Preparation and Antitumor Activity of 2″-*O*-, 3″-*O*- and 2″,3″-Di-*O*-substituted Derivatives of Etoposide

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The 2″-*O*-, 3″-*O*- and 2″,3″-di-*O*-substituted derivatives (4a—p) of etoposide were prepared by nucleophilic substitution of 4′-*O*-benzyloxycarbonyletoposide (2) followed by deprotection. Controlled reaction (a limited amount of reagents and low temperature) was required for preparing the mono-*O*-substituted derivatives.

In terms of ED_{125} values, doses which show 125% of T/C against P388 leukemia in mice, both the 2″-*O*-acetate (4a, ED_{125} = 0.18 mg/kg) and 3″-*O*-acetate (4b, 0.23 mg/kg) were nearly as active as etoposide (1, 0.19 mg/kg), while the 2″,3″-di-*O*-acetate (4c, 1.9 mg/kg) was somewhat less potent. In the replacement with other substituents, antitumor activity of the 2″-*O*-substituted derivatives was affected much more by the difference of the substituents as compared with that of the corresponding 3″-*O*-substituted derivatives. In the 2″,3″-di-*O*-substituted derivatives, the activity was decreased additively on the substituents.

Keywords etoposide; 2″-*O*-acyletoposide; 3″-*O*-acyletoposide; antitumor activity; water-solubility

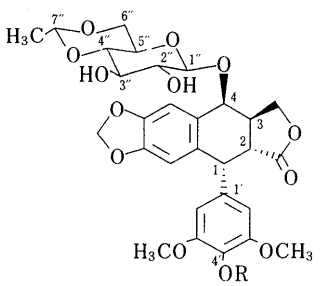
Introduction

Etoposide (VP-16, VePesid)[1-4] is a semisynthetic derivative of podophyllotoxin[5] (a naturally occurring lignan lactone extracted from the roots and rhizomes of *Podophyllum peltatum* and *P. emodi*) and is currently used in the treatment of small-cell lung cancer, testicular cancer, malignant lymphoma and other types of tumor. However, etoposide is poorly soluble in water and the complex drug formulation is required for intravenous and oral administrations. Our modification program on etoposide was intended to develop compounds showing improved water solubility, more potent antitumor activity and/or improved pharmacokinetics. The study was started with modification of the glucose moiety. Modification on the C-4″, C-6″-cyclic acetal moiety has already been studied extensively by Sandoz's chemists.[1] Recently, several amino sugar[6] or non-sugar[7a] analogues, five-membered ring C derivatives,[7b] ring E desoxy analogues,[7c] and a phosphorylated prodrug[8] have been prepared and their various antitumor activities investigated. We first investigated modification on the C-2″ and/or C-3″ OH groups of the glucose moiety by direct substitution of etoposide.

This paper describes the preparation and antitumor activity against P388 leukemia cells of the C-2″ and/or C-3″ substituted etoposide derivatives.

Results and Discussion

Chemistry The 4′-*O*-benzyloxycarbonyl etoposide (**2**, Fig. 1) was prepared according to the Kuhn's method[9] and used for the sugar modification as shown in Fig. 2. Acetylation of **2** with excess acetic anhydride in pyridine at room temperature predominantly gave the diacetate **3c** (yield 82%). In order to obtain the mono-*O*-acetates, the acetylation was carried out with a limited amount of acetic anhydride (1.8 eq) at low temperature (−10 °C) to give the mono-acetates **3a** (yield 18%) and **3b** (yield 9%), the diacetate **3c** (yield 1%) and recovered **2** (yield 42%). Hydrogenolysis of **3a**, **3b** and **3c** gave 2″-*O*-acetate (**4a**), 3″-*O*-acetate (**4b**) and 2″,3″-di-*O*-acetate (**4c**) of etoposide, respectively.



1: R=H (etoposide)
2: R=CO-O-$CH_2C_6H_5$

Fig. 1. Etoposide (**1**) and 4′-*O*-Benzyloxycarbonyl Etoposide (**2**)

Mono- and di-*O*-benzoates of etoposide (**4d—f**), were similarly prepared by the controlled benzoylation of **2** with benzoyl chloride followed by deprotection. Formylation of **2** with a mixture of 49% formic acid and acetic anhydride could not be controlled to afford mono-*O*-formyl derivatives, but always gave the 2″,3″-di-*O*-formate (**4g**).

The *O*-mesyl (**4h—j**) and *O*-tosyl (**4k—m**) derivatives were also prepared similarly by the condensation of **2** with mesyl chloride and tosyl chloride, respectively, followed by deblocking.

In an early stage of the modification program by Senter *et al.*, they informed that etoposide 4′-phosphate[8] was soluble in water with retention of the antitumor activity of **1**. Therefore, the introduction of acidic functions such as phosphate and sulphate was attempted to the C-2″ and/or C-3″ positions.

The reaction of **2** with phosphorus oxychloride (1.5 eq) in dry pyridine–methylene chloride (1 : 1) at 0 °C followed by quenching with benzyl alcohol gave three products, which were separated by silica gel column chromatography and subsequently deblocked by hydrogenolysis to give the water-soluble products, **4n**, **4o** and **4p** (Table I). Compounds **4n** and **4o** were the 2″-phosphate and 3″-phosphate, respectively. In contrast with the *O*-acyl and *O*-sulfonyl derivatives described above, the third product **4p** was not the diphosphate, but bis(3″-*O*-etoposidyl)-phosphate according to the mass and proton nuclear magnetic resonance (^{1}H-NMR) spectral study (Fig. 3).

For the preparation of the sulfate derivatives, **2** was treated with pyridine–sulfur trioxide complex and then

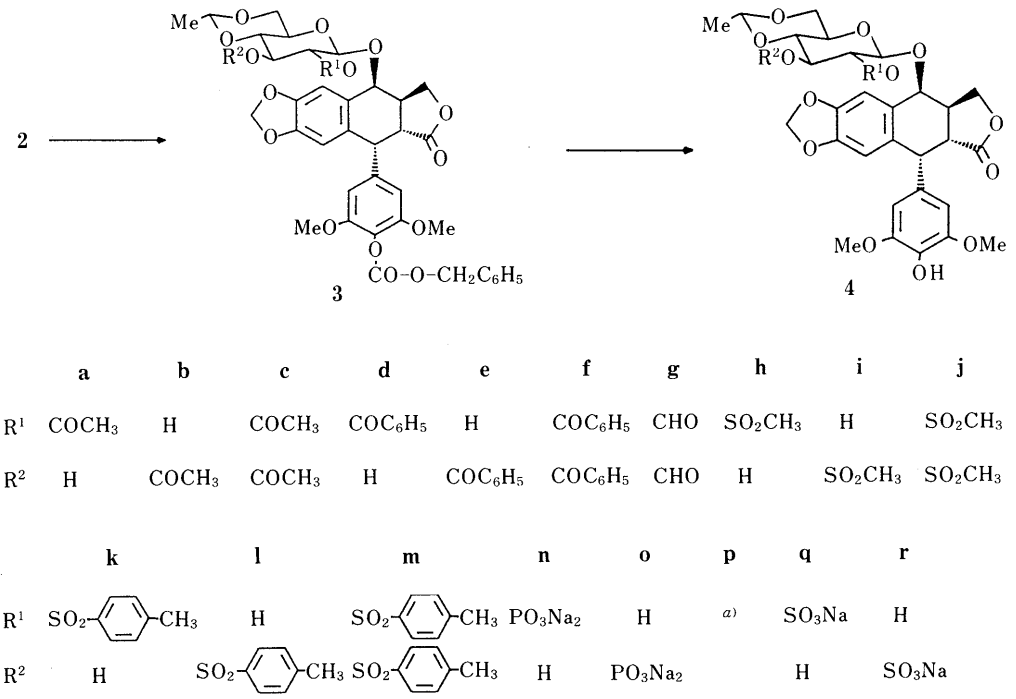
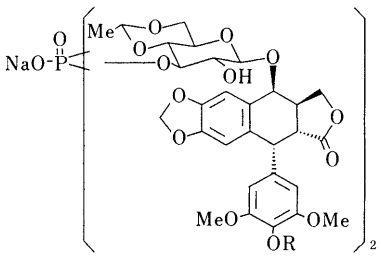


Fig. 2. Preparation of 2″-*O*- and/or 3″-*O*-Substituted Derivatives (**4**) of Etoposide (**2**)
a) See Fig. 3.

TABLE I. Water-Solubility of Etoposide Derivatives

Compound	Solubility in water (mg/ml at 24 °C)
4n	>100
4o	>100
4p	23
4q	>100
4r	72
Etoposide	0.1



3p: R=CO-O-$CH_2C_6H_5$
4p: R=H

Fig. 3. Bis(3″-*O*-etoposidyl)phosphates (**3p** and **4p**)

subjected to hydrogenolysis and chromatographic separation on a C_{18}-reverse phase column. In this case only mono-sulfates, **4q** and **4r**, were isolated, and neither the disulfate nor the bis-etoposidyl derivative were observed. Both **4q** and **4r** were readily soluble in water.

Antitumor Activity The etoposide derivatives prepared in this study were assessed with *in vitro* cytotoxicity against B16 melanoma cells and *in vivo* antitumor activity in P388 leukemia-bearing mice. The results are shown in Table II.

Clear structure–activity relationships were observed in terms of ED_{125} values in anti-P388 *in vivo* activity. In the 2″-*O*-substituted derivatives, the difference of the substituents considerably affected the ED_{125} values. The 2″-*O*-acetate (**4a**) was nearly as effective as etoposide, while the 2″-*O*-benzoate (**4d**) was less potent than **4a**. The 2″-*O*-mesylate (**4h**) was also less potent than **4a** although it was more potent than **4d**, whereas the 2″-*O*-tosylate (**4k**) was nearly inactive. The 2″-*O*-phosphorylate (**4n**) and 2″-*O*-sulfonate (**4q**) were also inactive.

On the other hand, the 3″-*O*-substituted derivatives (**4b**, **e**, **i**, **l**, **o**) except for the 3″-*O*-sulfonate (**4r**) retained anti-P388 *in vivo* activity, although the difference of the substituents affected the activity to some extent (for example, the 3″-*O*-acetate (**4b**) and 3″-*O*-mesylate (**4i**) were more effective than the 3″-*O*-benzoate (**4e**) and 3″-*O*-tosylate (**4l**), respectively).

Exceptional inactivity of the 3″-*O*-sulfonate (**4r**) might be related to the detoxication mechanism of etoposide, because glucronate conjugates (at both sugar and phenolic sites) and sulfonate conjugate (at phenolic site) have been reported as metabolites of etoposide.[10]

In the 2″,3″-di-*O*-substituted derivatives, the activity was affected additively on the substituents. Therefore, the disubstituted derivatives (**4c**, **f**, **j**, **m**) were less potent than any of the corresponding mono-substituted derivatives. The 2″,3″-di-*O*-formate (**4g**) possessing the smallest substituent in this series of compounds was the most potent among the disubstituted derivatives. This means that the mono-*O*-formates would be at least as potent as, or probably more potent than mono-*O*-acetates (**4a**, **b**), although we could not isolate any of mono-*O*-formates in this study.[11]

In terms of maximum T/C values in anti-P388 *in vivo* activity, **4a**, **4e** and **4g** showed better activity than etoposide. Especially, when **4a** and **4g** were given at a dose of

TABLE II. Antitumor Activity of the 2″- and/or 3″-*O*-Substituted Derivatives (**4a—r**) of Etoposide

Compound	Substituent	Cytotoxicity *vs.* B16-F10 IC_{50}[a] (μg/ml)	Anti-P388 activity: Dose (mg/kg) Range	Anti-P388 activity: Dose (mg/kg) Optimal	Anti-P388 activity: T/C % at optimal dose	Anti-P388 activity: ED_{125}[b] (mg/kg)
4a	2″-*O*-Ac	1.2	0.1—120	120	>435 (2/4)[c]	0.18
4b	3″-*O*-Ac	1.4	0.1—120	120	205	0.23
4c	2″,3″-Di-*O*-Ac	2.7	0.1—120	120	240	1.9
4d	2″-*O*-Bz	0.92	0.1—120	120	145	28
4e	3″-*O*-Bz	0.17	0.1—120	60	420 (1/4)	0.44
4f	2″,3″-Di-*O*-Bz	4.4	0.1—120	120	125	120
4g	2″,3″-Di-*O*-CHO	2.0	0.1—120	120	>500 (3/4)	0.64
4h	2″-*O*-Ms	29	0.3—120	30—60	130	10
4i	3″-*O*-Ms	0.32	0.1—120	120	175	0.84
4j	2″,3″-Di-*O*-Ms	33	0.1— 30		115	>30
4k	2″-*O*-Ts	5.4	0.1— 30		115	>30
4l	3″-*O*-Ts	0.07	0.1—120	60	195	1.3
4m	2″,3″-Di-*O*-Ts	30	0.1— 30		105	>30
4n	2″-*O*-PO_3Na_2	20	0.1— 30		120	>30
4o	3″-*O*-PO_3Na_2	2.2	0.1—240	120	188	0.37
4p	3″-*O*-P(O ONa)(3″-*O*-ETD)[d]	37	0.1— 30	30	125	30
4q	2″-*O*-SO_3Na	>100	0.1— 30		105	>30
4r	3″-*O*-SO_3Na	>100	0.1—120		105	>120
Etoposide		0.21	0.1—240	120	288 (2/14)	0.19

a) Concentrations causing 50% inhibition of cell growth relative to that of untreated cells. *b*) Dose in mg/kg/injection, Q1D × 1, i.p., which shows 125% of T/C. *c*) No. of 50-d survivors/No. of mice tested. *d*) 3″-*O*-ETD = etoposide-3″-*O*-.

120 mg/kg, 2 and 3 of 4 mice treated, respectively, were completely cured and still survived at the end of the experimental period (50 d).

The results of the *in vitro* cytotoxicity showed similar structure–activity relationships on the position of the substituents. Generally the 3″-*O*-substituted derivatives were much more cytotoxic than the corresponding 2″-*O*-substituted ones, while the disubstituted compounds were the least toxic in the same substituent series. In contrast with the *in vivo* activity, the *O*-benzoates and *O*-tosylates were more potent than the corresponding *O*-acetates and *O*-mesylates, respectively, in terms of the *in vitro* cytotoxicity.

Based on the studies described above, the following conclusions can be postulated:

1. It is notable that the acetyl and formyl functions (**4a, e, g**) enhanced remarkably the antitumor *in vivo* activity compared to **1** having free hydroxy groups at 2″-*O*- and 3″-*O*-positions. Other 3″-*O*-substituted derivatives (**4b, e, i, l**) also retained the activity. However, when substituted with benzoyl and tosyl groups at 2″-*O*-position, the effect of their substituents resulted in extremely reducing both *in vitro* and *in vivo* antitumor activities.

2. Of the water-soluble derivatives, the 3″-phosphate (**4o**) retained the *in vivo* antitumor activity. However, the corresponding 2″-phosphate (**4n**) and the sulfates (**4q, r**) were not effective to anti-P388 *in vivo* activity.

Experimental

Melting points were determined with a Yanagimoto micro hotstage apparatus and are uncorrected. Infrared (IR) spectra were recorded on a JASCO IR Report-100 spectrometer and ultraviolet (UV) spectra on a Shimadzu UV-260 spectrophotometer. NMR spectra were recorded on a JEOL CL-60HL, Varian FT-80A or JEOL GX-400 spectrometer. Mass spectra (MS) were measured on a JMS-AX505H mass spectrometer.

Acetylated Products (3a—c) of 2 (Typical Procedure for a Controlled Substitution of C-2″ and/or C-3″ OHs of Etoposide) To a cooled (−10 °C) solution of **2** (142 mg, 0.2 mmol) in pyridine (8 ml) was added 4-dimethylaminopyridine (5 mg) and acetic anhydride (20 μl, 0.18 mmol). The mixture was stirred for 30 min at −10 °C, and then an additional amount of acetic anhydride (20 μl, 0.18 mmol) was added. After being stirred for 30 min at −10 °C, the reaction mixture was diluted with CH_2Cl_2 and washed successively with water, 5% HCl, aq. $NaHCO_3$, and water. The organic phase was dried over anhydr. Na_2SO_4, then filtered off. The filtrate was concentrated *in vacuo* to afford a colorless powder (117 mg), which contained four components (silica gel) thin layer chromatography (TLC): *Rf* 0.56, 0.46, 0.34 and 0.24; hexane : acetone = 1 : 1). The mixture was separated by silica gel column chromatography (2.5% MeOH–CH_2Cl_2). Fractions showing a spot of *Rf* 0.56 were combined and evaporated *in vacuo* to give 1 mg (1%) of **3c** . Similarly fractions of *Rf* 0.46, 0.34 and 0.24 afforded **3b** (13 mg, 9%, 3″-*O*-acetyl), **3a** (26 mg, 18%, 2″-*O*-acetyl) and **2** (60 mg, 42%) as colorless powder, respectively.

3a: mp 144—147 °C. IR ν_{max}^{KBr} cm^{-1}: 3400 (br), 1740, 1600. UV λ_{max}^{MeOH} nm (ε): 291 (4200). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.39 (3H, d, J = 5.1 Hz, 7″-CH_3), 1.91 (3H, s, $OCOCH_3$), 2.50 (1H, br, 3″-OH, exchangeable with D_2O), 2.83 (1H, m, 3-H), 3.19 (1H, dd, J = 5.1, 14.1 Hz, 2-H), 3.31 (1H, dt, J = 4.7, 9.4 Hz, 5″-H), 3.37 (1H, t, J = 9.4 Hz, 4″-H), 3.60 (1H, t, J = 10.0 Hz, 6″-H_{ax}), 3.66 (6H, s, 2 × OCH_3), 3.82 (1H, t, J = 9.0 Hz, 3″-H), 4.19 (1H, dd, J = 4.7, 10.3 Hz, 6″-H_{eq}), 4.22 (1H, t, J = 9 Hz, 11′-H), 4.41 (1H, dd, J = 9.0, 10.7 Hz, 11-H), 4.61 (1H, d, J = 5.1 Hz, 1-H), 4.71 (1H, d, J = 7.7 Hz, 1″-H), 4.76 (1H, q, J = 5.1 Hz, 7″-H), 4.83 (1H, d, J = 3.4 Hz, 4-H), 4.85 (1H, dd, J = 7.7, 9.2 Hz, 2″-H), 5.25 (2H, s, $COCH_2C_6H_5$), 5.99 (2H, dd, J = 1.3, 5.1 Hz, O–CH_2O), 6.26 (2H, s, 2′-H and 6′-H), 6.55 (1H, s, 8-H), 6.79 (1H, s, 5-H), 7.33—7.43 (5H, m, $COCH_2C_6H_5$). *Anal.* Calcd for $C_{39}H_{40}O_{16}$: C, 61.25; H, 5.27. Found: C, 60.84; H, 5.25.

As the ^{1}H-NMR values of the aglycone moiety of the following derivatives were similar to those of **3a**, their values were omitted from the experimental section.

3b: mp 226—228 °C. IR ν_{max}^{KBr} cm^{-1}: 3500 (br), 1770, 1600. UV λ_{max}^{MeOH} nm (ε): 291 (3920). ^{1}H-NMR (80 MHz, $CDCl_3$) δ: 1.32 (3H, d, J = 5 Hz, 7″-CH_3), 2.10 (3H, s, $OCOCH_3$), 2.58 (1H, br, 2″-OH, exchangeable with D_2O), 3.3—3.5 (2H, m, 4″-H and 5″-H), 3.4 (1H, m, 2″-H), 3.6 (1H, m, 6″-H_{ax}), 4.15 (1H, m, 6″-H_{eq}), 4.6 (1H, m, 7″-H), 4.66 (1H, d, J = 8 Hz, 1″-H), 5.07 (1H, t, J = 9 Hz, 3″-H). *Anal.* Calcd for $C_{39}H_{40}O_{16} \cdot 1/2H_2O$:

C, 60.54; H, 5.34. Found: C, 60.27; H, 5.26.

3c: mp 287—289 °C. IR ν_{max}^{KBr} cm^{-1}: 3500 (br), 1770, 1610. UV λ_{max}^{MeOH} nm (ε): 290 (3950). ^{1}H-NMR (60 MHz, $CDCl_3$) δ: 1.34 (3H, d, J=5 Hz, 7″-CH_3), 1.83 and 2.04 (6H, each s, 2×$OCOCH_3$), 3.3—3.6 (2H, m, 4″-H and 5″-H), 3.6 (1H, m, 6″-H_{ax}), 4.0—5.0 (2H, m, 6″-H_{eq} and 7″-H), 4.66 (1H, d, J=8 Hz, 1″-H), 4.91 (1H, m, 2″-H), 5.20 (1H, t, J=9 Hz, 3″-H). *Anal.* Calcd for $C_{41}H_{42}O_{17}$: C, 61.04; H, 5.25. Found: C, 60.64; H, 5.36.

Compound **3c** was also prepared by the following procedure. To a solution of **2** (500 mg, 0.69 mmol) in pyridine (10 ml) was added acetic anhydride (1 ml) and the mixture was stirred for 4 h at room temperature. The reaction mixture was quenched with MeOH (2 ml), diluted with dichloromethane, and then washed with water, 5% HCl, and water, successively. The organic layer was dried over anhydr. Na_2SO_4, then filtered off. The filtrate was concentrated *in vacuo* to obtain a colorless solid (**3c**, 551 mg) which was recrystallized from MeOH to give colorless crystals (455 mg, 82%).

2″-*O*-Acetyletoposide (4a) (Typical Procedure for Deprotection of the 4′-*O*-Cbz Group of Etoposide Derivatives) A stirred solution of **3a** (25 mg, 0.03 mmol) in EtOH–acetone (4 : 1, 15 ml) was hydrogenated for 1.5 h in the presence of 10% Pd–C (20 mg) at 1 atm, and then the catalyst was filtered off. The filtrate was concentrated *in vacuo* to obtain a colorless solid (21 mg, *ca.* 100%), which was crystallized from AcOEt–MeOH to give the pure **4a** as colorless crystals. mp 144–147 °C. $[\alpha]_D^{23}$ −68° (c=0.1, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3450 (br), 1770, 1740, 1610. UV λ_{max}^{MeOH} nm (ε): 240 (sh, 12900), 284 (4030). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.39 (3H, d, J=5.1 Hz, 7″-CH_3), 1.91 (3H, s, $OCOCH_3$), 2.50 (1H, d, J=3.3 Hz, 3″-OH, exchangeable with D_2O), 3.31 (1H, dt, J=4.8, 9.2 Hz, 5″-H), 3.37 (1H, t, J=9.2 Hz, 4″-H), 3.59 (1H, t, J=10.0 Hz, 6″-H_{ax}), 3.82 (1H, dt, J=3.3, 9.2 Hz, 3″-H), 4.19 (1H, dd, J=4.8, 10.0 Hz, 6″-H_{eq}), 4.72 (1H, d, J=8.1 Hz, 1″-H), 4.76 (1H, q, J=5.1 Hz, 7″-H), 4.85 (1H, m, 2″-H). *Anal.* Calcd for $C_{31}H_{34}O_{14}$: C, 59.04; H, 5.43. Found: C, 58.65; H, 5.46.

3″-*O*-Acetyletoposide (4b) Compound **3b** (10 mg, 0.01 mmol) was hydrogenated to give **4b** (9 mg, *ca.* 100%), as colorless powder. mp 226—228 °C. $[\alpha]_D^{23}$ −87° (c=0.1, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3450 (br), 1760, 1610. UV λ_{max}^{MeOH} nm (ε): 240 (sh, 12610), 285 (3960). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.35 (3H, d, J=5.1 Hz, 7″-CH_3), 2.14 (3H, s, $OCOCH_3$), 2.44 (1H, d, J=2.9 Hz, 2″-OH, exchangeable with D_2O), 3.40 (1H, m, 5″-H), 3.41 (1H, t, J=9.2 Hz, 4″-H), 3.50 (1H, ddd, J=2.9, 7.7, 9.2 Hz, 2″-H), 3.56 (1H, t, J=10.0 Hz, 6″-H_{ax}), 4.18 (1H, dd, J=4.8, 10.0 Hz, 6″-H_{eq}), 4.69 (1H, q, J=5.1 Hz, 7″-H), 4.71 (1H, d, J=7.7 Hz, 1″-H), 5.12 (1H, t, J=9.2 Hz, 3″-H). *Anal.* Calcd for $C_{31}H_{34}O_{14}\cdot 1/2H_2O$: C, 58.21; H, 5.52. Found: C, 58.22; H, 5.42.

2″,3″-Di-*O*-acetyletoposide (4c) Compound **3c** (405 mg, 0.5 mmol) was hydrogenated to give **4c** (360 mg, *ca.* 100%) as colorless crystals. mp 287—289 °C. $[\alpha]_D^{23}$ −74° (c=0.1, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3500 (br), 1760, 1610. UV λ_{max}^{MeOH} (ε): 240 (sh, 12780), 285 (4060). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.34 (3H, d, J=4.8 Hz, 7″-CH_3), 1.83 and 2.05 (6H, each s, 2×$OCOCH_3$), 3.38 (1H, dt, J=4.8, 9.5 Hz, 5″-H), 3.47 (1H, t, J=9.5 Hz, 4″-H), 3.59 (1H, t, J=10.0 Hz, 6″-H_{ax}), 4.20 (1H, dd, J=4.8, 10.0 Hz, 6″-H_{eq}), 4.69 (1H, q, J=4.8 Hz, 7″-H), 4.79 (1H, d, J=8.1 Hz, 1″-H), 4.91 (1H, dd, J=8.1, 9.4 Hz, 2″-H), 5.21 (1H, t, J=9.5 Hz, 3″-H). *Anal.* Calcd for $C_{33}H_{36}O_{15}$: C, 58.93; H, 5.39. Found: C, 58.56; H, 5.41.

Benzoylated Products (3d—f) of 2 A mixture of **2** (500 mg, 0.7 mmol) and benzoyl chloride (100 μl, 0.86 mmol) in pyridine (5 ml) was stirred at room temperature for 3d. The crude product containing three new components was separated by silica gel column chromatography (40% hexane–acetone) to obtain **3f** (30 mg, 5%), **3e** (227 mg, 40%), **3d** (70 mg, 12%) and recovered **2** (215 mg, 43%).

3d: IR ν_{max}^{KBr} cm^{-1}: 3400 (br), 1770, 1720, 1600. UV λ_{max}^{MeOH} nm (ε): 281 (4050). ^{1}H-NMR (80 MHz, $CDCl_3$) δ: 1.41 (3H, d, J=5.1 Hz, 7″-CH_3), 2.75 (1H, br, 3″-OH), 3.2—3.8 (1H, m, 3″-H), 4.77 (1H, q, J=5 Hz, 7″-H), 5.01 (1H, d, J=8 Hz, 1″-H), 5.17 (1H, t, J=8 Hz, 2″-H), 7.3—7.9 (10H, m, 2×C_6H_5). *Anal.* Calcd for $C_{44}H_{42}O_{16}$: C, 63.92; H, 5.12. Found: C, 63.80; H, 5.17.

3e: IR ν_{max}^{KBr} cm^{-1}: 3500 (br), 1770, 1600. UV λ_{max}^{MeOH} nm (ε): 225 (sh, 30890), 281 (4300). ^{1}H-NMR (80 MHz, $CDCl_3$) δ: 1.28 (3H, d, J=5 Hz, 7″-CH_3), 3.60 (1H, m, 2″-H), 4.68 (1H, q, J=5 Hz, 7″-H), 4.74 (1H, d, J=8 Hz, 1″-H), 5.35 (1H, t, J=9 Hz, 3″-H), 7.4—8.0 (10H, m, 2×C_6H_5). *Anal.* Calcd for $C_{44}H_{42}O_{16}\cdot H_2O$: C, 62.55; H, 5.25. Found: C, 62.73; H, 5.29.

3f: IR ν_{max}^{KBr} cm^{-1}: 1770, 1730, 1600. UV λ_{max}^{MeOH} nm (ε): 227 (sh, 40270), 282 (4550). ^{1}H-NMR (80 MHz, $CDCl_3$) δ: 1.31 (3H, d, J=5 Hz, 7″-CH_3), 4.50 (1H, q, 7″-H), 4.93 (1H, d, J=8 Hz, 1″-H), 5.32 (1H, t, J=8 Hz, 2″-H), 5.55 (1H, t, J=8 Hz, 3″-H), 7.3—7.9 (10H, m, 2×C_6H_5). *Anal.* Calcd for $C_{51}H_{46}O_{17}$: C, 65.80; H, 4.55. Found: C, 65.28; H, 4.98.

2″-*O*-Benzoyletoposide (4d) Compounds **3d** (54 mg, 0.07 mmol) was hydrogenated to give **4d** (40 mg, 88%) as colorless powder. mp 289—292 °C. $[\alpha]_D^{23}$ −89° (c=0.1, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3450, 1760, 1730. UV λ_{max}^{MeOH} nm (ε): 283 (4470). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.41 (3H, d, J=5.1 Hz, 7″-CH_3), 2.74 (1H, d, J=3.3 Hz, 3″-OH), 3.40 (1H, dt, J=4.8, 10.0 Hz, 5″-H), 3.47 (1H, t, J=9.2 Hz, 4″-H), 3.64 (1H, t, J=10.3 Hz, 6″-H_{ax}), 3.98 (1H, dt, J=3.3, 9.2 Hz, 3″-H), 4.23 (1H, dd, J=4.8, 10.3 Hz, 6″-H_{eq}), 4.80 (1H, q, J=5.1 Hz, 7″-H), 4.89 (1H, d, J=7.7 Hz, 1″-H), 5.07 (1H, dd, J=7.7, 9.2 Hz, 2″-H), 7.4—7.8 (5H, m, COC_6H_5). *Anal.* Calcd for $C_{36}H_{36}O_{14}\cdot H_2O$: C, 60.84; H, 5.11. Found: C, 61.10; H, 5.40.

3″-*O*-Benzoyletoposide (4e) Compound **3e** (34 mg, 0.04 mmol) was hydrogenated to give **4e** (27 mg, 95%) as a colorless solid. mp 188—191 °C. $[\alpha]_D^{23}$ −80° (c=0.1, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3400 (br), 1730. UV λ_{max}^{MeOH} nm (ε): 223 (35900), 280 (4030). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.31 (3H, d, J=5.1 Hz, 7″-CH_3), 2.58 (1H, br, 2″-OH), 3.47 (1H, m, 5″-H), 3.59 (1H, t, J=9.4 Hz, 4″-H), 3.61 (1H, t, J=10.0 Hz, 6″-H_{ax}), 3.67 (1H, br t, J=8.0 Hz, 2″-H), 4.22 (1H, dd, J=5.0, 10.0 Hz, 6″-H_{eq}), 4.72 (1H, q, J=5.1 Hz, 7″-H), 4.79 (1H, d, J=7.7 Hz, 1″-H), 5.38 (1H, t, J=9.2 Hz, 3″-H), 7.4—7.8 (5H, m, COPh). *Anal.* Calcd for $C_{36}H_{36}O_{14}\cdot 2H_2O$: C, 59.31; H, 4.98. Found: C, 59.14; H, 5.10.

2″,3″-Di-*O*-benzoyletoposide (4f) Compound **3f** (130 mg, 0.14 mmol) was hydrogenated to give **4f** (112 mg, *ca.* 100%) as colorless powder. mp >295 °C. $[\alpha]_D^{23}$ −24° (c=0.2, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 1770, 1730. UV λ_{max}^{MeOH} nm (ε): 226 (sh, 24200), 281 (3470). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.33 (3H, d, J=4.8 Hz, 7″-CH_3), 3.56 (1H, dt, J=9.5, 10.0 Hz, 5″-H), 3.67 (1H, t, J=10.0 Hz, 6″-H_{ax}), 3.69 (1H, t, J=9.5 Hz, 4″-H), 4.28 (1H, dd, J=4.4, 10.3 Hz, 6″-H_{eq}), 4.73 (1H, q, J=4.8 Hz, 7″-H), 4.98 (1H, d, J=8.1 Hz, 1″-H), 5.35 (1H, dd, J=8.1, 9.7 Hz, 2″-H), 5.67 (1H, t, J=9.5 Hz, 3″-H), 7.3—8.0 (10H, m, 2×COC_6H_5). *Anal.* Calcd for $C_{43}H_{40}O_{15}\cdot H_2O$: C, 63.39; H, 5.20. Found: C, 62.99; H, 4.99.

2″,3″-Di-*O*-formyletoposide (4g) To a solution of **2** (100 mg, 0.14 mmol) in pyridine (0.7 ml) was added dropwise a mixture of 99% formic acid (1.4 ml) and acetic anhydride (0.56 ml) at 0 °C. The reaction mixture was stirred at room temperature for 4 h, and was then diluted with dichloromethane and washed with water. The organic phase was dried over anhydr. Na_2SO_4, then filtered off. The filtrate was concentrated *in vacuo* to obtain a colorless solid (122 mg), which was purified by a silica gel column (MeOH : CH_2Cl_2 = 1 : 50) to give pure **3g** (91 mg, 84%) as amorphous powder. ^{1}H-NMR (80 MHz, $CDCl_3$) δ: 1.35 (3H, d, J=5 Hz, 7″-CH_3), 3.6—2.6 (6H, m), 3.66 (6H, s, 3′,5′-OCH_3), 5.23 (2H, s, $PhCH_2CO$), 5.4—4.0 (9H, m), 5.97 (2H, s, OCH_2O), 6.23 (2H, s, 2′-H and 6′-H), 6.53 (1H, s, 8-H), 6.72 (1H, s, 5-H), 7.36 (5H, s, $PhCH_2CO$), 8.04 and 7.80 (each 1H, s, CHO).

Hydrogenation of **3q** (71 mg, 0.09 mmol) gave **4g** (59 mg, *ca.* 100%) as colorless powder. mp 278—280 °C. $[\alpha]_D^{23}$ −81° (c=0.1, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3400, 1760, 1740, 1610. UV λ_{max}^{MeOH} nm (ε): 236 (sh, 14100), 285 (4230). ^{1}H-NMR (80 MHz, $CDCl_3$) δ: 1.35 (3H, d, J=5 Hz, 7″-CH_3), 3.7—2.6 (6H, m), 3.76 (6H, s, 3′,5′-OCH_3), 5.6—4.1 (6H, m), 5.98 (2H, s, OCH_2O), 6.23 (2H, s, 2′-H and 6′-H), 6.53 (1H, s, 8-H), 6.72 (1H, s, 5-H), 8.04 and 7.83 (each 1H, s, CHO). *Anal.* Calcd for $C_{31}H_{32}O_{15}$: C, 57.26; H, 5.00. Found: C, 57.09; H, 5.00.

Mesylation Products of 2 (3h—j) A mixture of **2** (1.00 g, 1.4 mmol) and mesyl chloride (270 μl, 2.8 mmol) in dry pyridine (50 ml) was stirred overnight at room temperature. After the usual treatment of the reaction mixture, the crude product containing three components was separated by silica gel column chromatography to give 440 mg (36%) of 4′-*O*-benzyloxycarbonyletoposide 2″,3″-dimesylate (**3j**), 314 mg (32%) of the 3″-mesylate (**3i**) and 248 mg (26%) of the 2″-mesylate (**3h**), respectively, as colorless powder.

3h: mp 159—161 °C, hygroscopic. *Anal.* Calcd for $C_{38}H_{40}O_{17}S\cdot 3H_2O$: C, 55.13; H, 5.11; S, 3.87. Found: C, 55.29; H, 5.00; S, 4.00.

3i: mp 167—170 °C. *Anal.* Calcd for $C_{38}H_{40}O_{17}S\cdot 1/2H_2O$: C, 56.36; H, 4.98; S, 3.96. Found: C, 56.23; H, 5.06; S, 4.14.

3j: mp 226—228 °C. *Anal.* Calcd for $C_{39}H_{42}O_{19}S\cdot 1/2H_2O$: C, 52.76; H, 4.77; S, 7.22. Found: C, 52.78; H, 4.77; S, 7.31.

Etoposide 2″- and/or 3″-Mesylates (4h—j) Hydrogenation of **3h** (30 mg, 0.04 mmol), **3i** (60 mg, 0.07 mmol) and **3j** (100 mg, 0.11 mmol) gave the 2″-mesylate (**4h**, 23 mg, 94%), the 3″-mesylate (**4i**, 49 mg, 98%) and the 2″,3″-dimesylate (**4j**, 76 mg, 93%), respectively.

4h: mp 179—181 °C. $[\alpha]_D^{23}$ −69° (c=0.1, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3500 (br), 1770, 1620. UV λ_{max}^{MeOH} nm (ε): 240 (sh, 12200), 285 (3950). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.39 (3H, d, J=5.1 Hz, 7″-CH_3), 2.84 (3H, s, SO_3CH_3), 3.3 (1H, m, 5″-H), 3.35 (1H, t, J=9.0 Hz, 4″-H), 3.60 (1H, t, J=10.0 Hz, 6″-H_{ax}), 3.86 (1H, t, J=9.0 Hz, 3″-H), 4.22 (1H, dd, J=5.0, 10.0 Hz, 6″-H_{eq}), 4.41 (1H, dd, J=7.7, 9.2 Hz, 2″-H), 4.69 (1H, d, J=7.7 Hz,

1″-H), 4.75 (1H, q, J=5.1 Hz, 7″-H). Fast atom bombardment mass spectrum (FAB-MS) m/z: 666 $(M)^+$,

4i: mp 226—229 °C. $[\alpha]_D^{23}$ −77° (c=0.1, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3500 (br), 1770, 1610. UV λ_{max}^{MeOH} nm (ε): 740 (sh, 12900), 286 (3950). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.38 (3H, d, J=4.8 Hz, 7″-CH_3), 2.77 (1H, d, J=3.3 Hz, 2″-OH), 3.13 (3H, s, SO_3CH_3), 3.36 (1H, m, 5″-H), 3.49 (1H, t, J=9.2 Hz, 4″-H), 3.59 (1H, t, J=10.3, 6″-H_{ax}), 3.61 (1H, m, 2″-H), 4.21 (1H, dd, J=4.8, 10.4, 10.4 Hz, 6″-H_{eq}), 4.65 (1H, t, J=9.2 Hz, 3″-H), 4.70 (1H, d, J=7.7 Hz, 1″-H), 4.74 (1H, q, J=5.1 Hz, 7″-H). FAB-MS m/z: 666 $(M)^+$.

4j: mp 206—209 °C. $[\alpha]_D^{23}$ −84° (c=0.1, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3400 (br), 1760, 1620. UV λ_{max}^{MeOH} nm (ε): 240 (sh, 13300), 285 (4010). ^{1}H-NMR (60 MHz, $CDCl_3$) δ: 1.33 (3H, d, J=5 Hz, 7″-CH_3), 2.93 and 3.06 (each 3H, s, SO_3CH_3), 3.3—3.7 (3H, m, 4″-H, 5″-H and 6″-H_{ax}), 4.1—4.6 (3H, m, 2″-H, 3″-H and 6″-H_{eq}), 4.64 (1H, d, J=8 Hz, 1″-H) 4.73 (1H, q, J=5 Hz, 7″-H). FAB-MS m/z: 744 $(M)^+$.

Etoposide 2″- and/or 3″-Tosylates (4k—m) A mixture of **2** (2.2 g, 3 mmol), 4-dimethylamino pyridine (200 mg) and tosyl chloride (840 mg, 4.6 mmol) in dry pyridine (50 ml) was stirred for 3d at room temperature. After a conventinal treatment of the reaction mixture, the crude solid (2.35 g) containing three components was separated by silica gel column chromatography (2% MeOH–CH_2Cl_2) to give 602 mg (19%) of 4′-*O*-benzyloxycarbonyletoposide 2″,3″-di-*O*-tosylate (**3m**), 690 mg (26%) of the 3″-tosylate (**3l**) and 156 mg (6%) of the 2″-tosylate (**3k**) as hygroscopic colorless powder, respectively.

Hydrogenation of **3k** (100 mg, 0.09 mmol), **3l** (47 mg, 0.05 mmol) and **3m** (80 mg, 0.08 mmol) gave the 2″-tosylate (**4k**, 90 mg, *ca.* 100%), the 3″-tosylate (**4l**, 42 mg, *ca.* 100%) and the 2″,3″-ditosylate (**4m**, 72 mg, *ca.* 100%), respectively.

4k: mp 210—212 °C. IR ν_{max}^{KBr} cm^{-1}: 3500 (br), 1780, 1600. UV λ_{max}^{MeOH} nm (ε): 243 (sh, 4560), 282 (1450). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.24 (3H, d, J=5.1 Hz, 7″-CH_3), 2.38 (3H, s, SO_2PhCH_3), 3.29 (1H, t, J=9.0 Hz, 4″-H), 3.4 (1H, m, 5″-H), 3.52 (1H, t, J=10.0 Hz, 6″-H_{ax}), 3.67 (1H, t, J=9.0 Hz, 3″-H), 4.09 (1H, dd, J=4.7, 10.0 Hz, 6″-H_{eq}), 4.15 (1H, t, J=9.0 Hz, 2″-H), 4.74 (1H, q, J=5.1 Hz, 7″-H), 4.96 (1H, d, J=8.1 Hz, 1″-H). FAB-MS m/z: 742 (M^+).

4l: mp 175—178 °C. $[\alpha]_D^{23}$ −90° (c=0.1, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3500 (br), 1780, 1600. UV λ_{max}^{MeOH} nm (ε): 245 (sh, 10260), 284 (3900). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 0.96 (3H, d, J=4.7 Hz, 7″-CH_3), 2.38 (3H, s, SO_2PhCH_3), 3.29 (1H, dd, J=7.7, 9.0 Hz, 2″-H), 3.39 (1H, dd, J=4.7, 9.4 Hz, 5″-H), 3.43 (1H, t, J=9.0 Hz, 4″-H), 3.48 (1H, t, J=9.8 Hz, 6″-H_{ax}), 4.08 (1H, dd, J=4.7, 10.0 Hz, 6″-H_{eq}), 4.55 (1H, q, J=4.7 Hz, 7″-CH_3), 4.62 (1H, t, J=9.0 Hz, 3″-H), 4.70 (1H, d, J=7.7 Hz, 1″-H). FAB-MS m/z: 742 $(M)^+$.

4m: mp 169—171 °C. $[\alpha]_D^{23}$ −93° (c=0.1, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3400 (br), 1770, 1600. UV λ_{max}^{MeOH} nm (ε): 245 (sh, 11750), 284 (3840). ^{1}H-NMR (60 MHz, $CDCl_3$) δ: 0.85 (3H, d, J=5 Hz, 7″-CH_3), 2.42 (6H, s, 2×Ts), 3.3—3.6 (3H, 4″-H, 5″-H and 6″-H_{ax}), 4.1—4.8 (5H, m). FAB-MS m/z: 896 $(M)^+$.

Phosphorylation Products of 2 (3n—p) To a cooled (0 °C) solution of **2** (1.511 g, 2.1 mmol) in dry methylene chloride (10 ml) and dry pyridine (10 ml) was slowly added phosphorus oxychloride (280 μl, 3 mmol) and the mixture was stirred at 0 °C for 45 min. Then benzyl alcohol (620 μl, 6 mmol) was added in one portion to the reaction mixture at 0 °C. After stirring at room temperature for 2 h, the mixture was diluted with methylene chloride (50 ml), washed with water, and dried over anhydr. Na_2SO_4. The organic solvent was evaporated *in vacuo* to give 2.40 g of a crude semi-solid containing three components (silica gel TLC: *Rf* 0.6, 0.54 and 0.46), which was separated by silica gel column chromatography (1% MeOH–CH_2Cl_2). Fractions showing a spot of *Rf* 0.66 were combined and evaporated *in vacuo* to give 435 mg (21%) of **3o** (3″-phosphate). Similarly, fractions of *Rf* 0.54 and 0.46 afforded **3p** (352 mg, 21%, dimer) and **3n** (262 mg, 13%, 2″-phosphate), respectively.

3n: mp 107—110 °C. IR ν_{max}^{Nujol} cm^{-1}: 3300 (br), 1760, 1600. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.41 (3H, d, J=5.1 Hz, 7″-CH_3), 3.3 (1H, m, 5″-H), 3.36 (1H, t, J=9.2 Hz, 4″-H), 3.57 (1H, t, J=10.3 Hz, 6″-H_{ax}), 3.87 (1H, dt, J=2.3, 9.2 Hz, 3″-H), 4.18 (1H, dd, J=5.0, 10.3 Hz, 6″-H_{eq}), 4.18 (1H, t, J=8.1 Hz, 2″-H), 4.46 (d, J=2.3 Hz, 3″-OH), 4.69 (1H, d, J=7.7 Hz, 1″-H), 4.76 (1H, q, J=5.1 Hz, 7″-H). FAB-MS m/z: 983 $(M+H)^+$. *Anal.* Calcd for $C_{51}H_{51}O_{18}P \cdot 3H_2O$: C, 59.07; H, 4.96. Found: C, 59.21; H, 5.02.

3o: mp 115—117 °C. IR ν_{max}^{Nujol} nm^{-1}: 3300 (br), 1760, 1600. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.29 (3H, d, J=5.1 Hz, 7″-CH_3), 3.32 (1H, m, 5″-H), 3.45 (1H, t, J=9.2 Hz, 4″-H), 3.57 (1H, t, J=10.3 Hz, 6″-H_{ax}), 3.60 (1H, dt, J=1.8, 7.7 Hz, 2″-H), 4.02 (d, J=1.8 Hz, 2″-OH), 4.19 (1H, dd, J=5.1, 10.3 Hz, 6″-H_{eq}), 4.42 (1H, t, J=9.2 Hz, 3″-H), 4.63 (1H, d, J=7.7 Hz, 1″-H), 4.71 (1H, q, J=5.1 Hz, 7″-H). FAB-MS m/z: 983 $(M+H)^+$. *Anal.* Calcd for $C_{51}H_{51}O_{18}P$: C, 62.32; H, 5.23. Found: C, 61.81; H, 5.17.

3p: mp 175—178 °C. IR ν_{max}^{Nujol} cm^{-1}: 3350 (br), 1760, 1600. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.19 and 1.26 (6H, each d, J=5.1 Hz, 2×7″-CH_3), 3.3 (2H, m, 2×5″-H), 3.42 and 3.45 (2H, each t, J=9.5 Hz, 2×4″-H), 3.5 (2H, m, 2×2″-H), 3.55 (2H, t, J=10.3 Hz, 2×6″-H_{ax}), 4.18 (2H, m, 2×6″-H_{eq}), 4.52 (2H, m, 2×3″-H), 4.60 and 4.62 (2H, each d, J=7.3 Hz, 2×1″-H), 4.65 and 4.68 (2H, each q, J=5.1 Hz, 2×7″-H). FAB-MS m/z: 1596 $(M+H)^+$. *Anal.* Calcd for $C_{81}H_{81}O_{32}P \cdot 3H_2O$: C, 60.02; H, 5.18. Found: C, 60.28; H, 5.07.

3″-*O*-Disodiophosphoryletoposide (4o) A solution of **3o** (270 mg) in MeOH (20 ml) and AcOEt (20 ml) containing 10% palladium on carbon (50 mg) was hydrogenated for 1 h. The catalyst was removed by filtration and the filtrate was concentrated *in vacuo* to give 188 mg of a semi-solid, which was triturated with AcOEt to give 145 mg (79%) of light brown powder. A 135-mg sample of the above product was dissolved in aq. $NaHCO_3$ (33.6 mg in 2 ml H_2O) and lyophilized to give 140 mg of **4o** as gray powder. mp 262—263 °C (dec.). $[\alpha]_D^{23}$ −79° (c=0.1, H_2O). IR ν_{max}^{KBr} cm^{-1}: 3377 (br), 1762, 1615. UV λ_{max}^{MeOH} nm (ε): 238 (sh, 13000), 285 (4010). ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 1.21 (3H, d, J=4.8 Hz, 7″-CH_3), 3.1—3.3 (3H, m, 2″-H, 4″-H and 5″-H), 3.47 (1H, br t, J=10.0 Hz, 6″-H_{ax}), 4.05 (1H, m, 6″-H_{eq}), 4.3 (1H, m, 3″-H), 4.54 (1H, d, J=7.7 Hz, 1″-H), 4.69 (1H, q, J=5.2 Hz, 7″-H). FAB-MS m/z: 713 $(M+H)^+$.

2″-*O*-Disodiophosphoryletoposide (4n) According to the above procedure, 97 mg of **3n** was hydrogenated to give 70 mg (*ca.* 100%) of **4n** as gray powder. mp 225—257 °C (dec.). $[\alpha]_D^{23}$ −62° (c=0.1, H_2O). IR ν_{max}^{KBr} cm^{-1}: 3460 (br), 1772, 1615. UV λ_{max}^{MeOH} nm (ε): 240 (sh, 12600), 284 (3740). ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 1.20 (3H, d, J=4.8 Hz, 7″-CH_3), 3.1—3.3 (2H, m, 4″-H and 5″-H), 3.5 (2H, m, 3″-H and 6″-H_{ax}), 4.06 (2H, m, 2″-H and 6″-H_{eq}), 4.53 (1H, d, J=7.5 Hz, 1″-H), 4.64 (1H, q, J=4.8 Hz, 7″-H). FAB-MS m/z: 713 $(M+H)^+$.

Sodium Bis(etoposidyl-3″-*O*-)phosphate (4p) A 135 mg sample of **4** was hydrogenated to give 60 mg (58%) of **4p** as light gray powder. mp 265—266 °C (dec.). $[\alpha]_D^{23}$ −59° (c=0.1, H_2O). IR ν_{max}^{KBr} nm^{-1}: 3460 (br), 1772, 1620. UV λ_{max}^{MeOH} nm (ε): 240 (sh, 24600), 285 (7730). ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 1.27 (6H, d, J=5.2 Hz, 2×7″-CH_3), 3.1—3.3 (6H, m, 2×(2″-H, 4″-H and 5″-H)), 3.66 (2H, m, 2×6″-H_{ax}), 4.06 (2H, m, 2×6″-H_{eq}), 4.47 (2H, d, J=7.5 Hz, 2×1″-H), 4.74 (1H, m, 7″-H). FAB-MS m/z: 1262 $(M+H)^+$.

Sodium 2″- and 3″-Etoposide Sulfates (4q and 4r) To a solution of **2** (500 mg, 0.69 mmol) in dry pyridine (20 ml) under argon was added sulfur trioxide–pyridine complex (330 mg, 2.07 mmol). After stirring at room temperature for 3 h, an additional sulfur trioxide–pyridine complex (250 mg, 1.59 mmol) was added and stirred at room temperature for 15 h. The mixture was concentrated *in vacuo* below 40 °C to give a crude oil (1.2 g), which was chromatographed on a silica gel column (2% MeOH–CH_2Cl_2) to afford a mixture of the 2″- and 3″-sulfate (411 mg). A stirred solution of the mixture (400 mg, 0.49 mmol) in EtOH–acetone (4 : 1, 15 ml) was hydrogenated with 10% palladium on carbon (400 mg). The catalyst was removed by filtration and the filtrate was concentrated *in vacuo* to give 370 mg of a mixture of the regioisomers, which was separated by C_{18}-reverse phase column chromatography to afford 52 mg (16%) of the 3″-sulfate (**4r**), 107 mg (32%) of the 2″-sulfate (**4q**) and 138 mg of a mixture of **4r** and **4q**. The 3″-sulfate was passed through a column of Dowex 50W (Na^+) resin with water as eluant and the water-soluble fractions were lyophilized to give the corresponding sodium salt of **4r** as colorless powder. Similarly, the sodium salt of **4q** was obtained as colorless powder.

4r: mp 223—225 °C. $[\alpha]_D^{23}$ −68° (c=0.1, H_2O). IR ν_{max}^{KBr} cm^{-1}: 3440, 1770, 1620. UV λ_{max}^{MeOH} nm (ε): 240 (sh, 11600), 284 (3670). ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 1.23 (1H, d, J=5.1 Hz, 7″-CH_3), 3.29 (1H, t, J=9.5 Hz, 4″-H), 3.30 (1H, t, J=9.0 Hz, 2″-H), 3.36 (1H, dd, J=4.8, 9.9 Hz, 5″-H), 3.49 (1H, t, J=9.9 Hz, 6″-H_{ax}), 4.05 (1H, dd, J=4.8, 9.9 Hz, 6″-H_{eq}), 4.20 (1H, t, J=9.5 Hz, 3″-H), 4.71 (1H, q, J=5.1 Hz, 7″-H), 4.82 (1H, d, J=7.5 Hz, 1″-H). FAB-MS m/z: 691 $(M+H)^+$.

4q: mp 246—247 °C. $[\alpha]_D^{23}$ −77° (c=0.1, H_2O). IR ν_{max}^{KBr} cm^{-1}: 3450, 1770, 1620. UV λ_{max}^{MeOH} nm (ε): 240 (sh, 11200), 285 (3650). ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 1.22 (1H, d, J=5.1 Hz, 7″-CH_3), 3.24 (1H, m, 5″-H), 3.28 (1H, t, J=9.0 Hz, 4″-H), 3.54 (1H, t, J=10.0 Hz, 6″-H_{ax}), 3.6 (1H, m, 3″-H), 3.85 (1H, t, J=8.0 Hz, 2″-H), 4.11 (1H, dd, J=4.5, 10.0 Hz, 6″-H_{eq}), 4.52 (1H, d, J=7.7 Hz, 1″-H), 4.74 (1H, q, J=5.1 Hz, 7″-H). FAB-MS m/z: 691 $(M+H)^+$.

***In Vitro* Cytotoxicity** For the *in vitro* cytotoxicity experiment, murine

melanoma B16-F10 cells were grown and maintained in Eagle's minimum essential medium (Nissui) containing kanamycin (60 μg/ml), which was supplemented with heat-inactivated fetal calf serum (10%), L-glutamine (292 μg/ml) and 10 mM MEM non-essential amino acids (0.6%), at 37°C under humidified atmosphere of 5% CO_2 and 95% air. Exponentially growing B16-F10 cells were harvested, counted and suspended in the culture medium at a concentration of 3.0×10^4 cells/ml. The cell suspension (180 μl) was planted into wells of a 96-well microtiter plate and incubated for 24 h. Test compounds (20 μl) were added to the wells and the plates were further incubated for 72 h. The cytotoxic activity was colorimetrically determined at 540 nm after staining viable cells with a neutral red solution.

***In Vivo* Antitumor Activity** *In vivo* antitumor activity was tested in the lymphocytic leukemia P388 system. Female CDF_1 mice were inoculated intraperitoneally with 0.4 ml of diluted ascitic fluid containing 10^6 P388 cells per mouse (day 0). Test compounds were intraperitoneally administered to the mice on day 1 (Q1D × 1) and animals were observed for 50d. The percent increase of median survival time (MST) of treated animals (T) over that of untreated control animals (C) was determined and reported as T/C%. Compounds showing T/C% values of 125 or greater are considered to have significant antitumor activity.

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References and Notes

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11) We have attempted another method of monoformylation with acetic-formic anhydride using the 2″- or 3″-*tert*-butyldimethylsilyl ethers of **2**. After desilylation of the monoformyl derivatives, both 2″- and 3″-monoformates obtained were so unstable that they could not be isolated as pure forms.