Lepadiformine: A Case Study of the Value of Total Synthesis in Natural Product Structure Elucidation

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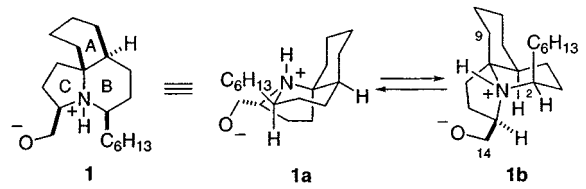
ABSTRACT
Since the emergence of routine X-ray crystallography and high-field FT NMR in the mid-twentieth century, the importance of total synthesis in structure elucidation has become underappreciated by most organic chemists. However, the limitations and fallibility of spectral methodology has recently been highlighted by the mischaracterization of a number of complex natural products, the correct structures of which were all ultimately assigned by total synthesis. This Account describes how total synthesis was not only instrumental in disproving the erroneously assigned structure of the marine alkaloid, lepadiformine, but also was also pivotal in establishing the correct structure and absolute configuration.

Introduction

From the earliest days of organic chemistry until the middle of the twentieth century, chemical degradation of natural products was of major importance not only for its primary objective of elucidating structure, but also for providing insight into the reactivity and chemical behavior of various structural and functional types. For many years the main role of total synthesis was to provide the final and conclusive proof of structure. However, with the advent of routine X-ray crystallography and high-field FT NMR in the late 1960s, chemical degradation all but disappeared, and the argument that total synthesis was a necessary structural confirmation became much less compelling. As synthetic chemists, we now recognize that natural product synthesis has replaced chemical degradation as a primary platform for basic discovery in organic chemistry. Although one certainly cannot dispute that research in natural products synthesis has indeed been the impetus for many fundamental discoveries (e.g., the vitamin B_{12} synthesis and Woodward−Hoffmann rules),[1] the ongoing importance of total synthesis in the realm of structure elucidation is probably underappreciated by most organic chemists today. Examples abound of the limitations and fallibility of spectral methodology in establishing natural product structures. Recent notable cases where some of these shortcomings were clearly demonstrated include the mischaracterization of the diazonamides (an incorrect structure determination by X-ray!),[2a] cylindrospermopsin,[2b] the sclerophytins,[2c,d] and batzelladine F.[2e] In all of these instances, the correct structures were ultimately determined by total synthesis. This Account tells the story of another such case involving the marine alkaloid lepadiformine. The research described here shows how total synthesis was instrumental both in disproving the initial erroneous structural assignment and in finally establishing the actual structure and absolute configuration of this metabolite.



1 1a 1b

FIGURE 1.

Background. Several years ago we became interested in a class of alicyclic marine alkaloids upon seeing a publication by Biard and co-workers describing the isolation and a postulated structure for a compound which they named lepadiformine.[3] This material was obtained by HCl extraction of the methylene chloride-soluble portion of the marine tunicate *Clavelina lepadiformis* (Muller) collected in the Mediterranean off the coast of Tunisia,[3a] and later from *Clavelina moluccensis* (Sluiter) obtained near Djibouti.[3b] Lepadiformine was found to have moderate in vitro cytotoxic activity against several tumor cell lines,[3a] as well as various cardiovascular effects in vivo and in vitro.[3b] On the basis of a series of proton and carbon NMR experiments, the Biard group suggested the tricyclic lepadiformine structure **1**, which contains an unusual zwitterionic vicinal amino alcohol moiety (Figure 1). It should be noted that the A/B ring system of **1** is a *cis*-1-azadecalin, and initial NMR NOE experiments seemed to suggest that this alkaloid had the conformation shown in **1a**. However, prior to starting the synthetic work described here, we performed molecular mechanics calculations which indicated that the 1-azadecalin prefers the alternative chair-chair form to that postulated, with the alkaloid conformation **1b** actually being about 3 kcal/mol more stable than **1a**.

The putative structure **1** would put lepadiformine into a small class of alkaloids known as the cylindricines. In

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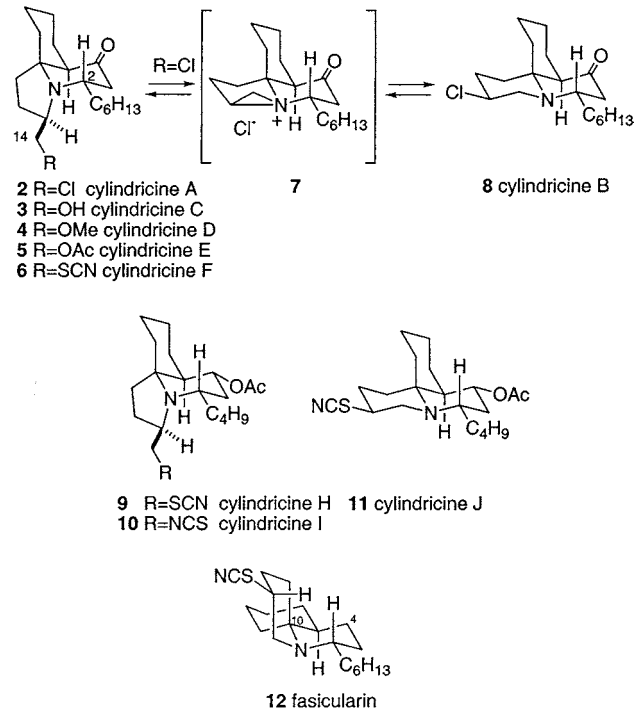
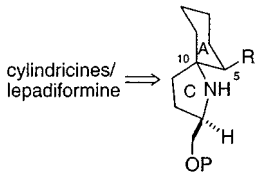


FIGURE 2.

the early 1990s, Blackman and co-workers reported the isolation of several cylindricines from the marine ascidian *Clavelina cylindrica*, collected at various sites off the coast of Tasmania.[4] The most abundant of these alkaloids are cylindricines A (**2**) and B (**8**), whose structures were established by a combination of spectroscopic methods and X-ray crystallography of their picrate salts (Figure 2). Interestingly, these two compounds exist in solution as a 3:2 equilibrium mixture, presumably interconverting ring C through the aziridinium intermediate **7**, a fact probably of relevance to the biogenesis of these natural products.[4a] Subsequent investigations led to the isolation of some additional minor compounds having the cylindricine A pyrroloquinoline framework and stereochemistry, but differing in the functionality at C-14. These alkaloids include cylindricines C (**3**), D (**4**), E (**5**), and F (**7**). In addition, a few alkaloids were found which possess a butyl chain at C-2 rather than the hexyl group [e.g., cylindricines H (**9**) and I (**10**)]. Similarly, compounds in the cylindricine B pyridoquinoline series exist which also have a butyl appendage, such as cylindricine J (**11**). It might be noted that X-ray crystal structure and NMR data, in addition to molecular mechanics calculations,[4c] indicated that these molecules prefer to exist in the *cis*-1-azadecalin conformations shown, corresponding to lepadiformine conformer **1b**.

In 1997, a congeneric tricyclic compound, fasicularin, was isolated from the ascidian *Neptheis fasicularis* collected in Pohnpei, Micronesia.[3] NMR and NOE experiments led to the assignment of the structure and relative stereochemistry of fasicularin as depicted in **12**. Therefore, the alkaloid belongs to the cylindricine B pyridoquinoline series but is epimeric at the C-10 quaternary center, thereby giving it a *trans*-1-azadecalin A/B ring fusion. This compound, like lepadiformine, lacks the C-4 oxygenation

Scheme 1



found in the cylindricine series. Fasicularin was also of considerable interest in view of its biological activity against a DNA repair-deficient strain of yeast, as well as its cytotoxicity against Vero cells.

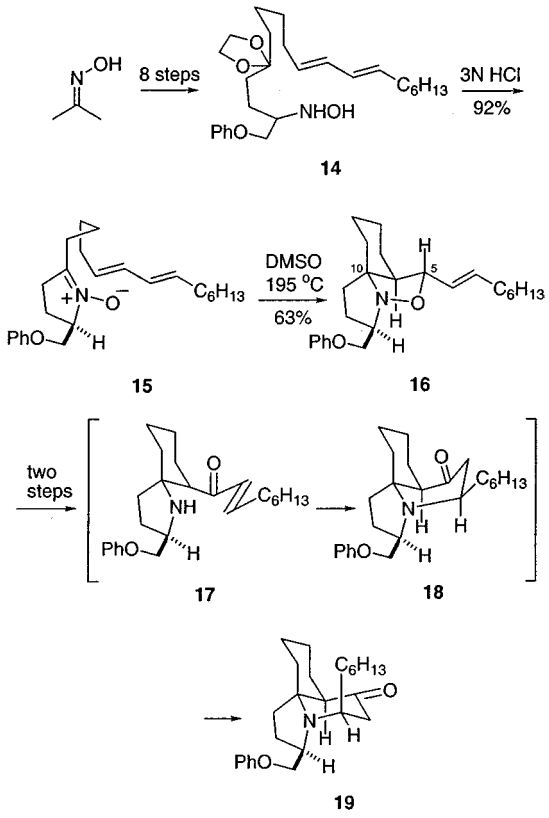
Disproof of the Biard Lepadiformine Structure via Synthesis. In 1996, prompted by the novel structures of these marine metabolites along with their interesting biological activity, we became involved in a program directed toward a stereoselective total synthesis of the proposed structure for lepadiformine, **1**.[6,7] We entered this arena with some trepidation, since there were concerns about the validity of the structural assignment. However, we believed that we could develop general methodology which would also be applicable to preparation of the cylindricines, whose constitutions were more firmly grounded. We thus chose to adopt a strategy which would lead initially to an A/C spirocycle like **13**, containing both the C-10 quaternary center and a manipulable substituent at C-5, which would provide a handle for construction of the remaining B ring with the requisite stereochemistry to generate a *cis*-1-azadecalin (Scheme 1). The key step in this process was to be an intramolecular nitrone/diene dipolar cycloaddition.[8]

The required nitrone **15** was easily prepared by acid-promoted cyclization of hydroxylamine ketal **14**, which was in turn synthesized in eight straightforward steps from acetone oxime (Scheme 2).[7] We were pleased to find that heating **15** in DMSO at 195 °C led to a single [3+2]-cycloadduct **16** as the only isolable product. This reaction presumably occurs via a conformation like that shown in structure **15**, thereby providing bicyclic adduct **16** which contains the quaternary C-10 center and the appropriate stereochemistry at C-5 for construction of the *cis*-1-azadecalin system of **1**.

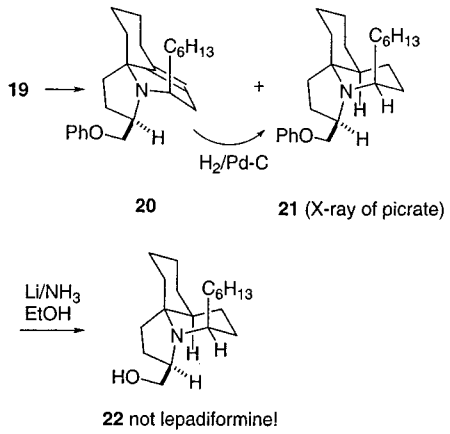
To continue the synthesis, cleavage of the N−O bond of this isoxazolidine could be effected cleanly with Zn/HOAc to give the corresponding amino alcohol, and subsequent oxidation with Dess−Martin reagent led directly to ketone **19**, presumably via the enone intermediate **17**. Tricycle **19** was a single stereoisomer whose configuration and conformation were assigned as shown based upon 2D NMR experiments (NOESY, HMQC, and ^{13}C Inadequate), along with the results of subsequent transformations (vide infra). The formation of the C-2 axial epimer here was not surprising, since stereoelectronic considerations dictate that the conjugate addition of the amino group to the enone in **17** must occur through a transition state leading initially to a boat B-ring ketone **18**, which then ring flips to the tricycle **19**.

Surprisingly, Clemmensen reduction of **19** provided a mixture of the desired deoxygenated tricyclic amine **21**

Scheme 2

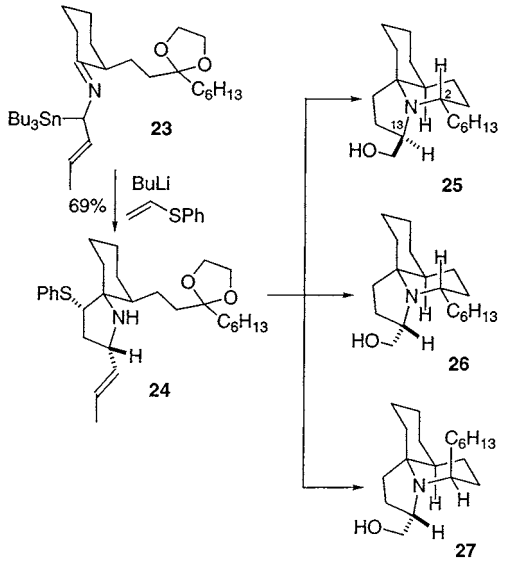


Scheme 3



(6%), along with the alkene **20** as the major product (51%) (Scheme 3). It was possible, however, to stereoselectively hydrogenate the olefin moiety of **20** to produce the requisite saturated intermediate **21**. To confirm the structure and stereochemistry of compound **21**, a single-crystal X-ray analysis was performed on its picrate salt. Interestingly, in the crystal, tricycle **21** has a conformation like that shown in **1b**, as was predicted by molecular modeling. Finally, the phenyl protecting group of **21** was removed by a Birch reduction/acid hydrolysis, providing racemic tricyclic amino alcohol **22**. At this point it became clear via a direct comparison of the proton and carbon NMR spectra of this material, as well as its hydrochloride salt, with those of the natural product kindly supplied by Professor Biard, that the compounds were in fact different. Moreover, there was no indication that our synthetic

Scheme 4

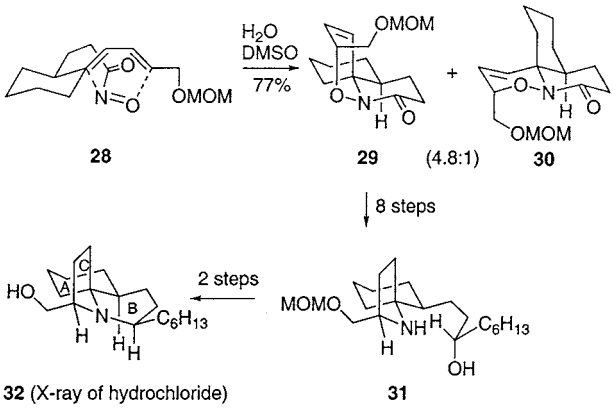


tricycle **22** tends to exist in the zwitterionic form proposed by the Biard group for lepadiformine (cf. **1**). It should also be noted that shortly after our work appeared, the Kibayashi group described a total synthesis of structure **1** using an intramolecular acylnitroso Diels−Alder reaction as the key step and confirmed that it was not identical to lepadiformine.[10a]

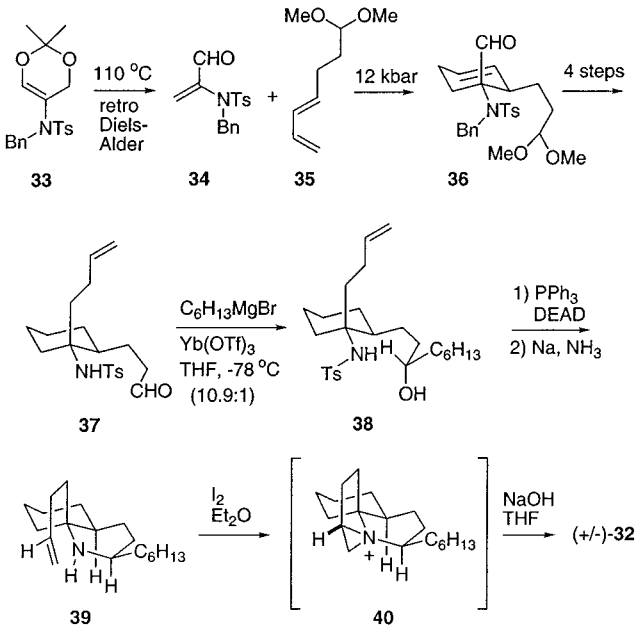
While our research described above was ongoing, the Pearson group was also involved in studies on the synthesis of the proposed lepadiformine structure **1**. The basic strategy to be used in their approach involved construction of an A/C-ring spirocycle via an intermolecular [3+2]-cycloaddition of a 2-azaallyl anion that had been pioneered in the Pearson labs.[9] Thus, imine stannane **23** was prepared and was subsequently treated with *n*-butyllithium in the presence of phenyl vinyl sulfide to afford adduct **24** as a single stereo- and regioisomer in 69% yield (Scheme 4). Unfortunately, spirocycle **24** had the incorrect C-10/C-13 relative stereochemistry to prepare the Biard structure **1**. It was possible, however, to convert intermediate **24** to the bis-epi-diastereomer **25**, as well as stereoisomers **26** and **27**. Significantly, none of these compounds, nor their hydrochlorides, was identical to the natural alkaloid. Thus, Pearson firmly established by synthesis that lepadiformine is not a stereoisomer of **1** at either C-2 or C-13.

At this point we were left with several alternatives with regard to the possible structure of lepadiformine. One was that its atomic connectivity is quite different than that of **1**. Another was that lepadiformine is actually epimeric to **1** at the quaternary carbon (C-10), thereby putting it into the same *trans*-1-azadecalin stereochemical series as fasicularin (**12**), but having the C-ring contracted to form a pyrroloquinoline skeleton (cf. **2** and **8**). Should this latter possibility in fact be the case, the question then still remained as to the configuration at C-2 and C-13. Since we felt there were too many unanswered questions about the alkaloid to proceed, synthetic work in this area was temporarily suspended.

Scheme 5



Scheme 6

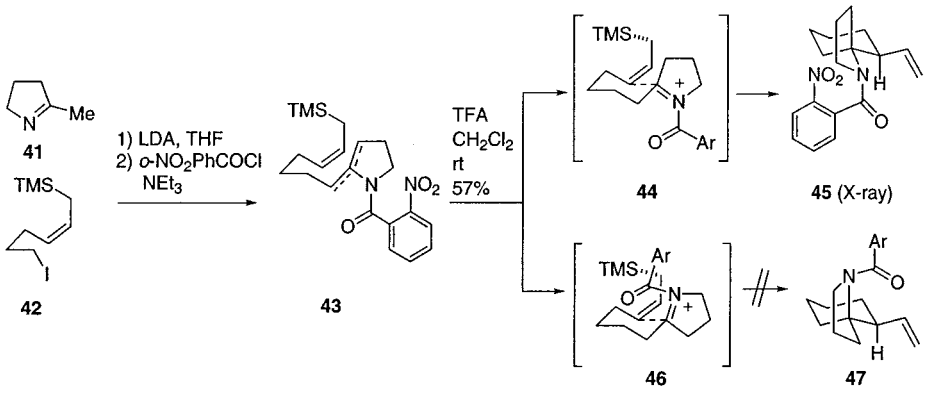


Determination of the Correct Structure of Lepadiformine by Kibayashi via Total Synthesis. In 2000, Kibayashi and co-workers used their intramolecular acylnitroso Diels−Alder strategy, which had previously been applied to prepare the Biard lepadiformine structure **1** (vide supra), to achieve the first total synthesis of fasicularin (**12**).[10b,11] In the course of these studies, some C-2/C-13 stereoisomeric C-ring contracted analogues of **12** which have the *trans*-1-azadecalin A/B-ring substructure were synthesized, with the intent of possibly unravelling the constitution of lepadiformine. Thus, acylnitroso diene **28**, generated by oxidation of the corresponding hydroxamic acid, was found to undergo a stereoselective cycloaddition via the preferred conformation shown to afford a 4.8:1 mixture of tricycles **29** and **30** (Scheme 5). The major cycloadduct **29** could be converted in several steps to amino alcohol **31** and was then stereoselectively cyclized to eventually produce racemic compound **32**. Comparison of the hydrochloride salt of tricyclic amine **32** with natural lepadiformine showed them to be identical. In addition, X-ray analysis of synthetic **32** hydrochloride indicated that the B-ring of the alkaloid exists in a twist boat conformation! Natural lepadiformine is therefore simply the hydrochloride salt of structure **32**, not a zwitterion as first postulated by the Biard group. In retrospect, this result is not at all surprising since lepadiformine was reportedly isolated via evaporation of an HCl extract.[3a] Interestingly, the HCl salt of synthetic racemic **32** is crystalline, whereas natural lepadiformine (also the hydrochloride) is an oil.

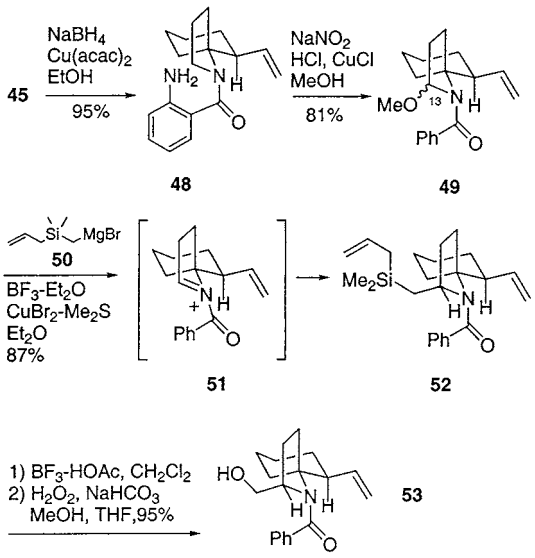
Second Generation Syntheses of Lepadiformine. Funk Approach. Shortly after the appearance of the Kibayashi paper with the revised lepadiformine structure, the Funk group here at Penn State began to investigate an approach to **32** using their novel amidoacrolein-based cycloaddition methodology as a pivotal step.[12] Their synthesis commenced with easily prepared sulfonamido dioxin **33**, which upon thermolysis in refluxing toluene undergoes a facile retro hetero-Diels−Alder reaction to afford amidoacrolein **34** (Scheme 6). This compound was then induced to react with 1,3-diene acetal **35** under high pressure to afford exclusively the endo Diels−Alder adduct **36** in high overall yield. This key reaction serves to set the C-5/C-10 stereochemistry of the alkaloid. Compound **36** was then converted in a few steps to aldehyde **37**. After some experimentation, it was found that hexylmagnesium bromide could be added to this aldehyde stereoselectively in the presence of stoichiometric amounts of ytterbium triflate to afford a 10.9:1 mixture of the desired alcohol **38** along with its epimer. This stereochemical result was rationalized via a chelation-control model involving the sulfonamide, aldehyde, and Grignard reagent. Mitsunobu closure of sulfonamide alcohol **38** with inversion, followed by removal of the sulfonamide moiety then provided tricycle **39**. Annulation of the remaining C-ring could be achieved stereoselectively simply by treating amino alkene **39** first with iodine to probably form aziridinium intermediate **40** and then with base which led directly to racemic lepadiformine (**32**). Interestingly, the regioisomeric ring opening product which would be the result of nucleophilic attack at the more substituted carbon of the aziridinium compound was not produced (cf. cylindricine A/B equilibrium). Thus, this elegant, highly stereoselective total synthesis was achieved in about a dozen steps from readily available dioxin **33**.

Weinreb Synthesis. We also began to investigate a new approach to racemic lepadiformine (**32**) soon after the Kibayashi structure appeared. The plan was to develop a novel stereoselective intramolecular allylsilane/*N*-acyliminium ion spirocyclization strategy for A/C-ring construction, along with the application of our radical-translocation methodology for generation of *N*-acylimines by remote amide oxidation.[13,14] Our synthesis commenced with commercially available 2-methyl-1-pyrroline (**41**), which was metalated and then *C*-alkylated with iodide **42** (Scheme 7). The crude product of this reaction was *N*-acylated with *o*-nitrobenzoyl chloride to afford a mixture of regioisomeric enamides **43**, which without separation was subsequently treated with trifluoroacetic acid. We were quite gratified to find that the desired spirocycle **45** was produced as a single isomer with the requisite C-5/

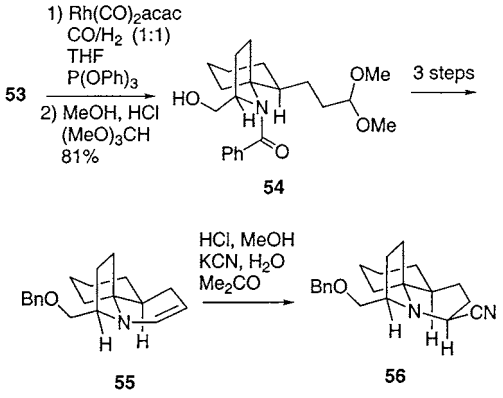
Scheme 7



Scheme 8



Scheme 9



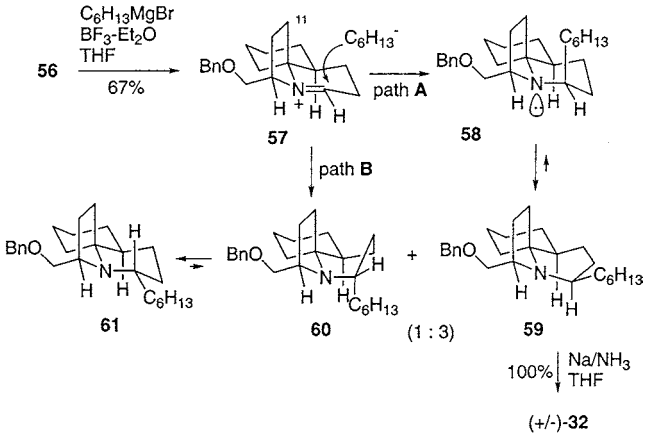
C-10 stereochemistry for lepadiformine. The structure of this cyclization product was firmly established by X-ray crystallography. We believe the spirocycle **45** forms via the preferred *N*-acyliminium ion allylsilane conformation **44**. Alternative conformer **46**, which would afford the isomeric spirocycle **47**, is probably destabilized relative to **44** because of an unfavorable steric interaction between the *N*-*o*-nitrobenzoyl group and the allylsilane moiety.

Using our methodology, we next turned to functionalization of compound **45** at C-13 in order to provide a handle for introducing the hydroxymethyl group of the alkaloid. To effect this transformation, the nitro functionality of **45** was first reduced to the *o*-aminobenzamide **48**, which was then subjected to the general conditions we developed several years ago for conversion of such functionality to an α-methoxybenzamide ($NaNO_2$, HCl, dry MeOH, cat. CuCl) to cleanly produce **49** (Scheme 8).[10] It was then possible to alkylate the acyliminium ion **51**, derived from α-methoxybenzamide **49**, with the cuprate from (allyldimethylsilyl)methylmagnesium bromide (**50**) to afford an 87% yield of a 7:1 mixture of epimeric products, with the desired silane **52** being the major stereoisomer. This transformation presumably occurs via the preferential attack of the cuprate from the least hindered face of the *N*-acyliminium salt **51**. A Tamao oxidation then allowed us to convert silane **52** to the key C-13 hydroxymethyl compound **53**.

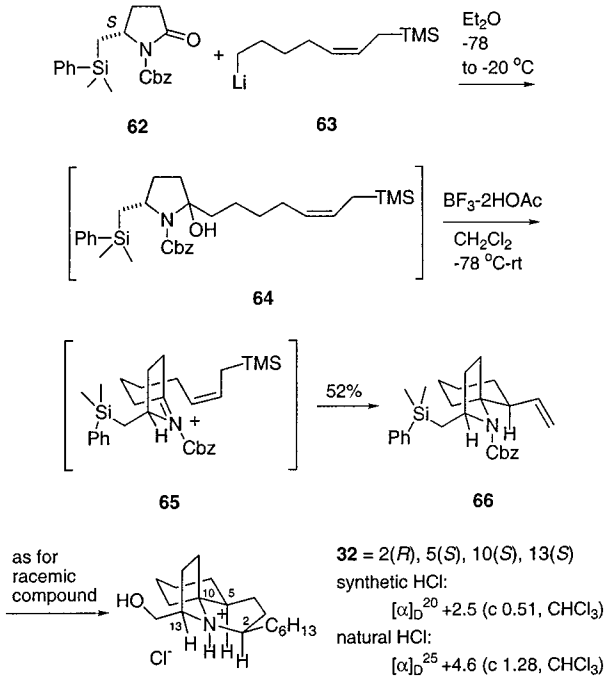
The next phase of the synthesis involved construction of the lepadiformine B-ring, along with introduction of the C-2 hexyl chain. Thus, terminal alkene **53** was first hydroformylated to produce an aldehyde which was converted to the dimethyl acetal **54** (Scheme 9). Basic hydrolysis of the benzoyl group of **54** then provided the corresponding amino alcohol, which was converted to the corresponding amine benzyl ether and then treated with acid to afford the unstable tricyclic enamine **55**. This enamine could be converted to the α-amino nitrile, to which we tentatively assigned the stereostructure **56** which contains a twist boat B-ring.

It was found that we could introduce the C-2 hexyl chain by simply treating the crude amino nitrile **56** with commercially available hexylmagnesium bromide in the presence of boron trifluoride etherate in THF which produced a 3:1 mixture of the desired C-alkylation product **59** along with its C-2 epimer **61** (Scheme 10). Stereoelectronic principles nicely summarized by Stevens many years ago can be used to rationalize the results of this reaction.[15] Thus, anti-periplanar addition of the Grignard reagent to the derived iminium salt **57** from the preferred "axial" direction (path **A**) would generate an initial chair B-ring as in **58**, and this compound would then undergo conformational inversion to the more stable lepadiformine twist boat conformation **59**. Nucleophilic attack on the iminium species **57** from the opposite face (path **B**), however, will initially produce an unfavorable B-ring boat

Scheme 10



Scheme 11



60, which ring flips to the more stable chair conformer **61** now having an equatorial C-2 hexyl group. In simple systems, path **A** is ordinarily preferred to a substantial degree over path **B**.[27,28] However, in the case of iminium ion **57**, perhaps a developing severe 1,3-diaxial interaction between the incoming nucleophile and the bridge carbon (C-11) is responsible for the lower stereoselectivity. To complete the total synthesis, reductive cleavage of the benzyl ether group from compound **59** afforded racemic lepadiformine (**32**). In summary, we were able to achieve a total synthesis of racemic lepadiformine in about fifteen steps starting from commercially available imine **41**.

Assignment of the Absolute Configuration of Lepadiformine by Synthesis. Despite the fact that a considerable amount of work had been done on the cylindricine/lepadiformine/fasicularin group of alkaloids, the absolute configuration of these compounds was not known.[16] Originally, Biard had found that lepadiformine had a sodium D-line rotation of zero, but upon later reexamination a small positive rotation [($[\alpha]_D$ +4.6 (c 1.28, $CHCl_3$)] was observed.[17] Although it seemed rather unlikely that lepadiformine was racemic, we felt that the very small optical rotation, combined with the errors inherent in these measurements, made this at least a possibility. It was decided that the best way to answer the question as to the absolute configuration of the metabolite was by total synthesis of one of the two possible enantiomers of **32**. Our plan was to appropriately modify the spirocyclization strategy used in the racemic series and apply it in a chiral system. We also decided to use a sterically bulky group to block one face of the system in this key cyclization step (vide infra). We chose as a starting material *N*-Cbz lactam **62** which contains a large silylmethyl substituent, which can be easily prepared from *S*-pyroglutamic acid (Scheme 11). Addition of lithium reagent **63** to compound **62** and immediate treatment of unstable intermediate **64** with boron trifluoride acetic acid complex gave spirocycle **66** as a single stereoisomer. This cyclization probably occurs via an *N*-acyliminium ion that has a conformation like that shown in **65** (cf. Scheme 7), where attack by the allylsilane occurs on the face opposite the silylmethyl group. Compound **66** was then converted to lepadiformine using methodology which we developed in the racemic series. Our synthetic alkaloid had a small positive rotation ($[\alpha]_D$ +2.5 (c 0.51, $CHCl_3$)) similar to that of the natural product. To make a more definitive comparison, however, natural lepadiformine supplied by Professor Biard, our synthetic enantiomerically pure lepadiformine, and racemic synthetic material were all converted to the corresponding Mosher esters and analyzed by NMR. It was clear from these results that the natural alkaloid is indeed enantiomerically pure and that it corresponds to the totally synthetic lepadiformine which was prepared from *S*-pyroglutamic acid. Thus, natural lepadiformine has the 2(*R*), 5(*S*), 10(*S*), 13(*S*) configuration. It will be interesting to see if the cylindricines and fasicularin also fall into this same enantiomeric series.

Conclusion

Undoubtedly in the future, the total synthesis of natural products will continue to play a pivotal role in basic discovery in organic chemistry. Furthermore, as highlighted in this Account, total synthesis will also maintain its importance in structure elucidation because, although powerful, physical methods of structure determination do have shortcomings. Not only have frequent misassignments of structure been made, certain stereochemical issues are often difficult or impossible to evaluate by spectral methodology alone.[18] This is not to say that total synthesis should be an obligatory confirmation of structure, but in those cases where a synthesis has been achieved, side-by-side comparison with naturally derived material should be done, if at all possible. This type of review should not only catch errors, but will also provide useful feedback to those doing natural product structure elucidation, thereby making physical methodology even more reliable.

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References

(1) The role of synthesis in modern organic chemistry was nicely articulated by Eschenmoser over a quarter of a century ago, and these comments are still relevant and timely: Eschenmoser, A. On Organic Natural Product Synthesis and Vitamin B_{12}. In *Proc. Robert A. Welch Found.* **1975**, *18*, 269−278.

(2) (a) Li, J.; Burgett, A.; Esser, L.; Amezcua, C.; Harran P. G. Total Synthesis of Nominal Diazonamides: Part 2. On the True Structure and Origin of Natural Isolates. *Angew. Chem., Intl. Ed. Engl.* **2001**, *40*, 4770−4773. See also: Ritter, T.; Carreira, E. M. The Diazonamides: The Plot thickens. *Angew. Chem., Int. Ed. Engl.* **2002**, *41*, 2489−2495. (b) Heintzelman, G. R.; Fang, W.-K.; Keen, S. P.; Wallace, G. A.; Weinreb, S. M. Stereoselective Total Syntheses and Reassignment of Stereochemistry of the Fresh Water Cyanobacterial Hepatotoxins Cylindrospermopsin and 7-Epicylindrospermopsin. *J. Am. Chem. Soc.* **2002**, *124*, 3939. (c) Bernardelli, P.; Moradei, O. M.; Friedrich, D.; Yang, J.; Gallou, F.; Dyck, B. P.; Doskotch, R. W.; Lang, T.; Paquette, L. A. Total Asymmetric Synthesis of the Putative Structure of the Cytotoxic Diterpenoid (−)-Sclerophytin A and of the Authentic Natural Sclerophytins A and B. *J. Am. Chem. Soc.* **2001**, *123*, 9021−9032. (d) Overman, L. E.; Pennington, L. D. Total Synthesis of the Supposed Structure of (−)-Sclerophytin A and an Improved Route to (−)-7-Deacetoxyalcyonin Acetate. *Org. Lett.* **2000**, *2*, 2683−2686. (e) Cohen, F.; Overman, L. E. Enantioselective Total Synthesis of Batzelladine F: Structural Revision and Stereochemical Definition. *J. Am. Chem. Soc.* **2001**, *123*, 10782−10783.

(3) (a) Biard, J. F.; Guyot, S.; Roussakis, C.; Verbist, J. F.; Vercauteren, J.; Weber, J. F.; Boukef, K. Lepadiformine, a New Marine Cytotoxic Alkaloid from *Clavelina lepadiformis* Muller. *Tetrahedron Lett.* **1994**, *35*, 2691−2694. (b) Juge, M.; Grimaud, N.; Biard, J. F.; Sauviat, M. P.; Nabil, M.; Verbist, J. F.; Petit, J. Y. Cardiovascular Effects of Lepadiformine, an Alkaloid Isolated from the Ascidians *Clavelina lepadiformis* (Muller) and *C. moluccensis* (Sluiter). *Toxicon* **2001**, *39*, 1231−1237.

(4) (a) Blackman, A. J.; Li, C.; Hockless, D. C. R.; Skelton, B. W.; White, A. H. Cylindricines A and B, Novel Alkaloids from the Ascidian *Clavelina cylindrica*. *Tetrahedron* **1993**, *49*, 8645−8656. (b) Li, C.; Blackman, A. J. Cylindricines C−G, Perhydropyrrolo[2,1-*j*]quinolin-7-one Alkaloids from the Ascidian *Clavelina cylindrica*. *Aust. J. Chem.* **1994**, *47*, 1355−1361. (c) Li, C.; Blackman, A. J. Cylindricines H−K, Novel Alkaloids from the Ascidian *Clavelina cylindrica*. *Aust. J. Chem.* **1995**, *48*, 955−965.

(5) Patil, A. D.; Freyer, A. J.; Reichwein, R.; Carte, B.; Killmer, L. B.; Faucette, L.; Johnson, R. K.; Faulkner, D. J. Fasicularin, a Novel Tricyclic Alkaloid from the Ascidian *Nephteis fasicularis* with Selective Activity Against a DNA Repair-Deficient Organism. *Tetrahedron Lett.* **1997**, *38*, 363−364.

(6) For other syntheses of cylindricines which appeared during the course of this work, see: (a) Snider, B. B.; Liu, T. Synthesis of (±)-Cylindricines A, D, and E. *J. Org. Chem.* **1997**, *62*, 5630−5633. (b) Molander, G. A.; Ronn, M. Total Synthesis of (−)-Cylindricine C. *J. Org. Chem.* **1999**, *64*, 5183−5187. (c) Liu, J. F.; Heathcock, C. H. Total Synthesis of (±)-Cylindricines A and B. *J. Org. Chem.* **1999**, *64*, 8263−8266.

(7) Werner, K. M.; De los Santos, J. M.; Weinreb, S. M.; Shang, M. A Convergent Stereoselective Synthesis of the Putative Structure of the Marine Alkaloid Lepadiformine via an Intramolecular Nitrone/1,3-Diene Dipolar Cycloaddition. *J. Org. Chem.* **1999**, *64*, 686−687. Werner, K. M.; De los Santos, J. M.; Weinreb, S. M.; Shang, M. An Intramolecular Nitrone-Olefin Dipolar Cycloaddition-Based Approach to Total Synthesis of the Cylindricine and Lepadiformine Marine Alkaloids. *J. Org. Chem.* **1999**, *64*, 4865−4873.

(8) For a prior example of an intramolecular nitrone/conjugated diene cycloaddition, see: Holmes, A. B.; Hughes, A. B.; Smith, A. L.; Williams, S. F. *N*-Alkenyl Nitrone Dipolar Cycloaddition Routes to Piperidines and Indolizidines. Part 3. Approach to the Gephyrotoxin Ring System. *J. Chem. Soc., Perkin Trans. 1* **1992**, 1089−1099. Intermolecular 1,3-dipolar cycloaddition reactions of nitrones with conjugated dienes leading to indolizidines and quinolizidines have also been reported: Tufariello, J. J.; Dyszlewski, A. D. A Nitrone Cycloaddition to a 1,4-Disubstituted-buta-1,3-diene. A Synthesis of 5-(3-Furyl)-8-methyl-octahydroindolizidine. *J. Chem. Soc., Chem. Commun.* **1987**, 1138−1140, and references cited.

(9) (a) Pearson, W. H.; Barta, N. S.; Kampf, J. W. Synthetic Studies on the Perhydropyrrolo[2,1-j]quinoline Marine Alkaloids Lepadiformine and Cylindricine C Using a 2-Azapentadienyl Anion Cycloaddition. Synthesis of 2,13-Diepilepadiformine (or 2-Epi-11-deoxycylindricine C). *Tetrahedron Lett.* **1997**, *38*, 3369−3372. (b) Pearson, W. H.; Ren, Y. Synthetic Studies on Lepadiformine Using the 2-Azaallyl Anion Methodology. *J. Org. Chem.* **1999**, *64*, 688−689.

(10) (a) Abe, H.; Aoyagi, S.; Kibayashi, C. Total Synthesis of the Proposed Structure of Lepadiformine via Intramolecular N-Acylnitroso Diels−Alder Reaction. *Tetrahedron Lett.* **2000**, *41*, 1205−1208. (b) Abe, H.; Aoyagi, S.; Kibayashi, C. First Total Synthesis of the Marine Alkaloids (±)-Fasicularin and (±)-Lepadiformine Based on Stereocontrolled Intramolecular Acylnitroso-Diels−Alder Reaction. *J. Am. Chem. Soc.* **2000**, *122*, 4583−4592.

(11) See also: Maeng, J.-H.; Funk, R. L. Total Synthesis of (±)-Fasicularin via a 2-Amidoacrolein Cycloaddition. *Org. Lett.* **2002**, *4*, 331−333.

(12) Greshock, T. J.; Funk, R. L. Total Synthesis of (±)-Lepadiformine via an Amidoacrolein Cycloaddition. *Org. Lett.* **2001**, *3*, 3511−3514.

(13) (a) Sun, P.; Sun, C.; Weinreb, S. M. A New Total Synthesis of the Marine Tunicate Alkaloid Lepadiformine. *Org. Lett.* **2001**, *3*, 3507−3510. (b) Sun, P.; Sun, C.; Weinreb, S. M. Stereoselective Total Syntheses of the Racemic Form and the Natural Enantiomer of the Marine Alkaloid Lepadiformine via a Novel N−Acyliminium Ion/Allylsilane Spirocyclization Strategy. *J. Org. Chem.* **2002**, *67*, 4337−4345.

(14) (a) Han, G.; McIntosh, M. C.; Weinreb, S. M. A Convenient Synthetic Method for Amide Oxidation. *Tetrahedron Lett.* **1994**, *35*, 5813−5816. (b) Han, G.; LaPorte, M. G.; McIntosh, M. C.; Weinreb, S. M.; Parvez, M. Exploratory Synthetic Studies of the α-Methoxylation of Amides *via* Cuprous Ion-Promoted Decomposition of *o*-Diazobenzamides. *J. Org. Chem.* **1996**, *61*, 9483−9493. Chao, W.; Weinreb, S. M. Radical-Based Methodology for Efficient Generation of Acyclic *N*-Acylimines. *Tetrahedron Lett.* **2000**, *41*, 9199−9204.

(15) Stevens, R. V. Nucleophilic Additions to Tetrahydropyridinium Salts. Applications to Alkaloid Syntheses. *Acc. Chem. Res.* **1984**, *17*, 289. See also: Polniaszek, R. P.; Belmont, S. E. Enantioselective Total Syntheses of Indolizidine Alkaloids 167B and 209D. *J. Org. Chem.* **1990**, *55*, 4688−4693.

(16) An enantioselective total synthesis of (−)-cylindricine C starting from (*S*)−(−)-1,2,4-butanetriol has been reported by Molander.[6b] However, the optical rotation of the natural product has never been determined, and a sample of the alkaloid is not available. Therefore, the absolute configuration of this compound cannot be established at present.

(17) Biard, J. F. Personal communication, 2001.

(18) See for example: Furstner, A.; Radikowski, K.; Wirtz, C.; Goddard, R.; Lehmann, C. W.; Mynott, R. Total Syntheses of the Phytotoxic Lactones Herbarumin I and II and a Synthesis-Based Solution of the Pinolidoxin Puzzle. *J. Am. Chem. Soc.* **2002**, *124*, 7061−7069 and references cited.

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