ASYMMETRIC DIHYDROXYLATION ONTO THE α,β-UNSATURATED CARBOXYLIC ESTER DERIVATIVES OF CAMPTOTHECIN

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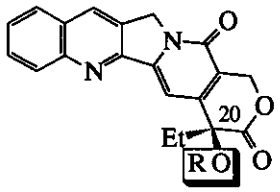
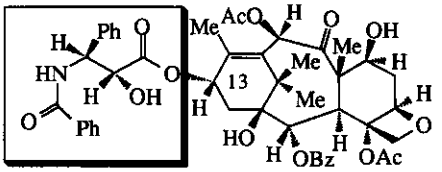
Abstract - Dihydroxyalkanoic ester derivatives (**4a,b**) and (**5a,b**) of 20*S*-camptothecin (**1**) were diastereoselectively synthesized by exploiting osmium-catalyzed asymmetric dihydroxylation based on the Sharpless procedure. The absolute configuration of the newly formed chiral centers, the 2' 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,[1] exhibited potent antitumor activity against various cell lines and in animal screens.[2] Only the 20*S*-enantiomer (**1**) exhibited antitumor activity[3] and its mode of action was found to trap a cleavable complex between topoisomerase I and DNA.[4] 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.[5] Among these derivatives, irinotecan hydrochloride showed clinically useful activity against lung, uterine, and ovarian tumors.[5a]

In 1991, we reporteded a useful information on the development of new podophyllotoxin and epipodophyllotoxin derivatives bearing the ester moiety of long chain fatty acids.[6] 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.[7] Thus, we have investigated chemical modification of α,β-unsaturated carboxylic esters (**3a,b**) of 20*S*-camptothecin (**1**) and their antitumor activity.



1 R = H



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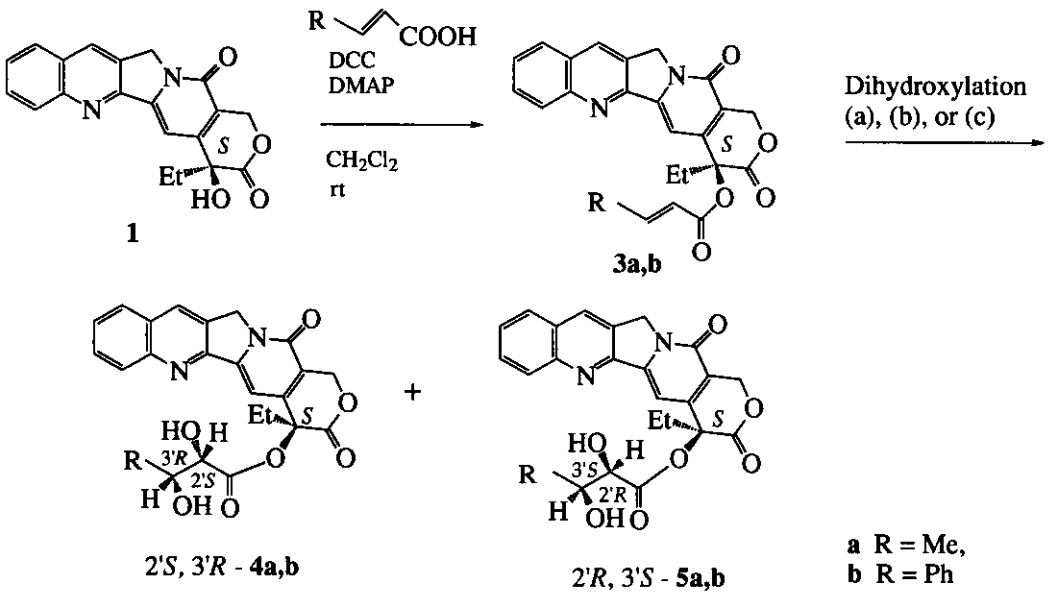
Scheme 1



Reagents and conditions for dihydroxylation :
(a) OsO_4 (0.05 mol eq), NMO (1.5 mol eq), Me_2CO : H_2O = 10 : 1, 0℃; (b) $(DHQD)_2$-PHAL (0.01 mol eq), $K_3Fe(CN)_6$ (3 mol eq), K_2CO_3 (3 mol eq), $K_2OsO_2(OH)_4$ (0.002 mol eq), $MeSO_2NH_2$ (1 mol eq), *t*-BuOH : H_2O = 1 : 1, 0 ℃; (c) $(DHQ)_2$-PHAL (0.25 mol eq), OsO_4 (0.05 mol eq), NMO (1.5 mol eq), Me_2CO : H_2O = 10 : 1, 0 ℃

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_2Cl_2 to give the corresponding esters (**3a,b**) as colorless needles [**3a** : mp 215-218°C (MeOH), **3b** : mp 285-288°C ($CHCl_3$-MeOH)] in each 95% yield. Subsequently, asymmetric dihydroxylation onto **3a,b** in the presence of a catalytic amount of chiral ligand $(DHQD)_2$-PHAL and $(DHQ)_2$-PHAL,[8] was efficiently performed on the basis of the Sharpless procedure[8] [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 $(DHQD)_2$-PHAL or $(DHQ)_2$-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, $CHCl_3$-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 ($CHCl_3$-hexane), $[\alpha]_D^{26}$ -40° (*c* 0.5, $CHCl_3$-MeOH (4 : 1))], [**4b**: mp 175-176°C (MeOH), $[\alpha]_D^{26}$ -62° (*c* 0.5, $CHCl_3$-MeOH (4 : 1))], and [**5b**: mp 172-174°C (MeOH), $[\alpha]_D^{26}$ +10° (*c* 0.5, $CHCl_3$-MeOH (4 : 1))], respectively.

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

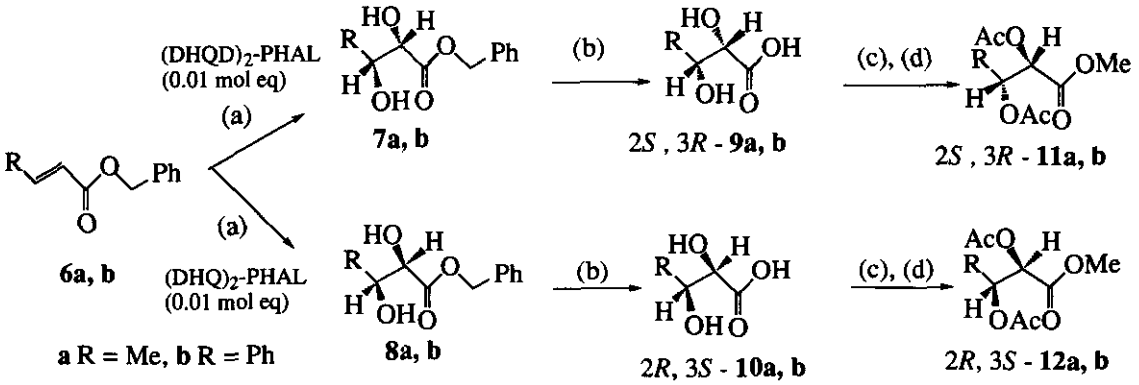
Entry	R	Conditions[1)]	Diastereomer ratio[2)]	Yild (%)[3)]
1	**3a**	(a)	**4a** : **5a** = 37 : 63[4)]	63
2	**3a**	(b)	**4a** : **5a** = 97 : 3[4)]	67
3	**3a**	(c)	**4a** : **5a** = 11 : 89[4)]	68
4	**3b**	(a)	**4b** : **5b** = 44 : 56[5)]	57
5	**3b**	(b)	**4b** : **5b** = 93 : 7[5)]	60
6	**3b**	(c)	**4b** : **5b** = 8 : 92[5)]	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_2Cl_2-2-propanol (98: 2), flow rate = 1.0 mL/min, UV detecter (254 nm). 5) Waters Puresil C_{18} 4.6X150 mm, MeCN-H_2O (35 : 65), flow rate = 1.0 mL/min, UV detecter (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 successfuly 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**)[9] and (2*R*, 3*S*-**10a**)[10] and two 2,3-dihydroxy-3-phenylpropanoic acids (2*S*, 3*R*-**9b**)[11] and (2*R*, 3*S*-**10b**)[11] were synthesized by exploiting the Sharpless' asymmetric dihydroxylation[8] 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[8] [See footnote (reagents and conditions (a)) in Scheme 2] in the presence of catalytic $(DHQD)_2$-PHAL (0.01 mol eq) gave chiral dihydroxy compound (**7a**) in 92% ee[12] and 76% yield. The similar dihydroxylation onto **6a** in the presence of catalytic $(DHQ)_2$-PHAL (0.01 mol eq) gave chiral dihydroxy compound (**8a**) in 90% ee[12] and 82% yield. The compound (**6b**) was also submitted to the same asymmetric dihydroxylation employing a catalytic amount (0.01 mol eq) of $(DHQD)_2$-PHAL or $(DHQ)_2$-PHAL as described above to afford each chiral dihydroxy compound, (**7b**: 94% ee,[12] 62% yield) or (**8b**: 90% ee,[12] 71% yield). Hydrogenolysis of all chiral benzyl dihydroxyalkanoates (**7a,b**) and (**8a,b**)

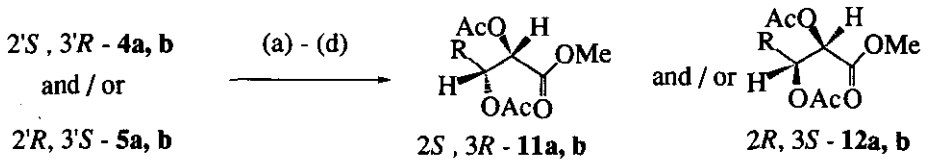
on 5% Pd-C in MeOH gave the corresponding known chiral carboxylic acids [2*S*, 3*R*-**9a** : 95% yield, colorless oil, $[\alpha]_D^{25}$ -17.00° (*c* 1; H_2O), lit.,[9] $[\alpha]_D^{25}$ -17.75° (*c* 1, H_2O); 2*R*, 3*S*-**10a** : 90% yield, colorless oil, $[\alpha]_D^{25}$ +15.0° (*c* 2.0, H_2O), lit.,[10] $[\alpha]_D^{20}$ +15.9° (*c* 2.1, H_2O); 2*S*, 3*R*-**9b** : 95% yield, mp 166-167°C (H_2O), $[\alpha]_D^{25}$ -39.0° (*c* 1, H_2O), lit.,[11] mp 166-167°C (H_2O), $[\alpha]_D^{20}$ -39.6° (*c* 1, H_2O); 2*R*, 3*S*-**10b** : 91% yield, mp 166-167°C (H_2O), $[\alpha]_D^{25}$ +40.0° (*c* 1, H_2O), lit.,[11] mp 166-167°C (H_2O), $[\alpha]_D^{20}$ +39.6° (*c* 1, H_2O)]. 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_2N_2 in MeOH) followed by acetylation (Ac_2O in pyridine). Each peak on the HPLC chart[12] 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) $K_3Fe(CN)_6$ (3 mol eq), K_2CO_3 (3 mol eq), $K_2OsO_2(OH)_4$ (0.002 mol eq), $MeSO_2NH_2$ (1 mol eq), *t*-BuOH : H_2O = 1 : 1, 0 ℃; (b) 5% Pd-C, MeOH;
(c) CH_2N_2 , MeOH; (d) Ac_2O (excess), pyridine

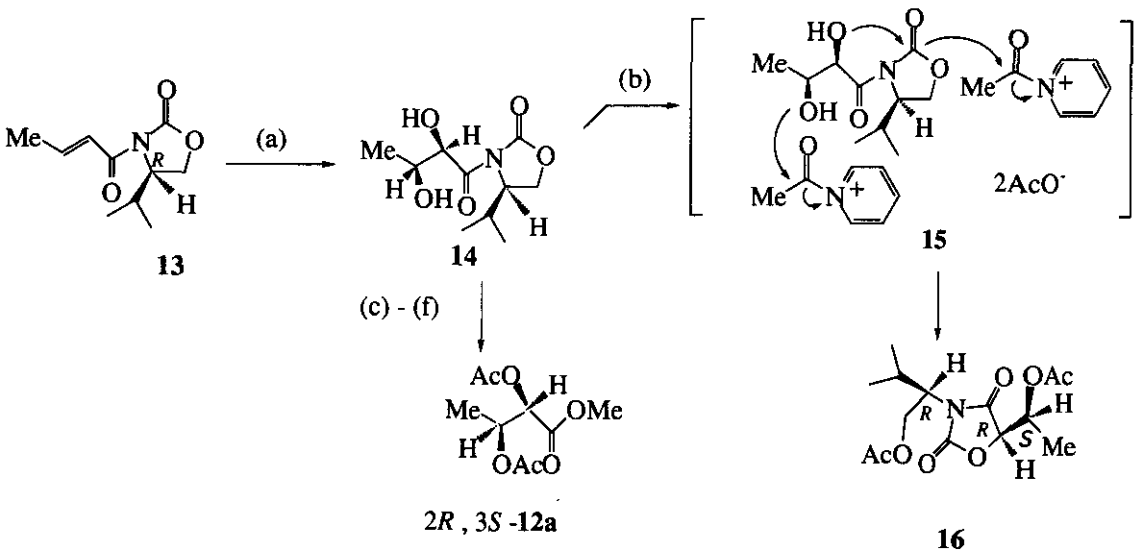
Scheme 3



Reagents and conditions :
(a) 5% NaOH, MeOH; (b) Amberlite IR 120B(H); (c) CH_2N_2,MeOH;
(d) Ac_2O (excess), pyridine

In the course of the stereochemistry determination of 2*S*,3*R*-**11a**,**b** and 2*R*,3*S*-**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 $(DHQ)_2$-PHAL to give 2*R*,3*S*-**14** in 90% de[13] and 86% yield (Scheme 4).

Scheme 4



Reagents and conditions :
(a) $(DHQ)_2$-PHAL (0.01 mol eq), $K_2OsO_2(OH)_4$ (0.002 mol eq), $K_3Fe(CN)_6$ (3 mol eq), K_2CO_3 (3 mol eq), $MeSO_2NH_2$ (1 mol eq), *t*-BuOH : H_2O = 1 : 1, 0 ℃; (b) Ac_2O (excess), pyridine; (c) 5% NaOH, MeOH; (d) Amberlite IR 120B(H); (e) CH_2N_2, MeOH; (f) Ac_2O (excess), pyridine

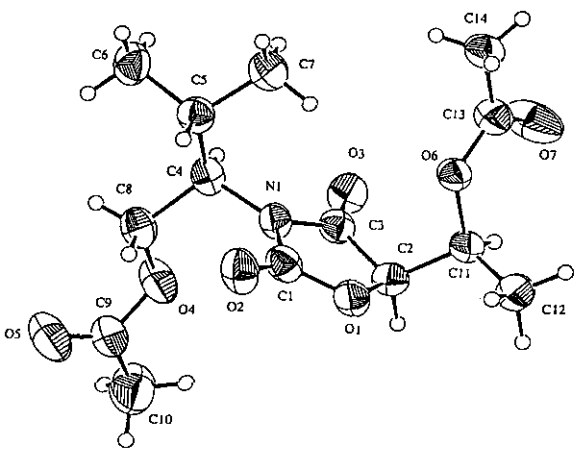


Figure 1. ORTEP drawing of the crystallographic stracture of **16**.

Surprisingly, usual acetylation of **14** with Ac_2O in pyridine gave an unexpected diacetate [**16**, 53% yield, mp 95-96°C (MeOH-H_2O)] of which was established by the X-Ray analysis (Figure 1).[14] 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.[15] 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)].[15]

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

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12. HPLC Analysis : Daicel chiralcel OD-H 4.6X150 mm, n-hexane-2-propanol (97 : 3), flow rate = 0.5 mL / min, UV detecter 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 detecter 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), α = 9.361 (3) Å, b = 8.064 (1) Å, c = 11.445 (4) Å, β = 106.665 (5)°, Z = 2, Dcalc = 1.265 g/cm^3, V = 827.5700 Å^3, 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 20*S*-camptothecin esters and the related compounds will be published as part of a forthcoming paper.

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