Natural Products

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Total Synthesis of Gracilamine**

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Abstract: *The total synthesis of gracilamine, a pentacyclic Amaryllidaceae alkaloid, was achieved from simple building blocks. The synthesis features a mild photo-Nazarov reaction, intramolecular 1,4-addition, and an intramolecular Mannich reaction. This approach not only confirms the C6 stereochemistry of natural gracilamine, and also provides a novel solution to prepare its derivatives and structurally related natural products.*

Amaryllidaceae plants have proven to be an important source of natural products with appealing structures and promising biological activities.[1] Gracilamine (**1**), a member of the *Amaryllidaceae* alkaloid family, was isolated from *Galanthus gracilis* collected from a mountain in Turkey by Ünver and Kaya in 2005 (Figure 1).[2] The structure and relative stereochemistry of **1**, except for the configuration of the hydroxy group on C6, were determined by NMR spectroscopy. However, the biological activities of this potentially valuable natural product are unknown because of its scarcity in nature.[2] In 2012, Ma and co-workers reported the first total synthesis of **1** using a biomimetic intramolecular [3+2] cycloaddition.[3] This synthetic breakthrough not only suggested how the compound is produced in nature, but it also provided evidence of the relative stereochemistry of C6. Since our research group is devoted to the synthesis of bioactive natural products, we set out to develop a new strategy to solve the efficiency of the chemical synthesis of **1** and facilitate the preparation of its analogues and derivatives for medicinal studies. We report herein the total synthesis of **1** using a photo-Nazarov reaction,[4] Michael addition, and an intramolecular Mannich reaction as key steps. Our synthesis provides additional evidence to support the C6 stereochemistry of natural product reported by Ma and co-workers.[3]

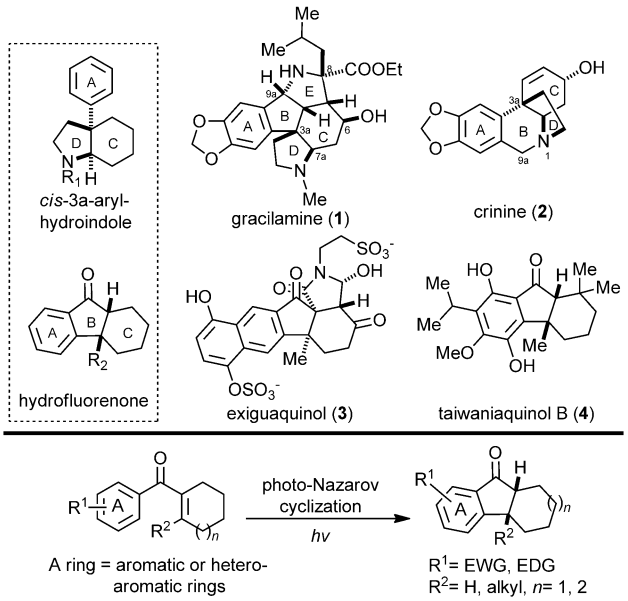

Figure 1. *Amaryllidaceae* alkaloids, hydrofluorenone-containing natural products, and the photo-Nazarov reaction. EDG = electron-donating group, EWG = electron-withdrawing group.

Gracilamine (**1**) possesses a fused pentacyclic ring comprising rings A–E and 7 stereocenters. including two quaternary centers (C3a and C8). It contains the same *cis*-3a-aryl-hydroindole skeleton (rings A, C, D) found in traditional *Amaryllidaceae* alkaloids[1] such as crinine (**2**).[5] Ünver and Kaya proposed that **1** forms through the condensation and [3+2] cycloaddition of **2** and leucine, followed by oxidation of the products.[2] Alternatively, **1** can be regarded as an aminal derivative of hydrofluorenone containing natural products (rings A–C). Similar compounds include exiguaquinol (**3**)[6] and taiwaniaquinol B (**4**).[7]

We have systematically studied the scope of the photo-Nazarov reaction of vinyl aryl ketones for constructing hydrofluorenone and related structures (Figure 1).[8] In contrast to the conventional Nazarov reaction we found that this photo-Nazarov reaction provides effective solutions under milder reaction conditions at neutral or basic pH, and we have used this reaction to synthesize **4**.[8] As a part of our continued studies, we report herein the construction of a *cis*-3a-aryl-hydroindole skeleton, thereby providing a new approach to synthesizing gracilamine (**1**) and traditional *Amaryllidaceae* alkaloids.

We envisioned that both **1** and **2** could be derived from the tetracyclic intermediate **5**, which contains rings A–D, a *cis*-3a-aryl-hydroindole skeleton, and a hydrofluorenone motif (Scheme 1). We proposed to construct the E ring of **1** through reductive amination of **5** with leucine and subsequent C5–C8

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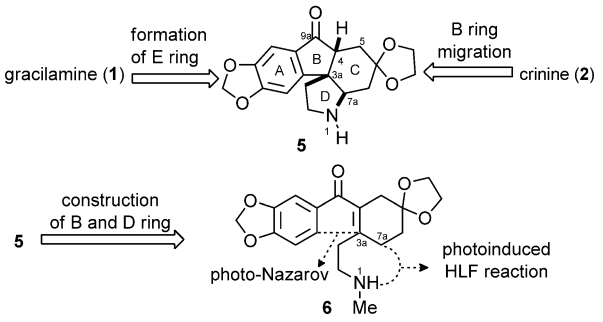
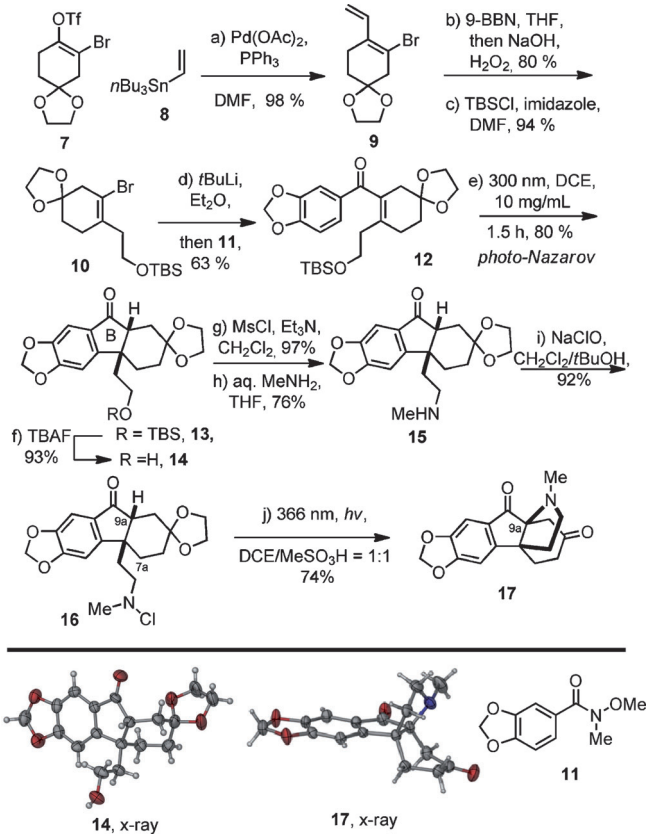


Scheme 1. Retrosynthetic analysis.

bond formation by intramolecular oxidative coupling.[9] The B ring of crinine (**2**) could be prepared through a ring migration process involving cleavage of the C4–C9a bond in **5** and reformation of the C9a–N1 bond. We hoped to generate the B and D rings of **5** using a photo-Nazarov reaction[4,8] and photoinduced Hofmann–Löffler–Freytag (HLF) reaction[10] of the enone **6**.

Our synthesis began with construction of the core skeleton (rings A–D) of **1** based on our retrosynthetic analysis (Scheme 2). The bromoenol triflate **7**, prepared using a reported three-step process,[11] underwent palladium-catalyzed Stille coupling with tributyl(vinyl)stannane (**8**) to furnish **9** in 98% yield. This compound was efficiently transformed into the vinyl bromide **10** in a two-step process involving hydroboration/oxidation and protection. Lithium–bromide exchange of **10** with *t*BuLi and subsequent reaction with the Weinreb amide **11** generated the enone **12**, the precursor of the Nazarov reaction for constructing the B ring. The traditional Nazarov reaction of aromatic vinyl ketones requires strong Lewis or Brønsted acids and high temperature, thus making the reaction unsuitable for substrates with acid-sensitive groups. Under our optimized photo-Nazarov protocol,[8] we irradiated a solution of **12** in dichloroethane under $\lambda = 300$ nm light for 1.5 hours at room temperature to obtain the desired hydrofluorenone **13** in 80% yield. Selective removal of the TBS group with TBAF gave **14**, the relative configuration of which was determined unambiguously by X-ray crystallographic analysis. The primary hydroxy group of **14** was then converted into the methyl amine **15**, which was treated with NaClO to give the corresponding N-chloro derivative **16** in good yield. We planned to use the photo-induced HLF reaction to functionalize C7a through an intramolecular 1,5-hydrogen atom transfer. After extensively surveying reaction conditions (see details in the Supporting Information), we found that photolysis of **16** in the presence of various acids always gave **17** as the only cyclized product. The structure of **17** was confirmed by single-crystal X-ray analysis.

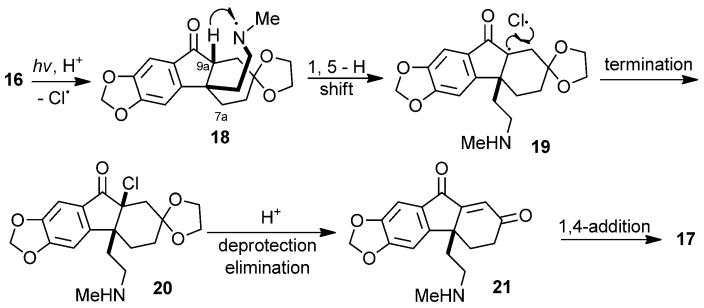
We postulate that the photoactivated homolytic cleavage of the N–Cl bond of **16** gave the nitrogen-centered radical **18**, which then underwent a 1,5-H shift from H9a instead of from H7a (Scheme 3). This regioselective hydrogen migration may be driven by the formation of the tertiary radical **19**, which is stabilized by an electron-withdrawing group on C9a.



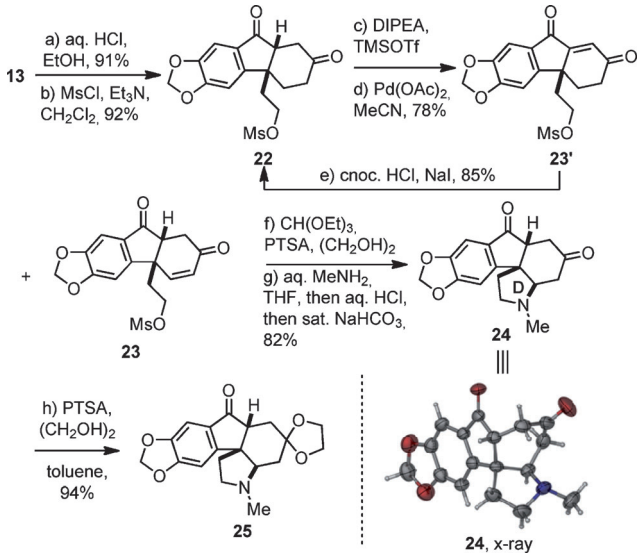
Scheme 2. Construction of the B ring using photo-Nazarov reaction. Reagents and conditions: a) $Pd(OAc)_2$ (0.14 equiv), PPh_3 (0.28 equiv), **8** (1.2 equiv), 25 °C, DMF, 3.5 h, 98%; b) 9-BBN (1.2 equiv), 25 °C, THF, 12 h; then NaOH (3 M), H_2O_2 (30%), reflux, 1 h; 80%; c) TBSCl (1.1 equiv), imidazole (1.5 equiv), 25 °C, DMF, 30 min, 94%; d) *t*BuLi (3.0 equiv), 1 h, then **11** (3.0 equiv), 1 h, −78 °C, Et_2O, 63%; e) 300 nm, 25 °C, DCE, 1.5 h, 80%; f) TBAF (2.0 equiv), 25 °C, THF, 1 h, 93%; g) MsCl (1.1 equiv), Et_3N (1.5 equiv), 0 °C, CH_2Cl_2, 1 h, 97%; h) aq. $MeNH_2$/THF (*v*/*v*) = 1:1, 60 °C, 10 h, 76%; i) aq. NaClO (30%), 25 °C, CH_2Cl_2/*t*BuOH (*v*/*v*) = 1:1, 1 h, 92%; j) 366 nm, 25 °C, DCE/$MeSO_3H$ (*v*/*v*) = 1:1, 0.5 h, 74%. 9-BBN = 9-borabicyclo[3.3.1]nonane, DCE = 1,2,-dichloroethane, DMF = *N*,*N*-dimethylformamide, Ms = methanesulfonyl, TBAF = tetra-*n*-butylammonium fluoride, TBS = *tert*-butyldimethylsilyl, THF = tetrahydrofuran.

Intermolecular chlorine abstraction converted **19** into **20**, which was then transformed into **17** after acetal removal, elimination, and 1,4-addition.

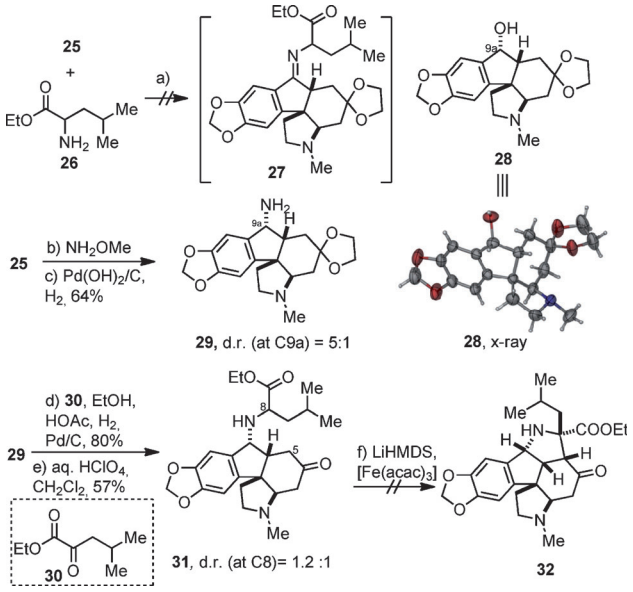
We then turned our attention to construct the D ring using an intramolecular Michael addition (Scheme 4). The diketone



Scheme 3. Proposed process of the HLF reaction of **16**.



Scheme 4. Construction of the D Ring. Reagents and conditions: a) cnoc. HCl/EtOH (v/v) = 1:5, 24 °C, 1 h, 91 %; b) MsCl (1.1 equiv), Et_3N (1.5 equiv), 0 °C, CH_2Cl_2, 30 min, 92 %; c) DIPEA (6.0 equiv), TMSOTf (3.0 equiv), −40 °C to 0 °C, CH_2Cl_2, 3 h; d) $Pd(OAc)_2$ (1.1 equiv), 25 °C, MeCN, 8 h, 60 % for **23**, 18 % for **23′** over 2 steps; e) conc. HCl (8.0 equiv), NaI (8.0 equiv), 25 °C, acetone, 30 min, 85 %; f) $CH(OEt)_3$ (20.0 equiv), $(CH_2OH)_2$ (20.0 equiv), PTSA (0.02 equiv), 18 °C, CH_2Cl_2, 3 h; g) aq. $MeNH_2$/THF (v/v) = 1:5, 60 °C, 10 h, then conc. HCl, 0.5 h, then sat.$NaHCO_3$, 24 °C, THF, 82 % over 2 steps; h) PTSA (1.2 equiv), $(CH_2OH)_2$ (50.0 equiv), reflux, toluene, 1 h, 94 %. DIPEA = diisopropylethylamine, PTSA = *para*-toluenesufonic acid, Tf = trifluoromethanesulfonyl, TMS = trimethylsilyl.

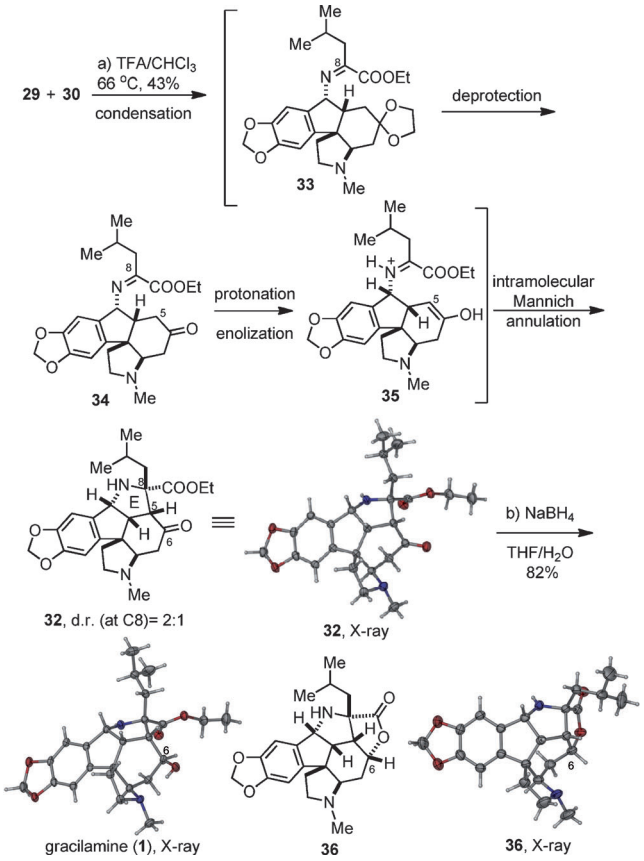


Scheme 5. Construction of the E ring using oxidative coupling. Reagents and conditions: a) **26** (3.0 equiv), $NaBH_3CN$ (1.5 equiv), 90 °C, MeOH/HOAc (v/v) = 5:1, 12 h; b) $NH_2OMe{\cdot}HCl$ (3.0 equiv), 70 °C, Pyridine, 12 h; c) $Pd(OH)_2/C$, H_2 (1 atm), 65 °C, aq. $NH_3{\cdot}H_2O$/EtOH (v/v) = 1:5, 12 h, d.r. (at C9a) = 5:1: 64 % over 2 steps; d) **30** (2.0 equiv), Pd/C, H_2 (1 atm), 25 °C, EtOH, 2 h, d.r. (at C8) = 1.2:1, 80 %; e) aq. $HClO_4$, 0 °C, CH_2Cl_2, 45 min, 57 %; f) LiHMDS (3.0 equiv), $[Fe(acac)_3]$ (2.2 equiv), −78 °C to 25 °C, THF, 1 h. acac = acetylacetonate, HMDS = hexamethyldisilazide.

22 was prepared from **13** by deprotection and mesylation. Then it was transformed into its corresponding TMS-enol ether and oxidized to the enone **23** and its regioisomer **23′** through a Saegusas–Ito oxidation. The regioisomer **23′** was recovered and transformed into **22** in 85 % yield by treatment with NaI and conc. HCl.[12] Sequential protection, amination, and 1,4-addition of **23** gave the core skeleton **24** in 82 % overall yield. The the structure of **24** was determined by single-crystal X-ray analysis. Selective protection of the carbonyl group on C6 in **24** gave **25** efficiently.

We initially tried to introduce the leucine motif through direct reductive amination involving **25** and the leucine ethyl ester **26** (Scheme 5). However, the condensation of **25** with **26** to form the imine intermediate **27** proved difficult because of steric effects. Adding reductant led to formation of the alcohol **28**, the structure and stereochemistry of which was confirmed by X-ray analysis. As an alternative to inefficient direct reductive amination, condensation of **25** with *O*-methylhydroxylamine yielded the corresponding O-methyl oxime intermediate, which was reduced by $Pd(OH)_2$-promoted hydrogenation to the amine **29** (d.r. = 5:1 at C9a). Reaction of **29** with the α-keto ester **30** in the presence of acetic acid gave an imine intermediate, which underwent efficient hydrogenation and acetal removal to give **31** as a mixture of inseparable diastereomers (d.r. = 1.2:1 at C8). We then investigated the closure of the E ring, that is, formation of a C5–C8 bond, through intramolecular oxidative coupling. Unfortunately, we did not obtain the desired product after testing several bases and various oxidants developed by the groups of Baran[9] and Ma.[9] Instead, we recovered **31** as a single diastereomer. We postulate that under these alkaline conditions, **31** underwent a facile deprotonation of the secondary amine and enolization of the ketone on the C ring, thereby preventing further enolization of the ester motif. Subsequent an intramolecular hydrogen shift occurred rapidly and racimized the C8.

Since the condensation of **29** and **30** gave an imine intermediate, we were intrigued by the possibility of trapping the iminium cation by an intramolecular Mannich reaction[13] (Scheme 6). To our delight, heating a solution of **29** and **30** in TFA/CH_3Cl (1:1, v/v) at 66 °C directly gave the cyclized product **32** as two diastereomers (d.r. = 2:1 at C8). This procedure involved the condensation of two fragments, **29** and **30**, acetal removal, acid-promoted iminium cation formation, and the intramolecular Mannich annulation involving the iminium cation and enol (**33**→**34**→**35**→**32**). The E ring, including a quaternary center (C8), was efficiently constructed in this process through the formation of a C8–N and a C5–C8 bond. X-ray analysis of **32** gave a structure consistent with that reported by Ma and co-workers,[3] thus allowing us to confirm unambiguously its relative stereochemistry. Reduction of the C6 carbonyl group of **32** with $NaBH_4$ in THF/H_2O (v/v = 4:1) gave gracilamine (**1**) and the lactone **36** in 82 % combined yield.[14] The relative stereochemistry of both **1** and **36** was confirmed by X-ray analysis. The formation of **36** suggested that the α-hydroxy group on C6 should easily undergo the intramolecular esterification



Scheme 6. Construction of the E Ring using a Mannich reaction. a) **30** (3.0 equiv), 66 °C, TFA/$CHCl_3$ 1:1 (*v*/*v*), 38 h; d.r. (at C8) = 2:1, 43% combined yield; b) $NaBH_4$ (3.0 equiv), 0 °C to 24 °C, THF/H_2O 4:1 (*v*/*v*), 1 h, 82% combined yield. TFA = trifluoroacetic acid

because of the favorable configuration, while the β-hydroxy group proved stable during the reduction. This finding further supports the C6 configuration of natural gracilamine, which matched with the conclusion reported by Ma and co-workers.[3]

In summary, we have accomplished the total synthesis of gracilamine, a pentacyclic *Amaryllidaceae* alkaloid. Our synthetic approach relies on three key ring-forming steps: 1) a mild photo-Nazarov reaction to form the B ring, 2) 1,4-addition to form the D ring, and 3) intramolecular Mannich reaction to form the E ring. Our research findings further confirm the C6 configuration of natural gracilamine. The synthetic strategies developed here should facilitate production of a variety of gracilamine derivatives and structurally related natural products, thus leading to biological studies.

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[14] We initially tried the reduction of **32** using the procedure reported by Ma and co-workers (Ref. [3]). We obtained gracilamine (**1**) and also observed the formation of the lactone **36** in our case. To determine the relative stereochemistry of **36**, we screened the reaction onditions and found water plays a key role in this reaction. Reduction using THF/water as cosolvent gave **1** and **36** (d.r. = 1.3:1 at C6), which lay the foundation for the structure–activity relationship (SAR) studies of gracilamine (**1**).