N^1-(2-furanidyl)-5-fluorouracil (Tegafur) is a potent inhibitor of mammalian cell growth in clinical use. However, their side effects, such as hot sensation and

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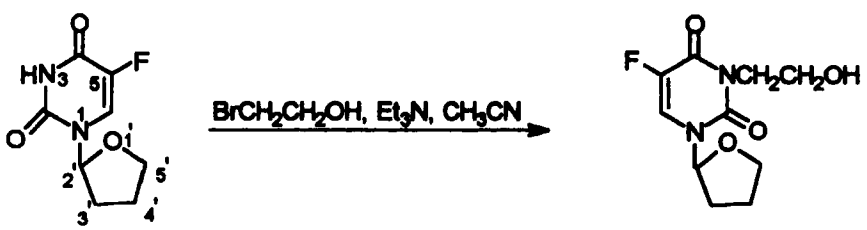
pollakiuria syndrome, encourage people to develop a better new drug.[6] So is our aim to synthesize highly biologically active and less toxic new phospholipid conjugates of 5-fluorouracil derivatives.

RESULTS AND DISCUSSION

Tegafur (**1**) is one of the 5-fluorouracil antitumor agents. In order to improve its antitumor activity, we attempted to synthesize its conjugates of glycerophospholipids. According to Scheme 1, the alkylation of tegafur (**1**) with bromoethanol was carried out in acetonitrile in the presence of triethylamine at room temperature for 48 hours, and the yield was only 78% due to less active N^3 position of the uracil ring[7].

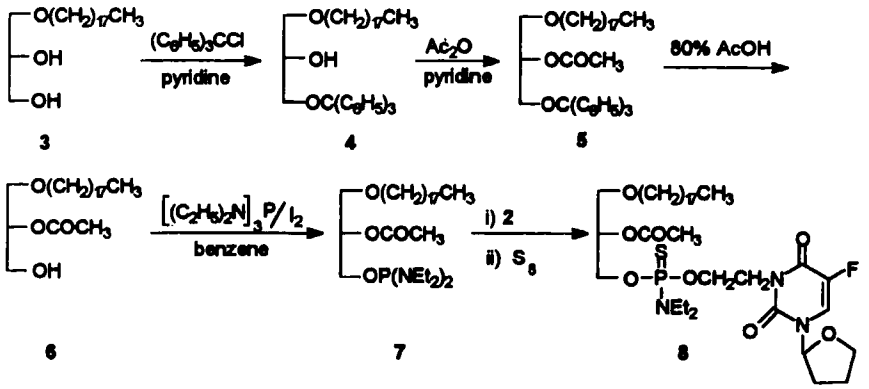
Scheme 2 described the synthesis of (rac-1-O-octadecyl-2-O-acetylglyceryl-3-O-)-[1-(N^1-(2-furanidyl)-5-fluorouracil-N^3-)ethyl-2-O-](N,N-diethylamido)thiophosphate (**8**). The primary hydroxyl group of **3** was converted to the corresponding trityl ether **4** by allowing **3** to react with triphenylmethyl chloride in anhydrous pyridine in excellent yield as reported earlier[8] with some modifications of solvent and purification. Acid-catalyzed cleavage of the trityl ether **5** obtained from the acetylation of **4** in 80% acetic acid at 60°C yielded the alcohol **6** as a waxy product[9] in 52% yield after isolation by column chromatography.

Compound **8** was obtained in 55% yield by a one-pot (two-step) reaction from rac-1-O-octadecyl-2-O-acetylglycerol (**6**) by means of hexaethylphosphorous triamide, activated by iodine, as a phosphorylating reagent under mild conditions according to the procedure of Stamalov[10]. Thus, the activated phosphoramidite was reacted with the alcohol **6** in stoichiometric amounts at 65°C to give the bis(N,N-diethylamido)-phosphite (**7**) in nearly quantitative yield. The consecutive treatment of the intermediate (**7**) with an equivalent amounts of the



1 **2**

SCHEME 1

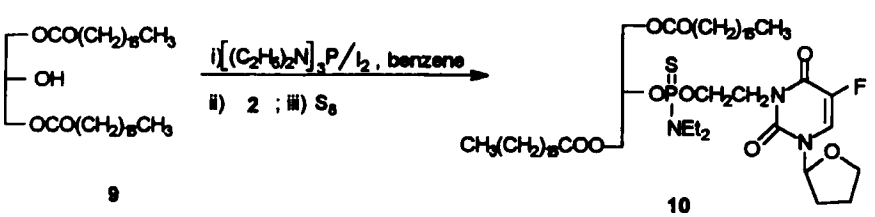


SCHEME 2

hydroxyethyltegafur (**2**) and sulfur at 65°C for 5 hr. and 30 min respectively afforded glyceroamidothiophosphate (8), which was isolated by column chromatography.

According to Scheme 3, (rac-1,3-O-distearoylglyceryl-2-O-)-[1-(N^1-(2-furanidyl)-5-fluorouracil)-N^3-)ethyl-2-O-]-[N,N-diethylamido)-thiophosphate (**10**) was synthesized from rac-1,3-O-distearoylglycerol (**9**)[11] and N^1-(2-furanidyl)-N^3-(2-hydr-oxyethyl)-5-fluorouracil (**2**) by using the same procedure as for preparation of **8**. The yield was only 42% because of severe steric hindrance of the free hydroxy group in **9**.

Physicochemical properties and biological activities of glyceroamidothiophosphates **8** and **10** are under investigation. These results will be discussed in detail in another paper.



SCHEME 3

EXPERIMENTAL

IR spectra were recorded on NICOLET 5DX instrument. NMR spectra were taken on a BRUKER AC-P200 spectrometer, TMS as an internal standard for ^1H NMR and 85% H_3PO_4 as an external standard for ^{31}P NMR. Elemental analyses were carried out with a YANACO CHN CORDER MT-3 elementary analyser. Melting points were uncorrected.

Benzene and pyridine were distilled from sodium and potassium hydroxide respectively before being used. Petroleum ether refers to a fraction bp 60–90°C. Column chromatography was carried out with silica gel H (10–40 μm).

Hexaethylphosphorous triamide was prepared according to literature[12] and freshly distilled.

N^1-(2-Furanidyl)-N^3-(2-Hydroxyethyl)-5-Fluorouracil (2)

A solution of 10.0g(0.05 mol) of N^1-(2-furanidyl)-5-fluorouracil (**1**), 7.5g (0.06 mol) of 2-bromoethanol and 6.1g(0.06 mol) of triethylamine in 100 ml of acetonitrile was stirred for 48h at r.t. The solvent was evaporated and the residue was extracted with ethyl acetate. The extract was dried ($MgSO_4$) and concentrated *in vacuo* to give a crude product, which was recrystallized from benzene to afford white crystals (9.5g, 78%). m.p. 88–90°C; $C_{10}H_{13}FN_2O_4$. Calcd: C, 49.18; H, 5.37; N, 11.47, Found: C, 49.32, H, 5.20; N, 11.58. ^1H NMR($CDCl_3$) δ 1.79–2.46(4H, m, 3′,4′-CH_2), 3.84(2H, t, J = 4.9Hz, NCH_2), 3.99(1H, m, 5′-Ha), 4.19(2H, t, J = 4.9Hz, HO$\underline{CH_2}$), 4.20(1H, m, 5′-Hb), 5.96(1H, brd, 2′-H), 7.40(1H, d, $J_{H\text{-}F}$ = 5.4Hz, 6-H).

Rac-1-O-Octadecyl-3-O-Tritylglycerol (4)

The compound was prepared according to a literature procedure[8] with modifications. A solution of 17.2g(0.05 mol) of rac-1-O-octadecylglycerol (**3**) and 17.0g(0.06 mol) of trityl chloride in 100ml of anhydrous pyridine was stirred at 70°C for 24h. The solvent was evaporated *in vacuo*, and the residue was dissolved in 200 ml of diethyl ether. The ether layer was washed with 5% hydrochloric acid and water, and dried over $MgSO_4$. The solvent was evaporated, and the oily residue was dissolved in 100 ml of dichloromethane. After cooling at −15°C overnight, the precipitated tritylalcohol was filtered off, and the filtrate was evaporated. The crude product remained was recrystallized from petroleum ether to yield a white solid (28.1g, 96%). TLC: R_f = 0.31 (petroleum ether/ethyl acetate, 20:1, v/v). m.p. 58–59°C (lit.[13] m.p. 59.5–60.5°C); ^1H NMR($CDCl_3$) δ

0.86(3H, t, CH_3), 1.24(30H, brs,$(CH_2)_{15}$), 1.54(2H, quintet, $OCH_2\underline{CH_2}(CH_2)_{15}CH_3$), 3.18(2H, d, J = 6.3Hz, CH_2OTr), 3.37–3.54(4H, m. CH_2OCH_2), 3.93(1H, quintet, CH), 7.24–7.44(15H, m, $(C_6H_5)_3$).

Rac-1-O-Octadecyl-2-O-Acetyl-3-Tritylglycerol (5)

A mixture of 5.9g(0.01 mol) of rac-1-O-octadecyl-3-O-tritylglycerol (**4**), 20 ml of anhydrous pyridine and 6 ml of acetic anhydride was stirred at r.t. for 40 h. Then, 50 ml of water was added, and the mixture was extracted with chloroform. The organic layer was washed with 5% hydrochloric acid and water, dried with $MgSO_4$, and evaporated *in vacuo*. The residue was recrystallized from petroleum ether to give white solid (6.3g, ~ 100%) R_f value: 0.66(petroleum ether/ethyl acetate, 20:1, v/v)m.p.49–50 °C; $C_{42}H_{60}O_4$ Calcd: C, 80.21; H, 9.62; Found: C,80.34;H,9.55. ^{1}H NMR($CDCl_3$) δ 0.86(3H, t, CH_3), 1.24(30H, brs, $(CH_2)_{15}$), 1.54(2H, quintet, $OCH_2\underline{CH_2}(CH_2)_{15}CH_3$), 2.08(3H, s, $COCH_3$), 3.20–3.80(6H, m, $\underline{CH_2}CH\underline{CH_2}O\underline{CH_2}$), 5.17(1H, quintet, CH), 7.24–7.44(15H, m, $(C_6H_5)_3$).

Rac-1-O-Octadecyl-2-O-Acetylglycerol (6)[9,13]

Compound (**6**) was prepared by a literature procedure[14] from **5**: yield 52%; R_f value: 0.23(petroleum ether/ethyl acetate, 20:1v/v) m.p. 45–46 °C(lit.[13] m.p. 47 °C).

(rac-1-O-Octadecyl-2-O-Acetylglyceryl-3-O-)-[1-(N^1-(2-Furanidyl)-5-Fluorouracil-N^3-) Ethyl-2-O-]-(N,N-Diethylamido)-Thiophosphate (8)

A mixture of 0.39g (1 mmol) of rac-1-O-octadecyl-2-O-acetylglycerol (**6**), 0.26g (1.05 mmol) of hexaethylphosphorous triamide and 0.013 g (0.05 mmol) of iodine in 25 ml of anhydrous benzene was stirred at 65 °C for about 30 min until the solution became clear. 0.24g(1 mmol) of N^1-(2-furanidyl)-N^3-(2-hydroxyethyl)-5-fluorouracil (**2**) was then added, and the mixture was continuously stirred at 65 °C for 5h. Then 0.033 g(1.05 mmol) of sulfur was added and the reaction system was kept under the same conditions for 30 min. The solvent was evaporated *in vacuo*, and the residue was chromatographed by silica gel column using petroleum ether—ethyl acetate (5:1) as the eluent. A colourless oily product (0.42g, 55.1%) was obtained. R_f value: 0.59(petroleum ether/ethyl acetate, 5:1 v/v). $C_{37}H_{67}FN_3O_8PS$. Calcd: C, 58.17; H, 8.84; N, 5.50. Found: C, 58.23; H, 8.66; N, 5.41. IR ν_{max}(liq) 1742, 1715, 1677, 1660, 1464, 1263, 1220, 1076, 1023, 790, 754, 718 cm^{-1}; ^{1}H NMR($CDCl_3$) δ 0.83(3H, t, J = 6.0Hz,

CH_3), 1.02(6H, t, J = 6.9Hz, $N(CH_2\underline{CH_3})_2$), 1.21(30H, brs, $(CH_2)_{15}$), 1.51(2H, m, $OCH_2\underline{CH_2}$),2.01(3H, s, $COCH_3$),1.79–2.46(4H, m, 3′,4′-CH_2),3.12(4H, m, $N(\underline{CH_2}CH_3)_2$), 3.37–3.64(6H, m, $\underline{CH_2}CH\underline{CH_2}O\underline{CH_2}$), 3.98(1H, m, 5′-Ha), 4.02–4.33(5H, m, 5′-Hb, OCH_2CH_2N), 4.57(1H, quintet, CH), 5.92(1H, brs, 2′-H), 7.34(1H, d, $J_{H\text{-}F}$ = 5.4Hz, 6-H); ^{31}P NMR($CDCl_3$) δ 77.63, 77.52, 76.99.

(Rac-1,3-O-Distearoylglyceryl-2-O-)-[1-(N^1-(2-Fluranidyl)-5-Fuorouracil-N^3-) Ethyl-2-O-]-(N,N-Diethylamido)-Thiophosphate (10)

The glyceroamidothiophosphate **10** was synthesized and purified by using rac-1,3-O-distearoylglycerol [11](**9**, 0.624g, 1.0 mmol) and N^1-(2-furanidyl)-N^3-(2-hydroxyethyl)-5-fluorouracil(**2**, 0.244g, 1.0 mmol) in the same way as described for compound **8**. The product was recrystallized from methanol to give white crystals(0.42g, 42%). R_f value: 0.79(petroleum ether/ethyl acetate, 5:1, v/v). m.p.44–46 °C. $C_{53}H_{97}FN_3O_9PS$ Calcd: C, 63.51; H, 9.75; N, 4.19. Found: C, 63.64; H, 9.51; N, 3.99. IR ν_{max}(KBr) 1733, 1652, 1463, 1259, 1160, 1068, 1020, 786, 752, 716 cm^{-1}. ^1H NMR($CDCl_3$) δ 0.85(3H, t, J = 6.0Hz, CH_3), 1.03(6H, t, J = 6.8Hz, $N(CH_2\underline{CH_3})_2$), 1.23(56H, brs, 2 × $(CH_2)_{14}$), 1.58(4H, m, 2 × $COCH_2\underline{CH_2}$), 2.29(4H, m, 2 × $COCH_2$), 1.83–2.45(4H, m, 3′,4′ -CH_2), 3.14(4H, m, $N(\underline{CH_2}CH_3)_2$), 3.86–4.39(10H, m, $\underline{CH_2}CH\underline{CH_2}$, 5′-$CH_2$, OCH_2 CH_2N), 4.68(1H, quintet, CH), 5.96(1H, brs, 2′-H), 7.36(1H, d, $J_{H\text{-}F}$ = 5.5Hz, 6-H). ^{31}P NMR($CDCl_3$) δ 77.78, 77.67.

Acknowledgement

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