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New synthesis of all the four stereoisomers of indolizidine 209D and their affinity for nicotinic acetylcholine receptor

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Abstract

Both enantiomers of a 2-(4-pentenyl)pyrrolidine derivative **4** (65–90% *ee*), prepared via the asymmetric dihydroxylation (AD) of terminal olefin **2**, underwent a second AD to provide all of the four stereoisomers of indolizidine 209D **1** with enantiomeric enhancement (92–98% *ee*). The affinity of **1** for nicotinic acetylcholine receptor was evaluated. © 1998 Elsevier Science Ltd. All rights reserved.

1. Introduction

The indolizidine ring system is found in many biologically active and structurally interesting alkaloids.[1] Among these alkaloids, the poison-dart frog alkaloids,[2] 3,5-disubstituted[3] and 5,8-disubstituted indolizidines[4] appear to represent an atypical and potent class of noncompetitive blockers for muscle-type and ganglionic nicotinic receptor-channels. However, the biological activity for simpler 5-substituted indolizidines such as indolizidine 209D **1**[2] isolated from skin extracts of neotropical members of the Dendrobatidae family of frogs has not been investigated (Fig. 1). So far, among four stereoisomers of **1**, the synthesis of (5*R*,9*R*)-(−)-[5] and (5*S*,9*R*)-indolizidine (+)-209D[6] have been reported. In contrast, the synthesis of the other two isomers have never been reported. Accordingly, we were stimulated to develop a comprehensive synthetic program for these alkaloids. Our interest in this field has focused on the potential strategies based on the enantiomeric enhancement[7] caused by the two-fold or more application of the Sharpless asymmetric dihydroxylation (AD) reaction.[8] In this report, we describe a new synthesis of all the four stereoisomers of **1** with high enantiomeric purity via an iterative AD reaction together with their binding test for the nicotinic acetylcholine receptor (nAChR).

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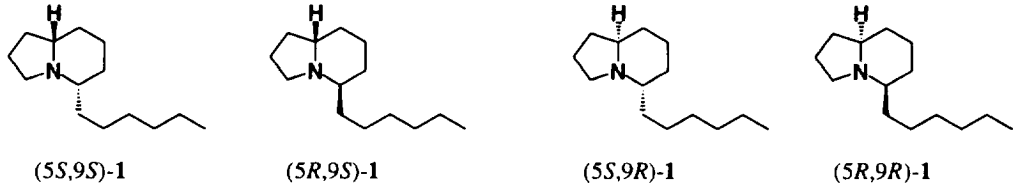
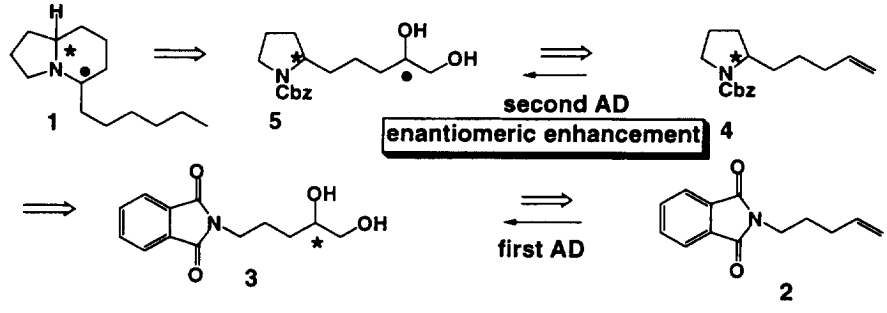


Fig. 1.

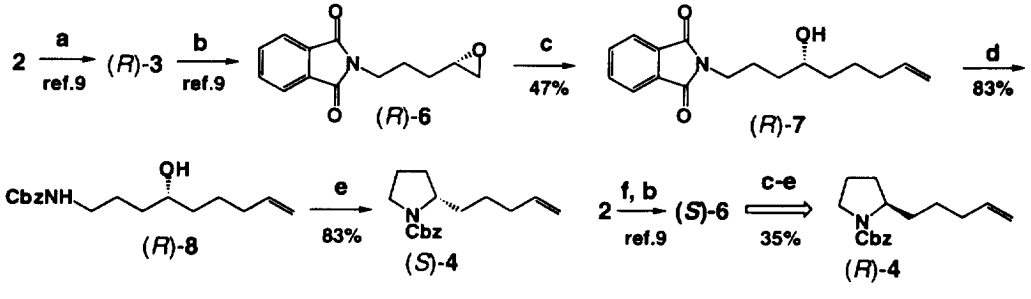
2. Results and discussion

As shown in Scheme 1, we considered that the two stereogenic centers in **1** would be installed with high enantio-enhancement via a sequence of two-fold AD reactions starting with olefins **2** and **4**. Recently we have reported a general route to 2-substituted pyrrolidines starting from *N*-pentenylphthalimide **2**.[9]



Scheme 1.

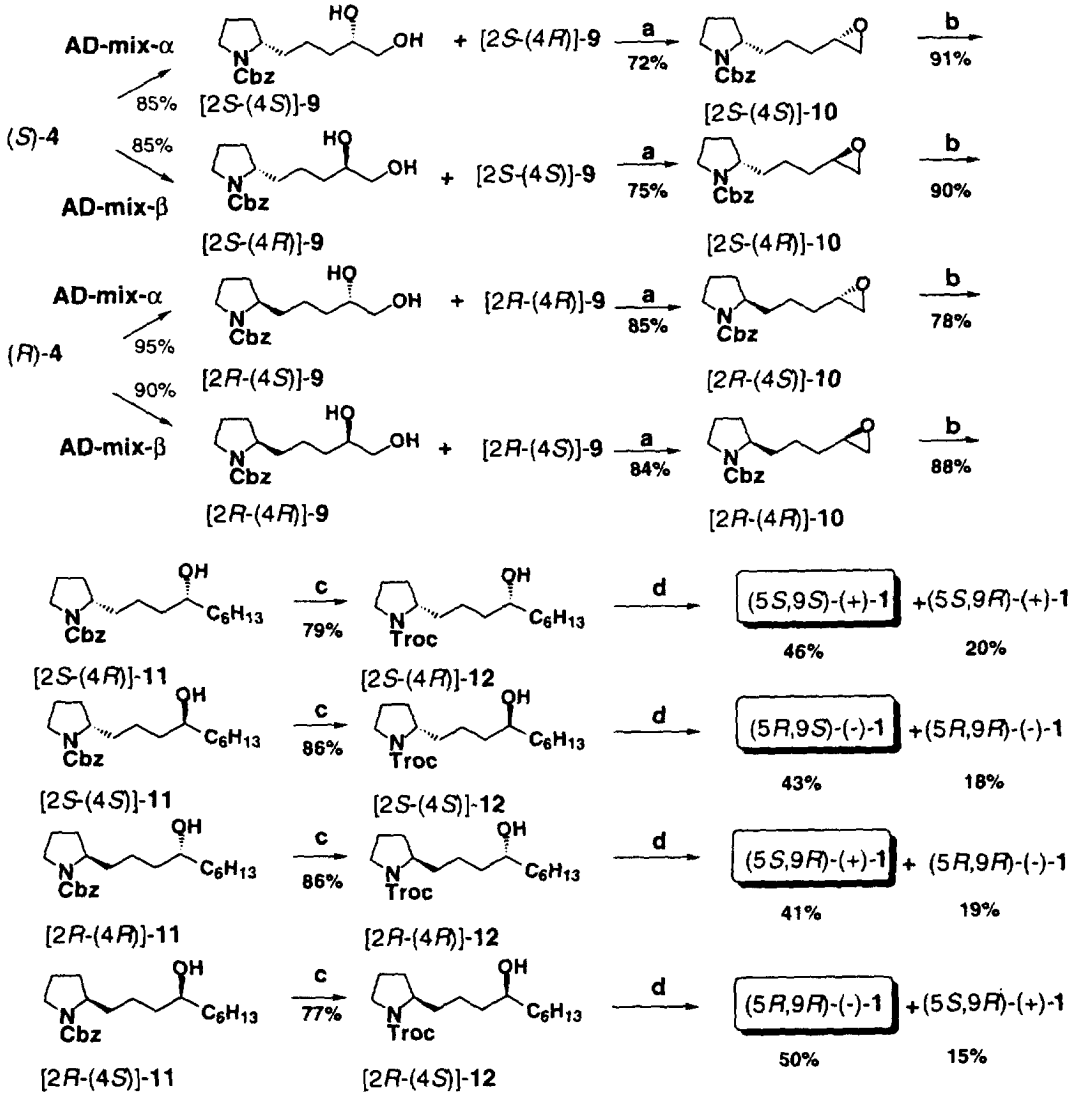
According to this procedure, the olefin **2** was successively subjected to $(DHQ)_2$-PYR[10] ligand-induced AD reaction, epoxidation,[11] and regioselective cleavage of the resulting epoxide ring of (*R*)-**6** with homoallylmagnesium bromide in combination with cuprous iodide to give the alcohol **7** in 34% overall yield from **2**. The phthalimide was transformed by a two-step sequence (1. hydrazine; 2. CbzCl/NaOH) into the *N*-benzyloxycarbonyl group (*N*-Cbz) (*R*)-**8**. Mesylation of (*R*)-**8** followed by cyclization with sodium hydride afforded the pyrrolidine (*S*)-**4** in 69% overall yield. In a similar sequence of reactions, the $(DHQD)_2$-PYR ligand-derived diol (*S*)-**3** was transformed into (*R*)-**4** in 35% overall yield. The enantiomeric purities of (*S*)-**4** and (*R*)-**4** were estimated to be 65% and 90%, respectively, on the basis of the *ee*s of (*R*)-**3** and (*S*)-**3** (Scheme 2).[12]



Scheme 2. (a) AD-mix-α [$(DHQ)_2$-PYR ligand]. (b) (1) $(CH_3O)_3CCH_3$/PPTS; (2) CH_3COBr; (3) K_2CO_3/MeOH. (c) Homoallylmagnesium bromide/CuI. (d) (1) NH_2NH_2; (2) CbzCl/NaOH. (e) (1) MsCl/pyridine; (2) NaH. (f) AD-mix-β [$(DHQD)_2$-PYR ligand]

With both enantiomers of *N*-Cbz-pyrrolidines **4** in hand, we turned our attention to the iterative AD to effect enantiomeric enhancement. The second AD [$(DHQ)_2$-PYR ligand] reaction at the terminal olefin in (*S*)-**4** was carried out to afford a diastereomeric mixture of the diols [2*S*-(4*S*)]-**9** and [2*S*-(4*R*)]-**9**.

Unfortunately, at this stage the diastereomers of **9** were inseparable. The diastereomeric mixtures of four diols **9** prepared from (*S*)-**4** and (*R*)-**4** by the AD reaction using two kinds of chiral ligands were converted into the four epoxides **10** by Sharpless's one-pot procedure[11] in a three-step sequence in good yields. Only major diastereomers of **10** are shown in Scheme 3.



Scheme 3. (a) (1) $(CH_3O)_3CCH_3$; (2) CH_3COBr; (3) K_2CO_3/MeOH. (b) pentylmagnesium bromide/CuI. (c) (1) H_2/$Pd(OH)_2$; (2) TrocCl/K_2CO_3. (d) (1) MsCl/pyridine/cat. DMAP; (2) 10% Cd–Pb/1N NH_4OAc

The regioselective cleavage of the resulting epoxide rings in **10** with *n*-pentylmagnesium bromide in the presence of cuprous iodide gave the secondary alcohols **11**[13] in good yields. The *N*-protecting group exchange of Cbz for 2,2,2-trichloroethoxycarbonyl (Troc) in **11** was carried out in a two-step sequence [1. H_2/$Pd(OH)_2$; 2. TrocCl/K_2CO_3] to afford the Troc carbamates **12**.[13] After mesylation of [2*S*-(4*R*)]-**12**, *N*-deprotection of the resulting mesylate with 10% Cd–Pb[14] gave desired (5*S*,9*S*)-(+)-**1**

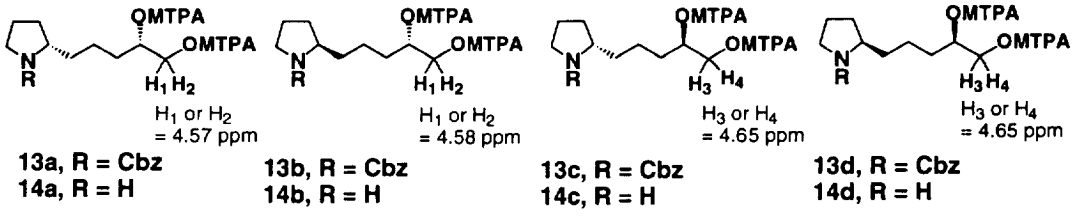


Fig. 2.

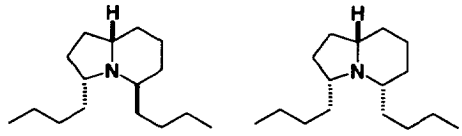
{$[\alpha]_D^{25}$ +81.8 (*c* 0.54; CH_2Cl_2)} in 46% yield and (5*S*,9*R*)-(+)-**1** {$[\alpha]_D^{25}$ +2.83 (*c* 0.35; CH_2Cl_2)} in 20% yield. Having obtained this result, a similar sequence with [2*S*-(4*S*)]-**12**, [2*R*-(4*R*)]-**12**, and [2*R*-(4*S*)]-**12** provided (5*R*,9*S*)-(−)-**1** {$[\alpha]_D^{25}$ −9.55 (*c* 1.16; CH_2Cl_2)}, (5*S*,9*R*)-(+)-**1** {$[\alpha]_D^{25}$ +9.86 (*c* 0.84; CH_2Cl_2)}, lit.[5a] {$[\alpha]_D$ +10.1} and (5*R*,9*R*)-(−)-**1** {$[\alpha]_D^{25}$ −89.6 (*c* 1.88; CH_2Cl_2)}, lit.[6d] {$[\alpha]_D$ −87.6} as the major products together with (5*R*,9*R*)-(−)-**1** {$[\alpha]_D^{25}$ −19.3 (*c* 0.71; CH_2Cl_2)}, (5*R*,9*R*)-(−)-**1** {$[\alpha]_D^{25}$ −23.7 (*c* 0.50; CH_2Cl_2)}, and (5*S*,9*R*)-(+)-**1** {$[\alpha]_D^{25}$ +2.33 (*c* 0.31; CH_2Cl_2)}, as the minor products, respectively.

Although the *ee*s of the four stereoisomers of **1** were not determined directly, they were indirectly calculated by ^{1}H NMR analysis of the corresponding bis-Mosher ester **14** [obtained by esterification of the diol **9** with (*S*)-MTPA-Cl followed by debenzyloxycarbonylation of the resulting bis-MTPA ester **13**] as follows. We made the assumption that the second AD reaction of **4** and its enantiomer contaminant occur with the same *ee* for both enantiomers. In addition, we expected that the chemical shifts of methylene protons at C_5 of the side chain are the same values in both **14a** and **14b** as well as in both **14c** and **14d** by NMR spectroscopy because of the remoteness between the 2 position of the pyrrolidine and the 4 position of the side chain. Fortunately, the signals of H_1 or H_2 of **14a,b** and H_3 or H_4 of **14c,d** were readily distinguishable by ^{1}H NMR (500 MHz) (Fig. 2). On the basis of this assumption, the ratio of **14a,b** to **14c,d** would refer to the diastereoselectivity of the second AD reaction. In the case of using two consecutive reactions with AD-mix-α [first AD (65% *ee*); second AD (68% de, a+b:c+d=84:16)], the ratio of the four stereoisomers was calculated to be [2*S*-(4*S*)]-**9**:[2*S*-(4*R*)]-**9**:[2*R*-(4*S*)]-**9**:[2*R*-(4*R*)]-**9**=(82.5×84=6930):(82.5×16=1320):(17.5×84=1470):(17.5×16=280). Accordingly, the *ee* of (5*S*,9*S*)-(+)-**1** derived from [2*S*(4*S*)]-**9** was calculated to be 92%. Based on a similar calculation, the *ee*s of the other three major products (5*R*,9*S*)-(−)-, (5*S*,9*R*)-(+)-, and (5*R*,9*R*)-(−)-**1** were evaluated to be 94%, 97%, and 98%, respectively.

With all the four homochiral indolizidine 209D **1** isomers in hand, our attention was directed toward their biological activity. The interaction of the four stereoisomeric indolizidines **1** with binding sites on carbamylcholine-activated nicotinic acetylcholine receptor (nAChR) channel complex from *Torpedo californica* electric organ was investigated using the radiolabeled probe, [^{3}H]-thienyl-cyclohexylpiperidine ([^{3}H]-TCP).[15] The K_i values for inhibition of [^{3}H]-TCP by **1**, compared to those of 3,5-disubstituted indolizidines **15** and **16** (Fig. 3),[3] are shown in Table 1. As a result, the configuration of **1** had little effect on the ion channel interactions. Although the affinities for the nAChR channel complex of **1** lacking a substitution at C_3 compared to those of 3,5-disubstituted indolizidines **15** and **16** were somewhat diminished, it was found that the substituent at C_3 was not necessarily required for this affinity.

Table 1
Evaluation of the affinity of **1** for the nAChR of *Torpedo californica*

compounds	(5*S*,9*S*)-**1**	(5*S*,9*R*)-**1**	(5*R*,9*S*)-**1**	(5*R*,9*R*)-**1**	**15**	**16**
K_i, μM	0.42	0.54	0.67	0.62	0.42	0.37



15 (racemate) **16** (racemate)

Fig. 3.

In summary, we have developed general access to 5-substituted indolizidines starting from an achiral *N*-pentenylphthalimide **2**. The two stereogenic centers in **1** were constructed with high enantioenhancement via a sequence of two-fold AD reactions. In practice, we demonstrated the asymmetric synthesis of all four stereoisomers of indolizidine 209D **1**, of which the affinity for the nAChR channel of *Torpedo californica* was tested. Further application of this methodology (the enantiomeric enhancement via the two-fold or more AD reactions) to asymmetric synthesis of other biologically active compounds is under investigation.

3. Experimental

3.1. General

Infrared spectra (IR) were measured with a Perkin–Elmer 1600 series FTIR spectrophotometer. Proton magnetic resonance (^{1}H NMR) spectra were recorded either at 300 MHz on a Varian Gemini-300, or 500 MHz on a Varian Unity-500 with $CHCl_3$ (7.26 ppm) as an internal standard. Carbon-13 NMR spectra were recorded at 75 or 125 MHz with $CDCl_3$ (77.2 ppm) as an internal standard unless otherwise specified. Mass spectra (MS) and high resolution mass spectra (HRMS) were measured on a JEOL JMS D-200 spectrometer. Optical rotations were measured on a JASCO DIP-140 instrument. Column chromatography was performed on silica gel (Fuji-Division BW-200 or Merck 60 (No. 9385)) with a medium pressure apparatus using a mixture of ethyl acetate:hexane as eluant unless otherwise specified. The extracts were dried over Na_2SO_4 unless otherwise specified.

3.2. (R)-2-(4-Hydroxy-8-noneyl)-1H-isoindole-1,3(2H)-dione (R)-7

To a slurry of CuI (2.50 g, 13.1 mmol) in THF (22.4 ml) was added a 0.658M 3-butenylmagnesium bromide–THF solution (34.8 ml, 22.9 mmol) at −78°C with stirring. After stirring for 1 h at −45°C, a solution of (*R*)-**6**[9] (3.0 g, 13.1 mmol) in THF (19 ml) was slowly added. The mixture was gradually warmed to −35°C, stirred for 3 h, and quenched with sat. NH_4Cl. The mixture was diluted with ether, washed with brine, dried, and evaporated. The residue was chromatographed using 25% ethyl acetate:hexane as eluant to give (*R*)-**7** (1.75 g, 47%) as an oil; $[\alpha]_D^{25}$ −0.72 (*c* 2.230; $CHCl_3$); IR cm^{-1} (neat) 3462, 2934, 1771, 1714, 1437, 1398, 1362, 1050, 720; ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.38–1.56 (6H, m), 1.71–2.07 (5H, m), 3.62–3.65 (1H, m), 3.71 (2H, t, *J*=7.3 Hz), 4.92–5.01 (2H, m), 5.74–5.82 (1H, m), 7.69–7.72 (2H, m), 7.81–7.84 (2H, m); ^{13}C NMR (125 MHz, $CDCl_3$) δ 25.0, 25.1, 33.8, 34.5, 37.1, 38.1, 71.4, 114.8, 123.3, 132.2, 134.1, 138.8, 168.7; HRMS calcd for $C_{17}H_{21}O_3N$ (M^+) 287.1521, found 287.1512.

3.3. (S)-2-(4-Hydroxy-8-nonenyl)-1H-isoindole-1,3(2H)-dione (S)-7

By a procedure similar to that for the preparation of (*R*)-**7**, the reaction of (*S*)-**6** (3.6 g, 15.6 mmol) with CuI (3.0 g, 15.6 mmol) and 1M homoallylmagnesium bromide–THF (31 ml, 31 mmol) gave (*S*)-**7** (2.38 g, 53%): $[\alpha]_D^{25}$ +1.08 (*c* 3.10; $CHCl_3$).

3.4. (R)-1-(Benzyloxycarbonyl)aminonon-8-en-4-ol (R)-8

A solution of (*R*)-**7** (223 mg, 0.81 mmol) and hydrazine hydrate (43 μl, 0.89 mmol) in ethanol (2.5 ml) was heated for 6 h at 90°C. To the mixture was added conc. HCl (0.4 ml) at room temperature. The mixture was filtered through Celite. The filtrate was evaporated to leave the hydrochloride salt. To a mixture of the salt and 2N NaOH (0.8 ml) was added benzyloxycarbonyl chloride (166 μl, 0.81 mmol) at 0°C. The mixture was stirred for 2 h at rt and extracted with ether. The extract was successively washed with 20% $KHSO_4$, satd $NaHCO_3$, brine, dried, and evaporated. The residue was chromatographed using 13% ethyl acetate:hexane as eluant to give (*R*)-**8** (195 mg, 83%) as an oil; $[\alpha]_D^{25}$ −0.76 (*c* 1.145; $CHCl_3$); IR cm^{-1} (neat) 3334, 2932, 1699, 1455, 1137, 911; ^1H NMR (500 MHz, $CDCl_3$) δ 1.42–1.75 (9H, m), 2.07 (2H, t, *J*=6.6 Hz), 3.19–3.26 (2H, m), 3.62 (1H, s), 4.93–5.04 (3H, m), 5.10–5.19 (2H, m), 5.80–5.85 (1H, m), 7.28–7.37 (5H, m); ^{13}C NMR (125 MHz, $CDCl_3$) δ 25.0, 26.2, 33.7, 34.2, 37.0, 41.0, 66.5, 71.0, 114.5, 127.9, 128.3, 136.5, 138.5, 156.5; HRMS calcd for $C_{17}H_{25}O_3N$ (M^+) 291.1835, found 291.1852.

3.5. (S)-1-(Benzyloxycarbonyl)aminonon-8-en-4-ol (S)-8

By a procedure similar to that for the preparation of (*R*)-**8**, the reaction of (*S*)-**7** (597 mg, 15.6 mmol) with hydrazine monohydrate (110 μg, 2.3 mmol) in EtOH (3.2 ml) gave the amine, which was benzyloxycarbonylated with benzyloxycarbonyl chloride (330 μl, 2.3 mmol) and 2N NaOH (2.2 ml) to yield (*S*)-**8** (447 mg, 73%). $[\alpha]_D^{25}$ +0.86 (*c* 1.225; $CHCl_3$).

3.6. (S)-N-Benzyloxycarbonyl-2-(4-pentenyl)pyrrolidine (S)-4

To a mixture of (*R*)-**8** (600 mg, 2.1 mmol) and DMAP (38 mg, 0.31 mmol) in pyridine (4.2 ml) was added methanesulfonyl chloride (0.24 ml, 3.1 mmol) at 0°C. After being stirred for 2 h at the same temperature, the mixture was diluted with ether and then acidified with 20% $KHSO_4$. The organic layer was successively washed with H_2O and brine, dried, and evaporated. The residue was purified by chromatography to yield the mesylate (783 mg) as an oil. To a suspension of NaH (109 mg, 2.7 mmol) in THF (9.3 ml) was added a solution of the mesylate (783 mg) in THF (9.3 ml) at 0°C. The mixture was stirred for 15 h at 50°C, quenched with sat. NH_4Cl, and diluted with ether. The mixture was successively washed with water and brine, then the mixture was dried, and evaporated. The residue was chromatographed using 10% ethyl acetate:hexane as eluant to give (*S*)-**4** (476 mg, 83%) as an oil; $[\alpha]_D^{25}$ +27.23 (*c* 1.145; $CHCl_3$); IR cm^{-1} (neat) 2929, 1700, 1187, 912; ^1H NMR (500 MHz, $CDCl_3$) δ 1.21–1.44 (3H, m), 1.67–1.71 (2H, m), 1.80–2.12 (5H, m), 3.38–3.51 (2H, m), 3.86 (1H, t, *J*=4.3 Hz), 4.92–5.03 (2H, m), 5.10–5.20 (2H, m), 5.73–5.85 (1H, m), 7.25–7.40 (5H, m); ^{13}C NMR (125 MHz, $CDCl_3$) δ 23.2, 24.0, 25.7, 25.8, 30.0, 30.7, 31.8, 33.6, 33.8, 34.0, 34.2, 46.4, 46.7, 57.4, 58.1, 66.6, 66.8, 114.7, 128.0, 128.6, 128.7, 137.3, 139.0, 155.0; HRMS calcd for $C_{17}H_{23}O_2N$ (M^+) 273.1729, found 273.1715.

3.7. (R)-N-Benzyloxycarbonyl-2-(4-pentenyl)pyrrolidine (R)-4

By a procedure similar to that for the preparation of (*S*)-**4**, the reaction of (*S*)-**8** (822 mg, 2.8 mmol) with methanesulfonyl chloride (0.33 ml, 4.2 mmol) in the presence of pyridine (5.8 ml) and DMAP (51 mg, 0.421 mmol) gave the mesylate, which was cyclized with 60% NaH (144 mg, 3.6 mmol) in THF (12 ml) to yield (*R*)-**4** (692 mg, 90%); $[\alpha]_D^{25}$ −39.94 (*c* 1.25; $CHCl_3$).

3.8. [2S-(4S)]-N-Benzyloxycarbonyl-2-(4,5-dihydroxypentyl)pyrrolidine [2S-(4S)]-9

The pyrrolidine (*S*)-**4** (583 mg, 2.13 mmol) was added to a mixture of AD-mix-α (2.77 g), prepared from $K_2OsO_4 \cdot 2H_2O$ (11 mg), $(DHQ)_2PYR$ (0.138 g), $K_3Fe(CN)_6$ (15.1 g), and K_2CO_3 (6.35 g) by a known procedure,[10] in *tert*-BuOH (10.2 ml), and H_2O (10.2 ml) at 0°C. After the reaction mixture was stirred for 24 h at the same temperature, sodium sulfite (3.2 g) was added to the mixture. After stirring for 30 min, the mixture was filtered through a Celite pad. The organic layer was separated, and the aqueous layer was extracted with ethyl acetate three times. The combined organic layers were washed with brine, dried, and evaporated. The residue was chromatographed using 90% ethyl acetate:hexane as eluant to yield a mixture of [2*S*-(4*S*)]-**9** and [2*S*-(4*R*)]-**9** (556 mg, 85%) as an oil; IR cm^{-1} (neat) 3419, 2940, 1682, 1417, 1359, 1107, 698; ^1H NMR (500 MHz, $CDCl_3$) δ 1.23–1.49 (5H, m), 1.66 (1H, d, *J*=6.0 Hz), 1.81–1.92 (4H, m), 3.36–3.68 (7H, m), 3.84 (1H, d, *J*=17.1 Hz), 5.06–5.16 (2H, m), 7.27–7.35 (5H, m); ^{13}C NMR (125 MHz, $CDCl_3$) δ 22.1, 22.2, 22.3, 23.1, 23.7, 29.9, 30.5, 32.7, 32.8, 33.0, 33.8, 34.5, 34.6, 46.3, 46.6, 57.2, 57.31, 57.7, 57.9, 66.6, 66.7, 66.8, 66.9, 71.8, 72.1, 127.8, 128.0, 128.5, 136.88, 136.93, 155.2; HRMS calcd for $C_{17}H_{25}O_4N$ (M^+) 307.1783, found 307.1777.

3.9. [2S-(4R)]-N-Benzyloxycarbonyl-2-(4,5-dihydroxypentyl)pyrrolidine [2S-(4R)]-9

By a procedure similar to that for the preparation of [2*S*-(4*S*)]-**9**, the AD reaction of (*S*)-**4** (432 mg, 1.58 mmol) with AD-mix-β [$(DHQD)_2PYR$ ligand] (2.05 g) in a mixture of *tert*-BuOH (7.5 ml) and H_2O (7.5 ml) gave [2*S*-(4*R*)]-**9** (413 mg, 85%).

3.10. [2R-(4S)]-N-Benzyloxycarbonyl-2-(4,5-dihydroxypentyl)pyrrolidine [2R-(4S)]-9

By a procedure similar to that for the preparation of [2*S*-(4*S*)]-**9**, the AD reaction of (*R*)-**4** (750 mg, 2.7 mmol) with AD-mix-α [$(DHQ)_2PYR$ ligand] (3.6 g) in a mixture of *tert*-BuOH (13 ml) and H_2O (13 ml) gave [2*S*-(4*R*)]-**9** (790 mg, 95%).

3.11. [2R-(4R)]-N-Benzyloxycarbonyl-2-(4,5-dihydroxypentyl)pyrrolidine [2R-(4R)]-9

By a procedure similar to that for the preparation of [2*S*-(4*S*)]-**9**, the AD reaction of (*R*)-**4** (455 mg, 1.66 mmol)) with AD-mix-β [$(DHQD)_2PYR$ ligand] (2.1 g) in a mixture of *tert*-BuOH (7.9 ml) and H_2O (7.9 ml) gave [2*R*-(4*R*)]-**9** (413 mg, 90%).

3.12. [2S-(4S)]-N-Benzyloxycarbonyl-2-(4,5-epoxypentyl)pyrrolidine [2S-(4S)]-10

A mixture of [2*S*-(4*S*)]-**9** (556 mg, 1.81 mmol), pyridinium *p*-toluenesulfonate (PPTS) (36 mg, 14.5 mmol), and trimethyl orthoacetate (276 μl, 2.17 mmol) in CH_2Cl_2 (2.8 ml) was stirred for 1.5 h at rt. After the solvent was removed by rotary evaporation, CH_2Cl_2 (2.8 ml) and acetyl bromide (161 μl,

2.17 mmol) were successively added to the resulting residue. After vigorous stirring, the mixture was evaporated. Methanol (6.1 ml) and K_2CO_3 (325 mg) were successively added to the resulting residue. After stirring for 2.5 h, the mixture was quenched with satd NH_4Cl, and extracted with CH_2Cl_2. The extracts were washed with brine, dried, and evaporated. The residue was chromatographed using 18% ethyl acetate:hexane as eluant to yield [2*S*-(4S)]-**10** (379 mg, 72%) as an oil; IR cm^{-1} (neat) 2945, 1698, 1411, 1187, 769; ^1H NMR (500 MHz, $CDCl_3$) δ 1.26–1.59 (5H, m), 1.62–1.68 (2H, m), 1.71–1.96 (3H, m), 2.32–2.37 (1H, m), 2.60–2.82 (2H, m), 3.26–3.37 (2H, m), 3.75 (1H, br s), 5.09–5.20 (2H, m), 7.30–7.37 (5H, m); ^{13}C NMR (125 MHz, $CDCl_3$) δ 22.5, 22.7, 22.9, 23.0, 23.8, 29.8, 30.6, 32.2, 32.4, 32.6, 33.8, 34.4, 46.2, 46.5, 46.9, 47.0, 52.0, 52.2, 57.1, 57.8, 66.3, 66.5, 127.55, 127.60, 128.2, 136.9, 154.6; HRMS calcd for $C_{17}H_{23}O_3N$ (M^+) 289.1678, found 289.1681.

*3.13. [2S-(4R)]-N-Benzyloxycarbonyl-2-(4,5-epoxypentyl)pyrrolidine [2S-(4R)]-**10***

By a procedure similar to that for the preparation of [2*S*-(4*S*)]-**10**, [2*S*-(4*R*)]-**9** (88 mg, 0.29 mmol) was treated successively with PPTS (0.6 mg, 2.29 μmol), trimethyl orthoacetate (43 μl, 0.34 mmol), acetyl bromide (25 μl, 0.34 mmol), and K_2CO_3 (52 mg, 0.38 mmol) to give [2*S*-(4*R*)]-**10** (62 mg, 75%).

*3.14. [2R-(4S)]-N-Benzyloxycarbonyl-2-(4,5-epoxypentyl)pyrrolidine [2R-(4S)]-**10***

By a procedure similar to that for the preparation of [2*S*-(4*S*)]-**10**, the reaction of [2*R*-(4*S*)]-**9** (790 mg, 2.57 mmol) with PPTS (5.0 mg, 20 μmol), trimethyl orthoacetate (390 μl, 3.1 mmol), acetyl bromide (230 μl, 3.1 mmol), and K_2CO_3 (456 mg, 3.3 mmol) gave [2*R*-(4*S*)]-**10** (629 mg, 85%).

*3.15. [2R-(4R)]-N-Benzyloxycarbonyl-2-(4,5-epoxypentyl)pyrrolidine [2R-(4R)]-**10***

By a procedure similar to that for the preparation of [2*S*-(4*S*)]-**10**, the reaction of [2*R*-(4*R*)]-**9** (456 mg, 1.48 mmol) with PPTS (2.5 mg, 10 μmol), trimethyl orthoacetate (230 μl, 1.78 mmol), acetyl bromide (130 μl, 1.78 mmol), and K_2CO_3 (266 mg, 1.92 mmol) gave [2*R*-(4*R*)]-**10** (359 mg, 84%).

*3.16. [2S-(4R)]-N-Benzyloxycarbonyl-2-(4-hydroxydecyl)pyrrolidine [2S-(4R)]-**11***

To a slurry of CuI (147 mg, 0.77 mmol) in THF (1.5 ml) was added a 0.642M pentylmagnesium bromide–THF solution (2.4 ml, 1.54 mmol) at −78°C with stirring. After stirring for 1 h at −45°C, a solution of [2*S*-(4*S*)]-**10** (224 mg, 0.77 mmol) in THF (1.3 ml) was slowly added. The mixture was gradually warmed to −35°C, stirred for 3.5 h, and quenched with sat. NH_4Cl. The mixture was diluted with ether, washed with brine, dried, and evaporated. The residue was chromatographed using 20% ethyl acetate:hexane as eluant to give [2*S*-(4*R*)]-**11** (254 mg, 91%) as an oil; IR cm^{-1} (neat) 3446, 2928, 1698, 1415, 1358; ^1H NMR (500 MHz, $CDCl_3$) δ 0.88 (3H, t, *J*=8.3 Hz), 1.25–1.49 (16H, m), 1.68 (1H, s), 1.79–1.93 (4H, m), 3.39–3.61 (3H, m), 3.86 (1H, br s), 5.09–5.20 (2H, m), 7.27–7.39 (5H, m); ^{13}C NMR (125 MHz, $CDCl_3$) δ 14.4, 22.6, 22.9, 23.3, 24.0, 25.9, 29.6, 30.1, 30.8, 32.1, 34.1, 34.9, 37.3, 37.36, 37.42, 37.7, 37.8, 37.9, 46.4, 46.7, 57.4, 57.9, 66.6, 66.7, 66.8, 71.7, 71.8, 127.9, 128.0, 128.4, 137.9, 155.0; HRMS calcd for $C_{22}H_{35}O_3N$ (M^+) 361.2617, found 361.2617.

3.17. [2S-(4S)]-N-Benzyloxycarbonyl-2-(4-hydroxydecyl)pyrrolidine [2S-(4S)]-11

By a procedure similar to that for the preparation of [2*S*-(4*R*)]-**11**, the reaction of [2*S*-(4*R*)]-**10** (207 mg, 0.715 mmol) with 0.642M pentylmagnesium bromide–THF solution (2.23 ml, 1.43 mmol) in the presence of CuI (207 mg, 0.72 mmol) gave [2*S*-(4*S*)]-**11** (251 mg, 90%).

3.18. [2R-(4R)]-N-Benzyloxycarbonyl-2-(4-hydroxydecyl)pyrrolidine [2R-(4R)]-11

By a procedure similar to that for the preparation of [2*S*-(4*R*)]-**11**, the reaction of [2*R*-(4*S*)]-**10** (553 mg, 1.9 mmol) with 0.642M pentylmagnesium bromide–THF solution (5.92 ml, 3.8 mmol) in the presence of CuI (361 mg, 1.9 mmol) gave [2*R*-(4*R*)]-**11** (534 mg, 78%).

3.19. [2R-(4S)]-N-Benzyloxycarbonyl-2-(4-hydroxydecyl)pyrrolidine [2R-(4S)]-11

By a procedure similar to that for the preparation of [2*S*-(4*R*)]-**11**, the reaction of [2*R*-(4*R*)]-**10** (338 mg, 1.2 mmol) with 0.642M pentylmagnesium bromide–THF solution (3.74 ml, 2.4 mmol) in the presence of CuI (229 mg, 1.2 mmol) gave [2*R*-(4*S*)]-**11** (380 mg, 88%).

3.20. [2S-(4R)]-N-(2,2,2-Trichloroethoxycarbonyl)-2-(4-hydroxydecyl)pyrrolidine [2S-(4R)]-12

A mixture of [2*S*-(4*R*)]-**11** and its minor diastereomer (96 mg, 0.27 mmol) and $Pd(OH)_2$ (9.6 mg) in MeOH (1.3 ml) was stirred for 15 h under a hydrogen atmosphere. The mixture was filtered through a Celite pad, and the filtrate was evaporated. To the residue dissolved in a mixture of CH_2Cl_2 (2.2 ml) and H_2O (0.7 ml) was successively added K_2CO_3 (75 mg, 0.54 mmol) and 2,2,2-trichloroethyl chloroformate (TrocCl) (41 ml, 0.30 mmol) at 0°C. After being stirred at rt for 15 h, the mixture was extracted with CH_2Cl_2. The extracts were dried and evaporated. The residue was chromatographed using 15% ethyl acetate:hexane as eluant to give [2*S*-(4*R*)]-**12** (85 mg, 79%). IR cm^{-1} (neat) 3446, 2928, 2857, 1717, 1411, 1123; ^1H NMR (300 MHz, $CDCl_3$) δ 0.88 (3H, br t, *J*=6.3 Hz), 1.28–1.50 (15H, m), 1.65–2.01 (5H, m), 3.38–3.57 (4H, m), 3.87–3.90 (1H, m), 4.60–4.90 (2H, m); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.4, 22.1, 22.5, 22.70, 22.73, 22.9, 23.3, 24.0, 25.8, 25.88, 25.91, 29.6, 30.1, 30.2, 30.7, 32.1, 33.71, 33.9, 34.6, 34.8, 37.3, 37.36, 37.42, 37.6, 37.7, 37.76, 37.82, 37.9, 46.6, 47.1, 56.7, 57.9, 58.0, 58.1, 58.2, 58.3, 71.8, 71.9, 74.8, 75.08, 77.4, 95.9, 153.02, 153.06; HRMS calcd for $C_{17}H_{29}O_3NCl_3$ (M^+-H) 400.1213, found 400.1197.

3.21. [2S-(4S)]-N-(2,2,2-Trichloroethoxycarbonyl)-2-(4-hydroxydecyl)pyrrolidine [2S-(4S)]-12

By a procedure similar to that for the preparation of [2*S*-(4*R*)]-**12**, the hydrogenolysis of [2*S*-(4*S*)]-**11** and its diastereomer (228 mg, 0.63 mmol) in the presence of $Pd(OH)_2$ (23 mg) in MeOH (3.0 ml) under a hydrogen atmosphere gave the corresponding amine, which was trichloroethoxycarbonylated with TrocCl (196 μl, 0.70 mmol) using K_2CO_3 (174 mg, 1.25 mmol) to yield [2*S*-(4*S*)]-**12** (218 mg, 86%).

3.22. [2R-(4R)]-N-(2,2,2-Trichloroethoxycarbonyl)-2-(4-hydroxydecyl)pyrrolidine [2R-(4R)]-12

By a procedure similar to that for the preparation of [2*S*-(4*R*)]-**12**, the hydrogenolysis of [2*R*-(4*R*)]-**11** and its diastereomer (534 mg, 1.48 mmol) in the presence of $Pd(OH)_2$ (53 mg) in MeOH (7.0 ml)

under a hydrogen atmosphere gave the corresponding amine, which was trichloroethoxycarbonylated with TrocCl (220 μl, 1.63 mmol) using K_2CO_3 (408 mg, 2.96 mmol) to yield [2*R*-(4*R*)]-**12** (513 mg, 86%).

3.23. *[2R-(4S)]-N-(2,2,2-Trichloroethoxycarbonyl)-2-(4-hydroxydecyl)pyrrolidine [2R-(4S)]-12*

By a procedure similar to that for the preparation of [2*S*-(4*R*)]-**12**, the hydrogenolysis of [2*R*-(4*S*)]-**11** and its diastereomer (249 mg, 0.69 mmol) in the presence of $Pd(OH)_2$ (25 mg) in MeOH (3.3 ml) under a hydrogen atmosphere gave the corresponding amine, which was trichloroethoxycarbonylated with TrocCl (100 μl, 1.63 mmol) using K_2CO_3 (190 mg, 1.38 mmol) to yield [2*R*-(4*S*)]-**12** (215 mg, 77%).

3.24. *(5S,9S)-(+)-Indolizidine 209D (5S,9S)-(+)-1 and (5S,9R)-(+)-indolizidine 209D (5S,9R)-(+)-1*

Methanesulfonyl chloride (MsCl) (19 μl, 0.25 mmol) was added to a mixture of [2*S*-(4*R*)]-**12** containing its diastereomer (67 mg, 0.166 mmol), DMAP (2.9 mg, 0.024 mmol) and pyridine (0.3 ml) at 0°C. After being stirred for 2 h, the mixture was diluted with ether at 0°C and quenched with 20% $KHSO_4$. The mixture was successively washed with water and brine and then dried and evaporated. The residue was chromatographed using 10% ethyl acetate:hexane as eluant to give the corresponding mesylate, which was dissolved in THF (1.3 ml). To the solution were added 1N NH_4OAc (1.3 ml) and 10% Cd–Pb couple (221 mg, 1.66 mmol). After being stirred for 15 h, the mixture was basified with 2N NaOH and filtered through Celite. The filtrate was extracted with CH_2Cl_2 three times. The extracts were dried with K_2CO_3 and evaporated. The residue was chromatographed using 1–3% EtOAc:hexane as eluant to give (5*S*,9*S*)-(+)-**1** (16 mg, 46%) as the major diastereomer and (5*S*,9*R*)-(+)-**1** (7 mg, 20%) as the minor diastereomer.

(5*S*,9*S*)-(+)-**1**; $[\alpha]_D^{25}$ +81.8 (*c* 0.540; CH_2Cl_2), lit.[6d] $[\alpha]_D^{20}$ −87.6 (*c* 1; CH_2Cl_2); IR cm^{-1} (neat) 2929, 2857, 2780; ^1H NMR (500 MHz, $CDCl_3$) δ 0.89 (3H, t, *J*=7.1 Hz), 1.13–1.45 (13H, m), 1.62–1.90 (9H, m), 1.97 (1H, q, *J*=9.0 Hz), 3.27 (1H, td, *J*=9.0 Hz, 2.10 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 14.3, 20.6, 22.8, 24.9, 26.0, 29.9, 30.7, 31.0, 31.2, 32.0, 34.8, 51.7, 64.1, 65.2; HRMS calcd for $C_{14}H_{27}N$ (M^+) 209.2143, found 209.2178.

(5S,9R)-(+)-**1**; $[\alpha]_D^{25}$ +2.83 (*c* 0.345; CH_2Cl_2), lit. [5a] $[\alpha]_D^{24}$ +10.1 (*c* 0.42; CH_2Cl_2); ^1H NMR (500 MHz, $CDCl_3$) δ 0.90 (3H, t, *J*=7.1 Hz), 1.13–1.19 (1H, m), 1.21–1.86 (19H, m), 2.53 (1H, br s), 2.65–2.70 (1H, m), 2.86 (1H, t, *J*=8.3 Hz), 2.91–2.95 (1H, m).

3.25. *(5R,9S)-(−)-Indolizidine 209D (5R,9S)-(−)-1 and (5R,9R)-(−)-indolizidine 209D (5R,9R)-(−)-1*

By a procedure similar to that for the preparation of (5*S*,9*S*)-(+)-**1**, the reaction of [2*S*-(4*S*)]-**12** containing its diastereomer (67 mg, 0.166 mmol) with MsCl (62 μl, 0.80 mmol) using pyridine (1.0 ml) and DMAP (10 mg, 0.08 mmol) as bases gave the corresponding mesylate, which was deprotected with 10% Cd–Pb couple (706 mg, 5.3 mmol) in a mixture of 1N NH_4OAc (4.1 ml) and THF (4.1 ml) to yield (5*R*,9*S*)-(−)-**1** (48 mg, 43%) and its diastereomer (5*R*,9*R*)-(−)-**1** (20 mg, 18%).

(5*R*,9*S*)-(−)-**1**; $[\alpha]_D^{25}$ −9.55 (*c* 1.165; CH_2Cl_2), lit.[5a] $[\alpha]_D^{24}$ +10.1 (*c* 0.42; CH_2Cl_2); IR cm^{-1} (neat) 2927, 2856; ^1H NMR (500 MHz, $CDCl_3$) δ 0.90 (3H, t, *J*=7.5 Hz), 1.13–1.20 (1H, m), 1.21–1.84 (19H, m), 2.45 (1H, br s), 2.65 (1H, q, *J*=8.1 Hz), 2.83 (1H, td, *J*=8.8, 3.20 Hz), 2.91–2.95 (1H, m); ^{13}C NMR (125 MHz, $CDCl_3$) δ 14.3, 19.4, 21.0, 22.9, 23.6, 27.7, 27.8, 29.9, 29.9, 30.7, 31.3, 32.1, 49.0, 55.4, 55.7; HRMS calcd for $C_{14}H_{27}N$ (M^+) 209.2144, found 209.2155.

(5*R*,9*R*)-(−)-**1**; $[\alpha]_D^{25}$ −19.3 (*c* 0.710; CH_2Cl_2), lit.[6d] $[\alpha]_D^{20}$ −87.6 (*c* 1; CH_2Cl_2); ^1H NMR (500 MHz, $CDCl_3$) δ 0.89 (3H, t, *J*=6.4 Hz), 1.16–1.89 (22H, m), 1.99 (1H, d, *J*=8.3 Hz), 3.28 (1H, br s).

3.26. (5S,9R)-(+)-Indolizidine 209D (5S,9R)-(+)-1 and (5R,9R)-(−)-indolizidine 209D (5R,9R)-(−)-1

By a procedure similar to that for the preparation of (5*S*,9*S*)-(+)-**1**, the reaction of [2*R*-(4*R*)]-**12** containing its diastereomer (171 mg, 0.42 mmol) with MsCl (49 μl, 0.63 mmol) using pyridine (0.79 ml) and DMAP (8 mg, 0.06 mmol) as bases gave the corresponding mesylate, which was deprotected with 10% Cd–Pb couple (554 mg, 4.2 mmol) in a mixture of 1N NH_4OAc (3.3 ml) and THF (3.3 ml) to yield (5*S*,9*R*)-(+)-**1** (36 mg, 41%) and its diastereomer (5*R*,9*R*)-(−)-**1** (17 mg, 19%).

(5*S*,9*R*)-(+)-**1**; $[\alpha]_D^{25}$ +9.86 (*c* 0.840; CH_2Cl_2), lit.[5a] $[\alpha]_D^{24}$ +10.1 (*c* 0.42; CH_2Cl_2); IR cm^{-1} (neat) 2927, 2857; ^1H NMR (300 MHz, $CDCl_3$) δ 0.87 (3H, t, *J*=6.6 Hz), 1.07–1.84 (20H, m), 2.38–2.48 (1H, m), 2.57–2.66 (1H, m), 2.78–2.83 (1H, m), 2.86–2.92 (1H, m); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.4, 19.7, 21.2, 23.0, 23.6, 27.9, 28.0, 30.0, 30.9, 31.6, 32.2, 49.0, 55.3, 55.7; HRMS calcd for $C_{14}H_{27}N$ (M^+) 209.2144, found 209.2124.

(5*R*,9*R*)-(−)-**1**; $[\alpha]_D^{25}$ −23.65 (*c* 0.496; CH_2Cl_2), lit.[6d] $[\alpha]_D^{20}$ −87.6 (*c* 1; CH_2Cl_2); ^1H NMR (300 MHz, $CDCl_3$) δ 0.88 (3H, t, *J*=6.6 Hz), 1.05–2.04 (23H, m), 3.27–3.33 (1H, m).

3.27. (5R,9R)-(−)-Indolizidine 209D (5R,9R)-(−)-1 and (5S,9R)-(+)-indolizidine 209D (5S,9R)-(+)-1

By a procedure similar to that for the preparation of (5*S*,9*S*)-(+)-**1**, the reaction of [2*R*-(4*S*)]-**12** containing its diastereomer (199 mg, 0.49 mmol) with MsCl (57 μl, 0.74 mmol) using pyridine (0.92 ml) and DMAP (9 mg, 0.07 mmol) as bases gave the mesylate, which was deprotected with 10% Cd–Pb couple (662 mg, 4.97 mmol) in a mixture of 1N NH_4OAc (3.9 ml) and THF (3.9 ml) to yield (5S,9R)-(−)-**1** (52 mg, 50%) and its diastereomer (5*S*,9*R*)-(+)-**1** (15 mg, 15%).

(5*R*,9*R*)-(−)-**1**; $[\alpha]_D^{25}$ −89.64 (*c* 1.880; CH_2Cl_2), lit.[6d] $[\alpha]_D^{20}$ −87.6 (*c* 1; CH_2Cl_2); IR cm^{-1} (neat) 2929, 2857, 2780; ^1H NMR (300 MHz, $CDCl_3$) δ 0.87 (3H, t, *J*=6.6 Hz), 1.06–1.49 (13H, m), 1.58–2.00 (10H, m), 3.25 (1H, td, *J*=8.8 Hz, 2.2 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.4, 20.7, 22.9, 25.0, 26.1, 30.0, 30.8, 31.1, 31.3, 32.1, 34.9, 51.8, 64.1, 65.2; HRMS calcd for $C_{14}H_{27}N$ (M^+) 209.2143, found 209.2175.

(5*S*,9*R*)-(+)-**1**; $[\alpha]_D^{25}$ +2.33 (*c* 0.305; CH_2Cl_2), lit.[5a] $[\alpha]_D^{24}$ +10.1 (*c* 0.42; CH_2Cl_2); ^1H NMR (300 MHz, $CDCl_3$) δ 0.88 (3H, t, *J*=6.6 Hz), 1.07–2.04 (20H, m), 2.46–2.54 (1H, m), 2.62–2.71 (1H, m), 2.80–2.94 (2H, m).

3.28. Bis-Mosher ester 14a from [2S-(4S)]-9

A solution of [2*S*-(4*S*)]-**9** (18 mg, 59 μmol) and DMAP (17 mg, 141 μmol) in THF (0.3 ml) was treated with (*S*)-Mosher's chloride (*S*)-MTPA-Cl, (22.5 μl, 134 μmol) at 25°C, and the reaction mixture was stirred for 30 h. After dilution with ether, the mixture was washed with 20% $KHSO_4$, water, and satd $NaHCO_3$, dried and evaporated to afford the bis-Mosher ester **13a** (41 mg) of [2*S*-(4*S*)]-**9**. A suspension of **13a** (41 mg) and $Pd(OH)_2$ (4.1 mg) in MeOH (0.3 ml) was stirred under a hydrogen atmosphere for 15 h. After filtration of the mixture through a Celite pad, the filtrate was evaporated to **14a**. ^1H NMR (500 MHz, $CDCl_3$) δ 4.57 (0.84H, dd, *J*=12.2, 2.8 Hz, C*H*$_2$OMTPA), 4.64 (0.16H, dd, *J*=12.2, 2.8 Hz, C*H*$_2$OMTPA).

3.29. Bis-Mosher ester **14b** from [2R-(4S)]-**9**

By a procedure similar to that for the preparation of bis-Mosher ester **14a**, the reaction of [2*R*-(4*S*)]-**9** (17 mg, 54 μmol) with (*S*)-MTPA-Cl (27 μl, 160 μmol) and DMAP (26 mg, 210 μmol) in THF (0.29 ml) afforded the bis-Mosher ester **13b**, which was deprotected with hydrogenolysis using $Pd(OH)_2$ (4.3 mg) to yield **14b**. ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.58 (0.79H, dd, *J*=12.2, 2.8 Hz, C*H*$_2$OMTPA), 4.66 (0.21H, dd, *J*=12.2, 2.8 Hz, C*H*$_2$OMTPA).

3.30. Bis-Mosher ester **14c** from [2S-(4R)]-**9**

By a procedure similar to that for the preparation of bis-Mosher ester **14a**, the reaction of [2*S*-(4*R*)]-**9** (17 mg, 54 μmol) with (*S*)-MTPA-Cl (27 μl, 160 μmol) and DMAP (26 mg, 210 μmol) in THF (0.29 ml) afforded the bis-Mosher ester **13c**, which was deprotected with hydrogenolysis using $Pd(OH)_2$ (4.3 mg) to yield **14c**. ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.58 (0.12H, dd, *J*=12.2, 2.8 Hz, C*H*$_2$OMTPA), 4.65 (0.88H, dd, *J*=12.2, 2.8 Hz, C*H*$_2$OMTPA).

3.31. Bis-Mosher ester **14d** from [2R-(4R)]-**9**

By a procedure similar to that for the preparation of bis-Mosher ester **14a**, the reaction of [2*R*-(4*R*)]-**9** (19 mg, 60 μmol) with (*S*)-MTPA-Cl (30 μl, 180 μmol) and DMAP (29 mg, 240 μmol) in THF (0.32 ml) afforded the bis-Mosher ester **13d**, which was deprotected with hydrogenolysis using $Pd(OH)_2$ (4.1 mg) to yield **14d**. ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.57 (0.18H, dd, *J*=12.4, 2.8 Hz, C*H*$_2$OMTPA), 4.65 (0.82H, dd, *J*=12.2, 2.8 Hz, C*H*$_2$OMTPA).

3.32. Evaluation of the affinity of **1** for the nAChR of Torpedo californica

3.32.1. Assay protocol

Membranes enriched in nAChR are harvested from homogenates of *Torpedo californica* electric organs by differential centrifugation as described in the literature. Binding of [^{3}H]-TCP (sp. act 57.6 Ci/mmole) to carbamylcoline-activated nAChR is used as a functional binding assay as described by Kats et al.[15]. [^{3}H]-TCP is available from the National Institute of Drug Abuse.

3.32.2. Binding assay

Test compounds **1** were dissolved in DMSO to make 100 mM stocks. A second stock of 1 mM in buffer (50 mM Tris HCl, pH=7.4) is prepared by diluting 10 μl DMSO stock into 1 ml buffer. Stocks of 100 times the desired test concentrations were prepared by further dilutions with buffer. 25 μg of *Torpedo* membranes were added to the final volume of 250 μl of buffer containing [^{3}H]-TCP, 100 μM carbamylcoline, and the test drug **1** at varied concentrations. Nonspecific binding is determined in presence of 5 mM amantadiene HCl. Bound [^{3}H]-TCP is separated by vacuum filtration over GF/B filters presoaked in 0.05% polyethyleneimine. Incubation time was 30 s at 23°C. Radioactivity retained on the filters, after washing with 5 ml ice cold buffer, was quantitated by liquid scintillation. All tests were performed in triplicate and data reported as % of control. Six concentrations were selected for each test compound covering a range 1000 times the lowest effective concentration. The percent inhibition of carbamylcholine stimulated [^{3}H]-TCP binding was plotted vs concentration. Each compound was tested twice and means +SEM from 6 values at each concentration were used to generate the concentration–response function. The IC_{50} values determined graphically from the log

concentration–response curves were then used to obtain the inhibitory equilibrium constant (K_i) using the Cheng–Prusoff equation (K_i=IC_{50}/1+[D]/K_D). The results are shown in Table 1.

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