Enantioselective Total Syntheses of Indolizidine Alkaloids 167B and 209D

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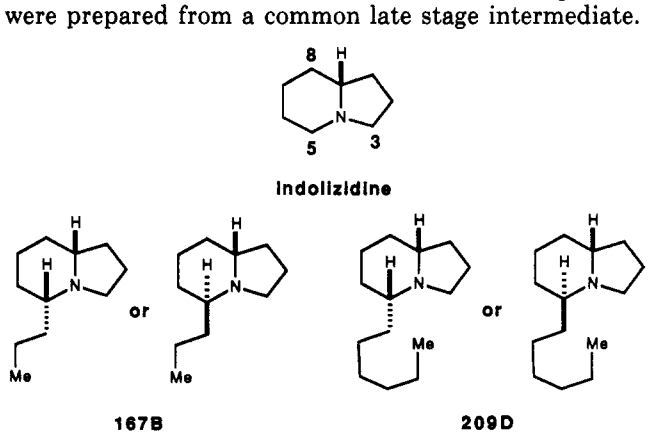
Enantioselective total syntheses of the indolizidine alkaloids **167B** and **209D** are described. The syntheses proceed via a common late-stage intermediate, amino nitrile 8. Each alkaloid was prepared in 10 steps and approximately 23% overall yield from (*S*)-(−)-α-phenethylamine. The configurations of these materials were determined by a combination of one- and two-dimensional NMR experiments.

The indolizidine (also referred to as "bicyclic gephyrotoxin") alkaloids are a family of 22 natural products isolated from the skin secretions of neotropical frogs.[1] The simplest bicyclic gephyrotoxin alkaloids possess a single substituent at C5 of the indolizidine skeleton, while more complex members are substituted at either C3 and C5 or C5 and C8. These indolizidine alkaloids represent a class of noncompetitive blockers of neuromuscular transmission, based on the reported ability of several members of the family to antagonize binding of labeled histrionicotoxin to the acetylcholine receptor (AChR) complex from *Torpedo californica* electric organ.[2]

The structural assignments of several bicyclic gephyrotoxin alkaloids are at present very tentative. For example, alkaloids **167B** and **209D** are very minor trace constituents which have been detected in only one population of frogs. Since these materials occur in such trace amounts, they have not been isolated and fully characterized. The single piece of physical evidence upon which their structure is tentatively assigned are mass spectral parent ions at *m/z* 167 and 209 and a common base peak at *m/z* 124. The base peak at *m/z* 124 is characteristic of 5-substituted indolizidines. The absolute configurations of **167B** and **209D** were inferred by analogy to the structurally related indolizidine **223AB** whose absolute stereochemistry is known.[3] In an attempt (1) to unambiguously assign the correct structures for these natural products, (2) to develop a general protocol for the preparation of structurally related and more complex indolizidine and quinolizidine alkaloids, and (3) for a collaborative effect with Dr. John Daly's group at the National Institutes of Health to probe the dependence of indolizidine stereochemistry on binding to the AChR complex, we undertook the syntheses of alkaloids **167B** and **209D**. We report herein practical, concise, enantioselective total syntheses of two diastereoisomers corresponding to the gross structures of the indolizidine alkaloids **167B**[4] and **209D**. These four compounds were prepared from a common late stage intermediate.



† Charles R. Hauser Fellow 1989–1990.

The syntheses begin by thermal condensation[5] of (*S*)-(−)-α-phenethylamine and succinic anhydride (Scheme I). As reported previously,[5] reduction of the imide **1**[6] with lithium triethylborohydride[7] produced a 95:5 mixture of hydroxylactam diastereoisomers **2** in high yield. Purification and diastereomer separation was achieved by simple recrystallization. Thus, after two recrystallizations the major hydroxylactam diastereomer was isolated in 92% yield from imide **1**. Reaction of hydroxylactam **2** with allyltrimethylsilane in the presence of stannic chloride at −22 °C produced an 82:18 mixture of allyllactam diastereomers **3a** and **3b** in 97% yield. The absolute configuration of the major diastereomer **3a** was assigned unambiguously by chemical correlation with (*S*)-(+)-2-pyrrolidinone-5-acetic acid as reported in ref 5. Reduction of the mixture of allyllactams **3a–b** was achieved with lithium aluminum hydride in refluxing THF.[8] The resultant olefinic amines **4** were then hydrozirconated (1.3 equiv of Cp_2ZrHCl, C_6H_6, 25 °C, 10 h) and carbonylated[9] (1 atm CO, 25 °C, 5 h) to afford a mixture of homologated aldehydes **5** (68%). Experimentally, we found that a modified workup of the hydrozirconation reaction which involved stirring the hydrolysis mixture with excess aqueous disodium–EDTA for several hours solubilized the voluminous emulsion of zirconium salts, making product isolation much easier. It was also necessary to recrystallize the zirconocene dichloride prior to preparation of the zirconocene chloride hydride[10] in order to reproducibly obtain the carbonylated amines **5**. The aldehydes **5** were then converted to the corresponding dimethyl acetals **6a** and **6b** with excess trimethyl orthoformate in acidic methanol.[11] The major isomer **6a** ($[\alpha]_D$ −67.6° (*c* 1.1, CH_2Cl_2)) and minor isomer **6b** ($[\alpha]_D$ +1.7° (*c* 1.1, CH_2Cl_2))

(1) Daly, J. W.; Spande, T. F. In *Alkaloids: Chemical and Biological Perspectives*; Pelletier, S. W., Ed.; Wiley: New York, 1986; Vol. 4, Chapter 1.
(2) Aronstam, R. S., Daly, J. W.; Spande, T. F.; Narayanan, T. K.; Albequerque, E. X. *Neurochemical Res.* **1986**, *11*, 1227.
(3) Royer, J.; Husson, H.-P. *Tetrahedron Lett.* **1985**, *26*, 1515. Tokuyama, T.; Nishimori, N.; Karle, I. L.; Edwards, M. W.; Daly, J. W. *Tetrahedron* **1986**, *42*, 3453.
(4) A racemic synthesis of **167B** has appeared: Smith, A. L.; Williams, S. F.; Holmes, A. B.; Hughes, L. R.; Lidert, Z.; Swithenbank, C. *J. Am. Chem. Soc.* **1988**, *110*, 8696. Recent synthesis of (±)-**223AB**: Watanabe, Y.; Iida, H.; Kibayashi, C. *J. Org. Chem.* **1989**, *54*, 4088. Brandi, A.; Cordero, F.; Querci, C. *J. Org. Chem.* **1989**, *54*, 1748. Broka, C. A.; Eng, K. K. *J. Org. Chem.* **1986**, *51*, 5045. Edwards, O. E.; Greaves, A. M.; Sy, W. W. *Can. J. Chem.* **1988**, *66*, 1163. For a comprehensive review of earlier synthetic work, see ref 1.
(5) Polniaszek, R. P.; Belmont, S. E.; Alvarez, R. *J. Org. Chem.* **1990**, *55*, 215.
(6) Wakabayashi, T.; Saito, M. *Tetrahedron Lett.* **1977**, 93.
(7) Chen, J.; Browne, L. J.; Gonnela, N. C. *Chem. Commun.* **1986**, 905.
(8) Hart, D. J.; Tsai, Y.-M. *J. Am. Chem. Soc.* **1984**, *106*, 8209.
(9) Bertelo, C. A.; Schwartz, J. *J. Am. Chem. Soc.* **1975**, *97*, 228.
(10) Buchwald, S. L.; LaMaire, S. J.; Nielsen, R. B.; Watson, B. T.; King, S. M. *Tetrahedron Lett.* **1987**, *28*, 3895.
(11) Mori, K.; Kato, M.; Kuwahara, S. *Justus Liebigs Ann. Chem.* **1985**, 861.

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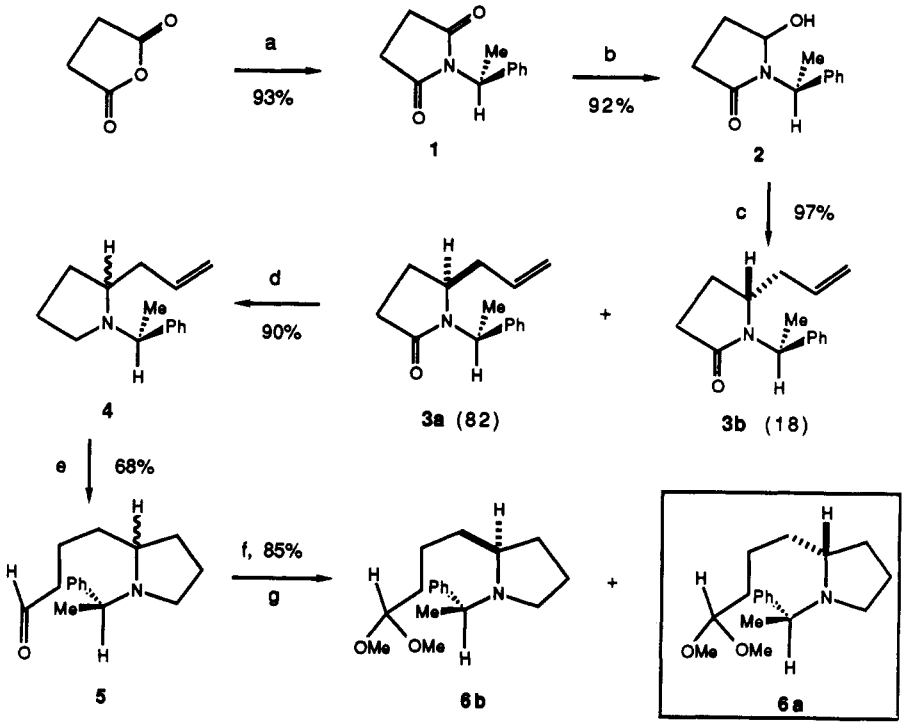
Scheme I[a]



[a] Reagents: (a) (*S*)-(−)-α-phenethylamine, 180 °C, neat, 20 h; (b) $LiEt_3BH$, THF, −78 °C; (c) $SnCl_4$, CH_2Cl_2, −22 °C, allyltrimethylsilane, 3.5 h; (d) 8 mol equiv of $LiAlH_4$, THF, 66 °C, 2 h; (e) 1.3 equiv of Cp_2ZrHCl, C_6H_6, 25 °C, 10 h, then 1 atm CO, 5 h, then Na_2–EDTA(aq); (f) excess $(MeO)_3CH$, MeOH, 1.05 PPTS, reflux, 9.5 h; (g) medium-pressure liquid chromatography.

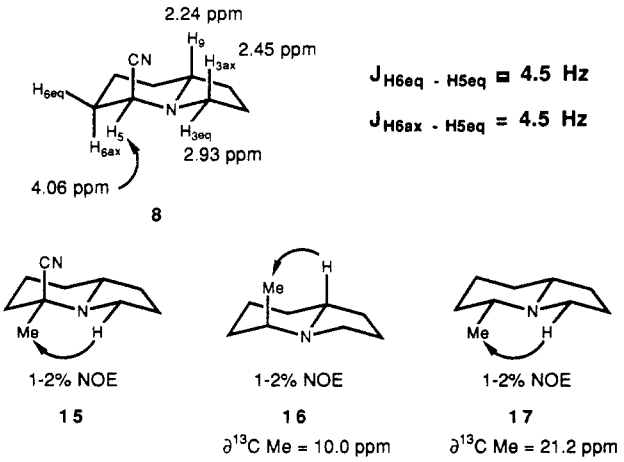
were separable by medium-pressure liquid chromatography. Scheme II reports the final steps of stereoselective syntheses of both diastereomers of **167B** and **209D** based on the major amino acetal **6a**.

The phenethyl moiety of amine **6a** (Scheme II) was hydrogenolyzed in the presence of 10% Pd/C and ammonium formate in methanol.[12] The acetal of the resultant secondary amine **7** was then hydrolyzed in the presence of hydrogen cyanide.[13] Under these conditions, the transient iminium ion was trapped by cyanide producing amino nitrile **8** as a single stereoisomer. The presence of a moderately strong Bohlmann band in the infrared spectrum of **8** at 2810 cm^{-1} implied that this substance possessed a trans ring fusion.[14] According to the Bohlmann correlation, an indolizidine will possess one or more strong infrared absorptions between 2700 and 2800 cm^{-1} when two or more hydrogens attached to carbon atoms adjacent to nitrogen are oriented trans and diaxial to the nitrogen lone electron pair. The aminonitrile **8** satisfies this stereoelectronic criterion only when the ring fusion is trans.

The appearance of the Bohlmann band of **8** at the unusually high frequency of 2810 cm^{-1} can be rationalized by invoking delocalization of the nitrogen lone electron pair into the C5-CN σ* orbital. Such delocalization would reduce the amount of donation of the nitrogen lone electron pair into the antibonding orbitals linking both C3 and C9 to their axial hydrogens.[15] This phenomenon should result in an increase in the C3–$H3_{ax}$ and C9–$H9_{ax}$ bond strengths and a higher frequency Bohlmann band. The stereochemical assignment of **8** appears reasonable since the trans-fused form of the parent ring system, indolizidine, has been estimated to be more stable than cis-fused indolizidine by approximately 2.4 kcal/mol,[16] and the nitrile group possesses a small effective steric size (the *A* value for CN is 0.2).[17] Furthermore, Husson et al. have observed that 6-substituted 2-cyanopiperidines prefer conformations in which the cyano group is axial. These authors noted that there is a gain in stabilization energy when the 2-cyano group is axial and likened this preferred orientation of the cyano group in these piperidines to the "anomeric effect" observed in pyranose sugars.[18] Returning to consideration of amino nitrile **8**, additional support for the 5*R*,9*R* stereochemical assignment was provided by off-resonance ^1H homodecoupling experiments. Sequential irradiation of each of the H6 protons of the indolizidine ring revealed that the geminal H5–H6 coupling constants were 4.5 and 4.5 Hz, respectively. These values are consistent with those expected for $H5_{eq}$–$H6_{ax}$ and $H5_{eq}$–$H6_{eq}$ coupling constants.

The amino nitrile **8** served as a pivotal precursor of both diastereomers of alkaloids **167B** and **209D**. Thus, deprotonation of amino nitrile **8** with LDA (THF, −78 → 0 °C)[19] followed by alkylation with either propyl bromide or hexyl bromide afforded alkylated amino nitriles **9** (84%) and **10** (85%). Each alkylation reaction produced only one of the two possible diastereomeric products. The orientation of the alkyl groups in **9** and **10** was assigned as equatorial, by analogy to results obtained in alkylation reactions of structurally related cyclic α-amino nitriles.[20] Rigorous

(12) Ram, S.; Ehrenkaufer, R. E. *Synthesis* **1988**, 91.
(13) Royer, J.; Husson, H.-P. *Tetrahedron Lett.* **1985**, *26*, 1515.
(14) (a) Bohlman, F. *Chem. Ber.* **1958**, *91*, 2157. (b) Theobald, A. E.; Lingard, R. G. *Spectrochim. Acta* **1968**, *24A*, 1245. (c) Sonnett, P. E.; Oliver, J. E.; Netzel, D. A.; Mendoza, R. *J. Heterocycl. Chem.* **1979**, *16*, 1041. (d) Sonnett, P. E. *J. Heterocycl. Chem.* **1975**, 289. (e) Lüning, B.; Leander, C. *Acta Chem. Scand.* **1967**, *21*, 2136.
(15) Hamlow, H. P.; Okuda, S.; Nakagawa, N. *Tetrahedron Lett.* **1964**, 2553.
(16) Aaron, H. S.; Ferguson, C. P. *Tetrahedron Lett.* **1968**, 6191.
(17) Jensen, F. R.; Bushweller, C. H. *Adv. Alicycl. Chem.* **1971**, *3*, 139.
(18) Bonin, M.; Romero, J. R.; Grierson, D. S.; Husson, H.-P. *J. Org. Chem.* **1984**, *49*, 2392.
(19) Stork, G.; Ozorio, A. A.; Leong, A. Y. W. *Tetrahedron Lett.* **1978**, 5175.



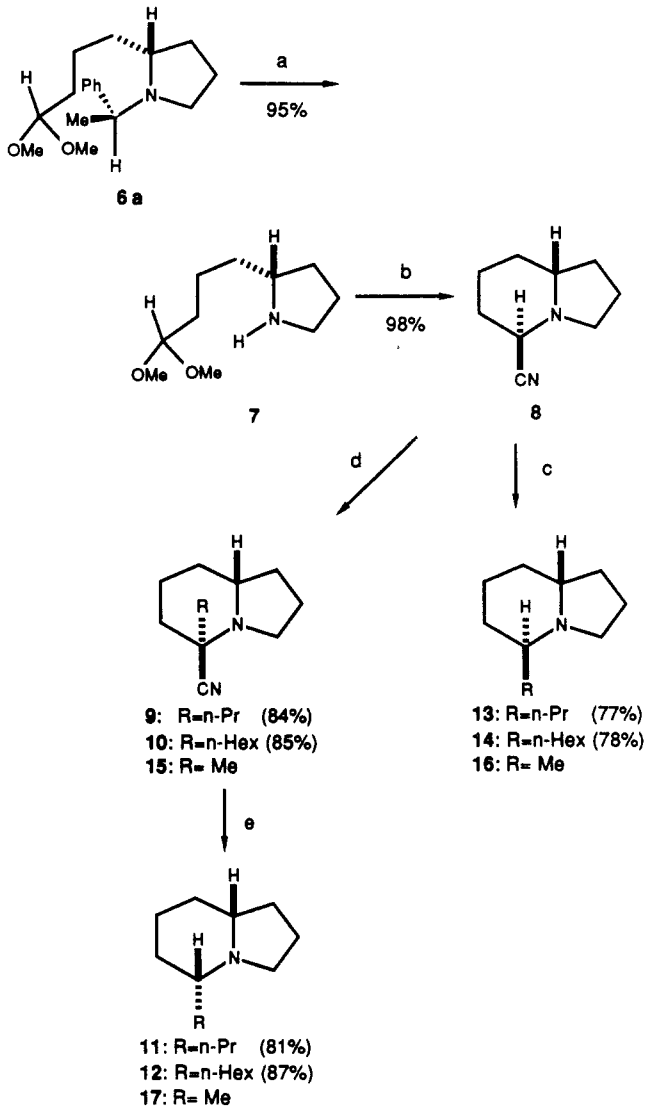
support for these assignments was provided by a combination of chemical and multinuclear NMR experiments (vide infra). Reduction of the amino nitriles with sodium borohydride in ethanol[21] at ambient temperature produced amines **11** (81%) and **12** (87%). Alternatively, reaction of cyano amine **8** with either propylmagnesium bromide or hexylmagnesium bromide[22] afforded amines **13** (77%) and **14** (78%).

The stereochemical and conformational assignments for compounds **8–14** were supported by rigorous one- and two-dimensional nuclear magnetic resonance experiments on these substances and their methyl-substituted congeners **15–17**. The important chemical shift data for positions 3, 5, and 9 of the indolizidine skeleton of these materials is presented in Table I. The relevant spectral data for indolizidine[23,24] is listed in Table I for purposes of comparison.

As articulated earlier, we assign an axial orientation to the nitrile moiety of α-amino nitrile **8**. Proton magnetic resonance studies of the trans-fused forms of indolizidine[14c-e,23] and quinolizidine[15] have shown that the resonances of equatorial hydrogens α to the nitrogen resonate at substantially lower field than do α-axial hydrogens. The hydrogen attached to position 5 of α-amino nitrile **8** resonated at 4.06 ppm. The equatorial hydrogen attached to C3 resonated at 2.93 ppm and the axial C3 hydrogen resonated at 2.45 ppm. The C3-H resonances were coupled to one another as proven by a homodecoupling experiment. Finally, the hydrogen attached to C9 appeared as a discrete multiplet at 2.24 ppm. Support for these assignments was provided by a combination of ^{1}H–^{13}C cross-correlation of spin-decoupled (CCSD or HETCOR)[25] and APT (attached proton test for ^{13}C)[26] NMR spectral experiments. Thus in the CCSD experiment, the furthest downfield proton (δ 4.06) correlated to the carbon assigned as C5, the coupled proton resonances at 2.93 and 2.45 correlated to a single carbon assigned as C3, and the proton resonance at 2.24 ppm correlated with a carbon assigned as C9. The attached proton test (APT) spectrum was fully consistent with these assignments. Thus, carbons assigned as C5 and C9 appeared as negative intensity signals (methine carbons), while the C3 resonance appeared as a positive peak (methylene carbon). All the rest of the compounds appearing in Table I were analyzed in exactly the same manner.

Inspection of Table I reveals an upfield shift of the ^{13}C resonances of both C3 and C9 in amino nitrile **8** relative to indolizidine, which is consistent with the axial orientation of the nitrile moiety in **8** (steric compression shift of C3 and C9). Alkylation of α-amino nitrile **8** produced compounds **15**, **9**, and **10**. The stereochemical assignment of **15** was supported by an NOE experiment. Thus, irradiation of the C3-H_{eq} signal at 3.05 ppm resulted in a 1–2% enhancement of the methyl group attached to C5. This observation is consistent with an equatorially oriented methyl group in **15**. As documented in Table I, the consistency in chemical shift values for the hydrogens attached to C3 and C9, and the regularity of ^{13}C chemical shifts for positions, 3, 5, and 9 in compounds **15**, **9**, and **10**, is indicative that each of these compounds possesses the same configuration at C5.

Scheme II[a]



[a] Reagents: (a) 10% Pd/C, MeOH, NH_4HCO_2, 25 °C, 3 h; (b) KCN, HCl-H_2O-CH_2Cl_2, 25 °C, 3 h; (c) RMgBr, Et_2O, 25 °C, 7 h; (d) LDA, THF, -78 to 0 °C, then RBr, -78 to 0 °C; (e) 7 equiv of $NaBH_4$, EtOH, 25 °C, 20 h.

(20) Bonin, M.; Besselievre, R.; Grierson, D. S.; Husson, H.-P. *Tetrahedron Lett.* **1983**, *24*, 1493. Bonin, M.; Romero, J. R.; Grierson, D. S.; Husson, H.-P. *Tetrahedron Lett.* **1982**, *23*, 3369. Zeller, E.; Grierson, D. S. *Heterocycles* **1988**, *27*, 1575.
(21) Grierson, D. S.; Vuilhorgne, M.; Husson, H.-P.; Lemoine, G. *J. Org. Chem.* **1982**, *47*, 4439.
(22) Bruylants, P. *Bull. Soc. Chim. Belg.* **1924**, *33*, 467. Paukstelis, J. V., Cook, A. G., Ed.; *Enamines: Synthesis, Structure, and Reactions*, 2nd ed.; Marcel Dekker: New York, 1988; Chapter 6.
(23) Ringdahl, B.; Pinder, A. R.; Pereira, W. E., Jr.; Oppenheimer, N. J.; Craig, J. C. *J. Chem. Soc. Perkin Trans. 1* **1984**, 1.
(24) Wenkert, E.; Bindra, J. S.; Chang, C.-J.; Cochran, D. W.; Schell, F. M. *Acc. Chem. Res.* **1974**, *7*, 46.
(25) Maudsley, A. A.; Müller, L.; Ernst, R. R. *J. Magn. Reson.* **1977**, *28*, 463. Bodenhausen, G.; Freeman, R. *J. Magn. Reson.* **1977**, *28*, 471.
(26) Patt, S. L.; Shoolery, J. N. *J. Magn. Reson.* **1982**, *46*, 535.

Table I[a]

compound	C3-H_{eq}	C3-H_{ax}	C5-H	C9-H	^{13}C3	^{13}C5	^{13}C9
indolizidine[b]	3.06	2.08	3.11,[c] 1.98[d]	–	53.9	52.7	64.1
8	2.93	2.45	4.06	2.24	51.2	51.7	58.5
15	3.05	2.28	–	2.21	48.1	62.6	60.0
9	3.06	2.30	–	2.26	47.7	62.7	60.0
10	3.06	2.30	–	2.27	47.7	62.8	60.0
16	2.78	2.50	3.20	2.40	49.1	50.0	54.6
13	2.71	2.53	2.83	2.35	48.8	55.3	55.1
14	2.78	2.60	2.88	2.42	48.8	55.5	55.1
17	3.18	1.93	1.96	–	51.8	58.9	64.8
11	3.21	1.92	–	–	51.6	63.8	65.1
12	3.22	1.93	–	–	51.6	63.9	65.1

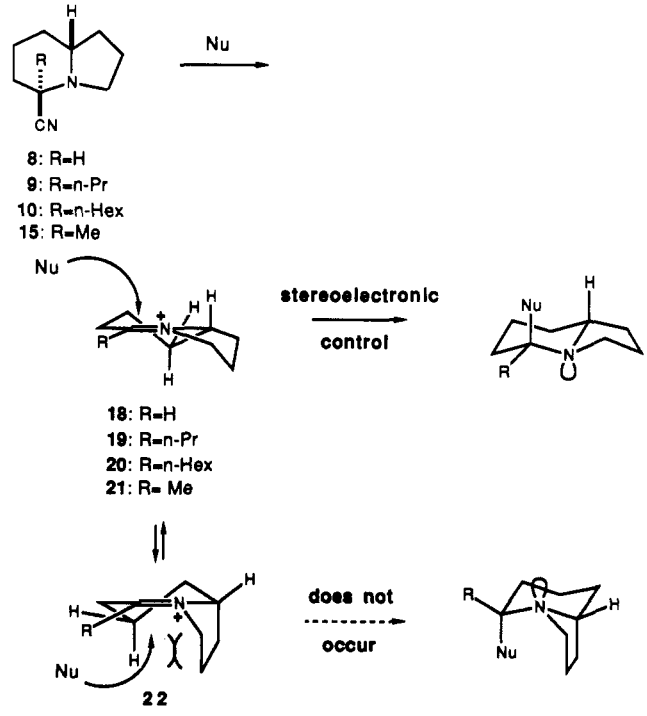
[a] Values of chemical shift are reported in ppm from tetramethylsilane. [b] Values taken from refs 23 and 24. [c] Equatorial hydrogen. [d] Axial hydrogen.

Reaction of the α-amino nitrile **8** with methyl, propyl, or hexyl Grignard reagents produced compounds **16**, **13**, and **14**, respectively. Indolizidine **16** displayed a moderately strong Bohlmann band at 2800 cm^{-1}, consistent with the presence of a trans-fused indolizidine skeleton. In this regard, each substituted indolizidine derivative which we prepared possessed distinct resonances for each of the hydrogens attached to C3. Furthermore, the more downfield signal due to the equatorial hydrogen always appeared as a triplet of doublets while the upfield signal arising from the axial hydrogen attached to C3 always appeared as a quartet. Oppenheimer and Craig,[23] in a thorough investigation of the ^{1}H NMR spectrum of indolizidine, consider such a spectral pattern to be diagnostic for trans-fused indolizidine ring systems. We are therefore led to believe that compounds **8**–**17** all exist in the trans-fused form. The ^{13}C chemical shift of the methyl group of **16**, which appeared at 10 ppm, was consistent with an axial orientation of this group.[14c] Further evidence for an axial C5 methyl group in a trans-fused form of the indolizidine ring system was provided by the upfield (steric compression) shift of ^{13}C resonances for carbon atoms C3 and C9 (relative to indolizidine), and a 1–2% NOE effect between the methyl group and axial C9-H. The similarities in ^{13}C chemical shift for positions 3 and 9 in compounds **16**, **13**, and **14** is consistent with the 5*S*,9*R* configurations assigned to these structures.

Reduction of α-amino nitriles **9**, **10**, and **15** produced amines **11**, **12**, and **17**. Indolizidine **17** possessed a very strong Bohlmann band at 2800 cm^{-1}, consistent with a trans ring fusion. A small (2%) NOE between the equatorial hydrogen attached to C3 and the methyl group attached to C5 is consistent with an equatorial orientation of this methyl group. The regularity of the ^{13}C chemical shift of positions C3 and C9 in **17**, **11**, and **12** is indicative that each of these compounds possesses the same configuration at C5.

As mentioned earlier, the gross structures of natural **167B** and **209D** were inferred by analysis of their mass spectra. Having prepared both diastereomers of the presumed gross structures, we anticipated an unambiguous structure proof of each alkaloid by direct comparison of the synthetic materials with the unfractionated frog extracts by gas chromatography. However, these alkaloids were detected in only one set of frog specimens which were collected and analyzed in the 1970s. Unfortunately, when these same unfractionated frog extracts were analyzed by GC–MS in 1989, the very trace alkaloids **167B** and **209D** could no longer be detected.[27] Thus, a direct comparison of the synthetic materials with the natural products by GC co-injection was not possible. We have found experimentally that the C5 epimers of each compound (**11** and **13**; **12** and **14**) possess virtually identical mass spectral fragmentation patterns. Independent comparison of the mass spectra derived from each diastereomer of the synthetic materials with GC–mass spectral data obtained from the original frog extracts by Daly's group at the NIH confirmed that the correct gross structures of the natural products are as originally formulated.[27] However, due to the virtually identical mass spectra displayed by isomers **11** and **13**; **12** and **14**, the relative stereochemical assignments of both **167B** and **209D** are still uncertain.

By analogy to the insightful stereoelectronic principles developed by Stevens,[28] we reason that the high stereoselectivity associated with both the hydride reduction of amino nitriles **9** and **10** and Grignard addition to amino nitrile **8** is a result of stereoelectronically controlled nucleophilic addition to iminium ions **18**, **19**, and **20**. The borohydride reductions (MeOH, 25 °C) of **9** and **10** and addition of propylmagnesium bromide (THF, 25 °C) to amino nitrile **8** were stereospecific. Addition of hexylmagnesium bromide to **8** resulted in a 98:2 ratio of **14** and **12**.



In summary, the first enantioselective total syntheses of the indolizidine alkaloids **167B** and **209D** have been achieved. Based on spectroscopic evidence, each diaste-

(27) Daly, J. W., personal communication. We thank Dr. Daly and Dr. Tom Spande for carrying out these comparisons.

(28) Stevens, R. V. *Acc. Chem. Res.* **1984**, *17*, 289.

reomer appears to exist in a chair, envelope-trans-fused indolizidine conformation. These findings may impact upon eventual AChR complex binding affinity studies. The methodology described in this report is being applied to more complex members of the indolizidine family.

Experimental Section[29]

(5*S*)-(−)-1-((1*S*)-1-Phenylethyl)-5-(1-prop-2-enyl)-2-pyrrolidinone (3a) and Diastereomer 3b. These compounds were prepared from hydroxylactam **2** as described previously.[5] A modified workup technique involving quenching of the reaction mixture with 1 N HCl avoided formation of an emulsion and led to a higher isolated product yield of 97% after chromatography.

(2*S*)-(−)-1-((1*S*)-1-Phenylethyl)-2-(1-prop-2-enyl)-pyrrolidine (4a) and Diastereomer 4b. Lactams **3a** and **3b** (1.00 g, 4.37 mmol, 82:18 diastereomeric ratio) were dissolved in THF (40 mL), $LiAlH_4$ (1.33 g, 8 equiv) was slowly added, and the reaction mixture was refluxed for 2 h. After cooling to room temperature, Et_2O (50 mL) was added, followed by water (1.1 mL), 3 M NaOH (1.1 mL), and additional water (2 mL). The solution was stirred for 2 h, filtered, and concentrated, affording amines **4**: 839 mg (90%) as a clear oil. **Major diastereomer 4a**: IR (NaCl) 1640 (m) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.40–7.20 (m, 5 H, ArH_5), 5.78 (m, 1 H, C_7-H), 5.05 (br d, 1 H, J = 16 Hz, C = C*H*H, trans), 5.01 (d, 1 H, J = 9 Hz, C=CH*H*, cis), 3.87 (q, 1 H, J = 7 Hz, ArCH), 2.86 (m, 1 H, C_2-H), 2.60 (m, 1 H), 2.45 (m, 1 H), 2.36 (m, 1 H), 2.07 (m, 1 H), 1.70 (m, 2 H), 1.56 (m, 2 H), 1.46 (d, 3 H, J = 7 Hz, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 136.4, 128.1, 128.0, 126.9, 116.1, 59.7, 59.3, 49.4, 38.9, 29.7, 22.4, 21.9; mass spectrum (CI, NH_3), m/e 216 (MH^+); $[\alpha]_D$ = −80.1° (*c* 1, CH_2Cl_2). Anal. Calcd for $C_{15}H_{21}H$: C, 83.67; H, 9.83. Found: C, 83.70; H, 9.86.

(2*S*)-(−)-2-(4-Oxobut-1-yl)-1-((1*S*)-1-phenylethyl)-pyrrolidine (5a) and Diastereomer 5b. Zirconocene chloride hydride[10] (1.01 g, 3.91 mmol) was suspended in benzene (5 mL), alkenes **4a** and **4b** (646 mg, 3.01 mmol) in benzene (8 mL) were added, and the mixture was stirred for 10 h. Carbon monoxide gas was bubbled through the solution for 10 min, and the suspension was stirred under CO (balloon pressure) for 5 h. The reaction was quenched with 0.2 M Na_2EDTA (60 mL), stirred 12 h, basified with 1 M NaOH, and extracted with Et_2O (5 × 50 mL). The combined organic extracts were dried (Na_2SO_4), filtered, and concentrated, and the residue was flash chromatographed on silica gel with a gradient of EtOAc to 60:40 EtOAc–MeOH, affording 500 mg (68%) of an oil. **Major diastereomer 5a**: IR (CH_2Cl_2) 1720 (s) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 9.78 (s, 1 H, CHO), 7.40–7.12 (m, 5 H, ArH_5), 3.82 (q, 1 H, J = 7 Hz, ArCH), 2.84 (td, 1 H, C_2-H), 2.54–2.38 (m, 3 H), 2.32 (q, 1 H, J = 8 Hz, C_5-H_a), 1.80–1.42 (m, 7 H), 1.43 (d, 3 H, J = 7 Hz, CH_3), 1.30 (m, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 202.7, 142.1, 128.1, 128.0, 126.9, 59.5, 59.4, 49.1, 44.1, 34.0, 30.2, 22.5, 21.7, 19.0; mass spectrum (CI, NH_3), m/e 246 (MH^+); $[\alpha]_D$ = −72.2° (*c* 0.9, CH_2Cl_2). Anal. Calcd for $C_{16}H_{23}NO$: C, 78.32; H, 9.45. Found: C, 78.36; H, 9.35.

(2*S*)-(−)-2-(4,4-Dimethoxybutyl)-1-((1*S*)-1-phenylethyl)-pyrrolidine (6a) and Diastereomer 6b. Aldehydes **5a** and **5b** (500 mg, 2.04 mmol) were dissolved in MeOH (18 mL), trimethyl orthoformate (8 mL) and pyridinium *p*-toluenesulfonate (550 mg, 1.05 equiv) were added, and the solution was refluxed for 9.5 h. 1 N NaOH (25 mL) was added, the product was extracted with CH_2Cl_2 (7 × 20 mL), and the combined organic layers were dried (Na_2SO_4), filtered, and concentrated. The residue was flash chromatographed on silica gel with a gradient of 95:5 to 60:40 EtOAc–MeOH, affording 507 mg (85%) of an oil. The diastereomers were separated using medium-pressure liquid chromatography (Merck LoBar B, 90:10 EtOAc–Hex). **Major diastereomer 6a**: IR (CH_2Cl_2) 1490 (w) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.35–7.20 (m, 5 H, ArH_5), 4.37 (t, 1 H, J = 6 Hz, C_9-H), 3.88 (q, 1 H, J = 7 Hz, ArCH), 3.32 (s, 6 H, OMe + OMe), 2.86 (m, 1 H, C_2-H), 2.44 (m, 1 H), 2.30 (q, 1 H, J = 7.5 Hz), 1.80–1.20 (m, 10 H), 1.45 (d, 3 H, J = 7 Hz $CHCH_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 142.0, 128.2, 127.9, 126.8, 104.6, 59.8, 59.3, 52.8, 52.7, 49.0, 34.4, 32.9, 30.2, 22.4, 21.7; mass spectrum (CI, NH_3) m/e 292 (MH^+); $[\alpha]_D$ = −67.6° (*c* 1.1, CH_2Cl_2). Anal. Calcd for $C_{18}H_{29}NO_2$: C, 74.18; H, 10.03. Found: C, 73.96; H, 9.95. **Minor diastereomer 6b**: IR (NaCl) 1545 (w) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.37–7.15 (m, 5 H, ArH_5), 4.24 (t, 1 H, J = 5.5 Hz, C_9-H), 3.71 (q, 1 H, J = 6.5 Hz, ArCH), 3.37 (s, 6 H, OMe + OMe), 2.78 (ddd, 1 H, J = 9, 6.5, 3.5 Hz), 2.69 (m, 1 H), 2.43 (q, 1 H, J = 8 Hz), 1.85–1.60 (m, 5 H), 1.51–1.03 (m, 5 H), 1.33 (d, 3 H, J = 6.5 Hz, $CHCH_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 145.5, 128.1, 127.8, 126.7, 104.5, 61.2, 60.4, 52.7, 49.8, 34.5, 32.6, 30.2, 23.1, 21.6, 17.3; mass spectrum (CI, NH_3), m/e 292 (MH^+); $[\alpha]_D$ = +1.7° (*c* 1.1, CH_2Cl_2).

(2*S*)-(−)-2-(4,4-Dimethoxybutyl)pyrrolidine (7). Amine **6a** (269.6 mg, 0.93 mmol) and ammonium formate (466 mg, 8 equiv) were dissolved in MeOH (13 mL), 10% Pd/C (100 mg) was added, and the mixture was stirred for 3 h. The suspension was filtered through Celite and concentrated, 1 N NaOH (8 mL) was added, and the mixture extracted with CH_2Cl_2 (5 × 20 mL). The combined organic extracts were dried (Na_2SO_4), filtered, and concentrated, affording 164.3 mg (95%) of an oil: IR (NaCl) 3300 (br) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 4.36 (t, 1 H, J = 5.5 Hz, C_9-H), 3.31 (s, 6 H, OMe + OMe), 3.04–2.88 (m, 2 H), 2.82 (m, 1 H), 1.94–1.16 (m, 10 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 104.1, 60.0, 53.0, 52.8, 44.3, 32.1, 31.9, 30.4, 23.6, 21.9; mass spectrum (CI, NH_3), m/e 188 (MH^+); $[\alpha]_D$ = −11.7° (*c* 1, CH_2Cl_2). Anal. Calcd for $C_{10}H_{21}NO_2$: C, 64.13; H, 11.30. Found: C, 64.22; H, 11.21.

(5*R*,9*R*)-(+)-5-Cyanoindolizidine (8). Acetal **7** (181.3 mg, 0.97 mmol) was dissolved in CH_2Cl_2/H_2O (7 mL each), KCN (780 mg, 12 equiv) was added, and the pH was adjusted to 3–4 with concentrated HCl and stirred for 4.5 h. After basification with 2 N NaOH, the mixture was extracted with CH_2Cl_2 (5 × 20 mL), and the combined organic layers dried (Na_2SO_4), filtered, and concentrated to afford 142.2 mg (98%) of an oil: IR (NaCl) 2810 (m), 2225 (w) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 4.06 (t, 1 H, J = 4.5 Hz, C_5-H), 2.93 (td, 1 H, J = 8.5, 3 Hz, C_3-H_e), 2.45 (q, 1 H, J = 8.5 Hz, C_3-H_a), 2.24 (m, 1 H, C_9-H) 1.95–1.06 (m, 10 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 116.5, 58.5, 51.7, 51.2, 30.7, 30.3, 28.5, 20.9, 20.5; mass spectrum (EI), m/e 150 (M^+); $[\alpha]_D$ = +2.9° (*c* 1, CH_2Cl_2). Anal. Calcd for $C_9H_{14}N_2$: C, 71.96; H, 9.39. Found: C, 71.82; H, 9.51.

(5*R*,9*R*)-(−)-5-Cyano-5-propylindolizidine (9). Diisopropylamine (38 μL, 0.27 mmol) was dissolved in THF (3 mL) and cooled to 0 °C, *n*-BuLi (0.12 mL, 0.27 mmol, 2.3 M in hexane) was added, and the mixture was stirred for 30 min. After the mixture was cooled to −78 °C, amine **8** (20 mg, 0.13 mmol) in THF (2 mL) was added, and the mixture warmed to 0 °C and stirred 30 min. After the mixture was cooled again to −78 °C, propyl bromide (91 μL, 1 mmol) was added, and the mixture warmed to 0 °C, stirred 30 min, and quenched with 1 N NaOH (8 mL). After extraction with CH_2Cl_2 (3 × 20 mL), the combined organic layers were dried (Na_2SO_4), filtered, and concentrated to afford 21.5 mg (84%) of an oil: IR (NaCl) 2820 (m), 2210 (w) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 3.06 (td, 1 H, J = 8.5, 3.5 Hz, C_3-H_e), 2.30 (q, 1 H, J = 8.5 Hz, C_3-H_a), 2.26 (m, 1 H, C_9-H), 1.93–1.02 (m, 14 H), 0.93 (t, 3 H, J = 7 Hz, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 118.9, 62.7, 60.0, 47.7, 40.9, 33.8, 30.5, 21.9, 20.2, 17.0, 14.3; mass spectrum (CI, NH_3), m/e 193 (MH^+); $[\alpha]_D$ = −38.7° (*c* 1, CH_2Cl_2). Anal. Calcd for $C_{12}H_{20}N_2$: C, 74.95; H, 10.48. Found: C, 74.89; H, 10.69.

(5*R*,9*R*)-(−)-5-Cyano-5-hexylindolizidine (10). Synthesized according to the procedure for **9** (using *n*-hexyl bromide), affording 81 mg (85%) of an oil: IR (NaCl) 2820 (m), 2210 (w) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 3.06 (td, 1 H, J = 8.5, 3.5 Hz, C_3-H_e), 2.30 (q, 1 H, J = 8.5 Hz, C_3-H_a), 2.27 (m, 1 H, C_9-H), 1.94–1.05 (m, 20 H), 0.86 (t, 3 H, J = 7 Hz, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 118.9, 62.8, 60.0, 47.7, 38.8, 33.8, 31.6, 30.5, 29.4, 23.6, 22.6, 21.9, 20.2, 14.1; mass spectrum (CI, NH_3), m/e 208 ($(MH - HCN)^+$); $[\alpha]_D$ = −30.9° (*c* 1, CH_2Cl_2). Anal. Calcd for $C_{15}H_{26}N_2$: C, 76.87; H, 11.18. Found: C, 76.62; H, 11.01.

(5*R*,9*R*)-(−)-5-Propylindolizidine (11). Cyanoamine **9** (21 mg, 0.11 mmol) in EtOH (2 mL) was added to $NaBH_4$ (20 mg, 5 equiv) in EtOH (2 mL) at 0 °C, and the mixture warmed to room temperature and stirred 20 h. The reaction was quenched with 1 N NaOH (8 mL) and extracted with CH_2Cl_2 (3 × 20 mL), and the combined organic layers were dried (Na_2SO_4), filtered, and

(29) A general experimental protocol has recently been published: ref 5. The ^1H–^{13}C correlation NMR experiments were obtained on a GE QE-300 (MHz) instrument.

concentrated, to afford 14.9 mg (81%) of an oil: IR (NaCl) 2780 (m) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 3.21 (td, 1 H, J = 9, 2 Hz, C_3-H_e), 1.92 (q, 1 H, J = 9 Hz, C_3-H_a), 1.85-1.05 (m, 16 H) 0.86 (t, 3 H, J = 7 Hz, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 65.1, 63.8, 51.6, 37.0, 31.1, 30.9, 30.6, 24.8, 20.5, 19.2, 14.6; mass spectrum (CI, NH_3) m/e 168 (MH^+); $[\alpha]_D$ = -111.3° (c 1.3, CH_2Cl_2). Anal. Calcd for $C_{11}H_{21}N$: C, 78.98; H, 12.65. Found: C, 79.10; H, 12.55.

(5*R*,9*R*)-(-)-5-Hexylindolizidine (12). Prepared according to the procedure for **11**, affording 62 mg (87%) of an oil: IR (NaCl) 2780 (s) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 3.22 (td, 1 H, J = 9.5, 2 Hz, C_3-H_e), 1.93 (q, 1 H, J = 8.5 Hz, C_3-H_a), 1.90-1.03 (m, 22 H), 0.89 (t, 3 H, J = 7 Hz, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 65.1, 63.9, 51.6, 34.7, 31.9, 31.1, 30.9, 30.6, 29.8, 25.9, 24.8, 22.7, 20.5, 14.2; mass spectrum (CI, NH_3), m/e 210 (MH^+); $[\alpha]_D$ = -80.4° (c 1, CH_2Cl_2). Anal. Calcd for $C_{14}H_{27}N$: C, 80.31; H, 13.00. Found: C, 80.11; H, 13.20.

(5*S*,9*R*)-(-)-5-Propylindolizine (13). Cyanoamine 8 (71.5 mg, 0.48 mmol) was dissolved in Et_2O (10 mL) and cooled to 0 °C, propylmagnesium chloride (2.1 mL, 7 eq, 1.6 M in Et_2O) was added, and the solution was stirred for 12 h at room temperature. After quenching with saturated NH_4Cl (10 mL) and basification with 1 N NaOH, the aqueous layer was extracted with CH_2Cl_2 (5 × 20 mL), and the combined organic layers were dried (Na_2SO_4), filtered, and concentrated. The residue was flash chromatographed on silica with 90:10 EtOAc-MeOH to afford **13**: 61.1 mg (77%), oil; IR (NaCl) 2800 (m) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 2.83 (td, 1 H, J = 8.5, 3.5 Hz, C_5-H), 2.71 (td, 1 H, J = 9, 3 H, C_3-H_e), 2.53 (q, 1 H, J = 9 Hz, C_3-H_a), 2.35 (m, 1 H, C_9-H), 1.75-0.98 (m, 14 H), 0.81 (t, 3 H, J = 7 Hz, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 55.3, 55.1, 48.8, 31.4, 30.7, 27.7, 25.8, 20.95, 20.91, 19.4, 14.5; mass spectrum (CI, CH_4), m/e 168 (MH^+); $[\alpha]_D$ = -1.7° (c 1.1, CH_2Cl_2). Anal. Calcd for $C_{11}H_{21}N$: C, 78.98; H, 12.65. Found: C, 79.12; H, 12.49.

(5*S*,9*R*)-(+)-5-Hexylindolizidine (14). Synthesized according to the procedure for **13** (using *n*-hexylmagnesium bromide), affording 64 mg (78%) of an oil: IR (NaCl) 2800 (m) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 2.88 (m, 1 H, C_5-H), 2.78 (td, 1 H, J = 8.5, 3 Hz, C_3-H_e), 2.60 (q, 1 H, J = 8.5 Hz, C_3-H_a), 2.42 (m, 1 H, C_9-H), 1.82-1.20 (m, 19 H), 1.12 (ddd, 1 H, J = 22, 12, 4.5 Hz), 0.86 (t, 3 H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 55.5, 55.1, 48.8, 32.0, 31.3, 30.7, 29.7, 27.73, 27.66, 23.4, 22.7, 20.9, 19.4, 14.2; mass spectrum (CI, NH_3), m/e 210 (MH^+); $[\alpha]_D$ = +8.1° (c 1, CH_2Cl_2). Anal. Calcd for $C_{14}H_{27}N$: C, 80.31; H, 13.00. Found: C, 80.09; H, 12.76.

(5*R*,9*R*)-5-Cyano-5-methylindolizidine (15): IR (NaCl) 2830 (s), 2230 (w) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 3.05 (td, 1 H, J = 8.5, 3.5 Hz, C_3-H_e), 2.28 (q, 1 H, J = 8.5 Hz, C_3-H_a), 2.21 (m, 1 H, C_9-H), 1.91-1.00 (m, 10 H), 1.42 (s, 3 H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 119.1, 62.6, 60.0, 48.1, 37.4, 30.5, 26.5, 22.0, 20.0; mass spectrum (CI, NH_3), m/e 138 ($(MH - HCN)^+$).

(5*S*,9*R*)-5-Methylindolizidine (16): IR (NaCl) 2810 (m) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 3.20 (m, 1 H, C_5-H), 2.78 (td, 1 H, J = 9, 3 Hz, C_3-H_e), 2.50 (q, 1 H, J = 9 Hz, C_3-H_a), 2.40 (m, 1 H, C_9-H), 1.81-1.00 (m, 10 H), 0.94 (d, 3 H, J = 7 Hz, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 54.6, 50.0, 49.1, 31.43, 31.40, 30.4, 20.8, 19.3, 10.0; mass spectrum (CI, NH_3), m/e 140 (MH^+).

(5*R*,9*R*)-5-Methylindolizidine (17): IR (NaCl) 2800 (s) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 3.18 (td, 1 H, J = 9, 2 Hz, C_3-H_e), 1.96 (m, 1 H, C_5-H), 1.93 (q, 1 H, J = 9 Hz, C_3-H_a), 1.84-1.10 (m, 10 H), 1.06 (d, 3 H, J = 6.5 Hz, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 64.8, 58.9, 51.8, 34.3, 31.1, 30.6, 24.8, 21.2, 20.4; mass spectrum (CI, NH_3), m/e 140 (MH^+).

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The Automated Synthesis of Sulfur-Containing Oligodeoxyribonucleotides Using 3*H*-1,2-Benzodithiol-3-one 1,1-Dioxide as a Sulfur-Transfer Reagent

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Several polysulfides were tested as potential sulfur-transfer reagents during the automated synthesis of oligodeoxyribonucleoside phosphorothioates via the "deoxyribonucleoside phosphoramidite" approach. The thiosulfonate 3*H*-1,2-benzodithiol-3-one 1,1-dioxide (**12**) was particularly efficient as a sulfurizing reagent. A 0.2 M solution of **12** in acetonitrile converted the dinucleoside monophosphite triesters **13a-d** into the corresponding phosphorothioates **15a-d** within 30 s in near quantitative yields. This reagent led to rapid, efficient (stepwise yields of 99%), and reliable automated synthesis of phosphorothioate oligomers (28-mers) complementary to the mRNA of the HIV-1 *rev* gene, carrying either exclusively or a predetermined number of P(S) linkages. Additionally, oligomers exposed to prolonged treatment (24 h) with the sulfurizing reagent did not show any detectable modification of the nucleosidic bases.

Introduction

Nucleoside phosphorothioates are isoelectronic analogues of natural nucleotides in which a nonbridging oxygen atom of the phosphate group is replaced by a sulfur atom.[1] When introduced on either the α- or β-phosphate of a nucleoside triphosphate this modification confers chirality at phosphorus, thereby providing an excellent tool for the determination of the stereochemical course of many enzymatic nucleotidyl and phosphoryl transfer reactions.[1,2] Moreover, the introduction of the above isoelectronic modification on the internucleotidic phosphate linkages of oligodeoxyribonucleotides considerably enhances their

(1) Eckstein, F. *Angew. Chem., Int. Ed. Engl.* **1983**, *22*, 423-506.

(2) Eckstein, F. *Ann. Rev. Biochem.* **1985**, *54*, 367-402.