Product Analyses of Ozone Mediated Nitration of Benzimidazole Derivatives with Nitrogen Dioxide: Formation of 1-Nitrobenzimidazoles and Conversion to Benzotriazoles

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Several benzimidazole derivatives having electron-withdrawing or -donating substituent(s) at the benzene moiety were used as models of the imidazole moiety of purine bases and their nitration with nitrogen dioxide and ozone (so-called *Kyodai* nitration) were examined. Products were extracted from the reaction mixture with AcOEt and their structures were analyzed. 1-Nitrobenzimidazole derivatives and unexpected 1-nitrobenzotriazole derivatives were identified. Although the yields of 1-nitrobenzimidazole derivatives were quite low, these were all new compounds that could be obtained only by *Kyodai* nitration. It was speculated that benzotriazoles were formed *via* 1-nitrobenzimidazoles and subsequent nitration toward benzotriazoles resulted in the formation of 1-nitrobenzotriazoles.

Key words nitration; nitrogen dioxide; benzimidazole; N-nitration; conversion; benzotriazole

Many types of DNA modification by chemical carcinogens and mutagens have been reported and it is well known that the imidazole moiety of guanine is one of the major reaction sites on this base; alkylation occurs at the N7 position, and arylamination and hydroxylation at the C8 position.[2] A recent report described nitration of the guanine moiety of DNA at the C8 position by peroxynitrite ($ONOO^-$), which is formed in living cells by the reaction of nitric oxide (NO) with superoxide anion radical.[3] Nitration of pyrimidine bases using a variety of nitrating agents ($NO_2^+BF_4^-$,[4] NO_2-$OCOCF_3$,[5,6] NO_2O_3,[7] and $Cu(NO_3)_2 \cdot 3H_2O/Ac_2O$[8]) have been well studied, however, reports on nitration of purine bases are very few. Koomen and coworkers recently obtained 2-nitroadenosine by nitration of N^6,N^6,$O^{2'}$,$O^{3'}$,$O^{5'}$-pentaacetyladenosine with tetrabutylammonium nitrate/trifluoroacetic anhydride and subsequent deacetylation.[9] Sodum *et al.* reported recently on the products of the reaction of 2′-deoxyadenosine with peroxynitrite.[10] They showed that the main product was 2′-deoxy-8-oxoadenosine, and only suggested that the minor one to be some nitrated derivative. Subsequent to their report, we described the preparation of 2′-deoxy-2-nitroadenosine by nitration of N^6,$O^{3'}$,$O^{5'}$-triacetyl-2′-deoxyadenosine with $Cu(NO_3)_2/Ac_2O$ and subsequent deacetylation.[11] As a part of our study on the reactivity of the imidazole moiety of purine bases, we have used benzimidazoles as models.[12—14] This time, we examined the nitration of benzimidazoles by ozone-mediated nitration with nitrogen dioxide. This method was developed by Suzuki *et al.*[15] and termed *Kyodai* nitration. The main feature of this method is that nitration proceeds efficiently under mild conditions by radical mechanisms which are different from the ordinary ones that involve NO_2^+ as the reactive species. *Kyodai* nitration proceeds as follows; O_3 oxidizes NO_2 to form NO_3 that has strong oxidizing potency, then NO_3 oxidizes the substrate (ArH) to form the cation radical, $ArH^{\cdot +}$, to which NO_2 couples to form $ArHNO_2^+$ which finally forms the nitrated product $ArNO_2$ by losing H^+.[15] Nitration of imidazole itself with the *Kyodai* nitration system is reported to produce 4-nitroimidazole and 1,4-dinitroimidazole.[7] In this study, we used benzimidazole derivatives as substrates for *Kyodai* nitration. Although the yields of the products were quite low, they were determined to be 1-nitrobenzimidazoles and 1-nitrobenzotriazoles. The 1-nitrobenzimidazole derivatives obtained were all new compounds that could be obtained only by *Kyodai* nitration. It was speculated that benzotriazoles were formed *via* 1-nitrobenzimidazoles and subsequent nitration toward benzotriazoles resulted in the formation of 1-nitrobenzotriazoles. With respect to the ring conversion from benzimidazole to benzotriazole, we previously reported an another example; treatment of 1-amino-3-methylbenzimidazolium chloride with lead(IV) acetate, an oxidizing agent, resulted in the formation of 1-methylbenzotriazole.[16] Such conversion also proceeded in 7-amino-9-ethylguaninium chloride and formed 8-aza-9-ethylguanine.[16] From these results, we propose that this kind of conversion from imidazole to triazole may occur in purine bases in DNA as secondary DNA damage after DNA has been nitrated or aminated by certain carcinogens/mutagens.

Results and Discussion

***Kyodai* Nitration of Benzimidazoles** As substrates, five benzimidazole derivatives (**1a**—**e**) having electron-withdrawing or -donating substituent(s) at the benzene moiety were employed (Table 1). *Kyodai* nitration was carried out as described in the Experimental section with NO_2 (11—36 mol eq) and O_3 (we refer to this nitrating system as NO_2/O_3), and products that were extracted with AcOEt were analyzed. *Kyodai* nitration of benzimidazole (**1a**) (Exp. 1) gave 1-nitrobenzimidazoles (**2**) [1-nitrobenzimidazole (**2a**), 1,5-dinitrobenzimidazole (**2b**) and 1,6-dinitrobenzimidazole (**2c**)] and 1-nitrobenzotriazoles (**3**) [1-nitrobenzotriazole (**3a**), 1,5-dinitrobenzotriazole (**3b**) and 1,6-dinitrobenzotriazole (**3c**)], although the yields of the products were quite low (less than 3%) as shown in Table 1. Products **3b** and **3c** could not be separated and were obtained as a mixture. As other products, 5-nitrobenzimidazole (**1b**) and 5-nitrobenzotriazole (**4b**) were obtained in a trace yield. When the reaction was carried out with a large amount of NO_2/O_3 and a longer reaction time (Exp. 2), only nitrated benzotriazoles **3a**—**c** were obtained and not nitrated benzimidazoles (**2**). Structures of the prod-

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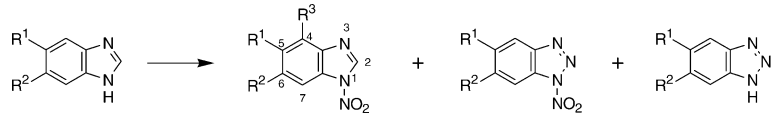
Table 1. *Kyodai* Nitration of Benzimidazole Derivatives



	R¹	R²
a	H	H
b	NO_2	H
c	NO_2	NO_2
d	CH_3	H
e	CH_3	CH_3

1

	R¹	R²	R³
a	H	H	H
b	NO_2	H	H
c	H	NO_2	H
d	NO_2	NO_2	H
e	NO_2	CH_3	H
f	CH_3	NO_2	H
g	CH_3	CH_3	NO_2
h	CH_3	CH_2NO_2	NO_2
	or CH_2NO_2	CH_3	NO_2

2

	R¹	R²
a	H	H
b	NO_2	H
c	H	NO_2
d	NO_2	NO_2
e	CH_3	NO_2
f	NO_2	CH_3

3

	R¹	R²
a	H	H
b	NO_2	H
c	NO_2	NO_2
d	CH_3	NO_2

4

Exp. No.	S.M.	Condition		Product (yield)		
		NO_2 (mol eq)	Time (min)	1-Nitrobenzimidazoles (**2**)	1-Nitrobenzotriazoles (**3**)	Others[a)]
1	**1a**	11	60	**2a** (<3%), **2b** (<3%), **2c** (<3%)	**3a** (<3%), **3b** (<3%), **3c** (<3%)	**1b**, **4b**
2	**1a**	22	90	—	**3a** (2.1%), **3b** (8.3%), **3c** (8.3%)	
3	**1b**	14	30	**2b** (1.6%), **2c** (0.8%)	**3b** (4.3%), **3c** (7.0%)	**4b**
4	**1b**	36	80	—	**3b** (18%), **3c** (8.4%)	
5	**1c**	22	30	**2d** (33%)	—	
6	**1c**	28	30	**2d** (7.4%)	**3d** (7.8%)	**4c**
7	**1d**	18	40	**2e** (5.3%), **2f** (0.2%)	**3e** (1.4%), **3f** (1.7%)	**4d**
8	**1e**	36	80	**2g** (1.2%), **2h** (0.9%)	—	

a) A trace of the yield.

ucts **2b** and **2c** were determined by NMR spectroscopy where the 7-H proton of **2c** (δ 9.02) resonated at lower field than the 4-H proton of **2b** (δ 8.75). Similarly, the assignment of the chemical shifts of the mixture of **3b** and **3c** in NMR spectra was carried out. When the nitration of **1a** was performed with nitric acid/sulfuric acid, the common method of nitration where NO_2^+ is the reactive species, products **1b**, 5,6-dinitrobenzimidazole (**1c**) and 4,6-dinitrobenzimidazole were obtained as reported.[17] This indicates that *Kyodai* nitration of **1a** proceeded by different mechanisms and gave different products. *Kyodai* nitration of **1b** (Exp. 3) gave 1-nitrobenzimidazoles (**2b**, **c**) and 1-nitrobenzotriazoles (**3b**, **c**). Another product, compound **4b**, was obtained in a trace yield. The reaction with a large amount of NO_2/O_3 and a longer reaction time (Exp. 4) gave only 1-nitrated benzotriazols (**3b**, **c**) as had the reaction with **1a** (Exp. 2). Even when CH_2Cl_2 was used as a solvent instead of CH_3CN, no change was observed. When **1b** was allowed to react with several nitrating agents such as H_2SO_4/HNO_3, NO_2BF_4, HNO_3/AC_2O, $Cu(NO_3)_2$/trifluoroacetic anhydride or NH_4NO_3/trifluoroacetic anhydride, only in the cases of H_2SO_4/HNO_3 and NO_2BF_4 were products **1c** and 4,6-dinitrobenzimidazole obtained in a good yield, however, none of the 1-nitrobenzimidazole derivatives that were obtained by *Kyodai* nitration were obtained in these reactions. *Kyodai* nitration of **1c** (Exp. 5) gave 1,5,6-trinitrobenzimidazole (**2d**) as a sole product in a 33% yield, which was the highest yield among the benzimidazole derivatives examined. The reaction with a large amount of NO_2/O_3 (Exp. 6) gave **2d** together with 1,5,6-trinitrobenzotriazole (**3d**). 5,6-Dinitrobenzotriazole (**4c**) was also obtained in a trace yield. *Kyodai* nitration of 5-methylbenzimidazole (**1d**) (Exp. 7) gave 1-nitrobenzimidazoles (**2**) [6-methyl-1,5-dinitrobenzimidazole (**2e**) and 5-methyl-1,6-dinitrobenzimidazole (**2f**)] and 1-nitorbenzotriazoles (**3**) [5-methyl-1,6-dinitrobenzotriazole (**3e**) and 6-methyl-1,5-dinitrobenzotriazole (**3f**)]. *Kyodai* nitration of 5,6-dimethylbenzimidazole (**1e**) (Exp. 8) gave only 1-nitrobenzimidazoles (**2**) [5,6-dimethyl-1,4-dinitrobenzimidazole (**2g**) and 5(or 6)-methyl-1,4-dinitro-6(or 5)-(nitromethyl)benzimidazole (**2h**)], and no benzotriazole derivatives were observed in spite of the higher amount of NO_2/O_3 and the longer reaction time used in the reaction. As shown in Table 1, although the yields of the products were quite low (0.2—18%), except in the case of **1c** (yield of **2d** was 33%), it seemed that benzimidazoles with more electron-withdrawing substituents tended to give a higher yield of products. In the reaction using the NO_2/O_3 system, the starting material was almost all consumed under the conditions employed. This indicates that unidentified products, including the decomposed products of 1-nitrobenzimidazole and 1-nitrobenzotriazole derivatives, existed in the aqueous fraction. We will describe later the reactivity of benzimidazoles (**1**) toward *Kyodai* nitration and the stability of the produced 1-nitrobenzimidazole derivatives.

Pathways for Formation of Products by *Kyodai* Nitration Pathways for the formation of products from **1a** by *Kyodai* nitration were considered (Fig. 1). Nitration of **1a** gave products **2a**—**c** and **3a**—**c** as already described. In addition to these products, formations of **1b** and **4b** were confirmed by HPLC. When the nitration of **1b** was carried out, products **2b**, **c** and **3b**, **c** were obtained, and formation of **4b** was also confirmed. When the nitration of benzotriazole (**4a**) was carried out, products **3a**—**c** and **4b** were obtained. When the nitration of **4b** was performed, products **3b**, **c** were obtained. Although we could not detect the formation of **4a** in the reaction mixture of **1a**, we could easily propose pathways for the formation of products, as shown in Fig. 1, based on

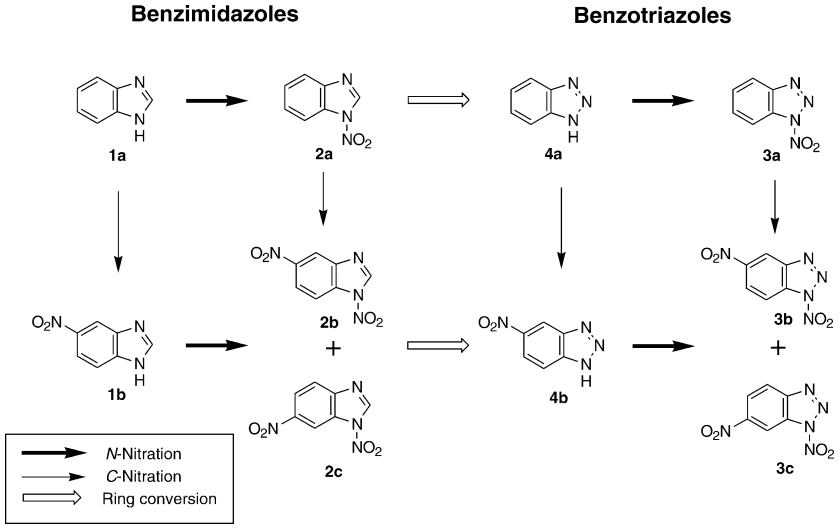


Fig. 1. Proposed Pathways for Formation of Products

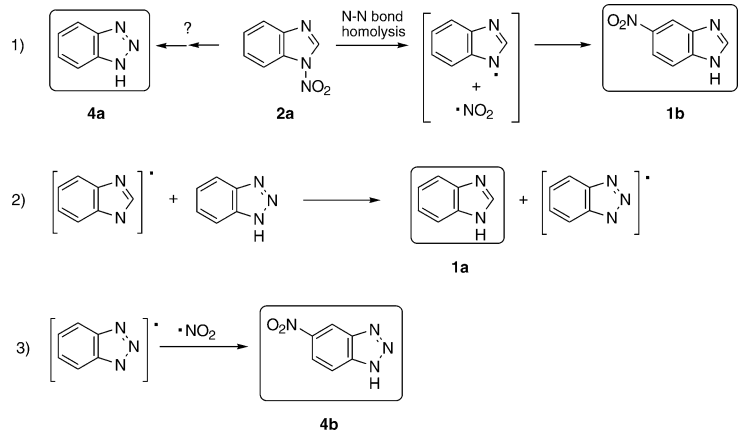


Fig. 2. Decomposition of 1-Nitrobenzimidazole (**2a**) in Isopropanol

the results described above. It is evident that ring conversion from benzimidazole to benzotriazole occurs from the 1-nitroimidazoles, however, we are unable as yet to elucidate the mechanism responsible. Trials to clarify these mechanisms will be described later.

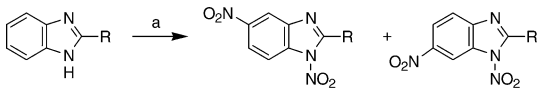
Stability (Decomposition) of 1-Nitrobenzimidazoles Compound **2a** was dissolved in isopropanol and left at room temperature for 3 d. The color of the solution changed from pale yellow to brown. Products were then analyzed by HPLC (data not shown) and more than ten peaks were observed. Among them, peaks of **1a**, **1b**, **4a** and **4b** were identified by comparing their retention times and UV absorption spectra with those of the authentic specimens. Formation of these products is proposed in Fig. 2. Two key steps are considered; one is the homolysis of the N–NO_2 bond of **2a** to form benzimidazole radical and NO_2 radical, and the other is the conversion of **2a** to **4a** as shown in line 1. Radical coupling between imidazole radical and NO_2 radical at the 5-C position may form **1b**. Compound **4b** may be formed by radical coupling between NO_2 radical and benzotriazole radical formed by a radical exchange reaction between benzimidazole radical and benzotriazole as shown in lines 2 and 3. Similar decomposition was observed when compounds **2b** and **2d** were each dissolved in isopropanol and the solution was left at room temperature for a few days. Thus, among the many decomposed products, compounds **1b** and **4b** were identified from **2b**, and compounds **1c** and **4c** were identified from **2d**. In addition to these, 4-isopropyloxy-5,6-dinitrobenzimidazole was identified in the case of **2d**. Based on these results, we re-considered the reaction pathways shown in Fig. 1; the benzotriazole ring formation and C-nitration (formations of **1b**, **4a** and **4b** from **2a**) could proceed even without NO_2/O_3. The decomposition also proceeded when 1-nitrobenzimidazoles (**2**) were dissolved in MeOH instead of isopropanol. Compounds **2b**—**d** and **2i**, **j** were then each dissolved in MeOH and the decrease in the starting materials at 37 °C was analyzed by HPLC (the preparation of compounds **2i**, **j** will be described later in Table 3). Half-lives of the compounds were shown in Table 2. Decomposition occurred very easily with half lives of 12 h for **2b**, 5 h for **2c** and 8—12 h for **2d**. These results showed that 1,6-dinitro derivatives (**2c**, **j**) were more labile than the corresponding 1,5-dinitro derivatives (**2b**, **i**) and the phenyl group at the 2-position (**2i**, **j**) made the compound more labile. Among the decomposed compounds, **4b** was formed from both compounds **2b**, **c**. Similarly, compound **4c** was also detected in the decomposed products from **2d**. As for **2i** and **2j**, the main decomposed product was 5-nitro-2-(*o*- or *m*-nitrophenyl)benzimidazole. It is worth

Table 2. Decomposition of 1-Nitrobenzimidazoles[a)]

Compound	$T_{1/2}$ (h)
2b	12
2c	5
2d	8—12[b)]
2i	3.8
2j	1.2

a) Compound dissolved in MeOH was left at 37 °C. Decrease in the compound was analyzed by HPLC. *b*) Data varied.

Table 3. *Kyodai* Nitration of 2-Substituted Benzimidazoles



R	Yield (%)	
Phenyl	12	3.7
CH_3	1.8	1.2

a; NO_2 (7 mol eq)/O_3 in CH_3CN, 0 °C, 30 min.

noting that ring-conversion product **4b** was not detected in the decomposition products from 2-substituted benzimidazoles, **2i**, **j**. When compound **2d** in MeOH was kept at a higher temperature of 70 °C and the products were analyzed by HPLC, the starting material disappeared at 2.5 h and peaks of intermediate-A and **1c** (the N1 de-nitro compound of **2d**) appeared as the main products. After that, the intermediate-A decomposed to many products including **4c** with time.

Stability of 1-nitrobenzimidazoles (**2**) in acidic and alkaline conditions was also examined by HPLC using **2d**. When **2d** was dissolved in MeOH containing 0.01 M NaOH and left at 37 °C, the starting material changed at once to an intermediate-A, and this intermediate changed to mainly an unknown compound-B within 5 h. When **2d** was dissolved in MeOH containing 0.1 M HCl and left at 37 °C, the starting material changed simultaneously to two intermediates, which decomposed further to mainly two unknown compounds-B, C. (these were not compounds shown in Table 1) with a half-life of 30 min.

***Kyodai* Nitration of 2-Substituted Benzimidazoles** In the course of the conversion from 1-nitrobenzimidazole to benzotriazole, the carbon at the 2-position must be eliminated. In order to clarify the reaction mechanisms, we used 2-substituted benzimidazole to determine if 2-substituted benzimidazole would give benzotriazole derivatives by *Kyodai* nitration and to examine the structures of the eliminated products when the conversion proceeded. We used 2-phenylbenzimidazole (**1f**) as a substrate and its *Kyodai* nitration was carried out with 22 mol eq of NO_2 for 2 h. Products were analyzed by HPLC after they were extracted with AcOEt, however, many peaks appeared that prevented the identification of the products. When these products was dissolved again in AcOEt and left at room temperature for 2 d, formations of both **3b** and **3c** were detected by HPLC. The possible products that will be formed by the elimination of C2-carbon are benzoic acid, benzaldehyde, toluene and benzyl alcohol, but none of these were detected by HPLC. In order to obtain 1-nitro derivatives of **1f**, *Kyodai* nitration of **1f** with a lower amount of NO_2/O_3 (7 mol eq of NO_2 for 30 min) was examined, and products 1,5-dinitro-2-phenylbenzimidazole (**2i**) and 1,6-dinitro-2-phenylbenzimidazole (**2j**) were obtained in 12% and 3.7% yields, respectively (Table 3). Under these conditions, benzotriazole derivatives were not detected. Products **2i** and **2j** were labile and decomposed rapidly when they were dissolved in $CHCl_3$, AcOEt or MeOH and left at room temperature (half lives of **2i**, **j** in MeOH were already described and shown in Table 2). Under the same nitration conditions described for **1f**, 2-methylbenzimidazole (**1g**) gave 2-methyl-1,5-dinitrobenzimidazole (**2k**) and 2-methyl-1,6-dinitrobenzimidazole (**2l**) in 1.8% and 1.2% yields, respectively. It is not clear why the yield of products from **1g** was lower than that from **1f** nevertheless the products **2k** and **2l** are more stable than products **2i** and **2j** in MeOH. Products **2k** and **2l** that formed in the reaction mixture may have undergone a further reaction with NO_2/O_3 to form other unidentified products.

Table 4. Reactivity of Benzimidazoles in *Kyodai* Nitration

S.M.	Decrease (%) in starting benzimidazole					
	NO_2 (7 mol eq)			NO_2 (14 mol eq)		
	10 min	20 min	40 min	5 min	10 min	15 min
1a	35	47	75	40	50	75
1b	75	88	100	74	100	100
1d	32	72	100	50	80	100

Reactivity of Benzimidazole Derivatives with *Kyodai* Nitration Since we knew the structure and characteristics of the products obtained by *Kyodai* nitration of benzimidazoles, we carefully re-examined the reactivity of benzimidazoles with this nitration. Benzimidazoles **1a**, **1b** and **1d** were used and their decrease was determined by HPLC using 7 and 14 mol eq of NO_2. As shown in Table 4, starting materials were consumed very quickly with a half-life of less than around 20 min and 10 min by treatments with 7 mol eq and 14 mol eq of NO_2, respectively. Among the compounds employed, the order of the decrease was **1b**>**1d**>**1a**, however, differences were not remarkable. This indicates that no electronic effect was present in the reaction. Although the starting material was completely consumed quite rapidly, the fact that the yields of 1-nitrobenzimidazoles and 1-nitrobenzotriazoles were very low suggests that the products formed decomposed rapidly through further reaction in the NO_2/O_3 system.

Trials for Clarifying Decomposition Mechanisms of 1-Nitrobenzimidazoles Using HPLC and NMR The decomposition processes in MeOH were studied using compound **2d** which gives simpler products for analyses by HPLC and NMR. Ten milligrams of **2d** were dissolved in 10 ml of MeOH and the solution was kept at 37 °C. Products were quantified with time using HPLC. The peak of **2d** (t_R 13.5 min) decreased with time while that of an intermediate-I (t_R 8.0 min) increased. After 17 h, the starting material had been completely consumed and the intermediate-I appeared as a main peak. MeOH was removed carefully to obtaine yellowish white residues. NMR spectra of the residues in CD_3OD showed only two CH protons [δ 7.99 (s) 1H, 7.03 (s) 1H] which were not those of **1c**. This means that one of the

three CH protons of **2d** [δ 9.04 (s) 1H, 8.69 (s) 1H, 8.44 (s) 1H] was missing. This intermediate-I decomposed easily by handling in an attempt at recrystallization for X-ray analysis. Among the decomposed products, compound **4c** was found. Decomposition of **2d** was also carried out by placing this compound in an NMR tube in CD_3OD at 37 °C and by taking the NMR spectra with time. Signal heights of the three CH protons of **2d** decreased with time and peaks of intermediate-I [δ 8.08 (s) 1H, δ 7.98 (s) 1H, 7.04 (s) 1H] appeared. Considering the NMR spectrum of the intermediate-I obtained in the previous experiment, the signal δ 8.08 must be the signal originated from the C2 hydrogen. However, the structure of this product could not be elucidated. Further study is required to clarify these reaction mechanisms.

Stability of 1-Nitrobenzotriazole In contrast to 1-nitrobenzimidazoles (**2**), 1-nitrobenzotriazoles (**3**) are stable in solution in organic solvent but labile in solid states; when the mixture of **3b** and **3c** was stocked in the solid state under argon atmosphere in a refrigerator for a few weeks, brown gas appeared in the stocked tube and it was identified as being NO_2 gas by Kitagawa's NO_2 gas detection test tube. Compound **3d** also decomposed to liberate NO_2 in solid states.

Speculation for Ring Conversion As shown in Table 1, the yield of 1-nitrobenzotriazoles (**3**) was increased by using a high amount of NO_2/O_3 and a longer reaction time. In addition, as we described in the section on "Stability of 1-nitrobenzimidazoles", benzotriazoles (**4**) were partly formed in the process of the decomposition of 1-nitrobenzimidazoles (**2**). Although 1-nitrobenzotriazoles were formed by nitration of benzotriazoles in the NO_2/O_3 system, it is not yet clear whether this system participates in ring conversion from 1-nitrobenzimidazoles to benzotriazoles. On the other hand, the decomposition of **2i** and **2j** in MeOH did not give 5-nitrobenzotriazole, while treatment of 5-nitro-2-phenylbenzimidazole (**1f**) with an excess amount of NO_2/O_3 did. These results suggested that alternative conversion mechanisms may be involved.

When imidazole itself was used as a substrate and examined by *Kyodai* nitration, no triazole derivatives were found. For ring conversion from imidazoles to triazoles, imidazole ring opening is the initial step, and subsequent ring closure completes the conversion. In benzimidazoles, ring closure seems to proceed more easily than in imidazoles.

We previously reported an example of the ring conversion from imidazoles to triazoles; treatment of 7-amino 9-ethylguaninium chloride and 1-amino-3-methylbenzimidazolium chloride with lead(IV) acetate, the oxidizing agent, resulted in the formation of 9-ethyl-7-azaguanine and 1-methylbenzotriazole, respectively.[16] From those findings together with the results of this study, we propose that this kind of conversion from imidazole to triazole may occur in purine bases in DNA as secondary DNA damage after DNA has been nitrated or aminated by certain carcinogens/mutagens.

Kyodai nitration of 1-methylbenzimidazoles and 9-ethylguanine derivatives is in progress.

Experimental

^{1}H-NMR spectra were recorded on JEOL Lambda 400 and 500 spectrometers, and chemical shifts are expressed in parts per million downfield from internal tetramethylsilane. Mass spectra were obtained with a JEOL JMS-SX 102A spectrometer. HPLC analyses were carried out using a Shimadzu LC-10AD apparatus equipped with a photodiode array UV detector SPD-M6A. A Merck LiChrospher 100RP-18(e) column (4×250 mm) was used and eluted with a solvent system of 50% MeOH in 1/15 M phosphate buffer (pH 6.8) at a flow rate of 0.8 ml/min. Melting points were measured with a Yanagimoto micro-melting point apparatus MP-500D and are uncorrected. Flash column chromatography was carried out with Merck silica gel 60 (40—63 μm). Nitrogen dioxide was purchased from Sumitomo Seika Chemical Co. Ltd. For the generation of ozone, a Nippon Ozone Co. Ltd. type ON-1-2 apparatus was used.

General Procedure for *Kyodai* Nitration of Benzimidazole Derivatives Generally, a previously reported method[7] was used with modification. Benzimidazole derivative (10 mmol) in 100 ml CH_3CN was placed in a three-necked 200 ml flask fitted with a gas inlet tube and a vent which permits waste gas to escape. The mixture was cooled to 0 °C in an ice bath, while a stream of ozonized oxygen (ozone generated at 29 mmol h^{-1} under an oxygen flow of 50 dm^3 h^{-1} at an applied voltage of 100 V) was introduced, with vigorous stirring, through the gas inlet tube which dipped below the surface of the liquid in the flask. An aliquot of liquid NO_2 (ρ=1.29), which was prepared from NO_2 in a bomb at under −20 °C, was then added dropwise into the solution. Throughout the reaction, ozonized oxygen was fed continuously. After an appropriate time, the reaction was quenched with saturated aqueous $NaHCO_3$. After CH_3CN was removed by evaporation, products were extracted with AcOEt (100 ml×3). The AcOEt layer was washed with brine and dried over anhydrous $MgSO_4$, and the solvent was removed by evaporation. The residue was subjected to flash column chromatography.

***Kyodai* Nitration of Benzimidazole (1a)** (Exp. 1): Benzimidazole (**1a**) (1180 mg, 10 mmol) in 100 ml CH_3CN was allowed to react with NO_2 (2 ml, 56 mmol) for 30 min. NO_2 (2 ml, 56 mmol) was further added and the mixture was allowed to react for another 30 min. Products were separated by flash column chromatography (silica gel, AcOEt/hexane 1 : 8→2 : 3→AcOEt only) to yield 1-nitrobenzimidazole (**2a**), 1,5-dinitrobenzimidazole (**2b**), 1,6-dinitrobenzimidazole (**2c**), l-nitrobenzotriazole (**3a**), and a mixture of 1,5-dinitrobenzotriazole (**3b**) and 1,6-dinitrobenzotriazole (**3c**). Yields of products were very low as shown in Table 1. The ratio of **3b** and **3c** was determined by comparing the height of their NMR signals. As other products, 5-nitrobenzimidazole (**1b**) and 5-nitrobenzotriazole (**4b**)[18] were obtained in a trace yield.

(Exp. 2): Benzimidazole (**1a**) (1180 mg, 10 mmol) in 100 ml CH_3CN was allowed to react with NO_2 (4 ml, 112 mmol) for 1 h. NO_2 (4 ml, 112 mmol) was further added and the mixture reacted for another 30 min. Products obtained were **3a** (<3%) and a mixture of **3b** (<3%) and **3c** (<3%) as shown in Table 1.

1-Nitrobenzimidazole (**2a**): ^{1}H-NMR ($CDCl_3$) δ: 8.73 (s, 1H, H-2), 8.10 (m, 1H, H-7), 7.83 (m, 1H, H-4), 7.54 (dt, 1H, J=7.8, 1.2 Hz, H-6), 7.48 (dt, 1H, J=7.8, 1.2 Hz, H-5). EI-MS m/z: 163 (M^+), 117 (M^+-NO_2). HR-EI-MS m/z: 163.0377 (M^+) (Calcd for $C_7H_5N_3O_2$: 163.0382). Decomposition occurred gradually when isopropanol solution was left at room temperature.

1,5-Dinitrobenzimidazole (**2b**): Pale yellow prisms (hexane/CH_2Cl_2). mp 151—153 °C. ^{1}H-NMR ($CDCl_3$) δ: 8.89 (s, 1H, H-2), 8.75 (d, 1H, J=2.0 Hz, H-4), 8.48 (dd, 1H, J=9.1, 2.2 Hz, H-6), 8.25 (d, 1H, J=9.0 Hz, H-7). EI-MS m/z: 208 (M^+). HR-EI-MS m/z: 208.0288 (M^+) (Calcd for $C_7H_4N_4O_4$: 208.0233). *Anal.* Calcd for $C_7H_4N_4O_4$: C, 40.40; H, 1.94; N, 26.92. Found: C, 40.12; H, 2.19; N, 26.52.

1,6-Dinitrobenzimidazole (**2c**): ^{1}H-NMR ($CDCl_3$) δ: 9.02 (d, lH, J=2.2 Hz, H-7), 8.95 (s, 1H, H-2), 8.42 (dd, 1H, J=9.0, 2.2 Hz, H-5), 7.98 (d, 1H, J=8.8 Hz, H-4). EI-MS m/z: 208 (M^+). HR-EI-MS m/z: 208.0244 (M^+) (Calcd for $C_7H_4N_4O_4$: 208.0233). Decomposition occurred gradually when the MeOH solution was left at room temperature.

1-Nitrobenzotriazole (**3a**)[19]: White needles (hexane/CH_2Cl_2). mp 75—77 °C (lit.[19] 73 °C). ^{1}H-NMR ($CDC1_3$) δ: 8.20—8.14 (m, 2H, H-4, H-7), 7.81 (t, 1H, J=7.3 Hz, H-5 or H-6), 7.63 (t, 1H, J=7.3 Hz, H-5 or H-6). EI-MS m/z: 164 (M^+), 118 (M^+-NO_2). HR-EI-MS m/z: 164.0338 (M^+) (Calcd for $C_6H_4N_4O_2$: 164.0334). *Anal.* Calcd for $C_6H_4N_4O_2$: C, 43.91; H, 2.46; N, 34.14. Found: C, 43.88; H, 2.54; N, 34.06.

A Mixture of 1,5-Dinitrobenzotriazole (**3b**) and 1,6-Dinitrobenzotriazole (**3c**): White needles (isopropanol). mp 89—l04 °C. ^{1}H-NMR ($CDCl_3$): Two sets of signals due to compounds **3b** and **3c** were observed. 1,5-Dinitrobenzotriazole (**3b**): δ 9.06 (d, 1H, J=2.0 Hz, H-4), 8.51 (dd, 1H, J=9.0, 2.0 Hz, H-6), 8.38 (d, 1H, J=9.0 Hz, 7-H). 1,6-Dinitrobenzotriazole (**3c**): δ 9.11 (d, 1H, J=2.0, H-7), 8.71 (dd, 1H, J=9.0, 2.0 Hz, H-5), 8.32 (d, 1H, J=9.0 Hz, H-4). EI-MS m/z: 209 (M^+), 163 (M^+-NO_2). HR-EI-MS m/z: 209.0182 (M^+) (Calcd for $C_6H_3N_5O_4$: 209.0185). *Anal.* Calcd for $C_6H_3N_5O_4$: C, 34.46; H, 1.45; N, 33.49. Found: C, 34.74; H, 1.55; N, 33.34. The product decomposed when stored in a solid state in a refrigerator for a month.

***Kyodai* Nitration of Benzimidazole Derivatives Having Substituent(s) at the Benzene Moiety and of Benzotriazole Derivatives. *Kyodai* Nitration of 5-Nitrobenzimidazole (1b)** (Exp. 3): 5-Nitrobenzimidazole (**1b**) (810 mg, 5 mmol) in 60 ml CH_3CN was allowed to react with NO_2 (2.5 ml, 70 mmol) for 30 min. Products were separated by flash column chromatography (silica gel, AcOEt : hexane=3 : 8→AcOEt only) to yield **2b** (1.6%), **2c** (0.8%) and a mixture of **3b** (4.3%) and **3c** (7.0%). 5-Nitrobenzotriazole (**4b**) was also obtained in a trace yield.

(Exp. 4): 5-Nitrobenzimidazole (**1b**) (810 mg, 5 mmol) in 60 ml CH_3CN was allowed to react with NO_2 (3.2 ml, 90 mmol) for 40 min. NO_2 (3.2 ml, 90 mmol) was further added and the mixture reacted for another 40 min. The products obtained were a mixture of **3b** (18%) and **3c** (8.4%).

***Kyodai* Nitration of 5,6-Dinitrobenzimidazole (1c)** (Exp. 5): 5,6-Dinitrobenzimidazole (**1c**) (208 mg, 1 mmol) in 30 ml CH_3CN was allowed to react with NO_2 (0.8 ml, 22 mmol) for 30 min. Products were separated by flash column chromatography (silica gel, AcOEt : hexane=1 : 2) to yield 1,5,6-trinitrobenzimidazole (**2d**) (33%).

1,5,6-Trinitrobenzimidazole (**2d**): Pale yellow plates (CH_2Cl_2/hexane). mp 146.2—147.5 °C. ^{1}H-NMR ($CDCl_3$) δ: 9.04 (s, 1H, H-2), 8.69 (s, 1H, H-7), 8.44 (s, 1H, H-4). EI-MS *m/z*: 253 (M^+), 208 (M^+-NO_2+1). HR-EI-MS *m/z*: 253.0078 (M^+) (Calcd for $C_7H_3N_5O_6$: 253.0083). *Anal.* Calcd for $C_7H_3N_5O_6$: C, 33.22; H, 1.19; N, 27.67. Found : C, 33.25; H, 1.53; N, 28.04.

(Exp. 6): 5,6-Dinitrobenzimidazole (**1c**) (208 mg, 1 mmol) in 30 ml CH_3CN was allowed to react with NO_2 (1.0 ml, 28 mmol) for 30 min. Products obtained were 1,5,6-trinitrobenzimidazole (**2d**) (7.4%) and 1,5,6-trinitrobenzotriazole (**3d**) (7.8%). 5,6-Dinitrobenzotriazole (**4c**)[20] was also obtained in a trace yield.

1,5,6-Trinitrobenzotriazole (**3d**): ^{1}H-NMR ($CDCl_3$) δ: 8.88 (s, 1H, H-7), 8.69 (s, 1H, H-4). EI-MS *m/z*: 254 (M^+), 208 (M^+-NO_2). HR-EI-MS *m/z*: 254.0033 (M^+) (Calcd for $C_6H_2N_6O_6$: 254.0036,).

***Kyodai* Nitration of 5-Methylbenzimidazole (1d)** (Exp. 7): 5-Methylbenzimidazole (**1d**) (396 mg, 3 mmol) in 30 ml CH_3CN was allowed to react with NO_2 (1.9 ml, 54 mmol) for 40 min. Products were separated by flash column chromatography (silica gel, AcOEt : hexane=1 : 8→1 : 5) to yield 6-methyl-1,5-dinitrobenzimidazole (**2e**) (5.3%), 5-methyl-1,6-dinitrobenzimidazole (**2f**) (0.2%) and a mixture of 5-methyl-1,6-dinitrobenzotriazole (**3e**) (1.4%) and 6-methyl-1,5-dinitrobenzotriazole (**3f**) (1.7%). A trace amount of 5-methyl-6-nitrobenzotriazole (**4d**) was also obtained.

6-Methyl-1,5-dinitrobenzimidazole (**2e**): ^{1}H-NMR ($CDCl_3$) δ: 8.87 (s, 1H, H-2), 8.26 (s, 1H, H-4). 8.13 (s, 1H, H-7), 2.67 (s, 3H, CH_3). EI-MS *m/z*: 222 (M^+), 176 (M^+-NO_2). HR-EI-MS *m/z*: 222.0389 (M^+) (Calcd for $C_8H_6N_4O_4$: 222.0389).

5-Methyl-1,6-dinitrobenzimidazole (**2f**): ^{1}H-NMR ($CDCl_3$) δ: 8.81 (s, 1H, H-2), 8.46 (s, 1H, H-7), 8.07 (s, 1H, H-4), 2.78 (s, 3H, CH_3).

A Mixture of 5-Methyl-1,6-dinitrobenzotriazole (**3e**) and 6-Methyl-1,5-dinitrobenzotriazole (**3f**): Yellow oil. ^{1}H-NMR ($CDCl_3$): Two sets of signals due to the compounds **3e** and **3f** were observed. 5-Methyl-1,6-dinitrobenzotriazole (**3e**): δ 8.78 (s, 1H, H-7), 8.13 (s, 1H, H-4). 6-Methyl-1,5-dinitrobenzotriazole (**3f**): δ 8.64 (s, 1H, H-4), 8.17 (s, 1H, H-7). EI-MS *m/z*: 223 (M^+), 177 (M^+-NO_2). HR-EI-MS *m/z*: 223.0344 (M^+) (Calcd for $C_7H_5N_5O_4$: 223.0342).

5-Methyl-6-nitrobenzotriazole (**4d**): ^{1}H-NMR ($CDCl_3$) δ: 8.36 (s, 1H, H-7), 8.31 (s, 1H, H-4), 2.59 (s, 3H, CH_3). EI-MS *m/z*: 178 (M^+).

***Kyodai* Nitration of 5,6-Dimethylbenzimidazole (1e)** (Exp. 8): 5,6-Dimethylbenzimidazole (**1e**) (439 mg, 3 mmol) in 50 ml CH_3CN was allowed to react with NO_2 (1.9 ml, 54 mmol) for 40 min. NO_2 (1.9 ml, 54 mmol) was further added and the mixture was allowed to react for another 40 min. Products were separated by flash column chromatography (silica gel, AcOEt/hexane 1 : 6→1 : 4→1 : 2→$CHCl_3$/MeOH 19 : 1) to yield 5,6-dimethyl-1,4-dinitrobenzimidazole (**2g**) and 5(or 6)-methyl-1,4-dinitro-6(or 5)-(nitromethyl)benzimidazole (**2h**).

5,6-Dimethyl-1,4-dinitrobenzimidazole (**2g**): White powder. ^{1}H-NMR ($CDCl_3$) δ: 8.71 (s, 1H, H-2), 8.05 (s, 1H, H-7), 2.54 (s, 3H, CH_3-5), 2.40 (s, 3H, CH_3-6). EI-MS *m/z*: 236 (M^+), 190 (M^+-NO_2). HR-EI-MS *m/z*: 236.0540 (M^+) (Calcd for $C_9H_8N_4O_4$: 236.0546).

5(or 6)-Methyl-1,4-dinitro-6(or 5)-(nitromethyl)benzimidazole (**2h**): ^{1}H-NMR ($CDCl_3$) δ: 8.82 (s, 1H, H-2), 8.31 (s, 1H, H-7), 5.66 (s, 2H, CH_2NO_2), 2.53 (s, 3H, CH_3).

***Kyodai* Nitration of 2-Phenylbenzimidazole (1f)** 2-Phenylbenzimidazole (**1f**) (388 mg, 2 mmol) in 50 ml CH_3CN was allowed to react with NO_2 (0.5 ml, 14 mmol) for 30 min. Products were separated by flash column chromatography (silica gel, AcOEt/hexane 1 : 6→1 : 4→1 : 2) to yield 1,5-dinitro-2-phenylbenzimidazole (**2i**) (12%) and 1,6-dinitro-2-phenylbenzimidazole (**2j**) (3.7%).

1,5-Dinitro-2-phenylbenzimidazole (**2i**): ^{1}H-NMR ($CDCl_3$) δ: 8.69 (d, 1H, *J*=2.2 Hz, H-4), 8.44 (dd, 1H, *J*=9.0, 2.2 Hz, H-6), 8.20 (d, 1H, *J*=9.0 Hz, H-7), 7.78—7.76 (m, 2H, H-2′, H-6′), 7.62—7.54 (m, 3H, H-3′, H-4′, H-5′). EI-MS *m/z*: 239 (M^+-NO_2+H). The product decomposed rapidly when $CHCl_3$, AcOEt or MeOH solution was left at room temperature.

1,6-Dinitro-2-phenylbenzimidazole (**2j**): ^{1}H-NMR ($CDCl_3$) δ: 8.99 (d, 1H, *J*=2.2 Hz, H-7), 8.42 (dd, 1H, *J*=8.8, 2.2 Hz, H-5), 7.92 (d, 1H, *J*=8.8 Hz, H-4), 7.81—7.76 (m, 2H, H-2′, H-6′), 7.63—7.54 (m, 3H, H-3′, H-4′, H-5′). EI-MS *m/z*: 239 (M^+-NO_2+H). The product decomposed rapidly when $CHCl_3$, AcOEt or MeOH solution was left at room temperature.

***Kyodai* Nitration of 2-Methylbenzimidazole (1g)** 2-Methylbenzimidazole (**1g**) (264 mg, 2 mmol) in 50 ml CH_3CN was allowed to react with NO_2 (0.5 ml, 14 mmol) for 30 min. Products were separated by flash column chromatography (silica gel, AcOEt/hexane 1 : 4→$CHCl_3$/MeOH 19 : 1) to yield 2-methyl-1,5-dinitrobenzimidazole (**2k**) (1.8%) and 2-methyl-1,6-dinitrobenzimidazole (**2l**) (1.2%).

2-Methyl-1,5-dinitrobenzimidazole (**2k**): ^{1}H-NMR ($CDCl_3$) δ: 8.60 (d, 1H, *J*=2.0 Hz, H-4), 8.38 (dd, 1H, *J*=9.0, 2.2 Hz, H-6), 8.18 (d, 1H, *J*=9.0 Hz, H-7), 3.00 (s, 3H, CH_3). EI-MS *m/z*: 222 (M^+), 176 (M^+-NO_2). HR-EI-MS *m/z*: 222.0387 (M^+) (Calcd for $C_8H_6N_4O_4$: 222.0387).

2-Methyl-1,6-dinitrobenzimidazole (**2l**): ^{1}H-NMR ($CDCl_3$) δ: 8.97 (d, 1H, *J*=2.0 Hz, H-7), 8.37 (dd, 1H, *J*=9.0, 2.2 Hz, H-5), 7.92 (d, 1H, *J*=8.8 Hz, H-4), 3.02 (s, 3H, CH_3). EI-MS *m/z*: 222 (M^+), 176 (M^+-NO_2). HR-EI-MS *m/z*: 222.0374 (Calcd for $C_8H_6N_4O_4$: 222.0387).

***Kyodai* Nitration of Benzotriazole (4a)** Benzotriazole (**4a**) (595 mg, 5 mmol) in 50 ml CH_3CN was allowed to react with NO_2 (2.0 ml, 56 mmol) for 60 min. Products were separated by flash column chromatography (silica gel, $CHCl_3$→$CHCl_3$: MeOH=9 : 1) to yield **3a** (13%) and a mixture of **3b** and **3c** (41%).

***Kyodai* Nitration of 5-Nitrobenzotriazole (4b)** 5-Nitrobenzotrizole (**4b**) (820 mg, 5 mmol) in 50 ml CH_3CN was allowed to react with NO_2 (2.0 ml, 56 mmol) for 60 min. Products were separated by flash column chromatography (silica gel, $CHCl_3$→$CHCl_3$: MeOH=9 : 1) to yield a mixture of **3b** and **3c** (40%).

Decomposition Products from 1-Nitrobenzimidazoles that were Left in Some Solvent Compound **2d** was dissolved in isopropanol and left at room temperature.

4-Isopropyloxy-5,6-dinitrobenzimidazole: ^{1}H-NMR ($CDCl_3$) δ: 7.94 (s, 1H), 7.83 (s, 1H), 5.46—5.37 (m, 1H, OC<u>H</u>$(CH_3)_2$), 1.50 (d, 6H, *J*=6.1 Hz, OCH(<u>CH</u>$_3)_2$). EI-MS *m/z*: 266 (M^+), 224 ($M^+-CH(CH_3)_2+H$), 208 ($M^+-OCH(CH_3)_2$).

Compound **2i** and **2j** were each dissolved in MeOH and left at room temperature.

5-Nitro-2-(*o*- or *m*-nitrophenyl)benzimidazole[21]: ^{1}H-NMR (CD_3OD) δ: 8.61 (d, 1H, *J*=2.2 Hz, H-4), 8.36 (dd, 1H, *J*=9.0, 2.2 Hz, H-6), 8.17—8.15 (m, 2H), 7.87 (d, 1H, *J*=9.0 Hz, H-7), 7.72—7.66 (m, 2H). MS *m/z*: 284 (M^+), 239 (M^+-NO_2+H). The position of the nitro group is *ortho* or *meta* could not be determined for the lack of the available data.[21]

Reactivity of Benzimidazole Derivatives The starting material (1 mmol) in 25 ml CH_3CN was allowed to react with NO_2 (0.25 ml, 7 mmol or 0.5 ml, 14 mmol) and O_3. Twenty μl of the reaction mixture was taken up at an appropriate time and neutralized with 20 μl of saturated aqueous $NaHCO_3$ solution. After the sample was diluted with distilled water, product analyses were carried out with HPLC.

References and Notes

1) The author passed away on June 9, 2002 at the age of 63 due to illness. This report is her posthumous work.
2) Hemminki K., Koskinen M., Rajaniemi H., Zhao C., *Regul. Toxicol. Pharmacol.*, **32**, 264—275 (2000).
3) Yermilov V., Rubio J., Ohsima H., *FEBS Lett.*, **376**, 207—210 (1965).
4) Huang G.-F. (Kohda K.), Torrence P. F., *J. Org. Chem.*, **42**, 3821—3824 (1977).
5) Ariza X., Bou V., Vilarrasa J., *J. Am. Chem. Soc.*, **117**, 3665—3673 (1995).
6) Ariza X., Farras J., Serra C., Vilarrasa J., *J. Org. Chem.*, **62**, 1547—1549 (1997).
7) Suzuki H., Nonoyama N., *J. Chem. Res.* (*S*), **1996**, 244—245 (1996).
8) Giziewicz J., Wnuk S. F., Robins M. J., *J. Org. Chem.*, **64**, 2149—2151 (1999).
9) Deghati P. Y., Wanner M. J., Koomen G.-J., *Tetrahedron Lett.*, **41**, 1291—1295 (2000).
10) Sodum R. S., Fiala E. S., *Chem. Res. Toxicol.*, **14**, 438—450 (2000).

11) Kaiya T., Tanaka H., Kohda K., *Nucleosides Nucleotides Nucleic Acids*, **21**, 427—433 (2002).
12) Kaiya T., Fujiwara T., Kohda K., *Chem. Res. Toxicol.*, **13**, 993—1001 (2000).
13) Kaiya T., Aoyama S., Kohda K., *Bioorg. Med. Chem. Lett.*, **8**, 625—630 (1998).
14) Kaiya T., Ohta M., Kohda K., *Tetrahedron*, **49**, 8795—8804 (1993).
15) Mori T., Suzuki H., *Synlett*, **1995**, 383—392 (1995).
16) Kaiya T., Aoyama S., Kohda K., *Bioorg. Med. Chem. Lett.*, **9**, 961—964 (1999).
17) Ficken G. E., Fry D. J., *J. Org. Chem.*, **28**, 736—738 (1963).
18) Kulibabina T. N., Kuzilina E. V., Malinina L. A., Pevzner M. S., *Khim. Geterotsikl. Soedin.*, **11**, 1570—1572 (1976). (CAN 86: 89711, AN 1977: 89711).
19) Habraken C. L., Cohen-Fernandes P., *J. Org. Chem.*, **36**, 3084—3086 (1971).
20) Coburn M. D., *J. Heterocyclic Chem.*, **10**, 743—746 (1973).
21) Il'ina V. A., Maslennikova T. A., Gal'pern G. M., Filatova N. N., *Zhurnal Analiticheskoi Khimii*, **36**, 1820—1823 (1981). (CAN 96: 14803, AN 1982: 14803).