Total synthesis: an enabling science

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Abstract
An improved synthesis for tryptophan-dehydrobutyryne diketopiperazine (TDD), a co-metabolite of the hybrid polyketide/non-ribosomal peptide hangtaimycin, starting from l-tryptophan is presented. Comparison to TDD isolated from the hangtaimycin producer Streptomyces spectabilis confirmed its S configuration. The X-ray structure of the racemate shows an interesting dimerisation through hydrogen bridges. The results from bioactivity testings of hangtaimycin, TDD and the hangtaimycin degradation product HTM$_{222}$ are given.

Introduction
Hangtaimycin (1, Scheme 1) was first isolated from Streptomyces spectabilis and shown to possess weak antimicrobial activity against Bacillus subtilis [1]. Together with a structural revision from 29Z to 29E configuration and further biological evaluation of its hepatoprotective properties, its biosynthetic gene cluster was recently identified [2]. The biosynthetic machinery is composed of a hybrid trans-acyltransferase (trans-AT, [3,4]) polyketide synthase (PKS) and non-ribosomal peptide synthase (NRPS) [2] with a dehydrating bimodule [5,6] involved in the installation of the remaining Z-configured double bond within the polyketide backbone [7]. Furthermore, a cytochrome P450 monoxygenase was recently shown to be responsible for the oxidation of deoxyhangtaimycin (3), a compound with antiviral activity, to 1 [8]. The thereby installed
hemiaminal function is also the breaking point for 1 into a larger lactone-polyene peptide fragment and a smaller fragment HTM$_{222}$ (2, named after its molecular mass of 222 Da) [2]. Another hangtaimycin co-metabolite in *S. spectabilis* [9] is tryptophan-dehydrobutyryl diketopiperazine (TDD, 4) that was already isolated several decades before the discovery of 1, and likewise reported to have no antibacterial activity [9]. The initially published structure was that of (E)-4 [9], but later revised as that of (Z)-4. The same compound is also observed in *S. olivaceus* [10] and was reported to function as a competitive inhibitor of glutathione S-transferase [11], which may be a result of a thiol addition of glutathione to the Michael acceptor in 4. While the relative and absolute configuration of hangtaimycin have not yet firmly been established, 2 is known to be S-configured and is derived from an l-alanine unit [2]. TDD (4) was recently suggested to be R-configured, containing a δ-tryptophan unit, based on a comparison of the optical rotation of the isolated compound ([α]$_D^{20} = -12.67$, $c$ 1.1, 95% EtOH [1]) to 4 synthesised from l-tryptophan ([α]$_D^{21} = +13$, $c$ 0.03, EtOH [12]), but the melting point of the synthetic material (mp 191–192 °C, for the compound numbered (Z)-32 in ref. [12]) did not match that of isolated TDD (mp 121–123 °C [9]), and conclusively the compounds that have been compared cannot be the same. This prompted the authors of the synthetic study to conclude on the need for a structural revision of 4 [12], with unclear reasoning for the newly assigned structure. However, this newly suggested structure of 4 is not reflected in the structure of 1 [1,2] and not supported by bioinformatic analysis of its biosynthetic gene cluster [2], although it seems reasonable to consider 4 as a degradation product of 1. Moreover, the originally reported optical rotation of 4 is positive ([α]$_D^{24.5} = +10.0$, $c$ 1.1, 95% EtOH [9]), in contrast to the later reported negative value mentioned above [1]. In order to resolve the confusion, we have reisolated 4 from *S. spectabilis* and report on an improved synthesis. Furthermore, the results from bioactivity testing with 1, 2 and 4 are discussed.

**Results and Discussion**

**Synthesis of TDD**

The first synthetic route towards 4 started from l-tryptophan (5) that was converted through a standard transformation into the methyl ester 6 and then through sequential reductive aminations with benzaldehyde and paraformaldehyde into 7 (Scheme 2) [13]. Cleavage of the benzyl group by catalytic hydrogenation afforded 8 that was coupled with tert-butylxoy-carbonyl (Boc)-protected threonine using bis(2-oxo-3-oxazolidinyl)phosphinocarbonyl (BOP)-Cl [14,15] and Hünig’s base to give 9. Cleavage of the Boc group with 5% TFA followed by
basic treatment resulted in the cyclisation to the dioxopiperazine 10. Acetylation and subsequent treatment with LiClO₄ and DBU is a common strategy for the dehydration of serine and threonine units in peptides [16], but unfortunately the acetylation of 10 failed. Interestingly, the direct treatment of 10 with LiClO₄ and DBU under prolonged reaction times (3 days) resulted in the elimination of water. This reaction proceeded with a high diastereoselectivity (Z/E = 8:1), giving access to 4 in a satisfactory yield of 29% over 6 steps. However, the optical rotation of the obtained material showed only a small positive value ([α]D²⁵ = +1.9, c 0.27, EtOH), suggesting that 4 had undergone racemisation during the prolonged basic treatment with DBU in the last step. This was confirmed by HPLC analysis on a chiral stationary phase, showing that the obtained target compound 4 was nearly racemic (Figure 1A).

Because of the configurational instability of 4 under base treatment, we aimed at an approach for the final elimination step.

**Scheme 2: First synthetic route towards TDD (4).**

**Figure 1:** HPLC analyses of (Z)-4 on a chiral stationary phase. A) Nearly racemic 4 from the first synthetic route (Scheme 2), B) enantiomerically enriched 4 (80% ee) from the second synthetic approach (Scheme 3), C) enantiomerically enriched 4 (90% ee) obtained under optimised conditions for the elimination reaction of 14 (Scheme 3), and D) natural enantiomerically pure (S)-TDD isolated from *S. spectabilis.*
using milder conditions (Scheme 3). The newly developed synthesis started from 7 that was Boc-protected at the indole to yield 11. Removal of the benzyl group by catalytic hydrogenation to 12 was followed by coupling with benzylloxycarbonyl (Cbz) and methoxymethyl (MOM)-protected threonine to give 13. Removal of the Cbz group by catalytic hydrogenation proceeded with spontaneous cyclisation to 14. With this material, the elimination of the MOM group smoothly proceeded by treatment with KH and 18-crown-6 in THF at 25 °C to 15, that upon removal of the Boc group with TFA and 1,3-dimethoxybenzene [17] gave (Z)-4 as a single diastereomer through anti elimination. Overall, TDD was obtained from L-tryptophan in a high yield of 37% over eight linear steps. HPLC analysis on a chiral stationary phase showed that 4 obtained through this second route was enantiomerically enriched (80% ee by peak integration, Figure 1B). Further improvement of the enantiomeric excess of 4 (90% ee, Figure 1C) was possible by performing the elimination reaction with 14 and KH and 18-crown-6 under ice cooling. This helped to suppress basic racemisation of 4, but required prolonged reaction times and gave a slightly diminished yield for 15 (70%), lowering the overall yield of TDD to 33% over eight steps. The major enantiomer of 4 obtained from this second route was identical to natural TDD (Figure 1D) which is thus S-configured, i.e., derived from L-tryptophan. Moreover, the olefinic double bond in 4 is Z-configured as indicated by a strong NOESY correlation between the amide NH and the neighbouring methyl group.

The structure of TDD

These findings not only challenge the originally assigned structure of (E)-4 [9] and confirm the structural revision of (Z)-4 [1], but also question the suggested structural revision that placed the N-methyl group at the other nitrogen of the dioxopiperazine moiety [12]. Moreover, the confusing situation about the absolute configuration and optical rotation are resolved through this work, clearly showing S-configuration for 4 that exhibits a negative optical rotation ([α]D25 = –15.5, c 0.102, MeOH). The reason for the varying melting points for 4 in the literature is unclear, but we noticed a pronounced difference in the crystallisation behaviour of racemic and enantiomerically pure 4. While (rac)-4 readily formed crystals (mp 134–136 °C), several attempts to crystallise (S)-4, a material that was obtained as a viscous oil, failed. The X-ray crystallographic analysis of (rac)-4 showed an interesting dimer interaction of its enantiomers through hydrogen bridges between the amide (NH-CO) groups (Figure 2, for crystallographic parameters cf. Supporting Information File 1, Table S1), that may support its easy crystallisation in comparison to enantiomerically enriched 4.

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**Scheme 3:** Second synthetic route towards TDD (Z)-4.
Bioactivity testing

Previous reports have mentioned that TDD (4) exhibits no antibacterial activity, without providing information about the test organisms used [9]. For this reason, and because of the above-mentioned confusions about the true nature of 4 in the previous literature, the bioactivity of natural (enantiomerically pure) 4 isolated from *S. spectabilis* was reinvestigated. For comparison, synthetic (rac)-(Z)-4 and its stereoisomer (rac)-(E)-4 (Scheme 2) were included in the bioactivity testing, as well as the previously synthesised HTM$_{222}$ (2) and 1 isolated from *S. spectabilis*. Neither 2 nor any of the stereoisomers of 4 showed antibacterial effects against a panel of Gram-positive and Gram-negative organisms (Supporting Information File 1, Table S2). Only 1 exhibited concentration-dependent growth retardation of the Gram-positive species *Bacillus subtilis* 168 and *Acinetobacter baumannii* 09987 (Figure 3A and B). However, growth inhibition was not strong enough to yield a clear MIC value, as the determination of a MIC requires complete cures of the same enantiomer can only lead to a chiral dimer that, if formed at all, may crystallise less efficiently.

Note that the dimer between the two enantiomers of 4 is achiral which allows for a regular packing of (rac)-4 in the crystal. In contrast, a hypothetical similar interaction between two molecules of the same enantiomer can only lead to a chiral dimer.
inhibition of visible bacterial growth and residual growth occurred up to the highest concentration tested (256 μg/mL). In the Gram-negative Escherichia coli, the outer membrane protects the cells from the impact of I. When the integrity of the outer membrane was compromised by adding the outer-membrane permeabilizing polymyxin B nonapeptide (PMBN, 10 μg/mL), a MIC of 128 mg/mL was achieved (Figure 3C).

We also investigated whether the reported inhibition of glutathione S-transferase [11] is a result of a Michael addition of glutathione to TDD. However, no reaction occurred between glutathione and TDD in DMF/H2O (1:1) under prolonged stirring at room temperature. Also the addition of base (NEt2) did not promote the reaction. Therefore, the mode of action of TDD towards glutathione S-transferase needs further investigation.

Conclusion
We have established an efficient synthesis of TDD that makes this compound available from L-tryptophan with a high yield of 33% (90% ee) over eight linear steps, establishing S configuration for the natural product from S. spectabilis that is likely reflected in the corresponding portion of hangtaimycin. A key step in the synthesis is the elimination of a MOM group using KH and 18-crown-6 that must be carried out with care, because TDD easily undergoes racemisation under basic conditions. The X-ray analysis showed an interesting dimer interaction of the enantiomers in racemic TDD through hydrogen bridges, that reflected in the corresponding portion of hangtaimycin. A key step in the synthesis is the elimination of a MOM group using KH and 18-crown-6 that must be carried out with care, because TDD easily undergoes racemisation under basic conditions. The X-ray analysis showed an interesting dimer interaction of the enantiomers in racemic TDD through hydrogen bridges, that may support its much easier crystallisation in comparison to enantiomerically pure or enriched TDD. In fact, the obtained viscous oils did not crystallise, suggesting that the previously reported melting point of 121–123 °C [9] that is close to our measured melting point for (rac)-4 (134–136 °C) may have been measured for isolated material after it had undergone (partial) racemisation. The reported inactivity of 4 against bacteria was confirmed in this study, and also 2 is an inactive metabolite of S. spectabilis, while for 1 moderate growth retardation against A. baumannii and B. subtilis, and growth inhibition against PMBN-treated E. coli was observed. However, the low activity of 1 in these assays suggests that the natural function of this structurally remarkable compound awaits future clarification.

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Supporting Information
Supporting Information File 1
Experimental, analytical and X-ray data as well as copies of NMR spectra.
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Abstract

This review summarizes examples for the application of vicinal ketoesters such as α-ketoesters, mesoxalic esters, and α,β-diketoesters as key intermediates in the total synthesis of natural products utilizing their electrophilic keto group as reactive site. Suitable key reactions are, e.g., aldol additions, carbonyl ene reactions, Mannich reactions, and additions of organometallic reagents. The vicinal arrangement of carbonyl groups allows the stabilization of reactive conformations by chelation or dipole control.

Introduction

Vicinal ketoesters contain a carbonyl group adjacent to an ester group. One keto group results in α-ketoesters 1 and two vicinal keto groups lead to α,β-diketo esters 2 (Scheme 1). On the other hand, two carboxylic acid functionalities adjacent to a keto group result in mesoxalic diesters 3, or mesoxalic ester amides 4. The increased electrophilicity of the keto group and the high density of these complex functional groups make such structures attractive as key intermediates for the total synthesis of natural products [1]. Thus, the high electrophilicity of the central carbonyl group in α,β-diketoesters 2 allows the formation of stable hydrates 5. In case of an enolizable position enolization (2→6) is facilitated.

The chemistry of vicinal polycarbonyl compounds such as vic-diketoesters has been investigated in depth by Wasserman, Parr [2] and Gleiter, Rubin [3]. Important contributions for the use of α,β-diketoesters in stereoselective transformations came from Doyle’s group [4,5]. One remarkable example is the diastereoselective intramolecular aldol addition of ketones such as 7 (Scheme 2) [5]. Brønsted-acid catalysis leads via a transition state 8 to the aldol 9, while the use of chelating Lewis acids results via 10 in the epimeric aldol 11.

This review is a collection of total syntheses of natural products where vicinal keto esters were used as key intermediates.
Scheme 1: Structures of vicinal ketoesters and examples for their typical reactivity.

Scheme 2: Doyle’s diastereoselective intramolecular aldol addition of α,β-diketoester.

For reasons of clarity and better comparability all syntheses are strongly summarized highlighting the key step only.

The presentation of the examples is structured in three parts:

1. α-Ketoesters as key intermediates: (+)-euphorikanin A, (−)-preussochromone A, (−)-preussochromone D, (−)-jiadifenoxolane A, palau’amine, jatrophen, (−)-hopeanol, (+)-campthotecin, isoretronecanol, corynoxine, (+)-gracilamine, (−)-irofulven.

2. Mesoxalic diester and ester amides as key intermediates: (+)-awajanomycin, (−)-aplaminal, cladoniamide G.

3. α,β-Diketoesters as key intermediates: preussochromones E and F.

Review

1. α-Ketoesters as key intermediates: (+)-Euphorikanin A

In the final step of the synthesis of (+)-euphorikanin A (16), an ingenane-derived diterpenoid with a 5/6/7/3-fused tetracyclic carbon skeleton, Carreira et al. used an intramolecular nucleophilic addition of an alkenyl metal species to the α-ketoester 15 (Scheme 3) [6]. The ketoester 15 was synthesized by a chiral pool approach starting from (+)-3-carene derived cycloheptenone 13 [7,8] and aldehyde 12 (accessible from (R)-Roche ester [9]) via the γ-lactone 14. The ketoester moiety was established by an enolate hydroxylation with Davis’ oxaziridine and subsequent oxidation using Dess–Martin periodinane. Initial attempts for the key step (15 → 16) like a Nozaki–Hiyama–Kishi reaction failed, but lithium–halogen exchange using t-BuLi at low temperatures gave the desired vinyllithium intermediate I which successfully added to the desired α-carbonyl group.

(−)-Preussochromone A

In 2020, the Koert group disclosed the synthesis of (−)-preussochromone A (24), a fungal metabolite with a highly substituted tetrahydrothiopyran core annulated to a chromene [10]. The tetrahydrothiopyran ring was closed by a Lewis-acid-promoted cycloisomerization of the α-ketoester 22, which can be described as a Friedel–Crafts-type reaction or an aldol reaction of an S,O-ketene acetal (Scheme 4). The re-
Required ketoester 22 was synthesized from sulfonylchromone 20, accessible from dihydroxyacetophenone 19 and thiol 18 derived from known alcohol 17 [11,12]. DMP oxidation of $\alpha$-hydroxyester 21 and subsequent cycloisomerization led to the desired cyclization product 23 via transition state 21 in a dr of 5:1. Final deprotection gave preussochromone A (24).
(-)-Preussochromone D
A similar approach was chosen in the synthesis of the structurally related natural product preussochromone D (30) reported by Koert et al. [13]. The synthesis commenced with the efficient production of alcohol 26 from 5-hydroxy-4H-chromen-4-one (25, Scheme 5) [14]. The ketoester moiety was build up via oxidation and nucleophilic addition of methyl diazoacetate, yielding alcohol 27. Subsequent oxidation gave α-ketoester 28 which was used in an intramolecular, Lewis acid-mediated aldol reaction, presumably via tridentate complex transition state III, to give diol 29 as a single diastereomer. Inversion of the secondary alcohol and deprotection gave preussochromone D (30).

(-)-Jiadifenoxolane A
The Illicium sesquiterpenes containing a seco-preizazaane carbon framework are highly oxidized, structurally complex natural products. Maimone et al. published a remarkable synthesis of the Illicium sesquiterpene (-)-jiadifenoxolane A (36), starting from the abundant sesquiterpene (+)-cedrol (31, Scheme 6) [15]. Through a series of finely tuned CH oxidations, cedrol (31) was converted to the lactone 32. In a single step, using Riley oxidation conditions, the methyl ketone moiety was transferred to the α-ketoester 33. Reduction, lactonization, and elimination gave the ketoesters-derived enol 34. Oxidation of the latter compound to the α-keto-β-hydroxy ester IV using DMO and subsequent heating in PhCF₃ triggered an α-ketol rearrangement which led to ketol V. Diastereoselective reduction gave α,β-dihydroxyester 35 which was converted to (-)-jiadifenoxolane A (36) in five further steps.

Palau’amine
Palau’amine (45), a dimeric pyrrole-imidazole-bisguanidine alkaloid, was first isolated from the marine sponge Stylotella aurantium in 1993 [16,17]. It received considerable attention from the synthetic community because of its broad range of biological activities and complex structure. In an early endeavour of L. Overman et al. in 1997 [18] towards the originally proposed structure of palau’amine (44), a [3 + 2]-dipolar cycloadition of α-ketoester 41 and the thiosemicarbazide 42-derived azomethine imine VI to the triazacyclopenta(cd)pentalenene 43 was utilized as a key step (Scheme 7) [18-21].

The α-ketoester 41 was accessible from amide 38, which in turn was obtained from allylic alcohol 37. Oxidation and Horner–Wadsworth–Emmons reaction with phosphonate 39 delivered the silyl enol ether 40, which was deprotected and
Scheme 6: Synthesis of an α-ketoester through Riley oxidation and its use in an α-ketol rearrangement in the synthesis of (−)-jiadifenoxolane A (36) [15].

cyclized via a Grubbs metathesis to α-ketoester 41. Subsequent cycloaddition delivered the advanced intermediate 43 in an efficient and elegant way.

Jatropha-5,12-diene
Towards the total synthesis of natural and unnatural jatrophane diterpenes, Hiersemann et al. used a highly efficient, intramolecular carbonyl-ene reaction of α-ketoester 49 (Scheme 8) [22]. The ketoester was synthesized by a Horner–Wadsworth–Emmons reaction of phosphonate 48 with aldehyde 47. Enantiopure aldehyde 47 was easily accessible from oxazolidinone 46 via Evans-aldol chemistry [23]. Heating of the α-ketoester 49 led to the highly substituted cyclopentanol 50 in a good dr of ≈5:1 (minor diastereomer not shown) via transition state VII where pseudo-1,3-strain is minimized. Nineteen further steps were necessary to give the naturally occurring jatrophen 51.

(−)-Hopeanol
In the synthesis of the polyphenolic natural product (−)-hopeanol (59), Nicolaou et al. used an α-ketoester moiety as a precursor for an intramolecular Friedel–Crafts cyclization (Scheme 9) [24]. Therefore, phenylacetaldehyde 52 was converted to the alcohol 53, which was esterified with the α-ketoacid 54 to give ketoester 55. Grignard addition to the keto carbonyl and subsequent TBS deprotection delivered the tertiary alcohol 56, which was dehydroxylated to the diastereomeric cations VIII and IX. Friedel–Crafts reaction gave diastereomeric lactones 57 and 58. The major diastereomer 58 could be converted to the complex polyphenol (−)-hopeanol (59) in seven further steps.

(+)-Camptothecin
In the formal synthesis of the pentacyclic, antiproliferative quinoline alkaloid camptothecin (65), Peters et al. used an α-ketoester moiety in an auxiliary controlled approach towards the only stereogenic center present in the natural product (Scheme 10) [25]. First, the ketoacid 60 was esterified with 8-phenylmenthol (61) to yield the α-ketoester 62, followed by nucleophilic addition of isopropenylmagnesium bromide to give α-hydroxyester 63 in excellent yield and diastereoselectivity. Eight additional steps gave the bicyclic compound 64 which was already known from previous camptothecin syntheses.
Isoretronecanol

The α-ketoester moiety can also be used in photochemical reactions, as shown by Gramain et al. in the synthesis of the pyrrolizidine alkaloid (rac)-isoretronecanol (69, Scheme 11) [26]. A Claisen condensation of the lithium enolate of N-acetyl-pyrrolidine (66) with diethyl oxalate gave the ketoester 67. Irradiation of compound 67 with a medium pressure mercury lamp in Pyrex® glassware triggered a 1,6-HAT leading to biradical X which combined to the racemic pyrrolizidine 68 as a 1:1 mixture of diastereomers. Three more steps gave the target compound 69 in 31% overall yield.

Corynoxine

Hiemstra et al. used the α-ketoester moiety for different purposes in the syntheses of a range of oxindole alkaloids. The start of the synthesis of (rac)-corynoxine (76) was the conversion of tryptamine (70) to oxindole 71, which was used in a chemoselective Mannich reaction with aldehyde 72, introducing the α-ketoester moiety (Scheme 12) [27].

The major trans-isomer 73 was further converted to the natural products corynoxine and rychnophylline. The minor cis-isomer 74 was used in an intramolecular Tsuji–Trost reaction, where the ketoester served as a nucleophile, which build up the piperidine ring and selectively set the desired cis-substitution. Subsequent transesterification gave the α-ketoester 75, which was used in a Wittig reaction. The undesired Z-configured double bond was isomerized to the E-alkene and final hydrogenation delivered corynoxine (76).

(+)-Gracilamine

The Mannich reaction was also used by Nagasawa et al. as a key step in the synthesis of (+)-gracilamine (83), a penta-
Scheme 8: Intramolecular diastereoselective carbonyl-ene reaction of an \(\alpha\)-ketoester in the synthesis of jatrophane diterpenoids [22].

Scheme 9: Grignard addition to an \(\alpha\)-ketoester and subsequent Friedel–Crafts cyclization in the synthesis of (-)-hopeanol (59) [24].
cyclic alkaloid isolated from the plant *Galanthus gracilis*, (Scheme 13) [28]. The synthesis started from readily available sesamol (79) and imine 78 which gave the advanced intermediate 80 in ten steps. An intramolecular Mannich reaction of compound 80 with α-ketoester 81 furnished compound 82 with the last ring of the target (+)-gracilamine (83), which was accessible after two further steps.

(−)-Irofulven

Irofulven (87) is a highly cytotoxic, semisynthetic drug obtained from the illudin sesquiterpene family. In a de novo synthesis towards (−)-irofulven (87), Movassaghi et al. used a Cu(II)-catalyzed asymmetric aldol reaction of O-silyl ketene S,O-acetal 84 with methyl pyruvate (85) to enantioselectively install the crucial tertiary TMS-protected alcohol in ester 86 (Scheme 14) [29]. Eleven further steps gave (−)-irofulven (87).

2. Mesoxalic diesters and ester amides as key intermediates

(+)-Awajanomycin

Diethyl mesoxalate (90a) is a valuable building block due to the high density of carbon atoms in high oxidation states. As a vic-ticarbonyl compound, its central keto group is an especially potent electrophile. The Koert group used this reactivity in their synthesis of (+)-awajanomycin (92), a marine natural product with a γ-lactone-δ-lactam core structure (Scheme 15) [30,31]. Key step was an asymmetric allylboration of diethyl mesoxalate (90a) with boronate 89, which was easily accessible through a Matteson homologation of dichloromethyl boronate 88. The reaction of (Z)-alkenyl boronate 89 with mesoxalate 90a delivered product 91 through the six-membered transition state XI. Eight further steps accomplished the total synthesis of (+)-awajanomycin (92).
(--)-Aplaminal
Dimethyl mesoxalate (90b) was used by Smith and Liu in the synthesis of the cytotoxic metabolite (--)-aplaminal (96), which was isolated from the sea hare *Aplysia kurodai* [32]. The natural product is characterized by a triazabicyclo[3.2.1]octane, where each bridge possesses a nitrogen atom. The synthesis commenced with N-Boc-serine (93) which was converted to secondary aniline 94 in three steps (Scheme 16). Subsequent deprotection and condensation with dimethyl mesoxalate (90b) gave imidazolidine 95. With compound 95 at hands, five further steps gave (--)-aplaminal (96) in a good overall yield of 19%.

Cladoniamide G
The unsymmetrical mesoxalic acid amide 102 was used by Koert et al. in the racemic synthesis of the bisindole alkaloid (rac)-cladoniamide G (103, Scheme 17) [33]. The synthesis...
Scheme 14: Enantioselective aldol reaction using an α-ketoester in the synthesis of (-)-irofulven (87) [29].

Scheme 15: Allylboration of a mesoxalic acid ester in the synthesis of (+)-awajanomycin (92) [30, 31].

Scheme 16: Condensation of a diamine with mesoxolate in the synthesis of (-)-aplaminal (96) [32].
started with benzaldehyde 97 and indole 99 which were converted to the indole building blocks 98 and 100, respectively. These were connected to bisindole 101, which reacted with mesoxalic ester amide 102 in a Friedel–Crafts reaction followed by a spontaneous lactamization to give (rac)-cladoniamide G (103). The mesoxalic ester amide 102 was synthesized from malonyl chloride 104 through amidation and Regitz diazotransfer, yielding diazo compound 105. Subsequent oxidation and dehydration of the resulting hydrate through short-path distillation gave the desired vic-tricarbonyl compound 102.

3. α,β-Diketoesters as key intermediates
Preussochromone E and F
In a short and enantioselective total synthesis of preussochromone E (110) and F (109), Koert et al. used the complex vic-tricarbonyl compound 108 to set two stereogenic centers and correct one via an intramolecular aldol addition (108 → 109; Scheme 18) [34]. The vic-tricarbonyl compound 108 was synthesized via DMDO oxidation from α-diazo-β-ketoester 107, which was easily accessible from 5-methoxy-4H-chromen-4-one (106). The thermodynamically controlled basic intramolecular aldol addition of compound 108 using the bulky amine base 2,6-di-tert-butyl-4-methylpyridine (DTBMP) led to epimerization of the methyl group and cyclization, giving preussochromone F (109) as single isolable diastereomer probably via transition state XII. The subsequent reduction of compound 109 gave preussochromone E (110).

Conclusion
The variety of examples prove that vicinal ketoesters are valuable synthetic intermediates for the synthesis of complex target structures such as natural products. α-Ketoesters, mesoxalic esters, and α,β-diketoesters can be used bearing an electrophilic keto group as reactive site. The vicinal arrangement of carbon-yl groups allows the stabilization of reactive conformations by chelation or dipole control. Suitable key reactions are e.g., aldol additions, carbonyl ene reactions, Mannich reactions, and addi-
Scheme 18: The thermodynamically controlled, intramolecular aldol addition of a vic-tricarbonyl compound in the synthesis of preussochromones E (110) and F (109) [34].

The presented examples may encourage the use of vicinal ketoesters in future applications, in particular in the field of natural product synthesis.

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Enantioselective total synthesis of putative dihydrorosefuran, a monoterpen with an unique 2,5-dihydrofuran structure

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Abstract
An original synthesis of the structure of dihydrorosefuran, a compound allegedly identified in Artemisia pallens and Tagetes mendocina, has been developed. The key steps in the five-step 36% overall yield synthesis are a CpTiCl₂ mediated Barbier-type allenylation of a linear aldehyde and the formation of a 2,5-dihydrofuran scaffold through a Ag(I)-mediated cyclization. Neither of the reported spectral data for dihydrorosefuran match those of the synthetic product, suggesting that the isolated compound from Tagetes mendocina is in fact the natural product rosiridol, while the real structure of the product from Artemisia pallens remains unknown.

Introduction
Artemisia pallens is an aromatic plant from southern India whose essential oil, known as Davana oil, has shown increasing interest mainly for its use in some beverages, cakes, pastries, etc., as well as in the perfumery industry [1]. In addition, A. pallens has been used in Indian traditional medicine (Ayurveda) for the treatment of measles, cough, cold, depression, diabetes, and high blood pressure [2]. More recently other biological activities have been reported, such as the blood glucose lowering effect of A. pallens [3,4], and its anti-asthmatic potential [5].
Results and Discussion

Our synthetic strategy is based on two metal-mediated steps (Scheme 1). In this way, we thought that the 2,5-dihydrofuran structural motif that is found in the target molecule 1 could be prepared through a Ag(I)-induced intramolecular addition of the hydroxy group to the terminal double bond of the allene in compound 3. Another key step is the Ti(III)-mediated straightforward synthesis of this α-hydroxyallene, which could be achieved through a regioselective Barbier-type coupling of a propargylic halide (1-bromo-2-butyne) with the aldehyde 4 mediated by the organometallic half-sandwich complex [CpTi^{III}Cl_2] [11,12].

Following this retrosynthetic proposal, our route starts from ethyl 4-oxobutanoate (4) [13] which was prepared by ozonolysis of commercially available ethyl pent-4-enoate (Scheme 2). Coupling of the aldehyde 4 with 1-bromobut-2-ynyl in the presence of CpTi^{III}Cl_2 (generated in situ by reduction of CpTiCl_3 with Mn) afforded α-hydroxyallene 3. We have recently described that this Barbier-type reaction affords α-hydroxyallenes as major products, mixed with smaller amounts of homopropargylic alcohols, either if the reaction is performed with stoichiometric amounts of CpTiCl_3 or if catalytic amounts are used [12]. However, using the particular substrates in this approach, the allenic compound 3 was exclusively formed, in a satisfactory 81% yield [14]. It is also important to control the pH during the reaction workup, as some contamination of the product with lactone 5 can arise at low pH values, which goes in detriment of the yield. The 2,5-dihydrofuran ring in target compound 1 was obtained through a Ag(I)-mediated intramolecular addition of the hydroxy to the allene group, a process that transformed allene 3 into compound 2. The isopropenyl residue of the target compound 1 was assembled through a two-step sequence. The first one was the addition of an excess of methylmagnesium bromide to the ester 2, that completed the carbon skeleton. The second step was the pH-controlled regioselective dehydration of the tertiary alcohol 6 with amberlyst-15® leading to the monoterpen 1. Other systems tested for the elimination of the hydroxy group in 6 were pyridinium p-toluenesulfonate (PPTS) and camphorsulfonic acid (CSA), that gave poorer results, failing to afford a single product. On the other hand, lactone 5 could also be transformed into alcohol 6 through a simple change in the order of the reactions: addition of methylmagnesium bromide to 5 afforded 7, which was then transformed into 6 by the Ag(I)-mediated cyclization (Scheme 2).

Once we had synthesized racemic compound 1, we designed a chiral version using a stereoselective kinetic resolution of allenol 3 via lipase AK-catalyzed acetylation [15]. In this way, unaltered, (−)-hydroxyallene 3 could be separated from (+)-acetyl derivative 9 through standard column chromatography.
phy (Scheme 3). Enantiomeric excesses of (−)-3 and (+)-9 were determined by chiral HPLC analyses. Analysis of the NMR data of the Mosher’s derivatives of 8 suggested (S) configuration for the alcohol (−)-3 [16].

On the other hand, enantiopure acetate (+)-(R)-9 was transformed into diol (+)-(R)-7 by the addition of an excess of MeMgBr. Finally, these enantiopure compounds, α-hydroxyallene (−)-(S)-3 and diol (+)-(R)-7, can be used to prepare both enantiomers of compound 1 following the procedures shown in Scheme 2.

Unfortunately, once the racemic synthesis was successfully completed and the chiral design was fulfilled, it was found that the spectroscopic data of compound 1 did not match neither with those published for the allegedly dihydorosefuran isolated from Artemisia pallens nor with those reported for the compound from Tagetes mendocina (see Table 1 and Table 2). The 13C NMR data of compound 1 are quite similar to those of the natural product isolated from T. mendocina, except for the signals of the oxygenated carbons (C2 and C5). The same behavior pattern can be observed in the 1H NMR data. This made us think that the natural product of T. mendocina could have an acyclic skeleton instead of a dihydrofuran one. For this reason, we propose this compound should be the diol called rosiridol (Table 1), a substance that has been isolated from other natural sources [18,19], whose structure was also confirmed by total synthesis some years ago [20]. Comparison of NMR data (Table 1) confirmed the initial suspicion. We are still intrigued about the real structure of the natural product isolated from A. pallens. However, it must be considered that this product was elucidated using low frequency NMR machines, which suggests that further research on the chemical composition of this oil is needed.

![Scheme 3: Racemic resolution of alleno 3 and synthesis of derivatives. a) Lipase AK, vinyl acetate, t-BuOMe, 30 °C, (−)-(S)-3: 46%, 90% ee, (+)-(R)-9: 39%, 95% ee; b) N,N′-dicyclohexylcarbodiimide (DCC), dimethylaminopyridine (DMAP), (S) or (R)-(−)-α-methoxy-α-(trifluoromethyl)phenylacetic acid, 66% [17], c) MeMgBr (5 equiv), Et2O, 67%.

| Table 1: 1H NMR data of isolated and synthetic products.α |
|----------------------------------------------------------|
| sources of claimed dihydorosefuran                        |
| A. pallensa,c [7,8]                                       |
| T. mendocina[9]                                           |
| this work synthesis                                      |
| literature synthesis                                     |
| H2  4.04 (td, J = 8.0, 1.0 Hz)                            |
| H4  5.07 (td, J = 8.0, 1.0 Hz)                            |
| H5  4.55 (dd, J = 7.0 Hz)                                 |
| H1′ 2.30 (m)                                              |
| H2′ 5.32 (t, J = 7.0 Hz)                                 |
| H4′ 1.67 (s)                                              |
| H5′ 1.60 (s)                                              |
| H1′ 2.05 (s)                                              |
| T. mendocina                                             |
| 4.03 (t, J = 8 Hz)                                       |
| 5.66 (t, J = 8 Hz)                                       |
| 4.21 (dd)                                                |
| 2.24 (m)                                                |
| 5.13 (t, J = 8 Hz)                                       |
| 1.75 (s)                                                 |
| 1.66 (s)                                                 |
| 2.05 (s)                                                 |
| 5.51 (br s)                                              |
| 4.62–4.53 (m)                                            |
| 2.41 (m)                                                 |
| 2.20 (dd, J = 14.0, 7.0, 6.5 Hz)                         |
| 5.21 (t, hept, J = 6.9, 1.5 Hz)                          |
| 1.74 (s)                                                 |
| 1.66 (s)                                                 |
| 1.69 (s)                                                 |
| 1.73 (d, J = 1.1 Hz)                                     |
| 2.82–2.19 (m)                                            |
| 5.13–5.09 (m)                                            |
| 1.67 (s)                                                 |

αCDCl3 in all cases; bArbitrary numbering for comparison purposes; c80 MHz; d400 MHz; e500 MHz.
Conclusion

In summary, we have proved that the two-step sequence Ti(III) allenylation–Ag(I) cyclization is a simple and efficient strategy for the preparation of the 2,5-dihydrofuran moiety present in many natural products. In fact, we have achieved the total synthesis of the 2,5-dihydrofuran structure 1. After systematic data analysis of our prepared compound and those in the literature, it can be concluded that the proposed structure of the product isolated from *Artemisia pallens* oil, dihydrorosefuran, is not correct. In addition, it is clear that the compound isolated from *Tagetes mendocina* is the acyclic diol named rosiridol.

Experimental

**Ti-induced allenylation of ethyl 4-oxobutanoate (4)**

Under an Ar atmosphere, dry THF (8 mL) that was deoxygenated prior to use was added to a mixture of CpTiCl₃ (329 mg, 1.50 mmol) and Mn dust (165 mg, 3.00 mmol) resulting in a green suspension. Then, a solution of ethyl 4-oxobutanoate (4, 196 mg, 1.50 mmol) and 1-bromobut-2-yn (0.27 mL, 3.00 mmol) in THF (2 mL) was dripped and the mixture was stirred for 2.5 hours. The mixture was filtered, diluted with EtOAc, washed with 3% HCl and brine, and dried (anhydrous MgSO₄), and the solvent was removed. The residue was purified by flash chromatography (n-hexane/EtOAc 8:2) to afford ethyl 4-hydroxy-5-methylhepta-5,6-dienoate (3, 225 mg, 81%) isolated as light yellow oil. IR (ATR) ν (cm⁻¹): 3434, 2972, 2928, 1581, 1573, 1436, 1374, 1172, 1028, 925, 853; ¹H NMR (300 MHz, CDCl₃) δ 4.77 (dq, J = 2.3, 3.2 Hz, 2H), 4.12 (q, J = 7.1 Hz, 2H), 4.05 (m, 1H), 2.42 (t, J = 7.2 Hz, 2H), 2.18 (s, 1H), 2.04–1.77 (m, 2H), 1.70 (td, J = 0.5, 3.2 Hz, 3H), 1.25 (t, J = 7.1 Hz, 3H) ppm; ¹³C NMR (75 MHz, CDCl₃, DEPT) δ 204.8 (C), 174.0 (C), 101.6 (C), 77.0 (CH₂), 71.5 (CH), 60.5 (CH₂), 30.4 (CH₂), 30.0 (CH₂), 14.5 (CH₃), 14.2 (CH₃) ppm; HRMS–ESI (Q-TOF, m/z): [M + H]⁺ calcd for C₁₀H₁₇O₃, 185.1178; found, 185.1158. A lactone is formed as side product (0–10%) when the HCl solution used for the workup has a concentration higher than 3%. Compound 5: IR (ATR) ν (cm⁻¹): 2982, 2927, 2960, 1762, 1427, 1331, 1162, 974, 918, 855; ¹H NMR (300 MHz, CDCl₃) δ 4.89 (m, 1H), 4.86 (m, 2H), 2.55 (m, 2H), 2.30 (m, 2H), 1.79 (t, J = 3.1 Hz, 3H) ppm; ¹³C [¹H] NMR (75 MHz, CDCl₃, DEPT) δ 206.0 (C), 177.0 (C), 98.0 (C), 80.4 (CH), 77.5 (CH₂), 28.5 (CH₂), 26.1 (CH₃), 15.0 (CH₃) ppm; HRMS–ESI (Q-TOF, m/z): [M + H]⁺ calcd for C₉H₁₇O₂, 139.0759; found, 139.0782.

Silver(I)-promoted cyclization of ethyl 4-hydroxy-5-methylhepta-5,6-dienoate (3)

A solution of the allenol 3 (65 mg, 0.35 mmol) in acetone (2 mL) was added to a suspension of AgNO₃ (120 mg, 0.70 mmol) in acetone (1.5 mL) in the absence of light, and the mixture was stirred at 40 °C overnight. Brine was added and the mixture was extracted with Et₂O. The organic phase was dried over anhydrous MgSO₄, and concentrated under reduced pressure. The residue was purified by silica gel flash column chromatography (n-hexane/EtOAc 9:1) to afford ethyl 3-(3-methyl-2,5-dihydrofuran-2-yl)propanoate (2, 57 mg, 88%) isolated as colorless oil. IR (ATR) ν (cm⁻¹): 2969, 2927, 2849, 1731, 1442, 1376, 1251, 1160, 1092, 1026, 895, 734; ¹H NMR (300 MHz,
Supporting Information

Supporting Information File 1
Experimental procedures, characterization of other substances, and copies of IR, NMR spectra and HPLC chromatograms.

[https://www.beilstein-journals.org/bjoc/content supplementary/1860-5397-18-132-S1.pdf]

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Preparation of an advanced intermediate for the synthesis of leustroducsins and phoslactomycins by heterocycloaddition

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Abstract

A convergent strategy for the synthesis of leustroducsins and phoslactomycins has been designed, relying on the synthesis and the coupling of three main fragments. The central fragment was synthesized via a regio- and stereoselective nitroso Diels–Alder reaction with an enol phosphate, followed by reductive cleavage of the phosphate to the ketone \(11b\). Coupling studies of this fragment with the lactone fragment was accomplished in a stereoselective fashion through a vinyllithium intermediate. An advanced synthetic intermediate was then obtained after functional group transformation.

Introduction

Leustroducsins 1a–c and phoslactomycins 2a–f are a family of closely related natural products extracted from *Streptomyces platensis* (leustroducsins) or *Streptomyces nigresens* (phoslactomycins) [1-4]. The main difference within this large family is the presence of an additional ester substituent on the terminal cyclohexane ring. Common structural motifs include a polyunsaturated acyclic chain with an unsaturated lactone ring and an amine-containing side chain (Figure 1).

These natural products have attracted much attention due to their original structure and to their activity as inhibitors of the serine/threonine phosphatase enzyme PP2A [5,6]. Therefore, phoslactomycins [7-12] and leustroducsins [13-17] have been subject of extensive synthetic studies.

In a project related to the synthesis of leustroducsins and phoslactomycins, we have designed a convergent synthetic strategy involving the preparation and the coupling of three main fragments (Figure 2): the lactone fragment 3, the central fragment 4 and the cyclohexane fragment 5. We have previously described the enantioselective synthesis of the lactone fragment 3 [18]; we now disclose the synthesis of the oxazi-
none 4 and attempts for coupling both fragments for the synthesis of an advanced intermediate.

The synthetic strategy for the synthesis of the central fragment takes advantage of the proximity between the terminal amino function and the hydroxy function at C9. It was anticipated that both functions could arise from the cleavage of a N–O bond from an 1,2-oxazine, itself obtained by a nitroso Diels–Alder reaction from a chiral nitroso derivative and a functionalized diene (Figure 3). The nitroso Diels–Alder cycloaddition reaction has been well studied and has been used as a powerful tool for synthesis [19-22].

We have reported extensive studies on the regio-and stereoselectivity of nitroso Diels–Alder reactions between various nitroso derivatives and functionalized dienes [23]. These studies led to the selection of enol phosphates as ketone precursors for the diene functionalization. Enol phosphates display several advantages over the related enol silyl ethers [24,25]: they are more stable towards acidic conditions, their electronic character contributes to high regioselectivity in cycloaddition reactions, and they can be converted to many other functions, including their hydrolysis to ketones [26]. In the other hand, we have shown that the Wightman reagent, a chiral chloronitroso deriviative [27], led to a complete regio- and stereoselective reaction with functionalized dienes (Scheme 1). The chiral auxiliary contributes to both regioselectivity and stereoselectivity. After hydrolysis of the chiral auxiliary and Boc-protection of the nitrogen atom, cycloadduct 8 was obtained in 55% yield and 90% ee. Therefore, the combination of both these reagents
Figure 3: strategy for the synthesis of central fragment 4: nitroso Diels–Alder reaction.

Scheme 1: A highly regio-and stereoselective nitroso Diels–Alder cycloaddition between Wightman’s reagent 6 and a dienic enol phosphate.

Results and Discussion

Asymmetric cycloaddition

Preliminary studies for the conversion of enol phosphate to the corresponding ketone were accomplished using an unprotected primary alcohol. However, it appeared that hydroxy group protection was necessary: control experiments made on the racemic cycloadduct 8 showed that basic hydrolysis of the enol phosphate led to the cyclic hemiacetal 9 in modest yield (Scheme 2).

Therefore, compound 8 was protected as silyl or benzyl ether using standard techniques. Unfortunately, no hydrolysis under several basic conditions provides the target ketone, no conversion and/or decomposition being observed (Scheme 3).

Enol phosphates can be hydrolysed under basic, acidic or reductive conditions [26]. Although acidic conditions could not be used due to the lability of the nitrogen Boc-protecting group, we found that the TIPS-protected cycloadduct 10b could be cleanly transformed into the ketone 11b with excess Red-Al [28], together with a small amount of the over reduced alcohol 12b,
which could be reoxidized to 11b (Scheme 4). Other substrates failed to deliver appreciable yields of the ketone under the same conditions.

These studies validate the role of TIPS ether as protecting group for the primary alcohol. At this stage we wondered whether it was possible to perform the whole synthetic sequence with this protecting group. Accordingly, the enol phosphate 13 was synthesized in five steps (26% overall yield) from 1,4-butanediol (Scheme 5). Since cycloaddition with the Wightman reagent 6 releases hydrogen chloride in the reaction medium, it was found necessary to add a small amount of calcium carbonate. Optically active cycloadduct 10b was obtained in 73% yield and 86% ee after nitrogen protection as its Boc-carbamate. Ketone
Scheme 6: Synthesis and derivatization of the lactone fragment.

We first attempted the coupling with the terminal alkyne 19, anticipating the possibility of reducing the triple bond after coupling reaction. In agreement with literature precedents, we chose LiHMDS for deprotonation of 19 [30,31]. However, condensation of the corresponding lithium acetylide to the ketone 11b gave modest and non-reproducible yields of the desired product 22 (Scheme 7, Table 1). The configuration of the newly created stereogenic center was undetermined.

We have therefore completed a quick, efficient and selective access to the central core of leustroducsins/phoslactomycins using an asymmetric nitroso Diels–Alder reaction. This fragment displays a ketone function that will be used for coupling with the lactone fragment 3 by generation of the tertiary alcohol.

Studies in fragment coupling

We have previously reported the synthesis of the lactone fragment by catalytic asymmetric [2 + 2] cycloaddition followed by ring extension [18]. The initial product was the TMS-acetylene 18 which could be easily desilylated to give 21. However, model studies for coupling revealed the incompatibility of the lactone function; therefore, it was reduced with DIBAL-H then transformed into 19 by a one pot acetalization–desilylation procedure (91:9 mixture of diastereomers) [17]. Hydrozirconation followed by treatment with iodine furnished the target vinyl iodide 20 (Scheme 6); iodination with NIS, as previously described [29], gave lower yields.

Table 1: Coupling reaction between alkyne 19 and ketone 11b.

| entry | n₁ | n₂ | conditions                     | yield |
|-------|----|----|--------------------------------|-------|
| 1     | 1  | 1,2| –78 °C, 15 min, then rt, 8 h   | 21%   |
| 2     | 1  | 1,2| –78 °C, 2 h, then rt, 16 h     | 16%   |
| 3     | 1,5| 1,8| –78 °C, 2 h, then rt, 3 h      | 24%   |
| 4     | 1,5| 1,6| –78 °C, 2 h, then rt, 4 h      | 39%   |

These experiments showed the necessity to perform a fast reaction in order to avoid degradation. The optimal amount of base was found to be 1.6 equivalents (Table 1, entry 4). Higher

11b was obtained by Red-Al reduction in identical yield to the racemic equivalent.
amounts lowered the yields (Table 1, entry 3), probably due to competitive enolization of the cyclic ketone. Excess alkyne was also necessary, as low yields were obtained when using equimolar amounts of both 19 and 11b (Table 1, entries 1 and 2).

These disappointing results with alkyne 19 prompted us to investigate the coupling with an organometallic reagent derived from vinyl iodide 20. This reagent was already synthesized and coupled with acyclic ketones in previous syntheses of leustroducins or phoslactomycins [7-17]. Thus, treatment of 20 with n-butyllithium in THF gave the organometallic intermediate which was condensed onto ketone 11b (Scheme 8, Table 2). Since no product was obtained under these standard conditions, we considered the use of additives in order to make the organolithium intermediate more nucleophilic. However, no reaction was observed when ZnMe$_2$ (which was used in the synthesis of leustroducin B by Trost and co-workers [17]) was added; trimethylaluminum and cerium chloride also failed to promote the reaction. However, switching the solvent from THF to toluene afforded 21% of product 23 with CeCl$_3$ as additive. It appeared that the solvent had more influence on the course of the reaction than the metal. Indeed, reaction between vinyl iodide and ketone with n-BuLi in toluene [32] without any additive gave a reproducible 46% yield of 23. Optimal conditions were obtained using 1.8 equivalents of vinyl iodide and 1.7 equivalents of BuLi (Table 2, entry 6).

It was difficult at this stage to determine the stereoselectivity of the coupling reaction since the starting acetal in 20 was a mixture of diastereomers. Therefore, we decided to oxidize the acetal in 23 to the corresponding lactone (Scheme 9). The acetal was first hydrolyzed to the hemiacetal 24 in quantitative yield. Oxidation of 24 proved delicate due to the lability of the tertiary allylic alcohol, and the presence of acid-sensitive protecting groups. Several conditions were tested: silver oxide on celite [33] failed to give any conversion. PCC with sodium acetate [34] gave only traces of the target lactone 25. However, the use of the Jones’ reagent gave reproducible yields of 25, together with the deprotected alcohol 26. Under optimized conditions (1.15 equiv, 15 min) a combined 46% yield could be obtained. Higher equivalents of the oxidizing reagents or longer reaction time considerably lowered the yields.

NMR analysis of products 25 and 26 showed these compounds were obtained as single diastereomers, thus indicating the complete stereoselectivity of the coupling reaction. This validates the overall strategy for the synthesis of leustroducins or phoslactomycins by the synthesis of a central cyclic core and its coupling with the other fragments.

**Conclusion**

We have synthesized an advanced intermediate for the total synthesis of leustroducins and phoslactomycins using a highly regio- and stereoselective nitroso Diels–Alder reaction, and a coupling reaction between a ketone and a vinyl lithium reagent. This strategy offered quick and stereoselective access to an advanced precursor to these natural products. Further studies concerning the completion of the total synthesis via the preparation and coupling of the fragment 5 is under study in our laboratory.
Experimental

Unless otherwise stated, all reactions were conducted in oven-dried glassware under an atmosphere of dry argon. Tetrahydrofuran was distilled over sodium/benzophenone ketyl under argon. Acetonitrile, dichloromethane, DMSO, DMF and toluene were distilled over calcium hydride under argon. All other reagents were used as received. Chromatographic purifications refer to flash chromatography on silica gel.

**1H NMR spectra** were measured at 250, 300, 360 or 400 MHz using CDCl₃ as solvent using residual chloroform (7.26 ppm) as an internal reference.

**13C NMR spectra** were measured at 62.5, 75 or 90 MHz using residual chloroform (77.1 ppm) as an internal reference. High-resolution mass spectrometry (HRMS) analyses were conducted with electro spray ionization (ESI).

**6-Triisopropylsilyloxyhex-1-en-3-one (16):** A solution of oxalyl chloride (0.49 mL, 5.75 mmol, 1.5 equiv) in dichloromethane (12 mL) was cooled to −78 °C and DMSO (0.82 mL, 11.49 mmol, 3 equiv) was added over 5 min. After 15 min, a solution of the alcohol [35] (1.044 g, 3.83 mmol) in dichloromethane (5 mL) was added over 5 min. The reaction mixture was stirred for 30 min at −78 °C before addition of triethylamine (2.7 mL, 19.15 mmol, 5 equiv). The cooling bath was removed and the solution was allowed to warm to rt in 30 min. It was then poured into diethyl ether (50 mL) and the solution was successively washed with saturated aqueous CuSO₄ solution (4 × 12.5 mL), saturated aqueous NH₄Cl solution (3 × 12.5 mL), dried (MgSO₄), filtered and concentrated under reduced pressure to give a brown oil (1.021 g, 99%). 

**Rf:** 0.59 (10% AcOEt/cyclohexane); **1H NMR** (300 MHz, CDCl₃) δ 6.32 (dd, J = 17.7, 10.2 Hz, 1H), 6.19 (dd, J = 17.7, 1.5 Hz, 1H), 5.78 (dd, J = 10.2, 1.5 Hz, 1H), 3.68 (t, J = 6 Hz, 2H), 2.68 (t, J = 7.2 Hz, 2H), 1.86−1.77 (m, 2H), 1.00 (m, 21H) ppm; **13C NMR** (75 MHz, CDCl₃) δ 200.8, 136.7, 127.9, 62.4, 35.9, 27.2, 18.0, 12.0 ppm; HRMS (m/z): [M + Na]⁺ calcd 293.1907; found, 293.1898.

**6-R-(diethoxyphosphoryloxy)-6-(trisopropylsilyloxy)hexa-1,3-dien (17):** A 0.5 M solution of potassium hexamethyldisilazide in toluene (4.4 mL, 2.22 mmol, 1.2 equiv) was added to a cooled (−78 °C) solution of diethyl chlorophosphate (0.27 mL, 1.85 mmol) in anhydrous THF (7 mL). A solution of the enone [16] (500 mg, 1.85 mmol) in THF (6 mL) was then slowly added. The solution was stirred 30 min at −78 °C, then 1 h at 0 °C and then 1 h at rt, before being poured in diethyl ether (35 mL). The solution was washed with 5% aqueous ammonia solution (18 mL). The aqueous layer was extracted with diethyl ether (3 × 35 mL) and the combined organic layers were dried (MgSO₄), filtered and concentrated under reduced pressure to give a brown oil. Purification by column chromatography (25% AcOEt/cyclohexane) gave the enol phosphate [17] as a yellow oil (200 mg, 26%). 

**Rf:** 0.47 (30% AcOEt/cyclohexane); **1H NMR** (360 MHz, CDCl₃) δ 6.15 (dd, J = 17.3, 10.8 Hz, 1H), 5.47 (d, J = 17.3 Hz, 1H), 5.29 (dt, J = 7.2, 1.4 Hz, 1H), 5.08 (d, J = 10.8 Hz, 1H), 4.15−4.12 (m, 4H), 3.71 (t, J = 6.5 Hz, 2H), 2.48 (2dt, J = 7.2, 6.5 Hz, 2H), 1.31 (dt, J = 6.8, 1.1 Hz, 6H), 1.01 (m, 21H) ppm; **13C NMR** (90 MHz, CDCl₃) δ 146.2, 131.9, 118.0, 114.2, 64.4, 62.4, 30.0, 18.0, 16.2, 12.0 ppm; HRMS (m/z): [M + H]⁺ calcd 407.2377; found, 407.2359.

**6R-R-(diethoxyphosphoryloxy)-6-(2-(trisopropylsilyl)oxy)ethyl)-3,6-dihydro-2H-1,2-oxazine-2-carboxylate (10b):** A solution of the enol phosphate [17] (420 mg, 1.03 mmol) in chloroform (1.8 mL) was added to a solution of the Wightman reagent 6 (981 mg, 2.06 mmol, 2 equiv), calcium carbonate (206 mg, 2.06 mmol, 2 equiv) and water (40 µL,
2.06 mmol, 2 equiv) in isopropanol (1.8 mL). The mixture was stirred at rt for 30 h. Water (0.75 mL) was added and the solution stirred for additional 1 h. The pH was adjusted to 8 by addition of saturated aqueous NaHCO₃ solution (1.6 mL), and a solution of Boc-O (899 mg, 4.12 mmol, 4 equiv) in chloroform (0.8 mL) was added. The solution was stirred at rt for 64 h and poured into a mixture of water (37 mL) and dichloromethane (74 mL); the layers were separated and the aqueous layer extracted with dichloromethane (3 × 74 mL). The combined organic layers were dried (MgSO₄), filtered and concentrated under reduced pressure. Purification of the crude product by column chromatography (30% AcOEt/cyclohexane) gave the cycloadduct 10b as a yellow oil (404 mg, 73%). Rf 0.42 (30% AcOEt/cyclohexane). ¹H NMR (360 MHz, CDCl₃) δ 5.69 (m, 1H), 4.57 (broad d, J = 9.4 Hz, 1H), 4.16 (q, J = 7.2 Hz, 4H), 4.12–4.00 (m, 2H), 3.99–3.82 (m, 2H), 2.03–1.85 (m, 2H), 1.47 (s, 9H), 1.34 (t, J = 7.2 Hz, 6H), 1.05 (m, 21H) ppm; ¹³C NMR (90 MHz, CDCl₃) δ 154.8, 146.8, 105.1, 81.7, 75.2, 64.8, 59.3, 43.7, 33.7, 28.4, 18.1, 16.2, 12.1 ppm; HRMS (m/z): [M + Na]+ calcd 560.2779; found, 560.2780; [α]D²⁰ +37.4 (c 0.5, CH₂Cl₂).

(5S,6R)-5-Ethyl-6-ethylidene-5,6-dihydro-2H-pyran-2-one (21): Caesium fluoride (290 mg, 1.91 mmol, 1.3 equiv) was added to a solution of the lactone 18 [6] (327 mg, 1.47 mmol) in anhydrous acetonitrile (15 mL). The solution was stirred at rt; after 2 h 20 min, additional CsF (112 mg, 0.74 mmol, 0.5 equiv) was added. After a total time of 3 h 30 min, the solution was portioned between diethyl ether (70 mL) and water (35 mL). The layers were separated, the organic layer was washed with saturated aqueous NaCl solution (35 mL). The combined aqueous layers were extracted with diethyl ether (2 × 70 mL). The organic layers were combined, dried (MgSO₄), filtered and concentrated under reduced pressure. Purification of the residue by column chromatography (25% Et₂O/pentane) gave 21 as a yellow oil (171 mg, 77%). Rf 0.03 (30% Et₂O/pentane). ¹H NMR (250 MHz, CDCl₃) δ 6.79 (dd, J = 9.8, 3.5 Hz, 1H), 6.05 (dd, J = 10.0, 2.0 Hz, 1H), 5.16 (dd, J = 4.8, 2.3 Hz, 1H), 2.68–2.59 (m, 1H), 2.56 (d, J = 2.0 Hz, 1H), 1.86–1.62 (m, 2H), 1.04 (t, J = 7.3 Hz, 3H) ppm; ¹³C NMR (90 MHz, CDCl₃) δ 162.5, 148.8, 120.3, 77.4, 76.6, 70.7, 38.2, 22.6, 10.9 ppm; HRMS (m/z): [M + Na]+ calcd 173.0573; found, 173.0572; [α]D²⁰ +132.0 (c 1.0, CH₂Cl₂).

(2R,3S,6RS)-3-Ethyl-2-ethylidene-3,6-dihydro-2H-pyran (19): This compound was prepared according to reference [18]. A solution of the lactone 18 (1.23 g, 5.53 mmol) in anhydrous dichloromethane (10 mL) was cooled to −78 °C and a solution of DIBAL-H in toluene (1.2 M, 6 mL, 7.19 mmol, 1.3 equiv) was added dropwise. The reaction mixture was stirred at −78 °C for 30 min then poured into a NaHCO₃ solution (5 mL). The layers were separated and the aqueous layer extracted with ethyl acetate (3 × 10 mL). The combined organic layers were dried (MgSO₄), filtered and concentrated under reduced pressure. The residue (1.3 g) was redissolved in anhydrous methanol (25 mL) and paratoluene sulfonic acid hydrate (53 mg, 0.277 mmol, 0.05 equiv) was added. After stirring 1 h at rt, solid K₂CO₃ (1.53 g, 11.06 mmol, 2 equiv) was added and the mixture stirred overnight at rt. Diethyl ether (50 mL) was added and the solution washed with water (2 × 50 mL). The organic layer was dried (MgSO₄), filtered and carefully concentrated under reduced pressure.
pressure. Purification by column chromatography (5% Et₂O/pentane), gave 19 as a colourless oil (866 mg, 94%, 91/9 mixture of stereoisomers). Analytical data were in agreement with literature data [18].

(2R,3S,6RS)-3-Ethyl-2-((E)-2-iodovinyl)-6-methoxy-3,6-dihydro-2H-pyran (20): This compound was prepared according to reference [18].

A solution of the alkyne 19 (300 mg, 1.80 mmol) in anhydrous dichloromethane (4.2 mL) was added dropwise to a suspension of Cp₂ZnHCl (696 mg, 2.70 mmol, 1.5 equiv) in anhydrous dichloromethane (9 mL). After stirring at rt for 15 min, a solution of iodine (777 mg, 3.06 mmol, 1.7 equiv) in anhydrous dichloromethane (9 mL) was added dropwise until a light brown solution was obtained. The reaction mixture was hydrolyzed by successive addition of a saturated aqueous Na₂S₂O₃ solution (25 mL) and water (9 mL). The layers were separated and the organic layer was washed with water (9 mL). The combined aqueous layers were back-extracted with diethyl ether (2 × 40 mL). The combined organic layers were dried (MgSO₄), filtered and concentrated under reduced pressure. Purification of the residue by column chromatography (2.5% Et₂O/pentane) gave 20 as a yellowish oil (347 mg, 65%, 91/9 mixture of stereoisomers). Analytical data were in agreement with literature data [18].

Coupling reaction between vinyl iodide 20 and ketone 11b: A solution of the vinyl iodide 20 (283 mg, 0.962 mmol, 1.8 equiv) in anhydrous toluene (2 mL) was cooled to −78 °C, and a n-butyllithium solution (2.3 M in hexanes, 0.39 mL, 0.909 mmol, 1.7 equiv) was added dropwise. The solution was stirred for 30 min at −78 °C then a solution of ketone 11b (215 mg, 0.535 mmol, 1 equiv) in toluene (3.8 mL) was slowly added. The reaction was stirred at −78 °C for 45 h than slowly warmed to rt over 20 h. The reaction as quenched by addition of a saturated aqueous NH₄Cl solution (3.8 mL) was slowly added. The reaction mixture was filtered and concentrated under reduced pressure. Purification of the residue by column chromatography (25 to 40% AcOEt/cyclohexane) gave first the protected lactone 25 as a sticky yellow oil (42 mg, 29% over two steps), further elution with 100% AcOEt gave the unprotected alcohol 26 (18 mg, 17%).

tert-Butyl (6R)-5-(((E)-2-((2S,3S)-3-ethyl-6-oxo-3,6-dihydro-2H-pyran-2-yl)vinyl)-5-hydroxy-6-(2-((trisopropylsilyl)oxy)ethyl)-1,2-oxazinan-2-carboxylate (25): Data for 25: Rf: 0.10 (30% AcOEt/cyclohexane); 1H NMR (360 MHz, CDCl₃) δ 6.97 (dd, J = 9.7, 5.5 Hz, 1H), 6.05 (d, J = 9.7 Hz, 1H), 5.95 (dd, J = 15.5, 4.2 Hz, 1H), 5.82 (dd, J = 15.5, 1.4 Hz, 1H), 5.02 (ddd, app td, J = 4.2, 4.2, 1.4 Hz, 1H), 3.99–3.90 (m, 3H), 3.76–3.69 (m, 1H), 3.55 (td, J = 13.1, 2.7 Hz, 1H), 2.44–2.37 (m, 1H), 1.90–1.70 (m, 2H), 1.67–1.57 (m, 3H), 1.49 (s, 9H), 1.45–1.39 (m, 1H), 1.05 (m, 21H), 0.93 (t, J = 7.5 Hz, 3H) ppm; 13C NMR (62.5 MHz, CDCl₃) δ 163.9, 155.1, 150.1, 135.5, 125.1, 121.0, 82.6, 81.8, 79.8, 70.8, 59.0, 42.3, 39.4, 35.9, 31.3, 28.4, 21.8, 18.1,12.0,11.1 ppm; HRMS (m/z): [M + Na]⁺ calcld 576.3327; found, 576.3330; [α]D₂⁰ +86.3 (c 1.1, CH₂Cl₂).

tert-Butyl (6R)-5-(((E)-2-((2S,3S)-3-ethyl-6-oxo-3,6-dihydro-2H-pyran-2-yl)vinyl)-5-hydroxy-6-(2-hydroxyethyl)-1,2-oxazinan-2-carboxylate (26): Data for 26: Rf: 0.38 (80% AcOEt/cyclohexane); 1H NMR (400 MHz, acetone-d₆) δ 7.09 (dd, J = 10.0, 5.2 Hz, 1H), 6.02 (dd, J = 15.6, 5.5 Hz, 1H), 5.97 (dd, J = 10.0, 1.2 Hz, 1H), 5.85 (dd, J = 15.6, 1.2 Hz, 1H), 5.06 (dd, J = 5.5, 4.0,1.2 Hz, 1H), 4.27 (s, exchangeable with D₂O, 1H), 3.96–3.91 (m, 2H), 3.74–3.66 (m, 2H), 3.63–3.59 (m, 1H), 2.61–2.53 (m, 1H), 1.95–1.83 (m, 2H), 1.73–1.67 (m, 1H), 1.67–1.55 (m, 2H), 1.49 (s, 9H), 1.47–1.38 (m, 1H), 0.94 (t, J = 7.6 Hz, 3H) ppm; 13C NMR (100 MHz, acetone-d₆) δ 163.83,
156.0, 151.0, 137.2, 126.1, 121.2 ppm; HRMS (m/z): [M + Na]+ calc 420.1993; found, 420.1970.

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Solid-phase total synthesis and structural confirmation of antimicrobial longicatenamide A

Takumi Matsumoto, Takefumi Kuranaga*, Yuto Taniguchi, Weicheng Wang and Hideaki Kakeya*

Abstract

Longicatenamides A–D are cyclic hexapeptides isolated from the combined culture of *Streptomyces* sp. KUSC_F05 and *Tsukamurella pulmonis* TP-B0596. Because these peptides are not detected in the monoculture broth of the actinomycete, they are key tools for understanding chemical communication in the microbial world. Herein, we report the solid-phase total synthesis and structural confirmation of longicatenamide A. First, commercially unavailable building blocks were chemically synthesized with stereocontrol. Second, the peptide chain was elongated via Fmoc-based solid-phase peptide synthesis. Third, the peptide chain was cyclized in the solution phase, followed by simultaneous cleavage of all protecting groups to afford longicatenamide A. Chromatographic analysis corroborated the chemical structure of longicatenamide A. Furthermore, the antimicrobial activity of synthesized longicatenamide A was confirmed. The developed solid-phase synthesis is expected to facilitate the rapid synthesis of diverse synthetic analogues.

Introduction

Naturally occurring bioactive compounds can serve as both drug leads and research tools for chemical biology [1]. Because the rediscovery rate of these compounds has increased in the last few decades, new approaches to explore natural products are in demand [2]. To this end, the combined-culture strategy has been applied to discover new natural products. For example, the mycolic acid-containing bacterium *Tsukamurella pulmonis* TP-B0596 can influence the biosynthesis of cryptic natural products [3]. Additionally, we have developed several highly sensitive labeling reagents to detect and identify scarce and cryptic natural products [4]. Integrating the combined-culture strategy and new labeling reagents has led to the detection and structural determination of several unprecedented secondary metabolites [5–7].
Longicatenamides A–D (1–4, Figure 1) are cyclic hexapeptides isolated from the combined-culture of *Streptomyces* sp. KUSC_F05 and *T. pulmonis* TP-B0596 [8]. The planar structures were determined by analyzing two-dimensional (2D) nuclear magnetic resonance (NMR) spectra and mass spectrometry (MS) data, and the absolute configurations of their constituent amino acids were elucidated by using highly sensitive reagents that we recently developed [4]. Among the isolated longicatenamides, compound 1 exhibits weak but preferential antimicrobial activity against *Bacillus subtilis*. Because peptides 1–4 are not detected in the monoculture broth of *Streptomyces* sp. KUSC_F05, they are key tools for understanding chemical communication in the microbial world. To elucidate the role of compounds 1–4 in the microbial world, developing a strategy to synthesize compounds 1–4, including future derivatization to produce probe molecules, is required. Herein, we report the total synthesis of peptide 1 by Fmoc-based solid-phase peptide synthesis [9] and the evaluation of its antimicrobial activity.

The retrosynthesis of peptide 1 is displayed in Scheme 1. First, the cyclic peptide 1 was linearized by retrosynthesis, and acid-labile protecting groups were attached onto the reactive side chain. The biomimetic synthesis of cyclic peptides often enables efficient synthesis [12,13] and provides insights into the biosynthesis pathways of these peptides [14]. However, the biosynthetic gene clusters of compounds 1–4 remain unidentified. Therefore, the least sterically hindered amine, namely the amino group of glycine, was selected as a nucleophile of the cyclization reaction in this study. Second, to realize the solid-phase synthesis, the C-terminus of the linear peptide was connected to 2-chlorotrityl resin [15] to give resin-bound peptide 5. Peptide 5 was divided into six building blocks 6–11 by retrosynthesis.

At the beginning of the total synthesis, commercially unavailable building blocks 7 and 10 were chemically constructed from readily available starting materials. The synthesis of building block 10 commenced with the synthesis of compound 15 through Wittig reaction of Garner’s aldehyde (13) [16], which was readily obtained from tert-butyloxycarbonyl (Boc)-protected *d*-serine 12 (Scheme 2). Treatment of the olefin 15 with trifluoroacetic acid (TFA) cleaved the Boc protecting group and the acetonide to deliver unsaturated amino alcohol 16. The amino group in 16 was protected by the fluorenylmethyloxycarbonyl (Fmoc) protecting group for solid-phase peptide synthesis, and then hydrogenation of the double bond in 17 provided intermediate 18. Oxidation of the alcohol 18 to acid 10 was realized with the combination of Dess–Martin oxidation [17,18] and Pinnick oxidation [19].

Another unusual amino acid 7 was also synthesized from *d*-serine (20, Scheme 3). The SnCl₂-catalyzed coupling reaction [20] between 21 and 22 afforded β-keto ester 23, which...

![Figure 1: Structures of longicatenamides A–D (1–4).](attachment:image.png)
Scheme 1: Retrosynthesis of longicatenamycin A (1).

was then reduced to the corresponding β-hydroxy ester 24 by K-Selectride (dr > 20:1), and subsequent acidic removal of the acetonide furnished diol 25. The stereochemistry of the newly generated hydroxy group was determined using the modified Mosher’s method [21]. Protection of diol 25 by tert-butyl(dimethyl)silyl (TBS) group followed by selective depro-
tection of the primary alcohol led to 27. Finally, acid 7 was obtained from alcohol 27 through the same two-step oxidation used to obtain compound 10.

Having synthesized the necessary building blocks, we turned our attention to construct resin-bound peptide 5 (Scheme 4). The assembly of this hexapeptide started with the loading of Fmoc-ᴅ-Trp(Boc)-OH (6) onto 2-chlorotrityl resin with iPr₂NEt, which was followed by piperidine treatment to liberate resin-bound amine 29. Then, five rounds of DIC/Oxyma-mediated amidation [22] and Nα-deprotection with piperidine led to resin-bound peptide 5. Treatment of 5 with TFA/CH₂Cl₂ 1:99 released 30 into the solution without unmasking the acid-labile protecting groups of the side chains. Subsequently, peptide 30 was cyclized by the action of PyBOP/HOAt [23,24] followed by treatment with TFA/iPr₂SiH/H₂O 95:2.5:2.5 to provide crude 1. After reversed-phase high-performance liquid chromatography (HPLC) purification, longicatanamide A (1) was obtained with 36% yield over 15 steps starting from 6.

The NMR spectra of synthesized compound 1 agreed with those of natural 1. At this stage the confirmation of the identity of natural and synthesized compounds by structural determination using NMR spectroscopy is often difficult because the NMR spectra of peptidic products vary depending on the conditions in the NMR tube [25-29], such as concentration, pH, and purity. Thus, analyses of the NMR spectra of peptides sometimes lead to incorrect structural determination even though total synthesis of the proposed structures is successful [30]. In this study, synthesized and natural 1 were compared by collecting LC–MS data. As displayed in Figure 2, the retention time of synthesized 1 was identical to that of natural 1. These results confirmed total synthesis of 1 and supported our proposed structure of isolated natural 1.

Total synthesis sometimes invalidates the reported biological activity of the isolated natural product, which could be due to the presence of uncharacterized impurities. To verify the bioactivity of 1, the antimicrobial activity of synthesized 1 was preliminary tested in this study following a previously reported
method [8], revealing that chemically synthesized 1 (minimum inhibitory concentration (MIC) = 50 µM) exhibited moderate but selective antimicrobial activity against *Bacillus subtilis* similar to natural 1 (MIC = 100 µM).

**Conclusion**

We accomplished solid-phase total synthesis of longicarenamide A (1). Initially, commercially unavailable building blocks 7 and 10 were chemically synthesized with stereocontrol. Then, the peptide chain was elongated by Fmoc-based solid-phase peptide synthesis. Finally, the cyclization of the peptide chain followed by simultaneous cleavage of all protecting groups in the solution phase afforded target compound 1. The comparison of the chromatograms of synthesized and natural 1 corroborated the chemical structure of 1. Further studies of the structure–activity relationship, identification of
biosynthetic gene clusters, and detailed investigations of the combined-culture production of longicatenamides are currently underway in our laboratory.

Supporting Information
Supporting Information File 1
Experimental procedures and compound characterization data. [https://www.beilstein-journals.org/bjoc/content/supplementary/1860-5397-18-166-S1.pdf]

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Figure 2: LC–MS extracted ion chromatograms (EICs) of synthesized and natural 1. Column: Imtakt Cadenza CD-C18 3 × 150 mm; eluent: MeCN/H2O/TFA 30:70:0.05, isocratic, 0.2 mL/min; 40 °C.
Formal total synthesis of macarpine via a Au(I)-catalyzed 6-endo-dig cycloisomerization strategy

Jiayue Fu¹,²,³, Bingbing Li¹,²,³, Zefang Zhou¹,²,³, Maosheng Cheng¹,³, Lu Yang*¹,²,³ and Yongxiang Liu*¹,²,³

Abstract
The formal total synthesis of macarpine was accomplished by the construction of a naphthol intermediate in Ishikawa’s synthetic route with two different synthetic routes. The convergent synthetic strategies feature the utilization of Au(I)-catalyzed cycloisomerizations of a 1,5-enyne and alkynyl ketone substrates, which were prepared by Sonogashira coupling reactions.

Introduction
Benzo[c]phenanthidine alkaloids are an ancient and influential category of isoquinoline alkaloids, mainly found in Papaveraceae and Rutaceae (Scheme 1) [1,2]. According to their oxidation states, benzo[c]phenanthidine alkaloids can be divided into two types: partially hydrogenated base and fully aromatized base, in which natural fully aromatic alkaloids can be further classified into three subclasses: O₄-base, O₅-base, and O₆-base [3].

Among these alkaloids macarpine is the most oxidized tetracyclic alkaloid with many bioactivities, including anesthesia, anticancer, anti-inflammatory [4-8], insecticidal, fungicidal, etc [9]. In addition to the above-mentioned activities, macarpine was also used as a DNA probe for flow cytometry and fluorescence microscopy due to its fluorescent properties [10]. Despite some research on the activities of macarpine had been performed, a more in-depth evaluation of the biological activities was still limited due to the need of its isolation from natural sources. Inspired by the requirement of further biological evaluation, the chemical syntheses of macarpine have been developed rapidly in the last three decades.
synthetic routes were completed in the last step by constructing ring B or ring C. Some representative examples and their key strategies are summarized in Scheme 2. In 1989, Hanaoka and co-workers developed the total synthesis of macarpine by Hofmann elimination from protoberberine by introducing rings B and C (Scheme 2a) [11]. In 1995, Ishikawa and co-workers accomplished the total synthesis via a Reformatsky reaction and aromatic nitration through the building of rings B and C (Scheme 2b) [12]. In 2010, Echavarren and co-worker completed the formal total synthesis via a Au(I)-catalyzed cyclization (Scheme 2c) [13]. In 2018, Pabbaraja and co-workers disclosed the synthesis of macarpine by constructing ring C through the domino Michael addition/S_N_Ar reaction of nitromethane to an ynone precursor (Scheme 2d) [14].

Results and Discussion

The efforts on developing efficient synthetic strategies to access macarpine never ceased during the last decades, and we have joined this meaningful research. Herein, a strategy involving the synthesis of an intermediate reported by Ishikawa in 1995 in the total synthesis of macarpine [12] is proposed via a Au(I)-catalyzed cycloisomerization reaction.

With the building blocks 5 and 8 in hand, ketone 9 was prepared via a palladium-catalyzed Sonogashira coupling reaction in a yield of 95%. The precursor 10 for the gold(I)-catalyzed [19-24] cycloisomerization was then synthesized by treating ketone 9 with sodium bis(trimethylsilyl)amide (NaHMDS) and tert-butylimethyldisilyl chloride (TBSCI) (Scheme 5).
Scheme 2: Representative synthetic strategies for macarpine (1).

a) Hofmann elimination (Hanaoka)

b) Reformatsky reaction (Ishikawa)

c) Au(I)-catalyzed cyclization (Echavarren)

d) domino Michael addition/SeAr (Pabbaraja)

Scheme 3: Retrosynthetic analysis of marcarpine precursor 12 for a partial synthesis.
To find the best cycloisomerization conditions, the 1,5-enyne substrate 10 was subjected to different reaction conditions as listed in Table 1. It was observed that [1,3-bis(2,6-diisopropylphenyl)imidazol-2-ylidene]gold(I) chloride (IPrAuCl) itself failed to catalyze the cycloisomerization (Table 1, entry 1). Evaluation of a number of silver salts illustrated that silver hexafluoroantimonate (AgSbF6) was the optimal additive to activate the gold catalyst (Table 1, entries 2, 3, and 7). Screening of the other ligands of Au(I) catalysts, including triphenylphosphane (Ph3P), [1,1'-biphenyl]-2-yl-di-tert-butylphosphane (JohnPhos) dicyclohexyl(2',4'-diisopropyl-3,6-dimethoxy-[1,1'-biphenyl]-2-yl)phosphane (BrettPhos) (Table 1, entries 4–6) revealed that 1,3-bis(2,6-diisopropylphenyl)imidazol-2-ylidene (IPr) was still the best one (Table 1, entry 7). Neither decreasing nor increasing the loading of the catalyst gave better yields (Table 1, entries 8 and 9). Examination of the reaction time showed that 2 h was the shortest reaction time and that extending the reaction time did not help to increase the yield (Table 1, entries 10 and 11). Lowering or raising the reaction temperature resulted in lower yields (Table 1, entries 12 and 13). The solvent had less effect on the reaction, and combining various factors, DCM was used for the reaction (Table 1, entries 14 and 15). When AgSbF6 was utilized as the sole catalyst, no product was generated indicating cationic Au(I) was the true catalyst (Table 1, entry 16). A control experiment using 2,6-di-tert-butylpyridine as a proton scavenger in the IPrAuCl/AgSbF6 system provided the product in good yield, which excluded the influence of trace amounts of acids on the reaction (Table 1, entry 17).

The Au(I)-catalyzed cycloisomerization reaction of substrate 10 occurred under the catalysis of 5 mol % [1,3-bis(2,6-diisopropylphenyl)imidazol-2-ylidene]gold(I) chloride (IPrAuCl) and 5 mol % silver hexafluoroantimonate (AgSbF6) [25,26] in anhydrous DCM at room temperature for 2 h forming a benzene ring smoothly, leading to the exclusive formation of biaryl intermediate 11 in a yield of 82%. It is worth noting that the methoxy substitution in the substrate played a crucial role in controlling the selectivity of the cycloisomerization according to our previous study [15]. It was rationalized that the methoxy substitution in the substrate played a crucial role in controlling the selectivity of the cycloisomerization according to our previous study [15]. It was rationalized that the electron-donating phenyl ring enabled the coordination of the alkyne with the Au+ complex in the α-position, which promoted the silyl ether to attack the β-position of the alkyne to promote a 6-endo-dig cyclization. Next, compound 11 was subjected to a
Table 1: Optimization of the Au(I)-catalyzed cycloisomerization conditions.

| entry | catalyst          | solvent | additive | T (°C) | yield (%) |
|-------|-------------------|---------|----------|--------|-----------|
| 1     | IPrAuCl           | DCM     | –        | 23     | 0         |
| 2     | IPrAuCl           | DCM     | AgOTf    | 23     | 61        |
| 3     | IPrAuCl           | DCM     | AgCO₂CF₃| 23     | 23        |
| 4     | Ph₃PAuCl          | DCM     | AgSbF₆   | 23     | 77        |
| 5     | JohnPhosMeCNAuSbF₆| DCM     | –        | 23     | 64        |
| 6     | BrettPhosAuCl     | DCM     | AgSbF₆   | 23     | 45        |
| 7     | IPrAuCl           | DCM     | AgSbF₆   | 23     | 82        |
| 8     | IPrAuCl           | DCM     | AgSbF₆   | 23     | 68ᵃ       |
| 9     | IPrAuCl           | DCM     | AgSbF₆   | 23     | 82ᵇ       |
| 10    | IPrAuCl           | DCM     | AgSbF₆   | 23     | 57ᶜ       |
| 11    | IPrAuCl           | DCM     | AgSbF₆   | 23     | 81ᵈ       |
| 12    | IPrAuCl           | DCM     | AgSbF₆   | 0      | 63        |
| 13    | IPrAuCl           | DCM     | AgSbF₆   | 40     | 72        |
| 14    | IPrAuCl           | toluene | AgSbF₆   | 23     | 82        |
| 15    | IPrAuCl           | THF     | AgSbF₆   | 23     | 80        |
| 16    | –                 | DCM     | AgSbF₆   | 23     | 0         |
| 17    | IPrAuCl           | THF     | AgSbF₆   | 23     | 80ᵉ       |

ᵃ3 mol % IPrAuCl and 3 mol % AgSbF₆ were used.ᵇ10 mol % IPrAuCl and 10 mol % AgSbF₆ were used.ᶜThe reaction was run for 1 h.ᵈThe reaction was run for 3 h.ᵉ5 mol % 2,6-di-tert-butylpyridine was added. IPr = [1,3-bis(2,6-diisopropylphenyl)imidazol-2-ylidene]. JohnPhos = [(1,1'-biphenyl)-2-ylditetrt-butylphosphane]. BrettPhos = [dicyclohexyl(2',4'-diisopropyl-3,6-dimethoxy-[1,1'-biphenyl]-2-yl)phosphane].

Scheme 6: Formal total synthesis of macarpine (1).
solution of tetrabutylammonium fluoride (TBAF) in tetrahydrofuran (THF), resulting in the formation of naphthol 12 [12,13], a key intermediate in the previous total synthesis of macarpine (1) reported by Ishikawa (Scheme 6).

To simplify the synthetic procedure, a more straightforward strategy was proposed by using alkynyl ketone 9 [27-29] as the substrate for the gold-catalyzed cycloisomerization in the presence of protic acid. It was supposed that alkynyl ketone 9 would undergo enolization under the acidic conditions, followed by a gold-catalyzed cycloisomerization to provide the naphthol 12. To test the idea, alkynyl ketone 9 was subjected to different reaction conditions as listed in Table 2. It was observed that both the acids and the temperatures had a great influence on the cycloisomerization. An attempt was also made by using only p-toluenesulfonic acid (TsOH) in the cycloisomerization step, but no corresponding product was obtained. Finally, the optimal conditions for the Au(I)-catalyzed cycloisomerization of alkynyl ketone 9 were determined as to stir the substrate under the catalysis of 5 mol % [IPrAuCl/AgSbF6 with 2 equiv of TsOH as the additive at 70 °C for 2 h (Table 2, entry 3). It is notable that our synthetic route to naphthol 9 is shorter and proceeds with higher yield (5 steps, 59% yield) than Ishikawa’s route (9 steps, 13% yield).

**Conclusion**

In summary, the formal total synthesis of the natural product macarpine was achieved through two synthetic routes by synthesizing Ishikawa’s naphthol intermediate via Au(1)-catalyzed cycloisomerizations. Compared to the route reported in the literature, these routes are more concise and easier to perform. This gold-catalyzed strategy provides a new approach to macarpine and related benzo[c]phenanthridine alkaloids and the application of this strategy to access benzo[c]phenanthridine derivatives and further assessments of their bioactivities are currently in progress in our laboratory.

| entry | acid     | solvent | T (°C) | yield (%) |
|-------|----------|---------|--------|-----------|
| 1     | TsOH     | DCM     | 23     | 0         |
| 2     | TsOH     | DCM     | 40     | 0         |
| 3     | TsOH     | DCE     | 70     | 73        |
| 4     | TFA      | DCE     | 70     | 50        |
| 5     | AcOH     | DCE     | 70     | 24        |
| 6     | PhCO2H   | DCE     | 70     | 39        |

**Supporting Information**

Supporting Information File 1

Synthetic procedures and characterization data for compounds 3–5, 8–12, and their 1H NMR and 13C NMR spectra.

[https://www.beilstein-journals.org/bjoc/content/supplementary/1860-5397-18-169-S1.pdf]

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Synthetic study toward the diterpenoid aberrarone

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Abstract

An approach to aberrarone, an antimalarial diterpenoid natural product with tetracyclic skeleton is reported. Key to the stereoselective preparation of the 6-5-5 tricyclic skeleton includes the mediation of Nagata reagent for constructing the C1 all-carbon quaternary centers and gold-catalyzed cyclopentenone synthesis through C–H insertion.

Introduction

Marine natural products have found myriad use in new drug development, exemplified by ET-743 and eribulin [1]. Back in 1990s, Rodriguez and co-workers isolated a rich array of terpenoid natural products from the Caribbean sea whip, *Pseudopterogorgia elisabethae* with unprecedented carbon skeleton, most of which showed antitumor, antituberculosis and antimalarial activities [2-6]. Among these structurally intriguing natural products, aberrarone (1) shows antimalarial activity against the chloroquine-resistant strain of *Plasmodium falciparum* (IC₅₀ = 10 μg/mL) [7]. Structurally, aberrarone possesses an unusual tetracyclic carbon skeleton yet-to-be found in *Pseudopterogorgia elisabethae* species, although related cyclohexane-angularly-fused triquinane systems have been found in waihoensene (3), conidiogenone (4), lycopodium alkaloids magellamine (5) and lycojaponicumin C (6) (Figure 1). Its seven stereogenic centers, including two all-carbon quaternary moieties collectively render aberrarone as an attractive but challenging synthetic target. Its congener elisabanolide (2) with a lactone in the D ring shows their potential biosynthetic relationship [2]. These natural products have been popular synthetic targets mainly due to their intriguing structural features. For example, several total syntheses of 3–6 have been reported [8-29]. Previously, two synthetic studies of aberrarone were reported [30,31] and more recently, Carreira and co-workers reported [32] the first total synthesis of aberrarone through an impres-
A cascade reaction including a gold-catalyzed Nazarov cyclization, a cyclopropanation followed by intramolecular aldol reaction to forge the A, B and D rings. Impressed by the structural features and biological profiles, our group embarked a project on the total synthesis of this natural product. Herein, we report our stereoselective synthesis of its 6-5-5 tricyclic skeleton.

![Diagram showing retrosynthetic analysis](image)

Our retrosynthetic analysis is shown in Scheme 1. For the formation of the D ring with one quaternary carbon stereocenter and 1,2-diketo moiety, Nazarov cyclization [33] of 7 was proposed for synthesizing this challenging moiety. The corresponding precursor cyclopentenone 8 may be prepared from alkynone 9 through a gold-catalyzed C–H insertion [34]. Alkynone 9 could be achieved through functional transformation from 10, which itself would be prepared through methylation and conjugate addition from Pauson–Khand adduct 11. This cyclopentenone could be readily accessed from 1,7-enzyme 12 which could be obtained through the reported procedure [35] from the commercially available 5-hexenoic acid.

**Results and Discussion**

Our synthetic route commenced from known compound 12 which is readily accessed from 5-hexenoic acid through a reported procedure [35]. In the mediation of Co$_2$(CO)$_8$, the 6-5 bicyclic skeleton [36] was constructed with the right configuration at C6, and the explanation of this stereoselectivity is possible through the conformation of 14 where the OTBS group is in pseudoequatorial position (Scheme 2). Therefore, the Pauson–Khand reaction proceeded to afford 11 containing an α-H at C6. From this intermediate, to our delight, the stereoselective attachment of the requisite methyl group through the corresponding lithium enolate occurred from the convex face of the bicyclic ring system [37]. After these two continuous stereo-centers were successfully installed, the expected challenging all-carbon quaternary center at C1 was constructed utilizing the Nagata reagent (Et$_2$AlCN). By using this strategy, the stereogenic center at C1 was synthesized, along with a smooth attachment of the cyanate group served for further functional group transformation to construct the C ring through C–H insertion. The stereochemistry finding of this conjugate addition from the convex face of the 6-5 ring system was further confirmed through X-ray crystallographic analysis.
With the key intermediate 10 in hand, we were in a position to test the planned two-step transformation including the palladium-catalyzed reductive cross coupling with HCO₂H followed by Pd/C-catalyzed hydrogenation. To our surprise, the hydrogenation turned out to be a difficult transformation due to the steric hindered environment of the trisubstituted double bond, mainly caused by the bulky OTBS group. However, direct subjection of compound 16 to hydrogenation [38] afforded reduction of both triflate and double bond. The plausible pathway for this facile transformation might proceed with first hydrogenation followed by the substitution of the labile triflate ester (for details, see Supporting Information File 1). Moving forward, compound 17 was further converted into alkynone 9 through DIBAL-H reduction, nucleophilic addition and Dess–Martin oxidation. At this stage, the pivotal C–H insertion step was tried under the reported conditions [34], and cyclopentenone 8 was successfully obtained. Further study with cross coupling or halogen–magnesium exchange shows this moiety is inert for functional group transformation. The attempt for constructing the D ring is currently undergoing.

Conclusion
In summary, we have developed an approach to assemble the tricyclic skeleton of aberrarone through stereoselective methylation, conjugate addition and gold-catalyzed C–H insertion from the readily accessed cyclopentenone. Further work to access natural product aberrarone from the key intermediate cyclopentenone 8 is currently underway, and will be reported in due course.
Abstract
The first syntheses of the amino acids (−)-halichonic acid and (−)-halichonic acid B have been achieved in ten steps starting from commercially available (−)-α-bisabolol. The optimized synthetic route includes a new purification method for isolating (−)-7-amino-7,8-dihydrobisabolene in enantiomerically pure form via recrystallization of its benzamide derivative. The key intramolecular aza-Prins reaction forms the characteristic 3-azabicyclo[3.3.1]nonane ring system of halichonic acid along with the lactonized form of halichonic acid B in an 8:1 ratio. Optical rotation measurements confirmed that these synthetic compounds were in fact the enantiomers of the natural products, establishing both the relative and absolute configurations of the halichonic acids.

Introduction
Marine sponges produce a large number of structurally diverse natural products, including many that exhibit biological activity [1-3]. In 2019, Tsukamoto and co-workers isolated the amino-bisabolene sesquiterpenoid halichonic acid ( (+)-1 ) from the sponge Halichondra sp. (Figure 1) [4]. This amino acid natural product features a rigid 3-azabicyclo[3.3.1]nonane ring system containing four stereogenic centers within the piperidine ring. In 2021, the same group re-isolated ( +)-1 from the sponge Axinyssa sp. along with the structurally related compound halichonic acid B ( (+)-2 ) [5]. Structurally, ( +)-2 is a pipecolic acid derivative containing a cyclohexenyl ring as a substituent group. This compound also features four stereogenic centers (three of which are located within the piperidine ring) and a tertiary alcohol. The structures of compounds ( +)-1 and ( +)-2 were elucidated through a combination of HRMS and NMR spectroscopy, while the relative configuration of each compound was established through nuclear Overhauser effect (NOE) correlations. Additionally, the absolute configuration of each compound was determined based on calculated electronic circular dichroism (ECD) spectra that were compared to the experimental ECD spectra of ( +)-1 and ( +)-2. Although these natural products did not exhibit antimicrobial activity or cytotoxicity against HeLa cells, their biological activities in other assays have not yet been investigated.
Our group was particularly interested in the structure of halichonic acid ((+)-1), which shares the same bridged bicyclic ring system found in many of the Aristotelia alkaloids [6]. Since our lab recently reported a synthesis of the natural product aristotelin [7], we viewed the halichonic acids as ideal targets to extend the scope of our synthetic methodology. Given the structural similarity between compounds (+)-1 and (+)-2 (and the fact that they were co-isolated from the same sponge), Tsukamoto et al. proposed that these natural products could be derived from a common biosynthetic pathway starting from farnesyl pyrophosphate and glycine [5]. This prompted us to investigate a biomimetic synthesis in which the halichonic acids could be prepared from a common imine intermediate via divergent intramolecular aza-Prins cyclizations [8]. Herein, we report the first syntheses of the enantiomers of these natural products (i.e., (−)-1 and (−)-2), confirming the structural assignments of the halichonic acids and establishing their absolute configurations.

Results

Our synthetic route began with the readily available sesquiterpenoid (−)-α-bisabolol (3), as shown in Scheme 1. In 2013, Shenvi and co-workers reported an operationally simple and high-yielding method for converting tertiary alcohols (including 3) to the corresponding primary amines via the intermedacy of an isonitrile [9]. This four-step procedure was conveniently carried out on a multigram scale, affording (−)-7-amino-7,8-dihydrobisabolene (4) and its C7-epimer as an 83:17 mixture of diastereomers in 87% overall yield. Unfortunately, these diastereomers were not separable by conventional column chromatography. Although this diastereomeric mixture could be converted into a variety of amine derivatives (e.g., hydrochloride salt, mandelic acid salt, phthalimide, ketopinic acid amide, salicylaldehyde imine, p-toluenesulfonamide, acetamide, etc.), all attempts to separate the resulting isomers (which were oils) were similarly unsuccessful.

Seeking an alternative to column chromatography, we decided to prepare solid derivatives of 4 (and its C7-epimer) that could be purified by recrystallization. Although the α-bromoacet-
Having finally separated the C7-diastereomers, we anticipated that the amide 5 could be hydrolyzed to give a single enantiomer of amine 4. However, we found that amide 5 was remarkably resistant to hydrolysis, even under forcing conditions. For example, no amide hydrolysis was observed in concentrated aqueous NaOH solution at reflux (with or without an organic co-solvent), and slow decomposition occurred under acidic conditions at elevated temperatures. Alternative methods to cleave the benzamide using sodium peroxide [11] or triethylammonium tetrafluoroborate [12] were also unsuccessful, giving either no reaction or significant decomposition, respectively. At this stage, we started to investigate alternative methods to cleave the amide via reduction. Achieving selective C–N-bond cleavage of amides under reductive conditions is still a largely unsolved problem since a C–O-bond cleavage is typically the preferred mode of reactivity, especially when using hydride reducing agents [13]. Nevertheless, specialized conditions for achieving C–N-bond cleavage of amides using SmI₂ [13], Tf₂O/ Et₃SiH [14], and stoichiometric Schwartz’s reagent [15] have been reported; however, none of these methods was successful in reducing amide 5 to the desired amine 4.

Although there is one literature example of directly reducing a benzamide with diisobutylaluminum hydride (DIBAL) to achieve C–N-bond cleavage [16], we observed exclusive over-reduction of compound 5 under these conditions to form the corresponding N-benzylamine, even at −78 °C. We next investigated the reducing agent sodium bis(2-methoxyethoxy)aluminum hydride (Red-Al®), which is a convenient alternative to LiAlH₄ that exhibits high solubility in organic solvents and is also known to reduce amides [17]. When a solution of amide 5 in toluene was treated with an excess of Red-Al® at 0 °C, rapid gas evolution (likely H₂) occurred. However, no reduction of the amide was observed, even after stirring at room temperature for 24 hours. In an effort to “salvage” the reaction by reducing the amide to the corresponding N-benzylamine (which could potentially be oxidized to the corresponding imine with IBX [18] and subsequently hydrolyzed to give 4), we added excess DIBAL and allowed the reaction mixture to stir at room temperature for an additional 24 hours. Upon quenching the reaction with a saturated aqueous solution of potassium sodium tartrate (Rochelle’s salt), we were astonished to observe the clean formation of imine 6. Presumably, the combination of Red-Al® and DIBAL reacts with amide 5 to form a stable tetrahedral intermediate that collapses to 6 upon aqueous workup. This type of direct amide semi-reduction using aluminum hydride reagents is, to the best of our knowledge, previously unknown within the chemical literature and is especially notable since it does not require cryogenic temperatures. Efforts to further investigate the scope of this unique transformation are currently underway in our laboratory and will be reported in due course.

Attempts to purify imine 6 by column chromatography on silica gel resulted in extensive decomposition. Therefore, the crude imine was immediately hydrolyzed using aqueous citric acid [14], affording (−)-7-amino-7,8-dihydroisabalone (4) as a single stereoisomer in 90% yield over the two steps. The enantiomer of 4 is itself a natural product with cytotoxic, antifungal, and antimicrobial properties [10,19-22]. Notably, (+)-4 was also co-isolated with compounds (−)-1 and (−)-2 in sponge extracts, suggesting that these compounds may share a common biosynthetic pathway [4,5]. Both enantiomers of 4 have been previously synthesized [9,23,24], and this compound has also been prepared in racemic form [25]. To supply the final two carbon atoms found in the halichonic acids, amine 4 was condensed with a solution of ethyl glyoxylate in toluene, giving imine 7 in 95% yield. We found that imine 7 could be purified by column chromatography on silica gel if the mobile phase contained approximately 2% triethylamine as a basic additive. However, it is also possible to use crude 7 in the subsequent cyclization step without significantly affecting isolated yields. 1H NMR analysis showed that 7 was formed as a single geometrical isomer; although the imine configuration was not rigorously established, we have assigned it as the (E)-isomer, as is commonly observed in aldime formation.

The stage was now set for the key intramolecular aza-Prins reaction that would form the bicyclic structures of the halichonic acids (Scheme 2). When a solution of imine 7 in chloroform was treated with a large excess (85–100 equiv) of formic acid at room temperature, we were pleased to observe the formation of bicyclic compound 8 as the major product in 64% yield. Notably, 8 is the ethyl ester of (−)-halichonic acid and features the characteristic 3-azabicyclo[3.3.1]nonane ring system found in this natural product. However, we were intrigued that a competing cyclization process also formed isomeric lactones 9 and 10 in 8% yield and 11% yield, respectively. 1H NMR analysis confirmed that compounds 9 and 10...
were both trans-fused 6/5 bicycles based on the magnitude of the vicinal coupling constant between the two methine hydrogens at the ring fusion ($^{3}J = 12.9$ Hz). The rigid nature of the trans-fused 6/5 ring system results in distinct conformers for 9 and 10; fortunately, this allowed for the unambiguous assignment of the relative configurations of these diastereomers via NMR based on nuclear Overhauser effect (NOE) correlations (see the Supporting Information File 1 for additional details). This analysis showed that the minor product 9 corresponded to the lactone of (−)-halichonic acid B.

At this point, all that remained to complete the syntheses of the halichonic acids was hydrolysis of compounds 8 and 9 to form the corresponding amino acids. Thus, treating bicycle 8 with aqueous lithium hydroxide resulted in hydrolysis of the ethyl ester, and subsequent neutralization with pH 7 phosphate buffer afforded halichonic acid (−)-1 in 88% yield after purification by column chromatography. Similarly, hydrolysis of lactone 9 under analogous conditions afforded halichonic acid B ((−)-2) in 76% yield. $^{1}$H and $^{13}$C NMR data for the synthetic compounds (−)-1 and (−)-2 were identical to those reported for the halichonic acids, confirming the proposed structures of these natural products. However, the observed optical rotations of these synthetic compounds were of opposite sign to those reported for the halichonic acids. Since we synthesized the enantiomers of these natural products, the absolute configurations of (+)-1 and (+)-2 assigned by Tsukamoto et al. have now been experimentally confirmed [4, 5]. For the sake of comparison, the diastereomeric lactone 10 was also hydrolyzed under the same conditions to form the “unnatural” product 11 in 70% yield, which we have designated (−)-isohalichonic acid B. Although the NMR spectra of (−)-2 and 11 are quite similar, we did note a
significant difference in the $^{13}$C NMR chemical shift of the C7-methyl group, which appears at δ = 20.7 in (−)-2 and δ = 14.5 in 11.

**Discussion**

Rationalizing the outcome of the aza-Prins reaction leading to the formation of ethyl ester 8 and isomeric lactones 9 and 10 (Scheme 2) provides an interesting exercise in acyclic conformational analysis. Three divergent mechanistic pathways can be formulated by considering the different conformers of protonated imine 7, namely iminium ions 12a–c (Scheme 3). In each case, the chair-like transition state of the intramolecular aza-Prins reaction is controlled by the C7-stereogenic center, which bears a methyl group, the electrophilic site (the iminium ion), and two possible nucleophilic sites (a prenyl group and a trisubstituted alkene within a cyclohexene ring).

In conformer 12a, the prenyl group occupies a pseudo-axial position, the methyl group occupies a pseudo-equatorial position, and the trisubstituted alkene within the six-membered ring serves as the nucleophile. It is important to note that in this chair-like conformer, the ethyl ester group at C2 assumes a pseudo-equatorial position. Although an alternative boat-like conformer is also possible (which would ultimately lead to the C2-epimer of 8), the resulting transition state is presumably much higher in energy. In practice, the intramolecular aza-Prins reaction of 12a forms a new carbon–carbon bond to generate a rigid 3-azabicyclo[3.3.1]nonane ring system (13). Although

![Scheme 3: Proposed intermediates for the intramolecular aza-Prins reaction leading to the formation of ethyl ester 8 and isomeric lactones 9 and 10.](image-url)
several different fates could be envisioned for this carbocation (e.g., a nucleophilic attack of formic acid to give a formate ester), only alkene formation was observed in this system. Interestingly, the deprotonation step is completely regioselective, giving the more highly substituted endocyclic trisubstituted alkene found in 8 as opposed to the isomeric exocyclic 1,1-disubstituted alkene [7,8]. Alternative mechanistic pathways involving (1) deprotonation to form a bridgehead alkene, or (2) intramolecular nucleophilic attack of the ethyl ester to form a lactone are not possible in this system due to the rigid geometric constraints of the 3-azabicyclo[3.3.1]nonane ring system. In any case, this aza-Prins reaction is by far the preferred mode of cyclization of iminium ion 12 based on the isolated yield of 8 (64%), ultimately leading to the carbon skeleton found in the natural product halichonic acid ((+)−1).

In conformer 12b, the cyclohexenyl ring system occupies a pseudo-axial position, the methyl group occupies a pseudo-equatorial position, and the trisubstituted alkene of the prenyl group serves as the nucleophile. The chair-like transition state of the intramolecular aza-Prins reaction allows both the ethyl ester and the resulting tertiary carbocation to occupy equatorial positions (14), establishing the observed trans-relationship between these groups while simultaneously setting two new stereogenic centers. As before, one can envision several different fates for the tertiary carbocation present in 14. Although elimination to form an alkene or intramolecular nucleophilic attack by formic acid (ultimately giving a formate ester) are reasonable mechanistically, only the intramolecular nucleophilic attack by the carbonyl group of the pendent ethyl ester was observed in this system to form the resonance-stabilized oxocarbenium ion 16. Subsequent loss of the ethyl group (either as ethyl formate upon solvolysis with the formic acid co-solvent or as ethanol upon aqueous workup) gives lactone 9, which features a strained trans-fused 6/5 ring system. Although this lactone survives aqueous workup at neutral pH, it is rapidly hydrolyzed under basic conditions (Scheme 2) to form the enantiomer of halichonic acid B ((−)−2). It is interesting to note that halichonic acid B exists exclusively as an open-chain 4-hydroxyxycarboxylic acid even though the corresponding γ-lactones typically form spontaneously. Indeed, no lactone formation was observed from (−)-2 even upon purification by column chromatography on silica gel, reflecting the highly strained nature of trans-fused lactone 9.

Finally, conformer 12c is similar to 12b in that the trisubstituted alkene of the prenyl group once again serves as the nucleophile; however, the methyl group now occupies a pseudo-axial position, and the cyclohexenyl ring system occupies a pseudo-equatorial position. In this case, the aza-Prins reaction forms trans-fused lactone 10 via an analogous intramolecular nucleophilic attack of the ethyl ester on the intermediate tertiary carbocation 15 to give oxocarbenium ion 17. In comparing conformers 12b and 12c, it appears that the chair-like transition state 12c should be lower in energy since the more sterically demanding cyclohexenyl ring is located in a pseudo-equatorial position. Although we did observe a slightly higher yield of 10 as compared to 9 (11% vs 8%), these values are sufficiently close to make any conclusions regarding the effects of conformational preferences on reactivity tenuous at best. However, it is interesting to note that the enantiomer of 11 has not been co-isolated as a natural product along with compounds (+)−1 and (−)−2. If the biosyntheses of these natural products does occur through a common iminium ion intermediate, then our isolation of 11 suggests that the key aza-Prins cyclization is enzyme-mediated rather than spontaneous.

Conclusion

In summary, we have synthesized the enantiomers of halichonic acid and halichonic acid B in 10 steps starting from commercially available (−)-α-bisabolol (3). An important intermediate in our route was (−)-7-amino-7,8-dihydroisabolene (4), which was prepared in enantiomerically pure form following recrystallization of a diastereomeric mixture of the corresponding benzamides. A common imine intermediate (7) underwent two different intramolecular aza-Prins reactions in the presence of formic acid to give the ethyl ester of (−)−1 and the lactone of (−)−2 in 64% yield and 8% yield, respectively. Subsequent hydrolysis of these intermediates under basic conditions afforded (−)-halichonic acid and (−)-halichonic acid B, confirming the proposed structures of the natural products. Efforts to investigate the biological activities of compounds (−)−1 and (−)−2 and their synthetic analogs are currently underway in our laboratory.

Supporting Information

The supporting information file contains detailed experimental procedures, full characterization data and copies of 1H and 13C NMR spectra for all new compounds, and complete NMR spectral assignments for compound (−)-1, (−)-2, 8, 9, 10, and 11. A tabular comparison between the NMR data reported for natural products (+)−1 and (−)−2 and that obtained for their synthetic enantiomers (−)−1 and (−)−2 is also provided.

Supporting Information File 1

Experimental procedures, characterization data and copies of 1H and 13C NMR spectra. [https://www.beilstein-journals.org/bjoc/content/supplementary/1860-5397-18-174-S1.pdf]
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Total synthesis of grayanane natural products

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Abstract

Grayananes are a broad family of diterpenoids found in Ericaceae plants, comprising more than 160 natural products. Most of them exhibit interesting biological activities, often representative of Ericaceae use in traditional medicine. Over the last 50 years, various strategies were described for the total synthesis of these diterpenoids. In this review, we survey the literature for synthetic approaches to access grayanane natural products. We will focus mainly on completed total syntheses, but will also mention unfinished synthetic efforts. This work aims at providing a critical perspective on grayanane synthesis, highlighting the advantages and downsides of each strategy, as well as the challenges remaining to be tackled.

Introduction

The Ericaceae are a large plant family, with over 4250 known species all around the world [1]. While Ericaceae’s toxicity has been known since at least 400 BC, barks, leaves and flowers from these plants are still commonly used in traditional medicine in Asia, Europe and America, mainly for their anti-inflammatory and analgesic properties, but also to treat different conditions (e.g. arthritis, hypertension, diabetes, lung, liver and gastrointestinal disorders), and for crop protection [2].

Among natural products formed by Ericaceae, grayananes are a wide diterpenoid family whose biological activities are often representative of Ericaceae’s use in traditional medicine [3-5]. In particular, many grayananes were found to have analgesic [6], antifeedant [7-9], antituberculosis [10], cytotoxic [11] and antioxidant [12] properties. The grayanane family comprises more than 160 natural products, and new members are isolated every year. For instance, 13 new grayanane natural products were reported since 2020 [13-16].

Grayanane diterpenoids all share the same tetracyclic skeleton, with 5, 7, 6 and 5-membered carbocycles commonly named A, B, C and D (Figure 1). The diversity in this family arises from different oxidation states at positions 2, 3, 5, 6, 7, 10, 14, 15, 16, and 17 which can bear free, acylated or glycosylated alcohol, olefin, ketone or epoxide functionalities.

From a biosynthetic point of view, grayananes arise from an oxidative rearrangement of the ent-kaurane skeleton (Scheme 1).
The diversity is generated by cytochromes P450 (CYP) enzymatic oxidation of the grayanane skeleton [17].

The biological activities and low extraction yields have prompted various research groups to undertake the total synthesis of grayanane natural products. In this work, we will survey the literature for synthetic routes to grayanane diterpenoids, including uncompleted approaches. The review is chronologically organized, starting from the earliest synthetic efforts from 1971 to the latest in 2022. In a last part we will present unfinished syntheses.

Review
Early syntheses by Matsumoto and Shirahama

The first synthetic approach towards a grayanane natural product was reported by Matsumoto in the 70s, using a relay approach. The authors first reported in 1972 the synthesis of grayanotoxin II from a degradation product 1 obtained in a few steps from grayanotoxin II itself (Scheme 2) [18]. Later on in 1976 [19], the same authors reported the synthesis of intermediate 1 from the known phenanthrene derivative 2 [20]. The tricyclic compound 2 was converted to 3 through a 6-step sequence involving reduction of the aromatic ring and oxidation of the enone to a dienone. The resulting dienone 3 underwent a key photoinduced santonin-like rearrangement in the presence of acetic acid, furnishing 4 in high yield. It should be noted that the group of Hiraoka had previously reported a similar rearrangement for the synthesis of a grayanane-type skeleton [21]. Further methylation and protecting group interconversions lead to an advanced tricyclic structure 5, which could be further elaborated into relay intermediate 1.

Although Matsumoto’s approach does not fit with the requisites of modern organic synthesis, as it requires over 40 steps to synthesize grayanotoxin II and was performed in racemic form (in the case of the formal synthesis), it represents an impressive piece of synthetic work. Achieving the synthesis of such a com-
plex structure represented a challenging puzzle at that time, and was brilliantly solved by Matsumoto and co-workers.

Almost 20 years later, in 1994, Shirahama’s group published the total synthesis of another member of the grayananes: grayanotoxin III [22]. One of the keys of their synthesis was the stereocontrolled 7-membered ring closure through a SmI$_2$-promoted pinacol coupling. This reaction had been previously described by the same group and proved to be highly effective [23]. For rings A, C and D, other SmI$_2$-promoted steps were employed, giving respectively the cyclopentane A ring moiety and the bicyclo[3.2.1]octane CD ring system with the correct configuration.

The synthesis started from commercially available (S)-2-((p-toluenesulfonyl)oxy)-1-propanol (6) which was converted to (R)-2-(benzyloxy)propionaldehyde (7) by a sequence involving formation of the a phenyl sulfide through an epoxide intermediate, protection of the secondary alcohol as a benzyl ether, oxidation of the sulfur and Pummerer rearrangement (Scheme 3). A
Wittig reaction gave compounds 8, as a 10:1 separable diastereomeric mixture. A diastereoselective Diels–Alder cycloaddition followed by oxidation of the resulting epimeric mixture gave substituted cyclohexanone 9, corresponding to the future C ring [24]. After deprotonation, the C3 position was stereoselectively alkylated using propargyl bromide, and the benzyl protecting group was cleaved with FeCl3, leading to spontaneous lactone closure. A Luche reduction stereoselectively converted enone 10 to the corresponding allylic alcohol, followed by a Au-catalyzed alkyne hydration, providing hemiketal 11. This intermediate was in equilibrium with hydroxy-ketone 12, which was suitable for a SmI2-promoted cyclization, affording intermediate 13 selectively, already bearing rings C and D. The selectivity was achieved by chelation of the Sm(III) intermediate with hydroxy groups present on the structure. As the direct coupling with the A-ring precursor failed, a strategy to build this part was developed, starting with a sequence involving a protection of the alcohols as MOM ethers, lactone hydrolysis, esterification, and Jones oxidation, affording intermediate 14 with a good 79% yield over 4 steps. Next, the methyl ketone was converted to an enol triflate, and then coupled with Li2CuCN(CH2SPh)2. A reduction of the ester with DIBAL, followed by Dess–Martin oxidation and Wittig reaction lead to the formation of 15. This intermediate was coupled with an (R)-epoxide in presence of s-BuLi, and intermediate 16 with E configuration was then obtained by a (PhS)2-accelerated 1,3-sulfide shift. The A ring was then cyclized by a sequence consisting of protection of the alcohol, oxidative cleavage of the PMB protecting group, Dess–Martin oxidation, and SmI2-induced cyclization. This last step was highly selective, giving solely the intermediate 17. The synthesis was then pursued by the hydroboration–oxidation of the monosubstituted alkene, followed by stereoselective epoxidation of the 1,1-disubstituted olefin and reductive epoxide ring-opening giving triol 18. After oxidation of the primary and the secondary alcohols with Dess–Martin periodinane, the remaining tertiary alcohol was protected as a MOM ether and the silyl ether protecting group was removed. The obtained intermediate 19 was then a suitable starting material for the SmI2-promoted pinacol coupling, directed by the free hydroxy group, affording a complete selectivity in the formation of the 7-membered ring B. The synthesis of grayanotoxin III was then achieved by acetylation of the secondary alcohols, oxidative cleavage of the MOM protecting groups followed by hydrolysis of the acetyl protecting groups, affording the desired product with spectral data identical to reported natural samples.

Shirahama’s synthesis was an illustrative example that rings A, B and the bicycle CD of grayananes could all be obtained by SmI2-promoted steps, obtaining excellent selectivity in all cases. Alternatively, the same year, the authors published a vinyl radical cyclization occurring in presence of n-Bu3SnH, providing a stereoselective access to the bicyclo[3.2.1]octane unit corresponding to the CD rings [25].

**Newhouse’s synthesis of principinol D**

In 2019, Newhouse’s group published a total synthesis of principinol D [26], a compound isolated from *Rhododendron principis* in 2014 [27,28]. Compared to grayanotoxin III, principinol D displays an inverse configuration at C1 as well as an exo-olefin at C10–C20. The strategy developed by the group relied on the obtainment of two distinct fragments, a racemic bicyclo[3.2.1]octane unit 25 corresponding to rings C and D, and an enantioenriched cyclopentyl aldehyde derivative 29, corresponding to ring A. These fragments were successfully coupled under basic conditions, and the ring B was later reductively closed using SmI2.

The synthesis of fragment 25 began with commercially available cyclohexenone (21), which underwent a copper-catalyzed vicinal difunctionalization with vinylmagnesium bromide and DMPU and trapping using methyl cyanoformate, leading to the formation of ketoester 22 (Scheme 4). This intermediate was then allylated, the ester group selectively reduced with Zn(TMP)2 and LiBH4, and the resulting primary alcohol was protected as a TBS ether, providing intermediate 23 as a single diastereomer. This key intermediate 23 was then submitted to a Ni-catalyzed α-vinylation and direct TBS deprotection giving the bicyclo[3.2.1]octane subunit with a good yield of 74%. A sequence involving diastereoselective reduction of ketone 24 with SmI2, Appel reaction to convert the primary alcohol to the corresponding primary alkyl iodide followed by a MOM protection of the secondary alcohol afforded fragment 25 with 73% yield over 3 steps. On the other hand, the enantioenriched cyclopentyl aldehyde fragment 29 was obtained starting from commercially available 2,2-dimethylcyclopentane-1,3-dione (26). The dione was submitted to a sequence involving a monoreduction, protection of the alcohol as a TBS ether, α,β-desaturation, dithiane addition, MOM protection and dithiane deprotection.

The fragment 25 was lithiated with t-BuLi and the fragment 29 was then added, forming the coupling product as a 5:5:1:1 separable mixture of diastereomers (Scheme 5). The desired diastereomer 30 was isolated with a yield of 26%. The secondary alcohol was protected as a MOM ether and the allylic silyl ether was converted to an enone. A selective oxidative cleavage, only affecting the monosubstituted alkene, led to the formation of 31, which underwent a key SmI2-promoted seven-membered ring closure, giving a single diastereomer. The stereochemistry and absolute configuration of the obtained tetracyclic structure 32 was confirmed by NOESY NMR and X-ray crystallography.
Some additional modifications were required on the structure to synthesize principinol D: oxidation of the secondary alcohol to the corresponding ketone was achieved using Dess–Martin periodinane with a pyridine buffer. Addition of Me₃SiCH₂Li efficiently afforded the Peterson adduct 33. The 1,1-disubstituted alkene was then submitted to Mukaiyama hydration to form the tertiary alcohol, in presence of Mn(dpm)₃, PhSiH₃ and O₂. Then, the ketone was selectively reduced in the presence of LiEt₃BH, while the Peterson adduct was eliminated concurrently, upon heating. Finally, treatment with H₂SO₄ allowed
total deprotection of the MOM ethers, leading to the formation of prinicipinol D, in complete correspondence with reported spectral data.

Newhouse’s synthesis represents an efficient access to grayananes, relying on two accessible fragments. Remarkably, although the strategy is different from that of Shirahama as it involves different retrosynthetic disconnections, it makes use of similar tactics through the use of a SmI$_2$-promoted cyclization. This first total synthesis of principinol D, in 19 steps as the longest linear sequence, is asymmetric even though a separation of the mixture of diastereomers resulting from fragment coupling is necessary. The SmI$_2$-mediated reductive ring-closure of the 7-membered ring is among the most remarkable steps of the synthesis, along with the Ni-catalyzed formation of the bicyclo[3.2.1]octane unit. It should be noted that due to the SmI$_2$-mediated ring-closure’s stereochemical outcome, this synthesis can only be applied to compounds with an $R$-configured $C_1$ stereocenter, which are epimers of the vast majority of grayanane structures.

Ding’s synthesis of rhodomolleins XX and XXII

Shortly after Newhouse’s synthesis, Ding’s group reported a synthetic strategy to access rhodomollein XX and XXII [29,30]. These natural products have the particularity of displaying an enone moiety on ring A. Ding’s approach involves the construction of a tetracyclic structure where rings A and B had the correct arrangement, while rings C and D form a bicyclo[2.2.1]octane structure [31]. The correct bicyclo[3.2.1]octane structure was obtained after a key reductive epoxide opening/Dowd–Beckwith rearrangement cascade.

The synthesis started from 3-hydroxy-2-methoxybenzaldehyde (34), which was converted into Grignard reagent 35 and added onto 3-methylbut-2-enal (Scheme 6). A sequence involving Claisen rearrangement, Roskamp homologation, diazo transfer and intramolecular cyclopropanation led to intermediate 37. The hydroxy group on C$_6$ was introduced after cyclopropane ring-opening, ketone protection, epoxidation and reductive ring-opening of the resulting epoxide. A one-pot $\beta$-keto phos-
phonate formation/Horner–Wadsworth–Emmons reaction with formaldehyde afforded 38, a precursor for the key oxidative dearomatization-induced Diels–Alder cycloaddition. Treatment of 38 with TBAF followed by PhIr(OAc)_2 led to the formation of 39, having the A and B ring correctly arranged. The product was obtained in 70% yield, along with 25% of an undesired diastereoisomer. The dimethoxy functionality was reduced in the presence of Kagan’s reagent and DMD could induce an epoxidation on the strained olefin. From intermediate 40, the key reductive epoxide opening/Dowd–Beckwith rearrangement cascade could be performed in the presence of an in situ-generated Ti(III) catalyst. The main side-product of this reaction was due to a simple reductive opening of the epoxide (15%). From 41 having the correct tetracyclic skeleton, a transient protection followed by Petasis olefination, deprotection, selenide-mediated α,β-dehydrogenation and Mukaiyama oxidation afforded an advanced intermediate 42 bearing most of the target’s functionalities. A sequence of enol-ether formation/Grignard addition lead to intermediate 43, from which simple acidic treatment led to rhodomollanol XXII, while α-oxidation in the presence of rhenium oxide followed by acidic work-up afforded rhodomollolin XX.

Interestingly, Ding’s synthesis constitutes an efficient approach (22 and 23 steps) to access grayananes with a cyclopentenone moiety on the A ring. It should be noted that although this is a racemic synthesis, intermediate 37 was also synthesized in enantioenriched form using a chiral copper catalyst for the cyclopropanation and a chiral auxiliary on the ester moiety. Moreover, the same group reported a related approach for the synthesis of various diterpenoids including rhodomollanol, an abeo-grayanane natural product [32,33].

Luo’s synthesis of grayanotoxin III, principinol E and rhodomollolin XX

In 2022, Luo et al. described an efficient and enantioselective synthetic route based on a convergent strategy to accomplish the synthesis of principinol E, grayanotoxin III and rhodomollolin XX [34]. The key steps include i) a tandem reaction combining organocatalytic Mukaiyama aldol and intramolecular Hosomi–Sakurai reactions in a one-pot manner; ii) a 7-membered cyclization based on a bridgehead tertiary carbocation intermediate forging the B ring; iii) redox manipulations and a 1,2-migration as final steps. The synthesis started from (S)-ketone 44 which was prepared via asymmetric CBS reduction of diketone 26 (Scheme 7). Firstly, this (S)-ketone 44 was transformed into dimethylacetal 45 by Vilsmeier reaction followed by aldehyde protection in 54% yield over two steps. A Mukaiyama aldol reaction between trimethylsilyl enol ether 46 and dimethylacetal 45 followed by Sakurai cyclization provided an inseparable mixture of C⁹ epimers (dr = 2:1). A catalyst optimization showed that chiral squaramide 47 developed by Jacobsen’s group significantly accelerated the Mukaiyama reaction compared to TMSOTf or TiCl₄ thanks to chiral hydrogen bond-donor effect [35]. After Sakurai cyclization promoted by EtAlCl₂, the desired product 48 was obtained with the required diastereoselectivity in 58% on a 3 g scale. Subsequently, vinyl halide 48 was converted to diene 50 by Suzuki coupling with potassium vinyltrifluoroborate (49) in 90% yield (Scheme 8). The C⁷–C⁸ bond formation from a bridgehead carbocation was a real challenge to close the 7-membered ring. To achieve this, the secondary alcohol was oxidized by DMP, the tertiary alcohol was triflated, 4-phenylpyridine was added and the mixture was heated at 80 °C for 14 h. The intermediate carbocation was trapped by the terminal olefin, generating a dienone 51 after deprotonation at the relatively acidic position C². A singlet oxygen ene reaction involving the electron-rich olefin allowed the formation of an aldehyde, which was directly cleaved by an iridium-catalyzed deformylation, affording 52 in one-pot [36]. Deprotonation with KHMDS allowed the formation of an electron-rich diene which could again react with singlet oxygen by diastereoselective cycloaddition followed by C¹⁵–C¹⁶ epoxidation with m-CPBA. Reductive cleavage of the O–O bond by Zn/AcOH treatment afforded epoxide 53 as a single diastereomer in two steps and 65% yield. The correct bicyclo[3.2.1]octane was obtained by Wagner–Meerwein epoxide rearrangement promoted by EtAlCl₂. Two separable alkene regioisomers 54 and 55 were
obtained in 19% and 50% yield, respectively. A metal-catalyzed hydrogen atom transfer (MHAT) allowed 54 to be partially converted to 55 via Shenvi’s isomerization [37]. Selective TBS protection on the bicyclo[3.2.1]octane moiety and ketone methylation gave access to 56. Directed C1–C5 vanadium-mediated epoxidation followed by DBU treatment and TBS deprotection afforded 57 in one pot. The tertiary alcohol 58 was obtained as a single diastereomer after hydration of position C18. Subsequent reduction with DIBAL-H gave the desired alcohol on the A ring in 75% yield. The C3 epimer was also obtained in 4% yield and confirmed by X-ray diffraction. Hydrogenation of the sterically hindered C1–C2 alkene was accomplished using a combination of Mn(dpm)3 and Ph(iPrO)SiH2, providing grayanotoxin III in 51% yield.

The authors also achieved the synthesis of principinol E and rhodomoline XX by slight modifications of the late-stage functional group transformations. The synthesis of principinol E was performed in 6 steps starting from 60, which was obtained by protection of 55 (Scheme 9). As before, directed epoxidation of C1–C5 in compound 60 was employed to form an α, β-epoxyketone intermediate which underwent SmI2 reduction at −78 °C, giving access to the desired syn diastereomer with good selectivity (dr = 8.5:1). The free alcohol was then protected with an ethoxymethyl ether (EOM). Finally, Peterson olefination, stereoselective carbonyl reduction and TBS deprotection afforded principinol E in 53% yield over 4 steps. For the synthesis of rhodomollein XX, addition of methyllithium to 54 followed by Mukaiyama hydration using Mn(dpm)3 afforded 62. To acquire rhodomollein XX, an additional oxidation state at the C2 position was required. Luo’s team used Davis’ oxaziridine followed by treatment with K2CO3 to equilibrate the hydroxyketone, delivering an inseparable epimeric mixture (1:3) of 63 in 31% overall yield. An additional TBS protection allowed

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**Scheme 8:** Luo’s total synthesis of grayanotoxin III.
separation of the epimers. After acidic treatment, pure rhodomollein XX and 3-epi-rhodomollein were obtained in 31% and 34% yield in two steps, respectively.

Luo’s synthetic work offers a powerful approach to synthesize grayanane natural products. The synthesis is both efficient (18 to 20 steps) and flexible, as demonstrated by the synthesis of 3 natural products, using pivotal intermediates 54 and 60.

**Other synthetic efforts**

In 2011, Williams and co-workers reported an efficient synthetic route detailing the central core construction of pierisformaside C, which is the first grayanane isolated displaying three central double bonds (Scheme 10) [38]. The team’s objective was to develop a synthetic pathway also giving access to several diterpene glycosides close to pierisformaside C in order to study the biological activity of this family. Their strategy was based on a common forward intermediate and a late construction of the central seven-membered ring.

In the beginning of the synthesis, the authors followed the strategy developed previously by Marinovi’s group to form the bicyclo[3.2.1]octane moiety [39]. The synthesis started from 64 with a one-pot Birch reduction/alkylation with vinyl bromide 65, affording 66 in 68% yield over two steps. Next the construction of the bicyclo[3.2.1]octane 67 was achieved by a radical cyclization using n-Bu3SnH in refluxing toluene. A sequence involving an ester reduction, Ley–Griffith oxidation and Seyferth–Gilbert homologation with Bestmann–Ohira reagent allowed to obtain the alkynyl bicyclo[3.2.1]octane 69. On the other hand, the five-membered triflate 71 was synthesized from diketone 26 in 5 steps and 37% overall yield. Both fragments were assembled by a Sonogashira cross-coupling, affording 72 in 72% yield. In a first attempt, TBS protection was considered on the bicyclo[3.2.1]octane. However, later in the strategy, the deprotection presented some difficulties, and the authors decided to investigate the use of a free ketone. The partial hydrogenation of alkyne 72 proved to be inefficient, due to a lack of chemoselectivity involving competitive olefin reduction on the bicyclo[3.2.1]octane. To overcome the over-oxidation, 72 was treated with m-CPBA, providing epoxide 73 as the main product in 71% yield (dr = 6:1). Lindlar hydrogenation of the alkyne and cyclization proceeded smoothly, and the tetracyclic skeleton 74 was obtained in moderate yield. However, the synthesis of pierisformaside C was never completed. The missing transformations include the removal of the ketone on the C ring, epoxide reductive opening, formation of the B ring exo-olefin, and glycosylation.
In 2021, Hong et al. presented a synthetic effort focused on the synthesis of rhodojaponin III B–C rings [40]. The authors employed a Mn(III)-mediated intramolecular radical cyclization of an alkynyl ketone as the key step. The synthesis started by a Cu-catalyzed conjugate addition of the vinyl Grignard reagent, followed by TMS α-propargylation under basic conditions, affording the TMS-alkynyl ketone 76 as the major diastereomer (Scheme 11). Originally a Au-catalyzed Conia-ene-type cyclization, classically considered as a reliable method for the construction of bridged bicyclic structures [41], was envisaged. However, using a gold(I) catalyst the desired 5-exo-dig cyclization failed and only a 6-endo-dig cyclization was observed. Thus, Hong et al. explored a Mn(III)-mediated radical cyclization, an approach which had been previously reported by Jia and co-workers during the synthesis of glaucocalyxin A [42]. After treatment with Mn(OAc)$_3$, the desired 5-exo-dig cyclization product 77 was obtained in 43% yield as an E/Z mixture. The TMS group was removed under acidic conditions. Then, a wide range of reducing agents was explored for the stereoselective ketone reduction. However, only the undesired diastereomer was obtained. Knowing that the correct diastereomer could be obtained in the presence of a primary alcohol instead of the ester moiety, as described by Newhouse previously [26], the authors performed a complete reduction of the carbonyl moieties using LiAlH$_4$ and TBS protection of the primary alcohol, affording intermediate 78 with the wrong configuration at the secondary alcohol stereocenter. After reoxidation and deprotection of the primary alcohol, SmI$_2$ reduction finally afforded the desired diastereomer 79. This synthetic work highlights again the challenge of the bicyclo[3.2.1]octane construction, especially regarding the diastereocontrol at the secondary alcohol moiety. To date, this approach towards rhodojaponin III was never concluded.

**Conclusion**

Over the past 50 years, the synthesis of grayanane natural products has attracted the interest of many synthetic chemists, leading to the development of five total synthesis approaches, as well as two unfinished syntheses [43]. Table 1 summarizes the completed total syntheses in terms of steps, yields, advantages and drawbacks. The clear superiority of recent syntheses beauti-
Table 1: Summary of previous syntheses.

| Synthesis               | Number of steps | Overall yield | Advantages          | Drawbacks                        |
|-------------------------|-----------------|---------------|---------------------|----------------------------------|
| Matsumoto (1972–1976) [18,19] | >40             | <0.0005%      | –                   | many steps, racemic              |
| Shirahama (1994) [22]    | 38              | 0.05%         | enantioselective    | many steps                       |
| Newhouse (2019) [26]     | 19              | 0.4%          | convergent, few steps| limited to C\textsuperscript{1} epimers of the general structure |
| Ding (2019) [31]         | 22–23           | 1.2–1.4%      | few steps, highest yielding | limited to C\textsuperscript{1}–C\textsuperscript{5} dehydrogenated grayananes |
| Luo (2022) [34]          | 18–20           | 0.01–0.5%     | few steps, flexible  | lower yields                     |
grayanane family were synthesized, out of the more than 160 compounds known to date. Thus, we anticipate that in the future, organic chemists will keep focusing on highly enantioselective, efficient and flexible synthetic strategies towards grayanane natural products.

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See for a new total synthesis published by the group of Jia during the editing process of this article, following their work on the total synthesis of glaucocalyxin A [42].

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