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Messages from Nature – How CHEMICAL Synthetic Biology Could Look Like

Andreas Kirschning*, Olena Mancuso, Jekaterina Hermane, and Gerrit Jürjens

Institute of Organic Chemistry
and Center of Biomolecular Drug Research (BMWZ),
Leibniz University Hannover, Schneiderberg 1b, 30167 Hannover, Germany

E-Mail: *andreas.kirschning@oci.uni-hannover.de

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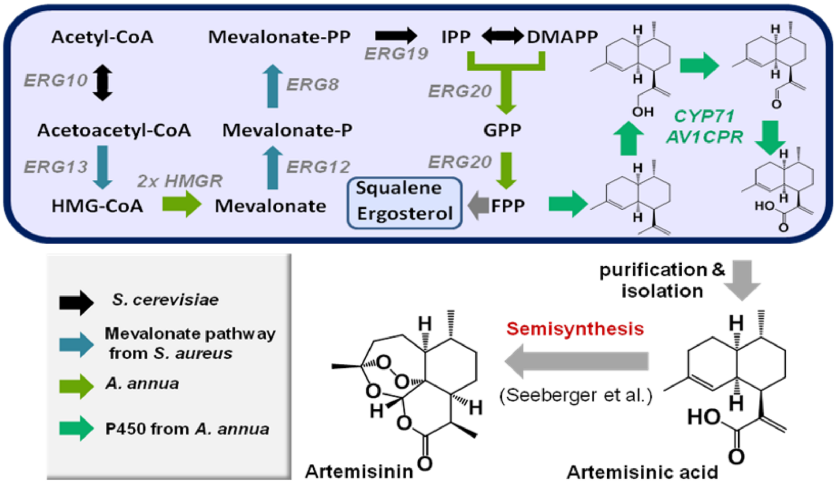
Introduction

The term synthetic biology is a relative new expression for an emerging topic at the interface between chemistry and biology. Other, related fields may include medicinal chemistry, bioorganic chemistry and chemical biology; all these subfields are still based on chemical synthesis. Likewise one can see this trend of diversification in the field of biotechnology which has been complemented with terms like metabolic engineering or combinatorial biosynthesis. Many of these terms are not that strictly defined and substantially overlap and redundancy has to be acknowledged. There is still some debate of what synthetic biology covers. In short, one may describe it to assemble and merge genes *in vitro* to create a new useful biological system [1, 2].

The field of synthetic biology is mainly driven by biologists and biotechnologists. It seems as if in these early days of this modern type of biotechnology the expertise of synthetic chemists as well as the opportunities associated with chemical synthesis are overlooked or ignored. The question may be raised whether the *de novo* establishment of biosynthetic pathways will consequently lead to new derivatives of a target molecule or will only provide a better access to a known natural product. One can also doubt that by including enzymatic cassettes that are not on the given natural biosynthetic pathway will provide a wide variety

of new derivatives. If derivatives are required e. g. for improving certain desirable properties particularly in the arena of medicinal chemistry, chemical synthesis still provides the largest flexibility.

In scheme 1 this is exemplified for artemisinin, a diterpene that has emerged as one of the most effective antimalarial drugs [3]. It is produced by *Artemisia annua* but isolated in amounts that makes this drug rather costly in view of the economic situation in countries where malaria is most abundant [4]. Keasling *et al.* reported on the production of the antimalarial drug precursor artemisinic acid in engineered yeast which has become one of the representative examples for synthetic biology and can be regarded to be one of the initial milestones [5]. However, their work provided a biosynthetic precursor, namely artemisinic acid which is not active as an antimalarial drug. It was the Seeberger group [6] that recently provided an elegant flow approach relying on a short chemical sequence that likely will pave the way to produce artemisinin using Keasling's engineered organism and Seeberger's semisynthesis. This example illustrates without adressing the issue of accessing new derivatives, how synthetic biology or metabolic engineering will beneficially work hand in hand with chemical synthesis to practically solve synthetic challenges like the medically important natural product artemisinin.



Scheme 1. Production of artemisinin by combining of biosynthetic modification of *Saccheromyces cerevisiae* with semisynthesis.

Indeed, natural products have recently proven to open up new avenues in emerging areas of biomedical research. Gupta and coworkers studied the effect of small molecules on the development of cancer stem cells [7]. These cells have become a hot topic in oncological research because their resistance towards current cancer therapies including chemo- and radiation therapy [8]. For that purpose a compound library containing about 16.000 entities was screened for its selective inhibitory properties on cancer stem cells. The assay revealed

32 active compounds from which four natural products, namely Salinomycin (**1**), Abamectin (**2**), Nigericin (**3**) and Etoposide (**4**) (Fig. 1) turned out to be the most active ones. Noteworthy, except for Etoposide, all of these known molecules are polyketide-derived.

In this report selected showcases from our laboratories which are far from covering the broad scope of possible and thinkable strategies, will demonstrate, that the liaison of chemical synthesis and modern metabolic engineering provides great future prospects for expanding the opportunities for natural products and derivatives particularly in the field of medicinal chemistry and pharmaceutical research.

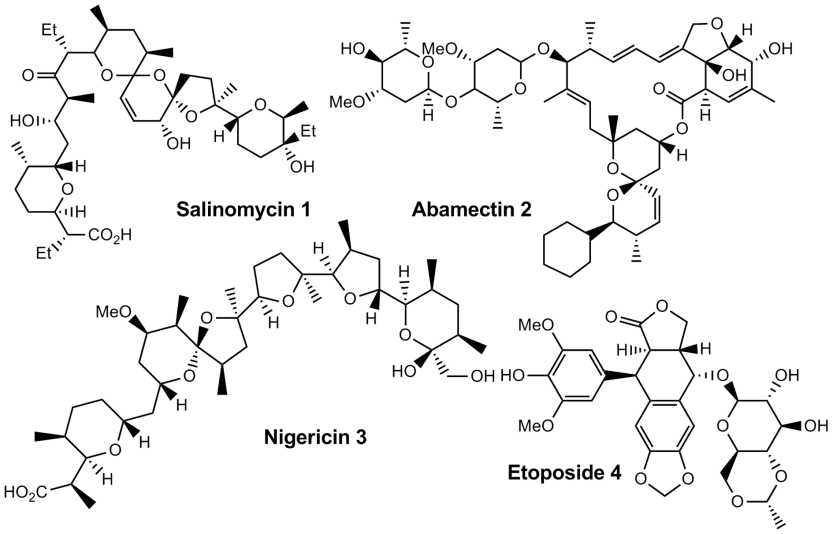


Figure 1. Structures of Salinomycin (**1**), Abamectin (**2**), Nigericin (**3**) and Etoposide (**4**).

Mutational Biosynthesis (Mutasynthesis)

In 1969, Rinehart and Gottlieb introduced the term mutational biosynthesis in a rather visionary account in which they speculated on opportunities of how to manipulate biosynthetic machineries on a genetic level [9]. Their proposals and ideas were visionary because at that time the techniques of molecular biology and metabolic engineering did not exist to interfere with the genome with the precision of today's routines. In fact, these opportunities were supposed to come decades later. Mutational biosynthesis or in short mutasynthesis comprises the generation of mutants of a producer organism blocked in the formation of a key biosynthetic building block required for assembly of the end-product [10]. The administration of potentially altered building blocks, so-called mutasynthons, to the blocked mutant may result in new metabolites which are isolated and biologically evaluated (Fig. 2).

Importantly, the authors stressed the need of isolation and biological evaluation of the new derivatives formed. This is a very important aspect if mutasynthesis wants to become an established and respected method because it competes with pure chemical methods such as semi and total synthesis and only to a minor extent it also plays a role in elucidating details on biosynthetic pathways.

Nowadays, a much deeper understanding of biosynthetic pathways of complex secondary metabolites has been gained. More importantly, tools in molecular biology and genetic engineering are now in hand [11], that allow to carry out natural product synthesis similar to total synthesis programs that are flexibilized by merging chemical synthesis with biosynthesis, so that natural products may return back on the agenda of drug development [12]. Conceptually the best of both worlds, the flexibility of chemical synthesis for introducing structural changes and the preciseness of biosynthetic pathways are chosen and combined for a given target molecule.

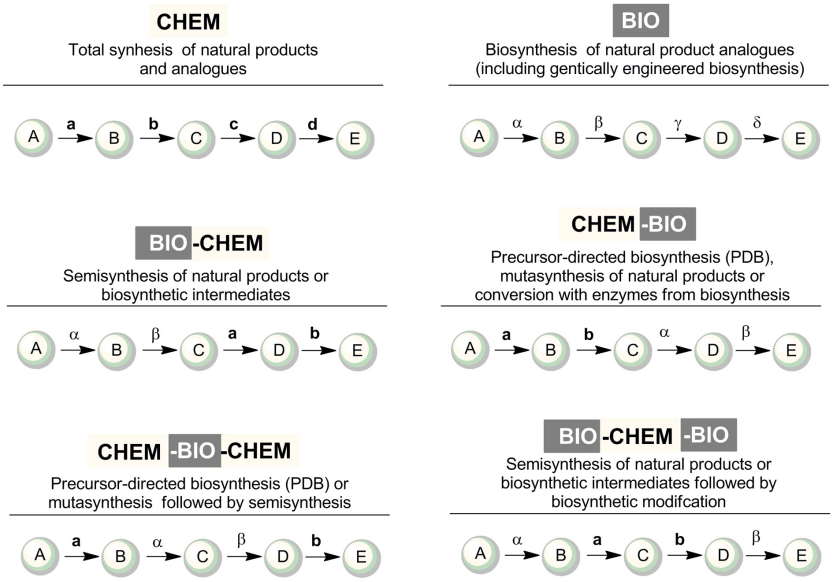


Figure 2. Classification of "total synthesis" approaches towards natural product analogues and libraries based on chemical and biological methods and combinations (these are selected combinations but other combinations like BIO-BIO-CHEM can also be envisaged; (a-d = chemical reagents or catalysts; α-δ = enzymes; A = starting material; B-D = synthetic and/or biosynthetic intermediates; E = natural product or derivative) [13].

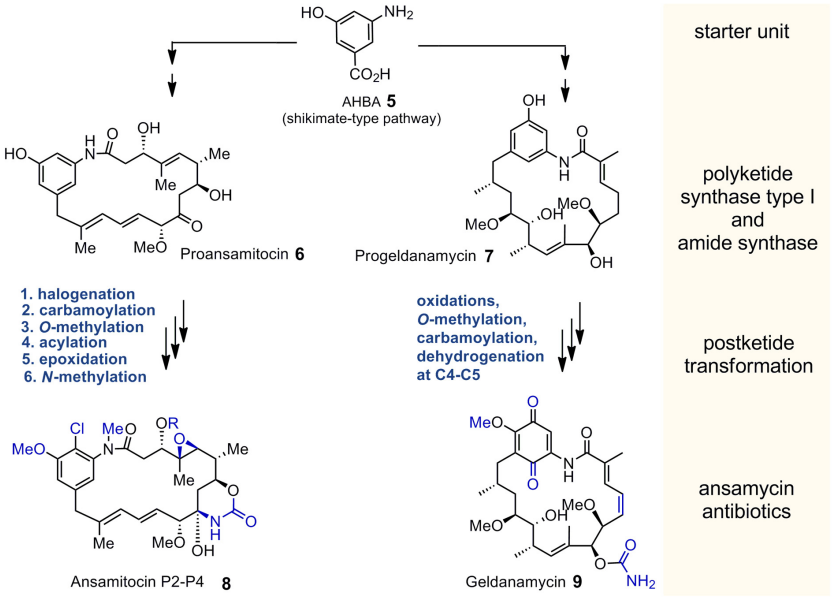
Recently, we suggested a simple classification for visualizing the synthetic opportunities of hybrid techniques (Fig. 2) [13]. It mainly focuses on synthetic strategies. Two abbreviations serve to distinguish between chemical synthesis labelled CHEM and biotransformations performed by overexpressed enzymes or biosynthetic multienzyme cassettes called BIO.

This for example means that a BIO-CHEM synthesis is simply a semisynthetic derivatization of a starting material obtained from a natural source or by fermentation. This creates structural diversity which is further expanded by the extended BIO-CHEM-BIO approach. This would mean that a semisynthetically modified natural product is subjected to an enzymatic biotransformation or to a whole cell fermentation [13].

The Ansamycin Antibiotics

A showcase for hybrid CHEM-BIO total syntheses

An important group of secondary metabolites are the ansamycins which are macrolactams, that contain an aryl, naphtyl or quinone chromophor which is part of a polyketide-type ansa chain. The chain is attached via the meta positions to the chromophor. Famous examples are rifamycin, geldanamycin (9) and maytansin [14]. One member of an aryl ansamycin antibiotic are the maytansinoids which exhibit cytotoxic activity, evident in the growth inhibition of different tumor cell lines and human solid tumors at very low concentrations (10^{-3} to 10^{7} μg/ml) [14]. Their antimitotic mode of action is based on the interaction with β-tubulin. It prevents polymerisation of tubulin and thereby promoting depolymerisation of microtubules. The maytansinoids currently attract high clinical interest as ‘‘warheads’’ in tumor-targeted immunoconjugates [15]. Maytansin was isolated from plant material while later the closely related ansamitocins (8), were found in the bacteria like *Actinosynnema pretiosum*. The biosynthesis of the ansamitocins involves several unusual steps of which the aromatic biosynthetic starter 3-amino-5-hydroxybenzoic acid (AHBA) (5) supplied by a dedicated biosynthetic pathway [16] (Scheme 2) is an important feature. Furthermore, a separate amide synthase acting after polyketide synthase processing provides proansamitocin (6). This cyclization is another key enzymatic process of the ansamycin antibiotics before the resulting core structure 6 is further decorated by a set of tailoring enzymes.



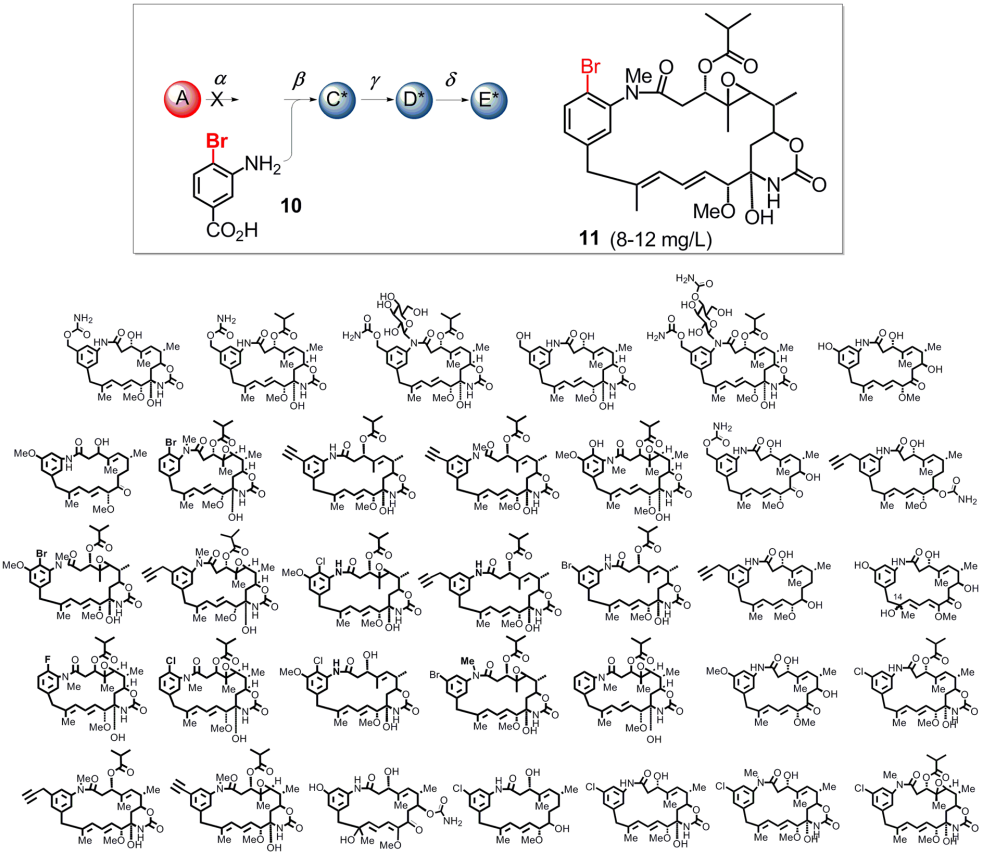
Scheme 2. Short overview over the biosynthesis of the two ansamycin antibiotics ansamitocin and geldanamycin.

Likewise, geldanamycin (**9**) is another important member of the ansamycins, and it is produced by *Streptomyces hygroscopicus* var. *geldanus*. Geldanamycin is a potential anti-tumor drug [17] which binds to the *N*-terminal ATP-binding domain of heat shock protein 90 (Hsp90) and consequently it inhibits the ATP-dependent chaperone activities [18]. Most unnatural geldanamycin derivatives reported to date are aminated in the 17 position by a semisynthetic step [19]. The biosynthesis of geldanamycin (**9**) is closely related to ansamitocin P-3 (**8**). It is also based on a PK synthase with AHBA (**5**) as the starter unit and a stand-alone amide synthase for cyclisation. Individual post-PKS tailoring enzymes follow formation of progeldanamycin (**7**) that differ from those occuring in *A. pretiosum* terminate geldanamycin biosynthesis (Scheme 2).

Geldanamycin is the best studied example of ansamycin antibiotics being modified by mutasynthesis as well as other metabolic engineering approaches [20 – 22], while so far only our group has been involved in this arena to create libraries of ansamycin antibiotics [23, 24].

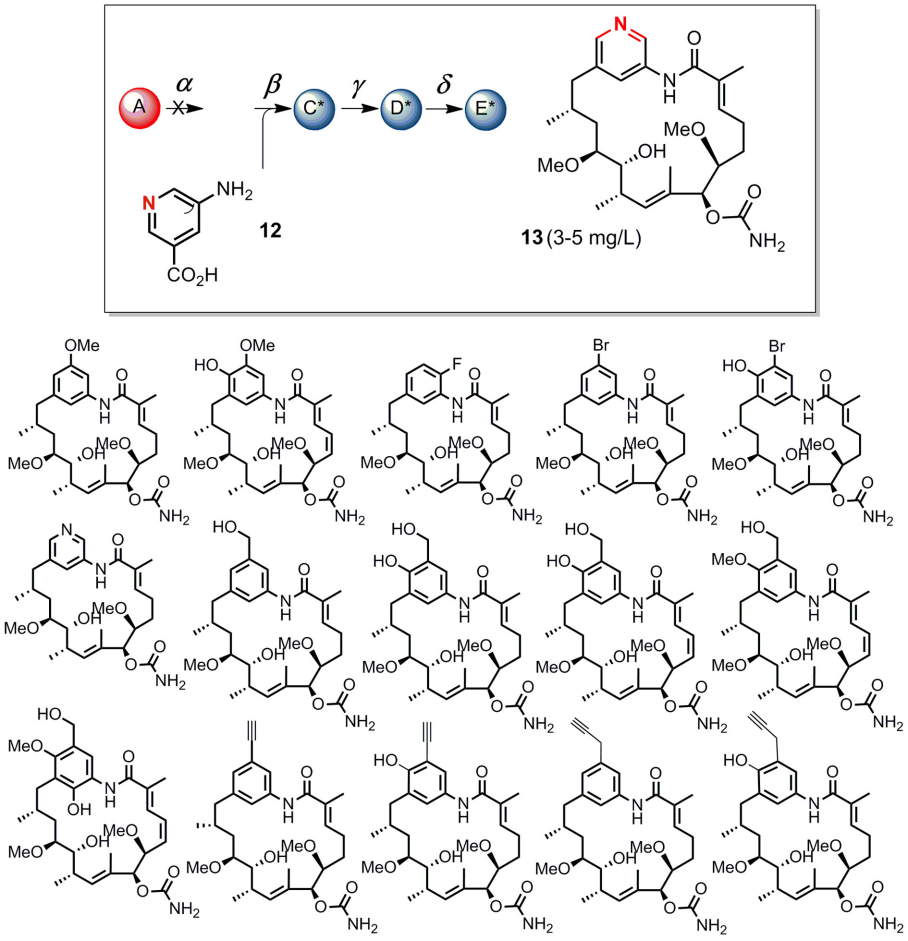
Indeed, ansamycin antibiotics are ideally suited for mutasynthetic generation of analogues as AHBA (**8**), the starter unit exerts biosynthetic uniqueness. For this purpose a knock-out strain of *A. pretiosum* has to be generated that is blocked in the biosynthesis of AHBA. By doing so, one does not interfere with the essential primary metabolic pathways in the microorganism. Gratifyingly, the AHBA loading domain of the ansamitocin PKS turned

out to accept and process a structurally diverse number of 3-aminobenzoic acids as exemplified for aminobenzoic acid (**10**) which was smoothly transformed into ansamitocin derivative **11** (Scheme 3) [25, 26].



Scheme 3. Mutasynthetic preparation of ansamitocin derivative **11** from mutasynthon **10** and representative examples of new ansamiton derivatives obtained by mutasynthesis using a AHBA blocked mutant of *A. pretiosum*.

In a similar manner, the geldanamycin producer *S. hygroscopicus* var. *geldanus* was genetically manipulated and exploited in mutasynthetic fermentations by supplementing 3-aminobenzoic acid derivatives such as **12** (Scheme 4) [23]. In fact, aza-geldanamycin derivative (**13**) represents the first example of a mutaproduct that was generated from feeding an aromatic heterocycle to an AHBA blocked mutant.

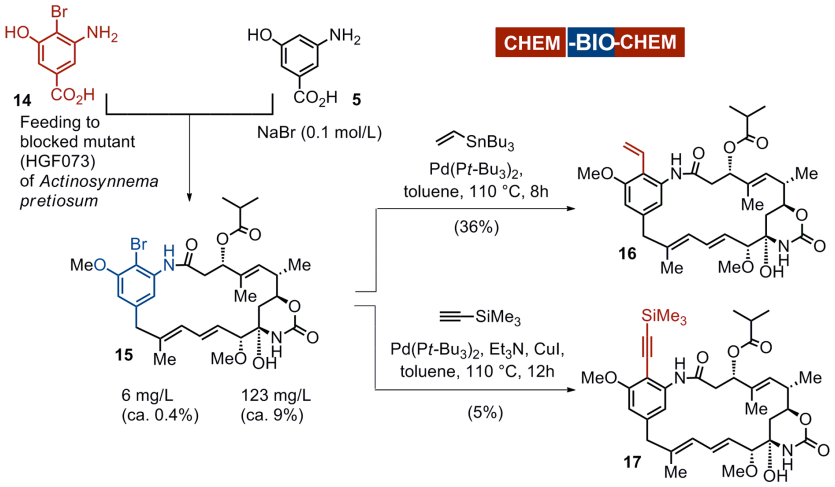


Scheme 4. Mutasynthetic preparation of aza geldanamycin derivative **13** from mutasynthon **12** and representative examples of new geldanamycin derivatives obtained by mutasynthesis using a AHBA blocked mutant of *S. hygroscopicus*.

Mutasynthesis followed by semisynthesis (CHEM-BIO-CHEM)

In order to broaden the options for diversity oriented synthesis the concept of mutasynthesis can be extended in a combination with semisynthesis (Fig. 2). This strategy requires, that the product formed from mutasynthesis bears a new, chemically useful functionality, which then serves for semisynthetic derivatizations. The CHEM-BIO-CHEM approach requires that the mutasynthesis provides sufficient amounts of the new natural product for chemical synthesis. It needs to be noted that this requirement is not always fulfilled with mutasynthesis.

An early example is shown in Scheme 5. Feeding of 3-amino-4-bromo-5-hydroxy benzoic acid **14** to the AHBA(-) blocked mutant of *A. pretiosum* [27] furnished the bromo-AP-3 derivative **15** in very good yield. Alternatively, the substrate selectivity of the perhalogenase can be extended to bromination when the fermentation broth was supplemented with sodium bromide and AHBA **5.** Bromo-AP-3 **15** is a key precursor for semisynthetic derivatisations particularly when Pd-catalysed reactions such as the Stille and the Sonogashira cross coupling reactions are exploited. With the Stille coupling the vinyl group was introduced to furnish vinyl AP-3 **16** [25]. Alkinylation of the aromatic moiety was achieved with TMS acetylene which gave ansamitocin P-3 derivative **17** (Scheme 5). It is noteworthy, that despite the substantial alterations in the aromatic unit of AP-3, two of these new ansamitocin derivatives **15** and **16** show strong cytotoxic activity (< 20 nM) towards a classical panel of cell lines.

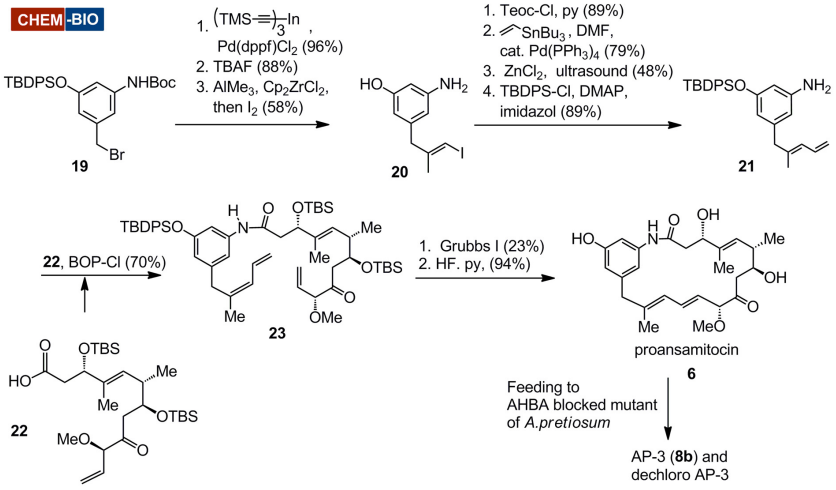


Scheme 5. Combined mutasynthesis/biosynthesis and semisynthesis for the preparation of ansamitcon derivatives **16** and **17** [25].

Proansamitocin – chemical and biochemical synthesis and feeding studies

Proansamitocin **6**, the first macrolactam biosynthetic intermediate, is another versatile starting point for performing semisynthetic derivatisations (Scheme 2). In contrast to the mutasynthetic strategies described in schemes 3 and 4 where the aromatic moiety in ansamitocin was chemically modified, proansamitocin paves the way to carry out semisynthesis on functional groups that are located in the ansa chain. In order to assure that complex mutasynthons like proansamitocin **6** and semisynthetic products derived therefrom are able to penetrate the bacterial membrane and are further processed inside, we prepared proansamitocin **6** by total synthesis and fed it to the AHBA blocked mutant of *A. pretiosum*.

The total synthesis is briefly depicted in Scheme 6. It starts from benzyl bromide **18** [28] which was converted by a two-step process into vinyl iodide **20**, involving an alkynylation using alkynylindium **19** followed by a carbomethylation and *ipso*-iodination. The diene required for the alkene-diene ring closing metathesis was set up by a Stille reaction. Next, the major part of the ketide-based ansachain **22** [29], which had been prepared by conventional stereocontrolled reactions common in polyketide synthesis, was introduced via the amino group. The resulting amide **23** was then subjected to ring closing metathesis conditions using the Grubbs 1 catalyst which after desilylation yielded proansamitocin **6** (starting material and a small amount of *E,Z* diene were also isolated). It needs to be mentioned, that this is a rare example of a complex alkene diene ring closing metathesis reaction. The key experiment, feeding of pronsamitocin **6** to the AHBA blocked mutant of *A. pretiosum* was successful in that the last steps of the biosynthesis took place and AP-3 **8b** and its dechloro derivative were formed and isolated [29]. This experiment paved the way to prepare analogues of proansamitocin in order to utilize the tailoring enzymes of ansamitocin biosynthesis.



Scheme 6. Total synthesis of proansamitocin **6** and feeding to ΔAHBA blocked mutant of *A. pretiosum*.

Next, we utilised a new blocked mutant of *A. pretiosum* that is unable to carry out postketide modifications [blocked in *asm12* (chlorination) and *asm21* (carbamoylation)] which provided proansamitocin **6** [27, 30] in good yield (Fig. 3). Additionally, small amounts of 10-*epi*-proansamitocin **24** and *O*-methyl proansamitocin **25** were isolated. Very likely **24** may arise from α-epimerization of the keto group at C-9 during work-up. In addition, the *O*-methyl transferase (Asm7), that remained intact in the blocked mutant, is still able to operate on proansamitocin to a minor degree. Finally, two separable diastereomeric byproducts **26a**,**b** were isolated that contained an additional hydroxyl group at C-14 and the diene moiety, which may have resulted from an intermediate oxirane at C 13-C 14.

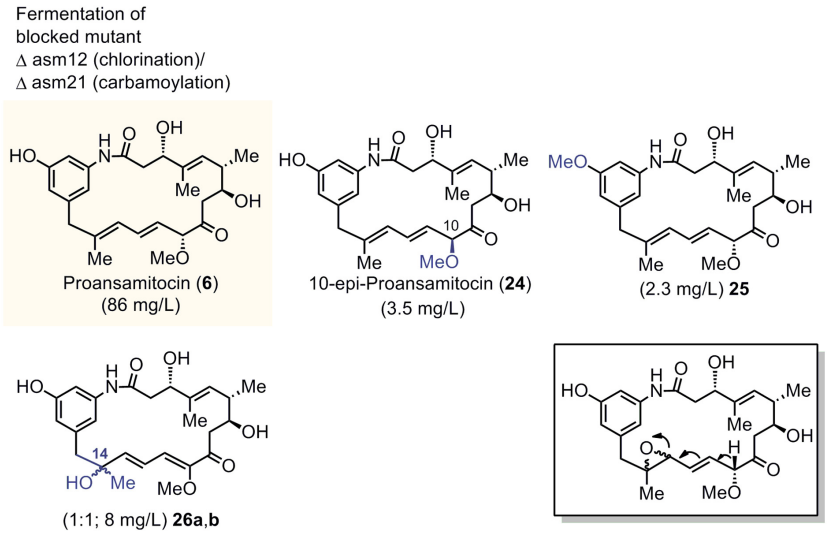
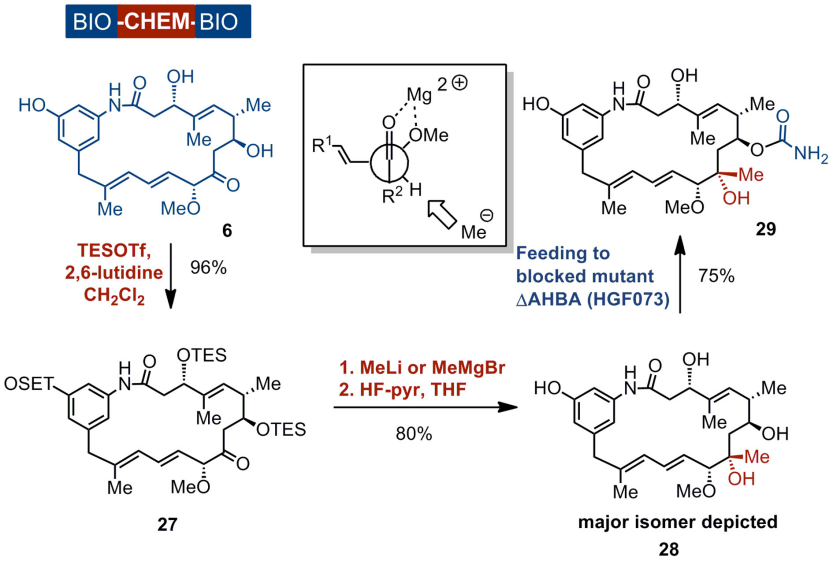


Figure 3. Structures of proansamitocin 6, 10-*epi*-proansamitocin 24, *O*-methyl proansamitocin 25 and oxidation by-products 26a,b.

Biosynthesis followed by semisynthesis and mutasynthesis (BIO-CHEM-BIO)

With sufficient amounts of proansamitocin **6** in hand, semisynthetic derivatisation in the ansa chain of ansamitocin was pursued. For example, nucleophilic methylation of the keto group at C-9 in per-*O*-silylated proansamitocin **27** yielded the methyl-branched proansamitocins **28a**,**b** (5:1 for MeLi or 10:1 for MeMgBr, respectively) (Scheme 7) [27].

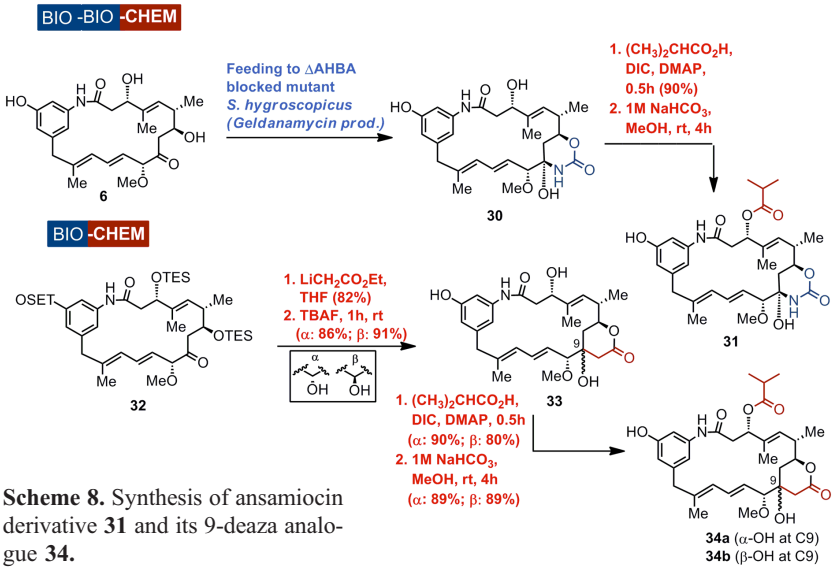


Scheme 7. Semisynthesis of C-9 branched proansamitocin derivative **29** starting from proansamitocin following a BIO-CHEM-BIO approach.

We postulate that the above conformer (framed) is enforced by the chelating properties of the neighbouring methoxy group. This chelation is expected to control the facial selectivity of the nucleophilic attack. When this derivative was supplemented to the AHBA blocked mutant of *A. pretiosum*, carbamoylation of the hydroxyl group at C7 took place and provided the proansamitocun derivative **29** in good yield. In this example two blocked mutants served as synthetic tool while flexible derivatisation was achieved by chemical synthesis.

It is well accepted that the isobutyrate group at C3 in AP-3 is essential for its strong antiproliferative activity, while it has been debated, whether the cyclic carbamate has pharmacophoric properties. Therefore, we first devised a synthetic sequence that allows to introduce esters at C-3 in proansamitocin and its analogues. Being generally applicable this two-step protocol is represented for the preparation of ester **29** starting from the major diastereomer **28a**,**b.** This synthetic route was exploited to address one SAR issue that had remained to be part of an open debate for several decades.

What is the pharmacological role of the cyclic carbamate moiety in AP-3?



Scheme 8. Synthesis of ansamiocin derivative **31** and its 9-deaza analogue **34.**

Thus, besides the simplest AP-3 derivative that bears both the ester side chain as well as the carbamate moiety, we also prepared the carbaanalogues **34** by semisynthetic modification of per-*O*-silylated proansamitocin **32** (Scheme 8). First, the Claisen-reaction gave diastereomeric lactons **33a**,**b** after fluoride-induced desilylation. Then, acylation at C3 was carried out as developed for the preparation of carbamate **31.** As a result, this short sequence yielded the deaza analogues **34a**,**b.** Comparative evaluation of the antiproliferative activities of these three derivatives **31** and **34a,b** unravelled that the carbaanalogue **34a** with α-orientation of

the hydroxyl group at C 9 shows antiproliferative activity in the lower nanomolar range which is in the similar range as was found for the carbamate **31.** In contrast, the 9-epimer **34b** is substantially less active

Targeting tumors: Conjugate assembly by a CHEMBIOCHEM approach

The antitumor activity of the maytansinoids was extensively evaluated in human clinical trials [31], but although potent *in vitro*, the maytansinoids displayed a poor therapeutic window *in vivo*. By forming conjugates with monoclonal antibodies [32], vitamins such as folic acid [33] and others [34] that bind to specific markers on the surface of tumor cells, ansamitocins and related highly cytotoxic agents show improved selectivity towards tumors associated with reduced cytotoxicity. Recently, the humanized anti-HER2 mAb antibody trastuzumab was conjugated with maytansine and this construct reached late clinical trials for the treatment of HER2+ metastatic breast cancer [35].

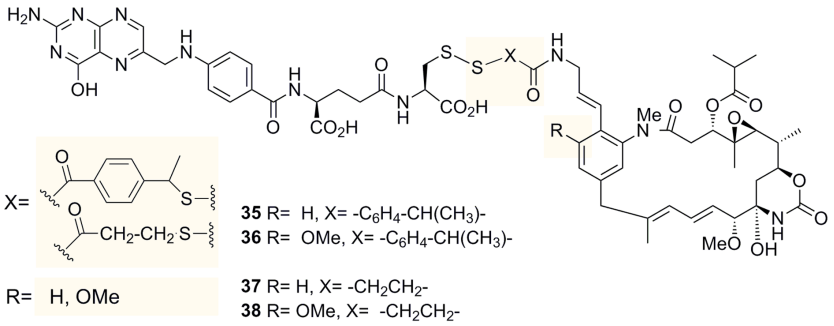
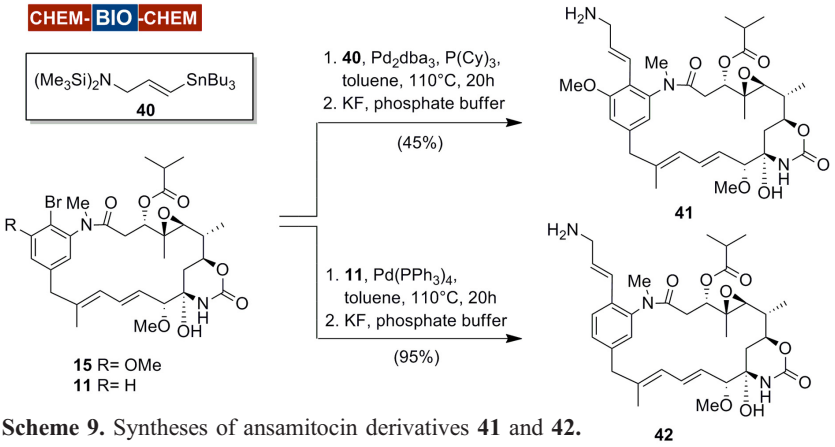


Figure 4. Folic acid/AP-3 conjugates **35-38** prepared by a combined synthetic/mutasynthetic approach.

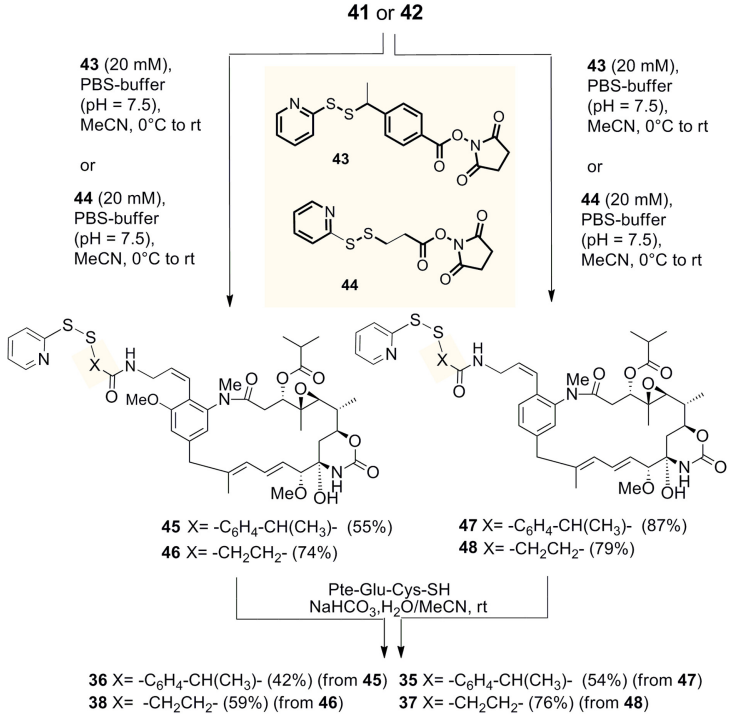
The vitamin folic acid is a promising ligand for selective delivery of attached therapeutic agents to tumor tissues [36]. Many different cancer cells do overexpress folic acid receptors (FR) on their cell surfaces [37]. Folic acid-drug conjugates targeting FR are commonly constructed of three components, the the folic acid, the cytotoxic drug, and the linker connecting the drug to the tumor-specific ligand. The latter should be able to undergo a release mechanism of the active drug [38]. It is established that a folate-disulfide-drug conjugate can undergo reduction after endocytosis [36].

We prepared four new tumor specific folic acid/ansamitocin conjugates **35-38** (Fig. 4) and utilised a synthetic strategy based on the combination of mutasynthesis and semisynthesis. The two bromo-ansamitocin derivatives **15** and **11** are accessible by mutasynthesis or by a modified fermentation protocol, respectively (Scheme 9). An allyl amine linker **40** was introduced under Stille conditions [39] to yield modified ansamitocins **41** and **42.**



Scheme 9. Syntheses of ansamitocin derivatives **41** and **42.**

The amino group was transformed into the pyridyl disulfide derivatives **45**-**48** with doubly activated 3-(2-pyridyldithio)-propionic ester (SPDP) **43** and 4-[1-(2-pyridyldithio)-ethyl]-benzoic ester (SMPT) **44**, respectively (Scheme 10). Standardised coupling steps introduced the pteroic acid/glutamic acid/cysteine (Pte-Glu-Cys-SH) unit to the modified ansamitocins yielding the target conjugates **35**-**38.**

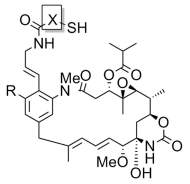


Scheme 10. Preparation of folic acid/AP3 conjugates **35**-**38.**

These conjugates as well as those that are expected to be generated after internalization of the folic acid/ansamitocin conjugates into the cancer cell and reductive cleavage of the disulfide linkage 49 – 51 [40] were evaluated with respect to their antiproliferative activity. The latter were prepared from the corresponding pyridyl disulfides after treatment with dithiothreitol (DTT) in PBS-buffered acetonitrile. All three thiols exerted good to strong antiproliferative activities against selected cell lines including a lung carcinoma A-549 devoid of membrane-bound folic acid receptors (FR) (Table 1). These modified ansamitocins are about 10 – 100 fold less active than ansamitocin P3 (8b) but are still suited to serve as an anticancer agents.

Table 1. Antiproliferative activity IC_{50} [nM] of ansamitocin derivatives **49** – **51** in SW-480, A-549 and HCT-116 cells (FR-: devoid of membrane-bound folic acid receptors).

Cell line	origin	49	50	51
SW-480	*colon carcinoma*	5	6	42
KB-3 – 1	*cervix carcinoma*	4	4	14
A-549 (FR-)	*lung carcinoma*	6	24	420
HCT-116	*colon carcinoma*	8	10	49



49 R= H, X= -C_6H_4-$CH(CH_3)$-
50 R= OMe, X= -C_6H_4-$CH(CH_3)$-
51 R= H, X= -CH_2CH_2-

Conjugates **35-38** were tested to two cancer cell lines. Cervix carcinoma KB-3 – 1 (FR+) contained membrane-bound folic acid receptors whereas lung carcinoma A-459 (FR-) was deficient in this respect. All four conjugates showed strong antiroliferative activity for the former cell line but were inactive towards the latter FR- cell line (Table 2). The values of cytotoxicity favorably match with those for thiols **49-51** (Table 1) the supposed products resulting from *in vivo* reductive cleavage of **35-38.**

Table 2. Antiproliferative activity IC_{50} [nM] of folate-AP-3 conjugates **22-25.** (KB-3 – 1 cells: folate receptor positive and A-549 cells: folate receptor negative).

Cell line	Origin	35	36	37	38
KB-3 – 1 (FR+)	*cervix carcinoma*	13	8	21	7
A-549 (FR-)	*lung carcinoma*	$> 10^4$	$> 10^4$	$> 10^4$	$> 10^4$

We regard this project as a proof for the power of combining mutasynthesis and semisynthesis in order to access complex natural product architectures and a proof that these conjugates are able to exert selectivity.

New macrocyclic architectures by mutaysynthesis

The combination of chemical synthesis and biotransformations using engineered organisms can even pave new avenues towards novel macrocyclic backbones. When an AHBA(-) mutant of *Streptomyces hygroscopicus var. geldanus* was supplemented with hydroxymethy-

laminobenzoic acid (52) several new metabolites were formed, isolated and fully characterised (Fig. 5) [24]. Five metabolites 53 – 57 can be put in a logical biogenetic order. Thus, the timing of biosynthetic events of the tailoring biotransformations can be postulated in that after ring-closure of the progeldanamycin-analogue by the amide synthase, carbamoylation, oxidation at C 17, 4,5-desaturation and O-methylation take place. The final tailoring modification is the oxidation at C 21. Importantly, also three additional mutaproducts 58a-58c were isolated which are 20-membered macrolactones, compared to the 19-membered macrolactam ring in geldanamycin (9) (Fig. 6). Clearly, the amide synthase, that is responsible for macrolactam formation, is also able to cyclise the PKS-bound seco acid via the mere nucleophilic benzylic alcohol.

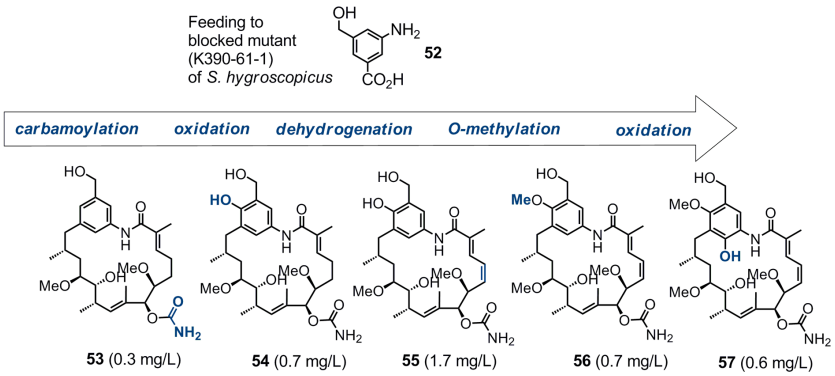


Figure 5. Mutasynthesis with *S. hygroscopicus* (strain K390 – 61 – 1) using 3-amino-5-hydroxymethylbenzoic acid **52** and the proposed sequence of tailoring biotransformations.

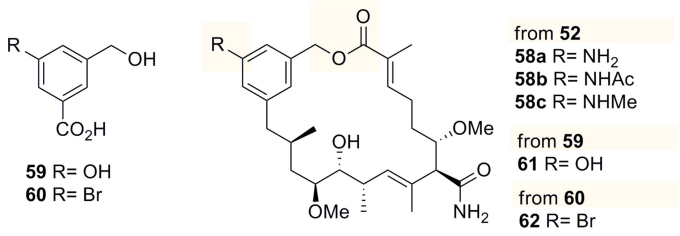


Figure 6. Formation of 20-membered macrolactones after feeding benzyl alcohols **52**, **59** and **60** to a AHBA blocked mutant of *S. hygroscopicus*.

Conclusions

The new term synthetic biology covers an emerging research field that combines the investigative nature of biology with the constructive nature of engineering. Efforts in synthetic biology have largely focused on the creation and perfection of genetic devices and small modules that are constructed from these devices. Artemisinic acid, that was presented as a show case for the preparation of small molecules, is an illustrative example, where the latest approaches and genetic techniques of metabolic engineering will lead to in the near future (Scheme 1).

It is also an illustrative example that the combination of metabolic engineering with chemical synthesis holds much larger future prospects, because in fact artemisinin is the active metabolite. Furthermore, chemical synthesis is able to broaden the chemical space of metabolites thus creating larger structural diversity. The present account covers only one aspect of this emerging field in that only the chemical utility of mutant strains that are specifically blocked in one location of the biosynthetic gene cluster is discussed. Many more combinations of chemical synthesis with metabolically engineered "biofactories" can be envisaged, particularly as synthetic biology currently sets out to create more flexible arrangements and combinations of biosynthetic elements. Ideally, this development will create custom made or "programmable" metabolic pathways in an effort to reach similar flexibility as is typical for chemical synthesis but with larger preciseness. Eventually, the preparation of a target molecule will rely on a tool box composed of biological and chemical synthetic methods that will be combined in a highly flexible manner for a given synthetic problem.

Synthesis quo vadis? A CHEMICAL Synthetic Biology is definitely one answer.

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