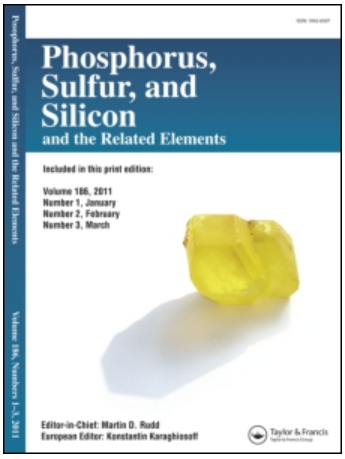

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SYNTHESIS OF NOVEL OPTICALLY ACTIVE CYCLIC PHOSPHOLIPID CONJUGATES OF TEGAFUR AND URIDINE STARTING FROM L-SERINE

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SYNTHESIS OF NOVEL OPTICALLY ACTIVE CYCLIC PHOSPHOLIPID CONJUGATES OF TEGAFUR AND URIDINE STARTING FROM L-SERINE

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Starting from L-serine, cyclic thiophosphoramidate conjugates (**2** and **3**) of Tegafur and uridine were synthesized via a multiple-step procedure of esterification, cyclic phosphorylation, and sulfurization, etc. L-serinoate was N-alkylated, then cyclized with phosphorus oxychloride, and further reacted with N^3-(2-hydroxyethyl) Tegafur to afford cyclic phospholipid conjugate **4**. The resultants (**2**, **3**, and **4**) were successfully separated in the form of pure diastereomer by column chromatography on silica gel. Their configurations were discussed and assigned according to their NMR spectra. The asymmetric induction effects of the carbon-based chiral centre on the diastereomer preference were also observed in these two synthetic phosphorylation cyclizations. The bioassay on their antitumor activities is under investigation.

Keywords: L-serinoate; Phosphorylation cyclization; Phospholipid; Antitumor activity

INTRODUCTION

Phospholipid not only possesses extensive physiological activities, but also serves as an efficient drug carrier. The conjugates consisting of phospholipid and drugs especially with antitumor activities can be used as prodrugs, which are able to be readily transported to the target sites tumor cells by means of phospholipid's supporting function, and then liberate free drug molecules generating desired medicinal effects. This approach is

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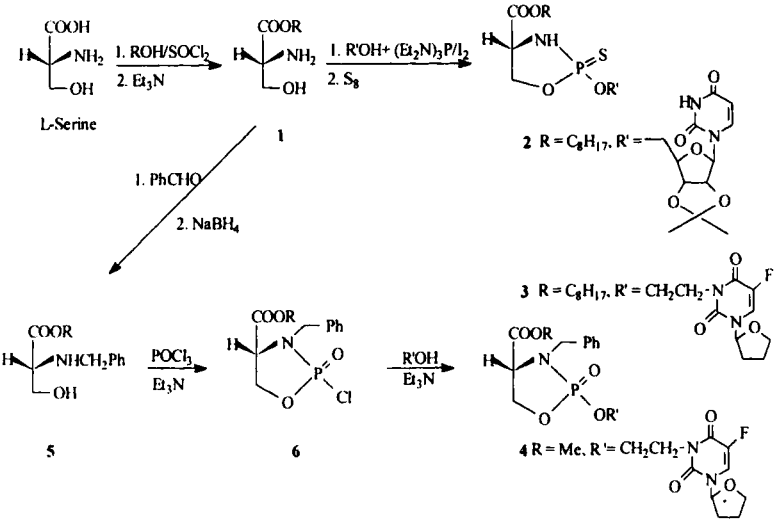
expected to achieve such purposes of improving therapeutic effects and diminishing normal tissue toxicity.

Previously we have reported the syntheses and preliminary antitumor activities of some new types of cyclic glycerophosphatide conjugates with Tegafur derivative N^1-(2-furanidyl)-N^3-(2-hydroxyethyl)-5-fluorouracil and some other nucleoside antitumor agents[1–5] Those reported compounds were all prepared and evaluated as antitumor agents in racemic forms. In this paper, it is reported the synthesis of a novel type of optically active cyclic phospholipid derivatives of Tegafur and uridine starting from L-serine. All of these derivatives have a heteroatom nitrogen at C_2 position and one alkoxy carboxy group as the replacement of hydroxy-derived group at C_1 position. Although there is a remarkable structural difference between this type of conjugates and traditional phospholipid, they still remain the main carbon backbone of phospholipid. Moreover, these conjugates possess another feature that phosphorus atom is chiral. All of their diastereomers were successfully isolated, thus, this provides a possibility for studying on the stereoselectivity of a pair distereomers to the antitumor activity.

Chlorination of L-serine, followed by esterification with alcohols afforded L-serinoate **1**. Direct cyclization of **1** with I_2-activated hexaethyl phosphorous triamide, followed by condensation with uridine or Tegafur derivatives provided three-coordinated cyclic phosphorus compounds, which were sulfurized with elemental sulfur S_8 to give four-coordinated cyclic thiophosphates **2** and **3**. Another cyclic phosphate **4**was synthesized from L-serinoate **1**. **1** was N-benzylated into N-benzyl L-serinoate **5**, which was cyclized with phosphorus oxychloride giving cyclic chloridate **6**. 6 reacted with Tegafur derivative yielding **4**.

In above synthetic reactions, the asymmetric cyclizations of L-serinoate **1** with $(Et_2N)_3P$ / I_2 and N-benzyl L-serinoate **5** with $POCl_3$ were investigated. The asymmetric induction effect of chiral carbon centre to phosphorus atom was evaluated in terms of diastereomeric excess percentage (de%), which can be calculated from ^{31}P NMR intensities of a pair of diastereomers mixture. In the above-mentioned cyclizations, the induction effect is poor, and crude products **2, 3**, and **4** as diastereomeric mixture were obtained with 8.2%, 30.0% and 17.6% de values, respectively. Fortunately, **2, 3** and **4** were all separated in individual diastereomer form by column chromatography on silica gel. Their related data of physical prop-



erties and analyses are listed in Table I and Table II. Their antitumor activity is under investigation in our laboratory.

EXPERIMENTAL

^{1}H, ^{13}C and ^{31}P NMR were recorded in $CDCl_3$ as solvent on FX-90Q and AC-P200 instruments using TMS as internal standard for ^{1}H, ^{13}C NMR, and 85% H_3PO_4as external standard for ^{31}P NMR. Elemental analyses were conducted on MF-3 automatic analyzer. IR-spectra were measured on Nicolet 5DX IR-spectrometer. Melting points were determined on MP-500 melting point apparatus. Optical rotations were measured on a Perkin-Elmer 241MC polarimeter. All temperatures and pressures were uncorrected.

2.1 The following intermediates were prepared as described in literatures

2', 3'- isopropylidene uridine[6]

Obtained from the reaction of uridine with triethyl ortho-formate in acetone in the presence of catalytic amount of p-toluenesulfonic acid. Yield 92%, m.p. 163–165 °C

TABLE I The data of Compds. **2, 3,** and **4**

m.p. (°C)	$[\alpha]_D$	$^{31}PNMR\ \delta(ppm)$	*% de*	*Yield (%)*[a]	*Elementary*		*Analys*
					C%	*H%*	*N%*
					Calc. (Found)	*Calc. (Found)*	*Calc. (Fo*
30–32	−10.8	85.86	8.2	34.9	49.20 (49.30)	6.42 (6.84)	7.48 (7.1
43–45	−11.2	86.85	8.2	41.9	49.20 (49.12)	6.42 (6.43)	7.48 (7.5
thick liq.	−10.3	85.95	30.0	32.5	48.37 (48.28)	6.33 (6.85)	8.06 (7.9
thick liq.	−20.1	87.32	30.0	38.4	48.37 (48.43)	6.33 (6.18)	8.06 (7.1
thick liq.	−26.0	20.20	17.6	26.2	50.70 (50.65)	5.0 (5.14)	8.45 (8.4
thick liq.	+16.7	20.87	17.6	31.9	50.70 (50.94)	5.03 (5.12)	(8.45) (8.

yield.

TABLE II ^{1}HNMR and IR data of Compds. **2, 3, 4**

^{1}HNMR,δ(ppm), ($CDCl_3$/TMS)	IR (cm^{-1}), film or KBr			
	P=S or P=O	P-O-C	P-N	C=O
.83(t,3H), 1.23(brs, 10H), 1.31(s,3H), 1.53(s,3H), 1.63(m,2H), 4.10 (t,1H), .30~4.38(m,8H), 4.85 (dd,2H), 5.82(dd,2H), 7.45(d,1H), 9.60(s,1H)	712	1064, 1153	969	1686
.85(t,3H), 1.25(brs,10H), 1.32(s,3H), 1.54(s,3H), 1.65(m,2H), 4.16(t,1H), .23~4.55(m,8H), 4.88 (dd,2H), 5.72(dd,2H), 7.38(d,1H), 9.43(s,1H)	711	1061, 1152	962	1683
.67(t,3H), 1.07(brs,10H), 1.46(m,2H), 1.75~2.30 (m,4H), 3.77(t,2H), .94(m,1H), 4.02~4.37(m,6H), 5.79(brs,1H), 7.22(d,1H)	755	1074, 1172	968	1655, 1676, 172
.85(t,3H), 1.25(brs,10H), 1.60(m,2H), 1.83~2.33 (m,4H), 3.93(t,2H), .66(m,1H), 4.11~4.54(m,6H), 5.95(brs, 1H), 7.36(d, 1H)	755	1077, 1114	962	1654, 1676, 171
.84~2.59(m,4H), 3.66(s,3H), 3.73(t,2H), 3.93 (dt, 1H), 4.16~4.44(m,8H), .88(brs,1 H), 7.24~7.38 (m,6H)	1262	1072, 1166	929	1653, 1711, 174
.92~2.48(m,4H),3.69(s,3H),3.72(t,2H),3.97 (dt, 1H), 4.15~4.51(m,8H), .94(brs,1H), 7.24~7.39 (m,6H)	1263	1073, 1168	929	1653, 1713, 174

N^3-(2-hydroxyethyl) Tegafur[7]

Prepared from the reaction of Tegafur with 2-bromo ethanol in the presence of triethylamine in acetonitrile as solvent. Yield 78%, m.p. 89–91°C.

N-Octyl L-serinoate hydrochloride[8]

prepared from the reaction of L-serine with an excess of n-octyl alcohol and thionyl chloride. Yield 88.7%, m.p. 72–74 °C.

Methyl L-serinoate hydrochloride[8]

Obtained from L-serine, an excess of methanol and thionyl chloride. Yield 93.5%, m.p. 161–163 °C.

Methyl N-benzyl L-serinoate[9]

A solution of methyl L-serinoate hydrochloride in methanol was neutralized with equivalent triethylamine, then reacted with benzaldehyde, followed by reduction with sodium borohydride to afford the desired product. Yield 71.8%, m.p. 31–33 °C.

Hexaethyl phosphorous triamide[10]

Prepared from the reaction of phosphorus trichloride with diethylamine in petroleum ether. Yield 61.5%, b.p. 74–76°C / 13.3 pa, n^{25}_D 1.4711.

2.2 Preparation of Compound 2

To a solution of n-octyl L-serinoate hydrochloride (0.53 g, 2.1 mmol) in 15 mL of methylene chloride, triethylamine (0.22 g, 2.2 mmol) was added at room temperature. The reaction mixture was then stirred at 20–30 °C for 4 h. After evaporation of methylene chloride under reduced pressure, 15 mL of anhydrous benzene was incorporated with the residue, and triethylamine hydrochloride precipitate was filtered off and washed with a small amount of anhydrous benzene. The filtrate was collected as a benzene solution of n-octyl L-serinoate for the next step.

A mixture of hexaethyl phosphorous triamide (0.52 g, 2.1 mmol) and 50 mL of anhydrous benzene was heated to 70 °C with stirring. Iodine (0.025 g) was added and reacted for 15 min at 70 C, and then 2′,3′-isopropylidene uridine (0.568 g, 2.0 mmol) was added and reacted for additional 2 h at the same temperature. The benzene solution of n-octyl L-serinoate

prepared above was dropwise added and the resulting reaction mixture was stirred at 70–80 °C for 2 h. Elemental sulfur powder (0.067 g, 2.1 mmol) was added and reacted for 0.5 h. After cooling to room temperature, a sample was taken from the reaction mixture for ^{31}P NMR test, which disclosed that the diastereomeric excess percentage of the desired product **2** was 8.2%. After removal of solvent under reduced pressure, the crude resultant was purified and isolated by column chromatography on silica gel (petroleum ether – ethyl acetate, gradient elution) to afford two fractions. **2**a, 0.22 g, isolated yield 34.9%, white solid, m.p. 30–32°C, $[\alpha]_D$ −10.8° (c = 0.60, $CHCl_3$), TLC R_f = 0.43 (petroleum ether (60 – 90°) / ethyl acetate 1:1 (v/v)), ^{31}P NMR: δ 85.86 ppm. **2**b, 0.26 g, isolated yield 41.9%, white solid, m.p. 43–45 C, $[\alpha]_D$ −11.2° (c = 0.67, $CHCl_3$), TLC R_f = 0.34 (petroleum ether (60 – 90°) / ethyl acetate 1:1 (v/v)), ^{31}P NMR: δ 86.85 ppm.

2.3 Preparation of Compound 3

Crude compound **3** was obtained from the same procedure as that for compound **2** except that N^3-(2-hydroxyethyl) Tegafur (0.488 g, 2.0 mmol) was used as substrate instead of 2′,3′-isopropylidene uridine. Its ^{31}P NMR showed that the diastereomeric excess percentage was 30.0%. Chromatographic isolation gave two fractions. **3**a, 0.18 g, isolated yield 32.5%, pale yellow viscous oil, $[\alpha]_D$ −10.3° (c = 1.80, $CHCl_3$), TLC R_f = 0.64 (petroleum ether (60 – 90°) / ethyl acetate 1:1 (v/v)), ^{31}P NMR: δ 85.95 ppm. 3b, 0.22 g, isolated yield 38.4%, pale yellow viscous oil, $[\alpha]_D$ −20.1° (c = 6.80, $CHCl_3$), TLC R_f = 0.56 (petroleum ether (60 – 90°) / ethyl acetate 1:1 (v/v)), ^{31}P NMR: δ 87.32 ppm.

2.4 Preparation of Compound 4

Methyl N-benzyl L-serinoate (2.10 g, 10 mmol) was dissolved in 40 mL of anhydrous toluene and cooled to 0 °C with stirring, and triethylamine (3 mL) was added. Then a solution of phosphorus oxychloride in 10 mL of anhydrous toluene was dropwise added and the reaction was stirred at room temperature for 2 h. After evaporation of solvent under reduced pressure (vapor temperature not above 40 °C), crude product cyclic phosphorochloridate **6** as a diastereomeric mixture was obtained with 17.6% de value based on ^{31}P NMR spectrum. The diastereomers were separated by

column chromatography on silica gel (200 – 300 mesh, petroleum ether / ethyl acetate, gradient elution). **6a**, 1.3 g, ^{31}P NMR: δ 23.42 ppm; **6b**, 1.4 g, ^{31}P NMR: δ 23.78 ppm, total yield 94.7% (lit.[9]: ^{31}P NMR: δ 23.68 and 23.92 ppm, respectively, yield 92%).

To a solution of N^3-(2-hydroxyethyl) Tegafur (0.50 g, 2.0 mmol) dissolved in 20 mL of chloroform, a solution of **6a** (0.58 g, 2.0 mmol) in 5 mL of chloroform was dropwise added, followed by triethylamine (0.3 mL). The reaction mixture was stirred at room temperature overnight, and then washed with water (2 × 15 mL), dried over Na_2SO_4, and evaporated in vacuo to yield crude product, which was purified by column chromatographic method (200 – 300 mesh silica gel, petroleum ether / ethyl acetate, gradient elution) to afford 0.26 g of **4a** as a pale yellow viscous oil, yield 26.2%, $[\alpha]_D$ −26.0° (c = 0.60, $CHCl_3$), ^{31}P NMR: δ 20.20 ppm.

Similarly, **4b** (0.32 g) was prepared as a pale yellow viscous oil from **6b** (0.58 g, 2.0 mmol) in 31.9% isolated yield, $[\alpha]_D$ +16.7° (c = 0.60, $CHCl_3$), ^{31}P NMR: δ 20.87 ppm.

RESULTS AND DISCUSSION

The title compounds **2, 3** and **4** obtained herein are all low melting point or viscous substances and unsuitable for growth of single crystal for X-ray diffraction. Consequently we could not determine the conformations of these five-membered phosphorus heterocycles and their absolute configurations of phosphorus atom using X-ray diffraction analysis. Fortunately, with the help of known methods reported in literatures we can preliminarily assign their configurations on the basis of their NMR data.

Bentrude et al.[11] have reported when a exocyclic group on phosphorus atom (not P=O or P=S) resides in the axial position in 1,3,2-oxazaphosphorinane molecules, the ^{31}P NMR chemical shift is less than that in the equatorial position, namely, $\delta(^{31}P_a) < \delta(^{31}P_e)$. Subsequently Thompson et al.[9] employed this conclusion for the absolute configuration assignment of some 1,3,2-oxazaphospholidines and also acquired consistent results. Among our prepared compounds **2, 3** and **4**, there is a considerable ^{31}P NMR chemical shift difference between each pair of diastereomers. Chemical shift δ values of diastereomers **2a**, **3a** and **4a** (**a** series) are about 1 ppm less than those of their counterparts **2b**, **3b** and **4b** (**b** series),

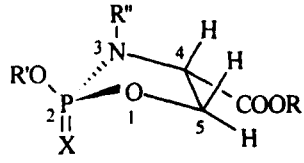
namely, **a** series appear approximately 1 ppm upfield relative to **b** series on their ^{31}P NMR spectra. According to the conclusions reported by Bentrude[11] and Thompson[9] et al., **a** series diastereomers should respond to the exocyclic group on phosphorus atom trans to the carboxy ester moiety at C_4 position (7a) while **b** series isomers should correspond to the exocyclic group cis to the carboxy ester (7b). Consequently the absolute configuration at phosphorus atom can be also figured out (Table III).

Thompson et al.[9] have also found that there is a correlation between the ^{13}C NMR chemical shift of ring carbon C_4 and molecular configuration (trans / cis) in 1,3,2-oxazaphospholidines (**7**, R'' = $PhCH_2$, × = O). The C_4 chemical shift value δ of trans isomer is approximately 1 ppm larger than that of cis isomer. This correlation is demonstrated in the case of compound **4** in the present work. The C_4 ^{13}C NMR δ value of trans isomer **4a** is 55.88 ppm while that of cis isomer **4b** is 55.00 ppm. However, this correlation is inverse in the cases of compounds **2** and **3**. This result is presumably due to substituent difference and conversion of P=O into P=S. The C_4 chemical shift δ values of trans isomers **2a** and **3a** are approximately 0.5 ppm smaller than those of the corresponding cis isomers **2b** and **3b** (Table III).

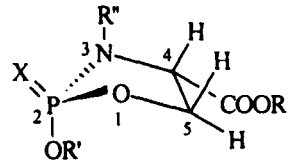
TABLE III ^{31}P, ^{1}H and ^{13}CNMR and Configuration of compds. **2, 3, 4**

Compds.	*^{31}P,δ (ppm)*	*^{1}H, δ (ppm) at C_4*	*^{13}C,δ (ppm) of C_4*	*config. (cis/trans)*	*Config. at P*
2a	85.86	4.10	55.50	trans	R
2b	86.85	4.16	56.21	cis	S
3a	85.95	3.94	55.81	trans	S
3b	87.32	4.06	56.29	cis	R
4a	20.20	3.93	55.88	trans	S
4b	20.87	3.97	55.00	cis	R

Additionally we have noted a slight trend of C_4-H proton chemical shifts in ^{1}H NMR spectra of the compounds **2, 3** and **4**. For cis isomers **2b** – **4b**, the C_4 hydrogen cis to the (thio)phosphoryl group P=O(S) trends to be deshielded presumably owing to the anisotropy of phosphoryl group. Its proton signal appears relatively downfield with a little bigger chemical



7a trans



7b cis

shift δ value than that of the corresponding trans isomer. Although the C_4-H proton chemical shift data listed in Table III consistently reveal this trend, their chemical shift difference Δδ is generally too small (0.04 – 0.12 ppm). Therefore, this trend is unsuitable for assigning the stereochemistry of related system.

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