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Asymmetric Total Synthesis of Gracilamine and Determination of Its Absolute Configuration

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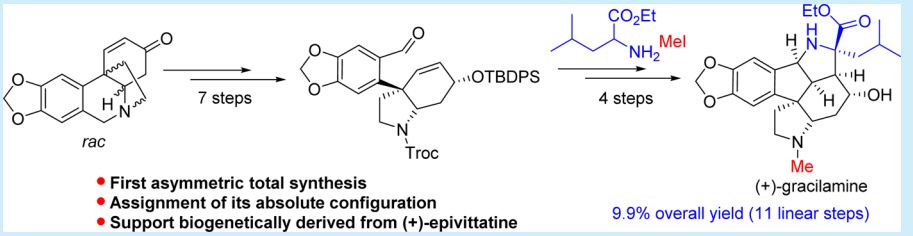
Supporting Information



ABSTRACT: (+)-Gracilamine, a biologically attractive and structurally unique pentacyclic Amaryllidaceae alkaloid, was biomimetically synthesized in 11 linear steps in 9.9% overall yield from the known racemic oxocrinine. The synthesis features an asymmetric hydrogenation, a ring-opening/benzylic oxidation/cyclization sequence, and a biomimetic intramolecular cycloaddition. This total synthesis not only allows the assignment of its absolute configuration, but also provides experimental support for the hypothesis that naturally occurring (+)-gracilamine is biogenetically derived from the crinine-type alkaloid (+)-epivittatine.

Natural products, such as the Amaryllidaceae alkaloids, are an invaluable source of potential leads for drug discovery.[1] Amaryllidaceae alkaloids are a large class of structurally complex molecules isolated from plants of the Amaryllidaceae family,[2] extracts of which have long been used as traditional remedies for treatment of various diseases.[3] Gracilamine (**1**, Scheme 1) is an unusual pentacyclic dinitrogenous Amaryllidaceae alkaloid isolated by Ünver and Kaya in 2005 from *Galanthus gracilis*, which grows in Turkey.[4] The structure and relative stereochemistry of gracilamine (except for the configuration of the hydroxyl-substituted carbon atom on the cyclohexane ring) were determined by NMR spectroscopy and recently confirmed by total synthesis and X-ray crystallography,[5] but the absolute configuration of the molecule remains to be determined. In addition, because **1** was isolated from the source of plant by an ethanol extraction process, the ethyl ester unit associated with it is probably an artifact of the isolation process, the actual natural product being either the corresponding free acid or another ester.[5c] More importantly, its biological activities have not been evaluated because only a limited amount of the optically pure sample has been obtained from the natural source (3.8 mg of **1** was isolated from 5.25 kg of dried, powered *G. gracilis*).[4]

Ünver and Kaya proposed that gracilamine is biosynthesized from a crinine- or tazettine-type alkaloid via an intramolecular [3 + 2] cycloaddition reaction with leucine to form the pentacyclic skeleton.[4] On the basis of this proposal and the fact that tazettine-type alkaloids are derived from crinine-type alkaloids,[6] Jin suggested a biosynthetic pathway involving a crinine-type alkaloid (Scheme 1a).[7] Specifically, biocatalytic oxidation/hydrolysis of the benzylic C−N bond of a crinine-type alkaloid such as (+)-epivittatine (**2**)[8] provides aldehyde **3**. Reaction of **3** with leucine followed by intramolecular [3 + 2] cycloaddition of the resulting imine (**4**) affords **5**, which has a pentacyclic skeleton. Esterification of the carboxyl group and selective methylation of the less hindered pyrrolidine ring of **5** yield gracilamine.

On the basis of this hypothetical biosynthetic pathway, Ma et al.[5a] completed the total synthesis of gracilamine in 2012. They used a key intermediate **6** without a fused pyrrolidine ring and completed the synthesis of **1** in racemic form through a biomimetic intramolecular [3 + 2] cycloaddition to assemble its two fused five-membered BE-rings (Scheme 1b). This highly elegant work provides the first solid evidence supporting the proposed biosynthetic pathway. Just recently, another precise biomimetic synthesis was reported by Banwell et al. which also supports the hypothesis of an intramolecular [3 + 2] cycloaddition to install the fused five-membered BE-rings of **1**.[5c] They employed an aldehyde **8** containing C3a-arylhexahydroindole unit (ACD-rings), which was synthesized by a palladium-catalyzed intramolecular Alder-ene reaction, as a key intermediate (Scheme 1b). However, because the absolute configuration of gracilamine remains unknown, the biogenetic precursor of the molecule has not been conclusively identified.

The unique fused pentacyclic structure bearing seven contiguous stereocenters, two of which are quaternary centers, and the potential biological activities of gracilamine have

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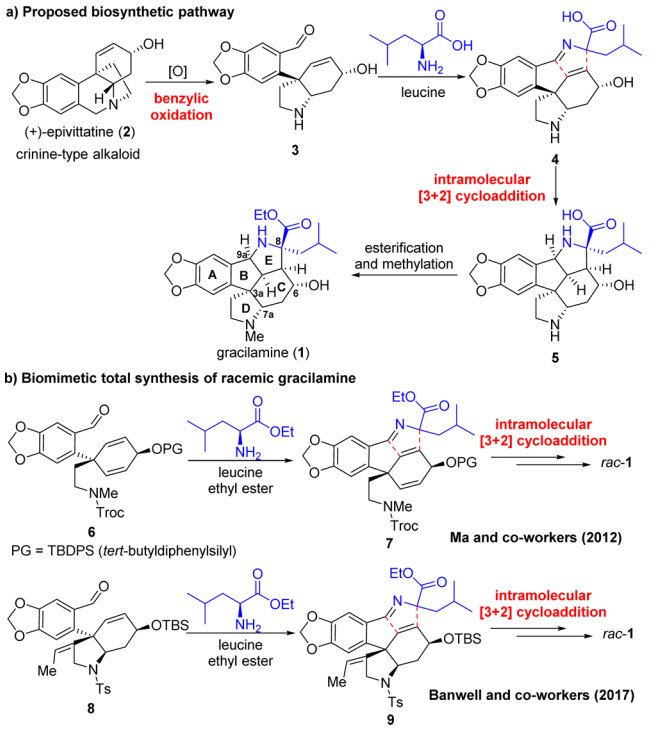
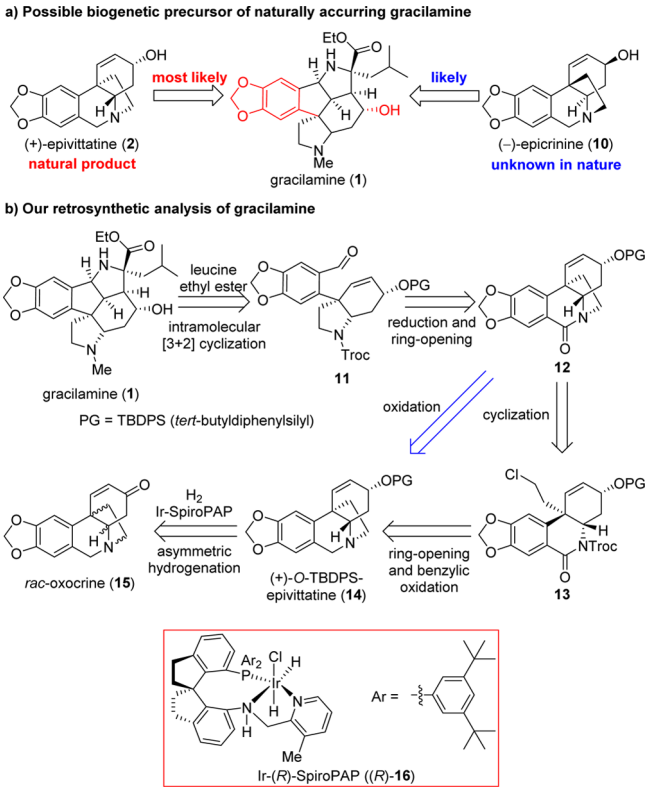
Scheme 1. Proposed Biosynthetic Pathway and Biomimetic Total Synthesis of Gracilamine



Scheme 2. Possible Biogenetic Precursors and Our Retrosynthetic Analysis of Gracilamine



recently also attracted the attention of other synthetic research groups. For example, Gao et al.[5b] reported a total synthetic strategy featuring a photo-Nazarov reaction, intramolecular hetero-Michael, and intramolecular Mannich reaction to construct the core structure of **1**. Following the work of Gao et al., Snyder et al.[9] described a formal synthesis of **1** using an intramolecular Diels–Alder reaction as the key step to install the octahydroindole-fused tetracyclic ABCD-skeleton. Yu et al.[10] developed an asymmetric route to synthesize the cyclic diketone bearing a tricyclic ABD-core framework, the key intermediate of Gao's synthesis of **1**, using a rhodium-catalyzed [3 + 2 + 1] cycloaddition of an optically active 1-yne–vinylcyclopropane and CO.[11] In addition, Adrio, Carretero, and co-workers reported catalytic asymmetric construction of the tricyclic ABD-core of **1** through a copper-catalyzed 1,3-dipolar cycloaddition of an azomethine ylide and an activated 1,3-diene.[12] However, the asymmetric total synthesis of **1** has not been accomplished to date. In this paper, we report the first asymmetric total synthesis of **1** based on the hypothetical biosynthetic pathway and the determination of the absolute configuration, as well as the biogenetic precursor of naturally occurring (+)-**1**.

On the basis of evidence that the hydroxyl and piperonyl groups on the cyclohexene ring of **1** are *trans* to each other, we deduced that naturally occurring (+)-epivittatine (**2**)[8] is the most likely biogenetic precursor of (+)-**1**, although its antipodal isomer, (−)-epicrinine (**10**),[13] which has not yet been discovered in nature, is also a possible precursor (Scheme 2a). Thus, we envisaged a new biomimetic synthetic route to (+)-**1** via *N*-Troc-protected aldehyde **11** (Scheme 2b) instead of the aldehyde **3**, the intermediate in the biosynthetic pathway proposed by Jin.[7] Aldehyde **11**, the key compound for the intramolecular [3 + 2] cycloaddition to form the fused pentacyclic ring of gracilamine, would be prepared by reduction of the amide group of tetracyclic compound **12** to the corresponding hemiaminoacetal group and subsequent ring opening with TrocCl. Compound **12** would be synthesized by removal of the Troc group from the amide of **13** and formation of the five membered aza ring via a cyclization reaction. Compound **13** would be obtained by ring opening of (+)-*O*-TBDPS-epivittatine ((+)-*trans*-**14**),[5a] followed by benzylic oxidation. The compound (+)-*trans*-**14** would be obtained by asymmetric hydrogenation of racemic *rac*-oxocrinine (**15**) catalyzed by chiral iridium catalyst Ir–(*R*)-SpiroPAP ((*R*)-**16**),[8d] which was highly efficient for the asymmetric hydrogenation of ketones, ketoesters, enones, and esters.[14] Thus, with a catalytic asymmetric hydrogenation of racemic *rac*-oxocrinine (**15**), a sequence of ring-opening/benzylic oxidation/cyclization, and a biomimetic intramolecular [3 + 2] cycloaddition the gracilamine **1** could be synthesized in optically active form. Note that Ma et al. have tried to synthesize **1** by means of a similar hypothetical biosynthetic pathway, but they eventually abandoned this route because they were unable to obtain the key aldehyde intermediate, an analogue of **11**, from *rac*-oxocrinine (**15**) with their designed one-pot ring opening/debenzylation strategy;[5a] in addition, if compound **12** could be obtained from direct benzylic oxidation of compound **14**, a more concise route may be possible.

We found that asymmetric hydrogenation of *rac*-**15**[15] catalyzed by (*R*)-**16** and subsequent protection with TBDPSCl afforded *O*-TBDPS-protected (+)-epivittatine ((+)-*trans*-**14**, 43%, 93% ee) and *O*-TBDPS-protected (−)-crinine[16] ((−)-*cis*-**14**, 38%, 97% ee) on a gram scale (Scheme 3).[8d] We initially converted (+)-*trans*-**14** to tetracyclic compound (+)-**12** via direct benzylic oxidation, but all attempts were unsuccessful.[17] The reason for this is likely owing to the effect of steric

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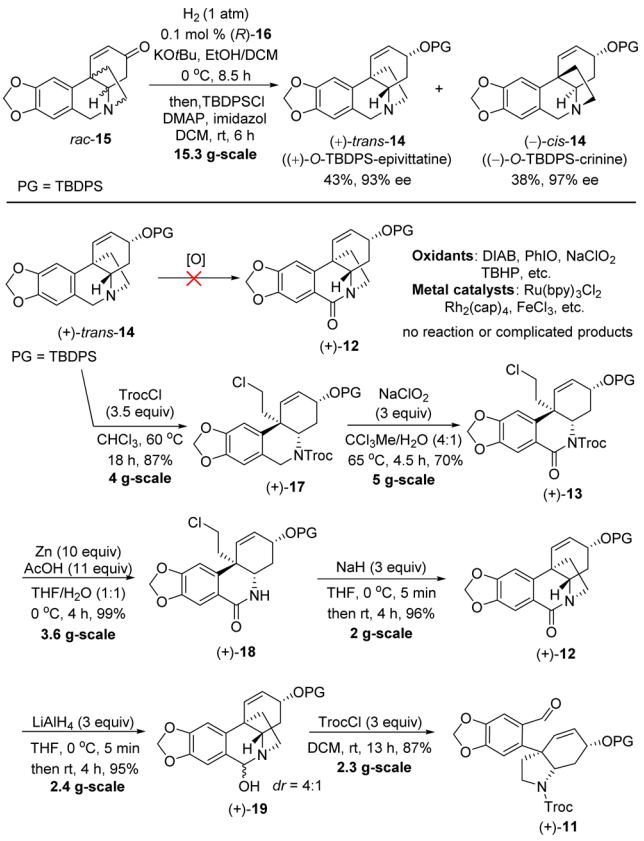
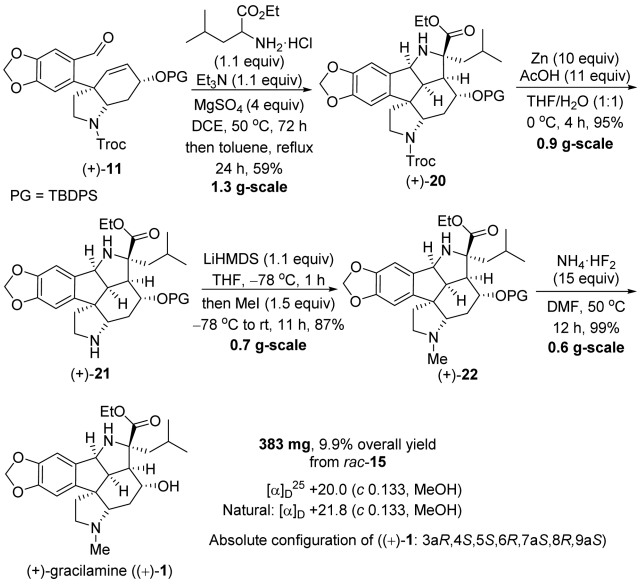
Scheme 3. Asymmetric Synthesis of *N*-Troc-Protected Aldehyde 11



hindrance of the 5,10b-enthano bridge on benzylic hydrogens. Thus, we then tried selective opening of the *aza*-five membered ring of (+)-*trans*-**14**, followed by benzylic oxidation and cyclization. The conversion of (+)-*trans*-**14** to (+)-**12** was accomplished on a multiple-gram scale in high yield (Scheme 3). The (+)-*trans*-**14** was converted to (+)-**17** in 87% yield by reaction with TrocCl (2,2,2-trichloroethyl chloroformate). Fortunately, we found that the $NaClO_2$ is a suitable oxidant for the benzylic oxidation of (+)-**17**, yielding (+)-**13** in 70% yield according to Zhang's protocol.[18] After removal of the Troc group of (+)-**13** with Zn/HOAc and subsequent cyclization in the presence of NaH, (+)-**12** was obtained in 95% yield (two steps). Reduction of (+)-**12** with $LiAlH_4$ afforded **19** as a 4:1 mixture of diastereoisomers in 95% yield. Reaction of (+)-**19** with TrocCl afforded *N*-Troc-protected aldehyde (+)-**11** in 87% yield.

Condensation of aldehyde (+)-**11** with leucine ethyl ester hydrochloride, followed by intramolecular [3 + 2] cycloaddition of the resulting imine intermediate according to Ma's procedure,[5a] produced (+)-**20** as a single isomer in 59% yield (Scheme 4). Treatment of (+)-**20** with Zn/HOAc to remove the Troc group and selective methylation of the resulting unprotected pyrrolidine ring with MeI afforded (+)-**22** in 83% yield (two steps). Deprotection of the hydroxyl group of (+)-**22** with $NH_4{\cdot}HF_2$ afforded (+)-gracilamine ((+)-**1**). The NMR spectroscopic data and the optical rotation ($[\alpha]_D^{25}$ +20.0 (*c* 0.133, MeOH); natural: $[\alpha]_D$ +21.8 (*c* 0.133, MeOH)) of our synthetic sample were identical to those reported for naturally occurring (+)-gracilamine.[4] Thus, we completed the synthesis of (+)-**1** in an overall yield of 9.9% in 11 steps from known *rac*-oxocrinine (*rac*-**15**).[19] This represents the first

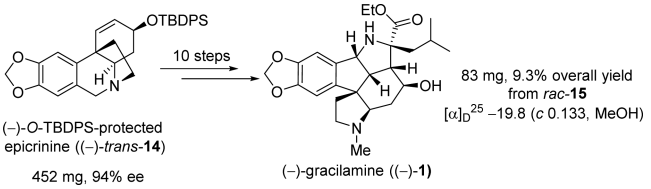
Scheme 4. Asymmetric Synthesis of (+)-Gracilamine



asymmetric total synthesis of (+)-gracilamine. The absolute configuration of (+)-**1** was established to be (3a*R*,4*S*,5*S*,6*R*,7a*S*,8*R*,9a*S*) as depicted in Scheme 4 on the basis of the known structure of (+)-epivittatine (**2**).[8c,d] This result suggests that **2** is the biosynthetic precursor of naturally occurring (+)-gracilamine.

For assessment of biological activity, we also synthesized (−)-gracilamine ((−)-**1**) from *O*-TBDPS-protected (−)-epicrinine ((−)-*trans*-**14**), which was obtained in the step of asymmetric hydrogenation of *rac*-**15** with (*S*)-**16** (Scheme 5). By using the procedure described for (+)-gracilamine, we synthesized (−)-gracilamine in 9.3% overall yield.

Scheme 5. Asymmetric Synthesis of (−)-Gracilamine



In conclusion, the first asymmetric total synthesis of (+)-gracilamine has been achieved. Using an iridium-catalyzed asymmetric hydrogenation of a cyclohexenone as the key step, and the following ring-opening/benzylic oxidation/cyclization sequence, in addition to a biomimetic intramolecular [3 + 2] cycloaddition, we synthesized (+)-gracilamine in 11 linear steps in 9.9% overall yield from known racemic oxocrinine (15 linear steps in 6.3% overall yield from commercially available materials; lit.[5] 1.4−4.5% overall yield/17−18 linear steps). The absolute configuration of naturally occurring (+)-gracilamine was assigned. This work provides experimental support for the hypothesis that naturally occurring (+)-gracilamine is biogenetically derived from the crinine-type alkaloid (+)-epivittatine. The reported reaction sequence lends itself to the production of analogues that might be useful for probing the structure−activity relationship profile of this class of compound. These works are now in progress in our laboratory.

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■ ASSOCIATED CONTENT

Supporting Information

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Experimental procedures and characterization data for the intermediates and products (PDF)

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Notes

The authors declare no competing financial interest.

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