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Syntheses of antiangiogenic or cytotoxic natural products: Fumagillin and bengacarboline*

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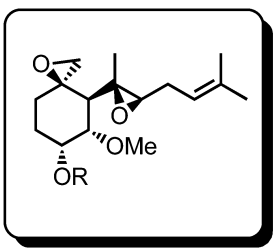
Abstract: Syntheses of fumagillol, a precursor of antiangiogenic sesquiterpene fumagillin, and of cytotoxic marine alkaloid bengacarboline are described.

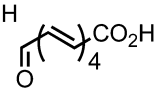
Keywords: fumagillol; antiangiogenic; sesquiterpene; fumagillin; bengacarboline.

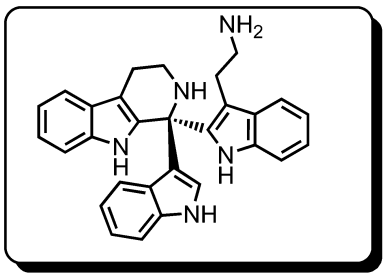
A novel formal synthesis of fumagillol **1a**, the direct precursor of fumagillin **1b**, an antiangiogenic sesquiterpene, and the first total synthesis of a cytotoxic marine alkaloid bengacarboline **2** are presented and discussed herein (Scheme 1).

Fumagillin **1b**, isolated in 1951 by Elbe and Hanson [1] from the microbial organism *Aspergillus fumigatus*, is representative of a class of sesquiterpenes like ovalicin **3** [2] and FR 65814 **4** [3], which displays interesting biological activities. Fumagillin itself was first described as an antimicrobial agent, but more recently Folkman and coworkers [4] discovered that this compound is a potent and selective inhibitor of angiogenesis.



Fumagillol **1a** : R = H
Fumagillin **1b** : R =





Bengacarboline **2**

Scheme 1 Syntheses of antiangiogenic or cytoxic compounds: Fumagillin and bengacarboline.

The same activity has been reported later for ovalicin **3** [5], which, as FR 65814 **4**, is also a potent immunosuppressor [3]. More recently, semisynthetic compound TNP-470 **5** [6,7] and other analogs showed a better therapeutic index than fumagillin **1b**. The recent discovery that methionine amino-

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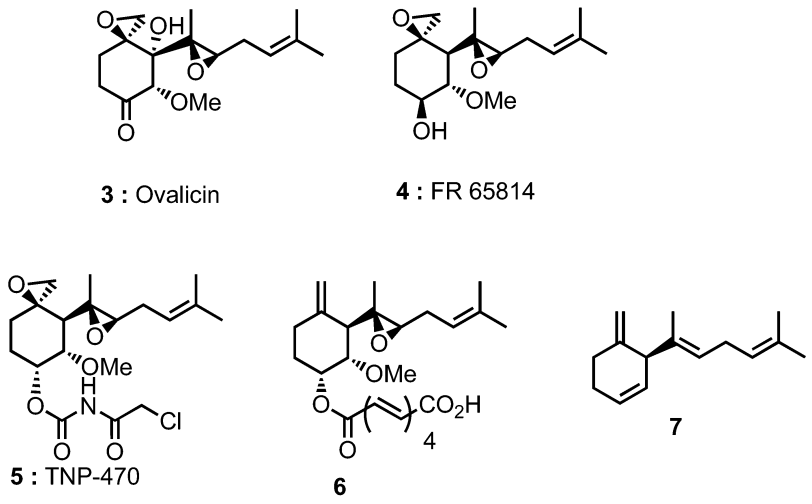
peptidase II (MetAp-II) is selectively inhibited by fumagillin **1b** [8], and the X-ray structure of the covalent complex between fumagillin **1b** and MetAp-II [9] increases the interest of the synthesis of fumagillin **1b** and of new fumagillin analogs [10–12].

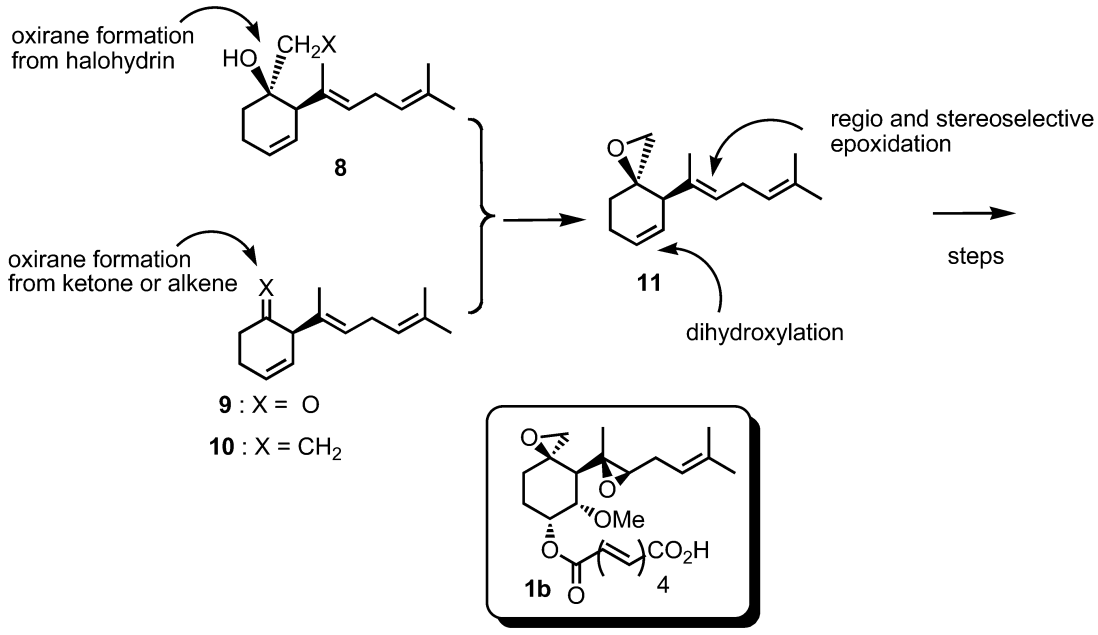


Scheme 2

After the pioneering synthesis of racemic fumagillin **1b** by Corey in 1972 [13], this area of research remained dormant for 25 years. But in the past 5 years, three syntheses of (–)-fumagillin **1b** or (–)-fumagillol **1a**, the direct precursor of fumagillin **1b** [14–16,17b], as well as two syntheses of racemic fumagillin **1b**/fumagillol **1a** [17a,18] have been published.

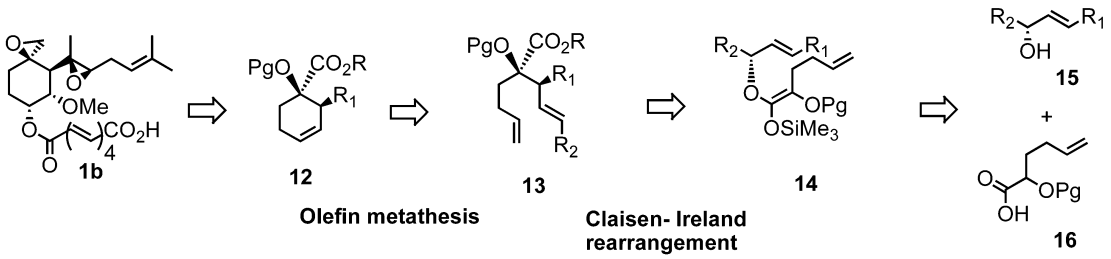
From a biogenetic point of view, it has been demonstrated that fumagillin **1** and ovalicin **2** have as a common precursor *cis*-farnesyl pyrophosphate [19,20]. This biogenetic pathway has been recently reinforced by the discovery of a new metabolite **6** in the fermentation broth producing fumagillin **1b** [21,22].

The structure of fumagillin **1b** is characterized by the presence of six stereogenic centers, a functionalized *cis*-diol, and two epoxides. The nucleophilic attack of histidine 231 of MetAp-II on spiro epoxide gave rise to the formation of a covalent bond, which was shown in the X-ray structure of the complex between **1b** and MetAp-II [9]. From a synthetic point of view, a possible precursor of fumagillin could be the terpene-like derivative **7** characterized by the presence of four double bonds. The selective functionalization of three of these double bonds can be considered as the main challenge in the synthesis of **1b**. Owing to the relative reactivity of these double bonds, the sequence of functionalization of each alkene is also fundamental (Scheme 3).



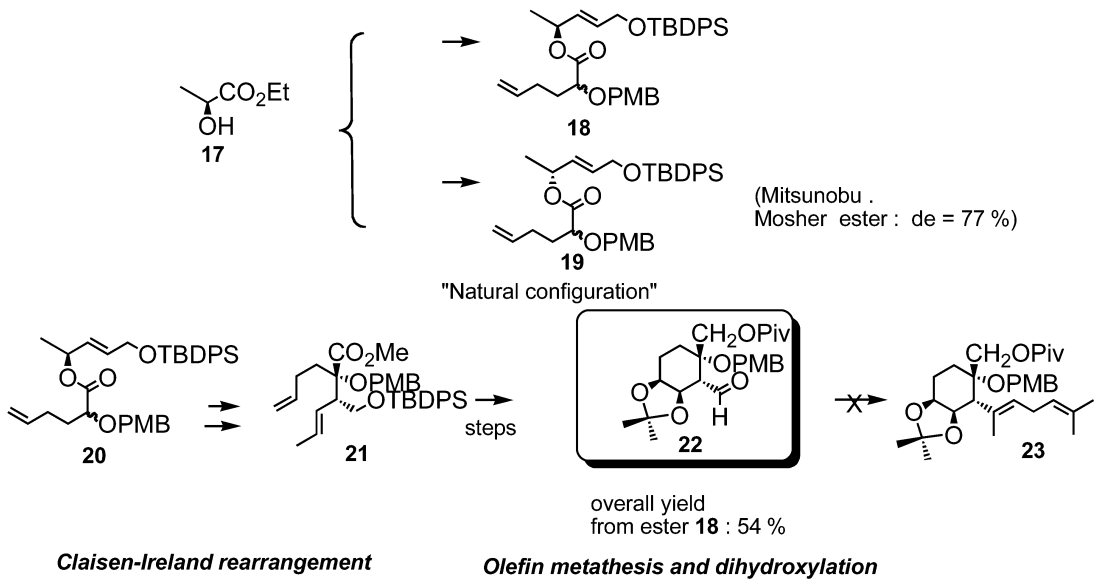
Scheme 3 Main steps in fumagillin synthesis.

Our initial plan for the synthesis of fumagillin **1b** involved in a key step a Claisen–Ireland rearrangement [23] of the silylketene acetal derivative **14** followed by ring-closing metathesis [24]. The stereogenic center in the starting allylic alcohol **15** should allow the control of all of the stereogenic centers in the target molecule.



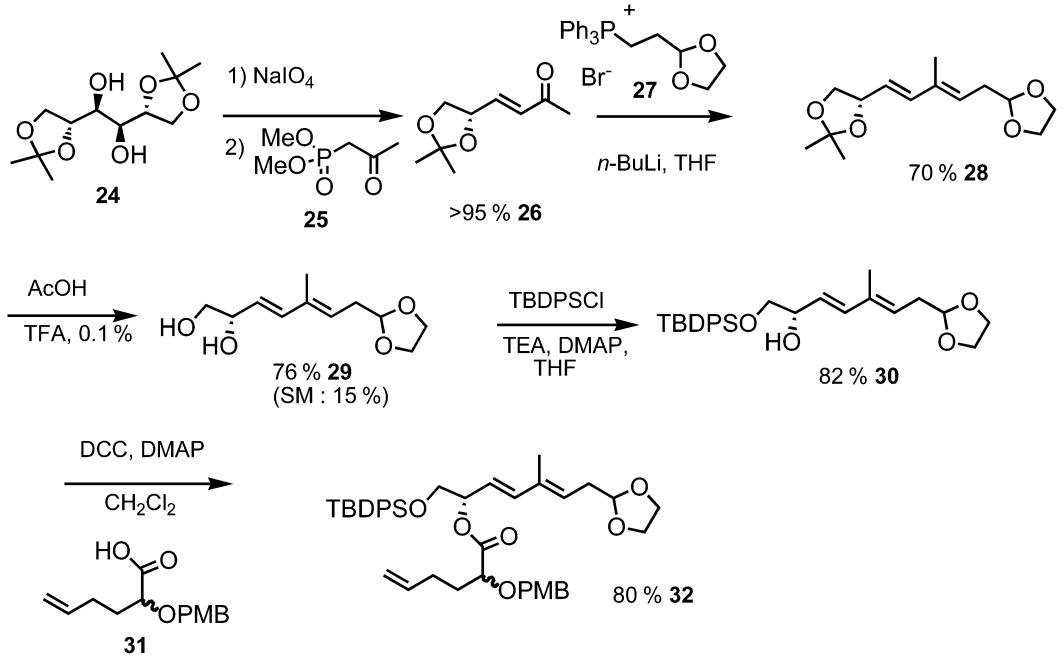
Scheme 4 Fumagillin retrosynthetic scheme.

In a first approach, ethyl lactate **17** was used as chiral pool starting material. Natural configuration was obtained after the Mitsunobu reaction. However, inversion of the configuration was not complete, and at this stage, a model study was developed in the antipodal series. Selective Claisen–Ireland rearrangement followed by ring-closing metathesis and further transformations afforded aldehyde **22** in high overall yield [25]. This approach was thwarted in the following step because of the instability of this compound under mild basic conditions, which precluded the introduction of the acetylenic unit (Scheme 5). Exploration of a more convergent approach in which one of the side-chain trisubstituted double bonds could be introduced in an early stage of the synthesis was consequently studied.



Scheme 5 First approach. Chiral pool: ethyl lactate.

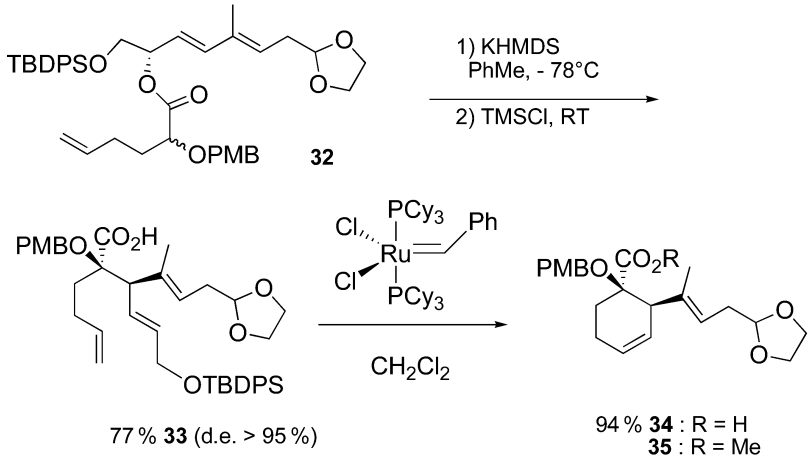
As illustrated in Scheme 6, di*iso*propylidene mannitol **24** was used as starting material. A known sequence of reactions afforded ketone **26** in high yield [26,27]. A second olefination [28] followed by a chemoselective hydrolysis gave rise to dioxolanyl diol **29**. A selective protection of the primary alcohol and esterification of the secondary alcohol led to the target ester **32**.



Scheme 6 A more convergent approach. Chiral pool: di*iso*propylidene mannitol.

On the other hand, the acid derivative **31** was obtained by oxidation ($NaClO_2$, NaH_2PO_4, methyl-2-butene, *t*BuOH–H_2O, yield >95 %) of the corresponding aldehyde previously described by Evans' group [29]. Esterification between **30** and **31** afforded ester **32** in 52 % overall yield from di*iso*propylidene mannitol **24** (Scheme 6).

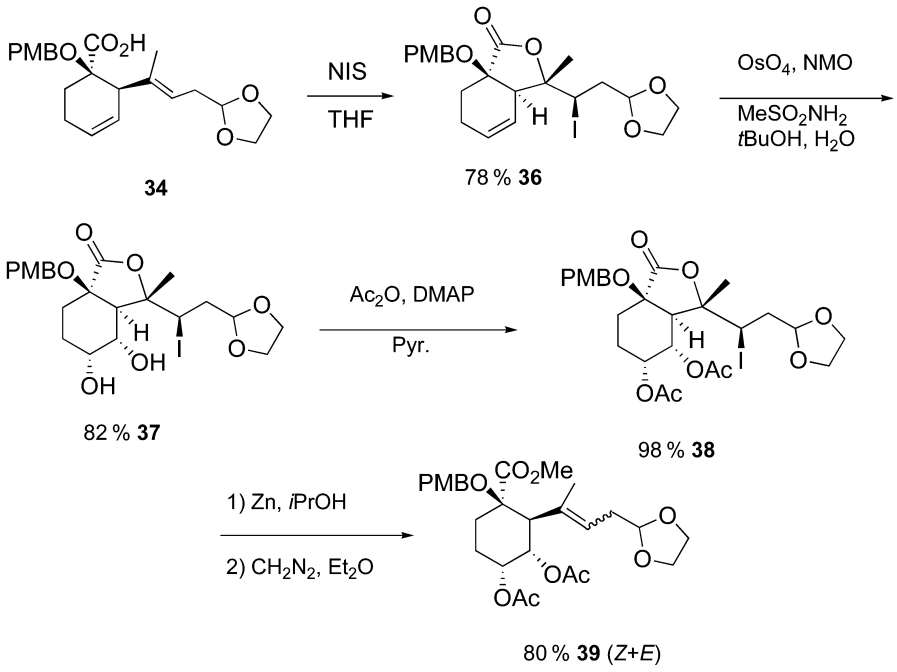
A Claisen–Ireland rearrangement under the same conditions used in our first approach [25] afforded after hydrolysis in high yield and selectivity the rearranged acid **33**. A *Z*-chelated enolate and a chair-like intermediate are probably the reason for the observed selectivity (Scheme 7). Ring-closing metathesis gave rise in nearly quantitative yield to the cyclohexene derivative **34**. This sequence of reaction can be performed in a one-pot process without loss of yield.



Scheme 7 Claisen–Ireland rearrangement and ring-closing metathesis.

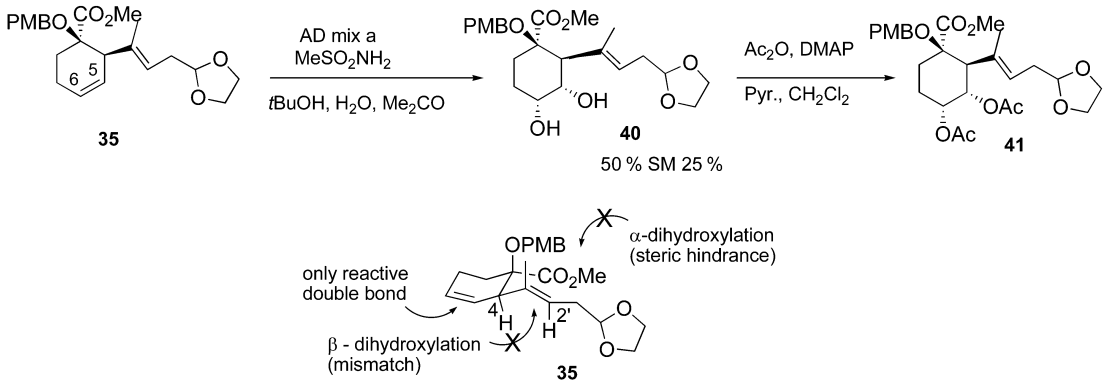
With acid **34** and the corresponding methyl ester **35** in hand, dihydroxylation of the theoretically less reactive disubstituted double bond and epoxidation of the side-chain trisubstituted double bond were next examined. According to the previous synthesis [17], this reaction sequence has to be made in this order. The presence of alcohol at C5 in the homoallylic position is necessary for stereogenic control of the side-chain epoxidation.

An internal protection of the trisubstituted double bond was examined for the first time. Accordingly, iodo lactonization of acid derivative **34** afforded compound **36** [30]. Selective dihydroxylation followed by diol **37** acetylation gave rise in good overall yield to lactone diacetate derivative **38**. Retrolactonization was promoted by zinc powder. After treatment with diazomethane, careful examination of the NMR spectra of the resulting ester **39** revealed, however, that a mixture of *Z*- and *E*-isomers constituted this compound (Scheme 8).



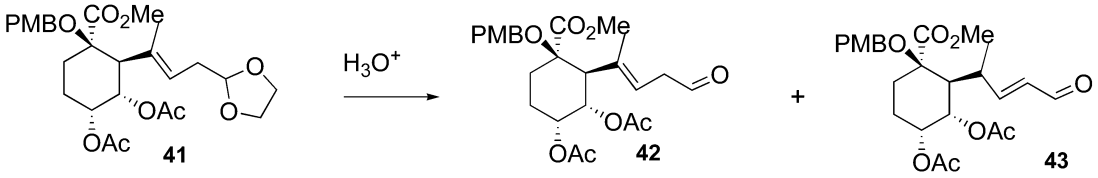
Scheme 8 Internal protection of the trisubstituted double bond.

The use of the Sharpless asymmetric dihydroxylation process offered another possibility to overcome this problem of relative reactivity between the two double bonds. Examination by NMR of the possible conformation in compound **35** showed a spatial proximity between C_4–H and $C_{2'}$–H [31]. It was anticipated that AD mix α [32], reagent for a selective α-dihydroxylation, could not react with the trisubstituted double bond, for steric reasons with the α-face, and for mismatch reasons on the β-face. The only reactive double bond would be the C_5–C_6 disubstituted double bond. This hypothesis was fulfilled by experimental results. Thus, dihydroxylation of **35** with the above reagent afforded cleanly the anticipated diol **40** with some amount of starting material. Interestingly, dihydroxylation without chiral ligand afforded a mixture of the two regioisomeric diols and a small amount of tetrol, demonstrating the role of the chiral ligand in the selective reaction (Scheme 9).



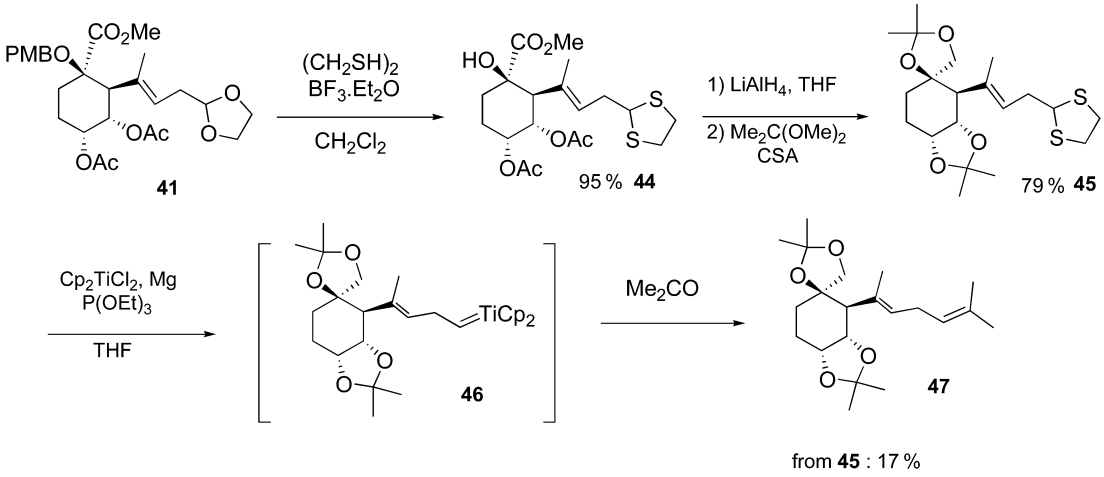
Scheme 9 Regio- and stereoselective dihydroxylation.

Classical hydrolysis of the dioxolane moiety in diacetate derivative **41** proved to be more difficult than expected. Despite various reaction conditions, aldehyde **42** was always contaminated with the re-conjugated isomer **43** (Scheme 10).



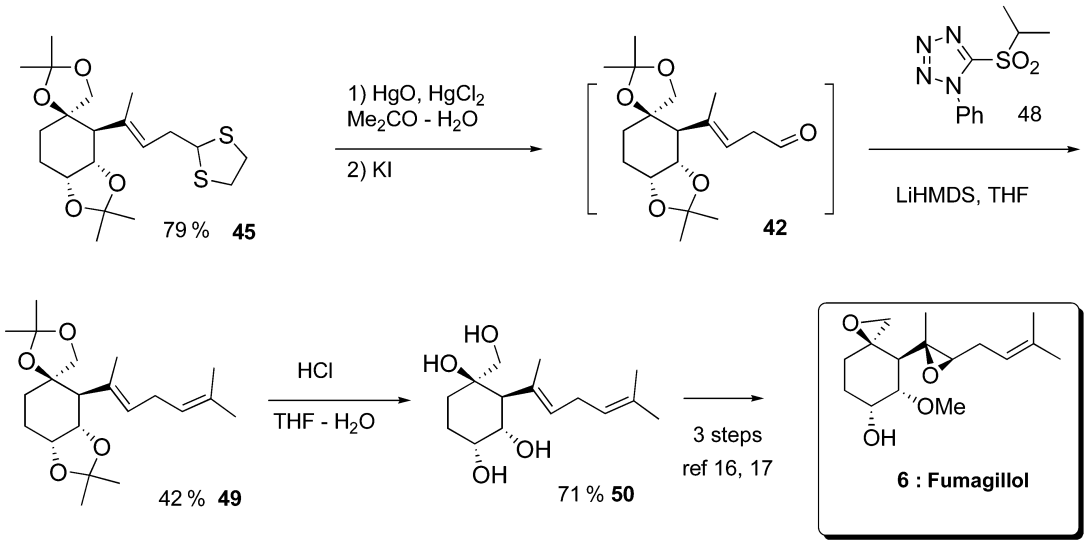
Scheme 10 Attempted hydrolysis.

To overcome this difficulty, dioxolane in **41** was transformed in dithioacetal **44** [33]. Gratifyingly, the PMB protecting group was cleaved in the same operation. Reduction of the esters groups in **44** was followed by protection of the alcohols. The resulting compound **45** was for the first time submitted to Takeda's reaction condition [34], the reaction of choice for direct transformation of a dithioketal into a homologous alkene. Unfortunately, the yield of this transformation remained unacceptably low (Scheme 11).



Scheme 11 Takeda's olefination.

The Julia–Kocienski olefination [36] was studied as a two-step alternative. Mild hydrolysis of the dithiocetal moiety in **45** was performed in the presence of mercury salts [37]. The aldehyde intermediate **42** was not isolated, but directly engaged in the following step. Inverse addition of the anion of sulfonyltetrazole **48** gave rise to the anticipated unconjugated diene **49** in 42 % yield for two steps. Hydrolysis of the protecting group afforded tetrol **50**, identical in all respect to the compound described by Sorensen [17]. As far as this tetrol has been converted in a three-step sequence into fumagillol **1a**, this work constitutes a new formal synthesis of this direct precursor of fumagillin **1b** itself.

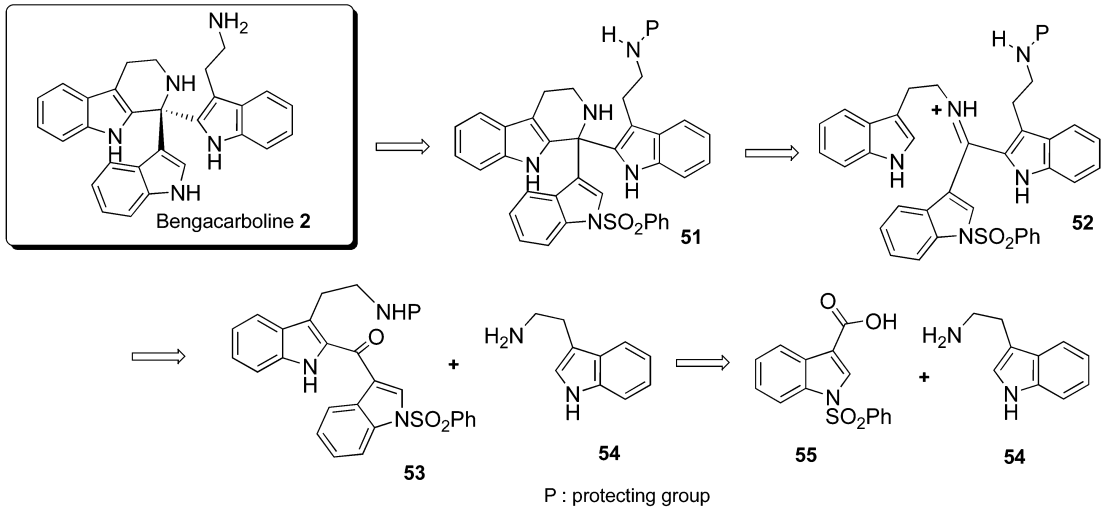


Scheme 12 Julia–Kocienski olefination and completion of the synthesis.

The main features of this synthesis are a Claisen–Ireland rearrangement—ring-closing metathesis, chemo- and stereoselective dihydroxylation, and a Julia–Kocienski olefination.

Bengacarboline **2**, a marine alkaloid extracted from the Fijian ascidian *Didemnum* sp., was described six years ago by C. M. Ireland and colleagues [38]. This alkaloid is an inhibitor of topoisomerase II and displays an in vitro toxicity with a mean of 0.9 μg/ml (0.4 μmol/ml) toward 26 cell-line tumor panel. The structure of bengacarboline **2** is characterized by a tetrahydro-β-carboline core-linked at C1 with tryptamine and indol units. Absolute configuration of the quaternary center in bengacarboline **2** has not yet been determined, but the circular dichroism (CD) spectrum of this alkaloid showed a strong positive peak at 247 nm.

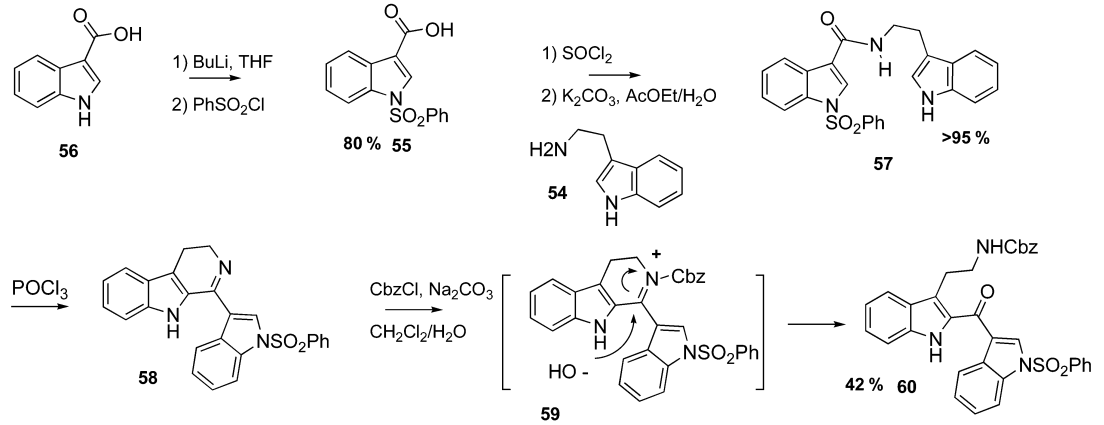
The original structure as well as the interesting biological activity of bengacarboline **2** led us to plan a total synthesis of this alkaloid following the retrosynthetic analysis shown in Scheme 13. We envisioned making the quaternary center by a nucleophilic attack of the indole unit at the intermediate iminium salt **52** in a Pictet–Spengler-type cyclization [39]. The imine precursor of the iminium **52** should be obtained by condensation between the keto derivative **53** and tryptamine **54**. In the first set of reactions, ketone **53** could be prepared using indole-3-carboxylic derivative **55** and an additional tryptamine unit **54**.



Scheme 13 Bengacarboline: Retrosynthetic scheme.

The preparation of the keto derivative **53** is summarized in Scheme 14. Protection of nitrogen in indole-3-carboxylic acid **56** affording the *N*-sulfonyl derivative **55** [40] was followed by acid chloride formation and acylation of tryptamine.

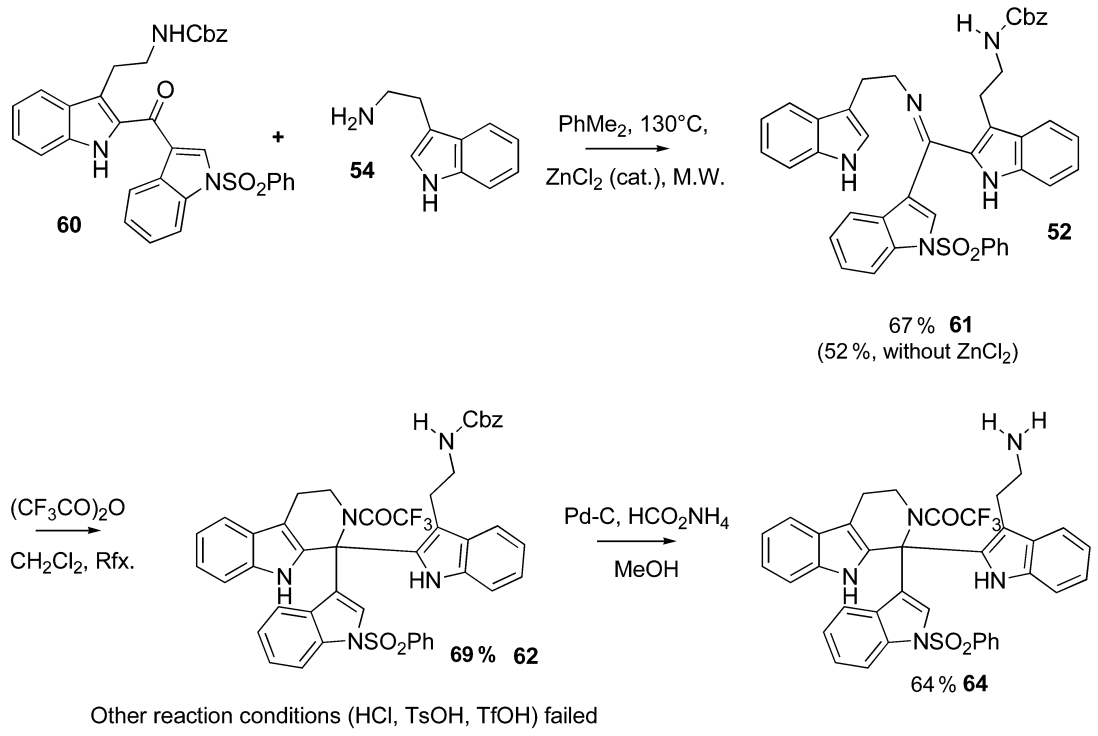
Amide **57** was isolated in good overall yield. This compound was in turn submitted to a Bischler–Napieralski cyclization in the presence of phosphorous oxychloride [41]. The resulting dihydro-β-carboline **58** was then acylated with carbobenzyloxy chloride under Schotten–Baumann conditions. Nucleophilic addition of hydroxy anion on the acyl iminium intermediate **59** was followed by spontaneous ring opening, affording the anticipated keto derivative **60** in 42 % overall yield from **57** (Scheme 14) [42].



Scheme 14 Synthesis and cleavage of the tetrahydrocarboline intermediate.

Condensation of tryptamine **54** with the keto compound **60** using a wide range of classical reaction conditions failed. Fortunately, the use of microwave irradiation in 1,4-xylene in the presence of zinc dichloride afforded the expected imine derivative **61** in 67 % yield (Scheme 15) [43].

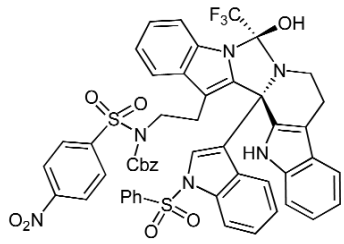
The construction of the quaternary center, a cornerstone in this synthesis, was next examined. However, no cyclization was observed under a variety of reaction conditions in the presence of Brønstedt or Lewis acids [43]. We hypothesize that the inactivity of the imine toward nucleophilic at-



Scheme 15 Construction of the quaternary center.

tack of indole could be due to several factors such as instability of the imine group, steric hindrance, and conjugation of the imine with indole nitrogens despite the presence of a sulfonyl protecting group for one of them. We therefore turned our attention to the use of trifluoroacetic anhydride, which could generate a highly electrophilic acyl iminium intermediate. Under reflux in dichloromethane, this reagent afforded the expected tetrahydro-β-carboline **62** as trifluoroacetamide derivative (Scheme 15).

At this stage, we were not able to crystallize compound **62** for X-ray analysis to secure the formation of the quaternary center. However, *N*-sulfonylation with 4-nitrophenylsulfonyl chloride afforded compound **63**, which crystallized as small needles by diffusion of pentane in an ethyl acetate solution of **63**. The structure of compound **63** is shown in Fig. 1. Unexpectedly, an additional ring was formed in **63** by nucleophilic attack of the indole nitrogen of the tetrahydro-β-carboline moiety on trifluoroacetamide carbonyl.



63

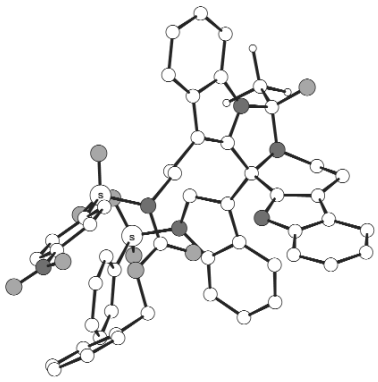
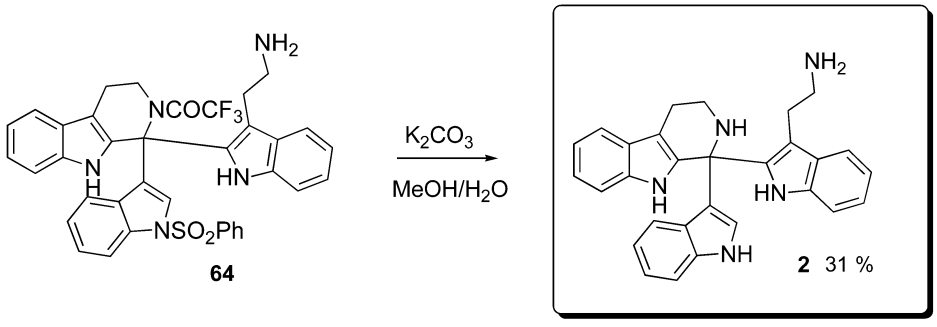


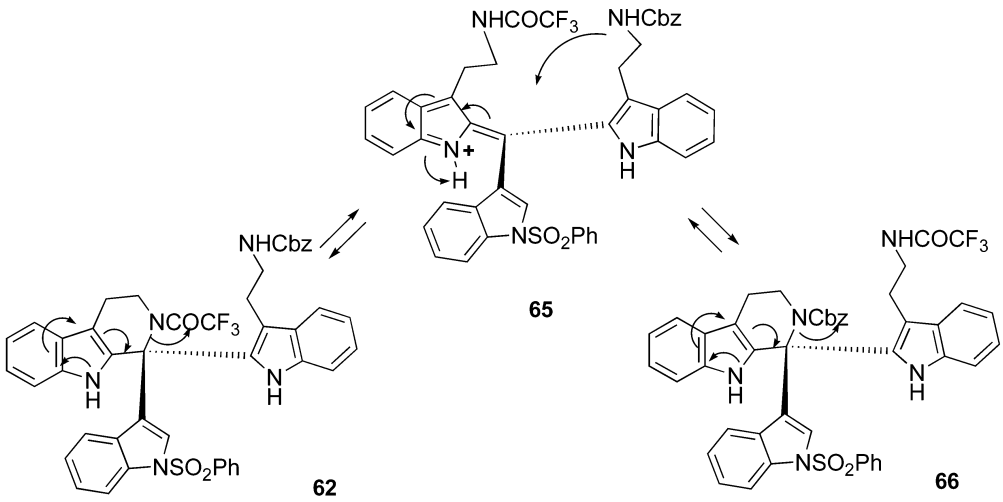
Fig. 1

Final deprotections in compound **62** were then performed in two steps. Hydrogenolysis of the carbobenzyloxy group under classical conditions generated compound **64**. In a final step, alkaline hydrolysis of both trifluroacetyl and phenyl sulfonyl groups afforded (±)-bengacarboline **2**. The modest yield observed during the last step is probably due to the instability of bengacarboline **2** in alkaline media (Scheme 16) [38].



Scheme 16 Final deprotection.

During hydrogenolysis of the Cbz-protecting group in **62**, we also observed the formation of a new compound **66**, obtained by an apparent exchange of the protecting group between trifluoroacetamide and Cbz carbamate. A tetrahydro carboline ring opening followed by a competitive ring closure between the tryptamine nitrogens in the iminium intermediate **65** as depicted in Scheme 17 is more likely. If this equilibrium can be performed with an acidic catalysis in the presence of an enantiomerically pure acid, such as camphorsulfonic acid, it opens the way to a possible dynamic resolution of racemic bengacarboline **2**. This point is under investigation.



Scheme 17 Equilibration: Formal exchange of protecting group.

In summary, a first total synthesis of (±)-bengacarboline **2** has been achieved. The main features of this synthesis are the use of microwave irradiation for the preparation of an imine intermediate and the use of trifluoroacetic anhydride to promote a Pictet–Spingler-type cyclization.

ACKNOWLEDGMENTS

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