

## ASYMMETRIC DIHYDROXYLATION ONTO THE $\alpha,\beta$ -UNSATURATED CARBOXYLIC ESTER DERIVATIVES OF CAMPTOTHECIN

Keiko Tagami,\*<sup>a</sup> Shuzo Takagi,<sup>a</sup> Shigeki Sano,<sup>b</sup> Motoo Shiro,<sup>c</sup> and Yoshimitsu Nagao\*<sup>b</sup>

<sup>a</sup> Faculty of Pharmaceutical Sciences, Mukogawa Women's University, 11-68 Koshien Kyuban-cho, Nishinomiya Hyogo 663, Japan

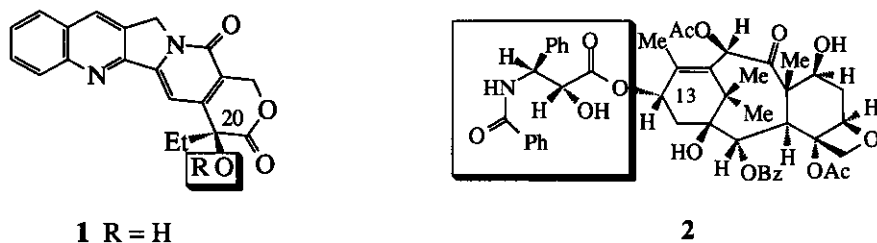
<sup>b</sup> Faculty of Pharmaceutical Sciences, The University of Tokushima, Sho-machi, Tokushima 770, Japan

<sup>c</sup> Rigaku Corporation, 3-9-12 Matsubara-cho, Akishima, Tokyo 196, Japan

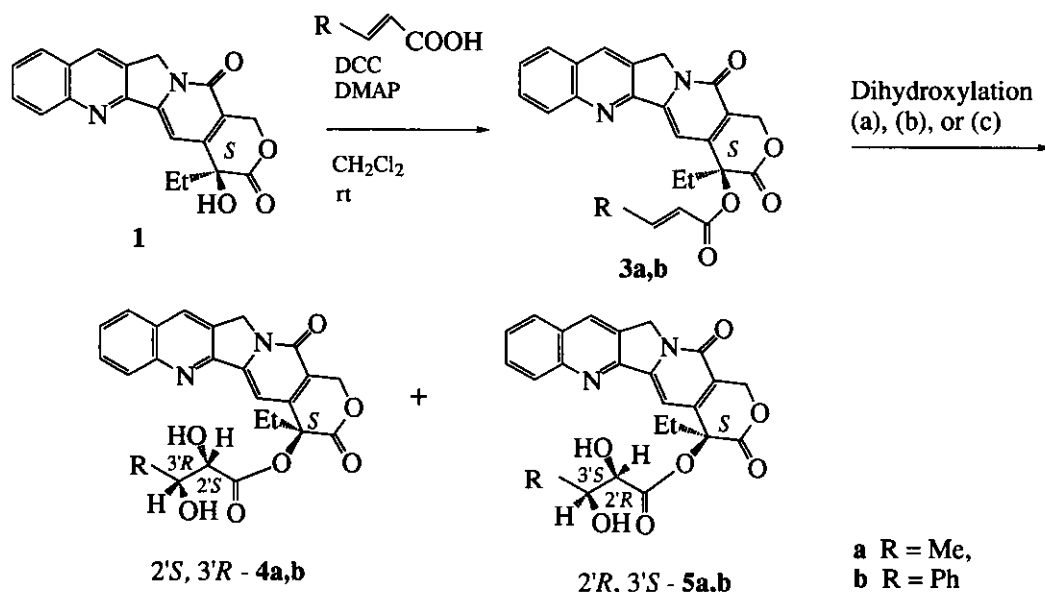
**Abstract** - Dihydroxyalkanoic ester derivatives (**4a,b**) and (**5a,b**) of 20*S*-camptothecin (**1**) were diastereoselectively synthesized by exploiting osmium-catalyzed asymmetric dihydroxylation based on the Sharpless procedure. The absolute configuration of the newly formed chiral centers, the 2' and 3' positions of the 20-alkanoyl side chain of **4a,b** and **5a,b** was determined by the chemical correlation with the known chiral dihydroxyalkanoic acids.

20*S*-Camptothecin (**1**), a pentacyclic alkaloid isolated from *Camptotheca acuminata* by Wall and co-workers in 1966,<sup>1</sup> exhibited potent antitumor activity against various cell lines and in animal screens.<sup>2</sup> Only the 20*S*-enantiomer (**1**) exhibited antitumor activity<sup>3</sup> and its mode of action was found to trap a cleavable complex between topoisomerase I and DNA.<sup>4</sup> However, several problems such as severe toxicity and poor water solubility prevented its application as a clinical antitumor agent. Therefore, many derivatives of 20*S*-camptothecin (**1**) have been synthesized and investigated toward the efficient antitumor agents.<sup>5</sup> Among these derivatives, irinotecan hydrochloride showed clinically useful activity against lung, uterine, and ovarian tumors.<sup>5a</sup>

In 1991, we reported a useful information on the development of new podophyllotoxin and epipodophyllotoxin derivatives bearing the ester moiety of long chain fatty acids.<sup>6</sup> Hydroxy group at C-20 of 20*S*-camptothecin (**1**) should be essential for antitumor activities. In spite of that, there is few attempts to modify the hydroxy group at C-20 of 20*S*-camptothecin (**1**). The acyl side chain at C-13 of taxol (**2**) should also play an important role for the excellent antitumor activity.<sup>7</sup> Thus, we have investigated chemical modification of  $\alpha,\beta$ -unsaturated carboxylic esters (**3a,b**) of 20*S*-camptothecin (**1**) and their antitumor activity.



## Scheme 1



## Reagents and conditions for dihydroxylation :

(a) OsO<sub>4</sub> (0.05 mol eq), NMO (1.5 mol eq), Me<sub>2</sub>CO : H<sub>2</sub>O = 10 : 1, 0°C; (b) (DHQD)<sub>2</sub>-PHAL (0.01 mol eq), K<sub>3</sub>Fe(CN)<sub>6</sub> (3 mol eq), K<sub>2</sub>CO<sub>3</sub> (3 mol eq), K<sub>2</sub>OsO<sub>2</sub>(OH)<sub>4</sub> (0.002 mol eq), MeSO<sub>2</sub>NH<sub>2</sub> (1 mol eq), *t*-BuOH : H<sub>2</sub>O = 1 : 1, 0 °C; (c) (DHQ)<sub>2</sub>-PHAL (0.25 mol eq), OsO<sub>4</sub> (0.05 mol eq), NMO (1.5 mol eq), Me<sub>2</sub>CO : H<sub>2</sub>O = 10 : 1, 0 °C

Esterification at the C-20 hydroxy group of **1** was readily done by treatment with *E*-crotonic acid (3 mol eq) and *E*-cinnamic acid (3 mol eq) in the presence of dicyclohexylcarbodiimide (DCC, 3 mol eq) and 4-dimethylaminopyridine (DMAP, 0.2 mol eq) in CH<sub>2</sub>Cl<sub>2</sub> to give the corresponding esters (**3a,b**) as colorless needles [**3a** : mp 215-218°C (MeOH), **3b** : mp 285-288°C (CHCl<sub>3</sub>-MeOH)] in each 95% yield. Subsequently, asymmetric dihydroxylation onto **3a,b** in the presence of a catalytic amount of chiral ligand (DHQD)<sub>2</sub>-PHAL and (DHQ)<sub>2</sub>-PHAL,<sup>8</sup> was efficiently performed on the basis of the Sharpless procedure<sup>8</sup> [See footnote (reagents and conditions (a)-(c)) in Scheme 1] to furnish the corresponding dihydroxy derivatives (**4a,b**) and (**5a,b**) in 57-68% yields (Table 1). Diastereoselectivity of the dihydroxylation products (**4a,b**) without use of the chiral ligand was very poor (Entries 1 and 4). However, when catalytic (DHQD)<sub>2</sub>-PHAL or (DHQ)<sub>2</sub>-PHAL was employed, the desired asymmetric dihydroxylation proceeded in a

fairly good (Entry 3) or an excellent (Entries 2, 5, and 6) diastereoselective manner to give a mixture of **4a** and **5a** or a mixture of **4b** and **5b**, respectively (Table 1). Pure compound [**4a**: mp 178-180°C (MeOH),  $[\alpha]_D^{26}$  -74° (c 0.5, CHCl<sub>3</sub>-MeOH (4 : 1))] was obtained by repeated recrystallization of the mixture of **4a** and **5a** (97 : 3) in MeOH. Similar recrystallization of other diastereomeric mixtures furnished the corresponding pure compounds, [**5a**: mp 165-167°C (CHCl<sub>3</sub>-hexane),  $[\alpha]_D^{26}$  -40° (c 0.5, CHCl<sub>3</sub>-MeOH (4 : 1))], [**4b**: mp 175-176°C (MeOH),  $[\alpha]_D^{26}$  -62° (c 0.5, CHCl<sub>3</sub>-MeOH (4 : 1))], and [**5b**: mp 172-174°C (MeOH),  $[\alpha]_D^{26}$  +10° (c 0.5, CHCl<sub>3</sub>-MeOH (4 : 1))], respectively.

**Table 1.** Asymmetric dihydroxylation of **3a,b**.

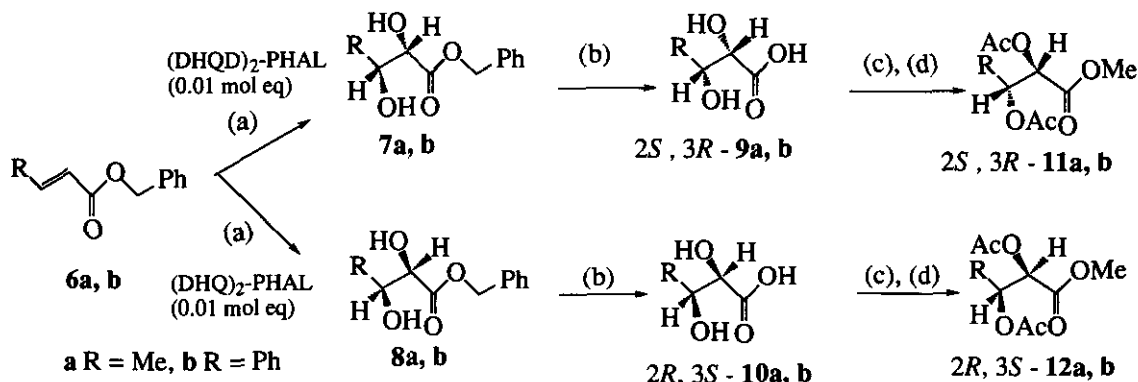
Entry	R	Conditions <sup>1)</sup>	Diastereomer ratio <sup>2)</sup>	Yild (%) <sup>3)</sup>
1	<b>3a</b>	(a)	<b>4a</b> : <b>5a</b> = 37 : 63 <sup>4)</sup>	63
2	<b>3a</b>	(b)	<b>4a</b> : <b>5a</b> = 97 : 3 <sup>4)</sup>	67
3	<b>3a</b>	(c)	<b>4a</b> : <b>5a</b> = 11 : 89 <sup>4)</sup>	68
4	<b>3b</b>	(a)	<b>4b</b> : <b>5b</b> = 44 : 56 <sup>5)</sup>	57
5	<b>3b</b>	(b)	<b>4b</b> : <b>5b</b> = 93 : 7 <sup>5)</sup>	60
6	<b>3b</b>	(c)	<b>4b</b> : <b>5b</b> = 8 : 92 <sup>5)</sup>	57

1) See footnote in Scheme 1. 2) Determined by HPLC analysis. 3) Yield of the diastereomeric mixture. 4) Waters Nova-pak Silica 3.9X150 mm, CH<sub>2</sub>Cl<sub>2</sub>-2-propanol (98 : 2), flow rate = 1.0 mL/min, UV detector (254 nm). 5) Waters Puresil C<sub>18</sub> 4.6X150 mm, MeCN-H<sub>2</sub>O (35 : 65), flow rate = 1.0 mL/min, UV detector (254 nm).

The absolute configuration of the newly formed chiral centers C-2' and C-3' of the dihydroxyalkanoyl moiety in all products (**4a,b**) and (**5a,b**) was successfully determined by their chemical correlation as shown in Schemes 2 and 3. First of all, known two 2,3-dihydroxybutanoic acids (2*S*, 3*R*-**9a**)<sup>9</sup> and (2*R*, 3*S*-**10a**)<sup>10</sup> and two 2,3-dihydroxy-3-phenylpropanoic acids (2*S*, 3*R*-**9b**)<sup>11</sup> and (2*R*, 3*S*-**10b**)<sup>11</sup> were synthesized by exploiting the Sharpless' asymmetric dihydroxylation<sup>8</sup> onto the corresponding *E*-crotonic acid benzyl ester (**6a**) or *E*-cinnamic acid benzyl ester (**6b**) as follows. Dihydroxylation of **6a** under the Sharpless procedure<sup>8</sup> [See footnote (reagents and conditions (a)) in Scheme 2] in the presence of catalytic (DHQD)<sub>2</sub>-PHAL (0.01 mol eq) gave chiral dihydroxy compound (**7a**) in 92% ee<sup>12</sup> and 76% yield. The similar dihydroxylation onto **6a** in the presence of catalytic (DHQ)<sub>2</sub>-PHAL (0.01 mol eq) gave chiral dihydroxy compound (**8a**) in 90% ee<sup>12</sup> and 82% yield. The compound (**6b**) was also submitted to the same asymmetric dihydroxylation employing a catalytic amount (0.01 mol eq) of (DHQD)<sub>2</sub>-PHAL or (DHQ)<sub>2</sub>-PHAL as described above to afford each chiral dihydroxy compound, (**7b**: 94% ee,<sup>12</sup> 62% yield) or (**8b**: 90% ee,<sup>12</sup> 71% yield). Hydrogenolysis of all chiral benzyl dihydroxyalkanoates (**7a,b**) and (**8a,b**)

on 5% Pd-C in MeOH gave the corresponding known chiral carboxylic acids [ 2*S*, 3*R*-**9a** : 95% yield, colorless oil,  $[\alpha]_D^{25} -17.00^\circ$  (*c* 1; H<sub>2</sub>O), lit.,<sup>9</sup>  $[\alpha]_D^{25} -17.75^\circ$  (*c* 1, H<sub>2</sub>O); 2*R*, 3*S*-**10a** : 90% yield, colorless oil,  $[\alpha]_D^{25} +15.0^\circ$  (*c* 2.0, H<sub>2</sub>O), lit.,<sup>10</sup>  $[\alpha]_D^{20} +15.9^\circ$  (*c* 2.1, H<sub>2</sub>O); 2*S*, 3*R*-**9b** : 95% yield, mp 166-167°C (H<sub>2</sub>O),  $[\alpha]_D^{25} -39.0^\circ$  (*c* 1, H<sub>2</sub>O), lit.,<sup>11</sup> mp 166-167°C (H<sub>2</sub>O),  $[\alpha]_D^{20} -39.6^\circ$  (*c* 1, H<sub>2</sub>O); 2*R*, 3*S*-**10b** : 91% yield, mp 166-167°C (H<sub>2</sub>O),  $[\alpha]_D^{25} +40.0^\circ$  (*c* 1, H<sub>2</sub>O), lit.,<sup>11</sup> mp 166-167°C (H<sub>2</sub>O),  $[\alpha]_D^{20} +39.6^\circ$  (*c* 1, H<sub>2</sub>O)]. The four chiral dihydroxyalkanoic acids (**9a,b**) and (**10a,b**) were converted to the corresponding diacetoxyalkanoic methyl esters (2*S*, 3*R*-**11a,b**) and (2*R*, 3*S*-**12a,b**) by their conventional methylation (CH<sub>2</sub>N<sub>2</sub> in MeOH) followed by acetylation (Ac<sub>2</sub>O in pyridine). Each peak on the HPLC chart<sup>12</sup> of the compounds (2*S*, 3*R*-**11a,b**) and (2*R*, 3*S*-**12a,b**) obtained from alkaline hydrolysis (5% NaOH) of 20*S*-camptothecin derivatives (2'*S*, 3'*R*-**4a,b**) and (2'*R*, 3'*S*-**5a,b**) followed by neutralization (Amberlite IR 120B(H)) and some other reactions of the resultant dihydroxyalkanoic acids as shown in Scheme 3; was identified with that of the same compounds derived from the authentic chiral dihydroxyalkanoic acids (2*S*, 3*R*-**9a,b**) and (2*R*, 3*S*-**10a,b**) as shown in Scheme 2.

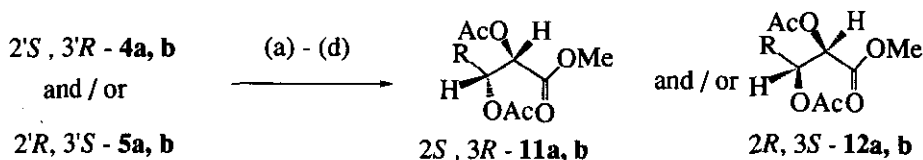
## Scheme 2



Reagents and conditions :

- (a) K<sub>3</sub>Fe(CN)<sub>6</sub> (3 mol eq), K<sub>2</sub>CO<sub>3</sub> (3 mol eq), K<sub>2</sub>OsO<sub>2</sub>(OH)<sub>4</sub> (0.002 mol eq), MeSO<sub>2</sub>NH<sub>2</sub> (1 mol eq), *t*-BuOH : H<sub>2</sub>O = 1 : 1, 0 °C; (b) 5% Pd-C, MeOH; (c) CH<sub>2</sub>N<sub>2</sub>, MeOH; (d) Ac<sub>2</sub>O (excess), pyridine

## Scheme 3

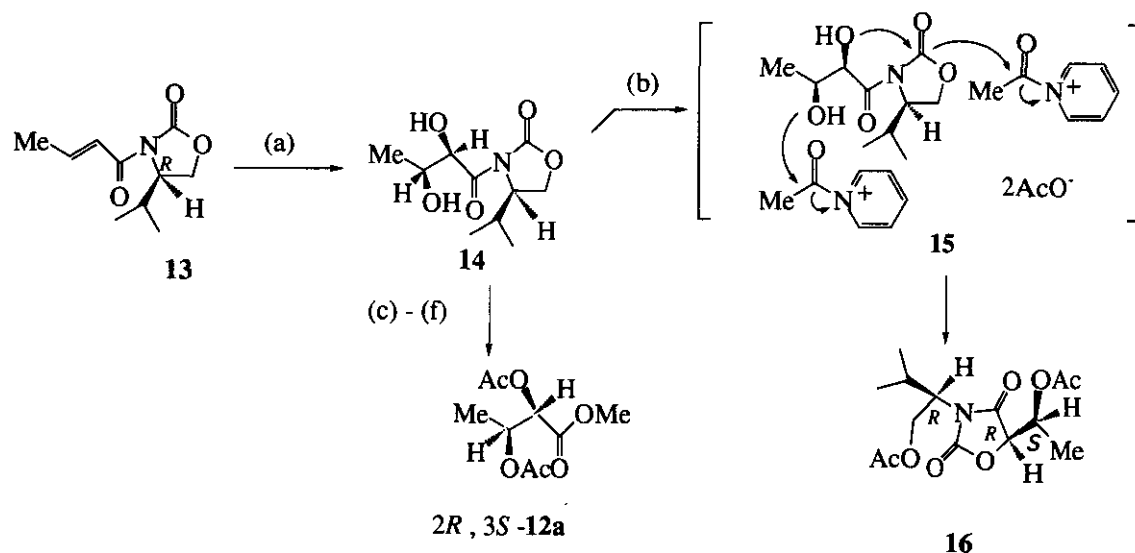


Reagents and conditions :

- (a) 5% NaOH, MeOH; (b) Amberlite IR 120B(H); (c) CH<sub>2</sub>N<sub>2</sub>, MeOH; (d) Ac<sub>2</sub>O (excess), pyridine

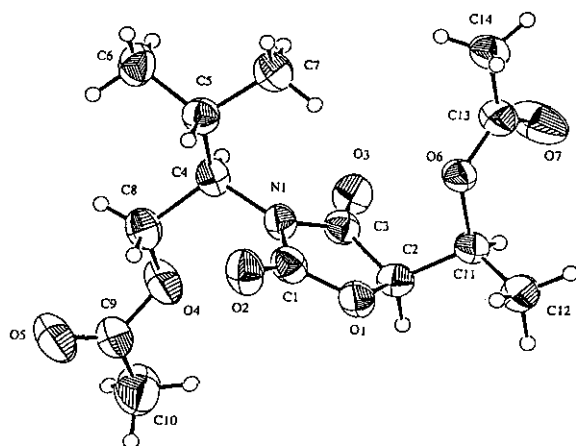
In the course of the stereochemistry determination of *2S,3R*-**11a,b** and *2R,3S*-**12a,b** as described above, we carried out tentatively diastereoselective dihydroxylation onto 3-crotonyl-4*R*-isopropyl-1,3-oxazolidin-2-one (**13**) under the reaction conditions with same reagents systems. Highly diastereoselective dihydroxylation onto **13** was achieved only in the case with catalytic (DHQ)<sub>2</sub>-PHAL to give *2R,3S*-**14** in 90% de<sup>13</sup> and 86% yield (Scheme 4).

Scheme 4



## Reagents and conditions :

- (a) (DHQ)<sub>2</sub>-PHAL (0.01 mol eq),  $\text{K}_2\text{OsO}_2(\text{OH})_4$  (0.002 mol eq),  $\text{K}_3\text{Fe}(\text{CN})_6$  (3 mol eq),  $\text{K}_2\text{CO}_3$  (3 mol eq),  $\text{MeSO}_2\text{NH}_2$  (1 mol eq), *t*-BuOH : H<sub>2</sub>O = 1 : 1, 0 °C; (b)  $\text{Ac}_2\text{O}$  (excess), pyridine; (c) 5% NaOH, MeOH; (d) Amberlite IR 120B(H); (e)  $\text{CH}_2\text{N}_2$ , MeOH; (f)  $\text{Ac}_2\text{O}$  (excess), pyridine

Figure 1. ORTEP drawing of the crystallographic structure of **16**.

Surprisingly, usual acetylation of **14** with  $\text{Ac}_2\text{O}$  in pyridine gave an unexpected diacetate [**16**, 53% yield, mp 95-96°C (MeOH-H<sub>2</sub>O)] of which was established by the X-Ray analysis (Figure 1).<sup>14</sup> This diacetate (**16**) could be derived from **14** via a plausible pathway (**15**). Here, the absolute configuration of 2*R*, 3*S*-**12a** was directly determined by the chemical correlation of **14** to it as shown in Scheme 4.

Interestingly, both diastereomeric 2',3'-dihydroxybutanoyl esters (2'*S*,3'*R*-**4a**) and (2'*R*,3'*S*-**5a**) exhibited fairly strong antitumor activities against p388 lymphocytic leukemia inoculated into mice [**4a** : T/C = 175 % (200 mg/kg), 175 % (100 mg/kg), and 142 % (50 mg/kg); **5a** : T/C = 99 % (250 mg/kg), 210 % (125 mg/kg), and 148 % (62.5 mg/kg)]. However, both diastereomeric 2',3'-dihydroxy-3-phenylpropanoyl esters (2'*S*, 3'*R*-**4b**) and (2'*R*, 3'*S*-**5b**) did not exhibit any antitumor activity.<sup>15</sup> The antitumor activities of new compounds (2'*S*, 3'*R*-**4a**) and (2'*R*, 3'*S*-**5a**) must be better than those of 20*S*-camptothecin [1: T/C = 65 % (100 mg/kg), 88 % (50 mg/kg), and 180 % (25 mg/kg)].<sup>15</sup>

Thus, this convenient asymmetric dihydroxylation method seems to be available for chemical modification of various drugs, which is currently undertaken in our research groups.

## REFERENCES AND NOTES

1. M. E. Wall, M. C. Wani, C. E. Cook, K. H. Palmer, A. T. McPhail, and G. A. Sim, *J. Am. Chem. Soc.*, 1966, **88**, 3888.
2. Reviews, a) A. G. Schultz, *Chem. Rev.*, 1973, **73**, 385. b) C. R. Hutchinson, *Tetrahedron*, 1981, **37**, 1047.
3. M. C. Wani, A. W. Nicholas, and M. E. Wall, *J. Med. Chem.*, 1987, **30**, 2317.
4. For discussions on topoisomerase I inhibitors and leading references, see : D. E. Berry, L. MacKenzie, E. A. Shultis, J. A. Chan, and S. M. Hecht, *J. Org. Chem.*, 1992, **57**, 420.
5. a) T. Kunimoto, K. Nitta, T. Tanaka, N. Uehara, H. Baba, M. Takeuchi, T. Yokokura, S. Sawada, T. Miyasaka, and M. Mutai, *Cancer Res.*, 1987, **47**, 5944. b) M. C. Wani, A. W. Nicholas, and M. E. Wall, *J. Med. Chem.*, 1986, **29**, 2358. c) B. C. Giovanella, J. S. Stehlin, M. E. Wall, M. C. Wani, A. W. Nicholas, L. F. Liu, R. Silber, and M. Potmesil, *Science*, 1989, **246**, 1046. d) M. Sugimori, A. Ejima, S. Ohsuki, K. Matsumoto, Y. Kawato, M. Yasuoka, H. Tagawa, and H. Terasawa, *Heterocycles*, 1994, **38**, 81 and references cited therein.
6. Y. Nagao, J. Musutafa, S. Sano, M. Ochiai, T. Tashiro, and S. Tsukagoshi, *Med. Chem. Res.*, 1991, **1**, 295.
7. a) F. Gueritte-Voegelein, V. Senilh, B. David, D. Guenard, and P. Potier, *Tetrahedron*, 1986, **42**, 4451. b) L. Mangatal, M. T. Adeline, D. Guenard, and F. Gueritte-Voegelein, *ibid.*, 1989, **45**, 4177.
8. H. C. Kolb, M. S. VanNieuwenhze, and K. B. Sharpless, *Chem. Rev.*, 1994, **94**, 2483.
9. F. W. Bachelor and G. A. Miana, *Can. J. Chem.*, 1969, **47**, 4089
10. Y. Izumi, S. Tatsumi, and M. Imaida, *Bull. Chem. Soc. Jpn.*, 1966, **39**, 2223.
11. a) C. N. Riiber, *Ber.*, 1915, **48**, 823. b) A. Collet, *Bull. Soc. Chim. Fr.*, 1975, 215.
12. HPLC Analysis : Daicel chiralcel OD-H 4.6X150 mm, n-hexane-2-propanol (97 : 3), flow rate = 0.5 mL / min, UV detector 254 nm (**11b**, **12b** and diacetate of **7a,b** and **8a,b**), 210 nm (**11a** and

12a).

13. HPLC Analysis : Waters Nova pak Silica 3.9X159 mm , n-hexane-2-propanol (95 : 5), flow rate = 1 mL / min, UV detector 210 nm.
14. The crystallographic data of compound (16) are as follows.  $C_{14}H_{21}NO_7$ , FW = 315.32, monoclinic, Space Group  $P2_1$  (# 4),  $\alpha = 9.361$  (3) Å,  $b = 8.064$  (1) Å,  $c = 11.445$  (4) Å,  $\beta = 106.665$  (5)°,  $Z = 2$ ,  $D_{calc} = 1.265$  g/cm<sup>3</sup>,  $V = 827.5700$  Å<sup>3</sup>,  $R = 0.056$ .
15. The authors express their appreciation to Dr. T. Yamori ( Cancer Chemotherapy Center, Tokyo, Japan) for his kind antitumor testing. Full detail of the antitumor activity of all 20S-camptothecin esters and the related compounds will be published as part of a forthcoming paper.

Received, 19th June, 1997