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Chemoenzymatic Approaches toward Dechloroansamitocin P-3†

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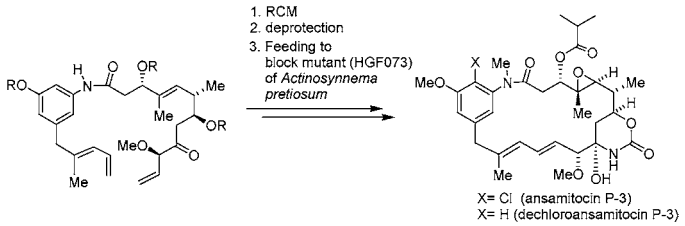
ABSTRACT



The enantioselective total synthesis of proansamitocin, a key biosynthetic intermediate of the highly potent antitumor agent ansamitocin P-3, is described which bears a diene-ene RCM as the key macrocyclization step. Feeding of proansamitocin to an AHBA block mutant *Actinosynnema pretiosum* (HGF073) yielded ansamitocin P-3 as well as dechloroansamitocin P-3, the latter also being formed upon fermentation in the presence of 3-amino-5-methoxybenzoic acid.

Maytansine, first isolated from the Ethiopian plant *Maytenus serrata*,[1,2] and the related ansamitocins P-1 to P-4,[3−5] which are of microbial origin (*Actinosynnema pretiosum*), consist of a 19-membered macrolactam ring and differ in the side chain at C-3. 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) by inhibiting tubulin polymerization. However, the clinical development of maytansinoids had to be stopped in phase II[2a,6] due to gastrointestinal side effects and neurotoxicities.[4b,7]

Total synthesis approaches[5,8] contributed little to our knowledge of the structure−activity relationships; this

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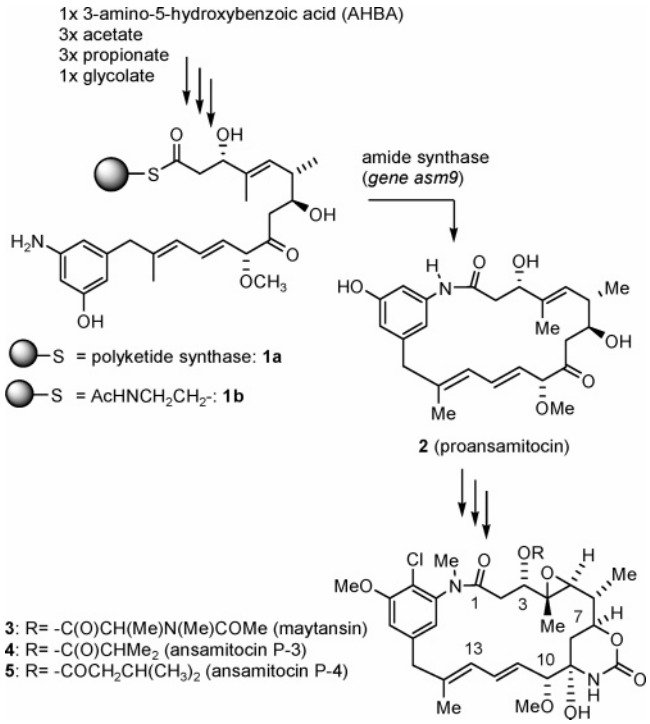
information was basically collected from semisynthetic work starting with the natural products.[2a,e]

As a result of detailed biosynthetic studies on ansamycin antibiotics[9−13] including ansamitocin P-3, Floss and co-workers designed a block mutant (HGF073) of *Actinosynnema pretiosum* which is unable to biosynthesize the starter unit, 3-amino-5-hydroxybenzoic acid (AHBA),[9] of the type I modular polyketide synthase. This synthase is responsible for assembling the carbon framework (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*)[12] to yield the cyclic 19-membered macrocyclic lactam, proansamitocin **2** (Scheme 1).[13]

Scheme 1. Biosynthesis of Maytansin **3** and Ansamitocins **4**, **5** via *seco*-Proansamitocin **1** and Proansamitocin **2**

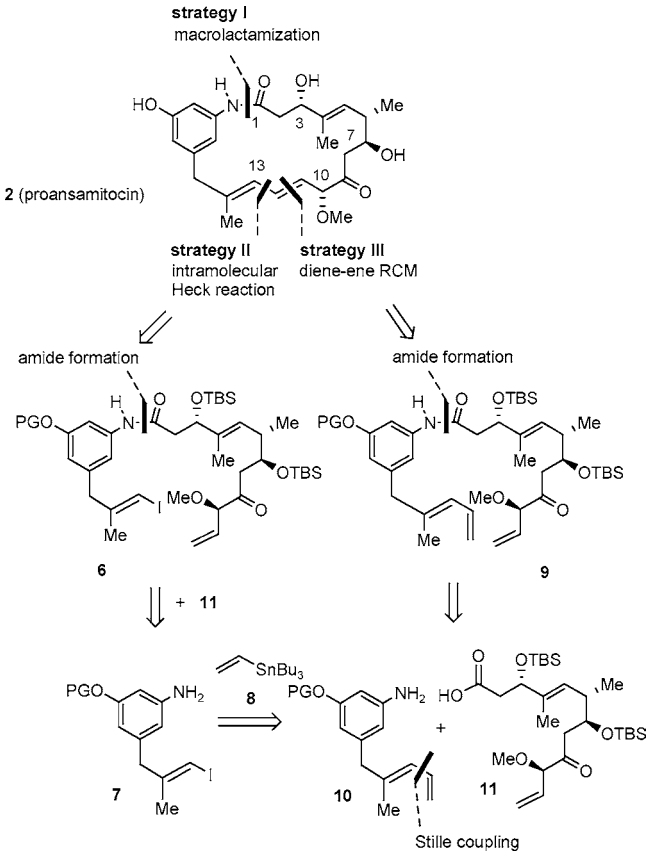


Recently, we initiated a research program dedicated to synthetically exploit genetically engineered microorganisms such as the AHBA block mutant (HGF073)[14] for chemoenzymatically generating new analogues of pharmaceutically highly potent secondary metabolites like the ansamitocins. As part of these studies we disclosed the total synthesis of the *N*-acetylcysteamine derivative of *seco*-proansamitocin **1b**,[15] which is the SNAC-analogue for the natural substrate of the cyclizing amide synthase (*asm9*).[16]

We now report on the first synthesis of proansamitocin **1b** the product of the amide synthase which we wish to utilize in screening for the amide synthase and in chemoenzymatic studies with strain HGF073.

Analysis of **2** led us to consider three strategies for macrocyclization. While macrolactamization (strategy I) is a biomimetic approach the other two concepts (intramolecular Heck-reaction (strategy II)[17] and diene−ene ring closing metathesis[18] (RCM) (strategy III)) are based on transition metal catalysis and would, compared to the first approach, provide more synthetic novelty (Scheme 2). In fact, we found

Scheme 2. Macrocyclization Strategies (I−III) and Retrosynthetic Analysis for Strategies II and III[a]



[a] PG = protecting group. TBS = *tert*-butyldimethylsilyl.

that macrolactamization of related acyclic aniline precursors (ansamitocin and ansatrienine) was not successful and proceeded only in low yields.[19] A suitable retrosynthetic

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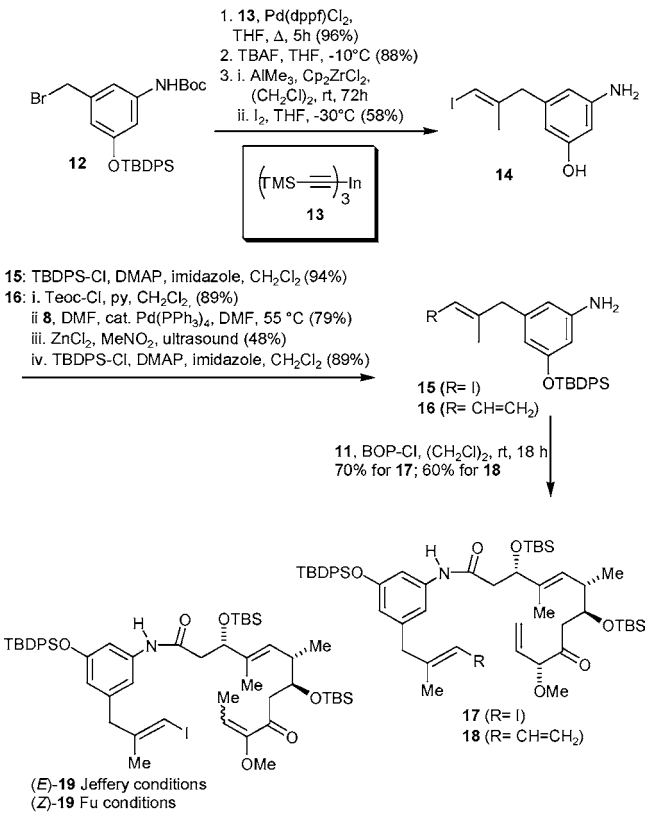
Scheme 3 [a]



[a] dppf = bis(diphenylphosphinoyl)ferrocene, TMS = trimethylsilyl, TBAF = tetra-*n*-butylammonium fluoride, Cp = cyclopentadienyl, TBDPS= *tert*-butyldiphenylsilyl, Teoc = trimethylsilylethoxycarbonyl, BOP-Cl = *N*,*N*-bis(2-oxo-3-oxazolidinyl)-phosphonic chloride.

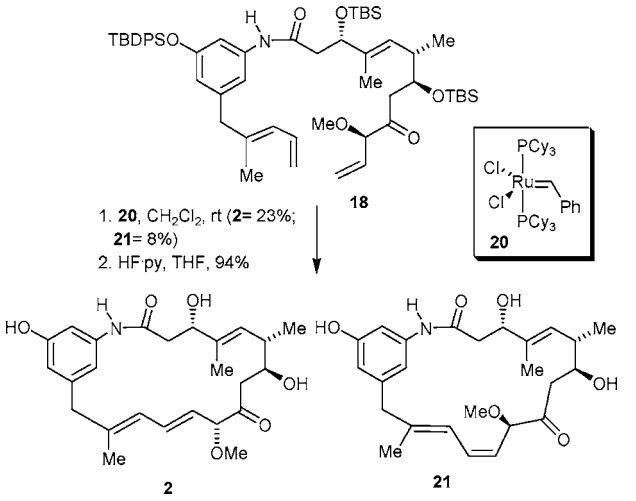
precursor for the Heck-strategy II is vinyl iodide **6**, which derives from the advanced ketide fragment **11**. This had been prepared by us before as part of our total synthesis of the SNAC-ester **1b**,[15] and aniline **7**. Vinyl iodide **7** is also the starting material for the diene−ene RCM strategy III, which relies on the intermediate Stille coupling product **10**. This compound is planned to be coupled with fragment **11** so that amide **9** serves as the RCM precursor.

In principle, the two required aromatic building blocks **15** and **16** had to be prepared from the same starting benzyl bromide **12**. It was transformed into vinyl iodide **14** (after Pd-catalyzed alkynylation with alkynylindium **13**,[20] desilylation, Negishi-type methyl metalation of intermediate alkyne,[21] followed by iodination; Scheme 3). Vinyl iodide **14** was then transformed into the two free aniline derivatives **15** and **16**, respectively. **15** was simply prepared after *O*-silylation while the latter sequence included exhaustive Teoc-protection, a Stille coupling for constructing the diene unit in **16**, and deprotection followed by *O*-silylation. Now the stage was set to achieve intermolecular amide formation. BOP-chloride[22] turned out to be the best coupling reagent for coupling carboxylic acid **11** with both anilines **15** and **16**, respectively, to yield the corresponding amides **17** and **18**.

Despite the fact that intermolecular Heck coupling between fragments **11** and **14** proceeds well under Jeffery conditions,[23,24] we were unable to obtain the expected Heck cross-coupling product by Pd(0)-catalyzed macrocyclization of vinyl iodide **17**, while the Jeffery conditions (K_2CO_3, Bu_4NCl, cat. $Pd(OAc)_2$, NEt_3, DMF, rt) led to migration of the terminal olefinic double bond furnishing the (*E*)-configured α,*β*-unsaturated ketone **19**, perhaps because of the basic conditions. However, the Fu conditions ($Pd_2(dba)_3$, P(*t*-Bu)$_3$, Cy_2NMe, dioxane, 110 °C)[25] generated the (*Z*)-stereoisomer of **19**, so that a simple base-mediated isomerization most likely has to be excluded.

We were delighted to find that the diene−ene RCM concept (strategy III) turned out to be successful when Grubbs 1 catalyst **20** was employed (Scheme 4). The Grubbs

Scheme 4. Diene−Ene RCM of 19[a]



[a] Cy = cyclohexyl.

2 catalyst did not afford RCM products. Besides unreacted starting material (∼30%), we isolated the RCM products as

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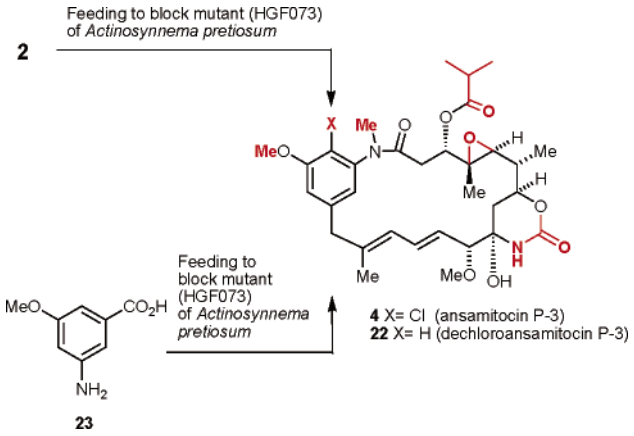
a mixture of stereoisomers, the (*E*,*E*-)-isomer being favored (~3:1). Removal of the silyl protection finally afforded proansamitocin **2**[26] and the (*E*,*Z*)-isomer **21**.[27]

We then conducted preliminary feeding experiments to test whether complex substrates such as the synthetic proansamitocin **2** are accepted and processed by *Actinosynnema pretiosum*. Compound **2** (2.6 μmol) was fed in three equal portions 72, 96, and 120 h after inoculation to a 25 mL culture of *Actinosynnema pretiosum* mutant HGF073, which lacks the ability to synthesize AHBA (Scheme 5).[14] Parallel

Scheme 5. Feeding Experiments (Functional Groups Introduced Are Labeled in Red)[a]



[a] DDQ = dichlorodicyanoquinone, DCC = dicyclohexylcarbodiimide, DMAP = 4-dimethylaminopyridine, Pyr = pyridine.

fermentations were carried out with the wild-type strain and with mutant HGF073 supplemented with AHBA and without supplementation. The cultures were harvested after 7 days and extracted with ethyl acetate. The extract was subjected to electrospray ionization mass spectrometry (ESI-MS) and revealed formation of AP-3 **4** along with a new metabolite (parent ions at *m*/*z* 601 $(M + H)^+$ and *m*/*z* 624 $(M + Na)^+$) that is consistent with dechloroansamitocin P-3 **22**. We could not obtain confirmation of the structure using NMR spectroscopy. Therefore, we tested whether dechloroansamitocin P-3 **22** can be prepared directly by feeding 3-amino-5-methoxybenzoic acid **23** (37.5 μmol) to a culture of *Actinosynnema pretiosum* mutant HGF073. After workup and HPLC purification, 1.5 mg (2.5 μmol) (from 250 mL of fermentation broth) of **22** was isolated as pure material. In tests with cultured human tumor cell lines it showed strong antiproliferative activity with IC_{50} values down to 10 pg/mL (Table 1).

Table 1. Antiproliferative Activity IC_{50} [ng/mL][28]

cell line	origin	**4**	**22**
KB-3-1	cervix carcinoma	0.11	0.5
U-937	lymphoma	0.0035	0.01
PC-3	prostate carcinoma	0.035	0.08
A-431	epidermoid carcinoma	0.05	0.25
A-498	kidney carcinoma	1.1	9
SK-OV-3	ovarian carcinoma	0.03	0.1

In conclusion, we achieved the first total synthesis of proansamitocin **2** and showed that such a complex biosynthetic intermediate can successfully be fed to a AHBA block mutant of *Actinosynnema pretiosum* thereby reestablishing AP-3 production. Formation of the biologically highly active byproduct dechloroansamitocin P-3 **22** was independently confirmed by mutasynthesis feeding 3-amino-5-methoxybenzoic acid.

In principle, these results pave the way to prepare many new AP-3 derivatives by feeding simple as well as advanced derivatives of biosynthetic intermediates. Additionally, with proansamitocin in hand, we will be able to screen for the amidase (gene *asm9*).

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

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(26) ^1H and ^{13}C NMR data were identical in every respect with those reported for proansamitocin **2**, a fermentation byproduct (ref 16).

(27) Yields refer to isolated yields of pure isomers **2** and **21**. These were collected after several chromatographic runs, which included preparative HPLC.

(28) Proansamitocin showed no antiproliferative activity. Like AP-3 (IC_{50} = 0.015 ng/ml) dechloroansamitocin P-3 **22** (IC_{50} = 0.15 ng/ml) also showed strong antiproliferative activity for primary human endothel cells.