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Synthesis of the *N*-Acetylcysteamine Thioester of *seco*-Proansamitocin

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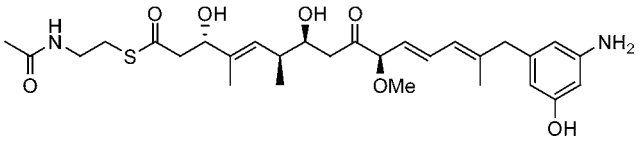
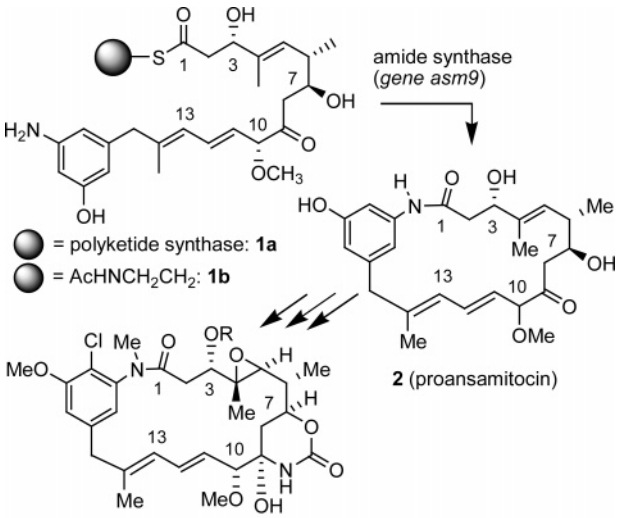
ABSTRACT



The enantioselective total synthesis of the *N*-acetylcysteamine thioester of *seco*-proansamitocin, a key biosynthetic intermediate of the highly potent antitumor agent ansamitocin, is described, which twice utilizes the Nagao acetate aldol reaction, as well as an indium-mediated alkynylation of a benzyl bromide followed by carboalumination. The key step is a Heck reaction between two terminal alkenes for merging the two major fragments.

Maytansin **3**, first isolated from the Ethiopian plant *Maytenus serrata*[1,2] and the related ansamitocins P-1−P-4 (see **4** and **5** in Scheme 1),[3,4] which are of microbial origin (*Actinosynnema pretiosum*), are members of the growing ansamycin family of antibiotics.[5] They consist of a 19-membered macrolactam ring and differ in the side chain at C-3. Whereas the ansamitocins **4** and **5** are fatty acid esters of maytansinol, the maytansins **3** commonly carry modified amino acid side

Scheme 1



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(4) A mutant of *A. pretiosum* spp. *auranticum* provided 15 additional ansamitocines: (a) Izawa, M.; Tanida, S.; Asai, M. *J. Antibiot.* **1981**, *34*, 496−506. (b) Komoda, Y.; Kishi, T. Maytansinoids. In *Anticancer Agents Based on Natural Product Models*; Douros, J., Cassady, J. M., Eds.; Academic Press: New York, 1980; pp 353−389.

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chains. Preclinical studies[6] demonstrated that maytansinoids are strong antitumor agents as a result of binding to tubulin, thereby shutting down tubulin polymerization.[7] They inhibit growth of different leukaemia cell lines as well as human solid tumors at very low concentrations (10^{-3} to 10^{-7} μg/mL). Despite promising toxicity tests in different animal models,[6] the clinical development of maytansinoids had to be stopped in phase II[2a,8] because of gastrointestinal side effects and neurotoxicities.[4b,6,9]

However, given the high intrinsic potency of this class of natural products, efforts to develop them into clinically useful agents still continue. Particularly promising are the initial results toward increasing their selectivity by conjugating the ansamitocins to tumor-targeted antibodies.[10]

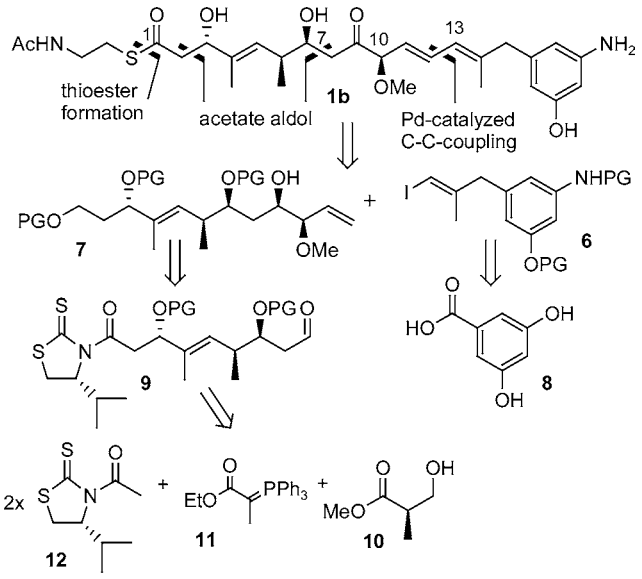
The potent antitumor activity of the maytansinoids stimulated substantial synthetic work that from 1980 on led to several total syntheses.[11] Because of their complexity these syntheses contributed little to our knowledge of the structure−activity relationships; this was basically collected from semisynthetic work starting with the natural products.[2a,e]

However, recent cloning and sequencing of the ansamitocin (*asm*) biosynthetic gene cluster from *Actinosynnema pretiosum* by Floss and co-workers[12] has paved the way for the detailed analysis of ansamitocin biosynthesis at the genetic and biochemical levels. This may provide a tool for the chemoenzymatic synthesis of maytansine analogues carrying backbone structural modifications that are not easily accessible by chemical means. The biosynthesis involves the assembly of the carbon framework on a type I modular polyketide synthase[13] from 3-amino-5-hydroxybenzoic acid (AHBA)[14] through chain extension by one "glycolate", three propionate, and three acetate units. The last PKS module holds the *seco*-proansamitocin **1a**, which is released and cyclized by an amide synthase (gene *asm9*)[15,16] to yield the cyclic 19-membered macrocyclic lactam, proansamitocin **2** (Scheme 1).[17] A closer study of the substrate specificity of this key enzyme could pave the way for a chemoenzymatic strategy toward new macrocyclic analogues of proansamitocin.

Therefore, we first decided to prepare the *N*-acetylcysteamine derivative **1b** to explore whether it is a substrate for this cyclase before generating analogues of *seco*-proansamitocin to study the substrate specificity of the amide synthase.

Retrosynthetic analysis of **1b** led us to vinyl iodide **6** and alkene **7**, which are supposed to be connected by Pd(0)-catalyzed cross-coupling, preferentially by the Heck reaction (Scheme 2).

Scheme 2 [a]



[a] PG = protective group.

Fragment **6** originates from commercially available 3,5-dihydroxybenzoic acid (**8**). Alkene **7** is further simplified to aldehyde **9**, which is disconnected to the three starting building blocks **10**−**12**.

This strategy has to make use of two asymmetric acetate aldol reactions and requires an optimized protecting group strategy, because a thioester moiety, a keto, a phenolic, and an amino group have to be taken into consideration. Furthermore, the target molecule is prone to conjugation of the diene unit with the keto group, epimerization at C-10, and β-elimination next to the thioester.

Thus, starting from benzoic acid **8**, intermediate benzyl bromide **13** was prepared by a set of standard reactions that

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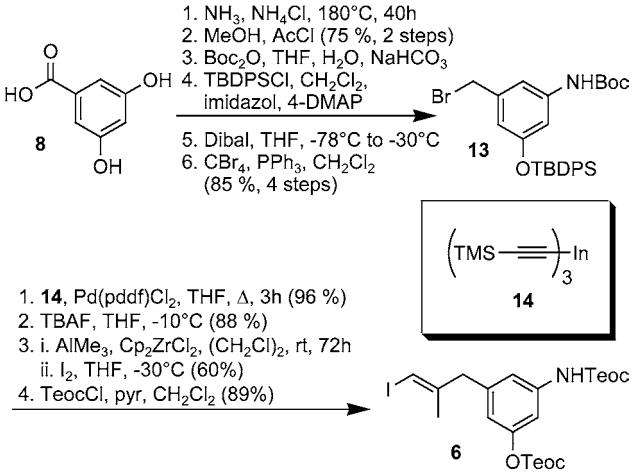
involved introduction of the amino group,[18] protection of the phenolic and amino groups, and transformation of the carboxyl group into the primary bromide (Scheme 3). Among

Scheme 3 [a]



[a] TBDPS = *tert*-butyldiphenylsilyl, Boc = *tert*-butoxycarbonyl, Dibal = Diisobutylaluminum hydride, Cp = cyclopentadienyl, dppf = bis(diphenylphosphino)ferrocene, Teoc = trimethylsilylethoxycarbonyl.

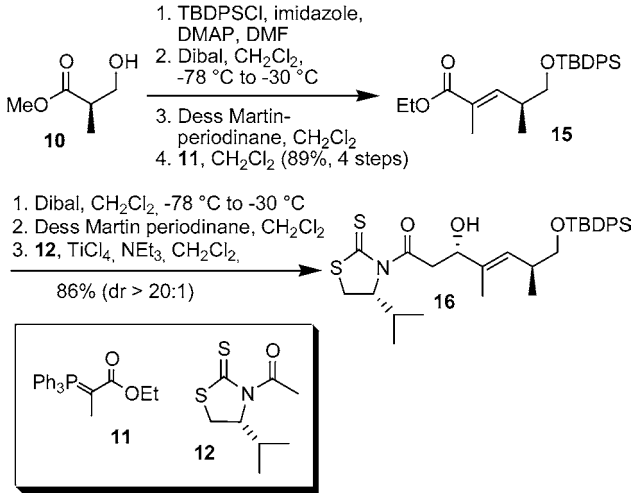
several attempts to substitute benzyl bromide **13** with an alkyne moiety, only the palladium-catalyzed reaction with indium derivative **14** was successful.[19] After desilylation, a carboalumination[20] during which the Boc group was lost followed by reprotection allowed creation of the vinyl iodide **6** in good yield and with good stereoselectivity.

Synthesis of the second major building block started from Roche ester **10**, which was transformed into ester **15** by a set of standard steps (Scheme 4).[21]

The ethyl ester was then transformed into the corresponding aldehyde group[22] which was subjected to the Nagao acetate-aldol protocol utilizing the thiazolidin-2-thione derivative **12**.[23] Alternatively to the sensitive and costly Sn(II) triflate, in the present case Ti(IV) chloride was employed as enolizing agent,[24] providing aldol product **16**.

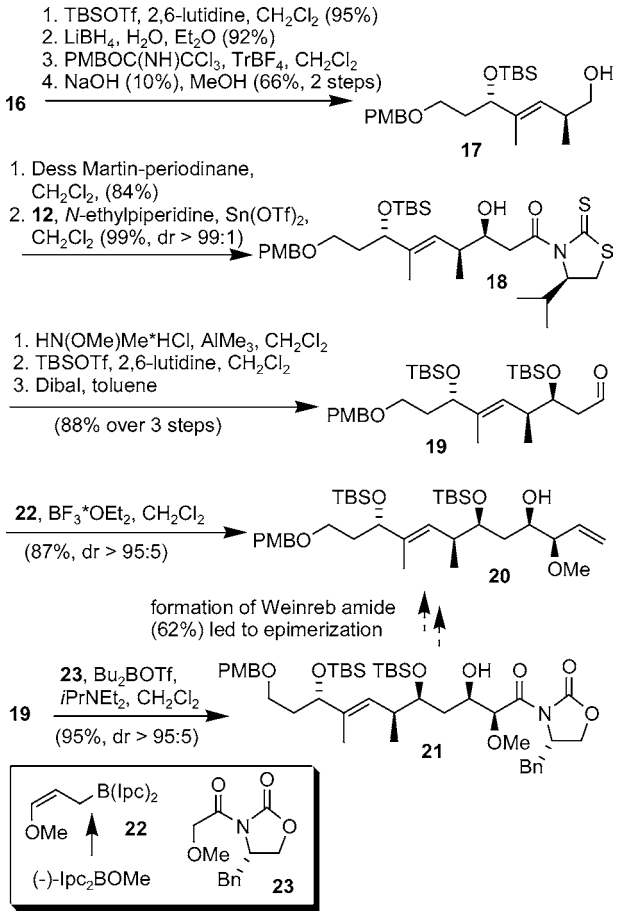
C-1 of proansamitocin, now established in **16**, was transformed into the PMB-protected alcohol, opening the option of elongating fragment **17** at the opposite terminus

Scheme 4



(Scheme 5). After formation of the intermediate aldehyde a second acetaldol reaction was performed, again employing thiazolidine-2-thione **12** as chiral auxiliary.[23] This time, tin(II) triflate in the presence of *N*-ethyl piperidine was the

Scheme 5 [a]



[a] PMB = *p*-methoxybenzyl, TBS = *tert*-butyldimethylsilyl, Tf = trifluoromethylsulfonyl.

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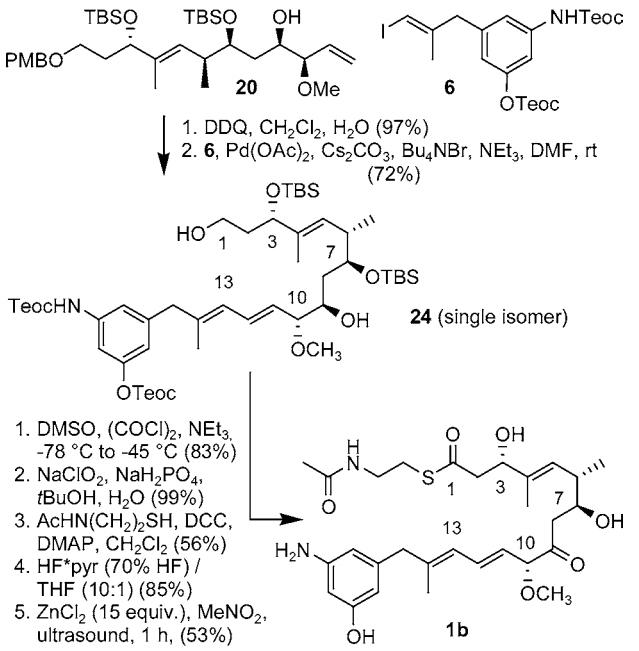
reagent system of choice (99%, dr > 99:1). A standard sequence (Weinreb amide formation, protection and Dibal reduction) yielded aldehyde **19**, which was transformed into alkene **20** by two alternative procedures. On the basis of the protocol first developed by Brown et al.,[25] the *Z*-configured vinyl methyl ether **22** was added with Lewis acid promotion with high diastereoselectivity (dr > 95:5) to aldehyde **19**, which yielded allyl methyl ether **20** in one step. Alternatively, aldehyde **19** was subjected to an Evans aldol reaction, using the boron enolate derived from the α-methoxy acetate **23**.[26] The resulting aldol product **21** was obtained in excellent yield and with high diastereoselectivity. However, transformation into the corresponding Weinreb amide proceeded only in moderate yield (62%) and with epimerization, so this route was not further elaborated. Removal of the PMB protection at C-1 allowed analysis of the configurations at C-7 and C-9[27] and set the stage for merging both main fragments by means of the Heck reaction, employing the Jeffery protocol (Scheme 6).[28] This procedure yielded the coupling product **24** in 72%, which contains the complete carbon backbone of *seco*-proansamitocin. Simultaneous oxidation of both free alcohol groups and further oxidation of the aldehyde moiety to the carboxylic acid was followed by SNAC ester formation. In the following, the silyl ethers could only be removed by treatment with the HF·pyr complex. Basic desilylating agents (e.g., TBAF) led to decomposition. Finally, $ZnCl_2$-mediated removal of both Teoc groups[29] under ultrasound conditions afforded the SNAC-ester of *seco*-proansamitocin **1b**.

In conclusion, we achieved the first total synthesis of *seco*-proansamitocin activated as its *N*-acetylcysteamine thioester, which will be tested as a substrate for the amide synthase of *Actinosynnema pretiosum*. The synthetic approach allows the introduction of other aryl groups at a late stage of the synthesis. This will be important for studying the substrate specificity of this enzyme. Details on these studies will be reported in a full account.

Scheme 6 [a]



[a] DDQ = dichlorodicyano quinone, DCC = dicyclohexyl carbodiimide, DMAP = 4-dimethylamino pyridine, Pyr = pyridine.

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Supporting Information Available: Descriptions of experimental procedures for compounds and analytical characterization. This material is available free of charge via the Internet at http://pubs.acs.org.

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