# Synthetic Studies on Lepadiformine Using the 2-Azaallyl Anion Method

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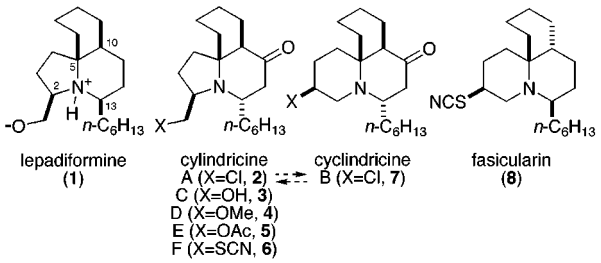
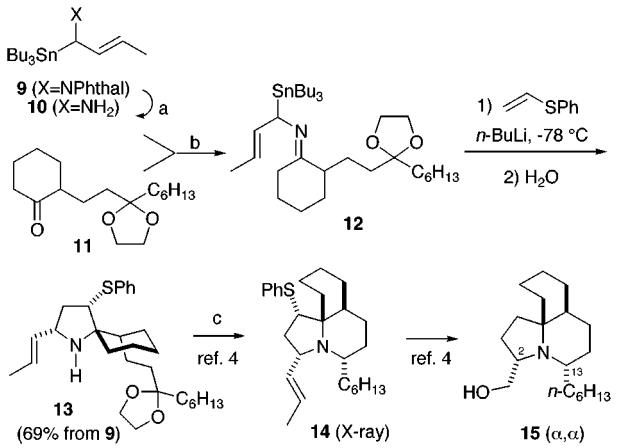
Lepadiformine (**1**),[1] the cylindricines (**2**−**7**),[2] and fasicularin (**8**)[3] are structurally related marine alkaloids isolated recently from the ascidians *Clavelina lepadiformis, Clavelina cylindrica,* and *Nephteis fasicularis,* respectively. Lepadiformine is cytotoxic against KB, HT29, P388, P388 doxorubicin-resistant, and NSCLC-N6 tumor cell lines.[1] Cylindricines A and B, which exist in equilibrium, cause mortality in a brine shrimp bioassay.[2a] Fasicularin is cytotoxic to Vero cells ($IC_{50}$ = 14 mg/mL) and was found to act as a DNA-damaging agent by its action against a DNA repair-deficient yeast strain.[3] The structure of lepadiformine rests upon MS, NMR, and IR analysis. Its absolute configuration is not known, although curiously, its optical rotation is zero. The *β*-amino alcohol portion of lepadiformine is proposed to exist in a novel zwitterionic form on the basis of IR analysis. Derivatization has not been possible, thwarting the preparation of crystalline materials suitable for X-ray crystallographic analysis.[1] The perhydropyrrolo[2,1-*j*]quinoline skeleton of **1**−**6** and the perhydropyrido[2,1-*j*]quinoline skeleton of **7** and **8** are new structural classes. The perhydropyrrolo-[2,1-*j*]quinoline skeleton has seen recent synthetic activity; we recently disclosed the total synthesis of 2,13-diepilepadiformine,[4] and Snider and Liu[5] reported total syntheses of cylindricines A (**2**), D (**4**), and E (**5**). The perhydropyrrolo-[2,1-*j*]quinoline skeleton has been accessed by Snider and Liu, since cylindricine A (**2**) exists in equilibrium with cylindricine B (**7**). We report herein the synthesis of three of the four possible diasteromers of lepadiformine (**1**) at C(2) and C(13). Since Weinreb et al. have now synthesized **1** and found it to be different from natural lepadiformine,[6] our work serves to show that natural lepadiformine is not a diastereomer of **1** at C(2) and C(13).




An improved synthesis of 2,13-diepilepadiformine **15** (the "α,α" diastereomer at C-2 and C-13) is shown in Scheme 1. While this route mirrors our previously published route,[4] a new protocol for the key 2-azaallyl anion cycloaddition[7] is featured. Hydrazinolysis of the phthalimide **9** afforded the amine **10**, which was immediately condensed with the ketone **11** in the presence of trimethylaluminum in benzene. Without further manipulation, the resultant mixture was diluted with THF, cooled to −78 °C, and treated with phenyl vinyl sulfide and *n*-butyllithium. Aqueous workup gave the cycloadduct **13** in 69% isolated yield (overall from **9**) as a single stereo- and regioisomer. The aluminum byproducts from the imine condensation apparently do not interfere with the transmetalation/cycloaddition process. This process is more reproducible and proceeds in higher yield than in our previous work, where we isolated the imine **12** prior to its use in the cycloaddition reaction, and has proven useful in other 2-azaallyl anion cycloadditions in our laboratories. The cycloadduct **13** had been previously converted to 2,13-diepilepadiformine **15**, notably proceeding through **14**, whose relative configuration was assigned by X-ray crystallography. Compound **15** was found to exhibit dramatically different $^1H$ and $^{13}C$ NMR spectra than those obtained from examination of a sample of natural lepadiformine.[8] We then turned our attention to adjusting the configuration at C(2) using epimerization techniques.

**Scheme 1. Synthesis of the 2α,13α-Diastereomer 15**[a]




[a] Key: (a) $N_2H_4{\cdot}H_2O$, EtOH, reflux; (b) $AlMe_3$, PhH, 0 °C; (c) 10% aqueous HCl, THF; $NaBH_4$, MeOH (58%).

In preparation for oxidative cleavage of the propenyl group of **13**, we encountered considerable difficulty in protecting the secondary amino group. Ultimately, a formyl group was successfully introduced, producing **16**. Marshall oxidative cleavage[9] of **16** produced the ester **17**, where the sulfide had been oxidized to the sulfoxide. Thermolysis of **17** gave the dihydropyrrole **18a** as a single stereoisomer. Epimerization of **18a** with DBU led to a 1:1.4 equilibrium mixture of **18a** and **18b**, which was not separated. Hydrogenation of this

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(5) Snider, B. B.; Liu, T. *J. Org. Chem.* **1997**, *62*, 5630−5633.

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(7) Pearson, W. H.; Szura, D. P.; Postich, M. J. *J. Am. Chem. Soc.* **1992**, *114*, 1329−1345.

(8) We thank Professor Biard for a sample of natural lepadiformine and for helpful discussions regarding its isolation and manipulation.

(9) Marshall, J. A.; Garofalo, A. W. *J. Org. Chem.* **1993**, *58*, 3675−3680.

(10) In Biard's work on the isolation of lepadiformine (ref 1), it was found that after column chromatography, "the main fractions contain two minor, but inseparable, byproducts in addition to lepadiformine. They convert into lepadiformine on evaporation of an acidic solution (methanol/aqueous 1 N HCl; 99:1) of the mixture, affording lepadiformine **1** as a colorless oil". Apparently, lepadiformine isolated by this procedure is not a hydrochloride salt. The mixture obtained from the debenzylation of **25** exhibited similar behavior and was also subjected to acidic treatment, converting the mixture of unknown substances into a single compound, **27**, not identical with an authentic sample of natural lepadiformine provided by Professor Biard. While the $^1H$ NMR spectrum of **27** is similar to that of **1**, it is not identical. Further, the $^{13}C$ NMR spectra of **27** and **1** are very different.

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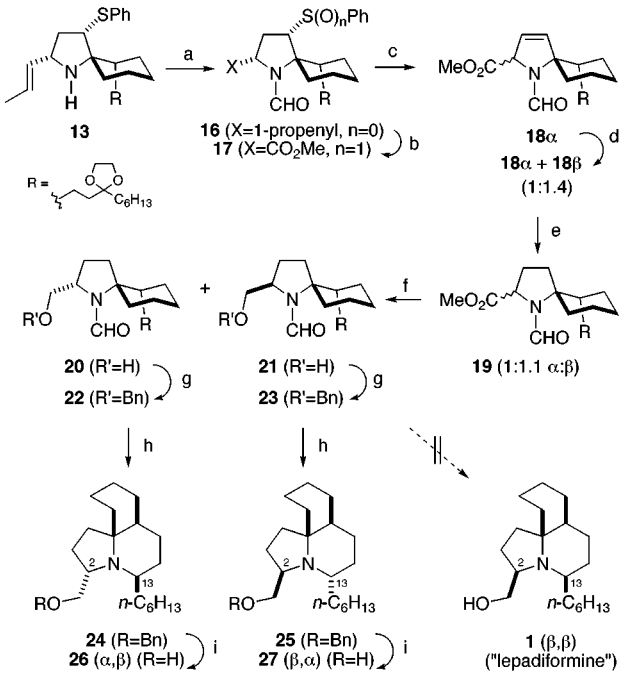
**Scheme 2. Synthesis of the 2α,13*β*- and 2*β*,13α-Diastereomers 26 and 27**[a]




[a] Key: (a) $MeCO_2CHO$, $NEt_3$, $CH_2Cl_2$, 0−25 °C (91%); (b) $O_3$, NaOH, MeOH, $CH_2Cl_2$, −78 °C; (c) $Na_2CO_3$, toluene, reflux (46% from **16** plus 6% of unreacted **17**); (d) DBU, THF, reflux; (e) 40 psi $H_2$, Pd/C, MeOH; (f) $LiBEt_3H$, THF, 0 °C (73% from pure **18**α, 1:1 mixture of **20**:**21**, separated); (g) NaH, THF; BnBr, *n*-$Bu_4NI$; 92% for **22**, 97% for **23**; (h) MeLi, THF; HCl, THF; $NaBH_4$, MeOH; 53% for **24**, 50% for **25**; (i) Raney Ni, $H_2$, EtOH; 100% for both **26** and **27**.

mixture produced **19**, which was subjected to hydride reduction followed by separation to afford the alcohols **20** and **21**. A variety of deformylation conditions failed to deprotect these compounds. However, O-benzylation to give **22** and **23** followed by deformylation with methyllithium proceeded smoothly to produce the amino alcohols (not shown), which were subjected to ketal removal and reductive amination to afford the tricycles **24** and **25**, each as single diastereomers. Debenzylation of **24** gave 2-epilepadiformine (the "α,*β*" diastereomer), which was clearly different from both natural lepadiformine and the previously synthesized α,α diastereomer **15**.[4] Debenzylation of **25** gave 13-epilepadiformine, again different from natural lepadiformine and the diastereomers **15** and **24**.[10] Surprisingly, none of the *β*,*β* diastereomer (**1**) was detected in the latter sequence.

During the course of exploring other options for generating **1** from **21** or related compounds, we became aware of the synthesis of **1** by Weinreb and co-workers, who found the synthetic material to be different from natural lepadiformine.[6] Since the structure of lepadiformine was assigned mainly by NMR spectroscopy and that assignment is now in question, it is natural to suspect that lepadiformine is actually a diastereomer of **1**. Thus, our work serves to rule out the possibility that lepadiformine is a diastereomer at C(2) or C(13). Thus, one must conclude that lepadiformine is either epimeric at C(10) (and perhaps other stereocenters) as in fasicularin **8** or it has a different connectivity altogether. Clearly, the structure of lepadiformine must be revised.

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**Supporting Information Available:** Experimental details for the preparation of new compounds and photocopies of $^1H$ and $^{13}C$ NMR spectra for compounds without elemental analysis.

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