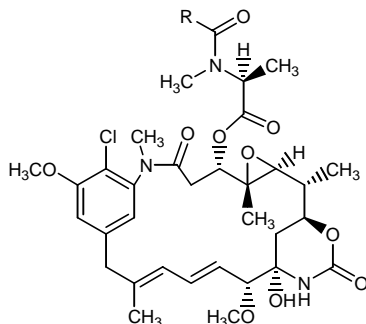


Maytansine

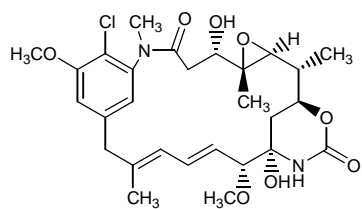
Maytansine is an ansa macrolide derived primarily via a polyketide pathway. Maytansine was first isolated in 1972 from the African plant, *Maytenus ovatus* (Celastraceae), later renamed *Maytenus serrata*, by Kupchan and co-workers in a yield of 0.2 mg/kg of dried plant material. It showed antileukemic activity against the P388 lymphocytic leukemia in mice at the $\mu\text{g}/\text{kg}$ level over a 50-100 fold dose range, and cytotoxicity against the KB cell culture derived from a human epidermoid carcinoma of the mouth (ED_{50} 10^{-4} - 10^{-5} $\mu\text{g}/\text{ml}$). A search for a better source of maytansine led to *Maytenus buchananii*, another member of the Celastraceae family, which provided maytansine in a yield of 1.5 mg/kg, along with several other homologues (maytansinoids). Other plants in the Celastraceae family would later prove to be better sources of maytansine and its homologues, yielding up to 12-15 mg/kg, but the plants were less common. In order to obtain sufficient maytansine for preclinical pharmacology testing and clinical trials, two large scale collections of *Maytenus buchananii* were made, approximately 7,000 lb in 1973 and 15,000 lb in 1976. The maytansine isolated from these collections was sufficient to provide material for preclinical toxicology and clinical trials. The cost of this maytansine was estimated at \$75,000/g.

Screening of maytansine in animal systems showed significant activity at $\mu\text{g}/\text{kg}$ doses against the P388 lymphocytic leukemia (142% average increase in life span (ILS); this is equivalent to expressing the result as a T/C (test/control) of 242), the L1210 leukemia (49% ILS), the B16 melanoma (57% ILS), the the Lewis lung carcinoma (32% ILS), and colon 26 (31% ILS) systems. Maytansine acts as a mitotic inhibitor by inhibiting the polymerization of tubulin thus interfering with the formation of microtubules in the cell nucleus. It inhibits DNA, RNA, and protein synthesis, with the greatest effect seen in the inhibition of DNA synthesis. The relatively impressive activity in animal tests made maytansine a leading candidate for rapid advancement into clinical trials. In preclinical toxicity studies, maytansine caused some acute toxicity, reversible chronic toxicity in numerous tissues which was dose related, mild neurotoxicity, dose related teratogenicity, and inflammation and fibrosis at the injection site. None of these toxicities were sufficient to prevent initiation of Phase I clinical trials. These trials were begun in 1975. The toxicities determined in the clinical trials were gastrointestinal toxicity which was dose related and dose limiting, hepatic toxicity which was again dose related, neurotoxicity, both central and peripheral. The maximum tolerated dose was 0.5-2 mg/meter². Responses were seen in patients with acute lymphocytic leukemia, breast carcinoma, ovarian cancer, thymoma, melanoma, and non-small scale lung cancer. However, after advancing into Phase II clinical trials, maytansine was found to have little or no anticarcinogenic effect in most patients at the maximum tolerated dose. While gastrointestinal toxicity was manageable, the neurotoxicity at the site of injection was not. The pain was evidently so severe that patients refused additional treatments with maytansine. As a result, maytansine was dropped from clinical trials.

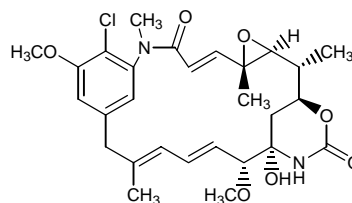


<u>Name</u>	<u>R</u>	<u>P388 <i>in vivo</i> (T/C)</u>	<u>KB <i>in vitro</i></u>
Maytansine	CH ₃	220 @ 25 $\mu\text{g}/\text{kg}$	10^{-6} $\mu\text{g}/\text{ml}$
Maytanbutine	CH(CH ₃) ₂	190 @ 0.8 $\mu\text{g}/\text{kg}$	10^{-6} $\mu\text{g}/\text{ml}$
Maytanprine	CH ₂ CH ₃	154 @ 1.6 $\mu\text{g}/\text{kg}$	10^{-7} $\mu\text{g}/\text{ml}$
Maytanvaline	CH ₂ CH(CH ₃) ₂	187 @ 12.5 $\mu\text{g}/\text{kg}$	10^{-7} $\mu\text{g}/\text{ml}$

The maytansine story is important for what was determined about the structure-activity relationships in this complex molecule. These relationships were determined by testing naturally-occurring homologues, as well as derivatives prepared synthetically. The first site studied for its relation to the activity was C(3) with its ester moiety. In the course of the original investigations, homologues of maytansine with different N-acyl groups on the C(3) *l*-alanyl ester were isolated. These were named maytanbutine, maytanprine, and maytanvaline. All of these ester homologues have approximately the same activity as maytansine. At the same time, homologues without the C(3) ester were also isolated, e.g., maytansinol and maysine. (Maytansinol could also be prepared synthetically by reductive hydrolysis of any of the C(3) ester homologues with lithium aluminum hydride.) These derivatives had significantly reduced activity, and these results indicated that a C(3) ester was required for optimal activity.

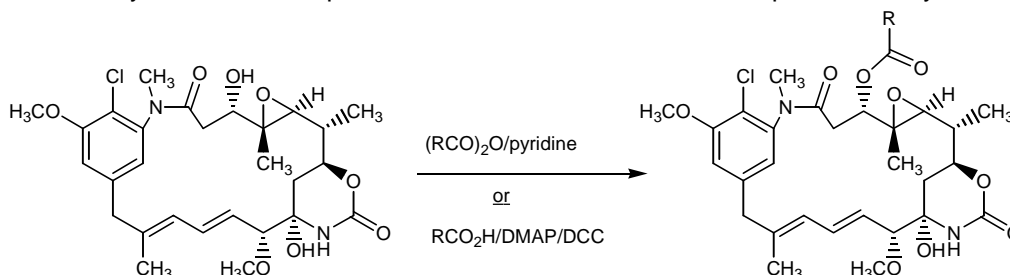


Maysinol
P388 *in vivo*: T/C <125 @ 3.1-400 µg/kg
9KB *in vitro*: ED₅₀ 10⁻¹ µg/ml



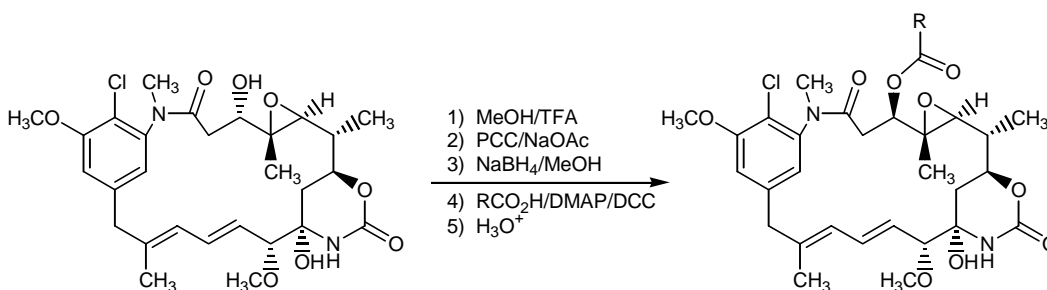
Maysine
P388 *in vivo*: T/C <125 @ 0.2-100 µg/kg
9KB *in vitro*: ED₅₀ 10⁻² µg/ml

The availability of maytansinol permitted the synthesis of derivatives at C(3), particularly simple aliphatic esters such as the acetate (also a naturally occurring homologue named maytanacine) and the propionate. Shortly thereafter, Japanese researchers were able to produce maytansinoids from a

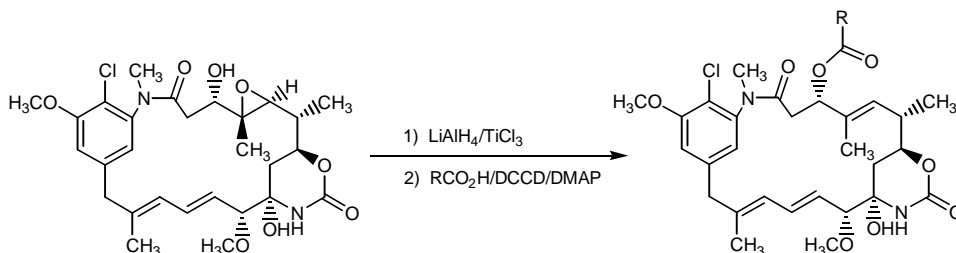


Nocardia cell culture on a large scale. Their compounds, which did not have the amino acid ester, were named ansamitocins. This procedure provided maytansinol in quantities sufficient to synthesize large numbers of different C(3) esters. All of the esters were active at levels equivalent to maytansine, confirming that the structure of the C(3) ester was not important to the activity. These results also reinforced the proposal that the C(3) ester is involved in a function like transport which would not be significantly affected by small changes in structure.

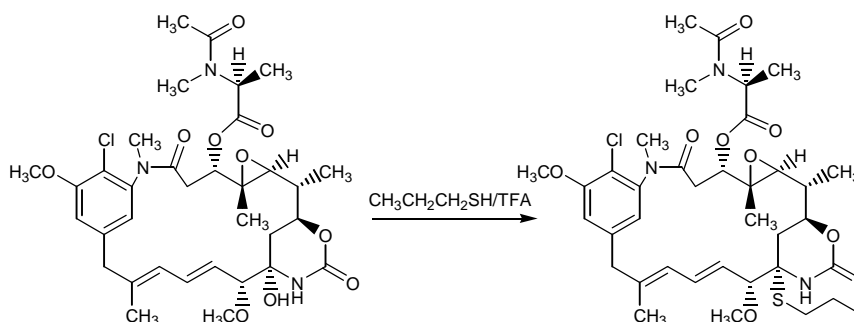
The stereochemistry of the ester at C(3) could also be inverted by first protecting the C(9) carbinol moiety of maytansinol as the methyl ether, then oxidizing the C(3) alcohol, reducing with sodium borohydride, acylating, and hydrolyzing the C(9) ether. The C(3) *epi*-esters did not inhibit tubulin polymerization, indicating that the stereochemistry at C(3) is crucial to the activity of the maytansinoids.



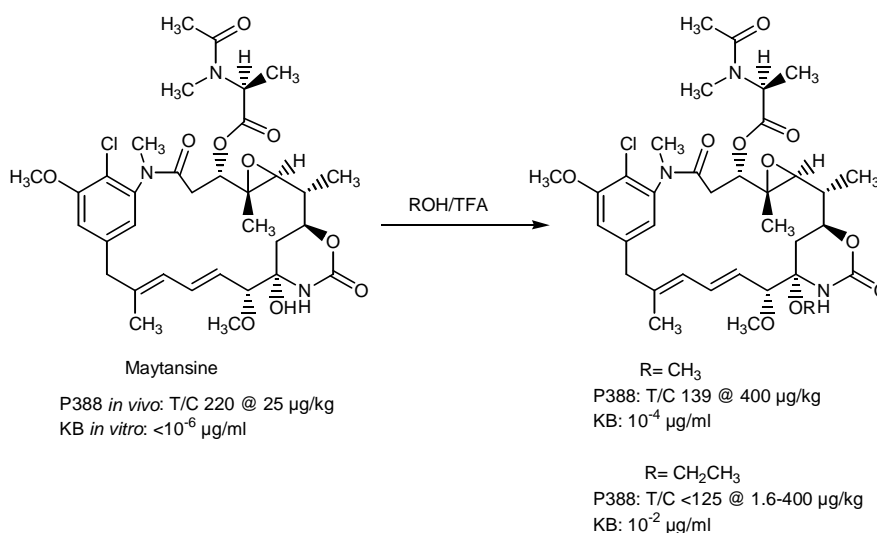
To determine the role of the C(4)-C(5) epoxide, maytansinol was deoxygenated to the C(4)-C(5) alkene by treatment with lithium aluminum hydride/titanium trichloride and then acylated. The 4,5-deoxymaytansinoids prepared by this method had activity comparable to the analogous maytansinoids with the epoxide.



The next functional group to consider for involvement in the biological activity is the C(9) carbinolamide moiety. This is a somewhat unusual grouping, and the presence of a tertiary carbinol suggested immediately that this would be a potential site for alkylation by a biological macromolecule such as a protein. To investigate this possibility, Kupchan and co-workers examined the reactivity of maytansine and its homologues with a mimic for such an alkylation, propanethiol. They determined that the maytansinoids readily reacted with propanethiol under acidic conditions to give the 9-thiopropyl ether, as shown for maytansine. This was a general reaction for maytansinoids with the carbinolamide moiety.

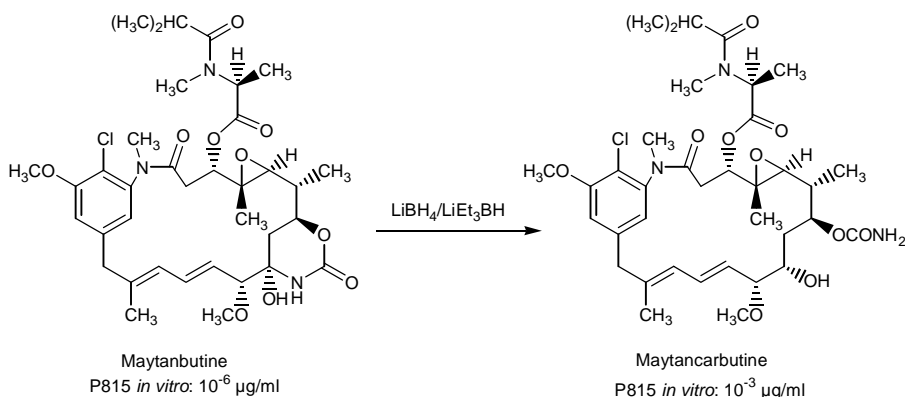


Maytansine could also be converted easily to the C(9) ether by the reaction of an alcohol under acidic conditions. The ethers formed in this reaction were less active than the parent maytansinoid, as shown for



the methyl and ethyl ethers of maytansine, but did show some activity. This may be attributed to hydrolysis of the ether *in vivo*.

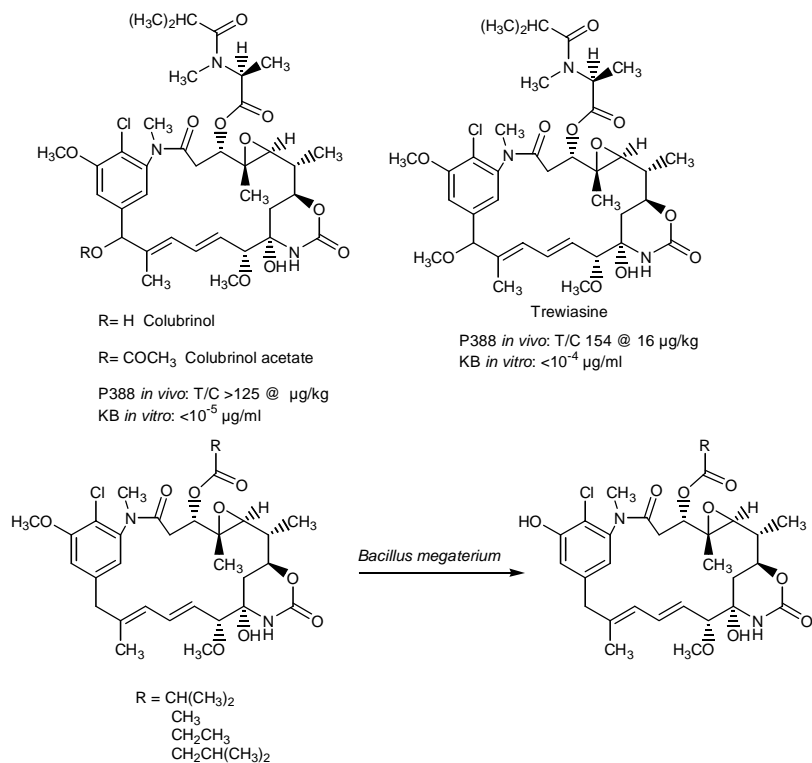
It was later found that the carbinolamide moiety would react under mild reductive conditions to cleave the C(9)-N bond, resulting in compounds such as maytancarbutine (from maytanbutine). These compounds, which contain a secondary carbinol rather than a more reactive tertiary carbinol, are



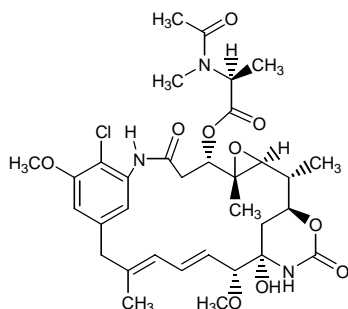
approximately 1000 times less active than the parent compound. All of these results demonstrate that the C(9) carbinolamide is required for optimal antileukemic activity in the maytansinoids.

The C(15) position is a methylene in maytansine and most of the homologues, however, colubrinal and colubrinal acetate from *Colubrina texensis* and trewasine from *Trewia nudifolia* all have oxygen moieties at this position. Each of these has activity similar to maytanbutine, showing that this site has little to do with the biological activity.

The C(20) methoxyl, as an aromatic methoxyl, is expected to be relatively inert and would not be expected to influence activity significantly. However, this methoxyl was converted to the phenol using several different ansamitocins and the bacterium *Bacillus megaterium*. The phenolic compounds were more active *in vivo* against the P388 and L1210 leukemias than were the parent compounds. Thus, the C(20) methoxyl evidently inhibits the activity of the maytansinoids.



There are several naturally occurring homologues which have the C(18) N-CH₃ replaced with a N-H moiety, e.g., normaytansine. There does not appear to be a significant change in the activity due to this change.



Normaytansine

P388 *in vivo*: T/C 181 @ 100 µg/kg
 KB *in vitro*: <10⁻³ µg/ml

Catalytic hydrogenation of the diene in the southwest quadrant of maytansine produces a tetrahydromaytansine. While not screened for antileukemic activity, this derivative does demonstrate similar brine shrimp lethality to that observed for maytansine. Thus, the diene does not appear to be required for activity.

As a result of testing of the naturally occurring and semi-synthetic homologues of maytansine, and a few other experiments not mentioned, the structure-activity relationships of maytansine have been fairly well worked out. They are summarized as shown. If maytansine had shown better activity in the clinical trials, these SARs would be important in preparing second and third generation homologues to improve the activity and reduce the toxicity of this compound.

Maytansine Structure-Activity Relationships

