Chemical Modification of an Antitumor Alkaloid, 20(*S*)-Camptothecin: Glycosides, Phosphates and Sulfates of 7-Ethyl-10-hydroxycamptothecin[1)]

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Water-soluble derivatives having the lactone ring intact were synthesized starting from 7-ethyl-10-hydroxycamptothecin (1). Glycosides (2) of the phenolic hydroxyl group of 1 were obtained by reaction with acetylated α-bromosugars in acetone or aqueous acetone in the presence of potassium carbonate, followed by deprotection. Phosphates (3) were prepared by reaction of 1 with phosphoryl chloride in pyridine or with dibenzylchlorophosphoridate. Sulfates (4) were obtained by reaction of 1 with sulfur trioxide–pyridine complex in the presence of a tertiary amine. The organic ammonium salts of monophosphate (3p) and sulfates (4a and 4b) showed significant activity against L1210 *in vivo*.

Keywords 20(*S*)-camptothecin; 7-ethyl-10-hydroxycamptothecin; water-soluble derivative; glycoside; phosphate; sulfate; L1210

20(*S*)-Camptothecin is an antitumor alkaloid which was first isolated from *Camptotheca acuminata* (Nyssaceae) by Wall and co-workers in 1966.[2a)] This alkaloid showed significant inhibitory activity toward leukemia L1210 in mice and Walker 256 sarcoma in rats. A clinical trial was carried out on its water-soluble sodium salt in which the lactone ring was cleaved by sodium hydroxide, but severe toxicity of the salt to the bone marrow and bladder ruled out its usefulness in cancer therapy.[2b,3)]

We are conducting a study on chemical modification of 20(*S*)-camptothecin as a starting material to obtain less toxic derivatives. We earlier reported the synthesis and antitumor activities of derivatives in which the quinoline moiety was modified.[1a)] A program was initiated to synthesize water-soluble derivatives with the lactone ring intact, a structure said to be essential for the activity.[3)] We selected 7-ethyl-10-hydroxycamptothecin (**1**) as the starting material, because **1** showed significant cytotoxicity against KB cells and leukemia L1210 cells *in vitro* and a high therapeutic index, (*i.e.* (maximum tolerance dose/minimum effective dose)) toward leukemia L1210 in mice, and it is also a potent inhibitor of deoxyribonucleic acid (DNA) topoisomerase I.[4)] Irinotecan hydrochloride (CPT-**11**) is one of the derivatives synthesized on this line, in which the phenolic hydroxyl group of **1** is linked to piperidino-piperidine through a carbamate bond. This was suggested to be effective for lung, uterine and ovarian cancers and phase II clinical trials are now being conducted on other cancers.[1b,5)]

In this report we wish to describe the synthesis and antitumor activities of water-soluble derivatives, glycosides, phosphates and sulfates of **1**.

Chemistry Glycosides (**2**) were obtained by reaction of **1** with acetylated α-bromosugars[6)] in acetone or aqueous acetone in the presence of potassium carbonate, followed by deprotection. In the condensation process with protected sugars, glucoside (**2a**) and maltoside (**2b**) were obtained in modest yield, but the yields were very low in the cases of glucuronide (**2c**) and 2-deoxy-2-aminoglucoside (**2d**). Because α-bromosugars were employed for the glycosidation of **1**, all the derivatives obtained here have β-configurations, and their structures were confirmed from the coupling constants of the anomeric protons (about 7 Hz).

The glycosides did not show sufficient water-solubility for the antitumor test (**2e**, 0.58 mg/ml; **2f**, 1.54 mg/ml at 25 °C), whereas glucuronide (**2g**) dissolved in water give an acidic solution.

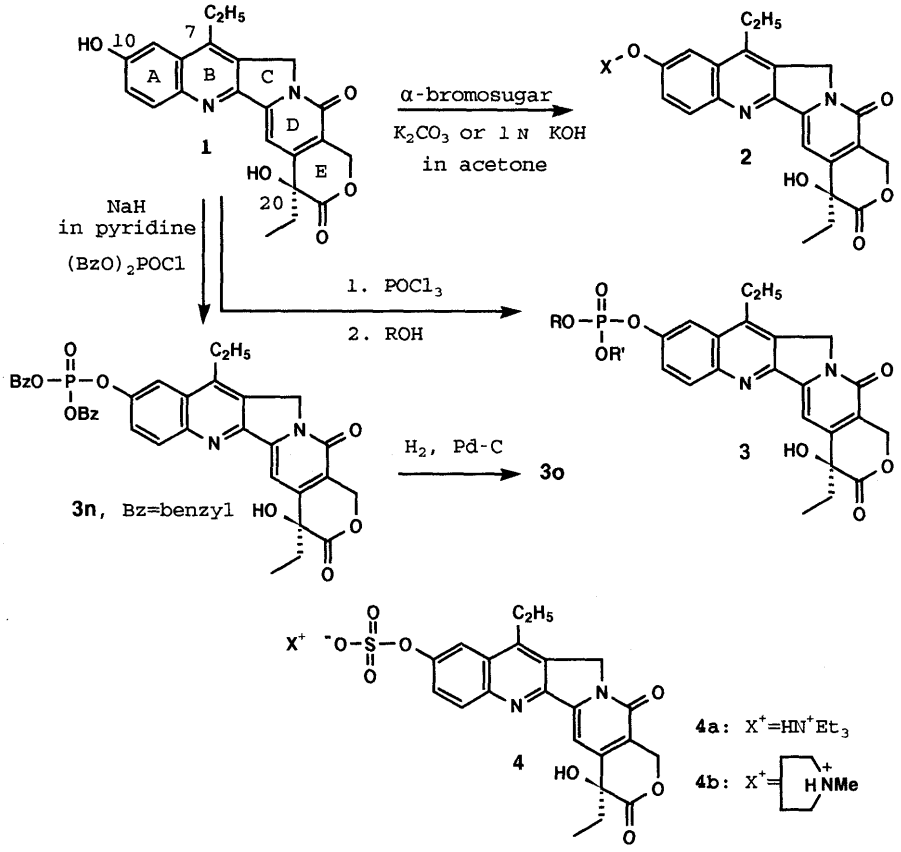
Phosphates (**3**) were prepared by reaction of **1** with phosphoryl chloride in pyridine. The dichlorophosphoridate of **1** was trapped with an alcohol or phenol to yield di- and triesters, concomitantly. The diesters were purified through reverse-phase column chromatography (ODS: octadecylsilanized silica gel column, 60—200 mesh, with aqueous methanol). Reaction of **1** with dibenzylchlorophosphoridate[7)] gave a dibenzyl triester (**3n**), which was purified through silica gel column chromatography and then debenzylated by catalytic hydrogenation to yield the monoester (**3o**), and its triethyl ammonium salt (**3p**) was isolated. The diesters (except stearyl ester **3g**) gave clear aqueous solutions as their sodium salts. Table I shows the substituents and ^{31}P-nuclear magnetic resonance (^{31}P-NMR) chemical shifts of **3**.

Sulfates (**4**) were synthesized by reaction of **1** with sulfur trioxide–pyridine complex[8)] in the presence of a tertiary amine (triethylamine or *N*-methylpyrrolidine). The

TABLE I. Substituents and ^{31}P-NMR Chemical Shifts (δ) of Phosphate, **3**

Compd. No.	R	R′	(δ ppm)[a)]
3a	CH_3	H	−4.24
3b	C_2H_5	H	−4.69
3c	C_2H_5	C_2H_5	−5.10
3d	C_3H_7	H	−5.46
3e	iso-C_3H_7	H	−6.10
3f	C_4H_9	H	−5.51
3g	$C_{18}H_{37}$	H	−6.37
3h	Cyclohexyl	H	−6.02
3i	Cyclohexylmethyl	H	−5.34
3j	$C_6H_5CH_2$	H	−4.61
3k	$C_6H_5CH_2CH_2$	H	−5.77
3l	$CH_2CH=CH_2$	H	−5.11
3m	C_6H_5	H	−10.57
3n	$C_6H_5CH_2$	$C_6H_5CH_2$	−5.19
3o	H	H	−4.96

a) 85% H_3PO_4 was used as the external standard.



2a: X=β-D-glucosyl, tetraacetate
2b: X=β-D-maltosyl, heptaacetate
2c: X=β-D-glucuronyl, triacetate, Me ester
2d: X=β-D-2-deoxy-2-trifluoro-acetaminoglucosyl, triacetate
2e: X=β-D-glucosyl
2f: X=β-D-maltosyl
2g: X=β-D-glucuronyl
2h: X=β-D-2-deoxy-2-aminoglucosyl-

3a: R=Me, R'=H
3b: R=Et, R'=H
3c: R=Et, R'=Et
3d: R=Pr, R'=H
3e: R=iso-Pr, R'=H
3f: R=Bu, R'=H
3g: R=$C_{18}H_{37}$, R'=H
3h: R=cyclohexyl, R'=H
3i: R=cyclohexylmethyl, R'=H
3j: R=Bz, R'=H
3k: R=$C_6H_5CH_2CH_2$, R'=H
3l: R=$CH_2CH{=}CH_2$, R'=H
3m: R=Ph, R'=H
3n: R=Bz, R'=Bz
3o: R=H, R'=H
3p: R=Et_3NH^+, R'=H

Chart 1

TABLE II. Antitumor Activity (L1210 in Mice) of Camptothecin Derivatives, **2**, **3** and **4**

Compd. No.	*T/C* %[a] (Cured mice)								
	Total dose (mg/kg)								
	3.12	6.25	12.5	25	50	100	150	200	400
1[b]	120[c]	128[d]	138[e]	158	158	169	180	111	—
2a[f]	—[g]	—	—	124	162	189	—	405 (2/6)[h]	391 (2/6)
2e[f]	—	—	114	144	179	348 (2/6)	—	236	—
2f[f]	—	—	—	119	119	132	—	144	204
2g	—	—	100	110	114	—	—	—	—
3a	100	—	110	—	113	—	—	117	—
3b	108	—	113	—	117	—	—	128	—
3c[f]	129	—	182	—	393 (3/6)	—	—	102	—
3d	—	—	—	122	136	138	—	138	—
3e	100	—	110	—	119	—	—	123	—
3f	111	—	122	—	133	—	—	138	—
3g[f]	116	—	140	—	233	—	—	502 (5/6)	—
3h	107	112	119	123	132	146	—	151	—
3i	—	—	—	131	144	149	—	157	—
3j	—	113	—	129	—	138	—	—	—
3k	—	—	—	126	131	144	—	144	—
3l	114	114	119	123	132	132	—	151	—
3m	110	112	116	123	135	144	—	160	—
3o	109	116	116	127	136	171	—	184	53
3p	—	—	—	120	160	193	—	442 (3/6)	—
4a	109	118	142	169	191	202	—	280 (1/6)	—
4b	118	120	158	184	216	253	—	380 (3/6)	—

a) $T/C\% = (\text{mean survival time of tested mice})/(\text{mean survival time of control mice}) \times 100$. *b*) Administered as sodium salt. *c*) 2.5 mg/kg. *d*) 5 mg/kg. *e*) 10 mg/kg. *f*) Administered in suspension. *g*) Not tested. *h*) Number of cured mice/number of mice tested.

products were isolated as organic ammonium salts through ODS column chromatography. They dissolved in water to give clear neutral aqueous solutions.

The nuclear magnetic resonance (NMR) spectra of the synthesized derivatives herein are summarized in Table III in Experimental.

Biological Results and Discussion

Antitumor activities of the derivatives against leukemia L1210 in mice are shown in Table II, compared with the activity of the lactone-ring-cleaved sodium salt of **1**.

Glucosides (**2a** and **2e**), triphosphate (**3c**) and stearyl diester (**3g**) exhibited noticeable activities, but these derivatives were administered as suspensions. Glucuronide (**2g**) which was identified as a metabolite of CPT-**11** in rats[9] showed no activity. Diesters (**3a**, **3b**, **3d**—**f**, **3h**—**m**) dissolved in water as their sodium salts were weaker than that of the sodium salt of **1**. The sodium salt of monophosphate **3o** showed activity equivalent to that of **1** and was higher than the diesters, while organic ammonium salts **3p**, **4a** and **4b** showed significant activity.

Compounds **2**, **3** and **4** are suggested to be hydrolyzed enzymatically to yield **1**.[10] Lipophilic salts, **3p**, **4a** and **4b**, seem to persist for a longer period, and a higher concentration of **1** is maintained than by administration of the sodium salt of **1**. That gives significant activity to these lipophilic derivatives since **1** is said to exert its activity restricted-time-dependently.[11]

Experimental

Melting points (with decomposition) were uncorrected. ^{1}H- and ^{31}P-NMR spectra were determined with a JEOL GX-400 (400 MHz) spectrometer with tetramethylsilane as the internal standard (for ^{1}H) and 85% phosphoric acid as the external standard (for ^{31}P). Infrared (IR) spectra were recorded on a Shimadzu IR 435 spectrophotometer, while mass spectra (MS) were measured with a Hitachi M-80B Mass Spectrometer.

Glycoside (2, Method A) A mixture of **1** (1.57 g, 4.0 mmol), bromosugar (15.0 mmol) and K_2CO_3 (2.35 g, 17.0 mmol) in acetone (300 ml) was refluxed with vigorous stirring for 6 h. The mixture was filtered by suction, and the filtered material was washed with $CHCl_3$ (200 ml × 3). The filtrate and the washings were combined and evaporated. The residue was purified through silica gel column chromatography with 3% MeOH–$CHCl_3$. Compounds **2a**, **2b** and **2c** were obtained by this method.

2a: Yield 24.6%. Colorless needles, mp 191—192 °C ($CHCl_3$–*n*-C_6H_{14}). IR (KBr) *v*: 1745, 1655, 1600, 1220, 1030 cm^{-1}. SI-MS *m/z*: 723 $(M+H)^+$. *Anal.* Calcd for $C_{36}H_{38}N_2O_{14}\cdot H_2O$: C, 58.38; H, 5.44; N, 3.78. Found: C, 58.53; H, 5.27; N, 3.45.

2b: Yield 27.0%. Yellow powder, mp 157—161 °C (EtOH). IR (KBr) *v*: 1740, 1650, 1600, 1225, 1025 cm^{-1}. SI-MS *m/z*: 1011 $(M+H)^+$. *Anal.* Calcd for $C_{48}H_{54}N_2O_{22}\cdot 2H_2O$: C, 55.07; H, 5.58; N, 2.68. Found: C, 55.07; H, 5.42; N, 2.86.

2c: Yield 3.4%. Colorless needles, mp 179—182 °C (EtOH). IR (KBr) *v*: 1740, 1645, 1590, 1205, 1025 cm^{-1}. SI-MS *m/z*: 709 $(M+H)^+$. *Anal.* Calcd for $C_{35}H_{36}N_2O_{14}\cdot 3/2H_2O$: C, 57.14; H, 5.34; N, 3.81. Found: C, 56.94; H, 5.08; N, 3.75.

2d (Method B) To a solution of **1** (1.00 g, 2.55 mmol) in 0.1 N KOH (64 ml), a solution of the bromosugar (5.10 mmol) in acetone (30 ml) was added and the mixture was stirred at ambient temperature for 3 h. The precipitate was filtered and the filtrate was evaporated *in vacuo*. The residue was purified in the same manner as described above.

2d: Yield 2.1%. Pale yellow powder, mp 148—151 °C ($CHCl_3$–*n*-C_6H_{14}). IR (KBr) *v*: 1745, 1645, 1595, 1225, 1030 cm^{-1}. SI-MS *m/z*: 776 $(M+H)^+$.

Deprotection of 2a and 2b To a suspension of an acetylated glycoside (1.00 mmol) in absolute MeOH (100 ml), 28% NaOMe solution (0.5 ml) was added dropwise while stirring. The stirring was continued at ambient temperature for 30 min and then water (2 ml) was added. The mixture was neutralized with ion exchange resin (H^+), and the resin was filtered through filter paper. The filtrate was evaporated *in vacuo*, and the residue was recrystallized from an appropriate solvent.

2e: Yield 89.1%. Pale yellow needles, mp 232—233 °C (MeOH). IR (KBr) *v*: 3370, 1715, 1645, 1595 cm^{-1}. SI-MS *m/z*: 555 $(M+H)^+$. *Anal.* Calcd for $C_{28}H_{30}N_2O_{16}\cdot H_2O$: C, 58.74; H, 5.63; N, 4.89. Found: C, 58.36; H, 5.31; N, 4.81.

2f: Yield 94.7%. Colorless needles, mp 220—221 °C (EtOH). IR (KBr) *v*: 3380, 1730, 1650, 1580 cm^{-1}. SI-MS *m/z*: 717 $(M+H)^+$.

Deprotection of 2c To a suspension of **2c** (440 mg, 0.62 mmol) in acetone (30 ml), 0.2 N NaOH (18.7 ml) was added dropwise, and the mixture was stirred at ambient temperature for 2 h. The mixture was acidified with 1 N HCl to about pH 5 and then evaporated *in vacuo*. The residue was passed through an ODS chromatographic column with H_2O–MeOH (7 : 3). **2g**: yield 59.2%. Pale yellow powder, mp 226—229 °C (EtOH–Et_2O). IR (KBr) *v*: 3385, 1735, 1655, 1620, 1600 cm^{-1}. SI-MS *m/z*: 569 $(M+H)^+$. *Anal.* Calcd for $C_{28}H_{28}N_2O_{11}\cdot 7/2H_2O$: C, 53.25; H, 5.59; N, 4.44. Found: C, 53.48; H, 5.41; N, 4.77.

Deprotection of 2d To a suspension of **2d** (50 mg, 0.06 mmol) in MeOH (5 ml), 0.1 N NaOH (3.9 ml) was added dropwise and the mixture was stirred at ambient temperature for 2.5 h. The mixture was acidified with 0.1 N HCl (6 ml) and then evaporated *in vacuo*. The residue was purified as described above. **2h**: yield 61.8%. Pale yellow powder, mp 223—226 °C (EtOH–*n*-C_6H_{14}). IR (KBr) *v*: 3365, 1730, 1650, 1620, 1585 cm^{-1}. SI-MS *m/z*: 554 $(M+H)^+$. *Anal.* Calcd for $C_{28}H_{32}ClN_3O_9\cdot 3/2H_2O$: C, 54.50; H, 5.72; N, 6.81. Found: C, 54.82; H, 5.85; N, 6.51.

Phosphate (3, General Procedure) To a solution of **1** (400 mg, 1.02 mmol) in pyridine (64 ml), $POCl_3$ (0.38 ml, 4.08 mmol) was added dropwise while held in an ice bath with stirring. The stirring was continued at ambient temperature for 1.5 h and an alcohol or phenol (12.24 mmol) was added. The mixture was stirred for an additional 1.5 h and water (0.4 ml) was added. The resulting mixture was stirred at ambient temperature for 30 min and then evaporated to dryness. The residue was purified through an ODS chromatographic column with H_2O–MeOH (7 : 3) to give a diester.

Triester (**3c**) was purified as followed. The ODS column separated diester (**3b**) was further eluted with MeOH, and the eluate was collected and evaporated to dryness under reduced pressure. The residue was purified through silica gel column chromatography with a 3% MeOH–$CHCl_3$.

3a: Yield 52.0%. Yellow powder, mp 196—197 °C (EtOH–*n*-C_6H_{14}). IR (KBr) *v*: 1740, 1660, 1600, 1230, 1085 cm^{-1}. SI-MS *m/z*: 487 $(M+H)^+$.

3b: Yield 21.6%. Yellow powder, mp 194—196 °C (EtOH–*n*-C_6H_{14}). IR (KBr) *v*: 1740, 1655, 1595, 1225, 1075 cm^{-1}. SI-MS *m/z*: 501 $(M+H)^+$.

3c: Yield 30.2%. Yellow powder, mp 210—211.5 °C ($CHCl_3$–*n*-C_6H_{14}). IR (KBr) *v*: 1755, 1660, 1615, 1050, 1020 cm^{-1}. MS *m/z*: 528.1659 (M^+) for $C_{26}H_{29}N_2O_8P = 528.1627$.

3d: Yield 27.9%. Yellow powder, mp 181—183 °C (EtOH–*n*-C_6H_{14}). IR (KBr) *v*: 1750, 1660, 1600, 1230, 1000 cm^{-1}. SI-MS *m/z*: 515 $(M+H)^+$.

3e: Yield 36.1%. Yellow powder, mp 165 °C (EtOH–*n*-C_6H_{14}). IR (KBr) *v*: 1745, 1655, 1595, 1230, 1000 cm^{-1}. SI-MS *m/z*: 515 $(M+H)^+$.

3f: Yield 31.0%. Yellow powder, mp 158 °C (EtOH–*n*-C_6H_{14}). IR (KBr) *v*: 1745, 1655, 1600, 1230, 1030 cm^{-1}. SI-MS *m/z*: 529 $(M+H)^+$.

3g: Yield 24.1%. Yellow solid, mp 245 °C ($CHCl_3$–*n*-C_6H_{14}). IR (KBr) *v*: 1745, 1655, 1590, 1230, 1100 cm^{-1}. SI-MS *m/z*: 725 $(M+H)^+$.

3h: Yield 20.9%. Yellow powder, mp 223 °C (EtOH–*n*-C_6H_{14}). IR (KBr) *v*: 1740, 1655, 1595, 1225 cm^{-1}. SI-MS *m/z*: 555 $(M+H)^+$.

3i: Yield 29.1%. Yellow powder, mp 169—172 °C (EtOH–*n*-C_6H_{14}). IR (KBr) *v*: 1745, 1655, 1600, 1235, 1160, 1030 cm^{-1}. SI-MS *m/z*: 569 $(M+H)^+$.

3j: Yield 38.5%. Yellow powder, mp 172—175 °C (MeOH–Et_2O). IR (KBr) *v*: 1735, 1645, 1585, 1230, 1080 cm^{-1}. SI-MS *m/z*: 563 $(M+H)^+$.

3k: Yield 36.2%. Yellow powder, mp 113—115 °C (iso-PrOH–*n*-C_6H_{14}). IR (KBr) *v*: 1745, 1660, 1600, 1230 cm^{-1}. SI-MS *m/z*: 577 $(M+H)^+$.

3l: Yield 28.5%. Yellow powder, mp 184—186 °C (MeOH–Et_2O). IR (KBr) *v*: 1740, 1655, 1600, 1230, 1025 cm^{-1}. SI-MS *m/z*: 513 $(M+H)^+$.

3m: Yield 43.3%. Yellow powder, mp 182—185 °C (EtOH–*n*-C_6H_{14}). IR (KBr) *v*: 1735, 1650, 1585, 1230, 1190, 1085 cm^{-1}. SI-MS *m/z*: 549 $(M+H)^+$.

Monoester 3o To a solution of **1** (5.00 g, 12.74 mmol) in dry pyridine (800 ml), NaH (60% in oil, 2.55 g, 63.75 mmol) was added in an ice-bath with stirring. The mixture was stirred at ambient temperature for 1 h. A benzene solution of $(C_6H_5CH_2O)_2P(O)Cl$ (prepared *in situ* by reaction of dibenzylphosphonate with *N*-chlorosuccinimide, 25.55 mmol) was added dropwise over 30 min, and the resulting mixture was stirred for 1 h. The precipitate was filtered, and the filtrate was evaporated to dryness *in vacuo*. The residue was purified through silica gel column chromatography with 3% MeOH–$CHCl_3$ to give **3n** (1.94 g, 23.4% yield). Colorless needles, mp 125—128 °C (EtOH). IR (KBr) *v*: 1745, 1655, 1605 cm^{-1}. MS *m/z*: 652.1972 (M^+) for $C_{36}H_{33}N_2O_8P = 652.2018$.

A solution of **3n** (1.50 g, 2.30 mmol) in a 1 : 1 mixture of EtOH and

TABLE III. ^{1}H-NMR Chemical Shifts (δ) of Camptothecin Derivatives, **2**, **3** and **4**

2a	A 1.04 (3H, t, J=7 hz), 1.40 (3H, t, J=8 Hz), 1.81—1.98 (2H, m), 2.06 (3H, s), 2.07 (3H, s), 2.08 (3H, s), 2.11 (3H, s), 3.07—3.24 (2H, m), 3.83 (1H, s), 3.98 (1H, ddd, J=3, 5, 12 Hz), 4.22 (1H, dd, J=3, 12 Hz), 4.33 (1H, dd, J=5, 12 Hz), 5.20—5.42 (4H, m), 5.25 (2H, s), 5.31 (1H, d, J=16 Hz), 5.75 (1H, d, J=16 Hz), 7.51 (1H, dd, J=3, 9 Hz), 7.60 (1H, d, J=3 Hz), 7.64 (1H, s), 8.20 (1H, d, J=9 Hz)
2b	A 1.04 (3H, t, J=7 Hz), 1.42 (3H, t, J=8 Hz), 1.83—1.96 (2H, m), 2.02 (3H, s), 2.04 (3H, s), 2.05 (3H, s), 2.07 (3H, s), 2.08 (3H, s), 2.09 (3H, s), 2.11 (3H, s), 3.08—3.26 (2H, m), 3.82 (1H, s), 3.93—4.07 (2H, br m), 4.08 (1H, dd, J=2, 12 Hz), 4.19 (1H, dd, J=9 Hz), 4.27 (1H, dd, J=4, 12 Hz), 4.31 (1H, dd, J=4, 12 Hz), 4.55 (1H, dd, J=3, 12 Hz), 4.88 (1H, dd, J=4, 11 Hz), 5.08 (1H, dd, J=10 Hz), 5.20 (1H, dd, J=8 Hz), 5.26 (2H, s), 5.31 (1H, d, J=16 Hz), 5.38 (1H, d, J=8 Hz), 5.36—5.44 (2H, m), 5.49 (1H, d, J=4 Hz), 5.75 (1H, d, J=16 Hz), 7.49 (1H, dd, J=3, 9 Hz), 7.59 (1H, d, J=3 Hz), 7.64 (1H, s), 8.19 (1H, d, J=9 Hz)
2c	A 1.03 (3H, t, J=7 Hz), 1.40 (3H, t, J=8 Hz), 1.81—1.97 (2H, m), 2.08 (3H, s), 2.09 (3H, s), 2.12 (3H, s), 3.14 (2H, q, J=8 Hz), 3.73 (3H, s), 3.98 (1H, s), 4.33 (1H, d, J=10 Hz), 5.24 (2H, s), 5.29 (1H, d, J=16 Hz), 5.36—5.49 (4H, m), 5.73 (1H, d, J=16 Hz), 7.48 (1H, dd, J=3, 9 Hz), 7.61 (2H, br s), 8.13 (1H, d, J=9 Hz)
2d	A 0.96 (3H, t, J=7 Hz), 1.32 (3H, t, J=8 Hz), 1.79—1.93 (2H, m), 2.10 (3H, s), 2.11 (3H, s), 2.12 (3H, s), 2.85—2.99 (1H, m), 3.00—3.14 (1H, m), 4.04 (1H, ddd, J=3, 5, 10 Hz), 4.25 (1H, dd, J=3, 12 Hz), 4.33 (1H, br), 4.37 (1H, dd, J=5, 12 Hz), 4.49 (1H, q-like, J=8, 10 Hz), 4.88 (1H, d, J=19 Hz), 5.04 (1H, d, J=19 Hz), 5.26 (1H, dd, J=9, 10 Hz), 5.27 (1H, d, J=16 Hz), 5.45 (1H, d, J=8 Hz), 5.47 (1H, dd, J=9, 10 Hz), 5.71 (1H, d, J=16 Hz), 7.35 (1H, dd, J=3, 9 Hz), 7.49 (1H, d, J=3 Hz), 7.63 (1H, br), 7.65 (1H, s), 8.08 (1H, d, J=9 Hz)
2e	B 0.88 (3H, t, J=7 Hz), 1.31 (3H, t, J=8 Hz), 1.78—1.96 (2H, m), 3.12—3.25 (3H, br m), 3.32—3.39 (2H, br m), 3.44—3.54 (2H, br m), 3.70—3.80 (1H, m), 4.68 (1H, t, J=5 Hz), 5.10 (1H, d, J=5 Hz), 5.14 (1H, d, J=8 Hz), 5.16 (1H, d, J=5 Hz), 5.32 (2H, s), 5.43 (3H, s), 6.50 (1H, s), 7.29 (1H, s), 7.58 (1H, dd, J=2, 10 Hz), 7.74 (1H, d, J=2 Hz), 8.11 (1H, d, J=10 Hz)
2f	B 0.88 (3H, t, J=7 Hz), 1.32 (3H, t, J=8 Hz), 1.78—1.95 (2H, m), 3.06—3.83 (14H, m), 4.53 (1H, t, J=6 Hz), 4.68 (1H, t, J=6 Hz), 4.92 (1H, d, J=5 Hz), 4.94 (1H, d, J=6 Hz), 5.09 (1H, d, J=3 Hz), 5.24 (1H, d, J=8 Hz), 5.32 (2H, s), 5.43 (2H, s), 5.50 (1H, t), 5.56 (1H, d, J=5 Hz), 5.67 (1H, t), 6.50 (1H, s), 7.29 (1H, s), 7.58 (1H, dd, J=3, 9 Hz), 7.73 (1H, d, J=3 Hz), 8.11 (1H, d, J=9 Hz)
2g	B 0.88 (3H, t, J=7 Hz), 1.30 (3H, t, J=8 Hz), 1.78—1.95 (2H, m), 3.07—3.47 (5H, m), 3.74 (1H, d, J=10 Hz), 5.11—5.25 (1H, br), 5.21 (1H, d, J=7 Hz), 5.30 (2H, s), 5.43 (3H, s), 6.50 (1H, s), 7.29 (1H, s), 7.60 (1H, dd, J=3, 9 Hz), 7.73 (1H, d, J=3 Hz), 8.11 (1H, d, J=9 Hz)
2h	B 0.88 (3H, t, J=7 Hz), 1.32 (3H, t, J=8 Hz), 1.78—1.96 (2H, m), 2.86 (1H, t-like, J=9 Hz), 3.18 (2H, q, J=8 Hz), 3.46—3.79 (2H, m), 3.73—3.83 (1H, m), 4.71 (1H, br t), 5.11 (1H, d, J=8 Hz), 5.15—5.22 (1H, br), 5.31 (2H, s), 5.43 (2H, s), 6.50 (1H, s), 7.29 (1H, s), 7.62 (1H, dd, J=3, 9 Hz), 7.78 (1H, d, J=3 Hz), 8.12 (1H, d, J=9 Hz)
3a	B 0.89 (3H, t, J=7 Hz), 1.31 (3H, t, J=8 Hz), 1.78—1.94 (2H, m), 3.12 (2H, q, J=8 Hz), 3.55 (3H, d, J=11 Hz), 5.27 (2H, s), 5.38 (1H, d, J=16 Hz), 5.43 (1H, d, J=16 Hz), 6.46 (1H, br s), 7.30 (1H, s), 7.70 (1H, dd, J=2, 9 Hz), 7.91 (1H, d, J=2 Hz), 8.06 (1H, d, J=9 Hz)
3b	B 0.89 (3H, t, J=7 Hz), 1.13 (3H, t, J=7 Hz), 1.32 (3H, t, J=8 Hz), 1.78—1.94 (2H, m), 3.11 (2H, q, J=8 Hz), 3.80—3.91 (2H, m), 5.27 (2H, s), 5.38 (1H, d, J=16 Hz), 5.43 (1H, d, J=16 Hz), 6.45 (1H, s), 7.29 (1H, s), 7.69 (1H, dd, J=2, 9 Hz), 7.90 (1H, d, J=2 Hz), 8.03 (1H, d, J=9 Hz)
3c	B 0.89 (3H, t, J=7 Hz), 1.28—1.37 (9H, m), 1.81—1.93 (2H, m), 3.18 (2H, q, J=7 Hz), 4.19—3.80 (4H, m), 5.32 (2H, s), 5.39 (1H, d, J=16 Hz), 5.44 (1H, d, J=16 Hz), 6.46 (1H, br s), 7.33 (1H, s), 7.69—7.75 (1H, m), 7.96—8.02 (1H, m), 8.20 (1H, d, J=9 Hz)
3d	B 0.82—0.94 (6H, m), 1.29 (3H, t, J=7 Hz), 1.54—1.65 (2H, m), 1.79—1.95 (2H, m), 3.12 (2H, q, J=7 Hz), 3.96 (2H, q-like, J=7 Hz), 5.28 (2H, s), 5.42 (2H, s), 6.32—6.70 (1H, br), 7.29 (1H, s), 7.70 (1H, dd, J=2, 9 Hz), 7.92 (1H, br s), 8.13 (1H, d, J=9 Hz)
3e	B 0.89 (3H, t, J=7 Hz), 1.24 (6H, d, J=6 Hz), 1.31 (3H, t, J=7 Hz), 1.80—1.93 (2H, m), 3.12 (2H, q, J=7 Hz), 4.51—4.67 (1H, m), 5.28 (2H, s), 5.39 (1H, d, J=17 Hz), 5.44 (1H, d, J=17 Hz), 6.58—6.60 (1H, br), 7.30 (1H, s), 7.69 (1H, dd, J=2, 9 Hz), 7.92 (1H, br s), 8.12 (1H, d, J=9 Hz)
3f	B 0.79 (3H, t, J=7 Hz), 0.88 (3H, t, J=7 Hz), 1.20—1.37 (5H, m), 1.41—1.51 (2H, m), 1.79—1.94 (2H, m), 3.12 (2H, q, J=8 Hz), 3.78 (2H, br s), 5.29 (2H, s), 5.38 (1H, d, J=16 Hz), 5.43 (1H, d, J=16 Hz), 6.46 (1H, s), 7.28 (1H, s), 7.72 (1H, br d, J=9 Hz), 7.91 (1H, br s), 8.04 (1H, d, J=9 Hz)
3g	B 0.84 (3H, t, J=7 Hz), 0.88 (3H, t, J=7 Hz), 0.88—1.55 (32H, br m), 1.31 (3H, t, J=8 Hz), 1.78—1.92 (2H, m), 3.13 (2H, br q, J=7 Hz), 3.53—4.00 (2H, br), 5.26 (2H, br s), 5.37 (1H, d, J=17 Hz), 5.43 (1H, d, J=17 Hz), 6.46 (1H, s), 7.28 (1H, s), 7.70—7.98 (2H, br m), 8.03 (1H, d, J=9 Hz)
3h	B 0.88 (3H, t, J=7 Hz), 1.05—1.70 (13H, m), 1.77—1.94 (2H, m), 3.11 (2H, br q, J=7 Hz), 4.24—4.35 (1H, br), 5.28 (2H, s), 5.42 (2H, s), 6.49 (1H, br s), 7.29 (1H, s), 7.69 (1H, br d, J=9 Hz), 7.93 (1H, br s), 8.12 (1H, br d, J=9 Hz)
3i	B 0.76—1.41 (5H, m), 0.88 (3H, t, J=7 Hz), 1.30 (3H, t, J=7 Hz), 1.46—1.75 (6H, br m), 1.77—1.97 (2H, m), 3.11 (2H, br q, J=7 Hz), 3.78 (2H, t-like, J=7 Hz), 5.28 (2H, s), 5.42 (2H, s), 6.36—6.63 (1H, br), 7.29 (1H, s), 7.69 (1H, dd, J=2, 9 Hz), 7.91 (1H, br s), 8.12 (1H, d, J=9 Hz)
3j	B 0.88 (3H, t, J=7 Hz), 1.26 (3H, t, J=7 Hz), 1.77—1.95 (2H, m), 3.06 (2H, q, J=7 Hz), 4.90 (2H, d, J=7 Hz), 5.26 (2H, s), 5.39 (1H, d, J=16 Hz), 5.43 (1H, d, J=16 Hz), 6.47 (1H, br s), 7.20—7.37 (6H, m), 7.69 (1H, dd, J=2, 9 Hz), 7.88 (1H, d, J=2 Hz), 8.03 (1H, d, J=9 Hz)
3k	B 0.89 (3H, t, J=7 Hz), 1.28 (3H, t, J=7 Hz), 1.80—1.95 (2H, m), 2.89 (2H, t, J=7 Hz), 3.10 (2H, q, J=7 Hz), 4.19 (2H, q-like, J=7 Hz), 5.29 (2H, s), 5.40 (1H, d, J=16 Hz), 5.45 (1H, d, J=16 Hz), 6.40—6.60 (1H, br), 7.10—7.32 (5H, m), 7.30 (1H, s), 7.63 (1H, dd, J=3, 9 Hz), 7.89 (1H, br s), 8.09 (1H, d, J=9 Hz)
3l	B 0.88 (3H, t, J=7 Hz), 1.30 (3H, t, J=7 Hz), 1.79—1.94 (2H, m), 3.11 (2H, br q, J=7 Hz), 4.52 (2H, br s), 5.16 (1H, d, J=10 Hz), 5.28 (2H, s), 5.32 (1H, d, J=17 Hz), 5.42 (2H, s), 5.87—6.00 (1H, m), 6.31—6.69 (1H, br), 7.29 (1H, s), 7.69 (1H, br d, J=10 Hz), 7.92 (1H, br s), 8.12 (1H, d, J=10 Hz)
3m	B 0.88 (3H, t, J=7 Hz), 1.25 (3H, t, J=7 Hz), 1.77—1.95 (2H, m), 3.06 (2H, q, J=7 Hz), 5.28 (2H, s), 5.42 (2H, s), 7.00—7.45 (6H, m), 7.70 (1H, dd, J=2, 10 Hz), 7.89 (1H, br s), 8.09 (1H, d, J=10 Hz)
3n	B 0.88 (3H, t, J=7 Hz), 1.22 (3H, t, J=7 Hz), 1.80—1.94 (2H, m), 3.07 (2H, q, J=7 Hz), 5.26 (4H, d, J=9 Hz), 5.33 (2H, s), 5.44 (2H, s), 6.53 (1H, s), 7.32 (1H, s), 7.34—7.43 (10H, m), 7.70 (1H, dd, J=2, 10 Hz), 7.90 (1H, br s), 8.19 (1H, d, J=10 Hz)
3o	B 0.88 (3H, t, J=7 Hz), 1.31 (3H, t, J=7 Hz), 1.78—1.96 (2H, m), 3.15 (2H, q, J=7 Hz), 5.32 (2H, s), 5.43 (2H, s), 6.40—6.67 (1H, br), 7.30 (1H, s), 7.70 (1H, dd, J=2, 10 Hz), 7.94 (1H, br s), 8.17 (1H, d, J=10 Hz)
3p	B 0.88 (3H, t, J=7 Hz), 1.17 (9H, t, J=7 Hz), 1.30 (3H, t, J=8 Hz), 1.78—1.95 (2H, m), 2.90—3.10 (8H, m), 5.24 (2H, s), 5.39 (1H, d, J=16 Hz), 5.43 (1H, d, J=16 Hz), 6.36—6.61 (1H, br), 7.26 (1H, s), 7.69 (1H, dd, J=2, 9 Hz), 7.87 (1H, d, J=2 Hz), 8.02 (1H, d, J=9 Hz)
4a	B 0.89 (3H, t, J=7 Hz), 1.18 (9H, t, J=7 Hz), 1.33 (3H, t, J=8 Hz), 1.79—1.96 (2H, m), 3.05—3.20 (8H, m), 5.31 (2H, s), 5.40 (1H, d, J=16 Hz), 5.45 (1H, d, J=16 Hz), 6.30—6.65 (1H, br), 7.32 (1H, s), 7.73 (1H, dd, J=3, 9 Hz), 7.97 (1H, d, J=3 Hz), 8.09 (1H, d, J=9 Hz), 8.68—9.02 (1H, br)
4b	B 0.89 (3H, t, J=7 Hz), 1.32 (3H, t, J=8 Hz), 1.79—1.99 (6H, m), 2.81 (3H, s), 3.14 (2H, q, J=8 Hz), 3.31 (4H, br s), 5.32 (2H, s), 5.41 (1H, d, J=16 Hz), 5.45 (1H, d, J=16 Hz), 6.48 (1H, s), 7.31 (1H, s), 7.73 (1H, dd, J=3, 9 Hz), 7.96 (1H, d, J=3 Hz), 8.10 (1H, d, J=9 Hz)

A: In $CDCl_3$. B: In DMSO-d_6.

dioxane (300 ml) containing 10% Pd–C (300 mg) was shaken in a hydrogenation flask under ambient H_2 pressure for 2 h. The mixture was filtered through filter paper and the filtrate was evaporated to dryness *in vacuo*. The residue was recrystallized to give **3o** (1.08 g, 99.4% yield). Yellow powder, mp 221—223 °C (EtOH–*n*-C_6H_{14}). IR (KBr) *v*: 1740, 1655, 1590, 1225, 1155 cm^{-1}. SI-MS *m/z*: 473 $(M+H)^+$.

Triethylammonium Salt 3p To a solution of **3o** (800 mg, 1.69 mmol) in MeOH, Et_3N (171 mg, 1.69 mmol) was added. The mixture was evaporated to dryness under reduced pressure. The residue was recrystallized to give **3p** (860 mg, 88.6% yield). Pale yellow powder, mp 191—196 °C (EtOH–Et_2O). IR (KBr): 1745, 1660, 1603, 1235, 1160 cm^{-1}. SI-MS *m/z*: 574 $(M+H)^+$.

Sulfate (4, General Procedure) To a suspension of **1** (1.20 g, 3.06 mmol) in $CHCl_3$ (100 ml) containing a tertiary amine (10.4 eq), SO_3–pyridine (10.4 eq) was added dropwise while stirring. The stirring was continued at ambient temperature until the reaction was completed (monitored by TLC). The mixture was evaporated *in vacuo* and the residue was purified through an ODS chromatographic column with H_2O–MeOH (7 : 3).

4a: Yield 69.7%. Yellow powder, mp 125—128 °C (MeOH–Et_2O). IR (KBr) *v*: 1735, 1655, 1583, 1272, 1255, 1235, 1049 cm^{-1}. SI-MS *m/z*: 574 $(M+H)^+$. *Anal.* Calcd for $C_{28}H_{35}N_3O_8S \cdot 3/2H_2O$: C, 55.99; H, 6.38; N, 7.00. Found: C, 56.35; H, 6.07; N, 6.92.

4b: Yield 45.2%. Pale yellow powder, mp 114—116 °C (MeOH–Et_2O). IR (KBr) *v*: 1742, 1655, 1590, 1560, 1265, 1230 cm^{-1}. SI-MS *m/z*: 558 $(M+H)^+$. *Anal.* Calcd for $C_{27}H_{31}N_3O_8S \cdot 3/2H_2O$: C, 55.47; H, 5.86; N, 7.19. Found: C, 55.61; H, 5.66; N, 7.18.

Antitumor Activity L1210 leukemia cells (10^5) were implanted intraperitoneally (i.p.) in 7-week-old CDF_1 female mice on day 0; 6 mice were used at each dose. The samples were administered i.p. on days 1, 5, and 9. The control mice were injected with saline. Cured mice were counted on day 40.

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