Total synthesis of the reported structure of 13a-hydroxytylophorine

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The first total synthesis of the reported structure of 13a-hydroxytylophorine was accomplished. The key step was an unprecedented NaBH4-promoted one-pot reductive cyclization cascade that efficiently yielded a hydroxyl azonane intermediate. The indolizidine framework was obtained by means of oxidation and a subsequent unexpected protecting-group migration. This total synthesis revealed that the reported structure of the naturally isolated compound is incorrect.

Phenanthroindolizidine alkaloids, which are isolated from plants of the Cynanchum, Pergularia, and Tylophora genera, as well as some genera of the Asclepiadaceae family1,2, are a group of pentacyclic natural products. To date, more than 60 members of this group have been isolated and characterized, and they have been the subject of considerable research attention because they exhibit a variety of bioactivities3–6, including anticancer, anti-inflammatory, and antifungal activities. For example, (R)-tylophorine and (R)-antofine show potent anticancer activity against human lung cancer cells (A549), with 50% growth inhibition concentrations (G100) of 0.50 and 0.01 μM, respectively7. In addition, the synthetic analogue DCB-3503, for example, is reported to have an average G100 of approximately 30 nM in 60 cell lines8 (Fig. 1). Apart from these common C13a-H compounds, a unique compound with a C-13a hydroxyl group, 13a-hydroxytylophorine (1), was isolated from Tylophora hirsuta by Bhutani et al9,10 in 1984.

The 13a-hydroxytylophorine molecule has an aza-hemiacetal motif, and because aza-hemiacetals are usually acid- and base-sensitive, an effective method for construction of this motif constitutes the major challenge in the synthesis of 13a-hydroxytylophorine. Although numerous synthetic approaches to 13a-H phenanthroindolizidine alkaloids have been reported11–15, along with a few syntheses of 13a-methyl compounds16–19, the total synthesis of 13a-hydroxytylophorine has not yet been achieved. As part of our ongoing research20–23 on the synthesis of phenanthroindolizidine alkaloids and systematic evaluation of their biological activities, we herein report the first total synthesis of the reported structure of 13a-hydroxytylophorine.

Results

Our retrosynthetic analysis is presented in Fig. 2A. To circumvent the problem of the lability of the aza-hemiacetal motif, we decided that a novel strategy involving late-stage transannular aza-hemiketalization reaction of amino ketone 2 to form the N–C13a bond would make sense. However, synthesis of the strained phenanthro-annulated nine-membered-ring azonane motif in 2 presented a challenge. Traditional strategies for the preparation of this core architecture involve direct methods such as N-substitution reactions24 (e.g., Mitsunobu reaction and alkylation), lactamization25, and ring closure metathesis26. Indirect methods involving ring expansion27–29 via fragmentation of azabicycles offered another option, although syntheses using such methods tend to be complex. We suspected that the rigidity and steric bulk of the phenanthrene ring might render the direct methods unfeasible for our purposes. In addition, because the ring-expansion strategy might be favoured by release of transannular strain, we adopted this indirect strategy.

We expected that amino ketone 2 could be obtained by oxidation of alcohol 3, which could be prepared from phenanthro-δ-lactone 4 by a ring-expansion reaction. Compound 4 would be prepared by a simple homologation reaction of functionalized precursor 5, which would in turn be synthesized via a coupling reaction between known dibromo compound6,30 6 and 4-azido-aldehyde 7. As mentioned above, the ring-expansion reaction is the key step in this strategy. We hoped to prepare key hydroxy-substituted azonane intermediate 3 in one pot by
means of a reductive cyclization cascade reaction of lactone 4 (Fig. 2B). Specifically, in the presence of a suitable reductant, lactone 4 would be reduced to lactol 3, which would undergo rapid intramolecular attack by the amino group formed in situ to generate hemiaminal 9. Hemiaminal 9 would then be further reduced to required compound 3. That is, the azonane ring of 3 would be constructed from a six-membered-ring lactone with the necessary oxygen atom in the correct position.

We began by constructing the required lactone skeleton (Fig. 3). To make the functionalized precursor of 4, we used an umpolung strategy. An acyl anion equivalent, namely, tert-butyldimethylsilyl-protected cyanohydrin 10, was prepared in 85% yield via reaction of known 4-azido-aldehyde 7 with NaCN and TBSCI in the presence of a catalytic amount of ZnI₂. Cyanohydrin 10 was then smoothly coupled with phenanthryl bromide 6 in the presence of LiHMDS as a base. The resulting coupled adduct was treated directly with TBAF to afford ketone 11 in 77% yield over two steps. Subsequent reduction with NaBH₄ gave alcohol 5 in 97% yield. Next, we needed to install the last one-carbon unit of the target molecule framework. Knowing that lithium-halogen exchange can be used to generate a phenanthryl anionic species, we planned to introduce the necessary carbonyl group via an intramolecular acyl rearrangement from the oxygen atom of the hydroxyl group to the phenanthrene ring. Treatment of alcohol 5 with dimethylcarbamoyl chloride in the presence of KHMDS smoothly gave carbamate 12 in 90% yield. Satisfyingly, reaction of 12 with n-BuLi effected the desired acyl rearrangement to afford carbamoyl compound 13. Subsequent lactonization catalysed by CSA (camphorsulfonic acid) gave phenanthro-δ-lactone 4 in 63% yield over two steps. With 4 in hand, we explored our proposed one-pot reductive cascade sequence.

Initially, we speculated that when the tethered azido group was reduced to an amino group by a suitable reductant, intramolecular transamidation might occur to produce nine-membered-ring lactam 15. However, we were concerned that direct transamination might be unfavourable owing to the transannular strain in the

Figure 1. Representative phenanthroindolizidine alkaloids.

Figure 2. (A) Retrosynthetic analysis of 13a-hydroxytylophorine 1 and (B) proposed one-pot reductive cyclization cascade.
nine-membered ring. In fact, we found that under common azide reduction conditions (10% Pd/C, H₂), amino lactone 14 was obtained (77% yield) instead of lactam 15 (Fig. 4). Therefore, we explored alternative conditions for the desired intramolecular transamidation of 14. No reaction occurred under either basic conditions (DBU, TEA, NaOMe, and K₂CO₃) or acidic conditions (CSA and HCl). On the basis of these results, we suspected that the lactone centre of 14 was not sufficiently electrophilic to undergo either inter- or intramolecular attack by the amino group. Therefore, we decided to transform the lactone to the corresponding lactol, which would be more susceptible to intramolecular attack. Unlike the transamidation product (lactam 15), the product of reaction of the lactol, that is, 3, would not have an acyl group, so further transformation would be unnecessary.

Because DIBAL-H is commonly used to prepare lactols, we used this reagent in our initial attempts to reduce lactone 4. However, none of the desired product was detected (Table 1, entry 1). We screened several other hydride reductants (NaBH₄, LiBH₄, NaBH(OAc)₃ and NaBH₃CN) and found that only NaBH₄ 33,34 in methanol gave any of the desired product, which was obtained in 20% yield along with recovered substrate (entries 2–5). Increasing the reaction temperature to 50 °C increased the yield to 40% (entry 9). Screening of various solvents revealed that methanol was indispensable; ethanol, isopropanol, and THF were not acceptable substitutes (entries 5–8). Finally, we found that reaction in 1:4 (v/v) THF/MeOH at 50 °C afforded the required azonane in 84% yield (entry 10). The reaction was relatively clean, and no intermolecular reaction product was observed. We suggest that the added THF improved the solubility of the substrate, which is only poorly soluble in methanol. We speculated that methanol also enhanced the reductive ability of NaBH₄ and, moreover, that methanol facilitated the difficult transformation by stabilizing newly formed lactol 8 (see Fig. 2B) and promoting rapid intramolecular attack by the amino to form hemiaminal 9 (which was detected by high-resolution mass spectrometry and ¹H NMR). Hemiaminal 9 was then reduced to desired product 3. Detailed information regarding the proposed mechanism is presented in the supplementary materials. To our delight, upstream azide 4 could be transformed to 3 in 74% yield under the same conditions if the amount of reductant was doubled and the reaction time was extended (entry 11). Owing to the tendency of 14 to decompose, the one-pot reduction of 4 was more favourable. In summary, we developed an unprecedented one-pot reductive cyclization cascade to construct the desired strained nine-membered ring from a six-membered-ring lactone under mild conditions with readily available, inexpensive NaBH₄. The whole procedure was efficient and operationally simple.

With the azonane architecture built, we were able to obtain (±)-tylophorine easily in 82% yield by means of a transannular Mitsunobu reaction in the presence of PPh₃ and DIAD (Fig. 5). The ¹H NMR and ¹³C NMR spectra of the synthetic (±)-tylophorine were consistent with those of standard samples prepared previously in our lab35,36. Thus, the synthesis described herein confirmed the structure of 3. To convert 3 to 13α-hydroxytylophorine, we needed to oxidize the hydroxyl group selectively. We envisioned that the formation of a quaternary ammonium salt might prevent undesired oxidation of free amine. We attempted to use Jones reagent to accomplish this transformation, but the reaction yielded a complex mixture. Other oxidative reaction conditions (IBX/HCl and HOAc/PIDA/DCM) also failed to afford the desired alkaloid. Therefore, we protected the free amino group of 3 by treating it with Boc₂O and trimethylamine, which afforded 16 in 91% yield. When 16 was treated with...
Dess–Martin periodinane, however, only a trace of desired product 17 was obtained, and the major product was instead 13a-O-Boc-tylophorine (18) (78% yield; 1H NMR, 13C NMR, and two-dimensional NMR spectra and HRMS data are available in the supplementary materials). That is, a one-pot oxidation and unexpected Boc migration took place. We assumed that this transformation proceeded by initial oxidation of the alcohol by DMP to afford expected product 17. Promoted by the release of transannular strain, 17 underwent rapid migration of the Boc group from the nitrogen atom to the oxygen atom. Steric hindrance between the phenanthrene ring and the

Table 1. Optimization of one-pot reductive cyclization conditions.

| Entry | Reductant | Solvent   | Temp | Yieldb |
|-------|-----------|-----------|------|--------|
| 1     | DIBAL-H   | DCM       | −78 °C → rt | ND*    |
| 2     | LiBH₄     | THF       | 0 °C → rt | NR     |
| 3     | NaBH₄(OAc)₃ | DCM       | 0 °C → rt | NR     |
| 4     | NaBH₄,CN  | DCM/TFA   | 0 °C → rt | ND     |
| 5     | NaBH₄     | MeOH      | 0 °C → rt | 20%e   |
| 6     | NaBH₄,iPrOH | EtOH     | 0 °C → rt | mixf   |
| 7     | NaBH₄     | iPrOH     | 0 °C → rt | NR     |
| 8     | NaBH₄     | THF       | 0 °C → reflux | NR     |
| 9     | NaBH₄     | MeOH      | 0 °C → 50 °C | 40%f   |
| 10    | NaBH₄     | THF/MeOH  | 0 °C → 50 °C | 84%    |
| 11    | NaBH₄     | THF/MeOH  | 0 °C → 50 °C | 74%    |

*aReaction conditions: 14 (0.50 mmol, 1 equiv), reductant (5 equiv), solvent (20 mL, 0.025 M), start at 0 °C and then increase to the required temperature, usually < 0.5 h.* Isolated yields are provided. NR, no reaction; ND, not detected. *DIBAL-H (2.5 equiv) was added at −78 °C under argon. *A complex mixture was obtained. *Substrate was recovered. *The reaction was conducted in 1:4 (v/v) THF/MeOH. *Azide 4 (0.50 mmol, 1 equiv) was used as the substrate, and 10 equiv of reductant was used.
bulky N-Boc group in 17 may also have contributed to the driving force for this transformation. Compound 17 was actually observed by thin-layer chromatography when the reaction was conducted at $-15^\circ$C, but it was soon transformed to compound 18 at room temperature or when in contact with silica gel. Finally, having obtained the indolizidine moiety in this unexpected way, we generated 13a-hydroxytylophorine (1) smoothly in 91% yield by removing the protecting group under mild conditions (TMSOTf, 2,6-lutidine).

To our disappointment, we found that the $^1$H NMR spectroscopic data for our synthetic 13a-hydroxytylophorine (1) differed considerably from the data for the sample isolated by Bhutani et al. Therefore, we conducted a detailed comparison of the chemical shifts of the protons of our synthetic 1 and the chemical shifts reported for the naturally isolated compound (Fig. 6). In particular, we noted that the chemical shifts of the aromatic protons of our synthetic sample differed markedly from those of the isolated compound, and the shifts of the four methoxyl group were slightly different as well.

We considered many factors that are known to affect chemical shift, such as sample concentration, the identity of the counterion, the presence of acid in the solvent, the presence of impurities in the sample, and the extent of CDCl$_3$ decomposition.\textsuperscript{37,38} We also conducted further experiments to find an explanation for the observed differences. Acidity can sometimes affect $^1$H NMR spectra, especially the spectra of alkaloids, and such affects...
have been reported previously for phenanthroindolizidine alkaloids. Therefore, we conducted an acid titration as described previously. The addition of incremental amounts of acid (TFA) to the sample resulted in dramatic changes in the chemical shifts, especially those of the aromatic protons (Fig. 7). However, our spectroscopic data did not match the reported data, so we concluded that the presence of acidic impurities in the isolated sample or in the NMR solvent were not responsible for the discrepancies. Moreover, the reported data were incomplete (for example, information on test conditions, chemical shifts of aliphatic protons, 13C NMR data, and a copy of the 1H NMR spectrum were unavailable). Therefore, we suspect that the reported structure of 13a-hydroxytylophorine is incorrect and should be revised.

Conclusions

In summary, we accomplished the first total synthesis of the reported structure of 13a-hydroxytylophorine in 17.2% overall yield. The route features an unprecedented NaBH4-promoted one-pot reductive cyclization cascade sequence for the construction of a nine-membered-ring azonane with a hydroxyl group at the desired position. The required oxygenated indolizidine framework was constructed via a sequence involving DMP-promoted oxidation and a subsequent unexpected Boc migration. In addition, the -tylophorine was synthesized via a transannular Mitsunobu reaction from a common intermediate. This total synthesis revealed that the reported structure of the naturally isolated product is incorrect. Structure modification and biological evaluation of synthetic 13a-hydroxytylophorine are in progress and will be reported in due course.

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Author Contributions

Q.M.W. and Y.C.G. conceived the concept and directed the project; H.Z. and B.S. designed the synthetic route; H.Z., G.L. and M.D. performed all of the synthesis and standard characterization. The manuscript was written by H.Z. and revised by Y.X.L., B.S. and Y.C.G., All authors discussed the results and commented on the manuscript.

Additional Information

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