Synthesis of 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

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Bivalent molecules of bis-imidacloprid with 2–10 alkylene tethers as well as tethers containing an ethylene, ethynylene, phenylene and oxide joint were prepared. These dimeric chloronicotinyl molecules were highly insecticidal against American cockroaches on injection at 2–30 nanomolar doses. The minimum lethal dose of the most potent hexamethylene derivative was close to that of imidacloprid, and the potency was augmented up to about thirty-five-fold following pretreatment with metabolic inhibitors, while the binding affinity to \[^{3}H\]imidacloprid-binding sites on the nicotinic acetylcholine receptor was weaker than that of imidacloprid by a factor of 160. The hexamethylene derivative elicited impulses in cockroach central nerves with an initial excitation and subsequent block at a potency comparable to imidacloprid.

Key words: neonicotinoid insecticides, imidacloprid, American cockroach, insecticidal activity, nerve-excitatory activity, alkylene-tethered bis-imidacloprid.

INTRODUCTION

Imidacloprid (1) and related neonicotinoids represent a novel and distinct chemical class of insecticides with remarkable crop-protection performances.\(^1\) Biochemical and electrophysiological experiments are elucidating their agonistic action on the nicotinic acetylcholine receptor (nAChR), and contrivances of structural variations from the prototype are developing promising products with a general insecticidal structure (Fig. 1).\(^2\)–\(^6\) Structure-activity relationship studies have been conducted on each part of the prototype molecule: the substituents on the pyridine ring, the length between the pyridine and the imidazolidine rings, the functional groups conjugated with the imidazolidine ring, the \(N3\)-area around the imidazolidine ring, and cyclic or acyclic modification of the imidazolidine ring. A study regarding the \(N3\)-area on the imidazolidine ring revealed that alkyl, alkenyl, and aralkyl substituents decrease the insecticidal activity as well as nerve-excitation potency, and the activity drop is greater as the introduced longer alkyl chain is longer.\(^7\)–\(^8\)

Recently Pang et al. fashioned a bifunctional ligand that is an extraordinarily potent inhibitor of acetylcholine esterase by using a string of methylene group to couple two molecules of an existing drug.\(^9\),\(^10\) This highlight of drug design provided us an incentive to study the physiological properties of tethered bivalent ligands composed of nAChR agonist neonicotinoid molecules. We initiated a study with imidacloprid coupling through an alkylene liaison between two \(N3\)-atoms of the imidazolidine rings. The alkylene represents 2–10 carbons and those entailing a phenylene, ethynylene, ethynylene or oxide filler. The prepared bivalent molecules were highly insecticidal against American cockroaches on injection. The results were quite different in the presence of metabolic inhibitors from those for monovalent \(N3\)-alkyl derivatives.\(^7\) Further, some selected ligands displayed strong binding affinity to \[^{3}H\]imidacloprid-binding sites on housefly nAChR and...
agonistic nerve-excitatory profiles. In this paper, we describe the synthesis of this new type of compound and interesting biological activities.

MATERIALS AND METHODS

1. Synthesis of Compounds

All melting points (mp) are uncorrected. NMR spectra were obtained with a Varian Gemini 2000 C/H instrument (400 MHz). The chemical shifts were recorded in δ (ppm) and the coupling constants J in Hz. IR spectra were measured as KBr discs with a JASCO A-100 spectrometer. Mass spectra were recorded (El, 70 eV) with a Shimadzu QP 1000 mass spectrometer. The physical and spectral data for the prepared compounds are given in Tables 1-3.

1.1 Representative preparative procedures

1.1.1 1,8-Bis[(6-chloronicotinyl)-2-nitroiminoimidazolidin-3-yl]octane (8)

Imidacloprid (1.03 g, 4 mmol) was added to a suspension of sodium hydride (60% oil dispersion, 0.17 g, 42.5 mmol) in 10 ml of DMF, and the mixture was stirred at room temperature for 1 hr. The cooled reaction mixture in an ice-water bath was treated dropwise with a solution of 1,8-diiodooctane (0.67 g, 2 mmol) in 10 ml of DMF in half an hour. The ice-bath was set aside and the stirring was continued at room temperature for 4 hr. The DMF was distilled off under reduced pressure and the residue was dissolved in water and chloroform. The chloroform phase was separated and dried over anhydrous MgSO₄. After evaporation of the solvent, the semi-solid residue was subjected to column chromatography (silica gel). The fractionation was carried out first with ethyl acetate as eluent until imidacloprid was no longer detected in the fraction on HPLC, then by elution with a mixture of ethyl acetate and ethanol (15:1 v/v). The product was recrystallized from ethanol. The yield was 0.95 g (77%).

Compounds 3-b and 10 were prepared by similar procedures, and compounds 7, 9 and 13 and compounds 14 and 15 were prepared from a,ω-bistosylates and a,a'-dichlorides, respectively, in place of diiodides.

1.1.2 1,4-Bis[(6-chloronicotinyl)-2-nitroiminoimidazolidin-3-yl]-(2E)-butene (II)

A mixture of imidacloprid (3.37 g, 12 mmol), 1,4-dibromo-2-(E)-butene (1.60 g, 6 mmol) and potassium carbonate (1.83 g, 12 mmol) in 30 ml of acetonitrile was refluxed for 19 hr. After evaporation of the solvent, the residue was taken up with chloroform, which was washed with water and dried over anhydrous MgSO₄. After evaporation of the solvent, the residue was subjected to column chromatography (silica gel). The fractionation was carried out first with ethyl acetate as eluent until imidacloprid was no longer detected in the fraction on HPLC, then by elution with a mixture of ethyl acetate and ethanol (15:1 v/v). The product was recrystallized from ethanol. The yield was 0.22 g (7%).

Compound 12 was prepared similarly.

1.1.3 1,2-Bis[(6-chloronicotinyl)-2-nitroiminoimidazolidin-3-yl]ethane (2)

A suspension of imidacloprid (0.13 g, 0.5 mmol), ethane 1,2-bistosylate (92 mg, 0.25 mmol) and potassium carbonate (160 mg, 1.16 mmol) in 5 ml of a mixture of THF and DMF (9:1 v/v) was refluxed for 50 hr. After evaporation of THF, the residue was subjected to column chromatography (silica gel). The fractionation was carried out first with ethyl acetate as eluent until imidacloprid was detected in the fraction on HPLC, then with a mixture of ethyl acetate and ethanol (10:1 v/v). The product was recrystallized from ethanol. The yield was 30 mg (11%).

2. Biological Tests

2.1 Chemicals

[3H]Imidacloprid (1.11 TBq/mmol) was purchased from Amersham Pharmacia Biotech, Buckinghamshire, UK. Reagent-grade piperonyl butoxide (PB) purchased from Tokyo Kasei Kogyo Co., Tokyo, Japan was used as an inhibitor of oxidative metabolism. NIA 16388 (propargyl propyl benzenephosphonate; NIA), originally an inhibitor of the hydrolytic metabolism of pyrethroids, was the same sample used in our previous study. The insecticidal assay against male adult American cockroaches, Periplaneta americana L., was conducted as described previously. Various volumes (1-10 μl) of each compound dissolved in DMSO containing some amount of methanol were injected into the abdomen of a cockroach. The volume of the DMSO fraction was up to 5 μl. Organic solvents alone in this range did not have a toxic effect. Details of the dosage were fundamentally the same as described previously. The doses were varied by 1.25. In some experiments, a methanol solution (1 μl) containing PB (50 μg), NIA (50 μg) or a mixture of PB (50 μg) and NIA (50 μg) was injected 1 hr before injection of the test compound. The metabolic inhibitors in these amounts did not have a toxic effect. Details of the dosage were fundamentally the same as described previously. The doses were varied by 1.25. In some experiments, a methanol solution (1 μl) containing PB (50 μg), NIA (50 μg) or a mixture of PB (50 μg) and NIA (50 μg) was injected 1 hr before injection of the test compound. The metabolic inhibitors in these amounts did not have a toxic effect. Three insects were used to test each dose of compound and were kept at 22–25°C for 24 hr after injection. The minimum dose at which two of three insects were considered to be killed was taken as the minimum lethal dose (MLD in moles). Paralyzed
insects were also counted as killed, because they did not recover. The MLD values for the test compounds are listed in Table 4. Each value is the mean of at least two experiments with a deviation of 0.64 to 1.6-fold.

2.3 Binding assay

The preparation of the housefly-head membrane fraction was fundamentally the same as that described previously.15,16) Housefly heads were homogenized in buffer A (100 mM sodium phosphate buffer, pH 7.4) containing 0.32 M sucrose and 0.1 mM EDTA using a glass homogenizer with a motor. The homogenate was filtered through three layers of cheesecloth to remove debris. The 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 buffer, pH 7.4, containing 50 mM sodium chloride and 0.1% (w/v) Triton X-100). The suspension was used for the binding assay. The protein concentration was measured using a bicinchoninic acid protein-assay kit (Pierce Chemical Co., Rockford, IL) with bovine serum albumin as a standard.

The binding assay was conducted as reported previously.17) The housefly membrane fraction (3 mg/ml protein, 20 µl) was placed in a glass tube (12 X 75 mm) containing 2 µl of test compound dissolved in DMSO. After incubation at 24°C for 3-5 min, [3H]imidacloprid (50 nM), which was prepared by dilution with buffer B, was added so as to make its final concentration 10 nM. The mixture was further incubated at 24°C for 60 min. The reaction was terminated by filtration through a Unifilter GF/B, which had been treated with a washing buffer consisting of 10 mM sodium phosphate buffer (pH 7.4) and 50 mM sodium chloride. The tube was rinsed with the washing buffer (1 ml X 2 times). Then, the filter was washed with the washing buffer (2.5 ml X 4 times) and dried under an infrared lamp for about 30 min. After addition of Aquasol-2 (2 ml) over the filter in a vial, measurements of radioactivity were made with a liquid scintillation counter (2500TR, Packard Instrument Co., Meriden). The molar concentration of test compounds required for 50% inhibition of the specific binding of [3H]imidacloprid, IC50 (M), was determined by a nonlinear regression analysis using PRISM (Graphpad Software Inc., San Diego, CA). The IC50 values for tested compounds are given in Table 4 along with the standard deviation from two determinations.

2.4 Neurophysiological assay

The neurophysiological test of the compounds was conducted as described previously.7,13,14) In brief, a nerve preparation containing the abdominal fifth and sixth ganglia of a male adult American cockroach was placed in a saline solution. One of the bundles of the nerve cord on the thoracic side was taken up with saline into a glass tube, in which a silver wire was set as an electrode. As the reference electrode, another wire was set around the cut end of the tube. The silver wires were thinly coated with silver chloride. The number of spontaneous discharges that were larger than approximately 15 µV was consecutively counted with a pulse counter (MET-1100, Nihon Koden, Tokyo, Japan) over 30-sec periods. The frequency was usually quite high for a few minutes after setting, and then normally subsided. When the frequency decreased at around a range of 30-500 counts per 30 sec for 5 min or more, the saline solution was exchanged for saline containing test compound dissolved in methanol or containing DMSO. The final concentration of a mixture of these two organic solvents was lower than 1% (v/v), which did not affect the nerve activity. Measurements were conducted at 22-25°C.

RESULTS AND DISCUSSION

1. Chemistry

The alkylene-tethered bis-imidacloprid was obtained by double substitution of a,a-diiodo- or bistosylalkylenes with two molar equivalents of imidacloprid in a slight excess of sodium hydride in DMF (Fig. 2). The substitutions at the benzylic sites went smoothly with the chlorides using potassium carbonate in refluxing acetonitrile, affording derivatives (14 and 15). The preparation of the ethylene derivative (2) failed either in the aforementioned base-solvent systems or in THF alone.

![Fig. 2 Synthesis of alkylene-tether bis-imidacloprid.](image-url)
as solvent, resulting in the recovery of imidacloprid. We managed to isolate the product only using potassium carbonate in a mixture of THF and DMF (9:1 v/v). The isolation yields of the products were low, partly because we eluted so exhaustively with ethyl acetate lest residual imidacloprid should mingle with the product.

In the mass spectra of the products, the parent peak was detected at low intensity in the ethylene derivative (2). The other products gave only fragments after scission of the fragile N-NO₂ as observed in imidacloprid and related molecules (Table 1). The major fragment of the 6-chloronicotinyl ion of 126 m/z is a good indicator of this class of molecules. The ¹H- and ¹³C-NMR spectra showed chemical shifts and coupling patterns for the chloronicotinyl imidazolidine moieties similar to those of imidacloprid N₃-alkyl derivatives (Tables 2 and 3).

### Table 1 Melting points (mp) and mass spectra (MS) of the prepared products.

<table>
<thead>
<tr>
<th>No.</th>
<th>mp (°C)</th>
<th>Formula (M.W.)</th>
<th>MS (m/z)</th>
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</thead>
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<tr>
<td>2</td>
<td>197-198</td>
<td>C₉H₁₇ClN₂O₄ (387.4)</td>
<td>537 (M⁺, 3%), 448 (M⁺-2Na⁺, 17%), 322 (50%), 237 (70%), 224 (80%), 126 (100%)</td>
</tr>
<tr>
<td>3</td>
<td>139-141</td>
<td>C₉H₁₇ClN₂O₄ (387.4)</td>
<td>460 (10%), 336 (63%), 251 (19%), 237 (29%), 223 (23%), 126 (100%)</td>
</tr>
<tr>
<td>4</td>
<td>186-188</td>
<td>C₈H₁₅ClN₂O₄ (365.4)</td>
<td>476 (9%), 350 (100%), 266 (10%), 224 (16%), 198 (28%), 126 (91%)</td>
</tr>
<tr>
<td>5</td>
<td>140-142</td>
<td>C₈H₁₅ClN₂O₄ (379.4)</td>
<td>490 (51%), 363 (100%), 224 (11%), 153 (17%), 126 (46%)</td>
</tr>
<tr>
<td>6</td>
<td>139-141</td>
<td>C₈H₁₅ClN₂O₄ (379.4)</td>
<td>504 (9%), 378 (94%), 224 (18%), 167 (9%), 126 (100%)</td>
</tr>
<tr>
<td>7</td>
<td>syrup</td>
<td>C₈H₁₅ClN₂O₄ (407.5)</td>
<td>406 (98%), 224 (28%), 126 (100%)</td>
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<tr>
<td>8</td>
<td>syrup</td>
<td>C₈H₁₅ClN₂O₄ (421.5)</td>
<td>406 (99%), 224 (31%), 126 (100%)</td>
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<tr>
<td>9</td>
<td>syrup</td>
<td>C₈H₁₅ClN₂O₄ (435.6)</td>
<td>420 (100%), 224 (24%), 126 (85%)</td>
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<tr>
<td>10</td>
<td>77-79</td>
<td>C₈H₁₅ClN₂O₄ (498.6)</td>
<td>434 (92%), 224 (26%), 126 (100%)</td>
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<td>11</td>
<td>199-202</td>
<td>C₈H₁₅ClN₂O₄ (563.4)</td>
<td>472 (64%), 346 (77%), 263 (28%), 224 (22%), 210 (19%), 126 (100%)</td>
</tr>
<tr>
<td>12</td>
<td>207-209</td>
<td>C₈H₁₅ClN₂O₄ (561.4)</td>
<td>398 (100%), 315 (42%), 300 (20%), 224 (10%), 210 (53%), 187 (63%), 126 (94%)</td>
</tr>
<tr>
<td>13</td>
<td>128-131</td>
<td>C₈H₁₅ClN₂O₄ (609.5)</td>
<td>520 (12%), 394 (14%), 252 (36%), 225 (21%), 126 (100%)</td>
</tr>
<tr>
<td>14</td>
<td>150-153</td>
<td>C₈H₁₅ClN₂O₄ (613.5)</td>
<td>398 (55%), 314 (16%), 210 (53%), 187 (53%), 126 (63%), 105 (100%)</td>
</tr>
<tr>
<td>15</td>
<td>204-206</td>
<td>C₈H₁₅ClN₂O₄ (613.5)</td>
<td>398 (100%), 314 (40%), 301 (21%), 210 (25%), 187 (63%), 126 (94%), 104 (65%)</td>
</tr>
</tbody>
</table>

a) Found compositions for C, H, N were within 0.3% of the calculated values.

### Table 2 ¹H NMR spectral data of the newly prepared compounds.

<table>
<thead>
<tr>
<th>No.</th>
<th>NCH₂YCH₂N</th>
<th>Chemical shifts (δ ppm)</th>
</tr>
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<tr>
<td>2°</td>
<td>3.50 (4H, s)</td>
<td>3.67 (m), 3.78 (m)</td>
</tr>
<tr>
<td>3°</td>
<td>2.00 (2H, qnt, J=7.0), 3.36 (4H, t, J=7.0)</td>
<td>3.66 (m), 3.80 (m)</td>
</tr>
<tr>
<td>4°</td>
<td>1.51 (4H, bs), 3.21 (4H, bs)</td>
<td>3.61 (m), 3.73 (m)</td>
</tr>
<tr>
<td>5°</td>
<td>1.51 (4H, m), 1.61 (4H, m)</td>
<td>3.66 (m), 3.80 (m)</td>
</tr>
<tr>
<td>6°</td>
<td>1.34 (4H, m), 1.62 (4H, m)</td>
<td>3.63 (m), 3.77 (m)</td>
</tr>
<tr>
<td>7°</td>
<td>1.32 (6H, m), 1.61 (4H, m), 3.32 (4H, t, J=7.0)</td>
<td>3.66 (m), 3.79 (m)</td>
</tr>
<tr>
<td>8°</td>
<td>1.50 (6H, m), 1.60 (4H, m), 3.26 (4H, t, J=7.0)</td>
<td>3.60 (m), 3.76 (m)</td>
</tr>
<tr>
<td>9°</td>
<td>1.33 (6H, m), 1.60 (8H, m), 3.33 (4H, t, J=13.2)</td>
<td>3.62 (m), 3.78 (m)</td>
</tr>
<tr>
<td>10°</td>
<td>1.28 (12H, m), 1.60 (4H, m), 3.30 (4H, t, J=7.0)</td>
<td>3.60 (m), 3.75 (m)</td>
</tr>
<tr>
<td>11°</td>
<td>3.83 (4H, d, J=3.6), 5.71 (2H, t, J=3.6)</td>
<td>3.62-3.75 (4H, m)</td>
</tr>
<tr>
<td>12°</td>
<td>4.16 (4H, s)</td>
<td>3.68 (m), 3.81 (m)</td>
</tr>
<tr>
<td>13°</td>
<td>1.75 (4H, tt, J=6.0/8.0), 3.27 (4H, t, J=6.0), 3.57 (4H, t, J=5.8)</td>
<td>3.65 (m), 3.77 (m)</td>
</tr>
<tr>
<td>14°</td>
<td>4.45 (4H, s)</td>
<td>3.60-3.70 (4H, m)</td>
</tr>
<tr>
<td>15°</td>
<td>4.51 (4H, s)</td>
<td>3.70-3.77 (4H, m)</td>
</tr>
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</table>

a) In DMSO-d₆. b) In CDCl₃. c) In acetone-d₆.
2. Biological Activity

Insecticidal activity against the American cockroach was measured by injection to obtain the effect at the target site under conditions that minimize factors relating to the penetration through insect cuticle membranes and transport in the body as much as possible. As a whole, the set of alkylene-linked bivalent compounds were highly insecticidal in the treatment without synergists. For compounds 2-10, the MLD values varied from 2.2 nmol (compound 6) to 11 nmol (compound 4) in a small range with a factor of about 5. The potency of the most active compound 6 was very close to that of imidacloprid (1) (Table 4). The potency difference among the present compounds is much smaller than that of the corresponding imidacloprid N3-alkyl derivatives, where longer alkyl chains reduce the activity greatly. Considering the pronounced decrease in the MLD for N3-n-hexyl imidacloprid, 1/200 that for imidacloprid, it is surprising that the present alkylene compounds were highly active, despite bearing a substituent with a 2-3 fold larger molecular weight. The high level of activity was maintained in ethynylene- (12) and oxygen atom-inserted derivatives (13), while the activity of the ethylene compound (11) was rather low. The meta- (14) and para-phenylenes (15) decreased the insecticidal potency to about one-tenth that of the most active compound 6. We could not, however, discern any definite relationship between the insecticidal potency and the length of the tethers, even though the potency appears to decrease from a peak for compound 6 as the chains become longer or shorter.

We found previously a small but significant synergistic effect of PB and greater effects of NIA or a combination of PB with NIA on the insecticidal actions of
neonicotinoids.\textsuperscript{7,13,14} We tested the synergistic effect for selected compounds with each of PB and NIA and for all of the compounds with a mixture of PB and NIA. Here also PB generally increased the potency to a smaller degree and NIA or a mixture of PB and NIA to a greater degree. With PB, the synergistic ratio was 1–4 among the compounds tested, except for compound 13. PB is a poly-ether and an inhibitor of the microsomal P450 oxidase systems.\textsuperscript{19} For this reason, the synergistic effect of PB on the ether-type compound 13 might be more remarkable. With NIA, the synergistic ratio was around 10–20. With both synergists, the MLD values came into a range of 0.16–1.5, being 2.2–21 times the value for imidacloprid (1), except for compound 4. Synergistic ratios determined with both synergists could be used to divide compounds into three groups. The first group comprises compounds having a very small value, including 2–4 and 6. Among these, compounds 2–4 have a short polymethylene group. The second group consists of only compound 15, the synergistic ratio of which was more than that of imidacloprid (1). The other nine compounds have a value of 10–30. Imidacloprid is metabolized by hydrolysis of the N-N\textsubscript{O\textsubscript{2}} bond\textsuperscript{20} and is oxidized at the imidazolidine ethylene to give the less active compounds.\textsuperscript{21} The effect of the synergists on the insecticidal activity of the present set of compounds was, however, notably lower than that of imidacloprid except for compound 15, indicating that the metabolism of these alkylene derivatives was reduced. We have observed a similarly reduced synergism for N3-alkyl derivatives compared to imidacloprid.\textsuperscript{7,13} This is a typical result; the synergist ratios calculated from the reported molar insecticidal potencies without a mixture of PB and NIA to those with the synergists for imidacloprid and N-methylimidacloprid are 16 to 1\textsuperscript{7} and 50 to 2.\textsuperscript{13} Also there is a report about the synergistic ratio in the activity against the housefly with two sites on the pentameric subunits.\textsuperscript{26} This time was defined as zero and the number of discharges was similarly counted.

The binding affinity of a ligand to the receptor of the compound can be taken as one of the closest approximations of intrinsic potency. Recently, \textsuperscript{3}H} imidacloprid has been serving as a suitable ligand for neonicotinoid insecticides owing to their superior affinity to n\textsubscript{AChR}.\textsuperscript{23,24} In this experiment using housefly-head membrane preparations, the binding potency in terms of the IC\textsubscript{50} for imidacloprid was 0.02 \textmu M, the same as that obtained by another group.\textsuperscript{23} Among the dimeric compounds tested, the most potent was 6, which was less potent than imidacloprid by a factor of 160 (Table 4). The potency was decreased by shortening (compound 2) or elongating the tether of the dimeric molecule (compounds 10 and 13) by a factor of up to 25. In contrast, the insecticidal different activity among these four compounds was within a factor of four. Moreover, the insecticidal activity of imidacloprid (1) was 15 times that of these four dimeric compounds, whereas the binding activity of imidacloprid was about 6000 times higher than that of the least active compound, 2. The considerable drop in the binding potency on appending a long alkyl chain to the N3 of imidacloprid is consistent with the prediction by three-dimensional QSAR procedures indicating an unfavorable field around this region for a molecule to bind to the recognition site on n\textsubscript{AChR}.\textsuperscript{15,16} It is however conspicuous that the rate of decrease is much smaller than 1/2800 for N-methyl imidacloprid.\textsuperscript{23} It is generally assumed that the primary role of the tether in a bivalent drug is to reduce the entropy loss that would occur upon the binding of two independent monomeric units.\textsuperscript{25} This reduction in entropy loss may provide for a hexamethylene-linked bivalent molecule (6) to interact with two sites on the pentameric subunits.\textsuperscript{26}

Compound 6 was chosen for the neurophysiological experiments of discharges was similarly counted. After counting the cumulative number of spontaneous discharges every 30 sec for 5 min, the saline was changed with saline containing the compound at 9.00 \times 10^{-7} (\bullet) or 3.00 \times 10^{-4} M (\bigcirc). This time was defined as zero and the number of discharges was similarly counted.

\textbf{Fig. 3} Time courses of the effects of compound 6 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 changed with saline containing the compound at 9.00 \times 10^{-7} (\bullet) or 3.00 \times 10^{-4} M (\bigcirc). This time was defined as zero and the number of discharges was similarly counted.
experiments on a nerve preparation containing the abdominal fifth and sixth ganglia of a male adult American cockroach. Figure 3 shows time-dependent changes in the frequency of firing in nerve cords treated with compound 6 at two concentrations, where each symbol indicates cumulative counts for every 30 sec in each nerve preparation. Each time-response relation was taken from one nerve preparation. Similar results were obtained from different preparations (data not shown). The biphasic episode of the increase in frequency followed by subsidence to a control level or lower (Fig. 3) was similar to that of imidacloprid in Fig. 4. Similar effects have been found for nitromethylene compounds, where the initial increase in the frequency is caused by action on AChR and the following block is due to the desensitization of the receptor.27,28 Application of a concentration as low as 9.00 × 10⁻⁷ M of compound 6 had a comparable effect to imidacloprid for a brief but intense period of spontaneous discharges, immediately followed by subsidence. The times for maximum frequency and the subsequent block induced by the probes were concentration-dependent; both induction times were significantly shorter at the higher than lower concentration. Such a pattern of eliciting excitatory and blocking effects dependent on the concentrations and the exposure time appears common to the nerve induction by neonicotinoid molecules.7,13,14,28-30 Quantitative analysis of the relationship between the insecticidal and neurophysiological activities as was conducted previously7,13,14 should provide more important information on the present set of compounds.

In conclusion, alkylene-tethered bis-imidacloprid displayed potent insecticidal activity without an apparent dependence on tether length. Hexamethylene compound 6 elicited nerve excitation at almost comparable concentrations to imidacloprid. Despite the potency drops in the affinity to nAChR, the amplitude was still far greater than that of N3-methyl imidacloprid. These results suggest that alkylene-linked bivalent molecules have different physiological actions on insects from monovalent N3-alkyl derivatives of imidacloprid.

**ACKNOWLEDGMENTS**

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**REFERENCES**

要約
高い殺虫・神経興奮活性およびニコチン性アセチルコリン受容体でのイミダクログリッド結合部位への親和性をもつアルキレンビスイミダクログリッドの合成

剤を種々、尾根木村、林西臥樹、酒井有規

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アルキレン鎮（Cn: n=2－10）、およびフェニレン、C= C= C= C= C= C=とエーテル結合を介在したアルキレン鎮で連結されたビスイミダクログリッドを合成した。これらのクロニコチニル2量体化合物は、注射法によるワモンゴキブリに対する殺虫試験において、最少死詰苗が2〜30ナノモルと非常に高い活性を示した。その中で一番効果の高かったヘキサメチレン化合物の活性は、イミダクログリッドとほぼ同等であった。しかし代謝阻害剤の併用によりイミダクログリッドでは約35倍活性が上昇したのに対し、ヘキサメチレン化合物では8倍にすぎなかった。イエバニ頭部膜分の[3H]イミダクログリッド結合ニコチン性アセチルコリン受容体への結合実験では、ヘキサメチレン化合物のIC50はイミダクログリッドの160倍であり、それだけ結合活性は弱かった。ゴキブリ中枢神経に対しては、最初興奮を誘起したのち遮断効果をもたらした。その効果の発現状況の変化は、イミダクログリッドの場合とほぼ同等であった。