Syntheses and Properties of 1-Methyl-3-phenylaminobenzimidazolium Salts, Models of DNA Adducts of N7-Arylaminodeoxyguanosinium Salt

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When arylaminating carcinogens are administered to cells, they mainly generate the C8-arylamino-2′-deoxyguanosine adduct in DNA. A mechanism for this was proposed in which N7-arylaminated 2′-deoxyguanosine acts as an intermediate; however, it remained unclear whether this is actually the case. To elucidate the mechanisms involved in the generation of this adduct, a series of 5-substituted 1-methylbenzimidazole derivatives were used as models of the imidazole moiety of 2′-deoxyguanosine. Syntheses of a series of 5-substituted (CH_3, H, F, CF_3, or NO_2) 1-methyl-3-phenylaminobenzimidazolium salts (**7**) and their related compounds were carried out, and the chemical characteristics of these products were examined. Heating compound **7** at 80 °C for 48 h in H_2O/MeOH provided 5-substituted 1-methyl-2-oxo-2,3-dihydrobenzimidazoles but only when this compound contained a CF_3 or NO_2 substituent. Compound **7** decomposed in alkaline media, and its rate of decomposition increased when this compound had a stronger electron-withdrawing substituent. The product obtained under these conditions was 4-substituted N^1-methyl-2-phenylazoaniline. On the other hand, when 1-methyl-3-(4-nitrophenylamino)benzimidazolium salt was treated under the same conditions as described above, it generated a demethylated product, 1-(4-nitrophenylamino)benzimidazole, when heated in H_2O/MeOH and N^1-formyl-N^1-methyl-2-phenylazoaniline when treated in alkaline media. When the chemical characteristics of 3-phenylamino and 3-amino groups were compared using 3-substituted 1-methyl-5-(trifluoromethyl)benzimidazoles, the 3-phenylamino derivative was found to be more reactive.

Introduction

Carcinogens such as arylamines and nitroarenes are eventually metabolized to their ultimate form, *N*-aryl-*O*-acylhydroxylamine, before they react with cellular components. Arylnitrenium ion (*1*), the reactive species derived from *N*-aryl-*O*-acylhydroxylamine, reacts with DNA to generate several types of DNA adducts. C8-Arylamino-2′-deoxyguanine is the major adduct (Figure 1); however, it is not clear whether the arylnitrenium ion attacks the C8 position directly. To elucidate the mechanisms involved, we previously used NH_2OSO_3H (HAOS)[1] and *O*-(2,4-dinitrophenyl)hydroxylamine as simple models of *N*-aryl-*O*-acylhydroxylamine and examined their reactions toward guanosine (Guo) (*2*−*4*). We found that the reaction of HAOS with Guo at pH 2−4 gave 8-NH_2-Guo (*2*) and demonstrated that 8-NH_2-Guo was formed via an intermediate, N7-NH_2-Guo (*5*) (Figure 1). From these results, together with what we knew about the chemical characteristics of N7-NH_2-Guo (*4*), we speculated that the formation of C8-arylaminodeoxyguanosine might proceed via N7-arylamination. We also reported previously that the guanine-N7 adduct of 4-nitroquinoline 1-oxide (4NQO) was generated when poly(G) was treated with the ultimate form of 4NQO in vitro (*6*) (Figure 1).

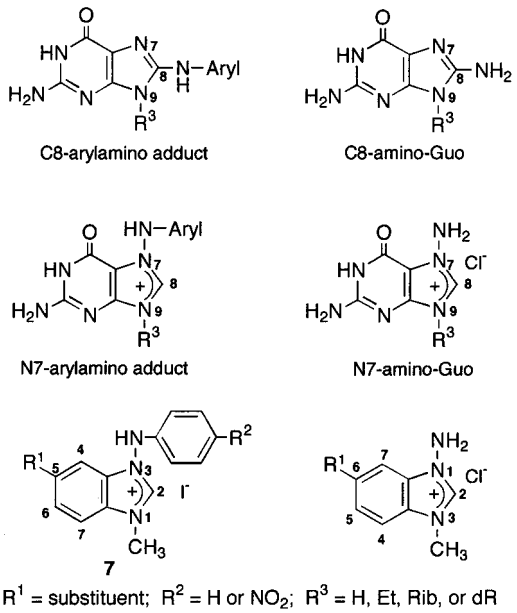

Figure 1. Structures of C8- and N7-(arylamino or amino)-guanines and benzimidazoles with corresponding substituents at the imidazole moiety.

Subsequently, data supporting the idea of an N7-arylamino intermediate were reported by the research groups of Guengerich (*7*) and Novak (*8*). On the other hand, McClelland et al. (*9*) reported recently that arylnitrenium

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[1] Abbreviations: HAOS, NH_2OSO_3H; Guo, guanosine; 4NQO, 4-nitroquinoline 1-oxide; dG, 2′-deoxyguanosine.

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ion attacks directly at the C8 position of 2′-deoxyguanosine (dG) based on the results of the experiments using flush photolysis of dG to generate C8-arylamino-dG. We intended to clarify the possibility of N7-adduct formation from a different point of view by synthesizing N9-substituted N7-phenylaminoguaninium derivatives and examining their chemical characteristics. Several attempts to prepare N9-substituted N7-phenylaminoguaninium derivatives, the simplest models of N9-substituted N7-arylaminoguaninium derivatives, were then examined; however, all attempts were unsuccessful. We had also used a series of 1-methylbenzimidazole derivatives as models of the imidazole moiety of purine (*10*−*12*) and studied the formation and characterization of 1-amino-3-methylbenzimidazolium salt derivatives (*10*, *12*) (Figure 1). In the study presented here using benzimidazole derivatives, we succeeded in preparing 1-methyl-3-phenylaminobenzimidazolium salts, the equivalents of N9-substituted N7-phenylaminoguaninium salts. In this paper, we describe the syntheses and chemical characteristics of a series of 5-substituted 1-methyl-3-phenylaminobenzimidazolium iodide derivatives (**7**) (Figure 1).

Experimental Procedures

^{1}H and ^{13}C NMR spectra were recorded on a JEOL EX 270, GSX 400, or ALPHA 500 spectrometer, and chemical shifts were expressed in parts per million using Me_4Si as the internal standard. Mass spectra were obtained with a JEOL JMS-DX300 spectrometer, and IR spectra were recorded on a JASCO IR-700 spectrometer. HPLC analyses were carried out using a Shimadzu LC-10AD apparatus equipped with an SPD-M6A, a photodiode array UV detector. Melting points were measured with a Yanagimoto micro melting point apparatus and are uncorrected. Silica gel 60 and 60 PF_{254} (Merck) were used for column and preparative thin-layer chromatography (PLC), respectively.

Syntheses of 5-Substituted 1-Methyl-3-phenylaminobenzimidazolium Iodide (7).

(1) 4-Methyl-2-nitrobenzanilide (2a). A mixture of 4-methyl-2-nitroaniline (**1a**, 10.0 g, 65.7 mmol), benzoyl chloride (10 mL, 86.9 mmol), and pyridine (20 mL) was left at 25 °C for 15 h. Water (100 mL) was then added, and the resulting precipitates were collected. Recrystallization of the precipitates from benzene gave 12.6 g (75%) of **2a** as yellow needles: mp 152−154 °C [lit. (*13*), 146−148 °C]; ^{1}H NMR ($CDCl_3$) δ 2.42 (s, 3H, CH_3), 7.54 (m, 3H, 5-H, 3′-H, 5′-H), 7.61 (dt, 1H, J = 1.5, 7.3 Hz, 4′-H), 7.99 (dd, 2H, J = 1.5, 7.0 Hz, 2′-H, 6′-H), 8.08 (d, 1H, J = 1.1 Hz, 3-H), 8.88 (d, 1H, J = 8.8 Hz, 6-H), 11.25 (br s, 1H, NH).

(2) 2-Amino-4-methylbenzanilide (3a). Compound **2a** (6.00 g, 23.4 mmol) was dissolved in 600 mL of MeOH. After 654 mg of 5% Pd-C had been added to the solution, **2a** was hydrogenated at room temperature and atmospheric pressure. After the calculated amount of hydrogen had been taken up, Pd-C was removed by filtration, and the mother liquor was evaporated to dryness. Recrystallization of the residue from MeOH gave 4.34 g (82%) of **3a** as colorless needles: mp 202−204 °C [lit. (*14*), 193−194 °C]; ^{1}H NMR ($CDCl_3$) δ 2.28 (s, 3H, CH_3), 3.85 (br s, 2H, NH_2), 6.66 (d, 1H, J = 7.7 Hz, 5-H), 6.67 (s, 1H, 3-H), 7.17 (d, 1H, J = 7.7 Hz, 6-H), 7.49 (m, 2H, 3′-H, 5′-H), 7.56 (t, 1H, J = 7.3 Hz, 4′-H), 7.74 (br s, 1H, NH), 7.91 (d, 2H, J = 7.7 Hz, 2′-H, 6′-H).

(3) 4-Methyl-2-phenylazobenzanilide (4a). Compound **3a** (10.1 g, 44.8 mmol) was dissolved in a mixture of EtOH (90 mL) and AcOH (160 mL). Nitrosobenzene (4.80 g, 44.8 mmol) was then added, and the solution was left at 25 °C for 15 h. After the solvent had been removed by evaporation, the product was purified by column chromatography (silica gel, *n*-hexane → 1/19 $CHCl_3$/*n*-hexane → 1/13 $CHCl_3$/*n*-hexane → 1/9 $CHCl_3$/*n*-hexane) to obtain 7.13 g (51%) of **4a** as an orange-colored powder: mp 121−123 °C; ^{1}H NMR ($CDCl_3$) δ 2.43 (s, 3H, CH_3), 7.36 (dd, 1H, J = 1.5, 8.8 Hz, 5-H), 7.55 (m, 6H, 3′-H, 4′-H, 5′-H of Bz, 3″-H, 4″-H, 5″-H of PhN_2), 7.78 (d, 1H, J = 1.5 Hz, 3-H), 7.89 (dd, 2H, J = 1.5, 7.6 Hz, 2″-H, 6″-H), 7.98 (dd, 2H, J = 1.5, 7.6 Hz, 2′-H, 6′-H), 8.78 (d, 1H, J = 8.8 Hz, 6-H), 11.31 (br s, 1H, NH).

(4) 4-Methyl-2-phenylazoaniline (5a). Compound **4a** (6.00 g, 19.0 mmol) was dissolved in 10 mL of MeOH containing 10% KOH, and the mixture was heated under reflux for 5 h. After the solvent had been removed by evaporation, the product was recrystallized from MeOH to obtain 3.82 g (95%) of **5a** as orange-colored needles: mp 123−125 °C; ^{1}H NMR ($CDCl_3$) δ 2.31 (s, 3H, CH_3), 5.63 (s, 2H, NH_2), 6.70 (d, 1H, J = 8.4 Hz, 6-H), 7.05 (m, 1H, 5-H), 7.40 (t, 1H, J = 7.3 Hz, 4′-H), 7.48 (m, 2H, 3′-H, 5′-H), 7.63 (br s, 1H, 3-H), 7.83 (d, 2H, J = 8.4 Hz, 2′-H, 6′-H); MS m/z 211 (M^+).

(5) 6-Methyl-1-phenylaminobenzimidazole (6a). Compound **5a** (500 mg, 2.37 mmol), AcOH (2.5 mL), 37% HCHO (0.25 mL, 3.34 mmol), and concentrated HCl (0.25 mL) were mixed, and the solution was heated at 80 °C for 1 h. After the solvent had been removed by evaporation, the product was separated by column chromatography (silica gel, $CHCl_3$) to obtain 80 mg (15%) of **6a**. Recrystallization from MeOH gave colorless needles: mp 234−236 °C; ^{1}H NMR (Me_2SO-d_6) δ 2.37 (s, 3H, CH_3), 6.45 (d, 2H, J = 8.5 Hz, 2′-H, 6′-H), 6.84 (t, 1H, J = 7.9 Hz, 4′-H), 7.03 (s, 1H, 7-H), 7.07 (d, 1H, J = 8.5 Hz, 5-H), 7.19 (m, 2H, 3′-H, 5′-H), 7.59 (d, 1H, J = 8.5 Hz, 4-H), 8.26 (s, 1H, 2-H), 9.49 (br s, 1H, NH). Anal. Calcd for $C_{14}H_{13}N_3$: C, 75.31; H, 5.87; N, 18.82. Found: C, 75.09; H, 5.96; N, 18.77.

(6) 1,5-Dimethyl-3-phenylaminobenzimidazolium Iodide (7a). Compound **6a** (112 mg, 0.50 mmol) was dissolved in 6 mL of MeOH. CH_3I (125 μL, 2.00 mmol) was then added, and the mixture was heated at 50 °C for 12 h. Further addition of CH_3I (125 μL) and a subsequent 12 h reaction were repeated three times (totals of 500 μL of CH_3I and 48 h of reaction). After the solvent had been removed by evaporation, the product was separated by PLC (silica gel, 9/1 $CHCl_3$/MeOH), yielding 86 mg (47%) of **7a**. The starting material was recovered (37 mg, 33%). Recrystallization of **7a** from MeOH yielded a white powder: mp 254.5−256.0 °C; ^{1}H NMR (Me_2SO-d_6) δ 2.45 (s, 3H, 5-CH_3), 4.10 (s, 3H, N-CH_3), 6.79 (d, 2H, J = 8.3 Hz, 2′-H, 6′-H), 6.99 (t, 1H, J = 7.4 Hz, 4′-H), 7.28 (m, 2H, 3′-H, 5′-H), 7.34 (s, 1H, 4-H), 7.55 (d, 1H, J = 8.6 Hz, 6-H), 8.01 (d, 1H, J = 8.6 Hz, 7-H), 10.11 (s, 1H, 2-H, H−D exchange occurred with the addition of D_2O), 10.22 (br s, 1H, NH); ^{13}C NMR (Me_2SO-d_6) δ 21.0 (5-CH_3), 33.7 (1-CH_3), 111.6 (4-C), 113.4 (2′-C, 6′-C), 113.8 (7-C), 122.1 (4′-C), 128.4 (6-C), 129.4 (3′-C, 5′-C, 7a-C), 130.4 (3a-C), 137.5 (5-C), 144.3 (2-C), 145.3 (1′-C); FAB MS m/z 238 [$(M - I)^+$], 147 [$(M - I)^+ -$ PhN].

(7) 2-Nitrobenzanilide (2b). A mixture of 2-nitroaniline (**1b**, 3.00 g, 21.7 mmol), pyridine (5 mL), and benzoyl chloride (4 mL, 34.4 mmol) was left at 25 °C for 15 h. Water (50 mL) was then added, and the resulting precipitates were collected. Recrystallization of the precipitates from MeOH gave 5.08 g (97%) of **2b** as yellow needles: mp 95−96 °C [lit. (*15*), 94 °C]; ^{1}H NMR ($CDCl_3$) δ 7.23 (m, 1H, 4-H), 7.55 (t, 2H, J = 7.3 Hz, 3′-H, 5′-H), 7.62 (t, 1H, J = 7.3 Hz, 4′-H), 7.72 (m, 1H, 5-H), 8.00 (d, 2H, J = 7.3 Hz, 2′-H, 6′-H), 8.29 (d, 1H, J = 8.5 Hz, 3-H), 9.01 (d, 1H, J = 8.5 Hz, 6-H), 11.36 (br s, 1H, NH).

(8) 2-Aminobenzanilide (3b). Compound **2b** (1.00 g, 4.13 mmol) was dissolved in 50 mL of MeOH. After 100 mg of 5% Pd-C had been added to the solution, **2b** was hydrogenated for 15 h. Pd-C was removed by filtration, and the mother liquor was evaporated to dryness. Recrystallization of the residue from MeOH gave 701 mg (80%) of **3b** as colorless needles: mp 157−158 °C [lit. (*15*), 151−152 °C]; ^{1}H NMR ($CDCl_3$) δ 3.87 (br s, 2H, NH_2), 6.85 (dd, H, J = 1.2, 7.9 Hz, 3-H), 6.85 (m, 1H, 5-H), 7.10 (m, 1H, 4-H), 7.33 (d, 1H, J = 6.7 Hz, 6-H), 7.49 (t, 2H, J = 7.3 Hz, 3′-H, 5′-H), 7.56 (t, 1H, J = 7.3 Hz, 4′-H), 7.87 (br s, 1H, NH), 7.90 (d, 2H, J = 7.3 Hz, 2′-H, 6′-H); MS m/z 197 (M^+).

(9) 2-Phenylazobenzanilide (4b). Compound **3b** (500 mg, 2.36 mmol) was dissolved in a mixture of EtOH (1 mL) and

AcOH (1.75 mL). After nitrosobenzene (253 mg, 2.36 mmol) had been added, the solution was left at 25 °C for 15 h. The resulting precipitate (orange-colored needles) of **4b** was then collected (484 mg, 68%): mp 127−128 °C [lit. (15), 122 °C]; ^{1}H NMR ($CDCl_3$) δ 7.26 (m, 1H, 4-H), 7.55 (m, 7H, 5-H, 3′-H, 4′-H, 5′-H of Bz, 3″-H, 4″-H, 5″-H of PhN_2), 7.89 (dd, 2H, J = 1.5, 7.8 Hz, 2″-H, 6″-H), 7.99 (m, 3H, 3-H, 2′-H, 6′-H), 8.91 (d, 1H, J = 8.3 Hz, 6-H), 11.49 (br s, 1H, NH).

(10) 2-Phenylazoaniline (5b). Compound **4b** (8.59 g, 28.5 mmol) was dissolved in 200 mL of MeOH containing 10% KOH, and the mixture was heated under reflux for 6 h. After the solvent had been removed by evaporation, the product was separated by column chromatography (silica gel, *n*-hexane → 8/2 *n*-hexane/$CHCl_3$ → 1/1 *n*-hexane/$CHCl_3$ → $CHCl_3$). Collection and evaporation of the fractions containing **5b** yielded 4.73 g (84%) of orange-colored columns: mp 56−57 °C [lit. (*16*), 59 °C]; ^{1}H NMR ($CDCl_3$) δ 5.88 (br s, 2H, NH_2), 6.77 (d, 1H, J = 8.3 Hz, 6-H), 6.82 and 7.21 (each m, each 1H, 4-H and 5-H), 7.42 (t, 1H, J = 7.3 Hz, 4′-H), 7.49 (t, 2H, J = 7.3 Hz, 3′-H, 5′-H), 7.83 (dd, 1H, J = 1.2, 7.3 Hz, 3-H), 7.84 (d, 2H, J = 7.3 Hz, 2′-H, 6′-H).

(11) 1-Phenylaminobenzimidazole (6b). Compound **5b** (1.00 g, 5.07 mmol), AcOH (5 mL), 37% HCHO (0.5 mL, 6.68 mmol), and concentrated HCl (0.5 mL) were mixed, and the solution was heated at 80 °C until its color had changed from red to pale brown. After it had cooled, water (50 mL) was added and the product was extracted with $CHCl_3$. The product was then purified by column chromatography (silica gel, 19/1 $CHCl_3$/*n*-hexane → $CHCl_3$) to obtain 448 mg (42%) of **6b**. Recrystallization from MeOH gave colorless crystals: mp 224 °C; ^{1}H NMR (Me_2SO-d_6) δ 6.48 (d, 2H, J = 7.6 Hz, 2′-H, 6′-H), 6.85 (t, 1H, J = 7.6 Hz, 4′-H), 7.18 (t, 2H, J = 7.6 Hz, 3′-H, 5′-H), 7.24 (m, 3H, 4-H, 5-H, 6-H), 7.74 (m, 1H, 7-H), 8.37 (s, 1H, 2-H), 9.54 (br s, 1H, NH); ^{13}C NMR (Me_2SO-d_6) δ 109.8 (7-C), 112.4 (2′-C, 6′-C), 120.0 (4′-C), 120.5 (4-C, 5-C, or 6-C), 122.1 (4-C, 5-C, or 6-C), 123.0 (4-C, 5-C, or 6-C), 129.2 (3′-C, 5′-C), 132.9 (7a-C), 141.5 (3a-C), 144.7 (2-C), 147.4 (1′-C); MS m/z 209 (M^+). Anal. Calcd for $C_{13}H_{11}N_3$: C, 74.62; H, 5.30; N, 20.08. Found: C, 74.63; H, 5.35; N, 19.93.

(12) 1-Methyl-3-phenylaminobenzimidazolium Iodide (7b). Compound **6b** (105 mg, 0.502 mmol) was dissolved in 10 mL of MeOH. CH_3I (438 μL, 7.04 mmol) was then added, and the mixture was left at 25 °C for 4 days. After the solvent had been removed by evaporation, the product was separated and purified by PLC (silica gel, 9/1 $CHCl_3$/MeOH) to obtain 76 mg (43%) of **7b**. Recrystallization from MeOH gave pale yellow columns: mp 223.5−225.0 °C; ^{1}H NMR (Me_2SO-d_6) δ 4.13 (s, 3H, CH_3), 6.82 (d, 2H, J = 7.8 Hz, 2′-H, 6′-H), 7.00 (t, 1H, J = 7.3 Hz, 4′-H), 7.29 (m, 2H, 3′-H, 5′-H), 7.53 (d, J = 8.3 Hz, 4-H), 7.64 (t, J = 8.3 Hz, 5-H), 7.73 (t, J = 8.3 Hz, 6-H), 8.13 (d, 1H, J = 8.3 Hz, 7-H), 10.11 (s, 1H, 2-H, H−D exchange occurred with the addition of D_2O), 10.22 (br s, 1H, NH); ^{13}C NMR (Me_2SO-d_6) δ 33.7 (1-CH_3), 112.6 (4-C), 113.6 (2′-C, 6′-C), 114.2 (7-C), 122.2 (4′-C), 126.9 (6-C), 127.1 (5-C), 129.4 (3′-C, 5′-C), 130.0 (3a-C), 131.2 (7a-C), 144.8 (2-C), 145.3 (1′-C); FAB MS m/z 224 [$(M - I)^+$], 133 [$(M - I)^+ -$ PhN]. Anal. Calcd for $C_{14}H_{14}IN_3$: C, 47.88; H, 4.02; N, 11.97. Found: C, 47.78; H, 4.01; N, 12.13.

(13) 2-Amino-4-fluoroacetanilide (3c). 4-Fluoro-2-nitroacetanilide (**2c**, 5.00 g, 25.2 mmol, Tokyo Chemical Industry, Co., Ltd.) was dissolved in 200 mL of MeOH. After 400 mg of 5% Pd-C had been added to the solution, **2c** was hydrogenated. Pd-C was removed by filtration, and the mother liquor was evaporated to dryness. Recrystallization of the residue from AcOEt gave 4.14 g (98%) of **3c** as colorless needles: mp 126−129 °C; ^{1}H NMR (Me_2SO-d_6) δ 2.01 (s, 3H, CH_3), 5.15 (br s, 2H, NH_2), 6.28 (dd, 1H, J = 2.7, 8.5 Hz, 5-H), 6.46 (dd, 1H, J = 2.7, 11.3 Hz, 3-H), 7.06 (dd, 1H, J = 6.4, 8.5 Hz, 6-H), 9.02 (br s, 1H, NH); MS m/z 168 (M^+), 126 ($M^+ - C_2H_2O$). Anal. Calcd for $C_8H_9FN_2O$: C, 57.13; H, 5.39; N, 16.66. Found: C, 57.40; H, 5.42; N, 16.34.

(14) 4-Fluoro-2-phenylazoacetanilide (4c). Compound **3c** (168 mg, 1.00 mmol) was dissolved in a mixture of EtOH (0.4 mL) and AcOH (0.6 mL). Nitrosobenzene (117 mg, 1.09 mmol) was then added, and the solution was left at 25 °C for 15 h. After the solvent had been removed by evaporation, the product was purified by PLC (silica gel, $CHCl_3$) to obtain 91 mg (35%) of **4c**. Recrystallization from MeOH gave orange-colored needles: mp 158.5−159.5 °C; ^{1}H NMR (Me_2SO-d_6) δ 2.19 (s, 3H, CH_3), 7.42 (m, 1H, 4′-H), 7.43 (dd, 1H, J = 3.1, 9.5 Hz, 3-H), 7.62 (m, 3H, 5-H, 3′-H, 5′-H), 8.04 (dd, 2H, J = 1.5, 8.1 Hz, 2′-H, 6′-H), 8.20 (dd, 1H, J = 5.5, 9.2 Hz, 6-H), 10.01 (br s, 1H, NH); MS m/z 257 (M^+), 152 ($M^+ -$ PhN_2). Anal. Calcd for $C_{14}H_{12}FN_3O$: C, 65.36; H, 4.70; N, 16.34. Found: C, 65.13; H, 4.66; N, 16.29.

(15) 4-Fluoro-2-phenylazoaniline (5c). Compound **4c** (273 mg, 1.06 mmol) was dissolved in 15 mL of MeOH containing 10% KOH, and the mixture was heated under reflux for 1.5 h. After the solvent had been removed by evaporation, the residue was applied to a silica gel column (1/1 *n*-hexane/$CHCl_3$) to remove salts, and the product was further purified by PLC (silica gel, 1/1 *n*-hexane/$CHCl_3$) to yield 215 mg (94%) of **5c** as red needles: mp 58−60 °C; ^{1}H NMR (Me_2SO-d_6) δ 6.58 (br s, 2H, NH_2), 6.89 (dd, 1H, J = 5.2, 9.1 Hz, 6-H), 7.13 (m, 1H, 5-H), 7.35 (dd, 1H, J = 3.0, 10.1 Hz, 3-H), 7.48 (dd, 1H, J = 1.5, 7.3 Hz, 4′-H), 7.55 (m, 2H, 3′-H, 5′-H), 7.92 (dd, 2H, J = 1.5, 7.7 Hz, 2′-H, 6′-H); MS m/z 215 (M^+), 110 ($M^+ -$ PhN_2); HRMS m/z M^+ calcd for $C_{12}H_{10}FN_3$ 215.0859, found 215.0860.

(16) 6-Fluoro-1-phenylaminobenzimidazole (6c). Compound **5c** (300 mg, 1.39 mmol), AcOH (1.5 mL), 37% HCHO (0.15 mL, 2.00 mmol), and concentrated HCl (0.15 mL) were mixed, and the solution was heated at 80 °C for 15 min. After it had cooled, water (15 mL) was added and the product was extracted with $CHCl_3$. The product was purified by PLC (silica gel, 19/1 $CHCl_3$/MeOH) to obtain 100 mg (31%) of **6c**. Recrystallization from MeOH gave colorless columns: mp 175.5−177.5 °C; ^{1}H NMR (Me_2SO-d_6) δ 6.48 (dd, 2H, J = 0.9, 8.5 Hz, 2′-H, 6′-H), 6.87 (dd, 1H, J = 0.9, 7.3 Hz, 4′-H), 7.02 (dd, 1H, J = 2.4, 8.5 Hz, 7-H), 7.21 (m, 3H, 5-H, 3′-H, 5′-H), 7.75 (dd, 1H, J = 4.9, 8.9 Hz, 4-H), 8.39 (s, 1H, 2-H), 9.52 (br s, 1H, NH); MS m/z 227 (M^+). Anal. Calcd for $C_{13}H_{10}FN_3$: C, 68.71; H, 4.44; N, 18.49. Found: C, 68.98; H, 4.48; N, 18.38.

(17) 5-Fluoro-1-methyl-3-phenylaminobenzimidazolium Iodide (7c). Compound **6c** (454 mg, 2.00 mmol) was dissolved in 15 mL of MeOH. CH_3I (600 μL, 9.64 mmol) was then added, and the mixture was heated at 50 °C for 12 h. Further addition of CH_3I (600 μL) and a subsequent 12 h reaction were repeated three times. After the solvent had been removed by evaporation, the product was separated and purified by column chromatography (silica gel, $CHCl_3$ → 19/1 $CHCl_3$/MeOH → 9/1 $CHCl_3$/MeOH) to obtain 381 mg (55%) of **7c**. Recrystallization from EtOH gave colorless plates: mp 198−200 °C; ^{1}H NMR (Me_2SO-d_6) δ 4.13 (s, 3H, CH_3), 6.82 (d, 2H, J = 8.3 Hz, 2′-H, 6′-H), 7.02 (t, 1H, J = 7.3 Hz, 4′-H), 7.30 (m, 2H, 3′-H, 5′-H), 7.46 (dd, 1H, J = 2.4, 7.8 Hz, 4-H), 7.66 (m, 1H, 6-H), 8.21 (dd, 1H, J = 4.2, 9.0 Hz, 7-H), 10.16 (s, 1H, 2-H, H−D exchange occurred with the addition of D_2O), 10.21 (br s, 1H, NH); ^{13}C NMR (Me_2SO-d_6) δ 34.0 (1-CH_3), 99.4 (4-C), 113.7 (2′-C, 6′-C), 115.7 (6-C), 116.4 (7-C), 122.3 (4′-C), 128.0 (7a-C), 129.4 (3′-C, 5′-C), 130.9 (3a-C), 145.1 (1′-C), 146.1 (2-C), 160.7 (5-C); FAB MS m/z 242 [$(M - I)^+$], 151 [$(M - I)^+ -$ PhN]. Anal. Calcd for $C_{14}H_{13}FIN_3 \cdot {}^2/_3EtOH$: C, 46.05; H, 4.29; N, 10.51. Found: C, 45.82; H, 4.03; N, 10.33.

(18) 2-Nitro-4-(trifluoromethyl)acetanilide (2d). A mixture of 2-nitro-4-(trifluoromethyl)aniline (**1d**, 5.00 g, 24.3 mmol, Tokyo Chemical Industry, Co., Ltd.), AcOH (10 mL), and Ac_2O (10 mL) was heated under reflux for 5 h. After the mixture had been evaporated, the residue was recrystallized from MeOH to obtain 4.9 g (82%) of **2d**: mp 115 °C [lit. (*17*), 112−113 °C]; ^{1}H NMR (Me_2SO-d_6) δ 2.12 (s, 3H, CH_3), 7.88 (d, 1H, J = 8.5 Hz, 6-H), 8.08 (dd, 1H, J = 2.1, 8.5 Hz, 5-H), 8.27 (br s, 1H, 3-H), 10.60 (br s, 1H, NH).

(19) 2-Amino-4-(trifluoromethyl)acetanilide (3d). Compound **2d** (4.87 g, 19.6 mmol) was dissolved in 500 mL of MeOH. After 400 mg of 5% Pd-C had been added to the solution, **2d**

was hydrogenated. Pd-C was removed by filtration, and the residue was evaporated to dryness. Recrystallization of the residue from MeOH gave 3.85 g (90%) of **3d** as colorless needles: mp 130−131 °C; ^{1}H NMR (Me_2SO-d_6) δ 2.07 (s, 3H, CH_3), 5.36 (br s, 2H, NH_2), 6.83 (dd, 1H, J = 1.8, 7.9 Hz, 5-H), 7.02 (d, 1H, J = 1.8 Hz, 3-H), 7.49 (d, 1H, J = 7.9 Hz, 6-H), 9.24 (br s, 1H, NH); MS m/z 218 (M^+), 176 ($M^+ - C_2H_2O$). Anal. Calcd for $C_9H_9F_3N_2O$: C, 49.54; H, 4.16; N, 12.84. Found: C, 49.32; H, 4.26; N, 12.59.

(20) 2-Phenylazo-4-(trifluoromethyl)acetanilide (4d). Compound **3d** (436 mg, 2.00 mmol) was dissolved in a mixture of EtOH (0.8 mL) and AcOH (1.2 mL). Nitrosobenzene (236 mg, 2.20 mmol) was then added, and the solution was left at 25 °C for 15 h. After the solvent had been removed by evaporation, the product was purified by PLC (silica gel, $CHCl_3$) to obtain 113 mg (18%) of **4d**. Recrystallization from AcOEt gave orange-colored needles: mp 209−210 °C; ^{1}H NMR (Me_2SO-d_6) δ 2.26 (s, 3H, CH_3), 7.63 (m, 3H, 3′-H, 4′-H, 5′-H), 7.90 (dd, 1H, J = 2.1, 8.8 Hz, 5-H), 7.94 (br s, 1H, 3-H), 8.11 (m, 2H, 2′-H, 6′-H), 8.55 (d, 1H, J = 8.8 Hz, 6-H), 10.29 (br s, 1H, NH); MS m/z 307 (M^+), 202 ($M^+ - PhN_2$). Anal. Calcd for $C_{15}H_{12}F_3N_3O$: C, 58.63; H, 3.94; N, 13.68. Found: C, 58.42; H, 3.98; N, 13.64.

(21) 2-Phenylazo-4-(trifluoromethyl)aniline (5d). Compound **4d** (230 mg, 0.749 mmol) was dissolved in 20 mL of MeOH containing 10% KOH, and the mixture was heated under reflux for 1 h. After the solvent had been removed by evaporation, the product was separated by PLC (silica gel, 1/1 *n*-hexane/$CHCl_3$) to obtain 180 mg (91%) of **5d** as orange-colored columns: mp 73−74 °C; ^{1}H NMR (Me_2SO-d_6) δ 7.04 (d, 1H, J = 8.9 Hz, 6H), 7.41 (br s, 2H, NH_2), 7.49 (dd, 1H, J = 2.1, 8.9 Hz, 5-H), 7.52 (m, 1H, 4′-H), 7.57 (m, 2H, 3′-H, 5′-H), 7.90 (d, 1H, J = 2.1 Hz, 3-H), 7.99 (d, 2H, J = 7.3 Hz, 2′-H, 6′-H); MS m/z 265 (M^+), 160 ($M^+ - PhN_2$); HRMS m/z M^+ calcd for $C_{13}H_{10}F_3N_3$ 265.0827, found 265.0825.

(22) 1-(Phenylamino)-6-(trifluoromethyl)benzimidazole (6d). Compound **5d** (265 mg, 1.00 mmol), AcOH (1 mL), 37% HCHO (0.1 mL, 1.34 mmol), and concentrated HCl (0.1 mL) were mixed, and the solution was heated at 80 °C for 15 min. After it had cooled, water (20 mL) was added and the product was extracted with $CHCl_3$. The product was then purified by PLC (silica gel, 19/1 $CHCl_3$/MeOH) to obtain 193 mg (70%) of **6d**. Recrystallization from MeOH gave colorless columns: mp 172−174 °C; ^{1}H NMR (Me_2SO-d_6) δ 6.61 (d, 2H, J = 8.1 Hz, 2′-H, 6′-H), 6.95 (t, 1H, J = 7.3 Hz, 4′-H), 7.26 (m, 2H, 3′-H, 5′-H), 7.62 (dd, 1H, J = 1.2, 8.5 Hz, 5-H), 7.74 (d, 1H, J = 1.2 Hz, 7-H), 7.98 (d, 1H, J = 8.5 Hz, 4-H), 8.63 (s, 1H, 2-H); MS m/z 277 (M^+). Anal. Calcd for $C_{14}H_{10}F_3N_3$: C, 60.65; H,3.63; N, 15.16. Found: C, 60.64; H, 3.72; N, 15.14.

(23) 1-Methyl-3-(phenylamino)-5-(trifluoromethyl)benzimidazolium Iodide (7d). Compound **6d** (485 mg, 1.75 mmol) was dissolved in 10 mL of MeOH. CH_3I (438 μL, 7.04 mmol) was then added, and the mixture was heated at 50 °C for 1 day. Further addition of CH_3I (438 μL) and a 1 day reaction were repeated three times. After the solvent had been removed by evaporation, the product was separated and purified by column chromatography (silica gel, $CHCl_3$ → 19/1 $CHCl_3$/MeOH → 9/1 $CHCl_3$/MeOH) to obtain 334 mg (46%) of **7d**. Recrystallization from EtOH gave colorless plates: mp 204−206 °C; ^{1}H NMR (Me_2SO-d_6) δ 6.87 (d, 2H, J = 8.1 Hz, 2′-H, 6′-H), 7.03 (t, 1H, J = 7.4 Hz, 4′-H), 7.30 (m, 2H, 3′-H, 5′-H), 7.91 (s, 1H, 4-H), 8.13 (d, 1H, J = 8.9 Hz, 6-H), 8.41 (d, 1H, J = 8.9 Hz, 7-H), 10.28 (br s, 1H, NH), 10.35 (s, 1H, 2-H, H−D exchange occurred with the addition of D_2O); ^{13}C NMR (Me_2SO-d_6) δ 34.2 (1-CH_3), 110.3 (4-C), 113.8 (2′-C, 6′-C), 116.2 (7-C), 122.5 (4′-C), 123.7 (6-C), 124.6 (5-C), 127.3 (5-CF_3), 129.4 (3′-C, 5′-C), 130.0 (3a-C), 133.7 (7a-C), 145.1 (1′-C), 147.7 (2-C); FAB MS m/z 292 [$(M - I)^+$], 201 [$(M - I)^+ - PhN$]. Anal. Calcd for $C_{15}H_{13}F_3IN_3 \cdot 0.25H_2O$: C, 42.52; H, 3.21; N, 9.92. Found: C, 42.33; H, 3.26; N, 9.94.

(24) 4-Nitro-2-phenylazoanilne (5e). 4-Nitro-1,2-phenylenediamine (1.99 g, 13.0 mmol, Aldrich) was dissolved in a mixture of AcOH (7.8 mL) and EtOH (5.2 mL), and nitrosobenzene (1.53 g, 14.3 mmol) was then added. After the reaction mixture had been left at 25 °C for 15 h, the solvent was removed by evaporation, and the product was separated by column chromatography (silica gel, 6/4 *n*-hexane/$CHCl_3$). Recrystallization of the product from *n*-hexane/$CHCl_3$ gave 642 mg (20%) of **5e** as orange-colored needles: mp 155−156 °C; ^{1}H NMR (Me_2SO-d_6) δ 6.99 (d, 1H, J = 9.1 Hz, 6-H), 7.58 (m, 3H, 3′-H, 4′-H, 5′-H), 7.93 (br s, 2H, NH_2), 8.05 (d, 2H, J = 7.0 Hz, 2′-H, 6′-H), 8.08 (dd, 1H, J = 2.7, 9.1 Hz, 5-H), 8.46 (d, 1H, J = 2.7 Hz, 3-H); ^{13}C NMR (Me_2SO-d_6) δ 116.6 (6-C), 118.1 (3-C), 122.7 (2′-C, 6′-C), 127.3 (5-C), 129.2 (3′-C, 5′-C), 131.2 (4′-C), 133.3 (2-C), 136.1 (4-C), 151.5 (1-C), 151.9 (1′-C); MS m/z 242 (M^+), 165 ($M^+ - C_6H_5$), 137 ($M^+ - PhN_2$); HRMS m/z M^+ calcd for $C_{12}H_{10}N_4O_2$ 242.0804, found 242.0804.

(25) 6-Nitro-1-phenylaminobenzimidazole (6e). Compound **5e** (121 mg, 0.50 mmol), AcOH (1 mL), 37% HCHO (0.1 mL), and concentrated HCl (0.1 mL) were mixed, and the solution was heated at 80 °C for 15 min. After it had cooled, water (20 mL) was added and the product was extracted with $CHCl_3$. The product was purified by PLC (silica gel, 19/1 $CHCl_3$/MeOH) to obtain 48 mg (38%) of **6e**. Recrystallization from AcOEt gave yellow needles: mp 194−195 °C; ^{1}H NMR (Me_2SO-d_6) δ 6.56 (m, 2H, 2′-H, 6′-H), 6.91 (t, 1H, J = 7.3 Hz, 4′-H), 7.23 (m, 2H, 3′-H, 5′-H), 7.96 (d, 1H, J = 8.8 Hz, 4-H), 8.13 (d, 1H, J = 2.4 Hz, 7-H), 8.17 (dd, J = 2.4, 8.8 Hz, 5-H), 8.79 (s, 1H, 2-H), 9.73 (br s, 1H, NH); ^{13}C NMR (Me_2SO-d_6) δ 106.4 (7-C), 112.6 (2′-C, 6′-C), 117.8 (5-C), 120.7 (4-C), 121.2 (4′-C), 129.4 (3′-C, 5′-C), 132.4 (7a-C), 143.3 (6-C), 145.8 (3a-C), 146.9 (1′-C), 149.3 (2-C). Anal. Calcd for $C_{13}H_{10}N_4O_2$: C, 61.41; H, 3.96; N, 22.04. Found: C, 61.54; H, 3.97; N, 21.82.

(26) 5-Nitro-1-methyl-3-phenylaminobenzimidazolium Iodide (7e). Compound **6e** (75 mg, 0.30 mmol) was dissolved in 2 mL of MeOH. CH_3I (75 μL, 1.20 mmol) was then added, and the mixture was heated at 50 °C for 12 h. Further addition of CH_3I (75 μL) and a subsequent 12 h reaction were repeated three times. After the solvent had been removed by evaporation, the product was separated by PLC (silica gel, 9/1 $CHCl_3$/MeOH) to obtain 36 mg (31%) of **7e**. Compound **7e** was unstable and decomposed gradually during purification by PLC: ^{1}H NMR (Me_2SO-d_6) δ 4.18 (s, 3H, CH_3), 6.91 (d, 2H, J = 8.4 Hz, 2′-H, 6′-H), 7.04 (t, 1H, J = 7.3 Hz, 4′-H), 7.31 (m, 2H, 3′-H, 5′-H), 8.33 (d, 1H, J = 2.2 Hz, 4-H), 8.39 (d, 1H, J = 9.3 Hz, 7-H), 8.57 (dd, J = 2.2, 9.3 Hz, 6-H), 10.30 (br s, 1H, NH), 10.41 (s, 1H, 2-H, H−D exchange occurred with the addition of D_2O); ^{13}C NMR (Me_2SO-d_6) δ 34.4 (1-CH_3), 108.9 (4-C), 113.9 (2′-C, 6′-C), 115.9 (7-C), 122.1 (6-C), 122.7 (4′-C), 129.5 (3′-C, 5′-C), 129.8 (3a-C), 134.9 (7a-C), 145.0 (1′-C), 145.8 (5-C), 149.3 (2-C).

(27) 1-Methyl-3-(4-nitrophenylamino)benzimidazolium Iodide (7f). A mixture of 1-acetylaminobenzimidazole (*18*) (**8**, 500 mg, 2.85 mmol), K_2CO_3 (100 mg), and 1-fluoro-4-nitrobenzene (0.6 mL, 5.66 mmol) in *n*-propanol (25 mL) was heated under reflux for 24 h. After the solvent had been removed by evaporation, the product was separated by PLC (silica gel, 19/1 $CHCl_3$/MeOH) to obtain 195 mg (27%) of 1-(4-nitrophenylamino)-benzimidazole (**9**) as a yellow powder: ^{1}H NMR ($CDCl_3$) δ 6.62 (d, 2H, J = 8.5 Hz, 2′-H, 6′-H), 7.24 (d, 1H, J = 7.9 Hz, 4-H), 7.32 and 7.36 (each m, each 1H, 5-H, 6-H), 7.85 (d, 1H, J = 7.9 Hz, 7-H), 8.05 (s, 1H, 2-H), 8.14 (d, 2H, J = 8.5 Hz, 3′-H, 5′-H).

1-(4-Nitrophenylamino)benzimidazole (**9**, 206 mg, 0.810 mmol) was dissolved in MeOH (5 mL). A large excess of CH_3I (10 mL) was then added, and the solution was heated at 50 °C for 3 days. After the solvent had been removed by evaporation, the product was separated by column chromatography (silica gel, $CHCl_3$ → 9/1 $CHCl_3$/MeOH) to obtain 254 mg (79%) of 1-methyl-3-(4-nitrophenylamino)benzimidazolium iodide (**7f**). Recrystallization from MeOH gave pale yellow plates: mp 201.5−203.5 °C; ^{1}H NMR ($CDCl_3$) δ 6.62 (d, 2H, J = 8.5 Hz, 2′-H, 6′-H), 7.24 (d, 1H, J = 7.9 Hz, 4-H), 7.32 and 7.36 (each m, each 1H, 5-H, 6-H), 7.75 (br s, 1H, NH), 7.85 (d, 1H, J = 7.9 Hz, 7-H), 8.05 (s, 1H, 2-H), 8.14 (d, 2H, J = 8.5 Hz, 3′-H, 5′-H); ^{13}C NMR ($CDCl_3$) δ 33.9 (1-CH_3), 112.3 (7-C), 113.0 (2′-C, 6′-C), 114.3 (4-C), 125.7 (3′-C, 5′-C), 127.1 (6-C), 127.4 (5-C), 129.8 (3a-C), 131.2 (7a-C),

141.4 (4′-C), 144.9 (2-C), 151.1 (1′-C); FAB MS *m/z* 269 [(M − I)$^+$], 137.

Heat Treatment of Compound 7 in H_2O/MeOH and Formation of 5-Substituted 1-Methyl-2-oxo-2,3-dihydrobenzimidazole (10d,e). Compound **7** (0.01 mmol) dissolved in 2 mL of H_2O/MeOH (1/1 v/v) was heated at 80 °C for 48 h in a round-bottomed flask fitted with a condenser. Product analysis was carried out by HPLC. The Merck LiChrosphere 100 RP-18e, 4 mm × 250 mm column was eluted at a flow rate of 0.6 mL/min with a 0.15 M phosphate buffer (pH 7.0)/MeOH system by applying a linear gradient of MeOH (20% at 0 min to 80% at 30 min), followed by 80% MeOH. Products were observed with only **7d** and **7e**, and were 1-methyl-2-oxo-5-(trifluoromethyl)-2,3-dihydrobenzimidazole (**10d**) and 1-methyl-5-nitro-2-oxo-2,3-dihydrobenzimidazole (**10e**) (*11*), respectively.

1-Methyl-2-oxo-5-(trifluoromethyl)-2,3-dihydrobenzimidazole (10d). Compound **7d** (70 mg, 0.17 mmol) was dissolved in a solution of H_2O (5 mL) and MeOH (1 mL), which was then heated at 80 °C for 7 days. Products were separated by PLC (silica gel, 9/1 $CHCl_3$/MeOH). Yields of 1-methyl-2-oxo-5-(trifluoromethyl)-2,3-dihydrobenzimidazole (**10d**) and starting material were 11 mg (35%) and 43 mg (61%), respectively: ^{1}H NMR for **10d** (*19*) (Me_2SO-d_6) δ 3.40 (s, 3H, CH_3), 6.98 (d, 1H, J = 8.1 Hz, 7-H), 7.28 (s, 1H, 4-H), 7.33 (d, 1H, J = 8.3 Hz, 6-H), 9.36 (br s, 1H, NH); HRMS *m/z* M$^+$ calcd for $C_9H_7F_3N_2O$ 216.0511, found 216.0509.

Rate of Decomposition of Compound 7 in Alkaline Media. MeOH solutions (20 mM) of compounds **7a**, **7b**, and **7d** were prepared. After an equal volume of aqueous 0.1 N NaOH had been added, the solution was kept at 40 °C. At appropriate times, 100 μL of the solution was removed, and 100 μL of aqueous 0.1 N HCl was added to stop the decomposition. MeOH (800 μL) was then added, and the solution was subjected to HPLC analysis. The Merck LiChrosphere 100 RP-18e, 4 mm × 250 mm column was eluted at a flow rate of 0.6 mL/min with a 1/15 M phosphate buffer (pH 6.8)/MeOH system by applying a linear gradient of MeOH (50% at 0 min to 80% at 15 min), followed by 80% MeOH.

Alkaline Treatment of Compound 7 and Formation of 4-Substituted *N*^{1}-Methyl-2-phenylazoaniline (11a−e). Compounds **7a**−**e** (0.075 mmol) were dissolved in a solution of aqueous 1 N NaOH (3 mL) and MeOH (4.5 mL), and this was left at 20 °C for 5 h. The products were extracted with $CHCl_3$ (3 × 30 mL), dried over $MgSO_4$, and separated by PLC (silica gel, $CHCl_3$). 4-Substituted *N*^{1}-methyl-2-phenylazoaniline (**11a**−**e**), the major product which eluted the earliest, was collected.

^{1}H NMR for **11a** (R = CH_3) ($CDCl_3$) δ 2.26 (s, 3H, 4-CH_3), 2.91 (s, 3H, N-CH_3), 6.63 (d, 1H, J = 8.5 Hz, 6-H), 7.08 (dd, 1H, J = 1.2, 8.5 Hz, 5-H), 7.30 (t, 1H, J = 7.3 Hz, 4′-H), 7.40 (m, 2H, 3′-H, 5′-H), 7.59 (br s, 1H, 3-H), 7.73 (d, 2H, J = 7.6 Hz, 2′-H, 6′-H), 8.31 (br s, 1H, NH); MS *m/z* 225 (M$^+$); HRMS *m/z* M$^+$ calcd for $C_{14}H_{15}N_3$ 225.1267, found 225.1265.

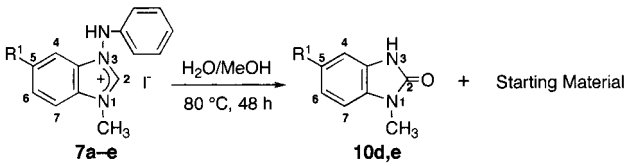
^{1}H NMR for **11b** (R = H) ($CDCl_3$) δ 3.00 (s, 3H, CH_3), 6.78 (d, 1H, J = 7.6 Hz, 6-H), 6.79 and 7.32 (each t, each 1H, each J = 7.6 Hz, 4-H and 5-H), 7.39 (t, 1H, J = 7.3 Hz, 4′-H), 7.48 (t, 2H, J = 7.3 Hz, 3′-H, 5′-H), 7.81 (d, 2H, J = 7.3 Hz, 2′-H, 6′-H), 7.86 (d, 1H, J = 7.6 Hz, 3-H), 8.69 (br s, 1H, NH); MS *m/z* 211 (M$^+$); HRMS *m/z* M$^+$ calcd for $C_{13}H_{13}N_3$ 211.1111, found 211.1113.

^{1}H NMR for **11c** (R = F) ($CDCl_3$) δ 2.99 (s, 3H, CH_3), 6.71 (dd, 1H, J = 4.6, 9.3 Hz, 6-H), 7.09 (m, 1H, 5-H), 7.41 (t, 1H, J = 7.1 Hz, 4′-H), 7.49 (m, 2H, 3′-H, 5′-H), 7.55 (dd, 1H, J = 3.2, 9.5 Hz, 3-H), 7.82 (d, 2H, J = 7.6 Hz, 2′-H, 6′-H), 7.89 (br s, 1H, NH); MS *m/z* 229 (M$^+$); HRMS *m/z* M$^+$ calcd for $C_{13}H_{12}FN_3$ 229.1017, found 229.1019.

^{1}H NMR for **11d** (R = CF_3) ($CDCl_3$) δ 2.97 (d, 3H, J = 5.1 Hz, CH_3), 6.76 (d, 1H, J = 9.0 Hz, 6-H), 7.36 (t, 1H, J = 7.1 Hz, 4′-H), 7.43 (m, 3H, 5-H, 3′-H, 5′-H), 7.76 (d, 2H, J = 7.8 Hz, 2′-H, 6′-H), 8.06 (s, 1H, 3-H), 8.88 (br d, 1H, NH); MS *m/z* 279 (M$^+$); HRMS *m/z* M$^+$ calcd for $C_{14}H_{12}F_3N_3$ 279.0984, found 279.0977.

^{1}H NMR for **11e** (R = NO_2) ($CDCl_3$) δ 3.11 (d, 3H, J = 5.1 Hz, CH_3), 6.79 (d, 1H, J = 9.4 Hz, 6-H), 7.51 (m, 3H, 3′-H, 4′-H, 5′-H), 7.85 (dd, 2H, J = 1.1, 8.1 Hz, 2′-H, 6′-H), 8.20 (dd, 1H, J = 2.6, 9.4 Hz, 5-H), 8.79 (d, 1H, J = 2.6 Hz, 3-H), 9.31 (br, 1H, NH); MS *m/z* 256 (M$^+$); HRMS *m/z* M$^+$ calcd for $C_{13}H_{12}N_4O_2$ 256.0960, found 256.0961.

Table 1. Heat Treatment of Compound 7 in H_2O/MeOH[a]

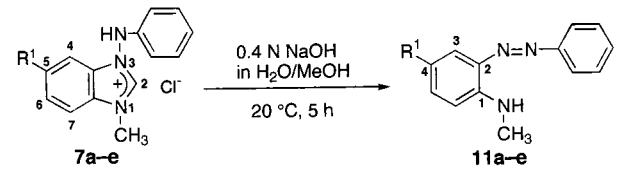


7a–e 10d,e

R^1	**10**	SM	R^1	**10**	SM
(a) CH_3	0%	100%	(d) CF_3	7%	67%
(b) H	0%	100%	(e) NO_2	51%	26%
(c) F	0%	100%			

[a] Compound **7** (0.01 mmol) was dissolved in 2 mL of H_2O/MeOH (1/1 v/v), and the mixture was heated at 80 °C for 48 h in a round-bottomed flask fitted with a condenser. Product analysis was carried out by HPLC.

Table 2. Alkaline Treatment of Compound 7[a]



7a–e 11a–e

R^1	**11**	R^1	**11**
(a) CH_3	53%	(d) CF_3	85%
(b) H	47%	(e) NO_2	75%
(c) F	82%		

[a] Compound **7** (0.075 mmol) was dissolved in a mixture of aqueous 1 N NaOH (3 mL) and MeOH (4.5 mL), and the solution was left at 20 °C for 5 h. Products were extracted with $CHCl_3$ and separated by HPLC.

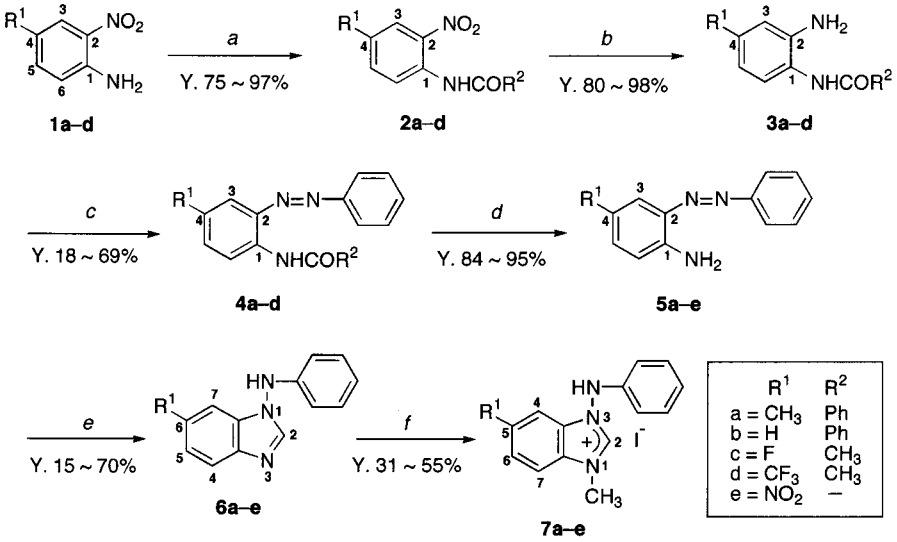
Properties of 1-Methyl-3-(4-nitrophenylamino)benzimidazolium Iodide (7f). Compound **7f** was treated using conditions similar to those used for compounds **7a**−**e** (Tables 1 and 2).

(1) Heat Treatment in H_2O/MeOH. Compound **7f** (102 mg, 0.257 mmol) was dissolved in 5 mL of H_2O/MeOH (1/1 v/v), and the mixture was heated at 80 °C for 2 days in a round-bottomed flask fitted with a condenser. The product was separated by PLC (silica gel, 9/1 $CHCl_3$/MeOH). 1-(4-Nitrophenylamino)benzimidazole (**9**) was obtained in a 6.8 mg (10.4%) yield, and the starting material was recovered in a 90.5 mg (91%) yield.

(2) Alkaline Treatment. Compound **7f** (19.8 mg, 50.0 μmol) was dissolved in a solution of aqueous 1 N NaOH (2 mL) and MeOH (3 mL), and this was then left at 20 °C for 5 h. After the solution had been neutralized with 1 N HCl, the products were extracted with $CHCl_3$ and purified by PLC (silica gel, 9/1 $CHCl_3$/MeOH). *N*^{1}-Formyl-*N*^{1}-methyl-2-(4-nitrophenylazo)aniline (**12**), the major product, was obtained in a 12.3 mg (87%) yield together with a trace amount of *N*^{1}-methyl-2-(4-nitrophenylazo)-aniline (**11f**). Data for **12**: ^{1}H NMR ($CDCl_3$) δ 3.46 (s, 3H, CH_3), 7.45 (d, 1H, J = 7.7 Hz, 6-H), 7.46 and 7.65 (each m, each 1H, 4-H and 5-H), 7.89 (d, J = 8.1 Hz, 3-H), 8.02 (d, 2H, J = 9.2 Hz, 2′-H, 6′-H), 8.30 (s, 1H, CHO), 8.39 (d, 2H, J = 9.2 Hz, 3′-H, 5′-H); MS *m/z* 284 (M$^+$); IR (KBr, cm^{-1}) 1681 (C═O), 1517 (NO_2). Data for **11f**: ^{1}H NMR ($CDCl_3$) δ 3.05 (s, 3H, CH_3), 6.82 (d, 1H, J = 8.6 Hz, 6-H), 6.83 and 7.38 (each m, each 1H, 4-H and 5-H), 7.88 (d, J = 8.0 Hz, 3-H), 7.92 (d, 2H, J = 9.0 Hz, 2′-H, 6′-H), 8.35 (d, 2H, J = 9.2 Hz, 3′-H, 5′-H), 9.20 (br s, 1H, NH); MS *m/z* 256 (M$^+$).

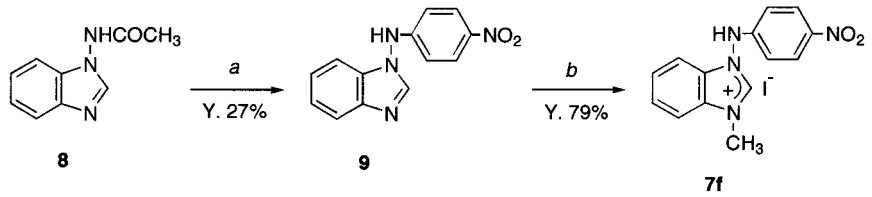
1-(*N*-Acetyl-*N*-phenylamino)-3-methylbenzimidazolium Iodide (13). A solution containing compound **6b** (1.50 g, 7.17 mmol), acetic anhydride (4 mL, 42.4 mmol), and pyridine

Scheme 1[a]



[a] (a) BzCl/pyridine for **1a**,**b** and Ac_2O/AcOH for **1c**,**d**; (b) H_2, Pd-C; (c) Ph-NO, AcOH, EtOH; (d) KOH/MeOH; (e) HCHO/concentrated HCl/AcOH, 80 °C; (f) CH_3I/MeOH.

Scheme 2[a]



[a] (a) *p*-F-Ph-NO_2/K_2CO_3/DMF; (b) CH_3I/MeOH.

(4 mL) was left at 20 °C for 4 days. After the solvent had been removed by evaporation, the residue was recrystallized from MeOH to give 1.06 g (59%) of 1-(*N*-acetyl-*N*-phenylamino)-benzimidazole (**14**) as white needles: mp 161.5−163.0 °C; ^{1}H NMR ($CDCl_3$) δ 2.05 (bs, 3H, CH_3), 7.39 (m, 7H, 5-H, 6-H, Ph-H), 7.45 (d, 1H, J = 7.6 Hz, 4-H), 7.86 (d, 1H, J = 7.6 Hz, 7-H), 8.09 (s, 1H, 2-H); MS *m*/*z* 251 (M^+). Anal. Calcd for $C_{15}H_{13}N_3O$: C, 71.70; H, 5.21; N, 16.72. Found: C, 71.79; H, 5.29; N, 16.69. Compound **14** (1.01 g, 4.02 mmol) was dissolved in 20 mL of MeOH. CH_3I (0.5 mL, 8.04 mmol) was then added, and the mixture was heated at 50 °C for 10 h. After the solvent had been removed by evaporation, the product was separated by column chromatography (silica gel, $CHCl_3$ → 19/1 $CHCl_3$/MeOH → 9/1 $CHCl_3$/MeOH). Recrystallization from EtOH gave 415 mg (27%) of 1-(*N*-acetyl-*N*-phenylamino)-3-methylbenzimidazolium iodide (**13**) as a yellow powder: mp 201−203 °C; ^{1}H NMR (Me_2SO-d_6) δ 2.18 (s, 3H, N-CH_3), 4.16 (s, 3H, CH_3CO), 7.55 (m, 3H, 2′-H, 4′-H, 6′-H), 7.78 (m, 2H, 5-H, 6-H), 7.95 (m, 2H, 3′-H, 5′-H), 8.12 (d, 1H, J = 8.5 Hz, 4-H), 8.27 (dd, 1H, J = 2.1, 7.0 Hz, 7-H), 10.42 (s, 1H, 2-H, H−D exchange occurred with the addition of D_2O); FAB MS *m*/*z* 266 [$(M - I)^+$]. Anal. Calcd for $C_{16}H_{16}IN_3O$: C, 48.85; H, 4.07; N, 10.71. Found: C, 48.64; H, 4.14; N, 10.74.

1-Amino-3-methyl-6-(trifluoromethyl)benzimidazolium Chloride (15). 1-Methyl-5-(trifluoromethyl)benzimidazole (100 mg, 0.500 mmol) and *O*-(2,4-dinitrophenyl)hydroxylamine (370 mg, 1.86 mmol) were dissolved in 10 mL of DMF, and the mixture was left at 40 °C for 48 h. After DMF had been removed by evaporation, 10 mL of aqueous 1 N HCl was added, and the mixture was washed with AcOEt (3 × 5 mL). The aqueous layer was evaporated to dryness, and residues were recrystallized from MeOH to yield 16.1 mg (13%) of 1-amino-3-methyl-6-(trifluoromethyl)benzimidazolium chloride (**15**): mp 243.5−245.5 °C; ^{1}H NMR (Me_2SO-d_6) δ 4.05 (s, 3H, CH_3), 7.07 (br s, 2H, NH_2), 8.06 (d, 1H, J = 8.8 Hz, 5-H), 8.26 (d, 1H, J = 8.8 Hz, 4-H), 8.35 (s, 1H, 7-H), 9.96 (s, 1H, 2-H); FAB MS *m*/*z* 216 [$(M - Cl)^+$].

Results and Discussion

Syntheses and Spectroscopic Data of Compound 7. Syntheses of 1-methyl-3-phenylaminobenzimidazolium iodide derivatives **7a**−**f** were achieved in two ways as shown in Schemes 1 and 2. The syntheses of compounds **7a**−**e** using a cyclization procedure are demonstrated in Scheme 1. Compounds **5a**−**d** were prepared in several steps starting from compound **1** or **2**. Compound **5e** was selectively prepared by the reaction of 4-nitro-1,2-phenylenediamine with nitrosobenzene. Cyclization of **5a**−**e** with HCHO gave **6a**−**e**, respectively, and subsequent treatment with CH_3I resulted in selective methylation at the N3 position to afford **7a**−**e**, respectively. Compound **7e** is unstable and decomposed gradually during purification by PLC. The synthesis of compound **7f** by direct nitrophenylation is demonstrated in Scheme 2. As shown, 1-acetylaminobenzimidazole (**8**) was directly 4-nitrophenylated at the *N*-amino group with 4-nitrofluorobenzene to afford 1-(4-nitrophenylamino)benzimidazole (**9**), and its subsequent methylation with CH_3I gave **7f**. In this case, a large excess of CH_3I was required to obtain the product. When 1-aminobenzimidazole was used instead of **8**, no reaction proceeded. Assignment of ^{1}H and ^{13}C NMR signals of compounds **5e** and **6e** was carried out by means of HMBC correlation. Assignment of ^{1}H and ^{13}C NMR signals of compounds **7a**−**f** was carried out using ^{1}H−^{13}C COSY.

The spectroscopic data of compound **7** are described in Experimental Procedures. In ^{1}H NMR spectra, the 2-H

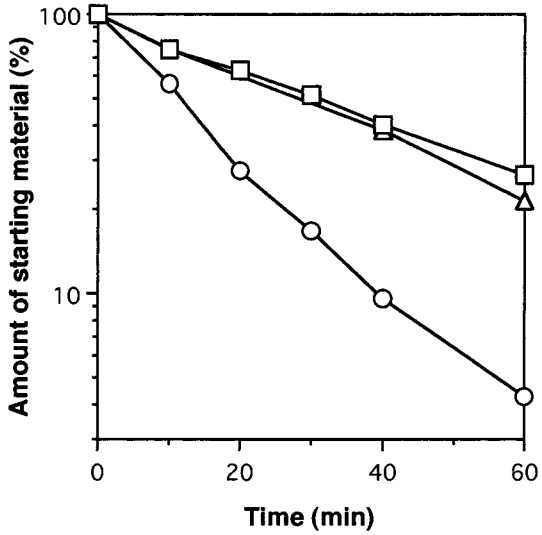


Figure 2. Rates of decomposition of compounds **7a**, **7b**, and **7d** (10 mM) under conditions of 0.05 N NaOH in H_2O/MeOH (1/1 v/v) at 40 °C. The decrease in the amount of starting material was measured by HPLC: **7a** (□), **7b** (△), and **7d** (○).

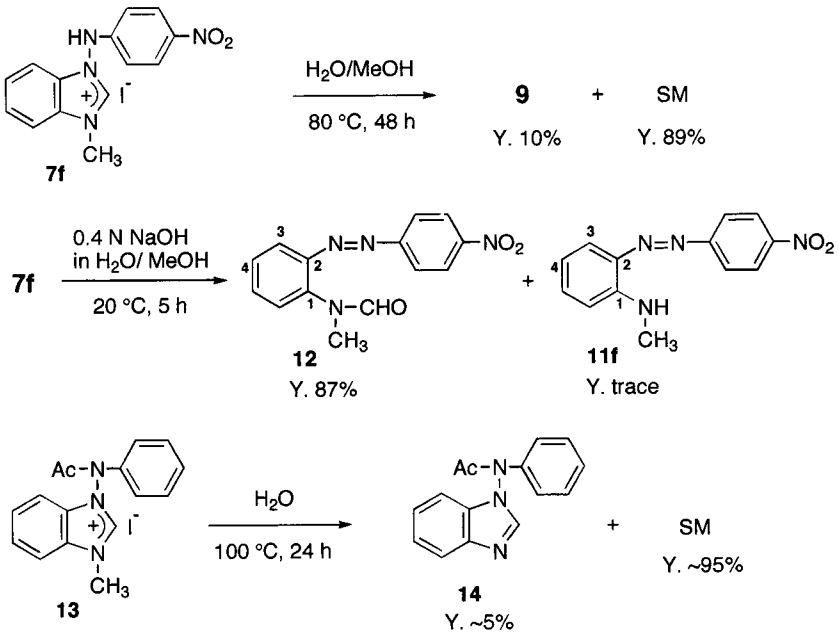
protons of **7a**−**e** resonated at a lower field (δ 10.02−10.41) and were exchanged for D with the addition of D_2O. The chemical shift values of these protons increased with compounds having a more electron-withdrawing substituent. When the chemical shift values of these 2-H protons were plotted against Hammett's σ values, a very good correlation was observed with σ_{para} ($r^2 = 0.984$), but there was less of a correlation with σ_{meta} ($r^2 = 0.886$). When similar plots were made of the chemical shift values of the 2-C of **7a**−**e** from ^{13}C NMR spectra and σ values, very good correlations were observed with both σ_{para} ($r^2 = 0.968$) and σ_{meta} ($r^2 = 0.961$). FAB MS spectra of compound **7** showed the characteristic peaks of $(M - I)^+$ and $[(M - I)^+ - PhN]$.

Stability of Compound 7. To compare levels of stability among compounds **7a**−**e**, they were first treated under mild conditions, such as heating at 80 °C for 48 h in H_2O/MeOH (1/1 v/v), and the products were analyzed by HPLC (Table 1). Product formation was observed with only **7d** and **7e**, the compounds with a strong electron-withdrawing substituent. The products were 2,3-dihydro-2-oxo-5-(trifluoromethyl)benzimidazole (**10d**) and 2,3-dihydro-5-nitro-2-oxobenzimidazole (**10e**), respectively. The yield of the latter (51%) was higher than that of the former (7%), which indicates that compound **7e** which has a stronger electron-withdrawing nitro substituent is more unstable (or reactive). For structural identification of compound **10d**, a large amount of this compound was obtained by a large-scale reaction of **7d** under more extreme conditions, and its structure was then determined on the basis of spectroscopic data. The structure of compound **10e** was identified by comparing spectral data with those of the authentic sample (*11*). The mechanisms responsible for the formation of 2-oxo derivatives (**10d**,**e**) involved nucleophilic attack of OH^- at C2 of **7d**,**e**, and subsequent removal of the phenylamino group.

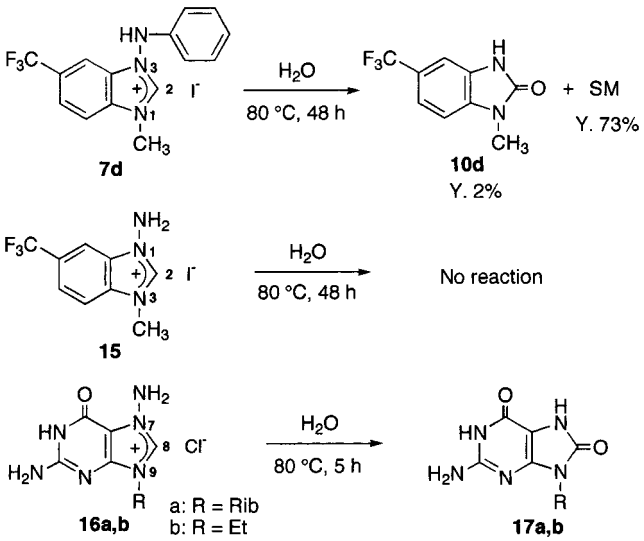
The stability of compound **7** at 40 °C under stronger alkaline conditions (0.05 N NaOH) was examined using **7a**, **7b**, and **7d**, and the reduction in the amount of compound **7** was examined by HPLC. As shown in Figure 2, the amount of compound **7** decreased according to pseudo-first-order kinetics, with the amount of compound **7d** decreasing the fastest ($k = 5.90 \times 10^{-2}$ min^{-1} and $t_{1/2} = 11.8$ min). No remarkable difference was observed between **7a** and **7b**, and their k and $t_{1/2}$ values were 2.11×10^{-2} min^{-1} and 32.9 min and 2.51×10^{-2} min^{-1} and 27.6 min, respectively.

Products Formed by Alkaline Treatment of Compound 7. Compounds **7a**−**e** were left at 20 °C for 5 h in a solution of H_2O/MeOH containing 0.4 N NaOH. Products were separated and purified by PLC. Major products were 4-substituted N^1-methyl-2-phenylazoanilines (**11**) (Table 2), the derivatives of compound **5**, and their structures were determined by NMR and MS spectroscopy. The mechanisms responsible for product formation may involve OH^- attack at the C2 of compound **7a**−**e**, with subsequent ring opening of the imidazole moiety and removal of the formyl group.

Scheme 3



Scheme 4



Properties of 1-Methyl-3-(4-nitrophenylamino)-benzimidazolium Iodide (7f). Compound **7f** has the same structure as compound **7b**, except that a nitro group is present at the para position of the phenylamino group. When compound **7f** was treated under the same conditions as for compound **7a**−**e** and the product was analyzed, it was found that different types of reactions had proceeded. Heating **7f** in H_2O/MeOH at 80 °C afforded a demethylated product **9** in a 10% yield (Scheme 3). Unlike the 1-phenylaminobenzimidazolium moiety, the 1-(4-nitrophenylamino)benzimidazolium moiety may be a good leaving group and afforded compound **9** and MeOH by heating in H_2O/MeOH. Similar types of reactions were observed when 1-(*N*-acetyl-*N*-phenylamino)-3-methylbenzimidazolium iodide (**13**) was heated at 100 °C in H_2O for 1 day, conditions under which demethylation proceeded to give 1-(*N*-acetyl-*N*-phenylamino)benzimidazole (**14**). The *p*-nitro group of **7f** and the acetyl group of **13** serve to decrease the electron density of the $\equiv N^+-N=$ moiety, which may favor a good leaving group status for the benzimidazolium moiety. Alkaline treatment of compound **7f** also gave a different type of product **12** in which the formyl group remained, together with a trace amount (~5%) of **11f**, the usual deformylated product.

Comparison of the Reactivity between the 3-Phenylamino and 3-Amino Derivatives of 3-Substituted 1-Methyl-5-(trifluoromethyl)benzimidazolium Salts (7d, 15). We reported previously on the chemical characteristics of N7-aminoguanosinium salt (*2*, *4*, *5*) and 1-amino-3-methylbenzimidazolium salts (*10*). Carcinogens such as arylamines and nitroarenes generate arylaminated adducts in cellular DNA. Therefore, the effect of the phenyl moiety at the amino group on the reaction was examined using compounds **7d** and **15**. Both compounds were heated at 80 °C for 2 days in H_2O, and the products were analyzed. As already shown, compound **7d** gave small amounts (2%) of **10d**; however, compound **15** was stable under these conditions. On the other hand, 7-aminoguanosinium salt (**16a**) and 7-amino-9-ethylguaninium salt (**16b**) gave 7,8-dihydro-8-oxoguanosine (**17a**) and 7,8-dihydro-9-ethyl-8-oxoguanine (**17b**), respectively, in almost quantitative yields under the same conditions (*4*, *10*). Values for the chemical shift in Me_2SO-d_6 of 2-H of compounds **7d** and **15** were 10.23 and 9.96 ppm, respectively. The phenyl group appears to withdraw electrons, facilitating the attack of OH^- at the C2 position. On the other hand, the value of the chemical shift of 8-H of compound **16** was 8.9−9.2 ppm, and this value is smaller than that of compound **15**. Nevertheless, the reaction of compound **16** proceeded more rapidly. Factors other than electronic mechanisms may be involved in the reaction.

In this study, we elucidated some of the chemical characteristics of the imidazole moiety of benzimidazole derivatives; however, we could not detect the formation of 1-methyl-2-phenylaminobenzimidazoles, the products formed by transfer of the *N*-phenylamino group to carbon. The question of whether the N to C transfer reaction requires a guanine structure or a polycyclic aryl moiety remains. Quite recently, a report was published by Guengerich's group focusing on a similar subject using N7-aminoguanosinium salt (*20*). This group also failed to clarify the mechanisms involved, despite their many attempts. Many gaps remain in our understanding of this topic, but further effort may finally ensure that we reach our goal.

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Supporting Information Available: Correlation curves between the values of the chemical shift of 2-H or 2-C of compounds **7a**−**e** and Hammett's σ_{para} (Figure S1). This material is available free of charge via the Internet at http://pubs.acs.org.

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