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Structural Requirements for Antileukemic Activity among the Naturally Occurring and Semisynthetic Maytansinoids[1a,2]

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In an effort to determine the structural requirements for the significant antileukemic, cytotoxic, antitubulin, and antimitotic activity exhibited by the novel ansa macrolide, maytansine (1), four new C-3 ester and six new C-9 ether homologues were synthesized. The biological activities of these compounds were assayed and compared to the activities of previously reported, naturally occurring maytansinoids. From the data, it is apparent that presence of the C-3 ester is necessary for significant activity, and variations in the ester group are not accompanied by marked changes in activity. However, elimination of the ester group, as in maytansinol (7), maysine (8), normaysine (9), and maysenine (10), results in a significant decrease in biological activity. Blockage of the C-9 carbinolamide via etherification markedly reduces antileukemic and cytotoxic activity and slightly reduces antitubulin activity but has relatively little effect on antimitotic acivity against sea urchin eggs. Thus, a free carbinolamide at C-9 is advantageous for optimal activity.

Earlier work in this laboratory on the potent antileukemic ansa macrolides from the genera *Maytenus* and *Putterlickia*[3] in the plant family Celastraceae led to the isolation of maytansine (1), maytanbutine (2), maytanprine (3), maytanvaline (4), maytanbutacine (5), maytanacine (6), maytansinol (7), maysine (8), normaysine (9), and maysenine (10).[4-8] Considerable biological[9-16] and chemical[17] interest in the maytansinoids continues, and maytansine (1) is currently undergoing clinical trials under the auspices of the National Cancer Institute. Maytansinoids 1–6 showed excellent antileukemic activity against P-388 lymphocytic leukemia in the mouse (PS) and potent cytotoxicity against cells derived from human carcinoma of the nasopharynx (KB).[18] In contrast, 7–10 showed greatly diminished activity against both PS and KB relative to 1–6. Since 7–10 all lack the C-3 ester moiety, an ester at C-3 appears to be necessary for antileukemic activity.

In order to further investigate the structure–activity relationships among the maytansinoids, several modified C-3 esters were prepared from maytansinol (7).[7,8] For the current work, maytansinol (7) was prepared by treatment of maytanbutine (2) with lithium aluminum hydride in dry tetrahydrofuran at −23 °C.[19] Extensive preparative thin-layer chromatography (PTLC) of the reaction mixture yielded 7 in 40% yield.[20]

The C-3 trifluoroacetate ester 14 was prepared in 30% yield by treatment of 7 with trifluoroacetic anhydride–trifluoroacetic acid.[22] However, the most efficient method for preparation of other C-3 esters proved to be treatment

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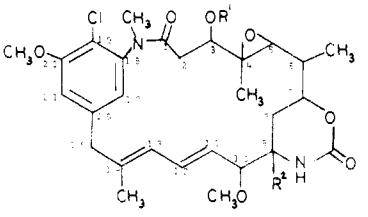
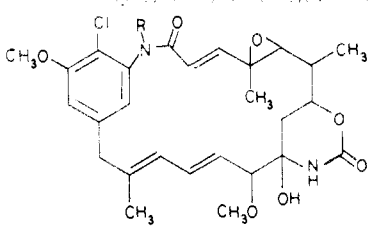
of maytansinol (7) with pyridine and the appropriate symmetrical anhydride.[7,8,21] Thus, maytanacine (6) was prepared from 7 and pyridine–acetic anhydride in 53% yield, and the C-3 propionate 11 was obtained in 38% yield from treatment of 7 with pyridine–propionic anhydride. To determine the effect of additional potential alkylating sites on the activity, both the C-3 bromoacetate 12 (43%) and the C-3 crotonate 13 (13%) esters were prepared from 7 and the appropriate anhydride.



1 R = COCH(CH3)N(CH3)COCH3; R1 = OH
3 R = COC=(CH3)N(CH3)COCH(CH3)2; R1 = OH
4 R1 = COCH(CH3)N(CH3)COCH2CH(CH3)2; R2 = OH
6 R1 = COCH3; R2 = OH
7 R1 = R2 = OH
11 R1 = COCH2CH3; R2 = OH
12 R1 = COCH2Br; R2 = OH
13 R1 = COCH=CHCH3; R2 = OH
14 R = COCF3; R1 = OH
15 R1 = COCH(CH3)N(CH3)COCH3; R2 = OCH2CH2CH2Br
16 R1 = COCH(CH3)N(CH3)COCH3; R2 = OCH3
17 R1 = COCH(CH3)N(CH3)COCH3; R2 = OCH2CH3
18 R1 = COCH(CH3)N(CH3)COCH(CH3)2; R2 = OCH3
19 R1 = COCH(CH3)N(CH3)COCH(CH3)2; R2 = OCH2CH3
20 R1 = COCH(CH3)N(CH3)COCH3; R2 = SCH2CH2CH3
21 R1 = COCH(CH3)N(CH3)COCH(CH3)2; R2 = SCH2CH2CH3



8 R = CH3
9 R = H

Some aspects of the mechanism(s) of biological action of the maytansinoids have been elucidated,[9-16] but a complete explanation is by no means at hand. Since the C-9 carbinolamide moiety has been proposed[6] to function as an alkylating function intimately involved in the antitumor activity of maytansine(1), we sought to determine what effect modification of this site would have on the activity. Consequently, several C-9 ethers (16–19) were prepared. Maytansinoid C-9 ethers were originally synthesized by treatment of the maytansinoid with the appropriate alcohol and *p*-toluenesulfonic acid in dichloromethane.[4,8] However, the low yields, generally <15%, prompted modification of this method. Treatment of maytansine (1) with the appropriate alcohol and trifluoroacetic acid in dry benzene under nitrogen in a sealed system for 3 days increased the yield of maytansine 9-(3)-bromopropyl ether (15) from 15 to 55%.[8] Ethers 16 (25%), 17 (56%), 18 (28%), and 19 (37%) were prepared in the same manner from the appropriate maytansinoids.

Maytansinoids 7–10, which lack the C-3 ester, show no antileukemic activity (Table I) over a wider dose range than the dose range required for activity of homologues 1–6 and 11–13.[23] Replacement of the C-3 amino acid ester with a simple alkyl ester (6, 11) or with an alkyl ester having potential alkylating sites (12, 13) has little effect upon the PS activity but apparently does increase the dose required for optimal activity. The lack of antileukemic activity exhibited by the C-3 trifluoroacetate ester (14) is presumably due to in vivo hydrolysis to inactive maytansides. Compounds 1–14 all show significant cytotoxicity against KB cells (Table II), but maytansinoids which lack the C-3 ester are less active than those with esters.

The importance of the carbinolamide moiety to the antileukemic activity is illustrated by the testing data for maytansinoids 16–21 vs. data for maytansine (1) and maytanbutine (2). When the C-9 alcohol of the carbinolamide is converted to an ether, as in the case of ethers 16–21, a marked decrease in antileukemic activity (Table I) is observed. The ethers begin to show activity (PS) only at doses ca. 400-fold greater than the minimum dose required for the parent maytansinoid.[23] Ethers 16–21 also show a significant decrease in cytotoxicity against KB cells compared to 1 and 2. The ready conversion of maytansinoid C-9 esters back to the parent maytansinoids[8] suggests that the activity shown by the ethers may be due to in vivo hydrolysis.

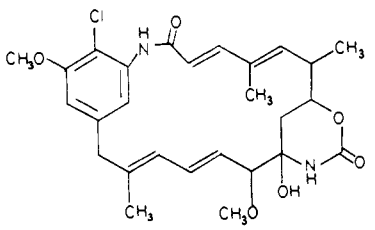
Since maytansine (1) is known to inhibit mitosis in sea urchin eggs and to inhibit the polymerization of brain tubulin,[9] several additional maytansinoids were assayed using these systems (Table III) to determine whether there are consistent effects on all dividing systems. Brain tubulin was isolated and purified through two cycles of polymerization and depolymerization using techniques described by Shelanski et al.[24] Extent of polymerization was monitored by measuring turbidity as a function of time (in the absence of glycerol).[25] Since only small quantities of some of the derivatives were available, complete inhibition curves could not be obtained for all compounds with the result that precise quantitative comparison could not be obtained. To overcome this problem use was made of the observation that all of the compounds inhibited tubulin polymerization to some degree and no one compound was more than 4–5 times more potent than any other. Thus, it was possible to choose both a tubulin concentration and a derivative concentration such that 10–90% inhibition of polymerization was obtained for all compounds tested. The formula used in Table III then gives a correct relative ordering of potencies in inhibition of tubulin polymerization and is a semiquantitative measure of true potency. If all compounds interacted with tubulin in strict Michaelis–Menten fashion, the function would yield true relative potencies as long as the measurements were in the linear portion of the curve. Our conditions approximate this. All experiments with tubulin and sea urchin eggs were done double blind and repeated three times.

The absence of an ester group at the C-3 carbon in maysine (8) and normaysine (9) reduces the potency of the compounds relative to maytansine (1) to about one-fourth to one-fifth. An important exception is maysenine (10) which lacks the epoxy group in the C-3 region [more variability was obtained with maysenine (10) in the tubulin experiments than with any other compound]. All derivatives which have a functional group on the C-3 carbon are more potent than maysine (8) and normaysine (9), those with C-3 esters being the most potent of all. Blocking the C-9 carbinolamide reduces the potency of the parent compound but does not completely abolish its activity. The C-9 derivatives of maytansine (1) and maytanbutine (2) show a systematic decrease in potency in the same relative order, Table III.

From these results it can be seen that the C-3 ester linkage appears to be important in tubulin–maytansinoid interaction. The presence of the free C-9 carbinolamide group also appears to aid the ability of maytansinoids to

inhibit tubulin polymerization, suggesting that the compounds may interact with tubulin at two sites. However, blocking the carbinolamide group does not strongly affect antitubulin activity, suggesting that the carbinolamide is of secondary importance in relation to the C-3 ester for antitubulin activity.

Sea urchin eggs were obtained and handled as described by Remillard et al.[9] and Wang et al.[26] Concentrations of drugs required for complete inhibition of cell division are given in Table III. Compounds **7**–**9**, which do not possess an ester group on C-3, are between 20 and 2000 times less potent as inhibitors of cleavage than are any other compounds in the group tested, in agreement with the results on the three other systems investigated. The large differences in potency of the compounds with C-3 esters compared to maysine in the sea urchin egg system as well as in other cellular systems tested suggest that the esters may be involved in a transport process. These variations in potency would otherwise be difficult to account for solely on the basis of the quantitative differences in effect on tubulin. Maysenine (**10**) is the exception since it would not be expected to be more potent than normaysine (**9**) based on its lack of a C-3 ester group.



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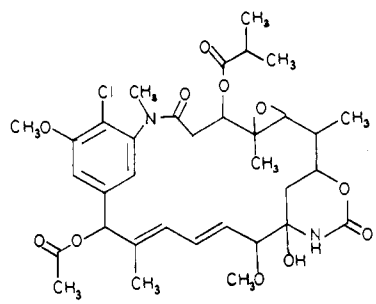

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Surprisingly, blockage of the carbinolamide group by etherification of C-9 does not result in a significant decrease in ability to inhibit cleavage in sea urchin eggs, in contrast to the effects with in vivo leukemic cells, KB cells in culture, and isolated tubulin. To pursue this further, we performed preliminary experiments with Chinese hamster ovary cells (CHO) in culture and have found maytanbutine (**2**) to be at least 20-fold more active than maytanbutine methyl ether (**18**) in inhibiting cell division. Thus, both KB (Table II) and CHO cells in culture and in vivo leukemic cells apparently make a much greater distinction between compounds blocked at the C-9 position than do dividing sea urchin eggs. Given the fact that all of the C-9 blocked derivatives are less active in inhibiting polymerization of tubulin than are the parent compounds and thus should be less potent in inhibition of spindle formation in cell division, it appears likely that eggs, but not leukemic cells, KB cells, and CHO cells, possess a means of hydrolyzing the C-9 blocking group, thus freeing the parent compound. Such variation of putative hydrolyzing activity among cell types could be of therapeutic importance; if specific tumors possessed significantly higher degrees of such activity than do normal cells, considerably higher doses of the C-9 blocked compounds could be safely administered.

Interestingly, in three systems tested (KB cells, sea urchin eggs, and in vitro tubulin—but not the in vivo P-388 leukemic system) maysenine (**10**) is more potent than normaysine (**9**) (about 50 times for KB cells, 200 times for sea urchin eggs, and 4 times for tubulin, Tables II and III) from which it differs only by lacking an epoxide group between carbons 4 and 5. This suggests the interesting possibility that, in some systems, the epoxide group may partially inhibit the effectiveness of the maytansinoid compounds.

In summary, as ester group at C-3 appears to be necessary for potent antitubulin, antimitotic, and antileukemic activity. Blockage of the carbinolamide group by etherification of the C-9 hydroxyl decreases but does not abolish antitubulin activity, has no discernable effect on antimitotic activity against sea urchin eggs, and decreases in vivo antileukemic activity and antimitotic activity against cells in culture by at least 20 to as much as several hundred times. Maytansine (**1**) continues to be of great interest due to the very low doses required (<25 μg/kg) for significant antileukemic activity and the wide effective dose range (0.4–25 μg/kg), and work continues in an effort to further elucidate the structure–activity relationships among this important class of compounds.

Experimental Section

Melting points were determined on a Fisher-Johns melting point apparatus. Ultraviolet absorption spectra were determined on a Beckmann Model DK-2A recording spectrophotometer. Infrared spectra were determined on a Perkin-Elmer Model 257 recording spectrophotometer. NMR spectra were determined on a JEOL PS-100p FT NMR spectrometer interfaced to a Texas Instruments JEOL 980A computer, with tetramethylsilane as an internal standard. Mass spectra were determined on Hitachi Perkin-Elmer Model RMU-6E and AEI Model MS-902 spectrometers at the University of Virginia and at the Research Triangle Center for Mass Spectrometry. Values of $[\alpha]_D$ were determined on a Perkin-Elmer Model 141 automatic polarimeter. Microanalyses were carried out by Spang Microanalytical Laboratory, Ann Arbor, Mich. All thin-layer chromatography was carried out on commercially prepared plates and visualization was effected with short-wavelength UV or with iodine spray. "ChromAR" refers to ChromAR 7GF plates by Mallinckrodt, and "silica gel" refers to silica gel 60 F-254 plates by E. M. Reagents. Maytansinoids **1**–**5** and **8**–**10** were isolated and characterized as previously reported in ref 8.

Maytansinol (7). A mixture of maytanbutine (**2**, 40 mg, 0.0556 mmol) and excess lithium aluminum hydride was stirred in dry tetrahydrofuran (4 mL) at −23 °C (carbon tetrachloride–dry ice bath) for 3 h. Ethyl acetate (40 mL) was added, followed by 10 mL of pH 6.8 phosphate buffer,[27] and the mixture was further extracted with ethyl acetate (4 × 10 mL). The extracts were combined, dried over sodium sulfate, and brought to dryness. The residue (45 mg) was submitted to PTLC on ChromAR, developed with 5% methanol–chloroform twice to give 21 mg of material which was further purified by PTLC on ChromAR, developed with 3% isopropyl alcohol–ethyl acetate twice. The major band (17.2 mg) was chromatographed over a very short column of aluminum oxide (activity II–III), packed in dichloromethane with the product eluted with 5% methanol–dichloromethane, to give 16.0 mg of maytansinol. Precipitation from dichloromethane–hexane afforded white, solid **7** (12.5 mg, 40%): mp 173–174.5 °C; $[\alpha]^{23}_D$ −309° (*c* 0.110, $CHCl_3$); UV (EtOH) 232 nm (ϵ 32 700), 244 (sh, 30 800), 252 (31 600), 281 (5810), 288 (5700); IR (KBr) 5.85, 6.06, 6.35 μ; NMR ($CDCl_3$) δ 0.84 (3 H, s, C-4-CH_3), 1.32 (3 H, d, J = 6 Hz, C-6-CH_3), 1.68 (3 H, s, C-14-CH_3), 3.20 (3 H, s, C-1-NCH_3), 3.36 (3 H, s, C-10-OCH_3), 3.44 (1 H, br s, C-3-OH), 3.54 (1 H, br s, C-9-OH), 3.98 (3 H, s, C-20-OCH_3), 4.36 (1 H, m, C-7-H), 5.53 (1 H, dd, $J_{10,11}$ = 9, $J_{11,12}$ = 15 Hz, C-11-H), 6.19–6.39 (3 H, C-12-H, C-13-H, C-9-NH), 6.81, 7.05 (2 H, d, $J_{17,21}$ = 1.5 Hz, C-17-H, C-21-H), 1.30–3.55 (10 H, C-2-H_2, C-3-H, C-5-H, C-6-H,

Table I. Antileukemic Activity of the Maytansinoids toward the P-388 System[d]

Compd	Dose, μg/kg	Toxicity day survivors[a]	Animal wt diff[b]	T/C, %[c]	Compd	Dose, μg/kg	Toxicity day survivors[a]	Animal wt diff[b]	T/C, %[c]
1	100.0	6/6	−0.4	<80	9	100.0	6/6	1.2	95
	50.0	6/6	0.3	125		50.0	6/6	−0.3	110
	25.0	6/6	0.6	220		25.0	6/6	−0.5	110
	12.5	6/6	1.6	190		12.5	6/6	−0.5	110
	6.2	6/6	1.3	195		6.2	6/6	0.4	115
	3.1	6/6	−0.1	165		3.1	6/6	−0.2	115
	1.6	6/6	1.6	155		1.6	6/6	0.0	100
	0.8	6/6	1.4	145		0.8	6/6	−0.1	90
	0.4	6/6	1.7	125		0.4	6/6	0.1	100
2	50.0	1/6	0.1	<80		0.2	6/6	1.0	100
	25.0	6/6	0.1	<80	10	200.0	6/6	1.2	104
	12.5	6/6	0.1	120		100.0	6/6	0.8	120
	6.2	6/6	−1.3	155		50.0	6/6	1.8	120
	3.1	6/6	−0.9	145		25.0	6/6	−0.8	108
	1.6	6/6	0.0	155		12.5	6/6	0.7	<80
	0.8	6/6	0.1	190		6.2	6/6	0.7	87
	0.4	6/6	0.4	155		3.1	6/6	1.4	95
	0.2	6/6	0.1	165		1.6	6/6	1.0	104
	0.1	5/6	0.3	125	11	200.0	2/4	−3.5	<80
3	50.0	0/6	−1.2	<80		100.0	4/4	−2.0	<80
	25.0	6/6	−2.2	<80		50.0	4/4	−0.9	198
	12.5	6/6	−1.3	104		25.0	4/4	−0.5	153
	6.2	6/6	−2.1	125		12.5	4/4	−0.3	163
	3.1	6/6	−0.6	150		6.2	4/4	−1.0	140
	1.6	6/6	−1.2	154		3.1	4/4	1.3	131
	0.8	6/6	−1.1	145		1.6	4/4	−0.8	129
	0.4	6/6	−0.8	137		0.8	4/4	−0.5	110
	0.2	6/6	−1.1	120	12	200.0	4/4	−2.5	<80
	0.1	6/6	−1.1	137		100.0	4/4	−1.3	232
4	50.0	6/6	−0.5	<80		50.0	4/4	−0.3	189
	25.0	6/6	−0.4	116		25.0	4/4	−0.5	162
	12.5	6/6	1.3	187		12.5	4/4	−0.3	153
	6.2	6/6	0.8	154		6.2	4/4	0.2	146
	3.1	6/6	0.2	158		3.1	4/4	−0.2	130
	1.6	6/6	−1.3	154		1.8	4/4	−0.6	131
	0.8	6/6	0.7	133		0.9	4/4	−0.4	129
	0.4	6/6	−1.1	125	13	200.0	3/4	−1.8	210
	0.2	6/6	−0.6	125		100.0	4/4	−3.6	210
	0.1	6/6	1.4	120		50.0	4/4	−3.5	210
5	50.0	6/6	−1.0	<80		25.0	4/4	−3.6	173
	25.0	6/6	−0.9	170		12.5	4/4	−2.5	170
	12.5	6/6	−0.8	179		6.2	4/4	−3.0	160
	6.2	6/6	−0.9	166		3.1	4/4	−1.7	150
	3.1	6/6	−0.4	150		1.6	4/4	−4.5	150
	1.6	6/6	−0.7	162		0.8	4/4	−4.5	150
	0.8	6/6	−1.0	137	14	200.0	4/4	−1.9	140
	0.4	6/6	−0.6	133		100.0	4/4	0.3	108
	0.2	6/6	−1.3	125		50.0	4/4	−0.4	130
	0.1	6/6	−0.5	125		25.0	4/4	−1.0	130
6	200.0	4/4	−3.7	100		12.5	4/4	0.1	120
	100.0	4/4	−0.6	230		6.2	4/4	0.3	108
	50.0	4/4	−0.3	183		3.1	4/4	0.2	100
	25.0	4/4	−1.2	173		1.6	4/4	0.1	100
	12.5	4/4	0.3	158		0.8	4/4	−0.8	110
	6.2	4/4	0.5	150	16	400.0	6/6	−0.9	139
	3.1	4/4	−1.0	133		200.0	6/6	−0.7	93
	1.5	4/4	−1.1	150		100.0	6/6	−0.9	104
	0.8	4/4	0.0	110		50.0	6/6	−2.3	101
7	400.0	4/4	−2.6	102	17	400.0	6/6	−0.6	100
	200.0	4/4	−1.8	112		200.0	6/6	0.3	98
	100.0	4/4	1.2	105		100.0	6/6	−2.0	105
	50.0	4/4	−0.4	112		50.0	6/6	−0.8	98
	25.0	4/4	0.5	94		25.0	6/6	−0.3	116
	12.5	4/4	1.5	102		12.5	6/6	−0.3	101
	6.2	4/4	0.5	102		6.2	6/6	−1.3	99
	3.1	4/4	0.9	102		3.1	6/6	−0.7	99
8	100.0	6/6	1.0	110		1.6	6/6	−0.1	99
	50.0	6/6	2.6	<80	18	400.0	6/6	−3.0	136
	25.0	6/6	1.7	<80		200.0	6/6	−0.9	100
	12.5	6/6	1.0	<80		100.0	6/6	−1.0	99
	6.3	6/6	0.3	<80		50.0	6/6	−2.0	99
	3.1	6/6	0.4	90		25.0	6/6	−0.5	96
	1.1	6/6	2.0	90		12.5	6/6	−2.1	99
	0.8	6/6	2.8	90		6.2	6/6	−0.6	100
	0.4	6/6	2.5	90		3.1	6/6	−1.1	99
	0.2	6/6	2.1	90		1.6	6/6	−1.8	101

Table I (*Continued*)

Compd	Dose, μg/kg	Toxicity day survivors[a]	Animal wt diff[b]	T/C, %[c]	Compd	Dose, μg/kg	Toxicity day survivors[a]	Animal wt diff[b]	T/C, %[c]
19	400.0	6/6	−0.9	149	**21**	400.0	6/6	0.6	151
	200.0	6/6	0.3	134		200.0	6/6	−2.4	151
	100.0	6/6	3.4	109		100.0	6/6	−1.6	127
	50.0	6/6	1.3	100		50.0	6/6	0.4	118
20	400.0	6/6	−0.7	114		25.0	6/6	0.7	101
	200.0	6/6	0.1	114		12.5	6/6	0.6	95
	100.0	6/6	2.8	114		6.2	6/6	−0.1	<80
	50.0	6/6	−1.3	111		3.1	6/6	0.6	97
						1.6	6/6	0.8	100

[a] (Number of survivors on toxicity day)/(number of mice started on test). Toxicity day is normally 4 days after the first injection and survivors are recorded on this day as a measure of drug toxicity. [b] The difference, in grams, between the weights of test and control animals. [c] T/C is the ratio (expressed as a percent) of the median survival time of the treated group of mice divided by the median survival time of the control group. Compounds are considered active by the NIH protocol if T/C ≥ 125%. [d] See ref 18.

Table II. Cytotoxicity of the Maytansinoids toward KB Cells[b]

Compd	ED_{50},[a] μg/mL	Compd	ED_{50},[a] μg/mL
1	0.0000061	11	0.00021
2	0.0000036	**12**	0.00044
3	0.00000014	13	0.00023
4	0.00000023	14	0.01
5	0.0000015	**16**	0.00019
6	0.0000018	**17**	0.011
7	0.19	**18**	0.00016
8	0.025	**19**	0.0012
9	0.019	20	0.00018
10	0.002	21	0.00031

[a] The ED_{50} is the calculated effective dose which inhibits growth to 50% of control growth. The value given is a typical result from several tests on each compound. [b] See ref 18.

C-8-H_2, C-10-H, C-15-H_2); mass spectrum *m/e* 503.2075 (M^+ − H_2O − HNCO; calcd for $C_{27}H_{34}ClNO_6$, 503.2074).

Maytanacine (6). Maytansinol (**7**, 1.5 mg, 0.0027 mmol) was treated with 1 mL of acetic anhydride–pyridine (1:1) at 53 °C for 18 h. The reaction mixture was brought to dryness and the residue was chromatographed on ChromAR developed with 5% methanol–chloroform. The major band was removed, eluted with 10% methanol in ethyl acetate, and evaporated to a white solid. Crystallization from dichloromethane–hexanes gave **6** (0.8 mg, 48%): mp 234–237 °C; $[\alpha]^{23}_D$ −119° (*c* 0.100, $CHCl_3$); UV (EtOH) 233 nm (ε 30 300), 242 (sh, 28 000), 252 (27 900), 281 (5360), 289 (5360); IR (KBr) 5.70, 5.80, 6.00, 6.34 μ; NMR ($CDCl_3$) δ 0.84 (3 H, s, C-4-CH_3), 1.28 (3 H, d, *J* = 6 Hz, C-6-CH_3), 1.69 (3 H, s, C-14-CH_3), 2.18 (3 H, s, C-3-$OCOCH_3$), 2.05–2.30 (1 H, C-2-H), 2.46 (1 H, dd, $J_{2,3}$ = 12, $J_{2,2}$ = 14 Hz, C-2-H), 2.89 (1 H, d, $J_{5,6}$ = 9 Hz, C-5-H), 3.18 (3 H, s, C-1-NCH_3), 3.36 (3 H, s, C-10-OCH_3), 3.52 (1 H, d, $J_{10,11}$ = 9 Hz, C-10-H), 3.10–3.60 (3 H, C-9-OH, C-15-H_2), 3.99 (3 H, s, C-20-OCH_3), 4.16 (1 H, m, C-7-H), 4.92 (1 H, dd, $J_{2,3}$ = 3, 12 Hz, C-3-H), 5.48 (1 H, dd, $J_{10,11}$ = 9, $J_{11,12}$ = 15 Hz, C-11-H), 6.10–6.59 (3 H, C-9-NH, C-12-H, C-13-H), 6.84, 6.76 (2 H, s, C-17-H, C-20-H), 0.80–2.50 (3 H, C-6-H, C-8-H_2); mass spectrum *m/e* 545.2180 (M^+ − H_2O − HNCO; calcd for $C_{29}H_{36}ClNO_7$, 545.2180), 485.1969 (M^+ − H_2O − HNCO − CH_3COOH; calcd for $C_{27}H_{32}ClNO_5$, 485.1969). Anal. Calcd for $C_{30}H_{39}ClN_2O_9$:

Table III

Compd	$1 - \frac{\text{abs}^a \text{ maytansinoid}}{\text{abs control}}$ / $1 - \frac{\text{abs maytansine}}{\text{abs control}}$	Concn[b] (mol/L) required for inhibn of sea urchin egg mitosis	Cytotoxicity[c] toward KB cells (ED_{50}, mol/L)
1	1.0	10^{-7}	8.8×10^{-12}
17	0.6	10^{-7}	1.5×10^{-8}
20	0.5	10^{-8}	2.4×10^{-10}
16	0.5	10^{-8}	2.7×10^{-10}
15	0.3	N.T.	N.T.
2	1.3	10^{-8}-10^{-9}	5×10^{-12}
19	0.8	10^{-7}	1.6×10^{-9}
21	0.6	10^{-8}	4.0×10^{-10}
18	0.6	10^{-8}	2.1×10^{-10}
7	0.3	10^{-5}	3.4×10^{-7}
11	0.9	10^{-8}-10^{-9}	3.4×10^{-10}
12	0.8	10^{-8}	6.4×10^{-10}
14	N.T.[d]	N.T.	1.5×10^{-8}
13	N.T.	N.T.	3.6×10^{-10}
8	0.3	10^{-5}-10^{-6}	4.5×10^{-8}
9	0.3	10^{-5}-10^{-6}	3.5×10^{-8}
3	1.0	10^{-8}	2.0×10^{-13}
4	0.9	10^{-8}	3.1×10^{-13}
5	0.9	10^{-7}	2.2×10^{-12}
6	1.0	10^{-8}	3.0×10^{-11}
10	0.9	10^{-8}	3.9×10^{-9}

[a] Abs = turbidity of polymerized tubulin[25] measured as the absorbance at 310 nm and 37 °C using a Beckmann Model 25 spectrophotometer. [b] Concentrations of, e.g., 10^{-8}-10^{-9} M mean that the eggs cleaved at 10^{-8} but not at 10^{-9}; concentrations of, e.g., 10^{-8} M mean that halving the concentration allowed cleavage to occur. [c] KB cell data from Table II have been converted to moles per liter. KB cells are considerably more sensitive to maytansinoid compounds than sea urchin eggs or CHO cells (see text). The basis for this difference is not known. [d] N.T. = not tested.

C, 59.35; H, 6.48; N, 4.61. Found: C, 59.19; H, 6.39; N, 4.69.

Maytansinol 3-Propionate (11). A solution of maytansinol (21.3 mg, 0.038 mmol), dry pyridine (400 μL), and propionic anhydride (200 μL, 203 mg, 1.56 mmol) was heated in a sealed flask at 45 °C under nitrogen for 72 h. After cooling, the excess pyridine and propionic anhydride were removed on a rotary evaporator; the residue was taken up in 5 mL of 1 N hydrochloric acid and extracted with methylene chloride (3 × 5 mL). The combined dichloromethane layers were dried over anhydrous sodium sulfate and then evaporated to dryness. The residue was subjected to PTLC on ChromAR developed with 5% methanol–ethyl acetate. The major band was removed, eluted with 10% methanol–ethyl acetate, and evaporated to give a white solid. Crystallization of this solid from dichloromethane–hexanes yielded white crystals of **11** (9.0 mg, 38%): mp 187.2–188.6 °C; $[\alpha]^{22}_D$ −119° (*c* 0.133, $CHCl_3$); UV (EtOH) 233 nm (ε 31300), 252 (28400), 280 (5610), 288 (5540); IR (KBr) 2.91, 3.41, 5.71, 5.75, 6.00, 6.34, 6.85 μ; NMR ($CDCl_3$) δ 0.83 (3 H, s, C-4-CH_3), 1.19 (3 H, t, J = 7 Hz, C-2′-CH_3), 1.27 (3 H, d, J = 7 Hz, C-6-CH_3), 1.68 (3 H, s, C-14-CH_3), 2.18 (1 H, dd, $J_{2,3}$ = 3, $J_{2,2}$ = 14 Hz, C-2-H), 2.44 (2 H, q, J = 7 Hz, C-2′-H_2), 2.52 (1 H, dd, $J_{2,3}$ = 12, $J_{2,2}$ = 14 Hz, C-2-H), 2.91 (1 H, d, $J_{5,6}$ = 9 Hz, C-5-H), 3.16 (3 H, s, C-1-NCH_3), 3.20 (1 H, d, $J_{15,15}$ = 13 Hz, C-15-H), 3.36 (3 H, s, C-10-OCH_3), 3.49 (1 H, d, $J_{15,15}$ = 13 Hz, C-15-H), 3.50 (1 H, d, $J_{10,11}$ = 9 Hz, C-10-H), 3.53 (1 H, br s, C-9-OH), 3.99 (3 H, s, C-20-OCH_3), 4.16 (1 H, m, C-7-H), 4.87 (1 H, dd, $J_{2,3}$ = 3, 12 Hz, C-3-H), 5.48 (1 H, dd, $J_{10,11}$ = 9, $J_{11,12}$ = 15 Hz, C-11-H), 6.28 (1 H, s, C-9-NH), 6.35 (1 H, dd, $J_{11,12}$ = 15, $J_{12,13}$ = 10 Hz, C-12-H), 6.10–6.60 (1 H, C-13-H), 6.80, 6.83 (2 H, C-17-H, C-20-H), 0.50–2.50 (3 H, C-6-H, C-8-H_2); mass spectrum m/e 620.2504 (M^+; calcd for $C_{31}H_{41}ClN_2O_9$, 620.2500), 559.2330 ($M^+ - H_2O$ − HNCO; calcd for $C_{30}H_{38}ClNO_7$, 559.2336), 485, 470, 450. Anal. Calcd for $C_{31}H_{41}ClN_2O_9$: C, 59.94; H, 6.65; N, 4.51. Found: C, 59.79; H, 6.69; N, 4.62.

Maytansinol 3-Bromoacetate (12). A chloroform (4 mL) solution of maytansinol (20.4 mg, 0.036 mmol), dry pyridine (400 μL), and bromoacetic anhydride (42.7 mg, 0.16 mmol) was heated at 45 °C under nitrogen for 18 h. The solution was cooled and evaporated to dryness, and the residue was partitioned between 1 N hydrochloric acid (5 mL) and dichloromethane (3 × 5 mL). The combined dichloromethane layers were dried over anhydrous sodium sulfate and evaporated to dryness. The residue was subjected to PTLC on ChromAR developed with 5% methanol–ethyl acetate. The major band was removed, eluted with 10% methanol–ethyl acetate, and evaporated to yield a white solid. Crystallization from dichloromethane–hexanes afforded white crystals of **12** (10.7 mg, 43%): mp >300 °C; $[\alpha]^{22}_D$ −149° (*c* 0.107, $CHCl_3$); UV (EtOH) 233 nm (ε 24900), 252 (22300), 280 (4600), 289 (4470); IR (KBr) 2.91, 3.40, 5.68, 5.81, 6.01, 6.32 μ; NMR ($CDCl_3$) δ 0.86 (3 H, s, C-4-CH_3), 1.29 (3 H, d, J = 6 Hz, C-6-CH_3), 1.69 (3 H, s, C-14-CH_3), 3.18 (3 H, s, C-1-NCH_3) 3.37 (3 H, s, C-10-OCH_3), 3.53 (1 H, d, $J_{10,11}$ = 9 Hz, C-10-H), 3.83 (2 H, s, C-2′-H_2), 3.99 (3 H, s, C-20-OCH_3), 4.21 (1 H, m, C-7-H), 5.01 (1 H, dd, $J_{2,3}$ = 3, 12 Hz, C-3-H), 5.54 (1 H, dd, $J_{10,11}$ = 9, $J_{11,12}$ = 15 Hz, C-11-H), 6.34 (1 H, s, C-9-NH), 6.20–6.70 (2 H, C-12-H, C-13-H), 6.86, 7.12 (2 H, s, C-17-H, C-21-H), 1.50–4.00 (9 H, C-2-H_2, C-5-H, C-6-H, C-8-H_2, C-9-OH, C-15-H_2); mass spectrum m/e 623.1293 ($M^+ - H_2O$ − HNCO; calcd for $C_{29}H_{35}BrClNO_7$, 623.1286), 485, 470, 450. Anal. Calcd for $C_{30}H_{38}BrClN_2O_9$: C, 52.52; H, 5.58; N, 4.08. Found: C, 52.42; H, 5.66; N, 4.11.

Maytansinol 3-Crotonate (13). A solution of maytansinol (8.0 mg, 0.0142 mmol), dry pyridine (150 μL), and crotonic anhydride (75 μL, 76.35 mg, 0.496 mmol) was heated at 65 °C under nitrogen for 20 h. Excess pyridine was removed on the rotary evaporator, the residue was partitioned between 1 N hydrochloric acid (5 mL) and dichloromethane (3 × 5 mL), and the combined dichloromethane layers were dried over anhydrous sodium sulfate. After removal of the dichloromethane, the residue was subjected to PTLC on ChromAR developed with 5% methanol–ethyl acetate. The two major bands were removed, eluted with 10% methanol–ethyl acetate, and evaporated to give white solids. The lower R_f band proved to be maytansinol (**7**, 2.2 mg). The higher R_f band was passed through a short plug of alumina, eluted with 10% methanol–dichloromethane, evaporated, and crystallized from dichloromethane–ether to give **13** (1.2 mg, 13.4%) as white crystals: mp 214.1–216.0 °C; $[\alpha]^{24}_D$ −70° (*c* 0.037, $CHCl_3$); UV (EtOH) 231 nm (ε 26400), 252 (20700), 280 (4410), 289 (4410); IR (KBr) 2.92, 3.41, 5.71, 5.80, 6.03, 6.35 μ; NMR ($CDCl_3$) δ 0.84 (3 H, s, C-4-CH_3), 1.28 (3 H, d, J = 6 Hz, C-6-CH_3), 1.61 (3 H, s, C-14-CH_3), 1.64 (3 H, d, J = 7 Hz, C-3′-CH_3), 3.18 (3 H, s, C-1-NCH_3), 3.35 (3 H, s, C-10-OCH_3), 3.99 (3 H, s, C-20-OCH_3), 4.14 (1 H, m, C-7-H), 4.83 (1 H, m, C-3-H), 5.20–5.90 (2 H, m, C-11-H, C-2′-H), 6.18 (1 H, s, C-9-NH), 6.72, 6.83 (2 H, s, C-17-H, C-20-H), 6.10–6.60 (3 H, C-12-H, C-13-H, C-3′-H), 1.50–3.70 (10 H, C-2-H_2, C-5-H, C-6-H, C-8-H_2, C-9-OH, C-10-H, C-15-H_2); mass spectrum m/e 571.23184 ($M^+ - H_2O$ − HNCO; calcd for $C_{31}H_{38}ClNO_7$, 571.23367), 485.19582 ($M^+ - H_2O$ − HNCO − $C_4H_6O_2$; calcd for $C_{27}H_{32}ClNO_5$, 485.196895), 470, 450.

Maytansinol 3-Trifluoroacetate (14). A solution of maytansinol (2.0 mg, 0.0035 mmol) in dichloromethane (0.25 mL) under nitrogen was cooled to −10 °C. Fifteen drops of trifluoroacetic anhydride and 3 drops of trifluoroacetic acid were added, and the reaction mixture was stirred for 15 min; 5 mL of dichloromethane was then added and the solution was washed with 5 mL of 5% sodium bicarbonate and 5 mL of saturated sodium chloride solution and dried over anhydrous sodium sulfate. The dichloromethane was evaporated and the residue was subjected to PTLC on ChromAR, developed with 2% methanol–chloroform. The major band was eluted with 10% methanol–ethyl acetate and crystallized from dichloromethane–carbon tetrachloride to give 0.7 mg of **14** (30%): mp 162.2–163.0 °C; $[\alpha]^{23}_D$ −289° (*c* 0.080, $CHCl_3$); UV (EtOH) 232 nm (ε 34600), 240 (sh, 32000), 252 (30300), 281 (5800), 288 (5700); IR (KBr) 2.92, 3.41, 5.58, 5.80, 6.01, 6.35 μ; NMR ($CDCl_3$) δ 0.87 (3 H, s, C-4-CH_3), 1.30 (3 H, d, J = 7 Hz, C-6-CH_3), 1.67 (3 H, s, C-14-CH_3), 2.27 (1 H, dd, $J_{2,3}$ = 3, $J_{2,2}$ = 15 Hz, C-2-H), 2.60 (1 H, dd, $J_{2,3}$ = 12, $J_{2,2}$ = 15 Hz, C-2-H), 2.87 (1 H, d, $J_{5,6}$ = 9 Hz, C-5-H), 3.16 (1 H, d, $J_{15,15}$ = 13 Hz, C-15-H), 3.17 (3 H, s, C-1-NCH_3), 3.35 (3 H, s, C-10-OCH_3), 3.49 (1 H, d, $J_{10,11}$ = 9 Hz, C-10-H), 3.58 (1 H, s, C-9-OH), 3.79 (1 H, d, $J_{15,15}$ = 13 Hz, C-15-H), 3.99 (3 H, s, C-20-OCH_3), 4.26 (1 H, m, C-7-H), 5.01 (1 H, dd, $J_{2,3}$ = 3, 12 Hz, C-3-H), 5.47 (1 H, dd, $J_{10,11}$ = 9, $J_{11,12}$ = 12 Hz, C-11-H), 6.55–6.09 (2 H, C-12-H, C-13-H), 6.26 (1 H, s, C-9-NH), 6.84, 6.80 (2 H, 2 s, C-17-H, C-20-H), 2.0–1.0 (3 H, C-6-H, C-8-H_2); mass spectrum m/e 600.1976 ($M^+ - H_2O$ − HNCO + H^+; calcd for $C_{29}H_{34}F_3ClNO_7$, 600.1975), 599.1927 ($M^+ - H_2O$ − HNCO; calcd for $C_{29}H_{33}F_3ClNO_7$, 599.1897).

Maytansinoid Ethers. General Procedure. A solution of the maytansinoid (ca. 10 mg), the appropriate alcohol (200 μL, dried over activated 3Å molecular sieves), and trifluoroacetic acid (2 drops from a Pasteur pipet) in dry benzene (200 μL) in a 1-dram vial was allowed to stand under nitrogen at room temperature in a sealed (parafilm) 1-oz jar ca. one-third full of activated 3Å molecular sieves. During the course of the reaction, benzene (200 μL) and the alcohol (200 μL) lost by evaporation were replenished. After 3 days the solution was chromatographed on silica gel 60 F-254 (two 0.25 mm × 20 × 20 cm plates) using 4% 2-propanol–dichloromethane. Bands corresponding to the desired ether and to the unreacted maytansinoid were eluted (30% methanol–chloroform, ca. 20 mL) and filtered and the filtrate was concentrated. Recovered maytansinoid was recycled once to obtain additional ether. The combined maytansinoid ether samples were rechromatographed using 8% 2-propanol–methylene chloride, eluted, filtered, concentrated, dissolved in dichloromethane, filtered, concentrated, dried overnight in vacuo (1.5 mm at room temperature), and crystallized from dichloromethane–ether–hexanes.

Maytansine Methyl Ether (16). Maytansine (1, 10.3 mg) gave **16** (2.6 mg, 25%): mp 237–238 °C; UV (EtOH) 232 nm (ε 25800), 240 (23700), 253 (23500), 280 (5150), 288 (5080); IR ($CHCl_3$) 5.74, 5.82, 5.87, 6.04, 6.12, 6.35, 9.33 μ; NMR ($CDCl_3$) differs from that of maytansine (1)[8] in the appearance of δ 3.41 (3 H, s, C-9-OCH_3) and disappearance of δ 3.52 (C-9-OH) signals; mass spectrum m/e 706.3104 (M^+ + H; calcd for $C_{35}H_{49}ClN_3O_{10}$, 706.3106).

Maytansine Ethyl Ether (17). Maytansine (1, 26.7 mg) gave **17** (13.1 mg, 56.6%; 65% based on 1 not recovered): mp 224–225 °C; UV (EtOH) 233 nm (ε 28300), 242 (sh, 26000), 253 (27850), 280 (5390), 288 (5610); IR ($CHCl_3$) 5.75, 5.82, 5.87, 6.04, 6.12, 6.35 μ; NMR ($CDCl_3$) differs from that of maytansine (1) in the appearance of δ 1.14 (3 H, t, J = 7 Hz, CH_2CH_3) and δ 3.68 (2 H, q, J = 7 Hz, $-OCH_2CH_3$) and the disappearance of δ 3.52 (C-9-OH) signals; mass spectrum m/e 719.3179 (M^+; calcd for

$C_{36}H_{50}ClN_3O_{10}$, 719.3184), 673, 630, 485, 470, 450.

Maytanbutine Methyl Ether (18). Maytanbutine (**2**, 11.4 mg) gave **18** (3.2 mg, 28%): mp 232–233 °C; $[\alpha]^{23}_D$ −154° (*c* 0.0176, $CHCl_3$); UV (EtOH) 233 nm (ε 30500), 240 (28100), 253 (28200), 280 (5970), 288 (5930); IR ($CHCl_3$) 5.74, 5.82, 5.87, 6.04, 6.12, 6.35, 9.33 μ; NMR differs from that of maytanbutine (**2**)[8] in the appearance of a signal at δ 3.41 (3 H, s, C-9-OCH_3) and the disappearance of the δ 3.52 (C-9-OH) signal; mass spectrum *m/e* 734.3416 (M^+ + H; calcd for $C_{37}H_{53}ClN_3O_{10}$, 734.3419).

Maytanbutine Ethyl Ether (19). Maytanbutine (**2**, 11.1 mg) gave **19** (4.3 mg, 37%): mp 166–168 °C; UV (EtOH) 233 nm (ε 27200), 242 (25300), 253 (25800), 280 (5340), 288 (5130); IR ($CHCl_3$) 5.74, 5.87, 6.04, 6.12, 9.33 μ; NMR differs from that of maytanbutine (**2**) at δ 3.70 (3 H, m, C-9-OCH_2CH_3 superimposed on C-15-H), at δ 1.0 (OCH_2CH_3 triplet buried in complex methyl signals), and at δ 3.52 (no C-9-OH signal); mass spectrum *m/e* 748.3586 (M^+ + H; calcd for $C_{38}H_{55}ClN_3O_{10}$, 748.3586).

Maytansine 9-*n*-Propyl Thioether (20). A solution containing maytansine (**1**, 10.5 mg), propanethiol (100 μL), trifluoroacetic acid (4 drops), and methylene chloride (0.8 mL) was stirred 15 h at room temperature. Excess dichloromethane, trifluoroacetic acid, and propanethiol were evaporated, and the residue was subjected to PTLC on silica gel 60 F-254 twice developed with 8% 2-propanol–methylene chloride. The major band was eluted with 30% methanol–chloroform and crystallized from dichloromethane–ether–hexanes to give 6.1 mg of **20** (49%): mp 218–220 °C; UV (EtOH) 234 nm (ε 24800), 245 (23600), 255 (25400), 280 (5150), 289 (4910); IR ($CHCl_3$) 5.76, 5.88, 5.94, 6.04, 6.12, 6.37, 9.36 μ; NMR ($CDCl_3$) δ 0.983 (3 H, t, *J* = 7 Hz, $-SCH_2CH_2CH_3$); mass spectrum *m/e* 750.3200 (M^+ + H; calcd for $C_{37}H_{53}ClN_3O_9S$, 750.3190).

Maytanbutine 9-Thiopropyl Ether (21). Compound **21** was prepared in same manner as **20** from 6.49 mg of **2** to give 3.89 mg (55%) of **21**: mp 203–206 °C; $[\alpha]^{23}_D$ −97.4° (*c* 0.0421, $CHCl_3$); UV (EtOH) 233 nm (ε 25500), 245 (24100), 255 (25900), 281 (5150), 289 (4900); IR ($CHCl_3$) 2.92, 3.34, 3.37, 3.41, 3.47, 3.54, 5.74, 5.86, 6.03, 6.12, 6.34, 6.86, 8.48, 9.26 μ; mass spectrum *m/e* 778.3451 (M^+ + H; calcd for $C_{39}H_{57}ClN_3O_9S$, 778.3504), 701, 658, 657, 485, 470, 450.

References and Notes

(1) (a) Tumor Inhibitors. 124. For part 123, see S. M. Kupchan, J. W. Ashmore, and A. T. Sneden, *Phytochemistry*, in press. (b) Deceased Oct 19, 1976.

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