

Accounts

Total Synthesis of Bioactive Tricyclic Marine Alkaloids, Lepadiformine and Related Compounds

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The first total synthesis of tricyclic marine alkaloids (±)-fasicularin (**2**) and (±)-lepadiformine (**5**) has been accomplished. The key common strategic element for the synthesis is the stereocontrolled intramolecular hetero-Diels–Alder reaction of an *N*-acylnitroso moiety with an exocyclic diene with or without bromine substitution to control the syn-facial or anti-facial selectivity, respectively. This reaction leads to the trans- or cis-fused decahydroquinoline ring systems **13** or **23** involving the simultaneous introduction of the nitrogenated quaternary center in a single step. On further elaboration of the six-membered or five-membered ring A, the trans-fused adduct **13** provided either (±)-fasicularin (**2**) or (±)-lepadiformine (**5**). The hydrochloride salt of synthetic (±)-**5** was found to be identical with the isolated natural sample of lepadiformine, however, the tricyclic amino alcohol **4** that has the proposed structure of lepadiformine in a non-zwitterionic form, derived from the cis-fused adduct **23**, was found to be different from lepadiformine by spectral comparison. These results thus unambiguously established the relative stereochemistry of lepadiformine, formerly assigned incorrectly, as shown by **5**. The synthesis of natural (−)-enantiomer of lepadiformine was then undertaken using a highly efficient protocol involving a new variant of the *N*-acyliminium ion-initiated intramolecular spirocyclization in which a conjugated diene was exploited as a π nucleophile. Thus, the synthesis is accomplished in nine steps with 31.4% overall yield, making this the most selective and shortest synthesis of lepadiformine to date. Direct comparison of chiral HPLC analysis of synthetic (−)-lepadiformine and the natural product allowed to assign the absolute configuration of natural lepadiformine to be 3*R*,5*S*,7a*R*,11a*R*.

Tunicates (ascidians) have been proven to be a particularly rich source of a variety of structurally fascinating and bioactive nitrogen compounds.[1] Since the first members were reported in 1993, 11 cylindricines A–K have been identified from the Tasmanian ascidians *Clavelina cylindrica* as new marine alkaloids[2] with a tricyclic ring system unprecedented among natural products, consisting of the perhydropyrrolo[2,1-*j*]quinoline or the perhydropyrido[2,1-*j*]quinoline. Shortly after the first isolation of cylindricines A (**1a**) and B (**1b**),[2a] Biard and co-workers reported the isolation and structure elucidation of a closely related marine alkaloid, named lepadiformine, from the marine tunicate *Clavelina lepadiformis* collected in the Mediterranean near Tunisia[3a] in 1994 and later from *Clavelina moluccensis* found along the Djibouti coast.[3b] It was found to be moderately cytotoxic toward various tumor cell lines in vitro. Moreover, a recent study indicated that lepadiformine is very active in the cardiovascular system in vivo and in vitro and suggested that it has antiarrhythmic properties.[3b] On the basis of extensive spectral analysis, this alkaloid was assigned the unusual zwitterionic structure **3**. Although its specific rotation value in a chloroform solution was reported to be zero, it is believed that lepadiformine is not racemic (Fig. 1).

In addition to these tricyclic alkaloids, fasicularin (**2**) was discovered in 1997 by Patil and co-workers[4] from the Micronesian ascidian *Nephteis fasicularis*, which has selective activity against a DNA repair-deficient organism and is cytotoxic to Vero cells. The structure and relative stereochemistry of **2** were deduced on the basis of NMR studies, though the absolute configuration is still unknown.

The novel structural features and biological significance of these tricyclic alkaloids represent a promising new class of nitrogen heterocycles biosynthesized by ascidians (such as indolizidines, quinolizidines, and decahydroquinolines), and have attracted the increasing attention of organic chemists during the past several years. Until our project that aimed at the total synthesis of lepadiformine and related tricyclic alkaloids started in 1997, some approaches for the total syntheses of cylindricines A, D, and E (**1a**, **1d**, and **1e**)[5] had been developed, but no synthetic investigations had been reported on lepadiformine or on fasicularin.

Recently, we achieved the first total synthesis of racemic fasicularin and lepadiformine, the latter of which led to a revision of the proposed structure **3** of lepadiformine to **5**.[6] We wish to describe some of the details of our recent investigations in this area. The enantioselective synthesis of (−)-lepadiformine[7] that allowed the establishment of the absolute configuration of the

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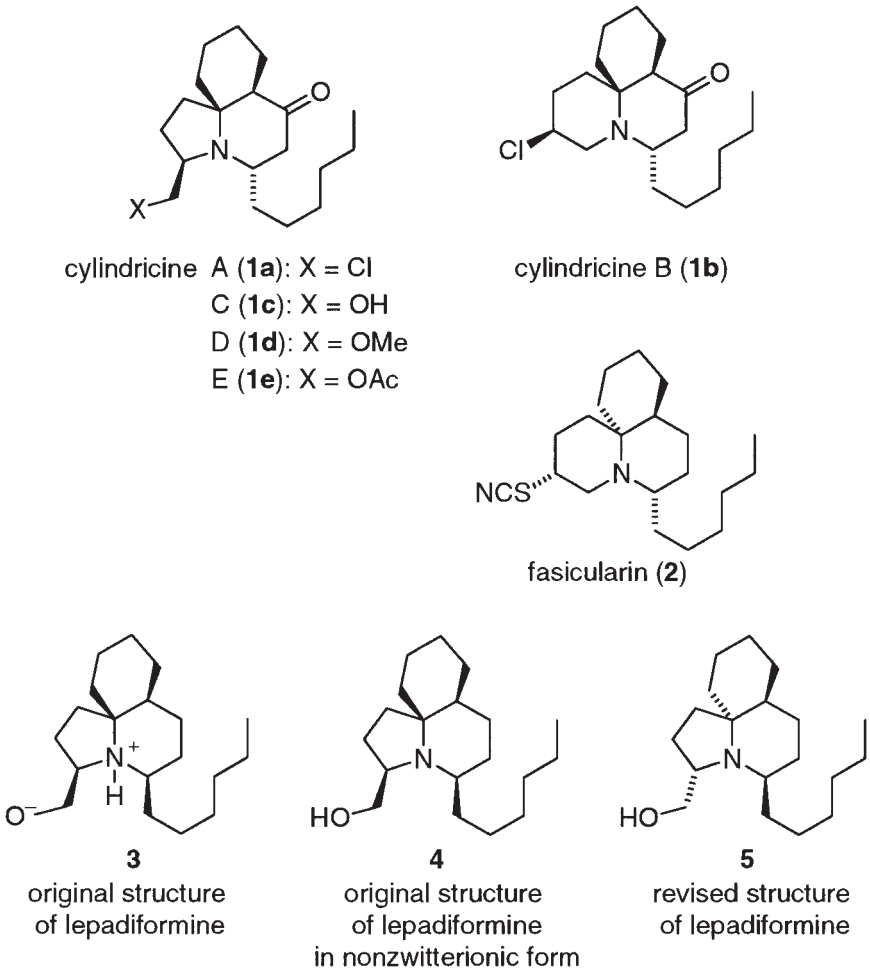


Fig. 1.

natural lepadiformine will also be outlined.

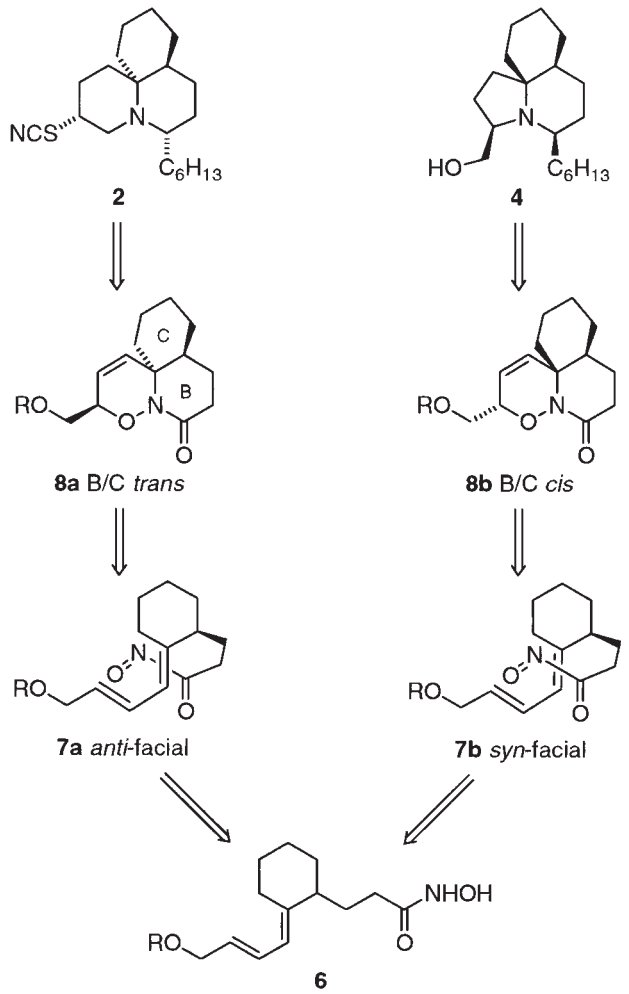
1. Synthetic Design

At the outset of our project, since the above arguments pertaining to the lepadiformine structure had not been published, we planned to synthesize lepadiformine based on **4** having the stereochemistry originally assigned. Also, we became interested in fasicularin (**2**) as a target for synthesis. Since these compounds **2** and **4** comprise a perhydroquinoline ring system, which is a common core unit present in this class of alkaloids, with trans and cis ring fusions, respectively, it presented an opportunity for the diastereoselective construction of the perhydroquinoline skeleton. Accordingly, the route for the synthesis of **2** and **4** we envisaged is shown in Scheme 1. Conceptually, this approach involves as the key feature an intramolecular hetero-Diels–Alder reaction using an *N*-acylnitroso compound **7**, generated from a hydroxamic acid **6**, wherein anti and syn-facial additions are formally possible through endo transition states **7a** and **7b** to form B/C trans- and B/C cis-fused cycloadducts **8a** and **8b**, respectively, which correspond to fasicularin (**2**) and compound **4**.

The hetero-Diels–Alder reaction of *N*-acylnitroso compounds has been widely investigated and has been proved to be useful for natural products synthesis because of its potential for further structural elaboration.[8,9] However, there was only one example of the use of an exocyclic diene as a diene component in the intramolecular acylnitroso-Diels–Alder reaction for the formation of a fused 7/6 ring system.[10] In this case, syn-facial stereochemistry was predominant as a result of the approach of the acylnitroso moiety to the exocyclic diene moiety from the same face to the anchor position of the tether, albeit in low selectivity. These findings suggest that the nitroso–diene intermediate generated from **6** prefers a syn-facial approach via **7b** to form the B/C cis-fused adduct **8b** rather than the B/C trans-fused adduct **8a**. Inspection of molecular models, however, suggests that the anti-facial transition state conformation **7a** leading to trans selectivity is the energetically most favorable among the possible conformations (vide infra).

2. Diastereofacial Intramolecular Hetero-Diels–Alder Reaction of Acylnitroso Compounds

We thus began our synthetic method by examining the cycloaddition of the acylnitroso compound **12** (Scheme 2). Upon oxidation of the hydroxamic acid **11**, prepared from the 2-substituted cyclohexanone **9** in 10 steps, with Pr_4NIO_4 under the conventional nonaqueous conditions using $CHCl_3$ at 0 °C, the in situ generated acylnitroso compound **12** was subjected to intramolecular [4 + 2] cycloaddition to yield the B/C trans-fused and cis-fused tricyclic lactams, **13** and **14**, with low diastereoselection of 2.1:1 in 58% combined yield (see Table 1, entry 1).



Scheme 1.

The use of water as the solvent for Diels–Alder reaction improves rate, yield, and selectivity owing to hydrophobic effect on a reactant encapsulated in a cavity surrounded by a hydrogen-bonding network of water molecules.[11] Indeed, our previous studies revealed that the use of aqueous conditions for intramolecular acylnitroso-Diels–Alder reaction effects significant enhancement of the diastereoselectivity due to the hydrophobic effect.[12] Accordingly, the cycloaddition of **11** was carried out in aqueous media; the results obtained are collected in Table 1 (entries 2–5). As can be seen, employing these aqueous conditions significantly enhanced the desired trans selectivity (4.5:1–4.8:1) as well as the yields (75–84%).

The trans facial preference observed in the cycloaddition of **11** can be rationalized in terms of endo transition states **12**. The syn-facial transition state conformer **12B** leading to the cis-fused adduct **14** would produce repulsive interactions between the ring methylene group at C3 and the nitroso-containing tethering side chain and also between the C5-methylene group and the nitrogen atom (Chart 1). The other possible syn-facial conformer **12C** leading to the cis-fused adduct **14** would be disfavored due to the tethering side chain placed into an axial position. Thus, avoidance of these unfavorable steric interactions should lead to the most favored anti-facial conformer **12A**, in which the tethering side chain is equatorially disposed, affording the trans-fused adduct **13**. The hydrophobic effect in this case may have contributed to stabilizing the more compact endo transition state conformer **12A** rather than **12B** and **12C**, on enhancement of the trans selectivity as well as the yield.

To the best of our knowledge, the predominant formation of the trans-adduct via such an anti-facial transition state with the dienophile approaching from the face opposite to the anchor position of the tether has not been previously recognized in an intramolecular Diels–Alder reaction.[13]

From these results, we expected that the cycloaddition using the nitroso–diene compound with bromine substitution at the diene tether would lead to the preferential formation of the B/C cis-fused adduct **16a**, since a syn-facial transition state **15A** with the tethering 2-alkyl side chain adopting an axial position to avoid the 1,3-allylic strain[14] with the bromine atom is predicted to be energetically more favorable than an anti-facial one **15B** leading to the B/C trans-fused adduct **16b** (Chart 2). We thus next turned our attention to the cycloaddition using the bromine-substituted nitroso–diene compound.

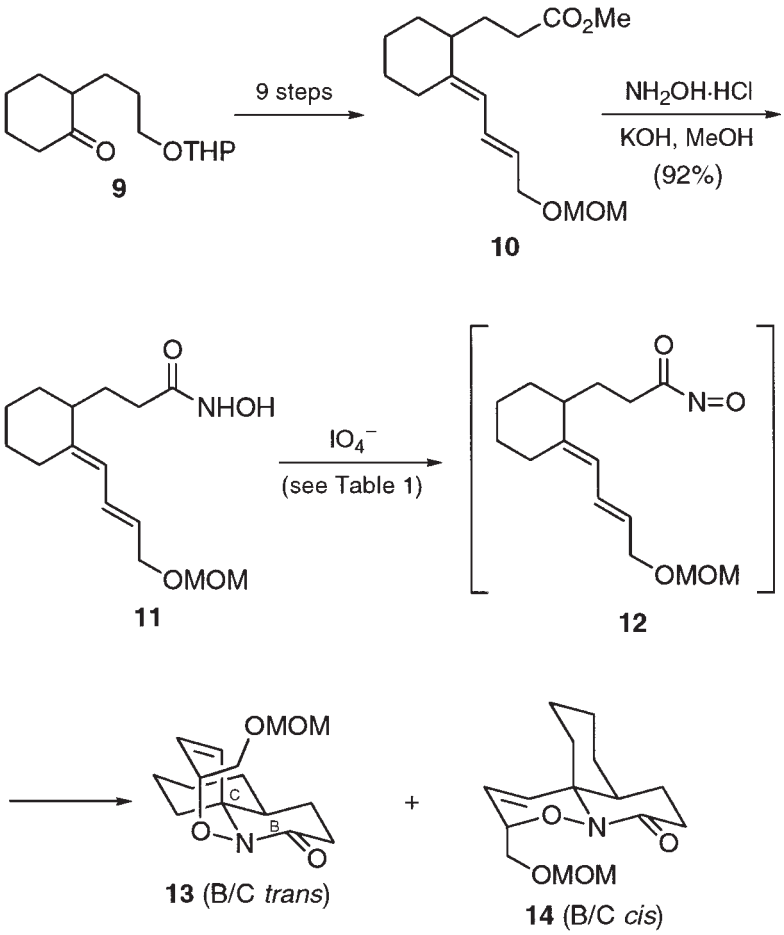
The ketone **17** underwent Horner–Emmons olefination followed by DIBALH reduction to give the unsaturated aldehyde **18**, which was subjected to anti-addition of bromine followed by base-induced dehydrobromination to form the (*Z*)-exocyclic olefin and converted to the bromo diene **19** (Scheme 3). After transformation of **19** to the ester **20** in 5 steps, treatment with hydroxylamine provided the hydroxamic acid **21**.

Exposure of **21** to Pr_4NIO_4 in a $CHCl_3$ solution at room temperature allowed the acylnitroso–diene **22** generated in situ to undergo intramolecular cycloaddition; however, although the TLC analysis revealed that the starting material disappeared within 20 min, the reaction produced only a poor yield (8%) of the cycloadducts **23** and **24** in a 2.6:1 ratio, though the desired cis-fused cycloadduct **23** was predominantly formed as expected. Neither prolonging the reaction time at room temperature nor heating at reflux temperature improved the yield of the cycloadducts; in the latter case, rapid decomposition of the substrate resulted. The poor yield in this cyclization would

Table 1. Intramolecular Diels–Alder Reaction of the *N*-Acyl Nitroso Compound[a)]

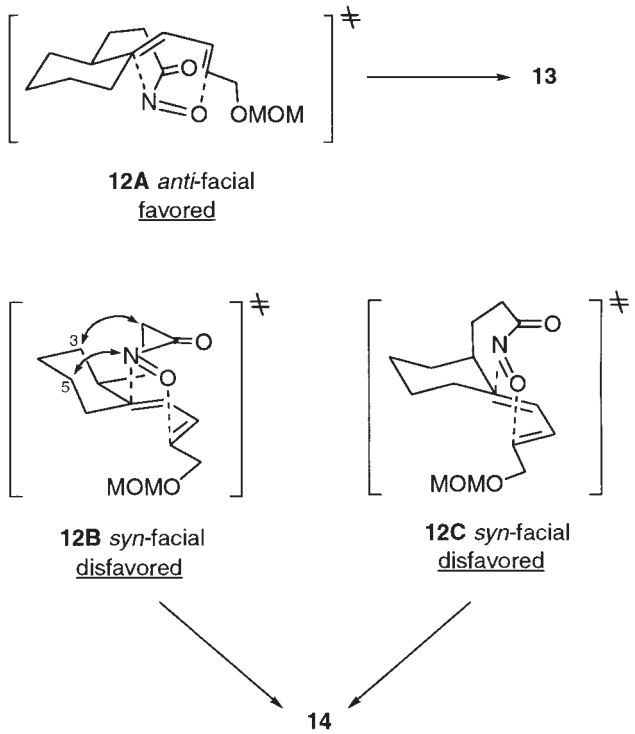
Entry	Periodate	Solvent	**13**:**14**[b)]	Yield/%[c)]
1	Pr_4NIO_4	$CHCl_3$	2.1:1	58
2	Pr_4NIO_4	H_2O–MeOH (5:1)	4.5:1	80
3	Bu_4NIO_4	H_2O–MeOH (5:1)	4.5:1	84
4	Bu_4NIO_4	H_2O–DMF (5:1)	4.7:1	75
5	Bu_4NIO_4	H_2O–DMSO (5:1)	4.8:1	77

a) All reactions were carried out by treatment of the hydroxamic acid **11** with the periodate at 0 °C for 30–45 min. b) The ratios were determined by HPLC analysis. c) Isolated combined yields.



9 **10** **11** **12** **13** (B/C *trans*) **14** (B/C *cis*)

Scheme 2.



12A *anti*-facial favored

12B *syn*-facial disfavored

12C *syn*-facial disfavored

14

Chart 1.

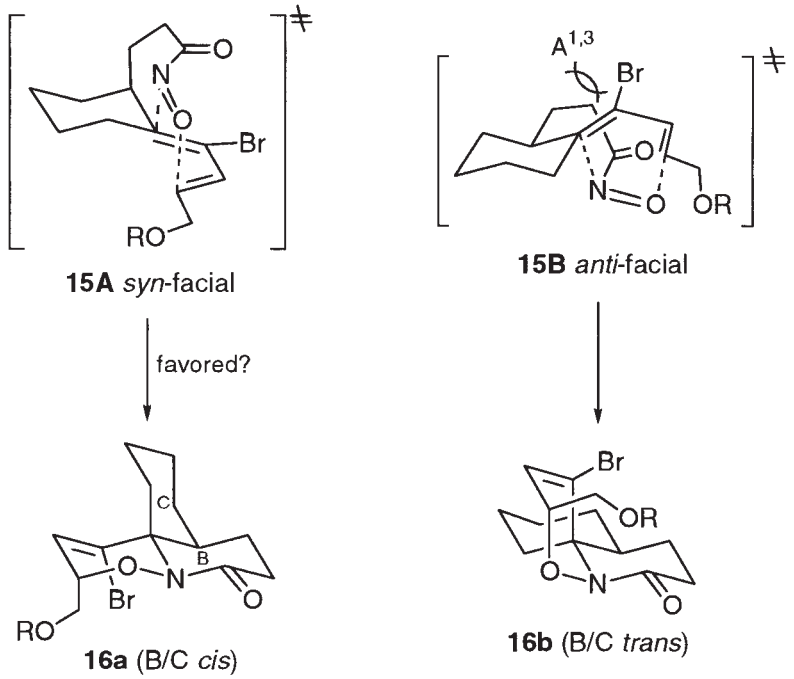
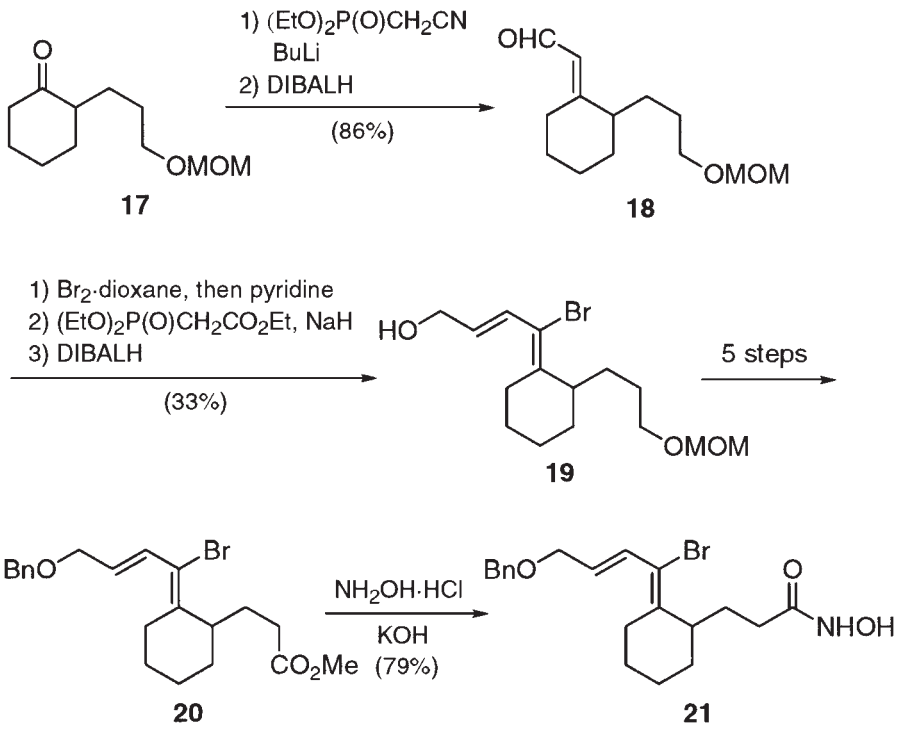
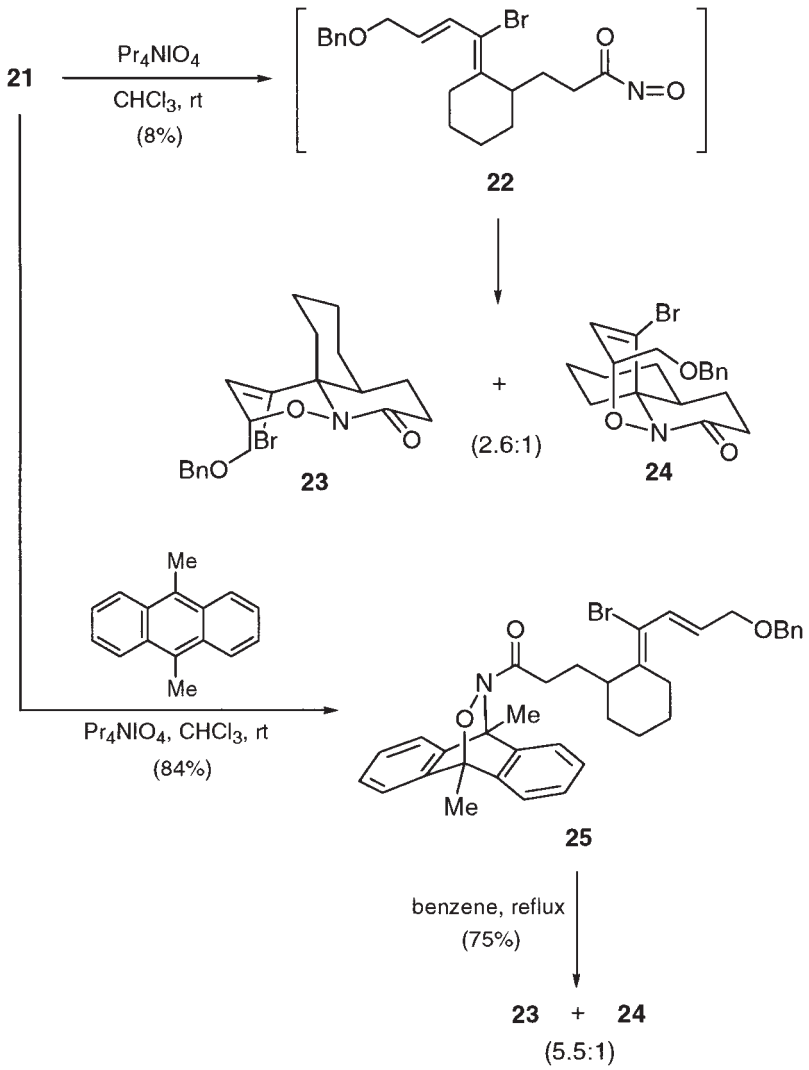


Chart 2.



Scheme 3.

be attributed to a significant decrease of the reactivity of the acylnitroso compound **22** due to the attachment of an electron-withdrawing bromine atom to the diene moiety, which would lead to decomposition of the in situ generated **22** with properties associated with the RCO–N=O species, namely, they are extremely labile and short-lived.[9a] To overcome these problems including the inherent disadvantage of the acylnitroso compound, we sought to utilize the 9,10-dimethylanthracene adduct **25**, which was considered to be a stable acylnitroso equivalent.[15] Thus, upon exposure of **21** to the same oxidation conditions (Pr_4NIO_4, $CHCl_3$, room temperature) in the presence of 9,10-dimethylanthracene, intermolecular cycloaddition reaction smoothly proceeded to form the adduct **25** in 84% yield (Scheme 4). Thermolysis of **25** in refluxing benzene caused a retro Diels–Alder reaction to regenerate the intermediate acylnitroso–diene **22**, which immediately underwent intra-



Scheme 4.

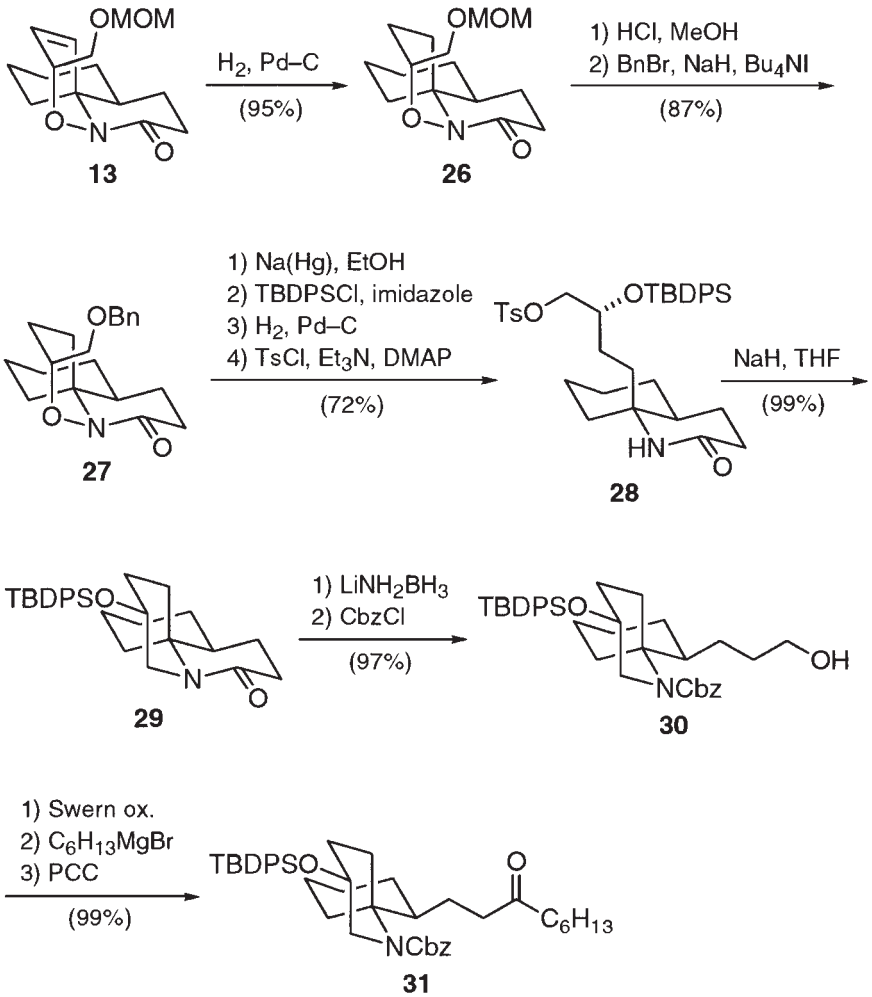
molecular cycloaddition under the reaction conditions. It afforded the cycloadducts **23** and **24** in 75% yield and in a 5.5:1 ratio favoring the B/C cis-fused adduct **23** in contrast to the facial selectivity (anti) observed in the cycloaddition with the non-brominated acylnitroso–diene **12** described above.

3. Synthesis of (±)-Fasicularin

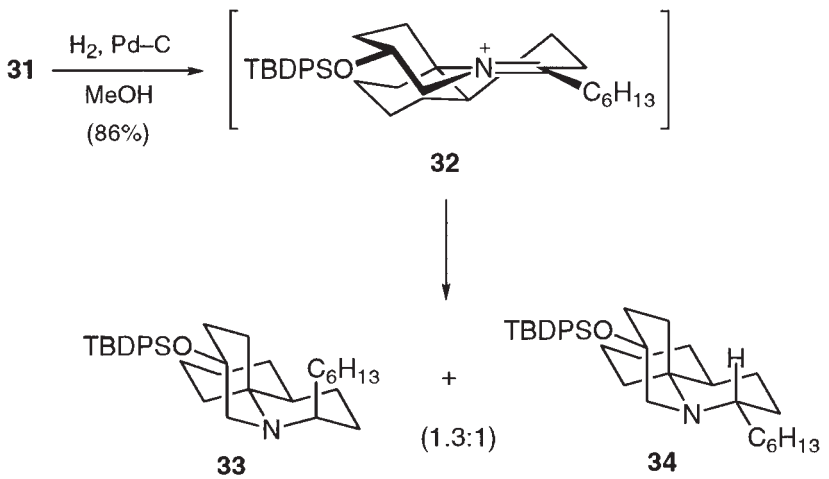
Having obtained the desired trans-fused cycloadduct **13** through the face-selective anti-addition described above, we initially focused our efforts on the total synthesis of fasicularin (**2**). After catalytic hydrogenation of the double bond of **13**, the hydroxy-protecting group was changed from MOM to benzyl to give **27**, which was subjected to N–O bond cleavage by treatment with sodium amalgam and converted to the tosylate **28** (Scheme 5). Cyclization of **28** to the tricyclic lactam **29** was accomplished using sodium hydride in THF at reflux to give an essentially quantitative yield. Since attempts to introduce the hexyl side chain into the lactam ring in **29** were unsuccessful, ring-opening of the lactone ring was deemed necessary for the attachment of the hexyl side chain. Thus, **29** was exposed to $LiNH_2BH_3$, prepared from $BH_3 \cdot NH_3$ and BuLi,[16] leading to reductive ring-opening,[17] and it underwent subsequent N-protection to yield the alcohol **30**. Swern oxidation of **30** followed by sequential addition of the hexyl Grignard reagent and PCC oxidation provided the ketone **31**.

Palladium-catalyzed hydrogenation of **31** resulted in ring closing via hydrogenolytic cleavage of the Cbz group followed by hydrogenation of the in situ generated iminium ion **32** to form the tricyclic products **33** and **34** in slight preference for the undesired isomer **33** (1.3:1, 86% combined yield) with the configuration at the hexyl group inconsistent with that of fasicularin (Scheme 6).

The formation of the desired isomer **34** was thus found to be less favored probably due to the steric hindrance in an intermediary iminium ion **32** that disturbs hydrogen delivery from the sterically congested β face. To circumvent this problem associated with the face selectivity of the hydrogenation, we envisaged the use of a substrate bearing a hydroxy group to be bound to the catalytic surface during hydrogenation so as to enforce the addition of hydrogen from the sterically hindered β face.[18] For this purpose, the silyl protecting group in **31** was removed and subsequent inversion of configuration at the secondary alcohol center in **35** using the Mitsunobu procedure[19] led to the epimerized alcohol **36** (Scheme 7). We first exam-

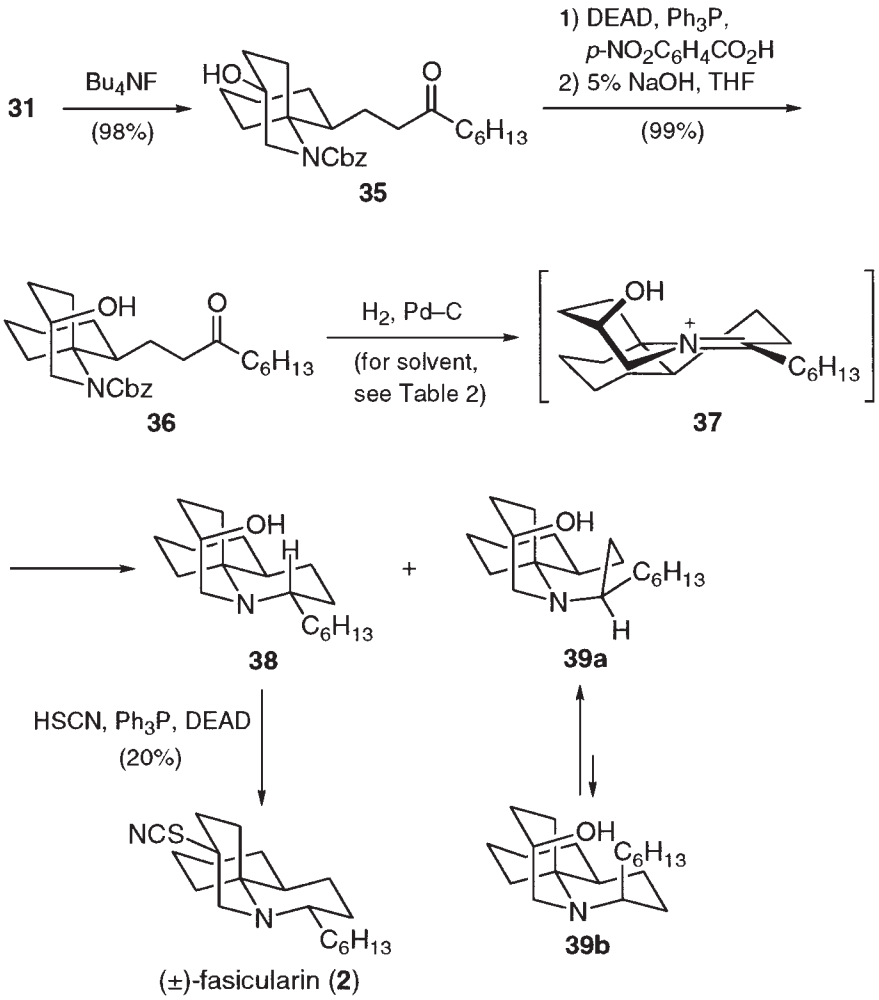


Scheme 5.



Scheme 6.

ined the reductive cyclization of **36** in ethanol, which proceeded under the hydrogenolytic conditions with palladium on carbon to provide a 1:1.7 mixture of the tricyclic products **38** and **39** in 63% combined yield favoring the undesired 6β-hexyl isomer **39** (Table 2, entry 1). The use of ethyl acetate as a solvent resulted in the preferential formation of **38** as well with a slight decrease of the **38**/**39** ratio (1:1.3) (entry 2). The results indicate that the use of a polar solvent does not lead to the face selectivity of the hydrogenation in the desired sense, presumably due to the competitive association of the solvent molecule with the metal surface, which diminishes the directing effect of the hydroxy group in the iminium intermediate **37**. We envisaged



Scheme 7.

Table 2. Cyclization of **36** via Intramolecular Reductive Amination

Entry	Solvent	**38**:**39**[a)]	Yield/%[b)]
1	EtOH	1:1.7	63
2	AcOEt	1:1.3	66
3	benzene	3.4:1	65
4	cyclohexane	5.2:1	62

a) The ratios were based on the isolated products. b) Isolated combined yield.

the hydrocarbons as nonpolar solvents, which do not compete for binding sites of the catalytic surface, thus enforcing the hydroxy group–catalyst association and thereby favoring formation of the desired 6α-hexyl isomer **38**.[20] Therefore, the catalytic hydrogenation of **36** was carried out using benzene and cyclohexane, whereby the stereochemical outcome of the cyclization was found to be reversed as expected, affording the desired 6α-hexyl isomer **38** that predominated with ratios of 3.4:1 and 5.2:1, respectively, over its 6-epi isomer **39** (entries 3, 4).

The 6α-hexyl side chain occupying the equatorial position postulated for **38** was irrevocably confirmed by the X-ray crystal structure analysis (Fig. 2). We expected that the 6β-hexyl isomer **39** would adopt the conformation **39a** in a twist-boat

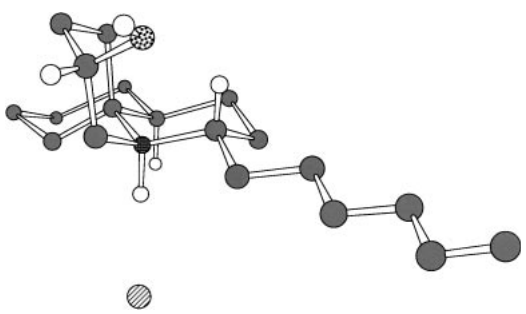


Fig. 2. The X-ray structure (Chem3D representation) of **38**·HCl.

form for ring B leaving the hexyl group in the equatorial position to avoid severe steric interaction between the β-hexyl group and ring A bearing the β-hydroxy group present in the chair conformation **39b**. This is in agreement with molecular mechanics (MM3) calculations (Molecular Mechanics, Version 4.0, CAChe system, Oxford Molecular Group, Inc.), which show that the twist-boat conformation **39a** is ca. 2.0 kcal/mol more stable than the chair conformation **39b**.

For the final thiocyanation step, we expected to introduce the thiocyanato group with the correct stereochemistry via a nucleophilic displacement reaction of the mesylate of **38** in an S_N2

fashion. However, all attempts using KSCN under various conditions gave only elimination and decomposition products. To circumvent this problem in this thiocyanation strategy, an alternative route to convert **38** directly into (±)-fasicularin (**2**) was next sought. Treatment of **38** with the isothiocyanatophosphonium salt[21] resulted in no reaction (at −45 °C to room temperature) or in formation of a small amount of the isothiocyanate (at 60 °C); however, a Mitsunobu condensation (Ph_3P, DEAD, benzene)[22] with thiocyanic acid proceeded with complete inversion of configuration at the reaction center to provide (±)-fasicularin (**2**), albeit in low yield (20%) and with concomitant formation of an elimination product (54%) and an isothiocyanate (2%). The synthetic material of (±)-**2** so obtained showed ^{1}H and ^{13}C NMR spectra in full agreement with those of natural fasicularin, which verified the structure and relative stereochemistry proposed in the literature[4] for the natural product.

4. Synthesis of the Putative Structure of Lepadiformine

The next object of our research was the synthesis of the putative structure **4** of lepadiformine in the nonzwitterionic form utilizing the above-described B/C cis-fused cycloadduct **23** including a small amount of the trans-fused isomer **24**, which were obtained as an inseparable 5.5:1 diastereomeric mixture. Reduction of the double bond, debenzylation, and debromination of this mixture were accomplished by hydrogenation over palladium on carbon in the presence of Et_3N in a single operation, affording the cis-fused isomer after chromatographic separation. Subsequent reductive N–O bond cleavage using sodium amalgam gave the 1,2-diol **40**, which was converted to the epoxide **41** via selective mesylation of the primary alcohol function followed by alkaline treatment (Scheme 8). Treatment of **41** with sodium hydride in refluxing THF caused selective intramolecular 5-exo-epoxy ring-opening to form the tricyclic lactam in preference to the 6-endo-epoxy ring-opening. After protection of the hydroxy group as the MOM ether, reductive lactam ring-opening of **42** was effected using $LiNH_2BH_3$, leading to the azaspiro alcohol which underwent N-protection to give **43**. Swern oxidation of **43** and subsequent addition of hexylmagnesium bromide followed by PCC oxidation afforded the ketone **44**. Cyclization of **44** could be successfully performed via intramolecular reductive amination under catalytic hydrogenation conditions to produce **46** as a single isomer. The preferential formation of this diastereomer can be explained by invoking an iminium intermediate **45** in which hydrogenation should occur on the less hindered α face.

Finally, removal of the MOM protecting group from **46** under methanolic HCl conditions and subsequent saturated Na_2CO_3 workup provided the tricyclic amino alcohol **4**. It possessed the stereostructure proposed for lepadiformine, which was found to exist in a non-zwitterionic form as shown rather than the proposed zwitterionic structure **3**. The spectral properties (^{1}H and ^{13}C NMR) for both the free base and the hydrochloride salt of the synthetic material, however, were clearly different from those reported[3a] for natural lepadiformine.

5. Total Synthesis of (±)-Lepadiformine

While our work was in progress, Weinreb et al.[23] reported the synthesis of the putative structure **4** of lepadiformine and found their synthetic material to be different from natural lepadiformine. At the same time, Pearson et al.[24] described the synthesis of a series of the tricyclic amino alcohols **47**–**49** constituting the *cis*-perhydroquinoline ring system which corresponds to three of the four possible diastereoisomers of **4** at C3 and C5 (Fig. 3); however, none of these compounds was found to be compatible with lepadiformine. These results called into question the validity of the published structure of lepadiformine and also ruled out the possibility that lepadiformine consists of the *cis*-perhydroquinoline ring system, suggesting that it must constitute the trans-fused perhydroquinoline ring system as in fasicularin. Furthermore, the cis relationship between the C3-hydroxymethyl group and the C11 methylene group has previously been defined by NOESY correlation.[3a] Considering these findings and the NMR spectral evidence, two structures **50** and **5** both including the trans-fused decahydroquinoline framework can be reasonably proposed for lepadiformine (Fig. 4).

It was envisioned that these compounds **50** and **5** could be constructed by utilizing the above-described tricyclic lactam **26**, the intermediate for the synthesis of fasicularin, as the starting material having the trans-fused octahydroquinolinone unit. To investigate this approach, **26** was subjected to reductive cleavage of the N–O bond followed by mesylation to give the mesylate **52**, which upon treatment with *t*-BuOK furnished the tricyclic lactam **53** (Scheme 9). Reductive lactam ring-opening of **53** using $LiNH_2BH_3$ followed by N-protection

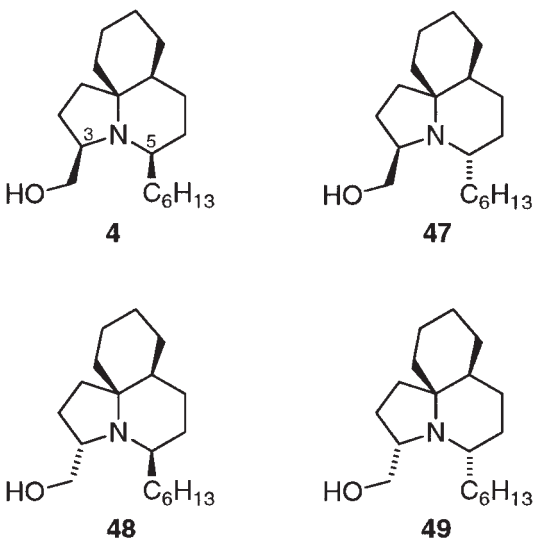


Fig. 3. The *cis*-perhydroquinoline series of the tricyclic amino alcohols.

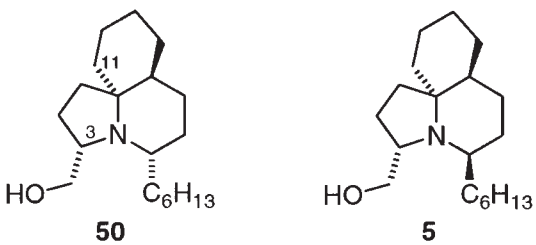
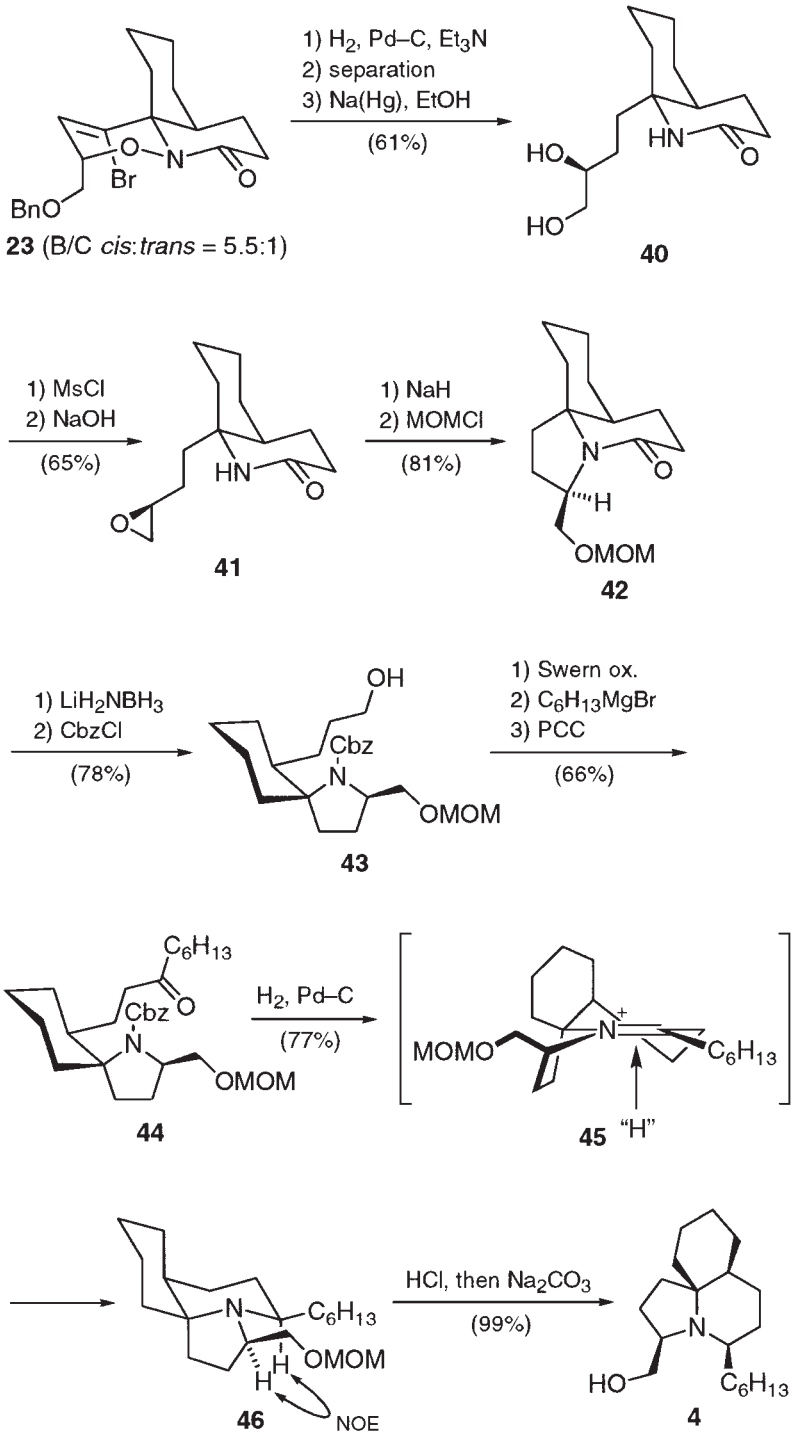


Fig. 4. The *trans*-perhydroquinoline series of the tricyclic amino alcohols with the C3-hydroxymethyl and the C11-ring methylene groups in the cis relationship.

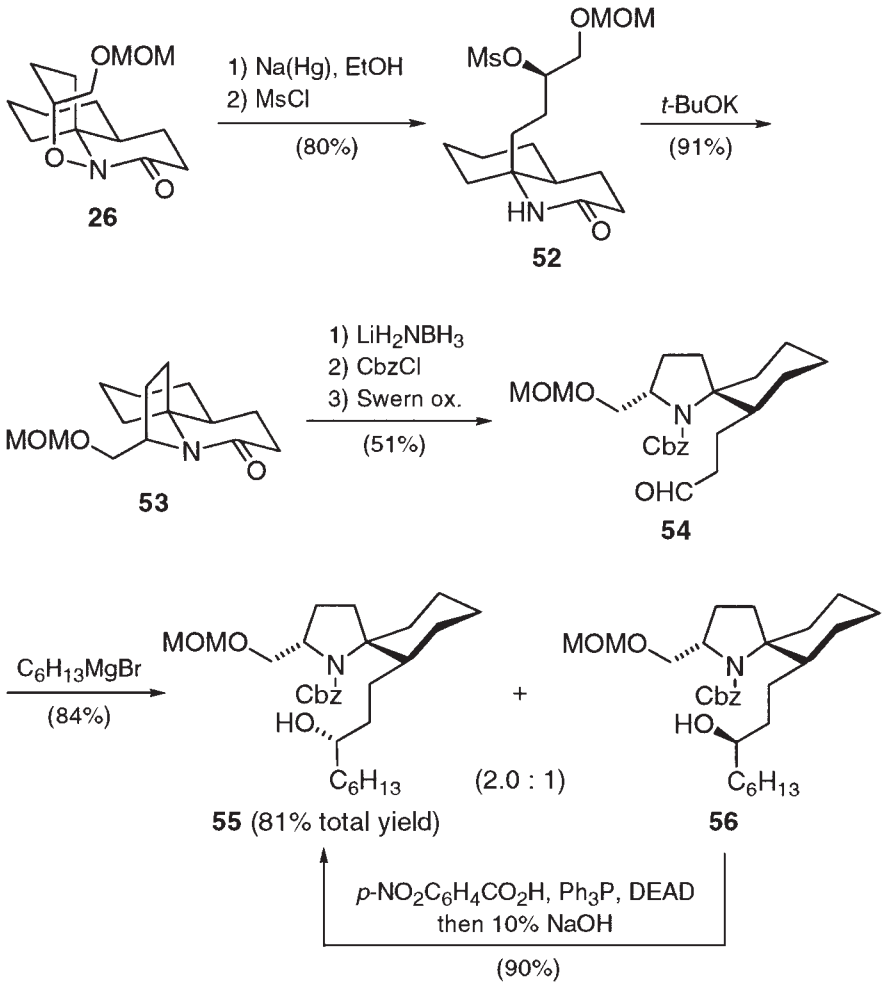


Scheme 8.

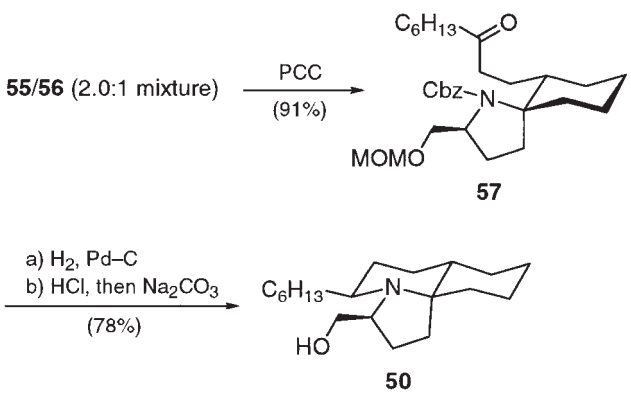
and Swern oxidation provided the azaspirocyclic aldehyde **54**. Addition of the hexyl Grignard reagent to **54** afforded the epimeric secondary alcohols **55** and **56** with a 2.0:1 ratio favoring the α-isomer **55** in 84% combined yield. The minor β-isomer **56** could be converted to the α-isomer **55** by inversion of the hydroxy configuration using the Mitsunobu procedure in 90% yield; thus, the total yield of **55** from the aldehyde **54** was 81%.

After PCC oxidation of the 2.0:1 epimeric mixture of the alcohols **55** and **56** described above, the resulting ketone **57** underwent ring closure upon catalytic hydrogenation to form the tricyclic compound. Subsequent removal of the MOM protecting group under the standard acidic conditions afforded the tricyclic amino alcohol **50** (Scheme 10). The spectra (^{1}H and ^{13}C NMR) of both the free base and the hydrochloride salt of **50** were found to be different from those reported[3] for natural lepadiformine.

Our next concern was the synthesis of another target **5** by utilizing the α-alcohol **55**. Thus, after hydrogenolytic removal of the Cbz group, the resulting amino alcohol **58** was exposed to CBr_4 and Ph_3P. This led to smooth dehydrocyclization[25] with



Scheme 9.



Scheme 10.

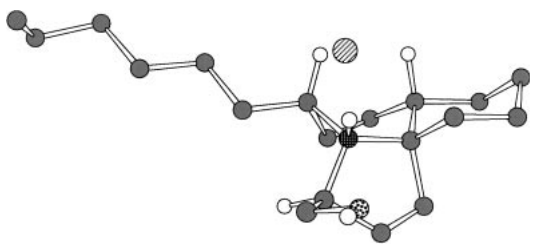
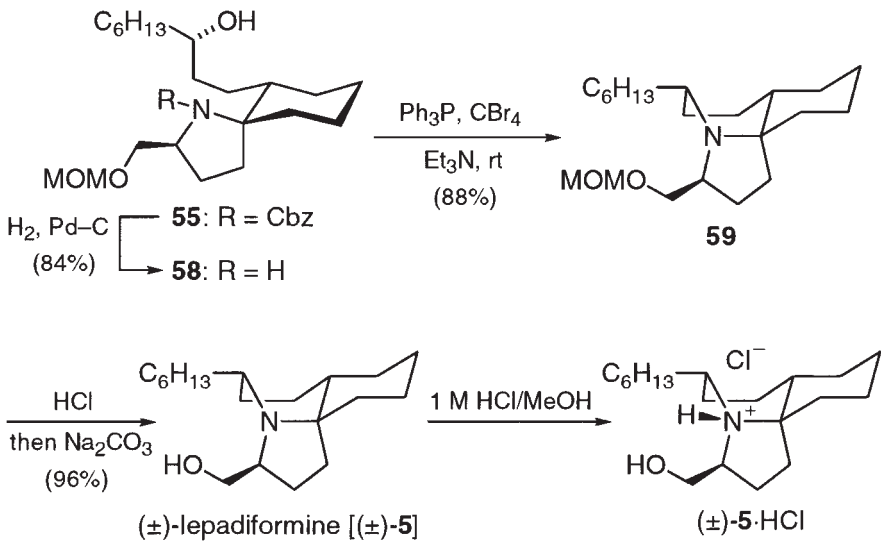
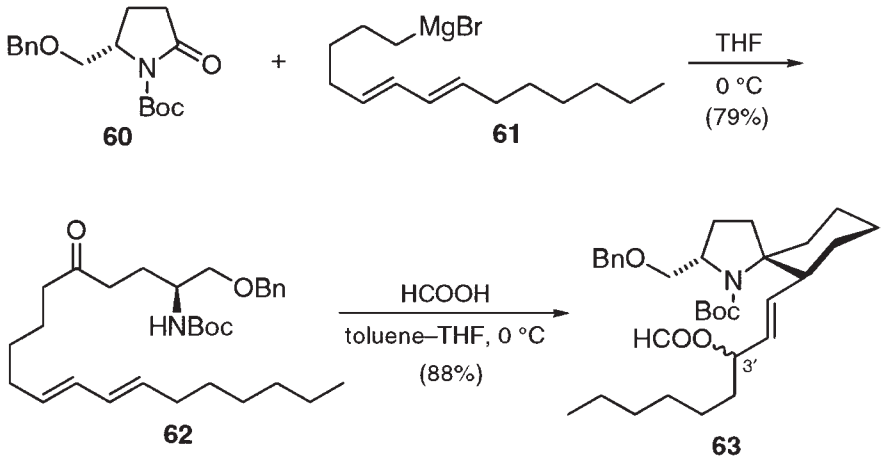


Fig. 5. The X-ray structure (Chem3D representation) of synthetic (±)-lepadiformine hydrochloride [(±)-**5**·HCl].

complete inversion of the configuration at C3′ to form **59** (Scheme 11). Deprotection of the MOM protecting group with concentrated HCl in methanol and subsequent basic treatment provided (±)-**5** as an oil. Further treatment of this material with methanolic HCl, followed by evaporation of the solvent, resulted in the hydrochloride salt of (±)-**5** as a solid. This provided single crystals from recrystallization in ether, thus allowing structural assignment to be unambiguously secured by X-ray analysis, which revealed the stereochemistry of (±)-**5**·HCl with the B ring in a somewhat unusual boat (twist-boat) form with the preferred adoption of an equatorial orientation of hexyl side chain (Fig. 5). Although both ^{1}H and ^{13}C NMR spectral data for synthetic (±)-**5** as the free base were distinctly different from those published[3a] for natural lepadiformine, measurement of the ^{1}H and ^{13}C NMR spectra of the synthetic hydrochloride salt (±)-**5**·HCl allowed direct comparison with the spectra on natural lepadiformine kindly provided by Professor Biard, revealing an exact match. This finding strongly implies that the structure of natural lepadiformine reported in the literature[3a] was actually that of the hydrochloride salt; this is understandable because the isolation of the natural alkaloid was made by evaporation of an acidic solution (MeOH–1 M HCl,



Scheme 11.



Scheme 12.

99:1) of the chromatography fraction. These results therefore clearly indicate that structural formula **3** involving the zwitterionic structure originally assigned to natural lepadiformine should be revised to **5** as shown.

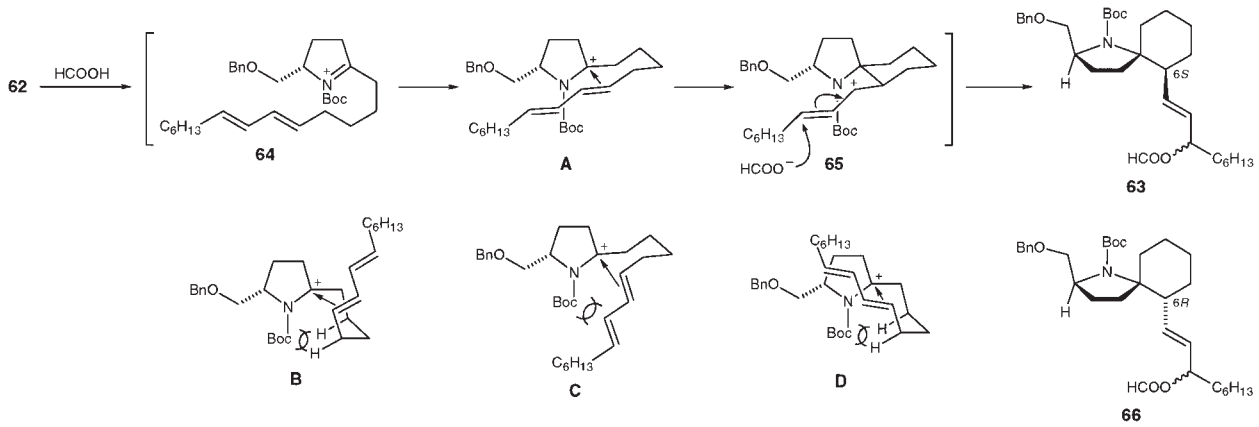
6. Total Synthesis of (−)-Lepadiformine

After the above establishment of the relative stereochemistry of lepadiformine, two syntheses of racemic lepadiformine were reported by Weinreb[26] and Funk[27] based on spirocyclization of an allylsilane–*N*-acyliminium ion and amidoacrolein-derived Diels–Alder reaction, respectively. However, because the natural product is not crystalline and its crystalline derivatives could not be prepared, efforts to obtain an X-ray structure of natural lepadiformine for the determination of the absolute configuration have so far been unsuccessful.[28] This prompted us to undertake the enantioselective synthesis of lepadiformine and to determine the absolute configuration of the natural product.

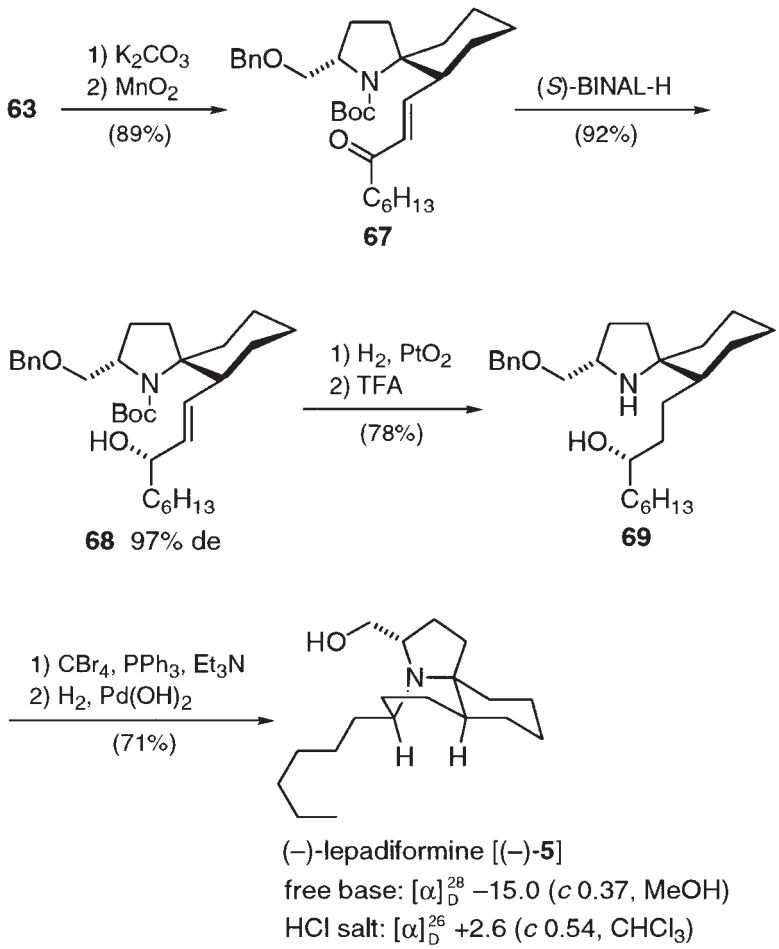
A crucial element in our approach to the target compound was the *N*-acyliminium ion-initiated olefin cyclization to elaborate the azaspirocyclic core of lepadiformine (**5**). Such cyclizations, which lead to spirocyclic compounds, were initially developed by Speckamp et al.[29] and Evans et al,[30] and were recently applied successfully in Weinreb's synthesis[26] of (±)-**5**. Described herein is a new variant of the *N*-acyliminium-ion-initiated intramolecular spirocyclization in which a conjugated diene was exploited as a π nucleophile; the method has been proved to be quite effective for the highly stereoselective and extremely short approach to **5**.

Our synthesis commenced with the known (*S*)-*N*-Boc-2-pyrrolidinone **60**[31] which upon treatment with the (5*E*,7*E*)-tetradeca-5,7-dienyl Grignard reagent **61** underwent selective attack at the endocyclic (ring) carbonyl group[32] to yield the ketone **62** (Scheme 12). When a solution of **62** in toluene–THF (95:5) was treated with formic acid at 0 °C for 2 h and then neutralized with aqueous NH_3, in situ generation of the *N*-acyliminium ion followed by spirocyclization proceeded with synchronous formation of the new C–O bond at C3′ to furnish the 1-azaspirocyclic formate ester **63** in 88% yield. Notably, the spirocyclization of the *N*-acyliminium ion generated from **62**, which bears a conjugated diene, proceeded quite smoothly and was completed in a short time, in marked contrast to the case with the reported spirocyclization of *N*-acyliminium ions that bear nonconjugated olefins which requires a long reaction time.[29,30]

The 6-*exo*-*trig* ring closure[31] gave **63** with complete regio-



Scheme 13.



Scheme 14.

control, as predicted by considering the initially formed carbocation, which is stabilized through resonance with the olefin π-bond in the alkene side chain. In this manner, the nucleophilic attack of the olefin moiety occurred exclusively at the sterically less hindered β face (opposite the 5-benzyloxymethyl group) of the 1-pyrroline ring, with exclusive introduction of the desired (6*S*)-chirality. The concomitant formate substitution at C3′ occurred with low diastereoselectivity (1.6:1 favoring the 3′β-formate) as determined by HPLC analysis (vide infra).

Exclusive preferential formation of the (6*S*)-isomer **63** can be understood by comparison of the stabilities of the configuration of the six-membered chairlike π-complexes **A**–**D**, which arise from the in situ generation of the *N*-acyliminium ion **64** as shown in Scheme 13. In the transition states **C** and **D**, which lead to the (6*R*)-isomer **66**, the *N*-Boc group displays unfavorable nonbonded interactions with the axially oriented diene moiety and the 1,3-diaxial hydrogens of the newly formed cyclohexane ring, respectively. The latter steric interaction be-

tween the *N*-Boc group and the 1,3-diaxial hydrogens is also present in the transition state **B**, which leads to the (6*S*)-isomer **63**. Neither of these interactions is present in the transition state **A**; therefore, of the four possible chairlike transition states **A**–**D**, **A** is the least disfavored and leads to the observed (6*S*)-product **63**.

The formate ester **63**, which is epimeric at C3′, thus obtained underwent basic hydrolysis and then MnO_2 oxidation to form the α,β-conjugated ketone **67** (Scheme 14). Reduction of **67** to the corresponding alcohol with $BH_3 \cdot THF$ (THF, 0 °C) was almost nonstereoselective (**68**/C3′-epimer 1.15:1). (*S*)-BINAL-H diastereoselective reduction[32] provided the (3′*S*)-alcohol **68** in 92% yield with 97% de, which then underwent hydrogenation over PtO_2 in ethyl acetate followed by removal of the Boc protecting group. The resultant amino alcohol **69** was subjected to cyclodehydration using CBr_4 and PPh_3 with complete inversion of the configuration at C3′ and then hydrogenolytic removal of the benzyl protecting group to furnish lepadiformine (**5**), whose spectral properties were identical in all respects with those of an authentic sample of racemic lepadiformine (±)-**5** previously prepared by us. The optical rotation of synthetic alkaloid **5** was measured: $[\alpha]_D^{28}$ −15.0 (*c* 0.37, MeOH) for the free base (oil) and $[\alpha]_D^{26}$ +2.6 (*c* 0.54, $CHCl_3$) for the hydrochloride salt (colorless gum). However, it was not possible to determine the absolute stereochemistry of natural lepadiformine by comparison of the optical rotations, since, as described above, the natural product (actually the hydrochloride salt) had been reported to have no optical rotation in chloroform.

The absolute stereochemistry was assigned successfully by comparison of synthetic and natural lepadiformine on a chiral HPLC column. In the chiral HPLC analysis, the authentic sample of racemic lepadiformine (±)-**5** gave peaks at different retention times [(+)-**5**, 45 min; (−)-**5**, 48 min] (Fig. 6). Comparison of retention time and co-injection revealed that the synthetic (−)-enantiomer corresponds to the natural product, thus allowing the absolute stereostructure of natural lepadiformine to be assigned as 3*S*,5*R*,7a*S*,11a*S*.

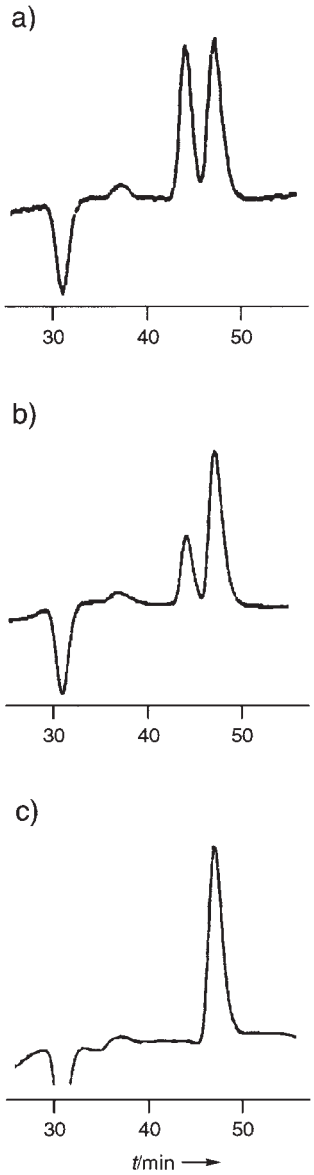


Fig. 6. HPLC chromatogram of lepadiformine on a Daicel Chiralpak OD: a) racemate; b) mixture of racemate and natural; c) mixture of synthetic (−)-enantiomer and natural. Conditions: mobile phase, hexane–2-propanol–Et_2NH (500:10:1); flow rate, 0.1 mL min^{-1}; detection, RI (refractive index).

7. Conclusion

The first total synthesis of tricyclic marine alkaloids, (±)-fasicularin (**2**) and (±)-lepadiformine (**5**), has been achieved. The key common strategic element for the synthesis is the stereocontrolled intramolecular hetero-Diels–Alder reaction of an *N*-acylnitroso moiety with an exocyclic diene with or without bromine substitution to control the syn-facial or anti-facial selectivity, respectively, thereby leading to the trans- or cis-fused decahydroquinoline ring systems **13** or **23** involving the simultaneous introduction of the nitrogenated quaternary center in a single step. On further elaboration of the six-membered or five-membered ring A, the trans-fused adduct **13** provided either (±)-fasicularin (**2**) or (±)-lepadiformine (**5**). The hydrochloride salt of synthetic (±)-**5** was found to be identical with the isolated natural sample of lepadiformine; however, the tricyclic amino alcohol **4** having the proposed structure of lepadiformine in a non-zwitterionic form, derived from the cis-fused adduct **23**, was found to be different from lepadiformine by spectral comparison. These results thus unambiguously established the relative stereochemistry of lepadiformine, formerly assigned incorrectly, as shown by **5**.

Having thus established the relative stereochemistry of lepadiformine, we next extended our study to the enantioselective synthesis of lepadiformine and to the determination of the absolute configuration of the natural product. Accordingly, cyclization of the keto amide **62** bearing the conjugated diene was performed using formic acid as the key step; here an intramolecular conjugated diene cyclization of the in situ generated *N*-acyliminium ion **64** proceeded very efficiently to form the 1-azaspiro formate ester **63** in a single step. The total synthesis of lepadiformine (**5**) was accomplished as the natural (−)-enantiomer with a high degree of stereoselectivity in nine steps, with an overall yield of 31.4%, starting from the (*S*)-*N*-Boc-pyrroli-

done **60**; this constitutes the shortest route and the highest overall yield in the lepadiformine synthesis to date.[33] The present synthesis thus allowed the absolute configuration of natural lepadiformine to be assigned as 3*R*,5*S*,7a*R*,11a*R*.

We are grateful to Professor J. F. Biard (Université de Nantes) for a sample of natural lepadiformine and to Professor D. J. Faulkner (Scripps Institution of Oceanography, University of California at San Diego) for providing authentic spectra of ^{1}H and ^{13}C NMR of facicularin. We also thank Mr. S. Igarashi and Dr. K. Yoza (Bruker Japan Co., Ltd.) for X-ray crystallographic analysis of synthetic (±)-lepadiformine hydrochloride salt. This work was supported in part by a Grant for Private Universities provided by the Ministry of Education, Culture, Sports, Science and Technology, and the Promotion and Mutual Aid Corporation for Private School of Japan.

References

1 For recent reviews on marine natural products, see: a) B. S. Davidson, *Chem. Rev.*, **93**, 1771 (1993). b) D. J. Faulkner, *Nat. Prod. Rep.*, **11**, 355 (1994). c) D. J. Faulkner, *Nat. Prod. Rep.*, **12**, 223 (1995). d) D. J. Faulkner, *Nat. Prod. Rep.*, **13**, 75 (1996). e) D. J. Faulkner, *Nat. Prod. Rep.*, **15**, 113 (1998). f) D. J. Faulkner, *Nat. Prod. Rep.*, **16**, 155 (1999).

2 a) A. J. Blackman, C. Li, D. C. R. Hockless, B. W. Skelton, and A. H. White, *Tetrahedron*, **49**, 8645 (1993). b) C. Li and A. J. Blackman, *Aust. J. Chem.*, **47**, 1355 (1994). c) C. Li and A. J. Blackman, *Aust. J. Chem.*, **48**, 955 (1995).

3 a) J. F. Biard, S. Guyot, C. Roussakis, J. F. Verbist, J. Vercauteren, J. F. Weber, and K. Boukef, *Tetrahedron Lett.*, **35**, 2691 (1994). b) M. Juge, N. Grimaud, J. F. Biard, M. P. Sauviat, M. Nabil. J. F. Verbist, and J. Y. Petit, *Toxicon*, **39**, 1231 (2001).

4 A. D. Patil, A. J. Freyer, R. Reichwein, B. Cartre, L. B. Killmer, L. Faucette, R. K. Johnson, and D. J. Faulkner, *Tetrahedron Lett.*, **38**, 364 (1997).

5 a) For synthesis of (±)-cylindricines A, D, and E, see: B. B. Snider and T. Liu, *J. Org. Chem.*, **62**, 5630 (1997). b) For synthesis of (−)-cylindricine C, see: G. A. Molander and M. Rönn, *J. Org. Chem.*, **64**, 5183 (1999). c) For synthesis of (±)-cylindricines A and B, see: J. F. Liu and C. H. Heathcock, *J. Org. Chem.*, **64**, 8263 (1999).

6 a) H. Abe, S. Aoyagi, and C. Kibayashi, *Tetrahedron Lett.*, **41**, 1223 (2000). b) H. Abe, S. Aoyagi, and C. Kibayashi, *J. Am. Chem. Soc.*, **122**, 4583 (2000).

7 H. Abe, S. Aoyagi, and C. Kibayashi, *Angew. Chem., Int. Ed.*, **41**, 3017 (2002).

8 For our own achievements in the development of the intramolecular acylnitroso-Diels–Alder approach for the synthesis of nitrogen-containing natural products, see: C. Kibayashi and S. Aoyagi, *Synlett*, **1995**, 873.

9 For reviews on acylnitroso-Diels–Alder reaction, see: a) G. W. Kirby, *Chem. Soc. Rev.*, **6**, 1 (1997). b) S. M. Weinreb and R. P. Staib, *Tetrahedron*, **38**, 3087 (1982). c) D. L. Boger and S. M. Weinreb, "Hetero Diels–Alder Methodology in Organic Synthesis," Academic Press, San Diego (1987). d) S. M. Weinreb, "Comprehensive Organic Synthesis," ed by B. M. Trost, I. Fleming, and L. A. Paquette, Pergamon Press, Oxford (1991), Vol. 5, pp. 401–449. e) J. Streith and A. Defoin, *Synthesis*, **1994**, 1107. f) M. Orena, "Methods of Organic Chemistry (Houben-Weyl)," 4th ed, ed by G. Helmchen, R. W. Hoffmann, J. Mulzer, and E. Schaumann, Thieme, Stuttgart (1995), Vol. E21e, pp. 5547–5587.

10 a) T. P. Burkholder and P. L. Fuchs, *J. Am. Chem. Soc.*, **110**, 2341 (1988). b) T. P. Burkholder and P. L. Fuchs, *J. Am. Chem. Soc.*, **112**, 9601 (1990).

11 For a review, see: A. Lubineau, J. Augé, and Y. Queneau, *Synthesis*, **1994**, 741.

12 M. Naruse, S. Aoyagi, and C. Kibayashi, *Tetrahedron Lett.*, **35**, 595 (1994).

13 For reviews on intramolecular Diels–Alder reaction, see: a) W. Oppolzer, *Angew. Chem., Int. Ed. Engl.*, **16**, 10 (1977). b) G. Brieger and J. N. Bennett, *Chem. Rev.*, **80**, 63 (1980). c) A. G. Fallis, *Can. J. Chem.*, **62**, 183 (1984). d) E. Ciganek, *Org. React.*, **32**, 1 (1984). e) D. Craig, *Chem. Soc. Rev.*, **16**, 187 (1987). f) W. R. Roush, "Comprehensive Organic Synthesis," ed by B. M. Trost, I. Fleming, and L. A. Paquette, Pergamon Press, Oxford (1991), Vol. 5, pp. 513–550. g) D. Craig, "Methods of Organic Chemistry (Houben-Weyl)," 4th ed, ed by G. Helmchen, R. W. Hoffmann, J. Mulzer, and E. Schaumann, Thieme, Stuttgart (1995), Vol. E21c, pp. 2872–2904.

14 a) F. Johnson, *Chem. Rev.*, **68**, 375 (1968). b) R. W. Hoffmann, *Chem. Rev.*, **89**, 1841 (1989).

15 a) G. W. Kirby and J. G. Sweeny, *J. Chem. Soc., Chem. Commun.*, **1973**, 704. b) J. E. T. Corrie, G. W. Kirby, and R. P. Sharma, *J. Chem. Soc., Chem. Commun.*, **1975**, 915. c) G. Keck, *Tetrahedron Lett.*, **19**, 4767 (1978).

16 A. G. Myers, B. H. Yang, and D. J. Kopecky, *Tetrahedron Lett.*, **37**, 3623 (1996).

17 For recent application of this reaction to lactam ring-opening, see: S. Aoyagi, R. Tanaka, M. Naruse, and C. Kibayashi, *J. Org. Chem.*, **63**, 8397 (1998).

18 For a comprehensive review on heteroatom-directed organic reactions, see: A. H. Hoveyda, D. A. Evans, and G. C. Fu, *Chem. Rev.*, **93**, 1307 (1993).

19 O. Mitsunobu, *Synthesis*, **1981**, 1.

20 For a discussion of the solvent effect on stereochemical outcome of directed catalytic hydrogenation, see: H. W. Thompson, E. McPherson, and B. L. Lences, *J. Org. Chem.*, **41**, 2903 (1976).

21 a) Y. Tamura, T. Kawasaki, M. Adachi, M. Tanio, and Y. Kita, *Tetrahedron Lett.*, **18**, 4417 (1977). b) J. Burski, J. Kieszkowski, J. Michalski, M. Pakulski, and A. Skowronska, *Tetrahedron*, **39**, 4175 (1983).

22 This procedure was based upon the following: H.-R. Sliwka and S. Liaaen-Jensen, *Tetrahedron: Asymmetry*, **4**, 361 (1993).

23 a) K. M. Werner, J. M. De los Santos, S. M. Weinreb, and M. Shang, *J. Org. Chem.*, **64**, 686 (1999). b) K. M. Werner, J. M. de los Santos, S. M. Weinreb, and M. Shang, *J. Org. Chem.*, **64**, 4865 (1999).

24 a) W. H. Pearson, N. S. Barta, and J. W. Kampf, *Tetrahedron Lett.*, **38**, 3369 (1997). b) W. H. Pearson and Y. Ren, *J. Org. Chem.*, **64**, 688 (1999).

25 Y. Shishido and C. Kibayashi, *J. Org. Chem.*, **57**, 2876 (1992).

26 P. Sun, C. Sun, and S. M. Weinreb, *Org. Lett.*, **3**, 3507 (2001).

27 T. J. Greshock and R. L. Funk, *Org. Lett.*, **3**, 3511 (2001).

28 Many attempts at obtaining a crystalline derivative of natural lepadiformine (provided by Professor Biard as an oil) suitable for X-ray crystallography were unsuccessful (W. Pearson, personal communication).

29 a) H. E. Schoemaker and W. N. Speckamp, *Tetrahedron Lett.*, **19**, 1515 (1978). b) H. E. Schoemaker and W. N. Speckamp, *Tetrahedron Lett.*, **19**, 4841 (1978). c) H. E. Schoemaker and W. N.

Speckamp, *Tetrahedron*, **36**, 951 (1980).

30 D. A. Evans and E. W. Thomas, *Tetrahedron Lett.*, **20**, 411 (1979).

31 a) T. Katoh, Y. Nagata, Y. Kobayashi, K. Arai, J. Minami, and S. Terashima, *Tetrahedron Lett.*, **34**, 5743 (1993). b) T. Katoh, Y. Nagata, Y. Kobayashi, K. Arai, J. Minami, and S. Terashima, *Tetrahedron*, **50**, 6221 (1994).

32 R. Noyori, I. Tomino, and Y. Tanimoto, *J. Am. Chem. Soc.*, **101**, 3129 (1979).

33 In addition to the syntheses of (±)-lepadiformine (Refs. 26 and 27), very recently the enantioselective synthesis of (−)-lepadiformine has been reported. See: P. Sun, C. Sun, and S. M. Weinreb, *J. Org. Chem.*, **67**, 4337 (2002).

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