Natural Products

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Potentially Biomimetic Total Synthesis and Relative Stereochemical Assignment of (±)-Gracilamine**

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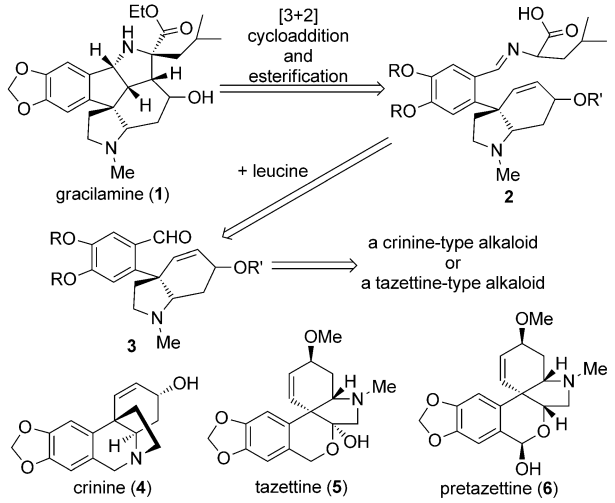
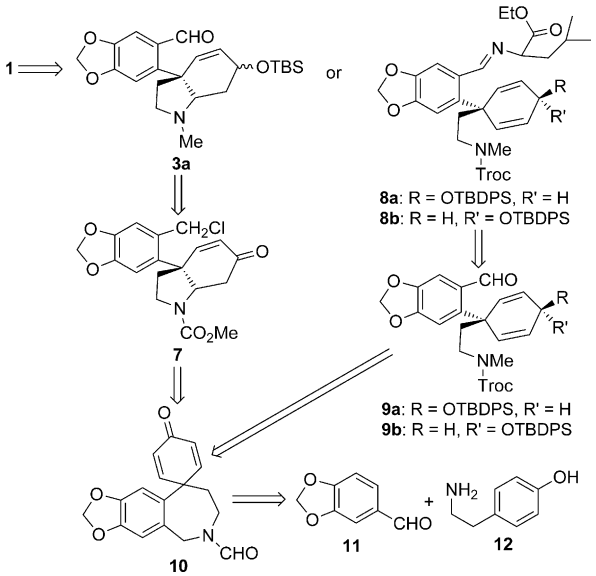
Plants from the *Amaryllidaceae* family are spread all over the world and have long been recognized for their medicinal properties.[1] A great number of pharmacologically active alkaloids have been isolated from these plants, including galanthamine,[2] lycorine,[3] plicamine[4] and tazettine.[5] These alkaloids have shown significant biological effects, ranging from antitumor, antiviral, and antiinflammatory activities to immunostimulatory and acetylcholinesterase inhibitory activities.[1–6] The structural diversity and important biological activities of these natural products have attracted great attention from the synthetic chemistry community, particularly from within the pharmaceutical industry.[1–5]

In 2005, gracilamine (**1**, Scheme 1), a structurally novel pentacyclic dinitrogenous alkaloid was isolated by Ünver and Kaya from an Amaryllidacae species, *Galanthus gracilis*, collected from a Turkish mountain.[7] The general structure of this alkaloid was initially established by extensive spectroscopic analysis. The relative stereochemistry of its hydroxy-bearing carbon atom, however, was not assigned. Additionally, because of the limited amount of gracilamine available from natural sources, its biological activities have not yet been evaluated. These issues, together with an interesting hypothesis concerning the biosynthesis of gracilamine (see below), prompted us to initiate a synthetic study to this alkaloid.



Scheme 1. Structures of selected *Amaryllidaceae* alkaloids and previously proposed biosynthetic pathway of gracilamine.

In their report on the isolation of gracilamine,[7] Ünver and Kaya suggested that gracilamine may be biosynthesized by an intramolecular [3+2] cycloaddition of imine **2** and subsequent esterification. The imine **2** could be generated through condensation of leucine with aldehyde **3**. The formation of aldehyde **3** might then result from enzymatic oxidation of an alkaloid related to crinine (**4**),[8] tazettine (**5**),[5] or pretazettine (**6**).[5] Based on this hypothesis, we investigated two routes for the potentially biomimetic synthesis of gracilamine (Scheme 2): one using the aldehyde **3a** as a key intermediate, which could be prepared from benzyl chloride **7** by hydrolysis and subsequent oxidation; another through assembly of imines **8a**/**8b** by condensation of aldehydes **9a**/**9b** with leucine ethyl ester. We believed that both **7** and **9** could be obtained from spirocyclic compound **10**, a known compound that has been prepared from piperonal **11** and tyramine **12** through an intramolecular phenolic oxidative coupling as the key step.[9] Our findings from both routes are disclosed herein.



Scheme 2. Retrosynthetic analysis of gracilamine. TBDPS = *tert*-butyldiphenylsilyl; TBS = *tert*-butyldimethylsilyl.

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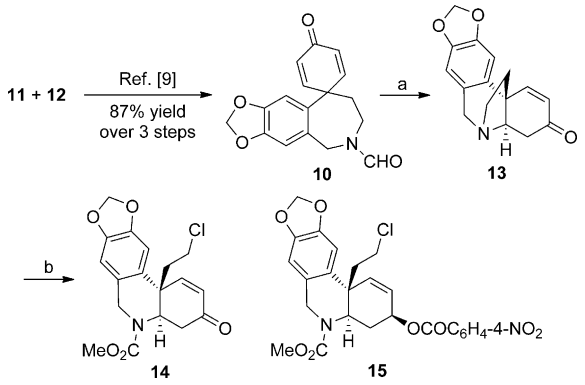
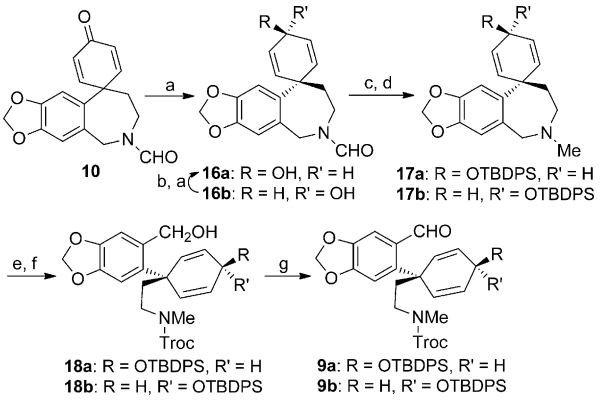
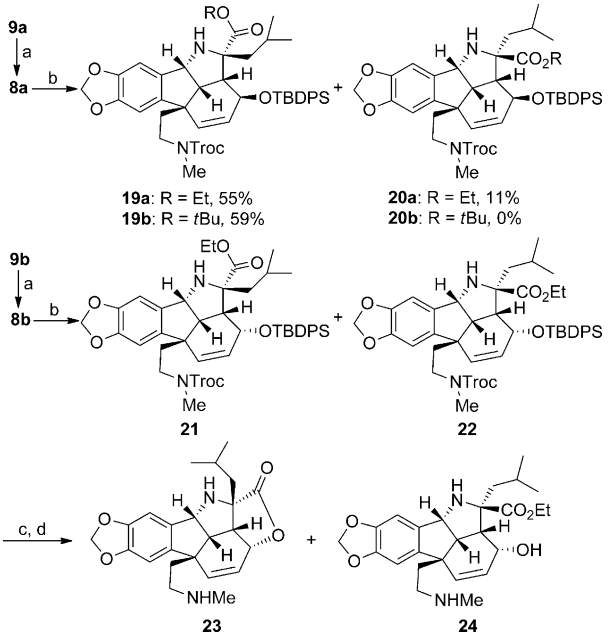


Scheme 3. Reagents and conditions: a) 10% KOH, MeOH, 61%; b) $ClCO_2Me$, 55% (89% yield based on recovered starting material).



Scheme 4. Reagents and conditions: a) $NaBH_4$, $CeCl_3 \cdot 7H_2O$, 0°C, 95%; b) MnO_2, CH_2Cl_2, 100%; c) $LiAlH_4$, THF, 94%; d) TBDPSCl, imidazole, CH_2Cl_2, 98%; e) TrocCl, Et_3N, CH_2Cl_2; f) $AgNO_3$, acetone, H_2O, 68% yield over two steps; g) DMP, CH_2Cl_2, 95%. DMP = Dess–Martin periodinane; Troc = 2,2,2-trichloroethoxycarbonyl chloride.



Scheme 5. Reagents and conditions: a) Leucine ethyl ester hydrochloride salt (or leucine *tert*-butyl ester hydrochloride salt), Et_3N, $MgSO_4$, CH_2Cl_2; b) toluene, reflux; c) Zn, AcOH; d) TBAF, THF. TBAF = tetra-*n*-butylammonium fluoride.

Following Node's procedure, the spirocyclic compound **10** was prepared in three steps and 87% overall yield from piperonal **11** and tyramine **12** (Scheme 3).[9] Cleavage of the amide bond in **10** through concomitant intramolecular 1,4-addition using K_2CO_3 afforded tertiary amine **13**. Our initial plan was to carry out a one-step ring opening and debenzylation reaction[10] to convert **13** into the benzyl chloride **7**. If this strategy had worked, we would have been able to quickly and efficiently obtain the desired aldehyde **3a**. Unfortunately, treatment of **13** with methyl chloroformate gave compound **14** as a single product, the structure of which was confirmed by X-ray structural analysis of its derivative, **15**.[11] The formation of **14** indicated that ring-opening occurred at the undesired site. The driving force for ring-opening at the undesired site appears to be greater strain release from opening the bridge in the five-membered ring over the bridge in six-membered ring.

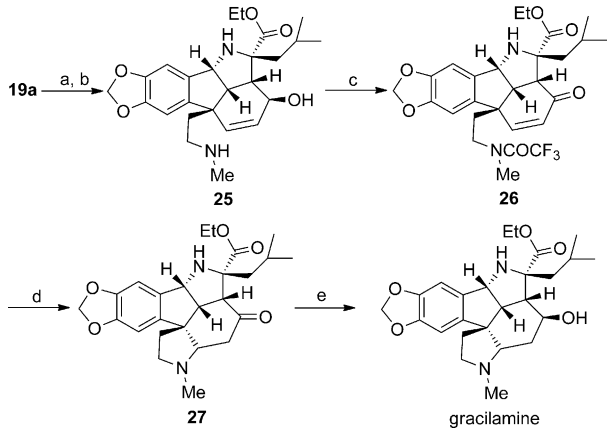
The failure to obtain the desired debenzylation product **7** prompted us to try debenzylation/ring-opening reaction prior to formation of the strained five-membered bridging ring. Accordingly, reduction of **10** with $NaBH_4$ afforded alcohols **16a**[11] and **16b** in a 1:1 ratio and 95% combined yield (Scheme 4). Reduction of **16a** with $LiAlH_4$ followed by protection with TBDPSCl delivered tertiary amine **17a**. Gratifyingly, ring-opening of **17a** with TrocCl/NEt_3 provided the desired benzyl chloride, which was immediately treated with $AgNO_3$ in aqueous solution to provide benzyl alcohol **18a** in 68% overall yield. Oxidation of **18a** with Dess–Martin periodinane then furnished aldehyde **9a**. Following the same procedure, aldehyde **9b** was obtained from diastereomeric alcohol **16b** in 51% overall yield. We note that these two aldehydes should serve as valuable building blocks for the assembly of other *Amaryllidaceae* alkaloids, such as augustamine-type molecules[12] and other graciline-type compounds.[13]

Condensation of aldehyde **9a** with leucine ethyl ester hydrochloride salt produced the imine intermediate **8a** (Scheme 5). The stage was then set for the crucial intramolecular [3+2] cycloaddition. At the outset, this was a challenging task as only a few reports regarding the [3+2] cycloaddition of non-polar olefins and azomethine ylides exist.[14] It is noteworthy that attempts to use Lewis acids, such as silver and copper salts, to promote the intramolecular [3+2] cycloaddition failed to give any desired products under typical reaction conditions.[15] After some experimentation, we were pleased to find that the cycloaddition could be achieved by refluxing imine **8a** in toluene,[14] simultaneously creating two rings and four stereogenic centers. Only the stereochemistry at the newly formed quaternary stereocenter was unpredictable, the stereochemistry at the other three stereocenters should be well-controlled because of large energetic preference for the formation of two *cis*-fused five-membered rings. Indeed, as expected, only two isomers, **19a** and **20a**, were isolated in 66% combined yield. In the subsequent studies we were pleased to find that the major

isomer **19a** was the isomer needed to synthesize gracilamine. The stereoselectivity at the newly formed quaternary stereocenter appears to be partially controlled by steric interactions with the bulky silyl ether group, as evident from the fact that the imine derived from the bulkier leucine *tert*-butyl ester produced **19b** exclusively.

When aldehyde **9b** was utilized, a mixture of diastereomers **21** and **22** was obtained after intramolecular [3+2] cycloaddition. Because these isomers could not be separated directly by column chromatography, we decided to carry out further conversions. Removal of the protecting groups in **21** and **22** through sequential treatment with zinc in acetic acid and TBAF provided lactone **23** and alcohol **24** in a ratio of 1:5.6. The stereochemistry at the newly formed quaternary carbon center of **24** was not the one we desired, while attempts to open the lactone ring of **23** under typical conditions failed to give the corresponding alcohol. These problems made aldehyde **9b** useless for the synthesis of the target molecule. Thus, in subsequent studies, we recycled early intermediate **16b** to its isomer **16a** (Scheme 4).

The completion of the synthesis of gracilamine is shown in Scheme 6. Cleavage of the Troc group in **19a** with Zn/HOAc followed by removal of the silyl protecting group with TBAF



Scheme 6. Reagents and conditions: a) Zn, AcOH, 94%; b) TBAF, THF, 85%; c) $(CF_3CO)_2O$, DMSO, Et_3N, −78 °C→RT, 75%; d) NaOEt, EtOH, 64%; e) $NaBH_4$, MeOH, 85%. DMSO = dimethyl sulfoxide.

provided amino alcohol **25** in 80% overall yield. Oxidation of allyl alcohol **25** to the corresponding enone, which should undergo spontaneous intramolecular 1,4 addition to construct the second pyrrolidine ring, proved to be difficult; **25** was found to be inert to several oxidants (such as MnO_2 and pyridinium chlorochromate). Only a Swern oxidation with $(CF_3CO)_2O$ and DMSO succeeded in oxidizing the alcohol moiety. In this case, amide formation took place to produce enone **26**. Fortunately, cleavage of the amide by treatment with NaOEt in ethanol delivered the cyclization product **27**.[11] Finally, reduction of **27** with $NaBH_4$ furnished the target molecule, whose structure was confirmed by X-ray analysis.[11] Because the analytical data of our synthetically obtained gracilamine were in agreement with those reported for naturally ocurring gracilamine, we could fully establish the relative configuration of gracilamine (Scheme 6).

In conclusion, we have achieved the first total synthesis of gracilamine in 17 linear steps and 4.5% overall yield (10.3% overall yield from the early intermediate **16a**). The synthesis features a potentially biomimetic intramolecular [3+2] cycloaddition to assemble its two fused five-membered rings and a debenzylation/ring-opening reaction to obtain the aldehyde intermediate. The success of our potentially biomimetic synthesis provides a circumstantial evidence to support the biosynthesis pathway of gracilamine proposed by Ünver and Kaya. Our synthetic route offers a powerful approach for preparing gracilamine and its analogues, which are valuable for biological studies. Additionally, some synthetic intermediates presented here are useful for assembling other *Amaryllidaceae* alkaloids. These investigations are actively pursued in our laboratory and the progress toward these goals will be disclosed in due course.

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