Total synthesis: an enabling science

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Synthesis of tryptophan-dehydrobutyryne diketopiperazine and biological activity of hangtaimycin and its co-metabolites

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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 HTM₂₂₂ 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 monooxygenase was recently shown to be responsible for the oxidation of deoxyhangtaimycin (3), a compound with antiviral activity, to 1 [8]. The thereby installed
The hemiaminal function is also the breaking point for \( \text{HTM}_{222} \) (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 \( D \)-tryptophan unit, based on a comparison of the optical rotation of the isolated compound \( ([\alpha]_D^{20} = -12.67, c 1.1, 95\% \text{ EtOH} [1]) \) to 4 synthesised from \( L \)-tryptophan \( ([\alpha]_D^{21} = +13, c 0.03, \text{EtOH} [12]) \), 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 \( ([\alpha]_D^{24.5} = +10.0, c 1.1, 95\% \text{ EtOH} [9]) \), 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 tests 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 \( tert \)-butyloxy-carbonyl (Boc)-protected threonine using bis(2-oxo-3-oxazolidinyl)phosphinic chloride (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 LiClO$_4$ 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 LiClO$_4$ 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 ($[\alpha]_D^{25} = +1.9$, 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.
using milder conditions (Scheme 3). The newly developed synthetic route 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 benzylxycarbonyl (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]25D = –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.
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 HTM222 (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

cules 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 mole-

Figure 2: X-ray structure of (rac)-4.

Figure 3: Bioactivity testing with hangtaimycin (1). A) Growth retardation of model species B. subtilis 168 and of B) nosocomial pathogen A. baumannii 09987 monitored by recording the turbidity increase of growing culture aliquots exposed to a concentration series of 1 every 10 min over 24 h. An increase in the optical density at 600 nm (OD_{600}) reflects biomass production. At concentrations of ≥64 µg/mL 1 precipitated, leading to elevated OD_{600} values at the beginning of the experiment that were unrelated to growth. The curves reflect the mean values of three separate cultures and standard deviations are depicted by black bars. C) MIC assay against E. coli. The MIC is defined as the lowest concentration of an antibacterial agent inhibiting visible bacterial growth (no turbidity detected by the naked eye) after overnight incubation. Only when the outer membrane was permeabilised by polymyxin B nonapeptide (PMBN), 1 inhibited growth of E. coli sufficiently to yield a clear MIC. GC, growth control (no inhibitor), SC, sterile control (no bacteria), DMSO, culture medium supplemented with 1% DMSO, reflecting the DMSO concentrations in the hangtaimycin-containing samples.
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 TDD easily undergoes racemisation under basic conditions. The reported inactivity of 4 against bacteria was confirmed in this study, and also 2 is an inactive metabolite of S. spectabilis, while for I moderate growth retardation against A. baumannii and B. subtilis, and growth inhibition against PMBN-treated E. coli was observed. However, the low activity of I in these assays suggests that the natural function of this structurally remarkable compound awaits future clarification.

Supporting Information

Supporting Information File 1
Experimental, analytical and X-ray data as well as copies of NMR spectra.
[https://www.beilstein-journals.org/bjoc/content/supplementary/1860-5397-18-120-S1.pdf]

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Vicinal ketoesters – key intermediates in the total synthesis of natural products

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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 chromone [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 sulfonylchromenone 20, accessible from dihydroxyacetophenone 19 and thiol 18 derived from known alcohol 17 [11,12]. DMP oxidation of α-hydroxyester 21 and subsequent cycloisomerization led to the desired cyclization product 23 via transition state II in a dr of 5:1. Final deprotection gave preussochromone A (24).

**Scheme 3:** Synthesis of euphorikanin A (16) by intramolecular, nucleophilic addition [6].

**Scheme 4:** Ketoester cycloisomerization for the synthesis of preussochromone A (24) [10].
(-)-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 built up via oxidation and nucleophilic addition of methyl diazoacetate, yielding alcohol 27. Subsequent oxidation gave alpha-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-prezizaane 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 alpha-ketoester 33. Reduction, lactonization, and elimination gave the ketoesters-derived enol 34. Oxidation of the latter compound to the alpha-keto-beta-hydroxy ester IV using DMDO and subsequent heating in PhCF_3 triggered an alpha-ketol rearrangement which led to ketol V. Diastereoselective reduction gave alpha,beta-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 cycloaddition of alpha-ketoester 41 and the thiosemicarbazide 42-derived azomethine imine VI to the triazacyclopenta[cd]pentalene 43 was utilized as a key step (Scheme 7) [18-21].

The alpha-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 5: Diastereoselective, intramolecular aldol reaction of an alpha-ketoester 28 in the synthesis of (-)-preussochromone D (30) [13,14].
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 isopropylmagnesium 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-acetylpyrrolidine (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 α-ketoester in the synthesis of jatropane diterpenoids [22].

Scheme 9: Grignard addition to an α-ketoester and subsequent Friedel–Crafts cyclization in the synthesis of (−)-hopeanol (59) [24].
Scheme 10: Diastereoselective addition to an auxiliary modified α-ketoester in the formal synthesis of (+)-camptothecin (65) [25].

Scheme 11: Intramolecular photoreduction of an α-ketoester in the synthesis of (rac)-isoretronecanol (69) [26].

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 CuII-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-tricarbonyl 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 hand, 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].
Scheme 17: Synthesis of mesoxalic ester amide 102 and its use in the synthesis of (rac)-cladoniamide G (103) [33].

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 carbonyl 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].

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ations of organometallic reagents. 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 monoterpenene 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 CpTi\textsubscript{III}Cl\textsubscript{2} 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].

A component responsible for the fresh and floral odor of the essential oil was isolated from Davana oil and was assigned as a 2,5-dihydrofuran monoterpenoid (compound 1 in Scheme 1) and named dihydrorosefuran [6-8]. Furthermore, the same structure was attributed to an isolated substance from the Argentinean herb Tagetes mendocina [9], although not all of its spectroscopic features did match point by point with those previously reported. This made us think that this could be a case of misassigned natural product [10], hence we decided to perform its total synthesis.
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 [CpTiCl₂] [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-yn in the presence of CpTiCl₂ (generated in situ by reduction of CpTiCl₃ 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 CpTiCl₃ 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 monoterpenone 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 allanol 3 via lipase AK-catalyzed acetylation [15]. In this way, unaltered, (−)-hydroxyallene 3 could be separated from (+)-acetyl derivative 9 through standard column chromatog-
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-hydroxylallene (−)-(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 $^{13}$C 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 $^1$H 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 allenol 3 and synthesis of derivatives.](attachment:image)

**Table 1**: $^1$H NMR data of isolated and synthetic products.

<table>
<thead>
<tr>
<th>sources of claimed dihydorosefuran</th>
<th>A. pallens$^{a,c}$ [7,8]</th>
<th>T. mendocina$^d$ [9]</th>
<th>this work synthesis$^e$</th>
<th>literature synthesis$^f$ [20]</th>
</tr>
</thead>
<tbody>
<tr>
<td>H2</td>
<td>4.04 (td, $J = 8.0$, 1.0 Hz)</td>
<td>4.03 (t, $J = 8$ Hz)</td>
<td>4.67 (br s)</td>
<td>4.02–3.99 (m)</td>
</tr>
<tr>
<td>H4</td>
<td>5.07 (td, $J = 8.0$, 1.0 Hz)</td>
<td>5.66 (t, $J = 8$ Hz)</td>
<td>5.51 (br s)</td>
<td>5.67–5.62 (m)</td>
</tr>
<tr>
<td>H5</td>
<td>4.55 (d, $J = 7.0$ Hz)</td>
<td>4.21 (dd)</td>
<td>4.62–4.53 (m)</td>
<td>4.24–4.15 (m)</td>
</tr>
<tr>
<td>H1'</td>
<td>2.30 (m)</td>
<td>2.24 (m)</td>
<td>2.41 (m), 2.20 (ddd, $J = 14.0$, 7.0, 6.5 Hz)</td>
<td>2.28–2.19 (m)</td>
</tr>
<tr>
<td>H2'</td>
<td>5.32 (t, $J = 7.0$ Hz)</td>
<td>5.13 (t, $J = 8$ Hz)</td>
<td>5.21 (t, hept, $J = 6.9$, 1.5 Hz)</td>
<td>5.13–5.09 (m)</td>
</tr>
<tr>
<td>H4'</td>
<td>1.67 (s)</td>
<td>1.75 (s)</td>
<td>1.74 (s)</td>
<td>1.73 (d, $J = 1.1$ Hz)</td>
</tr>
<tr>
<td>H5'</td>
<td>1.60 (s)</td>
<td>1.66 (s)</td>
<td>1.66 (s)</td>
<td>1.64 (s)</td>
</tr>
<tr>
<td>H1''</td>
<td>2.05 (s)</td>
<td>1.69 (s)</td>
<td>1.72 (s)</td>
<td>1.67 (s)</td>
</tr>
</tbody>
</table>

$^a$CDCl$_3$ in all cases; $^b$Arbitrary numbering for comparison purposes; $^c$80 MHz; $^d$400 MHz; $^e$500 MHz.
Conclusion
In summary, we have proved that the two-step sequence Ti\textsuperscript{III} allenylation–Ag\textsuperscript{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 \textit{Artemisia pallens} oil, dihydrorosefuran, is not correct. In addition, it is clear that the compound isolated from \textit{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 CpTiCl\textsubscript{3} (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-yne (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 MgSO\textsubscript{4}), 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\textsuperscript{-1}): 3434, 2972, 2928, 1958, 1723, 1436, 1374, 1172, 1028, 925, 853; \textsuperscript{1}H NMR (300 MHz, CDCl\textsubscript{3}) 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; \textsuperscript{13}C NMR (75 MHz, CDCl\textsubscript{3}, DEPT) δ 204.8 (C), 174.0 (C), 101.6 (C), 77.0 (CH\textsubscript{2}), 71.5 (CH), 60.5 (CH\textsubscript{2}), 30.4 (CH\textsubscript{2}), 30.0 (CH\textsubscript{2}), 14.5 (CH\textsubscript{3}), 14.2 (CH\textsubscript{3}) ppm; HRMS–ESI (Q-TOF, m/z): [M + H]\textsuperscript{+} calcd for C\textsubscript{10}H\textsubscript{12}O\textsubscript{3}, 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\textsuperscript{-1}): 2982, 2927, 1960, 1772, 1427, 1331, 1162, 974, 918, 855; \textsuperscript{1}H NMR (300 MHz, CDCl\textsubscript{3}) 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; \textsuperscript{13}C [\textsuperscript{1}H] NMR (75 MHz, CDCl\textsubscript{3}, DEPT) δ 206.0 (C), 177.0 (C), 98.0 (C), 80.4 (CH), 77.5 (CH\textsubscript{2}), 28.5 (CH\textsubscript{2}), 26.1 (CH\textsubscript{3}), 15.0 (CH\textsubscript{3}) ppm; HRMS–ESI (Q-TOF, m/z): [M + H]\textsuperscript{+} calcd for C\textsubscript{8}H\textsubscript{11}O\textsubscript{2}, 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 AgNO\textsubscript{3} (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 Et\textsubscript{2}O. The organic phase was dried over anhydrous MgSO\textsubscript{4}, 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\textsuperscript{-1}): 2969, 2927, 2849, 1731, 1442, 1376, 1251, 1160, 1092, 1026, 895, 734; \textsuperscript{1}H NMR (300 MHz,
Synthesis of 2-methyl-4-(3-methyl-2,5-dihydrofuran-2-yl)butan-2-ol (6)

Under an N₂ atmosphere, methylmagnesium bromide (3 M in Et₂O, 0.075 mL, 0.23 mmol) was added to dry Et₂O (1.5 mL). A solution of ethyl 3-(3-methyl-2,5-dihydrofuran-2-yl)propanoate (2, 16 mg, 0.087 mmol) in Et₂O (1 mL) was added dropwise and the reaction mixture was stirred for 3 hours at room temperature. The reaction was quenched with saturated NH₄Cl and extracted with EtOAc. The combined organic layer was washed with saturated NaHCO₃, brine, and dried over anhydrous MgSO₄. The solvent was evaporated in vacuum and the residue was purified by column chromatography (n-hexane/EtOAc 7:3) to give 2-methyl-4-(3-methyl-2,5-dihydrofuran-2-yl)propanoate (6, 169 mg, 90%) isolated as colorless oil. IR (ATR) ν (cm⁻¹): 3425, 2969, 2922, 2852, 1636, 1444, 1382, 1280, 975, 850, 778; ν (cm⁻¹) (ν−OH): 3066, 2965, 2918, 2843, 1668, 1445, 1377, 1080, 975, 778; ¹H NMR (500 MHz, CDCl₃) δ 137.8 (C), 120.7 (CH), 87.7 (CH), 74.5 (CH₂), 70.4 (C), 38.5 (CH₂), 29.5 (CH₃), 29.4 (CH₂), 28.3 (CH₂), 12.5 (CH₃) ppm; HRMS–ESI (Q-TOF, m/z): [M + H⁺] calculated for C₁₀H₁₉O₂, 171.1385; found, 171.1368.

Synthesis of 3-methyl-2-(3-methylbut-2-en-1-yl)-2,5-dihydrofuran (1)

The reaction of amberl Kylest®-15 (dry, 97 mg) and 2-methyl-4-(3-methyl-2,5-dihydrofuran-2-yl)butan-2-ol (6, 97 mg, 0.57 mmol), based on the previously reported literature procedure [21], afforded 3-methyl-2-(3-methylbut-2-en-1-yl)-2,5-dihydrofuran (1, 64 mg, 74%) isolated as colorless oil. IR (ATR) ν (cm⁻¹): 3066, 2965, 2918, 2843, 1668, 1445, 1377, 1080, 975, 778; ¹H NMR (500 MHz, CDCl₃) δ 5.51 (br s, 1H, H4), 4.68 (br s, 1H), 4.58 (m, 2H), 2.05 (br s, 1H), 1.82 (m, 1H), 1.72 (br s, 3H), 1.57 (m, 3H), 1.24 (s, 6H) ppm; HRMS–ESI (Q-TOF, m/z): [M + H⁺] calculated for C₁₀H₁₃O, 153.1274; found, 153.1262.

References
13. Smith, A. B., III; Fukui, M.; Vaccaro, H. A.; Empfield, J. R. 

14. Reaction performed with stoichiometric amounts of CpTiCl₂.


doi:10.1038/nprot.2007.354

17. Under this standard esterification conditions, some lactonization 
leading to compound 5 is observed.


doi:10.1016/j.tet.2012.06.083

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Abstract

A convergent strategy for the synthesis of leustroducins 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 vinillylithium intermediate. An advanced synthetic intermediate was then obtained after functional group transformation.

Introduction

Leustroducins 1a–c and phoslactomycins 2a–f are a family of closely related natural products extracted from Streptomyces platensis (leustroducins) 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 leustroducins [13-17] have been subject of extensive synthetic studies.

In a project related to the synthesis of leustroducins 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-
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 6, a chiral chloronitrosodi- 

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
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
11b was obtained by Red-Al reduction in identical yield to the racemic equivalent.

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.

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.

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

<table>
<thead>
<tr>
<th>entry</th>
<th>n1</th>
<th>n2</th>
<th>conditions</th>
<th>yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>1,2</td>
<td>–78 °C, 15 min, then rt, 8 h</td>
<td>21%</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>1,2</td>
<td>–78 °C, 2 h, then rt, 16 h</td>
<td>16%</td>
</tr>
<tr>
<td>3</td>
<td>1.5</td>
<td>1,8</td>
<td>–78 °C, 2 h, then rt, 3 h</td>
<td>24%</td>
</tr>
<tr>
<td>4</td>
<td>1.5</td>
<td>1,6</td>
<td>–78 °C, 2 h, then rt, 4 h</td>
<td>39%</td>
</tr>
</tbody>
</table>

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...
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 ZnMe2 (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 CeCl3 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 vinyllithium 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.
Scheme 9: Oxidation of the acetal to the lactone.

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 CDCl$_3$ 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 $-78 \, ^\circ \text{C}$ and DMSO (0.82 mL, 11.49 mmol, 3 equiv) was added over 5 min. After 15 min, a solution of the alcohol 15 [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 $-78 \, ^\circ \text{C}$ 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 CuSO$_4$ solution (4 × 12.5 mL), saturated aqueous NH$_4$Cl solution (3 × 12.5 mL), dried (MgSO$_4$), filtered and concentrated under reduced pressure to give a brown oil (1.021 g, 99%). R$_f$: 0.59 (10% AcOEt/cyclohexane); $^{1}$H NMR (300 MHz, CDCl$_3$) $\delta$ 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; $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 200.8, 136.7, 127.9, 62.4, 35.9, 27.2, 18.0, 12.0 ppm; HRMS (m/z): [M + Na]$^+$ calc 293.1907; found, 293.1898.

(3Z)-3-Diethylphosphoryloxy-6-triisopropylsilyloxyhexa-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 ($-78 \, ^\circ \text{C}$) 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 $-78 \, ^\circ \text{C}$, 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 (MgSO$_4$), 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%). R$_f$: 0.47 (30% AcOEt/cyclohexane); $^{1}$H NMR (300 MHz, CDCl$_3$) $\delta$ 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; $^{13}$C NMR (90 MHz, CDCl$_3$) $\delta$ 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]$^+$ calc 407.2377; found, 407.2359.

(6R)-tert-Butyl 5-(diethoxyphosphoryloxy)-6-(2-((triisopropylsilyloxy)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 was 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 Boc₂O (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, 560.2775; δ (90 MHz, CDCl₃) 7.2 Hz, 6H), 1.05 (m, 9H), 1.34 (t, J = 7.3 Hz, 3H) ppm; δ (150 MHz, CDCl₃) 14.9 min, tr (S) = 16.2 min).

(6R)-tert-Butyl 5-oxo-6-(2-((triisopropylsilyl)oxy)ethyl)-1,2-oxazinane-2-carboxylate (11b): A solution of the cycloadduct 10b (404 mg, 0.751 mmol) in anhydrous THF (12 mL) was cooled to 0 °C and a 3 M solution of Red-Al in toluene (1 mL, 3 mmol, 4 equiv) was rapidly added. After stirring 30 min at 0 °C, the reaction was hydrolyzed by addition of an saturated aqueous NH₄Cl solution (4 mL). The solution was concentrated under reduced pressure, the residue taken up with dichloromethane (10 mL) and filtered, washing with dichloromethane (3 × 5 mL). The filtrate was concentrated under reduced pressure to give a yellow oil (300 mg) consisting in a mixture of the ketone 11b and the over-reduced alcohol. This mixture was carried into the next step without further purification.

DMSO (0.16 mL, 2.241 mmol, 3 equiv) was added dropwise to a cooled (-78 °C) solution of oxalyl chloride (0.1 mL, 1.121 mmol, 1.5 equiv) in dichloromethane (3.4 mL). After stirring 15 min at -78 °C, a solution of the crude product from reduction reaction (300 mg) in dichloromethane (2 mL) was added dropwise. After 30 min at -78 °C, triethylamine (0.52 mL, 3.735 mmol, 5 equiv) was added. The cooling bath was removed and the solution stirred at rt for 40 min, before being poured into diethyl ether (45 mL). The solution was successively washed with saturated aqueous CuSO₄ solution (4 × 10 mL) and saturated aqueous NH₄Cl solution (3 × 10 mL), then dried (MgSO₄), filtered and concentrated under reduced pressure. The residue was purified by filtration through a short plug of silica gel, eluting with ethyl acetate. Concentration under reduced pressure gave the pure ketone 11b as an orange oil (255 mg, 84% over two steps). Rf 0.29 (10% AcOEt/cyclohexane). ¹H NMR (250 MHz, CDCl₃) δ 4.49 (dd, J = 8.3, 3.8 Hz, 1H), 4.18–4.08 (m, 1H), 3.94 (m, 4H), 2.67 (t, J = 7.0 Hz, 2H), 2.16–2.03 (m, 1H), 1.99–1.85 (m, 1H), 1.51 (s, 9H), 1.05 (m, 21H) ppm. ¹³C NMR (90 MHz, CDCl₃) δ 206.6, 154.9, 85.1, 82.3, 59.0, 45.0, 36.5, 32.2, 28.4, 18.1, 12.1 ppm; HRMS (m/z): [M + Na]⁺ calcd 424.2490; found, 424.2480; [M + Na]⁺ +37.4 (c 0.5, CH₂Cl₂).

(5S,6R)-5-Ethyl-6-ethyl-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 partitioned 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 0.33 (30% Et₂O/pentane).

(2R,3S,6RS)-3-Ethyl-2-ethyl-5-methoxy-3,6-dihydro-2H-pyran (19): This compound was prepared according to reference [18].
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 Cp₂ZrHCl (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). The layers were separated and the aqueous layer extracted with ethyl acetate (2 × 8 mL) and diethyl ether (2 × 8 mL). The combined organic layers were dried (MgSO₄), 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-((triisopropylsilyl)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 (dd, dd, t, 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]⁺ calc 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,
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Solid-phase total synthesis and structural confirmation of antimicrobial longicatenamide A

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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 stereoc control. 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 component 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.

Results and Discussion

Although the solution-phase total synthesis of an analogue of longicatenamycin A has been reported [10], a solid-phase strategy can facilitate the production of a wider variety of analogous compounds than solution-phase synthesis [11]. Consequently, in this study, compound 1 was synthesized using a solid-phase strategy for future derivatization to produce probe molecules for deciphering microbial chemical communication.

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
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-D-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, longicatenamide 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 \( \text{I} \) (minimum inhibitory concentration (MIC) = 50 \( \mu \)M) exhibited moderate but selective antimicrobial activity against \textit{Bacillus subtilis} similar to natural \( \text{I} \) (MIC = 100 \( \mu \)M).

**Conclusion**
We accomplished solid-phase total synthesis of longicatenamidine A (1). Initially, commercially unavailable building blocks 7 and 10 were chemically synthesized with stereocore. 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.
28. Ma, B.; Banerjee, B.; Litvinov, D. N.; He, L.; Castle, S. L. 


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Formal total synthesis of macarpine via a Au(I)-catalyzed 6-endo-dig cycloisomerization strategy

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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.

The benzo[c]phenanthidine skeleton consists of a phenanthridine (rings A, B, C) and a benzene (ring D), and most of the
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 nitrosation 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/SNAr 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.

Retrosynthetically, the target molecule macarpine (1) could be disconnected into naphthol 12 (Scheme 3), a key intermediate reported by Ishikawa in the total synthesis of macarpine. This intermediate could be synthesized from silyl enol ether compound 10 via the Au(I)-catalyzed cycloisomerization reaction developed by our group [15]. The compound 10 could be constructed by the Sonogashira coupling reaction from readily prepared iodoarene 8 [12,16] and ketone 5, which could be synthesized by using cheap 6-bromopiperonal (2) as the starting material.

To attempt the proposed synthetic strategy, ketone 5 and iodoarene 8 were prepared by following the synthetic route outlined in Scheme 4. Ketone 5 was prepared in a four-step procedure. Firstly, a Sonogashira coupling between 6-bromopiperonal (2) and trimethylsilylacetylene was performed to furnish aldehyde 3 [17,18] in 89% yield. A following nucleophilic addition reaction of aldehyde 3 by methylmagnesium bromide (MeMgBr) gave alcohol 4 in 99% yield, which was oxidized by pyridinium chlorochromate (PCC) leading to the formation of ketone 5 and the deprotection of the silyl group was accomplished in the presence of potassium carbonate (K2CO3) and methanol to provide the terminal alkyne 5 in 96% yield in two steps. The iodoarene 8 [12,16] was facilely synthesized from sesamol (6) via methylation and iodination in an overall yield of 67%.

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-butylimethylsilyl 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/S$_N$Ar (Pabbaraja)

Scheme 3: Retrosynthetic analysis of marcarpine precursor 12 for a partial synthesis.
To find the best cycloisomerization conditions, the 1,5-enzyme 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 (AgSbF$_6$) 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 (Ph$_3$P), [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 AgSbF$_6$ was utilized as the sole catalyst, not any 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/AgSbF$_6$ 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 (AgSbF$_6$) [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 methoxy substitution in the substrate played a crucial role in controlling the selectivity of the cycloisomerization according to our previous study [15].
Table 1: Optimization of the Au(I)-catalyzed cycloisomerization conditions.

<table>
<thead>
<tr>
<th>entry</th>
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<th>solvent</th>
<th>additive</th>
<th>$T$ (°C)</th>
<th>yield (%)</th>
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<td>23</td>
</tr>
<tr>
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</tr>
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<td>82$^b$</td>
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<td>17</td>
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<td>THF</td>
<td>AgSbF$_6$</td>
<td>23</td>
<td>80$^e$</td>
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</table>

$^a$3 mol % IPrAuCl and 3 mol % AgSbF$_6$ were used. $^b$10 mol % IPrAuCl and 10 mol % AgSbF$_6$ were used. $^c$The reaction was run for 1 h. $^d$The reaction was run for 3 h. $^e$5 mol % 2,6-di-tert-butylpyridine was added. IPr = [1,3-bis(2,6-diisopropylphenyl)imidazol-2-ylidene]. JohnPhos = [[1,1'-biphenyl]-2-yldi-tert-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(I)-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.

### Table 2: Reaction condition optimization of the cycloisomerization of alkynyl ketone 9.

<table>
<thead>
<tr>
<th>entry</th>
<th>acid</th>
<th>solvent</th>
<th>T (°C)</th>
<th>yield (%)</th>
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<td>DCM</td>
<td>23</td>
<td>0</td>
</tr>
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<td>2</td>
<td>TsOH</td>
<td>DCM</td>
<td>40</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>TsOH</td>
<td>DCE</td>
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<td>5</td>
<td>AcOH</td>
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<td>70</td>
<td>24</td>
</tr>
<tr>
<td>6</td>
<td>PhCO₂H</td>
<td>DCE</td>
<td>70</td>
<td>39</td>
</tr>
</tbody>
</table>

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

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References

Synthetic study toward the diterpenoid aberrarone

Liang Shi‡1, Zhiyu Gao‡1, Yiqing Li1, Yuanhao Dai1, Yu Liu1, Lili Shi*2 and Hong-Dong Hao*1,2

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-
sive 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.

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-enyne 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 stereocenters 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 cyano 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.
Scheme 1: Synthesis of (−)-7-amino-7,8-dihydrobisabolene (4) and its conversion to cyclization precursor 7.
amide [10] and p-bromobenzamide derivatives of these amines are solids, no change in dr was observed upon recrystallization from a variety of solvents. Fortunately, we ultimately found success with the benzamide derivative 5, which could be prepared from the mixture of 4 and its C7-epimer in 93% yield upon treatment with benzyl chloride. Recrystallization of the resulting mixture of diastereomeric amides from cyclohexane improved the dr from 83:17 to 95:5 (as determined by 1H NMR based on integration of the C7-methyl signals). A second recrystallization from cyclohexane afforded 5 as a single stereoisomer (>99:1 dr) with 42% overall recovery of material (corresponding to 51% recovery of the major diastereomer 5).

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-dihydrisabolene (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 intramolecularaza-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 $\delta = 20.7$ in (−)-2 and $\delta = 14.5$ 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
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 oxocarbonylum 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-hydroxycarboxylic 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 oxocarboxylic acid even though the corresponding γ-lactones typicaly form spontaneously. Indeed, no lactone formation was observed even upon purification by column chromatography on silica gel, reflecting the highly strained nature of the trisubstituted alkene of the prenyl group once again serving as the nucleophile. In this case, the 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).

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-dihydroabisabolene (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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