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Chemical Modification of Ansamitocins. III. Synthesis and Biological Effects of 3-Acyl Esters of Maytansinol

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Several semisynthetic maytansinoids that differ in the structure of the acyl group at the C₃ position were prepared by acylation of maytansinol (3) using appropriate carboxylic acids or their active derivatives, and the effects of the compounds on the growth of *Tetrahymena pyriformis* and the survival of tumor-bearing mice were determined.

Among these analogs, the C₃ esters having a straight chain aliphatic acyl (11, 12), cycloalkanecarbonyl (18—20) or phenylacetyl group (22), and those having a 2-(*N*-acetyl-*N*-methyl)amino hexanoyl (7) or 2-(*N*-acetyl-*N*-methyl)aminophenylpropionyl group (8), strongly inhibited the growth of *T. pyriformis* and exhibited potent activity against B16 melanoma in mice. The potencies were similar to those of maytansine and ansamitocin P-3. The most striking result was the finding that the phenylglycinate (31) was superior to maytansine in terms of its broader effective dose range against ip B16 melanoma and P388 leukemia in mice; however, higher doses of the phenylglycinate were required.

Keywords—ansamitocin; maytansine; maytansinoid; esterification; *Tetrahymena pyriformis*; tubulin polymerization; B16 melanoma; P388 leukemia; antitumor activity; structure-activity relationship

The complex chemistry and the potent activity of maytansine^{1,2)} and its congeners^{3,4)} make them an especially interesting family of compounds for studies of structure-activity relations. The structure and anticancer activity have been reported for a number of maytansinoids.^{2,4,5)} However, previous studies have dealt almost exclusively with maytansinoids of plant origin; little work has been done on semisynthetic compounds.

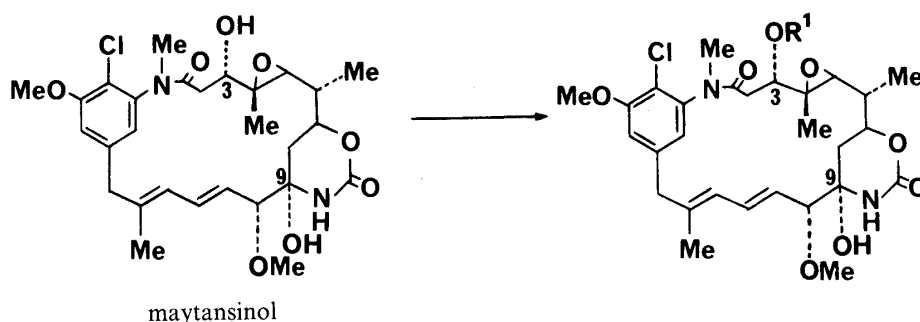
A recent paper⁶⁾ from this laboratory reported the synthesis of 4,5-deoxymaytansinoids that have antitumor activities almost equal to those of the corresponding maytansinoids. Subsequently, our studies on the synthesis and biological properties of 3-epimaytansinoids revealed that the appropriate stereochemistry of the C₃ acyloxy group is essential for the activity and that a change from the α - to the β -configuration leads to loss of the inhibitory activities.⁷⁾

Maytansinoids that differ in the structure of the C₃ acyloxy side chain have different levels of activity,^{2,5)} and this suggests that further chemical modification of the C₃ acyloxy group might result in compounds with superior activity. As an extension of our studies aimed at synthesizing better chemotherapeutic agents against human cancer, we tried to carry out systematic modifications of ansamitocin P-3 by introducing a variety of substituents at the C₃ hydroxyl group of maytansinol (3).

Chemistry

In order to study the structure-activity relationship, various semisynthetic maytansinoids with different C₃ acyl side chains were synthesized as shown in Chart 1. The structures of the

acylation of maytansinol



method	reagents	R ¹
A	R ² -COOH, DCCD, ZnCl ₂	-COR ²
B	R ² -COOH, DCCD, DMAP	-COR ²
C	R ² -NCO, ZnCl ₂	-CONHR ²
D	R ² -OCOC ₂ H ₅ , <i>n</i> -BuLi	-COOR ²

Chart 1

compounds are listed in Table I. The key intermediate was maytansinol (3), which was prepared by reductive cleavage²⁾ of ansamitocin P-3. Since the C₃ hydroxyl group of 3, the sterically hindered alcohol, shows relatively sluggish reactivity, and since the maytansinoids are generally less stable in solutions containing an acid or a base, the choice of reaction conditions, depending upon the chemical nature of the selected acylating agent, might be important. Thus, conversion of 3 to the C₃ esters was carried out by four different methods.

Method A—Maytansine analogs possessing a variety of 2-(*N*-acyl-*N*-alkyl)-aminoacyloxy groups at the C₃ position (4–8) were synthesized by esterification of maytansinol (3) with a selected *N*-acyl-*N*-alkylamino acid in the presence of dicyclohexylcarbodiimide (DCCD) and zinc chloride.⁸⁾ In this method, the reaction probably proceeds through the formation of an oxazolonium salt, the reactive cyclic intermediate, which, on subsequent coupling to the C₃ hydroxyl group, yields the desired products.⁹⁾ This method is not applicable to the reaction using *N*-acetylproline, which never forms the oxazolonium ion. The C₃ *N*-acetylproline ester (9), however, was synthesized according to Method B, described below. As in the case of the esterification of maytansine (1), the reaction of 3 with *N*-acyl-*N*-alkylamino acid gave two diastereoisomeric products with the D- and L-aminoacyl groups. The L-isomer, configurationally the same as that of maytansine,^{8,10)} was isolated by chromatography and evaluated biologically.

Method B—This method was the most convenient for preparing the C₃ esters (9–28, 31 and 32). It involved the treatment of 3 with a selected carboxylic acid in the presence of DCCD and 4-dimethylaminopyridine (DMAP),⁸⁾ satisfactory results were obtained when the reaction was accomplished with an excess of these three reagents in an inert solvent. Method B was used for the synthesis of the majority of the C₃ esters listed in Table I.

Method C—Maytansinol 3-phenylcarbamate (29) was synthesized by the reaction of 3 with phenylisocyanate in the presence of zinc chloride. Maytansinol (3) and isocyanates did not react under the usual conditions,¹¹⁾ where a base was used as a catalyst, but did react smoothly when a Lewis acid,¹²⁾ especially zinc chloride, was used to give the desired carbamoyl esters in good yield. Although carbamoyl esters other than 29 are not described here, method C has been used extensively for carbamoylation of 3.

Method D—This method of preparing alkylcarbonates of 3 involves reacting the C₃ hydroxyl of 3 with *n*-butyl lithium, followed by reaction with an alkyl chloroformate. Maytansinol 3-isopropyl carbonate (30) was synthesized according to this method. The

reaction of alcohols with alkyl chloroformates using a base as an HCl-acceptor, the usual method for preparing disubstituted carbonates, was not applicable to the reaction of **3**, presumably because of the poor reactivity of its C₃ hydroxyl group.

Maytansinol (**3**) is a diol possessing hydroxyl groups at the C₃ and C₉ positions. However, these four methods never afforded diesters under the usual esterification conditions. This can be explained by assuming that the diesters of **3** are highly labile and would decompose rapidly. The C₃ esters were purified by column chromatography and their purities were checked by high performance thin-layer chromatography (HPTLC) and high performance liquid chromatography (HPLC) analyses. The structures, including the site of esterification, were determined by mass spectrum (MS) and the proton nuclear magnetic resonance (¹H-NMR) spectroscopy in comparison with the spectra of structurally known maytansinoids, e.g., **1** and **2**.

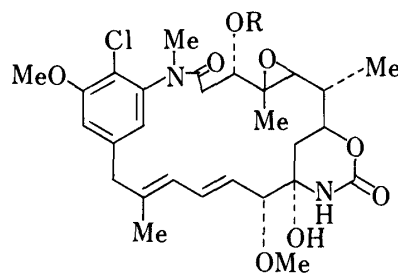
Structure-Activity Relations

The biological activities of semisynthetic maytansinoids with a variety of acyloxy side chains at the C₃ position were evaluated. The inhibition of tubulin polymerization (microtubule formation), the cytotoxicity and the antitumor activities of maytansine (**1**)²⁾ and ansamitocin P-3 (**2**)^{4,5,13)} have been extensively studied. The semisynthetic compounds listed in Table I inhibited the growth of *Tetrahymena* and the cilia regeneration of deciliated *Tetrahymena*, presumably by inhibiting tubulin polymerization, as do the naturally occurring maytansinoids, **1** and **2**. Twenty-nine new semisynthetic maytansinoids bearing a variety of C₃ acyloxy side chains could be conveniently classified into the following eight groups according to the structure: 2-(*N*-acyl-*N*-alkyl)aminoacryl esters (**4**—**9**), simple alkanooates (**10**—**17**), cycloalkanecarboxylates (**18**—**20**), alkanooates substituted with a phenyl or phenoxy group (**21**—**24**), heteroaromatic carboxylates (**25**—**28**), carbamate (**29**), carbonate (**30**), and α -substituted phenylacetates (**31**, **32**).

The modification of the C₃ *N*-acyl-*N*-alkylaminoacyloxy side chain gave new congeners of maytansine (**1**). The C₃ *N*-acetyl-*N*-methylamino acid esters (**7** and **8**) with the L-configuration potently inhibited *Tetrahymena*; the potencies were comparable to those of **1** and **2**. A change in the *N*-acyl group of the *N*-acyl-*N*-alkylamino acid moiety to benzoyl or phenoxyacetyl, as exemplified by **4** and **5**, resulted in products that retained strong activities, whereas the alteration of the *N*-alkyl group of the amino acid moiety to higher alkyl, such as benzyl (**6**) or cycloalkyl (**9**), greatly reduced the activities. These results suggest that variation of the *N*-acyl group does not affect the activity, but that the steric bulkiness of the *N*-alkyl group profoundly influences the activity.

Replacement of the C₃ side chain of **1** by simple alkanoyloxy groups gave a series of ansamitocin homologs. The compounds with a straight chain alkanoyl group containing four to seven carbon atoms (**10**—**13**) and those with a cycloalkanecarbonyl group containing five to seven carbon atoms (**18**—**20**) showed *in vitro* activities comparable to that of **2**. In both cases, an increase in the carbon atoms from the optimal number of five or six tended to lower the activity (**14**—**17**). The carboxylic acid esters having a benzene ring (**21**—**24**) showed high levels of activity. Among these compounds, **22** and **24** were found to be the most potent, and the activity was slightly greater than that of **1** or **2**. With the exception of the α -hydroxyphenylacetate (**32**) whose activity was comparable to that of **1**, the other classes of compounds—the C₃ heterocycle carboxylates (**25**—**28**), the phenylcarbamate (**29**), isopropyl carbonate (**30**) and the α -aminophenyl acetate (**31**)—have lower *in vitro* activities. The *in vitro* systems were used as bioassay tools to screen for *in vivo*-active C₃ esters.

The C₃ esters were selected for *in vivo* screening on the premise that those which exhibit potent *in vitro* activity are more likely to show *in vivo* anticancer activity. The murine B16 melanoma was used as a primary *in vivo* screen to find candidates for further development

TABLE I. Synthetic Methods for Maytansinoids and Inhibitory Activities against *Tetrahymena pyriformis*^{13,14)}

Compound	R	Method of preparation ^{a)}	MIC (μg/ml)	
			Growth inhibition	Inhibition to cilia regeneration
1	-COCH(CH ₃)N(CH ₃)COCH ₃	ref. 8	2—4	≤ 0.5
2	-COCH(CH ₃) ₂	ref. 4	2	≤ 0.5
3	-H	ref. 4	> 4	> 2
4	-COCH(CH ₃)N(CH ₃)COC ₆ H ₅	A	2—4	1
5	-COCH(CH ₃)N(CH ₃)COCH ₂ OC ₆ H ₅	A	1—2	1
6	-COCH(CH ₃)N(CH ₂ C ₆ H ₅)COCH ₃	A	> 4	> 2
7	-COCH[CH ₂ CH(CH ₃) ₂]N(CH ₃)COCH ₃	A	2	≤ 0.5
8	-COCH(CH ₂ C ₆ H ₅)N(CH ₃)COCH ₃	A	≤ 1	≤ 0.5
9	-COCHN(COCH ₃)CH ₂ CH ₂ CH ₂ -	B	> 4	> 2
10	-CO(CH ₂) ₂ CH ₃	B	2	≤ 0.5
11	-CO(CH ₂) ₃ CH ₃	B	1—2	≤ 0.5
12	-CO(CH ₂) ₄ CH ₃	B	1—2	≤ 0.5
13	-CO(CH ₂) ₅ CH ₃	B	1—2	1
14	-CO(CH ₂) ₆ CH ₃	B	2—4	> 2
15	-CO(CH ₂) ₈ CH ₃	B	> 4	> 2
16	-CO(CH ₂) ₁₁ CH ₃	B	> 4	> 2
17	-CO(CH ₂) ₁₄ CH ₃	B ^{b)}	> 4	> 2
18	-COCHCH ₂ CH ₂ -	B	4	≥ 2
19	-COCHCH ₂ CH ₂ CH ₂ -	B	≤ 1	0.5—1
20	-COCHCH ₂ CH ₂ CH ₂ CH ₂ CH ₂ -	B	≤ 1	≤ 0.5
21	-COC ₆ H ₅	B	4	> 2
22	-COCH ₂ C ₆ H ₅	B	≤ 1	≤ 0.5
23	-COCH ₂ CH ₂ C ₆ H ₅	B	2—4	1—2
24	-COCH ₂ OC ₆ H ₅	B	≤ 1	≤ 0.5
25	-CO- 	B	≥ 4	2
26	-CO- 	B	≥ 4	> 2
27	-CO- 	B	> 4	> 2
28	-CO- 	B	> 4	> 2
29	-CONHC ₆ H ₅	C	> 4	> 2
30	-COOCH(CH ₃) ₂	D	≥ 4	2
31	-COCH(NH ₂)C ₆ H ₅	B ^{b)}	> 4	> 2
32	-COCH(OH)C ₆ H ₅	B ^{b)}	2—4	0.5—1

a) See the text for methods A—D.

b) Deprotection procedure (acid hydrolysis of C₉-O-methyl ether, cleavage of *tert*-butoxycarbonyl group with CF₃COOH and alkaline hydrolysis of trifluoroacetyl group) is included; see the text for experimental methods.

TABLE II. Antitumor Activity against B-16 Melanoma

Compound	R	Dose ($\mu\text{g/kg/d}$)	Life span prolongation T/C %
1	$-\text{COCH}(\text{CH}_3)\text{N}(\text{CH}_3)\text{COCH}_3$	100	75
		50	175
		25	203
		12.5	192
2	$-\text{COCH}(\text{CH}_3)_2$	50	230
		25	230
		12.5	207
		6.25	189
7	$-\text{COCH}[\text{CH}_2\text{CH}(\text{CH}_3)_2]\text{N}(\text{CH}_3)\text{COCH}_3$	200	65
		100	202
		50	209
		25	171
8	$-\text{COCH}(\text{CH}_2\text{C}_6\text{H}_5)\text{N}(\text{CH}_3)\text{COCH}_3$	200	95
		100	205
		50	208
		25	211
11	$-\text{CO}(\text{CH}_2)_3\text{CH}_3$	100	152
		50	186
		25	216
		12.5	200
12	$-\text{CO}(\text{CH}_2)_4\text{CH}_3$	200	127
		100	189
		50	189
		25	158
13	$-\text{CO}(\text{CH}_2)_5\text{CH}_3$	400	165
		200	158
		100	158
		50	140
17	$-\text{CO}(\text{CH}_2)_{14}\text{CH}_3$	400	211
		200	177
		100	160
		50	124
18		100	210
		50	198
		25	193
		12.5	167
19		100	79
		50	190
		25	186
		12.5	193
20		200	158
		100	193
		50	188
		25	193
21	$-\text{COC}_6\text{H}_5$	400	154
		200	151
		100	151
		50	143
22	$-\text{COCH}_2\text{C}_6\text{H}_5$	50	186
		25	218
		12.5	196
		6.25	176

TABLE II. (continued)

Compound	R	Dose ($\mu\text{g/kg/d}$)	Life span prolongation $T/C\%$
24	$-\text{COCH}_2\text{OC}_6\text{H}_5$	100	182
		50	199
		25	176
		12.5	188
25		200	101
		100	199
		50	179
		25	189
26		400	48
		200	173
		100	176
		50	166
27		800	137
		400	120
		200	143
		100	123
28		800	143
		400	114
		200	124
		100	126
29		400	161
		200	151
		100	133
		50	125
30	$-\text{COOCH}(\text{CH}_3)_2$	400	130
		200	171
		100	171
		50	150
31		800	197
		400	224
		200	221
		100	203
32		100	75
		50	179
		25	209
		12.5	191

One-half ml of 1:4 tumor homogenate of B-16 melanoma in 0.9% NaCl solution was inoculated intraperitoneally into C57BL/6 $\times$ DBA/2 F1 mice on day 0. The drugs listed in Table II were injected intraperitoneally into the mice (5 and 25 mice per treated group and control group, respectively) daily for 9 consecutive days starting on day 1. Median survival times of each tested group (T) of 5 mice and the control group (C) were calculated. All these procedures are based on the protocol of the Developmental Therapeutics Program of NCI.¹⁴⁾

studies. The results are shown in Table II which includes the *in vivo* activities of maytansine (1) and ansamitocin P-3 (2), for comparative purposes.

As shown in Table II, a change in the structure of the C_3 ester group changed the activity of the compounds, though almost all of them were active against B16 melanoma in mice. Among the maytansine congeners that inhibited *T. pyriformis*, the 2-(*N*-acetyl-*N*-methyl)-amino-4-methylpentanoate (7) and the 2-(*N*-acetyl-*N*-methyl)amino-3-phenylpropionate (8) were subjected to *in vivo* antitumor screening. Both showed potent activity, comparable to that of maytansine (1). A class of *n*-alkanoic acid esters, especially those with an acyl group

TABLE III. Antitumor Activities of **1** and **31** against ip Implanted B-16 Melanoma^{a)} and P-388 Leukemia in Mice^{b)}

Compound	B-16 Melanoma		P-388 Leukemia	
	Dose ($\mu\text{g/kg/d}$)	Life span prolongation $T/C\%$	Dose ($\mu\text{g/kg/d}$)	Life span prolongation $T/C\%$
Maytansine (1)	100	75	50	100
	50	175	25	178
	25	203	12.5	196
	12.5	192	6.25	174
	6.25	166	3.12	141
	3.12	166	1.56	139
			0.78	113
Maytansinol 3-phenylglycinate (31)	800	197	800	266
	400	224	400	221
	200	221	200	223
	100	203	100	200
	50	197	50	194
	25	191	25	180
	12.5	156	12.5	160
	6.25	144	6.25	150
	3.12	132	3.12	140
	1.56	103		

a) See the footnote in Table II.

b) Tumor cells (1×10^6) were transplanted intraperitoneally into C57BL/6 $\times$ DBA/2 F1 female mice on day 0. The drugs (**1** and **31**) were injected intraperitoneally into the mice (5 and 25 mice per treated group and control group, respectively) daily for 9 consecutive days starting on day 1. Median survival times of each tested group (T) of 5 mice and the control group (C) were calculated. All these procedures are based on the protocols of the Developmental Therapeutics Program of NCI.¹⁴⁾

containing five to seven carbon atoms (**11**, **12**) showed high potency in this test system, although none showed greater efficacy than ansamitocin P-3 (**2**). The doses required for optimal *in vivo* activity tended to increase progressively with increase in the number of carbon atoms of the C_3 acyloxy side chain. The C_3 hexadecanoyl ester (**17**), despite its low *in vitro* activity, has a relatively high life-prolonging effect on B16-bearing mice at much greater doses than those of **2**. The C_3 cycloalkanecarboxylic acid esters, including those with cyclopropane (**18**), cyclobutane (**19**), and cyclohexane (**20**) rings showed potent *in vivo* activity but no distinct correlation was observed between their *in vitro* and *in vivo* activities. The C_3 benzoate (**21**), phenoxyacetate (**24**), and phenylacetate (**22**) showed potent *in vivo* activity; the potencies increased in this order and were approximately parallel to the order of the *in vitro* activities. The C_3 esters of heteroaromatic carboxylic acids (**25**–**28**) showed relatively lower activity both *in vitro* and *in vivo*. The exceptional cases are the C_3 2-furoate (**25**) and 2-pyridinecarboxylate (**26**), which showed moderate *in vivo* activity. Antitumor tests on the C_3 phenylcarbamate (**29**) and isopropylcarbonate (**30**) gave positive results, but the potencies were modest even at high doses. This finding suggests that the C_3 acyl ester function can be replaced by a carbamoyl or carbonyl ester without a great loss of the biological activities.

A study was made of the influence on the *in vivo* activity of chemical modification of the phenylacetyl moiety of **22**, because the overall drug lipophilicity–hydrophilicity balance is considered to be an important determinant of antitumor potency.¹⁴⁾ The introduction of a hydrophilic group, *e.g.*, amino or hydroxyl, into the α -position with respect to the phenylacetyl group gave the C_3 phenylglycinate (**31**) and the C_3 mandelate (**32**). Based on the results described below, the introduction of the amino group into the C_3 phenylacetate was found to

be much better than the introduction of the hydroxyl group for *in vivo* activity. The C₃ mandelate (**32**), which possessed strong *in vitro* activity, showed *in vivo* activity with a potency similar to that of the C₃ phenylacetate (**22**). However, the most dramatic finding was the *in vivo* antitumor activity of the C₃ phenylglycinate (**31**), which had a very low activity in the *in vitro* test. The life-prolonging effect on mice bearing B16 melanoma and those with P388 leukemia was comparable to those of **1** and **2**. Although the optimal doses were much greater (0.025–0.8 mg/kg/d), the effective dose ranges in the two assay systems were broader than that of **1**, as shown in Table III. The C₃ phenylglycinate (**31**) showed a 2–4 times better therapeutic ratio than maytansine (**1**). The above observations suggest that one cannot predict the degree of *in vivo* activity simply on the basis of the *in vitro* activity, but it seems likely that compounds giving low *in vitro* activity have *in vivo* activity only at doses much greater than the dose required for maytansine (**1**) or ansamitocin P-3 (**2**). The evaluation of compound **31** in additional systems employing different treatment routes and schedules, or in other tumor systems that include solid tumors remains to be done.

Further work is in progress in this laboratory aimed at finding new chemotherapeutic agents useful in cancer treatment among this important class of compounds.

Experimental

All melting points were measured on a Yanagimoto hot plate apparatus model MP-S3, and are uncorrected. Infrared (IR) spectra were recorded on a Hitachi 215 spectrometer. Mass spectra were determined with a JMS-01SC spectrometer equipped with a direct inlet system. ¹H-NMR spectra were obtained using Varian XL-100-12 and Varian EM-360 instruments: chemical shifts (δ) are reported in ppm downfield from internal TMS. For analytical thin layer chromatography, HPTLC pre-coated Kieselgel 60 F₂₅₄ (E. Merck, Art. 5642) was used. Reversed-phase HPLC analysis was performed on a Waters ALC/GPC 204 instrument using a C₁₈ μ -Bondapak column (Waters Associates, #27324). Preparative column chromatography was carried out using Kieselgel 60 (E. Merck, Art. 7743).

Maytansinol 3-Carboxylic Acid Esters. General Procedure Method A (4–8)—A solution of maytansinol (1 mmol), a selected *N,N*-disubstituted amino acid (4.5 mmol), dicyclohexylcarbodiimide (DCCD, 5.4 mmol) and ZnCl₂ (2.4 mmol) in CH₂Cl₂ (100 ml, dried over 3 Å molecular sieves) was stirred at room temperature for 30 min then small amounts of the amino acid (0.5 mmol), DCCD (0.53 mmol) and ZnCl₂ (0.53 mmol) were added and the whole was stirred for 2 h. The reaction mixture was filtered to remove a precipitate, then the filtrate was washed with water (40 ml $\times$ 2) and the separated organic layer was dried over MgSO₄ and evaporated to dryness *in vacuo*. The residue was chromatographed on a silica gel column using aqueous ethyl acetate as an eluent. The D- and L-diastereoisomers were separately eluted and subsequent work-up gave the desired L-diastereoisomer, which has an L-aminoacyloxy side chain (configurationally the same as that of maytansine (**1**)), along with the D-isomer. The yield based on **3** and spectral data for each L-diastereoisomer (**4–8**) are as follows.

Maytansinol 3-*N*-Benzoyl-*N*-methyl-L-alaninate (4**)**—21.6% yield. mp 188–195 °C (dec.). IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 1750, 1720, 1710, 1670, 1630, 1400, 1080. MS *m/e*: 710 (M⁺ – 43), 691 (M⁺ – 61), 677, 660, 657, 650, 485, 470, 450. ¹H-NMR (CDCl₃, 90 MHz) δ : 0.85 (3H, s), 1.28 (3H, d), 1.62 (3H, br s), 1.66 (3H, s), 2.85 (3H, s), 3.04 (3H, s), 3.36 (3H, s), 3.97 (3H, s), 6.82 (1H, d), 6.91 (1H, d), 7.34 (5H, s).

Maytansinol 3-*N*-Phenoxyacetyl-*N*-methyl-L-alaninate (5**)**—32.9% yield. mp 162–167 °C. IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 1750, 1710, 1700, 1670, 1660, 1460, 1080. MS *m/e*: 722 (M⁺ – 61), 707, 690, 680, 485, 470, 450. ¹H-NMR (CDCl₃, 90 MHz) δ : 0.80 (3H, s), 1.25 (3H, d), 1.33 (3H, d), 1.63 (3H, br s), 2.92 (3H, s), 3.16 (3H, s), 3.34 (3H, s), 3.96 (3H, s), 4.67 (2H, d), 6.7–7.3 (7H, m).

Maytansinol 3-*N*-Acetyl-*N*-benzyl-L-alaninate (6**)**—19.4% yield. mp 174–177 °C. IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 1750, 1730, 1710, 1670, 1650, 1580, 1080. MS *m/e*: 706 (M⁺ – 61), 691, 663, 503, 485, 470, 450. ¹H-NMR (CDCl₃, 90 MHz) δ : 0.86 (3H, s), 1.29 (3H, d), 1.36 (3H, d), 1.69 (3H, s), 2.17 (3H, s), 3.17 (3H, s), 3.35 (3H, s), 3.98 (3H, s), 4.56 (2H, s), 6.76 (1H, d), 6.84 (1H, d), 7.31–7.39 (5H, m).

Maytansinol 3-*N*-Acetyl-*N*-methyl-L-leucinate (7**)**—45.7% yield. mp 172–175 °C (dec.). IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 1750, 1720, 1710, 1670, 1650, 1580, 1080. MS *m/e*: 733 (M⁺), 716, 690, 672, 657, 640, 637, 630, 616, 485, 470, 450. ¹H-NMR (CDCl₃, 90 MHz) δ : 0.80 (3H, s), 0.93 (3H, d), 1.00 (3H, d), 1.28 (3H, s), 1.67 (3H, br s), 2.13 (3H, s), 2.83 (3H, s), 3.19 (3H, s), 3.35 (3H, s), 3.97 (3H, s), 6.82 (1H, d), 6.85 (1H, d).

Maytansinol 3-*N*-Acetyl-*N*-methyl-L-phenylalaninate (8**)**—18.1% yield. mp 188–193 °C (dec.). IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 1750, 1720, 1710, 1670, 1650, 1580, 1080. MS *m/e*: 706 (M⁺ – 61), 691, 674, 671, 664. ¹H-NMR (CDCl₃, 90 MHz) δ : 0.83 (3H, s), 1.27 (3H, d), 1.65 (3H, br s), 1.87 (3H, s), 2.71 (3H, s), 3.16 (3H, s), 3.35 (3H, s), 3.95 (3H, s), 6.77 (2H, d), 7.03–7.43 (5H, m).

Maytansinol 3-Carboxylic Acid Esters. General Procedure Method B (9—28, 31 and 32)—A solution of maytansinol (1 mmol), an appropriate carboxylic acid (6 mmol), DCCD (6 mmol) and 4-dimethylaminopyridine (2 mmol) in CH_2Cl_2 (200 ml, dried over 3 Å molecular sieves) was stirred at room temperature for 1 h. The reaction mixture was then filtered to remove a white precipitate and the filtrate was evaporated to dryness *in vacuo*. The residue was chromatographed on silica gel using MeOH in CHCl_3 as an eluent to afford the desired product. In cases with an asymmetric centre in the C_3 acyloxy side chain (9, 31 and 32), the L-diastereoisomer was separated chromatographically. The yield and physicochemical data for each L-isomer are as follows.

Maytansinol 3-N-Acetyl-L-prolinate (9)—18.4% yield. mp 195—198 °C (dec.). IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 1760, 1730, 1720, 1670, 1630, 1580, 1430, 1170, 1090. MS m/e : 642 ($\text{M}^+ - 61$), 639, 627, 607, 600, 528, 485, 470, 450. $^1\text{H-NMR}$ (CDCl_3 , 90 MHz) δ : 0.89 (3H, s), 1.28 (3H, d), 1.70 (3H, br s), 2.17 (3H, s), 3.17 (3H, s), 3.37 (3H, s), 3.98 (3H, s), 6.73 (1H, d), 6.85 (1H, d).

Maytansinol 3-Butyrate (10)—54.2% yield. The physicochemical properties of this compound were identical with those of an authentic sample.⁴⁾

Maytansinol 3-Valerate (11)—28.8% yield. mp 168—169 °C. IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 1730, 1710, 1660, 1650, 1580, 1460, 1090, 1080. MS m/e : 587 ($\text{M}^+ - 61$), 572, 555, 552, 545. $^1\text{H-NMR}$ (CDCl_3 , 90 MHz) δ : 0.83 (3H, s), 0.96 (3H, t), 1.2—1.8 (4H, m), 1.26 (3H, d), 1.68 (3H, br s), 2.23—2.56 (2H, m), 3.18 (3H, s), 3.37 (3H, s), 3.97 (3H, s), 6.54 (1H, s), 6.83 (2H, s).

Maytansinol 3-Hexanoate (12)—29.3% yield. mp 164—166 °C. IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 1730, 1710, 1660, 1650, 1580, 1460, 1100, 1080. MS m/e : 615 ($\text{M}^+ - 47$), 601 ($\text{M}^+ - 61$), 586, 569, 566, 559, 531. $^1\text{H-NMR}$ (CDCl_3 , 90 MHz) δ : 0.83 (3H, s), 0.87 (3H, t), 1.05—1.8 (9H, m), 1.69 (3H, s), 2.2—2.54 (4H, m), 3.16 (3H, s), 3.97 (3H, s), 6.79 (1H, d), 6.81 (1H, s), 6.82 (1H, d).

Maytansinol 3-Heptanoate (13)—20.4% yield. mp 161—162 °C. IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 1730, 1710, 1660, 1650, 1580, 1460, 1100, 1080. MS m/e : 676 (M^+), 659, 633, 615 ($\text{M}^+ - 61$), 600, 583, 580, 573, 545. $^1\text{H-NMR}$ (CDCl_3 , 90 MHz) δ : 0.84 (3H, s), 0.88 (3H, t), 1.1—1.8 (11H, m), 1.70 (3H, br s), 2.2—2.54 (3H, m), 3.15 (3H, s), 3.37 (3H, s), 3.99 (3H, s), 6.30 (1H, s), 6.80 (1H, d), 6.83 (1H, d).

Maytansinol 3-Octanoate (14)—39.2% yield. mp 154—156 °C. IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 1730, 1710, 1660, 1650, 1580, 1460, 1100, 1080. MS m/e : 629 ($\text{M}^+ - 61$), 614, 597, 594, 587, 559. $^1\text{H-NMR}$ (CDCl_3 , 90 MHz) δ : 0.83 (3H, s), 0.87 (3H, t), 1.1—1.8 (13H, m), 1.68 (3H, s), 2.2—2.7 (4H, m), 3.15 (3H, s), 3.37 (3H, s), 3.97 (3H, s), 6.77 (1H, d), 6.80 (1H, s), 6.83 (1H, d).

Maytansinol 3-Decanoate (15)—35.0% yield. mp 139—140 °C. IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 1730, 1710, 1660, 1650, 1580, 1460, 1100, 1080. MS m/e : 657 ($\text{M}^+ - 61$), 642, 625, 622, 615, 587, 559. $^1\text{H-NMR}$ (CDCl_3 , 90 MHz) δ : 0.83 (3H, s), 0.85 (3H, t), 1.05—1.85 (17H, m), 1.70 (3H, br s), 2.2—2.7 (4H, m), 3.16 (3H, s), 3.37 (3H, s), 3.97 (3H, s), 6.64 (1H, s), 6.80 (1H, d), 6.86 (1H, d).

Maytansinol 3-Tridecanoate (16)—30.1% yield. mp 131—133 °C. IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 1730, 1710, 1660, 1650, 1580, 1460, 1080. MS m/e : 717 ($\text{M}^+ - 43$), 699 ($\text{M}^+ - 61$), 684, 667, 664, 657, 629, 590, 552, 485, 470, 450. $^1\text{H-NMR}$ (CDCl_3 , 90 MHz) δ : 0.83 (3H, s), 0.87 (3H, t), 1.1—1.8 (23H, m), 1.68 (3H, br s), 2.2—2.7 (4H, m), 3.16 (3H, s), 3.37 (3H, s), 3.98 (3H, s), 6.66 (1H, s), 6.80 (1H, d), 6.87 (1H, d).

Maytansinol 3-Palmitate (17)—a) By esterification of 3. 16.7% yield. mp 118—121 °C. IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 1730, 1710, 1660, 1650, 1580, 1460, 1100, 1080. MS m/e : 741 ($\text{M}^+ - 61$), 726, 706, 699, 564, 485, 470, 450. $^1\text{H-NMR}$ (CDCl_3 , 90 MHz) δ : 0.81 (3H, s), 0.86 (3H, t), 1.26 (29H, m), 1.67 (3H, br s), 3.14 (3H, s), 3.34 (3H, s), 3.97 (3H, s), 6.78 (1H, d), 6.82 (1H, d).

b) By esterification of maytansinol C-9-O-methyl ether. Esterification of the C_9 -O-methyl ether of 3⁷⁾ (73.8 mg) with palmitic acid by using Method B gave the corresponding 3-palmitate (101 mg) in 97% yield. This was dissolved in 50% aq. MeOH (15 ml) and treated with 2 N HCl (2 ml) at room temperature for 10 h. The mixture was neutralized with solid NaHCO_3 and, after evaporation of methanol *in vacuo*, was extracted with ethyl acetate. The extract was dried over MgSO_4 and evaporated to dryness *in vacuo*. The residual solid was chromatographed on silica gel using aqueous ethyl acetate as an eluent. After work-up, the desired product (17) was obtained as a colorless solid (33.4 mg, 33.6%). The physicochemical data were identical with those of the product obtained directly from 3, as described above.

Maytansinol 3-Cyclopropanecarboxylate (18)—17.9% yield. mp 182—187 °C (dec.). IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 1730, 1710, 1660, 1650, 1580, 1460, 1100, 1080. MS m/e : 632 (M^+), 615, 589, 571 ($\text{M}^+ - 61$), 556, 539, 529, 501, 485, 470, 450. $^1\text{H-NMR}$ (CDCl_3 , 90 MHz) δ : 0.78—1.80 (5H, m), 0.85 (3H, s), 1.23 (3H, d), 1.70 (3H, br s), 3.18 (3H, s), 3.36 (3H, s), 3.98 (3H, s), 6.30 (1H, s), 6.84 (1H, d), 6.91 (1H, d).

Maytansinol 3-Cyclobutanecarboxylate (19)—22.1% yield. mp 187—190 °C (dec.). IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 1730, 1710, 1660, 1650, 1580, 1450, 1090, 1080. MS m/e : 646 (M^+), 628, 603, 585 ($\text{M}^+ - 61$), 570, 553, 550, 485, 470, 450. $^1\text{H-NMR}$ (CDCl_3 , 90 MHz) δ : 0.86 (3H, s), 1.25 (3H, d), 1.67 (3H, br s), 1.79—2.62 (6H, m), 3.13 (3H, s), 3.36 (3H, s), 3.96 (3H, s), 6.50 (1H, s), 6.76 (1H, d), 6.82 (1H, d).

Maytansinol 3-Cyclohexanecarboxylate (20)—24.3% yield. mp 202—206 °C (dec.). IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 1730, 1710, 1660, 1650, 1580, 1460, 1090, 1080. MS m/e : 674 (M^+), 659, 657, 631, 613 ($\text{M}^+ - 61$), 598, 578, 571, 543, 503, 502, 485, 470, 450. $^1\text{H-NMR}$ (CDCl_3 , 90 MHz) δ : 0.85 (3H, s), 1.1—2.3 (10H, m), 1.24 (3H, d), 1.69 (3H, br s), 3.13 (3H, s),

3.36 (3H, s), 3.97 (3H, s), 6.32 (1H, s), 6.45 (1H, d), 6.84 (1H, d).

Maytansinol 3-Benzoylate (21)—24.1% yield. mp 184–187 °C (dec.). IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 1730, 1720, 1660, 1580, 1270, 1100, 1080. MS m/e : 688 (M^+), 625, 607 ($M^+ - 61$), 592, 575, 572, 565, 502, 485, 470, 450. $^1\text{H-NMR}$ (CDCl_3 , 90 MHz) δ : 0.85 (3H, s), 1.30 (3H, d), 1.68 (3H, br s), 3.17 (3H, s), 3.23 (3H, s), 3.98 (3H, s), 6.20 (1H, s), 6.85 (1H, d), 7.09 (1H, d), 7.46–8.17 (5H, m).

Maytansinol 3-Phenylacetate (22)—58.7% yield. mp 180–182 °C (dec.). IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 1740, 1730, 1710, 1670, 1650, 1580, 1450, 1100, 1080. MS m/e : 621 ($M^+ - 61$), 606, 589, 586, 579, 503, 485, 470, 450. $^1\text{H-NMR}$ (CDCl_3 , 90 MHz) δ : 0.86 (3H, s), 1.27 (3H, d), 1.69 (3H, br s), 3.00 (3H, s), 3.40 (3H, s), 3.93 (3H, s), 6.60 (1H, s), 6.83 (1H, s), 7.28 (5H, br s).

Maytansinol 3-(3-Phenyl)propionate (23)—9.7% yield. mp 160–162 °C (dec.). IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 1730, 1710, 1660, 1650, 1580, 1450, 1100, 1080. MS m/e : 696 (M^+), 653, 635 ($M^+ - 61$), 485, 470. $^1\text{H-NMR}$ (CDCl_3 , 90 MHz) δ : 0.80 (3H, s), 1.27 (3H, d), 1.65 (3H, br s), 2.6–3.4 (4H, m), 3.10 (3H, s), 3.22 (3H, s), 3.95 (3H, s), 6.27 (1H, s), 6.63 (1H, d), 6.78 (1H, d), 7.1–7.4 (5H, m).

Maytansinol 3-Phenoxyacetate (24)—49.0% yield. mp 175–177 °C (dec.). IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 1740, 1730, 1710, 1670, 1580, 1460, 1090, 1080. MS m/e : 654, 637 ($M^+ - 61$), 622, 595, 485, 470, 450. $^1\text{H-NMR}$ (CDCl_3 , 90 MHz) δ : 0.83 (3H, s), 1.28 (3H, d), 1.66 (3H, br s), 2.82 (3H, s), 3.37 (3H, s), 3.95 (3H, s), 4.72 (2H, q), 6.32 (1H, s), 6.6–7.4 (7H, m).

Maytansinol 3-(2-Furyl)carboxylate (25)—30.0% yield. mp 180–189 °C (dec.). IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 1730, 1720, 1700, 1660, 1580, 1300, 1110, 1090, 1080. MS m/e : 615, 613, 597 ($M^+ - 61$), 582, 565, 562, 555, 545, 502, 485, 470, 450. $^1\text{H-NMR}$ (CDCl_3 , 90 MHz) δ : 0.82 (3H, s), 1.26 (3H, d), 1.67 (3H, br s), 3.17 (3H, s), 3.26 (3H, s), 4.00 (3H, s), 6.68 (1H, d), 6.84 (1H, d), 7.35 (1H, m), 7.53 (1H, m), 7.72 (1H, m).

Maytansinol 3-(2-Pyridine)carboxylate (26)—35.1% yield. mp 190–193 °C (dec.). IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 1730, 1720, 1710, 1660, 1580, 1440, 1100, 1080. MS m/e : 669 (M^+), 651, 626, 608 ($M^+ - 61$), 593, 576, 566, 502, 485, 470, 450. $^1\text{H-NMR}$ (CDCl_3 , 90 MHz) δ : 0.84 (3H, s), 1.31 (3H, d), 1.64 (3H, br s), 3.17 (3H, s), 3.20 (3H, s), 3.99 (3H, s), 6.20 (1H, s), 6.21 (1H, d), 6.83 (1H, d), 7.57 (1H, m), 7.90 (1H, m), 8.17 (1H, m), 8.35 (1H, m), 8.66 (1H, m).

Maytansinol 3-(3-Pyridine)carboxylate (27)—31.2% yield. mp 184–187 °C (dec.). IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 1730, 1720, 1700, 1670, 1650, 1590, 1580, 1280, 1110, 1080. MS m/e : 669 (M^+), 626, 608 ($M^+ - 61$), 593, 576, 573, 566, 513, 502, 485, 470, 450. $^1\text{H-NMR}$ (CDCl_3 , 90 MHz) δ : 0.90 (3H, s), 1.29 (3H, d), 1.69 (3H, br s), 3.15 (3H, s), 3.25 (3H, s), 4.00 (3H, s), 6.18 (1H, s), 6.86 (1H, d), 6.96 (1H, d), 7.45 (1H, dd), 8.32 (1H, m), 8.84 (1H, dd), 9.28 (1H, d).

Maytansinol 3-(4-Pyridine)carboxylate (28)—24.0% yield. mp 185–187 °C (dec.). IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 1730, 1710, 1700, 1670, 1650, 1580, 1280, 1110, 1100, 1080. MS m/e : 608 ($M^+ - 61$), 593, 576, 575, 573, 566, 502. $^1\text{H-NMR}$ (CDCl_3 , 90 MHz) δ : 0.86 (3H, s), 1.29 (3H, d), 1.68 (3H, br s), 3.17 (3H, s), 3.24 (3H, s), 3.98 (3H, s), 6.18 (1H, s), 6.85 (1H, d), 6.93 (1H, d), 7.86 (2H, m), 8.84 (2H, m).

Maytansinol 3-Phenyl Carbamate (29)—Method C: Phenyl isocyanate (23.8 mg, 0.2 mmol) and ZnCl_2 (30 mg, 0.22 mmol) were added to a solution of maytansinol (56.4 mg, 0.1 mmol) in CH_2Cl_2 (10 ml, dried over 3 Å molecular sieves). After being stirred at room temperature for 3 h, the reaction mixture was washed with water, dried over MgSO_4 and evaporated to dryness *in vacuo*. The residue was chromatographed on a silica gel column using aqueous ethyl acetate as an eluent to afford **29** (46 mg, 85.5%). mp 186–187 °C (dec.). IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 1730, 1710, 1700, 1670, 1650, 1580, 1480, 1100, 1080. MS m/e : 622 ($M^+ - 61$), 503, 485, 470. $^1\text{H-NMR}$ (CDCl_3 , 90 MHz) δ : 0.86 (3H, s), 1.25 (3H, d), 1.68 (3H, br s), 3.16 (3H, s), 3.28 (3H, s), 3.96 (3H, s), 6.36 (1H, d), 6.79 (1H, s), 7.1–7.5 (5H, m).

Maytansinol 3-Isopropyl Carbonate (30)—Method D: A solution of maytansinol (384 mg, 0.68 mmol) in dry THF (14 ml) was treated with 15% w/w *n*-BuLi in *n*-hexane (2.9 ml) under a nitrogen atmosphere at -30 – -40 °C for 15 min, and then isopropyl chloroformate (830 mg, 6.8 mmol) was introduced into the mixture. After being stirred at -30 °C for an additional 15 min, the reaction mixture was quenched with sat. aq. NaCl (2 ml). The separated organic layer was washed with sat. aq. NaCl, dried over MgSO_4 and evaporated *in vacuo*. The residue was chromatographed on a silica gel column using 2.5% MeOH in CHCl_3 as an eluent to afford **30** (72 mg, 16%). mp 148–150 °C (dec.). IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 1750, 1730, 1710, 1660, 1580, 1260, 1110, 1100, 1080. MS m/e : 650 (M^+), 589 ($M^+ - 61$), 574, 555. $^1\text{H-NMR}$ (CDCl_3 , 100 MHz) δ : 0.82 (3H, s), 1.28 (6H, d), 1.52 (3H, d), 1.69 (3H, br s), 3.16 (3H, s), 3.35 (3H, s), 3.98 (3H, s), 6.82 (1H, d), 7.00 (1H, d).

Maytansinol 3-L-Phenylglycinate (31)—Maytansinol 3-*N*-*tert*-butoxycarbonyl-L-phenylglycinate (210 mg), prepared by esterification of maytansinol (3) with *N*-*tert*-butoxycarbonyl phenylglycine in 65% yield according to method B, was dissolved in CH_2Cl_2 (2.5 ml) and treated with a 50% solution of CF_3COOH in CH_2Cl_2 (4 ml) on an ice bath. Then the ice bath was removed and the mixture was allowed to stand at room temperature for 15 min. The reaction mixture was poured into ice-water (8 ml) and neutralized with 5% aq. NaHCO_3 . The organic layer was taken and the aqueous layer was extracted with CHCl_3 (5 ml $\times$ 2). The combined organic layers were washed with water, dried over MgSO_4 and evaporated to dryness *in vacuo*. The residual solid was chromatographed on a silica gel column using 5% aqueous CH_3CN as an eluent to afford the desired product (81 mg, 44.0%) as a colorless powder. mp 186–188 °C (dec.). IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 1750, 1720, 1710, 1670, 1580, 1450, 1430, 1400, 1090, 1080. MS m/e : 636 ($M^+ - 61$), 574, 485. $^1\text{H-NMR}$ (CDCl_3 , 90 MHz) δ : 0.80 (3H, s), 1.27 (3H, d), 1.63 (3H, br s), 2.66 (3H, s), 3.36 (3H, s), 3.91 (3H, s),

6.71 (1H, d), 6.83 (1H, d), 7.36 (3H, s).

Maytansinol 3-L-Mandelate (32)—After esterification of maytansinol (56.4 mg) with *O*-trifluoroacetyl-DL-mandelic acid (144.8 mg) according to method B, the protective trifluoroacetyl group was removed directly by treating the reaction mixture with sat. aq. NaHCO_3 at room temperature for 30 min. The organic layer was separated and the aqueous layer was extracted with CHCl_3 (5 ml $\times$ 2). The combined organic layer and extracts were dried over MgSO_4 and evaporated to dryness *in vacuo*. The diastereomeric mixture was separated by chromatography on a silica gel column using 3% MeOH in CHCl_3 as an eluent to afford the desired product (**32**, 24 mg, 34.4%) with L-configuration at the mandelic acid moiety.^{8,9)} mp 168–170 °C. IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 1760, 1730, 1710, 1670, 1650, 1580, 1450, 1090, 1080. MS m/e : 637 ($\text{M}^+ - 61$), 485. $^1\text{H-NMR}$ (CDCl_3 , 90 MHz) δ : 0.78 (3H, s), 1.28 (3H, d), 1.60 (3H, br s), 2.70 (3H, s), 3.96 (3H, s), 5.20 (1H, s), 7.37 (5H, s).

Minimum Inhibitory Concentration against *Tetrahymena pyriformis*—Minimum inhibitory concentration (MIC) values of the semisynthetic maytansinoids against *T. pyriformis* were determined by a broth dilution method as described by Tanida *et al.*¹³⁾ The results are listed in Table I.

Cilia Regeneration Inhibition of Deciliated *T. pyriformis*—The cilia regeneration inhibition was determined by the method described by Tanida *et al.*¹⁵⁾

Therapeutic Test—C57 BL/6 $\times$ DBA/2 F1 mice were inoculated intraperitoneally with 0.5 ml of 1:4 tumor homogenate of B16 in 0.9% NaCl solution, as described by Geran *et al.*¹⁶⁾ The test compounds suspended in Tween 80-saline solution by careful grinding were injected ip into the mice (5 mice/group) daily for 9 consecutive days starting 24 h after tumor transplantation. Test doses were determined on the basis of acute toxicities; when the data were not available, 0.8 mg/kg was selected as the highest initial dose. Median survival time was calculated, and the antitumor activities of the test compounds were assessed in terms of T/C %. The median survival time of the control mice was about 17.4 d, and antitumor activity was considered to be positive when T/C % was over 125%. The results are summarized in Tables II and III.

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