[Chem. Pharm. Bull. 34(2) 694—700 (1986)]

Conformational Analysis of Tetragastrin in Comparison with Antigastric 5,1-Benzothiazocines

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(Received June 21, 1985)

Conformational analysis of the minimum active unit of gastrin, tetragastrin (Trp–Met–Asp–Phe–NH_2), led to the formulation of common hypothetical receptor binding moieties with 5,1-benzothiazocines, these moieties being a benzene ring, a nonbasic nitrogen and a hydrophobic group. A molecular mechanics calculation study of tetragastrin was carried out in an attempt to find a stereochemical correlation with a representative 5,1-benzothiazocine, RS-2039, which had been structurally elucidated by X-ray crystallographic analysis. Several stable conformers of tetragastrin were discovered to have a close approximation of the 3-dimensional array of receptor binding moieties to that of RS-2039. It has thus been theoretically demonstrated that gastrins and 5,1-benzothiazocines could bind with an identical receptor.

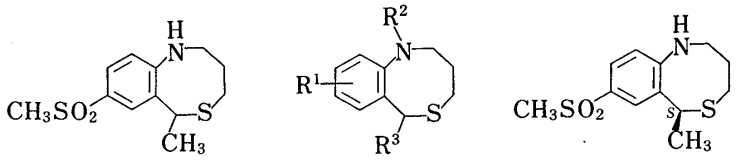
Keywords——gastrin; tetragastrin; antigastrin; conformational analysis; molecular mechanics; ECEPP; 5,1-benzothiazocine; 3-dimensional binding moiety

In the previous paper[1] gastrin (**4**) and antigastric 5,1-benzothiazocines (**2**)[2] (Chart 1) were postulated to have stereochemically common essential binding moieties consisting of three functional groups. Conformational analysis of a model compound of the gastrin active site, Ac–Trp–Met–NHMe, by molecular mechanics led to the formulation of hypothetical binding moieties identical with those of a representative 5,1-benzothiazocine, RS-2039 (**1**).

6-Methyl-8-methylsulfonyl-1,3,4,6-tetrahydro-2*H*-5,1-benzothiazocine, RS-2039 (**1**) and structurally related derivatives (**2**) were found to have antagonistic activity against pentagastrin (**5**) in rats and dogs.[3] The (*S*)-isomer (**3**) of **1** has been structurally elucidated by X-ray crystallographic analysis, while X-ray results on an acyl derivative of **3** were obtained in our earlier study.[1]

On the other hand, intensive studies of the structure–activity relationships of tetragastrin (**6**) and related peptides have been carried out. Finally, the Asp carboxyl group and one of the Phe amide protons in **6** were shown to be essential for the peptide to have high activity, while the Trp, Met and Phe side chain moieties played a part only in the binding characteristics.[4]

Considering the structural similarity of tetragastrin to RS-2039 (**1**), the benzene ring, the nitrogen atom, and the sulfur and the neighboring methylene groups at positions 3 and 4 in the latter are presumed to correspond stereochemically to the benzene ring and the nitrogen atom in the indole ring, and the sulfur atom and the neighboring methylene groups at the positions γ and ε in the former (Fig. 1). In other words, the above benzene ring, nitrogen atom, and sulfur and the neighboring hydrophobic groups in both molecules were postulated to be essential binding moieties and were assumed to bind with an identical receptor. In the present study a sulfur atom and the neighboring two methylene groups were assigned as the hydrophobic binding moiety, while in the previous study[1] only a sulfur atom was taken as one of the binding moieties. It is considered to be more reasonable that those nonpolar groups rather than the sulfur atom have general hydrophobic interactions with a possible nonpolar cavity in the gastrin receptor. Therefore, the hypothetical essential binding moieties in the



RS-2039 (**1**) **2** **3**

gastrin (**4**) Glp–Gly–Pro–Trp–Leu–Glu–Glu–Glu–Glu–Glu–Ala–Tyr(SO$_3$H)–Gly–Trp–Met–Asp–Phe–NH$_2$

pentagastrin (**5**) Boc–β-Ala–Trp–Met–Asp–Phe–NH$_2$

tetragastrin (**6**) Trp–Met–Asp–Phe–NH$_2$

Chart 1

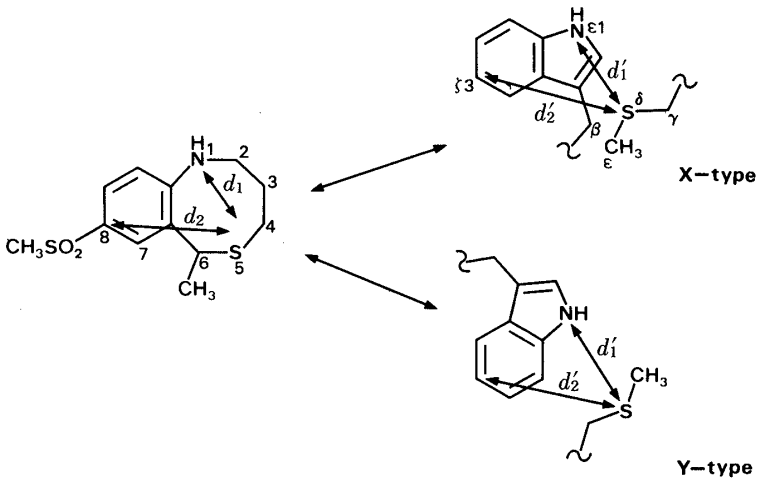


Fig. 1. Correlation of Essential Binding Moieties for Estimating Similarity between **1** and **6**

In the X-type arrangement, the sulfur atom is located close to Cβ of Trp, whereas it is located at the other side of the indole ring in the Y-type. d_1 refers to the distances between N1 and C3, C4 and S5 and d_2 refers to the distances between C8 and C3, C4 and S5. d_1' (to be compared with d_1) is assigned as the distance between Sδ of Met and Nε1 of Trp, and d_2' (to be compared with d_2) is assigned as the distance between Sδ of Met and Cζ3 of Trp.

stable conformations of **1** and **6** are stereochemically compared.

Methods

1) Generation of Initial Structures by the Monte Carlo Method——In an oligopeptide such as tetragastrin (**6**) there are so many bonds capable of free rotation that too many initial structures have to be taken into account. Therefore, randomly selected initial structures from among those to be considered were analyzed in place of calculations of whole conformers, that is, the Monte Carlo method was employed as described below.

In the case of the main chain, each ϕ and ψ around one residue were divided by 20° in the range of −180—180° to give $18^2 = 324$ combinations, from which conformers were selected at random (Fig. 2). Energetically favored combinations[5] or those found frequently in proteins were weighted to be selected with higher probabilities as shown in Fig. 3, although the weightings were not definitive. Each χ of the side chains and θ were selected randomly among angles such as 60, 180 and 300° which correspond to energetically favored *gauche* or *trans* conformers. By this method, initial conformers with lower potential energies could be generated very effectively and thus a large number of diverse conformations could be examined. This new algorithm to generate starting conformations was incorporated in the ECEPP program,[6] with which energy calculations were performed on a VAX11/780 computer.

2) Energy Minimizations of Tetragastrin——After energy minimizations of all the various conformations

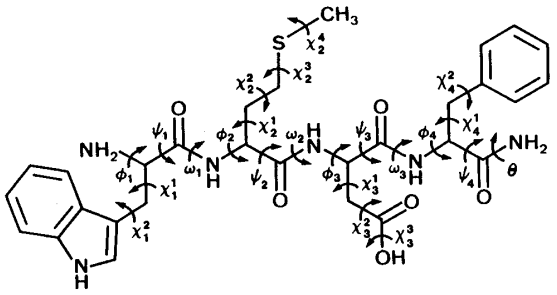


Fig. 2. Structural Diagram of Tetragastrin (**6**), Showing Torsional Angle Labels

Generation of initial conformations was performed in the following way. Combinations of ϕ_1 and ψ_1 were not weighted, while those of ϕ_2 and ψ_2, ϕ_3 and ψ_3 and ϕ_4 and ψ_4 were weighted as shown in Fig. 3. ω_1, ω_2 and ω_3 were fixed at 180°. Each χ in the side chains and θ were selected randomly among the angles shown in Table I.

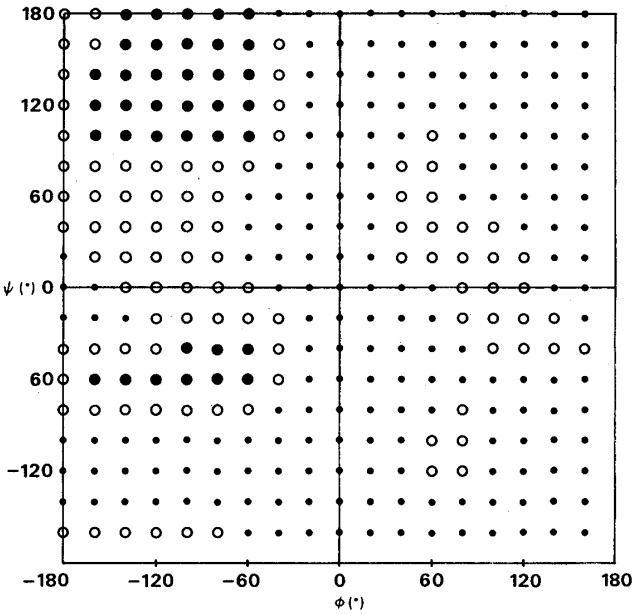


Fig. 3. Map of Weights for Combinations of ϕ and ψ

Weights are as follows. ● = 1.0, ○ = 0.67 and • = 0.33. Combinations marked ● occur 3 times as frequently as those marked •.

generated, stable conformers of tetragastrin (**6**) might be evaluated with respect to similarities to benzothiazocine and relative energy values. This method, however, is not so practical because energy minimization consumes too much time compared with the evaluation of similarities and relative energy values. In our study a more practical method, described below, was employed.

Energy minimizations were performed on the randomly-generated starting conformations having lower energies in order to estimate the lowest, that is, the global minimum energy (Results and Discussion 2)). On the other hand, energy minimizations were carried out on the conformers having an array of stereochemical binding moieties similar to that of 5,1-benzothiazocines and having relatively lower energies. The resulting conformers, with energies close to the global minimum energy previously obtained, were examined to determine whether they had similarities to benzothiazocine in terms of the essential binding moieties (Results and Discussion 3)).

3) Examination of Stereochemical Similarity between Essential Binding Moieties of Tetragastrin (6) and Those of RS-2039 (1)——Two types of arrangements (X-type and Y-type) in tetragastrin (**6**) were considered to be possible, which were similar to RS-2039 (**1**) in terms of the essential binding moieties based on an examination of molecular models (Fig. 1). In order to select conformers to be energetically examined, similarity between the two arrangements of the essential binding moieties of **6** and **1** was estimated by comparing the distance between two specific moieties in the former with that in the latter; an approximate estimation was sufficient as the coordinates of **6** would change after minimizations. In view of the calculation time it was desirable to examine both X-type and Y-type arrangements of **6** at the same time.

Therefore, the following equations were employed to examine similarity.

$$\mathrm{Min}(d_1)-\delta \leqq d_1' \leqq \mathrm{Max}(d_1)+\delta \quad (1)$$

$$\mathrm{Min}(d_2)-\delta \leqq d_2' \leqq \mathrm{Max}(d_2)+\delta \quad (2)$$

If d_1', the distance between Nε1 of Trp and Sδ of Met, and d_2', the distance between Cζ3 of Trp and Sδ of Met, satisfied Eqs. 1 and 2, respectively, the array of essential binding moieties of tetragastrin was considered possibly similar to that of RS-2039 (**1**). Min(d_1) was the minimum of the distances, $\overline{\mathrm{N1C3}}$, $\overline{\mathrm{N1C4}}$ and $\overline{\mathrm{N1S5}}$, while Max(d_1) was the maximum of them. Min(d_2) was the minimum of the distances, $\overline{\mathrm{C8C3}}$, $\overline{\mathrm{C8C4}}$ and $\overline{\mathrm{C8S5}}$, while Max(d_2) was the maximum of them. δ refers to the allowance of difference of distances. Equations 1 and 2 could be applied to both (*S*)- and (*R*)-isomers of **1**. The estimation of d_i' based on Eqs. 1 and 2 was newly embodied in the ECEPP program.[6]

Results and Discussion

1) The Coordinates of Essential Binding Moieties of RS-2039 (1) in Stable Conformations

The structure of the (*S*)-isomer (**3**) of **1** was elucidated by X-ray crystallographic analysis,[7] which showed the coexistence of two almost identical conformers, A and B. In fact, the two conformers are so similar that superimposition in terms of all atoms excluding hydrogens showed an average difference of 0.1 Å, as shown in Fig. 4. The conformer of an acyl derivative of **3** discussed in the previous paper[1] was compared with conformers A and B, showing similar stereochemistry except for puckering at C2 which is not among the essential binding moieties. Though there might be two kinds of stable conformations of RS-2039, the coordinates of the essential binding moieties of both would be almost the same. Therefore, we considered the coordinates of the essential binding moieties of A and B, and the antipodes of these conformers to be those of stable conformations. These were then used in the following calculations.

2) Estimation of the Lowest Energy of Tetragastrin (6)

Using the procedure in Methods 1), in generation of the initial structures, combinations of ϕ_i and ψ_i except for those of ϕ_1 and ψ_1 in the N-terminal were weighted and each χ in the side chains and θ were selected from among the angles shown in Table I, while ω_1, ω_2 and ω_3 were set to 180°. Under these conditions 20000 initial structures were randomly generated and energy minimizations were carried out on 77 conformers with energies less than 50 kcal/mol while keeping ω_1, ω_2 and ω_3 fixed. The three conformers with the lowest energies are shown in Table II and conformers 1 and 2 are illustrated in Fig. 5. The global minimum energy of tetragastrin was estimated to be −13.3 kcal/mol.

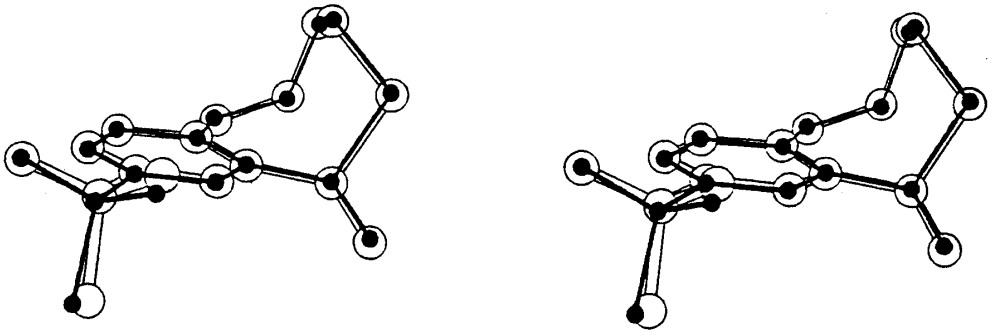


Fig. 4. Stereoview of the Superimposed Conformer A on B of RS-2039 (**1**)

The mean value of the distances between all atoms excluding hydrogens of conformer A and the corresponding atoms of conformer B is 0.1 Å.

TABLE I. Starting Torsional Angles (°) of χ and θ of Tetragastrin (**6**) in the Generation of Initial Conformers

χ_1^1	χ_1^2	χ_2^1	χ_2^2	χ_2^3	χ_2^4	χ_3^1	χ_3^2	χ_3^3	χ_4^1	χ_4^2	θ
60	30	0	0	60	0	60	30	60	60	30	60
180	90	60	60	180	180	180	150	180	180	150	180
300	150	120	120	300		300	270	300	300	270	300
	210	180	180								
	270	240	240								
	330	300	300								

TABLE II. Torsional Angles of Tetragastrin (**6**) with the Minimum Energies

Conformer No.	Energy (kcal/mol)	Torsional angles (°)																		
		ϕ_1	ψ_1	χ_1^1	χ_1^2	ϕ_2	ψ_2	χ_2^1	χ_2^2	χ_2^3	χ_2^4	ϕ_3	ψ_3	χ_3^1	χ_3^2	χ_3^3	ϕ_4	ψ_4	χ_4^1	χ_4^2	θ
1	−13.3	−76	150	178	75	−80	107	−74	72	−179	180	−75	103	54	119	179	−85	−35	−179	−57	116
2	−12.8	57	167	62	91	−72	114	−172	176	−179	−179	−164	154	61	81	177	−149	154	0	−63	−78
3	−12.4	51	138	168	69	−83	94	−68	−61	178	180	−69	−40	179	83	179	−154	148	0	−179	−104

TABLE III. Torsional Angles of Tetragastrin (**6**) with Lower Energies Showing Similarity to RS-2039 (**1**) in Terms of Essential Binding Moieties

Conformer No.	$\Delta E^{a)}$ (kcal/mol)	Torsional angles (°)																		
		ϕ_1	ψ_1	χ_1^1	χ_1^2	ϕ_2	ψ_2	χ_2^1	χ_2^2	χ_2^3	χ_2^4	ϕ_3	ψ_3	χ_3^1	χ_3^2	χ_3^3	ϕ_4	ψ_4	χ_4^1	χ_4^2	θ
4	3.6	43	166	−171	67	−59	129	−81	70	−179	60	63	31	−154	104	6	−164	81	179	−177	−111
5	3.9	170	−14	62	83	−66	153	−70	87	168	178	−75	94	−63	89	180	−81	−35	1	−55	−63
6	4.6	67	24	62	90	−158	159	52	−90	175	60	−99	137	−177	−99	180	−159	158	180	54	−93
7	5.9	64	70	−178	71	48	52	−68	77	86	56	−166	158	62	76	17	−74	−38	−179	−179	78

a) Difference from the global minimum energy (−13.3 kcal/mol).

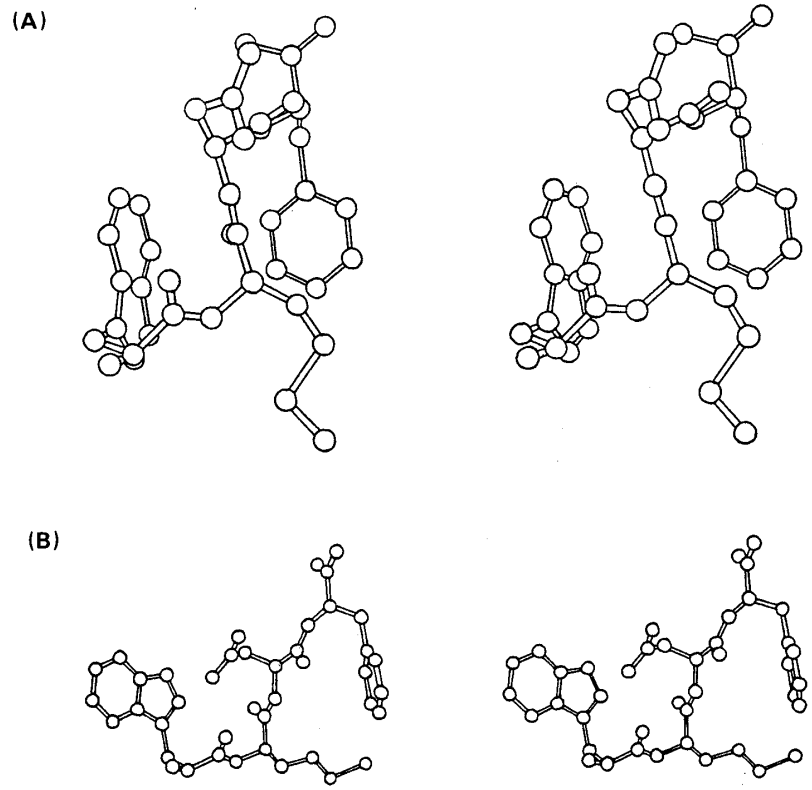


Fig. 5. Conformations of Tetragastrin (**6**) with the Lowest Energies
(A) conformer 1; (B) conformer 2.

3) Energy Calculation of Tetragastrin (6) to Search for Conformers with Essential Binding Moieties Stereochemically Similar to Those of RS-2039 (1)

According to Methods 3), Min(d_1), Max(d_1), Min(d_2) and Max(d_2) in Eqs. 1 and 2 were calculated to be 2.5, 3.7, 4.9 and 5.7 Å, respectively based on conformers A and B. Using a tentative value of 1.1 Å for δ, Eqs. 1 and 2 became Eqs. 3 and 4.

$$1.4\,\text{Å} \leqq d_1' \leqq 4.8\,\text{Å} \tag{3}$$

$$3.8\,\text{Å} \leqq d_2' \leqq 6.8\,\text{Å} \tag{4}$$

Starting structures numbering 300000 were generated in the same manner as described in 2). Energy minimizations were performed on 118 conformers which satisfied Eqs. 3 and 4, and had energies less than 150 kcal/mol while keeping ω_1, ω_2 and ω_3 fixed. Table III summarizes the minimized conformers with ΔE (difference from the global minimum energy previously estimated, that is, -13.3 kcal/mol) less than 6.0 kcal/mol which also satisfy Eqs. 3 and 4.

Superimpositions of four conformers in Table III and RS-2039 (**1**) were attempted in order to examine similarities more precisely. Each conformer was only overlapped with conformer A in terms of essential binding moieties because conformers A and B had almost identical conformations. As for the sulfur atom and neighboring hydrophobic groups, superposition of any two adjacent atoms in each moiety was considered to be sufficient for matching. In other words, Cγ–Sδ and Sδ–Cε of **6** were overlapped with C3–C4 and C4–S5 of **1** to find the best matching. There were consequently 8 combinations of superposition, taking (*S*)- and (*R*)-isomers of **1** into account.

The results are given in Table IV. These indicate that conformers 5 and 6 closely matched RS-2039 (**1**) with respect to essential binding moieties, with a mean displacement of about 0.3 Å between the corresponding moieties. Figure 6 illustrates conformer 5 superimposed on

TABLE IV. Energies and Stereochemical Aspects of the Four Stable Conformers of Tetragastrin (**6**)

Conformer No.	Energy (kcal/mol)	ΔE[a] (kcal/mol)	DISP[b] (Å)	Arrangement[c]	Configuration[d]
4	−9.7	3.6	0.9	X-type	*S*
5	−9.4	3.9	0.2	Y-type	*R*
6	−8.7	4.6	0.3	Y-type	*R*
7	−7.4	5.9	0.6	X-type	*S*

a) Difference from the global minimum energy (−13.3 kcal/mol). *b*) Mean value of the distances between essential binding moieties of **6** and the corresponding moieties of **1**, when they are superimposed. *c*) Arrangement type of **6** which correlates with **1**. *d*) Configuration of **1** which is superimposed on **6**.

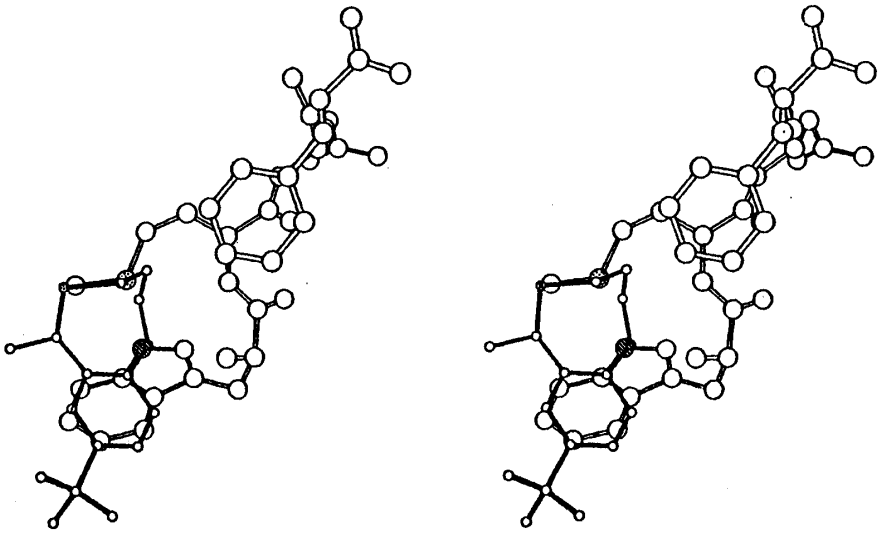


Fig. 6. Superposition of Conformer 5 (Open Bonds) and **1** (Solid Bonds)

Nitrogens and sulfurs in the essential binding moieties are represented by hatched circles and by dotted circles, respectively. Cε–Sδ of conformer 5 is overlapped with C3–C4 of **1**. Hydrogens are excluded from the examination of superposition.

the (*R*)-isomer of **1**. The corresponding atoms and groups are found to be well fitted. These theoretical results suggest that gastrin and 5,1-benzothiazocines could bind with an identical receptor in that they have similar arrays of the essential binding moieties. The present results may provide a simple template for the design of small-molecular gastric inhibitors, which might be valuable in the treatment of peptic ulcers.

Acknowledgment Atomic coordinates for **3** were kindly supplied by Mr. S. Sato of the Analytical and Metabolic Research Laboratories. We would also like to thank Drs. Tomita and Kobayashi, and Mr. Ryokai for discussions and encouragement.

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