Syntheses of Cyclomarins – Interesting Marine Natural Products with Distinct Mode of Action towards Malaria and Tuberculosis

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Published as part of the 50 Years SYNTHESIS – Golden Anniversary Issue

Abstract The cyclomarins are cyclic heptapeptides from marine streptomycetes containing four rather unusual amino acids. Interestingly, the cyclomarins address two completely different targets: ClpC1, a subunit of the caseinolytic protease of Mycobacterium tuberculosis (MTB), as well as PfAp3Aase of Plasmodium falciparum. Therefore, the cyclomarins are interesting lead structures for the development of drugs targeting tuberculosis and malaria. As a result, several synthetic protocols towards the synthesis of these unusual building blocks as well as the natural products themselves have been developed, which will be discussed in this review.

Key words natural products, cyclopeptides, cyclomarins, total synthesis, malaria, tuberculosis

1 Introduction

From the last century to the present, an uncountable number of peptidic secondary metabolites have been isolated from microorganisms such as bacteria or fungi. Due to their diverse and unique structures, they exhibit a remarkable range of biological activities. This fact is not surprising since they are the highly optimized result of millions of years of evolutionary selection. Thus, natural products play an important role in drug discovery, either as direct drug candidates or as lead structures. Nevertheless, it should be mentioned, that the isolation from complex mixtures often cannot provide the desired natural product in amounts suitable for structure elucidation or complete biological evaluation. Therefore, innovative methods are required to produce the desired natural product by total synthesis. In addition, structural modifications provide important insights into the structure–activity relationship (SAR).
An outstanding example is a small family of peptide natural products, the cyclomarins A–C (1–3), isolated in 1999 from the marine streptomycete strain CNB-982 (Figure 1). The crude extract of a sample, collected from sediments at May Bay, showed moderate cytotoxicity against human colon cancer cells (HCT-116). Furthermore, the organism produced the novel cyclopeptides under saline culture conditions, whereas the main metabolite cyclomarin A (1) shows an IC₅₀ value of 2.6 µM against various cancer cell lines. In addition to the anticancer properties, anti-inflammatory and antiviral activities were also observed. Moreover, in 2011 Schmitt, Camacho, and co-workers from Novartis reported antibacterial activity against Mycobacterium tuberculosis (Mtbc) in concentrations of 0.3 µM and 2.5 µM in culture broth medium and human-derived macrophages, respectively. Interestingly, cyclomarin A (1) retains antitubercular activity against several resistant strains and also inhibits nonreplicating intracellular bacteria in macrophages with a mortality rate of 90% (after 5 d incubation) with a drug concentration of 2.5 µM. This fact suggested that a completely new molecular target structure might be involved. Target profiling by chemical proteomics identified the ClpC1 subunit of the caseinolytic protease (Clp), where Schanda and co-workers, in 2018, determined the mode of action by NMR spectroscopic and crystallographic investigations. In addition, cyclomarin A (1) shows an impressive antiparasitic activity against Plasmodium falciparum (Pfalcp), the pathogen of malaria. By inhibition the growth of the blood stages of Pfalcp, cyclomarin A (1) binds a dimer of diadenosine triphosphate hydrolyses (PfAp3Aase) and thereby prevents the formation of the enzyme-substrate complex.

As already mentioned, cyclomarin B (2) and C (3) were also isolated in 1999, but in significantly smaller quantities. In the course of a broad screening for biologically active substances from the culture broth of the streptomycete strain BCC26924, cyclomarin C (3) was isolated in sufficient quantities and biologically evaluated. Thus, cyclomarin C (3) shows antitubercular activity (MIC = 0.10 µg/mL, strain H37Ra) as well as antiplasmodial activity against multidrug-resistant strain K1 with an IC₅₀ value of 0.24 µg/mL. Furthermore the analogous cyclomarin D (4) (Figure 1), in principle N-desmethylcyclomarin C, was identified in the culture medium of a Palau-derived actinomycete species (Salinospora arenicola CNS-205). In 2008, the structurally related metabolites cyclomarazins A (5) and B (6) (Figure 1) were also isolated. It is thought that the cyclomarins and cyclomarazins have a common biosynthetic origin due to the similarity of two noncanonical amino acids. The biosynthesis of these compounds was investigated in detail by Moore and co-workers, 16–18

Beside the three proteinogenic amino acids (L-Ala, L-Val, N-Me-L-Leu), the 21-membered cyclopeptides 1–4 differ by slight variation in the methylation and oxidation pattern of the noncanonical amino acids (blue), which were identified using 1D and 2D NMR techniques. Determination of the absolute stereochemistry was achieved by X-ray crystal structure of the diacetate derivative of cyclomarin A (1). Some of the unusual amino acids, especially the β-methoxyphenylalanine moiety and derivatives thereof are not only incorporated in cyclomarins, they also can be found as key structural motifs in a variety of biologically active compounds, ranging from discokiolides and callipelmins to more complex cyclic depsipeptides, such as neamphamide A, papuamides, or mirabamides. The β-hydroxytryptophan unit can be found in two variations, either with a N-tet-t-prenyl residue or the oxidized N’-[25]-2,3-epoxy-1,1-dimethyl[propyl] substituent, which is also a structural motif of another antitubercular class of natural products, the ilamycins. Furthermore the δ-hydroxyleucine fragment is found in BZR-cotoxin II and in leucosbatins, whereas the 2-amino-3,5-dimethylhex-4-enoic acid is unique to cyclomarins.

Soon after the isolation of the cyclopeptides, the first synthesis of the noncanonical amino acids of cyclomarin A (1) were reported by Yokokawa and co-workers. Due to the highly complex heptapeptidic structure with 12 or 13 stereogenic centers and the four nonproteinogenic amino acid building blocks, it is hardly surprising, that already in 2004 a synthetically access to cyclomarin C (3) was achieved via a convergent strategy by Yao and co-workers. Just over ten years later in 2016, Barbie and Kazmaier accomplished the synthesis of the natural products cyclomarin A (1), C (3), and D (4) and, as a simplified derivative, deoxycyclomarin C in a linear approach.

**Figure 1** Chemical structures of cyclomarins and cyclomarazins
In this review we will highlight the synthetic efforts towards these interesting natural products, beginning with the synthetic approaches towards the four noncanonical amino acids, and ending with the total synthesis of the natural products and derivatives, which will be discussed in detail.

2 Synthesis of the Building Blocks

2.1 (2S,3R)-β-Methoxyphenylalanine

The first reported synthetic access by Yokokawa and co-workers\(^\text{29}\) to β-methoxyphenylalanine was achieved by using Schöllkopf auxiliary 7 (Scheme 1).\(^\text{35}\) Here, 7 was first deprotonated at low temperature with \(\text{BuLi}\). After C\(\text{Tl}(\text{NET}_2)\)_3 mediated transmetalation and reaction with benzaldehyde, the secondary alcohol 8 was obtained in diastereomerically pure form in 78% yield.\(^\text{36}\) Subsequent O-methylation using Meerwein salt and Proton sponge® gave methyl ether 9.\(^\text{37}\) Removal of the chiral auxiliary with aqueous TFA and final amine protection provided building block 10 in a high yield of 92%.\(^\text{29}\)

The synthesis route of Hajra and co-workers also used an auxiliary approach (Scheme 2).\(^\text{38}\) The Evans auxiliary of cinnamic acid 11 was treated with bromine in a silver(I)-mediated halomethoxylation (via 12) to give the desired methyl ether as a diastereomeric mixture, from which 13 was isolated in 64% yield. This was followed by an S\(_{\text{N}}\text{2}\) reaction with sodium azide to provide syn product 14;\(^\text{39}\) the auxiliary was finally cleaved, giving acid 15. To get the desired β-methoxyphenylalanine, the azide group just needs to be converted into an amino group.

Joullié and co-workers followed an entirely different approach based on a diastereoselective Grignard reaction towards the literature-known Lajoie’s serine aldehyde 16 (Scheme 3).\(^\text{40}\) Via addition of phenylmagnesium bromide, the syn amino alcohol 17 was obtained in good diastereoselectivity, but unfortunately in moderate yield. Next, the methyl ether 18 was generated also here by the use of Meerwein salt. Finally, the amino acid 19 was obtained by Cbz deprotection via hydrogenation and cleavage of the orthoester under aqueous acidic conditions.

2.2 (2S,4R)-γ-Hydroxyleucine

Yokokawa and co-workers also utilized an auxiliary-based approach for the synthesis of the 6-hydroxyleucine building block (Scheme 4).\(^\text{29}\) For this purpose, the silyl-protected Roche ester 20 was reduced to the corresponding aldehyde using DiBAL-H. Subsequent Horner–Wadsworth–Emmons reaction with phosphonate A gave the α,β-unsaturated amide 21 in 79% yield over both steps.\(^\text{42}\) After hydrogenation of the double bond in 21, 22 was deprotonated using KHMD at \(-78\,^\circ\text{C}\); quenching the potassium enolate with 2,4,6-trisopropylphenylsulfonyl azide (trisyl azide) provided the diastereomerically pure azide 23;\(^\text{43,44}\) subsequent hydrogenation of the azide group with palladium in the presence of Boc\(_2\)O afforded the protected amine 24 in good yield. Oxidative cleavage of the Evans auxiliary,\(^\text{45}\) N-methylation, and esterification gave the desired product 25 in 85% yield over three steps.

According to Joullié and co-workers the protected (S)-pyroglutamic acid 26 was used as starting material (Scheme 4).\(^\text{46}\) α-Methylation of 26 gave 27 in a diastereomeric ratio of 7:1 in favor of the undesired diastereomer. However, to obtain the correct configuration in the product, the stereo-
genic center was inverted under basic conditions. The diastereomeric ratio of 28 was now 3.1:1 for the favored diastereomer. Acyclic building block 29 was obtained after hydrolysis with LiOH. To generate the desired alcohol, acid 29 was first activated as the mixed anhydride and then reduced with NaBH₄. Additional protection gave TBS ether 30.

For the final N-methylation, 30 was first deprotonated with excess NaHMDS and then treated with methyl iodide. The fully protected δ-hydroxyleucine 31 was thus obtained in 57% yield without epimerization.

Besides the ex-chiral pool strategy, Joullié and co-workers developed a synthetic method based on an asymmetric Evans alkylation and an asymmetric Strecker reaction as key steps (Scheme 5). First, deprotonation of 32, followed by the addition of allyl iodide gave oxazolidine 33 with a diastereomeric excess of >96%. Cleavage of the chiral Evans auxiliary under reductive conditions afforded alcohol 34, which was subsequently protected as benzyl ether 35. Next, the terminal double bond was oxidized in a one-pot sequence via dihydroxylation/periodate cleavage to obtain the corresponding aldehyde 36. Subsequent condensation with an enantiopure (+)-(S)-p-toluenesulfimamide B in the presence of Ti(OEt)₄ gave the desired chiral sulfinimine 37. Carbonyl addition of Et₂AlCN provided amino nitrile 38 in 92% yield with a diastereomeric excess of 91%. Final cleavage of the sulfinyl auxiliary and hydrolysis of the nitrile group under acidic conditions afforded the benzyl-protected methyl ester of (2S,4R)-δ-hydroxyleucine 40.

The synthesis of Chandrasekhar and co-workers in 2011 was achieved from amino lactone 41, which was prepared according to the literature (Scheme 6). Reductive ring opening of lactone 41 gave the amino alcohol, which subsequently protected as dimethoxazolidine 42. Next the primary alcohol function was silylated with TBDPSCI and imidazole. After removal of the oxazolidine under acidic con-
ditions, the resulting alcohol 43 was oxidized to the corresponding acid using (diacetoxyiodo)benzene and a catalytic amount of TEMPO in 71% yield. Final N-methylation afforded the building block 44.50

Scheme 6 Synthesis of δ-hydroxyleucine from an amino lactone via a dimethoxyxazolidine (Chandrasekhar)

In 2018, Zwick III and Renata reported the syntheses of δ-hydroxyleucine 46 via selective chemoenzymatic C–H functionalization (Scheme 7).51 Thereby, they used an α-ketoglutarate-dependent dioxygenase (Fe/αKG) GriE as biocatalyst for the C5-hydroxylation of 45 to obtain the desired δ-hydroxyleucine 46 in 90% yield and perfect diastereoselectivity.

Scheme 7 Chemoenzymatic approach to δ-hydroxyleucine (Renata)

2.3 (25S,3R)-N’-[(2R)-2,3-Epoxy-1,1-dimethylpropyl]-3-hydroxytryptophan

For the third noncanonical amino acid unit Yokokawa and co-workers52 used a literature-known protocol to gain access to N-propargylindoline 49 (Scheme 8).45 Here, indoline (47) was reacted with 3-acetoxy-3-methylbut-1-ynyl (48) in a copper(I) chloride mediated substitution reaction to give N-propargylindoline 49 in excellent yield. Subsequent Lindlar hydrogenation afforded the N-tert-prenylated indoline 50, which was oxidized with MnO2 to the N-tert-prenylated indole 51. In the subsequent Vilsmeier–Haack formylation, indole-3-carbaldehyde 52 was isolated quantitatively. Subsequently, the double bond in the tert-prenyl group was transformed into chiral diol 53 via Sharpless dihydroxylation;45 using the cinchona alkaloid based ligand (DHQD)$_2$Pyr, the enantiomeric excess was 85%. Next, the primary alcohol function of 53 was tosylated and converted under basic conditions into the epoxide 54. Additional Horner–Wadsworth–Emmons reaction with triethyl phosphonoacetate provided the required E-configured olefin 55. Final Sharpless aminohydroxylation53 released the desired β-hydroxytryptophan derivative 56 in moderate yield. In this reaction the regio- and diastereoselectivity were controlled by the cinchona alkaloid based ligand (DHQD)$_2$AQN, which gave product 56 with a diastereoselectivity of 95%.

In addition to this synthesis, a further partial synthesis was developed by Spinella and co-workers, that gave access to substrates for Sharpless aminohydroxylations. They used a transition-metal-catalyzed cross-coupling reaction as the key step (Scheme 8).52 The synthesis started with bromide 57. Sharpless dihydroxylation of bromide 57 gave diol 58 with 76% ee, which was converted into acetal 59 in an overall yield of 85% over two steps. Building block 59 was then reacted in a Heck coupling with ethyl acrylate to give α,β-unsaturated ester 61. However, very high amounts of catalyst were required and the desired product 61 was still only obtained in 36% yield. Furthermore, large amounts of the reduced indole 60 were formed as a side product. Alternative approaches using the analogous iodoindole or an acrylic stannane in the Stille coupling were also not successful. Thus, an alternative oxidative coupling of the nonhalogenated indole 60 with acrylic ester was investigated.53 After optimization of reaction conditions, the desired building block 61 was finally obtained in a very good yield of 86%. After cleavage of the acetal, the epoxide 62 was synthesized according to the protocol of Yokokawa and co-workers.29

Joullié and co-workers also reported a synthesis for this building block (Scheme 9).51 In analogy to their synthesis of β-methoxyphenylalanine, the hydroxytryptophan moiety was prepared by Grignard addition of an arylmagnesium to the Lajoie serine aldehyde 16. The required tert-prenylindole 51 was synthesized according to Yokokawa and co-workers.29 tert-Prenylindole 51 was converted by NBS-mediated bromination into 57, which was treated with magnesium turnings to give the corresponding organomagnesium species; subsequent addition of this nucleophile to the aldehyde 16 gave the secondary alcohol 63 in moderate yield, but in a good diastereoselectivity of 85%.

Furthermore, long reaction times and high temperatures were required to gain the desired organometallic species; therefore the use of iodoindole building block 64 was appropriated (Scheme 10). In analogy to the first proposed synthesis of this building block, the epoxide moiety was prepared by Sharpless dihydroxylation, followed by tosylation and subsequently elimination. It was found that the dihydroxylation of 64 provided 65 with 91% ee, while the formation of the epoxide 66 proceeded in comparable yields to the first synthesis. Next, halogen–metal exchange using nBuLi at low temperature and subsequent transmetalation with magnesium bromide gave the corresponding organomagnesium compound 67, which reacted with aldehyde 16 to give secondary alcohol 68. Despite the good diastereoselectivity, the yield was quite low. An additional disadvantage in the synthesis route is the fact that two equivalents of 67 need to be used with the aldehyde 16. The secondary alcohol 68 was subsequently protected as TBS ether 69. Af-
ter cleaving the orthoester under acetic acid conditions and saponification with lithium hydroxide, the acid was converted into its methyl ester 70.

A similar strategy for the synthesis of N’-tert-prenyl-tryptophan 78 was chosen by Barbie and Kazmaier (Scheme 10).54 Here, a simple one-pot protocol without an additional purification step gave iodoindole 72 in 94% yield.

To improve the selectivity of the chelate-controlled carbon-yl addition towards aldehyde 74, a protocol described by Knochel and co-workers was used.55 The corresponding zinc reagent 73, formed via transmetalation of the in situ formed Grignard reagent from 72 in the presence of LiCl, reacted with 74 to give the desired alcohol 75 in high yield and excellent diastereoselectivity. While the introduction of an orthogonal protecting group, e.g. MOM or TBDPS, failed, a second TBS group was chosen. Benzyl cleavage under reductive conditions provided the fully protected derivative 76 in 89% over two steps. With 76 in hand, the chemoselective reverse N’-prenylation was achieved by using a protocol by Baran and co-workers to obtain 77.56 Selective deprotection of the primary OH function with ammonium fluoride and subsequent two-step oxidation sequence of Parikh–Doering reaction57 and NIS-mediated oxidation 58 gave the fully protected N’-tert-prenylated tryptophan 78.

Scheme 8 Two synthetic routes to the β-hydroxytryptophan moiety (Yokokawa and Spinella)

Scheme 9 Grignard addition of N-tert-prenylindol-3-ylmagnesium bromide to the Lajoie serin aldehyde (Joullié)
2.4 (2S,3R)-2-Amino-3,5-dimethylhex-4-enoic Acid

Because of its unique structure, so far there is just one published fragment synthesis of the aminohexenoic acid 84 by Yokokawa and co-workers in 2002 (Scheme 11). Here, the synthetic sequence started with the commercially available aspartic acid derivative 79. This was converted into the corresponding alcohol after activation as a mixed anhydride and then reduced with NaBH₄; subsequent acid-catalyzed intramolecular transesterification generated lactone 80 in 52% yield. Next, the lithium enolate of 80 was reacted with methyl iodide at low temperatures to give 81 with a diastereomeric ratio of 10:1 for the favored diastereomer. DIBAL-H reduction of 81 to the corresponding lactol and subsequent Wittig reaction gave the oxazolidinone 82 in good yield. In the next step, oxazolidinone 82 was Boc-protected and converted into the primary alcohol 83 by hydrolysis. Final pyridinium dichromate (PDC) mediated oxidation gave the desired Boc-protected aminohexenoic acid 84 in 88% yield.

3 Total Synthesis of Cyclomarin C by Yao and Co-workers

Yao and co-workers started their synthesis of the β-methoxyphenylalanine derivative with tert-butylamide 85 derived from phthaloyl-protected L-phenylalanine. To obtain the oxygen function in the β-position, 85 was first brominated in the benzyl position via Wohl–Ziegler reaction...
(dr 1:1) \(^{61}\) Subsequent silver(I)-mediated nuclophilic substitution with water yielded the syn diastereomer preferentially. After removal of the undesired stereoisomer, \(O\)-methylation with silver(I) oxide and methyl iodide provided methyl ether \(^{86}\). The phthaloyl group was then removed with hydrazine. Hydrolysis of the amide and final Boc protection gave \(\beta\)-methoxyphenylalanine \(^{87}\) in a good yield of 79% over three steps.

The route for the preparation of the \(\delta\)-hydroxyleucine unit is also based on classical auxiliary chemistry, starting from \(^{88}\), which was initially prepared by a reported procedure\(^{62}\) (Scheme 13). After acidic hydrolysis of the tert-butyl ester and activation by oxalyl chloride, the generated acid dide provided methyl ether \(^{89}\). Diastereoselective azidation with trisyl azide after enolization of \(^{89}\) provided azide \(^{90}\). This was then protected in situ by catalytic hydrogenation with paloxazolidinone \(^{\text{Cl}}\) was reacted with lithiated Evans auxiliary \(^{\text{ester}}\) and activation by oxalyl chloride, the generated acid \(^{\text{dure62\text{ (Scheme 13).}}\)} After acidic hydrolysis of the from \(^{\text{unit is also based on classical auxiliary chemistry, starting}}\) with high ee. Subsequent protecting group manipulations of the secondary alcohol and saponification of the acetyl group provided the primary alcohol \(^{97}\). Subsequent Swern oxidation\(^{60}\) and methylene-Wittig reaction\(^{60}\) gave the tert-prenylated derivative \(^{98}\) in 50% yield for both steps. Via final protection group transformations gave building block \(^{99}\) for the peptide coupling.

To obtain the last non-proteinogenic amino acid \(^{105}\), Kazmaier’s asymmetric chelate enolate-Claisen rearrangement with quinidine as chiral alkaloid ligand was used (Scheme 14).\(^{63}\) Deprotonation of the ester \(^{100}\) and transmetalation to the chelated aluminum species yielded amino acid derivative \(^{101}\) via a [3,3]-sigmatropic rearrangement with high ee. Subsequent protecting group manipulations were necessary to suppress partial racemization of the \(\alpha\)-stereogenic center. Ozonolysis of the terminal double bond was converted into the hemiaminal \(^{92}\) using paraformaldehyde. Ring opening by ionic hydrogenation\(^{64}\) with triethylsilane and TFA and subsequent Boc protection provided the desired building block \(^{93}\) in quantitative yield.

Also in their synthetic route towards the \(N^\prime\)-tert-prenylated tryptophan building block \(^{99}\), the asymmetric Sharpless aminohydroxylation was used as key step (Scheme 13). First, the \(\alpha,\beta\)-unsaturated ester \(^{95}\) was generated in two steps by formylation of indole derivative \(^{94}\) followed by Horner–Wadsworth–Emmons reaction in 81% yield. Subsequent reaction with CBzNCla in the presence of catalytic amounts \(\text{K}_2\text{[OsO}_2\text{(OH)}_4]\) and \((\text{DHQD})_2\text{AQN}\) gave the 3-hydroxtryptophan derivative \(^{96}\) in moderate yield and 86% ee, however the regioselectivity was not reported. Silyl protection of the secondary alcohol and saponification of the acetyl group provided the primary alcohol \(^{97}\). Subsequent Swern oxidation\(^{60}\) and methylene-Wittig reaction\(^{60}\) gave the tert-prenylated derivative \(^{98}\) in 50% yield for both steps. Via final protection group transformations gave building block \(^{99}\) for the peptide coupling.

\[ \text{Scheme 12: Synthesis of} \ \beta\text{-methoxyphenylalanine} \]

\[ \text{Scheme 13: Evans diastereoselective azidation and Sharpless aminohydroxylation as key steps for the} \ \delta\text{-hydroxyleucine unit and} \ N^\prime\text{-tert-prenylated tryptophan} \]
followed by Wittig reaction provided the desired derivative 104 in only 31% yield. Final protecting group interconversions delivered Fmoc-amino acid 105.

3.1 Macrocyclization Studies and Completion of Total Synthesis

With all noncanonical amino acid building blocks in hand, several strategies for the construction of the linear heptapeptide scaffold and the macrolactamization of cyclomarin C were examined (Figure 2) in which four different sites for cyclization were investigated with the goal to minimize the number of linear steps. Because of its acid lability, the β-hydroxytryptophan moiety should be installed as late as possible.

First Yao and co-workers investigated a sequence based on a retrosynthetic peptide bond cleavage between the β-hydroxytryptophan and the aminohexenoic acid unit (route A). This resulted in linear precursor 106, which was synthesized in a [3+3+1] peptide fragment strategy. While the coupling of the individual peptide fragments proceeded without major problems, the C-terminal saponification of the methyl ester in 106 and subsequent cyclization under various conditions, unfortunately provided the desired product in trace amounts only. So, in alternative linear pre-
In a third attempt, the ring closure was planned between the L-alanine and the δ-hydroxylysine moiety (route C). However, this route failed already at a very early stage, the N-terminal deprotection; under the basic conditions a spontaneous cyclization to give diketopiperazine 109 took place (Scheme 15).

To circumvent additional disappointment in route D, the linear heptapeptide precursor was cyclized between the aminohexenoic acid and the N-Me-leucine moiety, using a [4+3]-coupling strategy for the synthesis of linear precursor 118. To prevent strong basic conditions for C-terminal deprotection, the methyl ester function was replaced by a terminal allyl ester. Thus, the synthesis of the desired peptide sequence started with the construction of tetrapeptide 114.

δ-Hydroxylysine 93 was coupled under standard conditions with allyl L-alaninate (110) to dipeptide 111 (Scheme 16). After N-terminal deprotection with TFA in dichloromethane, building block 99 was attached to give tripeptide 112 using a Bop-Cl/DIPEA protocol.65 Fmoc cleavage with 10% piperidine in dichloromethane and additional EDC/HOBt mediated coupling64 with aminohexenoic acid building block 105 afforded tetrapeptide 113 in 72% yield. Final allyl ester cleavage under Pd catalysis 64 led quantitatively to acid 114. The tripeptide 117 was also synthesized via standard peptide couplings.
For the [3+4] fragment coupling, removal of the N-Boc-protecting group of 116 with 15% TFA afforded the second precursor 117. The fragment coupling of 114 and 117 was performed using EDC/HOAt to give 118 in excellent yield (Scheme 17); with HOBt significantly lower yields were achieved. The protecting groups were subsequently removed and macrocyclization under high dilution conditions using PyBOP/DIPEA provided protected cyclomarin C (119) in 63%. Finally, treatment of 129 with K₂CO₃ in methanol at room temperature afforded the natural product cyclomarin C (3) in 80% yield after preparative HPLC.

### Total Synthesis of Cyclomarin A and C by Barbie and Kazmaier

In accordance with Yao and co-workers, Barbie and Kazmaier chose the same macrocyclization site for the synthesis of the cyclomarins A and C, but used a linear strategy for the generation of the required heptapeptides. The tryptophan building blocks need to be incorporated as late as possible in the synthesis from a common pentapeptide precursor.

The synthesis of the β-methoxyphenylalanine building block 123 should be facilitated by chelate-controlled arylmetal addition to a protected (R)-serinaldehyde (Scheme 18), which was obtained from ester 120 by reduction with one equivalent of DIBAL-H. In the subsequent reaction with a phenyl titanium species, generated from PhMgBr and Ti(Oi-Pr)₄, the desired product 121 was obtained as a single diastereomer. Subsequent O-methylation and cleavage of the silyl ether with TBAF provided the primary alcohol 122 in nearly quantitative yield. Final oxidation with NaClO₂ in the presence of catalytic amounts of TEMPO and (diacetoxyiodo)benzene led quantitatively to the required amino acid 123.

For the synthesis of the δ-hydroxyleucine 127, the silyl-protected Roche ester 124 was used as the starting material, which was reduced to the corresponding aldehyde via DIBAL-H and then reacted with phosphonoglycine ester E to give the protected α,β-unsaturated amino acid 125 (Scheme 19). By using the chiral phosphoramidite ligand (R)-MONOPHOS, the dehydroamino acid 125 was stereoselectively hydrogenated, providing the desired amino acid ester 126 in good yield and high enantioselectivity. Saponification and subsequent N-methylation gave the amino acid building block 127 almost quantitatively.
A new synthetic strategy was chosen for the tryptophans because of the acid lability of the β-hydroxy group (Scheme 20). According to a method of Stanley and co-workers, which allows a regioselectively prenylation of electron-deficient indoles, indole-3-carboxylic acid ester 128 was nearly quantitatively N'-tert-prenylated. Subsequently, the methyl ester 129 was saponified and after acidification the free acid was decarboxylated; iodination with N-iodosuccinimide (NIS) provided the common precursor 64 in 94% yield over three steps.

The organozinc species 131 was generated from iodoindole 64 using a literature-known protocol, which was then added to the previously prepared protected D-serine aldehyde at low temperature. Thus, amino alcohol 134 was obtained in good yield and satisfactory diastereomeric ratio. After silyl protection of the secondary alcohol 134, the primary OH function were selectively deprotected with ammonium fluoride to give 135. Alcohol 135 was finally transformed into methyl ester 136 in a two-step sequence of Parikh–Doering reaction and NIS-mediated oxidation.
Scheme 21  Synthesis of protected aminohexenoic acid via asymmetric ester enolate Claisen rearrangement

Scheme 22  Peptide coupling steps towards the linear precursors and completion of the natural product synthesis via macrolactamization
In order to obtain the epoxidized β-hydroxytryptophan 140, building block 64 was converted by a sequence of Sharpless dihydroxylation followed by tosylation and elimination into epoxide 66 with acceptable selectivity (Scheme 20). Subsequently, the epoxyindoline 66 was lithiated with BuLi at -78 °C and transmetalated with ZnBr₂. Then, zinc reagent 137 was reacted with the aldehyde from 132, whereby the desired product 138 was formed in moderate yield, but with perfect stereoselectivity. The completion of building block 140 was analogous to 136.

Starting material for the last building block, the γδ-unsaturated amino acid 145, was the racemic allyl alcohol 141, which was subjected to a enzymatic kinetic resolution and reacted with Boc-protected glycine (Scheme 21). Ester enolate Claisen rearrangement of the enantiomerically enriched ester 142 gave the desired amino acid ester 143 with perfect chirality transfer and very high diastereoselectivity. Ozonolysis of the double bond led to the corresponding aldehyde, which was then transformed into ester 144 in a Julia–Kocienski olefination with sulfone F.

4.1 Peptide Coupling and Macrolactamization

With all the building blocks in hand, the successive assembly of the linear heptapeptides 152a and 152b began with the formation of the dipeptide 147 (Scheme 22) using 2-bromo-1-ethylpyridinium tetrafluoroborate (BEP) as the coupling reagent. Hydrogenolytic cleavage of the Cbz protecting group required a hydrogen pressure of 20 atm and two equivalents of HCl were added to avoid formation of the diketopiperazine. The activated acid 123 was thus coupled with the Cbz salt of the dipeptide in 96% yield to give the tripeptide 148. Standard peptide couplings were used to build up pentapeptide 150. For the incorporation of the sensitive β-hydroxytryptophan building blocks 136 and 140 a BEP protocol was used. Both hexapeptides were obtained in satisfactory yield of 58% (151a) and 69% (151b). The Alloc protecting group was removed via palladium-catalyzed deprotection under Pd catalysis and subsequent EDC/HOBt mediated coupling afforded the desired heptapeptides 152a and 152b. Saponification of the methyl esters followed by N-terminal deprotection under Pd catalysis gave the free linear heptapeptides, which cyclized according to the method described by Yao and co-workers. Under these conditions, the O-silylated cyclopeptides 153a and 153b were obtained in good yield. Final two-stage deprotection with ammonium fluoride and TBAP provided cyclomarin A (1) in 51% and cyclomarin C (3) in 67% yield.

5 Conclusion

In summary, suitable protocols for all noncanonical amino acids were established by a couple of research groups, especially by Yokokawa and Joullié. Macrolactamization studies of Yao and co-workers identified the best position site for macrocyclization. So, the first synthesis of cyclomarin C (3) was thus achieved by a convergent strategy under high dilution conditions. Barbé and Kazmaier achieved the first total synthesis of cyclomarin A (1) in a linear straightforward route. In addition, this synthesis afforded by slight modifications also the natural product cyclomarin C (3) and simplified derivatives thereof for SAR studies, which are currently under investigation.

Acknowledgment

We thank Dr. Philipp Barbé for his enthusiasm and outstanding contribution.

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