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Asymmetric Chloronicotinyl Insecticide, 1-[1-(6-Chloro-3-pyridyl)ethyl]-2-nitroiminoimidazolidine: Preparation, Resolution and Biological Activities toward Insects and Their Nerve Preparations

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The asymmetric chloronicotinyl insecticide, 1-[1-(6-chloro-3-pyridyl)ethyl]-2-nitroiminoimidazolidine, was prepared, and the absolute configurations of the enantiomers were determined by an X-ray analysis. The insecticidal activity against the housefly measured with metabolic inhibitors showed the (*S*) enantiomer to be slightly more active than the (*R*) isomer. Electrophysiological measurements on the American cockroach central nerve cord showed the compounds to elicite the impulses and subsequently blocked them. The neuroblocking potency of the (*S*) isomer was 5.9 μM, while that of the (*R*) isomer was as high as 73 μM. The molar concentrations required for 50% inhibition of the specific binding of [^{3}H]imidacloprid to the housefly head membrane preparation were respectively 0.19 μM and 0.95 μM for the (*S*) and (*R*) isomers. This enatioselectivity ratio was smaller than 35 for nicotine isomers but greater than 2 for epibatidine isomers.

Key words: imidacloprid; asymmetric chloronicotinyl insecticide; insecticidal activity; neuroblocking potency; binding affinity

Chloronicotinyl/neonicotinoid insecticides are a rapidly growing chemical class of insecticides. Since imidacloprid (**1**) was introduced to the Japanese market in 1992, this new class has now a 15% share of the world insecticide market.[1–3] One of the factors that is raising chloronicotinyls to a new major insecticide class is its new insecticidal target with highly intrinsic insecticidal potency.

Biochemical and electrophysiological studies are providing evidence for its agonistic action on the insect nicotinic acetylcholine receptors (nAChR), and some of its neurobiological patterns are shared with the two typical nAChR agonists, nicotine (**2**) and epibatidine (**3**).[4] Indeed, these molecules possess the common structural features of a 6-chloro-3-pyridyl moiety and an amine nitrogen atom at almost the same internitrogen distance. However, there is a striking difference between them. Nicotine and epibatidine have an asymmetric carbon adjacent to the pyridine ring, while imidacloprid is a dissymmetric molecule. The different biological profiles of levorotatory natural (*S*)-nicotine and (1*R*,2*R*,4*S*)-epibatidine from dextrorotatory unnatural isomers have been subjects of neurophysiological, insecticidal and pharmaceutical research.[5–7]

We were interested in if and how the chloronicotinyl molecule would show any different biological action if a chiral center was introduced. For this purpose, we chose imidacloprid, the most prominent compound in this class, as the target molecule and introduced a methyl group to the site corresponding to the asymmetric center of the natural ligands. This paper describes the preparation of **4**, the racemic modification subsequently designated as Me-IMI, its optical resolution, determination of the absolute configuration, insecticidal activity against the housefly by the injection method, nerve excitation potency with the American cockroach central nerve cord, and binding affinity of each enantiomer to the [^{3}H]imidacloprid ([^{3}H]IMI)-binding site(s) on nAChR of the housefly (Fig. 1).

Materials and Methods

Instrumental analyses. All melting point (mp) data are uncorrected. NMR spectra were obtained with a Varian Gemini 2000 C/H instrument (400 MHz) in $CDCl_3$. Chemical shifts were recorded in δ (ppm), and coupling constants J in Hz. IR spectra were

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measured as KBr discs (solid sample) or neat on an NaCl pellet (liquid sample) with a Jasco A-100 spectrometer. Mass spectra were recorded with a Shimadzu QP 1000 mass spectrometer with EI at 70 eV. Preparative HPLC was carried out with Jasco PU-987 pump connected to a Jasco UV-970 spectrometer (254 nm) and Chiralpak AD column (10ϕ × 250 mm, Daisel Chemical Ind., Osaka) in a column chamber (Jasco CO-965).

Preparation of Me-IMI (4) and its optical resolution (Fig. 2).

3-Acetyl-6-chloropyridine (6). A 1.0 M solution of methylmagnesium bromide in THF (200 ml, 0.2 mol) was added dropwise under argon over 3 h to a stirred solution of 6-chloronicotinoyl chloride (**5**; 35.0 g, 0.2 mol) and tris(acetylacetonato)iron(III) (1.8 g, 0.005 mol) in 500 ml of THF at −30°C. After completing the addition, stirring was continued for 10 min at the same temperature, before a 30% aqueous NaOH solution (30 ml) was added dropwise to the vessel, the cooling bath was set aside, and stirring was continued until the reaction contents reached room temperature. The upper THF layer was separated, washed with brine (~1 ℓ) and dried over anhydrous $MgSO_4$. The lower layer, after being combined with this brine and mixed with benzene (300 ml), was filtered on Celite. The benzene layer was separated, washed with fresh brine and dried over anhydrous $MgSO_4$. Distilling off the THF and benzene and subsequent recrystallization of the combined residue from methanol afforded 20.0 g (64.7% yield) of a colorless powder of mp 94°C. IR ν_{max} (film) cm^{-1}: 1670, 1570, 1360, 1260, 1010, 840; EI-MS (m/z): 157 ($M^+ + 2$, 18%), 155 (M^+, 35%), 142 (39%), 140 (100%), 114 (22%), 112 (53%), 76 (26%); ^{1}H-NMR δ: 2.64 (3H, s), 7.45 (d, 1H, J=8.2), 9.20 (1H, dd, J=2.3, 8.2), 8.94 (1H, d, J=2.3); ^{13}C-NMR δ: 26.7, 124.5, 131.2, 138.1, 150.2, 155.7, 195.4. *Anal.* Found: C, 54.28; H, 4.07; N, 8.91%. Calcd. for C_7H_6ClNO: C, 54.04; H, 3.89; N, 9.01%.

1-(6-Chloropyridyl)ethanol (7). To a suspension of 3-acetyl-6-chloropyridine (4.4 g, 0.028 mol) in 30 ml of ethanol and 10 ml of water was added powdered sodium borohydride (2.2 g, 0.058 mol) in small portions (1 h), and the resulting mixture was stirred overnight at room temperature. The solvents were evaporated at below 50°C. The residue was diluted with a mixture of chloroform (10 ml) and water (20 ml), and the chloroform layer was separated. The aqueous phase was extracted with chloroform (3 × 10 ml). The combined chloroform layers were washed once with brine and then dried over anhydrous $MgSO_4$. Evaporating the solvent and subsequent distillation of the remaining liquid afforded 3.08 g (69.2% yield) of a colorless oil of bp 109–110°C at 0.05 mmHg. IR ν_{max} (film) cm^{-1}: 3350 (broad), 1460, 1380, 1105; EI-MS (m/z): 154 ($M^+ - 1$, 10%), 140 (27%), 112 (8%), 68 (11%), 40 (100%); ^{1}H-NMR δ: 1.49 (3H, d, J=6.6), 3.4 (1H, bs), 4.93 (1H, q, J=6.6), 7.28 (1H, d, J=8.4), 7.70 (1H, dd, J=8.4, 2.6), 8.28 (1H, d, J=2.6); ^{13}C-NMR δ: 25.2, 67.2, 124.1, 136.4, 140.3, 147.1, 150.1. *Anal.* Found: C, 53.89; H, 4.80; N, 9.10%. Calcd. for C_7H_8ClNO: C, 53.35; H, 5.11; N, 8.89%.

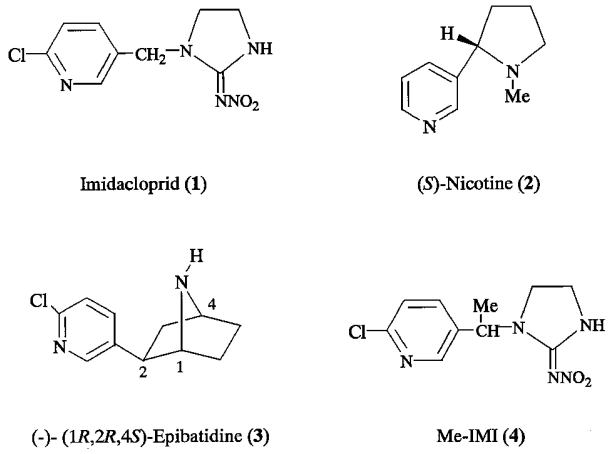


Fig. 1. Imidacloprid, (*S*)-Nicotine, (−)-(1*R*,2*R*,4*S*)-Epibatidine and (*S*)-Methylimidacloprid (Me-IMI).

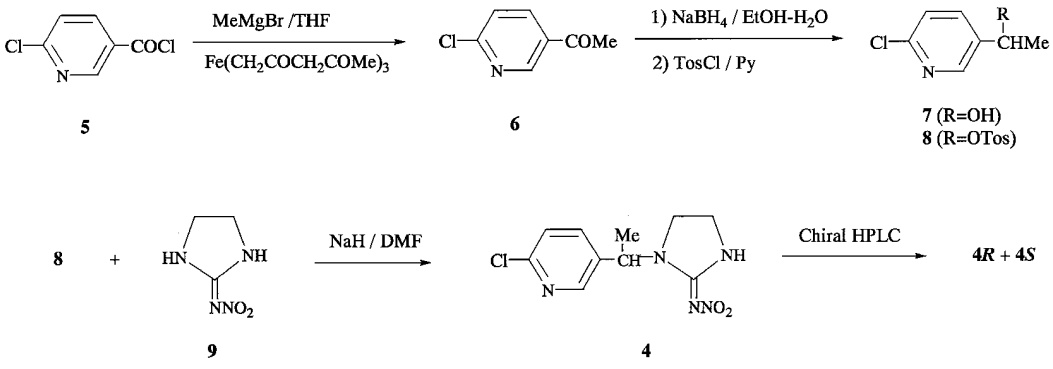


Fig. 2. Preparation Scheme for Me-IMI (**4**) and Optical Resolution.

1-(6-Chloropyridyl)ethyl p-tosylate (8). To a solution of alcohol **7** (3.02 g, 0.02 mol) and pyridine (4.74 g, 0.06 mol) in 20 ml of dry chloroform was added *p*-toluenesulfonyl chloride (7.62 g, 0.04 mol) in portions while ice cooling. After standing in a refrigerator overnight, the mixture was poured into a mixture of chloroform and water (1:1, v/v). The chloroform phase was separated and dried over anhydrous $MgSO_4$. The solvent was evaporated at 20–40°C, and the residue was successively washed with chilled ether and hexane. Tosylate **8** (6.04 g, 97.2% yield) was obtained as a colorless solid of mp 98°C. IR ν_{max} (KBr) cm^{-1}: 1590, 1570, 1460, 1360, 1320, 1190, 1175, 1095, 1020, 910, 815; EI-MS (*m/z*): 313 ($M^+ + 2$, 5.2%), 311 (M^+, 14.4%), 158 (38.1%), 141 (100%); ^{1}H-NMR δ: 1.62 (3H, d, $J = 6.6$), 2.42 (3H, s), 5.57 (1H, q, $J = 6.6$), 7.20 (1H, $J = 8.4$), 7.25 (2H, d, $J = 8.4$), 7.53 (1H, dd, $J = 8.4, 2.1$), 7.65 (2H, d, $J = 8.4$), 8.16 (1H, d, $J = 2.1$); ^{13}C-NMR δ: 21.6, 22.9, 77.2, 109.9, 124.2, 127.7, 129.8, 134.0, 136.6, 145.0, 147.7, 151.6. *Anal.* Found: C, 53.99; H, 4.38; N, 4.50%. Calcd. for $C_{14}H_{14}ClNO_3S$: C, 53.93; H, 4.53; N, 4.49%.

1-[1-(6-Chloro-3-pyridyl)ethyl]-2-nitroiminoimidazolidine (4). A mixture of tosylate **8** (1.7 g, 5.5 mmol), 2-nitroiminoimidazolidine (0.72 g, 5.5 mmol) and potassium carbonate (0.85 g, 6.2 mmol) in acetonitrile was heated under reflux for 8 h. The cooled reaction mixture was filtered from the inorganic solids, the filtrate was concentrated, the residue was extracted with chloroform, and the chloroform was evaporated. The residue was subjected to silica gel column chromatography with a mixture of chloroform and ethanol (9:1, v/v) as the eluent. Repeated washing of the solid with ether and subsequent recrystallization from methanol afforded the product as a colorless powder of mp 138–142°C in a 9.2% yield (137 mg). The sample for the biological tests showed the following analytical data. $R_f = 0.53$ in $CHCl_3$/EtOH (9:1, v/v); IR ν_{max} (KBr) cm^{-1}: 3400, 1555, 1540, 1430, 1300, 1255, 1220, 1100; EI-MS (*m/z*): 272 ($M^+ + 3$, 3.2%), 270 ($M^+ + 1$, 19.3%), 225 (36%), 223 (100%), 142 (16%), 140 (51.3%); ^{1}H-NMR δ: 1.61 (3H, d, $J = 7.3$), 3.22–3.28 (1H, m), 3.47–3.82 (3H, m), 5.55 (1H, q, $J = 7.3$), 7.35 (1H, d, $J = 7.7$), 7.67 (1H, dd, $J = 7.7, 2.5$), 8.19 (1H, bs), 8.38 (1H, d, $J = 2.5$); ^{13}C-NMR δ: 15.7, 40.6, 41.4. 48.8, 124.5, 133.3, 138.1, 148.3, 160.8. *Anal.* Found: C, 44.60; H, 4.51; N, 26.10%. Calcd. for $C_{10}H_{12}ClN_5O_2$: C, 44.53; H, 4.49; N, 25.97%.

Optical resolution of racemate 4 by HPLC. A 500-μl aliquot of a 1 mg/ml solution of **4** in hexane/ethanol (40:60, v/v) was injected into a preparative HPLC system under the following conditions: solvent system, hexane/ethanol (40:60, v/v); flow rate (isocratic), 2.0 ml/min; column chamber temperature, 25°C. The two substances obtained from the fractions with retention times of 13.1 min and 16.2 min were recrystallized from methanol. The melting point of the former compound, designated as **4A**, was 117–119°C, and of the latter, designated **4B**, was 116–118°C. Each isomer sample for the biological tests showed no peaks due to the antipode isomer or any other contamination from the HPLC results.

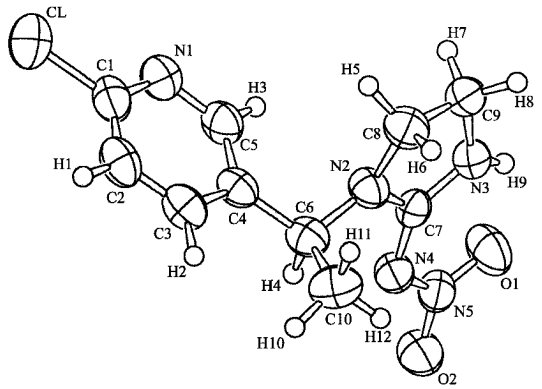


Fig. 3. Crystal Structure of (*S*)-Me-IMI.

X-ray analysis of 4A. Single crystals were grown by slowly evaporating a methanol solution of **4A** at room temperature. Crystallographic data: space group $P2_12_12_1$, a = 13.159(1) Å, b = 14.020(1) Å, c = 6.528(1) Å, V = 1204.4(1) Å^3; Z = 4; $Dx = 1.49$ g cm^{-3}. Intensity data were collected at 293 K by a Rigaku AFC7R diffractometer with graphite-monochromated Cu-Kα radiation ($\lambda = 1.54178$ Å). Of 1342 unique reflections, 1201 reflections with $I > \sigma(I)$ were used for a structural determination. The structure was solved by the direct method (SAPI91; Fan Hai-Fu, 1991; Structure Analysis Programs with Intelligent Control, Rigaku Co., Tokyo) and refined by full-matrix least squares. The absolute configuration was determined by comparing 53 Bijvoet pairs. The final *R* values were 0.037 for the (*S*) configuration and 0.047 for the (*R*) configuration. The significance of this *R*-value difference was justified on the basis of Hamilton's *R*-factor-ratio test at a significance level $\gg 0.995$. The molecular structure and the numbering scheme for the (*S*)-isomer (**4A**) with the selected atom distances and angles, and interatomic distances, torsion and interplane angles are shown in Fig. 3 and Table 1.

Biological tests.

Chemicals. [^{3}H]IMI (1.11 TBq/mmol) was purchased from Amersham Pharmacia Biotech (Buckinghamshire, UK). Reagent-grade piperonyl butoxide (PB) purchased from Tokyo Kasei Kogyo Co. (Tokyo) was used as an inhibitor of oxidative metabolism. NIA 16388 (propargyl propyl benzenephosphonate; NIA), an inhibitor of the hydrolytic metabolism of pyrethroids,[8] was the same sample as that used in our previous study.[9] Before

conducting a series of insecticidal assays, its purity was checked by TLC, no minor spot being found on a silica gel 60 F_{254} plate (Merck Co., Germany). Moreover, the synergistic ratio with stored NIA for imidacloprid was confirmed to be within the range reported.[10]

Insecticidal assay. The method for the insecticidal assay against houseflies was essentially the same as that previously reported.[11] In brief, a methanol solution (1 μl) containing NIA (0.2%, w/v) and PB (0.2%, w/v) was topically applied to the abdomen of female houseflies that had been anesthetized with carbon dioxide. After 1 h at 25°C, a 50% aqueous ethanol solution (0.22 μl) of a test compound at various concentrations was injected into the dorsal side of the thorax. One hour later, again at 25°C, the number of dead and/or paralyzed flies was counted. The 50% effective dose, ED_{50} (mol/insect), was evaluated from the dose-response relationship by the probit method.[12,13] The ED_{50} value is defined as the insecticidal activity.

Neuroblocking test. The neuroblocking activity of each test compound was measured in a similar way to that previously reported.[10,14–16] In brief, the excised abdominal central nerve cord of an adult male American cockroach was cut out between the fourth and fifth ganglia. The nerve preparation containing the fifth and sixth ganglia was placed in a chamber containing a saline solution (pH 7.3).[14] One of two bundles divided from the thoracic side of the nerve cord was tightly taken up with saline into a glass tube, in which a chlorinated silver wire had been set as the electrode. As the reference electrode, another silver wire was set outside the tube in the chamber. The number of spontaneous discharges that were larger than 15 μV was consecutively counted with a pulse counter (MET-1100, Nihon Koden, Tokyo) over several 30-sec periods. The saline solution was exchanged for another one containing a test compound dissolved in methanol. The final concentration of methanol was lower than 1% (v/v), which did not affect the neural activity of the compounds. Measurements were conducted at 22–25°C.

The potency of each compound was next estimated.[15,16] The time (t min) at which the frequency first decreased below 10 counts/30 sec within 30 min after starting the treatment with a compound was determined for each nerve preparation. Those nerve preparations that produced an extraordinarily high or low value in terms of t were not included for the subsequent analysis. The irregularly blocked nerve preparations were usually less than two to obtain three useful data. The concentration-blocking time relationship with at least three nerve preparations was determined for each point, enabling the concen-

Table 1. Selected Interatomic Distances, and Bond, Torsion and Interplane Angles[a]

Distance (Å)		Bond angle (deg)		Torsion angle (deg)[b]	
CL-C1	1.731(3)	C6-N2-C7	123.4(2)	N1-C5-C4-C6	177.1(3)
O1-N5	1.235(4)	C6-N2-C8	123.2(3)	N2-C6-C4-C3	−152.2(3)
O2-N5	1.235(3)	C7-N2-C8	110.1(2)	N2-C6-C4-C5	31.0(4)
N1-C1	1.321(5)	C7-N3-C9	111.1(2)	N2-C7-N3-C9	−3.2(3)
N1-C5	1.340(4)	N1-C5-C4	125.6(3)	N2-C7-N4-N5	177.3(2)
N2-C6	1.460(4)	N5-N4-C7	116.8(2)	N2-C8-C9-N3	−19.3(3)
N2-C7	1.350(4)	N2-C6-C4	109.7(2)	N3-C7-N2-C8	−10.7(3)
N2-C8	1.467(4)	O1-N5-O2	121.7(2)	N3-C7-N4-N5	−4.8(5)
N3-C7	1.327(3)	N2-C6-C10	111.6(3)	N4-C7-N2-C6	7.7(4)
N3-C9	1.462(4)	O1-N5-N4	123.4(2)	C3-C4-C6-C10	−25.7(4)
N4-N5	1.345(3)	C4-C6-C10	114.7(3)	C4-C6-N2-C7	−137.7(3)
N4-C7	1.344(3)	O2-N5-N4	114.9(2)	C4-C6-N2-C8	65.0(4)
C3-C4	1.393(5)	N2-C7-N3	110.2(2)	C5-C4-C6-C10	157.4(3)
C4-C6	1.520(4)	N2-C7-N4	116.6(2)	C6-N2-C8-C9	179.0(3)
C6-C10	1.537(5)	N3-C7-N4	133.2(2)	C7-N2-C6-C10	94.2(3)
C8-C9	1.523(5)	N2-C8-C9	101.9(3)	C8-N2-C6-C10	−63.1(4)
N1-N2	4.112	θ_g[c]	70.6		
N1-C7	5.123				

[a] Estimated standard deviations in the least significant figure are given in parentheses.
[b] The sign is positive if, when looking from atom 2 to atom 3, the clockwise motion of atom 1 would be superimposed on atom 4.
[c] Interplane angle between the pyridine and imidazolidine rings.

Table 2. Insecticidal and Binding Affinity of Methylimidacloprid, Nicotine, Epibatidine and Imidacloprid

Compound	Insecticidal activity[a]	ED_{50} (fmol/insect)[b]	Neuroblocking potency	Binding of [^{3}H]IMI
	Alone	+(PB+NIA)	BC (μM)	IC_{50} (μM)[c]
(*RS*)-Me-imidacloprid	26 (±5)	1.7 (±0.5)	15±5	0.21 (±0.06) (2)
(*S*)-Me-imidacloprid	45 (±30)	1.1 (±0.7)	5.9±1.5	0.19 (±0.04) (2)
(*R*)-Me-imidacloprid	55 (±5)	2.3 (±0.5)	73±27	0.95 (±0.18) (3)
(*S*)-nicotine				11.9 (1)
(*R*)-nicotine				422 (1)
(+)-epibatidine[d]				2.79 (1)
(−)-epibatidine[e]				1.34 (1)
imidacloprid	2.2 (±0.0)	0.26 (±0.09)	2.3±0.6	0.02 (±0.01)[f]

[a] Injection to *M. domestica*.
[b] Standard deviation for two runs in parentheses.
[c] Standard deviation or width from the average or single value for the number of experimental runs indicated in parentheses.
[d] (1*S*,2*S*,4*R*) isomer.
[e] (1*R*,2*R*,4*S*) isomer.
[f] Cited from ref. 18.

tration of each compound, BC (M), at which t attained 10 min to be found. The BC values for the test compounds are listed in Table 2.

Binding assay. The preparation of the housefly-head membrane fraction was fundamentally the same as that described previously.[11,17,18] Housefly heads were homogenized in buffer A (100 mM sodium phosphate at pH 7.4 containing 0.32 M sucrose and 0.1 mM EDTA) by using a glass homogenizer driven by a motor. The homogenate was filtered through three layers of cheesecloth to remove the debris. The resulting filtrate was centrifuged at 700 g for 10 min, and the supernatant was further centrifuged at 125,000 g for 60 min. The pellet was suspended in buffer B (10 mM sodium phosphate at pH 7.4, containing 50 mM sodium chloride and 0.1% (w/v) Triton X-100). This suspension was used for the binding assay. The protein concentration was measured by a bicinchoninic acid protein-assay kit (Pierce Chemical Co., Rockford, IL, USA) with bovine serum albumin as a standard.

The binding assay was conducted according to the procedure reported.[18] The housefly membrane fraction (3 mg/ml of protein, 200 μl) was placed in a glass tube (12 × 75 mm) containing 2 μl of a test compound dissolved in DMSO. After incubating at 24°C for 3–5 min, [^{3}H]IMI (50 μl), which had been prepared by diluting with buffer B, was added to give a final concentration of 10 nM. The mixture was further incubated at 24°C for 60 min. The reaction was terminated by filtration through a GF/B Unifilter which had been treated with a washing buffer consisting of 10 mM sodium phosphate at pH 7.4 and 50 mM sodium chloride. The tube was rinsed with the washing buffer (2 × 1 ml). The filter was then was washed with the washing buffer (4 × 2.5 ml) and dried under an infrared lamp for about 30 min. After adding of 2 ml of Aquasol-2 over the filter in a vial, radioactivity was measured with a liquid scintillation counter (2500TR, Packard Instrument Co., Meriden, ID, USA). The molar concentration of test compounds required for 50% inhibition of the specific binding of [^{3}H]IMI, IC_{50} (M), was determined by a nonlinear regression analysis using PRISM (Graphpad Software Inc., San Diego, CA, USA). The IC_{50} values for compounds are given in Table 2 along with the standard deviation for some compounds.

Results

Preparation

The synthesis of racemic modification **4** is outlined in Fig. 2. It is worth noting that the selective conversion of 6-chloronicotinoyl chloride **5** to 3-acetyl-6-chloropyridine **6**, with retention of the ring chlorine atom, was better carried out by the Grignard reaction with the addition of tris(acetylacetonato)iron(III)[19] than by the other methods tried.

The optical resolution of the racemic modification to enantiomers **4A** and **4B** was successfully conducted by HPLC with an amylose carbamate-coated silica gel column.

Crystal and molecular structure of enantiomer 4A

The asymmetric carbon of **4A** was in the (*S*) configuration, and the methyl group was located in the space between H2 and the nitroimino N4 nitrogen (Fig. 3). The chloropyridyl and imidazolidine planes were angled at 70.6° through a methylene bridge to avoid steric repulsion between pyridyl H2/H3 and imidazolidine H5/H6. The conjugated nitroguanidine moiety was made up of a coplanar nitroimine-amine π-electron network with shorter C7-N2 (1.35 Å)/C7-N3 (1.33 Å) bonds than the normal C-N (amine) (1.47 Å) but close to C=N (imine) (1.33 Å) resulting from transfer of the lone-pair electrons on the amines to the nitro group. Another particular feature of the enamine part was the *E*-configuration about the exo-cyclic C7=N4 bond. The preference for this geometry was due to the larger steric increment of the picolyl group in addition to intramolecular hydrogen bonding between the N3 amine hydrogen and the nitro oxygen forming a six-membered hydrogen bond ring. The N1-N2 distance was calculated to be 4.11 Å. These features are similar to those of imidacloprid.[20]

Insecticidal activity, neurophysiological effects and binding potency

Racemic Me-IMI had lower insecticidal activity and binding affinity than imidacloprid, but the potency was sufficiently high to elucidate the biological difference among the configurations.

The ED_{50} values of the Me-IMI isomers against the housefly were at femto molar levels, the (*S*) enantiomer appearing to be slightly more toxic than the (*R*) isomer. Synergist PB affects the activity by inhibiting the microsomal P450 oxidase system,[21] and the effects of NIA on the activity were presumably due to the inhibition of microsomal *N*-dealkylation and hydrolysis according to the literature.[8] In the present tests, the effect of a combination of these two synergists was 15-fold for the racemate, 41-fold for the (*S*) form and 24-fold for the (*R*) form. This synergic ratio was greater than 8 for imidacloprid, reflecting the likelihood that the incorporated α-methyl and/or methine carbon is likely to have been oxidatively metabolized. It may be also said that the activity of the (*S*) form was more than that of the antipode. The enhancing effect of NIA on the insecticidal activity of chloronicotinyl compounds has been reported elsewhere.[10,22,23]

Electrophysiological measurements of the compound acting at the central nervous system provide another evaluation of the activity at the target site. Those compounds with greater nerve-exciting poten-

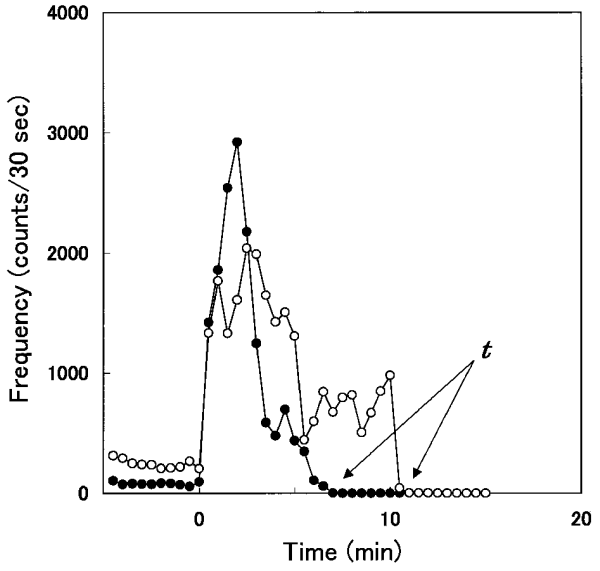


Fig. 4. Time-course Characteristics of the Effect of (*R*)-Me-IMI on Spontaneous Discharges in the Excised Central Nerve-cords of American Cockroaches.

After counting the cumulative number of spontaneous discharges every 30 sec for 5 min, the saline was exchanged with saline containing the test compound at 87 μM (○) and 170 μM (●). The time of exchange is defined as the zero time, and the number of discharges was similarly counted. Arrows indicate the "blocked" points.

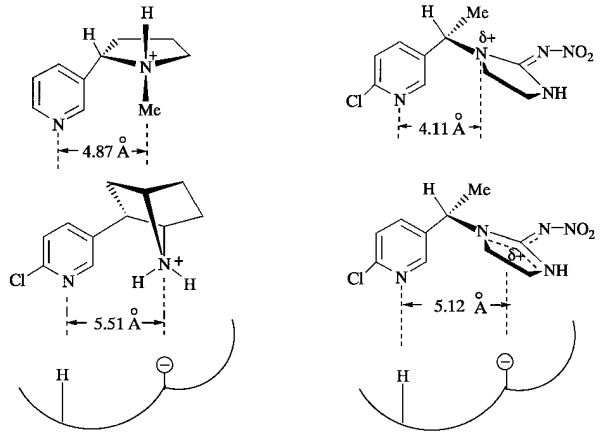


Fig. 5. Binding Models for (*S*)-Nicotine, (−)-(1*R*,2*R*,4*S*)-Epibatidine and (*S*)-Me-IMI.

cy elicited a higher frequency and a shorter time for subsiding at the same doses. Figure 4 shows time-dependent change in the frequency of firing in nerve cords treated with (*R*)-Me-IMI **(4B)** at two concentrations. Before treatment with **4B**, the nerve preparation gave spontaneous discharges with a low frequency. Application of the test compound immediately increased the level of impulses and their frequency, which then subsided to a level lower than that of the control. This biphasic characteristic of the nerve response is similar to that of imidacloprid.[18] The neuroblocking activity of racemic Me-IMI in terms of BC was lower than that of imidacloprid by a factor of 10 (Table 2). The potency difference in the neuroblocking action is obvious between the enantiomers, and (*S*)-Me-IMI was approximately ten times more active than the antipode.

In the binding experiment with the housefly membranes, Me-IMI displaced the labeled imidacloprid at as a low concentration for IC_{50} as 0.21 μM for the racemate, which was only ten times greater than that of imidacloprid, while epibatidine binds with an IC_{50} value 70–140 times greater than that and nicotine needed as much as a 595–21,100 times higher concentration to displace the [^{3}H]IMI. It is notable that nicotine stereoselectively competed at [^{3}H]IMI binding sites with a 35-fold difference in IC_{50} values between the (*R*) and (*S*) isomers, while Me-IMI discriminated five times the enantioselective affinity, this being only modest in the case of epibatidine.

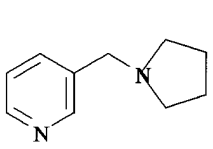
Discussion

The functional architecture and diversity of insect nAChRs, a target of neonicotinoids, are poorly understood relative to those of mammals. Based on the accumulated structures of this class, several pharmacophore models have been proposed[20,24–26] by referring to the previous models for nAChR agonists.[27,28] These models commonly require two elements, a) a nitrogen atom conjugated to a strongly electron-withdrawing group like a nitroimino moiety and b) a hydrogen-bond acceptor like the pyridine nitrogen atom at a 4–5 Å internitrogen distance. The nitrogen atom signified in a) is electron deficient and has an electronic nature common to the pyrrolidinyl nitrogen of nicotine or to the flagpole nitrogen of epibatidine which is fully ionized to the ammonium ion in the physiological fluid.

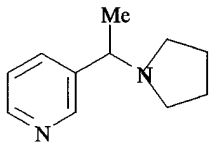
Glennon and co-workers have re-examined the pharmacophore models based on a comparative molecular field analysis (CoMFA) of a variety of nAChR ligands, including conformationally flexible and rigid skeletons, and have induced a new relationship between the internitrogen distance and the nAChR affinity, whereby the affinity can be better explained by a parabolic relationship with the internitrogen distance, the optimal distance being *ca* 5.3 Å.[29] The calculated internitrogen distance for epibatidine of 5.51 Å is close to the optimal distance, while this distance for nicotine is as short as 4.87 Å,[30] which reflects well the essentially lower affinity of nicotine than epibatidine. The internitrogen distance for Me-IMI was calculated as 4.11 Å from crystallographic data, which is even shorter than that for nicotine. However, the atomic charge map for the Me-IMI molecule shows that the positive charge potential is not centered at the N2-atom but rather is delocalized over the guanidine moiety (N2-C7-N3) (Akamatsu, unpublished results). It would therefore

be rational to take a value close to 5.12 Å for N1-C7 instead of internitrogen as the distance in question, and this distance accounts well for the high affinity by the Glennon model. Figure 5 shows the binding model based on the concept by Beer and Reich, using Glennon's steric parameters for nicotine and epibatidine and our X-ray analysis data for compound **4**.

The foregoing argument entitles Me-IMI to be classified as an agonist. The question is, then, why is the affinity of Me-IMI inferior to that of imidacloprid? The results of an experiment by Glennon and co-workers on an optional isomer of nicotine suggest an answer to this question. The binding value (*Ki*) of 3-pyridylmethylpyrrolidine (**10**) was 30 nM with



10



11

(*RS*)-[^{3}H]nicotine, when employing the rat whole brain, while methylated compound **11** gave a value as large as 1,300 nM.[31] The decrease in potency by the incorporated α-methyl group can be ascribed to steric hindrance in the approach of the ligand to the receptor binding site. Similar steric impairment is presumed for Me-IMI bound to insect nAChR.

Where does the different enantioselectivity in binding originate? The binding affinity to the [^{3}H]IMI binding site at housefly nAChR of the (*S*) isomer of Me-IMI was 5-fold larger than that of the (*R*) isomer, whereas for nicotine, the potency of the natural (*S*) form was as much as 35 times that of the unnatural (*R*) form, and we can also regard the natural (1*R*,2*R*,4*S*) form as being more potent for epibatidine. Given that the chiral center of a ligand stereospecifically approaches the receptor, the presence of some stereoselective activity in these chiral molecules is understandable. Furthermore, there is the finding that nornicotine (desmethyl nicotine) did not bind in an enantioselective fashion,[29] which suggests that the *N*-methyl group of nicotine must interact with a lipophilic segment on the receptor in conjugation with contact involving the asymmetric atom. In Me-IMI, the imidazolidine ethylene part corresponds to this methyl group, whereas the corresponding site in epibatidine is flagpole NH_2 which is less likely to give up the lipophilic interaction with the receptor. There has been a report that distinct stereoselectivity among the enantiomers emerged in *N*-methyl epibatidine.[32] Another aspect for this different enantioselectivity is that, in nicotine, the distance from the asymmetric carbon to the ammonium nitrogen atom is shorter than that in Me-IMI, where the Coulombic-binding center deviates a little from the nitrogen atom toward the nitroimino carbon as already discussed, and the corresponding distance in epibatidine is far greater than in the others. The closer to the cationic binding scaffold, the more the stereoconfiguration at the chiral center should be influential. These factors combined would account for the difference in enantioselectivity among them.

We show two representative types of neurophysiological effects for the Me-IMI analogues, excitatory and blocking in Fig. 4. In our quantitative analyses of the relationships between the insecticidal and neurophysiological activities for a set of imidacloprid and related compounds, the blocking effect generally provides a more reliable parameter than the exciting effect to describe the variations in the insecticidal activity.[15] The ten-times-higher activity of (*S*)-Me-IMI than that of the (*R*) isomer in the present neuroblocking tests agrees with the characteristics observed for the insecticidal activity (Table 2). A conflicting feature is the smaller factor by two of the insecticidal activity than would be expected from the neuroblocking activity. One possible explanation for this is that not only the neuroblocking effect but also the neuroexcitatory effect makes some contribution to the insecticidal potency. A difference in the insect species used is another possibility. When a test compound is injected into the insect body, the compound must be affected by such factors as metabolic inactivation (or activation) and transportation to and interaction with the target sites to have a fatal effect on the insect. Even though the measurements were conducted under conditions that would minimize as much as possible the factors due to metabolism by using PB and NIA, a possible remaining metabolic factor and some other factors might have overshadowed the enantioselectivity in the insecticidal effect. In fact, a straightforward estimate involving the neonicotinoid-specific binding sites of the present binding potency gave clear-cut proof for stereoselectivity among the enantiomers.

To summarize, the crystallographic data for Me-IMI possessing a chiral carbon at the "benzylic" site show a similar push-pull molecular framework to that of imidacloprid. The (*S*) enantiomer had 5-fold higher affinity to the [^{3}H]IMI binding site of housefly nAChRs than the antipode, with similar enantioselective characteristics, but a much smaller ratio than nicotine, of 35-fold preference for the (*S*) isomer. Epibatidine showed only twice the enantioselectivity for the (1*R*,2*R*,4*S*) isomer. This selectivity difference among these molecules is ascribed to the distance between the chiral carbon and the positive charge center, as well as to the presence of the pertinent lipophilic residue around the binding center.

Acknowledgments

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References

1) Kagabu, S., Chloronicotinyl insecticides-discovery, application and future perspective. *Rev. Toxicol.*, **1**, 75–129 (1997).
2) Elbert, A., Nauen, R., and Leicht, W., Imidacloprid, a novel chloronicotinyl insecticide: biological activity and agricultural importance. In ''Insecticides with Novel Modes of Action, Mechanism and Application'', eds. Ishaaya, I., and Degheele, D., Springer, Berlin, pp. 50–73 (1998).
3) Yamamoto, I., and Casida, J. E., Nicotinoid Insecticides and the Nicotinic Acetylcholine Receptor, Springer, Berlin (1999).
4) Nauen, R., Ebbinghaus-Kintscher, U., Elbert, A., Jeschke, P., and Tietjen, K., Acetylcholine receptors as sites for developing neonicotinoid insecticides. In ''Biochemical Sites of Insecticide Action and Resistance'', ed. Ishaaya, I., Springer, Tokyo, pp. 77–105 (2000).
5) Ducat, M., Damaj, M. I., Glassco, W., Dumas, D., May, E. L., Martin, B. R., and Glennon, R. A., Epibatidine: a very high affinity nicotine-receptor ligand. *Med. Chem. Res.*, **4**, 131–139 (1993).
6) Badio, B., and Daly, J. W., Epibatidine: a potent analgetic and nicotinic agonist. *Mol. Pharmacol.*, **45**, 563–569 (1994).
7) Tomizawa, M., Otsuka, H., Miyamoto, T., Eldefrawi, M. E., and Yamamoto, I., Pharmacological characteristics of insect nicotinic acetylcholine receptor with its ion channel and the comparison of the effect of nicotinoids and neonicotinoids. *J. Pesticide Sci.*, **20**, 57–64 (1995).
8) Suzuki, T., and Miyamoto, J., Metabolism of tetramethrin in houseflies and rats *in vitro*. *Pestic. Biochem. Physiol.*, **4**, 86–97 (1974).
9) Nakagawa, S., Okajima, N., Nishimura, K., Fujita, T., and Nakajima, M., Quantitative structure-activity studies of substituted benzyl chrysanthemates. 1. Correlation between symptomatic and neurophysiological activities against American cockroaches. *Pestic. Biochem. Physiol.*, **17**, 243–258 (1982).
10) Nishimura, K., Kanda, Y., Okazawa, A., and Ueno, T., Relationship between insecticidal and neurophysiological activities of imidacloprid and related compounds. *Pestic. Biochem. Physiol.*, **50**, 51–59 (1994).
11) Nishiwaki, H., Nakagawa, Y., Takeda, D. Y., Okazawa, Y., Akamatsu, M., Miyagawa, H., Ueno, T., and Nishimura, K., Binding activity of substituted benzyl derivatives of chloronicotinyl insecticides to housefly-head membranes, and its relationship to insecticidal activity against the housefly *Musca domestica*. *Pest Manag. Sci.*, **56**, 875–881 (2000).
12) Finney, D. J., Probit Analysis, Cambridge Univ. Press, Cambridge, UK (1952).
13) Sakuma, M., Probit analysis of preference data. *Appl. Entomol. Zool.*, **33**, 339–347 (1998).
14) Nishimura, K., Tanaka, M., Iwaya, K., and Kagabu, S., Relationship between insecticidal and nerve excitatory activities of imidacloprid and its alkylated congeners at the imidazolidine NH site. *Pestic. Biochem. Physiol.*, **62**, 172–178 (1998).
15) Kiriyama, K., Iwaya, K., Kagabu, S., and Nishimura, K., Relationship between insecticidal and nerve activities of an imidacloprid-related nitromethylene compound and its *N*-alkyl congeners. *J. Pesticide Sci.*, **26**, 55–59 (2001).
16) Kagabu, S., Azuma, A., and Nishimura, K., Insecticidal and neuroblocking activities of thiacloprid and its acyclic analogues and their related cyanoguanidine derivatives. *J. Pesticide Sci.*, **27**, 267–271 (2002).
17) Okazawa, A., Akamatsu, M., Ohoka, A., Nishiwaki, H., Cho, W.-J., Nakagawa, Y., Nishimura, K., and Ueno, T., Prediction of the binding mode of imidacloprid and related compounds to housefly head acetylcholine receptors using three-dimensional QSAR analysis. *Pestic. Sci.*, **54**, 134–144 (1998).
18) Kagabu, S., Iwaya, K., Konishi, H., Sakai, A., Itazu, Y., Kiriyama, K., and Nishimura, K., Synthesis and alkylene-tethered bis-imidacloprid derivatives as highly insecticidal and nerve-exciting agents with potent affinity to [^{3}H]imidacloprid-binding sites on nicotinic acetylcholine receptor. *J. Pesticide Sci.*, **27**, 249–256 (2002).
19) Fiandanese, V., Marchese, G., Martina, V., and Ronzini, L., Iron catalyzed cross-coupling reactions of acyl chloride with Grignard reagents. *Tertrahedron Lett.*, **25**, 4805–4808 (1984).
20) Kagabu, S., and Matsuno, H., Chloronicotinyl insecticides. 8. Crystal and molecular structures of imidacloprid and analogous compounds. *J. Agric. Food Chem.*, **45**, 276–281 (1997).
21) Casida, J. E., Mixed-function oxidase involvement in the biochemistry of insecticide synergists. *J. Agric. Food Chem.*, **18**, 753–772 (1970).
22) Liu, M.-Y., Lanford, J., and Casida, J. E., Relevance of [^{3}H]imidacloprid binding site in housefly head acetylcholine receptor to insecticidal activity of 2-nitromethylene- and 2-nitroimino-imidazolidines. *Pestic. Biochem. Physiol.*, **46**, 200–206 (1993).
23) Miyagawa, H., Sato, K., Nishiwaki, H., Nakagawa, Y., and Miyashita, M., Metabolism of imidacloprid in houseflies and effect of synergists. 10th International Congress on Chem. Crop Protect. **Aug. 2002**, Basel, Switzerland, Abstract No. 3d, 12.
24) Yamamoto, I., Yabuta, G., Tomizawa, M., Saito, T., Miyamoto, T., and Kagabu, S., Molecular mechanism for selective toxicity of nicotinoids and neonicotinoids. *J. Pesticide Sci.*, **20**, 33–40 (1995).
25) Nakayama, A., and Sukekawa, M., Quantitative correlation between molecular similarity and receptor-binding activity of neonicotinoid insecticides. *Pestic. Sci.*, **52**, 104–110 (1998).
26) Matsuda, K., Buckingham, S. D., Kleier, D., Rauh,

J. J., Grauso, M., and Sattelle, D. B., Neonicotinoids: insecticides acting on insect nicotinic acetylcholine receptors. *Trends Pharmacol. Sci.*, **22**, 573–579 (2001).
27) Beers, W. H., and Reich, E., Structure and activity of acetylcholine. *Nature*, **228**, 917–922 (1970).
28) Sheridan, R. P., Nilakantan, R., Dixon, J. S., and Venkataraghavan, R., The ensemble approach to distance geometry: application to the nicotinic pharmacophore. *J. Med. Chem.*, **29**, 899–906 (1986).
29) Glennon, R. A., Herndon, J. L., and Dukat, M., Epibatidine-aided studies toward definition of a nicotine receptor pharmacophore. *Med. Chem. Res.*, **4**, 461–473 (1994).
30) Glennon, R. A., and Dukat, M., Nicotine receptor ligands. *Med. Chem. Res.*, **6**, 465–486 (1996).
31) Glennon, R. A., Maarouf, A., Fahmy, S., Martin, B., Fan, F., Yousif, M., Shafik, R. M., and Dukat, M., Structure-affinity relationships of simple nicotine analogs. *Med. Chem. Res.*, **2**, 546–551 (1993).
32) Badio, B., Shi, D., Garraffo, H. M., and Daly, J. W., Antinoceptive effects of the alkaloid epibatidine: further studies on involvement of nicotinic receptors. *Drug Dev. Res.*, **36**, 46–59 (1995).