SYNTHESIS OF TAXOIDS II. SYNTHESIS AND ANTITUMOR ACTIVITY OF WATER-SOLUBLE TAXOIDS†

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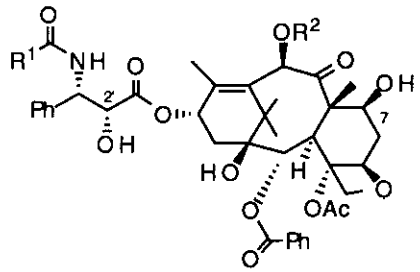
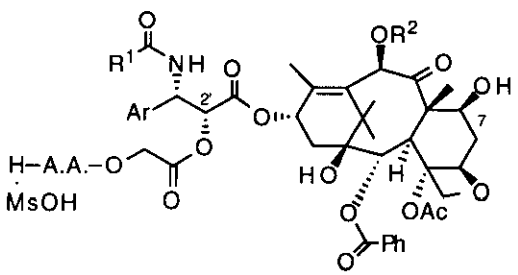
Abstract — Synthesis of novel taxoid water-soluble prodrugs that have 2'-substituted amino acid derivatives with spacer is described. Enantioselective synthesis of the C-13 side chains proceeded through the asymmetric dihydroxylation. Several compounds had good solubility in saline and showed potent antitumor activity against B16 melanoma in mice.

Paclitaxel (**1**), a diterpene natural product, was isolated in 1971 from the bark of *Taxus brevifolia*[1] and is regarded as one of the most promising antitumor drugs-available,[2] especially for the treatment of ovarian, breast and lung cancer. Docetaxel (**2a**),[3] a semisynthetic analogue of paclitaxel (**1**) is two times as active as paclitaxel



1 R^1 = Ph, R^2 = Ac (Paclitaxel)
2a R^1 = *tert*-BuO, R^2 = H (Docetaxel)



3 R^1 = Ph, R^2 = Ac
4 R^1 = *tert*-BuO, R^2 = H
A.A. = amino acid

Scheme 1

†This paper is dedicated to the memory of Emeritus Professor Shun-ichi Yamada (Tokyo University).

(**1**) in an *in vitro* tubulin assay as an inhibition of microtuble depolymerization.[4] In spite of its excellent antitumor activity, there are considerable difficulties in employing **1** and **2a** in the clinical. One of the major problems is its poor solubility in water (e.g. **1**,< 0.004 mg/mL; **2a** , < 0.05 mg/mL), creating formulation difficulties for intravenous administration. Due to the poor solubility, cremophore EL (in the case of **1**) or Tween 80 (in the case of **2a**) is presently used as solubilizing agents.[5] However, these agents induce adverse effects such as hypersensitivity reactions including hypotension, urticaria and dyspnea. These side effects can be attenuated by employing antihistaminic agents such as dexamethasone and diphenhydramine, but the inescapable fact is that this results in additional medication, discomfort and cost to the patient.

A number of groups have recently reported the synthesis and biological evaluation of water-soluble prodrugs of paclitaxel (**1**).[2,6] Mathew *et al.* have reported the synthesis of several compounds with amino acid substituents at 2'- *and/or* 7-positions of **1**.[6a] Among them, a few of the 2'-paclitaxel (**1**) esters exhibited potent antitumor activity, however, they were quite reactive and underwent chemical hydrolysis readily.[6] On the other hand, 7-paclitaxel (**1**) esters were quite stable chemically, being strongly resistant to enzymatic cleavage, but had weak antitumor activity.

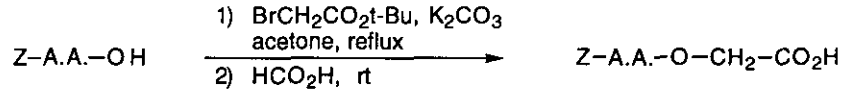
Consequently, we focused on synthesizing water-soluble analogues with substitutions at the 2'-position of taxoids and speculated that the poor chemical stability of 2'-amino acid derivatives of **1** was caused by steric repulsion of bulky groups at 2'- and 3'-positions of **1** and electronic effect of amino group. We designed and synthesized novel 2'-substituted water-soluble prodrugs (**3**) and (**4**) (**Scheme 1**) , which have amino acid derivatives with a glycolate spacer (to evade the steric repulsion and electronic effect of amino group), and examined antitumor activity against B16 melanoma in mice.

CHEMISTRY

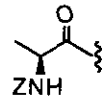
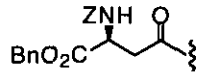
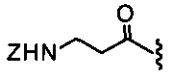
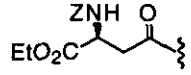
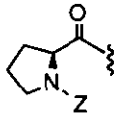
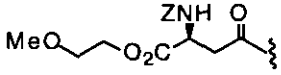
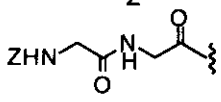
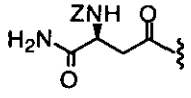
Aminoacyloxyacetic acid derivatives (**5**), the water-soluble auxiliaries, were synthesized as follows. Benzyloxycarbonyl (Z) - protected amino acids (**6**) were treated with *tert*-butyl bromoacetate in the presence of potassium carbonate (K_2CO_3) in refluxing acetone,[7] followed by deprotection of *tert*-butyl group using formic acid to give **5** in good yields (**Table1**).

First, we synthesized water-soluble prodrugs of paclitaxel (**1**). The hydroxy group at 2'- position of **1** is more reactive than the sterically hindered 7- hydroxy group. Therefore, it is possible to use this difference in reactivity to introduce **5** at the 2'- position of **1**. Compound (**1**) reacted with **5** in the presence of *N,N'*-dicyclohexyl-carbodiimide (DCC) gave the desired 2'-ester of **1** quantitatively, followed by palladium catalyzed hydrogenation in tetrahydrofuran (THF) in the presence of methanesulfonic acid (MsOH) gave **3a** and **3b** in

Table 1. Synthesis of 2'-side chains (**5**)

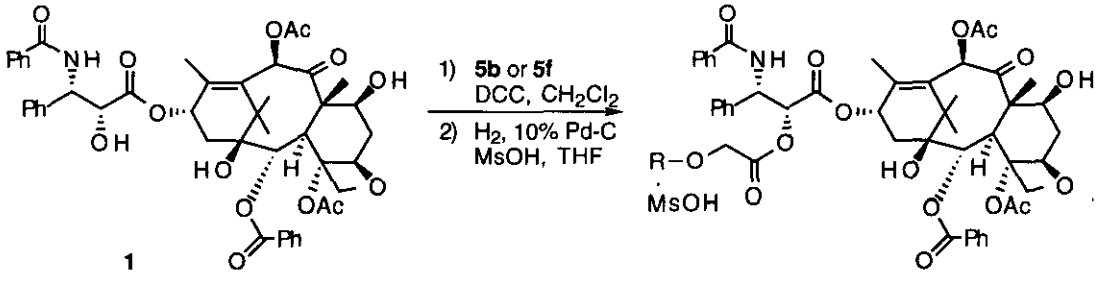


6 **5**

compd. No.	Z–A.A.	Y = (%)	compd. No.	Z–A.A.	Y = (%)
5a		84	**5e**		67
5b		82	**5f**		85
5c		99	**5g**		73
5d		63	**5h**		58

excellent yields (**Scheme 2**). The solubility of **3a** and **3b** in saline fell short of our expectations (< 0.1 mg/mL).

We then synthesized water-soluble docetaxel derivatives (**4**) as shown in **Schemes 3** and **4**. We have already established the efficient and convenient synthesis of **2a** and its fluoro- substituted derivatives.[8] Namely, the reaction of aryl orthoacetates (**7**) with acetyl bromide readily proceeded regioselectively to afford the desired aryl bromides (**8**) in the presence of tin(II) or zinc bromide as a catalyst (e.g. R=H; **8/9** = >100, without Lewis acid[9] **8/9** = 5) (**Scheme 3**). By using the improved process, we tried to synthesize the methoxy substituted analogues (**8**, R= 2-OMe and 4-OMe). However, in the presence of Lewis acid, the reaction of

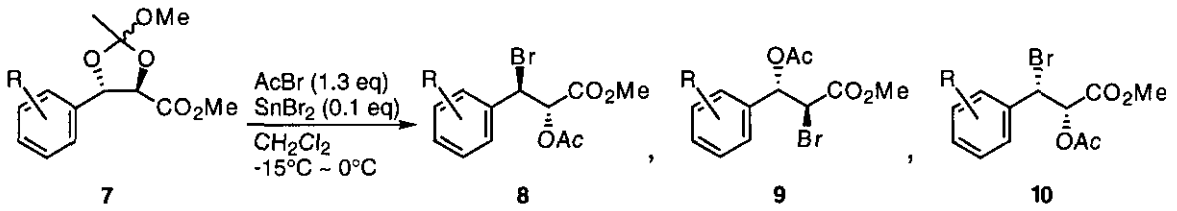


1

3a R = H_2N Y = 93% (2 steps)

3b R = EtO_2C Y = 99% (2 steps)

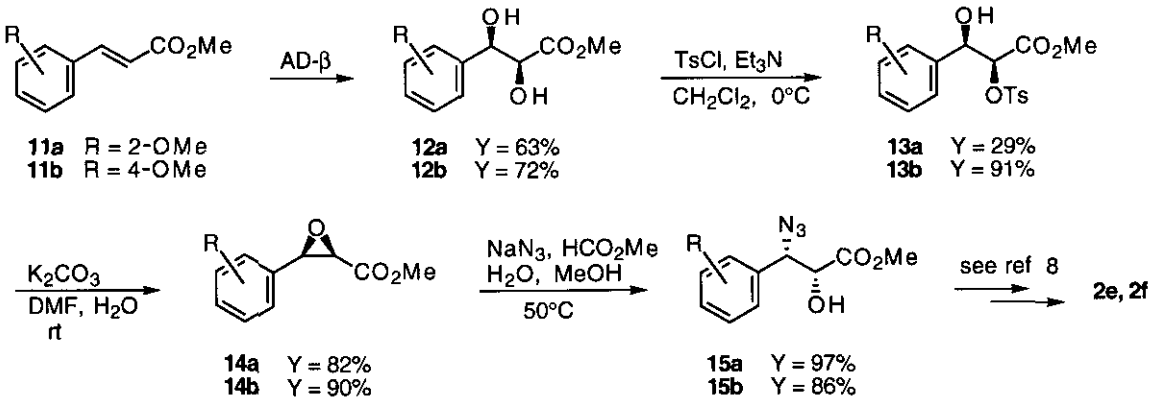
Scheme 2 solubility in saline: **3a**, **3b** < 0.1 mg/mL



7 8 9 10

Scheme 3

compounds containing electron donating group such as methoxy on the benzene ring of cyclic orthoacetates with acetyl bromide afforded the diastereoisomer (**10**) as a side product (**Scheme 3**). Generation of **10** would be considered to result from the participation of carbocationic character on the benzilic position.[10] Anyway, **2e** and **2f** were synthesized by an alternative method as shown in **Scheme 4**.[11] The methyl cinnamates (**11a, 11b**) were subjected to the asymmetric dihydroxylation (AD) process (AD-β)[12] at room temperature to give the enantiomerically pure (2S,3R) - diols in good yields (**12a**, 63% ; **12b**, 72%). The diols (**12**) were converted to the monotosylates (**13**) by reaction with tosyl chloride in the presence of triethylamine (Et_3N) at 0°C (**13a**, 29% ; **13b**, 91%), which were treated with K_2CO_3 in *N,N*-dimethylformamide (DMF) in the presence of small amount of water to afford the *cis*-glycidic esters (**14**) in good yields (**14a**, 82% ; **14b**, 90%). Then, **14** reacted with sodium azide in aqueous methanol in the presence of methyl formate at 50°C yielded the hydroxy azide



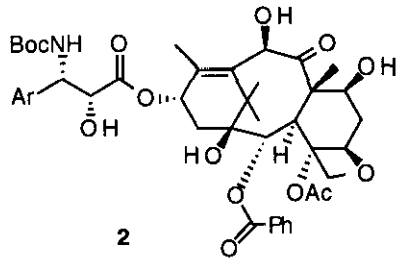
11a R = 2-OMe
11b R = 4-OMe

12a Y = 63%
12b Y = 72%

13a Y = 29%
13b Y = 91%

14a Y = 82%
14b Y = 90%

15a Y = 97%
15b Y = 86%



2

2a Ar = Ph (Docetaxel)
2b Ar = 4-F-Ph
2c Ar = 3-F-Ph
2d Ar = 2,4-diF-Ph
2e Ar = 2-OMe-Ph
2f Ar = 4-OMe-Ph

Scheme 4

esters (**15**) in good yields (**15a**, 97% ; **15b**, 86%). Conversion of **15** to **2e** and **2f** was carried out in the same manner as **2a** ~ **2d** (see ref. 8).

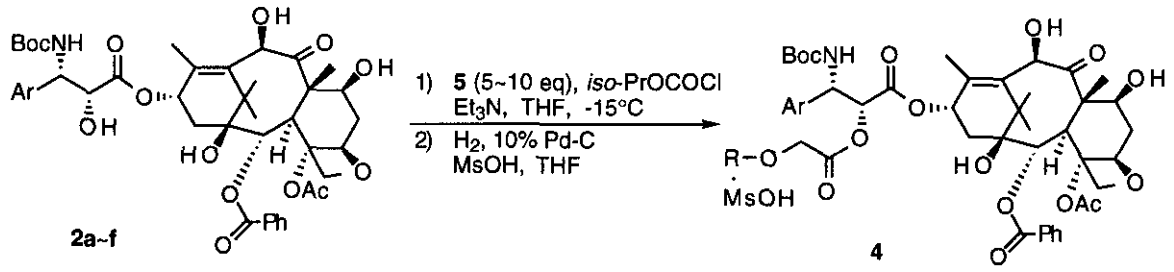
The hydroxy group at 2'-position of **2** was also considered more reactive than 7- and 10-hydroxy groups. Thereupon, we examined the esterification of **2** in the same manner for the preparation of **3** as described above. Compound (**2**) was reacted with **5** in the presence of DCC to give the desired 2'-ester of **2** as the major product with an appreciable amount of the unwanted esters (2',7- and 2',10-diesters) and the starting material (**2**). So far, we have not obtained the evidence which elucidated the reason for less selective esterification of **2**. Therefore, we examined further studies for finding desirable esterification condition. Among various reactions, mixed anhydride method was found to be the best. Compound (**2**) was treated with the mixed anhydrides that were prepared from the aminoacyloxyacetic acids (**5**) and *iso*-propyl chloroformate in THF in the presence of Et_3N at -15°C to afford 2'-esters predominantly. The final deprotection was performed in the same manner for the preparation of **3**, and the desired deprotected compounds (**4a-l**) were obtained as the methanesulfonate with high purity (>95%) as shown in **Table 2**. The methanesulfonic acid salt of 2'-esters (**4a-l**) had the greatly improved solubility in saline (0.5 ~ > 10 mg/ mL).

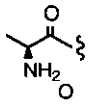
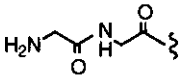
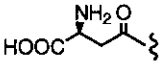
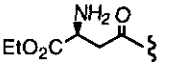
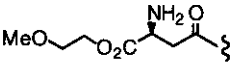
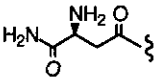
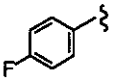
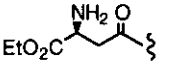
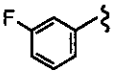
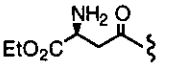
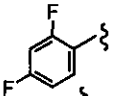
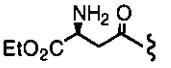
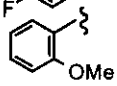
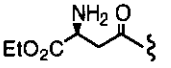
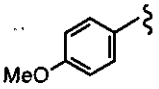
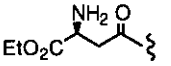
ANTITUMOR ACTIVITY AND DISCUSSION

The water-soluble derivatives (**3** and **4**) were tested for antitumor activity against B16 melanoma in mice. First, antitumor activities against B16 melanoma (implanted intraperitoneally) were examined by intraperitoneally administration and paclitaxel (**1**) and docetaxel (**2a**) were included for the comparison (**Table 3**). Several compounds we synthesized had similar potent antitumor activity as **1** and **2a**. Correlation between antitumor activity and water solubility was not recognized. Among the various 2'-amino acid derivatives side chain, aspartate derivatives (**4d-g**) showed potent antitumor activity. Compounds (**4h-l**), which had the fluoro- or methoxy- substituents on the benzene ring of 3'-position, did not improve the antitumor activity.

Next, the selected compounds were examined for antitumor activity against B16 melanoma (implanted subcutaneously) in mice by intravenous administration with **1** and **2a** as the comparision (**Table 3**). Several compounds (**4**) had potent antitumor activity, while **1** exhibited weak activity. In particular, in the case of **4e**, a 100% cure rate was observed at optimal dose (OD), and resulted in a 60% cure rate at 1/2 OD. Namely, compound (**4e**) had more potent antitumor activity against B16 melamoma than that of **1** and **2a**. Furthermore, water-soluble prodrugs (**4**) were found to be chemically stable in saline (e.g. **4e** was dissolved in saline and standed at room temperature for 20 h, 98.4% of **4e** remained). Further chemical stability studies and enzymatic studies are ongoing.

Table 2 Synthesis of water-soluble derivatives (**4**)



Entry	2	5	Product 4				
			compd. No.	Ar =	R =	Y = (%) (2 steps)	solubility[a]
1	**2a**	**5a**	**4a**	Ph		69	B
2	**2a**	**5c**	**4b**	Ph		54	B
3	**2a**	**5d**	**4c**	Ph		38	A
4	**2a**	**5e**	**4d**	Ph		56	B
5	**2a**	**5f**	**4e**	Ph		47	B
6	**2a**	**5g**	**4f**	Ph		68	A
7	**2a**	**5h**	**4g**	Ph		67	C
8	**2b**	**5f**	**4h**			68	C
9	**2c**	**5f**	**4i**			30	C
10	**2d**	**5f**	**4j**			54	C
11	**2e**	**5f**	**4k**			35	A
12	**2f**	**5f**	**4l**			54	A

a) Solubility in saline, A; >10 mg/mL, B; 1~10 mg/mL, C; 0.5~1 mg/mL

Table 3 Antitumor activity of water-soluble taxoids against B16 melanoma

compd. No.	ip-ip[a]			sc-iv[b]		
	OD (mg/kg)[c]	ILS (%)[d]	cure[e]	OD (mg/kg)[c]	ILS (%)[d]	cure[e]
3a	25	74.7	0/5		N.T.[f]	
3b	50	61.3	0/5		N.T.[f]	
4a	25	150.7	1/5		N.T.[f]	
4b	25	168.6	0/5		N.T.[f]	
4c	25	147.0	0/5		N.T.[f]	
4d	12.5	107.3	0/5	6.3	149.2	3/5
4e	12.5	111.4	3/5	25		5/5
4f	25	113.9	2/5		N.T.[f]	
4g	12.5	129.2	1/5	6.3	198.3	3/5
4h	3.1	135.5	1/5	25	192.8	1/5
4i	3.1	132.6	0/5	25	45.8	4/5
4j	3.1	132.6	0/5		N.T.[f]	
4k	25	41.7	0/5	6.3	35.6	0/5
4l	25	131.6	0/5	25	140.7	0/5
1 (Paclitaxel)	3.1	61.0	3/5	1.6	5.5	0/5
2a (Docetaxel)	12.5	121.1	3/5	12.5	149.9	2/5

a) B16 melanoma cells were inoculated intraperitoneally, and each compound was administered *i.p.* on days 1 to 4. b) B16 melanoma cells were inoculated subcutaneously, and each compound was administered *i.v.* on days 1 to 5. c) Optimal dose of drug . d) Increase in life span of mice when treated at the optimal dose. ILS(%) = (mean survival time of treated group (except cure) / that of control group - 1) × 100 e) The mice survived on days 90, and the tumor was undetectable. f) Not tested.

In conclusion, we have synthesized several water-soluble taxoids (**4**). These compounds had moderate to good solubility in saline. Moreover, several compounds showed potent antitumor activity against B16 melanoma, compared with **1** and **2a**.

EXPERIMENTAL

Melting points were determined with a Büchi 535 melting point apparatus. All melting points are uncorrected. IR spectra were obtained with an Analect FT-IR spectrophotometer. ^{1}H-NMR were measured with a Varian Gemini-300 or a JEOL JNM-GSX-400 spectrometer. MS were recorded with a Hitachi RMu-6 or a JEOL JMS-HX 100 mass spectrometer. Microanalysis were performed on a Perkin-Elmer 240B C.H.N. analyzer. Optical rotations were measured with a Horiba SEPA-200 high sensitive polarimeter. Silica gel (SiO_2) 60K-230 (230-430 mesh) (Katayama) was used for column chromatography. In general, reactions were carried out in dry solvents under argon atmosphere.

General Procedure for the Synthesis of *N*-Benzyloxycarbonylamino-α-acyloxyacetic Acids (5): To a solution of *N*-benzyloxycarbonylamino acids (**6**, 126 mmol) in acetone (500 mL) were added *tert*-butyl bromoacetate (29.4 g, 151 mmol) and K_2CO_3 (34.8 g, 252 mmol). The mixture was heated to reflux for 18 h. The mixture was cooled to rt and concentrated *in vacuo*. To the residue were added ethyl acetate and water. The organic layer was separated, and washed with water and brine, and dried over Na_2SO_4. After the solvent was removed *in vacuo*, the residue was purified by column chromatography on SiO_2 using ethyl acetate / hexanes as an eluent to give *tert*-butyl α-acyloxyacetates. To the *tert*-butyl α-acyloxyacetate was added formic acid (250 mL). The mixture was stirred at rt for 18 h. The formic acid was removed *in vacuo*. To the residue was added *iso*-Pr_2O-hexanes and the mixture was stirred vigorously for 1 h. The resulting precipitates were collected by filtration to give **5**.

Physical data of **5** are summarized in **Table 4**.

Methyl (2*S*, 3*R*)-2,3-Dihydroxy-3-(2-methoxyphenyl)propionate (12a): To a solution of $K_3Fe(CN)_6$ (64.8 g; 197 mmol), K_2CO_3 (27.2 g; 197 mmol) and $(DHQD)_2PHAL$ (511 mg; 0.656 mmol) in *tert*-butanol-water (1 : 1, 650 mL) was added osmium tetraoxide (34 mg; 0.134 mmol) with stirring. To the mixture was added **11a** (12.6 g; 65.6 mmol). The mixture was stirred at rt for 18 h. To the reaction mixture was added Na_2SO_3 (98 g), and the mixture was stirred at rt for 30 min. The mixture was diluted with ethyl acetate and the organic layer was separated. The aqueous layer was extracted with ethyl acetate. The organic layers were combined and washed with brine, and dried over Na_2SO_4. After the solvent was removed *in vacuo*, the residue was purified by column chromatography on SiO_2 using ethyl acetate / hexanes (3 : 2) as an eluent to give **12a**

(9.35 g, Y = 63 %) as a colorless powder. mp 80.5-81.5°C (ethyl acetate-hexanes). $[\alpha]_D^{20}$-18.6° (c 1.03, $CHCl_3$). IR (Nujol): 3510, 3360, 1730 cm^{-1}. FAB-MS m/z: 227 (MH^+). ^{1}H-NMR ($CDCl_3$)δ: 2.98 (1H, d, *J* = 7.7 Hz, D_2O exchangeable (exch.), 3-OH), 3.06 (1H, d, *J* = 6.1 Hz, D_2O exch., 2-OH), 3.82 (3H, s, OMe), 3.86 (3H, s, OMe), 4.48 (1H, dd, *J* = 6.1, 2.9 Hz, 2-H), 5.32 (1H, dd, *J* = 7.7, 2.9 Hz, 3-H), 6.87-7.43 (5H, m, Ar-H).

Methyl (2*S*, 3*R*)-2,3-Dihydroxy-3-(4-methoxyphenyl)propionate (12b) was obtained in the same manner. Y = 72 %. mp 108-109°C (ethyl acetate-hexanes). $[\alpha]_D^{20}$-2.39° (c 1.00, MeOH). IR (Nujol): 3500, 3320, 1710 cm^{-1}. FAB-MS m/z: 249 (M^++Na). ^{1}H-NMR ($CDCl_3$)δ: 2.71 (1H, d, *J* = 6.6 Hz, D_2O exch., 3-OH), 3.12 (1H, d, *J* = 6.2 Hz, D_2O exch., 2-OH), 3.81 (3H, s, OMe), 3.81 (3H, s, OMe), 4.33 (1H, dd, *J* = 6.2, 2.9 Hz, 2-H), 4.96 (1H, dd, *J* = 6.6, 2.9 Hz, 3-H), 6.86-6.94 (2H, m, Ar-H), 7.29-7.36 (2H, m, Ar-H).

Methyl (2*S*,3*R*)-3-Hydroxy-3-(4-methoxyphenyl)-2-(tosyloxy)propionate (13b): To a solution of **12b** (10.11 g, 44.7 mmol) in dichloromethane (220 mL) at 0°C were added Et_3N(6.79 g, 67.1 mmol) and tosyl chloride (8.78 g, 46.1 mmol) with stirring. After being stirred for 40 h at 0°C, the mixture was poured into ice-water and extracted with ethyl acetate. The organic layer was washed with brine, and dried over Na_2SO_4. The solvent was removed *in vacuo*, and the residue was purified by column chromatography on SiO_2 using ethyl acetate / hexanes (1 : 1) as an eluent to give **13b** (15.51 g, Y = 91 %) as a powder. mp 85.0-87.0°C (ethyl acetate-hexanes). IR (Nujol): 3500, 1740cm^{-1}. FAB-MS m/z: 381 (MH^+). ^{1}H-NMR ($CDCl_3$)δ: 2.42 (4H, s, Me and OH), 3.60 (3H, s, OMe), 3.79 (3H, s, OMe), 4.88 (1H, d, *J* = 4.9 Hz, 2-H), 5.04 (1H, d, *J* = 4.9 Hz, 3-H), 6.7-7.7 (8H, m, Ar-H).

Methyl (2*S*, 3*R*)-3-Hydroxy-3-(2-methoxyphenyl)-2-(tosyloxy)propionate (13a) was obtained in the same manner. Y = 29 %. mp 161.0-163.0°C (ethyl acetate-hexanes). IR (Nujol): 3520, 1760 cm^{-1}. FAB-MS m/z: 403 (M^++Na). ^{1}H-NMR ($CDCl_3$)δ: 2.40 (3H, s, Me), 2.86 (1H, d, *J* = 8.2 Hz, D_2O exch., OH), 3.73 (3H, s, OMe), 3.73 (3H, s, OMe), 5.18 (1H, d, *J* = 3.5 Hz, 2-H), 5.34 (1H, dd, *J* = 8.3, 3.5 Hz, 3-H), 6.67-7.45 (8H, m, Ar-H).

Methyl (2*R*, 3*R*)-3-(2-Methoxyphenyl)oxiranecarboxylate (14a): A solution of **13a** (6.48 g, 17.0 mmol) and water (1.5 mL) in DMF (100 mL) was treated with K_2CO_3 (7.05 g, 51.0 mmol). After being stirred for 70 h at rt, the mixture was poured into ice-water and extracted with ethyl acetate. The organic layer was washed with brine, and dried over Na_2SO_4. The solvent was removed *in vacuo*, and the residue was purified by column chromatography on SiO_2 using ethyl acetate / hexanes (1 : 2) as an eluent to give **14a** (2.93 g, Y = 82 %) as an oil. IR (Neat): 1750, 1730 cm^{-1}. FAB-MS m/z: 209 (MH^+). ^{1}H-NMR ($CDCl_3$)δ: 3.54 (3H, s,

OMe), 3.84 (3H, s, OMe), 3.86 (1H, d, J = 4.7 Hz, 2-H), 4.40 (1H, d, J = 4.7 Hz, 3-H), 6.84 (1H, m, Ar-H), 6.94 (1H, m, Ar-H), 7.27 (1H, m, Ar-H), 7.41 (1H, m, Ar-H).

Methyl (2*R*, 3*R*)-3-(4-methoxyphenyl)oxiranecarboxylate (14b) was obtained as an oil in the same manner. Y = 90 %. IR (Neat): 1750 cm^{-1}. FAB-MS m/z: 209 (MH^+). ^{1}H-NMR ($CDCl_3$)δ: 3.58 (3H, s, OMe), 3.79 (3H, s, OMe), 3.80 (1H, d, J = 4.8 Hz, 2-H), 4.21 (1H, d, J = 4.8 Hz, 3-H), 6.83-6.90 (2H, m, Ar-H), 7.30-7.37 (2H, m, Ar-H).

Methyl (2*R*, 3*S*)-3-Azido-2-hydroxy-3-(2-methoxyphenyl)propionate (15a): A solution of **14a** (2.40 g, 11.5 mmol) in methanol-water (8 : 1, 70 mL) was treated with methyl formate (12 mL) and sodium azide (3.75 g, 57.6 mmol) and then stirred at 50°C for 19 h. The mixture was poured into ice-water and extracted with ethyl acetate. The organic layer was washed with brine, and dried over Na_2SO_4. The solvent was removed *in vacuo*, and the residue was purified by column chromatography on SiO_2 using ethyl acetate / hexanes (1 : 3) as an eluent to give **15a** (2.80 g, Y = 97 %) as an oil. IR (Neat): 3480, 2110, 1740 cm^{-1}. FAB-MS m/z: 252 (MH^+). ^{1}H-NMR ($CDCl_3$)δ: 2.99 (1H, d, J = 6.9 Hz, D_2O exch., OH), 3.86 (3H, s, OMe), 3.87 (3H, s, OMe), 4.46 (1H, dd, J = 7.3, 2.6 Hz, 2-H), 5.39 (1H, d, J = 2.6 Hz, 3-H), 6.92 (1H, m, Ar-H), 7.03 (1H, m, Ar-H), 7.33 (1H, m, Ar-H), 7.54 (1H, m, Ar-H).

Methyl (2*R*, 3*S*)-3-Azido-2-hydroxy-3-(4-methoxyphenyl)propionate (15b) was obtained as an oil in the same manner. Y = 86 %. IR(Nujol): 3480, 2060, 1760, 1740 cm^{-1}. FAB-MS m/z: 252 (MH^+). ^{1}H-NMR ($CDCl_3$)δ: 3.08 (1H, d, J = 6.8 Hz, D_2O exch., OH), 3.82 (3H, s, OMe), 3.84 (3H, s, OMe), 4.36 (1H, dd, J = 6.8, 2.9 Hz, 2-H), 4.81 (1H, d, J = 2.9 Hz, 3-H), 6.90-7.00 (2H, m, Ar-H), 7.30-7.50 (2H, m, Ar-H).

The conversion of **15a** and **15b** to **2e** and **2f**, respectively, was in the same manner for the preparation of **2a-2d** (see ref. 8). Physical data of **2e** and **2f** are described as follows.

2e: Amorphous powder. IR (Nujol): 3440, 1710 cm^{-1}. FAB-MS m/z: 838 (MH^+). ^{1}H-NMR ($CDCl_3$)δ: 1.13 (3H, s, 15-Me), 1.27 (3H, s, 15-Me), 1.32 (9H, s, Boc), 1.58 (1H, d, J = 7.0 Hz, D_2O exch., 7-OH), 1.65 (1H, s, D_2O exch., 1-OH), 1.76 (3H, s, 8-Me), 1.85 (1H, m, 6-H), 1.93 (3H, d, J = 1.3 Hz, 12-Me), 2.05-2.64 (3H, m, 6-H and 14-H), 2.45 (3H, s, Ac), 3.24 (1H, d, J = 5.2 Hz, D_2O exch., 2'-OH), 3.92 (1H, d, J = 7.3 Hz, 3-H), 3.94 (3H, s, OMe), 4.09 (1H, d, J = 8.4 Hz, 20-H), 4.26 (1H, d, J = 1.8 Hz, D_2O exch., 10-OH), 4.26 (1H, m, 7-H), 4.31 (1H, d, J = 8.4 Hz, 20-H), 4.70 (1H, m, 2'-H), 4.96 (1H, m, 5-H), 5.21 (1H, d, J = 1.8 Hz, 10-H), 5.46 (2H, m, 3'-H and 3'-NH), 5.68 (1H, d, J = 7.3 Hz, 2-H), 6.29 (1H, m, 13-H), 6.93 (1H, m, 3'-Ar-H), 7.00 (1H, m, 3'-Ar-H), 7.24-7.33 (2H, m, 3'-Ar-H), 7.47 (2H, m, 2-Ar-H), 7.60

(1H, m, 2-Ar-H), 8.11 (2H, m, 2-Ar-H).

2f: Amorphous powder. IR (Nujol): 3400, 1730, 1710 cm^{-1}. FAB-MS m/z: 838 (MH^+). ^{1}H-NMR ($CDCl_3$)δ: 1.13 (3H, s, 15-Me), 1.23 (3H, s, 15-Me), 1.34 (9H, s, Boc), 1.64 (1H, m, D_2O exch., 7-OH), 1.72 (1H, s, D_2O exch., 1-OH), 1.75 (3H, s, 8-Me), 1.85 (1H, m, 6-H), 1.86 (3H, s, 12-Me), 2.27 (2H, m, 14-H), 2.37 (3H, s, Ac), 2.57 (1H, m, 6-H), 3.42 (1H, d, J = 5.6 Hz, D_2O exch., 2'-OH), 3.78 (3H, s, OMe), 3.91 (1H, d, J = 6.9 Hz, 3-H), 4.19 (1H, d, J = 8.4 Hz, 20-H), 4.22 (1H, d, J = 1.7 Hz, D_2O exch., 10-OH), 4.20-4.30 (1H, m, 7-H), 4.31 (1H, d, J = 8.4 Hz, 20-H), 4.57 (1H, m, 2'-H), 4.94 (1H, m, 5-H), 5.17 (1H, m, 3'-H), 5.21 (1H, d, J = 1.6 Hz, 10-H), 5.38 (1H, m, 3'-NH), 5.68 (1H, d, J = 6.9 Hz, 2-H), 6.20 (1H, m, 13-H), 6.87-6.94 (2H, m, 3'-Ar-H), 7.28-7.32 (2H, m, 3'-Ar-H), 7.45-7.53 (2H, m, 2-Ar-H), 7.61 (1H, m, 2-Ar-H), 8.08-8.13 (2H, m, 2-Ar-H).

General Procedure for the Synthesis of 3: A mixture of Paclitaxel (Indena® , purity > 98 %) (150 mg, 0.176 mmol), **5** (0.528 mmol) and DCC (127 mg, 0.616 mmol) in dichloromethane (8 mL) was stirred at rt for 26 h. The precipitate was removed by filtration, and the filtrate was concentrated *in vacuo*. The residue was purified by column chromatography on SiO_2 using ethyl acetate / hexanes (1 : 2) as an eluent to give 2'-esters. The 2'-ester was dissolved in THF (10 mL). To the solution was added methanesulfonic acid (19 mg, 0.198 mmol) and 10 % palladium on activated carbon (50 mg), and the mixture was hydrogenated at rt for 3 h. The catalyst was removed by filtration, and the filtrate was concentrated to 2 mL *in vacuo*. To the concentrated solution was added Et_2O and the mixture was stirred at rt for 1 h. The resulting precipitate was collected by filtration to give **3** as an amorphous powder.

General Procedure for the Synthesis of 4: A mixture of **5** (2.5 mmol), Et_3N (253 mg, 2.5 mmol) and *iso*-propyl chloroformate (306 mg, 2.5 mmol) in THF (10 mL) was stirred at -15°C for 30 min, then **2** (0.50 mmol) in THF (10 mL) was added. The mixture was stirred at -15°C for 5 h. The mixture was poured into ice-aqueous sodium bicarbonate and extracted with ethyl acetate. The organic layer was washed with water and brine, and dried over Na_2SO_4. The solvent was removed *in vacuo*, and the residue was purified by column chromatography on SiO_2 using ethyl acetate / hexanes (2 : 1) to give 2'-esters. The 2'-ester was hydrogenated in the same manner as described for the preparation of **3**, to give **4** as an amorphous powder.

Analytical and physical data of **3** and **4** are summarized in **Table 5**.

Antitumor Activity against B16 Melanoma: Male BDF1 mice (5 weeks old) were inoculated intraperitoneally, or subcutaneously in the left inquinal region, with B16 melanoma cells (20 % homogenate / 0.15 mL / body) on day 0. Each compound was administered daily *i.p.* or *i.v.* as a single injection (0.2 mL /

Table 4 Physical data for **5**

compd. No.	mp	IR (cm^{-1}) ν	FAB-MS m/z	^{1}H-NMR ($CDCl_3$) δ
5a	oil	3350, 1750	282 (MH^+)	1.47 (3H, d, J = 7.3 Hz, Me), 4.49 (1H, qd, J = 7.3, 7.1 Hz, CH), 4.63 (1H, d, J = 16.2 Hz, $OC\underline{H}_2COOH$), 4.78 (1H, d, J = 16.2 Hz, $OC\underline{H}_2COOH$), 5.11 (2H, m, $PhC\underline{H}_2$), 5.37 (1H, d, J = 7.1 Hz, NH), 7.25-7.42 (5H, m, Ph)
5b	56-57°C (toluene-*iso*-Pr_2O)	3350, 1760, 1730, 1690	282 (MH^+)	2.54-2.69 (2H, m, CH_2), 3.42-3.57 (2H, m, CH_2), 4.66 (2H, s, $OC\underline{H}_2COOH$), 5.09, 5.14 (total 2H, br s, $PhC\underline{H}_2$), 5.46, 6.24 (total 1H, br s, NH), 7.29-7.37 (5H, m, Ph)
5c	oil	1760, 1710, 1660	308 (MH^+)	1.83-2.10 (2H, m, CH_2), 2.11-2.34 (2H, m, CH_2), 3.45-3.69 (2H, m, CH_2), 4.41-4.85 (3H, m, CH and $OC\underline{H}_2COOH$), 5.05-5.22 (2H, m, $PhC\underline{H}_2$), 7.27-7.38 (5H, m, Ph)
5d	137-138°C (THF-Et_2O)	3350, 3280, 1760, 1740, 1690, 1660	325 (MH^+)	(DMSO-d_6) 3.66 (2H, d, J = 6.4 Hz, CH_2) , 3.95 (2H, d, J = 5.9 Hz, CH_2), 4.60 (2H, s, $OC\underline{H}_2COOH$), 5.03 (2H, s, $PhC\underline{H}_2$), 7.22-7.42 (5H, m, Ph), 7.50 (1H, t, J = 6.4 Hz, NH), 8.34 (1H, t, J = 5.9 Hz, NH), 13.0 (1H, br s, COOH)
5e	75-77°C (toluene-hexanes)	3330, 1740, 1705, 1690	416 (MH^+)	2.97 (1H, dd, J = 17.0, 4.7 Hz, CH_2), 3.14 (1H, dd, J = 17.0, 4.7 Hz, CH_2), 4.47 (1H, d, J = 16.4 Hz, $OC\underline{H}_2COOH$), 4.60 (1H, d, J = 16.4 Hz, $OC\underline{H}_2COOH$), 4.73 (1H, m, CH), 5.10 (2H, s, $PhC\underline{H}_2$), 5.18 (2H, s, $PhC\underline{H}_2$), 5.92 (1H, d, J = 8.6 Hz, NH), 7.33 (10H, m, Ph)
5f	66-67°C (toluene-hexanes)	3350, 1760, 1735, 1710, 1700	354 (MH^+)	1.25 (3H, t, J = 7.0 Hz, $CO_2CH_2C\underline{H}_3$), 2.98 (1H, dd, J = 17.0, 5.0 Hz, CH_2), 3.13 (1H, dd, J = 17.0, 5.0 Hz, CH_2), 4.21 (2H, q, J = 7.0 Hz, $CO_2C\underline{H}_2CH_3$), 4.60-4.70 (3H, m, CH and $OC\underline{H}_2COOH$), 5.12 (2H, s, $PhC\underline{H}_2$), 5.89 (1H, br d, J = 8.4 Hz, NH), 7.35 (5H, m, Ph)
5g	oil	3330, 1750	384 (NH^+)	2.98 (1H, dd, J = 17 0, 4.7 Hz, CH_2), 3.15 (1H, dd, J = 17.0, 4.7 Hz, CH_2), 3.35 (3H, s, OMe), 3.59 (2H, m, $C\underline{H}_2OMe$), 4.30 (2H, m, $C\underline{H}_2CH_2OMe$), 4.62 (2H, m, $OC\underline{H}_2COOH$), 4.71 (1H, m, CH), 5.11 (2H, s, $PhC\underline{H}_2$), 6.00 (1H, br d, J = 8.5 Hz, NH), 7.34 (5H, m, Ph)
5h	74-76°C (EtOH-Et_2O-hexanes)	3440, 3320, 3240, 1730 1700, 1670	325 (MH^+)	2.65 (1H, dd, J = 16.4, 8.5 Hz, CH_2), 2.82 (1H, dd, J = 16.4, 5.3 Hz, CH_2), 4.30 (1H, m, CH), 4.54 (2H, s, $OC\underline{H}_2COOH$), 4.90 (1H, d, J = 8.1 Hz, NH), 5.03 (2H, s, $PhC\underline{H}_2$), 7.27-7.43 (6H, m, Ph and NH_2), 7.13 (1H, br s, NH_2)

Table 5 Analytical and physical data for **3** and **4**

compd.	IR (cm^{-1})	FAB-MS	Formula	Analysis (%) Calcd (Found)			^{1}H-NMR (DMSO-d_6)
No.	ν	m/z (MH^+)		C	H	N	δ
3a	3320, 1750, 1720	983	$C_{52}H_{58}N_2O_{17}$ • CH_3SO_3H	58.99 (58.73	5.79 5.81	2.60 2.41)	0.99 (3H, s, 15-Me), 1.02 (3H, s, 15-Me), 1.49 (3H, s, 8-Me), 1.60-1.80 (3H, m, 7-OH, 1-OH and 6-H), 1.76 (3H, s, 12-Me), 2.11 (3H, s, 10-Ac), 2.22 (3H, s, 4-Ac), 2.10-2.20 (3H, m, 6H and 14-H), 2.33 (3H, s, Ms), 2.73 (2H, m, 2'-C<u>H</u>$_2$NH$_3^+$), 2.97 (2H, m, 2'-C<u>H</u>$_2$CH$_2$NH$_3^+$), 3.55 (1H, d, J = 7.0 Hz, 3-H), 3.80 (3H, br s, 2'-NH$_3^+$), 4.00-4.10 (3H, m, 7-H and 20-H), 4.80-4.95 (3H, m, 2'-OC<u>H</u>$_2$CO$_2$ and 5-H), 5.20-5.35 (3H, m, 2'-H, 2-H and 3'-H), 5.75-5.80 (1H, m, 13-H), 6.28 (1H, s, 10-H), 7.20-8.00 (15H, m, Ar-H), 9.27 (1H, d, J = 8.4 Hz, 3'-NH)
3b	3600-3200 (br) 1750, 1720	1055	$C_{55}H_{62}N_2O_9$ • CH_3SO_3H	58.43 (58.31	5.78 5.92	2.43 2.18)	0.99 (3H, s, 15-Me), 1.02 (3H, s, 15-Me), 1.23 (3H, t, J = 7.1 Hz, C<u>H</u>$_3$-CH$_2$O), 1.49 (3H, s, 8-Me), 1.59-1.79 (3H, m, 7-OH, 1-OH and 6-H), 1.77 (3H, s, 12-Me), 2.11 (3H, s, 10-Ac), 2.20 (3H, s, 4-Ac), 2.10-2.20 (3H, m, 6-H and 14-H), 2.33 (3H, s, Ms), 2.97-3.08 (2H, m, C<u>H</u>$_2$CHNH$_3^+$), 3.55 (1H, d, J = 7.0 Hz, 3-H), 3.80-4.25 (6H, m, CH$_3$C<u>H</u>$_2$O, 20-H, 7-H and 2'-H), 4.40 (1H, br s, C<u>H</u>NH$_3^+$), 4.82-4.91 (2H, m, OCH$_2$CO$_2$), 4.95 (1H, m, 5-H), 5.32 (1H, d, J = 7.0 Hz, 2-H), 5.50-5.54 (1H, m, 3'-H), 5.75-5.81 (1H, m, 13-H), 6.28 (1H, s, 10-H), 7.16-8.08 (15H, m, Ar-H), 8.42 (3H, br s, NH$_3^+$), 9.26 (1H, d, J = 8.5 Hz, 3'-NH)
4a	3420, 1760, 1710	937	$C_{48}H_{60}N_2O_{17}$ • CH_3SO_3H • $2H_2O$	55.05 (54.92	6.41 6.51	2.62 2.43)	0.98 (6H, s, 15-Me), 1.38 (9H, s, Boc), 1.40-1.82 (3H, m, 6-H and 14-H), 1.47 (3H, d, J = 7.3 Hz, C<u>H</u>$_3$CHNH$_3^+$), 1.51 (3H, s, 8-Me), 1.69 (3H, s, 12-Me), 2.21 (3H, s, Ac), 2.17-2.30 (1H, m, 6-H), 2.32 (3H, s, Ms), 3.61 (1H, d, J = 6.7 Hz, 3-H), 3.98-4.04 (3H, m, 20-H and 7-H), 4.26 (1H, br s, OH), 4.44 (1H, br s, OH), 4.75 (1H, m, C<u>H</u>NH$_3^+$), 4.87-5.10 (7H, m, 5-H, OH, 10-H, 2'-H, 3'-H and C<u>H</u>$_2$CO$_2$), 5.39 (1H, d, J = 7.2 Hz, 2-H), 5.80 (1H, m,13-H), 7.10-8.00 (11H, m, Ar-H and 3'-NH), 8.40 (3H, br s, NH$_3^+$)

4b	3400, 1760, 1720	963	$C_{50}H_{62}N_2O_{17}$ • CH_3SO_3H • $1.5H_2O$	56.40 (56.19	6.40 6.32	2.58 2.39)	0.98 (6H, s, 15-Me), 1.38 (9H, s, Boc), 1.51 (3H, s, 8-Me), 1.68 (3H, s, 12-Me), 1.71-2.42 (8H, m, 6-H, 14-H and $C\underline{H}_2C\underline{H}_2CH$), 2.21 (3H, s, Ac), 2.31 (3H, s, Ms), 3.18-3.45 (2H, m, $C\underline{H}_2NH_2^+$), 3.62 (1H, d, J = 7.3 Hz, 4-H), 3.97-4.07 (1H, m, 7-H), 4.01 (1H, br s, OH), 4.01 (1H, d, J = 7.2 Hz, 20-H), 4.05 (1H, d, J = 7.2 Hz, 20-H), 4.40-4.49 (1H, m, $C\underline{H}NH_2^+$), 4.53-4.62 (1H, m, 2'-H), 4.86-4.99 (3H, m, 5-H and $OC\underline{H}_2CO_2$), 5.02-5.14 (3H, m, 10-H, OH and OH), 5.19 (1H, br d, J = 7.6 Hz, 3'-H), 5.40 (1H, d, J = 7.2 Hz, 2-H), 5.72-5.83 (1H, m, 13-H), 7.13-7.22 (1H, m, 2-Ar-H), 7.32-7.47 (4H, m, 2-Ar-H), 7.62-7.77 (3H, m, 3'-Ar-H), 7.91 (1H, br d, J = 8.9 Hz, 3'-NH), 7.95-8.05 (2H, m, 3'-Ar-H), 9.00 (1H, br s, NH_2^+), 9.50 (1H, br s, NH_2^+)
4c	3400, 1760, 1710	980	$C_{49}H_{61}N_3O_{18}$ • CH_3SO_3H • $3H_2O$	53.14 (53.01	6.33 6.30	3.72 3.50)	0.98 (6H, s, 15-Me), 1.38 (9H, s, Boc), 1.51 (3H, s, 8-Me), 1.60-1.90 (3H, m, 6-H and 14-H), 1.68 (3H, s, 12-Me), 2.18-2.28 (1H, m, 6-H), 2.22 (3H, s, Ac), 2.30 (3H, s, Ms), 3.40 (3H, br s, NH_3^+), 3.59-3.70 (3H, m, 3-H, $C\underline{H}_2NH_3^+$), 3.96-4.06 (4H, m, 7-H, 20-H, OH), 4.11 (2H, d, J = 5.8 Hz, $NHC\underline{H}_2$), 4.44 (1H, br s, OH), 4.87-5.10 (7H, m, 5-H, 10-H, 2'-H, 3'-H, $OC\underline{H}_2CO_2$ and OH), 5.40 (1H, d, J = 7.0 Hz, 2-H), 5.76-5.81 (1H, m, 13-H), 7.18-7.21 (1H, m, 3'-Ar-H), 7.25-7.52 (4H, m, 3'-Ar-H), 7.63-7.70 (2H, m, 2-Ar-H), 7.70-7.77 (1H, m, 2-Ar-H), 7.90-8.05 (3H, m, 2-Ar-H, 3'-NH), 8.82-8.86 (1H, m, NH)
4d	3400, 1740, 1710	981	$C_{49}H_{60}N_2O_{19}$ • CH_3SO_3H • $3H_2O$	53.09 (52.80	6.23 6.20	2.47 2.44)	0.98 (6H, s, 15-Me), 1.38 (9H, s, Boc), 1.50-1.55 (1H, m, 14-H), 1.52 (3H, s, 8-Me), 1.60-1.72(1H, m, 6-H), 1.70 (3H, s, 12-Me), 1.78-1.87 (1H, m, 14-H), 2.23 (3H, s, Ac), 2.25-2.35 (1H, m, 6-H), 2.30 (3H, s, Me), 2.96-3.05 (2H, m, $C\underline{H}_2CHNH_3^+$), 3.63 (1H, d, J = 7.0 Hz, 3-H), 3.97-4.05 (3H, m, 7-H and 20-H), 4.20-4.26 (1H, m, $C\underline{H}NH_3^+$), 4.41 (1H, br s, OH), 4.79-5.01 (5H, m, 5-H, $OC\underline{H}_2CO$, OH and OH), 5.02-5.12(1H, m, 3'-H), 5.08 (1H, s, 10-H), 5.18 (1H, d, J = 7.4 Hz, 2'-H), 5.40 (1H, d, J = 7.2 Hz, 2-H), 5.76-5.83 (1H, m, 13-H), 7.14-7.22 (1H, m, 3'-Ar-H), 7.29-7.45 (4H, m, 3'-Ar-H), 7.60-7.70 (2H, m, 2-Ar-H), 7.70-7.77 (1H, m, 2-Ar-H), 7.89 (1H, d, J = 9.3Hz, 3'-NH), 7.92-8.01 (2H, m, 2-Ar-H), 8.29 (4H, br s, NH_3^+ and CO_2H)

4e	3400, 1750, 1720	1009	$C_{51}H_{64}N_2O_{19}$ • CH_3SO_3H • $2H_2O$	54.73 (54.61	6.36 6.38	2.45 2.38)	0.98 (6H, s, 15-Me), 1.23 (3H, t, J = 7.1 Hz, $C\underline{H}_3CH_2O$), 1.38 (9H, s, Boc), 1.51 (3H, s, 8-Me), 1.59-1.79 (1H, m, 14-H), 1.69 (3H, s, 12-Me), 1.79-1.90 (1H, m, 6H), 2.20-2.30 (2H, m, 6-H and 14-H), 2.22 (3H, s, Ac), 2.30 (3H, s, Ms), 3.01 (1H, dd, J = 17.6, 5.5 Hz, $C\underline{H}_2CHNH_3^+$), 3.09 (1H, dd, J = 17.6, 5.5 Hz, $C\underline{H}_2CHNH_3^+$), 3.62 (1H, d, J = 7.0 Hz, 3-H), 3.97-4.07 (3H, m, 7-H and 20-H), 4.17-4.28 (2H, m, $C\underline{H}_2CH_3$), 4.38-4.45 (2H, m, $C\underline{H}NH_3^+$ and OH), 4.80-5.20 (8H, m, 5-H, 10-H, 2'-H, 3'-H, OH, OH and OCH_2CO_2), 5.40 (1H, d, J = 7.0 Hz, 2-H), 5.76-5.82 (1H, m, 13-H), 7.18 (1H, m, 3'-Ar-H), 7.35-7.44 (4H, m, 3'-Ar-H), 7.62-7.70 (2H, m, 2-Ar-H), 7.71-7.75 (1H, m, 2-Ar-H), 7.90 (1H, d, J = 8.6 Hz, NH), 7.94-8.00 (2H, m, 2-Ar-H), 8.44 (3H, br s, NH_3^+)
4f	3420, 1755, 1715	1039	$C_{52}H_{66}N_2O_{20}$ • CH_3SO_3H • $2H_2O$	54.35 (54.13	6.37 6.34	2.39 2.32)	0.98 (6H, s, 15-Me), 1.37 (9H, s, Boc), 1.48-1.54 (1H, m, 14-H), 1.51 (3H, s, 8-Me), 1.60-1.70 (1H, m, 6-H), 1.69 (3H, s, 12-Me), 1.78-1.84 (1H, m, 14-H), 2.23 (3H, s, Ac), 2.23-2.30 (1H, m, 6-H), 2.30 (3H, s, Ms), 3.01 (1H, dd, J = 17.6, 5.5 Hz, $C\underline{H}_2CHNH_3^+$), 3.09 (1H, dd, J = 17.6, 5.5 Hz, $C\underline{H}_2CHNH_3^+$), 3.26 (3H, s, OMe), 3.54-3.59 (2H, m, $MeOC\underline{H}_2$), 3.62 (1H, d, J = 7.0 Hz, 3-H), 3.99-4.05 (3H, m, 7-H and 20-H), 4.26-4.32 (2H, m, $MeOCH_2C\underline{H}_2$), 4.41-4.47 (2H, m, $C\underline{H}NH_3^+$ and OH), 4.80-4.90 (3H, m, 5-H and $OC\underline{H}_2CO_2$), 4.94 (1H, m, OH), 4.99 (1H, m, OH), 5.07 (1H, s, 10-H), 5.04-5.12 (1H, m, 3'-H), 5.17 (1H, d, J = 7.0 Hz, 2'-H), 5.40 (1H, d, J =7.0 Hz, 2-H), 5.74-5.80 (1H, m, 13-H), 7.18 (1H, m, 3'-Ar-H), 7.29-7.41 (4H, m, 3'-Ar-H), 7.65 (2H, m, 2-Ar-H), 7.73 (1H, m, 2-Ar-H), 7.90 (1H, d, J = 9.0 Hz, 3'-NH), 7.98 (2H, m, 2-Ar-H), 8.43 (3H, br s, NH_3^+)
4g	3400, 1740, 1700	980	$C_{49}H_{61}N_3O_{18}$ • CH_3SO_3H • $3H_2O$	53.14 (53.06	6.33 6.21	3.72 3.65)	0.98 (6H, s, 15-Me), 1.38 (9H, s, Boc), 1.52 (3H, s, 8-Me), 1.54-1.70 (2H, m, 6-H and 14-H), 1.70 (3H, s, 12-Me), 1.78-1.93 (1H, m, 14-H), 2.20 (3H, s, Ac), 2.20-2.30 (1H, m, 6-H), 2.31 (3H, s, Ms), 2.94 (1H, dd, J = 17.3, 7.7 Hz, $C\underline{H}_2CHNH_3^+$), 3.06 (1H, dd, J = 17.3, 4.8 Hz, $C\underline{H}_2CHNH_3^+$), 3.62 (1H, d, J = 7.0 Hz, 3-H), 3.97-4.09 (4H, m, 7-H, 20-H and $C\underline{H}NH_3^+$), 4.43 (1H, br s, OH), 4.74-4.96 (4H, m, $OC\underline{H}_2CO_2$, OH and OH), 4.99 (1H, m, 5-H), 5.05 (2H, m, 10-H and 3'-H), 5.19 (1H, d, J = 7.4 Hz, 2'-H), 5.40 (1H, d, J = 7.0 Hz, 2-H), 5.80 (1H, m, 13-H), 7.19 (1H, m, 3'-Ar-H), 7.33-7.48 (4H, m, 3'-Ar-H), 7.62-7.69 (3H, m, 2-Ar-H and BocN$\underline{H}$), 7.69-7.77 (1H, m, 2-Ar-H), 7.83-7.93 (2H, m, NH_2), 7.99 (2H, m, 2-Ar-H), 8.09 (3H, br s, NH_3^+)

4h	3440~3400, 1750, 1710	1027	$C_{51}H_{63}N_2O_{19}F$ • CH_3SO_3H • $2.5H_2O$	53.46 (53.42	6.21 6.06	2.39 2.35)	1.02 (6H, s, 15-Me), 1.25 (3H, t, J = 7.0 Hz, $C\underline{H}_3CH_2$), 1.39 (9H, s, Boc), 1.55 (3H, s, 8-Me), 1.69 (2H, m, 6-H and 14-H), 1.73 (3H, s, 12-Me), 1.95 (1H, m, 14-H), 2.27 (3H, s, Ac), 2.30 (1H, m, 6-H), 2.33 (3H, s, Ms), 3.03 (1H, dd, J = 17.7, 6.0 Hz, $C\underline{H}_2CHNH_3^+$), 3.11 (1H, dd, J = 17.7, 5.6 Hz, $C\underline{H}_2CHNH_3^+$), 3.68 (1H, d, J = 7.0 Hz, 3-H), 4.05 (3H, m, 7-H and 20-H), 4.24 (2H, m, $CH_3C\underline{H}_2$), 4.42 (1H, m, $C\underline{H}NH_3^+$), 4.52 (1H, s, OH), 4.79-5.00 (5H, m, 5-H, $OC\underline{H}_2CO_2$, OH and OH), 5.11 (1H, s, 10-H), 5.17 (1H, m, 3'-H), 5.24 (1H, d, J = 7.0 Hz, 2'-H), 5.45 (1H, d, J = 6.9 Hz, 2-H), 5.86 (1H, m, 13-H), 7.26 (2H, m, 3'-Ar-H), 7.45 (2H, m, 3'-Ar-H), 7.62 (2H, m, 2-Ar-H), 7.73 (1H, m, 2-Ar-H), 7.91 (1H, d, J = 9.5 Hz, 3'-NH), 8.00 (2H, m, 2-Ar-H), 8.43 (3H, br s, NH_3^+)
4i	3420, 1750, 1710	1027	$C_{51}H_{63}N_2O_{19}F$ • CH_3SO_3H • $2.5H_2O$	53.46 (53.41	6.21 6.18	2.39 2.29)	0.99 (6H, s, 15-Me), 1.23 (3H, t, J = 7.1 Hz, $C\underline{H}_3CH_2O$), 1.37 (9H, s, Boc), 1.52 (3H, s, 8-Me), 1.62-1.66 (2H, m, 6-H and 14-H), 1.71 (3H, s, 12-Me), 1.89-1.95 (1H, m, 14-H), 2.24-2.30 (1H, m,6-H), 2.26 (3H, s, Ac), 2.30 (3H, s, Ms), 3.01 (1H, dd, J = 17.6, 5.7 Hz, $C\underline{H}_2CHNH_3^+$), 3.09 (1H, dd, J = 17.6, 5.2 Hz, $C\underline{H}_2CHNH_3^+$), 3.64 (1H, d, J = 7.7 Hz, 3-H), 3.99-4.05 (3H, m, 7-H and 20-H), 4.22 (2H, q, J = 7.1 Hz, $CH_3C\underline{H}_2O$), 4.40 (1H, m, $C\underline{H}NH_3^+$), 4.48 (1H, s, OH), 4.77-5.05 (4H, m, 5-H, 2'-H and $OC\underline{H}_2CO_2$), 5.08-5.27 (4H, m, 10-H, 3'-H, OH and OH), 5.42 (1H, d, J = 7.0 Hz, 2-H), 5.82 (1H, m, 13-H), 7.04 (1H, m, 3'-Ar-H), 7.14 (1H, m, 3'-Ar-H), 7.33 (1H, m, 3'-Ar-H), 7.43 (1H, m, 3'-Ar-H), 7.60 (2H, m, 2-Ar-H), 7.72 (1H, m, 2-Ar-H), 7.89 (1H, d, J = 10.3 Hz, 3'-NH), 7.99 (2H, m, 2-Ar-H), 8.41 (3H, s, $N\underline{H}_3^+$)
4j	3420, 1760, 1720	1045	$C_{51}H_{62}N_2O_{19}F_2$ • CH_3SO_3H • $2H_2O$	53.06 (52.99	5.99 5.92	2.38 2.35)	1.01 (3H, s, 15-Me), 1.03 (3H, s, 15-Me), 1.21 (3H, t, J = 7.1 Hz, $C\underline{H}_3CH_2O$), 1.36 (9H, s, Boc), 1.53 (3H, s, 8-Me), 1.67 (1H, m, 6-H), 1.74 (3H, s, 12-Me), 1.94 (1H, m, 14-H), 2.08 (1H, m, 14-H), 2.23 (3H, s, Ac), 2.27 (1H, m, 6-H), 2.31 (3H, s, Ms), 3.01 (1H, dd, J = 17.5, 5.5 Hz, $C\underline{H}_2CHNH_3^+$), 3.09 (1H, dd, J = 17.5, 5.4 Hz, $C\underline{H}_2CHNH_3^+$), 3.69 (1H, d, J = 6.9 Hz, 3-H), 4.04 (3H, m, 7-H and 20-H), 4.20 (2H, q, J = 7.0 Hz, $CH_3C\underline{H}_2O$), 4.39 (1H, m, $C\underline{H}NH_3^+$), 4.64 (1H, s, OH), 4.79 (1H, d, J = 16.5 Hz, $OC\underline{H}_2CO_2$), 4.90 (1H, d, J = 16.2 Hz, OCH_2CO_2), 4.92 (1H, m, 5-H), 4.98 (1H, br s, OH), 5.02 (1H, br s, OH), 5.11 (1H, s, 10-H), 5.16 (1H, d, J = 5.8 Hz, 2'-H), 5.45 (1H, d, J = 7.1 Hz, 2-H), 5.53 (1H, dd, J = 9.6, 5.8 Hz, 3'-H), 5.89 (1H, m, 13-H), 7.18-7.32 (2H, m, 3'-Ar-H), 7.60 (2H, m, 2-Ar-H), 7.70 (2H, m, 2-Ar-H and 3'-Ar-H), 8.00 (3H, m, 2-Ar-H and 3'-NH), 8.44 (3H, br s, $N\underline{H}_3^+$)

4k	3400, 1750, 1710	1039	$C_{52}H_{66}N_2O_{20}$ • CH_3SO_3H • $3H_2O$	53.53 (53.48	6.44 6.40	2.36 2.31)	1.00 (3H, s, 15-Me), 1.03 (3H, s, 15-Me), 1.21 (3H, t, J = 7.0 Hz, $C\underline{H}_3CH_2O$), 1.36 (9H, s, Boc), 1.53 (3H, s, 8-Me), 1.70 (1H, m, 6-H), 1.74 (3H, s, 12-Me), 1.80-2.10 (2H, m, 6-H and 14-H), 2.24 (3H, s, Ac), 2.28 (1H, m, 6-H), 2.30 (3H, s, Ms), 3.00 (1H, dd, J = 17.7, 5.9 Hz, $C\underline{H}_2CHNH_3^+$), 3.06 (1H, dd, J = 17.7, 5.9 Hz, $C\underline{H}_2CHNH_3^+$), 3.67 (1H, d, J = 6.9 Hz, 3-H), 3.83 (3H, s, MeO), 4.00-4.10 (3H, m, 7-H and 20-H), 4.20 (2H, q, J = 7.0 Hz, $CH_3C\underline{H}_2$), 4.38 (1H, m, $C\underline{H}NH_3^+$), 4.59 (1H, s, OH), 4.73 (1H, d, J = 16.5 Hz, $OC\underline{H}_2CO_2$), 4.86 (1H, d, J = 16.5 Hz, $OC\underline{H}_2CO_2$), 4.84-5.01 (3H, m, 5-H, OH and OH), 5.10-5.13 (2H, m, 10-H and 2'-H), 5.43 (1H, d, J = 7.1 Hz, 2-H), 5.65 (1H, dd, J = 8.0, 5.8 Hz, 3'-H), 5.83 (1H, m, 13-H), 7.02 (2H, m, 3'-Ar-H), 7.20 (1H, m, 3'-Ar-H), 7.51 (1H, m, 3'-Ar-H), 7.62 (2H, m, 2-Ar-H), 7.75 (2H, m, 2-Ar-H and 3'-NH), 8.00 (2H, m, 2-Ar-H), 8.40 (3H, br s, $N\underline{H}_3^+$)
4l	3400, 1750, 1710	1039	$C_{52}H_{66}N_2O_{20}$ • CH_3SO_3H • $3H_2O$	53.53 (53.40	6.44 6.38	2.36 2.29)	1.00 (6H, s, 15-Me), 1.22 (3H, t, J = 7.1 Hz, $C\underline{H}_3CH_2$), 1.36 (9H, s, Boc),1.52 (3H, s, 8-Me), 1.60-1.80 (2H, m, 6-H and 14-H), 1.71 (3H, s, 12-Me), 1.90-2.00 (1H, m, 14-H), 2.21-2.31 (1H, m, 6-H), 2.28 (3H, s, Ac), 2.31 (3H, s, Ms), 3.01 (1H, dd, J = 17.7, 5.5 Hz, $C\underline{H}_2CHNH_3^+$), 3.09 (1H, dd, J = 17.7, 5.5 Hz, $C\underline{H}_2CHNH_3^+$), 3.50 (3H, s, MeO), 3.67 (1H, d, J = 7.1 Hz, 3-H), 4.00-4.09 (3H, m, 7-H and 20-H), 4.22 (2H, m, $CH_3C\underline{H}_2O$), 4.39 (1H, br s, OH), 4.40 (1H, t, J = 5.5 Hz, $C\underline{H}NH_3^+$), 4.81 (1H, d, J = 16.5 Hz, $OC\underline{H}_2CO_2$), 4.91 (1H, d, J = 16.5 Hz, $OC\underline{H}_2CO_2$), 4.92 (1H, m, 5-H), 5.00 (1H, br s,OH), 5.09 (3H, m, 10-H, 3'-H and OH), 5.16 (1H, d, J = 6.7 Hz, 2'-H), 5.43 (1H, d, J = 7.1 Hz, 2-H), 5.80 (1H, m, 13-H), 6.92 (2H, d, J = 8.7 Hz, 3'-Ar-H), 7.28 (2H, d, J = 8.5 Hz, 3'-Ar-H), 7.61 (2H, m, 2-Ar-H), 7.70 (1H, m, 2-Ar-H), 7.77 (1H, br d, J = 9.3 Hz, 3'-NH, 7.99 (2H, m, 2-Ar-H), 8.43 (3H, br s, $N\underline{H}_3^+$)

body), from days 1 to 4 or 1 to 5. Compounds were dissolved in saline (or EtOH-Tween 80- saline). Each group except the control group consisted of five mice; the control group consisted of ten mice. Increase in life span was determined by comparing the mean survival time of treated group with that of control group.

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