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Synthesis of 1,2- and 1,3-Cyclic Phospholipid Conjugates of N^1-(2-Furanidyl)-N^3-(2-hydroxyethyl)-5-fluorouracil

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ABSTRACT: *The conjugates of cyclic glycerolphospholipids and N^1-(2-furanidyl)-N^3-(2-hydroxyethyl)-5-fluorouracil have been synthesized by a one-pot procedure. The hexaethylphosphorus triamide activated by a catalytic amount of iodine was used as the phosphorylating and cyclizing reagent.* © 1998 John Wiley & Sons, Inc. Heteroatom Chem 9:295–298, 1998

INTRODUCTION

Liposome technology has provided a powerful tool for efficient drug delivery and targeting [1], as a number of pharmaceuticals have been encapsulated in liposome forms or attached onto the surface of liposomes by a labile bond. The liposomes with inhibitors displayed on the surface could either directly interact with the targeted surface receptor via a multivalent contact or serve as prodrugs for a sustained release of inhibitor upon enzymatic hydrolysis in vivo, e.g., by cellulur lipases and phospholipases [2,3]. Various types of 5-fluorouracil derivatives have been widely used as effective chemotherapeutics against neoplastic cells. For example, 1,3-bis(hydroxyalkyl)-5-fluorouracil has been described as an antitumor compound [4], 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 promotion of a hot sensation and pollakiuria syndrome, have encouraged medicinal chemists to develop a better drug [5]. Thus, it is our aim to synthesize highly biologically active and less toxic new cyclic phospholipid conjugates of 5-fluorouracil derivatives. Such cyclic glycerophospholipids play an important functional role in cell membranes in maximizing the activity of enzymes [6]. The contemporary concepts of the biological importance of cyclic phosphorus systems are closely related to studies in the field of cell "second messenger" signal transduction, tumor promotion, and cancer therapy [7]. Such kinds of compounds may be of interest not only in chemistry but in biochemistry and pharmacology as well.

RESULTS AND DISCUSSION

Tegafur **1** is one of the known 5-fluorouracil antitumor agents. In order to improve its antitumor activity, we attempted to synthesize its conjugates of cyclic glycerophospholipids. According to Scheme 1, the alkylation of Tegafur **1** with bromoethanol was carried out as described in a previous article [8].

Scheme 2 describes the synthesis of 1,2- and 1,3-cyclic monoalkyl (acyl)-rac-glycerothiophosphates of N^1-(2-furanidyl)-N^3-(2-hydroxyethyl)-5-fluoroura-

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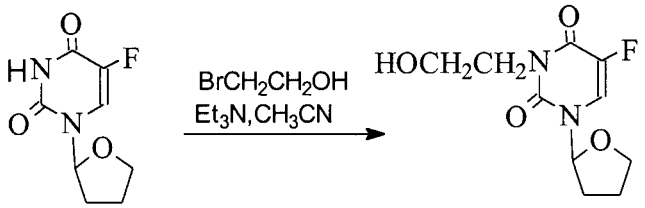


1 **2**

SCHEME 1

cil **9, 10,** and **11.** 1,3-Benzylidenglycerol **3** was prepared as described by J. D. Van Roon [9]. Compound **3** reacted with palmitoyl chloride in the presence of pyridine at room temperature for 12 hours to give **4** or reacted with sodium hydride, then with 1-bromohexadecane to give **5.** Catalytic hydrogenaton of **4** and **5** in the presence of 5% Pd-C gave **6** and **7,** respectively, in excellent yields. Compound **9** was obtained in 48% yield by a one-pot (two-step) reaction from rac-2-O-palmitoylglycerol **6** by use of hexaethylphosphorous triamide, activated by iodine, as a phosphorylating reagent under mild conditions according to the procedure of Stamatov [10]. Thus, the activated hexaethylphosphorous triamide was reacted with N^1-(2-furanidyl)-N^3-(2-hydroxyethyl)-5-fluorouracil **2** in stoichiometric amounts at 65°C to give the bis(N,N-diethylamido) phosphite **8** in nearly quantitative yield. The consecutive treatment of the intermediate **8** with an equivalent amount of dihydroxyl compound **6** and sulfur at 65°C for 5 hours and 30 minutes, respectively, afforded the target compound **9,** which was isolated by column chromatography. Compounds **10** and **11** were synthesized by using the same procedure as for the preparation of **9.**

EXPERIMENTAL

IR spectra were recorded on a Nicolet 5DX instrument. NMR spectra were taken on a Bruker AC-P200 spectrometer, TMS being used as an internal standard for ^{1}H NMR and 85% H_3PO_4 as an external standard for ^{31}P NMR spectroscopy. Elemental analyses were carried out with a Yanaco CHN Corder MT-3 elementary analyzer. Melting points are uncorrected.

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

Hexaethylphosphorous triamide was prepared according to the literature [11] and freshly distilled.

rac-2-O-Palmitoyl-1,3-benzylidenglycerol **4**

Compound **4** was prepared in the same way as described in the literature [12] from **3**: yield 84%; mp 64–66°C (Ref. [12], mp 63.5°C).

rac-2-O-Hexadecyl-1,3-benzylidenglycerol **5**

The compound was prepared according to the literature [13], with some modification. A solution of 3.36 g (20 mmol) **3** in 30 mL of THF was added to 0.7 g (80%) of sodium hydride in 20 mL of THF at 0°C. The mixture was stirred at room temperature for 30 minutes, and 7.4 g (21 mmol) of hexadecyl bromide in 10 mL of THF was added. The solution was stirred at Rt for 48 hours. The mixture was worked up by successively adding methanol (1 mL) and water (100 mL), followed by extraction with ether (2 × 50 mL). The ether extract was dried over magnesium sulfate and concentrated *in vacuo.* The product was recrystallized from ethanol to afford 6.4 g, a white solid, yield 80%, mp 48–50°C (Ref. [13], mp 48–50°C).

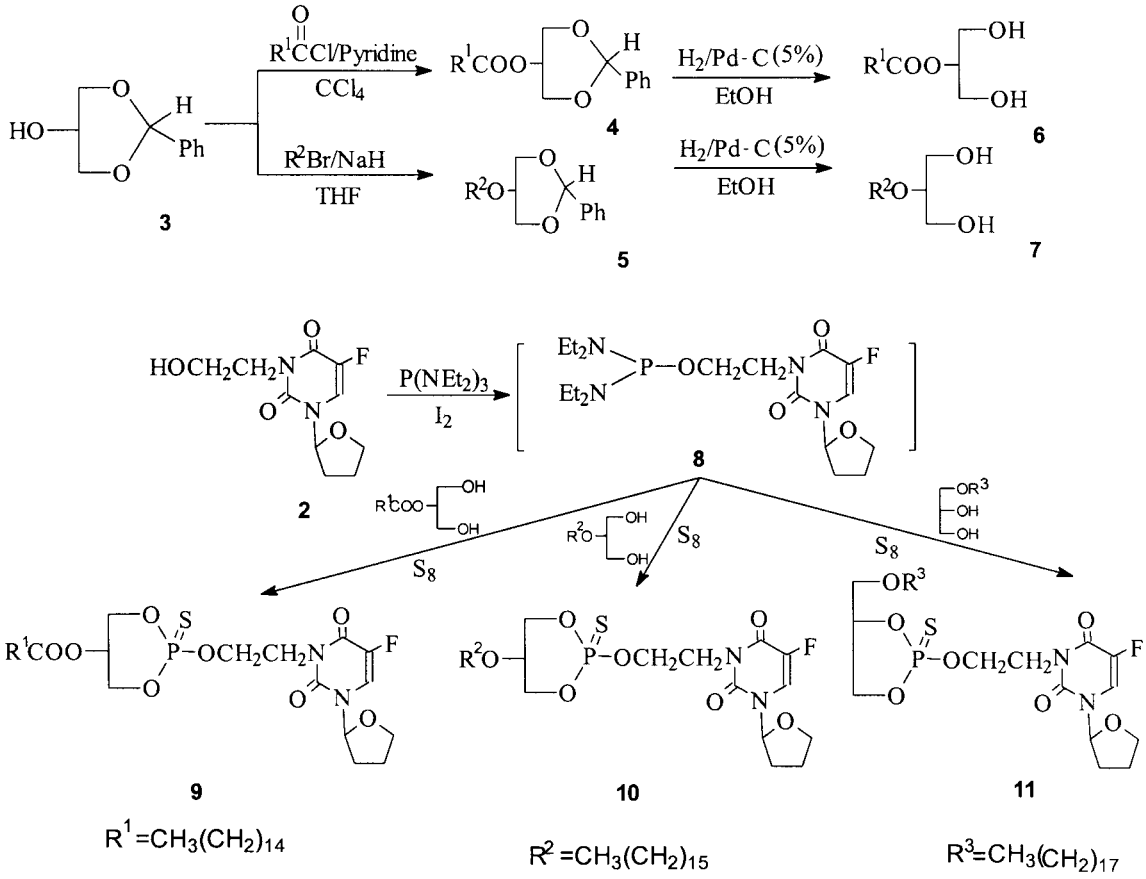
rac-2-Palmitoylglycerol **6**

Compound **4** (4.2 g, 10 mmol) was subjected to hydrogenolysis [0.5 g of Pd–C(5%) in 300 mL of absolute EtOH] in a Parr shaking hydrogenator (Rt, 30 psig). After about 2 hours, the catalyst was filtered off and the solvent was removed on a rotary evaporator to give 3.6 g of a white solid that was recrystallized from ethanol to afford 3.0 g of compound **6,** yield 90%, mp 67–68°C (Ref. [12], mp 69°C).

The same method was used to prepare compound **7:** yield, 92%; mp 53–54°C (Ref. [13], mp 55°C).

2-[1-(N^1-(2-Furanidyl)-5-fluorouracil-N^3-)ethyl-2-O-]-2-thio-5-palmitoyl-1,3,2-dioxaphosphorinane **9**

A mixture of iodine (0.026 g, 0.1 mmol) and the tris(N,N-diethyl)amide of phosphorous acid (0.519 g, 2.1 mmol) in benzene (50 mL) was heated at 65°C in a stream of nitrogen for about 15 minutes. After the precipitate had dissolved, N^1-(2-furanidyl)-N^3-(2-hydroxyethyl)-5-fluorouracil **2** (0.490 g, 2.0 mmol) was added, and the reaction system was heated at 65°C for 3 hours. 2-Palmitoyl-rac-glycerol **6** (0.640 g, 2.0 mmol) was then added, and the reaction mixture was heated at 65°C for another 3 hours. Sulfur (0.067 g, 2.1 mmol) was added, and the reaction system was kept under the same conditions for 30 minutes. The solvent was evaporated in vacuo, and the residue was



SCHEME 2

chromatographed by use of a silica gel column with petroleum ether–ethyl acetate (1:1) as the eluent. A colorless oily product (0.61 g, 48%) was obtained. R_f value: 0.55 (petroleum ether/ethyl acetate, V/V, 1:1). $C_{29}H_{48}FN_2O_8PS$. Calcd: C, 54.89; H, 7.57; N, 4.42. Found: C, 54.92; H, 7.52; N, 3.99. IR (liq): 1734, 1674, 1464, 1259, 1071, 1022, 786, 767, 716 cm^{-1}; ^1H NMR ($CDCl_3$): 0.83 (3H, t, J = 6.0 Hz, CH_3), 1.20 [24H, brs, $(CH_2)_{12}$], 1.57 (2H, m, $OCH_2\underline{CH^2}$), 1.79–2.46 (4H, m, 3′,4′-CH_2), 2.33 (2H, t, $CH_2\underline{CO}$), 3.27–3.50 (4H, m, $CH_2\underline{CH}\underline{CH_2}$), 3.98(1H, m, 5′-$H_a$), 4.02–4.33 (5H, m, 5′-$H_b$, OCH_2CH_2N), 4.58 (1H, quintet, CH), 5.96(1H, brs, 2′-H), 7.35 (1H, d, J_{HF} = 5.4 Hz, 6-H); ^{31}P NMR ($CDCl_3$, δ): 63.24, 63.30.

2-[1-(N¹-(2-Furanidyl)-5-fluorouracil-N³-)ethyl-2-O-]-2-thio-5-hexadecyl-1,3,2-dioxaphosphorinane **10**

By use of N¹-(2-furanidyl)-N³-(2-hydroxyethyl)-5-fluorouracil **2** (0.490 g, 2.0 mmol) and rac-2-O-hexadecylglycerol (0.663 g, 2.1 mmol), compound **10** was synthesized and then purified in the same way as described for compound **9**. $C_{29}H_{50}FN_2O_7PS$. Calcd.: C, 56.13; H, 8.06; N, 4.52. Found: C, 56.20; H, 8.02; N, 4.48. IR (liq.): 1714, 1674, 1464, 1262, 1072, 1022, 839, 767, 656 cm^{-1}; ^1H NMR ($CDCl_3$, δ): 0.85 (3H, t, J = 6.0 Hz, CH_3), 1.22 [26H, brs, $(CH_2)_{13}$], 1.51 (2H, m, $OCH_2\underline{CH_2}$), 1.79–2.46 (4H, m, 3′,4′-CH_2), 3.52 (2H, t, CH_2O), 3.12–3.40 (4H, m, $\underline{CH_2}CH\underline{CH_2}$), 3.98 (1H, m, 5′-$H_a$), 4.02–4.33 (5H, m, 5′-$H_b$, OCH_2CH_2N), 4.58 (1H, quintet, CH), 5.96 (1H, brs, 2′-H), 7.35 (1H, d, J_{HF} = 5.4 Hz, 6-H); ^{31}P NMR ($CDCl_3$, δ): 63.27, 63.23.

2-[1-(N¹-(2-furanidyl)-5-fluorouracil-N³-)ethyl-2-O-]-2-thio-4-octadecoxy-methyl-1,3,2-dioxaphospholane **11**

By use of N¹-(2-furanidyl)-N³-(2-hydroxyethyl)-5-fluorouracil **2** (0.490 g, 2.0 mmol) and *rac*-1-O-octadecyl-*rac*-glycerol (0.690 g, 2.1 mmol), compound **11** was synthesized and then purified in the same way as described for compound **9**. $C_{31}H_{54}FN_2O_7PS$. Calcd.: C, 57.41; H, 8.33; N, 4.32. Found: C, 57.20; H, 8.32; N, 4.38. IR (liq.): 1713, 1664, 1464, 1262, 1172,

1075, 996, cm^{-1}; ^1H NMR ($CDCl_3$, δ): 0.85 (3H, t, J = 6.0 Hz, CH_3), 1.22 [30H, brs, $(CH_2)_{15}$], 1.52 (2H, m, $OCH_2\underline{CH_2}$), 1.79–2.46 (4H, m, 3′,4′-CH_2), 3.52 (2H, t, CH_2O), 3.12–3.40 (4H, m, $\underline{CH_2}CH\underline{CH_2}$), 3.98 (1H, m, 5′-$H_a$), 4.02–4.33 (5H, m, 5′-$H_b$, OCH_2CH_2N), 4.58 (1H, quintet, CH), 5.96 (1H, brs, 2′-H), 7.35 (1H, d, J_{HF} = 5.4 Hz, 6-H); ^{31}P NMR ($CDCl_3$, δ): 84.41, 84.22.

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