

Synthesis of Poison-Frog Alkaloids and Their Pharmacological Effects at Neuronal Nicotinic Acetylcholine Receptors

Naoki Toyooka^{*1}, Hiroshi Tsuneki¹, Soushi Kobayashi¹, Zhou Dejun¹, Masashi Kawasaki², Ikuko Kimura¹, Toshiyasu Sasaoka¹ and Hideo Nemoto¹

¹Graduate School of Medicine and Pharmaceutical Sciences, University of Toyama, Japan

²Faculty of Engineering, Toyama Prefectural University, Japan

Abstract: The flexible and efficient enantioselective synthesis of poison-frog alkaloids has been described using the highly stereoselective conjugate addition reactions as the key step. Several 5,8-disubstituted indolizidines and 1,4-disubstituted quinolizidines have been synthesized according to this strategy. Furthermore, 5,6,8-trisubstituted indolizidine type of poison-frog alkaloid **223A** and unique tricyclic poison-frog alkaloid **205B** have also been synthesized by sequential use of the above key conjugate addition reaction. Investigations of inhibitory effects of synthetic poison-frog alkaloids on neuronal nicotinic acetylcholine receptors have been conducted, and we found that most of the synthetic compounds showed inhibitory effects on the neuronal nicotinic acetylcholine receptors. Especially, the 5,8-disubstituted indolizidine **235B'** inhibited the $\alpha 4\beta 2$ -neuronal nicotinic acetylcholine receptors in highly subtype-selective manner. These results suggested that the synthetic alkaloid **235B'** is a promising lead compound for the drugs designed to treat cholinergic disorders such as autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE).

1. INTRODUCTION

A diverse array of biologically active alkaloids has been detected in amphibian skin, which contains over 20 structural classes and over 800 alkaloids [1]. Most of these alkaloids appear to be derived from dietary sources such as ants, mites, beetles and millipedes [2]. Many of these poison-frog alkaloids are expected to show interesting biological activities such as inhibitory effects on the neuronal nicotinic acetylcholine receptors [3]. However, these alkaloids have been isolated in insignificant amounts from the amphibian skin. As a consequence, the need for the development of the efficient synthetic strategy of poison-frog alkaloids has arisen for the determination of the structure of natural products as well as the investigations for their biological activities. In this review, we wish to report the synthetic efforts of these alkaloids and their pharmacological effects at neuronal nicotinic acetylcholine receptors.

2. SYNTHESIS OF 5,8-DISUBSTITUTED INDOLIZIDINE AND 1,4-DISUBSTITUTED QUINOLIZIDINE TYPE POISON-FROG ALKALOIDS [4, 5]

The 5,8-disubstituted indolizidines are one of the extensive subclasses of poison-frog alkaloids, and about 80 examples of indolizidines have been detected to date [1]. On the other hand, about 20 examples of 1,4-disubstituted quinolizidines have been assigned [1].

We envisioned the efficient and flexible synthesis of 5,8-disubstituted indolizidine and 1,4-disubstituted quinolizidine type poison-frog alkaloids as shown in Fig. 1.

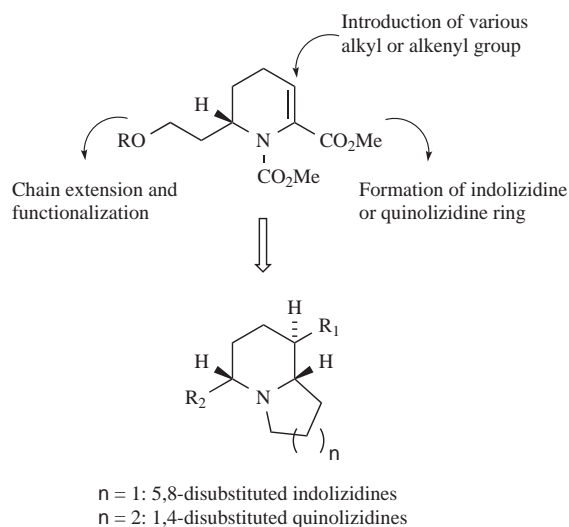
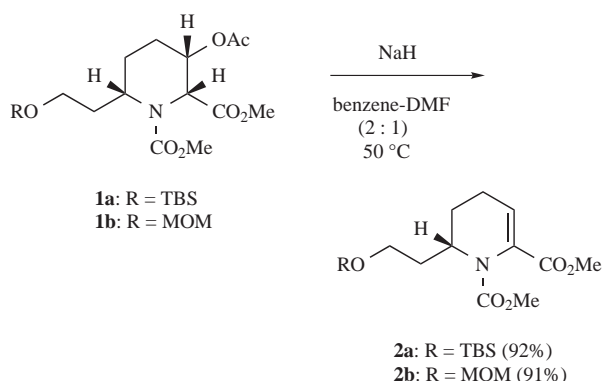
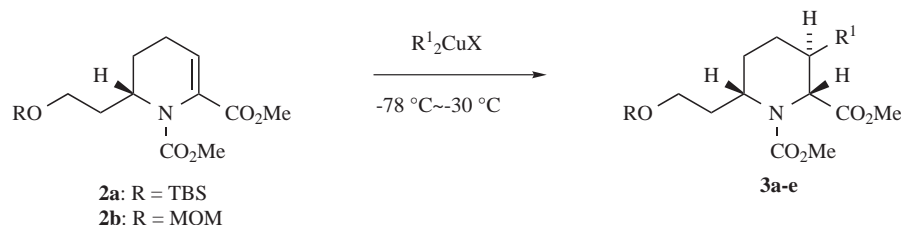


Fig. (1). Strategy for the construction of 5,8-disubstituted indolizidine or 1,4-disubstituted quinolizidine ring core.



Scheme 1. Synthesis of enaminoesters **2a** and **2b**.

*Address correspondence to this author at the Graduate School of Medicine and Pharmaceutical Sciences, University of Toyama, Sugitani 2630, Toyama 930-0194, Japan; Tel: 81-76-434-7532; Fax: 81-76-434-4656; E-mail: toyooka@pha.u-toyama.ac.jp

Table 1. Construction of the Trisubstituted Piperidines **3a-e**

	R¹	X	R	Solvent	Yield (%)
3a	Me	Li	TBS	Et ₂ O	92
3b	Et	MrBr	TBS	THF	96
3c	vinyl	Li	MOM	Et ₂ O	91
3d	allyl	MgCl	MOM	THF	80
3e	<i>n</i> -Bu	Li	TBS	Et ₂ O	94

The starting enaminoesters **2a-b** were synthesized from the optically active trisubstituted piperidines **1a-b** [6] by the elimination of acetic acid with base.

The key Michael-type conjugate addition reaction of **2** proceeded smoothly and the desired trisubstituted piperidines (**3a-e**) were obtained in high yield in each case as a single isomer. The results are summarized in Table 1.

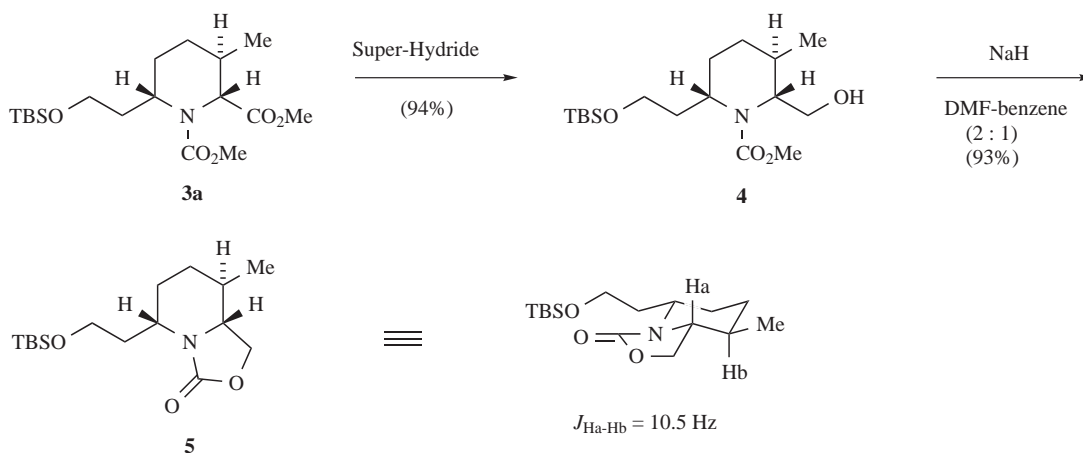
The stereochemistry of the adducts was determined by the analysis of the coupling constant in the ¹H NMR spectrum of the oxazolizinone **5** derived from **3a** shown in Scheme 2.

The stereochemical course of the above conjugate addition reaction was rationalized as follows.

The conformation of **2** is restricted not only to **B** but also to **A** owing to the A^(1,3) strain [7] between the methoxycarbonyl moiety on the nitrogen and the side chain on the α-position. The anion attacks from the stereoelectronically preferred α-axial orientation [8], resulting in the protonation of the enolate to give rise to trisubstituted piperidine as a single isomer. This stereoselectivity is also explained by Cieplak's hypothesis [9].

With the requisite piperidines **3a-e** being obtained, we next focused on the synthesis of indolizidine and quinolizidine type of poison-frog alkaloids. Carbon-chain elongation at the 2-position of the alcohol **4** was performed by Swern oxidation, followed by Horner-Emmons reaction to afford the α,β-unsaturated ester **6**, which was transformed into the MOM ether **7** in three-step sequence. The removal of the silyl group in **7** provided the alcohol **8**, whose hydroxyl group was converted to iodide *via* the corresponding methanesulfonate to provide iodide **9**. The cross coupling reaction of **9** with allylmagnesium chloride in the presence of CuI afforded the olefin **10**. Removal of methoxycarbonyl group using the Corey's procedure [10] and the treatment of the resulting amine with the acid provided the amino alcohol **11**, which had been previously synthesized by another route and then converted to the indolizidines **207A** and **209B** by Kibayashi [11].

In a similar manner, the iodide **9** was transformed into olefin **12**. The application of the Kibayashi indolizidine closure [11] to **12** gave rise to indolizidine **235B'**. The absolute stereochemistry of natural **235B'** was determined to be 5*R*, 8*R*, 9*S* by this chiral synthesis.

**Scheme 2.** Determination of the stereochemistry of the piperidine **3a**.

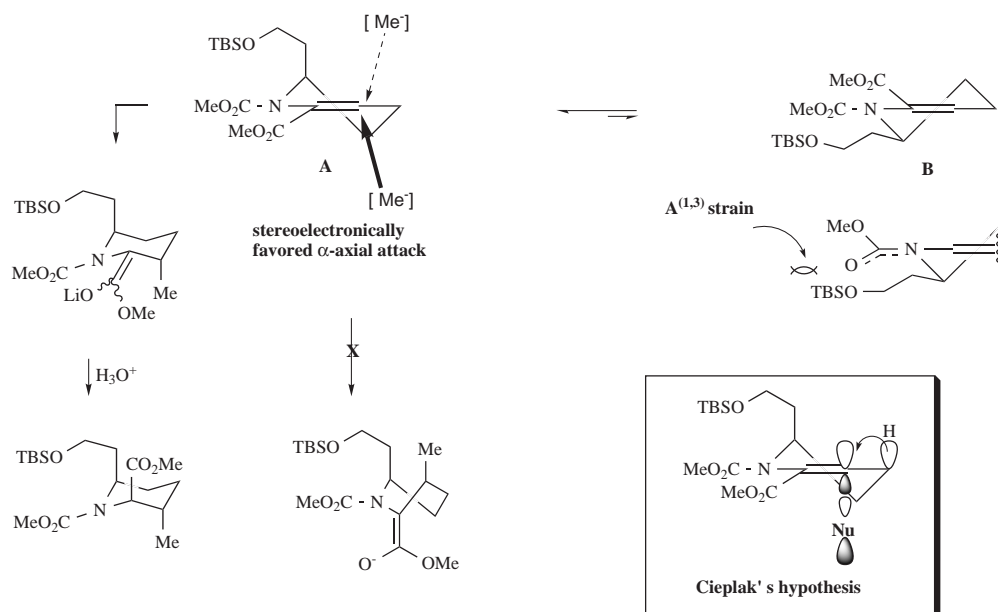
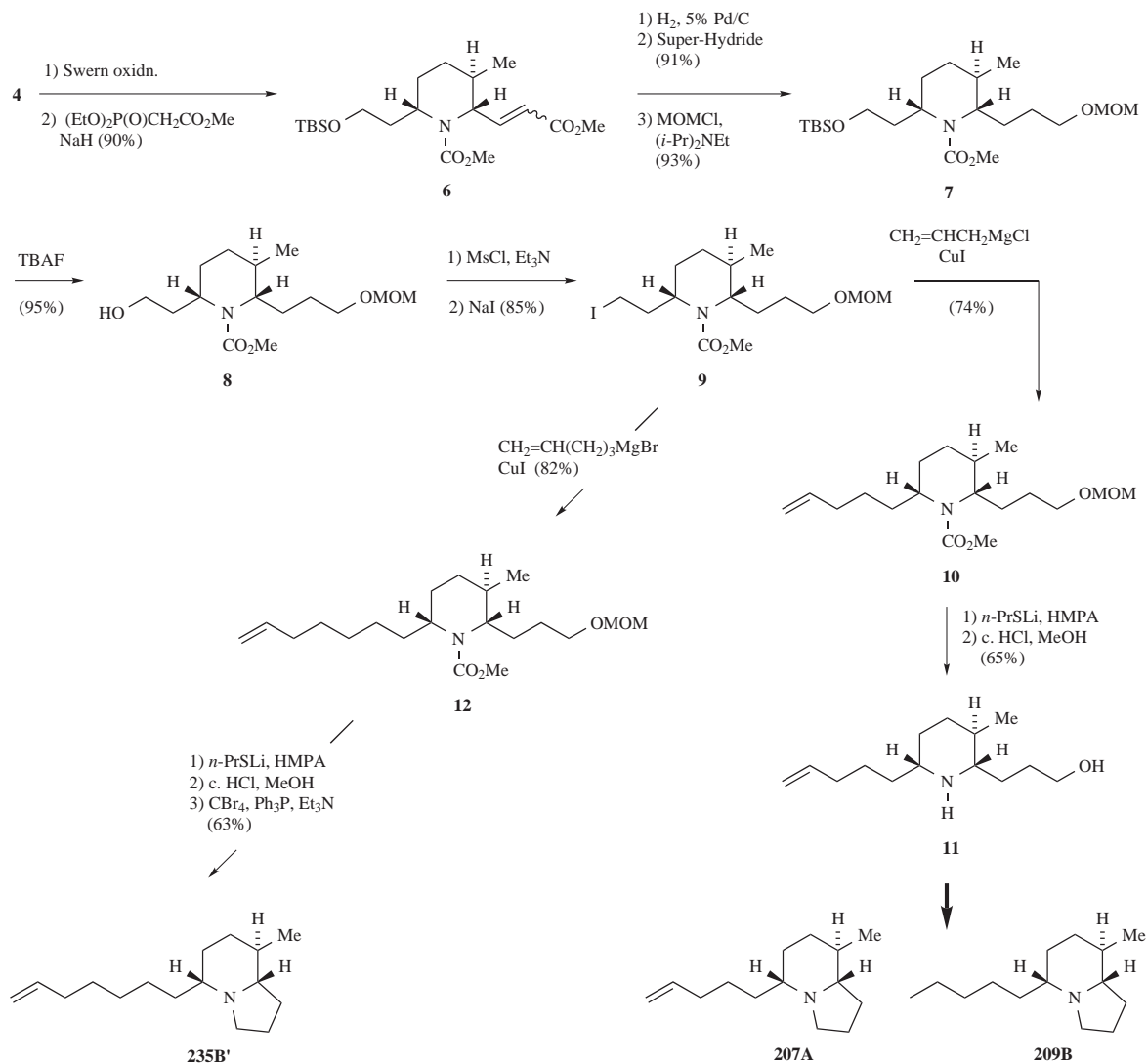
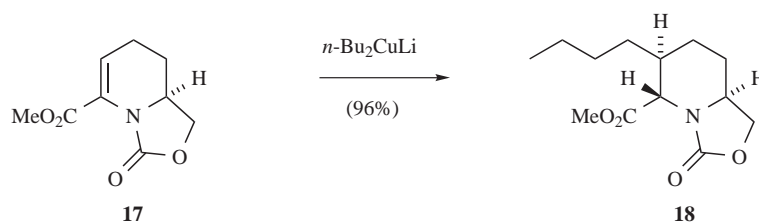


Fig. (2). Stereoselectivity of the key Michael-type conjugate addition reaction of **2**.



Scheme 3. Total synthesis of the indolizidine **235B'** and formal synthesis of the indolizidines **207A** and **209B**.



Scheme 5. Michael-type conjugate addition reaction of the enaminoester **17** bearing the oxazolizinone ring.

Natural **223I** showed weak absorbance in the Bohlmann band region on GC-FTIR, suggesting a 5, 9*E*-relative stereochemistry. To determine the relative stereochemistry of natural **223I**, we planned the stereoselective chiral syntheses of two 5, 9*E* diastereomers of **223V** (**15**, **16**).

The synthesis of **15** began with the cyclic enaminoester **17** [14]. The key conjugate addition reaction of **17** with dibutyl lithium cuprate proceeded smoothly to afford the adduct **18** again as a single isomer.

Stereoelectronically
favored axial attack
of *n*-Bu anion

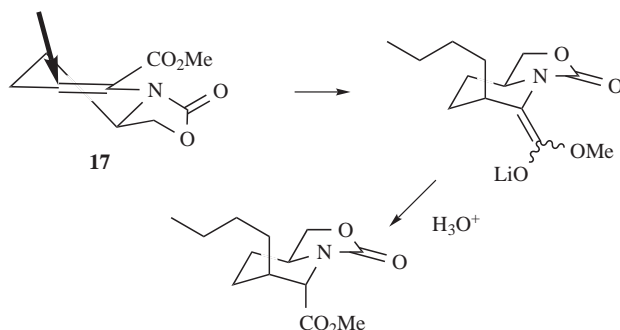
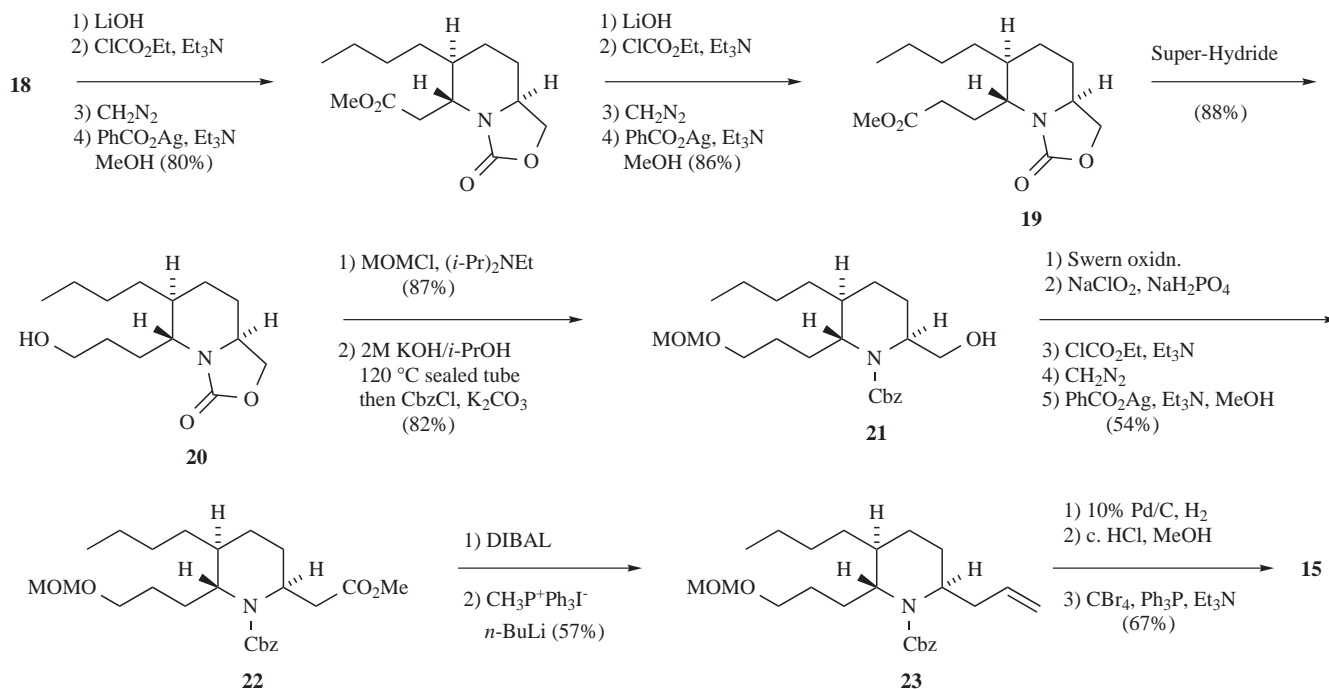


Fig. (4). Stereochemical course of the Michael-type conjugate addition reaction of **17**.



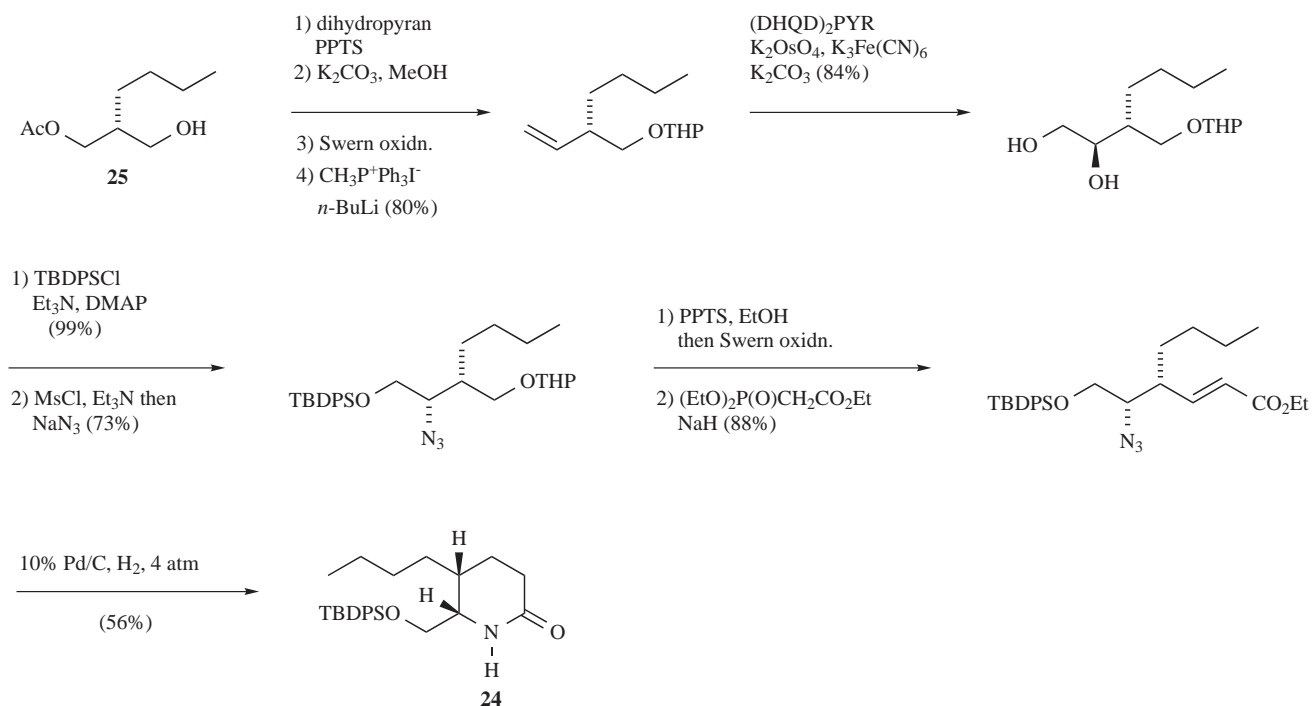
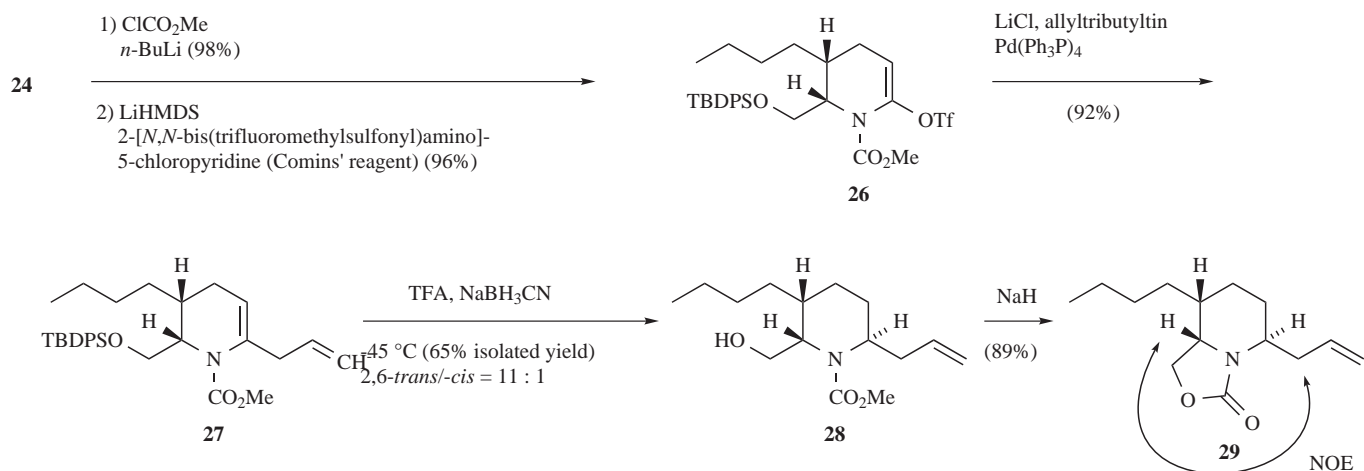
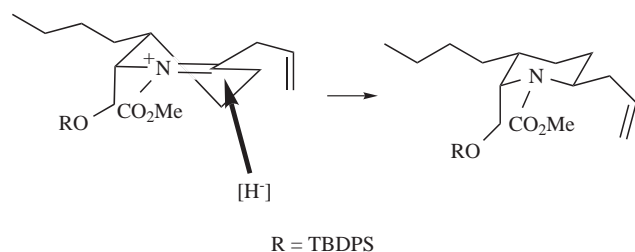
Scheme 6. Synthesis of one of the possible structure **15** for the alkaloid **223I**.

The stereoselectivity of the conjugate addition reaction of **17** can also be explained by stereoelectronic effect [8].

Carbon-chain elongation of **18** was performed by Arndt-Eistert sequence to give the homologated ester **19**, whose ester moiety was reduced with Super-Hydride to provide alcohol **20**. Protection of the hydroxyl group, followed by hydrolysis and the treatment of the resulting amino alcohol with CbzCl afforded the carbamate **21**. The alcohol **21** was converted to the homologated ester **22**, which was transformed into olefin **23**. Hydrogenation of **23** over 10% Pd/C and indolizidine ring closure reaction furnished **15**.

On the other hand, another stereoisomer **16** was synthesized starting from the piperidone **24**, derived from the chiral acetate **25** [15] as shown in Scheme 7.

After conversion of **24** to the methyl carbamate, which was treated with Comins' triflating reagent [16], yielded the enol triflate **26**. The triflate **26** was subjected to a Still-type coupling reaction [17] to provide olefin **27**. A stereoselective reduction of **27** with NaBH₃CN under acidic conditions gave rise to the reduction product as a 11 : 1 mixture, and the major isomer **28** was isolated in 65% yield. The stereochemistry of **28** was determined to be that of the desired 2,6-*trans* isomer, based upon the NOE spectrum of the corresponding oxazolizinone **29**.

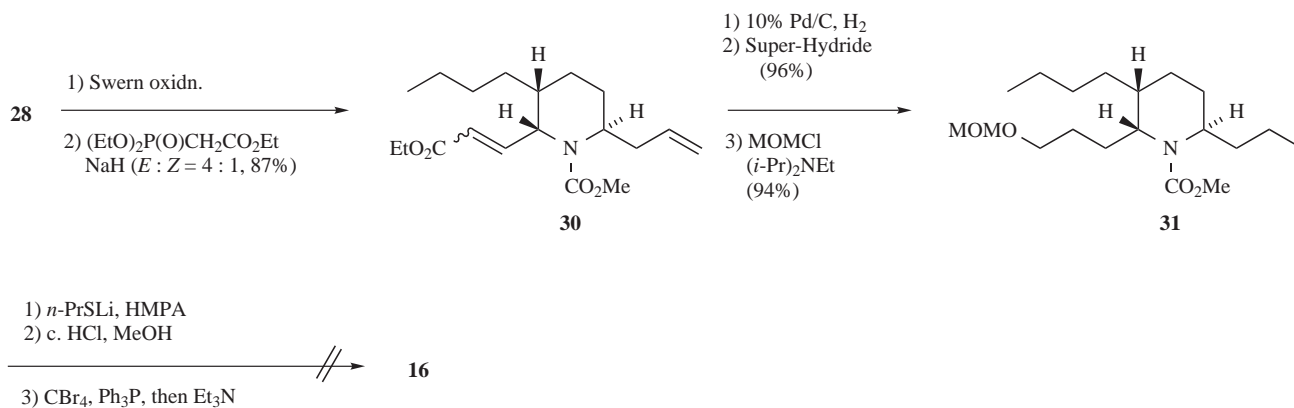
**Scheme 7.** Synthesis of *cis*-substituted piperidone **24**.**Scheme 8.** Synthesis of trisubstituted piperidine **28** and determination of its stereochemistry.**Fig. (5).** Stereoselectivity of the reduction of the iminium salt derived from **27**.

The stereochemical course of the hydride attack on the iminium salt generated from **27** under the acidic condition can be rationalized by $A^{(1,2)}$ strain and a stereoelectronic effect as shown in Fig. 5.

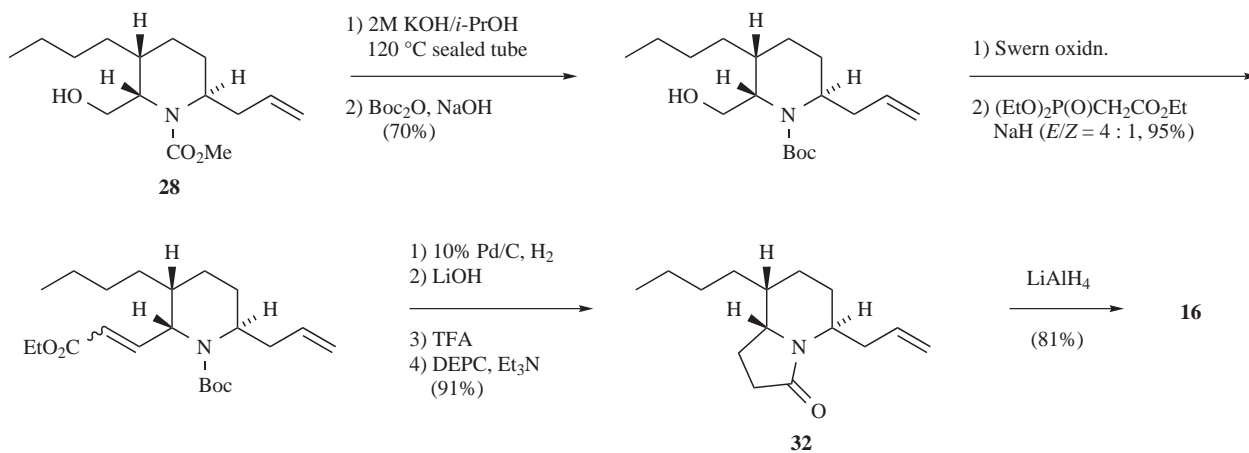
Swern oxidation of **28**, followed by Horner-Emmons reaction of the resulting aldehyde gave the unsaturated ester **30**, which was converted to MOM ether **31** by means of hydrogenation of both double bonds, reduction of the ester moiety and protection of the resulting alcohol. The ether **31** was then subjected to indolizidine ring closure, but no indolizidine formation was observed.

Next we examined the synthesis of **16** via the lactam **32**, which was derived from olefin **28**. The carbamate moiety of **28** was changed to Boc group, and then the carbon-chain elongation, followed by the formation of the lactam ring using the Shioiri's reagent [18] provided **32**. Reduction of the lactam moiety furnished the desired **16**.

Synthesis of both 5, 9*E*-indolizidines (**15**, **16**) provided clear proof that alkaloid **223I** was not a 5, 9-disubstituted indolizidine. Both **15** and **16** had an appreciable



Scheme 9. Attempt to construct the indolizidine **16** via the piperidine **31**.



Scheme 10. Synthesis of another possible structure **16** for the alkaloid **223I**.

retro-Diels-Alder fragment at m/z 96, while natural **223I** did not. The structure of natural **223I** should be revised, but further studies are required for the determination of the structure of natural product.

4. SYNTHESIS AND THE DETERMINATION OF THE ABSOLUTE STEREOCHEMISTRY OF QUINOLIZIDINE **207I** [19]

As mentioned in section 2, comparison of synthetic **14** with natural **207I** revealed that the two are not identical although both GC-FTIR spectra of **14** and natural **207I** are very similar including the Bohlmann band region.

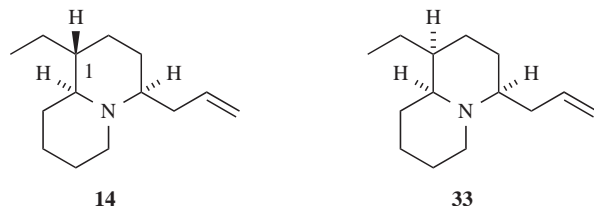


Fig. (6). Revised structure **33** for the quinolizidine **207I**.

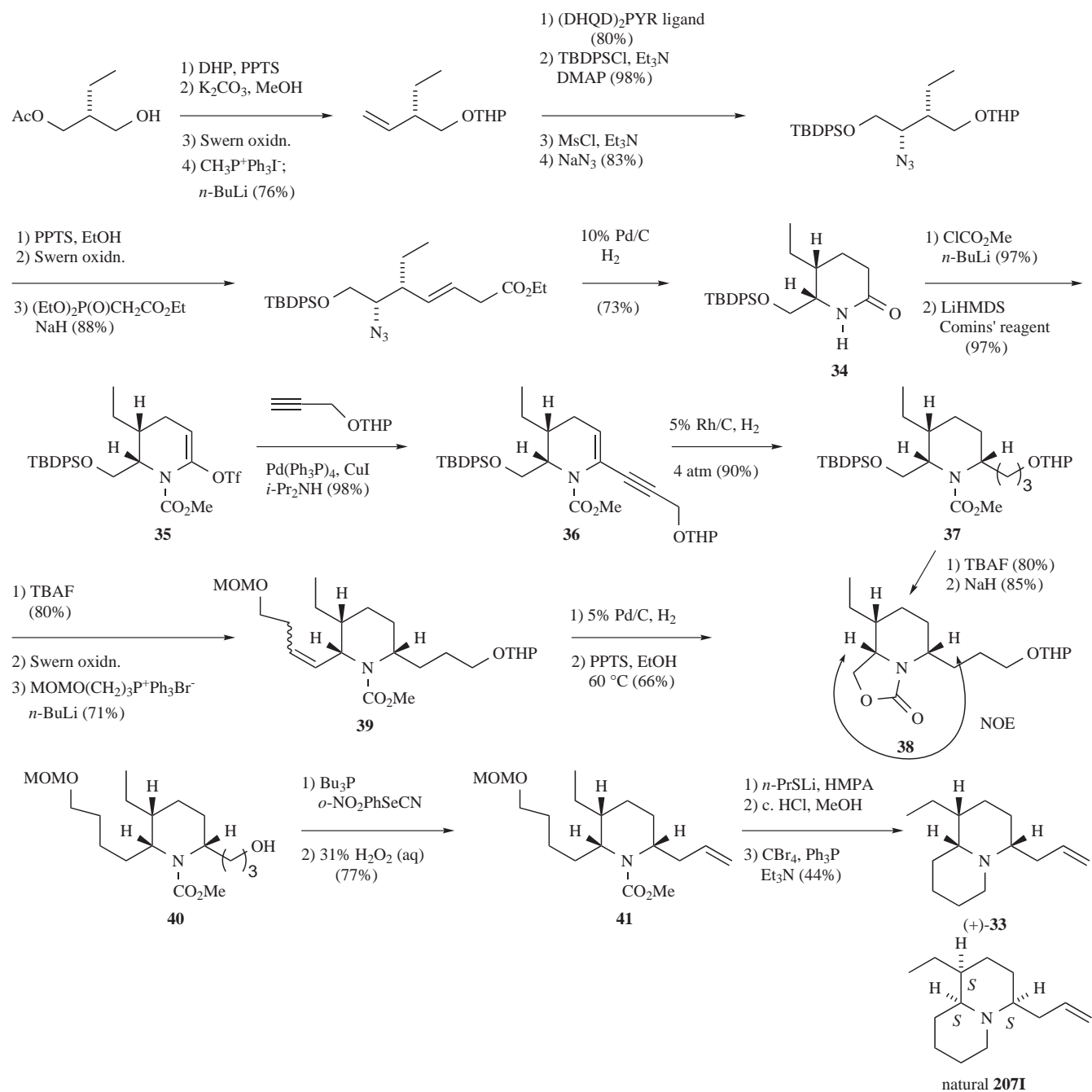
The relative stereochemistry of natural **207I** is anticipated to be the 1-epimer **33**. In 1999, Rassat *et al.* reported the first synthesis of **33** [20]. The FTIR spectrum of **33** was observed to be identical to that of natural **207I** in all respects. Furthermore, the synthetic racemate could be separated on a β -dextrin chiral column [21]. We planned the chiral synthe-

sis of **33** to determine the absolute stereochemistry of natural **207I**.

The synthesis began with the chiral piperidone **34**, derived from the known monoacetate [22], which was converted to enol triflate **35**. Sonogashira coupling [17] of **35** gave **36**. Hydrogenation of both double and triple bonds over Rh/C under the medium pressure afforded the piperidine **37**. The stereochemistry of **37** was determined to be all *cis* by the NOE experiment of the oxazolizone **38**. The piperidine **37** was converted to alcohol **40** via olefin **39**. Swern oxidation of **40**, followed by the Wittig reaction of the resulting aldehyde afforded olefin **41**. Finally, three-step quinolizidine ring closure was performed to provide the quinolizidine (+)-**33**. The GC analysis of synthetic (+)-**33** using a β -dextrin chiral column revealed that the natural **207I** is the antipode of (+)-**33**, and possesses the $1S$, $4S$, $10S$ absolute configurations as shown in Scheme 11.

5. SYNTHESIS AND THE STRUCTURAL REVISION OF THE INDOLIZIDINE **223A** [23, 24]

The alkaloid **223A**, isolated from the skin extract of a Panamanian population of the frog *Dendrobates pumilio* Schmidt (Dendrobatidae) in 1997, is the first member of the 5,6,8-trisubstituted indolizidine type poison-frog alkaloid [25]. Now about 70 alkaloids of this class have been detected [1]. The relative stereochemistry of **223A** was established to be **42**, based upon GC-MS, GC-FTIR, and ^1H NMR spectral studies [25].



Scheme 11. Synthesis of the antipode (+)-33 of natural 207I and the determination of the absolute stereochemistry of natural 207I.

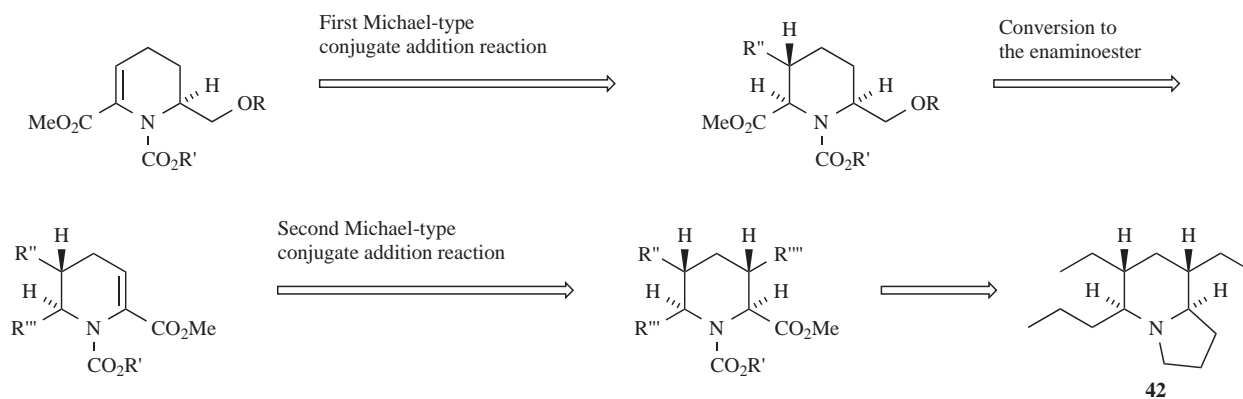
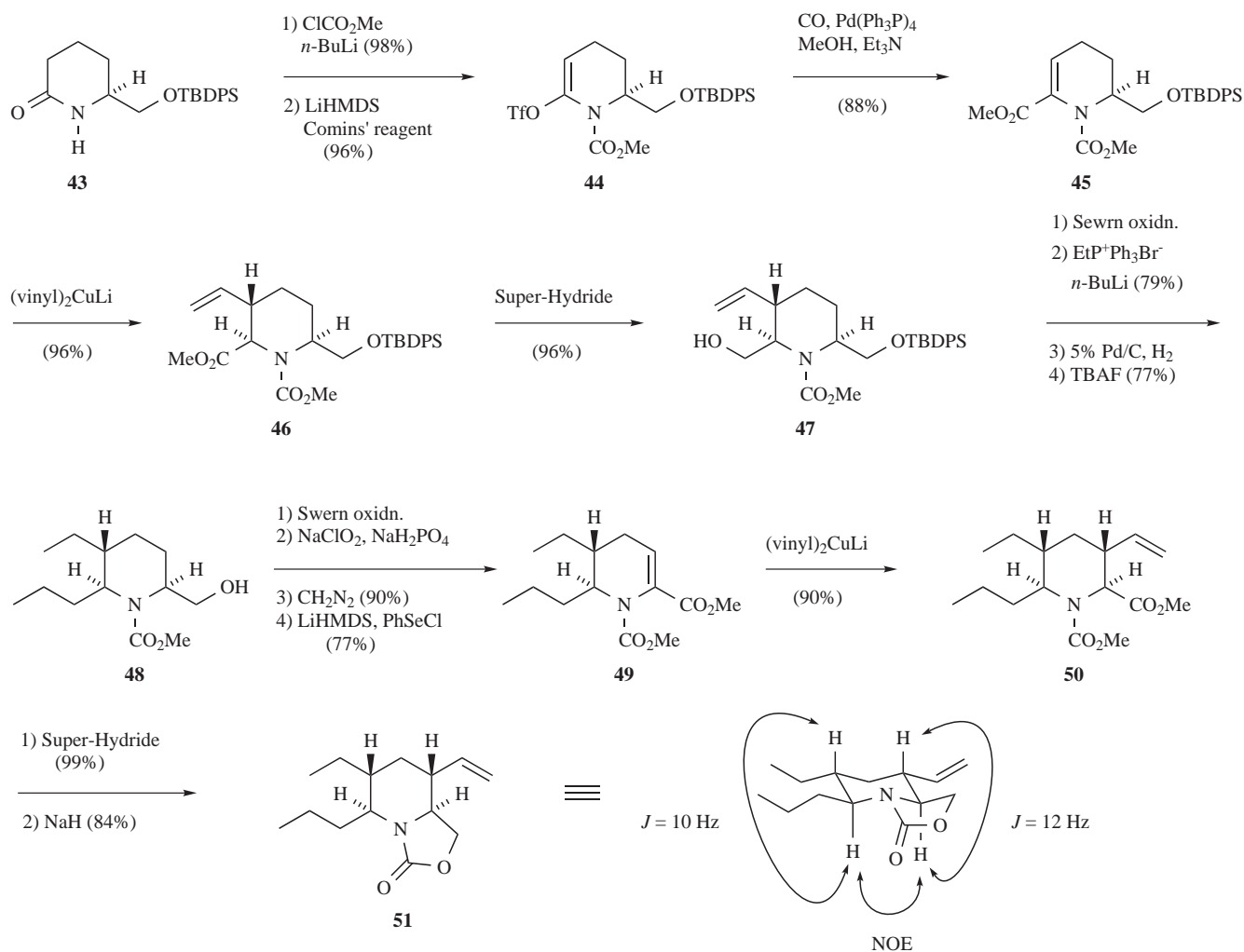


Fig. (7). Strategy for the construction of trisubstituted indolizidine ring system 42.



Scheme 12. Synthesis of key tetrasubstituted piperidine **50** by sequential use of the Michael-type conjugate addition reaction and the determination of the stereochemistry.

We examined the synthesis of **42** by sequential use of Michael-type conjugate addition reaction as shown in Fig. 1 to form the tetrasubstituted piperidine ring system as the key step.

The synthesis began with chiral amide **43**, which was converted to cyclic enaminoester **45** via the enol triflate **44**. The first Michael-type conjugate addition reaction of **45** proceeded smoothly to give rise to the adduct **46** as a single isomer. Reduction of the ester moiety with Super-Hydride afforded the alcohol **47**, which was transformed into the alcohol **48** by Swern oxidation of **47**, Wittig olefination of the resulting aldehyde, and hydrogenation, followed by the treatment with TBAF. Two-step oxidation of **48** and the treatment of the resulting carboxylic acid with diazomethane gave the corresponding ester, which was converted to the enaminoester **49** using Rubio's protocol [26]. The second Michael-type conjugate addition reaction of **49** proceeded to afford the adduct **50**, again as a single stereoisomer. The stereochemistry of **50** was determined by the coupling constants and NOE of the oxazolizinone **51**.

The stereoselectivity of the second and key Michael-type conjugate addition reaction can be explained by the $A^{(1,3)}$

strain [7] and the stereoelectronic effect [8] as shown in Fig. 8.

It is noteworthy that the stereochemical course of this addition reaction is controlled by the stereoelectronic effect despite severe 1,3-diaxial steric repulsion between the axial ethyl group at the 5-position and the incoming vinyl anion. This remarkable stereoselectivity can also be explained by Cieplak's hypothesis [9].

Elaboration of the adduct **50** into the indolizidine **42** is shown in Scheme 13.

Reduction of the ester moiety of **50**, and Swern oxidation of the resulting alcohol, followed by Horner-Emmons reaction afforded the unsaturated ester **52**. Hydrogenation of **52** and reduction of the resulting ester gave the corresponding alcohol, whose hydroxyl group was protected as the MOM ether to give rise to **53**. Finally, deprotection of the methoxycarbonyl group, cleavage of the MOM ether, treatment of the resulting amino alcohol with CBr_4 and Ph_3P followed by Et_3N furnished **42**.

The ^1H and ^{13}C NMR and FTIR spectra of **42** were neither identical to that for the natural product, nor was the GC retention time. The close similarity of the Bohlmann bands

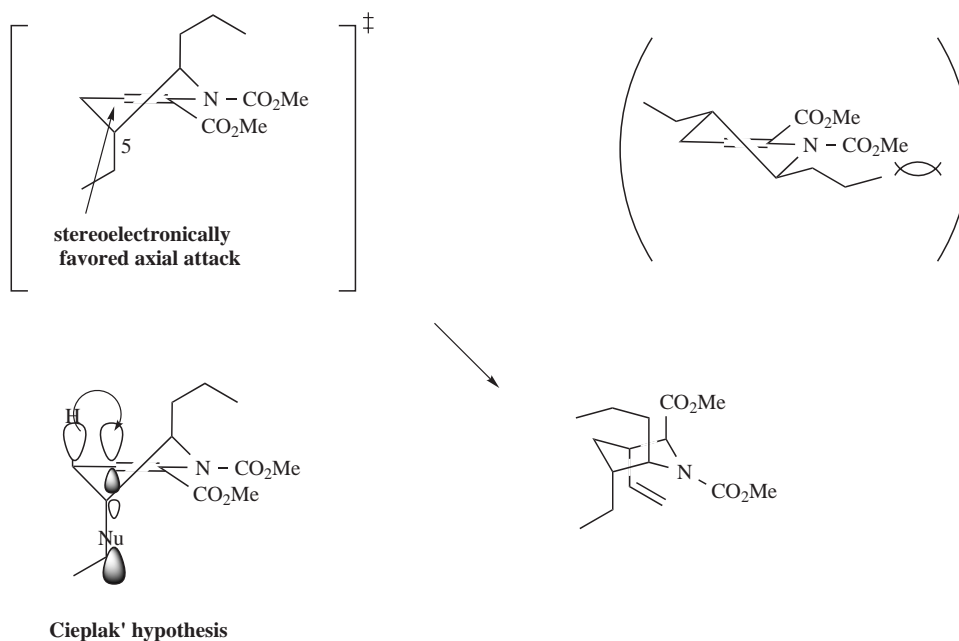
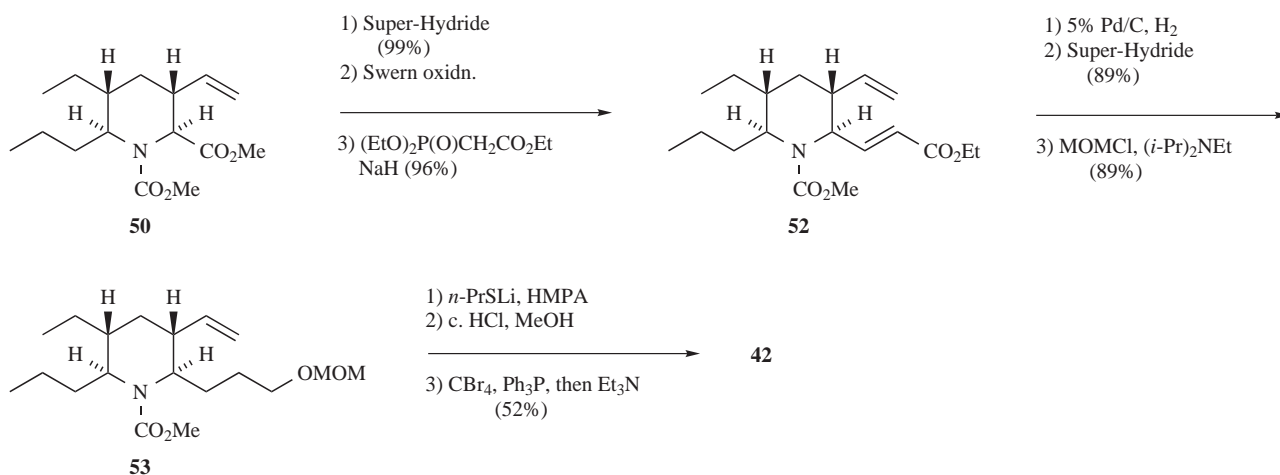


Fig. (8). Explanation of the stereoselectivity of the second Michael-type conjugate addition reaction.



Scheme 13. Completion of the synthesis of the proposed structure **42** for natural **223A**.

in the vapor phase FTIR spectra of **42** and natural **223A** indicated the same 5,9*Z* configuration for both compounds. In ^1H NMR spectra, synthetic DCI salt of **42** showed a nicely separated large quartet-like signal at δ 1.01 with a J of 12.5 Hz for H-7 axial proton. This observation means that the quartet-like signal with three large and approximately equal couplings for the H-7 axial proton must include two *trans*-diaxial vicinal couplings with H-6 and H-8 protons and one geminal coupling with the H-7 equatorial proton, and thus both ethyl-appendages at the 6- and 8-positions should be of the equatorial orientation. A quartet at this chemical shift was not seen in the natural product. On the other hand, the H-5 proton in **42** and natural **223A** was a doublet of triplet with J values of 11, 4.7 Hz, respectively, in the ^1H NMR spectrum. We concluded that the hindered rotation at C-5 in the C-6 epimer **54** of proposed structure for natural **223A** (**42**) leads to a large (11-Hz J_{5-10}) coupling constant and does not reflect an originally assumed *trans*-diaxial J_{5-6} coupling.

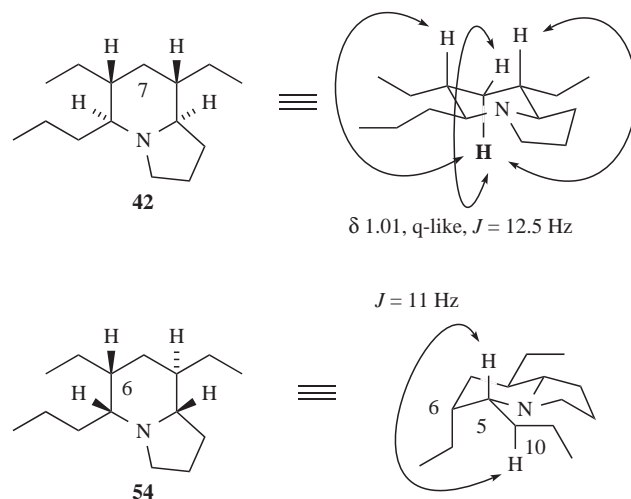
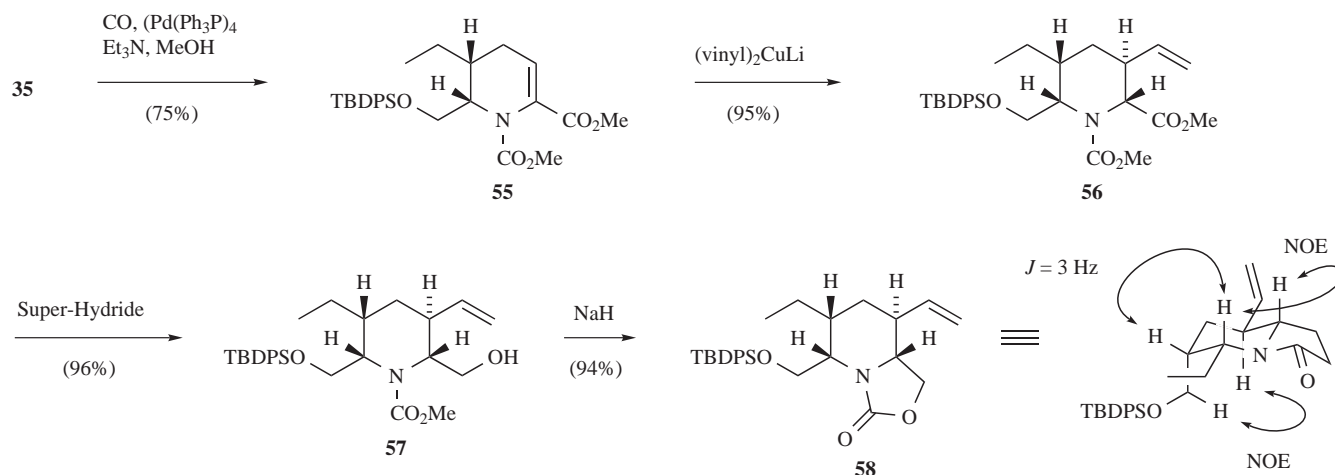


Fig. (9). Consideration of the real structure of natural **223A** by NMR studies.



Scheme 14. Synthesis of tetrasubstituted piperidine ring system **56** for the synthesis of revised structure **54** of natural **223A** and determination of its stereochemistry.

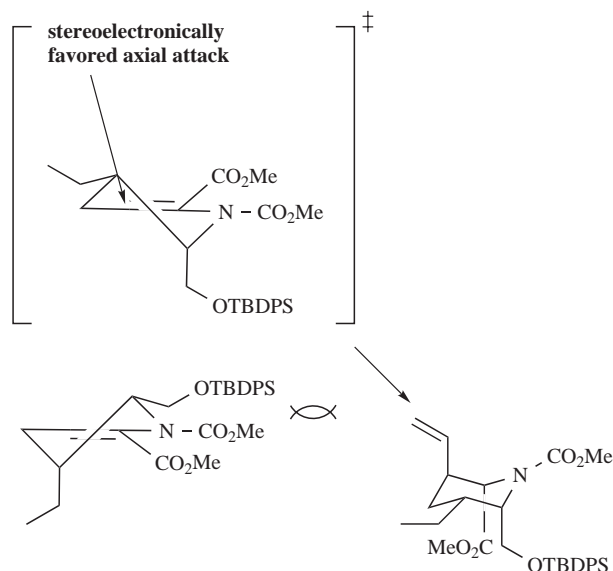
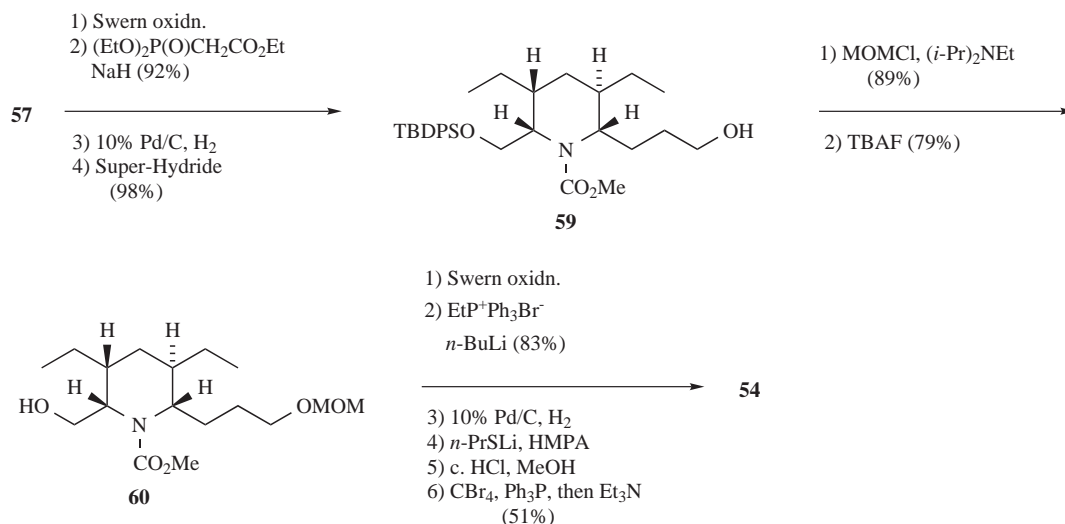


Fig. (10). Stereoselectivity of the Michael-type conjugate addition reaction of **55**.

Therefore, we commenced the synthesis of the epimer **54** from enol triflate **35**. The palladium catalyzed CO insertion reaction of **35** provided the enamoester **55**, which was subjected to Michael-type conjugate addition reaction to afford the adduct **56** again as a single isomer. The stereochemistry of **56** was determined by the indicated coupling constant and NOE experiments of the corresponding oxazolizone **58** derived from **56** via the alcohol **57**.

Stereoselectivity of this addition reaction can also be rationalized by the $A^{(1,3)}$ strain [7] and stereoelectronic effect [8] as shown in Fig. 10.

The synthesis of **54** was accomplished via the alcohols **59** and **60** in the same manner as used in the synthesis of **42** in Scheme 13. The spectral data of synthetic **54** were completely identical to that of the natural product. Thus, the structure of natural **223A** was revised to **54**, and the relative stereochemistry of this natural product was determined to be $5R^*$, $6R^*$, $8R^*$, $9S^*$ by the present chiral synthesis. After completion of our total synthesis, several syntheses and synthetic studies on the alkaloid **223A** were reported [27].



Scheme 15. Completion of the total synthesis of the revised structure **54** of natural **223A**.

6. SYNTHESIS AND THE DETERMINATION OF THE ABSOLUTE STEREOCHEMISTRY OF TRICYCLIC ALKALOID 205B [28, 24]

The alkaloid **205B**, one of the poison-frog alkaloids isolated from the skin extracts of the Panamanian frog *Dendrobates pumilio*, possesses an unusual and unique 8b-azaacenaphthylene ring system.

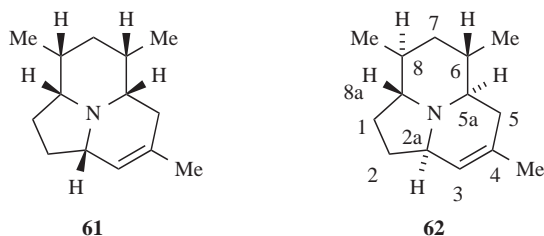


Fig. (11). First proposed structure **61** and revised structure **62** of **205B**.

The structure of this alkaloid was first proposed to be **61** [29] and recently revised to be **62** based on FTIR, NMR and

MS data [30]. This alkaloid contains five asymmetric centers in its compact, fourteen-carbon-atom frame; however, no synthesis of this unique alkaloid has been reported, and its absolute stereochemistry is still unknown.

As shown in Fig. 7, we established the stereocontrolled construction of tetrasubstituted piperidine ring system and its application to the synthesis of the proposed structure for natural **223A** as discussed in section 5. We envisioned the total synthesis of the alkaloid **205B** using the above strategy as the key step. For the synthesis of **205B**, it was essential to construct the tetrasubstituted piperidine ring core **C**, which would be synthesized by the second Michael-type conjugate addition reaction with opposite stereochemistry as shown in Fig. 12.

For this purpose, we designed the enaminoester having the cyclic carbamate **63** for the substrate for the second conjugate addition reaction. First, Michael-type conjugate addition reaction of **45** gave the adduct **64** as a single isomer, which was converted to oxazolizinone **65** in a two-step sequence. Cleavage of the silyl ether in **65** with TBAF, and

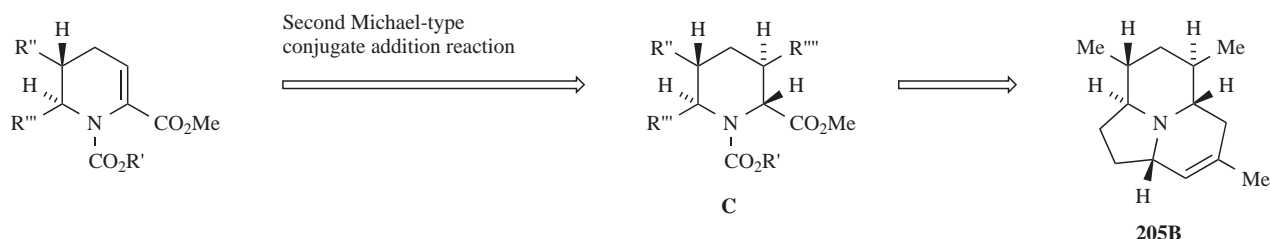
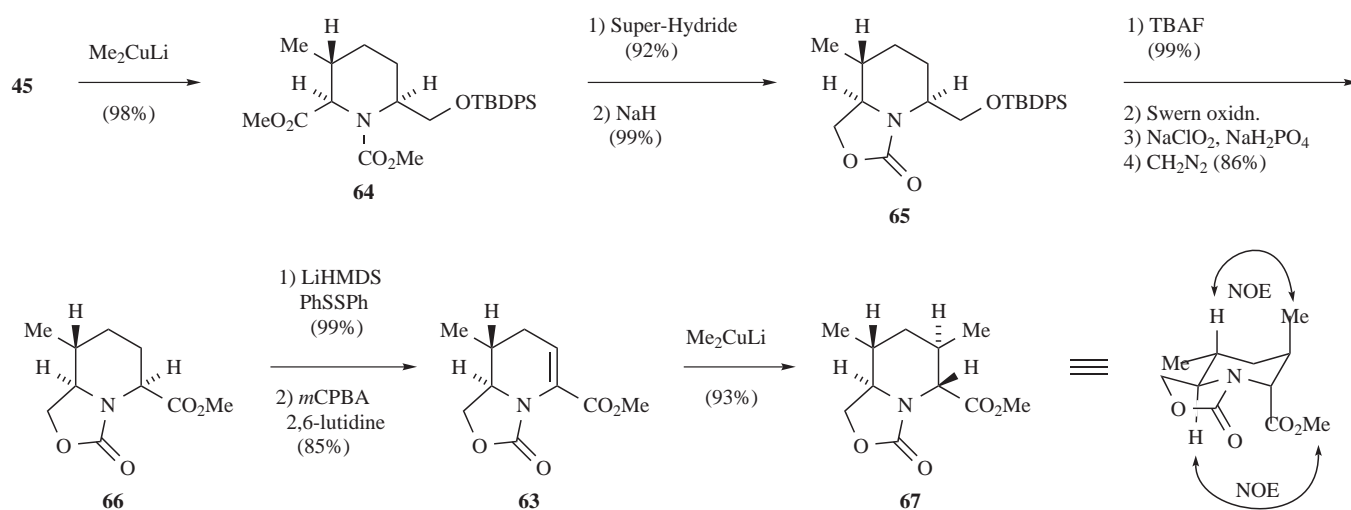


Fig. (12). Strategy for the synthesis of **205B**.



Scheme 16. Construction of the tetrasubstituted piperidine system **67** and determination of its stereochemistry.

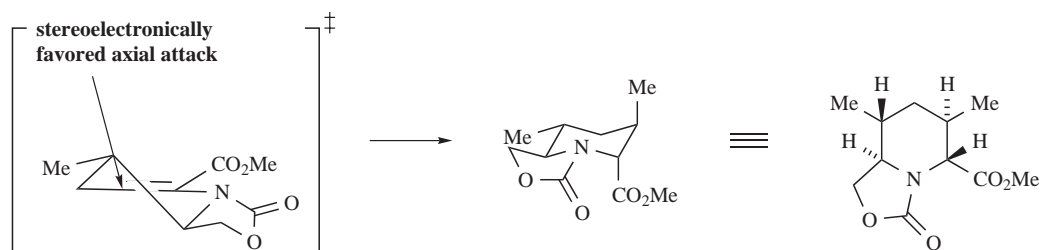


Fig. (13). Stereoselectivity of the Michael-type conjugate addition reaction of enaminoester **63**.

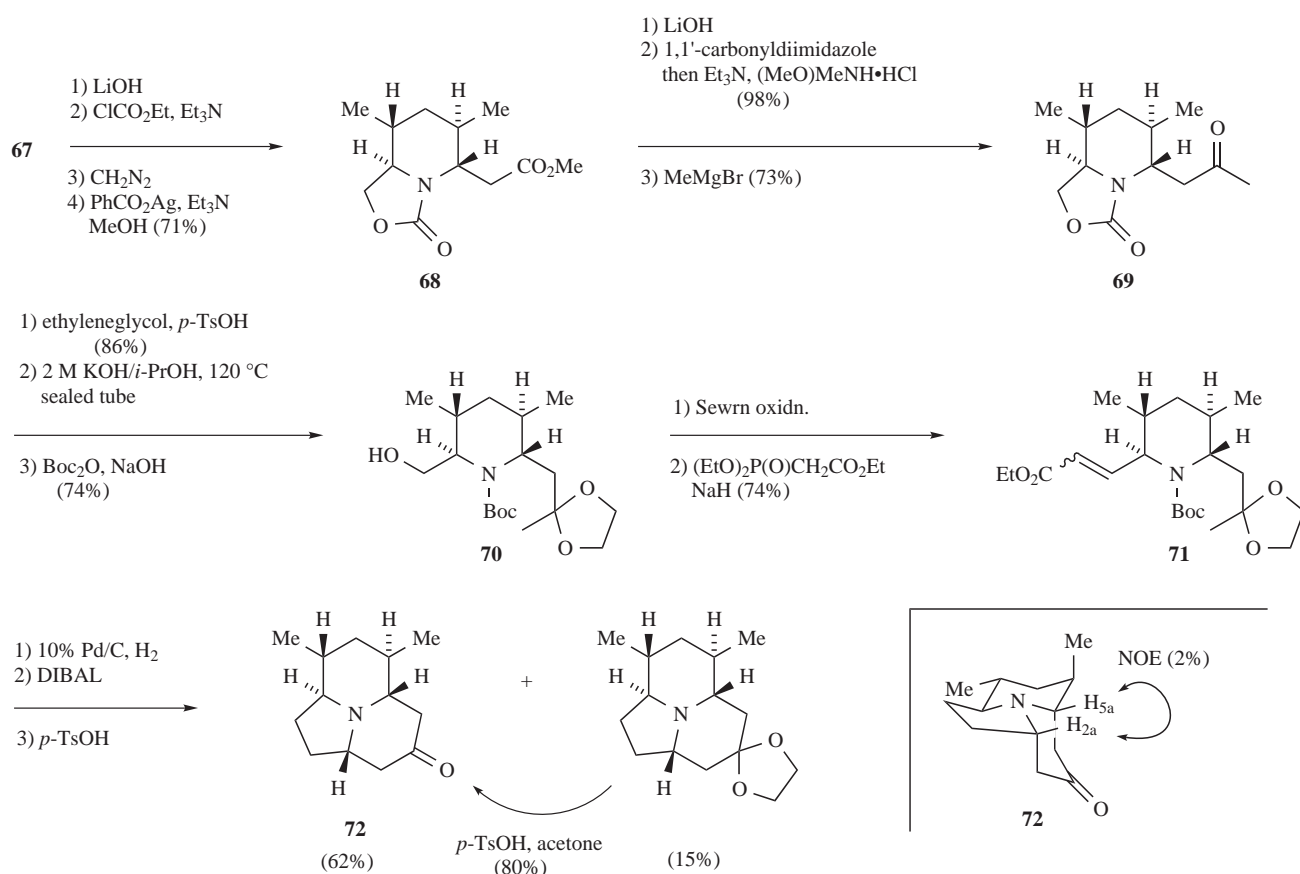
two-step oxidation of the resulting alcohol, followed by the treatment with CH_2N_2 provided the corresponding methyl ester **66**. This ester was transformed into the desired **63** using the Matsumura's protocol [31]. The key and second Michael type conjugate addition reaction of **63** with dimethylolithium cuprate proceeded smoothly to give rise to the adduct **67** again as a single isomer. The stereochemistry of **67** was confirmed by the NOEs shown below.

The observed stereoselectivity of the second conjugate addition reaction can be explained by the stereoelectronic effect [8] illustrated in Fig. 13.

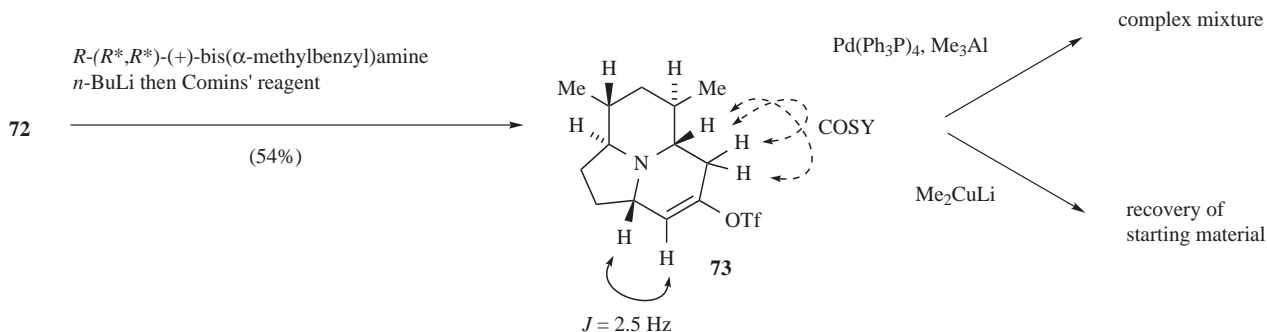
An Arndt-Eistert sequence was used for the carbon-chain extension of **67** to afford the homologated ester **68**, which was transformed into ketone **69** by the reaction of the corresponding Weinreb's amide [32] with the Grignard reagent. Protection of the carbonyl group as an acetal with ethylene

glycol, followed by hydrolysis of the oxazolizinone ring with the base and the protection of the resulting amine with Boc_2O provided the alcohol **70**. Carbon-chain elongation of **70** was performed by Swern oxidation and Horner-Emmons reaction of the resulting aldehyde to give rise to unsaturated ester **71**. Hydrogenation of **71**, half-reduction of the resulting ester to aldehyde and acid-catalyzed intramolecular Mannich-type cyclization of the resulting amine provided the key tricyclic ketone **72** along with its acetal, which was converted to **72** by the treatment with acid. The stereochemistry at the newly formed position in **72** was confirmed by the NOE between H_{2a} and H_{5a} .

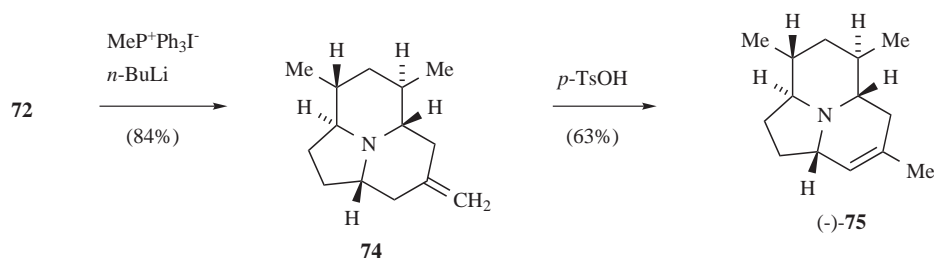
With the requisite ketone **72** in hand, we next focused our attention on the final step for the completion of total synthesis of the alkaloid **205B**. Regioselective enolization of **72** in the presence of a chiral lithium amide base was performed to



Scheme 17. Synthesis of key tricyclic core **72** for the synthesis of **205B**.



Scheme 18. Attempt to conversion of **72** to **205B** via the enoltriflate **73**.



Scheme 19. Completion of the total synthesis of **205B** and determination of the absolute stereochemistry of natural product.

provide the enol triflate as a 3 : 1 mixture of regioisomers, and the major isomer **73** was isolated in 54% yield. The triflate **73** was confirmed to be a desired isomer by the NOE and COSY spectral data. Attempts to convert **73** into the alkaloid **205B** under Takai and Nozaki's protocol [33] or McMurry's reaction [34] conditions led only to a complex mixture or recovered starting material, respectively.

On the other hand, the acid-catalyzed isomerization of the exo-olefin **74**, derived from **72** by Wittig reaction was quite effective, and led to the desired endo-olefin **75** in 63% isolated yield [35]. The spectroscopic data of **75** were identical to that for the natural **205B**. The absolute stereochemistry of natural **205B** was unambiguously determined to be an antipode of our synthetic (+)-**75** by the comparison of optical rotations. Quite recently, Smith's group completed the synthesis of natural enantiomer of the alkaloid (-)-**205B** (**62**) [36].

7. NICOTINIC ACETYLCHOLINE RECEPTORS AS THERAPEUTIC TARGETS IN NEUROLOGICAL DISORDERS

Cholinergic nicotinic neurotransmission is one of the most important neuronal pathways that regulate the physiological processes of reward, cognition, learning and memory [37,38]. Acetylcholine (ACh) released from cholinergic neurons is the neurotransmitter acting on the nicotinic acetylcholine receptors (nAChRs) in many regions of the central nervous system and peripheral nervous system. It has become increasingly evident that the impairment of the nicotinic neurotransmission is associated with various neurological disorders, such as Tourette's syndrome, autism, Alzheimer's disease, Parkinson's disease, schizophrenia and epilepsy [39,40]. Selective nicotinic agonists and antagonists would therefore be very valuable, and are being investigated for possible use in these devastating disorders [41,42].

Nicotinic receptors are ligand-gated ion channels composed of five subunits. The best characterized nAChRs are those being expressed at the neuromuscular junction, i.e. muscle nAChR composed of two $\alpha 1$ subunits and one each of $\beta 1$, δ , and either γ or ϵ [38,42]. The subunit combinations of neuronal nAChRs are much more complex than those of muscle nAChRs, because neuronal nAChRs are known to be pentameric combinations of various subunits, of which 12 ($\alpha 2$ - $\alpha 10$ and $\beta 2$ - $\beta 4$) have been so far identified [42,43]. In fact, different subtypes (e.g. $\alpha 2\beta 2$, $\alpha 3\beta 2$, $\alpha 4\beta 2$, $\alpha 6\beta 2$, $\alpha 2\beta 4$, $\alpha 3\beta 4$, $\alpha 4\beta 4$, $\alpha 6\beta 4$ and $\alpha 7$) have different biophysical and pharmacological properties [40]. These complexities pose difficulties for the development of novel selective nicotinic ligands. Recently, different categories of nAChR subtypes have been created, based on their pharmacology, function

and location. Three major subtypes in the mammalian nervous system are those containing the $\alpha 7$ subunit ($\alpha 7^*$), the $\beta 2$ subunit ($\beta 2^*$), or the $\beta 4$ subunit ($\beta 4^*$) [44]. In particular, $\alpha 4\beta 2$ receptors and $\alpha 7$ homomeric receptors are the most abundant native nAChRs in the central nervous system, whereas $\alpha 3\beta 4$ receptors are the predominant form involved in autonomic ganglionic transmission [38,45].

8. THE FINDING OF SUBTYPE-SELECTIVE NICOTINIC BLOCKERS FROM FROG SKIN ALKALOIDS [46]

As mentioned above, extracts from the skin of certain poison frogs provide a variety of pharmacologically active alkaloids [1,47]. Interestingly, a large number of frog alkaloids have been shown to interact with nAChRs: for instance, epibatidine as a nicotinic agonist, and histrionicotoxins, pumiliotoxins and indolizidines as nicotinic antagonists [47-49]. In terms of the subtype selectivity, however, most frog alkaloids have long remained to be characterized. Recently, an effort has been made to clarify the selectivity of frog alkaloids across several recombinant nAChRs ($\alpha 4\beta 2$, $\alpha 7$, $\alpha 3\beta 2$, $\alpha 3\beta 4$ and $\alpha 4\beta 4$) expressed in *Xenopus* oocytes, using electrophysiological technique [46].

The alkaloid (-)-**235B'**, a 5,8-disubstituted indolizidine, acts as a noncompetitive nicotinic antagonist and inhibits carbamylcholine-elicited $^{22}\text{Na}^+$ -influx via nAChR channels in PC12 cells [49]. This alkaloid is a highly potent blocker of $\alpha 4\beta 2$ nAChRs expressed in oocytes ($\text{IC}_{50} = 0.07 \mu\text{M}$) (Fig. 14, Table 2). The sensitivity of (-)-**235B'** for $\alpha 4\beta 2$ receptors is comparable to that of the best characterized antagonist of $\alpha 4\beta 2$ nAChRs, dihydro- β -erythroidine (DH β E, $\text{IC}_{50} = 0.11 \mu\text{M}$) [50]. Moreover, (-)-**235B'** blocks $\alpha 4\beta 2$ receptors more effectively than $\alpha 7$ receptors (6-fold), $\alpha 3\beta 2$ receptors (40-fold), $\alpha 3\beta 4$ receptors (50-fold) and $\alpha 4\beta 4$ receptors (54-fold). The rank order of potency of (-)-**235B'** is $\alpha 4\beta 2 > \alpha 7 > \alpha 3\beta 2 > \alpha 3\beta 4 \approx \alpha 4\beta 4$, whereas that of DH β E is $\alpha 4\beta 4 > \alpha 4\beta 2 > \alpha 3\beta 2 > \alpha 3\beta 4 \approx \alpha 7$ [50].

In general, open channel blockers exhibit both a voltage-dependent and a use-dependent inhibition of nAChRs [51]. Consistent with the criteria, (-)-**235B'** predominantly blocks the currents through $\alpha 4\beta 2$ receptor-channels that are more frequently open both at higher ACh concentrations (Fig. 15) and at hyperpolarized potentials (Fig. 16). In addition, the $\alpha 4\beta 2$ currents elicited by repetitive ACh pulses are progressively inhibited by (-)-**235B'** in a use-dependent manner (Fig. 17). Therefore, it is most likely that (-)-**235B'** behaves as an open channel blocker of the $\alpha 4\beta 2$ nAChR.

The 5,8-disubstituted indolizidine (-)-**223V** (also called as **I**, which had been expected as **223W** in [46]) has a 5,9-*cis*

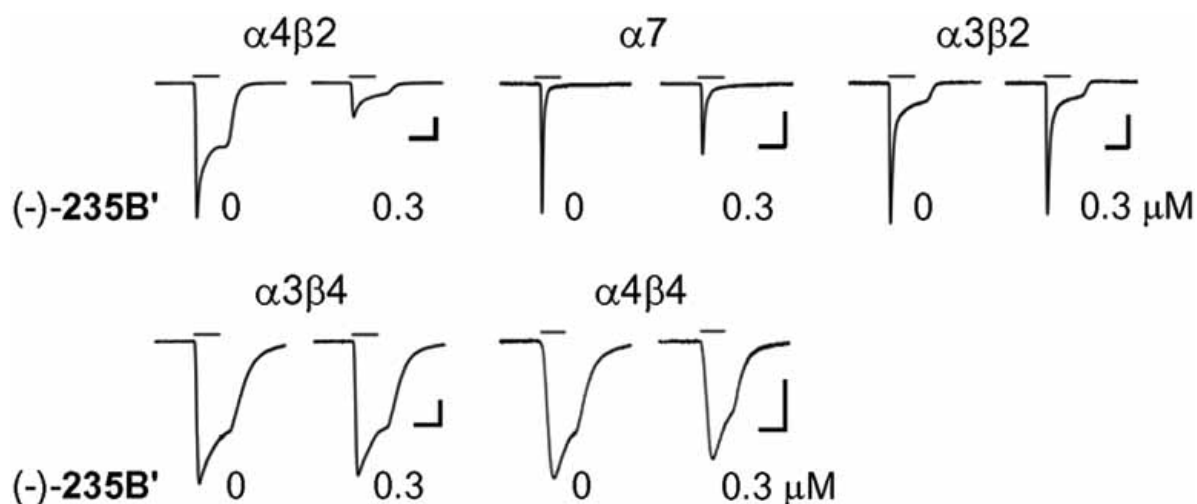


Fig. (14). Inhibitory effects of (-)-235B' on ACh-induced currents in *Xenopus* oocytes expressing recombinant nicotinic receptors. Reproduced, with permission, from [46].

(5,9Z) structure, as well as (-)-235B', and indolizidines **15** ((+)-8,9-diepi-223V, also called as **II** in [46]) and **16** ((-)-9-epi-223V, also called as **III** in [46]) are the stereoisomers that have 5,9-*trans* (5,9E) structures. The indolizidine (-)-223V blocks the responses mediated by $\alpha4\beta2$ receptors and $\alpha7$ receptors similarly, whereas **15** and **16** are more potent on $\alpha7$ receptors than $\alpha4\beta2$ receptors (Table 2). Therefore, the 5,9-*cis* (5,9Z) structure appears to be important for acting on $\alpha4\beta2$ receptors with high affinity. On the other hand, (-)-223V has a negligible effect on the $\alpha3\beta4$ receptor responses at a high concentration (10 μM), whereas the responses through this receptor are substantially blocked by **15** ($\text{IC}_{50} = 14.7 \mu\text{M}$) and are potently blocked by **16** ($\text{IC}_{50} = 3.0 \mu\text{M}$).

The 5,6,8-trisubstituted indolizidine (-)-223A (1-10 μM) exhibits the blocking effects on the ACh-elicited currents mediated by $\alpha4\beta2$ and $\alpha7$ receptors to a similar extent, whereas (+)-6-epi-223A (10 μM) has a negligible effect on

these receptors responses (Table 2). These findings suggest that the alkaloid (-)-223A binds to these receptors in a stereoselective manner. The blocking effect of (-)-223A on $\alpha3\beta4$ currents is similar to that of (+)-6-epi-223A.

The 1,4-disubstituted quinolizidine (-)-1-epi-207I blocks $\alpha7$ receptor responses ($\text{IC}_{50} = 0.6 \mu\text{M}$), with 9-fold higher sensitivity than the blockade of $\alpha4\beta2$ receptor responses and 15-fold higher than that of $\alpha3\beta4$ receptor responses (Table 2). The alkaloid (+)-207I equally blocked the responses mediated by $\alpha4\beta2$ and $\alpha7$ receptors.

When the oocytes expressing $\alpha7$ nAChRs were treated with 8*b*-azaacenaphthylene (+)-205B (3 μM), which is the unnatural enantiomer, the peak amplitude of the ACh-elicited currents was markedly decreased, whereas the $\alpha4\beta2$ and $\alpha3\beta4$ currents were not strongly affected. As summarized in Table 2, (+)-205B blocks the $\alpha7$ receptor-mediated currents ($\text{IC}_{50} = 2.5 \mu\text{M}$) with 5-fold higher sensitivity than the blockade of the $\alpha4\beta2$ and $\alpha3\beta4$ recep-

Table 2. Potency of Blocking Effects of Frog Skin Alkaloids on Recombinant Nicotinic Receptors Expressed in *Xenopus* oocytes

	$\alpha4\beta2$	$\alpha7$	$\alpha3\beta4$
	1 μM ACh	100 μM ACh	100 μM ACh
	IC ₅₀ , μM		
(-)-235B'	0.07	0.4	3.8
(-)-I	6.0	3.4	>10.0
(+)-II	16.8	2.5	14.7
(-)-III	20.1	1.8	3.0
(+)-6-epi-223A	>30.0	>30.0	15.1
(-)-223A	4.5	4.2	14.1
(-)-1-epi-207I	5.2	0.6	8.8
(+)-207I	5.0	3.4	N.D.
(+)-205B	13.5	2.5	11.3

IC₅₀: concentration causing 50% inhibition. N.D.: not determined. Reproduced, with permission, from [46].

tor-mediated currents. Thus, (+)-**205B** can selectively block $\alpha 7$ receptor among the major types of nAChRs.

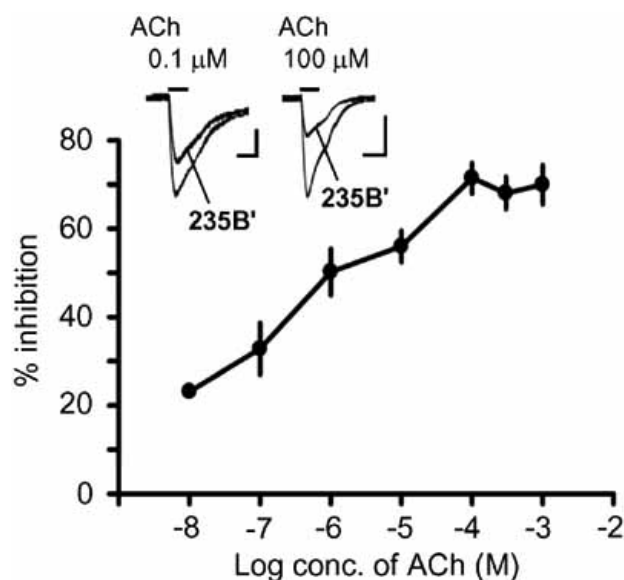


Fig. (15). The percentage inhibition by (-)-**235B'** (0.1 μM) of $\alpha 4\beta 2$ nAChR currents elicited by different concentrations of ACh. Horizontal bars, 5s. Vertical Scale bars, 150 nA for 0.1 μM ACh and 1000 nA for 100 μM ACh. Reproduced, with permission, from [46].

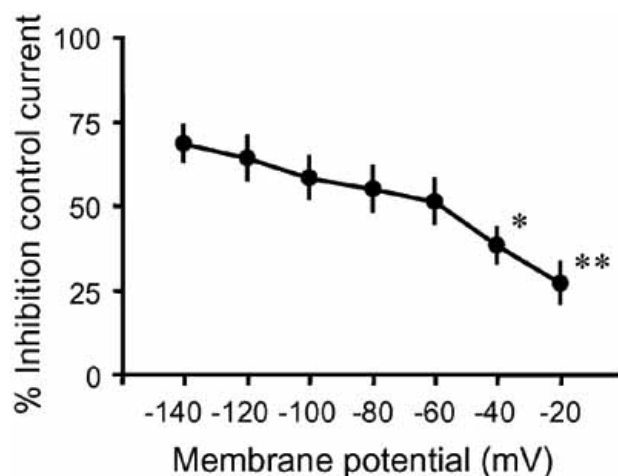


Fig. (16). Voltage-dependent blockade of $\alpha 4\beta 2$ nAChR currents by (-)-**235B'** (0.1 μM). * $P < 0.05$, ** $P < 0.01$, vs. the values at -140 mV. Reproduced, with permission, from [46].

9. PHARMACOLOGICAL SIGNIFICANCE OF SELECTIVE INHIBITION OF NICOTINIC RECEPTORS DISPLAYING A GAIN OF FUNCTION

As one of the most frequent neurological disorders, epilepsy affects 0.5-1% of the world's population [52]. Autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE) is an idiopathic epilepsy, with various symptoms such as nocturnal paroxysmal dystonia, paroxysmal nightmare, paroxysmal arousals and nocturnal wanderings [52]. Several genetic studies reveal that ADNFLE is associated with mutations of the $\alpha 4$ and $\beta 2$ nAChR subunits. Until now, six dif-

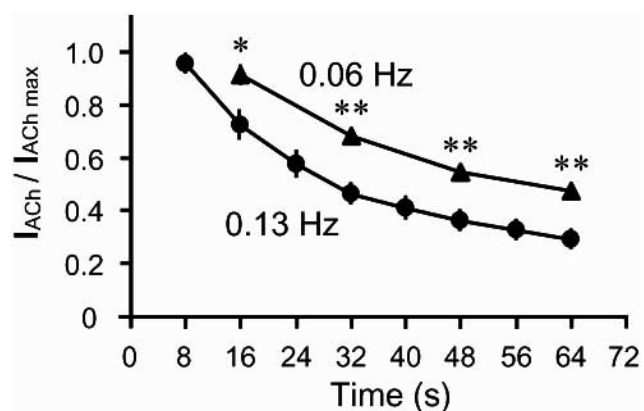


Fig. (17). Use-dependent blockade of $\alpha 4\beta 2$ nAChR currents by (-)-**235B'** (0.1 μM). * $P < 0.05$, ** $P < 0.01$, vs. the values at the higher frequency (0.13 Hz) of ACh pulses. Reproduced, with permission, from [46].

ferent mutations have been identified [53,54]: five missense mutations [$\alpha 4$ (S248F), $\alpha 4$ (S252L), $\alpha 4$ (T261I), $\beta 2$ (V287M) and $\beta 2$ (V287L)] and one small insertion [$\alpha 4$ (L259-260ins)]. Importantly, all these mutations are located within the second transmembrane domain that forms ion channel pore, thereby disrupting the activity of nAChRs [55,56]. Several electrophysiological studies have demonstrated that all mutations in the $\alpha 4$ subunit gene cause a loss of function of the nAChR, whereas mutations in the $\beta 2$ subunit gene exert a gain of function of the receptor [52,53]. Thus, the mutations of the $\alpha 4$ and $\beta 2$ subunits might cause the same phenotype *via* independent mechanisms. Despite these controversies, drug discovery research focuses on some common characteristics shared by all the mutants identified. One of the notable common characteristics is the increase in ACh sensitivity [55]. Moreover, the findings that carbamazepine (an anti-epileptic drug, also known as a noncompetitive blocker of nAChRs) can inhibit seizures in ADNFLE patients strengthen the hypothesis that a gain of function of these mutant receptors leads to the neural network dysfunction involved in the ADNFLE seizures [57]. Therefore, it has been proposed that the most promising therapeutic strategy for the treatment of ADNFLE would be to reduce the enhanced response of the mutant $\alpha 4\beta 2$ nAChRs [57].

To accomplish the goal of selectively inhibiting only mutant receptors, which display enhanced agonist sensitivity, open channel blockers may be valuable. Open channel blockers bind to ionic pore of nAChRs and inhibit the ionic flux through the receptor-channels by steric hinderance. This type of blocker could selectively inhibit the mutant receptors involved in ADNFLE through the following mechanism: (1) since the $\alpha 4$ mutant receptors are hypersensitive to ACh, open channel blocker could effectively block the mutant receptors activated by low concentrations of ACh, without acting at wild-type nAChRs that are not activated; (2) open channel blockers could more easily gain access to the channel pore of the $\beta 2$ mutant receptors, which are desensitized slowly, as compared with wild-type receptors (see [58]). Since the alkaloid (-)-**235B'** is an open channel blocker of $\alpha 4\beta 2$ nAChR, this will be an ideal compound for blocking the mutant receptors. Thus, we expect that the approach based on the frog alkaloids can provide lead compounds for new drugs to treat cholinergic disorders, such as ADNFLE.

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REFERENCES

- [1] Daly JW, Spande TF, Garraffo HM. Alkaloids from Amphibian Skin: A tabulation of Over Eight-Hundred Compounds. *J Nat Prod* 2005; 68: 1556-75.
- [2] Daly JW, Kaneko T, Wilham J, *et al.* Bioactive Alkaloids of Frog Skin: Combinatorial Bioprospecting Reveals That Pumiliotoxins Have an Arthropod Source. *Proc Natl Acad Sci USA* 2002; 99: 13996-14001.
- [3] Daly JW. Nicotinic Agonists, Antagonists, and Modulators from Natural Sources. *Cell Mol Neurobiol* 2005; 25: 513-52.
- [4] Momose T, Toyooka N. Asymmetric Synthesis of the Indolizidine Alkaloids 207A, 209B, and 235B: 6-Substituted 2,3-Didehydropiperidine-2-carboxylate as a Versatile Chiral Building Block. *J Org Chem* 1994; 59: 943-5.
- [5] Toyooka N, Tanaka K, Momose T, Daly JW, Garraffo HM. Highly Stereoselective Construction of *trans*(2,3)-*cis*(2,6)-Trisubstituted Piperidines: An Application to the Chiral Synthesis *Dendrobates* Alkaloids. *Tetrahedron* 1997; 53: 9553-74.
- [6] Momose T, Toyooka N, Jin M. Asymmetric Twin-Ring Differentiation by Lipase-Catalyzed Enantioselective Reaction of the Ring-Crossed *meso* Glycol: Asymmetric Synthesis of a Highly Functionalized Piperidine from the Conjoined Twin Piperidine System. *Tetrahedron Lett* 1992; 33: 5389-90.
- [7] Hoffmann RW. Allylic 1,3-Strain as a Controlling Factor in Stereoselective Transformations. *Chem Rev* 1989; 89: 1841-60.
- [8] Deslongchamps P. In: *Stereoelectronic Effects in Organic Chemistry*. New York, Pergamon. 1983; 209-90.
- [9] Cieplak AS. Stereochemistry of Nucleophilic Addition to Cyclohexanone. The Importance of Two-Electron Stabilizing Interactions. *J Am Chem Soc* 1981; 103: 4540-52.
- [10] Corey EJ, Yuen P. A Short, Stereospecific Route to Chiral *trans*-2,6-Disubstituted Quinuclidines. *Tetrahedron Lett* 1989; 30: 5825-28.
- [11] Shishido Y, Kibayashi C. Enantiogenic Total Syntheses of (-)-Indolizidines (Bicyclic Gephyrotoxins) 205A, 207A, 209B, and 235B *via* the Intramolecular Diels-Alder Reaction of a Chiral *N*-Acylnitroso Compound. *J Org Chem* 1992; 57: 2876-82.
- [12] Toyooka N, Nemoto H, Kawasaki M, Garraffo HM, Spande TF, Daly JW. Enantioselective Syntheses of Two 5,9*E* Diastereomers of 223V, an Alkaloid from the Poison Frog *Dendrobates Pumilio*. *Tetrahedron* 2005; 61: 1187-98.
- [13] Daly JW, Spande TF, Garraffo HM. Unpublished results.
- [14] Toyooka N, Okumura M, Nemoto H. Stereodivergent Process for the Synthesis of the Decahydroquinoline Type of *Dendrobatid* Alkaloids. *J Org Chem* 2002; 67: 6078-81.
- [15] This chiral acetate was synthesized by the lipase-mediated transesterification of the corresponding *meso*-diol in 95% ee, its absolute stereochemistry was determined by its transformation into the known 2-methyl-1-hexanol.
- [16] Comins DL, Dehghani A. Pyridine-Derived Triflating Reagents: An Improved Preparation of Vinyl Triflates from Metallo Enolates. *Tetrahedron Lett* 1992; 33: 6299-302.
- [17] Foti CJ, Comins DL. Synthesis and Reactions of α -(Trifluoromethanesulfonyloxy) Encarbamates Prepared from *N*-Acyl lactams. *J Org Chem* 1995; 60: 2656-57; Nicolaou KC, Shi G-Q, Namoto K, Bernal F. Synthesis of *N*-Heterocycles *via* Lactam-Derived Ketene Amino Phosphates. Asymmetric Synthesis of Cyclic Amino Acids. *Chem Commun* 1998; 1757-8.
- [18] Yamada S, Kasai Y, Shioiri T. Diethylphosphoryl Cyanide. A New Reagent for the Synthesis of Amides. *Tetrahedron Lett* 1973; 1595-8.
- [19] Toyooka N, Nemoto H. First Enantioselective Synthesis of (+)-Quinolizidine 207I: Determination of the Absolute Stereochemistry. *Tetrahedron Lett* 2003; 44: 569-570.
- [20] Michel P, Rassat A. Total Synthesis of (\pm)-Quinolizidine 207I, an Alkaloid from *Mantella Baroni*, a Madagascan Mantelline Frog. *Chem Commun* 1999; 2281-2.
- [21] Michel P, Rassat A, Daly JW, Spande TF. A Stereospecific Synthesis of (\pm)-5,8-Disubstituted Indolizidines and (\pm)-1,4-Disubstituted Quinolizidines Found in Poison Frog Skin. *J Org Chem* 2000; 65: 8908-18.
- [22] Izquierdo I, Plaza MP, Rodriguez M, Tamayo J. Chiral Building-Blocks by Chemoenzymatic Desymmetrization of 2-Ethyl-1,3-propanediol for the Preparation of Biologically Active Natural Products. *Tetrahedron: Asymmetry* 1999; 10: 449-55.
- [23] Toyooka N, Fukutome A, Nemoto H, *et al.* Synthesis of Alkaloid 223A and a Structural Revision. *Org Lett* 2002; 4: 1715-18.
- [24] Toyooka N, Fukutome A, Shinoda H, Nemoto H. Stereodivergent Synthesis of the 2,3,5,6-Tetrastituted Piperidine Ring System: An Application to the Synthesis of Alkaloids 223A and 205B from Poison Frogs. *Tetrahedron* 2004; 60: 6197-216.
- [25] Garraffo HM, Jain P, Spande TF, Daly JW. Alkaloid 223A: The First Trisubstituted Indolizidine from *Dendrobatid* Frogs. *J Nat Prod* 1997; 60: 2-5.
- [26] Ezquerra J, Escribano A, Rubio A, Remuinan MJ, Vaquero JJ. 4-Benzyl-2,3-didehydropicolinate as a Homochiral Template for Michael Additions. Synthesis of Enantiopure α -Allokainoids, β -Kainoids, 2,3-Methanoproline and Other 3,4-Disubstituted Proline. *Tetrahedron: Asymmetry* 1996; 7: 2613-26.
- [27] Pu X, Ma D. Asymmetric Total Synthesis of (-)-Alkaloid 223A and Its 6-Epimer *J Org Chem* 2003; 68: 4400-5; Harris JM, Padwa A. A Flexible Approach toward Trisubstituted Piperidines and Indolizidines: Synthesis of 6-*epi*-Indolizidine 223A. *J Org Chem* 2003; 68: 4371-81; Kumareswaran R, Gallucci J, Rajanbabu TV. Tuning the Acceptors in Catalyzed Cyclizations Initiated by Allenes. Silyl-stannylation/Cyclization of Allene-Aldehydes for Synthesis of Polyalkylated Indolizidines Including 223A Congeners *J Org Chem* 2004; 69: 9151-8; Zhu W, Dong D, Puy X, Ma D. An Efficient Sequential Reaction Process to Polysubstituted Indolizidines and Quinolizidines and Its Application to the Total Synthesis of Indolizidine 223A *Org Lett* 2005; 7: 705-8; Davis FA, Yang B. Asymmetric Synthesis of α -Substituted β -Amino Ketones from Sulfinimines (*N*-Sulfinyl Imines). Synthesis of the Indolizidine (-)-223A. *J Am Chem Soc* 2005; 127: 8398-407.
- [28] Toyooka N, Fukutome A, Shinoda H, Nemoto H. Total Synthesis of the Antipode Alkaloid 205B. *Angew Chem Int Ed* 2003; 42: 3808-10.
- [29] Tokuyama T, Nishimori N, Shimada A, Edwards MW, Daly JW. New Classes of Amidine, Indolizidine and Quinolizidine Alkaloids from a Poison-Frog *Dendrobates Pumilio* (Dendrobatidae). *Tetrahedron* 1987; 43: 643-57.
- [30] Tokuyama T, Garraffo HM, Spande TF, Daly JW. A Revised Structure for Alkaloid 205B, a Novel 8 β -Azaacenaphthylene from a Poison Frog. *An Soc Quim Argent* 1998; 86: 291-8.
- [31] Matsumura Y, Inoue M, Nakamura Y, Talib IL, Maki T, Onomura O. A Convenient Method for Synthesis of Optically Active 2,3-Methanopipicolic Acid. *Tetrahedron Lett* 2000; 41: 4619-22.
- [32] Nahm S, Weinreb SM. *N*-Methoxy-*N*-methylamides as Effective Acylating Agents. *Tetrahedron Lett* 1981; 22: 3815-8.
- [33] Takai K, Sato M, Oshima K, Nozaki H. Cross-Coupling Reaction between Enol Phosphates and Organoaluminum Compounds in the Presence of Palladium (0) Catalyst. *Bull Chem Soc Jpn* 1984; 57: 108-15.
- [34] McMurry JE, Scott WJ. A New Method of Olefin Synthesis. Coupling of Lithium Dialkylcuprates with Enol Triflates. *Tetrahedron Lett* 1980; 21: 4313-6.
- [35] Calculations were performed by using the B3LYP method in Gaussian98 with the 6-31 + G** basis set. The desired isomer 75 is more stable than the olefinic isomer by 2.01 kcal mol⁻¹.
- [36] Smith III AB, Kim D-S. Total Synthesis of the Neotropical Poison-Frog Alkaloid (-)-205B. *Org Lett* 2005; 7: 3247-3250; A General, Convergent Strategy for the Construction of Indolizidine Alkaloids: Total Syntheses of (-)-Indolizidine 223AB and Alkaloid (-)-205B. *J Org Chem* 2006; 71: 2547-2557.
- [37] Changeux JP, Bertrand D, Corringier PJ, *et al.* Brain nicotinic receptors: structure and regulation, role in learning and reinforcement. *Brain Res Brain Res Rev* 1998; 26: 198-216; Dani JA. Overview of nicotinic receptors and their roles in the central nervous system. *Biol Psychiatry* 2001; 49: 166-74.
- [38] Lindstrom JM. Nicotinic acetylcholine receptors of muscles and nerves: comparison of their structures, functional roles, and vulnerability to pathology. *Ann NY Acad Sci* 2003; 998: 41-52.

- [39] Weiland S, Bertrand D, Leonard S. Neuronal nicotinic acetylcholine receptors: from the gene to the disease. *Behav Brain Res* 2000; 113: 43-56.
- [40] Gotti C, Clementi F. Neuronal nicotinic receptors: from structure to pathology. *Prog Neurobiol* 2004; 74: 363-96.
- [41] Lloyd GK, Williams M. Neuronal nicotinic acetylcholine receptors as novel drug targets. *J Pharmacol Exp Ther* 2000; 292:461-7.
- [42] Dani JA, De Biasi M, Liang Y, *et al.* Potential applications of nicotinic ligands in the laboratory and clinic. *Bioorg Med Chem Lett* 2004; 14: 1837-9.
- [43] Hogg RC, Raggenbass M, Bertrand D. Nicotinic acetylcholine receptors: from structure to brain function. *Rev Physiol Biochem Pharmacol* 2003; 147: 1-46.
- [44] Alkondon M, Albuquerque EX. Diversity of nicotinic acetylcholine receptors in rat hippocampal neurons. I. Pharmacological and functional evidence for distinct structural subtypes. *J Pharmacol Exp Ther* 1993; 265: 1455-73; Zoli M, Léna C, Picciotto MR, Changeux JP. Identification of four classes of brain nicotinic receptors using $\beta 2$ mutant mice. *J Neurosci* 1998; 18: 4461-72.
- [45] Clarke PB. Nicotinic modulation of thalamocortical neurotransmission. *Prog Brain Res* 2004; 145: 253-60.
- [46] Tsuneki H, You Y, Toyooka N, *et al.* Alkaloids indolizidine 235B', quinolizidine 1-epi-207I, and the tricyclic 205B are potent and selective noncompetitive inhibitors of nicotinic acetylcholine receptors. *Mol Pharmacol* 2004; 66: 1061-9.
- [47] Daly JW, Garraffo HM, Spande TF. Alkaloids from amphibian skins, in *Alkaloids: Chemical and Biological Perspectives*, 1999; Vol. 13, (Pelletier SW ed) pp 1-161, Pergamon Press, New York.
- [48] Spivak CE, Maleque MA, Oliveira AC, *et al.* Actions of the histri-
nicotins at the ion channel of the nicotinic acetylcholine receptor and at the voltage-sensitive ion channels of muscle membranes. *Mol Pharmacol* 1982; 21: 351-61; Warnick JE, Jessup PJ, Overman LE, *et al.* Pumiliotoxin-C and synthetic analogues. A new class of nicotinic antagonists. *Mol Pharmacol* 1982; 22: 565-73; Aronstam RS, Daly JW, Spande TF, Narayanan TK, Albuquerque EX. Interaction of gephyrotoxin and indolizidine alkaloids with the nicotinic acetylcholine receptor-ion channel complex of Torpedo electroplax. *Neurochem Res* 1986; 11: 1227-40.
- [49] Daly JW, Nishizawa Y, Padgett WL, *et al.* 5,8-Disubstituted indolizidines: a new class of noncompetitive blockers for nicotinic receptor-channels. *Neurochem Res* 1991; 16: 1213-8.
- [50] Chavez-Noriega LE, Crona JH, Washburn MS, Urrutia A, Elliott KJ, Johnson EC. Pharmacological characterization of recombinant human neuronal nicotinic acetylcholine receptors $\alpha 2\beta 2$, $\alpha 2\beta 4$, $\alpha 3\beta 2$, $\alpha 3\beta 4$, $\alpha 4\beta 2$, $\alpha 4\beta 4$ and $\alpha 7$ expressed in *Xenopus* oocytes. *J Pharmacol Exp Ther* 1997; 280: 346-56.
- [51] Buisson B, Bertrand D. Open-channel blockers at the human $\alpha 4\beta 2$ neuronal nicotinic acetylcholine receptor. *Mol Pharmacol* 1998; 53: 555-63; Pintado AJ, Herrero CJ, Garcia AG, Montiel C. The novel $\text{Na}^+/\text{Ca}^{2+}$ exchange inhibitor KB-R7943 also blocks native and expressed neuronal nicotinic receptors. *Br J Pharmacol* 2000; 130: 1893-902.
- [52] Combi R, Dalpra L, Tenchini ML, Ferini-Strambi L. Autosomal dominant nocturnal frontal lobe epilepsy: a critical overview. *J Neurol* 2004; 251: 923-34.
- [53] Sutor B, Zolles G. Neuronal nicotinic acetylcholine receptors and autosomal dominant nocturnal frontal lobe epilepsy: a critical review. *Pflugers Arch* 2001; 442: 642-51.
- [54] Raggenbass M, Bertrand D. Nicotinic receptors in circuit excitability and epilepsy. *J Neurobiol* 2002; 53: 580-9.
- [55] Bertrand D, Picard F, Le Hellard S, *et al.* How mutations in the nAChRs can cause ADNFLE epilepsy. *Epilepsia*, 2002; 43 (Suppl 5): 112-22.
- [56] Rózycka A, Trzeciak WH. Genetic basis of autosomal dominant nocturnal frontal lobe epilepsy. *J Appl Genet* 2003; 44: 197-207.
- [57] Hogg RC, Bertrand D. Neuronal nicotinic receptors and epilepsy, from genes to possible therapeutic compounds. *Bioorg Med Chem Lett* 2004; 14: 1859-61.
- [58] De Fusco M, Becchetti A, Patrignani A, *et al.* The nicotinic receptor beta 2 subunit is mutant in nocturnal frontal lobe epilepsy. *Nat Genet* 2000; 26: 275-6.