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Molecular Editing

Probing the Biology of Natural Products: Molecular Editing by Diverted Total Synthesis

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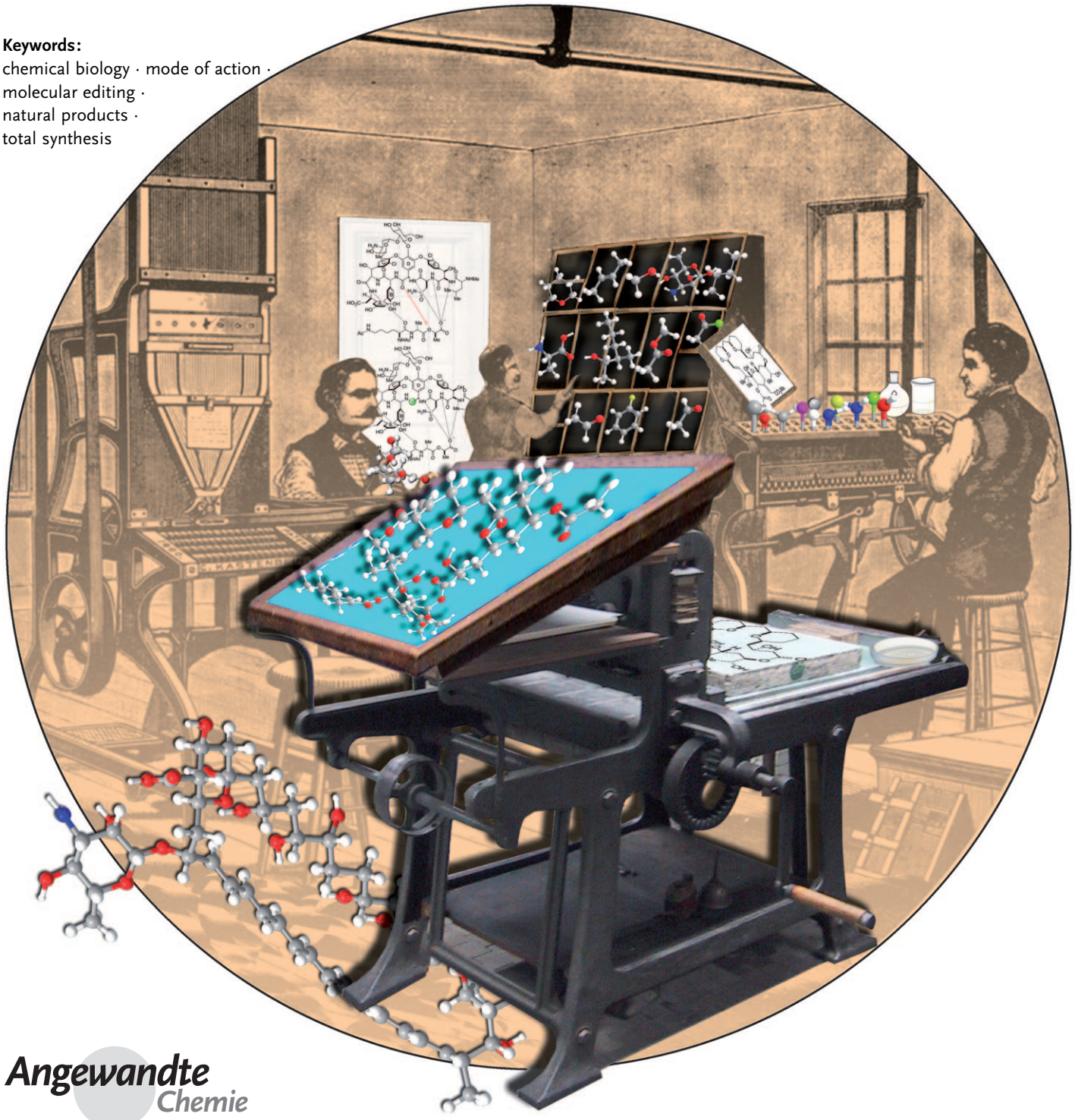


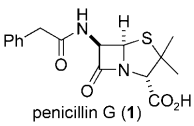
The systematic modification of natural products through diverted total synthesis is a powerful concept for the systematic modification of natural products with the aim of studying mechanistic aspects of their biological activity. This concept offers far-reaching opportunities for discovery at the interface of biology and chemistry. It is underpinned by the power of chemical synthesis, which manifests itself in the ability to modify structure at will. Its implementation, when combined with innovative design, enables the preparation of unique mechanistic probes that can be decisive in differentiating and validating biological hypotheses at the molecular level. This Review assembles a collection of classic and current cases that illustrate and underscore the scientific possibilities for practitioners of chemical synthesis.

From the Contents

1. Introduction

Historically, the primary raison d'être for the design and implementation of total syntheses was structural determination. Additional important objectives include: 1) the evaluation and validation of new synthetic methods, 2) the development of innovative strategies, 3) the generation of quantities of material that is otherwise not readily available from natural sources so as to enable biological studies, and 4) the preparation of analogues with improved pharmacological properties. This Review focuses on a much rarer aspect of total synthesis, namely the design and synthesis of molecular probes—analogues of the natural products—specifically crafted to investigate the function of the natural product and ultimately broader questions in biology or medicine. In a 2006 review, Wilson and Danishefsky coined the expression "*molecular editing through divergent total synthesis*"[1] for the study of mechanistic aspects of biological activity. This feature of chemical synthesis underscores what has been stated to be one of the unique characteristics of the chemical sciences, namely the ability to create new forms of matter.

Perhaps one of the first observations that led to a profound understanding of the mechanism of a natural compound was the finding that penicillin (**1**) could be inactivated by treatment with base.[2] However, structural data was lacking, and the significance of this observation initially escaped attention. Only when Hodgkin determined the structure of penicillin V by X-ray diffraction in 1949[3] did the missing piece of the puzzle fall into place. The realization that the β-lactam ring possessed high chemical reactivity was a prime factor in identifying it as the pharmacophore.



penicillin G (**1**)

Only decades later did the advent of modern synthetic methods allow the rational design and preparation of modified natural products with the goal of identifying various mechanisms of action. Indeed, the ever-growing number of synthetic methods has the effect that the preparation of increasingly complex natural products becomes not only possible, but even practical.

In 1828, the German physician and chemist Friedrich Wöhler disclosed the chemical synthesis of urea from silver cyanate and ammonium chloride in a flask.[4] Urea had been discovered as a major component in mammalian urine by the French chemist Hilaire Rouelle in 1773. As every scientist learns early in his/her education, this observation sealed the date of vitalism. Wöhler's total synthesis of "synthetic" urea demonstrated that an organic compound could be prepared not only by a living organism but also by the experimental prowess of a chemist. Consequently, Wöhler is considered the father of organic chemistry and the fascination of organic chemists with natural products.

In the following sections, we present a number of classic and contemporary examples that illustrate the progress and contributions made by diverted total synthesis to the field of natural products synthesis. Importantly, these examples have been selected on the basis of the synthetic work providing

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insight into modes of action or opening new vistas in fundamental biology.

2. Neocarzinostatin Chromophore

Neocarzinostatin (Figure 1) was isolated from a *Streptomyces carzinostaticus* F-41 fermentation broth and found to exhibit potent antitumor properties.[5] It was soon shown to inhibit DNA synthesis by DNA scission.[6] Neocarzinostatin was initially characterized as a polypeptide.[7] Only years later

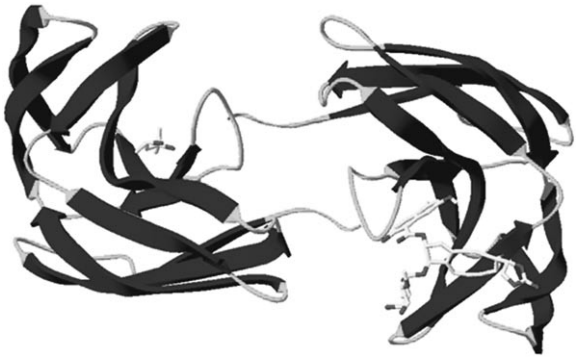
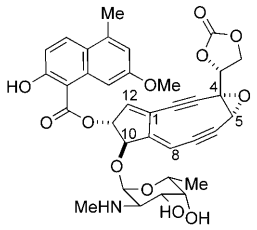


Figure 1. Structure of the neocarzinostatin complex, with the neocarinostatin chromophore (**2**) on the right (stick model).[12]

was the existence of the neocarzinostatin chromophore (**2**) discovered.[8] Subsequently, **2** was shown to be the instigator/mediator of the cytotoxic effects of the neocarzinostatin complex.[9] The structure of the complex was first established by using NMR spectroscopy[10,11] and later confirmed by X-ray crystallography.[12] The function of the protein, retroactively named apo-neocarzinostatin, is to serve as a carrier that protects the sensitive chromophore **2** from premature activation before reaching its target.[13] It binds **2** with an affinity constant $K_d \approx 10^{-10}$ M.

The ability of the neocarzinostatin chromophore to sever DNA strands was studied extensively before its mechanism of activation was proposed. An important observation was that the presence of thiols increases the activation rate by three orders of magnitude.[14,15]

In 1987 the Goldberg research group published a mechanism for DNA scission (Scheme 1).[16] According to this



neocarzinostatin chromophore (**2**)

proposal, hydrogen abstraction by a radical derived from the neocarzinostatin chromophore takes place at those positions of the deoxyribose that lead to the more stable radicals, that is, at the more highly substituted carbon atoms 1′, 5′, as well as at 4′. Trapping of the resulting radicals **4**–**6** by oxygen leads to oxygen-centered radicals that may increase damage by abstracting further hydrogen atoms from a neighboring deoxyribose. In any event, peroxide species are eventually reduced to alcohols by the cells protective mechanisms. This, however, facilitates DNA scission at the oxidized positions to form a legion of breakdown products, the major ones of which are shown in Scheme 1. Notably, some cancer types are inherently hypoxic, and, thus, the fate of the initially formed carbon-centered radicals **4**–**6** should be different in such cells. Indeed, the formation of adducts between **2** and DNA has been observed in the absence of oxygen or at high thiol concentrations.[15]

DNA scission takes place with some base specificity with more than 75% of the DNA lesions taking place at T and A bases.[17] There is, however, little sequence specificity, as with other enediynes (see Section 3). The majority of the lesions are of the single-strand type, while a lower percentage of lesions are of the double-stranded type. Double-stranded lesions take place with some specify at the 1′- and 5′-positions of AGC*-*TCG sequences and at the 5′- and 4′-positions of the AGT*-*TCA sequences (* marks the affected position). It is believed that these lesions are more important for cytotoxicity than the single-stranded type as they are more difficult to repair.[17]

Throughout the initial investigations the identity of the hydrogen-abstracting reagent remained unknown. In 1987, Myers published a study that postulated the mechanism shown in Scheme 2.[18] This hypothesis was based on the earlier observations mentioned above as well as NMR data for the then unidentified chromophore degradation products.

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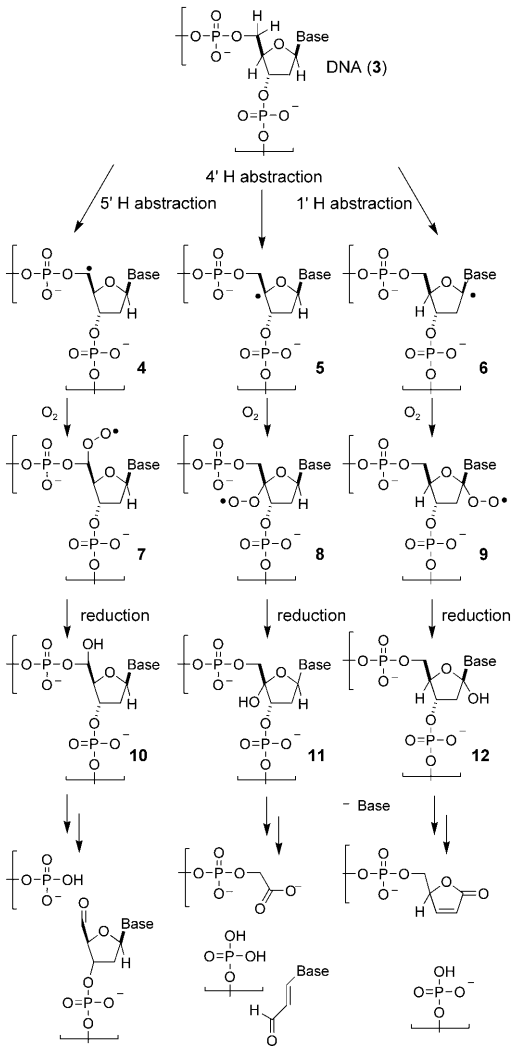
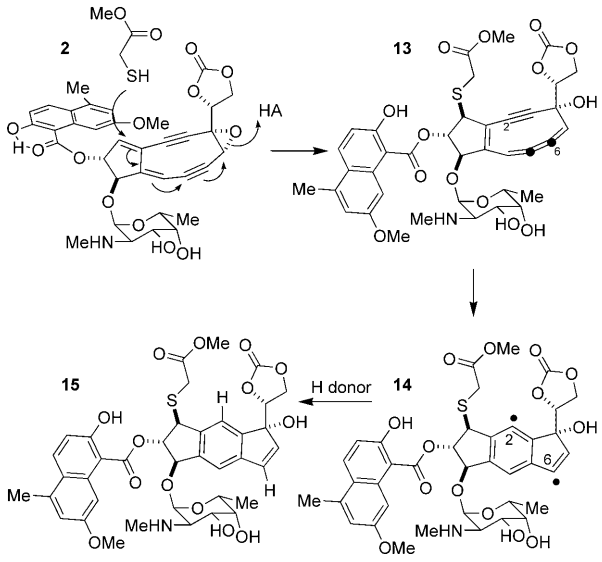
Alex M. Szpilman completed his MSc at the Technical University of Denmark, where he worked with Professor John Nielsen. He then moved to the Weizmann Institute of Science, where he received his PhD under the supervision of Professor Mario D. Bachi. After postdoctoral work in the group of Professor Erick Carreira at the ETH Zurich, he joined the Technion-Israel Institute of Technology as a Senior Lecturer.



Scheme 1. Degradation of DNA strands induced by hydrogen abstraction.



Scheme 2. Mechanism of thiol-induced activation. In the cell, DNA acts as the hydride source (see Scheme 1).

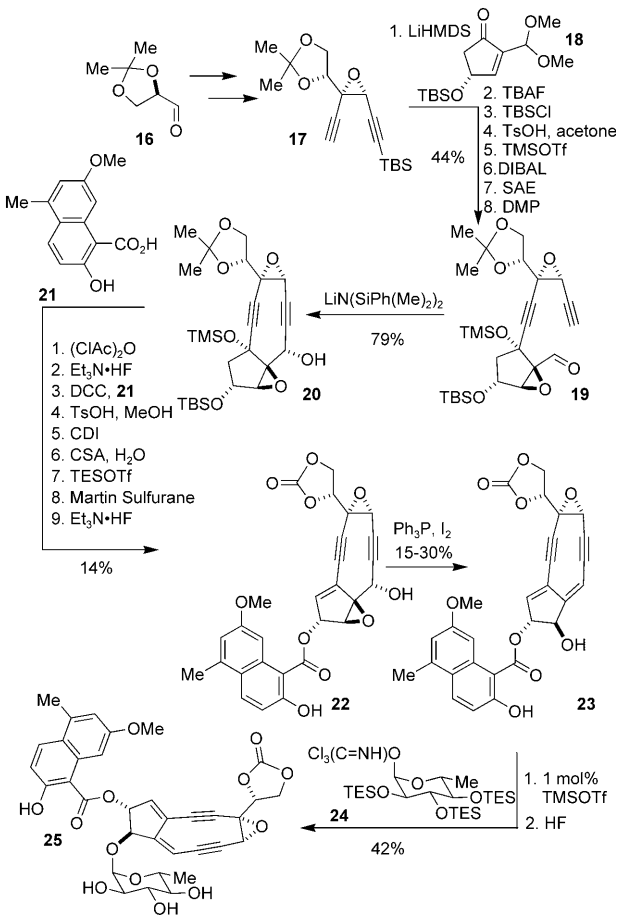
Accordingly, Myers proposed that thiols would attack the C12 alkene terminus of the cross-conjugated system, thereby leading to the formation of a highly strained cummulene system with concomitant opening of the epoxide **13**. This would be followed by a Bergman-type cyclization[19] to afford C2,C6-diradical **14**. This compound would serve as the perpetrator of the hydrogen abstraction shown in Scheme 1, thus leading to reduced compound **15**. Needless to say, **14** would also be capable of recombining with thiol radicals or radicals such as **4**–**6**. Thus, the sequence of events depicted in Schemes 1 and 2 would also account for the reported formation of such adducts.[15]

Shortly after proposing the mechanism, Myers et al. reported an investigation involving the isolation and structural characterization of the hitherto postulated end product **15**,[11,20] which provided additional support for the mechanism. The use of deuterated thiols in the decomposition reaction led to the incorporation of deuterium at positions 2 and 6. Careful NMR spectroscopic studies allowed the identification of the cummulene thiol adduct **13** formed between **2** and methyl thioglycolate at −70 °C. Heating the adduct in the presence of a hydrogen donor resulted in its conversion into **15**.[21]

The X-ray crystallographic structure of the neocarzinostatin complex[12] raised questions regarding the role of the carbohydrate residue. It had been noted that the methylamino group rests over C12 in the protein-bound form, and it had been speculated that this might serve to protect the electrophilic system from nucleophiles. Furthermore, there was speculation that the sequence specificity in the cleavage of double-stranded DNA stemmed from a sequence-specific recognition by the carbohydrate moiety. Studies using a simplified TBS-protected analogue of aglycone **23** had already shown that the glycoside residue served to accelerate attack by thiols.[22] As part of their efforts towards the total synthesis of **2**, Myers et al. targeted the aglycone for synthesis (Scheme 3).[23]

The synthesis commenced from protected glyceraldehyde, which was rapidly converted into enediyne **17** in a sequence that included a Sharpless asymmetric epoxidation to set the configuration of the stereocenter in the epoxide unit. The unprotected alkyne was converted into its lithium salt, which added to ketone **18**[24] with a selectivity of 20:1. After conversion into bisepoxide **19**, cyclization was effected in high yield by formation of the lithium acetylide. The free alcohol was protected as the chloroacetate ester. The hydroxy group at C11 was deprotected and esterified to naphthol **21** by using DCC. The ester was converted into **22** in a sequence that included dehydration of the tertiary alcohol and formation of the carbonate with carbonyldiimidazole. Finally, the aglycone was formed by a spectacular tandem iodination/iodo-elimination reaction. The yields of this process were 15–30 %.

With the aglycone **23** in hand, it proved possible to evaluate the role of the aminoglycoside moiety.[25] As the yield of the final step shown in Scheme 3 indicates, the aglycone was exceedingly unstable, especially in neat form. It could be stabilized by the addition of 5-*tert*-butyl-4-hydroxy-2-methylphenyl sulfide (Kishi's radical scavenger).[25,26] This observation, and the fact that the much more stable parent chromophore **2** could be stabilized in the same manner,



Scheme 3. Synthesis of neocarzinostatin aglycon **23** and desmethylamino-neocarzinostatin chromophore **25**.

suggested that the instability of the neocarzinostatin chromophore might be due to radical attack at C12. Thus, the positioning of the sugar residue may serve to protect the unsaturated system from attack by radicals. This observation also had synthetic ramifications. The addition of Kishi's radical scavenger during workup of the final synthetic step in combination with a number of other practical tricks allowed isolation of the aglycone **23** in 71% yield.[27] Careful evaluation of the reactivity of the aglycone towards thiols revealed it to react significantly slower than the parent compound. This further supports the role of the methylamino group as an internal base able to activate thiols towards nucleophilic attack at C12.

To further study this possibility, the desmethylamino-neocarzinostatin analogue **25** was prepared by glycosidation of the aglycone **23** with trichloroacetimidate **24** as shown in Scheme 3.[28] In contrast to aglycone **23**, hydroxy-desmethylamino-neocarzinostatin chromophore **25** proved highly stable at room temperature. Significantly, while **2** reacts with methyl thioglycolate at −78 °C, no reaction ensued when **25** was subjected to the same conditions at room temperature. Only in the presence of triethylamine could cyclization be induced. This further cements the role of the aminosugar as an internal base that accelerates the specific attack of thiols at C12. In stark contrast, this same moiety appears to stabilize the system by preventing attack by radicals.

Aglycone **23** was also used to address the issue of sequence specificity in double-stranded lesions.[25] In contrast to, for example, calicheamicin γ^I1 (see Section 3), the sugar moiety is not responsible for sequence recognition. The profile of DNA lesions caused by the aglycone **23** was largely identical to that of the parent compound **2**. Accordingly, it was surmised that the sugar moiety has little significance for DNA specificity. More likely it is the naphthol moiety of **2** that intercalates and that this event takes place primarily at T or A bases. Neocarzinostatin remains a molecule of great interest and continues to be used as a platform for new anticancer treatments.[29,30]

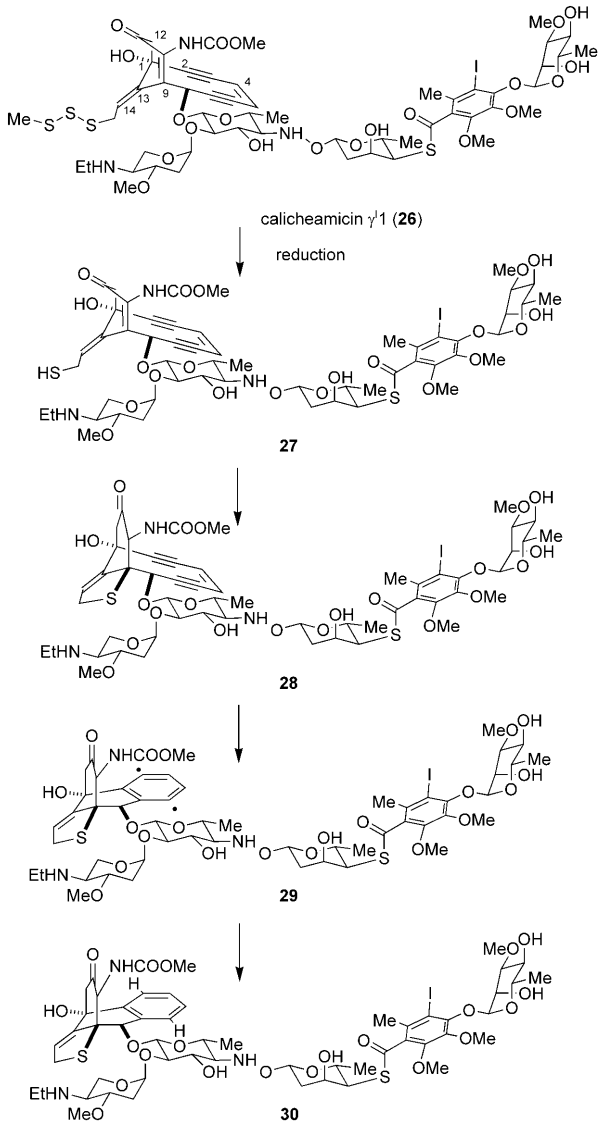
3. Calicheamicin γ^I1

The potent antitumor agent calicheamicin γ^I1 (**26**) was isolated from *Micromonospora echinospora* ssp. Calichensis and shown to be a potent cytotoxic agent.[31,32] In analogy to neocarzinostatin chromophore (**2**), calicheamicin γ^I1 (**26**) cleaves DNA. However, in contrast, to neocarzinostatin, it has a propensity to cleave DNA in a highly sequence-specific manner.[33]

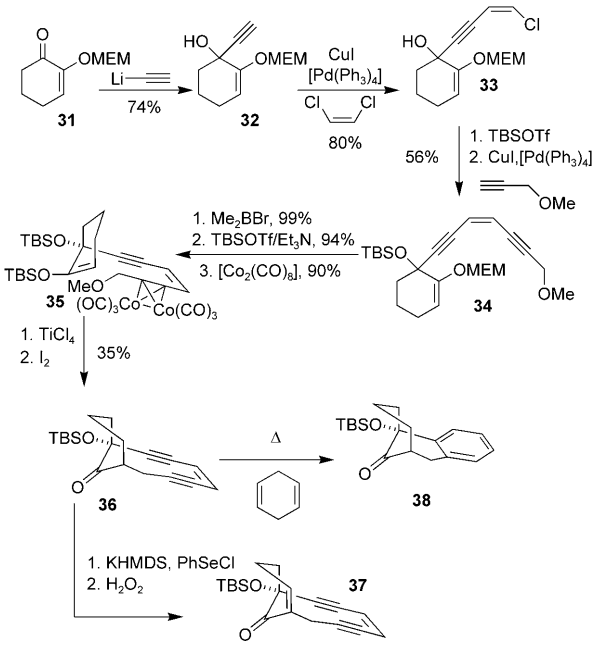
Given the structural resemblance of neocarzinostatin (**2**) and **26**, a similar mechanism of action was proposed (Scheme 4).[31] Accordingly, the trisulfide is first cleaved reductively, for example, by endogenous thiols, to produce thiol **27**. This triggers the conjugate addition of the free thiol at C9 of the enamide ketone to produce adduct **28**. Concomitant rehybridization from sp^2 to sp^3 at C9 brings the two alkyne units of the enediyne system closer together, thereby providing a low-energy pathway for cyclization. This Bergman-type cyclization leads to the formation of diradical species **29**, which is capable of abstracting hydrogen from DNA (Scheme 1). The reduced form **30** was isolated and identified.[31]

Mirroring the earlier work by Myers et al., the Townsend research group was able to corroborate the formation of adduct **28** by performing the reduction of **26** with tributylphosphine in [D_4]methanol at −67 °C.[34] The adduct was relatively stable at this temperature, but upon warming to −11 °C in the presence of methyl thioglycolate it underwent smooth conversion into the deuterated form of **30**, which could be isolated in 70% yield. In contrast, a simple ten-membered monocyclic enediyne that was able to cleave DNA was stable at room temperature, but underwent Bergman cyclization at 37 °C with a half-life of 11.8 h.[35] Apparently, the bicyclic system not only confers stability to **26** but also accelerates the Bergman cyclization in its reduced form **27**.

The relationship between the cyclization rate and the hybridization state at the bridgehead carbon atom was examined by Magnus et al. in a model study.[36] They prepared two different simplified models of the aglycone, that is, **36** and **37** (Scheme 5) which differed only in the hybridization of C9. Interestingly, the C9(sp^3) analogue **36** underwent Bergman cyclization at 82 °C. In contrast, the sp^2-hybridized analogue **37** was recovered unchanged under these conditions. Fur-



Scheme 4. Structure and mechanism of activation of calicheamicin γ^{I}1 (**26**).



Scheme 5. Synthesis of aglycone analogues **36** and **37** with different hybridization at C9, according to Magnus et al.

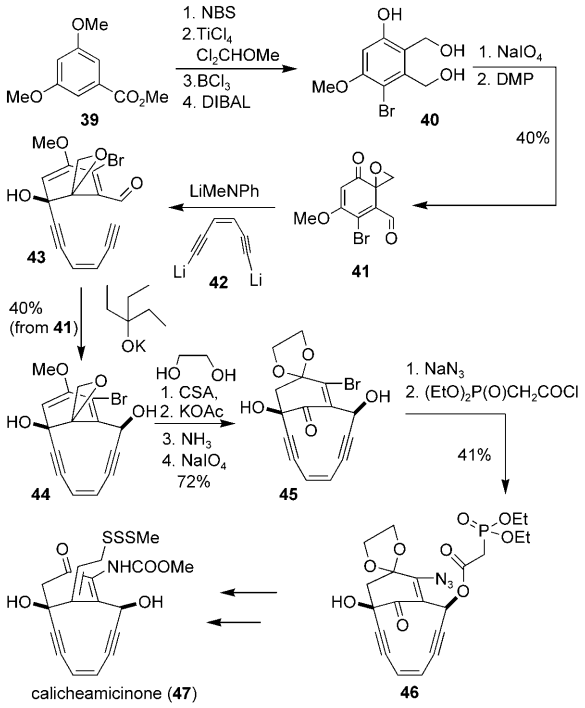
Figure 2. Sequence recognition of calicheamicin γ^{I}1 (**26**; underlined) and sites of double-stranded DNA cleavage (arrows).

thermore, the reduction of **36** with DIBAL led to the corresponding alcohol, which rapidly underwent aromatization. In view of these findings it is tempting to speculate that Bergman cyclization is inhibited when C9 is sp^2 hybridized since cyclization would involve an energy penalty arising from the formation of a highly strained bicyclic system with a bridgehead double bond.[37]

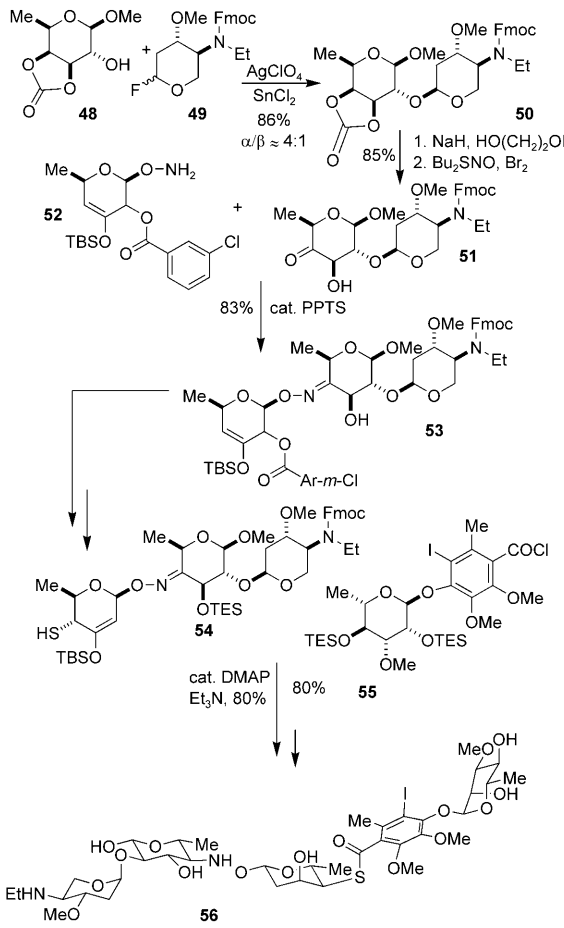
Zein et al. established in a series of publications that **26** cleaves DNA in a highly sequence-specific manner.[33] Cleavage was found to take place in a single- to double-stranded manner in a 1:2 ratio.[38] Hydrogen abstraction took place mainly at the "*5′ C terminus of a TCCT sequence and three nucleotides toward the 3′ side of the 3′ G in the complementary AGGA box*" (Figure 2). This specificity was further confirmed by studying the cleavage of a series of DNA dodecamers.[39] Other authors obtained evidence that calicheamicin γ^{I}1 (**26**) abstracts hydrogen from deoxyribose primarily at the 5′ and 4′ site.[40] It was suggested that sequence recognition was a process of induced fit.[41]

These findings spurred synthetic efforts by several research groups which finally resulted in the total synthesis of calicheamicin γ^{I}1 (**26**) by the Nicolaou and Danishefsky research groups.[42–44] The elegant synthesis of the aglycone **47** in racemic form by Danishefsky and co-workers is shown in Scheme 6.[44e]

The synthesis commenced from commercial ester **39**. Selective bromination was followed by formylation. By using the formyl group as a directing group, the *ortho* methoxy group could be selectively cleaved with BCl_3. Reduction with DIBAL afforded the unstable diol **40**, which upon exposure to sodium periodate cyclized to form an epoxyquinone. A second oxidation, this time with the Dess–Martin periodinane, afforded aldehyde **41**. Taking advantage of in situ protection of the aldehyde as the *gem*-amino alkoxide, the bislithioenedyne **42** was added selectively to the vinylogous ester carbonyl to form **43**. The tertiary alcohol was protected in situ as a TMS ether. Deprotonation of the terminal alkyne with potassium ethylpentoxide led to cyclization and the formation of enediyne **44** in 40% yield from **41**. The methyl enol ether was transformed into a dioxolane by the action of CSA in ethylene glycol. Opening of the epoxide with potassium acetate followed by hydrolysis with ammonia gave a diol, which was cleaved by sodium periodate to



Scheme 6. Synthesis of racemic calicheamicinone (**47**) according to Danishefsky et al.



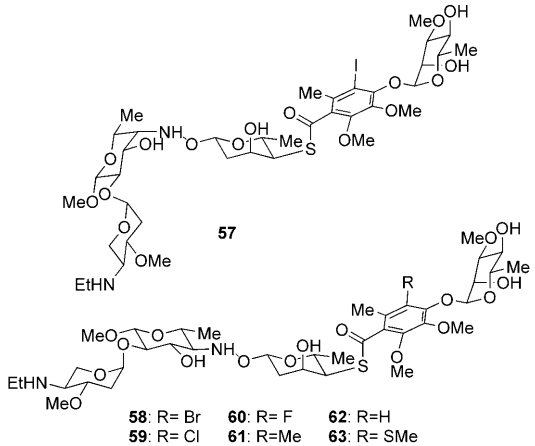
Scheme 7. Synthesis of the oligosaccharide moiety **56** according to Nicolaou et al.

afford enone **45**. This enone facilitated the introduction of the requisite methyl carbamate. Accordingly, sodium azide added to the enone in a conjugate fashion. Subsequent elimination of bromide reconstituted the enone system. Acylation of the free propargylic alcohol with diethyl phoshonoacetic acid chloride set the stage for the introduction of the methylene unit by an intramolecular Horner–Wadsworth–Emmons (HWE) reaction. With the backbone secured, a number of relatively simple steps led to the formation of calicheamicinone (**47**).

Danishefsky and co-workers studied the DNA-cleaving properties of the racemic and resolved aglycones, and found that triggering cyclization in the presence of DNA led to DNA cleavage with a lower fraction of double-stranded DNA damage than single-stranded damage.[44e] In addition, there was no observable sequence specificity. These observations supported the importance of the oligosaccharide moiety for sequence recognition, as proposed earlier by Schreiber and co-workers.[45]

As a part of the total synthesis of **26**, the oligosaccharide **56** was prepared as a separate entity. The synthesis of **56** by Nicolaou et al. is shown in Scheme 7.[43a] With the oligosacharide in hand, it was possible to study dodecamers complexed with **56** in solution by NMR spectroscopy.[46] Nicolaou and co-workers studied the interactions between **56** or **26** with a DNA dodecamer by examining changes in the chemical shift. These studies indicated that the oligosaccharide contains sufficient structural elements to bind selectively to DNA. Nonetheless, the oligosaccharide binds to the TCCT sequence with a lower affinity than the parent compound calicheamicin $\gamma^{I}1$ (**26**).

Schreiber and co-workers proposed that that the iodine atom played a specific role in binding to an NH_2 group of guanidine groups.[45] This conjecture was examined experimentally independently by the research groups of Nicolaou, Joyce,[46] and Kahne.[47] The research groups of Nicolaou and Joyce prepared oligosaccharide analogues **57**–**62** and studied their properties in DNAase I footprinting and DNA binding studies. These footprinting studies showed that neither **57** nor **62** were able to bind specifically to DNA sequences readily



58: R= Br **60**: R= F **62**: R=H
59: R= Cl **61**: R=Me **63**: R= SMe

recognized by the native oligosaccharide **56**. Furthermore, decreasing the size and increasing the electronegativity of the halide led to an incremental loss of the DNA binding affinity. Interestingly, **61** was a weaker binder than even the chloride and fluoride analogues **59** and **60**, respectively. This finding would indicate that electronegativity was more important than steric size for binding. These studies demonstrated the importance of the overall shape of the oligosaccharide unit and specifically the configuration of the -NH-O unit. The latter finding was also supported by computational studies by Kahne and co-workers.[47] These studies showed that the NH-O unit in the oligosaccharide plays a critical role in placing the two halves of the sugar moiety in such a way that predisposes it for binding to the minor groove of DNA.[47] In contrast to the findings of Nicolaou and co-workers, these authors found that the steric bulk of the iodine was of crucial importance. Thus, they prepared the methylthio analogue of oligosaccharide **56**, that is, **63**.[48] The methylthio group was suggested to have a similar size, but different electronic properties than an iodine atom. Notably, analogue **63** was able to bind to the calicheamicin TCCT recognition site, albeit with a threefold lower affinity. In contrast, **63** was unable to bind to a TTTT site to which the native oligosaccharide **56** was able to bind. Thus, it would appear that the major role of the iodine atom is to constrict rotation about the aryl glycoside linkage, thereby restricting the number of available conformations of the oligosaccharide moiety. Contemporaneously, Kahne and co-workers studied long-range NOE interactions between **26** and a bound DNA fragment (Figure 3).[49] These studies indicated

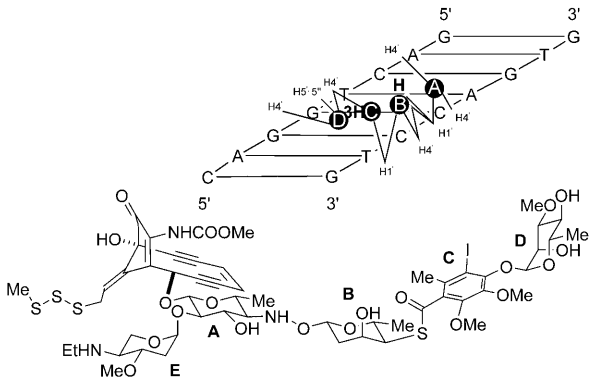


Figure 3. Schematic representation showing intermolecular NOE NMR signals. The letters refer to the various rings in the structure of calicheamicin $\gamma^{I}1$ (**26**).

the relative position of the sugar subunits of the DNA coil. Studies on **26** culminated in the first solution-phase structure obtained by NMR spectroscopy (Figure 4)[50] and shows how calicheamicin $\gamma^{I}1$ (**26**) binds to the minor groove of DNA. The aglycone moiety protrudes out in the vicinity of the DNA backbone, prepositioning it for hydrogen abstraction.

The findings described above have been used to prepare DNA-cleaving calicheamicin–oligosaccharide conjugates.[51] For example, Danishefsky and co-workers have prepared a daunorubicin calicheamicin oligosaccharide hybrid.[52] Nicolaou et al. designed and prepared calicheamicin $\theta^{I}1$, a fully

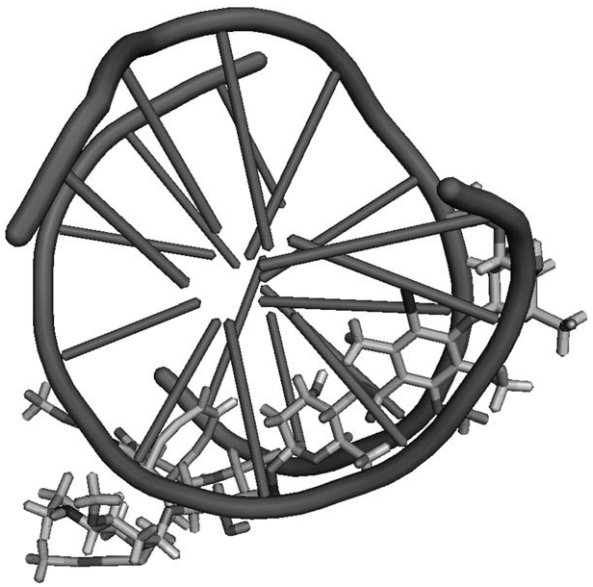


Figure 4. Solution-phase structure of the complex of **26** with a 23mer hairpin duplex of DNA. Calicheamicin $\gamma^{I}1$ (**26**) is shown as a stick model.[50]

synthetic analogue of **26**, which was shown to be a potent and sequence-specific DNA scissor.[53] Furthermore, Nicolaou et al. prepared and studied tail-to-tail and head-to-tail dimers of the oligosaccharide.[54] These dimers were capable of recognizing and specifically binding to DNA sequences of eight DNA base pairs.

Calicheamicin $\gamma^{I}1$ (**26**) itself is too toxic for clinical use. However, gemtuzumab ozogamicin, a conjugate of a monoclonal antibody and a semisynthetic derivative of calicheamicin $\gamma^{I}1$, was until recently in clinical use for the treatment of myeloid leukemia.[55] This powerful agent targets the CD33 antigen found on the surface of more than 80% of cancerous myeloid leukemia cells.[56]

4. FK506

The body's immune system mounts a powerful defense that is capable of combating severe diseases and maintaining physical health despite unceasing attack by foreign organisms and toxic substances from the surroundings. The inherent power of this intricate system is all the more devastating when it turns upon the body itself, causing afflictions collectively termed autoimmune diseases. The immune system must also be restrained in the case of organ transplantation, when a diseased organ is surgically exchanged for a healthy donor organ. Ironically, it is the ability of the immune system to recognize the transplanted tissue as foreign that must be dulled to prevent organ rejection. The molecules shown in Figure 5 belong to a select group capable of performing this vital service.[57]

Cyclosporin A (**64**) was first identified from a Norwegian soil sample in 1974[58] and was approved for clinical use in 1983.[57] FK506, a much more powerful immunosuppressive agent, was isolated from a *Streptomyces tsukubaensis* found in

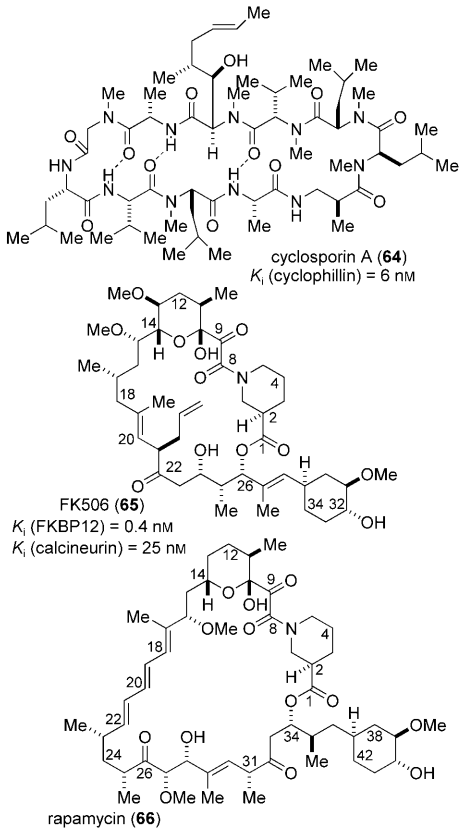
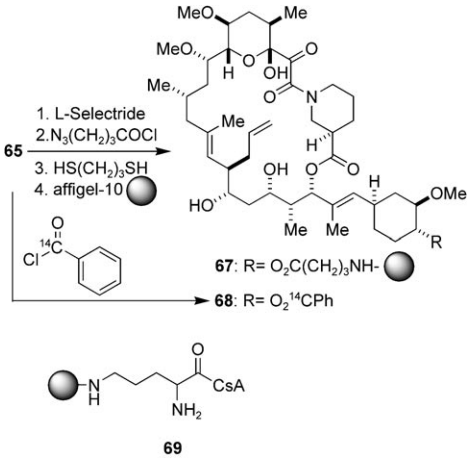


Figure 5. Structures of T-cell activation inhibitors cyclosporine (**64**), FK506 (**65**), and rapamycin (**66**).

a Japanese soil sample.[59] It too entered clinical use, winning approval from the American Food and Drug Administration (FDA) in 1994. In contrast, rapamycin was discovered in 1975 (from an Easter Island soil sample),[60] but the study of its immune-suppressing properties commenced later, and it won FDA approval for use in 1997. The mechanism of action remained open, even after these drugs won clinical approval. Through a monumental interdisciplinary effort in collaboration with the Crabtree and Clardy research groups, Schreiber and co-workers were able to elucidate many aspects of the mechanism of action of FK506 and subsequently that of rapamycin (**66**) and cyclosporin A (**64**). For this, they used an extensive array of chemical and biological tools, such as enzyme assays, affinity chromatography, and diverted total synthesis.

Through clever structural modification of FK506 by semisynthesis, Schreiber and co-workers were able to produce an affigel-based affinity matrix **67** for FK506 as well as a ^{14}C40-labeled FK506 derivative **68** (Scheme 8).[61] An affinity matrix for cyclosporine A, that is, **69** was also prepared. By using these affinity matrices they were able to isolate the known 14 kDa cyclosporin A binding protein cyclophillin[62] and a new 14 kDa protein that binds specifically to FK506 (**65**). This protein, named FKBP (FK506 binding protein), was later shown to exist in several isoforms, some of which are ubiquitous and some that have been found only in mitochondria. Subsequently, rapamycin (**66**) was shown to also bind FKBP. Cyclosporin A (**64**) on the other hand binds to the



Scheme 8. Synthesis of affinity matrices **67** of FK506 and **69** of cyclosporin A (CsA). The ^{14}C-labeled **68** was used in competitive binding studies.

aptly named cyclophillin proteins. By using ^{14}C-labeled **68** in a displacement assay it could be shown that **64** did not bind to FKBP. FK506 (**65**) also did not displace **64** from cyclophillin. Interestingly, both FKBP and cyclophillin have *cis-trans* peptidyl-prolyl isomerase (PPIase) activity (Figure 6). PPIases are able to catalyze the folding of proteins into their secondary structure. Studies on FKBP–peptide interactions suggested that a leucine–proline dipeptide would adopt a twisted amide conformation with a characteristic 90° angle between the proline plane and carbonyl group during the isomerization reaction.[63] FK506 was shown to inhibit the isomerase activity of FKBP by binding noncovalently to the active site. From this it was inferred that the C8–C9 α-keto amide of FK506 served as a twisted amide surrogate when binding to FKBP.[64] Indeed, on binding to the FKBP PPIase site, FK506 adopts the conformation shown in Figure 6c. Curiously, proteins whose folding was catalyzed by FKBP and cyclophillin were found to reach their natural state even in the absence of FKBP. Moreover, the catalytic effect is small, at most speeding up the folding by one order of magnitude. This value was too small to account for the rates observed in the T-cell activation cascade. This led to the suspicion that the cycloisomerase activity of FKBP and cyclophillin is incidental and unrelated to the biological target being suppressed by **64**–**66**.

A putative binding domain was identified on the basis of the above observations, the structural similarity between rapamycin and FK506, and solution-state NMR studies on their FKBP complexes.[65] Compound 506BD (**78**), a macrocyclic molecule that preserves the binding domain of FK506 and whose cyclic structure was designed to ensure the 90° angle required for optimal binding to FKBP, was synthesized (Scheme 9). It was anticipated that a 506BD complex with FKBP would differ enough from the FK506/FKBP complex to report on the importance of inhibition of PPIase activity. Interestingly, the validity of these assumptions was only confirmed later when Schreiber and co-workers secured X-ray crystallographic data of FK506 bound to the catalytic site of FK506 (Figure 7).[66]

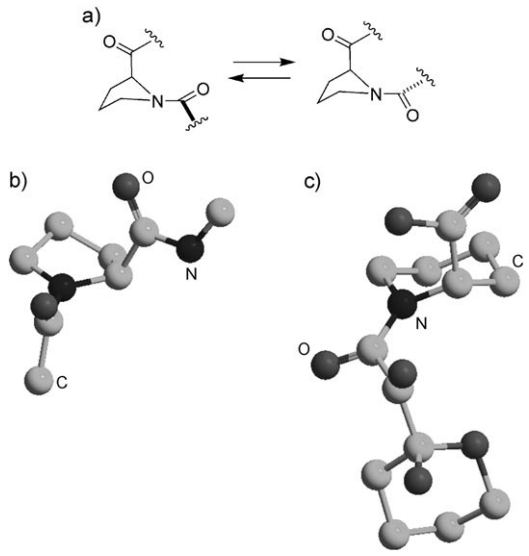


Figure 6. a) Equilibrium catalyzed by the immunophilins cyclophillin and FKBP. b) Transition-state structure stabilized by immunophilins; c) partial structure of FK506.

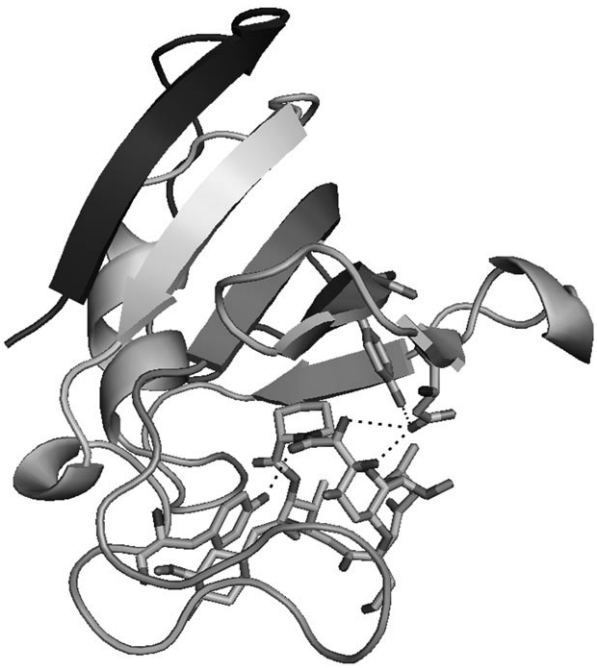
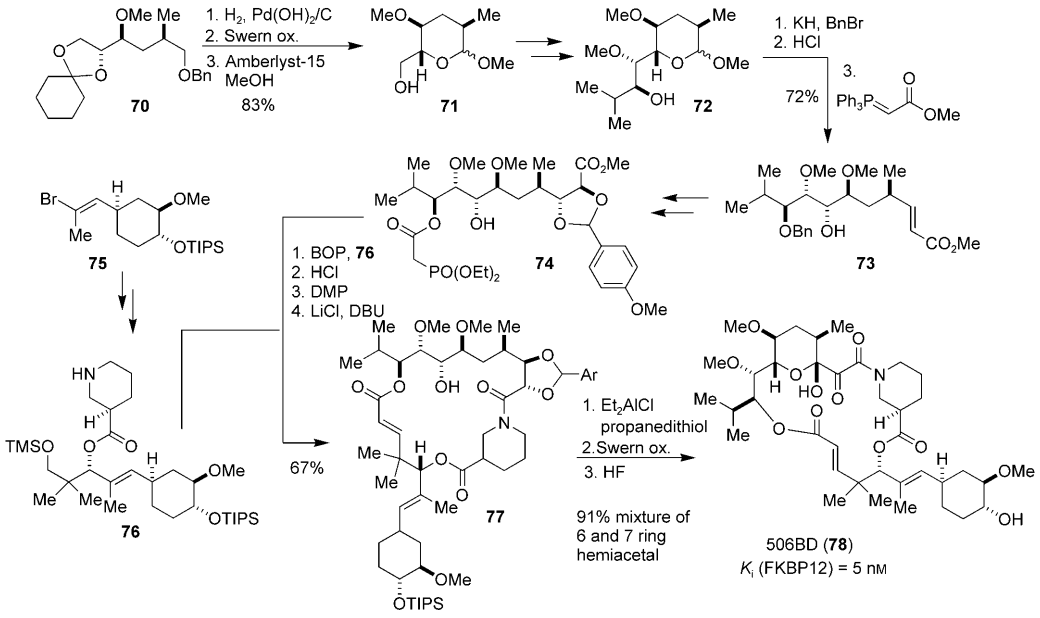


Figure 7. X-ray diffraction structure of FK506 (**65**) bound to its target FKBP. Hydrogen-bonding interactions are indicated. The 90° angle of the α-amide carbonyl group can be clearly seen (compare with Figure 6c). Stick model: ■ C, ■ O, ■ N..

The synthesis[65] of 506BD (**78**) took advantage of experience and materials that had been obtained during the total synthesis of FK506.[67,68] Compound **71**, which corresponds to the hemiacetal of the binding domain, was prepared starting from known **70**. Removal of the benzyl group, oxidation to the aldehyde, and treatment with acid and methanol led to the formation of cyclic hemiacetal **71**. After introduction of the isopropyl side chain, the methyl ketal protecting group was removed by the action of acid. In the next step, a Wittig reaction extended the chain by three carbon atoms. This reaction took advantage of the equilibrium between the cyclic hemiacetal and open-chain aldehyde to allow the latter to react with the Wittig reagent. Cyclohexane **75**, an intermediate in the total synthesis of FK506 by Schreiber and co-workers,[68] was converted into **76**. Subsequently, **75** and **76** were coupled using the BOP reagent and then macrocyclization was effected by a HWE reaction under Roush–Masamune conditions. The PMB acetal of **77** was cleaved by treatment with diethylaluminum chloride and propanedithiol. Oxidation of the free C9 and C10[69] hydroxy groups led to formation of the C10 hemiketal. Final deprotection using HF afforded 506BD (**78**) in high yield, but as a mixture of six- and seven-membered cyclic hemiacetals. The seven-membered ring hemiacetal proved to be a poor binder for FKBP in vitro, but its formation could be suppressed, thereby allowing the study of the binding properties of 506BD.



Scheme 9. Synthesis and binding properties of 506BD (**78**).

506BD (**78**) was shown to be a strong inhibitor of FKBP isomerase activity (K_i = 5 nM).[70] A different analogue which did not

include the cyclohexane appendage in its backbone as well as the seco form of 506BD were very poor inhibitors. 506BD was able to displace tritium-labeled FK506 with a K_d value of 20 nM. Importantly, 506BD showed no immunosuppressive properties. Indeed, 506BD is an antagonist of the immunosuppressive effect of FK506 and rapamycin. Thus, it could be surmised that FK506 contained two, but not necessarily mutually exclusive, moieties: a) a binding domain responsible for binding to FKBP and b) another yet unidentified domain (the "effector" domain) responsible for expressing the immunosuppressive effect of the FK506/FKBP complex. A similar finding was obtained by Nicolaou and co-workers from the study of FKBP with a synthetic rapamycin binding domain.[71]

Since the PPIase function of FKBP could be sequestered from its T-cell activation inhibition it was suggested that the complex binds to a different target and that this target was responsible for transmitting the immune-suppressing signal. Indeed, both the rapamycin/FKBP complex and FK506/FKBP complexes were shown to bind to larger protein units (see below).[72] Subsequently, the protein complex containing bound FK506 was identified as a hybrid of FKBP and calcineurin (Figure 9). The cyclophillin–cyclosporin A complex also binds to calcineurin.[73] However, it was not clear which portion of FK506 was the "effector" motif that would allow binding of the FK506/FKBP complex to calcineurin.

By using site-specific mutagenesis Schreiber and co-workers identified that binding to the Gly89-Ile90 unit of FKBP was essential for eliciting calcineurin inhibition.[74] With these data, and based on the X-ray data (Figures 7 and 8), an acyclic analogue SBL506 (**89**) was designed in the hope of retaining those binding interactions with FKBP that were crucial for calcineurin inhibition.[75]

The synthesis of SBL506 (**89**) commenced from the readily available imide **79**. Evans methylation took place with excellent stereoselectivity. The Evans auxiliary was reductively cleaved and the resulting alcohol oxidized to the aldehyde oxidation state by PCC. Brown allylation afforded *syn*-product **80** in excellent yield and stereoselectivity. Fragment **81** was conjugated to **80** by the action of EDC/DMAP, and a Lemieux–Johnson type oxidation afforded aldehyde **82**. The diallyl ketone moiety of **84** was introduced by a highly *syn*-selective Mukaiyama aldol reaction. A one-pot TBS protection/Boc cleavage then afforded **84** (Scheme 10).

The synthesis of the complementary C8–C17 fragment **87** also relied on an Evans aldol reaction to establish the correct configuration at C11. Fragments **84** and **85** were conjugated by PyBrop amide formation (Scheme 11). The final segment of the synthesis relied strongly on the previous total synthesis of FK506,[68] and afforded SBL506 (**89**) in a total of 25 linear steps in good yield. Remarkably, while SBL506 was a poor inhibitor of FKBP PPIase activity, the resulting complex was capable of binding to calcineurin at nanomolar concentrations (approximately 13-fold less than FK506). This finding further showed that the activity of FK506 could be derived from only a subdomain of its structure. Additionally, this result also opened up the possibility of the synthesis of synthetic analogues of FK506 with improved pharmacological properties.

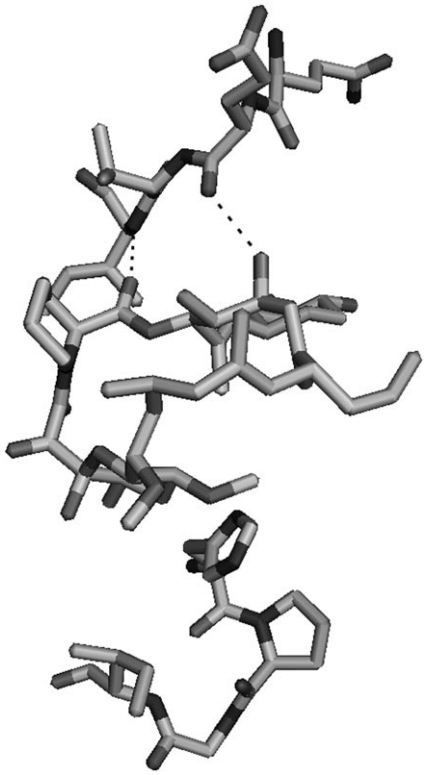
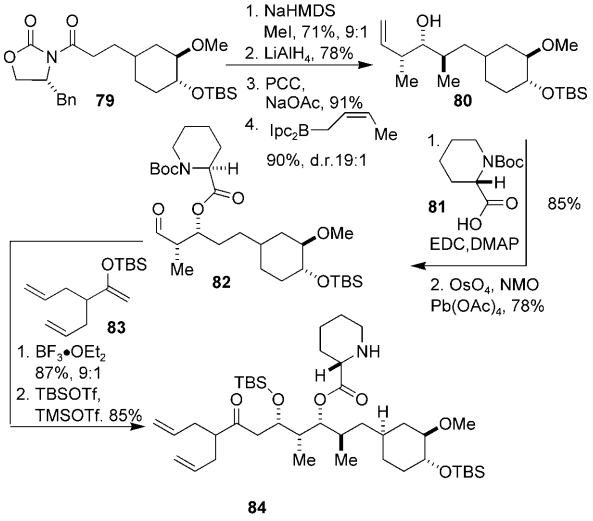
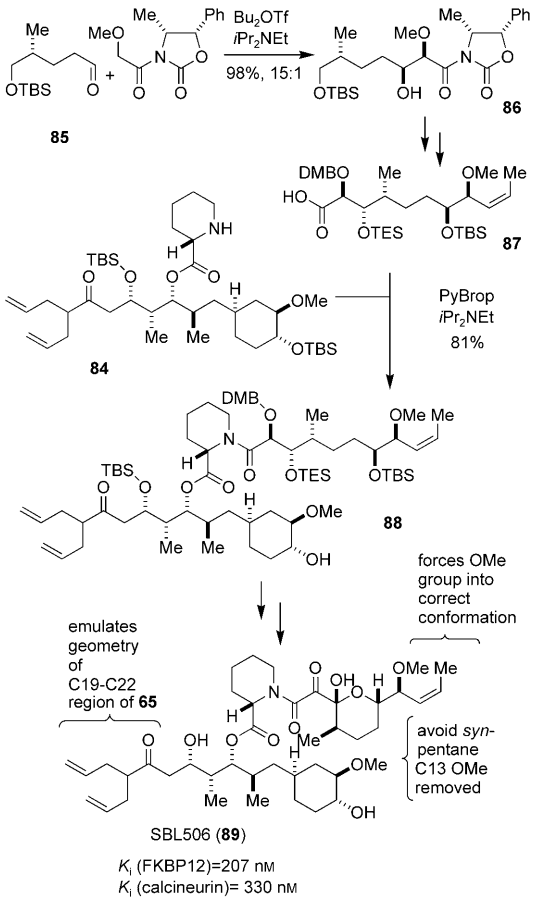


Figure 8. Interaction of FK506 with the Gln53-Glu54-Val55-Ile-56 (top) and His87-Pro88-Gly89-Ile90 residues (bottom) believed to be part of the composite surface structure of the FK506–FKBP complex responsible for calcineurin binding. Compare with Figure 7. ■ C, ■ O, ■ N.



Scheme 10. Synthesis of the effector domain of SBL506. (**84**)

A simplified overview of the findings presented here is given in Figure 9.[76] Calcineurin is a calcium-dependent phosphatase responsible for dephosphorylating the NF-ATC factor. NF-ATC is the one of the two subunits of the NF-AT transcription factor that is localized in the cytosol. NF-AT is believed to regulate the transcription of the crucial cytokine IL-2 gene.[77] In contrast to FK506/FKBP, the rapamycin/



Scheme 11. Completion of the synthesis and biological properties of SBL506 (**89**).

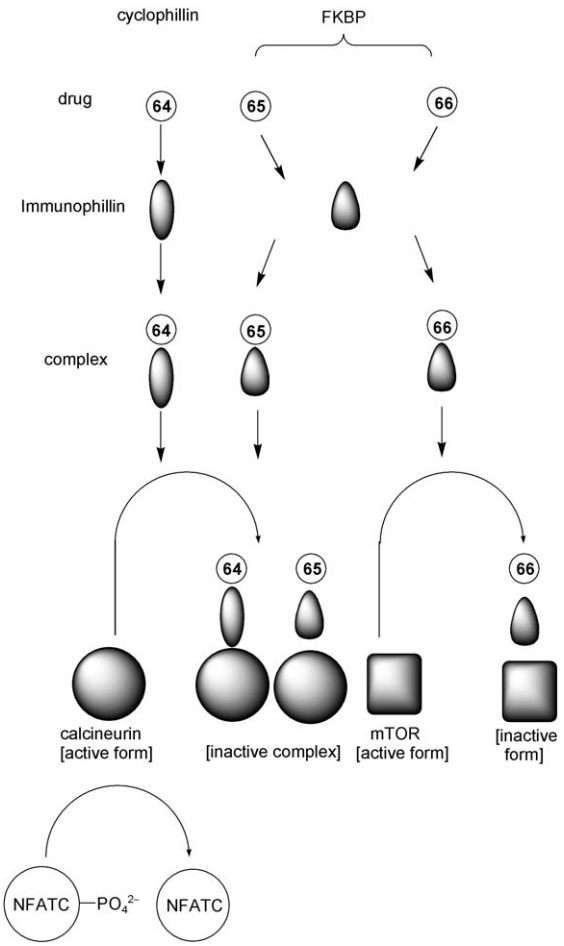


Figure 9. Simplified overview of the initiation of the T-cell inactivation pathway.

FKBP complex was found to bind to a different protein, aptly named mTOR (mammalian target of rapamycin).[78] This protein is also a protein kinase which regulates cell proliferation and mRNA translation (Figure 9).

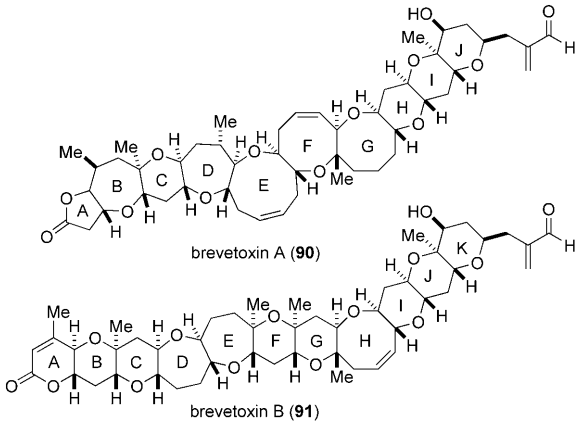
Much additional work was needed and is still ongoing to develop an understanding of all the intricate details involved in the processes discussed. Indeed, novel properties of cyclosporine, FK506, and rapamycin are still being discovered.[79] However, it is fair to say that the results presented here represent an extraordinary odyssey in chemical and biological discovery, and one that illustrates the power of organic synthesis, genetic engineering, and structural biology.

5. Brevetoxin B

The brevetoxins belong to the family of marine "red-tide" ladder polyethers. In addition, to brevetoxin A (**90**) and B (**91**), the family includes the ciguatoxins and the largest nonpeptide natural product to be isolated to date, maitotoxin.

The brevetoxins are neurotoxic agents and bind to binding site 5 of the α subunit of voltage-gated sodium channels (Figure 10).[80] In doing so, they cause a number of effects: a) a shift of activation potential to lower values; b) occurrence of subconductance states; c) induction of longer mean open times of the channel; and d) inhibition of channel inactivation. Collectively, these effects depolarize nerve cells and lead to increased sodium flow.[81] Structurally, brevetoxin A and B have a number of similarities; however, their binding affinity and ability to cause each of the noted effects differ.

Gawley et al. studied and compared the inhibitory effect and conformations of **90** and **91** and related congeners in a series of publications.[82,83] The A ring and the position of the carbonyl group was found to be essential for activity.[84] Their



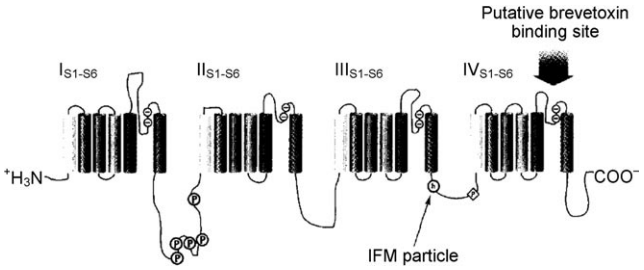


Figure 10. Secondary structure of the α subunit of the voltage-gated sodium channel. The subunit comprises four homologous domains and six subdomains with intracellular and extracellular loops. The position of the IFM particle, which is thought to be involved in inactivation of the open channel, is shown. The amino acid residues forming the IFM particle are represented by (h). A protein kinase C phosphorylation site is indicated by (P). Reprinted from Ref. [80].

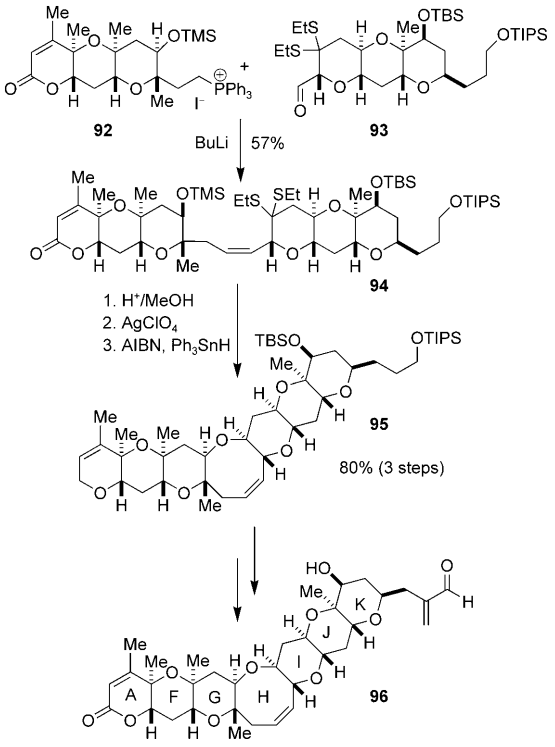
findings underscored the similarities between the HIJ moiety of brevetoxin A (**90**) and the IJK rings of brevetoxin B (**91**). The medium-sized rings found in brevetoxin A impart it with much greater conformational flexibility than brevetoxin B. Nevertheless, docking of the lowest energy conformations of **90** and **91** showed that several conformations shared the same space when the HIJ and IJK rings where superimposed. On the basis of these studies they put forward a postulate regarding the structural requirement for activity of ladder polyether toxins: "… *the common pharmacophore for the toxins that bind to site 5 is a roughly cigar-shaped molecule, 30 Å long, bound to its receptor primarily with hydrophobic and non-polar hydrogen bond donors near the site of the A-ring carbonyls.*"[83]

En route to their successful total synthesis[85,86] of brevetoxin B, the Nicolaou research group purposefully veered off the track to test the validity of this hypothesis.[87] The truncated brevetoxin [AFGIJK] **96** was designed to test and confirm the findings noted above. Thus, it included the vital A ring, and was designed to have an overall conformation strongly resembling brevetoxin B. However, its overall length would be only 20 Å.

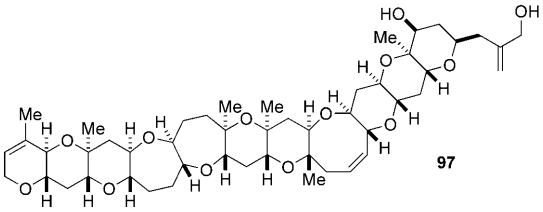
The two tricyclic precursors **92** and **93** were quickly assembled from known intermediates in the brevetoxin B synthesis[88] and coupled through a Wittig olefination reaction (Scheme 12). The eight-membered H ring was formed by silver-induced thiohemiacetal formation and the acetal function reduced to the ether under free-radical conditions.[87] Truncated brevetoxin [AFGHIJK] **96** was then completed in a series of steps in which the protection groups were removed, and the oxidation stage of the A ring and the K ring side chain adjusted.

The properties of **96** and whether it could exert the noted effects of brevetoxin B on the ion channel were then studied in receptor-binding assays and electrophysiological measurements.[80] These studies also examined semisynthetic brevetoxin analogue **97**, in which the carbonyl group of the unsaturated lactone in A ring had been reduced to the allylic ether and the aldehyde to the alcohol.

Truncated analogue **96** was able to shift the activation potential downwards by 10 mV, albeit only at a concentration of 1 μM. However, it was unable to induce the other three



Scheme 12. Central steps in the synthesis of truncated brevetoxin B [AFGHIJK] by Nicolaou et al.



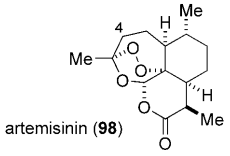
effects of brevetoxin B noted above. In contrast, the reduced analogue **97** binds to the binding site less effectively (IC_{50} = 73 nM) compared to **90** (IC_{50} = 36.5 nM), but does inhibit the inactivated state of the sodium channel. However, binding does not result in longer mean open times of the channel.

Although these results were not unambiguous, they did point to the importance of the length of the molecule and thus indicated that brevetoxin B (**91**) would be in an essentially linear conformation on binding to the ion channel. The results with semisynthetic **97** indicated the importance of the A ring carbonyl group and pointed to the possibility of a hydrogen-bonding interaction between the carbonyl group of the A ring and a hydrogen donor in the binding site. Moreover, since the different effects were not a function of a single structural feature of brevetoxin it should be possible to prepare brevetoxin antagonists. Indeed, this has proven to be the case.[89]

6. Artemisinin

A series of ingenious investigations were carried out by O'Neill and Posner to elucidate the mode of action of the antimalarial endoperoxides artemisnin (qinghaosu).[90]

Artemisinin (**98**) was isolated from a plant extract of *Artemisia annua* used as a traditional prescription for fever in China.[91] The identification of the endoperoxide moiety as the pharmacophore was based on the observation that reduction of the peroxide bond led to inactive products.[92] Iron-mediated homolytic cleavage of the peroxide bond was quickly identified as the trigger of the event that brings about parasite death.[92–94] A key finding by Meshnick et al. was that radicals were formed upon reaction of artemisinin with heme.[95]



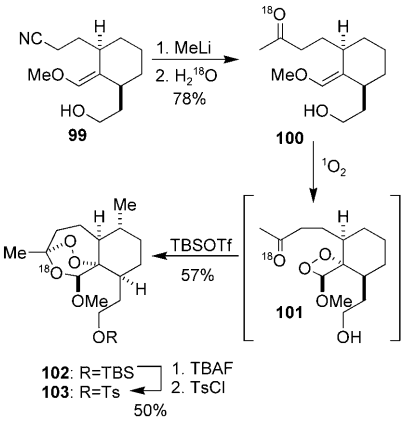
artemisinin (**98**)

Studies with semisynthetic radioactively labeled artemisinin showed that a major part of the radioactively labeled material ended up bound to hemozoin.[96] In infected red blood cells, hemoglobin is degraded and utilized as an amino acid source by the *Plasmodium* parasite. The resulting free heme is deposited as hemozoin and later excreted. This has led to the notion that heme may be toxic to the parasite. Recently, it has been shown that artemisinin (**98**) binds to PfATP6 in an iron(II)-dependant manner. PfATP6 is a parasite-specific calcium-dependent ATP phosphatase belonging to the sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) family.[97] In contrast, **98** did bind to human analogues of the SERCA enzyme or site-directed mutants of PfATP6.

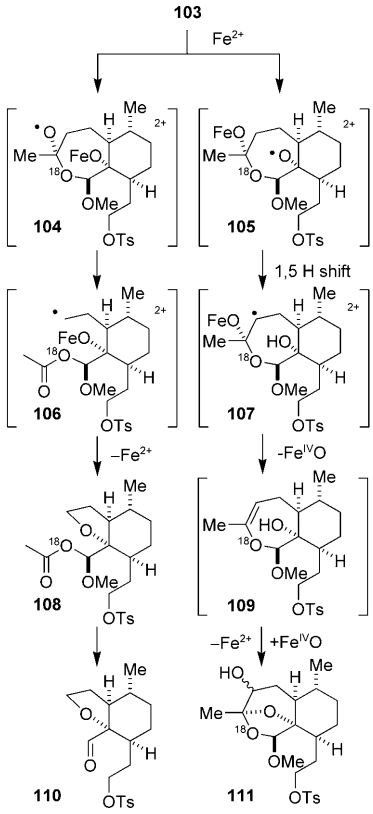
Efforts directed at identifying the reactive species responsible for the alkylating properties of artemisinin and related endoperoxides are discussed in this section. It should be noted that this subject remains highly controversial. We focus on the most widely accepted mechanism based on carbon-centered radicals as intermediates and how synthetic chemistry played a major role in establishing this sequence of events.[98]

Relying on the experience garnered from earlier total syntheses of **98** as well on their own efforts to prepare active analogues,[99] Posner and Oh prepared an active ^{18}O-labeled analogue of artemisinin as shown in Scheme 13.[100] Nitrile **99** was treated with methyllithium and the ^{18}O label was introduced by hydrolysis of the resulting imine using ^{18}O-labeled water. A key step was the introduction of the endoperoxide unit. A [2+2] cycloaddition of **100** to singlet oxygen afforded the unstable intermediate **101**. Exposure to *tert*-butyldimethylsilyl triflate resulted in a rearrangement taking place that led to the formation of the trioxabicyclo-[3.2.2]nonane motif.

Exposure of **103** to iron salts or hemin/PhSH (presumably in situ formed heme) led to the formation of a mixture of three products **108**, **110**, and **111** (Scheme 14).[100] The product ratios varied with the reaction conditions; however, the overall yield was generally 60–70%. Although the electrophilic properties of aldehyde **108** cannot be ignored, most interest focused on the putative, highly reactive carbon-centered radicals **106** and **107**. These radicals could potentially abstract hydrogen from parasite protein or serve as an alkylating agent. However, no evidence for their formation was provided in this study. Also, little attention was paid to the mechanism of the regeneration of iron(II), although this is a prerequisite to account for the observation that only catalytic amounts of iron(II) are necessary for complete reduction of artemisinin (**98**).

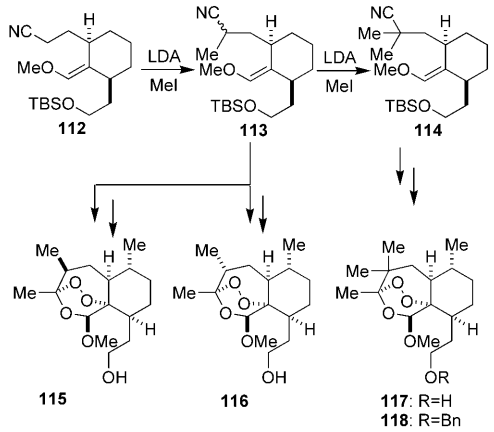


Scheme 13. Synthesis of ^{18}O-labeled analogue **103** of artemisinin (**98**).



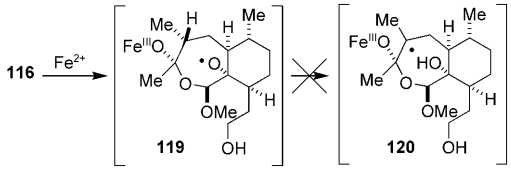
Scheme 14. Reaction of **103** with iron(II). Counterions are omitted for clarity.

Four analogues of **103** were synthesized to provide support for the 1,5-hydrogen abstraction step (**105** to **107**).[101] Although compounds **115–118** (Scheme 15) are structurally very similar to **98** and **103** (and each other), their antimalarial activities are dramatically different. Compound **115** is as active an antimalarial in vitro as artemisinin (**98**) at 4.5 ng mL^{-1} and 8 ng mL^{-1}, respectively. In contrast, none of the other methylated derivatives including **116** (the C4 epimer of **115**) show any measureable activity. An



Scheme 15. Synthesis of analogues **115**–**118**.

explanation for this observation is shown in Scheme 16. While other possibilities cannot be ruled out, the lack of activity can be speculated to arise from the inability of the putative

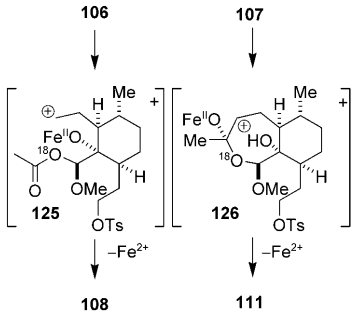


Scheme 16. Precluded intramolecular 1,5-hydride abstraction.

intermediate **119** to undergo 1,5-hydride abstraction to afford C4 carbon-centered radical **120** (compare to **105** in Scheme 14). Notably, despite being inactive, **116** underwent electron-transfer-induced degradation by the action of heme. However, this reaction led to the formation of a tetrahydrofuran product similar to **11** as the only product.

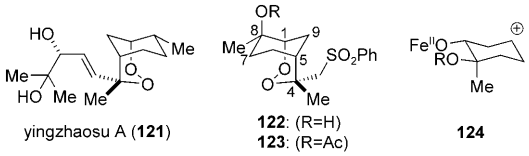
Many studies have been undertaken in the last decade to elucidate the identity of the alkylating species.[90,98] Indeed, it has been shown that products of the alkylation of heme by **98** are derived from radicals, such as **106**.[102] Other studies have addressed potential oxidative damage as a source of parasiticidal effects. Posner et al. have provided evidence in favor of the formation of a high-valent iron(IV) species,[103] as indicated in Scheme 14 (**107** to **109**). However, other researchers have contested this view and the importance of such processes remain unclear.[104]

Bachi et al. have studied the reaction of potent analogues **122** and **123**[105] of the natural endoperoxides yingzhaosu A (**121**)[106] with iron(II) salts. These studies indicated that the initially formed carbon-centered radicals are oxidized by the juxtaposed iron(III) ion formed in the activation step to afford carbocations **124**.[107] While no study has been conducted to evaluate these findings experimentally in the case of artemisinin (**98**) and its congeners, it is possible that similar processes may take place. The mechanism shown in Scheme 17 has recently been formulated on the basis of the observations of the Bachi research group.[90] This mechanism would account for the formation of the products shown in Scheme 14 and explain the catalytic role of iron(II). It is



Scheme 17. Possible involvement of carbocations **125** and **126** in the formation of products **108** and **111**.

important to note that since carbon-centered radicals are nucleophilic in nature, strongly electrophilic carbocations, if formed, are more likely to alkylate proteins.



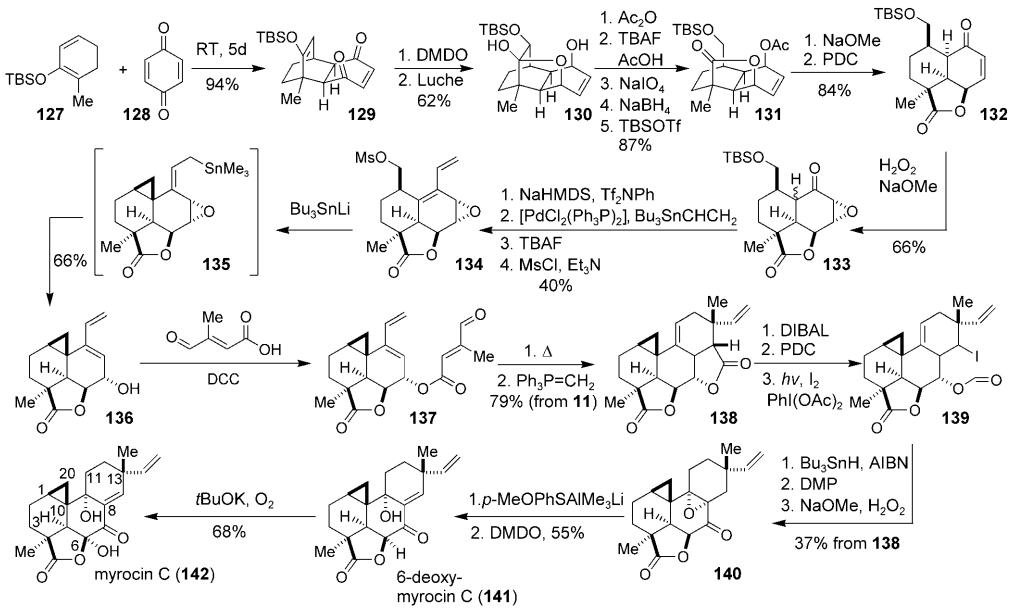
Artemisinin and artemisinin dimers also exhibit potent cytotoxicity against cancer cells. While the mechanism of this effect remains largely unresolved, there is evidence that it also depends on the endoperoxide pharmacophore.[90] Recently, it has been observed that inhibition of NO formation takes place in cancer cells upon exposure to artemisnin derivatives.[108] Thus, the biological mechanisms of endoperoxides remain an attractive avenue for further discovery.

7. Myrocin C

Myrocin C (**142**) was isolated from *Myrothecium verrucaria* and was shown to be an active anticancer agent with the ability to significantly extend the lifetime of mice.[109] This attracted the attention of Danishefsky and co-workers, who went on to develop a total synthesis of **142** (Scheme 18).[110,111]

This synthesis is a study in perseverance under difficult circumstances. After several unproductive attempts, a Diels–Alder reaction involving 1,4-benzoquinone (**128**) and electron-rich cyclohexadiene **127** was successfully applied to prepare compound **129**. This cyclohexenedione probably owes its curious stability to its boatlike conformation. Deprotonation, an event that would inevitably lead to aromatization, is retarded, since in this confirmation the orbital overlap necessary for deprotonation cannot be achieved.

A Rubottom oxidation followed by reduction under Luche conditions led to compound **130**. The isolation of **130** in its stable hemiacetal form allowed differentiation of the various alkoxy functions and facilitated the $NaIO_4$-induced oxidative cleavage of the bridge to give **131**. Compound **132** was formed after two further steps. In contrast to **129**, this



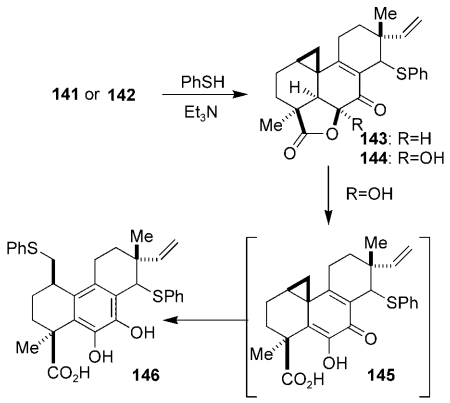
Scheme 18. Total synthesis of myrocin C (**142**).

have access to both myrocin C (**142**) and its desoxy analogue **141**.[113] Exposing **141** to thiophenol under mild basic conditions led to the formation of the conjugate addition product **143** in excellent yield (Scheme 19). In contrast, the reaction of **142** under identical conditions led to the formation of bisthiophenol adduct **146** in 63% yield. Given the isolation of stable compound **143**, the formation of **146** can be assumed to proceed via a similar intermediate **144**. However, by virtue of the C6 hydroxy group, **144** is able to undergo a cascade

compound was highly prone to aromatization. Hence, the double bond was oxidized to the epoxide. Conversion into diene **134** set the stage for formation of the cyclopropyl ring. Treating **134** with lithium trimethylstannane led to the formation of **136** presumably via intermediate **135**.[111] This remarkable reaction probably owes its success to the conformational predisposition of the system for cyclopropane formation.

The third cyclohexyl ring was formed by an intramolecular Diels–Alder reaction, and the resulting bislactone **138** was reduced to the bishemiacetal. Selective oxidation of the C5 hemiacetal was achieved with PDC. The origin of the selectivity in this reaction remains unclear.[111] The remaining acetal at C21 was cleaved oxidatively using DIAB and iodine to give **139** in 96% yield. Conversion into epoxide **140** was achieved in three steps. Deiodonation under standard conditions led to the reductive transfer of the vinyl group at C13 to C14. This side reaction presumably proceeds via an intermediate cyclopropyl species. The side reaction could be suppressed by running the reaction in neat tri-*n*-butyltin hydride. Concomitant deformylation took place during the reaction. Oxidation of the free alcohol gave the ketone. A tandem double bond isomerization/epoxidation sequence was then brought about by the joint action of sodium methoxide and hydrogen peroxide to afford compound **140**. Opening of the epoxide took place by aluminum-assisted nucleophilic attack of the thiophenol anion at the sterically hindered C8. Subsequent *syn* elimination of the sulfoxide then afforded 6-desoxymyrocin (**141**), an important compound highlighted in the discussion below. Finally, exposure of the enolate of this compound afforded myrocin C (**142**).

Inspired by their concurrent work on the mode of action of the mitomycins,[112] the Danishefsky research group had proposed that myrocin C might act as a bifunctional electrophile for the cross-alkylation of DNA strands. To investigate this, they had purposely designed their synthetic route so as to



Scheme 19. Model study on the ability of myrocin C to act as a bisalkylating agent.

of events that lead to aromatization of the central cyclohexane ring and addition of a second equivalent of thiophenol. These results indicate that the cytotoxicity of myrocin C (**142**) may be due to its ability to function as a DNA cross-linking reagent.

Myrocin C (**142**) has itself not been the subject of clinical studies; however it is plausible that progress in synthetic methods may one day make the preparation of analogues possible.[114] If so, the described studies will play a key role in their design.

8. Bryostatin

Bryostatin 1 (**147**; Figure 11) was originally isolated in 1968 from *Bugula neritina*, a marine bryozoan. Its structure, however, remained ambiguous until it was resolved in 1982 by the Clardy research group.[115] The isolation and structure of bryostatin 2 was reported the following year,[116] and to date

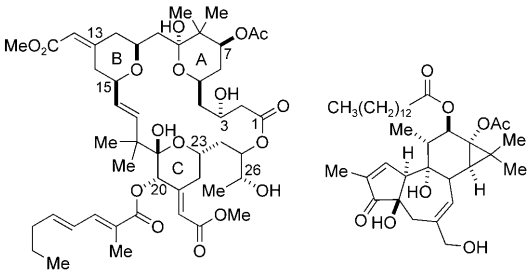


Figure 11. Structure of bryostatin 1 (**147**) and phorbol 12-myristate 13-acetate (**148**; PMA).

more than 20 members of the bryostatin family have been identified.[117] With the exception of bryostatin 16 and 17 (which have a double bond between C19 and C20), the bryostatins differ structurally by the ester substituents at C7 and C20. The low bio-availability of bryostatins has led to further studies into their origin. These studies implicate bacteria living in symbiosis with the bryozoan as the actual source of bryostatin 1.

The bryostatins inhibit the protein kinase C family of 1,2-diacyl-*sn*-glycerol (DAG) activated serine/threonine phosphorylases. The PKC family has been implicated in numerous cell-type-dependent signaling pathways, some of which are intimately involved with phorbol ester (for example, **148**) induced tumor promotion.[118] The PKC family consists of more than 14 isozymes, which are divided into the conventional (cPKC), novel (nPKC), and atypical classes (aPKC). The conventional PKC isozymes (α, βI, βII, and γ) require Ca^{2+} ions for activation, while the so-called novel PKCs (δ, ε, η, and θ) are calcium independent. Notably, the activators and indeed the substrate bind to different domains within the PKC structure. For atypical PKCs (ζ and -λ/ι) the DAG binding site is absent and they do not bind bryostatin (**147**) or phorbol esters (for example, **148**). Since activation of different PKC isozymes has been invoked in a number of diseases, including cancer, heart, diabetes, and Alzheimer's disease, the discovery and development of selective PKC inhibitors is of great importance.[119] Upon binding, bryostatin 1 (**147**) activates PKC, but only induces a limited number of the effects compared to phorbol.[120]

Despite their immensely difficult isolation from natural sources, these properties make the bryostatins attractive leads for developing treatments for human disease. This spurred the development of total syntheses of bryostatins 2,[121] 3,[122] 7,[123] and 16[124] as well as numerous synthetic approaches.[125,126] These monumental efforts have, however, not solved the limited supply problem. To meet these challenges, Wender initialized a research program that has spanned 20 years and led his research group and others to develop numerous simplified analogues with potent PKC binding properties and selectivity.

In collaboration between the Wender, Pettit, and Blumberg research groups, a model was developed that compared the structures of DAG, PMA, and bryostatins.[127] An important finding was that the bryostatins bound with nanomolar affinities to a mix of PKCs. For example, **147** binds with a K_i value of 1.35 nM (Figure 11). Computer-assisted conformational analysis based on the crystal structures of structures of bryostatin 1 (**147**), PMA (**148**), and DAG led to the realization that certain heteroatoms in all three compounds would overlap on binding to PKC (Figure 12).[127] Further refinements of this model a decade later[120,128] led to the hypothesis that the bryostatin structure could be divided into a binding domain (C15–C27), which would include the crucial heteroatoms, and a spacer domain (C1–C14), whose purpose was to constrict the binding domain into the active conformation. With this hypothesis in mind it would prove possible to design analogues (termed bryologues) that would bind to PKC, but in contrast to the bryostatins themselves, would be accessible in large quantities through a relatively low number of synthetic steps.

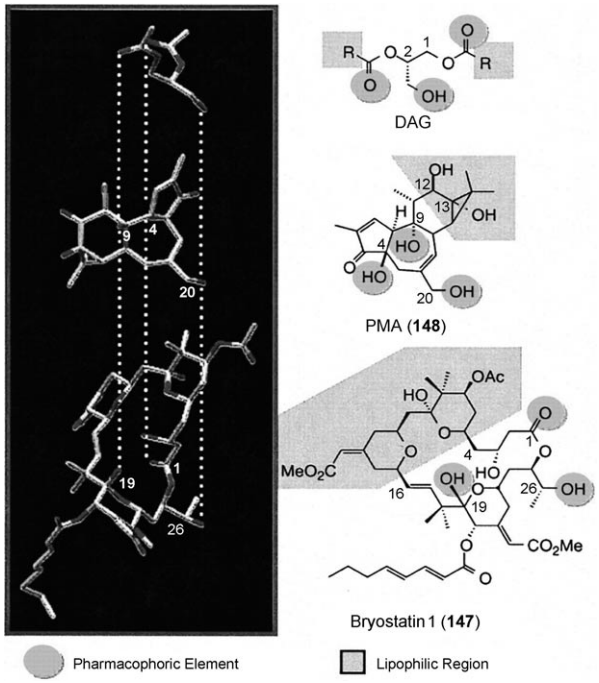
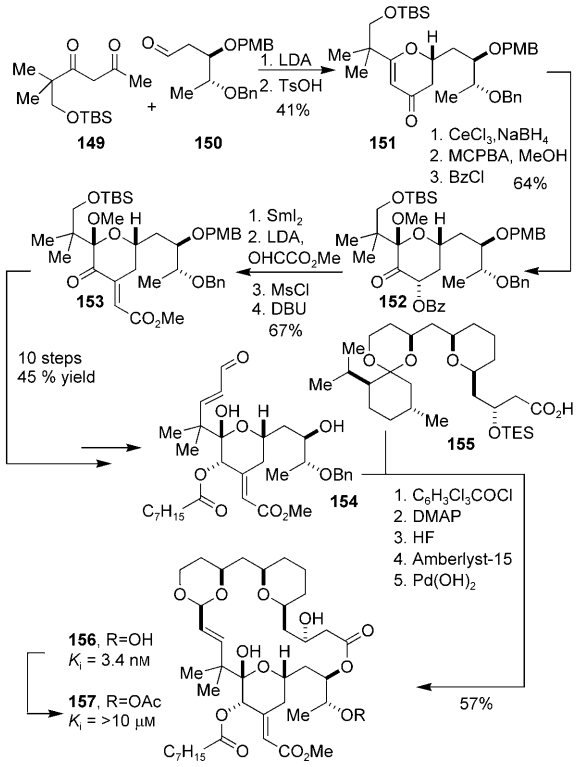


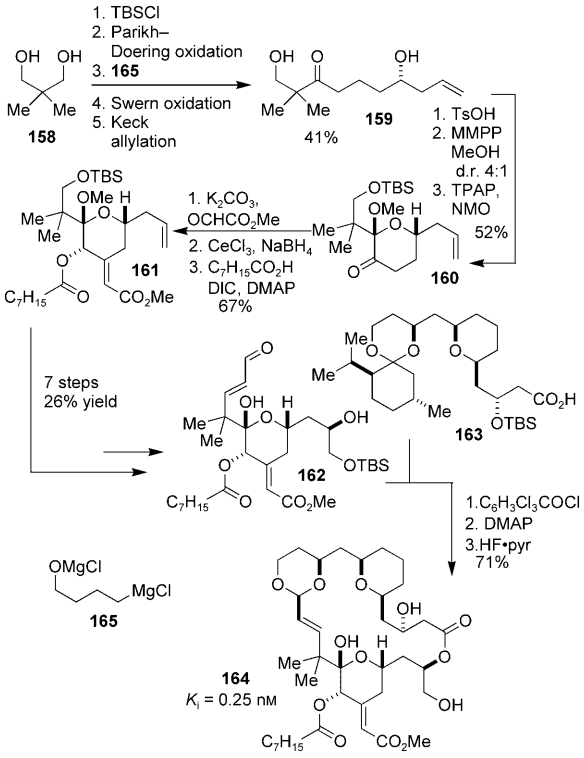
Figure 12. Comparison of the structures of bryostatin 1 (**147**), PMA (**148**), and DAG. Reprinted from Ref. [120].

Proof of concept was achieved with the synthesis of a series of compounds with low nanomolar binding affinities for PKC.[120,129] This generation of analogues had much simplified A and B rings. The synthetic strategy towards these compounds is exemplified by the synthesis of bryologue **156**, the most active of this first generation of bryologues (Scheme 20).[129]

The key bryostatin binding element in the form of aldehyde **154** was synthesized in a 19 step sequence. Construction of aldehyde **154** began by addition of the dianion of **149** to aldehyde **150**. The 1:1 mixture of the resulting diastereomeric hydroxy ketones was cyclized and dehydrated under acidic conditions, which resulted in the formation of **151** in 41% yield along with its C23 epimer in similar yield. Reduction under Luche conditions set the stage



Scheme 20. First generation total synthesis of bryologues **156** and **157** according to Wenders et al.



Scheme 21. Total synthesis of the second generation bryologue **164** according to Wenders et al.

for a directed epoxidation. The epoxide opened up in situ to the corresponding methyl hemiacetal. The resulting alcohol was protected as the benzoyl ester in preparation for a samarium(II) iodide induced deoxygenation. Reaction of the resulting ketone with glyoxalic acid methyl ester gave a hydroxy ester that was dehydrated in a two-step sequence. A number of seemingly trivial steps then led to compound **154**. The secondary alcohol was esterified with acid **155** according to the Yamaguchi protocol.

Macrocyclization was then achieved by treating the seco compound with Amberlyst-15. This reaction was a cornerstone of the synthetic strategy since it allowed an efficient and uncomplicated late-stage macrocyclization. Accordingly, this step was conspicuous in the synthesis of a majority of the bryologues prepared by the Wender group.

The benzyl ether protecting group on the secondary alcohol at C26 was cleaved to afford bryologue **156**, which showed impressive binding affinity to PKC. The importance of the hydrogen bonding between PKC and the C26-hydroxy group was highlighted by selective acetylation. The resulting bryologue **157** was essentially devoid of activity. Notably, the structural simplifications of the bryologues compared to the bryostatins allow the synthesis to be achieved in less than 40 total steps.

If the secondary alcohol at C26 corresponds to the primary alcohol at C20 of phorbol (Figure 12), a reasonable expectation would be that excising the C27 methyl group could afford more active compounds. This conjecture found support through the synthesis and study of bryologue **164** (Scheme 21).[130] Bryologue **164** was indeed more active than both **156** and bryostatin 1 (**147**). A number of improvements were evident in this route. For example, the attachment and dehydration of glyoxalic acid methyl ester was achieved in a single operation by reaction with **160** in the presence of K_2CO_3.

The ready availability of synthetic bryologues **164** facilitated studies on their binding to PKC isozymes. For example, they were shown to bind in the cysteine-rich domain of PKC isozymes and promote PKC translocation.[131,132] In addition, they were shown to activate the RasGRP1 pathway.[133]

With economy of steps always a prime concern for synthesis design in the Wender research group,[118] more efficient syntheses of the spacer and binding domain were developed to facilitate the synthesis of larger quantities of brylogues as well as second-generation analogues.[134] Recent studies have examined the significance of the A ring[135] and B ring[136] as well as the C20[137] and C7 side chains.[138]

Inspired by these efforts, other research groups have recently joined the the area.[139] Trost et al. have prepared an interesting ring-expanded analogue **166** through a strategy involving a metathesis macrocyclization.[140] This compound showed potent cytotoxicity against the NCI ADR cancer cells (Figure 13). Interestingly, the C16–C17 *cis* isomer also obtained in the key ring-closing metathesis step was ninefold less active. Subsequent work by Trost and Dong led to a total synthesis of bryostain 16.[124]

Recently, Keck et al. developed a Prins-type macrocyclization strategy towards simplified bryostatin congeners.[141] The application of this strategy to synthesize biologically active bryologues was recently disclosed by the Wender and

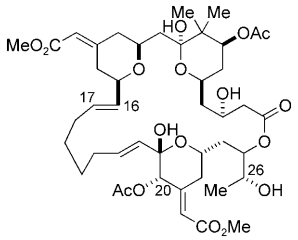


Figure 13. Bryologue **166** prepared by Trost et al.

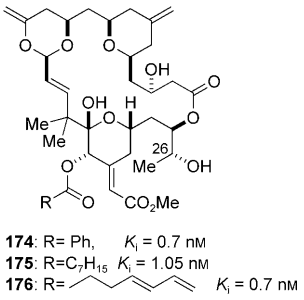
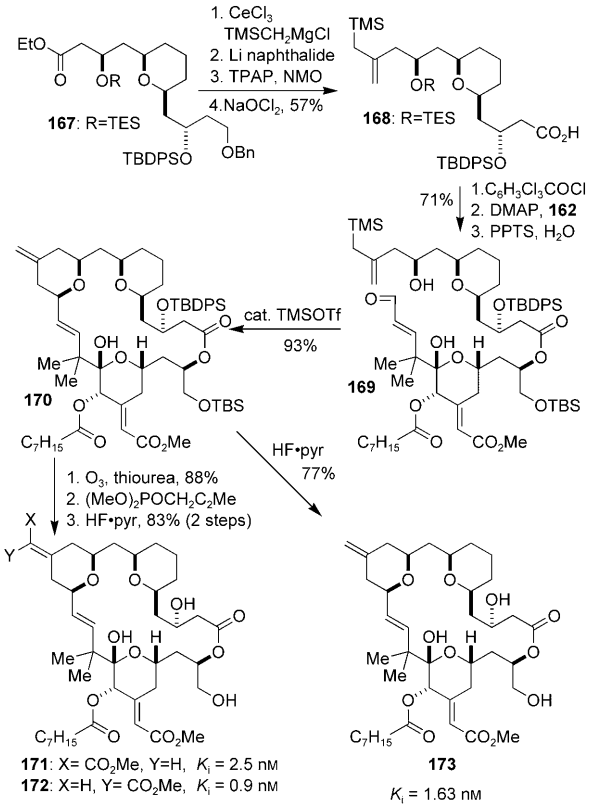


Figure 14. Bryologues **174–176** created by Keck et al.

Keck research groups (Scheme 22, Figure 14).[142,143] In the study by Wender et al., the ester functionality of **167** was converted into an allylsilane according to Bunnel's protocol

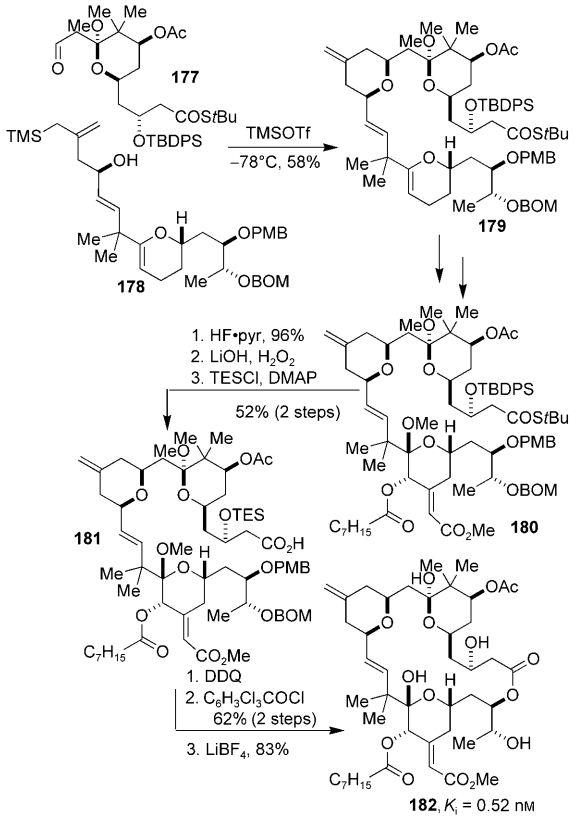


Scheme 22. Total synthesis of third generation bryologues **171–173** according to Wenders et al.

($CeCl_3$, 2 equiv $TMSCH_2MgCl$).[142] Further elaboration into carboxylic acid **168** was followed by coupling to alcohol **162** under Yamaguchi conditions. Macrocyclization and formation of the B ring was then achieved by a TMS triflate induced oxo-Prins reaction of the aldehyde and allyl silane groups. Three different bryologues were prepared from the product **170**. All of these compounds were more active than bryologue **164** (which had a K_i value of 3.1 nM in the same assay).

This study was published back-to-back with a report by the Keck research group[143] in which the preparation of bryologues **174–176** (Figure 14), according to a similar strategy to that shown in Scheme 22, was disclosed. These bryologues all bind to PKC with low nanomolar binding affinities. However, their effect in cell proliferation and attachment assays showed them to be more akin to PMA (**148**) than to bryostatin 1 (**147**).

Strikingly, the Keck group has recently shown that while not essential for the strength of PKC binding, retaining the A ring of bryostatin 1 (**147**) is key to the different biological responses of cells on exposure to PMA (**148**) and bryostatin 1 (**147**).[144] To this end, they assembled bryologue **182** which differed only in the lack of the C13 carboxymethyl group from brytostatin 1 (**147**; Scheme 23). The synthesis started from known compounds **177** and **178**. In a remarkable reversal of their earlier strategy, the two fragments were coupled by an intermolecular TMS triflate induced oxo-Prins reaction,



Scheme 23. Synthesis of decarboxymethylbryostatin 1 (**182**) according to Keck et al.

which proceeded in 58% yield and excellent stereoselectivity. Subsequent functional-group transformations led to **180**. This sequence relied in part on the findings of Wender et al., such as the one-step introduction of the C21 methylene carboxymethyl group (Scheme 20) as well as the setting of the C20 stereogenic center (Scheme 21). The TBDPS group was removed and then the C1 *tert*-butyl thioester was cleaved by exposure to lithium peroxide. The C3 alcohol was reprotected as a TES ether to afford **181**. The C25 alcohol was deprotected with DDQ and the crucial macrocyclization was effected using the Yamaguchi reagent, and global deprotection achieved using lithium tetrafluoroborate. Bryologue **182** had a profile very similar to bryostatin 1 (**147**) in U937 cell attachment and proliferation assays. Further investigations have revealed that antagonism appears to depend on either both or one of the C9 hemiacetal group and the C8 *gem*-dimethyl of the A ring.[145]

Spanning more than 20 years, the efforts to develop synthetically accessible bryologues with superior PKC binding illustrates the application of chemical biology to the design and exploration of molecular properties. The development of multiple synthetic strategies towards the bryologues shown in Schemes 20–23, illustrates the progress in synthetic methodology and mechanistic understanding of bryostatin/PKC binding over the last decade.

9. Vancomycin

Vancomycin (**183**) belongs to the family of glycopeptide antibiotics and was discovered in 1952 from a Borneoan soil sample by Kornfield at the Eli Lilly company.[146,147] Its potent antibacterial properties led it to being approved for clinical use in 1958. However, a number of toxic side effects led to its use being restricted to treat infections from penicillin-resistant bacteria, notably *Staphylococcus aureus*.

The structure was unknown at the time of approval because of its structural complexity and the limitations of analytical methods of the day. Indeed, the first significant advance towards elucidating its structure came only in 1978 with the X-ray structure of CDP-I, a degradation product[148] of vancomycin.[149] Further structural revisions based on NOE NMR data[150,151] and careful amino acid analysis were needed to arrive at the correct structure.[151] Final confirmation was achieved by X-ray crystallography only in 1996.[152]

Seminal studies by Perkin and co-workers showed that vancomycin binds with a high affinity to terminal D-alanyl-D-alanine dipeptide residues found at the cross-linking precursor site of peptidoglycan (Figure 15).[153] These studies were carried out by simply adding various dipeptides to vancomycin–bacteria suspensions. Remarkably, addition of **184** led to the complete inhibition of the bactericidal effect of vancomycin.[153] It was suggested that **183** binds to the D-Ala-D-Ala residue through hydrogen bonding,[154] and solution-phase NMR studies confirmed this model (Figure 16).[155] Additionally, the chlorine atoms of vancomycin (**183**) were shown to be important for achieving conformational rigidity.[156]

It is believed that vancomycin, by binding to the D-Ala-D-Ala residue, inhibits cross-linking, an imperative step in cell-wall synthesis (Figure 15).[146] The combination of its restricted use and inherent properties has meant that the emergence of resistance towards vancomycin (**183**) has been slow, and only recently have a significant number of clinical cases been reported. In anticipation of this event, Walsh and co-workers initiated studies on vancomycin resistance. They were able to show that resistant bacteria strains synthesize peptidoglucan chains with the terminal D-alanine residue substituted by a D-lactate, as in **186**.[157]

It has been shown that while the aminosugar moiety is not crucial for vancomycin's antibacterial activity, it is important in restricting the conformation of vancomycin and is important for its tendency to homodimerize noncovalently.[158] Glycopeptide antibiotics that are able to dimerize have a

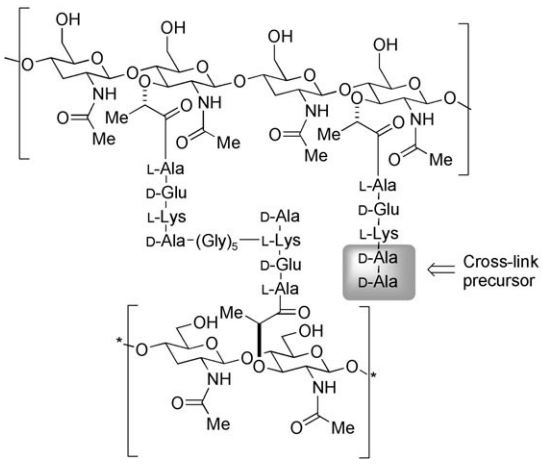


Figure 15. Structure of the cross-linked peptidoglycan polymer of *S. aureus*. The site of the cross-link is shown in green.

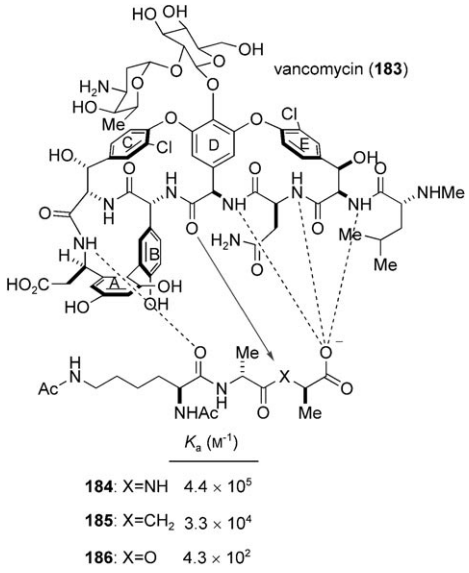


Figure 16. Structure of vancomycin (**183**), a binding model for the Ac_2-L-Lys-D-Ala-D-Ala residue (**184**), methylene analogue **185**, and the Ac_2-L-Lys-D-Ala-D-Lac residue (**186**). The dotted arrows show intermolecular hydrogen bonding. The solid arrow shows the repulsion case of compound **186**.

higher activity against vancomycin-resistant bacteria strains, and a large number of covalently linked vancomycin dimers have been developed and screened for improved antibacterial activity.[159] Dimeric vancomycin is believed to be able to overcome resistance through cooperative effects (see below).[160]

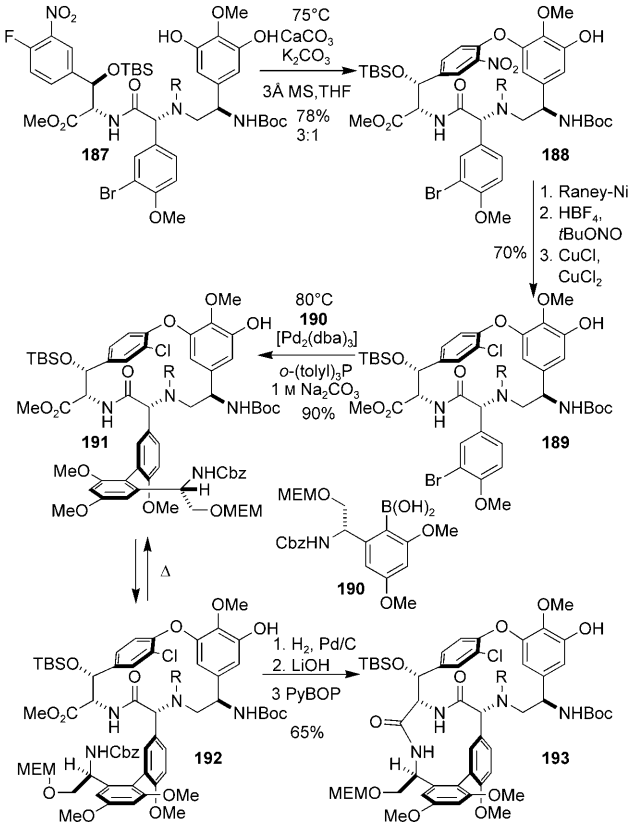
The intriguing properties and monumental challenge of assembling vancomycin has inspired several syntheses of the aglycone,[161,162] as well as a single total synthesis of the complete natural product.[163] Boger et al. has also disclosed the syntheses of the aglycones of teicoplanin and ristocetin, related natural products.[164]

Emulating the earlier work by Perkins, Boger and coworkers prepared and tested tripeptides **184–186** and measured their affinity for vancomycin (**183**).[165] Lactate residue **186** binds 1000-fold weaker to vancomycin than parent D-Ala peptide **184**. By studying the interaction of methylene analogue **185** with vancomycin **183**, these researchers attempted to quantify the loss of affinity on going from the attractive NH-carbonyl hydrogen-bond interaction in **184** to the repulsive interaction between the carbonyl and ester lone pairs of electrons in **185** (Figure 16). These studies indicated that the repulsion between the lone pairs of electrons was responsible for a major part of the loss of affinity.

Based on these findings and their experience in the synthetic realm of vancomycin chemistry, Crowley and Boger designed and prepared fully synthetic [Ψ[CH_2NH]Tpg^4] vancomycin aglycon **197** (Schemes 24 and 25).[166] This compound was designed to eliminate the repulsive interaction between lone pairs of electrons on the vancomycin carbonyl group and the terminal lactate residue. It was hoped that by replacing the carbonyl group in residue 4 with a methylene group, activity against vancomycin-resistant species would be restored, while activity against ordinary bacteria would be retained. Importantly, it proved impossible to prepare **197** by semisynthesis.[166]

A major problem in the synthesis of vancomycin is the assembly of the macrocyclic AB, DE, and CD biaryl systems. Atropisomeric mixtures usually ensue, which require either separation or equilibration. The Boger research group had pioneered an approach that relied on the thermal equilibration of atropisomers to shuttle all the material towards the desired product. Thus, a key consideration in the planning of the synthesis of vancomycin (**183**) was the order of forming the macrocyclic rings. Specifically, it had been necessary first to form the biaryl system with the highest activation barrier for equilibration. Computational work and model studies had determined the following order for equilibration: E_a(DE ring system) = 18.7 kcal mol^{-1}) < E_a(AB biaryl precursor) = 25.1 kcal mol^{-1}) < E_a(CD ring system) = 30.4 kcal mol^{-1}).[162,167] A similar strategy was, therefore, invoked for the synthesis of **197**.[166]

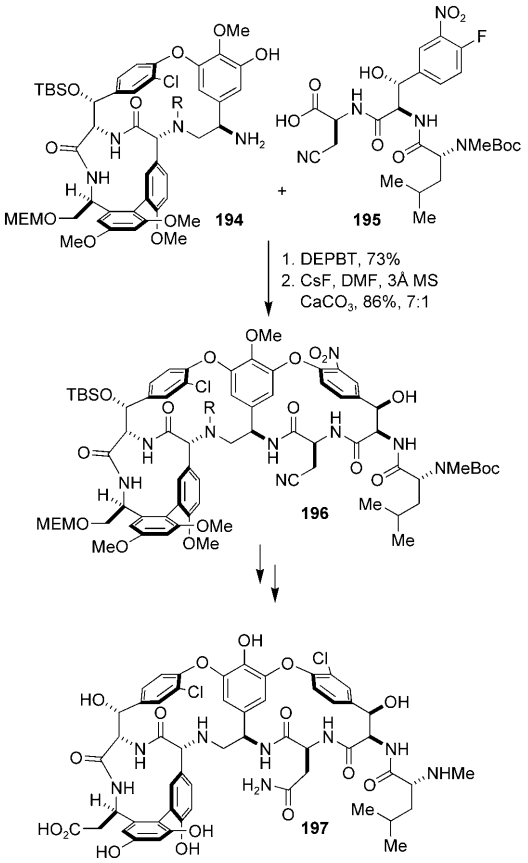
The synthesis began with the preparation of **187**, a compound analogous to an intermediate in the vancomycin synthesis but for the amide group of residue 4, which had been replaced by a protected amine.[166] Cyclization by aromatic substitution afforded the CD biaryl system, with the desired atropisomer **188** as the major product in a selectivity of 2.5:1–3:1. Reduction of the nitro group and Sandmeyer chlorination afforded **189**. Curiously, it proved impossible to equilibrate the CD atropisomers of both **188** and **189**. This is apparently due to the steric demand imposed by the methylcarbamate group. Palladium-catalyzed coupling of **190**, an intermediate from the vancomycin aglycone synthesis, with **189** afforded the AB biaryl system as a 1:1.3 mixture. In this case, equilibration proved possible, thus ensuring the conservation of material. The remaining steps to **193**, including macrolactamization, proceeded uneventfully (Scheme 24).



Scheme 24. Assembly of the ABCD system. R = COOMe.

The coupling of **194** with **195**, another intermediate from the vancomycin synthesis, brought together all the heavy atoms found in the vancomycin aglycone backbone. Formation of the DE ether afforded the desired *P* atropisomer in excellent yield (86%) and selectivity (7:1). The final steps to [Ψ[CH_2NH]Tpg^4] vancomycin aglycone **197** included substitution of the nitro group for a chloride atom, adjustment of oxidation stages, and global deprotection. All these transformations proceeded in good yield (Scheme 25).

The binding affinity of the [Ψ[CH_2NH]Tpg^4] vancomycin aglycone was compared with that of vancomycin aglycone and vancomycin itself. The compounds were tested for binding to bis-acetyl-L-Lys-D-Ala-D-Ala (**184**) as well as bis-acetyl-L-Lys-D-Ala-D-Lac (**186**). Remarkably, **197** binds to **184** only an order of magnitude less strongly than vancomycin (**183**) or its aglycone. This difference presumably reflects the actual loss of the stabilizing hydrogen bond formed between native



Scheme 25. Completion of the synthesis of **197**. R = COOMe.

vancomycin (**183**) and the NH group of the terminal alanine of **184**. Even more impressively, **197** binds more strongly to **186** by more than two orders of magnitude than the parent vancomycin congeners.

When **197** was tested against a vancomycin-resistant (Van A) strain of *Enteroccocus faecalis*, [Ψ[CH_2NH]Tpg^4] vancomycin aglycone **197** was able to inhibit growth with a MIC value of 31 μg mL^{-1}. In contrast, vancomycin (**183**) and its aglycone were only able to inhibit growth at mg mL^{-1} concentrations.

This study elegantly reconfirmed the vancomycin binding model and served to show that vancomycin resistance can be overcome with appropriately designed structural modifications to the vancomycin backbone. While, sadly, the complexity of vancomycin still precludes the large-scale synthesis of analogues, it is conceivable that analogues similar to the [Ψ[CH_2NH]Tpg^4] vancomycin aglycone **197** may be prepared by genetic engineering of the vancomycin-producing organism. With the specter of vancomycin resistance becoming a clinical reality, the development of new antibiotics remains a challenge for science. The challenge is being met with semisynthetic vancomycin derivatives and structurally related glycopeptide natural products. Compounds currently in advanced clinical trials or that have already received approval include oritavancin,[168] telavancin,[169] and dalbavancin.[170] These compounds all have a high dimerization affinity and are capable of binding to the cell membrane through lipophilic (aliphatic or biphenyl) substituents appended at the nitrogen atom of the aminosugar. However, these compounds may in fact work through a recently discovered alternative mechanism of action[171] that relies on the aminosugar substituents as a pharmacophore and involves direct inhibition of transglycosylase, an enzyme in the peptidoglycan biosynthesis.[172] Indeed, it has been argued that homodimerization and membrane binding is too insignificant a factor to account for their activity against vancomycin-resistant baceteria strains.[173]

10. Butylcycloheptylprodigiosin

The prodigiosin alkaloids constitute a class of numerous naturally occurring alkaloids characterized by the presence of three linked pyrrole subunits in their backbone. Their biological portfolio includes impressive immunosuppressant properties at low concentrations in addition to anticancer properties at higher concentrations.[174] Importantly, they work in a synergistic manner with immunosuppressants FK506, rapamycin, and cyclosporin. As one would surmise from the presence of three closely linked pyrrole fragments in their molecular backbone, the prodigiosins are excellent ligands for metal ions such as copper(II). The anticancer properties of the prodigiosins has been proposed to stem from their ability to undergo oxidation by copper(II) to form a radical cation and ultimately to cleave DNA. This damage could conceivably follow a pathway such as that shown in Scheme 1. Importantly, copper(II) is found in higher concentrations in the nuclei of cancerous cells than in those of healthy cells. However, other properties, including their ability to act as membrane proton shuttles, may also be of significance.[174]

The ackwardly named butylcycloheptylprodigiosin (**199**; Figure 17) was until recently mainly noted for the controversy regarding its existence, having on the one hand been isolated from two different Streptomyces species and described by Gerber and Stahly[175] and Floss and co-workers[176] as having the structure **199**, and on the other hand alleged to actually be streptorubin (**198**) by Weyland and co-workers.[177] The claim by Weyland and co-workers was, however, based on the isolation of prodigions from a different Streptomyces strain. Fürstner et al. successfully settled the dispute by preparing **199**.[178] A series of analogues **200**–**203** were also accessed by diverted total synthesis.[179]

The synthetic design was based on the preparation of the versatile building block **211** (Scheme 26). This compound could be converted into both the natural product **199** as well as its analogues **200**–**203** through Suzuki coupling with the appropriate aryl boron reagent. The synthesis commenced from the known compound **204**, itself made on a gram scale from readily available cyclooctanone by a classical ring expansion/bromination/elimination sequence. Selective 1,2-reduction using DIBAL led to the allylic alcohol, which was converted into its acetate ester. Next a Tsuji–Trost allylation of methyl acetoacetate was carried out. Simply heating the ketoester to 180 °C in DMSO led to decarboxylation and afforded methylketone **205** in excellent yield. The ketone was first converted into the oxime and then acylated with

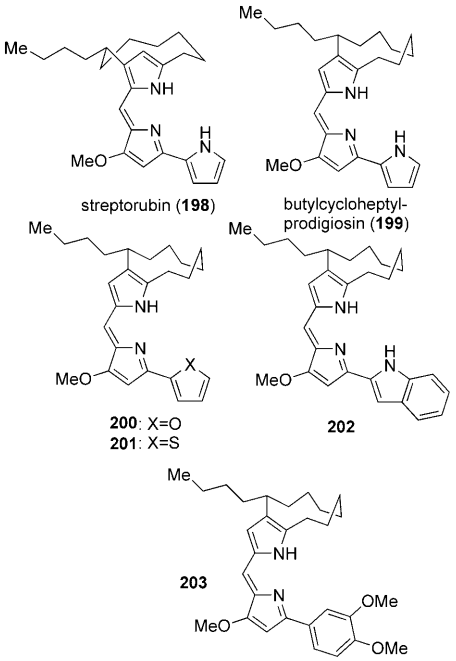
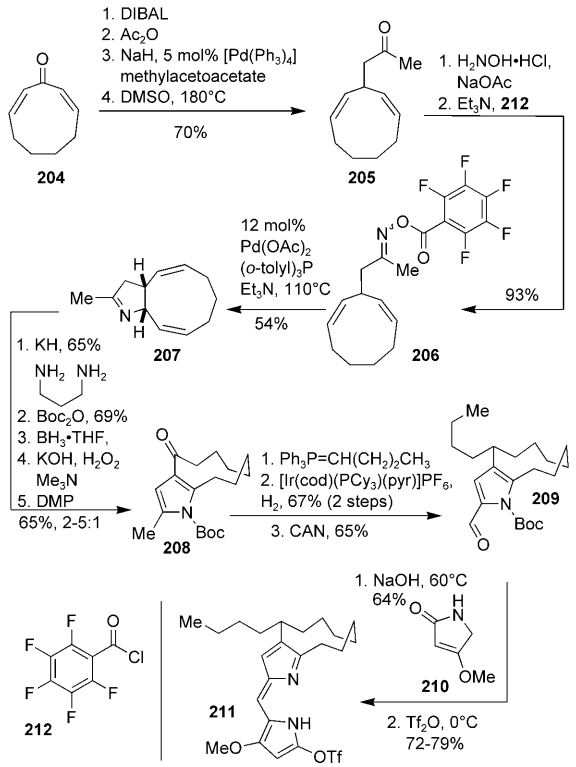


Figure 17. Structure of naturally occurring streptorubin (**198**), butylcycloheptylprodiogiosin (**199**), and designed analogues **200**–**203.**



Scheme 26. Synthesis of the key intermediate **211**.

pentafluorobenzoyl chloride in preparation for a Narasaka–Heck cyclization.[180] This key step was carried out using substoichiometric amounts of palladium(II) acetate and tris-*o*-toluoylphosphine as the ligand and afforded the bridged bicyclic **207** in 54% yield.[178,179]

Next, the first pyrrolidine ring was converted into a pyrrole by base-induced isomerization of the double bonds. Interestingly, this afforded a single product, presumably reflecting the thermodynamic stability of the compound. The product proved prone to decomposition, and hence it was immediately protected, and the double bond conjugated to the pyrrole converted into ketone **208** by using a hydroboration/oxidation sequence. The ketone was subjected to olefination to introduce the C_4 side chain. A subsequent hydrogenation took place primarily from the exocyclic face to furnish the core structure as a single diastereoisomer in 67% yield over two steps. An oxidation with CAN afforded aldehyde **209** which was condensed with unsaturated lactam **210**. Finally, triflation of the lactam led to key intermediate **211** in a process that subtly rearranged the extended conjugated π system. This compound was subsequently converted into the racemic natural product **199** in good yield by Suzuki coupling with the appropriate boron reagent. Detailed NMR spectroscopic studies clearly established that **199** is identical to an authentic sample of the material of Floss and co-workers and different from streptorubin (**198**).[178] With an access path to butylcycloheptylprodigiosin effectively paved, Fürstner et al. synthesized congeners **200**–**203**.[179] Examination of **199** and its analogues showed that the presence of the terminal pyrrole ring is indispensable for Cu^{II}-dependent DNA strand cleaving properties. Bacteriophage plasmid DNA suffered extensive single-strand cleavage in the presence of **199** and copper(II) acetate. Under identical conditions, the isoelectronic non-pyrrole analogues **200**–**203** showed only trace amounts of single-strand DNA cleavage.[178,179]

The prodiogiosin story underscores the enduring power of chemical synthesis for the unambiguous determination of the structure of a natural product which is otherwise in dispute. In addition, this work allowed molecular editing of the structure of butylcycloheptylprodigiosin (**199**), thereby providing the first studies aimed at understanding the mode of action of this potent cytotoxic agent at the molecular level.

11. Largazole

The discovery by Luesch and co-workers of the potent and selective cytotoxic agent largazole (**213**)[181] spurred a bout of activity amongst synthetic chemists in early 2008 (Figure 18), and within a few months multiple total syntheses were reported.[182–188] The rush was partly brought on by the large difference in growth inhibition activity exhibited by largazole against cancer cells over normal cells (about an order of magnitude).[181] As part of their synthetic work, the Luesch research group identified histone-deacetylase (HDAC) as the biological target of largazole.[182]

Histone deacetylase (HDAC) belongs to a family of zinc-containing enzymes that, among other roles, catalyze the deacetylation of chromatine. The reverse process is catalyzed by histone acetylases (HAT). In vivo, HDACs form part of a multifunctional protein complex that plays an important role in the regulation of gene expression. HDAC is overexpressed in many cancer cell types.[189] It has recently been shown that

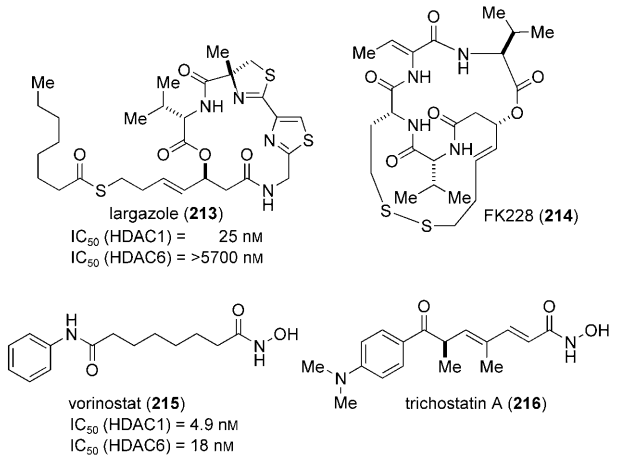


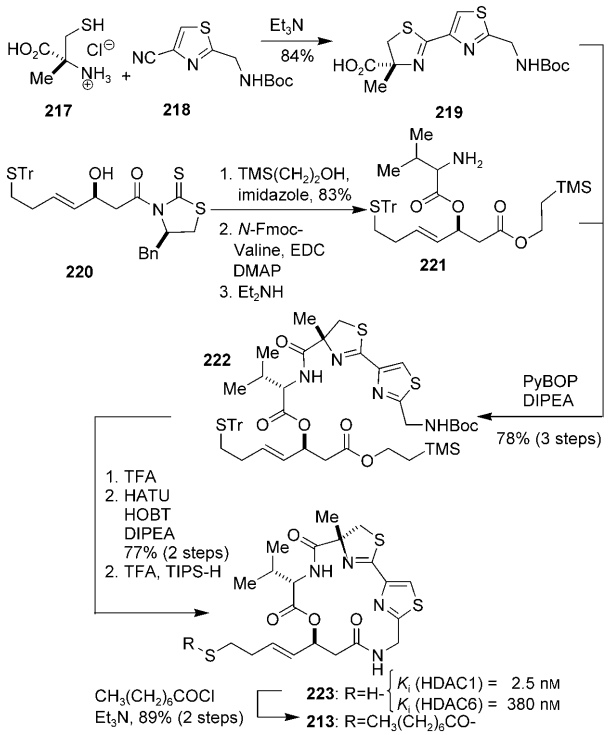
Figure 18. Structure of largazole, structurally related compound FK228, and classic HDAC inhibitors vorinostat and trichostatin.

HDAC inhibition leads to strong inhibition of the growth of tumors.[190] Vorinostat (**215**), a HDAC inhibitor, has received FDA approval for clinical treatment of various forms of lymphoma. The basis for growth inhibition remains unclear, but is likely due to epigenetic pathways.[191]

A complicating feature of HDAC inhibition is that a multitude of HDAC isozymes exist. Thus far, 18 isozymes have been identified. They are classified into four subclasses, each of which differs in their function and localization within the cell. Inhibition of the type I class of HDAC (which includes HDAC1, HDAC2, HDAC3, and HDAC8) has been linked to the anticancer effects described above.[191] In contrast, inhibition of the other three HDAC classes has been postulated to lead to the toxic side effects such as fatigue and thrombocytopenia exhibited by, for example, the FDA-approved drug vorinostat (**215**) or the natural product trichostatin A (**216**).[192]

Largazole was shown by Luesch and co-workers to be both an exceedingly potent HDAC inhibitor as well as to be highly selective for the HDAC class I of isozymes (Figure 18).[182] Several of the cited publications correctly, independently, and in parallel identified the largazole thioester as an in situ hydrolyzable function that would generate the active thiol form of largazole.[182–184]

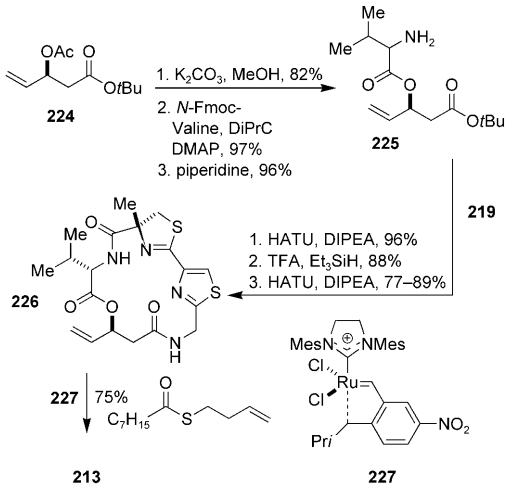
The synthesis by Schreiber, Williams, and co-workers, shown in Scheme 27 is representative of many of the published synthetic efforts. A key factor in their study as well as that of Luesch and co-workers was the similarities between FK228 (**214**),[193] a known HDAC inhibitor, and largazole (**213**; Figure 18). Specifically, they realized that the (*S*)-3-hydroxy-7-mercaptohept-4-enoic acid moiety was present in both compounds, which led to the conjecture that the two compounds may share the same target and biological mechanism. The synthesis by Williams and co-workers commenced from α-methylcysteine, which was condensed with **218** to afford thiazole-dihydrothiazole **219** (Scheme 27). The Crimmins aldol product **220** was converted into **221** in three steps and then coupled with **219** using PyBOP. The trimethylsilylethyl ester and the Boc group were removed under acidic conditions and the free amine and carboxylic



Scheme 27. Synthesis of largazole (**213**) and its free thiol **223** by Schreiber, Williams, and co-workers.[183]

acid were then coupled. The trityl group was removed to afford the free thiol. This compound was tested for HDAC inhibition and was shown to be more potent than largazole itself, but also less selective for HDAC1 (a class I HDAC) relative to HDAC6 (a class II HDAC). Similar results were reported by the Luesch[182] and Cramer[184] research groups. Ye and co-workers independently developed a route to largazole (**213**) that was akin to that shown in Scheme 27.[188]

Several syntheses (including those of Luesch,[182] Cramer,[184] Phillips,[186] and later efforts by the Schreiber/Williams team[194]) relied on the assembly of the macrocyclic ring bearing a terminal methylene group. The methylene group served as a reactive locus for appending various side chains, thereby leading to access to an array of analogues through diverted total synthesis. The synthesis by Cramer and co-workers started from **224**, a compound accessible by enzymatic resolution of the corresponding racemate (Scheme 28). The valine side chain was attached using DiPrC and the Fmoc protecting group was removed by the action of piperidine. Compound **219** was coupled to **225**, the Boc group removed, and macrocyclization induced using HATU to afford key intermediate **226**. The side chain of largazole was then attached by cross-metathesis using ruthenium catalyst **227**. Similar strategies where developed by the research groups of Phillips[186] and Luesch.[181] Gosh and Kulkarni also used **224** as a starting material and employed a cross-metathesis reaction, but in their case the side chain was appended before macrocyclization.[185] Compound **226** served as a common intermediate for the diverted total synthesis of an array of analogues with different side chains. As shown by the research groups of Luesch, Phillips, and Cramer, complete



Scheme 28. Synthesis of largazole (**213**) according to Cramer and co-workers.

loss of selectivity is observed in the absence of a thiol group at the crucial locus.

Although the pharmacophore of largazole (and FK228) is clearly the C4 thiol, HDAC selectivity appears to be a function of the macrocyclic ring. the research groups of Cramer[184] and Luesch[195] disclosed the synthesis of a series of analogues with modification in the length of the linker between the thiol and the alkene groups. These data showed that two methylene groups found in largazole (**213**) is indeed the optimal chain length (see Figure 19a for the results of Luesch and co-workers). Any deviation led to significant loss of HDAC inhibition. Luesch and co-workers also showed that the valine can be replaced by an alanine without any great loss in activity or selectivity. Schreiber, Williams, and co-workers have reported that substituting the oxygen atom of the (*S*)-3-hydroxy-7-mercaptohept-4-enoic acid moiety for an NH group leads to significant loss of activity (one order of magnitude).[196] However, the free thiol still retains significant activity. In addition, they have shown that substitution of methylcysteine for cysteine leads to no loss of selectivity.[194] Importantly, substitution of the thiazole ring for a pyridine ring leads to a compound which is four times more active than largazole, but is still highly selective (Figure 19b).[194]

Largazole (**213**) constitutes an important lead in the search for new and selective anticancer agents. While largazole itself would probably be a poor drug candidate, because of its hydrolytic instability and absorption, significant efforts are underway to solve these issues. In the short time since its discovery, pioneering studies by research groups around the globe have contributed towards a better understanding of the structural features that are important for its activity.

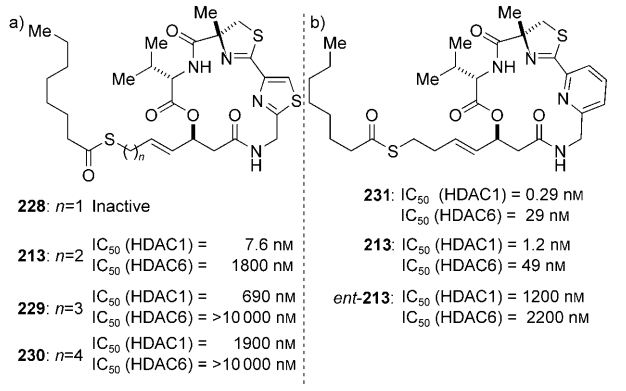
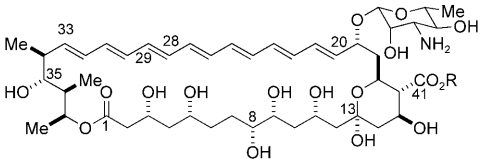


Figure 19. IC_{50} values for selected analogues of laragzole (**213**) as well as its enantiomer. a) Luesch and co-workers.[195] b) Williams, Schreiber, and co-workers.[194]

12. Amphotericin B

Amphotericin B (AmB, **232**) is an antifungal agent first isolated five decades ago from a Venezuelan strain of *Streptomyces nodosus*.[197] As a consequence of its potent antifungal properties, it was rapidly introduced into the clinic.[198] Recently, it has also found use as a treatment for Leishmaniasis. Since its discovery, more than 8000 articles have been published on all aspects of its chemical, physical, and biological properties. Despite these massive efforts, its mechanism of action remains controversial.[199] In the most commonly accepted mechanism, 4–12 amphotericin B (**232**) molecules self-assemble in the fungal membrane to form a transmembrane ion channel.[200] This is commonly referred to as the barrel-stave model (Figure 20). Electrolyte efflux with concomitant loss of oxidation potential then leads to cell death. The formation of ion channels has been shown to be more efficient in ergosterol-containing membranes (for example, fungal membranes) than in cholesterol-containing membranes (for example, mammalian cell membranes).[199] An alternative theory suggests that the polyene functionality participates in redox processes that lead to cell death through oxidative stress.[201]



Several attempts to elucidate the connection between molecular structure and activity have been reported. Early studies naturally focused on functionalizing those structural elements that could be selectively modified through semisynthesis, that is, the hemiacetal, carboxylic acid, and free amine. Since amide derivatives are inactive, the presence of a basic amino group appears to be crucial. Recently, studies by Carreira and co-workers have shown that appending two linear alkyl amines to the mycosamine leads to analogues with higher antifungal activity and lower toxicity in vitro than **232**.[202] Most ester derivatives retain activity and often have

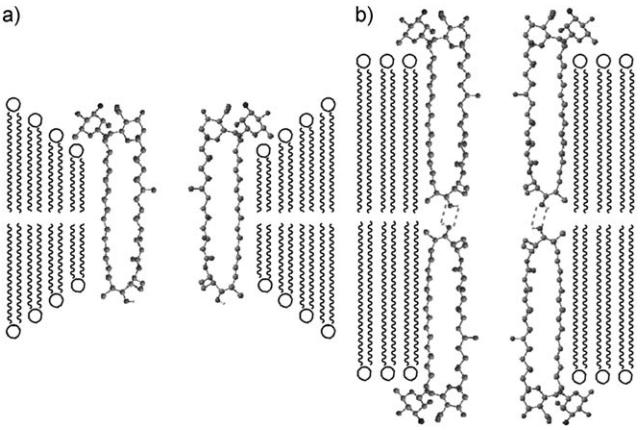
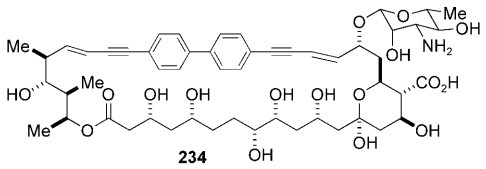


Figure 20. Model of the proposed ion channel depicted as a cross-section: a) monomeric and b) dimeric arrangement, with hydrogen-bonding interactions indicated.

improved pharmacological and toxicity profiles, while derivatization of the hemiacetal has more ambiguous consequences. The fact that esters are known to retain their activity has long argued against any special role of the carboxylate.

Rychnovsky and co-workers prepared semisynthetic derivative **234**, in which the polyene had been replaced by a diyne-diarene unit of roughly the same length.[203] This compound was devoid of antifungal activity, thus highlighting the significance of an uncompromised polyene moiety. Earlier studies had shown that hydrogenation of the polyene also led to loss of activity.[204] It should be noted that other mycosamine macrolides that lack the C28–C29 double bond (for example, nystatin) are active antifungal agents.



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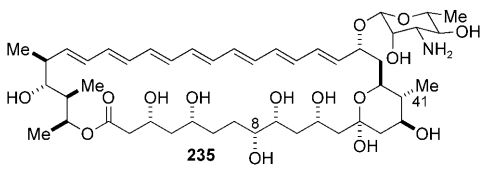
Caffrey et al. have characterized the amphotericin B polyketide synthase,[205] and through genetic engineering prepared 7- and 15-oxoamphotericin B, 8-deoxyamphotericin B,[206] and 41-descarboxy-41-methyl-amphotericin B (**235**).[207] All these compounds showed activity comparable to that of the parent compound. Compounds lacking the mycosamine unit were devoid of activity.

Other advances in mechanistic understanding have been obtained by Murata and co-workers. Incubation of the AmB-producing strain with ^{13}C-labeled propionic acid resulted in



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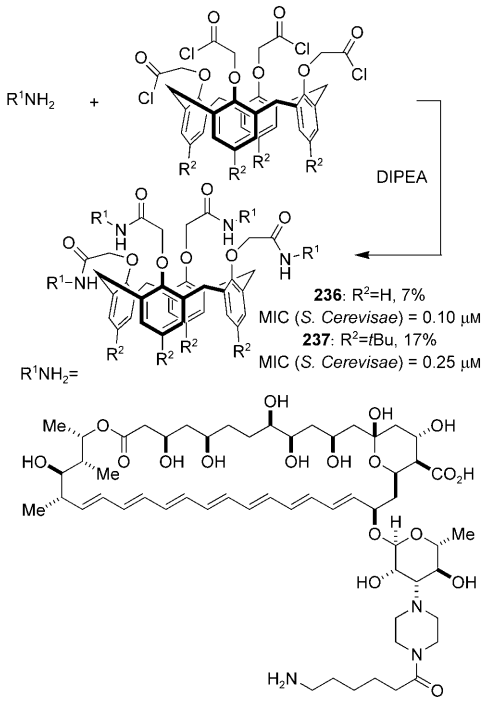
introduction of the ^{13}C label at positions C38, C39, C40, and C41.[208] Uniformly labeled AmB has also been prepared. A C28-fluorinated analogue was prepared by semisynthesis.[209] The availability of these compounds allowed the study of their interactions with synthetic membranes by using solid-state NMR and CD spectroscopy.[210] These studies indicated that ion-channel assemblies appear to be surrounded by ergosterol molecules rather than having the ergosterol molecules inserted between two neighboring amphotericin B molecules. Other studies have examined the orientation of amphotericin B (**232**) in membranes.[211]

Recent research by Carreira abd co-orkers has focused on the properties and uses of amphotericin B conjugates.[212] An amphotericin B–calix[4]arene conjugate was designed to emulate a preassembled ion channel and was tested in a variety of assays (Scheme 29). Conjugates **236** and **237**



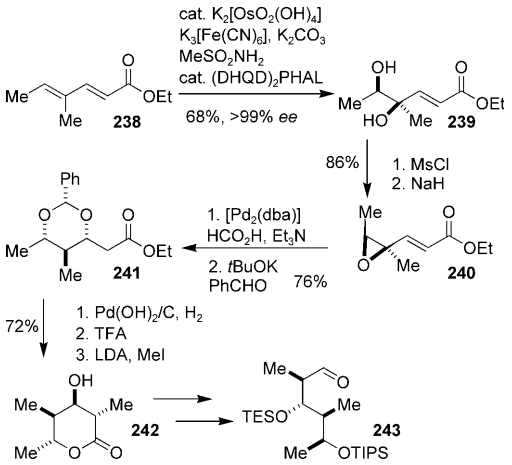
Scheme 29. Synthesis of calix[4]arene–Amb conjugates **236** and **237**.

showed antifungal activity comparable to that of AmB (**232**). In addition, the ability of **236** and **237** to cause potassium efflux from large unilamellar vesicles was also examined by using potassium-selective electrodes. Notably, the conjugates were capable of causing efflux of potassium ions with high efficiency.[212]

It has been postulated that molecular editing of the structure of amphotericin B (**232**) through diverted total synthesis would provide a powerful means to create molecular probes for its mechanism of action—free of the restrictions imposed by alternative methods. At the same time, it was apparent that this was bound to be a sizable effort because of the structural complexity of amphotericin B (**232**). Indeed, only one total synthesis of **232** has been completed to date.[213–215] Accordingly, efforts in this area have aimed at a

general, versatile strategy that would allow the rapid synthesis of a collection of probes.[216] Efforts from the Carreira research group towards achieving this goal are outlined in Schemes 30, 31, and 32.

The synthesis of the C33–C38 chain of AmB is shown in Scheme 30.[217] Sharpless dihydroxylation of diene **238** led to diol **239** in high enantiomeric purity. The diol was converted

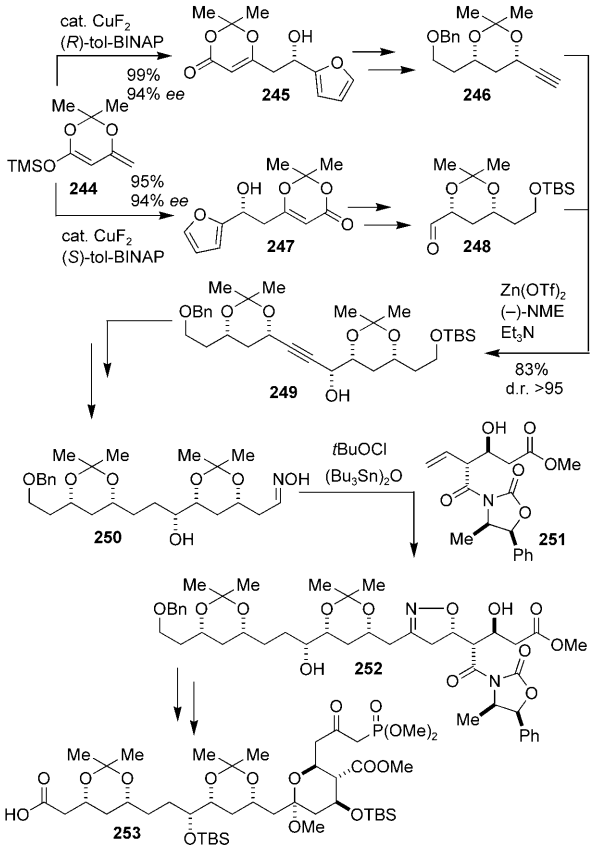


Scheme 30. Synthesis of the C33–C38 fragment **243**.

into an epoxide which was opened chemoselectively under transfer hydrogenation conditions. Tandem acetal formation/conjugate addition using benzaldehyde afforded **241**. Hydrogenative removal of the acetal was followed by lactone formation. Fráter–Seebach methylation then introduced the methyl group and set the C34 stereogenic center. Lactone **242** was then converted into the protected C33–C38 fragment **243**.

An outline of the synthesis of the C1–C20 fragment is shown in Scheme 31. This synthesis relied on the use of recently developed methods to achieve efficient and convergent assembly of this fragment. The configuration of the stereocenters at C5 and C11 were set through catalytic asymmetric aldol reactions.[218] Subsequent transformations led to compounds **246** and **248**, which were coupled using the asymmetric zinc acetylide addition reaction to afford propargylic alcohol **249** in excellent yield and stereoselectivity.[219] Notably, this method was able to overcome the intrinsic bias of the substrates to give the diastereoisomer with the opposite configuration. The alkyne **249** was converted into oxime **250**, which served as a precursor for a nitrile oxide that participated in a cycloaddition with alkene **251**, as described by McGarvey et al.[220] This reaction proceeded in high yield and diastereoselectivity. The product isoxazoline **252** was then converted into the C1–C20 fragment **253**.

A synthesis of a suitably reactive mycosamine precursor (**259**) was then developed. The synthesis (Scheme 32) proceeded in 17 steps from D-glucose and permitted the preparation of **259** in gram quantities.[221] Thus, a key requisite of the synthetic strategy had thereby been met: access to gram-scale amounts of all the three key precursors **243**, **253**, and **259** of amphotericin B (**232**). The stage was thus set for designing and preparing synthetic probes for the mode of action of amphotericin B (**232**).

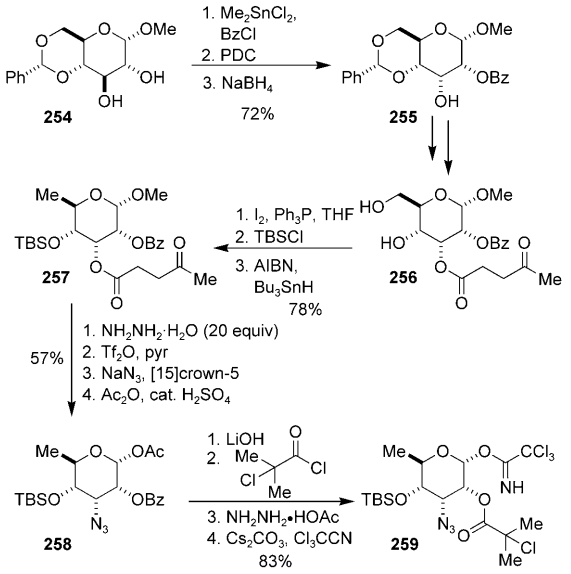


Scheme 31. Synthesis of the C1–C20 subunit **253**.

An important component of the controversy surrounding the barrel–stave model is the discrepancy between the overall length of amphotericin B (**232**) at 21 Å and that of the typical fungal cell membrane at 40 Å. This divergence has been explained by the contraction of the cell membrane around the site of the ion channel (Figure 20a) or the formation of larger ion channels consisting of two identical subunits oriented towards each other in a tail to tail fashion (Figure 20b).[222] The C35 hydroxy unit has been singled out and hypothesized to be essential for the formation and stabilization of these dimeric ion channels through hydrogen bonding.

The 35-deoxy analogue of amphotericin B (**232**) has been selected as a target for synthesis to examine the validity of the various models. By eliminating the putative hydrogen bond believed to be necessary for stabilizing the postulated dimeric channel, critical information and insight was expected regarding the relative importance of the models shown in Figure 20a,b.

The necessary 35-deoxy-polyene-polypropionate moiety **265** was prepared starting from chiral (*S*)-3-hydroxybutyric acid ethyl ester (**260**).[216] Hydroxy ester **260** underwent Fráter–Seebach alkylation in high diastereoselectivity and 92% yield, and the ester was reduced with lithium aluminum hydride (Scheme 33). The resulting diol was selectively converted into a primary iodide under Appel's conditions. Finally, the secondary alcohol was protected as the TES ether. Iodide **261** was then used to alkylate the lithium enolate of **262** according to Myers' protocol to afford **263** in 71% yield

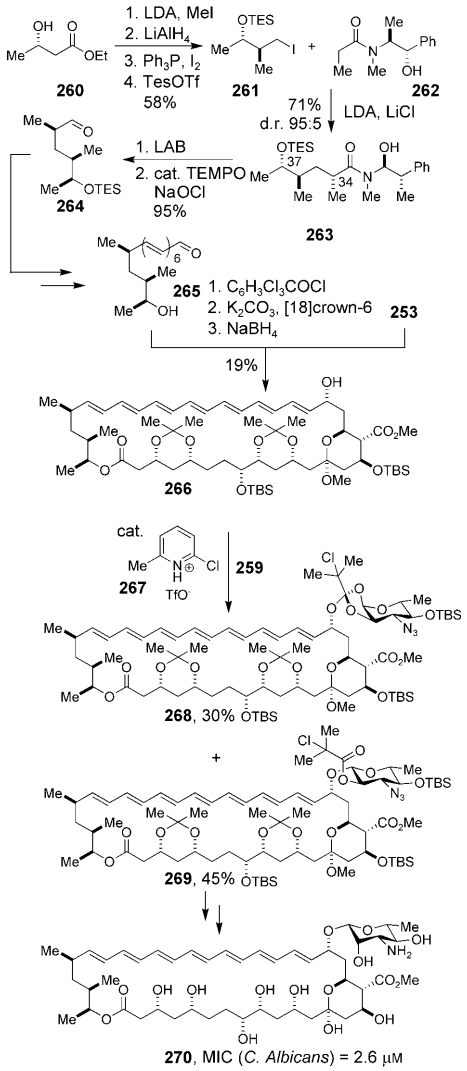


Scheme 32. Synthesis of the mycosamine donor **259**.

and a diastereomeric ratio of 95:5. The Myers' auxiliary was removed under reductive conditions and the product oxidized under Anneli's conditions to produce aldehyde **264**. This compound was then converted into the aldehyde **265** through two sequential chain extensions. Esterification of **253** with **265** was achieved by using a one-pot modification of the Yamaguchi protocol. Macrocyclization was effected under mild basic conditions (K_2CO_3 60 °C). Finally, 35-deoxyamphoteronolide (**266**) was obtained by stereoselective and chemoselective reduction using $NaBH_4$.

A final obstacle remained: the introduction of the mycosamine appendage. Despite the passage of 20 years since the first synthesis of amphotericin B (**232**), this feat remained as challenging as ever. The known protocol relied on the use of a trichloroacetimidate donor bearing an acetate ester to ensure β-glucosidation through anchimeric assistance. This reaction was, however, known to produce the orthoester as a major side product and proceed with low conversion. On the basis of the realization that the orthoester likely results from attack by the aglycone on the putative acetoxonium carbon atom of the reactive intermediate in the reaction, donor **259** was designed.[223] By using mildly acidic catalyst **267**, glycoside formation was optimized and full conversion was achieved. This glycosidation protocol has been shown to be of general value for the glycosidation of sterically hindered alcohols.[224] With the molecular backbone fully assembled, the synthesis of 35-deoxyamphotericin B methyl ester (**270**) was completed in short order (Scheme 33).[223]

Direct comparison of the antifungal activity of 35-deoxyamphotericin B methyl ester (**270**) to amphotericin B methyl ester (**233**) showed a 20-fold drop in activity.[223] Furthermore, **270** has severely diminished ability to induce the leakage of K^+ compared to **233** (Figure 21). Collectively, these data support the intermediacy of a dimeric transmembrane ion (Figure 20b) as being important for the mechanism of action of amphotericin B. This result is likely to influence future developments of new amphotericin B types of drugs.



Scheme 33. Completion of the synthesis of 35-deoxyamphtericin B methylester **236**.

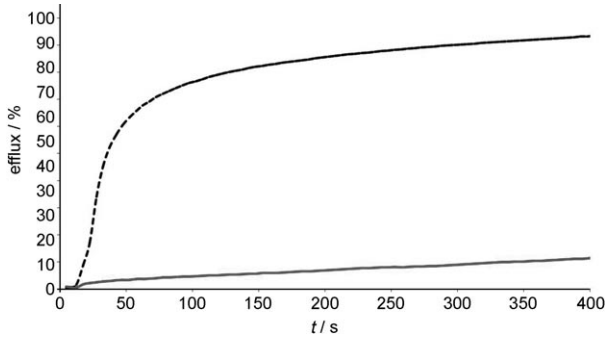


Figure 21. KCl efflux from LUVs induced by **270** (——) and **233** (-----) measured by potentiometry. The substrate was added externally (as a solution in DMSO) to afford a final concentration of 1 μM. LUVs with a diameter of 100 nm and containing 13% ergosterol and 87% POPC in their membranes were utilized.

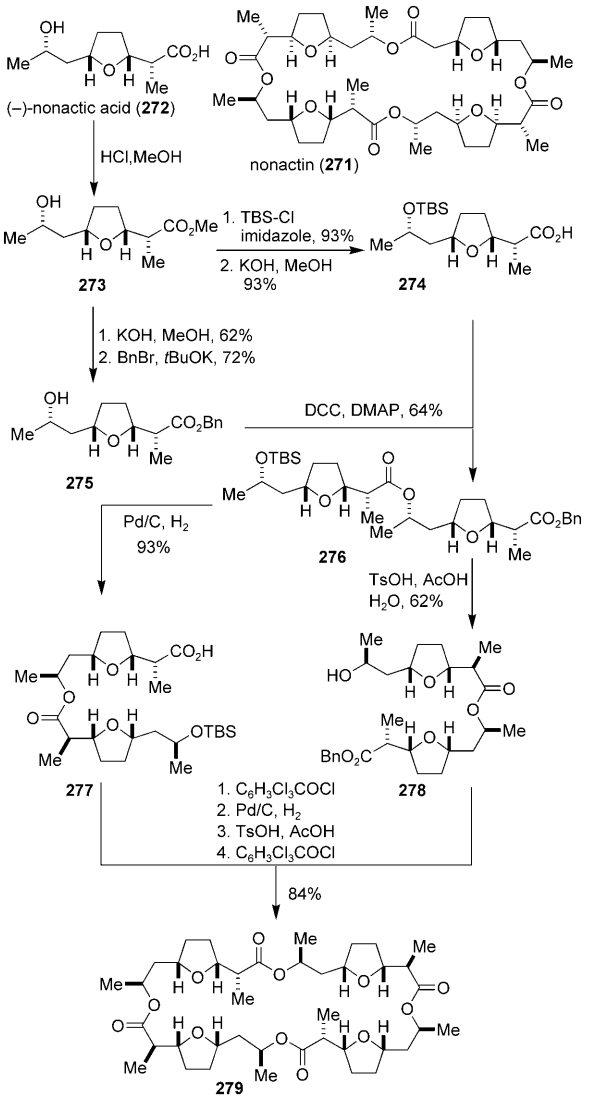
13. Nonactin

Nonactin (**271**) is an ionophore antibiotic first isolated from *Streptomyces griseus* ETH A7796 in 1955.[225] Nonactin owes its antimicrobial activity to its ability to passively shuttle bound cations[226] across mitochondria cell walls, thereby altering the electrochemical gradient essential for biological processes. Strikingly, the structure of nonactin (**271**) is made up of two mirror-image forms of nonactic acid (**272**). Hence, to accomplish the biosynthesis of nonactin the producing organism must synthesize the enantiomeric constituents (−)-nonactic acid (**272**) and (+)-nonactic acid (*ent*-**272**) in a chemically compartmentalized manner.[227] Two total syntheses of nonactic acid have been reported.[228]

The research group of Priestly has recently disclosed an intriguing study on the properties of (−)-nonactin (**279**), which comprises only the (−)-nonactic acid (**272**).[229] The enantiomer *ent*-**279** was also prepared from (+)-nonactic acid (*ent*-**272**). (−)-Nonactic acid (**272**) was isolated directly from a culture broth of *S. griseus* Δ*nonD*, a mutant strain of the nonactin-producing bacteria unable to convert **272** into nonactin (**271**). The compound was then esterified and protected in two different ways to provide the substrates for formation of the two halves of the macrocycle (Scheme 34). Accordingly, TBS protection of the alcohol **273** and basic hydrolysis of the methyl ester afforded **274**. Benzyl ester **275** was formed by first performing the basic hydrolysis and then specifically alkylating the carboxylate with benzyl bromide. These two compounds (**274** and **275**) were then coupled (DCC) in 62% yield. The product, **276**, was then converted into two opposing halves of the macrocycle. Hydrogenative debenzylation afforded **277** and removal of the TBS group under acidic conditions afforded **278**. Treating **277** with the Yamaguchi reagent afforded the activated mixed anhydride, which when treated with **278** led to formation of the expected ester. The benzyl ester and TBS ether were cleaved in high yield, and then macrocyclization took place in the presence of the Yamaguchi reagent to afford the optically active (−)-nonactin (**279**).

The *ent*-**279** analogue was prepared in an identical fashion starting from *ent*-**272**. This compound was prepared directly from nonactin (**271**). Hydrolysis afforded a racemic mixture of **272**, which was then resolved by kinetic oxidative resolution by *Rhodococcus*, a gram-positive bacteria. With the two enantiomers of **279** in hand, Priestly and co-workers were able to compare their binding to alkali-metal ions with that of nonactin.

As expected, natural nonactin (**271**) was shown to be a bactericidal agent with MIC values of 1–2 μM against a range of gram-positive bacteria, including methicillin-resistant *Staphylococcus aureus*. In stark contrast, synthetic **279** and *ent*-**279** were completely inactive. Microcalorimetry afforded binding constants for nonactin (**271**) and (−)-nonactin (**279**) association to potassium and sodium ions: Nonactin has a 900-fold higher binding constant to potassium compared to **279**, which is derived from (−)-nonactin. Finally, molecular modeling studies suggested that the reason for the poorer binding to potassium ions may result from steric interactions between opposing moieties of the macrocycle assembled from homochiral constituents that are absent in the heterochiral natural product (Figure 22). These interactions force the ligand into a conformation that is less favorable for binding metal ions.



Scheme 34. Structure of nonactin (**271**) and synthesis of its (−) analogue **279**.[229]

This study elegantly illustrates the power of the full arsenal of genetically engineered bacteria strains, physical and biophysical analytical methods, modeling studies, and chemical synthesis. The findings of Priestly and co-workers provide an important piece of the puzzle in the mechanism of action of nonactin (**271**) and guidelines for the design of ionophores.

14. Conclusion

In this Review we have presented a selection of case studies in which the synthesis of natural products has been critical to address important questions in biology. The mechanisms of action span a wide spectrum of molecular

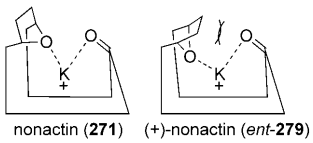


Figure 22. Models of the binding of potassium ions to nonactin (**271**) and (+)-nonactin (*ent*-**279**). Steric interaction would occur if *ent*-**279** was to adopt a similar conformation to **271**. Modified from Ref. [229].

partners in the cell, including DNA, proteins, and lipids. In addition, the molecules discussed have a high degree of structural diversity: polyketides, sesquiterpenes, and depsipetides isolated from terrestrial and marine sources. An important lesson stands out from this cornucopia: the synthetic chemistry of natural products constitutes a powerful and veritable asset in the study of their biological mechanisms of action. The ability to chemically modify any functional group of a molecule virtually at will, while at times laborious and taxing, is thus a valuable alternative to semisynthesis and genetic engineering. This process of "molecular editing" can provide access to tailor-made structures crafted by the researcher that would otherwise be intractable by the alternative methods. Additionally, non-natural analogues of natural products as synthesis targets provide a wealth of challenges and opportunities for innovation beyond those presented by the products of secondary metabolism, namely, natural products.[230] Natural products have served as veritable targets that provide challenges to chemists with an interest in synthesis. The traditional domain of natural products synthesis has been largely defined and driven by the structures that are the product of biosynthesis. In the words of Corey, "*rigorous analysis of a complex synthetic problem … produces superlative returns … Molecular complexity can be used as an indicator of the frontiers of synthesis, since it often causes failures which expose gaps in existing methodology.*"[231] This statement has repeatedly proven itself successful in multiple scenarios. A notable example has been highlighted by Eschenmoser: "*The research area of natural product synthesis requires and provides such knowledge in exceptional breadth. It is therefore particular fitting that it was the protagonist of modern natural product synthesis who triggered the final breakthrough of the use of the quantum mechanical model of structure and reactivity in organic chemistry, an advance that parallels the establishment of the classical structure theory, the tetrahedral model of carbon, the octet rule, and conformational analysis.*"[232] Indeed, natural products themselves will continue to inspire and drive the field of chemical synthesis.

This Review has highlighted research in which synthesis has played a key role in deciphering questions pertaining to the molecular mode of action. Although the activity may have parallels to, and is not inconsistent with, structure–activity studies, it sets itself apart in that the goal is the elucidation of the mode of action and not directed to the discovery of new therapeutic agents or potent drugs. The term "diverted total synthesis" captures the essence and mechanics of this research, as it relates to the traditional role natural products have played for synthetic chemists. However, although the adjective "diverted" may connote, or suggest, an endeavor that is off the main track or aimed at an alternative, secondary goal, the examples discussed in this Review underscore that this is not the case. Close inspection and study of the work of the pioneers in the field, some of which have been highlighted, reveal that in searching for synthetic challenges the modern practitioner need not be limited by structures that are merely the products of evolutionary, biological contingency. Consequently, the chemist may play an active role in defining new targets inspired by the natural products, thereby formulating the questions that broadly drive science. The ensuing research activity is inherently multidisciplinary in that it necessarily entails multiple means and aims that include questions in synthesis as well as in biology and medicine. In so doing, the synthesis enterprise not only endures in its more traditional role in advancing chemical breakthroughs, but has also expanded its reach with respect to new opportunities in science. Lehn[233] has reminded us that the essence of chemical science can find full expression in the words of Leonardo da Vinci: "*Where nature finishes producing its own species, man begins, using natural things and in harmony with this very nature, to create an infinity of species.*" This captures the power of synthetic chemistry and should increasingly be the purview of its practitioners. Indeed, it is evidently clear that chemical space is considerably more vast than the range covered by biology. With the advent of increasingly more sophisticated methods and strategies in the fields of organic, computational, and analytical chemistry as well as biology, it is a brave new world that awaits us with a myriad of new problems to challenge and inspire the synthetic chemist.[234] It is our hope that the present Review will attract more chemists to take advantage of the opportunities their trade affords them to investigate deeply into the chemical problems offered by biological systems.

Abbreviations

AIBN	Azobis(isobutyronitrile)
Bn	Benzyl
Boc	*tert*-Butyloxycarbonyl
BOM	Benzyloxymethyl
Bz	Benzoyl
CAN	Cerium ammonium nitrate
CBz	Carbobenzyloxy
CDI	Carbonyldiimidazole
cod	1,5-Cyclooctadiene
CSA	Camphorsulfonic acid
DAG	Diacylglycerol
dba	Dibenzylideneacetone
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
DCC	Dicyclohexyl carbodiimide
DIC	Diisopropylcarbodiimide
DDQ	2,3-Dichloro-5,6-dicyano-1,4-benzoquinone
DEPBT	3-(Diethoxyphosphoryloxy)-1,2,3-benzotriazin-4(3*H*)-one
$(DHQD)_2PHAL$	Dihydroquinidine 1,4-phthalazinediyl diether

DIAB	(−)-3-*exo*-(Dimethylamino)isoborneol
DIBAL	Disobutylaluminum hydride
DIPEA	*N*,*N*-Diisopropylethylamine
DiPrC	Diisopropylcarbodiimide
DMAP	Dimethylaminopyridine
DMB	3,4-Dimethoxybenzyl
DMDO	Dimethyldioxirane
DMP	Dess–Martin periodinane
EDC	1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride
Fmoc	9-Fluorenylmethoxycarbonyl
HATU	*N*-[1-(Dimethylamino)-1*H*-1,2,3-triazole-[4,5-*b*]pyridin-1-ylmethylene]-*N*-methylmethanaminium hexafluorophosphate
HMDS	1,1,1,3,3,3-Hexamethyldisilazide
HOBT	1-Hydroxy-1*H*-benzotriazole
Ipc	Isopinocampheyl
LDA	Lithium diisopropylamide
LUV	Large unilamellar vesicles
MCPBA	*meta*-Chloroperbenzoic acid
MEM	2-Methoxyethoxymethyl
MIC	Minimum inhibitory concentration
MMPP	Magnesium monoperoxyphthalate
MOM	Methoxymethyl
Mes	Mesityl
Ms	Mesyl
NBS	*N*-Bromosuccinimide
NME	*N*-Methylephedrine
NMO	*N*-Methylmorpholine-*N*-oxide
PCC	Pyridinium chlorochromate
PDC	Pyridinium dichromate
PMB	*para*-Methoxybenzyl
POPC	1-Palmitoyl-2-oleoyl-*sn*-glycerophosphocholine
PyBOP	Benzotriazolyl-1-oxy-tripyrrolidinophosphonium hexafluorophosphate
PyBrop	Bromotri(pyrrolidino)phosphonium hexafluorophosphate
Pyr	Pyridine
SAE	Sharpless asymmetric epoxidation
TBAF	Tetrabutylammonium fluoride
TBDPS	*tert*-Butyldiphenylsilyl
TBS	*tert*-Butyldimethylsilyl
TEMPO	2,2,6,6-Tetramethylpiperidine-1-oxyl
TES	Triethylsilyl
Tf	Triflate
TFA	Trifluoroacetic acid
TIPS	Triisopropylsilyl
TMS	Trimethylsilyl
tol-BINAP	2,2′-Bis(di-*p*-tolylphosphanyl)-1,1′-binaphthyl
TPAP	Tetra-*n*-propylammonium perruthenate
Tr	Triphenylmethyl
Ts	Toluene-4-sulfonyl

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